Technical Report No. 18-04

Aquatic Biomonitoring at the Arctic-Bornite Prospect, 2017

by Parker T. Bradley

March 2018

Alaska Department of Fish and Game **Division of Habitat**

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)

Measures (fisheries)

fork length	FL
mideye-to-fork	MEF
mideye-to-tail-fork	METF
standard length	SL
total length	TL

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

TECHNICAL REPORT NO. 18-04

AQUATIC BIOMONITORING AT THE ARCTIC-BORNITE PROSPECT, 2017

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Cover: Dolly Varden from Subarctic Creek, upper site, September 20, 2017. Photograph by Parker T. Bradley

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Introduction

The Ambler mining district is located in northwest Alaska in the Kobuk River drainage along the southern end of the Brooks Range (Figure 1). There are two primary deposits currently being explored by Trilogy Metals (formally NovaCopper). The Bornite deposit is located approximately 17 km north of Kobuk, and the Arctic deposit is located approximately 37 km northeast of Kobuk. The Bornite deposit contains primarily copper while the Arctic deposit contains copper, lead, zinc, silver and gold. The Bornite deposit occurs in the drainage of Ruby Creek while the Arctic deposit occurs in the upper end of Subarctic Creek. Both Ruby and Subarctic creeks are tributaries to the Shungnak River, which flows into the Kobuk River. A large waterfall in the lower Shungnak River is believed to prevent upstream passage of fish into the area so no anadromous fish occur near the sites (Figure 2). All fish occurring in the area complete their life cycle within the Shungnak River drainage.

All sample sites, except one, occurred in the Shungnak River drainage. Riley Creek, which flows into the Kogoluktuk River, was selected to sample as it is being considered as an option for a tailings facility.

Figure 1. Location of the Arctic and Bornite deposits in northwest Alaska.

Figure 2. Waterfall on the Shungnak River blocking fish passage upstream, July 21, 2016.

Aquatic baseline work conducted in the area in 2010 focused on macroinvertebrate and fish species present (Tetra Tech, 2011). The fish species documented in the 2010 survey were Arctic grayling (*Thymallus arcticus*), round whitefish (*Prosopium cylindraceum*), slimy sculpin (*Cottus cognatus*), and Dolly Varden (*Salvelinus malma*). Trilogy Metals contracted the ADF&G Division of Habitat to continue the aquatic sampling beginning in 2016. The ADF&G study plan was based on aquatic biomonitoring the Division of Habitat routinely conducts at various large hard rock mines in the state. Three primary types of data were collected: periphyton, aquatic invertebrates, and fish which included samples for whole body element analyses and genetic analysis.

This report will summarize the samples that were collected by ADF&G in 2017, as well as water quality data that were collected by Trilogy Metals in 2017, and the genetic analysis conducted by USFWS Conservation Genetics Laboratory.

Methods

Sampling Overview

Based on results from 2016, some sample sites were moved in 2017, some new sites were added, and two additional sampling events occurred (Figure 3; Table 1).

The first sampling event occurred June 14–15, 2017. The objective was to capture Dolly Varden fry using aquatic invertebrate drift nets to determine where spawning occurred.

The second sampling event occurred July 17–22, 2017. At each location replicate samples of the aquatic community were performed; including aquatic invertebrates, periphyton, and fish.

The third sampling event occurred September 19–21, 2017. The objective was to determine Dolly Varden distribution, determine their spawning condition, and obtain additional fish for element analysis. An aerial survey was also conducted on the Kogoluktuk River from Riley Creek to the Kobuk River to look for anadromous whitefish, chum salmon, and Dolly Varden.

A majority of the effort was located in Ruby and Subarctic creeks as they are locations where there may be changes to the system once mining begins. The reference site was sampled again in 2017, and is located in the upper Shungnak River. The Riley Creek sample site was moved further downstream in 2017 to increase likelihood of capturing fish. Additionally, a new sample site was added in Jay Creek should mining activities occur in that drainage.

The objectives of the biological monitoring program were to document in-situ productivity of aquatic communities downstream of potential project facilities.

Water Quality

Trilogy Metals has collected water quality data from many locations throughout the Arctic-Bornite Prospect project area. The prior report summarized all water quality data collected since 2008 (Bradley 2017a). This report summarizes only data collected in 2017. These data were provided to ADF&G and were compiled and graphed showing median, minimum, and maximum values (Appendix 1). Only water quality data from locations in close proximity to the 2017 sample sites were used. Depending on the sample site, three to five water samples were collected from April to December 2017.

Periphyton

Periphyton, or attached micro-algae, are sensitive to changes in water quality and are often used in monitoring studies to detect early changes in aquatic communities (Ott et al. 2010). The presence of periphyton in a stream system is evidence of in-situ productivity (Ott et al. 2010). Periphyton samples were collected at seven locations around the Arctic Bornite area (Table 1; Figure 3).

Ten flat rocks, each larger than 25 cm^2 were collected from submerged areas at each site. A 5-cm x 5-cm square of high density flexible foam was placed on the rock. All the material around the foam was scrubbed off with a toothbrush. The foam square was then removed from the rock, and that section of the rock was brushed onto a 0.45 µm glass fiber filter receptacle attached to a hand vacuum pump. Material from the toothbrush was also rinsed onto the filter. The water was extracted from the periphyton using a hand vacuum pump. Just before all the water was pumped through the filter, one to two drops of magnesium carbonate ($MgCO_3$) was added to the water which prevented acidification and additional conversion of chlorophyll-a to phaeophytin.

Filters from each rock were folded in half, with the sample material on the inside, and placed in individual dry coffee filters. All ten coffee filters were placed in a zip-lock bag containing desiccant to absorb any remaining moisture. The bags were then wrapped in aluminum foil to prevent light from reaching the samples, placed in a cooler with ice packs, then transferred to a freezer at the Arctic Bornite camp. Samples were kept frozen until they were analyzed at the ADF&G laboratory in Fairbanks. Additional details regarding periphyton sampling and analysis methods can be found in ADF&G Technical Report No. 17-09 (Bradley 2017b).

Table 1. Arctic-Bornite sampling locations (WGS 84), 2017.

Water Body/ Sample Site	Latitude	Longitude	June larval sampling	July periphyton/ invertebrate/ minnow traps	July Minnow traps	July Fyke nets	September minnow traps
Shungnak R.							
USR	67.24337	-156.61642	---	X	---	---	---
Subarctic Cr.							
SAC 1	67.16942	-156.62933	---	---	---	X	---
SAC 2	67.17115	-156.61711	X	X	---	---	X
SAC 3	67.15987	-156.56725	X	---	X	---	X
SAC 4	67.16383	-156.43503	X	---	X	---	X
SAC 5	67.19272	-156.39093	X	X	---	---	X
Ruby Cr.							
RBC 1	67.11395	-156.91669	---	---	---	X	---
RBC 2	67.10038	-156.92242	X	X	---	---	---
RBC 3	67.06826	-156.94514	---	---	X	---	X
RBC 4	67.04689	-156.93333	---	---	X	---	---
RBC 5	67.04247	-156.93744	X	X	---	---	---
Jay Cr.							
JC	67.08035	-156.94437	---	X	---	---	X
Riley Cr.							
RIC	67.04265	-156.69231	X	X	---	---	X

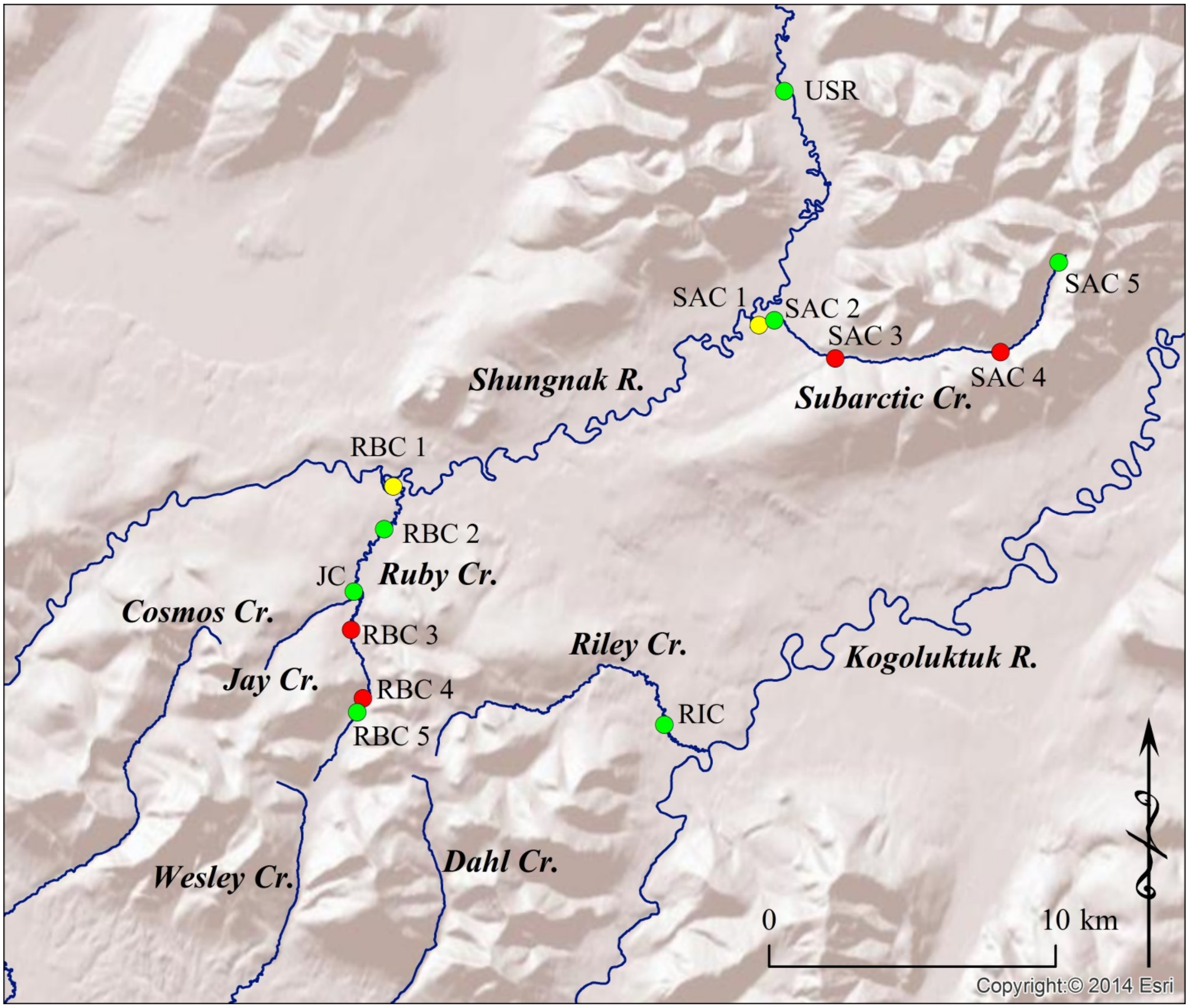

Figure 3. Periphyton, aquatic invertebrate, and minnow trap sample sites (green), fyke net sample sites (yellow), and minnow trap sample sites (red), 2017.

Aquatic invertebrates

At each sample site, five drift nets were installed along a transect perpendicular to the flow (Table 1; Figure 4). The drift nets were 45.7 cm wide, 30.5 cm tall with 363 µm mesh size. At each net, water depth and water velocity were measured using a Marsh McBirney FH950 flow meter. By using these parameters the volume of water sampled by each net could be calculated. After one hour, the nets were removed and material was flushed into the cod end by splashing water on the outside of the net. The cod end contents were then removed and placed in individual pre-labeled Nalgene bottles. Denatured ethyl alcohol was added to each Nalgene to preserve the samples. Samples were sorted and invertebrates identified to the lowest taxonomic level, typically family or genus by a private aquatic invertebrate lab in Fairbanks. Because invertebrates belonging to the orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and

Trichoptera (caddisflies) (EPT) are sensitive to differences in water quality, percent composition of EPT's were calculated and compared to groups of other invertebrates, which are less sensitive.

Figure 4. Drift nets set for capturing aquatic invertebrates in Subarctic Creek (SAC 2) July 18, 2017.

Fish

From June 14-15, larval fish sampling occurred at a total of seven sites in Subarctic Creek, Ruby Creek, and Riley Creek (Table 1). Five drift nets were installed along a transect perpendicular to the stream flow (identical to the aquatic invertebrate methods). The substrate was disturbed by kicking from about 20 meters upstream of the net, down towards the net opening. The nets were immediately pulled and put on the shoreline. The contents from each net were put in a sieve and material was sifted through in an attempt to find larval fish.

The primary fish sampling event occurred July 17–22. At each sample site, after the periphyton and aquatic invertebrates were sampled, ten minnow traps were baited with cured salmon roe in a perforated plastic bag and placed upstream and downstream of the periphyton and aquatic insect sampling locations (Table 1). Traps were placed in a variety of habitats, including cut banks, pools, and near submerged woody debris. Traps were allowed to soak overnight and checked about 24 hours later. All captured fish were measured for fork or total length. Some fish were retained for whole body element analyses. Those fish were handled wearing class 100 nitrile gloves, and placed in individual pre-labeled plastic zip-lock bags. The fish were placed in a cooler with ice packs and transferred to a freezer in the camp. The samples remained frozen until they were to be analyzed by ACZ Laboratories. Unfortunately, in transit to the lab, the carrier lost track of the cooler containing fish for six days and they became unsuitable for element analysis. They were eventually returned to Fairbanks where otoliths were removed for age analysis. Additionally, fin clips were taken from 50 Dolly Varden in Subarctic Creek for genetic analysis. Fin clips were stored in individually labelled 1.2ml cryovials filled with silica desiccant. Samples were sent to the USFWS Conservation Genetics Laboratory, assayed for genetic variation, and compared to anadromous Dolly Varden from the Kobuk River (Appendix 4).

In addition to the minnow traps, fyke nets were set in lower Ruby Creek and lower Subarctic Creek during the July sampling event (Table 1, Figure 5). Two nets were set at each location in such a way to catch fish moving both upstream and downstream. Nets were allowed to sit for about 24 hours.

The fall sampling event occurred September 19–21. A total of seven sites were sampled for fish with minnow traps (Table 1). Similar handling methods were used for fish being retained for whole body element analysis. Additionally, some fish were retained for dissection to determine spawning condition. An aerial survey was also flown on the Kogoluktuk River September 21 from Riley Creek to the mouth at the Kobuk River.

Figure 5. Fyke nets in lower Subarctic Creek (SAC 1) July 21, 2017.

Results and Discussion

Water Quality

A summary of sample dates and water quality results are shown in Appendix 1. Alaska Department of Environmental Conservation (ADEC) water quality standards are presented for some metals for both acute (24 hr) and chronic (one month) aquatic life exposure limits (Appendix 1). Most of the water quality sites occur at the same sites as the periphyton, aquatic invertebrate, and fish sampling occur. However, the water quality data from the Shungnak River used in these results were collected near the mouth of Subarctic Creek, not at the reference site further upstream (USR).

In general, median cadmium values in 2017 were low and at or below the detection value of 0.015 µg/L in upper Ruby Creek and lower Riley Creek (Figure 6). The highest median values occurred in the Shungnak River. Acute and chronic water quality standards for aquatic life for cadmium depends on water hardness. Based on water hardness values, cadmium concentrations were below the acute and chronic standards at all sites for each sampling event, except for one sample in lower Subarctic Creek in July (Appendix 1).

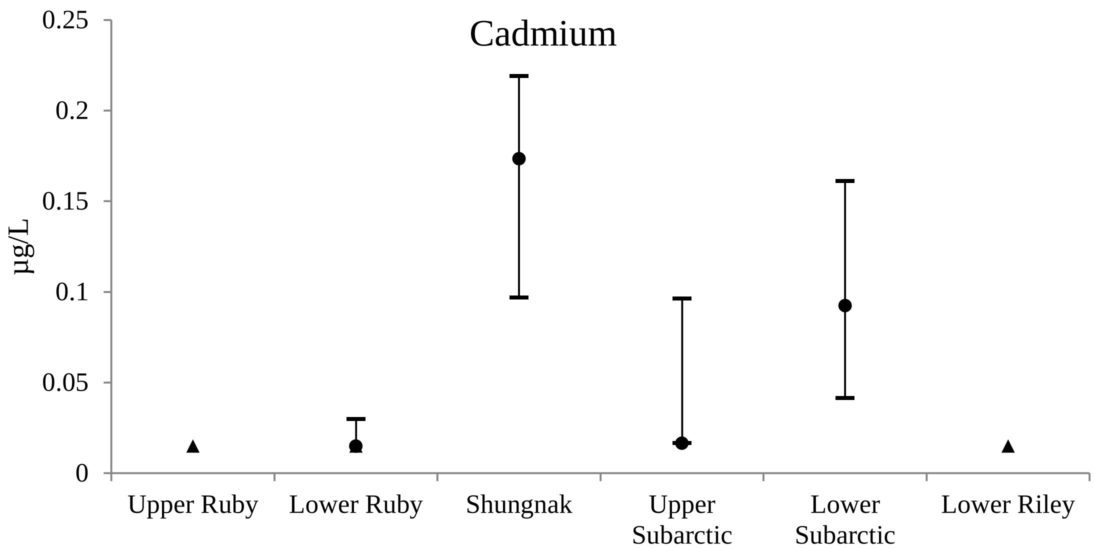

Figure 6. Median, minimum, and maximum cadmium concentrations at sample sites in 2017. Dots represent median values and triangles represent value at or below the detection limit.

Median selenium values were very low and similar among all sample sites (Figure 7, Appendix 1). Some locations had minimum values at or below the detection limit of 0.31 µg/L. All values were well below the current water quality standard for aquatic life which is 20 µg/L for acute and 5 µg/L for chronic.

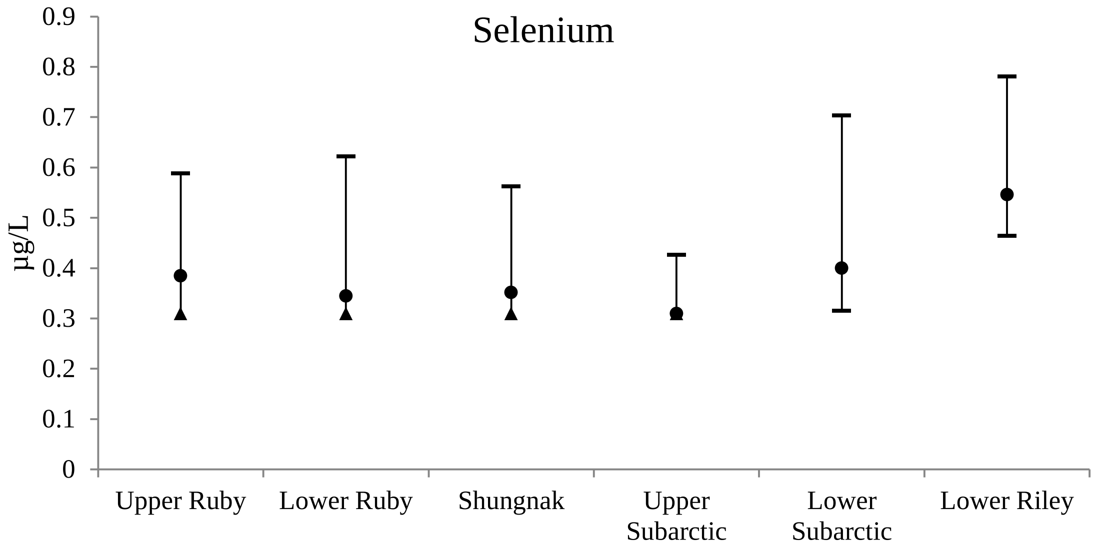

Figure 7. Median, minimum, and maximum selenium concentrations at sample sites in 2017. Dots represent median values and triangles represent value at or below the detection limit.

Median copper values were relatively similar among all sites and ranged from 0.263 μg/L at upper Subarctic Creek to 1.175 μg/L at lower Subarctic Creek (Figure 8, Appendix 1). The highest maximum value for copper was 3.220 μg/L and occurred in lower Subarctic Creek in July. Acute and chronic water quality standards for aquatic life for copper depend on water hardness. Based on water hardness values, copper concentrations were below the acute and chronic standards at all sites for each sampling event (Appendix 1).

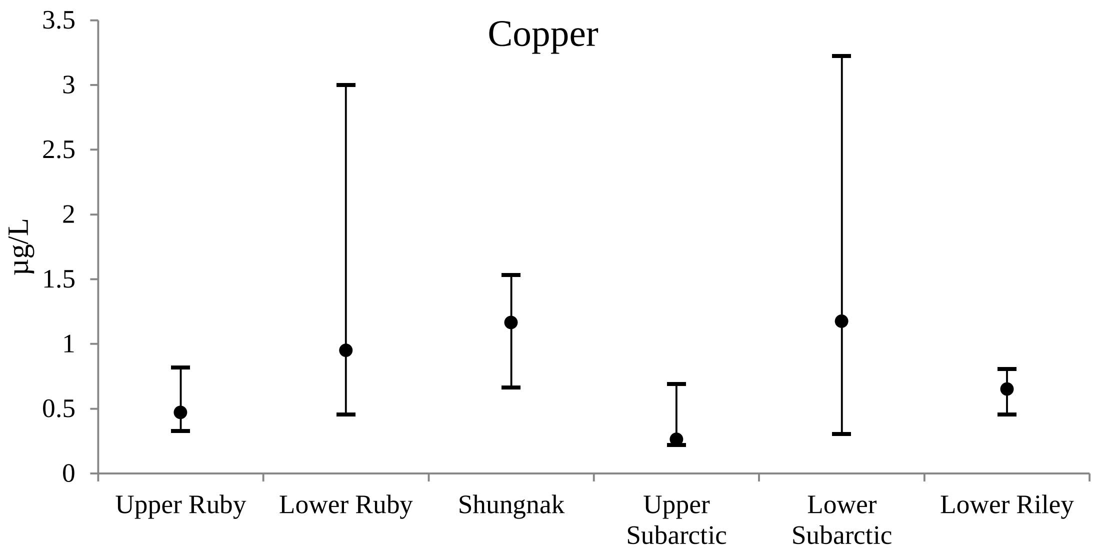

Figure 8. Median, minimum, and maximum copper concentrations at sample sites in 2017. Dots represent median values.

Mercury values were low at all sample sites (Figure 9). Median mercury values ranged from 0.5 ng/L at upper Ruby Creek, upper Subarctic Creek, and the Shungnak River to 0.744 ng/L at lower Ruby Creek. The highest maximum value (2.25 ng/L) occurred at lower Ruby Creek. All mercury values were well below the water quality standards for aquatic life for mercury which are 2,400 ng/L for acute and 12 ng/L for chronic.

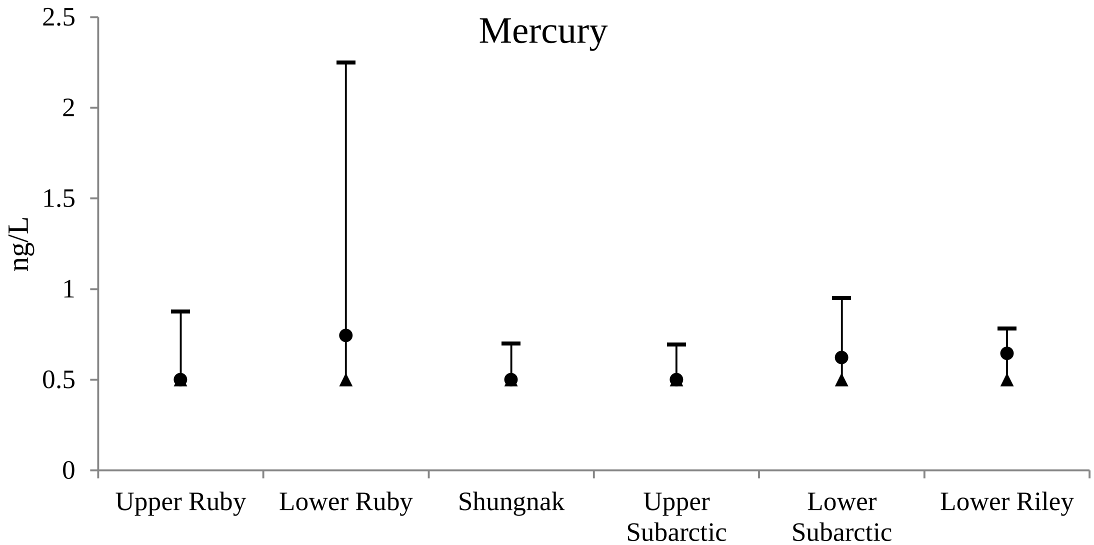

Figure 9. Median, minimum, and maximum mercury concentrations at sample sites in 2017. Dots represent median values, triangles indicate value at or below the detection limit.

Median zinc concentrations were lowest in lower Riley Creek (1.15 µg/L) and highest in lower Subarctic Creek (13.4 µg/L) (Figure 10). Overall, zinc concentrations were very low and well below the water quality standard for aquatic life, which depends on water hardness (Appendix 1).

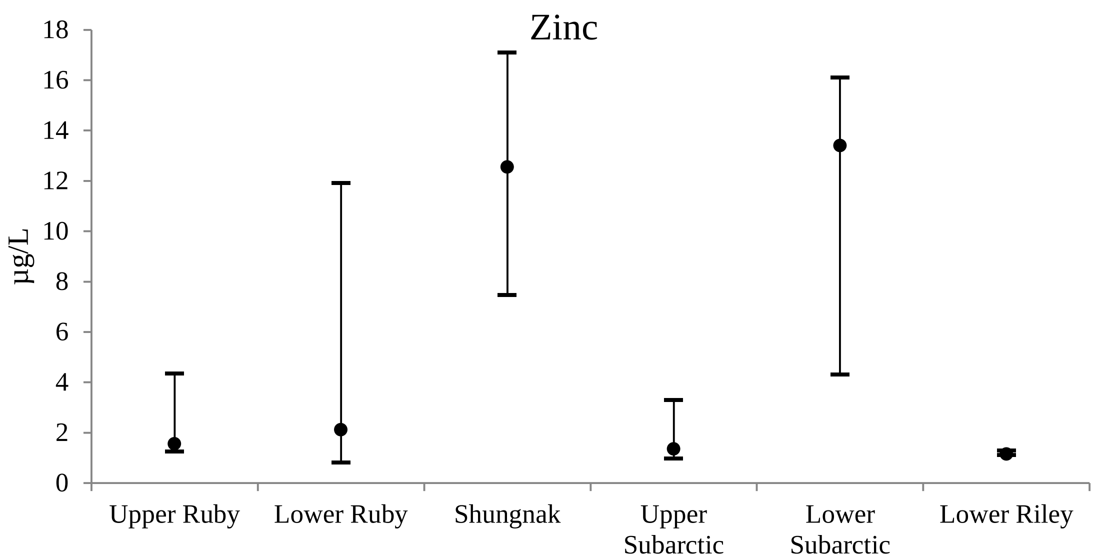

Figure 10. Median, minimum, and maximum zinc concentrations at sample sites in 2017. Dots represent median values.

Total Dissolved Solids (TDS) followed a similar pattern in 2017 with historic data. Lowest median values occurred in upper Subarctic Creek (55 mg/L) and lower Subarctic Creek (53 mg/L) and highest values occurred in upper Ruby Creek (163 mg/L) (Figure 11, Appendix 1).

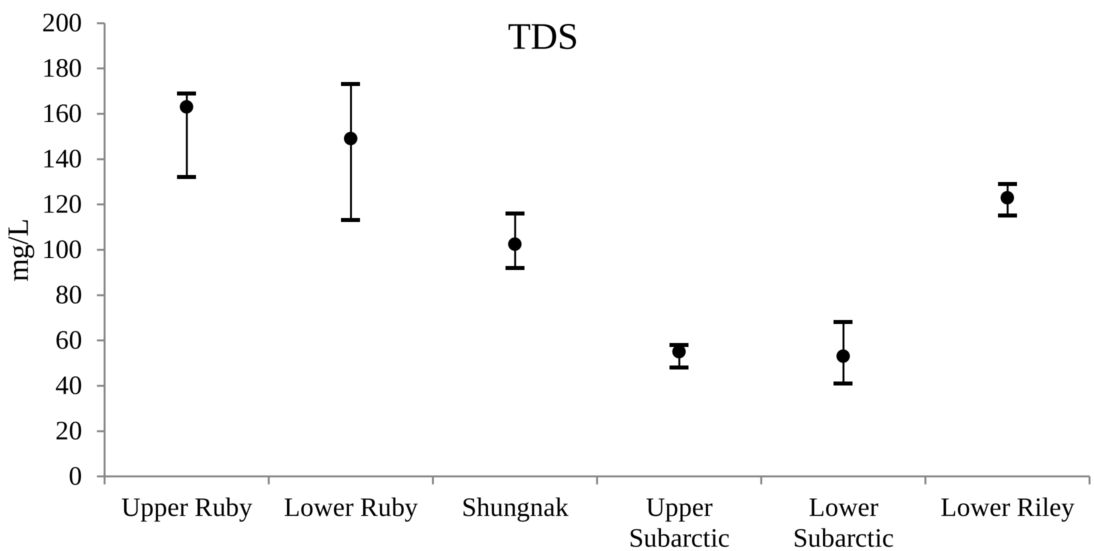

Figure 11. Median, minimum, and maximum TDS concentrations at sample sites in 2017. Dots represent median values.

Periphyton

Mean chlorophyll-a concentrations were highest in upper Ruby Creek (10.5 mg/m^2) and lowest in lower Subarctic Creek (SAC 2) (1.0 mg/m^2) (Figure 12). The mean chlorophyll-a concentrations at the remaining sites ranged from 1.4 mg/m^2 to 9.5 mg/m^2. These results are similar to those found in 2016 (Bradley 2017a).

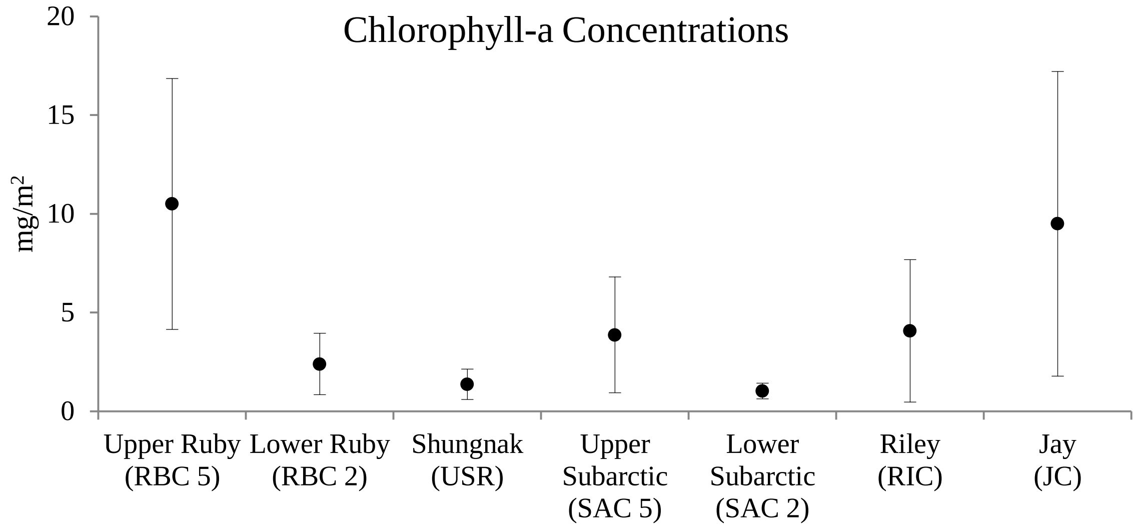

Figure 12. Mean chlorophyll-a concentrations (± 1 SD), July 2017.

Aquatic Invertebrates

The upper Ruby Creek sample site was moved about 0.5 km upstream from the 2016 location (RBC 4) to a more suitable sample site (RBC 5). Upper Ruby Creek is characterized by beaver pond habitats, deep water, dense vegetative cover, short channels between beaver dams, and minimal gravel/cobble. The new sample site for 2017 was located in a channel between beaver dams and consisted of gravel/cobble. Densities in upper Ruby Creek were the highest among the sample sites and averaged 24.6 aquatic invertebrates/m^3 of water (Figure 13). A total of 26 taxa were identified in the upper Ruby Creek samples. Samples were dominated by other invertebrates (56%), which were primarily Ostracods and Cladocerans, followed by aquatic diptera (33%) which were primarily chironomids, and EPT species (11%) (Table 2, Figure 14).

Lower Ruby Creek where sampling took place was characterized by pool/riffle habitat, shallower water, gravel/cobble substrate, and grass riparian habitats. Densities in lower Ruby Creek averaged 8.5 aquatic invertebrates/m^3 of water (Figure 13). A total of 23 taxa were identified in lower Ruby Creek. Samples were dominated by aquatic diptera (60%) which were primarily chironomids, followed by other species (36%), and EPT species (4%) (Table 2, Figure 14). These results are relatively similar to aquatic invertebrate data from 2016 (Bradley 2017a).

The Shungnak River had the highest species richness among all the sample sites with 29 total different taxa identified, yet one of the lowest densities at 3.9 aquatic invertebrates/m^3 (Table 2; Figure 13). This site also had the highest percentage of EPT species at 49%, most of which were comprised of species in the order Ephemeroptera (Figure 14). The remaining samples were comprised of aquatic diptera (33%), primarily chironomids, and other species (18%).

The average aquatic invertebrate density in upper Subarctic Creek was 11.1 aquatic invertebrates/m^3 (Figure 13), which is much lower than was found in 2016 (23.9 aquatic invertebrates/m^3). However, similar to 2016, this site had the lowest species richness with 16 total taxa identified. Aquatic dipteras, mostly chironomids, accounted for an average of 49%, EPT accounted for approximately 3%, and other species, primarily Ostracods, accounted for 48% (Table 2, Figure 14). Very large numbers of Cladocerans were captured in 2016; however, none were captured in 2017. This sample site was located a few hundred yards below the origin of the creek, which abruptly forms when water goes from subsurface to surface flows.

Lower Subarctic Creek has the lowest average aquatic invertebrate density at 3.8 aquatic invertebrates/m^3 (Figure 13). Aquatic invertebrates were comprised of aquatic diptera (70%), most of which were chironomids, EPT species (14%), and other species (16%) (Table 2, Figure 14).

The Riley Creek site was moved about 7 km downstream to a site more easily accessible. This new site was characterized by riffle/pool habitat with gravel and cobble. It is also much wider with less dense vegetation on the shoreline. The average aquatic invertebrate density in Riley Creek was 5.2 aquatic invertebrates/m^3 (Figure 13). A total of 22 taxa were captured represented primarily by aquatic diptera (86%), followed by other species (8%), then EPT species (6%) (Table 2, Figure 14). Though the aquatic invertebrate density was lower than 2016, the composition of catches was relatively similar.

Jay Creek was a new site sampled this year. It is characterized by riffle/run habitats with very dense vegetation and canopy cover barely wide enough for the five drift nets. The second highest average aquatic invertebrate densities occurred here with 12.8 aquatic invertebrates/m^3 (Figure 13). Aquatic diptera dominated the community composition (75%), most of which were chironomids (Table 2, Figure 14). Other species made up 22% of catches followed by a small percentage of EPT species (3%).

Table 2. Total taxa and percent community composition of aquatic invertebrates by site, July 2017.

	Upper Ruby (RBC 5)	Lower Ruby (RBC 2)	Shungnak (USR)	Upper Subarctic (SAC 5)	Lower Subarctic (SAC 2)	Riley (RIC)	Jay (JC)
Total taxa	26	23	29	16	22	22	24
% other	56%	36%	18%	48%	16%	8%	22%
% Ephemeroptera	10%	4%	46%	3%	13%	5%	2%
% Plecoptera	<1%	<1%	2%	0%	1%	1%	<1%
% Trichoptera	1%	<1%	<1%	<1%	<1%	<1%	1%
% Aquatic Diptera	33%	60%	33%	49%	70%	86%	75%

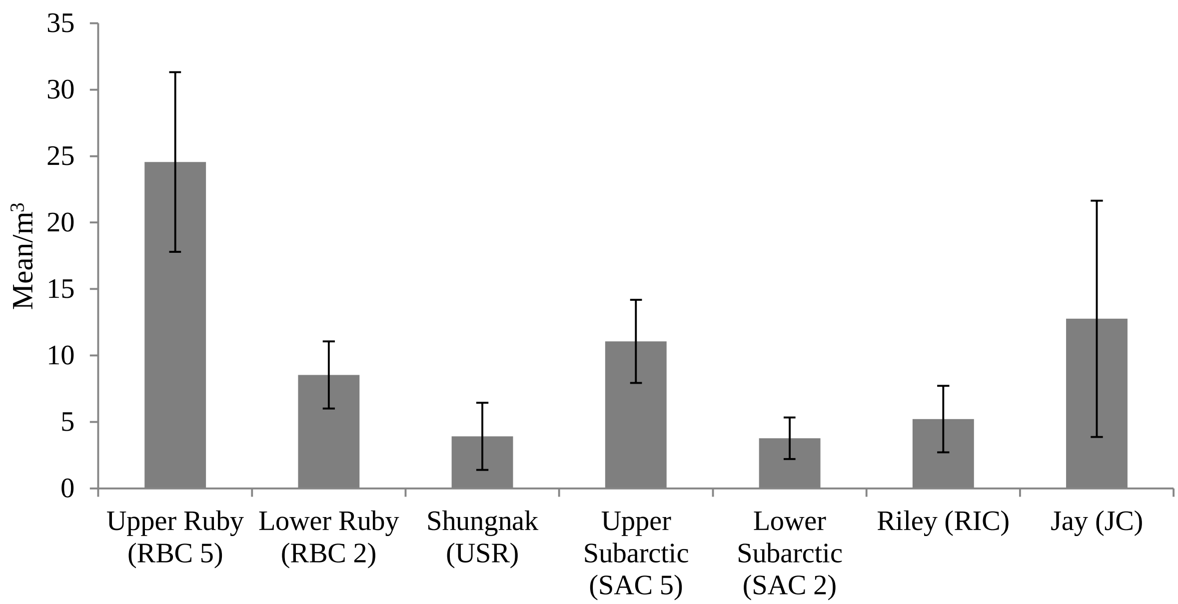

Figure 13. Mean number of aquatic invertebrates/m^3 (± 1 SD) at each sample site, July 2017.

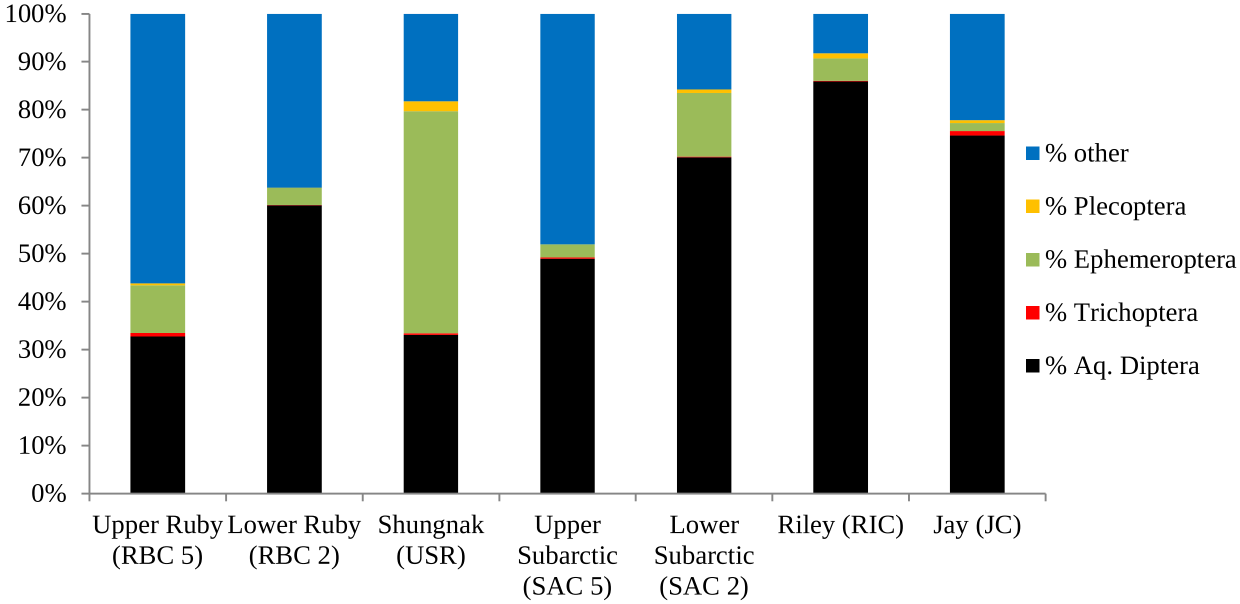

Figure 14. Mean percent EPT, aquatic diptera, and other species in the aquatic invertebrate samples, July 2017.

Fish Captures

June larval sampling

No larval fish were captured with the aquatic invertebrate drift nets in June. It is unknown if the sampling time frame occurred before or after Dolly Varden hatched. However, one larval fish was captured during aquatic invertebrate sampling in July in upper Ruby Creek (RBC 5) (Figure 15). It was about 7 mm in length, still attached to its egg, and too small to identify. The fish was sent to the University of Alaska Fairbanks genetics lab where they confirmed it was a slimy sculpin (Andres Lopez, University of Alaska Museum Curator of Fishes, personal communication). Capture of a larval fish confirms slimy sculpin spawn in this location.

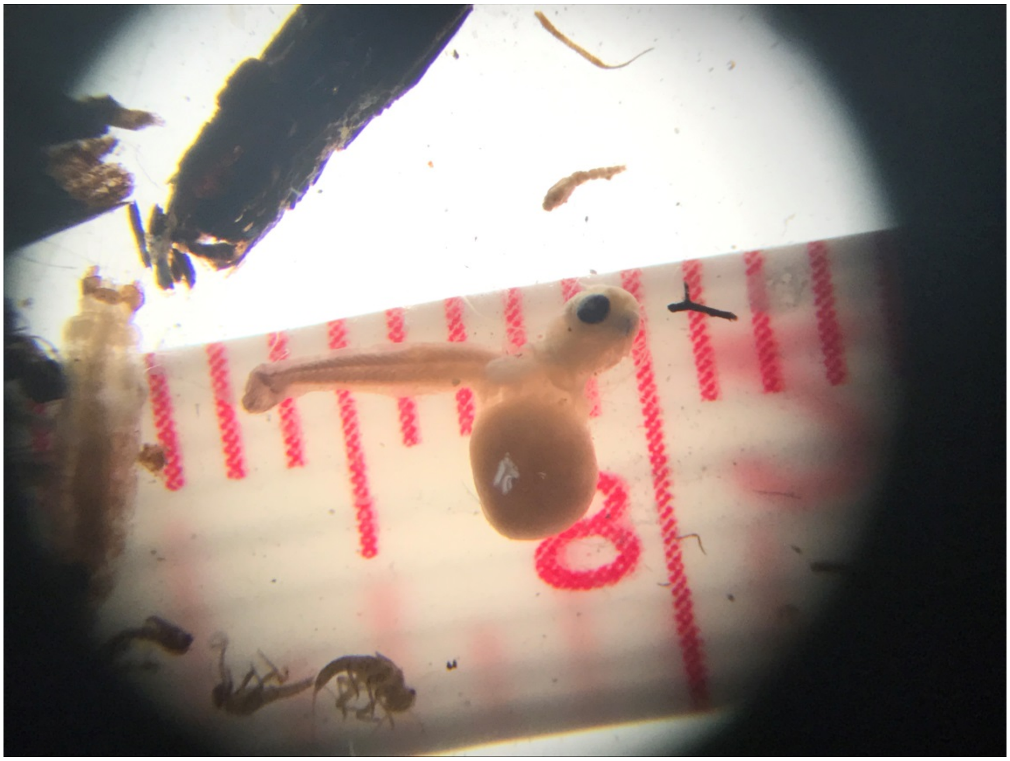

Figure 15. Larval slimy sculpin captured in aquatic invertebrate drift nets in upper Ruby Creek (RBC 5), July 20, 2017

July Minnow Traps

Throughout Ruby Creek, slimy sculpin dominated catches, followed by Dolly Varden (Table 3). Two sample sites were new for 2017; RBC 3 and RBC 5. Most of the slimy sculpin were captured at RBC 3, which is located by the bridge over Ruby Creek that provides access from the camp to the airport. There are many beaver dams in this drainage which may impede upstream passage of fish. The beaver dams create deep pools so it is likely fish occurring here are able to overwinter in upper Ruby Creek. Ten slimy sculpin from RBC 2, four Dolly Varden from RBC 2, 15 slimy sculpin from RBC 3, and six Dolly Varden from RBC 4 were originally retained for element analysis, but instead used for aging.

Table 3. Number, mean length, and length range of slimy sculpin and Dolly Varden captured in minnow traps at 11 sites, July 17–22, 2017.

Sample Site	Slimy Sculpin			Dolly Varden		
	Number captured	Mean total length (mm)	Length range (mm)	Number captured	Mean fork length (mm)	Length range (mm)
Shungnak R.						
USR	1	75	---	9	77	65–100
Subarctic Cr.						
SAC 2	2	54	41–67	8	106	72–135
SAC 3	---	---	---	7	131	104–162
SAC 4	---	---	---	15	120	64–157
SAC 5	---	---	---	40	118	72–154
Ruby Cr.						
RBC 2	17	64	42–81	4	114	80–136
[1]RBC 3	81	72	46–93	---	---	---
[2]RBC 4	69	65	42–102	7	142	108–175
RBC 5	61	73	40–110	---	---	---
Jay Cr.						
JC	---	---	---	11	119	84–161
Riley Cr.						
[3]RIC	8	65	45–89	16	112	82–129

[1]Eight traps instead of 10 were used
[2]Traps were reset for an additional 24 hours to increase sample size of fish for element analysis
[3]One 86 mm Arctic grayling was also captured at this location

In the Shungnak River, one 75 mm slimy sculpin and nine Dolly Varden were captured averaging 77 mm (range 65–100 mm) (Table 3). The Dolly Varden were originally retained for element analysis, but instead used for aging.

In Subarctic Creek, two additional sites (SAC 3 and SAC 4) were added to better understand fish distribution through Subarctic Creek. Dolly Varden dominated catches in Subarctic Creek, with only two slimy sculpin being captured at the lowest sample site, SAC 2 (Table 3). Generally, catches of Dolly Varden increased with elevation, with the highest catch rates occurring at SAC 5 at the top of the drainage. One young-of-the-year Dolly Varden (34 mm) was observed in a shallow isolated pool at SAC 2 and captured by hand (Figure 16). This sample site is only 1 km from the Shungnak River, though it is likely this fish originated upstream suggesting spawning may occur in Subarctic Creek. Fifteen Dolly Varden from SAC 5, 10 Dolly Varden from SAC 2, and one slimy sculpin from SAC 2 were retained for whole body element analysis, but instead used for aging. The genetic analyses of the 50 Dolly Varden from Subarctic Creek showed that they are reproductively isolated from and contain less genetic variation than anadromous Dolly Varden sampled from the Kobuk River. A detailed description of the genetic analysis and results is provided in Appendix 4.

Figure 16. Young-of-the-year Dolly Varden captured in lower Subarctic Creek (SAC 2), July 19, 2017

In 2017, the Riley Creek sample site was moved further downstream to increase the likelihood of capturing fish. A total of eight slimy sculpin, 16 Dolly Varden, and one Arctic grayling (86 mm), were captured at RIC (Table 3). Twelve Dolly Varden and three slimy sculpin were retained for whole body element analysis, but were instead used for aging. Additionally two Dolly Varden were retained for determining age and maturity status.

The Jay Creek sample site was new for 2017. A total of 11 Dolly Varden were captured averaging 119 mm (range 84–161 mm) (Table 3). Jay Creek is a small tributary to Ruby Creek and characterized by riffle run habitat and dense vegetation cover.

Fyke nets

Two fyke nets were set in Ruby Creek at RBC 1 on July 20 and checked about 24 hours later on July 21. A total of 84 Arctic grayling, 50 longnose suckers (*Catostomus catostomus*), 15 slimy sculpin, six round whitefish, and one Dolly Varden were captured. Roughly equal numbers of Arctic grayling were captured moving upstream as downstream (Figure 17). In 2016, a majority of the Arctic grayling captured were likely age 0 (49–66 mm) (Bradley 2017a). However, no age-0 Arctic grayling were captured during this year's sampling. Most of the Arctic grayling captured were likely age-1 or age-2 fish. Longnose suckers also made up a substantial amount of catches, which have not previously been documented in any sampling in the Shungnak River drainage. A majority of the longnose suckers were juveniles likely age 0 or age 1 and moving upstream (Figure 18). In 2016, large numbers of age-0 round whitefish were captured at this location (52–66 mm); however, the few round whitefish captured this year were likely age 1 (92–117 mm).

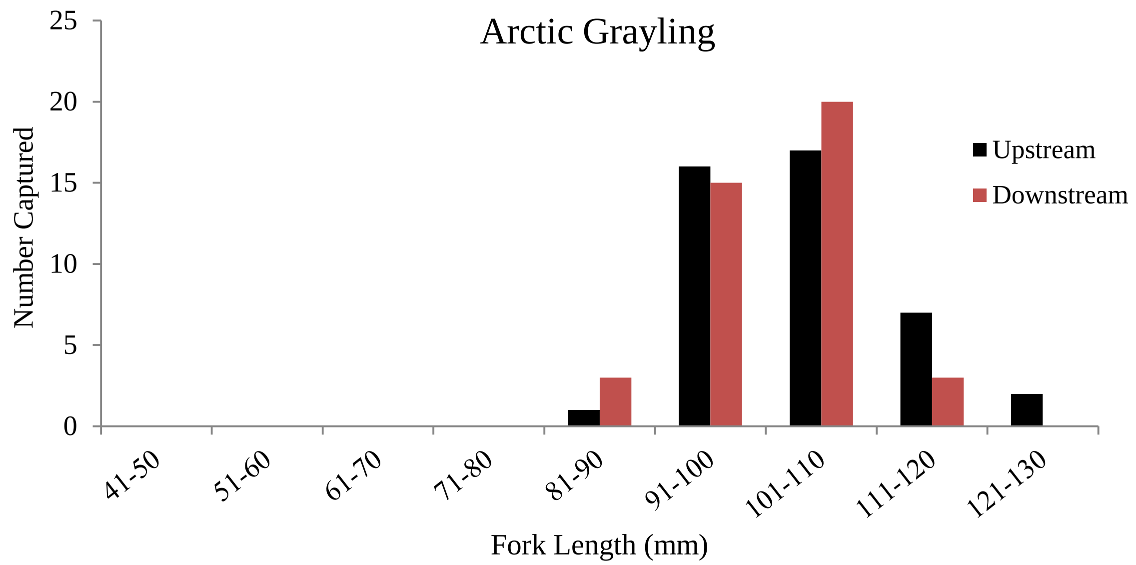

Figure 17. Length frequency distribution of Arctic grayling captured in lower Ruby Creek (RBC 1) and direction of movement, July 2017

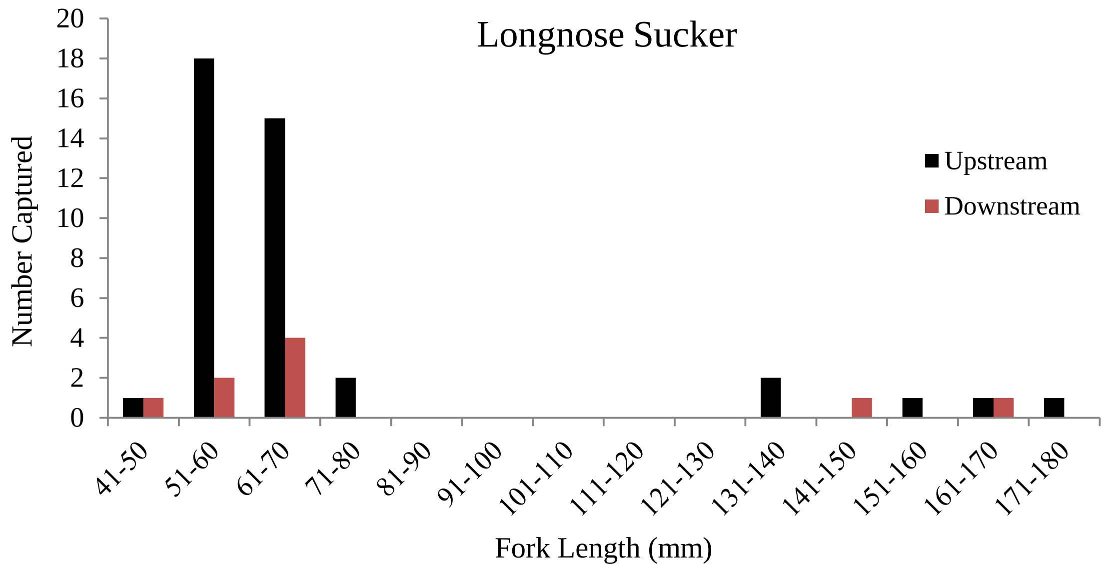

Figure 18. Length frequency distribution of longnose suckers captured in lower Ruby Creek (RBC 1) and direction of movement, July 2017.

Two fyke nets were set in Subarctic Creek (SAC 1) on July 20 and checked about 24 hours later on July 21. In the net designed to capture fish moving upstream, only 2 slimy sculpin were captured (42 and 45 mm). In the net set to capture downstream moving fish, 26 Arctic grayling were captured averaging 109 mm (range 87–142 mm). Fifteen were retained for whole body element analysis, but instead used for aging along with five fish retained specifically for aging that were not lost in the shipping delay.

September Minnow Traps

Minnow traps were set in seven locations in September to determine distribution of Dolly Varden in Subarctic Creek, and obtain fish for whole body element analysis. Catches were substantially lower than the July sampling (Table 4).

In Subarctic Creek, the highest numbers of Dolly Varden were captured at the lowest site (SAC 2) and the furthest upstream site (SAC 5), with sizes of fish increasing from downstream to upstream (Table 4). A total of 20 Dolly Varden were retained from Subarctic Creek, 10 for whole body element analysis, and 10 for dissection to determine age and assess maturity status.

In Ruby Creek, where 81 slimy sculpin were captured at RBC 3 in July, only one 75 mm slimy sculpin was captured in September (Table 4). It is likely they had either moved to another location already for overwintering, or they were not as susceptible to minnow traps because of reduced metabolism. The one sculpin captured was retained for whole body element analysis.

Fish captures in Jay Creek were comprised of only two slimy sculpin (78 and 90 mm) and one 79 mm Alaska blackfish (*Dallia pectoralis*) (Figure 19). One of the slimy sculpin was unusually light colored when compared to other slimy sculpin captured (Figure 19).

Riley Creek fish captures were much lower than the July sampling event, and were comprised of one slimy sculpin and three Dolly Varden (Table 4). The three Dolly Varden were retained for aging and to assess maturity.

Table 4. Number, mean length, and length range of slimy sculpin and Dolly Varden captured in minnow traps at seven sites, September 19–21, 2017.

Sample Site	Slimy Sculpin			Dolly Varden		
	Number captured	Mean total length (mm)	Range (mm)	Number captured	Mean fork length (mm)	Range (mm)
Subarctic Cr.						
SAC 2	2	72	63–81	11	89	73–107
SAC 3	---	---	---	5	97	74–120
SAC 4	---	---	---	1	121	---
SAC 5	---	---	---	13	118	91–143
Ruby Cr.						
[1]RBC 3	1	75	---	---	---	---
Jay Cr.						
[2]JC	2	84	78–90	---	---	---
Riley Cr.						
RIC 2	1	86	---	3	127	113–148

[1]Eight traps instead of 10 were used
[2]One 79 mm Alaska blackfish was also captured at this location

Figure 19. Alaska blackfish (left) and unusually light colored slimy sculpin (right) captured in Jay Creek (JC) September 21, 2017.

Fish Metals

Fish retained for element analysis are listed in Appendix 2 and results for each fish are listed in Appendix 3. Unfortunately, 97 fish captured during the July sample were delayed by the carrier in shipment and arrived at the lab in an unusable condition. Because the bony structures remained intact, the fish were returned to Fairbanks for aging. All fish used for whole body element analysis were captured in September, when catches were much lower. However, results provide a good continuation for a baseline data set regarding element concentrations in fish and similar patterns occurred compared to 2016 data. Similar elements have been examined in whole body juvenile Dolly Varden around the state including Tulsequah Chief Mine, the Pebble prospect, Red Dog Mine, Greens Creek Mine, and Kensington Gold Mine and provide a good data set for comparative purposes (Legere and Timothy, 2016).

Cadmium concentrations were highest, but variable, in the fish from upper Subarctic Creek, which averaged 0.69 mg/kg (Figure 20). This value is slightly lower than the 2016 value which averaged 1.05 mg/kg. Similar to 2016, slightly lower cadmium concentrations were found in the fish at the lower site in Subarctic Creek. The one slimy sculpin from Ruby Creek contained 0.34 mg/kg of cadmium.

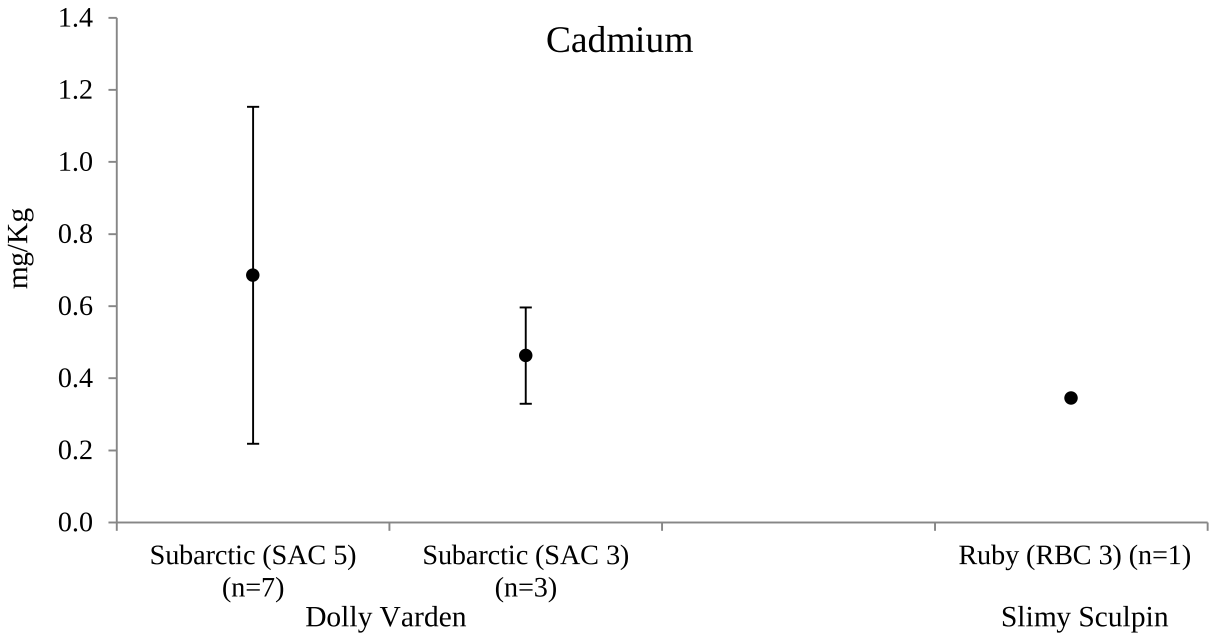

Figure 20. Mean whole body dry weight concentrations of cadmium (mg/kg) (± 1 SD) in Dolly Varden and slimy sculpin from various sample sites, September 2017.

Mean copper concentrations at upper Subarctic (SAC 5) in 2017 were similar to those found in 2016 (Figure 21). At the lower sample site (SAC 3), mean values were slightly higher in 2017 (6.95 mg/kg) than 2016 (4.10 mg/kg). The copper concentration from the one sculpin in Ruby Creek was also higher in 2017 (8.37 mg/kg) than the mean value found in 2016 (2.79 mg/kg). These results found for Dolly Varden at Arctic-Bornite are similar to other locations from across the state (Legere and Timothy, 2016).

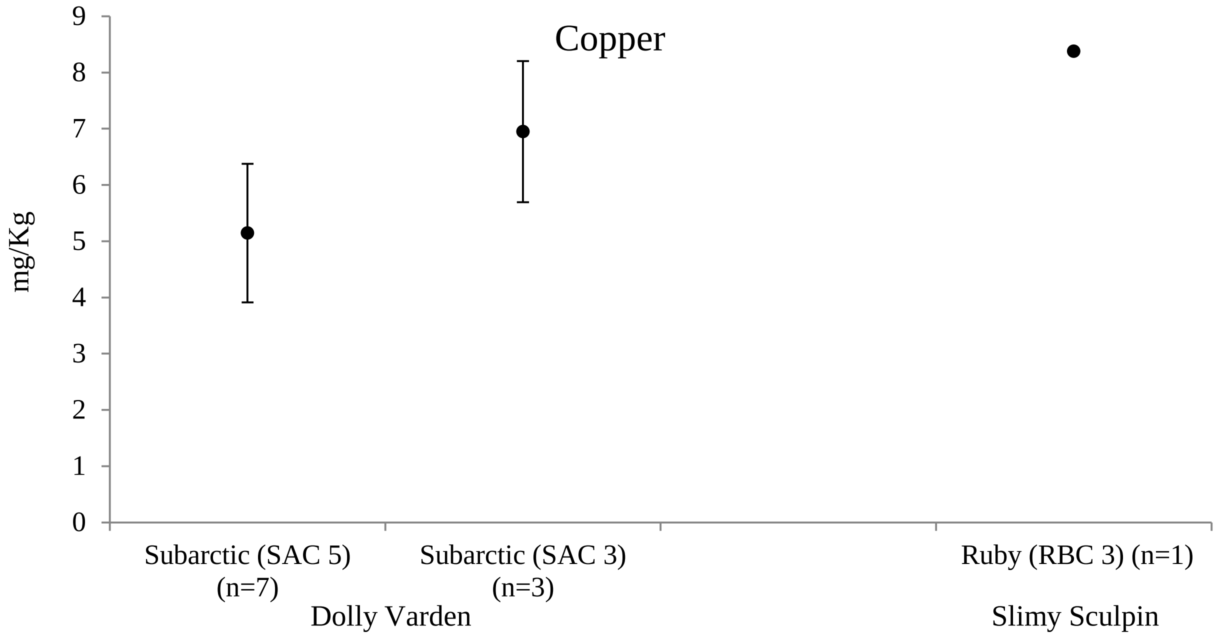

Figure 21. Mean whole body dry weight concentrations of copper (mg/kg) (± 1 SD) in Dolly Varden and slimy sculpin from various sample sites, September 2017.

Mean mercury concentrations were similar in Dolly Varden in Subarctic Creek between 2017 and 2016 (Figure 22). These values in Dolly Varden are comparable to the values found at other mines and prospect sites across the state (Legere and Timothy, 2016). The highest value for mercury was seen in the slimy sculpin in upper Ruby Creek.

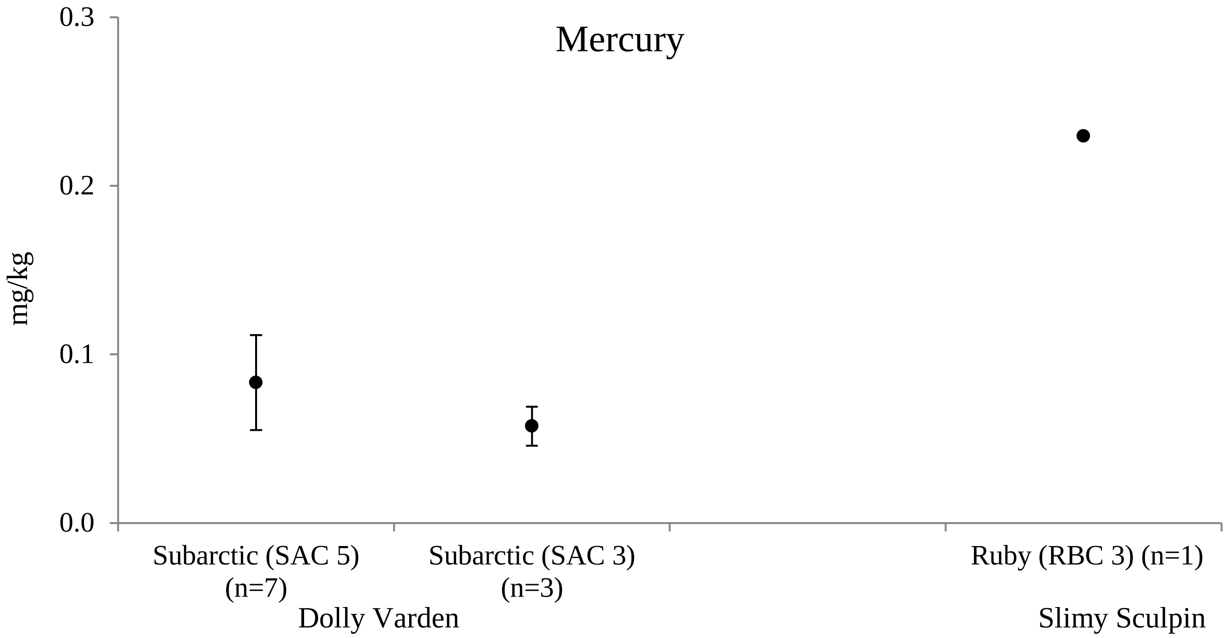

Figure 22. Mean whole body dry weight concentrations of mercury (mg/kg) (± 1 SD) in Dolly Varden and slimy sculpin from various sample sites, September 2017.

Similar to 2016, selenium concentrations were nearly the same among fish at all sample sites (Figure 23). These values are slightly higher than those found at Tulsequah Chief Mine and the Pebble Prospect, and comparable to those found in juvenile Dolly Varden at Red Dog Mine, Greens Creek Mine, and Kensington Gold Mine (Legere and Timothy, 2016).

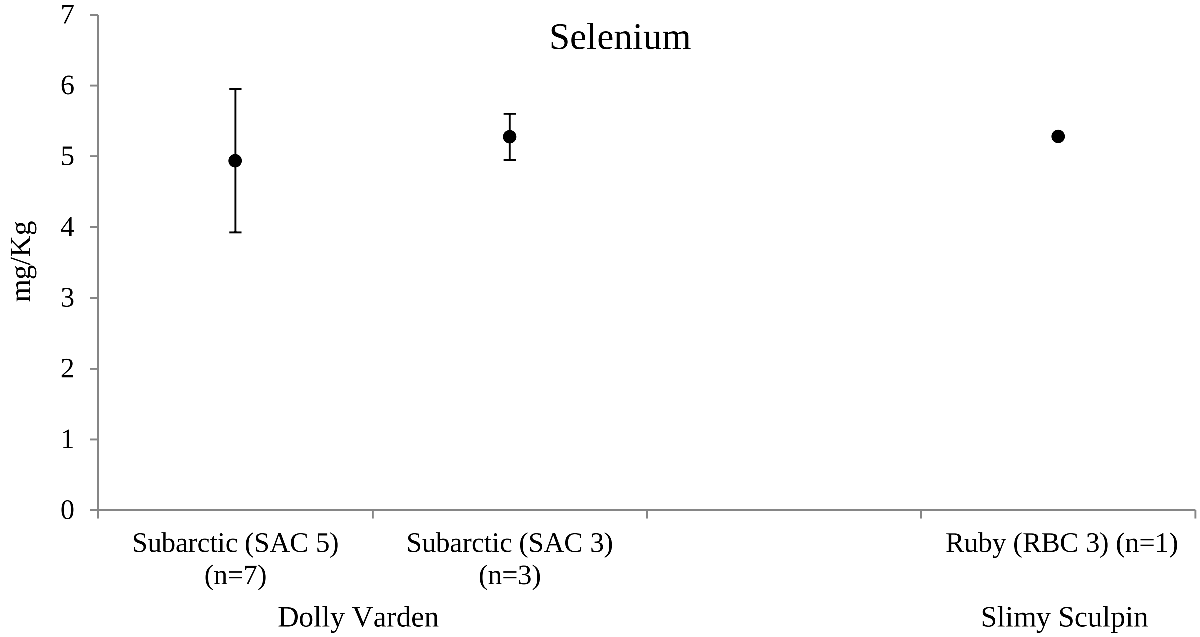

Figure 23. Mean whole body dry weight concentrations of selenium (mg/kg) (± 1 SD) in Dolly Varden and slimy sculpin from various sample sites, September 2017.

Higher mean zinc concentrations were found in Dolly Varden in lower Subarctic Creek (SAC 3) (197.21 mg/kg) than upper Subarctic Creek (SAC 5) (118.66 mg/kg) (Figure 24). These are very similar values and a similar pattern that was found in 2016. The zinc concentration in the slimy sculpin was higher in 2017 (212.32 mg/kg) than the mean value found in 2016 in Ruby Creek (128.32 mg/kg). These values all fall within the range of concentrations found in Dolly Varden in other regions of the state (Legere and Timothy, 2016).

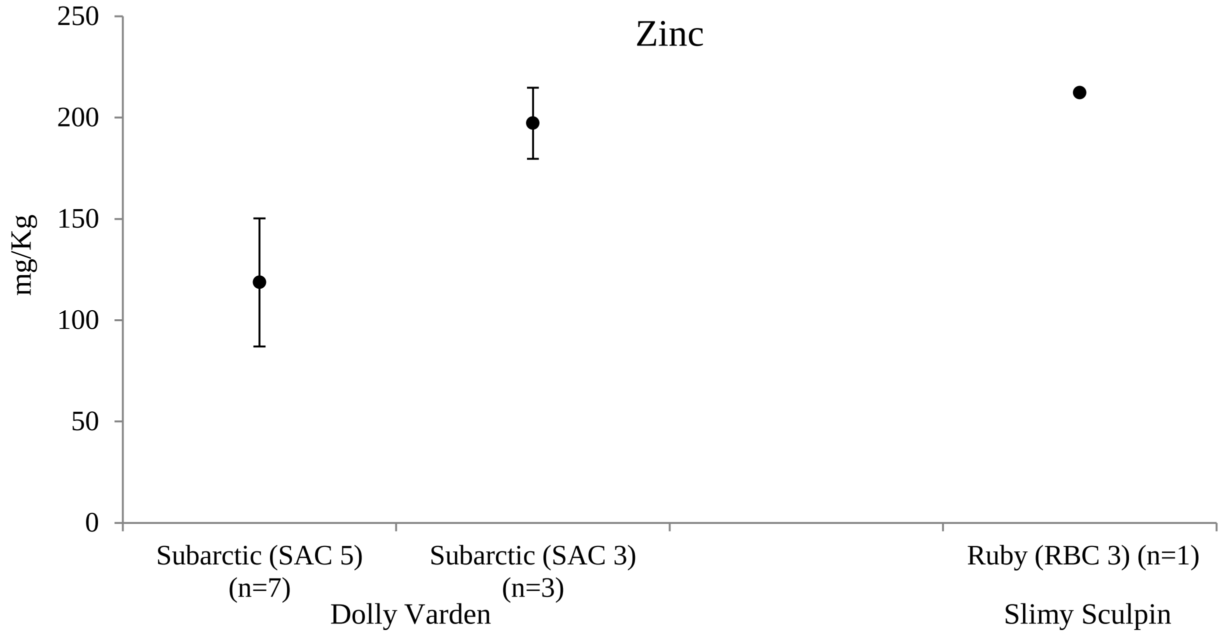

Figure 24. Mean whole body dry weight concentrations of zinc (mg/kg) (± 1 SD) in Dolly Varden and slimy sculpin from various sample sites, September 2017.

Fish Age and Maturity

Dolly Varden

Dolly Varden retained for aging ranged from 1 to 5 years old (Table 5, Figure 25). Length-weight relationships show the largest fish occurred in Ruby Creek with the smallest occurring in the Shungnak River (Figure 26). Dolly Varden from Ruby Creek ranged 1 to 3 years old and had the highest length at age for those age classes (Table 5). All fish captured in Riley Creek were age 2, and ranged in length from 93-148 mm. Two fish were retained from Riley Creek in July for determining maturity status. One was immature (115 mm) while one was a mature male (116 mm). Additionally, during the September sampling, three fish were retained from Riley Creek. Two were immature (113 and 120 mm) and one was a mature male (148 mm), all of which were age 2.

Table 5. Age and lengths of Dolly Varden captured in Ruby Creek, Riley Creek, Subarctic Creek, and the Shungnak River July and September, 2017.

	Ruby			Riley			Subarctic			Shungnak		
Age	N	Mean Length (mm)	Length Range (mm)	N	Mean Length (mm)	Length Range (mm)	N	Mean Length (mm)	Length Range (mm)	N	Mean Length (mm)	Length Range (mm)
1	3	101	80-116				3	97	85-107	3	74	71-79
2	4	129	109-139	14	116	93-148	3	93	84-100	2	99	97-100
3	2	146	132-157				6	119	107-134			
4							16	126	111-143			
5							3	117	105-124			

In Subarctic Creek, six age classes were captured, including the presumed age-0 fish (34 mm). Age 4 was the most common age class of fish that were retained. There is lots of overlap in lengths between the age classes. Ten fish captured in September were assessed for maturity. Two fish captured at the lowest sample site (SAC 2) were both immature and age 1. The remaining eight fish captured at SAC 3, SAC 4, and SAC 5 were age-4 males in spawning condition, with the exception of one age-4 female. The female appeared to not be in ripe condition (Figure 27).

Dolly Varden retained from the Shungnak River were all age-1 and age-2 fish (Table 5). Though sample size was low, age-1 fish had the smallest lengths compared to age-1 fish from the other sample sites (Figure 25).

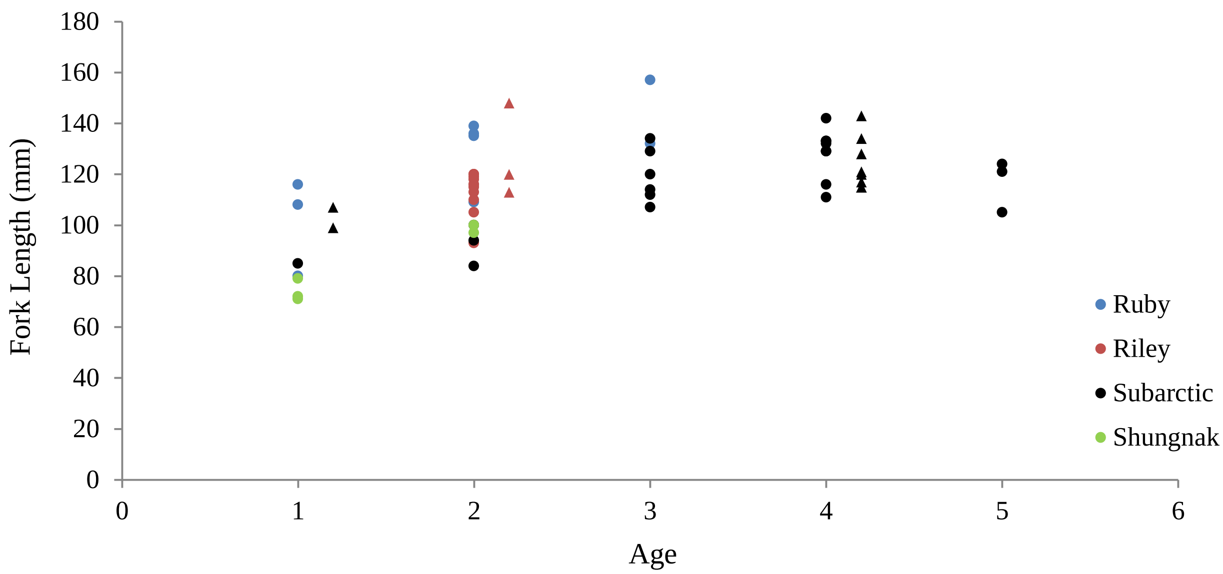

Figure 25. Age-length relationship for Dolly Varden captured in Ruby Creek, Riley Creek, Subarctic Creek, and the Shungnak River in July (dots) and September (triangles), 2017.

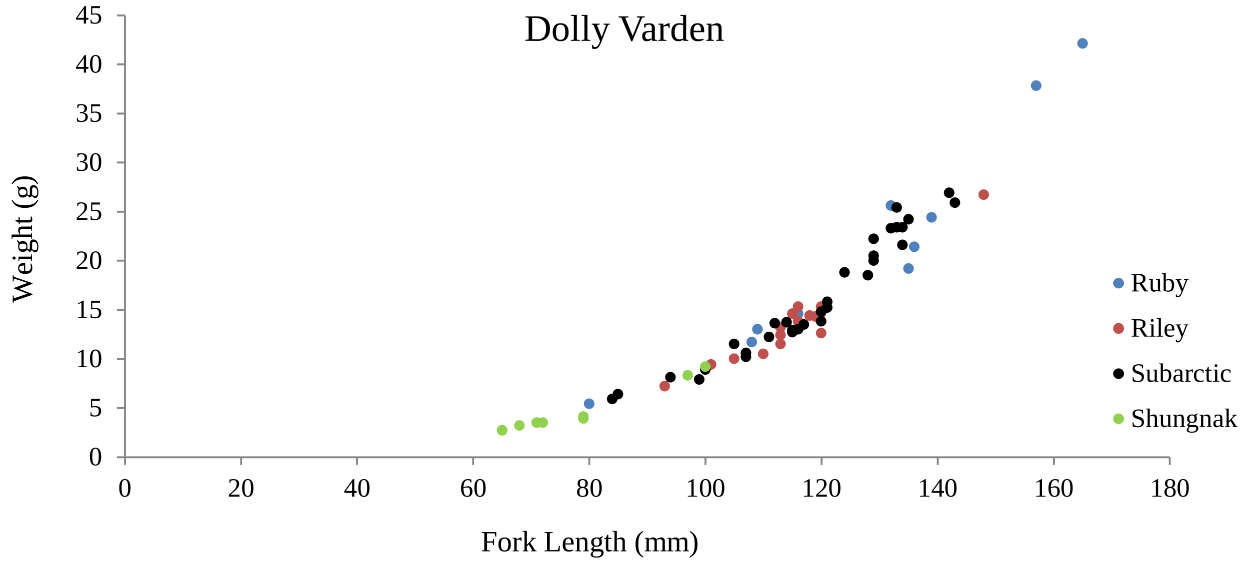

Figure 26. Length-weight relationship for Dolly Varden captured in Ruby Creek, Riley Creek, Subarctic Creek, and the Shungnak River July and September, 2017.

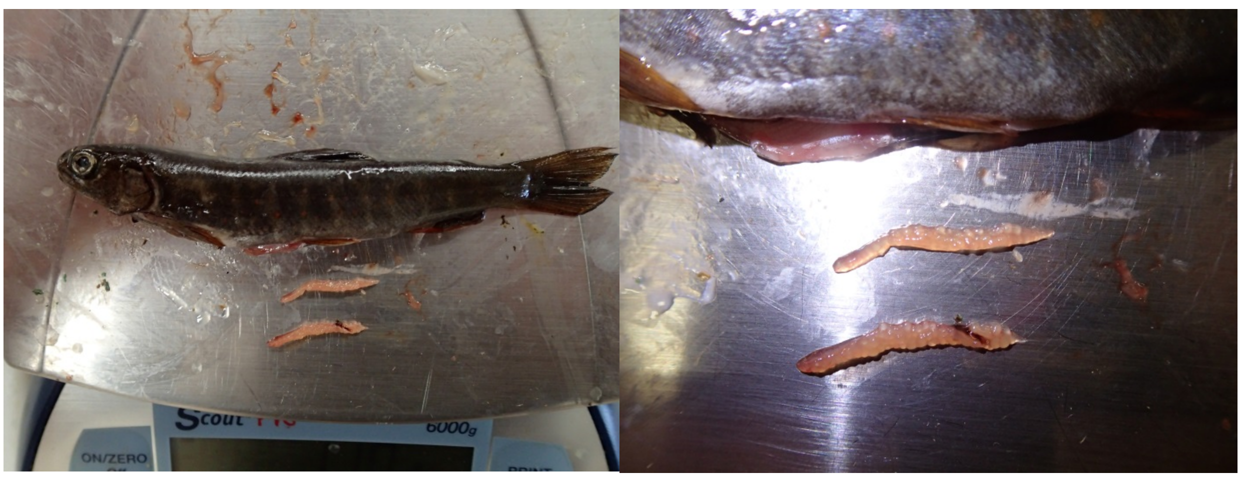

Figure 27. Age-4 female Dolly Varden captured September 20, 2017 in Subarctic Creek (SAC 5) with eggs removed and placed below fish.

Slimy Sculpin

Slimy sculpin ranged in age from 3 to 11 years old, and lengths ranged from 62 to 85 mm (Figure 28). There was extensive overlap in lengths of each age class, making age classification based on length difficult. Similar results have been documented on slimy sculpin ages in the Chena River near Fairbanks (Sonnichsen 1981).

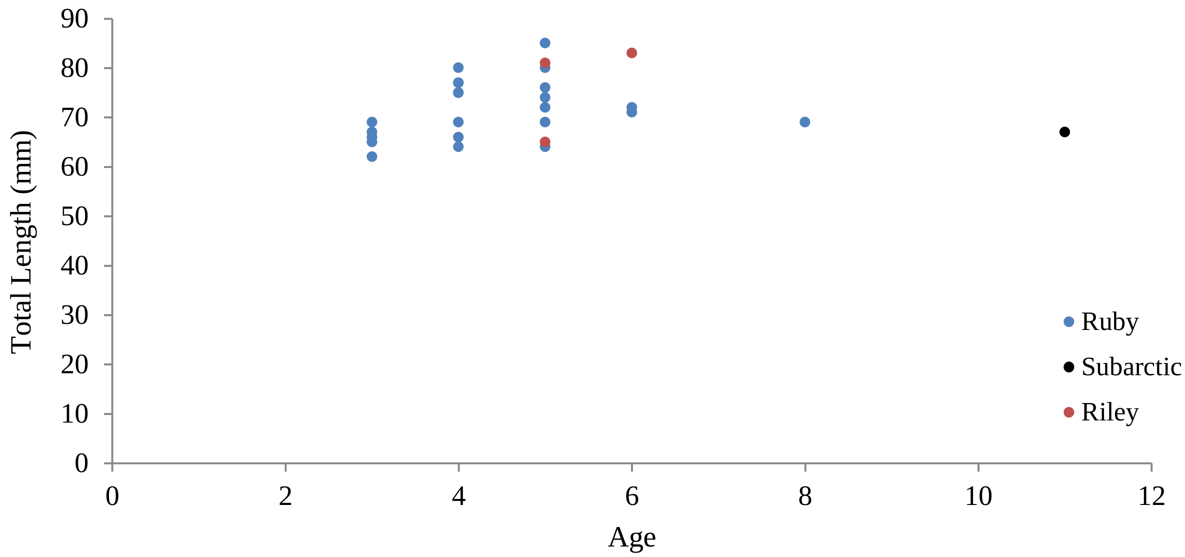

Figure 28. Age-length relationship of slimy sculpin captured July 18–21, 2017 in Ruby, Subarctic, and Riley creeks.

Arctic Grayling

Of the 12 Arctic grayling that were aged from lower Subarctic Creek (SAC 1), 11 were age 1 averaging 104 mm in length (range: 83–117 mm). Only one fish was age 2, and was 142 mm in length.

Fall Aerial Survey

An aerial survey was flown on the Kogoluktuk River on September 21 from near Riley Creek, downstream to its confluence with the Kobuk River. The flight was conducted using an R-44 helicopter. Water was high and murky so visibility was poor. Only one chum salmon was observed near the mouth at coordinates 66.92686, -156.75194.

Conclusion

Despite being isolated from the Kobuk River by a large waterfall, the Shungnak River drainage supports self-sustaining populations of Arctic grayling, Dolly Varden, round whitefish, slimy sculpin, longnose sucker, and Alaska blackfish. Slimy sculpin occurred at every sample site other than the three upper sample sites in Subarctic Creek (SAC 3, 4, and 5). With the capture of a larval slimy sculpin in upper Ruby Creek, spawning is confirmed in that reach.

Similar to 2016, catches in Subarctic Creek were dominated by Dolly Varden. The upper site had the lowest aquatic invertebrate species richness among all sample sites again, but the third highest density. It is likely the Dolly Varden move into the upper reaches of Subarctic Creek to feed on the abundant aquatic insects, and with the confirmation of fish in pre-spawning condition in September, they likely remain there to spawn. Future work will likely focus on further confirmation of Dolly Varden spawning in upper Subarctic Creek, which would require another spring survey targeting newly hatched fish. Based on ages and assessment of maturity status from 2016 and 2017, male and female Dolly Varden mature between ages 3 and 4. No Dolly Varden older than 5 have been documented in Subarctic Creek, so it's likely fish only live for one to two years after maturing.

The Dolly Varden captured in Riley Creek in July and September have the potential to be anadromous as no permanent physical barrier exists downstream. Of the 14 fish aged, all were age 2 which is consistent with rearing anadromous fish. Due to decomposition of fish originally to be used for whole body element analysis, only five fish not sent to the lab could be assessed for maturity. Three were immature, and two were mature males in spawning condition. If some of these fish are anadromous, Riley Creek may serve as spawning habitat for resident Dolly Varden and rearing habitat for anadromous juveniles. However, the presence of small, sexually mature males does not prove there is a self-sustaining resident population of Dolly Varden in Riley Creek. Many anadromous populations of Dolly Varden contain "residual" males that never migrate to the ocean, but instead spend their entire life cycle in freshwater. These males act as sneaker males and spawn with anadromous females (Armstrong and Morrow 1980). Future fish sampling in Riley Creek will likely involve spring, summer, and fall surveys to better assess fish use along with taking genetic samples. With the baseline genetic information on the resident Dolly Varden in Subarctic Creek showing they are reproductively isolated and contain less genetic variation than anadromous Dolly Varden from the Kobuk River, genetics from Riley Creek could provide insight to whether some are anadromous or not. Additional fall aerial surveys in the Kogoluktuk River to look for anadromous Dolly Varden would help confirm the presence or absence of anadromous fish.

In 2016, fyke net catches in Ruby Creek were dominated by age-0 Arctic grayling and round whitefish. However, this year, all Arctic grayling and round whitefish were likely age 1+. The only young-of-the-year fish captured were longnose suckers, which is a species not previously documented in the Shungnak drainage. Based on catches in 2016 and 2017, it is likely Arctic grayling, round whitefish, and longnose suckers spawn upstream of Ruby Creek in the Shungnak River drainage.

If future aquatic sampling is planned, we recommend continuation of periphyton and aquatic invertebrate sampling. Additional fish work should be focused on continuing our understanding of how and when fish utilize target areas around the Arctic and Bornite deposits. Obtaining greater sample sizes for fish whole body element analysis, collecting fin clips from Dolly Varden for genetics analysis, and conducting fall aerial surveys are additional recommendations.

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Appendix 1. Water quality data from 2017 from six sample sites. Only metals data used in fish whole body element analyses are shown. Acute and chronic water quality standards for aquatic life are shown for cadmium, copper, and zinc, which are dependent on water hardness.

Location	Date Collected	Cadmium µg/L	Cadmium Acute Limit µg/L	Copper Chronic Limit µg/L	Copper µg/L	Copper Acute Limit µg/L	Copper Chronic Limit µg/L	Mercury ng/L	Selenium µg/L	Zinc µg/L	Zinc Acute Limit µg/L	Zinc Chronic Limit µg/L	Hardness $CaCO_3$ mg/L	TDS mg/L
Upper Ruby	4/27/2017	*0.015			0.33	20.38	13.12	*0.5	*0.31	1.56	167.98	167.98	149	169
Upper Ruby	7/18/2017	*0.015			0.47	18.96	12.28	0.605	0.329	1.25	157.41	157.41	138	163
Upper Ruby	8/23/2017	*0.015			0.41	17.14	11.21	*0.5	0.547	1.43	143.77	143.77	124	136
Upper Ruby	9/18/2017	*0.015			0.53	15.18	10.04	0.876	0.385	1.66	128.89	128.89	109	132
Upper Ruby	12/2/2017	0.015	2.98	0.35	0.82	19.09	12.36	*0.5	0.588	4.36	158.38	158.38	139	166
Lower Ruby	4/27/2017	*0.015			0.46	20.25	13.04	*0.5	0.345	0.81	167.02	167.02	148	173
Lower Ruby	7/24/2017	0.0298	1.90	0.25	3.00	12.58	8.47	2.25	*0.31	4.24	108.86	108.86	89.3	119
Lower Ruby	8/26/2017	0.0165	2.52	0.31	1.17	16.36	10.75	0.612	0.409	2.12	137.85	137.85	118	149
Lower Ruby	9/22/2017	*0.015			0.95	16.10	10.59	0.744	*0.31	11.90	135.87	135.87	116	113
Lower Ruby	11/30/2017	*0.015			0.57	19.35	12.51	0.749	0.622	1.32	160.31	160.31	141	160
Upper Shungnak	4/27/2017	0.097	1.77	0.24	0.67	11.80	7.99	*0.5	0.334	8.39	102.74	102.74	83.4	108
Upper Shungnak	7/22/2017	0.130	1.78	0.24	0.88	11.81	8.00	*0.5	0.369	7.45	102.84	102.84	83.5	116
Upper Shungnak	8/24/2017	0.219	1.68	0.23	1.45	11.20	7.62	*0.5	0.563	17.10	98.02	98.02	78.9	97
Upper Shungnak	9/20/2017	0.217	1.57	0.22	1.53	10.54	7.21	0.701	*0.31	16.70	92.84	92.84	74	92
Upper Subarctic	7/21/2017	0.0165	0.89	0.14	0.22	6.21	4.46	*0.5	*0.31	0.96	57.68	57.68	42.2	55
Upper Subarctic	8/21/2017	0.0963	1.05	0.16	0.69	7.29	5.16	*0.5	0.426	3.29	66.60	66.60	50	58
Upper Subarctic	9/20/2017	0.0166	0.86	0.14	0.26	6.06	4.36	0.695	*0.31	1.35	56.41	56.41	41.1	48
Lower Subarctic	4/27/2017	0.0415	1.08	0.16	0.31	7.42	5.25	*0.5	0.704	4.32	67.72	67.72	51	68
Lower Subarctic	7/19/2017	0.1610	0.98	0.15	3.22	6.82	4.86	0.95	0.315	16.10	62.74	62.74	46.6	62
Lower Subarctic	8/24/2017	0.0829	0.81	0.13	1.02	5.71	4.14	*0.5	0.402	11.50	53.48	53.48	38.6	44
Lower Subarctic	9/20/2017	0.1020	0.77	0.13	1.33	5.42	3.94	0.746	0.398	15.30	51.01	51.01	36.5	41
Lower Riley	7/19/2017	*0.015			0.45	14.92	9.88	0.645	0.546	1.15	126.89	126.89	107	129
Lower Riley	8/22/2017	*0.015			0.65	15.58	10.28	*0.5	0.781	1.29	131.89	131.89	112	123
Lower Riley	9/20/2017	0.015	2.10	0.27	0.81	13.77	9.19	0.783	0.464	1.12	118.09	118.09	98.3	115

* Indicates value at or below detection limit.

Appendix 2. List of fish retained for whole body element analysis.

Sample ID	Stream	Sample Site	Date Collected	Fish Spp[1]	Length (mm)	Weight (g)	Metals to be analyzed: Cu	Hg	Se	Cd	Zn
092017LMSADV01	Subarctic	SAC 3	9/20/2017	DV	108	11	x	x	x	x	x
092017LMSADV02	Subarctic	SAC 3	9/20/2017	DV	91	7.1	x	x	x	x	x
092017LMSADV04	Subarctic	SAC 3	9/20/2017	DV	93	7.3	x	x	x	x	x
092017USADV01	Subarctic	SAC 5	9/20/2017	DV	100	10	x	x	x	x	x
092017USADV02	Subarctic	SAC 5	9/20/2017	DV	118	16.7	x	x	x	x	x
092017USADV04	Subarctic	SAC 5	9/20/2017	DV	123	19	x	x	x	x	x
092017USADV05	Subarctic	SAC 5	9/20/2017	DV	111	13.9	x	x	x	x	x
092017USADV07	Subarctic	SAC 5	9/20/2017	DV	133	27.4	x	x	x	x	x
092017USADV11	Subarctic	SAC 5	9/20/2017	DV	106	11.3	x	x	x	x	x
092017USADV12	Subarctic	SAC 5	9/20/2017	DV	91	6.9	x	x	x	x	x
092117RubySS01	Ruby	RBC 3	9/21/2017	SS	75	4.6	x	x	x	x	x

[1] Dolly Varden (DV) and slimy sculpin (SS)

Appendix 3. Results for whole body element analysis. Results used in this report are highlighted in yellow. Note, results for Mercury are listed in ng/g in this table, but converted to mg/kg throughout the report.

Subarctic Creek

Sample ID	Species[1]	Sample Site	Collect Date	Analyte	Method	Wet Wt. Result	QUAL[2]	Units	MDL[3]	PQL[3]	%solids	Dry Wt. Result	Dry Wt MDL[3]
092017LMSADV01	DV	SAC 3	9/20/2017	Cadmium	M6020 ICP-MS	0.07	B	mg/Kg	0.02	0.1	24.4	0.29	0.08
092017LMSADV02	DV	SAC 3	9/20/2017	Cadmium	M6020 ICP-MS	0.11		mg/Kg	0.02	0.1	22.4	0.49	0.09
092017LMSADV04	DV	SAC 3	9/20/2017	Cadmium	M6020 ICP-MS	0.13		mg/Kg	0.02	0.09	21.3	0.61	0.09
092017USADV01	DV	SAC 5	9/20/2017	Cadmium	M6020 ICP-MS	0.19		mg/Kg	0.02	0.1	24	0.79	0.08
092017USADV02	DV	SAC 5	9/20/2017	Cadmium	M6020 ICP-MS	0.04	B	mg/Kg	0.02	0.1	22	0.18	0.09
092017USADV04	DV	SAC 5	9/20/2017	Cadmium	M6020 ICP-MS	0.08	B	mg/Kg	0.02	0.09	18.6	0.43	0.11
092017USADV05	DV	SAC 5	9/20/2017	Cadmium	M6020 ICP-MS	0.06	B	mg/Kg	0.02	0.09	18.6	0.32	0.11
092017USADV07	DV	SAC 5	9/20/2017	Cadmium	M6020 ICP-MS	0.14		mg/Kg	0.02	0.1	18.9	0.74	0.11
092017USADV011	DV	SAC 5	9/20/2017	Cadmium	M6020 ICP-MS	0.13		mg/Kg	0.02	0.08	21	0.62	0.10
092017USADV012	DV	SAC 5	9/20/2017	Cadmium	M6020 ICP-MS	0.42		mg/Kg	0.02	0.1	24.5	1.71	0.08
092017LMSADV01	DV	SAC 3	9/20/2017	Copper	M6020 ICP-MS	1.62		mg/Kg	0.09	0.4	24.4	6.64	0.37
092017LMSADV02	DV	SAC 3	9/20/2017	Copper	M6020 ICP-MS	1.93		mg/Kg	0.08	0.4	22.4	8.62	0.36
092017LMSADV04	DV	SAC 3	9/20/2017	Copper	M6020 ICP-MS	1.19		mg/Kg	0.07	0.4	21.3	5.59	0.33
092017USADV01	DV	SAC 5	9/20/2017	Copper	M6020 ICP-MS	1.07		mg/Kg	0.09	0.5	24	4.46	0.38
092017USADV02	DV	SAC 5	9/20/2017	Copper	M6020 ICP-MS	0.99		mg/Kg	0.09	0.4	22	4.50	0.41
092017USADV04	DV	SAC 5	9/20/2017	Copper	M6020 ICP-MS	1.45		mg/Kg	0.07	0.3	18.6	7.80	0.38
092017USADV05	DV	SAC 5	9/20/2017	Copper	M6020 ICP-MS	0.84		mg/Kg	0.07	0.4	18.6	4.52	0.38
092017USADV07	DV	SAC 5	9/20/2017	Copper	M6020 ICP-MS	0.86		mg/Kg	0.09	0.5	18.9	4.55	0.48
092017USADV011	DV	SAC 5	9/20/2017	Copper	M6020 ICP-MS	0.86		mg/Kg	0.06	0.3	21	4.10	0.29
092017USADV012	DV	SAC 5	9/20/2017	Copper	M6020 ICP-MS	1.49		mg/Kg	0.09	0.5	24.5	6.08	0.37
092017LMSADV01	DV	SAC 3	9/20/2017	Mercury	M7473	14.1	B	ng/g	3.32	16.6	24.4	57.79	13.61
092017LMSADV02	DV	SAC 3	9/20/2017	Mercury	M7473	16	B	ng/g	3.76	18.8	22.4	71.43	16.79
092017LMSADV04	DV	SAC 3	9/20/2017	Mercury	M7473	9.16	B	ng/g	3.61	18.05	21.3	43.00	16.95
092017USADV01	DV	SAC 5	9/20/2017	Mercury	M7473	14.4	B	ng/g	3.26	16.3	24	60.00	13.58
092017USADV02	DV	SAC 5	9/20/2017	Mercury	M7473	12.6	B	ng/g	3.39	16.95	22	57.27	15.41
092017USADV04	DV	SAC 5	9/20/2017	Mercury	M7473	23.3		ng/g	3.43	17.15	18.6	125.27	18.44
092017USADV05	DV	SAC 5	9/20/2017	Mercury	M7473	18.4		ng/g	3.68	18.4	18.6	98.92	19.78
092017USADV07	DV	SAC 5	9/20/2017	Mercury	M7473	22		ng/g	3.87	19.35	18.9	116.40	20.48
092017USADV011	DV	SAC 5	9/20/2017	Mercury	M7473	15.9	B	ng/g	3.76	18.8	21	75.71	17.90
092017USADV012	DV	SAC 5	9/20/2017	Mercury	M7473	12.1	B	ng/g	3.15	15.75	24.5	49.39	12.86
092017LMSADV01	DV	SAC 3	9/20/2017	Moisture Content	D2216-80	75.6		%	0.1	0.5	24.4		
092017LMSADV02	DV	SAC 3	9/20/2017	Moisture Content	D2216-80	77.6		%	0.1	0.5	22.4		
092017LMSADV04	DV	SAC 3	9/20/2017	Moisture Content	D2216-80	78.7		%	0.1	0.5	21.3		
092017USADV01	DV	SAC 5	9/20/2017	Moisture Content	D2216-80	76		%	0.1	0.5	24		
092017USADV02	DV	SAC 5	9/20/2017	Moisture Content	D2216-80	78		%	0.1	0.5	22		
092017USADV04	DV	SAC 5	9/20/2017	Moisture Content	D2216-80	81.4		%	0.1	0.5	18.6		
092017USADV05	DV	SAC 5	9/20/2017	Moisture Content	D2216-80	81.4		%	0.1	0.5	18.6		
092017USADV07	DV	SAC 5	9/20/2017	Moisture Content	D2216-80	81.1		%	0.1	0.5	18.9		
092017USADV011	DV	SAC 5	9/20/2017	Moisture Content	D2216-80	79		%	0.1	0.5	21		
092017USADV012	DV	SAC 5	9/20/2017	Moisture Content	D2216-80	75.5		%	0.1	0.5	24.5		
092017LMSADV01	DV	SAC 3	9/20/2017	Selenium	M6020 ICP-MS	1.19		mg/Kg	0.02	0.06	24.4	4.88	0.08
092017LMSADV02	DV	SAC 3	9/20/2017	Selenium	M6020 ICP-MS	1.18		mg/Kg	0.02	0.05	22.4	5.27	0.09
092017LMSADV04	DV	SAC 3	9/20/2017	Selenium	M6020 ICP-MS	1.21		mg/Kg	0.02	0.05	21.3	5.68	0.09
092017USADV01	DV	SAC 5	9/20/2017	Selenium	M6020 ICP-MS	1		mg/Kg	0.02	0.06	24	4.17	0.08
092017USADV02	DV	SAC 5	9/20/2017	Selenium	M6020 ICP-MS	0.83		mg/Kg	0.02	0.05	22	3.77	0.09
092017USADV04	DV	SAC 5	9/20/2017	Selenium	M6020 ICP-MS	0.89		mg/Kg	0.02	0.04	18.6	4.78	0.11
092017USADV05	DV	SAC 5	9/20/2017	Selenium	M6020 ICP-MS	1.29		mg/Kg	0.02	0.05	18.6	6.94	0.11
092017USADV07	DV	SAC 5	9/20/2017	Selenium	M6020 ICP-MS	1		mg/Kg	0.02	0.06	18.9	5.29	0.11
092017USADV011	DV	SAC 5	9/20/2017	Selenium	M6020 ICP-MS	1.16		mg/Kg	0.02	0.04	21	5.52	0.10
092017USADV012	DV	SAC 5	9/20/2017	Selenium	M6020 ICP-MS	1		mg/Kg	0.02	0.06	24.5	4.08	0.08
092017LMSADV01	DV	SAC 3	9/20/2017	Zinc	M6020 ICP-MS	42.1		mg/Kg	0.4	1	24.4	172.54	1.64
092017LMSADV02	DV	SAC 3	9/20/2017	Zinc	M6020 ICP-MS	47.5		mg/Kg	0.4	1	22.4	212.05	1.79
092017LMSADV04	DV	SAC 3	9/20/2017	Zinc	M6020 ICP-MS	44.1		mg/Kg	0.4	0.9	21.3	207.04	1.88
092017USADV01	DV	SAC 5	9/20/2017	Zinc	M6020 ICP-MS	18.2		mg/Kg	0.5	1	24	75.83	2.08
092017USADV02	DV	SAC 5	9/20/2017	Zinc	M6020 ICP-MS	23.8		mg/Kg	0.4	1	22	108.18	1.82
092017USADV04	DV	SAC 5	9/20/2017	Zinc	M6020 ICP-MS	26.8		mg/Kg	0.3	0.9	18.6	144.09	1.61
092017USADV05	DV	SAC 5	9/20/2017	Zinc	M6020 ICP-MS	33		mg/Kg	0.4	0.9	18.6	177.42	2.15
092017USADV07	DV	SAC 5	9/20/2017	Zinc	M6020 ICP-MS	21.3		mg/Kg	0.5	1	18.9	112.70	2.65
092017USADV011	DV	SAC 5	9/20/2017	Zinc	M6020 ICP-MS	26		mg/Kg	0.3	0.8	21	123.81	1.43
092017USADV012	DV	SAC 5	9/20/2017	Zinc	M6020 ICP-MS	21.7		mg/Kg	0.5	1	24.5	88.57	2.04

[1]Dolly Varden (DV)
[2]B=Analyte concentration detected at a value between MDL and PQL. The associated value is an estimated quantity.
[3]MDL=Method Detection Limit. PQL=Practical Quantitation Limit

Ruby Creek

Sample ID	Species[1]	Sample Site	Collect Date	ANALYTE	Method	Wet Wt. Result	QUAL[2]	UNITS	MDL[3]	PQL[3]	% solid	Dry Wt. Result	Dry Wt. MDL[3]
092117RUBYSS01	SS	RBC 3	9/20/2017	Cadmium	M6020 ICP-MS	0.07	B	mg/Kg	0.02	0.1	20.3	0.34	0.10
092117RUBYSS01	SS	RBC 3	9/20/2017	Copper	M6020 ICP-MS	1.7		mg/Kg	0.1	0.5	20.3	8.37	0.49
092117RUBYSS01	SS	RBC 3	9/20/2017	Mercury	M7473	46.6		ng/g	2.65	13.25	20.3	229.56	13.05
092117RUBYSS01	SS	RBC 3	9/20/2017	Selenium	M6020 ICP-MS	0.92		mg/Kg	0.02	0.06	20.3	4.53	0.10
092117RUBYSS01	SS	RBC 3	9/20/2017	Zinc	M6020 ICP-MS	43.1		mg/Kg	0.5	1	20.3	212.32	2.46
092117RUBYSS01	SS	RBC 3	9/20/2017	Moisture Content	D2216-80	79.7		%	0.1	0.5	20.3		0.49

[1]Slimy Sculpin (SS)

[2]B=Analyte concentration detected at a value between MDL and PQL. The associated value is an estimated quantity.

[3]MDL=Method Detection Limit. PQL=Practical Quantitation Limit

Appendix 4: Genetic Variation in Dolly Varden Collected in Subarctic Creek, 2017[1]

Introduction

A pilot study was conducted to evaluate genetic variation in Dolly Varden sampled in Shungnak River above the waterfall and compare it to anadromous Dolly Varden sampled in two tributaries lower in the Kobuk River. Waterfalls acting as barriers to migration are a primary cause of genetic divergence among fish populations within rivers. Genetic variation is typically reduced in populations above waterfalls due to founder events at the time of isolation or long-term reduction in population size following isolation (e.g., Crispo et al. 2006; Gomez-Ichida et al. 2009; Whitely et al. 2010; Carim et al. 2016). Genetic divergence of populations located above waterfalls is typically increased because gene flow is limited across the migration barrier (e.g., Costello et al. 2003; Gomez-Ichida et al. 2009). Gene flow, should it occur, has been documented more frequently from upstream of the waterfall to downstream than the reverse (Crispo et al. 2006; Whitely et al. 2010).

The effect of waterfalls on genetic population structure has been detected in numerous salmonids, including Dolly Varden. Dolly Varden isolated by waterfalls in streams of the North Slope of Alaska and Yukon, Canada had lower levels of heterozygosity and allele richness than their downstream counterparts (Everett et al. 1997; Harris et al. 2015). Waterfalls were an important source of genetic divergence in this region, as well as in Prince William Sound (Currens et al. 2003). Everett et al. (1997) suggested that gene flow occurring from populations above waterfalls to those below could lessen signals of population divergence. However, Harris et al. (2015) did not find evidence for first generation migrants originating from above waterfalls in samples of fish below the waterfall in two rivers in Canada, though some evidence of historical gene flow was detected.

Dolly Varden were sampled in Subarctic Creek, a tributary located above the waterfall on Shungnak River, to determine 1) do Subarctic Creek Dolly Varden contain less genetic variation than samples of anadromous Dolly Varden collected in Salmon and Tutuksuk rivers, tributaries of the mid Kobuk River, and 2) are Dolly Varden in Subarctic Creek divergent from the lower river tributaries?

Methods

Genetic variation was assayed at 11 microsatellite loci in 50 Dolly Varden collected on 19 July 2017 in Subarctic Creek. Loci assayed were *Ogo1A* (Olsen et al. 1998); *Sfo18* (Angers et al. 1995); *Smm-3*, *-5*, *-10*, *-17*, *-21*, *-22*, and *-24* (Crane et al. 2004); and Smm-*41* and *-44* (USFWS, unpublished). Total genomic DNA was isolated from fin tissue sampled from each fish using the Qiagen 96-well DNeasy® procedure. Polymerase chain reaction (PCR) amplifications were

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carried out in 10 ul reaction volumes using 30–60 ng DNA, 1.5–2.5 mM $MgCl_2$, 0.8–1 mM dNTPs, 0.1–0.6 µM labeled forward primer, 0.1–0.6 µM reverse primer, and 0.025–0.05 U/µl *Taq* polymerase, after adjusting DNA concentrations to 30 ng/l with a Perkin Elmer Victor 2030 microplate reader and Janus liquid handler. Applied Biosystems Veriti thermal cyclers were used for PCR with 1 cycle of 2 min at 92^o; 30 cycles of 15 sec at 92^o, 15 sec at 55^o-60^o, and 30 sec at 72^o, and a final extension for 10 min at 72^o. The PCR products were separated with an Applied Biosystems 3730 Genetic Analyzer using a polymer denaturing capillary system. Size scoring was completed using GeneMapper® Software ver 5.0. Applied Biosystems GeneScan™-600 LIZ® size standard, 20-600 bases, were loaded as internal size standards. Two researchers scored alleles independently. Samples with score discrepancies between researchers were re-amplified at the loci in question and rescored.

The program FSTAT ver 2.9.3.2 (Goudet 2001) was used to test for deviation of genotypic frequencies from Hardy-Weinberg expectation and genotypic disequilibrium and to estimate expected heterozygosity and allelic richness. Genetic data from fish collected from Subarctic Creek were combined with data collected from two tributaries of the Kobuk River in 2016, Salmon River N=112 and Tutuksuk River N=78 (Savereide and Crane 2017). The program FSTAT was used to estimate F_{ST} and to test for allele frequency heterogeneity between pairs of collections. The loss of heterozygosity and allelic richness in Subarctic Creek compared to Salmon River and Tutuksuk River was calculated following Whitely et al. (2010), which assumes that contemporary genetic variation within populations located below barriers are representative of the genetic variation present above the barrier before isolation. STRUCTURE 2.3.4 (Pritchard et al. 2000; Falush et al. 2003) was used to infer K, the number of Hardy-Weinberg linkage-equilibrium groups without assuming population membership a *priori*. The program defaults of an admixture model with correlated allele frequencies were used in 5 independent runs, with each run consisting of 100,000 MCMC samples after a burn in of 100,000, and varying K from 1-5. The program STUCTURE HARVESTER (Earl and vonHoldt 2012) was used to collate the results of the STRUCTURE runs and infer K using the ΔK method (Evanno et al. 2005). STRUCTURE was used to determine if individuals with migrant ancestry from Subarctic Creek were present in either of the below barrier tributaries following Pritchard et al. (2000); this analysis assumes all genetic groups present in the Kobuk River are represented. This STRUCTURE run included prior population information as determined above; individuals assigned with cluster proportion $q > 0.8$ to their sampling locality were considered residents, individuals with $q < 0.2$ were considered immigrants, and individuals with $0.2 < q < 0.8$ were considered to have immigrant ancestry (Bergl and Vigilant 2007).

Results

Genotypic frequencies of Dolly Varden sampled from Subarctic Creek did not deviate from Hardy-Weinberg proportions (P=0.33). Significant genotypic disequilibrium was detected in two locus pairs (P<0.05), but these did not remain significant after Bonferroni adjustment for 36 multiple tests. Expected heterozygosity ranged from 0.55 in Subarctic Creek to 0.63 in both

Salmon River and Tutuksuk River, an average reduction of 13%. Allelic richness ranged from 6.15 in Subarctic Creek to 9.26 in Tutuksuk River, an average reduction of 36%. Six alleles over three loci were observed in Subarctic Creek samples that were not seen in the below barrier collections.

Significant allele frequency heterogeneity was detected between all population pairs (*P*=0.02). Pairwise F_{ST}s between Subarctic Creek and below-barrier collections were 0.067 and 0.062; F_{ST} between Salmon River and Tutuksuk River was 0.012. The highest posterior probability in all five STRUCTURE runs was for K=2 genetic clusters, also supported by ΔK (Appendix Figure 1). Using sampling localities corresponding to above- and below barrier as a prior for the final STRUCTURE run showed all except two individuals had q>0.8 from their sampling locality. These individuals were sampled in Tutuksuk River, with q-values of 0.66 and 0.55 suggestive of immigrant ancestry.

Discussion

The presence of waterfalls in a river system can limit dispersal, resulting in reduced genetic variation and increased genetic divergence in populations above the waterfall relative to populations below (Koizumi et al. 2006, Crispo et al. 2006). The pilot study for Dolly Varden in Subarctic Creek was no exception, with genetic diversity patterns in Kobuk River samples following these general expectations.

First, there was a signal of reduced within-population variation in Dolly Varden sampled from Subarctic Creek relative to two tributaries lower in the Kobuk River. Samples collected in Subarctic Creek averaged a 36% loss in allelic richness relative to samples collected in Salmon River and Tutuksuk River, and a 13% loss in gene diversity. These numbers fall near the low end of the range of values (17%-100% for gene diversity, 30%-100% for allele richness) reported for cutthroat trout *Onchorhynchus clarkii clarkii* in 8 streams used to assess the maintenance of genetic variation in isolated populations (Whitely et al. 2010). Possible reasons for comparably higher levels of retention of genetic variation in Subarctic Creek Dolly Varden are habitat quantity/availability or the possibility of multiple populations of Dolly Varden in the Shungnak River drainage connected through gene flow. Whitely et al. (2010) found that there was a negative correlation between habitat size and the loss of genetic variation, particularly in stream lengths less than approximately 2km. Subarctic Creek alone exceeds 12km. Dolly Varden were detected in Shungnak River upstream of Subarctic Creek and in Ruby Creek downstream of Subarctic Creek (Bradley 2017a), suggesting the possibility of interconnected habitat patches. Further, if there are multiple spawning populations of Dolly Varden in the Shungnak River, gene flow among these populations may aid in maintaining genetic variation.

Second, there was a signal of increased genetic divergence of Dolly Varden sampled from Subarctic Creek relative to two tributaries in the mid-Kobuk River. Pairwise F_{ST} was far greater between Subarctic Creek and the two lower Kobuk River tributaries (~0.06) than between the Salmon and Tutuksuk rivers (~0.01). This is similar to patterns observed in Dolly Varden in Prince William Sound, where genetic differentiation of populations across waterfalls was twice that of populations located below waterfalls (Currens et al. 2003). Without *a priori* population definition, the program STRUCTURE detected two genetic clusters in the set of samples from the Kobuk River, corresponding to above- and below waterfall localities. The average proportion of the above-barrier group genome assigned to samples collected in Subarctic Creek was 96%, while the average proportion of the below-barrier genome assigned to Salmon River and Tutuksuk River samples was 86%, suggesting a signal of greater gene flow downstream of Shugnak River. These proportions are very similar to those reported by Whitely et al. (2010), where 15% of the genome of cutthroat trout sampled below waterfalls in 8 streams in Southeast Alaska originated from the adjacent above-waterfall population, yet only 3% in the reverse direction. Assymetric, downstream migration has been heavily documented in stream-dwelling fishes, with and without the presence of physical barriers to migration (e.g., Crispo et al. 2006, Hänfling and Weetman 2006, Whitely et al. 2010). When using population information as a prior, two individuals with migrant ancestry were detected among the Tutuksuk River samples, but no individuals of migrant ancestry were detected in Subarctic Creek.

In conclusion, Dolly Varden from Subarctic Creek showed reduced levels of within population variation and increased genetic divergence relative to Dolly Varden from two tributaries of the middle Kobuk River. Further sampling of Dolly Varden in the Shungnak River drainage to determine if further population subdivision exists would allow an assessment of the mechanisms maintaining genetic variation in Subarctic Creek. Further sampling of Dolly Varden in the Kobuk River, particularly tributaries in the upper portion of the drainage, would allow further assessment of the factors leading to genetic divergence (e.g., distance between populations versus barriers to migration), and patterns of gene flow in the drainage.

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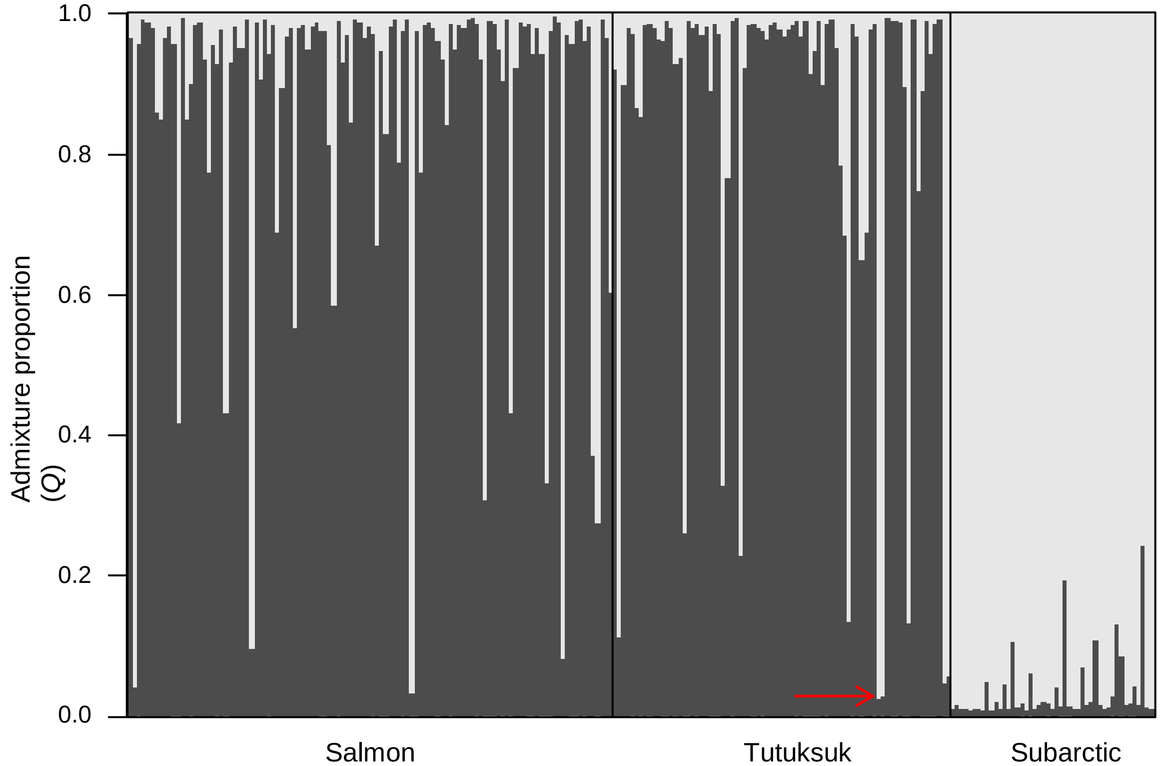

Appendix Figure 1. Proportion of the genome belonging to two genetic groups estimated by STRUCTURE for samples of Dolly Varden collected from three tributaries of the Kobuk River: Salmon River, Tutuksuk River, and Subarctic Creek. Each column represents a sampled fish; dark grey corresponds to the genetic group associated below the Shungnak River, and light grey corresponds to the genetic group associated above the waterfall on Shungnak River. The two individuals identified by the arrow are possibly of migrant ancestry.