

DEVELOPMENTS IN FISH CULTURE

COMPARE FERTILIZATION SUCCESS WHEN MIXING EGGS AND MILT BY HAND  
STIRRING VERSUS POURING FROM ONE PAN TO ANOTHER

INVESTIGATORS

JOHN B. SHRABLE  
DANIEL J. ABEYTA  
JIM McFALL  
DAVID G. NOBLE

U.S. FISH & WILDLIFE SERVICE  
ENNIS NATIONAL FISH HATCHERY  
180 FISH HATCHERY RD.  
ENNIS, MT 59729

SEPTEMBER 9, 1998

## INTRODUCTION

A 0.75% saline solution is routinely used as a fertilization medium for rainbow trout (*Oncorhynchus mykiss*) eggs at the Ennis National Fish Hatchery. Brown, et al (1994), increased sperm motility time 23.5% from 23.0 seconds in fresh spring water at 54° F to 28.4 seconds in a 0.75% saline solution of fresh spring water. This allows more time for fertilization to occur. The saline solution also prevents precipitation and coagulation of yolk protein from broken eggs which reduces fertilization success by blocking the micropyle and inhibiting sperm motility and penetration. Because of the short duration of sperm motility it is imperative that the eggs and milt be mixed thoroughly and as quickly as possible after the milt is introduced into the fertilization medium. E. van Heerden et al., 1993 found that the average duration of sperm motility (when 50% of the spermatozoa cease forward motion) was 19.20 seconds in fresh stream water at 50, F and 34.0 seconds in a buffered Borax-boric acid diluent. The egg-diluent ratio is also important to fertilization success. Billard (1975b) recommended a 3 to 1 egg-diluent ratio, in other words, 3 liters of eggs to one liter of diluent, providing a concentration of 10-20 eggs/ml of diluent.

## OBJECTIVE

The objective of this experiment was to determine if fertilization rate could be changed by changing the method of mixing eggs and milt, in this case by pouring the mixture from one pan to another versus mixing eggs and milt by hand.

## MATERIALS AND METHODS

Ten ERD-96 females were spawned into a pan with 1 liter (L) of 0.75 % saline solution and mixed thoroughly. These eggs were then split equally into two pans with 1 L. of saline in each pan. Twenty milliliters (ml) of precollected and pooled milt from 20 ERD-96 males was added to each group. Milt was mixed with the eggs in one pan by thorough hand stirring. The other pan of eggs and milt was mixed by pouring back and forth 3 times from one pan to another. Both groups of eggs were rinsed within two minutes of fertilization and then water hardened for 30 minutes in a 50 parts per million (PPM) iodine solution. After water hardening, each pan of eggs was split into 3 replicates of test jars for incubation.

The eggs were shocked on day 15 and picked on day 16. The blanks were sample counted in 3 - 100 egg samples from each replicate of good eggs after being electronically picked. Both good and bad eggs in each replicate were then counted with an electronic egg counter.

A one-way analysis of variance (ANOVA) test using the computer program Statistix 4.1 was used to compare the means for significant statistical differences between the two groups.

## RESULTS

The percentages of eyed eggs for all three replicates of both groups were very high ranging from 94.63 % to 97.49% (Table 1). The one-way ANOVA and Least Significant Difference (T) comparison of means indicate no significant difference between the groups of means at the .01 level (Tables 2 & 3).

Table 1. Comparison of percent fertilization of rainbow trout eggs when eggs and milt are mixed by hand stirring versus pouring the eggs and milt mixture from one pan to another.

METHOD	TOTAL EGGS	TOTAL EYED	NET
			PERCENT EYED
HAND MIX	5,922	5,721	96.60
HAND MIX	6,278	6,113	97.37
HAND MIX	7,616	7,425	97.49
TOTALS/AVE.	19,816	19,259	97.15*
POURING	6,637	6,281	94.63
POURING	6,671	6,424	96.30
POURING	7,563	7,316	96.73
TOTALS/AVE.	20,871	20,021	95.89*

(\*) common superscripts denote no significant difference at the 0.01 level.

Table 2. One-way ANOVA for mixing eggs and milt by hand stirring versus pouring.

SOURCE	DF	SS	MS	F	P
Between	1	2.40667	2.40667	3.29	0.1440
Within	4	2.92773	0.73193		
Total	5	5.33440			

Table 3. Least Significant Difference (T) pairwise comparison of mean percent eyed eggs fertilized by mixing eggs and milt by hand versus pouring from one pan to another.

HOMOGENOUS

VARIABLE	MEAN	GROUPS
HAND	97.153	I
POUR	95.887	I

There are no significant pairwise differences among the means at the 0.01 level.

CONCLUSION

From this investigation we have concluded that mixing milt and eggs by hand can yield fertilization percentages comparable to those achieved by mixing milt and eggs by pouring. However, we recommend mixing milt and eggs by pouring to eliminate the risk of reduced

fertilization caused by individual variability in how quickly and how thoroughly the mixing is performed.

#### LITERATURE CITED

Billard, R., 1975b. Artificial Insemination in Salmonids, Bull. Fr. Piscic. 527:121-135

Brown, Daniel R., John B. Shrable and Wesley H. Orr, 1994. Unpublished. The use of various fertilization media and their effects on rainbow trout gametes. Ennis National Fish Hatchery, Ennis, Montana.

Heerden, Eurika van, Johan H.J. Van Vuren and Gert J. Steyn, 1993. Development and evaluation of sperm diluents for the artificial insemination of rainbow trout (*Oncorhynchus mykiss*). Aquatic Living Resour. vol.6, 57-62.

Statistix, version 4.1

