Acclimation potential of *Acipenser persicus* post-larvae to abrupt or gradual increase in salinity

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Summary

Besides commercial aquaculture another major aim of sturgeon culture is in support of restocking programmes. Identifying the appropriate size, age and location for release of post-larvae or fingerlings is very important to achieve success. We investigated the potential acclimation ability of *Acipenser persicus* post larval (245 ± 21.53 mg) to abrupt changes of salinity. Reared post larval (salinity 0.5) were exposed, either abruptly or gradually to brackish water (salinities: 2, 4, 6, 8, and approximately 12.5) for 5 days (120 h). Daily survival rate, gill chloride cell counts, and variation in glomeruli size were measured during exposure periods in both trails. These parameters were compared at the start and end of each trial. No mortality was observed during gradual adaptation to brackish water while chloride cell counts differed significantly (P < 0.05) at 6 ppt vs 12 ppt. In the gradual exposure trial, glomeruli sizes displayed a reduction with salinity increase from 8 to 12, this result was observed again between 96 and 120 h exposure to (12.5) in the abrupt exposure trial. In addition, post-larvae exposed to a gradual salinity increase had significantly higher final weights than those exposed to an abrupt salinity increase. Based on our result, it seems that *A. persicus* post-larvae exhibit some acclimation ability to gradual salinity change.

Introduction

Institutions operating under the Iranian Fisheries Organization are releasing annually about 14 millions fingerlings of the Persian sturgeon, *Acipenser persicus*, (weight 2.5–3.0 g) into the southern region of the Caspian Sea. It was previously unknown whether direct release of post-larvae could result in good survival in coastal brackish waters or whether this fish could tolerate abrupt transfer to low salinity concentrations. Osmotic balance depends on specialized tissues and organs such as gills, intestine and kidney that are controlled by physiological and hormonal functions (Evans, 1980, 1993; Varsamos et al., 2005). It is suggested that sturgeons have an osmoregulation similar to teleosts when exposed to different salinities (McEnroe and Cech, 1985; Natochin et al., 1985; Shelukhin et al., 1990; Krayushkina et al., 2001). Gills are heavily involved in osmoregulation by secretion of monovalent ions (Evans, 1993). The salinity effects on morphology and development of gill epithelia and chloride cells have extensively been investigated (Cataldi et al., 1995; Kültz and Somero, 1995; Altinok et al., 1998; McKenzie et al., 1999; Krayushkina et al., 2001; Carmona et al., 2004). Exposure to high salinity resulted in an increase in the number of chloride cells and in Na⁺-K⁺-ATPase activity (Hwang et al., 1989). Under saline conditions, an increase in size and number of chloride cells was observed in Beluga, *Huso huso*, (Krayushkina et al., 1976) and in the Gulf sturgeon, *A. oxyrinchus desotoi* (Altinok et al., 1998). Uchida et al. (1996) recognized two types of chloride cells (MRCs) in the gills of Chum salmon, *Oncorhynchus keta*, post-larvae, suggesting that in fresh water MRCs develop in both, lamellae and filaments of the gills. However, MRCs are only on the gill filaments in brackish water (Uchida et al., 1996, 2000). These results indicated that MRCs may operate as ion absorbers in fresh water, while MRCs in gill filaments act as salt secretor in seawater (Fosket and Schelley, 1982; Varsamos et al., 2005). The other important organ involved in osmoregulation is the kidney. It has been shown that kidney’s glomeruli surface is involved in filtration (Hickman and Trump, 1969). Even the kidneys of some fishes become agglomerate when being acclimated to sea water (Brown and Rankin, 1999). Previous studies have shown that salinity tolerance in anadromous fish depends on age [e.g., American shad, *Alosa sapidissima* (Zydlewski and McCormick, 1997a,b), or size for example in Atlantic salmon, *Salmo salar*, (McCormick and Naiman, 1984)]. Several studies demonstrated size dependent salinity tolerance potential in sturgeon (Brannon et al., 1985; Jenkins et al., 1993). For example, early salinity tolerance of two sizes (10 and 30 g) of 1-year-old juvenile white sturgeons, *Asipeser transmontanus*, differed when exposed abruptly to diluted sea water (0, 8, 16, 24 and 32 ppt), showing that at the same age salinity tolerance was higher in heavier fish (Mojazi Amiri et al., 2009). Others also found age dependent acclimation effects to salinity change (McEnroe and Cech, 1985). European Atlantic sturgeon, *Acipenser sturio*, juveniles could survive in 3–8 ppt NaCl concentrations, but died shortly after transfer to full strength sea water at the age of 2 years (Holcik, 1989). Although 4-year-old fish were able to live in the ocean (salinity 33), they could easily adjust themselves to different salinity environments at the age of 7 years (Magnin, 1963c). However, if the physiological mechanisms respond to changing environmental salinities in an size-age-dependent fashion, survival can be threatened if transferred too early. Therefore, this study was designed to test the salinity tolerance of *A. persicus* post-larvae with the objective to determine physiological indicators for best strategies to release early stages in coastal and estuarine environments of variable salinity.

Materials and methods

Two trials were conducted to test the physiological responses of *A. persicus* post-larvae to abrupt vs gradual exposure to
Caspian Sea coastal salinity. Post-larvae reared in freshwater (salinity 0.5) [20 days post-hatching, $245 \pm 22$ mg (n = 7)] were randomly distributed into nine tanks (three treatments with three replicates) at densities of 30 fish per tank. Different salinities were provided by dilution of coastal Caspian Sea water (salinity approximately 12.5) with freshwater. Post-larvae reared in fresh water (0.5) were used as controls. Salinity was determined with a digital salinometer (Cond 330i / set WTW, Germany). In the first trial, post-larvae were transferred abruptly from freshwater (n = 30, three replicates) to a salinity of approximately 12.5. In the second trial, post-larvae were stocked (n = 30, three replicates) in freshwater (salinity 0.5), then salinity was gradually increased within 5 days (2 units per day up to salinity 8), when finally the salinity reached Caspian coastal sea water strength (day 5). Post-larvae were fed with Daphnia at a rate of 2–3% of body weight (wet weight) per day, delivered in three portions. The survival rate and final weights were determined at the end of each trial. Three post-larvae were sacrificed daily after each stage of increasing salinity; they were fixed for histology using conventional methodology (Altinok et al., 1998; Carmona et al., 2004). Also three replicate samples were taken after 96 and 120 h in the abrupt exposure trial to Caspian Sea water salinity (approximately 12.5) to investigate salinity effects on chloride cell counts and glomeruli sizes.

Chloride cells were counted along the base of the gill arches and between the lamellae for each salinity trial and the control. Changes in minor and major diameters of the glomeruli (μm) were determined during the trials (five glomeruli of each of the three histological slide per salinity step for both, gradual and abrupt trials were measured).

Data analysis
Statistics were conducted using the SPSS package ver. 10.0 (SPSS Inc., Chicago, IL, USA). After checking for normality and homogeneity of variance, data were subjected to a one-way analysis of variance (ANOVA). Student t-test was used to test for significant differences between means of post-larvae weight among the two tests and the control group.

Results
Survival rate
The survival of A. persicus post-larvae in the abrupt exposure trial is presented in Fig. 1. After abrupt transfer to Caspian Sea water (approximately 12.5), post-larvae swam faster than in fresh water and started to circle around the tank, suggesting behavioral stress. After 1 day, swimming ceased in most of them and specimens remained on the bottom of the tank, while subsequently starting to die. On day 5, only 7–8 of the originally 30 post-larvae survived. Mean weight of post-larvae at the end of the first trail was $299.7 \pm 23.1$ mg. In contrast, no mortality was observed in the gradual exposure trial when salinity was gradually increased from 0.5 to approximately 12.5 (coastal Caspian sea water). They fed normally, displaying a mean weight of $353.8 \pm 56.8$ mg on day 5 (end of trial). Final mean weight in the control group was $392.5 \pm 56.8$ mg. A statistically difference between gradual exposure and the control groups was documented (P < 0.05) while mean weight differed (P < 0.05) between abrupt exposure and control group (Table 1). Thus, mortality occurred only in the abrupt exposure trial to salinity of 12. Apparently, dead post-larvae have not been feeding for some time as all stomachs were empty. There were also visible indications (redness under the skin) of inflammation and bleeding in the anus region.

Chloride cells
In both salinity trials (abrupt and gradual salinity transfer), chloride cell counts increased initially, with cells developing in the interspaces between gill lamellae, rather than on the gill filaments. In the abrupt transition trial, chloride cell counts increased significantly (P < 0.05) on the base of the gill arches and in the interspaces between the 10 gill lamellae (after 96 h), reaching on average 106 in number (Fig. 2). No further changes were observed up to 120 h, while some abnormalities were seen in gills after 120 h (Fig. 3). In the gradual transition trial, the number of chloride cells increased, reaching 126 cells (salinity of 6; at 72 h), but gradually decreasing thereafter (96 h) to about 103 cells as salinity increased to Caspian coastal sea water (approximately 12.5) (Fig. 4).

Size of glomeruli
Kidney histology showed that the glomerulus sizes in A. persicus post-larvae decreased with increasing salinity in the gradual trial, and this was significant at salinities higher than 6 compared to the mean value for the control group. No significant mean size differences were seen between salinities of 2, 4 and 6 (Fig. 5). Nonetheless, some evidence of glomeruli degradation was evident, even at lower salinity (Fig. 6). In the

![Fig. 1. Survival rate as a function of time after abrupt transfer of post-larvae from freshwater to brackish water (salinity approximately 12.5) during an exposure period of about 5 days. Values represent means and standard deviation (three replicates; initial n = 30 per replicate)](image)

![Fig. 2. Chloride cell counts (n = 3 per replicate) per gill arch base vs time in the abrupt transition trial from freshwater to a salinity of about 12.5. Columns represent means; bars Standard Deviations; different letters indicate significant differences at the end of experiment (120 h exposure)](image)
abrupt trial, a significant decrease was evident only after 96 and 120 h exposure to Caspian Sea salinity (approximately 12.5; Fig. 7).

Discussion

Adriatic sturgeon, *Acipenser naccari*, showed that this species is hypotonic to seawater (Sulak et al., 2007), while also under constant physiological stress when osmoregulating in saline waters (McKenzie et al., 2001a). Jenkins et al. (1993) described that 17-day-old Shortnose sturgeon, *Acipenser brevirostrum*, could tolerate salinity exposure up to 5 for a period of 96 h without mortalities, but at a salinity of 20 stress signs occurred almost immediately and no larvae survived the treatment after 6 h. However, Brannon et al. (1985) found that white sturgeon larvae and post-larvae could not tolerate and survive in salinities over 16. McEnroe and Cech (1985) found that a gradual transfer of *A. transmontanus* juveniles from fresh water to a salinity of 15 increased the survival rate. However, no juveniles survived abrupt transfers to a salinity of 25. Specimens seemed to grow better in freshwater rather than in brackish water during early post-larvae development stage when comparing the final weight of both trails (gradual or abrupt salinity exposure) and the control group. The reason may be that osmoregulation is metabolically costly, especially for sturgeon juveniles (Singer and Ballantyne, 2002), and these costs are lowest in freshwater during juvenile stages (Jarvis et al., 2001). A higher standard metabolic rate (30%) and subsequently lower growth rate (17%) was found in YOY Adriatic sturgeons reared in a salinity of 11 compared to the freshwater control group (McKenzie et al., 2001a). In this study, exposure times (max 5 days) were short, so that differences may have been affected not so much by growth but more by differential mortality of smaller post-larvae or feeding cessation during abrupt salinity change.

Gill chloride cells responded in both salinity trials by increasing in number mostly by the third day. Similar findings have been reported by Altinok et al. (1998) and others.
changes. For instance, kidneys secrete bivalent ions (especially 
either due to decrease in glomeruli surface or due to structural 
Baustian et al., 1997). The urea filtration rate (UFR) decreases 
observed as previously reported (Nishimura and Imai, 1982; 
and that various stages of glomeruli degradatio would be 
mossambicus (Hwang and Wu, 1988), 
anadromous American shad, 
may not be able to adjust to abrupt salinity increase. In the 
increase in gill chloride cell counts improves. Thus post-larvae 
reported in teleosts and Adriatic sturgeon (Zydlewski and 
compared to freshwater adapted specimens. Our observations 
(McKenzie et al., 1999; Krayushkina et al., 2001; Carmona 
changes in MRCs size was not evaluated in the present study. 
The mechanisms of differential salinity adaptation require 
may be hypothesized that glomeruli filtration rate is low (0.5–1 ml kg 
filtration rate is low (0.5–1 ml kg 
the river before entering to the bay, suggesting that a period of 
period of juveniles will continue up to 24 days in fresh water 
gradual exposure, but not during abrupt exposure. 
Also, in ecological studies on Gulf Sturgeon the migration 
propagation center (SMSPC) for their kind help during the 
protection Center (SMSPC) located in the southern region of the Caspian Sea (Gorgan Province). 

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