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# A COMPARISON OF BENTHIC MACROINVERTEBRATE ASSEMBLAGES AMONG KRYAL AND RHITHRAL LAKE OUTLETS IN THE NORTH CASCADE MOUNTAINS

By

Kelley L. Turner

Accepted in Partial Completion of the Requirements for the Degree of Master of Science

Moheb A. Ghali, Dean of the Graduate School

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Chair, Dr. Robin A. Matthews

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#### **MASTER'S THESIS**

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# A COMPARISON OF BENTHIC MACROINVERTEBRATE ASSEMBLAGES AMONG KRYAL AND RHITHRAL LAKE OUTLETS IN THE NORTH CASCADE MOUNTAINS

A Thesis Presented to The Faculty of Western Washington University

In Partial Fulfillment Of the Requirements for the Degree Masters of Science

> by Kelley L. Turner May 2009

#### ABSTRACT

This study compares the physico-chemical conditions and composition of benthic macroinvertebrates from five rhithral (snowmelt-fed) and five kryal (glacially-fed) lake outlet streams in the North Cascade Mountains, WA. Non-metric, non-parametric cluster and association analysis (NMCAA) clearly separated outlet streams of kryal and rhithral origin based on physico-chemical and taxon variables. Kryal lake outlets were characterized by lower water temperatures, unstable in-stream channels and higher turbidity, discharge and fine substrates than rhithral sites. A total of 24,985 specimens representing 93 macroinvertebrate taxa were collected. Rhithral lake outlets had significantly higher densities and supported more taxa than kryal sites (9,049 ind./m<sup>2</sup> and 77 taxa versus 821 ind./m<sup>2</sup> and 35 taxa). Chironomidae were the dominant taxon amongst all sites, although densities and taxa richness were one-third in the kryal lake outlets when compared to rhithral sites. Rhithral lake outlets contained higher densities of non-insect taxa such as Acari, Oligochaeta, Nemathelminthes, Planariidae and crustaceans. Water temperature, stream discharge and turbidity were the variables most strongly correlated to density and taxa richness. My results suggest that glacial presence was the dominant factor influencing instream environmental conditions and subsequently macroinvertebrate assemblages of alpine lake outlet streams.

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#### **1** INTRODUCTION

#### 1.1 ALPINE STREAM TYPES

Alpine environments represent the upper limits of terrestrial and freshwater ecosystems in both a physical and biological sense, generally characterized by exposed rock surfaces, steep gradients, extreme seasonality and highly specialized biotic communities adapted to these conditions. Within these environments, between the permanent snowline and treeline, three general types of stream systems have been defined based upon their primary water source: kryal streams, which are glacially-fed; rhithral streams, which are dominated by seasonal snowmelt; and krenal streams, which are groundwater fed (Steffan 1971, Ward 1994, Brittain and Milner 2001). The difference in water sources among these streams affects their physical and chemical properties which in turn affect the resident macroinvertebrate communities (Milner and Petts 1994, Tockner et al. 1997, Brittain et al. 2000, Füreder et al. 2000, Füreder et al. 2001, Lods-Crozet et al. 2001, Hieber et al. 2005).

#### 1.2 DIFFERENCES BETWEEN KRYAL, KRENAL, AND RHITHRAL STREAMS

Among these stream types, kryal streams embody the harshest, most dynamic alpine environment for biota, especially in the summer when there are high diel fluctuations in water temperature, discharge, and concentrations of suspended solids and nutrients as glaciers melt during the day and refreeze at night (Milner and Petts 1994, Ward 1994, Füreder et al. 2001). In comparison, rhithral streams generally have less pronounced fluctuations in discharge and higher maximum temperatures (Zbinden et al. 2008). In a study conducted in the Swiss Alps, Hieber et al. (2002) found that levels of ammonia, nitrate, particulate phosphorus and total suspended solids were also significantly higher in glacial-fed systems than those dominated by snowmelt.

The differences between kryal and rhithral streams are also seen when comparing macroinvertebrate fauna, diversity, and abundance. In general, alpine streams are located on the declining limb of the harshness-diversity curve, where environmental harshness is high and taxonomic diversity is low (Figure 1; Tockner et al. 1997). The harshness-diversity curve states that species diversity peaks at intermediate environmental disturbance levels when conditions favor the coexistence of competitive and disturbance-tolerant species (Mackey and Currie 2001). Within this characterization, however, the range of environmental harshness and its affect on macroinvertebrate diversity is also dependent on the primary water source. Rhithral streams have been found to have more diverse and taxon-rich assemblages than kryal streams (Hieber et al. 2005). Füreder et al. (2001) found that although the Chironomidae (Diptera) represent one of the major taxa in glacial stream systems, their taxa richness was one-half to one-third compared to krenal streams during the summer months.

#### 1.3 LAKE OUTLETS

Recent research has also focused on the physical, chemical, and biotic characteristics of alpine lake outlets, a transition zone between lentic ("still water") and lotic ("flowing water") environments (Wotton 1995, Kownacki et al. 1997, Donath and Robinson 2001, Hieber et al. 2001, 2002, and 2005, Brunke 2004, Boggero and Lencioni 2006, Maiolini et al. 2006). Lake outlets are a unique habitat within a stream system that is strongly influenced by the

characteristics of the lake that feeds them (Richardson and Mackay 1991, Donath and Robinson 2001). Although the macroinvertebrate fauna of lake outlets is most similar to streams and rivers, the characteristics of the lake (i.e. size, temperature, allochthonous inputs) can affect the lake outlet community (Wotton 1995).

Lakes generally stabilize outlet streams by increasing water temperature and decreasing daily temperature and discharge fluctuations, especially in glacially-fed systems (Donath and Robinson 2001, Hieber et al. 2002). In the summer, warm epilimnic surface water discharging into the outlet may increase macroinvertbrate metabolism (Richardson and Mackay 1991) and digestive function (Wotton 1995). Lakes also contribute food sources to outlet streams in the form of detritus from decomposition of dead biota, faecal material, and dissolved organic matter (Wotton 1988). Research has shown that non-glacial lake outlets had more diverse and abundant macroinvertebrate communities than glacial-fed lake outlets (Füreder et al. 2001, Hieber et al. 2005, Maiolini 2006).

#### 1.4 GLACIER RETREAT IN THE NORTH CASCADES

Approximately 10% of the earth's land surface is now covered by glaciers as opposed to 32% during the glacial maxima of the Pleistocene era (1,808,000 to 11,550 Years Before Present; Brittain and Milner 2001). Over the last century, there has been a general pattern of glacial retreat in most regions of the world (IAHS (ICSI) – UNEP – UNESCO 2008). Glaciers in the North Cascades lost on average more than 9.5 meters in thickness and 20 to 40% of their volume between 1984 and 2005 (Pelto 2006). Similar to the harsh environmental conditions for macroinvertebrates induced by glacial melt during warm summer days, increased overall

melting may result in reduced stream temperatures, decreased channel stability, and altered sediment loads that could potentially reduce the diversity of macroinvertebrate communities in kryal streams (Brittain and Milner 2001). As warming continues, the contribution of meltwater from glaciers and snowpack will decrease as these sources are exhausted earlier in the summer or disappear entirely, resulting in a less harsh physico-chemical stream habitat (Hannah et al. 2007). Subsequently, species from lower altitudes may colonize these streams. Although local in-stream diversity and abundance may increase, the loss of kryal habitats may lead to an overall reduction in regional-scale diversity (Brown et al. 2007). Long-term monitoring of changes could be used to document effects of climate change (Brittain and Milner 2001).

#### 1.5 OBJECTIVES

The main objective of my study was to compare benthic macroinvertebrate assemblages between kryal and rhithral lake outlets in the North Cascades, Washington. Krenal streams were not included in the study due to time and access limitations. When differences were found between kryal and rhithral macroinvertebrate assemblages, I sought to determine which, if any, physico-chemical parameters were influencing these differences. Glacial and alpine lake outlet research has been centered on European systems (Kownacki et al. 1997, Brittain and Milner 2001, Donath and Robinson 2001, Hieber et al. 2001, 2002, and 2005, Brunke 2004, Boggero and Lencioni 2006, Maiolini et al. 2006). My study will broaden the geographical scope of glacial and alpine lake outlet research and provide a base-line of kryal and rhithral lake outlet data for the North Cascades.

#### 2 MATERIALS AND METHODS

#### 2.1 REGIONAL DESCRIPTION

The northern-most portion of the Cascade Mountains, known as the North Cascades, differ from the southern Cascades not only by their characteristic jagged profile, but also by their geologic composition. Large portions of the North Cascades are comprised of large granitic batholiths and folded, partially metamorphosed, ancient sedimentary rock that stretch over 150 km from Snoqualmie Pass in Washington State to British Columbia's Fraser River valley. They are bounded to the west by the Puget Sound coastal lowlands and to the east by the Okanogan Highlands and Columbia River. The North Cascades are noted for their steep relief, rising to peaks over 3,000 m, remote terrain, and glaciated landscape. They drain thousands of kilometers of streams into several major watersheds including the Fraser, Stehekin, Nooksack, and Skagit. Due to their orientation and proximity to the Puget Sound and Pacific Ocean, the North Cascades intercept moisture coming in from the Pacific, creating a distinct west to east precipitation pattern. Annual precipitation on the west side of the mountains averages 280 cm while at Lake Chelan on the east side the average is less than 90 cm (Weisburg 1988).

#### 2.2 SITE DESCRIPTIONS

Study sites were located at or above treeline between 1117 and 1755 m on the western slope of the North Cascade Mountain range within the North Cascades National Park (NOCA) boundary (Figure 2). Sites included 10 lake outlets comprised of five kryal and five rhithral systems (Table 1). All lake outlets were first-order streams (Wetzel 2001). All sites were located within the subalpine/alpine ecoregions of the park where continuous forest is

replaced by alpine heaths, meadows, barren rock and permanent snow and ice fields (Weisburg 1988). Alpine lakes and streams in the North Cascades were historically fishless. To represent these ecosystems, outlet streams fed by fishless lakes were chosen as study sites. Fishless status was determined by reviewing NOCA's compilation of stocking records and fish observations (National Park Service 2008). Kryal lake outlet sites were located in five different catchments throughout the western region of the park, while four of the five rhithral lake outlet sites were in the Little Beaver Creek catchment. Two of these sites, Upper Middle and Lower Middle, are a chain of lakes. Upper Middle lake outlet is the inlet to Lower Middle lake.

I worked in conjunction with NOCA's aquatics program to select and sample my study sites. Each summer NOCA samples near-shore benthic invertebrates and water chemistry of 20 alpine lakes for long-term monitoring. To enhance the understanding of these lake systems, three of my 10 sites were lake outlets from these lakes (EP-11-01, Lousy, and Price). Due to difficult access, four of my study sites (Ouzel, M-25-01, EP-11-01, and Lousy) were accessed via helicopter. At these sites, habitat measurements and macroinvertebrate and water samples were collected by NOCA's aquatic biologists Ashley Rawhouser and Carmen Welch. I hiked into the remaining six sites.

#### 2.3 PHYSICAL MEASUREMENTS

Geographic location, lake area, and site elevation of each study site were determined using Geographic Information Systems (GIS) data from NOCA and Western Washington University (ESRI, ArcGIS 9.2). Watershed characteristics are reported in Table 1. The remaining physico-chemical measurements and macroinvertebrate sampling were conducted between 31 August and 20 September 2007.

#### 2.3.1 Channel Stability

Channel stability is the measure of a stream channel's resistance to changes in flow and sediment load and its capacity to adjust and recover from these changes (Pfankuch 1975). Channel stability is a key factor affecting the instream habitat conditions for benthic macroinvertebrates and affects the community structure of alpine and especially glacial streams (Milner and Petts 1994, Burgherr and Ward 2000). Numerous channel stability indices exist and each index relies on different sets of field measurements to determine stability in relation to a "stable" reference stream (Rosgen 2001). We assessed channel stability index (PSI) to classify my stream reaches into four categories of channel bottom stability ( $\leq 15$  excellent, 16 - 30 good, 31 - 45 fair, 46 - 60 poor). This index is based on several qualitative observations of the stream bottom including rock angularity, consolidation, size distribution and aquatic vegetation cover (Table 2). I chose this index because it focused on instream habitat, was a quickly assessed in the field, and was used by Hieber et al. (2002) in a similar study in the Alps.

#### 2.3.2 Discharge

Lake outlet discharge (cubic meters per second; CMS) measurements were made at each study site using the neutrally buoyant object procedure (U.S. EPA 2004). At each site a segment of the stream reach was selected that was deep and long enough to float a small stick

between 10 and 30 seconds. Average width and depth of the float segment was determined from measurements of one to three channel cross-sections depending on the variability in channel width and depth throughout the segment. A stopwatch was used to measure the average time required to float the object through the segment based on three trials. Discharge in cubic meters per second was determined based on the following equation (U.S. EPA 1997):

Flow = ALC/T

Where:

A = average cross-sectional area of the stream (average width times average depth) (m) L = float length (m) C = 0.8 (correction factor for rocky bottom streams)

T = average time (seconds)

#### 2.3.3 Substrate

The relative percent cover of each substrate type was visually estimated for the entire stream reach. Substrate types were delineated based upon size fractions and included bedrock (>4906 mm), boulders (256 to 4096 mm), cobble (65 to 256 mm), gravel (2 to 65 mm), sand 0.06 to 2 mm, and silt (< 0.06 mm). I reduced the number of substrate variables tested in principal components and non-metric conceptual clustering analyses to focus on those variables which most strongly affect macroinvertebrate communities. I combined percent cover of sand and silt to create a percent fine sediment variable and combined percent cover of boulder and bedrock substrate to create a percent boulder/bedrock variable.

#### 2.4 WATER CHEMISTRY

#### 2.4.1 *Field Methods*

Water chemistry measurements and samples were collected midway along the stream reach between the location of the third and fourth macroinvertebrate samples. Water temperature and dissolved oxygen were measured in the field using a Yellow Springs Instrument (YSI) field meter or a field thermometer and Hach Dissolved Oxygen kit (Model OX-2P). Water samples for alkalinity, conductivity, pH, turbidity, total phosphorus, total nitrogen, and total suspended solids were collected in clean, acid-washed (2 N HCl) 1-Liter polyethylene bottles. Due to the remote location of the study site and short holding times, water samples for anions, soluble reactive phosphate, nitrate, and ammonia were filtered through glass fibre filters (Whatman GF/F filters; 0.45-µm) in the field using a Nalgene filtering apparatus. Filtered water for anion analysis was stored in a 60-mL polyethylene bottle that had not been acid washed. Filtered water for soluble reactive phosphate and nitrate analysis was collected in a clean, acid-washed (2 N HCl) 60-mL polytheylene bottle. Prior to collecting each sample, bottles were rinsed three times with filtered stream water. Sample bottles were filled to the rim and care was taken to not introduce any particulate matter into the sample. For ammonia analysis, 25 mL of the filtered stream water was collected in a clean, acid-washed, graduated tube and acidified to a pH less than 2 with 0.5 mL of 1 N HCl. For chlorophyll a analysis, 500 mL of stream water was filtered through a 0.45-µm glass fiber filter in the field, and the filter was stored in a 25-mL plastic scintillation vial. To minimize post-collection photosynthesis, stream water was filtered in the dark by placing a piece of clothing over the filtering apparatus. In addition, we wrapped each scintillation vial in foil. All water samples were stored in cooler bags or dry bags packed with snow and kept in cold water until they

were transported to the Institute for Watershed Studies (IWS) at Western Washington University in Bellingham, WA, a Washington State Department of Ecology accredited laboratory. No more than three days elapsed between water collection and storage in the laboratory.

#### 2.4.2 Laboratory Processing

Each sample was analyzed for pH, conductivity, phosphorus (total phosphorus and soluble reactive phosphate), nitrogen (total nitrogen, nitrate, and ammonia), turbidity, total suspended solids, alkalinity, and chlorophyll *a*. Water samples were processed according to the *Standard Methods for the Examination of Water and Wastewater* (Eaton et al. 2005; Table 3). Laboratory analysis measured combined concentrations of nitrate and nitrite. In freshwater systems nitrite is readily oxidized to form nitrate and only represents a small fraction of the combined concentration. For ease of reading in the remainder of this document, I will use the term nitrate to describe the combined concentration of nitrate and nitrite. Turbidity, total suspended solids, conductivity, pH, and alkalinity were measured at IWS within seven days of collection. Cholorophyll *a* analysis was conducted within one month of sample collection. Soluble reactive phosphate, total phosphorus, ammonia, and nitrate were analyzed within 45 days of collection at IWS.

Anions (Br, Cl, Fl, NO<sub>3</sub>, and SO<sub>4</sub>) were initially measured within 50 days of collection using high performance ion chromatography (HPIC). Most of the sample concentrations from this analysis were below the standard curve for each of the tested anions. Anions were reanalyzed in January 2009 using lower standard concentrations. Sulfate measurements for three lake outlet sites were within the standard curve range for both 2007 and 2009 analyses. The difference between 2007 and 2009 sulfate concentrations for these three sites ranged from 0.0 to -0.85 ppm. The 2007 sulfate concentrations were used for analysis for these three sites. Data analyzed in 2009 were used for the remaining sites and anions. Due to the long time between collection and analysis, anion results were statistically analyzed separately from the other physico-chemical parameters.

#### 2.4.3 *Quality Control*

For quality assurance, duplicate field samples were collected at 10% of the study sites. Field duplicates test the accuracy associated with sample collection. In the laboratory, duplicate laboratory analyses were run on 10% of the samples to determine the accuracy of laboratory methods. For nutrient analysis, two internal check standards (20 and 80% of the calibration curve) were measured to verify that analytical precision and calibration biases were acceptable. No quality control measures were taken for temperature and dissolved oxygen measurements.

#### 2.5 MACROINVERTEBRATES

#### 2.5.1 *Field Sampling*

Stream reaches were 30 m long and began 10 m downstream from the lake outlet. For nine of the ten stream reaches we collected six replicate macroinvertebrate samples. Due to late summer low stream flow at Tapto, West, I collected only three replicate macroinvertebrate samples. Macroinvertebrate sampling was started at the downstream end of the stream reach working upstream. Samples were collected from riffle/fast- moving habitats. The sampling locations along the stream reach were chosen using a random number table. One random number between 0 and 10 was generated for each sample to be collected and was multiplied by 10 to represent the percent upstream along the stream reach length the sampler would be placed (PNAMP 2006). At all sampling sites the sampler was placed in the thalweg (deepest section) of the stream. For example, if the random number 1 was generated, the sampler was placed in the thalweg of the stream 10% upstream of the total length of the stream reach (3 m). If a sampling location was not suitable for sampling due to low flow, bedrock substrate, or pool habitat, another random number was generated until a suitable habitat was found. Tapto West and Upper Tapto were dominated by bedrock and boulder substrate and sampling locations were not selected randomly, but rather by locating the only available sampling locations for that stream reach.

At each sampling location, benthic macroinvertebrates were collected using a Surber sampler (0.09 m<sup>2</sup>, 500-µm mesh). The Surber sampler was placed in the streambed with the open end facing upstream and the bottom frame of the sampler flush with the streambed. Within the frame, all large stones and debris were picked up and carefully rubbed by hand so that any attached animals were swept downstream into the net; the stones were then discarded out of the frame. After all of the larger stones had been removed from the frame, we stirred the remaining gravel and sand with a trowel to a depth up to 10 cm starting at the upstream end of the sampling frame (PNAMP 2006). When boulders and bedrock prevented us from disturbing the substrate to a depth of 10 cm, we agitated the substrate as deep as possible. Macroinvertebrate samples were transferred to 500-mL Whirl-pak storage bags and fixed with 95% non-denatured ethanol. All macroinvertebrate samples were stored in cooler bags

or dry bags packed with snow and kept in cold water until they were transported to IWS where they were topped off with 95% ethanol.

#### 2.5.2 Laboratory Processing

At the onset of the laboratory processing, seven macroinvertebrate samples were sent to Rhithron Associates, Inc. (Missoula, MT) to be sorted and identified. Rhithron Associates provided a reference collection from these samples that I used to familiarize myself with common North Cascades taxa. I sorted the remaining 50 macroinvertebrate samples using a dissecting microscope by separating each macroinvertebrate specimen from the substrate and vegetation in the sample and placing it in a 70% ethanol and glycerin solution for later identification. One Petri dish of sample material was sorted at a time and rescanned for any overlooked specimens after the initial sorting. Each specimen was identified to the lowest possible taxonomic level. Most macroinvertebrate specimens were identified to genus or species, depending on the availability of taxonomic keys. All Chironomidae larvae were identified by Rhithron Associates to the species or genus level.

#### 2.5.3 Quality Control

To determine sorting efficacy, sample residue from 10% of the sorted samples was randomly selected to be re-sorted. Of the selected samples, a 20% aliquot of each residue was thoroughly re-sorted to check for overlooked specimens. In addition, 10% of the sorted samples were randomly selected to be re-identified and enumerated. Twelve percent of the samples collected were sorted and identified by Rhithron Associates. All mature Chironomidae larvae were also identified by Rhithron Associates, representing 16% of the

total number of macroinvertebrate specimens collected. As an additional quality control measure, representatives from 71% of the taxa I identified were sent to taxonomists for verification. Representatives of all Trichoptera taxa were verified by Robert Wisseman (Aquatic Biology Associates, Inc.). Ephemeroptera, Plecoptera, Coleoptera, Acari, and non-midge Diptera representatives, along with representatives from all Chironomidae pupae, were sent to Rhithron Associates, Inc. for taxonomic verification. Taxa identifications were corrected, if necessary, after verification. Taxonomic resources I used for macroinvertebrate identification included: Pennak (1989), Thorp and Covich (1991), Merritt and Cummins (1996), Wiggins (1998), Stewart and Stark (2002), and Adams et al. (2004).

#### 2.6 DATA SCREENING

Ammonia and soluble reactive phosphate measurements were below detection limits (10 and 3  $\mu$ g/L, respectively) at all sites and excluded from analysis (Table 4). Some lake outlet sites had concentrations below detection limits for the remaining nutrient parameters, but were included for rank-based tests (PCA and NMCAA) because the majority of the sites had concentrations above detection limits. Total suspended solid concentrations were below detection limits (2.6 mg/L) at all lake outlet sites and excluded from analysis.

#### 2.7 STATISTICAL ANALYSES

#### 2.7.1 Physico-chemical Parameters

Physical and chemical parameters were analyzed with graphics and with both confirmatory and exploratory statistics. Physico-chemical differences between kryal and rhithral lake outlet sites were described with box plots and Kruskal-Wallis tests with Wilcoxon contrasts (Kruskal and Wallis 1952). The Kruskal-Wallis test is a non-parametric method for testing equality of population medians among groups using ranked data.

I used principal components analysis (PCA) to determine if lake outlet sites ordinated by primary water source based on physico-chemical variables. This linear model searches for combinations of variables that explain the most variance in the data. The first principal component accounts for as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible (Pearson 1901). All physico-chemical data were  $log_{10} (x + 1)$  transformed prior to conducting the ordination in order to improve the fit.

To explore clustering patterns within the physico-chemical data set, I used non-metric conceptual clustering with association analysis (NMCAA) based on RIFFLE software (Matthews and Hearne 1991). Non-metric conceptual clustering utilizes all levels of data without requiring transformation, is able to detect and control for noisy variables, and is not sensitive to missing data. Non-metric conceptual clustering is a powerful tool that does not use distance metrics, but rather evaluates each variable independently and compares it to an evolving clustering scheme based on common properties. Non-metric conceptual clustering searches the dataset iteratively and arrives a final cluster solution when the largest number of variables are associated with the strongest cluster solution. Chi-squared tests ( $P \le 0.05$ ) were conducted on the generated clusters to measure the degree of association with physico-chemical parameters. Non-metric conceptual clustering also measures the proportional

reduction in error (PRE) for each variable reporting how strong a particular variable is in predicting cluster membership.

#### 2.7.2 Macroinvertebrates

#### 2.7.2a Assemblage Composition

Macroinvertebrate density per square meter was calculated for each sample and averaged for the number of samples taken per sampling reach. Total, non-insect, and Chironomidae density and taxa richness were compared using nonparametric Kruskal-Wallis tests with Wilcoxon contrasts (Kruskal and Wallis 1952). I also visually compared the distribution of taxa that were only present at either rhithral or kryal lake outlet sites.

#### 2.7.2b Functional Feeding Groups

Taxa were placed into functional feeding groups of collector filterer, collector gatherer, macrophyte shredder, shredder, scraper, predator, parasite, omnivore, and unknown feeding group based on Merritt and Cummins (1996) with modifications by Wisseman and Doughty (2004). I used graphical analyses to examine the differences in functional feeding groups between rhithral and kryal lake outlet sites.

#### 2.7.2c Habit Types

Taxa were placed into habit types of skater, floater, planktonic, diver, swimmer, clinger, sprawler, climber, burrower, attacher, miner, and hyporheic based on Merritt and Cummins (1996) with modifications by Wisseman and Doughty (2004). Habits describe the mode by which macroinvertebrates maintain their location within the aquatic habitat. Determining the

habit types of macroinvertebrates can also tell something about environmental conditions where they were collected (i.e. substrate, flow, turbulence; Merritt and Cummins 1996). I used graphical analyses to examine the differences in habit types between rhithral and kryal lake outlet sites.

#### 2.7.2d Cluster Analysis

Differences in macroinvertebrate assemblage composition among sites were analyzed using NMCAA for macroinvertebrate density per square meter values for each taxon. Based on NMCAA results, I conducted nonparametric Kruskal-Wallis tests with Wilcoxon contrasts (Kruskal and Wallis 1952) between kryal and rhithral sites for taxon variables with strong predictive power of cluster membership based upon their PRE scores.

In addition, I used hierarchical clustering (Euclidean distance and Ward's linkage) to classify taxa presence/absence data. Although the Euclidean distance metric is designed for continuous data, it is acceptable for use with binary data when there are many variables, a trait common to macroinvertebrate studies (Pielou 1984). Ward's linkage is a minimum variance clustering method and is a useful metric when clusters are of equal size, such as in this study (Everitt 1993).

#### 2.7.3 Invertebrate-habitat relationship

I used non-metric conceptual clustering to determine if combined physico-chemical and macroinvertebrate data would cluster based on primary water source. To explore the relationship between environmental conditions and macroinvertebrate communities, I ran Kendall's correlation analysis (Kendall 1938) on all physico-chemical variables and selected macroinvertebrate indices (Total, EPT, Non-Insect, and Chironomidae densities and Total, EPT, Non-insect, and Chironomidae taxon richness).

### **3 RESULTS AND DISCUSSION**

#### 3.1 PHYSICO-CHEMICAL PARAMETERS

#### 3.1.1 Principal Components Analysis

Principal Components Analysis clearly ordinated kryal and rhithral sites based on physicochemical variables (Figure 3). Sixty percent of the cumulative variance was explained by the first two principal components. Turbidity was the variable with the greatest influence in moving sample ordinations toward the west on PC1 and percent fine substrate was the most influential variable for movement south on PC2. Kryal sites were ordinated along these variables with higher turbidity and percent fine substrates than the rhithral sites. Kryal lake outlets were also ordinated with higher nitrate and alkalinity concentrations, increased stream discharge, lake surface area, and lower in-stream stability. Rhithral sites were ordinated with higher percentages of boulder and bedrock substrate, total nitrogen and chlorophyll *a* concentrations and water temperature.

#### 3.1.2 Cluster Analysis

To explore the physico-chemical differences between lake outlet sites, I also conducted NMCAA. Non-metric conceptual clustering of physico-chemical variables also clearly separated kryal and rhithral lake outlet sites. Chi-squared testing for association between

cluster classifications identified that 35% of the NMCAA iterations were significantly associated with kryal and rhithral water sources (chi-square = 6.4, df =1, P = 0.01). Of the twenty non-metric conceptual clustering iterations, this significant cluster solution was repeated seven times with no misclassifications (100% accuracy).

Eleven of the nineteen physico-chemical variables had principal reduction in error scores equal to or greater than 0.50 and were selected as the best indicators of water source (Table 5). A PRE score of 0.50 indicates that the ability to predict variables based upon cluster membership into kryal and rhithral categories is increased by 50% over an uninformed prediction based purely on chance. Three of these variables, water temperature, dissolved oxygen, and stream discharge, had PRE scores of 1.0. In the case of water temperature, dissolved oxygen, and stream discharge, the ability to predict variables based upon cluster membership was increased by 100% over an uninformed prediction. Randomizing the physico-chemical data set and producing no significant clusters associated with kryal and rhithral primary water source. This result suggests that the cluster membership into kryal and rhithral categories was not likely to occur by chance.

#### 3.1.3 Descriptive Statistics, Boxplots, and Multisample Tests

Ordination and cluster analysis highlighted several physico-chemical variables that separated kryal and rhithral lake outlet sites. For these and the other physico-chemical variables I used descriptive statistics, boxplots, and Kruskal-Wallis tests to detail the differences between kryal and rhithral lake outlets.

#### 3.1.3a Geographical Location

Although not statistically different (Figure 4, Table 6) as separate variables, study site elevation and longitude were top variables predicting non-metric conceptual clustering membership (Table 5). Rhithral study sites were generally higher and less variable in elevation than kryal sites (Figure 5). This may be due in part to the closer geographical distances of four of the five rhithral sites, which were all located in the Little Beaver Creek drainage (Figure 2).

#### 3.1.3b Lake Surface Area

Although no statistically significant differences were found between kryal and rhithral lake surface area (Figure 4, Table 6), the variable had a PRE score of 0.60 for the physicochemical NMCAA (Table 5). Overall, lakes feeding kryal outlet streams were larger in surface area than rhithral lakes. Lakes influence the channel stability, primary productivity, and discharge and thermal regimes of outlet streams (Milner and Petts 1994). The degree of this influence may be related to lake size (Gustafson 2008).

#### 3.1.3c Discharge

Lake outlet discharge was significantly higher at kryal sites than at rhithral sites (Figure 4, Table 6). In an alpine environment, discharge is primarily affected by water source and seasonal changes in weather. In a kryal stream, discharge slowly increases over the spring as first snow and then glacial meltwater enter the system. After the surrounding snowpack has melted away, glacial melt is the primary source of water into the stream. Discharge reaches a peak mid-summer when the higher zones of the glacier also begin melting. In the Northern Hemisphere, this peak in discharge usually occurs in July. In contrast, rhithral streams can have a peak in discharge as early as April then decline over the summer as winter snowpack melts away (Milner and Petts 1994).

The timing and intensity of these discharge peaks may be mitigated by the presence of a lake upstream, which can stabilize the discharge regime of outlet streams (Spence and Hynes 1971, Milner and Petts 1994). At my lake outlet sites, discharge was measured in late August and September, well past the predicted peaks for both stream types. The rhithral streams had mostly returned to their baseflow, with some sites in the Little Beaver Creek basin barely flowing. The higher discharge measured at the kryal sites in late summer indicates that the year-round permanence of a glacier contributes meltwater to these streams throughout the entire summer season.

#### 3.1.3d Pfankuch Bottom Index of Channel Stability

Discharge also has a strong influence over the physical characteristics of lotic environments. The volume and velocity of water work to form and alter stream channels. Kryal streams were significantly more unstable than corresponding rhithral streams based on the PSI (Figure 4, Table 6). Channel stability in kryal streams rated from poor to good (values 60 - 16), whereas rhithral streams ranged from good to excellent ( $30 - \leq 15$ ). These results support similar findings from studies in Europe that also found kryal streams to be more unstable (Milner and Petts 1994, Ward 1994, Hieber et al. 2001). The seasonal and diel changes in discharge from glacial meltwater may influence in-stream stability through shifting channels and altering bedloads (Milner and Petts 1994, Brittain and Milner 2001).

#### 3.1.3e Substrate

When evaluated individually, percent silt, boulder, and bedrock substrates were significantly different between kryal and rhithral lake outlet sites (Figure 5, Table 6). Percent silt and boulder substrates were higher in kryal lake outlets, while percent bedrock was higher in rhithral sites. For PCA and NMCAA analyses, these variables were reduced to percent fine sediment (silt plus sand) and percent boulder/bedrock. Percent fine sediment was a top variable in both PCA (Figure 3) and NMCAA analyses (Table 5). Kryal outlet streams had significantly higher percent fine substrates than rhithral outlet streams, likely due to the contribution of rock flour from melting glaciers (Figure 4, Table 6; Milner and Petts 1994).

#### 3.1.3f Water Temperature and Dissolved Oxygen

Water temperature has been found to be one of the leading physico-chemical characteristics distinguishing kryal from rhithral streams with kryal streams typically not exceeding  $10^{\circ}$  C during summer months (Milner and Petts 1994). At my kryal sites water temperatures ranged from 2.2 to  $7.2^{\circ}$  C (median =  $4.8^{\circ}$  C), whereas rhithral sites had water temperatures ranging from 9.5 to  $12.0^{\circ}$  C (median =  $10.2^{\circ}$  C; Table 4). Not only was water temperature significantly lower at kryal lake outlets (Figure 4, Table 6), but it was one of the top non-metric conceptual clustering variables predicting cluster membership (Table 5). Kryal sites have cold water temperatures throughout the summer because of the continued contribution of glacial meltwater (Milner and Petts 1994). Kryal lake outlets were more oxygenated due to cooler temperatures and higher discharge although, overall, rhithral sites were still near or at oxygen saturation ((9 - 10 mg/L or 90 - 100%; Figure 4, Table 6).

#### 3.1.3g Turbidity, Total Suspended Solids and Chlorophyll a

Turbidity and total suspended solids (TSS) are both measures of suspended solids in the water column. Turbidity measures the amount of light scattered from a sample (more suspended particles cause greater scattering), whereas TSS measures the weight of the particles per volume of water (Wetzel 2001). Based upon a review of kryal research, Milner and Petts (1994) found that kryal stream turbidity and TSS concentrations in the summer were generally greater than 30 NTU and 20 mg 1<sup>-1</sup>, respectively, from the contribution of rock flour. Rock flour (aka. glacial flour) is finely ground, clay-sized particles of rock that are produced as glaciers cause rocks to grind beneath them during advance and retreat. When glacial ice melts during the summer these particles become suspended and distributed throughout the stream.

Median turbidity concentration at my kryal lake outlet sites was 12.6 NTU, lower than the typical concentration of 30 NTU, although significantly higher than rhithral sites (Figure 4, Table 6). Turbidity was also a top variable in both PCA (Figure 3) and NMCAA analyses (Table 5). In this study, TSS concentrations were below detection limits and excluded from analysis (Table 4). The fact that turbidity values were measurable and TSS was not may be due to the limitations of field research in a remote, mountainous location. As a result of weight restrictions, only 500 mL of water was carried out to filter for total suspended solids. This volume turned out to be insufficient to yield detectable levels of suspended solids. The lower than typical values for both turbidity and TSS at my kryal lake outlets was most likely due to rock flour settling out in the lake before discharging into the outlet stream. Although below detection limits, I ran analyses to determine if there were any patterns in the TSS data

relative to primary water source. Lake outlet TSS was positively correlated to turbidity (Kendall's  $\tau = 0.73$ , P = 0.002) and significantly higher in kryal lake outlet sites (Kruskal-Wallis chi-squared = 5.8, df = 1, P = 0.02).

Chlorophyll *a* is a measure of algal productivity. High turbidity can reduce primary production by limiting the penetration of light into the water column (Milner and Petts 1994). Although not significantly different between sites (Figure 4, Table 6), non-metric conceptual clustering found that chlorophyll *a* was an important indicator variable identifying water source (Table 5). Overall, chlorophyll *a* was higher and more variable at rhithral lake outlets than kryal lake outlets. Warmer water temperatures along with greater light penetration in rhithral lake outlets likely created a more hospitable environment for primary production than kryal sites. The presence of aquatic mosses at rhithral lake outlet sites in comparison to little or no aquatic moss found at kryal sites (personal observation) further support these findings.

#### 3.1.3h Nutrients: Nitrogen and Phosphorus

Overall, nutrient concentrations were low at all lake outlet sites indicating these are oligotrophic (low productivity) systems limited by nitrogen and phosphorus (Table 4). Although not statistically significant, nitrate was one of the top physico-chemical variables predicting kyral and rhithral cluster membership (Table 5). In an alpine ecosystem, nitrogen enters aquatic environments primarily although meltwater from glaciers and snowpacks (Barica and Armstrong 1971, Malard et al. 1999). Nitrogen is deposited onto the tops of glaciers and snowpack through atmospheric deposition and is stored underneath them as organic matter decomposes. Phosphorus is the nutrient primarily limiting biological

productivity in aquatic ecosystems (Wetzel 2001). Research has linked particulate phosphorus concentration with seasonal and diel discharge pulses in kryal streams (Tockner et al. 1997, Hieber et al. 2001). Phosphorus tends to bind to the surface of small inorganic and organic matter and is often moved from terrestrial to aquatic environments through runoff (Wetzel 2001). Phosphorus has been found to be correlated with total suspended solid concentration in other alpine streams (Hieber et al. 2001). The overall low values of both TSS and phosphorus at my lake outlets may be because oligotrophic alpine lakes often act as sinks rather than sources of particles (Hieber et al. 2001).

#### 3.1.3i Alkalinity, pH, and Conductivity

Alkalinity, pH, and conductivity are related in aquatic systems (Matthews et al. 2006). Conductivity and pH are measures of dissolved ions in the water. Conductivity measures the ability of a solution to carry an electrical flow (Wetzel 2001). Acidity, reported as pH, is determined by measuring the proton activity of hydrogen ions in a solution (National Research Council 1983). Alkalinity is the ability of a solution to neutralize (buffer) acids and is usually related to the levels of carbonate ions (carbonic acid, carbonate, and bicarbonate) in the water. Higher carbonate levels in water result in a higher buffering capacity (Wetzel 2001).

The range of pH values at all lake outlet sites was 4.8 to 6.7 (median 6.2; Table 4), and values were not significantly different between kryal and rhithral lake outlets (Table 6). The pH of natural waters ranges from < 2 to 12, where pH 7 is neutral, values below 7 are increasingly acidic and values above 7 increasingly basic (Wetzel 2001). In natural waters,
lethal effects of acidity typically occur at a pH of 4.5 (Wetzel 2001), although negative effects in biological communities are seen starting at pH values just below 6.0 (Mills and Schindler 1986). Rhithral sites Upper Tapto, Tapto West and Lower Middle and kryal site M-25-01 all had pH values below 6.0 (Table 4).

Overall, all lake outlets had low alkalinities (< 5.5 mg/L) indicating that they have low buffering capacity and are vulnerable to acidification (Table 4). Alkalinity was significantly correlated to pH (Kendall's  $\tau$  = 0.87, P = 0.0001), and those sites with the lowest pH (< 6.0) also had the lowest alkalinities (< 1 mg/L). Although not significantly different, alkalinities were generally higher at kryal sites than rhithral sites (median values 2.8 and 0.6 mg/L as CaCO<sub>3</sub>, respectively; Table 6). In their survey of 54 North Cascades lakes, Loranger et al. (1986) found that bedrock appeared to be the controlling factor for surface water acid neutralizing capacity. Acid neutralizing capacity is closely related to alkalinity and is a measure of the overall buffering capacity of a solution to acidification. Slow weathering processes and the infrequent occurrence of carbonate rocks such as limestone and dolomite in the North Cascades result in low buffering capacity of surface waters (Loranger et al. 1986, Holloway 1993).

Conductivity was not significantly different between kryal and rhithral sites (Table 6). Conductivity has been defined as one of the important water quality variables characterizing kryal streams (Milner and Petts 1994). Conductivity of ice melt is usually less than 10  $\mu$ S cm<sup>-1</sup>, but can reach values up to 50  $\mu$ S cm<sup>-1</sup> through ionic enrichment from groundwater inputs (Milner and Petts 1994). Conductivity at kryal lake outlet sites was below 50  $\mu$ S cm<sup>-1</sup> ranging from 3.8 to 24.2  $\mu$ S cm<sup>-1</sup> (median = 16.8  $\mu$ S cm<sup>-1</sup>; Table 4). Conductivity at rhithral lake outlets ranged between 4.3 and 76.6  $\mu$ S cm<sup>-1</sup> (median = 9.3  $\mu$ S cm<sup>-1</sup>).

Conductivity was highest at the rhithral site Lower Middle (76.6  $\mu$ S cm<sup>-1</sup>), which also had the lowest pH and alkalinity measurements (4.8 and  $\leq$  0.1 mg/L, respectively; Table 4). This lake outlet was unusual in that there was iron hydroxide (FeOH) precipitate covering all substrate in the stream along with the littoral edge of the upstream lake (Figure 6). High iron concentration in the lake outlet could explain the high conductivity measurement. Reddish, iron-rich rocks were seen surrounding this lake. An input of groundwater, rich in iron, may be the cause of the iron precipitate in Lower Middle lake and outlet stream. An alternate explanation could be the low pH at this site may be releasing iron from terrestrial soils and lake sediments into the lake and outlet stream (Schindler 1988). In comparison, Upper Middle lake and its outlet stream that feed into Lower Middle lake did not show any iron precipitate and had pH, alkalinity, and conductivity values of 6.2, 2.4 mg/L, and 26.8  $\mu$ S cm<sup>-1</sup>, respectively (Table 4).

# 3.1.3j Anions

There were no statistically significant differences between kryal and rhithral lake outlets for fluoride, chloride, sulfate, and nitrate anions (Table 6). Bromide anions were not detected at any of the lake outlet stream sites (Table 4).

# **3.2 MACROINVERTEBRATES**

#### 3.2.1 Assemblage Composition

A total of 93 macroinvertebrate taxa were represented by 24,985 specimens collected from all lake outlet sites. Of the 93 taxa collected, 27 were from the orders Ephemeroptera, Plecoptera, or Trichoptera (EPT), 47 were Diptera, and 19 were Collembola, Coleoptera, Hemiptera and non-insects (Table 7). Rhithral lake outlets had significantly higher densities and supported more taxa than kryal sites (Figure 7). A total of 77 macroinvertebrate taxa were collected at rhithral lake outlet sites and the mean density of individuals per square meter was 9,049. At kryal lake outlets, 35 taxa were collected and the mean macroinvertebrate density was 821 individuals/m<sup>2</sup>. Chironomidae was the numerically dominant taxon amongst all sites, although densities and taxon richness were 1/3 in the kryal lake outlets when compared to rhithral sites. Rhithral lake outlets contained significantly higher densities and numbers of non-insect taxa such as Acari (mites), Oligochaeta (segmented worms), Nemathelminthes (nematodes), Planariidae (planaria) and crustaceans (copepods) (Figure 7).

#### 3.2.2 Trophic Structure

Collector-gatherers were the dominant functional feeding group at both rhithral and kryal lake outlets (Table 8). These results reflect the large number of collector-gatherer chironomid taxa found at these sites. Omnivores were the second most dominant functional feeding group at the kryal sites (29%), most likely driven by the large numbers of the plecopteran *Taenionema* sp. Banks collected at the Ouzel Lake outlet. Parasites and predators were the second and third most dominant functional feeding group at the rhithral outlets (12 and 10%,

respectively). Parasites were composed of the large numbers of Acari and Nematodes collected at these sites. Acari, commonly known as mites, are a diverse group and depending on the species can be classified as either parasite or predator. For this analysis I placed half of the Acari collected in the parasite FFG and the other half in the predator FFG. The predators at rhithral lake outlets were composed of Acari, Coleoptera, Diptera, Plecoptera, and Trichoptera species.

Functional Feeding Groups (FFG) describe the way in which an organism feeds and can help explain food web interactions within a stream. Filter feeder taxa typically dominate low to mid-elevation lake outlets (Richardson and Mackay 1991). In alpine lake outlets, however, this pattern does not always hold true. Some alpine studies have found filter-feeders to be the dominant taxa (Kownacki et al. 1997, Donath and Robinson 2001), while others found collectors and collector-gatherers to be the dominant functional feeding groups (Hieber et al. 2005, Hamerlík et al. 2006). High concentration of seston (suspended particles) flowing from the upstream lake is the factor most often attributed to high densities of filter feeders in outlet streams (Richardson and Mackay 1991). The low concentrations of seston flowing out of many oligotrophic alpine lakes may explain why filter feeders are not always dominant in these outlets (Hamerlík et al. 2006). Low TSS, turbidity, and cholorphyll *a* concentrations at my lake outlets support this theory. In addition, particulates tend to settle out of the water column in slow moving water such as that found in lakes. As water speeds up during the transition from lentic to lotic environments in a lake outlet, less seston may settle.

# 3.2.3 Habit Types

Sprawlers were the dominant habit taxa type for kryal lake outlets (55%) followed by burrowers (24%) and clingers (10%; Table 9). The habit for almost half of specimens (49%) from the rhithral lake outlets is unknown due to a lack of habitat information on many benthic invertebrate taxa. Of the taxa with known habit types, the top three were sprawlers (21%), clingers (17%), and burrowers (8%). Many of the chironomid taxa collected at both kryal and rhitral sites were sprawlers. Sprawlers crawl on substrates such as rocks, fine sediments, woody debris, and leaf packs in running and still waters. Chironomid sprawlers often reside in porous areas of rocks or debris and may become partially covered with sediment. Clingers are able to remain stationary on substrates in flowing water usually due to specialized grasping claws, hooks, or ventral attachment discs. Burrowers are benthic insects that burrow and live in the soft bottom substrates of slower moving sections or bank areas of streams (Merritt and Cummins 1996).

## 3.2.4 Cluster Analysis

The non-metric conceptual clustering results suggest that benthic invertebrate communities were responding to the different primary water sources. Chi-square testing for association between cluster classifications identified that 60% of non-metric conceptual clustering iterations were significantly associated with kryal and rhithral water sources (chi-square = 3.75, df = 1, P = 0.05). Three different significant cluster solutions each repeated four times were derived from the 20 non-metric conceptual clustering iterations (Table 10). Classification accuracy was similar among the cluster solutions with only one stream site misclassified in each cluster solution (90% accuracy). Different stream sites were

misclassified in each of the three cluster solutions. Tapto West was misclassified as a kryal site in cluster a, Lower Middle was misclassified as a kryal site in cluster b and M-25-01 was misclassified as a rhithral site in cluster solution c.

The misclassification of these three stream sites can be understood by exploring the variables with the highest PRE scores for each of the three different cluster solutions. In cluster solution *a* Tapto West was misclassified as a kryal stream. In this cluster solution *Eukiefferiella claripennis* Group Lundbeck and *Setvena* sp. Illies were the top two variables with PRE scores of 1.0. *Eukiefferiella claripennis* Gr. was not found in any of the kryal sites (Table 7). At Tapto West, *Eukiefferiella claripennis* Gr. density was 7.4 individuals m<sup>2</sup>, whereas the other 4 rhithral sites had a median density of 39 individuals m<sup>2</sup>. Similarly, *Setvena* sp., were not found at any of the kryal streams sites or at Tapto West.

Lower Middle was misclassified in cluster solution *b*. Nine invertebrate taxon variables had PRE scores of 1.0: Acari, *Corynoneura* sp. Winnertz, early instar Ephemeroptera, early instar Plecopterea, Harpacticoida, *Polycelis coronata* Girard, *Rhyacophila rotunda* Gr. Banks, *Setvena* sp., and *Zapada columbiana* Claassen (Table 10). Harpacticoida, *Rhyacophila rotunda* Gr., *Setvena* sp., and *Zapada columbiana* were not found at any of the kryal lake outlet sites and their densities were lower at Lower Middle relative to the other rhithral sites.

In cluster solution *c*, M-25-01 was misclassified as a rhithral site. *Megarcys* sp. Klapálek and *Rhithrogena* sp. Eaton were the top two PRE variables with scores of 1.0 in this cluster solution (Table 10). In both cases no specimens from these taxa were found at M-25-01 or

any of the rhithral sites. M-25-01, located in the Diobsud Creek drainage, is a unique site. This kryal lake has recently emerged from Bacon Glacier over the past 60 years as the glacier has receded (Figure 9). The few summers that the lake has been ice-free have left little time for invertebrates to colonize the lake and outlet stream as shown by the small numbers of specimens collected (15 ind.) represented by only five taxa (Oligochaeta, Nematoda, and the chironomids *Diamesa* sp. Meige, *Dipocladius* sp.Kieffer, *Orthocladius* sp. Wulp).

For each taxon variable, I averaged the PRE scores for all three cluster solutions to determine which variables had a mean PRE score equal to or above 0.5. When combined, 17 of the 118 invertebrate taxon variables had mean PRE scores equal to or above 0.5 and possessed the highest predictive power in assigning sites into kryal and rhithral categories (Table 5). The highest mean PRE score for the combined cluster solutions was 0.78 for early instar Chironomidae densities. Although numerous at all sites, chironomid densities were significantly higher at rhithral lake outlets, especially for early instar larvae (Figure 8). Randomizing the macroinvertebrate data weakened the results producing no significant clusters associated with kryal and rhithral primary water source. This provides further evidence that cluster membership into kryal and rhithral categories was not likely to occur by chance.

#### 3.2.5 *Hierarchical Clustering*

Hierarchical clustering of presence/absence macroinvertebrate data reinforced non-metric conceptual clustering results that primary water source was a determinant in structuring

macroinvertebrate communities. Kryal and rhithral sites were clearly separated using squared Euclidean distance with Ward's Method (Figure 10).

## 3.2.6 Comparison of Macroinvertebrate Distributions

Of the 93 taxa collected from all study sites, 55 were only found at rhithral outlet streams (Table 11). Twenty-five of these 55 taxa were in the Chironomidae family. The chironomids *Eukiefferiella claripennis* Gr. and *Rheocricotopus* sp. Thienemann and Harnisch were collected at all five rhithral sites; *Zavrelimyia* sp.Kittkau and *Corynoneura* sp. were found at four sites; and *Eukieffella brehmi* Group Gowin, *Psectrocladius* sp. Kieffer, and *Micropsectra* sp. Kieffer at three sites. The remaining 48 chironmid taxa were collected at all rhithral sites. From the EPT taxa, *Zapada columbiana* was found at all rhithral lake outlets and *Setvena* sp. and *Rhyacophila rotunda* Gr. were found at four of the five rhithral outlets. Springtails from the Poduridae family were found at four rhithral sites and those from the Isotomidae family were found at three lake outlets.

Twelve macroinvertebrate taxa were only collected at kryal lake outlets (Table 11). Of these 12, two taxa, the plecopteran *Megarcys* sp. and ephemeropteran *Rhithrogena* sp. were found at all five kryal lake outlets. The Ephemeropteran *Epeorus* spp. (*E. deceptivus* McDunnough, *E. grandis* McDunnough or both) and the chironomid *Parothocladius* sp. were found at three of the five sites. The remaining eight taxa were collected at only one or two kryal outlet streams.

When comparing the distribution of taxa between kryal and rhithral lake outlet streams, two divergent groupings become apparent. The perlodid *Megarcys* sp. was only collected at kryal lake outlets, while *Setvena* sp. was only found at rhithral sites. Anatomically, these genera are very similar separated primarily by an extra set of gills on *Megarcys* sp. (Merritt and Cummins 1996). Both of these taxa are clingers adapted to life in fast-moving water. Some differences exist between these genera, *Megarcys* are omnivores, whereas, *Setvena* are predators. *Megarcys* is also cold stenotherms; capable of living and growing within a limited range of temperatures, making them suitable for a glacial environment. Similarly, *Zapada oregonensis* Gr. was only collected at kryal sites, while *Zapada columbiana* was only found at rhithral sites. These nemourids are anatomically separated by a slight difference in their gill structure with *Zapada columbiana* having a constriction in its neck gills while *Zapada oregonensis* Gr. does not. Both taxa are shredders and sprawler/clingers, and mostly grow during the fall (Adams et al. 2004).

The fact that there is no overlap in distribution between these two closely related taxonomic groups suggests that primary water source is the driving factor. However, I did not find any literature suggesting that *Megarcys* and *Zapada oregonensis* Gr. are only present in kryal systems or that *Zapada columbiana* and *Setvena* are not. At Mt. Rainier National Park (MORA), also located in the Cascades, *Megarcys* sp., *Setvena* sp., *Zapada oregonensis* Gr., and *Zapada columbiana* were collected in both glacial and non-glacial streams and rivers (Kondratieff and Lechleitner 2002).

If primary water source is not excluding these taxa, perhaps they have yet to disperse to all of the various study sites. In the alpine environment, migration via larval drift or adult flight is difficult due to the low biogeographical connectivity between mountainous watersheds (Monaghan et al. 2005) and the short ice-free season. Glaciers blanketed the region during the most recent glacial event, the Wisconsin glaciation, which began about 80,000 years ago and ended around 10,000 years ago (USGS 2003). Four of the five rhithral sites are located within the Little Beaver Creek drainage, making dispersal between these sites easier. However, both Setvena and Zapada columbiana were also collected at Upper Thornton lake outlet, over 20 kilometers from the other study sites. In addition, the kryal sites were spread out throughout the entire western slope of the park suggesting that these taxa had equal opportunity to also disperse to the rhithral lake outlet sites. Elevation could also possibly be driving these distribution patterns. In general, rhithral lake outlets were higher in elevation than kryal sites and Megarcys and Zapada oregonensis Gr. could be replaced by Setvena and Zapada columbiana with increasing elevation. Zapada oregonensis Gr. was not collected above 1,208 m. *Megarcys*, however, was found at elevations 192 m higher than sites where Setvena was collected.

It could be that these taxa have a preference for primary water source but will colonize outside of these groups. Mt. Rainier National Park has more biogeographical connectivity than the multiple peaks in NOCA because its watersheds all originate off one volcanic peak. This landscape feature may explain why these taxa have already dispersed throughout MORA, but have not yet done so in NOCA. Macroinvertebrate sampling from more streams throughout the park may help to determine if indeed primary water source is influencing distribution.

# 3.3 INVERTEBRATE-HABITAT RELATIONSHIP

#### 3.3.1 Cluster Analysis

The non-metric conceptual clustering clearly separated outlet streams of kryal and rhithral origin based on physico-chemical and taxon variables. Chi-square testing for association among the cluster classifications identified that 35% of the non-metric conceptual clustering trials were significantly associated with kryal and rhithral sources (chi-square = 6.4, df =1, P = 0.01). Of the twenty NMCAA iterations, one significant cluster solution was derived and repeated seven times with no misclassifications of lake outlet sites (100% accuracy).

Twenty-nine of the 137 physico-chemical and invertebrate variables had PRE scores that were equal to or above 0.50 and were considered the variables with the best predictive power for clustering kryal and rhithral sites (Table 5). Ten of the 11 top physico-chemical variables and 15 of the 17 top macroinvertebrate variables were also selected in the combined data set. Of the combined data set, dissolved oxygen, early instar Chironomidae, stream discharge and water temperature were the variables with the highest PRE values at 1.0. Randomizing the combined data set weakened the results producing no significant clusters associated with kryal and rhithral primary water source. This provides further evidence that cluster membership into kryal and rhithral categories was not likely to occur by chance.

## 3.3.2 Correlation Analysis

To understand how lake outlet physico-chemical parameters potentially affected macroinvertebrate communities at my study sites, I ran Kendall's non-parametric correlation analysis on all physico-chemical variables and selected macroinvertebrate indices (total, EPT, non-insect, and Chironomidae densities and total, EPT, non-insect, and Chironomidae taxon richness). Multiple significant correlations were found between invertebrate indices and physico-chemical variables (Figure 11, Table 12).

# 3.3.2a Water Temperature

Water temperature was positively correlated to total and non-insect densities and total and non-insect taxon richness (Figure 11, Table 12). In my study, lake outlet water temperature appeared to be a dominant physico-chemical variable driving differences in macroinvertebrate assemblages between kryal and rhithral sites. It was significantly lower in kryal lake outlets, was one of the top variables predicting clustering in non-metric conceptual clustering, and was significantly correlated to several invertebrate indicies (Figure 4 and 14, Tables 5, 6, and 10). Water temperature has long been recognized as one of the primary physical habitat factors influencing macroinvertebrate distribution and diversity (Vannote and Sweeney 1980, Vannote et al.1980, Ward 1985) especially in alpine streams (Milner and Petts 1994). In lotic waters, water temperature controls the rates of primary and secondary production, including the growth rates of macroinvertebrates. Water source is the primary driver of water temperature in alpine streams (Brown et al. 2003).

## 3.3.2b pH, Alkalinity and Turbidity

Richness of EPT taxa increased with both pH and alkalinity concentrations (Figure 11, Table 12). These results suggest that EPT taxa are particularly sensitive to acidic environments. Total and non-insect densities and EPT richness of taxa were negatively correlated to turbidity levels (Figure 11, Table 12). High turbidity can affect macroinvertebrate communities by reducing primary production through decreased light penetration, though discharge regime and channel stability must also be considered (Milner and Petts 1994).

## 3.3.2c Discharge and Pfankuch Bottom Index of Channel Stability

Non-insect taxa richness decreased with channel stability and densities decreased with increased discharge (Figure 11, Table 12). High discharge may lead to substrate and channel movement within the stream and limit the types of macroinvertebrates present because of the challenge in adapting to this physically shifting environment. High discharge in the summer may also make it difficult for macroinvertebrates to emerge (Milner and Petts 1994). The channel bottom of a stream is a completely aquatic environment and the location where macroinvertebrates feed, develop, and seek shelter. Based on the PSI, low channel stability implies not only movement of substrate within the channel, but a low resistance to and recovery from the forces stream flow may exert on it (Pfankuch 1975). If the stream bottom is frequently altered, it may reduce macroinvertebrate diversity to a few specialized taxa that are adapted to highly dynamic disturbance environment (Milner and Petts 1994). Some non-insect taxa, such as oligochaetes and nematodes, burrow into the stream bottom and may be adversely affected by frequent disturbance. In this study, high discharge and low channel stability were associated with kryal lake outlets. These features are characteristic of kryal

habitats and the lack of non-insect taxa present in is similar to studies from Europe (Hieber et al. 2005).

#### 3.3.2d Percent Fine Sediment

Non-insect taxon richness was negatively correlated with the percentage of fine sediment composing the stream substrate (Figure 11, Table 12). As the percentage of fines increased, non-insect taxon richness decreased. Fine sediments, such as silt and sand, may fill the interstitial spaces between larger pieces of gravel, decreasing the amount of available oxygen reaching macroinvertebrates living there. The character and type of substrate also affects the ability of insects to adhere, cling, burrow and build (Mackay 1977, Minshall 1984).

## 3.3.2e Lake Surface Area

Previous research has shown significant correlation between lake area and outlet chironomid taxa richness (Bitušík et al. 2006) while another study found that lakes with areas greater than 5 ha were colonized by more EPT taxa than smaller lakes (Hamerlík et al. 2006). The only significant correlation between lake surface area and macroinvertebrate density and richness indices in my study was a negative correlation between lake surface area and EPT density (Figure 11, Table 12). I found that as lake size increased EPT density decreased, showing the opposite effect of what Hamerlík et al. (2006) reported, probably because in my study, the larger lakes were glacially-fed.

## 3.3.2f Dissolved Oxygen

Dissolved oxygen concentrations ranged from 9 to 13 mg/L in all stream sites indicating oxygen saturation (Table 4). Ecologically, the difference between kryal and rhithral lake outlet dissolved oxygen concentrations would not be considered significant; however, dissolved oxygen was continually found to be a top variable determining clustering patterns in NMCAA (Table 5). In addition, dissolved oxygen concentration was significantly higher in kryal lake outlets than rhithral lake outlets (Figure 4, Table 6). To explore if there could be ecologically significant validity to this clustering, I ran Kendall's non-parametric correlation analysis on dissolved oxygen and all macroinvertebrate taxa collected at my study sites.

Sixteen of the 118 taxa had significant correlations with dissolved oxygen ( $P \le 0.05$ ; Figure 12). *Microspectra* sp., *Rheocricotopus* sp., *Zapada columbiana*, Poduridae, Harpacticoida G. O. Sars, and early instar Chironomidae densities increased with decreasing dissolved oxygen concentrations. *Megarcys* sp. and *Rhithrogena* sp. on the other hand were positively correlated with dissolved oxygen concentration. These results suggest that *Megarcys* sp. and *Rhithrogena* sp. thrive in a stream saturated with oxygen, while the other taxa are sensitive to saturation or more tolerant of lower oxygen environments. It is important to note, however, that with the exception of early instar Chironomidae, all of these taxa were found either at rhithral or kryal lake outlet sites, but not at both (Table 11). The correlations found between dissolved oxygen and macroinvertebrate taxa may have more to do with the multiple physico-chemical differences between kryal and rhithral lake outlets than to a true ecological significance. For example, at our lake outlet sites, dissolved oxygen was negatively

correlated to elevation (Kendall's  $\tau = -0.51$ , P  $\leq 0.05$ ), because with increasing altitude, the relative pressure decreases reducing gas solubility. In general, rhithral lake outlet sites were higher in elevation and had significantly higher dissolved oxygen concentrations. More research considering multiple physico-chemical variables and including a broader range of dissolved oxygen concentrations is needed to verify these results.

### 3.3.3 Predictive Model for Kryal Benthic Invertebrate Assemblages

From a synthesis of European and Alaskan literature, Milner and Petts (1994) first proposed and then modified (Milner et al. 2001) a conceptual model to predict the structure of macroinvertebrate assemblages in glacial streams based upon water temperature and channel stability (Figure 13). The model is only applied to the summer meltwater season when glacial melt is driving daily fluctions in abiotic variables that have a strong influence on macroinvertebrate communities (Milner et al. 2001).

In kryal streams when maximum summer water temperatures do not exceed 2° C and channel stability is low, chironomids from the family Diamesinae and especially those from the genus *Diamesa* dominate the macroinvertebrate community. *Diamesa* are generally cold adapted (Oliver 1983) and possess strong posterior proleg claws enabling them to grip substrate in the strong currents from cold glacial meltwaters (Milner and Petts 1994). As temperatures rise between 2 and 4 °C, chironomids from the Orthocladiinae subfamily begin to colonize; if channel stability is increased, tipulids and oligochaetes may be added to the community. Above 4° C, other dipterans (Simuliidae and Empididae) along with ephemeropterans (Baetidae) and plecopterans (Perlodidae and Taeniopterygidae) may colonize. With

increasing temperatures and channel stability, additional EPT and dipteran taxa are added to the community (Milner and Petts 1994).

Although I could not directly compare my results to the model because I only took one water temperature measurement, I did use the model to determine if my study sites in the North Cascades held similar communities to kryal streams in Alaska and Europe. Of the chironomids collected in this study, kryal lake outlets were dominated by two chironomid subfamilies, Orthocladiinae (62%) and Diamesinae (38%), with midges from the *Diamesa* genus representing 36% of the total number of chironomids collected. In addition, several EPT (Ameletidae, Ephemereliidae, Heptageniidae, Capniidae, Nemouridae, Perlodidae, Taeniopterygidae, Limnephilidae) and Dipteran (Tipulidae) taxa were collected at most of the kryal lake outlet sites, along with non-insects Oligochaeta, Nematoda, *Polycelis coronata*, Cyclopoida Burmeister, Araneae, and Acari. Based on the conceptual model, the community structure found at my kryal lake outlet sites is most similar to those seen at kryal streams with maximum summer water temperature above 8° C and high channel stability.

From the one measurement taken I did not find water temperatures above 8° C at any of my kryal lake outlet sites, emphasizing the need for more comprehensive thermal regime measurements to develop and refine models of community structure (Table 4). Channel stability at my kryal sites ranged from 18 to 50 (good to very poor) and did not show any similar community structure patterns in relation to channel stability as those found in the conceptual model (Figure 14). In addition, temperature and channel stability were not correlated at my kryal sites as shown in the conceptual model. Milner and Petts (1994)

acknowledged that their model would be modified by the presence of a lake, which can ameliorate physical conditions such as temperature and channel stability within the stream downstream (Milner et al. 2001). Studies including more kryal sites throughout the region would be needed to determine if this conceptual model is relevant to North Cascade glacial streams and outlets.

# 4 SUMMARY AND IMPLICATIONS

From my study it is clear that the primary water source has shaped the physical and chemical features of North Cascade lake outlet streams, which in turn have influenced macroinvertebrate assemblages. Similar to studies from Europe, the presence of a glacier was a major determinant driving several physical and chemical characteristics of lake outlet streams. As a glacier melts throughout the summer it contributes ice water and rock flour to streams and lakes downstream. Water temperatures decrease while discharge increases. Cold water temperatures and high discharge lead to saturation of dissolved oxygen. Diel and seasonal discharge fluctuations alter stream beds resulting in dynamic channel conditions. Rock flour increases turbidity in the water column reducing light penetration. This combination of frequently disturbed stream beds and reduced light penetration may lead to a decrease in primary production. As rock flour settles out into lake and stream beds, the percentage of fine sediments increases. These physico-chemical conditions create a harsh environment for benthic macroinvertebrate communities.

Hieber et al. (2005) modified a conceptual model first introduced by Poff (1997) describing the habitats of alpine stream systems as nested landscape filters that screen out regional species based upon their organismal traits (Figure 15). The assumption is that given a long enough period of time, all species are capable of dispersing to all locales in the region. The absence or low abundance of certain species at some locations must therefore be the result of selective filters. Filters are habitat features that can be defined at any scale (Poff 1997). Climate is the largest scale landscape filter determining the temperature and flow regime (permanent or intermittent) of alpine streams and thereby filtering out some taxa unsuited for these environments. Primary water source is the next filter shaping physico-chemical characteristics within the stream. The number and types of taxa passing through or blocked by this filter depend upon the water source. As applied to this study, kryal streams may be blocking out more taxa than rhithral streams, but let those pass that are tolerant to their harsh environmental conditions. Füreder (2007), found that taxa that are habitat specialists adapted to cold temperatures, low nutrient levels and high adult mobility are predominant in glacial streams. Channel type represents the next filter to the regional pool of alpine taxa. In this model lake outlets are classified as a channel type with the presence of an upstream lake affecting the water temperature, seasonality, and amount of transported organic matter in the outlet. At the smallest scale, differences in substrate or food resources can filter or favor certain taxa. Biotic interactions, touched upon but not modeled, may also constrain the distribution of certain taxa.

The one taxon passing through all of these filters at my lake outlet sites and dominating macroinvertebrate assemblages was Chironomidae. The family Chironomidae is the most widely distributed in freshwater ecosystems represented by approximately 15,000 species (Merritt and Cummins 1996). Chironomids evolved several hundred million years ago in

cold running water and have a wide range of tolerance from very sensitive to very tolerant (Lencioni and Rossaro 2005). On the tolerant end, some chironomid taxa are adapted to extreme environmental conditions such as desiccation, anoxia, low temperatures and freezing (Danks 1971, Danks and Oliver 1972). Their dominance in alpine ecosystems is in part due to their variety of adaptations to overwintering including their migration activity, cocoon building, supercooling, and freezing tolerance (Irons et al. 1993). Chironomidae is a diverse family with species filling many different niches in alpine streams. Understanding the habit type, function feeding group membership, phenology, and toleratnee to abiotic conditions of its species can help us to further understand alpine macroinvertebrate communities.

Alpine ecosystems are subject to high winds, cold temperatures, extended periods of snow cover and low humidity and precipitation. The combination of these conditions make alpine environments one of the most biologically demanding on the earth and sensitive indicators to climate change (Brown et al. 2007, Füreder 2007). Glacial retreat has been recorded in the North Cascades and is evident at one of my study sites, M-25-01, a newly formed lake that is a direct result of glacial retreat. This study provides evidence that glacial macroinvertebrate communities have unique species that differ from rhithral lake outlets within the North Cascades. Increased glacial meltwater from warming may alter macroinvertebrate communities as they shift from those associated with kryal water source to those associated with rhithral water source. A loss of kryal-based aquatic habitats may result in a loss of specialists and decreased regional biodiversity. Continued warming could also lead to a loss of permanent snowpack and those species associated with rhithral lake outlets. Long-term macroinvertebrate studies of North Cascade kryal and rhithral streams are needed to further

understand the function and structure of these groups and their response to effects of climate change.

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# 6 FIGURES



Figure 1. Modified harshness-diversity curve adapted from Tockner et al. (1997). Alpine streams are positioned on the descending limb of the harshness-diversity curve.



Figure 2. Map showing the locations of the rhithral and kryal lake outlet study sites.



Figure 3. Biplot of PCA results delineating lake outlet sites into rhithral (solid line) and kryal (dashed line) categories based on physico-chemical variables (Pearson 1901). Area = lake surface area, Elev = elevation, Turb = turbidity, Cond = conductivity, Alk = alkalinity, Chla = chlorophyll a, TP = total phosphorus, TN = total nitrogen, SRP = soluble reactive phosphorus, NO3 = nitrate/nitrite, Temp = water temperature, CMS = discharge, PSI = Pfankuch stability index, % Fines = % Fines, % BldBed = % Boulder & Bedrock. Physico-chemical variables latitude, longitude, lake surface, pH, and dissolved oxygen loaded along coordinates 0, 0 and are not shown.



Figure 4. Box plots comparing the median, 25th, and 75th percentiles (boxes), and the maximum and minimum values (whiskers) between kryal and rhithral lake outlet sites for physico-chemical variables with NMCAA PRE scores  $\geq 0.50$ . The PRE scores for physico-chemical and combined data set NMCAA are reported along with Kruskal-Wallis chi-squared significance tests (Kruskal and Wallis 1952). Line through Nitrate/Nitrate box plots denotes analysis detection limit.

\* indicates significantly different,  $P \le 0.05$ 



Nitrate/Nitrite (ug/L)





Lake Surface Area (acres)





%Fine Substrate\*



Figure 4 continued.



Figure 5. Box plots comparing the median, 25th, and 75th percentiles (boxes), and the maximum and minimum values (whiskers) between kryal and rhithral lake outlet sites for percent silt, boulder, and bedrock substrate variables. Kruskal-Wallis chi-squared significance tests results reported (Kruskal and Wallis 1952).

\* indicates significantly different,  $P \le 0.05$ 



Figure 6. Photographs of Lower Middle lake and outlet stream site. Iron hydroxide (FeOH) precipitate covered all substrate in the stream along with the littoral edge of the upstream lake.



Figure 7. Box plots comparing the median, 25th, and 75th percentiles (boxes), and the maximum and minimum values (whiskers) between kryal and rhithral lake outlet sites for macroinvertebrate richness and density data. Kruskal-Wallis chi-squared significance tests reported (Kruskal and Wallis 1952).

\* indicates significantly different,  $P \le 0.05$ )


Figure 8. Box plots comparing the median, 25th, and 75th percentiles (boxes), and the maximum and minimum values (whiskers) for kryal and rhithral lake outlet sites for macroinvertebrate taxon densities with NMCAA mean PRE scores  $\geq 0.50$  (Matthews and Hearne 1991). PRE scores for macroinvertebrate and combined data set NMCAA reported along with Kruskal-Wallis chi-squared significance tests (Kruskal and Wallis 1952).

\* indicates significantly different,  $P \le 0.05$ )



Figure 8 continued.











Rheocricotopus sp. (ind/sq m)\*



Figure 8 continued.





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Figure 9. Aerial photographs (above) of the emergence of M-25-01 lake over the past 60 years. Below is a recent photograph of receding Bacon Glacier and the ice-free lake taken in September, 2007.



Figure 10. Cluster dendrogram of lake outlet study sites based on benthic macroinvertebrates. Euclidean distance measure and Ward's linkage metrics (Everitt 1993) were used on presence/absence data.



Figure 11. Statistically significant ( $P \le 0.05$ ) Kendall's correlation analyses (Kendall 1938) between physico-chemical variables and macroinvertebrate density and richness indices. Correlation statistic and p-value reported. No EPT taxa were collected at kryal site M-25-01, therefore this site is not plotted against pH, alkalinity, and lake surface area for EPT taxon richness or density.



Figure 11 continued.



Figure 11 continued.



Figure 12. Dissolved oxygen concentration (mg/L) and macroinvertebrate taxon density (ind./square meter) for lake outlet sites for statistically significant ( $P \le 0.05$ ) Kendall's correlation analyses (Kendall 1938).



Figure 12 continued.



Figure 13. Milner et al.'s (2001) modified conceptual model describing the likely first appearance of macroinvertebrate taxa along and upstream-downstream continuum from the glacier margin with increasing water temperature and channel stability during the melt season for European glacier-fed rivers. Arrows indicate taxa that may be found across this temperature range in other geographical areas -SA = South America, NA = North America.



water Temperature (C)									
EP-11-01	Ouzel	M-25-01	Lousy	Price					
				Х					

Cyclopoidae					Х
Nematoda	Х	Х	Х		Х
Oligochaeta	Х		Х	Х	Х
P. coronata		Х		Х	Х
Acari	Х				
Araneae				Х	Х
Ameletidae	Х			Х	Х
Ephemerellidae	Х				
Capniidae		Х		Х	Х
Heptageniidae	Х	Х		Х	Х
Nemouridae	Х			Х	Х
Perlodidae	Х	Х		Х	Х
Taeniopterygidae	Х	Х		Х	
Limnephilidae	Х			Х	Х
Rhyacophilidae	Х				
Tipulidae	Х	Х			
Diamesiinae	Х	Х	Х	Х	Х
Orthocladiinae	Х	Х	Х	Х	Х
Tanypodinae		Х			

Figure 14. Plot of kryal lake outlet channel stability and water temperature measurements with table of macroinvertebrate taxa collected at each kryal lake outlet site. In comparison to Milner et al.'s (2001) modified conceptual model (Figure 13), my kryal lake outlet sites do not follow the same pattern of likely first appearance in relation to water temperature and channel stability.



Figure 15. Conceptual model of landscape filters (Hieber et al. 2005) determining assemblage composition in different alpine stream types. Each hierarchical level represents an environmental feature and the dominant habitat characteristics that further filter the invertebrate species. Macroinvertebrate drawings used with permission from the artist Christine Elder.

## 7 TABLES

Table 1. Location and general physical characteristics of the lake outlet study sites. Site codes are for lake outlets used in the text.

Site	Site Code	Туре	Elevation (m)	Latitude (UTM)	Longitude (UTM)	Lake Area (Hectares)	Discharge (CMS)	Pfankuch Index	%Fines	% Boulder/Bedrock
EP-11-01	EP-11-01	Kryal	1209	636832	5382068	4.3	3.77	23	7	66
Lousy	Lousy	Kryal	1117	624383	5410182	8.5	0.81	33	0	30
M-25-01	M-25-01	Kryal	1602	610362	5390356	15.6	0.19	18	60	20
Ouzel	Ouzel	Kryal	1728	627097	5424803	1.1	0.88	50	40	0
Price	Price	Kryal	1193	602078	5412477	21.3	1.12	33	6	8
Middle, Lower	MidLow	Rhithral	1707	620947	5415997	1.2	0.07	17	0	45
Middle, Upper	MidUp	Rhithral	1740	620657	5416104	1.8	0.04	15	0	18
Tapto, Upper	TapUp	Rhithral	1756	619540	5415758	4.1	0.01	17	0	12
Tapto, West	TapWest	Rhithral	1725	619168	5415773	0.9	0.02	17	0	33
Thornton, Upper	UpThorn	Rhithral	1536	622059	5394358	12.7	0.06	18	6	10

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Table 2. Pfankuch index of bottom stability (PSI; Pfankuch 1975) rating system for lake outlet sites. Totals from each of the rating categories were added together to determine the stream stability index for the stream reach ( $\leq 15$  excellent, 16 – 30 good, 31 – 45 fair, 46 – 60 poor).

Item Rated	Excellent		Good		Fair		Poor	
Rock Angularity	Sharp edges and corners, plane surfaces roughened	1	Rounded corners and edges, surfaces smooth and flat.	2	Corners & edges well rounded in two dimensions	3	Well rounded in all dimensions, surfaces smooth	4
Brightness	Surfaces dull, darkened, or stained, Generally. not "bright"	1	Mostly dull, but may have up to 35% bright surface.	2	Mixture, 50-50% dull and bright, $\pm$ 15% ie. 35-65%	3	Predominantly bright, 65%+, exposed or scoured surfaces.	4
Consolidation or Particle Packing	Assorted sizes tightly packed and/or overlapping	2	Moderately packed with some overlapping.	4	Mostly a loose assortment with no apparent overlap	6	No packing evident. Loose assortment, easily moved.	8
Bottom Size Distribution & %Stable Materials	No change in sizes evident. Stable materials 80-100%	4	Distribution shift slight. Stable materials 50- 80%	8	Moderate change in sizes. Stable materials 20-50%	12	Marked distribution change. Stable materials 0-20%	16
Scouring & Deposition	Less than 5% of the bottom affected by scouring and deposition	6	5-30% affected. Scour at constrictions and where grades steepen. Some deposition in pools.	12	30-50% affected. Deposits & scour at obstructions, constrictions, and bends. Some filling of pools.	18	More than 50% of the bottom in a state of flux or change nearly yearlong	24
Clinging Aquatic Vegetation (Moss & Algae)	Abundant. Growth largely moss-like, dark green, perennial. In swift water too.	1	Common. Algal forms in low velocity & pool areas. Moss here too and swifter waters.	2	Present but spotty, mostly in backwater areas. Seasonal blooms make rocks slick.	3	Perennial types scarce or absent. Yellow-green, short term bloom may be present	4
Totals	Excellent Column Total		Good Column Total		Fair Column Total		Poor Column Total	

TΓ

Table 3. Summary of analytical methods used for lake outlet water chemistry as outlined by the Institute for Watershed Studies. Nitrate/nitrite is a measure of combined concentrations of nitrate and nitrite

Analyte	Abbr.	Method Reference (AHPA 2005)	Detection or Sensitivity Limit
Alkalinity	Alk	SM2320, titration	$\pm$ 0.6 mg/L
Conductivity - lab	Cond	SM2510	<u>+</u> 0.8 µS/cm
Dissolved Oxygen - field	DO	SM4500-O G., membrane electrode (field meter) or drop count titration/modified Winkler	$\pm$ 0.1 mg/L (field meter) or 0.2 mg/L (drop count)
Nitrogen – ammonia	$\mathrm{NH}_3$	SM4500-NH <sub>3</sub> H., flow inject, phenate	10 µg Nitrogen/L
Nitrogen – nitrate/nitrite	$NO_3$	SM4500-NO <sub>3</sub> I., flow inject, Cd reduction	10 μg Nitrogen/L
Nitrogen – total	TN	SM4500-N C., flow inject, persulfate digest	20 µg -Nitrogen/L
pH – lab	pН	SM4500-H <sup>+</sup> , electometric lab	$\pm 0.1$ units
Phosphorus - soluble reactive	SRP	SM4500- P G., flow inject	3 μg Phosphorus/L
Phosphorus – total	ТР	SM4500- P H., flow inject, persulfate digest	5 μg Phosphorus/L
Temperature – field	Temp	SM2550 thermistor (field meter) or field thermometer	<u>+</u> 0.1 C
Total Suspended Solids	TSS	2540D	2.6 mg L-1
Turbidity	Turb	SM2130, nephelometric	<u>+</u> 0.2 NTU
Chlorophyll a	Chla	SM10200 H	$\pm 0.1 \text{ mg/m}^3$

Table 4. Water chemistry of lake outlet study sites. TSS, SRP, and NH3 were excluded from analysis. Temp = water temperature C°, DO = dissolved oxygen (mg/L), Alk.= alkalinity (mg/L, Cond. = Conductivity ( $\mu$ S/cm), TSS = total suspended solids (mg/L), Turb. = turbidity (NTU), Chla = Chlorophyll *a* (mg/m<sup>3</sup>), TP = total phosphorus ( $\mu$ g - P/L), SRP = soluble reactive phosphorus ( $\mu$ g - P/L), TN = total nitrogen ( $\mu$ g - N/L), NO3 = nitrate/nitrite ( $\mu$ g - N/L), NH3 = ammonia ( $\mu$ g - N/L), Br = bromide (mg/L), Cl = chloride (mg/L), Fl = flouride (mg/L), NO3-An = nitrate/nitrite (mg/L) anion analysis, SO4 = sulfate (mg/L).

	Site	Sampling Date	Туре	Temp	DO	pН	Alk.	Cond.	TSS*	Turb.	Chla	ТР	SRP*	TN	NO3	NH3*	Br	Cl	Fl	NO3-An	SO4
	EP-11-01	Aug 31, 2007	Kryal	2.2	11.9	6.7	4.7	16.8	0.0177*	17.5	0.12	20.21	2.90*	26.9	21.5	1.5*	0	0.09	0.00	0.00	0.51
	Lousy	Sept 11, 2007	Kryal	6.0	12.9	6.7	5.5	24.2	0.0295*	51.1	0.03	16.28	1.93*	6.7*	20.0	2.0*	0	0.14	0.00	0.08	0.66
	Ouzel	Sept 12, 2007	Kryal	4.3	11.8	6.2	1.3	3.8	0.0080*	9.5	0.14	7.06	2.30*	9.8*	13.0	-4.5*	0	0.17	0.01*	0.01	0.05
	Price	Sept 2, 2007	Kryal	7.2	11.4	6.5	2.8	11.0	0.0043*	12.6	0.59	6.42	1.60*	3.0*	0.5*	-3.3*	0	0.13	0.02	0.02	0.48
	M-25-01	Sept 20, 2007	Kryal	4.8	10.4	5.1	0.0*	18.6	0.0010*	0.9	0.18	0.02	-0.03*	18.7*	20.7	-4.7*	0	0.13	0.00	0.08	0.73
	Median			4.8	11.8	6.5	3.8	16.8	0.0080	12.6	0.14	7.06	2.1	9.8	20.0	1.8	0.0	0.13	0.00	0.02	0.51
	Standard Deviation			1.9	0.9	0.7	1.9	7.8	0.0121	19.3	0.2	8.14	0.6	9.7	8.9	0.4	0.0	0.03	0.01	0.04	0.26
	Middle, Lower	Sept 9, 2007	Rhithral	9.5	10.0	4.8	0.0*	76.6	0.0016*	3.2	0.02	17.73	1.64*	0.6*	4.6*	-4.9*	0	0.14	0.15	0.00	0.91
	Middle, Upper	Sept 9, 2007	Rhithral	9.5	10.0	6.2	2.4	26.8	0.0004*	0.4	1.20	7.00	3.31*	13.7*	1.1*	-1.1*	0	0.10	0.03	0.03	0.76
~1	Tapto, Upper	Sept 10, 2007	Rhithral	10.5	10.0	5.7	-0.4*	4.3	0.0007*	0.2	2.15	0.63	1.60*	44.1	2.8*	1.6*	0	0.12	0.00	0.00	0.34
6	Tapto, West	Sept 9, 2007	Rhithral	12.0	9.0	5.7	0.6	9.3	-0.0012*	0.4	0.30	0.52	0.79*	41.7	5.2*	7.2*	0	0.12	0.01*	0.01	0.57
	Thornton, Upper	Sept 15, 2007	Rhithral	10.2	10.0	6.2	1.4	5.5	0.0005*	0.2	0.21	4.34	0.71*	18.3*	0.6*	-4.7*	0	0.13	0.06	0.00	0.23
	Median			10.2	10.0	5.7	1.4	9.3	0.0006	0.4	0.30	4.30	1.6	18.3	2.8	4.4	0.0	0.1	0.0	0.00	0.57
	Standard Deviation			1.0	0.4	0.6	0.9	30.5	0.0007	1.3	0.89	7.09	1.0	18.7	2.0	4.0	0.0	0.0	0.1	0.01	0.28

\*Values below detection limits.

Table 5. Non-metric clustering PRE scores for the variables possessing the highest predictive power in assigning sites to kryal and rhithral lake outlet sites for benthic invertebrate, combined, and physico-chemical datasets (Matthews and Hearne 1991). Three significant cluster solutions were found for the benthic invertebrate dataset and the mean PRE score is reported. The combined and physico-chemical datasets each had a single significant cluster solution.

Benthic Invertebrate Dataset		Combined Dataset		Physico-Chemical Dataset	
Variable M	ean PRE Score	Variable	PRE	Variable	PRE
Early Instar Chironomidae	0.78	Dissolved Oxygen	1.00	Dissolved Oxygen	1.00
Acari	0.67	Early Instar Chironomidae	1.00	Stream Discharge	1.00
Corynoneura sp.	0.67	Stream Discharge	1.00	Water Temperature	1.00
Early Instar Ephemeroptera	0.67	Water Temperature	1.00	Pfankuch Stability Index	0.78
Early Instar Plecoptera	0.67	Acari	0.78	Chlorophyll a	0.60
Eukiefferiella claripennis Gr.	0.67	Corynoneura sp.	0.78	Elevation	0.60
Harpacticoida	0.67	Early Instar Ephemeroptera	0.78	Lake Surface Area	0.60
Megarcys sp.	0.67	Early Instar Plecoptera	0.78	Longitude (UTM Easting)	0.60
Polycelis coronata	0.67	Eukiefferiella claripennis Gr.	0.78	Nitrate/Nitrite	0.60
Rhithrogena sp.	0.67	Harpacticoida	0.78	Turbidity	0.60
Rhyacophila rotunda Gr.	0.67	Megarcys sp.	0.78	% Fine Sediment	0.50
Setvena sp.	0.67	Pfankuch Stability Index	0.78		
Zapada columbiana	0.67	Poduridae	0.78		
Zavrelimyia sp.	0.67	Polycelis coronata	0.78		
Isotomidae	0.51	Rheocricotopus sp.	0.78		
Poduridae	0.50	Rhithrogena sp.	0.78		
Rheocricotopus sp.	0.50	Rhyacophila rotunda Gr.	0.78		
		Setvena sp.	0.78		
		Zapada columbiana	0.78		
		Zavrelimyia sp.	0.78		
		Ameletus sp.	0.60		
		Chlorophyll a	0.60		
		Elevation	0.60		
		Lake Surface Area	0.60		
		Longitude	0.60		
		Nematoda	0.60		
		Nitrate/Nitrite	0.60		
		Oligochaeta	0.60		
		Turbidity	0.60		

Table 6. Kruskal-Wallis multisample tests with Chi-square approximation between kryal and rhithral lake outlet sites for physico-chemical variables (Kruskal and Wallis 1952). Soluble reactive phosphorus, ammonia, and total suspended solid concentrations were all below detection limit and not included in test.

Physico-chemical Parameter	Chi-Square	P-value
Elevation (ft)	3.15	0.08
Latitude (UTM)	0.27	0.60
Longitude (UTM)	1.32	0.25
Lake Surface Area (hectares)	1.84	0.17
Water Temperature (C°)	6.86	0.01*
Dissolved Oxygen (mg/L)	7.26	0.01*
Stream Discharge (CMS)	6.82	0.01*
Pfankuch Stability Index (PSI)	6.52	0.01*
pH	1.84	0.17
Specific Conductance (µS/cm)	0.01	0.92
Alkalinity (mg/L)	1.84	0.17
Turbidity (NTU)	5.77	0.02*
Chlorophyll $a (mg/m^3)$	1.32	0.25
Total Phosphorus (µg-P/L)	0.53	0.46
Total Nitrogen (µg-N/L)	0.53	0.46
Nitrate/Nitrite (µg-N/L)	2.45	0.12
% Silt Substrate	3.72	0.05*
% Sand Substrate	1.12	0.29
% Gravel Substrate	0.55	0.46
% Cobble Substrate	2.47	0.12
% Boulder Substrate	4.48	0.03*
% Bedrock Substrate	4.04	0.04*
% Fines (Silt & Sand)	4.05	0.04*
% Boulder/Bedrock	0.1	0.75
Flouride (mg/L)	2.97	0.08
Chloride (mg/L)	1.11	0.29
Bromide (mg/L)	NA	NA
Nitrate/Nitrite (mg/L)	2.22	0.14
Sulfate (mg/L)	0.27	0.60

\* indicates significantly different,  $P \le 0.05$ 

Phylum	Class	Order	Family (Subfamily)	Genus species	Kryal	Rhithral
Arthropoda	Insecta	Ephemeroptera	Ameletidae	Ameletus sp.Eaton	х	х
			Ephemerellidae	Drunella doddsi Needham	Х	
			Heptageniidae	Cinygmula sp. McDunnough	Х	
				Epeorus deceptivus McDunnough	Х	
				Epeorus grandis McDunnough	Х	
				Rhithrogena sp. Eaton	Х	
		Plecoptera	Capniidae	Capnura sp. Banks	Х	х
				Utacapnia sp. Nebeker and Gaufin	Х	
			Nemouridae	Podmosta sp. Ricker	Х	
				Zapada columbiana Claassen		х
				Zapada Oregonensis Gr. Claassen	Х	
				Megarcys sp. Klapálek	Х	
			Perlodidae	Setvena sp. Illies		Х
			Taeniopterygidae	Taenionema sp. Banks	Х	
		Trichoptera	Apataniidae	Apatania sp. Kolenati		Х
			Brachycentridae	Micrasema sp. McLachlan		Х
				Parapsyche elsis Milne		Х
			Hydropsychidae	Desmona mono Denning		Х
			Limnephilidae	Ecclisomyia sp.Banks	Х	Х
				Ecclisocosmoecus scylla Milne		Х
				Psychoglypha sp. Ross	Х	Х
			Rhyacophilidae	Rhyacophila brunnea Gr. Banks		Х
				Rhyacophila rotunda Gr. Banks		х
				Rhyacophila verrula Gr. Milne	х	х
			Uenoidae	Neothremma sp. Dodds & Hisaw		Х
		Diptera	Chironomidae			
			(Chironominae)	Micropsectra sp. Kieffer		Х
				Paracladopelma sp. Harnish		х
				Paratanytarsus sp. Bause		Х
				Polypedilum sp. Kieffer		Х
				Tanytarsus sp. Van Der Wulp		Х
			(Diamesinae)	Diamesa sp. Meige	х	Х
				Pagastia sp. Oliver		Х
				Pseudodiamesa sp. Goetghe buer	х	х
			(Orthocladiinae)	Chaetocladius dentiforceps Gr. Edwards	х	х
				Chaetocladius sp. Kieffer	х	х
				Corynoneura sp. Winnertz		Х
				Diplocladius sp. Kieffer	х	х
				Eukiefferiella brehmi Gr. Gowin		х
				Eukiefferiella claripennis Gr. Lundbeck		Х
				Eukiefferiella devonica Gr. Edwards		х
				Eukiefferiella gracei Gr. Edwards	х	х
				Heleniella sp. Saether		х
				Heterotrissocladius marcidus Walker		х
				Hydrobaenus sp. Fries	х	х
				Krenosmittia sp. Thienemann		х
				Limnophyes sp. Eaton		х
				Nanocladius parvulus Gr. Keiffer		х
				Nanocladius sp. Kieffer		х
				Orthocladius sp. Wulp	х	х
				Orthocladius (Euorthocladius) Thienemann	х	х
				Orthocladius (Orthocladius) Wulp	х	х

Table 7. List of all taxa found at kryal and rhithral lake outlet	sites.
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## Table 7 continued.

Phylum	Class	Order	Family (Subfamily)	Genus species	Kryal	Rhithral
Arthropoda	Insecta	Diptera	Chironomidae			
			(Orthocladiinae)	Parametriocnemus sp. Thienemann		х
				Parorinociaaius sp.	х	
				Psectrocladus sp. Kieller		X
				Pseudorthooladius sor		X
				Psilometriognamus sp. Spather		X
				Phonericationus an Thiomann and Harnisch		X
				Rheocricotopus sp. Thereinan and Harnisch		X
				Sworthooladius sp		X
				Thionomonuolla sp. Vieffer	v	X
				Tuatania sp. Kieffer	A V	A V
				Tvetenia bavarica Gr. Goetabebuer	A V	A V
			(Podonominae)	Parochlus sp. Edwards	л	A V
			(Tanynodinae)	Krenonelonia sp. Editkan		A V
			(Taliypoullae)	Procladius sp. Skuse		A V
				Thionomonnimula Gr		A V
				Zavralimvia sp. Kittkau		A V
			Tinulidae	Gonomvodes sp	v	л
			Tipuldae	Hesperaconona sp. Alexander	A V	
				Pedicia sn Latreille	~	v
				Molonhilus sp. Curtis	v	л
			Ceratopogonidae	Notophilus op. Cultis	А	
			Ceratopogoninae (subfamily)			v
			Contropogonniae (Subhanniy)	Atrichopogen sp		v
			Empididae	Clinocera sp. Meigen		A V
			Emplaidue	Oregageton sp. Schiper		x
			Simulidae	Simulium sp. Latreille		x
			Simulate	Piezosimulium sp		x
				Dicranota sp		x
		Hemintera	Heteroptera (suborder)	Distantian op.		x
		Tiempteru	Homontera (suborder)			x
		Coleontera	Dytiscidae			
		concoptulu	(Dytiscinae)			x
			()	Agabus sp. Leach		x
				Hydrocolus sp. Roughlev and Larson. Alarie and Roughlev		x
				Hyerotus sp. Stephens		x
				Stictotarsus striatellus LeConte		x
			Hydroporinae			
			(Hydroporinae)			х
			Hydrophilidae Latreille			х
			(Sphaeridiinae)			х
			Staphylinidae Latreille			x
			The second se	Stenus sp. Latreille		х
	Arachnidae	Acari (subclass)		1		х
		Araneae				х
	Entognatha	Collembola	Isotomidae			х
	0		Poduridae			х
	Maxillopoda	Harpacticoida G. O. Sars				х
	1	Cyclopoida Burmeister			х	х
		Calanoida G. O. Sars				х
Annelida	Oligochaeta				х	х
Platyhelminthes	Turbellaria	Tricladida	Planariidae	Polycelis coronata Girard	х	х
Nematoda					x	x

Table 8. Relative percentage of functional feeding groups (FFG) present at kryal and rhithral lake outlet streams, based on abundance (Merritt and Cummin 1996 with modifications by Wisseman and Doughty 2004).

FFG	Kryal	Rhithral
Collector-Gatherer	51	65
Omnivore	29	3
Shredder	7	3
Unknown	4	6
Scraper	4	1
Predator	3	10
Parasite	2	12
Macrophyte Shredder	0	<1
Collector-Filterer	0	<1
Totals	100	100

Habit Type	Kryal	Rhithral
Sprawlers	54	21
Burrowers	24	8
Unknown	11	49
Clingers	10	17
Attachers	0	1
Swimmers	<1	4
Hyporheic	<1	<1
Totals	100	100

Table 9. Relative percentage of habit groups present at kryal and rhithral lake outlet streams, based on abundance (Merritt and Cummin 1996 with modifications by Wisseman and Doughty 2004).

Table 10. Contingency tables of the significant (chi-squared,  $P \le 0.05$ ) site classifications for benthic macoinvertebrate NMCAA cluster solutions (Matthews and Hearne 1991). Three different cluster solutions were significantly associated with kryal and rhithral water sources. In each cluster solution a different lake outlet site was misclassified. Tapto West was misclassified as a kryal site in cluster *a*, Lower Middle was misclassified as a kryal site in cluster *b* and M-25-01 was misclassified as a rhithral site in cluster solution *c*. Below each contingency table are the associated PRE values for the variables with scores > 0.50.

Cluster Solution	а		Cluster Solution <i>b</i>	2		Cluster Solution c				
Tapto West Mise	classified		Lower Middle Mi	sclassified		M-25-01 Misclassified				
Category	Cluster 1	Cluster 2	Category Cluster !		Cluster 2	Category	Cluster 1	Cluster 2		
Kryal	6	0	Kryal	6	0	Kryal	4	0		
Rhithral	0	4	Rhithral	0	4	Rhithral	0	6		
Variable		PRE	Variable		PRE	Variable		PRE		
Eukiefferiella cla	aripennis Gr.	1.0	Acari		1.0	Megarcys sp.		1		
Setvena sp.		1.0	Corynoneura sp.		1.0	Rhithrogena	sp.	1		
Early Instar Chir	onomidae	0.78	Early Instar Ephe	meroptera	1.0	Early Instar C	hironomidae	0.78		
Isotomidae		0.71	Early Instar Pleco	ptera	1.0	Parorthoclad	ius sp.	0.71		
Acari		0.5	Harpacticoida		1.0	Acari	0.5			
Corynoneura sp	•	0.5	Polycelis coronat	а	1.0	Corynoneura	0.5			
Early Instar Ephemeroptera 0.5		0.5	Rhyacophila rotu	<i>nda</i> Gr.	1.0	Cyclopoidae		0.5		
Early Instar Plecoptera 0.5		0.5	Setvena sp.		1.0	Diamesa sp.		0.5		
Harpacticoida		0.5	Zapada columbia	na	1.0	Early Instar E	phemeroptera	0.5		
Megarcys sp.		0.5	Ameletus sp.		0.78	Early Instar P	lecoptera	0.5		
Orthocladius (O	rthocladius)	0.5	Early Instar Chiro	nomidae	0.78	Eukiefferiella	0.5			
Poduridae		0.5	Oligochaeta		0.78	Eukiefferiella	0.5			
Polycelis corona	ta	0.5	Eukiefferiella bre	hmi Gr.	0.71	Eukiefferiella	0.5			
Pseudodiamesa	sp.	0.5	Isotomidae		0.71	Harpacticoida	L	0.5		
Rheocricotopus	sp.	0.5	Micropsectra sp.		0.71	Micropsectra	sp.	0.5		
Rhithrogena sp.		0.5	Cyclopoidae		0.5	Orthocladius	(Orthocladius)	0.5		
Rhyacophila roti	<i>unda</i> Gr.	0.5	Eukiefferiella cla	ripennis Gr.	0.5	Poduridae		0.5		
Rhyacophila ver	<i>rula</i> Gr.	0.5	Eukiefferiella gra	<i>cei</i> Gr.	0.5	Polycelis core	onata	0.5		
Tvetenia sp.		0.5	Megarcys sp.		0.5	Rheocricotop	us sp.	0.5		
Zapada columbi	ana	0.5	Orthocladius (Or	thocladius)	0.5	Rhyacophila H	Rotunda Gr.	0.5		
Zavrelimyia sp.		0.5	Poduridae		0.5	Setvena sp.		0.5		
			Rheocricotopus s	p.	0.5	Thienemannie	ella sp.	0.5		
			Rhithrogena sp.		0.5	Zapada colun	ıbiana	0.5		
			Thienemanniella	sp.	0.5	Zavrelimyia s	sp.	0.5		
			Zavrelimyia sp.		0.5					

Table 11. Non-overlapping taxa in kryal and rhithral lake outlet streams. The number of sites at which each taxon was present is also reported.

Rhithral	No. of Sites	Rhithrol	No. of Sites
	THE OF SILES	Continued	THU. UI SILES
Zapada columbiana	5	Clinocera sp	1
Satvana sp	1	Oreganton sp.	1
bervenu sp.	7	Simulium sp.	1
трісиортера		Piezosimulium sp.	1
Rhyacophilla rotunda Gr	4	Dicranota sp.	1
Rhyacophilla houmaa Gr.	4	Dicranola sp.	1
Na sthe server a se	2	COLEODTED A	
Neothremma sp.	2		2
Apatania sp.	1	<i>Hygrotus</i> sp.	<u>∠</u>
<i>Micrasema</i> sp.	1	Agabus sp.	1
Desmona mono	1	Dytiscinae	1
Parapsyche elsis	1	Stictotarus striatellus	1
Ecclisocosmoecus scylla	1	Sphaeridiinae	1
		Stenus sp.	1
DIPTERA		Hydrocolus sp.	1
Chironomidae	_		
Eukiefferiella claripennis Gr.	5	OTHER	
Rheocricotopus sp.	5	Harpacticoida	5
Zavrelimyia sp.	4	Poduridae	4
Corynoneura sp.	4	Isotomidae	3
Eukieffella brehmi Gr.	3	Heteroptera	1
Psectrocladius sp.	3	Homoptera	1
Micropsectra sp.	3	Calanoida	1
Eukiefferiella devonica Gr.	2		
Heterotrissocladius marcidus	2	Kryal	No. of Sites
Parochlus sp.	2	PLECOPTERA	
Rheocricotopus fuscipes Gr.	2	Megarcys sp.	5
Heleniella sp.	1	Zapada oregonensis Gr.	2
Krenosmittia sp.	1	Utacapnia sp.	2
Krenopelopia sp.	1	Taenionema sp.	2
Limnophyes sp.	1	Podmosta sp.	2
Nanocladius sp.	1	·	
Pagastia sp.	1	EPHEMEROPTERA	
Parametriocnemus sp.	1	Rhithrogena sp.	5
Paratanytarsus sp.	1	Epeorus spp.	3
Polypedilum sp.	1	Drunella doddsii	1
Procladius sp	1	Cinvemula sp	1
Psectrocladius sordidellus Gr	1	emygnum sp.	-
Pseudorthocladius sp	1	DIPTERA	
Psilometriocnemus sp.	1	Chironomidae	
Synorthocladius sp.	1	Parorthocladius sp	3
Tanytarsus sp.	1	Tinulidae	5
Thienemannimvia Gr	1	Gonomvodes sn	2
Paracladonalma sn	1	Hasparoconona sp.	2 1
Ather Dipterans	1	Molonkilus en	1
Constangening	2	<i>motopnitus</i> sp.	1
Dedicia en	∠ 2		
reaicia sp.	2		

Table 12. Results of significant (P  $\leq$  0.05) Kendall's correlation analyses between macroinvertebrate density and diversity indices and physico-chemical variables.

	Macroinvertebrate Indicies	Physico-chemical Variable	Kendall's τ	P-value
	Total Density	+water temp; -discharge; -DO; -turbidity	+0.58; -0.51; -0.55; -0.51	0.024; 0.047; 0.033; 0.047
	EPT Density	-lake surface area	-0.51	0.047
88	Non-Insect Density	+water temp; -discharge; -DO; -turbidity	+0.67; -0.51; -0.79; -0.60	0.007; 0.047; 0.002; 0.017
	Total taxon richness	+water temp	+0.57	0.024
	EPT taxon richness	+pH; +alkalinity +water temp; -discharge; -DO; -turbidity;	+0.71; +0.61 +0.77; -0.65; -0.56; -0.60;	0.007; 0.019 0.003; 0.01; 0.036; 0.022;
	Non-insect taxon richness	-PSI; -%Fines	-0.57; -0.63	0.047; 0.023

## A APPENDIX A: QUALITY CONTROL RESULTS

Appendix A.1. Macroinvertebrate sorting quality control results for 5 randomly selected macroinvertebrate samples. Data presented include number of specimens found in original sort, number of specimens found in re-sort of 20% of sample, and overall sorting efficacy. Median sorting efficacy was 93%.

Site	<b>Original Sort</b>	20% Re-sort Count	% Sorting Efficacy
Upper Middle - 3	806	24	85
Lower Middle - 2	186	1	97
Lousy - 1	49	0	100
Lousy - 2	58	1	91
Upper Thornton - 1	429	6	93

Appendix A.1a. Macroinvertebrate sample count quality control results for 5 randomly selected macroinvertebrate samples. Original and quality control counts presented for taxa found in each sample excluding Chironomidae subfamily, genera, and species counts which were counted by Rhithron Associates, Inc. Original counts were 99% accurate.

	Lousy - 1		Price - 1	Upper T	apto - 1	Upper T	Capto - 2	Upper Tapto - 6		
Taxa	Original	Quality Control	Original	Quality Control	Original	Quality Control	Original	Quality Control	Original	Quality Control
Heteroptera (sub-order)	0	0	0	0	1	1	0	0	0	0
Isotomidae (family)	0	0	0	0	0	0	1	1	0	0
Harpacticoida (order)	0	0	0	0	0	0	2	2	0	0
Calanoida (order)	0	0	0	0	0	0	1	1	0	0
Nematoda (phylum)	0	0	0	0	2	2	2	2	0	0
Polycelis coronata (Planariidae)	0	0	0	0	1	1	25	25	8	8
Araneae (Arachnidae)	1	1	0	0	0	0	0	0	0	0
Acari (Arachnidae)	0	0	0	0	22	22	4	4	5	5
Coleoptera (early instar)	0	0	0	0	1	1	0	0	0	0
Stictotarus striatellus (Dytiscidae)	0	0	0	0	2	2	1	1	3	3
Hydroporinae (Dytiscidae)	0	0	0	0	0	0	0	0	1	1
Ameletus (Ameletidae)	0	0	2	2	6	6	13	13	0	0
Rhithrogena (Heptageniidae)	4	4	0	0	0	0	0	0	0	0
Plecoptera (early instar)	1	1	0	0	0	0	3	3	0	0
Capniidae (family)	1	1	0	0	0	0	0	0	0	0
Utacapnia (Capniidae)	0	0	5	5	0	0	0	0	0	0
Nemouridae (early instar)	5	5	0	0	0	0	0	0	0	0
Zapada columbiana (Nemouridae)	0	0	0	0	3	3	55	55	0	0
Zapada oregonensis Gr. (Nemouridae)	3	3	0	0	0	0	0	0	0	0
Megarcys (Perlodidae)	2	2	1	1	0	0	0	0	0	0
Stevena (Perlodidae)	0	0	0	0	1	1	0	0	0	0
Taenionema (Taeniopterygidae)	1	1	0	0	0	0	0	0	0	0
Desmona mono (Limnephilidae)	0	0	0	0	0	0	0	0	1	1
Psychoglypha (Limnephilidae)	0	0	1	1	0	0	0	0	0	0
Rhyacophila rotunda Gr. (Rhyacophilidae)	0	0	0	0	2	2	1	1	0	0
Ceratopogoninae (Ceratopogonidae)	0	0	0	0	1	1	0	0	0	0
Chironomidae (early instar)	10	10	2	2	56	56	143	143	137	132
Total	28	28	11	11	98	98	251	251	155	150

A	pr	pendix A	2.	Summar	y of	`lab	and	fie	ld	dup	olicate	e water	qualit	y control	l result	s for	· 10%	6 of	the	sam	oles	colle	ected
					J									J									

Analyte	Site	Original	Lab Duplicate	Relative Difference (%)	Absolute Difference	Field Duplicate	Relative Difference (%)	Absolute Difference
pH	Upper Thornton	6.230	6.1500	1	0.08	6.030	3	0.200
Turbidity (NTU)	Upper Thornton	0.214	0.2210	3	0.01	0.266	24	-0.052
Conductivity (µS/cm)	Upper Thornton	5.500	5.5000	0	0.00	5.300	4	0.200
Alkalinity (mg/L)	Upper Thornton	1.400	1.4000	0	0.00	1.600	14	0.200
	Middle, Upper	2.400	2.4000	0	0.00			
Chlorophyll a (µg/L)	Upper Thornton	0.212	0.2474	14	0.04	0.289	36	0.080
Total Phosphorus (µg-P/L)	Upper Thornton	4.337*	4.0928*	6	0.24	4.465*	3	0.130
	Ouzel	7.064	7.3605	4	0.30			
Total Nitrogen (µg-N/L)	Upper Thornton	18.292*	18.8681*	3	0.58	1.399*	92	16.892
	Ouzel	9.758*	8.8161*	10	0.94			
Soluble Reactive Phosphate (µg-P/L)	Upper Thornton	0.708*	0.4321*	39	0.28	0.053*	92	0.657
Nitrate/Nitrite (µg-N/L)	Upper Thornton	0.624*	0.4694*	25	0.15	1.413*	128	0.790
Ammonia (µg/L)	Upper Thornton	-4.704*	-6.0678*	22	1.36	-0.948*	80	3.752
	Lousy	2.026	3.1983	37	1.17			
Total Suspended Solids (mg/m <sup>3</sup> )	Upper Thornton	0.001*	-0.0003*	60	0.001	0.001	125	0.001
Dissolved Oxygen (mg/L)	Upper Thornton	10.000	10.0000	0	0.00	10.000	0	0.000

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\* indicates concentration below detection limit

## **B** APPENDIX B: MACROINVERTEBRATE DATA

Appendix B. Macroinvertebrates collected at study site, EP-11-01, 30 August 2007. Sample represents the number of individuals collected in a composite of six Surber samples  $(0.54 \text{ m}^2)$ .

Macroinvertebrate Taxa	Sample
Nematoda	13
Oligochaeta	2
Acari	3
Ephemeroptera	
Ameletus sp.	1
Drunella doddsii	2
Cinygmula sp.	7
Epeorus grandis	1
Rhithrogena sp.	2
Plecoptera	
Podmosta sp.	1
Zapada oregonensis Gr.	130
Perlodidae (early instar)	8
Megarcys sp.	4
Taeniopterygidae (early instar)	3
Tricoptera	
Limnephilidae (early instar)	4
Rhyacophila verrula Gr.	6
Chironomidae	
Chaetocladius sp.	20
Diamesa sp.	117
Eukiefferiella gracei Gr.	34
Hydrobaenus sp.	14
Orthocladiinae (early instar)	7
Orthocladius sp.	375
Parorthocladius sp.	3
Pseudodiamesa sp.	9
Tipulidae (early instar)	1
Gonomyodes sp.	4
Hesperoconopa sp.	1
Molophilus sp.	1

	Sample								
Macroinvertebrate Taxa	1	2	3	4	5	6			
Oligochaeta	0	0	0	0	2	0			
Polycelis coronata	0	0	0	0	1	0			
Araneae	1	0	0	0	0	0			
Ephemeroptera (early instar)	0	0	0	1	0	0			
Ameletus sp.	0	0	0	1	0	0			
Epeorus deceptivus	0	10	1	1	0	1			
Rhithrogena sp.	4	5	3	2	5	1			
Plecoptera (early instar)	1	5	3	6	4	1			
Capniidae	1	0	0	0	0	0			
Utacapnia sp.	0	0	1	0	0	0			
Nemouridae (early instar)	5	0	0	0	0	0			
Podmosta sp.	0	0	0	1	0	0			
Zapada oregonensis Gr.	3	1	0	2	1	0			
Perlodidae (early instar)	0	0	3	6	0	1			
Megarcys sp.	2	2	18	1	2	3			
Taenionema sp.	1	0	0	1	0	0			
Tricoptera									
Ecclisomyia sp.	0	1	0	0	0	0			
Chironomidae (early instar)	10	3	0	25	2	0			
Chaetocladius dentiforceps Gr.	0	0	1	0	0	0			
Diamesa sp.	1	0	7	4	0	0			
Orthocladius sp.	19	22	11	33	5	8			
Orthocladius(Euorthocladius)	0	0	0	2	0	0			
Orthocladius(Orthocladius)	0	9	0	12	0	2			
Tvetenia bavarica Gr.	0	0	0	3	0	0			

Appendix B (continued). Macroinvertebrates collected at study site, Lousy, 11 September 2007. Units in numbers of macroinvertebrates per  $0.09 \text{ m}^2$ .

Appendix B (continued). Macroinvertebrates collected at study site, M-25-01, 20 September 2007. Sample represents the number of individuals collected in a composite of six Surber samples  $(0.54 \text{ m}^2)$ .

Macroinvertebrate Taxa	Sample
Nematoda	8
Oligochaeta	2
Chironimidae	
Diamesa sp.	1
Diplocladius sp.	1
Orthocladius sp.	3

Appendix B (continued). Macroinvertebrates collected at study site, Ouzel, 12 September
2007. Sample represents the number of individuals collected in a composite of six Surber
samples $(0.54 \text{ m}^2)$ .

Macroinvertebrate Taxa	Sample
Nematoda	18
Polycelis coronata	3
Ephemeroptera	1
Epeorus sp.	11
Rhithrogena sp.	10
Plecoptera	1
Plecoptera (early instar)	28
Capniidae	1
Capnura sp.	1
Megarcys sp.	6
Taenionema sp.	622
Chironomidae (early instar)	1
Diamesa sp.	252
Orthocladius sp.	4
Orthocladius (Orthocladius)	5
Parorthocladius sp.	39
Pseudodiamesa sp.	1
Thienemanniella sp.	1
Tvetenia sp.	8
Tvetenia bavarica Gr.	1
Tipulidae	
Gonomyodes sp.	10

	Sample						
Macroinvertebrate Taxa	1	2	3	4	5	6	
Cyclopoidae	0	0	1	0	0	0	
Nematoda	0	0	0	0	2	0	
Oligochaeta	0	1	0	0	11	0	
Polycelis coronata	0	2	0	1	4	0	
Araneae	0	1	0	0	0	0	
Ephemeroptera							
Ameletus sp.	2	4	2	2	2	1	
Rhithrogena sp.	0	1	0	0	0	0	
Plecoptera (early instar)	0	0	1	0	0	0	
Utacapnia sp.	5	1	5	2	4	0	
Megarcys sp.	1	0	0	2	1	1	
Tricoptera							
Ecclisomyia sp.	0	0	0	0	1	0	
Psychoglypha sp.	1	0	0	0	0	0	
Chironomidae (early instar)	2	0	2	4	72	22	
Parorthocladius sp.	3	2	3	0	0	1	
Pseudodiamesa sp.	0	0	4	0	1	1	
Tvetenia sp.	0	0	0	1	1	0	

Appendix B (continued). Macroinvertebrates collected at study site, Price, 1 September 2007. Units in numbers of macroinvertebrates per  $0.09 \text{ m}^2$ .

	Sample						
Macroinvertebrate Taxa	1	2	3	4	5	6	
Isotomidae	0	0	0	1	0	0	
Harpacticoida	8	19	134	90	6	104	
Cyclopoidae	1	2	4	3	5	5	
Nematoda	236	28	125	68	23	129	
Oligochaeta	14	63	120	37	7	33	
Polycelis coronata	11	13	48	100	3	29	
Acari	23	31	164	93	8	208	
Ephemeroptera	1	0	0	1	0	0	
Ephemeroptera (early instar)	1	3	4	1	0	2	
Ameletus sp.	1	3	12	16	1	2	
Plecoptera	0	1	2	1	1	0	
Plecoptera (early instar)	5	5	4	6	3	6	
Capnura sp.	0	0	1	0	0	0	
Zapada columbiana	18	22	54	66	4	116	
Perlodidae (early instar)	0	4	7	4	0	2	
Setvena sp.	0	0	5	0	0	2	
Trichoptera	0	1	2	0	0	0	
Micrasema sp.	0	0	1	0	Õ	0	
Ecclisomvia sp.	0	2	9	0	Õ	4	
Ecclisocosmoecus scylla	0	1	0	0	Õ	0	
Psychoglynha sp	Ő	0	1	Ő	Ő	Ő	
Paransyche elsis	1	Ő	0	2	Ő	Ő	
Rhvacophila rotunda Gr	0	0	1	4	0	1	
Rhyacophila verrula Gr	Ő	0	1	0	0	0	
Henoidae	0	1	0	0	0	0	
Neothremma sp	0	0	1	0	0	0	
Chironomidae	0	1	0	0	0	0	
Chironomidae (early instar)	55	87	972	211	10	270	
Chaetocladius sp	0	0	0	0	1	270	
Commongura sp.	0	2	2	5	3	3	
Diamasa sp	1	1	0	0	0	0	
Fukiefferiella brehmi Gr	0	1	1	0	0	2	
Eukiefferiella clarinennis Gr	16	1	1/	25	3	20	
Eukiefferiella devonica Gr	10	0	0	25	0	29	
Eukiefferiella aracei Gr	11	1	2	2	0	17	
Halavialla sp	0	1	2	2	0	0	
Kranosmittia sp.	1	0	2	1	0	1	
Micropsostra sp.	2	14	22	27	2	1 Q	
Nanoeladius sp.	5	2	10	10	1	8	
Nanocladius Sp.	0	0	19	10	1	0	
Nanociaalus Farvulus Gr.	0	0	1	0	0	0	
Orthogladius sp	5	10	170	12	4	27	
Orthogladius Sp.	2	40	170	45	4	21	
Orthociaalus(Euorthociaalus)	2	1	0	0	0	5	
Orthociaalus(Orthociaalus)	0	1	0	0	0	0	
Parametriocnemus sp.	0	0	1	1	0	1	
Pseudodiamesa sp.	0	3 14	1	20	10	1	
<i>Rheocricotopus</i> sp.	4	14	4	15	3	5	
<i>Synorinociaaius</i> sp.	0	10	29	2	2	1	
<i>i nienemanniella</i> sp.	0	1	0	U	1	1	
Intenemannimyta Gr.	0	1	2	0	0	3	
<i>Ivetenia</i> sp.	0	I	2	3	1	1	
i vetenia bavarica Gr.	1	0	0	0	1	U	
Zavrelimyia sp.	0	1	0	1	1	0	
Empididae	3	4	0	3	0	2	
Oreogeton sp.	0	1	0	0	0	0	
Simuliidae	0	0	2	0	0	1	

Appendix B (continued). Macroinvertebrates collected at study site, Upper Thornton, 15 September 2007. Units in numbers of macroinvertebrates per  $0.09 \text{ m}^2$ .
			San	ıple		
Macroinvertebrate Taxa	1	2	3	4	5	6
Heteroptera	1	0	1	0	0	0
Isotomidae	0	1	1	1	0	0
Poduridae	0	0	0	1	0	0
Harpacticoida	0	2	8	10	38	0
Cyclopoidae	0	0	2	4	1	0
Calanoida	0	1	0	0	1	0
Nematoda	2	2	10	10	2	0
Oligochaeta	0	0	0	3	0	0
Polycelis coronata	1	25	54	1	12	8
Acari	22	4	121	129	157	5
Coleoptera	0	0	0	1	0	0
Coleoptera (early instar)	1	0	0	1	0	0
Stictotarsus striatellus	2	1	16	26	9	3
Hydroporinae	0	0	0	8	6	1
Ephemeroptera	0	0	1	0	0	0
Ephemeroptera (early instar)	0	0	0	2	10	0
Ameletus sp.	6	13	5	23	26	0
Plecoptera (early instar)	0	3	22	1	4	0
Zapada columbiana	3	55	47	0	0	0
Setvena sp.	1	0	2	0	1	0
Trichoptera pupa	0	0	0	1	0	0
Desmona mono	0	0	5	23	1	1
Rhyacophila rotunda Gr.	2	1	6	0	0	0
Ceratopogonidae	1	0	3	17	3	0
Chironomidae (early instar)	56	143	1979	358	586	137
Chaetocladius dentiforceps Gr.	0	1	0	0	0	0
Corynoneura sp.	7	27	31	10	18	2
Eukiefferiella brehmi Gr.	0	4	0	0	2	0
Eukiefferiella claripennis Gr.	1	0	9	4	9	0
Heterotrissocladius marcidus	0	0	0	1	0	0
Hydrobaenus sp.	0	0	1	0	3	0
Orthocladius sp.	1	0	1	3	2	1
Orthocladius(Euorthocladius)	0	0	0	1	0	0
Paracladopelma sp.	0	0	0	1	0	0
Procladius sp.	1	0	0	0	0	0
Psectrocladius sp.	0	1	0	2	3	1
Pseudodiamesa sp.	5	10	2	14	13	8
Rheocricotopus sp.	0	0	0	0	1	0
Tvetenia sp.	0	0	2	2	1	0
Tvetenia bavarica Gr.	0	0	1	0	1	0
Zavrelimyia sp.	5	5	0	18	5	2

Appendix B (continued). Macroinvertebrates collected at study site, Upper Tapto, 9 September 2007. Units in numbers of macroinvertebrates per 0.09 m<sup>2</sup>.

	Sample					
Macroinvertebrate Taxa	1	2	3	4	5	6
Homoptera	0	1	1	0	0	0
Poduridae	1	1	1	0	0	0
Harpacticoida	2	2	4	0	0	1
Nematoda	61	107	100	26	74	7
Acari	1	1	0	2	1	0
Coleoptera						
Agabus sp.	0	0	0	0	1	0
Dytiscinae	0	0	0	0	1	0
Sphaeridiinae	1	0	0	0	0	0
Staphylinidae	0	1	0	0	0	0
Stenus sp.	0	1	0	0	0	0
Hydroporinae	1	0	0	0	0	0
Hygrotus sp.	1	0	0	0	0	0
Ephemeroptera						
Ameletus sp.	1	0	0	0	0	0
Zapada columbiana	0	1	0	0	2	0
Setvena sp.	1	0	0	0	0	1
Trichoptera						
Ecclisomyia sp.	1	0	0	0	0	0
Rhyacophila verrula Gr.	0	0	1	0	0	0
Chironomidae (early instar)	5	24	23	11	144	62
Chaetocladius sp.	1	0	0	0	2	0
Eukiefferiella claripennis Gr.	0	1	4	2	6	1
Eukiefferiella gracei Gr.	0	0	1	0	0	0
Krenopelopia sp.	1	0	0	0	0	0
Orthocladius sp.	2	1	0	0	1	5
Polypedilum sp.	1	0	0	0	0	0
Psectrocladius sp.	45	37	12	48	57	67
Psectrocladius sordidellus Gr.	2	0	0	0	0	0
Pseudodiamesa sp.	7	3	0	0	2	2
Pseudorthocladius sp.	0	0	0	1	0	0
Rheocricotopus sp.	0	0	1	1	3	0
Tvetenia sp.	0	4	0	0	1	1
Tvetenia bavarica Gr.	0	1	2	0	15	0
Tipuladae						
Pedicia sp.	1	0	0	0	1	1

Appendix B (continued). Macroinvertebrates collected at study site, Lower Middle, 9 September 2007. Units in numbers of macroinvertebrates per 0.09 m<sup>2</sup>.

	Sample					
Macroinvertebrate Taxa	1	2	3	4	5	6
Isotomidae	0	0	0	2	0	0
Poduridae	1	0	0	0	0	0
Harpacticoida	23	0	41	120	24	9
Nematoda	0	0	8	7	0	0
Oligochaeta	1	0	6	0	0	0
Polycelis coronata	5	8	15	21	40	38
Acari	61	31	154	186	129	22
Ephemeroptera (early instar)	0	0	0	3	2	0
Ameletus sp.	3	0	8	0	4	1
Plecoptera (early instar)	13	0	7	43	10	7
Zapada columbiana	4	36	7	19	1	0
Perlodidae (early instar)	4	16	19	20	0	0
Setvena sp.	13	18	10	12	7	3
Trichoptera						
Apatania sp.	0	0	2	0	12	9
Ecclisomyia sp.	0	0	0	0	0	2
Rhyacophilidae (early instar)	0	0	2	0	0	0
Rhyacophila brunnea Gr.	0	1	0	0	0	0
Rhyacophila rotunda Gr.	0	1	0	0	0	0
Rhyacophila verrula Gr.	5	3	12	15	7	4
Neothremma sp.	0	0	1	0	0	0
Chironomidae (early instar)	205	0	397	202	113	39
Corynoneura sp.	1	0	2	0	0	0
Diamesa sp.	0	1	0	0	0	0
Diplocladius sp.	1	0	0	0	0	0
Eukiefferiella claripennis Gr.	0	4	1	7	3	4
Eukiefferiella devonica Gr.	18	67	10	3	3	1
Eukiefferiella Gracei Gr.	19	74	16	6	0	2
Micropsectra sp.	3	0	1	4	2	2
Orthocladiinae (early instar)	0	28	0	0	0	0
Orthocladius sp.	22	18	17	7	5	2
Orthocladius (Euorthocladius)	0	0	1	1	0	0
Pagastia sp.	1	4	1	1	0	0
Parochlus sp.	0	0	0	2	0	0
Pseudodiamesa sp.	0	0	0	2	4	0
Rheocricotopus sp.	5	0	7	15	1	0
Thienemanniella sp.	0	0	0	0	1	1
Tvetenia sp.	44	0	6	26	4	6
Tvetenia bavarica Gr.	41	376	51	170	3	13
Zavrelimyia sp.	0	0	1	1	0	0
Empididae	0	0	2	0	0	0
Clinocera sp.	1	1	0	1	0	0
Tipulidae						
Dicranota sp.	0	0	1	0	1	0
Pedicia sp.	1	0	0	0	0	0

Appendix B (continued). Macroinvertebrates collected at study site, Upper Middle, 9 September 2007. Units in numbers of macroinvertebrates per  $0.09 \text{ m}^2$ .

		Sample	
Macroinvertebrate Taxa	1	2	3
Poduridae	0	1	2
Harpacticoida	0	11	227
Cyclopoidae	0	3	3
Nematoda	2	22	55
Oligochaeta	0	16	234
Polycelis coronata	2	1	75
Acari	3	37	1462
Coleoptera			
Dytiscidae	0	0	1
Hydrocolus sp.	0	0	1
Hygrotus sp.	0	10	17
Ephemeroptera	0	0	2
Ephemeroptera (early instar)	0	12	470
Ameletus sp.	23	29	60
Plecoptera (early instar)	0	1	211
Zapada columbiana	10	54	124
Perlodidae (early instar)	0	0	1
Trichoptera	Ū	0	-
Psychoglypha sp	0	1	1
Rhvacophila rotunda Gr	3	3	44
Ceratonogonidae	0	61	195
Atrichopogen sp	0	01	1
Chironomidae (early instar)	13	335	4015
Chaetocladius sp	0	0	1
Commongura sp.	0	44	86
Eukiefforiella brohmi Cr	1	44	00
Eukiefferiella elarinennia Gr.	1	0	2
Eukiefferiella araaai Gr	0	0	ے 1
Euklejjenena gracer GL.	0	0	1
Heleroirissociaalus marciaus	0	0	1
Limnophyes sp.	0	12	1
Micropsectra sp.	0	15	0/
Orthociaaius sp.	2	/	0
Orthociaaius (Orthociaaius)	0	0	1
Parametriocnemus sp.	0	0	3
Parocnius sp.	0	0	09
Psectrociaaius sp.	0	2	0
Psectrocladius soraidellus Gr.	0	0	2
Psilometriocnemus sp.	0	0	2
Rheocricotopus sp.	0	28	0
Rheocricotopus fuscipes Gr.	0	0	137
Tanytarsus sp.	0	3	0
Thienemanniella sp.	0	0	1
Tvetenia sp.	0	0	2
Tvetenia bavarica Gr.	0	10	493
Zavrelimyia sp.	5	37	71
Simuliidae	0	0	1
Simulium sp.	0	0	5
Piezosimulium sp.	0	0	1

Appendix B (continued). Macroinvertebrates collected at study site, Tapto West, 9 September 2007. Units in numbers of macroinvertebrates per  $0.09 \text{ m}^2$ .