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Original Article

Responses of beluga (*Huso huso*) to salinity exposure: a laboratory evaluation of the effect of field-based salinity levels on osmoregulatory characteristics and growth performance

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Abstract: There is a need for a better understanding of how sturgeon, especially hatchery reared juveniles, respond to salinity challenges. Therefore, here we examined the effects of different field-based salinities (Freshwater [FW] (0.5), 3, 6, 9 and 12 ppt) on osmoregulatory characteristics and growth performance of juvenile beluga sturgeon, *Huso huso*, (22.1±1.1 g body weight) over a 60-day period. Survival rate was relatively high in all treatments although there was a sign of adverse effects of salinity on the survival as fish at 12 ppt salinity. Growth performance was better in fish reared at 3 ppt, followed by 6, 9 and 12 ppt. Overall, an increase in plasma sodium, potassium, calcium, magnesium and glucose levels was found in association with the increase of salinity, while the FW control group maintained basal levels. Haematocrit levels were also affected by the salinity and the observed levels in FW, 3 and 6 ppt salinities were lower than other salinity concentrations. The results indicated that the beluga sturgeon juveniles are able to survive and acclimate to moderate salinities. Here, we also discussed the importance of evaluating and comparing specific mechanisms of acclimation in populations across brackish waters of the southern Caspian Sea as such investigations may aid and improve aquaculture strategies.

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Introduction

Sturgeons are migratory species that occur in major river systems of the northern hemisphere (Bemis and Kynard, 1997; Grande and Bemis, 1991), especially in the Caspian Sea basin. Most sturgeon species have been listed on the IUCN red list of endangered species due to the drastic declines in their wild populations (Birstein, 1993; Birstein et al., 1997; IUCN, 2012), and many are unfortunately at the brink of extinction. Overfishing, poaching, pollution and habitat degradation are the major threats to sturgeon longevity and sustainability (Pourkazemi, 2006; Ruban and Khodorevskaya, 2011). Many efforts have been made, especially in the past decade, to protect sturgeons, and aquaculture plans for restoration, conservation and commercial purposes have received the most attempt and attention. In Iran, Fisheries Organization has widely been involved in breeding programs to develop sturgeon aquaculture and restock sturgeon populations, so that thousands of sturgeon fingerlings are released into the Caspian Sea annually or kept for sturgeon rearing to meet meat and caviar demands.

Acipenseriformes are similar to teleosts with respect to the main features of their osmoregulation (Potts and Rudy, 1972; Krayushkina et al., 1995). Fish challenged with an altered environmental salinity must maintain their body osmolality and ionic balance. Therefore, it is necessary for both sturgeons and teleosts to maintain rather tight control of serum water and ion concentration for efficient physiological function when they move between fresh water and salt water (Natochin et al., 1985; Krayushkina et al., 1995). This is mainly accomplished by profound morphological and physiological changes, such as drinking rate (Tytler and Blaxter, 1988; Ura et al., 1996; Miyazaki et al., 1998), stress hormone levels,

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which can disturb hydromineral balance and blood parameters such as haematocrit (Woo and Chung, 1995; Wendelaar Bonga, 1997; Brown et al., 2001) and functions of the osmoregulatory surfaces (Hwang and Hirano, 1985; Hwang et al., 1989; Arai et al., 1997; Perry, 1998; Kelly and Woo, 1999).

There are a variety of studies assessing osmo-regulatory mechanisms and salinity tolerance in sturgeon species, and it has been documented that many sturgeons species are able to adapt different salinity ranges (McEnroe and Cech, 1985; Krayushkina, 1996; Krayushkina, 1998; Altinok et al., 1998; Martinz Alvarez et al., 2002; Jarvis and Ballantyne, 2003; Allen and Cech, 2007; He et al., 2009; Zhao et al., 2010). However, exposure length, fish size and age appear to play key roles in adaptation capacity as salinity tolerance may decrease especially when fish in smaller sizes are subjected to high salinities. It has previously been suggested that salinity can also affect fish growth, and an interaction between growth and salinity has been demonstrated in several fish species (Altinok and Grizzle, 2001; Wada et al., 2004; Martinez-Palácios et al., 2004; Tibblin et al., 2012). In tilapia, *Oreochromis mossambicus*, its rearing in sea water is resulted in an improved growth performance compared to freshwater (Kuwaye et al., 1993; Riley et al., 2003). Improved growth at intermediate salinity may be explained by a reduction of the metabolic cost for osmoregulation, whereas appetite and/or the endocrine system may also play a role (Boeuf and Payan, 2001). Sardella and Kultz (2009) demonstrated that green sturgeon, *Acipenser medirostris*, with a mean weight of 121 g were able to survive and acclimate following a salinity transfer and they observed minimal osmotic stress in the exposed fish. Juvenile Atlantic sturgeon, *A. oxyrinchus,* with a mean weight of 440 g were reared under three salinity conditions (0, 10, or 33 ppt) for 6 months and it was found that fish in 0 and 10 ppt grew more than those of 33 ppt (Allen et al., 2014). In contrast, after 30 days rearing, Zhao et al. (2010) did not observe significant effect of salinity exposure (up to 25 ppt) on the growth of 5-month-old Amur sturgeon, *A. schrenckii*, with a mean initial body weight of 106.8 g. However, growth performance in sturgeons under different saline environments appears to be species dependent and can also be impacted by the size of fish (Allen and Cech, 2007).

There are few studies on the effects of salinity on the Caspian Sea sturgeons, especially beluga sturgeon. Although previous studies have shown that sturgeons are capable of adopting different salinities, however, it has also been indicated that adaptation capacity in small juveniles reduces as they may not be able to tolerate even brackish waters (Jalali et al*.,* 2008). Thus, detailed information regarding the salinity effects and during a various exposure times can provide a better understanding of sturgeon responses to environmental challenges. It can also be a useful method for aiding aquaculture programs especially when the fish are kept for coastal-based-rearing and aquaculture purposes. Sea-cage-based and pen culture of sturgeons have recently been considered in the Iranian part of the Caspian Sea as it can provide an alternative to wild stocks and for supply of sturgeon meat and caviar. Thus, in this study we selected filed-based salinity doses of freshwater (FW), 3, 6, 9 and 12 ppt representing salinity ranges that the beluga are likely to encounter in the Iranian part of the Caspian Sea. We then aimed to assess osmoregulatory characteristics (including sodium, potassium, calcium and magnesium concentrations), survival and growth performance of beluga (*Huso huso*) juveniles to these salinities over two months, a relatively long experimental period.

Materials and Methods

Fish rearing conditions: Five months old beluga with a mean body weight of 22.1±1.1 g (mean±SD) were obtained from the Shahid Marjani Sturgeon Propagation Center (Aq Qala, Golestan Province, Iran). Fish were transferred to the Aquaculture Research Centre at the University of Gorgan, then stocked in five groups with triplicate per group (200 L tanks with a stocking density of 15 fish per tank), and cultured in FW (0.5), 3, 6, 9 and 12 ppt for 60 days. Acclimation to salinity was performed by increasing water salinity at an approximate rate of 3 ppt per day until reaching 12 ppt. Salinity levels were obtained by mixing dechlorinated tap water with salt, and measured by water checker (HORIBA U-10, Japan). Temperature was kept at 21°C, and supplemental aeration was also provided to maintain dissolved oxygen levels near saturation. The photoperiod was maintained at 13 h light /11 h dark. During the experiment, the fish were fed three times a day with the same commercial pellet diet (approximately 3% of body weight/day; 54% protein, 18% lipid, 11% ash and 0.3% fiber; Biomar Company). The amount of feed offered was daily recorded for food conversion ratio calculation. The tanks were siphoned daily to remove uneaten feed and feces. In each tank, half the water volume was renewed every day to assure water quality. It was tried to minimize any other stress during the entire period of the experiment.

Sampling and data analysis: At the end of the experiment, feeding was discontinued 24 hrs prior to measurements and all fish were weighed and growth parameters were calculated: specific growth rate ({[Ln final body weight-Ln initial body weight] × 100}/total days), feed conversion ratio (dry feed fed/body weight gain) and condition factor ({[body weight/body length (cm^3)] × 100}) (Lugert et al., 2014; Shalaby et al., 2006). Percent of survival were also calculated.

In order to evaluate the haematocrit (% PCV), ions (sodium [Na^+], potassium [K^+], calcium [Ca^{2+}] and magnesium [Mg^{2+}]) and glucose concentrations, the blood was collected from the caudal vein of individual fish employing heparinized syringes. Blood samples were centrifuged at 16000 g for 5 min in a clinical centrifuge (Hettich-D7200, Tuttlingen, Germany) for haematocrit evaluation. The blood plasma was decanted and pipetted into Eppendorf tubes and preserved at -20°C. In order to evaluate how different salinities impact plasma ion concentrations, sodium (Na^+) and potassium (K^+) concentrations were measured with flame photometer (Corning 405C: IRI). Magnesium (Mg^{2+}), calcium (Ca^{2+}) and glucose concentrations were measured with an absorption spectrophotometer (UNICO 3115233: USA) (Hoseini et al., 2011).

Statistical analysis: Data were initially checked for normality and homogeneity of variance (using Bartlett and Kolmogorov-Smirnov tests) and then salinity was considered as the independent variable and fish haematological, biochemical and growth parameters as the dependent variables. Data were analyzed by one-way analysis of variance (ANOVA) with Duncan's new multiple range tests (SPSS software version 18). Statistical values are expressed as mean±SD. The values of $P<0.05$ were considered significantly different.

Results

Biochemical and haematological variables: The blood plasma electrolytes, including sodium, potassium, calcium, magnesium and glucose concentrations were affected by increasing the salinity, and significant differences were observed among treatments ($P<0.05$). Plasma sodium concentration was higher in the fish reared at 9 and 12 ppt salinities and there was a decline in the levels of this parameter with decrease in salinity (Fig. 1a). Plasma potassium was also higher in the fish exposed to 9 and 12 ppt salinities (Fig. 1b). Plasma calcium and magnesium levels showed a relatively similar patterns, with high concentrations in the fish reared at 6, 9 and 12 ppt salinities (Fig. 1c-d). Glucose level in fish exposed to FW (0.5 ppt) , and 3 and 6 ppt salinities was lower compared to other treatments, and it raised at higher salinities (Fig. 1e). Haematocrit levels of fish reared at salinities of 0.5, 3 and 6 ppt were lower than those of 9 and 12 ppt salinities (Fig. 1f).

Survival and growth performance: Although survival was relatively high in all groups, there were some significant differences among salinity treatments in term of survival rate (Fig. 2), and fish subjected to 12 ppt salinity had lower survival compared to the fish exposed to other salinity levels ($P<0.05$). There was no significant difference in survival rate between salinities of 3, 6 ppt and 9 ppt. Growth parameters were significantly different between treatments ($P<0.05$; Table 1). Fish reared at lower salinities (FW, 3 and 6 ppt) showed higher final weight, final length and specific growth rates compared to the fish reared at higher salinities (Table 1, $P<0.05$). Condition factor

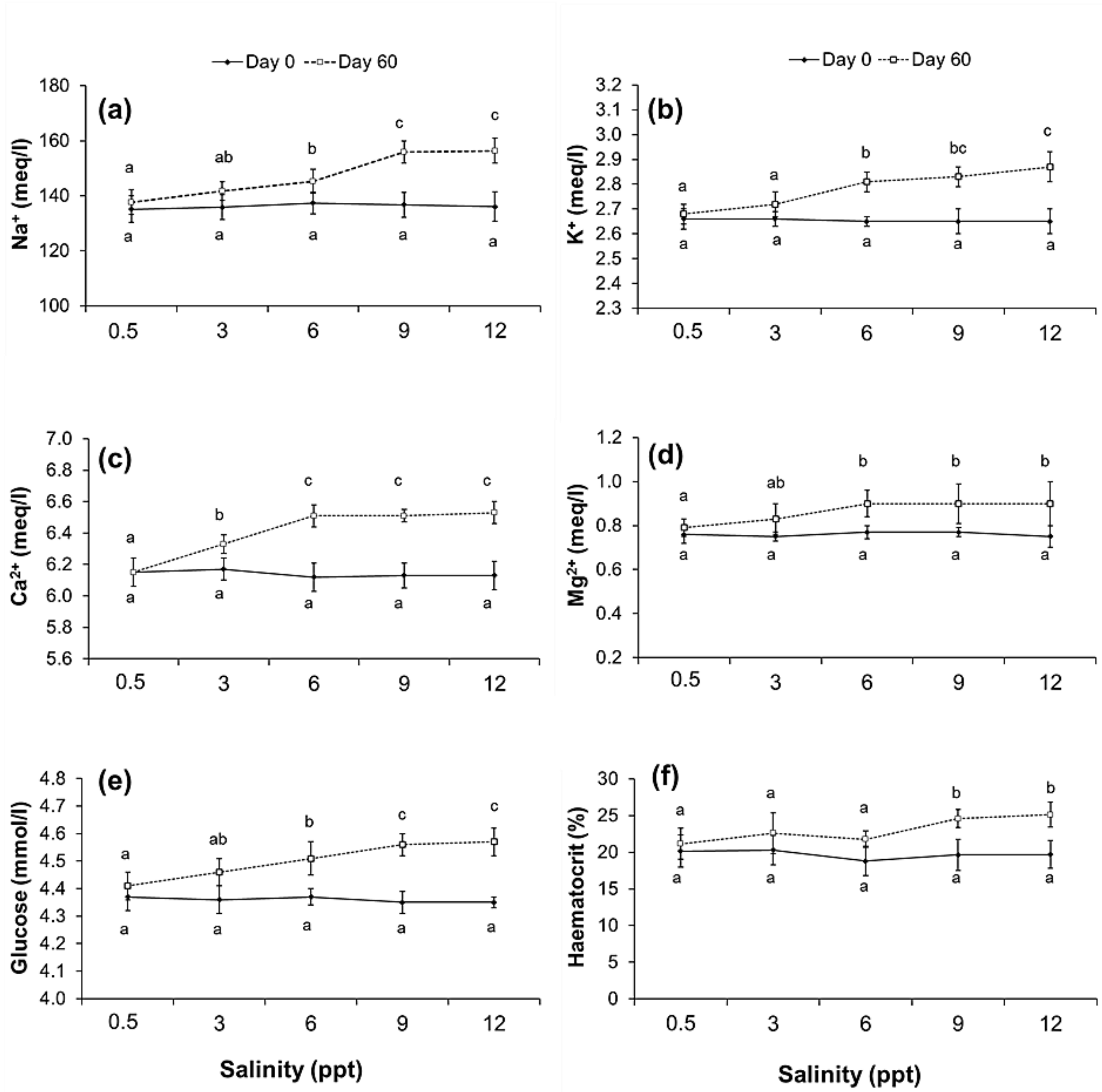

Figure 1. Trends in the levels of (a) sodium [Na^+], (b) potassium [K^+], (c) calcium [Ca^{2+}], (d) magnesium [Mg^{2+}], (e) glucose and (f) haematocrit in the blood samples of beluga sturgeon (*Huso huso*) on the 0th day and 60th day of the experiment. Data are presented as mean (±SD). Groups having different letters are significantly different ($P<0.05$).

Table 1. Growth parameters of juvenile beluga sturgeon (*Huso huso*) after a 60-day rearing period at various salinities.

Parameters	**Treatments**				
	Freshwater (0.5)	**Salinity 3**	**Salinity 6**	**Salinity 9**	**Salinity 12**
Initial weight (g/fish)	22.3±1.0^{a}	22.0±1.0^{a}	21.9±1.0^{a}	22.2±1.2^{a}	22.2±1.3^{a}
Final weight (g/fish)	102.2±3.7^{a}	105.5±2.6^{b}	103.3±3.6^{a}	97.8±3.1^{c}	97.0±2.7^{c}
Initial length (cm)	19.7±0.4^{a}	19.8±0.3^{a}	19.8±0.2^{a}	19.9±0.2^{a}	19.8±0.1^{a}
Final length (cm)	31.0±1.1^{a}	32.3±1.1^{b}	30.9±1.7^{a}	28.8±1.7^{c}	28.5±1.4^{c}
SGR[1]	2.53±0.01^{a}	2.61±0.02^{b}	2.57±0.01^{b}	2.46±0.03^{c}	2.45±0.01^{c}
FCR[2]	1.7±0.10^{a}	1.61±0.07^{a}	1.71±0.12^{a}	1.88±0.07^{b}	1.95±0.05^{b}
CF[3]	0.33±0.005^{a}	0.30±0.01^{b}	0.34±0.005^{a}	0.40±0.01^{c}	0.41±0.01^{c}

Note: [1]Specific growth rate, [2]food conversion ratio and [3]condition factor; Means in the same row with different superscripts are significantly different ($P<0.05$); Data are presented as mean ±SD

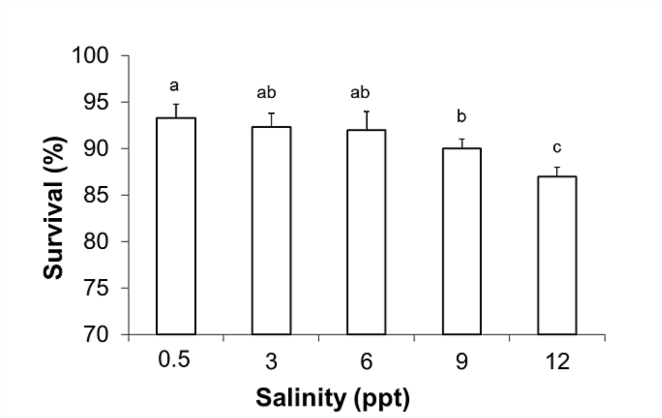

Figure 2. The beluga sturgeon (*Huso huso*) survival (%) exposed to different salinities over a 60-day experimental period. Data are presented as mean (±SD). Groups having different letters are significantly different (P<0.05).

in the fish exposed to 9 and 12 ppt salinities was higher than those of lower salinities (P<0.05). In addition, food conversion ratio increased when fish were subjected to 9 and 12 ppt (P<0.05).

Discussion

The results of the present study showed how juvenile beluga sturgeon respond when exposed to brackish water in terms of osmoregulatory ion concentrations, survival, and growth. Fish were acclimated to several salinity ranges (FW (0.5) to 12 ppt) by increasing water salinity at a rate of 3 ppt per day. These doses and rate of increase were chosen on the basis of conditions that juvenile beluga sturgeon may encounter in the Iranian part of the Caspian Sea especially if they are kept for pen- and cage-based culture. Although fish at low salinities showed a greater survival rate, the difference in survival between the lowest (FW) and the highest (12 ppt) salinities was 6.3% after 60 days. Thus, it appears that five-month-old juvenile beluga sturgeon is able to acclimate to brackish water with salinity doses close to the concentrations observed in the southern Caspian Sea. In this regard, previous studies also indicated that salinity tolerance in sturgeons is improved after acclimation to different salinity levels (McEnroe and Cech, 1985; Altinok et al., 1998; McKenzie et al., 2001). Such acclimation is probably needed to initiate enzymatic and cellular osmoregulatory changes necessary for withstanding osmotic challenge (Morgan et al., 1997; Morgan and Iwama, 1999).

Nonetheless, fish body size appear to largely influence the process of successful acclimation to salinity changes. Several studies have indicated that osmoregulatory abilities are positively correlated with fish size and larger fish show less sensitivity due to the structural and physiological developments during their ontogeny (McEnroe and Cech, 1987; Altinok et al., 1998; LeBreton and Beamish 1998; Cataldi et al., 1999; Allen and Cech, 2007; Jalali et al., 2010; Allen et al., 2011). These mechanisms, however, relatively differ amongst sturgeon species due mainly to the species specific osmoregulatory characteristics and life history. For example, juvenile green sturgeon, *A. medirostris*, are capable of surviving seawater (34 ppt) and near brackish water (15 ppt) concentrations even following an immediate transfer (Sardella and Kultz, 2009; Allen et al. 2011). But according to the previous investigation, abrupt transfer to such concentrations would lead a serious osmotic shock and catastrophic mortality in juvenile beluga sturgeon especially at small and fingerling range sizes (Jalali et al., 2010). This is probably related to the species evolutionary history. Green sturgeons move between oceanic waters and freshwater encountering an extended salinity ranges (0 to 35 ppt) within their habitats. In contrast, average salinity recorded in the northern and southern Caspian Sea respectively fall around 9 and 13 ppt (near an isosmotic medium), about a third salinity of most seawater. Therefore, compared to other sturgeon species rearing at higher salinities or migrating to oceans, Caspian Sea sturgeon including beluga sturgeon habitats occur within relatively limited salinity ranges and this is likely to affect Caspian sturgeon's salinity tolerance. However, it has generally been said that salt tolerance is poor for early life history stages of sturgeon. Therefore, more detailed assessment of how tolerance to a salinity gradient evolves in sturgeons could provide a better understanding of species and environmental associations. In addition, this would help successful aquaculture plans in producing sturgeon meat considering that high potential exist along the Caspian Sea to develop sturgeon aquaculture.

There are numerous similarities in osmoregulatory mechanisms between sturgeon and teleosts such as the composition of the blood parameters (i.e. plasma osmolality and electrolyte concentrations) in both fresh water and sea water. They are hyperosmotic with respect to fresh water and hyposmotic with respect to salt water (Holmes and Donaldson, 1969). In this experiment, we detected an increase in electrolyte concentrations with enhance in salinity. Our results indicated an elevation in haematocrit levels after 60 days exposure especially to 12 ppt salinity whereas these levels were low in the fish reared at lower salinities. A previous study indicated that haematocrit levels rose in juvenile shortnose sturgeon, *A. brevirostrum,* after 10 weeks in hyperosmotic conditions (Jarvis and Ballantyne, 2003). On the other hand, the haematocrit levels were not different between long-term fresh water and salt water-acclimated juvenile Adriatic sturgeon, *A. naccarii*, and Gulf sturgeons, *A. oxyrinchus* (Altinok et al., 1998; Martinez-Alvarez et al., 2002). It has been shown that stressful conditions can lead to alterations in haematocrit levels and an elevated haematocrit level can reflect a stress response in fish (Soivio and Nikinmaa, 1981; Maxime et al., 1990; Franklin et al., 1992). It may also reflect haemo-concentration or demand of fish to oxygen because of the increased respiratory demand caused by salinity exposure, although there is no evidence for this theory as this study did not evaluate oxygen demands in the exposed fish and such demand could also be species-dependent (Ern et al., 2014).

The results indicated that plasma sodium, potassium, calcium and magnesium contents increased when the fish were reared in brackish water, and ion concentrations were higher at 9 and 12 ppt treatments. These alternations can be due to changes in the water content in the blood, resulted from the change in environmental salinity as indicated in other salt water-exposed sturgeons (Plaut, 1998; Krayushkina, 1998; McKenzie et al., 1999; Martınez-Alvarez et al., 2002; He et al., 2009). Sturgeons hatch and grow at least initially in fresh water and thus, they regulate at elevated ion homeostasis levels in hyperosmotic salinities (McEnroe and Cech, 1987; Altinok et al., 1998; Krayushkina, 1998; Rodriguez et al., 2002). Changes in blood and osmotic parameters have been shown to be time-dependent as the levels return to steady state during the acclimation period when the fish remain at a constant salinity and reach homeostasis. When fish is exposed to the concentrated environment, it loses water and the content of the elements in the blood increases. Fish would consequently tend to ingest more water to dilute the level of the blood parameters (He et al., 2009; Allen and Cech, 2007). At the end, the contents of these parameters reach the constant levels as a consequence of the rest of the osmoregulatory mechanisms, which act to re-establish the extracellular volume salinity (Martınez-Alvarez et al., 2002; He et al., 2009). Glucose is an essential fuel for various tissues and its level in plasma was also higher in the fish-kept at 9 and 12 ppt treatments. It has previously been indicated that glucose showed both a rise (Bashamohideen and Parvatheswararao, 1972; Assem and Hanke, 1979) and a fall (Soengas et al., 1991; Krumschnabel and Lackner, 1993; Allen and Cech, 2007) during seawater adaptation. The plasma glucose proliferation can be affected by cortisol changes though cortisol level was not evaluated here. Nonetheless, there appears to be a high glucose demand to supply the energy by osmoregulatory mechanisms (Krum-schnabel and Lackner, 1993; Plaut, 1998), where upon glyconeogenesis even increases (Jürss and Bittorf, 1990).

Growth performance and food conversion ratio in the fish reared at low salinities (FW, 3 and 6 ppt) were significantly higher than those of fish reared at higher salinities. In the case of marine teleost fish, several authors reported better performance at intermediate salinities. Atlantic cod, *Gadus morhua*, larvae reared at 7, 14 and 28 ppt, showed higher growth rates at the intermediate salinity (14 ppt), possibly due to a more efficient conversion ratio (Lambert et al., 1994). Whitefish larvae, *Chirostoma estor estor*, exhibited greater specific growth rates at 10 and 15 ppt, compared to those at 0 and 5 ppt, but net production, based on survival and growth, was clearly superior at

10 ppt, with a very low response at 0 ppt (Martinez-Palácios et al., 2004). Wada et al. (2004) found that growth rates in spotted halibut, *Verasper variegatus*, juveniles kept at 8 and 16 ppt were higher than fish kept at 32 ppt (control) and at 4 ppt. Specific growth rates in Gulf sturgeon, *A. oxyrinchus,* were higher at 3 and 9 ppt compared to those of fresh water (Altinok and Grizzle, 2001). Jarvis et al. (2001) found that weight gain and feed conversion efficiency in juvenile shortnose sturgeon was the highest in fresh water, and the lowest at 20 ppt, the highest salinity tested. Juvenile Adriatic sturgeon, *A. naccarii,* had lower specific growth rates and food conversion efficiencies at 20 than 0 ppt (McKenzie et al., 1999) and lower specific growth rates at 11 than 0 ppt (McKenzie et al., 2001). A recent study by Allen et al. (2014) indicated that growth rate in Atlantic sturgeon was greater at 0 and 10 ppt than in seawater (33 ppt). The beluga sturgeon in this study also grew better in near freshwater and lower salinity medium. Physiological and structural changes in fish following exposure to higher salinities require energy which may negatively impact the growth due to an increased osmoregulatory costs. Although juveniles of many fish species grow optimally in intermediate salinities (Boeuf and Payan, 2001), the results obtained from sturgeon species vary probably due to life history, age/body size differences, species-specific morphophysiological mechanisms and osmoregulatory ability.

It can be concluded that juvenile beluga sturgeon are capable to acclimate, grow and survive in brackish water. This capability can lead the selection of beluga sturgeon as a good candidate for pen and cage culture in brackish water of the Iranian area of the Caspian Sea. Nonetheless, it should be noted that initial salinity adaptation plays a key role in survivorship and growth rates in saline environments.

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چکیده فارسی

پاسخ فیل ماهی (*Huso huso*) به شوری: ارزیابی آزمایشگاهی اثرات شوری مبتنی بر غلظت‌های محیط طبیعی روی تنظیم اسمزی و کارایی رشد

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چکیده:
درک بهتر چگونگی پاسخ ماهیان خاویاری به چالش محیطی شوری به‌ویژه در تاسماهیان جوان پرورشی ضروری است. در این مطالعه، اثرات شوری مبتنی بر غلظت‌های محیط طبیعی (۰/۵، ۳، ۶، ۹ و ۱۲ گرم در لیتر) روی تنظیم اسمزی و کارایی بچه فیل ماهی (*Huso huso*) (۱/۲۲ گرم وزن اولیه) در طی یک دوره ۶۰ روزه مورد بررسی قرار گرفت. نرخ ماندگاری در تمام دوره آزمایش نسبتاً بالا بود، اگرچه نشانه ای از عوارض جانبی شوری بر میزان بقای ماهیان در شوری ۱۲ گرم در لیتر مشاهده گردید. عملکرد رشد در ماهیان پرورش یافته در شوری ۳ گرم در لیتر بهتر از سایر گروه‌ها بود، و پس از آن ماهیان در شوری‌های ۶، ۹ و ۱۲ به‌ترتیب بیشترین رشد را داشتند. به‌طورکلی، افزایش سطح سدیم، پتاسیم، کلسیم، منیزیم و گلوکز در پلاسما در ارتباط با افزایش شوری مشاهده شد، در حالی که گروه کنترل (آب شیرین) سطوح اولیه این پارامترها را داشت. سطح هماتوکریت نیز توسط شوری تحت تاثیر قرار گرفت و تغییرات هماتوکریت در ماهیان گروه آب شیرین، شوری ۳ و ۶ پایین‌تر از سایر غلظت‌ها بود. نتایج نشان داد که بچه فیل ماهیان قادر به زنده ماندن و انطباق با شوری متوسط بودند. اهمیت ارزیابی و مقایسه مکانیسم‌های خاص سازگاری به شوری در آب لب‌شور منطقه جنوبی دریای خزر در این مطالعه مورد بحث قرار گرفت. نتایج حاصل از انجام چنین تحقیقاتی می‌تواند به بهبود استراتژی‌های آبزی‌پروری تاسماهیان کمک نماید.

کلمات کلیدی: بیوشیمی خون، ماهی خاویاری جوان، تنظیم اسمزی، سازگاری، پرورش تاسماهیان.