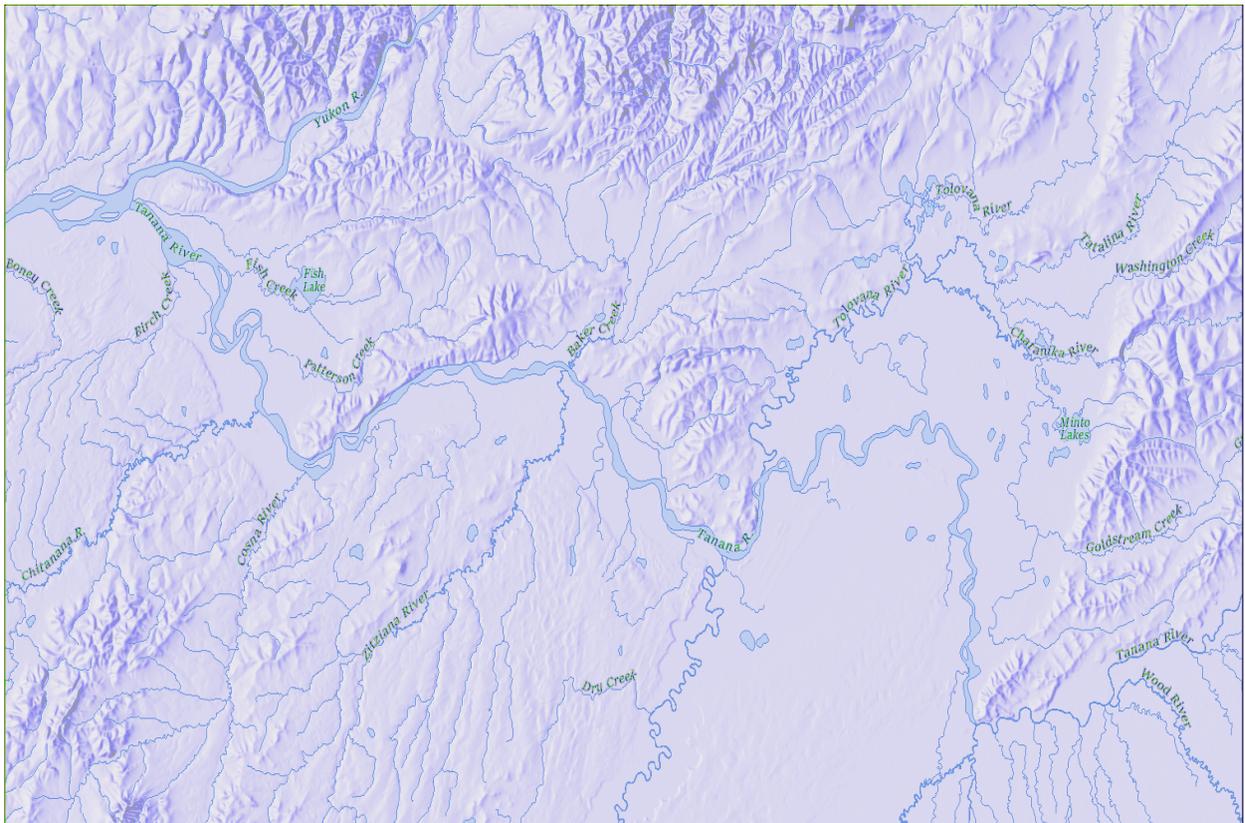


Nonnatal Stream use by Juvenile Salmonids in the Lower Tanana River

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Cover photo: Map of the lower Tanana River and its major tributaries. USGS National Map 3D Elevation Program (3DEP), National Hydrography Dataset. Data refreshed April 2020. Accessed April 28, 2021: https://viewer.nationalmap.gov/advanced-viewer/?p=default&b=base1&x=-17340905.087439593&y=9606757.230858238&l=5&v=US_Topo%3A1%3B2%3B0. Scale 1:1,161084.

Nonnatal Stream use by Juvenile Salmonids in the Lower Tanana River

Raymond F. Hander and Randal G. Loges

Abstract

In July 2016, we explored 19 nonnatal Tanana River tributary streams between the confluence of the Tanana and Chena rivers for the presence of juvenile Chinook Salmon *Oncorhynchus tshawytscha*. Only 8 of 19 streams were sampled because the remaining 11 streams consisted of inadequate rearing habitat. The eight streams were sampled 1 km upstream of the tributary and Tanana River interface. Average minnow trap hours per stream was 204 (range 118–230). Despite extensive sampling efforts, no juvenile Chinook Salmon were captured. Instead, we captured juvenile Longnose Sucker *Catostomus catostomus* ($n = 3$), Northern Pike *Esox lucius* ($n = 3$), and Coho Salmon *O. kisutch* ($n = 2$) in minnow traps in two of the eight sampled streams. We used single nucleotide polymorphism analysis of anal fin tissue and epidermal mucus swab samples to confirm field identification for captured Coho Salmon. Although juvenile Chinook Salmon rearing habitat in sampled portions of the eight streams was suboptimal, we recommend temporal sampling upper reaches of larger tributaries to the lower Tanana River to determine rearing habitat use.

Introduction

The Tanana River is a large glacial tributary to the Yukon River and drains approximately 114,000 km² (Brabets et al. 2000). Major regions that make up the drainage are the northern foothills of the Alaska Range, the Tanana-Kuskokwim lowland, and the Yukon-Tanana uplands. The Chena and Salcha rivers originate from the Yukon-Tanana uplands. Telemetry data suggests that the Chena and Salcha rivers have the two largest Chinook Salmon *Oncorhynchus tshawytscha* spawning populations in the Yukon River basin (Eiler et al. 2014). Other Chinook Salmon spawning streams in the Tanana River basin include Barton Creek, Bearpaw River, Birch Creek, Chatanika River, Clear Creek (Kantishna), Clear Creek (Tanana), Cosna River, Goodpaster River, McDonald Creek, McKinley River, Moose Creek, and Nenana River (Brown et al. 2017). Tanana basin origin Chinook Salmon make up approximately 22% of the Yukon River Chinook Salmon return (Eiler et al. 2014). Studies of juvenile salmonid distribution have concentrated on streams with documented occurrences of spawning fish such as the Chena and Salcha rivers (ADF&G 2019; Matter 2018). However, little information is available on the importance of nonnatal streams for rearing of juvenile Chinook Salmon in the Tanana River drainage. Erkinaro et al. (1997) and LaPerriere and Reynolds (1997) suggest small streams may be especially important for salmon but, are often poorly understood and lack the level of protection afforded to spawning areas. Investigations in the Canada and Alaska portion of the upper Yukon River drainage have documented extensive use of nonnatal streams by subyearling Chinook Salmon for feeding and overwintering (Bradford et al. 2001; Daum and Flannery 2011). After emergence from river gravel, stream-type Chinook Salmon typically disperse to suitable

rearing habitat, feed, and grow throughout the summer, overwinter in freshwater, and usually leave rearing areas for marine waters during the second or third year (Healey 1983, 1991). Previous life history and distribution studies have shown that in some stream-types, age-0 juveniles leave their natal streams to rear and overwinter in downriver, nonnatal habitats (Levings and Lauzier 1991; Scrivener et al. 1994; Bradford and Taylor 1997; Bradford et al. 2001, Daum and Flannery 2011). The mechanisms that cause some individuals to migrate out of their natal streams while others remain are largely unknown but appear to be controlled, at least in part, by some active behavioral response and not solely from passive displacement by the river current (Healey 1991; Taylor et al. 1994; Bradford and Taylor 1997). Possible explanations for these behavioral responses include phenotypic variability among individuals (Bradford and Taylor 1997), competition (Reimers 1968; Grant and Kramer 1990), and genetic predisposition (Healey 1991).

Declines in Yukon River Chinook Salmon have led to over a decade of commercial fishing closures and various management actions to restrict subsistence harvests (JTC 2019). Threats to freshwater habitat in the Tanana River drainage include anthropogenic activity, particularly in the Fairbanks metropolitan area, which has experienced an increase in human population, new housing, and associated infrastructure. Additionally, commercial development such as forestry, mining, and military training activity occur in the Chena and Salcha River watersheds. Development projects may lead to increased siltation, degradation of water quality and quantity, and reduction of rearing habitat for fish populations. Cumulative effects from these and other activities degrade fish habitat, thus negatively affecting Chinook Salmon production. Further, a decline in the quality and quantity of habitat in the Tanana River drainage may affect production and sustainability of numerous fish species that are important to subsistence, recreational, and commercial fisheries throughout the Yukon River drainage.

The Tanana River downstream of Nenana has not been explored for juvenile salmonid presence in nonnatal tributaries. Other studies concentrated in the Tanana River drainage between Big Delta and Nenana (Mecum 1984; Ott et al. 1998; Hemming and Morris 1999; Durst 2001; Hander and Legere 2012), however, they focused on sampling a wide variety of habitats and did not solely concentrate on nonnatal tributaries. The primary objectives of our study were to: 1) determine the presence of juvenile Chinook Salmon in nonnatal tributary streams between the confluence of the Chena and Tanana rivers and the mouth of the Tanana River; 2) determine their genetic region of origin; 3) determine their age structure; 4) determine the geographic distribution of juvenile Chinook Salmon between the confluence of the Chena and Tanana rivers and the mouth of the Tanana River and; 5) nominate streams to *The Catalog of Waters Important for the Spawning, Rearing or Migration of Anadromous Fishes* (AWC; ADF&G 2019) where juvenile salmon were found and not previously reported.

Study Area

The Tanana River is the second largest tributary to the Yukon River. It drains approximately 114,736 km² and contributes about 19% (Brabets et al. 2000; Figure 1) of the total flow of the Yukon River. The Tanana River basin experiences a continental climate with warm summers and cold winters (Shulski and Wendler 2007). Average high temperatures during mid-summer are about 21°C and average low temperatures during mid-winter are about -29°C. The average annual precipitation is approximately 25 cm, half of which is received during the summer months (Shulski and Wendler 2007).

The Tanana Lowlands region of Interior Alaska, defined by Wahrhaftig (1965), encompasses the area between the Tanana River and the Alaska Range and Wrangell Mountains to the south. High elevation mountain areas (1,830 to 2,745 m elevation or more) are dominated by glaciated valleys and small to extensive ice fields. Large valley glaciers emanate from this region feeding large, braided river systems. North of the Alaska Range are rolling foothills with elevations ranging from 215 to 365 m, followed by extensive lowlands extending further north to the Tanana River. Braided glacial streams originating in the Alaska Range flow northward across this lowland region and the entire area is underlain by permafrost. Flow in the smaller streams draining the lowland areas south of the Tanana River comes from clear groundwater springs and localized precipitation. These smaller streams generally flow northwesterly towards the Tanana River.

Streams flowing south from the Yukon-Tanana uplands to the Tanana River are fed almost entirely by snowmelt and rain. These northern streams can be clear or turbid depending on flow level or tannic if draining large wetland regions.

Methods

Stream Selection

We selected and explored 19 Tanana River tributary streams between the confluence of the Tanana and Chena rivers and the confluence of the Yukon and Tanana rivers for the presence of juvenile Chinook Salmon (Table 1; Figure 1). We selected tributaries based on AWC nomination status for Chinook Salmon and connectivity to the Tanana River. None of the selected streams are known to support spawning populations of Chinook Salmon (Barton 1984; Eiler et al. 2014; Johnson and Litchfield 2016). Only Baker Creek has an AWC nomination for adult Chinook Salmon present ($n = 3$) that were observed near the Elliot Highway in 2002 (Johnson and Litchfield 2016). We initially examined streams using remote sensed imagery and topographic maps to screen for probable connectivity to the Tanana River. It was apparent that some selected streams did not meet preferred habitat characteristics of juvenile Chinook Salmon that have been described by others (Hillman et al. 1987; Healey 1991; Roper et al. 1994; Bidlack et al. 2014). Nonetheless, we explored and sampled a wide variety of lower Tanana River tributaries.

Fish Sampling

We accessed streams by boat and foot, depending on wading ability, from July 7–14, 2016. Minnow traps (23 x 45 cm, 0.6 cm wire mesh, with 2.5 cm diameter openings) baited with cured salmon roe were the primary sampling gear. Traps were soaked overnight to accommodate diel foraging behaviors (Sagar and Glova 1988; Bradford and Higgins 2001). Catch per unit effort (CPUE) was calculated as fish per trap hour.

We used a handheld global positioning system (GPS; Garmin GPSMAP 76CSx) trip meter function for initial trap location and standard distance between traps. Boatable stream sampling began approximately 1 river kilometer (rkm) upstream from the Tanana River confluence to minimize influence of the Tanana River and provide a minimum upstream distance for fish presence in a tributary. We set 10 minnow traps approximately 100 m apart on alternating sides of streams.

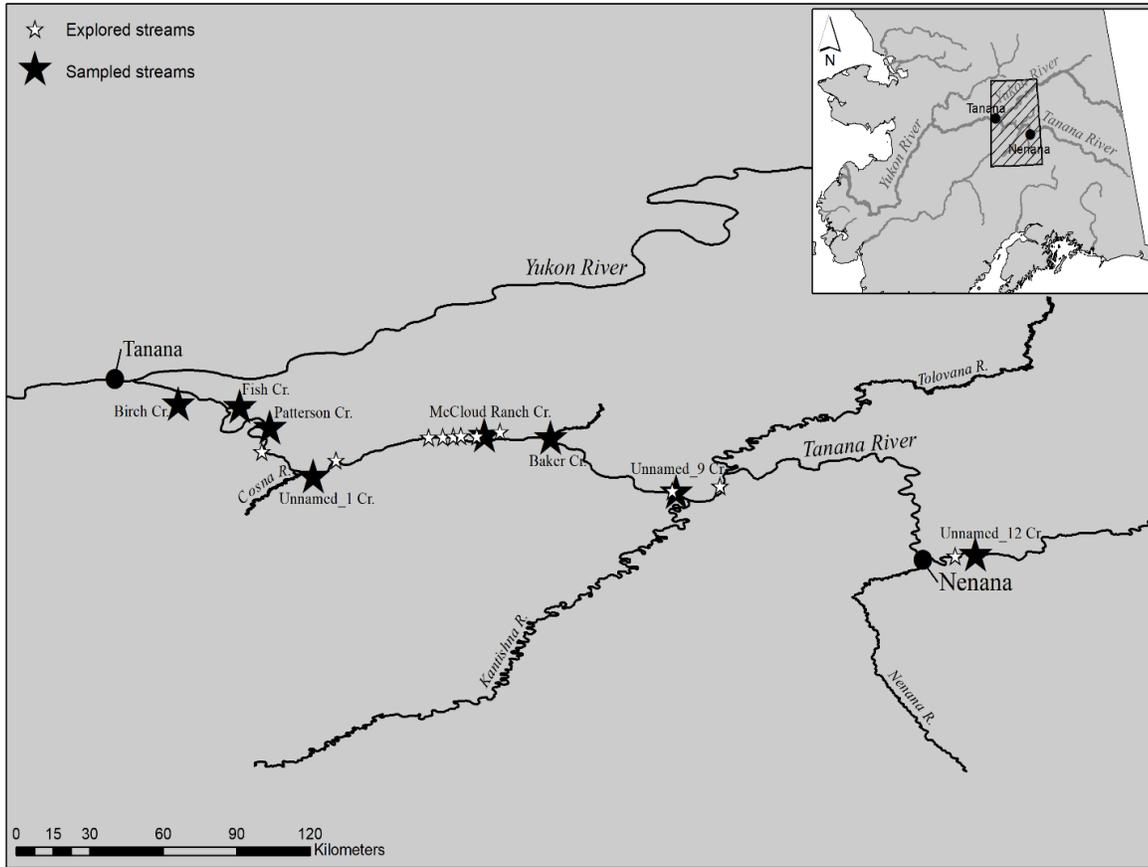


Figure 1.—The Tanana River drainage from near Nenana to the mouth of the Tanana River. Black stars indicate sampled streams and white stars are streams that were explored but not sampled.

We determined the initial sampling location for streams accessed by foot travel by locating the interface between Tanana River and sample stream water, i.e., glacial versus clear water. The first trap was set 100 m upstream from the aforementioned water interface and remaining traps were set approximately 100 m apart at opportunistic locations. Captured fish were field identified to species and life stage. We measured fish to the nearest 1 mm using fork length (FL). Fish life stage was determined through observation of characteristics such as length, and comparison of meristics from published literature. We externally examined all fish for secondary sex and life stage characteristics. After a period of recuperation in still water, we released fish into the stream at the trap site. We made nominations to the AWC based on fish captured in streams not previously reported to have juvenile salmon.

Genetic sampling for species identification

Juvenile Chinook and Coho salmon *O. kisutch* can have overlapping external meristics that may lead to field misidentification (McPhail and Lindsey 1970; Scott and Crossman 1973; Habicht et al. 2019). Both species spawn in Tanana River tributaries upstream of and within our study area and juveniles of both species are highly migratory. We used molecular DNA techniques in conjunction with field identification to confirm species assignments to avoid misidentification of juvenile salmon.

We used two non-lethal sampling methods to obtain genetic material for DNA analysis from two juvenile salmon. We removed anal fin tissue from juvenile salmon and stored it in 2-ml vials containing non-denatured ethanol. We collected anal fin tissue because of its tendency to regenerate quickly (Johnsen and Ugedal 1988) and its removal would least affect swimming performance (Webb 1975). In addition, we collected epidermal mucus samples from the ventral surface of juvenile salmon using Whatman™ OmniSwabs following methods developed by the Alaska Department of Fish and Game (2015; Appendix Figure A.1). The OmniSwabs were stored in 2-ml vials containing silica beads. We shook the vials to immerse each OmniSwab in the silica beads to enhance drying. We minimized sample cross-contamination to the extent possible by handling fish with clean (new) latex examination gloves and cleaning fish handling equipment between sampling events.

Table 1.—Tanana River tributary streams selected for exploration and fish sampling in 2016. Sampled streams are noted with a superscript “s”.

Stream Name	Latitude	Longitude
Birch Creek ^s	65.09512	-151.83357
Fish Creek ^s	65.07929	-151.62781
Patterson Creek ^s	65.00940	-151.52015
Chitanana River	64.92670	-151.52780
Unnamed_1 Creek ^s	64.84611	-151.33944
Unnamed_2 Creek	64.89833	-151.25528
Unnamed_3 Creek	64.97472	-150.91583
Unnamed_4 Creek	64.97639	-150.86389
Unnamed_5 Creek ⁵	64.97806	-150.82500
Unnamed_6 Creek	64.98056	-150.79694
Unnamed_7 Creek	64.97861	-150.73889
McCloud Ranch Creek ^s	64.98207	-150.71171
Cold Creek	64.99316	-150.65334
Baker Creek ^s	64.97138	-150.47398
Unnamed_8 Creek	64.79444	-150.02222
Unnamed_9 Creek ^s	64.79156	-150.00879
Unnamed_10 Creek	64.81044	-149.84653
Unnamed_11 Creek	64.57376	-148.98366
Unnamed_12 Creek ^s	64.58139	-148.92524

Genetic analysis

We followed two DNA extraction protocols contained in the QIAamp DNA Investigator Handbook (Qiagen 2020): “Isolation of Total DNA from Surface and Buccal Swabs” for the Whatman Omni Swab mucus samples, and “Isolation of Total DNA from Tissues” for the anal fin clip samples. We eluted mucus swab origin DNA with 50 µl of Buffer ATE and anal fin tissue origin DNA with 100 µl Buffer ATE. We used a ThermoFisher Scientific Qubit dsDNA HS Assay Kit (ThermoFisher Scientific 2015) to determine the DNA concentration of each sample.

We performed single nucleotide polymorphism (SNP) species identification using mitochondrial DNA TaqMan markers OKI1-OKI and OTSOKI1-OKI as described in Habicht et al. (2019). We ran polymerase chain reactions on a Quant Studio 12K Flex real-time thermocycler following the “wet DNA method” for a MicroAmp Fast Optical 96-Well Reaction Plate from the ThermoFisher Scientific TaqMan SNP Genotyping Assays User Guide (ThermoFisher Scientific 2017). We used ThermoFisher Scientific Quant Studio 12K Flex Software v1.4 (ThermoFisher Scientific

2019) to analyze Real-Time Rn data with Median (Rna to Rnb) and perform allelic discrimination; auto-calling alleles at 30 cycles with a quality value of 95. We included DNA from four positively identified adult Chinook Salmon and four positively identified adult Coho Salmon in the analysis as reference samples. We included a no template control (NTC) for each assay.

Aquatic habitat measurements

We qualitatively characterized water quality and aquatic habitat conditions at fish sampling sites. We recorded geospatial coordinates of sample sites in decimal degrees (to the 5th decimal) with handheld GPS units set for WGS84 datum. We determined stream gradient (%) of study streams using USGS National Map's Spot Elevation Tool (USGS 2020) for trap location elevations. Stream order was assigned based on Strahler (1957) using USGS 1:63,000 scale topographic maps and major stream type classification followed Rosgen (1996). We estimated drainage size using Google Earth's polygon measuring tool. Aquatic habitat characteristics were sampled for relative flow stage and water color, wetted width, water body type, dominant substrate composition, percent woody debris, and percent aquatic vegetation. We obtained water temperature (C) using Onset TidbiT v2 Temp Loggers by placing a logger in the most upstream and downstream traps. Digital photographs were taken of survey sites. Analysis of aquatic habitat data was limited to the characterization of point observations for stream conditions encountered during sampling.

Results

Stream selection

We sampled 8 of 19 selected Tanana River tributaries from Unnamed_12 Creek to the mouth of the Tanana River (Table 1; Figure 2) for the presence of juvenile Chinook Salmon. The remaining 11 streams varied in habitat quality and were explored, but they did not meet general criteria for preferred juvenile Chinook Salmon rearing habitat and were not sampled (Table 2). Exploration occurred up to 100 m upstream of the Tanana River confluence or the stream's interface with the Tanana River. Explored streams with varying characteristics including Tanana River influence dominating the stream with dense aquatic vegetation (low gradient), high gradient streams with large substrates, and heavily wooded streams with multiple blockages (Table 2; Figure 1).

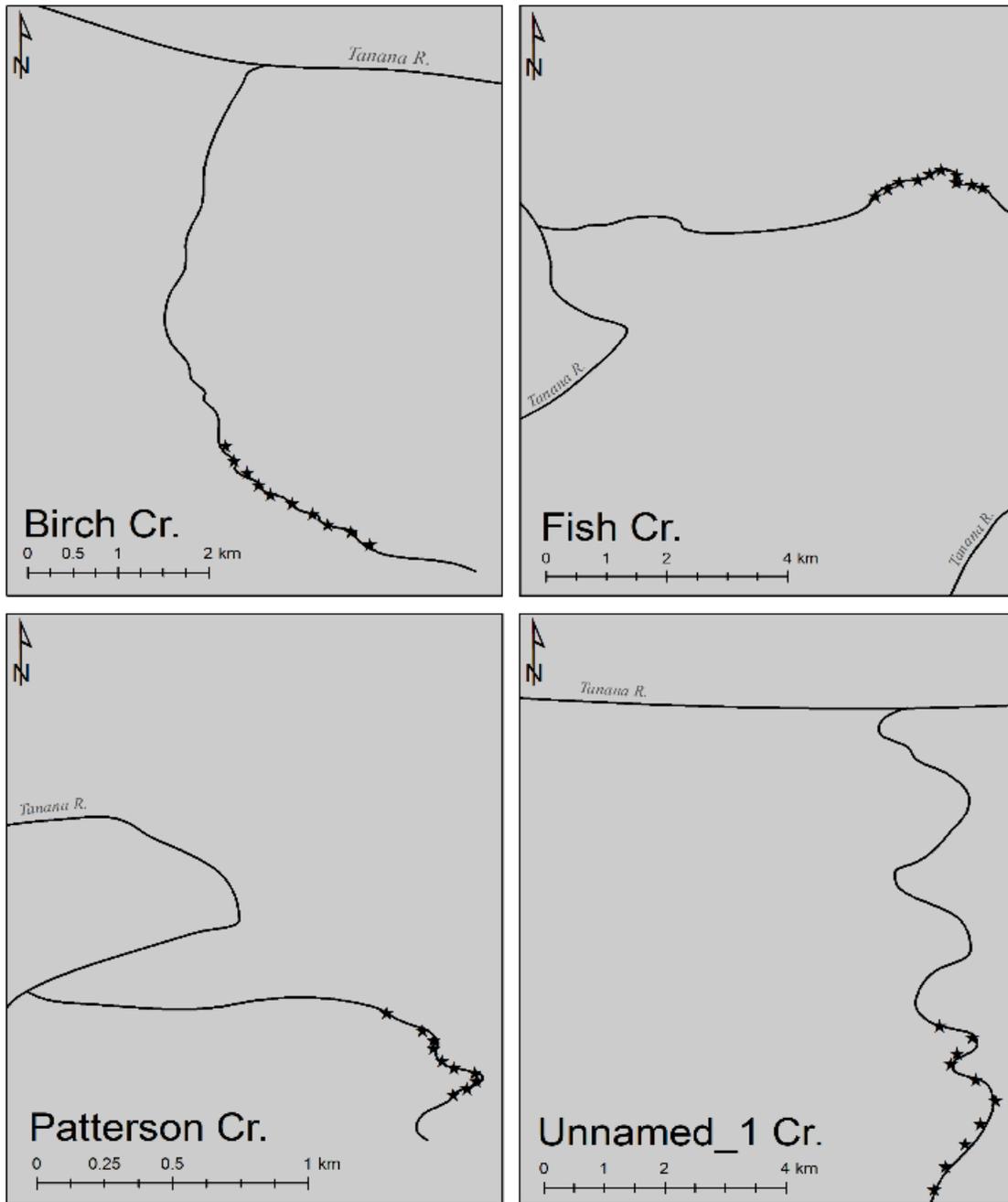


Figure 2.— Streams sampled for juvenile salmon. Black stars indicate individual trap locations. Map scales are variable.

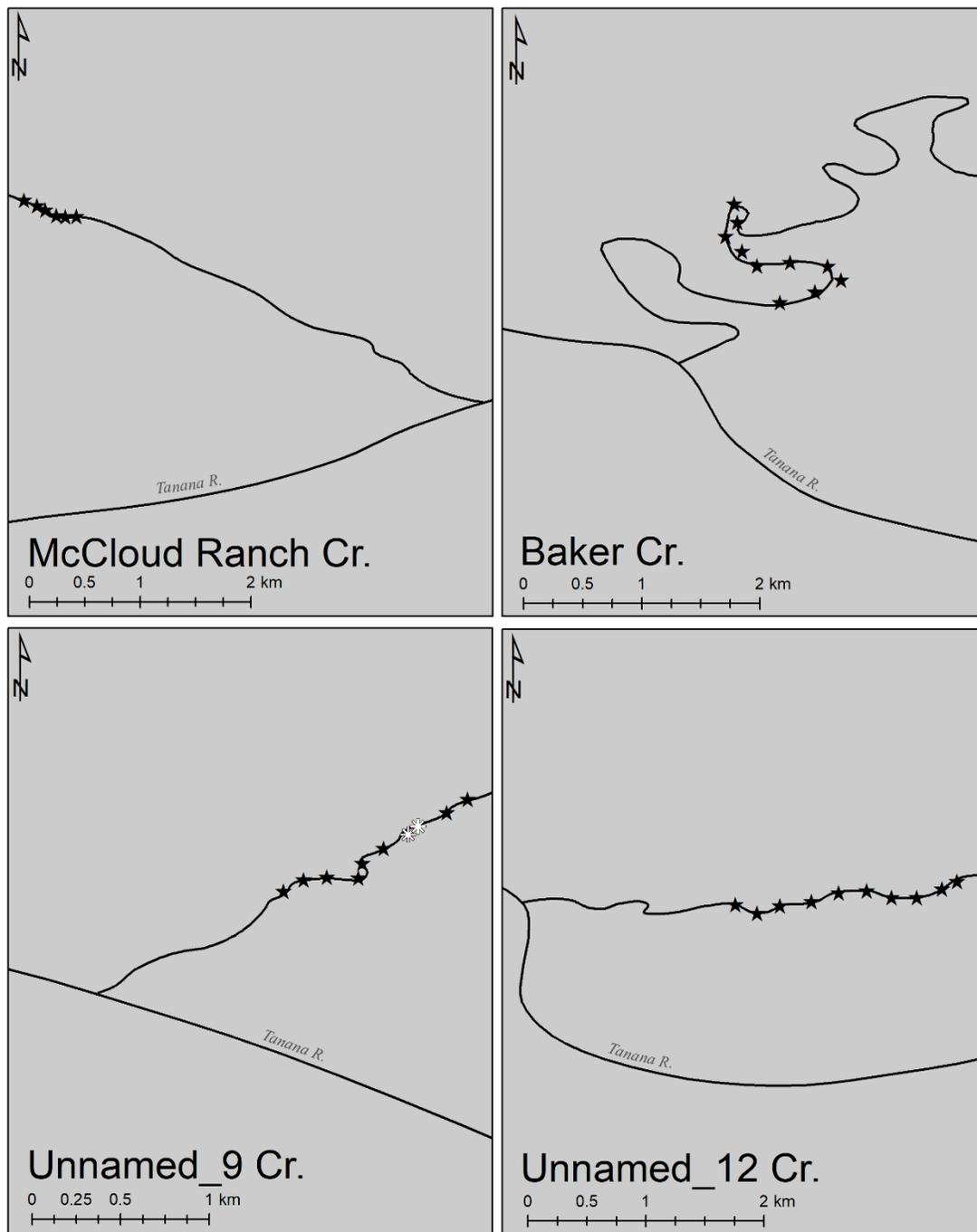


Figure 2 continued.— Streams sampled for juvenile salmon. Black stars indicate individual trap locations. The white symbols in Unnamed 9 Creek indicate juvenile Coho Salmon capture locations. Map scales are variable.

Table 2.— General description of Tanana River tributaries that were explored for juvenile salmon rearing habitat qualities.

Stream name	Stream habitat observation and estimated Rosgen channel type in ()
Chitanana River	Low gradient stream with little flow and silt substrate. Estimated 25 m wetted width within 100 m of its mouth (E).
Unnamed_2 Creek	Low gradient creek with little flow and ended in a wetland with dense vegetation. Estimated 2.5 m wetted width within 50 m of its mouth (E).
Unnamed_3 Creek	Small high gradient clear flowing creek. Estimated 1.5 m wetted width at its mouth (A).
Unnamed_4 Creek	Small high gradient clear flowing creek. Estimated 1.5 m wetted width within 50 m of its mouth (A).
Unnamed_5 Creek	Small high gradient creek with little flow and dense vegetation. Estimated 1.5 m wetted width within 50 m of its mouth (B).
Unnamed_6 Creek	Small moderate gradient, clearwater creek with numerous wood jam blockages. Estimated 1.5 m wetted width within 50 m of its mouth (B).
Unnamed_7 Creek	Small low gradient creek with little flow and ended in a wetland bog. Estimated 2 m wetted width within 100 m of its mouth (E).
Cold Creek	Small low gradient creek with little flow and dense aquatic vegetation. Estimated 1 m wetted width within 20 m of its mouth (E)
Unnamed_8 Creek	Small high gradient creek with large substrate. Estimated 2 m wetted width within 20 m of its mouth (A)
Unnamed_10 Creek	Low gradient Tanana River influenced dead end channel. Estimated 3 m wetted width within 50 m of its mouth (E)
Unnamed_11 Creek	Small low gradient creek with little flow and dense vegetation. Estimated 2 m wetted width within 50 m of its mouth (E)

Fish sampling

We sampled for the presence of juvenile Chinook Salmon in eight Tanana River tributaries July 7–14, 2016 (Table 3; Figure 2). Fish sampling was limited to 1 km sections relative to stream confluence with the Tanana River. Non-wadable streams produced no fish and the sampled area was dominated by inadequate quality rearing habitat for Chinook Salmon. Longnose Sucker *Catostomus catostomus* ($n = 2$; FL = 53 and 123 mm), Northern Pike *Esox lucius* ($n = 2$; FL = 80 and 120 mm) and Coho Salmon ($n = 2$; FL = 55 and 64 mm; both age-0; Howard et al. 2017; Sethi et al. 2017; Appendix Figure A.2) were captured in Unnamed_9 Creek. A Longnose Sucker ($n = 1$; FL = 73 mm) and Northern Pike ($n = 1$; FL = 89 mm) were captured in Unnamed_1 Creek (Table 4 and 5). No fish were captured in any of the remaining sampled streams (Table 4). Birch, Fish, Patterson, Baker, and Unnamed_12 creeks were not wadable and sampled by boat whereas Unnamed_1, McCloud Ranch, and Unnamed_9 creeks were accessed by wading. The mean number of trap hours per stream was 204.8 ($n = 8$; 95% SD = 36.7) and traps were soaked for a mean of 21.6 hours (95% SD = 1.3). Non-wadable sampled stream distance varied 990–1,790 m ($n = 5$; mean = 1,331 m; SD = 292.9; Table 3). Wadable sampled stream distance varied 290–880 m ($n = 3$; mean = 666 m; SD = 328.8). McCloud Ranch Creek (sample distance 290 m) was too shallow for minnow trap use above approximately 300 m from the initial downstream trap so six traps were set at 50 m intervals.

Table 3.— Sample stream, distance sampled, latitude, longitude, and sample site type for streams sampled during 2016. Location data is expressed in WGS 84 datum.

Sample stream	Distance sampled (m)	Latitude	Longitude	Sample site number	Sample site type ^a
Birch Creek	1,375	65.08790	-151.83367	1	FH
		65.08663	-151.83236	2	F
		65.08533	-151.83122	3	F
		65.08429	-151.83006	4	F
		65.08337	-151.82791	5	F
		65.08224	-151.82584	6	F
		65.08113	-151.82436	7	F
		65.08032	-151.82202	8	F
		65.07901	-151.82016	9	F
		65.08946	-151.83454	10	FH
Fish Creek	1,250	65.07960	-151.60825	1	FH
		65.08076	-151.60637	2	F
		65.08188	-151.60462	3	F
		65.08223	-151.60190	4	F
		65.08312	-151.60014	5	F
		65.08382	-151.59845	6	F
		65.08301	-151.59609	7	F
		65.08181	-151.59619	8	F
		65.08140	-151.59385	9	F
		65.08089	-151.59227	10	FH
Patterson Creek	1,790	65.00922	-151.49828	1	FH
		65.00645	-151.49293	2	F
		65.00492	-151.49127	3	F
		65.00365	-151.49128	4	F
		65.00166	-151.49000	5	F
		65.00053	-151.48828	6	F
		64.99972	-151.48513	7	F
		64.99849	-151.48496	8	F
		64.99724	-151.48628	9	F
		64.99629	-151.48836	10	FH
Unnamed_1 Creek	830	64.84520	-151.33874	1	FH
		64.84480	-151.33765	2	F
		64.84423	-151.33816	3	F
		64.84386	-151.33836	4	F
		64.84330	-151.33753	5	F
		64.84257	-151.33689	6	F
		64.84175	-151.33739	7	F
		64.84104	-151.33788	8	F
		64.84024	-151.33853	9	F
		64.83943	-151.33891	10	FH

Table 3.— continued.

Sample stream	Distance sampled (m)	Latitude	Longitude	Sample site number	Sample site type
McCloud Ranch Creek	290	64.98315	-150.71106	1	FH
		64.98311	-150.71193	2	F
		64.98323	-150.71268	3	F
		64.98366	-150.71359	4	F
		64.98401	-150.71425	5	F
		64.98447	-150.71531	6	F
Baker Creek	1,250	64.97484	-150.46877	1	F
		64.97565	-150.46612	2	F
		64.97656	-150.46414	3	F
		64.97759	-150.46514	4	F
		64.97789	-150.46800	5	F
		64.97763	-150.47051	6	F
		64.97873	-150.47166	7	F
		64.97988	-150.47291	8	F
		64.98095	-150.47203	9	F
		64.98238	-150.47227	10	FH
Unnamed_9 Creek	880	64.79182	-150.00742	1	FH
		64.79240	-150.00641	2	F
		64.79255	-150.00523	3	F
		64.79250	-150.00363	4	F
		64.79324	-150.00343	5	F
		64.79401	-150.00234	6	F
		64.79473	-150.00110	7	F
		64.79514	-150.00060	8	F
		64.79582	-149.99915	9	F
		64.79652	-149.99807	10	FH
Unnamed_12 Creek	990	64.58209	-148.89334	1	FH
		64.58031	-148.91023	2	F
		64.57964	-148.90857	3	F
		64.58021	-148.90684	4	F
		64.58056	-148.90442	5	F
		64.58121	-148.90234	6	F
		64.58137	-148.90022	7	F
		64.58086	-148.89832	8	F
		64.58086	-148.89640	9	F
		64.58150	-148.89449	10	FH

^a Abbreviations represent fish and habitat sample site (FH), fishing site only (F)

Table 4.— Sample date, trap number, minnow trap effort (h), species, number of fish captured (*n*), and catch per unit effort (CPUE) of all fish species captured in sampled Tanana River tributary streams where fish were captured, 2016. Streams with no captured fish have the total number of traps set and total trap hours calculated.

Stream and Date	Trap Number	Effort (h)	Species ^a	<i>n</i>	CPUE
Unnamed_1 Creek					
July 12	1	23			0.00 /h
	2	21			0.00 /h
	3	21	NOPI	1	0.05 /h
	4	21			0.00 /h
	5	21			0.00 /h
	6	21			0.00 /h
	7	21			0.00 /h
	8	21	LNSU	1	0.05 /h
	9	21			0.00 /h
	10	21			0.00 /h
Total		212			
Unnamed_9 Creek					
July 8	1	18			0.00 /h
	2	18	NOPI	1	0.06 /h
	3	20	NOPI	1	0.05 /h
	3		LNSU	1	0.05 /h
	4	20			0.00 /h
	5	20			0.00 /h
	6	20			0.00 /h
	7	20	LNSU	1	0.05 /h
	7		COHO	1	0.05 /h
	8	20	COHO	1	0.05 /h
	9	20			0.00 /h
	10	20			0.00 /h
Total	10 traps	196			
Birch Creek					
Total	10 traps	220			0.00 /h
Fish Creek					
Total	10 traps	212			0.00 /h
Patterson Creek					
Total	10 traps	230			0.00 /h
McCloud Ranch Creek					
Total	6 traps	118			0.00 /h
Baker Creek					
Total	10 traps	229			0.00 /h
Unnamed_12 Creek					
Total	10 traps	221			0.00 /h

Table 5.— Fish species captured in Unnamed_1 and Unnamed_9 creeks in 2016.

Family and scientific name	Common name	Unnamed 1 Creek	Unnamed 9 Creek
Catostomidae <i>Catostomus catostomus</i>	Longnose Sucker	X _j	X _j
Esocidae <i>Esox lucius</i>	Northern Pike	X _j	X _j
Salmonidae <i>Oncorhynchus kisutch</i>	Coho Salmon		X _j

j – juvenile life stage

Genetic analysis

Mucus swab samples (S1 and S2) and anal fin clip tissue samples (F1 and F2) were collected from each of two fish. Extracted DNA concentrations were 2.8 ng/μl (S1) and 3.6 ng/μl (S2) for the mucus swab samples and 12 ng/μl (F1) and 11 ng/μl (F2) for the anal fin tissue samples. All unknown samples were heterozygous at the OTSOKI1-OKI locus confirming they were either of Coho Salmon or Chinook Salmon origin (Figure 3). All unknown samples were homozygous for the Coho Salmon allele at the OKI1-OKI locus and therefore genetically determined to be of Coho Salmon origin (Figure 4).

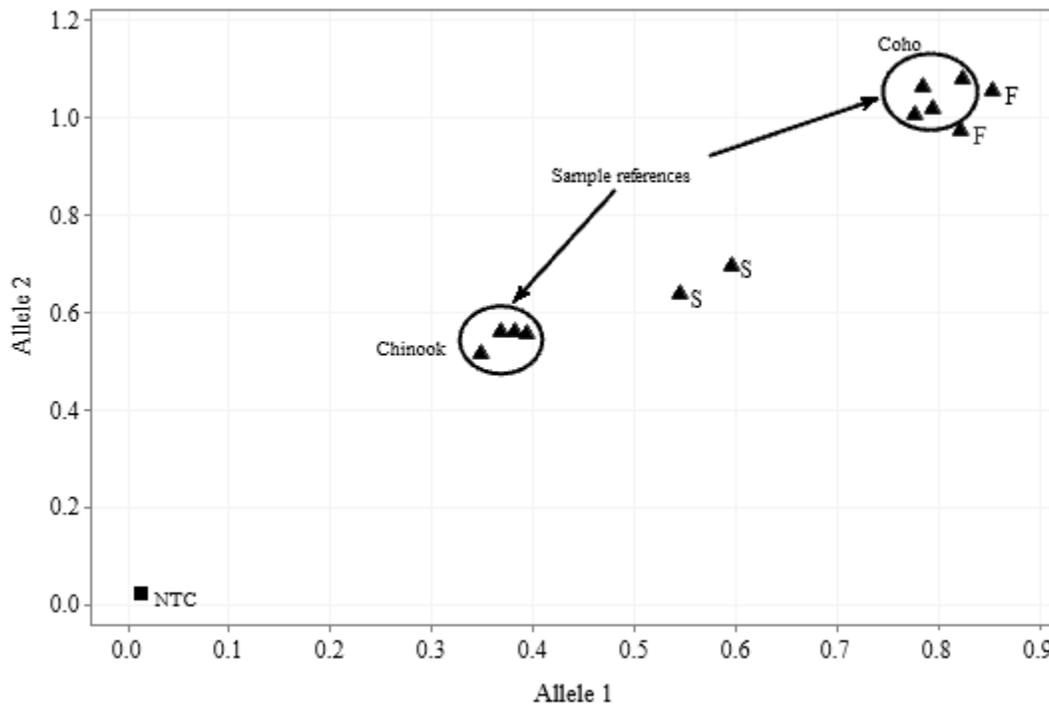


Figure 3.— All samples, references (adult Coho and Chinook Salmon) and unknown species samples (labeled; S=swab, F=fin), were heterozygous at the OTSOKI-OKI locus. The square point in the bottom left corner is a No Template Control (NTC).

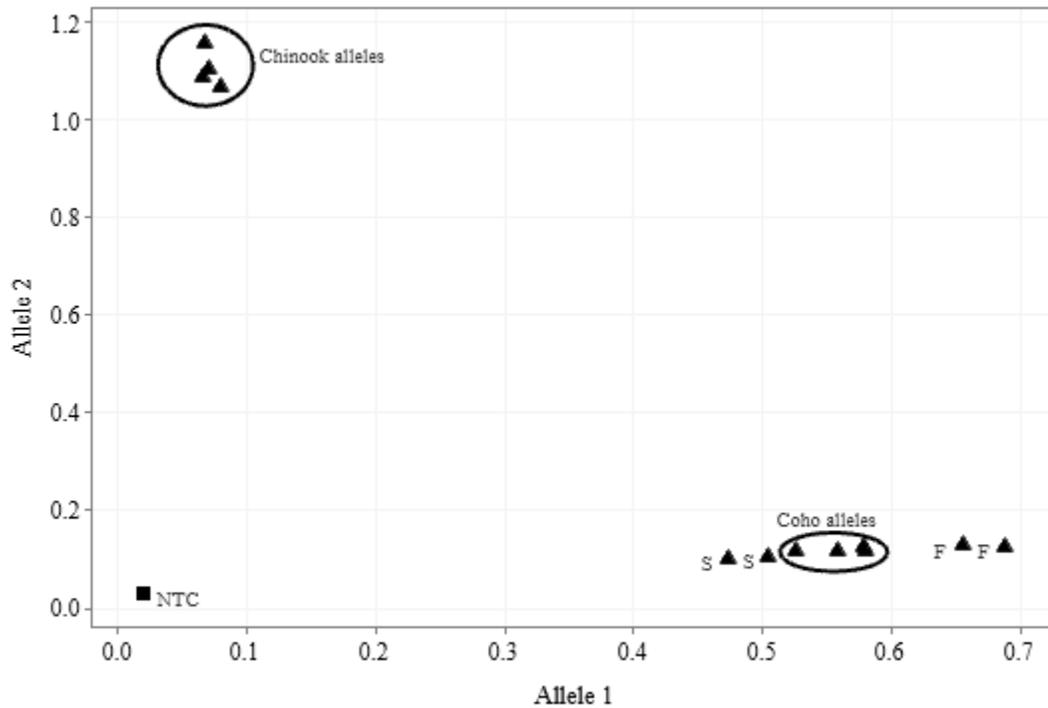


Figure 4.— All unknown species samples (labeled; S=swab, F=fin) were homozygous for Coho Salmon alleles at the OKI1-OKI locus. The square in the bottom left corner is a No Template Control (NTC.)

Aquatic habitat measurements

Birch Creek trap sites 1 and 10 were sampled for habitat characteristics on July 13, 2016 (Table 3 and 6; Figure 5). Birch Creek is a 2nd order stream and the gradient of the sampled portion is 0.07%. Birch Creek is a single channel stream dominated by a pool flow regime with a heavily vegetated riparian zone, 10% aquatic vegetation rating, and a Rosgen classification of F5. The mean water temperature at sample sites 1 and 10 were 16.6 and 17.0°C respectively. Birch Creek’s humic stained water prevented stream substrate observation.

Fish Creek trap sites 1 and 10 were sampled for habitat characteristics on July 12, 2016 (Table 3 and 6; Figure 5). Fish Creek is a 2nd order stream and the gradient of the sampled portion is 0.05%. Fish Creek is a single channel stream dominated by a pool flow regime with a heavily vegetated riparian zone, 50% aquatic vegetation rating, and a Rosgen classification of F5. Fish Creek’s dominant substrate was detritus (Table 6).

Patterson Creek trap sites 1 and 10 were sampled for habitat characteristics on July 12, 2016 (Table 3 and 6; Figure 5). Patterson Creek is a 2nd order stream and the gradient of the sampled portion is 0.17%. Patterson Creek is a single channel stream dominated by a pool flow regime with a heavily vegetated riparian zone, 75% aquatic vegetation rating, and a Rosgen classification of F5. The mean water temperature at sample sites 1 and 10 were 14.7 and 15.7°C respectively. Patterson Creek’s dominant stream substrate was detritus.

Unnamed_1 Creek trap sites 1 and 10 were sampled for habitat characteristics on July 11, 2016 (Table 3 and 6; Figure 5). Unnamed_1 Creek is a 2nd order stream and the gradient of the sampled portion is 1.20%. Unnamed_1 Creek is an incised (approximately 14 m from the stream surface to bank top) single channel stream dominated by a step pool flow regime with a

vegetated riparian zone and a Rosgen classification of G6c. The mean water temperature at sample site 1 was 9.4°C. Unnamed_1 Creek's dominant stream substrate at trap site 10 was silt. There was a 10% and 20% woody debris rating at trap sites 1 and 10. The water color at trap site 10 was humic but visibility was good.

McCloud Ranch Creek trap site 1 was sampled for habitat characteristics on July 10, 2016 (Table 3 and 6; Figure 5). McCloud Ranch Creek is a 1st order stream and the gradient of the sampled portion is 7.40%. McCloud Ranch Creek is a moderately incised single channel stream dominated by a step pool flow regime and a Rosgen classification of A5. The mean water temperature at sample site 1 was 5.0°C using a handheld thermometer. McCloud Ranch's dominant stream substrate at trap site 1 was sand. There was a 20% woody debris rating at trap site 1.

Baker Creek trap site 10 was sampled for habitat characteristics on July 9, 2016 (Table 3 and 6; Figure 5). Baker Creek is a 3rd order stream and the gradient of the sampled portion is 0.10%. Baker Creek is a single channel stream dominated by a pool flow regime with a heavily vegetated riparian zone and a Rosgen classification of C5c. The mean water temperature at trap site 10 was 14.0°C using an uncalibrated handheld thermometer. Baker Creek's humic stained water prevented stream substrate observation.

Unnamed_9 Creek trap sites 1 and 10 were sampled for habitat characteristics on July 9, 2016 (Table 3 and 6; Figure 5). Unnamed_9 Creek is a 2nd order stream and the gradient of the sampled portion is 1.10%. Unnamed_9 Creek is an incised single channel stream dominated by a step pool flow regime with a vegetated riparian zone and a Rosgen classification of E3. The mean water temperature at sample site 1 was 9.9°C. Unnamed_9 Creek's dominant stream substrate at trap site 1 was silt and trap site 10 was sand. There was a 10% and 20% woody debris rating at trap sites 1 and 10. The water color was humic but water visibility was good at both trap sites.

Unnamed_12 Creek trap sites 1 and 10 were sampled for habitat characteristics on July 7, 2016 (Table 3 and 6; Figure 5). Unnamed_12 Creek is a 2nd order stream and the gradient of the sampled portion is 0.03%. Unnamed_12 is a single channel stream dominated by a pool flow regime with a heavily vegetated riparian zone, 10% aquatic vegetation rating, and a Rosgen classification of C5. The mean water temperature at sample site 1 was 19.0°C. Unnamed_12's glacial influenced water and depth prevented stream substrate observation.

Table 6.— Habitat characteristics for sampled tributaries in the lower Tanana River, July 7–13, 2016. Table acronym: MNT=measurement not taken. Stream abbreviations: BC- Birch Creek, FC Fish Creek, PC - Patterson Creek, U1C - Unnamed_1 Creek, MCR - McCloud Ranch Creek, AC - Baker Creek, U9C - Unnamed_9 Creek, and U12C - Unnamed_12 Creek.

Stream - Date	Sample site	Stream stage	Water color	Water temp. (°C) (SD)	Wetted width (m)	Estimated Drainage size (ha)	Dominant substrate	% Woody debris	% Aquatic vegetation
BC- 7/13	1	medium	humic	16.6 (0.01)	19	29,800	MNT	0	10
BC-7/13	10	medium	humic	17.0 (0.01)	14		MNT	0	MNT
FC- 7/12	1	medium	humic	MNT	45	56,400	detritus	25	50
FC -7/12	10	medium	humic	MNT	42		detritus	25	50
PC- 7/12	1	medium	humic	14.7 (0.01)	80	35,400	detritus	5	75
PC- 7/12	10	medium	humic	15.7 (0.08)	33		detritus	5	80
U1C- 7/11	1	medium	humic	9.4 (0.01)	6	3,300	MNT	0	0
U1C- 7/11	10	medium	humic	MNT	2.5		silt	15	0
MRC- 7/10	1	medium	clear	5.0 ^a	1.4	560	sand	20	0
AC- 7/9	10	high	humic	14.0 ^a	48	149,400	MNT	2	MNT
U9C- 7/9	1	medium	humic	9.9 (0.01)	5	2,400	silt	10	0
U9C- 7/9	10	medium	humic	MNT	2		sand	20	0
U12C-7/7	1	high	glacial	19.0 (0.01)	30	4,600	MNT	5	5
U12C-7/7	10	high	clear	MNT	29		MNT	5	5

^a uncalibrated handheld thermometer



Figure 5.— Birch, Fish, Patterson, and Unnamed 1 creeks that were sampled for juvenile salmon.



Figure 5 continued.— McCloud Ranch, Baker, Unnamed 9, and Unnamed 12 creeks that were sampled for juvenile salmon.

Discussion

Overview

We explored 19 Tanana River nonnatal tributaries for suitable rearing habitat and presence of juvenile Chinook Salmon. Of the explored streams, eight were sampled using baited minnow traps. Although no juvenile Chinook Salmon were documented during this survey, we captured two Coho Salmon in Unnamed_9 Creek. Prior to this study, unnamed_9 Creek was not nominated in the AWC for any salmon life stage, so the captured fish likely migrated into the stream. Overall, Unnamed_9 and Unnamed_1 creeks were the only streams where fish were detected including juvenile Longnose Sucker and Northern Pike. Unnamed_9 Creek sampling resulted in an approved AWC nomination (number 21-007) for Coho Salmon rearing and added a new stream to the catalog.

Stream selection

Sampled Tanana River tributaries generally lacked characteristics associated with optimal juvenile Chinook Salmon rearing habitat. The combination of geomorphic and hydrologic factors in sampled Tanana River tributaries likely render habitat inhospitable to juvenile salmon (Platts 1979; Daum and Flannery 2011; Bidlack et al. 2014; Huntsman and Falke 2018). Future sampling in lower Tanana River tributaries should concentrate on upstream habitat that aligns with greater probability of juvenile salmonid occurrence. For example, Daum and Flannery (2011) documented juvenile Chinook Salmon in several Yukon River nonnatal tributaries; however, they avoided sampling streams with intermittent access to the Yukon River main stem and streams having a high potential for fish predators occupying the lower reaches, i.e., low gradient, deep and turbid water, and low water velocity. A similar strategy applied to lower Tanana River tributaries may elucidate juvenile Chinook Salmon distribution and their use of nonnatal stream habitat. However, the availability of lower Tanana River nonnatal tributary streams similar to those selected and sampled by Daum and Flannery (2011) are lacking. This study's exploration and sampling of nonnatal tributaries was not exhaustive and did not discover additional prospective streams to sample.

Fish sampling

Our results suggest that sampled off-channel habitats between the confluence of the Chena and Tanana rivers and the mouth of the Tanana River may not provide suitable rearing habitat for juvenile Chinook Salmon. Chinook Salmon smolts migrate from the Chena River from approximately mid-April through mid-June (Williamson 1984; Peterson 1997) and are typically age-1 (Skaugstad 1990; 1993; Eaton 2016). July sample timing for this study may have missed migrant Chinook Salmon using nonnatal tributary habitats. They also migrate from other natal streams such as the Salcha River and are present in the Tanana River for an unknown time period and may bypass tributary streams unless adequate habitat exists within proximity to the mainstem Tanana River. Most Yukon River Chinook Salmon stocks have a stream-type life history (Healy 1991) and thus expected to remain within their natal stream until smolting.

Our study did not identify rearing habitats for juvenile Chinook Salmon, however, it highlighted that these areas might serve as important rearing habitats for other Pacific salmon and resident fish species. Like Chinook Salmon, Coho Salmon fry emerge in the spring and spend one to two years in freshwater before migrating to saltwater to mature, with most Coho Salmon returning to spawn at age three (Eaton 2016). Capture of rearing Coho Salmon in Unnamed_9 Creek is

consistent with Hander and Legere (2012) and Gerken and Sethi (2013) in respect to using tributary environments for rearing during July. Similarly, Ott et al. (1998), Hemming and Morris (1999), Durst (2001), and Hander and Legere (2012) found juvenile Coho Salmon in the Tanana River and tributaries with length distributions that encompassed the size fish found in Unnamed_9 Creek but those catches mostly occurred in May and June. The aforementioned studies were also focused on varying fish habitat characteristics and documenting anadromous waters, not specifically on sampling nonnatal streams for the presence of juvenile Chinook Salmon.

Subsistence, commercial, and recreational harvest of Coho Salmon occur throughout the Yukon River drainage but there is little baseline information about their distribution. As juveniles, Coho Salmon are migratory and little is known about their use of nonnatal streams for rearing in the Yukon River drainage. In 2022, a collaborative Yukon River Coho Salmon radio-telemetry study led by the Alaska Department of Fish and Game and the U.S. Fish and Wildlife Service will begin to investigate spawner run timing and distribution. Information from that study may offer direction to discover additional juvenile Coho Salmon rearing habitat.

Genetic analysis

It is important to accurately identify species for nomination to the AWC and similar applications. Non-lethal anal fin clips and mucus swabs were used to obtain paired genetic samples from individual fish. Extracted DNA concentrations were greater from anal fin tissue than the mucus swab samples. Both anal fin clips and mucus swabs paired sample's SNP analysis was successful in discriminating between positively identified Chinook and Coho Salmon references samples. Genetic analysis can accurately discriminate between Pacific salmon species in a short time frame relative to more labor-intensive methods such as internal meristic counts (Hander and Legere 2012).

Habitat

All explored Tanana River tributaries lie in the broad depression of the Tanana-Kuskokwim Lowland and the entire area consists of permafrost (Brabets et al. 2000). All sampled streams with temperature measurements except for Unnamed_12 Creek, were within the range for habitation for rearing Chinook Salmon (Table 6; USEPA 2003). The mean drainage size of sampled Tanana River tributaries (Table 6) was not significantly different than that described by Daum and Flannery (2011; $T = 0.032$; $p = 0.376$). Stream order was significantly higher in the Yukon River tributaries ($n = 8$; $W = 95.5$, $p = 0.002$) than in sampled Tanana River tributaries. Platts (1979) found that stream order values of 3, 4, and 5 provided spawning and rearing areas for Chinook Salmon and Rainbow Trout *O. mykiss* and are commensurate with Daum and Flannery's (2011) stream order values. Their stream's substrate composition observations varied from more favorable sand and gravel to large cobble, which contrasts with predominantly detritus, silt, and sand observed in sampled Tanana River tributaries. Ultimately, the general structural complexity of the sampled areas of Tanana River tributaries was not conducive to rearing Chinook Salmon (Scrivener and Andersen 1982).

Conclusion

Sampled lower Tanana River tributaries generally lacked habitat quality adequate for rearing Chinook Salmon. Our sampling was limited to a single sampling event that occurred in the furthest lower reaches of some streams. Tributaries such as Fish and Baker creeks drain areas

where adequate rearing habitat in their upper reaches may occur and warrant investigation. Placer mining in the upper reaches of Fish and Baker creeks may have caused some habitat degradation. However, the lower reaches of these streams may be inhospitable for rearing salmon, e.g., lack of prey and an abundance of predators, to migrate through to potentially optimal upper stream-reach habitats. Additionally, temporal sampling during the open water season may increase the opportunity to capture fish that have varying life history strategies. More extensive sampling nearer the headwaters of the non-wadable streams may offer better quality salmonid rearing habitat. Wadable streams were suboptimal Chinook Salmon rearing habitat because the combination of necessary habitat characteristics was inadequate.

Future studies requiring accurate species identification could lessen impacts on fish resources by using non-lethal genetic sampling and analysis methods. Especially fish resources such as Yukon River Chinook Salmon that are currently experiencing sustained years of poor production.

Acknowledgements

The Northern Alaska Fish and Wildlife Field Office provided funding and administrative support and the U.S. Fish and Wildlife Service, Conservation Genetics Laboratory provided genetic species discrimination analysis for this project. Sincere appreciation is extended to those who contributed to this project: K. Sellmer for technical field assistance and field logistics, K. Drew for manuscript comments and map construction, and R.J. Brown and J. Wenburg for manuscript comments and suggestions.

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Non-lethal Juvenile Finfish OmniSwab Sampling for DNA Analysis

ADF&G Gene Conservation Lab, Anchorage

I. General Information

We use the mucus samples from juvenile fish using OmniSwab to determine the genetic characteristics and profile of a particular run or stock of fish. The most important thing to remember in collecting sample 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 fish.

II. Sampling Method



Figure 1



Figure 2

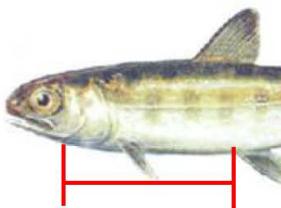


Figure 3

III. Supplies included with sampling kits:

1. OmniSwab – plastic applicator swab for collecting mucus from fish.
2. 2.0ml vials – pre-labeled individual vial and cap for sample storage.
3. Silica beads – vial pre-filled ½ silica beads/capped prior to sampling.
4. White boxes – storage for individual capped vials with silica beads.
5. Hinged plastic box – used while sampling, protects vials from rain.
6. Sampling instructions.

IV. Shipping: No special paperwork required for return shipment of these samples.

Return to ADF&G Anchorage lab: ADF&G – Genetics 333 Raspberry Road	Lab staff: 907-267-2247 Judy Berger: 907-267-2175 Anchorage, AK 99518
Freight code: _____	

Steps for taking mucus samples in 2.0ml vials:

- Organize work area prior to sampling.
- Hinged plastic box will hold up to 50 silica pre-filled vials. Works best with 40 vials or less so hinged lid can close easily between sampling events.
- Lift lid on white box, should be marker line upper left edge of box bottom; starting vial #1,2,3... left to right.
- Load plastic box with vial #s 1,2,3...in **consecutive** order. **All vials remain capped until sampling each fish**. Do not uncap vials ahead of time since silica will begin absorbing moisture. Want to minimize exposure time to moisture.
- Cover work area (cooler, tarp, rain coat, backpack, under tree) to protect samples from rain and/or direct sunlight.
- Wipe right hand dry before opening each OmniSwab to reduce excess water dripping on swab pad applicator.
- Dry hands, open OmniSwab by peeling package open at the handle end of swab and remove carefully.
- Pick up one fish and hold in palm of left hand with belly side up (Figure 1).
- **Do not touch swab pad applicator (Figure 2).**
- Sample location on fish is located between lower jaw and front of pelvic fin (Figure 3).
- Hold OmniSwab handle in right hand, **gently** rub the swab pad serrated edge against preferred area (Figure 3 and below):
 - Rub swab pad back/forth 8-10 times (back/forth=1 time).
 - **Very important to complete total 10 swab cycles on fish!**
- Be careful not to depress ejector tip while swabbing fish.
- Once sampling is complete, release fish back to the local stream or waterway.
- **Uncap vial** with dry hand after sample is taken. Tilt vial on slight angle making room for swab pad in silica beads and eject swab pad (using release button at tip) into one vial. Cap and swiftly shake capped vial to distribute silica beads around applicator pad to enhance drying process.
- **Place only one swab pad per vial!**
- Record metadata (vial #, date, location, lat/long, etc...) electronic copy preferred.
- Place each individual vial back into white storage box, working from vial #s 1,2,3...100 consecutively until the entire box of 100 vials are full.
- Swab pads will slowly dry inside capped vials and be dry by the end of the day.
- In field: store vial collection at room temperature away from heat and/or place in **dry** cooler or tote.
- In lab: Store in -20* freezer (lid on).

Appendix Figure A.1.—Non-lethal sampling method for juvenile finfish using a buccal swab technique developed by the Alaska Department of Fish and Game, Gene Conservation Laboratory.



Appendix Figure A.2.—Juvenile Coho Salmon captured in Unnamed_9 Creek in the lower Tanana River. The top image is the 55 mm fish captured in trap number 7 and the lower is the 64 mm fish captured in trap number 8.