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Chinook Salmon Genetics Sampling Along the Alaska Peninsula and Adjacent Areas Operational Plan, 2013

by

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and

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May 2013

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

Symbols and Abbreviations

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Weights and measures (metric)

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

Weights and measures (English)

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

Time and temperature

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

Physics and chemistry

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

General

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

Mathematics, statistics

all standard mathematical signs, symbols and abbreviations	
alternate hypothesis	H_A
base of natural logarithm	*e*
catch per unit effort	CPUE
coefficient of variation	CV
common test statistics	(F, t, χ^2, etc.)
confidence interval	CI
correlation coefficient (multiple)	R
correlation coefficient (simple)	r
covariance	cov
degree (angular)	°
degrees of freedom	df
expected value	*E*
greater than	>
greater than or equal to	≥
harvest per unit effort	HPUE
less than	<
less than or equal to	≤
logarithm (natural)	ln
logarithm (base 10)	log
logarithm (specify base)	$\log_2$, etc.
minute (angular)	'
not significant	NS
null hypothesis	H_O
percent	%
probability	P
probability of a type I error (rejection of the null hypothesis when true)	α
probability of a type II error (acceptance of the null hypothesis when false)	β
second (angular)	"
standard deviation	SD
standard error	SE
variance	
population	Var
sample	var

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ABSTRACT

Recent low runs of Chinook salmon *Oncorhynchus tshawytscha* in Alaska have resulted in an increased interest in developing a more comprehensive genetic baseline. A genetic baseline will allow for examination of potential impacts of fisheries on Chinook salmon stocks using mixed stock analysis. Potential hatchery contributions to fisheries will be determined using parental based tagging of Pillar Creek Hatchery Chinook salmon. There are several stocks in the Westward Region that do not have good representation in the genetic baseline, specifically on the north Alaska Peninsula and adjacent areas and near and on Kodiak Island. This document describes a plan to sample the Chinook salmon stocks in these areas to improve the genetic baseline.

Key words: Chinook salmon, genetic baseline, Chignik, Alaska Peninsula, Kodiak, sampling, single nucleotide polymorphism, parental based tagging, mixed stock analysis, MSA

INTRODUCTION

In recent years, Chinook salmon *Oncorhynchus tshawytscha* runs in Alaska have been lower than in the past, causing widespread concern and increased interest in determining the causal factors for the decline. One important tool for examining potential impacts of fisheries on Chinook salmon is mixed-stock analysis using genetic data (MSA). An essential component of MSA is a comprehensive genetic baseline. There are several stocks in the Westward Region that do not have good representation in the genetic baseline, specifically on the north Alaska Peninsula. Several stocks on the north side of the Alaska Peninsula have been historically observed during aerial surveys but are not represented in the genetic baseline. These stocks occur in remote locations and are generally small in size and difficult to access. While these stocks may not be large in magnitude, it is important to represent all stocks that may be present in commercial fisheries to help determine the causal factors for Chinook salmon declines.

A larger than expected percentage (14-27%) of Chinook salmon caught incidentally in the 2005-2010 Bering Sea pollock fishery were genetically identified as being of north Alaska Peninsula origin (NMFS 2009; Guyon et al. 2010a,b; Guthrie et al. 2012). Since the number of Chinook salmon caught in pollock fishery can be quite large (10,000 to 122,000 annually during 2003-2012; NMFS), the estimated contribution of Chinook salmon of north Alaska Peninsula origin suggests four possible explanations: 1) the estimated contribution of north Alaska Peninsula fish to the Bering Sea pollock fishery is biased high because Chinook salmon from adjacent areas are genetically similar, 2) these stocks are more vulnerable than other stocks to bycatch in the Bering Sea pollock fishery, 3) the relatively small population size of north Alaska Peninsula fish is underestimated, or 4) the productivity of Chinook salmon on the north Alaska Peninsula is higher than expected.

Most known stocks in the Kodiak Management Area and the Chignik Management Area have been adequately sampled (Table 1 – genetic inventory). There are no known stocks of Chinook salmon on the south side of the Alaska Peninsula other than the Chignik River. The purpose of this project is to obtain additional samples of known Chinook salmon stocks to expand the genetic baseline and to investigate the existence of additional stocks that are not currently known by the department.

Pillar Creek Hatchery in Kodiak initially used broodstock from Karluk River to initiate introduced runs at Monashka Creek, American River, and Olds River on the Kodiak road system. In recent years, broodstock has been collected from the three introduced runs, and each system is subsequently stocked annually with fry that are raised in the hatchery. The access to hatchery broodstock and/or progeny samples provides an opportunity to assess the potential for parental-based tagging (PBT; Anderson and Garza 2006) of the hatchery production in tandem with

traditional MSA. PBT would allow for the identification of hatchery offspring in all fisheries where Pillar Creek Chinook salmon may be harvested as each progeny is putatively tagged when all parental pairs are genotyped. The potential for this application depends on the ability of single nucleotide polymorphisms (SNP) sets to identify offspring based upon parental genotypes and known mating pairs. The Gene Conservation Laboratory is currently investigating a large suite of SNPs for such applications.

Funding to improve the Chinook salmon genetic baseline was approved by the Alaska Legislature in May of 2012. This document describes a plan for efforts during the summer of 2013 to continue this work.

OBJECTIVES

1. Fly aerial surveys on the Alaska Peninsula and adjacent areas to locate migrating or spawning Chinook salmon.
2. Collect genetic samples from spawning Chinook salmon on the Alaska Peninsula (concentrating on the north) and adjacent areas using a beach seine.
3. Collect genetic samples from juvenile Chinook salmon in specified locations on the Alaska Peninsula (concentrating on the north) and adjacent areas using baited minnow traps.
4. Distribute sampling kits to north Alaska Peninsula sport fishing guides and train them to collect genetic samples of Chinook salmon during sport fishing operations.
5. Collect genetic samples from adult and juvenile Chinook salmon originating from Pillar Creek Hatchery for evaluation of parental based tagging and augmentation of the baseline for MSA.

PROCEDURES

Most sampling during 2013 will be focused on the north Alaska Peninsula and adjacent areas and broodstock for Pillar Creek Hatchery. Other stocks in the Kodiak area are well represented in the genetic baseline and only a few need further sampling. Chignik River is also well represented in the genetic baseline and there are no known significant stocks on the South Alaska Peninsula.

LOGISTICS

Fuel

Aviation fuel will be ordered from Seattle in conjunction with the department's annual fuel order and delivered to Chignik Bay. A barge is chartered annually to move supplies from the Chignik Bay shipping location to the Chignik River Weir and the fuel will be included in that charter. Department pilots will then establish fuel caches with fixed-wing aircraft near remote airstrips, cinder blows, or beaches. Fuel will be cached in the Meshik River and Cinder River valleys and in the North Creek area. Additional caches will be established if Chinook salmon are observed or reported in unexpected areas.

Lodging

The sampling crew will establish lodging at the department's remote field offices in Port Moller, Cold Bay, and at the Chignik River Weir. Since logistics of packing food and supplies in a helicopter with limited space and purchasing food in remote areas with limited hours and availability are difficult, the remote field offices will purchase additional food with their annual orders and charge it to the Westward Chinook Genetics budget. Lodging for sampling adjacent

areas in southeastern Bristol Bay and southwestern Cook Inlet will be coordinated in King Salmon and Homer.

Crew

To limit the amount of gear and weight during long trips in the helicopter while transiting between base lodging locations, the core crew will be limited to the crewleader and helicopter pilot. One additional crew member will be picked up at the remote field offices thereby spreading out the work and limiting the budgetary impact of the additional crew member. Personnel are available in Chignik, Port Moller, and Cold Bay as well as from field camps at Nelson River, Bear Lake, Sandy River, and Ilnik River. Staff from the Gene Conservation Laboratory in Anchorage will also deploy sampling staff to one or more of the field offices. Many options for crew members will allow flexibility in sample collection planning and logistics. Staff from the King Salmon the Homer offices will either direct sampling or support sampling in the adjacent areas.

Transportation to sampling sites

A helicopter charter will be arranged so that the pilot will stay at the department field offices with the sampling crew. This is necessary because there are few lodging resources on the Alaska Peninsula and the pilot must remain flexible to dynamic conditions. A pilot familiar with the Alaska Peninsula area is necessary to ensure the safety and success of the charter. An R-44 helicopter is desired for this type of work due to the relatively low fuel consumption and lower charter cost relative to other helicopters. While an R-44 has a lower payload than many other helicopter charter services, much of the Chinook salmon sampling work will be aerial surveying and no large payloads are necessary in most cases. If the need arises for additional personnel or nets, a second R-44 or a larger Bell Jet Ranger helicopter can be chartered in the Alaska Peninsula area. Adjacent areas will be accessed by fixed wing on wheels or floats, R-44, or skiff.

Communication

The crewleader will keep their supervisor up to date on the daily sampling site plan as it changes throughout the season and the nightly crew lodging location. A flight or float plan including intended route, personnel on board, and return time will be posted in the field office that the crew departs from daily. A personal GPS locater will be in the craft during each trip that will allow tracking of the trip progress over the internet and short text messages to be sent and received by the crew.

SAMPLING

Beach Seining

The strategy for sampling each stock of fish will depend on the river conditions, number of fish, and their behavior. In general, a 23 m long spectra full-purse beach seine will be used to encircle schools of fish or will be held in a hook pattern and schools of fish will be herded into the net. Once the fish are encircled, the bottom of the net will be pursed up to minimize escape. The axillary process will be clipped from the left side of the fish using dog toenail clippers and preserved in a 250 ml bottle of ethanol (Figure 1). The fish will then be released, unharmed. As many fish as possible, up to 200 individuals, will be sampled from each river. The GPS location, river name, names of samplers, sample size collected and any other pertinent information will be

recorded for each sampling location. Approximately 24 hours after sampling, the ethanol in each bottle will be refreshed with new ethanol.

Juvenile Sampling

Minnow traps baited with salmon roe (disinfected with iodophor) will be anchored in areas where juvenile Chinook salmon are likely to be holding in the river. Areas that offer cover with slower moving, deeper water with woody debris are likely locations. Traps will be anchored in place during aerial survey trips and their GPS coordinates will be recorded. The traps will be checked 1 to 2 days later and, depending on the catch results, may be redeployed.

Juvenile Chinook salmon will be sampled from the trap catch by removing the pelvic or the caudal fin, depending on fish size (Figure 4). After sampling, fish sampled by removing the caudal fin will be sacrificed. Fins will be preserved in a 250 ml bottle filled with ethanol. Approximately 24 hours after sampling, the ethanol in each bottle will be refreshed with new ethanol.

Sport Fishermen Sampling

During 2012, several sport fishing lodges that target Chinook salmon were contacted about clipping axillary processes from fish while sport fishing. Most guides were receptive to the project and will likely be willing to participate again in 2013. Since guides located on the North Peninsula fly in to several different rivers and have opportunity to sample several different spawning stocks, their efforts will be particularly valuable. Many lodges on the North Peninsula employ several guides so several sampling kits will be prepared for each guide, allowing for sampling of each river that the guide is likely to catch Chinook salmon from.

JUNE TRIP

During the second half of June, an R-44 helicopter will be chartered to collect adult Chinook salmon samples with a beach seine, collect juvenile samples with minnow traps, and contact sport fishing guides and outfit them with sampling kits.

Extensive surveys will be performed on the North Alaska Peninsula to locate adult Chinook salmon migrating or spawning in freshwater. While the peak of the Chinook salmon migration typically occurs later in the season, these surveys will allow us to capture the early portions of the migrations and will be ideally timed if the 2013 run timing is earlier than normal.

Particular focus in effort will take place in the Cinder and Meshik rivers area; however, all streams that have the potential for spawning Chinook salmon will be surveyed. The location of the streams targeted for sampling are depicted in Figure 2.

Concurrent to the surveys for adults, minnow traps baited with salmon roe will be placed in areas of known juvenile concentrations (Anadromous Waters Catalog; Wagner and Lanigan 1988) and in areas that juvenile Chinook salmon are likely to concentrate. The minnow traps will be placed in rivers that are known to contain spawning Chinook salmon as well as in rivers that are typically too murky to enumerate escapement via aerial survey. It is unknown if these rivers contain spawning Chinook salmon and sample collection from juveniles may elucidate distribution information.

Concurrent to North Alaska Peninsula surveys, the crew will stop at all of the sport fishing lodges in the area to explain the project goals and, if the guides are willing, distribute sampling kits so that sport-caught fish can be sampled. Each fishing guide or lodge owner will be trained

on proper sampling procedures and a procedure sheet will be included in the sampling kits. Since most lodges on the North Alaska Peninsula are very remote and are only accessible by airplane, they also typically do fly-out fishing trips and fish for Chinook salmon in a variety of areas. This will allow our sampling effort to have a larger geographic and temporal range. Interviews will be conducted with all sport fishing guides to record Chinook salmon migration observations and trends to expand the department's knowledge of Chinook salmon distribution.

JULY TRIP

A second charter trip will be conducted in late July to coincide with the typical peak Chinook salmon spawning and migration time. While the objectives of this trip will be similar to those during June, more effort will be focused on sampling spawning adult Chinook salmon and less effort focused on sampling juveniles. Each sport fishing lodge will be visited to collect any samples that sport fishing guides have collected and to restock sampling supplies if necessary.

Department management biologists begin to conduct aerial surveys in July to assess escapement in most rivers on the Alaska Peninsula. Efforts will be made to sample adult Chinook salmon immediately after or concurrent to escapement assessment surveys to maximize the number of observers looking for Chinook salmon and increase the probability of encountering sufficient numbers of fish to sample.

During the July sampling trip, sport fishing guides, commercial fishermen, and department biologists will be interviewed to determine if there are any Chinook salmon stocks on the Alaska Peninsula that are not accounted for in the Anadromous Waters Catalog. Aerial surveys and sample collection if possible will occur on any new stocks revealed.

EXPLORATORY TRIPS

If time and budget allow, further aerial surveys and juvenile trapping will be conducted along the South Alaska Peninsula, Chignik, and Kodiak mainland areas to explore rivers that may contain spawning Chinook salmon. Rivers that may contain spawning Chinook salmon are the Alagogshak, Big, and Swikshak rivers (Figure 3). In addition, if fish are available, they will be captured in Dog Salmon Creek on the south side of Kodiak. Chinook salmon were transplanted from Karluk River to Dog Salmon Creek in 1966 through 1969 and have small, naturally propagating annual returns. Several rivers are very remote and receive very little aerial survey effort while some rivers are chronically murky and aerial surveys are ineffective to determine presence or absence of Chinook salmon. Further fuel caches will be established via floatplane if remote areas will be accessed by helicopter.

ADJACENT AREA TRIPS

Sampling of Chinook salmon from adjacent areas in southeastern Bristol Bay and in southwestern Cook Inlet will be conducted in June and July. In southeastern Bristol Bay, the King Salmon and Dog Salmon tributaries of the Ugashik River, the King Salmon tributary of the Egegik River, and the Big Creek tributary of the Naknek River will be sampled in July. In southwestern Cook Inlet McNeil and Little Kamishak rivers will be sampled in July.

PILLAR CREEK HATCHERY SAMPLING

In 2013 we will sample each adult used as the broodstock immediately prior to the egg take process. Axillary processes will be clipped in conjunction with the egg take at Monashka Creek where all captured broodstock are held in captivity to fully mature.

SAMPLE PROCESSING

All samples collected from the Alaska Peninsula and Kodiak will be brought back to the Kodiak ADF&G office. Samples from southeastern Bristol Bay will be brought back to the King Salmon ADF&G office. Samples collected in southwestern Cook Inlet will be brought back to the Homer ADF&G office. Samples will be organized, cataloged and packaged for commercial hazardous material shipping. At the end of the sampling season, the samples will be shipped to the Gene Conservation Laboratory in Anchorage for genetic analysis.

LABORATORY ANALYSIS

Samples will be inventoried and catalogued in the Gene Conservation Laboratory's Oracle database, LOKI, upon receipt in Anchorage. Collections with enough samples to reliably estimate allele frequencies (>50) will be selected for analysis. Laboratory analysis will include the extraction genomic DNA via Qiagen extraction kits. These samples will be genotyped for a suite of 45 single nucleotide polymorphisms (SNPs) chosen to best characterize Chinook salmon from this region. If this number of SNPs fails to adequately characterize Chinook salmon from this region, we will investigate the utility of genotyping samples for up to 192 SNPs. Genotypes will be imported into LOKI. A laboratory quality control (QC) analysis will include the re-extraction and genotyping of 8% of project individuals by staff not included in the original analysis. This QC analysis will identify and fix any catastrophic errors that occurred in the laboratory and measure background error rates in our genotyping process.

STATISTICAL ANALYSIS

Following retrieval of genotypes from LOKI, we will conduct a statistical QC to verify quality of the data. This analysis will identify and remove invariant SNPs, identify and remove individuals missing genotypes for greater than 20% of analyzed SNPs, non-Chinook salmon, and duplicate individuals. Further statistical QC will verify that collections conform to common population genetic expectations (Hardy-Weinberg and linkage equilibria) that are assumed in mixed stock analysis.

A series of analyses will be conducted to examine genetic variation among populations within the Alaska Peninsula region and adjacent areas. These analyses will include an examination of population genetic structure via genetic distances among populations (visualized on trees and multidimensional scaling plots), principal component analysis as well as the calculation of summary statistics (heterozygosities, *F*-statistics, etc.). These analyses will give us insight into the relationships among populations within this region and how fine of a scale mixed stock analysis might be appropriate within the region.

Further analyses will examine how these new collections correspond to others in a broader geographic context. These analyses of among region genetic structure will evaluate the utility of observed structure for mixed stock analysis. Tests of the genetic baseline of this larger geographic scope will include 100% proof tests as well as proof tests with stock compositions similar to those observed in the pollock fishery to evaluate the baseline's accuracy and precision.

Finally, the samples of Pillar Creek Hatchery broodstock will be analyzed for their potential in PBT analyses.

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FIGURES

Figure 1.–The location of the axillary process on the left ventral side of the fish.

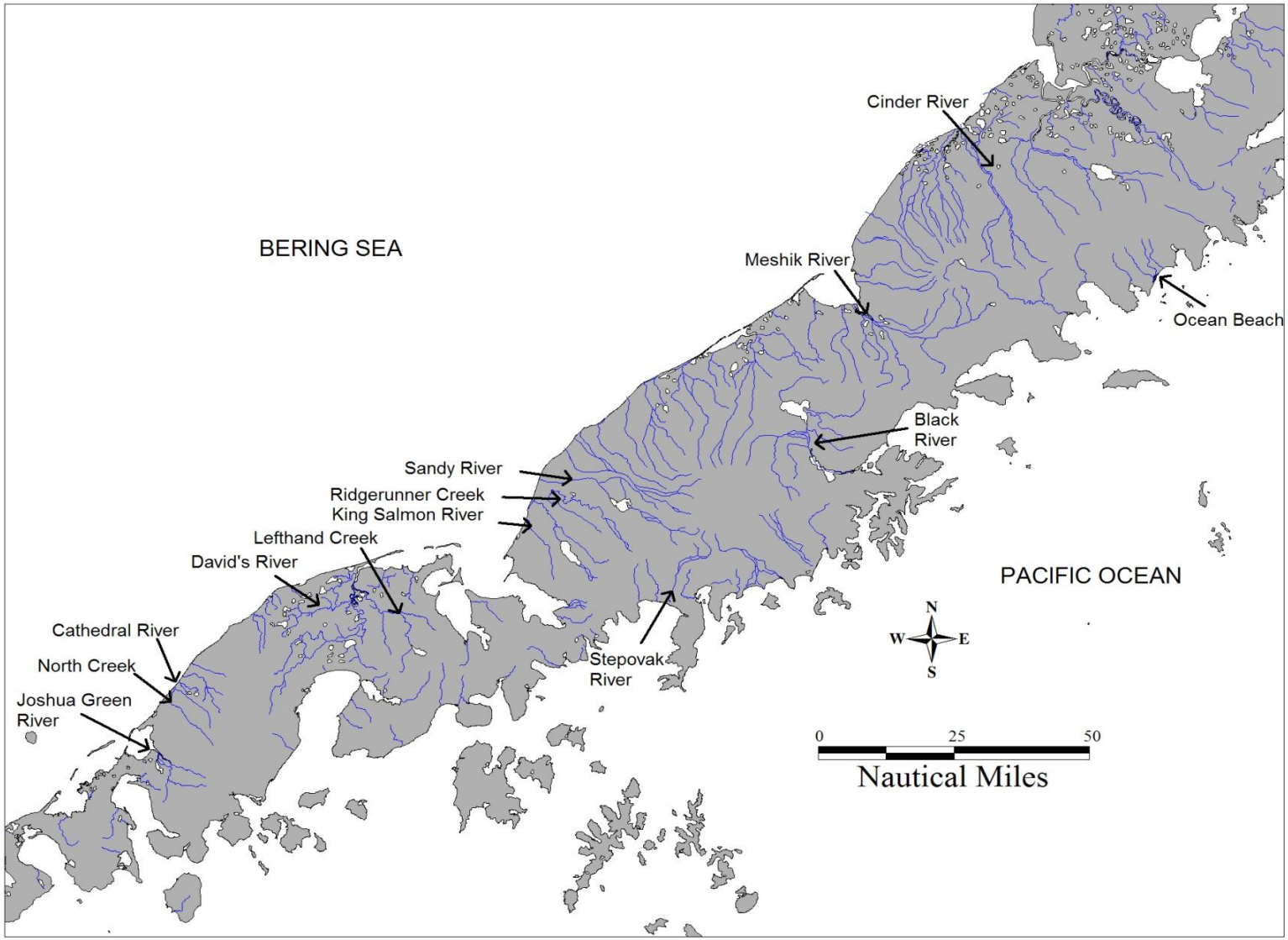

Figure 2.–Map of the Alaska Peninsula with possible Chinook salmon spawning stocks targeted for sampling in 2013 depicted.

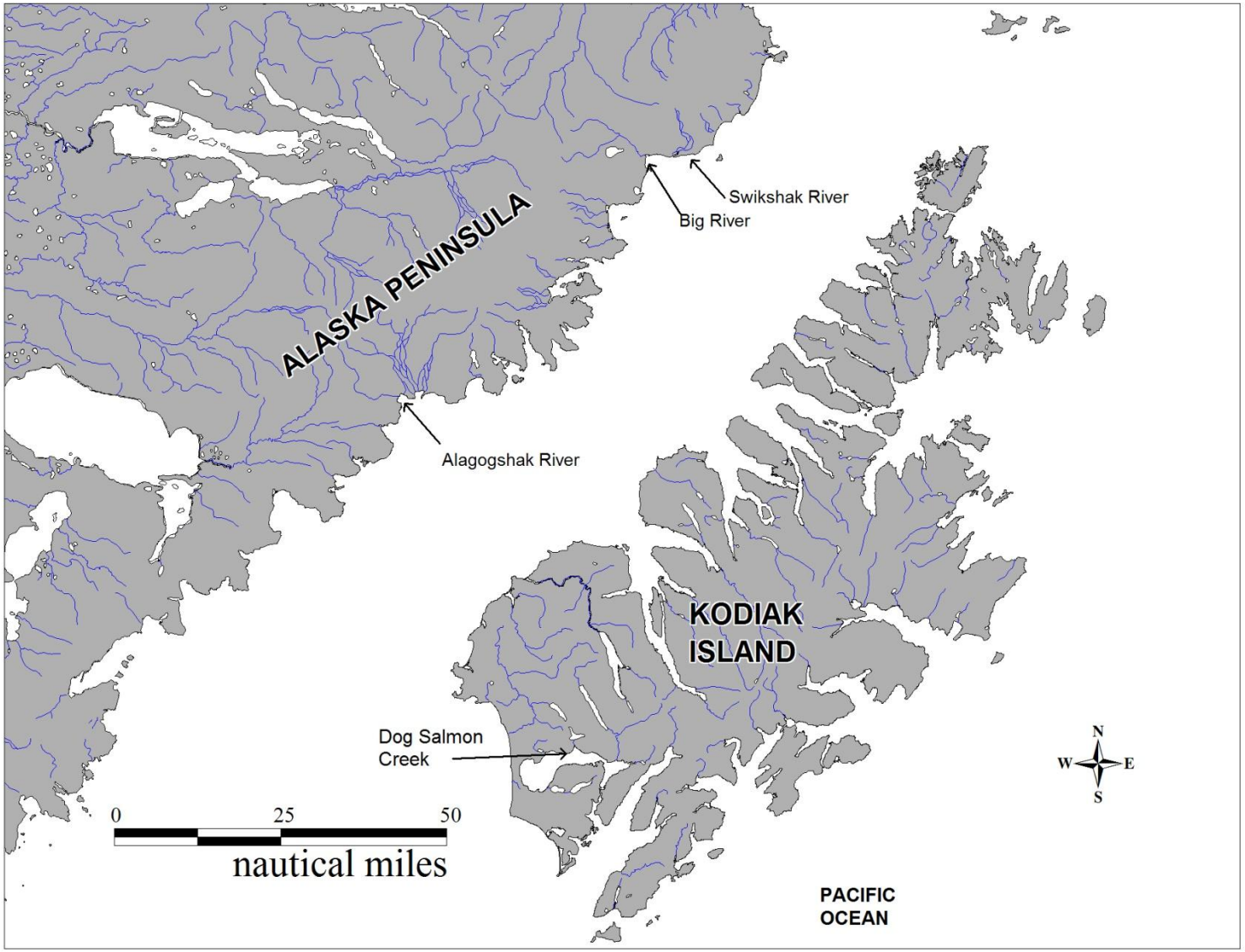

Figure 3.–Map of the Kodiak area with possible Chinook salmon spawning stocks targeted for sampling in 2013 depicted.

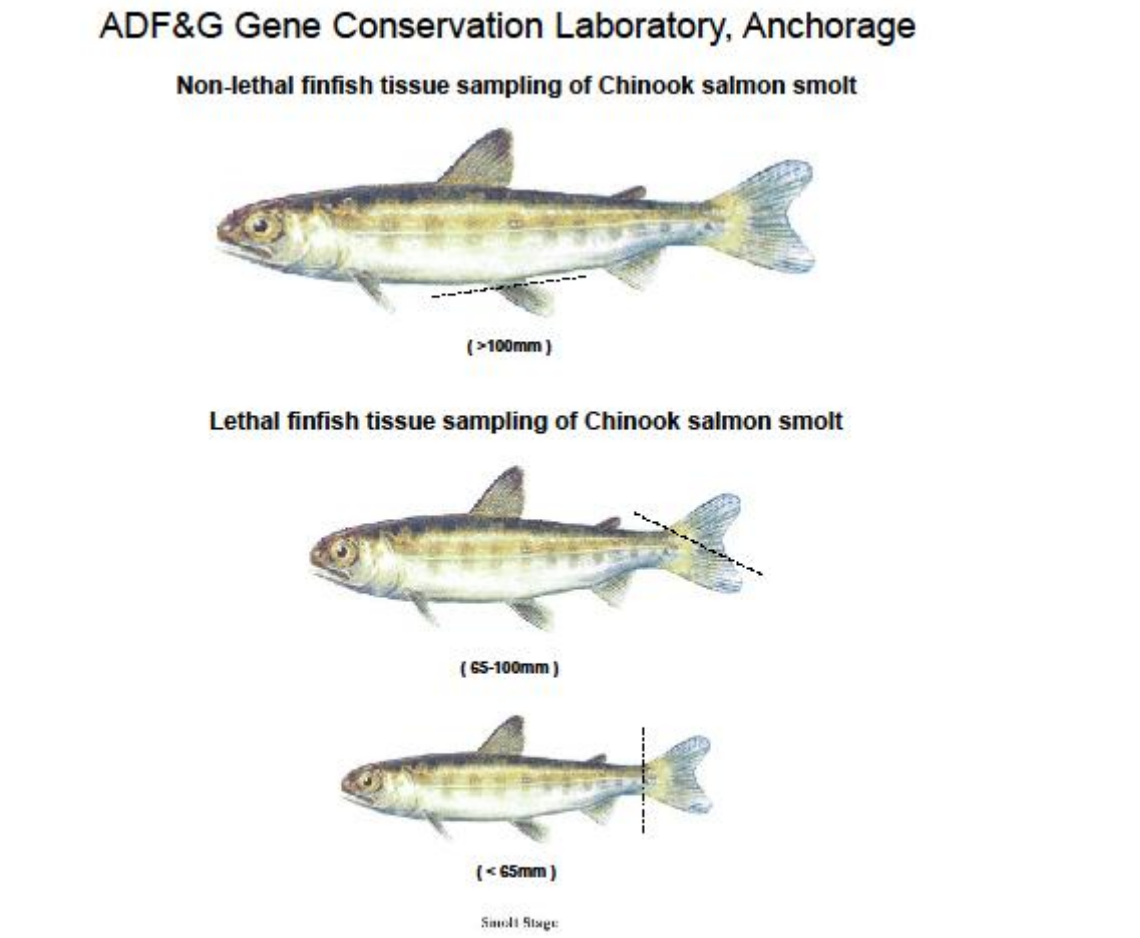

Figure 4.–Non-lethal and lethal sampling of Chinook salmon smolt (or fry) for tissues suitable for genetic analysis.