Manipulation of Hatchery Spawning Procedures to Improve Walleye Egg Fertility and Survival

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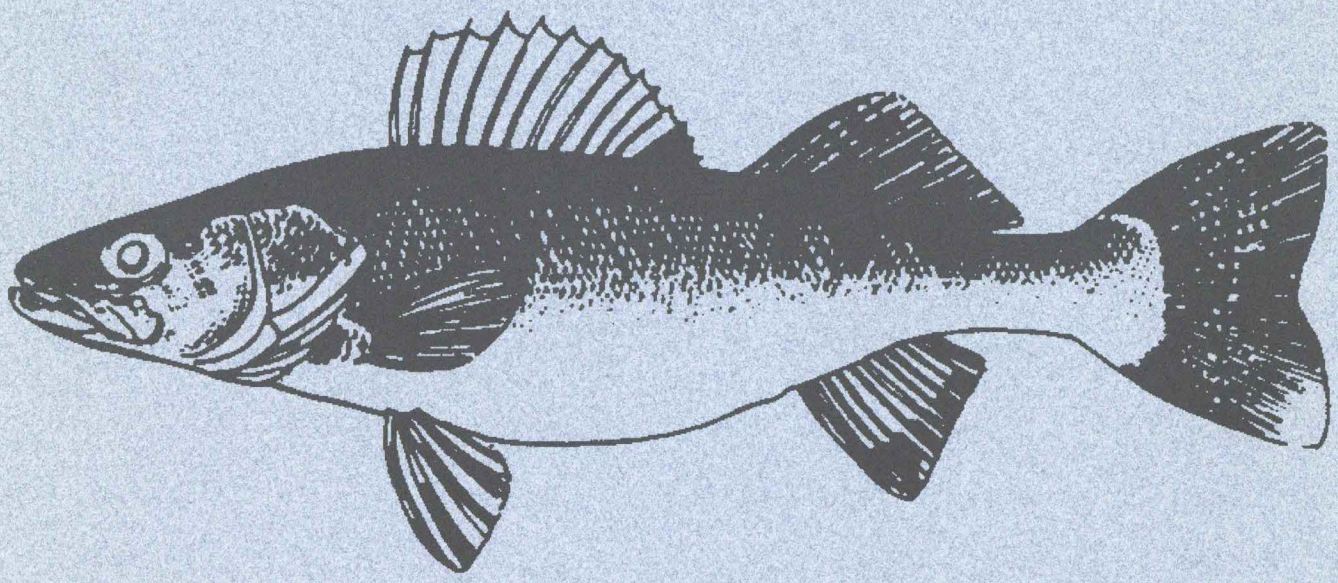

Iowa Department of Natural Resources
Larry J. Wilson, Director
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ABSTRACT

The purpose of this investigation was to study the effect of current spawning procedures on walleye egg survival, to reduce the effect of these procedures on survival, and to evaluate different techniques to improve egg fertility and subsequent survival and hatch. Findings indicate a 4.0 to 7.0 mm egg depth using 16-ounces of eggs is most desirable for good sperm cell distribution to the eggs. Also, 9.0 ml of extended walleye semen (3.0 ml undiluted semen) is an adequate amount for good fertilization. Semen should be added to the eggs in equal amounts two to three times during the fertilization process and at 30 second intervals. Eggs should be stirred in a clay solution a maximum of 2 to 3 minutes to reduce adhesiveness followed by a minimum of 4.0 hours of water hardening. Siphoning to remove dead eggs from the hatching jar should be performed carefully, otherwise, up to 8.0% live eggs may be removed from the jar. Following these procedures, mean walleye egg survival was increased 13.5% over previously used hatchery methods to 83.5%, which translates to at least 13.5 million more walleye sac-fry each year for stocking into Iowa waters.

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INTRODUCTION

The propagation of walleye (*Stizostedion vitreum vitreum)* is an important function of the Fisheries Bureau in the state of Iowa and fisheries departments of other states. Twenty-five states have native walleye populations, and 21 states support a program of walleye stocking to maintain and/or expand walleye fisheries. Fifteen state agencies have established new walleye fisheries where none existed, and because of their predatory nature, fisheries personnel in several states believe walleye are beneficial whether fisherman catch them or not (Prentice et al. 1977).

State fish hatcheries in Iowa annually produce 110,600,000 fish and walleye represent 92% of this output or 100 million fry and 975,000 fingerling fish. In addition, the demand for these hatchery walleye is expected to increase during the coming years. Present hatchery facilities and production capabilities will not satisfy the increased needs. Improved culture techniques could, however, benefit walleye production and partially resolve this problem.

Walleye egg fertility and hatch success, reported by fish culturists, varies greatly. According to Kleinert and Degurse (1968), the "wet method" of egg fertilization yielded a fertility rate of 72.1%. Using the "dry method" of spawning, Minnesota reported egg fertility averaged 61.1%; however, carefully fertilized egg samples taken from 227 females produced an average fertilization rate of 90.5% (Olson 1971). In agreement, egg fertility attained in Iowa's fish hatcheries ranged from 47.7 to 74% during normal hatchery operations and 75 to 95% during experimental trials (Moore 1984, 1985, 1986). Explanations for the variability in walleye egg fertility and percent hatch have been many; however, three stand out: 1) variability in quality of eggs and sperm (Hurley 1972); 2) temperature and temperature changes at the time of fertilization and incubation (Koenst and Smith 1976); and 3) culture techniques (Moore, 1984, 1985, 1986) (Olson, 1971).

Techniques that affect egg fertility and percent hatch include the "wet method" vs the "dry method" of fertilization (Hurley 1972; Olson 1971), the use of harsh materials or stirring to reduce adhesiveness (Olson 1971; Duman and Brand 1972; Colesante and Youmans 1983; Krise et al. 1986), and distribution of semen among eggs at the time of fertilization (Olson 1971; Moore 1984, 1985, 1986). Recent research of the problem focused on techniques and procedures used to reduce egg adhesiveness. The use of protease enzymes to eliminate this problem resulted in a percent hatch of 83 compared to 44 for eggs bathed in bentonite, 48 with continuously stirred eggs, and 64 for eggs subjected to tannic acid. In agreement, a 29% reduction in fertility was observed after eggs were "mucked" in clay (Olson 1971), while stirring and water hardening eggs in 300-400 mg/l tannic acid gave mean percent eye-up equal to or better than conventional methods (Colsante and Youmans 1983).

Fertilization and handling procedures are extremely important to egg fertility and survival. The objective of this project is to improve egg fertility and survival 10-15% above the present 70%. This will be accomplished by improving semen distribution throughout the egg mass and by reducing stress to eggs during various stages of handling, including efforts to reduce adhesiveness.

METHODS

Evaluation of Egg Handling Techniques

The techiques used during the "dry method" of walleye egg fertilization were evaluated. Steps included the following:

- eggs and semen were mixed in a dry pan
- water was added to activate the sperm cells
- eggs and sperm cells are gently swirled during a 3.0 minute fertilization period
- fertilized eggs were placed in a Fuller's Earth bath and stirred
- eggs were rinsed to remove clay
- eggs water hardened 4.0 hours before incubation

One ounce egg samples were taken after the 3.0 minute fertilization period, 2.0 to 10.0 minutes after initiation of the claying process, and upon completion of water hardening. Egg samples were placed on divided screen trays in a Heath incubator. Three to five days following fertilization, lots of 125 eggs were randomly selected from each one ounce sample and egg survival determined by microscopic evaluation.

Egg Depth Evaluation

Test 1: Three plastic containers of different size were used to evaluate the effect of egg depth on fertilization. Containers were sized to hold 6 ounces of eggs at depths of 4.0, 7.0, and 10.0 mm. One-milliliter of extended semen (Moore 1987) was added to each experimental egg lot.

Test, eggs were taken from one female, mixed and 6-ounce lots placed in fertilization containers. Semen, taken from two males prior to egg spawning, was mixed and extended one part semen to two parts extender. Eggs were processed by the "dry method" and jar incubated. After two to four days of incubation, aliquots of 125 eggs per jar were randomly selected for microscopic evaluation.

Sperm Cell Dilution Evaluation

Walleye semen was diluted with walleye extender at ratios of 1:2, 1:10, 1:15, and 1:20. Sperm cells were then assessed to determine motility and eggs were examined for fertility and survival.

The sperm cell motility study consisted of taking one milliliter samples of walleye semen, extended 1:2, and diluting these samples with additional extender to attain the needed rates of dilution. All samples were mixed with lake water to activate the sperm cells and initial motility evaluated at two and five minutes following the addition of water. Cell motility was evaluated with a compound microscope and stopwatch.

Semen mixed with walleye extender, at the above ratios, were used to fertilize homogenous 6-ounce lots of eggs. After eggs and diluted semen were mixed, 200 ml of lake water was added to activate the sperm cells. The mixture was stirred with a feather for 1 minute, gently agitated for 2 minutes and stirred in a fuller's earth slurry for two minutes. Eggs were jar incubated for 3 days and survival determined from aliquots of 125 eggs examined with a dissecting microscope.

Egg Dilution Evaluation

The technique of diluting walleye eggs with water (wet method) prior to the addition of semen was evaluated as a means of improving sperm cell distribution to eggs; hence egg fertility. Two homogenous 6-ounce lots of eggs were fertilized with 0.6 ml walleye semen extended 1:2. One lot of eggs was fertilized using the dry method, while the second egg lot was diluted with 150 ml of lake water just prior to the addition of semen. Egg depths of 4.0 mm and 7.0 mm were used in the test. Egg processing, incubation and fertility assessment were performed as previously described.

Sperm Cell Activator Evaluation

Salt plus urea, and dilute walleye extender plus urea, were tested as means of prolonging sperm cell motility. In addition, egg survival was examined to determine the benefit of these substances on the fertilizing capacity of sperm cells.

Combinations of these substances were diluted in 10 ml of lake water. A 0.75 ml subsample of the solution was added to 0.01 ml extended walleye semen. Motility of the activated semen was observed with a compound microscope at 100X power. The observation continued until all movement stopped and duration of activity was noted with the aid of a stop watch. The control used in the test was the motility period of sperm cells activated by lake water.

Fertilizing capacity of sperm cells plus activator was evaluated using 6-ounce egg lots, 7.0 mm in depth. Eggs in the control were fertilized by the dry method with 2.7 ml of standard 1:2 semen to walleye extender mixture, and sperm cells activated with 200 ml lake water. Eggs in test lots were fertilized in the same manner, except sperm cells were activated with either 200 ml of salt plus urea mixture, or 200 ml of dilute walleye extender plus urea.

Multiple Addition of Semen Evaluation
4-ounce Egg Lots

The addition of extended walleye semen to 4 ounce egg lots at multiple time intervals was investigated. Five homogenous egg groups were used, including a control, and four test lots. A 4.0 mm egg depth and the dry method of fertilization was used during testing. Eggs in the control lot were fertilized with 3.0 ml walleye semen extended 1:2 with walleye extender. Semen and eggs were mixed and sperm cells activated with 175 ml lake water.

Test 1: Four lots of eggs were each mixed with 1.0 ml of extended semen and sperm cells activated with 175 ml lake water. Two of these egg lots (T_1 and T_2) again received 1.0 ml additions of semen 30 seconds and 60 seconds following initial activation. The two remaining egg lots (T_3 and T_4) received three additional 1.0 ml aliquots of semen at 15, 30, and 60 seconds after initial activation for a total of 4.0 ml.

Test 2: In a second evaluation, egg lots used as the control were mixed with 3.0 ml extended walleye semen and sperm cells activated with 175 ml lake water. Two additional egg lots (T_1 and T_2) were initially mixed with 1.0 ml extended walleye semen and sperm cells activated with 175 ml lake water. These egg lots again received 1.0 ml additions of semen 30 and 60 seconds after initial activation. In a second test group, two egg lots (T_3 and T_4) were initially fertilized with 1.5 ml semen. This semen addition was repeated 30 and 60 seconds after initial sperm cell activation.

6-ounce Egg Lots

Test 1: Four homogenous 6-ounce egg lots were used. This provided a control and a test lot for two egg depths, 7.0 and 4.0 mm. Eggs in the control lots were mixed with 3.0 ml extended walleye semen and sperm cells activated with 175 ml lake water. Eggs in the test lots (T_1 and T_2) were initially mixed with 1.0 ml extended walleye semen and sperm cells activated with 175 ml lake water. The 1.0 ml semen addition was repeated 30 and 60 seconds following initial sperm cell activation.

Test 2: Three homogenous 6-ounce egg lots were used to provide a control and two test egg lots for T_1 and a control and two test egg lots for T_2. The control egg lot again was fertilized as in the above experiment, while test egg lots (T_1 and T_2) were initially mixed with 1.5 ml extended walleye semen. After initial sperm cell activation, the addition of 1.5 ml semen was repeated at either 30 (T_1) or 60 (T_2) seconds. The total extended semen added to an egg lot in this test was 3.0 ml.

8-ounce Egg Lots

Test 1: Three homogenous 8-ounce egg lots provided a control lot and two test lots. Eggs were maintained at a depth of 7.0 mm and sperm cells were activated with 200 ml lake water. Control eggs were mixed with 4.5 ml extended walleye semen and sperm cells activated. Test egg lot one (T_1) was mixed with 1.5 ml semen and test egg lot two (T_2) was mixed with 2.25 ml semen. After sperm cell activation, semen addition to T_1 and T_2 was repeated at 30 and 60 seconds. Total semen added was 4.5 ml in T_1 and 6.75 ml in T_2.

Test 2: The design of the second evaluation consisted of four 8-ounce egg lots, several volumes of extended walleye semen, use of 350 ml lake water to activate semen and an egg depth of 7.0 mm. Control eggs were mixed with 4.5 ml semen and sperm cells activated. Eggs in experimental lot one (T_1) were initially mixed with 1.5 ml semen. The addition of semen was repeated 30 and 60 seconds after sperm cell activation with a total of 4.5 ml semen used. Egg lot two (T_2) was mixed with 1.5 ml semen, and 30 seconds after activation received an additional 1.5 ml semen for a total of 3.0 ml. Egg lot three (T_3) received 2.25 ml semen initially and an additional 2.25 ml 30 seconds after activation for a total of 4.5 ml of semen.

16-ounce Egg Lots

Four homogenous 16-ounce egg lots were used to give a control and three test egg lots. Maximum egg depths of 4.0 to 6.0 mm were targeted for the control (C) and egg test lots one and two (T_1 and T_2) and a 10.0 mm egg depth for eggs in lot three (T3). Sperm cells in egg lots C, T_1 and T_2 were activated with 600 ml lake water. Control egg lots were fertilized with 9.0 ml extended walleye semen, then activated. Eggs in test lot one (T_1) were initially mixed with 3.0 ml extended semen and the addition of 3.0 ml of semen was repeated at 30 and 60 seconds after initial activation. Eggs in test lot two (T_2) were initially mixed with 4.5 ml extended semen and an additional 4.5 ml semen was added 30 seconds after initial sperm cell activation. The egg lot that most resembled the technique used in hatcheries (T_3) was mixed with 9.0 ml semen. Sperm cells in this lot were activated with 400 ml lake water in 1995, and 600 ml lake water in 1996.

The egg lot fertilization order for randomness was planned as follows:

Test No.	Egg Lot Test Order				
1	C	T_1	T_2	T_3	T_4
2	T_1	T_2	T_3	T_4	C
3	T_2	T_3	T_4	C	T_1
4	T_3	T_4	C	T_1	T_2
5	T_4	C	T_1	T_2	T_3

When Test No. 6 was reached, the test block began over as in Test No. 1. Also, when less than five egg lots were used, the egg test order rotated accordingly.

The dry method of fertilization was used as standard procedure in all egg fertilization evaluations, and included a 2 to 3 minute fertilization period, 2 minute claying in fuller's earth, and 4 hours of water hardening. Each egg group was incubated in a hatching jar or as 1-ounce subsamples incubated in a Heath tray incubator. As soon as viable eggs could be accurately determined (approximately 50 to 60 Tu°F), five 25 egg subsamples from each egg group were selected. A dissecting microscope was used to enumerate live and dead eggs.

Gamete Quality

The quality of eggs and semen used during walleye spawning was measured for the entire study. Egg survival, regardless of test, was compiled for the 1) first one-half of the spawning season and 2) second one-half of the spawning season and the data was tested for significance.

Egg Survival Prediction

Using the work of Heidinger et al. (1997), the procedure of using the percent survival of 1-ounce egg lots, incubated in Heath trays to 54 temperature units (TU), to predict the survival of eggs incubated in jars was investigated. Fifteen different egg groups were used in this investigation.

Sixteen ounce egg lots were fertilized using the dry method described previously. Extended walleye semen (4.5 ml) was mixed with walleye eggs and activated with 800 ml lake water. Approximately 30 seconds later an additional 4.5 ml extended walleye semen was added, and the mixture swirled for 2.0 minutes. After water hardening, a 1-ounce sample was taken and placed in a Heath incubator tray. The remaining eggs were placed in a conventional hatching jar. Approximately 2400 ml/min water was run through each jar until sampling at 54 TU, then 3000 ml/min until 200 TU were reached.

The number of eggs/linear millimeter was determined at <24 hour and at 200 TU by aligning eggs side by side in a "V" trough for a length of 50 mm. This was done to verify egg diameter changes during incubation.

The level of eggs in each jar was measured to the nearest millimeter by turning the water flow off, allowing the eggs to settle for 30 seconds, then measuring with a millimeter rule from the jar bottom to the egg level. These measurements were taken at three locations around the jar when the eggs were first placed in the jar then at 200 TU.

Dead eggs were siphoned off as needed. Random samples of 50 to 125 eggs were taken from siphoned eggs and observed under a dissecting microscope to determine the number of live eggs incidentally siphoned.

The 1-ounce egg group in the Heath tray incubator was sampled at approximately 54 TU. All eggs were siphoned from the tray and five 25 egg samples were randomly selected for observation under a dissecting microscope. Percent survival was calculated by dividing the number of live eggs per sample by the number of eggs in the sample. This gave a projected final egg survival. Corresponding eggs incubated in a hatching jar were sampled at approximately 200 TU. Measurements taken at this stage were 1) egg height in the jar in millimeters, 2) percent live eggs left in the jar by evaluating five 25 egg samples as above, and 3) number of eggs per linear millimeter.

Survival of the jar incubated eggs was predicted by the following formulas:

1) $B \times E = F$

2) $\dfrac{(A - F)(C)}{100} = G$

3) $\dfrac{(G + F)(D)}{100} \div A = \%$ Egg Survival

Where:
A = Egg height in hatching jar (mm) at <24 hr.
B = Egg height in hatching jar (mm) at approximately 200 TU.
C = % Live eggs siphoned from hatching jar.
D = % Live eggs left in hatching jar.
E = Number of eggs/mm at 200 TU ÷ number of eggs/mm at <24 h.
F= Adjusted egg height.
G= mm live eggs siphoned from hatching jar.

Data Analysis

Data were analyzed by Chi-square, independent t-test, and Mann-Whitney μ tests at the 0.05 alpha level.

FINDINGS

Evaluation of Egg Handling Techniques

Egg samples revealed survival rates of 71.3% immediately following the 3 minute fertilization process; 71.5% 2 minutes into the claying process; 68.4% 5 minutes into the claying process; and 71.0% after 4 hours of water hardening (Table 1). Testing showed no significant difference between sampling periods.

Table 1. *Percent survival of walleye eggs sampled at four stages of the walleye spawning process, Rathbun Fish Culture Research, 1988.*

Date	3 Min. After Fertilization	2 Min. in Clay	5 Min. in Clay	After Water Hardening
3-27	71.2	66.0	61.6	63.2
4-3	71.2	67.2	72.0	76.0
4-7	84.8	83.2	80.0	88.8
4-7	71.2	67.2	65.6	64.0
4-8	71.2	84.0	64.8	68.8
4-9	75.2	67.2	77.6	—
4-12	89.6	88.0	86.4	89.6
4-13	84.8	86.4	88.8	87.2
4-15	63.2	60.0	38.4	69.6
4-16	44.8	48.8	52.0	45.6
4-17	56.8	68.0	65.6	57.6
Mean	**71.3[a]**	**71.5[a]**	**68.4[a]**	**71.0[a]**

Mean values with the same letter were not significantly different.

Additional testing on the effect of stirring time on egg survival again revealed no difference between samples taken immediately after the 3 minute fertilization period (77.7%) and after the 5 minute clay stirring period (75.4%) (Table 2). If eggs were stirred in clay longer than 5 minutes, a significant reduction in egg survival occurred (70.5%) when compared to samples taken immediately after the 3 minute fertilization period (76.6%) (Table 3).

Egg Depth Evaluation

Test 1: Egg survival was strongly related to egg depth at the time of fertilization (P=0.003). As egg depth increased from 4.0 to 10.0 mm, mean egg fertility decreased from 86% to 65.3% (Table 4). Egg depth at the time of fertili-

Table 2. *Percent survival of walleye eggs sampled 3 minutes after initial fertilization and after 5 minutes of stirring in fuller's earth, Rathbun Fish Culture Research, 1988.*

Date	3 Min. after Fertilization	5 Min. Stir In Fuller' Earth
3-30	82.4	84.0
4-1	76.0	78.4
4-1	76.8	74.4
4-1	75.2	76.0
4-1	78.4	80.0
4-2	76.0	76.6
4-4	85.6	81.6
4-4	76.8	78.4
4-6	72.0	70.4
4-6	82.4	84.0
4-6	85.6	52.0
4-10	66.4	68.8
4-10	72.0	70.4
4-10	83.2	83.2
Mean	**77.7**	**75.4**

Table 3. *Percent survival of walleye eggs sampled 3 minutes after initial fertilization and after more than 5 minutes of stirring in fuller's earth, Rathbun Fish Culture Research, 1988.*

		>5 Minute Stir	
Date	3 Min. After Fertilization (A)	Time Stirred (Min:Sec)	Egg Survival (B)
4-5	83.2	5:10	84.8
4-6	85.6	5:42	76.8
4-8	74.4	6:00	70.4
4-7	67.2	6:36	74.4
3-30	81.6	7:00	71.2
4-3	71.2	7:30	66.4
4-10	68.0	7:32	70.6
4-17	76.8	7:54	70.4
4-7	83.2	8:50	75.2
4-12	81.6	9:27	69.6
4-13	72.8	10:00	66.4
4-12	68.0	10:01	56.8
4-4	76.8	10:30	72.8
4-9	88.8	10:52	71.2
4-13	71.2	11:58	67.2
4-11	74.4	14:25	64.0
Mean	**76.6**	**8:52**	**70.5**

Table 4. *Relationship between egg depth at the time of fertilization and total percent walleye egg survival, Rathbun Fish Culture Research, 1988.*

	Egg Depth		
Date	**4.0 mm**	**7.0 mm**	**10.0 mm**
4-7	97.6	70.4	56.0
4-9	84.8	59.2	80.8
4-10	80.8	—	77.6
4-11	92.0	47.2	44.0
4-11	93.6	87.2	64.8
4-12	88.0	80.0	44.8
4-13	87.2	95.2	86.4
4-13	70.4	76.8	62.4
4-14	94.4	86.6	79.2
4-16	69.6	83.2	55.2
4-19	88.0	84.8	67.2
Mean	**86.0[a]**	**77.1[b]**	**65.3[c]**

Mean values with the same letter were not significantly different.

zation did significantly impact egg survival when 4.0 and 7.0 mm depths were compared. The shallower depths produced significantly higher egg survival when compared to an egg depth of 10.0 mm .

Sperm Cell Dilution Evaluation

A loss in sperm cell motility was noted when the higher semen to walleye extender dilution ratios of 1:10, 1:15, and 1:20 were compared to the standard 1:2. An estimated 10% decline in motility was observed after semen had been stored two to three days. Observation with a microscope to determine if the higher dilution ratios were prematurely activating sperm cells proved negative.

Semen to extender dilution ratios had no significant impact on egg fertility when egg depth was 4.0 mm at the time of fertilization. Egg fertility rates at the 1:2, 1:10, 1:15, and 1:20 dilution ratios were 59.3%, 60.2%, 55.7%, and 57.6%, respectively.

At the 7.0 mm egg depth, the 1:15 dilution ratio fertilized significantly fewer eggs when compared to other ratios. Egg fertility rates were 82.1%, 81.4%, 74.4%, and 80.1% at semen dilution ratios of 1:2, 1:10, 1:15 and 1:20. Ten trials were conducted.

Egg Dilution Evaluation

No significant difference in egg fertility was observed when the dry method technique of egg fertilization was compared to diluting eggs with water just prior to adding semen. The fertilization rate at the 4.0 mm egg depth was 56.5% for the dry method and 60.1% for the wet method. At the 7.0 mm egg depth, fertilization was 83.7% for the dry method and 82.5% for the wet method.

The technique of diluting eggs with water in conjunction with the four previously mentioned semen dilution rates did not appreciably affect egg fertility. At the 7.0 mm egg depth, the 1:2 semen to extender ratio had the highest fertilization rate of 74.5% and was significantly higher than the 1:20 ratio (69.6%) (Table 5). Percent fertility rates of the 1:10 and 1:15 semen dilution ratios were not different compared to the 1:2 ratio. Fertility was 73.7 and 73.8, respectively.

Table 5. *Percent fertile walleye eggs per 150 egg sample using eggs diluted with water and four semen to extender dilution rates, Rathbun Fish Culture Research, 1989.*

	Semen to Extender Dilut			
Egg Depth	**(control) 1:2**	**1:10**	**1:15**	**1:20**
7 mm	74.5[a]	73.7[a]	73.8[a]	69.6[b]
4 mm	64.2[a]	55.8[b]	57.2[b]	60.2[a]

Values at a corresponding egg depth with the same letter were not significantly different.

The 1:2 (64.2%) semen dilution ratio fertilized a significantly higher percent of eggs than the 1:10 (55.8%) or the 1:15 (57.2%) ratios at the 4.0 mm egg depth, but was not different when compared to the 1:20 (60.2%) ratio.

A direct comparison of egg fertility between egg depths was not performed as the egg lots were unrelated.

Sperm Cell Activator Evaluation

Walleye sperm cells activated only with water were motile 26 to 57 seconds, with a mean life of 36.6 seconds. Salt (0.025 to 0.035 grams) plus urea (0.03 grams) dissolved in 10 ml lake water maintained sperm cell motility from 69 to 113 seconds. The optimum solution contained

Table 6. *Number of live walleye eggs per 125 egg sample when tests included multiple semen addition and egg depth was 4 mm, Rathbun Fish Culture Research, 1992.*

	0, 30, 60 Sec. Add.		0, 15, 30, 60 Sec Add.		
Date	**Test 1**	**Test 2**	**Test 3**	**Test 4**	**Control**
4-4	108	97	91	113	99
4-6	105	117	116	94	109
4-7	114	119	119	123	118
4-7	115	121	114	120	109
4-8	122	101	117	114	111
4-9	115	117	116	112	113
4-9	116	113	116	92	115
4-10	110	116	106	115	93
4-11	108	113	116	115	107
4-11	103	84	102	89	91
4-12	115	118	111	112	59
4-13	109	116	119	111	118
Mean of Data	**111.6**	**111.0**	**111.9**	**109.2**	**103.5**
Mean					
% Survival	**89.0[a]**		**88.4[a]**		**82.8[b]**

Mean values with the same letter were not significantly different.

eggs in test lots 1 and 2 were 69.3% and 72.0%, respectively, and 74.1% for eggs in the control.

Multiple Addition of Semen Evaluation

4-ounce Egg Lots

Test 1: Multiple addition of semen to the egg mass after initial sperm cell activation improved egg survival (Table 6). Combined percent survival and CI for test lots T_1 and T_2 was 89.0 ± 1.36; test lots T_3 and T_4 88.4 ± 1.17; and control egg lots 82.8 ± 1.97. Percent survival between test groups (T_1, T_2, and T_3, T_4) was not significantly different; however, egg survival in all test egg lots was significantly higher than

0.03 grams salt plus 0.03 grams urea. Walleye extender diluted by one-half plus 0.03 grams urea per 15 ml of dilution maintained sperm cell activity an average of 102 seconds. Salt and urea concentrates of 0.07 grams and 0.04 grams and higher gave no motility.

The use of salt and urea did not increase egg survival. Egg survival for test 1 and 2 egg lots was 62.6% (200 ml mixture of salt and urea) and 65.0%, respectively, and survival for control egg lots was 71.2%. Survival of the eggs in the control was significantly higher than eggs in lot 1. Diluted walleye extender plus urea did not significantly increase egg fertility and subsequent survival above that experienced by eggs in the control lots. Survival rates for

Table 7. *Number of live walleye eggs per 125 egg sample when treatments included multiple semen addition and 4-ounce egg lots; Rathbun Fish Culture Research, 1993.*

	1.0 ml Semen 0, 30, 60 Sec Add		1.5 ml Semen 0, 30, 60 Sec Add		3.0 ml Semen
Date	**Test 1**	**Test 2**	**Test 3**	**Test 4**	**Control**
4-7	102	118	112	115	70
4-8	121	120	107	111	110
4-9	102	106	96	105	99
4-10	120	118	110	118	104
4-10	112	103	113	105	92
4-11	114	114	113	113	106
4-12	116	115	100	111	112
4-13	111	110	108	93	100
4-14	104	103	98	97	73
4-16	94	112	108	112	88
4-17	74	96	108	106	85
4-18	117	109	122	112	97
4-23	108	99	80	101	81
Mean of Data	**107.3**	**109.5**	**105.7**	**106.8**	**93.6**
Mean					
% Survival	**86.7[a]**		**85.0[a]**		**74.8[b]**

Mean values with the same letter were not significantly different.

Table 8. *Number of live walleye eggs per 125 egg sample with 30 and 60 second multiple semen addition and two egg depths, Rathbun Fish Culture Research, 1991.*

	4 mm Egg Depth		7 mm Egg Depth	
Date	**Test**	**Control**	**Test**	**Control**
4-3	119	111	106	95
4-4	117	100	103	94
4-4	73	83	88	77
4-5	103	102	113	111
4-6	117	108	111	118
4-6	109	94	100	89
4-7	72	71	76	83
4-8	108	117	107	99
4-8	102	70	94	94
4-9	79	62	76	77
4-9	76	55	68	64
4-11	101	84	90	111
4-11	87	64	80	66
4-12	98	101	89	95
4-12	73	59	83	55
4-13	84	81	59	68
4-13	82	57	79	74
4-14	92	91	102	96
4-14	100	89	109	84
4-15	52	90	72	92
Mean of Data	**92.0**	**84.4**	**90.2**	**87.1**
Mean % Survival	**73.8[a]**	**67.5[b]**	**72.2[a,c]**	**69.7[b.c]**

Mean values with the same letter were not significantly different.

the control. No significant egg survival advantage was gained by adding 4.0 ml (T_3 and T_4) of extended semen to a 4-ounce egg mass over that attained by adding 3.0 ml (T_1 and T_2).

Test 2: The multiple addition of extended semen to walleye eggs improved egg survival compared to eggs in control lots (Table 7). All test lots (T_1, T_2, T_3, and T_4) had significantly higher egg survival than that observed in controls. Combined percent survival and confidence intervals for egg lots T_1 and T_2 was 86.7 ± 1.19, and egg lots T_3 and T_4, 85.0 ± 1.75. Survival and CI for control egg lots was 74.8% ± 2.15. No significant difference was noted in egg survival between lots T_1, T_2 and lots T_3, T_4. This indicated that the additional extended semen received by egg lots T_3 and T_4, was not beneficial.

6-ounce Egg Lots

Test 1: The addition of extended walleye semen to 6-ounce egg lots at 30 and 60 seconds after initial sperm cell activation improved egg survival only slightly (Table 8). Percent egg survival and CI for the 4.0 mm egg depth (73.8 ± 1.76) was significantly higher than that experienced in control lots (67.5% ± 1.87). Egg survival at the 7.0 mm egg depth (72.2% ± 1.97) was not significantly better than the control (69.7 % ± 1.84). Egg survival was not significantly different when egg depths of 4.0 and 7.0 mm were compared.

Test 2: The addition of 1.5 ml extended walleye semen to the 6-ounces of walleye eggs either 30 or 60 seconds after initial sperm cell activation again gave a minimal increase in survival (Table 9) (Table10). Mean egg survival for egg lot T_1 was 83.0% after the 30 second semen addition, and 75.5% for T_2 after the 60 second semen addition. Control egg lot survival for tests T_1 and T_2 was 80.8% and 69.9%, respectively. There was no significant difference in egg survival between test lots and controls.

Table 9. *Percent survival of walleye eggs when 1.5 ml semen was added to 6-ounce egg lots 30 seconds after initial sperm cell activation, Rathbun Fish Culture Research, 1990.*

Date	**Test 1**	**Test 1**	**Control**
4-5	86.4	88.0	85.6
4-6	84.0	79.2	72.0
4-8	88.0	78.4	70.4
4-10	63.2	84.0	89.6
4-12	85.6	93.6	95.2
4-14	86.4	89.6	89.6
4-16	82.4	76.8	64.0
Mean	**82.2[a]**	**84.1[a]**	**80.8[a]**
Mean of T_1	**83.0**		

Values with the same letter were not significantly different.

Table 10. *Percent survival of walleye eggs when 1.5 ml semen was added to 6-ounce egg lots 60 seconds after initial sperm cell activation, Rathbun Fish Culture Research, 1990 .*

Date	Test 2	Test 2	Control
4-6	70.4	79.2	66.4
4-7	79.2	77.6	64.0
4-9	64.0	75.2	78.4
4-11	88.8	86.4	84.0
4-13	54.4	50.4	54.4
4-15	77.6	79.2	58.4
4-19	82.4	79.2	84.0
Mean	**73.8[a]**	**75.2[a]**	**69.9[a]**
Mean of T_2	**75.5**		

Mean values with the same letter were not significantly different.

Table 11. *Number of live walleye eggs per 125 egg sample when treatments involved multiple semen addition and 8-ounce egg lots; Rathbun Fish Culture Research, 1993.*

	(T_1) 1.5 ml Semen	(T_2) 2.25 ml Semen	4.5 ml Semen
Date	0, 30, 60 Sec Add	0, 30, 60 Sec Add	Control
4-9	105	109	110
4-10	105	105	85
4-11	94	95	84
4-12	68	57	64
4-13	106	111	99
4-14	105	111	99
4-15	99	101	96
4-16	109	97	102
4-17	103	107	88
4-18	103	103	105
4-19	105	102	85
4-20	105	95	80
4-22	97	106	95
Mean of Data	**100.3**	**99.9**	**91.6**
% Survival	**80.2[a]**	**79.9[a]**	**73.3[b]**

Values with the same letter were not significantly different.

8-ounce Egg Lots

Test 1: Multiple addition of extended walleye semen to 8-ounce egg lots improved egg survival significantly over that attained in control lots. Percent egg survival for T_1 (1.5 ml) was 80.2 ± 1.99 and 79.9 ± 2.0 for T_2 (2.25) and these survivals were not significantly different. Survival of eggs in control lots was 73.3 ± 2.0 (Table 11).

Test 2: The addition of 1.5 ml extended walleye semen to an 8-ounce egg mass 30 and 60 seconds after initial sperm cell activation in T_1 and at 30 seconds in T_2 signifi-

Table 12. *Number of live walleye eggs per 125 egg sample when treatment included multiple semen addition and 8-ounce egg lots; Rathbun Fish Culture Research, 1994.*

	(T_1) 1.5 ml Semen Added	(T_2) 1.5 ml Semen Added	(T_3) 2.25 ml Semen Added	Control 4.5 ml
Date	0, 30, 60 Sec.	0, 30 Sec.	0, 30 Sec.	Semen
4-7	80	68	65	69
4-8	113	105	93	82
4-9	96	109	100	102
4-10	103	97	94	101
4-10	100	111	100	997
4-11	104	111	72	105
4-11	93	95	105	87
4-12	115	119	110	107
4-12	109	117	91	114
4-13	113	112	107	105
4-13	107	111	106	91
4-14	100	114	81	113
4-14	110	112	100	117
4-14	106	80	89	101
4-16	101	109	103	96
4-16	111	99	101	109
4-16	111	104	92	112
4-17	111	109	106	115
4-18	98	95	83	91
4-19	112	104	96	89
4-20	99	102	97	89
Mean of Data	**104.4**	**103.9**	**94.8**	**99.6**
% Survival	**83.5[a]**	**83.1[a]**	**75.8[b]**	**79.6[c]**

Values with the same letter were not significantly different.

cantly improved egg survival when compared to T_3 and control egg lots (Table 12). No significant difference in egg survival was found between T_1 and T_2. Survival in control egg lots, however, was significantly higher than T_3. Percent egg survival was 83.5, for T_1, 83.1 for T_2, 75.8 for T_3, and 79.6 for the control.

16-ounce Egg Lots (Combined 1995-1996 Data)

The addition of extended walleye semen to 16-ounces of walleye eggs in 1995 and 1996 gave significantly higher egg survival in tests T_1 and T_2 when compared to the control (Table 13). Percent egg survival for test lots T_1 and T_2 was 83.4 and 83.6, while the percent egg survival for control (C) was 79.3. No significant difference in egg survival was found between T_1 and T_2 tests.

T_3 produced significantly lower egg survival when compared to T_1, T_2 and the control. Egg survival obtained in T_3 (the old hatchery method of fertilizing eggs) was 70.2%. Standard deviation from the mean values showed less variability in egg survival in T_1 trials (9.5) and T_2 trials (10.1) than that found in the control (12.8) and T_3 trials (13.5).

Gamete Quality

Egg survival from walleye spawned during the first one-half of the spawning season was significantly higher than egg survival from walleye spawned during the second one-half of the spawning season. Mean survival of eggs taken during the first one-half of the spawning season was 82.0%, while survival of eggs taken later in the spawning season was 77.5%.

A stepwise regression conducted on five factors influencing egg fertility and survival (time of spawning season, semen volume, fertilization techniques, egg depth, and egg volume) indicated time of spawning season, egg depth, and fertilization technique as significantly important ($P<0.001$) ($R^2=.24$).

Egg Survival Prediction

No significant difference was found in egg survival predicted with eggs incubated in Heath trays to 54 TU compared to eggs incubated in a hatching jar to 200 TU. Mean percent survival predicted with Heath tray incubated eggs

Table 13. *Number of live walleye eggs per 125 egg sample when treatments included multiple addition of semen and 16-ounce egg lots; Rathbun Fish Culture Research Facility, 1995 and 1996. (Blanks indicate no test)*

Date	9.0 ml semen added once (C)	3.0 ml semen 0, 30, 60 sec. add (T_1)	4.5 ml semen 0, 30 sec. add (T_2)	9.0 ml semen added once (12 mm depth: T_3)
4-6	118	112	110	—
4-6	95	104	109	—
4-7	115	108	116	—
4-8	104	114	102	—
4-8	95	111	112	—
4-8	110	106	112	—
4-9	82	108	104	—
4-9	105	108	106	98
4-9	92	108	103	99
4-9	115	117	106	117
4-9	85	115	109	103
4-10	112	97	105	—
4-10	86	103	105	101
4-10	104	107	105	90
4-10	111	87	118	90
4-10	95	115	112	70
4-11	96	94	106	—
4-11	111	117	114	91
4-11	72	114	117	95
4-11	98	105	96	101
4-11	84	112	106	99
4-12	95	97	94	97
4-12	—	—	114	107
4-12	77	100	72	80
4-12	121	120	110	95
4-12	108	112	115	—
4-13	—	93	107	71
4-13	94	110	106	83
4-13	96	100	99	71
4-13	103	109	112	105
4-13	106	117	117	102
4-14	97	107	85	72
4-14	111	102	—	79
4-14	105	103	114	94
4-14	106	116	101	93
4-14	103	96	106	105
4-14	103	110	112	97
4-15	98	106	109	57
4-15	112	117	—	91
4-15	102	105	92	92
4-15	67	113	96	73
4-16	97	97	103	60
4-16	95	110	110	99
4-16	102	—	117	90
4-16	98	111	95	68
4-16	116	100	103	94
4-16	101	96	99	—
4-17	111	98	104	85
4-17	93	98	93	81
4-17	108	81	105	80
4-17	101	86	99	91
4-17	108	107	110	100
4-17	108	93	116	—
4-18	96	84	101	90
4-18	102	110	101	88
4-18	98	101	113	84
4-18	100	109	87	59
4-18	71	100	80	79
4-19	104	96	106	65
4-19	63	78	73	81
4-19	72	107	106	82
4-19	111	93	116	95
Mean % Survival	**99.1**	**104.2**	**104.5**	**87.8**
	79.3[b]	**83.4[a]**	**83.6[a]**	**70.2[c]**

Means with the same letter were not significantly different.

Table 14. *A comparison of eggs incubated to 54 temperature units to eggs incubated to approximately 200 temperature units at Rathbun Fish Culture Research Facility, 1997.*

Jar #	Egg/mm <24HR	Egg/mm 200 TU	% Change	Jar Egg Height <24hr (mm)	Jar Egg Height 200 TU (mm)	%Good Eggs Siphoned	% Survival Heath Incubator	Jar Adjusted % Survival
10	0.45	0.47	-4.0	129.3	108.3	7.8	81.6	82.6
15	0.46	0.48	-4.0	128.0	112.0	3.5	78.4	76.6
35	0.49	0.49	0.0	132.6	117.6	8.3	85.6	84.6
32	0.45	0.46	-2.0	133.3	126.6	3.3	95.2	91.7
43	0.50	0.51	-2.0	100.6	97.0	2.6	87.2	90.5
44	0.48	0.49	-2.0	108.0	105.0	7.0	88.0	93.6
37	0.47	0.48	-2.0	117.0	117.3	7.8	80.0	79.0
39	0.47	0.48	-2.0	135.6	123.3	6.6	68.8	87.2
4	0.46	0.47	-2.0	124.3	116.3	7.7	95.2	90.4
6	0.47	0.47	0.0	119.3	119.0	4.6	88.8	96.5
7	0.48	0.48	0.0	128.0	115.6	4.9	74.4	80.6
7B	0.47	0.47	0.0	125.6	113.6	4.0	86.4	89.1
10	0.47	0.48	-2.0	112.0	105.0	1.6	84.8	85.8
15	0.49	0.50	-2.0	116.6	109.6	2.6	92.0	89.7
32	0.50	0.50	0.0	122.6	120.0	8.0	89.6	93.3
Mean						**5.35**	**85.0**	**87.4**

was 85.0%, and 87.4% with eggs incubated in a hatching jar (Table 14). Egg size at 24 hours of age was not significantly different when compared to egg size at 200 TU. At 200 TU, the eyes were easily detectable on the developing embryo, but were not yet fully pigmented.

The sampling of eggs siphoned from the jars revealed that a mean of 5.35% of the eggs siphoned were live eggs. Percent live eggs incidentally siphoned ranged from 1.6 to 8.3 per jar.

DISCUSSION

The reduction in egg survival from 3 minutes post-fertilization to eggs stirred >5.0 minutes, indicated egg trauma was a major cause of reduced egg survival. This fact is in agreement with researchers that found abrasives and/or excessive stirring were detrimental to egg viability (Olson 1971, Hurley 1972, Colesante and Youmans 1983). Stirring eggs 5 minutes or less tended to reduce trauma and did not significantly reduce egg survival. Design of the test eliminated differences in stirring techniques that might be attributable to personnel assisting with the evaluation. The same individual handled and stirred all samples.

A reduction in egg depth at the time of fertilization from 10.0 mm to 4.0 mm increased egg fertility approximately 21.0%. Since these tests were performed using actual hatchery spawning procedures, and eggs jar incubated at least 3 days, all results are similar to that experienced in normal hatchery production figures. The percentages are in agreement with the 90% egg survival reported by Olson (1971) and Moore (1984, 1985, 1986) for small egg lots and concur with the hypothesis that distribution of quality semen to quality eggs is extremely important at the time of fertilization.

Even though motility evaluations showed little detrimental effect of increased semen dilution, only one fertility replication exhibited the desired 90.0% level of egg fertilization and survival. The 1:2 semen to extender dilution ratio, used at the Rathbun Hatchery, proved equal to or better than the other semen dilutions. This occurred whether used with the dry method or with eggs diluted with water prior to fertilization. In several comparisons during 1989, the 7.0 mm egg depth showed higher egg survival than the 4.0 mm egg depth. A closer examination indicated tests at the 7.0 mm egg depth were conducted during the first one-half of the spawning season and the 4.0 mm tests during the second one-half. Gamete quality may have influenced results.

Sperm cell motility enhancers appeared promising under the microscope but failed to give the desired rates of egg survival.

Mean egg survival was higher in all multiple semen addition tests conducted during 1991 and 1992. These results indicated a change in technique could produce increased egg survival. Mean egg survival was again higher in all multiple addition semen tests during 1993. A reduction in egg survival between 4-ounce egg lots and 8-ounce egg lots was noted indicating proper sperm cell distribution to the larger egg mass was very important. Similar results were again obtained in 1994 and indicated the problem had not been solved. A major finding occurred in 1995 and 1996 when the 16-ounce egg lots produced egg survival equal to that of the 8-ounce lots used in 1994. This clearly indicated egg survival on a hatchery production level could be improved. The highest egg survival found for 8-ounce egg lots was 83.5% compared to the egg survival rates of 84.4% (1995) and 83.2% (1996) in 16-ounce egg lots. Sixteen ounce egg lots fertilized using standard hatchery methods and a 12.0 mm egg depth, had egg survival rates of 68.6% in 1995 and 71.3% in 1996. Eggs fertilized with a multiple addition of semen had significantly higher survival than control or hatchery method fertilized eggs, thus indicating multiple addition of semen is best. The multiple addition of semen, plus reduced egg depth at fertilization, and reduced mechanical damage, has allowed an increase in egg survival of greater than 13.0% over previously used hatchery methods. After applying the techniques recommended in this publication, hatchery personnel achieved an overall egg eye up of 80.3% and % hatch of 78.1. Percent eye up ranged from 77.2 to 85.4. The reduction in gamete quality from the first one-half of the spawning season compared to the second one-half was detected from 640 egg survival observations and these findings are similar to those of Hurley (1972). The 4.5% decline in egg survival tends to indicate that continuous high egg survival may be impossible in the natural environment. It is obvious from this study that egg trauma, semen distribution, and gamete quality play important and cumulative roles in egg survival.

Confirming the ability to accurately predict final egg survival by evaluating eggs in the early stages of development is in ageement with Heidinger et al. (1997). The ability to predict egg survival gives increased credibility to this study because most results were based on work done with eggs in early development (ie 3 to 5 days). This ability also allows managers to predict egg hatch reasonably early in the spawning season.

The multiple addition of semen, plus reduced egg depth at fertilization, and reduced mechanical damage has allowed an increase egg survival of greater than 13.0% over previously used hatchery methods. After applying the techniques recommended in this publication, hatchery personnel achieved an overall egg eye up of 80.3% and % hatch of 78.1%. Percent eye up ranged from 77.2 to 85.4.

RECOMMENDATIONS

1. Use a 4.0 to 7.0 mm egg depth during the fertilization process, and if possible, limit egg amounts to 16-ounces during the fertilization process. A 16-ounce egg volumn should be marked on containers to insure the proper egg depth.

2. Use 9.0 ml of extended walleye semen/16-ounce egg lot. The actual amount of undiluted semen used in this recommendation is 3.0 ml, which is diluted with 6.0 ml walleye extender.

3. The extended walleye semen should be added to the eggs in equal measure three times during the fertilization process; 3.0 ml initially, at 30 seconds and at 60 seconds. A second option can be used which will require 4.5 ml extended semen added initially and at 30 seconds later.

4. Keep eggs in the fertilization pan 2.0 to 3.0 minutes and stir eggs in the "clay solutions" a maximum of 2.0 - 3.0 minutes to eliminate adhesiveness. Additional stirring exposes eggs to unnecessary mechanical damage.

5. Rinse eggs gently to remove excess clay and allow a minimum of 4 hours for water hardening before jar incubation.

6. Efforts should be made to collect adult walleye during the first portions of the spawning season to insure quality gametes.

7. Siphoning of dead eggs from hatching jars should be done with great care and patience. Hatch can be reduced by as much as 4.0% or more if siphoning is done improperly.

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