EFFECTS OF WATER SALINITY ON FERTILIZATION AND SIZE OF EGGS OF THE ASP (*ASPIUS ASPIUS* L.)

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Abstract

Effects of water salinity (1.0, 2.0, 3.0, 4.0 and 5.0 PSU) on the first stages of embryogenesis in the asp (*Aspius aspius* L.) were studied by microscope equipped with a digital imaging system and computer software. The results presented show that the number of eggs fertilized decreased when incubated in water with increasing salinity up to 5.0 PSU and that the size of eggs and of the perivitelline space in eggs incubated in saline water (1.0 – 5.0 PSU) were greater than in tap water (0.25 PSU).

Keywords

Asp, Aspius aspius , eggs, fertilization, salinity

INTRODUCTION

Embryonic development enables continuity of a species and so is an essential stage in the life cycle of fish. Variable, often unfavourable environmental conditions affecting the process of reproduction at that stage determine its success or failure. Water salinity is an important barrier to the spread of aquatic organisms, including fish. In evolution, fish had to develop different mechanisms to adapt and spread in waters with low and high concentrations of ions, i.e. freshwater and seawater, respectively, with up to 80.0 – 142.4 PSU in the latter [30]. Salinity of inland waters can result either from mixing of fresh river water with sea water, which creates estuarine

water [13], or from mixing with some anthropogenic pollutant, e.g. in water from mines, which is usually highly saline (even up to 42.0 PSU) [9] or with industrial wastes [24].

Advanced studies on the effects of water salinity on embryogenesis of various taxa of the fresh- and seawater fish were being done early in the 20th century in Russia. Olifan [28, 29] studied effects of water salinity on the spawn of common carp *Cyprinus carpio carpio* (Linnaeus, 1758), common bream *Abramis brama* (Linnaeus, 1758), Caspian roach *Rutilus rutilus caspius* (Jakovlev, 1970) and zander (pikeperch) *Stizostedion luciopoerca* (Linnaeus, 1758). Vernidub [41] determined effects of water salinity on particular stages of embryogenesis of European perch *Perca fluviatilis* (Linnaeus, 1758), common bream, zander and ruffe *Gymnocephalus cernuus* (Linnaeus, 1758). Pietrova [31] incubated eggs of European smelt *Osmerus eperlanus* (Linnaeus, 1758), European whitefish or lavaret *Coregonus lavaretus bäe*ri (Kessler, 1864) and Valaam whitefish *Coregonus lavaretus ladoga* (Poljakov, 1874) in solutions based on Ringer's solution, with salinity ranging between 5.5 and 33.0 PSU. In 1964 and 1966 Rykova made a series of studies on grass carp *Ctenopharyngodon idella* (Valenciennes, 1844) and silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1844). She found that when eggs of either species were incubated in saline water their perivitelline space decreased with increasing salinity of the water. Changes in size of the perivitelline space in eggs of common carp and silver carp incubated in saline water were also observed by Soin [38]. In eggs of common carp, the perivitelline space increased with increased salinity of water from 5.0 to 9.0 PSU, and decreased at higher PSU. In eggs of silver carp, the perivitelline space decreased with increased salinity of water.

In Poland, effects of various water salinity levels on embryogenesis of freshwater fish were studied by Dziekońska [11, 12]. Her studies concentrated on bream sperm and its fertilized spawn. Holliday and Jones [17, 18] concluded from advanced studies that the reproductive cells (eggs and sperm) of both fresh- and seawater fish, which are in general stenohaline, i.e. able to live only within a narrow range of water salinity, have various levels of tolerance of water salinity. Many scientists continued

studies on effects of water salinity on embryonic development in different taxa of fish. These studies concerned eggs of ruffe at 1.0 – 11.0 PSU [42], eggs of the spined loach *Cobitis taenia* (Linnaeus, 1758) [7], and spawn of vundu *Heterobranchus longifilis* (Valenciennes,1840) [14]. Effects of water salinity on embryogenesis of the Salmonidae family (sea trout, salmon) were studied by Xingfu et al. [43], Landergren and Vallin [23], and Bonisławska [8].

The asp is a freshwater fish that prefers clean and well oxidized water, flowing or standing (rivers, oxbows, lakes, dam reservoirs); it also lives in estuaries. Asp spawn is adhesive (a feature characteristic of eggs of fish in the Cyprinidae family) and its eggs are small, with diameter range 1.3 – 2.1 mm [2, 3, 5, 27]

Determining any effects of relatively low salinity of water (1.0 – 5.0 PSU, which is still more saline than fresh water, with 0.25 – 0.35 PSU) on the first stage of embryogenesis (fertilization, formation of the perivitelline space) may contribute to recognition of conditions for possible spawning of the asp in mesohaline waters (with salt concentration between 0.5 and 16.0 $g \cdot dm^{-3}$ [25].

MATERIALS AND METHODS

The study was carried out in March 2011. Gametes (eggs and sperm) of the asp were collected from mature specimens cultured indoors in concrete tanks. The eggs were obtained from females that were hormonally stimulated (by Ovopel). Ovopel was applied twice, with a 12-hour interval, with a simultaneous temperature increase of $2^{o}C$ within the 12 hours. The eggs were fertilized using the "dry" method in an isothermal laboratory at Department of Hydrobiology, Ichthyology and Biotechnology of Reproduction, West Pomeranian University of Technology in Szczecin. Tap water (0.25 PSU), settled by standing, and water with various concentrations of salt (1.0, 2.0, 3.0, 4.0 and 5.0 PSU) were used to activate the process of fertilization of the spawn. Fertilized eggs were incubated at 11 ± 0.2°C, in large 0.5 dm^3 glass crystallizing dishes filled with constantly aerated tap water (control) or saline water (treatment); the latter had the same salinity as that applied during fertilization. Water in the crystallizing dishes was exchanged systematically during the embryogenesis. Surviv-

al of gametes and the first stages of embryogenesis in the eggs were observed and recorded using a microscope (Nikon ECLIPSE TE-2000S) equipped with an imaging system (Nikon Coolpix5400 camera, digital cameras CCD Sony), computer, and cooling system providing a stable and optimal temperature during the observations. The images recorded were analysed with a computer and NIS Elements BR software which handles multi-dimensional imaging and can be used for measuring of eggs.

Measurements from 20 eggs were made for each treatment once absorption of water had finished. Two diameters of each egg and of its yolk were measured and averages were calculated [4]. The egg and yolk volumes were calculated according to the formula:

$$V = \frac{4}{3} \bullet \pi r^3 \quad \text{mm}^3$$

The size of the perivitelline space was expressed as a percentage from the perivitelline space part volume to egg volume ratio. The percentage of fertilized eggs was based on examination of 100 eggs at the blastopore closure stage. The results were processed using Statistica 9.0 PL software (StatSoft Poland). The statistical procedures applied to compare the size of eggs, egg cells, and yolk spheres between treatments and the control included one-way analysis of variance (ANOVA, $P < 0.01$) and Duncan's multiple range test ($P < 0.05$).

RESULTS

Successful fertilization of asp eggs incubated in water with salinity range between 1.0 – 5.0 PSU (including that with the highest salinity) was observed.

The highest percentage of fertilized eggs at the blastopore closure stage was recorded in the control, i.e. tap water with salinity 0.25 PSU (83.0%), and in water with salinity of 1.0 PSU (84.0%). A similar percentage of fertilized eggs was observed in water with salinity of 2.0 PSU (80.0%).

The percentage of the fertilized eggs decreased with increased salinity of water. Only 22.0% of eggs were fertilized at salinity of 5.0 PSU (Fig. 1).

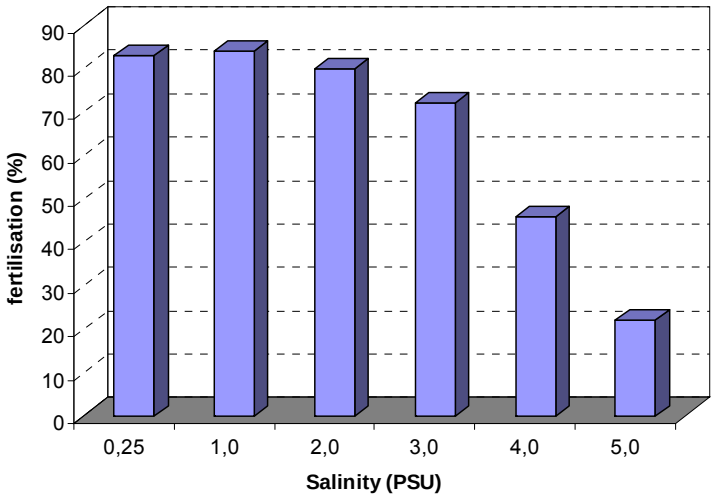

Fig.1. **The percentage of asp eggs fertilized when incubated in water with various salinity levels**

The size of the fertilized asp eggs was smallest (1.94 ± 0.04 mm) in the control (tap water). Their size (diameter and volume) differed significantly from that in treatments (Tab.1, Fig.2 a-f).

Table 1. **The size of fertilized asp eggs incubated in tap water and in water with increasing salinity (1.0 – 5.0 PSU) and ($\bar{x}$ ±SD)**

Dimensions	Tap water 0.25 PSU	Water salinity (PSU)				
		1.0	2.0	3.0	4.0	5.0
Egg diameter (mm)	1.94a ±0.04 min 1.86; max 1.99	2.07b ±0.04 min 2.00; max 2.13	2.05b ±0.02 min 2.02; max 2.07	2.06b ±0.04 min 1.98; max 2.11	2.07b ±0.05 min 1.99; max 2.13	2.06b ±0.03 min 2.00; max 2.12
Egg volume (mm^3)	3.80a ±0.22 min 3.39; max 4.12	4.68b ±0.26 min 4.20; max 5.03	4.54b ±0.10 min 4.35; max 4.66	4.59b ±0.30 min 3.89; max 4.94	4.72b ±0.29 min 4.16; max 5.08	4.53b ±0.17 min 4.21; max 4.81
No. of eggs incubated (n)	256	265	248	256	288	265

Means denoted with identical superscripts are not significantly different (Duncan's T test. $P<0.05$).

There were no significant differences in size and diameter of eggs incubated at salinity from 1.0 PSU to 5.0 PSU. The diameter of eggs incubated at increased salinity was about 0.12 mm more than the control (Tab. 1).

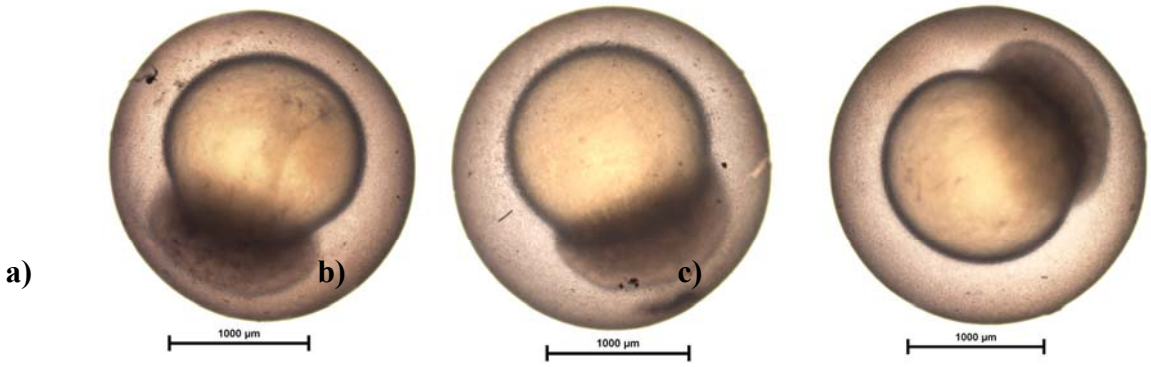

Fig. 2. **The size of asp eggs incubated in tap water (a) and at salinity of 2.0 PSU (b) and 4.0 PSU (c) at 1/3) epiboly**

The maximum volume of eggs incubated at increased salinity was 5.08 mm^3 and was greater than the control, in which maximum volume was 4.12 mm^3 (Tab.1). There were no significant differences in size of yolk spheres in eggs incubated in tap water and saline water (Tab. 2).

Table 2. **The size of the yolk sphere in asp eggs incubated in tap water and in water at increasing salinity (1.0 – 5.0 PSU) ($\bar{x}$ ±SD)**

Dimensions	Tap water 0.25 PSU	Water salinity (PSU)				
		1.0	2.0	3,0	4,0	5,0
Yolk sphere diameter (mm)	**1.43**[a] ±0.04 min 1.33; max 1.51	**1.43**[a] ±0.02 min 1.38; max 1.47	**1.44**[a] ±0.02 min 1.40; max 1.46	**1.45**[a] ±0.02 min 1.42; max 1,48	**1.45**[a] ±0.02 min 1.41; max 1.47	**1.43**[a] ±0.02 min 1.39; max 1.47
Yolk sphere volume (mm^3)	**1.54**[a] ±0.13 min 1.34; max 1.79	**1.53**[a] ±0.09 min 1.35; max 1.65	**1.53**[a] ±0.07 min 1.34; max 1.62	**1.59**[a] ±0.04 min 1.50; max 1.64	**1.58**[a] ±0.07 min 1.48; max 1.66	**1.53**[a] ±0.08 min 1.48; max 1.66

Means denoted with identical superscripts are not significantly different (Duncan's T test. $P<0.05$).

The size of the perivitelline space, which contains the developing embryo, increased distinctly in eggs with increasing salinity from 0.25 to 1.0 PSU (Fig. 3).

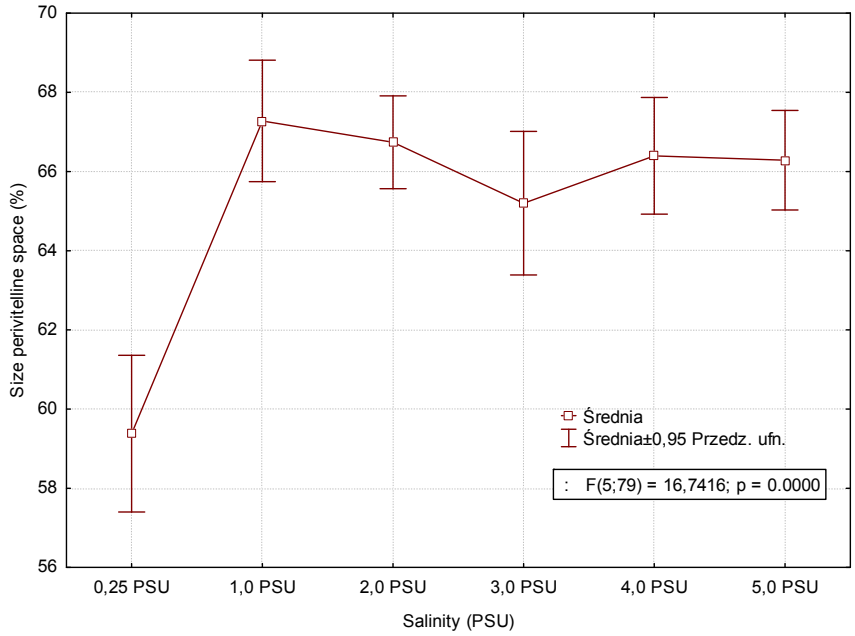

Fig.3. **The size of the perivitelline space in asp eggs incubated in tap water (0.25 PSU) and in water at increasing salinity (1.0 – 5.0 PSU) (ANOVA, *P*<0,01)**

The perivitelline space occupied 59.38 ± 3.57 % of the egg volume in eggs incubated in tap water, and from 65.20 ± 2.66% in eggs incubated at salinity of 3.0 PSU to 66.76 ± 2.03 % in eggs incubated at salinity of 1.0 PSU.

DISCUSSION

This study shows that increased water salinity, which was thought to create unfavourable conditions for asp development, did not prevent the process of egg fertilization. Negative effects of increased salinity of the habitat on the further stages of embryonic development of asp can not be excluded, however. The fertilization of asp eggs at increased salinity was possible because of specific properties of fish gametes, particularly the sperm. Studies have shown that sperm is less sensitive than eggs to higher concentration of salts. A moderate increase in water salinity does not affect activity of the sperm while the eggs become unable to be fertilized [11, 12, 19]. Sperm of the Caspian bream was still viable at salinity of 11.0 PSU while its eggs were unable to be fertilized at 8.0 PSU [19]. Sea trout sperm was still mobile, although only as vibration, at 14.0 PSU and all sperm mobility ceased at 16.0 PSU; only 1.0% of sea trout eggs were fertilized at 5.0 PSU and no fertilization was observed at 6.7 or 10.0 PSU [23].

The present results show that increasing the salinity of water used for incubation of asp spawn disrupted proper fertilization. This agrees with results of studies on other fish taxa [1, 7, 8, 14].

According to Olifan [28, 29], maximum salinity for successful embryogenesis in bream, roach and zander (pike-perch) is between 8.0 and 10.0 PSU. In a review of advanced studies by many authors, Chlebovič [10] gives the value of 8.0 PSU as the maximum for successful fertilization in freshwater bony fish. This disagrees with results of Dziekońska [12], who reported that fertilization of eggs of bream (from the Vistula Lagoon) is prevented in water with salinity of 3.7 and 5.61 PSU.

The reports referred to above suggest that maximum salinity for successful fertilization in freshwater fish depends on their population in an individual body of water and their resistance to water salinity. In the case of bream from the Caspian Sea, fertilization can occur at salinity of 7.0 – 10.0 PSU [19, 29], of bream from the Aral Sea at salinity of 8.6 – 10.0 PSU [6, 16], from the Azov Sea at salinity of 10.0 PSU [28] and from the Vistula Lagoon at the much lower salinity of 1.4 – 2.8 PSU [12].

The size of the asp eggs, and hence of the perivitelline space, was greater in water with salinity of 1.0 – 5.0 PSU than in tap water with lower salinity, although the size of yolk spheres was not affected. In contrast, the size of the eggs of carp studied by Soin [38] increased after incubation in moderate salinity and decreased at high salinity. The diameter of eggs from fresh water was 1.62 mm, and from saline water was 1.9 at 9.0 PSU and 1.65 at 30.0 PSU. Rykova [32, 33, 34, 35] and Soin [38] studied the pelagic spawn in the family Cyprinidae, e.g. silver carp, bighead carp and grass carp, finding a different reaction in which size of the perivitelline space decreased in water with increasing salinity.

Differences observed among various species of the family Cyprinidae in the reaction of their eggs to saline water at the stage of ‘hydration’ can be explained by differences in the ecology of their reproduction. The asp, as a lithophile and reophile species, spawns either in sandy or gravel bottoms with fast flowing water or in shallows or reservoirs filled with macrophytes and roots, while the phytophiles, silver carp, bighead carp and grass carp, produce spawn consisting of large eggs, which

measure 3.5-5.5 mm in diameter when swollen and can easily float at the bottom and central layers of reservoirs [20, 21, 22, 26]

Zotin [44, 45] concluded that salinity inhibits the process of secretion of hydrophilic colloids by the cortical alveoli (the cortical vesicles). In the family *Salmonidae*, e.g. landlocked salmon *Salmo salar morpha* sebago (Girard 1853) and brown trout *Salmo trutta morpha lacustris* L., and the family Acipenseridae (sturgeon), these colloids are known, after egg activation to be responsible for absorption of water and "filling" of the perivitelline space, affecting its size. Dziekońska [12] reported that when fertilization of bream eggs occurred at salinity of 3.7 and 5.6 PSU, both eggs and perivitelline space were smaller because of less absorption of water.

The formation of a larger or smaller perivitelline space can result from different (depending on the fish taxa) effects of salts (cations and anions occurring in absorbed water) on penetrability of the egg membranes. The effect can be similar to that observed after application of a stable magnetic field (1.5 and 2.0 mT), which increased the penetrability of egg membranes [15, 36, 37]. An effect of heavy metals on the size of eggs and the perivitelline space can not be excluded. Lead, for example, can combine with mucopolysaccharides secreted during the cortical reaction to form compounds which inhibit further secretion, preventing swelling of the eggs and "filling" of the perivitelline space with water [39, 40].

The present results suggest an effect of increased water salinity on the process of "egg hydration", which agrees in part with the results of Soin [38]. Despite the increase in living space in eggs incubated in water with salinity of 1.0 to 5.0 PSU the number of eggs with successful fertilization decreased. It can be concluded that the critical, maximum salinity of water for successful fertilization of the greatest number of eggs is 3.0 PSU.

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