THESIS

CHANGES IN TAXONOMIC AND FUNCTIONAL DIVERSITY OF AQUATIC

MACROINVERTEBRATES ALONG A GRADIENT OF STREAM SIZE AND FLOW

STABILITY IN THE NORTHEASTERN COLORADO ROCKY MOUNTAINS

Submitted by

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ABSTRACT

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While the pattern of aquatic macroinvertebrate communities along stream size gradients have been examined in past studies, it is usually in the context of the river continuum, moving along a stream network from the headwaters to large rivers. The effect of stream size among small headwater streams has received less attention. With increasing temperatures and decreasing snowfall predicted in the Colorado Rockies, streams in the area are likely to decrease in size and have an increased likelihood of flow cessation in especially dry years. To understand how these changes will affect aquatic macroinvertebrate communities, this study explored differences in species occurring in streams of differing size and flow stability. I examined the taxonomic and functional diversity of aquatic macroinvertebrates in 12 headwater streams in the Cache la Poudre watershed of northern Colorado. Each stream was assigned a stream "type" based on size (measured by discharge and drainage area) and the stability of the flow throughout the summer.

My results show that size was positively correlated with both taxonomic and functional richness. I found that the large streams with intermediate stability and small streams with stable flow had greater taxa and functional richness than did the small streams with intermediate flow and small streams with unstable flow, illustrating that flow stability is also important in determining macroinvertebrate communities. Certain species functional traits, such as inhabiting

erosional zones and filter-feeding were found to be associated with increasing stream size and stability.

I calculated β-diversity across the size and stability gradient and found that replacement of taxa (turnover) better explained among-site differences than did addition of taxa (nestedness). The specific taxa that prefer smaller streams were identified with indicator species analysis. Overall, these results indicate that the hotter and drier summers predicted by climate change models may lead to decreases in overall macroinvertebrate taxa and functional richness and potentially cause displacement of taxa as the smallest headwater streams become less stable.

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INTRODUCTION

Headwater streams serve an important role in river health, helping to maintain

biodiversity and providing linkages with terrestrial systems (Lowe & Likens, 2005, Finn *et al*.,

2011). Headwater streams comprise a conservatively-estimated 70% of river networks (Lowe &

Likens, 2005); and, within headwaters, a large amount of variation exists between streams.

While headwaters of all continents have been the focus of copious research, the definition of

what defines a headwater stream is still vague (Clarke *et al*., 2008). A meta-analysis of

headwater macroinvertebrate communities found that only 3 of the 24 studies examined fit their

stricter definition for headwater streams: Clarke *et al*. (2008) proposed the term headwater be

reserved for streams that drain a 0-order basin, are 1-2 order on a 1:24,000 map, or drainage area

less than 100 hectares. One ramification of this unclear definition is the omission of smaller

headwater streams from ecological research, many of which are not indicated on available maps

(Lowe & Likens, 2005).

The close ties of headwater streams with the terrestrial environment make them

vulnerable to rising temperatures and shifting climates. In the northern Colorado Rockies,

montane streams above 2000 m asl are fed, at least in part, from snowmelt, and streams with

watersheds reaching above 3000 m asl are almost entirely dependent on snowmelt for flow

(Kampf & Richer, 2014). Climate models for the northern Colorado Rockies predict a decrease

in snowfall and an increase in summer temperatures (Rasmussen *et al*., 2014). This will result in

peak summer snowmelt arriving anywhere from two weeks to a month earlier (Barnett *et al*., 2005; Rasmussen *et al*., 2014). There is evidence that snowmelt is already advancing in Colorado (Clow, 2010; Rood *et al.*, 2008). Models also predict the climate becoming more arid with less precipitation and a smaller fraction of the precipitation falling as snow (Rasmussen *et al*., 2014). These changes to the climate will likely lead to headwater streams with decreased flow, especially in late summer and fall, and to a disruption in macroinvertebrate communities that occupy those streams (Schlosser, 1987).

Understanding how communities currently differ along a gradient of stream size and flow will help inform what taxa and functional niches are most vulnerable to changing conditions. Stream size has long been recognized as a potential indicator of community composition in streams (Vander Vorste *et al*., 2017), especially when viewing a river system longitudinally. The River Continuum Concept (RCC) describes changes in both physical and biological attributes of streams and rivers when moving from the headwaters to large rivers, and it has become a foundational theory in stream ecology (Vannote *et al*., 1980). Stream size serves as the gradient the RCC uses to predict changes in energy sources and community composition. Since its inception, numerous studies have been conducted to test the hypotheses of the RCC, and many of them have found stream size to be an important predictor of macroinvertebrate communities (Heino *et al*., 2005; Paller *et al*., 2006). However, at this scale the effect of stream size is highly confounded by other environmental variables (Harrington *et al*., 2016). While informative of possible patterns along a size gradient, the RCC and accompanying research do not scale down to size differences among headwater streams (e.g., 1st-2nd order).

Stream size can be defined in numerous ways (e.g. wetted width, depth, discharge, stream order) (Vander Vorste *et al*., 2017), and one measure alone likely will not capture how the

variation in size affects aquatic communities. When comparing streams that fall along a stream-size gradient, there are two main components that change with size: the quantity of usable habitat and the quality of that habitat. For benthic macroinvertebrates, the quantity of usable habitat translates roughly to the area of sediment covered with water, often estimated by measurements of wetted width (Dewson *et al.*, 2007). The species-area relationship predicts that fewer species will be found in smaller areas (Arrhenius, 1921). Multiple studies have found habitat size to influence stream communities (Carrara *et al.* 2014; Cowx *et al.*, 1984; Stanley *et al.*, 1997). In many stream types, decreasing size can also lead to decreasing habitat heterogeneity which is hypothesized to affect macroinvertebrate richness. While habitat heterogeneity has been linked to taxa richness of individual streams (Miller *et al.*, 2007), there is still a paucity of evidence for the connection (Palmer *et al.*, 2010).

The quality of streams along a stream-size gradient is also important for shaping aquatic communities; most notably, stream flow. A well-accepted concept in stream ecology is that the flow regime influences the composition of a stream community (Poff, 1997; Poff *et al.*, 1997). While emphasis is often put on the importance of the high flows, low flows and flow cessation are receiving increasing attention (Datry *et al.*, 2014; Datry *et al.*, 2016). At its extreme, low flows can create intermittent streams which are defined by flow cessation during dry periods (Nadeau & Rains, 2007). For many streams, drying is a part of the annual flow regime, while other streams may only experience flow cessation during particularly dry years. Measures such as flow permanence are used to capture the variation in magnitude and frequency of drying events (Datry *et al.*, 2016). Smaller stream-sizes do not always indicate an increased risk of flow cessation, but smaller drainage areas mean less area to accumulate surface runoff from snowmelt or stochastic rain events which could increase the chances during dry years if no other source is

available. Even in the absence of complete drying, decreased flow affects aquatic communities. Smaller habitats from low flows have been shown to support shorter food webs (McHugh *et al.*, 2014).

Many metrics have been developed to quantify biodiversity. The most basic method involves looking at the number of taxa present in a community (taxa richness) for a given area; for this study, a stream reach. This measure of local diversity is known as alpha (α) diversity to set it apart from beta (β) diversity which is a measure of how communities differ between these patches (e.g. stream reaches) in a region (Whittaker, 1960). β-diversity is measured through pairwise comparisons between local communities to measure how dissimilar these communities are from each other (Anderson, 2001). While headwaters usually have lower α-diversity than mid-order streams, previous studies have found that headwater streams often house higher β-diversity (Finn *et al.*, 2011). This has even been found to be true in montane streams independent of the elevation of the headwaters (Harrington *et al*., 2016). The high heterogeneity among headwater streams and relative geological isolation from each other leads to this high β-diversity (Astorga *et al*., 2014; Finn *et al*., 2011, Harrington *et al*., 2016).

β-diversity is influenced by two phenomena: species turnover and nestedness (Podani, 2013). Species replacement often occurs along gradients (e.g. elevation, habitat size) where species are replaced by others due to dispersal limitations or differences in habitat preference (Heino *et al.*, 2015). High taxa replacement along a gradient can be a sign of local adaptations of species (Heino *et al.*, 2003). For example, a study in southeast Arizona found that most species occupying intermittent streams had adaptations for surviving dry conditions (e.g. egg diapause) that were not found in species occupying nearby perennial streams (Bogan *et al.*, 2013).

In contrast to species turnover, nestedness occurs when one site possesses higher taxa richness than another site, and the more depauperate site contains a subset of taxa found in the richer site (Ulrich *et al.*, 2009). Several studies have found evidence for nestedness along gradients of stream size and permanence (Arscott *et al.*, 2010; Datry *et al.,* 2014). For the mountain headwaters being used in this study, it is likely that nestedness will explain more of the pattern of β-diversity since many of the small streams do not dry with enough frequency to exert a strong selective pressure on the macroinvertebrates found in small streams for there to be specialized taxa in those streams.

An additional way of examining diversity is by focusing on the functional niches present at a site, known as functional diversity. Taxa are assigned functional traits based on knowledge of their habitat preference, morphology, and behaviors which can then be used to quantify their functional niche and the overall functional diversity (Poff *et al.,* 2006). When examining communities along a gradient, focusing on functional niches can be more informative than focusing on the taxa composition because it gives more insight to the mechanisms causing changes along the gradient (de Bello *et al.*, 2010; McGill *et al.*, 2006; Menezes *et al.,* 2010). The use of functional traits and functional diversity has also gained popularity in community ecology because it lessens the reliance on the taxonomic identity of regional taxa pools which differ from region to region and make comparisons difficult.

Past research on functional diversity of aquatic communities have detected several patterns along gradients of stream size and permanence. Studies of intermittent streams have found high abundances of taxa with the ability to either survive flow cessation through the ability to survive desiccation (Arscott *et al.*, 2010; Datry *et al.*, 2007; Griswold *et al.*, 2008), or to recolonize quickly after drying events through high female adult dispersal (Chester & Robinson,

2011). Many studies focus on how macroinvertebrates' mode of eating (functional feeding groups or guilds) changes along gradients (Heino, 2005, Vannote *et al*., 1980). One of the most prevalent patterns is a decrease in collector-filterers with decreasing stream size and permanence (Ledger *et al*., 2011; Santos & Stevenson, 2011). Changes in the composition of predators have also been detected: engulfer predators are typically seen decreasing along a stream permanence gradient while piercer predators increase (Ledger *et al*., 2011). Table 1 contains a complete list of the functional traits used in this study and their predicted responses along a stream size and permanence gradient.

The purpose of this study is to explore how benthic macroinvertebrate communities differ along a gradient of stream size and flow stability within the category of headwater streams. I predict that

1.) α-diversity will decrease with decreasing stream size and flow stability,

2.) functional diversity will decrease, with certain traits responding predictably to the size gradient (see Table 1), and lastly

3.) nestedness will be more prominent than species turnover when moving down the stream-size gradient.

To test these hypothesis, the macroinvertebrate communities of 12 montane streams were sampled three times between June 23 and October 10 of 2016.

METHODS

Site Selection

Sites were selected based on elevation and watershed area. StreamStats (https://streamstats.usgs.gov/) was used to select potential sites that were then visited and chosen

Table 1. *A priori* hypotheses of whether the proportion of selected traits will increase (↑), decrease (↓) or have no change (-) with decreasing stream size. The table is modeled after Bonada *et al*. (2007) and the trait states are taken from Poff *et al*. (2006). Thermal preference has three states in Poff *et al*. (2006) but only two are included here because no invertebrates in this study had strictly warm eurythermal preferences.

Trait	States	Code	Response	Rationale
Voltinism	Semivoltine (<1 generation/y)	Volt1	↑	Taxa with faster life cycles have greater probability of completing life cycles before stream conditions become unfavorable
	Univoltine (1 generation/y)	Volt2	-	
	Bi- or multivoltine (>1 generation/y)	Volt3	↓	
Development	Fast seasonal	Devl1	↑	Nonseason development and fast-seasonal development decrease the chances of being present in streams for unfavorably low flow conditions
	Slow seasonal	Devl2	↓	
	Nonseasonal	Devl3	↑	
Ability to Exit	Absent (excluding emergence)	Exit1	↓	Increased frequency of drying more likely to select for ability to exit unfavorable stream conditions
	Present	Exit2	↑	
Ability to survive desiccation	Absent	Desi1	↓	Increased frequency of drying more likely to select for ability to survive desiccation (Williams, 1996)
	Present	Desi2	↑	
Female Dispersal	Low (<1 km flight before laying eggs)	Disp1	↓	High dispersers more likely to recolonize small streams after a drying event
	High (>1 km flight before laying eggs)	Disp2	↑	
Occurrence in Drift	Rare (catastrophic only)	Drft1	-	Drift decreases with decreasing stream discharge, so smaller streams will have less drifting (Delucchi, 1989) but macroinvertebrates may drift to escape unfavorable conditions in drying reaches
	Common (typically observed)	Drft2	↑/↓	
	Abundant (dominant in drift samples)	Drft3	↑/↓	
Shape	Streamlined (flat, fusiform)	Shpe1	↓	Decreased streams will lessen the selection pressure for streamlined bodies
	Not streamlined (cylindrical, round, or bluff)	Shpe2	↑	
Respiration	Tegument	Resp1	↓	Lower flow conditions make oxygen harder to obtain from the water, favoring plastrons and spiracles (Bonada *et al*., 2007)
	Gills	Resp2	↓	
	Plastron, spiracle (aerial)	Resp3	↑	
Size at maturity	Small (<9 mm)	Size1	↑	Larger macroinvertebrates are more susceptible to drying disturbances and will be selected against in smaller streams (Ledger *et al*., 2011)
	Medium (9–16 mm)	Size2	-	
	Large (>16 mm)	Size3	↓	

Table 1 (cont.)

Rheophily	Depositional only	Rheo1	↑	Decreased discharge will select for ability to survive in depositional zones
	Depositional and erosional	Rheo2	-	
	Erosional	Rheo3	↓	
Thermal preference	Cold stenothermal or cool eurythermal	Ther1	↓	Temperature usually increases with decreasing stream size and permanence, filtering out taxa that must have cold temperatures (Rader & Belish, 1999)
	Cool/warm eurythermal	Ther2	↑	
Habit	Burrow	Habi1	↑	Stream drying events with select for burrowing into the hyporheic zone as a refuge
	Climb	Habi2	-	
	Sprawl	Habi3	-	
	Cling	Habi4	↓	Clinging will decrease with decreasing stream permanence because less food will be present in the water column
	Swim	Habi5	-	
	Skate	Habi6	-	
Trophic Habit	Collector-gatherer	Trop1	-	
	Collector-filterer	Trop2	↓	Decreased flow decreases the amount of food flowing from upstream (Grubbs, 2011)
	Herbivore (scraper, piercer, and shedder)	Trop3	↓	Decreased scrapers and shedder with decreased stream flow (Grubbs, 2011; Heino, 2005)
	Predator (piercer and engulfer)	Trop4	↑/↓	Piercer predators have been shown to increase with decreasing stream discharge while engulfers decrease (Ledger *et al*., 2011)
	Shredder (detritivore)	Trop5	↑	Shredders increase with decreasing stream size (Vannote *et al*., 1980)

based on accessibility. 12 streams were selected across an elevation range of 2036 to 2938 meters asl in the Cache la Poudre watershed in the northeastern Colorado Rockies. Two of the streams, Elkhorn and Little Beaver (abbreviated ELKH and LIBE for this study), were being monitored by a companion study (Rugenski & Flecker, *unpublished data*). These streams were larger than the other 10 included in the study: the average width of ELKH and LIBE was 431 cm while the average of the other 10 streams was 102 cm. The larger streams were included so comparisons between them and the smaller streams could be made.

At each stream, a 50-meter reach was measured and marked for use throughout the season. All macroinvertebrate and environmental data for a stream was taken within that reach. Data collection occurred between June 23 and October 10 of 2016.
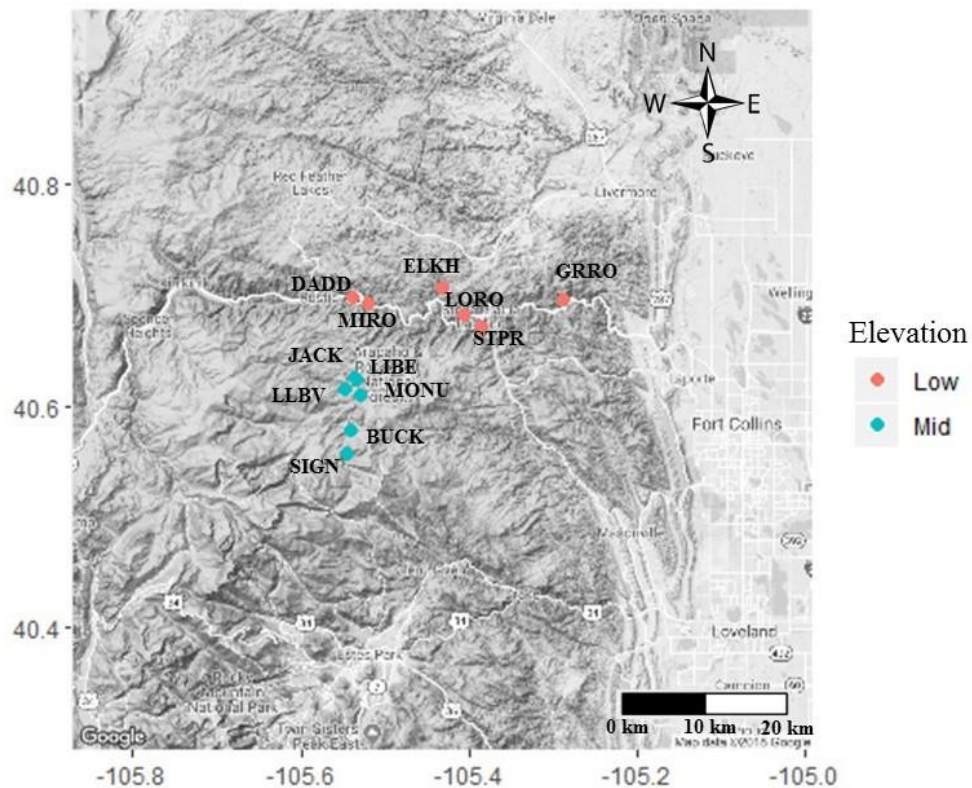
Fig. 1. A map of the study streams in the Cache la Poudre watershed. Blue dots indicate the sampling locations for streams that are 2450-2950 m asl (mid-elevation) and the red dots indicate streams that are 2000-2400 m asl (low-elevation)

Environmental Sampling

Each stream was visited an average of every two weeks, resulting in 7-8 measuring points for each site during the summer. The two largest streams (LIBE and ELKH) were an exception to this since those streams were already being monitored by a separate study (Rugenski & Flecker, *unpublished data*). Those sites were visited four times during the summer. Each time a stream was visited, pH, conductivity, and temperature were measured using multimeter and probe (Thermo Fisher Scientific, Waltham, Massachusetts). Discharge was measured by taking ten velocity measurements, spaced evenly along a cross-section of the stream, using a Schiltknecht MiniWater® 20 flow meter (Switzerland) having a 2.5 cm diameter propeller. Average depth measurements were collected by walking the length of the 50-meter reach and taking measurements haphazardly in a zigzag pattern. To obtain average width, ten width measurements were taken approximately every five meters. The two larger streams were not sampled for pH or conductivity, so those variables were excluded from the analyses which included those sites.

At three points during the field season, percent canopy cover was calculated using the IOS CanopyApp (Schloss *et al*., 2013). The percentage of cover was measured at the top, middle, and bottom of each 50-m reach. At each point, four canopy cover measurements were taken, one while facing upstream, downstream, left, and right. The resulting 12 measurements were then averaged. At one point during the season, average particle size (d50) of the sediment was taken by randomly selecting 250 mineral particles from the streambed and measuring them with a gravelometer. Each meter along the reach was classified as riffle or pool by sight halfway through the summer to obtain the percentage of riffles and pools.

The rate of decline of discharge throughout the summer was calculated for each stream by fitting an exponential decline model to the discharge measurements of the site. The resulting slope was used as the rate of decline (ROD) variable for the analyses. For most streams, seven discharge measurements taken throughout the summer were used for this analysis. However, for the sites LIBE and ELKH, only four measurements throughout the summer were available for the analysis. For this reason, the measurements of ROD for LIBE and ELKH were used for graphing (Fig. 2) but not in statistical tests. The stability of the discharge throughout the summer was estimated by calculating the percentage variation in discharge ("Q_{var}") (Smith & Wood, 2002). The following formula was used for the calculation:

$$Q_{var} = \frac{maxQ - minQ}{maxQ} \text{ x } 100$$

Q_{var} is also used as a metric for flow permanence (Smith & Wood, 2002); but, because most of the streams used in this study did not dry during the summer of observation, I am using it as a measure of how stable the flow was throughout the summer.

Streams were separated into stream "types: based on their size and the flow stability (Table 2). Stream size was determined by average discharge measured over the summer. The designation of "large" was assigned to the streams ELKH and LIBE whose average discharge were 0.131 m³/s and 0.042 m³/s, respectively. The remaining 10 streams all had average discharges less than 0.002 m³/s and were designated as "small". To separate the streams based on stability, the Q_{var} was plotted for each stream (see Fig. 2) to see how the streams clustered across the gradient. From this, the streams were categorized as either "stable" (Q_{var} < 90%), "intermediate" (90% < Q_{var} < 95%), "unstable" (Q_{var} > 95%), or "drying" (Q_{var} = 100%).

Table 2. Two variables, size and flow stability, were used to divide sites into stream types.

Variable	Description	Levels	Definition
Size	Discharge (m^3/s) averaged across the summer	Large (L)	Discharge > 0.004 m^3/s
		Small (S)	Discharge < 0.002 m^3/s
Flow Stability	Percentage of variation in discharge throughout the summer (Q_{var})	Stable (S)	Q_{var} < 90%
		Intermediate (I)	Q_{var} between 90% and 95%
		Unstable (U)	Q_{var} > 95%
		Drying (D)	Q_{var} = 100%

Five stream types were classified using this criteria: large-intermediate (LI), small-stable (SS), small-intermediate (SI), small-unstable (SU), and small-drying (SD) (Table 3). The streams in these categories clustered together when the variables discharge, Q_{var}, and ROD were plotted (Fig. 2). SD contained only one site, so it was excluded from most group analyses. Pairwise comparisons of environmental variables between sites were conducted by running a linear model with stream type as the explanatory variable. That model was then used to calculate estimated marginal means using the *emmeans* package in R (this method is abbreviated as "pairwise
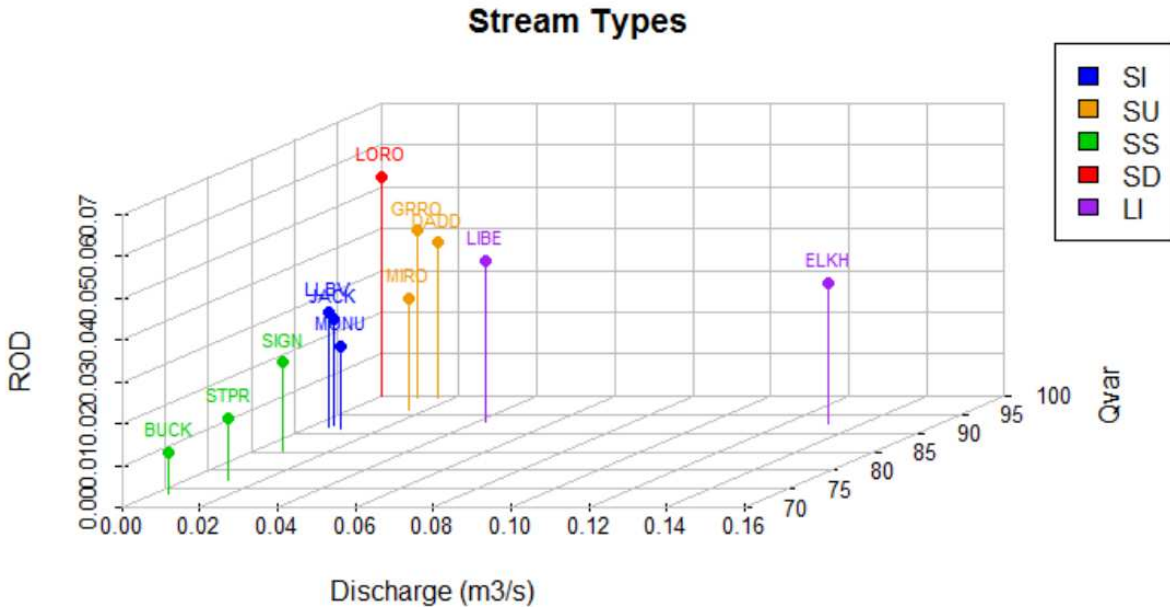
Figure 2. Graph of three discharge variables, average discharge, variation in discharge (Q_{var}), and the absolute value of ROD (rate of decline of discharge). Sites are colored by the stream type they were assigned.

Table 3. Averages of environmental variables for all sites. Conductivity, pH, and % riffles were not measured at the two LI streams. While depth measurements were taken at those sites, they were not divided between pools and riffles so are not included in this table. Rate of decline was not calculated for the LI streams because discharge was measured less frequently than the other sites.

Group	Site	Drain. Area (km²)	Elev. (m)	Discharge (m³/s)	Qvar (%)	d50	Temp. (°c)	Width (cm)	% Canopy Cover	Cond. (μS)	pH	Pool Depth (cm)	Riffle Depth (cm)	% Riffle	Rate of Decline (m³/s/day)
LI	ELKH	85.47	2044	0.1311	92.6%	90	16.65	423.76	64.9%	n/a	n/a	n/a	n/a	n/a	n/a
	LIBE	37.55	2428	0.0421	93.1%	90	11.15	438.93	4.0%	n/a	n/a	n/a	n/a	n/a	n/a
SS	BUCK	4.51	2816	0.0035	73.9%	32	8.34	91.08	39.5%	66.01	7.01	4.30	7.50	78%	0.010
	SIGN	2.72	2938	0.0077	85.2%	45	7.38	85.42	44.6%	41.43	6.30	6.39	15.96	56%	0.021
	STPR	5.18	2222	0.0109	77.4%	32	15.59	120.50	28.0%	299.20	8.33	5.54	11.10	76%	0.015
SI	JACK	5.91	2688	0.0060	91.9%	45	11.00	114.94	53.3%	121.86	7.51	4.91	8.81	74%	0.026
	LLBV	2.54	2679	0.0047	91.7%	32	9.60	73.85	55.5%	89.67	7.57	5.23	10.33	74%	0.027
	MONU	2.64	2600	0.0090	91.2%	64	13.25	154.61	38.0%	128.07	7.63	6.44	13.35	60%	0.020
SU	DADD	7.72	2478	0.0161	99.3%	45	11.29	114.17	54.4%	227.40	7.42	4.96	9.72	76%	0.037
	GRRO	3.26	2036	0.0104	99.5%	64	16.16	137.80	34.8%	245.06	8.10	4.71	16.13	56%	0.040
	MIRO	2.64	2414	0.0151	96.3%	16	11.71	59.03	45.9%	259.80	7.94	3.22	5.56	86%	0.027
SD	LORO	0.73	2094	0.0002	100.0%	32	19.11	66.09	8.5%	198.99	8.16	2.48	5.35	82%	0.052

comparisons" from here on) (Lenth, 2018). While still not ideal, this method allowed us to test if

SD was significantly different from other stream types even though it contains only one site. All

stream types differed significantly for at least one variable except for the comparisons of SU

with SD and SI (Table 4). However, I believe these groups are still significant because the

presence of compete flow cessation separates SD and, although not statistically significant, the

Table 4. *T-statistics (df = 6) and p-values (*below*) for pairwise comparisons of environmental*

variables between stream types. ROD comparisons were not performed on LI streams because

that measurement was not collected for those sites.

	LI-SD	LI-SI	LI-SS	LI-SU	SD-SI	SD-SS	SD-SU	SI-SS	SI-SU	SS-SU
Elevation	0.414	-1.643	-1.655	-0.075	-1.739	-1.748	-0.476	-0.013	1.561	1.573
	0.992	*0.524*	*0.518*	*1.000*	*0.478*	*0.474*	*0.987*	*1.000*	*0.566*	*0.559*
Discharge	2.725	3.387	3.354	2.834	-0.213	-0.239	-0.411	-0.037	-0.282	-0.249
	0.161	*0.075*	*0.078*	*0.142*	*0.999*	*0.999*	*0.992*	*1.000*	*0.998*	*0.999*
Average Width	11.057	12.869	13.498	11.321	-1.553	-1.057	-1.813	0.702	-0.468	-1.096
	0.000	***0.000***	***0.000***	***0.000***	*0.569*	*0.822*	*0.444*	*0.949*	*0.988*	*0.804*
%Canopy Cover	1.063	-0.797	-0.161	-0.511	-1.757	-1.255	-1.480	0.710	0.237	-0.398
	0.819	*0.923*	*1.000*	*0.983*	*0.469*	*0.725*	*0.607*	*0.947*	*0.999*	*0.993*
Qvar	-1.744	0.417	4.616	-1.956	2.179	5.499	0.147	4.695	-2.560	-6.759
	0.476	*0.992*	***0.020***	*0.383*	*0.300*	***0.009***	*1.000*	***0.019***	*0.195*	***0.003***
ROD	*n/a*	*n/a*	*n/a*	*n/a*	4.232	5.597	2.673	1.929	-2.205	-4.135
					0.021	***0.006***	*0.128*	*0.309*	*0.224*	***0.024***

stream types SU and SI clearly separate along the axis of Q_{var} (Fig. 2). Elevation and % canopy

cover were found to not differ significantly between any group.

Two correlation analyses were conducted on the environmental variables to remove

redundant variables (r > 0.75). The first analysis included all sites (Table 5), and the second

analysis was performed on just the "small" stream types with the two "large" streams excluded

(Table 6). This second analysis was conducted because more environmental variables were

Table 5. *Pearson's r for correlations between environmental variables with all sites included.*

	Q_{var}	Drainage Area (km^2)	Elevation (m)	d50	Temp (°C)	Discharge (m^2/s)	Width (cm)	Depth (cm)
Drainage Area	0.08							
Elevation	-0.46	-0.4						
d50	0.21	0.74**	-0.3					
Temp	0.41	0.27	-0.93****	0.21				
Discharge	0.11	0.99****	-0.44	0.69*	0.3			
Width	0.09	0.88***	-0.34	0.90****	0.19	0.82**		
Depth	0.06	0.82**	-0.21	0.91****	0.05	0.76**	0.96****	
Canopy Cover	-0.03	0.21	0.25	-0.08	-0.3	0.3	-0.13	-0.1

*$p < .05$. **$p < .01$. *** $p < .001$.

measured at the 10 "small" streams, and the LI sites were found to significantly pull some correlations in one direction or the other.

Macroinvertebrate Collection

Macroinvertebrates were collected three times during the summer: the end of June, beginning of August, and beginning of September. A separate sample was taken for pool and riffle features at each of the 12 sites for each time, resulting in a total of 72 samples. A D-frame kick net with 500μm mesh was used for two minutes for each sample. Samples were taken by disturbing the streambed with kicking motions and collecting dislodged

insects in the net, following a zigzag motion across the channel (Resh & Rosenberg, 1984). Care was taken to sample all habitat types within the channel. Samples were preserved in 100% ethanol and returned to the lab where a dissecting scope was used to sort macroinvertebrates from the sediment. The invertebrates were then identified to the lowest taxonomic unit needed for functional trait analysis (typically genus). Over 80,000 individuals were identified from 103 taxonomic groups (see Appendix 2).

Table 6. Pearson's r for correlations between environmental variables for the 10 "small" sites. More variables were measured for these streams than the "large" streams, and those variables are included in this analysis.

	Drainage Area (km²)	Elevation (m)	d50	Cond. (µS)	pH	Temp (°C)	Discharge (m²/s)	Width (cm)	Canopy Cover (%)	Pool Depth (cm)	Riffle Depth (cm)	% Riffle	Qvar (%)
Elevation (m)	0.17												
d50	0.11	-0.05											
Cond. (µS)	0.17	-0.85**	-0.14										
pH	-0.11	-0.88***	-0.1	0.82**									
Temp (°C)	-0.29	-0.93****	0.13	0.70*	0.85**								
Discharge (m²/s)	0.5	-0.14	0.05	0.56	0.12	-0.09							
Width (cm)	0.39	-0.14	0.85**	0.14	0.14	0.19	0.27						
Canopy Cover (%)	0.53	0.63	0.04	-0.33	-0.48	-0.78**	0.43	0.05					
Pool Depth (cm)	0.28	0.5	0.57	-0.37	-0.46	-0.44	0.21	0.61	0.43				
Riffle Depth (cm)	0.02	0.11	0.76*	-0.18	-0.3	-0.13	0.19	0.62	0.16	0.79**			
% Riffle	0.1	-0.16	-0.85**	0.32	0.37	0.12	-0.03	-0.61	-0.1	-0.72*	-0.96****		
Qvar (%)	-0.18	-0.48	0.21	0.34	0.34	0.43	0.22	-0.04	-0.01	-0.37	-0.07	0	
Rate of Decline (m³/s/day)	-0.27	-0.63	0.11	0.35	0.4	0.62	-0.09	-0.18	-0.36	-0.55	-0.16	0.09	0.87**

*p < .05. **p < .01. *** p < .001.

Each taxon was assigned functional traits based on Poff *et al*. (2006). For taxa collected that were not included in Poff *et al*. (2006), Merritt *et al*. (2006) and Dr. Boris Kondratieff were consulted (see Appendix 3). Only insect taxa with more than five individuals collected during the summer were used in the trait analyses (a total of 73 taxa). Two non-insect groups (flatworm *Polycelis coronata* and aquatic mite *Arrenurus*) were included because of their high abundance. 13 functional traits were selected for the analysis for a total of 39 trait states (see Table 1).

Alpha Diversity

The first question I wanted to answer was whether taxa diversity was significantly influenced by any environmental gradients and if this pattern was the same for macroinvertebrates found in riffles and pools. I combined the taxa abundance data from the three sample periods for riffles and pools for each site, resulting in one pool and riffle sample for each site. These data were then used to calculate taxon richness and Shannon's Diversity Index (H`). Taxa richness was chosen because of its ability to be compared with my measure of functional richness. H` was included because it accounts for abundance and changes to the evenness among groups.

A correlation analysis was conducted between the environmental variables and the two measures of diversity to test if any environmental gradients were influencing differences in diversity. Sites were then divided by stream type (see Table 2), and pairwise comparisons were run to test for difference in taxa richness and H` between stream types. SD was excluded from the analyses.

To test my prediction that the differences between streams of varying size and flow permanence would become more pronounced as the summer progressed, I combined the riffle and pool samples for each sampling time, resulting in one sample for each site at each sampling

17

point. Taxa richness and H` were calculated at each time point for each site. Separate pairwise comparisons were run for each sample period to see if taxa richness or H` differed between stream types.

A redundancy analysis (RDA) was performed to see how both sites and individual taxa were related to environmental variables. This constrained ordination method assumes that the variables (e.g. taxa and traits) respond linearly to environmental gradients. While this method is not ideal for large-scaled gradients where variables may have a unimodal response, I believed it was appropriate for the fine gradient of stream size (Finn & Poff, 2005; Legendre & Gallagher, 2001). Rare species were excluded from the RDA to make the results more comparable to the functional trait RDA (see below). The abundance data were transformed using the Hellinger method (Borcard *et al*., 2011; Legendre & Gallagher, 2001) before being used in the RDA. Two RDAs were performed. The first RDA included all sites and used only uncorrelated environmental variables which were discharge, elevation, % canopy cover, and Q_{var}. A second RDA was run that did not include the two LI sites. This was done so the additional variables only sampled at those sites could be included. The LI sites also strongly influenced correlation amongst some of the environmental variables, so excluding LI changed which variables were correlated (see Table 3). The environmental variables used in the second RDA were elevation, discharge, Q_{var}, ROD, % canopy cover, and average width. To make the second ordination more comparable with the first, four of these variables were selected using the *ordistep* function from the vegan package (Blanchet *et al*., 2008).

Lastly, an indicator species analysis (ISA) was performed to identify taxa that might be strongly associated with a stream type (Cáceres & Legendre, 2009). Taxa are scored by their specificity to a group (whether the taxa were found only in one stream type) and fidelity

(whether the taxa occurred in all the streams of a stream type). A permutation test was then conducted using the *multipatt* function from the indicspecies package in R to test the null hypotheses of there being no relationships between taxa and stream types (de Cáceres & Legendre, 2009). Stream type SD was excluded from this analysis because it contained only one site.

Functional Diversity

Functional diversity is comprised of several components, including richness, evenness, and divergence (Mason *et al*., 2005). Functional richness ("FRic") was measured as the number of unique functional trait state combinations, or functional niches, possessed by taxa in a community. This definition was suggested by Poff *et al*. (2006) and later used by Schmera *et al*. (2012). This measure is independent of abundance and is comparable to taxa richness. A total of 68 total functional niches were present. Functional evenness ("FEve") is a measure of how abundance is spread across the functional niches present (Mason *et al*., 2005). FEve is constrained between 1 and 0, with 1 indicating that the macroinvertebrate abundance was evenly spread across all functional niches present (Villéger *et al*., 2008). Functional divergence ("FDiv") is a measure of how different functional niches are from each other (Mason *et al*., 2005). For this study, I focused on FRic and FEve. Both were calculated using the *dbFD* function from the "FD" package in R (Laliberté *et al*., 2014) which utilizes a species abundance-by-site and trait state-by-species matrix to calculate the functional diversity indices.

To examine whether functional diversity was significantly influenced by any environmental gradients in riffle and pool habitats, I combined the taxa abundance data from the three sample periods for riffles and pools for each site. These combined data were used to calculate FRic and FEve. Correlation analyses were conducted with environmental variables to

detect environmental gradients influencing FRic and FEve. Streams were then split into the same stream types used in previous analyses. Pairwise comparisons were conducted to see if the mean values of FRic and FEve differed between stream types. To test whether the functional diversity of stream types diverged more as the summer progressed, the taxa abundance data were split by sample time and the pool and riffle components of each sample time were combined. FRic and FEve were calculated for each time point and then compared between groups through pairwise comparisons.

RDAs were run to ascertain how environmental variables influenced individual trait states and sites. To make these RDAs comparable to those based on taxonomic data, a method described by Finn *et al*. (2005) was used. The taxa-by-trait state matrix (76 taxa x 39 traits states) was multiplied by the species abundance matrix (12 sites x 76 taxa). The Hellinger transformation was then performed, followed by the RDA. Like with the taxa data, two RDAs were run, one including the LI streams and one using only the "small" streams.

I hypothesized that species traits would respond in a predictable manner to decreasing stream size. To test this, I calculated the proportion of each trait state in the samples of each stream. These proportions were compared between stream types using pairwise comparisons.

Beta Diversity

Community differences between sites were compared using the Jaccard dissimilarity index (Borcard *et al*., 2011; Podani, 2013). This index is based on presence-absence data, so the abundances of taxa present in each stream were not considered. Dissimilarity has two components. "Taxa replacement", also known as taxa turnover, measures the turnover in species across sites. "Richness differences" represent the difference between sites taxa richness. These are calculated as follows:

Dissimilarity $\qquad D_J = (b+c)/(a+b+c)$

Taxa Replacement $\qquad Repl_J = 2(\min\{b,c\}/(a+b+c)$

Richness Difference $\qquad RichDiff_J = |b-c|/(a+b+c)$

where a represents the taxa shared between the two sites (e.g. site 1 and 2) and b and c are the taxa unique to site 1 and site 2, respectively (see Fig. 3) (Podani & Schmera, 2011; Legendre, 2014). Each dissimilarity component was divided by the total number of taxa found at both sites so that differing overall richness between streams would not influence the results.

These values indicate the relative importance of taxa turnover (measured though the replacement component) and nestedness (estimated through the richness difference). Richness
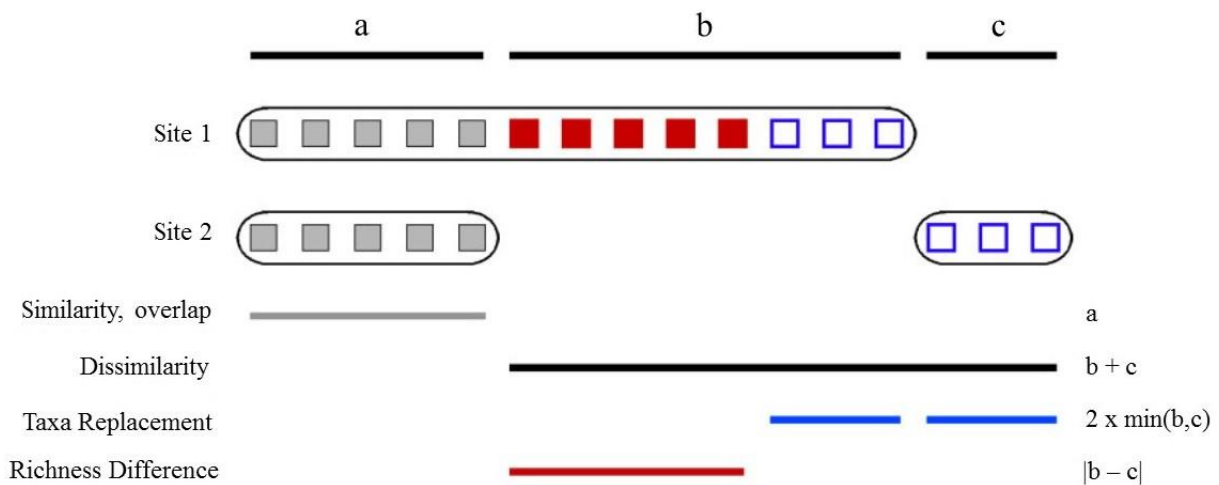
Fig. 3. A diagram of the components used to calculate taxa replacement (open blue squares) and richness difference (red squares) taken from Legendre 2014. When using Jaccard dissimilarity, each square represents a taxon found at both (grey squares) or only one of the sites (red and open blue squares).

difference is, however, not a direct measure of nestedness because it does not account for species present at both sites, but it is a convenient way to calculate the proportion of dissimilarity not caused by taxa turnover. If a high proportion of the dissimilarities between sites is due to replacement, it would indicate there may be small stream specialists; however, if richness differences contribute more to dissimilarities, it is an indication that small streams are likely

nested subsets of the taxa found in larger streams with higher stream permanence (Carvalho *et al.,* 2013). These values were plotted with the Jaccard similarity $(1 - D_J)$ in a triangle (simplex) plot (Podani & Schmera 2011) to illustrate the relative importance of each to β-diversity. β-diversity was broken down for each of the three sample periods. Sites were then separated into two elevation bands: low (2000-2400 m asl) and mid (2450-2950 m asl) in order to see if taxa turnover along an elevational gradient were affecting the results since certain taxa in the area are known to turnover along an elevation gradient (Polato *et al.,* 2017)

RESULTS

Environmental Variables

Correlation analysis of all 12 sites (including the two larger 3rd order sites) showed that that temperature and elevation were negatively correlated and drainage area, d50, discharge, width, and depth were all positively correlated (see Table 5). Q_{var} and percent canopy cover were not correlated with any other variables. When the correlation analysis was conducted only on the "small" streams, elevation was found to be negatively correlated with conductivity, pH, and temperature (see Table 6). The percentage of the 50 m reach that was riffle habitat (% riffle) was negatively correlated with d50 and pool and riffle depth, and d50 was found to be positively correlated with width and riffle depth. Lastly, the rate of decline of discharge and Q_{var} were positively correlated with each other (see Table 6).

Alpha Diversity

Taxa richness was positively correlated with drainage area (r = 0.56, p = 0.056) and discharge (r = 0.58, p = 0.047) for riffle habitats (Table 7). In pool habitats, taxa richness was negatively correlated with Q_{var} (r = -0.57, p = 0.051). Shannon's diversity (H`) was negatively

correlated with temperature in both riffle (r = -0.57, p = 0.051) and pool (r = -0.68, p = 0.014) habitats. H` was also positively correlated with canopy cover for riffles (r = 0.58, p = 0.049).

Table 7. Correlation coefficients (Pearson's r) and p-values (*below*) of taxa richness and Shannon's diversity index and environmental variables. Rate of Decline does not include the LI sites. P-values significant at α = 0.057 are bolded.

		Qvar	Drain. Area (km^2)	Elev. (m)	d50	Temp (°C)	Disch. (m^3/s)	Width (cm)	Depth (cm)	Canopy Cover (%)	ROD (m^3/s per day)
Taxa Richness	Riffle	-0.28	0.56	-0.33	0.29	0.21	0.58	0.47	0.53	0.04	-0.28
		0.3863	***0.0564***	*0.2872*	*0.3642*	*0.5065*	***0.0465***	*0.1193*	*0.0787*	*0.8925*	*0.4254*
	Pool	-0.57	0.23	-0.14	0.01	0.04	0.20	0.19	0.16	0.03	-0.37
		0.0508	*0.4698*	*0.6596*	*0.9647*	*0.8909*	*0.5382*	*0.5647*	*0.6244*	*0.9169*	*0.2893*
H`	Riffle	-0.29	0.37	0.36	0.11	-0.57	0.35	0.21	0.31	0.58	-0.41
		0.3578	*0.2365*	*0.2465*	*0.7268*	***0.0509***	*0.2588*	*0.5052*	*0.3280*	***0.0491***	*0.2446*
	Pool	-0.43	-0.13	0.51	-0.15	-0.68	-0.14	-0.10	-0.06	0.47	-0.64
		0.1587	*0.6858*	*0.0875*	*0.6347*	***0.0143***	*0.6540*	*0.7553*	*0.8451*	*0.1244*	*0.0460*

Pairwise comparisons showed that no stream type differed significantly in taxa richness or H` for riffles or pools. While not statistically significant, the average richness by stream type in figure 4 illustrate that the large and/or more stable stream types, LI and SS, had higher taxa richness than the small, less stable stream types, SI and SU. The stream type Small-Drying (SD) was not included because it contained only one stream. No pattern appeared when graphing the averages on H`.

When samples were broken down by sampling period, the stream types diverged as the summer progressed (Fig. 5). There was little difference between the averages of taxa richness for the first sample in late June. By the third sample, taken at the end of September, the stream types LI and SS had higher taxa richness than the stream types SI and SU. Pairwise comparisons showed that no comparisons were significant after adjusting for multiple tests; however, the difference in richness between SS and SU was marginally significant ($t_7 = 2.85$, p = 0.092). There were no significant differences at any sampling period for H`.
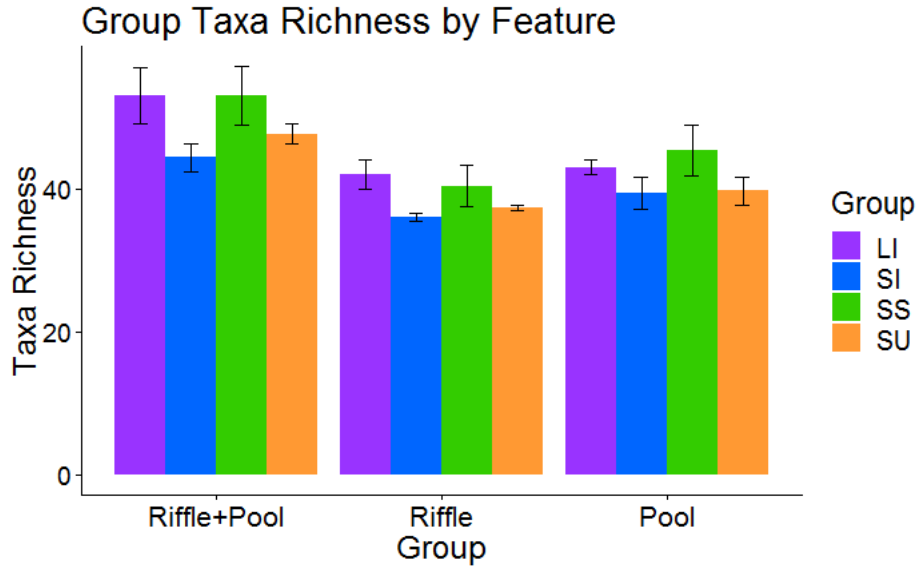
Fig. 4. Average taxa richness of groups with standard error. Results of pairwise comparisons show that no groups differ significantly for the pool, riffle, or riffle+pool samples. Stream types are labeled as LI = large-intermediate, SI = small-intermediate, SS= small-stable, SU = small-unstable. The stream type SD (small-drying) was not included because it contained only one stream.
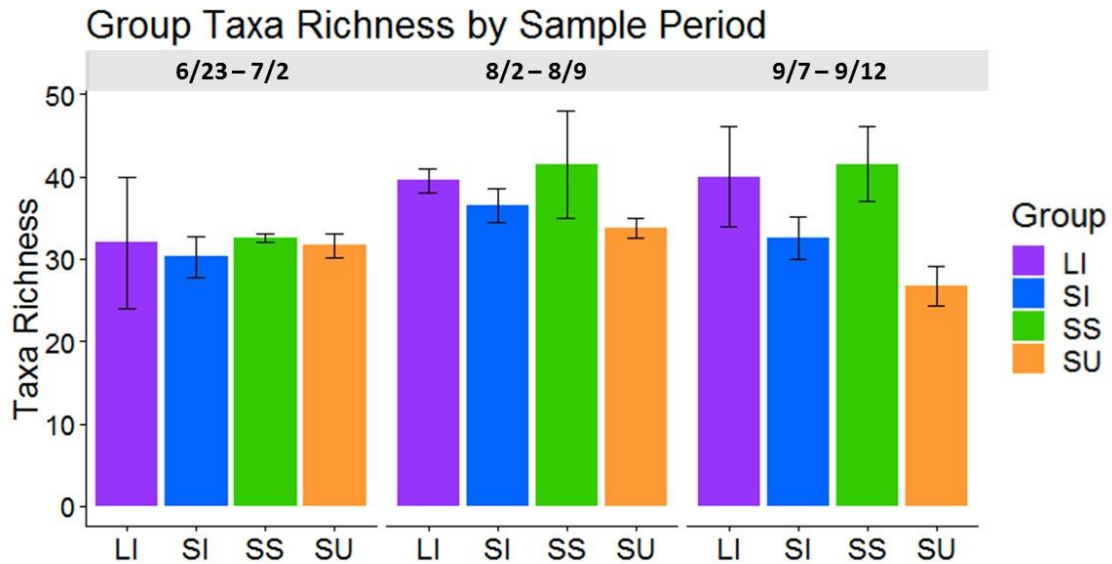
Fig. 5. Average taxa richness and standard error for each stream type at each sampling point. No stream types were significantly different for the first two samples. At the third sampling period, the difference between SS and SU was marginally significant (t_7 = 2.85, p = 0.092).

The RDA using all streams produced four constrained axes explaining 41% of the total variation between sites, with the first two axes explaining 17% and 11% (Fig. 6). Elevation, discharge, and Q_{var} contributed the most to the first axis, and % canopy cover contributed the most to the second. The streams did not cluster clearly by stream type in the correlation biplot, but the two "large" streams are both found associated with higher discharge. Elevation had the largest influence on the first axis which is evident by how the streams line up in almost perfect order of decreasing elevation from left to right (Fig. 6 a).

When the species were projected onto the distance biplot, there appeared to be equal amounts of spread along both axes (Fig. 6 b). The midges of the subfamilies Orthocladiinae and Chironominae were found highest on the second RDA axis, indicating that they were negatively associated with canopy cover. The flatworm *Polycelis coronata* and the stoneflies *Zapada* and *Sweltsa* were found farthest to the left, indicating a positive association with elevation and a possible negative association with Q_{var} and discharge.

In the second RDA using only the "small" streams, six environmental variables were used in the original ordination: % canopy cover, width, discharge, elevation, Q_{var}, and ROD. The variables elevation and Q_{var} were found to be the least influential using the *ordistep* function and removed for the final RDA (Blanchet *et al*., 2008). The four constrained axes explained 64% of the variation with the first two axes explaining 27% and 15% (Fig. 7). ROD and % canopy cover contributed the most to the first axis, and width and discharge contributed the most to the second. The streams of type SU and SD were separated from the streams of SS and SI along the gradient of ROD (Fig. 7a). The taxa were equally spread along the first and second axis for the RDA using small streams. Several mayfly genera, including *Cinygmula, Ameletus,* and

a.)

b.)
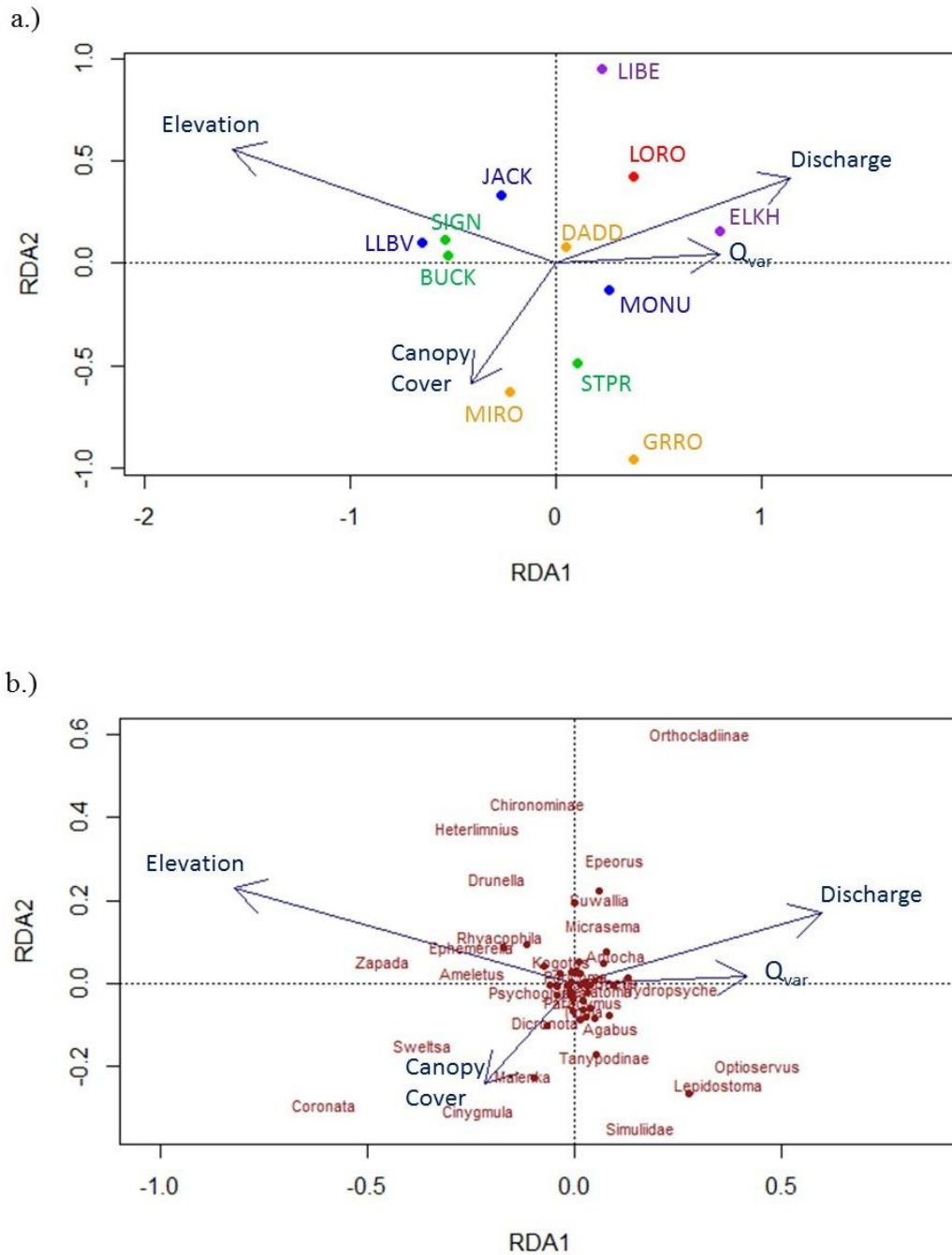
Fig. 6. The first two RDA axes when all sites were included for a correlation biplot (a) showing the correlations of environmental variables and sites and a distance biplot (b) showing how taxa relate to environmental variables. Not all taxa names could fit and still be legible, so preference was given to taxa that contributed the most to H`. The four constrained axes explained 41% of the variation in communities. Eigenvalues for the constrained axes: RDA1 = 0.052, RDA2 = 0.033, RDA3 = 0.024, RDA4 = 0.020.

a.)
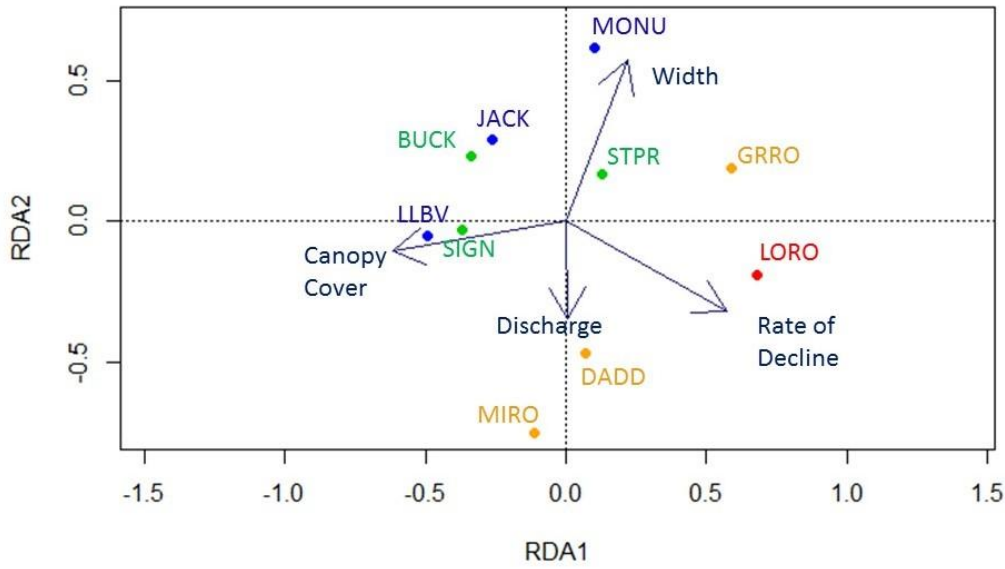
b.)
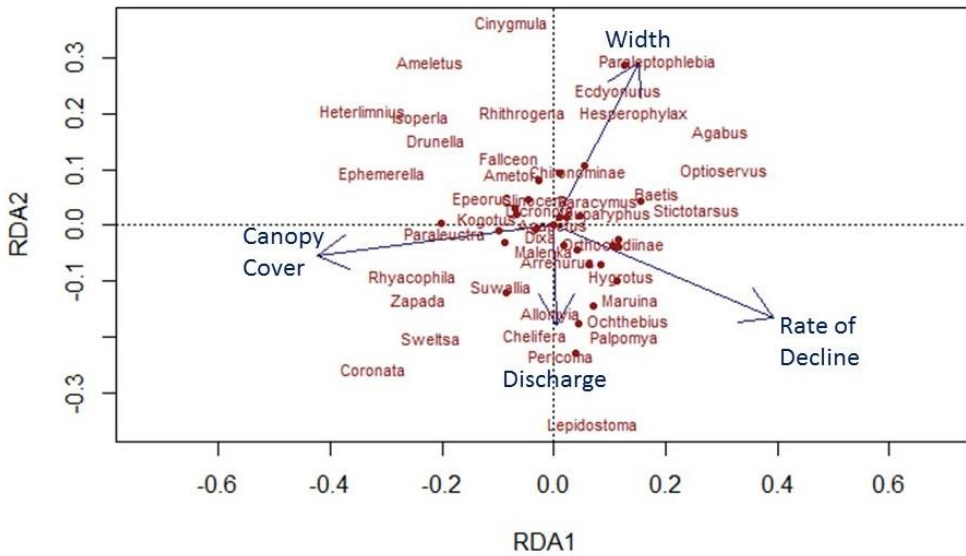
Fig. 7. The first two RDA axes using only the 10 "small" streams for a correlation biplot (a) showing the correlations of environmental variables and sites and a distance biplot (b) showing how taxa relate to environmental variables. Not all taxa names could fit and still be legible, so preference was given to taxa that contributed the most to H`. The four constrained axes explained 64% of the variation, with the first two axes explaining 28% and 15%, respectively. Eigenvalues for the constrained axes: RDA1 = 0.059 RDA2 = 0.033 RDA3 = 0.028 RDA4 = 0.018.

Paraleptophlebia, were found high on the second axis, indicating an association with increasing stream width.

The indicator species analysis revealed at least two indicator species for every stream type except SI that were significant at α = 0.05 after permutation testing (Table 8). The stream type SD was excluded from the analysis because it contained only one site. All indicator taxa found had perfect fidelity to the stream type, signifying that they were found in all streams within that stream type. The caddisflies, *Brachycentrus* and *Agapetus* also had perfect specificity, meaning that those genera only occurred in the large-intermediate (LI) streams. They were also the only caddisflies found to be indicator taxa. All other indicator taxa were found in multiple types of streams but still found to be significantly associated with a single stream type (see Table 7). In addition to the two caddisflies, the two Ephemeroptera (mayflies) *Rhithrogena* and *Epeorus*, the Plecoptera (stonefly) *Suwallia* and Diptera (true fly) *Antocha* were found to be indicators of the LI streams. The mayfly *Cinygmula* and stonefly *Malenka* were found to be indicators of the small-stable streams (SS), as well as the flatworm *Polycelis coronata*. The stream types small-unstable (SU) had two coleopterans (beetles) as indicators: the family Scritidae and genus *Ochthebius*. The beetles found in Scritidae are only aquatic during larva stages and are considered terrestrial as adults. Beetles in the genus *Ochthebius* are aquatic for all stages of their lives, and most of the *Ochthebius* collected during this study were adults. More discussion about the functional traits of these species can be found below.

Table 8. Indicator taxa for each stream type. S indicates the specificity of the taxa and F indicates the fidelity.

Group	Taxa	S	F	stat	P
LI	*Brachycentrus*	1	1	1	0.022
	Agapetus	1	1	1	0.022
	Antocha	0.9913	1	0.996	0.016
	Rhithrogena	0.7233	1	0.85	0.025
	Epeorus	0.6361	1	0.798	0.027
	Suwallia	0.619	1	0.787	0.023
SS	*Polycelis coronata*	0.6673	1	0.817	0.041
	Cinygmula	0.5955	1	0.772	0.017
	Malenka	0.6138	1	0.783	0.016
SI	n/a				
SU	Scirtidae	0.8889	1	0.943	0.023
	Ochthebius	0.6667	1	0.816	0.032

Functional Diversity

Functional richness (FRic) was found to be positively correlated with drainage area (r = 0.61, p = 0.035), discharge (r = 0.63, p = 0.028), and depth (r = 0.63, p = 0.027) for riffle samples (Table 9). For pool samples, FRic was negatively correlated with Q_{var} (r = -0.62, p = 0.033). Functional evenness (FEve) was not associated with any environmental variables for pools or riffles.

Pairwise comparisons revealed no significant difference between stream types for riffles, pools, or pool+riffle (Fig. 8). However, Figure 8 displays a similar pattern as the one found for taxa richness, where the larger and/or more stable stream types, LI and SS, have higher functional richness than stream types SI and SU. Functional evenness also did not differ significantly between any stream types for pools or riffles.

Table 9. Correlation coefficients (Pearson's r) and p-values (*below*) of function richness (FRic) and functional evenness (FEve) and environmental variables. Rate of Decline does not include the LI sites. P-values significant at α = 0.05 are bolded.

		Qvar	Drain. Area (km²)	Elev. (m)	d50	Temp (°C)	Disch. (m³/s)	Width (cm)	Depth (cm)	Canopy Cover (%)	Rate of Decline (m³/s/day)
FRic	**Riffle**	-0.31	0.61	-0.07	0.36	-0.10	0.63	0.55	0.63	0.25	-0.57
		0.3210	***0.0350***	0.8291	0.2453	0.7478	***0.0280***	0.0627	***0.0271***	0.4290	0.0852
	Pool	-0.62	0.26	0.09	0.14	-0.20	0.17	0.34	0.30	-0.11	-0.51
		0.0331	0.4110	0.7792	0.6655	0.5425	0.5893	0.2768	0.3505	0.7340	0.1341
FEve	**Riffle**	0.37	-0.05	-0.29	0.18	0.24	0.01	-0.09	-0.14	0.26	0.31
		0.2363	0.8746	0.3552	0.5720	0.4614	0.9648	0.7919	0.6611	0.4199	0.3813
	Pool	-0.02	0.34	0.31	0.49	-0.23	0.34	0.33	0.42	0.24	-0.25
		0.9618	0.2765	0.3285	0.1059	0.4697	0.2739	0.2895	0.1779	0.4582	0.4807

When FRic was broken down by sampling period, it was evident that stream types LI and SS began to separate in FRic from the stream types SI and SU as the summer progressed (Fig. 9). Pairwise comparisons showed that the no stream types were significantly different for the first two samples, and that the difference in FRic between SU and SS was marginally significant at the third sampling point ($t_7 = 2.86$, $p = 0.089$).
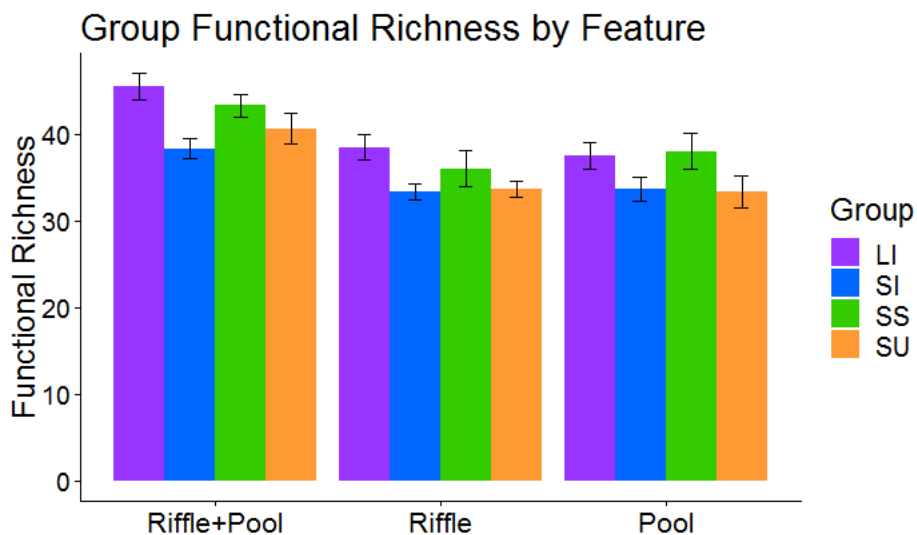
Fig. 8. Average functional richness and standard error for each stream type for pools, riffles, and riffle+pool. No stream types were significantly different for any of the samples; however, the same pattern found in taxa richness can be found, with stream types LI and SS having higher functional richness than the other stream types.

Group Functional Richness by Sample Period
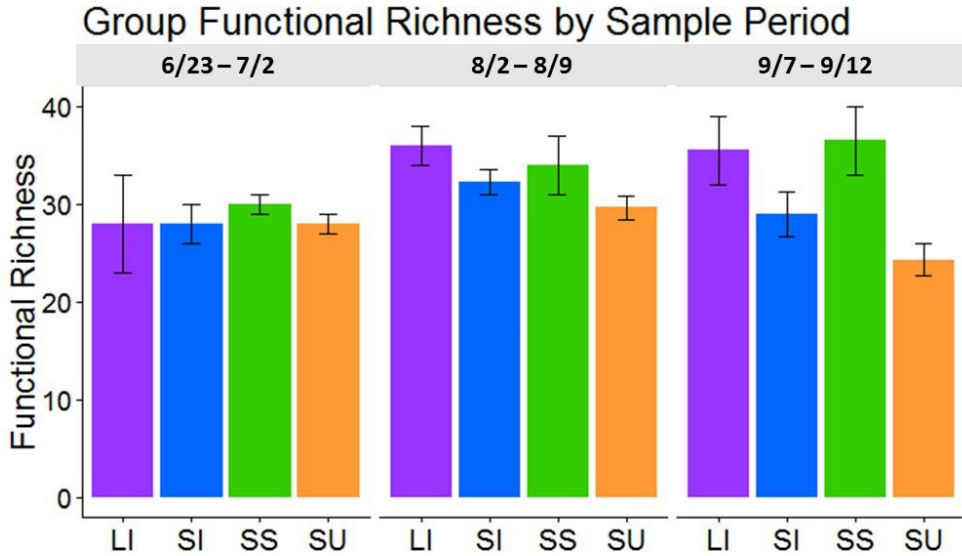
Fig. 9. Average functional richness and standard error for each stream type at each sampling point. No stream types were significantly different for the first two samples. At the third sampling period, the difference between SS and SU was marginally significant ($t_7 = 2.86$, $p = 0.089$).

The RDA using the functional trait state by site matrix for all sites resulted in four constrained axes explaining 38% of the variation, with the first two axes explaining 21% and 9% (Fig. 10). Canopy cover and elevation contributed the most to the first axis while discharge and Q_{var} contributed to the second. The LI streams ELKH and LIBE were closely correlated with increasing discharge, similar to the RDA using taxa data.

The distance biplot from the RDA shows an equal spread of trait states along both axes. Interestingly, the trait Rheo1 (depositional zones only) was found associated with increasing discharge, while Rheo3 (erosional zones only) and Trop2 (collector-filterer) were associated with decreasing discharge. This was an unexpected result and may be explained by unconstrained variation (62% of the variation among functional traits were not explained by the environmental variables used in the analysis).

a.)

b.)
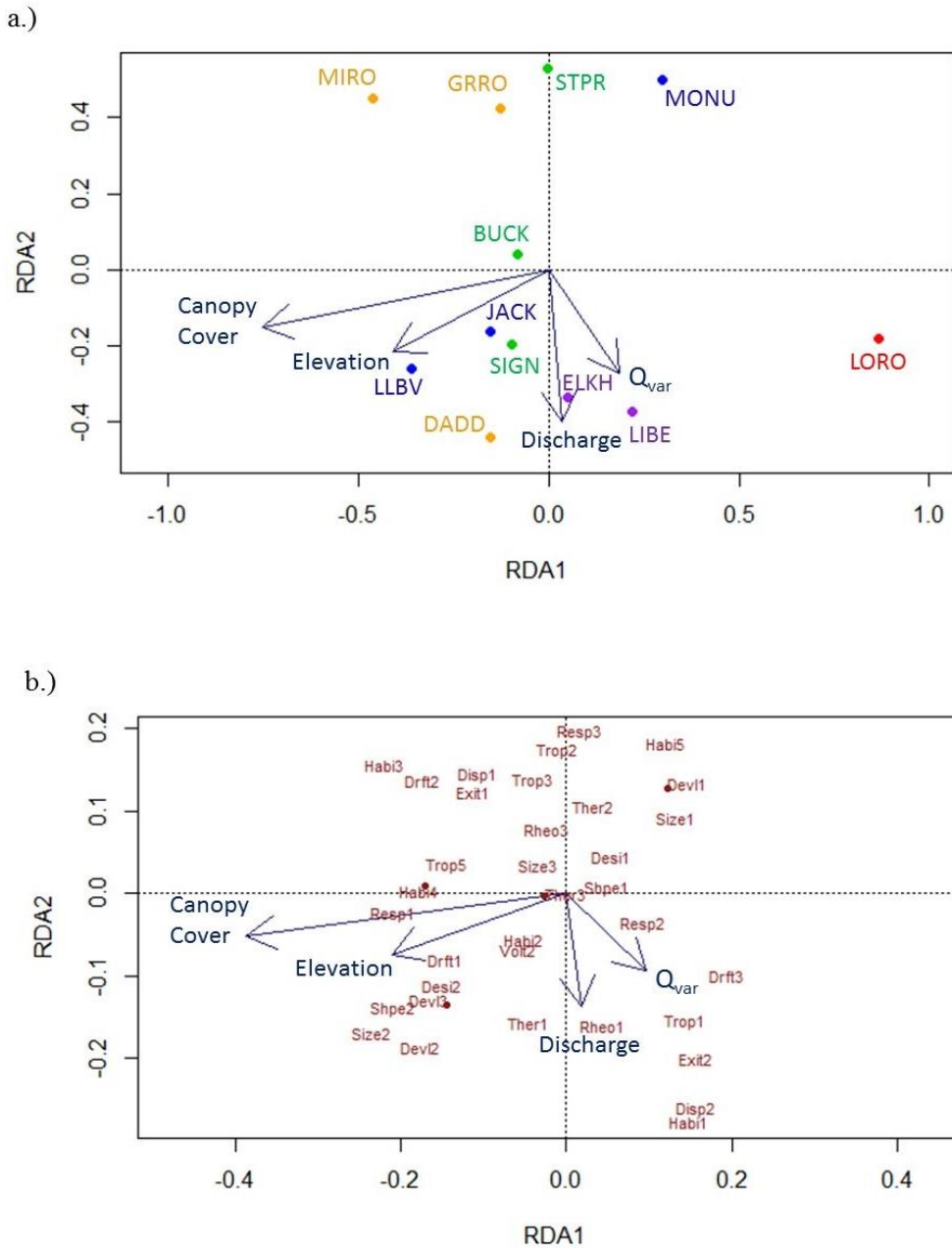
Fig. 10. The first two constrained axes for an RDA preformed using a trait state abundance by site matrix for all streams. The figure contains a correlation biplot (a) showing the correlations of environmental variables and sites and a distance biplot (b) showing how taxa relate to environmental variables. The four constrained axes explained 38% of the variation, with the first two axes explaining 21% and 9%, respectively. Eigenvalues for the constrained axes: RDA1 = 0.0088, RDA2 = 0.0039, RDA3 = 0.0019, RDA4 = 0.0012.

a.)

b.)
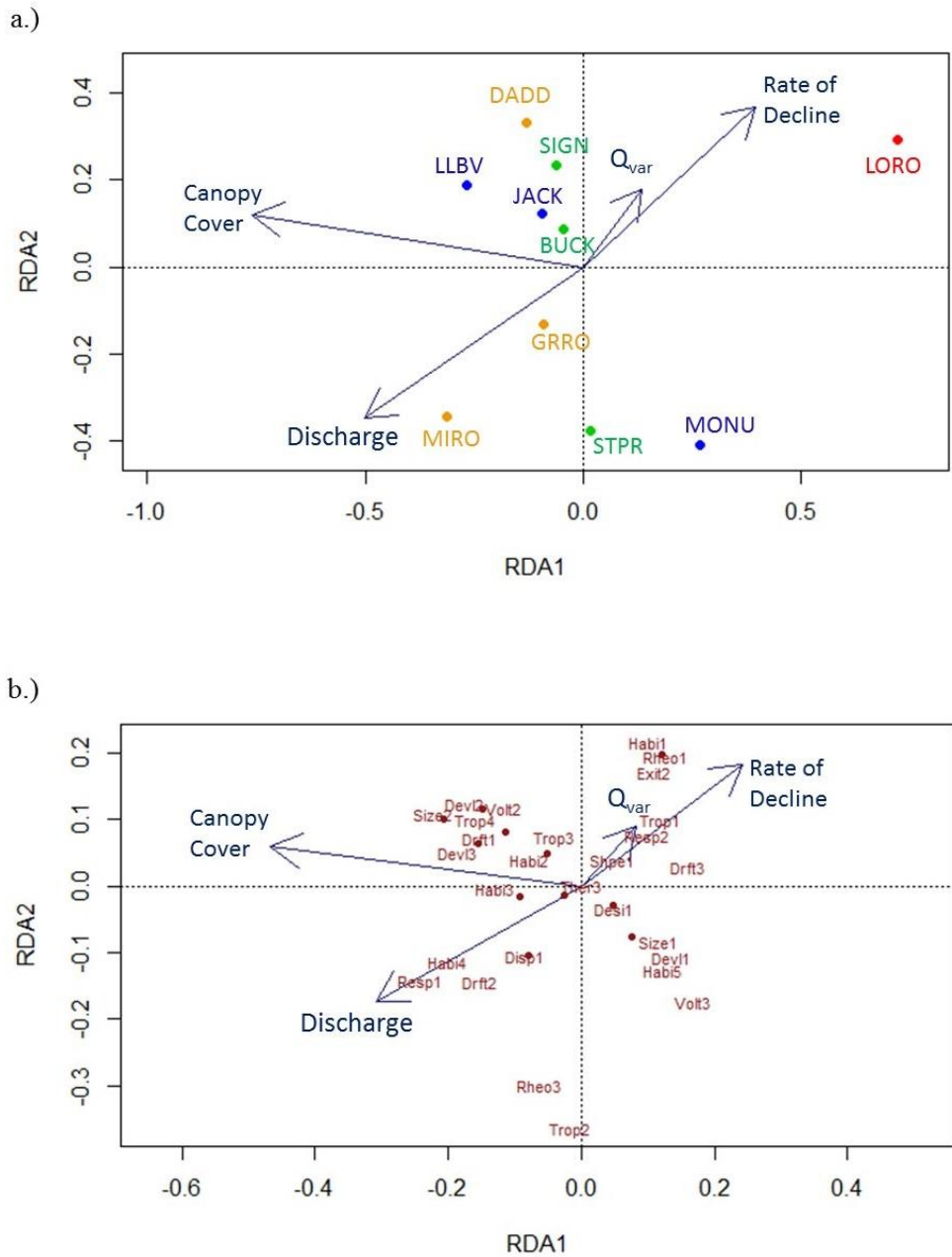
Fig. 11. The first two constrained axes for an RDA preformed using a trait state abundance by site matrix for only "small" streams. The figure contains a correlation biplot (a) showing the correlations of environmental variables and sites and a distance biplot (b) showing how taxa relate to environmental variables. The four constrained axes explained 66% of the variation, with the first two axes explaining 41% and 14%, respectively. Eigenvalues for the constrained axes: RDA1 = 0.017, RDA2 = 0.011, RDA3 = 0.0019, RDA4 = 0.0012.

An RDA for just the "small" streams was performed using all six uncorrelated variables, and four of these variables were selected using the *ordistep* function from the vegan package (Blanchet *et al.*, 2008). The variables elevation and width were found to be the least informative, leaving % canopy cover, discharge, ROD, and Q_{var} (Fig. 11). The resulting four constrained axes explained 66% of the variation, with the first two axes explaining 41% and 14%. Discharge and ROD contributed equally to both axes, and % canopy cover strongly influenced the first axis. Q_{var} had a smaller contribution in explaining variation.

The placement of traits in the ordination space was more logical in the RDA using only small sites than the RDA conducted using all sites, likely because the environmental variables explained significantly more of the variation. The first axis alone in the second RDA explained more than all constrained axes in the first RDA (41% versus 38%). Rheo3 (erosional zones only) and Trop2 (collector-filterer) were found positively associated with discharge and negatively associated with Q_{var} and ROD. Habi1 (burrowing), Rheo3 (depositional zones only), and Exit2 (ability to exit present) were all associated with increasing values of ROD and Q_{var}.

The frequency of individual trait states present at a site did not differ significantly between stream types for most traits. Contrary to my hypothesis, LI streams had more taxa capable of exiting the stream (not including adult emergence) than SU streams ($t_7 = 3.08$, p = 0.067). 44% of the taxa found in the LI stream LIBE had the ability to exit, which is dramatically higher than the average of 22% found stream types SS, SU, and SI. However, when the SD stream was included, results showed that 54% of its taxa had the ability to exit, significantly more than SS ($t_7 = 3.30$, p = 0.071), SI ($t_7 = 3.56$, p = 0.051), and SU ($t_7 = 3.91$, p = 0.033). The SD stream also had significantly more taxa with strong female dispersal than SS ($t_7 = 3.39$, p = 0.063), SI ($t_7 = 3.41$, p = 0.062), and SU ($t_7 = 3.88$, p = 0.035) and had more taxa abundant in

drift than SS ($t_7 = 3.58$, $p = 0.05$), SI ($t_7 = 3.44$, $p = 0.057$), and SU ($t_7 = 4.24$, $p = 0.022$). Fitting with my predictions, the SD stream had significantly more taxa that are found only in depositional areas of streams with 36% of its taxa preferring those conditions (comparison with LI – $t_7 = -4.039$ $p = 0.029$, SS – $t_7 = 4.264$, $p = 0.022$, SI – $t_7 = 3.844$, $p = 0.036$, and SU – $t_7 = 5.287$, $p = 0.007$).

When examining the functional trait niches of the indicator taxa identified for each stream type, several patterns became evident (Fig. 12). The only taxa that were found strictly in erosional areas (Rheo3) were indicators of the LI stream type. This makes sense because those streams have significantly higher discharge than the other streams. The two Coleopteran taxa that were indicators for the SU stream type were the only indicator taxa to use plastrons of spiracles (areal) for respiration (Resp3).

Beta Diversity

Analysis of the components of Jaccard dissimilarity revealed that taxa replacement, not nestedness, played the largest role in β-diversity. The Jaccard dissimilarity changed little between the different sampling periods with 0.564 for the first sample, 0.533 for the second sample, and 0.544 for the final sample. At each sampling point, taxa turnover (e.g. taxa replacement) accounted for the majority of the dissimilarity between sites. Taxa replacement accounted for 72% of the dissimilarity for the first sample period, 78% of the second sample period, and 66% of the third sample period (Fig 13).

When β-diversity was further broken down by elevation band, species turnover still explained most of the dissimilarity between streams. Species turnover made up 83% of the dissimilarity between mid-elevation streams and 76% between low-elevation streams (Fig. 14). This illustrated that taxa replacement along the elevation gradient is not responsible for all of the

a.) Large-Intermediate (LI)

b.) Small-Stable (SS)
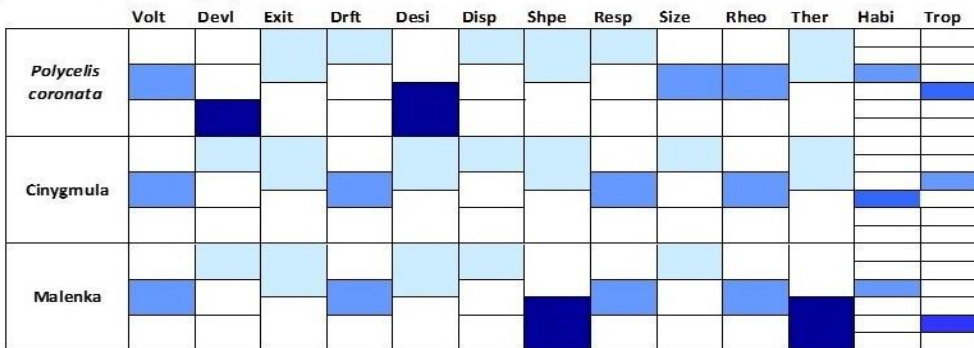
c.) Small-Unstable (SU)
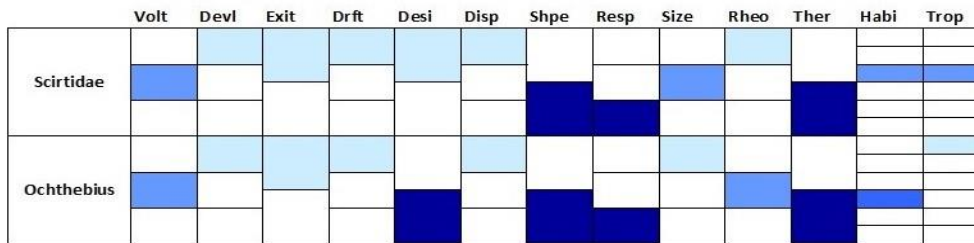
Fig. 12. Visual representations of the functional niches of the indicator taxa for a.) large-intermediate streams, b.) small-stable streams, and c.) small-unstable streams. Each trait is represented as a box that is divided into the number of states that trait has (e.g., voltinism has three states (Volt1, Volt2, Volt3), so its box is divided into three). The top box of each trait represents the first state (e.g., Volt1) then precedes in descending order. For example, *Brachycentrus* possesses the trait state Volt2 and Devl2. See Table 1 for a list of the traits.
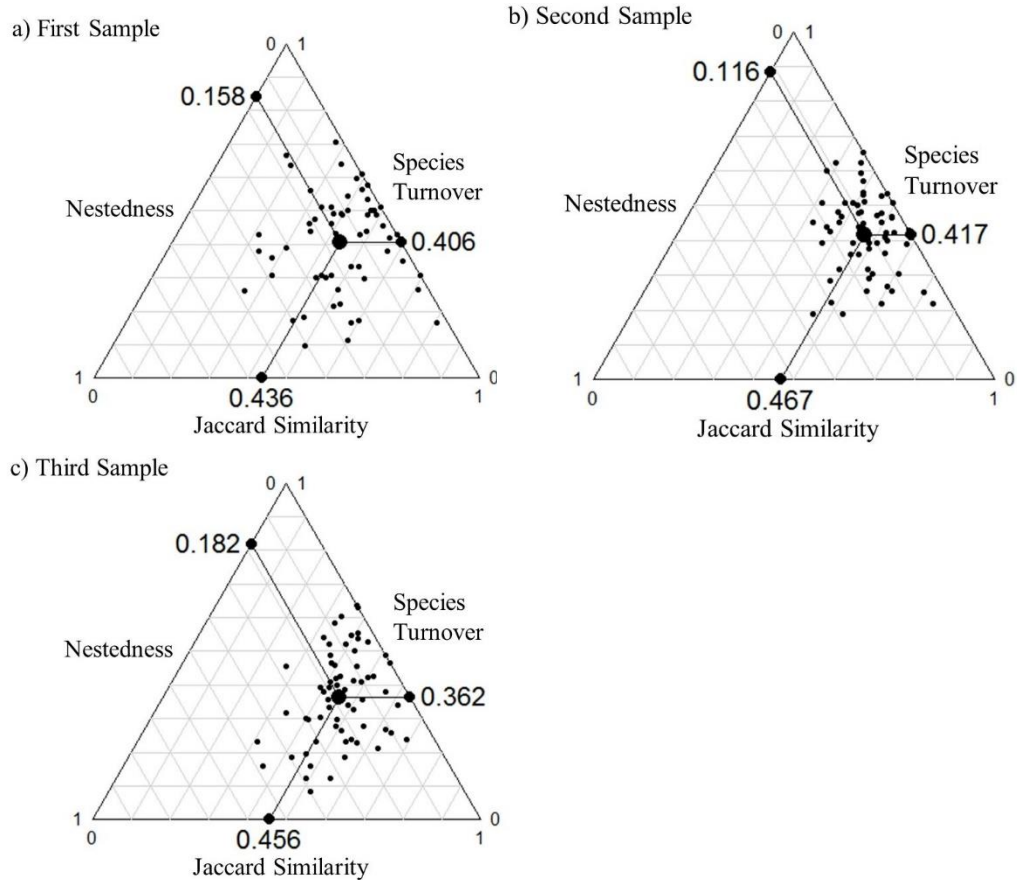
Fig. 13. Triangle (simplex) plots for the a) first, b) second, and c) third sample periods. Each point represents a pairwise comparison between two sites for taxa replacement, abundance difference, and Jaccard similarity. The bold numbers display the mean of each component for all pairwise comparisons.

species turnover. These results contradict my original hypothesis that nestedness would contribute more to β-diversity. Nestedness did not significantly influence β-diversity at either elevation ban and was lowest at the mid-elevation streams.

DISCUSSION

The goal of my study was to determine if subtle differences in stream size and flow stability affected aquatic macroinvertebrates communities in montane headwater streams. While many previous studies have examined the role stream size plays in macroinvertebrate communities in low-order streams (e.g. headwater, mid-order), few have focused on how macroinvertebrate communities change in the smallest of streams and whether flow stability
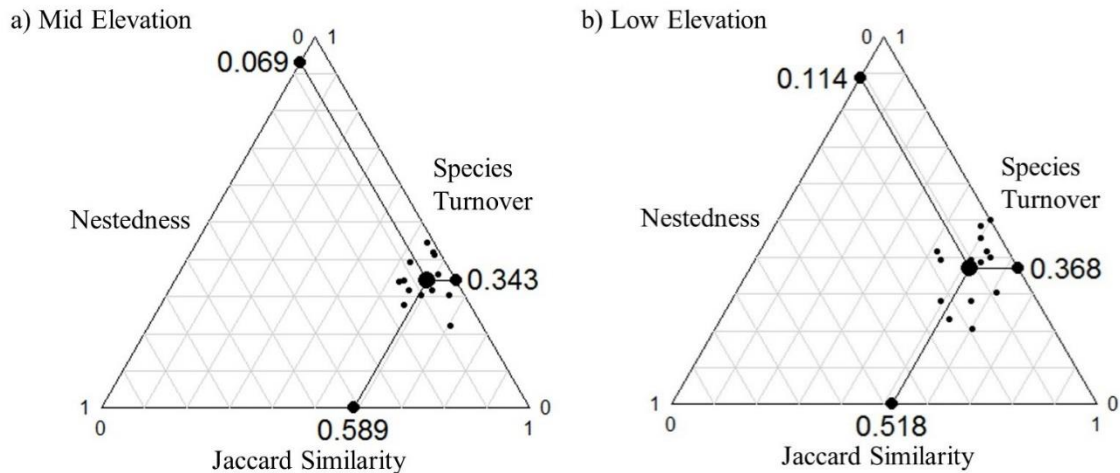
Fig. 14. Triangle (simplex) plots showing the Jaccard similarity and the nestedness and species turnover components of β-diversity for a) mid (2450-2950 m asl) and b) low elevation (2000-2400 m asl) sites.

influences macroinvertebrate community composition. My findings thus contribute to the basic understanding of macroinvertebrate distribution and abundance in small headwater streams, and they provide some clues as to how communities may change under warmer and drier conditions expected under rapid climate change.

Alpha Diversity

My analyses of taxa richness revealed some evidence to support my original hypothesis that α-diversity would decrease with decreasing stream size; however, this was only the case when looking at riffle communities and quantifying diversity using taxa richness (Table 7). Shannon's diversity index displayed no relationship with size variables. The variables drainage-basin area and discharge were positively correlated with increasing riffle richness, but stream width, another common measure of stream size, was not significantly correlated with taxa richness, suggesting that patterns along a stream-size gradient are dependent on the measure being used (Vander Vorste *et al*., 2017). My results agree with a previous study which focused on size differences within headwater streams and also found a positive correlation with taxa

richness and stream size (Brönmark *et al*., 1984), although their results revealed a stronger correlation between size and richness.

Comparisons between stream types showed that stream size was not the only factor affecting taxa richness; flow stability also influenced the richness of the streams. The richness of macroinvertebrates in pools had a negative correlation with Q_{var} and the small-stable (SS) streams supported similar taxa richness to the large-intermediate (LI) streams (Fig. 3). If the species-area relationship (Arrhenius 1921) was the only driver of taxa richness, LI would have higher taxa richness than the other stream types since those streams are significantly larger than the other streams in the study. It is also clear that the stream types SS and LI differentiate from the other stream types as the summer progresses. Herbst *et al*. (2018) found little difference between perennial and intermittent streams during years with average to above average flow, but a significant difference between perrenial and intermittent streams during dry years. This same pattern may be present at my streams, but at an annual scale rather than an interannual one where the effect of stream size and flow stability is only detectable after the high flows caused by snowmelt begin to subside.

A novel contribution of this study is the finding that flow stability has a large effect on the taxa richness and functional richness between very small streams. There were no significant differences in size between stream types SS, SU, and SI, yet SS streams had taxa richness more comparable to the LI streams than the other small streams. This pattern was true for both riffles and pools (Fig. 4). While flow permanence is known to have a large effect on shaping macroinvertebrate communities in small streams (Datry *et al*., 2007; Herbst *et al.,* 2018; Leigh & Datry, 2017; Ruegg & Robinson, 2004), this study is the first to show that the stability of flow on

a seasonal time scale can explain differences in community composition of small, permanent streams.

The RDA using taxa data from all sites showed that the midge subfamilies Orthocladiinae and Chironominae were negatively correlated with % canopy cover (Fig 6). This association was likely driven by the midges' association with the sites LIBE and LORO which had the least amount of canopy cover (see below). At the last sample period in September, 72% of the individuals collected in the pools of LORO were midges (Appendix 3). LORO was the only site to experience flow cessation in some reaches during 2016, and by the September sample, all of the riffle habitat towards the bottom of the 50-meter reach had dried, leaving isolated pools. Midges are known to be tolerant to many disturbances (Allan & Castillo, 2007; Rader & Belish, 1997), so it was not surprising to find them to be the most abundant in a stream with flow cessation.

Functional Diversity

Functional richness (FRic) followed the same patterns as taxa richness. The FRic found in riffles was positively correlated with drainage area and discharge, just like taxa richness. Additionally, FRic was found to be positively correlated with depth and negatively correlated with ROD in riffles. The size variables (drainage area, discharge, depth) had stronger correlations with FRic than taxa richness ($r > 0.6$), but they were still rather weak for discerning ecologically significant patterns. Functional evenness (FEve) was not correlated with any variables and did not differ between stream types at any point during the summer. Past research has also found FEve to be the least responsive measure of functional diversity to environmental gradients (Heino 2005).

The two RDAs performed using the trait-state abundance by site matrix varied largely in the total amount variation they explained despite both having four constrained axes. The RDA performed on all sites contained several counterintuitive patterns (Fig. 10), most notably the association of the trait Rheo1 (depositional zone only) with increasing discharge. Trop2 (collector-filterer) was found to be negatively associated with discharge, contradicting previous findings (Ledger *et al.,* 2011; Santos & Stevenson 2011). These interesting associations are likely a result of unconstrained variation in the ordination since only 38% could be explained by the environmental variables used. When the two LI sites were removed for the second ordination, 66% of the variation was constrained by the variables. In this RDA, Rheo1 was found to have a negative relationship with increasing discharge and Trop2 was found positively associated with discharge. One possible explanation for this sudden increase in constrained variation is the inclusion of the environmental variable rate of decline (ROD) in the second ordination which was not available for the LI sites. However, an equally viable explanation is that the variables measured in this study did not capture all the differences between the LI sites and the other smaller streams.

Functional richness was highly correlated with taxa richness, likely due to the method used to calculate FRic. FRic was measured by the number of unique trait combinations for the 13 traits selected for this analysis. A benefit of this measure is that it accounts for the inter-dependency traits have due to evolutionary constraints (Poff *et al*., 2006); however, it is strongly influenced by the number of traits selected for the analyses. Defining species using 39 total states of 13 traits ended up being a strict definition of functional richness; a total of 68 functional niches were present for 73 taxa. Heino (2005) also used unique trait combinations to quantify FRic, but they used only the traits of habit and trophic habit to define their niches, loosening the

specificity of each niche but losing information at the same time. While comparing FRic between stream types does not provide much additional information than just using taxa richness, functional traits still have the benefit of elucidating patterns along environmental gradients.

Several individual traits behaved as I hypothesized in response to stream size and permanence. In the RDA using trait-state abundance for the 10 "small" streams (Fig. 11), burrowing (Habi1), preference for dispositional zones (Rheo1) and having the ability to exit the stream (Exit2) were all associated with increasing ROD and Q_{var}. Burrowing has been shown to act as a refuge during low flow or flow cessation (Larned *et al*., 2010; Larsen & Ormerod, 2010), and having the ability to exit the water allows invertebrates to leave unfavorable drying conditions. On the same RDA, the use of tegument for respiration (Resp1) and the habit of clinging (Habi4) and both positively associated with increasing discharge, matching my predictions.

Canopy Cover

The percent of canopy cover (% canopy cover) was found to strongly influence the RDAs, and the H' of riffles was found to have a positive correlation with increasing % canopy cover (r = 0.58, p = 0.049). Past research has found an increase in taxa and functional richness with decreasing canopy cover (Heino 2005); however, those studies did not restrict their size gradient to headwater streams, and canopy cover typically decreases when entering mid-order streams (Vannote *et al.,* 1980), where there is also greater habitat volume and food resources. The three sites with the lowest % canopy cover in my study (LORO, LIBE, STRP) coincide with the streams affected at least in part by the 2012 High Park Fire. Minshall *et al*. (2001) found that it took five years for taxa richness in burned areas to recover to the level of reference streams. Taken four years after the fire, my samples could still reflect some residual effects from the fire.

Beta Diversity

I predicted that nestedness would be the primary driver of the β-diversity among these streams, i.e., smaller streams would contain a nested subset of the species found in larger streams (Arscott *et al*., 2010; Corti & Datry, 2015). This hypothesis was based on the idea that smaller size would lead to less habitat variability and thus fewer taxa, and that small streams would experience more frequent disturbance events (e.g. drying), resulting in more local taxa extinction events in those streams (Heino *et al.,* 2009).

Contrary to this hypothesis, I found that taxa turnover contributed more to β-diversity compared to nestedness for each sampling period (Fig. 13). Previous studies have also not found clear patterns of nestedness along environmental gradients (Heino *et al*., 2010; Monaghan *et al*., 2005). Heino *et al*. (2009) found that stream size was the physical variable most correlated with nestedness among their study streams; however, they found nestedness to be relatively weak amongst sites due to a number of idiosyncratic taxa. My results indicate that this was not the case for the streams used in this study, even when they were split into two elevation bands shown to be ecologically meaningful for small streams in this study area (Harrington *et al.*, 2016). While a pattern of nestedness along a size gradient has been found in other types of habitat (e.g. ponds) (McAbendroth *et al.,* 2005), the complexity of stream connectivity may prevent a nested pattern to form. Or, potentially, nestedness patterns may not emerge among sites that do not span a large size gradient, although this has not been tested.

CONCLUSION

Overall, these findings indicate that stream size, while correlated with taxa richness in some cases, is a relatively weak predictor of diversity on its own. The comparisons between stream "types" showed that stability of flow throughout the summer is also an important aspect for predicting the α and functional diversity of a stream (Fig. 4 & 8). Previous research on

intermittent streams has demonstrated that flow permanence can have a large impact on macroinvertebrate communities (Datry *et al*., 2014; Datry *et al*., 2016; Dewson *et al.,* 2007). My findings add to the evidence that, even in the absence of complete flow cessation or stream drying, the stability of stream flow can impact the diversity of taxa found in very small headwater streams.

Contrary to my predictions that small streams would contain nested subsets of the taxa found in larger streams, β-diversity among the streams was caused primarily through taxa turnover. This indicates that there may be some specialization taking place where certain taxa are more successful in the smaller headwater streams compared to the larger streams. Indeed, the indicator taxa in the small-unstable streams in the study were beetles in the family Scirtidae and the genus *Ochthebius* that have the ability to capture atmospheric oxygen through a plastron, which facilitates survival in drying streams. Because small streams are not nested subsets of larger streams, it is difficult to pinpoint what specific taxa will be the most vulnerable if larger headwater streams begin to decrease in size under new climatic conditions. Rheophilic taxa dependent on fast moving water for feeding and respiration may be the most vulnerable to decreased flow (Herbst *et al.,* 2018) unless they are able move to larger streams. This study does suggest, however, that even very small streams may support rheophilic taxa as long as they have seasonally stable flow. Climate change that causes small streams to lose flow stability (e.g., reduce volume in late summer) could be expected to show large reductions in taxa and functional richness.

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APPENDIX I

Appendix I. Names, coordinates, and dates macroinvertebrates were sampled for all sites.

Abbreviation	Name	Latitude	Longitude	Dates Sampled First	Second	Third
BUCK	Buckhorn Creek	40.57895	-105.54196	7/1/2016	8/8/2016	9/7/2016
DADD	Dadd Gulch	40.69829	-105.53913	6/29/2016	8/2/2016	9/11/2016
ELKH	Elkhorn Creek	40.70621	-105.43255	6/27/2016	8/2/2016	9/11/2016
GRRO	Grey Rock	40.69567	-105.28855	6/23/2016	8/3/2016	9/12/2016
JACK	Jack's Gulch	40.62558	-105.53707	6/30/2016	8/2/2016	9/9/2016
LIBE	Little Beaver Creek	40.62423	-105.5332	6/30/2016	8/9/2016	9/7/2016
LLBV	Tributary to Little Beaver	40.61584	-105.54857	7/2/2016	8/2/2016	9/9/2016
LORO	n/a	40.68253	-105.4074	6/27/2016	8/3/2016	9/12/2016
MIRO	n/a	40.69377	-105.51969	6/29/2016	8/2/2016	9/11/2016
MONU	Monument Gulch	40.61112	-105.52862	6/30/2016	8/8/2016	9/7/2016
SIGN	Signal Mountain	40.55682	-105.54471	7/1/2016	8/8/2016	9/7/2016
STPR	Stove Prairie	40.67162	-105.38524	6/25/2016	8/3/2016	9/12/2016

Appendix II. Trait states for taxa used in analyses. See Table 1 for definitions of trait states.

	Volt	Devl	Exit	Drft	Desi	Disp	Shpe	Resp	Size	Rheo	Ther	Habi	Trop
Ephemeroptera													
Ameletus sp.	2	1	1	1	1	1	1	2	1	2	1	5	1
Acentrella sp.	3	1	1	2	1	1	1	2	1	2	2	5	1
Baetis sp.	3	1	1	3	1	1	1	2	1	2	2	5	1
Fallceon quilleri	3	1	1	3	1	1	1	2	1	2	2	5	1
Drunella sp.	2	2	1	1	1	1	2	2	2	2	2	4	3
Ephemerella sp.	2	2	1	2	1	1	2	2	2	2	2	4	1
Timpanoga hecuba	2	2	1	2	1	1	2	2	1	2	1	4	1
Cinygmula sp.	2	1	1	2	1	1	1	2	1	2	1	4	3
Ecdyonurus sp.	2	1	1	2	1	1	1	2	1	2	2	4	3
Epeorus sp.	2	1	1	1	1	1	1	2	2	2	1	4	1
Rhithrogena sp.	2	1	1	2	1	1	1	2	2	2	1	4	1
Paraleptophlebia sp.	2	1	1	2	1	1	1	2	2	2	2	5	1
Plecoptera													
Suwallia sp.	2	2	1	2	1	1	1	1	2	2	2	4	4
Sweltsa sp.	1	2	1	2	1	1	2	1	2	2	2	4	4
Paraleuctra sp.	2	1	1	2	2	1	1	1	1	2	2	3	5
Malenka sp.	2	1	1	2	1	1	2	2	1	2	2	3	5
Zapada sp.	1	1	1	1	1	1	2	2	1	2	1	3	5
Isoperla sp.	2	2	1	2	2	1	2	1	2	2	2	4	4
Kogotus modesta	2	2	1	1	1	1	1	1	2	3	1	4	4
Trichoptera													
Brachycentrus sp.	2	2	1	1	1	1	2	2	2	3	2	4	2
Micrasema bactro	2	2	1	1	1	1	2	2	1	3	2	4	3
Agapetus sp.	2	2	1	1	2	1	2	1	1	3	1	4	3
Glossosoma sp.	2	2	1	1	1	1	2	1	1	3	2	4	3
Archtopsyche grandis	2	2	1	1	1	1	2	2	2	3	1	4	2
Hydropsyche sp.	2	2	2	2	1	2	2	2	2	3	2	4	2
Hydroptila sp.	2	2	1	2	1	2	1	2	1	2	2	4	3
Lepidostoma sp.	2	2	1	1	1	1	2	2	2	2	1	2	5
Oecetis sp.	2	2	1	1	1	2	2	2	2	2	2	4	4
Allomyia sp.	2	2	1	1	1	1	2	1	2	3	1	4	3
Ecclisomyia sp.	1	2	1	1	1	1	2	2	2	3	1	4	1
Hesperophylax sp.	2	2	1	1	1	1	2	2	3	2	2	3	5
Psychoglypha sp.	2	2	1	1	1	1	2	2	3	3	1	3	1
Dolophilodes	2	2	1	1	1	1	2	1	1	3	2	4	2
Rhyacophila alberta	2	2	1	1	1	1	2	1	2	3	2	4	4
Rhyacophila angelita	2	2	1	1	1	1	2	1	2	3	2	4	4
Rhyacophila brunnea	2	2	1	1	1	1	2	1	2	3	2	4	4
Rhyacophila verrula	2	2	1	1	1	1	2	1	2	3	2	4	3
Neophylax sp.	2	2	1	1	1	1	2	2	2	3	2	4	3
Neothremma alicia	2	2	1	1	1	1	2	2	1	3	1	4	3
Diptera													
Atrichopogon sp.	2	1	1	1	1	1	2	2	1	2	2	3	1
Palpomya sp.	2	1	1	1	1	1	2	2	1	2	2	1	4
Chironominae gn.	3	1	2	3	1	2	1	2	1	1	2	1	1
Diamesinae gn.	2	1	2	3	1	2	2	2	1	2	1	3	1
Orthocladiinae gn.	2	1	2	3	1	2	1	2	1	2	1	1	1
Tanypodinae gn.	2	1	2	3	1	2	1	2	1	2	2	3	4

Appendix II (cont.)

	Volt	Devl	Exit	Drft	Desi	Disp	Shpe	Resp	Size	Rheo	Ther	Habi	Trop
Dixa sp.	2	1	2	2	1	1	2	3	1	2	2	5	2
Chelifera sp.	2	2	2	1	1	1	2	1	2	2	2	3	4
Oreogeton sp.	2	2	2	1	1	1	2	1	2	3	2	3	4
Maruina sp.	2	1	1	1	1	1	1	1	1	3	1	4	3
Pericoma sp.	2	1	1	1	1	1	2	1	1	1	2	1	1
Ptychoptera sp.	2	1	1	1	1	1	2	3	3	3	2	1	1
Simuliidae gn.	3	1	1	2	1	1	1	1	1	3	2	4	2
Euparyphus sp.	2	2	1	1	2	1	1	3	2	2	2	3	1
Antocha sp.	2	2	1	1	2	1	2	2	2	3	2	4	1
Dicronota sp.	2	2	1	1	2	1	2	2	2	2	2	3	4
Hexatoma sp.	2	2	1	1	2	1	2	2	2	2	2	1	4
Limoniinae gn.	2	2	1	1	2	1	2	2	2	2	2	1	5
Ormosia sp.	2	2	1	1	2	1	2	2	2	1	2	1	1
Tipula sp.	2	2	1	1	2	1	2	2	2	2	2	1	5
Coleoptera													
Helichus sp.	1	2	1	1	1	1	2	3	1	3	2	4	3
Agabus sp.	1	2	2	1	1	2	1	3	2	2	2	5	4
Hygrotus sp.	1	2	2	1	1	2	1	3	2	1	2	5	4
Oreodytes sp.	1	2	2	1	1	2	1	3	2	1	2	5	4
Sanfilippodytes sp.	1	2	2	1	1	2	1	3	2	2	2	5	4
Stictotarsus sp.	1	3	1	2	2	1	2	1	1	2	2	4	1
Ochthebius sp.	2	1	1	1	2	1	2	3	1	2	2	4	1
Ametor sp.	2	1	1	1	2	1	2	3	2	1	2	4	1
Anacaena sp.	3	2	1	1	2	1	2	3	1	1	2	1	1
Laccobius sp.	2	2	1	1	2	1	2	3	1	1	2	1	3
Paracymus sp.	2	2	1	1	2	1	2	3	1	1	2	1	1
Scirtidae gn.	2	1	1	1	1	1	2	3	2	1	2	3	3
Other													
Arrenurus sp.	2	2	2	1	1	1	2	1	1	3	2	5	4
Polycelis coronata	2	3	1	1	2	1	1	1	2	2	1	3	4

Appendix III. Abundance of taxa collected at each site at each sampling period for riffles (R) and pools (P)

	BUCK						DADD						ELKH						GRRO					
	7/1/16		8/8/16		9/7/16		6/29/16		8/2/16		9/11/16		6/27/17		8/2/16		9/11/16		6/23/17		8/3/16		9/12/16	
	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P
Ephemeroptera																								
Ameletus sp.	1	32	4	80	25	163	-	-	-	-	-	1	-	7	-	-	-	3	-	7	-	-	-	-
Acentrella sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Baetis sp.	454	112	216	51	140	51	17	8	361	101	75	19	459	45	380	5	79	23	138	17	66	7	71	-
Fallceon quilleri	-	2	-	-	-	-	-	-	-	-	-	-	1	3	6	-	-	1	-	-	-	1	-	-
Drunella sp.	16	3	27	3	1	-	22	17	4	3	-	-	3	3	2	-	2	7	-	-	-	-	-	-
Ephemerella sp.	5	15	22	4	3	8	-	-	2	-	-	2	19	23	8	12	33	19	-	-	-	-	-	-
Timpanoga hecuba	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	2	-	1	-	-	-	-	-	-
Cinygmula sp.	237	47	246	19	174	57	-	-	-	-	-	1	3	9	3	-	48	6	229	424	212	-	15	14
Ecdyonurus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	86	-	-	-
Epeorus sp.	-	1	-	-	-	-	-	-	-	1	-	-	60	1	5	-	27	12	-	-	-	-	-	-
Rhithrogena sp.	-	-	10	-	-	1	-	-	-	-	-	-	-	-	23	-	14	7	-	-	-	-	-	-
Paraleptophlebia sp.	-	1	14	1	13	7	-	-	-	-	-	4	5	18	7	9	111	144	-	140	-	100	1	52
Plecoptera																								
Suwallia sp.	-	-	-	-	-	-	-	-	-	-	-	-	43	22	6	-	2	-	-	-	-	-	-	1
Sweltsa sp.	20	17	90	32	87	114	24	43	107	124	25	121	-	1	13	4	25	8	3	6	6	8	18	6
Paraleuctra sp.	1	-	6	2	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Malenka sp.	12	8	64	1	43	10	-	-	-	-	-	-	4	4	25	1	30	-	-	-	4	20	13	23
Podmosta sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Zapada sp.	14	9	281	4	76	46	-	-	2	-	-	-	-	-	-	-	-	-	-	-	2	3	-	-
Isoperla sp.	-	1	8	5	27	13	-	8	2	-	-	-	-	3	17	-	23	1	-	-	-	-	-	-
Kogotus modesta	4	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trichoptera																								
Brachycentrus sp.	-	-	-	-	-	-	-	-	-	-	-	-	8	-	10	-	4	-	-	-	-	-	-	-
Micrasema bactro	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	2	-	-	-	-	-	1	-
Agapetus sp.	-	-	-	-	-	-	-	-	-	-	-	-	23	-	-	-	-	-	-	-	-	-	-	-
Glossosoma sp.	1	-	-	-	-	-	-	-	-	-	1	-	-	1	1	-	1	-	-	-	2	-	-	-
Archtopsyche grandis	-	-	-	-	-	-	-	-	1	-	-	-	-	-	21	-	17	-	-	-	-	-	-	-
Hydropsyche sp.	-	-	-	-	-	-	-	-	-	-	-	-	49	14	73	3	234	2	-	-	2	-	1	-

	BUCK						DADD						ELKH						GRRO					
	7/1/16		8/8/16		9/7/16		6/29/16		8/2/16		9/11/16		6/27/17		8/2/16		9/11/16		6/23/17		8/3/16		9/12/16	
	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P
Hydroptila sp.	-	-	-	-	-	-	-	-	1	-	-	-	22	12	36	21	-	-	-	-	-	-	-	-
Lepidostoma sp.	-	5	3	-	1	2	35	139	24	98	3	14	45	209	30	8	207	76	4	103	7	43	-	7
Oecetis sp.	-	-	-	-	-	2	-	-	-	-	-	-	-	-	2	6	5	15	-	-	-	-	2	-
Allomyia sp.	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ecclisomyia sp.	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hesperophylax sp.	-	-	-	1	-	40	1	1	-	-	6	53	-	-	-	-	-	-	3	1	-	2	-	1
Psychoglypha sp.	-	1	2	-	-	32	-	-	4	20	1	2	-	2	-	-	-	-	-	1	-	11	-	-
Dolophilodes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	2	-	-
Rhyacophila sp.	14	14	59	3	15	2	3	3	-	-	1	-	3	-	15	-	10	-	-	-	-	-	-	-
Neophylax sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Neothremma alicia	6	4	25	3	40	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diptera																								
Atrichopogon sp.	-	-	10	-	-	7	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Palpomya sp.	-	-	3	3	-	2	1	1	3	28	-	23	-	-	-	2	-	1	-	10	-	1	-	-
Chironominae gn.	1	11	112	311	39	110	1	5	23	76	6	27	3	415	6	178	11	164	2	16	5	18	2	1
Diamesinae gn.	-	-	-	-	8	-	-	-	-	2	3	-	-	-	-	-	-	-	-	-	-	-	-	-
Orthocladiinae gn.	11	22	99	36	94	103	19	32	47	467	2	102	55	502	155	145	149	275	13	12	7	26	13	23
Tanypodinae gn.	-	13	1	2	-	6	-	4	1	2	-	22	-	6	1	3	-	7	-	46	3	44	1	47
Deuterophlebiidae gn.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dixa sp.	-	1	3	12	5	70	-	-	-	1	-	1	-	1	-	-	3	2	2	-	-	-	1	1
Dolichopodidae gn.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-
Chelifera sp.	-	-	-	-	-	-	2	-	-	1	-	-	-	-	2	-	1	-	-	-	-	-	-	-
Clinocera sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hemerodromia sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oreogeton sp.	-	-	-	-	-	1	2	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
Rhamphomyia sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ephydridae gn.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Maruina sp.	-	-	-	-	-	-	3	-	-	-	-	1	-	-	-	-	-	-	-	-	3	-	-	-
Pericoma sp.	-	-	2	-	-	1	-	-	3	4	6	50	-	-	-	-	4	-	-	-	-	1	9	-
Pteronarcella sp.	-	-	-	-	-	-	-	-	-	-	-	-	9	10	13	-	12	-	-	1	-	-	-	-
Ptychoptera sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Appendix III (cont.)

	BUCK						DADD						ELKH						GRRO					
	7/1/16		8/8/16		9/7/16		6/29/16		8/2/16		9/11/16		6/27/17		8/2/16		9/11/16		6/23/17		8/3/16		9/12/16	
	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P
Limnophora sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Simuliidae gn.	35	18	34	1	15	6	-	2	5	7	-	-	142	1	412	2	76	-	54	2	195	6	17	1
Euparyphus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	2	-	-	2	-
Forcipomyia sp.	-	-	-	4	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Odontomyia sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hybomitra sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Protoplasa sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Antocha sp.	-	-	-	-	-	-	-	-	-	-	-	-	1	-	7	1	28	14	-	-	-	-	-	-
Dicronota sp.	1	5	30	7	2	17	4	7	4	16	2	6	-	-	3	1	16	-	1	-	10	-	8	-
Erioptera sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hexatoma sp.	-	-	-	-	-	2	-	2	2	1	1	-	1	3	8	4	7	4	-	-	-	2	2	-
Limnophila sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Limonia sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Limoniinae gn.	-	-	-	-	-	-	4	2	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ormosia sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pedicia sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pseudolimnophora sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhabdomastix sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tipula sp.	-	-	-	-	-	-	-	-	1	3	1	-	-	-	-	-	8	-	2	2	-	-	-	-
Coleoptera																								
Helichus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Agabus sp.	-	-	-	1	-	-	-	-	-	3	-	-	-	3	-	-	-	-	2	14	6	4	-	-
Hygrotus sp.	-	2	-	-	1	-	-	1	-	-	-	-	-	1	-	-	-	-	2	1	-	-	-	-
Oreodytes sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Sanfilippodytes sp.	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Stictotarsus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	4	-	9	-	-
Dubiraphia sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Heterlimnius corpulentus	6	9	70	31	27	94	5	3	-	-	5	-	1	-	13	-	7	-	-	-	-	-	-	-
Narpus concolor	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-	-	-
Optioservus sp.	-	-	-	-	-	-	-	-	3	2	-	-	96	24	154	46	281	94	4	3	14	23	10	4
Zaitzevia parvula	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-

	BUCK						DADD						ELKH						GRRO					
	7/1/16		8/8/16		9/7/16		6/29/16		8/2/16		9/11/16		6/27/17		8/2/16		9/11/16		6/23/17		8/3/16		9/12/16	
	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P
Ochthebius sp.	-	-	-	-	-	-	1	1	-	-	2	-	-	-	-	-	1	-	5	-	-	-	-	-
Arrenurus sp.	2	9	41	12	-	7	-	20	14	20	-	21	2	-	4	20	13	10	-	14	38	9	6	1
Ametor sp.	-	-	-	-	-	4	-	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anacaena sp.	-	2	-	-	-	-	-	2	4	-	-	-	-	1	-	-	-	-	4	6	-	-	-	2
Helophorus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydrochus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Laccobius sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-
Paracymus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	2
Tropisternus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Scirtidae gn.	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	5	-
Odonata																								
Ophiogomphus severus	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Other																								
Oligochaeta	-	5	30	15	-	20	5	5	35	36	1	30	-	404	28	313	8	12	4	19	27	52	1	21
Pisidium sp.	-	-	-	-	-	1	-	3	-	-	-	5	-	35	-	4	-	1	-	5	1	59	1	-
Polycelis coronata	9	14	119	6	16	3	63	93	76	11	19	4	-	-	-	-	-	-	1	3	-	3	3	-
Physa sp.	-	-	1	1	-	-	-	-	-	2	-	-	-	-	-	-	-	-	11	80	80	576	-	-

	JACK						LIBE						LLBV						LORO					
	6/30/16		8/2/16		9/9/16		6/30/16		8/8/16		9/7/16		7/2/16		8/9/16		9/9/16		6/27/17		8/3/16		9/12/16	
	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P
Ephemeroptera																								
Ameletus sp.	-	2	-	8	1	6	-	-	-	3	2	3	-	1	4	57	14	135	-	-	-	-	-	-
Acentrella sp.	-	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-
Baetis sp.	257	142	150	91	295	11	734	-	211	66	24	37	90	348	163	118	36	10	372	50	420	211	504	534
Fallceon quilleri	5	40	-	5	-	-	-	1	-	-	-	-	-	4	-	1	-	-	-	-	-	-	-	-
Drunella sp.	61	31	23	12	10	-	32	2	52	9	28	7	29	96	65	9	14	2	-	-	-	-	-	1
Ephemerella sp.	490	20	105	98	42	7	14	1	46	22	1	9	290	679	208	95	14	49	-	-	2	1	-	-
Timpanoga hecuba	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8
Cinygmula sp.	85	38	31	8	34	2	27	-	39	10	17	2	46	134	117	27	63	7	-	-	1	-	4	-
Ecdyonurus sp.	-	45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Epeorus sp.	26	4	-	-	-	-	97	4	92	5	11	3	1	2	-	-	-	-	-	-	-	-	-	-
Rhithrogena sp.	-	-	-	-	-	-	16	-	2	4	24	25	-	-	-	-	-	-	-	-	-	-	-	-
Paraleptophlebia sp.	2	3	8	7	32	6	-	-	-	1	-	-	-	-	-	3	1	8	20	5	-	3	-	3
Plecoptera																								
Suwallia sp.	12	20	-	5	-	-	47	1	15	25	5	-	11	10	18	16	9	-	-	-	-	-	-	1
Sweltsa sp.	29	41	71	168	83	110	-	-	19	22	33	39	18	48	183	164	166	294	1	3	-	-	-	1
Paraleuctra sp.	-	-	7	6	-	-	-	-	1	-	4	4	-	-	7	3	3	1	-	-	-	-	-	-
Malenka sp.	52	4	20	11	5	-	-	1	2	1	1	-	5	13	23	6	37	2	-	-	4	6	58	28
Podmosta sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Zapada sp.	2	-	16	2	3	-	-	-	2	-	4	3	15	80	161	47	48	111	11	2	-	-	-	-
Isoperla sp.	2	2	7	24	-	26	1	-	8	4	16	6	-	3	58	19	6	38	-	-	-	-	-	-
Kogotus modesta	-	1	-	-	-	-	4	2	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-
Trichoptera																								
Brachycentrus sp.	-	-	-	-	-	-	-	1	4	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-
Micrasema bactro	-	-	-	-	-	-	-	-	3	4	17	44	-	-	-	-	-	-	-	-	-	-	-	-
Agapetus sp.	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glossosoma sp.	-	-	1	1	6	-	-	-	1	-	-	-	-	-	1	1	3	-	-	-	-	-	-	-
Archtopsyche grandis	-	-	-	-	-	-	-	-	10	1	7	2	-	-	-	-	-	-	-	-	-	-	-	-
Hydropsyche sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydroptila sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-
Lepidostoma sp.	7	13	-	4	-	1	3	3	16	12	-	5	6	25	1	24	1	8	2	-	1	-	2	2

Appendix III (cont.)

	JACK						LIBE						LLBV						LORO					
	6/30/16		8/2/16		9/9/16		6/30/16		8/8/16		9/7/16		7/2/16		8/9/16		9/9/16		6/27/17		8/3/16		9/12/16	
	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P
Oecetis sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Allomyia sp.	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ecclisomyia sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hesperophylax sp.	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	8
Psychoglypha sp.	-	2	-	3	-	-	1	-	-	3	-	-	-	2	-	-	-	12	-	-	-	-	-	-
Dolophilodes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Rhyacophila sp.	38	9	2	8	17	4	7	2	13	3	9	11	-	50	57	13	55	17	-	-	-	-	-	2
Neophylax sp.	-	-	-	-	-	-	-	-	6	12	8	39	-	-	-	-	2	-	-	-	-	-	-	-
Neothremma alicia	-	-	-	-	-	-	2	-	-	-	-	-	-	-	6	4	3	-	-	-	-	-	-	-
Diptera																								
Atrichopogon sp.	-	-	-	-	-	-	-	-	1	2	-	1	-	-	-	-	-	-	-	-	4	3	4	-
Palpomya sp.	-	-	-	6	-	2	-	-	5	3	-	-	-	1	7	9	-	46	12	2	9	24	1	57
Chironominae gn.	21	9	71	670	24	99	1	-	128	254	3	51	4	35	178	410	18	619	200	13	35	228	43	1375
Diamesinae gn.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Orthocladiinae gn.	38	26	35	112	16	12	20	-	1015	404	81	236	20	63	203	110	58	106	90	19	65	103	114	411
Tanypodinae gn.	1	28	5	13	1	25	2	-	1	5	-	1	-	6	-	5	-	18	3	-	12	23	6	54
Deuterophlebiidae gn.	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dixa sp.	1	-	1	4	-	-	-	-	-	-	-	-	1	1	6	21	4	7	5	1	4	-	5	3
Dolichopodidae gn.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Chelifera sp.	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Clinocera sp.	-	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hemerodromia sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oreogeton sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Rhamphomyia sp.	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Ephydridae gn.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Maruina sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	2	-	-
Pericoma sp.	-	-	-	-	-	-	-	-	-	1	2	17	-	-	-	1	-	15	-	-	-	-	-	1
Pteronarcella sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ptychoptera sp.	-	-	1	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Limnophora sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	3	1	-
Simuliidae gn.	73	85	8	-	3	-	3	-	27	1	211	20	82	211	45	27	16	3	-	-	12	-	-	1

	JACK						LIBE						LLBV						LORO					
	6/30/16		8/2/16		9/9/16		6/30/16		8/8/16		9/7/16		7/2/16		8/9/16		9/9/16		6/27/17		8/3/16		9/12/16	
	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P
Euparyphus sp.	-	-	-	123	-	-	-	-	-	6	-	-	-	-	-	-	-	-	-	1	2	1	-	-
Forcipomyia sp.	-	-	13	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	1	-	-	-
Odontomyia sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Hybomitra sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Protoplasa sp.	-	-	-	-	-	17	-	-	-	-	-	-	-	-	-	12	-	-	-	-	-	-	-	-
Antocha sp.	-	-	-	-	-	-	-	-	-	-	-	6	-	-	1	-	-	-	-	-	-	-	-	-
Dicronota sp.	2	7	4	17	3	1	-	-	3	1	2	3	-	4	27	20	1	10	-	-	1	-	2	1
Erioptera sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Hexatoma sp.	1	-	-	4	-	8	1	1	-	2	3	2	-	-	-	2	-	-	-	-	-	-	-	1
Limnophila sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Limonia sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	-	-	-	-	-	-	-
Limoniinae gn.	4	1	1	-	1	-	1	-	-	-	-	-	-	1	2	-	-	-	-	-	-	-	3	23
Ormosia sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12
Pedicia sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Pseudolimnophora sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhabdomastix sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tipula sp.	-	-	-	3	1	1	-	-	-	-	-	-	-	-	-	3	-	5	-	-	-	-	2	1
Coleoptera																								
Helichus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Agabus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18	6	8	5	3	3
Hygrotus sp.	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	2	-	-	4	-	-
Oreodytes sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sanfilippodytes sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	2	-	4
Stictotarsus sp.	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	3	10	34	2	13
Dubiraphia sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Heterlimnius corpulentus	137	42	141	290	127	210	19	5	198	176	49	123	2	20	121	63	15	70	-	-	-	-	-	-
Narpus concolor	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Optioservus sp.	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	7	-	-	3
Ochthebius sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Arrenurus sp.	2	1	5	35	2	2	5	1	39	68	6	76	2	28	55	61	9	32	4	1	9	4	2	4
Ametor sp.	-	-	-	1	1	-	-	-	-	-	-	-	-	-	3	1	-	-	-	-	-	-	-	-

Appendix III (cont.)

| | JACK | | | | | | LIBE | | | | | | LLBV | | | | | | LORO | | | | | |
| | 6/30/16 | | 8/2/16 | | 9/9/16 | | 6/30/16 | | 8/8/16 | | 9/7/16 | | 7/2/16 | | 8/9/16 | | 9/9/16 | | 6/27/17 | | 8/3/16 | | 9/12/16 | |
	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P
Anacaena sp.	6	5	1	2	-	-	-	-	-	1	-	-	2	2	-	1	-	-	3	-	7	14	4	-
Helophorus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydrochus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Laccobius sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	8
Paracymus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tropisternus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Scirtidae gn.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Odonata																								
Ophiogomphus severus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Other																								
Oligochaeta	5	2	3	6	1	-	-	-	29	6	2	3	-	22	12	8	1	17	-	-	-	6	-	1
Pisidium sp.	5	-	-	17	-	18	-	-	-	-	-	-	-	32	-	49	-	11	-	-	-	-	-	-
Polycelis coronata	6	-	5	2	3	-	-	-	-	-	-	-	66	311	614	272	78	76	-	-	-	-	-	-
Physa sp.	-	-	-	-	-	-	-	-	-	-	-	-	2	2	-	-	-	-	-	-	-	-	-	-

	MIRO						MONU						SIGN						STPR					
	6/29/17		8/2/16		9/11/16		6/30/16		8/8/16		9/7/16		7/1/16		8/8/16		9/7/16		6/25/17		8/3/16		9/12/16	
	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P
Ephemeroptera																								
Ameletus sp.	-	-	-	-	-	-	1	3	2	11	-	7	2	6	-	16	5	43	1	2	-	1	-	3
Acentrella sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Baetis sp.	118	75	223	125	139	42	464	99	1357	395	139	200	585	241	172	71	112	10	833	329	998	935	449	238
Fallceon quilleri	-	1	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	2
Drunella sp.	-	-	-	-	-	-	-	-	17	2	-	6	67	20	35	9	2	-	-	-	-	-	2	-
Ephemerella sp.	3	-	1	-	-	-	6	1	-	2	-	-	-	-	9	5	1	11	19	2	3	-	-	1
Timpanoga hecuba	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cinygmula sp.	-	1	2	-	-	-	32	7	-	-	-	35	372	320	258	193	129	27	5	3	17	7	361	215
Ecdyonurus sp.	-	-	-	-	-	-	-	-	1	3	-	-	-	-	-	-	-	-	-	-	-	-	2	-
Epeorus sp.	8	9	-	-	-	-	35	11	-	-	-	1	28	5	21	13	-	-	58	8	-	-	37	3
Rhithrogena sp.	-	-	-	-	-	-	-	-	10	-	-	7	8	-	11	-	4	-	1	-	-	-	6	-
Paraleptophlebia sp.	-	-	-	-	-	-	-	3	9	8	-	26	-	-	-	-	-	1	-	-	-	2	2	7
Plecoptera																								
Suwallia sp.	27	-	-	-	-	-	1	-	-	1	-	-	-	6	-	-	24	-	-	-	-	-	-	-
Sweltsa sp.	-	36	49	125	40	154	3	-	1	6	10	19	37	12	68	71	29	38	23	8	20	19	32	70
Paraleuctra sp.	-	1	2	-	-	-	-	-	-	-	-	-	6	-	-	2	3	-	-	-	1	-	8	1
Malenka sp.	33	17	138	65	151	45	26	4	4	3	12	29	155	14	229	40	172	11	9	-	137	56	324	120
Podmosta sp.	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Zapada sp.	-	-	51	104	-	-	-	-	1	4	-	-	278	32	271	83	132	32	4	9	21	5	1	-
Isoperla sp.	-	-	-	-	-	-	-	3	1	-	4	1	4	2	49	18	2	6	4	-	23	7	15	7
Kogotus modesta	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Trichoptera																								
Brachycentrus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Micrasema bactro	-	-	-	-	-	-	-	-	1	-	-	-	4	-	-	-	-	-	-	-	-	5	-	22
Agapetus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glossosoma sp.	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	2	-	-	-	6	11	13	20
Archtopsyche grandis	-	-	-	-	-	-	-	-	32	1	72	2	-	-	-	-	-	-	-	-	-	-	-	-
Hydropsyche sp.	-	-	-	-	-	-	4	-	3	9	113	14	-	-	-	-	-	-	11	-	12	1	18	-
Hydroptila sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lepidostoma sp.	43	73	7	15	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	3	21	-	22

	MIRO						MONU						SIGN						STPR					
	6/29/17		8/2/16		9/11/16		6/30/16		8/8/16		9/7/16		7/1/16		8/8/16		9/7/16		6/25/17		8/3/16		9/12/16	
	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P
Oecetis sp.	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Allomyia sp.	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ecclisomyia sp.	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	13	-	-	-	-	-	-	-
Hesperophylax sp.	-	-	-	-	-	-	-	-	9	61	9	5	-	-	-	4	-	-	14	1	-	4	2	24
Psychoglypha sp.	-	3	3	2	-	1	-	-	-	12	-	-	-	-	3	-	-	37	-	-	2	-	-	-
Dolophilodes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhyacophila sp.	4	8	5	5	6	4	-	-	-	1	-	-	45	7	49	12	14	8	1	-	-	3	17	6
Neophylax sp.	2	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	32	-	-	2	-	-	-	-
Neothremma alicia	-	1	-	-	-	-	-	-	1	2	-	-	35	3	40	29	-	5	-	-	-	-	-	-
Diptera																								
Atrichopogon sp.	-	-	1	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Palpomya sp.	-	-	2	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	1	1	1	6
Chironominae gn.	2	-	8	19	7	1	4	2	20	393	2	60	252	47	206	1166	2	111	-	3	3	49	10	86
Diamesinae gn.	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Orthocladiinae gn.	31	6	18	10	12	5	43	8	37	141	26	98	259	23	560	228	32	109	17	15	32	203	79	725
Tanypodinae gn.	-	1	-	1	-	-	-	1	2	4	-	2	9	2	2	21	-	5	-	-	-	3	-	30
Deuterophlebiidae gn.	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Dixa sp.	5	4	23	13	5	4	-	-	2	9	5	3	19	8	17	20	3	-	-	2	19	40	372	152
Dolichopodidae gn.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Chelifera sp.	1	-	-	2	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-
Clinocera sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hemerodromia sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Oreogeton sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhamphomyia sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ephydridae gn.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Maruina sp.	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	4	-	22	2	28	-
Pericoma sp.	-	-	3	2	-	-	-	-	-	-	-	-	-	-	17	6	-	4	-	-	-	-	-	1
Pteronarcella sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ptychoptera sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Limnophora sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Simuliidae gn.	312	36	382	113	15	-	661	33	788	58	15	33	157	9	23	6	15	1	66	40	2577	78	168	48

Appendix III (cont.)

	MIRO						MONU						SIGN						STPR					
	6/29/17		8/2/16		9/11/16		6/30/16		8/8/16		9/7/16		7/1/16		8/8/16		9/7/16		6/25/17		8/3/16		9/12/16	
	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P
Euparyphus sp.	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-
Forcipomyia sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	1	-	-	-	-	-	-	1
Odontomyia sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hybomitra sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Protoplasa sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Antocha sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dicronota sp.	4	-	11	8	5	-	29	15	10	7	9	4	6	-	5	1	-	3	3	-	2	28	13	12
Erioptera sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hexatoma sp.	-	-	-	4	1	-	1	-	-	-	-	-	-	-	-	1	-	1	-	3	-	3	4	13
Limnophila sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Limonia sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Limoniinae gn.	4	-	1	1	-	-	-	-	-	-	-	-	4	4	-	-	-	-	-	-	-	-	1	-
Ormosia sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pedicia sp.	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pseudolimnophora sp.	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Rhabdomastix sp.	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tipula sp.	1	-	-	-	-	1	-	-	-	-	1	-	-	-	3	1	-	1	2	-	1	3	1	7
Coleoptera																								
Helichus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	1
Agabus sp.	-	-	-	-	-	-	8	8	21	10	2	1	-	-	-	-	-	-	4	2	2	11	5	-
Hygrotus sp.	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Oreodytes sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
Sanfilippodytes sp.	-	2	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	3	-	-
Stictotarsus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-
Dubiraphia sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Heterlimnius corpulentus	1	1	-	1	-	-	4	2	-	7	5	-	1	-	1	2	1	2	22	2	-	29	-	40
Narpus concolor	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Optioservus sp.	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	8	26	88	80	-	61
Ochthebius sp.	-	-	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Arrenurus sp.	11	1	15	22	12	-	1	-	4	13	13	5	15	2	38	16	5	12	3	4	20	103	13	51
Ametor sp.	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	2	1

	MIRO						MONU						SIGN						STPR					
	6/29/17		8/2/16		9/11/16		6/30/16		8/8/16		9/7/16		7/1/16		8/8/16		9/7/16		6/25/17		8/3/16		9/12/16	
	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P
Anacaena sp.	1	2	-	1	-	-	-	1	3	-	-	-	1	-	-	-	-	-	2	1	-	7	2	-
Helophorus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Hydrochus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-
Laccobius sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Paracymus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Tropisternus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Scirtidae gn.	-	1	1	-	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Odonata																								
Ophiogomphus severus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Other																								
Oligochaeta	12	-	46	38	4	1	-	-	1	73	6	71	-	-	14	14	-	3	2	5	2	14	11	13
Pisidium sp.	-	-	-	1	-	-	-	-	-	-	-	-	4	-	3	1	-	1	-	-	-	-	-	-
Polycelis coronata	90	58	181	98	68	13	1	-	-	-	68	-	310	98	674	458	141	115	156	31	64	93	179	403
Physa sp.	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-