Baseline Biological Surveys in Wadeable Streams of the Kvichak and Nushagak Watersheds, Bristol Bay, Alaska

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Table of Contents

Tables

Figures

Appendices

Introduction

In May 2008, we began collecting an independent set of baseline biological data in wadeable streams of the Nushagak and Kvichak watersheds, focusing our efforts in and around the area's extensive mineral claims. In this effort we have sampled benthic macroinvertebrates and diatoms from a total of 78 streams over three years (2008, 2009, and 2010, Figures 1 and 2) during May and June. We have sampled five of these streams repeatedly (2008 – 2011) to evaluate interannual variation in biological communities (Figures 1 and 2). We also measured water quality in-situ and characterized stream physical habitat as covariates to help explain patterns in the biological data. This report describes the field and lab methods and the results of preliminary analyses relating biological communities to environmental gradients in Nushagak and Kvichak wadeable streams.

The primary purpose of monitoring biological communities is to track the quality of the aquatic environment (Barbour et al. 1999, Paulsen et al. 2008, Rosenberg et al. 2008). Biota integrate the effects of their physical and chemical environment over time, including stressors such as nutrient enrichment, toxic chemicals, increased temperature, and sedimentation and, therefore, offer information on perturbation not always possible with "snap shot" water chemistry measurements or discrete toxicity tests. For example, a survey of biological condition in wadeable streams throughout the 48 contiguous United States found that 28% of the stream length was in good condition, 25% was in fair condition, 42% was in poor condition, and that nutrient enrichment and sedimentation were the leading causes of biological degradation (Paulsen et al. 2008).

The importance of diatoms and macroinvertebrates in monitoring biological condition is eclipsed by their importance in aquatic food webs. Diatoms are photosynthetic and nutritious and vast numbers of them live in the slippery biofilm layer that covers streambeds. They are an important energy source in streams, especially in settings where an abundance of sunlight reaches the stream channel (Mulholland et al. 2001) as in the treeless uplands of the Bristol Bay region. Along with leaves and other organic matter that falls into streams, diatoms in the biofilm layer are major food sources for the macroinvertebrate community (Wallace and Webster 1996) which, in turn, is a major food source for juvenile salmonids rearing in streams (Nielsen 1992).

The Aquatic Ecology Program at the University of Alaska Anchorage's Alaska Natural Heritage Program (AKNHP) has been involved in biological monitoring of aquatic resources in Alaska for over 15 years. We developed standard operating procedures (SOPs) based on the U.S. Environmental Protection Agency's (USEPA) Rapid Bioassessment Protocols (Barbour et al. 1999) for use in Alaska's wadeable streams (Major and Barbour 2001). The SOPs include macroinvertebrate and diatom sampling and measurements of water quality and instream and riparian habitat. We have previously used these SOPs to guide the calibration of a macroinvertebrate biological assessment index for the Alexander Archipelago ecoregion (i.e., southeast Alaska; Rinella et al. 2005) and a macroinvertebrate and diatom index for the Cook Inlet Basin ecoregion (Rinella and Bogan 2007).

The main objective of this work is to characterize baseline habitat and biological conditions, including spatial and temporal patterns of variability, so that any future impacts from resource development or climate change can be recognized. In order to accomplish this objective, the following tasks have been completed and are summarized in this report:

1. Describe the physical and chemical characteristics of wadeable streams in the Nushagak and Kvichak watersheds.

2. Describe the macroinvertebrate and diatom communities in wadeable streams of the Nushagak and Kvichak watersheds.
3. Evaluate the dominant environmental gradients driving differences in stream biological community composition.
4. Describe the interannual variability in stream biological community composition for the five streams sampled over four years.
5. Discuss the ongoing development of RIVPACS models that could be used to predict the invertebrate and diatom community composition expected to occur at any given site. The biological integrity stream sites can be assessed by comparing the expected community to that actually observed in biological samples.

Figure 1. Sampling sites in the Nushagak and Kvichak watersheds.

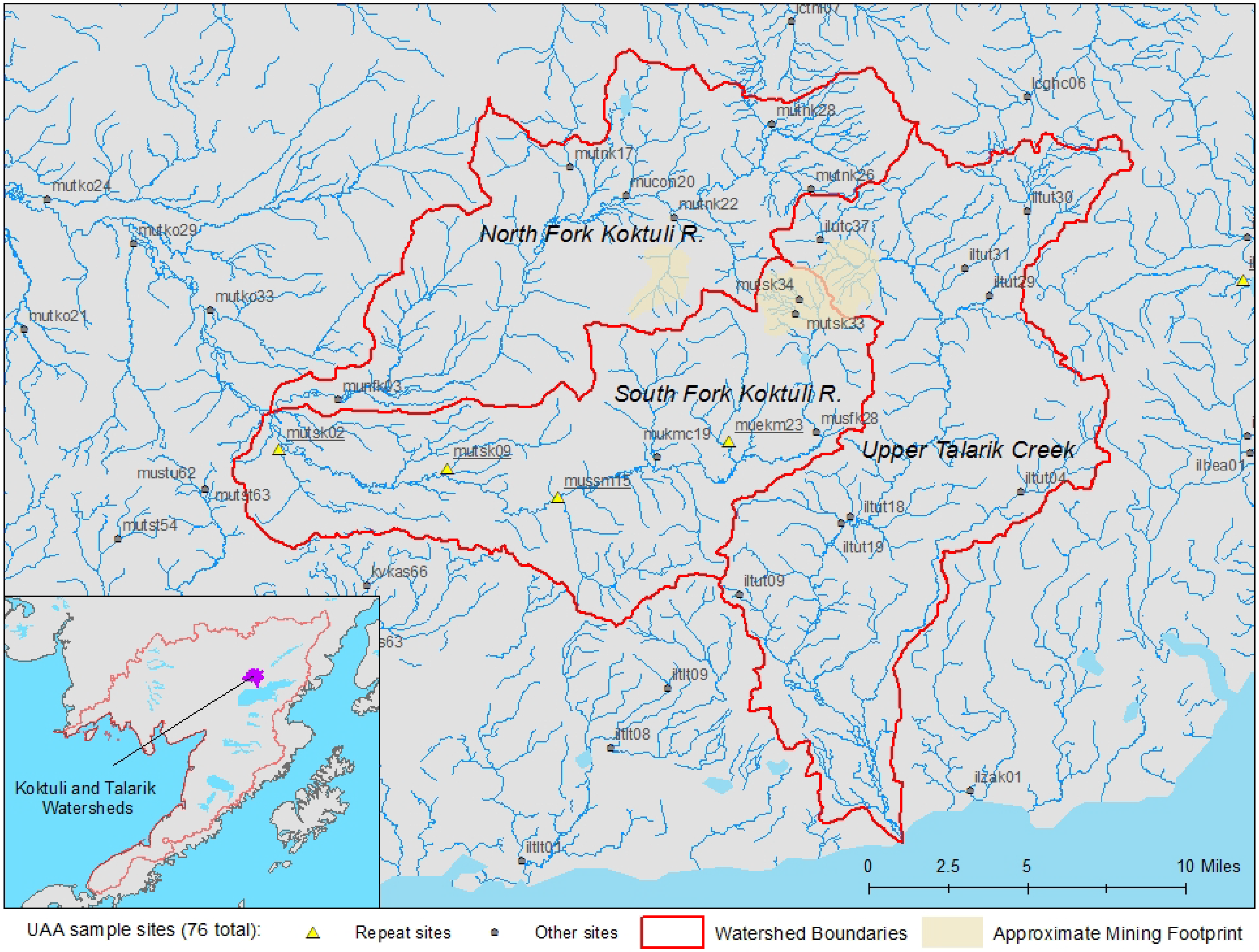

Figure 2. Subset of sampling sites in the Koktuli River and Upper Talarik Creek watersheds.

Methods

Study Area and Site Selection

The 78 sample sites are located within four major sub-watersheds delineated by USGS 8-digit Hydrologic Unit Codes (Upper Nushagak, Mulchatna, Lake Clark, and Lake Iliamna). In total, these sub-watersheds drain an area of approximately 50,000 km^2 and include the proposed Pebble Mine area, which is currently undergoing advanced exploration of copper, gold, and molybdenum deposits. The exploration sits about twenty miles west of the village of Nondalton, and is surrounded by additional mining claims on State of Alaska land, which comprise approximately 22% of the total area in the four sub-watersheds (Alaska Department of Natural Resources data).

Prior to each field season we selected sampling sites on wadeable steams in the Nushagak and Kvichak watersheds opportunistically based on the target sampling population and logistical considerations. Streams within our target population met the following criteria: 1500 feet (457 m) or less in elevation, hard-bottomed substrates, and wadeable along at least half of the stream reach length. We accessed streams by road, helicopter, float plane, power boat, raft, and hiking.

Water Quality and Physical Habitat

All water quality parameters were collected in-situ with a Hydrolab MiniSonde 4a multiprobe that was calibrated daily. These included temperature, dissolved oxygen, specific conductance, and pH.

Each sampling site consisted of a stream reach with a length of 150 meters or 40 times the average stream wetted width, whichever was greater. We subdivided each reach with 11 evenly spaced transects and measured the following at each: channel wetted width, bankfull width, the width of any mid channel bars, bank height, bank angle, and bank undercut distance. We measured riparian canopy coverage with six densiometer readings along each transect. We recorded water depth, substrate size class, and embeddedness at five points along each transect. Additionally, we recorded substrate size at midpoints between each of the 11 transects, for a total of 21 transects and 105 measurements. To characterize fish cover we estimated the extent of filamentous algae, macrophytes, big and small woody debris, live trees or roots, overhanging vegetation, undercut banks, and boulders at each transect using five areal cover classes (0%, <10%, 10-40%, 40-75%, or >75%). Between each of the 11 transects, we counted pieces of large woody debris within and above the bankfull channel according to several size classes. At the center transect, we measured stream discharge using the velocity-area method and a Marsh-McBirney flow meter. We characterized riparian vegetation cover separately for canopy, understory, and ground cover along the entire stream reach using five areal cover classes (0%, <10%, 10-40%, 40-75%, or >75%) and several types (deciduous, coniferous, mixed, or none). To characterize channel slope and sinuosity, we recorded the compass azimuth (aspect) and channel slope between each pair of transects.

Macroinvertebrates

Our field methods followed the sampling methods of Major and Barbour (2001), a modification of the USEPA Rapid Bioassessment Protocols for use in Alaska. We collected macroinvertebrate samples throughout a 100-m reach at each site with a 350-μm-mesh D-frame net. Each sample was a composite of 20 subsamples collected from various instream habitats in proportion to each habitat's abundance. Riffles were the predominant substrate sampled, with submerged streambanks and large woody debris comprising smaller portions. For riffle samples we disturbed an area of streambed approximately 0.14 m^2 (1.5 ft^2)to a depth of 10 cm (4 in.) and rubbed each cobble and boulder by hand to ensure all macroinvertebrates were dislodged and swept into the net by the stream's current. We sampled streambanks by making three successive sweeps of the net across a 0.14 m^2 (1.5 ft^2) area while rapidly jabbing the net into the substrate. We sampled woody debris by manually scouring a 0.14 m^2 (1.5 ft^2) area of wood immediately upstream of the net. We preserved all samples in the field with ethanol and returned them to UAA's Aquatic Ecology lab for processing. In the lab, we subsampled each macroinvertebrate sample to obtain a fixed count of 300 ±20% organisms to standardize the taxonomic effort across all sites. In addition, we conducted a five minute search through the remaining sample to select any large or 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, Merritt and Cummins 1996, Wiggins 1996, Thorpe and Covich 2001, Stewart and Oswood 2006).

Diatoms

Diatom sampling and processing followed UAA Aquatic Ecology Lab's protocols adopted from U.S. EPA methods. Each sampling reach consisted of four consecutive riffles. From each riffle we selected four stones (cobble or large gravel), ensuring that algal coverage on the stones was visually representative of the riffle at large. From a standardized area on each stone (4.5 cm diameter circle), we scrubbed the surface with a small brush and rinsed the algal layer into a washtub. For each stream, we composited algae from all stones (4 stones x 4 riffles) into a single sample which we preserved with Lugol's solution.

In the lab, we homogenized each sample and transferred 20 ml to a clean beaker. We added nitric acid and heat to digest the diatom protoplasm and other organic material, thereby clearing the diatoms for easier identification. We then neutralized the acid digested aliquots by a succession of dilutions, concentrated the cleared diatom frustules by allowing them to settle, and slide mounted the frustules using NAPHRAX mounting medium. For each sample site, we identified a fixed count of 600 diatom valves to species or lowest practical taxonomic level. We also scanned the slide and recorded any new taxa not discovered in the fixed count. The primary taxonomic references were Krammer and Lange-Bertalot (1986-1991) and Patrick and Reimer (1975).

Data Analysis

Our primary analytical goal was investigating the relationships between natural environmental gradients and stream macroinvertebrate and diatom communities. Prior to analyses, we explored and reduced the environmental and species abundance datasets. We examined environmental variables for outliers, normality, and co-linearity using pairwise plots. We combined substrate percent composition from the pebble counts based on size: sand and fines; coarse and fine gravel; and cobble, small, and large boulders. Bankfull width and wetted width were found to be strongly correlated, so we selected bankfull width for use in analyses because it does not depend upon the hydrologic conditions at the time of the field visit. We log transformed several variables to improve normality and facilitate the detection of relationships with biota (e.g. NMS axes in ordinations). These included bankfull width, discharge, shade, large woody debris, conductivity, slope, elevation, and cobble-boulder. A complete list of the environmental variables used for data analysis is provided in Table 1.

We used non-metric multidimensional scaling (NMS), an ordination method, to explore differences in diatom and macroinvertebrate community composition between streams. Ordinations are helpful for reducing complex, multivariate endpoints into a smaller set of axes (usually 2 or 3 dimensions). Prior to analysis, we removed rare taxa (<5% frequency and <2% abundance) to reduce noise in the data and clarify relationships between environmental variables and the biological communities (McCune and Grace 2002). Additionally, we log transformed [log10(x+1)] abundances to lessen the influence of abundant taxa. We used Bray-Curtis distances for the macroinvertebrate and diatom NMS ordinations and rotated each so that shade was loaded entirely onto Axis 1 to facilitate interpretation of environmental gradients driving the communities.

We fit environmental vectors using both continuous and factor variables to the NMS ordinations for interpretation of environmental gradients associated with differences in stream biological communities. For continuous variables, vectors point in the direction of largest change of the environmental variable and the length of the vector indicates the strength of the correlation between the ordination and the environmental variable. Vector-fitting assumes a linear relationship and p values were based on 1000 random permutations of the data. We explored the relationship between factor variables and the ordinations by overlaying symbols for the different classes. Vectors were scaled similarly across both the diatom and macroinvertebrate ordinations so that the strength of the relationship between an individual environmental variable and the ordination can be compared.

We used a separate NMS ordination to explore inter-annual differences in stream biological community composition for the five sites that were sampled repeatedly from 2008 to 2011. Successional vectors were added to the ordination to indicate the direction and magnitude of change in community composition for each site over time.

All statistical analyses were run in the R statistical platform (version 2.14.1; R Development Core Team, Vienna, Austria). The vegan library was used for the NMS analyses and correlations of environmental data to the ordinations using vectors (Oksanen et al. 2011).

Table 1. Environmental variables used for vector fitting with NMS ordinations

Variable	Type	Definition (units)	Transformation
sands and fines	continuous	substrate count of 105, percent and fines	none
coarse and fine gravel	continuous	substrate count of 105, percent gravels	none
cobble, small and large boulders	continuous	substrate count of 105, percent cobble, small boulder, and large boulder	log10
algae cover	class	algae cover class[1]	none
macrophyte cover	class	macrophyte cover class[1]	none
detritus cover	class	detritus cover class[1]	none
moss cover	class	moss cover class[1]	none
muck cover	class	muck cover class[1]	none
canopy cover	class	canopy cover classes[2]	none
canopy type	class	canopy cover types[3]	none
understory cover	class	under story cover classes[2]	none
understory type	class	understory cover types[3]	none
underherb cover	class	under herb cover classes[2]	none
conductivity	continuous	specific conductivity (μS)	log10
dissolved oxygen	continuous	dissolved oxygen (percent)	none
pH	continuous	pH	none
water temperature	continuous	water temperature (Celsius)	none
discharge	continuous	stream discharge (cubic feet per second)	log10
bankfull width	continuous	bankfull width (meters)	log10
large woody debris	continuous	count of large woody debris per 100 meters of stream	log10
shade	continuous	average shade (percent) based on 6 measurements at each transect	log10
slope	continuous	stream reach slope (percent)	log10
elevation	continuous	site elevation (feet)	log10
date	continuous	days since May 1	none
sub-watershed	class	four sub-watersheds	none
year	class	three years	none

[1] 0-none, 1-slight, 2-slight to moderate, 3-moderate, 4-moderate to heavy, 5-heavy

[2] 0.5 = 2.5%, 1 = 5%, 1.5 = 15%, 2 = 25%, 2.5 = 41%, 3 = 57.5%, 3.5 = 72.5%, 4 = 87.5%

[3] N = None, C = Coniferous, D = Deciduous, M = Mixed

Results

Water Quality and Physical Habitat

Water quality data are summarized in Table 2 for the four sub-watersheds and indicate that surface waters are cold and have neutral pH, low conductivity, and high dissolved oxygen. Very low water temperatures are from sites sampled early in a field season. Some streams have conductivity measurements much higher than the average indicating that groundwater may be an important input at those sites.

A summary of selected physical habitat parameters are provided in Table 3. All streams were below the target of 1500 feet (457 m) in elevation and streams in the Mulchatna River sub-watershed had the highest mean elevation. For all sites, the mean stream percent slope was between one and three percent. The steepest stream had a slope of 8% recorded at a tributary of the Chilikadrotna River. Streams overall averaged between five and seven meters in width, although the range was from less than one meter to over 16. Average discharges were similar across the four sub-watersheds and ranged from 28 to 44 cubic feet per second (cfs), although the largest streams in all four sub-watersheds had discharge from two to six times the mean. Sites in the Lake Clark sub-watershed had the highest shade and coverage of instream large woody debris. All four sub-watersheds had similar substrate composition as indicated by the mean percent of coarse and fine gravels.

Table 2. Summary of water quality data by sub-watershed. For each parameter, the sub-watershed average (and range) is provided.

Sub-watershed	Total Sites[1]	Water temp. (°C)	Dissolved oxygen (mg/L)	pH	Conductivity (µs/cm)
Lake Clark	18	5.9 (1.5-7.9)	11.9 (10.6-13.4)	7.4 (7.1-7.9)	59 (17-176.4)
Lake Iliamna	17	8.4 (3.6-17.3)	11.1 (8.4-13.1)	7.4 (6.9-7.9)	43.5 (22-67)
Mulchatna R.	34	7.8 (0.6-14.2)	11.2 (8.8-13.4)	7.2 (5.4-8.1)	47.5 (5-139.6)
Upper Nushagak R.	8	6.8 (3.8-10.4)	12 (10.5-13.1)	7.2 (6.6-7.6)	47 (19.8-87.4)

[1] One site was missing water chemistry and physical habitat data resulting in a total of 77 sites.

Table 3. Summary of selected physical habitat data by sub-watershed. For each parameter, the sub-watershed average (and range) is provided.

Sub-watershed	Total Sites[1]	Elevation (ft)	Slope (%)	Wetted width (m)	Discharge (cfs)	Shade (%)	LWD (no./100 m)	Gravels (%)
Lake Clark	18	438 (235-1210)	2.35 (0.6-4.7)	5.6 (0.7-10.7)	39.5 (3.7-127)	42 (3-96)	7.8 (0-34)	54 (27-82)
Lake Iliamna	17	471 (70-1015)	1.71 (0-3.5)	5.3 (1.1-15.8)	29.2 (4.7-98)	14 (1-47)	0.3 (0-3)	56 (31-73)
Mulchatna R.	34	816 (400-1395)	1.72 (0.3-8)	4.9 (1-13.7)	28.7 (1.8-248)	19 (0-73)	3 (0-32)	54 (0-86)
Upper Nushagak R.	8	305 (240-570)	1.35[2]	7.1 (1.9-16.7)	44 (2.0-121)	23 (8-56)	6.3 (0-27)	64 (59-74)

[1] One site was missing water chemistry and physical habitat data resulting in a total of 77 sites.
[2]Field measurement of channel slope was only completed at one site on the upper Nushagak River due to a faulty clinometer.

Macroinvertebrates

A total of 137 macroinvertebrate taxa were identified across all of the sites representing 116 genera and 38 families (see Appendix 1 for a complete taxa list). Sites showed a range of taxonomic richness, from a low of nine taxa in a tributary of the Chilikadrotna River to a high of 40 in a tributary of the Koksetna River in the Lake Clark sub-watershed (Table 4). Mean site richness was similar across the four sub-watersheds and ranged from 23 to 30 taxa. The density of organisms was highly variable within each of the four sub-watersheds and mean densities ranged from 1,700 to 3,800 per square meter of stream substrate. A list of the most common macroinvertebrate taxa (frequency > 50%) are provided in Table 5. Chironomidae (non-biting midges) were the most common family, with four of the top five most frequently encountered taxa across all of the sites. *Baetis* mayflies occurred in 88% of the sites and had the highest mean density of any taxa reaching almost 400 in each square meter. Water mites (Arachnida) and non-chironomid dipterans were also commonly encountered, in addition to one stonefly (Plecoptera), one caddisfly (Trichoptera), and an unknown number of different aquatic worms (classified to subclass Oligochaeta only).

Table 4. Taxonomic richness and density of macroinvertebrates by sub-watershed. For each parameter, the sub-watershed average (and range) is provided.

Sub-watershed	Total sites[1]	Richness (no. of taxa)	Density (no./m^2)
Lake Clark	18	26 (18-40)	1,688 (277-7,581)
Lake Iliamna	17	23 (13-35)	2,626 (576-8,306)
Mulchatna R.	34	27 (9-37)	2,813 (102-11,371)
Upper Nushagak R.	8	30 (25-35)	3,785 (1,713-7,460)

[1]77 total sites. One site was removed from macroinvertebrate analysis because it was missing environmental data and thus could not be used for relating to the ordination.

Table 5. Frequencies and densities of common macroinvertebrate taxa. Only taxa that have frequency greater than 50% are shown. Total frequency and frequency within each of the sub-watersheds is provided along with the mean density across all of the sites.

Higher taxonomy	Taxon name	Frequency (%)	Mean density (no/m^2)	Frequency in each sub-watershed (%)			
				Lake Clark	Lake Iliamna	Mulchatna River	Upper Nushagak R.
Diptera (Chironomidae)	*Micropsectra/ Tanytarsus*	97	295	89	94	100	100
Ephemeroptera	*Baetis bicaudatus*	88	387	78	88	88	100
Diptera (Chironomidae)	*Eukiefferiella*	87	107	94	82	79	100
Diptera (Chironomidae)	*Pagastia*	83	84	89	82	79	75
Diptera (Chironomidae)	*Cricotopus/ Orthocladius*	78	223	67	76	79	88
Arachnida	*Lebertia*	78	38	67	76	82	75
Ephemeroptera	*Cinygmula*	76	112	78	71	74	88
Diptera	*Simulium*	72	178	61	59	76	100
Arachnida	*Sperchon*	71	42	61	59	76	88
Arachnida	Hydracarina	70	32	56	71	68	100
Plecoptera	*Zapada*	70	76	78	71	62	75
Diptera (Chironomidae)	*Tvetenia*	67	43	83	53	65	63
Diptera	*Prosimulium*	66	56	56	71	71	50
Trichoptera	*Brachycentrus*	64	42	61	65	62	75
Clitellata	Oligochaeta	63	45	78	41	62	75
Diptera	*Dicranota*	54	8	78	35	56	25
Diptera	*Probezzia*	54	15	39	41	59	88

The NMS analysis of the macroinvertebrate community composition resulted in a three-dimensional solution with a final stress of 17.3. Overlay of the sub-watersheds on the ordination resulted in no clear distinction of compositional changes driven by sub-watershed membership (Figure 3). Sites sampled in 2010 tended to occur on the positive end of Axis 2 (not shown in the NMS) and year was the only factor variable significantly related to the macroinvertebrate ordination (Table 6). The macroinvertebrate ordination was rotated to match the dominant environmental gradient in the diatom ordination by loading the shade variable entirely onto Axis 1. Shade, canopy cover, and large woody debris were all positively associated with Axis 1, although the relationships were only marginally significant (*p-values* > 0.05 for shade and large woody debris, Table 6). Algae cover and water temperature were strongly negatively associated with Axis 1 and algae cover explained the most variance in macroinvertebrate community composition of all the environmental variables. The second axis in the macroinvertebrate ordination represented a weak gradient from smaller to larger streams with more detritus present in the small streams at the negative end. Axis 3 represented an elevation gradient with sites at higher elevations sampled later in the field season.

Table 6. Environmental variables significantly correlated to the macroinvertebrate ordination. Only variables with *p-values* < 0.1 are shown. Variables are sorted according to the strength of their correlation to the ordination (decreasing R^2).

Variable	R^2	***p*-value**
algae	0.30	0.001
date	0.26	0.001
slope	0.25	0.001
bankfull width	0.22	0.004
water temperature	0.22	0.001
discharge	0.19	0.004
elevation	0.17	0.016
year	0.16	0.001
canopy cover	0.15	0.015
detritus	0.14	0.022
shade	0.12	0.061
large woody debris	0.11	0.061
boulders	0.11	0.061
muck	0.11	0.086
aquatic grasses	0.11	0.072

The NMS ordination of the five repeat sampling sites shows that the magnitude of interannual changes in four of the sites were much larger than for site mutsk02 (Figure 4). The taxanomic stability observed in Mutsk02 probably relates to its hydrologic stability, as this stream appears to derive most of its flow from conspicuous groundwater seeps and its discharge was remarkably similar across the four years of sampling. Two of the sites in the Mulchatna sub-watershed shifted in a negative along Axis 2 across almost all four years (mutsk09 and mussm15). Two of the sites had similar patterns of change (iltnr19 and mustk02) since 2009, but of much different magnitudes. The fifth site, muekm23, shifted in an opposite direction as the other sites from 2008 to 2009, but also moved negatively along the second axis from 2009 to 2010 and 2010 to 2011. All five sites shifted in a negative direction along the second axis from 2010 to 2011. This shift was also of much lower magnitude than between other year pairs, suggesting more stable environmental conditions between 2010 and 2011.

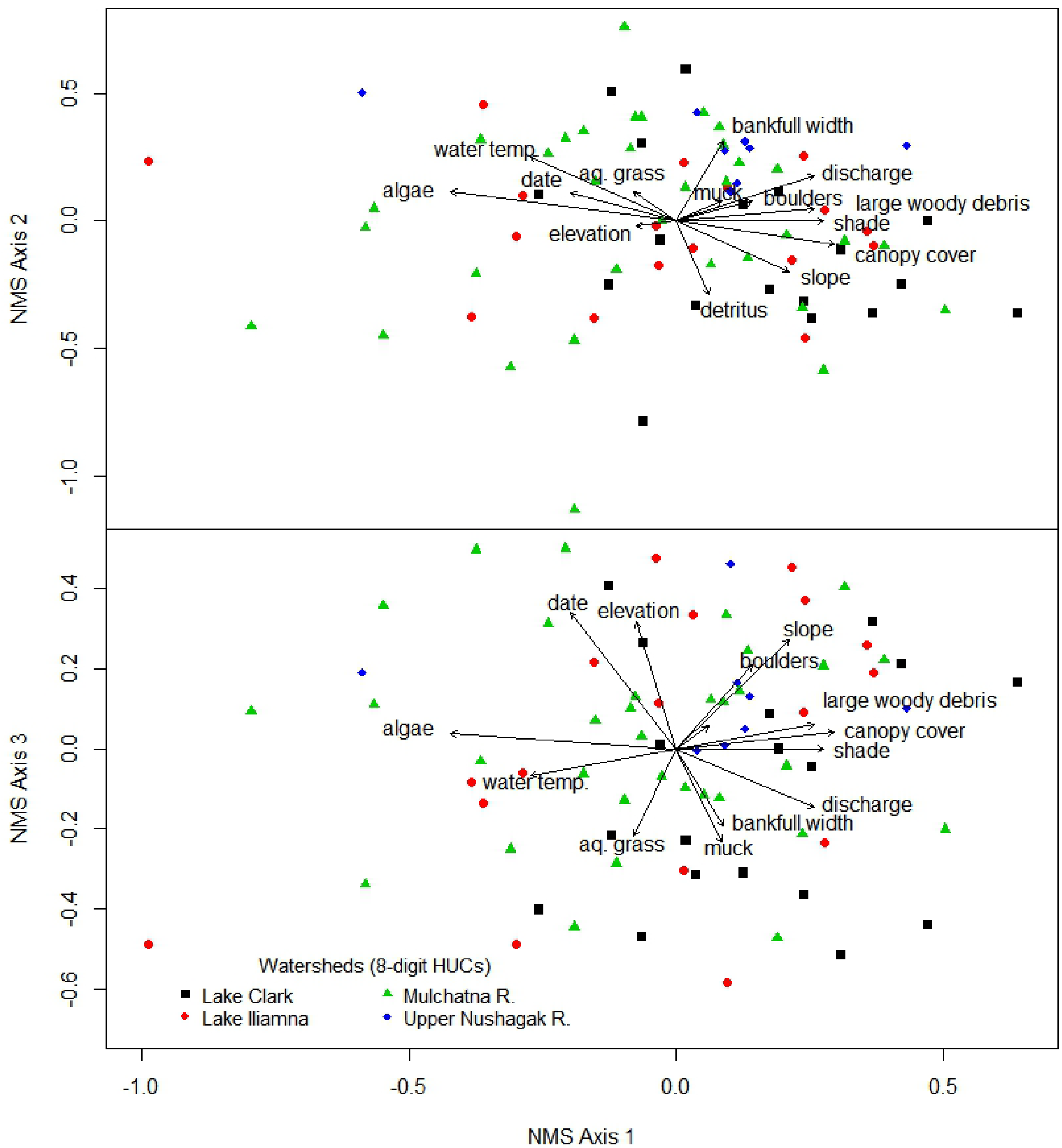

Figure 3. NMS ordination of macroinvertebrate community composition. The first axis represented a gradient of sites from less to more canopy cover. Streams with higher algal cover and warmer temperatures were at the negative end of the first axis. The second axis represented a weak gradient of stream size and the third axis represented an elevation gradient. Sub-watersheds generally had overlapping community composition, but sites sampled in 2010 (years are not shown here) tended to cluster on the high end of the second axis.

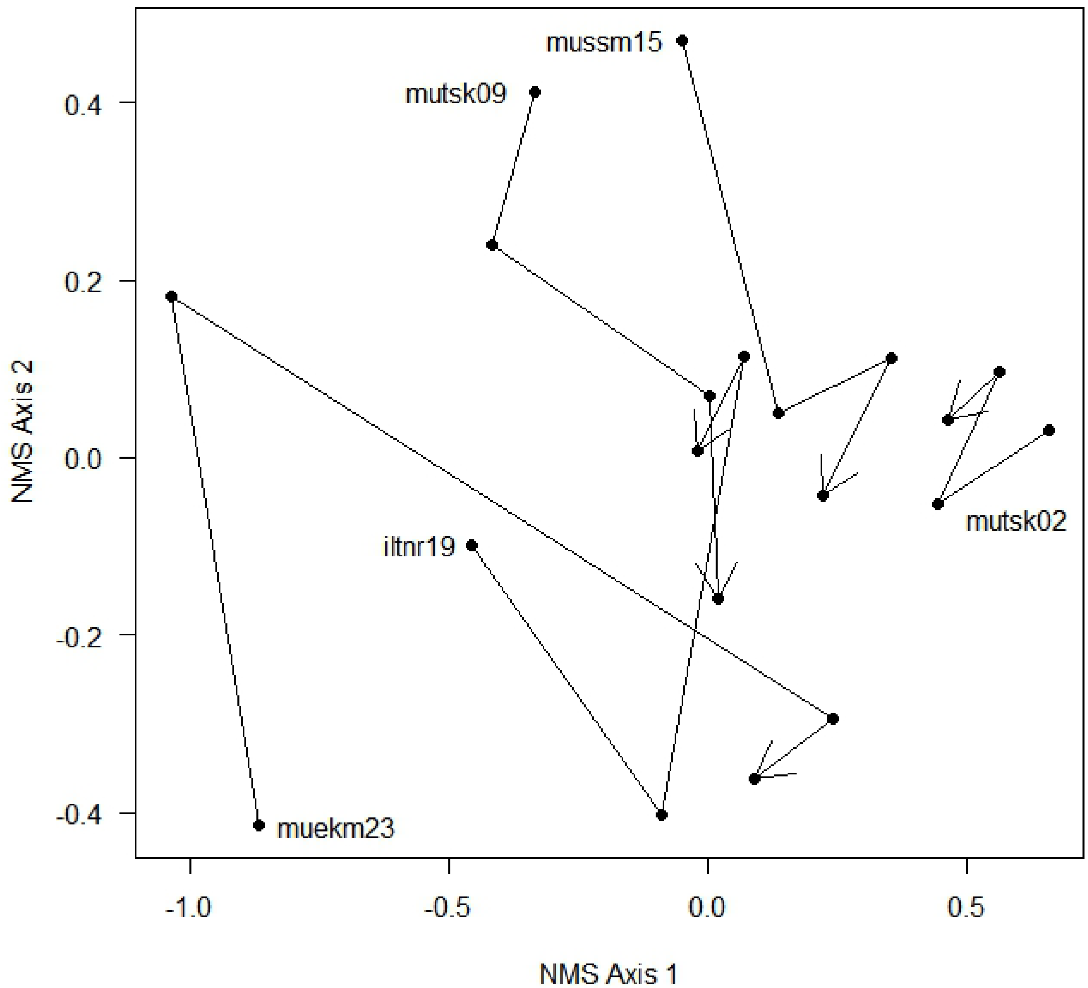

Figure 4. NMS ordination of macroinvertebrates for five repeat sampling sites. The arrows show the magnitude and direction of change in community composition over four years. The shift from 2010 to 2011 was the only interannual time period with a similar pattern across all sites. Changes in macroinvertebrate community composition indicated that some sites changed similarly over time (mutsk02 and iltnr19 in addition to mussm15 and mutsk09), while muekm23 generally experienced the largest changes in community composition.

Diatoms

Diatoms identified from the 600-valve fixed count represented 201 individual taxa. When the taxa identified in the secondary scan are included, the total taxonomic richness increased to 280, representing 71 genera and 28 families (see Appendix 2 for a complete taxa list). Mean diatom richness from the fixed count across the four sub-watersheds ranged from 26 to 35 (Table 7). Individual site richness ranged from a low of 14 at a tributary of Sixmile Lake (just upstream of its outlet to the Newhalen River) to a high of 51 at Rock Creek (just upstream of its confluence with Groundhog Creek), both of which are in the Lake Clark sub-watershed.

A total of 19 diatom taxa occurred at more than 50% of the sampled sites (Table 8). Only one taxon, *Achnanthidium minutissimum*, a common cosmopolitan species, had a mean abundance greater than 10%. Taxa from the family Fragilariaceae were the most commonly encountered, with *Fragilaria capucina, Fragilaria vaucheriae, Staurosirella pinnata, Meridion ciruculare, Synedra ulna, Hannaea arcus,* and *Diatoma mesodon* all occurring in more than 75% of the sites. In addition, *Tabellaria flocculosa,* recently reclassified from the Fragilariaceae was one of the most pervasive diatoms, encountered in 95% of sampled sites. The closely related diatoms, *Encyonema silesiacum* and *E.* minutum, were found in over 80% of sampled sites, while two mono-raphid diatoms, *Psammothidium subatomoides* and *Cocconeis placentula* were present in over 75% of sampled sites.

Table 7. Taxonomic richness of diatoms by sub-watershed.

Sub-watershed	Total sites[1]	Richness[2] (no. of taxa)
Lake Clark	18	27 (14-51)
Lake Iliamna	17	35 (25-46)
Mulchatna R.	33	30 (16-45)
Upper Nushagak R.	8	26 (17-36)

[1] 76 total sites. One site missing environmental data and one site lacking diatom identifications were removed from the diatom data analysis.

[2] Richness is based on the 600-valve fixed count and does not include taxa identified in the secondary scan.

Table 8. Frequencies and abundances of common diatom taxa. Only taxa that have frequency greater than 50% are shown. Total frequency and frequency within each of the sub-watersheds is provided along with the mean percent abundance across all of the sites.

Family	Species	Frequency (%)	Mean abundance (%)	Frequency in each sub-watershed (%)			
				Lake Clark	Lake Iliamna	Mulchatna R.	Upper Nushagak R.
Fragilariaceae	*Fragilaria capucina*	95	4	89	94	97	100
Tabellareaceae	*Tabellaria flocculosa*	95	6	94	94	94	100
Achnanthidiaceae	*Achnanthidium minutissimum*	92	17	100	100	91	63
Fragilariaceae	*Fragilaria vaucheriae*	88	2	83	88	91	88
Cymbellaceae	*Encyonema silesiacum*	87	2	94	82	82	100
Fragilariaceae	*Staurosirella pinnata*	86	6	83	94	82	88
Cymbellaceae	*Encyonema minutum*	80	2	67	94	88	50
Fragilariaceae	*Meridion circulare*	79	6	67	71	88	88
Fragilariaceae	*Synedra ulna*	79	2	72	82	79	88
Achnanthaceae	*Psammothidium subatomoides*	78	2	89	76	76	63
Fragilariaceae	*Hannaea arcus*	78	8	78	76	76	88
Fragilariaceae	*Diatoma mesodon*	76	7	56	71	91	75
Achnanthidiaceae	*Eucocconeis laevis*	75	2	100	71	73	38
Aulacoseiraceae	*Aulacoseira alpigena*	74	2	61	65	85	75
Cymbellaceae	*Reimeria sinuata*	71	3	83	88	58	63
Achnanthidiaceae	*Planothidium haynaldii*	67	3	39	76	76	75
Fragilariaceae	*Pseudostaurosira brevistriata*	63	4	50	76	61	75
Gomphonemataceae	*Gomphonema micropus*	57	1	61	53	55	63
Cocconeidaceae	*Cocconeis placentula*	53	2	83	65	33	38

The NMS analysis of the diatom community resulted in a three dimensional solution, which maximized the reduction in stress while still remaining interpretable. The final stress was 16.2. The four sub-watersheds and three years across which the study was conducted were initially used to interpret the NMS ordination. Both sub-watersheds and years were significantly correlated to the ordination, indicating that the sites from at least one sub-watershed and year were significantly different from the others in diatom community composition (Table 9 and Figure 5). Sites in the Upper Nushagak sub-watershed tended to be located on the negative end of the first axis, indicating they were sites with little shade or canopy. Also, sites in the Lake Clark sub-watershed tended towards the positive end of Axis 1 and negative end of Axis 2 indicating those sites had more shade and were larger streams (higher discharge and bankfull width). There was broad overlap in the sites sampled by year making it difficult to discern which year average was driving the weak correlation to diatom community composition (overlay not shown).

Table 9. Environmental variables significantly correlated to the diatom ordination. Only variables with *p-values* < 0.1 are shown. Variables are sorted according to the strength of their correlation to the ordination (decreasing R^2).

Variable	R^2	***p -value***
canopy cover	0.37	0.001
shade	0.35	0.001
understory cover	0.29	0.001
ph	0.25	0.002
large woody debris	0.23	0.001
slope	0.20	0.004
conductivity	0.20	0.002
sub-watershed	0.19	0.001
canopy type	0.15	0.003
discharge	0.14	0.035
bankfull width	0.14	0.036
water temperature	0.12	0.050
date	0.12	0.075
coarse and fine gravels	0.10	0.090
muck	0.10	0.080
aquatic grasses	0.10	0.097
year	0.10	0.007
understory type	0.08	0.002

The strongest environmental gradient explaining differences in diatom community composition included four variables associated with riparian vegetation: canopy cover, understory cover, shade, and large woody debris (Figure 5). Sites on the positive end of Axis 1 had high riparian and in-stream cover. Date was negatively correlated to the cover gradient, indicating that sites sampled later in the season may have been streams with more open canopies.

The second axis represented a weak environmental gradient with many variables loading more heavily on the third axis. Based on the few variables most strongly associated with the second axis, large streams with warmer temperatures occurred low on Axis 2. Other variables positively correlated with stream size, but which had stronger correlations to the third axis, included pH, conductivity, and muck. Axis 3 was most strongly driven by sites with high slope, pH, and conductivity.

The NMS ordination of the five sites that were sampled repeatedly from 2008 to 2011 resulted in a two dimensional solution with a stress of 11.5 (Figure 6). The proximity of data points representing an individual site over time indicates that diatom community composition is more stable within a stream reach than macroinvertebrates, despite the overlap across sub-watersheds. The successional vectors indicate that many of the sites shifted in a similar direction over time. From 2008 to 2009, the dominant direction of change was positively along the first and second axes (muekm23, mussm15, and mutsk09). A fourth site increased along the second axis, but decreased along the first axis and the fifth site showed a strong shift negatively along the first axis with no change along the second. Four sites decreased along the second axis from 2009 to 2010, while the fifth shifted mostly along the first axis. The pattern reversed for the years 2010 to 2011: four sites increased along the second axis, while one site increased along the first axis. Different sites were anomolous to the main pattern of change between years indicating that no single site was responding uniquely to interannual changes in environmental conditions.

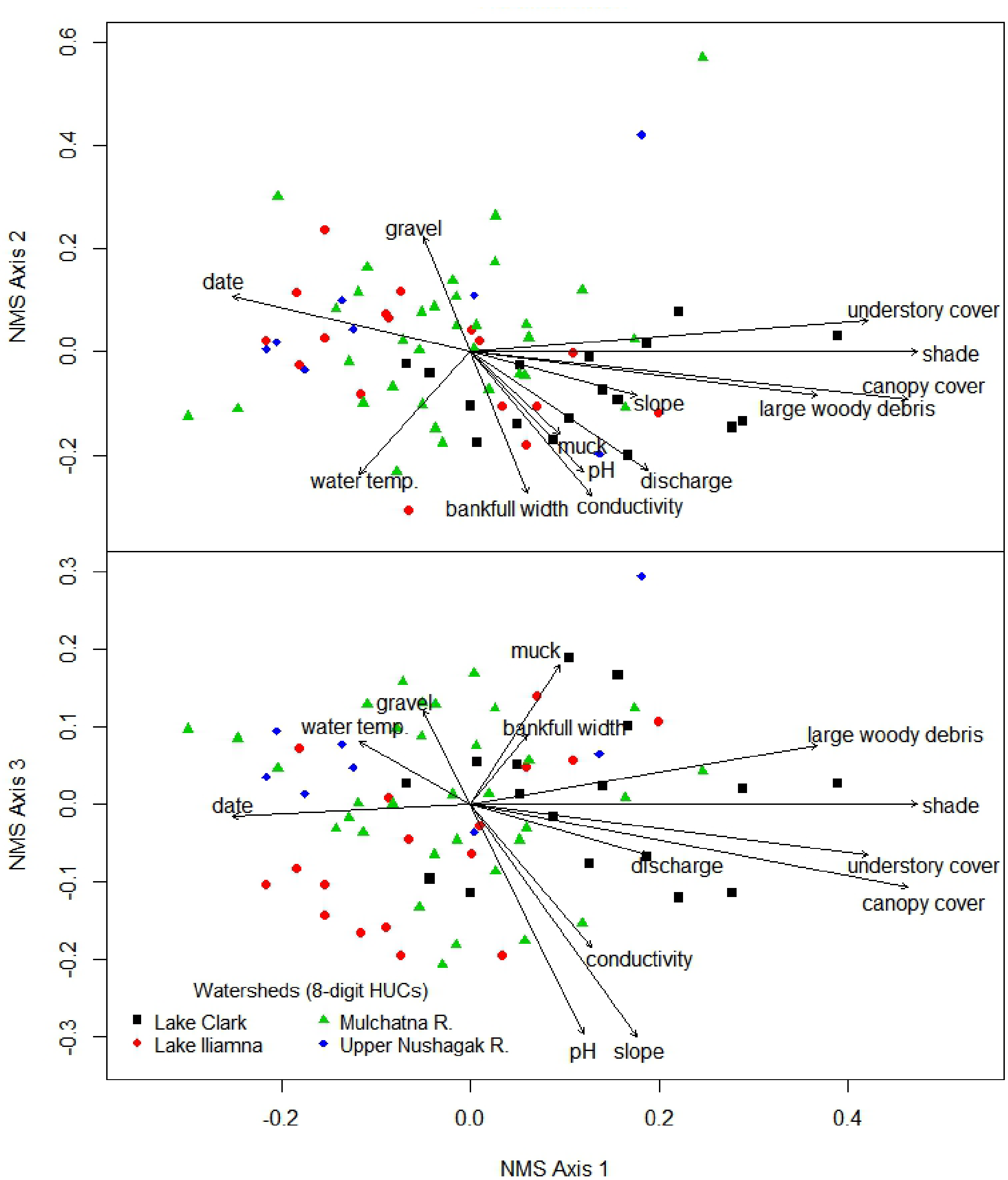

Figure 5. NMS ordination of diatom community composition. The first axis represents a gradient from sites with less to more canopy cover. Sites low on the second axis were larger streams, while sites low on the third axis were streams with steeper slopes leading to higher pH and conductivity. Sub-watersheds generally had overlapping community composition except for the Upper Nushagak River, which tended towards the low end of Axis 1 (open canopies).

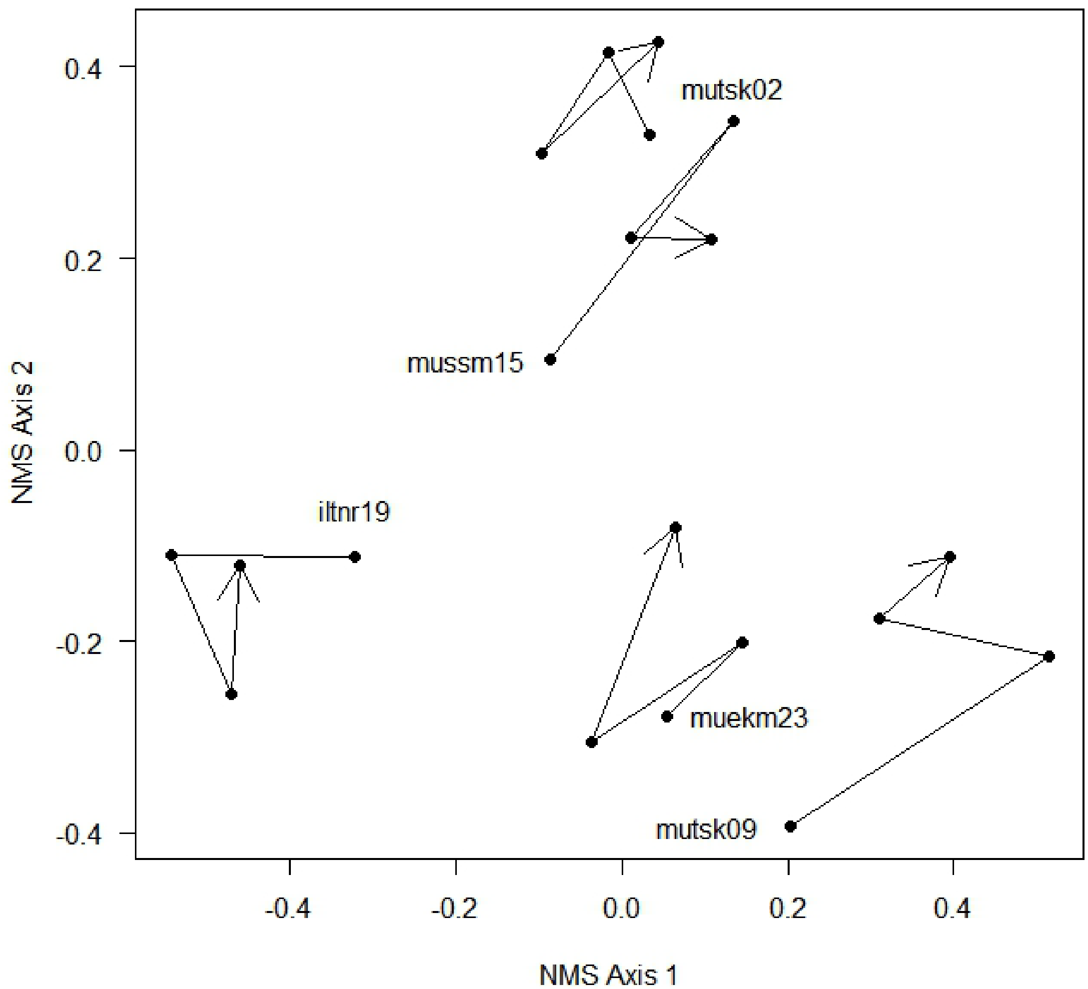

Figure 6. NMS ordination of diatoms for five repeat sampling sites. The arrows indicate the change in composition from 2008 to 2011. Generally, the majority of sites moved in similar directions between each sampling period. Within-site community composition was more stable for diatoms than for macroinvertebrates.

Discussion

Macroinvertebrate and diatom data are limited for southwestern Alaska, especially outside of the Pebble exploration area. The 78 streams sampled in the Lake Clark, Lake Iliamna, Upper Nushagak, and Mulchatna sub-watersheds of Bristol Bay provide an important baseline dataset describing aquatic habitats and macroinvertebrate and diatom communities present in the region. Water quality results indicate clear, cool, highly oxygenated water with a range in solute concentrations apparently corresponding to variable groundwater inputs. The sizes of streams sampled were well represented across the four sub-watersheds. Stream widths and discharge spanned a wide range indicating this baseline is representative of small to medium sized streams (likely 1st through 3rd order or higher). The streams provide a range of habitats with differences in both riparian canopy and in-stream cover. Since macroinvertebrate and diatom communities respond to changes in habitat or water quality, they are useful as biological indicators of stream health (Karr and Chu 1999). Should resource development, road construction, or other disturbances occur in the region, the frequencies and densities of these taxa will provide a helpful baseline for future monitoring, detection of ecological impacts, and as goals for any restoration efforts.

The dominance of Chironomidae in stream macroinvertebrate communities, as seen in this study, has been observed in other areas of Alaska. A meta-analysis found that dipterans constituted more than 60% of stream macroinvertebrate communities across much of Alaska and that 90% of these were Chironomidae (Oswood 1989). This study also found that mayflies (Ephemeroptera) constituted between 6-10% of stream macroinvertebrate communities and were most common in small rivers (Oswood 1989). All of southwest Alaska was omitted from the meta-analysis due to lack of data (Oswood 1989).

Diatom taxonomic richness in this study (mean=30) is comparable to wadeable reference streams in the nearby Cook Inlet region, where richness averaged 27 (n=18, Rinella and Bogan 2007). In Cook Inlet streams, diatom richness increased with anthropogenic landscape disturbance, presumably due to the associated increases in nutrient inputs to otherwise nutrient-poor streams (Rinella and Bogan 2007). All of the 19 most common diatom taxa found in the current study are non-motile, suggesting low levels of bedload sediment in these streams. None of these taxa have elevated organically bound nitrogen requirements or are indicators of enriched organic nitrogen, consistent with the low nitrogen concentrations in the streams in this region (Zamzow 2011). All of the 19 taxa have moderate to high oxygen requirements, preferring water with at least 50% dissolved oxygen saturation, while eight of the 19 taxa require dissolved oxygen levels close to 100% saturation. *Tabellaria flocculosa,* a diatom found to be sensitive to mining impacts (Cattaneo et al. 2004), was one of the two most pervasive diatoms in this study, present in 95% of sampled sites.

This dataset provides the foundation for describing associations between habitats and stream biological communities and the responses of taxa along the dominant environmental gradients in wadeable stream ecosystems. Further analysis could also allow for quantitative predictions of expected community composition across the region's wadeable streams, which will enhance the ability to detect changes in composition associated with habitat alteration. The dominant environmental gradient driving differences in macroinvertebrate and diatom community composition was represented by variation in riparian vegetation (shade and canopy cover), which was positively related to the amount of in-stream cover (count of large woody debris) and negatively related to the algal biomass on the substrates (algae cover class). The amount and type of riparian canopy cover affects the food resources available to macroinvertebrate consumers as some are specifically adapted to shredding or collecting organic materials, while others may be adapted for scraping algae in streams with more light availability (Cummins et al. 2008). The importance of canopy cover to diatom community composition may be related to a number of biotic and abiotic factors, such as grazing pressure from scrapers and temperature, which interact with and possibly override the effects of light availability (Hill et al. 1995).

The secondary environmental gradients associated with the community ordinations represented other physical and chemical habitat attributes important to macroinvertebrates and diatoms. For macroinvertebrates, the elevation gradient along the third axis likely reflected the effect of stream size on invertebrate taxa. Attributes associated with stream size independent of differences in riparian cover (represented on axis 1) include differences in substrate size and flow velocity. Macroinvertebrates adapted to faster flows and larger substrates may inhabit steep headwaters, while those adapted to smaller substrates and moderate flows may be found lower in the watershed. Natural variation in pH and conductivity were controlling diatom community change along the third axis in the diatom ordination. Potapova and Charles (2003) demonstrated that conductivity explained a statistically significant amount of variation in diatom community composition in U.S. rivers, while diatom pH tolerances have been established (Van Dam et al. 1994) and are widely used.

Additional exploration of the environmental gradients driving changes in stream biological communities should involve exploring the shape of the relationships (linear, non-linear, threshold, etc.) between the environmental variables and the community ordinations. Other variables, such as substrate, may have a non-linear relationship with the ordinations that may not have resulted in a significant correlation during vector fitting. In order to relate taxa to the environmental gradients, individual abundances can be overlaid on the ordinations to visualize frequency and abundance along the identified gradients. Weighted averages of taxa abundances are often used to identify their central tendency and can be combined with bootstrapped confidence intervals to determine the breadth of their habitat preference along the gradient (e.g. King et al. 2012). Cluster analysis of the streams into different habitat groups followed by indicator species analysis could be used as a basis to describe the stream types present in the sampled population and the taxa with high fidelity and abundance that occur in each of the habitat types.

The results of the community ordinations for the five sites sampled repeatedly from 2008 to 2011 indicate that macroinvertebrate community composition tends to have more variation within streams and over time than does the diatom community composition. The persistence (presence of taxa from year to year) and stability (presence combined with abundance over time) of stream macroinvertebrate communities has been shown to vary in relation to winter snowfall and channel stability in streams of Denali National Park (Milner et al. 2006). Further analysis of this dataset should include measuring the persistence and stability of both the macroinvertebrates and diatoms over the four year period to determine how inter-annual variation might affect their usefulness for interpreting changes to aquatic habitats at a later time (Milner et al. 2006, Scarsbrook 2002).

The highest priority for future field work should be ongoing data collection at the five repeat sites to characterize interannual variability in the region's biotic communities over longer time scales. Longer-term data sets are necessary for capturing the true range in interannual variation and the effects of natural (and potentially anthropogenic) disturbances and recovery (Jackson and Fureder

2006). Another priority should be to sample sites that will give more even coverage of the Kvichak and Nushagak watershed's wadeable streams. We will continue to refine the analyses described in this report and hope to publish a peer-reviewed paper using these data in the near future.

RIVPACS Modeling

We are currently working to develop RIVPACS (River Invertebrate Prediction and Classification System) models for macroinvertebrate and diatom communities in wadeable streams of the Nushagak and Kvichak watersheds. RIVPACS is a statistical approach that uses biological data from undisturbed reference sites to models the expected community composition in the region's streams based on their habitat conditions (see Wright et al. 2000). In essence, the model tells the user which invertebrate and/or diatom species are expected to occur at a site of interest, given the type of habitat found at that site. For example, steep, rocky stream reaches will naturally harbor different biological communities than silty, low-gradient streams, and the RIVPACS model accounts for this sort of habitat variation when calculating the expected community. Once a model is calibrated, the biological integrity of any stream site can be assessed by comparing the expected community to that actually observed in biological samples. Discrepancies between the expected and observed communities will indicate the level of biological impairment and often give insights into the type of stressor(s) responsible for the impairment.

We are modeling macroinvertebrate and diatom communities from 76 undisturbed streams in the Nushagak and Kvichak watersheds in a RIVPACs framework to calculate expected taxa in unsampled streams and provide a reference baseline in the event of future disturbances within these watersheds. The RIVPACS model development includes the following steps:

1. We used the macroinvertebrate and diatom datasets in a cluster analysis to identify groups of streams that harbor similar biological communities.
2. We then used non-metric multidimensional scaling (NMS) ordination on the same datasets and groups were overlaid on the ordination diagram to visually examine the fidelity of sites to their groups in species space.
3. We generated a suite of 28 environmental variables in GIS (See Table 10) and used them in random forest models to determine which habitat attributes explained variation in biological communities. The use GIS-based environmental variables allows the RIVPACS model to be calculated remotely (i.e., without on-the-ground habitat measurements), making it possible to map expected community composition for all target streams in GIS.
4. Finally, we are using R scripts (Van Sickle 2011) to examine model accuracy and precision. These scripts also include a function to predict expected taxa at new sites using the environmental variables.

All analyses were performed in R (R Development Core Team 2012), using the cluster, vegan, and random forest packages (Liaw and Wiener 2002, Maechler et al. 2012, Oksanen et al. 2012). Validation of the random forest model was conducted using the model.predict.RanFor.4.2 function (Van Sickle

2011). RIVPACS model development is ongoing, and we are receiving assistance from Lester Yuan at USEPA's Office of Research and Development. We expect the modeling to be completed during the fall of 2012 and we intend to publish a paper describing the completed models.

Table 10. Habitat variables calculated for each of the 76 Bristol Bay stream sites for use in RIVPACS model development.

Variable	Definition	Type	Units
Geography			
Elevation	Elevation from USGS National Elevation Dataset for Alaska (60 m DEM).	Continuous	Feet
Latitude	Collected in field using GPS and NAD83 datum.	Continuous	Decimal degrees
Longitude	Collected in field using GPS and NAD83 datum.	Continuous	Decimal degrees
Hydrology			
Watershed area*	Watersheds were created using ArcHydro and Spatial Analyst tools and the NED DEM.	Continuous	Km^2
Stream order	Stream order was assigned using the tool in spatial analyst based on the raster stream network created as part of watershed processing.	Continuous	NA
8-digit Hydrologic Unit Code	The 76 sampling sites occurred in four different USGS 8-digit HUCs: Upper Nushagak River, Mulchatna River, Lake Clark, and Lake Iliamna.	Class	NA
Lake density*	The density of lakes was calculated for each watershed using the area of the waterbodies in the USGS_National Hydrography Dataset (NHD).	Continuous	Proportion (0-1)
Connected lake within 1 km*	Sites that had a lake upstream and connected on the main river channel within 1 km of the sampling site. Lakes and rivers used for selection process were from the NHD.	Binary	0/1
Land cover	Land cover type at the sampling site from a mosaic land cover dataset for the entire state developed by the Alaska Natural Heritage Program (AKNHP).	Class	NA
Vegetation life form	Vegetation life form (forest, low shrub, herbaceous, etc.) at the sampling site from AKNHP land cover dataset.	Class	NA
Topography			
Watershed topographic wetness*	Topographic wetness was calculated as the ln(watershed area)/tan(slope) for every pixel in the watershed and averaged.	Continuous	NA
Watershed slope*	Percent slope was calculated using the slope	Continuous	Percent

	tool in Spatial Analyst from the NED DEM and averaged across the watershed.		
Climate*			
Annual temperature	Mean annual watershed temperature from Scenarios Network for Alaska and Arctic Planning (SNAP) historical climate data for 2000-2009.	Continuous	˚C
Annual temperature variation	Standard deviation of annual watershed temperature from SNAP historical climate data from 2000-2009.	Continuous	˚C
Spring temperature	Mean spring watershed temperature from SNAP historical climate data for 2000-2009.	Continuous	˚C
Summer temperature	Mean summer watershed temperature from SNAP historical climate data for 2000-2009.	Continuous	˚C
Fall temperature	Mean fall watershed temperature from SNAP historical climate data for 2000-2009.	Continuous	˚C
Winter temperature	Mean winter watershed temperature from SNAP historical climate data for 2000-2009.	Continuous	˚C
Annual precipitation	Mean annual watershed precipitation from SNAP historical climate data for 2000-2009.	Continuous	mm
Annual precipitation variation	Standard deviation of annual watershed precipitation from SNAP historical climate data for 2000-2009.	Continuous	mm
Spring precipitation	Mean spring watershed precipitation from SNAP historical climate data for 2000-2009.	Continuous	mm
Summer precipitation	Mean summer watershed precipitation from SNAP historical climate data for 2000-2009.	Continuous	mm
Fall precipitation	Mean fall watershed precipitation from SNAP historical climate data for 2000-2009.	Continuous	mm
Winter precipitation	Mean winter watershed precipitation from SNAP historical climate data for 2000-2009.	Continuous	mm
Other			
Year	Year sampled – 2008, 2009, or 2010.	Class	NA
Days since May 1	Number of days sampled after May 1.	Continuous	Days
Spawning sockeye	Is there documented sockeye spawning in the watershed? From the 2012 ADF&G Anadromous Waters Catalog.	Binary	0/1
Spawning salmon	Is there documented spawning of any anadromous fish in the watershed? From the 2012 ADF&G Anadromous Waters Catalog.	Binary	0/1

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