

TITLE

Viability of stored rainbow trout (*Onchorynchus mykiss*) milt and unfertilized green eggs held in ovarian fluid for various lengths of time.

INTRODUCTION

Frequently at the Ennis National Fish Hatchery fresh milt and unfertilized green rainbow trout eggs in ovarian fluid are shipped to research laboratories. Eggs and milt are typically shipped overnight delivery via Federal Express. On occasion, oversights in shipping occur and eggs and milt are delivered at various times later than expected. It would benefit both hatchery personnel and researchers to know the time frame of egg and milt viability. Using a domestic Arlee rainbow trout strain, the intent of this experiment is to fertilize green eggs at seven different time periods using both fresh and precollected/pooled milt.

OBJECTIVES

- 1) Determine the viability of green eggs stored in ovarian fluid for various lengths of time prior to fertilization.
- 2) Determine the viability of stored milt versus fresh milt.

MATERIALS AND METHODS

The experiment was initiated November 17, 1998 and continued through November 20. The four day study consisted of seven different fertilization time periods as follows:

Fertilization	Fertilization	Number of	Eggs/
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Date	Day	Time	Interval	Sub-samples	Sub-sample
11/17	Tuesday	0800	Initial	3	1000
11/18	Wednesday	0800	24 hrs.	6	1000
11/18	Wednesday	1600	32	6	1000
11/19	Thursday	0800	48	6	1000
11/19	Thursday	1600	56	6	1000
11/20	Friday	0800	72	6	1000
11/20	Friday	1600	80	6	1000

Eggs and ovarian fluid from eight two-year old Arlee females were air spawned into a plastic pan and mixed thoroughly. Each female produced approximately 5000 eggs resulting in a total of 40,000 eggs. Eggs and ovarian fluid were stored in the plastic pan and placed in an oxygen filled plastic bag to prevent dessication and held in a refrigerator at 2 degrees celsius for the various fertilization time periods. Using aspirators, 30 milliliters (ml) of milt was collected and pooled from 20 randomly selected two-year old Arlee males and served as the stored milt. The stored milt was held in an oxygen filled ziplock storage bag and refrigerated at 2 degrees celsius. The fresh milt was aspirated from the same 20 males at each fertilization period. Aspiration was used in order to prevent contamination of both the stored and fresh milt. To ensure all 20 males were equally represented, 1 ml of fresh milt was aspirated into a test tube from each male. This milt was then pooled and mixed thoroughly to fertilize at the desired sperm-egg concentration.

At each fertilization period, 3000 eggs were fertilized in 300 ml of 0.75% saline solution using fresh milt, and 3000 eggs were fertilized in the same manner using stored milt. Both the fresh and stored milt were applied at a rate of 3ml/3000 eggs. Each group of eggs was rinsed

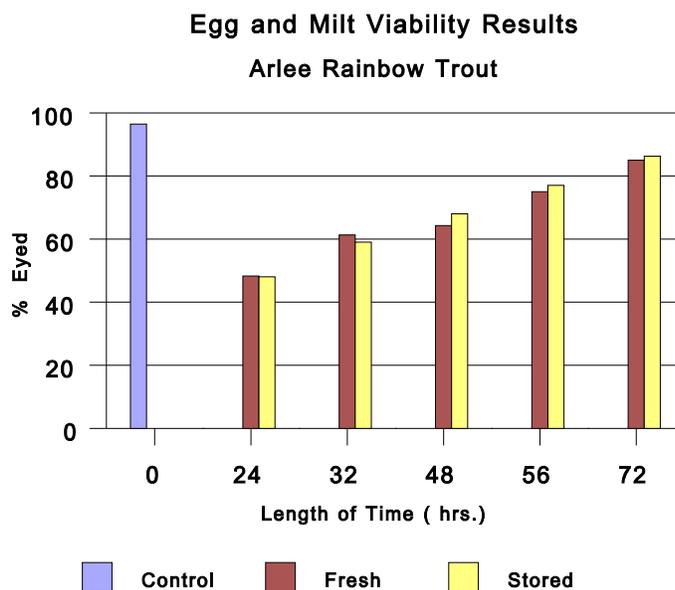
three times within two minutes after fertilization. Each group was then water hardened in a 50 parts per million (ppm) povidone iodine solution for 30 minutes.

After water hardening, each group of 3000 eggs was split evenly into 3 replicate sub-samples for incubation. All replicates of 1000 fertilized eggs were incubated for 14 days at 54 degrees Fahrenheit and shocked on day 15. On day 16, eggs were sorted with an electronic egg picker and both viable and dead eggs were counted with an electronic counter. Blanks were sample counted in 3 - 100 egg samples from each replicate to determine percent eyed.

The initial fertilization using fresh milt was conducted at 0800 hours on November 17 and served as the control group for the experiment. The second fertilization period was conducted 24 hours later. Subsequent fertilizations were then conducted at 8 and 16 hour intervals respectively.

One-Way Analysis Of Variance (ANOVA) and Least Significant Difference (LSD) statistical tests using the computer program Statistix (Version 2 for Windows) were used to analyze the data. These tests were used to determine significant statistical differences in mean

percent eyed eggs for each group at each fertilization period..



RESULTS

