Monitoring Stream Habitats and Biological Communities in the Lime Hills Ecoregion of Bristol Bay

Alaska Center for Conservation Science
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From left to right: Kristy Jeffries, Dustin Merrigan, Dan Bogan, Rebecca Shaftel, Sarah O'Neal, Tim Troll, and Stan Hermens (2018).

1 INTRODUCTION

The Kvichak and Nushagak watersheds support some of the largest sockeye salmon runs in Bristol Bay, which is the largest sockeye salmon producing region in the world (Ruggerone et al. 2010). Stream habitats in these watersheds support all five species of Pacific Salmon (*Oncorhynchus* sp.) in addition to at least 24 other resident and anadromous fish species (Wiedmer 2014). These valuable fishery resources are under threat from climate change and potential massive-scale mining. State and federal lands in these watersheds are open to mineral development and over 500 square miles are currently under lease to mining companies. In addition to mining, climate changes are already occurring in Western Alaska. Increased air temperatures (Chapin et al. 2014) and changes in seasonal precipitation (McAfee et al. 2013) will impact streams by decreasing the snowpack leading to changes in the seasonality and magnitude of discharge (Wobus et al. 2015) and a loss in thermal diversity across the stream network (Lisi et al. 2015). In anticipation of these threats, the Southwest Alaska Salmon Habitat Partnership's strategic conservation actions include steps such as developing long term water chemistry and water temperature monitoring networks.

In efforts to catalog biodiversity and baseline habitat conditions in the upper Nushagak and Kvichak watersheds, stream habitats and biological communities have been strategically monitored as part of several different projects over the last 12 years. The Alaska Center for Conservation Science (ACCS) at the University of Alaska Anchorage (UAA) monitored stream benthic communities (diatoms and macroinvertebrates) and physical and chemical conditions in 78 hard-bottomed wadeable streams from 2008-2010 (Bogan et al. 2018). Four tributary streams of the South Fork Koktuli River and one tributary of the Newhalen River were selected for long-term monitoring and have been sampled annually since 2008 (samples were last collected in 2019). Fish communities were sampled in 105 headwater streams by Dr. Carol Ann Woody and Sarah O'Neal from 2008 to 2010 and 168 km of streams were added to the Anadromous Waters Catalog, providing additional regulatory protections to these streams (Woody and O'Neal 2010). Fish have also been sampled over several years at the five long-term monitoring sites. The results of these monitoring programs provide important baseline information prior to impacts from climate change or mineral development, but because each project selected sample sites to meet different objectives, they may not represent the full range of biological and habitat diversity in these important watersheds.

A new monitoring plan was developed to address monitoring needs in the Lime Hills ecoregion of Bristol Bay, which includes the Mulchatna River watershed in the upper Nushagak basin and that portion of the Kvichak watershed north of Lake Iliamna and west of Lake Clark (Woody et al. 2014). The Lime Hills ecoregion also includes the extensive mining leases on State lands associated with the Pebble deposit and other mineralized areas. The plan recommended a probabilistic survey design where sites are selected randomly across the study area. Probabilistic surveys provide a better estimate of the full range of variation in wadeable stream habitats and biological communities than strategic sampling, which introduces bias into site selection. Additionally, probabilistic surveys allow results to be extrapolated to the entire population of wadeable streams. In 2015, the project team sampled 30 randomly selected sites (i.e. sites were selected randomly from the entire population of wadeable streams) and 10 strategically selected sites from streams in the Lime Hills Ecoregion of the Nushagak and Kvichak watersheds.

Both the historic datasets and the new probabilistic dataset described in this report provide useful baselines of current stream conditions from which we can measure change in the future. Stream biological communities offer information on perturbation not always obtained with discrete water chemistry measurements by integrating environmental conditions over time, thus providing a measure of the aggregate impact of multiple stressors on streams. Because different assemblages operate on different spatial scales and are sensitive to different types of impacts (Hughes et al. 2000), the use of multiple biological assemblages (e.g. macroinvertebrates, diatoms, and fish) in aquatic monitoring programs can enhance the ability to detect and diagnose ecological impairment (Karr and Chu 1999). There is a long history of biomonitoring using benthic macroinvertebrates and diatoms to document impacts to streams from land uses in the watershed, especially mining (Wagener and LaPerriere 1985, Clements and Carlisle 2000, Hirst et al. 2002, Milner and Piorkowski 2004, Hogsden and Harding 2012, Smucker et al. 2014) and urbanization (Cuffney et al. 2010, King and Baker 2010, Smucker et al. 2013). There is increasing evidence that stream communities are also indicators of climate change (Burgmer et al. 2007, Durance and Ormerod 2007, Heino et al. 2009, Lawrence et al. 2010, Piggott et al. 2014).

The objectives of this report include 1) describing the current conditions of stream physical habitat, water chemistry, fish, macroinvertebrates, and diatoms using data from probabilistically-selected sites sampled in 2015; and 2) selecting monitoring indicators that can be used to detect future changes from climate change and mineral development. All final datasets can be found on the ACCS Data Catalog. We are currently funded by the Bristol Bay Heritage Land Trust and the Alaska Department of Environmental Conservation to continue data analysis, including inter-annual data collected at ten sites through 2019. Future products will be provided on the Data Catalog for this project.

2 METHODS

2.1 Study area

We selected our study area as that part of the Lime Hills ecoregion that drains to Bristol Bay. The study area ranges from 40 to 1,000 feet in elevation and is bounded to the north by the watershed divide between the Nushagak and Kuskokwim rivers, to the east by the Alaska Range, to the south by Lake Iliamna, and to the west by the Mulchatna River (Figure 1). The State of Alaska is the major landowner across the study area and lands are susceptible to both mineral and other types of development, making monitoring of habitats and biodiversity a priority. The study area drains approximately 15,600 km^2 and includes a potential mining district that encompasses approximately 2,000 km^2. The Lime Hills ecoregion is characterized by rounded ridges and gently sloping valleys (Nowacki et al. 2001). There are no glaciers within the study area, although several of the large rivers contain glacial meltwater from their headwaters in the Alaska Range. Mixed forests occur at low elevations, whereas shrub and tundra vegetation are common throughout the study area. The towns of Iliamna, Newhalen, Nondalton, and Port Alsworth are all located in the southern portion of the study area.

Average monthly air temperatures for Iliamna are below freezing for five months of the year (November through March) with the coldest temperatures in January (-8.1˚C) and the warmest temperatures in July (13.4˚C, NCDC 1981-2010 monthly normals, Western Regional Climate Center). Annual precipitation averages 633 mm, with approximately one-third falling as rain during the summer months (June through August).

Several medium to large lakes are located on the eastern boundary of the study area within the Alaska Range: Turquoise Lake, Twin Lakes, and Lake Clark. Major rivers flowing from east to west out of the Alaska Range include (from north to south) the Mulchatna, Chilikadrotna, Kijik, and Tazimina Rivers. Other rivers whose source waters are entirely within the study area include the Chilchitna, Koksetna, Koktuli, Swan, and Stuyahok Rivers.

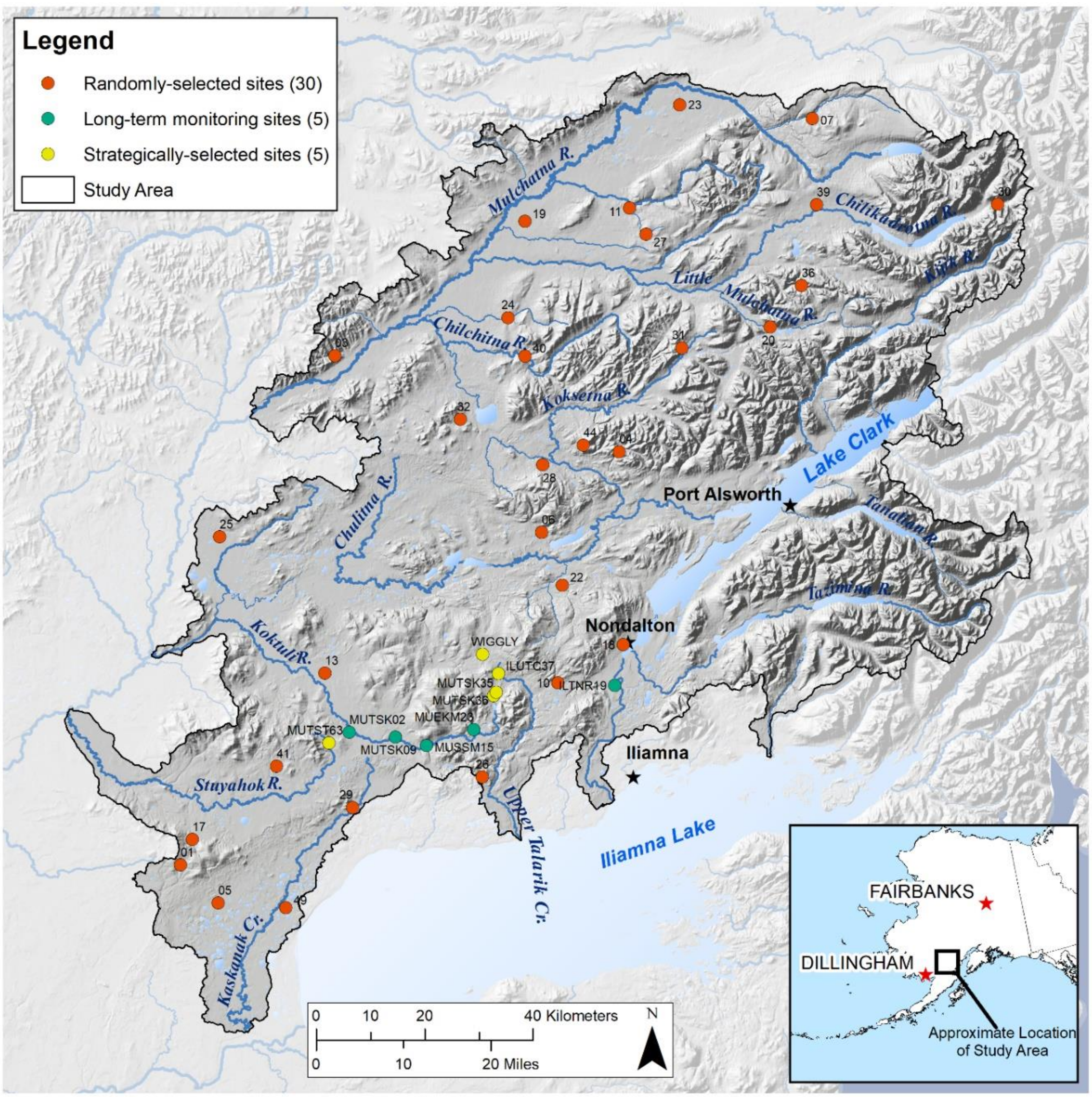

Figure 1: Study area and monitoring sites.

2.2 Study design

Our target population consisted of wadeable streams that provide habitat for Pacific salmon. We selected wadeable streams for several reasons: they comprise the majority of streams in the network, non-wadeable streams are difficult to sample and introduce safety and logistical concerns, sampling all streams would require a much larger sampling effort to capture the entire range of variation.

We used a synthetic stream network for the Nushagak and Kvichak watersheds created from a 30-meter digital elevation model for our sample frame (Woll et al. 2014). We selected 1st through 4th order streams that have a gradient of 10% or less to exclude non-wadeable rivers and high gradient habitats not generally used by Pacific salmon, respectively. We removed stream reaches that were inaccessible to salmon because they were located upstream of geologically fixed barriers (waterfalls) or reaches of gradient greater than 20%. We further refined the sample frame to exclude short (< 1 km in length) 1st order streams that most likely are non-existent or intermittent. The final sample frame included 12,434 km of streams and 72% were 1st or 2nd order headwaters.

We used a generalized random tessellation survey (GRTS) design (Stevens and Olsen 2004) to select 120 sample locations equally-distributed across small (1st and 2nd order) and large (3rd and 4th order) streams. Our goal was to sample 30 sites and we included oversample sites in the event that streams were non-target or not accessible. The GRTS survey design allowed for spatially balanced sampling when oversample sites were used.

All survey sites were evaluated in the office or in the field prior to sampling. A total of 30 sites were sampled (18 large streams and 12 small streams) out of 49 that were evaluated. The 19 evaluated sites were not sampled for several reasons: there was no stream in the vicinity of the sample site (i.e. map error), the sample point was in a wetland without a defined stream channel, the stream was not wadeable (i.e. too deep), or the stream channel was dry. The sampled population represents 56% of all streams in the sample frame, 95% CI [43%, 70%] and this proportion of the sample frame equals 6,991 km of streams in the study area, 95% CI [5,259 km, 8,723 km]. The streams in the sample frame not represented by this monitoring effort did not meet the definition of our target population for the reasons described above (evaluated sites that were not sampled).

Sample weights were used to estimate statistical parameters (means and standard deviations) for response variables using the spsurvey package and R statistical computing software (Kincaid and Olsen 2016, R Core Team 2017). Weights were initially calculated by dividing the target population extent (stream length) by the study design sample size and were adjusted after implementation of the study design to account for sites not sampled.

In addition to our 30 randomly-selected sites, we also sampled ten strategically-selected sites in the southwest portion of the study area. Five of these sites are long-term monitoring sites that have been sampled annually for stream macroinvertebrates and diatoms since 2008. An additional five sites were selected because they were either near to the Pebble deposit or previous sampling indicated acidic water chemistry, which indicated they likely drained mineralized areas. Biological communities at mineralized sites should reflect these conditions.

2.3 Field methods

One to two streams were sampled each day between June 1st and June 24th 2015. The index period was selected to capture base-flow conditions. All sampling was performed within the stream reach, which was defined as 40 times the average stream wetted width or 150 meters, whichever was greater.

2.3.1 Water chemistry

Upon arrival, water chemistry was measured in-situ at the center point of the stream reach (referred to as the X-site). A Hydrolab MS5 sonde was held in the stream at mid-depth and allowed to equilibrate until measurements had stabilized before recording dissolved oxygen (DO mg/L and % saturation), pH, temperature (°C), and specific conductance (µS/cm, EC25). The Hydrolab was calibrated each morning using a three-point pH calibration and a one-point calibration for specific conductance (1,000 µS/cm). The Hydrolab was calibrated at the site for dissolved oxygen using the percent saturation method.

Water samples were collected for dissolved and total metals, dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), and total dissolved nitrogen (TDN) at the X-site after in-situ measurements were complete. Samples were collected with nitrile gloves to avoid cross-contamination. Stream water was collected from the middle of the stream channel at mid-depth into a bottle or syringe while facing upstream. All containers were rinsed three times before filling. For dissolved metals, 40-mL of sample were filtered through a 0.45-µm clean syringe filter. The first 3-5 mL were discarded before filtering into the sample container. The samples for DOC, DIC, and TDN were sampled next using the same syringe and filter. Samples were filtered into a 40 mL carbon-free amber glass bottle and closed with a lid while avoiding introduction of air bubbles. The total metal samples were collected directly into the sample container without filtration. Field duplicates were collected at 10% of all sites to measure sample precision and handled identically to other samples.

An alkalinity sample was collected from the stream and carried to the field laboratory for processing. Alkalinity was measured each day of sampling using the inflection point titration method described in the USGS National Field Manual for the Collection of Water Quality Data (Chapter 6.6 Alkalinity and Acid-Neutralizing Capacity).

All water samples were kept cool using frozen gel packs in a cooler until the field crew returned to the base camp daily, whereupon they were refrigerated. A temperature blank was carried with the samples to assure that samples stayed below 7 °C. All water samples were transported in a cooler with gel packs to the Applied Science, Engineering, and Technology Laboratory (ASET) lab at UAA once in the middle of the field effort and once again at the end.

2.3.2 Physical habitat

Physical habitat data were collected using EPA's National Rivers and Streams Assessment methods (National Rivers & Streams Assessment (NRSA) 2013/14 Field Operations Manual Wadeable). Physical habitat measurements were collected at 11 equally-spaced transects along the stream reach and included wetted width, bankfull width, bankfull height, incision height, bank angle, undercut distance, canopy cover, fish cover, and riparian vegetation cover. Substrates were characterized at 105 points within each reach by size class. The deepest location in the stream (the thalweg), was measured at 100 to 150 locations longitudinally within each reach. Channel habitat types were also recorded at each thalweg measurement location. Large woody debris was counted based on four diameter and three length size classes both within and above the bankfull channel between transects along the entire reach. Physical habitat metrics for each stream reach were calculated using the aquamet library (received from Karen Blocksom, U.S. EPA, March 14, 2017) in R software.

2.3.3 Fish

Fish sampling followed modified U.S. EPA Environmental Monitoring and Assessment Protocols (McCormick and Hughes 1998) for electrofishing using a Smith-Root Model LR-24 backpack electro-fisher (Smith-Root, Vancouver, Washington). Stream specific conductance was used to set appropriate backpack electrofisher settings. Crews fished from the bottom of the reach upstream, discontinuously sampling all habitat types. Electrofishing time ranged from 16 to 33 minutes of shock time (as opposed to sampling duration) across 38 of the sampled sites. At two sites, shock times were four and eight minutes due to battery or other equipment issues. Captured fish were held in a 2-gallon bucket and identified to species, except for sculpin (*Cottus* sp.), lamprey (*Lamprey* sp.), and whitefish (*Coregonus* sp.), which were identified to the lowest known classification. A random subset of fish were weighed and measured at each site. Fish were released is less than 60 minutes after capture downstream of the sampling reach.

2.3.4 Macroinvertebrates and diatoms

Stream benthic communities (macroinvertebrates and diatoms) were also sampled according to the 2013/14 NRSA methods. Diatoms and macroinvertebrates were sampled in one habitat at each of 11 transects and composited for each site. Habitats were selected randomly at the left, center, or right sampling point (25%, 50%, and 75% of the wetted width) at each transect. Diatoms were sampled by collecting rock or wood substrate from the sampling point, placing an area delimiter over the substrate (12-cm^2), scrubbing with a toothbrush for 30 seconds, and rinsing the biofilm with a squirt bottle into a funnel placed inside a sample bottle, which was later preserved with Lugol's solution. Macroinvertebrates were sampled by placing a D-frame net on the bottom of the stream and disturbing all substrate in a one square foot area upstream so that it washed into the net. Net contents were composited into a bucket for elutriation before placement into sample bottles and preserving with 70% ethanol.

2.4 Laboratory methods

2.4.1 Water chemistry

Water samples were analyzed for dissolved metals, total metals, dissolved inorganic carbon, dissolved organic carbon, and total dissolved nitrogen according to standard operating procedures that followed EPA methods (Hagedorn 2015, Dodds and Hagedorn 2007). Dissolved and total metals included silver (Ag), aluminum (Al), arsenic (As), barium (Ba), beryllium (Be), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), antimony (Sb), selenium (Se), silicon (Si), thorium (Th), thallium (Tl), uranium (U), vanadium (V), and zinc (Zn). Major ions also analyzed with metals included calcium (Ca), potassium (K), magnesium (Mg), and sodium (Na).

Dissolved metals were analyzed without further preparation using Inductively Coupled Plasma Mass Spectrometer (Agilent 7500) with 7 level external calibration (0.1 to 500 ppb) and internal standard mix. The calibration was verified using international NIST standard (SRM 1640a) and continuous calibration standards every 10th sample.

Total metals were further processed in the laboratory. Samples were acidified to pH 2 using concentrated (68-72%) ultrapure nitric acid (HNO_3) and placed for 24 hours on a shaker and then filtered through a 0.45 µm syringe filter (GHP Acrodisc 25 mm) into a 15 mL auto-sampler vial. Samples were analyzed with the same instrument and methods as used for dissolved metals with a different calibration standard due to the potentially higher concentrations in total metals samples. A calibration standard with 10x higher concentration in Ca, K, Mg, Na, and Fe compared to the other trace metals was used making calibration concentrations ranging from 0.5 ppb to 10,000 ppb.

Dissolved organic carbon (DOC) samples were directly placed on the auto-sampler. 500 µL of sample was mixed to 1.5 %v/v with 2N HCl to remove bicarbonate. Ten microliters were injected into a quartz glass furnace and heated to 680 °C leading to total combustion of the sample and formation of CO_2 gas and water vapor. Water vapor was removed by vapor scrubber before CO_2 detection by infrared adsorption. Quantitation was performed using external seven level calibration. Blank samples and calibration verification samples were analyzed every 10th sample to monitor analytic performance.

Dissolved inorganic carbon was calculated from the difference between total dissolved carbon (TDC) and DOC. TDC was analyzed from the same sample vial as DOC following the same procedure but without acidification of the sample prior to analysis. DIC was then calculated as DIC = TDC – DOC.

Total dissolved nitrogen was analyzed together with TDC using a nitrogen detector that is in-line with the CO_2 detector.

2.4.2 Macroinvertebrates and diatoms

We preserved all benthic macroinvertebrate samples in the field with ethanol and processed them in UAA's Aquatic Ecology lab. In the lab, we subsampled each macroinvertebrate sample to obtain a fixed count of 500 ±20% organisms to standardize the taxonomic effort across all sites. Counts were adjusted to total counts using the proportion of the sample in the subsample (e.g. if 50% of the sample was subsampled, then counts were multiplied by two). In addition, we conducted a five-minute search through the remaining sample to select any large rare taxa that may have been missed during subsampling. We identified all insects to genus or lowest practical taxonomic level, including Chironomidae, and non-insects to a higher taxonomic level (usually family or order) using standard taxonomic keys (Weiderholm 1983, Pennak 1989, Wiggins 1996, Thorpe and Covich 2001, Stewart and Oswood 2006, Merritt and Cummins 2008). We converted total counts to densities by dividing by the area sampled (1.0 m^2).

For diatoms, each sample was homogenized and 20 ml were transferred to a clean beaker. Diatoms were cleared for easier identification by adding nitric acid and heat to digest the diatom protoplasm and other organic material. Acid-digested aliquots were neutralized by a succession of dilutions, cleared diatom frustules were concentrated by settlement, and slide-mounted using NAPHRAX mounting medium. A fixed count of 600 diatom valves was identified to species or lowest practical taxonomic level. The slide was scanned for any taxa not discovered in the fixed count. The primary taxonomic references used were Krammer and Lange-Bertalot (1986-1991), Patrick and Reimer (1975), Krammer (2000-2003) and Lange-Bertalot (2002 and 2011). Taxonomy was updated based on the California Academy of Sciences Catalogue of Diatom Names.

2.5 Indicator selection

We selected a suite of indicators from each of our five multivariate datasets that we expected would be sensitive to climate change or mineral development. Specific stressors from mineral development that we considered were decreased pH, increased specific conductance, increased sedimentation, potential for overall chemical pollution from mineral development, and thermal pollution through discharge of treated water. We also considered increased stream temperature from climate change. From water quality and physical habitat datasets, we selected a comprehensive suite of indicators that represent current chemical and physical habitat conditions and could be used as indicators for habitat changes in the future. For the biological communities, we selected indicators by 1) screening for taxa that occurred relatively frequently in our dataset (50% or more of sites), and 2) identifying taxa that were sensitive to one or more of the expected stressors identified above from either climate change or mineral development.

We expected that all our water chemistry parameters would be sensitive to changes from either mining or climate. Mineral development and the resulting leaching of mineralized rock could lead to increased dissolved and total metals concentrations, higher specific conductance, and lower pH. Climate change effects on stream thermal regimes include higher temperatures from increased solar radiation, decreased cold water inputs from a diminished snowpack, and lower base flows from increased evapotranspiration. Other parameters that may change include stream nutrient regimes (i.e. DOC, TOC, and TN), which may respond to landcover changes such as wetland drying or increased shrub and tree cover along streambanks. We summarized water chemistry data for all parameters with more than 50% of results greater than method detection limits.

We selected 28 physical habitat variables for analysis that captured elements of each stream's topography, geometry, pool habitats, fish cover types, substrate, and riparian vegetation. Stream topographic variables included elevation, slope, and watershed area. Stream geometry variables included mean bankfull width, mean bankfull width to depth ratio, sinuosity, mean thalweg depth, the standard deviation of thalweg depths, and the mean width by depth area. To describe residual pools, we included a count of all pools with depth greater than five centimeters, the maximum pool depth, and the pool density. We selected the median and standard deviation of particle diameters from the substrate measurements in addition to percent sands and fines to represent substrate composition. We also included the log relative bed stability, which is a ratio of the mean particle diameter divided by the critical particle diameter (Kauffmann et al. 2008). We included the fractional presence of seven fish cover types (based on observations on both banks at 11 transects): filamentous algae, macrophytes, boulders, live trees, large woody debris, overhanging vegetation, and undercut banks to describe habitat characteristics. Two other variables selected to describe habitat were percent of slow habitats (pools and glides) and volume of woody debris, normalized to reach length. For riparian vegetation, we included mean cover of the canopy (greater than five meters in height) and mean cover of all three vegetation strata, which includes canopy, mid layer, and ground layer. We also included the mean mid-channel canopy density from densiometer readings at each transect.

We selected three fish species as indicators for climate change and mineral development because they were commonly found in our stream sampling sites and have different habitat preferences: sculpin, coho salmon (*Oncorhynchus kisutch*), and Dolly Varden (*Salvelinus malma*). Sculpins are resident fish with established sensitivity to acidity and aluminum in streams (Baker et al. 1996, Kaeser and Sharpe 2001). Freshwater resident Dolly Varden are often found in small headwater streams. Coho salmon are anadromous, but juveniles rear in freshwater for one to three years before out-migrating to the ocean. Salmonids are also sensitive to copper toxicity in the low-hardness waters of Bristol Bay (McIntyre et al. 2008, Morris et al. 2019a, Morris et al. 2019b).

For the macroinvertebrate dataset, we utilized the Freshwater Biological Traits Database (Vieira et al. 2006) to explore taxa traits that indicate sensitivities to climate change and mineral development. Specifically, we linked the invertebrate genera to three traits: tolerances to temperature, pH, and pollution. For each genus, there were multiple entries in the database due to species-specific entries within a genus or entries from different data sources for the same species or genus. For numerical traits (e.g. pollution tolerance), we calculated the mean value across entries, and for categorical traits (e.g. thermal preference), we selected the mode, or category that was listed most frequently.

For the diatom dataset, we utilized the Algal Attributes database (Porter 2008), which classifies algal taxa by their physiological optima or tolerances to different water quality parameters. We linked diatom species to database entries, where available, and utilized tolerances to pH, specific conductance, and general pollution to identify indicator taxa most sensitive to climate change or mining development. We also identified taxa by their motility as an indicator of sensitivity to sedimentation.

2.6 Data analysis

We compared water quality results to two sets of standards, Alaska Water Quality Standards (AWQS) and the NOAA Screening Quick Reference Tables (Squirts). The aquatic life - freshwater use category was used for the AWQS. The median hardness across all 40 sites was used to calculate hardness-dependent standards (copper, nickel and zinc) for both AWQS and Squirts.

For each of the water quality, physical habitat, and species indicators, we used adjusted sample weights to estimate population parameters (means and standard deviations) for small streams, large streams, and all streams within our target population. We calculated the same parameters for our ten strategically-streams sampled near to the Pebble deposit and compared results.

3 RESULTS

3.1 Water chemistry

For several trace elements, the majority (> 50%) of results were below method detection limits and were not further summarized. This included dissolved and total silver, dissolved arsenic, dissolved and total beryllium, dissolved and total cadmium, dissolved and total cobalt, dissolved and total chromium, dissolved and total copper, total iron, total nickel, dissolved and total lead, dissolved and total selenium, total thorium, dissolved and total thallium, dissolved and total uranium, and dissolved zinc (Table 1).

Table 1: Number of sites with results below detection limits for 52 water quality parameters and 40 stream sampling sites. Laboratory detection limits are also provided.

Group	Parameter	Units	Detection limit	Count below detection limit
Major Ions	Ca	ug/L	154.98	0
Major Ions	Ca diss.	ug/L	1.31	0
Major Ions	K	ug/L	31.92	0
Major Ions	K diss.	ug/L	1.82	0
Major Ions	Mg	ug/L	37.11	0
Major Ions	Mg diss.	ug/L	0.65	0
Major Ions	Na	ug/L	100.00	0
Major Ions	Na diss.	ug/L	9.88	0
Metals	Ag	ug/L	5.30	40
Metals	Ag diss.	ug/L	1.26	40
Metals	Al	ug/L	3.74	2
Metals	Al diss.	ug/L	0.14	0
Metals	As	ug/L	0.14	10
Metals	As diss.	ug/L	1.60	34
Metals	Ba	ug/L	0.11	0
Metals	Ba diss.	ug/L	0.13	0
Metals	Be	ug/L	1.26	40
Metals	Be diss.	ug/L	1.74	40
Metals	Cd	ug/L	0.20	40
Metals	Cd diss.	ug/L	2.40	40
Metals	Co	ug/L	0.79	40
Metals	Co diss.	ug/L	0.18	38
Metals	Cr	ug/L	1.66	40
Metals	Cr diss.	ug/L	0.31	38
Metals	Cu	ug/L	0.31	25
Metals	Cu diss.	ug/L	0.36	40
Metals	Fe	ug/L	44.77	24
Metals	Fe diss.	ug/L	0.32	0
Metals	Mn	ug/L	0.73	13
Metals	Mn diss.	ug/L	0.05	0

Table 1 continued.

Group	Parameter	Units	Detection limit	Count below detection limit
Metals	Mo	ug/L	0.03	5
Metals	Ni	ug/L	0.47	38
Metals	Ni diss.	ug/L	0.11	7
Metals	Pb	ug/L	0.12	38
Metals	Pb diss.	ug/L	0.99	40
Metals	Sb	ug/L	0.15	0
Metals	Se	ug/L	0.69	39
Metals	Se diss.	ug/L	4.51	40
Metals	Si	ug/L	98.58	0
Metals	Th	ug/L	0.10	40
Metals	Tl	ug/L	0.12	40
Metals	Tl diss.	ug/L	1.21	40
Metals	U	ug/L	0.08	39
Metals	U diss.	ug/L	0.71	40
Metals	V	ug/L	0.32	18
Metals	V diss.	ug/L	0.16	10
Metals	Zn	ug/L	0.35	0
Metals	Zn diss.	ug/L	3.57	37
Nutrients	DIC	mg/L	0.50	0
Nutrients	DOC	mg/L	0.50	0
Nutrients	TN	mg/L	0.10	13

There were several exceedances of the acute and chronic AWQS for total aluminum, total copper, and total zinc. There were eight exceedances of the dissolved barium standard from the Squirts table (Table 2).

Table 2: Exceedances of water quality standards.

Parameter	Standard	Type	Number	Sites
Al	AWQS	chronic	2	AKBB-001, AKBB-028
Ba diss.	Squirts	chronic	14	AKBB-001, AKBB-003, AKBB-004, AKBB-010, AKBB-013, AKBB-017, AKBB-022, AKBB-024, AKBB-025, AKBB-030, AKBB-040, ILUTC37, MUTSK35, MUTSK36
Cu	AWQS	acute	2	AKBB-028, MUTSK36
Cu	AWQS	chronic	4	AKBB-007, AKBB-028, AKBB-032, MUTSK36
Zn	AWQS	acute	16	AKBB-001, AKBB-003, AKBB-005, AKBB-010, AKBB-011, AKBB-013, AKBB-017, AKBB-022, AKBB-029, AKBB-030, AKBB-032, AKBB-036, AKBB-041, AKBB-049, MUEKM23, MUTSK02
Zn	AWQS	chronic	16	AKBB-001, AKBB-003, AKBB-005, AKBB-010, AKBB-011, AKBB-013, AKBB-017, AKBB-022, AKBB-029, AKBB-030, AKBB-032, AKBB-036, AKBB-041, AKBB-049, MUEKM23, MUTSK02

Dissolved oxygen concentrations were high and pH values were circumneutral across all stream types: the strategically-selected streams close to the Pebble deposit, and small and large wadeable streams (Table 3). Across the entire population of wadeable streams, large streams tended to be warmer. The strategic streams were also warmer, which may be due to their lack of shade and lower elevation relative to other streams in the study area. Large streams had higher average specific conductance, but this parameter varied widely within stream types. All streams had low alkalinity indicating low buffering capacity.

Table 3: Summary statistics for water quality parameters for four different stream populations. Means are provided with standard deviation in parentheses.

Group	Parameter	Units	All streams	Small streams	Large streams	Strategic streams
In Situ Parameters	DO	mg/L	11.72 (1.22)	11.84 (1.15)	11.51 (1.31)	11.21 (0.86)
In Situ Parameters	pH	NA	7.31 (0.25)	7.27 (0.24)	7.37 (0.24)	7.19 (0.38)
In Situ Parameters	Specific cond.	µS/cm	59.7 (27.2)	56.33 (23.77)	65.64 (31.5)	56.64 (29.52)
In Situ Parameters	Temp.	°C	8.14 (3.96)	7.49 (3.09)	9.28 (4.93)	12.34 (4.21)
Metals	Al	ug/L	37.57 (44.9)	30.56 (29.66)	49.91 (61.46)	16.37 (11.87)
Metals	Al diss.	ug/L	16.51 (14.02)	17.87 (15.67)	14.11 (10.08)	10.81 (6.69)
Metals	Alkalinity	mg/L CaCo3	22.42 (10.21)	19.37 (9.05)	26.17 (10.31)	18.68 (11.26)
Metals	As	ug/L	0.59 (0.66)	0.34 (0.29)	1.01 (0.88)	0.33 (0.48)
Metals	Ba	ug/L	5.09 (6.27)	5.02 (7.52)	5.21 (2.99)	4 (2.45)
Metals	Ba diss.	ug/L	4.56 (5.71)	4.54 (6.82)	4.59 (2.88)	3.54 (2.27)
Metals	Ca	ug/L	7693.31 (3999.32)	7290.02 (3647.92)	8403.1 (4464.75)	6692.03 (3504.56)
Metals	Ca diss.	ug/L	6733.25 (3605.34)	6386.05 (3227.94)	7344.32 (4117.26)	5957.08 (3117.05)
Metals	Fe diss.	ug/L	96.31 (105.67)	96.93 (104.71)	95.21 (107.35)	105.36 (119.29)
Metals	K	ug/L	372.32 (217.28)	354.61 (249.33)	403.49 (139.19)	320.69 (160.11)
Metals	K diss.	ug/L	374.3 (219.11)	363.73 (253.34)	392.89 (137.87)	321.69 (162.91)
Metals	Mg	ug/L	1542.63 (839.43)	1419.89 (568.7)	1758.65 (1141.24)	1438.58 (1067.2)
Metals	Mg diss.	ug/L	1515.34 (849.47)	1409.55 (614.23)	1701.54 (1128.37)	1485.28 (1119.14)
Metals	Mn	ug/L	2.4 (3.86)	1.6 (1.82)	3.82 (5.68)	3.04 (3.48)
Metals	Mn diss.	ug/L	7.44 (8.81)	7.11 (8.95)	8.03 (8.54)	13.5 (16.99)
Metals	Mo	ug/L	0.23 (0.3)	0.17 (0.13)	0.32 (0.46)	0.63 (0.94)

Table 3 continued.

Group	Parameter	Units	All streams	Small streams	Large streams	Strategic streams
Metals	Na	ug/L	2317.01 (646.71)	2360.7 (739.41)	2240.11 (427.57)	2451.03 (918.41)
Metals	Na diss.	ug/L	2191.43 (612.37)	2241.88 (682.49)	2102.64 (450.37)	2413.7 (946.29)
Metals	Ni diss.	ug/L	0.2 (0.09)	0.21 (0.09)	0.18 (0.08)	0.17 (0.14)
Metals	Sb	ug/L	1.4 (0.28)	1.39 (0.19)	1.41 (0.38)	1.4 (0.32)
Metals	Si	ug/L	5077.42 (1495.29)	5182.45 (1563.83)	4892.55 (1346.56)	5269.96 (1460.5)
Metals	V	ug/L	0.47 (0.31)	0.44 (0.25)	0.51 (0.38)	0.41 (0.26)
Metals	V diss.	ug/L	0.31 (0.22)	0.34 (0.2)	0.25 (0.23)	0.35 (0.27)
Metals	Zn	ug/L	1149.71 (1672.76)	917.84 (1564.02)	1557.79 (1776.63)	282.36 (681.22)
Nutrients	DIC	mg/L	6.08 (2.04)	5.99 (1.89)	6.24 (2.27)	5.06 (2.73)
Nutrients	DOC	mg/L	3.93 (4.7)	4.88 (5.62)	2.26 (1.03)	2.01 (0.97)
Nutrients	TN	mg/L	0.21 (0.15)	0.21 (0.16)	0.21 (0.14)	0.14 (0.11)

3.2 Physical habitat

The ten strategic sites included three small streams (2nd order) and seven large streams (3rd and 4th order). Elevation of all sampled streams ranged from 34 to 770 m and there were no differences in mean elevations across large and small streams (Table 4). Mean watershed area was 6 km^2 for small streams and 42 km^2 for large streams. Only one sampled stream, the Chilchitna River, had a watershed area greater than 100 square kilometers (186 km^2). Both small streams and large streams had similar habitat characteristics and riparian vegetation cover. Small streams tended to be steeper and had smaller channel dimensions, leading to reduced depths, widths, ratios, and areas.

Table 4: Summary statistics for physical habitat metrics. Means are provided with standard deviation in parentheses. Details on metric calculations are provided in the methods.

Metric	Units	All streams	Small streams	Large streams	Strategic streams
Relative bed stability	unitless	-1.2 (0.8)	-1.3 (0.9)	-0.9 (0.5)	-0.8 (0.5)
Mid-channel canopy density	percent	30.8 (26.2)	33.5 (26)	26.1 (25.8)	8.7 (16.4)
Slow habitat	percent	46.4 (32.3)	45.6 (32.5)	47.8 (31.9)	46.2 (28.2)
Bankfull width to depth ratio	unitless	7 (3.3)	6 (2.8)	8.7 (3.5)	10.5 (5.7)
Standard deviation depth	m	12.8 (6.2)	11.1 (5.6)	15.7 (6.1)	13.7 (5.4)
Mean bankfull width	m	4.9 (3.6)	3.4 (2.1)	7.4 (4.3)	6.8 (4.6)
Mean depth	cm	34.6 (16.7)	28.4 (11.1)	45.4 (19.1)	34.4 (14)
Width x depth	m2	1.6 (2.2)	0.7 (0.6)	3.3 (2.9)	2.2 (2.1)
Algae	percent	0.2 (0.3)	0.1 (0.3)	0.3 (0.3)	0.3 (0.4)
Macrophytes	percent	0.8 (0.3)	0.9 (0.3)	0.7 (0.3)	0.8 (0.2)
Boulders	percent	0.9 (0.2)	0.9 (0.2)	0.8 (0.2)	0.8 (0.3)
Live trees	percent	0.5 (0.4)	0.5 (0.4)	0.4 (0.3)	0.5 (0.4)
Large woody debris	percent	0.1 (0.2)	0 (0.1)	0.3 (0.3)	0.1 (0.2)
Overhanging vegetation	percent	1 (0.1)	1 (0.1)	1 (0)	0.9 (0.1)
Undercut banks	percent	0.9 (0.2)	0.9 (0.1)	0.8 (0.3)	0.9 (0.1)
Woody debris volume	m	0.4 (0.9)	0.1 (0.3)	0.8 (1.2)	0.1 (0.1)
Pool density	cm	11 (8.6)	9.5 (8.1)	13.7 (8.8)	10.6 (4.6)
Pool count	count	14.9 (6.5)	16.8 (7.2)	11.7 (2.8)	12.9 (2.6)
Pool maximum depth	cm	51.6 (26.7)	47.5 (24.9)	59 (28)	54.6 (25.4)
Riparian canopy cover	percent	0.1 (0.1)	0 (0.1)	0.1 (0.1)	0 (0)
Riparian vegetation cover	percent	1.5 (0.3)	1.4 (0.3)	1.6 (0.3)	1.4 (0.3)
Sinuosity	unitless	1.3 (0.2)	1.3 (0.2)	1.4 (0.2)	1.4 (0.3)
Slope	percent	3.6 (2.9)	4.6 (3.3)	2 (0.8)	1.9 (0.8)

Table 4 continued.

Metric	Units	All streams	Small streams	Large streams	Strategic streams
Substrate D50	mm	1 (1.3)	0.8 (1.6)	1.4 (0.5)	1.2 (0.4)
Substrate standard deviation	percent	1.2 (0.3)	1.2 (0.4)	1.1 (0.2)	1 (0.2)
Sands and fines	percent	27.7 (22.2)	31.3 (25.2)	21.3 (13.2)	23.8 (14.3)
Elevation	meters	328.5 (206.4)	335.4 (222)	316.4 (174.9)	245.3 (74.3)
Watershed area	square kilometers	19.2 (31.8)	6.4 (10.5)	41.7 (42.3)	16.9 (15.8)

3.3 Fish

A total of 12 different freshwater fish were identified across 40 sites in 2015, which included juvenile sockeye and coho salmon (Table 5). The most common species were sculpin (80% of sites), Dolly Varden (62% of sites), and coho salmon (45% of sites).

Table 5: Frequencies of fish observations across 40 stream sampling sites.

Common Name	Scientific Name	Frequency
Alaska blackfish	*Dallia pectoralis*	4
Arctic grayling	*Thymallus arcticus*	2
Burbot	*Lota lota*	3
Coho salmon	*Oncorhynchus kisutch*	18
Dolly Varden	*Salvelinus malma*	25
Lamprey	Lamprey	4
Longnose sucker	*Catostomus catostomus*	1
Ninespine stickleback	*Pungitius pungitius*	6
Rainbow trout	*Oncorhynchus mykiss*	4
Sculpin	*Cottus* sp.	32
Sockeye salmon	*Oncorhynchus nerka*	3
Whitefish	*Coregonus* sp.	1

We used indicators of fish abundance and diversity at the 30 probabilistic sites to estimate population means and standard deviations across all sampled streams. Prior to estimation, fish counts were standardized by reach length and multiplied by 100 to account for differences in sampled length. Mean fish species richness across all sites was approximately three species and there were no differences across stream size. Sculpin had the highest densities across all streams, averaging approximately 30 fish per 100 m of stream reach, followed by coho salmon and Dolly Varden, which averaged 16 and 6 fish per 100 m, respectively (Table 6). All three species had similar densities across both stream sizes, although there was high variability within each stream size, indicating other stream attributes were driving habitat preferences.

Table 6: Summary statistics for fish species richness and mean densities for three fish taxa. Means are provided with standard deviation in parentheses.

Indicator	All streams	Small streams	Large streams	Strategic streams
Richness	2.7 (1.6)	2.7 (1.6)	2.6 (1.6)	3.2 (1.9)
Coho salmon	16.2 (38.5)	16.4 (38.8)	15.8 (37.9)	77.2 (169.5)
Dolly Varden	5.5 (7.6)	4.8 (5.8)	6.4 (9.7)	6.2 (7)
sculpin	29.7 (35.4)	26.8 (36.3)	34.2 (33.5)	42.2 (32.9)

3.4 Macroinvertebrates

A total of 120 genera were identified in the macroinvertebrate samples, 106 of which were insects. Of the remaining 14 genera identified, six were arachnids, two were amphipods, five were snails or clams, and one was a hydrozoan (Table 7). Insect diversity was spread across six different orders with dipterans exhibiting the highest diversity (70 genera), followed by Trichoptera (18), Plecoptera (11), Ephemeroptera (9), Coleoptera (3) and Collembola (1). The total number of unique taxa at each site ranged from 22 to 44 taxa.

Table 7: Macroinvertebrate diversity by family and genus for 40 stream sampling sites. Entries with zero families or genera could only be identified to a higher taxonomic classification.

Phylum	Class	Order	Number of families	Number of genera
Annelida	Hirudinea	NA	0	0
Annelida	Oligochaeta	NA	0	0
Arthropoda	Arachnida	Sarcoptiformes	0	0
Arthropoda	Arachnida	Trombidiformes	5	6
Arthropoda	Insecta	Coleoptera	2	2
Arthropoda	Insecta	Collembola	1	0
Arthropoda	Insecta	Diptera	8	69
Arthropoda	Insecta	Ephemeroptera	4	8
Arthropoda	Insecta	Plecoptera	5	10
Arthropoda	Insecta	Trichoptera	8	17
Arthropoda	Malacostraca	Amphipoda	2	2
Cnidaria	Hydrozoa	Anthoathecatae	1	1
Mollusca	Bivalvia	Pelecypoda	1	1
Mollusca	Gastropoda	Basommatophora	2	3
Mollusca	Gastropoda	Mesogastropoda	1	1
Platyhelminthes	Turbellaria	NA	0	0

Several genera that occurred in 50% or more of the sites indicated low tolerance to changes expected from climate or mineral development. Of the 106 insect genera in the dataset, 98 had listed pollution tolerance scores in the Traits database. Pollution tolerance scores are on a scale from 0 to 10, with 0 being extremely sensitive to pollution and 10 extremely tolerant. Specific taxa with low pollution tolerance (mean tolerance < 3) included the genera *Pagastia*, *Zapada*, *Dicranota*, *Cinygmula*, *Brachycentrus*, *Suwallia*, *Isoperla*, and *Rhyacophila* (Table 8).

Table 8: Pollution tolerance of macroinvertebrate genera in the macroinvertebrate traits database. Means and ranges were calculated across all entries in the database for each genus. Frequencies indicate the number of sites where that genus was observed out of 40 stream sampling sites.

Genus	Freq.	Mean value (range)
Baetis	38	4.42 (4-4.83)
Micropsectra	37	4.05 (1.1-7)
Eukiefferiella	36	5.7 (3.41-8)
Pagastia	36	1.4 (1-1.8)
Simulium	34	4.79 (4-5.58)
Corynoneura	32	6.5 (6-7)
Prosimulium	32	4.5 (4-5)
Tvetenia	32	4.33 (3.65-5)
Zapada	32	1.33 (1-2)
Cricotopus	31	5.97 (4.95-7)
Thienemanniella	31	6 (6-6)
Dicranota	30	2 (0-3)
Cinygmula	29	2 (0-4)
Onocosmoecus	28	3 (2-4)
Chelifera	27	6 (6-6)
Brachycentrus	25	0.71 (0-1.13)
Orthocladius	23	5.96 (5.93-6)
Suwallia	23	1.2 (1.2-1.2)
Isoperla	22	1.82 (1.65-2)
Parametriocnemus	22	4.35 (3.7-5)
Rhyacophila	22	1.37 (0.74-2)

Fifty-five of the 106 insect genera had pH preferences listed in the Traits database. Taxa classified as preferring alkaline, neutral, or alkaline-neutral conditions were considered potential indicators of acidity from mineral exploration. We excluded any taxa with records that indicated no strong preference for specific pH conditions. Indicator taxa for acidity included *Zapada*, *Brachycentrus*, and *Suwallia* (Table 9).

Table 9: pH preferences of macroinvertebrate genera in the macroinvertebrate traits database. Counts indicate the number of entries in the database by pH preference category and genus. Frequencies indicate the number of sites where that genus was observed out of 40 stream sampling sites.

Genus	Freq.	Acidic	Acid-Neutral	Neutral	Alkaline-Neutral	Alkaline	No strong preference
Baetis	38	0	2	0	10	1	2
Micropsectra	37	0	0	0	2	0	2
Eukiefferiella	36	0	0	0	0	0	2
Pagastia	36	0	1	0	0	0	0
Corynoneura	32	0	0	0	0	0	1
Zapada	32	0	0	0	4	0	0
Cricotopus	31	0	0	1	3	0	2
Thienemanniella	31	0	0	0	0	0	1
Onocosmoecus	28	0	0	0	0	0	1
Brachycentrus	25	0	0	0	2	0	0
Orthocladius	23	0	1	0	0	0	1
Suwallia	23	0	0	1	1	0	0
Isoperla	22	0	1	1	15	0	2
Parametriocnemus	22	0	0	0	0	0	1
Rhyacophila	22	1	6	2	7	1	19

In the Traits database, 71 insect genera had information on temperature preferences. Temperature classifications in the traits database ranged from cold to hot, in addition to no strong preference. Of the 16 genera that occurred at half or more of the sites with information on temperature preferences, only *Lebertia* and *Onocosmoecus* were listed as preferring exclusively cold or cold-cool thermal conditions (Table 10). For the remaining taxa, there were one or more records indicating no strong preference for specific thermal conditions or preference for warm or hot waters.

Table 10: Temperature preferences of macroinvertebrate genera in the macroinvertebrate traits database. Counts indicate the number of entries in the database by temperature preference category and genus. Frequencies indicate the number of sites where that genus was observed out of 40 stream sampling sites.

Genus	Freq.	Cold (<5 C)	Cold-cool (0-15 C)	Warm (15-30 C)	Hot (>30 C)	No strong preference
Baetis	38	0	9	2	0	14
Micropsectra	37	0	0	1	0	3
Eukiefferiella	36	0	2	1	0	0
Lebertia	35	0	1	0	0	0
Corynoneura	32	0	0	1	0	0
Zapada	32	0	4	0	0	2
Cricotopus	31	0	2	1	0	5
Thienemanniella	31	0	0	1	0	0
Cinygmula	29	1	5	0	0	1
Onocosmoecus	28	0	2	0	0	0
Brachycentrus	25	1	7	4	1	6
Orthocladius	23	0	0	1	0	1
Suwallia	23	0	1	0	0	3
Isoperla	22	0	2	1	0	22
Parametriocnemus	22	0	0	0	0	1
Rhyacophila	22	0	10	0	0	23

Our preliminary analysis of macroinvertebrate genera with traits indicating sensitivities to pH, pollution, or temperature resulted in ten indicator taxa. In addition to summarizing densities for these indicator taxa, we included species richness as an indicator for the macroinvertebrate community. Total genus richness was very similar across stream types and averaged 33 genera for all streams (Table 11). The indicator taxa that had the highest densities (> 50 organisms/m^2) included two that preferred small streams (*Lebertia* and *Zapada*), two that preferred large streams (*Cinygmula* and *Suwallia*), and two others that had high densities across all streams (*Brachycentrus* and *Pagastia*).

Table 11: Summary statistics for macroinvertebrate richness and indicator taxa densities. Means are provided with standard deviation in parentheses.

Indicator	All streams	Small streams	Large streams	Strategic streams
Richness	32.9 (5)	32.1 (4.6)	34.4 (5.2)	34.3 (5.9)
Brachycentrus	55.6 (86)	46.6 (67.8)	71.5 (109.2)	14.2 (17.4)
Cinygmula	92.1 (140.4)	38.1 (79.7)	187.1 (170.4)	59.7 (57.4)
Dicranota	13.3 (16.6)	10.3 (13.3)	18.5 (20.2)	24.9 (25)
Isoperla	5 (12.5)	3 (8.8)	8.5 (16.5)	7.9 (7.5)
Lebertia	82.1 (84.8)	105.4 (93.7)	41.1 (41.7)	94.7 (131.1)
Onocosmoecus	4.9 (11.3)	3.5 (5.8)	7.3 (16.8)	8.5 (13.6)
Pagastia	109.6 (129.7)	96.4 (132.8)	132.7 (120.6)	225.7 (227.2)
Rhyacophila	29.1 (47.9)	25 (37.5)	36.3 (61.4)	5.8 (13.6)
Suwallia	26.4 (44.5)	7.1 (19.6)	60.4 (54.6)	74 (100.2)
Zapada	286.9 (375.8)	352.2 (444.6)	172 (145.4)	85.2 (111.9)

3.5 Diatoms

A total of 312 diatom species were identified across all 40 sites sampled in 2015. Species richness across all sites varied from 24 to 61. Approximately 40% (125/312) of identified diatom taxa had information on pH tolerances in the USGS Algal Attributes database. Diatom autecological preferences for different pH levels were classified from 1 to 6: 1 and 2 are species that prefer acidic conditions, 3 through 5 are species that prefer circumneutral to alkaline conditions, and 6 are diatoms with a wide tolerance to different pH. We identified diatom species classified as 3 through 5 as sensitive to acidic conditions that could result from mineral development. Of the 19 species that occurred in at least half of the sites and had pH information in the database, the two most common species were indifferent to pH conditions, two species preferred slightly acidic conditions, and the remaining 15 species preferred circumneutral to alkaline conditions and could be used as indicators for changes in pH (Table 12).

Table 12: pH preferences of diatom species in the algal attributes database. Frequencies indicate the number of sites where each diatom was observed out of 40 stream sampling sites.

Taxon	Freq.	pH preference
Achnanthidium minutissimum (Kützing) Czarnecki	40	Indifferent
Aulacoseira alpigena (Grunow) Krammer	36	Indifferent
Staurosirella pinnata (Ehrenberg) Williams et Round	36	Alkaliphilous
Encyonema silesiacum (Bleisch) Mann	33	Circumneutral
Diatoma mesodon (Ehrenberg) Kützing	32	Circumneutral
Eucocconeis laevis (Østrup) Lange-Bertalot	31	Circumneutral
Gomphonema micropus Kützing	31	Alkaliphilous
Reimeria sinuata (Gregory) Kociolek et Stoermer	30	Circumneutral
Fragilaria capucina Desmazieres	26	Circumneutral
Ulnaria ulna (Nitzsch) Compére	26	Alkaliphilous
Hannaea arcus (Ehrenberg) Patrick	25	Alkaliphilous
Planothidium lanceolatum (Brébisson) Lange-Bertalot	24	Alkaliphilous
Nitzschia perminuta (Grunow) Peragallo	23	Alkaliphilous
Eunotia minor (Kützing) Grunow	22	Acidophilous
Rossithidium petersennii (Hustedt) Round et Bukhtiyarova	22	Circumneutral
Diatoma tenuis Agardh	21	Alkaliphilous
Meridion circulare (Greville) Agardh	21	Alkaliphilous
Gomphonema parvulum (Kützing) Kützing	20	Circumneutral
Psammothidium subatomoides (Hustedt) Bukhtiyarova et Round	20	Acidophilous

Twenty-nine of the 312 identified diatom species have documented specific conductance optima and were classified as preferring either high (> 500 uS) or low (< 200) specific conductance. Three common diatom species had documented specific conductance optima and all preferred low specific conductance and could be used as indicators for potential water quality impacts from mineral development (Table 13).

Table 13: Specific conductance preferences of diatom species in the algal attributes database. Frequencies indicate the number of sites where each diatom was observed out of 40 stream sampling sites.

Taxon	Freq.	Specific conductance preference
Diatoma mesodon (Ehrenberg) Kützing	32	Low
Fragilaria capucina Desmazieres	26	Low
Eunotia minor (Kützing) Grunow	22	Low

Pollution tolerance is documented for 33% (96/312) of identified diatom taxa in the USGS Algal Attributes database. Diatom autecological tolerances to pollution were classified as 1 - most tolerant, 2 - less tolerant, or 3 - sensitive to pollution. We identified diatom species classified as sensitive to pollution as indicators of water quality impacts that could result from mineral exploration. Of the 16 species that occurred in at least half of the sites and with known pollution tolerances, 11 species were classified as sensitive (Table 14).

Table 14: Pollution tolerances of diatom species in the algal attributes database. Frequencies indicate the number of sites where each diatom was observed out of 40 stream sampling sites.

Taxon	Freq.	Pollution tolerance
Achnanthidium minutissimum (Kützing) Czarnecki	40	Sensitive
Aulacoseira alpigena (Grunow) Krammer	36	Sensitive
Staurosirella pinnata (Ehrenberg) Williams et Round	36	Sensitive
Encyonema silesiacum (Bleisch) Mann	33	Sensitive
Diatoma mesodon (Ehrenberg) Kützing	32	Sensitive
Eucocconeis laevis (Østrup) Lange-Bertalot	31	Sensitive
Reimeria sinuata (Gregory) Kociolek et Stoermer	30	Sensitive
Fragilaria capucina Desmazieres	26	Less tolerant
Ulnaria ulna (Nitzsch) Compére	26	Less tolerant
Planothidium lanceolatum (Brébisson) Lange-Bertalot	24	Less tolerant
Nitzschia perminuta (Grunow) Peragallo	23	Sensitive
Rossithidium petersennii (Hustedt) Round et Bukhtiyarova	22	Sensitive
Diatoma tenuis Agardh	21	Less tolerant
Meridion circulare (Greville) Agardh	21	Sensitive
Gomphonema parvulum (Kützing) Kützing	20	Most tolerant
Psammothidium subatomoides (Hustedt) Bukhtiyarova et Round	20	Sensitive

Motility (ability to move) is describe for over half of all diatom species documented for this project. Of the 24 species that occurred in half or more of all sites, 20 are considered non-motile, making them sensitive to sedimentation (Table 15).

Table 15: Motility of diatom species in the algal attributes database. Frequencies indicate the number of sites where each diatom was observed out of 40 stream sampling sites.

Taxon	Freq.	Motility
Achnanthidium minutissimum (Kützing) Czarnecki	40	Non-motile
Aulacoseira alpigena (Grunow) Krammer	36	Non-motile
Staurosirella pinnata (Ehrenberg) Williams et Round	36	Non-motile
Encyonema silesiacum (Bleisch) Mann	33	Non-motile
Diatoma mesodon (Ehrenberg) Kützing	32	Non-motile
Tabellaria flocculosa (strain IV) sensu Koppen	32	Non-motile
Eucocconeis laevis (Østrup) Lange-Bertalot	31	Non-motile
Gomphonema micropus Kützing	31	Non-motile
Reimeria sinuata (Gregory) Kociolek et Stoermer	30	Non-motile
Fragilaria capucina Desmazieres	26	Non-motile
Ulnaria ulna (Nitzsch) Compére	26	Non-motile
Hannaea arcus (Ehrenberg) Patrick	25	Non-motile
Planothidium lanceolatum (Brébisson) Lange-Bertalot	24	Non-motile
Nitzschia perminuta (Grunow) Peragallo	23	Non-motile
Eunotia minor (Kützing) Grunow	22	Non-motile
Rossithidium petersennii (Hustedt) Round et Bukhtiyarova	22	Non-motile
Diatoma tenuis Agardh	21	Non-motile
Meridion circulare (Greville) Agardh	21	Non-motile
Gomphonema parvulum (Kützing) Kützing	20	Non-motile
Psammothidium subatomoides (Hustedt) Bukhtiyarova et Round	20	Non-motile

Our preliminary analysis of diatom species with sensitivities to pH, specific conductance, pollution, or sedimentation resulted in 21 indicator taxa. In addition to summarizing relative abundances for these indicator taxa, we included species richness as an indicator for the diatom community. Total diatom species richness was similar across small and large streams and averaged 44 species (Table 16). Species richness was lower at the strategically-selected sites near to the Pebble deposit and averaged 35 species. Species relative abundances were generally low (< 10%), although the most common diatom (*Achnanthidium minutissimum*) had mean relative abundance of 17% and another diatom found at 21 sites (*Staurosirella pinnata*) had a mean relative abundance of 11%.

Table 16 Summary statistics for diatom indicator taxa relative abundances (%). Means are provided with standard deviation in parentheses.

Indicator	All streams	Small streams	Large streams	Strategic streams
Richness	43.6 (9.3)	44.8 (8.9)	41.4 (9.7)	34.9 (8.3)
Achnanthidium minutissimum (Kützing) Czarnecki	17 (16.5)	14.9 (16.7)	20.8 (15.5)	19.2 (21.8)
Aulacoseira alpigena (Grunow) Krammer	3 (3.3)	3.3 (3.8)	2.5 (2.2)	1.1 (1.4)
Diatoma mesodon (Ehrenberg) Kützing	4.2 (4.9)	3.7 (4)	5 (6.1)	3 (6.5)
Diatoma tenuis Agardh	1.1 (2.4)	0.8 (1.5)	1.6 (3.5)	1.3 (2.4)
Encyonema silesiacum (Bleisch) Mann	3 (5.7)	0.9 (1.4)	6.8 (7.9)	2.4 (3)
Eucocconeis laevis (Østrup) Lange-Bertalot	1.2 (1.3)	1 (0.8)	1.5 (1.7)	0.8 (1.1)
Eunotia minor (Kützing) Grunow	1.5 (3.9)	2.2 (4.8)	0.3 (0.4)	0.6 (0.9)
Fragilaria capucina Desmazieres	1.1 (1.6)	1 (1.3)	1.3 (2)	2.4 (3.1)
Gomphonema micropus Kützing	3 (4.3)	1.9 (3.2)	4.8 (5.3)	2.2 (3.6)
Gomphonema parvulum (Kützing) Kützing	0.8 (1.7)	0.5 (1)	1.3 (2.4)	2.9 (7.2)
Hannaea arcus (Ehrenberg) Patrick	2.1 (4.9)	2.1 (5.8)	2.2 (2.5)	2.7 (3.1)
Meridion circulare (Greville) Agardh	1.2 (4.4)	0.3 (0.8)	2.8 (6.9)	0.6 (0.7)
Nitzschia perminuta (Grunow) Peragallo	0.6 (0.8)	0.6 (0.9)	0.4 (0.4)	0.5 (0.6)
Planothidium lanceolatum (Brébisson) Lange-Bertalot	2.2 (3.6)	2.9 (4.2)	0.9 (1)	2.6 (6.2)
Psammothidium subatomoides (Hustedt) Bukhtiyarova et Round	0.6 (0.7)	0.8 (0.8)	0.2 (0.3)	0.4 (0.5)
Reimeria sinuata (Gregory) Kociolek et Stoermer	1.8 (3.4)	1 (0.9)	3.3 (5.1)	2.3 (1.7)
Rossithidium petersennii (Hustedt) Round et Bukhtiyarova	1.1 (1.7)	1.2 (1.8)	0.8 (1.4)	0.3 (0.4)
Staurosirella pinnata (Ehrenberg) Williams et Round	10.6 (11)	13.3 (11.6)	5.8 (7.8)	4.6 (5.8)
Tabellaria flocculosa (strain IV) sensu Koppen	0.8 (0.7)	0.8 (0.8)	0.6 (0.6)	1.4 (1.7)
Ulnaria ulna (Nitzsch) Compére	0.8 (1.4)	0.4 (0.7)	1.5 (1.9)	1 (1.1)

4 CONCLUSIONS AND FUTURE WORK

Baseline monitoring is an urgent need for streams of the Nushagak and Kvichak watersheds due to rising temperatures and changing precipitation patterns from climate change in addition to current mineral exploration and potential mineral development. Monitoring conducted in 2015 resulted in water chemistry concentrations for 56 parameters, physical habitat measurements used to calculate over 300 metrics, and documentation of 12 freshwater fish species, 120 macroinvertebrate genera, and over 300 diatom species. Most importantly, this is the first probabilistic dataset for the region, from which we generated unbiased estimates for 31 water chemistry parameters, 28 physical habitat metrics, and 34 indicator taxa.

Our results showed that wadeable streams in the Lime Hills ecoregion are cold and pristine with very low concentrations of nutrients or trace metals, except in a few cases. Some metal concentrations exceeded criteria for aquatic life, most likely due to mineral deposits in the study area. Streams have extremely low alkalinity, indicating poor buffering capacity and high susceptibility of salmonids to low levels of copper and other potentially toxic metals (Morris et al. 2019a). Stream habitats are diverse and include deep, low-gradient streams with organic substrates and grasses and shrubs along the shoreline; small, steep streams with cobble and boulder substrates and thick shrub cover providing extensive shade; and wide shallow streams with gravel substrates and a mixture of grass, shrub, and tree cover at lower elevations. Fish diversity was low, but streams provided valuable habitat for sculpin, Dolly Varden, and coho salmon, which were frequently found throughout the study area. Both macroinvertebrate and diatom diversity was high and several taxa indicated low tolerance to pollution, acidity, sedimentation, specific conductance, and rising temperatures, making them useful indicators for both mineral development and climate change.

We are continuing this work by analyzing inter-annual variation in water chemistry, physical habitat, and biological indicators using ten sites sampled every summer through 2019. Results from the complete analysis will be submitted to a peer-reviewed journal for publication. All final datasets, analyses, and publications will be publicly shared on the ACCS data catalog.

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