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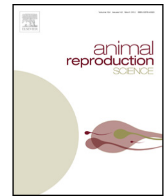

New technique for fertilizing eggs of burbot, asp and ide under hatchery conditions

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ABSTRACT

The development of a new protocol for egg fertilization may increase embryo survival and benefit the aquaculture process. In the present study, a new technique of partially adding sperm to activated eggs in the artificial fertilization of burbot (*Lota lota*), ide (*Leuciscus idus*) and asp (*Aspius aspius*) eggs was evaluated. If the same volume of sperm was divided into two or three parts and added to eggs in 30–60 s intervals, it significantly improved embryo survival at the eyed-egg-stage of development. In the present study, the periodic addition of spermatozoa to eggs affected fertilization (ide and asp) and embryo survival rates (ide, asp and burbot) and might be successfully applied under hatchery conditions.

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1. Introduction

Successful artificial reproduction and larviculture are the primary limitations in aquaculture diversification. The production of larvae depends on gamete quality and survival rates during the embryonic and larval periods. The fertilization rate is one of the most important factors and influences further production (Billard et al., 1995; Linhart et al., 2006). In freshwater finfish culture, the fertilization rate may depend on gamete qualities, the type of gamete applied, activating media, fertilization techniques and water variables such as temperature, pH, hardness, etc. (Żarski et al., 2012, 2014). In some species there could be a major impact on commercial or conservative aquaculture in many European countries, such as the burbot (*Lota lota*), northern pike (*Esox lucius*), asp (*Aspius aspius*), ide (*Leuciscus idus*), or dace (*Leuciscus leuciscus*) (Krejszeff et al., 2009; Targonska et al., 2010; Targońska et al., 2012; Nowosad et al., 2014), the percentage of fertilization (especially in commercial hatcheries) is usually poor. It stimulates the testing of new techniques to fertilize the eggs of these species. Targońska et al. (2008) showed that, for burbot and ide, the duration of spermatozoa motility was more closely correlated with fertilization rate than the percentage of motile spermatozoa. This suggests that the enhancement of the period spermatozoa are motile during gamete activation might influence embryo fertilization rate.

In many freshwater finfish species, including asp, dace, ide or burbot, the motility of spermatozoa in tap or hatchery water is short and usually does not exceed 30–40 s (Targońska et al., 2008; Alavi and Cosson, 2005; Khara et al., 2013). Research conducted on Eurasian perch (*Perca fluviatilis*) (Żarski et al., 2012) and some other fish species (Targonska et al., unpublished) showed that the insemination (and consequent fertilization) capacity of eggs is much longer than the time of spermatozoa motility during *in vitro* fertilization. For example, in the case of Eurasian perch, it is possible to fertilize eggs from 0 to 180 s after egg acti-

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vation (Żarski et al., 2012). In a similar time period, it is possible to fertilize eggs obtained from asp, ide, northern pike or dace which was consistent with the time of spermatozoa motility (about 30s). If the spermatozoa sample was contaminated (e.g. by urine or had contact with fluids like water), the period during which sperm motility is sustained was much shorter. Thus, during the mass egg fertilization process, large differences are observed between the period for which eggs remain fertile and the period of during which spermatozoa fertilization capacity is sustained.

In aquaculture, in a large number of cases, gametes were obtained using the “dry” method and were then mixed and a water or activation solution was added (Nowosad et al., 2014; Żarski et al., 2014). Thus, the gametes obtained from both sexes are activated at the same time. If spermatozoa motility (i.e., fertilization capacity) is much shorter than “egg activity” a poor fertilization rate might be the outcome. In the case of Eurasian perch, the percentage of egg fertilization was greater, if the spermatozoa was added to eggs with a minimum of 15 s delay in the eggs’ contact with water (Żarski et al., 2012). This suggests that artificial fish fertilization techniques might be improved. One possibility is to delay the process of spermatozoa contact with eggs after activation of the eggs. Another technique that could be employed is to lengthen the duration of gametes contact by partially adding the spermatozoa to the activated eggs.

The aim of this study was to assess the technique of egg fertilization of ide, asp and burbot using the periodic addition of spermatozoa to the eggs with a focus on the increase in fertilization and survival rates.

2. Materials and methods

Fish (wild spawners of asp, ide and burbot) were obtained from the natural waters of northern Poland. Spawning was induced artificially using the method described by Żarski et al. (2010) for burbot, by Targonska et al. (2010) for asp and by Targońska et al. (2012) for ide. Fish were stimulated hormonally with an intraperitoneal injection of Ovopel (D-Ala6, Pro9Net)-mGnRH + metoclopramide; (Unic-trade, Budapest, Hungary) at the base of the pelvic fin. The first dose was 0.2 pellet/kg of body weight (1 pellet of Ovopel contains 18–20 μg mammalian analogue of GnRH and 8–10 mg of metoclopramide; Horvath et al., 1997); after 24 h, another injection was performed with administration of 1 pellet/kg of body weight. The pellets were pulverized before application and then dissolved in saline solutions (0.9% sterile NaCl; Nowosad et al., 2014). The spermatozoa were collected from the male with sterile syringes, while care was taken to prevent its contamination with urine, feces or blood. The spermatozoa motility and period over which motility was sustained were determined using CASA (Cejko et al., 2010) and for further treatment, only the samples with a minimum of 70% motility and a 30–35 s range in the period spermatozoa motility was sustained were used. Eggs were collected individually in small plastic boxes. Before each manipulation, the ide and asp individuals that were spawning anaesthetized in a 2-fenoxyethanol solution (0.5 mL/L; Merck, Darmstadt, Germany) and for the burbot species were anesthetized using a MS-222 solution (150 ppm; Argent, USA). In each experiment, the eggs were obtained from a minimum of four females (and pooled) and the milt was stripped using syringes from a minimum of three males and pooled before using.

2.1. Experimental design

For each experiment and each species, about 3000 eggs were taken for each replicate. Several groups were established as:

Group C with about 3000 eggs being mixed with 150 μL of spermatozoa and 10 mL of tap (hatchery) water was then added at “zero” time;

Group G–30 with about 3000 eggs being mixed with 75 μL of sperm, 10 mL of tap (hatchery) water was subsequently added, and after 30 s the second portion of 75 μL of spermatozoa was added;

Groups G–60 with about 3000 eggs being mixed with 75 μL of sperm, 10 mL of tap (hatchery) water was subsequently added, and after 60 s the second portion of 75 μL of spermatozoa was added;

Groups G–30/60 with about 3000 eggs being mixed with 50 μL of sperm, 10 mL of tap (hatchery) water was subsequently added, and after 30 and 60 s, the second and third portions of 50 μL of spermatozoa were added.

Tap water had the following characteristics: conductivity (25 °C): 516; pH: 7.43; total water hardness: 255 mg/L $CaCO_3$; Na^+: 10.8 ppm; K^+: 4.9 ppm; Fe: 12 μg/L; Mn: 26 μg/L.

After 5 min of gamete activation, the eggs of asp and ide species was rinsed in Woynarovich solution (3 g NaCl and 4 g of urea in 1 L of hatchery water) for 60 min. Subsequently, there were two emersions (together 30 s) in tannin solution (0.6 g per 1 L of hatchery water) occurred. For the burbot species, the eggs were mixed with hatchery water for 30 min. The eggs were incubated in small (0.7 L) glass Weiss jars at a water temperature of 12.5 °C for the ide and asp species (Kupren et al., 2011) and in 2 °C for burbot eggs.

The survival rate was recorded at 24 and 72 h after egg fertilization and at the eyed-egg-stage for the ide and asp species, and at 24 and 144 h after egg fertilization and at the eyed-egg-stage for the burbot species. The experiment was conducted in triplicate.

2.2. Mass spawning application method

The second experiment was conducted using the ide and asp species. For each experiment and each species, about 100,000 eggs were obtained for each replicate. Several groups were established as:

Group C with about 100,000 eggs being mixed with 4.5 mL of spermatozoa and 500 mL of tap (hatchery) water being added at “zero” time;

Group T–30 with about 100,000 eggs being mixed with 2.25 mL of spermatozoa and 500 mL of tap (hatchery) water being added and after 30 s the second portion of 2.25 mL of spermatozoa was added;

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