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The effects of salinity on the fertilization rate and rearing of the Persian sturgeon (*Acipenser persicus*) larvae

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Abstract Persian sturgeon eggs were fertilized with different levels of salinities (0.5, 2, 4, 6, 8, 10, 12 ppt), and then each group was incubated in the same salinity until hatch. The fertility (%), hatching rate as well as larvae cumulative mortality rate and abnormality (%) were measured. Our Results revealed that Persian surgeon eggs could be fertilized in the different salinity concentrations but not more than 4 ppt. Moreover, hatching rate decreased with increase in salinities more than 2 and 4 ppt, respectively, and no larvae hatched in 6 ppt salinity. According to these results, the salinity tolerance threshold for Persian sturgeon larvae hatching in brackish water is less than 4 ppt.

Keywords Salinity · Fertilization · Egg · Mortality · Hatching · Abnormality

Introduction

Sturgeon culture either in fresh or in brackish water is promoting nowadays in Iran due to severe decrease of these fishes in the Caspian Sea. Persian sturgeon migrants, *Acipenser persicus,* as an anadromous fish, annually migrate to upstream of southern rivers of Caspian Sea to find proper spawning area. Construction of several types of preventive blocks (e.g., dams, bridges, etc.) across the migration ways and near the estuary caused several kinds of problems for natural propagation of Persian sturgeon. Although there are some reports on propagation techniques of Persian sturgeon restocking methods (Alavi et al. 2004; Abtahi et al. 2006), limited information is available on the environmental requirements. The possibilities of sturgeon spawning in brackish water and hatching of fertilized

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egg under this condition have not been cleared yet. It seems that the adaptability of each developmental stage to salinity depends on osmoregulation ability of embryos and larvae. Although the salinity tolerance and osmoregulation capability of sturgeon juveniles (Shelukhin et al. 1990; Jenkins et al. 1993; Ziegeweid 2008; Xugang et al. 2009; Mojazi Amiri et al. 2009) and adults (McEnroe and Cech 1985; Paschos et al. 1999; Krayushkina 2006) are well documented, the investigation of salinity effects on fertilization and early ontogenesis of sturgeons was in fancy (Cataldi et al. 1999; Khodabandeh et al. 2007). It has been reported by several researchers that sturgeon has a similar osmoregulatory mechanism with teleost (McEnroe and Cech 1985; Shelukhin et al. 1990; Krayushkina 2001). The mechanism of osmotic and ionic regulation of eggs and post-embryonic development of teleost fish (Alderdice 1988; Varsamos 2005) and the effects of salinity in some teleost species eggs and larvae have been reviewed before (Bohlen 1999; Weirich and Tiersch 1997, Phelps and Walser 1993; Fashina-Bombata and Busari 2003; Fuda et al. 2007). There are some evidences that indicate freshwater is critical for fertilization of all sturgeon species (Bemis and Kynard 1997), but low salinity tolerance during early developmental stages has been observed in some species (Bain et al. 2000). Dovel and Berggren (1983) reported the presence of sturgeon embryos far from Hudson River which includes some brackish water but others indicated mortality of *Acipenser transmontanus* and *Acipenser brevirostrum* at salinities over 5–10 ppt (Jenkins et al. 1993). Although the osmoregulation mechanism of Persian sturgeon fry and fingerlings has been investigated in two separate studies (Khodabandeh et al. 2009; Khatooni et al. 2011), the possibility of fertilization and embryo development in brackish water remained to be studied.

Embryonic and larval abnormalities are distinguished as a recurring problem in fish aquaculture that represents ethic and economic challenges for the industry (Takle et al. 2005). Some natural environmental factors, like salinity, pH, etc., are shown to be responsible for inducing deformities in fish embryos and/or larvae (Kjørsvik et al. 1990). Bune et al. (2000) reviewed the malformations occurrence in the embryonic and early larval stages under various conditions, such as natural environmental stressors, anthropogenic pollution, and hatchery rearing practices. Abnormality developments in late larval (i.e., post-yolk-sac feeding stage), the juveniles, or the adult stages have been described in the literatures for a variety of species (Gavaia et al. 2002, Fraser et al. 2004; Fraser and De Nys 2005). The sturgeons malformation caused by environmental condition was investigated by Dettlaf et al. (1992). In addition, different types of abnormalities in Sturgeon postembryonic ontogeny were studied by Ruban et al. (2006).

According to the above-mentioned facts, determination of the salinity effects on fertilization, hatchability rate, and potential tolerance of the embryo and larvae of Persian Sturgeon during early ontogeny would be applicable to understand the fate of spawning happens near the estuary. Thus, this study investigates the salinity threshold of Persian sturgeon embryo and larvae during early stages.

Materials and methods

Eggs and sperm were collected from spawners fish migrating to the rivers of Southern Caspian Sea (Golestan province, NorthEastern, Iran) during spawning season ($\sim$18 °C, March). Different levels of salinity treatments were obtained by diluting Caspian Sea water (12 $\pm$ 0.5 ppt) with fresh water (0.5 ppt). Water salinity was measured with a salinometer (WTW, Germany).

The effect of salinity on fertilization and short-term storage of Persian sturgeon eggs in brackish water

Eggs were divided into 50 g groups and then fertilized in 0.5 (fresh water as control), 2, 4, 6, 8, 10, 12 ppt salinity treatments. After removing adhesiveness with tannic acid (1 %), the triplicated groups of fertilized eggs were transferred into special recycle incubators with 0.5, 2, 4, 6, 8, 10, 12 ppt salinity (Fig. 1). Sperm motility duration was measured in each salinity treatment before fertilization under light microscopy (Microscope Olympus BX50 with dark-field condensor) and then fertilization rate calculated at second cleavage stage according to Dettlaff and Ginsburg (1992). Hatching was started on fifth day post-fertilization and lasted for 3 days (from fifth to seventh day) in 18 °C. Hatching rate was calculated based on the equation of the hatched larvae in each treatment/the numbers of eggs incubated in each treatment $\times 100$; the eggs number was calculated based on the equation (number of eggs in 1 g of eggs (Ca. 54–56) $\times$ 50 g).

The effects of salinity on short-term storage of Persian sturgeon larvae in brackish water

After hatching, larvae were stocked in tanks with the same salinity at which they were hatched and reared until the start of exogenous feeding which lasted for 12 days post-hatch (DPH). Cumulative abnormality and mortality rate of larvae were determined daily. Larvae mortality rates in various salinity levels were studied in three separate phases of Laval development including: 1. endogenous feeding (up to 7 dph); 2. exo-endogenous feeding stage (7–10 dph), and 3. exogenous feeding (10–12 dph).

Data analysis

Data were subjected to a one-way ANOVA to test difference between treatments. Tukey test was used for mean comparison among treatments ($P < 0.05$) (Zar 1994). Data are expressed as mean values $\pm$ SE. All statistical analysis was carried out using the statistic software SPSS version 11.0 (SPSS Inc., Chicago, IL, USA).

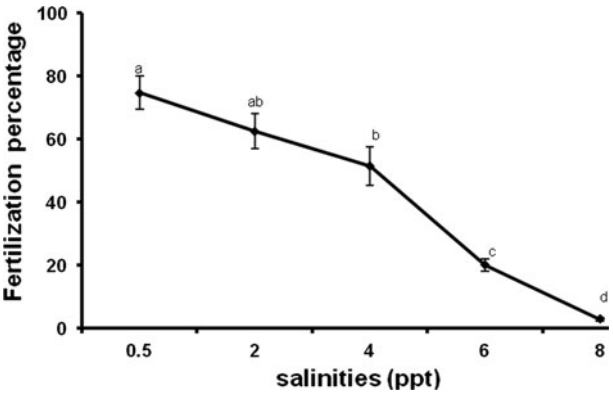

Fig. 1 The fertilization rate of Persian sturgeon eggs in different salinities. Different superscript letters indicate significant difference ($P > 0.05$). Data are presented as mean $\pm$ SE

Results

The effect of salinity on fertilization and short-term storage of Persian sturgeon eggs in brackish water

Sperm motility duration

Sperm motility period during exposure to various salinity levels are shown in Table 1. The motility duration decreased significantly with the increase of water salinity ($P < 0.05$). Absolutely no motility was observed in 10 and 12 ppt brackish water. No forward movements observed in 8 ppt and only their sperm tail was moving in the same place (Table 1).

Fertilization rate

Fertilization rate decreased significantly in 2 and 4 ppt treatments compared to control ($P < 0.05$). The reduction was more remarkable from 4 ppt onward and fertilization rate was near zero in 8–12 ppt treatments (Fig. 2).

Hatching rate

Hatching started on fifth day-post fertilization at 18 °C and lasted for 3 days in all treatments except 2 ppt which completed mostly in the sixth day. The hatchability of eggs incubated in 2 ppt and control group was 81 ± 3.1 and 85 ± 4.1 %, respectively, and was significantly higher than other treatments ($P < 0.05$). The hatching rate decreased by 54 ± 3.5 % in 4 ppt salinity and absolutely no hatching observed in 6 and 8 ppt (Fig. 3).

The effects of salinity on short-term storage of Persian sturgeon larvae in brackish water

The cumulative daily abnormality from hatching to the beginning of exogenous feeding was divided into three stages according to abnormality curve in the Fig. 3. In the first stage, 1–7 dph larvae (which were only dependent to the yolk sac absorption), larval abnormality increased gradually in all treatments but this increasing was more obvious in 4 ppt salinity. This trend prolonged in second stage (endo-exo feeding period) and was slower between 7 and 10 dph (Fig. 3). In the third stage (exogenous feeding), the abnormality increased in 10–12 dph larvae. Severe abnormalities were seen during developmental stages in 4 ppt fertilization and incubation treatment (Fig. 4). These abnormalities include abnormal ocular development (single eye larvae, and no eye larvae) and skeletal/morphological deformation (curved larvae, small head larvae) (Fig. 4).

Table 1 The effects of brackish water on duration of sperm motility in Persian Sturgeon

Salinity	0.5	2	4	6	8	10	12
Sperm motility duration (s)	90 ± 5^{a}	75 ± 8^{b}	38 ± 4^{c}	15 ± 9^{d}	10 ± 8^{d}	0^{e}	0^{e}

Data are presented as mean ± SE

Values in a row with the same superscripts denote no significant difference ($P > 0.05$)

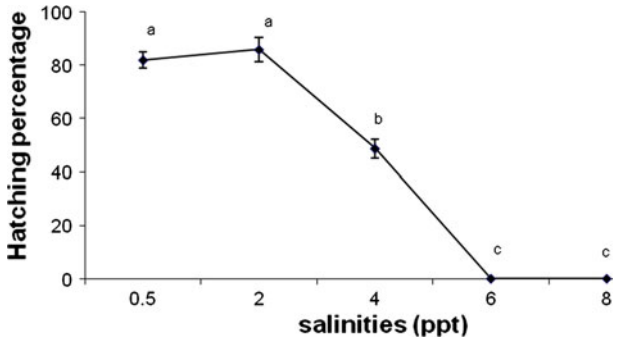

Fig. 2 The hatchability percent of Persian sturgeon eggs incubated in different salinities. Different superscript letters indicate significant difference ($P > 0.05$). Data are presented as mean ± S.E

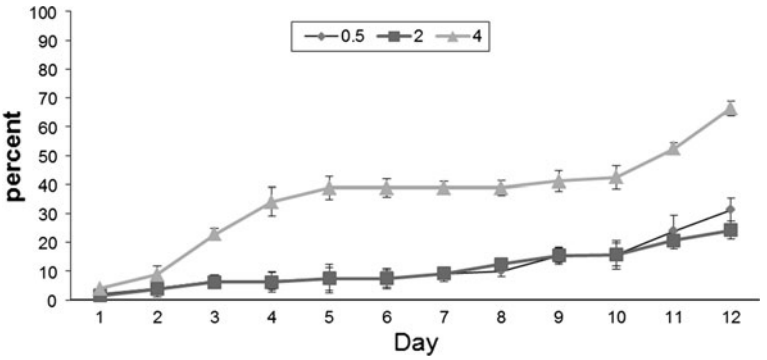

Fig. 3 The cumulative daily abnormality of *A. persicus* larvae in different salinities. Data are presented as mean ± SE

Daily cumulative mortality of larvae showed the same trend as abnormality during three stages of larval development. The mortality significantly increased in 4 ppt treatment. The result showed that the highest mortality rate occurs in the first stage of larvae development in 4 ppt treatment. Exogenous feeding in 4 ppt occurred one day later compare to other treatments (Fig. 5).

Discussion

This preliminary study investigates the possibility of fertilization, hatching, and rearing of the Persian sturgeon *Acipenser persicus* larvae in brackish water. The results showed that the water salinities more than 4 ppt significantly decreased Persian sturgeon fertilization rate. This reduction is possibly due to decrease of spermatozoa motility duration in brackish water. No motility of Persian sturgeon sperm observed in 8 ppt supports the hypothesis. Alavi et al. (2004) reported that osmolalities more than 100 mos mol kg had an inhibitory effect on sperm motility of Persian sturgeon. Likewise, in other sturgeons species such as shovelnose sturgeon (*Scaphirhynchus platorynchus*) and paddlefish (*Polyodon spathula*), 70–120 mosmol resulted in remarkable reduction in sperm motility

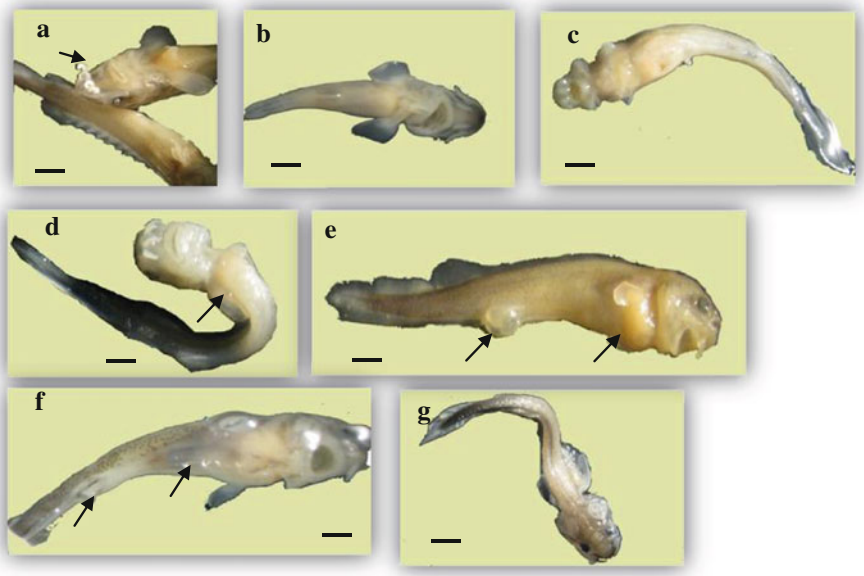

Fig. 4 Some severe abnormalities in post-larvae of Persian Sturgeon that fertilized and produced in 4 ppt salinity treatments. (**a** curved barbells, **b** abnormal mouth, **c** undeveloped head, **d** curved abnormal larvae with short barbells and short snout and unformed pectoral fin, **e** sever abnormal larvae with defection in hart and anal, **f** sever abnormal larvae with defections in heart and blood current system also defection in head, mouth and anal shape, **g** abnormal curved larvae with one pectoral fine (defection in left pectoral fin)). As it showed in the picture all these abnormal larvae have malnutrition and died after yolk sac absorption (*bar*: 1 mm)

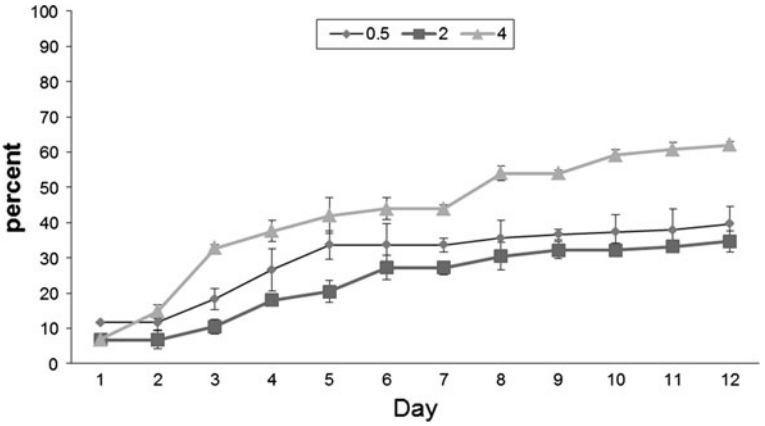

Fig. 5 The cumulative daily mortality of *A. persicus* larvae in different salinities. Data are presented as mean ± SE

(linhart et al. 1995). Our results were in accordance with critical salinity range (<8) reported for sperm motility in some fresh water fish species and high osmotic pressure (more than 120–400 mos mol kg) which inhibits sperm motility in freshwater fishes

(Plouidy and Billard 1982; Linhart et al. 1995; Alavi and Cosson 2006). Although Mg^{2+} (15 mM) showed negative impact on the motility of spermatozoa in *A. persicus* (Alavi et al. 2004), external Ca^{2+} ions act as a prerequisite for the initiation of motility of sperm in sturgeons (Linhart et al. 2002). It can be concluded that decrease of sperm motility in *A. persicus* is possibly due to increase in Mg^{2+} and decrease in Ca^{2+} ion content in environmental salinities, more than 4 ppt. Moreover, salinity affects water absorption during fertilization. It was found that even low concentration of NaCl would prevent water absorbance and subsequent previtelin space formation in many fresh water species (Zotin 1965). Also it has been reported that the chorion hardening in *Oncorhynchus* sp. would be stopped in salinities more than 3 ppt (Alderdice 1988). Phelps and Walser (1993) and Weirich and Tiersch (1997) were also reported the same results on the hatching, and survival of channel catfish of eggs and fry in 4 ppt salinity.

The results of this study indicate that the salinities more than 2 and 4 ppt significantly affect cumulative abnormality and mortality rate of Persian sturgeon larvae. Therefore, it seems that in the rivers where the fish could not migrate due to human made restrictions, if sturgeons spawn far from estuaries (salinity lower than 4 ppt) and the other conditions like oxygen content and proper food, larvae can be alive. Khodabande et al. (2007) also declared acclimation of Persian Sturgeons fry with 5 ppt salinity with cortisol hormone bathing. Other studies also demonstrated low salinity tolerance of sturgeon larvae (Crance 1987; Bain et al. 2000). Bath et al. (1981) collected the sturgeon larvae in the water with the salinity of 0–22 ppt, and Dovel and Berggren (1983) reported the sturgeon's embryos presence in far distances as 60–148 km from the sea of Hudson River which includes some areas of brackish water. However, Van Eenennaam et al. (1996) reported that the Atlantic sturgeon's *Acipenser oxyrinchus* embryos habitat is completely above the salt wedge. Shortnose Sturgeon (*Acipenser brevirostrum*) larvae and post-larvae mortality were reported in 5–10 ppt salinities (McEnroe and Chech 1985; Jenkins et al. 1993). Brannon et al. (1985) also found that salinities lower than 16 ppt kill white sturgeon larvae and fry, and Jenkins et al. (1993) reported that 17-day-old shortnose sturgeon fry were able to tolerate up to 5 ppt salinity with no mortalities for 96 h. It is well documented that salinity tolerance in sturgeon species larvae and juveniles is size- (Jenkins et al. 1993) and age-dependent (Cataldi et al. 1999; Mojazi Amiri et al. 2009) process. Therefore, as the fertilization and hatching rate significantly decreased gradually with the increase of salinity from 2 to 4 ppt, it could be concluded that the *A. persicus* eggs and embryo has medium salinity tolerance like most of sturgeons (Bain et al. 2000) and can tolerate the salinity less than 6 ppt.

In conclusion, our results indicated that the water with low salinity by 4 ppt can be used in Persian Sturgeon fertilization, and it seems that this may help to control the fungi infectious and reduce necessity for using common antifungus like formalin, etc. (Abtahi et al. 2006). Also, the results confirmed that the salinity threshold of spawning habitat for Persian Sturgeon must be less than 4 ppt and then brackish water can be considered in artificial propagation where accessing to fresh water is restricted.

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