Regional Operational Plan SF.3F.2014.02

Operational Plan: Region III Egg Takes for Stocked Fisheries Programs and Donor Stock Disease Screening

by

Cal Skaugstad,

and

April Behr

January 2015

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)

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

REGIONAL OPERATIONAL PLAN SF.3F.2014.02

OPERATIONAL PLAN: REGION III EGG TAKES FOR STOCKED FISHERIES PROGRAMS AND DONOR STOCK PATHOLOGY SCREENING

by

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Division of Sport Fish

January 2015

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This document should be cited as:
Skaugstad, C., and A. Behr. 2014. Operational plan: Region III egg takes for stocked fisheries programs and donor stock pathology screening. Alaska Department of Fish and Game, Regional Operational Plan ROP.SF.3F.2014.02, Anchorage.

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Signature Page

Project Title:	Region III egg takes for stocked fisheries programs and donor stock pathology screening
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Division, Region and Area	Sport Fish, Region III, Fairbanks
Project Nomenclature:	F-10-30; F-3-02
Period Covered	1 May, 2014–30 April, 2015
Field Dates:	1 May–17 October 2014
Plan Type:	Category I

Approval

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ABSTRACT

This project details the number of adult donors needed from wild stocks to provide sufficient fertilized gametes to meet Board of Fish (BOF) management objectives for stocked fisheries in Tanana River and Upper Copper/Upper Susitna management areas. We identify wild donor stocks for Arctic grayling (Chena River), Chinook salmon (Salcha River), and coho salmon (Delta Clearwater River) and describe capture locations and methods for each species. Also listed are wild donor stocks that require stock disease screening and family tracking. This operational plan provides detail necessary for Fish Transport Permits (FTP) that are required to capture, transport, and hold wild donors; and collect, fertilize, and transport gametes to Ruth Burnett Sport Fish Hatchery (RBSFH) in Fairbanks and William Jack Hernandez Sport Fish Hatchery (WJHSFH) in Anchorage.

Key words: Arctic grayling *Thymallus arcticus*, Chinook salmon, *Oncorhynchus tshawytscha,* coho salmon, *Oncorhynchus kisutch,* egg take, fish stocking, family tracking, stock disease history, hatchery, Chena River, Salcha River, Delta Clearwater River.

PURPOSE

The purpose of this project is to collect gametes from Arctic grayling, Chinook salmon, and coho salmon for rearing in the Ruth Burnett Sport Fish Hatchery in Fairbanks and William Jack Hernandez Sport Fish Hatchery in Anchorage and for subsequent outstocking into landlocked lake systems in the Tanana River and Upper Copper/Upper Susitna management areas.

OBJECTIVES

1. Arctic grayling - collect and artificially fertilize 118,700 eggs using 39 females and 39 males from the Chena River population.

2. Chinook salmon - collect and artificially fertilize 29,300 eggs using 8 females and 8 males from the Salcha River population. Up to 10 females and 10 males may be taken to account for culling due to BKD.

3. Chinook salmon - visually inspect all Chinook salmon donors for *Ichthyophonus*.

4. Coho salmon - collect and artificially fertilize 59,900 eggs using 40 females and 40 males from the Delta Clearwater River population.

5. Family tracking - Obtain kidney samples from all female Chinook and coho salmon donors to screen for bacterial kidney disease.

6. Stock disease history – Obtain kidney and ovarian samples from Arctic grayling, and Chinook and coho salmon for stock disease history.

METHODS

ARCTIC GRAYLING

Statewide Stocked Fisheries Program Requirement

The Chena River Arctic grayling population supplies gametes for both Region II and Region III stocked fisheries programs. Region II requires 116,200 fertilized eggs to produce 20,000 fingerlings (2g) in 2014 and 55,010 catchables (120g) in 2015. Region III requires 60,400 fertilized eggs to produce 13,600 fingerlings (2g) in 2014 and 46,800 catchables (120g) in 2015.

Donor and Gamete Objectives

Approximately 60,400 fertilized eggs are needed for Region III which will require 20 females and an equal number of males. These numbers were calculated using fecundity and hatchery survival values listed in Table 1 for RBSFH. WJHSFH uses different survival values and has requested fertilized eggs from 18 females and 18 males to meet fish production needs.

Table 1.–Donor and egg requirement for Arctic grayling egg take, Chena River, 2014.

Statewide Stocking Needs:	Region II		Region III
Fingerlings to stock in 2014 (3N)	9,500		10,500
Catchables to stock in 2015 (3N)	20,600		34,410
Statewide total number of fish to be produced from 2014 egg take		75,000	
Survival Estimates:			
Survival Green Egg to Ponding:			88%
Survival Fry to Fingerling:			88%
Survival Fingerling to Catchable:			95%
Number of Eggs Needed to produce (number rounded to nearest 100):			
Fingerlings (2014)			13,600
Catchables (2015)			46,800
	55,800		60,400
Statewide total number of eggs needed from 2014 egg take		116,100	
Females Required:			
Fecundity - Eggs/female:		3,100	
Total females required for spawning	18[a]		20
		38	

[a] Request from WJHSFH based on survival values specific to their Arctic grayling culture program.

Background

The Region III Sport Fish (SF) staff captures and holds Arctic grayling *Thymallus arcticus* for gamete collection to support Region II and III stocked fisheries programs. They also assist staff from Ruth Burnett Sport Fish Hatchery to artificially spawn fish. Adult Arctic grayling are collected from a wild population in the Chena River.

The last week in April or the first two weeks in May, a boat equipped with electrofishing gear will be used to capture adult Arctic grayling prior to spawning. The upper boundary of the capture area is about 7 km above the Nordale Road bridge and extends down to the bridge (Figure 1). Electrofishing will be confined to this 7 km area which will limit exposing most of the Arctic grayling population to electricity. The electrofishing boat will operate for 2 or 3 days and for not more than 2 hours per day. Each day we will make a single pass through the area along only one bank. The capture date depends on weather conditions that influence when river ice cover is broken and transported downstream making river navigation possible. Immediately following breakup, female Arctic grayling are within 3 to 7 days of spawning. Most males are ready to spawn.

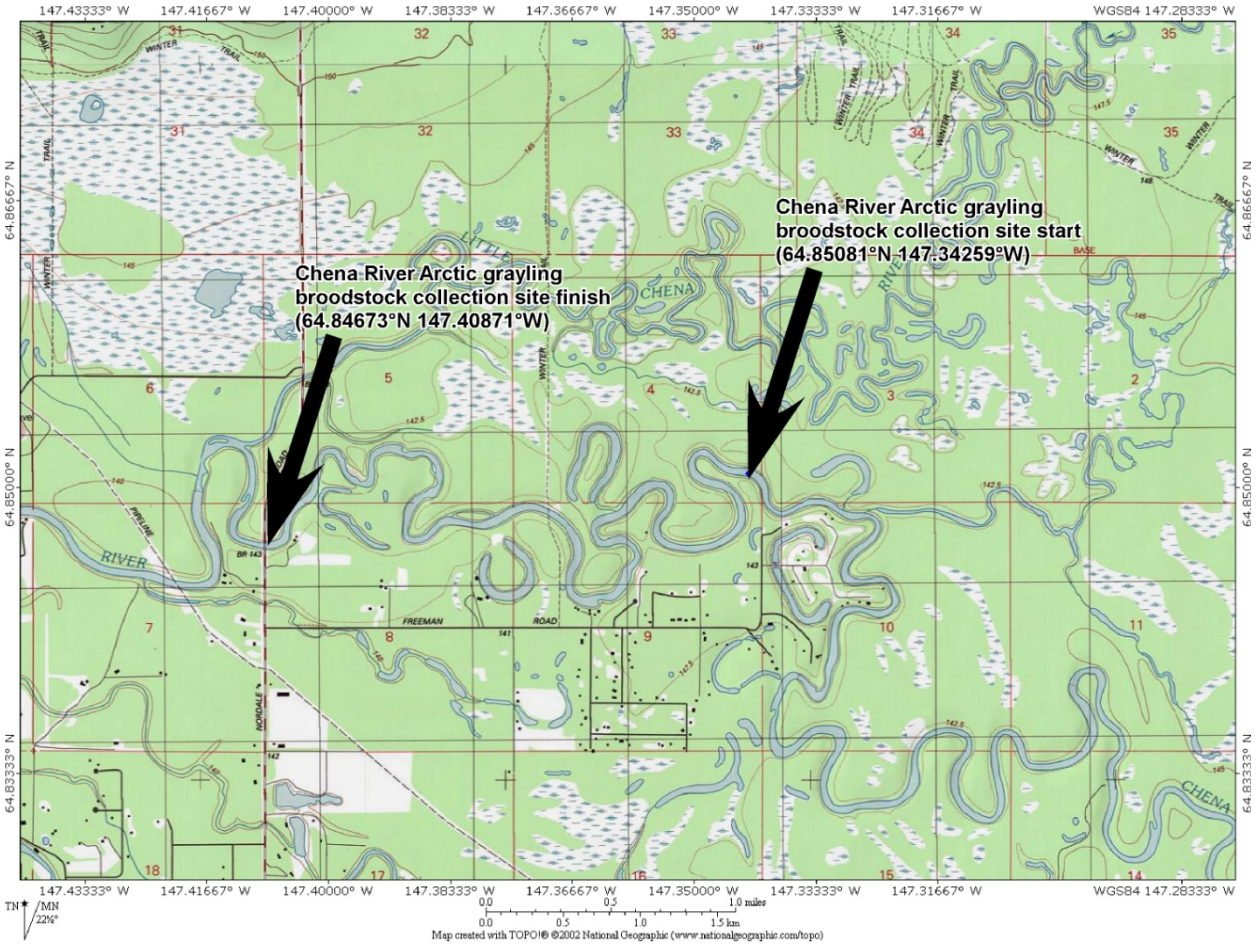


Figure 1.–Location of Arctic grayling capture area on the Chena River.

After fish are captured they are transferred to a fish transport truck and taken to Spafford's Pond (Figure 2), a gravel pit in Aurora subdivision at the East end of Hansen Road, Fairbanks, and put into net pens. The fish are held until the females are mature and can be artificially spawned. This may happen within a few days to two weeks. After gametes are collected all fish are returned live to the Chena River at Nordale Road Bridge. In Fairbanks, staff from RBSFH transport fertilized eggs to the hatchery. Fertilized eggs for WJHSFH are sent to Anchorage by

commercial airline. Staff from WJHSFH pick up the eggs at the airport and transport them to the hatchery for incubation and rearing.

Multiple egg takes may be necessary when at least 24 fish are ready to spawn and there is indication that some fish have begun to spawn in the holding pen. Waiting for additional fish to mature may result in not meeting egg-take goals because early maturing fish will release eggs prior to the egg take. Having two egg takes usually guarantees that egg goals are achieved without having to capture and hold a large number of extra fish for a single egg take. A second egg take usually follows within a few days.

We typically capture twice the number of females and males required to meet egg-take goals because we've found that at most only one-half of females will mature within a suitable period. About one-third of males won't produce milt or an amount too small to be useful. We want to have all egg takes completed within one week because we need to start other projects. We limit the number of fish that we capture and hold to lessen possible negative impact to the wild population. The Arctic grayling fishery in the Chena River is catch-and-release due to conservation concerns.

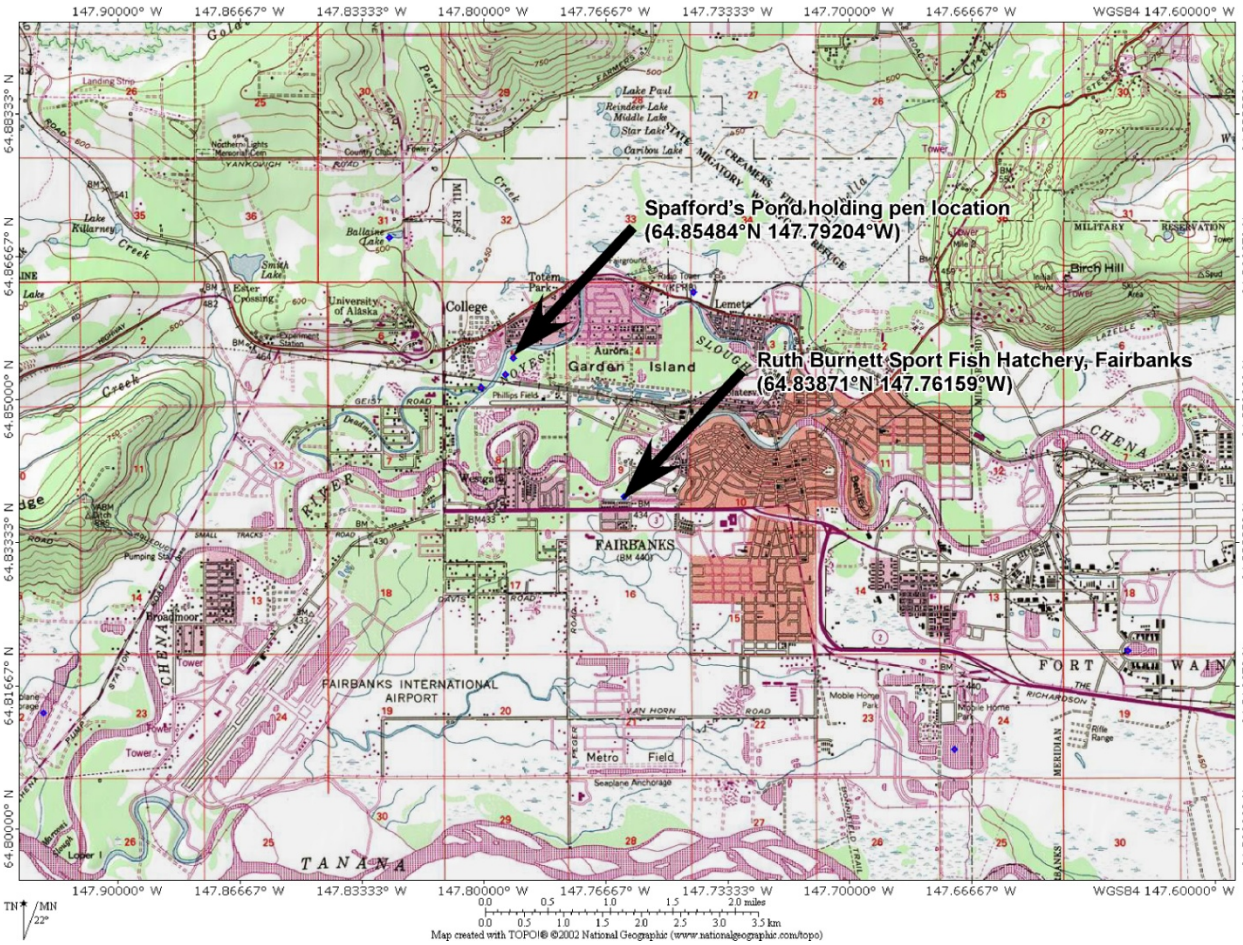


Figure 2.–Location of Spafford's Pond for holding Arctic grayling in net pens.

Timing for this egg take is critical because most spawning seems to occur within a two week period. Our ability to capture fish during a two week window is dependent on river ice conditions. Ice flowing down the river or blocking boat ramps will prevent or delay our ability to navigate the river when fish are spawning.

Other capture methods such as gill nets and fyke nets were considered but are not effective in the Chena River immediately following breakup because of high debris load. Arctic grayling and non-targeted species that are captured in gill nets are more likely to be injured around the gills from entanglement and when being removed from the net. Beach seines are not practical because Arctic grayling are dispersed. Adult salmon and rainbow trout are not present in the Chena River during this sampling event.

Gamete Collection and Fertilization Procedures

We will follow the procedures describe in *General Egg-Take Procedures for ADF&G Sport Fish Hatchery Program* (Appendix A). The procedure for Arctic grayling has been modified to include equipment and methods that we have found useful. Eggs from one female will be fertilized with milt from one male. A male will not be used to fertilize the eggs from multiple females.

Triploidy Induction

All fertilized eggs will be shocked using hydrostatic pressure to induce triploidy as describe in *Triploid Induction with Hydrostatic Pressure* (Appendix D). This document is a draft. We include it because the information and instruction is very important to field crews operating the hydrostatic chamber to induce triploidy. Portions of the document has been edited for clarity where wording was obviously in draft stage. Specific procedures for using hydrostatic pressure to induce triploidy in Arctic grayling and recent results are summarized by Loopstra and Hansen (2010).

Family Tracking and Pathology

Family tracking is not required for Arctic grayling. Kidney and ovarian samples are required for disease history (Table 2). We will follow procedures described in *Collection Protocol for Routine Broodstock Examination for ELISA/FAT Kidney Samples* (Appendix B) and *Sample Collection for Routine Broodstock - Examination for Virology* (Appendix C).

Table 2.–Sample size for disease screening of Arctic grayling, Chena River, 2014.

	Kidney	Ovarian Fluid
Male	24	
Female		23

CHINOOK SALMON

Region III Stocked Fisheries Program Requirement

A total of 18,000 catchable (120g) Chinook salmon are required for stocking Region III lakes in 2014.

Donor and Gamete Objectives

About 48,457 fertilized eggs are needed which will require at least 5 females and an equal number of males. These numbers were calculated using fecundity and hatchery survival rates listed in Table 3. All females will be screened for disease (family tracked) and eggs from fish identified having disease will be discarded. Up to 12 females and an equal number of males may be required to meet the objective of 48,500 fertilized eggs after disease screening. Because fecundity has a positive relation to fish size and the typical size range of Chinook salmon females is 750 to 1050 mm, we will decide in the field the number of females needed to achieve egg goals. We expect the total number of females needed to provide eggs will not exceed 12.

Table 3.–Donor and egg requirement worksheet for Chinook salmon egg take, Salcha River, 2014.

Catchables to stock in 2015	31,950
Survival Estimates:	
Egg to Eye	82%
Eye to Ponding	92%
Fry to Fingerling	92%
Fingerling to Catchable	95%
Total Number of Eggs Need:	48,500
Females Required:	
Fecundity - Eggs/female	6,500
Total females required for spawning	8

Background

The Region III Sport Fish staff captures and holds Chinook salmon for gamete collection to support Region III stocked fisheries programs. They also assist staff from the Ruth Burnett Sport Fish Hatchery to collect gametes. Chena River and Salcha River populations of Chinook salmon were selected by fishery managers as the primary and secondary brood sources to provide gametes for production at the Ruth Burnett Sport Fish Hatchery.

During week 1 or 2 in July, Chinook salmon adults will be captured using hook-and-line sport fishing gear at the mouth of the Salcha River. The capture area is about 1 km below the Salcha River/Richardson Highway bridge (Figure 3). The spawning condition of female Chinook salmon at this time is typically pre-spawning. Most males are ready-to-spawn. Females will be sorted and placed in separate net pens. Green females will be checked every second day for spawning condition. Males will be held in a third net pen. Fish will be held until females are ready to spawn.

If we fail to capture sufficient numbers of adult Chinook salmon using sport fishing gear we will use a boat equipped with electrofishing gear during week 3 or 4 in July. The capture area is about 35 km above the Salcha River/Richardson Highway bridge (Figure 3). The use of

electrofishing gear will be based on sufficient numbers of adult fish returning and we will limit the capture area to 25 to 45 km above the Salcha River/Richardson Highway bridge to limit the portion of the population exposed to electricity. We intend to make one pass through either the upper or lower section of the capture area each day. It will take about 6 hours to traverse a section. During a pass we will use electrofishing gear along only one bank. We expect to limit electrofishing to 4 days or less. This will minimize the number of fish exposed to electricity.

We will intentionally select larger females and males (> 950 mm) for spawning. Eggs will be fertilized on site near the Salcha River boat launch. Fertilized eggs will be transported by vehicle on the road system to RBSFH.

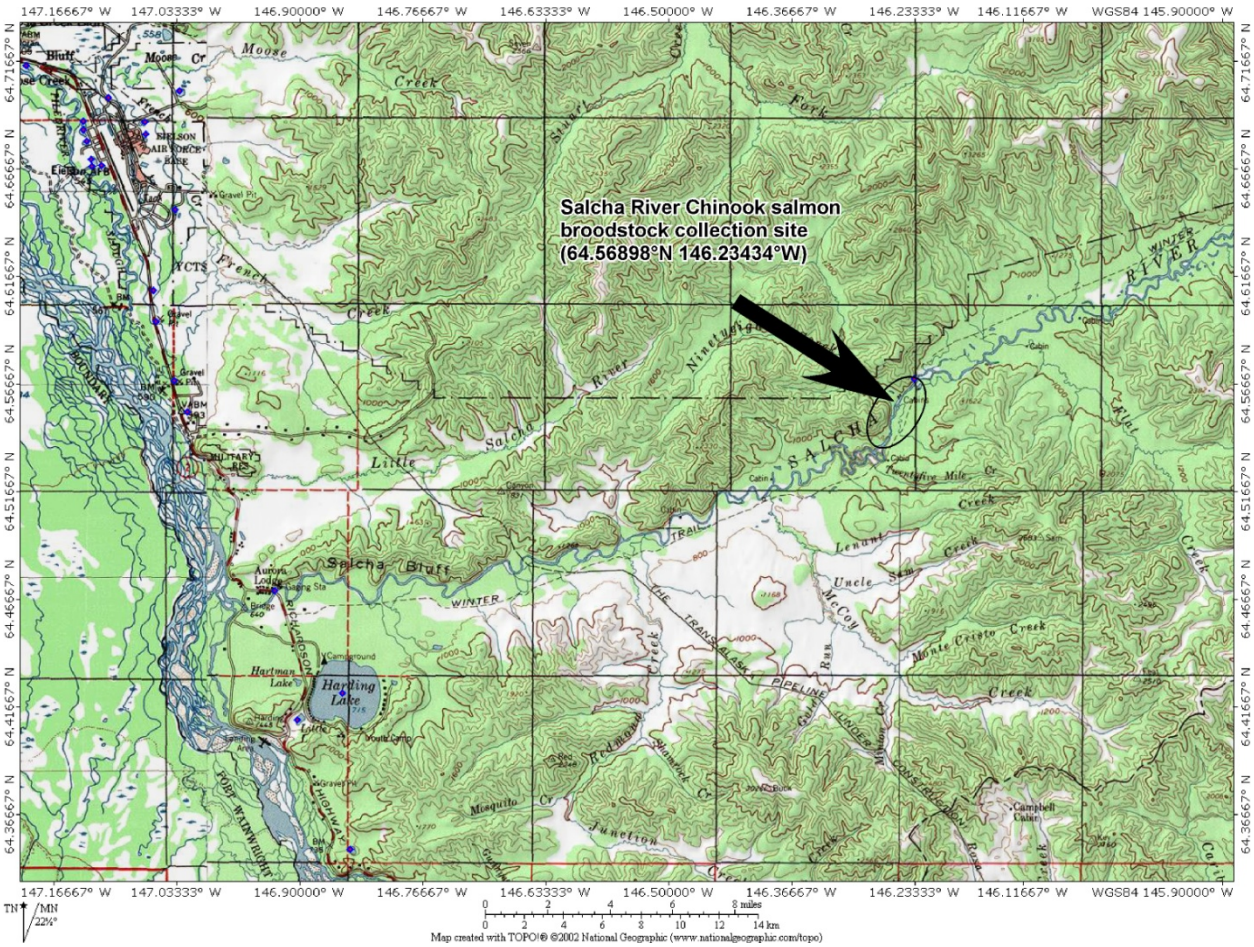


Figure 3.–Location of Chinook salmon capture area on the Salcha River.

Gamete Collection and Fertilization Procedures

We will follow procedures described in *Generalized Egg-Take Procedures for ADF&G Sport Fish Hatchery Program* (Appendix A). Prior to fertilizing eggs, we will visually inspected each donor pair for *Ichthyophonus*. Eggs from one female will be fertilized with milt from one male. A male will not be used to fertilize the eggs from multiple females.

Family Tracking and Disease Screening

Family tracking of donors is required along with additional disease screening of the wild population. We will follow procedures described in *Collection Protocol for Routine Broodstock Examination for ELISA/FAT Kidney Samples* (Appendix B) and *Sample Collection for Routine Broodstock - Examination for Virology* (Appendix C). We will take samples from all donors for disease screening and family tracking.

Table 4.–Sample size for disease screening and family tracking of Chinook salmon, Salcha River, 2014.

	Kidney	Ovarian Fluid
Male	all fish spawned	
Female	all fish spawned	all fish spawned

COHO SALMON

Region III SFP Requirement

A total of 86,500 fingerling (4g) coho salmon are required for stocking Region III lakes in 2014.

Donor and Gamete Objectives

About 106,700 fertilized eggs are needed which will require at least 27 females and an equal number of males. These numbers were calculated using fecundity and hatchery survival rates listed in Table 3. All females will be screened for disease (family tracked) and eggs from fish identified having disease will be discarded. Up to 32 females and an equal number of males may be required to meet the objective of 86,500 fertilized eggs after disease screening.

Background

The Region III Sport Fish staff captures and holds coho salmon for gamete collection to support Region III stocked fisheries programs. They also assist staff from the Ruth Burnett Sport Fish Hatchery to artificially spawn the fish. The Delta Clearwater River population of coho salmon was selected by fishery managers as the primary donor to provide gametes for production at the Ruth Burnett Sport Fish Hatchery.

The capture event will happen during week 1 or 2 in October. The capture area is about 1 km below the Delta Clearwater River State Park (Figure 4). We will capture the fish with a beach seine. The spawning condition of most female coho salmon at this time ranges from pre-spawner to ready-to-spawn. Most males are ready-to-spawn. The fish will be held in net pens until the females are ready to spawn. Females will be sorted for spawning condition and placed in separate net pens. Green females will be checked every second day for maturity. Males will be held in a third net pen.

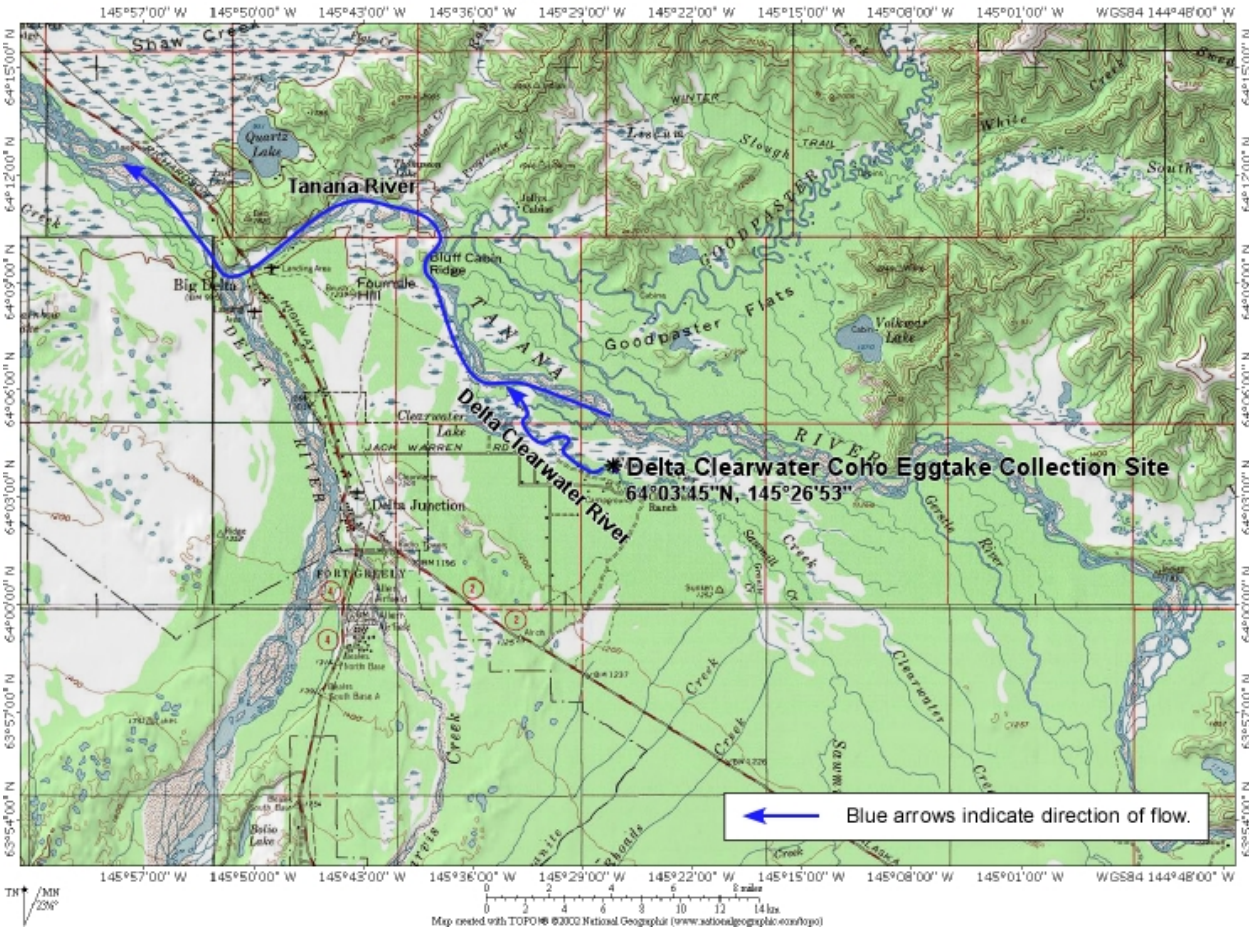


Figure 4.–Location of coho salmon capture area on the Delta Clearwater River.

Fish will be held in net pens near the capture site until ready to spawn. For gamete collection the fish will be transported up river to the Delta Clearwater River Park. Eggs will be fertilized on site or eggs and milt will be stored separately for later fertilization at the hatchery. The fertilized eggs or separated gametes will be transported on the road system to RBSFH.

Gamete Collection and Fertilization Procedures

We will follow the procedures describe in *Generalized Egg-Take Procedures for ADF&G Sport Fish Hatchery Program* (Appendix A). Eggs from one female will be fertilized with milt from one male. A male will not be used to fertilize the eggs from multiple females.

Table 5.–Donor and egg requirement worksheet for coho salmon egg take, Delta Clearwater River, 2014.

Fingerlings to stock in 2014	86,500
Survival Estimates:	
Egg to Eye	88%
Eye to Ponding	97%
Fry to Fingerling	95%
Total Number of Eggs Need:	106,700
Females Required:	
Fecundity - Eggs/female	4,000
Total females required for spawning	27

Family Tracking and Pathology

Family tracking of donors is required along with additional disease screening of the wild population. We will follow procedures described in *Collection Protocol for Routine Broodstock Examination for ELISA/FAT Kidney Samples* (Appendix B) and *Sample Collection for Routine Broodstock - Examination for Virology* (Appendix C). We will take samples from all donors for disease screening and family tracking.

Table 6.–Sample size for disease screening and family tracking of coho salmon, Delta Clearwater River, 2014.

	Kidney	Ovarian Fluid
Male		
Female	all fish spawned	23

FISH TRANSPORT PERMITS

These permits authorize ADF&G staff to capture, hold, spawn, and transport life fish and eggs as specified in *5 AAC 41.005. PERMIT REQUIRED. (a) Except as otherwise provided, a person may not transport, possess, export from the state, or release into the waters of the state, any live fish unless the person holds a fish transport permit issued by the commissioner (or authorized designee), and the person is in compliance with all conditions of the permit. (b) A fish transport permit authorizes only that operation specified in the permit. Any change of species, broodstock, or location requires a new permit. Any other change requires an amendment to the permit.*

Species	Hatchery	Permit Number	Valid Dates
Arctic Grayling	RBSFH	10A-0047	1/1/2010–12/31/2016
Arctic Grayling	WJHSFH	12A-0106	1/1/2012–12/31/2016
Chinook Salmon	RBSFH	10A-0038	1/1/2010–12/31/2014
Coho Salmon	RBSFH	10A-0043	1/1/2010–12/31/2014

SCHEDULE AND DELIVERABLES

All statistics from donor capture and spawning (e.g. capture dates, number of fish held and spawned, number of eggs collected, etc.) will be maintained at Fairbanks regional office and Ruth Burnett Sport Fish Hatchery. An annual performance report will summarize the results of this project.

CHENA RIVER ARCTIC GRAYLING EGG TAKE:

Dates	Activity
14–18 Apr 14	Inspect and prepare equipment.
21 Apr – 2 May 14	Inspect Chena River for ice status and migrating Arctic grayling.
5–9 May 14	Capture and hold Arctic grayling.
12–16 May 14	Egg take.

SALCHA RIVER CHINOOK SALMON EGG TAKE:

Dates	Activity
1–11 Jul 14	Inspect and prepare equipment.
14–25 Jul 14	Capture and hold Chinook salmon.
26–31 Jul 14	Egg take

DELTA CLEARWATER RIVER COHO SALMON EGG TAKE:

Dates	Activity
22–26 Sep 14	Inspect and prepare equipment.
6–10 Oct 14	Capture and hold coho salmon.
9-10 Oct 14	Egg take

These dates can shift forward or backward by one week depending on ice conditions, run timing, and maturation rates.

RESPONSIBILITIES

List of Personnel and Duties:

Cal Skaugstad: Fishery Biologist III, Supervisor for Stocked Fisheries Program - Region III. Fairbanks.

Duties: Supervision of all aspects of fish capture and gamete collection from wild donor populations in Region III. Participate in field work to capture, hold, and spawn fish. Coordinate with area managers (Commercial Fisheries and Sport Fish divisions) to determine if wild populations can support egg takes.

Supervise fieldwork to capture, hold, and spawn fish. Supervise work to monitor river conditions, prepare and store equipment. Direct and participate in field work to capture, hold, and spawn fish.

April Behr: Fishery Biologist II, Project Supervisor for Stocked Fisheries Program - Region III. Fairbanks.

Duties: Supervise fieldwork to capture, hold, and spawn fish. Supervise work to monitor river conditions, prepare and store equipment. Direct and participate in field work to capture, hold, and spawn fish.

Not filled: Fishery Technician II, Stocked Fisheries Program - Region III. Fairbanks.

Duties: Assist with fieldwork, equipment preparation and storage, egg take, and tissue sample collection.

Staff from RBSFH: Hatchery staff to assist with capture and to supervise and conduct egg-take activities. Provide all equipment necessary to conduct each egg take, and collect kidney and fluid samples. Transport fertilized Arctic grayling, and Chinook and coho salmon eggs to RBSFH.

Staff from Region III Stocked Fisheries Program: Transport fertilized Arctic grayling eggs scheduled for Region II to Fairbanks International Airport for flight to Anchorage.

Staff from WJHSFH: Retrieve fertilized Arctic grayling eggs from Anchorage International Airport and deliver to WJHSFH.

REFERENCE CITED

Loopstra, D., and P.A. Hansen. 2010. Induction of triploidy in Arctic grayling (Thymallus arcticus) using hydrostatic pressure. Alaska Department of Fish and Game, Fishery Data Series No. 10-55, Anchorage.

APPENDIX A:
GENERALIZED EGG-TAKE PROCEDURES FOR ADF&G SPORT FISH HATCHERY PROGRAM

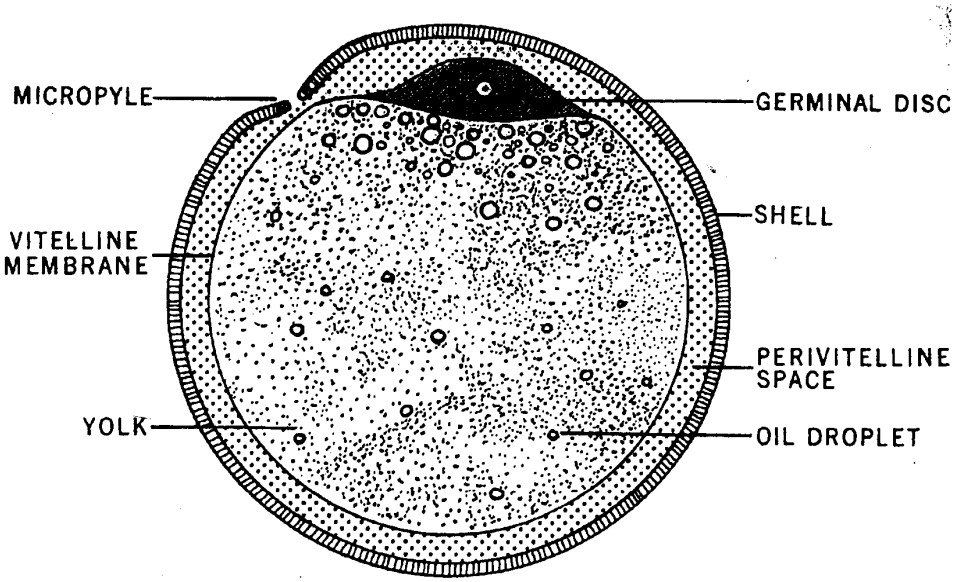
GENERAL EGG-TAKE PROCEDURES FOR ADF&G SPORT FISH HATCHERY PROGRAM

Proper planning and execution of an egg take is the first step in a successful fish production program. Poor quality eggs produce poor survival and poor quality fry. To help insure that all planning and egg-take work is done as effectively as possible, there should always be one designated supervisor for each egg-take project. This person is given the responsibility and authority to insure that quality eggs are collected whenever possible. It is then incumbent on the supervising staff member to become familiar with all aspects of a quality egg take and to keep this information in mind when making decisions. It is always appropriate to stop an egg take when questions arise. The following is an overview of current egg-take issues and procedures. These procedures are not carved in stone but there should be thorough review before any changes are made. Each egg-take location represents a unique set of circumstances and should be evaluated on a case by case basis for logistics though the basic biology remains the same.

REVIEW OF FERTILIZATION AND EARLY DEVELOPMENT

This section discusses aspects of fertilization and early embryology in salmon that are particularly important to egg fertility and survival to the eyed stage. For a more complete review see *Trout and Salmon Culture*, Earl Leitritz and Robert C. Lewis, 1980, Agricultural Sciences Publications, University of California, Berkeley, California 94720.

Schematic drawing of a salmon egg.



Cross sectional diagram of a salmon egg. (Source: Piper, R. G., et al., 1982. Fish Hatchery Management. United States Department of the Interior, Washington, D. C.)

The shell of the egg is rigid, but is porous to water. Sperm enters the egg through the micropyle in the shell. The perivitelline space surrounds the yolk and receives colloidal substances that are extruded from the yolk as soon as the egg comes in contact with water. By absorbing water, these colloidal substances enhance the diffusion of water into the perivitelline space, creating the

process of water hardening. Within 20 or 30 minutes of being placed in water, the volume of the egg will have increased by about 20% (its maximum size) as the colloids continue to absorb water and expand the perivitelline space. The micropyle is closed very early in the water hardening process.

After passing through the micropyle, sperm unites with the nucleus of the egg. Only one sperm cell can unite with the nucleus. The germinal disc is present in the egg before fertilization. Once the egg is exposed to water, the germinal disc becomes more pronounced. After the egg has been exposed to water for several hours, the germinal becomes quite pronounced whether or not fertilization has occurred. An unfertile germinal disc will remain in this condition unless the vitelline membrane is disrupted.

The vitelline membrane encases the yolk. This membrane is very sensitive to physical shock, especially as water hardening progresses. After water hardening is complete, the membrane is slightly less sensitive for several hours after which it becomes extremely sensitive again until the embryo reaches the eyed stage. If the vitelline membrane is disrupted, water will enter the yolk, the yolk will coagulate and the egg will be destroyed.

Sperm cells are relatively inactive and long lived while in seminal fluid. However, once they are exposed to ovarian fluid or water, they become more active and their lives are much shorter. In water, sperm cells swim very actively for about 15 seconds, then they begin to slow down. Within 90 seconds, most sperm cells are dead. Sperm cells are less active and longer lived in ovarian fluid. When eggs are in the presence of sperm, fertilization can occur either in ovarian fluid or water.

The first division of the zygote usually takes place within the first half day. The second division, the 4-cell stage, occurs between 5.5 and 6.5°C temperature units (CTUs) for Pacific salmon, and at 5.0 CTUs for rainbow trout. A CTU is 1°C for 24 hours. Thus a water temperature of 10°C for one day results in 10 CTUs. Or, 10°C for 14.4 hours is equal to 6 CTUs. As development progresses, the variation in development rate between different individual eggs increases. After the 4-cell stage, the variability is great enough that it is difficult to sample fertility until the somite is formed.

As the embryo develops, more cells are added to the vitelline membrane and it gradually becomes sturdier. When an egg reaches the eyed stage, the membrane is very resilient to physical shock. The membrane of infertile eggs or any eggs that died during early development will still be very sensitive. In many cases, the membrane in these eggs may have already ruptured causing the yolk to coagulate and turn white.

CRITICAL FACTORS AFFECTING FERTILITY AND EARLY SURVIVAL

The purpose of this section is to summarize the important factors impacting egg quality at egg take.

Success in achieving egg-take quality goals is measured by 4-cell stage fertility samples and by green egg to eyed egg survival. The final definitive measure of egg-take quality is the green egg to eyed egg survival.

Experience from the past demonstrates that essentially all the mortality measured at the eyed stage results during egg take. That is, of the small percentage of eggs in the hatchery that are dead at the eyed stage (3% to 3.5%), most were either infertile or were damaged during handling at egg take. This is not to say that there is a problem with the egg take. On the contrary, because the incubation system works so well, essentially all the live eggs placed in the hatchery at egg take, live to the eyed stage. Therefore, the green to eye survival may be used as a measure of egg-take quality. A production scale egg take that results in total survivals of 96% to 97% year after year is outstanding. This type of consistent performance requires careful attention to the mechanisms of infertility and egg mortality.

Infertility and physical damage are the two major causes of mortality at egg take. Let's examine infertility first. Infertility occurs when healthy sperm are unable to unite with the nucleus of a healthy egg. The major causes of infertility are listed below.

1. ***Eggs come into contact with water before fertilization, water hardening begins, the micropyle closes, and sperm cells are unable to enter the egg.*** Literature suggests that the micropyle closes within less than three minutes from the time water contacts the egg. This may occur either after the eggs are out of the female, or while the eggs are still in a fish that is dead and has been lying in water.
2. ***Foreign substances block the micropyle.*** Cytoplasm from broken eggs is thought to be the major cause of micropyle blockage. Some sources contend that blood and other tissues can also cause blockage. Foreign organic tissue also acts as a substrate for fouling fungus during incubation.
3. ***Sperm comes into contact with water prior to being introduced to eggs.*** Fifteen seconds after introduction to water, sperm cells begin to slow down. After 90 seconds, most cells are immotile.
4. ***Prior to the closing of the micropyle, eggs are only exposed to seminal fluid from an infertile male.***
5. ***A female has become over mature, the viability of her eggs is reduced***. Water may have entered a portion of the body cavity and some eggs may have started to water harden.
6. ***A fish is killed and is left lying around for a long time before spawning.*** Gametes are living cells and require oxygen. As the tissues of the fish become anoxic, the gametes loose viability. Sperm cells are especially sensitive to anoxia.

Physical damage to the egg can be caused by physical shock to the vitelline membrane, sunlight, excessive artificial light, or extreme temperature fluctuation. The major sources of physical damage at egg take are discussed below.

1. ***Physical shock and rupture of the vitelline membrane can occur at anytime, even in the fish.*** But the membrane becomes especially fragile within minutes of the first contact with water. For practical purposes this extremely sensitive period continues until the early eyed stage. If absolutely necessary, eggs may be handled in the incubators very gently for the period that extends from one hour after fertilization to six hours after fertilization. If eggs must be handled expect an increase in mortality.
2. ***Direct sunlight will destroy either fertilized or unfertilized eggs in minutes.*** Some sources claim that excessive exposure to direct artificial light, especially florescent light, can also damage eggs.
3. ***During the sensitive period, extreme temperature changes can damage the egg.*** Try to keep temperature changes to less than 2°C per hour.

Several general **procedural criteria** emerge from the analysis above.

1. Fish should be handled carefully.
2. Minimize the time between gamete removal from the fish and loading fertilized eggs into the incubator.
3. Sperm and eggs should be mixed together immediately after leaving the fish.
4. Keep debris and organic matter out of the gametes.
5. Sperm from the males should be added to each bucket of eggs. A male:female ratio of 1:1 or 2:3 should be maintained for each bucket. Functionally there can never be too much sperm added to the buckets of eggs. However, operationally too much sperm causes delays in the rinsing process. The number of males and females per bucket will vary with each species being spawned.
6. Eggs should be handled carefully, especially after water has been added.
7. The time between adding first water at the rinse tank and loading eggs in the incubator should be minimized.
8. Each female's eggs should be inspected for the presence of water hardened eggs before they are accepted and poured into the community bucket.
9. The eggs from each female should be mixed with the sperm from several males.
10. Never allow eggs to come in contact with direct sunlight. Limit exposure to artificial light as much as is practical.

The procedures presented in the rest of this chapter have been designed with the above criteria in mind.

EQUIPMENT LIST

The following equipment list is what is needed for one standard egg take. Special procedures such as kidney sampling are covered later, and their equipment list is covered in section on those procedures. Equipment should be inventoried and examined 2–3 weeks prior to the planned egg take to allow adequate time to replace or repair damaged or missing equipment.

All Egg Takes	**Quantity**
Agentyne (Any appropriate Iodophor)	2 gallons
Spawn table	1
Heavy Duty Production Dip Net 17" x 15" Small D	2
Egg Buckets With Lids	Enough for all eggs
Spawning Pans	2
DO Meter	1
Un-iodized salt (Sodium Chloride fine)	10 lbs
Chest Waders	2
Shelter for Spawn Station & Fertilization	1
Paper Towels (minimum)	3 rolls
Cloth Diapers to Dry Buckets & Pans	4
Cotton Gloves	2 dozen
Rubber Wristers	5
Zak Knives	5
Replacement blades for Zak Knives	1 dozen
Contained area for green hens	1
Killing Clubs	3
Thermometer	1
Batteries, electrolyte solution, & membrane for DO Meter	1 set of spares
Rack for Fish to be Spawned	1
Gauntlet style rubber gloves	14 pr.
Gott cooler for saline solution	1
Remote Egg Takes	
Pathogen Free Water	Enough for all fertilization & rinsing
Add'l Chest Waders or Hip Boots	1–10 Depends on situation
Thermometer, spare	1
Coolers, ice, and a pad between the ice & eggs	Enough to transport all eggs
Egg bags & rubber bands	Enough for all eggs

Sponges (Grayling & Char only)	4
Spawn hoops (Grayling & Char only)	4
Onsite Arctic Grayling Egg Takes	
Egg Collection Nets	2
Anesthetizing agent	1 bottle
Pressure Shocking Equipment	1
Sponges	4
Spawn hoops	4
Whirl pak bags, 2 oz	100

GENERAL EGG-TAKE PROCEDURES

Manpower requirements: These will vary somewhat depending on the design of the brood holding area, and its proximity to the spawning area. The manpower listed here is only a guide, to be adapted to each situation:

Brood sorters	2
Transport fish to spawn station	1
Spawn station	2
Fertilization, rinse, incubation	1
Fecundity, egg size sampling	1
Total	**7**

Triploid production	1–2
XX male gamete harvest	3–4
Pathology sampling	2–3
Total	**6–9**

All egg takes should follow the same general procedure to ensure the best fertility rates. Differences for each species will be noted as that species is discussed.

Holding Brood: Ideally broodstock will be held in a raceway while they mature. If a raceway is not available (such as at a remote location), a holding pen may be used. Crowding and sorting brood is easiest in a raceway with good fitting crowders, but holding pens may be made to work when necessary. Whatever the holding container, several parameters are important to monitor while holding brood for maturation.

- ***Oxygen levels:*** Remember that the gametes are living cells, and require adequate levels of oxygen to remain viable. Broodstock held in low oxygen conditions will become stressed, and this may affect gamete viability, or result in loss of broodstock. Flows and fish loading rates should be adjusted so that dissolved oxygen is not allowed to get below 8.0 ppm. This should be monitored on a daily basis.
- ***Brood mortality:*** Broodstock mortalities should be removed from the holding container and enumerated daily. Females may be cut open and examined for ripeness. This will help give an indication of when to start egg collection. If brood are dying due to a fungal, bacterial, or viral infection it is important to remove them as soon as possible, to prevent spreading the infection to the remaining brood, and possibly passing it on to the eggs. In cases of large numbers of morts, the mort accumulation on the end screen can decrease water flow out of the container. In a raceway this can result in water backing up and flooding out of the raceway. In a holding pen this can decrease the water exchange through the holding pen. Both of these situations will decrease oxygen in the holding unit, and may cause further mortality.

Sorting Brood: Ideally only ripe females are chosen for spawning. Workers inexperienced in checking females for ripeness should work with more experienced workers until they have a

good feel for checking. If there are questionable females, it is acceptable to send one or two to the spawning station and ask for feedback from the spawners. The brood sorters should send males to the spawners in a male:female ratio of 1:2 for salmon. For Arctic Char and Arctic Grayling the ratio should be 1:1, and for rainbow trout the ratio is three females to one male. All brood fish should be handled carefully, especially the females. **Careless tossing of females can break eggs, or cause internal bleeding. Blood clots, egg shells and yolk can impair fertilization by blocking the micropyle, thus preventing sperm from entering the egg.** Sorters must remember that eggs and sperm are living cells and require oxygen to remain viable. It is not acceptable to spawn a fish that has been dead for over 15 minutes (sperm is more sensitive to oxygen starvation than eggs are) For this reason the sorters must monitor the spawn station's pace, and not get too far ahead of them.

When sorting females, handle them carefully. As females ripen the body wall will become somewhat thinner. If the eggs are still in the skein the belly will feel hard. If the eggs are loose the belly will appear softer and looser. If ripe, the ventral surface should be round, and there may be some sagging around the vent. If green, the ventral surface may still be round, but will be harder.

A suitable holding place should be designated for the green hens. This should have adequate water exchange, be large enough to hold the anticipated number of green hens, and should be close enough to the sorting area so that the fish can be placed into the green holding pen. Tossing fish from a distance is not acceptable. Someone should be responsible for checking the green holding area periodically to ensure that it is not becoming over crowded, that the fish are in good condition, that oxygen concentrations remain above 8.0 ppm, and that the pen is secure.

Spawning: The goal of the spawners should be to insure that each egg is able to come in contact with viable sperm at fertilization. The following considerations will help to obtain optimal fertilization rates:

- Only use dry buckets to spawn into. Look around for splashing and make sure that no water is being splashed into any container that is holding gametes. Any water will cause sperm activation, and start the egg water absorption process. If the egg and sperm are not in direct contact with each other when they encounter water fertilization will not occur.

- Alternate males and females into the bucket to maximize the chances of egg-sperm contact. Once the correct number of fish have been spawned into the bucket, give the eggs and sperm a gentle stir with a bare hand. Be sure to mix up the eggs from the bottom of the bucket, and ensure that if the sperm becomes accidentally activated that the chance of egg-sperm contact is maximized.

- If a female is spawned and it is observed that over 10%–20% of the eggs are bad, it is preferable to discard all of the eggs from that female, if possible. There will probably be more eggs that have started absorbing water that are not visible yet. Dead eggs in an incubator provide the fungus *Saprolegnia sp*. a near perfect media to grow on. This fungus can also suffocate live eggs, if it gets out of hand within the incubator. For this reason it is preferable to not use any eggs from a female that has a significant number of bad eggs visible. If broodstock are abundant, it may be worth considering tightening up on the bad egg criteria even further. If female broodstock are limited, and/or fecundity is less than anticipated it may become necessary to take the eggs from females with a higher number of bad eggs, and then deal with the increased fungal clumping during incubation later.

- Keep gametes away from direct sunlight. The UV rays from the sun can damage both eggs and sperm. Spawning should take place in some sort of enclosed area, with protection from sunlight and the elements. In a remote setting a shelter can be made using tarps, or a portable awning or a tent.

- When spawning males, first send a test squirt of milt onto the floor to look for water or bile. If water is present continue sending milt to the floor until it is thick and all white. Only at this point should the milt be added to the egg bucket. If little or no good milt is obtainable from a male use a second male before adding any more eggs to the bucket. For spawning char and grayling, this step is not practical, as their milt is less abundant. For these species it is acceptable to squirt the milt directly into the bucket of eggs.

- **If gametes are being collected separately for fertilization back at the hatchery:** The milt should be collected in a dry whirlpak bag. No water should be allowed to enter the bag, and the bag of milt should be shielded from direct sunlight, splashing, and rain. Eggs should be collected in a dry zip lock bag or dry bucket. Ensure that no water enters the egg container, and that the eggs are protected from rain, splashing, and direct sunlight. The gametes should be stored in a cooler as soon as possible after leaving the fish. Ensure that the gametes are not stored directly on ice or snow, as this will cause them to cool down excessively.

Spawning salmon: Since salmon only spawn once in their lives, and then die, salmon are killed prior to spawning, usually by a blow to the head. To reduce the amount of blood in the eggs it is helpful to bleed the females prior to spawning. This is done by either cutting across the gills, or cutting across the caudal peduncle, and allowing the fish to bleed for a few minutes before being spawned. It is helpful to put the end of the fish that was cut downhill to facilitate the flow of blood. To spawn the females insert the zac knife into the vent and cut straight up, going around the pelvic fins. Be sure that the point of the zac knife is in contact with the body wall while cutting upward. If the point is held toward the skein it may puncture eggs while cutting upward. If the female is ripe and ready to spawn the eggs will fall out, if she is not quite ripe it may be necessary to gently tease the eggs out of the skeins. The carcasses are disposed of after spawning. Dog mushers may be contacted about taking the carcasses to feed their dogs. If the fish have been coded wire tagged, it is necessary to scan for adipose clips, and remove the heads of fish with clipped fins.

Spawning Arctic Grayling: As grayling are repeat spawners, all of the handling concerns for char and rainbow also apply. Currently grayling are live spawned by holding the fish securely and squeezing the eggs out of the vent of the female, or the milt out of the male's vent. No anesthetic is currently used on the grayling. With practice, an experienced spawner can hold the fish securely for the removal of the eggs without subjecting the fish to the additional stress of the anesthetic. Future consideration of the use of anesthetic and air spawning techniques should be evaluated carefully. It is possible that these techniques may cause the fish more stress, and result in a lower recovery rate from spawning, than current methods.

Each bucket of grayling eggs contains the gametes from five females and five males. After water hardening four buckets are put into one shipping container for transport to the hatchery for incubation (one shipping container contains eggs from 20 females). Grayling eggs are especially sticky, and care must be taken to ensure adequate mixing of eggs, sperm, and water at fertilization. Be sure that no eggs are sticking to the corners of the bucket during fertilization.

It is important to keep an accurate count of the number of fish handled. Males, ripe females, green females killed, and green females set aside for later spawning must all be counted, in addition to mortality of both males and females.

One bucket of eggs should equal one Heath Tray incubator. The number of fish in the bucket will vary with the species being spawned. The numbers below are guidelines, and may be adjusted for unusually high or low fecundity.

Species	# Females per Bucket
Rainbow Trout	10–12
Arctic Grayling	5
Chinook Salmon	3
Coho Salmon	3

Once filled, if the bucket of eggs and milt must be moved outside at all between spawning and loading into an incubator a lid should be placed over the bucket to keep out any rain and/or direct sunlight.

Fertilization and Rinsing: Once water is added to a bucket, the sperm are only active for less than one minute. It is important that when the sperm first encounter water that there are green eggs nearby ready to fertilize.

1. To avoid temperature shocks the water used for fertilization and rinsing should be within 2 degrees Celsius of the temperature of the eggs.
2. Gently add water to the bucket. Avoid hard flows and splashing of water hitting the eggs directly. It is best to let the water flow along the sides of the bucket and upwell under the eggs. This method provides the best mixing action, with the least force directly hitting the eggs. Add only enough water to cover the eggs by ½" to 1". Adding too much water will dilute the sperm, and adversely affect fertility. A saline solution may be used for activation to enhance sperm motility. The saline solution is made by adding 7 grams of salt (sodium chloride) to each liter of water. The saline solution not only makes the sperm more active, but they remain active for a longer period of time. This can significantly improve fertilization rates. The saline solutions temperature should be monitored. The use of 5 gallon Gott water coolers maintains constant temperature throughout the egg-take day.
3. Wait one minute for fertilization to take place.
4. Rinse out excess sperm, blood, egg shells, and any other debris that may be in the bucket at this time. This may take several rinses. As with fertilization, be careful to not hit the eggs directly with a hard flow of water. Gentle upwelling provides the best rinsing, and is best for the eggs.
5. After the eggs are rinsed add enough water to nearly fill the bucket for water hardening. For onsite egg takes the eggs can be loaded directly into incubators as described below. For remote egg takes place a lid on the bucket and set it in a dark or shaded place where it will not be disturbed while the eggs absorb water.

6. Put a tag on the bucket lid indicating the time that the bucket was set down. Water hardening takes approximately 2 hours, and is temperature dependant. It may take somewhat less time in warmer water, and longer in colder water.
7. If the eggs are to be shipped to the hatchery from a remote location, keep in mind that the temperatures should not change by more than 2°C. <u>If the eggs are to be shipped on ice, make sure that the egg temperature does not drop below 5°C.</u> Chinook eggs are especially sensitive to cold temperatures early on in their development, and can experience developmental problems if they are exposed to temperatures below 5°C in the first 60 TUs following fertilization. (The 5° rule will not apply with coho and char since their water temperatures are already at or below that level.

Incubator Loading: As with fertilization and rinsing, be sure that the incubators have been set up with water that is within two degrees Celsius of the egg temperature, so as to not temperature shock the eggs. Flows should be set at 5 gpm for each half stack. It is standard practice to disinfect all eggs after water hardening for 15 minutes in a 100 ppm iodine solution. This is especially important when receiving eggs from a remote location, and is good practice, even when processing eggs from an on-site egg take.

1. Mix up a barrel of 100 ppm iodine solution to disinfect the eggs. It is wise to have two different people independently double check the calculation for making this solution. Again, the water used must be within 2°C of the egg temperature.
2. Pull out the Heath tray to be loaded, and pour out the water, setting the top screen out of the way (on top of the stack is convenient).
3. Pour in enough of the iodine solution to fill up the tray approximately half way.
4. Decant all extra water out of the bucket of water hardened eggs. A perforated screen which fits in the bucket its useful for this process. Chinook salmon eggs arrive in plastic bags, instead of buckets. For Chinook eggs, the plastic bag is emptied into a cooler containing a net and filled with water that is within 2°C of the eggs. The incubator tray is set in the sink in the arrival area. The sink is partially filled with the 100 ppm iodophore solution. Eggs are removed from the cooler using a pre-marked colander and put into the incubator tray in the iodophore solution in the sink. The iodophore should just cover the eggs. Currently, three full colanders, and a fourth colander marked up to the line are loaded into one incubator tray. This should equal approximately 7,500 green Chinook salmon eggs. The tray is then carried into the incubation room and set into the iodine solution in the incubation stack, as outlined below.
5. Gently pour the eggs into the incubator with the iodine solution. It is all right to gently even out the egg distribution in the tray with your hand. Make sure that all eggs are covered by the iodine solution.
6. Push the tray back in far enough to get it out of your way, but not so far that any water flows through it. The incubator is to remain in this position for 15 minutes for disinfection to take place.
7. Mark the incubator with the time that disinfection is complete (15 minutes from when the eggs were added to the iodine solution).

8. When disinfection is complete, push the incubator the rest of the way into its stack. Watch that water starts to flow, and that the iodine is being rinsed out.

Clean Up: When the egg take is complete for the day all racks, tables, buckets, lids, pans, Zak knives, etc. must be washed to remove any organic material, and disinfected with 150 ppm iodophore solution, and rinsed with pathogen free water.

FECUNDITY SAMPLING

Accurate fecundity sampling is critical to ensure that egg-take goals are met and that survivals are good. If the sample indicates a lower than average number of eggs from each female then the egg take will fall short and production goals will not be met. If the sample indicates more eggs than the historic average from each female then incubators may become overloaded, and the entire crew will work longer and handle more fish than necessary.

Obtaining an accurate sample requires that the sample come as close to representing average production as is possible. The steps below for onsite egg takes will help to ensure that the sample is as accurate as is reasonably possible.

1. Tare the scale to a production egg-take bucket. Ensure that the bucket is completely dry when the scale is tared.
2. Give the pre-tared bucket to one of the spawn teams, and let them know that it is a sample bucket so that it does not get sent on for fertilization.
3. Ensure that the spawn team is using a random sample of fish, not especially large or small.
4. Ensure that the count of females spawned into the bucket is accurate.
5. For the purposes of the sample bucket, partial females should be avoided.
6. Weigh the bucket with eggs on the pre-tared scale and record the weight.
7. Stir the eggs with a dry hand to evenly mix eggs and ovarian fluid.
8. Fill each sample cup with approximately 100 eggs.
9. Return the bucket to the spawn station for addition of milt and fertilization.
10. Weigh the sample cups with eggs and record the total weight of the cup and eggs. After pouring out the eggs, weigh the empty cup. Use this weight as a tare weight. This ensures that any ovarian fluid clinging to the outside of the cup is included in the tare weight.
11. Count the number of eggs in each cup.
12. Determine the grams of eggs in each cup by subtracting the weight of the cup without eggs from the weight of the eggs and cup together.
13. Determine the average number of eggs per gram by dividing the sum number of eggs by the sum number of grams of eggs in the three samples.
14. Determine total number of eggs in the bucket by multiplying the eggs per gram by the total grams of eggs in the bucket.
15. Determine eggs per female by dividing the number of females spawned into the bucket into the total number of eggs in the bucket.

16. A minimum of one sample should be obtained for each two hour shift of egg collection, and a minimum of three samples should be taken for each day of spawning.
17. If there is more than one team spawning, be sure to sample from both teams. One team may be getting more eggs out of each female than the other.

Modifications for remote egg takes: There have been some occasions of increased mortality observed when eggs are sampled back at the hatchery after a remote egg take. It is preferable to sample the eggs at the remote site, if possible, following the procedures above. If this is not possible, then the procedures below can be used.

1. Tare a wet fine meshed net on the hanging scale.
2. Pour a bucket or bag with a known number of females into the pre-tarred net suspended in a cooler filled with tempered process water.
3. Hang the net full of eggs from the hanging scale, out of the water, allowing nearly all of the water to drain out of the net. Take care to not allow the eggs to be exposed to direct sunlight during this step.
4. When all water has drained, record the weight of eggs in the net.
5. Mix the eggs in the net and obtain three samples as described above.
6. The remainder of the steps are as listed above in steps 11 through 18.
7. For a full day's production a minimum of three samples should be taken. If the lot is very small, previous large lots have been adequately sampled, and there is no reason to suspect significant deviation (example: lots of partials or spawn outs), then 1 or 2 samples may be adequate, at the discretion of the fish culturist overseeing the egg receiving. Be sure to check with someone who was at the egg take, to ensure that there were no abnormal circumstances during the egg take, before deciding to decrease the sampling of the eggs.

Modifications for grayling egg takes: Grayling eggs are enumerated by volume instead of by weight. This is because the eggs are so small and sticky that is it easier on both the eggs and the fish culturist to handle them in water.

Enumeration by volume: This procedure is used for water hardened Arctic grayling eggs coming from remote egg takes.

1. A standard colander will be used to scoop a random sample of disinfected eggs from the fresh water bath and placed to drain off the water. The colander should be at least half full (~300 milliliters of eggs by volume), and it should be allowed to drain for at least 60 seconds.
2. A 25 ml. graduated cylinder will be filled with fresh water until the meniscus exactly reaches the 15 ml. level.
3. A spoon will be used to scoop drained eggs into the graduated cylinder from the colander until the meniscus rises at least to the 20 ml. level, but not higher than the 25 ml. level. The remaining drained eggs in the colander will be returned to the fresh water bath. The volume of water displaced by the added eggs will be recorded (e.g. 20 ml. (final meniscus reading) minus 15 ml. (original meniscus reading) + 5 ml. (volume of the water displaced by the drained eggs)).

4. The eggs in the graduated cylinder will then be spread out on a Heath tray lid screen and hand counted. The number will be recorded, and the sample eggs will be returned to the fresh water bath. Using the recorded figures for volume of water displaced and the number of eggs in the sample, the number of eggs per milliliter will be calculated (e.g.171 eggs/5ml. of water displaced=34.2 eggs/ml.) and recorded.
5. Steps 1 through 4 will be repeated a minimum of three times throughout the process of seeding the eggs into the incubators, taking care that the samples are random and from different baskets of disinfected eggs. All samples will be averaged and if any sample values are not within 5% of the average, more samples will be taken until an average value for egg volume can be accepted with confidence.

SPECIAL CONSIDERATIONS FOR BACTERIAL KIDNEY DISEASE

Bacterial Kidney Disease (BKD) has been a problem in Chinook salmon, coho salmon, and rainbow trout. It has been detected in Arctic char, and Arctic grayling, but to date, no epizootic has been observed in the latter two species. This bacterial pathogen can be transmitted both vertically and horizontally. Because the bacteria has the ability to reside inside the hosts cells, normal antibiotic treatments only reduce the incidence of the bacteria, but are not effective at eradicating it. Elimination of this bacteria requires a multi-pronged approach.

- First, when possible the broodstock should be injected with Erythromycin thiocyanate 2–3 weeks prior to spawning. This will decrease the incidence of bacteria present in the ovarian fluid and in the developing eggs.
- Second, the brood should be family tracked during eggtake. This requires collecting kidney samples from each female and having the ADF&G Pathology lab sample them for the causative agent of BDK (*Renebacterium salmoninarum*) using the ELISA test. Eggs from female brood that tests positive for this pathogen are destroyed.
- Third, progeny from adults that are known to be carrying this bacteria are fed a four week ration of Erythromycin thiocyanate as soon as they are able to consume the #1 crumble feeds.

Rainbow trout: All three of these steps are currently being implemented due to the Fort Richardson Hatchery captive brood rainbow trout being diagnosed with BKD in 2009. In addition, the rainbow trout captive broodstock are having Erythromycin thiocyanate incorporated into their diet each summer for four weeks. It is hoped that by implementing all of these procedures that we can eventually decrease the incidence of this bacteria to a negligible level in the captive rainbow trout brood by the first egg take at the William Jack Hernandez Sport Fish Hatchery (WJHSFH).

Chinook and coho salmon: These egg takes are conducted exterior to the hatchery using broodstock that is returning from the ocean. All females are to be family tracked each year. Currently the incidence of infection is low enough that broodstock injection has not been necessary. Feeding medicated feed to the fry shortly after emergence is only necessary if any progeny from females which test positive for the bacteria are kept in production. Normally, if all eggs from positive females are destroyed, then feeding the medicated feed is not necessary.

FAMILY TRACKING PROCEDURES AT EGGTAKE

Currently all species except the Arctic grayling are family tracked for BKD during egg takes. This requires that each female that is spawned be numbered. The eggs from each female must be tracked by being placed in an appropriately identified (ID number) container for transport and transferred to an appropriately ID numbered incubator. The kidney sample from each female must also be placed into a Whirlpak bag with the corresponding ID number. For family tracking to be effective, great care must be taken to ensure that ID numbers correspond to each step of the egg take (spawning female, kidney sample, transport container, and incubator). This may require slowing the pace of the egg take to ensure that all numbers match at each step of the family tracking process.

For all species eggs from females will be placed in separate transport containers and incubators. If any female tests positive for BKD the entire incubator of eggs is destroyed at the eyed egg stage of development.

When eggs are being family tracked normal egg take procedures are followed. Exceptions are noted below:

- Additional disinfection steps must be implemented during spawning to decrease the possibility of transferring the pathogen between egg transport containers. After eggs have been removed from a female everything that came into contact with the fish (zac knives, needles, spawners hands, etc.) must be disinfected in iodophore before being used on the next female.
- Care must be taken that the eggs are placed in the correct transport container in sequential order.
- Care must also be taken that eggs from each numbered transport container are placed into the same numbered incubator (bucket number 1 goes into incubator number 1, etc.)
- Before passing along any females to the kidney sampling team, the fish must be accurately numbered. This has been accomplished with pre-numbered popsicle sticks placed in the operculum or with pre-numbered zip ties through the operculum in the past. Any system that reliably keeps the number attached to the fish until kidney sampling is complete is acceptable.
- Kidney sampling procedures from the ADF&G Pathology Lab are describe in Appendix B.

Alaska Department of Fish & Game, Sport Fish Division
Eggtake Worksheet

Date: _____ Water Temp: _____

Species: _____ Time Started: _____

Location: _____ Time Completed: _____

Females

Ripe	Green	Bad	Morts

APPENDIX B:
COLLECTION PROTOCOL FOR ROUTINE BROODSTOCK EXAMINATION - ELISA/FAT KIDNEY SAMPLES

COLLECTION PROTOCOL FOR ROUTINE BROODSTOCK EXAMINATION - ELISA/FAT KIDNEY SAMPLES

SUPPLIES

2 oz. (3" x 5") white stripe Whirl-Pak® bags (many other brands of bags split during processing allowing for sample contamination). VWR-11216-772 or Fisher 01-812-6A. Please order your own.

Black VWR or Sharpie markers (other brands and colors wipe off the bag, pens, and pencils can tear the bag).

Tool for kidney removal. We suggest a baby spoon with sharpened edges. Other tools that have been found to work are scalpels and small melon-ballers.

3% iodophor solution (Betadine or Wescodyne) for disinfecting the tools, and a container with a sponge.

Brush for cleaning the tools.

Sample Submission Form to send with the samples.

Cooler for sample transport, with frozen gel packs.

Tray for placing numbered samples in order if sampling is for family tracking

PROCEDURES

1. Wipe all organic material off the tool.
2. Scrub the tool with a brush in 3% iodophor solution.
3. Wipe disinfectant off tool.
4. Cut 2 small pieces of kidney (1 x 1 cm), 1 anterior and 1 posterior, and put them both into Whirl-Pak® bag. The total weight of the sample should be 1–2 grams (use of a scale at first can be helpful to visualize what 1–2 grams of tissue should look like). Individual bags need to be labeled only if you are tracking individual fish (i.e. to discard infected gametes). Ensure that the Whirl-Pak bags are properly closed by pushing out most of the air and then folding the wire tie over the bag several times down the length of the bag. Then secure the ends of the wire ties to each other to hold the folded down bag in place.
5. Wipe any blood or other fluids off of the bag prior to storing it in the cooler
6. Repeat steps 1–3 between each fish so as not to cross contaminate the samples.
7. Single family tracking bags should be placed in order on a plastic tray that holds each bag in place. If this type of tray is not available, samples should be bundled into groups of 10 to facilitate processing at the lab (Ex: samples number 1–10 and 11–20 bundled together).

SHIPPING

Ship samples to the pathology lab fresh on ice or frozen. Samples may be frozen for months prior to shipment. Mark cooler "KEEP COOL." Please remember to call a courier/delivery service to arrange for sample delivery from the airport to the Anchorage or Juneau lab (we have had reliable service with Express Delivery in Anchorage (907) 562-7333). Also call the lab to let them know that samples are en route and the airbill number - **Anchorage Fish Pathology - (907) 267-2244; or in Juneau Fish Pathology - (907) 465-3577**

APPENDIX C:
COLLECTION PROTOCOL FOR ROUTINE BROODSTOCK EXAMINATION – VIROLOGY SAMPLES

COLLECTION PROTOCOL FOR ROUTINE BROODSTOCK EXAMINATION – VIROLOGY SAMPLES

SUPPLIES

Ice chest and cold packs	Screw cap centrifuge tubes VWR-#21008656, Fisher #05-539-5
Paper cups	Iodine disinfectant and/or 70% ethanol
Paper towels	Whirl-Pak® bags
Forceps	Zak® knife, scalpel and blades or filet knife
Labels and marking pen	A sharpened spoon is handy for removing kidney
Large plastic bags	Sample Submission Form

PROCEDURES

Ovarian fluids

1. Disinfect the outside of the fish with disinfectant and dry with a paper towel.
2. Partially strip a single fish's ovarian fluids (ripe or post-spawner) into paper cup avoiding the extrusion of blood and fecal material. Do not handle the lip or inside of the cup.
3. Crimp the edges of the cup to form a spout and pour the fluid into a centrifuge tube. This will "strain out" eggs. Discard cup after each fish.
4. Repeat for a total of at least 60 fish, 65 is preferable.
5. Cap the tubes tightly. Label rack with stock of fish, sample location and date. Place upright in orange rack in cooler with frozen cold packs. Keep cold but do not freeze.

Tissue

Occasionally tissues from adult fish are requested for virology processing. In this case, use disinfection procedures similar to those detailed for ELISA/FAT but include a kidney and spleen (sometimes liver is requested) sample and put them both into Whirl-Pak® bag for each fish.

SHIPPING

Pack all samples in an ice chest with frozen cold packs or ice packaged in a plastic bag. Samples should be packaged in such a way to remain at 4°C or less for a least overnight.

Close, seal, and label ice chest with "refrigerate- do not freeze" and "perishable".

Ship to the Fish Pathology Lab immediately, arranging for the sample to be kept cool. Samples should be sent for processing as soon as possible after collection. Tissues and ovarian fluids can be held at 4°C for up to 5 days. This can be discussed when you first notify the lab that you are taking samples.

Arrange for a courier/delivery service to deliver the samples to the lab from the airport or air charter arrival point (we have had reliable service with Express Delivery in Anchorage (907) 562-7333) and from DHL (907) 789-2187 in Juneau. Tell them the airline, freight or express, time of arrival and airbill number and please notify the **Fish Pathology Section in Anchorage - (907) 267-2244 or in Juneau - (907) 465-3577** of the sample arrival time. Do not assume that the samples will be adequately delivered if you do not specifically talk to a pathology staff person subsequent to sending samples.

We would like to receive these samples fresh if at all possible. Therefore, it would be best to take all of the samples on the same day. To minimize the impact on the fishery, the fish can all be postspawners and the kidneys can be taken from the males as well as the females. The females you sample for ovarian fluid can only be released if they are marked so that you do not sample the same fish a second time. If you take the samples early in the week they have a better chance of getting to the lab without complications. Let us know when you will be taking the samples if there are any potential problems, and we will get things worked out between us. Good luck and happy fishing.

APPENDIX D:
TRIPLOID INDUCTION WITH HYDROSTATIC PRESSURE
- DRAFT -

TRIPLOID INDUCTION WITH HYDROSTATIC PRESSURE - DRAFT –

Diane Loopstra (In Draft). Alaska Department of Fish and Game, William Jack Hernandez Sport Fish Hatchery, Anchorage.

Three variables (other than condition of the gametes) affect the success of inducing triploidy with hydrostatic pressure: Duration of shock, pressure used, and Centigrade Temperature Minutes (CTMs)—the number of minutes from fertilization to shock initiation x water temperature. These variables differ amongst species. Accepted shock durations, pressure, and CTMs are included in Table 1.

Table 7–Recommended shock duration, pressure, and CTMs for inducing triploidy with hydrostatic pressure in Arctic char, Arctic grayling, Chinook salmon, and coho salmon.

Species	Shock Duration	Pressure	CTMs
Arctic char	5 minutes	9,500 psi	250
Arctic grayling	5 minutes	8,500 psi	175
Chinook salmon	4 minutes	10,000 psi	400
Coho salmon	4 minutes	10,000 psi	360

DATA

Keep a data log sheet to track events. A sample log sheet is presented in Table 2. For each batch of eggs, record the following data:

- Fertilization (activation) time = when saline is added to the gametes.
- Shock Initiation Time = # of minutes (target CTMs / water temperature) from fertilization to shock initiation + fertilization time.
- Cross off events after they occur.

Table 2–Sample log sheet.

Shock event	1	2	3	4	5	6
Fertilization time	~~10:00~~	~~10:10~~	~~10:20~~	~~10:30~~	~~10:40~~	~~10:50~~
Shock initiation	~~10:50~~	~~11:00~~	~~11:10~~	~~11:20~~	~~11:30~~	11:40

The order of these events varies with species. Arctic char, coho salmon, and Chinook salmon eggs are disinfected before pressure shocking to reduce the risk of BKD cross contamination during the egg shocking process. For these species, the water hardening time includes the disinfection. It is also easier to use a timer (instead of writing down an end disinfection time for these species. See handling of eggs for BKD screening. Water hardening times are presented in Table 3.

USING THE TRC HYDRAULIC PRESSURE CHAMBER

Set up

1. Position the pressure chamber near the fertilization and water hardening areas.
2. Use the brakes to lock the unit in place.
3. Remove the pin near the top of the chamber that holds the chamber in place for transport.
4. Tilt the chamber into place and secure with pin near the base of the chamber.
5. Plug the power cord into an 110v outlet.

Pressurizing the chamber

1. Close the valve at the bottom of the chamber.
2. Turn the hold/return valve near the hydraulic fluid reservoir to read hold.
3. Open the valve near the top of the chamber.
4. Add water to just above the fill line inside the chamber.
5. Set the chamber lid in place by lining up the arrows in the open position.
6. Push the lid down until it stops. Water should flow in a steady stream from a small overflow hole near the top of the chamber and through the upper valve tube.
7. Turn the lid to the closed position; make sure the locking lever is in place.
8. Close the top valve.
9. Use the foot pedal to achieve the desired pressure in the chamber.
    a. Monitor the pressure gauge attached to the chamber, not the one on the hydraulic fluid reservoir.
10. To depressurize the chamber, slowly turn the hold valve to return. Leave in the return position for 2–3 minutes so the ram can fully retract.
11. Once the pressure gauge reads 0 psi:
    a. Open the upper valve on the chamber.
    b. Press down on the locking lever and rotate the lid to the open position.
    c. Pull up on lid to open the chamber.
    d. Trouble Shooting

Problem	Solution
Lid won't seat properly: Water flow from the top valve may be obstructed.	▪ Make sure the top valve is open. ▪ Check to see if the mesh bag is obstructing the overflow hole. ▪ Make sure the overflow exit tube isn't pinched off.
Lid is difficult to remove from chamber	• Lubricate O ring with food grade lubricant • Change O ring.
Chamber fails to pressurize and hold pressure	• Make sure all valves are closed • Check for hydraulic fluid leak.
Chamber fails to achieve desired pressure because ram didn't retract completely (if distance from ram to top of chamber is <22.5 inches	Place the return/hold lever in the return position for 2-3 minutes to allow all the hydraulic fluid to return to the fluid reservoir.
Mesh bag won't submerge properly	Air may be trapped beneath the bag, or if the bag is full the water may not easily displace around it. Forcing the bag into the chamber will crush eggs. Use your hand to reposition the eggs in the bag to one side of the chamber to allow air and/water to escape around the bag.

GAMETE HANDLING

Collect gametes separately. Check the temperature of the incoming eggs, and temper them to within 2°C of the water bath temperature if necessary. Once tempered, carefully transfer the eggs to a fertilization container if they were not transported in one.

- Approximately 30 seconds before the pre-determined fertilization time, add milt from the desired number of males to the eggs, and gently stir the milt into the eggs.
- At the pre-determined fertilization time, activate the sperm with a 7% saline (7 g NaCl/L of water) (the same temperature as the rinsing water) solution and gently stir to thoroughly mix gametes and saline.
- Record fertilization time on your log sheet.
- Let the eggs sit for approximately two minutes (one minute for grayling) before rinsing off excess milt and saline solution.
- For Arctic char, coho, and Chinook salmon:
  - Use a mesh bag for a chamber insert.
  - Gently pour the eggs into a bag that is partially submerged in a heath tray filled with 1:100 iodophor solution.
  - Secure the open end with a rubber band.
  - Set the timer for 15 minutes.

- After 15 minutes of disinfection, push the tray in to rinse the iodophor from the tray.

- For Arctic grayling:
  - Use a perforated 48 ounce nalgene bottle for a chamber insert
  - Gently pour the eggs from 4–5 females into the insert
    - Use a squirt bottle to move sticky eggs from fertilization container to the chamber insert.
  - Partially submerge the bottle with eggs into a cooler with water to begin the water hardening process and to maintain a constant temperature.
  - Do not disinfect grayling eggs during water hardening. Eggs will be disinfected at the hatcheries.
- Shock duration is typically 4 or 5 minutes; it takes at least 3 minutes to switch out chamber inserts, and prepare the pressure chamber between batches of eggs. Therefore, allow at least 8 minutes (10 is preferable) between each shock group. This means the fertilization times should not be closer than 8–10 minutes.
- Keeping the eggs in the insert under water, transfer the eggs in the insert to the chamber(s) a couple minutes prior to shock initiation.
- Start pressurizing the chamber about 50 seconds before the desired shock initiation time.

Handling eggs for BKD screening

Handling and processing gametes collected from adult Arctic char and coho and Chinook salmon undergoing family tracking for BKD screening requires extra care to prevent cross contamination. ***You must disinfect the eggs before they are pressure shocked.*** Set the water in the tempering trough and the sink to the same temperature as the incubation water temperature. If eggs arrive in 1–3 gallon buckets, carefully disinfect the outside of the bucket without getting disinfectant inside. You could temper the eggs by placing the bucket in an iodophor solution the same temperature as the water bath (suspended in the tempering trough). Place a weight on the bucket to keep it from floating or tipping over. Keep a bucket with a 1:50 iodophor solution near the fertilization area for disinfecting hands and tools between batches of fertilized eggs.

Time and Temperature

Use the target CTMs (Table 1) and the temperature of the water bath to calculate the number of minutes between fertilization and shock initiation. CTMs = (time in minutes) x (temperature of water bath in centigrade). For example: If the shock initiation time is 250 CTMs and the water is 5°C, the time in minutes from fertilization to shock initiation is 50 minutes (50 x 5 = 250).

To determine the number of minutes from fertilization to pressure-shock initiation:

1. Double click on the table.
2. Enter the required number of CTMs.
3. Enter the temperature of the water bath.
4. Hit enter.

Minute Calculator

CTMs needed	Water temperature	Minutes
		#DIV/0!

Shock Initiation

Follow "Pressurizing the Chamber" instructions above. Starting 2–3 minutes before the target shock initiation time, add water from the water bath to the pressure chamber up to the fill line. Gently remove the chamber insert (with eggs) from the water bath or Heath tray and submerge it into the pressure chamber. Seal the pressure chamber. The pressure chamber pressurizes from 0 psi to 10,000 psi in 55 seconds, so start increasing pressure approximately 1 minute before the target shock initiation time.

Shocking

Once the target pressure inside the chamber is achieved, start a timer set for the desired shock duration time. Monitor the pressure valve for any loss of pressure. If pressure decreases, check to make sure all valves are completely closed and use the step pedal to increase pressure to the target pressure. Once the desired shock duration has been achieved, depressurize the chamber as described in "Pressurizing the Chamber," and return the chamber insert to the water bath to continue the water hardening process.

SPECIES SPECIFIC TIPS AND INFORMATION

Arctic grayling

Arctic grayling eggs are pressure shocked at the egg take site in Fairbanks. Spawn the eggs from 4–5 females into a single container, and add the milt from at least 4–5 males. Gently mix the milt into the eggs, and add saline to activate the sperm. The addition of saline is the fertilization start time.

Once fertilized and activated, grayling eggs quickly become very sticky. Quickly rinse eggs twice and gently transfer them to the chamber insert (48 ounce Nalgene bottle). If you take too much time rinsing, the eggs set up like tapioca and you can't pour them. If necessary, use a squirt bottle to gently move remaining eggs into the chamber insert. Grayling eggs swell considerably during the water hardening process.

Because Arctic grayling develop quickly, they require fewer CTMs to reach the ideal stage for inducing triploidy than the other species do.

A 5000 W generator supplies the power to the pressure chamber. Start the generator before plugging in the pressure chamber's power cord. Perform a pressurization test with water to make sure the generator can handle the load of starting the pressure chamber's motor. This should also be performed in Anchorage if the chamber and generator are being transferred from Anchorage. If the equipment is in Fairbanks, it should also be tested prior to the egg take.

Altered timing and pressure: The ideal shock for Arctic grayling is 8,500 psi of pressure starting 175 CTMs post fertilization for 5 minutes. Other combinations that yielded 100% triploid rates but poorer survival rates are: 5 minute shock using 9,000 psi 9,500psi or 10,000 psi starting 100,

175, or 250 CTMs post fertilization; and a 3 or 7 minute shock using 8,500 psi of pressure starting 175 CTMs post fertilization. Eggs shocked at 100 CTMs had the poorest survival rates.

Chinook Salmon

Transport eggs in small (2–3 gallon) buckets or in 2 gallon Ziploc bags with a lot of room in the cooler. The buckets are easier to spawn into, but they take up more space when transporting. Don't squish bags into the coolers. Yolk from crushed eggs will plug the egg's micropyle and result in unfertilized eggs.

Fertilize the eggs from a single female with milt from two males. Use a single milt packet twice. Example: males 1 and 2 will fertilize eggs from females 1 and 2. The volume of water hardened eggs from a single Chinook salmon may be greater than the volume of the pressure chamber. (need to test this with the mesh bags) Divide the eggs from a single female into 2 mesh bag inserts. Secure the open end with a rubber band. Place the bags with the eggs into a heath tray for a 15-minute iodophor solution (1:100) disinfection. Push the incubation tray in to flush the iodophor solution from the eggs after disinfection is complete.

Altered timing and pressure: The ideal shock for Chinook salmon is 10,000 psi of pressure starting 400 CTMs post fertilization for 4 minutes. Other combinations that yielded 100% triploid rates but poorer survival rates are: 9,500 psi starting 400 CTMs post fertilization for 4 minutes, and 10,000 psi starting 400 CTMs post fertilization for 3 minutes.

Coho Salmon

Fertilize and shock the eggs from two coho salmon females together. The chamber insert is not large enough to hold the eggs of three females (typical number spawned together for diploid coho salmon). Transporting the eggs in small (2–3 gallon) buckets works best, but gallon Ziplocs work fine also. Fertilize the eggs of two females with milt from two males.

Altered timing and pressure: The ideal shock for coho salmon is 10,000 psi of pressure starting 360 CTMs post fertilization for 4 minutes. Other combinations that yielded 100% triploid rates but poorer survival rates are: 9,000 psi 9,500 psi or 10,000 psi of pressure for 4 minutes starting at 240 or 360 CTMs post fertilization.

APPENDIX E
FISH TRANSPORT PERMITS

STATE OF ALASKA

DEPARTMENT OF FISH AND GAME

Division of Commercial Fisheries

SEAN PARNELL, GOVERNOR

1255 W. 8TH Street
P.O. BOX 115526
JUNEAU, AK 99811-5526

PHONE: (907) 465-4210
FAX: (907) 465-2604

MEMORANDUM

TO:	David Bedford Deputy Commissioner	DATE:	5/18/12
THRU:	Sue Aspelund Deputy Director	FILE NUMBER:	SF Statewide Stocking Plan
		PHONE NUMBER:	465-4100
FROM:	Ron Josephson Mark Stopha	SUBJECT:	Amendment to Fish Transport Permit (FTP) 10A-0047

As required under 5 AAC 41.040(b) and (c), the following entries supersede or are added to fish transport permit (FTP) # 10A-0047

The Effective Period shall read: January 1, 2010 – December 31, 2016

Justification: This FTP allows for capture of Arctic grayling from the Chena River, transport to Spafford's Pond (Category 1 Tanana River Drainage) for holding in net pens until ready to spawn, pressure treatment of fertilized eggs to induce triploidy, and transport of up to 170 thousand fertilized eggs to Ruth Burnett Sport Fish Hatchery for incubation and rearing. Arctic grayling resulting from these eggs will be used to support the ADF&G's fish stocking program. This amendment was requested by April Behr in an email on April 27, 2012.

This amendment is to be attached to FTP # 10A-0047. All other terms of the FTP remain the same. All activities must be consistent with the original permit.

DISTRIBUTION:
CF Division Files
Ted Meyers
William Grant
Bonnie Borba
Cal Skaugstad
April Behr

FISH TRANSPORT PERMIT

Applicant
Alaska Department of Fish and Game

Organization
Division of Sport Fish

Mailing Address
1300 College Rd.
Fairbanks, AK 99701

Phone
(907) 459-7362

Species
Arctic Grayling
(*Thymallus arcticus*)
Eggs

Stock Origin/Original Donor Stock
Chena River
Tanana River Drainage

Proposed Stocking Location
Ruth Burnett Sport Fish Hatchery
Tanana River Drainage

Project summary- Summary statement of precisely what is being proposed.
An annual Chena River Arctic grayling egg take will be conducted in May. Up to 170,000 fertilized eggs are required to meet production goals at the Ruth Burnett Sport Fish Hatchery (RBSFH), and approximately 60 males and 60 females are required to obtain these eggs. Arctic grayling will be captured in the Chena River and transported to Spafford's Pond (Category 1 Tanana River Drainage) where they will be held in net pens until they are ready to spawn. Because not all captured fish are in spawning condition, up to twice the number of fish required for the egg take may be collected and held. After artificial spawning occurs, fertilized eggs will be pressure treated to induce triploidy. Up to 170,000 fertilized eggs will be transported to the Ruth Burnett Sport Fish Hatchery and all broodstock will be returned to the Chena River. Arctic grayling resulting from these eggs will be used to support the ADF&G's fish stocking program.

Permit # SF Statewide Stocking Plan

					For Department Use Only
_X_ State Fish Transport Permit		FTP Number	10A-0047		
Consistent with facility/project plans	Yes			No	
__ Private Nonprofit Hatchery Fish Transport Permit					
Consistent with PNP permit	Yes			No	
Requires Permit Alteration prior to review	Yes			No	
Continuation of project	Yes			No	
New Project	Yes			No	
__ Other -	Yes			No	
Status					
Forms Complete	Yes		No		Date
Disease History Complete	Yes		No		Date
In review process	DATE 12/16/09				
Returned to applicant	DATE 4/5/10				

5 AAC 41.005. PERMIT REQUIRED. (a) No person may transport, posses, export from the state, or release into the waters of the state, any live fish unless the person holds a fish transport permit issued by the Commissioner of his authorized designee. The Fish Transport Permit (FTP) is the single document, approved by the Commissioner of Alaska Department of Fish and Game (ADF&G), that allows for movements of fish and eggs on an interstate and intrastate basis.

SIGNATURE PAGE

Comments

		Agree	Disagree	Date	Comments Provided Yes	Comments Provided No
1.	Fish Health Services Pathologist - Division of Commercial Fisheries (CF) Signature Ted Meyers	X		2/23/10	X	
	Incomplete					
2.	Regional Resource Development Biologist - CF Bonnie Borba	X		3/5/10	X	
3.	Regional Supervisor - CF Bonnie Borba for John Linderman	X		3/5/10		X
4.	Regional Supervisor - Division of Sport Fish Don Roach	X		3/11/10		X
5.	Principal Geneticist - CF William Grant	X		2/22/10	X	
6.	Deputy Director - CF Sue Aspelund	X		3/11/10		X
7.	Commissioner Dale Bosfol	Approval ✓	Disapproval	Date 4/2/10		

FISH TRANSPORT PERMIT

FTP PERMIT NO. 10A-0047

Applicant/Organization:
Alaska Department of Fish and Game / Division of Sport Fish

Date:
December 9, 2009

Project Leader:
Cal Skaugstad / April Behr

Telephone No
(907) 459-7362

Effective Period:
January 1, 2010 through December 31, 2011

Species:
Arctic grayling eggs

Transport Date(s):
January 1, 2010 through December 31, 2011

Stock Origin/Original Donor Stock:
Chena River / Tanana River Drainage. See attached map.

Maximal Number Allowed:
170,000

Lifestage:
Eggs

Incubation and Rearing Location(s):
Ruth Burnett Sport Fish Hatchery. See attached map.

Release Location:
N/A

Purpose and Benefits:
To provide Arctic grayling for the statewide stocking program.

Evaluation Plans:
Survival and development of eggs and young fish will be evaluated as per standard hatchery procedures.

Is release site landlocked?
N/A

FISH TRANSPORT PERMIT

Applicant Alaska Department of Fish and Game	**Organization** Division of Sport Fish
Mailing Address 1300 College Rd. Fairbanks, AK 99701	**Phone** (907) 459-7362 **Species** Arctic Grayling (*Thymallus arcticus*) Eggs
Stock Origin/Original Donor Stock Chena River Tanana River Drainage	**Proposed Stocking Location** William Jack Hernandez Sport Fish Hatchery

Project summary- Summary statement of precisely what is being proposed.
An annual Chena River Arctic grayling egg take will be conducted in May. Up to 170,000 fertilized eggs are required to meet production goals at the Ruth Burnett Sport Fish Hatchery (RBSFH) and William Jack Hernandez Sport Fish Hatchery (WJHSFH). Approximately 60 males and 60 females are required to obtain these eggs. This FTP covers the transport of fertilized eggs to the WJHSFH. Arctic grayling will be captured in the Chena River and transported to Spafford's Pond (Category 1 Tanana River Drainage) where they will be held in net pens until they are ready to spawn. Because not all captured fish are in spawning condition, up to twice the number of fish required for the egg take may be collected and held. After artificial spawning occurs, fertilized eggs will be pressure treated to induce triploidy. Up to 170,000 fertilized eggs will be transported to the WJHSFH and all broodstock will be returned to the Chena River. Arctic grayling resulting from these eggs will be used to support the ADF&G's fish stocking program.

Permit # Statewide Stocking Plan

	For Department Use Only			
_x_ **State Fish Transport Permit**	**FTP Number**	**12A-0106**		
Consistent with facility/project plans	**Yes**	_____	**No**	_____
__ **Private Nonprofit Hatchery Fish Transport Permit**				
Consistent with PNP permit	**Yes**	_____	**No**	_____
Requires Permit Alteration prior to review	**Yes**	_____	**No**	_____
Continuation of project	**Yes**	_____	**No**	_____
New Project	**Yes**	_____	**No**	_____
__ **Other -**	**Yes**	_____	**No**	_____
Status				
Forms Complete	**Yes**	x	**No** _____	**Date** 5/2/12
Disease History Complete	**Yes**	_____	**No** _____	**Date** _____
In review process	**DATE** 5/2/12			
Returned to applicant	**DATE** 5/4/12			

5 AAC 41.005. PERMIT REQUIRED. (a) No person may transport, posses, export from the state, or release into the waters of the state, any live fish unless the person holds a fish transport permit issued by the Commissioner of his authorized designee. The Fish Transport Permit (FTP) is the single document, approved by the Commissioner of Alaska Department of Fish and Game (ADF&G), that allows for movements of fish and eggs on an interstate and intrastate basis.

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SIGNATURE PAGE

Comments

	Agree	Disagree	Date	Comments Provided Yes	Comments Provided No
1. Fish Health Services Pathologist - Division of Commercial Fisheries (CF)					
Signature Jayde Ferguson for Ted Meyers	x		5/2/12	x	
Incomplete					
2. Regional Resource Development Biologist - CF					
Bonnie Borba	x		5/4/12	x	
3. Regional Supervisor - CF					
Bonnie Borba for John Linderman	x		5/4/12	x	
4. Regional Supervisor - Division of Sport Fish					
Cal Skaugstad for Don Roach	x		5/2/12	x	
5. Principal Geneticist – CF					
William Grant	x		5/2/12	x	
6. Deputy Director - CF					
Sue Aspelund	X		5/4/12		X

7. Commissioner	Approval	Disapproval	Date
Cora Campbell	X		5/4/12

FISH TRANSPORT PERMIT

FTP PERMIT NO. 12A-0106

Applicant/Organization:
Alaska Department of Fish and Game / Division of Sport Fish

Date:
May 4, 2012

Project Leader:
April Behr

Telephone No
(907) 459-7362

Effective Period:
January 1, 2012 through December 31, 2016

Species:
Arctic grayling eggs

Transport Date(s):
January 1, 2012 through December 31, 2016

Stock Origin/Original Donor Stock:
Chena River / Tanana River Drainage. See attached map.

Maximal Number Allowed:
170,000

Lifestage:
Eggs

Incubation and Rearing Location(s):
William Jack Hernandez Sport Fish Hatchery. See attached map.

Release Location:
N/A

Purpose and Benefits:
To provide Arctic grayling for the statewide stocking program.

Evaluation Plans:
Survival and development of eggs and young fish will be evaluated per standard hatchery procedures.

Is release site landlocked?
N/A

FISH TRANSPORT PERMIT

Applicant Alaska Department of Fish and Game	**Organization** Division of Sport Fish
Mailing Address 1300 College Rd. Fairbanks, AK 99701	**Phone** (907) 459-7362 **Species** Chinook salmon (*Oncorhynchus tshawytscha*) Eggs
Stock Origin/Original Donor Stock Salcha River Tanana River Drainage	**Proposed Stocking Location** Ruth Burnett Sport Fish Hatchery Tanana River Drainage

Project summary- Summary statement of precisely what is being proposed.
An annual Chena River Chinook salmon egg take will be conducted in July and/or August depending on run timing. If Chena River Chinook salmon can not be used, fish will be collected from the Salcha River as a secondary broodsource. Up to 120,000 fertilized eggs are required to meet production goals at the Ruth Burnett Sport Fish Hatchery (RBSFH), and approximately 40 males and 40 females are required to obtain these eggs. Chinook salmon will be captured in the Salcha River and held in net pens until they are ready to spawn. Because not all captured fish are ready to spawn, up to twice the number of fish required for the egg take may be collected and held. The fish will be artificially spawned and the resulting eggs and milt will be transported in one of two ways, separately or as fertilized eggs. In both cases, after fertilization the eggs will be pressure treated to induce triploidy. Chinook salmon resulting from these eggs will be used to support the ADF&G's fish stocking program. Excess broodstock collected will be released back into the Salcha River.

Permit # SF Statewide Stocking Plan

					For Department Use Only
__ **State Fish Transport Permit**		**FTP Number**	**10A-0038**		
Consistent with facility/project plans	**Yes**	**X**		**No**	
__ **Private Nonprofit Hatchery Fish Transport Permit**					
Consistent with PNP permit	**Yes**			**No**	
Requires Permit Alteration prior to review	**Yes**			**No**	
Continuation of project	**Yes**			**No**	
New Project	**Yes**			**No**	
__ **Other -**	**Yes**			**No**	
Status					
Forms Complete	**Yes**		**No**		**Date**
Disease History Complete	**Yes**		**No**		**Date**
In review process	**DATE** 12/16/09				
Returned to applicant	**DATE** 3-1-10				

5 AAC 41.005. PERMIT REQUIRED. (a) No person may transport, posses, export from the state, or release into the waters of the state, any live fish unless the person holds a fish transport permit issued by the Commissioner of his authorized designee. The Fish Transport Permit (FTP) is the single document, approved by the Commissioner of Alaska Department of Fish and Game (ADF&G), that allows for movements of fish and eggs on an interstate and intrastate basis.

STAFF RECOMMENDATIONS

FTP PERMIT NO. 10A-0038

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Comments

	Agree	Disagree	Date	Comments Provided Yes	Comments Provided No
1. Fish Health Services Pathologist - Division of Commercial Fisheries (CF) Signature Ted Meyers	X		1/4/10	X	
Incomplete					
2. Regional Resource Development Biologist - CF Bonnie Borba	X		1/23/10	X	
3. Regional Supervisor - CF John Linderman	X		2/16/10	X	
4. Regional Supervisor - Division of Sport Fish Cal Skaugstad for Don Roach	X		1/8/10	X	
5. Principal Geneticist - CF Stew Grant	X		1/7/10	X	
6. Deputy Director - CF Sue Aspelund	X		2/19/10		X
7. Commissioner Del Beffort	Approval ✓	Disapproval	Date 2/26/10		

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FISH TRANSPORT PERMIT

FTP PERMIT NO. 10A-0038

Applicant/Organization:
Alaska Department of Fish and Game / Division of Sport Fish

Date:
December 10, 2009

Project Leader:
Cal Skaugstad / April Behr

Telephone No
(907) 459-7362

Effective Period:
January 1, 2010 through December 31, 2014

Species:
Chinook salmon eggs

Transport Date(s):
January 1, 2010 through December 31, 2014

Stock Origin/Original Donor Stock:
Salcha River / Tanana River Drainage. See attached map.

Maximal Number Allowed:
120,000

Lifestage:
Eggs

Incubation and Rearing Location(s):
Ruth Burnett Sport Fish Hatchery. See attached map.

Release Location:
N/A

Purpose and Benefits:
To provide Chinook salmon for the statewide stocking program.

Evaluation Plans:
Survival and development of eggs and young fish will be evaluated as per standard hatchery procedures.

Is release site landlocked?
N/A

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FISH TRANSPORT PERMIT

Applicant
Alaska Department of Fish and Game

Organization
Division of Sport Fish

Mailing Address
1300 College Rd.
Fairbanks, AK 99701

Phone
(907) 459-7362

Species
Coho salmon
(*Oncorhynchus kisutch*)
eggs

Stock Origin/Original Donor Stock
Delta Clearwater River
Tanana River Drainage

Proposed Stocking Location
Ruth Burnett Sport Fish Hatchery
Tanana River Drainage

Project summary- Summary statement of precisely what is being proposed.
An annual Delta Clearwater River coho salmon egg take will be conducted in October. Up to 290,000 fertilized eggs are required to meet production goals at the Ruth Burnett Sport Fish Hatchery (RBSFH), and approximately 80 males and 80 females are required to obtain these eggs. Coho salmon will be captured in the Delta Clearwater River and held in net pens until they are ready to spawn. Because not all captured fish are ready to spawn, up to twice the number of fish required for the egg take may be collected and held. Fish will be artificially spawned and the resulting eggs and milt will be transported in one of two ways, separately or as fertilized eggs. In both cases, after fertilization the eggs will be pressure treated to induce triploidy. Coho salmon resulting from these eggs will be used to support the ADF&G's fish stocking program. Excess broodstock collected will be released back into the Delta Clearwater River.

Permit # SF Statewide Stocking Plan

				For Department Use Only
__ State Fish Transport Permit		FTP Number	10A-0043	
Consistent with facility/project plans	Yes	X	No	
__ Private Nonprofit Hatchery Fish Transport Permit				
Consistent with PNP permit	Yes		No	
Requires Permit Alteration prior to review	Yes		No	
Continuation of project	Yes		No	
New Project	Yes		No	
__ Other -	Yes		No	
Status				
Forms Complete	Yes	No	Date	
Disease History Complete	Yes	No	Date	
In review process	DATE 12/16/09			
Returned to applicant	DATE 3-1-10			

5 AAC 41.005. PERMIT REQUIRED. (a) No person may transport, posses, export from the state, or release into the waters of the state, any live fish unless the person holds a fish transport permit issued by the Commissioner of his authorized designee. The Fish Transport Permit (FTP) is the single document, approved by the Commissioner of Alaska Department of Fish and Game (ADF&G), that allows for movements of fish and eggs on an interstate and intrastate basis.

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Comments

	Agree	Disagree	Date	Comments Provided Yes	Comments Provided No
1. Fish Health Services Pathologist - Division of Commercial Fisheries (CF) Signature Ted Meyers	X		2/3/10	X	
Incomplete					
2. Regional Resource Development Biologist - CF Bonnie Borba	X		2/4/10	X	
3. Regional Supervisor - CF John Linderman	X		2/16/10	X	
4. Regional Supervisor - Division of Sport Fish Cal Skaugstad for Don Roach	X		2/9/10	X	
5. Principal Geneticist - CF Stew Grant	X		2/3/10	X	
6. Deputy Director - CF Sue Aspelund	X		2/19/10		X

	Approval	Disapproval	Date
7. Commissioner	✓		2/26/10

FISH TRANSPORT PERMIT

FTP PERMIT NO. 10A-0043

Applicant/Organization:
Alaska Department of Fish and Game / Division of Sport Fish

Date:
December 10, 2009

Project Leader:
Cal Skaugstad / April Behr

Telephone No
(907) 459-7362

Effective Period:
January 1, 2010 through December 31, 2014

Species:
Coho salmon Eggs

Transport Date(s):
January 1, 2010 through December 31, 2014

Stock Origin/Original Donor Stock:
Delta Clearwater River / Tanana River Drainage. See attached map.

Maximal Number Allowed:
290,000

Lifestage:
Eggs

Incubation and Rearing Location(s):
Ruth Burnett Sport Fish Hatchery. See attached map.

Release Location:
N/A

Purpose and Benefits:
To provide coho salmon for the statewide stocking program.

Evaluation Plans:
Survival and development of eggs and young fish will be evaluated as per standard hatchery procedures.

Is release site landlocked?
N/A