

Initial salinity tolerance and ion-osmotic parameters in juvenile Russian Sturgeon, *Acipenser gueldenstaedtii*, Brandt, 1833

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Received: September 2010

Accepted: January 2011

Abstract

The salinity tolerance, hematological and hydromineral regulation capabilities of juvenile Russian sturgeon (*Acipenser gueldenstaedtii*) were investigated in different ages and sizes in freshwater (FW: <0.5‰), estuary water (EW: 9.5‰) and Caspian Sea water (C_SW: 12.5‰). The fish were directly transferred from FW to EW and C_SW. The possible repercussions of ion-osmoregulatory processes on some classical indicators were examined at the end of 168 hours fish salinity tolerance. The mortality was not more than 35% in EW and C_SW in both groups. The survival percent and salinity tolerance were increased with increment of age and size of fish. The functional mechanism of osmotic and ionic homeostasis were similar in all groups (p>0.05) but differed in experimental media (p<0.05). Significant differences were observed between the levels of plasma ion concentrations in different media (p<0.01). Plasma Na⁺, K⁺ and Ca⁺² concentrations were higher than those of FW media, but lower than in C_SW media (p<0.05). Plasma Mg⁺² concentrations were lower than those of FW and C_SW media, but near to EW media (p<0.05). The hematocrit mean cell volume decreased but mean cell hemoglobin concentration, osmolarity and levels of plasma cortisol were increased from FW media to C_SW media (p<0.05). The hemoglobin concentration (Hb), the amount of hemoglobin per erythrocyte, red and white blood cells count did not change (p>0.05). However, the results showed that the above parameters in fish fingerlings did not return to initial values in the new environment and then physiological changes happened.

Keywords: *Acipenser gueldenstaedtii*, Hematological parameters, Ion, Cortisol, Osmolarity

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Introduction

The Russian Sturgeon, *Acipenser gueldenstaedtii* is found in Iranian coastal waters from west to east (Astara River to Gorgan River) as reported by Abbasi et al., (1999) and Kiabi et al., (1999). Access to many rivers is now restricted by reduced water flow, river fragmentation, irrigation canals and pollution.

Most sturgeons are euryhaline, breeding in fresh water but spending most of their lives in the sea (Ta'ati et al., 2011). Some sturgeons, such as *Acipenser fulvescens* of Great Lakes and the paddlefishes of Mississippi basin, are confined to fresh water. The ability to live in sea water must have been acquired independently by the sturgeons but as far as it is known, fishes maintain similar blood concentrations equivalent to about 30‰ in both sea water and fresh water (Potts and Rudy, 1972;). Their activities differ among the organs and tissues of freshwater and marine fish (Wdzieczak et al., 1982), depending on feeding behaviour (Radi and Matkovic, 1988) and environmental factors (Winston and Di Giulio, 1991; Roche and Boge, 1996). Therefore, one of the important physiological changes in fish is their acclimation and survival at different degrees of salinity (Farabi et al., 2007; Farabi et al., 2009).

Some aspects of these osmoregulatory processes (plasma osmolarity, Na⁺, K⁺, Mg²⁺, Ca²⁺ concentrations, cortisol level, 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 the Caspian Sea (Krayushkina, 1974;

Krayushkina et al., 1996; Krayushkina and Semenova, 2006), in *A. transmontanus* (McEnroe and Cech, 1985), in *A. naccari* (Cataldi et al., 1995, 1998; Sanchez de Lamadrid et al., 2000), in *A. bravirostrum* and *A. oxyrhynchus* (Krayushkina, 1998; Krayushkina, et al., 2001), in *A. persicus* (Jabbarzadeh et al., 2000; Kazemi et al., 2003; Rad Sadeghi et al., 2009), in *Huso huso* and *A. nudiventris* from Southern parts of the Caspian Sea (Farabi et al., 2007 and 2009). The purpose of the present study was the determination of initial salinity tolerance and ion-osmotic parameters in *A. gueldenstaedtii* fingerlings (different age and size) at the time of releasing to the natural environment from Southern parts of the Caspian Sea.

Materials and methods

Fish were sampled from the fish hatchery center of Shaheed Marjani in the southern part of the Caspian Sea. The experiments were carried out at Caspian Sea Ecology Research Center located in Sari city. The juveniles used in this experiment comprised of different age and size after starting feeding (Table 1). All treatments had three replications with 30 fingerlings. The primary objectives of this study were to determine the early tolerance of Russian Sturgeon juveniles to different salinities: estuary water (EW: 9.5‰) and the Caspian Sea water (C_SW: 12.5‰) with the effects of age and size. Juveniles were transferred directly from freshwater (FW: <0.5‰) to saline water (EW and CSW), using FW as control. Juveniles were not fed throughout the experimental period.

Table 1: Juveniles' *A. gueldenstaedtii* in different age and size at the time of releasing to natural environment

Groups	I	II	III	IV	V
	sample size = 30				
Age(days)	35	35	50	50	65
Weight (g)	2.78±0.15 ^e	3.49±0.27 ^d	6.07±0.61 ^c	8.65±0.77 ^b	12.53±1.88 ^a
Length (cm)	8±0.47 ^e	8.61±0.26 ^d	11.16±0.59 ^c	12.66±0.57 ^b	14.18±0.66 ^a

Note: The values are means±SD. and different superscript letters within each row are significantly different ($p < 0.01$)

Survival of juveniles (JS) was estimated by recording the number of dead fish at 3, 6, 12, 24, 48, 72, 120, 144 and 168 hours during the experimental period (Table 1). At the initiation of each experimental period, the total length (TL) and weight (TW) of fish were measured by a calibrated board (± 1 mm) and digital balance (± 0.1 g), 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 group at the end of each experimental period after 168h, blood samples were drawn from the surviving individuals by cutting peduncles with a heparinized micro-capillary tube.

Red blood cells (RBC), white blood cells (WBC), hematocrit (Hct) and hemoglobin concentration (Hb) were recorded in each group at the end of each experimental period after 168h. The plasma samples were extracted using centrifuge (Hettich-D7200 Tuttlingen: Germany) at 453.6 g 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 16329.6 g 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 hemoglobin concentration (Hb) was measured using the cyanmethemoglobin method with spectrophotometry (CECIL- CE1020: Germany) at 540 nm; mean cell volume (MCV) was computed as $MCV = Hct * 10 * RBC^{-1}$, mean cell hemoglobin concentration (MCHC) as $MCHC = Hb * 100 * Hct^{-1}$, and the amount of hemoglobin per erythrocyte (MCH) as $MCH = Hb * 10 * RBC^{-1}$, (Ameri Mahabadi, 1999; Blaxhall and Daisley, 1973). Plasma osmolarity was measured using the cryoscopy method by an osmometer (Roebbling Nr.9610003.Type 13: Germany). Sodium and potassium concentrations were determined with flame photometer (Corning 405C: IRI); magnesium and calcium concentrations were measured with an absorption spectrophotometer (UNICO 3115233: USA). Plasma cortisol was assayed on a competitive enzyme immunoassay (Stat

fax – Avernest, 330plus: USA). This test was used with automatic instrument for ELISA kits on microplate. The water for the test was supplied from Caspian Sea offshore as well as the Tajan estuary. Water salinity was measured by a salinimeter (Electrosolemer, GM-65M: Russia). Every experimental tank was well aerated by using an aerator. The experimental tanks were maintained at room temperature ($20\pm 1^\circ\text{C}$) and their water was replaced every 12 h to prevent accumulation of ammonia and other metabolites. The differences in parameters were tested for significance by a one-way analysis of variance (ANOVA) using SPSS.V10. Subsequent significances between groups were delineated by Duncan's test. A value of $P<0.01$ or $P<0.05$ were taken as the significance level.

Results

The survival of juveniles was higher than 50% in all groups (different age and size) in EW and C_5W media with 168 hours exposure. The survival increased with the increment of age and size of fish after their transfer to EW and C_5W for 168 hours exposure time (Figure 2). The FW juveniles of *A. gueldenstaedtii* with different sizes and ages had a blood Hct of $32.64\pm 0.34\%$, an average plasma osmolarity of 243.96 ± 0.78 mOsm/l, and plasma ion concentrations of 124.28 ± 0.35 mEq/l for Na^+ , 1.77 ± 0.01 mEq/l for K^+ , 4.91 ± 0.01 mEq/l for Ca^{+2}

and 0.62 ± 0.01 mEq/l for Mg^{+2} (Table 2 and 3). At 168 hours after the transfer of FW fish to different salinities (EW and C_5W), plasma osmolarity, ion concentration, MCHC, plasma cortisol levels had increased and Hct, Hb, MCV had decreased significantly ($p<0.05$) (Table 2 and 3). The ion concentrations in blood plasma as well as the three media showed significant differences ($p<0.01$), (Table 3). There were no significant differences in ion plasma concentrations among different age and size groups in different media (FW, EW and C_5W), ($p<0.05$), (Table 3). Sodium concentration in plasma was higher than in FW and EW, but it was lower than in C_5W (Table 3). The potassium concentrations in plasma were higher than in FW, but it was lower than in EW and C_5W (Table 3). The calcium concentrations in plasma were higher than in FW and EW but, it was lower than in C_5W (Table 3). The magnesium concentrations in plasma were lower than in FW, EW and C_5W (Table 3). The significant differences of osmolarity has been shown between three experimental media and blood plasma ($p<0.01$), (Table 2). The blood plasma osmolarity of fish acclimated to FW was higher than the osmolarity of corresponding media, but it was lower than media of EW and C_5W (Table 2). Therefore, the juvenile's *A. gueldenstaedtii* were hypertonic in FW, while in FW and C_5W they were hypotonic.

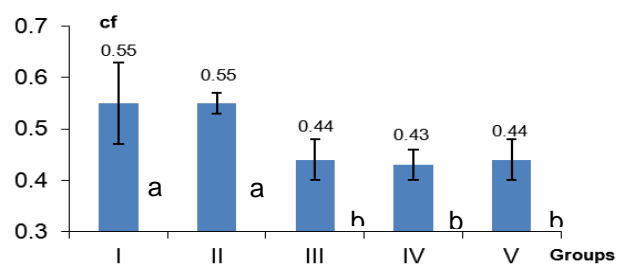


Figure 1: Comparative condition factor (CF) of juvenile *A. gueldenstaedtii* in different size/age at the start of test (The letters have shown significant difference: $p < 0.05$).

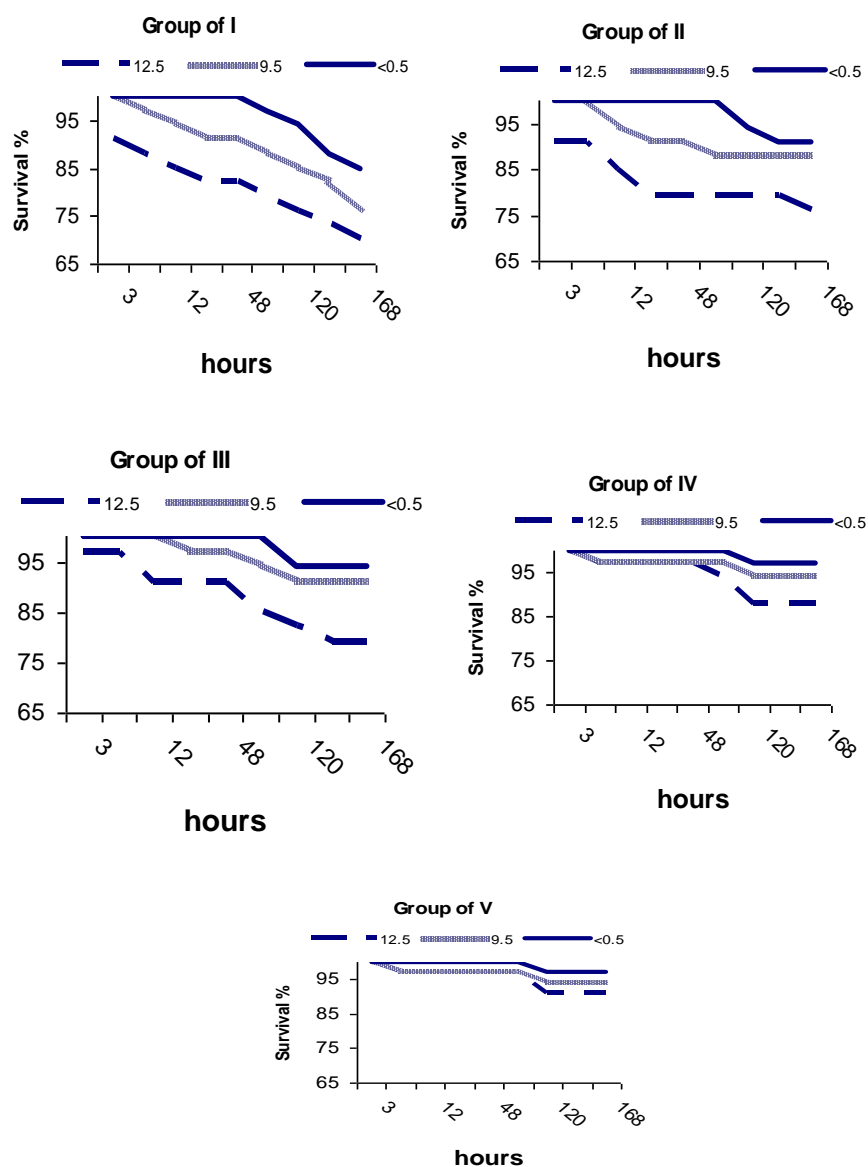


Figure 2: Survival of juvenile *A. gueldenstaedtii* in different size/age groups and different media (FW: <math>< 0.5\%</math>; EW: 9.5%; CSW: 12.5%) for 168 hours exposure (N=30, 3 replicates)

Table 2: Hematological parameters, concentration of cortisol and osmolarity in blood plasma of juvenile *A. guldenstaedtii* after acclimation for 168 hours to different media (FW :< 0.5‰; EW: 9.5‰; C_SW: 12.5‰)(Means ± SD)

Juvenile's group: n=10	Hct %	RBC 10 ³ cellsμl ⁻¹	WBC 10 ³ cellsμl ⁻¹	Hb g/dl	MCV fl	MCH pg	MCHC %	Cortisol ng/ml	Osmolarity mOsm/l
In FW: <0.5‰ and Osmolarity: 5±2 mOsm/l									
I .35days	32.80±1.55	626±38.35	14.5±3.34	5.30±0.85	524.51±13.95	84.32±9.37	16.11±2.04	19.59±1.7	244.5±4.01
II.35days	32.50±1.18	623±33.68	14.2±2.2	5.34±0.57	522.3±16.39	85.52±5.31	16.4±1.24	19.76±2.17	243.3±5.6
III .50days	31.1±0.99	623±65.83	14.2±2.89	5.39±0.76	535.26±42.38	86.32±6.08	16.24±1.93	19.55±1.86	243±3.13
IV .50days	32.2±1.03	639±80.2	14±2.74	5.36±0.39	508.82±43.85	84.42±5.74	16.63±0.78	19.39±1.17	244.2±5.54
V .65days	32.6±0.97	635±66.7	14.2±2.29	5.36±0.39	516.88±37.28	84.7±4.17	16.43±0.76	19.51±1.66	244.8±6.14
In EW: 9.5‰ and Osmolarity: 297.8±5.89 mOsm/l									
I .35days	29.9±1.1	620±44.72	14.1±3.24	5.29±0.9	483.56±21.49	84.95±9.72	17.63±2.46	32.15±2.93	280.6±3.24
II.35days	29.2±1.33	611±25.14	14.1±2.51	5.33±0.71	478.64±16.46	86.95±7.54	18.19±1.78	32.09±4.43	282.6±5.08
III .50days	30.1±1.66	620±69.44	14±2.16	5.38±0.68	488.59±32.28	86.74±4.47	17.82±1.39	32.25±4.35	280±5.75
IV .50days	30.2±0.79	628±56.92	14.2±2.59	5.26±0.75	483.49±32.26	83.47±5.69	17.37±2.08	32.4±3.37	283.9±3.78
V .65days	29.6±0.97	632±62.85	14.3±23.11	5.34±0.87	471.18±31.99	84.15±7.94	17.99±2.48	32.11±2.77	282.1±5.13
C_SW: 12.5‰ and Osmolarity: 407±2.92 mOsm/l									
I .35days	27.7±1.7	619±29.98	14.5±3.65	5.31±0.82	447.29±9.96	85.42±9.53	19.08±1.91	38.22±3.79	301.6±9.89
II.35days	26.9±1.91	632±25.73	14±2.54	5.29±0.97	425.23±15.5	83.29±12.46	19.54±2.48	38.34±2.76	303.1±8.4
III .50days	27±1.41	632±64.6	14.1±2.18	5.28±0.62	430±3385	83.45±2.78	19.51±1.6	38.61±2.96	300.9±8.41
IV .50days	26.6±1.43	633±34.65	14.1±2.77	5.26±0.49	420.33±9.01	82.98±4.43	19.76±1.24	38.47±4.6	303.2±6.75
V .65days	26.8±1.03	629±47.79	14.2±2.86	5.15±0.87	427.99±19.16	81.62±9.14	19.14±2.58	38.47±3.87	300.9±5.69

Table 3: Ion concentrations in blood plasma of juvenile *A. guldenstaedtii* and the exposure water after fish acclimation to different salinity for 168 hours. (Means ± SD)

Media	Fresh Water: <0.5‰ ^(c)				Estuary Water: 9.5‰ ^(b)				Caspian Sea Water: 12.5‰ ^(a)			
	Ion Concentration mEq/l				Ion Concentration mEq/l				Ion Concentration mEq/l			
Juvenile's group: n=10	Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺	Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺	Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺
I .35days	124.9±1.27	1.79±0.04	4.91±0.05	0.62±0.02	141.8±1.69	1.87±0.06	5.09±0.1	1.16±0.02	152.4±5.72	2.03±0.05	5.56±0.13	1.85±0.1
II.35days	124.2±2.86	1.77±0.06	4.92±0.07	0.62±0.09	141.8±3.26	1.86±0.07	5.11±0.11	1.16±0.05	152.8±5.69	2.01±0.05	5.59±0.14	1.84±0.11
III .50days	124.1±1.79	1.76±0.03	4.91±0.08	0.62±0.11	141.3±3.37	1.88±0.05	5.1±0.13	1.15±0.04	152.5±2.68	2.02±0.04	5.58±0.13	1.85±0.05
IV .50days	124.1±3.11	1.77±0.06	4.49±0.07	0.63±0.07	141.6±5.04	1.86±0.08	5.13±0.15	1.15±0.04	152.9±3.93	2.02±0.05	5.55±0.13	1.88±0.07
V .65days	124.1±3.11	1.76±0.05	4.49±0.11	0.63±0.08	141.7±5.14	1.86±0.07	5.12±0.12	1.16±0.03	152.8±3.88	2.01±0.05	5.59±0.15	1.86±0.08
Average:	124.28±0.35	1.77±0.01	4.91±0.01	0.62±0.01	141.64±0.21	1.87±0.01	5.11±0.02	1.16±0.01	152.68±0.22	2.02±0.01	5.57±0.02	1.86±0.02
Water: n=5	28.8±2.86 ^a	0.39±0.03 ^a	2.04±0.15 ^a	1.11±0.16 ^b	135.42±5.18 ^a	2.51±0.45 ^b	4.46±1.39 ^a	1.16±0.11 ^b	175.88±4.56 ^b	2.41±0.35 ^b	20.46±0.82 ^b	63.3±3.06 ^b
	^b	^b	^b	^a	^b	^a	^b	^a	^a	^a	^a	^a

Note: The values with different letter (between Average groups and water) within each column and first row (different media) have shown significantly difference (p<0.01, n= sample size)

Discussion

The main goal of sturgeon cultivation in the southern part of the Caspian Sea is producing fingerlings for restocking natural waters. The fingerling sturgeon needs to develop an ion-oregulatory mechanism to survive successfully in the time of migration from river to the sea. Fish cells must be in ion and osmotic equilibrium with environments. The environmental change can be considered as a potential source of stress and physiological changes (Donaldson, 1981).

The juvenile Russian sturgeon supported blood plasma osmolarity higher than FW and approximately similar to EW osmolarity, but lower than C_SW osmolarity (Table 2). The comparative results of this investigation in different salinities were similar to studies of *H. huso* and *A. nudiventeris* fingerlings in the southern part of the Caspian Sea (Farabi et al., 2007, 2009). But in this survey there were no statistically significant differences in size and age of *A. gueldenstaedtii* juveniles ($p > 0.05$). Also, the survival rate increased with increase in size and age (Farabi et al., 2007, 2009).

The ranking descending of ion plasma concentrations including sodium, calcium, potassium and magnesium in different media were similar to prior studies (Krayushkina, 1974; Krayushkina et al., 1996; Krayushkina and Semenova, 2006; Farabi et al., 2007, 2009). The potassium, calcium, magnesium and sodium blood concentrations gradually increased statistically significant from FW to EW and C_SW ($p < 0.05$), (Table 3). Also, the results showed that the juvenile Russian sturgeon regulated its blood ions concentration at lower than Levels of marine and brackish water teleost (Urist and Van de Putte, 1967; Holmes and Donaldson, 1969).

The Hct and MCV decreased and MCHC and plasma cortisol increased with increment of salinity ($p < 0.05$). Therefore, the Hb, MCH, RBC and WBC did not change ($p > 0.05$) in different size and ages of *A. gueldenstaedtii* juveniles (Chan et al., 1969; Mayer et al.,

1967; Nespolo and Rosenmann, 2002; Farabi et al., 2007 and 2009), (Table 2).

However, the results showed that the above parameters in *A. gueldenstaedtii* juvenile fingerlings did not return to initial values in the new environment and physiological changes happened. So, *Acipenser gueldenstaedtii* are able to regulate plasma ion and osmotic in the age of more than 35 days and weight of more than 2.78 ± 0.15 g.

Acknowledgements

Authors are grateful to all our colleagues in the Shaheed Marjani Sturgeon Propagation Center and the Caspian Sea Ecology Research center. Also our special appreciations go to Prof. Krayushkina and Dr. Hajimoradloo for their useful guidelines.

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تحمل اولیه شوری و پارامترهای یونی-اسمزی در تاسماهی روسی جوان *Acipenser gueldenstaedtii*, Brandt, 1833

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چکیده

تحمل شوری و توانائی تنظیم عوامل خونی و مواد معدنی در تاسماهی روسی با اندازه و سنین مختلف در آب شیرین (شوری: ۰,۵‰)، مصبی (شوری: ۹,۵‰) و لب شور دریای خزر (شوری: ۱۲,۵‰) مورد بررسی قرار گرفت. ماهیان مستقیماً از آب شیرین به آب مصبی و دریا انتقال داده شدند. پیامد های احتمالی فرآیند تنظیم یونی-اسمزی روی برخی از شاخص های وابسته در پایان ۱۶۸ ساعت تحمل شوری ماهیان مورد بررسی قرار گرفت. مرگ و میر در آب مصبی و دریا بیش از ۳۵٪ نبود. درصد بقاء و تحمل به شوری با افزایش سن و اندازه ماهی افزایش یافته بود. مکانیزم عملکردی هموستازی اسمزی و یونی در همه گروه ها مشابه بود ($p > 0,05$)، اما نسبت به شرایط محیطی مختلف، متفاوت بود ($p < 0,05$). تفاوت های معنی داری بین سطوح غلظت های یونی پلاسما در محیط های مختلف مشاهده گردید ($p < 0,01$). غلظت یون های سدیم، پتاسیم و کلسیم پلاسما ی آن ها در آب شیرین، بیشتر و در آب دریا کمتر بود ($p < 0,05$). غلظت یون منیزیم پلاسما ی آن ها در آب شیرین و دریا کمتر و نزدیک به محیط آب مصبی بود ($p < 0,05$). هماتوکریت و متوسط حجم سلولی کاهش، اما متوسط غلظت هموگلوبین سلولی، اسمولاریته و سطوح کورتیزول پلاسما از محیط آب شیرین به محیط آب دریا افزایش یافته است ($p < 0,05$). غلظت هموگلوبین، مقدار هموگلوبین در اریتروسیت و تعداد گلبول های قرمز و سفید تغییر نکرد ($p > 0,05$). بنابراین، نتایج نشان داد که پارامترهای فوق در بچه ماهیان در محیط جدید به سطوح مقادیر اولیه باز نگشت و در پی آن تغییرات فیزیولوژیک اتفاق افتاد.

واژگان کلیدی: تاسماهی روسی، پارامترهای هماتولوژی، یون، کورتیزول، اسمولاریته

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