

# Study on the Changes of Some Physiological Factors during Osmoregulation of Juvenile Persian Sturgeons (*Acipenser persicus*)

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**Abstract:** Experimental trials of acclimatization of juvenile Persian sturgeon, *Acipenser persicus* to different salinities of 0,5,10, 15<sub>ppt</sub> were studied. The juvenile Persian sturgeons in three weight classes (1.5, 3, 5g) have been transferred from freshwater to different concentrations of saline water. Na<sup>+</sup>, k<sup>+</sup>, Cl<sup>-</sup> concentrations of blood serum and the changes in the number and size of chloride cells were measured. Na<sup>+</sup> concentration showed an imperceptible trend and K<sup>+</sup> concentration had a decreasing one size of changed chloride cells in different salinities were 8.6-13 ± 0.3 μm and their numbers were (25-65±3) in 5 pairs of filaments. The results revealed that salinity tolerance was increased with the fish size. According to this study, there is a direct relation between the number and size of chloride cells and salinities and time exposure.

**KEY WORD:** Osmoregulation, Chloride Cell, Persian Sturgeon, *Acipenser persicus*

## Introduction

*Acipenser persicus* is a chondrosteian and semi-anadromous fish, habits mostly along the south coast of Caspian Sea. Today, its distribution range has been greatly reduced, due to the impact of factors such as pollution and overfishing. Propagation of fish and producing of numerous fingerlings for releasing to the Caspian Sea is a current national plan undertaken by the Iranian Fisheries Department. Juvenile sturgeons remain in freshwater from several months to several years, depending on the species (Doroshov, 1985) and then migrate to the sea. A study on the physiological conditions of juveniles during primary stages of life is being necessary to understand restocking management. Three recent studies

on the sturgeon's osmoregulation (Magnin, 1962 ; Urist & Van de putte, 1967 ; Potts & Rudy, 1972) have indicated that sturgeons are hypo-hyperosmotic regulators, as the teleosts are. However, a paucity of data and a lack of documentation regarding the environmental conditions needs further studies in chondrosteian osmoregulations. It seems, some sturgeon species have a narrow range of euryhalinity (Natochin *et al.*, 1985 ; Shelukin *et al.*, 1990). In teleosts organs which are involved in the osmoregulation are kidney, gills and digestive tract as well as in sturgeons (Cataldi *et al.*, 1995). In these studies, Na<sup>+</sup> concentration has shown an increasing trend in the first 12 hours and a decreasing trend after 24 hours (Kraushkina, *et al.*, 1978). Number and size of chloride cells also increased in sturgeons (Cataldi, *et al.*, 1995) and salmonid fishes (Clarke, 1982 ; Ura, *et al.*, 1997). In fact all of the studies in this area have been focused on the anadromous salmonids, as a results of their extensive hatchery culture and economic importance. In order to determine how juvenile sturgeons osmoregulate, and how this capacity for osmoregulation changes according to the development, this study was composed of three parts: study on plasma electrolytes, study on salinity tolerance in juveniles, and histological study on gills to determine changes in the number and size of the chloride cells.

## Materials and Methods

Juveniles of *A. persicus* from fish hatchery center were sampled and divided into three groups with the average weight of 1.5, 3 and 5g and then transferred to different salinity (0, 5, 10, 15<sub>ppt</sub>). This experiment had two replications in each treatment with 12 fingerlings.

Fingerlings were directly transferred into different concentrations of water salinity. Based on the fish weight, their density in each tank (25 lit volume) was about 125 ± 5g.

### **Water Supply:**

To have natural conditions, needed water was obtained from the Caspian Sea coast. Salinity of the sea water was about 8<sub>ppt</sub> (water was picked from the estuarine seaside). NaCl was used to prepare water with higher salinity and for low salinity, sea water was doused as an adequate supplement. Water salinity was measured by salinimeter (salinity Temperature Bridge Type M.C.5). Acclimation period was three days, sampling times were 3, 6, 12, 24, 72 hours after

acclimatization. Samples were taken from the blood for ion concentration and from gills for chloride cells observation.

### **Blood sampling:**

Blood was sampled by cutting the juvenile is peduncles. Totally 0.5<sup>cc</sup> of blood was supplied from ten fish. Blood samples were kept in 4<sup>°C</sup> overnight and then centrifuged at 3000 rpm for 20 min. The supernatant of serum was kept in (-20)<sup>°C</sup> up to measuring the ion concentration. Then Na<sup>+</sup> and k<sup>+</sup> were measured by Atomic absorption (Shimadzu-AA670/Japan) and Cl<sup>-</sup> concentration by Titrimetric-Method (Schales & Schales, 1998).

### **Gill sampling:**

Gill Fillaments were fixed in 4% formalin for several days. Then, they were embedded in parafin. Fillaments were sectioned to a thickness of 3 μm (with Rotary microtome) and stained with Hematoxylin-Eosin (H&E).

### **Statistics analysis methods:**

Kolmogrov-Smirnov Goodness of Fit test (P>0.05) was used to determine the normal distribution of data (Na<sup>+</sup>, k<sup>+</sup>, Cl<sup>-</sup> concentrations). Significant difference in ion concentration between different salinity for each weight class and also between different weight classes in the same salinity were determined by one-way analysis of variance (ANOVA).

## **Results**

During this study, mortality was not observed in 5<sub>ppt</sub> salinity in all weight classes. Mortality was observed in 1.5 and 3g in 10<sub>ppt</sub> salinity after 72 hours and in 15<sub>ppt</sub> salinity after 6 hours totaly (100%)(Fig.1&2). Mortality was observed in 15<sub>ppt</sub> salinity in 5g after 12 hours in all fish group (Fig.3). Na<sup>+</sup> concentrations showed an imperceptible trend (Fig. 4, 5 & 6) and K<sup>+</sup> concentrations, a decreasing trend (figures 7, 8 & 9).

Significant differences between Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> serum concentrations in different weights and salinity were determined (P<0.05). Serum Cl<sup>-</sup> concentration in 1.5, 3, 5g juveniles have had significant difference in all examined fish adapted in all salinity (P<0.05) . The same situation was seen in 3 and 5g fish. Na<sup>+</sup> and K<sup>+</sup>

serum concentrations in 1.5, 3 and 5g fish had significant difference in all salinity except 0 & 15, 5 & 10 and 10 & 15 ppt.

Significant differences of  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  concentrations between different weights in the same salinity have been illustrated in Fig. 10, 11 & 12.  $\text{Cl}^-$  concentration between 1.5 & 5g in 0 & 15ppt salinity showed a significant difference ( $P>0.05$ ).  $\text{K}^+$  concentration between 1.5 & 3g in salinity of 5ppt have significant difference ( $P>0.05$ ).  $\text{Na}^+$  concentration between 1.5 & 5g in salinity of 5 ppt have significant difference ( $P>0.05$ ).

Morphologically, a change in the chloride cell of gills was observed in different salinity after 72 hours. Number and size of chloride cells increased as the weight and salinity increased (Fig. 13,14,15, 16).

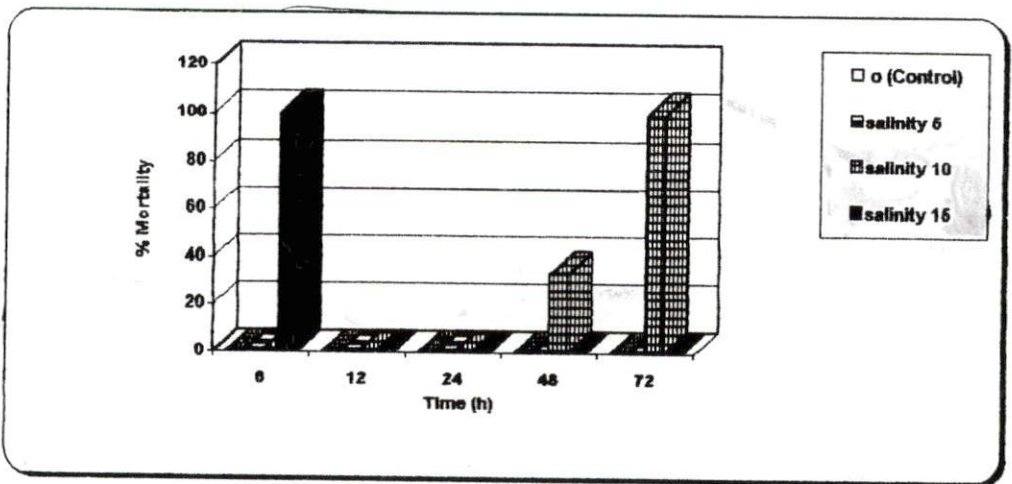


Fig. 1 : Mortality of juvenile Persian sturgeons (1.5g) at different exposure times (%).



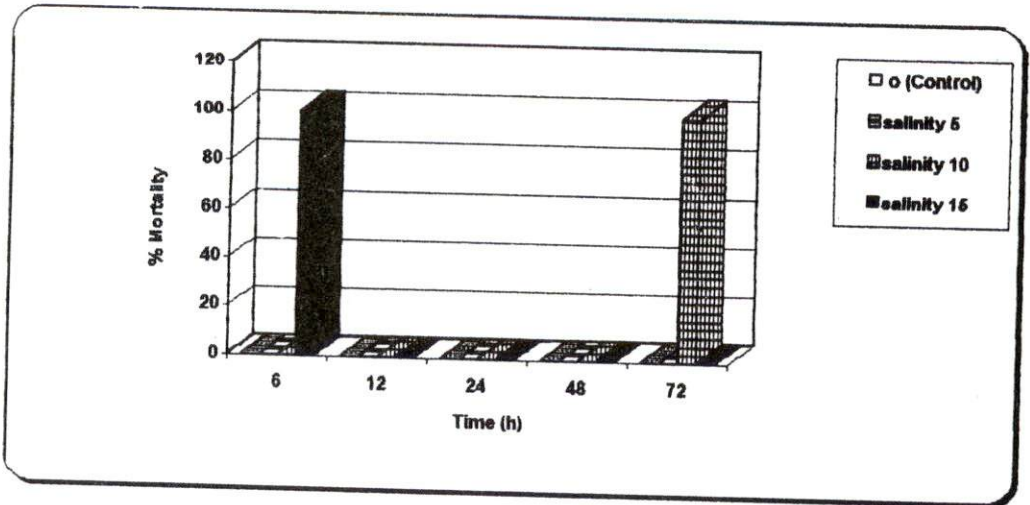


Fig. 2 : Mortality of juvenile Persian sturgeons (3g) at different exposure times (%).

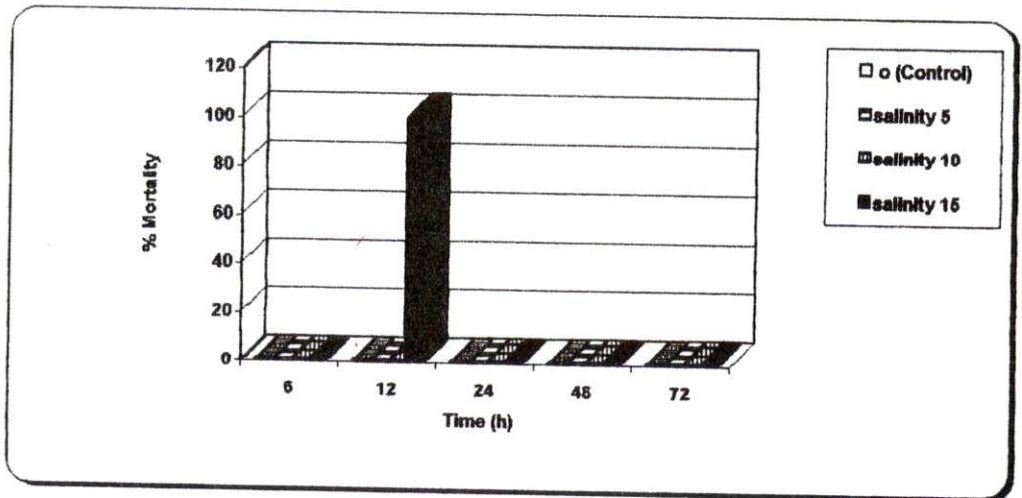


Fig. 3 : Mortality of juvenile Persian sturgeons (5g) at different exposure times (%).

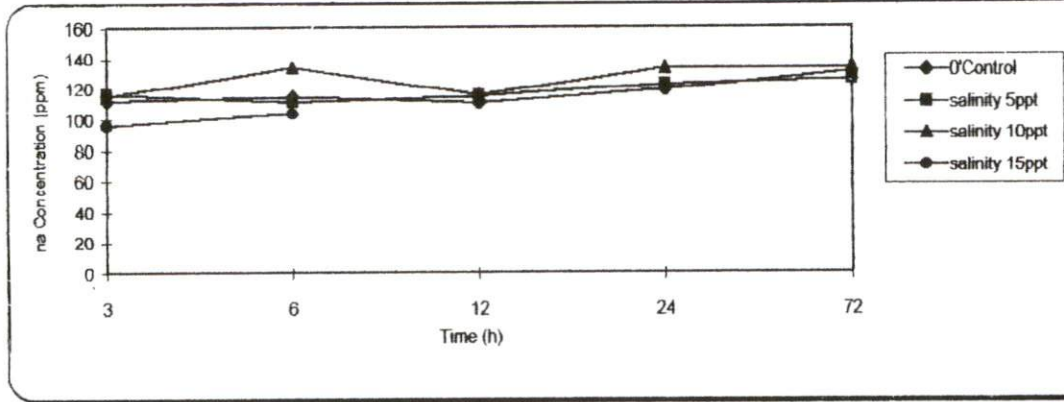


Fig. 4: Increasing and decreasing trend of Na<sup>+</sup> concentration during exposure time (1.5g).

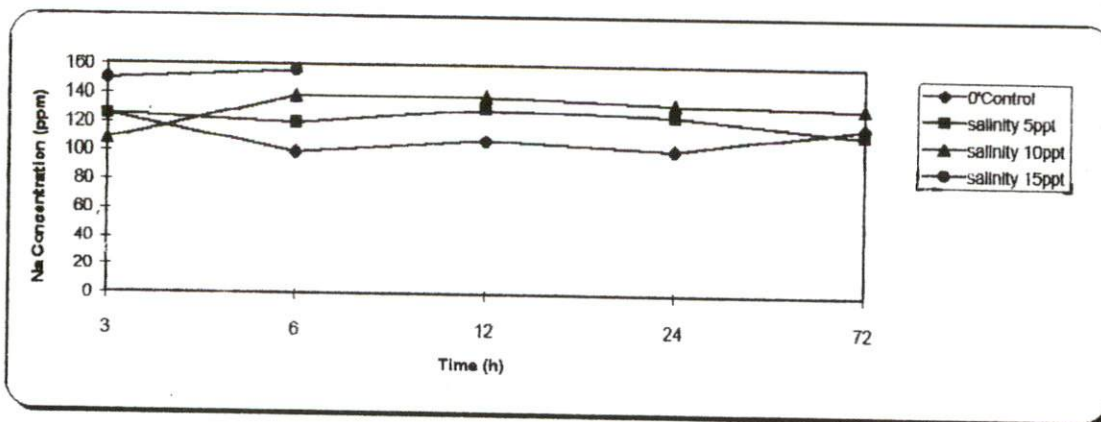


Fig. 5: Increasing and decreasing trend of Na<sup>+</sup> concentration during exposure time (3g).

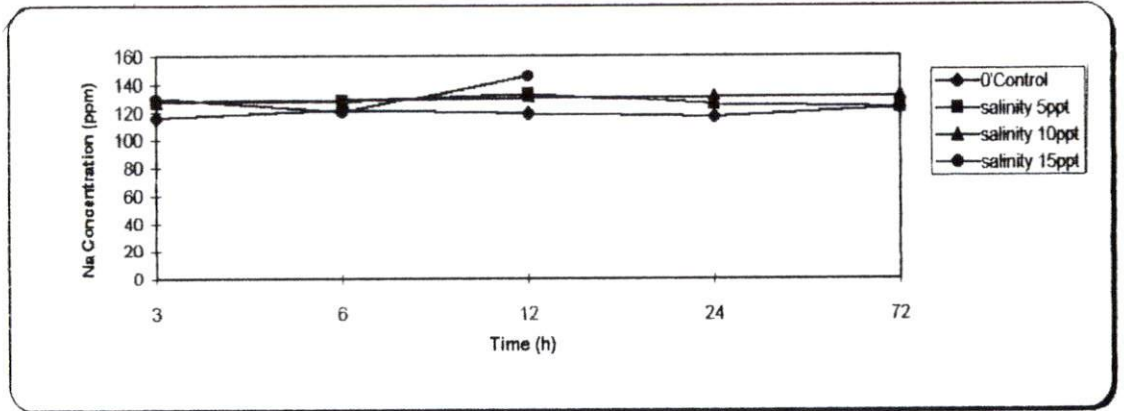


Fig. 6: Increasing and decreasing trend of Na<sup>+</sup> concentration during exposure time (5g).

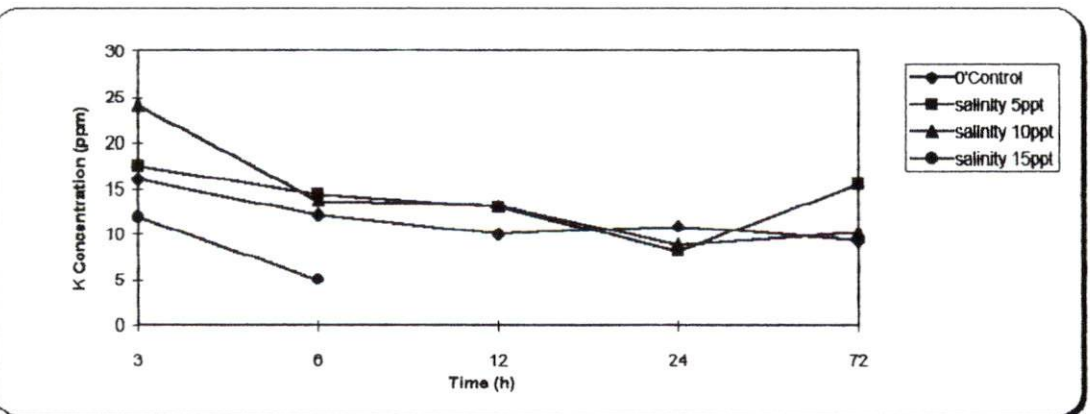


Fig. 7: Increasing and decreasing trend of K<sup>+</sup> concentration during exposure time (1.5g).

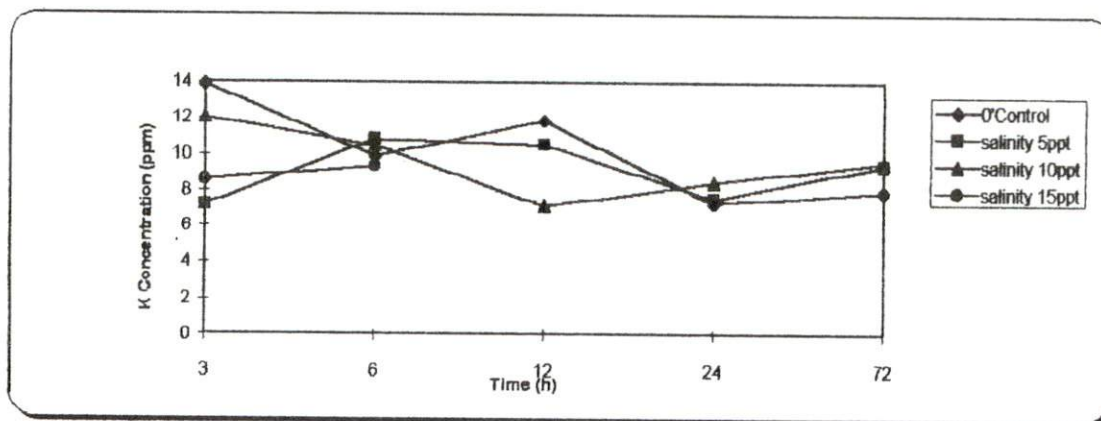


Fig. 8: Increasing and decreasing trend of  $K^+$  concentration during exposure time (3g).

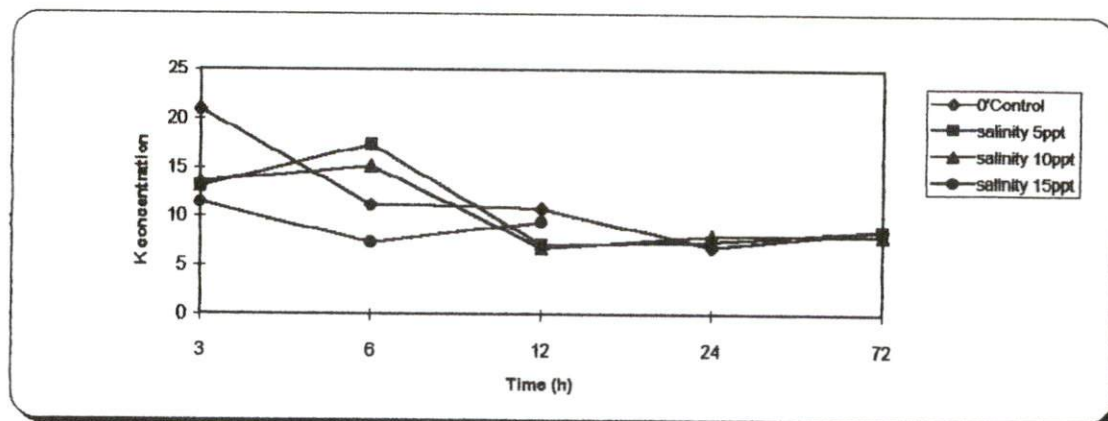


Fig. 9: Increasing and decreasing trend of  $K^+$  concentration during exposure time (5g).



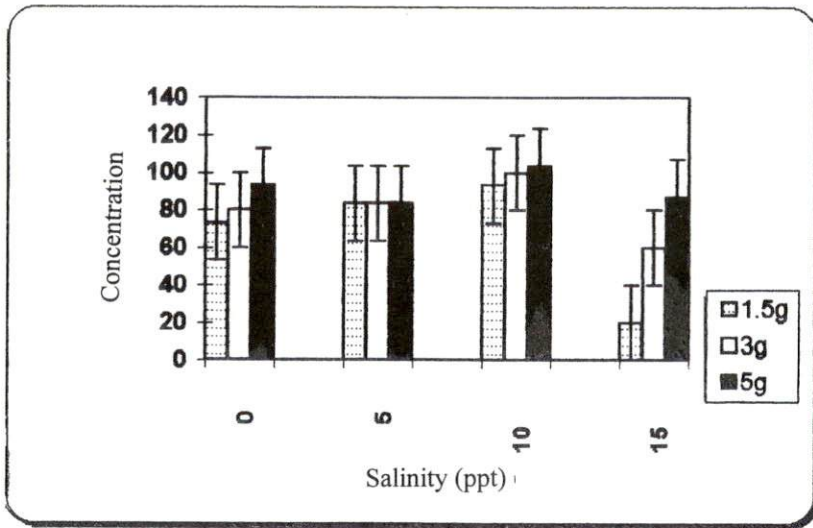


Fig.10: Cl<sup>-</sup> concentration in equal salinity in each weight class of Persian sturgeon

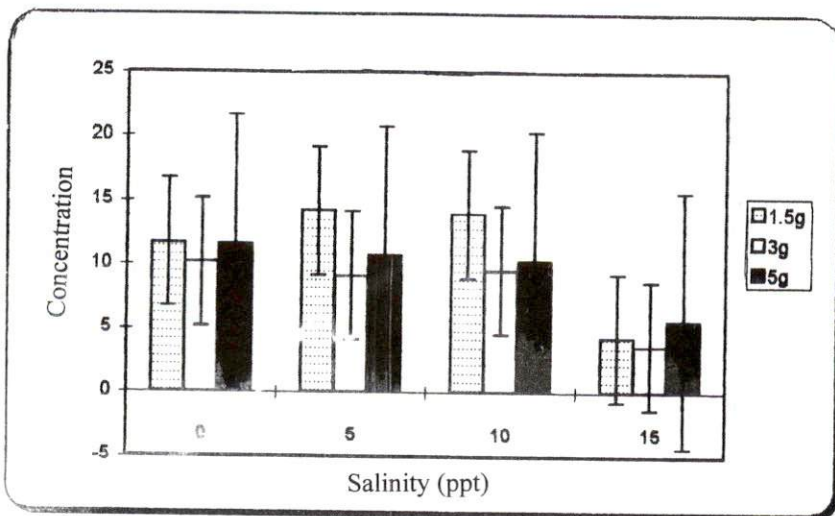


Fig.11: K<sup>+</sup> concentration in equal salinity in each weight class of Persian sturgeon

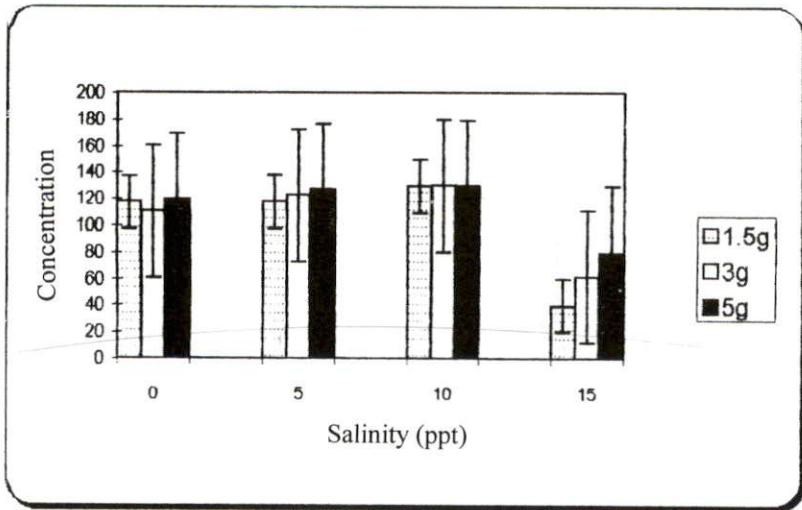


Fig.12: Na<sup>+</sup> concentration in equal salinity in each weight class of juvenile Persian sturgeon

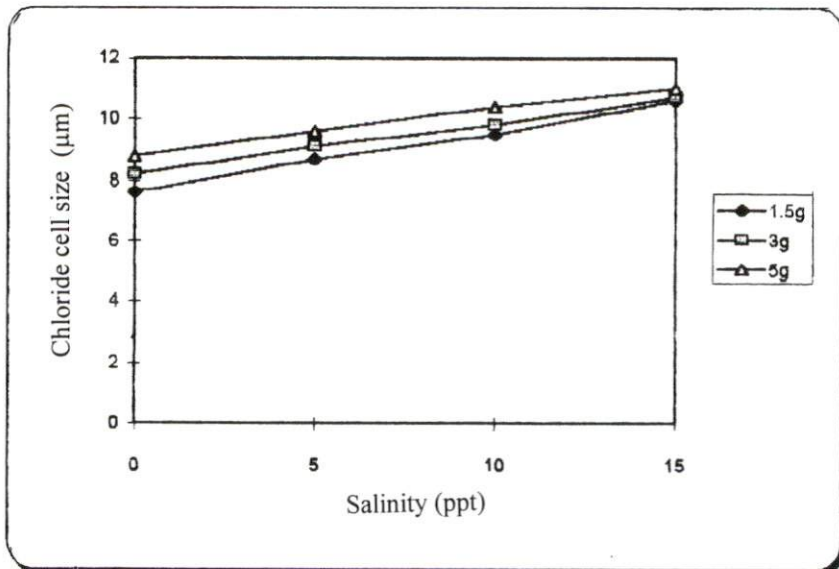


Fig.13: Changes of chloride cell size in different salinity exposed to juvenile Persian sturgeon (1.5, 3, 5g)

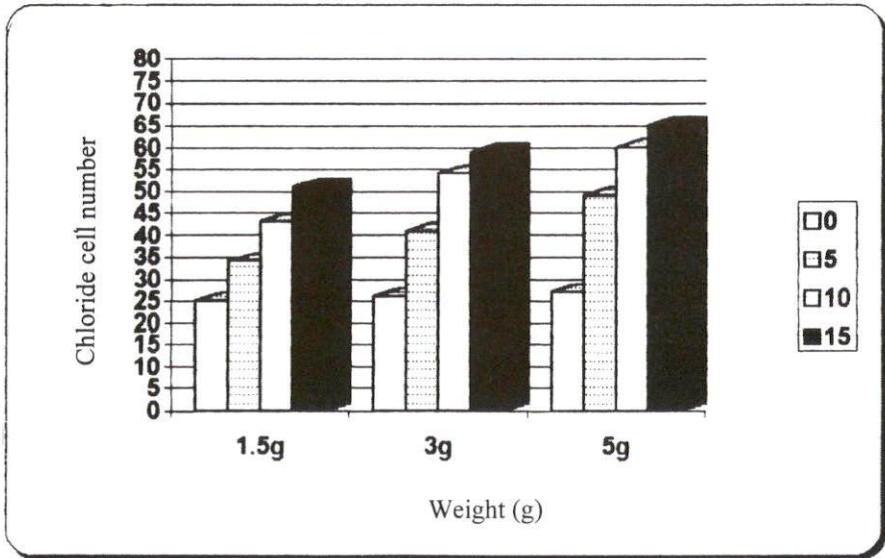


Fig.14: Changes of chloride cell number in different water salinity exposed to juvenile Persian sturgeon (1.5, 3, 5g)

## Discussion

*A. persicus*, a semi-anadromous fish, spends most of its life in the Caspian Sea. Recent studies on sturgeon's osmoregulation have indicated that sturgeons are hypo-hyperosmotic regulators, as the teleosts are (Magnin, 1962 ; Urist & Van de putte, 1967 ; Potts & Rudy, 1972). This study showed that the salinity tolerance is correlated with the size. It also showed that the mortality decreased with the increasing of fish size while salinity tolerance increased gradually. The same phenomenon was found in other sturgeon fish such as white sturgeon *A. transmontanus* (MacEnroe & Cech, 1985), *A. naccarii* (Cataldi, 1995), as in some teleosts: salmonids *i.e.* chum, sockeye, chinook, coho salmon and steelhead trout, (Clarke, 1982). Parry (1958, 60) concluded that the body size, not age, was the crucial factor in the development of salinity tolerance in salmonids. Our study indicated that *A. persicus* juveniles are able to tolerate salinity (about 5<sub>ppt</sub>). In other species of this genus Russian sturgeon, (*A. gueldenstaedtii*) in a water salinity of 5<sub>ppt</sub>, mortality was not observed, even at temperatures above 28°C and After 10 days, the mortality in this group was only 2% (Shelukin *et al.*, 1990). Although mortality was not reported in the salinity of 10.5<sub>ppt</sub>. (Krayushkina &

Moiseyanko, 1978).  $\text{Na}^+$  concentration had an imperceptible trend and  $\text{K}^+$  concentration had a decreasing trend, but  $\text{Na}^+$  and  $\text{K}^+$  concentration increased during the first 12 hours and then decreased after 12 hours in other sturgeons (Krayushkina & Moiseyanko, 1978). The same situation was reported in diadromous teleosts (Parry, 1985 & Houston, 1959). The osmoregulatory process in sturgeon is similar, in this respect, to the equivalent process in teleosts and differs from osmo-regulation in cartilaginous fishes (Krayushkina & Moiseyanko, 1978). In this study, it was shown that the number and size of chloride cells, were increased with weight and salinity. Such increased number and size of the interlamellar chloride cells were described for euryhaline teleosts during the adaptation to the seawater (Laurent & Hebebi, 1982). An increased number and size of chloride cells was also reported in *Oreochromis niloticus* and *O. mossambicus* (Cioni *et al.*, 1991). On the other hand, a degeneration and disappearance of lamellar chloride cells was reported for the Atlantic salmon during its migration to the sea (Borancin *et al.*, 1989). Recently, it has been reported that Chum salmon (*Oncorhynchus keta*) also had reductions in the numbers of cell mitochondria in salt water.

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