

FRED Reports

Viability of Cryopreserved
Chilkat River Chinook Salmon Milt
From Two Drainages
(Tahini River and Big Boulder Creek)

by
Scott Kelley

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TABLE OF CONTENTS

<u>Section</u>	<u>Page</u>
ABSTRACT.....	1
INTRODUCTION.....	1
METHODS.....	2
Spawning and Fertilization.....	2
Incubation.....	6
Analysis.....	7
RESULTS AND DISCUSSION.....	7
Results.....	7
Discussion.....	10
ACKNOWLEDGMENTS.....	11
REFERENCES.....	12

LIST OF TABLES

<u>Table</u>	<u>Page</u>
1. Remaining straws in liquid nitrogen and straws used for milt experiment for each chinook male.....	4
2. Results of viability test of cryopreserved Chilkat River chinook salmon milt.....	8

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
1. Variation among males and between stocks for survival to eye-up of pink salmon eggs fertilized with frozen Chilkat River chinook salmon milt.....	9

ABSTRACT

The viability of chinook salmon, *Oncorhynchus tshawytscha*, milt collected and cryopreserved from two Chilkat River tributaries during 1991 was tested. Eggs from 10 pink salmon females were pooled and fertilized with frozen or fresh chinook salmon milt and fresh pink salmon milt. Average survival to hatching for chinook x pink salmon controls was 53.8% and for pink x pink controls 42.9%. Average survival to hatching, expressed as a percent of chinook x pink controls, for the cryopreserved milt from Tahini River was 41.3% and from Big Boulder Creek was 65.5%.

KEY WORDS: Chinook salmon, *Oncorhynchus tshawytscha*, Chilkat River, cryopreservation, fertilization, percent survival.

INTRODUCTION

In response to a serious decline in Chilkat River chinook salmon, *Oncorhynchus tshawytscha*, populations the Alaska Department of Fish and Game (ADF&G) has taken several steps to protect and rehabilitate this stock (Josephson et al unpublished draft Chilkat River Chinook Salmon Plan). Among these steps are time, area, and gear restrictions on commercial fisheries; increasingly restrictive sport fishing regulations; artificial enhancement through hatchery culture and fry plants; and tissue sampling to establish a genetic baseline of the population using starch-gel electrophoresis. In addition, milt collected from wild chinook salmon males in 1991 was cryopreserved in large plastic straws (Wheeler and Thorgaard 1991).

The preserved sperm will constitute a "gene bank" that will preserve genetic variability inherent in the population.

This study was designed to test the effectiveness of the cryopreservation procedures used to freeze milt from chinook salmon from two Chilkat River tributaries, the Tahini River and Big Boulder Creek.

METHODS

Spawning and Fertilization

The experiment [was done at the Douglas Island Pink and Chum Salmon Incorporated (DIPAC) Gastineau Hatchery in Juneau, Alaska. Hatchery returns of pink salmon were selected as an egg source because of the lack of female chinook salmon.

To test for the effects of cryopreservation, and to control for possible hybrid effects, fresh chinook salmon milt was obtained from Gastineau Hatchery returns. Four chinook salmon males (rack returns) were removed from a pen in a raceway, dried thoroughly, and spawned into small Whirl-Pak™ bags. To control for possible egg quality effects, three pink salmon males (rack returns) were similarly spawned. The Whirl-Paks of fresh semen were then placed in a refrigerator at 4 degrees Celcius for short-term storage.

The eggs from 10 pink salmon females (rack returns) were stripped into a plastic colander to allow ovarian fluid drainage. The eggs were then carefully transferred into a completely dry 5-gal plastic

bucket and thoroughly mixed. A 100-egg sample was counted and placed in a 6-ounce Dixie Cup™. The cup was then cut off just above the egg level to form a measuring scoop that would hold approximately 100 eggs. One "scoop" of approximately 100 eggs was then placed into each of 80 full-size cups.

Randomly selected straws of cryopreserved milt from each of the chinook males were removed from the liquid nitrogen, one male's milt at a time. The milt from 10 Tahini River chinook salmon and 13 Big Boulder Creek chinook salmon was frozen in 1991. Immediately upon exposure of the frozen milt to ambient outside air temperatures there were popping noises. The noise was presumably the result of an undetermined number of the plastic straws splitting and cracking open because of the extreme temperature increase. The objective of this experiment was to thaw one straw from each male for use. In some cases, however, straws split open during the thawing process and were discarded. The number of straws remaining for each male and used for this experiment is summarized in Table 1. No effort was made to determine the number of straws that had split open in the canisters because we felt that the canisters should be replaced in the liquid nitrogen as quickly as possible to prevent thawing.

Table 1. Remaining straws in liquid nitrogen and straws of milt used in this study.

Male number ^a	Straws remaining ^b	Straws used
T1	8	1
T2	5	1
T3	4.5	1
T4	12	1
T5	9	1
T6	17	2
T7	7	1
T8	26	1
T9	12.5	2
T10	15.5	1
B1	14	1
B2	13	1
B3	5	2
B4	13.5	3
B5	13.5	1
B6	7.5	1
B7	5	2
B8	10	1
B9	15	1
B10	12	1
B11	27	1
B12	16	1
B13	6.5	1

^a T = Tahini River, B = Big Boulder Creek

^b Milt in each straw can fertilize approximately 500-800 chinook salmon eggs.

Unsplit straws for this study were put into a 5° C water bath to thaw. The water bath was kept at 5° C by adding ice cubes. A thermometer was kept in the bath for accurate monitoring of the temperature. After approximately 90 seconds the straws were cut on one end, inverted (the contents were prevented from pouring out by holding a finger over the open end), and the other end was cut. The straws were then emptied, one third of the contents into each of three replicate cups of eggs. Activator (recipe below) was added immediately thereafter, enough to just cover the eggs. After 5 minutes the eggs were rinsed and allowed to water harden for approximately half an hour.

Activator recipe:

-0.9% Sodium Chloride

-0.01M Tris Base

-0.02M Glycine

-Balance (to 1000 milliliters) distilled water

The fresh milt was taken from the refrigerator and examined for motility. Sperm from two of the four chinook males were motile as were sperm from two of the three pink salmon males. Sperm from one of the two pink salmon males were only slightly motile but the milt was used anyway. Milt which was non-motile was discarded. The milt from control chinook number 1 was divided into three cups of eggs, water was added, and the eggs were water hardened for approximately half an hour. Fresh chinook male number 2 was used

to fertilize the eggs in only two cups because of limitations in incubator space. Milt from pink salmon males 1 and 2 was used to fertilized three egg containers each. The total number of separate egg containers was 80 distributed equally in four incubator trays.

After water hardening each of the egg cups was emptied into a separate, randomly assigned, egg container that had been placed into a tray from a vertical-stack incubator. The egg containers were fabricated from plastic material normally used as downspouts to collect rain. Fiberglass screening was secured to one end with silicone adhesive. Twenty egg containers were placed in each tray. As the egg containers did not fit tightly batten material was placed between the egg containers and the wall of each tray to hold them in place. After water hardening for an additional half hour in a water bath the baskets were placed in a 100ppm Argentyne solution for 10 minutes for disinfection. After 10 minutes the baskets were transferred to the incubator cabinet. Flow rates were 8 liters per minute and hatchery water temperature at this time was 8.6° C.

Incubation

After the eggs eyed they were shocked and blank and dead eggs were removed and counted. At approximately 21 days post hatching any additional dead eggs or alevins were removed and counted. Survival to 21 days post hatching was determined (Table 2).

Analysis

Possible between-stock and male differences were tested by using the SAS General Linear Models procedure on the University of Alaska mainframe computer with the model:

$$P_{ijk} = \mu + S_i + M_{ji} + e_{ijk}$$

where P_{ijk} = percent survival to eye-up, S_i = effect of the i th stock and M_{ji} = effect of the j th male within the i th stock.

RESULTS AND DISCUSSION

Results

The average survival from fertilization to hatching (expressed as a percent of the survival of chinook x pink controls) for frozen milt was 43.1% for Tahini River and 65.5% for Big Boulder Creek. The results of the experiment are summarized in Table 2. Eggs fertilized with sperm from the pink salmon male, which were known to be poorly motile, had an average survival of only 15.6%. The average survival of pink salmon eggs fertilized with fresh chinook sperm was 53.8% with relatively low variability among replicates. The variability of the quality of sperm from different males within a stock is demonstrated in Figure 1.

There was no significant effect ($F_{3,23}=2.29$, $P < 0.1050$) of stock on survival to hatching between the frozen milt from Tahini River and Big Boulder Creek, and fresh milt from chinook salmon controls and pink salmon controls. There was a significant effect of males within a stock ($F_{23,53}=6.43$, $P < 0.0001$) on percent survival to eye-up.

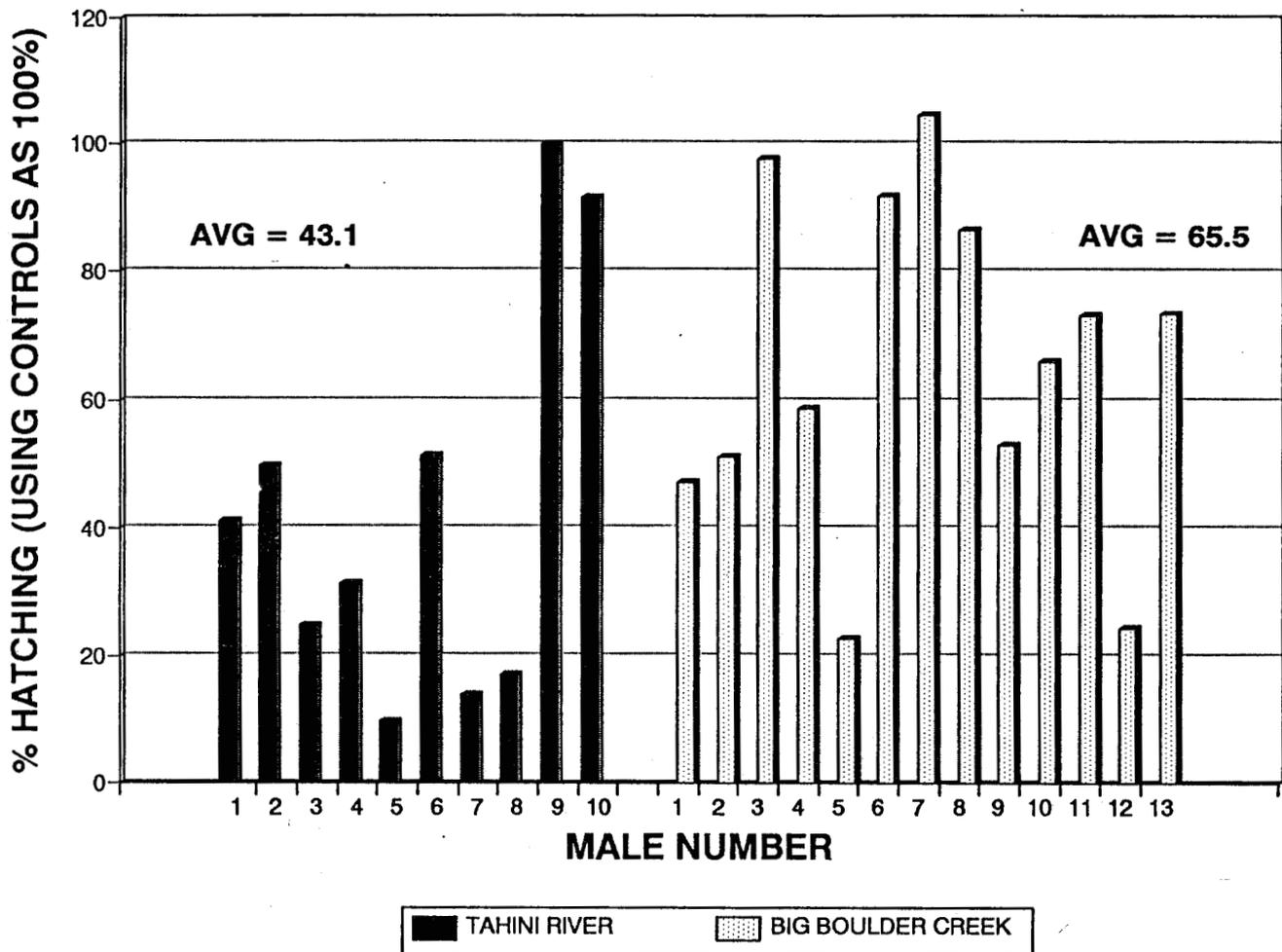
Table 2. Results of viability test of cryopreserved Chilkat River chinook salmon milt.

Male number ^a	Replicate % survival			Average % survival	Average survival (as a % of chinook x pink controls)
	1	2	3		
T1	48.7	17.0	2.4	22.7	41.3
T2	37.2	2.7	41.5	27.1	49.3
T3	1.8	35.3	3.2	13.4	24.4
T4	7.8	3.8	40.3	17.3	31.5
T5	0.8	12.9	2.0	5.2	9.5
T6	35.1	20.5	29.1	28.2	51.3
T7	12.9	4.9	5.1	7.6	13.8
T8	11.5	13.0	3.5	9.3	17.0
T9	63.9	44.7	57.6	55.4	100.7
T10	48.1	51.1	53.8	51.0	92.7
Average for all Tahini:				23.7	43.1
B1	30.8	37.9	11.7	26.8	48.7
B2	39.4	32.5	12.5	28.1	51.2
B3	66.4	33.6	61.4	53.8	97.8
B4	39.3	29.0	29.5	32.6	59.3
B5	3.1	19.6	15.0	12.6	22.8
B6	50.5	56.5	43.0	50.0	90.9
B7	51.8	66.7	54.4	57.6	104.8
B8	39.2	45.3	58.5	47.7	86.7
B9	28.7	23.1	34.7	28.9	52.5
B10	42.3	29.6	35.9	35.9	65.3
B11	51.5	29.7	40.3	40.5	73.7
B12	19.4	2.8	17.3	13.2	24.0
B13	38.2	27.4	55.8	40.4	73.5
Average for Big Boulder:				36.0	65.5
P1	73.0	70.8	67.1	70.3	
P2 ^b	5.7	26.3	14.7	15.6	
Average for pink controls:				42.9	
C1	56.1	52.3	57.7	55.3	
C2	56.2	48.5	-	52.4	
Average for chinook controls:				53.8	

^a T = Tahini River, B = Big Boulder Creek, P = Pink controls, C = Chinook controls.

^b Pink male which had poor motility.

Figure 1. Variation among males and between stocks for survival to hatching of pink salmon eggs fertilized with frozen Chilkat River chinook salmon milt.



Discussion

Based on the results of this experiment it appears that the initial attempt to cryopreserve chinook salmon milt under field conditions was moderately successful. The results were less than ideal however. The variability in survival among replicates for eggs fertilized by thawed sperm from individual males was very large. There were males whose sperm resulted in extremely poor overall egg survival to hatching. The mechanisms that led to these results are unclear.

The milt from the first six Tahini River males was kept on ice longer than milt from all the other frozen males. This was because the gametes from the first Tahini River milt and egg collection were transported to Juneau via state ferry from Haines, Alaska. All other milt was transported from Haines to Juneau via aircraft. All of the milt was not collected by the same individual. It is possible that the different methods of milt collection could have led to the variability in survival of eggs fertilized by milt from different males. Within-males variability could also have resulted from poor mixing of the milt and extender solution during fertilization. This could be resolved by mixing the contents of a straw prior to fertilization.

Milt from Big Boulder males one through six was collected directly into Whirl-Pak bags. All other milt was collected by inserting a large plastic pipet into the vent of the fish. There were no

obvious differences in survival between milt collected using either method. The pipet method and the direct method each had its advantages but field personnel greatly preferred the direct method.

The phenomenon of popping straws was very unsettling. If the cryopreserved milt is used for future fertilization, a technique must be developed to prevent any split straws from having contact with the water in the thawing bath. Possibly, a higher quality straw could be found that would reduce splitting.

Scheerer and Thorgaard (1989) suggested using theophylline in the activator solution. The absence of theophylline in our activator solution may also have resulted in less-than-desired survival.

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