

# Long Term Monitoring Plan for Wadeable Streams, Lime Hills Ecoregion, Kvichak and Nushagak River Watersheds

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14 March 2014



## ACKNOWLEDGEMENTS

The Bristol Bay Regional Seafood Development Association, The Bristol Bay Heritage Land Trust, The U. S. Fish and Wildlife Service National Fish Habitat Initiative and the Southwest Alaska Salmon Habitat Partnership provided funding for this project. Dr. Robert Hughes and Krista Bartz provided constructive critical reviews of the manuscript.

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# Long Term Monitoring of Salmon Ecosystems: a plan for headwater streams, Lime Hills ecoregion, Bristol Bay, Alaska

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## Introduction

Bristol Bay Alaska produces the world's largest most valuable all-wild salmon runs, averaging 38.7 million fish annually (20-year average 1991-2010; Jones et al. 2012) with an economic value of \$1.5 billion (2010 estimate, Knapp et al. 2013). About half the world's sockeye salmon supply comes from Bristol Bay, where salmon represent both economic and food security (Duffield et al. 2007, Jones et al. 2012). Salmon provide diverse ecosystem services and serve as an indicator of ecosystem health, as well as being culturally, recreationally, and nutritionally important to humans (Holmlund and Hammer 1999). Salmon are considered a keystone species (Willson and Halupka 1995, Levi et al. 2013); their abundance can influence productivity and distribution of more than 130 species including plants, fish, birds and mammals (Willson and Halupka 1995, Cedarholm et al. 2000).

Since the last glaciation about 10,000 years ago, Alaskan salmon successfully colonized and adapted to diverse natal habitats, including a range of water chemistry, temperature and other environmental parameters (Taylor 1991, Carlson and Seamons 2008, Woody et al. 2000, Fraser et al. 2011). Now hundreds of genetically and behaviorally distinct salmon spawning populations comprise Alaska's wild salmon runs (Hilborn et al. 2003, Ramstad et al. 2009, Jones et al. 2012, Woody *submitted*). Future fisheries sustainability is linked to conservation of wild salmon biodiversity, which enhances salmon evolutionary potential (Hilborn et al. 2003, Gustafson et al. 2007, Hutchinson 2008, Schindler et al. 2010). Studies of Bristol Bay salmon populations show that combined production is

remarkably stable over time because minor salmon producers under one climate regime became major producers under another, and vice versa (Hilborn et al. 2003). A meta-analysis indicates this reliable productivity is due, in part, to a “portfolio effect” whereby high biodiversity dampens annual variation in production, similar to earnings from a diverse financial portfolio (Schindler et al. 2010). High salmon biodiversity in Bristol Bay has thus far been conserved, as essential salmon habitats remain unaltered, sustainable fisheries practices are implemented and hatcheries are non-existent (Hilborn et al. 2003, Schindler et al. 2010). However, two factors have raised concern regarding long-term sustainability of salmon ecosystems in the region: proposed mineral development and climate change (Figure 1).

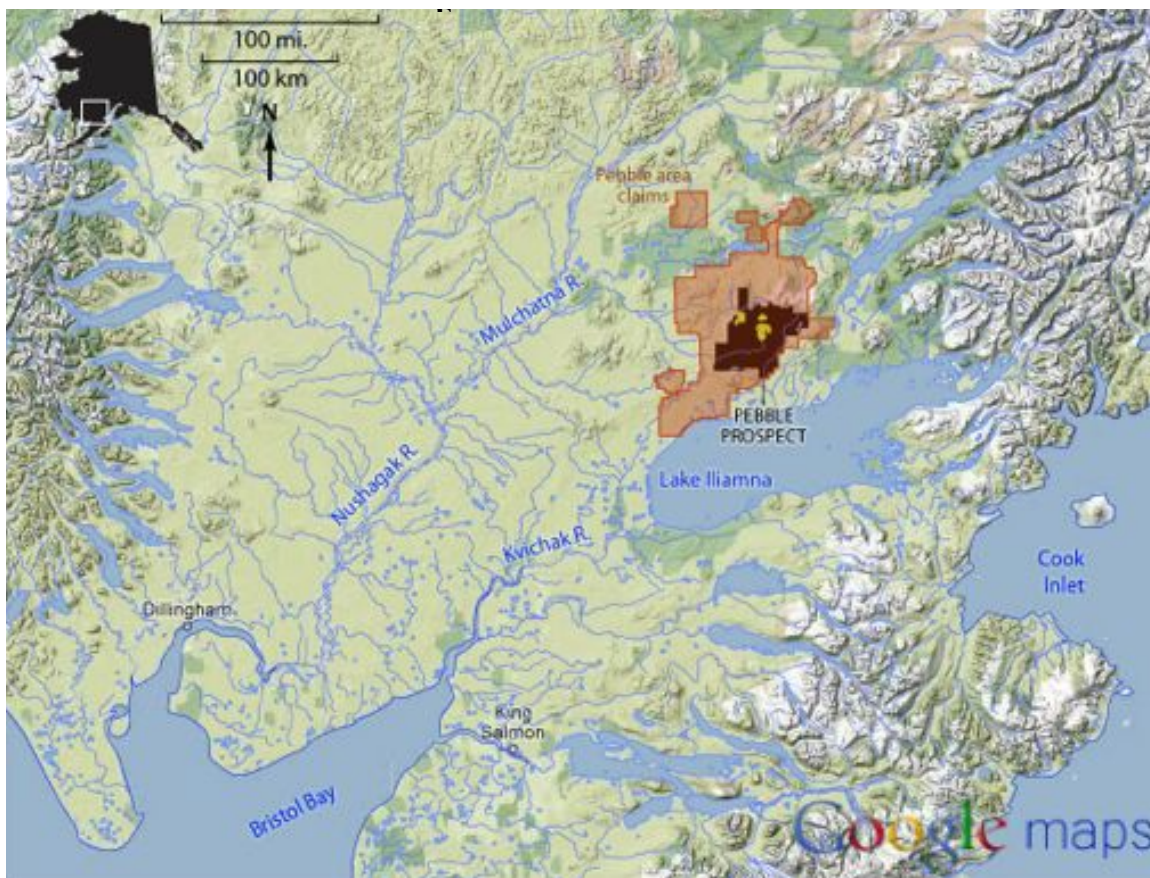


Figure 1. Bristol Bay, Alaska with detail of the Nushagak and Kvichak River watersheds. A potential State mining district (red) and a mineral prospect near permitting, Pebble, indicated.

Mineral interest and exploration in southwest Alaska accelerated the last decade (Szumigala 2012). In Bristol Bay, about 2000 km<sup>2</sup> of mine claims are now staked in a



potential hard-rock mining district that straddles a watershed divide and headwaters of the prolific Nushagak and Kvichak River drainages (Figure 2). These two river systems drain about half the Bristol Bay watershed and produce about half of Bristol Bay salmon (Jones et al. 2012). Headwater habitats in this region are currently intact, undisturbed and provide essential spawning and rearing habitat to salmon and other important subsistence fish species (Woody and O’Neal 2010, ADFG 2013). Headwaters are also critically important to downstream ecosystems, sustaining the productivity, function, and biocomplexity of navigable systems (Haig et al. 1998, Naiman et al. 1987, Wipfli and Gregovich 2002, Wipfli et al. 2007); they also comprise up to 80% of a stream network or watershed area (Naiman 1983, Benda et al. 2005).

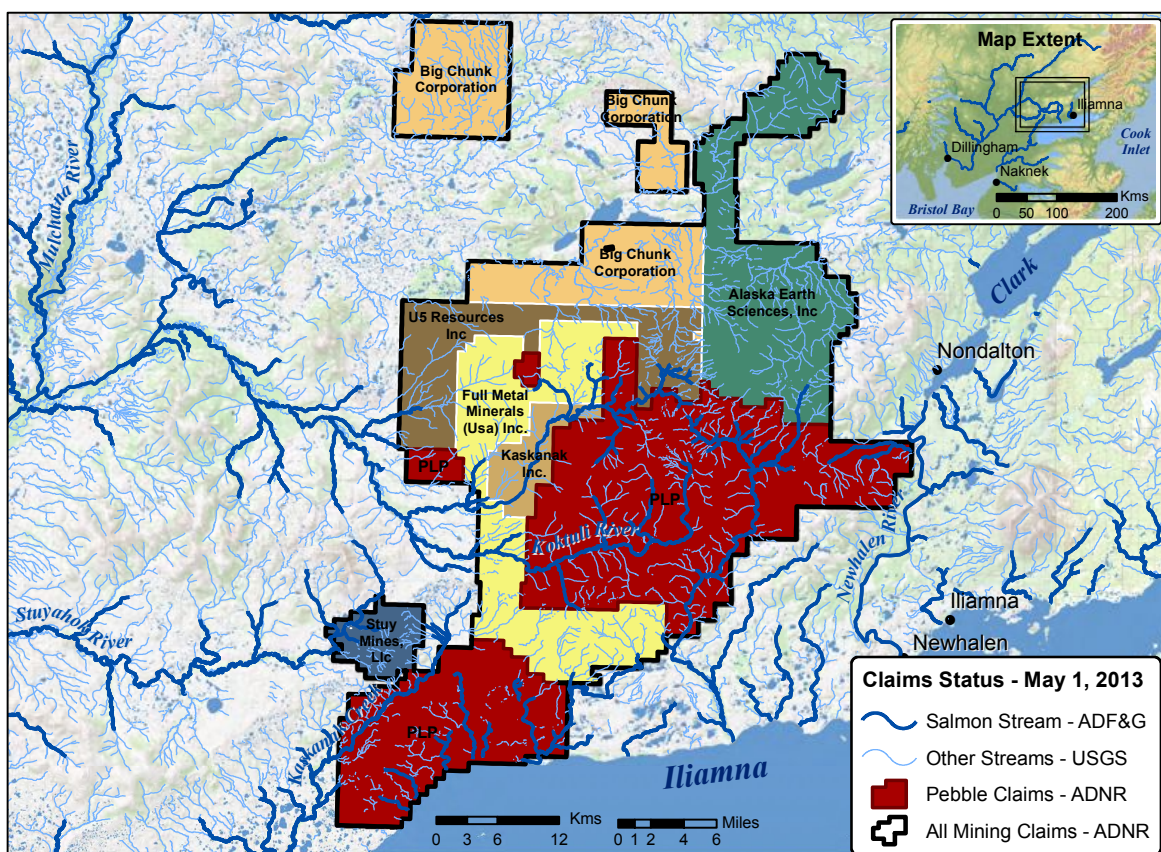


Figure 2. Mineral claims and known salmon streams in the proposed Bristol Bay mining district.

Several mineral deposits (Schmidt et al. 2007) are under exploration near the Alaska Native villages of Nondalton, Newhalen and Iliamna, including Pebble and Big Chunk (Figure 2). Due to their geology, mining of these deposits has potential to significantly alter area water chemistry. Specifically, porphyry copper mining (see Pebble, Figure 2;

Ghaffari et al. 2011, AKDNR 2013) poses Acid Rock Drainage (ARD) risks to Bristol Bay salmon systems. When large quantities of porphyry ore containing sulfide minerals (e.g. pyrite) are mined, then exposed to air and water they react to form acid; if the acid-generating capacity of the ore and waste exceeds the environment's acid-neutralizing potential, then acid and toxic metals can be mobilized into area waters (Ripley et al. 1996, EMBC 1997, Jimenez et al. 2009). ARD is highly acidic (pH ~2.5; EPA 2013), contains elevated metals, and has high specific conductance. ARD can contaminate both surface runoff and groundwater and can exceed tolerance thresholds of aquatic biota (Eisler 2000, Schowe et al. 2013), eliminating many species of algae, invertebrates, and fishes (Niyogi et al. 2002, Portner and Farrell 2008, Issak et al. 2010, USEPA 2013). ARD from mining can last decades, centuries or even thousands of years (Davis et al. 2000) and impacts can travel from the source downstream for great distances (EMCBC 1998).

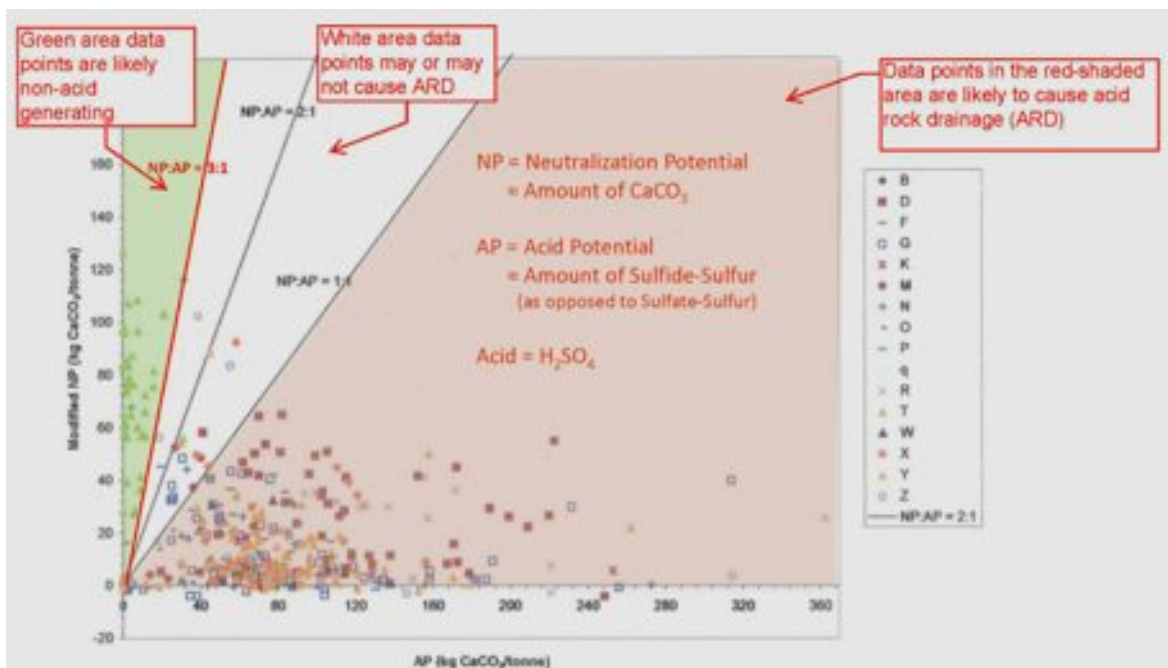


Figure 3. Acid generating potential (AP) of 399 samples from the Pebble West deposit, 1988-2003 (NDM 2005).

Geochemical studies of one Bristol Bay prospect indicate a relatively high potential for ARD if development proceeds (Figure 3; NDM 2005) while water quality studies show low acid buffering capacity and low concentrations of substances that ameliorate the toxicity of metals (Zamzow 2011). Considered in light of the region's highly permeable soils (Figure 4), extensive interchange between ground and surface waters, and inter-

watershed hydrologic exchange (Smith and McCreadie 2008), mining-altered water chemistry poses a risk to conservation of salmon ecosystem function, thus salmon biodiversity and sustainability.

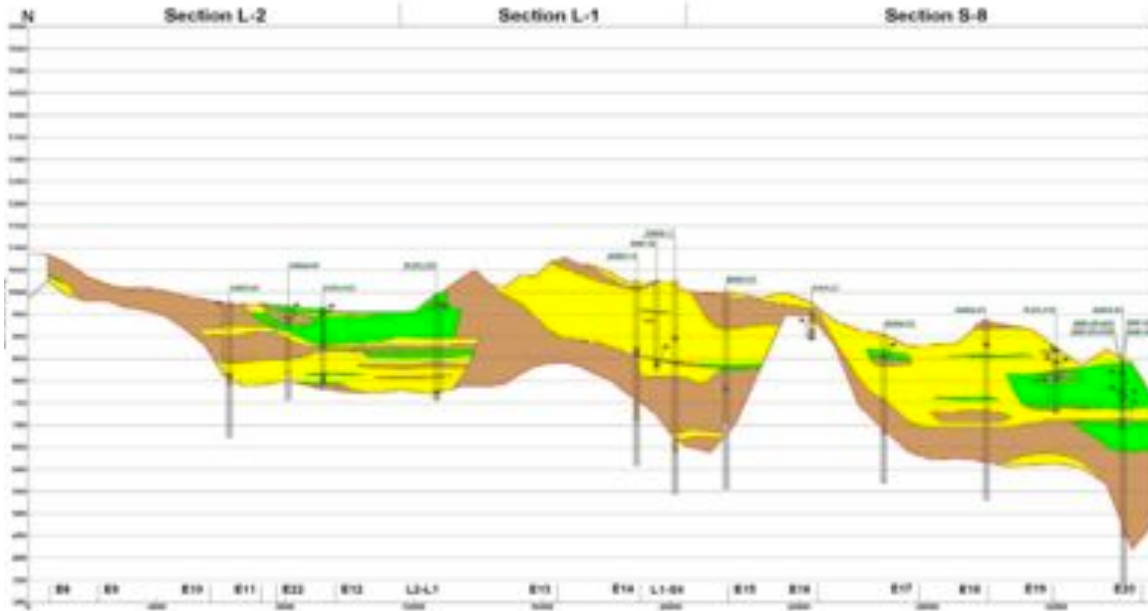


Figure 4. Hydrogeologic cross section from the Pebble prospect illustrating soil horizon complexity, and potential ARD flow paths (yellow). Colors represent different sediment layers with: brown=sand/silty sand; green=clay/silty clay; yellow=gravel/gravelly sand. Graphic from: Smith and McCreadie (2008).

Climate change is another factor that can impact salmon and the food webs that support them. Climate change predictions for coastal Alaskan watersheds indicate an average 1°C increase per decade and a 25-50% increase in precipitation by 2079-2099 (Maurer et al. 2007, IPCC 2007, Walsh et al. 2008). Through its profound effect on their physiology, temperature is a key determinant in the distribution and abundance of salmon and species that comprise their food chains, such as other fish, macroinvertebrates, and diatoms (Lessard and Hayes 2002, Parmesan and Yohe 2003, Perry et al. 2005, Daufresne et al. 2009). Universal ecological responses from warming include range shifts toward higher latitudes and altitudes as species' thermal tolerances are exceeded, as well as seasonal shifts in life-cycle events (Farrell et al. 2008, Daufresne et al. 2009, O'Gorman et al. 2012). Many freshwater species are limited in their dispersal ability and thus vulnerable to extirpation (Woodward et al. 2010). Warmer water also boosts the metabolic rates of fish and macroinvertebrates, increasing the amount of food required

for essential metabolic functions. This diminishes the amount of energy that can be allocated to growth when food is limited, but increases potential growth rates when food is abundant (Gibbons 1976, Wootton 1995). A decrease in body size with increasing temperature has been observed for some fish and macroinvertebrate species, although diatoms may be an exception (Larocque and Bigler 2004, Daufresne et al. 2009, Adams et al. 2013). Smaller body size of organisms comprising freshwater foodwebs has ecological implications for life histories, nutrient turnover, demography and trophic structure, through disruption of ecosystem function and services (Edeline et al. 2013). How Bristol Bay salmon and their major food chain components (diatoms, macroinvertebrates) may change is unclear as baseline and monitoring programs for change are inadequate.

Long term monitoring of freshwater biological communities will allow us to track the quality of the aquatic environment and detect changes in community structure associated with impacts from mining, climate change, and other perturbations (Barbour et al. 1999, Paulsen et al. 2008, Rosenberg et al. 2008). Biota integrate effects of their physical and chemical environment over time, including stressors such as toxic elements and increased temperature and, therefore, offer information on perturbations not always captured by "snap shot" water chemistry measurements or discrete toxicity tests. 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 (i.e., diatoms, macroinvertebrates, fish) can enhance the ability to detect and diagnose ecological impairment (Karr and Chu 1999), and most currently use multiple biological assemblages for water quality monitoring (USEPA 2002a). Diatoms (Figure 5) are relatively sedentary primary producers with very short life cycles (multiple generations/summer), which respond quickly to physical and chemical impacts. A considerable body of research has established diatom species optima for nutrients and trophic status (Van Dam et al. 1994) as well as diatom tolerance to acidification (Van Dam et al. 1994), organic pollution (Lange-Bertalot 1979, Palmer 1969), and sedimentation (Stevenson and Bahls 1999); these attributes can be quantified to detect general environmental and to diagnose specific causes of environmental impairment (Karr 1993).

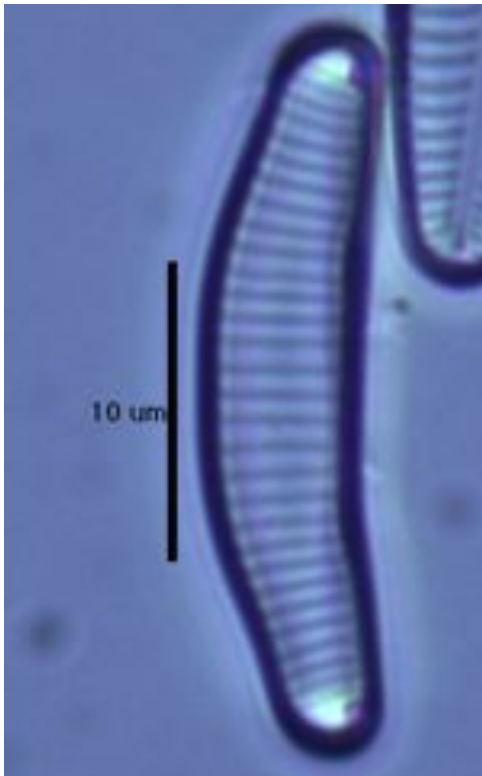


Figure 5. Diatoms, single-celled algae with silica cell walls, are useful biomonitors as they are sensitive to environmental degradation and can be diagnostic of causal factors. Species from the genus *Eunotia* (pictured) occur in higher abundance in acidic versus pH neutral habitats.

Macroinvertebrates are the most commonly used assemblage in aquatic monitoring (USEPA 2002a). They are relatively mobile, have relatively long life cycles (1-2 years) and demonstrated sensitivity to changes in ecological condition (Resh and Jackson 1993). They are considered good indicators of local conditions because they are diverse, even within functional groups, and show a wide range of tolerance to physical and chemical stressors (Barbour et al. 1999, Sloane and Norris 2003). Fish assemblages are also commonly used as bioindicators, they are highly mobile and are generally longer lived than most invertebrates; they can be better indicators of historic and chronic stressors and stressors that have regional impacts, such as habitat fragmentation (Karr 1981, Barbour et al. 1999, Townsend et al. 2003).

### Study Goals

The goals of our aquatic Long Term Monitoring (LTM) program are to characterize reference conditions, defined as the expected “normal” current condition of unimpaired streams, to use as a benchmark for detecting future changes in key indicators of 1<sup>st</sup> through 3<sup>rd</sup> order headwater streams of the Nushagak and Kvichak Rivers. The suite of indicators to be measured were chosen to detect and/or illuminate important changes in aquatic habitats expected to potentially arise due to (i) impacts from mining development or from (ii) climate change. This program will improve baseline characterization of these indicators by expanding on our previous targeted monitoring efforts in the region (Woody

and O’Neal 2010, Zamzow et al. 2011, Bogan et al. 2012a, 2012b, Rinella et al. in prep.) both in terms of providing a statistically sound basis for inference to the broader region and improving resolution in space and time.

The program will aid regulatory or re-active resource management decision making by increasing years of data for a more precise characterization of current conditions thus increasing statistical power to detect future changes, such as development impacts. The program’s suite of indicators were chosen to help identify specific pathways of insult should mine development proceed and thus illuminate the underlying source(s) of impact.

The program will aid future management of the region’s aquatic resources characterizing any systematic changes in these fundamental system features during a period when climate change is expected to increasingly impact basic hydrologic inputs and processes (Maurer et al. 2007, Walsh et al. 2008, IPCC 2007). The program’s suite of indicators will capture and reveal changes in the relationships between basic habitat features (pH, specific conductance, temperature, physical habitat) and assemblages of primary and secondary producers (diatoms, macroinvertebrates and fish).

### **Study Site**

In this study we will focus on a single ecoregion to both minimize the amount of climatic, geologic, and biological variability within a large area and increase our ability to detect changes associated with anthropogenic impacts (Hughes et al. 1994, Stoddard 2005). Our focus will be the Lime Hills ecoregion (Figure 6) of southwestern Alaska, just west of the Alaska Range (Nowacki et al. 2001). Repeated glacial advances sculpted the eastern portion of this ecoregion into tall rugged mountains and the western portion into gently rolling hills and horseshoe shaped valleys. Glacial moraines and alluvial deposits occur in river valleys; permafrost occurs in isolated masses. The region experiences a maritime climate, annually averaging from 56-70 cm precipitation and -3 to 0° C (Nowacki et al. 2001). Dominant vegetation consists of spruce, cottonwood and birch forests along river

valleys and lower mountains, with mixed shrub communities of willow, alder and birch below 1,200 m (Nowacki et al. 2001).



Figure 6. The study area (green) was defined by selecting USGS watersheds (10-digit HUCs) in the Lime Hills ecoregion draining to Bristol Bay. Existing 5 long term monitoring sites shown (dots).

## Sample Design

### Target population and Sample Frame

Our target population is wadeable streams that support Pacific salmon in the Bristol Bay drainage of the Lime Hills ecoregion (Figure 6). We have restricted our target population to wadeable streams for several reasons: non-wadeable streams are difficult to sample and introduce safety and logistical concerns, sampling the entire stream network would require a much larger sampling effort to capture all of the variation, and wadeable streams will be directly susceptible to impacts from mining and climate change. We will use spatial datasets in ArcGIS to create a sample frame that best approximates our target population. First- through third-order streams will be selected from the National Hydrography Dataset flow lines because we expect that larger systems will not be

wadeable. In addition, we will exclude streams above 10% gradient because Pacific salmon do not typically occupy habitats steeper than this (Bryant et al. 2004, King et al. 2012). We will stratify the NHD based on stream order so that we select roughly equal numbers of 1<sup>st</sup>-, 2<sup>nd</sup>-, and 3<sup>rd</sup>-order stream reaches. In total, our study area drains approximately 15,600 km<sup>2</sup> and includes a potential State mining district that encompasses approximately 2,000 km<sup>2</sup>.

### Sample Selection

EPA recommends the use of generalized random tessellation stratified (GRTS) survey designs over simple random sampling of aquatic resources because it selects sample sites that are spatially balanced across a study area (Stevens and Olsen 2004). GRTS survey designs have other benefits such as allowing for variable inclusion probabilities based on sampling strata and accommodating non-target or inaccessible points while maintaining a spatially balanced sample. EPA has produced an R library (spsurvey, Kincaid and Olsen 2013) that provides functions for design and analysis of probabilistic surveys of aquatic resources.

Once the sampling frame is developed in ArcGIS, we will use the spsurvey library in R to produce 150 sample sites using a GRTS survey design. The GRTS design will select all 150 of the sites at once and place them in order to achieve spatial balance. The 150 sites will cover our initial goal of sampling 30 sites in 2014 and provide oversample sites to use when a site cannot be sampled (e.g., very dense alder, etc.) or does not meet our target population definition. In addition, it will give us the opportunity to increase our sample size in the future, should additional sample sites be indicated by analysis. Based on previous sampling of wadeable streams in the Bristol Bay region, we expect that 25% of our streams may not meet our target population definition (Woody and O'Neal 2010). In order to be cost effective in our use of helicopter time, we will logistically plan to visit the first 38 sites in the GRTS sample in order to achieve a spatially balanced target of 30 sites. If more than 8 sites are not sampleable, we will visit additional oversample sites in order to achieve our target of 30 sites.



The oversample sites will be used when the reach associated with the sample site either is non-sampleable during the field visit or does not meet the target population definition. The sample site is located at the midpoint of the sample reach, which is defined as 40 stream widths or 150 m, whichever is greater. In some cases, temporary conditions may make the site non-sampleable, such as bear activity or high water flows. Depending upon the remaining time for sampling within our index period, these sites may be sampled at a later date. When the sample site does not meet our target population definition (i.e. > 50% of the reach is unwadeable or stream gradient > 10%), we will either shift the stream reach or move to an oversample site. The stream reach can be shifted upstream or downstream as long as the sample site remains within the stream reach, which places a maximum shift of ½ of the total reach length.

All field sampling will be conducted during the same index period every year. We have defined our index period to begin after spring snowmelt once streams have returned to baseflow conditions and to end prior to the return of adult salmon, since spawning disrupts stream substrates and the associated macroinvertebrate and diatom communities. This typically results in an index period that spans the month of June. When there is flexibility in our planning, we will monitor snowmelt and the associated peak discharge in gaged streams in the ecoregion (i.e. Koktuli River), and plan to begin sampling after stream levels have dropped.

We currently have data on macroinvertebrate and diatom communities from five sites sampled annually since 2008 (although only two sites were sampled in 2012). We plan to select 10 additional sites from our probabilistic sample and sample all 15 yearly through 2018 (Table 1). This will provide us with 11 years of data for five strategically placed sites downstream of mining claims and five years of data for 10 probabilistic sites located throughout our study area. We will also sample five select naturally mineralized sites to compare water quality, species richness and abundance with our five long-term non-mineralized sites.

## Sampling Schedule

The 30 randomly selected sites will be sampled during our index period (June) of 2014 for the entire suite of biological, physical, and chemical parameters following methods described below, along with the 5 sites that have been sampled annually since 2008, and 5 naturally mineralized sites for a total of 40 sites. We plan to resample our 20 long-term monitoring sites (5 strategic sites, 5 naturally mineralized sites and 10 probabilistic sites) for an additional four years (2015-2018) to capture inter-annual variation in water quality, physical habitat, and aquatic biological communities. In 2015, we plan to collect continuous data for water quality parameters at the 20 long-term monitoring sites between June and September to measure diurnal and seasonal variation in temperature, pH, and specific conductance. Additional probabilistic sampling will be dependent on funding availability.

**Table 1. June field sampling schedule, 2014-2018. Water quality sondes and HOBO thermistors will be deployed in June and retrieved prior to freeze-up. WQ = water quality (pH, DO, specific conductance); H = habitat, D = diatoms, M = macroinvertebrates, F = fish.**

	2014	2015	2016	2017	2018
30 probabilistic sampling sites	WQ, H, D, M, F				
10 inter-annual probabilistic sampling sites	WQ, H, D, M, F, HOBO	F, M, D, H, WQ sondes, HOBO	F, M, D, H, WQ, HOBO	F, M, D, H, WQ, HOBO	F, M, D, H, WQ, HOBO
5 established LTM sites in mining region	WQ, H, D, M, F, HOBO	F, M, D, H, WQ sondes, HOBO	F, M, D, H, WQ, HOBO	F, M, D, H, WQ, HOBO	F, M, D, H, WQ, HOBO
5 naturally mineralized sites	WQ, H, D, M, F, HOBO	F, M, D, H, WQ, HOBO	F, M, D, H, WQ, HOBO	F, M, D, H, WQ, HOBO	F, M, D, H, WQ, HOBO

## Measurement Methods

We have selected chemical, physical, and biological indicators to measure for wadeable streams that are likely to respond to changes in habitat and water quality associated with mineral development or climate change (Table 2).

**Table 2. Chemical, physical and biotic indicators to be used in data analysis.**

Measurement group	Indicator
Water quality: pH, dissolved oxygen, specific conductance	Daily average, monthly average
Water quality: temperature	Mean July temperature, maximum weekly average temperature, maximum weekly maximum temperature, cumulative degree days
Physical habitat	Channel stability, substrate size distribution
Diatoms	Diatom relative abundance by taxa, richness, $\alpha$ and $\beta$ diversity, persistence and stability; periphyton chlorophyll a and biomass
Macroinvertebrates	Relative abundance by taxa, richness, $\alpha$ and $\beta$ diversity, EPT taxa, persistence and stability
Fish	Relative abundance by species and age class, size at age, $\alpha$ and $\beta$ diversity, changes in abundance or presence by species and age class

### Water Quality

Water quality surveys in the region to date indicate surface waters are: circumneutral, of low specific conductance (median  $\leq 50 \mu\text{s}/\text{cm}$ ), low alkalinity (median  $\leq 30 \text{ mg}/\text{L CaCO}_3$ ), low hardness (median 11-15  $\text{CaCO}_3$  equivalent), with low dissolved organic carbon, and generally saturated  $\text{O}_2$  (Woody and O’Neal 2010, Zamzow 2011, Bogan et al. 2012a, 2012b, Rinella in prep.). We will continue to collect point measurements of water physicochemical parameters (pH, specific conductance, total dissolved solids, and dissolved oxygen) *in situ* at each of 40 surveyed sites in June using a Hydrolab Minisonde 5 multiprobe that is calibrated daily.

Relative to potential ARD should mining proceed, both sulfate and specific conductance are useful indicators of acid mine drainage as they are extremely sensitive to ARD even under high dilution. Specific conductance is very sensitive to sulfate ions. Therefore, as sulfate analysis in the field is difficult, specific conductance is easily measured and can be used as an indicator of sulfate concentration in both ARD and naturally mineralized surface waters (Cormier et al. 2013). Specific conductance can also serve as an indicator of heavy metal contamination, and can potentially be used to predict approximate

concentrations of key metals when water pH is within their respective solubility ranges (Gray 1996). We will test utility of remote sondes (pH, specific conductance) to continuously monitor during low flow by deploying a set at one easily accessible LTM site near the village of Nondalton in 2014. If we can successfully gather this information, we will expand ice-free continuous monitoring to our 20 LTM sites in 2015. Continuous monitoring will provide estimates of diurnal, monthly and seasonal variation in these key parameters.

Relative to climate change, a better understanding of current range of regional surface temperature variation (diurnal, monthly, seasonal) within and among years will be derived by deploying three remote temperature monitors (HOBOS, Onset Computer Corp.) at the 15 LTM sites during June through August 2014. Data will be downloaded prior to freezing and monitors redeployed to collect winter data. We will continuously monitor surface water temperature annually at all 20 LTM sites for the next 5 years.

### **Physical Habitat**

Physical habitat characterization will follow USEPA's Wadeable Streams Assessment protocols (USEPA 2004a), with some modifications to streamline field data collection as described below. Habitat characterization will focus on a stream reach with a length of 40 times the average wetted width, with a minimum reach length of 150 meters. We will subdivide the reach by marking 11 evenly spaced stream transects. At five points along each transect we will record water depth, substrate size class, and substrate embeddedness. Additionally, we will record substrate size at 5 points across the stream channel between each of the 11 transects, for a total of 21 transects and 105 substrate particles. Also at each transect we will measure wetted and bankfull channel dimensions, measure riparian canopy coverage, and the areal coverage of different types of fish cover (e.g., macrophytes, woody debris, overhanging vegetation, undercut banks). At the center transect in each reach we will measure stream discharge using the velocity-area method and a Marsh-McBirney flow meter. Between each of the 11 transects we will count pieces of large woody debris within and above the stream channel according to

several size classes. We will also measure the compass azimuth (aspect) and channel slope between each pair of transects to characterize channel slope and sinuosity. The above activities correspond to specific sections of USEPA's (2004) field data sheets. We will replace USEPA's (2004a) transect-based characterization of riparian vegetation and human influence with systematic photographic approach. We will take (and archive) 4 geo-referenced digital photos from each of the 11 habitat transects, one facing each upstream, downstream, and toward each stream. In addition, we will assign a Viereck level-3 vegetation classification (Viereck et al. 1992) to both banks of each stream reach

### Periphyton

Periphyton sampling will follow USEPA's Ecological Monitoring and Assessment Program protocols (and Peck et al. 2006, section 9). These samples will be used to characterize diatom community structure and, when funding permits, chlorophyll-a concentration and periphyton biomass. Periphyton samples will be taken from the streambed 1 m downstream of locations where each of the 11 macroinvertebrate subsamples is collected. In most cases samples will be taken from coarse substrates (i.e., rocks, wood) by removing the substrate from the stream, scrubbing a delimited area of 12 cm<sup>2</sup> with a hard-bristled toothbrush (using a new toothbrush at each stream), and rinsing the scrubbed material into a sample bottle. At any transects where coarse substrates are not available, we will use a syringe to vacuum the periphyton layer from a delimited area of 12 cm<sup>2</sup>. We will combine each of the 11 diatom sub-samples into a single composite sample, preserve them in the field with Lugol's solution, and return them to UAA's Aquatic Ecology lab for processing. In the lab, we will homogenize each sample and transfer them to 20-ml beakers. We will add nitric acid and heat to digest the diatom protoplasm and other organic material, thereby clearing the diatom valves for easier identification. We will then neutralize the acid-digested aliquots by a succession of dilutions, concentrate the cleared diatom valves by allowing them to settle, and slide mount the valves using NAPHRAX mounting medium. For each sample site, we will identify a fixed count of 600 diatom valves to species or lowest practical taxonomic level and then scan the slide to record any new taxa not discovered in the fixed count. Our primary taxonomic references will be Krammer and Lange-Bertalot (1986-1991) and

Patrick and Reimer (1975). For each chlorophyll-a and biomass analysis (when conducted), we will concentrate periphyton by filtering a 25-ml aliquot of the composite sample through a glass-fiber filter and retaining the filter for analysis (see Peck et al. 2006). Laboratory analysis of periphyton chlorophyll-a and biomass will follow standard methods (APHA 2012).

### Macroinvertebrate sampling

Our macroinvertebrate sampling and laboratory processing will also follow U.S. EPA's Wadeable Streams Assessment protocols (USEPA 2004a, b). We will collect one macroinvertebrate sub-sample at each of the 11 habitat transects using a 500- $\mu$ m-mesh D-frame net, alternatively sampling from near the left bank, the center of the stream, and near the right bank. We will collect samples by disturbing an area of streambed approximately 0.09 m<sup>2</sup> (1 ft<sup>2</sup>) for 30 seconds and rubbing each cobble and boulder by hand to ensure all macroinvertebrates were dislodged and swept into the net by the stream's current. We will combine each of the 11 macroinvertebrate sub-samples into a single composite sample, preserve them in the field with ethanol, and return them to UAA's Aquatic Ecology lab for processing. In the lab, we will subsample each macroinvertebrate sample to obtain a fixed count of 500  $\pm$ 20% organisms to standardize the taxonomic effort across all sites. In addition, we will conduct a five minute search through the remaining sample to select any large or rare taxa that may have been missed during subsampling. We will identify 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 (Wiederholm 1983, Smith 2001, Merritt et al. 2008, Wiggins 1996, Thorpe and Covich 2001, Stewart and Oswood 2006).

\*Our physical habitat activities correspond to the following sections on U.S. EPA's field data sheets (USEPA 2004): the "substrate cross-sectional information," "bank measurements," "fish cover/other," and "canopy cover measurements" sections of the Channel/Riparian Cross-Section Form; the "substrate" and "large woody debris" sections of the Thalweg Profile & Woody Debris Form, and the complete Slope and Bearing Form. We will replace the "visual riparian estimates" section of the Channel/Riparian Cross-

Section Form and omit the “thalweg profile” section of the Thalweg Profile & Woody Debris Form.

### **Fish Sampling**

Fish sampling will follow modified EMAP protocols (McCormick and Hughes 1998). Water conductivity will be measured and appropriate backpacker electro-fisher settings adjusted. Crews will fish upstream, discontinuously sampling all habitat types. Sampling duration will be from one to two hours depending on site characteristics; electro-fishing will be a minimum of 30 min. The objectives are to collect: 1) a representative fish assemblage sample, except for very rare species, 2) an unbiased measure of species proportional abundance, 3) relative abundance (# fish/electrofishing time) and 4) size and age data. Fish will be captured, identified, measured, weighed then scale or otolith samples collected for aging. All fish will be released after sampling. Voucher specimens will be collected for any unknown species.

### **Data Analysis**

The primary goal of establishing this monitoring program in the Bristol Bay region is to characterize current conditions of stream habitats and aquatic biota, including their spatial and inter-annual variability, so that we can detect trends associated with future mineral development or climate change. From our field data we will calculate a suite of indicators that quantify key aspects of physical, chemical and biological conditions in the region’s wadeable streams (Table x). There are several components of variation in ecological indicators that affect ability to detect trends on reasonable time scales (10-20 years): spatial variation across sites, inter-annual variation within a site, the interaction between spatial and inter-annual variation and residual variation (Larsen et al. 1995, Urquhart et al. 1998). Our first five years of data will provide us with an initial understanding of the important covariates driving spatial and inter-annual variation in our indicators, which will maximize our ability to detect changes over longer time scales.

## Analytical Objectives

1. *Baseline characterization.* We will provide unbiased estimates of indicator status and spatial and inter-annual variation across the study area. Indicators will be summarized for the 30 probabilistic sample sites and also for each of the long-term monitoring sites over the five-year period.
2. *Identify sensitive taxa and develop habitat suitability models.* We will use existing data sets (Woody and O'Neal 2010, Zamzow 2011, Bogan et al. 2012) to identify aquatic taxa that are sensitive to changes in water quality and physical habitat associated with mining and climate change and will develop habitat suitability models so that disappearance of these sensitive taxa from otherwise suitable habitats can be detected. The probabilistic sites will be used to assess performance of habitat suitability models.
3. *Investigate covariates to explain spatial and inter-annual variability.* Accounting for naturally-occurring environmental factors that contribute to variation in indicator values will enhance our ability to detect trends associated with mining and climate impacts. For the 30 probabilistic sites, we will examine local habitat variables that can be used to explain spatial variability for each indicator. Using existing datasets from five strategic sites for six years (2008-2013) we will explore effects of climate and habitat on inter-annual variation of macroinvertebrate and diatom communities. At end of five-year monitoring period (2018), we will conduct a combined analysis of covariates to explain spatial and inter-annual variation for all 15 sites. In addition, we will look for trends at five strategically placed sites given 11 years of monitoring and established covariates that can be used to reduce spatial and inter-annual variability.
4. *Evaluate monitoring indicators and refine sample design.* We will evaluate indicators for continued long-term monitoring based on their trend detection capability and consider alternative sampling designs that can be used to monitor both trends and status of wadeable streams in the future.

*Baseline Characterization.* For each indicator, we will calculate mean, median, standard deviation, and other parameters to represent the average condition and variability across



our population of wadeable streams using the 30 probabilistic sites. Graphical representations of averages and variability will also be presented using boxplots, cumulative distribution functions, or other plots. In order to characterize the current inter-annual variability for each indicator, we will calculate the same statistical parameters and present the same plots for each of our 10 probabilistic long-term monitoring sites.

*Identify sensitive taxa and develop habitat suitability models.* We will analyze existing macroinvertebrate and diatom datasets from this ecoregion to determine if any are sensitive to thresholds in the chemical or physical indicators associated with changes in mineral development or climate. These include, but are not limited to pH, sediment and temperature. It will be important to identify suitable habitat for our sensitive taxa so that we can identify distributional shifts or disappearances in the future due to changes in mineral development or climate.

In order to develop predictive models of habitat for sensitive taxa, we will require landscape predictors developed in a GIS framework. Landscape attributes that we expect are strongly related to reach-scale habitat conditions include topography, vegetation cover, surficial geology and climate. We will collect the best available source datasets and calculate a suite of predictors at both the site and landscape scale (e.g. stream reach gradient and average watershed slope). Random Forests will be used to develop predictive models for sensitive taxa in our study area based on its proven prediction accuracy and ability to handle interactions, non-linearity, spatial autocorrelation and other issues that are difficult to address in ecological modeling (Cutler et al. 2007, Evans et al. 2011). Either presence-absence (classification) or relative abundance (regression) may be modeled and performance evaluated using percent correctly classified or mean squared error, respectively. Our best test of model performance will be based on predictions for our 30 probabilistic sites and comparisons with field data. This information will help us determine if the models based on existing data accurately characterize suitable habitats within our wadeable streams target population. If

prediction accuracy for the probabilistic sites is poor, we will consider additional data collection from our oversample sites to increase our sample size and develop more accurate habitat suitability models. Final models will be used to predict and map suitable habitat for each sensitive taxa in our study area using GIS.

*Investigate covariates to explain spatial and inter-annual variability.* We will investigate habitat or landscape variables that can be used to explain spatial variability in our indicators from our 30 probabilistic sites using multiple regression analyses. Results from modeling spatial variability will be used to identify important spatial covariates that will inform a mixed model, which will be the foundation for detecting trends in the future (see below).

Based on previous work in Denali National Park, we expect that inter-annual variability in stream taxa presence or absence and relative abundance varies due to both climate and local habitat (Milner et al. 2006). We will conduct a preliminary analysis into factors affecting inter-annual variability using our existing dataset of five strategic sites with six years of data. Regional climate datasets will be collected for stream discharge (USGS), air temperature (Alaska Climate Data Center, ACDC), and winter snowpack (ACDC) to help explain coherent inter-annual variation. Habitat variables such as stream gradient, substrate size distribution, and channel stability will be calculated and also used as covariates. We will use a mixed model analysis for our macroinvertebrate and diatom indicators with climate and habitat predictors as fixed effects and repeated visits to the sites as a random effect.

At the end of our five-year baseline, we will build upon this initial analysis by analyzing a larger number of sites (15), examining fish communities and including predictors for spatial variability. These models will provide a current baseline of factors affecting variability across our wadeable stream habitats from year to year and can be used to detect trends in our indicators as we move forward. We can test the ability of our models

to detect trends by exploring data collected at the five strategically placed sites downstream of the State mining district, which will have 11 years of data by 2018.

*Evaluate monitoring indicators and refine sample design.* The ability to detect trends over time depends upon the amount of unexplained variation for each monitoring indicator. Our analyses will help us characterize which indicators will have high trend detection capability versus others, which may have too much noise to be useful for long-term monitoring. In addition, our investigation into sources of variability for our indicators will help us determine the best sampling design for long-term monitoring beyond our five year baseline. It will be important to consider alternative designs that include visits to new sites in order to monitor changes across the entire population of wadeable streams in our study area (Larsen et al. 1995).

All data analysis will be run using the R statistical platform (Team 2012) and several add-on packages, such as *vegan* (Oksanen et al. 2012), *randomForest* (Liaw and Wiener 2002) *raster* (Hijmans 2013), *nlme* (Pinheiro et al. 2009) and *gam* (Hastie 2013).

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