Regional Operational Plan SF.2A.2013.06

Operational Plan: Crooked Creek Chinook Salmon Enhancement Project

by

Jenny L. Cope

May 2013

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 figure 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

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CROOKED CREEK CHINOOK SALMON ENHANCEMENT PROJECT

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Alaska Department of Fish and Game, Division of Sport Fish, Soldotna

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Division of Sport Fish

May 2013

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This document should be cited as:
Jenny L. Cope. 2013. Crooked Creek Chinook salmon enhancement project. Alaska Department of Fish and Game, Division of Sport Fish, Regional Operational Plan ROP.SF.2A.2013.06, Soldotna.

Signature Page

Project Title:	Crooked Creek Chinook Salmon Enhancement Project
Project leader(s):	*Jenny L. Cope, Fishery Biologist I*
Division, Region and Area	Sport Fish, Region II, Soldotna
Project Nomenclature:	F-10-27
Period Covered	2013 - 2015
Field Dates:	June 1 – August 15
Plan Type:	Category II

Approval

Title	Name	Signature	Date
Project leader	Jenny L. Cope		
Biometrician	Anton Antonovich		
Research Coordinator	Jack Erickson		

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PURPOSE

The overall goal of this research program is to reconstruct naturally- and hatchery-produced returns of Chinook salmon to Crooked Creek such that a biological escapement goal (BEG) can be established.

BACKGROUND

Crooked Creek is a tannin-stained stream flowing into the glacial waters of the Kasilof River approximately 11 kilometers (km) upstream of the Kasilof River's mouth in Cook Inlet. The Kasilof River (flowing from its outlet at Tustumena Lake) is approximately 31 km to Cook Inlet (Figure 1). Its origin in the glaciers of the Kenai Mountains makes it turbid throughout the year. Four species of Pacific salmon: Chinook *Oncorhynchus tshawytscha*; coho *O. kisutch*; sockeye *O. nerka;* and pink *O. gorbuscha* are present in the drainage, as well as anadromous and resident rainbow trout *O. mykis*s, Dolly Varden *Salvelinus malma*, resident lake trout *S. namaycush* and round whitefish *Prosopium cylindraceum* (Johnson and Weiss 2006). Sport fisheries exist for all Pacific salmon species present, although most of the sport fishing effort is directed at early-run Chinook salmon destined for Crooked Creek. This operational plan describes Alaska Department of Fish and Game (ADF&G) Chinook salmon enhancement, escapement enumeration and biological sampling at the Crooked Creek Facility.

CROOKED CREEK FACILITY AND OPERATIONS

Crooked Creek originally had a stock of wild Chinook salmon, which has been supplemented with hatchery-produced Chinook salmon smolt of Crooked Creek origin. The stocking program began in 1974 and since then (except 1997 and 1998) the annual escapement has been monitored through a weir at the Crooked Creek Facility (Todd *Unpublished*). Naturally-produced fish (fish from naturally spawning parents) made up 96% of the escapement in 1978, but declined in proportion as hatchery production increased during the 1980s. Since 2002, the proportion of naturally-produced fish (fish denoted by the presence of an adipose fin) in the escapement has remained consistently higher than fifty percent and is likely to continue due to regulation changes affecting harvest in the Kasilof River sport fishery, and the reduction of the enhanced component (numbers of hatchery-produced smolt released). The Crooked Creek Hatchery Facility was operated by ADF&G until 1995, when Cook Inlet Aquaculture Association (CIAA) assumed operations. Escapement monitoring continued until 1997 when the facility was returned to ADF&G. There was no escapement monitoring at the Crooked Creek Facility during 1997 and 1998. During this time, smolt continued to be stocked despite inactivity at the facility. ADF&G resumed escapement monitoring in 1999. From 1988 to 1996, the number of naturally-produced Chinook salmon was held to approximately 700 fish in the spawning escapement of Crooked Creek upstream from the hatchery. The current management policy, adopted in 2001, requires ADF&G to achieve a sustainable escapement goal (SEG) at the Crooked Creek weir of 650-1,700 age-1.2+ naturally-produced adult Chinook salmon during the early run (Bue and Hasbrouck 2001).

Historically, hatchery-produced smolt from the Crooked Creek Chinook salmon stock have been stocked at different sites to create or enhance sport fisheries. Presently this stock is used to enhance Crooked Creek itself, one stocked lake on the Kenai Peninsula, and terminal fisheries in Resurrection Bay. From 1974 through 1994, broodstock collection and egg-takes were conducted at the Crooked Creek Hatchery. In 1995, broodstock collection moved to the Nick

Dudiak Fishing Lagoon (Homer Spit) where progeny from Crooked Creek Chinook salmon were returning. Adult fish were captured at the Homer Spit, transported to Elmendorf Hatchery, and held for egg-takes. Spawning success was low varying from 34% in 1995 to 66% in 1996 (D. Keifer, ADF&G, Elmendorf Hatchery, personal communication). Hormone ripening tests were conducted in 1997 and 1998 (at the Homer Spit) with generally poor results. Because of these problems and incidences of straying adult Chinook salmon, egg-takes and smolt imprinting were moved back to the Crooked Creek Facility. Starting in 1999, smolt were held at the facility for imprinting to address straying problems and egg-takes were conducted on-site to improve spawning success and fertilized gametes were taken to Fort Richardson Hatchery for incubation. Beginning in 2011, gametes are transported separately to William Jack Hernandez Sport Fish Hatchery (WJHSFH) where they are later fertilized and incubated.

Concerns about straying resulted in other changes in stocking policy for 2000 and subsequent years: (1) a decreased stocking level from approximately 210,000 smolt in 1999 to approximately 105,000 in subsequent years; and (2) marking all smolt with an adipose fin clip (AFC), a coded wire tag (CWT) and thermal otolith mark. In previous years the marking rate was highly variable, ranging from 12.5% to 50.0%. Currently the marking rate is estimated to be 100% for each of the three marks. In 2011, CWT's were discontinued; however adipose fin clips and thermal marks are used for marking hatchery-produced fish.

OBJECTIVES

The objectives of this study during 2013-2015 are to annually:

1. Census the escapement of naturally- and hatchery-produced Chinook salmon in Crooked Creek that pass through the weir from late May to the middle of August.
2. Estimate the age composition, sex composition, and age-by-sex composition of the naturally- and hatchery-produced Chinook salmon in Crooked Creek, such that the estimated proportions are within 10 percentage points of the true value 90% of the time.[1]

SECONDARY OBJECTIVES

In addition to the primary objectives outlined above, the following secondary objectives of this project are to annually:

1. Hold, imprint, and release approximately 105,000 Chinook salmon smolt at the Crooked Creek Facility in June.[2]
2. Collect, hold, and artificially spawn a minimum of 63 male and 63 female naturally- and hatchery-produced Chinook salmon adults returning to Crooked Creek during July.[3]
3. Collect a sufficient amount of fertilized eggs to release approximately 105,000 Chinook salmon smolt at Crooked Creek and up to 210,000 smolt for other releases.
4. Monitor upstream migration of returning adult sockeye salmon during the Chinook salmon run from late May to the middle of August.

[1] Until 2006, the criterion was within 0.075, 95% of the time. Simulations have shown that age composition sample sizes of less than 100 are sufficient to estimate stock-recruit parameters (Steve Fleischman, Fisheries Scientist, personal communication).

[2] Zero broodstock were collected in 2012 due to low abundance of naturally-produced Chinook salmon. Consequently, no eggs were collected to produce 105,000 smolt for stocking in 2013.

[3] This number is provided by William Jack Hernandez Sport Fish Hatchery staff and may change in response to stocking demands and production at other broodstock collection sites.

5. Summarize coded wire tags recovered from Chinook salmon stocked into Crooked Creek in previous years including recoveries found outside of the Kasilof River drainage.
6. Estimate the mean length-at-age of the naturally- and hatchery-produced Chinook salmon in Crooked Creek that pass through the weir from late May to the middle of August.
7. Collect axillary process tissue samples from age-1.2 and older naturally-produced and hatchery-produced Chinook salmon (target sample size of 200 each) migrating through the weir for inclusion in a genetic database of Cook Inlet Chinook salmon stocks. [4]
8. Collect otolith samples from naturally-produced Chinook salmon used for broodstock to determine if they were thermally marked and from another stocking location. [5]

METHODS

ESCAPEMENT SAMPLING

ADF&G personnel will monitor the weir from late May until approximately the middle of August or until the daily count of Chinook salmon through the weir is less than 1% of the cumulative seasonal count for three consecutive days. Fish will be allowed unobstructed passage through a chute located and attached to the gate in raceway 2 (Figure 2). An adjustable aluminum picket V-shaped gate attached to the exiting end of the passage chute will allow fish to exit upstream but not allow reentry.

A digital video recorder (DVR) will be used to record fish passage through the Crooked Creek Facility (Figure 2). The digital video system will be located in raceway 2 (Figure 2). One underwater video camera will be located inside a sealed video box that will be attached to the fish passage chute. The video box will be constructed of 3.2-mm aluminum sheeting and will be filled with filtered water to keep it submerged under the water in the raceway. Safety glass will be installed on the front of the video box to allow for a scratch free, clear surface through which video footage of passing fish will be captured. Video images will be recorded using a PC-based DVR located inside a building near the raceway. Two 12-volt underwater pond lights will be mounted inside the video box to provide a consistent source of light during all hours of the day and night.

Video information will be reviewed daily (Monday through Friday) by ADF&G personnel. All Chinook salmon will be examined for the presence of an AFC from recorded video footage. The hatchery contribution to the adult escapement into Crooked Creek can be obtained directly from the count of AFC Chinook salmon counted at the weir each year, since (1) all returning adults are from stocking release groups that were marked at a 100% rate, and (2) all Chinook salmon are inspected for AFC marks by examining recorded video footage. Determination of sex was attempted in 2008 and results illustrated the data collected was highly variable among video reviewers so this will not be conducted. Sex composition will be determined by biologically sampling the escapement (see Biological Sampling). Other species of adult fish such as Dolly Varden, rainbow trout or steelhead, pink salmon and sockeye salmon will be enumerated. Juvenile salmonids will not be identified or enumerated. Additionally, the entire return of coho

[4] The target sample size was provided by the ADF&G Gene Conservation Laboratory. This task will be conducted on odd years only.

[5] This task is a stipulation of the fish transport permit (FTP) covering egg takes at Crooked Creek. Otoliths from non-adipose fin clipped fish were collected in 2009 and 2010. No otoliths were collected in 2011 because there were no egg takes. The stipulation for the FTP is that this task be conducted for three years. No otoliths were collected in 2012. 2013 will be the last year this task will be conducted.

salmon will not be enumerated as they have periodically been in past years. This is in part due to the lack of personnel during coho salmon season and the amount of time required to review video footage containing unknown juvenile salmonids. All observed data will be recorded on the DVR Passage data form (Appendix A1). Chinook salmon longer than a 20-inch reference mark located within the DVR passage chute will be considered 2+-ocean fish, those shorter will be considered jacks (1-ocean Chinook salmon) [6]. Limited historical data from 1999, indicated that 1-ocean fish MEF lengths were in the 311-428 mm range (20 inches=508mm). We feel that the 20-inch reference mark used to enumerate jack Chinook salmon is a valid assumption for the length cutoff.

In the event of a DVR malfunction while staff are present, the gate to the upstream DVR passage chute and the swinging gate at the sampling structure will be closed immediately. All fish will be held in the tail trough and diverted to the sampling structure/box (Figure 2) for biological sampling, counting and passed upstream manually each day. ADF&G personnel will attend the weir every weekday until the DVR is operable. Staff will not be stationed at the weir after normal working hours or on weekends, but in the case of an electrical malfunction a battery backup and alarm system will be connected to a power source at the Crooked Creek Facility. This system will ensure a minimal amount of data is lost in the event of a power outage. An alarm system will automatically call ADF&G personnel and notify them of the problem and corrective measures will be taken immediately. The battery backup system should provide power to the DVR system until someone can get to the weir.

New weir designs were implemented during the 2009 and 2010 field seasons to allow for improved juvenile fish and emigrating steelhead kelt passage. A daily count will be kept of any steelhead and other fish mortalities as well as for emigrating fish that may be trapped, requiring assistance to pass the weir. The daily count data will be recorded on the Weir Mortalities and Trapped Emigrating Fish data form (Appendix A2). The weir will be cleaned to remove debris as necessary to ensure adequate water flow.

Due to gravel movement in an upstream braided channel above the Crooked Creek Facility, the water flow into the main water intake gate may be greatly diminished. In 2004, 2005, 2008 and 2011 ADF&G personnel obtained an ADF&G, Division of Habitat, Fish Habitat Permit and dredged this area to a depth of approximately four feet using a large track hoe. This dredging has increased the water flow into the main water intake gate, head trough and subsequently into raceways 1 and 2 (Figure 2). The creek inspection will be completed by late April and dredging will be completed by early May if it is needed and if snow depth and spring weather permits. Other measures will be taken to divert water as well. In the event of low water levels, a fence made with specialty fabric will be installed in Crooked Creek at a slight angle such that it parallels the current. It will divert water from the main channel to the channel that feeds water to the facility. An ADF&G, Division of Habitat, Fish Habitat Permit will be obtained for this activity as well.

Other changes were made to sampling operation and facility maintenance in 2009. Because the tail trough wall was identified as a life safety hazard in 2008, necessary measures were taken to enable the safety of personnel while handling and sampling fish. Instead of sampling and sorting fish in the tail trough, a sampling box and v-trap attached to the gate were installed in raceway 1.

[6] Sport fishing regulations define bag limits for Chinook salmon shorter or longer than 20 inches of length. Because of this, we consider Chinook salmon less than 20 inches to be jacks or 1-ocean Chinook salmon.

The box had an attached chute that provided ease in sorting fish. Fish were either passed into the broodstock collection area or back into the tail trough and through the DVR passage chute to be counted during video review. This feature allows easy and accessible handling of fish without putting individuals in the immediate vicinity of the tail trough wall. Additionally, the gate near the DVR box was moved closer to the sliding gate at the downstream edge of raceway 1. This helped direct Chinook salmon into the sampling box for entrapment (Figure 2). All incorporated structures will be utilized in the upcoming field seasons.

SMOLT IMPRINTING AND RELEASE

Dependent on spring weather and in preparation of DVR installation and smolt delivery, raceway 1 and 2 at the Crooked Creek Facility will have remaining ice removed and be cleaned using high-pressure water hoses. Once debris and sediments are removed, the raceways will be disinfected with a water and betadyne solution of 200 parts per million (Tesch, ADF&G, WJHSFH, personal communication). Preferably, this will be done on a sunny day to increase the effectiveness of the microbicide treatment. The raceway will then be flooded with water such that the water level is maintained within one foot of the top. One technician and the project biologist will be involved in the preparation.

Chinook salmon smolt (approximately 105,000 fish with an expected 100% mark: AFC and thermal otolith mark) will be transported from WJHSFH to the Crooked Creek Facility during the first week of June. A network of ultra-violet stabilized polyethylene fabric panels will be hung over the raceway to protect the imprinting smolt from feeding activities of birds and sun burn. ADF&G personnel will be on duty to feed the smolt a minimum of twice daily and monitor operations. Smolt will be held for approximately 7 days for imprinting. A daily smolt mortality census will be conducted and recorded on a Smolt Imprinting and Release data form (Appendix A3). Smolt will be examined for an AFC. If non-AFC fish are found in the mortality census, personnel will prepare them for storage and they will be sent to the ADF&G Coded Wire Tag Laboratory to identify if they have a thermal otolith mark. This will be done to confirm whether or not smolt are of hatchery- or naturally-produced origin. If mortality levels become a concern, the smolt may be released sooner. Other information including water temperature, dissolved oxygen content, and quantity of food fed will be recorded on a Smolt Imprinting and Release data form (Appendix A3). If ADF&G personnel encounter any problems with water flow into raceway 1 during the 7 days of imprinting, the gate to the DVR passage chute and the fish ladder located downstream of the weir will be closed and all adult fish species will not be allowed into the tail trough for passage.

After the smolt are released, raceway 1 will be dewatered, cleaned and disinfected in preparation for biological sampling and holding adult Chinook salmon for broodstock.

BIOLOGICAL SAMPLING

The gate to the DVR passage chute will be closed twice weekly which is tentatively scheduled to be on Mondays and Thursdays. Chinook salmon will be sampled for biological information the following day (Tuesdays and Fridays). Following sampling, Chinook salmon will either be placed into raceway 1 for holding as broodstock or into the tail trough (via the sampling chute) for upstream passage through the DVR. The water flow at the main water intake gate will be reduced for sampling purposes (Figure 2).

Adult Chinook salmon collected for sampling will be examined for sex, length (mid-eye to fork-of-tail to the nearest 1 mm), and the presence of an AFC. Scales will be removed and all data recorded on the Scale Sampling data form (Appendix A4). Age-1.1 Chinook salmon, primarily males of hatchery origin and easily identified by their small size (<20 inches total length), will be passed upstream without being sampled for age and length. Axillary process tissue samples will be collected from all naturally- and hatchery-produced Chinook salmon captured for biological sampling in 2013 and 2015. Tissue samples are taken on a biennial basis as recommended by the ADF&G Gene Conservation Laboratory, Division of Commercial Fisheries. Fish collected for broodstock and all mortalities will be recorded by sex and tallied on the Broodstock Collection data form (Appendix A5).

Sample size for estimation of the age and sex composition of the escapement of naturally-produced adult Chinook salmon was determined by applying a finite population correction factor (Cochran 1977) to the sample size given by Thompson 1987 as follows:

$$n_a = \frac{n_o}{1 + \frac{n_o - 1}{N}}, \quad \textbf{(1)}$$

where:

n_o = 101 adult Chinook salmon (Thompson 1987), and

N = total number of adult Chinook salmon expected to migrate past the weir.

Assuming that approximately 655 age-1.2+ naturally-produced adult Chinook salmon migrate to the weir during 2013[7], then 88 valid ages are required. Given that age cannot be determined on ~15% of the scale samples, sampling 104 adult Chinook salmon (~15% of projected return) would meet the stated objective criterion.

Assuming that approximately 210 age-1.2+ hatchery-produced adult Chinook salmon migrate to the weir during 2013[8], then 68 valid ages are required. Given that age cannot be determined on ~15% of the scale samples, sampling 80 adult Chinook salmon (~38% of projected return) would meet the stated objective criterion.

Accordingly, we can expect to obtain better than the desired precision for naturally-produced fish and slightly less than desired precision for hatchery-produced fish age and sex composition estimates by closing the gate for two 24-hour periods per week (= 48/24 x 1/7 = 29% of available time) and taking scale samples (see below) from every fish accumulated during that time.

The same method will be used to generate samples sizes in 2014 and 2015.

Three scales from the preferred area[9] will be collected from each adult Chinook salmon selected for age sampling (Welander 1940). Scales will be mounted on adhesive coated scale cards; the scales will be pressed such that impressions are made on acetate cards to allow aging postseason, following procedures described by Mosher (1969). Sex, length and marking status will be recorded for each fish by date on the Scale Sampling data form (Appendix A4).

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[7] Average of 2011-2012 naturally-produced fish to the weir = 655.

[8] Average of 2011-2012 hatchery-produced fish to the weir = 210.

[9] The preferred area for scale sampling is on the left side of the fish at a point on a diagonal line from the posterior insertion of the dorsal fin to the anterior insertion of the anal fin, two rows above the lateral line.

BROODSTOCK COLLECTION AND EGG-TAKES

Broodstock collection will begin in approximately late June or as soon as ripe fish start returning to the weir. Raceway 1 will be used to hold naturally-produced and hatchery-produced broodstock and the tail trough and raceway 2 will be used for passing non-ripe fish upstream. Any bright fish encountered during broodstock collection days will be immediately passed upstream as they have higher mortality rates when held. A minimum of 63 males and 63 females (WJHSFH request) will be used for the egg takes that will occur 2 or 3 times throughout July and possibly the first week of August. While fish are being held, raceway 1 will be partially covered by a network of polyethylene, ultra violet stabilized fabric panels to provide shelter from environmental conditions. Water temperature, mortalities and dissolved oxygen content will be recorded daily on the Broodstock Holding Hydrology and Mortality data form (Appendix A6).

Attempts will be made to collect sufficient numbers of naturally-produced adult Chinook salmon to ensure that there is enough progeny to be returned to Crooked Creek as smolt. Attempts will also be made to collect sufficient numbers of both naturally- and hatchery-produced adult Chinook salmon to support other stocking demands. If Crooked Creek naturally-produced Chinook salmon return is low, to the degree that sacrificing fish for egg takes will result in an escapement below the lower bound of the SEG (650), then marked (AFC) fish will be used exclusively as the source for the egg take. Hatchery-produced Chinook salmon progeny will not be used for restocking Crooked Creek in years when naturally-produced Chinook salmon return is low. Consequently, Crooked Creek may not be stocked with the total requested number of smolt. After the egg take, the heads of AFC marked fish will be removed and a cinch strap attached. These heads will be frozen and shipped to the ADF&G Coded Wire Tag Laboratory for verification of the smolt release location. Information on CWT fish will be recorded on the ADF&G Coded Wire Tag Laboratory CWT data form (see Appendix B1 for sampling instructions) and submitted to the ADF&G Coded Wire Tag Laboratory (the standard CWT form from the ADF&G Coded Wire Tag Laboratory will be used, but is not attached to this operational plan). Additionally, otoliths will be collected from all non-AFC Chinook salmon used for broodstock (see Appendix B2 for sampling instructions). The samples will be submitted to the ADF&G Thermal Mark Laboratory for analysis of hatchery-derived Chinook salmon strays.

Adult Chinook salmon being held for broodstock will be examined to determine sexual maturity; this will assist in setting dates for egg-takes. Egg takes are tentatively scheduled to begin in the middle of July and will be conducted weekly until desired numbers of fish have been artificially spawned. Two WJHSFH staff, 4 Soldotna staff and 2-3 Homer area office personnel will conduct the egg-takes. William Jack Hernandez Sport Fish Hatchery staff will provide necessary equipment for collecting gametes from broodstock. Eggs will be taken on-site following a limited Chinook salmon egg-take protocol (ADF&G 1983). Fish used for the egg-take will be sacrificed and recorded on the Egg Take data form (Appendix A7). The abdomen of the fish will be wiped with betadyne before removing the eggs. Separate gametes will be placed in sealed plastic bags. The gametes will be placed on ice in coolers for transport to WJHSFH the same day. The gametes will be fertilized at WJHSFH. Fish used for egg takes will be sampled for Infectious Hematopoietic Necrosis Virus (IHNV) and sampled for Bacterial Kidney Disease (BKD) by collecting ovarian fluid samples from females, and liver/kidney samples from males. These samples will be sent to the ADF&G Fish Pathology Laboratory for testing.

SOCKEYE SALMON

Small numbers of sockeye salmon arrive at the Crooked Creek weir from late July into September. Some sockeye salmon may pass upstream of the weir while the DVR is operating. Infectious Hematopoietic Necrosis Virus (IHNV) is commonly found in sockeye salmon (Meyers 2003). High densities of sockeye salmon on Chinook salmon spawning grounds can increase the potential spread of IHNV to Chinook salmon. Should Crooked Creek Chinook salmon stocks become infected with IHNV, the ability to use them for broodstock for Chinook salmon enhancement projects would be compromised. Since run timing of Crooked Creek sockeye salmon and Chinook salmon differ (sockeye salmon return late July through August and Chinook salmon June through early August), concerns of disease transmittal during broodstock collection periods are reduced (Meyers, Fish Pathology Laboratory, ADF&G, personal communication).

Sockeye salmon will be able to pass through the Crooked Creek facility freely although their passage will be recorded and enumerated using the DVR. On sampling days and broodstock collection days, any sockeye salmon encountered will be enumerated and destroyed. End of season sockeye salmon escapement summaries will be given to ADF&G Fish Pathology Laboratory and hatchery personnel for evaluation and programmatic recommendations will be solicited.

STRAYING OF CROOKED CREEK CHINOOK SALMON OF HATCHERY ORIGIN

In past years, CWT Chinook salmon stocked into Crooked Creek have been recovered at locations outside of the Crooked Creek and Kasilof River drainages. In the fall following the field season, the ADF&G Coded Wire Tag Laboratory database will be queried for all CWT recoveries of Chinook salmon originally released at Crooked Creek. These records will provide information about the location of the fish at the time of tag recovery and about potential straying into other systems.

DATA REDUCTION

Crooked Creek DVR counts, weir mortalities, smolt imprinting, broodstock collection, broodstock holding hydrology, egg take and ASL information will be recorded on specialized field data forms (Appendices A1-A7). Technicians will return data forms to the Soldotna office daily. The Project Biologist will examine all data forms for errors and enter the data electronically. The Project Biologist will convert the data to a fixed width comma separated values (.csv) modified mark sense format for analysis.

Data maps for all of the information collected in this project are shown in Appendix C. The project biologist will edit Crooked Creek biological and escapement data to ensure values of counts, age, and length-at-age are within regular bounds. The biologist will also prepare inseason data summaries daily, conduct postseason data analyses, and write the Division Fishery Data Series report. All Crooked Creek data will be in computer files and edited by 1 November. A final edited copy of all data files along with a data map will be sent to the Alaska Department of Fish and Game Research and Technical Services (RTS) for archiving.

DATA ANALYSIS

Separate analyses will be conducted for naturally- and hatchery-produced fish. The number of one-ocean age Chinook salmon (jacks) will be determined by examining recorded footage and comparing all passing fish to a 20-inch reference mark. Number of one-ocean age Chinook salmon, as well as fish of other age groups, serves an important role in brood table construction and subsequently escapement goal analysis.

The proportion of one-ocean age Chinook salmon for each group (naturally- versus hatchery-produced) will be calculated as a ratio of the number of jacks (N_{jack}) to the total number of Chinook salmon that passed through the weir (N_{weir}):

$$p_{jack} = \frac{N_{jack}}{N_{weir}} \tag{2}$$

The number of adult Chinook salmon, N_{adult}, in the weir count will be calculated by subtracting the number of jacks (N_{jack}) from the total number of Chinook salmon that passed through the weir (N_{weir}):

$N_{\text{adult}} = N_{\text{weir}} - N_{\text{jack}}$

The proportion of adult (ocean age-.2 and older) Chinook salmon which belongs to age/sex class j in each group (naturally- versus hatchery-produced) will be estimated using the following equations:

$$\hat{p}_j = \frac{n_j}{n}, \quad \text{and} \tag{3}$$

$$\text{var}(\hat{p}_j) = \left[1 - \frac{n}{N_{adult}}\right] \frac{\hat{p}_j(1-\hat{p}_j)}{(n-1)}, \tag{4}$$

where:

n_j = the number of adult Chinook salmon of age/sex class j,

n = the total number of adult Chinook salmon scale samples that could be aged, and

N_{adult} = the number of adult Chinook salmon in the weir count.

The number of adult Chinook salmon in age/sex class j (naturally- versus hatchery-produced) will be estimated as the product of a constant (N_{adult}) and a random variable ($\hat{p}_j$). The variance of this estimate will be calculated as:

$$\text{var}\left(\hat{N}_j\right) = N_{adult}^2 \left[\text{var}\left(\hat{p}_j\right)\right]. \tag{5}$$

Mean length-at-age of the naturally- and hatchery-produced Chinook salmon will be estimated by standard statistical techniques.

All components of hatchery release groups returning to Crooked Creek were 100% marked; therefore the number of marked Chinook salmon counted in the escapement is equal to the contribution of hatchery releases to the escapement.

The total number of spawners will be defined as the total escapement passed through the weir minus all one-ocean fish. The total number of adults that returned to the weir will be the sum of the total number of spawners, the number of adult Chinook salmon that died during holding, and the number used for egg-takes.

SCHEDULE AND DELIVERABLES

	Task	Date(s)	Responsibility
1.	Clean and disinfect raceways prior to smolt delivery.	May 24-28	Cope, Balise
2.	Install Crooked Creek weir	24-May	Cope, Balise
3.	Hold Chinook salmon smolt for imprinting and release	June 1 – June 7	Cope, Balise, Vacant
4.	Census all fish passed upstream of weir and sample adult Chinook salmon	June 7 – August 10	Cope, Balise, Vacant
5.	Crooked Creek Facility cleanup, winterization and monitor weir	August 1 – August 10	Cope, Balise, Vacant
6.	Weir removal	15-Aug	Cope, Balise
7.	Scale aging	1-Sep	Cope
8.	Data analysis and results	1-Nov	Cope
9.	Crooked Creek Chinook salmon escapement project FDS report	31-Dec	Cope
10.	Operational Plan	30-Apr	Cope

The results of this project will be presented in an Alaska Department of Fish and Game, Sport Fish Division, Fishery Data Series (FDS) Crooked Creek Chinook Salmon Enhancement Project.

RESPONSIBILITIES

Jenny Cope, Fishery Biologist I, Present – June 30:

This position is the Principal Investigator for this project and is responsible for overseeing the project development, data quality, data analysis, and report preparation.

The position will be responsible for hiring and training any new personnel and supervise two technicians. This person will be responsible for inseason data editing and reduction, postseason data analysis, and summary of the enhancement program, to be reported in an FDS report. This person will also be responsible for appropriate submittal of paperwork and Chinook salmon heads to the CWT Lab. They will also ensure all data is in proper RTS format and archived with RTS at the completion of the field season.

It will also be the responsibility of this position to keep their supervisor informed of any problems with equipment and/or personnel affecting the completion of this project. This individual will supervise crew activities involved with winterizing field equipment and the Crooked Creek Facility at the end of the season.

Inseason, this individual will enter Crooked Creek weir data into ADF&G's Internet "DocuShare", Region II Inseason Data, entitled: "Crooked Creek Weir Summary".

This position will write the project operational plan, FDS report, performance report and synopsis as well as manage the budget, prepare budget requests and perform midyear audits, write performance evaluations for technicians, apply for and renew fish transport permits and apply for fish habitat permits. This position also interacts with Anchorage hatchery staff in evaluation of the Crooked Creek enhancement program and coordinates activities associated with the Chinook salmon smolt release and the adult egg-take at Crooked Creek.

Anton Antonovich, Biometrician III, Not funded by this project:

This individual is responsible for the review of, consultation on, and approval of design and analytical procedures.

Vacant, Fish and Wildlife Technician II, June 1 – August 8

This individual will assist with (1) conducting a census of fish passed upstream of the weir at Crooked Creek and (2) biological sampling adult Chinook salmon. This individual will be responsible for conducting in-season Crooked Creek escapement counts either manually or using a DVR, pre and postseason cleaning and disinfecting of raceways, and preparation of the Crooked Creek Facility for winter. As time allows, this individual may be involved in some facility maintenance activities, such as painting buildings and vegetation control.

Kelly Balise, Fish and Wildlife Technician II, May 24 – August 15

This individual will assist with (1) conducting a census of fish passed upstream of the weir at Crooked Creek and (2) biological sampling adult Chinook salmon. This individual will be responsible for conducting in-season Crooked Creek escapement counts either manually or using a DVR, pre and postseason cleaning and disinfecting of raceways, and preparation of the Crooked Creek Facility for winter. As time allows, this individual may be involved in some facility maintenance activities, such as painting buildings and vegetation control.

BUDGET SUMMARY

Projected Costs:

Line	Category	FY13 Budget ($K)	FY14 Budget Request ($K)
100	Personnel Services	$91,200	$91,856
200	Travel	$900	$0
300	Contractual	$13,100	$12,900
400	Commodities	$3,100	$3,100
500	Equipment	$0	$0
Total			

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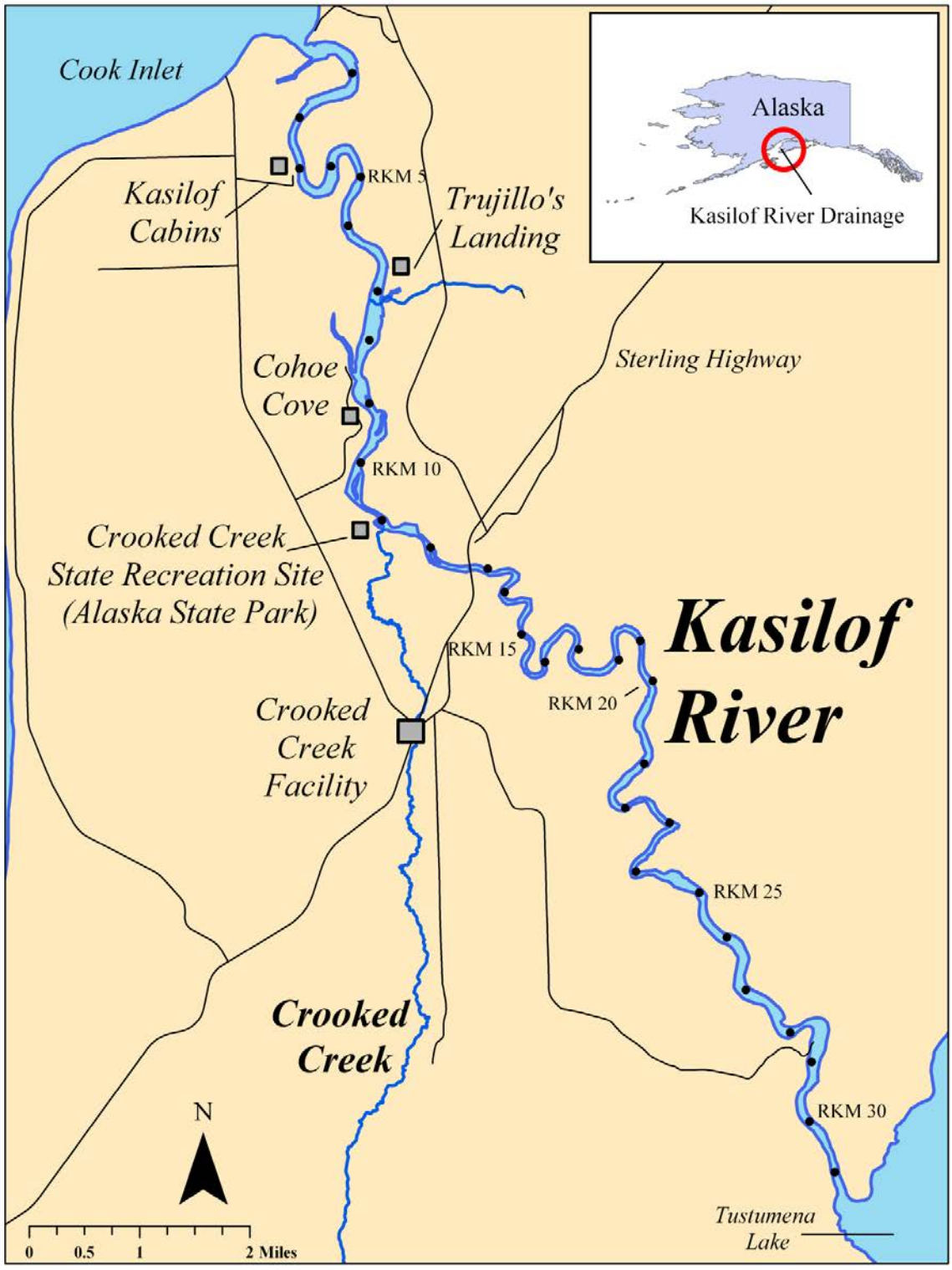

Figure 1.-Map showing Crooked Creek Facility, Kasilof River and river access locations.

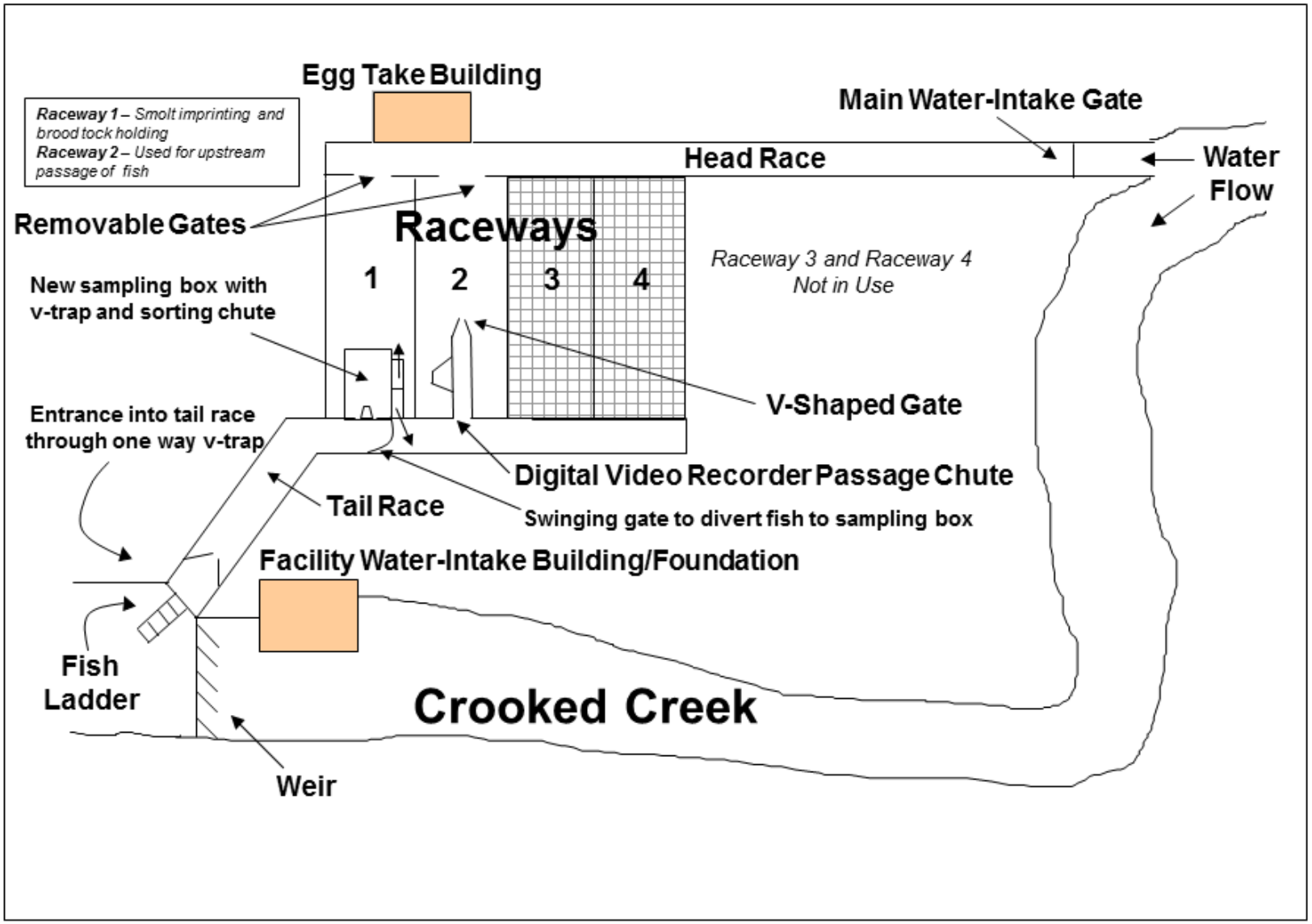

Figure 2.-Diagram of the layout of Crooked Creek Facility, weir and passage chute.

APPENDIX A - FISH ENUMERATION DATA FORMS

Appendix A1.–Crooked Creek Chinook Salmon Enhancement Project digital video recorder (DVR) passage data form.

DVR Passage

		Video Time	Video Time	Age 2+		Jacks						
Date	Observer	Start	Stop	Non-AFC	AFC	Non-AFC	AFC	DV	STHL/RT	Sockeye	Coho	Comments

Appendix A2.–Crooked Creek Chinook Salmon Enhancement Project weir mortalities and trapped emigrating fish data form.

Weir Mortalities and Trapped Emigrating Fish

Date	Time	Observer	Species	Daily Total	Comments

Appendix A3.–Crooked Creek Chinook Salmon Enhancement Project smolt imprinting and release data form.

Smolt Imprinting and Release

Raceway #1

Smolt arrival date: _____

No. of smolt: _____

Page _____ of _____ Smolt release date: _____

Date	Time	Observer	Water Temp (C)	Water DO (%)	Food (No. Scoops)	Mortalities in Raceway # 1 Non-AFC	AFC	Daily Total	Cumulative Total	Comments
Total:										

Appendix A4.–Crooked Creek Chinook Salmon Enhancement Project biological sampling data form.

Scale Sampling

Date: ________________________

Collectors: ________________________

Scale Card No.	Fish No.	Sex		AFC		Length	Age	Vial No.
	1	M	F	Y	N			
	2	M	F	Y	N			
	3	M	F	Y	N			
	4	M	F	Y	N			
	5	M	F	Y	N			
	6	M	F	Y	N			
	7	M	F	Y	N			
	8	M	F	Y	N			
	9	M	F	Y	N			
	10	M	F	Y	N			
Scale Card No.	Fish No.	Sex		AFC		Length	Age	Vial No.
	1	M	F	Y	N			
	2	M	F	Y	N			
	3	M	F	Y	N			
	4	M	F	Y	N			
	5	M	F	Y	N			
	6	M	F	Y	N			
	7	M	F	Y	N			
	8	M	F	Y	N			
	9	M	F	Y	N			
	10	M	F	Y	N			
Scale Card No.	Fish No.	Sex		AFC		Length	Age	Vial No.
	1	M	F	Y	N			
	2	M	F	Y	N			
	3	M	F	Y	N			
	4	M	F	Y	N			
	5	M	F	Y	N			
	6	M	F	Y	N			
	7	M	F	Y	N			
	8	M	F	Y	N			
	9	M	F	Y	N			
	10	M	F	Y	N			
Scale Card No.	Fish No.	Sex		AFC		Length	Age	Vial No.
	1	M	F	Y	N			
	2	M	F	Y	N			
	3	M	F	Y	N			
	4	M	F	Y	N			
	5	M	F	Y	N			
	6	M	F	Y	N			
	7	M	F	Y	N			
	8	M	F	Y	N			
	9	M	F	Y	N			
	10	M	F	Y	N			

Appendix A5.-Crooked Creek Chinook Salmon Enhancement Project broodstock collection data form.

Brood Stock Collection

Date: _____

Collectors: _____

Raceway #1 *(Chinook brood stock)*			
Non-AFC		**AFC**	
M	F	M	F
Mortalities:			

Appendix A6.-Crooked Creek Chinook Salmon Enhancement Project broodstock holding hydrology and mortality data form.

Brood Stock Holding Hydrology and Mortality

				Mortalities in Raceway # 1				
Date	Observer	Water Temp (C)	Water DO	Non-AFC	AFC	Daily Total	Cumulative Total	Comments
Total:								

Appendix A7.-Crooked Creek Chinook Salmon Enhancement Project egg take and artificially spawned count data form.

Egg Take

Date:

Personnel:

Non-AFC		**AFC**		**COMMENTS**
M	F	M	F	
Mortalities:				

APPENDIX B - CWT DATA, GENETICS AND OTOLITH COLLECTION INSTRUCTIONS

Appendix B1.–ADF&G coded wire tag sampling program instructions.

ALASKA DEPARTMENT OF FISH AND GAME

CODED WIRE TAG SAMPLING PROGRAM
DETAILED SAMPLING INSTRUCTIONS
RACK and ESCAPEMENT

SOUTH CENTRAL (COOK INLET), WESTWARD (KODIAK), AND AYK REGIONS

Introduction

Coded wire tags (CWT) recovered from properly designed and conducted studies can provide scientists, fishery managers and hatchery operators with data for evaluating and managing salmon stocks. The use of this stock identification tool has increased dramatically in the years since it was first introduced.

Sampling fish at hatchery racks, at weirs or during escapement surveys is the last of a series of sampling programs designed to look for, identify and collect heads of coded wire tagged fish. Tags recovered in commercial and sport fishery sampling programs expanded by catch/sample and release/tag ratios are coupled with tags recovered and expanded by escapement/sample ratios to produce overall survival estimates and to determine commercial and sport fishery exploitation rates.

General Instructions

All species of salmon and steelhead have been tagged in various areas of the state. Which species you check for missing adipose fins, the external mark indicating the presence of a CWT, is dependent on location and your project's goals, objectives and sampling design. **Individual project objectives, sampling design criteria and specific instructions for how, when, and where you conduct your sampling will be provided by the project leader or your supervisor.** When you observe an adipose clipped fish you must: complete a CWT Sampling form; insert a uniquely numbered cinch strap through the mouth and out the operculum; and collect the head. In some instances, your supervisor may instruct you to remove the heads of only a portion of the adipose clipped observed. **You should only sub-sample adipose clipped fish if specifically instructed to do so by your supervisor and if fewer heads collected randomly will provide you with data within acceptable confidence limits.**

Specific Instructions for Completion of CWT Sampling Form

Note: Specific data items listed on the CWT Sampling Form (sampling form) are identified in these instructions by the use of all capital letters. The sampling form and the specific instructions are divided into five major sections: General Information; Sampling Information; Area Information; Head Recovery Information and Comments. One sampling form will be completed for each head or group of heads recovered at a hatchery rack, weir, or stream survey site.

Only a single value for each requested data item is allowed. Only heads recovered from a single day at a single site should be recorded on one sampling form. **Samples from multiple days or locations cannot be listed on the same sampling form.** You may, however, have

multiple pages for a single sample. Heads not listed on a sampling form will not be processed by the Tag Lab.

General Sample Information Section

- **SAMPLE NUMBER:** This number identifies each unique sampling form in the CWT database. The supervisor has been given a sample number series to assign. If you do not know what sample number series to assign, please contact the Tag Lab.

- **PAGE____OF____PAGES:** additional pages will be required if more than 15 heads are recovered on a single day at a single site. Page numbers are specific to each individual sample; eg., a sample with 17 heads will have page 2 of 2 with the same sample number assigned to both sheets.

- **SOURCE:** circle one—if unsure, check with your supervisor or the Tag Lab for clarification.

  hatchery-rack (for sampling at a hatchery)—sometimes used if returning fish were produced at a hatchery.

  escapement-survey (for sampling at a weir, stream, river, lake or spawning grounds)

- **SURVEY SITE:** name of hatchery, stream, lake or weir surveyed

- **SAMPLE TYPE:** circle one

  Random samples are those samples where you actually count and inspect all or part of the returning fish for the presence of CWTs. If all returning fish are not inspected for CWTs, the data from the fraction of the return sampled can be used to make inferences about the unsampled return. Detailed instructions for random sampling procedures for your location will be given to you by the project leader or your supervisor. To ensure that a reliable estimate of marked and unmarked fish is attained, sampling must be done in the following two-step manner:

  First - **select fish you are going to inspect, count it**
  Second - **determine if the adipose fin is absent**

  You must first choose a fish to inspect, then look to see if the adipose fin is absent. Turn fish over if fin is not visible. Fish with partially regenerated adipose fins or poor quality marks should be set aside and treated as if coded wire tagged. **Complete a sampling form for each day and location sampled even if no adipose clips were observed.**

  Select samples are those heads that have been recovered from a source outside of a random sampling program. These heads would not have been recovered in your random sampling activities. For example, you are walking along a stream and happen to look down at a fish and see that it is clipped, then take the head. You are not actually looking for tagged fish. These recoveries cannot be used to make inferences about a larger unsampled population. Fish that have been sampled previously in the same year in the same system for CWT should be marked select so that they are not accounted for twice.

- **SAMPLER:** your last name.

- **DATE SAMPLED:** date fish are sampled by you. Heads sampled from only one day at one location can be listed on a single sampling form.

Sampling Information Section

For random samples only, a sampling form must be completed for each day and location fish were sampled even if no adipose clipped fish were observed. Random and select recoveries can not be listed on the same sampling form. Record for each species:

- **TOTAL # FISH COUNTED: count and record each fish, by species, you choose to inspect.** Included in that count will be both unclipped and adipose clipped fish. **Count only those fish you are sure either have or do not have an adipose fin. If you did not get a good look at the fin do not count that fish.**

- **# ADIPOSE CLIPS SEEN:** record by species the number of fish counted that are missing adipose fins. "Zero" adipose clips seen is a valid observation and must be recorded.

  Note: If your supervisor instructed you to collect only a portion of the heads of adipose clipped fish observed and counted (sub-sampling the heads) you should record the number of adipose clips observed and make a clear note in the COMMENTS section about the your sub-sampling activities. (For example you sampled 21 coho on August 23 at Elmendorf Hatchery (Ship Creek) and observed 8 adipose clipped coho. You were instructed by your supervisor to remove heads from only 3 fish. The remaining 5 adipose clipped fish were allowed to pass through the weir). For this example you would record the following (see Figure 2):

  TOTAL # FISH COUNTED = 21
  # ADIPOSE CLIPPED OBSERVED = 8*
  COMMENTS: **Sub-Sampled**
  3 heads taken
  5 heads not taken
  *8 total adipose clipped fish observed

  Tag Lab staff will assign phantom head numbers to the remaining 5 heads and list them as LOST (adipose clipped fish observed but not received at the Tag Lab for processing). This is still a **Random Sample** because you are accounting for all fish observed and counted and listing the ones that did not actually have the head removed.

- **WERE ALL SAMPLED?** circle **y**es or **n**o (for each species). It is vital that you count only those fish you are sure have or do not have an adipose fin, that you have actually determined this by visual sight. If you circle yes, you are stating that you looked at every single fish that possibly went by you in the stream, the weir, etc., and that you positively determined that each fish did or did not have a clip. This does not refer to the number of heads taken. Circle **yes** or **no**.

Area Information Section

- **AREA INFORMATION (DISTRICT-SUBDISTRICT):** for saltwater recoveries record commercial fishing district and subdistrict where fish were sampled/recovered. For freshwater samples, record the first five digits of the ANADROMOUS STREAM #. (e.g.; Wasilla Creek is 247-50, Nancy Lake is 247-41, Little Susitna River is 247-41, Kenai River is 244-30, etc.).

- **NAME of PLACE SURVEYED (HATCHERY OR STREAM):** location of facility, weir, stream, lake or spawning ground.

- **WATER TYPE:** were fish collected in saltwater or freshwater? Circle one.

- **ANADROMOUS STREAM # (freshwater-only)**: if these fish were sampled/recovered in freshwater, please enter the Anadromous Stream Catalog number listed in the latest edition of the "Catalog of Waters Important for Spawning, Rearing or Migration of Anadromous Fishes" published by the Department's Habitat and Restoration Division. This will be at least a ten digit number but could have as many as thirty-eight digits. If a catalog is unavailable, please call your local Habitat and Restoration Division office or the Tag Lab for assistance or be as descriptive as possible when you record the NAME OF HATCHERY OR STREAM. See attached list for ANADROMOUS STREAM #s.

Head Recovery Information Section

- ☑: each fish head should be checked off as it is boxed for shipment to the Tag Lab.

- **HEAD NUMBER*:** insert a pre-numbered cinch strap through the mouth and out the operculum (gill plate) of each head identified as bearing a CWT. Insert these so that the number can be read when the head is frozen. A series of cinch straps have been assigned to you for this specific project. Use them in numerical order. Cinch-up the strap and record its imprinted 6-digit number under HEAD NUMBER on the sampling form. If a cinch strap is missing from the sequence assigned to you, list that number(s) on the sampling form on which it should have appeared. The number along with the word "**Void**" should be written in the comments section of the sampling form.

  *Note: If you are using a cinch strap with only five digits or numbers simply insert a leading zero for the first digit.

- **SPECIES CODE:** Record species code of each adipose clipped fish using the following codes:

  410 = CHIN - king or Chinook salmon
  411 = JACK - king or Chinook salmon only; check with your site sampling supervisor for length criteria prior to selection and entry as a JACK (generally < 28 inches total length). Many projects do not use this designation and later sort the data based on length and age.
  420 = SOCK - sockeye or red salmon
  430 = COHO - coho or silver salmon
  440 = PINK - pink or humpback salmon
  450 = CHUM - chum or dog salmon
  540 = STHD - steelhead trout

- **MID-EYE TO FORK LENGTH:** record the length (mid-eye to the fork-of-tail), if measured, to the nearest millimeter (mm). See Figure 3.

- **CLIP:** note quality of adipose clip using the following codes:
  1 - OK (fish must be observed)
  2 - Questionable - partially regenerated or poor quality clip (fish must be observed)
  3 - Unknown (use for select samples where the fish is not observed by the sampler)

- **SEX:** record the sex of the fish using the following codes: (Note: Completion of this item is optional, however it is recorded on the CWT database if reported on the sampling form).
  F - female
  M - male

Comments Section

- **COMMENTS:** Record any comments you may have about the sample, or its irregularities in the comments section of the sampling form or on the back of the sampling form. If you write notes on the back, please indicate that we should "see back of the sampling form."

Head Preparation and Shipment Instructions

1. At the end of each day, check sampling forms.
   - Be sure that **all** data items have been completed.
   - Be sure all heads recovered are accounted for on the sampling forms for that day.
     - Be sure that all heads listed on sampling forms were retrieved, bagged, and are in a freezer.
2. Heads should be shipped to the Tag Lab periodically during the season, as often as once a week.
3. When collected, heads must be placed in an individual plastic bag, provided by the Tag Lab. Heads must be frozen (if a freezer is not available, preserved in borax or salt). Place individually bagged heads in large garbage bags inside a box (wet lock boxes not required if heads are double bagged). If time permits, please thoroughly rinse or remove gills from escapement fish. Residual sand and debris from the ground can cause problems with the magnetic detectors and false signals can occur when trying to dissect the tags.
4. Place all **original** sampling forms in a single plastic bag and place in the box with heads.
5. The person in charge of shipping heads to the Tag Lab will complete the HEAD SHIPMENT SUMMARY FORM and include it with the head shipment. Instructions for completion of that form will be sent to the person in charge of each project. In order to ensure that all heads are sent to Juneau, check off heads on sampling form as they are being boxed for shipment.
6. If your shipment includes more than one box, put data in one box and write **Data Enclosed** on the outside of the box.
7. Please number the boxes you ship to us. If you number the boxes 1 of 5, 2 of 5, etc. we can be sure that the air carrier gives us your complete shipment. It is also helpful to call or email the Tag Lab with the AWB number, number of boxes and estimated time of arrival into Juneau if you have access to a phone or computer.
8. Label the box sides with the words Keep Frozen or use Keep Frozen labels provided by the air carrier**.**
9. If you live in a community served by Alaska Airlines, send heads and data directly to the Tag Lab on that carrier. If you work in a community not served directly by Alaska Airlines send shipments to Juneau on a regularly scheduled commuter flight that transfers to Alaska Airlines.
10. Use shipping labels provided. Send heads **Prepaid** (see exception in #11 below) to:

Alaska Department of Fish and Game
CF Division, Mark, Tag, and Age Lab
P.O. Box 25526
Juneau, Alaska 99802-5526

CALL UPON ARRIVAL IN JUNEAU
(907) 465-3483

11. Heads recovered by the Northern Cook Inlet Urban Area Salmon Stocking Project and by the Kenai River Salmon Stock Assessment Project should send their heads to the Tag Lab **Freight Collect**. All other projects should send heads to us Prepaid.
12. **Heads shipped without data will not be processed.**
13. Please call if you have questions or if you need additional supplies. Thanks for your hard work and cooperation. Have a good season.

Appendix B2.–Collection of axillary process tissue samples for DNA analysis, ADF&G Gene Conservation Laboratory, Anchorage.

I. General Information

We will be using tissue samples from the axillary process from individual fish to determine the genetic characteristics and profile of a particular run or stock of fish. This is a non-lethal method of collecting tissue samples from adult fish for genetic analysis. The most important thing to remember in collecting samples is that **only quality tissue samples give quality results** so the fish tissues need to be as "fresh" and cold as possible at all times.

Sample preservative: Ethanol (ETOH) preserves tissues for later DNA extraction without having to store frozen tissues. Avoid extended contact with skin.

II. Supplies included with sampling kit:

1. Dog toenail clipper & scissors - use to cut off the axillary process (fleshy spine)
2. Cryovial- a small (2ml) plastic vial, pre-labeled with caps.
3. Cryovial rack- white plastic rack or neon box holds cryovials while sampling
4. Ethanol (ETOH) – bulk in Nalgene bottles
5. Squirt bottle – use to fill or "top off" each cryovial with ETOH
6. Paper towels – use to blot any excess water or fish slime off fin
7. Printout of sampling instructions
8. Data sheets or Rite-in-rain booklet
9. Gloves – lab gloves for decanting ethanol
10. Laminated "return address" labels

III. General set-up:

1. To insure that the tissues are kept fresh and cold, working fast is necessary. It is important to have your sampling area and supplies set up **before** the fish are caught.
2. Sample kits will come with pre-labeled and numbered cryovials for each individual fish (i.e. 1,2,3, ...). If not, label the empty plastic cryovials with the pre-printed labels in advance, with the adhesive labels provided in the sampling kit. Place the cryovials in the cryovial racks in an order that will allow you to work quickly. We find it easiest to set up ten individuals at a time.
3. Get set up in as comfortable a place as possible. You might use a portable table, piece of plywood, or anything to give you a surface at a good height.
4. Have the caps for the tubes set out along with the sampling tools provided.

IV. Sample procedure:

1. Tissue type: Axillary process samples should be "white" skeletal fleshy lobe just above the pelvic fin (see enclosed diagram). Pelvic or pectoral fin ray may be substituted if needed but **NO adipose tissue**.
2. Prior to sampling, fill the vials half way with ETOH. Fill only the vials that you will use for a particular sampling period.
3. Using dog toenail clippers or scissors, remove the entire axillary process or a portion of the lobe that will fit into the cryovial and place the tissue into the designated cryotube

labeled as follows (Fish #1 has it's tissue loaded in cryotube labeled # 1 etc.). If you have trouble getting the tissue into the tubes, cut it into smaller pieces.

4. To avoid any excess water, blood, dirt or fish slime in the vial, wipe the axillary process prior to sampling. Place axillary process tissue into ETOH. The tissue/ethanol ratio should be slightly less than 1:3 to thoroughly soak the tissue in the buffer.
5. Top up tubes with ETOH and screw cap on securely. Invert tube twice to mix ETOH and tissue. **It is important** to wipe your toenail clippers, other sampling tools and area off before sampling the next fish to avoid cross contamination between fish.
6. Discard remaining ethanol from the bulk bottle before shipping. **Tissue samples must remain in 2ml ethanol**, these small quantities do not require HAZMAT paperwork. Store vials containing tissues at room temperature, but away from heat. In the field: keep samples out of direct sun, rain and store capped vials in a dry, relatively cool location. Freezing the tissues collected in ETOH is not required.

V. Data to Record

Most field stations use electronic data recording devices. Otherwise, data forms are included in the sampling kit.

We appreciate your help with the sampling. If you have any questions, please give us a call.

VI. Shipping:

No HAZMAT paperwork is required for return shipment of these samples.

Ship samples to:

ADF&G – Genetics Lab	Lab staff: 1-907-267-2247
333 Raspberry Road	Judy Berger: 1-907-267-2175
Anchorage, Alaska 99518	Bill Templin: 1-907-267-2234

Shipping code:

Appendix B3.–ADF&G Salmon otolith sampling instructions and protocol.

SALMON OTOLITH PORT SAMPLING GUIDE

PURPOSE

Thermal marking is one of the methods being utilized to identify and manage hatchery-released salmonids. Thermal marking places a recognizable mark on the otoliths of a fish. This marking technique makes use of the natural growth tendencies of a developing otolith. When the embryonic fish are incubating in hatcheries, the water temperature is raised and lowered according to a pre-determined schedule that results in a predictable sequence of visibly enhanced growth increments or thermal rings. The appearance of thermal marks can be likened to the growth rings on a tree. Several thermal rings grouped closely together make up a band. Increasing the distance between these groups can create multiple bands. A discrete thermal mark is identified by counting the number of thermal bands present, and then counting how many thermal rings compose each band. For example in Figure 1a, there are 6 closely grouped rings that make up one band. In Figure 1b, there are still only six thermal rings, but this time they are grouped into two bands each composed of three rings.

Mark # 1	Mark # 2
IIIIII	III III
Figure 1a	**Figure 1b**

Fish have 3 pairs of otoliths - the sagittae, lapillae, and asteriscae. The sagittae are the largest and are what most people refer to as otoliths. When a sample of fish is collected, the sagittal otoliths are removed and sent to ADF&G s Thermal Mark Laboratory for processing. The left sagittal otolith is glued to a glass slide and then ground down on fine grit sandpaper. When the center of the otolith is reached, it is examined under a microscope for the presence of a thermal mark.

EQUIPMENT

DISSECTION TOOLS
1) Butcher knives with deep 6-8 blade 2) Forceps (fine point)
3) Cotton gloves
4) 96-cell otolith trays with compression plates & lids*****
5) Sample labels*****
6) Pencils
7) 5x7 Anti-skid matting
8) Paper towels
9) Brightly colored beads*****

OTOLITH CLEANING SUPPLIES
1) 1.0% Chlorine solution***** 2) De-chlor solution (0.7% sodium thiosulfate) *****
3) Water
4) Rubber bands (size 62)*****
5) 1000ml Nalgene bottles*****
6) 125ml Nalgene squirt bottles*****

SHIPPING SUPPLIES
1) Ziploc plastic bags
2) Packing tape
3) Packing boxes
4) Pre-addressed and numbered shipping labels*****

*** Provided by ADF&G Thermal Mark Laboratory**

DISSECTION

1. Collect a sample of fish heads from the appropriate fishing district.

2. Assemble the sample and dissection equipment in the location identified by processing plant personnel.

3. Fill out and place the adhesive tray label to the bottom of the 96-cell otolith sampling tray before it is filled with otoliths. **This step is critically important to maintaining the integrity of each sample!**

In the Port Sampling Supplies shipped to you, you will have several adhesive Tray Labels. Each label has a pre-assigned sample number printed in the upper right-hand corner (see diagram below). We prefer that you keep the tray numbers in sequential order throughout a given stat week and sampling season. **It is critical that each and every tray have a tray label attached**.

Because the sample number on each tray label is unique, you cannot use a label to identify more than one tray of 96 otoliths. Fill out each tray label completely with the appropriate fishery information. Use soft lead pencil not a pen because alcohol and water will dissolve ink! To avoid spilling otoliths from a tray, affix the label to the bottom of a tray ***before*** filling them with otoliths. **Do not place sample labels on the otolith tray lid because the lids can get separated from the sample tray!**

Never place otoliths from more than a one stat-week in a single tray. Otoliths from multiple statweeks should not be included in the same tray (e.g. one stat-week per tray)! Mixing otoliths from various statistical weeks results in massive confusion and an irrevocable loss of data. Even if you have a tray containing only ONE otolith for a Stat Week or to complete a Stat Week, send it that way.

4. To separate the messy job of cutting heads from the removal of otoliths (where some cleanliness is desired) cut the heads in batches of 20-40 using the following guidelines (Figs. 2 3):

a) Hold the fish head in front of you on its severed end, with the dorsal surface (top of the head) facing you. Flare out the gill plates to help stabilize it;

b) With left hand cupped against left side of fish face to stabilize it, and with knife in right hand (or opposite for lefties), begin a cut, down to about 1 deep, through the nose and such that if this cut line were to continue, it would go equilaterally between the left and right eye.

c) With your gloved left hand, grab the lower jaw and insert the point of the knife into the mouth and just below the thumb of your left hand (which is crosswise in the fish s mouth). Let the knife find your initial starter cut, then bear down on the knife so that the tip finds the table surface first, and then pivot the cut from this point down through the rest of the fish head.

d) The end product should be two halves split top-to-bottom between the eyes and still connected by the lower jaw. This allows for a fast way to cut heads and make their otoliths readily accessible.

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** Begin the first cut through the nose. Leave a small area attached at the base of the two halves.
** Allow the halves to open up, exposing the otolith cavities on either side.

5. After all of the fish heads have been cut, briefly clean the area and set up all the tools needed for otolith removal: 96-cell tray with pre-printed label, forceps, water squirt bottle, and towels.

6. Just before removal, fill each cell of the otolith tray with water so that residual blood and slime does not back into the crevices of the otolith.

7. Pick up a fish head and let the halves flop open. With forceps, remove the left sagittal otolith from its sagittal well . The sagittal well is a depression underneath the brain in the most posterior-ventral portion of the brain cavity. Place the otolith onto the back of your hand and then recover the right otolith from the right sagittal well (Fig. 4).

** Remove the otoliths from the sagittal wells with forceps, clean, and place in the tray.

8. Each otolith is encased in tissue. Tweeze the tissue off of each otolith, gently wipe off the blood, and place the pair of otoliths into the appropriate cell of the otolith tray. Note that the first specimen for every tray should go into the highlighted cell, A1 . Cells in a tray should be filled left to right by row (like reading a book): A1-A12, B1-B12 H1-H12. Do not fill cells in columns!

9. Repeat the dissection procedure until the tray(s) is (are) full and the sample is finished.

10. If one of the two otoliths is lost during the process, place one bead into the cell with the recovered otolith. If no otoliths are recovered from a fish head, discard that head. Do not skip a cell or place any beads into that cell unless there are AWL data matched to that fish head. If the otoliths are to be matched with other data in the sample collection, then place two beads in the cell to indicate that both otoliths are missing.

ALTERNATIVE DISSECTION METHOD

A second methods used to dissect and removal the otoliths is the flip top approach. In this method, the top of the head is removed by starting to cut at the top of both eyes. Then you slice back towards the body, but not beyond a line extending above the gill cover. With a twist of the knife, cut back towards the top of the head removing a wedge of tissue and bone. This will expose the cranial cavity. Remove the brain tissues so that both pairs of otoliths of can be extracted with forceps. The flip top method takes practice to obtain consistent cuts but tends to be less messy than the first approach.

** Make the cut just above eye-level.
** Cut all the way through and remove the skull-cap.
** Remove otoliths through a single opening into the brain cavity. They will be located on opposite sides of the opening.

CLEANING

1. Gently squirt the 1.0% chlorine solution into each cell with an otolith, filling each cell approx 1/2 full. Let sit for10 minutes. This helps to clear away blood and tissue. ***DO NOT*** let this sit for more than 30 minutes! Use caution when using squirt bottles of any solution: a forceful stream of water will cause the otoliths to jump from their cells and mix up the data!

2. Gently squirt the De-chlor solution to fill the cell. This deactivates the chlorine solution. You may move on to the next step without waiting because the de-chlorination is immediate.

3. Rinse with tap water from a squirt bottle. First, tap the tray gently against the counter to settle the otoliths in the very bottom of their cells. Check that they ve all fallen down. For those that don t, simply push them down with some forceps. Tip the tray 45-80 degrees and gently wipe the surface several times with the palm of your hand. Be careful not to draw the otoliths out of the cells by too fast a motion. This will help remove the excess De-chlor solution. Put the tray back on the counter and gently squirt tap water into the cells from the squirt bottle. Do some final wiping passes to remove water from the final rinse.

4. Dry the otoliths by letting them sit uncovered.

5. **Place *two* compression plates -- aligned with each other and so that they cover all 96-cells Then place a lid on the tray, and secure this with *three* rubber bands**!

SHIPPING

1. If there is any moisture left in the tray(s), wrap a few paper towels around them and place them inside a sealable plastic bag (Ziploc). Place the individual bags inside one more sealable plastic bag.

2. Pack the trays into a box, cushioning them with packing material.

3. Seal the box with tape and affix with the adhesive shipping labels provided:

SHIP TO: Alaska Department of Fish and Game
Otolith Processing Lab
ATTN: Kray Van Kirk
10107 Bentwood Place
JUNEAU, ALASKA 99801

4. Ship C.O.D. via ALASKA AIRLINES. If sending via the postal service, affix the correct postage.

APPENDIX C – CROOKED CREEK CHINOOK SALMON WEIR AND ASL DATA MAPS

Appendix C1.-Crooked Creek Chinook salmon weir and escapement data map.

Data Field Name	Width	Start Column	End Column	Comma Column	Codes/ Comments
Date Code	8	1	8	9	
Year	4	1	4		Four digit year
Month	2	5	6		Two digit month
Day	2	7	8		Two digit day
Var1	3	10	12	13	DVR count: Non-AFC age 2+ ocean
Var2	3	14	16	17	DVR count: AFC age 2+ ocean
Var3	3	18	20	21	DVR count: Non-AFC jacks
Var4	3	22	24	25	DVR count: AFC jacks
Var5	3	26	28	29	Upstream released or sampled: Non-AFC age 2+ ocean
Var6	3	30	32	33	Upstream released or sampled: AFC age 2+ ocean
Var7	3	34	36	37	Upstream released or sampled: Non-AFC jacks
Var8	3	38	40	41	Upstream released or sampled: AFC jacks
Var9	3	42	44	45	Facility mortalities: Non-AFC age 2+ ocean
Var10	3	46	48	49	Facility mortalities: AFC age 2+ ocean
Var11	3	50	52	53	Facility mortalities: Non-AFC jacks
Var12	3	54	56	57	Facility mortalities: AFC jacks
Var13	3	58	60	61	Pond 2 mortalities: Non-AFC age 2+ ocean
Var14	3	62	64	65	Pond 2 mortalities: AFC age 2+ ocean
Var15	3	66	68	69	Pond 2 mortalities: Non-AFC jacks
Var16	3	70	72	73	Pond 2 mortalities: AFC jacks
Var17	3	74	76	77	Brood stock collected: Non-AFC age 2+ ocean
Var18	3	78	80	81	Brood stock collected: AFC age 2+ ocean
Var19	3	82	84	85	Brood stock collected: Non-AFC jacks
Var20	3	86	88	89	Brood stock collected: AFC jacks

-continued-

Appendix C1.continued.-Crooked Creek Chinook salmon weir and escapement data map.

Data Field Name	Width	Start Column	End Column	Comma Column	Codes/ Comments
Var21	3	90	92	93	Brood stock released: Non-AFC age 2+ ocean
Var22	3	94	96	97	Brood stock released: AFC age 2+ ocean
Var23	3	98	100	101	Brood stock released: Non-AFC jacks
Var24	3	102	104	105	Brood stock released: AFC jacks
Var25	3	106	108	109	Brood stock mortalities: Non-AFC age 2+ ocean
Var26	3	110	112	113	Brood stock mortalities: AFC age 2+ ocean
Var27	3	114	116	117	Brood Stock mortalities: Non-AFC jacks
Var28	3	118	120	121	Brood stock mortalities: AFC jacks
DV	3	122	124	125	Dolly Varden
STH	3	126	128	129	Steelhead Trout
RT	3	130	132	133	Rainbow Trout
PS	4	134	137	138	Pink Salmon
SS	4	139	142	143	Coho Salmon
RS	4	144	147	end	Sockeye Salmon

Appendix C2.-Crooked Creek Chinook salmon ASL data map.

Data Field Name	Width	Start Column	End Column	Comma Column	Codes/ Comments
Date Code	8	1	8	9	
Year	4	1	4		Four digit year
Month	2	5	6		Two digit month
Day	2	7	8		Two digit day
(Blank)	2	10	11	12	
(Blank)	1	13	13	14	
Survey Area Code	2	15	16	17	P0 = Kenai Pennisula fresh water
Site Code	3	18	20	21	160 = Crooked Creek
(Blank)	2	22	23	24	
(Blank)	2	25	26	27	
Species	3	28	30	31	410 = Chinook
(Blank)	3	32	34	35	
(Blank)	3	36	38	39	
(Blank)		40	57	43,45,47,49,58	
(Blank)	2	59	60	61	
Sex	1	62	62	63	= M or F
AFC or Non-AFC	1	64	64	65	0=Non-AFC, 1=AFC
(Blank)	4	66	69	70	Length (mm)
(Blank)		71	86	76,81,84,87	
(Blank)	2	88	89	90	
(Blank)	5	91	95	96	
Scale Card Number	3	97	99	100	
Fish Number	1	101	102	103	Number on scale card (Values 1-10)
Age	2	104	105	106	column 104=Freshwater Age, column 105=Marine Age
Age error	1	107	107	end	R=Regen, M=Missing, I=Inverted, A=Absorbed, U=Unreadable, D=Dirty