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Annals of Aquaculture and Research

Research Article

An Evaluation of Three Walleye Egg De-Adhesion Suspensions

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Submitted: 16 August, 2026

Accepted: 02 November, 2026

Published: 03 November, 2026

ISSN: 2379-0881

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Keywords

• Walleye; De-Adhesion; Spawning; Mucking; Glycoprotein

Abstract

Artificial spawning and incubation requires the removal of the sticky, adhesive glycoprotein outer layer of walleye (*Sander vitreus*) eggs. This de-adhesion, or mucking, involves the use of substances applied in suspension or solution to eggs immediately after fertilization. This study evaluated the effect of three de-adhesion substances on walleye egg survival to hatch: diatomaceous earth, Fuller's earth, and bentonite. Walleye egg survival was significantly different among all three treatments, at 95% in the eggs mucked with Fuller's earth, compared to 80% in those mucked with bentonite clay, and 63% mucked with diatomaceous earth. All three substances effectively removed the glycoprotein layer, although bentonite was very difficult to remove from the fertilized eggs after the de-adhesion process. Based on the results of this study, the use of Fuller's earth is recommended for the de-adhesion of walleye eggs during artificial spawning.

INTRODUCTION

Walleye (*Sander vitreus*), a member of the family Percidae, have a native range covering a large portion of Canada and extending into the southern United States [1-3]. Because of their popularity with recreational anglers, they have been widely introduced throughout the United States [4,5]. However, walleye fisheries are frequently overexploited, which coupled with inconsistent or absent natural reproduction, requires them to be frequently maintained by hatchery rearing and stocking [6-9]. Approximately one billion hatchery-reared walleye are stocked annually in North America [1,10-12].

Artificial propagation of walleye typically begins by spawning wild broodstock [13]. Similar to many other fish species, walleye have eggs with a sticky, adhesive glycoprotein outer layer that must be removed to allow for the eggs to be hatchery-incubated [14]. During spawning, this de-adhesion, or mucking as it is commonly called, occurs immediately after egg fertilization. While adhesion can increase egg survival in natural situations [15], it reduces egg survival in hatchery incubation, with fused eggs more susceptible to oxygen deprivation and fungal infections, reducing survival [16,17].

Several substances in suspension and chemicals in solution have been used to remove the adhesive glycoprotein layer in walleye eggs. Substances such as bentonite, Fuller's earth, diatomaceous earth, starch, pond muck, or even milk have been used in suspension [17,18]. Chemicals used in solution include tannic acid and protease [19,20]. Fuller's earth and bentonite are most-commonly used [21], but diatomaceous earth has a history of use by the South Dakota Department of Game, Fish and Parks [22]. Only two controlled experiments have been published comparing walleye egg de-adhesion solutions. The first published study compared protease, tannic acid, and Fuller's earth and produced inconsistent results [16]. Within one egg pool with poor egg survival (<30%), no difference was observed among the treatments, while in another egg pool Fuller's earth yielded significantly higher egg survival [16]. Krise et al. [20], indicated significantly higher survival at 86% in walleye eggs treated with protease compared to that of tannic acid and bentonite at 64% and 44%, respectively. Although completely uncontrolled and anecdotal, Bullen [23], reported higher survival in walleye eggs when the adhesive glycoprotein layer was removed with Fuller's earth compared to bentonite.

There have been no studies evaluating the use of diatomaceous earth during walleye egg spawning. Thus, the objective of this study was to evaluate the effect of diatomaceous earth as a de-adhesion agent on walleye egg survival, in relation to the more commonly used Fuller's earth and bentonite suspensions.

MATERIALS AND METHODS

Gamete Collection

Eggs were obtained from feral adult female walleyes captured in fyke nets [22] from Lynn Lake, Day County, South Dakota, USA on 15 April 2025. The eggs from each female were manually stripped into a 30.48-m diameter x 10.16-cm deep plastic container. The container was filled to an egg depth of 7-to-8 mm [24]. If the spawn from an individual female did not fill the container to that depth, the spawn from an additional female was added. The plastic containers with the eggs were then placed into insulated coolers and covered with towels, on top of which was placed an ice-pack. The coolers with the plastic containers holding the eggs were then transported 30 minutes to Blue Dog State Fish Hatchery, rural Waubay, South Dakota, USA for fertilization and subsequent processing.

Milt was obtained from feral adult male walleyes captured in fyke nets from Blue Dog Lake, Day County, South Dakota, USA on 13 April 2025. The males were transported (10 minutes) to Blue Dog State Fish Hatchery and held in an outdoor raceway. Milt was obtained on 14 April 2025 via manual stripping. Milt from a minimum of three males was pooled. From this pool, 3 mL was removed by syringe and added to a tissue flask. Six mL of milt extender (1900 mL distilled H_2O, 0.234 g $CaCl_2 2H_2O$, 0.267 $MgCl_2 6H_2O$, 0.472 g Na_2HPO_4, 3.744 g KCl, 13.155 g NaCl, 20 g glucose, 0.2 g citric acid monohydrate, 0.539 g ampicillin, 40 mL NaOH solution [1.27 g NaOH 100 mL^{-1} H2O], 32 mL bicine solution [5.3 g bicine 100 mL^{-1} H_2O]) was added to the flask [24]. The flasks with extended milt were stored at 3.3°C until used to fertilized the eggs on 15 April 2025.

Fertilization

Fertilization, incubation, and experimentation occurred at Blue Dog State Fish Hatchery. Well water at 11°C, with a total hardness as $CaCO_3$ of 317 mg/L, alkalinity as $CaCO_3$ of 286 mg/L, pH of 7.7, and total dissolved solids of 402 mg/L was used during fertilization and extending throughout incubation.

This experiment began by sub-dividing the 30.48-m diameter x 10.16-cm deep plastic pan that contained the stripped eggs into three approximately equal parts, each of which was subsequently assigned to one of three treatments. The exact number or volume of each part was not determined, nor was the total egg quantity in each of the pans. Each subsequent pan was fertilized using the dry method, whereby eggs and milt are mixed prior to the addition of water to activate the milt [25]. One tissue flask of extended milt was added to each pan of eggs and the combined gametes were gently stirred for approximately 15 seconds to evenly distribute the milt. After stirring, 1 L of water was added to activate the milt. The pans were stirred for an additional 30 seconds.

De-adhesion

Each pan of newly-fertilized eggs was then assigned to one of three de-adhesion treatments: 83 g/L diatomaceous earth [22], 105 g/L Fuller's earth [26], or 166 g/L bentonite [20,21]. After the addition of the respective de-adhesion solutions, each pan was stirred with a feather for 2 minutes. The eggs were then rinsed and a small number (approximately 100) were placed into discretely labelled plastic bags containing well water for an hour for egg membrane separation (water-hardening). This process was repeated with three different egg pools (i.e. three pans were subdivided; n = 3).

Incubation

Eggs were incubated in Petri dishes at ambient temperature using the technique described by Voorhees and Barnes [27]. Twenty eggs were removed from the plastic bag, with ten eggs placed into each of two similarly labeled Petri dishes. Each dish contained 30 mL of well water. Dead eggs were removed and recorded every 3 days until the retinal pigments became visible at the eyed stage of egg development. After reaching the eyed stage, dead eggs and hatched fry were removed and recorded daily. Water in each Petri dish was also exchanged daily. Egg survival was calculated using the following formula: Survival (%) = 100 x [1 – (# dead eggs / 10)].

An error occurred when both Petri dishes from one of the Fuller's earth replicates were severely shocked. This replicate was removed from the study.

Data Analysis

Data were analyzed by one-way analysis of variance with mean comparisons performed using Fisher's protected least significant difference using the SPSS (24.0) statistical analysis program (IBM, Armonk, New York, USA). Percentage data were log transformed prior to analysis to stabilize the variances [28]. Significance was pre-determined at $p \leq 0.05$.

RESULTS

Egg survival was significantly different among all three treatments ($p = 0.002$). Mean ± SE percent egg survival in the Fuller's earth treatment was 95.0 ± 5.0, compared to 80.0 ± 0.0 in the bentonite treatment, and 63.3 ± 3.3 in the diatomaceous earth treatment.

DISCUSSION

The results of this study clearly indicate the superiority of Fuller's earth as an egg de-adhesion agent. Bullen [23], also observed that eggs mucked with Fuller's earth survived better than those mucked with bentonite, although this study was not a direct comparison between the two mucking suspensions. Fuller's earth is widely used in other species for egg de-adhesion, including common carp (*Cyprinus carpio*) [29] and many sturgeon species [18,30-33].

Although the survival of eggs where de-adhesion occurred using bentonite was not as high as Fuller's earth, it was not as low as diatomaceous earth. It did work as a de-adhesive. However, the bentonite suspension tended to clump into balls, making it very difficult to remove from the fertilized eggs. This alone makes it unsuitable for use at a production level of walleye spawning.

The relatively low egg survival observed with the diatomaceous earth is not surprising. Diatomaceous earth is made primarily from fossilized diatoms that are high in silica dioxide and highly abrasive [34]. Diatomaceous earth is also used as an insecticide because of its highly abrasive nature [35]. Rottmann et al. [36], advised against the use of diatomaceous earth for fish egg de-adhesion because the sharp edges of the particles have the potential to damage the egg membranes and decrease subsequent egg survival.

The use of chemical solutions in comparison to compound suspensions for egg de-adhesion has been examined in walleye [20,37], and other fish species [18,29]. Although chemical solutions are far superior for removing adhesiveness and much less time consuming than compound suspensions, they are also more expensive and have a much higher risk of considerable egg mortality if dosages and treatment times are not explicitly followed [18].

Although the 60% egg survival with diatomaceous earth was lower than that observed in the other two solutions, it was similar to the 50-to-60% hatch historically observed in South Dakota [38-43]. It is also similar to the walleye egg survival reported elsewhere [13,44,45]. Similarly, 95% egg survival for the walleye eggs in the Fuller's treatment is similar to the over 90% seen experimentally by Czesny et al. [46]. While Moore [47], reported 75% walleye egg survival while using Fuller's earth at a production hatchery, he also observed higher survival during experimentation compared to production. Krise et al. [20], reported 45% hatch from walleye eggs mucked in bentonite but also mentioned that the eggs were still adhesive after treatment and the eggs were stirred for much longer than the eggs in this experiment. Stirring time can be important, with a 7% decrease in survival occurring when eggs mucked with Fuller's earth were stirred for 5 minutes instead of 3 minutes [47].

Potential limitations with this study include the use of a greater than normal milt-to-egg mass ratio, which could have caused greater egg fertilization and thus higher survival than that seen in production [24]. In addition, although the milt was pooled prior to adding extender, fertilizing each lot with a different flask of extended milt may have possibly caused variability in fertilization success [48]. It is also possible, though unlikely, that slight variations in stirring times could have influenced the results of this study [49]. The observations in this study could also have been influenced by the chemistry of the water used during egg de-adhesion [18], as well as the genetics of the walleye strain used [50,51].

CONCLUSION

The very consistent results observed in this study strongly support the use of Fuller's earth suspensions for the de-adhesion of walleye eggs. Additional research to further validate these results using other walleye populations and water chemistries, with additional replication, would be beneficial. In addition, further research on the other components of walleye spawning, gamete transportation, egg fertilization, and incubation are also needed.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article

ACKNOWLEDGEMENTS

We thank all the South Dakota Department of Game, Fish and Parks staff who assisted in gamete collection for this experiment.

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