Technical Report No. 19-05

Aquatic Biomonitoring at the Arctic-Bornite Prospect, 2018

by Chelsea M. Clawson



October 2019

Alaska Department of Fish and Game



Habitat Section

Symbols and Abbreviations

The following symbols and abbreviations, and others approved for the Système International d'Unités (SI), are used without definition in reports by the Divisions of Habitat, Sport Fish and of Commercial Fisheries. All others, including deviations from definitions listed below, are noted in the text at first mention, as well as in the titles or footnotes of tables, and in figure or figure captions.

Weights and measures (metric)		General		Measures (fisheries)			
centimeter	cm	Alaska Administrative		fork length	FL		
deciliter	dL	Code	AAC	mideye-to-fork	MEF		
gram	g	all commonly accepted		mideye-to-tail-fork	METF		
hectare	ha	abbreviations	e.g., Mr., Mrs.,	standard length	SL		
kilogram	kg		AM, PM, etc.	total length	TL		
kilometer	km	all commonly accepted					
liter	L	professional titles	e.g., Dr., Ph.D.,	Mathematics, statistics			
meter	m		R.N., etc.	all standard mathematical			
milliliter	mL	at	@	signs, symbols and			
millimeter	mm	compass directions:		abbreviations			
		east	E	alternate hypothesis	H_A		
Weights and measures (English)		north	Ν	base of natural logarithm	е		
cubic feet per second	ft ³ /s	south	S	catch per unit effort	CPUE		
foot	ft	west	W	coefficient of variation	CV		
gallon	gal	copyright	©	common test statistics	$(F, t, \chi^2, etc.)$		
inch	in	corporate suffixes:		confidence interval	CI		
mile	mi	Company	Co.	correlation coefficient			
nautical mile	nmi	Corporation	Corp.	(multiple)	R		
ounce	07	Incorporated	Inc.	correlation coefficient			
pound	lh	Limited	Ltd.	(simple)	r		
quart	at	District of Columbia	D.C.	covariance	COV		
vard	yd vd	et alii (and others)	et al.	degree (angular)	0		
yard	yu	et cetera (and so forth)	etc.	degrees of freedom	df		
Time and temperature		exempli gratia		expected value	E		
day	d	(for example)	e.g.	greater than	>		
degrees Celsius	°C	Federal Information	. 8.	greater than or equal to	>		
degrees Eshrenheit	°F	Code	FIC	harvest per unit effort	- HPUE		
degrees kelvin	ĸ	id est (that is)	i.e.	less than	<pre>/// 01</pre>		
hour	h	latitude or longitude	lat. or long.	less than or equal to	~		
minute	min	monetary symbols	8.	logarithm (natural)	 In		
sacond		(U.S.)	\$. ¢	logarithm (base 10)	log		
second	3	months (tables and	-, -	logarithm (specify base)	log, etc		
Dhysics and shamistary		figures): first three		minute (angular)	10 <u>52</u> , etc.		
all stamic sumbals		letters	Ian Dec	not significant	NS		
all atomic symbols	AC	registered trademark	®	null hypothesis	Ц.		
	AC	trademark	тм	percent	11 ₀ %		
ampere	A	United States		probability	70 D		
calorie	cai	(adjective)	US	probability of a type Larror	r		
direct current	DC	United States of	0.5.	(rejection of the pull			
hertz	Hz	America (noun)	USA	(rejection of the num			
horsepower	hp		United States	hypothesis when true)	α		
hydrogen ion activity	рН	0.3.C.	Code	probability of a type II error			
(negative log of)		U.S. state	use two-letter	(acceptance of the hull	0		
parts per million	ppm	0.5. state	abbreviations	nypotnesis when faise)	p "		
parts per thousand	ppt,		(e.g., AK. WA)	second (angular)	CD.		
	‰			standard deviation	SD		
volts	V			standard error	SE		
watts	W			variance	••		
				population	Var		
				sample	var		

TECHNICAL REPORT NO. 19-05

AQUATIC BIOMONITORING AT THE ARCTIC-BORNITE PROSPECT, 2018

By

Chelsea M. Clawson Habitat Section, Fairbanks

Alaska Department of Fish and Game Habitat Section 1300 College Rd, Fairbanks, Alaska, 99701

October 2019

Cover: Dolly Varden from Subarctic Creek, upper site, July 27, 2018. Photograph by Audra Brase.

Technical Reports are available through the Alaska State Library, Alaska Resources Library and Information Services (ARLIS) and on the Internet: http://www.adfg.alaska.gov/index.cfm?adfg=habitat_publications.main.

This publication has undergone editorial review.

Note: Product names used in the publication are included for completeness but do not constitute product endorsement. The Alaska Department of Fish and Game does not endorse or recommend any specific company or their products.

Chelsea M. Clawson Alaska Department of Fish and Game, Habitat Section 1300 College Rd., Fairbanks, AK 99701-1599, USA

This document should be cited as:

Clawson, C. M. 2019. Aquatic Biomonitoring at the Arctic-Bornite Prospect, 2018. Alaska Department of Fish and Game, Technical Report No. 19-05, Fairbanks, Alaska.

The Alaska Department of Fish and Game (ADF&G) administers all programs and activities free from discrimination based on race, color, national origin, age, sex, religion, marital status, pregnancy, parenthood, or disability. The department administers all programs and activities in compliance with Title VI of the Civil Rights Act of 1964, Section 504 of the Rehabilitation Act of 1973, Title II of the Americans with Disabilities Act (ADA) of 1990, the Age Discrimination Act of 1975, and Title IX of the Education Amendments of 1972.

If you believe you have been discriminated against in any program, activity, or facility please write: ADF&G ADA Coordinator, P.O. Box 115526, Juneau, AK 99811-5526

U.S. Fish and Wildlife Service, 4401 N. Fairfax Drive, MS 2042, Arlington, VA 22203

Office of Equal Opportunity, U.S. Department of the Interior, 1849 C Street NW MS 5230, Washington DC 20240

The department's ADA Coordinator can be reached via phone at the following numbers:

(VOICE) 907-465-6077, (Statewide Telecommunication Device for the Deaf) 1-800-478-3648, (Juneau TDD) 907-465-3646, or (FAX) 907-465-6078

For information on alternative formats and questions on this publication, please contact:

ADF&G Division of Sport Fish, Research and Technical Services, 333 Raspberry Road, Anchorage AK 99518 (907) 267-2375

TABLE OF CONTENTS

Page
LIST OF TABLES
LIST OF FIGURES
ACKNOWLEDGEMENTS iv
INTRODUCTION
METHODS
Sampling Overview
Water Quality
Periphyton
Aquatic invertebrates
Fish
RESULTS AND DISCUSSION
Water Quality
Periphyton12
Aquatic Invertebrates
Fish Captures15
Fish Age and Maturity
Fish Metals25
CONCLUSION
LITERATURE CITED
APPENDIX 1. WATER QUALITY DATA FROM 2018
APPENDIX 2. LIST OF FISH RETAINED FOR WHOLE BODY ELEMENT ANALYSIS33
APPENDIX 3. RESULTS FOR WHOLE BODY ELEMENT ANALYSIS
APPENDIX 4. GENETIC VARIATION IN DOLLY VARDEN COLLECTED IN RED
ROCK CREEK, 2018

LIST OF TABLES

	Page
Table 1. Arctic-Bornite sampling locations (WGS 84), 2018.	5
Table 2. Mean chlorophyll-a concentrations ± 1 SD, 2016 to 2018	12
Table 3. Number, mean length, and length range of slimy sculpin and Dolly Varden	17
Table 4. Total number of Dolly Varden captured at each site	22
Table 5. Size, maturity and age of five Dolly Varden captured on Upper Subarctic Creek	24

LIST OF FIGURES

Page
Figure 1. Location of the Arctic and Bornite deposits in northwest Alaska 1
Figure 2. Waterfall on the Shungnak River blocking fish passage upstream, July 21, 2016 2
Figure 3. All locations sampled in 2018
Figure 4. Drift nets set for capturing aquatic invertebrates7
Figure 5. Fyke nets near the mouth of Ruby Creek July 21, 2018
Figure 6. Median, minimum, and maximum analyte concentrations at water quality sample sites,
2018
Figure 12. Mean number of aquatic invertebrates/m ³ at each sample site, 2016-2018 14
Figure 13. Mean percent EPT, aquatic diptera, and other species 14
Figure 14. Percent EPT and Chironomidae in the aquatic invetebrate samples
Figure 15. Young of the year Dolly Varden
Figure 16. Alaska blackfish and longnose sucker captured in Lower Ruby minnow traps 16
Figure 17. Series of small waterfalls on Red Rock Creek, and a Dolly Varden captured above the
falls on July 22, 201817
Figure 18. Length frequency distribution of Arctic grayling
Figure 19. Ruby Creek fyke net fish captures by species for 2016-2018
Figure 20. Mean daily discharge in Dahl Creek
Figure 21. Mature male Dolly Varden captured at the Mid Subarctic Creek sample site
Figure 22. Ripe female Dolly Varden on Lower Red Rock Creek with eggs
Figure 23. Ripe male Dolly Varden captured at Lower Center of the Universe Creek
Figure 24. Length frequency distribution of resident Dolly Varden
Figure 25. Mature female Dolly Varden, and an immature female Dolly Varden
Figure 26. Mean whole body dry weight concentrations of cadmium
Figure 27. Mean whole body dry weight concentrations of copper
Figure 28. Mean whole body dry weight concentrations of mercury
Figure 29. Mean whole body dry weight concentrations of selenium
Figure 30. Mean whole body dry weight concentrations of zinc

ACKNOWLEDGEMENTS

The author would like to thank Trilogy Metals and Cal Craig (of Trilogy Metals) for their logistical and financial support in monitoring fish and wildlife resources at the Arctic-Bornite Prospect.

Maria Wessel with the Alaska Department of Fish and Game (ADF&G) Habitat Section, provided assistance with laboratory work and field sampling, Audra Brase (ADF&G Habitat) assisted with field sampling, and Nora Foster of NRF Taxonomic Services was responsible for sorting and identification of aquatic invertebrates. Additionally, Penny Crane, Randal Loges, and Clarissa Zeller with the United States Fish and Wildlife Service (USFWS) Conservation Genetics Laboratory processed the Dolly Varden genetic samples and provided a summary of results.

Audra Brase and Dr. Al Ott (ADF&G Habitat) provided constructive reviews of this report.

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 about 17 km north of Kobuk in the Ruby Creek drainage, and the Arctic deposit is located approximately 37 km northeast of Kobuk in the upper end of the Subarctic Creek drainage. The Bornite deposit contains primarily copper while the Arctic deposit contains copper, lead, zinc, silver and gold. 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 prevents upstream passage of fish, so no anadromous fish occur near the sites (Figure 2). All fish in the area of the Bornite and Arctic deposits complete their life cycle within the Shungnak River drainage.

All sample sites except Riley Creek are in the Shungnak River drainage. Riley Creek, which flows into the Kogoluktuk River, was selected to sample as it is being considered as a possible location 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 presence (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 Habitat Section to continue aquatic sampling beginning in 2016. The ADF&G study plan was based on aquatic biomonitoring the Habitat Section 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 samples collected by ADF&G in 2018, as well as water quality data collected by Trilogy Metals in 2018, and the genetic analysis conducted by USFWS Conservation Genetics Laboratory.

METHODS

Sampling Overview

Based on results from 2017, some sample sites were eliminated, and some new sites were added in 2018 (Figure 3; Table 1). The Lower Middle Subarctic Creek (SAC 3) and the two Middle Ruby Creek sites (RBC 3 and RBC 4) were eliminated from the sampling roster since the remaining sites on each creek adequately captured the variability in habitats and aquatic conditions. Several new sites were added in the Red Rock Creek drainage, which is the next creek up the Shungnak River drainage from Subarctic Creek and could provide alternative habitat if Subarctic Creek is impacted by future mining activity.

The first sampling event occurred June 26 - 29, 2018. The objective was to capture Dolly Varden fry using aquatic invertebrate drift nets to determine where spawning occurred.

The second sampling event took place July 20 - 26, 2018. At each location replicate samples of the aquatic community were performed; including aquatic invertebrates, periphyton, and fish. Some fish were retained for whole body element analysis.

The third sampling event occurred September 18 - 20, 2018. The objective was to determine Dolly Varden distribution in the early fall, assess their spawning condition, and obtain additional fish for element analysis if needed. Caudal fin clips for genetic samples were also collected. Additionally, a small number of large, mature fish were retained for laboratory dissection to determine spawning condition, and their otoliths were extracted for age determination.

Sampling effort was concentrated in Ruby and Subarctic creeks as these locations are where there may be changes to the aquatic system based on projected mining development. The reference site in the upper Shungnak River was sampled again in 2018, as well as the Riley Creek and Jay Creek sites.

The objective of the biological monitoring program was to document in-situ productivity of aquatic communities in the vicinity of and 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 2016 report summarized all water quality data collected since 2008 (Bradley 2017a). This report summarizes only data collected in 2018. 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 near the 2018 sample sites were used. Depending on the sample site, two to four water samples were collected from March to December 2018.

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 eight locations around the Arctic-Bornite area (Table 1; Figure 3).

Ten flat rocks, each larger than 25 cm² 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 and washed back into the stream. The foam square was then removed from the rock, and that section of the rock was brushed and rinsed onto a 0.45 μ m 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 (MgCO₃) were added to the water to prevent 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 paper coffee filters. All ten coffee filters were placed in a zip-lock bag containing desiccant to absorb 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).

Water Body/ Sample Site	Latitude	Longitude	June larval/ minnow sampling	July periphyton/ invertebrate/ minnow traps	July minnow traps	July fyke nets	September minnow traps
Shungnak R.			10	1	ł		1
Upper	67.24404	-156.61598		X*			
Middle	67.16942	-156.62933				Х	
Subarctic Cr.							
Upper	67.19264	-156.39114	Х	Х			Х
Middle	67.15987	-156.56725	Х				Х
Lower	67.17198	-156.62079	Х	Х			Х
Mouth	67.16942	-156.62933				Х	
Ruby Cr.							
Upper	67.04078	-156.93936	Х	Х			
Lower	67.11140	-156.90843	Х	Х			
Mouth	67.11395	-156.91669				Х	
Red Rock Cr. ¹							
Upper	67.20915	-156.45293			Х		Х
Middle	67.19482	-156.52071			Х		Х
Lower	67.19324	-156.59906		Х			Х
Center of the							
Universe Cr. ¹							
Upper	67.20997	-156.40411					Х
Lower	67.21313	-156.42757					Х
Jay Cr.							
JC	67.08041	-156.94453		Х			
Riley Cr.	17 0 10 1 1	1.5.6.60.000					
RIC	67.04264	-156.69229		Х			
Road Xing 1	67.10086	-156.74783			Х		
Road Xing 2	67.13530	-156.70299			Х		

Table 1. Arctic-Bornite sampling locations (WGS 84), 2018.

* The Upper Shungnak sampling in July was periphyton and invertebrate only.

¹ All sample sites in Red Rock and Center of the Universe creeks were new in 2018.



Figure 3. All locations sampled in 2018. The approximate location of the Bornite and Arctic deposits are denoted by the green polygons. The Bornite deposit is in the Ruby Creek drainage and the Arctic deposit is in the Subarctic Creek drainage.

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 more sensitive to water quality, percent composition of EPT was calculated and compared to groups of other invertebrates, which are less sensitive.



Figure 4. Drift nets set for capturing aquatic invertebrates at the Upper Shungnak River reference site (July 22, 2018).

Fish

From June 29 – 29, larval fish sampling occurred at a total of five sites in Subarctic Creek and Ruby Creek (Table 1). Three 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. Additionally, two drift nets were placed upstream and allowed to fish passively (no kicking or disturbance) for one hour. Contents of all nets were removed and run through a series of sieves with decreasing mesh size and then examined for larval fish. We also placed 5 - 10 baited minnow traps at each site to determine if juvenile or adult Dolly Varden were present.

The primary fish sampling event occurred July 20 - 26. 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 invertebrate sampling locations (Table 1). Traps were placed in a variety of habitats, including cut banks, pools, and near submerged woody debris. Traps soaked overnight and checked about 24 hours later. All captured fish were measured for fork or total length, depending on species. 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 bags were placed in a cooler with ice packs and transferred to a freezer in the camp as soon as possible. The samples remained frozen until they were analyzed by ACZ Laboratories, Inc.

In addition to the minnow traps, fyke nets were set at the mouth of Ruby Creek, the mouth of Subarctic Creek, and on the Shungnak River upstream of the mouth of Subarctic Creek during the July sampling event (Table 1, Figure 5). Two nets were set at Ruby Creek to capture fish moving both upstream and downstream. A single net was set at Subarctic Creek to capture fish moving downstream, and a single net to capture fish moving upstream was set on the Shungnak River. Nets were fished for about 24 hours.

The fall sampling event occurred September 18 – 20. A total of seven sites were sampled for fish with minnow traps, using methods identical to the July sampling event (Table 1). Fifteen fish were retained from Red Rock Creek for whole body element analysis using the same methods as the July sampling. Five fish from Subarctic Creek were retained for dissection to determine spawning condition and age. Additionally, fin clips were taken from 50 Dolly Varden in Red Rock Creek for genetic analysis. Fin clips were stored in individually labelled 1.2 ml cryovials filled with silica desiccant. Samples were sent to the USFWS Conservation Genetics Laboratory, assayed for genetic variation, and compared to other Dolly Varden populations from the Kobuk River drainage (Appendix 4).



Figure 5. Fyke nets near the mouth of Ruby Creek July 21, 2018.

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 are at the same location as the periphyton, aquatic invertebrate, and fish sampling. However, the water quality data from the Shungnak River used in these results were collected just upstream of the mouth of Subarctic Creek, not at the reference site further upstream (Upper Shungnak River). Non-detect element data are graphically presented as the detection limit.

In general, median cadmium values in 2018 were low and similar to previous years (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 all sampling events except the June sample on the Shungnak River (Appendix 1). The result of 0.219 μ g/L slightly exceeded the chronic exposure limit of 0.210 μ g/L, but was still well under the acute exposure limit of 1.530 μ g/L.

Median selenium values were very low and similar among all sample sites (Figure 6, Appendix 1). All values were well below the current water quality standard for aquatic life which is $20 \,\mu g/L$ for acute exposure and $5 \,\mu g/L$ for chronic exposure.

Median copper values were relatively similar among all sites and ranged from $0.250 \mu g/L$ at Upper Subarctic Creek to $1.620 \mu g/L$ at the Shungnak River site (Figure 6, Appendix 1). The highest maximum value for copper was $1.820 \mu g/L$ and occurred at the Shungnak River site in June. 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 all sampling events (Appendix 1).

Mercury values were low at all sample sites (Figure 6). Median mercury values ranged from 0.687 ng/L at Upper Subarctic Creek to 1.006 ng/L at Lower Ruby Creek. The highest maximum value (2.030 ng/L) occurred at Upper Ruby Creek in August. 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.

Median zinc concentrations were lowest in Lower Riley Creek (3.10 μ g/L) and highest in the Shungnak River (17.60 μ g/L) (Figure 6). Overall, zinc concentrations were very low and well below the water quality standard for aquatic life, which depends on water hardness (Appendix 1).

Total Dissolved Solids (TDS) values in 2018 followed a pattern very similar to past years. Lowest median values occurred in Upper Subarctic Creek (56 mg/L) and Lower Subarctic Creek (53 mg/L) and highest values occurred in Upper Ruby Creek (167 mg/L) and Lower Ruby Creek (165 mg/L) (Figure 6, Appendix 1).



Figure 6. Median, minimum, and maximum analyte concentrations at water quality sample sites, 2018.

Periphyton

Mean chlorophyll-a concentrations were highest in Upper Ruby Creek (58.36 mg/m²) and lowest in Lower Red Rock Creek (0.14 mg/m^2) (Table 2). The mean chlorophyll-a concentrations at the remaining sites ranged from 0.38 mg/m^2 to 3.54 mg/m^2 . Mean chlorophyll-a concentrations decreased from the July 2017 values at all sites except Upper Ruby, where mean chlorophyll-a concentration was more than five times higher than 2017.

Site	2018	2017	2016
	Mean Chlorophyll-a	Mean Chlorophyll-a	Mean Chlorophyll-a
	(mg/m^2)	(mg/m^2)	(mg/m^2)
Upper Ruby	58.36 ± 26.56	10.50 ± 6.35	13.91 ± 5.34
Lower Ruby	1.31 ± 0.99	2.39 ± 1.55	3.87 ± 2.12
Shungnak	0.38 ± 0.43	1.37 ± 0.76	3.09 ± 2.44
Upper Subarctic	1.27 ± 0.61	3.87 ± 2.93	3.56 ± 1.20
Lower Subarctic	0.93 ± 0.34	1.03 ± 0.40	1.23 ± 0.36
Riley	1.42 ± 0.74	4.07 ± 3.61	7.24 ± 4.18
Jay	3.54 ± 2.70	$9.49 \hspace{0.1in} \pm 7.72 \hspace{0.1in}$	
Red Rock	0.14 ± 0.11		

Table 2. Mean chlo	rophyll-a concentrations \pm	1 SD	, 2016 to) 2018.
--------------------	--------------------------------	------	-----------	----------------

Aquatic Invertebrates

The Upper Ruby Creek site (UR) is characterized by beaver pond habitats, deep water, dense vegetative cover, short channels between beaver dams, and minimal gravel/cobble. The sample site is in a channel between beaver dams and was chosen for its gravel/cobble substrate. Aquatic invertebrate densities in Upper Ruby Creek were the highest among the sample sites and averaged 22.5 aquatic invertebrates/m³ of water (Figure 7). A total of 25 taxa were identified in the Upper Ruby Creek samples. Samples were dominated by aquatic Diptera (50%), which were primarily chironomids, and followed closely by other species (48%), which were primarily Ostracods and Cladocerans. Ephemeroptera, Plecoptera, and Tricoptera (EPT) only made up 3% of the sample (Figures 8 and 9).

The Lower Ruby Creek sample site (LR) is characterized by pool/riffle habitat, shallower water, gravel/cobble substrate, and grass riparian habitats. Densities in Lower Ruby Creek averaged 3.8 aquatic invertebrates/m³ of water (Figure 7). A total of 24 taxa were identified in Lower Ruby Creek. Samples were dominated by other species (47%) which were primarily Cladocerans and Acarians, followed by aquatic Diptera (40%), which were primarily chironomids, and EPT species (13%) (Figures 8 and 9).

The Shungnak River sample site (SH) is characterized by deep water, outside bend cut banks and inside bend gravel bars. The substrate is primarily gravel with some cobble. Species richness was the highest among all the sample sites with 34 total different taxa identified, yet it had the lowest density at 1.4 aquatic invertebrates/m³ (Figure 7). This site also had one of the highest percentages of EPT species at 30%, most of which were species in the order Ephemeroptera (Figures 8 and 9). The remaining samples were comprised of aquatic Diptera (37%), primarily chironomids, and other species (33%).

The Upper Subarctic Creek sample site (US) is in alpine tundra and is characterized by high gradient with step pools and large boulders. There are some shrubby willows along the banks, but most vegetation is limited to ground cover. The average aquatic invertebrate density in Upper Subarctic Creek in 2018 was 3.5 aquatic invertebrates/m³ (Figure 7), which is much lower than the density observed in 2016 (23.9 aquatic invertebrates/m³) and 2017 (11.1 aquatic invertebrates/m³). However, similar to past years, this site had the lowest species richness with 18 total taxa identified. Other species, primarily Ostracods, accounted for 69%, EPT accounted for 16%, and aquatic Diptera, mostly chironomids, accounted for 15% (Figures 8 and 9). Upper Subarctic Creek was one of only two sample sites where % EPT was higher than % chironomids. Very large numbers of Cladocerans were captured in 2016; however, none were captured in 2017 or 2018. This sample site is located a few hundred yards below the origin of the creek, which abruptly forms when water transitions from subsurface to surface flow.

The Lower Subarctic Creek site (LS) has a much lower gradient than the upper site, is wider, and is characterized by riffle/pool habitat with gravel/cobble substrate. The average aquatic invertebrate density was 2.6 aquatic invertebrates/m³ (Figure 7). Aquatic invertebrates were comprised of aquatic Diptera (46%), most of which were chironomids, other species (28%), most of which were Acarians, and EPT species (25%) (Figures 8 and 9).

The Riley Creek site (RI) is characterized by riffle/pool habitat with gravel and cobble. The average aquatic invertebrate density in Riley Creek was 2.6 aquatic invertebrates/m³ (Figure 7). A total of 25 taxa were captured represented primarily by aquatic Diptera (65%), followed by other species (21%), then EPT species (14%) (Figures 8 and 9).

The Jay Creek site (JAY) 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 6.5 aquatic invertebrates/m³ (Figure 7). Aquatic Diptera dominated the community composition (52%), most of which were chironomids, followed by other species (36%), and EPT (11%) (Figures 8 and 9).

The Lower Red Rock Creek sample site (RR) has similar habitat to the Lower Subarctic Creek site, with riffle/pool habitat and gravel/cobble substrate. The average aquatic invertebrate density

was 1.87 aquatic invertebrates/m³, the second lowest average density (Figure 7). However, this site had the highest percentage of EPT at 44%. The remaining aquatic invertebrates were comprised of other species (40%), most of which were Acarians and Ostracods, followed by aquatic Diptera (15%) (Figures 8 and 9).



Figure 7. Mean number of aquatic invertebrates/ m^3 (± 1 SD) at each sample site, 2016-2018.



Figure 8. Mean percent EPT, aquatic diptera, and other species in the aquatic invertebrate samples, 2016 to 2018.



Figure 9. Percent EPT and Chironomidae in the aquatic invertebrate samples at all sample sites, 2016 to 2018.

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, during the July fish sampling a small juvenile Dolly Varden was observed in a pool on the Middle Red Rock Creek minnow trap site and was captured by hand (Figure 10). This fish was approximately 20 mm long and was a young of the year Dolly Varden. This was the first confirmation of spawning Dolly Varden from our sampling sites.



Figure 10. Young of the year Dolly Varden captured by hand at Middle Red Rock Creek site on July 23, 2018.

June minnow traps

Minnow traps were set at Upper, Middle, and Lower Subarctic Creek, Upper Ruby Creek, and Lower Red Rock Creek. Three Dolly Varden ranging from 89 to 126 mm FL were captured at the Upper Subarctic site, no fish were captured at the Middle Subarctic site, and two slimy sculpin (50 and 62 mm TL), were captured at the Lower Subarctic site. Two slimy sculpin (50 and 62 mm TL) were captured at Upper Ruby. The minnow traps at Lower Red Rock Creek captured one slimy sculpin (46 mm TL) and three Dolly Varden that ranged from 78 to 122 mm FL.

July Minnow Traps

Throughout Ruby Creek, slimy sculpin dominated catches, followed by Alaska blackfish (*Dallia pectoralis*), Dolly Varden, and longnose sucker (*Catostomous catostomous*) (Table 3). This was the first time Alaska blackfish and longnose suckers have been captured in minnow traps in this creek (Figure 11). There are many beaver dams in this drainage which may impede upstream passage of fish, but may also provide overwintering habitat in Upper Ruby Creek. Fifteen slimy sculpin from Upper Ruby Creek and 12 slimy sculpin from Lower Ruby Creek were retained for element analysis.



Figure 11. Alaska blackfish (left) and longnose sucker (right) captured in Lower Ruby minnow traps on July 24, 2018.

Dolly Varden dominated catches in Subarctic Creek, with only four slimy sculpin captured at the lower sample site (Table 3). Most of the Dolly Varden were caught at the upper site. Fifteen Dolly Varden from the upper sample site were retained for whole body element analysis.

In 2017, the Riley Creek sample site was moved further downstream to increase the likelihood of capturing fish, and this same location was sampled in 2018. A total of 25 slimy sculpin and five Dolly Varden were captured (Table 3). Five Dolly Varden and nine slimy sculpin were retained for whole body element analysis.

The Jay Creek sample site was added in 2017 and was sampled again in 2018. A total of nine Dolly Varden were captured, and all nine were retained for whole body element analysis. Jay Creek

is a small tributary to Ruby Creek and is characterized by riffle run habitat and dense vegetation cover.

The three sites in Red Rock Creek were added in 2018 to ascertain if Red Rock Creek could provide viable fish habitat in case Subarctic Creek is impacted by mine development. Dolly Varden were captured at all three sample sites, even above a series of small waterfalls between the middle and upper sample sites (Figure 12). A total of 12 Dolly Varden and three slimy sculpin were captured, and six Dolly Varden were retained for element analysis.



Figure 12. Series of small waterfalls on Red Rock Creek, and a Dolly Varden captured above the falls on July 22, 2018.

Table 3. Number, mean length, and length range of slimy sculpin and Dolly Varden captured in minnow traps at 11 sites, July 20–26, 2018.

		Slimy Sculpin			Dolly Varden				
Sample Site	Number captured	Mean total length (mm)	Length range (mm)	Number captured	Mean fork length (mm)	Length range (mm)			
Subarctic Cr.									
Upper	0			35	115	72-152			
Lower	4	62	60-64	4	126	89-160			
Ruby Cr.									
Upper	21	74	28-95	1	170				
Lower ¹	15	65	52-76	0					
Red Rock Cr.									
Upper	0			2	145	136-153			
Middle	0			8	97	51-140			
Lower	3	65	63-67	2	109	108-110			
Jay Cr.	0			9	125	102-150			
Riley Cr. ²	25	64	45-93	5	106	66-133			
Road Xing 1	0			0					
Road Xing 2	9	74	59-103	0					

¹One longnose sucker and three Alaska blackfish were also captured at this site

²Traps were reset for an additional 24 hours to increase sample size of fish for element analysis

Fyke nets

Two fyke nets were set near the mouth of Ruby Creek on July 21 and checked 24 hours later on July 22. A total of 28 Arctic grayling, six round whitefish, one longnose sucker, one Dolly Varden, and one slimy sculpin were captured. Most of the Arctic grayling were captured moving upstream. In 2016, most of the Arctic grayling captured were likely age-0 (49 – 66 mm) (Bradley 2017a). However, no age-0 Arctic grayling were captured in 2017 (Bradley 2018) or during this year's sampling. Most of the Arctic grayling captured were likely age-1 or age-2 fish (Figure 13). In 2017, 50 longnose suckers were captured moving upstream in Ruby Creek, which was the first-time longnose suckers had been documented in any sampling in the Shungnak River drainage. (Bradley 2018). This year only one longnose sucker was captured at this location (52 – 66 mm), but in 2017 the few round whitefish captured were likely age-1 (92 – 117 mm). Similar to 2017, this year most of the round whitefish were likely age-1 (80 – 100 mm), but one large (445 mm) adult round whitefish was also captured, likely around age-10 (Alt 1977).



Figure 13. Length frequency distribution of Arctic grayling captured near the mouth of Ruby Creek 2016-2018.



Figure 14. Ruby Creek fyke net fish captures by species for 2016 - 2018.

One fyke net was set at the mouth of Subarctic Creek on July 21 and was checked 24 hours later on July 22. Only the downstream net was set, as the upstream net had very low catches in previous years. Two slimy sculpin (35 mm and 70 mm) were captured, as well as one Arctic grayling 143 mm in length.

In a new location this year, one fyke net was set in the Shungnak River just upstream from the mouth of Subarctic Creek on July 24 and was checked 24 hours later on July 25. This net was set to capture fish moving upstream, but only spanned approximately one-third of the river. The river was too deep, swift, and wide for a fyke to span the entire channel. A single Arctic grayling 155 mm in length was captured.

September Minnow Traps

Catches in 2018 were much higher than in 2017, likely due to the substantially lower water levels (Figure 15). Minnow trapping is generally more effective in lower water conditions.



Figure 15. Mean daily discharge in Dahl Creek, the nearest USGS gaged creek to the Arctic-Bornite sample area. Sampling occurred September 19 – 20 in 2017 and September 18 – 19 in 2018.

A total of 38 fish were captured at the Upper Subarctic Creek site, and five large (128 – 144 mm FL) fish were retained for dissection to determine spawning condition and age (Table 4). Some of the captured fish appeared to be in spawning condition with very bright spots and white fins, and 13 of the fish were ripe males, i.e. they expelled milt when gently squeezed (Figure 16). None of the fish spilled eggs when squeezed, but four of the fish were tentatively identified as female because they had large, soft bellies and did not produce milt.

Twenty-one fish were captured at the Middle Subarctic Creek site (Table 4). Eight fish were ripe males, and two were tentatively identified as not ripe females. A total of 16 Dolly Varden and two slimy sculpin were captured at the Lower Subarctic Creek site, and most of these fish were smaller than those captured farther upstream. One large (150 mm FL) fish was tentatively identified as a not ripe female.



Figure 16. Mature male Dolly Varden captured September 19, 2018 at the Middle Subarctic Creek sample site.

A total of 38 fish were captured at the Lower Red Rock Creek site (Table 4). Fifteen fish were retained for element analysis, and fin clips were taken from all 38 fish for genetic analysis. Seven fish were ripe males and two fish were ripe females (Figure 17). Both females that expelled eggs were 152 mm FL with eggs approximately 3.6 mm in diameter, which is consistent with the size of ripe resident Dolly Varden eggs in Southeast Alaska (Blackett 1973). Average fecundity for resident Dolly Varden in Southeast Alaska was 0.6 eggs per mm body length (Blackett 1973). Using this average fecundity-length relationship, a 152 mm female would have around 90 eggs. The presence of ripe females indicates that Dolly Varden are spawning near this sample location.



Figure 17. Ripe female Dolly Varden on Lower Red Rock Creek with eggs.

The Middle Red Rock Creek site is characterized by steep gradient and plunge pools. Six Dolly Varden were captured here, although minnow traps did not soak overnight. Fish here were the smallest on average across all eight sites, with a mean length of 84 mm (Table 4). None of the fish expelled milt or eggs when gently squeezed. Caudal fin clips were taken for genetic analysis from all six fish.

A total of 13 fish were captured at the Upper Red Rock Creek sampling location (Table 4). One fish was a ripe male, one was a not ripe female, and the others were immature. The remaining three fin clips for genetic analysis were collected here, for a total of 50 samples for genetic analysis.

The two sites on Center of the Universe Creek were added in 2018. Center of the Universe Creek is a tributary of Red Rock Creek that enters above the upper Red Rock Creek sampling location. Both sites are characterized by riffles and runs interspersed with pools. Substrate here is smaller gravel than at other downstream sites. A total of 21 Dolly Varden were caught at the Lower Center of the Universe Creek sample site, 14 of which were ripe males (Figure 18, Table 4).



Figure 18. Ripe male Dolly Varden captured at Lower Center of the Universe Creek

At the Upper Center of the Universe Creek site, 17 fish were caught and 12 of them were ripe males. Mean length for fish at the lower site was 128 mm, and mean length for fish at the upper site was 130 mm. Mean lengths for fish caught at both Lower and Upper Center of the Universe Creek sites were larger than at any of the other sites (Table 4).

A total of 173 Dolly Varden were captured over all eight sites, and ranged in length from 59 mm to 176 mm, with an average length of 118 mm. All size classes were represented, with an approximately normal distribution (Figure 19).

Table 4. Total number of Dolly Varden captured at each site, with mean fork length and length range for each site. Catch per unit effort (CPUE) is for 1 day (24 hours).

Site	# Captured	Mean FL (mm)	Range (mm)	CPUE
Lower Subarctic	16	104	72 - 150	19
Mid Subarctic	21	113	66 - 158	25
Upper Subarctic ¹	38	120	61 - 159	47
Lower Red Rock	41	123	74 - 176	47
Mid Red Rock	6	84	59 - 114	16
Upper Red Rock	13	109	68 - 156	14
Lower Center of the Universe ²	21	128	81 - 158	21
Upper Center of the Universe	17	130	80 - 160	17

¹ Two slimy sculpin were also captured at this site

² Only 8 minnow traps were deployed at this site



Figure 19. Length frequency distribution of resident Dolly Varden in the Subarctic and Red Rock drainages.

Fish Age and Maturity

Dolly Varden

Five large (128 – 144 mm FL) Dolly Varden were retained for dissection and aging from Subarctic Creek. They ranged from 5 to 7 years old (Table 5, Figure 20). Two of these fish were female; one had small, immature eggs and was determined unlikely to spawn that season, but the other one had large eggs. The whole fish weighed 32.12 g, and the eggs were 7.58 g, nearly 24% of the total body weight (Figure 20). This confirmed that Dolly Varden spawn near this sample location in Upper Subarctic Creek.

Table 5.	Size, maturity	and age of	five Dolly	Varden	captured or	n September	19, 2018 in
Upper S	ubarctic Creek.	•					

Sample ID	Weight (g)	Length (mm)	Sex	Mature	Age
09192018USADVA01	23.77	138	Male	yes	6
09192018USADVA02	32.12	144	Female	yes, ripe	6
09192018USADVA03	24.75	136	Male	yes	7
09192018USADVA04	21.78	128	Male	yes	5
09192018USADVA05	16.83	129	Female	no, small eggs	5



Figure 20. Mature female Dolly Varden (age-6) on the left, and an immature female Dolly Varden (age-5) on the right. Upper Subarctic Creek, September 19, 2018.

Fish Metals

Fish retained for element analysis are listed in Appendix 2 and results for each fish are listed in Appendix 3. 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 Mine and provide a good data set for comparative purposes (Legere and Timothy, 2016).

Cadmium concentrations were highest, but variable, in the fish from Lower Red Rock Creek, which averaged 0.99 mg/kg (Figure 21). Fish from Middle Red Rock Creek had similar concentrations, averaging 0.87 mg/kg. Sample collection on Red Rock Creek was new in 2018. Upper Ruby Creek slimy sculpin had the lowest cadmium concentrations, with an average of 0.03 mg/kg. Upper Ruby also had the lowest average cadmium concentration in 2016.

Mean copper concentrations were highest at Middle Red Rock Creek (6.26 mg/kg), followed closely by Lower Red Rock Creek (5.76 mg/kg) (Figure 22). Concentrations at all sites were similar to results found in 2016 and 2017. These results found for Dolly Varden at Arctic-Bornite are similar to other locations from across the state (Legere and Timothy, 2016).

Mean mercury concentrations found in 2018 were similar to results found in 2017 and 2016. (Figure 23). These values are comparable to the values found in Dolly Varden 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.

Selenium concentrations were nearly the same among Jay Creek, Lower Red Rock Creek, and Riley Creek Dolly Varden (Figure 24). The highest mean concentration was at Middle Red Rock Creek (6.72 mg/kg). 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 Mine (Legere and Timothy, 2016).

Higher mean zinc concentrations were found in Dolly Varden in Middle Red Rock Creek (202.48 mg/kg) than Lower Red Rock Creek (158.66 mg/kg) (Figure 25). These values all fall within the range of concentrations found in Dolly Varden in other regions of the state (Legere and Timothy, 2016).



Figure 21. Mean whole body dry weight concentrations of cadmium (mg/kg) (± 1 SD) in Dolly Varden and slimy sculpin from various sample sites, 2018.



Figure 22. Mean whole body dry weight concentrations of copper (mg/kg) (± 1 SD) in Dolly Varden and slimy sculpin from various sample sites, 2018.



Figure 23. Mean whole body dry weight concentrations of mercury (mg/kg) (± 1 SD) in Dolly Varden and slimy sculpin from various sample sites, 2018.



Figure 24. Mean whole body dry weight concentrations of selenium (mg/kg) (± 1 SD) in Dolly Varden and slimy sculpin from various sample sites, 2018.



Figure 25. Mean whole body dry weight concentrations of zinc (mg/kg) (± 1 SD) in Dolly Varden and slimy sculpin from various sample sites, 2018.

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.

Similar to previous years, 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 spawning condition in September, they likely remain there to spawn. In other populations of dwarf resident Dolly Varden, males mature as early as age 2 and almost all are mature by age 3, while females mature at ages 3 or 4 (McCart and Craig 1973, McCart and Bain 1974, Armstrong and Morrow 1980). The oldest

fish aged from Subarctic Creek was age 7. Dolly Varden in other resident populations have attained age 10, but few fish survive beyond age 5 (Armstrong and Morrow 1980).

This was the first year where Dolly Varden spawning location was confirmed, through the capture of a very small young of the year fish and capture of ripe females in September. So far, spawning has been confirmed in Upper Subarctic Creek and Lower Red Rock Creek, but it is likely that spawning occurs in other places like Center of the Universe Creek.

The Dolly Varden captured in Riley Creek in July have the potential to be anadromous as no permanent physical barrier exists downstream. 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 found in previous years 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 potentially involve surveys to better assess fish use along with genetic sampling to compare to Subarctic resident Dolly Varden and Kobuk drainage anadromous Dolly Varden. 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 were likely age 1+. The species and ages captured in the fyke nets have varied considerably over the three sample years. Future fish sampling could extend the period of fyke net operation to better capture the range of fish movement in these tributaries to the Shungnak River. Based on catches in 2016 to 2018, 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. Future fish work should be focused on expanding our understanding of how and when fish utilize target areas around the Arctic and Bornite deposits. Additional recommendations include obtaining greater sample sizes for fish whole body element analysis and conducting fall aerial surveys.

LITERATURE CITED

- Alt, K. T. 1978. Annual performance report for inventory and cataloging of sport fish and sport fish waters of Western Alaska. Alaska Department of Fish & Game, Volume 19, Fairbanks, AK.
- Angers, B., L. Bernatchez, A. Angers, and L. Desgroseillers. 1995. Specific microsatellite loci for brook charr reveal strong population subdivision on a microgeographic scale. Journal of Fish Biology 47 (Supplement A):177-185.
- Armstrong, R. H., and J. E. Morrow. 1980. The Dolly Varden charr, Salvelinus malma. Pages 99-140 in E. Balon, editor. Charrs: Salmonid fishes of the genus *Salvelinus*. Dr. W. Junk Publishers, The Hague, The Netherlands.
- Bergl, R. A., and L. Vigilant. 2007. Genetic analysis reveals population structure and recent migration within the highly fragmented range of the Cross River gorilla (*Gorilla gorilla diehli*). Molecular Ecology 16:501-516.
- Blackett, R. F. 1973. Fecundity of resident and anadromous Dolly Varden (Salvelinus malma) in southeastern Alaska. J. Fish. Res. Board Can. 30:543-548.
- Bradley, P. T. 2017a. Aquatic Biomonitoring at the Arctic-Bornite Prospect, 2016. Alaska Department of Fish & Game, Technical Report No. 17-06, Fairbanks, AK.
- Bradley, P. T. 2017b. Methods for aquatic life monitoring at the Red Dog Mine site. Alaska Department of Fish & Game, Technical Report No. 17-09, Fairbanks, AK.
- Bradley, P. T. 2018. Aquatic Biomonitoring at the Arctic-Bornite Prospect, 2017. Alaska Department of Fish & Game, Technical Report No. 18-04, Fairbanks, AK.
- Carim, K. J., L. A. Eby, C. A. Barfoot, and M. C. Boyer. 2016. Consistent loss of genetic diversity in isolated cutthroat trout populations independent of habitat size and quality. Conservation Genetics 17:1363-1376.
- Costello, A. B., T. E. Down, S. M. Pollard, C. J. Pacas, and E. B. Taylor. 2003. The influence of history and contemporary stream hydrology on the evolution of genetic diversity within species: an examination of microsatellite DNA variation in bull trout, *Salvelinus confluentus* (Pisces: Salmonidae). Evolution 57:328-344.
- Crane, P. A., C. J. Lewis, E. J. Kretschmer, S. J. Miller, W. J. Spearman, A. L. DeCicco, M. J. Lisac, and J. K. Wenburg. 2004. Characterization and inheritance of seven microsatellite loci from Dolly Varden, *Salvelinus malma*, and cross-species amplification in Arctic char, *S. alpinus*. Conservation Genetics 5:737-741.
- Crispo, E., P. Bentzen, D. N. Reznick, M. T. Kinnison, and A. P. Hendry. 2006. The relative influence of natural selection and geography on gene flow in guppies. Molecular Ecology 15:49-62.

- Currens, K. P., K. E. Griswold, and G. H. Reeves. 2003. Relations between Dolly Varden populations and between coastal cutthroat trout populations in Prince William Sound. Exxon Valdez Oil Spill Restoration Project Final Report (Restoration Project 98145). USDA Forest Service, Pacific Northwest Research Station, Corvallis, Oregon.
- Earl, D. A., and B. M. vonHoldt. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4:359-361.
- Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14:2611-2620.
- Everett, R. J., R. L. Wilmot, and C. C. Krueger. 1997. Population genetic structure of Dolly Varden from Beaufort Sea drainages of northern Alaska and Canada. American Fisheries Society Symposium 19:230-249.
- Falush, D., M. Stephens, and J. K. Pritchard. 2003. Inference of population structure: extensions to linked loci and correlated allele frequencies. Genetics 164:1567-1587.
- Gomez-Uchida, D., T. W. Knight, and D. E. Ruzzante. 2009. Interaction of landscape and life history attributes on genetic diversity, neutral divergence, and gene flow in a pristine community of salmonids. Molecular Ecology 18:4854-4869.
- Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). <u>http://www.unil.ch/izea/softwares/fstat.html</u>.
- Hänfling, B., and D. Weetman. 2006. Concordant genetic estimators of migration reveal anthropogenically enhanced source-sink population structure in the river sculpin, *Cottus* gobio. Genetics 173:1487-1501.
- Harris, L. N., R. Bajno, C. P. Gallagher, I. Koizumi, L. K. Johnson, K. L. Howland, E. B. Taylor, and J. D. Reist. 2015. Life-history characteristics and landscape attributes as drivers of genetic variation, gene flow, and fine-scale population structure in northern Dolly Varden (*Salvelinus malma malma*) in Canada. Canadian Journal of Fisheries and Aquatic Sciences 72:1477-1493.
- Koizumi, I., S. Yamamoto, and K. Maekawa. 2006. Decomposed pairwise regression analysis of genetic and geographic distances reveals a metapopulation structure of stream-dwelling Dolly Varden charr. Molecular Ecology 15:3175-3189.
- Legere, N. M. and J. Timothy. 2016. Tulsequah Chief acid mine drainage and Dolly Varden char whole body metals concentrations. Alaska Department of Fish & Game, Technical Report No. 16-06, Douglas, AK.
- Lopez. A. J. Personal Communication. University of Alaska Museum. Curator of Fishes and Associate Professor.

- McCart, P. and P. Craig. 1973. Life history of two isolated populations of Arctic char (*Salvelinus alpinus*) in spring-fed tributaries of the Canning River, Alaska. Journal of the Fisheries Research Board of Canada. 30:1215-1220.
- McCart, P. and H. Bain. 1974. An isolated population of Arctic char (*Salvelinus alpinus*) inhabiting a warm mineral spring above a waterfall at Cache Creek, Northwest Territories. Journal of the Fisheries Research Board of Canada. 31:1408-1414.
- Olsen, J. B., P. Bentzen, and J. E. Seeb. 1998. Characterization of seven microsatellite loci derived from pink salmon. Molecular Ecology 7:1087-1089.
- Ott, A. G., P. T. Bradley, and H. L. Scannell. 2016. Aquatic biomonitoring at Red Dog Mine, 2015. Alaska Department of Fish & Game, Technical Report No. 16-01, Fairbanks, AK.
- Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945-959.
- Savereide, J. W., and P. Crane. 2017. Genetic diversity of Dolly Varden populations in the Kobuk River. U.S. Fish and Wildlife Service, Office of Subsistence Management, Fishery Information Service Division, Final Technical Report for Study 16-03. Alaska Department of Fish and Game, Anchorage, Alaska.
- Sonnichsen, S. K. 1981. Ecology of slimy sculpin (*Cottus cognatus*) in the Chena River, Alaska. University of Alaska Fairbanks. Master's Thesis.
- Tetra Tech, Inc. 2011. Arctic Deposit Access Environmental Baseline Data Collection Aquatics. Ambler Mining District, Alaska. Prepared for: Nova Gold Resources Inc.
- Whitely, A. R., K. Hastings, J. K. Wenburg, C. A. Frissell, J. C. Martin, and F. W. Allendorf. 2010. Genetic variation and effective population size in isolated populations of coastal cutthroat trout. Conservation Genetics 11:1929-1943.

APPENDIX 1. WATER QUALITY DATA FROM 2018

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. The cadmium sample highlighted in yellow was the only sample that exceeded the more stringent chronic aquatic life exposure limit.

						Copper	Copper					Zinc Chronic/	
			Cadmium	Cadmium		Acute	Chronic					Acute	Hardness
Site	Collection	Cadmium	Acute Limit	Chronic	Copper	Limit	Limit	Mercury	Selenium	TDS	Zinc	Limit	CaCO3
Location	Date	(ug/L)	(ug/L)	Limit (ug/L)	(ug/L)	(ug/L)	(ug/L)	(ng/L)	(ug/L)	(mg/L)	(ug/L)	(ug/L)	(mg/L)
Upper	6/29/2018	0.025	2.980	0.350	0.341	19.090	12.360	0.939	0.500	156	3.10	158.380	139
Ruby	8/26/2018	0.025	3.070	0.350	0.392	19.610	12.660	2.030	0.435	179	5.00	162.230	143
	12/10/2018	0.025	3.130	0.360	0.500	20.000	12.890	0.606	0.459	176	5.00	165.110	146
	12/10/2018	0.025	3.290	0.370	0.500	20.900	13.420	*0.500	0.532	158	5.00	171.790	153
Lower	3/22/2018	0.025	3.110	0.360	0.699	19.870	12.820	0.562	1.000	178	3.10	164.150	145
Ruby	6/28/2018	0.025	2.720	0.320	0.896	17.530	11.440	1.140	0.500	148	3.98	146.710	127
	8/24/2018	0.025	2.480	0.300	1.080	16.100	10.590	1.280	0.500	152	5.00	135.870	116
	12/10/2018	0.025	3.090	0.350	0.542	19.740	12.740	0.871	0.592	187	5.00	163.190	144
Upper	6/27/2018	0.219	1.530	0.210	1.820	10.270	7.050	1.040	0.521	86	18.00	90.710	72
Shungnak	8/26/2018	0.227	1.810	0.240	1.420	12.010	8.120	0.513	0.595	104	17.20	104.400	85
Upper	3/25/2018	*0.015			0.250	7.700	5.420	*0.500	1.000	72	3.10	69.970	53
Subarctic	6/24/2018	0.025	0.710	0.120	0.323	5.070	3.710	0.889	0.500	38	3.10	48.030	34
	8/26/2018	0.025	0.950	0.150	0.249	6.600	4.720	0.773	0.450	56	5.00	60.910	45
	12/7/2018	0.017	1.050	0.160	0.250	7.290	5.160	0.601	0.500	56	5.00	66.600	50
Lower	3/24/2018	0.042	1.050	0.160	0.303	7.290	5.160	*0.500	1.000	73	4.49	66.600	50
Subarctic	6/27/2018	0.102	0.630	0.110	1.610	4.500	3.330	0.859	0.337	47	16.40	43.200	30
	6/27/2018	0.103	0.690	0.120	1.580	4.930	3.620	0.965	0.500	44	14.20	46.830	33
	8/26/2018	0.078	0.840	0.140	0.705	5.900	4.260	0.672	0.711	59	9.61	55.120	40
Lower	7/1/2018	0.025	2.110	0.270	0.513	13.870	9.250	0.890	0.825	106	3.10	118.800	99
Riley	7/1/2018	0.034	2.290	0.280	0.863	14.920	9.880	0.976	0.685	111	3.10	126.890	107
	8/28/2018	0.025	2.420	0.300	0.714	15.710	10.360	1.420	0.473	121	5.00	132.890	113

* Indicates value at or below detection limit.

APPENDIX 2. LIST OF FISH RETAINED FOR WHOLE BODY ELEMENT ANALYSIS

			Date	Fish	Length	Weight	М	etals t	o be a	analyz	ed
Sample ID	Stream	Site	Collected	Spp ¹	(mm)	(g)	Cu	Hg	Se	Cd	Zn
072418URUBSS01	Ruby	Upper	7/24/2018	SS	68	2.3	х	х	х	х	х
072418URUBSS02	Ruby	Upper	7/24/2018	SS	75	4.3	х	х	х	х	х
072418URUBSS03	Ruby	Upper	7/24/2018	SS	79	3.6	х	х	х	х	х
072418URUBSS04	Ruby	Upper	7/24/2018	SS	78	4.7	х	х	х	х	х
072418URUBSS05	Ruby	Upper	7/24/2018	SS	82	5.1	х	х	х	х	х
072418URUBSS06	Ruby	Upper	7/24/2018	SS	63	2.9	х	х	х	х	х
072418URUBSS07	Ruby	Upper	7/24/2018	SS	81	4.9	х	х	х	х	х
072418URUBSS08	Ruby	Upper	7/24/2018	SS	80	4.8	х	х	х	х	х
072418URUBSS09	Ruby	Upper	7/24/2018	SS	72	4.2	х	х	х	х	х
072418URUBSS10	Ruby	Upper	7/24/2018	SS	70	3.3	х	х	х	х	х
072418URUBSS11	Ruby	Upper	7/24/2018	SS	72	3.2	х	х	х	х	х
072418URUBSS12	Ruby	Upper	7/24/2018	SS	77	4.7	х	х	х	х	х
072418URUBSS13	Ruby	Upper	7/24/2018	SS	95	8	х	х	х	х	х
072418URUBSS14	Ruby	Upper	7/24/2018	SS	94	9.7	х	х	х	х	х
072418URUBSS15	Ruby	Upper	7/24/2018	SS	73	5	х	х	х	х	x
072418LRUBSS01	Ruby	Lower	7/24/2018	SS	66	2.5	х	х	х	х	х
072418LRUBSS02	Ruby	Lower	7/24/2018	SS	68	2.5	х	х	х	х	x
072418LRUBSS03	Ruby	Lower	7/24/2018	SS	65	2.4	х	х	х	х	х
072418LRUBSS04	Ruby	Lower	7/24/2018	SS	63	1.8	х	х	х	х	х
072418LRUBSS05	Ruby	Lower	7/24/2018	SS	63	2.7	х	х	х	х	х
072418LRUBSS06	Ruby	Lower	7/24/2018	SS	63	1.8	х	х	х	х	х
072418LRUBSS07	Ruby	Lower	7/24/2018	SS	75	4.1	х	х	х	х	х
072418LRUBSS08	Ruby	Lower	7/24/2018	SS	63	2	х	х	х	х	х
072418LRUBSS09	Ruby	Lower	7/24/2018	SS	76	4.2	х	х	х	х	х
072418LRUBSS10	Ruby	Lower	7/24/2018	SS	65	3.7	х	х	х	х	х
072418LRUBSS11	Ruby	Lower	7/24/2018	SS	66	3.3	х	х	х	х	х
072418LRUBSS12	Ruby	Lower	7/24/2018	SS	65	2.4	х	х	х	х	х
072218USADV01	Subarctic	Upper	7/22/2018	DV	144	28.3	х	х	х	х	х
072218USADV02	Subarctic	Upper	7/22/2018	DV	137	22.4	х	х	х	х	х
072218USADV03	Subarctic	Upper	7/22/2018	DV	124	18	х	х	х	х	х
072218USADV04	Subarctic	Upper	7/22/2018	DV	115	13.7	х	х	х	х	х
072218USADV05	Subarctic	Upper	7/22/2018	DV	106	9.6	х	х	х	х	х
072218USADV06	Subarctic	Upper	7/22/2018	DV	132	20.2	х	х	х	х	х
072218USADV07	Subarctic	Upper	7/22/2018	DV	114	13.9	х	х	х	х	х
072218USADV08	Subarctic	Upper	7/22/2018	DV	125	19.2	х	х	х	х	х
072218USADV09	Subarctic	Upper	7/22/2018	DV	125	16.7	х	х	х	х	х
072218USADV10	Subarctic	Upper	7/22/2018	DV	118	13.8	х	х	х	х	х
072218USADV11	Subarctic	Upper	7/22/2018	DV	116	13	х	х	Х	х	х
072218USADV12	Subarctic	Upper	7/22/2018	DV	121	16.1	х	х	Х	х	х
072218USADV13	Subarctic	Upper	7/22/2018	DV	124	18.2	х	х	Х	х	х
072218USADV14	Subarctic	Upper	7/22/2018	DV	119	15.5	х	х	х	x	х

0700101104 DV/15			7/00/0019	DV	115	144		I			I
072218USADV15	Dilas	Upper	7/22/2018		115	14.4	X	X	X	X	X
072418RILDV01	Biley	Lower	7/24/2018		114	14.4	X	X	X	X	X
072418KILDV02	Dilay	Lower	7/24/2018		108	9.4	X	X	X	X	X
072418RILDV03	Dilay	Lower	7/24/2018		155	21.9	X	X	X	X	X
072418KILDV04	Dilay	Lower	7/24/2018		108	2.4	X	X	X	X	X
072518RILDV05	Dilaw	Lower	7/25/2018		108	11.4	X	X	X	X	X
072418RILSS01	Riley	Lower	7/24/2018	22	/6	2.8	X	X	X	X	X
072418RILSS02	Riley	Lower	7/24/2018	22	66	2.4	X	X	X	X	X
072418RILSS03	Riley	Lower	7/24/2018	SS	12	4.3	X	X	X	X	X
072418RILSS04	Riley	Lower	7/24/2018	SS	61	1.6	X	X	X	X	X
072418RILSS05	Riley	Lower	//24/2018	SS	60	1.9	X	X	X	X	X
072518RILSS06	Riley	Lower	7/25/2018	SS	93	8	X	X	X	Х	Х
072518RILSS07	Riley	Lower	7/25/2018	SS	68	2.5	X	X	X	X	X
072518RILSS08	Riley	Lower	7/25/2018	SS	69	2.7	X	X	X	X	X
072518RILSS09	Riley	Lower	7/25/2018	SS	92	6.3	X	X	X	Х	Х
072418JAYDV01	Jay	Airstrip	7/24/2018	DV	138	21	X	х	x	х	х
072418JAYDV02	Jay	Airstrip	7/24/2018	DV	107	9.8	X	X	x	X	X
072418JAYDV03	Jay	Airstrip	7/24/2018	DV	102	8.5	X	X	x	х	X
072418JAYDV04	Jay	Airstrip	7/24/2018	DV	118	12.5	х	х	х	х	х
072418JAYDV05	Jay	Airstrip	7/24/2018	DV	138	22.3	х	х	х	х	х
072418JAYDV06	Jay	Airstrip	7/24/2018	DV	150	28.2	х	х	x	х	х
072418JAYDV07	Jay	Airstrip	7/24/2018	DV	110	9	х	х	х	х	х
072418JAYDV08	Jay	Airstrip	7/24/2018	DV	128	17.5	х	х	х	х	х
072418JAYDV09	Jay	Airstrip	7/24/2018	DV	136	19.1	х	х	x	х	х
072318MRRDV01	Red Rock	Middle	7/23/2018	DV	85	5.3	х	х	x	х	х
072318MRRDV02	Red Rock	Middle	7/23/2018	DV	125	13.5	х	х	x	х	х
072318MRRDV03	Red Rock	Middle	7/23/2018	DV	99	7.8	х	х	x	х	х
072318MRRDV04	Red Rock	Middle	7/23/2018	DV	140	26.3	х	х	х	х	х
072318MRRDV05	Red Rock	Middle	7/23/2018	DV	136	22.1	х	x	x	х	x
072318MRRDV06	Red Rock	Middle	7/23/2018	DV	90	5.5	х	x	x	х	x
091818LRRDV01	Red Rock	Lower	9/18/2018	DV	128	19.4	х	x	x	х	x
091818LRRDV02	Red Rock	Lower	9/18/2018	DV	109	12.1	х	х	x	х	х
091818LRRDV03	Red Rock	Lower	9/18/2018	DV	157	34.1	х	х	x	х	х
091818LRRDV04	Red Rock	Lower	9/18/2018	DV	154	31.2	х	х	x	х	х
091818LRRDV05	Red Rock	Lower	9/18/2018	DV	102	9.8	х	х	x	х	х
091818LRRDV06	Red Rock	Lower	9/18/2018	DV	109	12.1	х	х	х	х	х
091818LRRDV07	Red Rock	Lower	9/18/2018	DV	140	22.5	х	х	х	х	х
091818LRRDV08	Red Rock	Lower	9/18/2018	DV	121	15.5	х	x	х	x	x
091818LRRDV09	Red Rock	Lower	9/18/2018	DV	120	15.8	х	х	х	х	х
091818LRRDV10	Red Rock	Lower	9/18/2018	DV	140	21.7	х	х	х	х	х
091818LRRDV11	Red Rock	Lower	9/18/2018	DV	118	13.8	х	х	x	х	х
091818LRRDV12	Red Rock	Lower	9/18/2018	DV	116	12.4	х	х	x	х	х
091818LRRDV13	Red Rock	Lower	9/18/2018	DV	118	14.2	х	x	х	х	x
091818LRRDV14	Red Rock	Lower	9/18/2018	DV	110	10.5	х	х	x	х	х
091818LRRDV15	Red Rock	Lower	9/18/2018	DV	152	26.7	х	x	x	х	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.

Dolly	Varden

Sample ID	Site	Collection	Analyte	Method	Wet Wt. Result	Dry Wt. Result	MDL*	Dry Wt. MDL	PQL**	% Solid
Sample ID	Site	Date	Analyte	Wiethiou	(mg/Kg)	(mg/Kg)	(mg/Kg)	(mg/Kg)	(mg/Kg)	70 50lld
072418JAYDV01	JAY	7/24/2018	Cadmium	M6020B ICP-MS	0.069	0.27	0.007	0.027	0.04	25.6
072418JAYDV02	JAY	7/24/2018	Cadmium	M6020B ICP-MS	0.04	0.183	0.009	0.041	0.04	21.9
072418JA YDV03	JAY	7/24/2018	Cadmium	M6020B ICP-MS	0.06	0.271	0.008	0.036	0.04	22.1
072418JAYDV04	JAY	7/24/2018	Cadmium	M6020B ICP-MS	0.063	0.27	0.009	0.039	0.04	23.3
072418JAYDV05	JAY	7/24/2018	Cadmium	M6020B ICP-MS	0.026	0.102	0.007	0.028	0.03	25.4
072418JAYDV06	JAY	7/24/2018	Cadmium	M6020B ICP-MS	0.04	0.176	0.01	0.044	0.05	22.7
072418JAYDV07	JAY	7/24/2018	Cadmium	M6020B ICP-MS	0.068	0.288	0.008	0.034	0.04	23.6
072418JAYDV08	JAY	7/24/2018	Cadmium	M6020B ICP-MS	0.051	0.276	0.007	0.038	0.04	18.5
072418JAYDV09	JAY	7/24/2018	Cadmium	M6020B ICP-MS	0.046	0.196	0.008	0.034	0.04	23.5
072418JAYDV01	JAY	7/24/2018	Copper	M6020B ICP-MS	1.1	4.297	0.1	0.391	0.3	25.6
072418JAYDV02	JAY	7/24/2018	Copper	M6020B ICP-MS	1.3	5.936	0.1	0.457	0.4	21.9
072418JAYDV03	JAY	7/24/2018	Copper	M6020B ICP-MS	0.9	4.072	0.1	0.452	0.3	22.1
072418JAYDV04	JAY	7/24/2018	Copper	M6020B ICP-MS	1.5	6.438	0.1	0.429	0.4	23.3
072418JA YDV05	JAY	7/24/2018	Copper	M6020B ICP-MS	1.3	5.118	0.1	0.394	0.3	25.4
072418JAYDV06	JAY	7/24/2018	Copper	M6020B ICP-MS	0.9	3.965	0.2	0.881	0.4	22.7
072418JA YDV07	JAY	7/24/2018	Copper	M6020B ICP-MS	1.1	4.661	0.1	0.424	0.3	23.6
072418JA YDV08	JAY	7/24/2018	Copper	M6020B ICP-MS	0.6	3.243	0.1	0.541	0.3	18.5
072418JAYDV09	JAY	7/24/2018	Copper	M6020B ICP-MS	1	4.255	0.1	0.426	0.3	23.5
072418JA YDV01	JAY	7/24/2018	Mercury	M7473 CVAAS	0.036	0.139	0.003	0.011	0.01	25.6
072418JAYDV02	JAY	7/24/2018	Mercury	M7473 CVAAS	0.029	0.132	0.002	0.009	0.01	21.9
072418JAYDV03	JAY	7/24/2018	Mercury	M7473 CVAAS	0.023	0.103	0.002	0.008	0.01	22.1
072418JAYDV04	JAY	7/24/2018	Mercury	M7473 CVAAS	0.044	0.188	0.003	0.012	0.01	23.3
072418JAYDV05	JAY	7/24/2018	Mercury	M7473 CVAAS	0.035	0.136	0.002	0.006	0.01	25.4
072418JAYDV06	JAY	7/24/2018	Mercury	M7473 CVAAS	0.046	0.2	0.002	0.007	0.01	22.7
072418JAYDV07	JAY	7/24/2018	Mercury	M7473 CVAAS	0.04	0.167	0.003	0.011	0.01	23.6
072418JA YDV08	JAY	7/24/2018	Mercury	M7473 CVAAS	0.037	0.198	0.002	0.012	0.01	18.5
072418JA YDV09	JAY	7/24/2018	Mercury	M7473 CVAAS	0.045	0.191	0.002	0.008	0.01	23.5
072418JAYDV01	JAY	7/24/2018	Selenium	M6020B ICP-MS	0.99	3.867	0.01	0.039	0.04	25.6
072418JAYDV02	JAY	7/24/2018	Selenium	M6020B ICP-MS	1.25	5.708	0.02	0.091	0.04	21.9
072418JA YDV03	JAY	7/24/2018	Selenium	M6020B ICP-MS	1.11	5.023	0.02	0.09	0.04	22.1
072418JAYDV04	JAY	7/24/2018	Selenium	M6020B ICP-MS	1.11	4.764	0.02	0.086	0.04	23.3
072418JA YDV05	JAY	7/24/2018	Selenium	M6020B ICP-MS	1.39	5.472	0.01	0.039	0.03	25.4
072418JAYDV06	JAY	7/24/2018	Selenium	M6020B ICP-MS	1.38	6.079	0.02	0.088	0.05	22.7
072418JA YDV07	JAY	7/24/2018	Selenium	M6020B ICP-MS	1.39	5.89	0.02	0.085	0.04	23.6
072418JA YDV08	JAY	7/24/2018	Selenium	M6020B ICP-MS	1.09	5.892	0.01	0.054	0.04	18.5
072418JA YDV09	JAY	7/24/2018	Selenium	M6020B ICP-MS	1.22	5.191	0.02	0.085	0.04	23.5
072418JA YDV01	JAY	7/24/2018	Zinc	M6020B ICP-MS	14	54.688	0.6	2.344	1	25.6
072418JAYDV02	JAY	7/24/2018	Zinc	M6020B ICP-MS	41.2	188.128	0.7	3.196	2	21.9

Sample ID	Site	Collection Date	Analyte	Method	Wet Wt. Result (mg/Kg)	Dry Wt. Result (mg/Kg)	MDL* (mg/Kg)	Dry Wt. MDL (mg/Kg)	PQL** (mg/Kg)	% Solid
072418JA YDV03	JAY	7/24/2018	Zinc	M6020B ICP-MS	19.6	88.688	0.6	2.715	2	22.1
072418JAYDV04	JAY	7/24/2018	Zinc	M6020B ICP-MS	32.1	137.768	0.7	3.004	2	23.3
072418JAYDV05	JAY	7/24/2018	Zinc	M6020B ICP-MS	35.2	138.583	0.5	1.969	1	25.4
072418JAYDV06	JAY	7/24/2018	Zinc	M6020B ICP-MS	19.7	86.784	0.8	3.524	2	22.7
072418JAYDV07	JAY	7/24/2018	Zinc	M6020B ICP-MS	22.2	94.068	0.7	2.966	2	23.6
072418JAYDV08	JAY	7/24/2018	Zinc	M6020B ICP-MS	21.4	115.676	0.6	3.243	1	18.5
072418JAYDV09	JAY	7/24/2018	Zinc	M6020B ICP-MS	27.5	117.021	0.6	2.553	2	23.5
091818LRRDV01	LRR	9/18/2018	Cadmium	M6020B ICP-MS	0.14	0.625	0.01	0.045	0.05	22.4
091818LRRDV02	LRR	9/18/2018	Cadmium	M6020B ICP-MS	0.066	0.293	0.009	0.04	0.04	22.5
091818LRRDV03	LRR	9/18/2018	Cadmium	M6020B ICP-MS	0.389	2.103	0.009	0.049	0.04	18.5
091818LRRDV04	LRR	9/18/2018	Cadmium	M6020B ICP-MS	0.091	0.495	0.008	0.043	0.04	18.4
091818LRRDV05	LRR	9/18/2018	Cadmium	M6020B ICP-MS	0.12	0.561	0.01	0.047	0.05	21.4
091818LRRDV06	LRR	9/18/2018	Cadmium	M6020B ICP-MS	0.09	0.417	0.01	0.046	0.06	21.6
091818LRRDV07	LRR	9/18/2018	Cadmium	M6020B ICP-MS	0.15	0.765	0.01	0.051	0.05	19.6
091818LRRDV08	LRR	9/18/2018	Cadmium	M6020B ICP-MS	0.29	1.436	0.01	0.05	0.05	20.2
091818LRRDV09	LRR	9/18/2018	Cadmium	M6020B ICP-MS	0.151	0.825	0.007	0.038	0.04	18.3
091818LRRDV10	LRR	9/18/2018	Cadmium	M6020B ICP-MS	0.516	2.481	0.009	0.043	0.05	20.8
091818LRRDV11	LRR	9/18/2018	Cadmium	M6020B ICP-MS	0.208	0.924	0.008	0.036	0.04	22.5
091818LRRDV12	LRR	9/18/2018	Cadmium	M6020B ICP-MS	0.265	1.338	0.007	0.035	0.03	19.8
091818LRRDV13	LRR	9/18/2018	Cadmium	M6020B ICP-MS	0.169	0.797	0.007	0.033	0.04	21.2
091818LRRDV14	LRR	9/18/2018	Cadmium	M6020B ICP-MS	0.236	1.204	0.008	0.041	0.04	19.6
091818LRRDV15	LRR	9/18/2018	Cadmium	M6020B ICP-MS	0.116	0.614	0.007	0.037	0.04	18.9
091818LRRDV01	LRR	9/18/2018	Copper	M6020B ICP-MS	0.7	3.125	0.2	0.893	0.4	22.4
091818LRRDV02	LRR	9/18/2018	Copper	M6020B ICP-MS	1.7	7.556	0.1	0.444	0.4	22.5
091818LRRDV03	LRR	9/18/2018	Copper	M6020B ICP-MS	1.8	9.73	0.1	0.541	0.3	18.5
091818LRRDV04	LRR	9/18/2018	Copper	M6020B ICP-MS	0.7	3.804	0.1	0.543	0.3	18.4
091818LRRDV05	LRR	9/18/2018	Copper	M6020B ICP-MS	2	9.346	0.2	0.935	0.4	21.4
091818LRRDV06	LRR	9/18/2018	Copper	M6020B ICP-MS	1.5	6.944	0.2	0.926	0.4	21.6
091818LRRDV07	LRR	9/18/2018	Copper	M6020B ICP-MS	1.3	6.633	0.2	1.02	0.4	19.6
091818LRRDV08	LRR	9/18/2018	Copper	M6020B ICP-MS	0.7	3.465	0.2	0.99	0.4	20.2
091818LRRDV09	LRR	9/18/2018	Copper	M6020B ICP-MS	1.3	7.104	0.1	0.546	0.3	18.3
091818LRRDV10	LRR	9/18/2018	Copper	M6020B ICP-MS	0.9	4.327	0.1	0.481	0.4	20.8
091818LRRDV11	LRR	9/18/2018	Copper	M6020B ICP-MS	0.7	3.111	0.1	0.444	0.3	22.5
091818LRRDV12	LRR	9/18/2018	Copper	M6020B ICP-MS	1.4	7.071	0.1	0.505	0.3	19.8
091818LRRDV13	LRR	9/18/2018	Copper	M6020B ICP-MS	0.8	3.774	0.1	0.472	0.3	21.2
091818LRRDV14	LRR	9/18/2018	Copper	M6020B ICP-MS	1.1	5.612	0.1	0.51	0.3	19.6
091818LRRDV15	LRR	9/18/2018	Copper	M6020B ICP-MS	0.9	4.762	0.1	0.529	0.3	18.9
091818LRRDV01	LRR	9/18/2018	Mercury	M7473 CVAAS	0.016	0.073	0.002	0.008	0.01	22.4
091818LRRDV02	LRR	9/18/2018	Mercury	M7473 CVAAS	0.013	0.058	0.002	0.007	0.01	22.5
091818LRRDV03	LRR	9/18/2018	Mercury	M7473 CVAAS	0.038	0.207	0.002	0.011	0.01	18.5
091818LRRDV04	LRR	9/18/2018	Mercury	M7473 CVAAS	0.017	0.093	0.001	0.008	0.01	18.4
091818LRRDV05	LRR	9/18/2018	Mercury	M7473 CVAAS	0.018	0.085	0.002	0.009	0.01	21.4
091818LRRDV06	LRR	9/18/2018	Mercury	M7473 CVAAS	0	0	0.002	0.009	0.01	21.6

Sample ID	Site	Collection Date	Analyte	Method	Wet Wt. Result	Dry Wt. Result	MDL*	Dry Wt. MDL	PQL**	% Solid
091818LRRDV07	LRR	9/18/2018	Mercury	M7473 CVAAS	0.017	0.086	0.001	0.007	0.01	19.6
091818LRRDV08	LRR	9/18/2018	Mercury	M7473 CVAAS	0.015	0.075	0.002	0.009	0.01	20.2
091818LRRDV09	LRR	9/18/2018	Mercurv	M7473 CVAAS	0.015	0.08	0.002	0.009	0.01	18.3
091818LRRDV10	LRR	9/18/2018	Mercurv	M7473 CVAAS	0.013	0.06	0.004	0.018	0.02	20.8
091818LRRDV11	LRR	9/18/2018	Mercurv	M7473 CVAAS	0.02	0.088	0.002	0.009	0.01	22.5
091818LRRDV12	LRR	9/18/2018	Mercury	M7473 CVAAS	0.014	0.07	0.002	0.012	0.01	19.8
091818LRRDV13	LRR	9/18/2018	Mercury	M7473 CVAAS	0.015	0.072	0.002	0.01	0.01	21.2
091818LRRDV14	LRR	9/18/2018	Mercury	M7473 CVAAS	0.015	0.079	0.002	0.008	0.01	19.6
091818LRRDV15	LRR	9/18/2018	Mercury	M7473 CVAAS	0.011	0.058	0.002	0.011	0.01	18.9
091818LRRDV01	LRR	9/18/2018	Selenium	M6020B ICP-MS	1.12	5	0.02	0.089	0.05	22.4
091818LRRDV02	LRR	9/18/2018	Selenium	M6020B ICP-MS	0.9	4	0.02	0.089	0.04	22.5
091818LRRDV03	LRR	9/18/2018	Selenium	M6020B ICP-MS	1.3	7.027	0.02	0.108	0.04	18.5
091818LRRDV04	LRR	9/18/2018	Selenium	M6020B ICP-MS	0.91	4.946	0.02	0.109	0.04	18.4
091818LRRDV05	LRR	9/18/2018	Selenium	M6020B ICP-MS	0.97	4.533	0.02	0.093	0.05	21.4
091818LRRDV06	LRR	9/18/2018	Selenium	M6020B ICP-MS	0.93	4.306	0.02	0.093	0.06	21.6
091818LRRDV07	LRR	9/18/2018	Selenium	M6020B ICP-MS	1.8	9.184	0.02	0.102	0.05	19.6
091818LRRDV08	LRR	9/18/2018	Selenium	M6020B ICP-MS	1.13	5.594	0.02	0.099	0.05	20.2
091818LRRDV09	LRR	9/18/2018	Selenium	M6020B ICP-MS	0.93	5.082	0.01	0.055	0.04	18.3
091818LRRDV10	LRR	9/18/2018	Selenium	M6020B ICP-MS	1.42	6.827	0.02	0.096	0.05	20.8
091818LRRDV11	LRR	9/18/2018	Selenium	M6020B ICP-MS	0.95	4.222	0.02	0.089	0.04	22.5
091818LRRDV12	LRR	9/18/2018	Selenium	M6020B ICP-MS	1.21	6.111	0.01	0.051	0.03	19.8
091818LRRDV13	LRR	9/18/2018	Selenium	M6020B ICP-MS	0.94	4.434	0.01	0.047	0.04	21.2
091818LRRDV14	LRR	9/18/2018	Selenium	M6020B ICP-MS	1.16	5.918	0.02	0.102	0.04	19.6
091818LRRDV15	LRR	9/18/2018	Selenium	M6020B ICP-MS	1.05	5.556	0.01	0.053	0.04	18.9
091818LRRDV01	LRR	9/18/2018	Zinc	M6020B ICP-MS	38.7	172.768	0.8	3.571	2	22.4
091818LRRDV02	LRR	9/18/2018	Zinc	M6020B ICP-MS	26	115.556	0.7	3.111	2	22.5
091818LRRDV03	LRR	9/18/2018	Zinc	M6020B ICP-MS	48.6	262.703	0.7	3.784	2	18.5
091818LRRDV04	LRR	9/18/2018	Zinc	M6020B ICP-MS	32.9	178.804	0.6	3.261	2	18.4
091818LRRDV05	LRR	9/18/2018	Zinc	M6020B ICP-MS	31	144.86	0.8	3.738	2	21.4
091818LRRDV06	LRR	9/18/2018	Zinc	M6020B ICP-MS	23.4	108.333	0.9	4.167	2	21.6
091818LRRDV07	LRR	9/18/2018	Zinc	M6020B ICP-MS	34.4	175.51	0.8	4.082	2	19.6
091818LRRDV08	LRR	9/18/2018	Zinc	M6020B ICP-MS	19	94.059	0.8	3.96	2	20.2
091818LRRDV09	LRR	9/18/2018	Zinc	M6020B ICP-MS	32.2	175.956	0.6	3.279	1	18.3
091818LRRDV10	LRR	9/18/2018	Zinc	M6020B ICP-MS	25.1	120.673	0.7	3.365	2	20.8
091818LRRDV11	LRR	9/18/2018	Zinc	M6020B ICP-MS	25.6	113.778	0.6	2.667	2	22.5
091818LRRDV12	LRR	9/18/2018	Zinc	M6020B ICP-MS	35.8	180.808	0.5	2.525	1	19.8
091818LRRDV13	LRR	9/18/2018	Zinc	M6020B ICP-MS	28.3	133.491	0.6	2.83	1	21.2
091818LRRDV14	LRR	9/18/2018	Zinc	M6020B ICP-MS	48.2	245.918	0.6	3.061	2	19.6
091818LRRDV15	LRR	9/18/2018	Zinc	M6020B ICP-MS	29.6	156.614	0.6	3.175	1	18.9
072318MRRDV01	MRR	7/23/2018	Cadmium	M6020B ICP-MS	0.13	0.743	0.01	0.057	0.05	17.5
072318MRRDV02	MRR	7/23/2018	Cadmium	M6020B ICP-MS	0.271	1.39	0.007	0.036	0.04	19.5
072318MRRDV03	MRR	7/23/2018	Cadmium	M6020B ICP-MS	0.244	1.196	0.008	0.039	0.04	20.4
072318MRRDV04	MRR	7/23/2018	Cadmium	M6020B ICP-MS	0.13	0.699	0.009	0.048	0.05	18.6

Sample ID	Site	Collection Date	Analyte	Method	Wet Wt. Result (mg/Kg)	Dry Wt. Result (mg/Kg)	MDL* (mg/Kg)	Dry Wt. MDL (mg/Kg)	PQL** (mg/Kg)	% Solid
072318MRRDV05	MRR	7/23/2018	Cadmium	M6020B ICP-MS	0.151	0.812	0.007	0.038	0.03	18.6
072318MRRDV06	MRR	7/23/2018	Cadmium	M6020B ICP-MS	0.081	0.395	0.007	0.034	0.04	20.5
072318MRRDV01	MRR	7/23/2018	Copper	M6020B ICP-MS	1	5.714	0.2	1.143	0.4	17.5
072318MRRDV02	MRR	7/23/2018	Copper	M6020B ICP-MS	1.3	6.667	0.1	0.513	0.3	19.5
072318MRRDV03	MRR	7/23/2018	Copper	M6020B ICP-MS	1.3	6.373	0.1	0.49	0.3	20.4
072318MRRDV04	MRR	7/23/2018	Copper	M6020B ICP-MS	0.8	4.301	0.1	0.538	0.4	18.6
072318MRRDV05	MRR	7/23/2018	Copper	M6020B ICP-MS	1.7	9.14	0.1	0.538	0.3	18.6
072318MRRDV06	MRR	7/23/2018	Copper	M6020B ICP-MS	1.1	5.366	0.1	0.488	0.3	20.5
072318MRRDV01	MRR	7/23/2018	Mercury	M7473 CVAAS	0.013	0.071	0.002	0.01	0.01	17.5
072318MRRDV02	MRR	7/23/2018	Mercury	M7473 CVAAS	0.022	0.113	0.002	0.008	0.01	19.5
072318MRRDV03	MRR	7/23/2018	Mercury	M7473 CVAAS	0.016	0.078	0.002	0.009	0.01	20.4
072318MRRDV04	MRR	7/23/2018	Mercury	M7473 CVAAS	0.019	0.102	0.002	0.01	0.01	18.6
072318MRRDV05	MRR	7/23/2018	Mercury	M7473 CVAAS	0.013	0.071	0.002	0.009	0.01	18.6
072318MRRDV06	MRR	7/23/2018	Mercury	M7473 CVAAS	0.014	0.066	0.002	0.008	0.01	20.5
072318MRRDV01	MRR	7/23/2018	Selenium	M6020B ICP-MS	1.26	7.2	0.02	0.114	0.05	17.5
072318MRRDV02	MRR	7/23/2018	Selenium	M6020B ICP-MS	1.26	6.462	0.01	0.051	0.04	19.5
072318MRRDV03	MRR	7/23/2018	Selenium	M6020B ICP-MS	1.42	6.961	0.02	0.098	0.04	20.4
072318MRRDV04	MRR	7/23/2018	Selenium	M6020B ICP-MS	1.11	5.968	0.02	0.108	0.05	18.6
072318MRRDV05	MRR	7/23/2018	Selenium	M6020B ICP-MS	1.44	7.742	0.01	0.054	0.03	18.6
072318MRRDV06	MRR	7/23/2018	Selenium	M6020B ICP-MS	1.23	6	0.01	0.049	0.04	20.5
072318MRRDV01	MRR	7/23/2018	Zinc	M6020B ICP-MS	40.5	231.429	0.8	4.571	2	17.5
072318MRRDV02	MRR	7/23/2018	Zinc	M6020B ICP-MS	42.2	216.41	0.6	3.077	1	19.5
072318MRRDV03	MRR	7/23/2018	Zinc	M6020B ICP-MS	38.8	190.196	0.7	3.431	2	20.4
072318MRRDV04	MRR	7/23/2018	Zinc	M6020B ICP-MS	29.8	160.215	0.7	3.763	2	18.6
072318MRRDV05	MRR	7/23/2018	Zinc	M6020B ICP-MS	50	268.817	0.5	2.688	1	18.6
072318MRRDV06	MRR	7/23/2018	Zinc	M6020B ICP-MS	30.3	147.805	0.6	2.927	1	20.5
072418RILDV01	RIL	7/24/2018	Cadmium	M6020B ICP-MS	0.031	0.146	0.008	0.038	0.04	21.3
072418RILDV02	RIL	7/24/2018	Cadmium	M6020B ICP-MS	0.029	0.158	0.006	0.033	0.03	18.4
072418RILDV03	RIL	7/24/2018	Cadmium	M6020B ICP-MS	0.014	0.067	0.007	0.034	0.04	20.8
072418RILDV04	RIL	7/24/2018	Cadmium	M6020B ICP-MS	0.06	0.306	0.01	0.051	0.06	19.6
072518RILDV05	RIL	7/25/2018	Cadmium	M6020B ICP-MS	0.035	0.179	0.007	0.036	0.04	19.6
072418RILDV01	RIL	7/24/2018	Copper	M6020B ICP-MS	1.5	7.042	0.100	0.469	0.3	21.3
072418RILDV02	RIL	7/24/2018	Copper	M6020B ICP-MS	0.9	4.891	0.100	0.543	0.3	18.4
072418RILDV03	RIL	7/24/2018	Copper	M6020B ICP-MS	0.8	3.846	0.100	0.481	0.3	20.8
072418RILDV04	RIL	7/24/2018	Copper	M6020B ICP-MS	0.8	4.082	0.200	1.020	0.4	19.6
072518RILDV05	RIL	7/25/2018	Copper	M6020B ICP-MS	1.1	5.612	0.100	0.510	0.3	19.6
072418RILDV01	RIL	7/24/2018	Mercury	M7473 CVAAS	0.024	0.113	0.002	0.009	0.009	21.3
072418RILDV02	RIL	7/24/2018	Mercury	M7473 CVAAS	0.017	0.091	0.001	0.008	0.007	18.4
072418RILDV03	RIL	7/24/2018	Mercury	M7473 CVAAS	0.018	0.088	0.002	0.009	0.009	20.8
072418RILDV04	RIL	7/24/2018	Mercury	M7473 CVAAS	0.019	0.094	0.002	0.010	0.010	19.6
072518RILDV05	RIL	7/25/2018	Mercury	M7473 CVAAS	0.016	0.083	0.002	0.008	0.008	19.6
072418RILDV01	RIL	7/24/2018	Selenium	M6020B ICP-MS	1.340	6.291	0.020	0.094	0.040	21.3
072418RILDV02	RIL	7/24/2018	Selenium	M6020B ICP-MS	0.890	4.837	0.010	0.054	0.030	18.4

Sample ID	Site	Collection Date	Analyte	Method	Wet Wt. Result (mg/Kg)	Dry Wt. Result (mg/Kg)	MDL* (mg/Kg)	Dry Wt. MDL (mg/Kg)	PQL** (mg/Kg)	% Solid
072418RILDV03	RIL	7/24/2018	Selenium	M6020B ICP-MS	0.95	4.567	0.01	0.048	0.04	20.8
072418RILDV04	RIL	7/24/2018	Selenium	M6020B ICP-MS	0.9	4.592	0.02	0.102	0.06	19.6
072518RILDV05	RIL	7/25/2018	Selenium	M6020B ICP-MS	1.2	6.122	0.01	0.051	0.04	19.6
072418RILDV01	RIL	7/24/2018	Zinc	M6020B ICP-MS	24.3	114.085	0.6	2.817	2	21.3
072418RILDV02	RIL	7/24/2018	Zinc	M6020B ICP-MS	20.8	113.043	0.5	2.717	1	18.4
072418RILDV03	RIL	7/24/2018	Zinc	M6020B ICP-MS	15.8	75.962	0.6	2.885	1	20.8
072418RILDV04	RIL	7/24/2018	Zinc	M6020B ICP-MS	20.7	105.612	0.9	4.592	2	19.6
072518RILDV05	RIL	7/25/2018	Zinc	M6020B ICP-MS	20.5	104.592	0.6	3.061	1	19.6
072218USADV01	USA	7/22/2018	Cadmium	M6020B ICP-MS	0.07	0.293	0.01	0.042	0.06	23.9
072218USADV02	USA	7/22/2018	Cadmium	M6020B ICP-MS	0.103	0.417	0.007	0.028	0.04	24.7
072218USADV03	USA	7/22/2018	Cadmium	M6020B ICP-MS	0.114	0.518	0.007	0.032	0.04	22
072218USADV04	USA	7/22/2018	Cadmium	M6020B ICP-MS	0.13	0.528	0.01	0.041	0.05	24.6
072218USADV05	USA	7/22/2018	Cadmium	M6020B ICP-MS	0.109	0.441	0.008	0.032	0.04	24.7
072218USADV06	USA	7/22/2018	Cadmium	M6020B ICP-MS	0.143	0.574	0.007	0.028	0.04	24.9
072218USADV07	USA	7/22/2018	Cadmium	M6020B ICP-MS	0.095	0.426	0.008	0.036	0.04	22.3
072218USADV08	USA	7/22/2018	Cadmium	M6020B ICP-MS	0.08	0.386	0.008	0.039	0.04	20.7
072218USADV09	USA	7/22/2018	Cadmium	M6020B ICP-MS	0.13	0.588	0.01	0.045	0.05	22.1
072218USADV10	USA	7/22/2018	Cadmium	M6020B ICP-MS	0.111	0.496	0.009	0.04	0.04	22.4
072218USADV11	USA	7/22/2018	Cadmium	M6020B ICP-MS	0.161	0.685	0.007	0.03	0.04	23.5
072218USADV12	USA	7/22/2018	Cadmium	M6020B ICP-MS	0.16	0.669	0.01	0.042	0.05	23.9
072218USADV13	USA	7/22/2018	Cadmium	M6020B ICP-MS	0.079	0.346	0.008	0.035	0.04	22.8
072218USADV14	USA	7/22/2018	Cadmium	M6020B ICP-MS	0.106	0.449	0.007	0.03	0.03	23.6
072218USADV15	USA	7/22/2018	Cadmium	M6020B ICP-MS	0.06	0.284	0.01	0.047	0.06	21.1
072218USADV01	USA	7/22/2018	Copper	M6020B ICP-MS	0.7	2.929	0.2	0.837	0.5	23.9
072218USADV02	USA	7/22/2018	Copper	M6020B ICP-MS	1.4	5.668	0.1	0.405	0.3	24.7
072218USADV03	USA	7/22/2018	Copper	M6020B ICP-MS	0.6	2.727	0.1	0.455	0.3	22
072218USADV04	USA	7/22/2018	Copper	M6020B ICP-MS	0.9	3.659	0.2	0.813	0.4	24.6
072218USADV05	USA	7/22/2018	Copper	M6020B ICP-MS	0.6	2.429	0.1	0.405	0.3	24.7
072218USADV06	USA	7/22/2018	Copper	M6020B ICP-MS	1	4.016	0.1	0.402	0.3	24.9
072218USADV07	USA	7/22/2018	Copper	M6020B ICP-MS	1.2	5.381	0.1	0.448	0.3	22.3
072218USADV08	USA	7/22/2018	Copper	M6020B ICP-MS	0.9	4.348	0.1	0.483	0.3	20.7
072218USADV09	USA	7/22/2018	Copper	M6020B ICP-MS	1	4.525	0.2	0.905	0.4	22.1
072218USADV10	USA	7/22/2018	Copper	M6020B ICP-MS	1.3	5.804	0.1	0.446	0.3	22.4
072218USADV11	USA	7/22/2018	Copper	M6020B ICP-MS	1	4.255	0.1	0.426	0.3	23.5
072218USADV12	USA	7/22/2018	Copper	M6020B ICP-MS	0.6	2.51	0.2	0.837	0.4	23.9
072218USADV13	USA	7/22/2018	Copper	M6020B ICP-MS	0.6	2.632	0.1	0.439	0.3	22.8
072218USADV14	USA	7/22/2018	Copper	M6020B ICP-MS	0.7	2.966	0.1	0.424	0.3	23.6
072218USADV15	USA	7/22/2018	Copper	M6020B ICP-MS	0.5	2.37	0.2	0.948	0.4	21.1
072218USADV01	USA	7/22/2018	Mercury	M7473 CVAAS	0.026	0.107	0.003	0.013	0.02	23.9
072218USADV02	USA	7/22/2018	Mercury	M7473 CVAAS	0.022	0.091	0.003	0.013	0.02	24.7
072218USADV03	USA	7/22/2018	Mercury	M7473 CVAAS	0.017	0.075	0.003	0.014	0.02	22
072218USADV04	USA	7/22/2018	Mercury	M7473 CVAAS	0.014	0.058	0.003	0.01	0.01	24.6
072218USADV05	USA	7/22/2018	Mercury	M7473 CVAAS	0.013	0.053	0.003	0.011	0.01	24.7

Sample ID	Site	Collection	Analyte	Method	Wet Wt. Result	Dry Wt. Result	MDL*	Dry Wt. MDL	PQL**	% Solid
Sumpte ID	- She	Date			(mg/Kg)	(mg/Kg)	(mg/Kg)	(mg/Kg)	(mg/Kg)	,0 50 Hu
072218USADV06	USA	7/22/2018	Mercury	M7473 CVAAS	0.015	0.058	0.003	0.013	0.02	24.9
072218USADV07	USA	7/22/2018	Mercury	M7473 CVAAS	0.015	0.068	0.003	0.011	0.01	22.3
072218USADV08	USA	7/22/2018	Mercury	M7473 CVAAS	0.028	0.133	0.002	0.008	0.01	20.7
072218USADV09	USA	7/22/2018	Mercury	M7473 CVAAS	0.05	0.226	0.003	0.012	0.01	22.1
072218USADV10	USA	7/22/2018	Mercury	M7473 CVAAS	0.015	0.067	0.002	0.01	0.01	22.4
072218USADV11	USA	7/22/2018	Mercury	M7473 CVAAS	0.012	0.049	0.002	0.011	0.01	23.5
072218USADV12	USA	7/22/2018	Mercury	M7473 CVAAS	0.013	0.055	0.004	0.016	0.02	23.9
072218USADV13	USA	7/22/2018	Mercury	M7473 CVAAS	0.014	0.059	0.002	0.011	0.01	22.8
072218USADV14	USA	7/22/2018	Mercury	M7473 CVAAS	0.012	0.05	0.003	0.012	0.01	23.6
072218USADV15	USA	7/22/2018	Mercury	M7473 CVAAS	0.017	0.078	0.003	0.016	0.02	21.1
072218USADV01	USA	7/22/2018	Selenium	M6020B ICP-MS	1.08	4.519	0.02	0.084	0.06	23.9
072218USADV02	USA	7/22/2018	Selenium	M6020B ICP-MS	0.92	3.725	0.01	0.04	0.04	24.7
072218USADV03	USA	7/22/2018	Selenium	M6020B ICP-MS	0.91	4.136	0.01	0.045	0.04	22
072218USADV04	USA	7/22/2018	Selenium	M6020B ICP-MS	0.87	3.537	0.02	0.081	0.05	24.6
072218USADV05	USA	7/22/2018	Selenium	M6020B ICP-MS	0.92	3.725	0.02	0.081	0.04	24.7
072218USADV06	USA	7/22/2018	Selenium	M6020B ICP-MS	0.95	3.815	0.01	0.04	0.04	24.9
072218USADV07	USA	7/22/2018	Selenium	M6020B ICP-MS	0.78	3.498	0.02	0.09	0.04	22.3
072218USADV08	USA	7/22/2018	Selenium	M6020B ICP-MS	0.78	3.768	0.02	0.097	0.04	20.7
072218USADV09	USA	7/22/2018	Selenium	M6020B ICP-MS	0.97	4.389	0.02	0.09	0.05	22.1
072218USADV10	USA	7/22/2018	Selenium	M6020B ICP-MS	0.85	3.795	0.02	0.089	0.04	22.4
072218USADV11	USA	7/22/2018	Selenium	M6020B ICP-MS	0.78	3.319	0.01	0.043	0.04	23.5
072218USADV12	USA	7/22/2018	Selenium	M6020B ICP-MS	0.76	3.18	0.02	0.084	0.05	23.9
072218USADV13	USA	7/22/2018	Selenium	M6020B ICP-MS	0.71	3.114	0.02	0.088	0.04	22.8
072218USADV14	USA	7/22/2018	Selenium	M6020B ICP-MS	0.79	3.347	0.01	0.042	0.03	23.6
072218USADV15	USA	7/22/2018	Selenium	M6020B ICP-MS	0.88	4.171	0.02	0.095	0.06	21.1
072218USADV01	USA	7/22/2018	Zinc	M6020B ICP-MS	22.3	93.305	0.9	3.766	2	23.9
072218USADV02	USA	7/22/2018	Zinc	M6020B ICP-MS	22.2	89.879	0.6	2.429	1	24.7
072218USADV03	USA	7/22/2018	Zinc	M6020B ICP-MS	32.7	148.636	0.6	2.727	1	22
072218USADV04	USA	7/22/2018	Zinc	M6020B ICP-MS	31.5	128.049	0.8	3.252	2	24.6
072218USADV05	USA	7/22/2018	Zinc	M6020B ICP-MS	23.9	96.761	0.7	2.834	2	24.7
072218USADV06	USA	7/22/2018	Zinc	M6020B ICP-MS	28	112.45	0.6	2.41	1	24.9
072218USADV07	USA	7/22/2018	Zinc	M6020B ICP-MS	22.9	102.691	0.6	2.691	2	22.3
072218USADV08	USA	7/22/2018	Zinc	M6020B ICP-MS	27.6	133.333	0.6	2.899	2	20.7
072218USADV09	USA	7/22/2018	Zinc	M6020B ICP-MS	20.1	90.95	0.8	3.62	2	22.1
072218USADV10	USA	7/22/2018	Zinc	M6020B ICP-MS	27.4	122.321	0.7	3.125	2	22.4
072218USADV11	USA	7/22/2018	Zinc	M6020B ICP-MS	37.1	157.872	0.6	2.553	1	23.5
072218USADV12	USA	7/22/2018	Zinc	M6020B ICP-MS	31.7	132.636	0.8	3.347	2	23.9
072218USADV13	USA	7/22/2018	Zinc	M6020B ICP-MS	23.4	102.632	0.7	3.07	2	22.8
072218USADV14	USA	7/22/2018	Zinc	M6020B ICP-MS	26.4	111.864	0.5	2.119	1	23.6
072218USADV15	USA	7/22/2018	Zinc	M6020B ICP-MS	24.5	116.114	0.9	4.265	2	21.1
072218USADV14	USA	7/22/2018	Zinc	M6020B ICP-MS	24.5	116.114	0.9	4.265	2	23.0

*MDL = Method Detection Limit

**PQL = Practical Quantitation Limit

Sample ID	Site	Collection Date	Analyte	Method	Wet Wt. Result (mg/Kg)	Dry Wt. Result (mg/Kg)	MDL* (mg/Kg)	Dry Wt. MDL (mg/Kg)	PQL** (mg/Kg)	% Solid
072418RILSS01	RIL	7/24/2018	Cadmium	M6020B ICP-MS	0.03	0.13	0.01	0.043	0.06	23
072418RILSS02	RIL	7/24/2018	Cadmium	M6020B ICP-MS	0.03	0.106	0.01	0.035	0.06	28.3
072418RILSS03	RIL	7/24/2018	Cadmium	M6020B ICP-MS	0.02	0.075	0.01	0.037	0.05	26.7
072418RILSS04	RIL	7/24/2018	Cadmium	M6020B ICP-MS	0.06	0.224	0.01	0.037	0.06	26.8
072418RILSS05	RIL	7/24/2018	Cadmium	M6020B ICP-MS	0.06	0.207	0.01	0.034	0.06	29
072518RILSS06	RIL	7/25/2018	Cadmium	M6020B ICP-MS	0.019	0.079	0.009	0.037	0.04	24.1
072518RILSS07	RIL	7/25/2018	Cadmium	M6020B ICP-MS	0.01	0.035	0.01	0.035	0.05	28.2
072518RILSS08	RIL	7/25/2018	Cadmium	M6020B ICP-MS	0.02	0.077	0.01	0.038	0.05	26
072518RILSS09	RIL	7/25/2018	Cadmium	M6020B ICP-MS	0.04	0.19	0.01	0.047	0.05	21.1
072418RILSS01	RIL	7/24/2018	Copper	M6020B ICP-MS	0.6	2.609	0.2	0.87	0.5	23
072418RILSS02	RIL	7/24/2018	Copper	M6020B ICP-MS	0.6	2.12	0.2	0.707	0.5	28.3
072418RILSS03	RIL	7/24/2018	Copper	M6020B ICP-MS	0.7	2.622	0.2	0.749	0.4	26.7
072418RILSS04	RIL	7/24/2018	Copper	M6020B ICP-MS	1.1	4.104	0.2	0.746	0.5	26.8
072418RILSS05	RIL	7/24/2018	Copper	M6020B ICP-MS	0.8	2.759	0.2	0.69	0.5	29
072518RILSS06	RIL	7/25/2018	Copper	M6020B ICP-MS	0.6	2.49	0.1	0.415	0.4	24.1
072518RILSS07	RIL	7/25/2018	Copper	M6020B ICP-MS	0.6	2.128	0.2	0.709	0.4	28.2
072518RILSS08	RIL	7/25/2018	Copper	M6020B ICP-MS	0.7	2.692	0.2	0.769	0.4	26
072518RILSS09	RIL	7/25/2018	Copper	M6020B ICP-MS	0.8	3.791	0.2	0.948	0.4	21.1
072418RILSS01	RIL	7/24/2018	Mercury	M7473 CVAAS	0.094	0.408	0.002	0.007	0.01	23
072418RILSS02	RIL	7/24/2018	Mercury	M7473 CVAAS	0.039	0.139	0.003	0.01	0.01	28.3
072418RILSS03	RIL	7/24/2018	Mercury	M7473 CVAAS	0.027	0.101	0.003	0.012	0.02	26.7
072418RILSS04	RIL	7/24/2018	Mercury	M7473 CVAAS	0.044	0.166	0.003	0.013	0.02	26.8
072418RILSS05	RIL	7/24/2018	Mercury	M7473 CVAAS	0.028	0.097	0.002	0.008	0.01	29
072518RILSS06	RIL	7/25/2018	Mercury	M7473 CVAAS	0.08	0.332	0.002	0.008	0.01	24.1
072518RILSS07	RIL	7/25/2018	Mercury	M7473 CVAAS	0.03	0.107	0.003	0.012	0.02	28.2
072518RILSS08	RIL	7/25/2018	Mercury	M7473 CVAAS	0.027	0.102	0.002	0.008	0.01	26
072518RILSS09	RIL	7/25/2018	Mercury	M7473 CVAAS	0.022	0.102	0.002	0.011	0.01	21.1
072418RILSS01	RIL	7/24/2018	Selenium	M6020B ICP-MS	0.9	3.913	0.02	0.087	0.06	23
072418RILSS02	RIL	7/24/2018	Selenium	M6020B ICP-MS	1.33	4.7	0.02	0.071	0.06	28.3
072418RILSS03	RIL	7/24/2018	Selenium	M6020B ICP-MS	1.29	4.831	0.02	0.075	0.05	26.7
072418RILSS04	RIL	7/24/2018	Selenium	M6020B ICP-MS	1.26	4.701	0.02	0.075	0.06	26.8
072418RILSS05	RIL	7/24/2018	Selenium	M6020B ICP-MS	1.46	5.034	0.02	0.069	0.06	29
072518RILSS06	RIL	7/25/2018	Selenium	M6020B ICP-MS	0.74	3.071	0.02	0.083	0.04	24.1
072518RILSS07	RIL	7/25/2018	Selenium	M6020B ICP-MS	1.28	4.539	0.02	0.071	0.05	28.2
072518RILSS08	RIL	7/25/2018	Selenium	M6020B ICP-MS	1.03	3.962	0.02	0.077	0.05	26
072518RILSS09	RIL	7/25/2018	Selenium	M6020B ICP-MS	1.3	6.161	0.02	0.095	0.05	21.1
072418RILSS01	RIL	7/24/2018	Zinc	M6020B ICP-MS	28.2	122.609	0.9	3.913	2	23
072418RILSS02	RIL	7/24/2018	Zinc	M6020B ICP-MS	26	91.873	0.9	3.18	2	28.3
072418RILSS03	RIL	7/24/2018	Zinc	M6020B ICP-MS	21.5	80.524	0.8	2.996	2	26.7
072418RILSS04	RIL	7/24/2018	Zinc	M6020B ICP-MS	24.4	91.045	0.9	3.358	2	26.8
072418RILSS05	RIL	7/24/2018	Zinc	M6020B ICP-MS	19.9	68.621	0.9	3.103	2	29
072518RILSS06	RIL	7/25/2018	Zinc	M6020B ICP-MS	32.2	133.61	0.7	2.905	2	24.1
072518RILSS07	RIL	7/25/2018	Zinc	M6020B ICP-MS	24.8	87.943	0.9	3.191	2	28.2

Slimy Sculpin

Sample ID	Site	Collection Date	Analyte	Method	Wet Wt. Result (mg/Kg)	Dry Wt. Result (mg/Kg)	MDL* (mg/Kg)	Dry Wt. MDL (mg/Kg)	PQL** (mg/Kg)	% Solid
072518RILSS08	RIL	7/25/2018	Zinc	M6020B ICP-MS	22.8	87.692	0.8	3.077	2	26
072518RILSS09	RIL	7/25/2018	Zinc	M6020B ICP-MS	27.4	129.858	0.8	3.791	2	21.1
072418URUBSS01	URUB	7/24/2018	Cadmium	M6020B ICP-MS		0	0.01	0.035	0.06	28.8
072418URUBSS02	URUB	7/24/2018	Cadmium	M6020B ICP-MS	0.014	0.06	0.007	0.03	0.03	23.4
072418URUBSS03	URUB	7/24/2018	Cadmium	M6020B ICP-MS	0.03	0.136	0.01	0.045	0.05	22
072418URUBSS04	URUB	7/24/2018	Cadmium	M6020B ICP-MS	0.01	0.042	0.007	0.029	0.03	23.8
072418URUBSS05	URUB	7/24/2018	Cadmium	M6020B ICP-MS		0	0.006	0.023	0.03	25.7
072418URUBSS06	URUB	7/24/2018	Cadmium	M6020B ICP-MS		0	0.01	0.056	0.06	17.9
072418URUBSS07	URUB	7/24/2018	Cadmium	M6020B ICP-MS		0	0.008	0.03	0.04	26.3
072418URUBSS08	URUB	7/24/2018	Cadmium	M6020B ICP-MS	0.02	0.076	0.007	0.027	0.04	26.3
072418URUBSS09	URUB	7/24/2018	Cadmium	M6020B ICP-MS	0.014	0.065	0.009	0.042	0.04	21.6
072418URUBSS10	URUB	7/24/2018	Cadmium	M6020B ICP-MS		0	0.01	0.04	0.06	25.3
072418URUBSS11	URUB	7/24/2018	Cadmium	M6020B ICP-MS		0	0.01	0.042	0.05	23.7
072418URUBSS12	URUB	7/24/2018	Cadmium	M6020B ICP-MS		0	0.01	0.041	0.05	24.6
072418URUBSS13	URUB	7/24/2018	Cadmium	M6020B ICP-MS		0	0.008	0.034	0.04	23.3
072418URUBSS14	URUB	7/24/2018	Cadmium	M6020B ICP-MS		0	0.008	0.036	0.04	22.4
072418URUBSS15	URUB	7/24/2018	Cadmium	M6020B ICP-MS		0	0.008	0.035	0.04	22.8
072418URUBSS01	URUB	7/24/2018	Copper	M6020B ICP-MS	0.5	1.736	0.2	0.694	0.5	28.8
072418URUBSS02	URUB	7/24/2018	Copper	M6020B ICP-MS	0.4	1.709	0.1	0.427	0.3	23.4
072418URUBSS03	URUB	7/24/2018	Copper	M6020B ICP-MS	0.6	2.727	0.2	0.909	0.4	22
072418URUBSS04	URUB	7/24/2018	Copper	M6020B ICP-MS	0.4	1.681	0.1	0.42	0.3	23.8
072418URUBSS05	URUB	7/24/2018	Copper	M6020B ICP-MS	0.3	1.167	0.1	0.389	0.3	25.7
072418URUBSS06	URUB	7/24/2018	Copper	M6020B ICP-MS	0.5	2.793	0.2	1.117	0.5	17.9
072418URUBSS07	URUB	7/24/2018	Copper	M6020B ICP-MS	0.4	1.521	0.1	0.38	0.3	26.3
072418URUBSS08	URUB	7/24/2018	Copper	M6020B ICP-MS	0.7	2.662	0.1	0.38	0.3	26.3
072418URUBSS09	URUB	7/24/2018	Copper	M6020B ICP-MS	0.6	2.778	0.1	0.463	0.4	21.6
072418URUBSS10	URUB	7/24/2018	Copper	M6020B ICP-MS	0.4	1.581	0.2	0.791	0.5	25.3
072418URUBSS11	URUB	7/24/2018	Copper	M6020B ICP-MS	0.4	1.688	0.2	0.844	0.4	23.7
072418URUBSS12	URUB	7/24/2018	Copper	M6020B ICP-MS	0.5	2.033	0.2	0.813	0.4	24.6
072418URUBSS13	URUB	7/24/2018	Copper	M6020B ICP-MS	0.4	1.717	0.1	0.429	0.3	23.3
072418URUBSS14	URUB	7/24/2018	Copper	M6020B ICP-MS	0.3	1.339	0.1	0.446	0.3	22.4
072418URUBSS15	URUB	7/24/2018	Copper	M6020B ICP-MS	0.5	2.193	0.1	0.439	0.3	22.8
072418URUBSS01	URUB	7/24/2018	Mercury	M7473 CVAAS	0.069	0.241	0.004	0.013	0.02	28.8
072418URUBSS02	URUB	7/24/2018	Mercury	M7473 CVAAS	0.035	0.149	0.003	0.013	0.02	23.4
072418URUBSS03	URUB	7/24/2018	Mercury	M7473 CVAAS	0.032	0.146	0.002	0.009	0.01	22
072418URUBSS04	URUB	7/24/2018	Mercury	M7473 CVAAS	0.042	0.175	0.002	0.01	0.01	23.8
072418URUBSS05	URUB	7/24/2018	Mercury	M7473 CVAAS	0.028	0.109	0.002	0.009	0.01	25.7
072418URUBSS06	URUB	7/24/2018	Mercury	M7473 CVAAS	0.022	0.123	0.002	0.01	0.01	17.9
072418URUBSS07	URUB	7/24/2018	Mercury	M7473 CVAAS	0.025	0.096	0.002	0.007	0.01	26.3
072418URUBSS08	URUB	7/24/2018	Mercury	M7473 CVAAS	0.048	0.181	0.002	0.007	0.01	26.3
072418URUBSS09	URUB	7/24/2018	Mercury	M7473 CVAAS	0.033	0.151	0.002	0.011	0.01	21.6
072418URUBSS10	URUB	7/24/2018	Mercury	M7473 CVAAS	0.021	0.081	0.003	0.01	0.01	25.3
072418URUBSS11	URUB	7/24/2018	Mercury	M7473 CVAAS	0.04	0.168	0.003	0.014	0.02	23.7

Sample ID	Site	Collection Date	Analyte	Method	Wet Wt. Result (mg/Kg)	Dry Wt. Result (mg/Kg)	MDL* (mg/Kg)	Dry Wt. MDL (mg/Kg)	PQL** (mg/Kg)	% Solid
072418URUBSS12	URUB	7/24/2018	Mercury	M7473 CVAAS	0.025	0.103	0.003	0.011	0.01	24.6
072418URUBSS13	URUB	7/24/2018	Mercury	M7473 CVAAS	0.051	0.219	0.003	0.012	0.01	23.3
072418URUBSS14	URUB	7/24/2018	Mercury	M7473 CVAAS	0.044	0.194	0.002	0.01	0.01	22.4
072418URUBSS15	URUB	7/24/2018	Mercury	M7473 CVAAS	0.019	0.082	0.003	0.012	0.01	22.8
072418URUBSS01	URUB	7/24/2018	Selenium	M6020B ICP-MS	0.91	3.16	0.03	0.104	0.06	28.8
072418URUBSS02	URUB	7/24/2018	Selenium	M6020B ICP-MS	0.81	3.462	0.01	0.043	0.03	23.4
072418URUBSS03	URUB	7/24/2018	Selenium	M6020B ICP-MS	0.95	4.318	0.02	0.091	0.05	22
072418URUBSS04	URUB	7/24/2018	Selenium	M6020B ICP-MS	1.06	4.454	0.01	0.042	0.03	23.8
072418URUBSS05	URUB	7/24/2018	Selenium	M6020B ICP-MS	0.75	2.918	0.01	0.039	0.03	25.7
072418URUBSS06	URUB	7/24/2018	Selenium	M6020B ICP-MS	0.54	3.017	0.02	0.112	0.06	17.9
072418URUBSS07	URUB	7/24/2018	Selenium	M6020B ICP-MS	0.78	2.966	0.02	0.076	0.04	26.3
072418URUBSS08	URUB	7/24/2018	Selenium	M6020B ICP-MS	0.88	3.346	0.01	0.038	0.04	26.3
072418URUBSS09	URUB	7/24/2018	Selenium	M6020B ICP-MS	0.69	3.194	0.02	0.093	0.04	21.6
072418URUBSS10	URUB	7/24/2018	Selenium	M6020B ICP-MS	0.6	2.372	0.02	0.079	0.06	25.3
072418URUBSS11	URUB	7/24/2018	Selenium	M6020B ICP-MS	0.8	3.376	0.02	0.084	0.05	23.7
072418URUBSS12	URUB	7/24/2018	Selenium	M6020B ICP-MS	0.69	2.805	0.02	0.081	0.05	24.6
072418URUBSS13	URUB	7/24/2018	Selenium	M6020B ICP-MS	0.75	3.219	0.02	0.086	0.04	23.3
072418URUBSS14	URUB	7/24/2018	Selenium	M6020B ICP-MS	0.95	4.241	0.02	0.089	0.04	22.4
072418URUBSS15	URUB	7/24/2018	Selenium	M6020B ICP-MS	0.93	4.079	0.02	0.088	0.04	22.8
072418URUBSS01	URUB	7/24/2018	Zinc	M6020B ICP-MS	35	121.528	1	3.472	3	28.8
072418URUBSS02	URUB	7/24/2018	Zinc	M6020B ICP-MS	24.3	103.846	0.5	2.137	1	23.4
072418URUBSS03	URUB	7/24/2018	Zinc	M6020B ICP-MS	34	154.545	0.9	4.091	2	22
072418URUBSS04	URUB	7/24/2018	Zinc	M6020B ICP-MS	24.3	102.101	0.5	2.101	1	23.8
072418URUBSS05	URUB	7/24/2018	Zinc	M6020B ICP-MS	19.2	74.708	0.5	1.946	1	25.7
072418URUBSS06	URUB	7/24/2018	Zinc	M6020B ICP-MS	20	111.732	1	5.587	2	17.9
072418URUBSS07	URUB	7/24/2018	Zinc	M6020B ICP-MS	22.2	84.411	0.6	2.281	2	26.3
072418URUBSS08	URUB	7/24/2018	Zinc	M6020B ICP-MS	28.2	107.224	0.6	2.281	1	26.3
072418URUBSS09	URUB	7/24/2018	Zinc	M6020B ICP-MS	27.7	128.241	0.7	3.241	2	21.6
072418URUBSS10	URUB	7/24/2018	Zinc	M6020B ICP-MS	18	71.146	0.9	3.557	2	25.3
072418URUBSS11	URUB	7/24/2018	Zinc	M6020B ICP-MS	24.2	102.11	0.8	3.376	2	23.7
072418URUBSS12	URUB	7/24/2018	Zinc	M6020B ICP-MS	25.2	102.439	0.8	3.252	2	24.6
072418URUBSS13	URUB	7/24/2018	Zinc	M6020B ICP-MS	34.7	148.927	0.6	2.575	2	23.3
072418URUBSS14	URUB	7/24/2018	Zinc	M6020B ICP-MS	34.1	152.232	0.6	2.679	2	22.4
072418URUBSS15	URUB	7/24/2018	Zinc	M6020B ICP-MS	25.2	110.526	0.6	2.632	2	22.8

*MDL = Method Detection Limit **PQL = Practical Quantitation Limit

APPENDIX 4. GENETIC VARIATION IN DOLLY VARDEN COLLECTED IN RED ROCK CREEK, 2018¹

The Shungnak River is a tributary of the Kobuk River, flowing approximately 75 km before entering the Kobuk River downstream of the village of Shungnak. Though multiple tributaries of the Kobuk River support spawning populations of anadromous Dolly Varden *Salvelinus malma malma*, fish migration into the Shungnak River is impeded by a waterfall so that only stream-resident Dolly Varden are present. An aquatic biomonitoring project conducted in 2017 in the region detected resident Dolly Varden at sample sites in the Shungnak River mainstem, Ruby Creek, and Subarctic Creek (Bradley 2018). A genetic assessment of Dolly Varden sampled from Subarctic Creek in 2017 showed lower levels of within population variation and increased genetic divergence compared to Dolly Varden sampled from two tributaries (Salmon and Tutuksuk rivers) of the middle Kobuk River that support anadromous Dolly Varden (Bradley 2018). These genetic diversity patterns (increased divergence among populations, less genetic variation within populations above barriers) are typical where gene flow is limited across a migration barrier (e.g., Whitely et al. 2010).

Stream resident Dolly Varden in Alaska are typically found in the upper tributaries of major rivers, but their life history is poorly understood (Armstrong and Morrow 1980). Populations can be fully resident, i.e., never leaving their spawning tributary; or migratory, e.g., leaving headwater reaches to overwinter near tributary mouths (Armstrong and Morrow 1980). In Asia, partial migration has been described, where a segment of a population leaves the natal tributary to rear in other portions of the watershed (Armstrong and Morrow 1980, Koizumi et al. 2006a); these fish often attain larger sizes than their non-migratory counterparts (Koizumi et al. 2006a). If Dolly Varden adopt a migratory life history, they typically home to their natal tributary (Armstrong and Morrow 1908; Koizumi et al. 2006b). Genetic population structure can occur in freshwater salmonids within rivers, in part dependent on habitat size and complexity (Costello et al. 2003). Red Rock Creek, another tributary of the Shungnak River, was sampled in 2018 and compared to Dolly Varden from Subarctic Creek in 2017 (Bradley 2018) to determine if there is evidence of population subdivision within the Shungnak River.

Methods

Fin clips were collected from 50 Dolly Varden captured in baited minnow traps from 19-20 September 2018. Genetic variation was assayed at 11 microsatellite loci following the methods described in Bradley (2018). Data were added to data from resident Dolly Varden collected from Subarctic Creek (N=50) in 2017 and anadromous Dolly Varden collected from Salmon River and Tutuksuk River in 2016 (see Bradley 2018). Unless noted, all data analysis was conducted using the program FSTAT Version 2.9.4 (Goudet 2001). Observed genotypic frequencies in Red Rock

¹ By Penny Crane and Randal Loges, Conservation Genetics Laboratory, U.S. Fish and Wildlife Service, 1011 E. Tudor Road, Anchorage, AK 99503

Creek were tested to determine if they differed from Hardy-Weinberg expected frequencies and to test for genotypic disequilibrium between all pairs of loci. Population differentiation between Red Rock Creek and Subarctic Creek was tested. Gene diversity and allele richness based on a minimum sample size of N=40 were estimated for Red Rock Creek. Randomization tests were used to determine if gene diversity and allele richness in resident samples (Red Rock and Subarctic creeks) differed from anadromous samples (Salmon and Tutuksuk rivers). Lastly, the R package *hierfstat* (Goudet and Jombart 2018) was used to estimate pairwise F_{ST} for Red Rock Creek, Subarctic Creek, Salmon River, and Tutuksuk River according to the method of Weir and Cockerham (1984). A neighbor-joining tree was constructed from pairwise F_{ST}s to visualize genetic relationships among populations in the Kobuk River using the R package *ape* (Paradis and Schliep 2018).

Results

Nine of the eleven microsatellite loci assayed were polymorphic in Red Rock Creek Dolly Varden. One locus had genotypic frequencies deviating from Hardy-Weinberg proportions (P=0.03), but did not remain significant after Bonferroni adjustment for 9 multiple tests. Genotypic disequilibrium was detected in four locus pairs (P<0.05), but these did not remain significant after Bonferroni adjustment for 36 multiple tests. Gene diversity was 0.61 and allelic richness was 7.70 in Red Rock Creek. Gene diversity (0.58) and allele richness (6.92) in resident Dolly Varden from Red Rock Creek and Subarctic Creek did not differ from gene diversity (0.63) and allele richness (9.10) in anadromous Dolly Varden from Salmon River and Tutuksuk River (P<0.35).

Allelic heterogeneity was detected between Red Rock Creek and Subarctic Creek for eight of the nine polymorphic loci (P<0.004; overall P<0.001). Pairwise F_{ST} between Red Rock Creek and Subarctic Creek was 0.057; pairwise F_{ST}s between Red Rock Creek and anadromous samples from Salmon River and Tutuksuk River were 0.041 and 0.045. The neighbor-joining tree showed that Red Rock Creek and Subarctic Creek were divergent from each other as well as from Salmon and Tutuksuk rivers (Figure 1).

Discussion

Dolly Varden collected at Red Rock Creek were likely representative of a single, randomly mating population. There was no strong evidence for Hardy-Weinberg or genotypic disequilibrium that could be signs of multiple populations within this collection. It is not known if Dolly Varden in the Shungnak River system are migratory so the potential for mixing of tributary populations is unknown. However, samples were collected in September, corresponding to the spawning timing of river resident Dolly Varden (Armstrong and Morrow 1980), increasing the likelihood of collecting Dolly Varden natal to Red Rock Creek.

Significant genetic differentiation was detected between Red Rock Creek and Subarctic Creek, indicating that Shungnak River supports more than one breeding population of Dolly Varden. Population structure in resident salmonids is not uncommon, even at small spatial scales with no physical barriers to gene flow (e.g., Koizumi et al. 2006b) or if fish appear to be continuously

distributed within a watershed (e.g., Kazyak et al. 2015). The level of divergence between Red Rock Creek and Subarctic Creek was greater than that observed between anadromous Dolly Varden in Salmon River and Tutuksuk River (Figure 1), though the mouths of these tributary pairs are separated by similar distances, approximately 5 km. Harris et al. (2015) found global F_{ST} estimates to be greatest among Dolly Varden populations isolated by waterfalls, intermediate among resident populations, and the lowest among anadromous populations, illustrating the effect of physical barriers to migration and dispersal ability on population structure.

Estimates of within population variability, gene diversity and allele richness, were smaller in resident than anadromous populations, but not significantly so. Costello et al. (2003) found that the loss of within population variation can be buffered by increasing habitat size. Further, Dolly Varden have been detected at several locations in the Shungnak River and its tributaries (Bradley 2018). Low levels of gene flow through passive migration if fish are washed down stream, or straying if partial migration exists in these tributaries, may also be providing a buffer to the loss of within population variation.

Acknowledgements

We thank Jeff Olsen of the U.S. Fish and Wildlife Service, Conservation Genetics Laboratory and Chris Habicht of Alaska Department of Fish and Game Gene Conservation Laboratory for review. The findings and conclusions in this paper are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service. The use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Literature Cited

- Bradley, P. 2018. Aquatic biomonitoring at the Arctic-Bornite Prospect. Alaska Department of Fish and Game, Technical Report No.18-04, Fairbanks, AK
- Armstrong, R. H., and J. E. Morrow. 1980. The dolly varden charr, *Salvelinus malma*. Pages 99-140 in E. Balon, editor. Charrs: salmonid fishes of the genus Salvelinus. Dr. W. Junk Publishers, The Hague, The Netherlands.
- Costello, A. B., T. E. Down, S. M. Pollard, C. J. Pacas, and E. B. Taylor. 2003. The influence of history and contemporary stream hydrology on the evolution of genetic diversity within species: an examination of microsatellite DNA variation in bull trout, *Salvelinus confluentus* (Pisces: Salmonidae). Evolution 57:328-344.
- Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). <u>http://www.unil.ch/izea/softwares/fstat.html</u>.
- Goudet, J. and R. Jombart. 2018. hierfstat: estimation and tests of hierarchical F-statistics. http://www.r-project.org, <u>http://github.com/jgx65/hierfstat</u>.
- Harris, L. N., R. Bajno, C. P. Gallagher, I. Koizumi, L. K. Johnson, K. L. Howland, E. B. Taylor, and J. D. Reist. 2015. Life-history characteristics and landscape attributes as drivers of genetic variation, gene flow, and fine-scale population structure in northern Dolly Varden (*Salvelinus malma malma*) in Canada. Canadian Journal of Fisheries and Aquatic Sciences 72:1477-1493.

- Jombart, T. 2008. adegent: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403-1405.
- Kazyak, D. C., R. H. Hilderbrand, T. L. King, S. R. Keller, and V. E. Chhatre. 2016. Hiding in plain sight: a case for cryptic metapopulations in brook trout (*Salvelinus fontinalis*). PLoS ONE 11:e01146295. doi:10.1371/journal.pone.0146295.
- Koizumi, I., S. Yamamoto, and K. Maekawa. 2006a. Female-biased migration of streamdwelling Dolly Varden in the Shiisorapuchi River, Hokkaido, Japan. Journal of Fish Biology 68:1513-1529.
- Koizumi, I., S. Yamamoto, and K. Maekawa. 2006b. Decomposed pairwise regression analysis of genetic and geographic distances reveals a metapopulation structure of streamdwelling Dolly Varden charr. Molecular Ecology 15:3175-3189.
- Paradis, E., and K. Schliep. 2018. ape 5.0: an environment for modern phylogenetics and evolutionary analysis in R. Bioinformatics 35:526-528.
- Weir, B. S., and C. C. Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358-1370.
- Whitely, A. R., K. Hastings, J. K. Wenburg, C. A. Frissell, J. C. Martin, and F. W. Allendorf. 2010. Genetic variation and effective population size in isolated populations of coastal cutthroat trout. Conservation Genetics 11:1929-1943.



Figure 1. Neighbor-joining tree constructed from pairwise F_{ST} for Dolly Varden sampled from the Kobuk River drainage, 2016-2018. Dolly Varden from Subarctic Creek and Red Rock Creek are isolated by a waterfall in the Shungnak River and are resident, and Dolly Varden from Salmon River and Tutuksuk River are anadromous.