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Short communication

A novel method for rapid elimination of sturgeon egg stickiness using sodium hypochlorite

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ABSTRACT

Reducing egg stickiness is a crucial step in artificial culture of sturgeon eggs, with commonly used methods being time consuming or interfering with hatching. Sodium hypochlorite (SH) at varying concentrations and exposure times was tested on sterlet *Acipenser ruthenus* eggs, with 0.03% SH for 40 s effectively eliminating stickiness without impairment to eggs, embryo development, or hatching when compared with other de-adhesion methods (clay; NaCl, urea, and tannic acid). Field tests using sterlet, Siberian sturgeon *Acipenser baerii*, and Russian sturgeon *Acipenser gueldenstaedtii* showed similar numbers of larvae hatched using SH and clay. Immunohistochemistry revealed a protein carbonyl group only on the surface of eggs treated with SH, evidence of oxidation as the mode of action and indicating that oxidation did not affect inner egg layers or cytoplasm. Treatment with SH is a rapid, simple, and inexpensive method for de-adhesion of sturgeon eggs.

Statement of relevance: Sturgeons represent a valuable group of fish in aquaculture, especially for caviar, but also for meat production. This study describes a novel approach to sturgeon egg de-adhesion involving oxidation of egg surface. The method requires little time, is low cost, and disinfects and softens the egg surface, which facilitates hatching.

1. Introduction

Sturgeons are among the most ancient extant animal groups and are valued in aquaculture for caviar and for meat production. Many sturgeon species are critically endangered due to human activity including poaching, environmental pollution, habitat degradation, and construction of dams that interfere with their spawning migration (Havelka et al., 2011).

Breeding sturgeon is currently dependent on artificial propagation, which, for optimal results, requires removal of natural stickiness, without which eggs will aggregate and suffocate or may become infected by fungi and bacteria (Siddique et al., 2014). An effective egg de-adhesion method increases embryo survival and hatching (Linhart et al., 2000; Gela et al., 2003), benefiting both research facilities and fish farms. The procedure should not harden the egg envelope, as this may interfere with hatching, and, ideally, should also disinfect the eggs. Currently available methods can result in damage to sturgeon eggs and are time consuming, requiring approximately 45 min for each batch of eggs.

The goal of this study was to evaluate the use of a dilute solution of sodium hypochlorite as a novel technique to eliminate sturgeon egg stickiness.

2. Materials and methods

2.1. Testing in laboratory conditions

Gametes of three male and three female sterlet *Acipenser ruthenus* were collected according to Pšenička et al. (2010). The percent of motile spermatozoa was not lower than 80%. For each treatment, 1 g of eggs (n = ~100) from each female was fertilized by 50 μL of pooled sperm activated in 3 mL of dechlorinated (carbon filtration) tap water for 2 min in Petri dishes.

Fertilized eggs were exposed to sodium hypochlorite (SH) at concentrations of 0.00375, 0.0075, 0.015, 0.0225, 0.03, 0.0375, 0.045, and 0.0525% for 30, 40, 60, 90, and 120 s. The exposure was stopped by washing the eggs in a sieve three times with water. At 30 min post-fertilization, stickiness and damage to eggs were evaluated. Eggs adhering to the surface of the Petri dish or to other eggs were considered sticky. Eggs with deformed chorion or collapsed cytoplasm were counted as damaged.

The technique was compared with three other methods: 1) clay suspension for 45 min; 2) exposure to 0.04% solution of tannic acid for 10, 20, and 30 s with a water rinse between each exposure; and 3) the method described by Kowtal et al. (1986) based on NaCl, urea, and tannic acid. A control group of eggs was untreated. The eggs were placed in Petri dishes and incubated at 15 °C with water changed daily. Fertilization rates at the 2–8 cell stage (3 to 5 h post-fertilization) and hatching rates were recorded.

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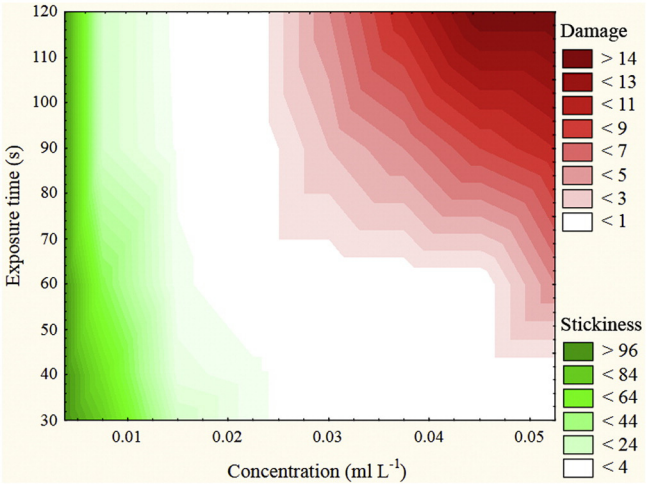

Fig. 1. Stickiness and damage of sterlet eggs (%) relative to SH concentration and exposure time.

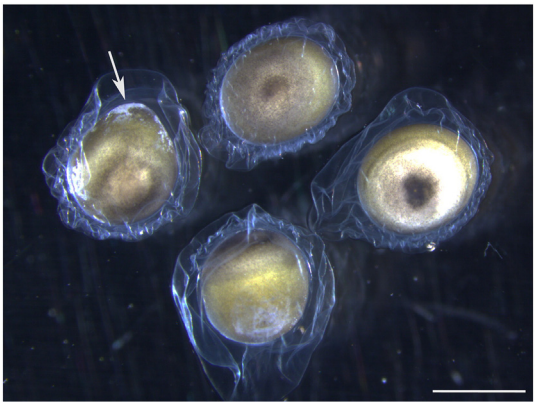

Fig. 2. Sterlet eggs with damaged chorion or collapsed cytoplasm (arrow) resulting from exposure to 0.1% SH for 60 s. Scale bar = 2 mm.

2.2. Testing in field conditions

The SH treatment was evaluated using eggs of three sterlet at the Genetic Fisheries Center in Vodňany, Czech Republic, and those of three Siberian sturgeon *Acipenser baerii* and two Russian sturgeon *Acipenser gueldenstaedtii* at the hatchery of Fischzucht Rhönforelle GmbH & Co. KG fish farm in Germany. Fifty grams of eggs were fertilized with 0.5 mL of pooled sperm from 3 males showing spermatozoon motility greater than 80%, activated in 75 mL of hatchery water. After 2 min, the fertilized eggs were poured through a sieve and then placed in a bowl with 50 mL of 0.03% SH solution for 40 s and mixed well. Subsequently, the eggs were placed in a sieve and washed three times in 100 mL of hatchery water. An equal volume of eggs was subjected to the standard clay treatment for 45 min. Eggs were incubated in hatching jars at 17 °C. Survival was assessed at the 2–8 cell stage, after hatching, and at 30 days post-fertilization (dpf), after conversion to dried food. The initiation of hatching was recorded in hours post-fertilization (hpf).

2.3. Protein carbonyl immunohistochemical staining

To test the mode of SH action on sturgeon eggs, treated and untreated eggs were processed according to a protein carbonyl immunohistochemical staining protocol based on 2,4-Dinitrophenylhydrazine derivation (Cosmo Bio Co., LTD., Product No. SML–R0IK04–EX) and visualized by the Avidin-Biotin System, Biotin Solutions (VECTOR, Inc.).

The data obtained from laboratory testing on sterlet were analyzed by one-way ANOVA and that from field testing on sterlet, Siberian, and Russian sturgeon by nested ANOVA, using Statistica for Windows, v. 9.1 (StatSoft, Inc., USA). Probability values of $P < 0.05$ were considered significant.

3. Results

Laboratory testing indicated that the optimal SH concentration and exposure time for egg de-adhesion without adverse effects was 0.03% for 40 s (Fig. 1). The eggs exposed to 0.1% SH for 60 s showed typical signs of damage such as deformed chorion or collapsed cytoplasm (Fig. 2). No significant differences in fertilization and hatching rate were observed among SH treatment, commonly used methods of sturgeon egg de-adhesion (clay, tannic acid and Kowtal's method), and non-treated controls (Table 1). The eggs in the evaluated treatments swelled differentially. Generally, eggs exposed to tannic acid, including Kowtal's method, swelled less than in other treatments, indicating hardening (Table 1, Fig. 3).

In field tests on three species at two hatcheries with different water sources, SH was at least comparable in effectiveness to the commonly used method of clay treatment, verifying its suitability for practical application. Fertilization rate, hatching rate, time of hatching, and number of surviving larvae 30 dpf for eggs treated with clay vs. SH in sterlet, Siberian, and Russian sturgeon is shown in Table 2. Although all parameters were slightly more favorable in SH-treated eggs, differences were not significant.

Immunohistochemical staining revealed aminoacyl carbonyl (product of protein oxidation) presence only on the surface of eggs treated by SH (Fig. 4), indicating oxidation to be the mode of de-adhesion. The layer containing the carbonyl group was not thicker than 2 μm. No indication of protein oxidation was found in deeper egg layers.

4. Discussion

Currently many methods of de-adhesion are in use. According to mode of action, they can be categorized as a) mechanical methods using a suspension or emulsion including clay, milk, and talc; b) use of enzymes such as alcalase, hyaluronidase, and trypsin; and c) chemical methods using urea, NaCl, and tannic acid (Woynarovich and Woynarovich, 1980; Linhart et al., 2000; Monaco and Doroshov, 1983). Modified versions of these methods have been applied in sturgeon culture, but it has not been possible to decrease the duration of

Table 1
Fertilization, hatching rate, and egg size with various methods of egg stickiness elimination in vitro in sterlet.

Method	Clay	Tannic acid	Kowtal's method	Sodium hypochlorite	Control
	Mean ± sd	Mean ± sd	Mean ± sd	Mean ± sd	Mean ± sd
Fertilization rate (%)	56.7a ± 25.0	68.3a ± 30.7	58.3a ± 29.3	69.0a ± 24.5	72.3a ± 28.1
Hatching rate (%)	48.3a ± 22.5	45.6a ± 25.2	43.3a ± 20.9	62.3a ± 23.0	52.6a ± 23.8
Egg size (mm)	32.5a ± 2.1	28.1c ± 1.8	28.7bc ± 2.0	32.2ab ± 2.2	32.3ab ± 1.9

Different letters within columns indicate significant differences, n = 3 for all applications.