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Original article

Effect of different levels of salinity on the viability of persian stuirgeon, *Acipenser persicus*, embryos during different developmental stages

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A B S T R A C T

Because of limited information about the developmental stages of *A. persicus* eggs and larvae in brackish water, the experiment conducted to compare the effects of salinity on ontogeny of Persian sturgeon embryos during incubation period. Fertilized eggs were divided into 50 gr groups and transferred directly into brackish water treatments with 0.5, 2, 4, 6, 8, 10 and 12 ppt salinity. Mortality and abnormality percent of embryos in three different developmental stages including gastrula, s-type heart formation and before hatch stage (35th embryonic stages) were measured and compared. Results showed that the mortality and abnormality of Persian Sturgeon embryos increased a long with increase in salinity but the embryos showed more tolerance during blastula and gastrula stages in lower salinity and larvae could not hatch in salinities more than 6 ppt. Results suggest that as the salinity increases more than 6ppt the embryonic development will be retarded or make it useless in Persian sturgeon. All in all, the salinity tolerable range during incubation period in Persian sturgeon is between 0.5- 6 ppt.

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1. Introduction

Sturgeon populations have suffered from over fishing, loss of habitat, and decrease of water quality (Smith and Dingley, 1984; Kynard, 1997). Annually they migrate upstream to find proper bed for spawning in fresh water. The factors which affect fish eggs mortality were divided in environmental and internal factors (Heath, 1992). Some studies have investigated the environmental factors impacts on abnormalities and morality during early developmental stages of sturgeon (Dettlaff and Ginsburg, 1992; Gvozdenko *et al.,* 1999; Hardy and Litvak, 2004; Ruban *et al.,* 2006) but the salinity effect on the developmental stages of Persian sturgeon (one of most important species of Caspian Sea) is not studied before. It was indicated that the salinity tolerance during early developmental stages depends on how internal fluids perform in various extents (Holliday, 1969; Alderdice, 1988). Salinity effects have been studied extensively in marine fish embryos and larvae (Young and Dueñas, 1993; Estudillo *et al.,* 2000; Berlinsky *et al.*, 2004; Cataldi *et al*., 2005; Yang and Chen, 2006; Jørgensen and Hansen, 2010), but they are limited in freshwater species (Phelps and Walser, 1993;Weirich and Tiersch**,** 1997; Gbulubo and Erondu, 1998; Bohlen, 1999; Sawant *et al.*, 2001; Fashina-Bombata and Busari, 2003; Albert *et al.,* 2004; Bonisławska, 2009). It has shown that some freshwater teleost eggs can be incubated and hatched in 5ppt salinity but fertility and eggs hatching rate of freshwater teleost decreases in saline water (Gbulubo and Erondu, 1998; Fashina-Bombata and Busari, 2003). The other report showed lower than 5ppt salinity tolerance in Zebrafish (*Brachydanio rerio*) (Sawant *et al.*, 2001). Rockwell (1956) reported 70-90 % mortality in Pacific salmon, *Oncorhynchus gorbuscha* and *O. keta* 19 to 40 days after incubation period in 12ppt salinity but, salinity less than 6ppt has not very lethal effect in these species. Rubin (1994) Also showed, (For the salinity range found in the Baltic Sea), a small negative influence on egg-to-fry survival of sea trout eggs in 6ppt salinity (11% more compare to freshwater) but it caused a delay in hatching time and duration of the hatching period was increased. On the other hand some studies reported the salinity effects on hatching rate of freshwater teleost. For example Spined loach (*Cobitis taenia*) embryos developed successfully in the range of 0.12 to 4.80ppt salinity (Bohlen, 1999). But at 6.00ppt the hatching was strongly reduced, and development failed at or above 7.20 ppt. Froelich and Engelhardt (1996) and Weirich and Tiersch (1997), reported that the low salinity incubation (as 2ppt) has control the fungus growth in koi carp (*C. caprio*) and Channel catfish (*Ictalurus punctatus*), also increases the hatching rate compare to control was reported but no effect on hatching time was reported in these species. Mortality occurs because of the embryo incapability to maintain osmotic pressure in normal rate in order to unbalanced ion gradient (Alderdice, 1988; Bunn *et al*., 2000). Holliday and Jones (1967) found that the egg salinity resistance was lowest in Blastula and gastrula in freshwater teleost eggs and Tylore (1971) found the most sensitivity just enclosing blastopore prior to hatch. However, there is limited information on how salinity affects developmental stages of sturgeons (Zotin, 1965; Jian-Yi *et al*., 2006). Zotin (1965) defined five phases of water uptake by embryos of Beluga and sevruga during developmental stages. First between fertilization and gastrulation which differs as rapid uptake, the second stage continued up to yolk plug closure which ceases water uptake, third followed up to heart angle formation that water uptake was rapid. Forth related to heart pulsation beginnings and he reported some water lost in this stage and in fifth period to hatch that no water is absorbed.

Thus, this study was conducted to investigate how salinity affects early developmental stages of the fertilized eggs and embryo of Persian sturgeon (*A. persicus*) during different developmental stages.

2. Materials and methods

Eggs and sperm were obtained from migratory breeders of southern region of Caspian Sea (Golestan province). The eggs were fertilized in fresh water (0.5ppt). After removing the eggs adhesiveness with tannic acid (1%) and water absorption (20 min), the fertilized eggs were divided into 50g groups (eggs per gr number, 45±2) and transferred directly into recycling incubators that designed for this study with 2, 4, 6, 8ppt salinity treatments. Caspian Sea water (12-12.5ppt) was diluted to provide brackish water treatment and fresh water used as a control (0.5ppt). The recycling incubator water exchanges two times every day. The eggs were treated by 1ppm formaldehyde just 2 times a day after each water exchange. Trial duration at the end of larvae hatching was 7 days at 18ºC. Mortality and abnormality percent were distinguished and determined according to Dettlaff and Ginsburg (1992) method during three developmental stages of gastrula, s-type heart formation and before hatch stages during exposure to the different salinity treatments. The salinity tolerance was compared in each stage between all treatments. Finally the hatching rate was measured [the numbers of hatched larvae/total eggs number)× 100] in each treatment. This experiment was done in a random projection including six treatments (2, 4, 6, 8, 10 and 12ppt) with three replications for each treatment. Data were analyzed with one way ANOVA and Tukey test used for comparing mean values in each treatment.

3. Results

Effects of Salinity on abnormality rate during incubation period:

The abnormalities were distinguished and classified in each of three developmental stages and the result showed in fig. 1 to fig.3. Result has shown that the effect of salinity on abnormality percent was different in each developmental stage (Table 1). For example the abnormality percent increased significantly in gastrula stage only in 6 and 8ppt treatments. The abnormality rate in s- type heart formation stage was significantly higher in all treatments compare to control but it was still below 20%. The abnormality rate increased significantly in 4 and 6ppt treatments before hatch stage.

Also our results showed that the salinity significantly affect abnormality percent during developmental stages of each treatment (*P*< 0.05). For example in 2ppt treatment the abnormality was increased significantly in s-type heart stage compare to gastrula stage but in 4ppt treatment the abnormality increased significantly in all three stages (*P*< 0.05). The significant decreased abnormality rate in 8ppt resulted from the high mortality of embryos in 8ppt treatment (Table 1).

Table 1
The effects of different salinity treatments on abnormality percentage during three developmental stages of Persian sturgeon embryo.

Treatments	**0.5**	**2**	**4**	**6**	**8**
Gastrula	2.56±0.6 ^{a}	4.33±1.0 ^{a*}	5.76±0.6 ^{a}	18.26±5.6 ^{b}	28.23±8.9 ^{c}
S- type heart formation	5.56±2.4 ^{a}	11.8±3.0 ^{b}	15.73±2.0 ^{bc*}	19.63±3.6 ^{c}	15.16±7.1 ^{bc}
Before hatch	8.16±3.8 ^{a}	14.66±4.5 ^{ab}	21.73±3.0 ^{b**}	30.33±5.4 ^{c*}	7.16±2.5 *

-Three stages of Persian sturgeon embryonic developmental stages include in: Gastrula, S-type heart formation and before hatching (in developmental stage 35) in each treatment.* means significant difference between values of three stages in a treatment. Different superscript letters indicate significant difference between treatments in each stage (*P*> 0.05).

These abnormalities were included some disruption in cell segmentation, retreated developing embryos and the yolk abnormal shortening in 6 and 8ppt in gastrula stage (Fig. 1), which resulted in the observance of sever deformity in embryos in higher developmental stages (deficiency in notochord, head formation etc.) (Fig. 2, 3). These kinds of abnormal embryos didn't hatch at the end of incubation period mostly in 8 and some in 6ppt (Fig. 3). All the embryos in 10 and 12ppt treatments were suppressed in blastula and beginning of gastrula stage and become dehydrated and died after this stage.

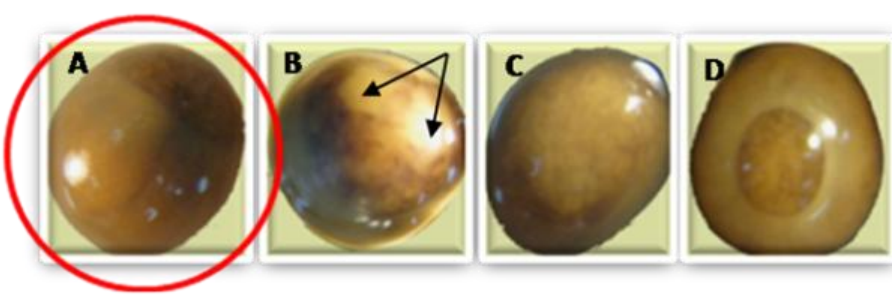

Fig. 1. Different abnormalities samples observed during incubation period of gastrula stage in salinities treatment. A: normal embryo in gastrula stage. B: abnormalities in segmentation (arrows pointed the areas which the cells dose not made normal divisions or the cells has been damaged, this kind of abnormalities was mostly observed in salinities more than 4ppt salinity).C: the abnormal embryos with shortened yolk sac compare to normal stage(it

was not numerous in salinities less than 6ppt). D: the developing retarded embryos compare to normal gastrula stage (this kind of abnormality was numerous in 8ppt treatment).

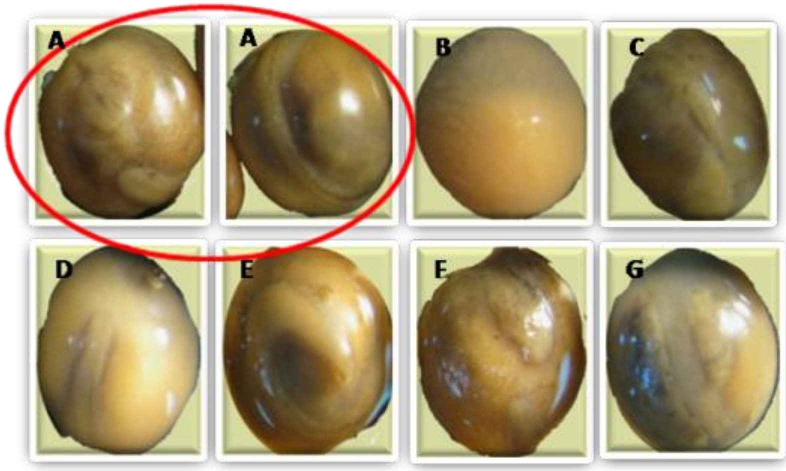

Fig. 2. Different abnormalities samples observed during incubation period of s-type heart formation stage (35 embryonic developmental stages according to Dettlaff and Ginsburg, 1992). A: the normal embryo in s-type heart formation stage. B: retarded embryo. C: deficiency in notochord formation. D: deformity in head and tail formation, E: lack of head and fore part of body and deformed tail. E: deformity in head (lengthened than normal). G: deformed embryo with deficiency in back bone.

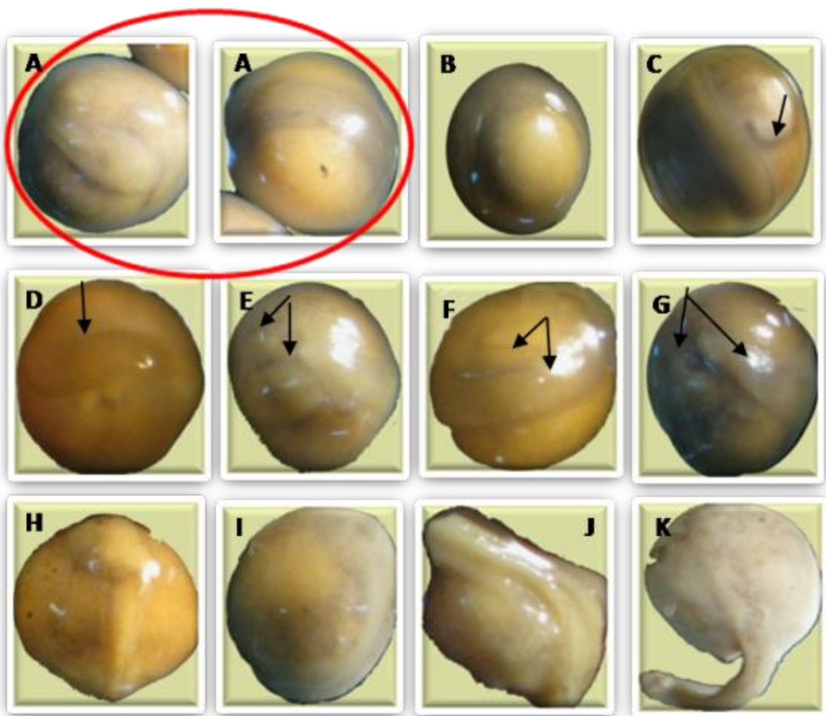

Fig. 3. Different abnormalities samples observed during incubation period of before hatching stage. A: normal embryo in before hatch stage, B: Yolk sac Vol. decreasing more than normal mood in this stage, C: the arrow pointed to presence of abnormal assemblage of cells on the yolk sac (the larvae which hatched with this kind of abnormality does not have any problem in their activity but the assemblage become thinner than compare to control in salinities more than 4ppt), D: the abnormal embryo without head and the for head parts. E: the

abnormal embryos which have two heads. F: the abnormal embryo with 2 spinal cords. G: the embryo with sever deformities on both head and tail. H: deformed retarded embryos which both head and tail parts are not form clearly. I: the retarded embryos which is not deformed yet in this stage. J and K: this samples are included the sever deformity in retarded embryos which was mostly seen in 8ppt salinity that no hatch was obtained.

3.1. Effects of Salinity on Mortality rate during incubation period

According to table 2 the effects of salinity on mortality rate in 2, 4, 6 and 8ppt treatments trough gastrula and s-type heart formation stages were as same as abnormality result in these stages. But in third stage salinity increase was significant just in 6 and 8ppt treatments ($P< 0.05$) (Table 2). The salinity effects on three studied developmental stages showed that the mortality was significantly increased in control and 2ppt treatments ($P< 0.05$) in before hatch stage but the mortality increasing in three other treatments (4, 6 and 8ppt) was only significant in s-type heart stage (Table 2).

Table 2
The effects of different salinity treatments on mortality rate during three developmental stages of Persian sturgeon embryo

Treatments	**0.5(control)**	**2**	**4**	**6**	**8**
Gastrula	18.06±0.9^{a}	19.08±1.4^{ab}	13.07±1.2^{a*}	25.93±3.1^{bc*}	31.03±3.4^{c*}
S-type heart formation	16.75±2.1^{a}	26.8±4.3^{b}	30.18±2.5^{b}	46±3.2^{c}	84.5±4.0^{d}
Before hatch	26.77±4.0^{a*}	30.71 ±3.8^{a*}	30.31±3.0^{a}	45.1±3.6^{b}	92.73±5.0^{c}

-Three stages of Persian sturgeon embryonic developmental stages include in: Gastrula, S-type heart formation and before hatching (in developmental stage 35) in each treatment. Asterisks mean significant difference between values of three stages in a treatment. Different superscript letters indicate significant difference between treatments in each stage ($P> 0.05$).

3.2. Egg hatchability

Hatching took place four days after fertilization (96h) and continued for three days more (7 d post-incubation) in all trials except 2ppt treatment which completed mostly in the day 6th.The hatchability of eggs incubated in 2ppt as well as control group ranged between 88.22 ±3.81% and 88.51±2.60%, respectively ($P< 0.05$). The hatching rate was recorded 84.19±3.2% in 4ppt treatment, while it was recorded 64.03%±1.8 in 6ppt treatments. Hatching did not occur in 8, 10 and 12ppt (Fig. 4). No Fungus was observed in salinities more than 2ppt.

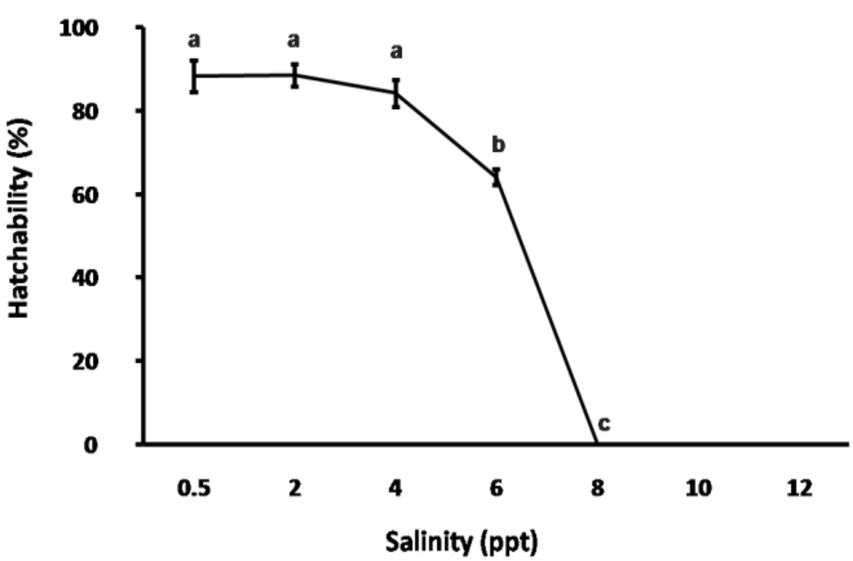

Fig. 4. The egg hatchability percent incubated in different salinities. Different superscript letters indicate significant difference between treatments in each stage ($P> 0.05$).

4. Discussion

Comparing the results of salinity effects on each stages of all (0.5, 2, 4, 6 and 8ppt) studied treatments showed that the embryos of *A. persicus* were more sensitive to salinity in gastrula stage in higher salinities like 6 and 8ppt, but they are more tolerable in gastrula stage as the abnormality and mortality rate were higher in heart formation and before hatch stage in lower salinity (2 and 4ppt). Zotin (1965) reported that the water absorption will cease in gastrula stage and it will start to absorb rapidly in heart formation stage again in Beluga (*Huso huso*). So the increasing of abnormality and mortality rate in all brackish water treatments comparing to control in heart formation stage confirm the presence of the same mechanism in Persian sturgeon. However, Holliday and Jones (1967) found that the egg salinity resistance was lowest in blastula and gastrula in freshwater teleost eggs and Tylore (1971) found the most sensitivity just enclosing blastopore prior to hatch. Sawant, *et al*. (2001) also reported increasing salinity potential along with developmental stage in zebra fish in low salinity as 2ppt (from embryonic cleavage up to gastrula stage) and cytological examinations indicated that higher salinity mainly impaired the nuclear division of the embryonic cells. Our result showed the more salinity tolerance in low salinity range in gastrula stage in Persian sturgeon but this potential decreased along with increase in salinity more than 6ppt. On the other hand, Dettlaff and Ginsburg (1992) declared that the mortality in these two stages will increase normally 2 times more than the gastrula stage because of the incubation condition. So increasing in mortality in the second developmental stage in 4, 6 and 8ppt treatments would be resulted from inappropriate incubation condition in these treatments. Rogers (1976) declared that some deficiency in embryo formation would occur in different salinity extent, and developmental process would become inefficient in teleost eggs. In Persian sturgeon it was shown that its embryos demonstrates a little adaptability during incubation period in higher salinity than freshwater. It seems that there wouldn't be another ion regulatory mechanism (chloride cell) like some of tolerable teleost in early developmental stages (Kaneko *et al.*, 2002). Decrease of hatching rate in salinities more than 6ppt and significant increase in mortality percent of *Acipenser persicus* embryo only in this treatment shows that the eggs could not tolerate salinities more than 6ppt during incubation stages. The same salinity potential was reported in some freshwater teleost eggs. For example Gbulubo and Erondu (1998) found the optimal salinity ranges for incubation 0.5 ppt and they found that hatching was significantly low above 5‰, no hatch was observed in 8ppt salinity in African catfish (*Heterobranchus longifilis*). Also the same salinity potential range was recorded in (up to 6ppt) spined loach and no hatch was observed in 8ppt (Bohlen, 1999).

Others reported some less salinity potential in fresh water embryos during incubation period. For example more than 2ppt in zebrafish (Sawant *et al.* 2001), 4.8 ppt in White fish (*Coregonus lavaretus*) (Albert *et al.,* 2004) and 3ppt in Sea trout (*Salmo trutta* L**.**) (Bonisławska, 2009) were recorded critical to hatch. Albert *et al*. (2004) found that the abnormality of Peipsi whitefish embryo was near 100% in 4 and 6ppt, but in 3ppt it was near 20%. Our result showed hatching occurs without delay in salinity treatments up to 6ppt compare to control but the duration of hatch decreased in 2ppt salinity (one day less than others). The reason would be decreasing of ion gradient of environment and the eggs which would reduce ion regulation cost in 2ppt salinity. Some authors have noticed the accelerated development of freshwater fish of moderate salinity (Lam and Sharma, 1985). The longest larvae of freshwater Ruffe (*Gymnocephalus cernuus*) were hatched in 2ppt salinity (Vetemaa and Saat, 1996). Also the common carp larvae weight hatched in 1.5-3ppt was more than freshwater embryos (Lam and Sharma, 1985). Also Albert *et al*., (2004) Declared that the salinity between 2 to 6.2ppt does not affect the development duration but the Peipsi whitefish larvae hatched earlier with increase in salinity. But in our result it was shown that the salinity affected the embryonic development in *A. persicus* and it was shown that the embryo were stopped development in blastula and beginning of gastrula stage and the deformed retarded embryos could not hatch even 8days after fertilization.

Consequently, it seems that increasing in salinity directly affects increasing mortality and abnormality in every three stages in 4, 6 and 8ppt treatments. However, the intensity of mortality in higher developmental level would be due to higher ion regulation expenses in higher salinity and increase of water salinity absorption. In addition, the suppression of eggs in early Blastula in 10-12ppt according to Zotin (1965) would be result from the water rapid uptake in first stage, and the low difference between mortality percent in s-type heart and prior to hatch stages was in order to water absorption ceasing in before hatch stage.

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