Technical Report No. 17-09

Methods for Aquatic Life Monitoring at the Red Dog Mine Site

A requirement of the 2017 APDES Permit AK0038652

by Parker T. Bradley

October 2017

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

Symbols and Abbreviations

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Weights and measures (metric)

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

Weights and measures (English)

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

Time and temperature

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

Physics and chemistry

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

General

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

Measures (fisheries)

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

Mathematics, statistics

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

TECHNICAL REPORT NO. 17-09

METHODS FOR AQUATIC LIFE MONITORING AT THE RED DOG MINE SITE

A Requirement of the 2017 APDES Permit AK0038652

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October, 2017

Cover: North Fork Red Dog Creek, July 2017, Drift Nets. Photograph by Parker T. Bradley

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Introduction

The Red Dog zinc (Zn) and lead (Pb) deposit is located in northwestern Alaska, about 130 km north of Kotzebue and 75 km inland from the coast of the Chukchi Sea (Figure 1). On July 28, 2017, the Alaska Department of Environmental Conservation (ADEC) issued Alaska Pollutant Discharge Elimination System (APDES) Permit No. AK0038652 (APDES Permit) to Teck Alaska Incorporated (Teck) to allow discharge of up to 2.418 billion gallons of treated effluent per year. The permit is effective September 1, 2017 through August 31, 2022. The APDES Permit requires bioassessment of periphyton, aquatic invertebrates, and fish in selected streams near the Red Dog Mine (Table 1).

Table 1. Location of APDES Sample Sites and Factors Measured.

Sample Site	Factors Measured
North Fork Red Dog Creek	Periphyton (chlorophyll-a concentrations) Aquatic invertebrates (taxonomic richness and abundance) Fish presence and use
Mainstem Red Dog Creek	Periphyton (chlorophyll-a concentrations) Aquatic invertebrates (taxonomic richness and abundance) Fish presence and use
Ikalukrok Creek	Periphyton (chlorophyll-a concentrations) Aquatic invertebrates (taxonomic richness and abundance) Fish presence and use

Teck is required by their APDES Permit to submit for approval an updated version of the Biomonitoring Plan – *ADF&G Methods for Aquatic Life Monitoring to Satisfy Requirements of 2010 NPDES Permit, Red Dog Mine Site (Revision #1)* (ADF&G 2010). This report is an update to that 2010 Biomonitoring Methods Report and is intended to satisfy the APDES biomonitoring plan update requirement. A complete description of the biomonitoring program is contained in Teck's Waste Management Plan dated November 2016 (SRK Consulting 2016). Teck's Monitoring Plan includes sample sites, sampling frequency, and parameters for all the aquatic sample sites (Table 2 and Figure 2). Field and laboratory procedures and quality control for all sites will be the same. All sampling is conducted when flow is present. Periphyton and aquatic invertebrates are sampled in early July. Fish sampling is conducted throughout the open water season. The "pond" referred to in Teck's Monitoring Plan is the freshwater reservoir, also referred to as Bons Pond. Teck's monitoring plan also is incorporated by reference into the

Alaska Department of Natural Resource's Reclamation Plan Approval (F20169958) dated September 28, 2016.

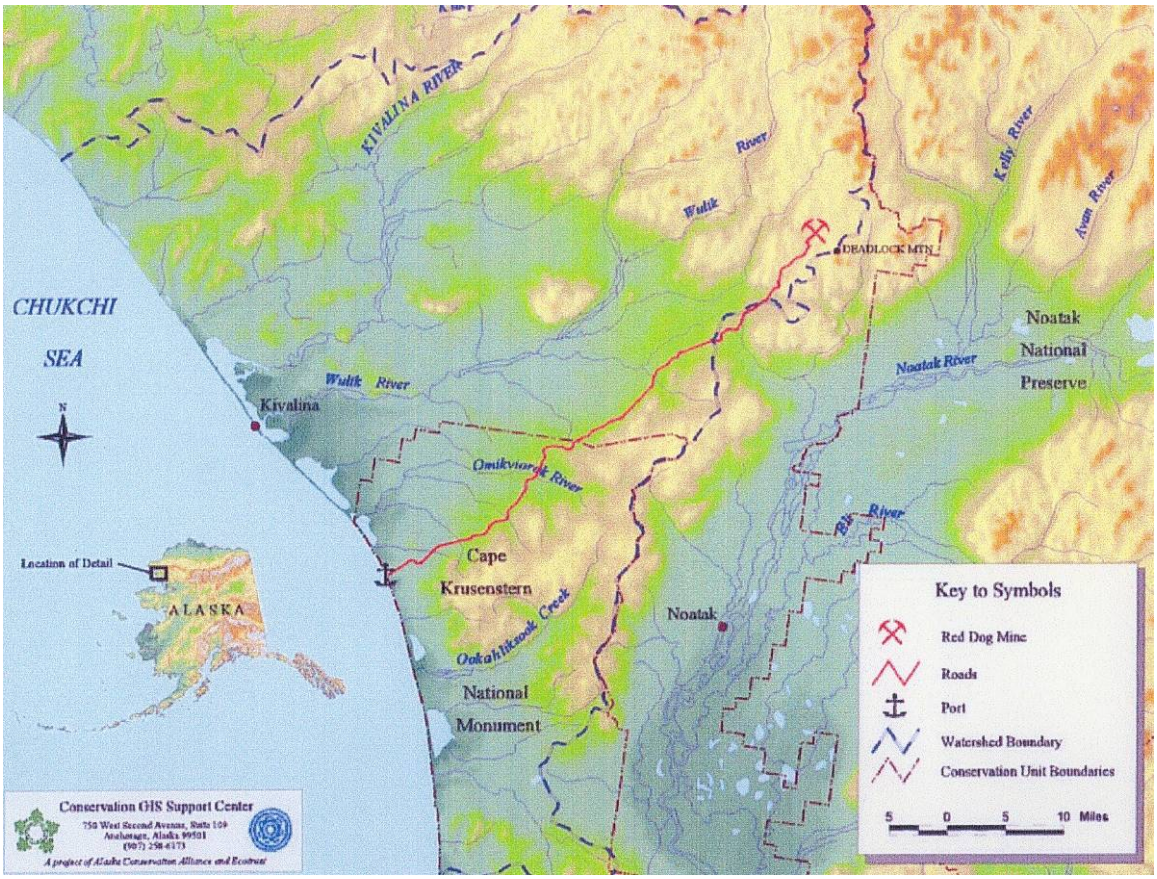

Figure 1. Location of the Red Dog Mine in northwestern Alaska. Map used with permission of Conservation GIS Support Center, Anchorage, Alaska.

Table 2. Location of Sample Sites and Factors Measured.

Location	APDES or WMP	Location Description	Sampling Frequency	Parameters
Wulik River	WMP	Kivalina Lagoon to about 10 km upstream of the mouth of Ikalukrok Creek	1/year	Fall aerial surveys for overwintering Dolly Varden
Ikalukrok Creek	WMP	Lower Ikalukrok Creek to mouth of Dudd Creek	1/year	Fall aerial surveys for adult chum salmon
Station 9	APDES/WMP	Ikalukrok Creek	1/year	Periphyton (as chlorophyll-a concentrations)
			1/year	Aquatic invertebrates (monitored for taxonomic richness, abundance, and density)
			1/year	Fish presence and use
Station 160	WMP	Lower Ikalukrok Creek	1/year	Periphyton (as chlorophyll-a concentrations)
			1/year	Aquatic invertebrates (monitored for taxonomic richness, abundance, and density)
			1/year	Fish presence and use
Station 20	WMP	Middle Fork Red Dog Creek	1/year	Periphyton (as chlorophyll-a concentrations)
			1/year	Aquatic invertebrates (monitored for taxonomic richness, abundance, and density)
Station 10	APDES/WMP	Mouth of Red Dog Creek	1/year	Periphyton (as chlorophyll-a concentrations)
			1/year	Aquatic invertebrates (monitored for taxonomic richness, abundance, and density)
			1/year	Fish presence and use
			1/year	Juvenile Dolly Varden elements in tissue (Zn, Pb, Se, Hg, and Cd)
Station 12	APDES/WMP	North Fork Red Dog Creek	1/year	Periphyton (as chlorophyll-a concentrations)
			1/year	Aquatic invertebrates (monitored for taxonomic richness, abundance, and density)
			1/year	Fish presence and use
			1/year	Record of spawning activity (Arctic grayling)
			Periodic	Capture/mark Arctic grayling
Buddy Creek	WMP	Below Falls, 1.5 km Downstream of Haul Road	1/year	Periphyton (as chlorophyll-a concentrations)
			1/year	Aquatic invertebrates (monitored for taxonomic richness, abundance, and density)
			1/year	Fish presence and use
			1/year	Juvenile Dolly Varden elements in tissue (Zn, Pb, Se, Hg, and Cd)
Buddy 221	WMP	Buddy Creek, Above Road	1/year	Periphyton (as chlorophyll-a concentrations)
			1/year	Aquatic invertebrates (monitored for taxonomic richness, abundance, and density)
Bons 220	WMP	Bons Creek, Below Pond	1/year	Periphyton (as chlorophyll-a concentrations)
			1/year	Aquatic invertebrates (monitored for taxonomic richness, abundance, and density)
Bons Above Pond	WMP	Above Pond	1/year	Periphyton (as chlorophyll-a concentrations)
			1/year	Aquatic invertebrates (monitored for taxonomic richness, abundance, and density)
			Periodic	Capture/mark Arctic grayling
Anxiety Ridge Creek	WMP	Haul Road Crossing	1/year	Fish presence and use
			1/year	Juvenile Dolly Varden metals in tissue (Zn, Pb, Se, Hg, and Cd)
Evaingiknuk Creek	WMP	East of Haul Road	1/year	Fish presence and use
Bons Reservoir	WMP	Above Reservoir Spillway	1/year	Juvenile Arctic grayling elements in tissue (Zn, Pb, Se, Hg, and Cd)
			1/year	Arctic grayling population estimate

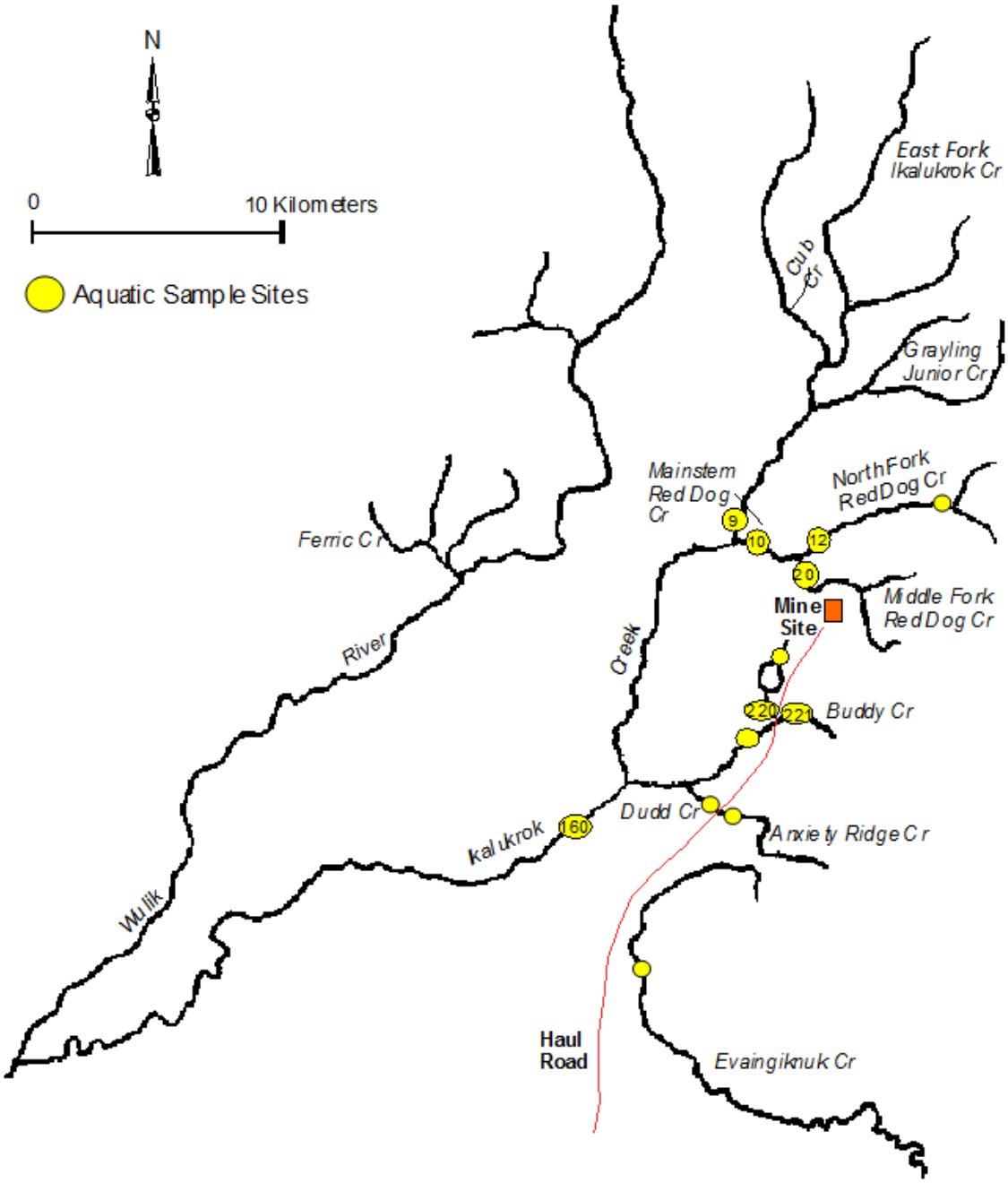

Figure 2. Location of sample sites (some have a Station #) in the Ikalukrok Creek and Evaingiknuk Creek (a tributary of the Noatak River) drainages.

Objectives, Methods, and Quality Control

Periphyton Standing Crop (as chlorophyll-a concentrations)

Objectives

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. The presence of periphyton in a stream system is evidence of continued in-situ productivity. Periphyton density will be monitored to detect changes in in-situ productivity in receiving waters downstream of the Red Dog Mine treated wastewater discharge. Reference sites will be sampled to detect variations due to other factors, including natural mineral seeps, climate, and thermal/hydraulic erosion.

Periphyton are sampled directly from cobble on the streambed. The periphyton are collected from a riffle area of submerged cobble, following the rapid bioassessment techniques of Barbour et al. (1997), but with more replicates per site to increase sample precision. The concentrations of chlorophyll-a are determined to estimate periphyton standing crop. Sampling is done once per year, during the period from late June through mid-July and only under low flows. Sampling during low flows allows us to ensure that the submerged cobble material has been wetted continuously for at least the last month.

Field Methods

Ten flat rocks larger than 25 cm^2 are collected from a submerged riffle area of the streambed and temporarily placed under water in the work area. Rocks are selected from an area of the stream where they are suspected to have been underwater for the last month. Rocks are removed from water generally deeper than 15 cm. A 5 cm x 5 cm square of high density flexible foam is placed in the middle portion of the rock surface that was facing up in the stream. The foam square is held in place while all material around the square is scrubbed with a toothbrush and rinsed from the rock with a squeeze bottle filled with clean water collected from the stream. This is done twice (Figure 3). The toothbrush is cleaned by thorough rinsing in the stream between each step. The foam square is removed from the rock and the portion of the rock that was covered by the foam square is brushed with a cleaned toothbrush and rinsed with clean water onto a 0.45 um glass fiber filter in a filter receptacle attached to a hand vacuum pump (Figure 4).

Figure 3. Brushing/removal of material from rock around the 5-cm x 5-cm flexible foam square.

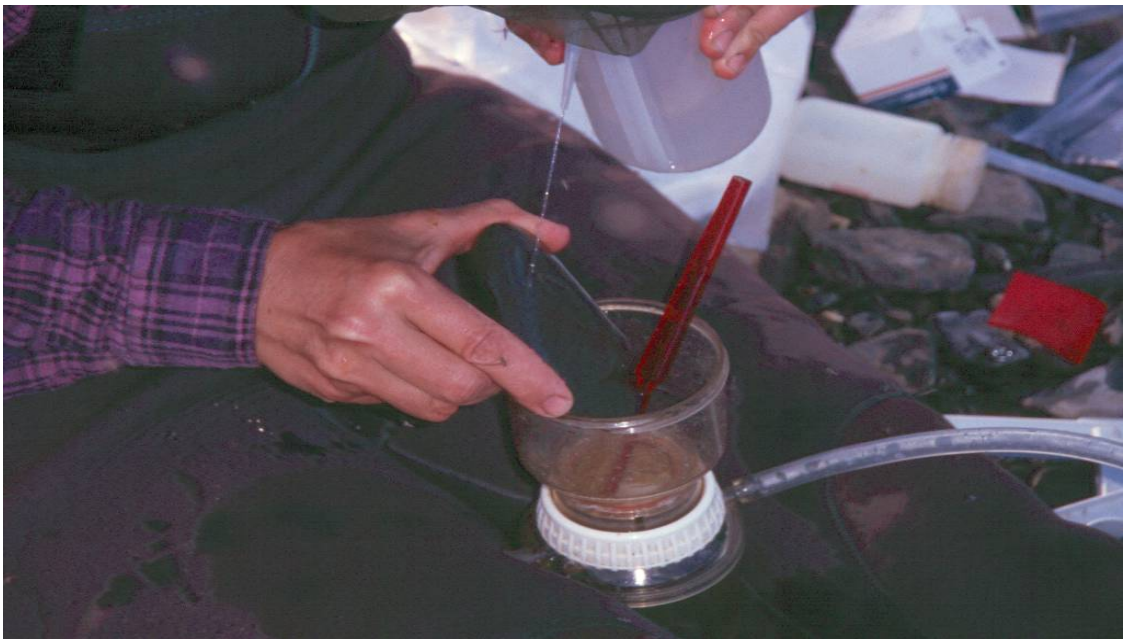

Figure 4. Removal of material from beneath the sample square and rinsing with clean water into the filter receptacle. Note toothbrush is placed in the filter receptacle since it contains part of the sample material.

The rock is brushed twice and the toothbrush is placed in the filter receptacle attached to the vacuum pump. After the rock has been cleaned, the material on the toothbrush is rinsed onto the

filter with clean stream water. Any material on the foam square, including that in contact with the rock, is not rinsed into the filter receptacle. However, the foam square is rinsed with clean water before being applied to the next rock.

Water is then extracted from the periphyton sample with the vacuum pump. After extracting most of the water (i.e., ¼ inch of water remains above the glass fiber filter), about 3 to 5 drops of a saturated solution of $MgCO_3$ is added to the sample (Figure 5). A saturated solution contains both a solid (solute) and a liquid (solvent), therefore the bottle of $MgCO_3$ should be well shaken before removing the solution with the eye dropper and applying it to the sample; care is taken to avoid applying the solid $MgCO_3$ to the sample. The $MgCO_3$ is added while gently swirling the sample to ensure the entire sample receives a light coating. Pumping continues until the water is gone and the glass fiber filter appears dry. The $MgCO_3$ is added to prevent acidification and additional conversion of chlorophyll-a to phaeophytin.

Figure 5. $MgCO_3$ is added to sample in the filter receptacle.

If the water has not moved through the filter within a few minutes, then a second glass fiber filter with another vacuum pump should be used and excess water transferred to the second filter receptacle. Each additional glass fiber filter required to collect the sample must be treated with $MgCO_3$ as outlined above.

The receptacle on top of the vacuum pump is then removed and the glass fiber filter is folded over, placing the sample material on the inside of the filter. If two glass fiber filters are used then these are placed face to face with the sample material on the inside. Alternatively, multiple glass fiber filters used for one rock can be folded separately, as above, but must be stored

together. The glass fiber filter(s) are then placed on a dry paper coffee filter (cone shaped) and the coffee filter is folded to cover the entire glass fiber filter(s) (Figure 6).

Figure 6. The folded glass fiber filter is placed in a coffee filter. The coffee filter is folded to completely cover the sample glass fiber filter.

The paper coffee filters are used to absorb any residual water that may be present. The coffee filters containing the glass fiber filter samples are then placed in a properly labeled, sealable plastic bag, silica gel desiccant is added, and the sample bag is placed in a light-proof container with ice. It is essential that filters be kept cool and dark while in the field to prevent sample degradation. Immediately upon return to a facility with a freezer, the samples are frozen and kept frozen until the samples are analyzed.

Laboratory Methods

A spectrophotometer is used to measure the concentrations of chlorophyll in the periphyton samples. To provide a chlorophyll sample for instrument calibration, fresh spinach leaves are placed in a 90% spectrophotometric grade acetone solution, covered in aluminum foil to ensure that they are not exposed to light and soaked overnight in a refrigerator. This concentration is used as the full strength control solution for instrument linear check dilutions. The control solution is diluted until meaningful absorption values are recorded.

Control dilutions ranging from full strength down to a solution with a concentration factor that produces chlorophyll a concentrations below our sample concentrations (typically concentration

factor 0.005) are analyzed on the spectrophotometer and total chlorophyll a, -b, and -c are calculated using the tri-chromatic equation. Tri-chromatic equations (according to Standard Methods, APHA 1992) are used to convert spectrophotometric optical densities (absorbance values) to total chlorophyll a, -b, and -c. Absorption values at 750 nm, 664 nm, 647 nm, and 630 nm are recorded. Calculated chlorophyll-a concentrations are then plotted against the known concentration as calculated from the concentration factors. The calculated and actual concentrations are compared to check for linearity. Three additional solutions of varying chlorophyll-a concentration are prepared. Ten samples are drawn from each solution and absorption values at the appropriate wave lengths to calculate chlorophyll a, -b, and -c are recorded. Descriptive statistics are calculated for each solution to determine detection limits.

Once the control solutions have been run, the periphyton samples are removed from the freezer, the glass fiber filters are cut into small pieces, placed in individual 15 ml centrifuge tubes with 10 ml of 90% spectrophotometric grade acetone, and soaked overnight in a dark refrigerator. Tubes are wrapped in aluminum foil to ensure they remain completely dark during the extraction. On the day following initial preparation, but within 24 hours of preparation, samples are placed in a centrifuge and spun at 1600 rpm for 20 minutes. Samples are then decanted individually into cuvettes and absorption values at 750 nm, 664 nm, 647 nm, and 630 nm are recorded on a split beam spectrophotometer. About 0.08 ml of 0.1N HCl acid is then added to each cuvette and the sample allowed to sit, in the dark, for 90 seconds. Absorption values at 750 nm and 665 nm are recorded.

The spectrophotometer is zeroed using a 90% acetone solution prior to analyzing samples and routinely checked throughout the sample run. Filter blanks also are processed and run on the spectrophotometer. Two new filters are placed on the laboratory bench prior to any sample preparation. One glass fiber filter is prepared as above, prior to preparation of samples and one filter is prepared after all sample filters are prepared. These filters serve as laboratory blanks to ensure samples are not being contaminated in the laboratory. Additionally, one sample per group of samples is decanted a second time from the 15 ml centrifuge tube and analyzed as a replicate to ensure repeatability.

Once all samples are analyzed, data are analyzed through the tri-chromatic equation to determine chlorophyll a, -b, and -c concentrations. Additionally, phaeophytin is calculated to determine if a chlorophyll-a conversion has occurred, and to correct for chlorophyll-a concentrations for the presence of phaeophytin.

Quality Control, Field

All steps identified in the methods section will be followed. Samples are placed in pre-labeled bags, fresh silica gel desiccant is added, and the sample bag is placed on ice in a small insulated container. Samples are immediately frozen upon return from the field within 6 to 8 hours of collection and are maintained in a frozen state until removed for analyses in the laboratory.

Quality Control, Laboratory

All steps identified in the methods section will be followed. All instrument self-checks and adjustments are conducted prior to analyzing samples. Fresh chlorophyll extractions are used to check the spectrophotometer for a linear response prior to each sample analysis. Samples with chlorophyll concentrations below the calibration point are reported as "non detectable."

Aquatic Invertebrates (Taxonomic Richness, Abundance, and Density)

Objectives

Aquatic invertebrate communities are sampled at selected sites in the Red Dog Mine area to document the biological integrity of these communities and to detect changes in in-situ productivity over time. Reference sites that are not influenced by the wastewater discharge are included. Reference sites are used to evaluate variations due to other factors, including natural mineral seeps, climate, and thermal/hydraulic erosion. The presence of mayflies (Ephemeroptera), stoneflies (Plecoptera), and caddisflies (Trichoptera) (EPT) are evidence of continued in-situ biological productivity. However, most sites in most years are dominated by midges (Chironomidae).

Field Methods

A modified rapid bioassessment technique developed by USEPA Barbour et al. (1997) is used to retain more quantitative features in the sampling program. The sampling techniques currently utilized at the Red Dog Mine are based on multiple trials of various field-sampling methods (kick nets, surber sampler, and drift nets) and the duration of time (1 or 24 hours for drift nets) drift nets are allowed to capture insects. The final determination of the sampling methods was based on the time required for sorting, identification, and counting of aquatic invertebrates captured, while still maintaining an adequately-sized and diverse sample of invertebrates. Based on multiple field tests, a 1 hour sample time was selected for drift nets as the best method for assessing the taxonomic richness, abundance, and density of aquatic invertebrates.

At each sample site, five drift nets are installed in riffle habitat. Nets are placed along a transect perpendicular to the flow (Figure 7) and are numbered from left (1) to right (5) facing upstream. All the streams sampled are wide enough to allow the placement of the five nets. The drift nets are 45.7 cm (18 in) wide by 30.5 cm (12 in) deep with 350 *u*m mesh size – nitex nylon for the bag portion of the net and stainless steel mesh for the collecting cod. The drift nets are placed with the long side on the stream bottom and the nets upright in the current (the metal stakes may need to be pounded into the substrate to secure the nets). The water depth at the inlet to the drift net and the average water velocity in the mouth of each net are measured and recorded with a Marsh-McBirney flowmeter.

Figure 7. Drift nets in lower Competition Creek, July 2017.

After 1 hour, the drift nets are removed and placed along the stream margin with the open end on the streambank and the cod end in the water to keep the sample wetted. Materials in the net are flushed into the cod end by splashing water on the outside of the net. After all debris and insects are rinsed from the net, the cod end is removed. The contents are rinsed out using a squirt bottled filled with denatured ethanol into a 500 ml Nalgene bottle labeled with the sample site and appropriate net number. Additional denatured ethanol is then added to completely submerge the sample. After the lid is put on, the sample bottle is inverted several times to ensure the alcohol is well mixed with the sample. Nets and cod ends should be well rinsed before deploying at the next site. The five labeled sample containers are then placed in a plastic bag.

For samples that contain much fine detritus or filamentous algae, which retain a lot of water, half the alcohol is decanted at the end of the day and replaced with fresh alcohol. The final concentration of alcohol should be approximately 80%. If the cod end is full of woody debris that won't fit easily in the Nalgene, sticks/leaves are rinsed and then discarded. If any juvenile fish (>50mm) are caught in the drift nets, they are identified & measured, then record in the data notebook and released.

Samples are packaged and shipped from the field to the laboratory via an air cargo flight.

Laboratory Methods

Each sample is drained of the denatured ethanol through a mesh sieve (350 *u*m) and then placed into a container and filled with water. Floating invertebrates are picked from the container until no more invertebrates are seen. Water is poured over the sample and the floating invertebrates poured into a mesh sieve (350 *u*m). Large pieces of debris are removed and flushed with water and the process is repeated until no more invertebrates are found in the debris. Care is taken to minimize invertebrates lost during the washing process.

The washed sample is then subsampled. Each sample is emptied onto a gridded tray and covered with water to assist with spreading the sample equally among the 30 squares. For very small samples, 15 of the 30 squares are used. Random numbers are selected to choose among the sample squares. The number of squares selected for the subsample is dependent upon the size of the sample; a larger sample would result in three squares in each subsample and five subsamples, whereas a smaller sample would result in five squares and three subsamples. Some samples with minimal material and invertebrates are analyzed without subsampling. Invertebrates from the subsamples are sorted, counted, and identified until the total sample exceeds the required 300 organisms (Barbour et al. 1997). The subsample of invertebrates that is sorted, counted, and identified is retained and stored in the laboratory. Notes are made to keep accurate track of how many subsamples are processed so that the total number of organisms by type can be calculated for each sample.

Quality Control, Field Sampling

All steps identified in the methods section are followed. Samples are placed in pre-labeled containers, transported back to the mine site, and later shipped by airfreight to Fairbanks. Samples are retained in one place until laboratory procedures are initiated.

Quality Control, Laboratory

All steps identified in the methods section are followed. Ten percent of the samples are selected and checked for identification and counting errors. Half of the samples checked are randomly chosen from samples available early in the process and the rest are selected towards the middle of the sorting and identification process. If problems are encountered early in the process, improvements are made in sorting and identification before the remaining samples are completed. Every effort is made to accurately sort and identify the invertebrates sampled. Verification of identifications is done by a Habitat Biologist experienced with invertebrate identification and the types of organisms found in streams at the Red Dog Mine.

Juvenile Dolly Varden and Arctic Grayling (Element Concentrations)

Objectives

Whole body analyses of juvenile Dolly Varden for Cadmium (Cd), Mercury (Hg), Lead (Pb), Selenium (Se), and Zinc (Zn) are conducted on fish collected in selected streams near the Red Dog Mine. The three streams are Mainstem Red Dog, Buddy, and Anxiety Ridge creeks. Juvenile Arctic grayling are collected from Bons Pond for whole body analyses for Cd, Hg, Pb, Se, and Zn. The objectives of the juvenile fish sampling are to build a database that can be used to determine differences among sample sites, species, and to evaluate changes of selected elements over time.

Field Methods

Juvenile Dolly Varden are collected in stream sample reaches in late-July to mid-August using minnow traps baited with salmon eggs. Juvenile Dolly Varden use of the sample sites is limited to the ice-free season as they outmigrate to overwintering areas in the fall. Sampling for juvenile Dolly Varden occurs in late summer, typically late July to mid-August. This sampling time is based on juvenile Dolly Varden distribution and abundance peaking just prior to decreasing water temperatures associated with freezeup (Ott and Morris 2005). Anxiety Ridge and Buddy creeks are reference sites, while Mainstem Red Dog Creek is potentially affected by mine-related activities associated with the tailing impoundment and treated wastewater. For Arctic grayling in Bons Pond the preferred sample time is early spring; juvenile Arctic grayling are year-round residents at the sample site so exposure time is known.

Sampling involves placement of 10 minnow traps per site (Figure 8). Minnow traps are used because they are very effective at catching juvenile Dolly Varden. Juvenile Dolly Varden are the primary target species because they are a ubiquitous species in the area and the species most susceptible to minnow traps.

Figure 8. Minnow trap being placed in Mainstem Red Dog Creek.

Plastic bait sacs containing treated salmon eggs are premade prior to field work. Salmon eggs are pretreated with a 1% solution of betadine for at least 10 minutes. Minnow traps are baited with the premade bait sacs by perforating the sac at the time the traps are set and placing them on the downstream side of the minnow trap. Rocks are picked from the streambed and placed in each minnow trap to both hold the trap and bait in place and to provide refuge for fish caught in the trap. Traps are placed in moving water and not in backwater areas or pools as juvenile Dolly Varden generally prefer higher velocity water. Traps are numbered for each sample reach and are fished for 24 hours. Each sample reach is established with an upper and lower point and 10 traps are placed in each sample reach. The exact location of traps varies annually due to changes in the stream and discharge at the sample time. Individual sites are marked in the field with flagging. When traps are checked, fish are removed, identified to species, measured to the nearest millimeter, and either retained for element analysis, or released back to the sample reach (Figure 9).

Figure 9. Juvenile Dolly Varden from Station 9 on Ikalukrok Creek, 2015.

Juvenile Dolly Varden between 90 and 140 mm fork length are selected for the whole body element analyses. Fork length and weight are recorded in the field. Selection of fish from this length range ensures that most of the fish are ages two or three. A maximum of fifteen juvenile Dolly Varden per sample reach are kept. Fish are handled with class 100 nitrile gloves and each fish is placed in an individually numbered plastic bag. Plastic bags are labeled with sample date, location, species, fish maturity, and an individual number [e.g., 081017MSRDDVJ1 = August 10, 2017, Mainstem Red Dog Creek (MSRD), Dolly Varden (DV), juvenile (J), and consecutive numbers for that sampling period]. The 15 plastic bags containing juvenile fish are placed in a larger sample bag that also is labeled with the sample location, and stored in an insulated container with an ice pack.

Juvenile Dolly Varden are transported back to the mine where they are immediately frozen. Fish are then packaged and shipped frozen to Fairbanks where they are placed in the low temperature freezers at the ADF&G office. The fish are kept in their sealed bag in a sealed container in the freezer at ADF&G until they are prepared for shipment to an analytical laboratory.

Juvenile Arctic grayling from Bons Pond between 150 and 200 mm fork length are selected for the whole body element analyses. All the same field protocols are used with the exception of the sampling method (angling and fyke nets) and the time frame (preferred sampling window is spring). Fish between 150 and 200 mm fork length are most likely age 2 or 3. There is no upstream movement of fish into Bons Pond due to an impassable falls at the end of the bypass channel that carries water around the dam. Therefore, these fish have spent their entire life in Bons Pond or the creeks that feed Bons Pond upstream of the freshwater dam.

Laboratory Methods

The frozen juvenile Dolly Varden and Arctic grayling are removed from the freezer and shipped via air to a private analytical laboratory. A catalog for each sample with an identification number is prepared and shipped with the samples. Whole body fish are analyzed for selected elements using U.S. Environmental Protection Agency standard methods.

Quality Control, Field Sampling

Juvenile fish are identified, measured, and weighed and placed in individually numbered plastic bags. Habitat Biologists with field experience ensure that all field procedures are followed. All fish are handled wearing class 100 nitrile gloves.

Quality Control, Laboratory

Frozen juvenile Dolly Varden and Arctic grayling are removed from the ADF&G low temperature freezer and shipped directly to a private analytical laboratory. A catalog for each sample with an identification number is prepared and shipped with the samples. Whole fish are analyzed for selected elements using U.S. Environmental Protection Agency standard methods.

Quality Control/Quality Assurance of Laboratory Analysis

The analytical laboratory provides quality assurance/quality control information for each analyte, including matrix spikes, standard reference materials, laboratory calibration data, sample blanks, and sample duplicates. All raw data, including laboratory calibration curves and internal quality control are included in the laboratory report.

Dolly Varden (Element Concentrations)

Objectives

Adult Dolly Varden (*Salvelinus malma*) from the Wulik River near Tutak Creek are collected to determine concentrations of selected elements in muscle, liver, kidney, and reproductive (ovary and testes) tissue. Elements selected for laboratory analyses include Cd, Copper (Cu), Pb, and Zn. Beginning in 1996, tissue samples also were analyzed for Se, and Hg was added in 2003. The objectives of this sampling are to compare element concentrations in Dolly Varden tissues to concentrations found prior to start up of the Red Dog Mine and to detect changes that may occur over time. Data are available, upon request, to other parties with interests in the Red Dog Mine and the Dolly Varden resource.

Dolly Varden adult samples are no longer a component of the APDES Permit, but are included in this report because Teck has included this sample effort in their Waste Management Monitoring Plan dated November 2016.

Field Methods

Individual adult Dolly Varden are caught by hook and line in the Wulik River near the mouth of Tutak Creek (Figure 10). Collections are made during early spring and late fall. The spring sampling period occurs just after breakup when water flows and discharge decrease, and just prior to fish leaving the Wulik River for the ocean; typically in early June. The fall sampling period takes place in September or October after fish have returned from the ocean to overwinter. When available, six adult Dolly Varden are kept and placed in individually labeled clean plastic bags from both the spring and fall sampling events. The bags are sealed with tie straps and are labeled with sample date, location, species, fish maturity, and an individual number [e.g., 091017WUDVA1 = September 10, 2017, Wulik River (WU), Dolly Varden (DV), adult (A), and consecutive numbers for that sampling period].

Figure 10. Dolly Varden, Wulik River near Tutak Creek, spring 2007.

Adult Dolly Varden are transported back to the mine where they are immediately frozen. The fish are then packaged and shipped frozen to Fairbanks where they are placed in the low temperature freezers at the ADF&G office. The fish remain in the sealed cooler in the freezer at ADF&G until dissections are performed.

Laboratory Methods

Upon removal from the freezer, adult fish are allowed to thaw for one to three hours so that the flesh and organs still contain ice crystals and are still partially frozen, but relatively easy to cut. Dissection of fish that are still partially frozen reduces the potential for contamination of the sample tissues with body fluids. The partially frozen tissue is relatively firm and more easily removed than completely thawed flesh.

Dolly Varden are measured (fork length), weighed, sexed, spawning condition noted, and otoliths removed (Figure 11). Otoliths are viewed under a microscope to determine both fresh and salt water age. The general condition of the fish and any abnormalities are noted during the necropsy.

Figure 11. Measuring a Dolly Varden.

Tissue samples of muscle (below the dorsal fin and above the lateral line with skin removed), kidney, liver (excluding bile tissue), and reproductive organs (i.e., both male and female if gonads are large enough to conduct element analyses) are removed using standard procedures to minimize contamination (Crawford and Louma 1993). About 5 g of each tissue are placed in pre-cleaned jars supplied by the analytical laboratory (EPA Series 300, Protocol C) and refrozen. Dissection scalpel blades are stainless steel and are cleaned after each tissue with ultra pure reagent grade nitric acid followed by rinsing with reverse osmosis water. One fish from each of

the spring and fall sampling events is selected for duplicate samples. Typically the largest fish is selected as organs will be larger and can provide enough tissue for two samples.

Frozen tissue samples are then shipped via air to a private analytical laboratory. A catalog for each sample with an identification number is prepared and shipped with the samples. Samples are analyzed for selected elements using U.S. Environmental Protection Agency standard methods.

Quality Control, Field Sampling

Each adult fish is immediately placed into a clean plastic bag after being caught. Fish are quickly rinsed in river water upon landing to ensure no foreign material is placed in the bag with the sample fish. The plastic bag is labeled with a sample identification number and placed in a cooler. Fish are transported back to the mine, frozen, and then shipped by air back to Fairbanks where they are immediately placed in a ultra-cool freezer.

Quality Control, Laboratory

All methods identified are closely followed to ensure that tissue samples are not contaminated. Class 100 nitrile gloves are worn during the laboratory procedures and dissecting instruments are cleaned after each tissue is removed. Liver bile ducts are not included in liver samples. Dissections are done on fish that are still partially frozen thus minimizing cross contamination by body fluids. All dissections are done by trained fisheries biologists. Otolith removal and reading are conducted by a biologist with extensive expertise with this procedure. Duplicate samples are taken from one of the larger adult fish to provide further quality control.

Only pre-cleaned bottles (Series 300, Protocol C) are used for fish tissues. After sample preparation, fish tissues are refrozen in an ultra-cool (-15°C) freezer until shipment to the analytical laboratory. Shipments are made early in the week and the laboratory pre-notified of the shipment to ensure that samples are received in a timely manner and in a frozen condition.

Chain of custody forms are prepared for each sample catalog. Samples will be numbered following the convention used by ADF&G since 1990.

Quality Control/Quality Assurance of Laboratory Analysis

The analytical laboratory provides quality assurance/quality control information for each analyte, including matrix spikes, standard reference materials, laboratory calibration data, sample blanks, and sample duplicates. All raw data, including laboratory calibration curves and internal quality control are included in the laboratory report. Blind duplicate tissues are submitted to the laboratory with each sample catalogue.

Fish Presence and Use

Objectives

The objectives of the fish monitoring study are to assess distribution and use of streams by Arctic grayling and Dolly Varden in the Red Dog Mine area. Fish monitoring focuses on the distribution and relative catch of juvenile Dolly Varden at selected sample sites and includes sites potentially affected by the mine as well as reference locations. The timing of the spawning run of Arctic grayling is annually monitored in North Fork Red Dog Creek (Figure 12). Additionally, the Arctic grayling population in Bons Pond (fish ≥ 200 mm) is estimated and the presence of age 0 Arctic grayling is assessed during early July sample events at all sites.

Figure 8. Fyke net in North Fork Red Dog Creek.

Field Methods

Field sampling focuses on the two most common fish species present in streams in the Red Dog Mine area: Arctic grayling and Dolly Varden. Fish sampling methods include visual and aerial surveys, angling, fyke nets, and minnow traps. Arctic grayling in the Red Dog Creek drainage are sampled during the spring spawning migration using fyke nets. Arctic grayling in Bons Pond are collected with both fyke nets and by angling throughout the open water season. Juvenile Dolly Varden sampling occurs in late summer using minnow traps baited with salmon roe. Informal observations of fish presence are also performed during the annual collection of aquatic invertebrates and periphyton.

The preferred sampling time for Arctic grayling use of North Fork Red Dog Creek is late May and early June to assess the spawning run of adults. Arctic grayling larger than 200 mm are marked with numbered Floy® T-bar anchor tags (Figure 13). The marking of Arctic grayling in Bons Pond follows the same protocol. Information on movements and use of various streams in the Ikalukrok Creek drainage is obtained from recaptured fish. Presence and length information on other species captured (e.g., slimy sculpin *Cottus cognatus*) is recorded.

Figure 13. Arctic grayling being measured just before tagging.

In early July, the presence and relative abundance of Arctic grayling fry is determined for Mainstem Red Dog and North Fork Red Dog creeks where historically most of the fry are found. Visual surveys for fry presence are conducted by walking along the stream and looking for fry along the edges and in backwaters. Recently emerged larval Arctic grayling also are collected in early July in invertebrate drift nets and are reported with the aquatic invertebrate data.

Sampling for juvenile Dolly Varden occurs in late summer, typically late July to mid-August. Juvenile Dolly Varden use of the sample sites is limited to the ice-free season as they outmigrate to overwintering areas in the fall. Late summer is the preferred sampling period; because it allows for maximum residency time for rearing juvenile Dolly Varden in that sample reach. Sampling methods were described in the previous section: *Juvenile Dolly Varden and Arctic Grayling (Element Concentrations).*

Quality Control, Field Sampling

All methods identified are followed by trained field biologists. Specific minnow trap sample reaches have been established at each site and the upstream and downstream limits of each reach remain unchanged (GPS located). Arctic grayling sampling is done in specified reaches of North Fork Red Dog Creek and in other areas in the Ikalukrok Creek drainage and in Bons Pond and Bons Creek. Fish biologists with multiple years of sampling experience perform all of the fieldwork.

Aerial Surveys, Dolly Varden and Chum Salmon

Objectives

The objective of monitoring overwintering Dolly Varden is to estimate the abundance and assess the distribution of overwintering Dolly Varden in the Wulik River. Changes in distribution of Dolly Varden during fall surveys with respect to the relative proportion upstream and downstream of the mouth of Ikalukrok Creek is determined. The objective of monitoring chum salmon in Ikalukrok Creek is to estimate the abundance and distribution of these fish from the mouth of Ikalukrok Creek to its confluence with Dudd Creek.

Field Methods

Wulik River aerial surveys are conducted between the mouth of the Wulik River to about 10 river km upstream of the confluence of the Wulik River with Ikalukrok Creek. Ikalukrok Creek surveys are done on the river from the mouth of Dudd Creek to the confluence of Ikalukrok Creek and the Wulik River. Surveys for chum salmon are conducted in September and Dolly Varden aerial work is performed in late September to early October, just prior to freezeup. Helicopters are used, if available, to fly the aerial surveys. Trip reports are prepared to summarize each field sampling effort.

Quality Control, Field Sampling

Fish biologists with experience in conducting aerial surveys perform the work. Generally, surveys are done by fish biologists who have conducted this same work in previous years.

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