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Walleye Egg Survival Was Unaffected by Four Semen Inclusion Techniques during Artificial Fertilization

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Abstract

Improving the percent hatch during artificial spawning of walleye (*Sander vitreus*) could save agencies substantial resources. Semen inclusion techniques used to artificially fertilize walleye eggs may have a subsequent influence on the percent hatch. We compared walleye egg survival to the early embryo stage across four semen inclusion techniques used during fertilization to determine if one or more of the techniques would result in a higher percent hatch. Six mL of freshly collected (within 2 h) semen was presented to common pools of walleye eggs separated into 250 mL lots by either 1) pouring semen on top of the eggs before sperm activation, 2) placing 3 mL of semen below and above the eggs before sperm activation, 3) pouring 3 mL of semen on top of the eggs, activating sperm, and adding the remaining 3 mL of semen after 30 s, or 4) activating the sperm by adding semen to 1 L of water, mixing and pouring the solution on the eggs within 3 s. All treatments received 1 L of filtered lake water for sperm activation and fertilization. Mean egg survival was similar ranging from 49.5% to 56.7% among the four techniques and was not significantly different across methods. These results suggest that any of the semen inclusion techniques will likely provide a similar percent hatch for walleye eggs when 250 mL of eggs, 6 mL of semen, and 1 L of water are used during fertilization.

Keywords

Walleye Egg Collection, Walleye Propagation, Walleye Spawning Operations

1. Introduction

Walleye (*Sander vitreus*) is the most popular sportfish among South Dakota an-

glers [1] and accounts for much of the $271 million that is generated annually by anglers fishing in South Dakota [2]. Maintaining or improving walleye populations is important from both an economic and quality of life perspective. As such, South Dakota Department of Game, Fish and Parks (SDGFP) annually propagates walleye to enhance populations throughout the state. Each spring SDGFP puts forth a large number of resources to obtain a predetermined walleye egg quota from wild broodstock populations in Lake Oahe and numerous waters in eastern South Dakota [3] [4] [5] [6]. Annual egg quotas have a 50% hatch built into the number of eggs needed to fulfill annual stocking request needs. Spawning procedures that enhance egg survival could lead to reduced egg quotas and provide improvements in the overall efficiency of the SDGFP walleye propagation program.

The propagation of walleye begins with the collection of gametes followed by artificial fertilization of the eggs. The "dry method" of fertilization is regularly used and involves keeping gametes free of water until both eggs and semen are in a pan [7]. Water is then added to initiate fertilization by activating the sperm. The semen inclusion technique prior to sperm activation has varied between two methods in SDGFP spawning operations. In one method, semen is expressed below and on top of the eggs while in the other, semen is only expressed on top of the eggs. No comparison between these two semen inclusion techniques has occurred to our knowledge. However, others have reported a 40% increase in percent hatch when semen inclusion is adjusted from expressing on top of the eggs (prior to sperm activation) to quickly stripping semen from two males into a container of water, mixing, and then pouring the activated sperm onto the eggs [8]. This technique may improve sperm dispersal throughout the eggs but may reduce the amount of time, the sperms are capable of fertilizing eggs [7]. Egg survival also was shown to increase when three additions of extended semen were added during the first minute of fertilization compared to a single addition of extended semen [9].

The lack of evaluation between semen inclusion methods currently used in SDGFP artificial walleye spawning operations combined with improved fertility and percent hatch metrics from other semen inclusion techniques were the impetus for this study. The study objective was to compare walleye survival to the early embryo stage among eggs that received differing semen inclusion techniques during fertilization. Ultimately our study goal was to potentially identify a semen inclusion method(s) that would result in an improved percent hatch for artificial fertilization of walleye in South Dakota.

2. Methods

Adult walleye brood stock were collected in overnight sets of modified-fyke nets (3/4 inch mesh) from the Lynn Lake complex (Lynn and Middle-Lynn Lakes, combined surface area = 1058 ha) in Day County, South Dakota, USA on April 21 and 22, 2021. Walleye semen was pooled from a minimum of six males before transferring to individual test tubes. This process was repeated until 32 test tubes

were each filled with 7 mL of semen. Test tubes were placed on ice and all semen was used for egg fertilization within 2 h of collection.

Walleye eggs were expressed from a minimum of four ripe (ovulating) females to create a common pool of eggs; this was repeated eight times to create eight distinct egg pools. From each common egg pool, four standard pans (269.8 mm diameter) received 250 mL of eggs (egg depth was approximately 4.4 mm). Each pan represented the experimental unit and received one of the four semen inclusion treatments (*i.e.*, treatment 1: 6 mL of semen on top of eggs before sperm activation, treatment 2: 3 mL of semen beneath and 3 mL of semen on top of the eggs before activation, treatment 3: two additions of 3 mL of semen on top of the eggs separated by 30 s with water being added following the first semen addition, and treatment 4: 6 mL of semen transferred to 1 L of water, mixed, and poured over the eggs within 3 s of activation). This process was repeated eight times resulting in each treatment being represented eight times. The four semen inclusion methods order of administration was randomized for each egg pool.

All sperm activation and fertilization occurred using 1 L of filtered (30 micron) lake water and each pan of eggs was stirred for 2 minutes using a turkey (*Meleagris* spp.) feather following the addition of water. One L of a diatomaceous earth solution (1:1 ratio by volume of diatomaceous earth and water) was then added and stirred for 2 minutes to remove egg adhesiveness. Eggs from individual pans were rinsed and placed in a 3.8 L plastic bag filled with filtered lake water and allowed to water harden for at least 1.5 h before being transported to the hatchery (Blue Dog State Fish Hatchery, Waubay, South Dakota).

At the hatchery, two random samples of eggs (≥150 eggs per sample) were removed from each bag and each sample was placed into a 2-L container filled with 1.5 L of well water (total hardness = 506 mg/L $CaCO_3$; alkalinity = 264 mg/L $CaCO_3$; pH = 7.5; total dissolved solids = 612 mg/L; dissolved oxygen 10 mg/L; temperature = 10.1˚C) where incubation occurred for 72 h to the early embryo stage. Dissolved oxygen remained above 7.2 mg/L in all incubation units while water temperature increased to 16.4˚C within 24 h and then remained constant.

The number of eggs surviving to the embryo stage was quantified by counting the number of embryos in each sample while viewed under a dissecting microscope (25× magnification). The average survival was determined for samples that were taken from the same bag to represent the survival for that experimental unit.

Data Analysis

Normality of percent viable egg data was tested with a Shapiro-Wilk test. We used a general linear model (GLM) to assess potential difference in percent egg viability across the four treatments. Common pool was included in the GLM as a blocking factor to account for potential differences in the eight common pools of eggs. All statistical tests were completed with Systat 13 (Systat Software Inc., Richmond, California) with a significance level of $\alpha = 0.05$.

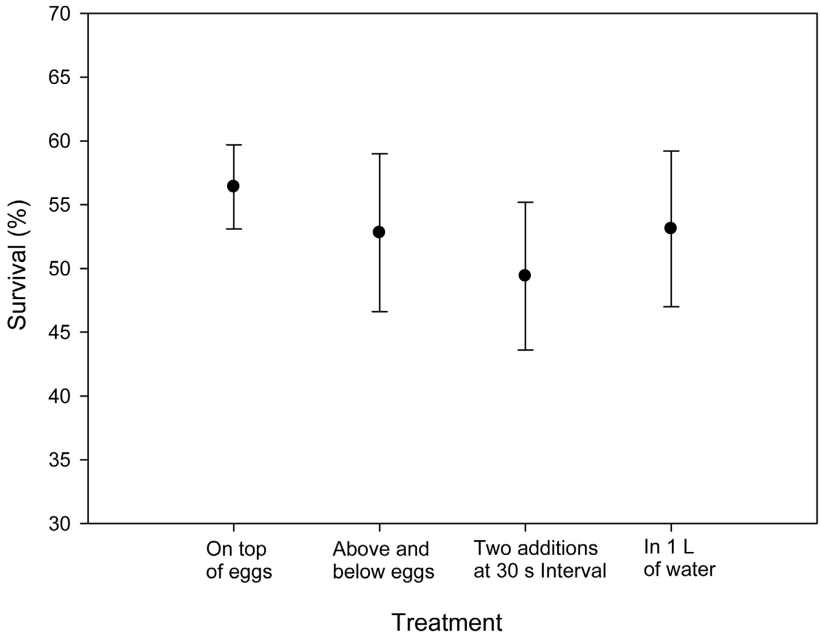

Figure 1. Mean (±SE) survival of walleye eggs to the early embryo stage (72 h) when fertilized by four differing semen inclusion methods: 1) adding 6 mL semen on top of the eggs before sperm activation, 2) placing 3 mL of semen below and above the eggs before sperm activation, 3) pouring 3 mL of semen on top of the eggs, activating sperm, and adding additional 3 mL of semen after 30 s, 4) activating the semen in 1 L of water, mixing and pouring the solution on the eggs within 3 s at Lynn Lake complex, South Dakota, in April 2021.

3. Results

The egg percent viability data followed a normal distribution (Shapiro Wilk test value = 0.946; $P = 0.112$). The blocking factor common pool was nonsignificant ($F_{7,22} = 0.195$, $P = 0.983$) in the GLM indicating that the eight common egg pools were similar. The percent egg survival to 72 h ranged from 49.4% to 56.4% with the best viability occurring when 6 mL of semen was added to the top of eggs before water activation (**Figure 1**). However, when percent egg survival was compared across the semen inclusion techniques there was no significant ($F_{3,22} = 0.016$, $P = 0.997$) difference in the four treatments.

4. Discussion

Semen inclusion techniques used in this study resulted in similar walleye egg survival across the four methods. The lack of a superior semen inclusion technique is different from what two previous studies have found. Two of the treatments used in this study (two additions at 30 s interval and adding to 1 L of water before adding to eggs) were chosen because of previous documentation of improved walleye egg survival [8]. A 40% increase of walleye egg percent hatch was observed after semen inclusion was adjusted from expressing on top of the eggs (prior to activating sperm) to quickly stripping semen from two males into a container of water and then presenting the diluted, activated sperm to the eggs

[8]. Although no significant difference in percent survival was observed in the present study, the percent egg survival when semen was mixed with 1 L of water and then added to the eggs was 3.3% lower than when semen was added to the top of eggs before activation. A 4% improvement in fertility rate occurred when three additions of 3 mL of extended semen were presented to walleye eggs at 30 s intervals compared to a single, 9 mL addition [9]. Conversely, our treatment of two, 3 mL semen additions at 30 s intervals provided the lowest percent survival of the four techniques in our study and the mean was 7.0% lower than when the semen was added to the top before activation.

The low volume of eggs used during fertilization may have contributed to the lack of differences observed in the four semen inclusion techniques used in this study. The 250 mL of eggs used in each pan equates to an approximate egg depth of 4.4 mm (based on pan dimensions) during fertilization. A reduction in walleye egg depth from 10 to 4 mm with the same volume of eggs resulted in improved egg fertility [9]. Similarly, lowering egg volume from 1055 mL (19 mm egg depth) to 390 mL (7 mm egg depth) during fertilization corresponded to improved walleye egg survival [10]. These studies suggest that sperm dispersal among the eggs is an important factor affecting egg fertilization. Our egg volume was substantially lower than previous studies making it more likely for sperm to encounter an egg.

In addition to egg depth and volume, sperm dispersal among the eggs could be influenced by the amount of semen and water that is used during fertilization. Previous fertilization studies have used 3 mL of walleye semen extended to a volume of 9.0 mL and then added anywhere from 0.4 to 1.0 L of water for fertilization [9] [10]. The current study used a total volume of 6 mL of walleye semen and 1 L of water during fertilization. An increased number of sperm would be expected in 6 mL of pure semen versus 9 mL of extended semen.

The four semen inclusion treatments evaluated in this study provided mean egg survivals that were similar to long-term percent hatch data (53%) for walleye eggs at Blue Dog State Fish Hatchery [3] [4] [5] [6]. The semen inclusion method used to artificially fertilize walleye eggs may not necessarily influence percent hatch, particularly when egg volume and depth are low (*i.e.*, 250 mL and 4.4 mm), semen volume is 6 mL, and water volume used during fertilization is 1 L. The efficiency of the semen inclusion method may be of higher importance when larger volumes of eggs are fertilized. The results of this study should be interpreted based on the relatively low volume of eggs (250 mL) that resulted in a shallow egg depth (4.4 mm), relatively high volume of freshly collected semen (6 mL), and the 1 L of water used during fertilization. Additional research should examine percent survival at higher egg volumes. Because we found no statistical difference in semen inclusion techniques, no change is currently recommended in how SDGFP conducts walleye artificial spawning.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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