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Research Article

Optimum weight of stellate sturgeon (*Acipenser stellatus*) fingerling to release into brackish water: plasma electrolytes, plasma hormones and histological observation

Keshtkar Langerudi E.[1]; Jamili Sh.[2*]; Ramezani-Fard E.[1]; Khoshnood Z.[3]

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Abstract

The present study was conducted to determine the appropriate weight to release the hatchery produced fingerling of stellate sturgeon, *Acipenser stellatus*, into its natural habitat. For this purpose, prolactin and cortisol hormones, plasma electrolytes and osmolality as well as histological changes in the gill and kidney of stellate sturgeon transferred from fresh to brackish water at different weight (0.5, 1, 2, and 3 g) were assessed during a one-week time course. A total of 2400 fingerlings with different weights (0.5, 1, 2, and 3 g) were equally assigned into 24 aquariums, each one containing 200 L of freshwater or brackish water. After 24 hours, prolactin showed the highest concentration in 0.5 and 3 g fish groups kept in both experimental waters. Cortisol exhibited a time-dependent increase in the 0.5 g group in both experimental waters as well as the 2 g larvae kept in the brackish water. Generally, lower levels of Mg^{2+}, Ca^{+2}; K^{+}, Na^{+}, and Cl^{-} were measured in the fish kept in freshwater aquariums. The lowest levels of osmolarity were found in the 0.5 g fingerling, especially those kept in the freshwater aquariums, whereas the highest levels were observed in 3 g groups in both experimental waters. Histological assessments illustrated that the volume of chloride cells in freshwater increased with increasing fish weight, whereas the cell count decreased. The same held true for glomerular capillary network size. Taken together, the evidence from this study suggests that stellate sturgeon fingerling less than 2 g are almost incapable to be transferred to brackish and saline environment, due to lack of kidney and gill development.

Keywords: Osmolarity, Cortisol and prolactin, Kidney and gill, *Acipenser stellatus* fingerling, Adaptation

1-Department of Marine Science, Science and Research Branch, Islamic Azad University, Tehran, Iran.
2-Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research Education and Extension Organization (AREEO), Tehran, Iran.
3-Department of Biology, Dezful Branch, Islamic Azad University, Dezful, Iran.
*Corresponding author's Email: shahlajamaili45@yahoo.com

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Introduction

Sturgeons (Acipenseriformes) are among the most valuable fish species exclusively habiting in the northern hemisphere. Currently, all of the living 27 species are endangered of extinction (Gong *et al*., 2019). The Caspian Sea is the natural habitat for five migratory sturgeon species, such as stellate sturgeon (*Acipenser stellatus*). Stellate sturgeon is distributed throughout the southern coast of the Caspian Sea as well as in the Black Sea and Azov Sea (Khoshnood *et al*., 2011; Vasilyeva *et al*., 2019).

This species migrates to freshwater for spawning and the fingerlings return to brackish waters. The migration from freshwater to higher salinity is accompanied by developing osmotic organs and some blood physiochemical adaptations and alterations (Demir *et al*., 2001; Khodabandeh *et al*., 2009). For sturgeons, salinity plays the prime role in the larval development and determines suitable ecophysiological circumstances at which the larvae can be released into the natural environments.

Given that the natural population of stellate sturgeon has been drastically declined during the last two decades, the fish is artificially spawned and restocked into the Caspian Sea. However, one of the main limiting factors for the restocking programs is mortality of the fish larvae and fry after releasing into the natural habitat (Suboski and Templeton, 1989; Olla *et al*., 1998; Khoshnood *et al*., 2010). The unsuccessful programs in restocking stellate sturgeon could be ascribed to the lack of information on the relationship among several biological and biochemical parameters, including weight, age, osmotic hormones, and number and size of chloride cells in the intestine and gill of the fish, before being released into the natural habitats. Therefore, the present study was aimed to determine the appropriate weight and age of the artificially hatched stellate sturgeon for releasing into the Caspian Sea.

Materials and methods

A total of 2400 stellate sturgeon fingerling with different weights (0.5, 1, 2, and 3 g) was used in freshwater and brackish water (7-9 ppt). The fish were assigned into two separated groups to be kept in one of the experimental waters and then transferred into 24 200-litter aquariums. The aquariums were filled with 150 L of freshwater or brackish water and equipped with a permanent aeration system. Each aquarium was used for 100 fingerlings for the main experiment. To prevent ammonia accumulation and other excretions from the fish, the water was exchanged about 30% every 24 h and the dead fish were recorded and removed. During the experiment (167 h), physiochemical parameters of the waters were measured every day and showed close values as follows: in freshwater (temperature, 25.8±1.09°C; dissolved oxygen, 7.3±0.5 mg/L; and pH, 7.8±0.2) and in the brackish water (temperature, 25.1±0.12°C; dissolved

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oxygen, 7.01±1.01 mg/L; and pH, 7.86±0.08).

Hematological and histological analyses

During the experiment, 10 fish per aquarium were randomly sampled at 24, 48 and 167 h and deeply anesthetized with clove powder (150 mg L^{-1}). To collect their blood, fish were decapitated and their body were embedded into 2 ml Eppendorf and centrifuged at 13,000 rpm for 20 min. The supernatant was collected to measure the concentration of plasma electrolytes [magnesium (Mg^{2+}), calcium (Ca^{2+}), chloride (Cl^{-}), potassium (K^{+}), and sodium (Na^{+})], osmolarity as well as prolactin and cortisol hormones (Tomas, 1998). Plasma osmolarity was measured in milliosmole per liter using a digital freezing osmometer (Nr. 9610003, Roebling, Germany). The concentration of cortisol and prolactin hormones was determined using the radioimmunoassay method (RIA) and ELISA kit, respectively. The kits were purchased from Pars Azmon Co. (Tehran, Iran).

For histological studies, the gill and kidney tissues were dissected and fixed in Bouin's fluid and stored in 70% ethanol. The samples were then dehydrated in an alcohol series (50%, 70%, 80%, and 96%) and then kept in chloroform for 30 min for two times. The samples were embedded in paraffin wax and slides of 5–7 µm were prepared. The prepared slides were stained by hematoxylin-eosin (H&E) and observed using light microscope and photographed (Joo *et al*., 2018).

Statistical analysis

Statistical analyses were performed with SPSS 16. All results are presented as the mean±standard deviation (S.D.). Significant differences were determined using one-way ANOVA, followed by the Duncan test to compare the differences between the experimental groups (p<0.05). T-test was also applied to compare each parameter between the fish kept in freshwater and brackish water (p<0.05).

Results

Findings concerning mortality revealed lower ability of the fingerlings with less weight after being transferred into the brackish water: 0.5 g, 19%; 1 g, 13%; 2 g, 9%; and 3 g, 7%.

Hormone alteration

Plasma prolactin and cortisol showed some time-and weight-dependent alterations after releasing into freshwater and brackish water (Fig. 1; overall alteration Fig. 4). The highest concentration of prolactin was measured 0.5 and 3 gr for larvae group kept in both experimental waters at the first sampling time (i.e., 24 h). The lowest fluctuation was observed for both 1-gram groups in freshwater and brackish water during the experiment.

Cortisol exhibited a time-dependent increase in the 0.5 g fish group in both experimental waters as well as the 2 g

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larvae kept in the brackish water (p<0.05). The lowest alterations were observed in the 3 g larvae group.

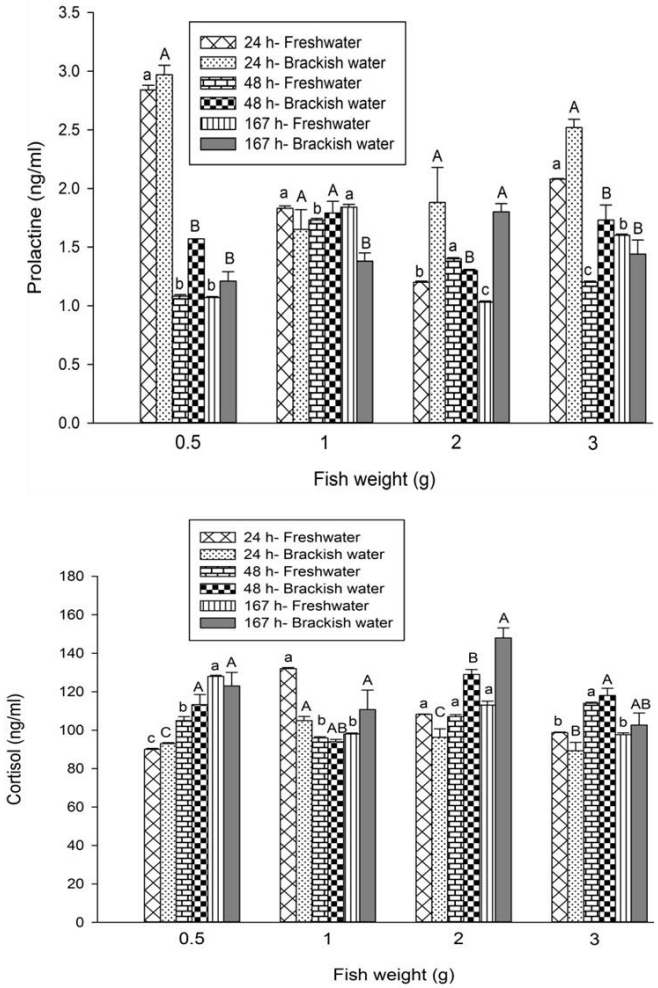

Figure 1: The concentration of plasma hormones (prolactin and cortisol) in stellate sturgeon fingerling with different weights (0.5, 1, 2, and 3 g) maintained in freshwater and brackish water for 167 h. The measurements were conducted at 24, 48, and 167 h after transferring the fish into aquariums containing freshwater and brackish water. Lowercase (a, b, and c) and uppercase (A, B, and C) letters represent the comparison between the fish kept in freshwater and brackish water, respectively.

Plasma electrolytes

The plasma Mg^{2+}, Ca^{2+}, Cl^{-}, K^{+}, and Na^{+} levels in the experimental fish fingerling are presented in Figure 2 (see overall alteration, Fig. 4). The concentration of Mg^{2+} in the 0.5 g larval group demonstrated a time-dependent increase in both freshwater and brackish water, yet showed a vice versa response in the other larvae groups in brackish water (p<0.05). Just similar to Mg^{2+}, Ca^{2+} concentration in 0.5 g fingerling increased during the experiment in both freshwater and brackish water, albeit no significant difference was observed between the two groups when compared together at each respective time (p<0.05). The lowest change was

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observed for 1 g fingerling in both freshwater and brackish water.

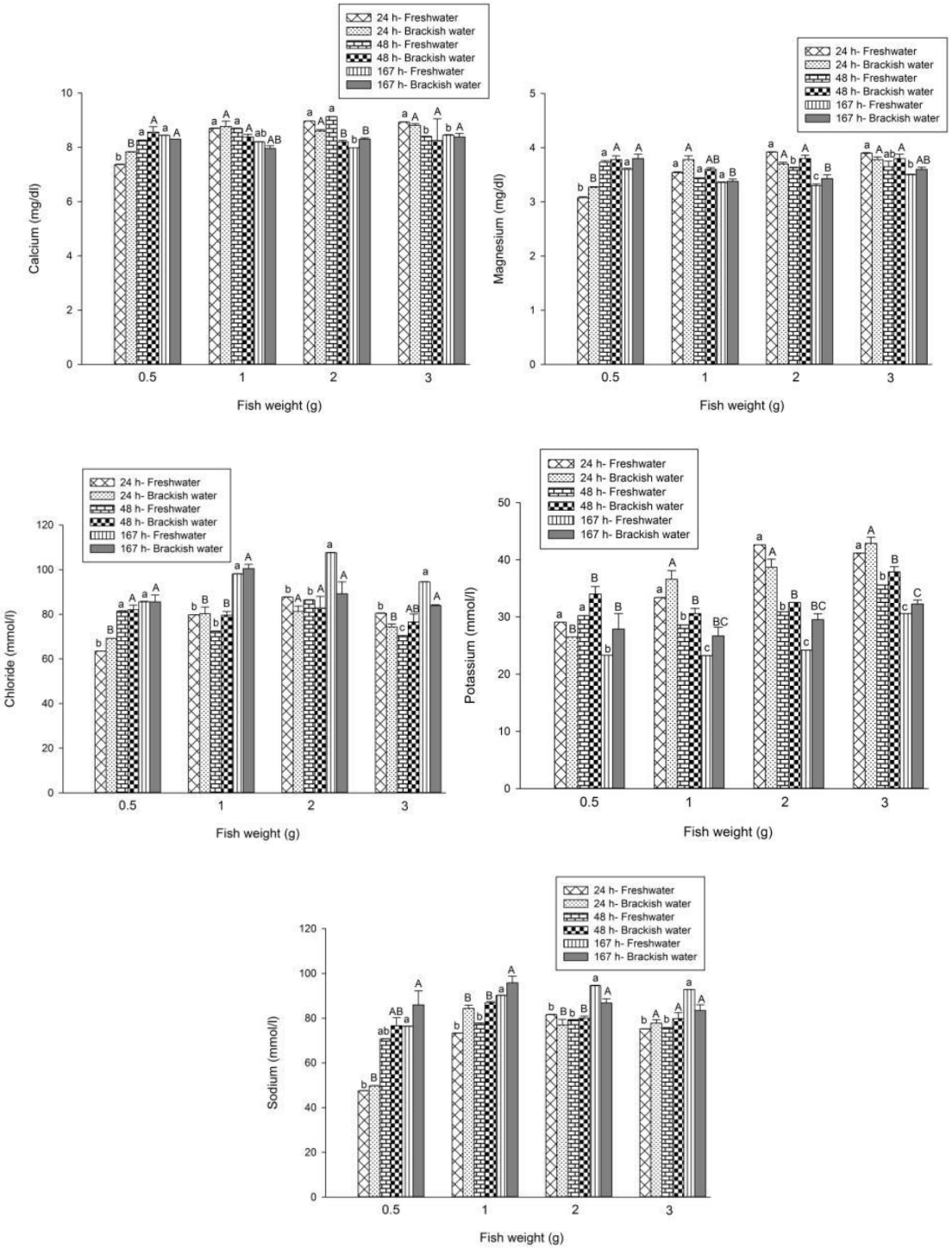

Figure 2: The concentration of plasma electrolytes (magnesium, calcium, chloride, potassium, and sodium) in stellate sturgeon fingerling with different weights (0.5, 1, 2, and 3 g) maintained in freshwater and brackish water for 167 h. The measurements were conducted at 24, 48, and 167 h after transferring the fish into aquariums containing freshwater and brackish water. Lowercase (a, b, and c) and uppercase (A, B, and C) letters represent the comparison between the fish kept in freshwater and brackish water, respectively.

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The plasma Cl^- concentration in the 0.5 g fingerling revealed a similar manner to Mg^{2+} and Ca^{2+}, with the lowest level measured at 24 h of the experiment. In 1 g and 3 g groups in both freshwater and brackish water, however, this electrolyte significantly increased at the last sampling time, although the highest concentration was found at 167 h in the 2 g fingerling kept in freshwater (p<0.05). The measured levels of K^+ illustrated a time-dependent decrease in the experimental groups, except in the 0.5 g group, and the highest levels were observed in 2 g and 3 g fingerling at 24 h of the experiment (p<0.05). Concerning Na^+, the lowest concentration was obtained in 0.5 g fish groups at 24 h in both used experimental waters. Over time, the electrolyte increased in 0.5 g and 1 gr fish groups in both freshwater and brackish water, and the highest level was observed in 1 g fingerling in brackish water at 167 h. In contrast, the lowest alterations of Na^+ were observed for the 2 g and 3 g fish fingerling during the experiment, although no significant difference was found between the respective weight fish in freshwater and brackish water during the experiment (p<0.05).

Blood osmolarity

The osmolarity measurements exhibited significant differences between the experimental fish groups (Fig. 3; overall alteration Fig. 4). The lowest levels of osmolarity were found in the 0.5 g fingerling, especially those kept in freshwater aquariums, whereas the highest levels were observed in 3 g groups in both experimental waters (p<0.05).

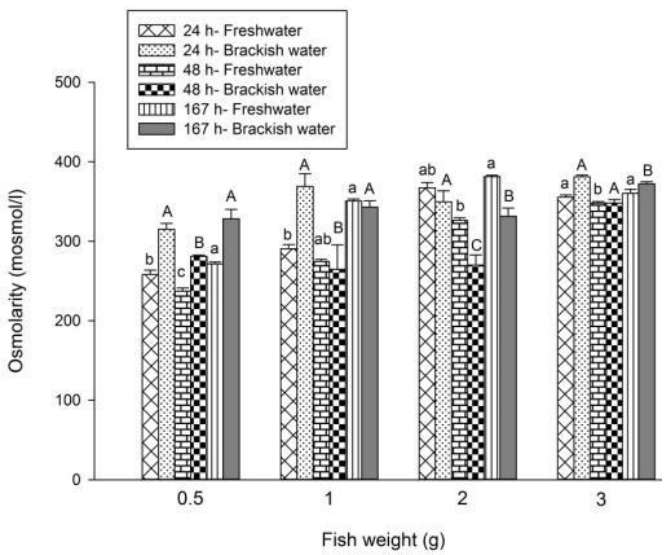

Figure 3: The plasma osmolarity of stellate sturgeon fingerling with different weights (0.5, 1, 2, and 3 g) maintained in freshwater and brackish water for 167 h. The measurements were conducted at 24, 48, and 167 h after transferring the fish into aquariums containing freshwater and brackish water. Lowercase (a, b, and c) and uppercase (A, B, and C) letters represent the comparison between the fish kept in freshwater and brackish water, respectively.

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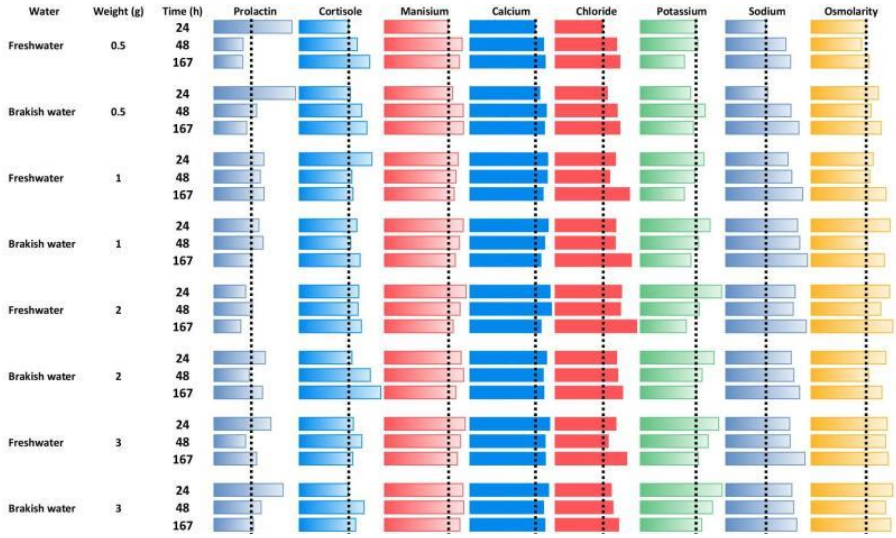

Figure 4: Overall alteration of prolactin, cortisol, magnesium, calcium, chloride, potassium, sodium, and osmolarity of plasma in stellate sturgeon fingerling with different weights (0.5, 1, 2, and 3 g) maintained in freshwater and brackish water for 167 h. The measurements were conducted at 24, 48, and 167 h after transferring the fish into aquariums containing freshwater and brackish water.

Alterations in chloride cell size and glomerulus number and diameter

After being transferred to brackish water as well as with increasing weight, the experimental fish fingerlings exhibited some changes in the volume and number of gill chloride cells and the renal glomeruli diameter. In the freshwater aquariums, the volume of chloride cells increased with increasing weight, from 5.63 in 0.5 g to 7.32 in 3 g, whereas the cell count decreased. The same held true for glomerular capillary network size, with being 91.76 µm and 185.68 µm in 0.5 g and 3 g fingerlings, respectively. Following exposure to brackish water, however, the diameter of both chloride and glomerular cells decreased with increasing weight; that is, the chloride cells exhibited diameter of 5.56 µm at 0.5 g and 4.53 µm at 3 g fingerlings, and the glomerular capillary network showed sizes of 85.34 and 49.71 µm at 0.5 and 3 g weights, respectively.

Histological changes in osmotic organs

Transferring the fish larvae from freshwater to brackish water caused some histological alterations in both kidney and gill tissues (Fig. 5-8). The inner space of the renal tubules in the fish kept in brackish water was larger than that of those held in freshwater. In brackish water, the number of glomeruli decreased with increasing salinity and age, when compared with the same sizes and weights in freshwater. Concerning the gill tissue, the number and size of chloride cells increased after transferring the larvae to brackish water. Their size showed a weight-dependent increase in freshwater, yet decreased in brackish water.

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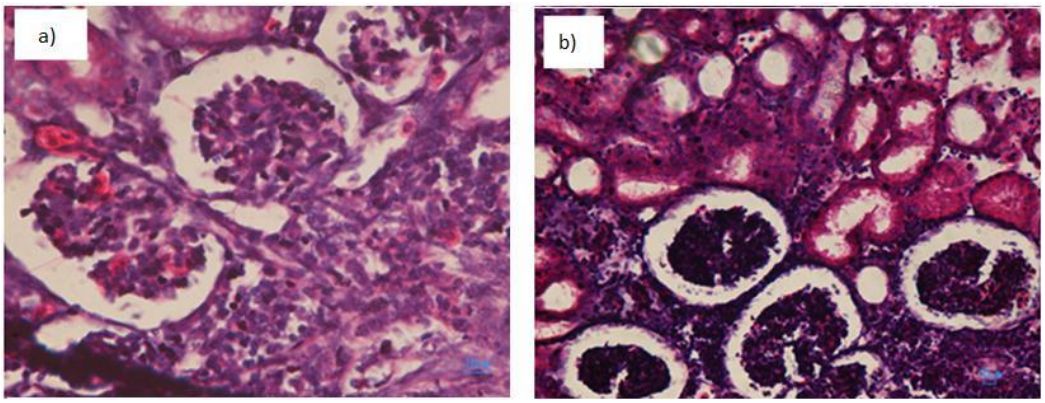

Figure 5: Histological alteration of kidney tissue of stellate sturgeon fingerling with different weights in freshwater: (a) 0.5 g and (b) 2 g. The inner space of renal tubules in the fish kept in freshwater was smaller than that of those held in brackish water and the number of glomeruli increased (Ob. 40x; H&E).

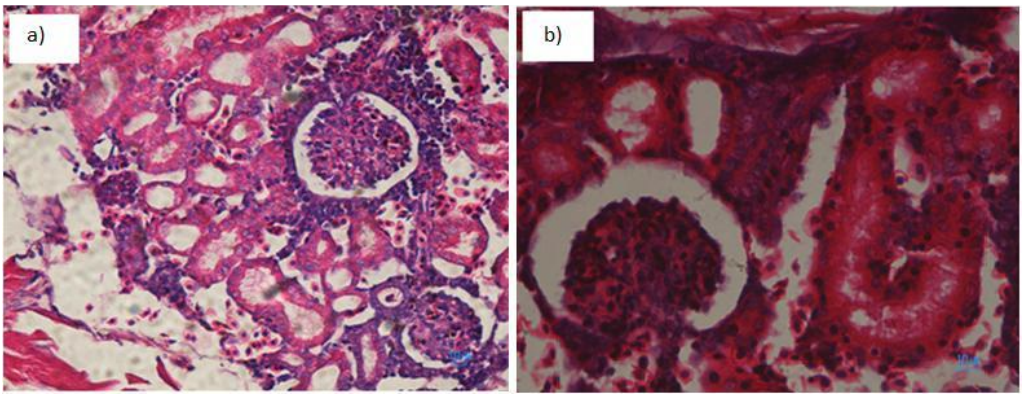

Figure 6: Histological section of kidney tissue of stellate sturgeon fingerling with different weights after transferring to brackish water for 167 h: (a) 0.5 g and (b) 2 g. The inner space of the renal tubules in the fish kept in brackish water was larger than that of those held in freshwater and the number of glomeruli decreased (Ob. 40x; H&E).

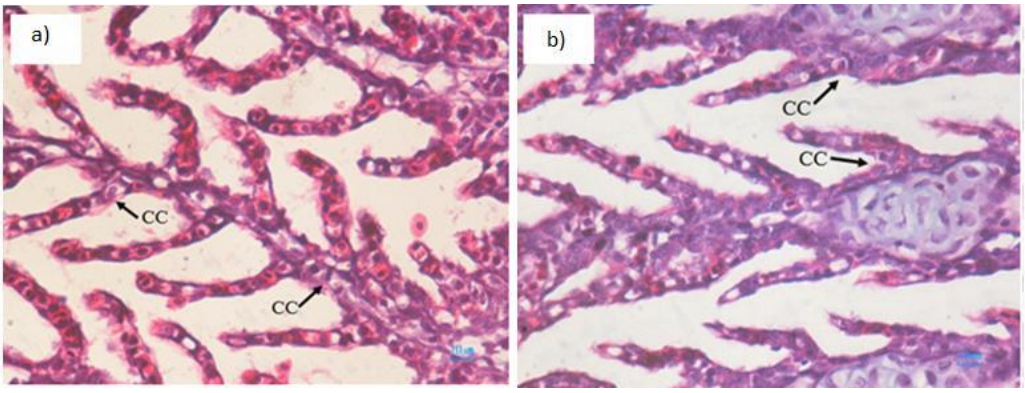

Figure 7: Histological section of gill tissue of stellate sturgeon fingerling with different weights in freshwater: (a) 0.5 g and (b) 2 g. In the freshwater, the volume of chloride cells increased with increasing weight, whereas the cell count decreased (Ob. 40x; H&E). CC: chloride cells.

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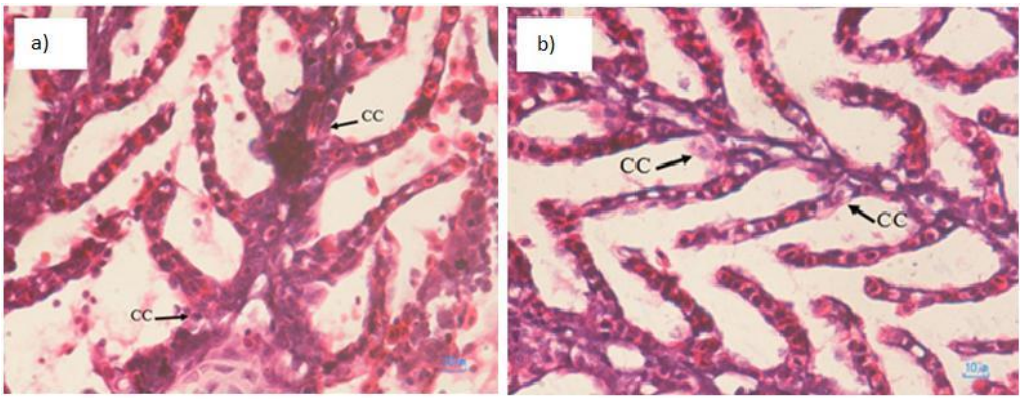

Figure 8: Histological section of gill tissue of stellate sturgeon fingerling with different weights after transferring to brackish water for 167 h: (a) 0.5 g and (b) 2 g. The number and size of chloride cells increased after transferring the larvae to brackish water (Ob. 40x; H&E). CC: chloride cells.

Discussion

In reviewing the literature, no data was found on the osmotic regulating system of stellate sturgeon fingerling before and after migrating from freshwater to brackish water or releasing into the second habitats. Overall, the data concerning mortality revealed a weight-dependent decrease with the highest and lowest mortality percentage for the fish with 0.5 g and 3 g, respectively. These findings could be attributed to the rather incapable osmotic regulatory system of the fish at lower weights to keep the homeostatic concentration of plasma electrolytes (such as sodium, calcium, and magnesium) upon introducing into brackish water. That is, the fish with less weights possess ontogenetically undeveloped osmoregulatory organs (such as gills, gut, kidney and urinary) and, in turn, have lower abundance and size of the Na^+-K^+-ATPase immunoreactive fluorescence chloride cells within the these organs. At lower weights, hence, lower expression and activity of different ion and electrolyte transporters, such as Na^+-K^+- ATPase (NKA) and Na^+-K^+-Cl^-(NKCC) co-transporter, in the chloride cells could reduce the tolerance of the fish fingerling at higher salinities when compared the fish with higher weights and more developed organs (Mancera *et al*., 1994; Salati *et al*., 2014; Lin *et al*., 2016). Support for this view has come from a report by Evangelista *et al.* (2019), who stated that the fish larvae at early stages cannot be cultured in brackish and saline waters.

The present research illustrated that the resistance of the larvae to salinity and their adaptation to higher osmotic conditions depend on age. Many researchers have reported similar findings on Adriatic sturgeon, *Acipenser naccarii* (Cataldi *et al*., 1998), Iranian sturgeon, *Acipenser persicus* (Jabbarzadeh Shiadeh *et al*., 2000; Kazemi *et al*., 2003), short snout sturgeon, *Acipenser brevirostrum* (Jenkins *et al*., 1993), and four species of Caspian sturgeons (Farabi *et al*., 2011). A time-dependent increase was observed in the concentration of Mg^{2+} in the 0.5 g larval group in both

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freshwater and brackish water, but an opposite response was found in the other larvae groups in brackish water. However, the level of sodium and chloride as well as plasma osmolarity of the fish larvae (especially up to 2 g) in freshwater were significantly lower than the respective ones in brackish water. These findings are consistent with those reported previously for white sturgeon by McEnroe and Cech (1985), for Chinese sturgeon (*Acipenser sinensis*) by He *et al*. (2009), for Iranian sturgeon by Kazemi *et al*. (2003), and for Adriatic sturgeon by Martinez-Alvarez *et al.* (2002). These results suggested that with increasing salinity the adaptation ability of these fish species is reduced. Furthermore, the lowest levels of osmolarity were found in the 0.5 gr fingerling, especially those kept in freshwater, whereas the highest levels were observed in 3 gr groups in both experimental waters. A possible explanation for these results may be ascribed to insufficient ability of the 0.5 g larval groups to regulate the osmotic electrolytes; that is, the rate activity of the chloride cells might be enhanced with increasing weight and age (Salman *et al*., 1998). When compared to lower salinities or freshwater, the metabolic value of osmotic regulation in brackish water is reduced because of the lower gradient of blood osmotic concentration (Lin *et al*., 2003). Consequently, adaptation to higher salinity could enhance the fish tolerance. Supportive evidence was observed previously through comparing the tolerance of the fish gradually adapted to higher salinities with that of those suddenly transferred to the new environmental conditions with different salinities (Güner *et al*., 2006). In addition, this fish species at larval stages exhibited higher levels of sodium, chlorine, and osmolarity, suggesting their relatively lower tolerant to higher salinities when compared with euryhaline fishes. The observed overall time- and weight-dependent increase in osmolarity, Na^+, K^+, Cl^- in all fish kept in both experimental water might be justified with compensatory response against osmotic shock or initial (de) hydration. These data corroborate the findings of Partridge and Lymbery (2008) who stated that osmotic shock could directly lead to inflow of electrolytes into the circulation system of fish.

In the present research, plasma prolactin and cortisol showed some time- and weight- dependent alterations after releasing into freshwater and brackish water. The highest concentration of prolactin was measured for 0.5 g and 3 g larval groups kept in both experimental waters at the first sampling time. Likewise, cortisol exhibited a time-dependent increase in the 0.5 g fish group in both experimental waters as well as the 2 g larvae kept in the brackish water, yet the lowest change was observed for 3 g larval group. Given that no justification was found for these findings, they therefore need to be interpreted with caution. In brackish water, however, the measured higher concentrations of

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prolactin in the larvae with more weights (at the first and last sampling times), when compared with that of kept in freshwater, could be attributed to its more functionality with increasing salinity.

Prolactin, as the most important osmotic and ionic regulating hormone in sturgeon fishes, reduces the activation of chloride cells and decreases their number in the fish adapted to freshwater. This hormone exhibited higher concentrations in the fish inhabited in brackish water, especially in those with 2 and 3 g weights at the first sampling time.

Cortisol not only plays an important role in regulating the function of chloride cells but also regulates their differentiation. In anadromous fish, moreover, the hormone stimulates morphological changes in the gill and kidney tissues. The histological observation showed larger inner space the renal tubules in the fish kept in brackish water than that of held in freshwater, but the number of glomeruli in brackish water decreased with increasing salinity and age. This finding, while preliminary, suggests that this fish at the early stages of life (at 0.5-2 g) is unable to migrate to higher salinities, even to brackish water. Although these results differ from a published study (Krayushkina *et al*., 2006) for Beluga (*Huso huso*), they are in agreement with those reported by Mosafer Khourjistani *et al*. (2008) who justified the greater area of glomerular network in low salinity to excessive excretion water (i.e., highly diluted urine) and, in turn, reabsorption of filtered ions.

Chloride cell sizes can indicate the ability of fish to actively regulate electrolytes through gill (Khoshnood *et al.,* 2010). The number and size of gill chloride cells increased after transferring the larvae to brackish water; that is, their size showed a weight-dependent increase in freshwater, yet decreased in brackish water. The observed different number and size of chloride cells could stem from different concentrations of cortisol hormone. Moreover, the observed size reduction of chloride cells in brackish water was consistent with previous findings for Iranian sturgeon by Pleasant *et al*. (2009) and for Adriatic sturgeon by Cataldi *et al*. (1998). Taken together, the evidence from this study suggests that stellate sturgeon fingerling less than 2 g are almost incapable to be transferred to brackish and saline environment, due to lack of kidney and gill development.

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