DEVELOPMENTS IN FISH CULTURE

EFFECT OF VARIED MILT VOLUME ON THE FERTILIZATION SUCCESS OF KAMLOOP RAINBOW TROUT EGGS

by
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Abstract

The effect of varied milt volume on fertilization success of Kamloop rainbow trout eggs. Opinion varies among fish culturists and researchers alike regarding ratios of milt, diluent and eggs necessary to obtain optimal fertilization success in rainbow trout (Oncorhynchus mykiss) and other salmonids. Concern over what is “minimal” and “excessive” in terms of milt volume has prompted this study.

Fertilization success in Kamloop strain rainbow trout at milt application rates of 1,5, and 10 times that normally employed at the hatchery were compared. In another test using the Erwin strain of rainbow trout, fertilization success was compared at “reduced” sperm concentrations of ½ and $\frac{1}{20}$ of amounts normally used. Five different diluents were tested at each sperm concentration. There was no significant effect of milt concentrations tested on fertilization rate measured at the eyed stage of embryonic development. Among the five diluents tested at two different milt concentrations, only water was significantly different. These findings indicate that in normal spawning situations, concern about an excess or deficiency of milt application is probably unwarranted.

Introduction

Spawning in wild salmonids is characterized by high concentrations of spermatozoa relative to the number of eggs on a redd. A spermatozoon has the ability to travel a distance of about 3 mm by flagellar motion. This is a shorter distance than the diameter of a single egg (4-6mm). Thus, eggs must be surrounded by spermatozoa in order to ensure the greatest chances for fertilization success (Billard 1992). Scott and Baynes (1980) in review of artificial insemination methods, reported that early studies indicated that 1 ml of semen per 1500-2000 eggs (approximately 10,000,000 sperm per egg) would yield maximum fertilization in salmonids. Billard et al. (1974) found that 70-80% fertilization could be achieved by adding 0.01 ml of semen to 10 ml of 20 mM carbonate-bicarbonate buffered (pH 9) saline and then added to batches of 800 eggs (approx 200,000 =1/50 of 10,000,000 sperm/egg). Erdahl and Graham (1987) reported that rainbow trout semen diluted at 1:8 with a seminal fluid mimicking extender gave results equal to control values of fertility obtained without semen dilution when semen was diluted 1:1 (extender:milt) fertility was higher than control values. Billard (1974, 1985) defined the minimum amount of sperm necessary to obtain optimum fertilization success with rainbow trout as 10-3 (1 ml semen:1 L diluent: 3 L of eggs). Which averages about 300,000 spermatozoa/egg. Billard (1974) also reported significantly lower fertilization when sperm in excess ($<^{10\text{-}2}$dilution) was mixed with eggs when compared to dilution rates of 10^{-2} and 10^{-3}. In a more recent investigation, Ciereszko and Dabrowski (1994) studied relationships between many aspects of rainbow trout semen and fertility. In one case, they varied milt volume using two concentrations; 0.1 ml and 0.02 ml on separate batches of (531 +/-23) eggs from the same female per 3 ml of 0.7% NaCl. No significant differences in fertilization success between the two milt volumes tested were

reported.

The objective of our study was to evaluate fertilization success of Kamloop strain rainbow trout eggs when applying milt at volumes 5 and10 times the concentration (1 ml semen: 50 ml diluent: 100 ml eggs) normally utilized in standard spawning methods at the Ennis National Fish Hatchery.

Materials and Methods

In February, 1994, during the peak of the spawning period milt was stripped from eighteen 3-yr old Kamloop rainbow trout males, pooled and transferred to 500 ml plastic containers and stored in an ice chest. Milt depth in each container did not exceed 1/8 inch. Approximately 80 ml of eggs were stripped from each of ten 3 yr old Kamloop females and pooled. The pooled lot was divided randomly into six replicates of approx 1000 eggs each. Two replicates each were subjected to either a 1 ml:50 ml:100 ml, 5 ml:50 ml:100 ml or a 10 ml:50 ml:100ml treatment (Milt volume: Diluent volume: Egg volume).

Each replicate of gametes, diluent, and eggs was gently mixed after combination, and allowed to stand 2-3 min then rinsed in fresh water. All replicates were then hardened in 50 mg/L iodophor solution for 30 min then transferred to individual upwelling incubators. On the fifteenth day following fertilization (330 TU) all eggs were exposed to mechanical shock and returned to individual incubation. On the following day all eggs in each replicate were picked (sorted) with the aid of an electronic picker (Jensorter Model JX-24, Jensorter Inc., 20225 Harvest Ln., Bend, OR, 97701) and counted with the aid of an electronic counter (Jensorter Model BC). Blank eggs (translucent without eye development) were separated, counted and removed manually.

Resulting data was recorded and later analyzed using SAS (1993) system of statistical analysis.

Results and Discussion

Milt volume did not significantly affect fertilization rate at dilution rates tested (Table 1). Treatment 18 (1 ml mi It: 50 ml diluent: 100 ml eggs) was considered to be the normal dilution utilized in spawning practices at the Ennis National Fish Hatchery. Increased milt application at 5 (58) and 10 (10S) times the normal (1S) quantity did not significantly alter fertilization rate ($P>0.05$). These findings resolved our concerns that increased milt volume might be deleterious to fertilization success.

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Treatment	Milt:Diluent:Eggs (1 ml sperm/1 of diluent/s1 eggs)	Eye-up%*	SD
1S	1ml:50ml:100ml=20 ml sperm/1 of diluent	81.3[a]	.6
5S	5ml:50ml:100ml sperm/1 of diluent	79.6[a]	3.0

10S	10ml:50ml:100ml=200 ml of sperm/1 of diluent	80.5[a]	.9

Table 1. Affect of Varied Milt Volume on the Fertilization Success of Kamloop Strain of Rainbow Trout Eggs.

*Like superscript denote no significant difference (P>0.05).

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