Methods for Aquatic Life Monitoring to Satisfy Requirements under 1998 NPDES Permit

NPDES AK-003865-2, Red Dog Mine Site

Submitted by:
Cominco Alaska Incorporated
Red Dog Mine
Kotzebue, Alaska

Prepared by:
Alaska Department of Fish and Game
Division of Habitat and Restoration
Fairbanks, Alaska
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Locations of Sample Sites and Factors Measured

Middle Fork Red Dog Creek	Periphyton (as Chlorophyll-a concentrations) Aquatic invertebrates: taxonomic richness and abundance
North Fork Red Dog Creek	Periphyton (as Chlorophyll-a concentrations) Aquatic invertebrates: taxonomic richness and abundance Fish presence and use
Mainstem Red Dog Creek	Periphyton (as Chlorophyll-a concentrations) Aquatic invertebrates: taxonomic richness and abundance Fish presence and use
Ikalukrok Creek, Stations 9, 7; and upstream of Dudd Creek	Periphyton (as Chlorophyll-a concentrations) Aquatic invertebrates: taxonomic richness and abundance Fish presence and use.
Ikalukrok Creek	Fall aerial survey of returning chum salmon
Wulik River	Metals concentrations in Dolly Varden gill, liver, muscle, and kidney. Fall aerial survey of overwintering Dolly Varden
Anxiety Ridge Creek	Fish presence and use
Evaingiknuk Creek	Fish presence and use
Buddy Creek	Fish presence and use

Locations of sites for aquatic sampling are shown on Figure 1, locations of Dolly Varden overwintering and chum salmon survey sites are shown on Figure 2. The sampling program will continue throughout the effective time of the 1998 NPDES permit and State 401 Certification.

Figure 1. Locations of sites for aquatic sampling.

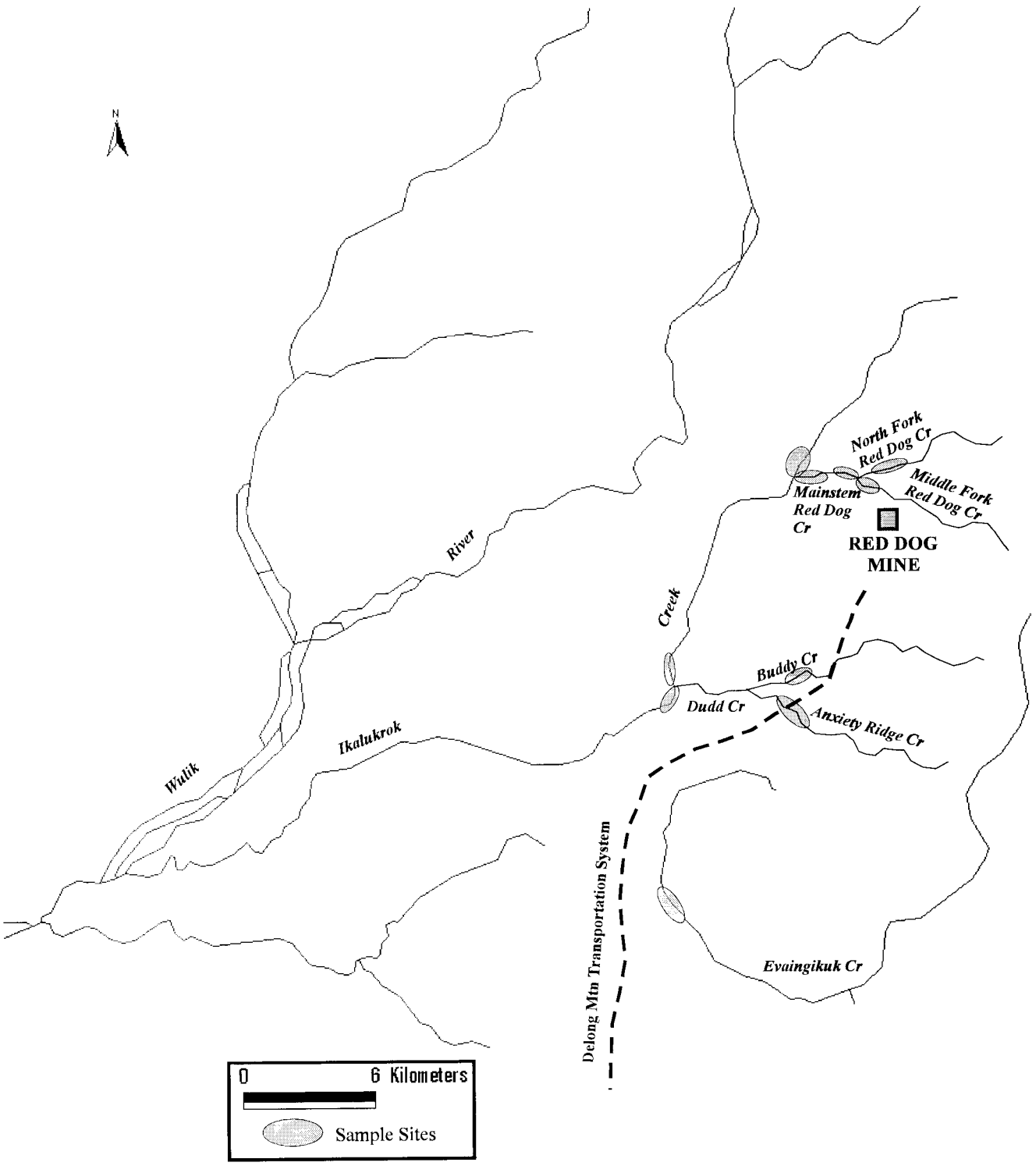
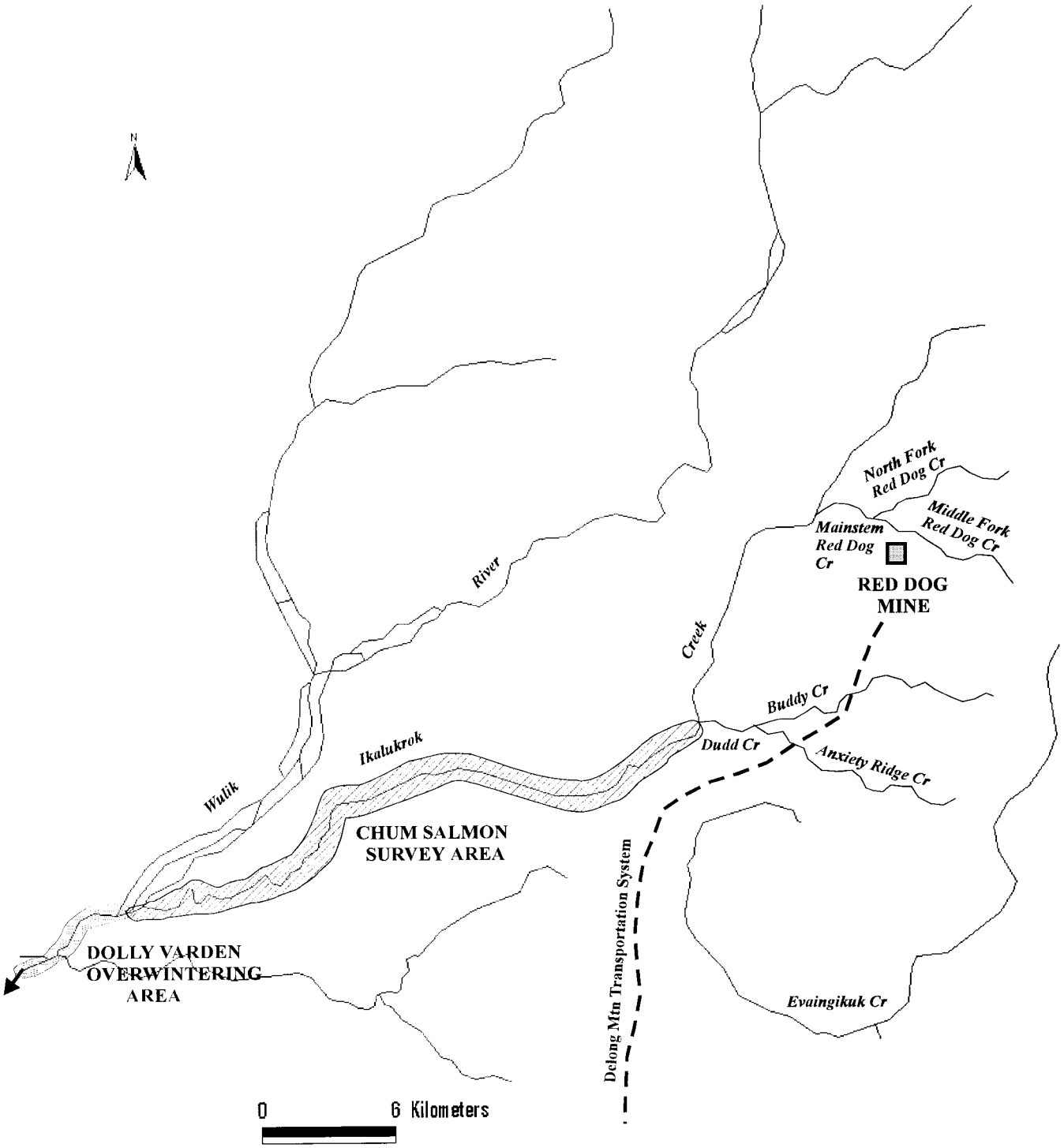


Figure 2. Locations of Dolly Varden overwintering and chum salmon survey sites



Periphyton Standing Crop (as Chlorophyll-a concentrations)

Objectives

Periphyton, or attached micro-algae, is sensitive to changes in water quality and is often used in monitoring studies to detect early changes in aquatic communities. The presence of periphyton in a stream system documents continued in-situ productivity. Periphyton density will be monitored to detect changes in in-situ productivity in receiving waters of the Red Dog Mine effluent. Reference sites will be sampled to detect variations due to other factors, such as climate.

Periphyton is sampled directly from cobble on the stream benthos. The periphyton is collected from a specific area of cobble, following the rapid bioassessment techniques of Barbour et al, but with more replicates per site to increase sample precision. The concentrations of chlorophyll-a are determined to estimate periphyton standing crop.

Sampling will be done once per year, during the period from late June through mid-July. Sampling will be done at low water periods.

Methods for Field Collection of Samples:

Ten rocks are collected from the stream benthos in each study reach. A 5-cm x 5-cm square of high density foam is placed on the rock. Using a small toothbrush, all material around the foam square is removed and rinsed away with clean water (Figure 3). The foam is removed from the rock and the rock is brushed with a clean tooth brush and rinsed onto a 0.45 um glass fiber filter, attached to a hand vacuum pump. After extracting as much water as possible, approximately 1 ml saturated $MgCO_3$ is added to the filter to prevent acidification and conversion of chlorophyll-a to phaeophytin. The dry filter is wrapped in a large filter (to absorb any additional water), labeled, placed in a sealable plastic bag, and packed over silicon gel desiccant. Filters are frozen in a light-proof container with desiccant.

Figure 3. Sampling for periphyton, or attached algae.

Laboratory Analysis

Filters are cut into small pieces and placed in a centrifuge tube with 10 ml of 90% buffered acetone. Extraction tubes are placed in a metal rack, covered with aluminum foil and held in a dark refrigerator for 24 hrs. After extraction, samples are read on a Shimadzu UV-1601 Spectrophotometer (1995) and a Turner Model 10 Fluorometer (1996). Trichromatic equations (according to Standard Methods, APHA 1992) are used to convert spectrophotometric optical densities to total chlorophyll-a. The Turner Fluorometer is calibrated with primary and secondary chlorophyll standards, according to Standard Methods (APHA 1992). A calibration curve is developed with chlorophyll-a standards using a spectrophotometer. New calibration curves are developed every year.

Quality Control of Field Sampling

Samples will be placed in pre-labeled bags, placed over fresh silica gel desiccant, and frozen. Samples will be kept frozen during transport to Fairbanks and until chlorophyll-a extraction is done.

Quality Control for Chlorophyll-a Determinations

Fresh chlorophyll-a standards will be used to calibrate the Fluorometer for each annual sampling event. Samples containing sufficient chlorophyll-a will be read on both the Fluorometer and the Spectrophotometer to check calibration curves. Samples with chlorophyll-a concentrations below the calibration point will be reported as "non-detectable."

Aquatic Invertebrates: Taxonomic Richness and Abundance

Objectives

Aquatic invertebrate communities will be sampled below the Red Dog Mine effluent to document the continued biological integrity of these communities and to detect changes in in-situ productivity. Reference sites will be used to detect variations due to other factors, such as climate.

Methods

We modified the rapid bioassessment techniques developed by USEPA (Barbour et al 1997) to retain more quantitative features of sampling. Benthic invertebrates will be collected in drift nets set within riffle habitats to standardize assessments among streams. Five drift nets will be installed at random locations in riffle habitat and left to collect invertebrates for one hour (Figure 4). Water velocity and depth will be measured at the mouth of each net to standardize numbers of insects by volume of water and to compare relative biomass of invertebrates among sites. Invertebrates will be transferred to individually labeled bags and preserved in 70% ETOH.

Invertebrate samples will be taken back to the laboratory and sorted. Samples are first washed with tap water into an enamel pan and the sample container examined for remaining invertebrates. Then samples are strained through a 90 *u*m mesh to remove water and placed in a glass dish. Acetate, cut to fit the diameter of the glass dish is used to divide the sample (Figure 5). Although Barbour et al (1997) recommends subsampling benthic invertebrates, we choose to split samples by 50%. This allowed us to standardize samples among streams by volume of water passing through each net and retain a more quantitative estimate of invertebrate density.

Sampling will be done once per year, during the period from late June through mid-July. Sampling will be done at low water periods.

Subsamples will be sorted from other detritus, placed in fresh 70% ETOH and identified to the lowest practical taxonomic level. Mature larvae and nymphs and adult forms of aquatic species usually can be identified to genus; immature larvae and nymphs are usually identified to family. Chironomidae will be identified to family.

Figure 4. Drift nets used to sample aquatic invertebrates.

Figure 5. Subsampling aquatic invertebrate samples.

Quality Assurance in the Field

Sample containers are pre-labeled with stream site name and date. Samples are assigned a discrete number that matches a particular net with measurements of stream velocity and flow. Nets are positioned in the stream to prevent interception of flow into downstream nets by staggering nets across the stream width. Where stream sites are too narrow to allow staggering, nets are distributed along the stream length, each net placed below a different riffle habitat.

Contents of the drift nets are washed with ETOH into sample containers. The end buckets of the drift nets are examined for remaining invertebrates.

Five replicate samples were collected from each site. A log will be maintained of all invertebrate samples and volumes of water flowing into each net.

Quality Assurance in the Laboratory

Quality Control for Sorting

Ten percent of the sorted samples will be examined by a senior biologist to determine the number of organisms missed by the sorter. If the QC sorter finds less than 10% of the total sample organisms remaining in the tray, the sample passed.

Quality Control for Taxonomy

A technician with training in entomology will do invertebrate identification; a senior biologist with extensive experience identifying invertebrates in Alaska will check the identification of at least 10% of the samples. A reference collection of identified taxa will be maintained. Because many of the aquatic insects in Alaska have not been identified to species, identification will be done to the family or genus level. Family level is used when insects are too immature to see identifying characteristics.

We sorted and identified both halves of three discrete samples from invertebrate drift samples collected in 1998 by Alaska Department of Fish and Game (ADF&G) (Appendix I) to test the accuracy of subsampling. The split samples were found to contain a sufficient representation of both numbers and taxa.

Metals Concentrations in Dolly Varden Tissues

Objective

Since 1990, ADF&G has sampled adult Dolly Varden from the Wulik River to determine the concentrations of Al, Cd, Cu, Pb, and Zn in muscle, gill, liver, and kidney tissue. Beginning in 1996, tissue samples also were analyzed for selenium. The objective of this sampling effort was to compare metals concentrations of fish tissues to concentrations found since beginning of operation of the Red Dog Mine and to detect any changes in concentrations of fish tissues that can be related to changes in metals concentrations in receiving waters below the mine. Sampling under the current NPDES permit for the Red Dog Mine effluent is a continuation of this effort.

Methods

Individual Dolly Varden will be caught by hook and line and placed in clean plastic containers and labeled with sample date and location. Collections will be done from the Wulik River below Tutak Creek in fall before freeze-up and in spring after break-up. When possible, six fish will be collected. Fish will be frozen before dissection and sample preparation. Upon removal from a freezer, fish will be measured to fork length, weighed, sex and spawning condition recorded, and otiliths removed to determine age. Tissue samples from muscle (from below the dorsal fin and above the lateral line), gill, kidney, and liver (excluding bile tissue) will be removed from partially thawed fish using standard procedures to minimize contamination (Crawford and Luoma 1993, Figure 6). About 10 g of each tissue will be placed in pre-cleaned jars (EPA Series 300, Protocol C) and refrozen. Tissue samples will be submitted to a private analytical laboratory. Samples were digested, freeze-dried, and analyzed for Al, Cu, Cd, Pb, Zn and Se using U.S. Environmental Protection Agency standard methods.

Quality Control for Collecting Fish Samples

Each fish will be immediately placed in a clean plastic bag after being caught. Fish will be labeled with time and location of collection and frozen as soon as possible. Fish will be kept frozen during transport to ADF&G for dissection.

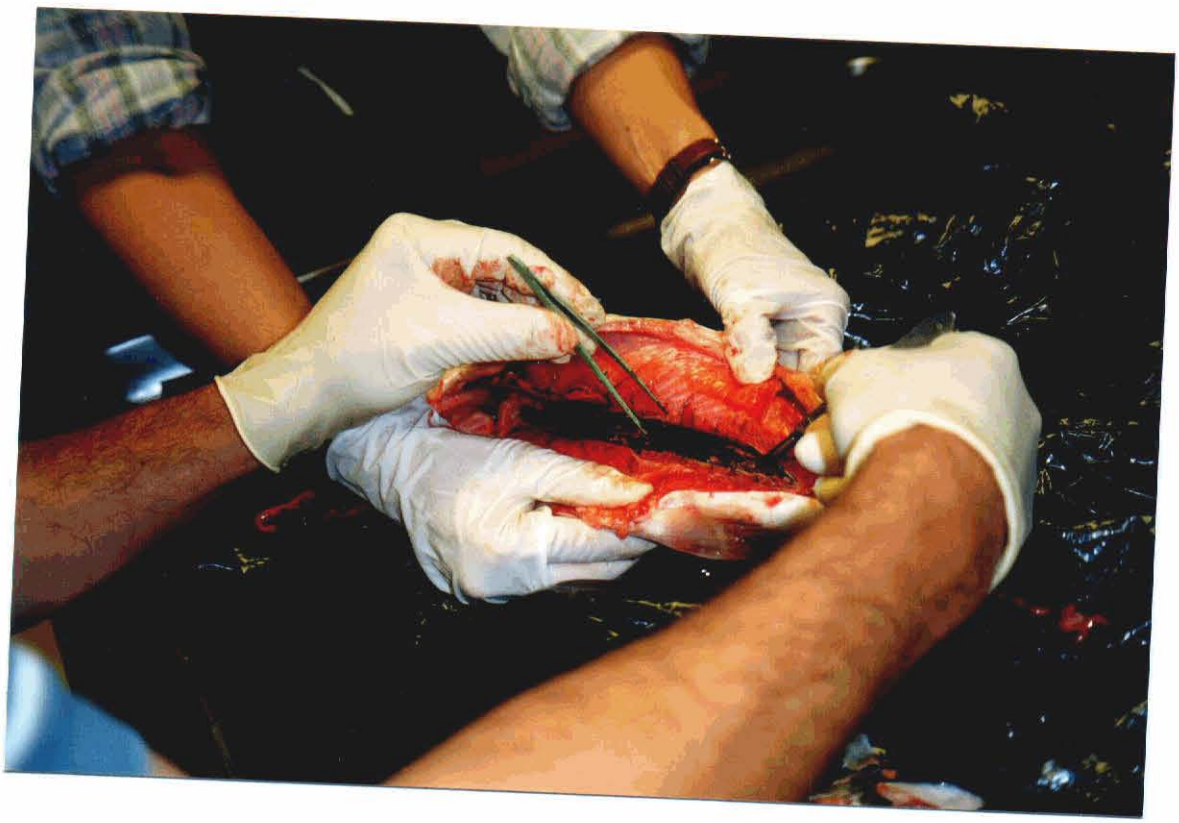


Figure 6. Dissection of fish tissues for metals analysis.

Quality Control in Preparing Fish Tissues

Each dissection instrument will be cleaned in ultra-pure nitric acid with two rinses in double distilled water before beginning dissection of a new tissue. No instruments will be used on successive tissues without cleaning. Fish will be dissected in a partially frozen condition to allow removal of discrete tissues without contamination by other tissues. Liver bile ducts will not be included in liver samples. Kidney tissues will be removed in a frozen condition to allow extraction of intact samples.

All dissections will be done by a trained fishery biologist. ADF&G's resident expert on char will do extraction and reading of otiliths.

Only pre-cleaned bottles (Series 300, Protocol C) will be used for fish tissues. After preparation of samples, fish tissues will be re-frozen in an ultra-cool (-30°C) freezer until shipment to the analytical laboratory. Shipments will not be made after mid-week to prevent samples arriving during week-end days. The analytical laboratory will be notified of any incoming shipments.

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

Date/Stream Code/Species Code/Age Code/Sample Number/Tissue Code

Quality Control / Quality Assurance of Laboratory Analysis

The analytic laboratory will provide 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 will be included in the laboratory report.

Blind duplicate tissues will be submitted to the laboratory with each sample catalogue.

Fish Presence and Use in Tributary Streams

Objectives

Fish monitoring will focus on the distribution and relative abundance of juvenile Dolly Varden and Arctic grayling downstream of the Red Dog Mine and in tributaries to waters potentially affected by the mine. Reference streams will be monitored to detect annual variations in distribution and abundance that are independent of mine operation.

Methods

Fish will be sampled two times per summer; preferred sampling times are late June and early August, using visual surveys, angling, and minnow traps. Juvenile fish will be collected in baited minnow traps. Minnow traps will be baited with fresh or preserved salmon eggs, contained in perforated plastic bags. Traps will be placed at numbered sites established and used by ADF&G since 1992. Traps will be placed in areas of moderate current to prevent traps from dislodging, and left to fish for about 24 hrs. All fish will be counted, identified, and measured to the nearest mm fork length (Figure 7). Numbers of fish will be compared within and among sample years.

Visual surveys and angling will be used to supplement sampling by minnow traps. Angling will be used to determine the presence of adult Arctic grayling throughout the drainage. Visual surveys will be used to document the presence of young-of-the-year Arctic grayling and Dolly Varden within the sample reaches. Recently emerged larval Arctic graying are opportunistically collected in invertebrate drift nets. Their presence and abundance will be recorded.

Quality Control of Fish Sampling

Specific trap locations established and used by ADF&G will be used for this study to allow comparisons with previous juvenile fish information. Identification and measurements of fish lengths will be done by a trained fishery biologist, with experience in northern regions.

Figure 7. Sampling and measuring juvenile fish.

Fall Aerial Survey of Overwintering Dolly Varden

Objective

The objective of monitoring overwintering Dolly Varden is to estimate the abundance and assess the distribution of overwintering adult Dolly in the Wulik River. Changes in the use of this river system (for example, relative proportion of fish upstream and downstream of Ikalukrok Creek) will be determined.

ADF&G has conducted a fall survey of overwintering Dolly Varden in the Wulik River since 1979, except in 1983, 1985, 1986, and 1990 when weather conditions did not permit aerial surveys (DeCicco 1997; Weber Scannell and Ott 1998). Sampling conducted under the current NPDES permit for the Red Dog Mine effluent will be compared to previous ADF&G surveys to detect any changes to the Dolly Varden adult population.

Methods

Surveys will be done from the mouth of the Wulik River to approximately five river miles upstream of the confluence of the Wulik River and Ikalukrok Creek. Surveys will be done during late September to early October, before freeze-up, with helicopter or fixed wing aircraft.

Quality Control for Dolly Varden Aerial Surveys

All surveys will be done by an experienced char biologist with specific training in conducting aerial surveys of riverine fish populations.

Chum Salmon Spawning

Objectives

The abundance and distribution of adult chum salmon spawning in Ikalukrok Creek downstream of Dudd Creek will be assessed using aerial surveys to document any changes in the use of this spawning area.

Methods

Aerial surveys will be conducted in mid-August by helicopter or small fixed wing aircraft. To the extent allowed by visibility and flow conditions of the river, adult salmon will be counted and identified to species. If observed, condition of fish (live, dead, actively spawning) will be noted.

Quality Control for Fall aerial survey of Returning Chum Salmon

All surveys will be done by an experienced salmon biologist with specific training in conducting aerial surveys of riverine fish populations.

Data Reporting

Reports of data will include trip reports summarizing field sampling efforts and preliminary findings. An annual report will be prepared and submitted to US Environmental Protection Agency, Alaska Department of Fish and Game, Alaska Department of Environmental Conservation, and other interested federal, state and private individuals summarizing fish, invertebrate, and periphyton data and relating these data to water quality conditions.

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Appendix 1. Evaluation of Similarity Among Invertebrate Subsamples.

Comparison of Aquatic Invertebrate Subsamples
(Comparison of 2 subsamples, each 1/2 of total sample)

Site Alvinella Site 3
Date July 1998
Net Number 2

	Subsample #1 (1/2 of sample) Observed	Subsample #2 (1/2 of sample) Observed	Total	Subsample #1 Expected	Subsample #2 Expected	Calculations for Chi Square	
Hymenoptera adults	4	3	7	3.479	3.521	0.08	0.08
Diptera adults	1	5	6	2.982	3.018	1.32	1.30
Alloperla	1	0	1	0.497	0.503	0.51	0.50
Baetis	27	31	58	28.824	29.176	0.12	0.11
Baetis adults	4	5	9	4.473	4.527	0.05	0.05
Capnia	12	11	23	11.430	11.570	0.03	0.03
Cinygmula	3	1	4	1.988	2.012	0.52	0.51
Homoptera	5	5	10	4.970	5.030	0.00	0.00
Chironomidae larvae	16	16	32	15.903	16.097	0.00	0.00
Nemoura	1	0	1	0.497	0.503	0.51	0.50
Chironomidae pupae	3	2	5	2.485	2.515	0.11	0.11
Staphylinidae	3	2	5	2.485	2.515	0.11	0.11
Simulidae	2	2	4	1.988	2.012	0.00	0.00
total	82	83	165				
					Chi-square =	6.63	

Chi-Square value = 6.63 12 df
Probability of ChiSquare with 12 df = 0.90
therefore samples are not different

Appendix 1. continued.

Comparison of Aquatic Invertebrate Subsamples
(Comparison of 2 subsamples, each 1/4 of total sample)

Site Alvinella Site 4
Date July 1998
Net Number 4

	Subsample #1 (1/4 of sample) Observed	Subsample #2 (1/4 of sample) Observed	Total	Subsample #1 Expected	Subsample #2 Expected	Calculations for Chi Square	
Hymenoptera adults	1	2	3	1.662	1.338	0.26	0.33
Diptera adults	6	6	12	6.646	5.354	0.06	0.08
Alloperla	0	0	0	0.000	0.000		
Baetis	4	2	6	3.323	2.677	0.14	0.17
Baetis adults	2	2	4	2.215	1.785	0.02	0.03
Capnia	7	5	12	6.646	5.354	0.02	0.02
Cinygmula	3	4	7	3.877	3.123	0.20	0.25
Homoptera	0	0	0	0.000	0.000		
Chironomidae larvae	9	4	13	7.200	5.800	0.45	0.56
Nemoura	1	2	3	1.662	1.338	0.26	0.33
Chironomidae pupae	2	0	2	1.108	0.892	0.72	0.89
Staphylinidae	0	1	1	0.554	0.446	0.55	0.69
Simulidae	1	1	2	1.108	0.892	0.01	0.01
total	36	29	65				
					Chi-square =	6.05	12 DF

Chi-Square value = 6.05
Probability of ChiSquare with 12 df = 0.90
therefore samples are not different

Appendix 1. continued.

Comparison of Aquatic Invertebrate Subsamples

Comparison of 1/2 of sample with total of 1/4 subsamples

Site Alvinella Site 4
Date July 1998
Net Number 4

	Subsample #1 (1/2 of sample) Observed	Subsample #2 (1/4+1/4 of sample) Observed	Total	Subsample #1 Expected	Subsample #2 Expected	Calculations for Chi Square	
Hymenoptera adults	7	3	10	4.602	4.398	0.56	0.58
Diptera adults	7	12	19	3.068	2.932	0.37	0.39
Alloperla	0	0	0	11.759	11.241	0.05	0.05
Baetis	3	6	9	9.203	8.797	0.35	0.37
Baetis adults	2	4	6	0.000	0.000		
Capnia	11	12	23	12.782	12.218	0.05	0.05
Cinygmula	11	7	18	6.135	5.865	1.34	1.40
Homoptera	0	0	0	2.045	1.955	0.00	0.00
Chironomidae larvae	12	13	25	0.511	0.489	0.51	0.53
Nemoura	9	3	12	3.068	2.932	0.28	0.30
Chironomidae pupae	2	2	4	68.000	65.000	0.00	0.00
Staphylinidae	0	1	1	0.000	0.000		
Simulidae	4	2	6	0.000	0.000		
total	68	65	133				
					Chi-Square =	7.18	

Chi-Square value = 7.18
Probability of ChiSquare with 12 df = 0.85
therefore samples are not different

Appendix 1. continued.

Comparison of Aquatic Invertebrate Subsamples

Site Red Dog Creek upstream of mine site
Date July 1998
Net Number 1

	Subsample #1 (1/2 of sample) Observed	Subsample #2 (1/2 of sample) Observed	Total	Subsample #1 Expected	Subsample #2 Expected	Calculations for Chi Square	
Hymenoptera adults	10	4	14	8.157	5.843	0.42	0.58
Diptera adults	27	10	37	21.557	15.443	1.37	1.92
Alloperla	0	0	0	0.000	0.000		
Baetis	44	32	76	44.278	31.722	0.00	0.00
Baetis adults	0	0	0	0.000	0.000		
Capnia	76	62	138	80.400	57.600	0.24	0.34
Cinygmula	1	1	2	1.165	0.835	0.02	0.03
Homoptera	10	10	20	11.652	8.348	0.23	0.33
Chironomidae larvae	19	18	37	21.557	15.443	0.30	0.42
Nemoura	0	1	1	0.583	0.417	0.58	0.81
Chironomidae pupae	2	0	2	1.165	0.835	0.60	0.83
Staphylinidae	0	0	0	0.000	0.000		
Simulidae	12	6	18	10.487	7.513	0.22	0.30
total	201	144	345				
					Chi-square =	9.57	12 DF
Chi-Square value =	6.05						
Probability of ChiSquare with 12 df = 0.65							
therefore samples are not different							