EYE-UP SUCCESS OF ARLEE RAINBOW TROUT EGGS AS AFFECTED BY STAGE OF EXPULSION

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At present, factors affecting egg quality in salmonids as well as other fishes are not well understood. Of concern to some fish culturists is whether or not egg quality (viability) remains uniform throughout the ″stripping episode.″ In-other-words, is the viability of the first eggs collected from a female the same as eggs from the middle and last stages of the spawn. This study provides evidence that significant differences ($P<0.01$) in egg viability, measured as eye-up success, can be expected from a particular female at different stages of egg expulsion. Although these findings may not significantly affect overall hatchery reproductive performance, they may impact studies conducted investigating reproductive performance. The methodology of studies evaluating egg viability should include procedures to account for variation in egg quality with respect to stage of expulsion.

Introduction

To date, there is limited data to explain variation in egg quality in rainbow trout. The majority of data available pertains to the effect of over-ripening on egg viability (Bromage et al. 1992). Kato and Kamler (1983) reported that water content of freshly fertilized rainbow trout eggs (non-water hardened) was negatively correlated with embryo survival to hatch. Springate et al. (1984) found maximum egg fertilization success could be attained if eggs were stripped 4-6 days post ovulation. According to Bromage et al. (1992), a slight reduction in fertilization rate results in eggs stripped immediately after ovulation because of under-ripeness.

Eggs that exhibit low mortality at fertilization and subsequent early life stages are generally considered to be high quality eggs. However, there is not a general consensus as to what mortality levels are permissible to qualify as high

quality eggs. In addition, there is no clear understanding of the factors within the parent fish or the egg itself which may affect egg quality (Bromage et al., 1992).

The objective of this study was to determine if eggs obtained from individual females are uniform in viability depending on the order of expulsion. That is, are the first eggs collected of equal viability as the last, as well as those collected in the middle of the spawn? Uniform egg quality from the first to the last few eggs expelled would allow for eggs to be stripped from females directly into treatment replicates in studies where reproductive performance is measured. Variation in egg viability introduced by order of expulsion would not allow for this simple procedure.

Materials and Methods

Six 2 -yr-old female Arlee rainbow trout were individually stripped by injecting compressed oxygen (air spawning) into the body cavity. The eggs were expelled in lines approximately 50 cm in length. Follow-up I1hand passes[l1] were not performed to remove the last few eggs remaining in the body cavity. The spawn from each female was divided into three approximately equal portions by order of expulsion (A; first 1/3; B second 1/3; C last 1/3). Each individual portion from each female was gently stirred then divided into duplicates of equal volume. This process resulted in a total of six lots of eggs from each female, two replicates from each of three stages of the spawn. Saline (100 ml of 0.75%) was added to each lot of eggs (36 lots), followed by two ml of pre-collected milt (pooled from 12 males). Each gamete mixture was gently, but thoroughly stirred and allowed to stand undisturbed for 1 min prior to rinsing in fresh water (54°F). A 50 mg/L iodophor solution (500 ml) was then added to each lot of eggs. Each lot remained in the iodophor solution for 30 min, and was then decanted and rehydrated with fresh water. Each of the 36 egg lots of was randomly assigned to individual upwell incubators (1 L capacity). All incubators received 1/2 gpm water flow. A formalin bath was administered daily (1200 mg/L for 15 min) to prevent fungal infection. Egg lots were mechanically shocked at the early eyed stage of embryonic

development (330 T.U.). On the following day eggs were sorted for viability with the aid of an electronic picker (Jensorter Model JX4, Jensorter Inc., 20225 Harvest Ln., Bend, OR 97701). Blank eggs (translucent without visual eye development) were manually removed from each lot and counted. Viable (eyed) eggs were counted with the aid of an electronic counter (Jensorter Model BC). Resulting data were analyzed using the Statistical Analysis System (SAS, 1986) All percentage data were arc-sine transformed prior to analysis.

Results

Egg viability measured as survival rate at the eyed stage of embryonic development was significantly affected ($P<0.000l$) by the stage of expulsion. Mean percent eye-up decreased with each successive stage of egg expulsion (Table 1). Frequency of blanks was not significantly affected by stage of expulsion. An interaction ($P<0.000l$) between stages of expulsion and individual females spawned was also observed (Table 2).

Discussion

In some cases the action of "hand" stripping can have a negative impact on egg viability. This is most likely to occur in eggs that are the last to be expelled, when several "passes" have been administered and broken or ruptured eggs are the result. Not only are these eggs lost, but egg yolk material (vitellus) from the broken eggs can hinder the fertilization of other eggs (Wilcox et alo, 1983). In this study, the last few eggs remaining in the body cavity were not removed by "hand stripping" and could not have impacted the data observed.

Conversely, reduced eye-up success in the last stages of expulsion in this investigation might have been due to "under-ripeness" (Bromage et al., 1992; Springate et al., 1984)

Eye-up success was unaffected by stage of expulsion in eggs collected from three of the females spawned in this study (Table 2). It is conceivable that these three females ovulated a few days before the others allowing for more

complete ripening. Another possibility might be that as a result of a greater time interval from ovulation, these three females produced more ovarian fluid than the others which facilitated more "egg mixing" action within the body cavity.

This study offers preliminary evidence that significant variation in egg quality can result in different segments of the spawn from a particular female. When measuring reproductive performance in salmonids, stripping eggs directly into randomly assigned replicates from a female could possibly bias test results. The authors suggest that when eggs are to be used for testing purposes, an individual female be fully stripped; the eggs gently stirred to homogenize the lot, and then randomly allocated to individual treatment replicates.

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Table 1. Mean percent eye-up of arlee strain rainbow trout eggs as affected by stage of expulsion.

Stage of expulsion	N	Mean Eye-up%**	SD
A (First 1/3 of spawn)	12	71.48[a]	31.05
B (Second 1/3 of spawn)	12	67.80[b]	32.03
C (Third 1/3 of spawn)	12	64.24[c]	29.76

*Effect of Stage: P<0.001; CV=6.42; R-Square=0.99

**Differing superscript denotes significant difference.

Table 2. Comparison of percent eye-up in eggs collected from arlee strain rainbow trout: Interaction between individual females and different stages of expulsion.

Female	Stage of expulsion	N	Eve-up%	SD
1	A	2	98.4	0.0
1	B	2	92.9	2.2
1	C	2	75.6	4.2
2	A	2	85.0	2.6
2	B	2	76.9	0.6
2	C	2	73.9	0.4
3	A	2	88.9	3.3
3	B	2	91. 8	2.5
3	C	2	88.0	0.2
4	A	2	36.5	10.2
4	B	2	28.5	7.3
4	C	2	29.9	9.5
5	A	2	95.7	0.4
5	B	2	94.6	1.1
5	C	2	96.5	1.4
6	A	2	24.4	0.9
6	B	2	22.4	0.4
6	C	2	21. 7	0.6

Effect of treatment: P>0.0001; CV=6.42; R-square=0.99