Seasonal changes of blood serum ions in Beluga (*Huso huso*) Cultured in Brackish Water

Alizadeh M. [*1]; Hedayati A.[2]; Bahmani. M.[3]

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1-Inland Saltwater Fish Research Station, PO BOX: 89715-1123, Bagh, Iran
2- Khorramshahr Marine Science University, Fishery Group, Khorramshahr, Iran.
3- Sturgeon International Research Institute, Rasht, Iran
* Corresponding author's email: m_alizadeh47@yahoo.com

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Living sturgeons (*Acipenseridae*) are representatives of the ancient group *Chondrostei*, which had a common origin with *Palaeonisci*, known from the Devonian period (Krayushkina & Semenova, 2004)**.** In spite of their freshwater origin, in their history, they occupied different salinities and at present, they can live in both fresh and seawater mediums.

Study of osmotic and ionic regulation in Acipenserids, that connect to marine conditions in different extents (freshwater, diadromous brackish water, diadromous seawater), make it possible to establish the dependence of functional level of osmotic and ionic homeostatic mechanisms from medium salinity in some acipenserids. It was ascertained that in higher salinities, the functional level of these mechanisms increases. So investigation of osmo-ion regulation is one of the most important problems in the culture of sturgeon in new mediums.

Diadromous sturgeons (group 3), Russian sturgeon (*A. gueldenstaedtii*), Persian sturgeon (*A. persicus*), Starred sturgeon (*A. stellatus*) and Beluga (*Huso huso*) have the ability to adapt to brackish water (12-18 ppt salinity) as firm osmo-regulators (Krayushkina, 1998). After transferring from freshwater to brackish water, these species support a blood serum osmolarity and ion concentration lower than the water ions and osmolarity in the environmental medium (Amini et al., 2005). The functional levels of osmoregulatory and ion regulatory systems in these species are not identical. The starred sturgeon is more euryhaline than the Russian sturgeon and the Beluga (Krayushkina, 1998).

The mechanism of hypo-osmotic regulation attains the highest development in diadromous marine sturgeons (group 4), Short nose sturgeon (*A. brevirostrum*) and Sharp snout sturgeon (*A. oxyrhynchus*), that are living in the ocean with a salinity of up to 33 ppt (Krayushkina, 1998).

This mechanism supports relative constant serum osmolarity under wide fluctuations of the environmental salinity.

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When environmental salinity was increased during study by 1000 mOsmo/l, only about 65-69 mOsm/l value of fish serum osmolarity was increased (Allen et al., 2006).

A major part of Beluga life is in brackish water, so it can tolerate this environment as a culture medium. Besides the use of brackish water as a new subject for commercial rearing (meat and caviar), in this study the osmolarity system in Beluga cultured in brackish water was examined to define the effects of brackish water on osmo-ion regulation mechanism and compare it to other culture mediums of this valuable fish.

This research was carried out in Inland Salt Water Fishes Research Station in Bafgh, Iran for one year. The experiments was performed in eight of 30-ton round-roofed cement ponds.They were equipped with a water distribution system and central aeration. The required water was supplied through a semi-deep well with variable salinity of 11-16 ppt. 75 four-year-old great sturgeons were selected randomly with a mean weight of about 11kg from a culturing population in a brackish water earth pond and they were transported to the cement ponds with five treatments and three replications per treatment. Fish were fed by BFT pellet food in protein and energy levels of 40% and 4500 kcal/kg, respectively.

During four sampling times at one year, 4-5 cc blood was extracted from the selected fish (Barannikova et al., 2002). Blood sampling was performed in caudal vessels using none-heparin zed syringes. Storage, centrifuging and pipe ting of the samples was carried out at -4 ˚C. Plasma was stored at -20 ˚C. (Chebanov and Ronald, 2001).

The water osmolarity and salinity were determined by OSMOMAT (through use of 10 micro liters of serum) and refractometer, respectively. Glucose was measured through GOD-POD method (Teuscher et al., 1997; Barham et al., 1972) by Auto-analyzer apparatus. Spectrophotometer was used for analyzing calcium and magnesium. Sodium and potassium were amounted by Flame photometry apparatus. The method for determining cortisol was Radio Immune Assay (RIA). The methods are initially explained by Young et al., 1983.

The experiment of profiles was done in duplicates. The SPSS software (version 10) was used for statistical analyses. Assignment of data correlation was done by Pearson and Kendal tests. Tukey and Duncan tests were used to compare mean differences. The significant differences were determined in %5 level.

Statistical results showed no significant correlation between serum ions and cortisol with osmolarity ($p > 0.05$) but only in calcium, an inverted significant correlation ($p>0.05$) was observed (Fig. 1). An inverted significant relation of water salinity with osmolarity was also demonstrated (sig = 0.02, r = -.0.39), so with increase in water salinity, serum osmolarity decreased (Fig. 2).

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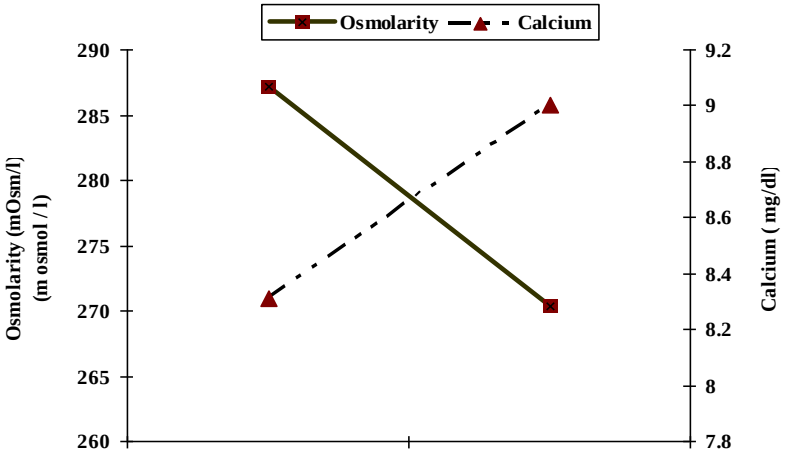


Figure 1: Relationship between serum calcium and osmolarity

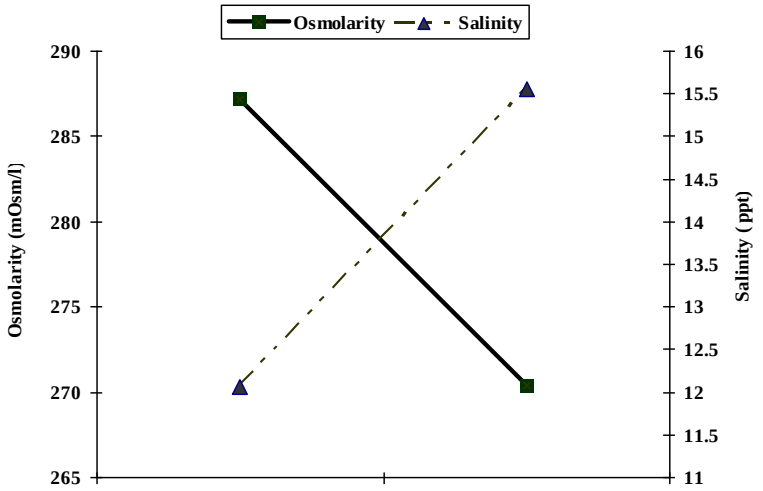


Figure 2: Relationship between water salinity and serum osmolarity

Among examined profiles, water salinity and serum calcium had more correlation, respectively; but they were inverted. Other blood ions (magnesium, sodium, potassium and glucose) and cortisol also had inverted correlation which with increase of water salinity, serum osmolarity and reciprocally blood ions and cortisol decreased (Fig 3, 4 and 5).

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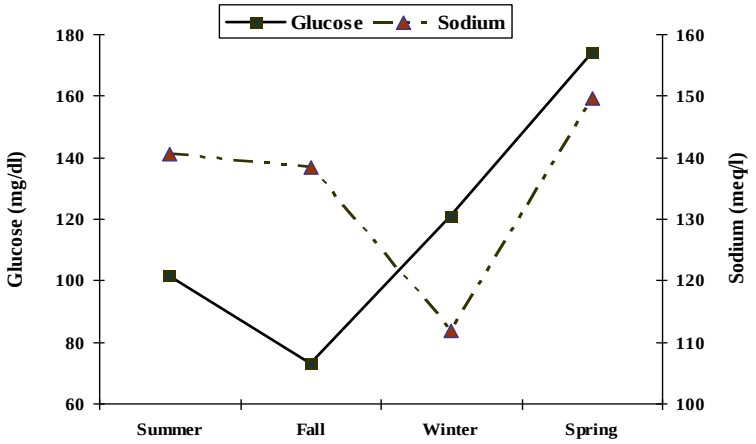


Figure 3: Exchange of serum glucose and sodium in different seasons

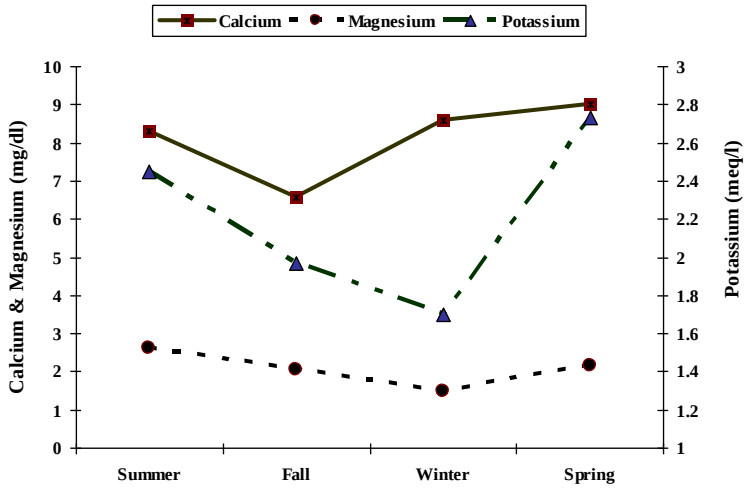


Figure 4: Exchange of serum calcium, magnesium and potassium in different seasons

During adaptation to seawater, sturgeons have different dynamics of cortisol and ion in their blood serum (Krayushkina and Semenova, 2005). The changes of these parameters explain osmo-regulatory mechanisms and ion regulatory systems in different species of Acipenserids.

The entrance of Acipenserids to sea and ocean waters was accompanied with the development of mechanisms of osmotic and ionic homeostasis. The development of hypo-osmotic regulation and the ability to change the regulation type contributed to the development of the diadromous mode of the life in Acipenserids and especially Beluga. So the Beluga definitely has the ability to grow in brackish water and could be used as a proficient species in brackish water mediums.

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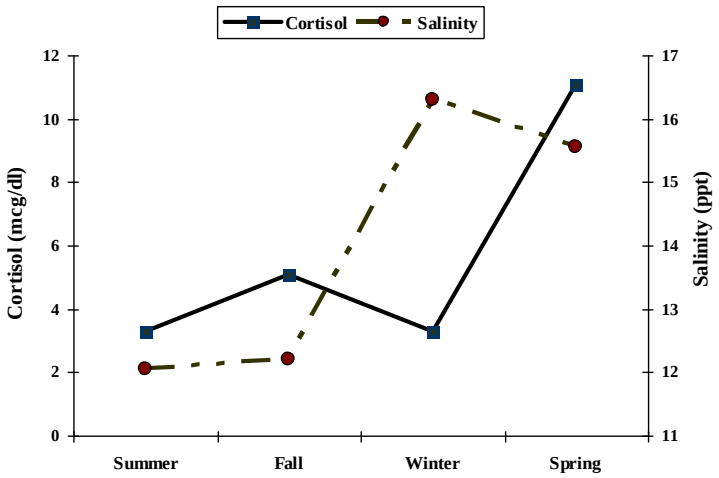


Figure 5: Exchange of serum cortisol and potassium in different seasons

Salinity is the stimulating factor for the evolution of osmo-regulatory and ion-regulatory systems in Acipenserids, so for evaluating these systems, attention to water salinity is essential (Krayushkina and Semenova, 2005). In addition, cortisol is a key hormone for adaptation to brackish water so measuring this hormone in order to investigate on osmo-regulation is important. Cortisol passes the signal to genome of cells-target in the osmo-regulatory organ (Krayushkina et al., 2005). By studying on osmo-ion regulation system in brood and reared juvenile of Iranian sturgeon, *Acipenser persicus*, it was found that osmolarity in sea, estuary and worksite brood is 305.29, 308.72 and 265.03 mOsm/l, respectively. Water osmolarity in estuaries was fewer than seawater but serum osmolarity in estuaries was higher than in seawater. It may result in non-complete adaptation of fishes to the estuary environment. In addition, it was clarified that by decreasing water salinity, osmolarity increased. Magnesium and sodium had important roles in the determination of serum osmolarity in fresh and brackish water (Kazemi *et al.*, 2005). Calcium had more effects on osmolarity than other ions that confirmed the role of different species and environmental conditions on the determination of osmolarity.

All Caspian sea sturgeons in one area may have the same ecological conditions but different osmotic pressure and blood serum ionic concentration are observed (Kazemi *et al.*, 2005). It seems that sturgeon characteristic is one of the most important factors to determine osmotic pressure amount and blood serum ionic concentration. In the present study, clarified Belugas are well adapted to brackish water and their serum osmolarity is suitable.

Amini et al. (2005) showed that serum osmolarity of Iranian sturgeon (*A. persicus*), is relatively higher than water which could be due to the higher amounts of sodium and potassium in blood than water while calcium and magnesium in blood were lower than water. In addition, they clarified that the regulator cells development of

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osmolarity is variable and depends on fish age (Amini *et al.,* 2005).

Examination of osmo-ion regulation mechanism in some immature sturgeon during adaptation to hyper osmotic condition and sea water after transition to freshwater showed that the function of homeostasis mechanism in various species is different. So with an increase in salinity, this mechanism will also be increased. Osmolarity of different species of sturgeons in seawater in Sterlet was 263 mOsm/l, 279 mOsm/l in Siberian sturgeon, in Russian sturgeon was 262 and in Beluga was 279 mOsm/l. During the adaptation of these species from sea water to brackish water, the range of osmolarity fluctuations have been decreased to 55.7% (147.1 mOsmo/l) for Sterlet, 18.8% (52.5 mOsm/l) and 17.8% (46.7 mOsm/l) for Siberian sturgeon and Russian sturgeon, respectively and eventually 14.4% (40.2 mOsm/l) for Beluga (Krayushkina *et al.,* 2005).

According to the results, it is demonstrated that osmo-regulation mechanism in Beluga is well developed and this fish adapted well to brackish water, so the culture of Beluga in brackish water has an economic justification.

Comparing the present study with previous studies indicated that tolerance of Beluga to brackish water is better than other sturgeons, because their serum osmolarity was similar to osmolarity in seawater, although environment osmolarity in brackish water is lower than seawater. Mediums with high salinity (hyper osmotic) have more osmolarity than mediums with low salinity (hypo osmotic) (Allen and Joseph, 2006). With an increase in serum osmolarity, the number of casualties increased (Askarian et al., 2006). Since osmolarity of Beluga in the present study was not increased, the brackish water environment can be considered as a suitable medium for culture of this species.

Previous studies about the effects of transition from freshwater to brackish water in some immature sturgeons specified that there are a lot of differences in hormone regulation for cortisol and ion homeostasis in different species of sturgeon (Krayushkina et al., 2005).

Osmo-regulation mechanism in different species and environmental conditions (especially salinity) is different and each medium has unique characteristics, so in the present study most correlations were related to calcium in which an increase in salinity, decreased osmolarity. Therefore, Ions and cortisol definitely have the most effect on osmo-regulation.

According to the results, there was an inverted correlation between cortisol and osmolarity that has also been confirmed in other species and conditions by other researchers. In addition, salinity, temperature, stress and other environmental factors influence the serum cortisol and ions but we could not exactly compare this profile with other results.

Since culture of Beluga in freshwater mediums produces some osmotic problems and leads to losing energy for osmo-regulation, and also according to obtained results by the present study, osmo-regulation mechanism in Beluga accomplishes more appropriately than most sturgeons,therefore, culture of this species in brackish water is

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justified economically. It seems that farming Beluga in mediums nearly like natural conditions such as inland brackish water ponds provide less energy consumption for growth development in this fish.

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