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Induction of Triploidy in Arctic Grayling (*Thymallus arcticus*) using Hydrostatic Pressure

by

Diane P. Loopstra

and

Patricia A. Hansen

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Weights and measures (metric)		General		Measures (fisheries)	
centimeter	cm	Alaska Administrative Code	AAC	fork length	FL
deciliter	dL	all commonly accepted abbreviations	e.g., Mr., Mrs., AM, PM, etc.	mid eye to fork	MEF
gram	g	all commonly accepted professional titles	e.g., Dr., Ph.D., R.N., etc.	mid eye to tail fork	METF
hectare	ha	at	@	standard length	SL
kilogram	kg	compass directions:		total length	TL
kilometer	km	east	E		
liter	L	north	N	Mathematics, statistics	
meter	m	south	S	<i>all standard mathematical signs, symbols and abbreviations</i>	
milliliter	mL	west	W	alternate hypothesis	H _A
millimeter	mm	copyright	©	base of natural logarithm	e
		corporate suffixes:		catch per unit effort	CPUE
Weights and measures (English)		Company	Co.	coefficient of variation	CV
cubic feet per second	ft ³ /s	Corporation	Corp.	common test statistics	(F, t, χ^2 , etc.)
foot	ft	Incorporated	Inc.	confidence interval	CI
gallon	gal	Limited	Ltd.	correlation coefficient (multiple)	R
inch	in	District of Columbia	D.C.	correlation coefficient (simple)	r
mile	mi	et alii (and others)	et al.	covariance	cov
nautical mile	nmi	et cetera (and so forth)	etc.	degree (angular)	°
ounce	oz	exempli gratia	e.g.	degrees of freedom	df
pound	lb	(for example)		expected value	E
quart	qt	Federal Information Code	FIC	greater than	>
yard	yd	id est (that is)	i.e.	greater than or equal to	≥
		latitude or longitude	lat. or long.	harvest per unit effort	HPUE
Time and temperature		monetary symbols (U.S.)	\$, ¢	less than	<
day	d	months (tables and figures): first three letters	Jan,...,Dec	less than or equal to	≤
degrees Celsius	°C	registered trademark	®	logarithm (natural)	ln
degrees Fahrenheit	°F	trademark	™	logarithm (base 10)	log
degrees kelvin	K	United States (adjective)	U.S.	logarithm (specify base)	log ₂ , etc.
hour	h	United States of America (noun)	USA	minute (angular)	'
minute	min	U.S.C.	United States Code	not significant	NS
second	s	U.S. state	use two-letter abbreviations (e.g., AK, WA)	null hypothesis	H ₀
Physics and chemistry				percent	%
all atomic symbols				probability	P
alternating current	AC			probability of a type I error (rejection of the null hypothesis when true)	α
ampere	A			probability of a type II error (acceptance of the null hypothesis when false)	β
calorie	cal			second (angular)	"
direct current	DC			standard deviation	SD
hertz	Hz			standard error	SE
horsepower	hp			variance	
hydrogen ion activity (negative log of)	pH			population	Var
parts per million	ppm			sample	var
parts per thousand	ppt, ‰				
volts	V				
watts	W				

FISHERY DATA SERIES NO. 10-55

INDUCTION OF TRIPLOIDY IN ARCTIC GRAYLING (*THYMALLUS ARCTICUS*) USING HYDROSTATIC PRESSURE

by
Diane P. Loopstra
Division of Sport Fish, Anchorage
and
Patricia A. Hansen
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

August 2010

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Diane P. Loopstra
Alaska Department of Fish and Game, Division of Sport Fish
333 Raspberry Road, Anchorage, Alaska 99518-1599, USA
and
Patricia A. Hansen
Alaska Department of Fish and Game, Division of Sport Fish, Research and Technical Services
333 Raspberry Road, Anchorage, Alaska 99518-1599, USA

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TABLE OF CONTENTS

	Page
LIST OF TABLES.....	ii
ABSTRACT	1
INTRODUCTION.....	1
METHODS.....	2
2004	2
Survival.....	3
Ploidy.....	3
2005.....	4
Survival.....	5
Ploidy.....	5
2006.....	5
Survival.....	6
Ploidy.....	6
RESULTS.....	6
2004	6
Survival.....	6
Ploidy.....	7
2005.....	8
Survival.....	8
Ploidy.....	8
2006.....	8
Survival.....	8
Ploidy.....	8
DISCUSSION.....	8
CONCLUSION	10
ACKNOWLEDGMENTS.....	10
REFERENCES CITED	11

LIST OF TABLES

Table		Page
1.	Shock initiation time, pressure, and shock duration used to achieve 100% triploid rates in Arctic char, coho salmon, and rainbow trout.	2
2.	Mean survival rates to the eyed-egg stage and to emergence relative to the control groups for Arctic grayling eggs pressure shocked in 2004, 2005, and 2006.	7
3.	Approximate Cumulative Temperature Units required to achieve the eyed-egg stage and hatching for rainbow trout, Arctic char, coho salmon, Chinook salmon, and Arctic grayling.	9

ABSTRACT

Hydrostatic pressure was applied to fertilized Arctic grayling (*Thymallus arcticus*) eggs in 2004, 2005, and 2006 to induce triploidy. In 2004, eggs collected from Chena River broodstock were pressure shocked for 5 minutes beginning at 100, 175, or 250 Cumulative Temperature Minutes (CTMs) post fertilization with 9,000, 9,500, or 10,000 psi of pressure. The three treatments shocked at 100 CTMs failed to achieve a minimum average eyed-egg survival rate criterion $\geq 70\%$ of the control group. All three treatments shocked at 175 CTMs achieved an average eyed-egg survival rate $\geq 70\%$ of the control group. The 9,000 and 9,500 psi treatments shocked at 250 CTMs achieved an average eyed-egg survival rate $\geq 70\%$ of the control group. Each treatment tested for ploidy in 2004 achieved a 100% triploid rate. In 2005, Arctic grayling eggs were pressure shocked at 175 CTMs post fertilization with 7,500, 8,500, or 9,500 psi of pressure for 5 minutes. Eggs shocked with 7,500 psi of pressure had a mean survival rate $< 70\%$ of the mean control group and were not tested for ploidy. Treatments shocked with 8,500 and 9,500 psi of pressure had mean survival rates to emergence $\geq 70\%$ of the control group, and both treatments achieved 100% triploid rates. In 2006, Arctic grayling eggs were pressure shocked at 175 CTMs post fertilization with 8,500 psi of pressure for 3, 5, or 7 minutes. Mean survival rates for the treatments were $\geq 70\%$ of the control group and a 100% triploid rate was achieved for each treatment.

Key words: triploid, flow cytometry, Arctic grayling, *Thymallus arcticus*, survival, hydrostatic pressure, aneuploid.

INTRODUCTION

The Alaska Department of Fish and Game (ADF&G) has historically stocked both landlocked and select nonlandlocked lakes with diploid Arctic grayling (*Thymallus arcticus*). Fish in these stocked lakes are subject to illegal transfer to other water bodies. In addition, nonlandlocked lakes may be flood prone or have intermittent or controlled outlets that potentially allow hatchery-released fish to escape and interact with adjacent native fish populations. Local broodstocks are used to minimize the genetic affects hatchery fish could have on native fish populations (ADF&G 1998). Stocking both landlocked and nonlandlocked lakes with sterile, triploid Arctic grayling would further reduce these affects. Triploid fish are incapable of reproduction, and therefore are unable to breed with native fish or establish a breeding population.

Heat shock, cold shock, hydrostatic pressure, and nitrous oxide treatments are used to produce sterile, triploid fish (Malison et al. 2001). These treatments prevent the occurrence of the second meiotic division, resulting in two sets of chromosomes being contributed by the female and one set from the male. Successful triploidy depends largely on three factors: shock initiation time (Cumulative Temperature Minutes (CTMs)), duration of the treatment (heat, pressure or nitrous oxide), and the intensity of the treatment (temperature or pressure).

With thermal shocking, egg placement within an egg mass affects the shock intensity and thus the treatment that each egg receives (Malison et al. 2001). Eggs near the center of an egg mass receive a less intense shock than eggs near the outside edge of the egg mass. One advantage to using hydrostatic pressure is that every egg in an egg mass receives the same treatment. Hydrostatic pressure shocking has also been used successfully to induce triploidy in salmonids including Arctic char (*Salvelinus alpinus*), coho salmon (*Oncorhynchus kisutch*), and rainbow trout (*O. mykiss*) (Table 1).

Table 1.-Shock initiation time, pressure, and shock duration used to achieve 100% triploid rates in Arctic char, coho salmon, and rainbow trout.

Species	Cumulative Temperature Minutes	Pressure (psi)	Duration (min)	Percent triploid
Arctic char	300	9,500	5	100
Coho salmon	210, 420	10,000	4	100
Rainbow trout	400	9,500	5	100

Source: O'Keefe and Benfey 1995; Teskeredzic et al. 1993; Yesaki et al. 1996

Shock initiation time (CTMs) – degrees centigrade multiplied by the number of minutes from fertilization to shock initiation – is based on the timing of the second meiotic division, which varies with species (Rottmann et al. 1991). Arctic grayling eggs require 100 Cumulative Temperature Units (CTUs) to achieve the eyed-egg stage, whereas Arctic char require approximately 230 CTUs, rainbow trout 240 CTUs, and coho salmon 265 CTUs (ADF&G Hatchery Database, unpublished). Therefore, it is likely that Arctic grayling eggs require fewer CTMs post fertilization to achieve the proper stage of development for inducing triploidy with pressure shocking than these other species.

A shock intensity (either duration or pressure) greater than needed to achieve a 100% triploid rate may generate a lower survival rate than a shock of less intensity that still achieves a 100% triploid rate. A shock intensity too low to achieve a 100% triploid rate may produce abnormal aneuploids (i.e., eggs with an incomplete set of chromosomes) (Chourrout 1984) which results in lower survival rates.

The goal of this project was to create triploid Arctic grayling using hydrostatic pressure shocking. The specific objectives were to:

1. Estimate the mean survival rate from fertilization to the eyed-egg stage in 2004 and to emergence in 2005 and 2006 for each treatment.

For those treatments with a minimum criteria survival rate greater than or equal to 70% of the control group survival rate:

2. Determine which treatment(s) produce the highest triploid rate.

METHODS

2004

Eggs and milt were collected from Chena River Arctic grayling broodstock on 13 May 2004. Eggs collected from 8 females were placed in individual plastic cups with lids and stored in a cooler on ice. Milt collected from 8 males was stored in separate vials in a cooler on ice. The gametes were transported to Fort Richardson Hatchery (FRH).

Before fertilization, a sample of milt from each vial was activated with a 7 ppt NaCl solution and examined with a microscope to verify the presence of motile sperm. Motile sperm were found in

only 4 of the vials, so only the milt from those 4 males was used for fertilization. The remaining 4 vials of milt were discarded. An estimated 7.5 ml of eggs from each of the 8 plastic cups were transferred to a plastic container and thoroughly mixed. About half of the egg mixture was transferred to a second container for fertilization. These eggs were used for the first replicate. Approximately an equal amount of milt from each of the 4 males was added and sperm was activated with the saline solution to enhance motility. Time of activation was recorded as the fertilization time. Excess sperm was rinsed away with 4.0°C water 1 minute post fertilization. For each replicate, an estimated 3 ml (approximately 257 to 407 eggs) of fertilized eggs were placed into each of 10 chamber inserts, one for each treatment and a control group. The inserts were submerged in a 4.0°C ±0.1°C water bath, and the eggs water hardened for 25 minutes (100 CTMs), 44 minutes (175 CTMs), or 62 minutes (250 CTMs) before they were pressure shocked.

Eggs were pressure shocked in three 24-ounce stainless steel chambers fitted with a double O-ring brass piston. Each chamber was filled with 4.0°C water and an insert for each replicate treatment was gently lowered into a chamber. The pistons were set in place and all air and excess water were expelled via a side relief valve before sealing the chambers. A 12-ton shop press with a 12-ton bottle jack was used to achieve 9,000 psi within one pressure chamber, a 15-ton shop press was used with a 20-ton jack to achieve 9,500 psi within a second pressure chamber, and 25-ton press was used with a 25-ton jack to achieve 10,000 psi in a third pressure chamber. Pressure was increased for approximately 1 minute to achieve the target pressure (9,000; 9,500; or 10,000 psi) within each chamber. The treatment began when the target pressure was achieved.

After 5 minutes, each chamber was rapidly depressurized, and the chamber inserts were transferred back to the 4.0°C water bath. At 75 minutes post fertilization, the chamber insert containing the control group eggs was placed in an unpressurized chamber. The control group eggs remained in the unpressurized chamber for 5 minutes before being returned to the 4.0°C water bath. At 90 minutes post fertilization, all eggs were transferred from the chamber inserts to individual incubation containers and disinfected with an iodophor solution (1:100 concentration) for 15 minutes.

Survival

At emergence, the dead eggs in each incubation container were enumerated and an attempt was made to determine how many of the dead eggs had achieved the eyed-egg stage. Live and dead alevin were also enumerated at emergence. Simple binomial proportions were used to calculate the survival rate for each group and treatment survival rates were estimated by averaging both replicates when possible. Survival rates relative to the control group were estimated for the first replicate 10,000 psi treatments using the first replicate control group survival rate. Survival rates for the second replicate 9,000 psi treatments were estimated using the second replicate control group survival rate. Survival rates for the 9,500 psi treatments were estimated using the mean control group survival rate.

Ploidy

Flow cytometry was used to analyze tissue cells for ploidy (Thorgaard et al. 1982). Tissue samples were preserved in 4',6-diamidino-2-phenylindole dihydrochloride hydrate (DAPI) and 10% dimethyl sulfoxide (DMSO) (Thorntwaite et al. 1980).

Tissue samples were analyzed from at least 45 alevin in each treatment that achieved a minimum survival rate criterion to the eyed-egg stage of ≥70% of the control group survival rate. Samples

from diploid fish were also collected to use as ploidy controls. Because triploid rates for Arctic grayling were unknown, a conservative acceptable triploid success rate of 80% was assumed. If the triploid rate of the first 10 samples of a treatment was less than 80%, then no further samples from that treatment were tested. Simple binomial proportions were used to calculate the triploid rate for each treatment and triploid rates were estimated by averaging across treatment replicates when possible.

2005

Eggs and milt were collected and stored in cups and vials as described for 2004 from Chena River Arctic grayling broodstock (8 females and 10 males) on 10 May 2005 and transported to FRH. A sample of milt from each vial was activated with a 7 ppt NaCl solution and examined with a microscope to verify the presence of motile sperm. Motile sperm were found in only 5 of the vials, so the milt from only those 5 males was used for fertilization. The remaining 5 vials of milt were discarded.

An estimated 4.0 ml of eggs from each of the 8 egg containers were transferred to a plastic container and thoroughly mixed. About half of the egg mixture was transferred to a second container for fertilization. These eggs were used for the first replicate. Approximately an equal amount of milt from each of the 5 males was added, and sperm was activated with the saline solution to enhance motility. Time of activation was recorded as the fertilization time. Excess sperm was rinsed away with 4.3°C water 1 minute post fertilization. Based on 2004 survival and triploid rates, all pressure-shock treatments in 2005 began at 175 CTMs, resulting in fewer treatments tested in 2005. For each replicate, an estimated 3.75 ml (approximately 311 to 439 eggs) of fertilized eggs were placed into each of 4 chamber inserts, one for each of the 3 treatments and a control. The chamber inserts were submerged in a 4.3°C ±0.1°C water bath. At 4.3°C, the eggs water hardened for approximately 40 minutes before they were pressure shocked at 175 CTMs.

Eggs were pressure shocked in three 24-ounce stainless steel chambers fitted with a double O-ring brass piston. Each chamber was filled with 4.3°C water and the chamber inserts for the first replicate were gently lowered into each chamber. Each chamber was sealed and pressurized as described for the 2004 treatments. The pressure shocking treatments began when the target pressure (7,500; 8,500; or 9,500 psi) was achieved within each chamber. After 5 minutes, each chamber was rapidly depressurized and the chamber inserts were transferred back to the 4.3°C water bath.

At 80 minutes post fertilization, the chamber insert containing the control group eggs was placed in an unpressurized chamber. The control group eggs remained in the unpressurized chamber for 5 minutes before being returned to the 4.3°C water bath. At 86 minutes post fertilization, all chamber inserts were transferred to an iodophor solution (1:100 concentration) for a 15-minute disinfection. Disinfected eggs were then transferred to an incubation container within a Heath tray.

The fertilization, pressure shocking, and disinfection processes were repeated for the second replicate treatments using milt from the same 5 males and the remaining egg mixture used in the first replicate.

Survival

Live and dead alevin and dead eggs were enumerated at emergence. Simple binomial proportions were then used to calculate the survival rate for each treatment and treatment survival rates were estimated by averaging both replicates. Mean survival rate to emergence relative to the mean control group survival rate was estimated for each treatment.

Ploidy

Tissue samples collected from Arctic grayling treatments were preserved and analyzed as described for 2004. Tissue samples were analyzed from at least 55 alevin in each treatment that achieved a mean survival rate to emergence $\geq 70\%$ of the mean control group survival rate. The acceptable triploid success rate for 2005 treatments was increased from 80% triploid to 95% triploid because treatments in 2004 achieved a 100% triploid rate. If the triploid rate of the first 5 samples of a treatment was significantly below 95% ($\alpha=0.05$), then sampling of that treatment was discontinued. Simple binomial proportions were used to calculate the triploid rate for each treatment and triploid rates were estimated by averaging across treatment replicates.

2006

Eggs and milt were collected and stored in cups and vials as described for 2004 from Chena River Arctic grayling broodstock (9 females and 10 males) on 19 May 2006 and transported to FRH. A sample of milt from each vial was activated with a 7 ppt NaCl solution and examined with a microscope to verify the presence of motile sperm. Sperm motility was verified in all 10 vials and 4 vials were randomly selected for fertilization. The remaining 6 vials were surplus and discarded.

An estimated 4.0 ml of eggs from each of the 9 egg containers were transferred to a plastic container and thoroughly mixed. About half of the egg mixture was transferred to a second container for fertilization. These eggs were used for the first replicate. Approximately an equal amount of milt from each of the 4 males was added and the sperm was activated with the saline solution to enhance motility. Time of activation was recorded as the fertilization time. Excess sperm was rinsed away with 4.4°C water 1 minute post fertilization. Based on 2004 and 2005 survival and triploidy rates, all pressure shock treatments in 2006 were initiated at 175 CTMs post fertilization with 8,500 psi of pressure for 3, 5, and 7 minutes.

For each replicate, an estimated 3.75 ml (approximately 313 to 400 eggs) of fertilized eggs were placed into each of 4 chamber inserts, one for each of the 3 treatments and a control group. The inserts were submerged in a 4.4°C $\pm 0.1^\circ\text{C}$ water bath. At 4.4°C, the eggs water hardened for approximately 40 minutes (175 CTMs) before they were pressure shocked.

Eggs were pressure shocked in three 24-ounce stainless steel chambers fitted with a double O-ring brass piston. Each chamber was filled with 4.4°C water and the chamber inserts containing the first replicate eggs were gently lowered into each chamber. Each chamber was sealed and pressurized as described for the 2004 and 2005 treatments. The pressure shock treatments began when the target pressure of 8,500 psi was achieved within each chamber. Eggs were pressure shocked for 3, 5, and 7 minutes. After each shock treatment, the chambers were depressurized and the eggs were transferred back to the 4.4°C water bath. At 80 minutes post fertilization, the chamber insert containing the control group eggs was placed in an unpressurized chamber. The control group eggs remained in the unpressurized chamber for 5 minutes. At 90 minutes post fertilization, all chamber inserts were transferred to an iodophor solution (1:100

concentration) for a 15-minute disinfection. Disinfected eggs were then transferred to an incubation container within a Heath tray.

The fertilization, pressure shocking, and disinfection processes were repeated for the second replicate treatments using milt from the same 4 males and the remaining egg mixture used in the first replicate.

Survival

Dead eggs were enumerated and removed from each incubation container 1 week before emergence to reduce the spread of fungus. At emergence, any remaining dead eggs, and live and dead alevin were enumerated. Simple binomial proportions were then used to calculate the survival rate for each treatment. Mean treatment survival rate to emergence relative to the mean control group survival rate was estimated for each treatment.

Ploidy

Tissue samples collected from Arctic grayling treatments were preserved and analyzed as described for 2004. Tissue samples were analyzed from at least 55 alevin in each treatment that achieved a mean survival rate to emergence $\geq 70\%$ of the mean control survival rate. If the triploid rate of the first 5 samples of a treatment was significantly below 95% ($\alpha=0.05$), then sampling of that treatment was discontinued. Simple binomial proportions were used to calculate the triploid rate for each treatment and triploid rates were estimated by averaging across treatment replicates.

RESULTS

2004

A needle valve failure prevented the pressure chamber in the first replicate 9,000 psi treatment group at 100 CTMs from holding at the desired pressure and the treatment was discontinued. The 9,500 and 10,000 psi treatments for the first replicate eggs were successfully completed. To include 9,000 psi treatments in the study, eggs in the second replicate were shocked at 9,000 and 9,500 psi. The 9,000 and 10,000 psi treatments were not replicated because of the valve failure in the third pressure chamber, so the reported survival and ploidy rates are for individual treatments. The 9,500 psi treatments were replicated and the reported survival rates are a mean of both treatment replicates.

Survival

Survival rates from green egg to eyed egg relative to the survival rate of the control group for treatments shocked at 100 CTMs post fertilization ranged from 63.5% for the individual 9,000 psi treatment to 69.7% for the individual 10,000 psi treatment (Table 2). Survival rates from green egg to eyed egg relative to the survival rate of the control group for treatments shocked at 175 CTMs post fertilization ranged from 77.4% for the individual 10,000 psi treatment to 91.0% for the individual 9,000 psi treatment. Survival rates from green egg to eyed egg relative to the survival rate of the control group for treatments shocked at 250 CTMs post fertilization ranged from 67.2% for the individual 10,000 psi treatment to 75.4% for the individual 9,000 psi treatment.

The survival rates to emergence for the two replicate control groups differed by 20.0 percentage points in 2004. A comparison of survival rates for the two groups (not relative to the control for treatments shocked with 9,500 psi of pressure) showed that the first replicate 175 CTM treatment

Table 2.-Mean survival rates to the eyed-egg stage and to emergence relative to the control groups for Arctic grayling eggs pressure shocked in 2004, 2005, and 2006.

Year	Treatment			Green egg to eyed egg		Green egg to emergence		Percent triploid
	CTMs	Pressure	Shock duration (min)	Survival	Survival relative to control	Survival	Survival relative to control	
2004	100	9,000	5	58.2%	63.5% ^a	32.3%	38.0% ^a	ND
	100	9,500	5	57.0%	64.9%	31.7%	44.0%	100.0% ^b
	100	10,000	5	58.9%	69.7% ^c	30.0%	46.0% ^c	ND
	175	9,000	5	83.5%	91.0% ^a	68.3%	80.3% ^a	100.0%
	175	9,500	5	76.6%	86.9%	55.1%	72.8%	100.0%
	175	10,000	5	65.4%	77.4% ^c	49.1%	75.4% ^c	100.0%
	250	9,000	5	69.2%	75.4% ^a	53.6%	63.0% ^a	100.0%
	250	9,500	5	65.3%	73.9%	52.9%	70.5%	100.0%
	250	10,000	5	56.8%	67.2% ^c	42.0%	64.5% ^c	ND
		Control group 1			84.5%		65.1%	
	Control group 2			91.8%		85.1%		
2005	175	7,500	5	ND	ND	64.5%	68.4%	ND
	175	8,500	5	ND	ND	73.5%	77.9%	100.0%
	175	9,500	5	ND	ND	73.0%	77.4%	100.0%
		Control group					94.3%	
2006	175	8,500	3	ND	ND	74.1%	81.2%	100.0%
	175	8,500	5	ND	ND	79.6%	87.2%	100.0%
	175	8,500	7	ND	ND	78.3%	85.8%	100.0%
		Control group					91.3%	

Note: CTMs = cumulative temperature minutes
 In 2004, 2005, and 2006, some data were not collected. ND = no data.
 Reported survival rates are means unless noted.

- ^a The 9,000 psi treatments were not replicated because of equipment failure. Survival rates are relative to control group 2.
- ^b Only the first replicate was tested for ploidy.
- ^c The 10,000 psi treatments were not replicated because of equipment failure. Survival rates are relative to control group 1.

survival rate was 21.3 percentage points lower than the second replicate. In the 250 CTM treatment, the survival rate for the first replicate was 13.7 percentage points lower than the second replicate. The survival rate to emergence for the first replicate 100 CTM treatment was 12.1 percentage points higher than the replicate 2 survival rate, but both survival rates were less than 40%.

Ploidy

There were 45 alevins tested for ploidy from all treatments (100 CTMs 9,500 psi first replicate; 175 CTMs 9,000 psi; 9,500 psi both replicates, and 10,000 psi; 250 CTMs 9,000 psi and

9,500 psi both replicates) that achieved the eyed-egg survival rate criteria of $\geq 70\%$ of the control group survival rate. Triploid rates were 100% for all treatments tested (Table 2).

2005

Survival

Mean survival rates relative to the control groups from green egg to emergence ranged from 68.4% for the 7,500 psi treatment to 77.9% for the 8,500 psi treatment (Table 2).

Ploidy

The 8,500 and 9,500 psi treatments achieved the minimum emergence survival criteria of $\geq 70\%$ of the control group. Tissue samples collected from 55 alevin in each treatment achieved a 100% triploid rate (Table 2).

2006

Survival

Mean survival rates relative to the control groups from green egg to emergence ranged from 81.1% for the 3 minute 8,500 psi treatment to 87.2% for the 5 minute 8,500 psi treatment (Table 2).

Ploidy

The 3-, 5-, and 7-minute duration 175 CTM 8,500 psi treatments achieved the minimum emergence survival criteria of $\geq 70\%$ of the control group. Tissue samples collected from 55 alevin in each treatment achieved a 100% triploid rate (Table 2).

DISCUSSION

Successfully inducing triploidy using hydrostatic pressure depends largely on shock initiation time, pressure intensity, and duration of treatment. Arctic grayling eggs require fewer Cumulative Temperature Units to reach the eyed-egg stage and hatch than eggs of rainbow trout, Arctic char, coho salmon, or Chinook salmon (Table 3). Therefore, to induce triploidy and achieve acceptable survival rates, it is reasonable that Arctic grayling eggs would also require fewer CTMs between fertilization and pressure shocking than these other species.

In 2004, nine combinations of shock initiation times and pressures were used to induce triploidy in Arctic grayling. The survival rates to emergence of less than 50% relative to the control groups for the all treatments at 100 CTMs suggested that the Arctic grayling eggs were not developed enough to survive the pressure treatment. The survival rates to emergence for the 250 CTM treatments were 2.3 to 17.3 percentage points lower than the survival rates of the 175 CTM treatments. Survival rates $< 70\%$ to emergence relative to the control groups for the 250 CTM 9,000 and 10,000 psi treatments suggest that some eggs at 250 CTMs were too well developed to survive the pressure treatment.

A difference of 20 percentage points in survival rate to emergence for the first replicate control group (65.1%) and the second replicate control group (85.1%) may indicate that egg handling and/or fertilization procedures were not identical for both replicates. Fertilization rates were not determined, but low milt volume (an estimated 0.2 ml each) and inexperience handling small eggs that become cohesive when sperm were activated by the addition of saline solution may

Table 3.-Approximate Cumulative Temperature Units required to achieve the eyed-egg stage and hatching for rainbow trout, Arctic char, coho salmon, Chinook salmon, and Arctic grayling.

Species	Number of thermal units to eyed-egg stage	Number of thermal units to hatching
Rainbow trout	250	300
Arctic char	225	400
Coho salmon	265	430
Chinook salmon	330	490
Arctic grayling	≈100	170

Source: ADF&G Sport Fish Hatchery Program. Fort Richardson Hatchery Incubation Procedures. <http://docushare.sf.adfg.state.ak.us/docushare/dsw/eb/View/Collection-1512> (accessed 6/22/2010).

have contributed to low fertilization and survival rates for some first replicate treatments. The 100% triploid rates achieved in 2004 confirmed that a 5-minute shock duration with 9,000 psi of pressure is intense enough to induce triploidy in Arctic grayling.

Dead eggs in each incubation container were connected by fungus making it difficult to differentiate between eyed eggs and eggs without eyes. Because the dead eggs were too fragile to rotate, eyed eggs may have been counted as eggs without eyes if they were not rotated so that the eyes were visible. Because of the difficulty in correctly identifying eyed eggs in 2004, eyed-egg survival rates were not estimated in 2005 and 2006.

Chourrout (1984) found that rainbow trout eggs shocked at lower intensities (either less pressure or shorter duration) experienced incomplete retention of the second polar body resulting in abnormal aneuploids. In 2005, we tested 5-minute shock durations at 175 CTMs with 7,500, 8,500, and 9,500 psi of pressure. Survival rates <70% to emergence relative to the control groups for the 7,500 psi treatments suggest that the treatment intensity may have been too low to complete retention of the second polar body. Treatments shocked with 8,500 psi achieved 100% triploid rates, and had a survival rate slightly (0.5 percentage point) higher than the 9,500 psi treatment. In 2006, 3-, 5- and 7-minute shock durations at 175 CTMs with 8,500 psi of pressure were tested. Mean survival rates to emergence for all 3 treatments were within 6.0 percentage points. Mean survival rate to emergence for the 3-minute treatment was 6.0 percentage points lower than that of the 5-minute treatment and 4.6 percentage points lower than that of the 7-minute treatment. The shock intensity of the 3-minute treatment may have contributed to the slightly lower survival rate. However, each treatment was intense enough to achieve a 100% triploid rate among the hatched Arctic grayling.

The 5-minute treatments shocked with 8,500 psi of pressure at 175 CTMs had a mean survival rate to emergence relative to the control groups of 77.9% in 2005 and 87.1% in 2006. In both years, chemical treatments to control fungal growth were discontinued just before hatching. Without treatment, fungus on dead eggs can spread to live eggs, possibly decreasing the survival rate. In 2004 and 2005, dead eggs were not removed from the incubation containers until emergence and fungus connected the dead eggs together. In 2006, dead eggs were removed from the incubation containers 1 week before emergence to reduce the spread of fungus. This may have increased survival rates in 2006.

CONCLUSION

All treatments tested for ploidy achieved a 100% triploid rate. Survival rates for the 8,500 and 9,500 psi treatments in 2005 were within 0.5 percentage points, and survival rates for the 5- and 7-minute treatments in 2006 were within 1.4 percentage points. Although survival rates are too close to definitively state which treatment achieved the highest survival rate, we will adopt the 5-minute shock treatment starting at 175 CTMs post fertilization with 8,500 psi of pressure as the accepted procedure for inducing triploidy in Arctic grayling.

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REFERENCES CITED

- ADF&G (Alaska Department of Fish and Game). 1998. Statewide Stocking Plan for Recreational Fisheries 1998 – 2002. Alaska Department of Fish and Game, Division of Sport Fish, Research and Technical Services, Anchorage.
- Chourrout, D. 1984. Pressure-induced retention of second polar body and suppression of first cleavage in rainbow trout: production of all-triploids, all-tetraploids, and heterozygous diploid gynogenetics. *Aquaculture* 36:111-126.
- Malison, J. A., J. A. Held, L. S. Weil, T. B. Kayes, and G. H. Thorgaard. 2001. Manipulation of ploidy in walleyes by heat shock and hydrostatic pressure shock. *North American Journal of Aquaculture* 63:17-24.
- O'Keefe, R., and T. Benfey. 1995. The production of triploid and sex-reversed arctic charr (*Salvelinus alpinus*). *Aquaculture* 137:157.
- Rottmann, R. W., J. V. Shireman, and F. A. Chapman. 1991. Induction and verification of triploidy in fish. Southern Regional Aquaculture Center. Publication No. 427.
- Teskeredzic, E., E. M. Donaldson, Z. Teskeredzic, I. I. Solar, and E. M. Lean. 1993. Comparison of hydrostatic pressure and thermal shocks to induce triploidy in coho salmon (*Oncorhynchus kisutch*). *Aquaculture* 117:47-55.
- Thorgaard, G. H., P. S. Rabinovich, M. W. Shen, G. A. E. Gall, J. Propp, and F. M. Utter. 1982. Triploid rainbow trout identified by flow cytometry. *Aquaculture* 29:305-310.
- Thornthwaite, J. T., E. V. Sugarbaker, and W. J. Temple. 1980. Preparation of tissues for DNA flow cytometric analysis. *Cytometry*. 1:229-237.
- Yesaki, T. Y., K. W. Scheer, and D. L. Greiner. 1996. Production-scale pressure shocking of rainbow trout (*Oncorhynchus mykiss*) in British Columbia. Pages 170-173 [In] Proceedings of the 47th Annual Northwest Fish Culture Conference, D. D. McKinlay, editor., Victoria BC
http://www.fishlib.org/library/Documents/NWFCC/proceedings_1996.pdf