Biological Recovery in an Urban Industrial Stream: Using the Reference Condition Approach to Assess the Current State of Junction Creek, Sudbury, Ontario

By

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Abstract

Benthic macroinvertebrate communities in Junction Creek, Sudbury, have recovered remarkably since the 1970s, following government initiatives to lower atmospheric pollution, the implementation of mine wastewater treatment, and Greater Sudbury's Regreening Program. To understand contemporary temporal and spatial patterns of biological condition in this stream system, I employed a number of benthic community metrics and the Reference Condition Approach. There was little evidence of temporal trends in the benthic macroinvertebrate communities across the 2003-2015 study period, however there was strong evidence of community composition, water and sediment quality differences among study sites.

This urban industrial stream is affected by multiple stressors (straightening, culverts, urban and mining pollutants, overflow from sewer outfalls, etc.), which accumulate as the water flows downstream. Lime-treated metal mining effluent forms the headwaters, which receive cold groundwater from the upper reaches and is further diluted by the Maley tributary before reaching the heavily urbanized city. In these upper reaches, community metrics that are indicative of sensitive organisms and higher diversity are elevated and metal levels are lowest within the study area. Biological and chemical conditions decrease slightly heading downstream, but are much better following the millions of dollars spent in 2001 on diverting acid mine drainage underground to be treated kilometers away before being discharged into the creek below the study area. Treated surface runoff from a historic mine site, and a portion of each the Clarabelle Mill property and slag storage area from one of the world's largest metal mining and smelting complexes in the world feed Nolin Creek, which then enters the stream through concrete box culverts that run beneath the city's downtown core before re-surfacing and flowing South of the city. Despite the recent appearance of fish within Nolin Creek, it is still a major source of contaminants to Junction Creek, with elevated water and sediment metal levels and low benthic macroinvertebrate community diversity and abundance.

This research showed that use of select benthic macroinvertebrate community metrics served as a better tool to assess biological conditions within the stream than comparison to near-pristine reference sites, but that local reference sites would serve as a best practise since they take account for naturally high regional metal levels, and the effects from decades of atmospheric pollution have had on Sudbury soil, lakes and streams. Additionally, test site benthic

macroinvertebrate metric scores were surprisingly similar to those of pristine reference sites, but abundance was much higher at all reference sites. The apparent similarities between test and reference biotic conditions may be due to high nutrients and ions found in urban environments, inappropriate matching of reference and test sites or numerous cumulative effects. It was therefore recommended that ecosystem processes and functioning be studied in order to obtain a more accurate view of the current biological condition of Junction Creek. Finally, aqueous metal levels have decreased substantially and benthic macroinvertebrate community abundance and richness have increased substantially in the last 50 years, but appear to be at a relatively stable point currently, although the variation in both metrics is generally higher than those of reference sites. Biological and chemical conditions will likely not improve until the major current stressors (residual contamination of sediments and soils, and addition of mining effluent) are removed and habitat is improved.

Keywords: Junction Creek, Mining, Restoration, Benthic Macroinvertebrates, Sudbury, Recovery, Reference Condition Approach, Bioassessment, Temporal Trends, Urban

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Many people have contributed to my thesis and experience with the Cooperative Freshwater Ecology Unit and through this single page of text, I cannot thank you all enough. First and foremost, I must thank my co-supervisor John Gunn, who has demonstrated patience and understanding throughout the last couple years and shown a keen interest in this project throughout its duration, which truly made my experience- I could not have done this without you. Secondly, I would like to extend my gratitude to my co-supervisor Brie Edwards, for jumping into this project near its end (but with much work left to do), providing indispensable statistical knowledge and assistance, and constant support even while on parental leave.

Completion of my thesis would not have been possible without the help of the numerous local sources of historical reports and expertise: Conservation Sudbury, Ontario Ministry of the Environment and Climate Change, and Junction Creek Stewardship Committee. I would like to recognize my final committee member Sarah Woods, who provided a wealth of knowledge regarding Junction Creek and its history. Additionally, I would like to thank Chantal Sarrazin-Delay and Bill Keller for sharing their knowledge with me, Emily Smenderovac and Michael Carson for their R wizardry, and members of the Ontario Ministry of the Environment and Climate Change and Ontario Ministry of Natural Resources and Fisheries at the Living with Lakes Centre for your help with field work and advice: Jocelyne Heneberry, Vanessa Bourne, Eric Wilcox and Lee Haslam. I would like to extend a special thank you to my boyfriend Trevor for his patience and continuous support over the last few years, especially during the final months while I lived between work and school.

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1 Introduction

In degraded urban ecosystems, restoration efforts may at times need to address the influence of multiple stressors, long-term accumulative effects on habitat conditions, as well as many significant and persistent societal considerations (e.g. public access, infrastructure and engineering for public safety, storm water management, flood control, vehicle and pedestrian crossings, etc.) that create unique flow regimes, nutrient conditions, water chemistry, etc. (Paul and Meyer 2001). Deciding on completion criteria for restoration projects in such managed and altered systems is therefore very challenging, however restoration ecologists still strive for best possible results, hoping to achieve as close to 'natural, self-sustaining and healthy ecosystems' as possible. This study addresses this restoration challenge by assessing recovery of key components of an urban industrial stream within the city of Greater Sudbury (herein referred to as Sudbury) Canada, home to one of the largest integrated mining complex in the world.

Since the commencement of mining in 1888, the massive roast yards and metal smelters in Sudbury have emitted more than 100 million tonnes of sulphur dioxide and tens of thousands of tonnes of Cu, Ni, and Fe into the atmosphere, and have created massive waste piles containing more than 500 million tons of acid generating tailings and waste rock (Potvin & Negusanti 1995; Wiseman and Michellutti 1995). As the mining and smelting activities increased into the 20th century Sudbury eventually became the largest point source of SO_2 in the world and this led to acidification of over 7,000 lakes within a 17,000 km^2 radius (Neary *et al*. 1990). Significant metal contamination of surface waters, soils and lake sediments also extended out approximately 30 kilometres from the point source (Gunn *et al.* 1995[a] and Gunn *et al.* 1995[b]).

The recovery of this severely damaged landscape was initiated by the government of Ontario through a series of control orders and regulations that began in 1970 and have resulted in

more than a 95% reduction in atmospheric pollutants. These progressive air pollution controls eventually led to some remarkable 'natural' (i.e. without direct treatment) aquatic and terrestrial recovery throughout the affected area (Keller *et al.* 1992, Keller *et al*. 1995, Gunn *et al*.1995[a], Keller *et al*. 2007). However, active land-reclamation efforts, including liming, fertilization and grassing of more than 3500 ha and planting of approximately 10 million trees, were key to the most visible "re-greening" of Sudbury (Gunn et al. 1995[a]). To date, much less research effort has been directly invested in recovery of fluvial environments in the area than of lakes and terrestrial systems.

The upper section of Junction Creek (herein referred to as Junction Creek) is an approximately 25 km stream that flows through the most heavily urbanized section of Sudbury and receives drainage from 5 tributaries that originate at many of the largest mining and waste storage areas in Sudbury (Fig. 2.1). Throughout the past 120 years the city has greatly altered the hydrology and habitat of the creek through dredging and straightening, adding many bridges, culverts and other road crossings, directing storm drain outfalls into the creek, building flood control reservoirs in the catchments (Conservation Sudbury additionally) and even diverting the stream underground in a lengthy box culvert below the downtown. Despite these engineered changes, today many sections of Junction Creek are aesthetically pleasing 'natural appearing areas' that the community values and has invested heavily in further restoration work (debris clean up, tree and shrub planting, creation of walking trails, etc.).

Prior to the commencement of restoration in the 1970s, Junction Creek received untreated wastewater from Copper Cliff, Frood-Stobie and Garson Mines (OWRC 1963), leading to extremely high metal concentrations in the water and sediments and low presence of benthic macroinvertebrates, as seen in Table 1.1 (OWRC 1966). During 1965 sampling events, pH

ranged from 3.4-9.9 in the system, and pH in Garson remained above 11 during 1972 due to liming (OWRC 1996 and MOE 1975). The stream was commonly used as a sewer outlet from the time urban development began until wastewater treatment facilities were put in place in the 1970's and the downtown portion of the creek was boxed to help alleviate flooding (OWRC 1966; Wallace and Thomson 1996).

Table 1.1 Historical water quality data and benthic macroinvertebrate community metrics from Junction Creek in June 1965 expressed in ppm, except pH, as per the original report (OWRC 1966). Methods and locations for benthic macroinvertebrate collection differ from the CABIN protocol and are therefore not directly comparable to the results of the current study.

Parameter	**Garson**	**Frood**	**Nolin**	**Main Branch**
Water Quality				
pH	7.2	4.9	6.5	7.2
Alkalinity	38.0	6.0	33.0	114.0
Calcium	234.0	173.0	156.0	65.0
Sulphate	650.0	870.0	765.0	570.0
TKN	4.1	5.3	5.9	61.0
Copper	0.1	0.4	1.4	1.7
Nickel	2.2	24.0	11.0	2.8
Benthic Macroinvertebrates				
Abundance	11	21	0	0
Family Richness	2	2	0	0
% EPT	0	0	0	0
% Chironomids	90	25	0	0

In the past 40 years industry has also invested heavily to reduce effluent inputs to Junction Creek, including the construction of liming plants to continuously treat their acid and metal contaminated effluent on three of the tributaries (Garson, Copper Cliff, and Nolin) and have diverted untreated effluent from a large acid –generating waste rock storage site on a fourth tributary: Frood (Gunn *et al.* 2010). These direct interventions by industry are in addition to the massive investments in atmospheric emission reductions and some extensive aerial liming and

seeding work in portions of the watershed. Finally, Junction Creek has also seen major environmental improvements through the construction of a municipal sewage plant (eliminating discharge of raw sewage), the removal of contaminated soil from a former creosote plant, the installation of bank erosion structures, etc.

Assessing recovery status or setting realistic restoration targets for such heavily manipulated systems is a challenge, especially in the case of a highly valued and accessible system like Junction Creek where community members have set very ambitious goals. For example, one of the major community goals is to restore the creek to a productive system for sensitive cold-water fish species such as brook trout (*Salvelinus fontinalis*). Additionally, naturally metal-rich sediments and an abundance of rock outcroppings make up the local landscape, which may call for Sudbury-specific management strategies.

My objective was to use available data from the Ontario Ministry of the Environment and Climate Change (OMOECC) biomonitoring program to identify contemporary recovery trends (i.e. towards an expected reference condition) at a variety of sites on Junction Creek (Garson to below just below downtown) in the past 13 years, and to assess the factors most strongly associated with recovery to assess the current state of the recovery. Therefore, the approach I have taken to evaluate this system is to employ the Reference Condition Approach (RCA) using benthic invertebrate communities as integrators and indicators of environmental change (Reynoldson *et al.* 1997, Norris *et al.* 2004) as well as comparison of benthic macroinvertebrate community metrics through time and among test sites.

2 Methods

2.1 Study System and Sampling Sites

The entire Junction Creek watershed has an area of approximately 320 km^2 from the headwaters to downstream McCharles Lake, with drainage entering the system from a variety of mining sites and active waste management areas (Fig. 1). The study focuses on the upper watershed, above the downtown portion of the city. This area contained 7 OMOECC monitoring sites with at least 8 years of assessment data. These sites cover the main branch, its headwaters in Garson, three tributary streams (Maley, Frood, and Nolin), and three downstream sites along the main branch that measure cumulative effects from the convergence of different tributaries. Junction 1 is downstream from where Garson and Maley tributaries converge; Junction 2 is situated downstream from where the Frood tributary joins and, Junction 3 is downstream of where the final tributary, Nolin Creek enters the system. See the Appendix A for photos and maps of each of these sites along with additional details of the local factors affecting each site. For example, the Garson site is below an active mine and associated wastewater treatment system, which employs hydroxide precipitation and settling for metal removal; Maley contains a flood control reservoir and has limited influence from mining (mine exploration sites); Frood has a second flood control reservoir and historically received AMD (Acid Mine Drainage) from a waste rock pile before its diversion in 2001 to be discharged and treated in Copper Cliff; and Nolin receives surface runoff from mining operations, requiring effluent water quality to be mitigated by a lime plant just upstream of the confluence of the Nolin East and West branches.

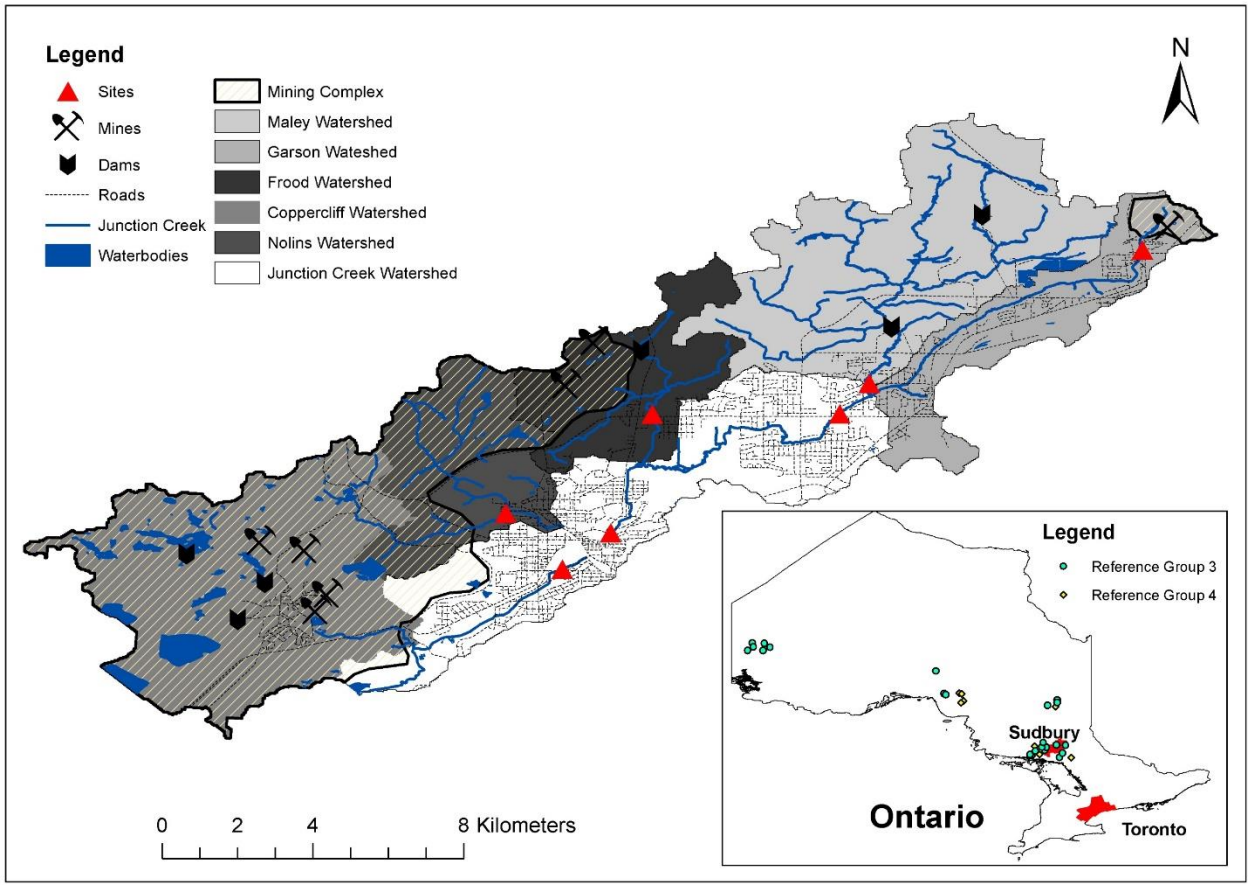

Figure 2.1 Map of Upper Junction Creek Study Area. Approximate area overlapping with the Copper Cliff Mining Complex and Garson Mine are included, as well as roads, to show the amount of land directly impacted by the mining and urbanization. Location of reference sites in Group 3 (n= 44) and Group 4 (n= 20) are shown in the lower right.

2.2 Sampling Methods

Water quality and benthic macroinvertebrate community data (samples taken from 2005-2014) for both the Junction Creek test sites and selected reference sites were obtained from the Canadian Aquatic Biomonitoring Network (CABIN) database, while the 2015 data was specifically collected for this project. The sites were all sampled in the fall (October 19-27) using consistent sampling protocols. Water chemistry, habitat characteristics and benthic macroinvertebrates were sampled from these 7 stream sites multiple times between 2005 and

2014 and all 7 sites were sampled in 2015. Stream sediment chemistry was only assessed in 2015. At each sampling event, benthic macroinvertebrates were collected first to avoid any impacts of disturbance by other aspects of the sampling. This was followed by sediment sample collection (in 2015) and habitat assessment which occurred within the same reach (defined as 6 times the stream width). Water chemistry samples were taken last, approximately 10-20 meters upstream of the benthos sampling area.

2.2.1 Benthic Macroinvertebrates

Benthic invertebrates were collected using a D net (mesh 400 um) following the standard CABIN protocol developed by Environment Canada for bioassessment (Environment Canada 2011). Briefly the 'Kick and Sweep Method' consisted of a zig-zag transect across the wadeable section of stream in an area that contained coarse substrate (gravel to large rocks). The transect proceeded from bank to bank for approximately three minutes, disturbing and kicking substrate to a depth of about 5-10 cm and capturing the released invertebrates with the net held downstream of the disturbed area (Environment Canada 2011). The 'kick area' encompassed as many types of microhabitat present at the site as possible. Immediately following the kick, benthic samples were transferred to plastic containers and 10% buffered formalin was added at a 1:3 ratio (1 part buffered formalin to 3 parts benthic sample), to preserve the sample and prevent losing organisms to predation. Samples were later sieved (400 μm mesh), transferred into 70% ethanol, and shipped to a certified taxonomist to be identified to the taxonomic level possible. The taxonomist used a Marchant box with 100 cells to homogenize and split the sample prior to identification. Cells were then randomly picked and each subsample (i.e. selected cell) was fully counted until a minimum count of 300 organisms had been identified. Specific details of the

subsampling and counting procedure are documented in the CABIN manual (Environment Canada 2014).

2.2.2 Physical and Chemical Sampling

A YSI probe was used to measure air and water temperature (°C), pH, conductivity (µs/m), dissolved oxygen (mg/L), and turbidity (NTU) as per the CABIN protocol (Environment Canada 2011). Water samples were collected and sent to the OMOECC environmental lab for analyses. Habitat characteristics (flow, maximum and average depth, bankfull and wetted width, substrate size, surrounding land use and riparian vegetation) were measured following the CABIN protocol. Additionally, sediments were collected in 2015 and analyzed for numerous elements and chemicals. Only chemicals listed as chemicals of concern (COCs within the Sudbury Soils Study (2009) are presented.

2.3 Data Management and Statistical Analyses

2.3.1 Benthic Macroinvertebrate Community Data

Benthic macroinvertebrate data was analyzed at the family level of taxonomic resolution (Bailey et al. 2001, Bowman and Bailey1996). Nine commonly used benthic macroinvertebrate community metrics were chosen for assessing temporal trends during the study period (2003-2015) and comparing among the 7 Junction Creek sites. These metrics were: Simpson Diversity, % chironomids, % EPT (Ephemeroptera, Plecoptera and Trichoptera), total abundance, Hilsenhoff Biotic Index (HBI), Shannon-Wiener Diversity (S-W Diversity), and Bray-Curtis

Distance, EPT richness and overall richness at the family level. Additionally, invertebrates were assigned to various functional feeding group (FFG; e.g. filterers, gatherers, predators, scrapers and shredders, and clingers). Because some benthic macroinvertebrates occupy different functional feeding groups depending on their life stage, or may be assigned to numerous functional feeding groups at once, when identification is at the family level, some redundancies may exist. For example, the entire family would be classified within a FFG even if only a few species within that family met this definition.

RCA Analysis

Because there was no predisturbance data or unimpacted reference sites within the Junction Creek watershed, test sites on Junction Creek were matched with reference groups within CABIN's Near North Ontario 2016 BEnthic Analysis of SedimenT (BEAST) Model based on the following environmental predictors: longitude, stream order, intrusive rock (%), metamorphic rock (%), sedimentary rock (%), precipitation in March (mm), precipitation in November (mm), minimum temperature in January (°C), minimum temperature in September (°C), minimum temperature in October (°C), and drainage area (km^2) (Novodorvsky and Bailey 2016). These 11 environmental predictors were chosen out of 148 candidate predictors following removal of predictors potentially affected by disturbance; removal of predictors that were not shared by all sites; and, forward- and backward-stepwise Discriminant Function Analysis using a cut-off tolerance of 0.1 (Novodorvsky and Bailey 2016). Note this model was generated for near pristine condition site in northern Ontario, sites that were meant for assessing the effects of climate change and industrial development in the future. Using these morphological or climate variables the BEAST model classified all test sites in Junction Creek as being within Reference

Group 3 (Garson, Maley, Frood and Nolin) or Reference Group 4 (Junction 1, Junction 2 and Junction 3) from the Near North Model of Novodorvsky and Bailey (2016).

Regressions on Time

For each selected metrics and FFGs (% filterers, gatherers, predators, scrapers and shredders, and # of clingers), regressions were performed on time. The functional feeding groups used by CABIN are described in detail in Merrit and Cummins 3rd Edition (1996). Shapiro tests were performed on each metric and FFG to test for normal distribution. Because the majority of metrics were not distributed normally, data was rank-transformed prior to performing the following two tests.

ANOVAs

Repeated Measures One Way ANOVAs were run for each metric and FFG to detect whether statistical differences in community metrics exist among the sites and to take account of the different time points during which these metrics were measured at each site. Post-hoc Tukeys tests were run on all benthic macroinvertebrate metrics to reveal where community metric dissimilarities resided between sites, with results displayed in Appendix B. Two types of Post-hoc Tukeys tests were run: (1) Tukeys HSD test, to group sites together where no significant difference in metric values resides; and (2) Tukeys Test to identify which sites hold significant differences in each metric.

NMDSs

Seven NMDS (Non-metric Multidimensional Scaling) ordinations were performed on the benthic count data of each site and the appropriate reference group sites using Bray-Curtis Distance. Four ellipses were then added to the ordination to represent four confidence intervals

of the reference data: 75% (similar to reference condition), 90% (mildly divergent), 99% (divergent), and 99.9% (highly divergent), following the CABIN BEAST (BEnthic Analysis of SedimenT) Assessment. Anything beyond the extent of the highly divergent ellipse was also seen to be highly divergent from the reference condition.

2.3.2 Water Chemistry Parameters and Quality

Water samples are analyzed for a suite of different chemical parameters, but for this study, focus was placed on a select list of chemicals of interest in the Sudbury area. These were the COCs (Contaminants of Concern from the Sudbury Soils Study and resulting Ecological Risk Assessment: aluminum, cadmium, cobalt, copper, iron, lead, nickel, and zinc (SARA Group 2009). Additional parameters used in the analysis were: DO (dissolved oxygen), pH, calcium, TP (total phosphorus), sodium and sulfate. Water chemistry data was unavailable for Junction 1, except for parameters measured in the field (pH and DO).

PCAs

Two PCAs (Principal Component Analyses) were conducted to assess variability in these parameters among sites through reducing the dimensionality of that data and thereby identifying which parameters are the most important predictors . The first was conducted to describe variation in water quality parameters among test sites (Fig. 3.8) and the second was conducted to describe the variation in water quality parameters among both test and reference sites (Fig. 3.9)

Db-RDAs

Distance-based Redundancy Analyses (db-RDAs) using Bray-Curtis Distance were employed to examine the variation between sites and years in relation to environmental conditions, including habitat characteristics (24 variables) and water chemistry parameters (19 variables) (Legendre and Legendre 1998, Legendre and Anderson 1996). Due to the number of variables considered, VIFs (Variance Inflation Factors) were used prior to analyses to detect multi-collinearity between parameters and remove redundant predictors. Parameters were backwards stepwise eliminated using a widely used VIF of ten (Craney and Surles 2002), which ultimately means that 90% of the variability in the ith independent variable is explained by the remaining variation in the model. Sulfate was included in the analysis despite having been eliminated due to its VIF, as was alkalinity, which had a VIF of 10.15. Following this analysis of VIFs, there were still 26 variables remaining, so two separate RDAs were run for habitat and water chemistry variables. Note; one site, Junction 1 had water chemistry data only for 2015. All other sites had complete data records.

Sediments were collected in 2015 and analyzed for numerous elements and chemicals, the results of which are displayed in Table 3.7 Only the metals listed as COCs within the Sudbury Soils Study are presented.

3 Results

3.1 Temporal Trends in Benthic Macroinvertebrate Communities

There was little evidence of any statistically significant temporal trends during the 2003-2015 study period when using all of the 9 community metrics or the 6 functional feeding group classification. In fact, in this total of 54 regression analysis over time, only 2 statistically

significant temporal trends were detected (Table 3.1, Fig.3.3). These were a significant increase in %EPT at the Junction 3 site (Fig.3.3) and a significant increase in one of the functional feeding groups (# clingers) in Junction 3 (Table 3.1). My overall conclusion was therefore that no significant temporal trends in the benthic invertebrate communities were occurring during the 2003-2015 study period with the exception of the Junction 3 findings, however 2/58 may be chance alone.

Table 3.1 R^2 values from benthic macroinvertebrate community metric regressions through time. Bold values are significant using an alpha of 0.05 and underlined values hold a high enough R^2 value to identify the direction of this trend.

Metric	Garson	Maley	Junction 1	Frood	Junction 2	Nolin	Junction 3
Abundance	0.2837	0.4519	0.5513	0.000684	0.2804	0.2702	0.3893
Bray-Curtis Distance	0.1572	0.4773	0.7668	0.00169	**0.5823**	0.2242	0.2441
HBI	**0.5338**	0.2111	0.8088	0.005567	**0.5473**	0.2371	**0.6276**
S-W Diversity	0.1383	0.2567	0.07692	0.002727	0.4188	0.003727	0.1731
Simpson's Diversity	0.2497	0.06749	N/A	0.0108	**0.6396**	0.02977	**0.5015**
Family Richness	0.2264	0.07999	0.05769	0.3056	0.03258	0.1632	**0.5538**
EPT Richness	0.2264	0.01688	0.03764	0.2098	0.03673	0.2526	**0.9231**
% EPT	0.008184	0.00453	0.1019	0.07955	**0.5288**	0.1495	**0.6528**
% Chironomids	0.07594	0.3764	0.004501	0.003903	0.473	0.009208	0.04788
% Filterers	0.0272	0.003386	N/A	0.09629	**0.6231**	0.2101	0.561
% Gatherers	0.03963	0.09092	0.4286	0.000846	**0.6831**	0.08126	0.4652
% Predators	0.484	**0.5872**	0.001052	0.0224	**0.6114**	0.04201	0.01012
% Scrapers	0.07705	0.006176	0.7953	0.00078	0.3123	0.06465	0.4115
% Shredders	0.1064	0.04166	0.003637	0.008863	0.4205	0.02762	0.3267
# Clingers	0.2536	0.009327	0.9231	0.3207	0.07872	0.009643	**0.8698**

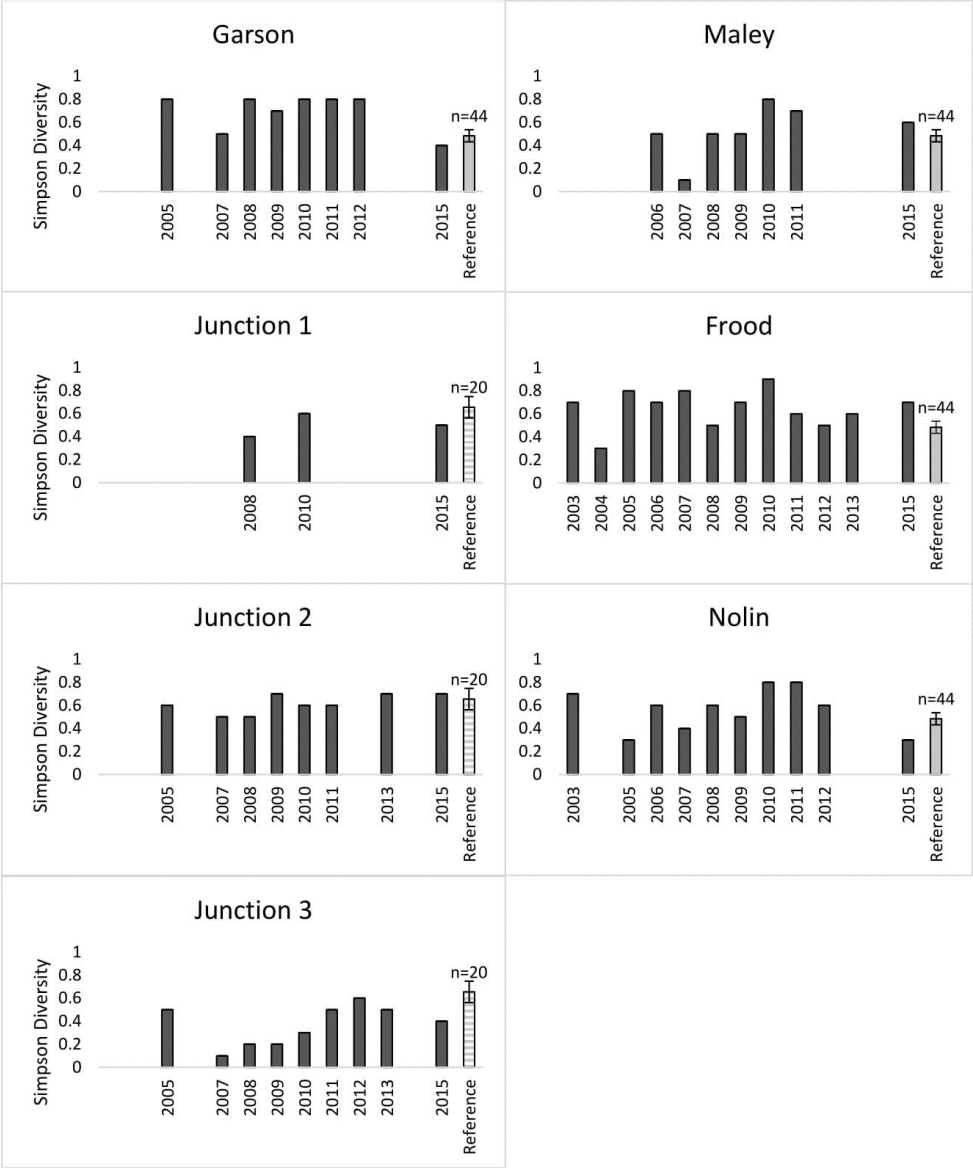

Figure 3.1 Simpson Diversity measures at each study site over time (2003-2015). Appropriate reference group averages with 95%confidence intervals are included with the total number of sites indicated in Group 3 (n=44) and Group 4 (n=20) reference sites.

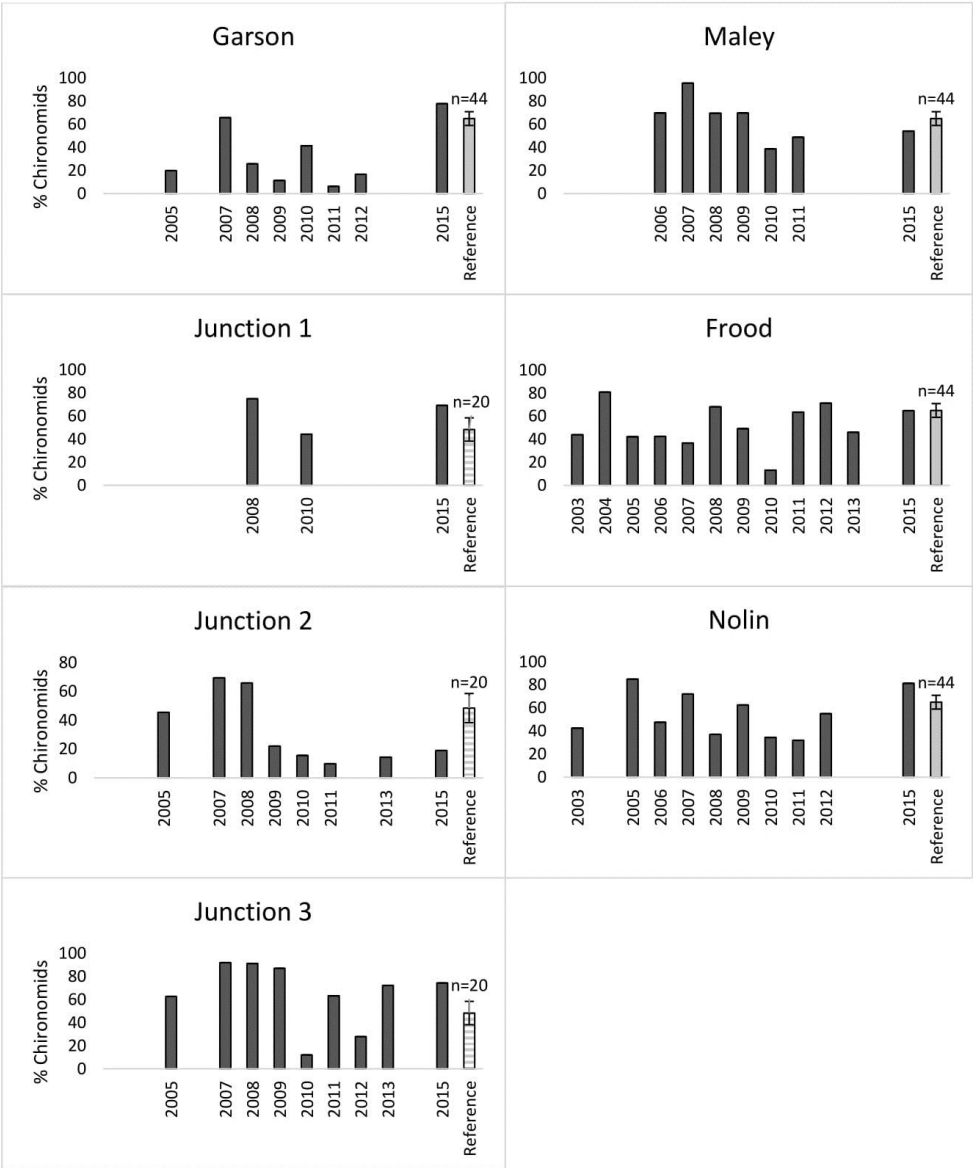

Figure 3.2 Percent chironomids at each study site over time (2003-2015). Appropriate reference group averages with 95%confidence intervals are included with the total number of sites indicated in Group 3 (n=44) and Group 4 (n=20) reference sites.

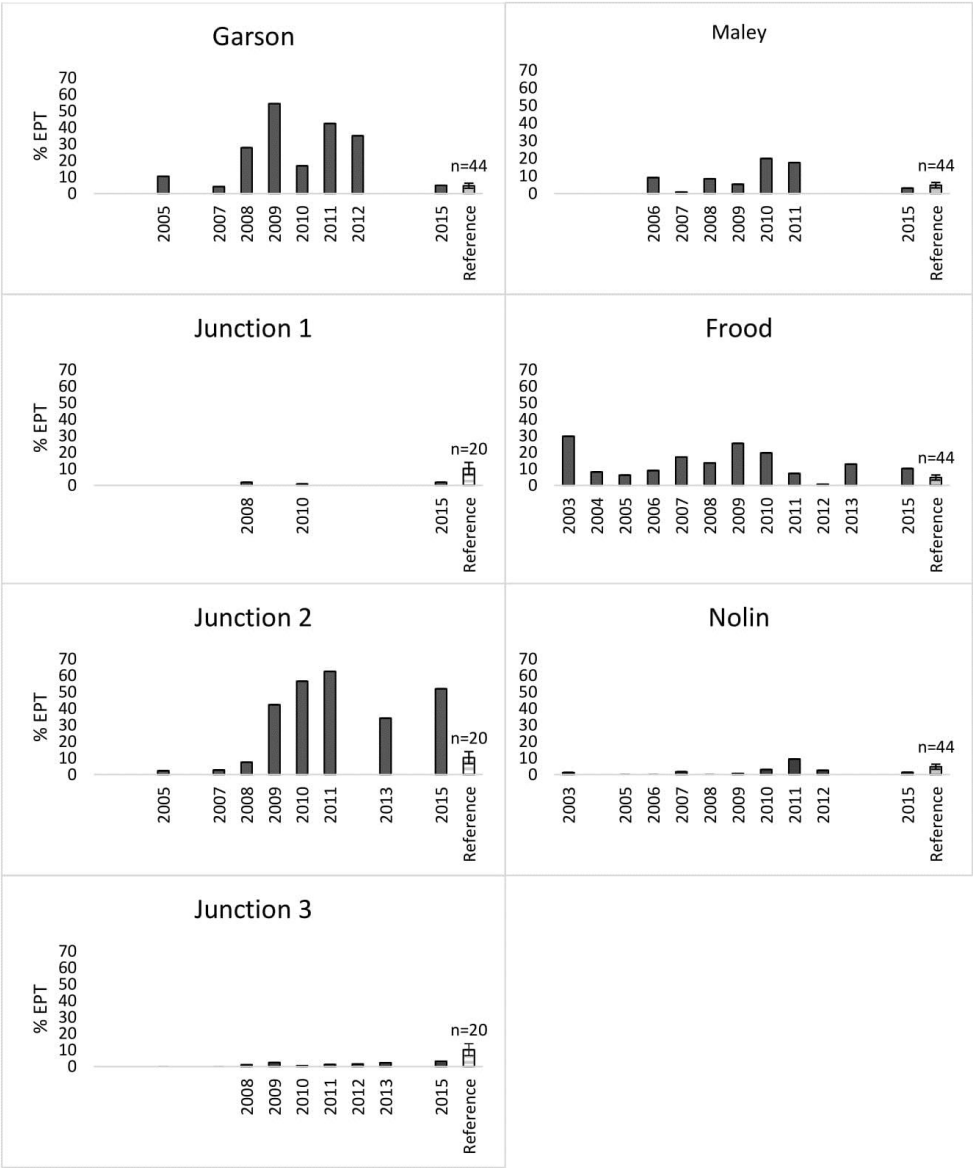

Figure 3.3 Percent EPT at each study site over time (2003-2015). Appropriate reference group averages with 95%confidence intervals are included with the total number of sites indicated in Group 3 (n=44) and Group 4 (n=20) reference sites.

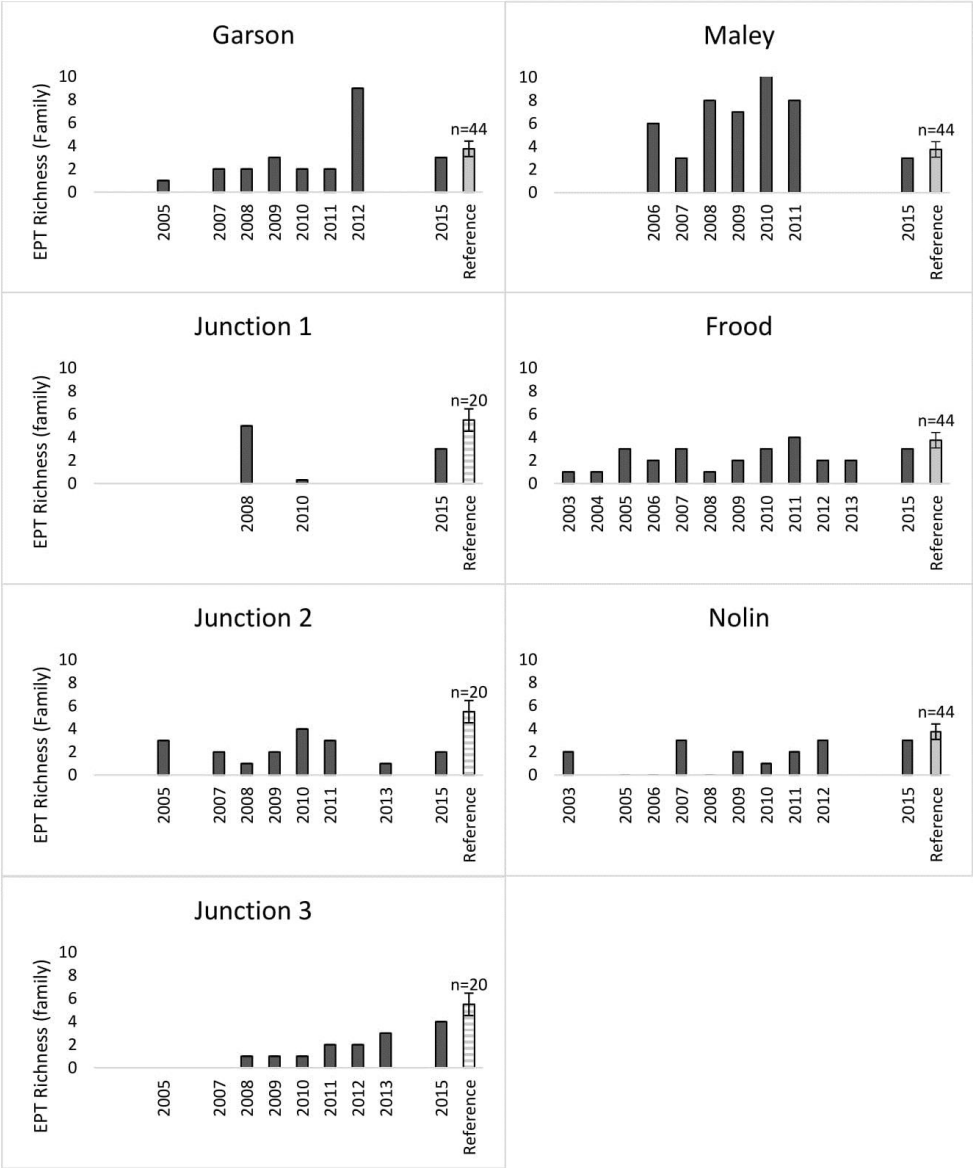

Figure 3.4 EPT Richness at each study site over time (2003-2015). Appropriate reference group averages with 95%confidence intervals are included with the total number of sites indicated in Group 3 (n=44) and Group 4 (n=20) reference sites.

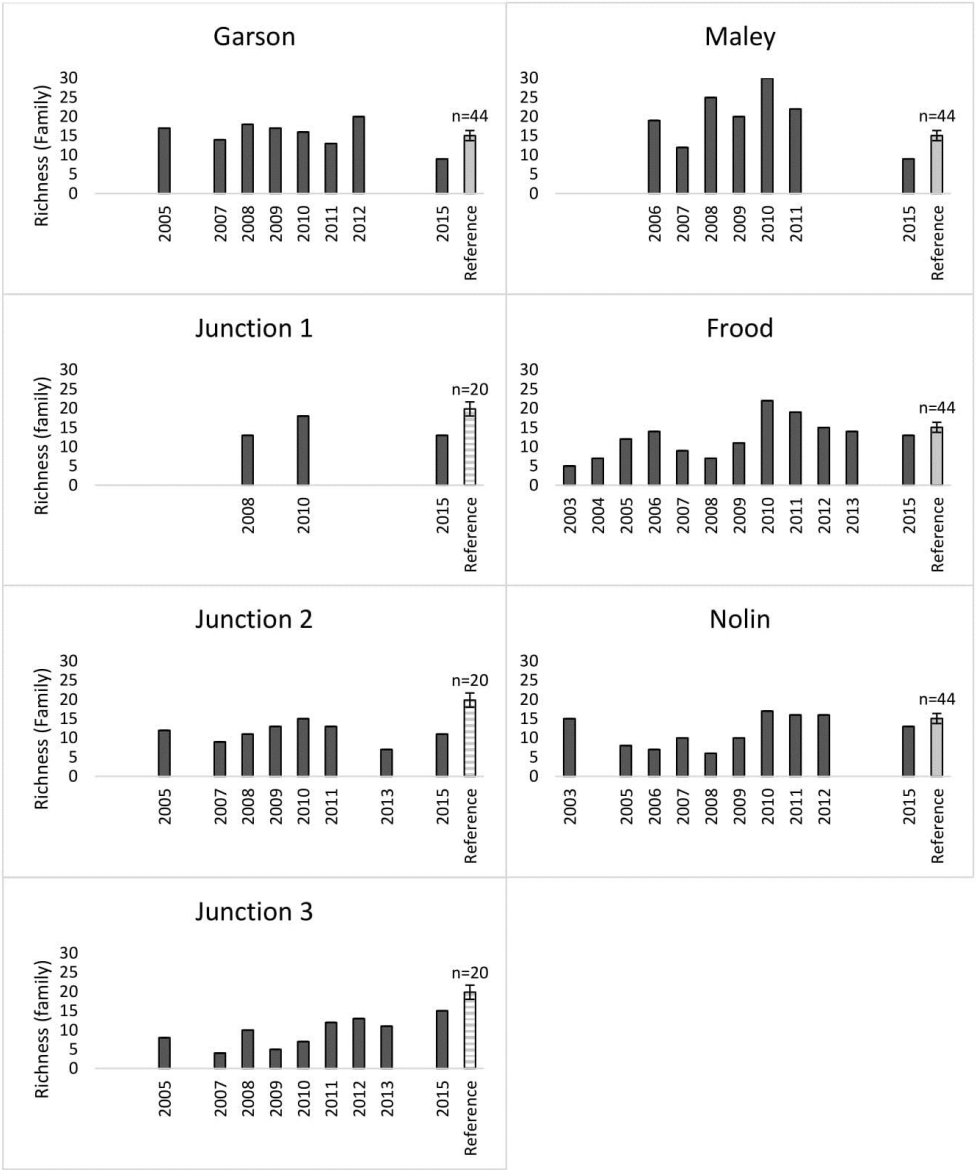

Figure 3.5 Overall richness (at the family level) at each study site over time (2003-2015). Appropriate reference group averages with 95%confidence intervals are included with the total number of sites indicated in Group 3 (n=44) and Group 4 (n=20) reference sites.

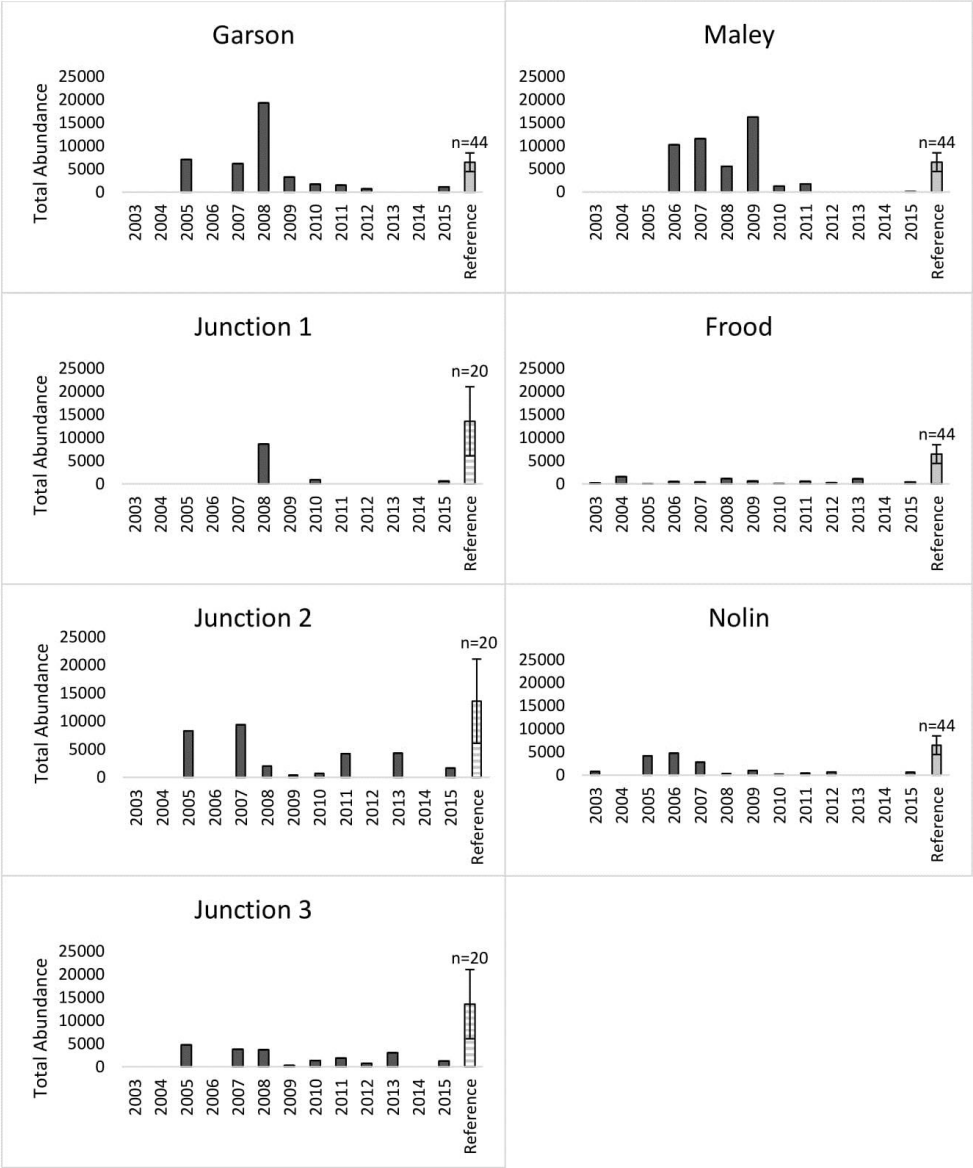

Figure 3.6 Total Abundance at each study site over time (2003-2015). Appropriate reference group averages with 95%confidence intervals are included with the total number of sites indicated in Group 3 (n=44) and Group 4 (n=20) reference sites.

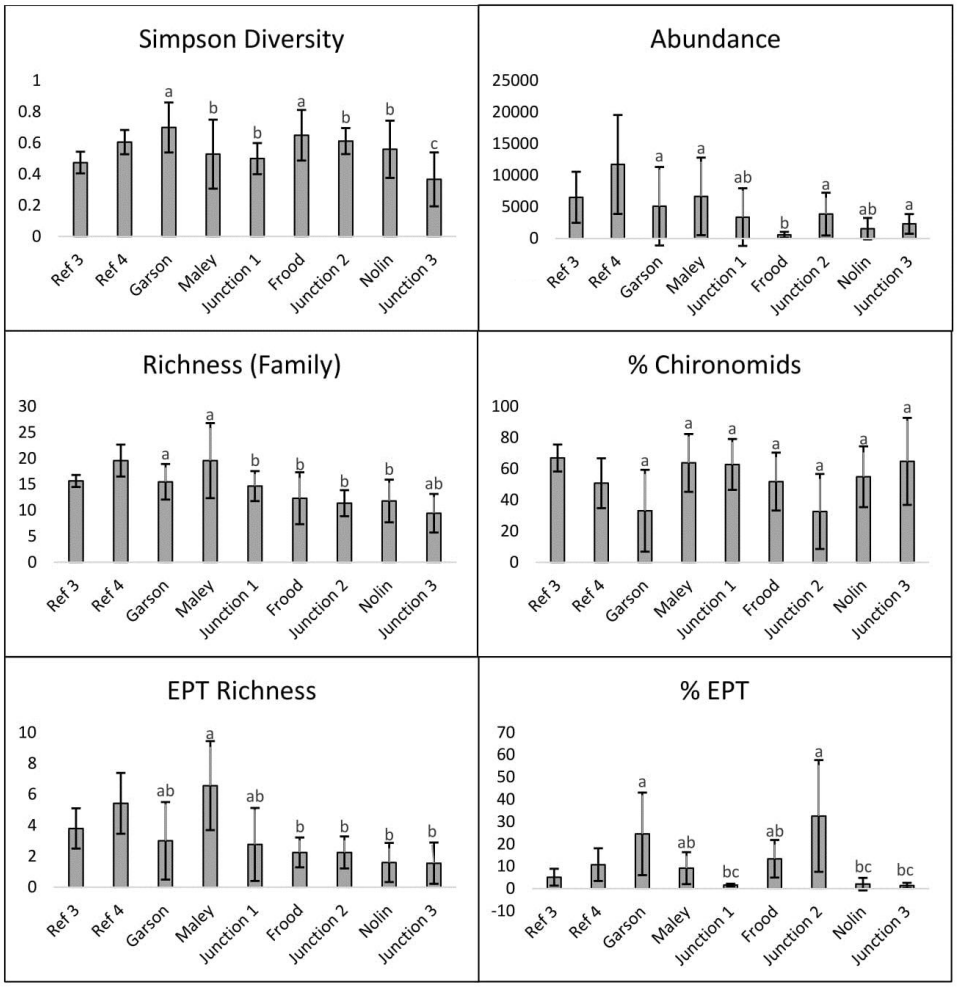

Figure 3.7 Benthic macroinvertebrate community metric averages with standard deviation by site and reference group. Test sites were grouped (a,ab,b,bc,c,cd,d) through performing a Tukeys HSD test. Test sites on tributaries (Garson, Maley, Frood and Nolin) belong to Reference Group 3 (n=44) and test sites along the main branch (Junction 1, Junction 2 and Junction 3) belong to Reference Group 4 (n=20).

3.2 Biotic and Chemical Variation Among Sites

Although linear trends over time were generally not significant, there is strong evidence of significant variation in benthic macroinvertebrate community composition, water and sediment quality among sites, the former displaying discrete differences between sites.

Copper and nickel concentrations were consistently above the Provincial Water Quality Objectives (PWQOs) of 5 µg/L 25 µg/L, respectively. Water metal levels were especially high at Nolin and Junction 3 (a mainstem site below the confluence with Nolin Creek), particularly copper (0.5-191.6 µg/L and 13-157 µg/L, PWQO= 5 µg/L), and cobalt (2.8-55.4 µg/L and 4.65-61.7 µg/L, PWQO= 0.9 µg/L), where there were fewer sensitive organisms and fewer invertebrates in general (Table 3.2 and Table 3.4). Cadmium is also high (PWQO is 0.2 µg/L) at Garson (0.10-260 µg/L), Junction 2 (0.04-450 µg/L), and Junction 3 (0.09-560 µg/L), the latter two of which also show elevated levels of aluminum (0.01-207.7 mg/L and 0.01-263.34 mg/L, Interim PWQO=75 µg/L), phosphorus (28-20200 µg/L and 20-100 µg/L, Interim PWQO= 30 µg/L) and lead (0.26-1120 µg/L and 0.2-1120 µg/L, Interim PWQO= 1-5 µg/L) in comparison with other test sites, as supported by the water chemistry PCA (Fig. 3.8).

Additionally, nickel, copper, pH and sodium seem to be driving benthic macroinvertebrate community composition within Frood and Nolin (Figure 3.8). Nickel (PWQO= 25 µg/L) and sulfate were consistently high at all test sites, except Maley (4.78-73.9 µg/L and 8.65-45.8 mg/L) which had the lowest metal concentrations of any test site. Metal and phosphorus concentrations are variable within sites, possibly reflecting seasonal flow rate changes due to major rain events and the spring freshet. Calcium is highest at Garson and Nolin, likely due to the treatment of metal mine effluent within these tributaries, with lower calcium values in Maley and Frood. Elements commonly elevated in urban environments (sodium and phosphorus) are significantly

higher at Frood, with high phosphorus concentrations also at Maley and Junction 2. Average sodium concentration was highest in Garson, although the maximum detected level was at Frood.

Table 3.2 Results of Repeated Measures One-way ANOVAs run on each community metric. Significance codes are listed at the bottom of the table. Metric averages by site are listed in rows 3-9 with 95% confidence intervals.

Metric	Signif.	Garson	Maley	Junction 1	Frood	Junction 2	Nolin	Junction 3
Abundance	**	5120.74 ± 5196.45	6679.61 ± 5679.35	3398.57 ± 11339.10	597.48 ± 294.78	3871.44 ± 2830.84	1562.12 ± 1213.27	2316.96 ± 1198.05
Bray-Curtis Distance	*	0.70 ± 0.16	0.61 ± 0.18	0.65 ± 0.53	0.82 ± 0.09	0.74 ± 0.19	0.63 ± 0.19	0.54 +/- 0.21
HBI	*	5.19 ± 1.21	4.53 ± 1.12	4.07 ± 1.46	5.23 ± 1.25	4.71 ± 1.04	6.75 ± 1.57	5.43 ± 1.74
S-W Diversity	***	0.70 ± 0.13	0.53 ± 0.20	0.50 ± 0.25	0.65 ± 0.10	0.61 ± 0.07	0.56 ± 0.13	0.37 ± 0.13
Simpson Diversity	**	1.61 ± 0.27	1.40 ± 0.58	1.07 ± 0.52	1.43 ± 0.25	1.29 ± 0.20	1.18 ± 0.29	0.83 ± 0.25
Family Richness	**	15.50 ± 2.86	19.57 ± 6.69	14.67 ± 7.17	12.33 ± 3.17	11.38 ± 2.09	11.80 ± 2.94	9.44 ± 2.85
EPT Richness	**	3.00 ± 2.10	6.57 ± 2.66	2.77 ± 5.86	2.25 ± 0.61	2.25 ± 0.87	1.60 ± 0.90	1.56 ± 1.03
% EPT	***	24.56 ± 15.47	9.19 ± 6.62	1.60 ± 1.51	13.39 ± 5.34	32.59 ± 20.94	2.03 ± 2.01	1.46 ± 0.89
% Chironomids	*	33.15 ± 21.96	63.84 ± 17.16	62.87 ± 40.57	51.88 ± 11.81	32.66 ± 20.14	54.95 ± 13.97	64.83 ± 21.48
% Filterers	***	28.63 ± 17.90	4.23 ± 4.53	0.70 ± 1.27	14.07 ± 6.33	39.3 ± 1 +/-25.64	4.10 ± 6.72	2.63 ± 1.06
% Gatherers	***	57.93 ± 22.89	82.86 ± 7.67	95.33 ± 2.74	63.47 ± 10.28	54.66 ± 27.81	81.75 ± 9.74	89.64 ± 5.74
% Predators	***	82.00 ± 11.91	76.60 ± 11.95	68.17 ± 39.30	91.89 ± 5.02	77.31 ± 11.82	95.00 ± 2.24	72.11 ± 20.62
% Scrapers	***	17.30 ± 6.94	7.23 ± 4.86	3.47 ± 5.10	10.89 ± 5.60	8.23 ± 9.12	23.61 ± 10.70	2.55 ± 1.75
% Shredders		1.63 ± 1.47	3.02 ± 1.78	1.83 ± 3.43	3.23 ± 2.96	7.64 ± 4.41	1.27 ± 0.74	2.31 ± 1.63
# Clingers	**	5.13 ± 2.77	8.14 ± 5.11	4.67 ± 2.87	3.42 ± 1.23	4.13 ± 1.13	2.71 ± 0.88	2.86 ± 1.24
Significance codes: 0 '*' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1**								

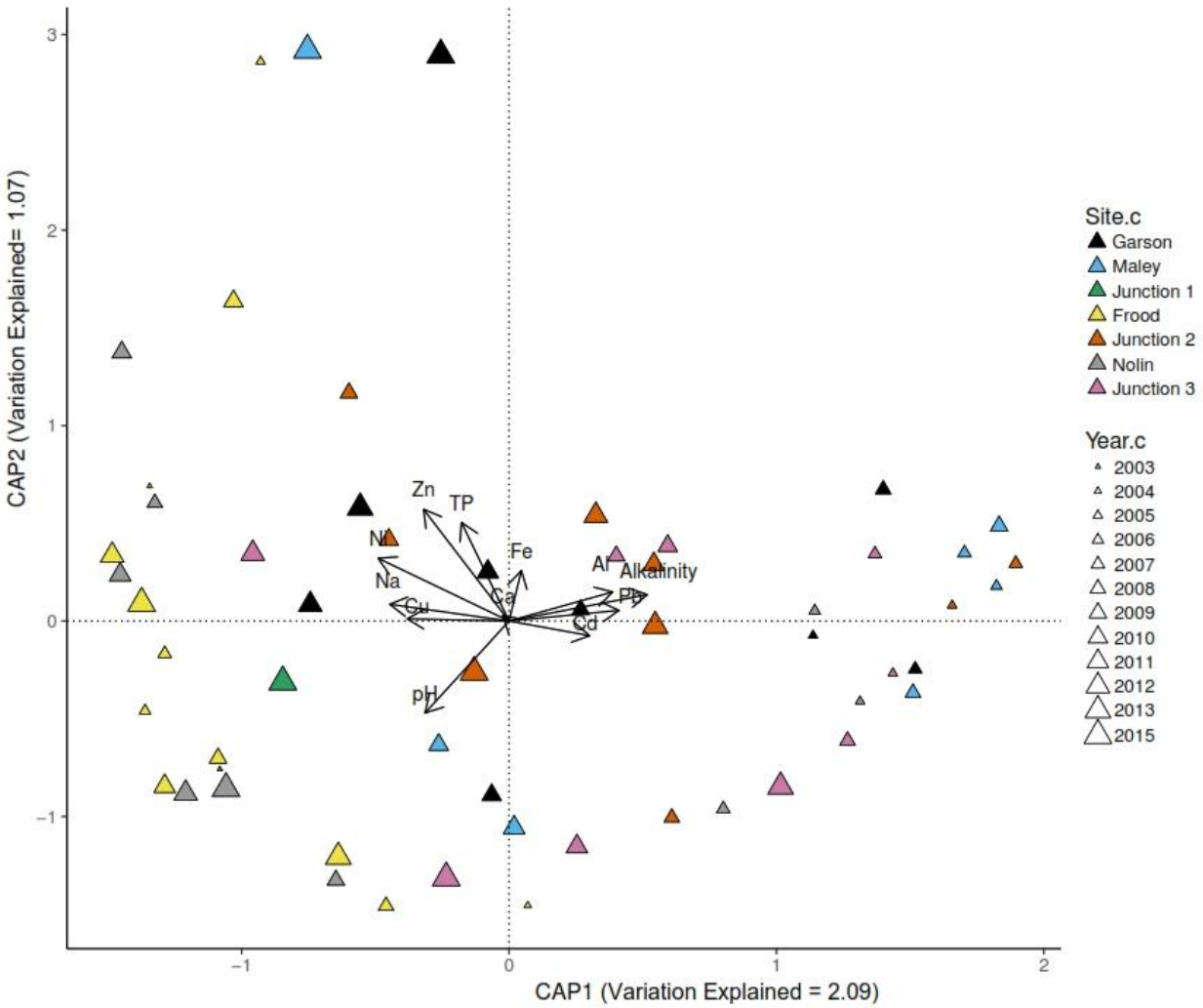

Figure 3.8 Distance-based RDA ordination of water chemistry variables analyzed in the lab. The db-RDA was performed following backwards stepwise elimination of variables using a VIF cut-off of 10. Each triangle is representative of one data point (year) per site, with colour indicative of site and size indicative of year.

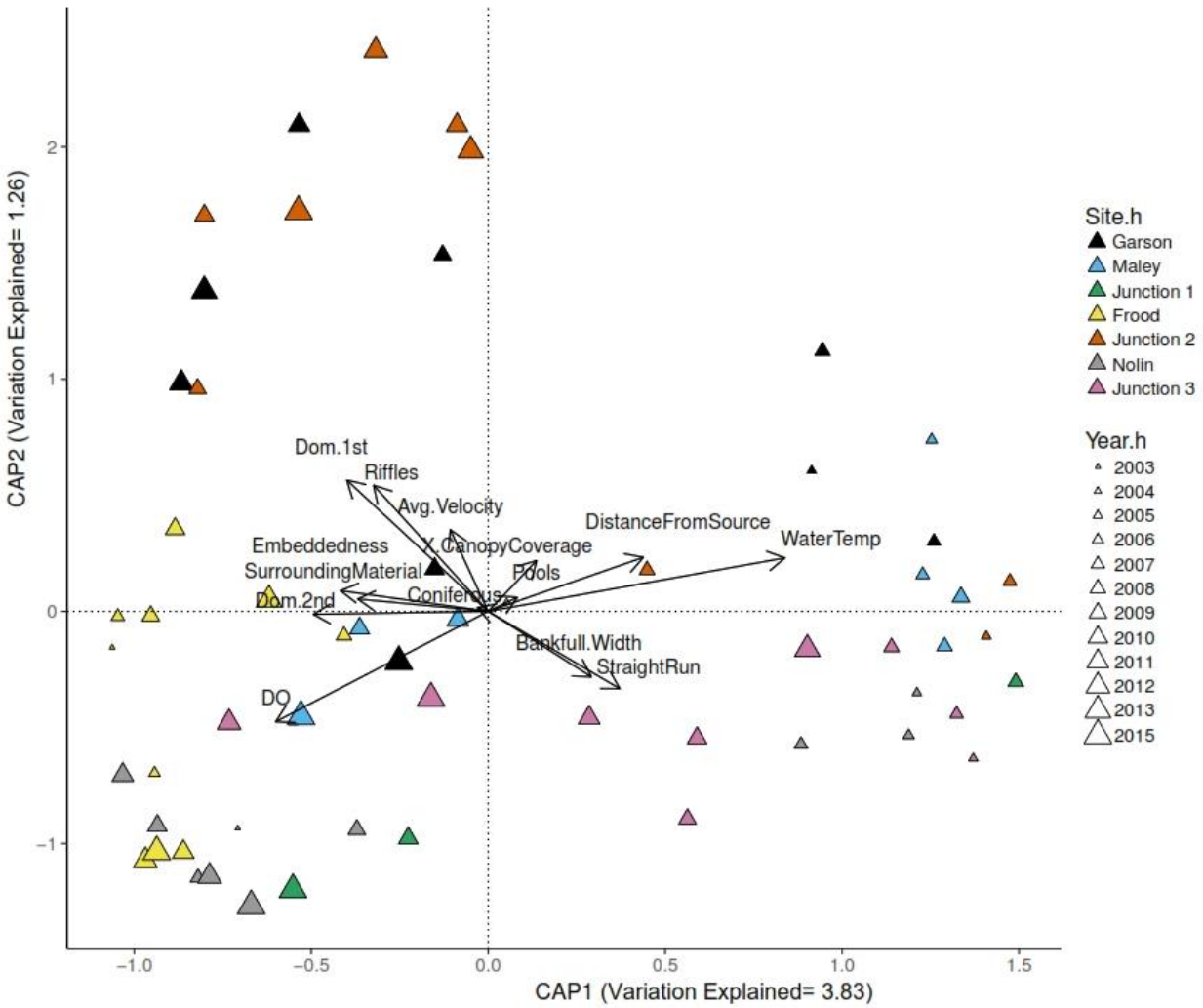

Figure 3.9 Distance-based RDA of habitat variables including DO. The db-RDA was performed following backwards stepwise elimination of variables using a VIF cut-off of 10. Each triangle is representative of one data point (year) per site, with colour indicative of site and size indicative of year.

Sediment chemistry reflects trends found in water chemistry; copper levels were above Provincial Sediment Quality Guidelines (PSQGs) Lowest Effect Level (16 ppm) at all sites and nickel levels were above the PSQG Severe Effect Level (75 ppm) at all sites, and high metal concentrations were observed at Frood, Nolin and Junction 3 (Table 3.6). Cobalt, copper, nickel, selenium and zinc levels are elevated at these sites, with copper concentrations above the PSQG Severe Effect Level, zinc concentrations above the PSQG Lowest Effect Level, and no PSQG for cobalt and selenium. Furthermore, lead levels are higher at Nolin (34 ppm) and Junction 3 (26.6 ppm), while arsenic concentration is high at Nolin (20.6 ppm), and cadmium concentrations are raised at Frood (0.78 ppm) and Nolin (2.01 ppm).

Table 3.3 2015 Sediment Chemistry Results in ppm. Note that no sediment sample was obtained from Junction 1 due to the depth (>1 m). Bolded values are above both Provincial Sediment Quality Guidelines for Metals and Nutrients Lowest Effect Levels (PSQG LELs).

Site	As	Cd	Co	Cu	Hg	Ni	Pb	Se	Zn
Garson	3.4	0.1	29.26	**62.3**	0.01	**210.3**	10.3	0.5	67.57
Maley	4.9	0.2	19.28	**44.9**	0.02	**90.3**	8.4	0.2	76.28
Frood	4.9	**0.78**	127.31	**362.3**	0.02	**656.3**	11.8	1.1	**150.97**
Junction 2	3.8	0.13	30.78	**66.4**	0.02	**97.5**	11.8	0.8	80.56
Nolin	**20.6**	**2.01**	200	**500**	0.01	**3000**	**34**	4	**336.5**
Junction 3	**6.6**	0.3	106.08	**500**	0.005	**1722.2**	**26.6**	5.7	**117.5**

Post hoc Tukeys tests showed that Frood generally had a lower abundance, % gatherers, % shredders, % scrapers and % EPT than other sites (Table 3.3). Similarly, J3 displayed significantly lower Simpson Diversity, Shannon-Wiener Diversity, richness, %EPT, % filterers, % gatherers, % predators and % scrapers. Tukeys tests revealed a higher EPT richness and # clingers at Maley; higher %EPT, % filterers, % gatherers and % scrapers at Garson; and, higher %EPT, % filterers, % predators and % scrapers at Junction 2. Junction 3 consistently has the

lowest mean for metric averages where a higher score is indicative of higher diversity and number of sensitive or specialist taxa (Shannon-Wiener Diversity, Simpson Diversity, family and EPT richness, % EPT, % filterers and % scrapers), and the highest % chironomids of all sites. Alternatively, Garson, Maley and Junction 2 have consistently high metrics associated with sensitive organisms and high diversity. Junction 1 seems to hold the most variability in community metrics, particularly abundance (Table 3.2).

Six metrics showed consistent differences between site conditions through time: Simpson Diversity, %EPT, % filterers, % gatherers, % predators and % scrapers (alpha of 0.95 in Table 3.2). These six metrics show the largest number of significant differences between specific sites (8-11), compared to the remaining metrics, which each diagnosed only 1-4 significant differences in means. There are 69 statistically significant differences between community metrics and FFG means between sites, of a possible 315 combinations (Table 3.3).

The following sites were commonly grouped together: Garson and Junction 2 (five times), Maley and Junction 2 (four times), Junction 1 and Junction 2 (five times), Junction 1 and Nolin (five times), and Junction 2 and Nolin (four times). These groupings can be seen in Appendix B.

The PCA on water chemistry (Fig.3.10 and table 3.4) found that all data points for Frood were grouped together and separated from other sites along the first axis. The relative positioning of test sites in ordination space was similar when considered alone (Fig. 3.8) and in the presence of reference sites (Fig. 3.9). In the test site only ordination (Fig. 3.8), PC1 explains 33.07% of variation and captures a decreasing gradient of calcium, potassium, magnesium and sulfate. PC2 (15.03% of the variation) is associated with increasing gradients of aluminum, cadmium and lead. Garson is grouped separately in ordination space along PC1, while Junction 2 and Junction 3 are grouped together along PC2.

With the addition of reference sites, there is less separation between test sites, but a close grouping of reference site 3 and 4 (Fig. 3.9). Garson remains closely associated with PC1, while Junction 2 and Junction 3 are grouped together along PC1.Consistent with the first PCA (test sites), in the second PCA (test and reference sites) PC1 (28.63 % of variation) also consists of a decreasing gradient in ions (calcium, potassium, magnesium, sodium), sulfate, molybdenum and lead. PC2 (18.55% of variation) consists of a negative gradient of aluminum, cadmium and lead.

Table 3.4 Principal Component Analysis PC1 and PC2 axes scores pertaining to Figure 3.14. Ordinations were performed on water chemistry parameters for all test sites, with one data point for Junction 1.

Chemical Parameter	PC1	PC2
DO	0.45627	-0.33078
Aluminum	-0.16102	1.16632
Calcium	-1.21044	-0.40631
Copper	0.40011	0.34502
Iron	0.79595	0.24601
Alkalinity	0.37908	0.07963
pH	0.53544	-0.46457
Potassium	-1.23102	-0.37039
Magnesium	-1.13094	-0.2635
Sodium	-0.72328	-0.25137
Nickel	0.14112	0.13681
Total Phosphorus	0.21027	-0.14797
Sulfate	-1.10723	-0.43953
Zinc	0.01247	0.33855
Cadmium	-0.39288	1.13207
Molybdenum	-0.91641	0.63977
Lead	-0.55586	1.17326

Table 3.5 Principal Component Analysis PC1 and PC2 axes scores pertaining to Figure 3.15. Ordinations were performed on water chemistry parameters for all test and reference sites, with one data point for Junction 1

Chemical Parameter	PC1	PC2
DO	-0.22556	0.49902
Aluminum	-0.75453	-1.20593
Calcium	-1.35941	0.56625
Copper	-0.71605	-0.09097
Iron	0.33183	-0.36180
Alkalinity	-0.24581	0.13382
pH	-0.45407	0.49447
Potassium	-1.40378	0.53337
Magnesium	-1.43176	0.45365
Sodium	-1.33977	0.40945
Nickel	-0.84437	0.17372
Total Phosphorus	-0.02655	0.18773
Sulfate	-1.26031	0.53998
Zinc	-0.73751	-0.10828
Cadmium	-0.89173	-1.19154
Molybdenum	-1.05575	-0.66248
Lead	-1.00457	-1.21642

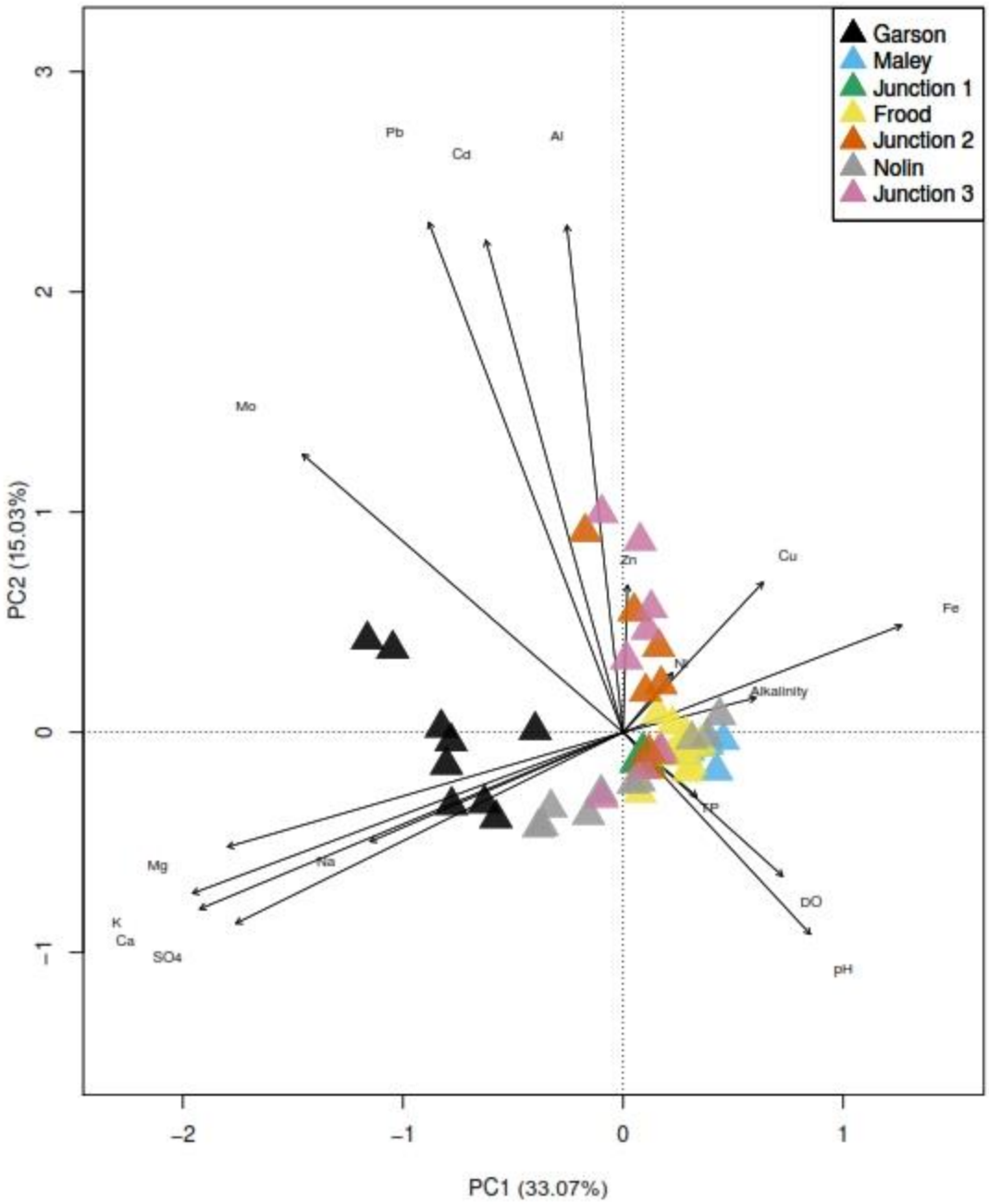

Figure 3.10 PCA of test site water chemistry data (17 parameters), with each triangle representing one data point (year). Only one data point is included for Junction 1 because water chemistry lab results exist for one year singularly (2015) despite three years of benthic macroinvertebrate sampling and in-field measurements of water parameters (DO and temperature).

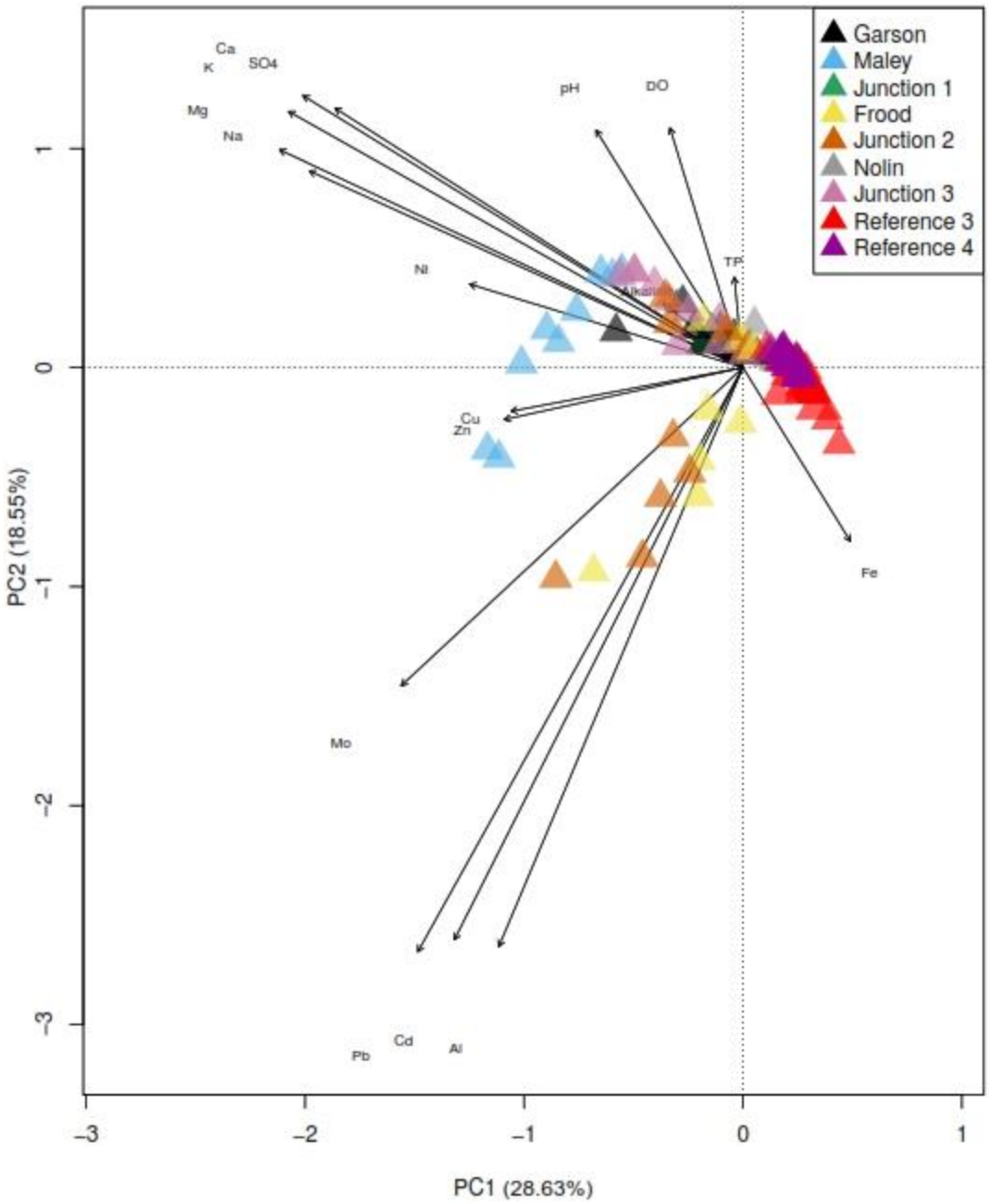

Figure 3.11 PCA of 17 of water chemistry parameters from both test and reference sites with each triangle representing one data point (year). Only one data point is included for Junction 1 because water chemistry lab results exist for one year singularly (2015) despite three years of benthic macroinvertebrate sampling and in-field measurements of water parameters (DO and temperature).

3.3 Proximity to Reference Conditions

All test site community metric results are generally similar to, or surpass, those of reference sites, except for total abundance (Fig. 3.1 – 3.7). Garson, Maley and Junction 2 typically hold similar benthic macroinvertebrate community metrics to those of the appropriate reference group conditions, and both Garson and Junction 2 community composition appear to be driven by substrate and presence of riffles (Figure 3.17). Maley was 'similar to reference' or 'mildly divergent' for all years except 2015, which was highly divergent. NMDS ordinations did not display temporal trends in any of the seven test sites, however some sites (Maley and Junction 1) did appear closer to reference condition than others. Five of nine Junction 3 sampling period community compositions are 'similar to reference', while the remaining four range from 'mildly divergent' to 'highly divergent'. The majority of samples for the remaining sites were plotted farther away from reference condition in ordination space.

Abundance at all test sites, particularly Frood, Nolin and Junction 3, puts the proportion-based community metrics into perspective. For example, %EPT at Frood in 2015 appears approximately four times greater than the average Reference Group 4 site, however the total abundance is only 1,660 compared to 12,876. Percentage of EPT at Garson, Maley, Frood and Junction 2 are all much higher than both reference groups. The trends in % EPT are not mirrored in EPT family richness, where only Maley is above reference and the average reference group values are not as low in comparison to test sites.

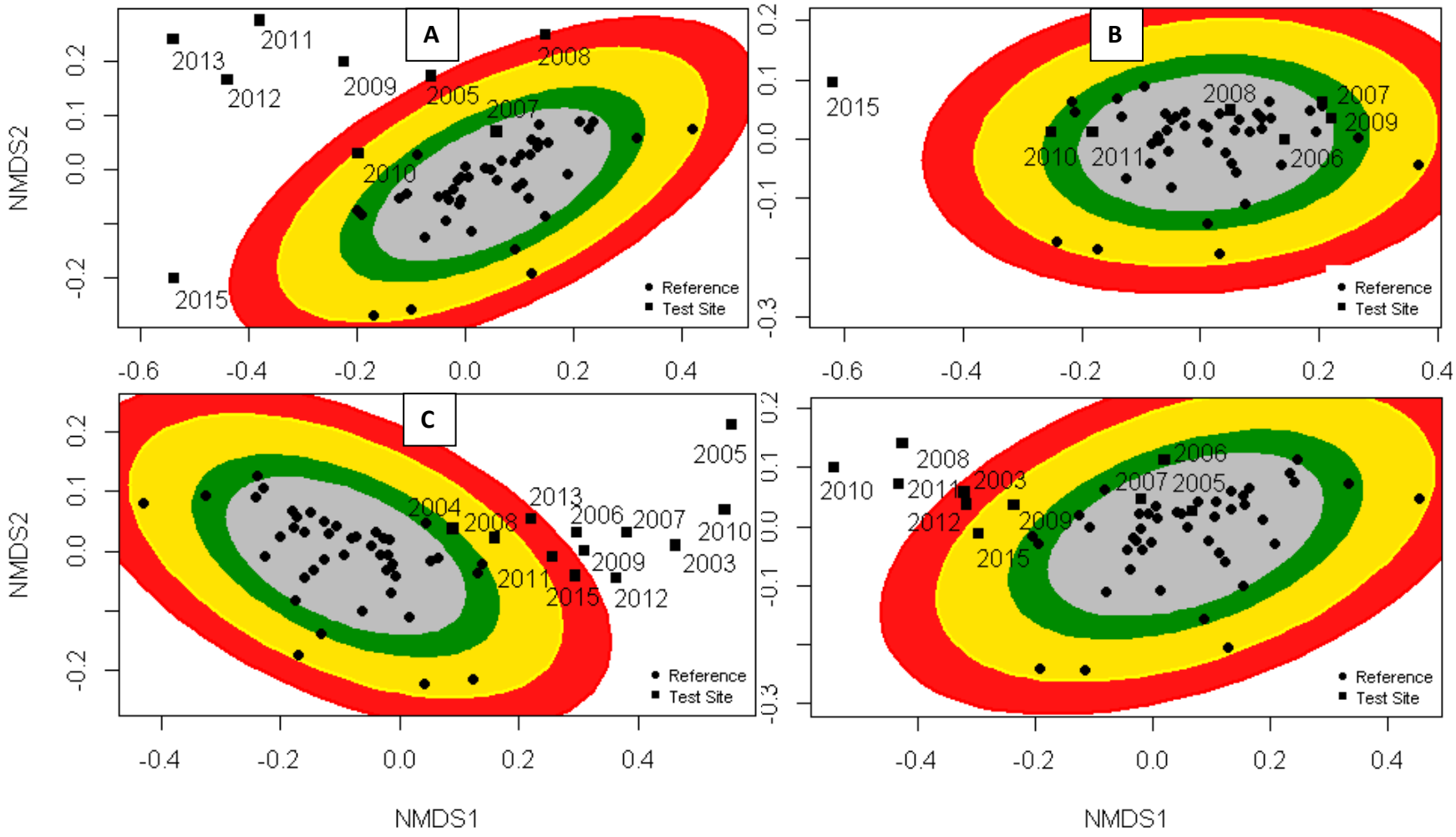

Figure 3.12 NMDSs for Reference Group 3 and individual test sites within this group, with A (Garson), B (Maley), C (Frood), and D (Nolin). Ellipses were created using reference site confidence intervals: 75% (grey), 90% (green), 99% (yellow) and 99.9% (red). Following the CABIN BEAST analysis, the coloured confidence interval ellipses are representative of communities that are 'similar to reference', 'mildly divergent', 'divergent', or 'highly divergent', respectively.

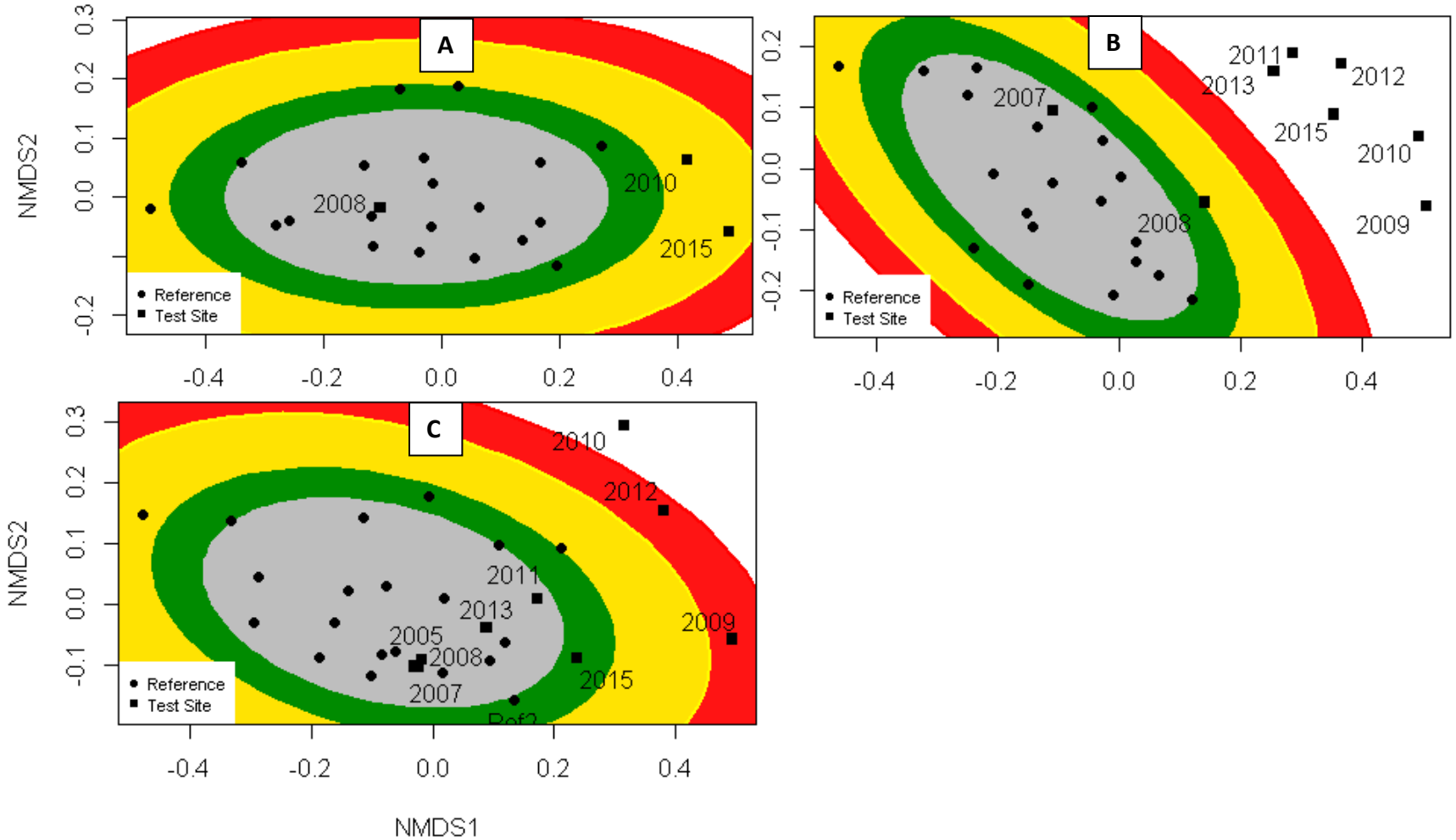

Figure 3.13 NMDSs for Reference Group 4 and individual test sites within this group, with A (Junction 1), B (Junction 2) and C (Junction 3). Ellipses were created using reference site confidence intervals: 75% (grey), 90% (green), 99% (yellow) and 99.9% (red). Following the CABIN BEAST analysis, the coloured confidence interval ellipses are representative of communities that are 'similar to reference', 'mildly divergent', 'divergent', or 'highly divergent', respectively.

4 Discussion

4.1 Temporal Trends in Benthic Macroinvertebrate Communities

Contrary to my hypothesis, the structure of benthic macroinvertebrate communities has not changed much within the 12-year study period, and no clear or significant temporal trends are apparent. However, both water quality and benthic macroinvertebrate communities have improved remarkably since the 1960's (Table 1.1). Additionally, both EPT Richness and # of Clingers were found to be increasing through time at Junction 3. This is an especially positive finding, as Junction 3 is the farthest downstream site within the study, receiving water from both the Frood and Nolin branches. Nevertheless, benthic macroinvertebrate indices measured that are positively correlated with healthy stream conditions (Simpson Diversity, abundance, richness, and % EPT) clearly place Junction 3 as consistently distant from reference conditions, although % EPT is increasing significantly.

Test site community metrics were generally found to be equal to, or above, those of reference sites, but a large range in each benthic macroinvertebrate community metric and water quality parameters was found at each test site (Figure 3.1 to Figure 3.7). With the continuation of mining and subsequent high metal levels in the sediments and water, current benthic macroinvertebrate communities may have reached a steady state, or a plateau in terms of recovery since the extreme conditions recorded in 1965. However, sites within the study area display high variability in community metrics in comparison to reference sites (Fig. 3.7), as well as fluctuating water quality parameters.

The majority of lotic recovery studies are designed to assess short-term acute events (i.e. spills) or follow the effects of management activities such as commissioning of a wastewater treatment facility or remediation of a catchment. These studies generally find that recovery of benthic macroinvertebrate communities can be rapid following a single disturbance event or treatment of effluent (Clements *et al.* 2010, Gunn *et al*. 2010), with biological conditions of sites closest to the point source improving the quickest (Cairns *et al.* 1971, Nelson and Roline 1996). In our study area, following the remediation efforts which began in the 1970's, this trend of delay with distance was in fact exhibited, and today Junction 3, the farthest downstream site of the study, is still one of the test sites farthest from reference condition. Additionally, Junction 3 is directly below the confluence of Nolin Creek with the main branch and is therefore the only test site within the study to receive this additional treated mining effluent from a creek which has been devoid of aquatic life within upper trophic levels for years. Cairns *et al.* (1971) concluded that the pace of recovery is dependent upon four factors: (1) severity and duration of the disturbance, (2) stressor number and type, (3) presence of recolonizing organisms, and (4) residual effects. A review of case studies built upon the above reasons associated with quick recovery of 'pulse' disturbance or disturbance following the implementation of pollution abatement programs to include: (1) accessibility of unaffected or undisturbed sources of colonizing organisms and refugia, (2) elevated flushing rate of lotic systems, (3) evolution of organism life history traits to adapt to such disturbances, and (4) supplementary life history traits allowing for rapid colonization and population (Yount and Niemi 1990). Contrarily, they revealed that 'press' disturbances, such as the continual addition of effluent from mining activities and urban runoff in the Junction Creek watershed, often deplete refugia, increase the

distance from which unaffected colonizers can travel, and alter the habitat both physically and chemically (Yount and Niemi 1990).

Moreover, it has been demonstrated that contaminated sediments can thwart recovery of lotic systems, sometimes for decades or more (Wallace 1990, Clements *et al*. 2010, Yount and Niemi 1990, Tolbert and Vaughan 1979, Soulsby *et al.* 1995, Besley and Chessman 2008). One study suggested that it was the saturation and subsequent mobilization of metals in Sudbury overburden and soil that sustains the high levels of metals in aquatic systems for a long period of time (Nriagu *et al.* 1998), while another proposed that acidity released from acid mine drainage-contaminated sediments would have toxic effects on benthic macroinvertebrate communities (for a long period) perhaps with effects more severe than the metal in the sediments (Dsa *et al.* 2008). There are also suggestions that chronic metal pollution can create a metal-tolerant benthic macroinvertebrate community that is sensitive to low pH (Courtney and Clements 2000) and reclamation efforts (Chadwick and Canton 1985). It has previously been proposed that absence of sensitive biota may be directly related to high toxicity of sediments within Junction Creek (Jaagumagi and Bedard 1999).

Other factors that delay recovery include impediments to dispersal and recolonization, due to drought, major rain events, pulse events, or surrounding land use types. Within the Junction Creek watershed, water quality has already been demonstrated to be adversely affected by land use and road density (Strangway 2015), while low permeability and a high amount of barren bedrock has been demonstrated to be negatively associated with benthic macroinvertebrate diversity, and positively correlated with increased levels of metals such as Ni and Cu (Davidson and Gunn 2012). In lotic systems it can also be difficult to detect recovery in the face of high seasonal or climatic variation (Clements *et al*. 2010, Niemi *et al.* 1990).

However, this issue may not lay with accounting for all this variation but perhaps with our expectations of what a benthic macroinvertebrate community should look like when we directly compare test sites to pristine locations or historic conditions.

Rather than compare current benthic macroinvertebrate communities to those of pristine locations, it may be more appropriate to acknowledge that the stream communities will not conform to those at remote locations as long as mining is active and urbanization continues to expand within the watershed. It has been suggested that interim types of benthic macroinvertebrate communities exist for the duration of a toxicants presence (Cairns *et al.* 1971) and that this altered community structure can be due to selection of favourable genotypes due to this presence or changes in physical habitat (Wallace 1990). Similarly, the disturbance hypothesis dictates a competitive hierarchy of species, where colonizing species dominate a system, eliminating resident species if disturbances are frequent enough to influence the original species composition (Resh *et al.* 1988). This absence of resident species lowers the overall diversity and richness, however, a disturbance regime that is intermediate in intensity and frequency, may allow both resident and colonizing species to remain in the ecosystem, producing a maximum species richness. This theory may account for the high taxa richness at all test sites (except Junction 3), which was found to be similar to that of reference sites (Fig. 3.1, Fig. 3.5 and Fig. 3.7), yet consistently lower abundance (Fig. 3.6 & Fig. 3.7) (Resh *et al.* 1988). Alternatively, the current macroinvertebrate communities may be representative of a 'Novel Ecosystem', in which both the abiotic and biotic conditions have been altered from those historically present, or a 'Hybrid Ecosystem', in which only abiotic or biotic conditions have been altered (Hobbs *et al.* 2009). With these types of systems gaining more attention recently, especially related to climate change, the traditional idea of management within a historical range

of variability may not be possible. Allison (2012) suggests two additional potential approaches for managing these systems: (1) use current and projected climate change to predict future conditions and what type of species will be best suited for them, and (2) actively use restoration to mitigate effects of climate change.

4.2 Selection of Reference Sites

Metrics that are indicative of healthier benthic macroinvertebrate communities were unexpectantly higher at many of the test sites than the references sites (Figures 3.1 to 3.5), which suggests we need a more in depth look at the reference sites selection process, as this is fundamental to any freshwater biomonitoring programs and employment of the Reference Condition Approach (Yates and Bailey 2010). Reference sites used in this study were part of the CABIN Near North 2016 model, which uses eleven habitat predictors to classify test sites against a modeled reference group: longitude, stream order, intrusive (%), metamorphic (%), sedimentary (%), precipitation in March, precipitation in November, temperature in January (min.), temperature in September (min.), temperature in October (min.) and drainage area (Novodorvsky and Bailey 2016). The number of environmental predictors was limited because of potential impacts that regional disturbances (i.e. Agriculture, logging, mining and urban) could have on them, with only 63 candidate predictors available of an original 148 environmental variables. It is possible that test sites may have been assigned to different reference groups if more habitat variables were included in the model. This possibility seems likely since only stream order and drainage area seem to have dictated the groupings, Group 4 having a larger mean stream order and drainage area (Novodorvsky and Bailey 2016). Because all test sites are within the City of Greater Sudbury, the remaining nine habitat variables should be similar among test sites. Additionally, Reference Group 3 and Group 4 have the lowest

average taxa richness and Bray-Curtis distance, which is driven by similar, and mostly tolerant, benthic macroinvertebrate taxa (Novodorvsky and Bailey 2016). All three test sites on the main branch of Junction Creek were assigned to Reference Group 4, which exhibits the highest average abundance of invertebrates (13,087, ranging from 903 to 41,300).

Because of Sudbury's unique geologic landscape, local 'least disturbed' sites within the Greater Sudbury region may provide a more logical reference condition with which to compare the Junction Creek test sites against. In order to encompass the habitat variability within the study area, it might be necessary to incorporate locations that contain naturally occurring low levels of copper- and nickel- rich sediments of the area, which is a signature of the ore deposits that created the mining activity in Sudbury in the first place. Examples include the reference sites used in Jennifer Davidson's 2002 M.Sc. thesis, titled "Applying the Reference Condition Approach to Monitor Invertebrates in Streams of the Sudbury Mining Area". Additionally, more local reference sites would account for the higher levels of SO_4, which is ubiquitous within the Sudbury landscape and incomparable to majority of regions. SO_4 concentrations in water were documented as being elevated as far as 140 km from Sudbury (Keller and Pitblado 1986). It is of course preferable to use reference sites form the local region that are as close as possible to "near-pristine" and this may be better done by including landuse activity data in the reference site selection process (Yates and Bailey 2010).

Two other ways of creating reference conditions when historic data is absent include: 1) 'hindcasting' the reference conditions (Kilgour and Stanfield 2006) or 2) determining them through adopting a paleolimnological approach (Thoms et al. 1999). Since the identification of local reference sites in 'near pristine' or 'least disturbed condition' can be challenging (Kilgour and Stanfield 2006, Yates and Bailey 2010) or local reference sites may not be abundant enough

to employ the RCA (Bailey *et al*. considers 25 reference sites to be the absolute minimum number sufficient for any study (2004)) Kilgour and Stanfield created models that can 'hindcast' the baseline condition of a stream using relationships between biophysical condition and landscape variables, particularly Percentage of Impervious Cover (2006). Likewise, Thoms et al. used paleolimnology to establish the historic physical/chemical/biological condition of a lowland floodplain river system in Australia, where it is also often hard to find reference sites (1999).

Because the majority of Ontario streams that are tributaries to Lake Ontario cannot be classified as 'minimally disturbed' or pristine, it is challenging to find enough sites with similar environmental variables to use the classic RCA approach (Kilgour and Stanfield 2006). Additionally, comparing test sites to reference sites within a different geographic location applies the inherent assumption of the RCA that sites consisting of the same habitat characteristics, or architecture, will hold the same ecological characteristics and communities. Essential environmental characteristics, such as sediment type and size, flow, depth and presence of riparian cover are not built into CABIN models because they are not fixed. When comparing these characteristics between reference sites in Group 3 and 4 and the seven test sites compared to them, major differences exist, some of which were found to be driving benthic macroinvertebrate communities in the db-RDA.

Utilizing one single reference database may have been effective elsewhere (Wright 1995), but in a location as unique as Sudbury, home to one of the largest copper deposits and mining complexes in the world, the Sudbury Igneous Complex, using reference sites from the pristine Far North may be inappropriate. As there are numerous CABIN reference models, and Wright suggests that different locations in the world may need numerous systems to maximize the fit between habitat characteristics and biological communities (1995), Greater Sudbury may

need its own reference model to correctly match reference and test sites. Furthermore, when comparing the efficiency of seven (established and novel) RCA methods to correctly classify 'validation' sites as being in referenced condition, Bailey *et al*. (2014) found that BEnthic Assessment of SedimenT (described in Reynoldson *et al.* 1995) approach, which is embedded in the CABIN protocol, had the highest rate of Type I Errors and consistently low statistical power. It was suggested that newer methods be integrated into current RCA programs (CABIN in this case) to develop more accurate predictive models, capable of better matching reference and test sites (Bailey *et al*. 2014).

4.3 Biotic and Chemical Differences between Sites

Variation in benthic macroinvertebrate communities among sites is directly reflective of water and sediment chemistry. Frood, Nolin and Junction 3 tended to have the lowest abundance levels of all test sites (Fig. 3.6), while Nolin and Junction 3 had lower % EPT (Fig, 3.4) and overall richness (Fig. 3.5). These three sites also had higher concentrations of cobalt, copper, and nickel concentrations in water (Table 3.3). Sediment chemistry results similarly show that zinc concentrations are above the Provincial Sediment Quality Guidelines for Metals and Nutrients LELs at all three sites, with arsenic and lead both surpassing the guidelines at Nolin and Junction 3, and cadmium surpassing it at Frood and Nolin (Table 3.6). Copper and nickel sediment concentrations surpassed the guidelines for all sites. High contaminant levels and low benthic macroinvertebrate community metrics indicative of an impaired system may be explained by cumulative impacts due to the lower locations of these sites within the study and the addition of surface runoff from mining operations that enter Nolin Creek and past AMD entering Frood (Cairns *et al.* 1971). Despite the AMD diversion and subsequent rapid recolonization in 2001

(Gunn *et al.* 2010), evidence suggests that the benthic macroinvertebrate community at Frood is still impacted although community metrics are generally still higher than those of Nolin and Junction 3 (Fig. 3.7). It may still take decades to see further changes in the benthic macroinvertebrate communities within these sites, as it has been revealed in a similar system that benthic and fish population measures did not recover to pre-industrial levels until 23-29 years following the cessation of mining activities (Tolbert and Vaughan 1979).

Maley and Garson tributaries appear to be in the best biological shape, which is intuitive based on the facts that Maley does not receive direct effluent from mines and the both test sites are upstream of the highly urban remainder of the watershed. Additionally, benthic macroinvertebrate metric regressions indicated that Junction 2 is moving closer to reference condition and displays additional recovery indicators including higher average %EPT and low % chironomids (Figure 3.7). The reasons for these trends may be due to habitat variables, such as presence of riffles, substrate type, riparian cover and presence of erosion control measures, rather than chemical parameters. For example, dominant 1st substrate, presence of riffles and average velocity seem to be driving the benthic macroinvertebrate communities of both Garson and Junction 2 in later years (Figure 3.18). Additonally, Junction 1 appears to be moving farther from reference condition through time, but this may be due to a lack of data; Junction 1 was sampled only three times, making it difficult to assess temporal trends within the site. High within-site variability of community composition also makes teasing apart temporal trends and comparing to reference condition difficult.

4.4 Additional Sampling Parameters and Study Design Limitations

There were inherent limitations in this study's ability to assess temporal changes, because of the limited choice of pre-existing study sites with sufficient data and the need to maintain a consistent CABIN protocol. Maley tributary has often been thought of us a reference watershed and was used a reference site within an earlier 2008 study (Weber *et al*. 2008), however it contains both a golf course and the historic Kirkwood Mine. The test site on the Maley tributary included in this study, and an additional monthly water sampling site, are both downstream of these potential point sources. Similarly, there is a regularly sampled test site immediately upstream of Kelly Lake far down in the watershed that might have been a good cumulative affects site, but unfortunately it is just below the input of both Copper Cliff Wastewater Treatment Plant, which makes it impossible to use to discern the effects of the changes over time in the upper watershed that was my focus.

Additionally, other studies documented concentrations of metals in Junction Creek water increase along a gradient downstream (Weber *et al.* 2008; Jaagumagi and Bedard 2002). As close as 60 days following an acid spill in the Clinch River, that killed all invertebrates within 11.7 miles downstream, Cairns *et al.* (1971) detected community diversity and presence of representative species comparable to that prior to the event.

Inclusion of organisms from differing trophic levels, such as microbes, periphyton and fish, may provide more information regarding the Junction Creek food web and potential reasons for the low abundance and diversity within benthic macroinvertebrate communities within future studies. Additionally, continuation of sediment sampling and metal analysis would provide temporal data for future studies to assess trends and relate them to those seen in benthic macroinvertebrate communities.

Metal bioavailability studies in benthos may also be useful with some earlier studies of fish (fathead minnows) suggesting that these metals in Junction Creek are readily taken up by the biota (Jaagumagi and Bedard 2002). Metal body burdens of creek chub and fathead minnow residing in Junction Creek were also studied by Weber *et al*. (2008), who found that multiple effluents from municipal and mine WWTPs did not seem to be affecting fish in a cumulative manner, with fish body burdens actually lower than expected partially due to high levels of ammonia and sulphate rendering metals as less bioavailable. Further, benthic macroinvertebrate metal body burdens could provide insight into the bioavailability of these metals, following a similar procedure to Cain *et al*. (2000), who performed the analyses on *Hydropsyche californica*. They discovered that the organisms contained elevated concentrations of some metals further downstream (120 km) than did the sediments.

4.5 Detecting Temporal Trends using a Multivariate Approach

Difficulty quantifying and evaluating recovery is typical in lotic environments (Clements *et al.* 2010, Niemi *et al.* 1990). Reasons for this include lack of pre-disturbance or pre-treatment data, and difficulty pinpointing the effectiveness of treatment efforts from natural variation, seasonal variation, weather events, climate and seasonal change, numerous urban influences (Clements *et al.* 2010, Niemi *et al.* 1990) and influences from contaminants that may even be present once the contaminant itself has been removed (Matthews *et al.* 1996). The path to recovery does not appear to be entirely linear (Figures 3.12 &3.13), as water quality is often improved immediately, followed by rapid recolonization of some benthic macroinvertebrates, but often delays in the recovery of overall community composition and abundance (Clements *et al.* 2010, Langford *et al.* 2009, Hoiland *et al.* 1994, Murphy *et al*. 2014). In fact, it has been suggested that

water chemistry variables must reach levels lower than the applicable thresholds before benthic macroinvertebrate communities can recover, and communities may still not be reflective of improved water quality until many decades later (Langford *et al.* 2009).

The biological and water chemistry data used in this study spans approximately 13 years, which may not be long enough to detect trends in benthic macroinvertebrate communities. Additionally, any benefits from restoration efforts (tree and shrub plantings and erosion control) are impossible to tease apart from confounding factors or potentially to even see within this length of time. Niemi *et al.* (1990) suggest that studying the effects of relatively discrete stressors within smaller systems may be the only possible avenue to evaluate recovery, and that examining the impacts of disturbance within systems impacted by mining activity is especially difficult due to a long trajectory to recovery.

4.6 Detecting Temporal Trends using Benthic Macroinvertebrate Community Metrics

Trends found using multimetric and multivariate approaches differed substantially; however, significant temporal trends over the 13 year study period were not detected using either method. In addition to measures of taxonomic recovery of benthic macroinvertebrates present, metrics that provide insight into the ability to survive (taxa density) and thrive (abundance) provide a measurement of functional recovery (Walter *et al.* 2012). In support of this, both measures were higher for Maley and Garson than other test sites, and lowest for Frood, Nolin and Junction 3 (Fig.3.7). Additionally, richness was lowest for Frood, Junction 2, Nolin and Junction 3 (Fig. 3.7), with Frood and Nolin generally grouped together in ordination space (Fig.315- Fig. 3.18). Furthermore, it was made obvious within this study that abundance differed greatly between the

test and reference sites (Fig. 3.6 and Fig. 3.7) while other metrics were surprisingly similar (Fig. 30.1- Fig. 3.7). However, it is possible that levels of ions and nutrients founds in urban environments may be playing a part in benthic macroinvertebrate community composition and abundance.

Niemi *et al.* (1993) determined the sample size necessary to determine numerous percentages of difference (5%, 20%, 40%, 60% and 80%) in benthic macroinvertebrate communities and found that % chironomids, generic richness and number of functional feeding groups present required the smallest sampling size, whereas total density of macroinvertebrates or chironomids required an unrealistic number of samples. Individual studies would therefore have to take cost of the sampling into consideration when determining methods and study design, as well as taxonomic resolution. It has been suggested that both multivariate and multimetric approaches be taken together to assess water quality using benthic macroinvertebrates (Reynoldson *et al*. 1997). The addition of functional indicators such as those included in the New Zealand Ministry for the Environment's 'Functional Indicators of River Ecosystem Health- An Interim Guide for Use in New Zealand' (2004) would be beneficial as they provide an integrated and complete picture of stream health beyond the presence of specific Functional Feeding Groups. Furthermore, it is suggested that numerous biological and chemical endpoints are necessary when assessing recovery of lotic systems due to their complex and variable nature (Adams *et al.* 2002, Walter *et al.* 2012).

4.7 Management Recommendations and Future Implications

Monitoring of long-term recovery in Junction Creek benthic macroinvertebrate communities following major changes in mining wastewater treatment and emissions reductions in the 1970's has shown that: (1) initial recovery quickly followed these treatment upgrades, but communities have had a relatively consistent composition throughout the most recent decade; (2) it is unlikely that benthic macroinvertebrate communities will become closer to reference conditions if the current water and sediment quality remain unchanged; and, (3) habitat differences among sites seem to be driving community composition rather than time, suggesting that they may play a larger role in driving community composition than initially thought. Additionally, the reference sites used in this study may not have been the most appropriate. It is evident that major manipulations of the study system and local contributions of point-source pollution are necessary to catalyze further recovery of the benthic macroinvertebrate communities within Junction Creek through increasing water quality. This is not to discount smaller-scale local restoration measures such as tree planting, which can increase the riparian area and improve soil stability. However, it may not be possible to statistically quantify or tease out the positive effects that such measures have had on the benthic macroinvertebrate communities.

Because Sudbury will remain a city centre and mining town for the foreseeable future, we know that treated effluent will continue to enter the stream and reaching PWQOs in sediments may not be attainable in the near future. At this time, I therefore recommend that sediment metal levels be incorporated into regular sampling programs to obtain temporal data and test whether sediment contamination is a major driver of benthic communities, impeding their recovery, as

found in other studies (Clements et al. 2010, Niemi et al. 1990, DeNicola and Stapleton 2002, Dsa et al. 2008 and Sullivan 2010).

Secondly, there may be additional reasons for the variability and delayed recovery of benthic macroinvertebrate communities in Junction Creek that have not yet been studied specifically. These possible reasons and factors include: level of streambed disturbance, lack of riffles, particle sizing, substrate instability and loading, and erosion. Water and sediment quality may also not be the sole factors inhibiting the recovery of benthic macroinvertebrate communities in Junction Creek, but further research regarding habitat factors will have to determine the feasible next steps taken towards its recovery. It may still be possible for the benthos of Junction Creek to obtain reference conditions if those conditions are based off local reference sites, or are instead based off baseline conditions revealed through modelling or paleolimnology (Kilgour and Stanfield 2006, Thoms 1999). Alternatively, formation of a long-term monitoring plan with specific restoration endpoints representing percentage of the reference condition metrics, such as with Silver Bow Creek (Sullivan 2010) make be more realistic and take account of the naturally high levels of copper, nickel and cadmium within Greater Sudbury.

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Appendix A: Study Site Locations and Photographs

Figure A1 Garson test site location, downstream of Garson Mine and within a residential community of Garson. Upstream of the site is a wetland, which receives treated effluent from Garson Mine. Prior to the wetland, the effluent is treated by the addition of lime and a polymer within a clarifier; precipitation of metals into a settling pond; running through a polishing pond, and; receiving, CO_2 through bubbling to adjust the pH. The site periodically floods due to beaver activity, despite controlled release of effluent from the mine. Samples are taken within the short reach between the wetland and twin corrugated steel pipes, which outlet approximately 300 meters downstream, at Birch Street. The Garson site has been sampled using the CABIN protocol since 2005 and serves as the headwaters of Junction Creek. Below this site, Garson Branch contains groundwater sources and potentially receives additional nutrients from Cedar Green Golfcourse.

Figure A2 Maley test site location is situated within a residential area near the end of the Maley Branch. When sampled, the urban influence was obvious from the number of bicycles and other large debris. Substrate of the study reach mainly consists of large cobble and large pebbles, surrounded by clay silt. Mining effluent does not directly enter the Maley Branch, but it does receive atmospheric deposition of SO2 from the mining stacks, as all waterbodies do within Greater Sudbury. The tributary may receive additional nutrients from Timberwolf Golfcourse, which is Northwest of the study site, and potential legacy pollutants from mine explorations sites and/or the historic Kirkwood Mine, closed in 1976. Water levels are controlled upstream by two dams. This site has been sampled using the CABIN protocol since 2006.

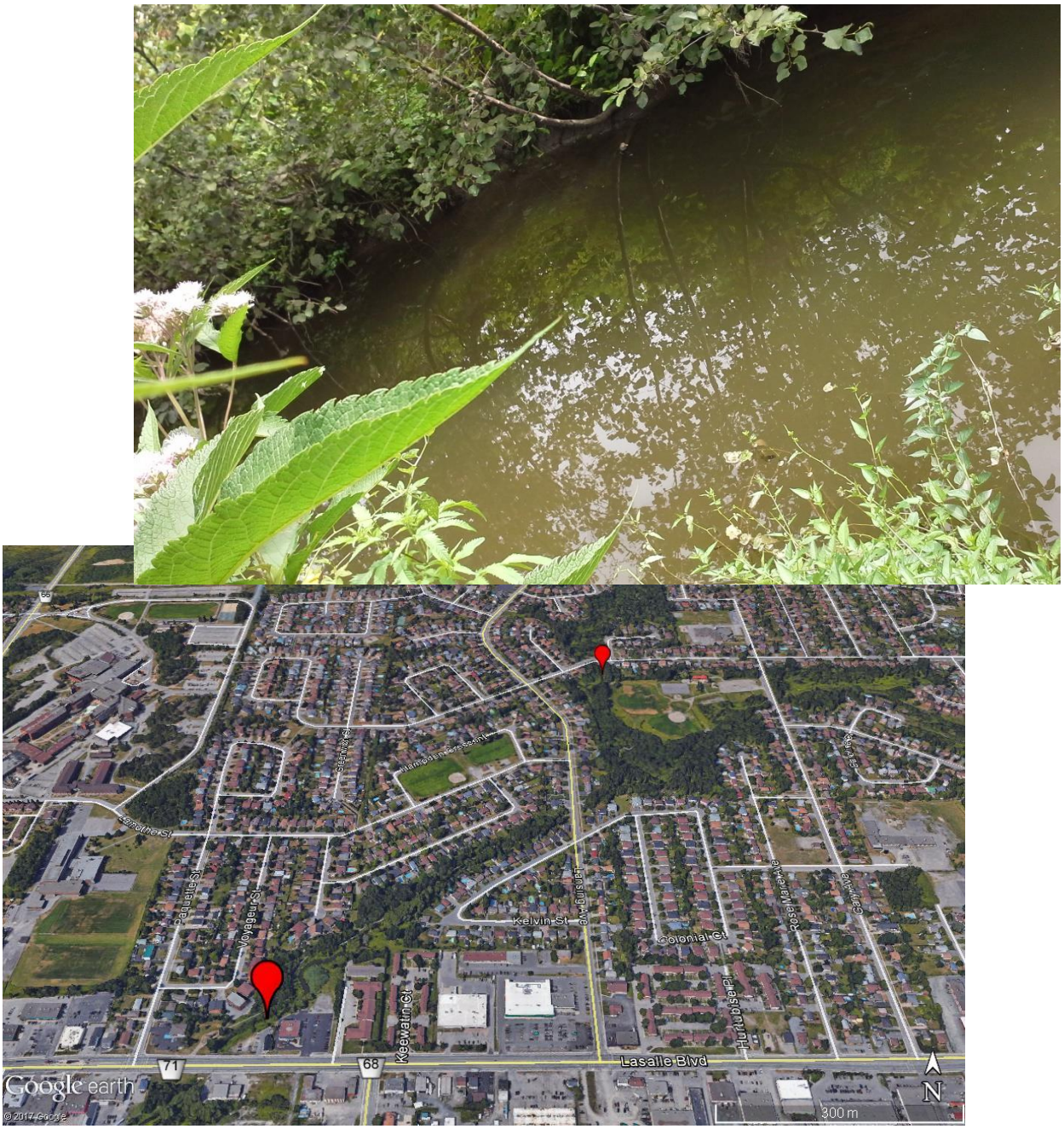

Figure A3 Junction 1 test site location, with Maley represented by the smaller symbol in the background. Junction 1 is the first test site along the main stretch of Junction Creek, downstream of where the Garson and Maley tributaries converge. Between the Maley and Frood tributaries, the main branch flows through a heavily urban environment, flowing under congested roads, which introduce their own contaminants, such as road salt. The test site makes up one of the deepest reaches of upper Junction Creek, where sampling the soft substrate was not possible. Canopy coverage was higher at this site due to the considerable number of overhanging shrubs. Junction 1 has been sampled using the CABIN protocol in 2008, 2010 and 2015.

Figure A4 Frood test site location adjacent to Lasalle Cemetery. This site is on the Frood Tributary, which historically received acid mine drainage runoff from mining waste rock containing high concentrations of sulphides, nickel and copper that was used to form a 1 km long airstrip near the Frood Stobie Mine (Gunn *et al*. 2010, Jaagumagi and Bedard 2002). The acid mine drainage runoff was diverted over the course 2000-2001 to travel approximately 1 km underground and emerged 7 km from the point source to be treated at Copper Cliff (Gun *et al*. 2010). Due to the importance of this site in measuring recovery following the diversion, it has been sampled the most frequently of all Junction Creek sites, both using the CABIN protocol (as of 2003) and another rapid bioassessment techniques, explained in Davidson 2002. Frood has been a focus of erosion control structure. Additionally, Nickeldale Dam controls water levels during the Spring freshet approximately 700 m upstream.

Figure A5 Junction 2 test site is situated on the main Junction Creek branch downstream of the convergence of the main branch and Frood tributary in the Ponderosa Wetland. Gabion baskets outline this short reach of the creek for erosion control, leading up to the large concrete box culvert that houses the creek as it travels nearly 1 km beneath downtown Sudbury. The box culvert was built in the mid 1960's to mitigate annual flooding. Junction 2 is one of the only reaches of upper Junction Creek with a presence of continuous riffles, which occur over the small and large cobble in the shallow water characteristic of the last 10-15 meters before the box culvert. This site has a higher canopy coverage than most sites in this study and has been sampled using the CABIN protocol since 2005.

Figure A6 Nolin Creek is comprised of an East and West Branch, which converge approximately 500 m downstream of Nolin test site, before entering a box culvert. This box culvert is nearly 1 km long and joins the main downtown box culvert, which is downstream of Junction 2. The site is partially surrounded by a residential area, but the water of Nolin West Branch comes from the slagpiles and tailings in the form of treated runoff. All runoff from Clarabelle and Nolin Reservoirs are treated, using the same method as Garson,at the Nolin Wastewater Treatment Facility. Nolin site is another well-sampled site as its headwaters is a mining wastewater treatment facility. It has been sampled using the CABIN protocol since 2003 and using a second rapid bioassessment technique (Davidson 2002) since 2001.

Figure A7 Junction 3 test site location, with Junction 2 represented by the smaller symbol in the background. Nolin Creek enters the concrete box culvert at the yellow star, where it travels for approximately 1 km before entering the main box culvert and Junction Creek. Junction 3 is meters from the outlet of the box culvert, as seen above. This site is characterized by shallow waters and a long bankfull width compared to other sites (11.5 m in October 2015). Small islands, sediment deposits, and riffles were all present. The ecological condition of this site is representative of the cumulative effects from all tributaries and point sources upstream, including the box culverts. Junction 3 has been sampled using the CABIN protocol since 2005.

Appendix B: Tukey's HSD Test and Tukey's Test Outputs

Table B1 Groupings from Tukey's HSD test.

	Garson	Maley	Junction 1	Frood	Junction 2	Nolin	Junction 3
Abundance	a	a	ab	b	a	ab	a
Bray Curtis Distance	a	a	a	a	a	a	a
HBI	ab	b	b	ab	b	a	ab
Simpson Diversity	a	ab	ab	a	ab	ab	b
S-W Diversity	a	ab	ab	a	ab	ab	b
Family Richness	a	a	ab	ab	ab	ab	b
EPT Richness	ab	a	ab	b	b	b	b
% EPT	a	ab	bc	ab	a	c	c
% Chironomids	a	a	a	a	a	a	a
% Filterers	a	bc	c	ab	a	abc	bc
% Gatherers	a	a	ab	b	a	ab	a
% Predators	abc	c	c	ab	c	a	bc
% Scrapers	ab	bcd	cd	abc	cd	a	d
% Shredders	a	a	a	a	a	a	a
# Clingers	ab	a	ab	b	ab	b	b

Table B2 Post Hoc Tukey's results following Repeated Measures One-way ANOVAs on community metrics.

Metric	Sites	z value	Significance
Abundance	Garson-Frood	3.662	**
	Junction 2-Frood	3.785	**
	Junction 3-Frood	3.256	*
	Maley-Frood	4.197	***
Bray-Curtis Distance	Junction 3-Frood	-3.231	*
HBI	Nolin-Maley	3.718	**
Simpson Diversity	Junction 3-Frood	-4.056	**
	Junction 3-Garson	-4.479	***
	Junction 3-Junction 2	-3.058	*
S-W Diversity	Junction 3-Frood	-4.4	***
	Junction 3-Garson	-4.998	***
	Nolin-Garson	-3.394	*
	Maley-Junction 3	3.339	*
Family Richness	Junction 3-Garson	-3.33	*
	Maley-Junction 3	3.897	**
EPT Family Richness	Maley-Frood	3.214	*
	Maley- Junction 3	4.261	***
	Nolin-Maley	-4.123	***
%EPT	Junction 3-Frood	-5.128	***
	Junction 1-Frood	-3.333	*
	Nolin-Frood	-5.056	***
	Junction 3-Garson	-5.978	***
	Junction 1-Garson	-4.117	***
	Nolin-Garson	-5.879	***
	Junction 3-Junction 2	-5.908	***
	Junction 1-Junction 2	-4.064	**
	Nolin-Junction 2	-5.764	***
	Maley-Junction 3	3.441	*
	Nolin-Maley	-3.324	*
% Chironomids	Junction 3-Junction 2	3.18	*
% Filterers	Junction 1-Frood	-3.608	**
	Junction 3-Frood	-3.354	*
	Maley-Frood	-3.63	*
	Junction 1-Garson	-4.416	***
	Junction 3-Garson	-4.506	***
	Maley-Garson	-4.509	**
	Nolin-Garson	-3.461	*
	Junction 2-Junction 1	4.156	***
	Junction 3-Junction 2	-4.106	***

Metric	Sites	z value	Significance
	Maley-Junction 2	-4.105	***
	Nolin-Junction 2	-3.093	*
% Gatherers	Junction 1-Frood	4.37	***
	Junction 3-Frood	4.855	***
	Nolin-Frood	3.249	*
	Junction 1-Garson	4.302	***
	Junction 3-Garson	4.574	***
	Nolin-Garson	3.043	*
	Junction 2-Junction 1	-3.968	**
	Junction 3-Junction 2	4.106	***
% Predators	Junction 1-Frood	-3.509	**
	Junction 2-Frood	-3.7	**
	Junction 3-Frood	-3.158	*
	Maley-Frood	-3.694	**
	Nolin-Garson	3.265	*
	Nolin-Junction 1	4.046	**
	Nolin-Junction 2	4.387	***
	Nolin-Junction 3	3.89	**
	Nolin-Maley	4.394	***
% Scrapers	Junction 3-Frood	-3.565	**
	Juntion 1-Garson	-3.277	*
	Juntion 2-Garson	-3.362	*
	Juntion 3-Garson	-5.23	***
	Nolin-Junction 1	3.822	**
	Nolin-Junction 2	4.177	***
	Nolin-Junction 3	6.136	***
	Nolin-Maley	3.479	*
# Clingers	Maley-Frood	3.219	*
	Maley-Junction 3	3.662	**
	Nolin-Maley	-3.806	**
Significance codes: 0 '*' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1**			