Regional Operational Plan No. ROP.SF.3F.2023.09

Operational Plan: Region 3 Egg Takes for Stocked Fisheries Programs and Donor Stock Disease Screening

by

April Behr

August 2023

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

Symbols and Abbreviations

The following symbols and abbreviations, and others approved for the Système International d'Unités (SI), are used without definition in the following reports by the Divisions of Sport Fish and of Commercial Fisheries: Fishery Manuscripts, Fishery Data Series Reports, Fishery Management Reports, and Special Publications. All others, including deviations from definitions listed below, are noted in the text at first mention, as well as in the titles or footnotes of tables, and in figures or figure captions.

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 NO. ROP.SF.3F.2023.09

OPERATIONAL PLAN: REGION 3 EGG TAKES FOR STOCKED FISHERIES PROGRAMS AND DONOR STOCK PATHOLOGY SCREENING

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Signature Page

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Approval

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ABSTRACT

This operational plan details the stocks and methods used to meet Region 2 and 3's requirements for fish production, disease screening, and family tracking. The number of adult donors needed from wild stocks to meet these requirements is described and resulting progeny will be released into local lakes to divert fishing pressure away from wild stocks and to provide additional sport fishing opportunities. We identify wild donor stocks for Arctic grayling *Thymallus arcticus* (Chena River), Chinook salmon *Oncorhynchus tshawytscha* (Chena River or Salcha River), coho salmon *Oncorhynchus kisutch* (Delta Clearwater River), and lake trout *Salvelinus namaycush* (Sevenmile Lake, Denali Highway). This operational plan also provides the information necessary for Fish Transport Permits (FTP) that are required to capture, transport, and hold wild donors; and to collect, fertilize, and transport gametes to Ruth Burnett Sport Fish Hatchery (RBSFH) in Fairbanks and to 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, lake trout, *Salvelinus namaycush*, fish stocking, family tracking, stock disease history, hatchery, Chena River, Salcha River, Delta Clearwater River, Sevenmile Lake Denali Highway.

PURPOSE

The purpose of this project is to collect gametes from Arctic grayling, Chinook salmon, coho salmon, and lake trout for rearing in the Ruth Burnett Sport Fish Hatchery (RBSFH) in Fairbanks and William Jack Hernandez Sport Fish Hatchery (WJHSFH) in Anchorage for subsequent outstocking into local lakes. Arctic grayling and lake trout progeny are released into lakes in the Tanana, Upper Copper/Upper Susitna, Kenai, Anchorage, and Mat-Su management areas. Chinook and coho salmon progeny are released into lakes in the Tanana and Upper Copper/Upper Susitna management areas. Species reared at the RBSFH and WJHSFH are subject to change annually pending budget and broodstock constraints and regional management needs; however, collection methods for all species are outlined in this plan to encompass all possible egg takes and pathology requirements.

OBJECTIVES

1. Arctic grayling–collect and artificially fertilize approximately 194,340 eggs annually using 60 females and 60 males from the Chena River population.
2. Chinook salmon–collect and artificially fertilize approximately 52,055 eggs annually using 8 females and 8 males from the Chena River or Salcha River population. Up to 10 females and 10 males may be taken to account for culling due to bacterial kidney disease (BKD).
3. Chinook salmon–visually inspect all Chinook salmon donors for *Ichthyophonus*.
4. Coho salmon–collect and artificially fertilize approximately 96,000–105,500 eggs annually using 34–37 females and 34–37 males from the Delta Clearwater River population. Up to 39 females and 39 males may be taken to account for culling due to BKD.
5. Lake trout–collect and artificially fertilize 53,924 eggs on alternate years using 54 females and 54 males from the Sevenmile Lake or Glacier Lake population.
6. Family tracking–Obtain kidney samples from all female Chinook and coho salmon donors to screen for BKD.

7. Stock disease history–Obtain kidney and ovarian samples from Arctic grayling, Chinook salmon, coho salmon, and lake trout for stock disease history as needed.

METHODS

Region 3 Sport Fish staff captures and holds Arctic grayling, Chinook salmon, coho salmon, and lake trout for gamete collection, and assists hatchery staff with artificially spawning fish. Adult fish from Chena River, Salcha River, Delta Clearwater River, and Sevenmile Lake or Glacier Lake wild populations are captured with an electrofishing boat or gill net and held in net pens until females are ready to spawn. The approximate egg and donor requirements to meet ADF&G hatchery production goals are outlined below with capture methods. These requirements will be checked and updated prior to each field season using scheduled fish releases listed in the most recent Statewide Stocking Plan.

We typically capture and hold up to twice the number of females and males required to meet egg take goals because we've found that only approximately 1/2 of females will mature within a suitable time, and because some males produce too little milt to be useful. For each project, our goal is to capture fish as close to spawning condition as possible to reduce holding times. We want donor collection and egg takes for each species to take no more than one week because we need to start other projects.

ARCTIC GRAYLING

Program Requirements

The Arctic grayling stocking program was suspended in 2019 due to budget cuts. When this program is active, the Chena River Arctic grayling population supplies gametes for both Region 2 and Region 3 stocked fisheries programs. Gametes are used for fingerling (2g) and catchable (120g) production at the RBSFH and WJHSFH. The number of each life stage fluctuates annually.

Donor and Gamete Objectives

Approximately 194,340 fertilized eggs are needed each year, 124,000 for Region 2 production and 70,340 for Region 3. Approximately 60 females and an equal number of males are needed. Donor requirements were calculated using fecundity and hatchery survival values listed in Table 1 for RBSFH. WJHSFH uses different survival values and requests fertilized eggs annually to meet their fish production needs.

Donor Collection

The last week in April or the first 2 weeks in May, a boat equipped with electrofishing gear will be used to capture adult Arctic grayling prior to spawning. Fish will be collected from a 7 km stretch of river immediately upstream from the Nordale Road 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 no more than 2 hours per day. Each day we will make a single pass through the capture area along one bank. Field dates will depend on weather conditions that influence when river ice is broken and transported downstream, making river navigation possible. Immediately following breakup, female Arctic grayling are within 3 to 7 days of spawning and most males are ready to spawn (ripe).

After fish are captured, they will be transferred to a fish transport truck and taken to a holding pond on Creamers Refuge (Figure 2) and put into net pens. The fish are held until the females are

mature and can be artificially spawned; this may take a few days to 2 weeks. The egg take will occur at the RBSFH. After gametes are collected, all fish will be returned live to the Chena River at the Nordale Road Bridge. Fertilized eggs for WJHSFH will be sent to Anchorage by commercial airline. Staff from WJHSFH will 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 2 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.

Timing for this egg take is critical because most spawning seems to occur within a 2-week period after ice out. Our ability to capture fish during a 2-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 also 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 are not present in the Chena River during this sampling event.

Gamete Collection and Fertilization Procedures

We will follow the procedures described 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 5 females will be fertilized with milt from 5 males. Fertilized eggs will be shocked using hydrostatic pressure to induce triploidy as described by Loopstra and Hansen (2010).

Family Tracking and Disease Screening

Family tracking is not required for Arctic grayling. The disease history for this stock needs to be updated every 5 years and is due when the next egg take occurs. Kidney and ovarian fluid samples will be collected from spawned fish until 60 samples each are obtained in a 5 consecutive year period. We will follow procedures described in *Collection Protocol for Routine Broodstock Examination - ELISA/FAT Kidney Samples* (Appendix B).

CHINOOK SALMON

Program Requirements

The Chena River or Salcha River Chinook salmon population supplies gametes for the Region 3 stocked fisheries program. Approximately 40,000 catchable (140g) Chinook salmon are produced at the RBSFH annually.

Donor and Gamete Objectives

About 52,055 fertilized eggs are needed each year to meet hatchery production goals, which will require at least 8 females and an equal number of males. These numbers were calculated using

fecundity and hatchery survival rates listed in Table 2. All females will be screened for disease (family tracked) and eggs from fish identified as having disease will be discarded. An additional 2 females (10 total) and an equal number of males may be required to meet the objective of 52,055 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 1,050 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.

Donor Collection

During the second or third week in July, Chinook salmon adults will be captured from a 20 km section of the Salcha River or Chena River. The capture area on the Salcha River is approximately 35 km upstream of the Richardson Highway Bridge (Figure 3). The capture area on the Chena River is directly above the Flat Creek Slough boat launch (MP 26.7 Chena Hot Springs Road, Figure 4). Fish will be captured using an electrofishing boat or a twisted multifilament gillnet with 18 cm stretch mesh measuring 3 m tall and 20 m long. The electrofishing boat will operate for up to 7 days and for no more than 4 hours per day. Each day we will make a single pass through the capture area. The gill net will be drifted through straight stretches where Chinook salmon are observed, snags are limited, and water depth is approximately 1–2 m. Spawning condition of female Chinook salmon at this time can vary from pre-spawning (green) to ripe, and most males are ready to spawn.

After fish are captured, they will be transferred to net pens and held until females are ready to spawn. Females will be sorted (green or ripe) 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. We will intentionally select larger females and males (> 950 mm) for spawning. Eggs and milt will be collected separately, transported by boat if necessary, and then by vehicle on the road system to the RBSFH where fertilization will occur.

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 inspect each donor pair for *Ichthyophonus*. We will not use gametes from infected fish. 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 for bacterial kidney disease (BKD) is required for Chinook salmon. The disease histories for the Chena River and Salcha River stocks need to be updated every 3 years; kidney samples from 60 males and ovarian fluid samples from 60 females are required to do this. Because fewer than 60 fish are spawned in a 3-year period, and because managers are reluctant to collect all samples in a single year, samples will be collected annually from all spawned fish. We will follow procedures described in *Collection Protocol for Routine Broodstock Examination - ELISA/FAT Kidney Samples* (Appendix B) and *Collection Protocol for Routine Broodstock Examination - Virology Samples* (Appendix C).

COHO SALMON

Program Requirements

The Delta Clearwater coho salmon population supplies gametes for the Region 3 stocked fisheries program. Approximately 96,000–105,500 fingerling (4g) coho salmon are produced at the RBSFH annually.

Donor and Gamete Objectives

About 125,845–138,298 fertilized eggs are needed to meet hatchery production goals, which will require 34–37 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 BKD (family tracked) and eggs from fish identified having disease will be discarded. An additional 2 females (36–39 total) and an equal number of males may be required to meet gamete requirements after disease screening.

Donor Collection

During the second week in October, coho salmon will be captured from the Delta Clearwater River using a beach seine. The capture area is about 1 km below the Delta Clearwater River State Park (Figure 5). The beach seine has 9 cm mesh (made from 3 mm black twisted nylon) and is 3 m tall and 46 m long, with a bag measuring approximately 4 m wide in the middle. Seining will occur by boat, with a minimum of 2 people positioned on shore, in areas where schools of coho salmon are observed, minimal snags are present, and water depth is less than 3 m. The spawning condition of most female coho salmon at this time ranges from pre-spawning to ripe. Most males are ready to spawn.

After fish are captured, they will be transferred to net pens and held until 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. Eggs and milt will be collected separately, and fertilization will occur at the RBSFH.

Gamete Collection and Fertilization Procedures

We will follow procedures described in *Generalized Egg-Take Procedures for ADF&G Sport Fish Hatchery Program* (Appendix A). Eggs from 2 females will be fertilized with milt from 2 males.

Family Tracking and Disease Screening

Family tracking of donors for BKD is required for coho salmon. The disease history for this stock needs to be updated every 5 years and is due the next time an egg take occurs. Kidney and ovarian fluid samples will be collected from spawned fish annually until 60 samples of each are obtained in a 5 consecutive year period. We will follow procedures described in *Collection Protocol for Routine Broodstock Examination - ELISA/FAT Kidney Samples* (Appendix B) and *Collection Protocol for Routine Broodstock Examination - Virology Samples* (Appendix C).

LAKE TROUT

Program Requirements

Sevenmile Lake supplies gametes for both Region 2 and Region 3 stocked fisheries programs. All lake trout are reared at the RBSFH and approximately 23,000 subcatchables (20g) are produced

on alternate years. Broodstock collection will occur in the fall in odd years and resulting progeny will be released in even years.

Donor and Gamete Objectives

Production levels of lake trout are low to minimize egg and donor requirements. Lake trout had not been produced since 2001, and triploid (3N) lake trout had never been produced by ADF&G staff, until the program was reinstated in 2019. Sevenmile Lake and Glacier Lake were identified by managers as potential donor stocks, and acceptable annual yields for these populations were calculated using the lake area model described by Burr (1991). Donor requirements and current harvest levels will not exceed acceptable annual yields for these locations (Table 4) and alternate year egg takes will be conducted to minimize the impact to wild populations as recommended by Parker and Wuttig (2000).

Approximately 39,670 fertilized eggs are needed on alternate years, 19,835 for Region 2 production and 19,835 for Region 3. Approximately 40 females and an equal number of males are needed. Donor requirements were calculated using fecundity and hatchery survival values listed in Table 5. These values are based on survival rates previously observed at the Fort Richardson and Ruth Burnett Sport Fish Hatcheries and may improve as culture methods are refined.

Donor Collection

The second to third week in September, fish will be collected from known spawning areas in Sevenmile Lake or Glacier Lake (Figure 6). Fish will be captured in the evening or at night using sinking and floating small mesh tangle nets (1 inch stretch, 1/2 inch bar, 150 feet long, and 16 feet deep). Tangle nets will be closely attended, and fish will be immediately removed following capture. This collection method has been utilized and proven successful during previous lake trout egg takes and assessment projects at Sevenmile Lake (Parker and Wuttig 2000). The spawning area in Sevenmile Lake is located in the northeast quartile (Parker and Wuttig 2000), and spawning fish have been captured and observed around the small islands at the south end of Glacier Lake (ADF&G unpublished data).

After fish are captured, they will be transferred to net pens and held until females are ready to spawn. Females will be sorted (green or ripe) and placed in separate net pens. We have found that lake trout are particularly susceptible to mortality if they are held for more than a few days. If a female is not expected to be ready to spawn within 3 days of capture, the fish will be released. Males will be held in a third net pen. Eggs and milt will be collected separately, transported by ATV if necessary, and then by vehicle on the road system to the RBSFH where fertilization will occur.

Gamete Collection and Fertilization Procedures

Eggs from 5 females will be fertilized with milt from 5 males. We will follow procedures described in *Generalized Egg-Take Procedures for ADF&G Sport Fish Hatchery Program* (Appendix A). Fertilized eggs will be shocked using hydrostatic pressure to induce triploidy as described by Kozfkay et al. (2005).

Family Tracking and Disease Screening

Family tracking is not required for lake trout. The disease history for this stock needs to be updated every 5 years and is due in 2025. Kidney and ovarian fluid samples will be collected from all spawned fish until 60 samples each are obtained in a 5 consecutive year period. We will follow

procedures described in *Collection Protocol for Routine Broodstock Examination - ELISA/FAT Kidney Samples* (Appendix B).

FISH TRANSPORT PERMITS

These permits authorize ADF&G staff to capture, hold, spawn, and transport live 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	Expiration Date
Arctic Grayling	RBSFH, WJHSFH	19A-0016(1), 12A-0106(2)	12/31/2031
Chinook Salmon	RBSFH	10A-0034(2) (Chena), 10A-0038(2) (Salcha)	12/31/2029
Coho Salmon	RBSFH	10A-0043(2)	12/31/2027
Lake Trout	RBSFH, WJHSFH	16A-0005(3) (Sevenmile) 19A-0010(1) (Glacier)	12/31/2030

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 the Fairbanks regional office and RBSFH. An annual Federal Aid performance report will summarize the results of this project.

Dates listed below can shift forward or backward by one week depending on river conditions, run timing, and maturation rates. Listed dates include the likely timing of project activities.

CHENA RIVER ARCTIC GRAYLING EGG TAKE

Dates	Activity
1–4 May	Inspect and prepare equipment.
7–9 May	Capture and hold Arctic grayling.
10–11 May	Egg take

CHENA RIVER OR SALCHA RIVER CHINOOK SALMON EGG TAKE

Dates	Activity
9–13 July	Inspect and prepare equipment.
16–18 July	Capture and hold Chinook salmon.
19–20 July	Egg take

DELTA CLEARWATER RIVER COHO SALMON EGG TAKE

Dates	Activity
1–5 October	Inspect and prepare equipment.
8–10 October	Capture and hold coho salmon.
11–12 October	Egg take

SEVENMILE LAKE OR GLACIER LAKE LAKE TROUT EGG TAKE

Dates	Activity
4–7 September	Inspect and prepare equipment.
10–19 September	Capture and hold lake trout.
20–21 September	Egg take

RESPONSIBILITIES

April Behr: Fishery Biologist 3, Project Supervisor for Stocked Fisheries and Resident Species Programs - Region 3. Fairbanks.

Supervise all aspects of fish capture and gamete collection from wild donor populations in Region 3. 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.

Kelly Mansfield: Fishery Biologist 2, Stocked Fisheries Program Biologist - Region 3. Fairbanks.

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

Vacant: Fishery Technician 2, Stocked Fisheries Program - Region 3. Fairbanks.

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

RBSFH: Hatchery staff will assist with capture and supervise and conduct egg-take activities. They will provide all equipment necessary to conduct each egg take and to collect kidney and fluid samples. They will also be responsible for transporting fertilized Arctic grayling, Chinook salmon, coho salmon, and lake trout eggs to RBSFH and for shipping fertilized Arctic grayling and lake trout eggs to WJHSFH.

REFERENCE CITED

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Burr, J. M. 2006. AYK Lake Trout Management Plan. Alaska Department of Fish and Game, Fishery Management Report No. 06-52, Anchorage.

Kozfkay, J. R., E. J. Wagner, D. Aplanalp. 2005. Production of triploid lake trout by means of pressure treatment. North American Journal of Aquaculture 67: 93-97.

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.

Parker, J. F., K. G. Wuttig. 2000. Stock assessment of lake trout in Sevenmile Lake, 1999. Alaska Department of Fish and Game, Fishery Data Series Number 00-37, Anchorage.

TABLES AND FIGURES

Table 1.–Approximate annual donor and egg requirement for Arctic grayling egg take, Chena River.

	Region 2[b]	Region 3[b]
Program Requirements:		
Fingerlings to stock even years (3N)	-	6,000
Catchables to stock annually (3N)	-	26,800
Survival Estimates:		
Survival Green Egg to Ponding:	-	80.0%
Enumeration Correction	-	70.0%
Survival Fry to Fingerling:	-	91.6%
Projected grade	-	90.0%
Survival Fingerling to Catchable:	-	98.4%
Number of Eggs Needed:	124,000[a]	70,340
Females Required:		
Fecundity (eggs/female):	-	3,300
Females required for spawning	38[a]	22

a. Historic request from WJHSFH using survival values specific to their Arctic grayling culture program.

b. The Arctic grayling program is currently inactive. Donor requirements listed are based on historic stocking requests.

Table 2.–Approximate annual donor and egg requirement for Chinook salmon egg take, Chena River or Salcha River.

	Region 3
Program Requirements:	
Catchables to stock annually(2N)	40,000
Survival Estimates:	
Survival Green to Eyed Egg	88.0%
Survival Eyed Egg to Ponding:	98.0%
Survival Fry to Fingerling:	99.4%
Projected grade	90.0%
Survival Fingerling to Catchable:	99.6%
Number of Eggs Needed:	52,055
Females Required:	
Fecundity (eggs/female):	6,600
Females required for spawning	8 (+2[a])

a. The eggs from 2 additional females will be collected in case disease screening results are positive.

Table 3.–Approximate annual donor and egg requirement for coho salmon egg take, Delta Clearwater River.

	Region 3
Program Requirements:	
Fingerlings to stock (2N)	96,000–105,500
Survival Estimates:	
Survival Green to Eyed Egg	97.0%
Enumeration Correction	85.0%
Survival Eyed Egg to Ponding:	97.7%
Survival Fry to Fingerling:	94.7%
Number of Eggs Needed:	125,845–138,298
Females Required:	
Fecundity (eggs/female):	3,750
Females required for spawning	34–37 (+2[a])

a. The eggs from two additional females will be collected in case disease screening results are positive.

Table 4.–Allowable annual yield of lake trout predicted from lake area and the most recent population estimates for mature fish in Sevenmile Lake and Glacier Lake.

	Sevenmile Lake	Glacier Lake
Lake Area (ha)	35	178
Assumed Average Size of Fish (kg)	0.7 [a]	0.9 [a]
Allowable Annual Yield	187 [a]	71 [a]
Population Size (of mature fish)	1,109 (SE=170) [b]	1,474 (SE=324) [c]

a. Burr 2006
b. Parker and Wuttig 2000
c Burr 1991

Table 5.–Approximate alternate year donor and egg requirement for lake trout egg take, Sevenmile Lake or Glacier Lake.

	Region 2	Region 3
Program Requirements:		
Subcatchables to stock every 4 years (3N)	11,500	11,500
Survival Estimates:		
Survival Green Egg to Ponding:	86 %	86 %
Survival Fry to Fingerling:	90 %	90 %
Survival Fingerling to Subcatchable:	98%	98%
Number of Eggs Needed:	19,835	19,835
Females Required:		
Fecundity (eggs/female):	1,000 [a]	1,000 [a]
Females required for spawning	20	20

[a.] Fecundity based on Sevenmile Lake stock.

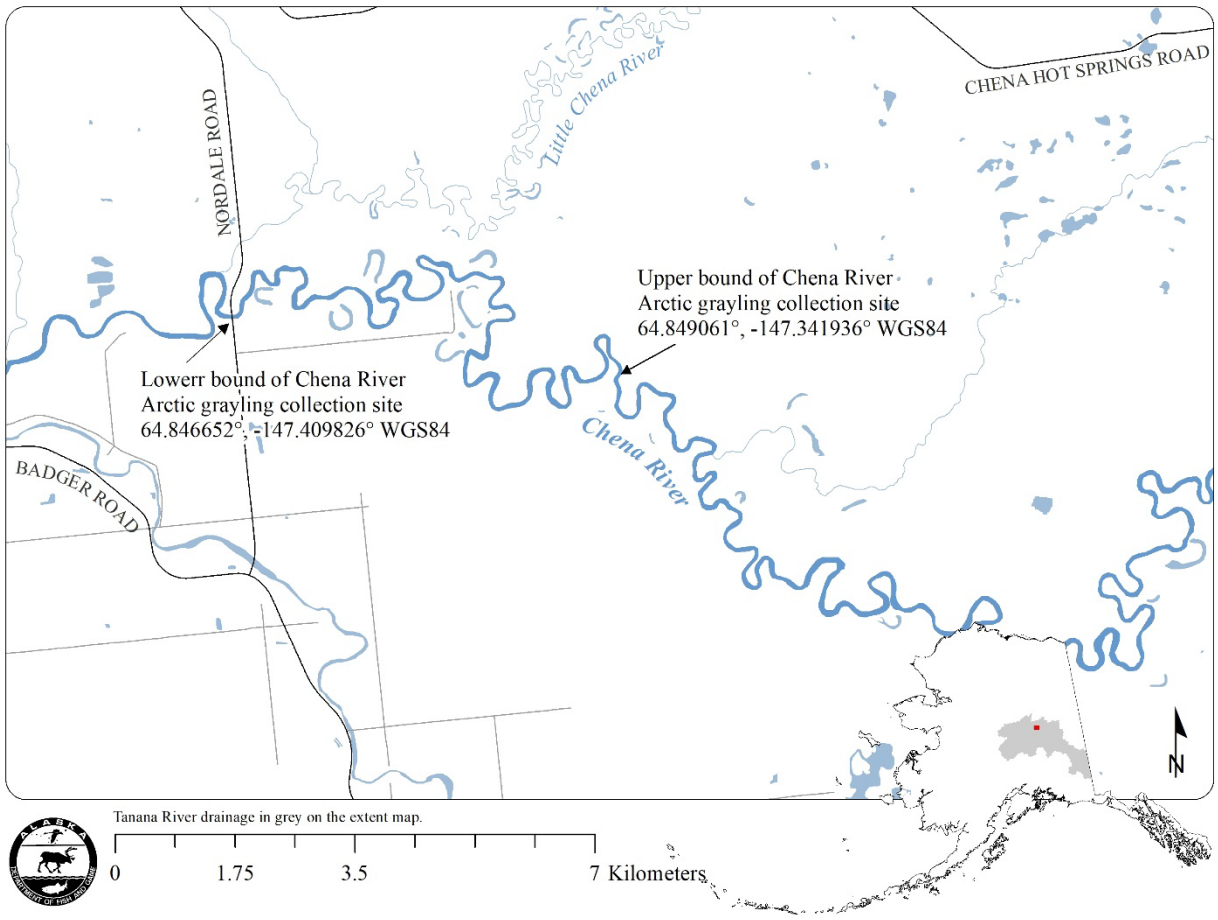


Figure 1.–Location of Arctic grayling capture area on the Chena River.

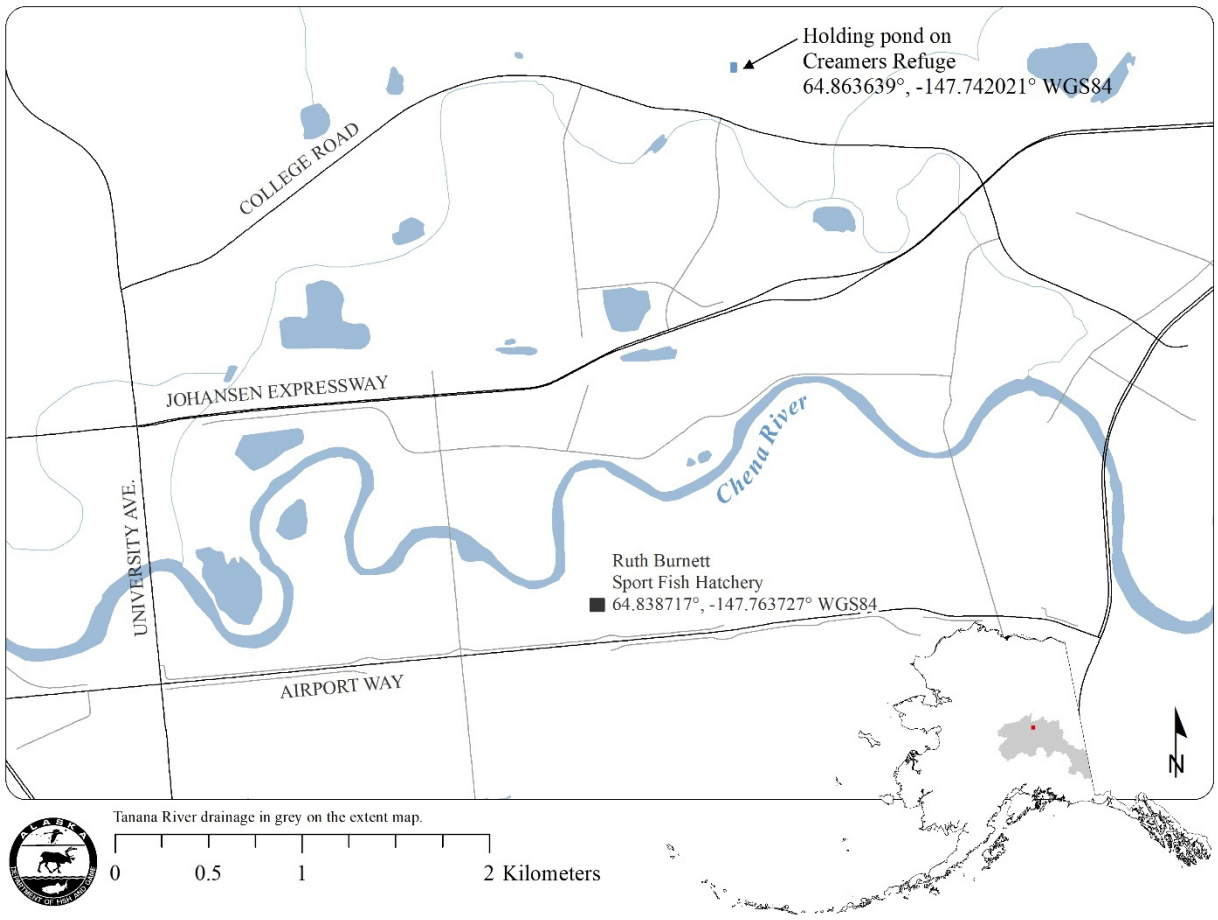


Figure 2.–Location of the holding pond on Creamers Refuge used for Arctic grayling and the Ruth Burnett Sport Fish Hatchery (RBSFH).

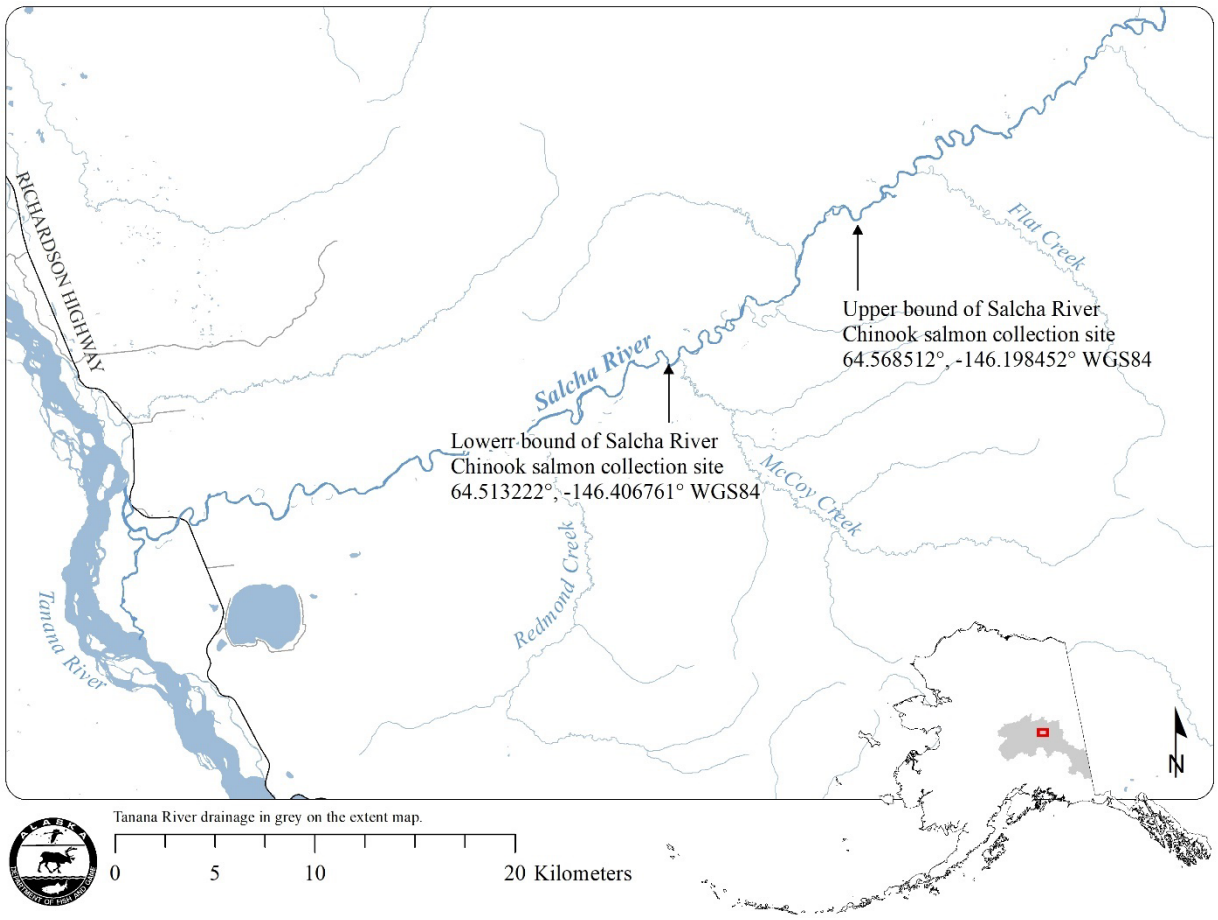


Figure 3.–Location of the Chinook salmon capture area on the Salcha River.

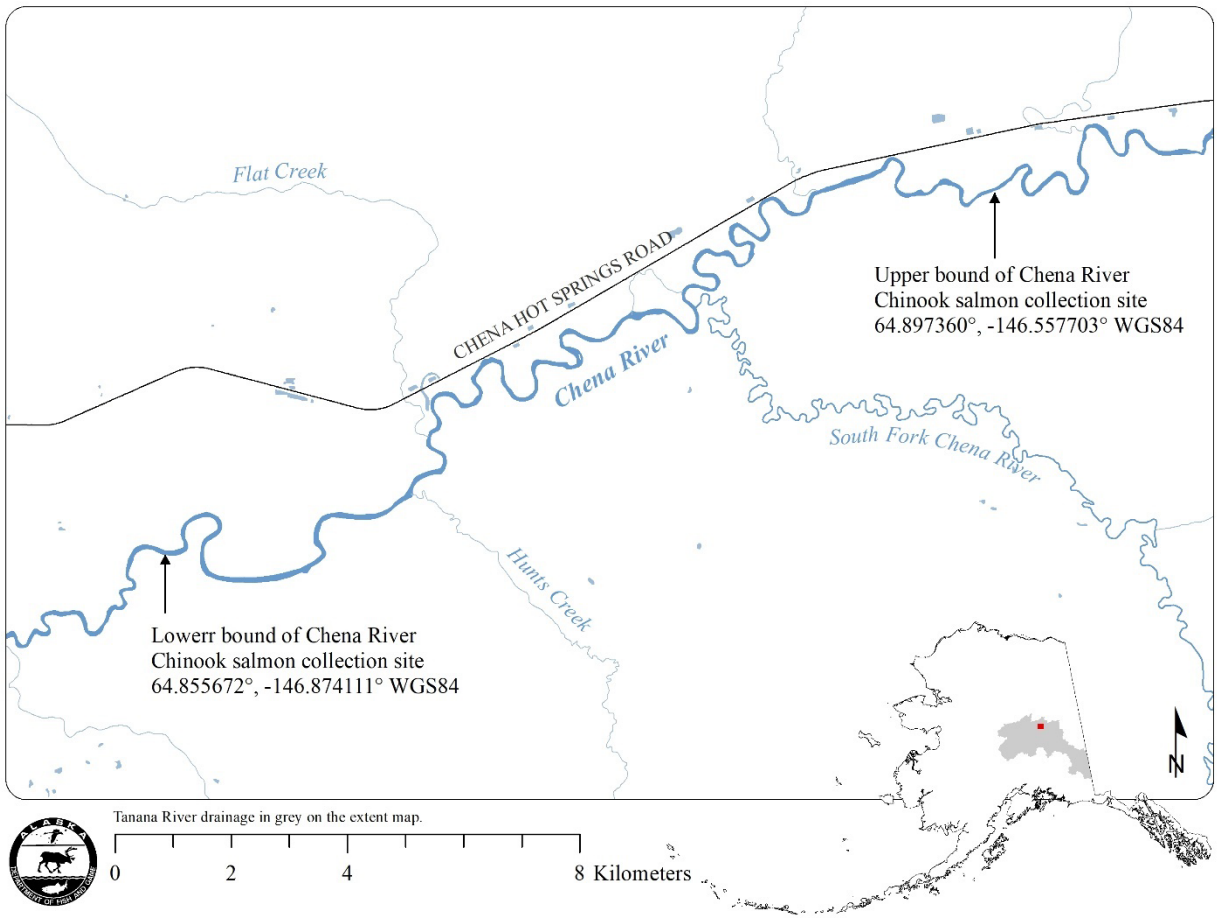


Figure 4.–Location of the Chinook salmon capture area on the Chena River. The blue arrow indicates the direction of river flow.

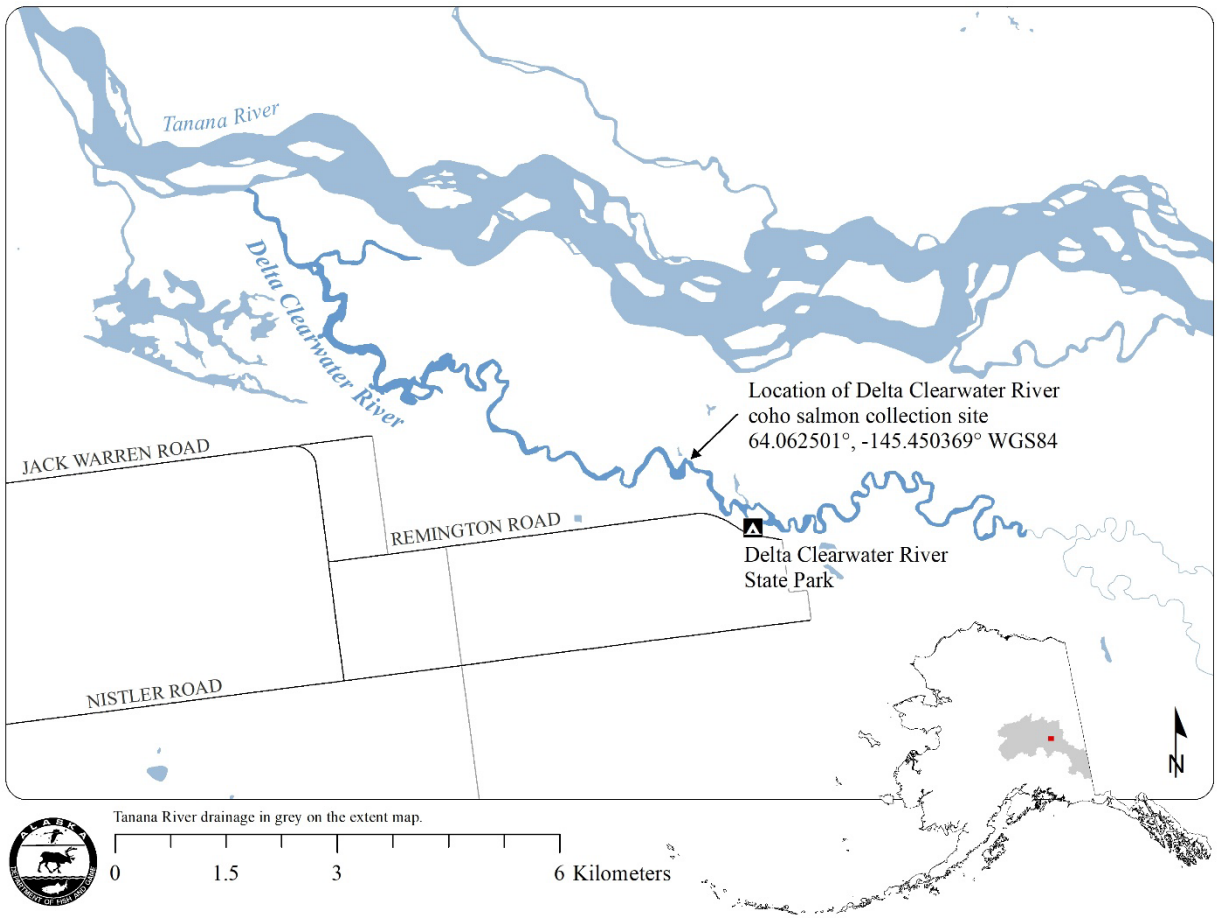


Figure 5.–Location of coho salmon capture area on the Delta Clearwater River. Blue arrows indicate direction of river flow.

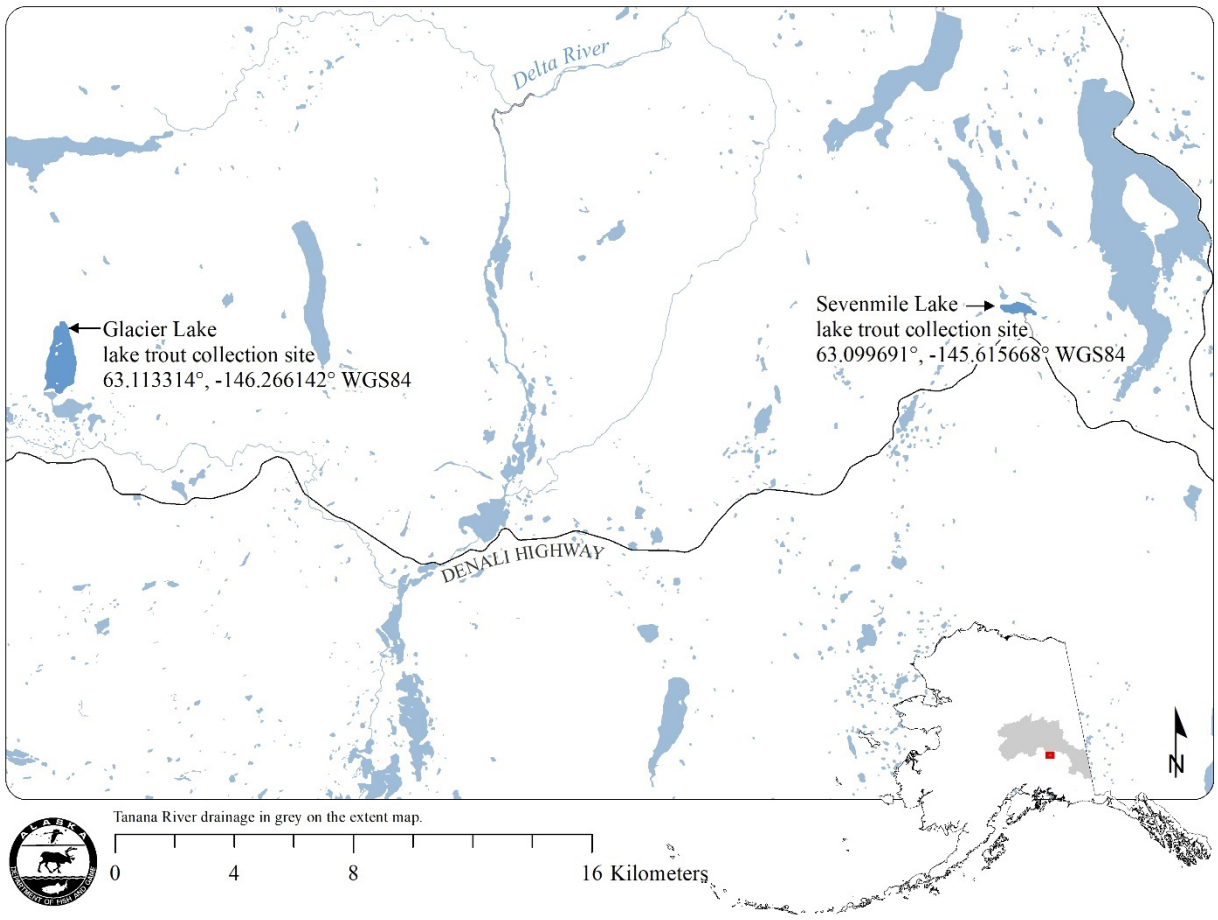


Figure 6.–Location of lake trout capture area at Sevenmile Lake or Glacier Lake.

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

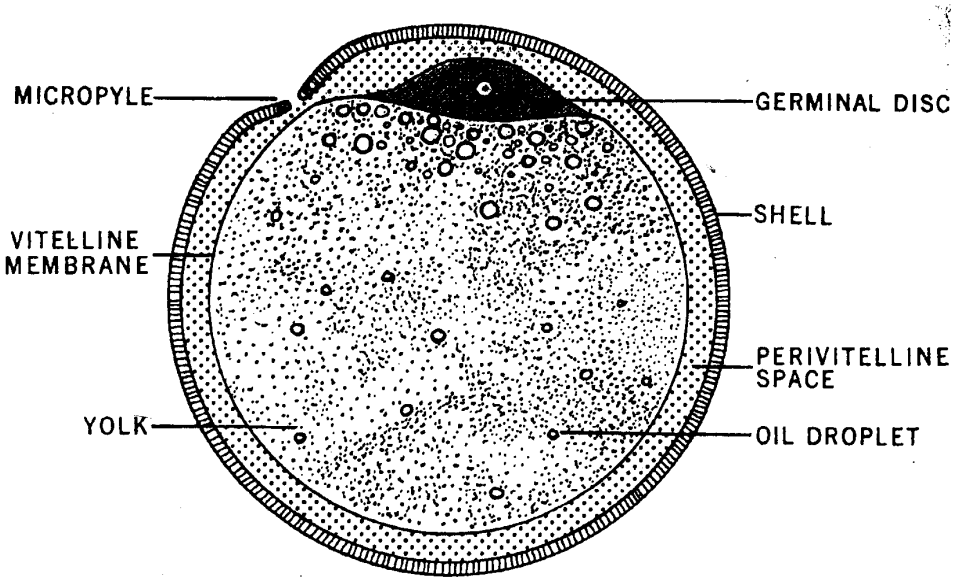
GENERALIZED EGG-TAKE PROCEDURES FOR ADF&G SPORT FISH HATCHERY PROGRAM

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 a 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°C 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. The variation in development rate between different individual eggs increases as development progresses. 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 2 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 3 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 any time, 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 1 to 6 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 the 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	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
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 1 or 2 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 1:3. 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.

Handle females carefully when sorting. 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 blood flow. 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 trout 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 5 females and 5 males. After water hardening 4 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 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 dependent. 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. If the eggs are to be shipped on ice, make sure that the egg temperature does not drop below 5°C. 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 CTUs following fertilization. (The 5° rule will not apply with coho and char because 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 2°C 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 2 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 is 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 egg temperature. 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, 3 full colanders, and a fourth colander filled up to a marked 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 the water as it starts to flow to ensure 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 on-site 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 cup with eggs.
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 3 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 2 hour shift of egg collection, and a minimum of 3 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 keep the eggs out of 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 3 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 3 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 ml 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/5 ml of water displaced=34.2 eggs/ml) and recorded.

5. Steps 1 through 4 will be repeated a minimum of 3 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 these 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.

1 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.

2 The brood should be family tracked during egg-take. 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 test positive for this pathogen are destroyed.

3 Progeny from adults that are known to be carrying this bacteria are fed a 4 week ration of Erythromycin thiocyanate as soon as they are able to consume the #1 crumble feeds.

Rainbow trout: All 3 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 4 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 EGG TAKE

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 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 described in Appendix B.

Alaska Department of Fish & Game, Sport Fish Division
Egg Take Worksheet

Date: _____ Water Temp: _____

Species: _____ Time Started: _____

Location: _____ Time Completed: _____

Females

Ripe	Green	Bad	Morts

Males

Ripe	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.

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 at 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.