Fishery Data Series No. 06-19

Triploidy Induction in Arctic Char *Salvelinus alpinus* using Heat Shocking and Pressure Shocking Techniques

by

Diane P. Loopstra

and

Patricia A. Hansen

April 2006

Alaska Department of Fish and Game **Divisions of Sport Fish and Commercial Fisheries**

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Weights and measures (metric)

centimeter	cm
deciliter	dL
gram	g
hectare	ha
kilogram	kg
kilometer	km
liter	L
meter	m
milliliter	mL
millimeter	mm

Weights and measures (English)

cubic feet per second	ft^3/s
foot	ft
gallon	gal
inch	in
mile	mi
nautical mile	nmi
ounce	oz
pound	lb
quart	qt
yard	yd

Time and temperature

day	d
degrees Celsius	°C
degrees Fahrenheit	°F
degrees kelvin	K
hour	h
minute	min
second	s

Physics and chemistry

all atomic symbols	
alternating current	AC
ampere	A
calorie	cal
direct current	DC
hertz	Hz
horsepower	hp
hydrogen ion activity (negative log of)	pH
parts per million	ppm
parts per thousand	ppt, ‰
volts	V
watts	W

General

Alaska Administrative Code	AAC
all commonly accepted abbreviations	e.g., Mr., Mrs., AM, PM, etc.
all commonly accepted professional titles	e.g., Dr., Ph.D., R.N., etc.
at	@
compass directions:	
east	E
north	N
south	S
west	W
copyright	©
corporate suffixes:	
Company	Co.
Corporation	Corp.
Incorporated	Inc.
Limited	Ltd.
District of Columbia	D.C.
et alii (and others)	et al.
et cetera (and so forth)	etc.
exempli gratia (for example)	e.g.
Federal Information Code	FIC
id est (that is)	i.e.
latitude or longitude	lat. or long.
monetary symbols (U.S.)	$, ¢
months (tables and figures): first three letters	Jan,...,Dec
registered trademark	®
trademark	™
United States (adjective)	U.S.
United States of America (noun)	USA
U.S.C.	United States Code
U.S. state	use two-letter abbreviations (e.g., AK, WA)

Measures (fisheries)

fork length	FL
mideye-to-fork	MEF
mideye-to-tail-fork	METF
standard length	SL
total length	TL

Mathematics, statistics

all standard mathematical signs, symbols and abbreviations	
alternate hypothesis	H_A
base of natural logarithm	*e*
catch per unit effort	CPUE
coefficient of variation	CV
common test statistics	(F, t, χ^2, etc.)
confidence interval	CI
correlation coefficient (multiple)	R
correlation coefficient (simple)	r
covariance	cov
degree (angular)	°
degrees of freedom	df
expected value	*E*
greater than	>
greater than or equal to	≥
harvest per unit effort	HPUE
less than	<
less than or equal to	≤
logarithm (natural)	ln
logarithm (base 10)	log
logarithm (specify base)	$log_{2,}$ etc.
minute (angular)	'
not significant	NS
null hypothesis	H_O
percent	%
probability	P
probability of a type I error (rejection of the null hypothesis when true)	α
probability of a type II error (acceptance of the null hypothesis when false)	β
second (angular)	"
standard deviation	SD
standard error	SE
variance	
population	Var
sample	var

FISHERY DATA REPORT NO. 06-19

TRIPLOIDY INDUCTION IN ARCTIC CHAR *SALVELINUS ALPINUS* USING HEAT SHOCKING AND PRESSURE SHOCKING TECHNIQUES

by
Diane P. Loopstra
Alaska Department of Fish and Game, Division of Sport Fish, Anchorage
and
Patricia A. Hansen
Alaska Department of Fish and Game, Division of Sport Fish, Research and Technical Services, Anchorage

Alaska Department of Fish and Game
Division of Sport Fish, Research and Technical Services
333 Raspberry Road, Anchorage, Alaska, 99518-1565

April 2006

Development and publication of this manuscript were partially financed by the Federal Aid in Sport Fish Restoration Act (16 U.S.C.777-777K) under Project F-32-11, Job No. F-2-5.

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Diane P. Loopstra
Alaska Department of Fish and Game, Division of Sport Fish, Region II
333 Raspberry Road, Anchorage, AK, 99518-1565
and
Patricia A. Hansen
Alaska Department of Fish and Game, Division of Sport Fish, Research and Technical Services
333 Raspberry Road, Anchorage, AK, 99518-1565

This document should be cited as:
Loopstra, D. P., and P. A. Hansen. 2006. Triploidy induction in Arctic char Salvelinus alpinus using heat shocking and pressure shocking techniques. Alaska Department of Fish and Game, Fishery Data Series No. 06-19, Anchorage.

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TABLE OF CONTENTS

Page

LIST OF TABLES....ii

ABSTRACT....1

INTRODUCTION....1

Objectives....2

METHODS....2

Heat Shocking....2
- Survival....3
- Ploidy....3

Pressure Shocking....4
- Survival....5
- Ploidy....5

RESULTS....5

Heat shocking....5
- Survival....5
- Ploidy....5

Pressure Shocking....5
- Survival....5
- Ploidy....7

DISCUSSION....7

ACKNOWLEDGMENTS....9

REFERENCES CITED....9

LIST OF TABLES

Table **Page**

1. The number of observed diploids per sample size resulting in a triploidization rate significantly less than 80%.....4
2. Survival and triploidization rates of each heat-shocked treatment group.....6
3. Average survival and triploidization rates for heat-shocked treatments.....6
4. Survival and triploidization rates of each pressure-shocked treatment group.....7
5. Average survival and triploidization rates for pressure-shocked treatments.....7

ABSTRACT

Heat shocking and pressure shocking techniques were applied to fertilized Arctic char *Salvelinus alpinus* eggs to induce triploidy. Eggs collected from 3-year-old Arctic char were heat shocked beginning 15 minutes post-fertilization in a 26°C or 28°C heat-bath for 10, 12, or 14 minutes. The number of surviving embryos in each treatment group was less than the minimum required sample number for ploidy testing. The eggs treated at 26°C for 14 minutes had the best average survival rate to the eyed egg stage relative to the control (31.9%) and a triploidy rate of 85.2%. The eggs treated at 28°C for 14 minutes had the highest triploidy rate at 100%, but a survival rate relative to the control of 3.2%. Eggs collected from 4-year-old Arctic char were pressure shocked beginning at 200, 250, 300, or 350 CTMs post fertilization, at 9,500 psi for a 5-minute duration. Average survival rate to the eyed egg stage of development ranged from 84.8% to 97.5% relative to the control, and average triploidization rates ranged from 98.9% to 100%.

Key words: triploid, flow cytometry, Arctic char, *Salvelinus alpinus*, survival, hydrostatic pressure, heat shocking.

INTRODUCTION

The Alaska Department of Fish and Game (ADF&G) classifies all water bodies considered for stocking into one of five categories. Category 1 lakes are landlocked or connected lakes from which fish cannot escape. Fish capable of reproduction may be stocked into these lakes. Category 2 lakes have intermittent outlets that may occur during periods of high water. These high water periods usually last for less than 2 weeks and the probability of escapement is low. Only sterile fish are stocked into these lakes due to potential problems for wild populations through genetic introgression, predation or competition. Currently Arctic char *Salvelinus alpinus* may only be stocked in category 1 lakes (ADF&G 1998). The induction of triploidy into Arctic char would allow the Arctic char stocking program to be expanded to include category 2 lakes.

Triploidy can be induced by subjecting fish eggs to a hydrostatic pressure shock treatment or heat shock treatment shortly after fertilization (Ihssen et al. 1990; Malison et al. 2001). The treatment prevents the second meiotic division, resulting in 2 sets of chromosomes being contributed by the egg cell, and one set from the sperm cell. Success in inducing triploidy depends largely on three factors: time of initiation, duration of the treatment (either heat or pressure), and the level of the treatment (either the temperature of the shock or the amount of pressure in the shock). Induction of triploidy using heat-shock procedures is not as well documented for Arctic char as it is for numerous other species of fish. Inducing triploidy in rainbow trout through heat shocking fertilized eggs has been performed annually at the ADF&G Fort Richardson Hatchery (FRH) since 1986. At FRH, triploidy is induced in rainbow trout by immersing the eggs in a 26°C water bath for 20 minutes, 20 minutes post fertilization (Brock et al. 1994). Triploidization rates are generally greater than 95%, and the production and stocking of these fish has been successful. The timing of the second meiotic division varies with species and water temperature (Rottmann et al. 1991). Galbreath and Samples (2000) found that in brook trout *Salvelinus fontinalis* heat-shock duration and temperature had a greater effect on survival rate and triploidization rate than time of initiation of the thermal shock procedure.

Hydrostatic pressure shocking has been used successfully to induce triploidy in other stocks of Arctic char (O'Keefe and Benfey 1995; Eric Johnson, Icy Waters Ltd., http://www.icywaters.com/index.html, personal communication). A 5-minute exposure of Arctic char eggs to a hydrostatic pressure shock of 9,500 psi beginning at 300-Centigrade temperature minutes (CTMs) post fertilization resulted in a 100% triploidy rate with survival rates between 85% and 95% (Eric Johnson, Icy Waters Ltd, http://www.icywaters.com/index.html, personal

communication). O'Keefe and Benfey (1995) achieved 100% triploidy rate in Arctic char eggs exposed to a hydrostatic pressure shock of 9,500 psi for a duration of 5 minutes beginning at 225 and 300 CTMs post fertilization.

OBJECTIVES

The goal of this project was to create triploid Arctic char using heat-shocking and pressure-shocking procedures. The specific objectives were to:

Heat shocking

1. Estimate the mean survival rate from fertilization to the eyed-egg stage of development for both shocking temperatures (26°C and 28°C) and a control.

For those groups that had a survival rate greater than or equal to 70% of the control:

2. Determine which of the six combination of heat-shock duration (10, 12, and 14 minutes) and shocking temperature (26°C and 28°C) produced the highest rate of triploidization.

Pressure shocking

3. Estimate the mean survival rate from fertilization to the eyed-egg stage of development for each treatment group (pressure shocking was initiated at 200, 250, 300, or 350 CTMs post fertilization) and a control.

For those groups that had a survival rate greater than or equal to 70% of the control:

4. Determine which treatment produced the highest rate of triploidization.

METHODS

HEAT SHOCKING

Eggs and milt were collected from 3-year-old captive Lake Aleknagik Arctic char broodstock on 30 October 2001 during the course of a production egg take at FRH. Approximately 60 ml of eggs collected from each of eight female Arctic char were thoroughly mixed together in a Ziploc®[1] bag. The bag of eggs was then stored in a cooler with ice. Milt collected from three Arctic char males was stored in individual vials on ice.

Approximately half of the eggs were placed into a fertilization container. These were the eggs for replicate 1. Milt from three males was thoroughly mixed with the eggs, and activated with a 120 mM solution of NaCl to enhance sperm motility. Excess sperm was rinsed away and 30 ml of fertilized eggs were gently poured into each of eight individual incubation units (four units per tray, two trays). Eggs were water hardened in 6.2°C water for 15 minutes.

Approximately 30 seconds before the heat-shocking treatment began, excess water was drained from the eggs. Exactly 15 minutes post fertilization the incubation trays containing heat shock egg groups were immersed in the two heat shock tanks, one set at 26°C and the other at 28°C. Eggs in both temperature treatments were heat shocked simultaneously. Exactly 10 minutes after immersion the 10-minute duration incubation units were removed from both heat tanks. A second incubation unit was removed from each tank exactly 12 minutes after immersion, and the last units were removed exactly 14 minutes after immersion. Water temperatures were

[1] Product names used in this report are included for scientific completeness but do not constitute product endorsement.

monitored throughout the heat-shock process, and thermostats were adjusted to maintain target temperatures. After the heat-shock the eggs were disinfected for 10 minutes using a 6.2^{o}C-iodophor solution (100 ppm).

The process was repeated for replicate 2 using milt from the same three males to fertilize the same mix of eggs.

Eggs for the replicate 1 control were accidentally heat shocked for 10 minutes. Eggs for the replicate 2 control remained in the 6.2^{o}C water bath while the treatment groups were being heat shocked. They were disinfected alongside the treatment eggs.

All eggs were incubated at 4.5^{o}C in Heath trays. To insure that mixing of alevins between treatments did not occur two Heath trays were used for each temperature and each Heath tray contained 4 separate incubation units (one for each of the three time treatments and the control).

Survival

At the eyed egg stage of development the eggs in each incubation unit were physically shocked by pouring them into a container of water from a height of approximately 1 foot to turn dead eggs white. Dead eggs were removed after 24 hours. Small egg size and dim lighting in the incubation area made it difficult to tell if the eggs that did not turn white were eyed, had microeyes, or were blank. Eggs were physically shocked a second time to turn more of the blank eggs white. All eggs that did not turn white were enumerated and returned to the incubation unit. Incubation units were inspected again at hatch. Dead eggs were removed and attempts were made to separate and enumerate them as either eyed, having microeyes, or blank. Live and dead alevin were enumerated at emergence.

Simple binomial proportions were used to calculate the survival rate for each group, and treatment survival rates were estimated by averaging across replicates.

Ploidy

Flow cytometry was used to analyze tissue cells for ploidy (Thorgaard et al. 1982). A tissue sample was collected from all surviving alevins in each treatment group for each replicate. Tissue samples were preserved in a solution containing the DNA binding fluorescent dye 4'-6-diamidino-2-phenylindole (DAPI) and dimethyl sulfoxide (DMSO) (Thornthwaite et al. 1980). Assuming a conservative triploidization rate of 80%, a sample size of 45 alevins would have a maximum error of 10%, 90% of the time (binomial distribution).

Each treatment group was tested for ploidy even though relative eyed egg survival rates were less than 70%, and fewer than 45 individuals existed in each treatment group.

If available, a minimum of 10 samples from each replicate treatment group were analyzed for ploidy. If the triploidization rate of the first 10 samples of a group was less than 60%, then no further samples from that group were tested. The criteria presented in Table 1 were used to determine if further samples needed to be processed.

Simple binomial proportions were used to calculate the triploidization rate for each group, and average triploidization rates were estimated by averaging across replicates.

Survival and triploidy averages were ranked to determine the most effective treatment.

Table 1.-The number of observed diploids per sample size resulting in a triploidization rate significantly less than 80%.

Observed Diploids	Sample Size
≥4	10
5	≤15
6	≤20
8	≤25
9	≤30
10	≤35
11	≤40
12	≤45

PRESSURE SHOCKING

Eggs and milt were collected from 4-year-old captive Lake Aleknagik Arctic char brood on 23 October 2002 during the course of a production egg take at FRH. Approximately 25 ml of eggs were collected from each of 10 females. Hatchery data indicated that 25 ml contained approximately 300 eggs. Eggs collected from the 10 females were thoroughly combined and stored in a cooler on ice. Milt collected from five males was examined for sperm motility and stored in separate vials in a cooler on ice.

Half of the eggs were transferred to a plastic container for fertilization. Milt from each of the five males was added, and sperm was activated with a 120 mM solution of NaCl to enhance motility. Time of activation was recorded as the fertilization time. Excess sperm was rinsed away with 5°C water 1 minute post fertilization.

The fertilized eggs were equally divided among five incubation units. The units were loaded into an incubation tray and submerged in a 5°C water bath. Temperature of the water bath remained at 5°C ± 0.1^{o}C for the duration of the water hardening process. At 5°C, the 200, 250, 300, and 350 CTM egg groups were water hardened for 40, 50, 60, and 70 minutes, respectively, before they were pressure shocked.

Eggs were shocked in a stainless steel pressure chamber with a double O-ring brass piston. The chamber was filled with 5°C water, and eggs from the 200 CTM group were added. The piston sealed the top of the chamber, and air and excess water were expelled via a side relief valve before closing the relief valve. A 20-ton jack was used with a 15-ton hydraulic press to achieve 9,500 psi within the pressure chamber. The 5-minute exposure to the pressure shocking began when 9,500 psi was achieved within the chamber. After 5 minutes the chamber was rapidly depressurized, and eggs were transferred back to the appropriate incubation unit in the 5°C water bath. These same procedures were repeated for each pressurized treatment group. At 105 minutes post fertilization the eggs for the control group were place in the chamber. The eggs remained in the unpressurized chamber for 5 minutes before transferring them to the appropriate incubation unit. At 115 minutes post fertilization all egg groups were disinfected with an iodophor solution (100 ppm) for 15 minutes.

All procedures were repeated using the second half of the fertilized eggs.

Each replicate had a separate Heath tray. Each tray contained five incubation units (four treatment groups and the control). All eggs were incubated at 4.5°C.

Survival

The eggs in each treatment group were physically shocked at the eyed egg stage of development to turn dead eggs white. Dead eggs and those with microeyes were removed after 24 hours. The live eyed eggs in each group were enumerated and returned to the appropriate incubation unit. Alevin mortalities were enumerated at ponding.

Survival rates were calculated in the same manner as the heat-shock treatment groups.

Ploidy

Tissue samples from the pressure-shocked Arctic char were collected, preserved and analyzed in the same manner as the heat-shocked Arctic char.

Treatment groups were tested for ploidy if their survival rate to the eyed egg stage of development was at least 70% of the survival rate of the control.

A minimum of 10 samples from each qualifying replicate treatment group was analyzed for ploidy (Table 1). The sampling criteria used with the heat-shocked eggs were applied to the pressure-shocked eggs.

Ploidy rates were calculated in the same manner as the heat-shocked ploidy rates.

Heat-shocked and pressure-shocked treatment groups were ranked by average percent survival to eyed egg stage, average percent survival to emergence, average percent survival from eyed egg stage to emergence, and the average percent triploids in the sample.

RESULTS

HEAT SHOCKING

Survival

Survival rate to the eyed egg stage of development was very low, ranging from 0.0% to 6.0% for the treatment groups, and from 13.0% and 14.4% for the control groups (Table 2). Average survival rates relative to the control to the eyed egg stage of development ranged from 3.2% to 33.6% (Table 3).

Ploidy

Embryos from each treatment group were tested for ploidy even though none of the treatment groups had enough surviving embryos to meet the minimum sample size requirements of 45 individuals. Triploidy rates for individual treatment groups ranged from 0% to 100% (Table 2). Average triploidy rates for each treatment ranged from 10% to 100% (Table 3).

PRESSURE SHOCKING

Survival

Survival rate to the eyed egg stage of development ranged from 49.1% to 69.4% for the treatment groups and from 66.2% to 68.7% for the control groups (Table 4). Average survival rates relative to the control to the eyed egg stage of development ranged from 84.8% to 97.8% (Table 5).

Table 2.-Survival and triploidization rates of each heat-shocked treatment group.

Replicate	Heat-Shock Temperature °C	Duration/ Quadrant[a] (Minutes)	% Survived to Eyed Egg Stage	% Survived to Emergence	% Survived from Eyed Egg to Emergence	% Triploids in Sample[b]
1	26	10	0.6%	0.0%	0.0%	NS
1	26	12	0.9%	0.6%	60.0%	100.0%
1	26	14	6.0%	5.8%	96.9%	93.5%
1	26	C-10	2.6%	2.6%	100.0%	0.0%
2	26	10	2.5%	2.0%	78.6%	20.0%
2	26	12	3.4%	2.2%	64.7%	72.7%
2	26	14	3.7%	2.8%	76.5%	76.9%
2	26	C	14.4%	13.5%	93.8%	
1	28	10	0.2%	0.0%	0.0%	NS
1	28	12	0.0%	0.0%	0.0%	NS
1	28	14	0.2%	0.2%	100.0%	100.0%
1	28	C-10	2.1%	1.9%	87.5%	100.0%
2	28	10	1.9%	1.9%	100.0%	100.0%
2	28	12	2.5%	2.1%	84.6%	63.6%
2	28	14	0.6%	0.6%	100.0%	100.0%
2	28	C	13.0%	12.8%	98.5%	

Note: C is control group.

[a] C-10 were control groups that were accidentally heat-shocked for 10 minutes.

[b] NS: 100% mortality, no samples collected for ploidy testing.

Table 3.-Average survival and triploidization rates for heat-shocked treatments.

Treatment °C-Minutes	Average Percent Survival: To Eyed Egg	Relative to Control	To Emergence	Relative to Control	From Eyed to Emergence	Relative to Control	Average Triploidy Rate
26-10[a,b]	1.9%	13.1%	1.5%	11.2%	59.5%	63.4%	10.0%
26-12	2.2%	15.0%	1.4%	10.2%	62.4%	66.4%	86.4%
26-14	4.8%	33.6%	4.3%	31.9%	86.7%	92.4%	85.2%
26-C	14.4%	100.0%	13.5%	100.0%	93.8%	100.0%	
28-10[a,b]	1.4%	11.0%	1.3%	9.9%	62.5%	63.5%	100.0%
28-12[b]	1.3%	9.7%	1.1%	8.3%	42.3%	43.0%	63.6%
28-14	0.4%	3.2%	0.4%	3.2%	100.0%	101.5%	100.0%
28-C	13.0%	100.0%	12.8%	100.0%	98.5%	100.0%	

Note: C is control group.

[a] Includes Replicate 1 control groups that were accidentally heat-shocked for 10 minutes.

[b] Average triploidy rate does not include treatment groups that could not be sampled for ploidy because of 100% mortality.

Table 4.-Survival and triploidization rates of each pressure-shocked treatment group.

Replicate	Treatment (CTMs)[a]	% Survived to Eyed Egg Stage	% Survived to Emergence	% Survived from Eyed Egg to Emergence	% Triploids in Sample
1	200	49.1%	49.1%	100.0%	100.0%
1	250	64.0%	64.0%	100.0%	100.0%
1	300	62.5%	61.8%	98.9%	100.0%
1	350	63.9%	63.1%	98.9%	100.0%
1	control	66.2%	66.2%	100.0%	
2	200	65.3%	64.9%	99.4%	100.0%
2	250	67.5%	67.2%	99.4%	100.0%
2	300	69.4%	68.0%	97.9%	100.0%
2	350	66.4%	66.1%	99.5%	97.8%
2	control	68.7%	68.3%	99.4%	

[a] Centigrade temperature minutes.

Table 5.-Average survival and triploidization rates for pressure-shocked treatments.

	Average Percent Survival						
	To Eyed Egg		To Emergence		From Eyed to Emergence		
Treatment (CTMs)[a]		Relative to Control		Relative to Control		Relative to Control	Average Triploidy Rate
200	57.2%	84.8%	57.0%	84.8%	99.7%	100.0%	100.0%
250	65.8%	97.5%	65.6%	97.5%	99.7%	100.0%	100.0%
300	65.9%	97.8%	64.9%	96.5%	98.4%	98.7%	100.0%
350	65.1%	96.6%	64.6%	96.1%	99.2%	99.4%	98.9%
control	67.5%	100.0%	67.2%	100.0%	99.7%	100.0%	

[a] Centigrade temperature minutes.

Ploidy

Each treatment group achieved the minimum acceptable relative eyed egg survival rate of at least 70%, and had at least 45 surviving embryos for ploidy testing. The triploidization rate was 100% for all treatment groups except one where it was 97.8% (Table 4). Average triploidization rates for each treatment ranged from 98.9% to 100% (Table 5).

DISCUSSION

Egg quality is thought to be a factor in triploid survival (Teskeredzic et al. 1993). The hatchery production eggs used in the heat-shocking experiment were collected from 3-year-old broodstock and averaged 16.3 eggs/gram (Fort Richardson Hatchery production records). Survival to the eyed egg stage for the eggs not used in the experiment was estimated at 30.9%. Survival to the eyed egg stage for heat-shock experiment control groups ranged from 13.0 to 14.4%. The difference in survival between the production eggs and the study control group eggs may be partially explained by the amount of handling that occurred during the egg picking process at the

eyed egg stage. A high percentage of blank eggs in the study groups failed to turn white after the first physical shock so eggs were shocked a second time and dead eggs were removed the following day. Production eggs were physically shocked only once.

None of the heat-shocked treatment groups in our study achieved the minimum acceptable survival rate of 70% relative to the control, and with replicates combined, four of the six treatments had fewer than 20 surviving alevin available for ploidy testing. Because of the low survival rates (0.4% to 4.8% to the eyed egg stage, 3.2% to 31.9% relative to control) in our heat-shocked treatment groups, trends in triploidy rates or mortality rates associated with heat shock temperature or duration could not be determined.

Gillet et al. (2001) reported relative eyed egg survival rates ranging from 80% to 97% for groups of Arctic char eggs pressure shocked at 320 CTMs. Average relative eyed egg survival rates ranged from 84.8% to 97.8% for our treatment groups shocked at 200–350 CTUs (Table 5). Replicate 1, 200 CTM treatment group was the first group of eggs to be pressure shocked. A portion of the eggs stuck to the bottom of the pressure chamber, and several methods of egg removal were tried before all eggs were successfully removed. The additional handling associated with the egg removal process may have damaged some eggs in this group resulting in a lower eyed-egg survival rate (Table 4). The replicate 2, 200 CTM group had a relative survival rate of 95%.

Production eggs for the pressure-shocking experiment were collected from 4-year-old broodstock, and averaged 11.9 eggs/gram (Fort Richardson Hatchery production records). Survival to the eyed egg stage for the production eggs not used in the experiment was 67.9%. This is comparable to the average survival to the eyed egg stage for the control groups in our study of 67.5%.

The first spawning of Arctic char brood fish held captive at FRH was in 2001. Only 3-year-old brood were available for spawning. In 2002 only 4-year-old captive brood were available for spawning

Eyed egg survival rates for eggs collected from wild Lake Aleknagik brood in 2001 and 2002 were 77.6%. Although size of eggs collected from wild fish is not presented here, the eggs were visibly larger than those collected from the hatchery broodstock. The age of the wild fish used for the 2001 and 2002 egg takes is not known, but is probably variable. Hatchery diet as well as age at spawning may contribute to the smaller egg size and lower survival rates of eggs collected from hatchery-spawned fish. Work is needed in the area of Arctic char broodstock management to improve egg survival rates.

Each of the pressure-shocked treatment groups achieved the minimum acceptable relative survival rate. Our triploidy rates of 100% for groups shocked at 250 or 300 CTMs are consistent with those reported by O'Keefe and Benfey (1995), and Eric Johnson, Icy Waters Ltd. (http://www.icywaters.com/index.html, personal communication). Triploidy rates of 100% were also achieved by groups shocked at 200 CTMs, and for the replicate 1, 350 CTM group. A 2.5-minute delay in pressurizing the eggs in replicate 2, 350 CTM treatment to 9,500 psi shifted the pressurization time to approximately 362.5 CTMs post fertilization. A triploidy rate of 97.8% for this group may be an indication that the second meiotic cell division in some individuals may begin to occur shortly after 350 CTMs are achieved. Gillet et al. (2001) reported triploidy rates ranging from 96% to 100% for Arctic char eggs shocked for 5 minutes at 400 CTMs using 650 bar (9,5430 psi) of pressure.

The results from each pressure-shocked treatment exceeded our minimum acceptable survival and triploidy rates. A ranking of the average survival rates from green egg to eyed egg, green egg to emergence, eyed egg to emergence, as well as triploidy rate averages indicates that pressure shocking eggs at 250 CTMs post fertilization was our most effective treatment.

ACKNOWLEDGMENTS

We would like to thank staff at Fort Richardson Hatchery for providing the Arctic char eggs, incubation space, and advice throughout this study. We would also like to thank Eric Johnson of Icy Waters for sharing his knowledge on pressure shocking Arctic char eggs, and the ADF&G genetics section for the use of the flow cytometer to process numerous samples.

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