Technical Report No. 26-03

Methods for Aquatic Life Monitoring at the Red Dog Mine Site

A Requirement of the 2026 APDES Permit No. AK0038652

by

Chelsea M. Clawson

April 2026

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

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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)

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. 26-03

METHODS FOR AQUATIC LIFE MONITORING AT THE RED DOG MINE SITE

A Requirement of the 2026 APDES Permit AK0038652

by
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April 2026

Cover: Aquatic invertebrate sampling using a Hess sampler, July 2025. Photograph by Lauren Yancy.

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INTRODUCTION

The Red Dog zinc and lead 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). Red Dog Mine began operation in 1989, about 10 years after baseline studies were initiated. Since development of the mine, the Alaska Department of Fish and Game (ADF&G) has conducted numerous studies of the aquatic communities in the Wulik River drainage. Initially, studies were short term in nature. In 1991, a 3-year study was initiated to document short-term changes in fish distribution during mine development and operation, focusing on the abundance and distribution of juvenile Dolly Varden (*Salvelinus malma*) and Arctic grayling (*Thymallus arcticus*), element concentrations in adult Dolly Varden in the Wulik River, and the number of Dolly Varden overwintering in the Wulik River (Ott and Weber Scannell 1994). In 1994, ADF&G began a 5-year study to investigate changes in fish distribution and species composition, relative abundance, and element concentrations in adult Dolly Varden tissues (Weber Scannell and Ott 1995, Ott and Weber Scannell 1996). In 1995 and 1996 an in-depth study of algal and aquatic invertebrate communities was conducted in creeks downstream of and adjacent to Red Dog Mine (Weber Scannell 1997). In 1997 and 1998, ADF&G sampled aquatic invertebrate and periphyton communities in streams that were directly exposed to mining activity of treated mine effluent (Weber Scannell and Andersen 2000).

The initial National Pollutant Discharge Elimination Permit (NPDES) AK-003865-2 was issued by the Environmental Protection Agency effective July 10, 1985. The NPDES permit was reissued effective August 28, 1998, and contained requirements for biomonitoring studies of fish and aquatic biota, and limited the annual discharge of treated effluent to no more than 2.418 billion gallons. The 1998 reissuance resulted in the establishment of consistent long-term biomonitoring sites that have been sampled annually since that time. The NPDES permit was updated and renewed in 2007, then temporarily withdrawn while the Supplementary Environmental Impact Statement was completed for the Aqqaluk Extension, development of a second mining pit. In 2008 and 2009, Teck Alaska Incorporated (Teck, the operator of Red Dog Mine) developed a comprehensive monitoring plan, which was incorporated into Waste Management Permit 0132-BA002 and NPDES Permit AK-003865-2, issued in December 2009 and January 2010, respectively. Effective April 1, 2013, the Alaska Department of Environmental Conservation (ADEC) assumed authority over the NPDES Permit as Alaska Pollution Discharge Elimination

Permit (APDES) AK0038652. On July 28, 2017, APDES AK0038652 was reissued by ADEC effective through August 31, 2022. This APDES permit was administratively extended on May 19, 2021, with a Compliance Schedule responding to region-wide permafrost thawing that elevated naturally occurring constituents in local waters, including the receiving waters of the mine's discharge.

On April 9, 2026, the ADEC reissued APDES Permit No. AK0038652 with an effective date of May 1, 2026, to Teck with an allowable discharge of up to 2.418 billion gallons of treated effluent per year into Middle Fork Red Dog Creek. The APDES Permit requires the continuation of the bioassessment program, including monitoring periphyton, aquatic invertebrates, and fish in selected streams near the Red Dog Mine (Table 1). Also contained in the APDES Permit is a 10-year Compliance Schedule for Teck to investigate, determine, and implement a long-term solution accounting for the loss of TDS assimilative capacity in Red Dog and Ikalukrok creeks due to naturally occurring permafrost degradation.

This report is an update to the 2017 Biomonitoring Plan (Bradley 2017). Teck is required by the APDES Permit to submit for approval an updated version of the Biomonitoring Plan – ADF&G Methods for Aquatic Life Monitoring. A complete description of the biomonitoring program is contained in Teck's Monitoring Plan as part of Waste Management Permit 2021DB0001 dated September 2021. Teck's Monitoring Plan includes sample sites, sampling frequency, and parameters for all the aquatic sample sites (Table 1 and Figure 2). All sampling is conducted when water flow is present. Periphyton and aquatic invertebrates are typically sampled in early July, and 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 is also incorporated by reference into the Alaska Department of Natural Resource's Reclamation Plan Approval (F20219958) dated September 23, 2021.

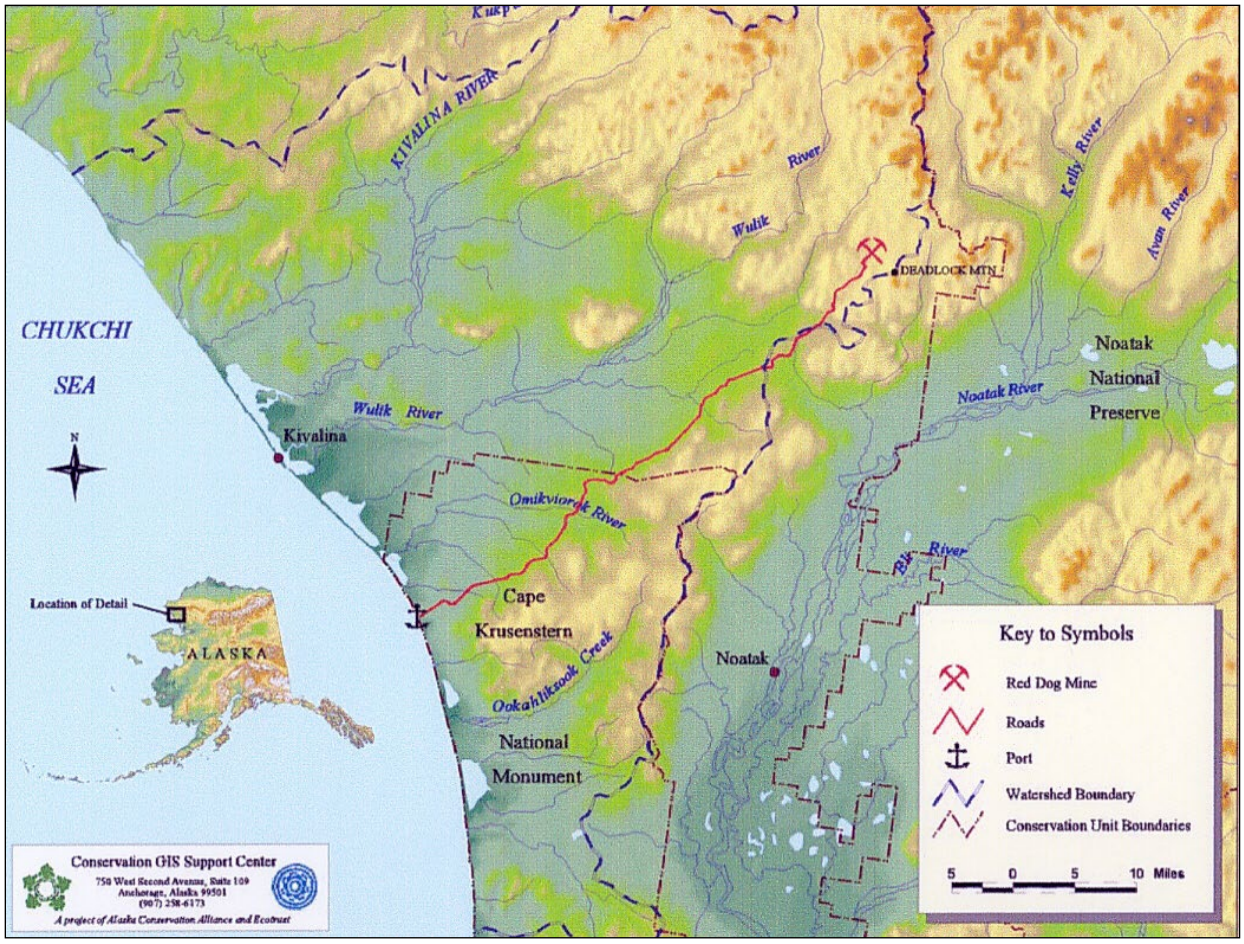

Figure 1.–Location of the Red Dog Mine in northwestern Alaska.[1]

[1] Map used with permission of Conservation GIS Support Center, Anchorage.

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

Location	APDES/WMP	Location Description	Parameters
Wulik River	WMP	Mouth to 10 km past mouth of Ikalukrok Creek	Fall aerial surveys for overwintering Dolly Varden
Ikalukrok Cr	WMP	Lower Ikalukrok Creek to mouth of Dudd Creek	Fall aerial surveys for adult chum salmon
Ikalukrok Station 9	APDES/ WMP	Ikalukrok Creek above confluence with Red Dog Creek	Periphyton (as chlorophyll-a concentration) Benthic macroinvertebrates Fish presence and use
Ikalukrok Station 160	WMP	Lower Ikalukrok Creek	Periphyton (as chlorophyll-a concentration) Benthic macroinvertebrates Fish presence and use
Middle Fork Red Dog Station 20	WMP	Middle Fork Red Dog Creek above fish weir	Periphyton (as chlorophyll-a concentration) Benthic macroinvertebrates
Red Dog Station 10	APDES/ WMP	Mouth of Red Dog Creek	Periphyton (as chlorophyll-a concentration) Benthic macroinvertebrates Fish presence and use Elements in whole body juvenile Dolly Varden
North Fork Red Dog Station 12	APDES/ WMP	North Fork Red Dog Creek above confluence with mainstem Red Dog	Periphyton (as chlorophyll-a concentration) Benthic macroinvertebrates Fish presence and use Record of spawning activity Capture/mark Arctic grayling
Upper NFRD	APDES	Upper North Fork Red Dog Creek, above Aqqaluk	Periphyton (as chlorophyll-a concentration) Benthic macroinvertebrates Fish presence and use
Red Dog Station 151	APDES	Upper mainstem Red Dog Creek	Fish presence and use
Buddy Creek	WMP	Below falls, about 1.5 km downstream of haul road	Periphyton (as chlorophyll-a concentration) Benthic macroinvertebrates Fish presence and use Elements in whole body juvenile Dolly Varden
Buddy Station 221	WMP	Buddy Creek above haul road	Periphyton (as chlorophyll-a concentration) Benthic macroinvertebrates
Bons Station 220	WMP	Bons Creek below pond	Periphyton (as chlorophyll-a concentration) Benthic macroinvertebrates
Bons	WMP	Bons Creek above pond	Periphyton (as chlorophyll-a concentration) Benthic macroinvertebrates
Anxiety	WMP	Anxiety Ridge Creek at haul road	Fish presence and use Elements in whole body juvenile Dolly Varden
Evaingiknuk	WMP	Evaingiknuk Creek	Fish presence and use
Bons Pond	WMP	Above reservoir spillway	Elements in whole body juvenile Arctic grayling Arctic grayling population estimate

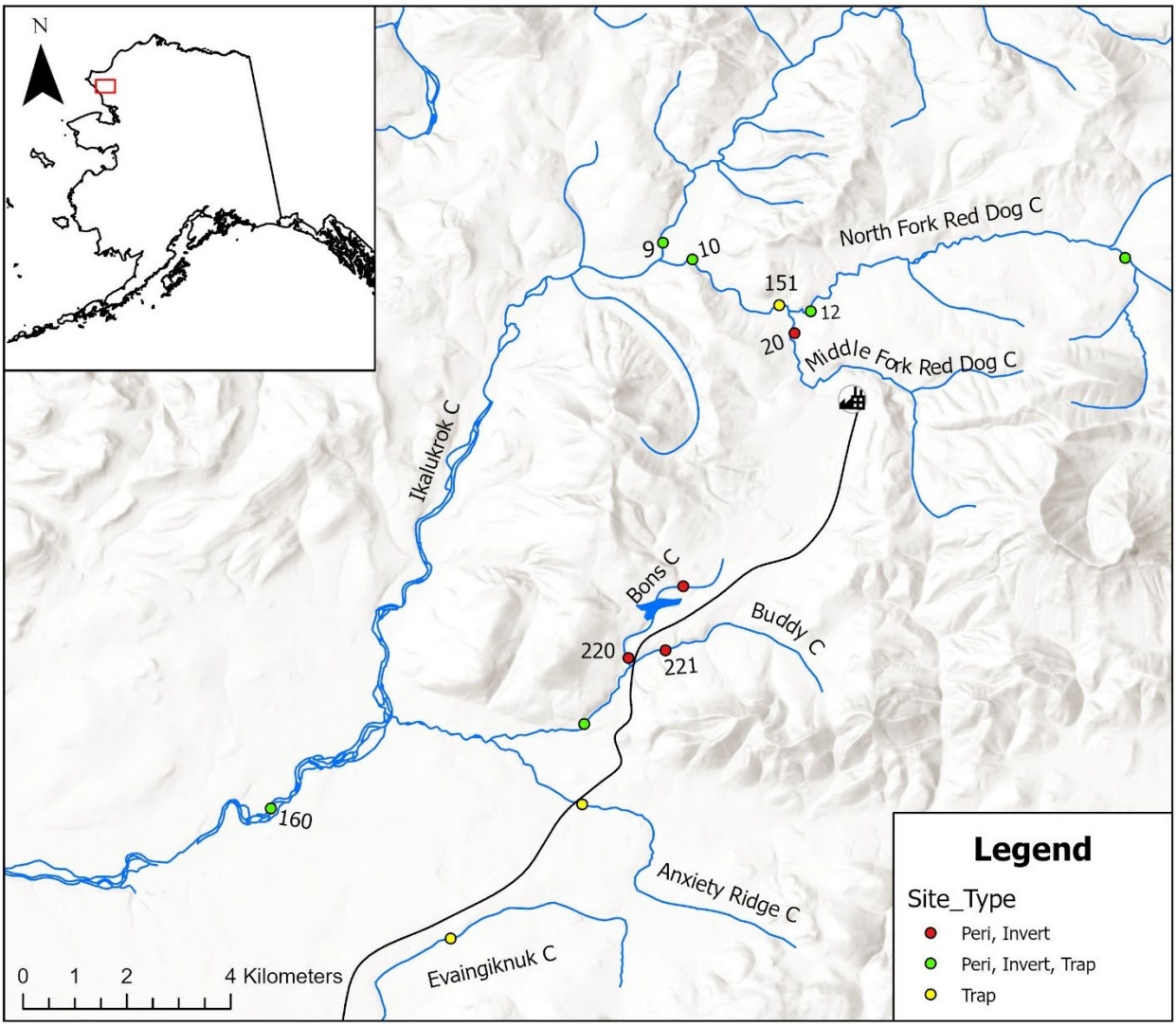

Figure 2.–Location of sample sites in the Ikalukrok Creek drainage and Evaingiknuk Creek.

PERIPHYTON STANDING CROP

OBJECTIVES

Periphyton is composed of chlorophyll producing organisms, such as algae, attached to submerged surfaces in a waterbody. Algal density and community structure are influenced by water and sediment quality through physical, chemical and biological factors that change throughout the year (Barbour et al. 1999). The concentration of chlorophyll-a pigments in periphyton samples provides an estimate of active algal biomass and is often used in monitoring studies to detect changes in aquatic communities. Periphyton density is monitored annually to detect changes in in-situ productivity in receiving waters downstream of the Red Dog Mine treated wastewater discharge.

Reference sites outside of the influence of mine discharge are also sampled to detect variations due to other factors, including input from natural mineral seeps, annual variation in precipitation and weather patterns, and/or thermal/hydraulic erosion. Sampling is performed once per year, during the period from late June through mid-July and typically under low to moderate stream flows.

FIELD METHODS

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. (1999), but with more replicates per site to increase sample precision. Ten flat rocks each 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 believed 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 at least 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 µm glass fiber filter in a filter receptacle attached to a hand vacuum pump (Figure 4 and Figure 5). The toothbrush is also rinsed into the filter receptacle after being used to scrub the sample area on the rock. 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.

Figure 3.–Brushing/removal of material from rock around the flexible foam square.

Figure 4. –Material left on the rock after brushing around the foam square.

Figure 5.–Removal of material remaining beneath the flexible foam 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.

Water is then extracted from the periphyton sample with the vacuum pump. After extracting most of the water (i.e., 0.5 cm of water remains above the glass fiber filter), 3 to 5 drops of a saturated solution of $MgCO_3$ are added to the sample. The $MgCO_3$ is added to prevent acidification and additional conversion of chlorophyll-a to phaeophytin. 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. 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 in a dry paper coffee filter and the coffee filter is folded to cover the entire glass fiber filter(s) (Figure 6). The paper coffee filters are used to absorb any residual water that may be present. If there are multiple filters from a single rock they are all placed in the same coffee filter. The ten coffee filters containing the glass fiber filter samples are then placed in a sealable plastic bag labeled with the sample site, silica gel desiccant is added to cover the folded coffee filters, and the sample bag is wrapped in aluminum foil to prevent light exposure. The foil wrapped sample bags are stored in a cooler with ice packs in the field. It is essential that filters be kept cool and dark while in the field to prevent sample degradation. To ensure quality control, 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 analysis in the laboratory.

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.

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 tri-chromatic equations. 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. For quality control, 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, and samples with chlorophyll concentrations below the detection limit determined during initial calibration are reported as “non detectable.”

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 measured and recorded

using the spectrophotometer. If a sample has detectable chlorophyll, 0.08 ml of 0.1N HCl acid is added to the sample to convert the chlorophyll to phaeophytin. After 90 seconds, absorption values at 750 nm and 665 nm are then measured and 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 during each day of sample analysis. 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 equations to determine chlorophyll a, -b, and -c concentrations. Additionally, phaeophytin is calculated to determine if a chlorophyll-a conversion has occurred, and to correct chlorophyll-a concentrations for the presence of phaeophytin.

AQUATIC INVERTEBRATES

OBJECTIVES

Aquatic invertebrate communities are sampled annually 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 outside of the influence of mine discharge are also sampled to detect variations due to other factors, including input from natural mineral seeps, annual variation in precipitation and weather patterns, and/or thermal/hydraulic erosion. The presence of mayflies (Ephemeroptera), stoneflies (Plecoptera), and caddisflies (Trichoptera) (EPT) are evidence of continued in-situ biological productivity. Sampling is performed once per year, during the period from late June through mid-July concurrent with periphyton sampling.

FIELD METHODS

A modified rapid bioassessment technique developed by Barbour et al. (1999) is used to retain more quantitative features in the sampling program. The sampling techniques currently utilized at the Red Dog Mine were refined after multiple trials of various field-sampling methods including kick nets, surber samplers, drift nets, and Hess samplers. The final determination of the sampling

method 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. Drift nets were used from 1996 to 2021. Beginning in 2022, Hess samplers were used instead of drift nets. Hess samplers are potentially more accurate at identifying the in-situ benthic community, rather than the drifting invertebrate community. This provides a more accurate baseline for evaluating changes at each site, rather than changes occurring upstream. Additionally, other baseline and aquatic biomonitoring programs throughout Alaska utilize Hess samplers, so the change in collection method makes data potentially more comparable across the state.

The Hess stream bottom sampler has a 0.086 m^2 sample area and material is captured in a 200 mL cod end–both constructed with 300 µm mesh net. At each sample site, a location is selected where 5 samples can be collected in a transect perpendicular to flow. If the stream is too narrow for a transect, samples are collected in multiple transects moving upstream, with care not to disturb the sample area before sampling. Samples are collected in flowing areas, not back eddies or pools. The Hess sampler is seated in the substrate with a twisting motion with the net portion extending downstream. Rocks within the Hess sampler are scoured by hand; and gravel, sand, and silt are disturbed to about 10 cm depth to dislodge macroinvertebrates into the net (Figure 7). Large rocks are examined for any clinging organisms, and any organisms present are removed into the Hess sampler (Figure 7).

The Hess sampler is then removed from the substrate, and any materials in the net are flushed into the cod end by splashing water on the net. The cod end is then removed, and the contents are rinsed out using a squirt bottle filled with denatured ethanol (greater than 70% solution) into a 500 ml Nalgene bottle labeled with the sample site and sample number. Additional denatured ethanol is then added if necessary to completely submerge the sample. After the lid is put on, the sample bottle is inverted several times to ensure the ethanol is well mixed with the sample. The Hess sampler and cod end are well rinsed before deploying at the next site. The five labeled sample containers are then placed in a labeled plastic bag. The sample bags are packed in vermiculite in a 4G cardboard box lined with a large plastic bag, then shipped to ADF&G or the invertebrate identification laboratory via cargo flight.

Figure 7.–Disturbing the substrate within the Hess sampler (left) and examining a rock for organisms (right).

LABORATORY METHODS

Each sample is drained of the denatured ethanol through a mesh sieve (350 µm) and then placed into a shallow container and filled with water. Floating invertebrates are picked from the container until no more invertebrates are seen. 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 if necessary. Samples with very low numbers of invertebrates do not require subsampling. Each sample is emptied onto a gridded tray and covered with water to assist with spreading the sample equally among the 30 squares. For 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. Invertebrates from the subsamples are sorted, counted, and identified until the total sample exceeds the required 300 organisms (Barbour et al. 1999). 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. For quality control, 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.

Invertebrates are identified to the lowest practicable taxonomic level. Aquatic invertebrates of the orders Ephemeroptera, Plecoptera, Trichoptera are identified to genus. Dipterans are identified to genus, except the nonbiting midges of the family Chironomidae. Copepoda, Collembola, and Coleoptera are identified to genus. Cladocera and Hydroida are identified to order. Oligochaeta, Ostracoda, Platyhelminthes, Nematoda, and Nematomorpha are identified to class level. Because invertebrates belonging to the orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) (EPT) are more sensitive to water quality, the total number of individual specimens of EPT are calculated and compared to groups of other invertebrates, which are less sensitive. The aquatic invertebrate density is calculated for each sample by dividing the number of BMI by 0.086 m^2, the Hess sampling area. Mean density is estimated for each site by calculating the mean density among the five samples. Taxa richness is reported as the number of taxonomic groups identified to the lowest practical level. Terrestrial organisms are excluded from all calculations.

ELEMENT CONCENTRATIONS IN JUVENILE FISH

OBJECTIVES

Whole body analyses of juvenile Dolly Varden for cadmium, mercury, lead, selenium, and zinc are conducted annually on fish collected in Mainstem Red Dog, Buddy, and Anxiety Ridge creeks near Red Dog Mine. Anxiety Ridge and Buddy creeks are reference sites, while Mainstem Red Dog Creek is potentially affected by mine-related activities associated with the tailings impoundment and treated wastewater.

Juvenile Arctic grayling are collected from Bons Pond for whole body analyses for cadmium, mercury, lead, selenium, and zinc. The objectives of the juvenile fish sampling are to build a database that can be used to identify potential differences among sample sites, fish species, and to evaluate changes in the concentrations of selected elements over time.

FIELD METHODS

Juvenile Dolly Varden

Juvenile Dolly Varden utilize the sampled creeks as rearing habitat during the ice-free season. They migrate downstream to Ikalukrok Creek and the Wulik River to overwinter just prior to freeze-up. 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 prior to decreasing water temperatures associated with freeze-up (Ott and Morris 2005).

Sampling involves placement of 10 minnow traps per site, each baited with cured salmon eggs (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 found throughout the drainages surrounding the mine and are easily caught in minnow traps.

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

Plastic bait sacs containing cured salmon eggs are prepared prior to field work. 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 typically placed in moderately moving water rather than backwater areas or pools as juvenile Dolly Varden generally prefer higher velocity water. 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 for ease of relocation the following day. Traps are fished for approximately 24 hours. When traps are checked, fish are removed to a bucket, identified to species, measured for fork length to the nearest millimeter, and either retained for element analysis, or released back to the sample reach (Figure 9). Data are recorded in the field.

Figure 9.–Juvenile Dolly Varden being measured.

Dolly Varden between 90 and 140 mm fork length are selected for the whole-body element analyses from Mainstem Red Dog Creek (Station 151 and Station 10), Buddy Creek, and Anxiety Ridge Creek (Table 2). Selection of fish from this length range ensures that most of the fish are age-2 or age-3. A maximum of fifteen juvenile Dolly Varden per sample creek are kept. Quality control measures to minimize contamination include using class 100 nitrile gloves to handle sample fish and placing each fish in an individually numbered plastic bag. Each plastic bag is

labeled with sample date, location, species, and an individual number. The 15 plastic bags containing juvenile fish are placed in a larger sample bag that also is labeled with the sample location, and immediately stored in a cooler with ice packs.

Retained 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

Juvenile Arctic grayling are annually collected for element analysis from Bons Pond, where they are year-round residents. 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. Fish are collected during the annual early spring Bons Creek Arctic grayling population estimate. All the same field protocols are used for both the juvenile Dolly Varden and Arctic grayling, except fyke nets and/or angling are used for the Arctic grayling rather than minnow traps, and they are sampled in the spring (May/June). Fifteen juvenile Arctic grayling between 150 and 200 mm fork length are retained for the whole-body element analyses, as these fish are most likely age-2 or age-3.

LABORATORY METHODS

The frozen juvenile Dolly Varden and Arctic grayling are removed from the freezer, weighed, and shipped directly via air to a private analytical laboratory with National Environmental Laboratory Accreditation Program (NELAC) certification. 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 (EPA 2014). Fish are analyzed for cadmium, copper, lead, selenium, and zinc using EPA Method M6020B ICP-MS. Fish are analyzed for mercury using EPA Method M7473 CVAAS. Moisture content is analyzed using EPA Method D2216-80.

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.

ADULT DOLLY VARDEN ELEMENT CONCENTRATIONS

OBJECTIVES

Adult Dolly Varden from the Wulik River are collected twice per year to determine concentrations of selected elements in muscle, liver, kidney, and reproductive (ovary and testes) tissue. Elements initially selected for laboratory analyses were cadmium, copper, lead, and zinc. Beginning in 1996, tissue samples also were analyzed for selenium, and mercury 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. Dolly Varden adult samples are not 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 September 2021.

FIELD METHODS

Individual adult Dolly Varden are caught by hook and line in the Wulik River, typically near the mouth of Tutak Creek (Figure 10). Collections are made annually in both the spring (May/June) and fall (September/October). The spring sampling period occurs after breakup once water flows and discharge decreases, 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. Seven adult Dolly Varden from each the spring and fall sampling events are kept and placed in individually labeled clean plastic bags. Fish are dispatched then quickly rinsed in river water to ensure no foreign material is placed in the bag with the sample fish. The bags are sealed with zip ties and labeled with sample date, location, species, fish maturity, and an individual number.

Figure 10.–Dolly Varden being caught at the mouth of Tutak Creek on the Wulik River, spring 2022.

The individually bagged Dolly Varden are placed in a cooler then 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 ADF&G freezer until dissections are performed.

LABORATORY METHODS

Upon removal from the freezer, adult fish are allowed to thaw for three to five hours so that the flesh and organs 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.

Each Dolly Varden is measured (fork length), weighed, sex determined, spawning condition noted, and otoliths removed (Figure 11). Otoliths are sectioned, mounted, and viewed under a microscope to determine age. The general condition of the fish and any abnormalities are noted during the dissection.

Figure 11.– Using a scalpel to cut out an inner muscle tissue sample on 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). Liver bile ducts are not included in liver samples. About 5 grams of each tissue are placed in pre-cleaned vials supplied by the analytical laboratory (EPA Series 300, Protocol C). Vials are individually bagged to reduce contamination in the case of vial leakage or breakage. 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. Class 100 nitrile gloves are worn during the laboratory procedures and dissecting instruments are cleaned after each tissue is removed for quality control. One fish from each of the spring and fall sampling events is selected for duplicate samples for additional quality control. Typically the largest fish is selected as organs will be larger and can provide enough tissue for two samples.

After sample preparation, the vials containing fish tissues are frozen in an ultra-cold (-15°C) freezer until shipment. Frozen tissue samples are 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 (EPA 2014). Fish tissues are analyzed for cadmium, copper, lead, selenium, and zinc

using EPA Method M6020B ICP-MS. Fish are analyzed for mercury using EPA Method M7473 CVAAS. Moisture content is analyzed using EPA Method D2216-80.

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.

FISH PRESENCE AND USE

OBJECTIVES

The objectives of the fish monitoring study are to assess distribution and use of streams by fish in the Red Dog Mine area. 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, minnow traps, and drift nets. Fish monitoring for Arctic grayling focuses on spawning occurrence in Red Dog Creek and population size in Bons Pond. Fish monitoring for Dolly Varden 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.

FIELD METHODS

Arctic grayling in Bons Pond are collected with both fyke nets and by angling throughout the open water season. Fyke net locations vary, but typically one net is set at the inlet where Bons Creek flows into Bons Pond, one net is set at the outlet channel of Bons Pond, and one net is set somewhere on the shore of Bonds Pond. Arctic grayling spawning occurrence in Mainstem and North Fork Red Dog creeks is assessed using visual surveys and/or angling in late May/early June. Informal observations of fish presence are also performed during the annual collection of aquatic invertebrates and periphyton.

The population of Arctic grayling ≥200 mm in Bons Pond is estimated annually in late May/early June, during their spawning period. Arctic grayling larger than 200 mm captured in Bons Pond are marked with numbered Floy® T-bar anchor tags (Figure 12).

The annual abundance of Arctic grayling ≥ 200 mm in Bons Pond is estimated using Chapman's modification of the Lincoln-Petersen two-sample mark-recapture model (Chapman 1951),

$$\widehat{N}_c = \left\{\frac{(n_1+1)(n_2+1)}{(m_2+1)}\right\} - 1$$

where $\widehat{N}_c$ = estimated population, n_1 = fish marked in first capture event, n_2 = fish captured during recapture event, and m_2 = fish captured during recapture event that were marked in the capture event.

Variance is calculated as (Seber 1982):

$$\text{var}(\widehat{N}_c) = \left\{\frac{(n_1+1)(n_2+1)(n_1-m_2)(n_2-m_2)}{(m_2+1)^2(m_2+2)}\right\}$$

The 95% CI for the population estimate is calculated as:

$$95\%\ \text{CI} = N_c \pm (1.960)\sqrt{\widehat{\text{var}}(\widehat{N}_c)}$$

Figure 12.–Arctic grayling being measured just before tagging.

In early July, the presence and relative abundance of Arctic grayling fry is assessed as conditions allow 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 may also be

collected in early July in drift nets. Drift nets are 45.7 cm (18 in) wide by 30.5 cm (12 in) deep with 350 µm mesh size with nitex nylon for the bag portion of the net and stainless steel for the collecting cod end. The drift nets are placed with the mouth facing upstream into the current and are held in place with metal stakes. Nets are placed opportunistically during invertebrate/periphyton sampling. After approximately 1 hour, nets are removed and the materials in the net are flushed into the cod end by splashing water on the outside of the net. The contents of the cod end are rinsed into a mesh sieve using a squirt bottle and visually examined for larval Arctic grayling in the field.

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 as it allows for maximum residency time for rearing juvenile Dolly Varden in that sample reach. Sampling methods for juvenile Dolly Varden are described in the previous section: Element Concentrations in Juvenile Fish.

AERIAL SURVEYS, DOLLY VARDEN AND CHUM SALMON

OBJECTIVES

The objective of the early fall aerial surveys of chum salmon in Ikalukrok Creek is to count the number of spawning salmon and determine the distribution of these fish from the mouth of Ikalukrok Creek to its confluence with Dudd Creek. The objective of the late fall aerial surveys of Dolly Varden in the Wulik River is to count the number of overwintering fish in the river and document their overwintering distribution.

FIELD METHODS

A helicopter is used to fly the aerial surveys at an elevation of 100 – 150 m above the water surface at approximately 15 km/h. Ideal survey conditions are lower flows with clear water, calm winds to minimize surface water disturbance, and clear skies or light cloud cover. The chum salmon survey is flown from the mouth of Dudd Creek downstream to the confluence of Ikalukrok Creek and the Wulik River in September, when chum salmon are actively spawning. Live fish and carcasses are counted separately. Beginning in 2019, increased turbidity in Ikalukrok Creek resulting from mineral seeps has impaired visibility in the creek, limiting or preventing aerial

survey counts of chum salmon. If this turbidity prevents comprehensive enumeration, the creek is still flown and any visible fish or carcasses on gravel bars are counted.

The Dolly Varden aerial survey is performed in late September to early October, just prior to freeze up. Ideally, this survey occurs as late in the year as possible to maximize the number of fish that have moved into the river to overwinter, but the survey must occur before the river begins to freeze up as ice interferes with the ability to see fish from the air. Wulik River aerial surveys are conducted between the mouth of the Wulik River to about 10 river km upstream of the confluence of Ikalukrok Creek with the Wulik River. The Wulik River is divided into 5 index survey zones (Figure 13). Zone 1 is from the Kivalina water intake pump upstream to Sivu Hill. Zone 2 is from Sivu Hill to Driver's Camp. The Wulik River is braided in this section, and each braid is flown during the survey. Zone 3 is from Driver's Camp to the mouth of Tutak Creek. Zone 4 is from the mouth of Tutak Creek to the most upstream mouth of Ikalukrok Creek. Zone 5 is from the mouth of Ikalukrok Creek upriver to the termination of the survey. The relative proportion of overwintering fish upstream and downstream of the mouth of Ikalukrok Creek is determined using the counts from each section. In some years since 2019, the turbidity originating from Ikalukrok Creek has impacted visibility in sections of the Wulik River. Even if turbidity prevents a complete count of the survey area, the standard survey route is flown and any impacts to visibility are noted.

SUMMARY

After annual field and laboratory studies are completed and full analyses performed, a technical report is produced which summarizes the results (ex. Clawson 2025). This report is peer reviewed and distributed to interested parties and is available to the public on the ADF&G website. Annual technical reports are also archived at the Alaska Resources Library and Information Services (ARLIS) and the Alaska State Library.

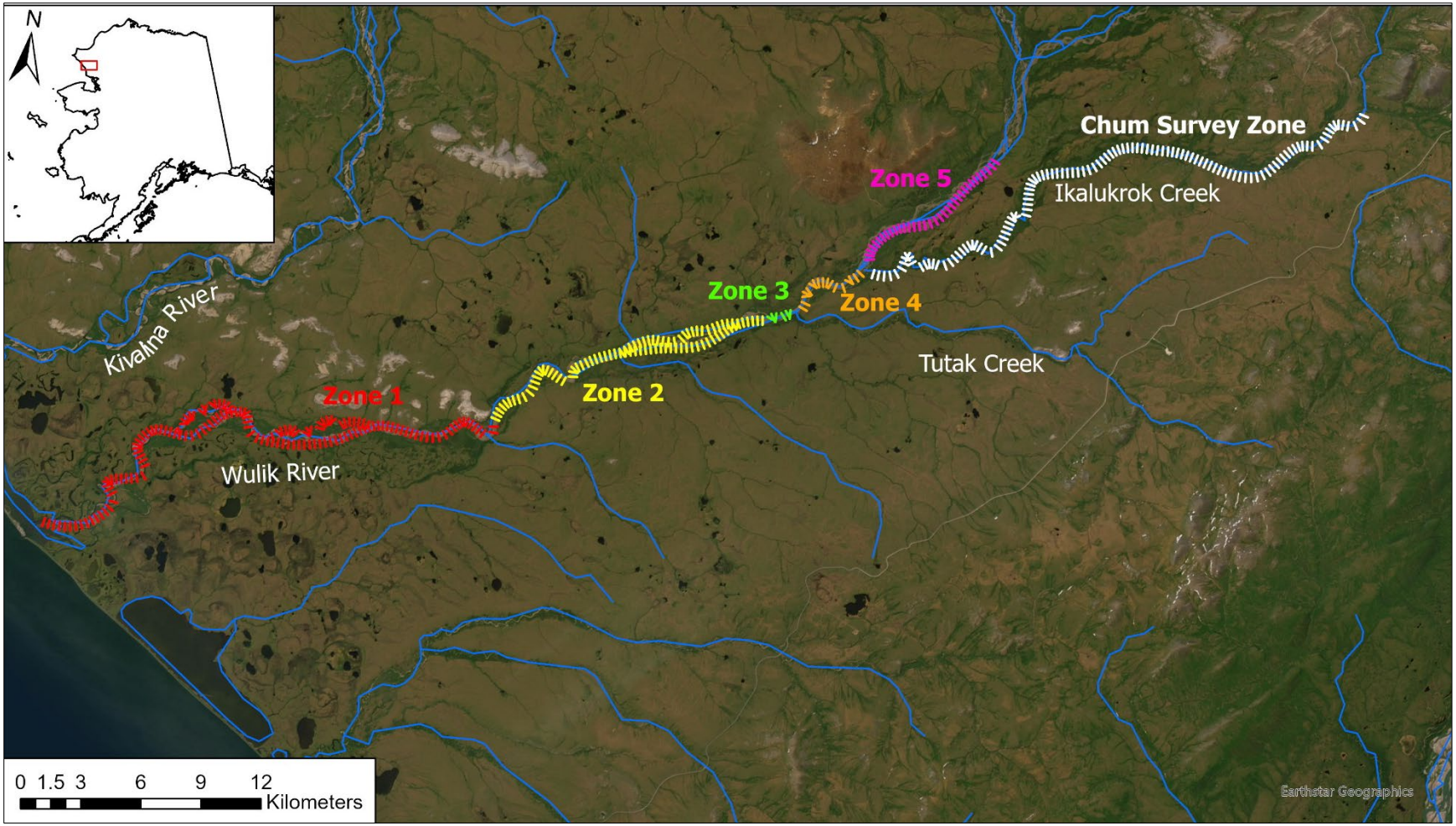

Figure 13.– Aerial survey zones for Dolly Varden overwintering on the Wulik River and chum salmon spawning in Ikalukrok Creek.

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