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FISHERIES DIVISION

TECHNICAL REPORT

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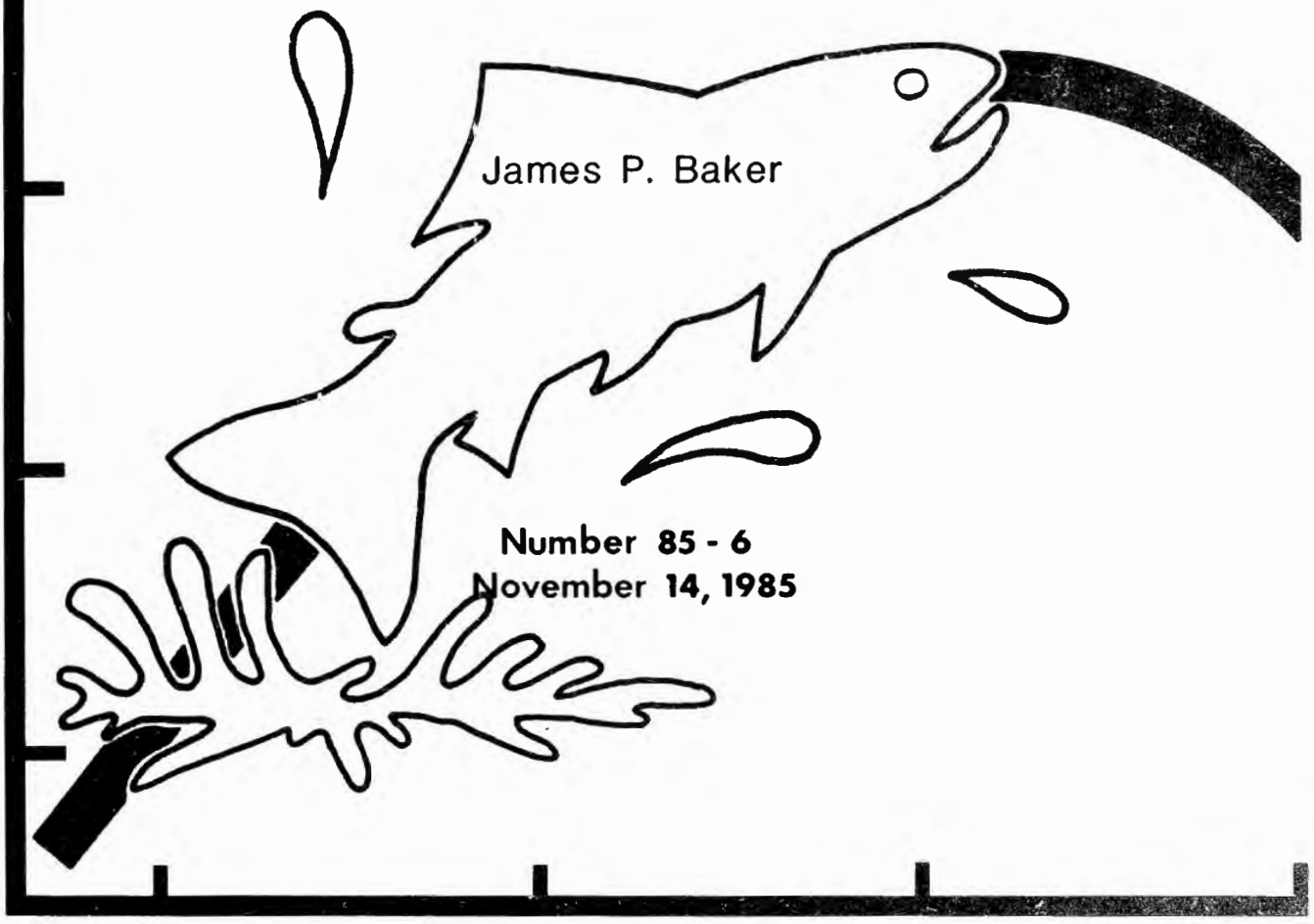

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AN EXAMINATION OF METHODS TO ELIMINATE ADHESIVENESS AND INCREASE SURVIVAL OF WALLEYE EGGS FOR HATCHERY PRODUCTION

James P. Baker

INTRODUCTION

One problem in the culture of walleye (Stizostedion vitreum) is that their eggs become sticky and clump together immediately after fertilization and prior to water hardening. If this adhesiveness is not eliminated, subsequent survival is greatly reduced due to inadequate exchange of gases and metabolites between the clumped eggs and the surrounding water and by proliferous fungal development. Various methods are employed by fish culturists to eliminate this adhesiveness. Suspensions of bentonite and fuller's earth are used to give the sticky eggs a coating of inert nonadhesive particles. Some culturists utilize tannic acid solutions to dissolve the glucoprotein coating which is responsible for the adhesive properties of the egg (Colesante and Youmans 1983). The United States Fish and Wildlife Service has reported increased hatching success through the use of protease solutions (Krise, unpublished 1984). Protease is an enzyme which also dissolves the glucoprotein coating on the egg. During the 1984 egg-take, an investigation was undertaken at the Wolf Lake State Fish Hatchery to find out which treatment, to prevent clumping, produced the highest rates of egg survival.

The walleye egg-take involves a large number of District fisheries personnel as well as the entire cultural staff of the hatchery. Eggs are taken, treated, and water hardened in the field and shipped to the hatchery for incubation. The Michigan Department of Natural Resources has been using fuller's earth to reduce egg adhesiveness for several years. Egg survival rates have varied slightly from year to year, but have averaged about 50%. The walleye program calls for the production of 50 million sac-fry annually, so approximately 100 million eggs must be taken. If the survival rate of the eggs could be increased significantly through a change in egg treatment procedures, it would not be necessary to take so many eggs and a substantial savings in man-hours should result.

METHODS AND MATERIALS

Walleye eggs were collected on two different dates from two different groups of fish. Fish in Trial Group 1 were collected in trap nets from Lake Macatawa, Ottawa County. Fish in Trial Group 2 were captured by electrofishing in the Muskegon River, Newago County. In each case, green females were captured, transported to the hatchery, and placed in holding tanks. Fish were examined for ripeness every 2 days. The fish were spawned when enough females were ripe to provide eggs for the experimental trials. In each trial group all spawning and fertilization were conducted in one session.

Two to three females (depending upon the size of the fish) were spawned into a single dry pan. Milt from an equal number of males was added, along with a small amount of water to activate the sperm, and the mixture was stirred briefly to distribute the sperm throughout the

eggs. The pans of eggs were then allowed to stand for approximately 1 minute to allow fertilization and micropyle closure. One minute after fertilization all eggs were combined in a larger pan and mixed thoroughly into a homogeneous batch. This helped eliminate variability in egg quality due to individual differences in spawning technique and possible differences in ripeness between individual fish.

Approximately equal volumes of eggs were dipped from the combined batch and placed in the test solutions. Concentrations and treatment procedures for each test solution are described below:

Solid protease: (Sigma No. P-4630 Crude Type 1 from bovine pancreas). One gram was mixed in 10 liters of water for a concentration of 100 mg/l.

Liquid protease: (Sigma No. P-8775 from Bacillus subtills). The stock solution contained 26 mg of protease per ml, so 38.5 ml of the stock solution was added to 10 liters of water to produce a concentration of 100 mg/l.

Tannic acid: (Sigma No. T-0125). Two and one-half grams of tannic acid was added to 10 liters of water to produce a concentration of 250 mg/l.

Fuller's earth: (Humco No. 0967). A handful of fuller's earth was added to a bucket of water, exactly as is done in current egg-handling procedures.

Eggs placed in the two protease solutions and the fuller's earth suspension were treated in the same manner. The eggs were kept in suspension by continuous gentle stirring for the first 5 minutes and then were stirred for 1 minute every 5 minutes thereafter until 1 hour had elapsed.

Eggs treated with tannic acid were stirred continuously for 4 minutes. Then the tannic acid solution was then poured off and the eggs were rinsed three times with clear water. Following rinsing, the eggs were placed in clear water and stirred for 1 minute every 5 minutes until 1 hour had elapsed.

After 1 hour, each lot of eggs was rinsed three times with clear water, placed in a hatching jar, and incubated in production hatching batteries at a water temperature of 11.5°C. A 1:600 formalin treatment was administered for 15 minutes daily to control fungus.

Walleye eggs become eyed in about 10 days when incubated at 11.5°C. Percentage of eye-up was determined after 10 days incubation for the trial group from Lake Macatawa and after 12 days for the Muskegon River trial group. The reason that the Muskegon River trial group was left 2 days longer is that the tenth day after egg-take fell on a weekend. I do not feel that the extra 2 days incubation made a difference in the ultimate results.

Simple random sampling was employed to determine the proportion of eyed eggs in each test lot. Based upon the estimated number of eggs in each test lot (Von Bayer method) and the expectation that 50% of the eggs would be eyed, it was determined statistically that a minimum

of 400 eggs must be examined in each test lot in order to obtain 95% confidence limits (B) upon the proportion of eye-up (p), where $n = Npq/ [(N-1) (B^2/4) + pq]$ (Mendenhall et al. 1971).

Each test lot was poured gently into a pan and thoroughly mixed to ensure the randomness of the sample. A random sample of several hundred eggs was pipetted from the pan to a petri dish half filled with water. The eggs were swirled gently to distribute them evenly over the bottom of the petri dish and the dish was set on a grid to facilitate numeration of the eggs. The numbers of eyed and dead eggs in each square of the grid were determined and successive squares were examined until the number of eggs examined exceeded 400. The proportion of eyed versus blank eggs was then calculated.

RESULTS AND DISCUSSION

Survival percentages to eye-up of walleye eggs subjected to the different treatments to prevent clumping are shown below:

	Source of eggs					
	Lake Macatawa			Muskegon River		
Treatment	N	Average	95% C.L.	N	Average	95% C.L.
Solid protease	417	27.1	22.1–32.1	406	37.9	32.9–42.9
Liquid protease	422	21.3	16.3–26.3	424	35.4	30.4–40.4
Tannic acid	432	21.8	16.8–26.8	415	25.5	20.5–30.5
Fuller's earth	402	22.6	17.6–27.6	445	48.1	43.1–53.1
Total	1,673	23.2	18.2–28.2	1,690	36.9	31.9–41.9

A simple test to determine whether one point estimate is significantly different from another is by examination of the 95% limits of the two point estimates. If they overlap, the estimates are not significantly different. Utilizing this test, it is obvious that there was no significant difference in the proportion of eyed eggs between any of the treatments in the Macatawa trial group. There was no apparent reason for the low proportion of eyed eggs in the Macatawa trial group, but previous experience with eggs taken from Lake Macatawa walleyes has shown eye-up and hatching success to be extremely variable. In all tests, the Macatawa trial group exhibited lower eye-ups than the Muskegon trial group.

In the Muskegon trial group, there was a significantly higher proportion of eyed eggs in the test lot treated with fuller's earth than in the lots treated with protease or tannic acid.

Eggs treated with the two protease solutions exhibited very soft shells. Many of the eggs broke during handling and some eggs hatched in the petri dish, making accurate enumeration difficult. It is likely that prolonged exposure of the eggs to the protease solutions caused deterioration of the egg shell. This condition may have caused the embryos to be damaged by the daily formalin treatment. Subsequent to the experiment, personal communication with William F. Krise indicated that the eggs should not be exposed to the protease solution for more than 15 minutes. However, it must also be noted that even after a full hour in the protease solutions, the eggs were found to be badly clumped when they were placed in the hatching jars.

The test lots treated with tannic acid exhibited the least tendency to clump in the hatching jars, and the eggs did not break with handling. However, the proportion of eyed eggs was low in both trial groups and was the lowest observed in the Muskegon trial group.

The test lots treated with fuller's earth showed little tendency to clump in the hatching jars and handled in a manner consistent with previous experience.

CONCLUSION

Based upon my findings, it appears that the use of protease and tannic acid to alleviate clumping of walleye eggs produces results no better than those obtained with fuller's earth. Furthermore, in the case of protease and tannic acid, the treatment solutions must be mixed very precisely and the time of treatment must be closely monitored in order to avoid damage (undue softening or hardening) to the egg shells. Such precision is seldom possible in the field under the conditions in which walleye eggs are taken. The use of fuller's earth required no such precision. Finally, the high cost of protease (about $7.00 per gram for the solid and $2.50 per gram for the liquid) in comparison to the relatively low cost of fuller's earth, makes its use in production quantities unattractive. The fuller's earth treatment is clearly the easiest procedure to employ under field conditions and, in one trial, eggs treated in this manner showed significantly higher survival than eggs treated with protease or tannic acid. Based upon these data, there is no evidence that a change in walleye egg treatment procedures is warranted.

It is indeed possible that the protease test lots were subjected to the treatment for too great a period of time, although even this extended time interval did not alleviate clumping. It is recommended that another limited test of protease, using a 15-minute treatment exposure, be conducted during the 1985 egg-take. This should help determine whether or not the low survival rate of the protease test lots was due to overexposure to the test solutions.

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