

Chinook Salmon Tissue Sample Collections for the Analysis of Yukon River DNA Baseline Samples in  
Alaska, 2015

Yukon River Restoration and Enhancement Fund  
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By:

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## Abstract

The purpose of this project is to continue to develop and refine genetic baselines for Yukon River Chinook and chum salmon stocks through collection and genetic analyses of tissue samples from representative spawning populations in the Yukon River. Continued development of the genetic baselines are necessary to obtain the most accurate allocations in mixed stock analysis, a critical tool for both inseason management and postseason evaluation of Yukon River salmon runs. This project consisted of the collection of Chinook salmon tissue samples in Alaska. These samples will be analyzed by the Alaska Department of Fish and Game's Gene Conservation Laboratory and will subsequently be added to the existing ADF&G and DFO baselines. Samples were collected from live fish and recently deceased fish with red gills, preserved in ethyl alcohol, and shared among the three genetics laboratories (ADF&G, DFO, & USFWS) which conduct mixed stock analyses of Yukon River salmon runs. This report covers samples collected and included in baselines in Alaskan tributaries only; Canadian sampling and baseline inclusions are reported separately. In 2015, a total of 50 Chinook salmon tissue samples were collected from one Alaska tributary spawning population, the Teedraanjik (Salmon Fork River). Genotypes from these samples will be used to improve the existing genetic baseline.

## Introduction

Management of Chinook and chum salmon in the Yukon River requires differentiation between stocks originating from the various tributaries in both the United States and Canada. Genetic stock identification (GSI) is effectively used to distinguish country of origin and broad and fine scale stock groupings of Chinook salmon caught in the commercial and subsistence fisheries on the Yukon River (e.g. Decovich and Howard 2011). Chum salmon can be genetically differentiated into summer and fall runs, with broad scale stock groupings in each (Flannery et al. 2007). Fundamental to accurate GSI is the development of a comprehensive baseline genetic database which represents all spawning stocks that potentially contribute to the mixed stock run or fishery. Genetic baselines for Yukon River salmon populations were originally constructed using allozyme markers starting in the late 1980's (e.g. Beacham et al. 1989). Single nucleotide polymorphisms (SNP) have been preferentially used as markers in the Chinook salmon baseline since 2004 (Smith et al. 2005), replacing the older allozyme database. At the beginning of the 2015 season, the Chinook baseline was comprised of 36 separate populations, and given adequate sample sizes, stocks can be identified to one of nine reporting groups (DeCovich and Howard 2011).

Similarly for chum salmon, a baseline using microsatellite markers was developed to replace the allozyme baseline around 2007 (Flannery et al. 2007). About 21 chum populations comprise the current chum baseline, from which stocks can be identified to one of two summer chum reporting groups and two Alaskan and two Canadian fall chum reporting groups. Although not part of this project, a large number of the Yukon chum salmon populations are also represented in the large Western Alaska Salmon Stock Identification Program (WASSIP) in the Coastal Western Alaska and Upper Yukon River reporting groups (Decovich et al., 2013).

Sampling salmon populations within the Yukon River drainage for genetic baselines is logistically difficult due to the large number of genetically discrete spawning populations distributed over a vast and remote region. Timing of spawning periods can be variable and flooding and turbidity during the spawning period may preclude sampling from occurring. For these reasons, samples are collected somewhat opportunistically, depending on run timing and environmental conditions, based on a priority list. The genetics sub-committee of the Yukon River Panel's (YRP) Joint Technical Committee (JTC) developed a

prioritized list for baseline collections, and it is updated annually and used as a guideline to direct sampling efforts each season (Appendix 1). High priority areas for sampling are those which could serve to further differentiate between genetically distinct groups and which contribute substantial numbers of spawners to Yukon River Chinook and chum salmon returns overall. This genetics baseline sampling project has been funded by the YRP's Restoration and Enhancement (R&E) fund since 2007, and has fostered partnerships between governmental agencies such as the Alaska Department of Fish and Game (ADF&G), the United States Fish and Wildlife Service (USFWS), and the Department of Fisheries and Oceans Canada (DFO), Alaska Native organizations such as the Tanana Chiefs Conference (TCC), Native Alaskan Tribes, and local resource users. Genetic baseline tissue samples have also been contributed by other projects and funding sources and samples may be collected opportunistically when another project is operating in an area from which samples are needed. Sampling may extend over a number of years to achieve sample sizes needed to distinguish among stock groups within an acceptable level of precision. Adding to and improving the Yukon River Chinook and chum salmon baselines is an ongoing process which will ultimately result in more accurate and timely management decisions.

### **Objectives**

1. Collect axillary fin tissue samples appropriate for genetic (DNA) analyses from Chinook salmon spawning in the Draanjik (Black River) and Teedraanjik (Salmon Fork River), and
2. Incorporate the sample genotypes into the existing baselines.

### **Methods:**

The TCC Wildlife and Parks (W&P) Department hired two fisheries technicians to collect Chinook salmon tissue samples for genetic analysis from priority Yukon River tributaries. The 2015 priority locations were the Teedraanjik (Salmon Fork River) and the Draanjik (Black River). The Yukon River Daily Update distributed by the ADF&G provides the approximate location of Chinook salmon pulses by date based on run timing at the Pilot Station Sonar project operated by the ADF&G (Appendix 2). Chinook salmon in the Yukon River are estimated to travel approximately 40 river miles per day (mpd) while migrating to their spawning grounds. Chinook salmon spawning in the Draanjik and Teedraanjik depart the Yukon River and enter the Porcupine River near Fort Yukon, Alaska. From there they migrate 20 river miles up the Porcupine River before entering the Draanjik, another 138 river miles upriver on the Draanjik prior to entering the Teedraanjik, and finally another 74 river miles upriver on the Teedraanjik before reaching the international border. The Teedraanjik continues into the Yukon, Canada with its headwaters located in the Ogilvie Mountains. This project was focused only on Alaskan Chinooks spawning downstream of the international border on the Teedraanjik. The last Chinook salmon pulse of the 2015 run was estimated to pass by Fort Yukon on July 19. Therefore, the majority of Chinook salmon were estimated to be present in the Teedraanjik approximately 5 to 6 days after passing Fort Yukon based on the distance to travel between Fort Yukon and the international border ( $n = 232$  river miles) and the estimated travel time ( $n = 40$  mpd).

The two priority locations were surveyed and sampled separately. The Teedraanjik was surveyed between July 23 and July 29, 2015. Sampling was conducted by the TCC fisheries biologist and two TCC fisheries technicians. Tissue collections on the Teedraanjik were completed while floating the river using one 16' Soar Pro Pioneer inflatable canoe and one 8' 4" Alpaca Mule inflatable kayak. A Cessna 185 with floats was chartered through Shadow Aviation for the put in flight. The goal was to land as close to the international border as possible. However, due to a lack of safe landing areas on the Teedraanjik near the international border, the actual put in location was 6.5 straight line miles downstream of the

international border on the Teedraanjik (66.500030°, -141.208130°, WGS84). Two flights were necessary for the put in. The first flight left Fairbanks on July 23, 2015 and provided transportation for the TCC fisheries biologist and the majority of the gear and supplies. The second flight (same day) picked up the two TCC technicians and the remaining gear and supplies in Circle, Alaska and flew them to the put in location. The field crew floated, surveyed, and sampled the Teedraanjik between the put in location and the confluence with the Draanjik for seven days. Jonas Carroll Sr. of Chalkyitsik was contracted by the TCC and met the field crew at the confluence with the Draanjik on July 29, 2015. Mr. Carroll provided transportation to Chalkyitsik via river boat. The TCC field crew overnighted in Chalkyitsik on July 29, and returned to Fairbanks on July 30, 2015 via Warbelow's Air.

To survey and sample the Draanjik, a Robinson R44 II helicopter was chartered through InFlight Helicopters between August 1 and August 3, 2015. On July 31, the TCC field crew drove a TCC pick-up truck with the gear and supplies to Circle where they met with the InFlight Helicopter pilot. The field crew and the pilot based operations out of Circle for each day of surveying and sampling. On August 1, aerial surveys were conducted on the upper Draanjik including Mountain Creek, Van Hatten Creek, Wood River, Bull Creek, and the Grayling Fork River. On August 2 and 3, due to water clarity in the Draanjik, aerial surveys were shifted to the Teedraanjik drainage to survey and sample the major tributaries of the Teedraanjik including Runt Creek, Drifting Snow Creek, Tetthajik Creek, and Kevinjik Creek, as well as the main stem of the Teedraanjik. The pilot returned to Fairbanks after sampling was complete on August 3, 2015. The TCC field crew departed Circle and arrived in Fairbanks on August 4, 2015.

Live fish, or recently deceased fish with red gills, were sampled on the spawning grounds. Collections were accomplished using rod and reel. Tissue samples were collected by clipping approximately a ½ - 1" portion of the pelvic axillary process of each individual fish according to the ADF&G Gene Conservation Laboratory's (GCL) protocol (Appendix 3). The sample size goal for Chinook salmon tissue samples in 2015 was to provide for a minimum of 50 samples per tributary, despite the need for 200 samples per tributary location to provide for optimal accuracy in identification of the baseline. Due to the sparseness of spawning salmon in these priority locations, samplers were instructed to collect as many samples as possible up to a maximum of 200 samples per location. Tissue samples were stored in bulk vials partially filled with anhydrous ethyl alcohol. The bulk vials were shipped to the ADF&G GCL. Samples were shared with the USFWS and with the DFO-Canada. The samples will be genotyped by the ADF&G GCL in late 2016, and will be used to improve the existing genetic baseline.

## **Results and Discussion**

In 2015, a total of 50 Chinook salmon axillary process tissue samples were collected. No samples were collected from the Draanjik (n = 0). Fifty samples were collected from the Teedraanjik (n = 50). Of the 50 samples collected in the Teedraanjik, the float trip resulted in 36 % of the samples (n = 18), and the aerial surveys resulted in 64 % of the samples (n = 32). Six samples were collected from the Tetthajik Creek, while the other 44 samples were collected from the main stem of the Teedraanjik (Table 1).

The Draanjik is a remote river system draining vast areas of lowlands within the Yukon Flats National Wildlife Refuge (YFNWR) and the Bureau of Land Management's (BLM) Eastern Interior region (Figure 1). During our aerial survey of the Draanjik on August 1, the river level was at or above bank full. The water clarity in the upper Draanjik, Bear Mountain Creek, Van Hatten Creek, Wood River, and Bull Creek was opaque and not suitable for visually locating Chinook salmon. Water clarity in the Grayling Fork River was transparent and ideal for visually locating salmon. However, no Chinook salmon were observed in

the Grayling Fork River. Therefore, aerial surveys on August 2 and August 3 focused on the clear water of the Teedraanjik drainage.

The Teedraanjik is also a remote system, originating in the Ogilvie Mountains in Yukon, Canada, and draining portions of the BLM's Eastern Interior region and the YFNWR (Figure 1). Aerial surveys were conducted on August 2 and August 3. On August 2, aerial surveys covered portions of the Teedraanjik tributaries that were not able to be sampled during the float trip. Aerial surveys were conducted on Runt Creek, Drifting Snow Creek, Tetthajik Creek, and Kevinjik Creek. Water clarity and visibility were ideal in all of the tributaries surveyed. No Chinook salmon were observed in Runt Creek, Drifting Snow Creek, and Kevinjik Creek. One Chinook salmon was observed in the Tetthajik Creek (n=1) approximately 1.9 straight line miles upstream from its confluence with the Teedraanjik (66.516370°, -141.754100°, WGS84). On August 3, an aerial survey was conducted on the main stem of the Teedraanjik. While conducting the aerial survey, we intentionally landed at known locations marked on our GPS units from the float trip where spawning Chinook salmon were observed or sampled. Water clarity and visibility were ideal, and Chinook salmon could easily be observed from the air. Spawning Chinook salmon were observed at various locations and samples were collected from locations with landing access for the R44 helicopter (Table 2). While 200 samples are needed for optimal accuracy, the 50 samples collected in 2015 from the Teedraanjik will be genotyped and used to improve the genetic baseline. Additional samples from this system will help to increase the accuracy and precision for this stock.

#### **Recommendations:**

Additional Chinook salmon tissue samples are needed from numerous spawning tributaries throughout the Yukon River drainage in order to complete and further refine the Yukon River Chinook salmon genetic baseline. Several spawning tributaries in need of additional samples have already been identified by the Yukon River's Joint Technical Committee (Appendix 1). Figure 2 illustrates the current known spawning tributaries that are in need of additional samples. Several of these unique spawning populations are currently unrepresented, while other populations require additional samples to refine the accuracy and precision of mixed stock analysis and their associated genetic reporting groups. It is recommended that additional tissue samples be collected from each of the spawning populations identified by the JTC. Furthermore, each spawning population should continue to be sampled until 200 tissue samples have been collected per spawning population.

#### **Acknowledgements**

We would like to express our gratitude to the Yukon River Panel's Restoration and Enhancement Fund for providing funding for this project. We wish to acknowledge the Chalkyitsik Village Council and the Circle Tribal Council for their support for this project. We would also like to thank the TCC employees Brian Lepping, Joe Matesi, and Nicole Farnham for their sampling efforts, and the TCC contractors Jonas Carroll Sr. (Chalkyitsik), Andy Greenblatt (Shadow Aviation), and Quintin Slade (InFlight Helicopters) for their logistical support. We also would like to thank the ADF&G GCL staff for their time in organizing sampling materials, intaking, processing, and analyzing samples. Finally, we would like to extend acknowledgement to our Canadian counterparts at the DFO and their contractors, who collected, processed, and analyzed samples from Canadian Yukon tributaries.

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Smith, C.T., W.D. Templin, J.E. Seeb, and L.W. Seeb. 2005. Single nucleotide polymorphisms provide rapid and accurate estimates of the proportions of U.S. and Canadian Chinook salmon caught in Yukon River fisheries. *North American Journal of Fisheries Management*, 25:3, 944-953.

# 2015 Chinook Salmon Tissue Sample Collections

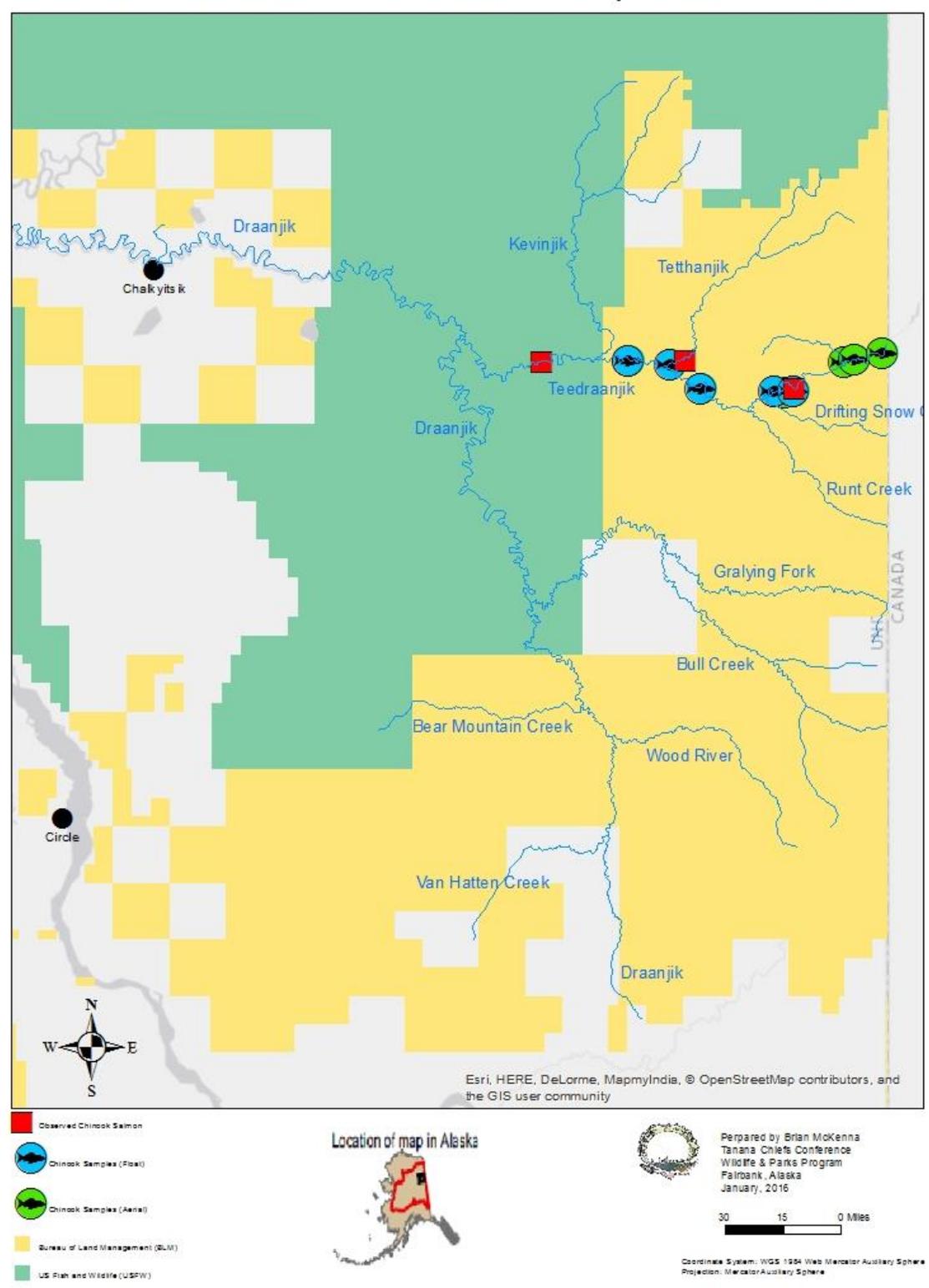


Figure 1 — Map illustrating the location of Chinook salmon tissue samples collected in the Teedraanjik in 2015.

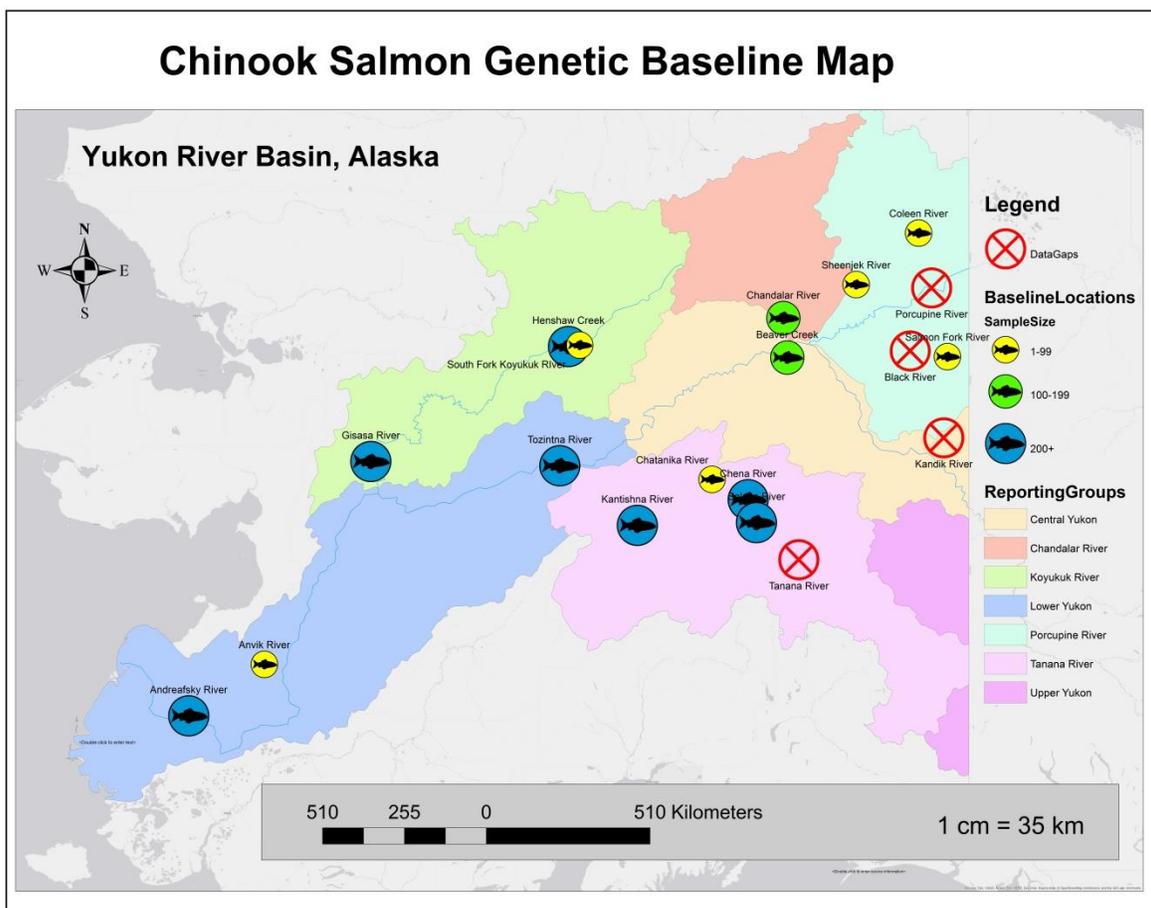


Figure 2 — Map illustrating tissue samples collected from individual baseline populations of spawning Chinook salmon within the Yukon River drainage. Note: the Central Yukon, Chandalar, and Porcupine reporting groups have not yet been separated via genetic analyses, and currently form the Upper U.S. Yukon reporting group as identified in DeCovich and Howard 2011.

Table 1 — Location and numbers of adult Chinook salmon genetic baseline tissue samples collected from Alaskan tributaries in 2015.

<b>Totals</b>	<b>Tributary Location</b>	<b>No. Samples</b>	<b>Latitude</b>	<b>Longitude</b>	<b>Method</b>
	Teedraanjik	13	66.516660	-141.966540	Aerial
	Teedraanjik	11	66.469830	-141.387540	Aerial
	Teedraanjik	4	66.513970	-141.165130	Aerial
	Teedraanjik	1	66.517560	-141.125320	Aerial
	Teedraanjik	3	66.527130	-141.020980	Aerial
	Teedraanjik	6	66.516660	-141.966540	Float
	Teedraanjik	1	66.474924	-141.698844	Float
	Teedraanjik	1	66.470120	-141.421110	Float
	Teedraanjik	4	66.470920	-141.352250	Float
<b>Sub-Total</b>	<b>Teedraanjik</b>	<b>N = 44</b>			
	Tetthajik Creek	6	66.509640	-141.809621	Float
<b>Sub-Total</b>	<b>Tetthajik Creek</b>	<b>N = 6</b>			
<b>Total</b>	<b>Teedraanjik &amp; Tetthajik Creek</b>	<b>N = 50</b>			
				<b>Aerial Total</b>	<b>N = 32</b>
				<b>Float Total</b>	<b>N = 18</b>

Table 2 — Additional locations and numbers of observed adult Chinook salmon. No samples were collected at these locations.

<b>Tributary Location</b>	<b>No. Observed</b>	<b>Latitude</b>	<b>Longitude</b>	<b>Method</b>
Teedraanjik	10	66.51317	-142.28636	Aerial
Teedraanjik	1	66.47501	-141.3461	Aerial
Tetthajik Creek	1	66.516370	-141.754100	Aerial

## Appendix 1 — 2015 R&amp;E Priorities for Chinook and Chum Salmon Genetic Baseline Tissue Collections.

## 2015 R&amp;E Priorities for adult Chinook genetic baseline tissue collections

Country	Main tributary	Branch tributary
US	Kandik River	
	Porcupine River	Coleen River Black River
	Tanana River	Chatanika River
Canada	Porcupine River	Miner River Fishing Branch
	Pelly River	Mainstem Population Ross River
	Stewart	McQuesten River Janet River
	Teslin River	Nisutlin River Morely River Jennings River
	White River	Nisling River Tincup River
	Yukon	Nordenskiold River North Big Salmon River

## 2015 R&amp;E Priorities for adult Chum genetic baseline tissue collections

Country	Main tributary	Branch tributary	Comments
US	Porcupine River		
	Big Salt River		
	Tanana River	Chena/Salcha Rivers	Distinguish between summer and fall runs
Canada	Pelly River		
	Stewart River		
	White River	Kluane Lake	
	Yukon	Klondike River Chandindu River Minto Tatchun Creek	

Appendix 2 — Approximate Location of Chinook Salmon Pulses by Date of the Pulse Past Sonar near Pilot Station Continuing up the Yukon River, 2015 (ADF&G Yukon Daily Update, July 20, 2015).

<b>Approximate Location of Chinook Salmon Pulses by Date of the Pulse Past sonar near Pilot Station Continuing Up the Yukon River, 2015.</b>									
COMMUNITY	RIVER MILES	DAYS BETWEEN SITES	FIRST FISH	EARLY FISH	PULSE 1	PULSE 2	PULSE 3	PULSE 4	PULSE 5
		Number of Days	1	4	4	5	2	5	
		Pulse Dates at Pilot	31-May	7-Jun	13-Jun	18-Jun	24-Jun	27-Jun	-
		Pilot Station Passage	-	4,534	15,479	27,541	9,817	28,077	-
	Travels in Miles Per Day	35	35	35	35	35	35	35	-
Emmonak	24	1.8	28-May	4-Jun	10-Jun	15-Jun	21-Jun	24-Jun	-
Mt Village	87	#DIV/0!	30-May	6-Jun	12-Jun	17-Jun	22-Jun	25-Jun	-
Pilot Station	123	0.0	31-May	7-Jun	13-Jun	18-Jun	24-Jun	27-Jun	-
Marshall	161	1.1	1-Jun	8-Jun	14-Jun	19-Jun	25-Jun	28-Jun	-
Russian Mission	213	2.6	3-Jun	10-Jun	16-Jun	21-Jun	27-Jun	30-Jun	-
Holy Cross	279	4.5	4-Jun	11-Jun	17-Jun	22-Jun	28-Jun	1-Jul	-
	<b>Travels in Miles Per Day</b>	<b>40</b>	<b>40</b>	<b>40</b>	<b>40</b>	<b>40</b>	<b>40</b>	<b>40</b>	-
Anvik	318	5.4	5-Jun	12-Jun	18-Jun	23-Jun	29-Jun	2-Jul	-
Grayling	336	5.9	5-Jun	12-Jun	18-Jun	23-Jun	29-Jun	2-Jul	-
Kaltag	450	8.7	8-Jun	15-Jun	21-Jun	26-Jun	2-Jul	5-Jul	-
Koyukuk	502	10.0	10-Jun	17-Jun	23-Jun	28-Jun	4-Jul	7-Jul	-
Galena	530	10.7	10-Jun	17-Jun	23-Jun	28-Jun	4-Jul	7-Jul	-
Ruby	581	12.0	12-Jun	19-Jun	25-Jun	30-Jun	6-Jul	9-Jul	-
Tanana	695	14.9	14-Jun	21-Jun	27-Jun	2-Jul	8-Jul	11-Jul	-
Rapids	731	15.8	15-Jun	22-Jun	28-Jun	3-Jul	9-Jul	12-Jul	-
Rampart	763	16.6	16-Jun	23-Jun	29-Jun	4-Jul	10-Jul	13-Jul	-
Stevens Village	847	18.7	18-Jun	25-Jun	1-Jul	6-Jul	12-Jul	15-Jul	-
<b>Fort Yukon</b>	<b>1,002</b>	<b>22.5</b>	<b>22-Jun</b>	<b>29-Jun</b>	<b>5-Jul</b>	<b>10-Jul</b>	<b>16-Jul</b>	<b>19-Jul</b>	-
Circle	1,061	24.0	24-Jun	1-Jul	7-Jul	12-Jul	18-Jul	21-Jul	-
Canadian Border	1,224	28.1	28-Jun	5-Jul	11-Jul	16-Jul	22-Jul	25-Jul	-
<b>Approximate Location of Chinook Salmon Pulses by Date of the Pulse Past sonar near Pilot Station Continuing Up the Tanana River (35 mpd), 2015.</b>									
Manley	765	16.9	16-Jun	23-Jun	29-Jun	4-Jul	10-Jul	13-Jul	-
Kantishna	793	17.7	17-Jun	24-Jun	30-Jun	5-Jul	11-Jul	14-Jul	-
Nenana/Toklat	860	19.6	19-Jun	26-Jun	2-Jul	7-Jul	13-Jul	16-Jul	-
Salcha River	965	22.6	22-Jun	29-Jun	5-Jul	10-Jul	16-Jul	19-Jul	-
Delta River	1,031	24.5	24-Jun	1-Jul	7-Jul	12-Jul	18-Jul	21-Jul	-
Bluff Cabin Slough	1,049	25.0	24-Jun	1-Jul	7-Jul	12-Jul	18-Jul	21-Jul	-

Appendix 3 — Non-lethal Bulk Sampling Finfish Tissues for DNA Analysis (ADF&G Gene Conservation Laboratory, Anchorage, Alaska).

## Non-lethal Bulk Sampling Finfish Tissues for DNA Analysis

ADF&G Gene Conservation Lab, Anchorage

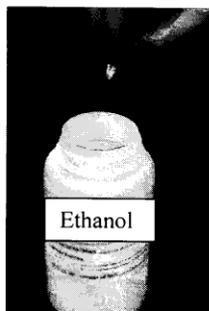
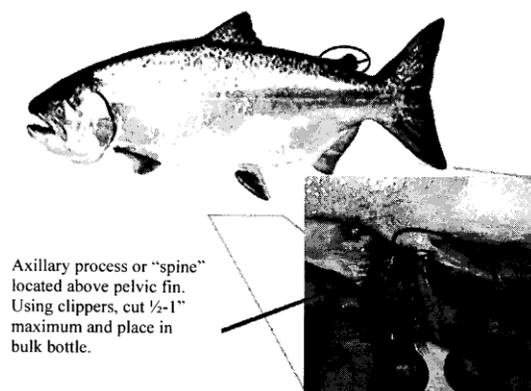
### I. General Information

We use axillary process samples 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**. If sampling from carcasses: tissues need to be as “fresh” and as cold as possible and recently moribund, do not sample from fungal fins.

### II. Sampling Method

**Preservative used: Isopropanol/Methanol/Ethanol (EtOH) preserves tissues for later DNA extraction. Avoid extended contact with skin.**

**Sampling instructions are written for (N=100 fish/125ml) bulk bottle. Steps for collecting axillary process tissues:**



SILLY: _____
Location: _____
Sample Date(s): ____/____/____
Sampler's name: _____
Total # fish sampled: _____
Latitude: _____
Longitude: _____
Species: _____
Comments: _____
ADF&G: Preserved in EtOH

#### Supplies included in sampling kit:

1. Clipper- used to cut a portion of **one** axillary process per fish.
2. Sample target: 100 axillary clips/125ml bulk bottle.
3. Labels on bulk sample bottles: Location, Sample date, Sampler, Total # fish sampled and comments (if any).
4. **1:125ml** wide mouth bottle(s) for EtOH “refresh” step.
5. Sampling instructions

- Wipe dry the axillary process “spine” prior to sampling to avoid getting excess water or fish slime into the 125ml bottle (see diagram).
- Clip off the axillary “spine” using dog nail clippers or scissors to get roughly a ½ - 1” **inch maximum** piece and/or about the size of a small fingernail.
- Place each tissue piece into bulk bottle (**place only one piece of axillary from each fish**).
- Repeat: **up to 100 fish /125ml bulk bottle** (into same bottle). If you don't reach this number of fish per location, that's ok. Maximum storage capacity 125ml bulk for proper preservation of axillary tissue is (N=100).
- Record on **each label**: Location, sampling date (mm/dd/yyyy), sampler's name(s), total number of fish sampled, latitude/longitude, and field notes (if any). Use pencil. This insures correct data with each collection bottle.
- If collection occurs over 4~5 day period, “refresh” EtOH at end of the collection.
- After the collection is complete and 24 hours have passed, “refresh” the axillary tissues as follows: carefully pour off ¾ EtOH and then pour fresh EtOH into sample bottle containing axillary clips. Cap and invert bottle twice mixing EtOH and tissue.
- Freezing not required, store sample bottle in upright cool location for good tissue quality.

<b>Return to ADF&amp;G Anchorage lab:</b>	ADF&G – Genetics 333 Raspberry Road Anchorage, Alaska 99518	Lab staff: 907-267-2247 Judy Berger: 907-267-2175 Freight code: _____
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