

University of Windsor

Scholarship at UWindsor

Electronic Theses and Dissertations

Theses, Dissertations, and Major Papers

1-1-2019

Bioassessment of Streams Within the Clay-Plains Region of Southwestern Ontario – Optimizing Sampling and Laboratory Assessment Methods

Alyssa Alves Frazao
University of Windsor

Follow this and additional works at: <https://scholar.uwindsor.ca/etd>

Recommended Citation

Frazao, Alyssa Alves, "Bioassessment of Streams Within the Clay-Plains Region of Southwestern Ontario – Optimizing Sampling and Laboratory Assessment Methods" (2019). *Electronic Theses and Dissertations*. 8164.

<https://scholar.uwindsor.ca/etd/8164>

This online database contains the full-text of PhD dissertations and Masters' theses of University of Windsor students from 1954 forward. These documents are made available for personal study and research purposes only, in accordance with the Canadian Copyright Act and the Creative Commons license—CC BY-NC-ND (Attribution, Non-Commercial, No Derivative Works). Under this license, works must always be attributed to the copyright holder (original author), cannot be used for any commercial purposes, and may not be altered. Any other use would require the permission of the copyright holder. Students may inquire about withdrawing their dissertation and/or thesis from this database. For additional inquiries, please contact the repository administrator via email (scholarship@uwindsor.ca) or by telephone at 519-253-3000ext. 3208.

BIOASSESSMENT OF STREAMS WITHIN THE CLAY-PLAINS REGION OF
SOUTHWESTERN ONTARIO – OPTIMIZING SAMPLING AND LABORATORY
ASSESSMENT METHODS

by

Alyssa A. Frazao

A Thesis
Submitted to the Faculty of Graduate Studies
Through the Department of Biological Sciences
In Partial Fulfilment of the Requirements for
The Degree of Master of Science at the
University of Windsor

Windsor, Ontario, Canada

2019

©2019 Alyssa A. Frazao

Bioassessment of Streams Within the Clay-Plains Region of Southwestern Ontario – Optimizing
Sampling and Laboratory Assessment Methods

by

Alyssa Frazao

APPROVED BY:

K. Stammler
Essex Region Conservation Authority

J. Gagnon
School of the Environment

K. Drouillard
School of the Environment

J.J.H. Ciborowski, Advisor
Department of Integrative Biology

December 18, 2019

Declaration of Originality

I hereby certify that I am the sole author of this thesis and that no part of this thesis has been published or submitted for publication.

I certify that, to the best of my knowledge, my thesis does not infringe upon anyone's copyright nor violate any proprietary rights and that any ideas, techniques, quotations, or any other material from the work of other people included in my thesis, published or otherwise, are fully acknowledged in accordance with the standard referencing practices. Furthermore, to the extent that I have included copyrighted material that surpasses the bounds of fair dealing within the meaning of the Canada Copyright Act, I certify that I have obtained a written permission from the copyright owner(s) to include such material(s) in my thesis and have included copies of such copyright clearances to my appendix.

I declare that this is a true copy of my thesis, including any final revisions, as approved by my thesis committee and the Graduate Studies office, and that this thesis has not been submitted for a higher degree to any other University or Institution.

Abstract

Evaluating the ecological condition of streams can be accomplished by assessing the community composition of macroinvertebrates whose differential sensitivity to perturbations reflect the conditions of their habitat. Two sampling protocols used to assess Ontario streams (Canadian Aquatic Biomonitoring Information Network (CABIN) (employed across Canada), and the Ontario Benthic Biomonitoring Network (OBBN)) recommend using D-framed dip nets (D-nets) to effectively assess streams, most of which have rapid flow and either hard bottoms or coarse sediment. I assessed the relative effectiveness of D-nets and Petite Ponar grabs to sample macroinvertebrates during the summer in 19 southwestern Ontario clay-plain streams, which typically have fine sediments and slow or nondetectable velocity. The two methods identified similar community composition; but the D-net captured more aquatic invertebrates and greater family richness than the Petite Ponar grabs.

Although both protocols recommend processing and subsampling samples using a Marchant Box I found that sorting up to 300 animals per size fraction of a series of nested sieves took approximately half the time, yielded significantly greater richness estimates and reduced the marked overestimates of abundance sometimes observed when subsampling to fixed counts with the Marchant Box. Effective bioassessment of southwestern Ontario clay plain streams can be achieved by collecting 2-3 jab-and-sweep D-net samples from glide region in late April-early May and processing subsamples separated into size fractions using nested sieves. Most streams sampled were dominated by tolerant organisms producing HBI scores ranging from 7-8. Tolerance scores for streams in Essex County were significantly higher than scores for streams in the Lower Thames Valley conservation region.

*To my family and friends,
for this journey would not be complete without you.*

Acknowledgements

There are so many people I would like to thank for their support throughout my thesis journey, but first and foremost, I would like to thank my parents and sister, Cathy. They have provided much love and support which I couldn't have gone without. I'd also like to thank Jan Ciborowski, for without his support and last-minute revisions, this thesis would not exist. Thank you, Jan, for seeing the potential scientist in me, guiding me through struggles, providing kind words, and having never-ending support.

The Ciborowski Lab as a whole is like another family to me. Li Wang, thanks for all the last minute mapping, weekly update reminders, and having such amazing advice for both my master's and other parts of my life. Michelle Dobrin (or should I say my future-self), thank you for believing in me, helping me develop my taxonomy skills, putting up with my silly questions, providing life lasting advice, and the list can go on and on. You've helped me so much to develop as a scientist, an artist, and just a better person all around, I can't thank you enough.

To the past fellow grad students, Jasmine St. Pierre, Kelly Menard, and Chantal Dings-Avery, thank you for showing me what it takes to be a good grad student. You were all great role models when I first started and thanks for giving a warm welcome to the lab. Danielle Gunsch, my partner in crime throughout my entire masters, I can't even. Thank you for showing me that no matter how tough life can get, you need to take time for yourself, and that trying new things can be fun, even when it's a huge step out of your comfort zone. You've done such a great job at hosting events and finding fun things to bring people together. Thank you for the Grad Bucket List journey and see you soon first in Vancouver, then in Hawaii. To Emilee Mancini and Nathan Tuck, you two are amazing people. You both have such great uplifting and positive spirits and I know

that whatever road life takes you, you will be super successful and will make a great impact on the world, you've been amazing lab-mates.

I've spent many evenings in the lab processing samples, but I couldn't have done it without Vanessa Francis. Vanessa, you have a beautiful soul. I am so lucky to have met a bright, smart, and fun person who has been with me through the good and bad times. I honestly couldn't have finished my thesis without you going through my endless samples.

To the original field crew, Claire Shrimpton and Daniel Picard. There are so many things you have both brought into my life, but the ones that stand out are the immense positivity and moments of laughter you two have given. Field work with you both was always so fun and I knew I could relax a bit because you both were always on track with tasks. I love how each of you has grown and I know that we will always be friends for life. Claire, see you in Hawaii with Danielle, Vanessa and Daniel, you can come too.

Others that have helped me with either my samples or with the field work include; Jordan Rideout, never stop making amazing jokes; Stephanie Johnson, you're an inspirational artist, never stop creating; Jess Robson, I can't wait to see what you do next, you are beautiful; Anique Gauvin, I'm honoured to have watched you grow into an amazing, strong person; Lyndon Barr, thank you for the chironomid mounting bonanza's and helping me become a better taxonomist; Justin Landry thank you for your hard work and helping me with my sediment data.

My two lovely best friends who have seen the best and the worst of me, Patricia Okpara and Catalina Fernandez. Thank you for being such amazing influential women who have helped me stay strong when I wanted to give up. I could not be the woman I am today without your love, sharing your life experiences, and participating in mind-opening conversations. I look forward to continuing our life-long journey with each other.

I'd also like to thank Chris Jones (OMNR) for providing the files and data on southwestern Ontario streams classified by physiographic region and land use stress, Jason Wintermute (Lower Thames Valley Conservation Authority) for providing long term data on discharge and temperature from the Thames River, to both Jason Wintermute and Katie Stammler (Essex Region Conservation Authority) for providing guidance on the locations and priorities for sampling stream sites within their jurisdictions, and to Katie Stammler for all your advice and for being such a huge role model and helping with thesis edits.

Last but not least, my sources of funding: This project was possible with funding from the Essex Region Conservation Authority, the Lower Thames Valley Conservation Authority, Ontario Graduate Scholarship; QE II- Graduate Scholarship in Science and Technology, and additional graduate and research assistantships from the University of Windsor.

Table of Contents

Declaration of Originality	iii
Abstract	iv
Dedication	v
Acknowledgements	vi
List of Tables	xi
List of Figures	xiii
Chapter 1: General Introduction	1
Project Summary and Objectives	1
Chapter 2: Comparison of Benthic Macroinvertebrate Field Collection Methods: Assessing Abundance, Richness and Community Composition from D-frame Sweep Net and Petite Ponar Grab Samples.	9
Introduction	9
<i>Stream Sampling Equipment</i>	10
Methods	11
<i>Study Sites</i>	11
<i>Habitat Assessment and Physicochemical Measurements</i>	12
<i>Macroinvertebrate Sampling</i>	13
Laboratory Procedures	15
<i>Preservation and Sorting</i>	15
Statistical Analyses	17
<i>Stream Specific Biodiversity</i>	17
<i>Invertebrate Community Composition</i>	17
<i>Effectiveness of Sampler using Bioassessment Measures.</i>	18
Results	19
<i>Biodiversity and Community Composition among Streams</i>	19
<i>Biodiversity differences due to Sampler Type</i>	21
<i>Sampler Effectiveness with Biotic Indices.</i>	38
Discussion	44
Conclusion	47
Chapter 3: Efficiency of Laboratory Macroinvertebrate Sample Processing; A Comparison of the Marchant Box and Nested Sieves Methods.	48
Introduction	48
<i>Great Lakes Region</i>	50
<i>International: Europe, New Zealand</i>	52
Methods	55
<i>Marchant Box Method</i>	56

<i>Nested Sieve Fractioning Method</i>	57
<i>Invertebrate Identification</i>	58
Statistical Analysis	59
<i>Subsampler Efficiency – Processing Time</i>	60
<i>Estimated Richness and Abundance</i>	60
<i>Community Composition</i>	61
Results	62
<i>Subsampler Efficiency – Processing Time</i>	62
<i>Richness and Abundance</i>	68
<i>Community Composition</i>	75
Discussion	76
Recommendations	80
Chapter 4: General Discussion	82
Project Overview	82
Major Findings and Recommendations for Regional Conservation Authorities	84
Limitations	85
Future Studies and Implications	86
References	89
Appendices	114
Appendix A: General Methods	103
<i>Environmental Surveys</i>	103
<i>Site Selection</i>	103
<i>Sampling Period</i>	108
<i>Macroinvertebrate Sampling</i>	110
Appendix B. Summary Tables of NMDS Analyses	115
Appendix C: Invertebrate Species List (2016)	118
Appendix D: 2016 Field Data	136
Appendix E: Invertebrate Species List (2017)	150
Appendix F: 2017 Field Data	190
Appendix G: Sediment Analysis Data (2017 Sampling Year)	226
Vita Auctoris	235

List of Tables

Table 2.1: Site names, coordinates and sampling date for 2016 benthic sampling year. Locations are illustrated in Fig. 1.3.....	16
Table 2.2: Main effects ANOVA table illustrating effects of sampler type and stream on family richness; samples pooled together based on sampler type.....	24
Table 2.3: The 5 most abundant taxa collected by each sampler in each stream. Abundances are based on values extrapolated from the mass of sorted detritus relative to the unsorted biomass in the 4.0, 1.0, and 0.5 mm sieve size fractions.....	28
Table 2.4: Table of significance values between difference samples using a t-test. Bolded numbers indicate statistical significance. ($\alpha=0.05$).....	33
Table 2.5: Hilsenhoff Biotic Index scores calculated from D-net, Ponar grab and combined samples for 2016 streams.....	39
Table 2.6: Paired comparison test of difference between HBI calculated from D-Net samples vs. Petite Ponar grab samples.....	42
Table 2.7. One-way ANOVA comparing Hilsenhoff Biotic Index scores for Essex Region Conservation Area streams (n=7) with Lower Thames Valley Conservation Area streams (n=12).....	42
Table 3.1. Summary of variables determined for assessment of processing efficiency of Marchant Box vs. Nested Sieve procedures.....	59
Table 3.2. Main Effects (unreplicated) ANOVA of effects of sampler type on Log ₁₀ transformed combined processing time.....	65
Table 3.3 Results of multiple regression analysis of the effects of subsampling method (Marchant Box = 1; Sieves = 0), sampling method (D-net =1; Petite Ponar = 0), habitat type (riffle = 1; pool =0) and detritus mass (grams) on Log-transformed total time required to process a sample (minutes; n=36 samples; R ² = 0.256).....	66
Table 3.4 Results of standard stepwise multiple regression analysis of the effects of the interaction between detritus mass (grams) with subsampling method (Marchant Box = 1; Sieves = 0), sampling method (D-net =1; Petite Ponar = 0), and habitat type (riffle = 1; pool =0) on total time required to process a sample (minutes; n=36 samples). R ² = 0.141.....	67
Table 3.5. Cumulative family richness when all 3 (D-net) and 5 (Petite Ponar) samples were pooled together (p>0.05).....	73

Table 3.6. Main Effects (unreplicated) ANOVA of effects of processing type on number of families recovered from sweep and Petite Ponar samples collected from 5 streams.....72

Table A.1. Sampling site, GPS coordinates, and sampling year.....113

List of Figures

- Figure 1.1: The physiographic regions of Southwestern Ontario. (Map Series: Physiographical Series, Ontario Department of Mines and Northern Affairs, Ontario Research Foundation, Maps 2224-2227. Physiographic Series, Ministry of Natural Resources, Ontario Research Foundation, Map 2228.)3
- Figure 2.1 Bar graph of the mean abundance of invertebrates (the sum of all individuals collected by each sampler) arithmetically averaged across all streams) collected using either the D-net (black) or the ponar (grey). Note the octave (Log_2) scale.22
- Figure 2.2: Arithmetic mean \pm SE invertebrate family richness of D-net and Ponar samplers.....23
- Figure 2.3: Number of families collected from D-net (red diamonds, $n=3$) and Petite Ponar grabs (blue triangles, $n=5$) as a function of stream-specific family richness. Dotted line represents equal richness. Each point represents one of the 19 streams sampled in 2016 (Table 2.1).25
- Figure 2.4 Number of families collected from D-net (red diamonds, $n=3$) and Petite Ponar grabs (blue triangles, $n=5$).. Dotted line represents equal richness.....26
- Figure 2.5 Rarefaction curve of the number of samples that should be collected in the field. Mean \pm SD values of richness based on 19 sites from 2016 sampling year. Letters represent a significant difference for that sample. Samples with the same letter indicates no significant difference between them. Calculations illustrated in Table 2.4.32
- Figure 2.6: NMDS ordination plot showing the relationship between D-net and ponar collections for each stream based on invertebrate community compositions. Stress = 11.25, dim=3. Lines connect the D-net and Ponar grab samples from each stream. Sampler type influences interpretation of stream community composition only for pairs of streams whose lines cross.36
- Figure 2.7: NMDS ordination plot showing the relationship between D-net and all samples collected (both D-net and ponar combined) for each stream based on invertebrate community compositions. Stress = 12.42, dim=3.37
- Figure 2.8: Scatterplot and regression of the Hilsenhoff scores for samples from 19 streams (ERCA: solid black circle; LTVCA: open black circles) collected using only the D-net compared to scores calculated for all samples from a stream combined (D-net and ponar samples combined)..40
- Figure 2.9: Scatterplot and regression of the Hilsenhoff scores for samples collected using only the ponar compared to scores for all samples combined (D-net and ponar samples combined)....41

Figure 2.10: The mean \pm SE of the Hilsenhoff Biotic Index Scores (HBI) between the Essex County and Lower Thames Regions (ERCA and LTVCA respectively). There is a significant difference between these regions (ANOVA, $F=5.028$, $p=0.039$).43

Figure 3.1: Scatterplot of the time taken to process one sample using nested sieves (squares and dotted line) and the Marchant box (triangles and solid line). The lines represent distance-weighted least squares fits through the data points (stiffness = 0.5).63

Figure 3.2: Mean \pm SE; $n=31$ processing time for samples using the Marchant Box and sieving subsampling methods (note the Log scale). There is a significant difference between sorting methods (ANOVA, $F(1,31) = 9.14.589$, $p=0.005$).64

Figure 3.3: The extrapolated abundance of invertebrates in sample (estimated from the subsampled fraction) as a function of the actual number for Marchant box and sieve processing methods. Each point represents one sample ($n=27$).....69

Figure 3.4. The mean(\pm SE) family richness found using the Marchant Box and nested sieves fractionation method. ($n=40$).71

Figure 3.5: Scatterplot and regression line of family richness estimated from Marchant Box and sieve fractionation processing methods vs. total number of families observed in source stream. The x-axis is a combination of both samplers. Each point represents the mean richness for 8 samples per stream using the respective subsampler. The dotted line represents equal richness for both axes. Sieves: $t=6.735$, $p>0.05$)73

Figure 3.6 Scatterplot and regression with polynomial fit of the absolute difference between the Marchant Box and Sieves to the Sieves as a percentage of the Marchant Box. Each point represents one sample..74

Figure 3.7: NMDS ordination of invertebrate community composition estimated from D-net samples collected from 5 streams, illustrating compositional differences inferred by subsampling methods.77

Figure A.2: Scatterplot of land use (areal percentage of land in row crops (X-axis; areal percentage of developed land) in the contributing watershed upstream of 809 stream sites within the Essex County region of Southwestern Ontario, and associated site numbers.106

Figure A.3: Scatterplot of land use (areal percentage of land in row crops (X-axis; areal percentage of developed land) in the contributing watershed upstream of 717 sites within the Lower Thames Valley region of Southwestern Ontario and associated site numbers.107

Figure A.4: Water temperature readings for the Thames River in Southwestern Ontario from 2007 to 2016. Black horizontal lines represent temperatures at 12°C and 20°C. Black vertical lines depict the sampling window.109

Figure A.5. Map of the Essex County and Lower Thames Valley region of Southwestern Ontario showing stream sites sampled in 2016 with the associated stream names. ($n=19$)111

Figure A.6. Map of the Essex County and Lower Thames Valley region of Southwestern Ontario showing stream sites sampled in 2016 and 2017 with the associated stream names.112

Chapter 1: General Introduction

Project Summary and Objectives

Streams are an important component of the ecosystem surrounding the Great Lakes. They are the channels that not only transport water into the lakes but also collect and carry the nutrients, chemicals, and organisms contributed along their length from the surrounding land. Varying geographical features along the length of streams alter macroinvertebrate community composition and functions (Vannote et al. 1980; Lenat and Crawford 1994; Sciera et al. 2008). It is important to recognize the contributing factors that affect macroinvertebrate community composition and abundance and how this can impact the Great Lakes (Økland and Økland 1986).

One way to assess streams is by studying their macroinvertebrate fauna as an indicator of the system's ecological condition or 'health', as well as the degree of anthropogenic effects (Resh et al. 1998; Bailey et al. 2004; Hilsenhoff 1982). Stream organisms vary in their tolerances to habitat perturbations. Consequently, the presence of sensitive macroinvertebrates implies that a stream is relatively unaffected by anthropogenic stress, whereas disturbed streams are dominated by tolerant organisms (Hilsenhoff 1987; 1977).

In Canada, two protocols have been developed and recommended to assess aquatic invertebrate communities in Ontario streams. Environment Canada has developed and oversees the Canadian Aquatic Biomonitoring Information Network (CABIN; Environment and Climate Change Canada, 2019), which is employed across Canada. The CABIN program maintains a national database that permits comparisons of multiple stream ecosystems across the regions because they prescribe use of a standardized set of sampling protocols (Reynoldson et al. 1999;

ECCC 2018). This allows for the comparison of data and streams themselves since collection methods are the same.

The Ontario Benthic Biomonitoring Network (OBBN) is a protocol co-founded by the Ontario Ministry of the Environment (MOE) and Environment Canada (Ecological Monitoring and Assessment Network – EMAN; Jones et al., 2004). Because it was derived from CABIN, OBBN has similar goals, including recommending use the of standardized methods, as well as providing a database to allow the comparison of data and focusing on providing a rapid bioassessment of the streams using macroinvertebrates as an ecological measure (Boyle 2003; Jones et al. 2007). Like CABIN, the OBBN describes methods to assess lakes and wetlands in addition to wadeable streams.

Both programs use Rapid Bioassessment Protocols (RBP; Plafkin et al. 1989; Barbour et al. 1999) for field sampling, meaning that they promote an efficient, easy and cost-effective approach to stream assessment (Resh and Jackson 1993; Buss and Vitorino 2010). Both CABIN and OBBN field methods are designed to effectively assess the fauna of wadeable streams that have relatively rapid flow and coarse sediment by using a D-framed sweep net. Yet, much of the southwestern Ontario landscape sits on a glacial-remnant clay plain (Figure. 1.1). The parent materials of the St. Clair Clay Plains and other clay plains largely dictate the sediment texture. Stony streams (coarse substrate) provide habitat for benthic invertebrates that can be disturbed during sampling, resulting in the invertebrates being dislodged and swept into a downstream net by the current (Knight and Gaufin 1967; OBBN 2007). Because topographic relief is minimal in the St. Clair Clay Plain region, stream velocities are slow, and often negligible during low discharge periods. Consequently, riffles and pools can be difficult or impossible to locate.

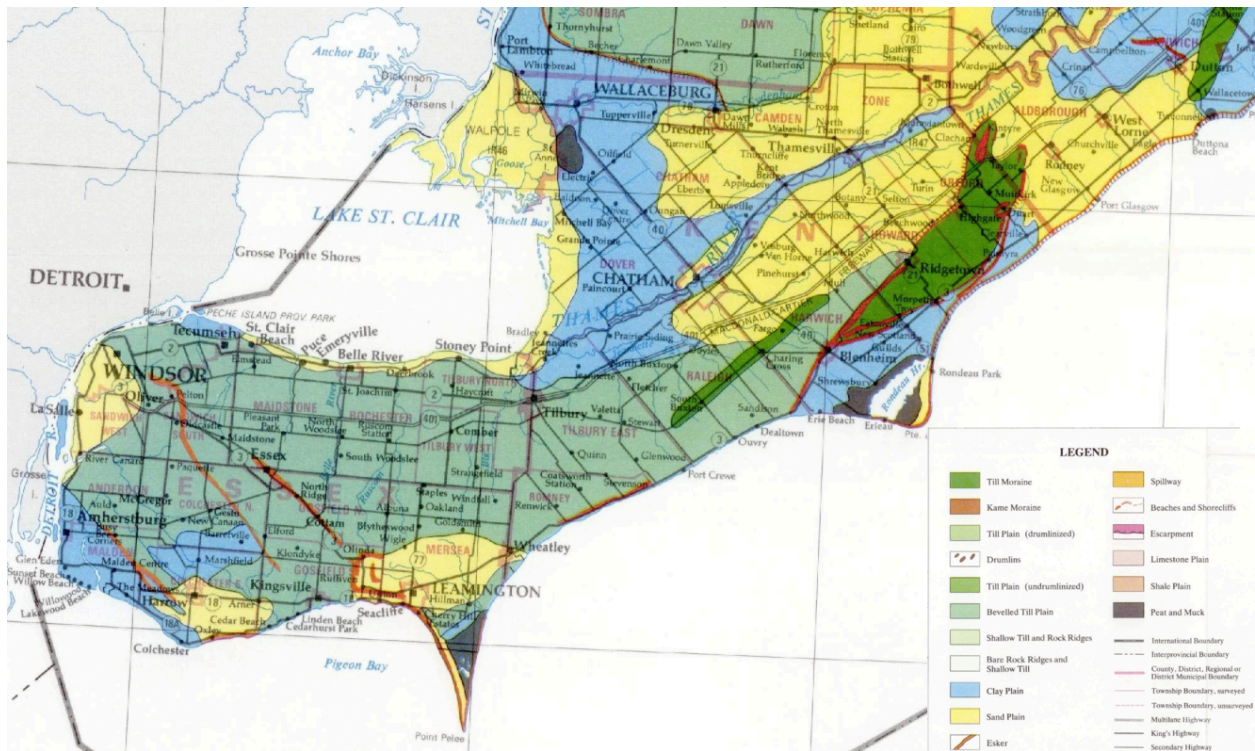


Figure 1.1: The physiographic regions of Southwestern Ontario. (Map Series: Physiographical Series, Ontario Department of Mines and Northern Affairs, Ontario Research Foundation, Maps 2224-2227. Physiographic Series, Ministry of Natural Resources, Ontario Research Foundation, Map 2228.)

Streams that have clay-dominated sediment are considered to be soft-bottomed (Stark 2001), a substrate that is more typical of a wetland or pond than of a river (Faulkner and Richardson 1989). Thus, in some respects southwestern Ontario streams are more similar to wetlands than to riffle-and-pool streams. The lack of discernable current and soft mud or clay substrate may compromise the D-net's effectiveness due to back flow and a tendency for the fine particles to clog the net. Therefore, the methods used to sample benthos in wetlands and ponds may be more effective than dip net sampling in these slow-flowing, soft bottomed streams. One such possible alternative to the D-net is the Petite Ponar grab since it is best used in low-flow, muddy areas (Elliott and Drake 1981).

Both the CABIN and OBBN protocols recommend using the Marchant Box (Marchant 1989) in the laboratory when subsampling is necessary to reduce the time devoted to sample processing. The Marchant Box was designed to process a whole sample by distributing the sample evenly into 100 cells, a subset of which are randomly selected and individually sorted to enumerate the invertebrates. However, this method can be time consuming and, if biased, can ultimately alter assessment of ecological condition (Valois et al. 2016). When a sample is comprised of a large amount of organic material it can then be difficult to distribute the material evenly among the cells when flipping the box upright. Furthermore, the Marchant Box method is a fixed-count protocol that requires examining cells until at least 300 organisms have been retrieved. Although the protocol is intended to reduce sorting time, the fixed-count stopping rule can result in the omission of rare macroinvertebrates when only a small number of cells are examined. The exclusion of rare and sensitive taxa can bias metrics of richness and ultimately bias assessments of a site's ecological condition.

An alternative procedure is the Nested Sieve-Fractioning approach (Ciborowski 1991; Bourassa and Morin 1995; Vinson and Hawkins 1996) whereby a sample is subdivided by particle size using a series of sieves. Each subsample is sorted independently according to size-specific criteria, which improves sorting efficiency and the detection of large, rarer taxa (Ciborowski 1991). For each size fraction at least 300 organisms may be counted but typically as the size fraction gets smaller there are more invertebrates. For the finer sieves (i.e. 0.05 mm) a ¼ of the size fraction can be counted. The detritus weight of what was unsorted can be compared to the sorted weight to estimate the individuals of the whole size fraction. This provides a better idea of what is in the entire sample rather than the sub-samples taken by the Marchant Box.

Around the world, agencies may use region-specific procedures to assess the streams within their jurisdictions. In the United States, the National Rivers and Streams Assessment (NRSA) collects information to describe the nation's stream and river ecological condition under the USEPA (Barbour et al. 1999). In Europe, multiple countries were involved in The Development and Testing of an Integrated Assessment System for the Ecological Quality of Streams and Rivers throughout Europe using Benthic Macroinvertebrates, (AQEM) project from 2000 to 2002 (AQEM 2002). These stream assessment procedures are now incorporated into the STAR (Standardization of River Classifications) project, which uses the rapid bioassessment protocol (Barbour et al. 1999). In 2002, the EU Water Framework Directive (WFD) was created to provide a collaborative effort amongst European countries to clean, protect and manage the waterbodies they share (EC 2000). In Canada, water quality guidelines were created to focus on the chemical, physical, and biological aspects of water quality, administered under the Canadian Water Quality Guidelines for the Protection of Aquatic Life (Canadian Council of Ministers of the Environment 2014; Reynoldson 2007).

For this study on the effectiveness of sample collection, I compared two alternative methods that accommodated the difficulties associated with sampling slow-flowing habitats that have soft substrates.

The objectives of my thesis were to

- a. propose field sampling protocols (timing, site selection, and intensity of sampling informed by OBBN and CABIN) suitable for conducting aquatic invertebrate bioassessments of low-gradient clay plain streams of southwestern Ontario;
- b. evaluate the effectiveness of two collection methods (D-frame kick net sampling and Petite Ponar grabs); and
- c. compare the efficiency of two laboratory subsampling and processing methods (Marchant Box vs. nested sieves) to determine which procedures can best characterize the streams' ecological condition.

I addressed these questions by using inventories of second to fifth-order streams for which the intensity of agricultural and rural/urban land use in contributing watersheds had been determined (Jones 2012) to stratified-randomly select a set of study streams representing the maximum range of potential disturbances to stream communities. Alterations in habitat, flow, and the materials transported in run-off due to human activity in watersheds can both directly and indirectly affect the invertebrate communities of receiving streams. Dance and Hynes (1980) found that two streams that were similar in community composition in 1840 had changed to having different communities due to changes in the surrounding agricultural land use. Similarly,

Stepenuck et al. (2002) found that the ecological condition of streams (measured in terms of the Hilsenhoff biotic index) became progressively poorer as urbanization increased.

With these guidelines, I sampled 19 streams in midsummer 2016 and 40 streams in April and May 2017. In 2017, I sampled within a seasonal timeframe based on long-term discharge/temperature records for the Thames River (see below) to ensure that discharge would be relatively high and that water temperatures below thresholds that might stimulate emergence of spring-developing aquatic insects.

The suitability of sampler type and intensity and habitat was assessed using the 2016 dataset. The efficiency of processing method was determined using samples from a subset of these streams by comparing sorting time, family richness and Hilsenhoff Biotic Index (HBI; Hilsenhoff 1987; Smith 2009) scores. Based on those findings, I subsequently processed triplicate D-net samples collected from the streams sampled in 2017 using the nested sieve protocol, and inferred stream condition from HBI scores for those samples (Appendix E).

This thesis is organized into 4 chapters. Chapter 1 introduces the research topic and describes my expectations. Chapter 2 compares the relative effectiveness of the CABIN and OBBN field protocols and the efficiency of two methods of sampling – a traveling sweep using a D-frame dip net, and Petite Ponar grabs. In Chapter 3, I assess the relative effectiveness of two methods of sample processing and subsampling – the Marchant Box method recommended by CABIN and OBBN, and the sieve fractionation method. In Chapter 4, I reiterate the strengths and weaknesses of the various protocols, recommend standard procedures for conducting macroinvertebrates rapid bioassessments, and identify future research needs. In Appendix B, I describe the general methods by which I selected sampling locations, determined the season during

which samples should be collected, and the environmental and biological sampling conducted during field visits. The relative condition of southwestern Ontario clay plain streams as summarized by HBI scores calculated from samples collected and processed according to the recommended procedures is documented in Appendix C.

Chapter 2: Comparison of Benthic Macroinvertebrate Field Collection Methods: Assessing Abundance, Richness and Community Composition from D-frame Sweep Net and Petite Ponar Grab Samples.

Introduction

The ecological status of streams and rivers is determined by its valley, including the surrounding land use and activities (Hynes 1975). Understanding the health of a region's watercourses is essential to conservation, preservation and restoration. The macroinvertebrate fauna is an especially good indicator of the system's ecological condition in relation to the degree of anthropogenic effects (Resh et al. 1998; Bailey et al. 2004; Hilsenhoff 1982). Benthic macroinvertebrates are indicative of stream water quality because they are small, which limits their mobility, and they are also a diverse group whose tolerances vary (Stewart and Loar 1994; Hynes 1960, 1970; Cummins 1979; Weber 1973; Platts et al. 1983; Patrick 1975). Consequently, the presence of pollution-sensitive macroinvertebrates at a site implies that a stream is relatively unaffected by anthropogenic stress, whereas disturbed streams are dominated by tolerant organisms (Hilsenhoff 1977; 1987).

Most methods recommended for assessing benthic macroinvertebrates are designed to sample relatively fast-flowing streams that have coarse substrate. However, these methods may not be equally effective for other stream types. Much of the southwestern Ontario landscape sits on a glacial-remnant alluvial clay plain. Consequently, streams are slow-flowing and have substrate composed largely of soft mud or clay, which in some respects are more similar to wetlands than to riffle-and-pool streams. The fauna of soft-bottomed streams tend to be dominated by invertebrates that are more tolerant of warm hypoxic conditions than the invertebrates of hard-bottom streams, and are less affected by sedimentation (Stark and Maxted 2007). Thus, the fauna expected to be found in stony reference streams are not very suitable indicators of conditions

expected in reference clay plain streams. In this study I compared two sampling protocols and equipment in streams in the Essex and Lower Thames Valley regions of southwestern Ontario, which flow through the St. Clair Clay Plain ecoregion (Baldwin et al. 2000; Richards et al. 1949). Because of the low relief of the region, the streams have little discharge and minimal velocity. Furthermore, many watercourses have been straightened to accommodate agricultural activity and resemble ditches rather than meandering natural streams, (Government of Canada: GeoGratis – Canada Base Map, 2010). Run-off from agricultural activity results in turbid water and significant sedimentation.

Stream Sampling Equipment

The guidelines and criteria by which to sample and assess macroinvertebrates vary among jurisdictions around the world (reviewed in detail in Chapter 1). Various samplers have been recommended for collecting invertebrates, yet these are typically used in fast flowing and rocky streams. They are less effective in atypical, slow flowing, and soft-substrate streams. I compared two samplers - D-frame sweep net and the Petite Ponar grab - as potential options to use in the clay-plain streams of southwestern Ontario.

In this study, I compared the D-framed sweep net using the jab and sweep method, and the Petite Ponar, since both instruments are used in still water, soft-bottom habitats (wetlands and lakes) similar to those of the Clay Plain streams and ditches of southwestern Ontario. The objective of this study was to contrast the sampling effectiveness of Petite Ponar grabs relative to D-frame net sampling in 19 southwestern Ontario streams. I predicted that:

1. The Petite Ponar grab would collect a representative benthic aquatic invertebrate sample in streams having soft sediment and little flow;
2. Invertebrate community composition would be better represented by ponar grab samples than by sweep net samples;
3. The relative effectiveness of sampler type (sweep net samples vs. Petite Ponar grabs) would depend on whether or not streams are stony (i.e., not within the clay plains) vs. silty or muddy (within the clay plain ecoregion). Sweep nets were expected to sample more effectively in streams outside of the clay plains, whereas the ponar was proposed to collect a more representative sample of the invertebrates in streams within the clay plains.

The findings of this study could result in a proposal to revise the methods for benthic macroinvertebrate sample collection be revised for slow-flowing, fine-sediment streams of the St. Clair Clay Plain region of southwestern Ontario.

Methods

Study Sites

For this part of the study, 19 streams were sampled in July and August 2016 in collaboration with the Essex Region Conservation Authority (ERCA) and the Lower Thames Valley Conservation Authority (LTVCA) to assess water quality and macroinvertebrate community composition in a cross-section of southwestern Ontario streams. Samples from a subset of these streams were chosen for the methods comparison study described herein.

Nineteen streams were visited -12 within the Lower Thames region, and 7 in the Essex County region of southwestern Ontario (Table 2.1; Fig. 1.1). As this was the first sampling season these sites were recommended by the Conservation Authorities administering each region.

Habitat Assessment and Physicochemical Measurements

On arrival, sites were inspected to confirm that they were accessible and wadeable. This was accomplished by either determining the water depth with a sweep net handle if there was a bridge or by entering the stream downstream of the sampling reach. Following inspection, stream habitat features were identified. A subjective visual assessment was made of the locations of riffles, runs and pools, meanders, location of the thalweg, and streambank/riparian features, as outlined in CABIN (Reynoldson et al. 2002) and OBBN (Jones 2015) guidelines. Where there was no evidence of rapidly flowing water at a site, the shallowest, most rapidly-flowing sections of the study area, containing the coarsest substrates were located and designated as riffles/glides (MPCA 2014). The areas immediately upstream and downstream of these locations were designated as pools - deeper, slower-flowing depositional zones that accumulate finer sediments (Hauer and Lamberti 2006). A sampling reach was on average 15 to 20 m in length for each site.

Environmental variables were sampled following protocols common to both CABIN and OBBN (Appendix A). Standard field-record sheets of both OBBN and CABIN (Appendix B) were used and completed on-site at the time of sampling. Measurements of stream temperature, dissolved oxygen concentration, electrical conductivity and pH were taken using a YSI Model 85 (Yellow Springs Instruments, Dayton, OH). All sections of the OBBN field sheets were completed; however, for CABIN field sheets, the sections labelled Slope, Velocity and Depth, and Substrate

Data were excluded. Slope could not be determined because the landscape is so flat. Furthermore, the 100-pebble count (designed to estimate particle size-frequency distribution of coarse substrates) was not conducted because pebbles were either rare or absent at sites. Instead, sediment samples were taken in a riffle and pool using a 4.5-cm diameter coring tube and processed in the laboratory using sediment particle size analysis procedures (Appendix F).

Upon arrival, after safety checks and habitat location assessments had been completed water quality measurements were made using a YSI Model 85 meter before entering the stream. Five Petite Ponar samples were collected and 3 D-net traveling sweep samples were collected in the riffle and pool as described below. Point measurements of stream depth were taken with a meter stick within each habitat (two riffles/glides, one pool) at the deepest point. Habitat assessment attributes such as reach data (i.e. habitat types present, canopy coverage, riparian vegetation) were noted, along with stream width and bankfull width. Velocity measurements were not collected in 2016.

Macroinvertebrate Sampling

A total of 8 samples were collected at each stream site, consisting of 3 D-frame sweep net samples and 5 Petite Ponar grab samples.

Petite Ponar grab Samples: The Petite Ponar grab collects a sample of substrate that is 15 cm x 15 cm in area and 15 cm deep (Mudroch and Azcue 1995). Samples were collected in a downstream-to-upstream order to minimize disturbance to the sediments prior to sampling. Grabs were collected from five locations in each stream; three in a pool, (one in the center and two along the edges of the stream) and two in a riffle/glide, (one-third of the distance from each streambank). This provided samples from across the range of habitat locations, ideally reflecting the diversity

of taxa present in the stream. Because all sites were wadeable, the sampler was placed on the stream bottom and manually pushed into the sediment rather than being dropped onto the substrate. The grab was then tripped by hand and manually closed. The sediment was then emptied into an enameled tray.

D-frame Sweep Net Samples: Sweep net samples were collected after Petite Ponar grab sampling had been completed, in two riffles/glides and one pool habitat. A 500- μ m mesh net was used, performing the kick-and-sweep procedure whenever there was noticeable velocity and coarse sediment. Alternatively, the jab-and-sweep method was used when there was little or no detectable flow and where fine sediment occurred (Stark et al. 2001). For both methods, sweeping was conducted with the net held slightly downstream while moving backwards in a zig-zag pattern across the stream for 3 min (Jones et al. 2004, Reynoldson et al. 1999). Area sampled varied depending on each site but averaged 2.75 m x 30 cm (the width of the net).

Each Petite Ponar sample was emptied into an enameled pan. The pan was topped up with stream water, the contents were swirled to suspend organic debris, and the water and debris were carefully poured into a 250- μ m mesh sieve bag. This ‘gold-panning’ procedure was repeated several times until only inorganic sediment remained in the pan. The sieve bag was repeatedly rinsed in the stream to remove fine sediments. Each D-net sample was rinsed in the stream while it was still in the D-net until most fine sediments passed. All sample contents were then placed individually in a labelled heavy-duty polyethylene soil bag, preserved with a formal-ethanol mixture (2.5:1 v/v 95% ethanol and 100% buffered formalin diluted 1:1 with stream water) (Pennak 1978; Edmunds et al. 1976; Wiggins 1927;1977; Krogmann and Holstein 2010) and the

bag was sealed with a twist-tie. Sample bags were returned to the laboratory where they were inventoried, heat-sealed to prevent leakage, and stored for later processing (Chapter 3).

Laboratory Procedures

Preservation and Sorting

In the laboratory, samples were processed and sorted in stratified-random order. Samples were emptied into a 0.180-mm mesh 20-cm diameter brass soil test sieve to drain and were then rinsed under running tap water to remove residual preservative. They were then subsampled according the Nested Sieving procedures as outlined in Chapter 3. Invertebrates recovered from sample debris were identified to at least the family level and stored in scintillation vials containing 70% ethanol.

Table 2.1: Site names, coordinates and sampling date for 2016 benthic sampling year. Locations are illustrated in Fig. A.3.

ERCA

Stream Name	Latitude	Longitude	Sampling Date
Belle River	42.251012	-82.714411	10-Aug-16
Little River	42.311337	-82.926891	05-Aug-16
Muddy Creek (M7)	42.080434	-82.489117	30-Aug-16
Sturgeon Creek (M5)	42.038942	-82.645428	25-Aug-16
Turkey Creek (M2)	42.244982	-83.065452	11-Aug-16
West Branch Drain	42.043116	-82.83671	11-Aug-16
Wigle Creek (E9)	42.029794	-82.773231	30-Aug-16

* Codes in brackets coincide with the Provincial (Stream) Water Quality Monitoring Network (PWQMN) for ERCA.

LTVCA

Stream Name	Latitude	Longitude	Sampling Date
Big Creek	42.190845	-82.47773	27-Jul-16
Hendry Drain	42.767545	-81.547026	11-Jul-16
McCarson Drain	42.517856	-82.015933	13-Jul-16
Natural Watercourse (Central)	42.675337	-81.616317	11-Jul-16
Natural Watercourse (Northeast)	42.737229	-81.48414	05-Jul-16
Newbiggen Creek	42.717937	-81.66988	11-Jul-16
Sharon Creek	42.87404	-81.400377	04-Jul-16
Sixteen Mile Creek	42.527415	-81.647913	12-Jul-16
South Dales Creek	42.106112,	-82.483699	07-Jul-16
Talbot Creek	42.681609	-81.374632	05-Jul-16
Two Creeks	42.117999	-82.461325	07-Jul-16
White Ash Creek	42.540209	-81.963236	13-Jul-16

Statistical Analyses

To assess the effectiveness of samplers, data from the 19 streams sampled in 2016 and sorted using Nested Sieves were analyzed. Analyses were conducted using STATISTICA 7.0 software, unless stated otherwise.

Stream Specific Biodiversity

The variability in family richness among streams was assessed from the 8 samples collected from the 19 streams (Appendix C). Family richness was calculated for each sample and collectively for each sampler (Petite Ponar vs. D-frame dip net). A two-way ANOVA was performed to estimate the among-stream variability and the effects of sampler type within streams on family richness. An Analysis of covariance (ANCOVA) was performed to compare family richness captured by each sampler type while accounting for differences in family richness among streams.

Invertebrate Community Composition

Both the abundance (numbers per sample) and relative abundance (Octaves – $\text{Log}_2(\text{percentage of a sample comprised of a family})$) of each taxon were tabulated. Non-metric Multidimensional Scaling (NMDS), with Bray-Curtis (Sorensen) distances, was used to portray similarity or dissimilarity between relative abundances of invertebrate collections for each sampler between different sites. Counts of all individuals belonging to a family that were collected by each sampler were pooled together for each site (i.e., specimens in the 5 Petite Ponar grabs from a stream were pooled, as were specimens from the 3 sweep samples). Relative abundances of families in each pooled sample were then calculated). Invertebrate families represented by fewer

than 50 individuals per sampler and site, or that had a frequency of occurring in 5 or fewer samples were considered outliers and were removed from the analysis so as to not skew the outcome. This resulted in 39 families being included in the analysis and the exclusion of 30 families that did not meet the inclusion criteria. The relative abundance (octaves) of each common family then was calculated. The octaves were calculated according to the formula

$$(4+\text{Log}_2(0.625+\text{RA}))*(\text{RA}>0)$$

where RA is relative abundance (percent). The constant (4) at the beginning of the formula was added to prevent negative numbers from occurring when relative abundance values were less than 0.0625 ($\text{Log}_2(0.0625) = -4$). The NMDS analysis was performed using PC-ORD Version 6 (McCune and Mefford 2011) and illustrated using the scatterplot feature of STATISTICA 7.

Effectiveness of Sampler using Bioassessment Measures.

A rarefaction curve was compiled to evaluate how many samples were necessary to reach the asymptotic family richness collected from a stream. The first point in each of the stream's rarefaction curve was the individual sample in each stream containing the highest richness. The second point was determined by identifying the sample from a stream yielding the greatest number of additional families and calculating the richness of the first and second samples combined. The third point in the cumulative richness curve consisted in the set of three samples yielding the greatest number of families per stream. The process was continued until all 8 samples, from both D-net and ponar samples from a stream had been incorporated into the cumulative curve. The progressive cumulative richness totals were then standardized by dividing the richness numbers by the overall family richness for a stream. Finally, the mean value and standard deviation were

calculated for each of the 8 cumulative values for the 19 streams sampled. A main-effects ANOVA between each of the samplers was calculated to determine their significance (Table 2.4 results).

An NMDS analysis with Bray-Curtis distances was performed to ordinate the community composition among all of the streams and to determine if there were differences in community composition collected by the two sampler types. Hilsenhoff Family Biotic Index tolerance scores were also calculated for each stream based on pooled samples collected using a D-net only, using ponars only, and using both samplers combined. Tolerance scores were based on the biotic tolerance values for New York State stream invertebrates (Smith et al. 2009), provided in the *Guide to Developing Conservation Authority Watershed Report Cards* provided by Katie Stammler (Essex Region Conservation Authority, pers. comm.). A scatterplot and regression of these tolerance scores was created to predict how well the actual tolerance score (based on D-net and ponar samples combined) was predicted by the samples collected using only one type of sampler. For each plot, the y-axis was the tolerance score based on all samples combined and the x-axis was the tolerance scores calculated for only one type of sampler. A scatterplot was created for each sampler individually.

Results

Biodiversity and Community Composition among Streams

Oligochaetes and chironomids were the most abundant taxa encountered (each totaling over 46,000 individuals), occurring at every site. The 5 most frequently encountered invertebrate families present among all the streams were Chironomidae, Oligochaeta (Naididae), Asellidae, Elmidae, and Sphaeriidae (last three all under 10,000 individuals). The overall number of taxa encountered in the study is summarized in Appendix C.

The streams within Essex and Lower Thames regions differed in that a larger proportion of LTVCA streams contained visible riffles (8/12) than did ERCA sites (1/7) (Appendix D). Streams in ERCA region had a mean±SD richness of 8.66±4.90 families (n=50) whereas LTVCA streams had 12.96±6.05 families (n=67; based on all 152 samples for each region).

Trends in biodiversity between ERCA and LTVCA streams (Appendix D) were similar, with some exceptions. McCarson Drain (LTVCA) was unique in supporting a variety of aquatic macrophytes. It was the only site at which emergent and rooted floating macrophytes were abundant. Sturgeon Creek had the lowest family richness (13), and McCarson Drain had the greatest number of families (43).

The invertebrates most frequently encountered in the streams regardless of abundance were Chironomidae, Oligochaeta, and Asellidae, each occurring in 19, 19, and 17 stream sites respectively. Chironomidae, Oligochaeta, Elmidae were the most abundant taxa in 17 streams. All the other macroinvertebrate families that we in the top 5 most frequently encountered were only captured in 6 or fewer streams (Table 2.3). Oligochaeta or Chironomidae were the most abundant taxa collected with the Petite Ponar grab; and were often more abundant than what the D-net collected. In contrast, the D-net samples typically had a larger variety of abundant taxa; Corixidae, Oligochaeta, Sphaeriidae, Chironomidae, Elmidae, Gammaridae. Most taxa were more abundant (total count) in the D-net samples than in Ponar grabs (Fig. 2.1) except for Chironomidae, Oligochaeta, and other invertebrates that are typically found within the substrate as opposed to being epibenthic.

Biodiversity differences due to Sampler Type

Overall, mean \pm SD family richness estimated from D-net samples (22.9 \pm 8.1; n=19) was greater than richness estimated from Petite Ponar grabs (20.4 \pm 7.3; Fig.2.1). Mean richness estimated from the 3 D-net samples exceeded richness estimated by the 5 Petite Ponar grabs in 16 of the 19 streams sampled (Fig. 2.1). Overall the difference in richness was statistically significantly different between sampler type when accounting for among stream variation (main effects ANOVA, F=2.78, $p>0.05$; Table 2.2). However, when among-stream variation in overall family richness was incorporated as a covariate, there was a significant effect of sampler type on richness (ANOVA, F=4.135, $p<0.04$; Table 2.3; Fig. 2.3). The slopes of the two regression equations were not significantly different (D-net: $y = -1.7475 + 0.8467*x$, and ponar: $y = -2.3343 + 0.78*x$). but the intercepts were significantly different (F= 4.136; $p = 0.048$; Table 2.3). Thus, across all the levels of among-stream richness 3 D-net samples collected a significantly larger proportion of the families present in a stream (85%) than the 8 Petite Ponar grabs (78%). A categorized scatterplot of family richness in individual samples illustrated the consistency of the differences (Fig. 2.3). The estimated percentage of the families collected by a single D-net (58%) and by a single ponar grab (44%) shows the D-net is closer to what is available in the entire stream (Figure 2.4).

Invertebrate Abundance Collected with D-net and Petite Ponar.

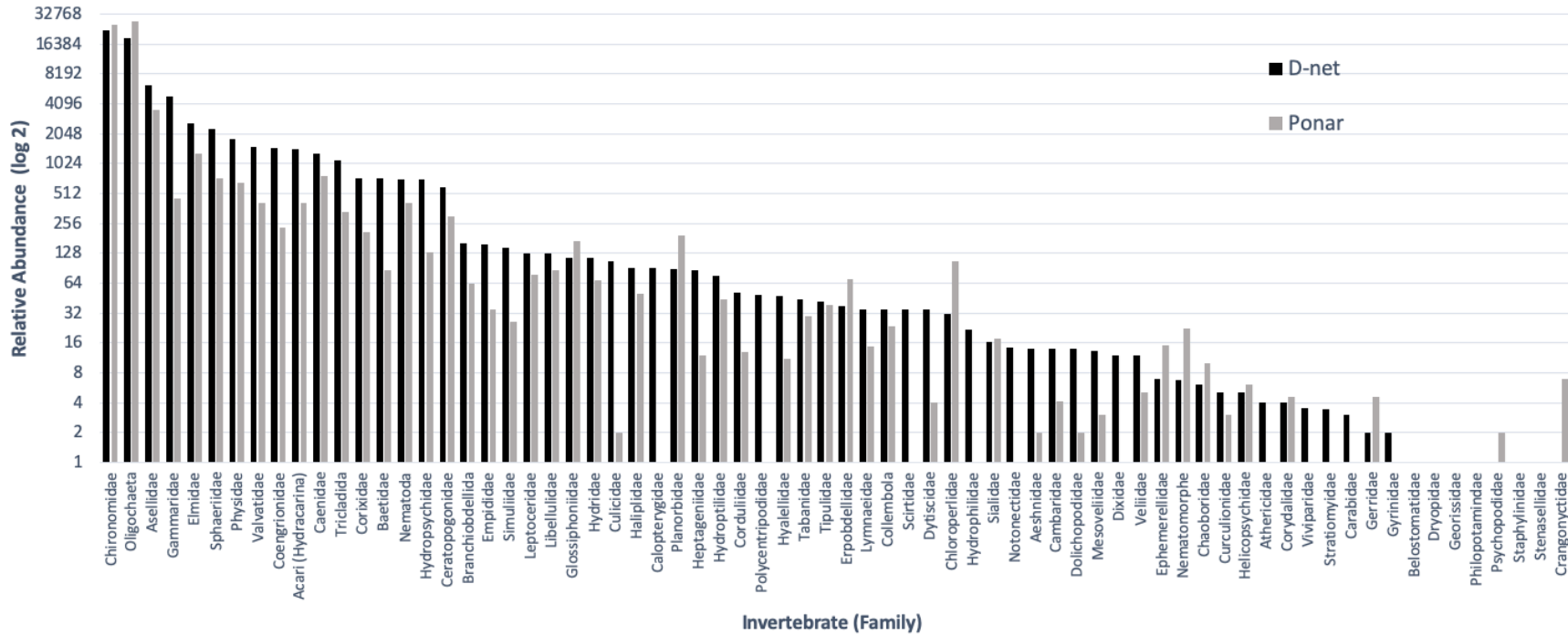


Figure 2.1 Bar graph of the mean abundance of invertebrates (the sum of all individuals collected by each sampler) arithmetically averaged across all streams) collected using either the D-net (black) or the ponar (grey). Note the octave (Log₂) scale.

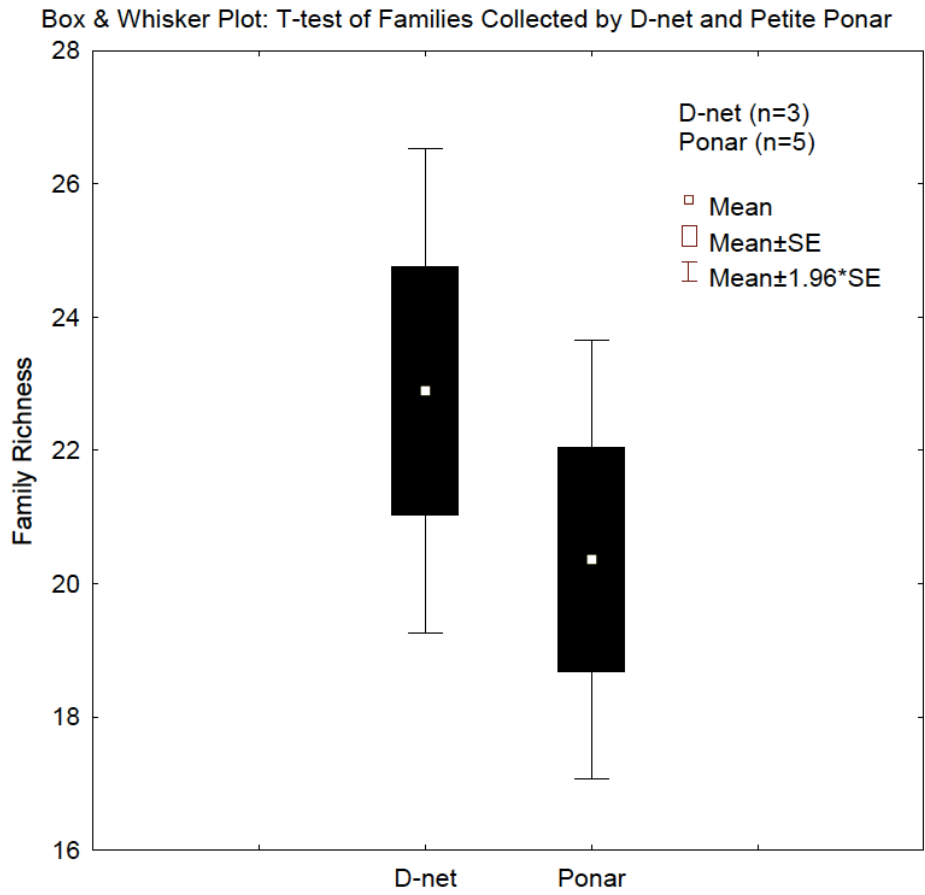


Figure 2.2: Arithmetic mean \pm SE invertebrate family richness of D-net and Ponar samplers. There is a significant difference between sampling devices (ANOVA, $F=4.14$, $p=0.050$).

Table 2.2: Main effects ANOVA table illustrating effects of sampler types and streams on family richness; samples pooled together based on sampler type.

Effect	D.F	SS	MS	F	<i>p</i>
Stream	18	1627.07	1627.07	110.98	0.001**
Sampler	1	60.63	60.63	4.14	0.050*
Discrepance	18	513.15	14.66		
Total	37	2200.85			

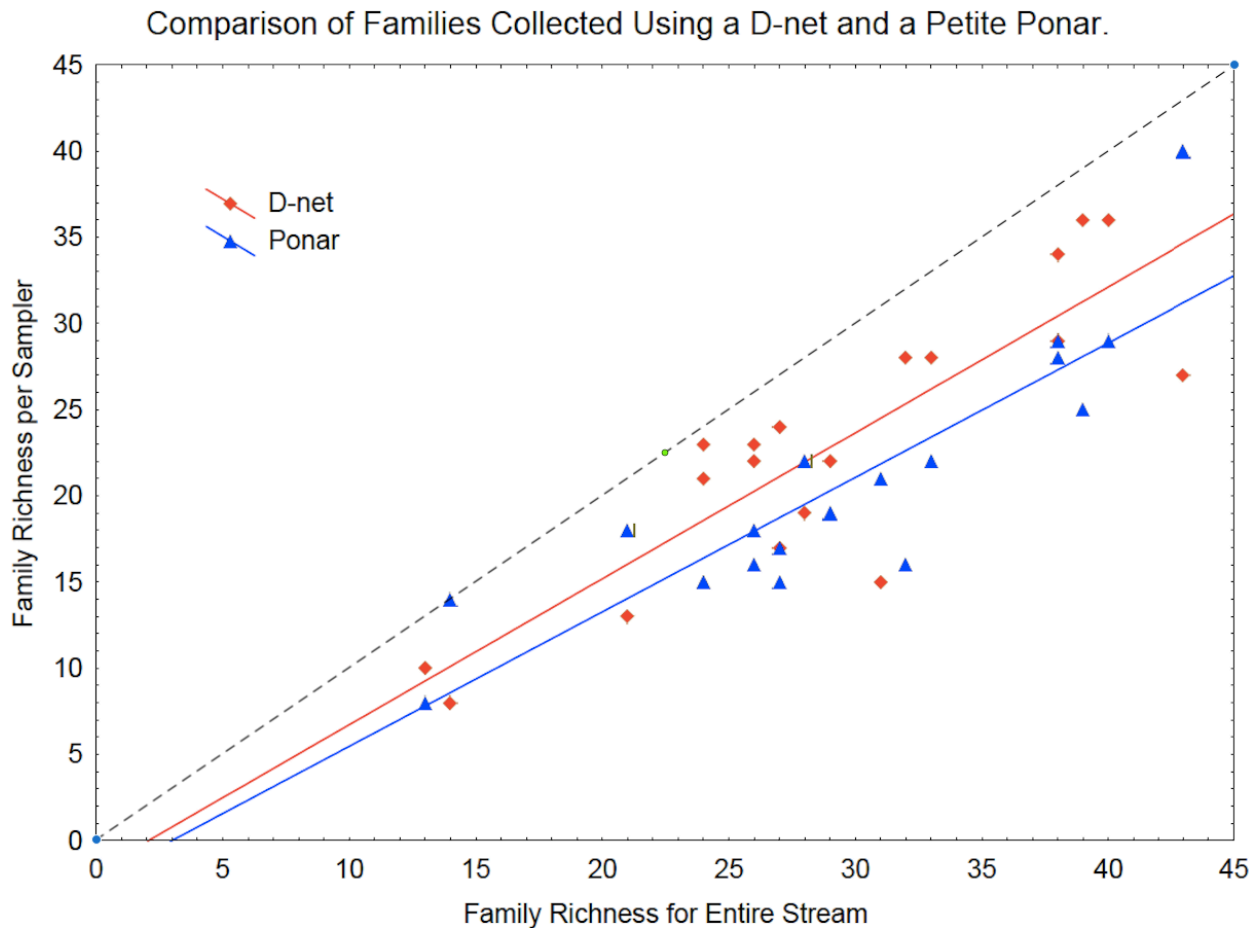


Figure 2.3: Number of families collected from D-net (red diamonds, n=3) and Petite Ponar grabs (blue triangles, n=5) as a function of stream-specific family richness. Dotted line represents equal richness. D-net: $R^2 = 0.75$; $SE = 7.89$; $y = -1.7475 + 0.8467x$. Petite Ponar: $R^2 = 0.78$; $SE = 10.07$; $y = -2.3343 + 0.78x$. Each point represents one of the 19 streams sampled in 2016 (Table 2.1).

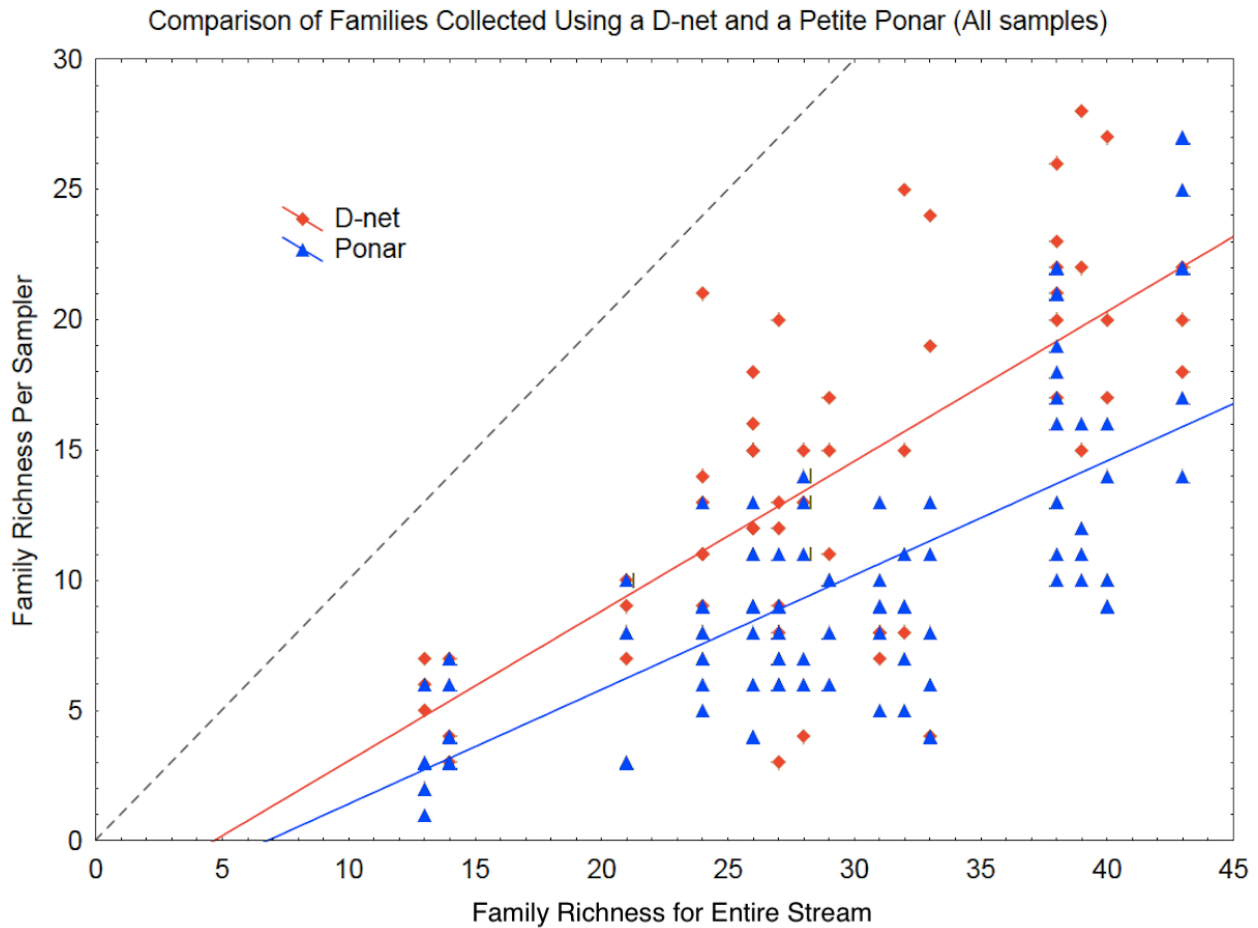


Figure 2.4 Number of families collected from D-net (red diamonds, n=3) and Petite Ponar grabs (blue triangles, n=5). Dotted line represents equal richness. D-net: $R^2=0.4883$; $SE=18.28$; $y = -2.6852 + 0.5751 \cdot x$. Petite Ponar: $R^2=0.5202$; $SE=20.33$; $y = -2.9754 + 0.4389 \cdot x$. Each point represents one sample collected in each stream of the 19 streams sampled in 2016.

Overall, the D-net collected more families in 13 streams and in only 5 streams did the ponar collect the most (mean \pm SD= 5.5 \pm 3.7; ANOVA, F=2.782). The largest difference in family richness between D-net and Petite Ponar samplers was observed where the Petite Ponar collected 13 more families than the D-net. The next greatest difference was where the D-net collected 12 more families than the Petite Ponar.

The 5 most common animals collected using the D-net were Chironomidae (22,546 individuals), Oligochaeta (18,798), Asellidae (6,597), Gammaridae (4,894), and Elmidae (2,765). The five most common for ponars were Oligochaeta (27,598), Chironomidae (25,343), Asellidae (3,516), Elmidae (1,276), and Caenidae (762). (Table 2.3).

According to the rarefaction curve (Figure 2.5) each of the three of the D-net samples provides the largest increase in the mean number of macroinvertebrate families, indicating that they would provide more information (invertebrates) than if the ponar samples were included next. The mean \pm SD proportion of the richness collected by D-net 1 (riffle habitat) was, 0.47 \pm 0.13; when combined with another sample (D-net 3 (riffle)) the mean \pm SD rose to 0.70 \pm 0.16, and if a third sample was combined (D-net 2 (pool)) the mean \pm SD was 0.77 \pm 0.17. D-net 1 is significantly different from any other sample, and D-net 3 is significantly different from all but D-net 2. A plateau is reached at Ponar 4, which was also in a riffle, and is not significantly different than the previous sample (D-net 2) as shown in Table 2.4. There was no significant difference among the cumulative values for any of the ponar samples.

Table 2.3: The 5 most abundant taxa collected by each sampler in each stream. Abundances are based on values extrapolated from the mass of sorted detritus relative to the unsorted biomass in the 4.0, 1.0, and 0.5 mm sieve size fractions.

ERCA

Belle River	D-net (91% of 734 animals)	Ponar (98% of 923 animals)
	1. Corixidae 333	1. Oligochaeta 555
	2. Chironomidae 154	2. Chironomidae 240
	3. Oligochaeta 131	3. Corixidae 52
	4. Elmidae 31	4. Elmidae 44
	5. Acari (Hydracarina) 18	5. Ceratopogonidae 14
Little River	D-net (99% of 4090 animals)	Ponar (96% of 8714 animals)
	1. Oligochaeta 3595	1. Oligochaeta 8299
	2. Chironomidae 475	2. Ceratopogonidae 42
	3. Sphaeriidae 12	3. Nematoda 28
	4. Ceratopogonidae 3	4. Sphaeriidae 16
	5. Nematoda 2	5. Planorbidae 4
Muddy Creek	D-net (88% of 3426 animals)	Ponar (97% of 1156 animals)
	1. Sphaeriidae 1533	1. Oligochaeta 693
	2. Oligochaeta 859	2. Sphaeriidae 326
	3. Nematoda 384	3. Asellidae 44
	4. Chironomidae 145	4. Nematoda 33
	5. Culicidae 101	5. Chironomidae 25
Sturgeon Creek	D-net (98% of 376 animals)	Ponar (100% of 660 animals)
	1. Oligochaeta 184	1. Oligochaeta 615
	2. Coenagrionidae 125	2. Chironomidae 37
	3. Chironomidae 47	3. Coenagrionidae 3
	4. Collembola 10	4.2 Nematomorphe 1
	5. Hydracarina 4	4.2 Tricladida 1
		4.2 Collembola 1
		4.2 Sphaeriidae 1
		4.2 Dolichopodidae 1
Turkey Creek	D-net (99% of 6920 animals)	Ponar (98% of 2678 animals)
	1. Oligochaeta 5495	1. Oligochaeta 1789
	2. Chironomidae 935	2. Chironomidae 717
	3. Ceratopogonidae 192	3. Nematoda 60
	4. Branchiobdellida 146	4. Ceratopogonidae 29
	5. Planorbidae 52	5. Branchiobdellida 21
West Branch Drain	D-net (87% of 1238 animals)	Ponar (97% of 3627 animals)
	1. Chironomidae 439	1. Oligochaeta 2317
	2. Asellidae 277	2. Chironomidae 977

	3. Oligochaeta 271	3. Sphaeriidae 76
	4. Gammaridae 56	4. Planorbidae 71
	5. Physidae 37	5. Elmidae 69
Wigle Creek	D-net (90% of 1228 animals)	Ponar (94% of 610 animals)
	1. Chironomidae 616	1. Chironomidae 295
	2. Oligochaeta 227	2. Oligochaeta 220
	3. Caenidae 130	3. Acari (Hydracarina) 30
	4. Coenagrionidae 82	4. Caenidae 14
	5. Elmidae 45	5. Hydridae 12

LTVCA

Big Creek	D-net (69% of 6015 animals)	Ponar (80% of 2017 animals)
	1. Oligochaeta 1450	1. Oligochaeta 870
	2. Coenagrionidae 988	2. Asellidae 288
	3. Physidae 817	3. Caenidae 199
	4. Gammaridae 532	4. Coenagrionidae 144
	5. Asellidae 371	5. Physidae 108
Hendry Drain	D-net (95% of 1049 animals)	Ponar (96% of 2682 animals)
	1. Chironomidae 546	1. Chironomidae 1323
	2. Oligochaeta 183	2. Oligochaeta 991
	3. Caenidae 112	3. Caenidae 119
	4. Elmidae 101	4. Elmidae 101
	5. Sphaeriidae 52	5. Nematoda 33
McCarson Drain	D-net (87% of 8857 animals)	Ponar (88% of 6620 animals)
	1. Chironomidae 3408	1. Chironomidae 3503
	2. Oligochaeta 1585	2. Oligochaeta 1452
	3. Valvatidae 1508	3. Valvatidae 403
	4. Tricladida 742	4. Tricladida 270
	5. Acari (Hydracarina) 471	5. Acari (Hydracarina) 209
Natural Watercourse (C)	D-net (98% of 846 animals)	Ponar (99% of 1774 animals)
	1. Chironomidae 663	1. Chironomidae 1059
	2. Oligochaeta 89	2. Oligochaeta 623
	3. Elmidae 60	3. Elmidae 41
	4. Acari (Hydracarina) 8	4. Nematoda 17
	5. Glossiphoniidae 6	5. Corixidae 9
Natural Watercourse (NE)	D-net (93% of 2842 animals)	Ponar (97% of 7820 animals)
	1. Chironomidae 2154	1. Chironomidae 6289
	2. Elmidae 185	2. Oligochaeta 919

	3. Oligochaeta 126 4. Asellidae 108 5. Corixidae 75	3. Elmidae 183 4. Asellidae 122 5. Corixidae 66
Newbiggen	D-net (83% of 2863 animals)	Ponar (90% of 1490 animals)
	1. Elmidae 781 2. Chironomidae 540 3. Hydropsychidae 395 4. Caenidae 387 5. Baetidae 264	1. Chironomidae 653 2. Elmidae 260 3. Caenidae 188 4. Oligochaeta 157 5. Chloroperlidae 83
Sharon Creek	D-net (67% of 1205 animals)	Ponar (93% of 1451 animals)
	1. Chironomidae 306 2. Tricladida 177 3.5 Baetidae 125 3.5 Hydropsychidae 125 5. Asellidae 77	1. Chironomidae 949 2. Oligochaeta 238 3. Hydropsychidae 67 4. Sphaeriidae 49 5. Asellidae 41
Sixteen Mile Creek	D-net (94% of 4502 animals)	Ponar (97% of 2587 animals)
	1. Gammaridae 3046 2. Oligochaeta 416 3. Chironomidae 403 4. Elmidae 265 5. Acari (Hydracarina) 86	1. Oligochaeta 1550 2. Chironomidae 706 3. Gammaridae 113 4. Elmidae 109 5. Sphaeriidae 33
South Dales Creek	D-net (94% of 14504 animals)	Ponar (97% of 15212 animals)
	1. Chironomidae 5475 2. Asellidae 3826 3. Oligochaeta 2763 4. Gammaridae 973 5. Elmidae 639	1. Chironomidae 6251 2. Oligochaeta 5573 3. Asellidae 2529 4. Elmidae 286 5. Gammaridae 151
Talbot Creek	D-net (96% of 905 animals)	Ponar (98% of 1355 animals)
	1. Chironomidae 355 2. Oligochaeta 221 3. Caenidae 125 4. Acari (Hydracarina) 123 5. Elmidae 47	1. Chironomidae 834 2. Oligochaeta 392 3. Caenidae 56 4. Elmidae 25 5. Acari (Hydracarina) 17
Two Creeks	D-net (95% of 9065 animals)	Ponar (93% of 2045 animals)
	1. Chironomidae 5386 2. Asellidae 1527 3. Oligochaeta 782 4. Physidae 743 5. Nematoda 149	1. Chironomidae 662 2. Physidae 503 3. Asellidae 392 4. Oligochaeta 309 5. Gammaridae 42

White Ash Creek	D-net (89% of 659 animals)	Ponar (82% of 690 animals)
	1. Elmidae 214	1. Chironomidae 445
	2. Simuliidae 131	2. Hydropsychidae 36
	3. Hydropsychidae 104	3. Elmidae 31
	4. Chironomidae 81	4. Oligochaeta 30
	5. Baetidae 57	5. Caenidae 24

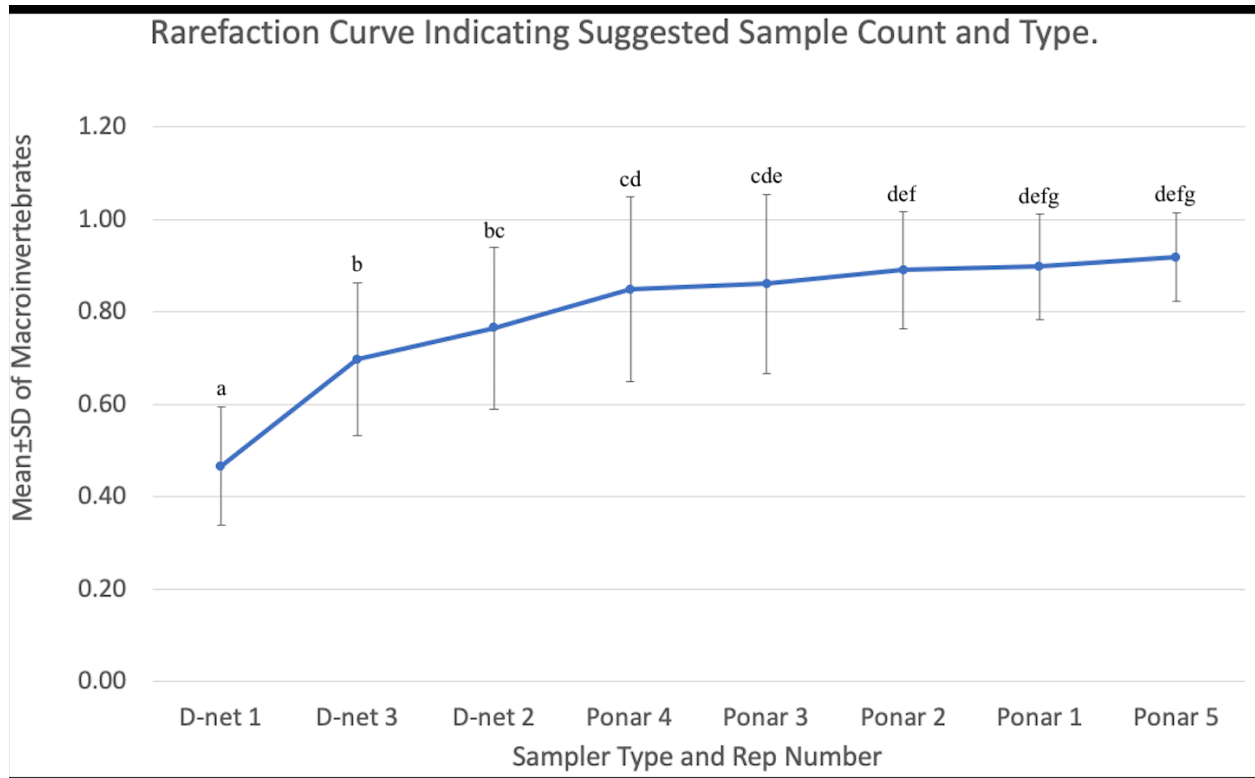


Figure 2.5 Rarefaction curve of the number of samples that should be collected in the field. Mean±SD values of richness based on 19 sites from 2016 sampling year. Letters represent a significant difference for that sample. Samples with the same letter indicates no significant difference between them. Calculations illustrated in Table 2.4.

Table 2.4: Table of significance values between difference samples using a t-test. Bolded numbers indicate statistical significance. ($\alpha=0.05$)

	D-net 1	D-net 3	D-net 2	Ponar 4	Ponar 3	Ponar 2	Ponar 1	Ponar 5
D-net 1		0.00003	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
D-net 3			0.22392	0.01557	0.00835	0.00029	0.00012	0.00002
D-net 2				0.18192	0.12191	0.01721	0.00951	0.00239
Ponar 4					0.85336	0.45094	0.35700	0.18330
Ponar 3						0.58129	0.47233	0.25546
Ponar 2							0.84139	0.45006
Ponar 1								0.56218
Ponar 5								

An NMDS ordination was performed to illustrate the relationship between the ponar and D-net invertebrate collections (Figure 2.6; stress=11.25). An NMDS represents the distribution of communities in relation to one another in a multidimensional space. It incorporates multiple variables in reduced dimensionality that is more easily interpreted. The dimensions then are a reflection of the variables that were more or less related with each invertebrate spreading them across the dimension. What is plotted are the streams associated with the invertebrates along that dimension. A cloud of points representing the different streams can be compared to each other.

The taxa whose relative abundances were most highly correlated with the two dimensions were Elmidae, Hydropsychidae, Baetidae, Empididae, Tipulidae, Simuliidae, Hydroptilidae, (positively associated with scores of Dimension one; Appendix B) and Branchiobdellida, and Oligochaeta (negatively correlated with scores of dimension one). Acari, Elmidae and Heptageniidae were most highly positively correlated with Dimension two scores, and Glossiphoniidae, Planorbidae, Ceratopogonidae, Asellidae, Physidae, Sphaeriidae, Erpobdellidae, Nematoda, Mesoveliidae, and Lymnaeidae were negatively correlate with scores of dimensions two . The vectors created by connecting the D-net sample point with its Petite Ponar counterpart for each stream tended to be oriented in a bottom left to top right direction, indicating that Elmidae, Hydropsychidae, Baetidae, Empididae, Tipulidae, Simuliidae, Hydroptilidae, Acari, and Heptageniidae were relatively more abundant in D-frame dip net samples than in Ponar grabs, whereas the Ponar grab samples had greater relative abundances of Branchiobdellida, Oligochaeta, Glossiphoniidae, Planorbidae, Ceratopogonidae, Asellidae, Physidae, Sphaeriidae, Erpobdellidae, Nematoda, Mesoveliidae, and Lymnaeidae.

The vectors for three pairs of streams cross each other (Sharon and 16 Mile Creek, West Branch and South Dales Creek, and Talbot Creek and Belle River), indicating that interpretation

of the community compositional similarities between these pairs would depend on the method by which they had been sampled. Otherwise, the ordination indicates that each stream was distinctive enough that Ponar Grab sampling collects community composition similar to D-net sampling. The fauna are similar in McCarson Drain and Turkey Creek between each sampler since their points are close together.

Three axes captured most of the variation in the macroinvertebrate communities in streams sampled in 2016 (n=19) across southwestern Ontario. Higher dimensions did not further reduce the stress to improve the model. The final instability for a 3-dimensional solution is 0.00000 and the number of iterations was 64.

An NMDS ordination plot was created for all of the samples showing the relationship between D-net for both the D-net and ponar combined (Figure 2.7). Only 1 pair of streams cross in this ordination (Sharon Creek and McCarson Drain), illustrating that the information provided by including Petite Ponar grab samples did not alter the pattern of community composition derived from D-net sampling alone. The final stress was = 12.42, for a 3-dimensional solution. South Dales Creek and Muddy Creek are the two streams that illustrate similarities in fauna collected since their points on the plot are close together.

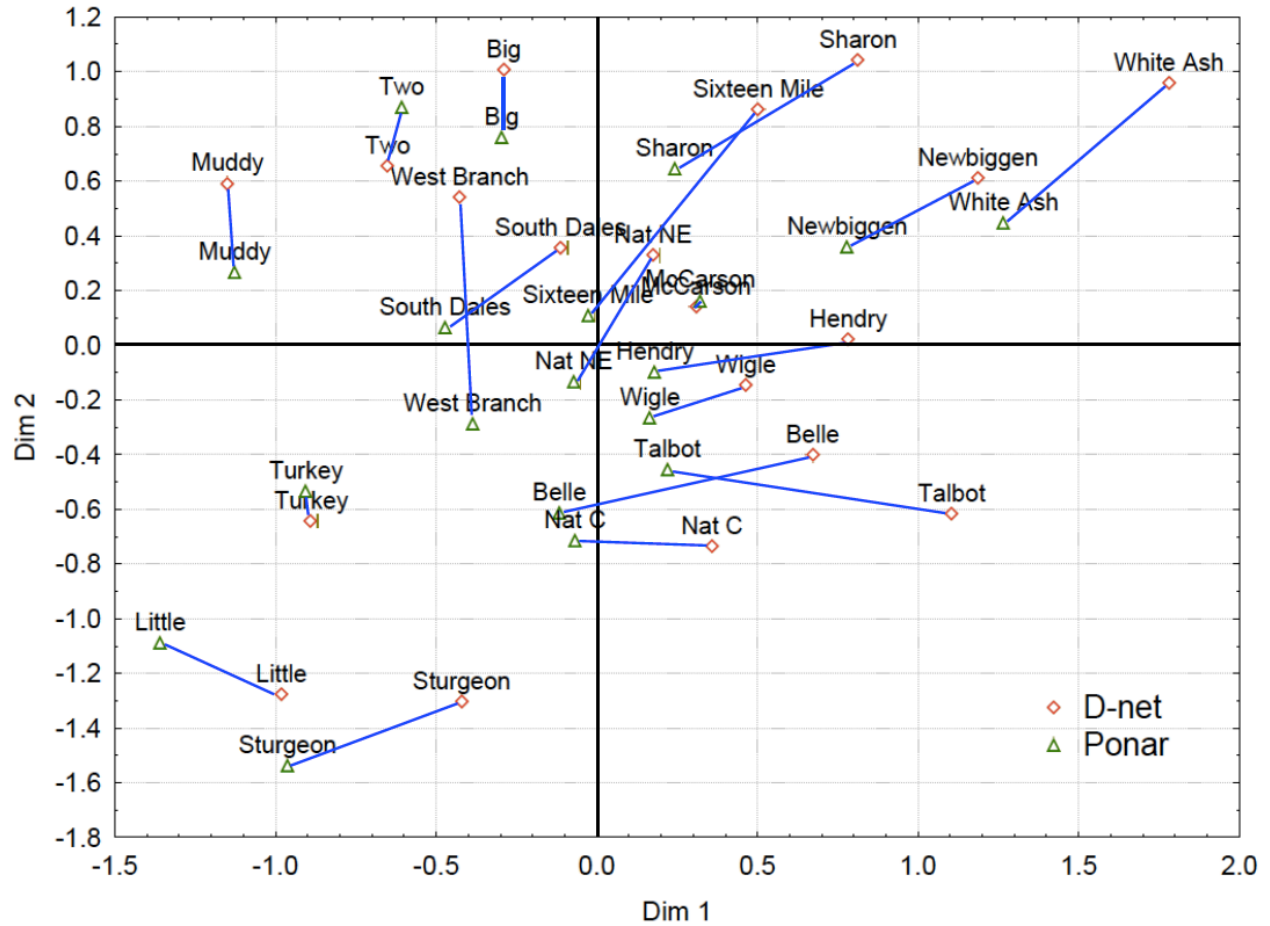


Figure 2.6: NMDS ordination plot showing the relationship between D-net and ponar collections for each stream based on invertebrate community compositions. Stress = 11.25, dim=3. Lines connect the D-net and Ponar grab samples from each stream. Sampler type influences interpretation of stream community composition only for pairs of streams whose lines cross.

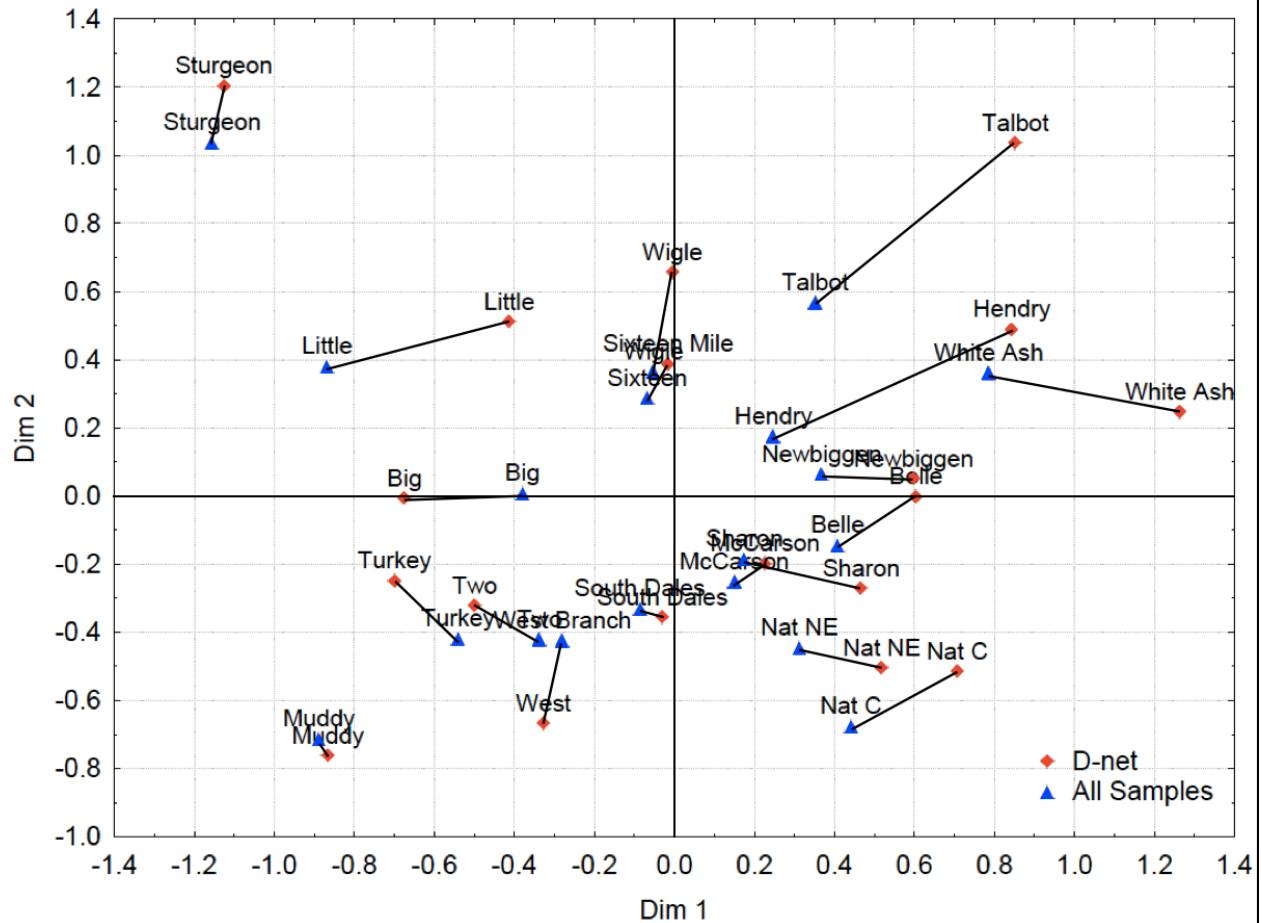


Figure 2.7: NMDS ordination plot showing the relationship between D-net and all samples collected (both D-net and ponar combined) for each stream based on invertebrate community compositions. Stress = 12.42, dim=3.

Sampler Effectiveness with Biotic Indices.

Table 2.5 shows the Hilsenhoff Biotic Index (HBI) scores calculated for the taxa found in each sampler separately and when they are combined. A scatterplot and regression based on these scores was performed for the D-net samples and the Petite Ponar grab samples individually compared to the scores found when all samples are combined (Figure 2.8 (D-net), Figure 2.9 (Ponar)). A paired comparison test showed there was a significant difference between the HBI scores of the D-net and the Petite Ponar samples ($p < 0.012$, Table 2.6).

A one-way ANOVA comparing HBI scores was performed for all the samples combined and it was found that there was a significant difference between streams located in the Essex region when compared to the streams found in the Lower Thames region (ANOVA, $p < 0.05$, Table 2.7, Figure 2.10).

Table 2.5: Hilsenhoff Biotic Index scores calculated from D-net, Ponar grab and combined samples for 2016 streams.

ERCA

Stream	D-net & Ponar combined	D-net only	Ponar only
Belle River	7.43	6.23	8.36
Little River	9.74	9.50	9.84
Muddy Creek	8.42	8.18	9.09
Sturgeon Creek	9.35	8.66	9.66
Turkey Creek	9.29	9.41	8.93
West Branch Drain	8.96	9.78	8.75
Wigle Creek	7.19	6.96	7.67

LTVCA

Big Creek	9.01	8.72	9.76
Hendry Drain	7.31	6.58	7.54
McCarson Drain	7.14	7.14	7.15
Natural Watercourse (C)	7.09	6.36	7.43
Natural Watercourse (NE)	6.56	6.44	6.60
Newbiggen	5.72	5.62	5.93
Sharon Creek	6.20	6.59	6.86
Sixteen Mile Creek	8.01	7.15	8.48
South Dales Creek	9.81	10.35	9.42
Talbot Creek	7.14	7.08	7.17
Two Creeks	8.58	8.40	9.42
White Ash Creek	5.63	5.32	5.93

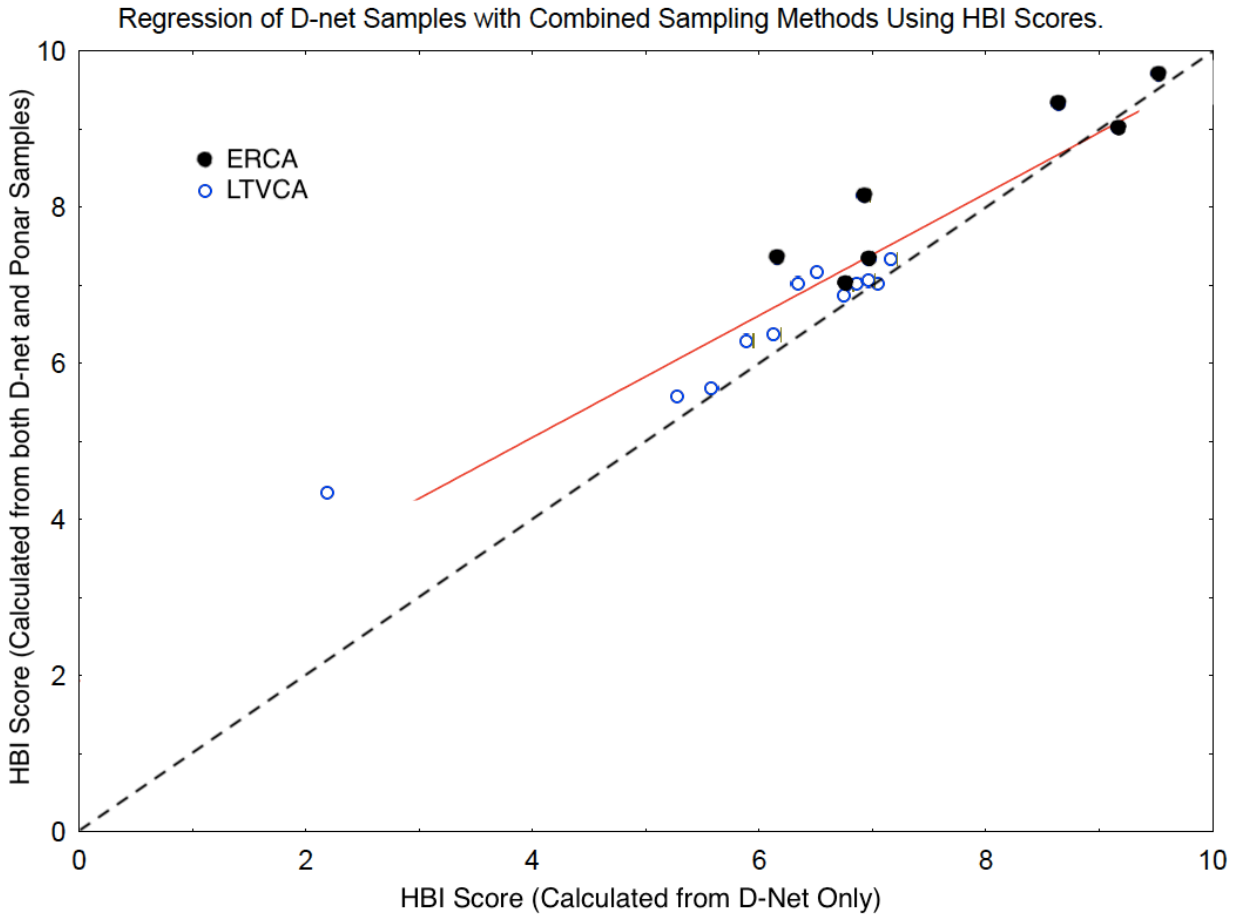


Figure 2.8: Scatterplot and regression of the Hilsenhoff scores for samples from 19 streams (ERCA: solid black circle; LTVCA: open black circles) collected using only the D-net compared to scores calculated for all samples from a stream combined (D-net and ponar samples combined). $Y = 1.929 + 0.7809x$. $R^2 = 0.8848$, $R = 0.9406$, $p = <0.001$ The dashed line represents expected perfect correspondence. $R^2 = 0.8923$, $r = 0.9446$, $p = 0.0000000001$, $y = 1.4108 + 0.8429x$.

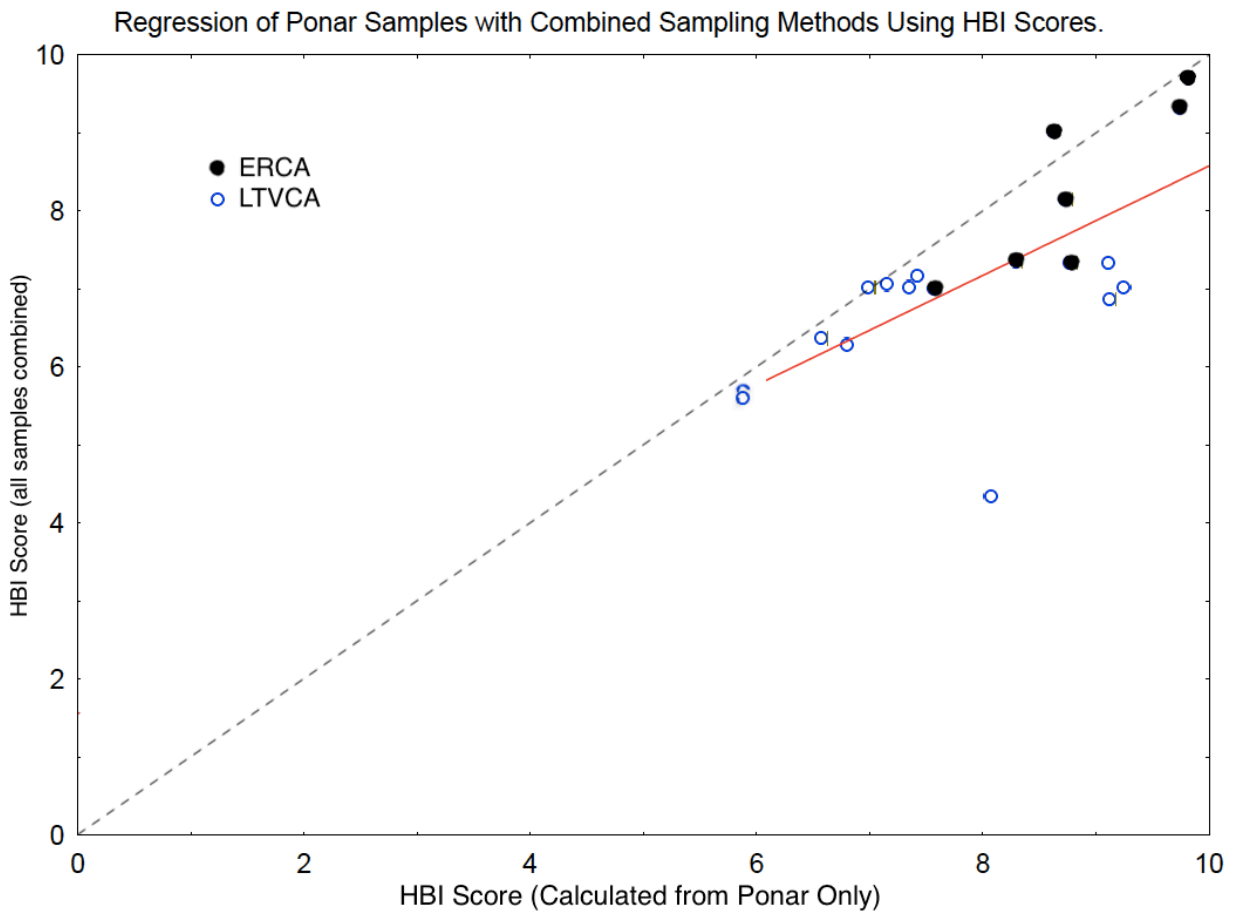


Figure 2.9: Scatterplot and regression of the Hilsenhoff scores for samples collected using only the ponar compared to scores for all samples combined (D-net and ponar samples combined). ERCA: solid black circles, LTVCA: open blue circles. $y=1.5605+0.7017*x$. $R^2 = 0.4453$, $R = 0.6673$, $p = 0.0018^{***}$

Table 2.6: Paired comparison test of difference between HBI calculated from D-Net samples vs. Petite Ponar grab samples. The mean (\pm SE) difference was -0.50 ± 0.18 (n=19), which was significantly different from zero $t = 2.8$, $p < 0.012$.

Mean Diff.	SD.	N	SE.	DF	t-value	p
-0.501053	0.778288	19	0.178552	18	-2.80621	0.011680

Table 2.7. One-way ANOVA comparing Hilsenhoff Biotic Index scores for Essex Region Conservation Area streams (n=7) with Lower Thames Valley Conservation Area streams (n=12)

	D.F.	SS	MS	F	p
ERCA vs. LTVCA	1	7.195	7.195	5.028	0.039
Error	17	24.328	1.431		
Total	18	31.523			

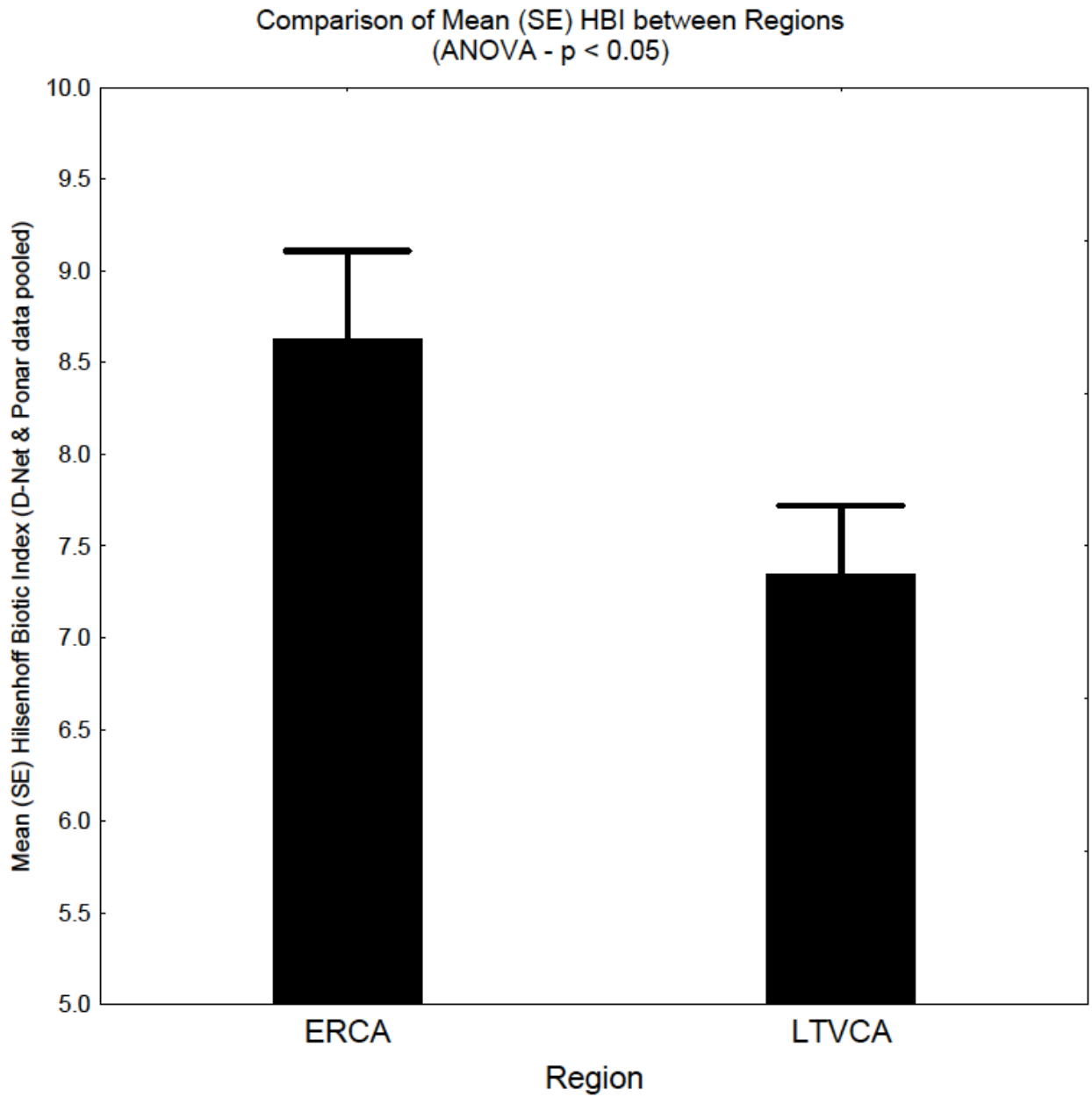


Figure 2.10: The mean \pm SE of the Hilsenhoff Biotic Index Scores (HBI) between the Essex County and Lower Thames Regions (ERCA and LTVCA respectively). There is a significant difference between these regions (ANOVA, $F=5.028$, $p=0.039$).

Discussion

Streams in the southwestern Ontario region are unique in that traditional samplers like the D-net may be difficult to use. Since streams in the study region do not follow the stereotypical rocky-substrate and instead have fine-grained, muddy substrates, it was thought that a Petite Ponar grab may be more effective than a D-frame net at collecting a representative sample of the macroinvertebrate community. There were highly significant differences in family richness which reflects inherent differences among streams, in that no two streams are exactly alike. Certainly, overall abundance of invertebrates varied greatly among streams (Table 2.3), and so D-net samples were found to collected significantly higher richness of invertebrates than the Petite Ponar grabs (ANOVA Table 2.2). It is noteworthy that even though more Ponar grab samples than D-net samples were collected, the D-net collected a greater number of invertebrate families per stream. Across all 19 streams the invertebrates that were collected using the D-net but not the Petite Ponar were Calopterygidae, Polycentropodidae, Notonectidae, Dixidae, Athericidae, Carabidae, Belostomatidae, Dryopidae, and Stenasellidae. The only family of invertebrates that was collected by the Petite Ponar but not the D-net was Crangonyctidae. (Figure 2.1). This may be because the D-nets sampled a variety of the microhabitats present across the entire width of the stream (on average 2.75 meters sampling track) and amongst the water column, whereas the Ponar grabs collected only a smaller portion of the stream's habitat diversity (collected 15x15x15 cm³ volume within the sediment, not along the water column and not along the entire width of the stream). Carter and Resh (2006) described larger samples as beneficial when collecting from microhabitat patches, allowing widespread degradation across the stream to be detected, which can be more informative than interpreting one sample representing an entire stream. Stark et al. (2001) also concluded that in soft-bottomed streams multiple habitats should be sampled using semi-

quantitative methods (i.e. kick-net) in an area of 3 m² since it may provide important information on environmental condition and no one area can represent the other microhabitats in that stream.

The rarefaction curve (Figure 2.5) illustrated that the D-net samples contributed the most to the biodiversity and richness estimates. A single D-net sample collected ~45% of the mean richness, 2 D-net samples collected an average of 70%, and 3 samples acquired just under 80%. Collecting until at least 70% of the total number of invertebrate taxa are detected is a common cut off point that allows a practical coverage of present taxa and minimizes the time spent going through samples (Mackey 2006). Furse (1981) found that within the first three samples, 62, 78, and 87% of families were collected, and Morgan and Eglishaw (1965) found that 51-87% of the total number of species were found within the first two kick-samples and an additional 9-36% were found in the next two samples. Thus, I recommend collecting 3 D-net samples from a stream -two in riffles and one in a pool because this strategy provides the greatest cumulative richness before reaching a plateau (approximately 90% of the total) and ensures that a representative sample of the invertebrate community is collected. Merritt and Cummins (1996) suggest that the numbers of samples depend on the site and the type of study, but generally with samples that have a low invertebrate density more samples are needed. Since the cumulative richness added by Petite ponar sample #4 was not significantly greater than from D-net sample #2 nor from any of the other Petite ponar samples (Table 2.4), I conclude that 3 samples per stream are sufficient for general bioassessment.

It also appears that samples taken in riffle/glide habitats have a higher mean richness than those taken in pool habitats. Even though riffles and pools were difficult to identify, they still seem to play a role in richness. Carter and Resh (2006) found that most sampling protocols (63.4%) only involved sampling from one habitat type (riffles: 25.6% and riffle and run: 24.4%) since these are

also considered the areas where there is high species richness. This is evident in Figure 2.5. However, since these are soft-bottomed streams, multiple microhabitats should be sampled as well (Carter and Resh 2006; Stark et al. 2001; Poulton et al. 2003).

Poulton et al. (2003) compared how well a rock basket artificial substrate, a kicknet, and a Petite Ponar performed - the former two methods in rocky habitats and the latter behind wing dikes. They found that kicknets collected a larger mean number of taxa that were in the community (88.4%) and that community composition was similar in rock baskets and kicknet methods, where 75.3% of the taxa were the same. The rock baskets and Ponar were similar in 73.1% of the taxa the yet was speculated that invertebrates captured with the Ponar grab in the slower flowing waters had been transported there due to drift (Poulton et al. 2003). Overall kicknets collected a higher richness in less-tolerant organisms, whereas the Ponar collected a higher-tolerant organisms, however the ponar collected the most unique taxa (Poulton et al. 2003). In contrast, I collected only one (relatively uncommon) family exclusively with Petite ponar sampling. In conclusion, the qualitative method of the D-net is more effective and provides a greater estimate of taxa richness than using the Petite Ponar grab. For the clay plains of Essex and Lower Thames, 3 samples should be collected, 2 in riffle habitats and one in a pool to generate a sufficiently representative collection of the macroinvertebrates present.

Examination of the most common taxa found among streams by each sampler indicated that more families were collected by the D-net than by the ponar. This was especially evident when only looking at the single most abundant family for each of the streams (Table 2.4, see also Figure 2.1, illustrating that chironomids and oligochaetes are the most abundant invertebrates). This may be because the Ponar collects quantitative samples and was consistently deployed in the same habitat among streams (within the sediment). Chironomidae can be abundant and species-rich,

especially in pool habitats (Ferrington et al. 1995), and in detritus and sand (Mackay 1969). They are also used as bioindicators of water quality due to their sensitivity (Richardson 1928), and the ratio of oligochaetes to chironomids is useful to locating areas of pollution (Saether 1979).

The NMDS analysis (Figure 2.6) illustrated the distinction between the D-net and the ponar grab. Since only a few site vectors crossed, it suggests the community composition identified by the two types of samplers are not distinct relative to among stream variation in community composition. The same pattern was evident when comparing all samples to the D-net (Figure 2.7). This finding is consistent with those of Poulton et al. (2003) since ponar and D-net richness, number of taxa and mean number of taxa were not statistically significant. These results suggest that since the samplers are similar in community composition that they collect, ponar grabs need not be included in sampling procedures. The paired-comparison analysis (Table 2.8) also suggests there is a significant difference in HBI tolerance scores between the two sampling methods. It suggests the score calculated from D-net samples is 0.5 units less (less tolerant overall score) than the score calculated from Petite Ponar grab samples from the same stream. This may be a result of the collection of invertebrates that have a higher tolerance in the Petite Ponar than in the D-net. For instance, Oligochaeta has a higher tolerance score (8) and was found more frequently in the Petite Ponar.

Conclusion

When comparing the effectiveness of the D-net and the Petite Ponar, results of the analyses indicate that the D-net is more suitable for rapid bioassessment since it collects a more representative sample of the stream community (greater proportion of the families present) than the ponar. Three D-net samples should be collected. Additional Petite Ponar grab samples need not be taken because they do not add family richness that the D-net samples don't already collect.

Chapter 3: Efficiency of Laboratory Macroinvertebrate Sample Processing; A Comparison of the Marchant Box and Nested Sieves Methods.

Introduction

When stream or wetland benthic materials are collected, the benthic macroinvertebrates are typically either hand-picked from the detritus in the field, while they are living, or the entire sample is preserved, and individuals are sorted from the sample in the laboratory. Field sorting is much more time-effective than lab processing. However, field-picked samples may not be representative of macroinvertebrate community composition. Large or active organisms are more likely to be seen and selected than smaller, inactive, or cryptic individuals (Payne 2017). Lab-sorted samples are less subject to these potential biases because sorting is normally done with the aid of a dissecting microscope. Different methods have been suggested or compared (Brinkman and Duffy 1996; Fairchild et al. 1987), with aims to reduce sample sorting time by using subsampling methods or devices, (Ciborowski 1991; Marchant 1989; Wrona et al. 1982; Hickley 1975), or fixed number counts (Barbour and Gerritsen 1996; Somers et al. 1998). Finding ways to reduce processing time allows more samples to potentially be included in study designs and/or reduces research costs (Brinkman and Duffy 1996).

Various subsampling methods have been proposed to reduce the amount of time required to sort a sample. Wrona et al. (1982) proposed using an Imhoff cone with an air supply at the tip of the cone to mix the invertebrates without damaging them. Although the subsampler is easy to build, this method requires a count of 100 individuals taken from at least 5 subsamples, which is a disadvantage because the combined subsamples will often yield a count of more than 100 individuals.

Hickley (1975) proposed placing the sample on a sieve resting on top of a subsampling chamber that would randomly split the sample. A lid with a hole in it allows water to be added, which will begin to bubble and gently separate the sample once compressed air is turned on. This allows invertebrates to be subsampled and avoids them becoming damaged during washing. Even though this method allows for the division of the sample, this apparatus is expensive to build.

In Ontario, two subsampling/sorting methods are commonly used - the Marchant Box (Marchant 1989) and a stack of nested sieves (e.g., Ciborowski 1991). The former is a plexiglass box that is subdivided internally into 100 cells in which components of a sample become evenly distributed and which can then be randomly selected for sorting. A count of 300 individuals must be collected and if this is reached before the contents of the 50th cell have been enumerated the sorting may stop; otherwise, the sorter must sort the materials within the entire box (CABIN 2011; Jones 2007). The advantage is that of only a subset of the sample needs to be sorted; yet, it is time-consuming to initially spread the sample evenly across the box, and subsequently to remove subsamples from the randomly selected cells. If a cell contains few invertebrates, then time that could otherwise be spent sorting is needed to remove the many individual subsamples from the box. The Marchant Box is also heavy and difficult to manipulate if one is to comply with the recommended methods of mixing the sample by repeatedly inverting the container. Furthermore, rare organisms (those that may be present in only a few cells) may not be encountered during the subsampling process and thus are not incorporated into the analyses. Depending on the metric used, this could affect the assessment of ecological condition.

An alternative approach is the Nested Sieve-Fractioning Method. This method involves devoting differential effort to sample fractions differing in particle size. The sample is elutriated in a pan and slowly poured into the topmost of a nested stack of sieves with mesh sizes of 4.00,

1.00, 0.50, and 0.25 mm. This not only prevents the sample's denser inorganic material (which remains in the pan for separate inspection) from being incorporated into the sample, but it also facilitates sorting because the materials in a single sieve are similar in size. This, in turn, should speed the inspection process, thus reducing processing time, allowing more samples to be sorted and hence increasing the precision of information about the stream (Vinson and Hawkins 1996; Colwell and Coddington 1995; Allanson and Kerrich 1961).

The frequency with which these two methods are used varies geographically and by jurisdiction, reflecting both the sampling environment (e.g. stream size and substrate characteristics) and the history of regional sampling programs.

Great Lakes Region

Sorting protocols used by agencies adjacent to southwestern Ontario were reviewed because the streams in these jurisdictions are likely to be similar to my study area. The Michigan Department of Environment, Great Lakes, and Energy may either hand pick invertebrates from samples in the field, or use the bucket-and-swirl method in the lab, whereby a sample is placed in a bucket and stirred, which suspends lighter, fine material and leaves coarse, inorganic materials on the bucket bottom (Michigan Department of Environment, 2008). Subsamples are taken from the bucket using a small, 1-mm mesh net and sorted for approximately 20 min until a total count of 300 ± 60 organisms are acquired (without the aid of a microscope). This procedure was derived from the United States Environmental Protection Agency (USEPA) Rapid Biological Assessment Protocols (Barbour et al. 1999), Ohio Environmental Protection Agency protocols (Ohio Environmental Protection Agency 1987a, 1987b, and 1987c), Illinois biological procedures, and tested by the Michigan Department of Environmental Quality (MDEQ). The protocols are

inexpensive, and flotation and elutriation methods such as this work well for highly inorganic samples (Rosenberg et al. 1998). However, these procedures are less effective for samples collected from areas where substrates are fine-grained (mud or clay). Instead, such samples may be field-rinsed through a sieve to eliminate inorganic materials, which can make up 50% of sample volume (Rossillon 1987; CABIN Field Manual 2009).

The Ohio EPA recommends sorting samples using a Caton tray (Caton 1991), sampling at least 10% of the tray, counting to at least 500 individuals, and using a microscope at 6X to 10X magnification. This is based on the RBPs of the US EPA (Barbour et al. 1999). The Caton tray is similar to the Marchant Box in that the sample is spread evenly over a grid within a container. In this case, a tray, where any overhanging material or material that crosses the grid can be cut, and a scoop is used to remove the contents from the randomly selected grids. Material is sorted under a dissection microscope (Barbour et al. 1999). Although this method counts to a larger number of individuals providing potentially greater richness, it is still time-consuming because only small sections of the Caton tray are sampled at a time. Another limitation is that multiple-sized organic particles are present, which makes it difficult for the sorter to inspect.

Texas is another jurisdiction in which stream substrates mostly comprise clay (Miller and White 1998). The Texas Commission on Environmental Quality (TCEQ) created the Surface Water Quality Monitoring Procedures, Volume 2 (2007). This protocol involves rinsing samples through a $\leq 595\mu\text{m}$ mesh, or a No. 30 sieve, or sieve bucket ($\leq 595\mu\text{m}$) to remove preservative and fine sediments. The material is then distributed evenly over the bottom of a white pan. Subsequently, a cookie cutter or Mason jar lid is used to isolate and allow removal of 4 subsamples, which are sorted beneath a stereo dissecting microscope to a count of 140 individuals (Surface Water Quality Monitoring Procedures, Volume 2, 2007). This approach is similar to the Caton tray

and the Marchant box in that subsamples (albeit large ones) are taken and examined. Although this method might be time-efficient, the 140-specimen count criterion might not represent the full diversity of the stream community composition. This is especially true when large, rare organisms, such as crayfishes, are present.

Several national programs have developed protocols that regions can employ, using various approaches. For example, Moulton et al. (2000) compared quantitative and qualitative sample sorting methods by the U.S. Geological Surveys National Water Quality Laboratory Biological Group. The qualitative method focuses on estimating the abundance of each taxon by sorting a sample for approximately 2 h, collecting only undamaged invertebrates. The sample is elutriated in a bucket, poured over 4.75-mm aperture sieve, and the coarse material retained is examined for 15 min. The remaining (finer) material is examined for 105 min. The quantitative method consists of collecting either 100 or 300 individuals from an elutriated sample placed on a gridded subsampling frame. However, because 3 subsampling frames and 2 estimation trays can be used, this leads to increasing variation amongst sorters. Moulton et al. (2000) even state that the number of possible combinations of frames and trays is too large, may have influence the analyses, and that a more standard approach is preferred.

International: Europe, New Zealand

Hasse et al. (2004) compared the *River InVertebrate Prediction And Classification System* (RIVPACS) (Wright 2000) and *The Development and Testing of an Integrated Assessment System for the Ecological Quality of Streams and Rivers throughout Europe using Benthic Macroinvertebrates/STANDARDISATION of River Classifications* (AQEM/STAR) protocols (Furse et al. 2006; STAR consortium 2003). RIVPACS is a model that uses environmental data to predict

the macroinvertebrate assemblage expected in a location in the absence of anthropogenic environmental stress, which is a tool to assess the quality of rivers (Wright 2000). To sort samples, the investigator hand-picks invertebrates in the lab from a fraction (i.e., $\frac{1}{2}$, or $\frac{1}{4}$) of a sample without magnification, and looks through the unsorted fraction for taxa that were not found in the sorted fraction (Haase et al. 2004). AQEM/STAR aims to standardize macroinvertebrate sampling protocol and assessments. In the sorting protocol, at least 700 individuals comprising at least $\frac{1}{6}$ th of the sample are identified without magnification (Haase et al. 2004). Estimates made using the RIVPACS were highly variable in terms of the fraction that had to be sorted; and the AQEM/STAR approach was very time-consuming. Haase et al. (2004) proposed an alternative modified AQEM/STAR method (MAS method), which consisted of using a 2-mm aperture sieve. This reduced sorting time and associated costs compared to RIVPACS and AQEM/STAR. This procedure is similar to using nested sieves in that similarly sized objects remain together, which eases processing.

New Zealand recognized the challenges and created a protocol to accommodate sampling soft-bottomed streams within their National River Water Quality Network (NRWQN). Maxted et al. (2003) observed a difference in abundance of invertebrates between soft-bottomed and hard bottomed streams, and consequently recommended that soft-bottomed samples should be entirely processed to increase information obtained. Stark et al. (2001) contrasted the three major methods of processing macroinvertebrates used by New Zealand biologists - full counts, fixed counts, and coded abundance (semi-quantitative assessments of samples from hard-bottomed streams). Stark et al. (2001) prepared a manual for the Ministry for the Environment of New Zealand and elaborated on all three methods; but only the first two will be summarized. The fixed count protocol consisted of counting up to 200 individuals and then scanning for rare taxa first by

washing the sample on a 0.5-mm aperture sieve (and 4.0 mm if desired), and distributing the retained material evenly in a white, gridded sorting tray (6 cm by 6 cm). Grids are randomly selected for examination (without a microscope) until 200 individuals are reached. The entire tray is subsequently examined for rare taxa. The second method is full count enumeration with a subsampling option. Stark et al. (2001) describe the latter as time-consuming and expensive but having the benefit of allowing for a direct measure of abundance and percent composition. Thus, it can be used for comparisons of abundance or calculation of metrics.

The full count protocol consists of pouring the sample through a stack of sieves (4.0, 2.0, 1.0, 0.5 mm) and inspecting the fractions to make sure materials are separated by size appropriately. The 4.0- and 2.0-mm size fractions are examined first, without the aid of a microscope. Subsampling is an option for reducing sorting time if more than 500 individuals are present (Stark et al. 2001). Thus, using sieves reduces sample sorting time and they have been recommended for samples from both coarse, and fine-grained substrates.

As reviewed above, although sample sorting protocols vary among programs, sieves are commonly used to speed the sorting process. Ciborowski (1991) found that for samples from two stony-bottomed streams, the processing time for samples that have abundant invertebrates can be greatly reduced by subsampling or simply using a smaller sampler. Allanson and Kerrich (1961) also recommended using sieves to reduce sorting time for samples collected from streams with sandy, muddy or stony substrates.

This study investigated which of two widely used laboratory processing protocols (Marchant box and nested sieve-fractioning) was better suited to sorting the invertebrates from clay plain stream samples. The efficiency (total processing time), effectiveness (number of

individuals and family richness) and accuracy (nearness of an estimate extrapolated from a subsample to the actual number) of the two subsampling techniques were compared.

The objectives were to:

1. Compare sample total processing times of each procedure. I predicted the Sieve-Fractioning Method to require less sorting time than the Marchant Box;
2. Compare the estimated family richness of samples processed by each method. I predicted the Sieve-Fractioning Method to have a larger family richness than the Marchant Box;
3. Assess the similarity in community composition estimated from samples processed by each method.
4. Determine the relative precision and accuracy of extrapolated counts estimated from subsamples using each procedure

Methods

Method comparisons were conducted on 40 samples (3 D-frame sweep and 5 Petite Ponar grab samples collected from each of 5 streams in 2016 - Big Creek, Little River, Sharon Creek, Sixteen Mile Creek, and White Ash Creek; Table 2.1 in Chapter 2). Samples were examined in stratified-random order. Each sample was first processed using the Marchant Box method (recommended by OBBN and CABIN). Subsequently, all invertebrates and detritus were recombined and processed according to the sieve-fractioning and subsampling method of Ciborowski (1991).

Marchant Box Method

A sample was washed through a 0.50-mm aperture soil test sieve stacked on top of a 0.25-mm sieve using a gentle stream of water from a faucet equipped with an aerator. Particles retained on the 0.25-mm sieve were archived due to time constraints but may be used for other future projects. Materials in the 0.50-mm sieve were rinsed into a Marchant box (Marchant 1989), which was then completely filled with water. The box lid was secured, and the entire box inverted to distribute the debris in the sample evenly in the water. The box was quickly returned to its upright position, allowing the sample contents to randomly settle into the 100 cells. A photo was taken after each inversion to illustrate how the sample may be distributed, and the number of inversions was recorded. The material from a randomly selected cell was removed with a pipette, transferred into a Petri plate, and all invertebrates were removed and identified at 10X magnification beneath a dissecting microscope. The procedure was repeated by sampling randomly selected cells (using numbers obtained from a random number generator) until the required number of animals was recovered. Wash time and sorting time were recorded to the nearest minute.

Sorting stopped once a total of 300 animals had been found, but only if this total was achieved by examining between 5 and 50 cells. The material from the cell in which the 300th organism was found was sorted entirely. If 300 individuals were found before the 5th cell, sorting continued until the biota in at least 5 cells had been enumerated. This did not occur in my study. If more than 50 cells had to be examined, detritus from the full complement of 100 cells was sorted. The sample (detritus + invertebrates from all cells) was then recombined and subsequently sorted using the Sieve-Fractioning Method.

Nested Sieve Fractioning Method

The sample was emptied from its storage bag into a white enameled tray containing 5 cm depth of tap water, and clumps of debris were gently teased apart with a pair of forceps. A nested stack of standard soil test sieves was placed in a sink and was used to split the sample into fractions sorted according to particle size. The stack was composed of a 4.00-mm, 1.00-mm, 0.50-mm and 0.25-mm US standard brass sieves. The 0.25-mm sieve was included to incorporate materials that did not pass through the D-net or the sieve bag while sampling. The tray contents were slowly poured through the top sieve. Additional water was repeatedly added to the tray to resuspend debris that remained on the bottom of the tray, and that water (and suspended debris) was also poured into the top sieve. Once all organic material from sample had been poured onto the top sieve, a gentle stream of running water was used to wash smaller particles through the largest-aperture sieve. Materials retained on the top sieve were then rinsed back into the pan, and the process was repeated to ensure that all fine material had passed through the coarsest sieve. Subsequently, the material remaining on the 4-mm sieve was rinsed into the enameled tray, the contents poured onto a 0.18-mm sieve (to drain off the water), and the material in that sieve was emptied into a Petri dish for later inspection under the microscope. The same steps were repeated for the 1.00-mm, 0.50-mm, and 0.25-mm size fractions. Depending on the volume of the entire sample, sieves could become clogged, causing them to begin to fill with water. In such cases, I carefully lifted and separated one sieve from another and allowed the water to drain into the sieve below.

Once the size fractions had been placed in individual Petri dishes, the materials were examined under the microscope. For this study, the 0.25-mm size fraction was archived (placed into a scintillation vial with ethanol for other potential projects).

For most samples, the entire subsample was sorted, but where there was a lot of detritus and more than 300 organisms were suspected to be in a size fraction, that size fraction was quarter-sampled using a right-angled plastic wedge that would isolate $\frac{1}{4}$ of the dish contents from the remainder. Typically, the 4-mm and 1-mm size fractions were completely sorted, and the 0.5-mm size fraction was quarter-sampled. All detritus aliquots (sorted or unsorted) were kept separate and placed in an oven at 70°C and dried to constant mass (at least 24 h). The masses of both the sorted and (if applicable) unsorted sample fractions were recorded. These proportions of detritus were then used to estimate the total number of invertebrates present in a size fraction by extrapolating the number of invertebrates in the sorted fraction of detritus to the total mass of detritus in the sample.

The total processing time needed to prepare and sort each sample (sum of washing time + handling time + sorting time to achieve the appropriate criterion) by each subsampling method was recorded to the nearest minute.

Invertebrate Identification

Invertebrates were identified to the family level of taxonomic resolution using keys of Merritt et al. (2008), typically as the sample was sorted. The identification times for each sample were recorded separately from the sorting time by using a stopwatch and recording the length of time it took for each. Invertebrates were stored in shell vials separated by fraction size and placed into scintillation vials for later verification. The time needed to identify taxa to the appropriate level of resolution is assumed to be independent of the processing method and thus was recorded, but not included in the comparison of lab techniques.

Statistical Analysis

Various measures of sample-processing efficiency were assessed. An effective process provides a precise and accurate estimate of the true number and kinds of organisms present, requiring the shortest possible period of time to sort organisms from detritus. Unfortunately, washing and sorting times were lost due to misplacing the written data for 14 of the 40 samples and a regression analysis was conducted to estimate these missing times. For each sample, I determined the following aspects of processing, identification and sample composition (Table 3.1):

Table 3.1. Summary of variables determined for assessment of processing efficiency of Marchant Box vs. Nested Sieve procedures.

Independent variables (units)	Dependent Variables (units)
<ul style="list-style-type: none"> • Stream name • Sampler type (D-frame net; Petite Ponar) • Processing method (Marchant box; nested sieves) • Detrital mass (g dry mass) • [Actual] abundance (total number of invertebrates in a sample) • [Actual] sample richness (total number of families observed in a sample) 	<ul style="list-style-type: none"> • Sample preparation time (min) • Sample sorting time (min) • Estimated invertebrate abundance (number of invertebrates in sample, extrapolated from subsamples where appropriate) • Estimated sample family richness (based on 300-animal count; families per sample) • Estimated streamwide family richness (based on processing method; families per stream) • NMDS axes of community composition (dimension score; sampler & process type specific)

Subsampler Efficiency – Processing Time

The relationship between the length of time taken to process each sample using the Marchant Box vs. sieve-fractionation method was assessed by regression. To account for variation in human factors, samples were sorted in a randomized order. A distance-weighted least squares line was fitted through the data to summarize the trend. Main effects (unreplicated; processing type x sample number) ANOVA was performed to compare the mean processing time of each procedure accounting for inter-sample variation. Because both processing times and the size of samples varied greatly, multiple regression analysis was performed to determine the degree to which other covariates influenced processing time (washing time + sorting time). The independent variables included both dummy variables (sorting method (Marchant Box vs. Sieves), sampling method (D-net vs. Petite Ponar), habitat type (riffle vs. pool)), stream sampled (4 variables to summarize the 5 streams) and quantitative variables (total detrital mass). In addition, variables representing several interactions (sorting method x detritus mass, sampler type x detritus) were included. Relationships were assessed by both forward and reverse stepwise regression. Because the same results were achieved by both methods, forward stepwise results are reported here.

Estimated Richness and Abundance

Abundance was expressed as the total number of invertebrates estimated to be present in a sample collected from a stream by one of the subsampling methods. Taxa were identified to family unless otherwise noted (depicted in Appendix C). Richness was variously expressed as the number of families present in a sample (sample richness; families per sample), the mean number of families collected by a particular sampler type in a particular stream (sampler richness; D-frame net vs. Petite Ponar grab), the cumulative number of families encountered in a particular stream (stream

richness; families per stream) or processing-specific richness (mean number of families observed per sample using the Marchant box procedure vs. nested sieve procedure). Regression analysis was used to estimate the relationship between estimated (extrapolated) abundance determined from the Marchant box procedure vs. the estimated (extrapolated) abundance determined from nested sieve procedure.

The relationship between best estimate of stream richness (based on the combination of subsamplers) compared to what is indicated by each subsampler individually was also considered. The absolute difference in abundance between the sieves and the Marchant Box relative to the estimated abundance from the sieves as a percent of the Marchant Box was also evaluated.

Community Composition

Community composition data were analyzed using the abundance and relative abundance using Octaves – $\text{Log}_2(\text{percentage of a sample comprising of a family})$ (as in Chapter 2). Non-metric multidimensional scaling (NMDS) with Bray-Curtis distances was performed to express community composition along a reduced number of biological axes and graphically illustrating the within vs. among sample differences of each subsampling procedure. The NMDS analysis was performed using PC-ORD Version 6 (McCune and Mefford 2011), and the scatterplot using STATISTICA 7.

Results

Subsampler Efficiency – Processing Time

The time in minutes was recorded for 40 samples collected with either a D-frame net or a Petite Ponar grab from among 5 streams for each processing method. Processing times that were not recorded for 12 out of 40 samples were interpolated using regression analysis to estimate what the time may have been to be included in the analysis. The equation used was The sieve-fractionation method was most efficient when samples required less than about 230 min of sorting time ((Sorting time = 229 + 0.1189 x mg detritus; Figure 3.1). A main effects ANOVA showed a significant difference between sorting times of the two subsampling methods (Figure 3.2 and Table 3.2: ANOVA, $F(1,31) = 9.15$, $p=0.005$).

Multiple regression was performed to determine whether sorting time was significantly influenced by subsampling method (Marchant Box vs. Sieves), field method (D-net vs. Petite Ponar), habitat type (riffle and pool), and detritus mass in a sample. Sorting time was independent of all variables except for detritus mass (Table 3.3). A simple regression with detritus mass (mg dry mass) was significant ($p=0.0095$) and the equation of the line is Sorting time (min) = 160.1841 + 4.6673 x Detrital mass (mg). Thus, at an increase in one gram of detritus will increase the total time to go through a sample by 5 minutes. Because detritus was significant at the $\alpha=0.05$ level, a standard stepwise regression analysis was conducted with interactions between sorting, sampling, and habitat with detritus. There was a significant effect of sorting method on sorting time, but it depended on the amount of detritus present in the sample (Table 3.4).

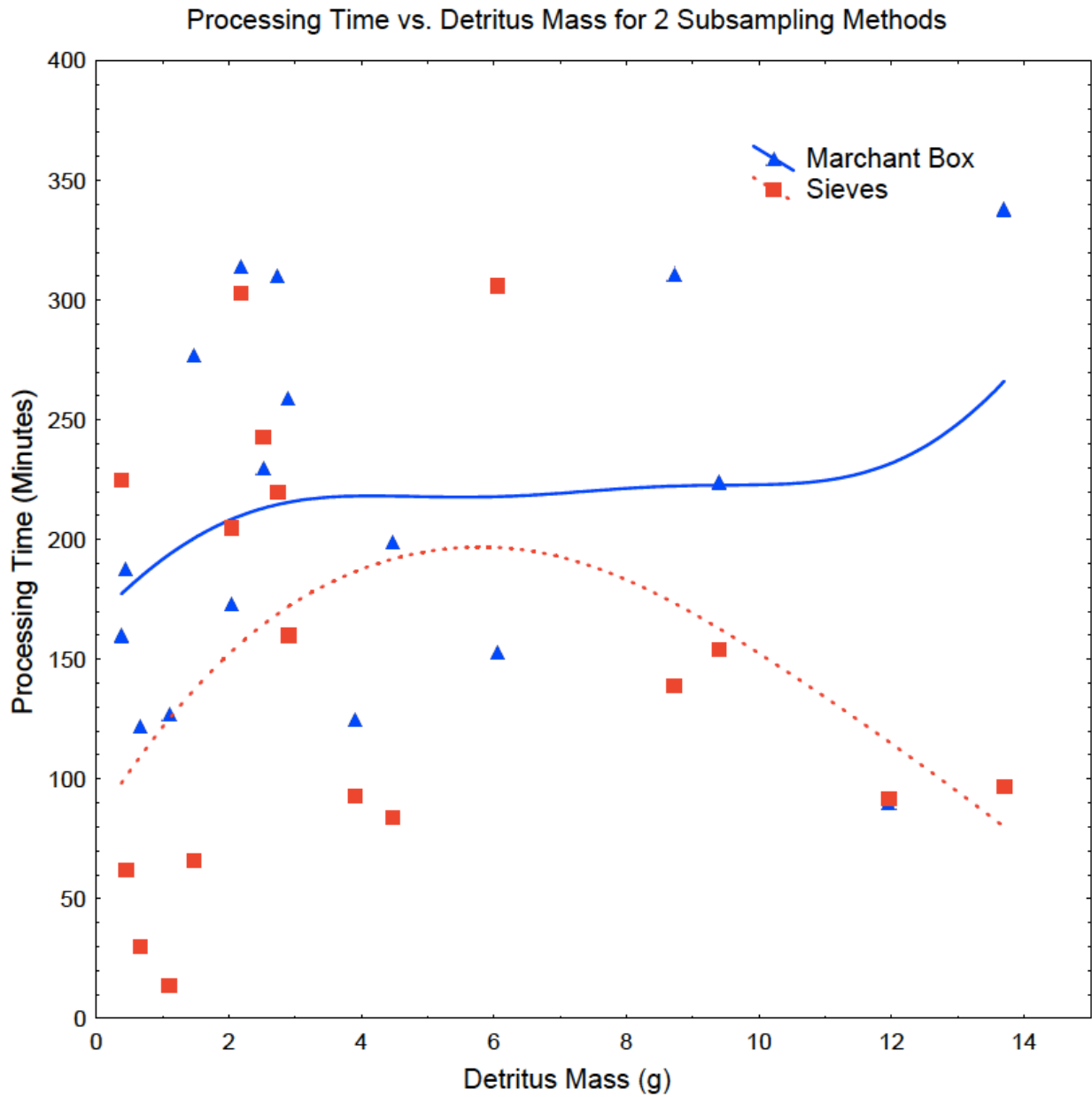


Figure 3.1: Scatterplot of the time taken to process one sample using nested sieves (squares and dotted line) and the Marchant box (triangles and solid line). The lines represent distance-weighted least squares fits through the data points (stiffness = 0.5).

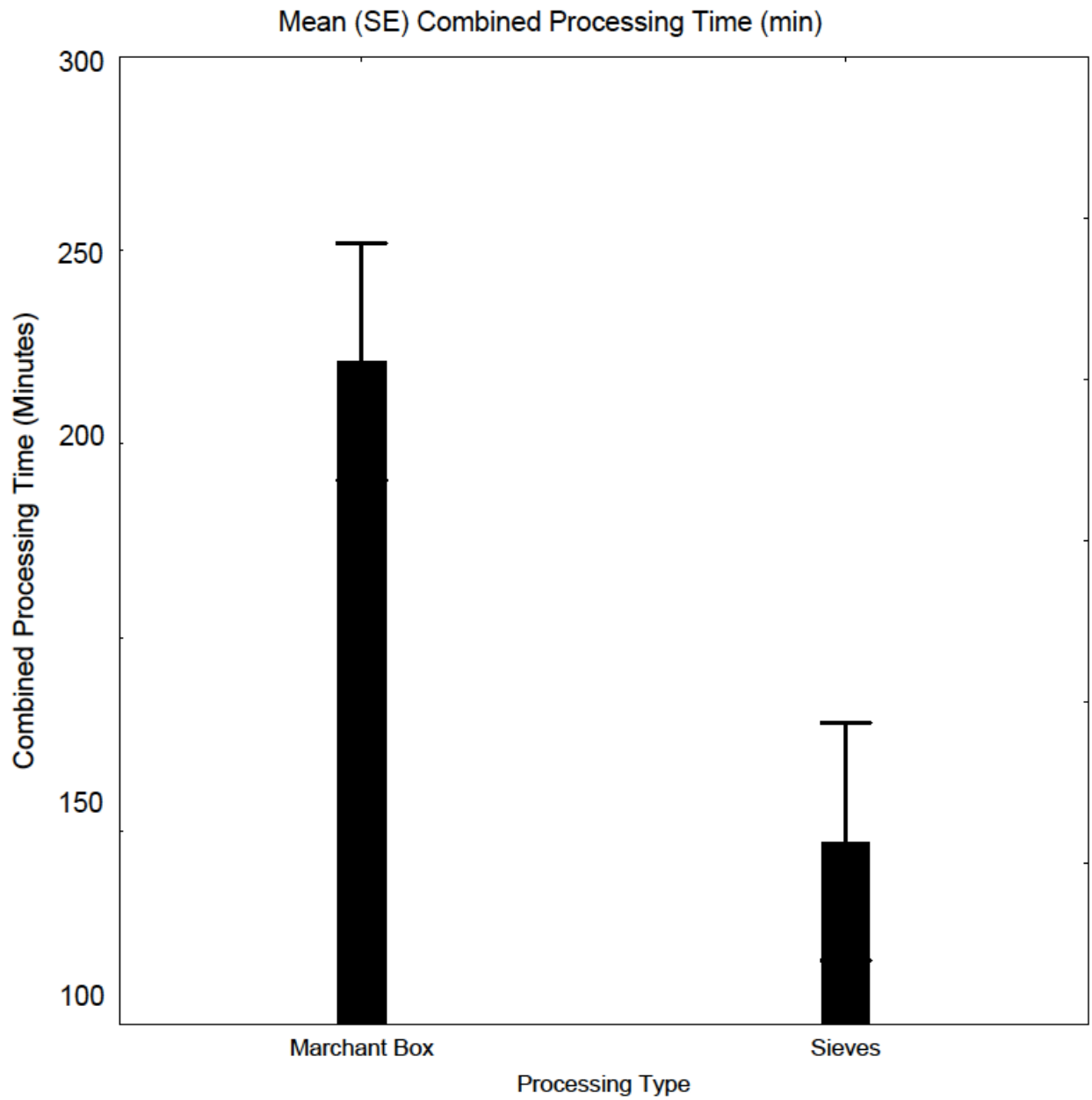


Figure 3.2: Mean \pm SE; n=31 processing time for samples using the Marchant Box and sieving subsampling methods (note the Log scale). There is a significant difference between sorting methods (ANOVA, $F(1,31) = 9.14.589$, $p=0.005$).

Table 3.2. Main Effects (unreplicated) ANOVA of effects of sampler type on Log₁₀ transformed combined processing time.

Effect	D.F.	SS	MS	F	p
Subsampler	1	0.9541	0.9541	9.139	0.005087
Sample	30	3.6086	0.1203	1.152	0.350312
Discrepance	30	3.1321	0.1044		
Total	61	7.6947			

Table 3.3 Results of multiple regression analysis of the effects of subsampling method (Marchant Box = 1; Sieves = 0), sampling method (D-net = 1; Petite Ponar = 0), habitat type (riffle = 1; pool = 0) and detritus mass (grams) on Log-transformed total time required to process a sample (minutes; n=36 samples; $R^2 = 0.256$)

n=36

Variable	All variables			
	Reg. Coeff.	S.E.	p	R ²
Intercept	123.691	32.662	0.00068	
Sorting Method	49.167	31.136	0.124	
Sampling Method	25.239	33.445	0.456	
HabitatType	-0.831	32.965	0.980	
Detritus Mass (g)	5.009	1.811	0.009	

Table 3.4 Results of standard stepwise multiple regression analysis of the effects of the interaction between detritus mass (grams) with subsampling method (Marchant Box = 1; Sieves = 0), sampling method (D-net =1; Petite Ponar = 0), and habitat type (riffle = 1; pool =0) on total time required to process a sample (minutes; n=36 samples). R2 = 0.141

n=35

Variable	All variables			
	Reg. Coeff.	S.E.	p	R ²
Intercept	169.139	18.71	0.000	
SortingxDetritus	8.870	4.272	0.047	
SamplingxDetritus	-4.675	9.109	0.612	
HabitatxDetritus	-1.769	8.569	0.838	

Richness and Abundance

The actual abundance of invertebrates in samples (those that were entirely sorted by at least one processing method) was compared to the estimated abundance extrapolated from the subsample of the other sorting method (Figure 3.3). The equation of the line for the Marchant box was $y = -102.6804 + 3.2853 * x$ and for the sieves was $y = -46.268 + 1.164 * x$. The Marchant box method sometimes greatly overestimated the abundance of invertebrates in a sample, whereas the nested sieve procedure slightly overestimated true abundance when over 300 individuals were in subsamples. The slopes of the two lines were highly significantly different from each other (Marchant Box: $R^2 = 0.22$, $p = 0.09$; Sieves: $R^2 = 0.89$, $p < 0.001$). The regression line for the sieve method was relatively unbiased (similar to the dashed line in Figure 3.3, which represents perfect prediction of invertebrate abundance in the whole sample extrapolated from the partial subsample).

A comparison of the richness estimated by the two sorting methods revealed that D-net samples processed by the sieving method collected more families than samples processed by the Marchant box in only 2 streams and equal in richness to the Marchant Box in one stream. Petite Ponar samples processed by the sieving method contained more families than when processed by the Marchant Box in 3 streams and an equal number in 1 stream (Table 3.5). There was a significant difference in richness between each sorting method (Main Effects ANOVA, $F_{(1,19)} = 5.959$, $p < 0.05$; Figure 3.4, Table 3.6).

The sieving method detected, on average, 94% of the families estimated to be present in a stream (estimated by regression; $y = -0.8341 + 0.9421 * x$, $SE = 0.139$, $R^2 = 0.9380$, $p = 0.0067$; Figure. 3.5). In contrast, the Marchant Box only detected about 83% of the families ($y = 0.0835 + 0.827 * x$, $SE = 0.118$, $R^2 = 0.9709$, $p = 0.0059$). Neither equation differed significantly from a slope of 1.0.

Invertebrate Abundance from Samples Sorted as a Whole Compared to the Estimated Abundance.

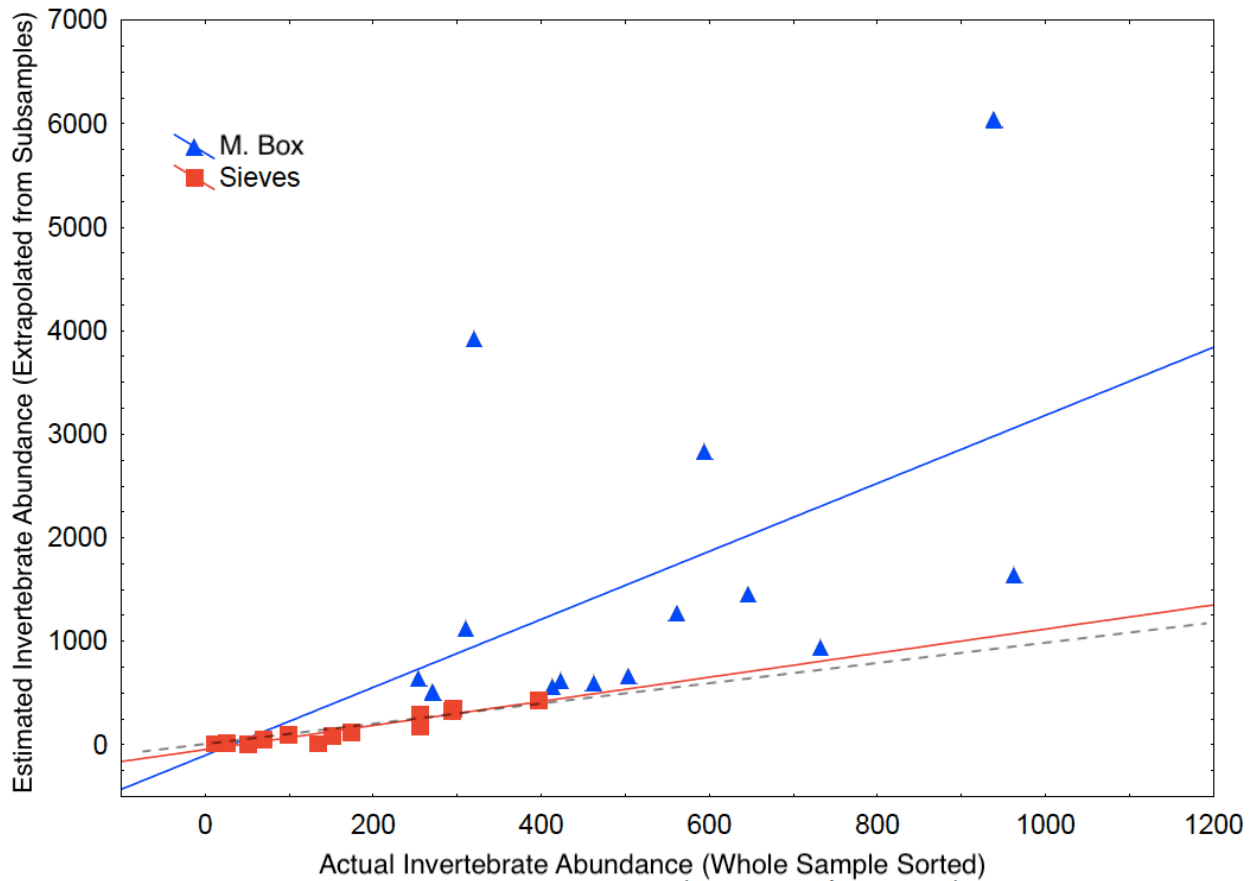


Figure 3.3: Estimated invertebrate abundance extrapolated from subsamples versus true abundance. Each point represents one sample (n=27). The solid blue line represents the projected estimates of the Marchant Box (Estimated abundance = $-102.6804 + 3.2853 \times \text{true abundance}$), and the solid red line represents that of the sieve method (estimated abundance = $46.0799 + 0.784 \times \text{true abundance}$). The dotted line indicates the expected extrapolated abundance in a sample if the subsampling method is unbiased (Extrapolated abundance = actual abundance).

Table 3.5. Cumulative family richness when all 3 (D-net) and 5 (Petite Ponar) samples were pooled together ($p>0.05$).

Stream	Sample Type			
	D-net (n=3)		Petite Ponar (n=5)	
	<u>Marchant Box</u>	<u>Sieves</u>	<u>Marchant Box</u>	<u>Sieves</u>
Big Creek	26	31	30	29
Little River	8	8	9	13
Sharon Creek	23	21	22	29
Sixteen Mile	15	18	13	14
White Ash Creek	24	23	20	20

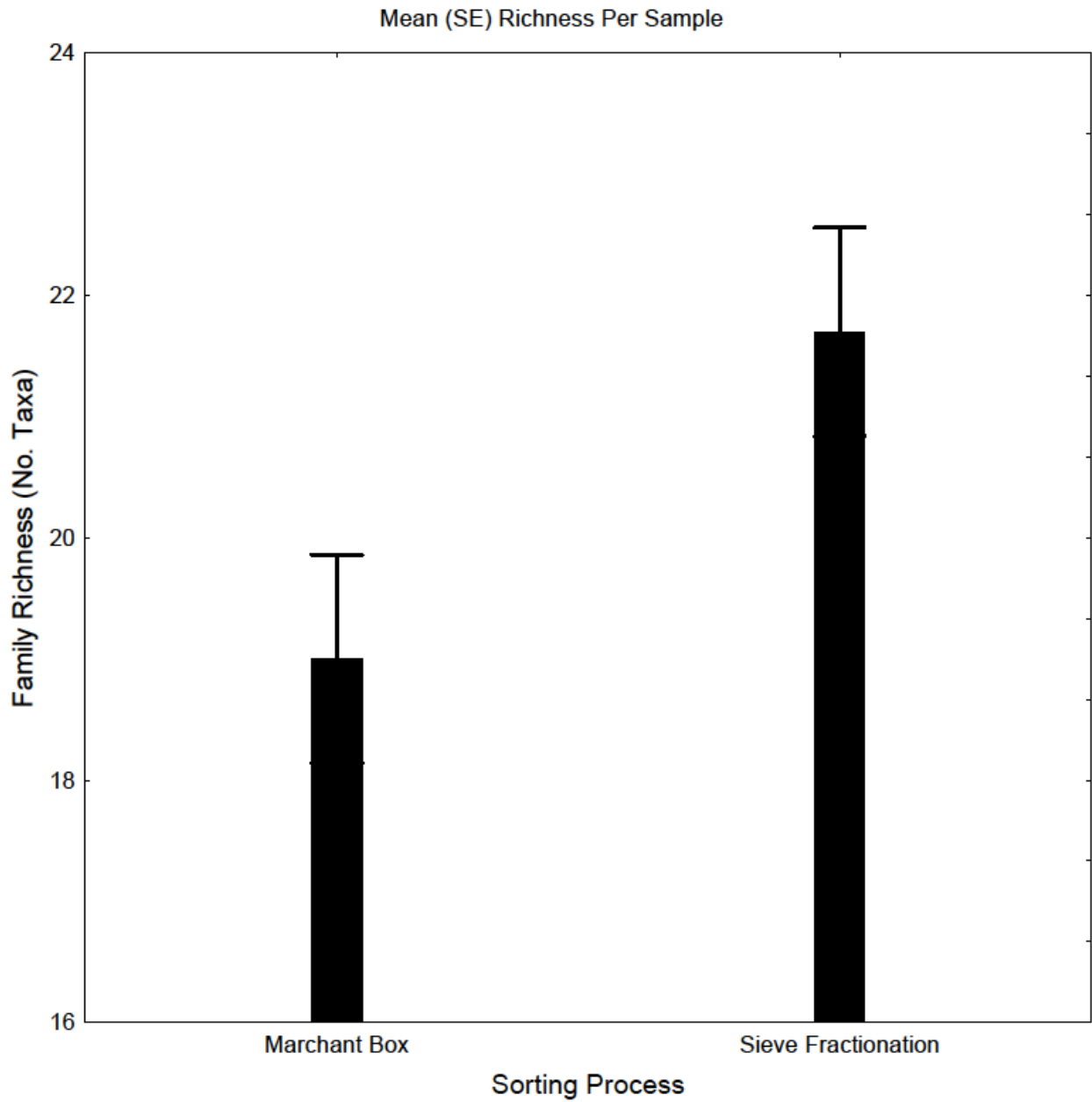


Figure 3.4. The mean(\pm SE) family richness found using the Marchant Box and nested sieves fractionation method. (n=40).

Table 3.6. Main Effects (unreplicated) ANOVA of effects of processing type on number of families recovered from sweep and Petite Ponar samples collected from 5 streams.

Effect	df	SS	MS	F	p
Sample	9	1079.050	119.894	19.601	0.000070
Process	1	36.450	36.450	5.959	0.037295
Discrepance	9	55.050	6.117		
Total	19	1170.550			

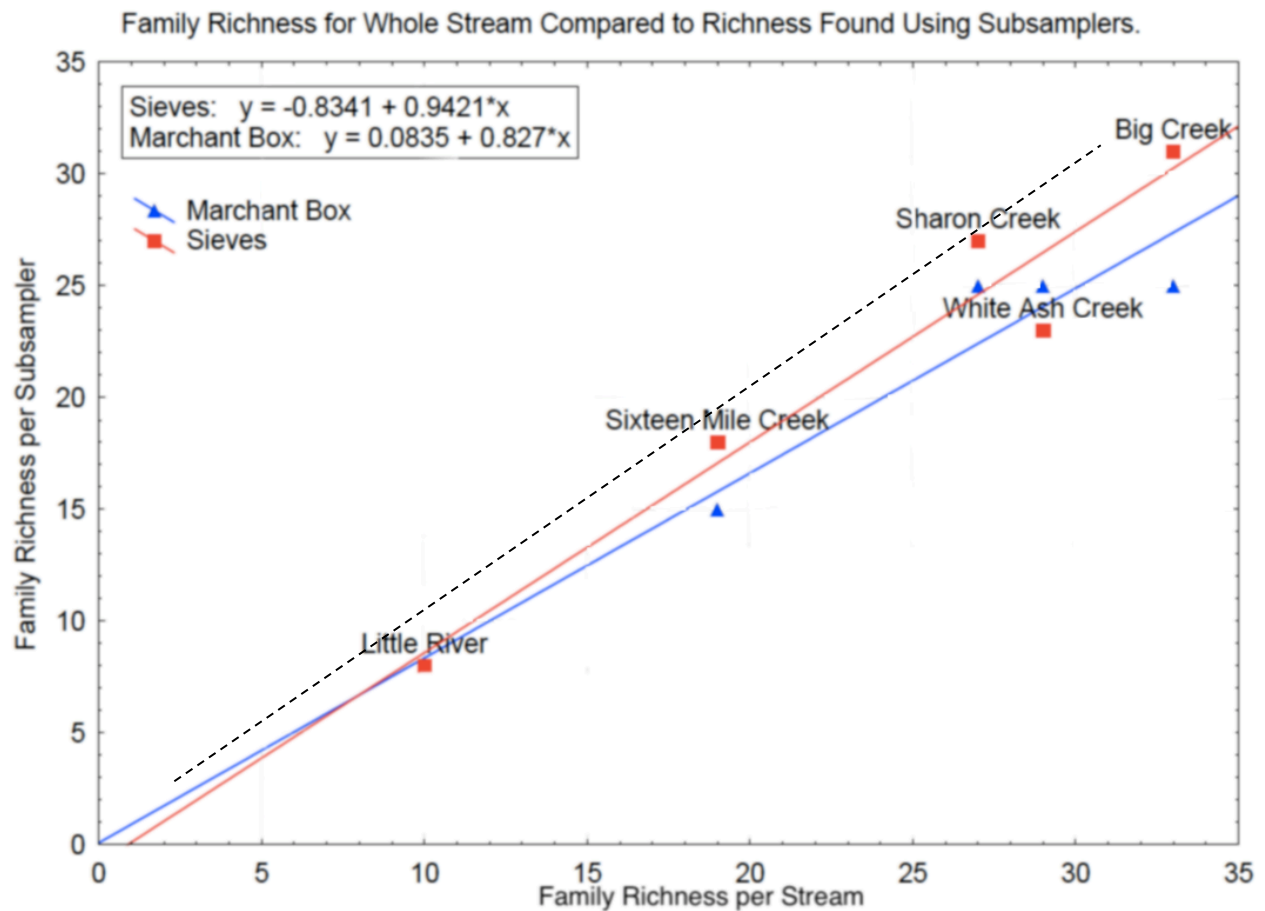


Figure 3.5: Scatterplot and regression line of family richness estimated from Marchant Box and sieve fractionation processing methods vs. total number of families observed in source stream. The x-axis is a combination of both samplers. Each point represents the mean richness for 8 samples per stream using the respective subsampler. The dotted line represents equal richness for both axes. The Sieve standard error is 0.139 and for the Marchant Box it is 0.118. The slope of the equation for both sorting methods is not significantly different from a slope of 1.0. (Marchant Box: $t=1.47$, $p>0.05$, Sieves: $t=0.41$, $p>0.05$)

The Absolute Difference in Estimated Abundance Between Two Sorting Methods Compared to Sieves as an Estimated Percentage of the Marchant Box.

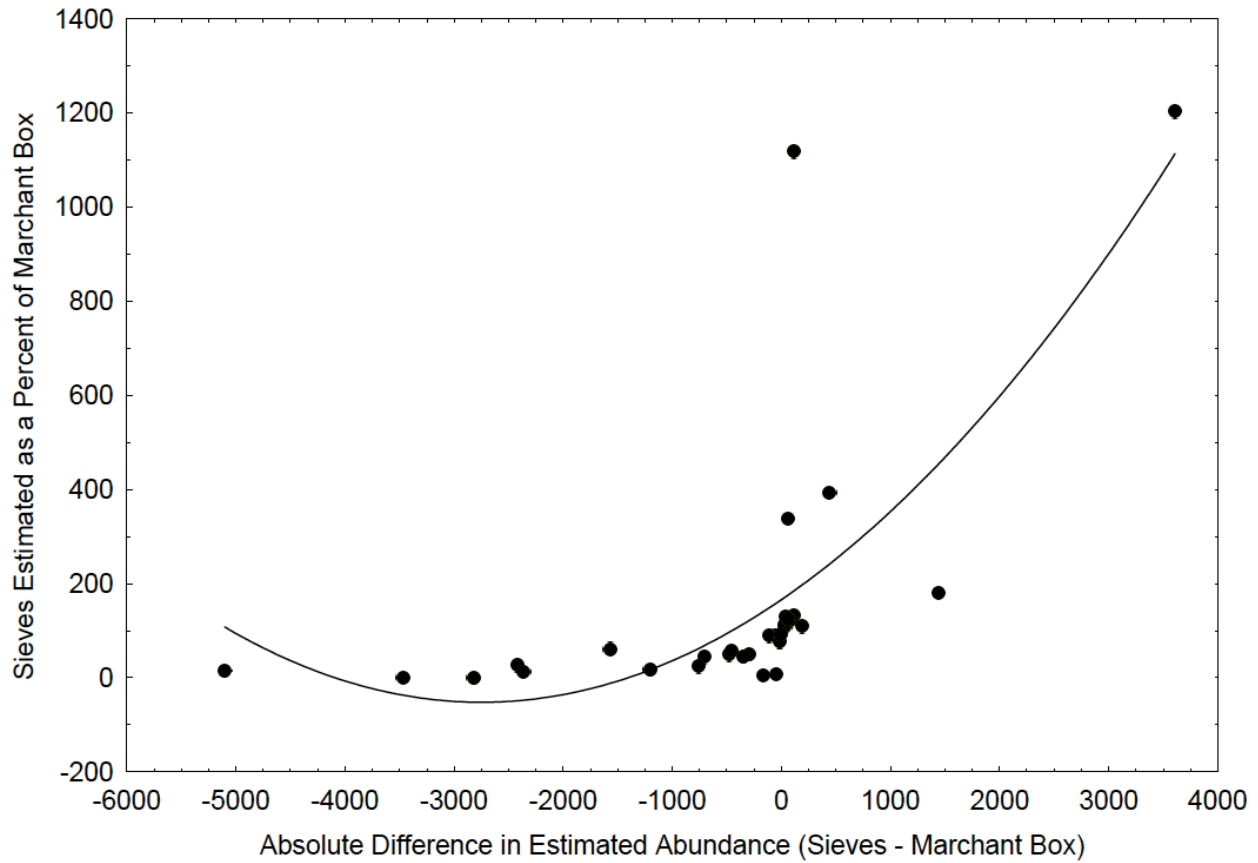


Figure 3.6 Scatterplot and regression with polynomial fit of the absolute difference between the Marchant Box and Sieves, to the Sieves as a percentage of the Marchant Box. Each point represents one sample. $R^2 = 0.2981$, $p=0.0015$, $y=208.9075+0.1009*x$.

Community Composition

Non-metric multidimensional scaling (NMDS) ordination of the invertebrate families collected in the D-net samples from the 5 streams represented community composition in 2 dimensions (stress=12.22). The similarity in community composition of Marchant Box vs. sieve subsampling was represented by connecting the two estimates from each sample with a line (Figure 3.7). Ellipses were drawn by eye to enclose the sample estimates from each of the 5 streams.

The NMDS indicated that community composition of each stream was relatively distinct from the others. There was little or no overlap among the ellipses for Little River and Sixteen Mile Creek, indicating that the fauna within these streams are different in composition from the communities of the other streams. White Ash Creek and Big Creek overlap, suggesting these two streams have similar community composition. Big Creek and Sharon Creek also overlap. The invertebrates whose relative abundances are positively associated with scores of Dimension 1 are Chironomidae and the dragonfly family Gomphidae, whereas many other families' relative abundances were negatively correlated with scores of Dimension 1 (Appendix B). The invertebrates whose relative abundances were positively correlated with scores of Dimension 2 were Corixidae, Elmidae, Heptageniidae, Hydropsychidae, Hydroptilidae, Simuliidae, and Tipulidae. Relative abundances of Oligochaeta were negatively correlated with scores of Dimension 2. Accordingly, one can infer that Sixteen Mile Creek is predominately influenced by oligochaetes, chironomids and Gomphidae. Little River is influenced by many invertebrates in the negative direction of Dimension 1, but minimally influenced by those associated with Dimension 2 (Appendix B). Sharon Creek is not dominated by any particular taxon. White Ash Creek and Big Creek are similar in composition, so their fauna are composed predominately of Corixidae, Elmidae, Heptageniidae, Hydropsychidae, Hydroptilidae, Simuliidae, and Tipulidae. Overall,

sorting method did not produce large differences in community composition except for samples from Big Creek (D-net sample 2 taken in pool habitat) and White Ash Creek (D-net sample 3 taken in a riffle).

Discussion

Minimizing sample sorting effort is one of the objectives of a rapid bioassessment protocol, (Resh et al. 1995; Barbour et al. 1999), because sample processing can be much more time-consuming than field collection. Several investigations have suggested that using sieves will reduce sorting time, (Ciborowski 1991; Vlek, Šporka, and Krno 2006; Barba et al. 2010). The goal of this chapter was to determine whether sieving procedures are more effective in sample processing than the recommended Marchant Box method (Marchant 1989; CABIN 2011; Jones et al. 2004, (OBBN)).

Comparisons of sorting times using the two subsampling methods indicate the sieve subsampling protocol is indeed more time-efficient than the Marchant Box. When using the sieves, a sample was completed in about 1 to 2 h, whereas the Marchant box typically took 4 h or more (Figure 3.1). The length of time taken to sort through a sample increased as a function of the amount of detritus in the sample until about 6 g of detritus. When more than 6 g of detritus occurred in a sample, the Marchant Box sorting time increased slightly, whereas sorting time required using the sieves method actually decreased, presumably because the finer sieve fractions could be subsampled. There was great variability in the time required to go through a sample.

I had hypothesized that invertebrate abundance may have been a contributing factor to overall processing time, as reported by Ciborowski (1991). However, multiple regression analysis indicated that this factor was not significant ($p=0.177$). This may be because the Marchant box

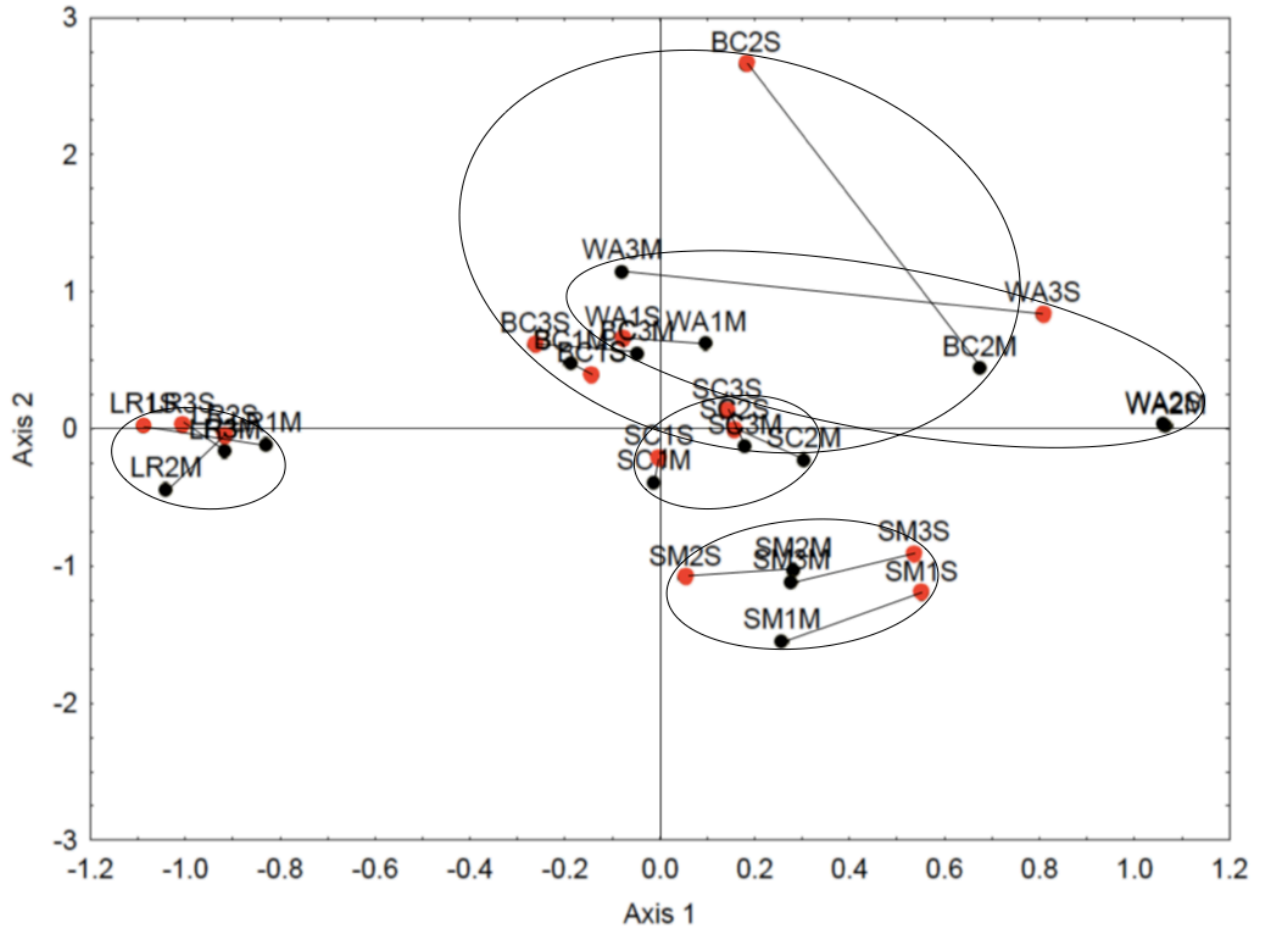


Figure 3.7: NMDS ordination of invertebrate community composition estimated from D-net samples collected from 5 streams, illustrating compositional differences inferred by subsampling methods. The red dots represent samples sorted using Sieve fractionation and the black dots connected to each red dot represents the same sample sorted using the Marchant box procedure. Ellipses enclose the replicate samples collected from each stream. (BC = Big Creek; LR = Little River; SC = Sharon Creek; SM = Sixteen Mile Creek; WA = White Ash Creek; #S = rep number of sample sorted by Sieves; #M = rep number of sample sorted by Marchant Box).

method uses a fixed count approach (300 individuals), whereas the sieve fractionation protocol is a fixed fraction subsampling method, so sieving only partially relies on the number of individuals present in the sample. Carter et al. (2006) found that fixed-count subsampling (typically 100 or 300 individuals but ranging up to 550) is the most common approach in Canada and the US. Carter, (2006) reported that almost 70% of studies reviewed reported using sieves to remove the large, rare organisms before going through the sample.

An important determinant of the length of time spent using the Marchant box method is the handling time spent removing materials from each cell. If few animals are present in a sample, then a considerable time is spent removing the cell contents and less time is devoted to searching for animals. The size fractionation achieved by the nested sieve method allows time to be spent primarily in sorting a single aliquot rather than having to handle many small subsamples, as is required using the Marchant box.

The amount of detritus in a sample was an important determinant of the time needed to sort samples, as has been frequently reported (Culp et al. 1983; Reice 1980). Each additional gram of detritus in a sample increased the sorting time by about 5 min using the sieve fractionation protocol (Table 3.3). In contrast, the Marchant box protocol required 8.87 minutes longer per gram of detritus to complete a sample (Table 3.4). This difference is especially noticeable for clay plain stream samples because the fine sediments or large quantities of debris can sometimes make it difficult to wash samples thoroughly. Overall, it takes significantly more time to extract materials from the Marchant box cells than it does to sort the contents of a sieve fraction in one go on a petri plate. Clearly, thorough field-washing of samples is an important aspect of removing as much fine sediment and detritus as possible. But when residual materials remain in samples, the sieve fractionation method is particularly more efficient than the Marchant Box.

A comparison of the estimated numbers of invertebrates in samples revealed that the Marchant Box sometimes markedly overestimated abundance compared to the sieving method (Figure 3.3). This was a function of the degree and method of subsampling. On average, sieves slightly overestimated the true abundance (by a factor of about 1.16 times; $R^2 = 0.94$), whereas the Marchant box overestimated abundance on average by a factor of 3.29 ($R^2 = 0.22$). This may be due to too few cells in the Marchant Box being sorted, producing an overestimate of the abundance since there is increased variability. For example, if 10 cells are sorted and 300 animals are found, the resulting estimated abundance is 3000, but if sorting continued it could be found that when the 50th cell is reached there are only 1000 individuals, which changes the estimated abundance from 3000 to 2000 organisms. The estimated abundance accuracy would increase if more cells were sorted since the values are closer to the true abundance. Courtemanch (1996) identified this limitation of the fixed count method with respect to invertebrate richness. He commented that a fixed number of organisms is not consistent because two communities cannot be assumed to be similar, and that when only collecting to a certain number the proportion of the total community that has been found is unknown; therefore, rare organisms may be missed, thus altering the estimate of biological integrity. Courtemanch (1996) discussed 3 options to resolve the short-coming - whole sample processing, two-phase processing, and serial processing. Of all of these, the sieving method is consistent with both the first and second approaches. When using the sieve fractionation approach, a larger proportion of the sample is processed. Furthermore, large organisms (in the coarsest size fraction) are collected first, and then the remainder of the sample is subsampled. The abundance of invertebrates in several samples was strongly overestimated by the Marchant box procedure (Figure 3.3), likely due to the very small proportion of cells that were examined by the time a stopping point (300 organisms) had been reached.

The result of the ordination analysis suggests that the community composition of Little River and Sixteen Mile Creek differed from the other streams, whereas White Ash Creek, and Big Creek were very similar in composition. The Sharon Creek ellipse slightly overlapped with that of Big Creek, so their community compositions are also similar. The dominant invertebrates in Sixteen Mile Creek were Chironomidae, Gomphidae, and Oligochaeta, suggesting that this stream is more degraded than the others. This is because these are deemed to be tolerant taxa, which can withstand degraded conditions, compared to the fauna that dominated the other streams which are not as tolerant. Although the family richness varied greatly among streams, the tolerance scores of the communities were relatively similar.

When looking at each of the streams individually there are a few distinct patterns that suggest the Marchant Box is different than sieves in Dimension 1 because the orientation between each of the connected points in Figure 3.7 tend to be separated in a left to right pattern. Distinct differences in sorting methods were seen in Big Creek sample 2 (D-net in pool habitat) and White Ash Creek sample 3 (D-net in riffle habitat)). In conjunction with the richness of each of the sorting methods, this coincides with the significant difference between what each collected (Table 3.6).

Recommendations

Several lines of evidence indicate that the sieve fractionation method is more effective than the Marchant box for processing samples collected from clay plain streams of southwestern Ontario. Although both subsampling techniques yield comparable overall community composition, the sieve method requires less washing and sorting time, recovers more specimens (leading to more precise abundance estimates) and greater family richness, and does not overestimate abundance of invertebrates. The efficiency of the sieve fractionation method can be

increased by ensuring that samples are thoroughly field-washed before preservation to ensure that fine materials are rinsed through the D-net and that large twigs and coarse detritus are removed from the sample. This also reduces the amount of preservative needed. Although both the Marchant box and sieve fractionation procedures characterize community composition similarly, the sieve methodology is preferable because it provides a more precise estimated abundance and is more time-efficient than the Marchant Box protocol.

Chapter 4: General Discussion

Project Overview

The objective of this study was to review, assess, and recommend sampling and sorting methods to optimize stream sample collections that would reflect the zoobenthic community composition of the streams in southwestern Ontario. Because streams in this region are slow flowing and have soft, largely clay-dominated sediments, currently used provincial and national protocols, which are designed for assessing faster-flowing, hard-bottomed streams, may not be appropriate or effective for the low gradient, fine-substrate systems of the St. Clair Clay Plain region. I compared two methods of sample collection from 19 streams, and two macroinvertebrate subsampling methods of processing the collections.

I found that using a D-framed sweep net as recommended by the Canadian Aquatic Biomonitoring Information Network (CABIN) and Ontario Benthic Biomonitoring Network (OBBN) protocols was as or more effective than using a Petite Ponar grab, even in locations that had deep water, muddy substrate and negligible flow. In the laboratory, the use of nested sieves was more time efficient and better represented the family richness of streams than the Marchant Box method recommended by CABIN and OBBN. Three D-frame sweep net samples per stream (two from riffles and one from a pool) each collected more invertebrates and a larger number of families than five Petite Ponar grabs. The samples from the 19 sites surveyed in 2016 were collected in July and August. During this season many other headwater streams that were visited had dried up, and this was a significant limitation to sampling during the summer. Consequently, I examined a 10-y temperature and discharge record for the Thames River to determine the duration and timing of spring melt conditions. On that basis, I determined the period of time during which discharge was likely to be low enough to permit safe access and yet cool enough (<20

degrees C) that eurythermic overwintering taxa were unlikely to have emerged. The recommended interval is between April 1st and May 7th. In 2017, samples were collected between April 18th and June 10th. Water temperatures were 20 degrees C or less in most of the streams at the time of sampling.

Of the two sample processing methods, the Marchant Box procedure took on average about 160 min longer per sample (62.5 percent longer) to process than the sieve fractionation protocol. The amount of detritus was a significant determinant of the variation in the time needed to go through a sample. There was a significant difference in the number of families recovered per sample by each method and the sieve fractionation procedure detected a higher proportion of the taxa found per stream (92%) than the Marchant Box method (82%), especially when macroinvertebrate densities were high. Although, an ordination of the two subsampling procedures found no evidence of bias in community composition, the sieve subsampling procedure produced consistently accurate estimates of actual macroinvertebrate density, whereas the Marchant Box procedure often greatly overestimated the number of animals in a sample. The differences in precision were associated with the proportion of a total sample that was sorted.

In conclusion, the D-framed sweep net and the nested sieves are the methods that should be used in the field and laboratory processing, respectively. These methods collect representative samples from the stream, allow efficient processing and provide a more accurate assessment of the streams than the complementary methods.

OBBN is the provincial protocol for benthic assessment and is the condensed version of CABIN, which is the national protocol. Because both approaches are available to use in the region, the question arises as to which protocol is best suited and most feasible for use in terms of efficiency and data collection. The stream habitat assessment features that OBBN does not require

include slope, velocity and pebble count (which are part of the CABIN protocol field data sheets) because these data are used in assigning test sites to the most appropriate suites of reference sites under the reference condition approach (Jones et al. 2007, Reynoldson 2002). Many streams in the Essex and Lower Thames region have minimal slope and negligible velocity (even during spring) due to the flatness of the local topography (Appendix B). Consequently, stream slope is likely best determined using map-based estimates of changes in elevation; and velocity records of <5 cm/s (the lowest effective reading of many current meters) will have to be used to estimate discharge of the sites that are sampled across the region. OBBN does, however, include elevation on the field data sheets. Because the streams are within the clay plains, it is impractical to conduct the pebble count protocol stipulated by CABIN. However, the sandy or muddy substrates can be sampled with sediment cores (which was done for 2017 field sampling), and particle size can be reported using the Wentworth scale after conducting appropriate particle size frequency analysis in the laboratory (Wentworth 1922). This was completed for 2017 samples, but due to time constraints, it has not been analyzed in conjunction with other factors that were presented in this thesis. Overall, there really is no true basis for comparison between CABIN and OBBN because there is no benthic information on reference streams in the clay plains regions. Yet, I recommend using the OBBN protocol to assess southwestern Ontario streams because it is a condensed rapid approach and does not include the sections of CABIN that would be difficult to assess.

Major Findings and Recommendations for Regional Conservation Authorities

The D-frame sweep net collected a higher abundance of invertebrates and greater family richness than the Petite Ponar grab samples. Although sweep-netting is a more qualitative method than fixed-area sampling with a Petite Ponar grab, the sweep net procedure collected

macroinvertebrates across the entire width of the stream and thus the fauna encountered were more representative of the range of microhabitats. Samples taken from both riffle (or glide) and pool areas are needed to represent the biodiversity that is present. Of the two types of sampling used to estimate community condition as represented by the Hilsenhoff Biotic Index, the D-net-derived estimates produced values closest to those determined from both D-net and Ponar grabs combined. However, NMDS ordination indicated that the type of sampler used had little influence on the interpretation of overall community composition among streams.

Of the two laboratory processing and subsampling methods, the Marchant box took about 160 minutes longer to complete a sample than the sieve fractionation method. The quantity of detritus significantly affected the time needed to complete a sample, even though both protocols entail subsampling of large samples. Ponar grabs required less time to sort, especially when processed using the sieve fractionation technique, because sieving removes residual clay in the samples during the washing process. Samples processed using the sieving procedure had significantly higher family richness, presumably because all large, rare individuals (those in the coarse size fraction) were found and identified. However, there was no significant difference in community composition between processes detected by the NMDS ordination relative to among-stream variation. Overall, the weight of evidence indicates that sieve fractionation is the more effective method, and it is highly recommended over the Marchant box for the Clay Plain streams assessed in this study.

Limitations

Limitations to this work include the relatively small number of samples for which data were available to compare the subsampling methods. Although 40 samples from 5 streams were used in

the analysis, the loss of the written records of detritus biomass data compromised the power of the analysis to identify the covariates that contributed most to variation in sorting time. Nevertheless, the data were sufficient to show that sieving is a more effective protocol than using the Marchant Box. However, to increase the power of the study more samples should be sorted through comparing both methods.

Another limitation is in my reporting identity of macroinvertebrates only to the family level of taxonomic resolution. I identified invertebrates to genus or species where possible, but very immature animals lack certain features needed for identification to the genus level. The family level of resolution is recommended for many studies because it reduces the time needed for identification providing more time in a limited budget for additional samples to be processed. The family level of resolution is reported to be sufficient for conducting multivariate analyses (Bowman and Bailey 1997) and for calculating tolerance indices (Hilsenhoff 2017), but researchers often advocate genus- or species-level identification for biomonitoring studies (Bailey et al. 2006). According to Milošević (2014) for any study the taxonomic resolution depends on where the threshold of information loss is acceptable. It should be at a level where the community can still be detected to have changes in response to differences in the environmental conditions it's subject to (Milošević et al. 2014).

Future Studies and Implications

Further research on factors influencing invertebrate community composition could include observing the effects of alterations to the stream- comparing the effects of excavation or maintenance of streams used as agricultural drains to streams that are left unaltered. Most streams in southwestern Ontario serve as agricultural drainage systems, and some are physically

manipulated. Ward-Campbell et al. (2017) studied the effects of excavation to fish communities in 8 southwestern Ontario streams. They found there was no significant difference in abundance or composition in fish communities (Ward-Campbell et al. 2017), possibly because fishes are able to travel larger distances than invertebrates. It would be interesting to assess the alterations in invertebrate community composition since it may be difficult for the slow-moving invertebrates (in comparison to faster moving fish) to repopulate the affected area.

Further research could also include developing a better understanding the relationship between macroinvertebrate community composition and the sediment type together with the role of sediment in determining the benthic community of clay plain streams, independent of anthropogenic activities. It would also be beneficial to understand the potential differences in the benthic community with respect to changes in season (Furse et al. 1984; Humphrey et al. 2000; Buss et al. 2015). Because streams of the clay plain are maintained largely by surface water rather than groundwater (due to clay's impermeability), these streams likely exhibit much greater seasonal variation in discharge and temperature than predominantly groundwater fed streams.

For this project, the 0.25-mm sieve size fraction was archived. Although this fraction contains many immature organisms (often dominated by small chironomids and oligochaetes) few programs report densities of such small individuals because sampling is conducted with 0.5-mm or coarser nets. However, examination of this size-fraction could provide complementary information about the zooplankton and benthic microcrustaceans.

This study provides important baseline information and an objective assessment of sampling methodology of special value to the Conservation Authorities of southwestern Ontario. Implementation of the recommended protocols will generate data that are representative of local streams and do so efficiently. The survey data collected by my study is of value in informing

regional authorities and the community at large about the ecological condition of southwestern Ontario's river systems, which have a role in and flow into larger systems, i.e. the Great Lakes. Although this study is a synopsis of only summer and spring conditions during 2016 and 2017, it provides an important baseline of the local fauna and a frame of reference against which to compare clay plain streams with other rivers of Ontario.

References

- Allan, J. D. (2004). Landscapes and riverscapes: the influence of land use on stream ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, 35, 257-284.
- Allanson, B. R., and Kerrich, J. E. (1961). A statistical method for estimating the number of animals found in field samples drawn from polluted rivers: With plate 3 und 2 figures on 1 folder. *Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen*, 14(1), 491-494.
- Anderson, J.T., Zilli, F.L., Montalto, L., Marchese, M.R., McKinney, M., Park Y.L. (2013). Sampling and Processing Aquatic and Terrestrial Invertebrates in Wetlands. In: Anderson J., Davis C. (eds) *Wetland Techniques*. Springer, Dordrecht
- AQEM (2002) *The AQEM sampling method to be applied in STAR*. Available from: <http://www.eu-star.at/pdf/AqemMacroinvertebrateSamplingProtocol.pdf>
- AQEM (Integrated Assessment System for the Ecological Quality of Streams and Rivers Throughout Europe Using Benthic Macroinvertebrates) Consortium. (2002). Manual for the application of the AQEM system: a comprehensive method to assess European streams using benthic macroinvertebrates, developed for the purpose of the Water Framework Directive. EVK1-CT1999-0002. AQEM, Essen, Germany.
- AQEM Consortium. (2002). Manual for the application of the AQEM system: a comprehensive method to assess European streams using benthic macroinvertebrates, developed for the purpose of the water framework directive, Version 1.0. EVK1-CT1999- 0002, AQEM, Essen, 198p.
- AQEM. Available from: <http://www.aqem.de/index.php>
- Bailey R.C., Norris R.H., Reynoldson T.B. (2004). *The Reference Condition Approach*. In: *Bioassessment of Freshwater Ecosystems*. Springer, Boston, MA
- Bailey, R. C., R.H. Norris, and T.B. Reynoldson. (2006). "Taxonomic Resolution of Benthic Macroinvertebrate Communities in Bioassessments." *Journal of the North American Benthological Society* 20 (2): 280–86. <https://doi.org/10.2307/1468322>.
- Bailey, R. C., Norris, R. H., and Reynoldson, T. B. (2001). Taxonomic resolution of benthic macroinvertebrate communities in bioassessments. *Journal of the North American Benthological Society*, 20(2), 280-286.
- Baldwin, D. J., Desloges, J. R., and Band, L. E. (2000). Physical geography of Ontario. *Ecology of a managed terrestrial landscape: patterns and processes of forest landscapes in Ontario*, 12-29.

- Beatriz, B., Larrañaga, A., Otermin, A., Basaguren, A., and Pozo, J. (2010). “The Effect of Sieve Mesh Size on the Description of Macroinvertebrate Communities.” *Limnetica* 29 (2): 211–20.
- Barbour, M. T., & Gerritsen, J. (1996). Subsampling of benthic samples: a defense of the fixed-count method. *Journal of the North American Benthological Society*, 15(3), 386-391.
- Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. (1999). Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency, Office of Water, Washington, D.C.
- Barbour, M.T., and Gerritsen, J. (2006). “Subsampling of Benthic Samples: A Defense of the Fixed-Count Method.” *Journal of the North American Benthological Society* 15 (3): 386–91. <https://doi.org/10.2307/1467285>.
- Batzer, D. P., Shurtleff, A. S., and Rader, R. B. (2001). Sampling invertebrates in wetlands. *Bioassessment and management of North American freshwater wetlands*. John Wiley and Sons, New York, 339-354.
- Berenzen, N., Lentzen-Godding, A., Probst, M., Schulz, H., Schulz, R., and Liess, M. (2005). A comparison of predicted and measured levels of runoff-related pesticide concentrations in small lowland streams on a landscape level. *Chemosphere*, 58(5), 683-691
- Binu, V. S., Mayya, S. S., and Dhar, M. (2014). Some basic aspects of statistical methods and sample size determination in health science research. *Ayu*, 35(2), 119–123. doi:10.4103/0974-8520.146202
- Bowman, M. F., and Bailey, R. C. (1997). Does taxonomic resolution affect the multivariate description of the structure of freshwater benthic macroinvertebrate communities? *Canadian Journal of Fisheries and Aquatic Sciences*, 54(8), 1802-1807.
- Boyle, T. (2003). Monitoring Aquatic Benthic Ecosystems of the Bruce Peninsula. Bruce Peninsula Biosphere Association.
- Brinkman, M. A., and Duffy, W. G. (1996). Evaluation of four wetland aquatic invertebrate samplers and four sample sorting methods. *Journal of Freshwater Ecology*, 11(2), 193-200.
- Brose, U., Jonsson, T., Berlow, E. L., Warren, P., Banasek-Richter, C., Bersier, L. F., Blanchard, J.L., Brey, T., Carpenter, S.R., Blandenier, F.C., Cushing, L., Dawah, H.A., Dell, T., Edwards, F., Harper-Smith, S., Jacob, U., Ledger, M.E., Martinez, N.D., Memmott, J., Mintenbeck, K., Pinnegar, J.K., Rall, B.C., Rayner, T.S., Reuman, D.C., Ruess, L., Ulrich, W., Williams, R.J., Woodward, G., Cohen, J.E. (2006). Consumer–resource body-size relationships in natural food webs. *Ecology*, 87(10), 2411-2417.

- Brown, A. V., Schram, M. D., and Brussock, P. P. (1987). A vacuum benthos sampler suitable for diverse habitats. *Hydrobiologia*, 153(3), 241-247.
- Buss, D. F., and Borges, E. L. (2008). Application of rapid bioassessment protocols (RBP) for benthic macroinvertebrates in Brazil: comparison between sampling techniques and mesh sizes. *Neotropical Entomology*, 37(3), 288-295.
- Buss, D. F., Carlisle, D. M., Chon, T. S., Culp, J., Harding, J. S., Keizer-Vlek, H. E., Robinson, W.A., Strachan, S., Thirion, C., and Hughes, R. M. (2015). Stream biomonitoring using macroinvertebrates around the globe: a comparison of large-scale programs. *Environmental monitoring and assessment*, 187(1), 4132.
- Buss, D.F., and Vitorino, A.S. (2010). Rapid Bioassessment Protocols using benthic macroinvertebrates in Brazil: evaluation of taxonomic sufficiency. *Journal of the North American Benthological Society*, 29(2):562-571
- CABIN (Canadian Aquatic Biomonitoring Network). (2011). CABIN Resources. <http://www.ec.gc.ca/rcba-cabin/default.asp?lang=En&n=74876ADD-1>
- CABIN National Database (<https://www.canada.ca/en/environment-climate-change/services/canadian-aquatic-biomonitoring-network/database.html>)
- Caires, A. M., and Chandra, S. (2012). Conversion factors as determined by relative macroinvertebrate sampling efficiencies of four common benthic grab samplers. *Journal of freshwater ecology*, 27(1), 97-109.
- Canadian Council of Ministers of the Environment, 2014 <http://ceqg-rcqe.ccme.ca/>
- Cao, Y., Larsen, D. P., and Thorne, R. S. J. (2001). Rare species in multivariate analysis for bioassessment: some considerations. *Journal of the North American Benthological Society*, 20(1), 144-153.
- Cao, Y., Williams, D. D., and Williams, N. E. (1998). How important are rare species in aquatic community ecology and bioassessment? *Limnology and Oceanography*, 43(7), 1403-1409.
- Carter, J. L., and Resh, V. H. (2001). After site selection and before data analysis: sampling, sorting, and laboratory procedures used in stream benthic macroinvertebrate monitoring programs by USA state agencies. *Journal of the North American Benthological Society*, 20(4), 658-682.
- Carter, J. L., Resh, V. H., Rosenberg, D. M., Reynoldson, T. B., Ziglio, G., Siligardi, M., and Flaim, G. (2006). Biomonitoring in North American rivers: a comparison of methods used for benthic macroinvertebrates in Canada and the United States. *Biological monitoring of rivers: applications and perspectives*, 203-228.
- Carter, J. L., and Resh, V. H. (2001). After site selection and before data analysis: sampling,

- sorting, and laboratory procedures used in stream benthic macroinvertebrate monitoring programs by USA state agencies. *Journal of the North American Benthological Society*, 20(4), 658-682.
- Carter, J. L., Resh, V. H., Rosenberg, D. M., Reynoldson, T. B., Ziglio, G., Siligardi, M., and Flaim, G. (2006). Biomonitoring in North American rivers: a comparison of methods used for benthic macroinvertebrates in Canada and the United States. *Biological monitoring of rivers: applications and perspectives*, 203-228.
- Caton, L. W. (1991). Improved subsampling methods for the EPA "Rapid Bioassessment" benthic protocols. *Bulletin of the North American Benthological Society*, 8(3), 317-319.
- Cheal, F., Davis, J. A., Growns, J. E., Bradley, J. S., and Whittles, F. H. (1993). The influence of sampling method on the classification of wetland macroinvertebrate communities. *Hydrobiologia*, 257(1), 47-56.
- Chirhart, J. (2003). Development of a macroinvertebrate index of biotic integrity (IBI) for rivers and streams of the St. Croix River Basin in Minnesota. St. Paul, MN: Minnesota Pollution Control Agency, Biological Monitoring Program. 1-41.
- Ciborowski, J. J.H. (1991). Estimating processing time of stream benthic samples. *Hydrobiologia*, 222(2), 101-107.
- Colwell, R. K., and J. A. Coddington. (1995). Estimating terrestrial biodiversity through extrapolation. Pages 101-118 in D. L. Hawksworth, (editor). *Biodiversity-measurement and estimation*. 1st edition. Chapman and Hall, London.
- Corbet, P. S. (1957, June). The Life-Histories Of Two Summer Species Of Dragonfly (Odonata: Coenagriidae). In *Proceedings of the Zoological Society of London* (Vol. 128, No. 3, pp. 403-418). Oxford, UK: Blackwell Publishing Ltd.
- Courtemanch, David L. (1996). "Commentary on the Subsampling Procedures Used for Rapid Bioassessments." *Journal of the North American Benthological Society* 15 (3): 381-85.
- Cuffney, T. F., Gurtz, M. E., and Meador, M. R. (1993). Methods for collecting benthic invertebrate samples as part of the National Water-Quality Assessment Program (Vol. 93, No. 406). Raleigh, North Carolina: US Geological Survey.
- Culp, J. M., Walde, S. J., and Davies, R. W. (1983). Relative importance of substrate particle size and detritus to stream benthic macroinvertebrate microdistribution. *Canadian Journal of Fisheries and Aquatic Sciences*, 40(10), 1568-1574.
- Cummins, K. W. (1979). The natural stream ecosystem. Pp. 7-24. In: Ward, J. V. and J. A. Stanford, eds., *The Ecology of Regulated Streams*. Plenum Press, NY. 398 p.
- Doberstein, C., J. Karr and L. Conquest, (2000). The effect of fixed-count subsampling on macroinvertebrate biomonitoring in small streams. *Freshwater Biology* 44: 355-371.

- Edmunds Jr, G. F., Jensen, S. L., and Berner, L. (1976). *The mayflies of north and central America*. University of Minnesota Press.
- Elliott, J.M., Drake, C.M. (1981). A comparative study of seven grabs used for sampling benthic macroinvertebrates in rivers. *Freshwater Biology* 11:99-120
- European Community (EC), (2000). Directive 2000/60/EC of the European parliament and of the council of 23 October 2000 establishing a framework for community action in the field of water policy. *Official Journal of the European Communities* L327, pp. 1–72.
- Fairchild, W. L., O'Neill, M. C. A., and Rosenberg, D. M. (1987). Quantitative evaluation of the behavioral extraction of aquatic invertebrates from samples of sphagnum moss. *Journal of the North American Benthological Society*, 6(4), 281-287.
- Faulkner, S. P., and Richardson, C. J. (1989). Physical and chemical characteristics of freshwater wetland soils. *Constructed wetlands for wastewater treatment*, 41-72.
- Ferrington Jr, L. C., Kavanaugh, R. G., Schmidt, F. J., and Kavanaugh, J. L. (1995). Habitat separation among Chironomidae (Diptera) in big springs. *Journal of the Kansas Entomological Society*, 152-165.
- Furse, M. T., Moss, D., Wright, J. F., and Armitage, P. D. (1984). The influence of seasonal and taxonomic factors on the ordination and classification of running-water sites in Great Britain and on the prediction of their macro-invertebrate communities. *Freshwater biology*, 14(3), 257-280.
- Furse, M. T., Wright, J. F., Armitage, P. D., and Moss, D. (1981). An appraisal of pond-net samples for biological monitoring of lotic macro-invertebrates. *Water Research*, 15(6), 679-689.
- Furse, M. T., Hering, D., Brabec, K., Buffagni, A., Sandin, L., & Verdonschot, P. F. (Eds.). (2009). *The ecological status of European rivers: evaluation and intercalibration of assessment methods* (Vol. 188). Springer Science & Business Media.
- Government of Canada. 2010 Atlas of Canada, 6th Edition. GeoGratis – Canada Base Map. <https://open.canada.ca/data/en/dataset/dc639a40-8893-11e0-96ca-6cf049291510>
- Haase, P., Lohse, S., Pauls, S., Schindehütte, K., Sundermann, A., Rolauuffs, P., and Hering, D. (2004). Assessing streams in Germany with benthic invertebrates: development of a practical standardised protocol for macroinvertebrate sampling and sorting. *Limnologica*, 34(4), 349-365.
- Haase, P., Murray-Bligh, J., Lohse, S., Pauls, S., Sundermann, A., Gunn, R., and Clarke, R. (2006). Assessing the impact of errors in sorting and identifying macroinvertebrate samples. In *The Ecological Status of European Rivers: Evaluation and Intercalibration of Assessment Methods* (pp. 505-521). Springer, Dordrecht.

- Haase, P., Pauls, S. U., Engelhardt, C. H., and Sundermann, A. (2008). Effects of sampling microhabitats with low coverage within the STAR/AQEM macroinvertebrate sampling protocol on stream assessment. *Limnologica*, 38(1), 14-22.
- Haase, P., S. Pauls, A. Sundermann and A. Zenker, (2004). Testing different sorting techniques in macroinvertebrate samples from running waters. *Limnologica* 34: 366–378.
- Hauer, F. R., Lamberti, G. A. 2011. *Methods in Stream Ecology*. Elsevier.
- Heatherly, T., Whiles, M. R., Royer, T. V., and David, M. B. (2007). Relationships between water quality, habitat quality, and macroinvertebrate assemblages in Illinois streams. *Journal of Environmental Quality*, 36(6), 1653-1660.
- Hess, A. D. (1941). *New limnological sampling equipment*. Limnological Society of America.
- Hickley, P. (1975). An apparatus for subdividing benthos samples. *Oikos*, 92-96.
- Hilsenhoff, W. L. (2017). An improved biotic index of organic stream pollution. *The Great Lakes Entomologist*, 20(1), 7.
- Hilsenhoff, W. L. (1977). Use of arthropods to evaluate water quality of streams [Wisconsin]. Technical Bulletin-Wisconsin Dept. of Natural Resources, Division of Conservation (USA).
- Hilsenhoff, W. L. (1982). Using a biotic index to evaluate water quality in streams. Madison, Wisconsin: Department of Natural Resources.
- Hilsenhoff, W. L. (1987). An improved biotic index of organic stream pollution. *Great Lakes Entomologist* 20.1: 31-40.
- Hornig, C. E., and Pollard, J. E. (1978). *Macroinvertebrate sampling techniques for streams in semi-arid regions: Comparison of the surber method and a unit-effort traveling kick method* (Vol. 1). Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory.
- Humphrey, C. L., Storey, A. W., and Thurtell, L. (2000). Ausrivias: Operator sample processing errors and temporal variability—implications for model sensitivity. Pages 143–163 in J. F.
- Hynes, H. B. N. (1960). *The Biology of Polluted Waters*. University of Toronto Press, Toronto, Canada. 202 p.
- Hynes, H. B. N. (1970). *The Ecology of Running Waters*. University of Toronto Press, Toronto, Canada. 555 p.
- Hynes, H.B.N. (1975). The stream and its valley. *Internationale Vereinigung für Theoretische und Angewandte Limnologie: Verhandlungen* 19:1-15.

- Illinois Environmental Protection Agency Bureau of Water. 2014 Illinois Water Monitoring Strategy for 2015-2020. Accessed 5 June 2019. <https://www2.illinois.gov/epa/Documents/epa.state.il.us/water/water-quality/monitoring-strategy/monitoring-strategy-2015-2020.pdf>
- Jones, C. Somers, K.M., Craig, B., Reynoldson, T.B. (2004). Ontario Benthos Biomonitoring Network protocol manual, version 1.0. Ontario Ministry of Environment.
- Jones, C. Somers, K.M., Craig, B., Reynoldson, T.B. (2007). Ontario Benthos Biomonitoring Network: Protocol Manual. Ontario Ministry of the Environment, Dorset, Ontario.
- Jones, R.C., and Clark, C.C., (1987). Impact of watershed urbanization on stream insect communities. *Journal of the American Water Resources Association*. 23(6): 1047–1055
- Jowett, I. G. (1993). A method for objectively identifying pool, run, and riffle habitats from physical measurements. *New Zealand journal of marine and freshwater research*, 27(2), 241-248.
- Jowett, I.G. (1993). A method for objectively identifying pool, run, and riffle habitats from physical measurements. *New Zealand Journal of Marine and Freshwater Research*, 27:2, 241-248
- Kang, S., and Lin, H. (2009). General soil-landscape distribution patterns in buffer zones of different order streams. *Geoderma*, 151(3-4), 233-240.
- Knight, A. W., and Gaufin, A. R. (1967). Stream type selection and associations of stoneflies (Plecoptera) in a Colorado River drainage system. *Journal of the Kansas Entomological Society*, 347-352.
- Krogmann, L., and Holstein, J. (2010). Preserving and specimen handling: Insects and other invertebrates. *Manual on Field Recording Techniques and Protocols for All Taxa Biodiversity Inventories*, 2, 463-481.
- Larsen, D. P., & Herlihy, A. T. (1998). The dilemma of sampling streams for macroinvertebrate richness. *Journal of the North American Benthological Society*, 17(3), 359-366.
- Lenat, D. R. (1988). Water quality assessment of streams using a qualitative collection method for benthic macroinvertebrates. *Journal of the North American Benthological Society*, 7(3), 222-233.
- Lenat, D. R., and Barbour, M. T. (1994). Using benthic macroinvertebrate community structure for rapid, cost-effective, water quality monitoring: rapid bioassessment. *Biological monitoring of aquatic systems*. Lewis Publishers, Boca Raton, Florida, 187-215.
- Lenat, D. R., and Crawford, J. K. (1994). Effects of land use on water quality and aquatic biota of three North Carolina Piedmont streams. *Hydrobiologia*, 294(3), 185-199.

- Lenat, D. R., and Resh, V. H. (2001). Taxonomy and stream ecology—the benefits of genus-and species-level identifications. *Journal of the North American Benthological Society*, 20(2), 287-298.
- Lewis, P. A., Mason Jr, W. T., and Weber, C. I. (1982). Evaluation of Three Bottom Grab Samplers for Collecting river Benthos'.
- Lisle, T. E, 1989. Using “Residual Depths” to Monitor Pool Depths Independently of Discharge. USDA Forest Service Res. Note, PSW-394, Pacific Southwest Experimental Station, Berkeley, 4 pp.
- Mackay, R. J. (1969). Aquatic insect communities of a small stream on Mont St. Hilaire, Quebec. *Journal of the Fisheries Board of Canada*, 26(5), 1157-1183.
- Mackey, A. P., Cooling, D. A., and Berrie, A. D. (1984). An evaluation of sampling strategies for qualitative surveys of macro-invertebrates in rivers, using pond nets. *Journal of Applied Ecology*, 515-534.
- Manual, F. (2009). The Canadian Aquatic Biomonitoring Network.
- Marchant, R. (1989). A subsampler for samples of benthic invertebrates. *Bulletin Australian Society of Limnology*, 12,49–52.
- Maxted, J. R., Evans, B. F., and Scarsbrook, M. R. (2003). Development of standard protocols for macroinvertebrate assessment of soft-bottomed streams in New Zealand. *New Zealand Journal of Marine and Freshwater Research*, 37(4), 793-807.
- McCune, B., and Mefford, M. J. (2011). *Multivariate Analysis of Ecological Data*, Version 6, MjM Software. *PC-ORD, Gleneden Beach, Oregon, USA*.
- Merritt, R. W., and Cummins, K. W. (Eds.). (1996). *An introduction to the aquatic insects of North America*. Kendall Hunt.
- Merritt, R. W., Cummins, K. W., and Berg, M. B. (2008). *An Introduction to the Aquatic Insects of North America*. 4th (Edition) Kendall Hunt Publishing. *Dubuque, Iowa, US. A*.
- Merritt, R. W., Cummins, K. W., and Berg, M. B. (2008). *An Introduction to the Aquatic Insects of North America*. 4th (Edition) Kendall Hunt Publishing. *Dubuque, Iowa, US. A*.
- Michigan Department of Environment, Great Lakes, and Energy (2008). *Qualitative Biological and Habitat Survey Protocols for Wadeable Streams and Rivers*. Water Bureau Policy and Procedures. Section 3103(1) of Part 31, Water Resources Protection, of the Natural Resources and Environmental Protection Act, 1994 PA 451, as amended (NREPA).
- Miller, D. A., and White, R. A. (1998). A conterminous United States multilayer soil characteristics dataset for regional climate and hydrology modeling. *Earth Interactions*, 2(2), 1-26.

- Milošević, D., Stojković, M., Čerba, D., Petrović, A., Paunović, M., and Simić, V. (2014). Different aggregation approaches in the chironomid community and the threshold of acceptable information loss. *Hydrobiologia*, 727(1), 35-50.
- Minnesota Pollution Control Agency 2017. Macroinvertebrate Data Collection Protocols for Lotic Waters in Minnesota: Sample Collection, Sample Processing and Calculation of Indices of Biotic Integrity for Qualitative Multihabitat Samples. <https://www.pca.state.mn.us/sites/default/files/wq-bsm3-12a.pdf>
- Minnesota Pollution Control Agency. 2014. Development of a macroinvertebrate-based Index of Biological Integrity for assessment of Minnesota's rivers and streams. Minnesota Pollution Control Agency, Environmental Analysis and Outcomes Division, St. Paul, MN. <https://www.pca.state.mn.us/water/water-quality-data> Accessed 5 June 2019.
- Monitoring and Assessment Section, Water Quality Planning Division, Texas Commission on Environmental Quality. Surface Water Quality Monitoring Procedures, Volume 2: Methods for Collecting and Analyzing Biological Assemblage and Habitat Data (RG-416) (June 2007) <https://www.tceq.texas.gov/publications/rg/rg-416/index.html> and https://www.tceq.texas.gov/waterquality/monitoring/swqm_procedures.html
- Moulton, I. I., Stephen, R., Carter, J. L., Grotheer, S. A., Cuffney, T. F., and Short, T. M. (2000). Methods of analysis by the US Geological Survey National Water Quality Laboratory-processing, taxonomy, and quality control of benthic macroinvertebrate samples (No. USGS-00-212). Department of the Interior Washington DC.
- Murdoch, A. and J. M. Azcue, (1995). Manual of Aquatic Sediment Sampling. Lewis Publishers, Boca Raton, 219 pp.
- Ohio Environmental Protection Agency. (1987)a. Biological Criteria for the Protection of Aquatic Life: Volume I. The Role of Biological Data in Water Quality Assessment. Ohio Environmental Protection Agency, Columbus, Ohio.
- Ohio Environmental Protection Agency. (1987)b. Biological Criteria for the Protection of Aquatic Life: Volume II. User's Manual for Biological Assessment of Ohio Surface Waters. Ohio Environmental Protection Agency, Columbus, Ohio.
- Ohio Environmental Protection Agency. (1987)c. Biological Criteria for the Protection of Aquatic Life: Volume III. Standardized Biological Field Sampling and Laboratory Methods for Assessing Fish and Macroinvertebrate Communities. Ohio Environmental Protection Agency, Columbus, Ohio.
- Ohio EPA. (2012). Field Evaluation Manual for Ohio's Primary Headwater Habitat Streams. Version 3.0. Ohio EPA Division of Surface Water, Columbus, Ohio. 117 pp.
- Økland, J., and Økland, K. A. (1986). The effects of acid deposition on benthic animals in lakes and streams. *Experientia*, 42(5), 471-486.

- Omernik, J. M., and Bailey, R. G. (1997). Distinguishing between watersheds and ecoregions 1. *JAWRA Journal of the American Water Resources Association*, 33(5), 935-949.
- Ontario Research Foundation. Physiographical Series, Ontario Department of Mines and Northern Affairs, Maps 2224-2227. Physiographic Series, Ministry of Natural Resources, Ontario Research Foundation, Map 2228.
- Patrick R. (1975). -Structure of stream communities. Pages 445-459 in *Ecology of species and communities*. Cody M. and Diamond J.M. (eds). Harvard University Press, Cambridge, MA.
- Payne, C., Ciborowski, J. J. H. (2017) Comparing the Efficacy of Field- and Laboratory- Sorted Benthic Macroinvertebrate Samples. Unpublished data.
- Pennak, R. W. (1955). Fresh-water invertebrates of the United States. The Ronald Press Co., New York, NY. 769 pages.
- Plafkin, J. L., Barbour, M. T., Porter, K. D., Gross, S. K. and Hughes, R. M.: (1989). Rapid Bioassessment Protocols for use in Streams and Rivers-Benthic Macroinvertebrates and Fish, United States Environmental Protection Agency, EPA/440/4-89/001.
- Platts, W. S., W. F Megahan, and G. W. Minshall. (1983). Methods for evaluating stream, riparian, and biotic conditions. General Technical Report No. INT-138, US Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station, Ogden, Utah.
- Poulton, B. C., Wildhaber, M. L., Charbonneau, C. S., Fairchild, J. F., Mueller, B. G., & Schmitt, C. J. (2003). A longitudinal assessment of the aquatic macroinvertebrate community in the channelized lower Missouri River. *Environmental Monitoring and Assessment*, 85(1), 23-53.
- Rabeni, C. F., and N. Wang. (2001). "Bioassessment of Streams Using Macroinvertebrates: Are the Chironomidae Necessary?" *Environmental Monitoring and Assessment* 71 (2): 177–85. <https://doi.org/10.1023/A:1017523115381>.
- Reice, S. R. (1980). The role of substratum in benthic macroinvertebrate microdistribution and litter decomposition in a woodland stream. *Ecology*, 61(3), 580-590.
- Resh V.H., Brown A.V., Covich A.P., Gurtz M.E., Li H.W., Minshall G.W., Reice S.R., Sheldon A.L., Wallace J.B. and Wissmar R.C. (1998) The role of disturbance in stream ecology. *Journal of the North American Benthological Society*, 7, 433– 455.
- Resh, V. H., Brown, A. V., Covich, A. P., Gurtz, M. E., Li, H. W., Minshall, W., Reice, S. R., Sheldon, A. L., Wallace, J. B., and Wissmar, R. C. (1988). The role of disturbance in stream ecology. *Journal of the North American Benthological Society*, 433-455.
- Resh, V. H., R. H. Norris, and M.T. Barbour. (1995). Design and Implementation of Rapid Assessment Approaches for Water Resource Monitoring Using Benthic Macroinvertebrates. *Australian Journal of Ecology* 20 (1): 108–21. <https://doi.org/10.1111/j.1442-9993.1995.tb00525.x>.

- Resh, V. H., and Jackson, J. K. (1993). Rapid assessment approaches to biomonitoring using benthic macroinvertebrates. *Chapman and Hall, New York(USA)*., 195-223.
- Reynoldson TB, Logan C, Pascoe T, Thompson SP. (2002). CABIN (Canadian Aquatic Biomonitoring Network) Invertebrate Biomonitoring Field and Laboratory Manual National Water Research Institute Environment Canada, Burlington, Ontario.
- Reynoldson, T. B., C. Logan, T. Pascoe, S. Thompson, S. Sylvestre, C. Mackinlay, and H. McDermott. (2007). CABIN Field Manual for Streams. Environment Canada.
- Reynoldson, T.B., Bombardier, M., Donald, D., O'Neill, H.J., Rosenberg, D.M., Shear, H., Tuominen, T. and Vaughan, H. (1999). Strategy for a Canadian Aquatic Biomonitoring Network. NWRI Contribution No. 99–248. 26 pp.
- Richards, N. R., A. G. Caldwell, and F. F. Morwick. (1949). *Soil survey of Essex County*. No. 11. Experimental Farms Service, Dominion Department of Agriculture and the Ontario Agricultural College.
- Richardson, R. E. (1928). The bottom fauna of the middle Illinois River, 1913-1925: its distribution, abundance, valuation, and index value in the study of stream pollution. *Illinois Natural History Survey Bulletin; v. 017, no. 12*.
- Riverwatch, H. (2000). Volunteer Stream Monitoring Training Manual. Indiana's Volunteer Stream Monitoring Program. *Natural Resources Education Center, Indianapolis, IN*.
- Roper, B. B., Kershner, J. L., Archer, E., Henderson, R., & Bouwes, N. (2002). An evaluation of physical stream habitat attributes used to monitor streams. *Journal of the American Water Resources Association, 38(6)*, 1637-1646.
- Rosenberg, D. M., and Resh, V. H. (1982). The use of artificial substrates in the study of freshwater benthic macroinvertebrates. *Artificial substrates*,175-235.
- Rosenberg, D. M., Davies, I. J., Cobb, D. G., and Wiens, A. P. (1998). Protocols for measuring biodiversity: Benthic macroinvertebrates in fresh waters. Freshwater Institute, Department of Fisheries and Oceans, Winnipeg, Manitoba.
- Rossillon, D. 1987. About the separation of benthos from stream samples. *Arch. Hydrobol.* 110:469-475. Smith, A. J., Duffy, B. T., and Heitzman, D. L. (2009). *Standard operating procedure: biological monitoring of surface waters in New York State*. New York State Department of Environmental Conservation, Division of Water.
- Saether, O.A. (1979). "Chironomid Communities as Water Quality Indicators." *Ecography* 2 (2): 65–74.

- Schloesser, D.W., Nalepa, T.F. (2002). Comparison of 5 benthic samplers to collect burrowing mayfly nymphs (*Hexagenia* species: Ephemeroptera: Ephemeridae) in sediments of the Laurentian Great Lakes. *Journal of the North American Benthological Society*, 21:487–501.
- Sciera, K. L., Smink, J. A., Morse, J. C., Post, C. J., Pike, J. W., English, W. R., ... and Klaine, S. J. (2008). Impacts of land disturbance on aquatic ecosystem health: quantifying the cascade of events. *Integrated environmental assessment and management*, 4(4), 431-442.
- Shackleford, B. (1988). Rapid bioassessment of lotic macroinvertebrate communities: biocriteria development. Arkansas Department of Pollution Control and Ecology, 45 pp
- Shostell, J. M., and Williams, B. S. (2005). A new benthic sampling device for soft sediments in shallow habitats. *Journal of Freshwater Ecology*, 20(3), 595-602.
- Singh, H., and Singh, A. (2013). Application of lean manufacturing using value stream mapping in an auto-parts manufacturing unit. *Journal of Advances in Management Research*, 10(1), 72-84.
- Smith, A. J., Duffy, B. T., and Heitzman, D. L. (2009). *Standard operating procedure: biological monitoring of surface waters in New York State*. New York State Department of Environmental Conservation, Division of Water.
- Somers, K. M., Reid, R. A., and David, S. M. (1998). Rapid biological assessments: how many animals are enough? *Journal of the North American Benthological Society*, 17(3), 348-358.
- STAR consortium. (2003). The AQEM sampling method to be applied in STAR. Available from <http://www.eu-star.at>
- Stark, J. D. (1993). Performance of the Macroinvertebrate Community Index: effects of sampling method, sample replication, water depth, current velocity, and substratum on index values. *New Zealand journal of marine and freshwater research*, 27(4), 463-478.
- Stark, J.D, Maxted, J.R. (2007). A user guide for the Macroinvertebrate Community Index. Prepared for the Ministry for the Environment. Cawthron Report No.1166. 58 p.
- Stark, J.D. Boothroyd, I.K.G. Harding, J.S. Maxted, J.R. Scarsbrook, M.R. (2001). Protocols for sampling macroinvertebrates in wadeable streams. Prepared for the Ministry for the Environment Sustainable Management Fund Project No. 5103
- Stewart, A. J., and Loar, J. M. (1994). Spatial and temporal variation in biomonitoring data. *Biological monitoring of aquatic systems*, 91-124.
- Stoddard, J. L., Larsen, D. P., Hawkins, C. P., Johnson, R. K., and Norris, R. H. (2006). Setting expectations for the ecological condition of streams: the concept of reference condition. *Ecological Applications*, 16(4), 1267-1276.

- Stoddard, J. L., Larsen, D. P., Hawkins, C. P., Johnson, R. K., and Norris, R. H. (2006). Setting expectations for the ecological condition of streams: the concept of reference condition. *Ecological Applications*, 16(4), 1267-1276.
- Surber, E. W. (1937). Rainbow trout and bottom fauna production in one mile of stream. *Transactions of the American Fisheries Society*, 66(1), 193-202.
- Surber, E. W. (1970). Procedure in taking stream bottom samples with the stream square foot bottom sampler. In *Proceedings of the 23rd Annual Conference of South East Association of Game and Fisheries Commission* (Vol. 23, pp. 589-591).
- Turner, R. E., and Rabalais, N. N. (2003). Linking landscape and water quality in the Mississippi River basin for 200 years. *Bioscience*, 53(6), 563-572.
- U.S. Geological Survey Open-File Report 00-212. US Geological Survey, Denver, Colorado.
- US Environmental Protection Agency (USEPA) National Rivers and Stream Assessment (NRSA). <https://www.epa.gov/national-aquatic-resource-surveys/what-national-rivers-and-streams-assessment#tab-1>
- USDA-NRCS, 2003 USDA-NRCS National Handbook of Conservation Practices USDA-NRCS, Washington, DC (2003) Available at: <http://www.nrcs.usda.gov/technical/Standards/nhcp.html> (accessed on 5 June 2019)
- USEPA. 2017. National Rivers and Streams Assessment 2018-19: Laboratory Operations Manual. EPA- 841-B-17-004. U.S. Environmental Protection Agency, Office of Water, Washington, DC.
- Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., and Cushing, C. E. (1980). The river continuum concept. *Canadian journal of fisheries and aquatic sciences*, 37(1), 130-137.
- Vinson, M. R., and Hawkins, C. P. (1996). Effects of sampling area and subsampling procedure on comparisons of taxa richness among streams. *Journal of the North American Benthological Society*, 15(3), 392-399.
- Vlek, H. E., Šporka, F., & Krno, I. J. (2006). Influence of macroinvertebrate sample size on bioassessment of streams. In *The Ecological Status of European Rivers: Evaluation and Intercalibration of Assessment Methods* (pp. 523-542). Springer, Dordrecht.
- Ward-Campbell, B., Cottenie, K., Mandrak, N. E., and McLaughlin, R. (2017). Fish assemblages in agricultural drains are resilient to habitat change caused by drain maintenance. *Canadian journal of fisheries and aquatic sciences*, 74(10), 1538-1548.
- Waters, T. F., and Knapp, R. J. (1961). An improved stream bottom fauna sampler. *Transactions of the American Fisheries Society*, 90(2), 225-226.

- Weber, C. I. (1973). Macroinvertebrates in biological field and laboratory methods for measuring the quality of surface waters and effluents. Nat. Environ. Res. Center, Cincinnati.
- Wentworth, C. K. (1922). A scale of grade and class terms for clastic sediments. *The journal of geology*, 30(5), 377-392.
- Wiggins, G.B. (1977). Larvae of the North American Caddisfly Genera (Trichoptera). Toronto: University of Toronto Press.
- Wiggins, J. H. (1927). U.S. Patent No. 1,651,051. Washington, DC: U.S. Patent and Trademark Office.
- Wright, D. W. Sutcliffe, and M. T. Furse (editors). Assessing the biological quality of fresh waters: RIV- PACS and other techniques. Freshwater Biological Association, Cumbria, UK.
- Wright, J.F. (2000) An introduction to RIVPACS. In: Wright, J.F. and Sutcliffe, D.W. and Furse, M.T. (eds.) Assessing the biological quality of freshwaters: RIVPACS and other techniques. Ambleside, UK, Freshwater Biological Association, pp. 1-24. (FBA Special Publications,8)
- Wrona, F. J., Culp, J. M., and Davies, R. W. (1982). Macroinvertebrate subsampling: a simplified apparatus and approach. *Canadian Journal of Fisheries and Aquatic Sciences*, 39(7), 1051-1054.
- Yellow Springs Instruments, Dayton, OH. Accessed September 2016. <https://www.ysi.com>
- Živić, I., Živić, M., Milošević, D., Bjelanović, K., Stanojlović, S., Daljević, R., and Marković, Z. (2013). The effects of geothermal water inflow on longitudinal changes in benthic macroinvertebrate community composition of a temperate stream. *Journal of Thermal Biology*, 38(5), 255-263

Appendices

Appendix A: General methods of field and laboratory sampling for 2016 and 2017.

Environmental Surveys:

During field trips, I measured and recorded data using standard field procedures and record sheets prescribed by both OBBN and CABIN. Field observations were completed and transcribed on-site at the time of sampling. Several procedures of the CABIN field surveys were omitted. In particular, slope could not be determined in the field because the landscape was essentially flat. The 100-pebble count (designed to estimate particle size-frequency distribution of coarse substrates) was not undertaken because pebbles were either rare or absent at sites. Instead, sediment cores were collected and used for laboratory sediment particle size analysis (see Appendix F).

Several stream habitat features are particularly important predictors of aquatic invertebrate community composition, including indices such as ‘residual depths’ (to determine the depth of pools; Lisle, 1987) or Froude number velocity/depth ratio as suggested by Jowett (1993). Although riffles and pools can easily be located in stony streams, Essex and Lower Thames Valley streams are often so slow-flowing and turbid that these features may be difficult to locate. As an alternative, stream width and depth were measured at several transects. Because riffles were typically absent, I located and sampled glides (MPCA, 2014), characterized as the shallowest, most rapidly flowing sections of a study area that contain the coarsest substrates.

Site Selection:

Streams were sampled within the St. Clair Clay Plain region of Essex County and the Lower Thames River valley. In 2016, 19 streams were sampled based on recommendations from

the Conservation Authority representatives for each region. The candidate stream sites for 2017 were selected from an inventory of 808 locations for Essex County and 716 locations for Lower Thames, compiled as part of the Ontario Benthic Biomonitoring Network (OBBN) program by Jones (2015). This extensive list consisted of second to fifth order streams in 31 physiographic regions in Southwestern Ontario. The sites had been selected based on accessibility and uniform spatial distribution across each region. Jones (2015) also calculated various catchment-scale attributes representing anthropogenic stresses, of which I used areal percentage of the drainage basin devoted to crop row agriculture (PCTCR) and areal percentage of the drainage basin used for municipal development (PCTDEV). Values for each site were determined using ArcMap 9.3.1 (ArcInfo) and the SOLRIS v.1.2 land inventory. I created a bivariate scatterplot of the two values for all streams in the inventory that occurred within the Essex region (Figure 1.2) and Lower Thames region (Figure 1.3).

In Essex County region (Figure 1.2), the overall level of land use for either municipal development or agriculture tended to be high, in that as the percentage of agriculture land use (crop row) increases, the percentage of development decreases. The percentage of land in row crops ranged from zero to 100%, and the proportion of land that was rural or municipal ranged from zero to over 80%. A similar trend is evident in the Lower Thames region. However, in this region all sites (except for one) had over 40% of the landscape devoted to row crops and (with one exception) less than 25% of the watershed area was developed for rural or urban use (Figure 1.3). Clearly, all of the sites identified in Essex (Figure 1.2) and Lower Thames Valley (Figure 1.3) are subject to some degree of disturbance as a consequence of agriculture and development. Nevertheless, I wished to sample streams that represented the broadest possible range of disturbances to ensure that my methodological comparisons pertained throughout the clay plain region. Accordingly, for

each of the stream locations illustrated in Figures 1.2 and 1.3, I calculated the Euclidean distance of the candidate sample sites from the graph's origin (the square root of the sum of the squares of the values of the two stress scores; "AgDev composite stress score"; Host et al. 2019) and arranged the sites in order of increasing stress for each region.

The list of sites within each region was then ordinated by composite stress score and the cumulative distribution was divided into deciles (10 sections). Two sites were then randomly selected from each decile using a random number generator to provide 20 candidate sampling sites per region. Therefore, in spring 2017, I sampled 20 sites in each region (Table 1.1), resulting in a total of 40 sites visited in 2017. Combined with 19 sites that were visited in the summer of 2016 for the pilot study, a total of 59 streams were sampled for this project (Figure 1.5, 1.6). Sites that were situated within 3 km of another site were replaced with other sites randomly selected from the same decile to ensure that coverage across each region as a whole was spatially relatively uniform.

ERCA Sites Agriculture Vs Development

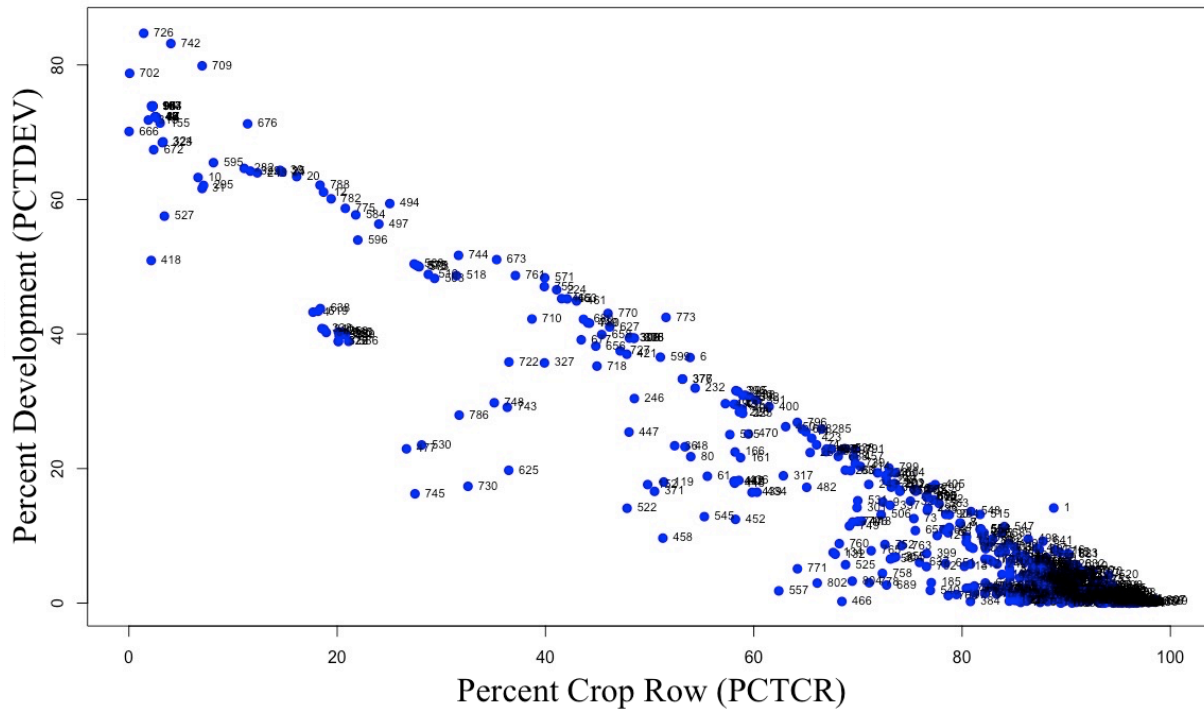


Figure A.2: Scatterplot of land use (areal percentage of land in row crops (X-axis; areal percentage of developed land) in the contributing watershed upstream of 809 stream sites within the Essex County region of Southwestern Ontario, and associated site numbers.

LTVCA Sites: Agriculture Vs Development

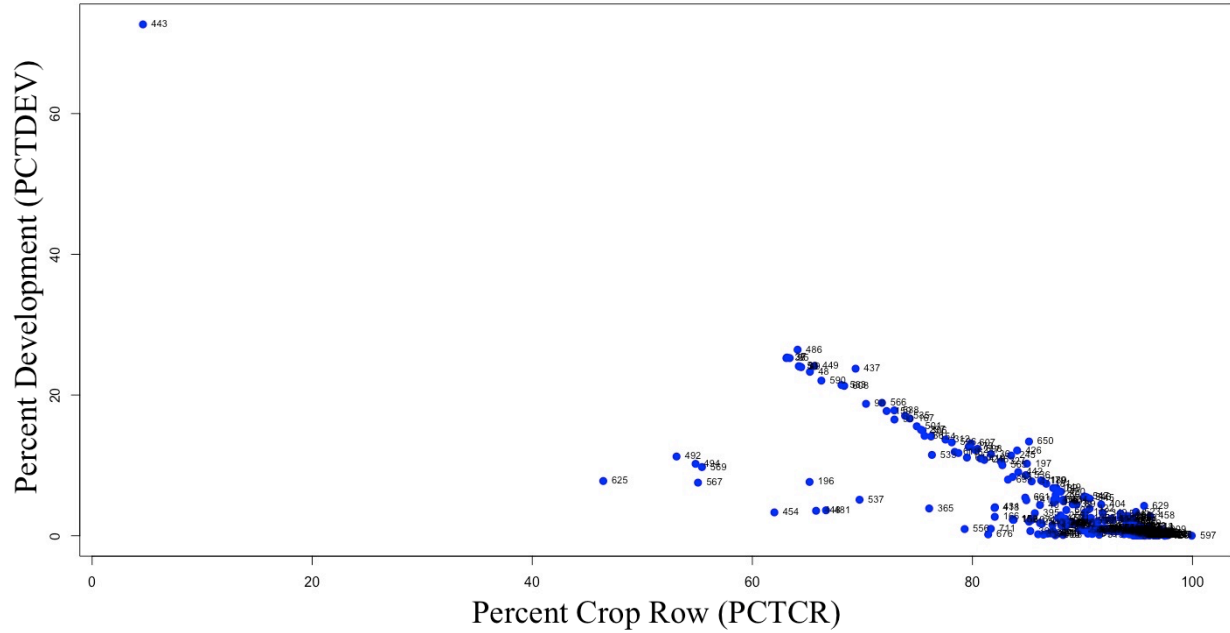


Figure A.3: Scatterplot of land use (areal percentage of land in row crops (X-axis; areal percentage of developed land) in the contributing watershed upstream of 717 sites within the Lower Thames Valley region of Southwestern Ontario and associated site numbers.

Sampling Period

Although CABIN recommends that sampling typically be conducted in the late summer or fall (when discharges typically become more stable and lower than earlier in the season), sampling in other seasons is permitted, as long as the timing of sampling is consistent from year to year. In contrast, OBBN allows sampling to be completed at any season and even lists costs and benefits for sampling in each season. OBBN also acknowledged that seasonal differences in abundance and the taxa captured can be expected due to variation in macroinvertebrate life histories. Most sediments of the Clay Plain region of southwestern Ontario are impermeable. Thus, streams are primarily surface-water fed. This makes them susceptible to very low summer discharges and high water temperatures. I wished to identify a sampling period that would ensure temperatures were low enough and discharges were high enough to support invertebrate fauna typical of perennial streams. To determine seasonal criteria for sampling streams I used a 10-y water temperature record provided by LTVCA for the Thames River. Data from 2007 to 2016 were plotted to illustrate variation in temperature by calendar date (Figure 1.4). To determine the time frame during which streams should be visited, the Thames River water temperature record (Figure 1.4) was analysed and compared to the temperatures at which many overwintering macroinvertebrate taxa emerge.

Most overwintering or early-spring developing taxa emerge when the water temperature reaches 12-24°C (Corbet, 1957; Trottier, 1973; Singh, 2008; Cushing, 2006; Becker, 2005; Milošević, 2013). Typically, the mean stream water temperature common macroinvertebrates of the region emerge is between 12°C and 20°C. As shown in Figure 1.4, that period in which sampling was therefore suggested to occur was from April to the beginning of May. Using the Thames River 10-year data I was able to determine which calendar dates would

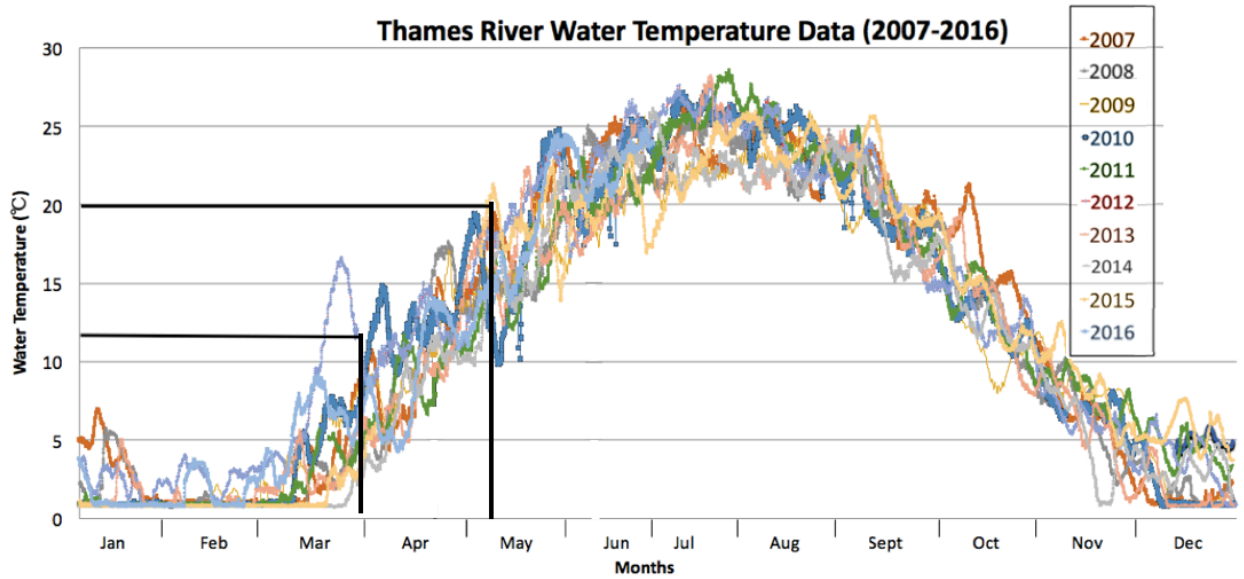


Figure A.4: Water temperature readings for the Thames River in Southwestern Ontario from 2007 to 2016. Black horizontal lines represent temperatures at 12°C and 20°C. Black vertical lines depict the sampling window.

best delineate the beginning and end of the sampling season. This was done by determining the date on which the water temperature first reached 12°C each year, and the last date in spring on which it reached 20°C for each year of the 10-y record. I then selected the first date on which the 12°C and 20°C temperatures were observed. On this basis I determined that the annual sampling period should begin in April and end at the end of the first week of May. The dataset on which this determination was based was interpreted in fall 2016 after the season's sampling had been concluded. Samples in 2016 were collected between July 4 and August 30 (Figure 1.4).

Macroinvertebrate Sampling

At each study site, macroinvertebrates were collected using two sampling instruments. Three samples were taken using a 30-cm wide, 0.50-mm mesh D-frame sweep net, and 5 Petite Ponar grab samples were collected (Chapter 2). Each sample was emptied into a 0.25-mm mesh sieve bag, which was repeatedly rinsed in the stream to remove fine sediments. All samples were placed in a labelled heavy-duty polyethylene soil bag, to which formalin-ethanol solution (2.5:1 v/v 95% ethanol and 100% buffered formalin diluted 1:1 with stream water) was added (Wiggins, 1977). Samples were returned to the laboratory where they were inventoried, heat-sealed to prevent leakage, and stored for later processing.

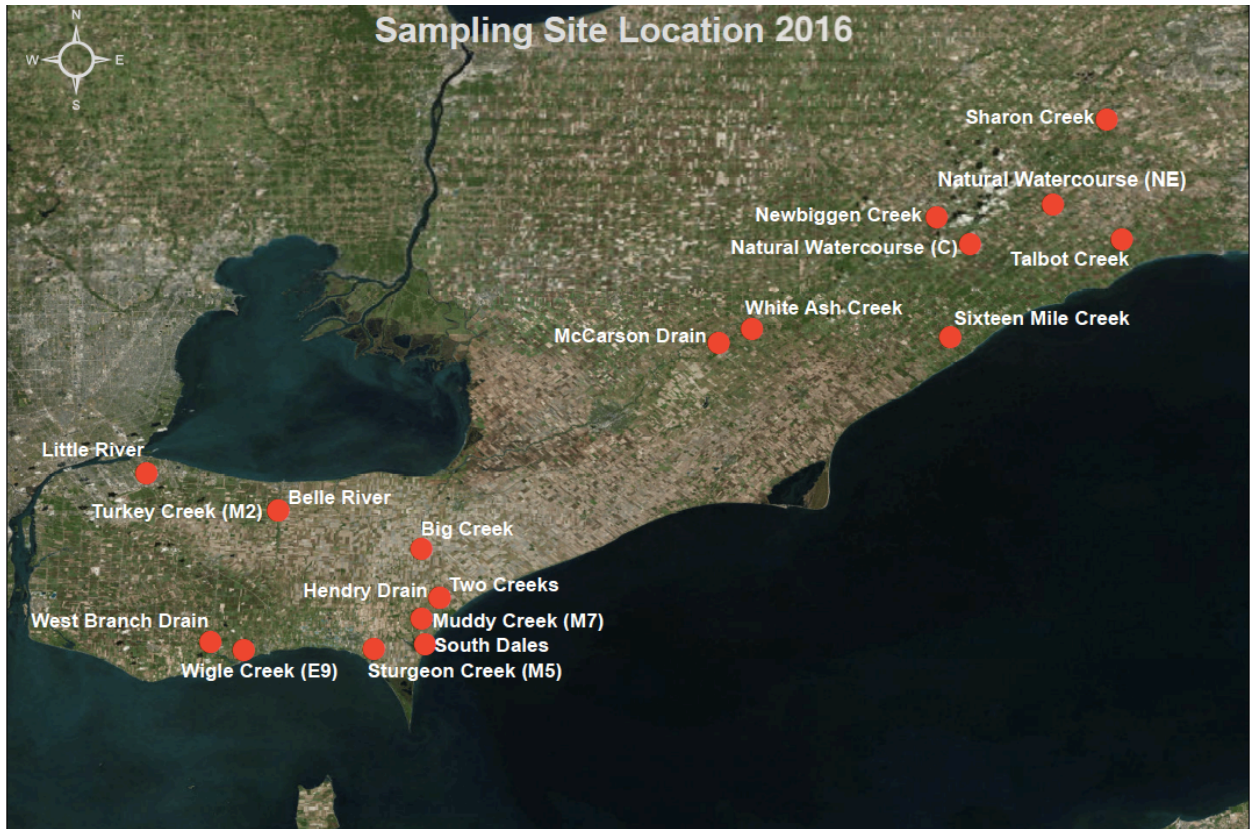


Figure A.5. Map of the Essex County and Lower Thames Valley region of Southwestern Ontario showing stream sites sampled in 2016 with the associated stream names. (n=19)

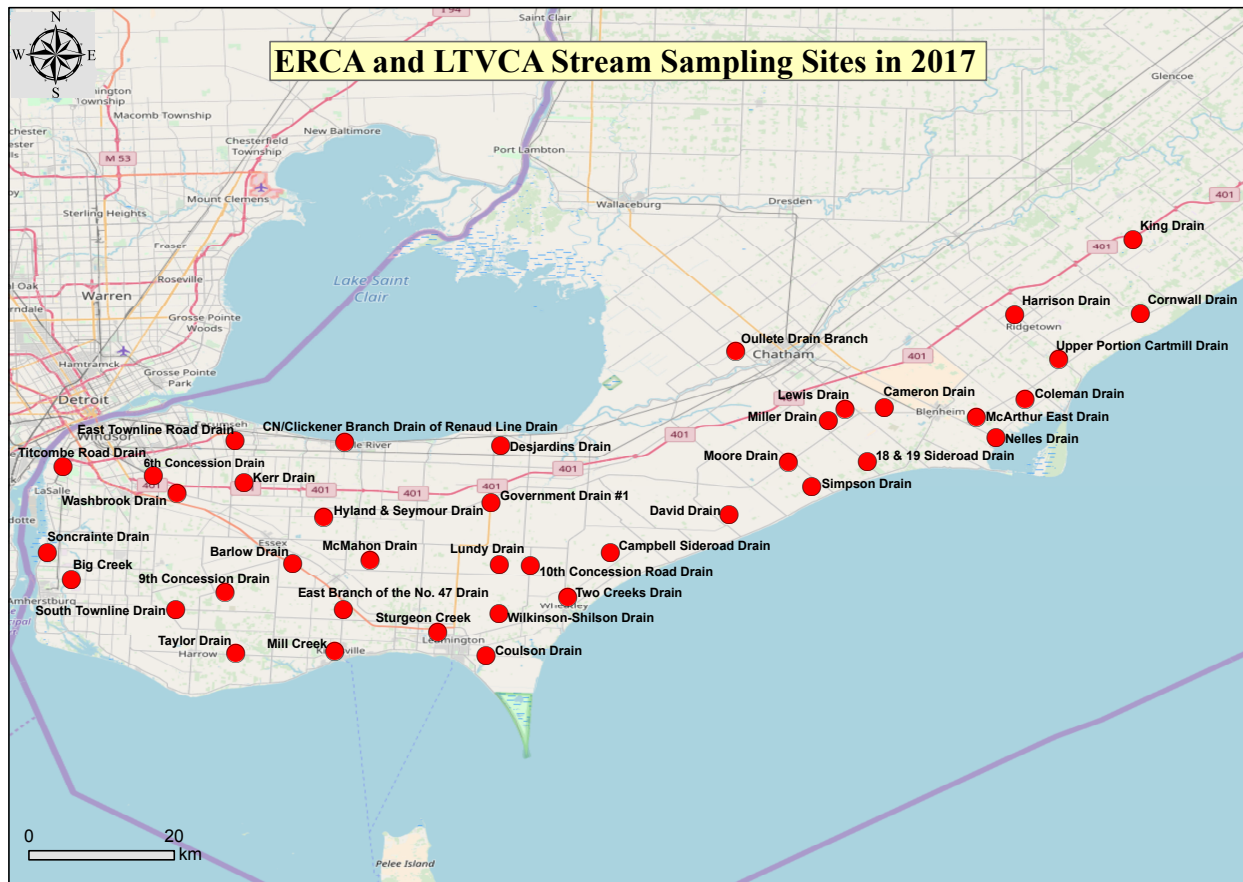


Figure A.6. Map of the Essex County and Lower Thames Valley region of Southwestern Ontario showing stream sites sampled in 2016 and 2017 with the associated stream names.

Table A.1. Sampling site, GPS coordinates, and sampling year.

<u>ERCA Sampling Sites</u>			
	Local Name	Latitude	Longitude
2016			
	Belle River	42.251012	-82.714411
	Turkey Creek (M2)	42.311337	-82.926891
	Little River	42.311337	-82.926891
	Wigle Creek (E9)	42.029794	-82.773231
	West Branch Drain	42.043116	-82.836710
	Sturgeon Creek (M5)	42.038942	-82.645428
	Muddy Creek (M7)	42.080434	-82.489117
2017			
	6 th Concession Drain	42.254569	-82.970914
	East Townline Road Drain	42.298428	-82.870118
	Washbrook Drain	42.233626	-82.941704
	Kerr Drain	42.246375	-82.858947
	Hyland & Seymour Drain	42.204002	-82.760201
	Barlow Drain	42.146528	-82.798416
	CN/Clickener Branch Drain of Renaud Line Drain	42.296646	-82.734088
	South Townline Drain	42.089439	-83.101978
	9 th Concession Drain	42.111351	-82.882197
	Soncrainte Drain	42.160257	-83.101978
	Titcombe Road Drain	42.266166	-83.083087
	Big Creek	42.126318	-83.072629
	Taylor Drain	42.035720	-82.869176
	Desjardins Drain	42.291887	-82.541526
	Sturgeon Creek	42.061702	-82.619199
	Wilkinson-Shilson Drain	42.084918	-82.543369
	Coulson Drain	42.032362	-82.559704
	Mill Creek	42.038161	-82.746721
	McMahon Drain	42.150684	-82.703198
	East Branch of the No 47 Drain	42.089470	-82.735803

Table A.1 (Cont'd). Sampling site, GPS coordinates, and sampling year

<u>LTVCA Sampling Sites</u>			
	Local Name	Latitude	Longitude
2016	Sharon Creek	42.874040	-81.400377
	Newbiggen Creek	42.717937	-81.669880
	Sixteen Mile Creek	42.527415	-81.647913
	Big Creek	42.190845	-82.477730
	Talbot Creek	42.681609	-81.374632
	White Ash Creek	42.540209	-81.963236
	South Dales	42.106082	-82.483055
	Two Creeks	42.117999	-82.461325
	McCarson Drain	42.517856	-82.015933
	Natural Watercourse (NE)	42.737229	-81.484140
	Natural Watercourse (C)	42.675337	-81.616317
	Hendry Drain	42.767545	-81.547026
2017	Two Creeks Drain	42.105010	-82.458764
	10 th Concession Road Drain	42.143959	-82.504970
	Lundy Drain	42.145377	-82.542982
	Campbell Sideroad Drain	42.159987	-82.405549
	David Drain	42.206589	-82.258832
	Government Drain #1	42.221670	-82.553368
	Simpson Drain	42.241828	-82.156474
	Moore Drain	42.272022	-82.185406
	18 & 19 Sideroad Drain	42.272089	-82.087772
	Nelles Drain	42.301417	-81.928453
	Lewis Drain	42.337092	-82.115209
	Cameron Drain	42.339162	-82.066617
	Coleman Drain	42.349840	-81.892920
	Upper Portion Cartmill Drain	42.399063	-81.850859
	Oullete Drain Branch	42.408963	-82.250456
	Harrison Drain	42.453757	-81.905205
	Cornwall Drain	42.455744	-81.749775
	King Drain	42.546554	-81.759285
	McArthur East Drain	42.327350	-81.952723

Appendix B: Summary Tables of NMDS Analyses

Table B1: Summary of Pearson correlations between relative abundances of families collected in D-net and Petite Ponar samples and their scores along NMDS axes as described in detail in Chapter 2 (ordination stress = 11.25). Bold-faced correlations are statistically significant ($p < 0.05$; uncorrected for multiple tests). Taxa names are sorted in decreasing order of their strength of correlation with then NMDS axis with which they are most highly associated.

Taxon	NMDS Axis 1	NMDS Axis 2	NMDS Axis 3
Elmidae	0.781	0.522	0.134
Hydropsychidae	0.760	0.178	-0.381
Baetidae	0.714	0.146	-0.205
Empididae	0.709	-0.067	-0.439
Tipulidae	0.675	-0.161	-0.362
Simuliidae	0.656	0.166	-0.266
Hydroptilidae	0.614	-0.167	-0.288
Leptoceridae	0.503	0.277	0.290
Tabanidae	0.446	-0.069	-0.280
Haliplidae	0.147	-0.058	0.031
Chloroperlidae	0.364	0.238	0.087
Branchiobdellidae	-0.550	-0.184	0.050
Oligochaeta	-0.797	-0.286	0.097
Acari	0.233	0.587	-0.107
Heptageniidae	0.410	0.503	-0.018
Caenidae	0.130	0.393	-0.029
Hydridae	0.011	-0.099	0.043
Gammaridae	0.361	-0.472	-0.403
Glossiphoniidae	0.113	-0.529	0.043
Planorbidae	-0.252	-0.529	-0.499
Ceratopogonidae	-0.260	-0.532	0.505
Asellidae	0.262	-0.534	-0.346
Physidae	0.200	-0.582	-0.339
Sphaeridae	0.230	-0.590	-0.034
Erpobdellidae	0.155	-0.622	-0.085
Nematoda	0.354	-0.648	0.204
Mesoveliidae	0.088	-0.655	-0.001
Lymnaeidae	0.068	-0.718	-0.050
Hyaellidae	-0.002	-0.412	0.602
Corixidae	0.139	0.187	0.581
Culicidae	-0.132	-0.462	0.572
Chironomidae	0.294	0.314	0.419

Nematomorpha	-0.262	-0.099	0.449
Cambaridae	0.262	-0.162	0.340
Hydrophilidae	0.175	-0.166	0.186
Collembola	-0.088	-0.240	-0.286
Tricladida	0.374	0.144	-0.395
Coenagrionidae	-0.333	-0.379	-0.415
Veliidae	0.293	-0.238	-0.470

Table B2: Summary of Pearson correlations between relative abundances of families identified using the Marchant Box and Nested Sieves protocols and their scores along NMDS axes as described in detail in Chapter 3 (ordination stress = 12.22). Bold-faced correlations are statistically significant ($p < 0.05$; uncorrected for multiple tests). Taxa names are sorted in decreasing order of their strength of correlation with then NMDS axis with which they are most highly associated.

Taxon	NMDS Axis 1	NMDS Axis 2
Chironomidae	0.555	-0.155
Leptoceridae	0.366	0.080
Sphaeriidae	0.027	0.011
Gomphidae	-0.420	-0.036
Gammaridae	-0.496	0.078
Glossiphoniidae	-0.514	0.011
Ceratopogonidae	-0.539	-0.187
Haliplidae	-0.579	0.105
Nematoda	-0.600	-0.144
Dytiscidae	-0.643	-0.026
Hydrophilidae	-0.673	0.012
Asellidae	-0.688	0.354
Corduliidae	-0.721	-0.031
Libellulidae	-0.746	-0.077
Baetidae	-0.748	0.214
Physidae	-0.749	-0.086
Caenidae	-0.823	-0.078
Coenagrionidae	-0.841	0.067
Tipulidae	0.128	0.826
Simuliidae	0.032	0.753
Corixidae	-0.158	0.636
Hydroptilidae	-0.057	0.487
Elmidae	0.050	0.400
Hydropsychidae	-0.046	0.396
Heptageniidae	-0.071	0.329
Siphonuridae	-0.030	0.295
Empididae	0.162	0.243
Polycentropodidae	-0.059	0.211
Planorbidae	-0.097	0.202
Hydracarina	0.128	0.159
Oligochaeta	-0.345	-0.751

Appendix C: List of taxa observed in at least one sample (3-D-net and 5 Petite Ponar grabs) in 19 streams sampled in 2016

<u>LTVCA</u>							Big Creek	Hendry Drain	McCarson Drain	Natural Watercourse (C)	Natural Watercourse (NE)	Newbiggen	Sharon Creek	Sixteen Mile Creek	South Dales Creek	Talbot Creek	Two Creeks	White Ash Creek	
PHYLUM																			
SUBPHYLUM																			
CLASS																			
ORDER																			
SUBORDER																			
Family																			
Subfamily																			
Genus																			
<i>species</i>																			
CNIDARIA																			
HYDROZOA																			
ANTHOATHECATA																			
Hydridae																			
Hydra							X	X	X	X	X	X	X				X		
NEMATODA							X	X	X	X	X	X	X			X	X	X	X
NEMATOMORPHA									X	X	X					X			
ANNELIDA																			
CLITELLATA																			
OLIGOCHAETA							X	X	X	X	X	X	X	X	X	X	X	X	X
ARHYNCHOBDELLIDA																			
ERPOBDELLIFORMES																			
Erpobdellidae							X		X	X								X	
<i>Erpobdella</i>																X		X	
<i>punctata</i>												X				X		X	
<i>Motobdella</i>									X							X			
<i>Other</i>															X				
BRANCHIOBDELLIDA									X				X		X	X	X		
RHYNCHOBDELLIDA														X					
Glossiphoniidae							X		X	X		X				X			
<i>Glossiphonia</i>																			

							<i>elegans</i>			X			X					
							<i>Helobdella</i>										X	
							<i>papillata</i>				X							
							<i>stagnalis</i>	X		X				X	X		X	
							<i>Placobdella</i>	X		X	X		X					
							<i>montifera</i>						X					
							<i>Other</i>											
							OTHER											
							MOLLUSCA											
							BIVALVIA											
							SPHAERIIDA											
							Sphaeriidae		X	X	X	X	X	X	X	X	X	X
							<i>Sphaerium</i>											
							<i>Musculium</i>											
							<i>Sphaerium/</i> <i>Musculium</i>	X						X	X			
							<i>Pisidium</i>	X		X	X	X	X		X	X		
							GASTROPODA											
							ARCHITAENIOGLOSSA											
							Viviparidae			X								
							<i>Bellamyia</i>			X								
							<i>Other</i>											
							BASOMMATOPHORA											
							Ancylidae											
							<i>Ferrissia/</i> <i>Laevapex</i>	X	X				X	X	X	X		X
							<i>Other</i>											
							Lymnaeidae	X							X		X	
							<i>Lymnaea</i>								X			
							<i>palustris</i>					X						
							<i>stagnalis</i>	X										
							<i>Other</i>											
							Physidae			X				X	X	X		X
							<i>Physa</i>	X		X		X	X		X	X	X	X
							<i>Other</i>											
							Planorbidae						X		X	X	X	
							<i>Helisoma</i>								X			
							<i>Other</i>											
							HETEROSTROPHA											

				Valvatidae		X		X				X							
				<i>Valvata</i>															
				<i>tricarinata</i>				X										X	
				<i>Other</i>															
PLATYHELMINTHES																			
				TURBELLARIA															
				TRICLADIDA															
				Planariidae		X	X	X		X	X	X						X	X
ARTHROPODA																			
				CHELICERATA															
				ARACHNIDA															
				TROMBIDIFORMES															
				HYDRACARINA		X	X	X	X	X	X	X		X	X	X	X	X	X
CRUSTACEA																			
				MAXILLOPODA															
				MALACOSTRACA															
				AMPHIPODA		X				X			X	X				X	
				Crangonyctidae															
				<i>Crangonyx</i>										X					
				Gammaridae			X	X		X	X	X		X				X	
				<i>Echinogammarus</i>															
				<i>ischnus</i>							X			X				X	
				<i>Gammarus</i>		X	X	X		X	X	X	X	X	X			X	
				<i>fasciatus</i>				X											
				<i>lacustris</i>															
				<i>tigrinus</i>				X											
				<i>Other</i>															
				Hyalellidae															
				<i>Hyalella</i>			X	X	X	X				X				X	
				<i>Other</i>															
DECAPODA																			
				Cambaridae					X	X	X	X		X					
				<i>Cambarus</i>															
				<i>Orconectes</i>										X	X				
				<i>Other</i>															
ISOPODA																			
				Asellidae										X				X	X
				<i>Caecidotea</i>		X		X		X	X	X	X	X	X	X	X	X	X

					Hebridae														
						<i>Merragata</i>													
						Gerridae			X										X
						Mesoveliidae			X										
						<i>Mesovelia</i>							X						X
						<i>Other</i>													
						Mesoveliidae/ Veliidae													
						Notonectidae		X	X										
						Veliidae							X						
						<i>Microvelia</i>						X	X		X			X	X
						<i>Rhagovelia</i>							X						
						<i>Other</i>													
						OTHER													
						MEGALOPTERA									X				
						Corydalidae													
						<i>Chauliodes</i>			X										
						Sialidae													
						<i>Sialis</i>			X		X								
						Other													
						COLEOPTERA													
						ADEPHAGA													
						Carabidae							X						
						Curculionidae		X					X		X		X	X	
						Dytiscidae		X	X										
						Agabinae													X
						<i>Agabus</i>													X
						<i>Hydrotrupes</i>													X
						Hydroporinae													X
						<i>Hydroporus</i>													X
						Matinae													
						<i>Matus</i>													X
						Other													
						Gyrinidae			X										
						Gyrininae													
						<i>Dineutus</i>													
						<i>Gyrinus</i>												X	
						Haliplidae		X	X						X				X
						<i>Brychius</i>			X										

						<i>Haliphus</i>				X								X		
						<i>Peltodytes</i>		X	X	X		X				X		X		
						Other														
					POLYPHAGA															
					Dryopidae															
					Elmidae															
							X	X	X	X	X	X	X	X	X	X	X	X	X	X
					Elminae															
						<i>Ancyronyx</i>				X		X								
						<i>Dubiraphia</i>		X	X	X	X	X	X	X	X	X	X	X	X	X
						<i>Dubiraphia/ Narpus</i>										X				
						<i>Maxronychus</i>									X					
						<i>Optioservus</i>						X			X	X				
						<i>Ordobrevia</i>					X	X								
						<i>Oulimnius</i>			X		X									
						<i>Stenelmis</i>			X	X			X	X	X					
					Hydrophilidae															
							X												X	
					Georissidae															
						<i>Georissus</i>		X							X					
					Hydrophilinae															
						<i>Berosus</i>		X											X	
						<i>Helochares</i>		X												
						<i>Tropisternus</i>		X		X										
					Helophorinae															
						<i>Helophorus</i>					X				X					
					Scirtidae															
					Staphylinidae															
								X	X											
					TRICHOPTERA															
					ANNULIPALPIA															
					Hydropsychidae															
							X		X			X	X	X	X			X	X	
					Hydropsychinae															
						<i>Cheumatopsyche</i>			X	X		X	X		X			X		
						<i>Hydropsyche</i>						X								
						Other														
					Macronematinae															
						<i>Smicridea</i>						X						X	X	
					Philopotamidae															
												X								
						<i>Chimarra</i>						X								
						Other														

				Polycentropodidae								X	X	X				
				Other														
				INTEGRIPALPIA														
				Hydroptilidae		X		X		X		X	X	X	X		X	X
				Hydroptilinae														
				<i>Hydroptila</i>				X				X		X			X	X
				<i>Oxyethira</i>				X										
				Other														
				Leptoceridae				X	X	X				X	X			X
				<i>Oecetis</i>			X	X				X		X	X		X	
				<i>Trianodes</i>				X										
				Other														
				Helicopsychidae								X						
				<i>Helicopsyche</i>								X						
				Other														
				DIPTERA														
				NEMATOCERA														
				Ceratopogonidae		X						X			X	X	X	
				Ceratopogoninae			X	X	X									
				<i>Bezzia/ Palpomyia</i>		X		X	X	X	X			X			X	
				<i>Culicoides</i>														
				<i>Serromyia</i>			X											
				<i>Stilobezzia</i>										X				
				Other														
				Chaoboridae													X	
				<i>Chaoborus</i>														
				<i>Mochlonyx</i>														
				Other														
				Chironomidae		X	X	X	X	X	X	X	X	X	X	X	X	X
				Culicidae					X									
				<i>Aedes</i>														
				<i>Anopheles</i>						X				X				
				<i>Culex</i>														
				Other														
				Dixidae														
				<i>Dixa</i>														
				Limoniidae														
				<i>Limonia</i>										X				

					Limoniinae														
					<i>Antocha</i>								X						
					Simuliidae			X			X	X					X	X	
					Tipulidae		X	X	X			X	X				X	X	
					Tipulinae														
					<i>Tipula</i>						X	X			X		X		
					ORTHORRHAPHA														
					Athericidae						X								
					<i>Atherix</i>										X				
					<i>Other</i>														
					Dolichopodidae						X							X	
					Empididae		X		X		X	X	X	X	X	X	X	X	X
					Psychodidae													X	
					Psychodinae			X											
					Other														
					Stratiomyidae						X								
					Tabanidae		X	X	X		X	X	X	X	X	X	X	X	X
					Chrysopsinae														
					<i>Chrysops</i>		X		X		X								

<u>ERCA</u>							Belle River	Little River	Muddy Creek	Sturgeon Creek	Turkey Creek	West Branch Drain	Wigle Creek
PHYLUM													
SUBPHYLUM													
CLASS													
ORDER													
SUBORDER													
Family													
Subfamily													
Genus													
<i>species</i>													
CNIDARIA													
HYDROZOA													
ANTHOATHECATA													
Hydridae													
Hydra								X					X
NEMATODA							X	X	X		X	X	X
NEMATOMORPHA							X			X	X	X	X
ANNELIDA									X				
CLITELLATA													
OLIGOCHAETA							X	X	X	X	X	X	X
ARHYNCHOBDELLIDA													
ERPOBDELLIFORMES													
Erpobdellidae									X			X	
<i>Erpobdella</i>													
<i>punctata</i>									X				
<i>Motobdella</i>													
<i>Other</i>													
BRANCHIOBDELLIDA											X	X	
RHYNCHOBDELLIDA													
Glossiphoniidae									X				
<i>Glossiphonia</i>													
<i>elegans</i>													

						<i>Helobdella</i>				X										
							<i>papillata</i>													
							<i>stagnalis</i>			X										
						<i>Placobdella</i>							X							
							<i>montifera</i>													
						<i>Other</i>														
						OTHER														
						MOLLUSCA														
						BIVALVIA														
						SPHAERIIDA														
						Sphaeriidae			X	X	X	X	X	X	X					
						<i>Sphaerium</i>														
						<i>Musculium</i>														
						<i>Sphaerium/</i>			X		X								X	
						<i>Musculium</i>														
						<i>Pisidium</i>					X	X							X	
						GASTROPODA														
						ARCHITAENIOGLOSSA														
						Viviparidae														
						<i>Bellamya</i>														
						<i>Other</i>														
						BASOMMATOPHORA														
						Ancylidae			X											
						<i>Ferrissia/</i>													X	X
						<i>Laevapex</i>														X
						<i>Other</i>														
						Lymnaeidae					X								X	
						<i>Lymnaea</i>					X									
							<i>palustris</i>												X	
							<i>stagnalis</i>													
						<i>Other</i>														
						Physidae					X									
						<i>Physa</i>			X	X				X	X				X	X
						<i>Other</i>														
						Planorbidae			X	X	X	X	X	X	X				X	X
						<i>Helisoma</i>														
						<i>Other</i>														
						HETEROSTROPHA														
						Valvatidae														

						<i>Valvata</i>													
							<i>tricarinata</i>												
						<i>Other</i>													
PLATYHELMINTHES																			
TURBELLARIA																			
TRICLADIDA																			
Planariidae																			
													X						X
ARTHROPODA																			
CHELICERATA																			
ARACHNIDA																			
TROMBIDIFORMES																			
HYDRACARINA																			
								X	X	X	X	X	X	X	X	X	X	X	X
CRUSTACEA																			
MAXILLOPODA																			
MALACOSTRACA																			
AMPHIPODA																			
								X		X	X					X			
Crangonyctidae																			
<i>Crangonyx</i>																			
Gammaridae																			
<i>Echinogammarus</i>																			
<i>ischnus</i>																			
<i>Gammarus</i>																			
<i>fasciatus</i>																			
<i>lacustris</i>																			
<i>tigrinus</i>																			
<i>Other</i>																			
Hyalellidae																			
<i>Hyalella</i>																			
								X		X			X	X	X				
<i>Other</i>																			
DECAPODA																			
Cambaridae																			
<i>Cambarus</i>																			
<i>Orconectes</i>																			
<i>Other</i>																			
ISOPODA																			
Asellidae																			
<i>Caecidotea</i>																			
								X	X	X	X	X	X	X					
<i>Other</i>																			

			Stenasellidae									
			OTHER									
		HEXAPODA										
			COLLEMBOLA			X		X		X		
			INSECTA									
			EPHEMEROPTERA									
			PISCIFORMA									
			Baetidae			X				X		
					<i>Baetis</i>							
					<i>Callibaetis</i>							
					<i>Cloeon</i>							
						<i>dipterum</i>	X					
					<i>Procloeon</i>							
					<i>Other</i>							
			Heptageniidae			X				X	X	
					<i>Stenacron</i>		X			X	X	
					<i>Stenonema</i>							
						<i>femoratum</i>						
					<i>Other</i>							
					Heptageniinae							
					<i>Macdunnoa</i>							
			Metretopodidae				X					
					<i>Other</i>							
			Siphonuridae									
			FURCATERGALIA									
			Caenidae									X
					<i>Caenis</i>		X		X	X	X	
			Ephemerellidae									
					<i>Hexagenia</i>							
						<i>limbata</i>						
					<i>Other</i>							
			ODONATA									
			ANISOPTERA									
			Aeshnidae									
					<i>Aeshna</i>							
					<i>Anax</i>							
					<i>Other</i>							
			Corduliidae									X

						<i>Cordulia</i>				X							
						<i>Other</i>											
						Libellulidae											
						<i>Erythemis</i>						X					
						<i>Libellula</i>											
						<i>Other</i>											
						Corduliidae/ Libellulidae											
						Other											
						ZYGOPTERA											
						Calopterygidae			X								X
						<i>Calopteryx</i>											X
						<i>Other</i>											
						Coenagrionidae			X	X	X	X	X	X	X	X	
						<i>Amphiagrion</i>											
						<i>Argia</i>								X	X		
						<i>Enallagma</i>						X					
						<i>Enallagma/</i> <i>Coenagrion</i>								X	X		
						<i>Ishnura</i>											X
						<i>Nehalennia</i>			X		X						
						<i>Other</i>											
						PLECOPTERA											
						Chloroperlidae											
						Other											
						THYSANOPTERA											X
						HEMIPTERA											X
						HETEROPTERA											
						Belostomatidae											
						Corixidae			X				X	X	X		
						Corixinae											
						<i>Callicorixa</i>											
						<i>Corisella</i>			X								
						<i>Dasycorixa</i>											
						<i>Hesperocorixa</i>											
						<i>Palmocorixa</i>			X								
						<i>Sigara</i>											
						<i>Trichocorixa</i>			X								
						<i>Other</i>			X								
						Hebridae											

					<i>Merragata</i>					X			
					Gerridae								X
					Mesoveliidae				X		X	X	
					<i>Mesovelia</i>				X		X	X	
					<i>Other</i>								
					Mesoveliidae/ Veliidae			X					
					Notonectidae								
					Veliidae								
					<i>Microvelia</i>						X	X	
					<i>Rhagovelia</i>								
					<i>Other</i>								
					OTHER								
					MEGALOPTERA								
					Corydalidae							X	
					<i>Chauliodes</i>							X	
					Sialidae								
					<i>Sialis</i>								
					Other								
					COLEOPTERA								
					ADEPHAGA								
					Carabidae								
					Curculionidae								
					Dytiscidae								
					Agabinae								
					<i>Agabus</i>								
					<i>Hydrotrupes</i>								
					Hydroporinae								
					<i>Hydroporus</i>								
					Matinae								
					<i>Matus</i>								
					Other								
					Gyrinidae								
					Gyrininae								
					<i>Dineutus</i>						X		
					<i>Gyrinus</i>								
					Haliplidae			X					
					<i>Brychius</i>								
					<i>Haliphys</i>								

					<i>Peltodytes</i>		X	X					
					Other								
				POLYPHAGA									
					Dryopidae								X
					Elmidae		X					X	X
					Elminae								
					<i>Ancyronyx</i>								
					<i>Dubiraphia</i>		X					X	X
					<i>Dubiraphia/ Narpus</i>								
					<i>Maxronychus</i>								
					<i>Optioservus</i>								
					<i>Ordobrevia</i>								
					<i>Oulimnius</i>								X
					<i>Stenelmis</i>								
					Hydrophilidae								
					Georissidae								
					<i>Georissus</i>								
					Hydrophilinae								
					<i>Berosus</i>								
					<i>Helochares</i>								
					<i>Tropisternus</i>								
					Helophorinae								
					<i>Helophorus</i>		X						
					Scirtidae		X		X			X	
					Staphylinidae								
				TRICHOPTERA									
				ANNULIPALPIA									
					Hydropsychidae								
					Hydropsychinae								
					<i>Cheumatopsyche</i>								
					<i>Hydropsyche</i>								
					<i>Other</i>								
					Macronematinae								
					<i>Smicridea</i>								
					Philopotamidae								
					<i>Chimarra</i>								
					<i>Other</i>								
					Polycentropodidae								

						<i>Antocha</i>											
						Simuliidae											
						Tipulidae				X				X			
						Tipulinae											
						<i>Tipula</i>											X
						ORTHORRHAPHA											
						Athericidae											
						<i>Atherix</i>											
						<i>Other</i>											
						Dolichopodidae						X					
						Empididae				X				X			X
						Psychodidae											X
						Psychodinae											
						Other											
						Stratiomyidae				X							
						Tabanidae				X							X
						Chrysopsinae											
						<i>Chrysops</i>											

Appendix D – 2016 Field Data.

Field measurements recorded at the 19 streams sampled in 2016.

**NR: no data were recorded during sampling.

Site Name	Water Temperature (°C)	Air Temperature (°C)	DO (mg/l)	Conductivity (uS/cm)	pH
ERCA					
Belle River	25.22	24.4	NR	NR	8.86
Little River	21.30	24.7	NR	NR	7.67
Muddy Creek	18.63	26.0	NR	3	8.28
Sturgeon Creek	22.27	29.0	NR	30.6	8.15
Turkey Creek	25.70	27.7	NR	1234	7.63
West Branch Drain	19.50	NR	NR	735	7.85
Wigle Creek	19.57	NR	54.3	NR	8.09
LTVCA					
Big Creek	21.60	29.0	0.34	NR	8.13
Hendry Drain	18.26	21.0	57.3	567	7.73
McCarson Drain	23.46	28.0	7.59	754	7.9
Natural Watercourse (C)	18.09	24.0	4.2	625	7.53
Natural Watercourse (NE)	20.20	29.8	10.08	671	7.98
Newbiggen	20.20	22.0	8.72	633	7.62
Sharon Creek	18.60	26.0	9.36	552	7.5
Sixteen Mile Creek	21.46	24.0	7.86	581	7.86
South Dales Creek	19.86	26.0	5.67	642	7.05
Talbot Creek	21.80	27.1	6.62	76.2	8.08
Two Creeks	18.00	25.5	7.72	487	6.97
White Ash Creek	19.80	25.5	7.62	612	8.62

Class	Description
1	Clay (hard pan)
2	Silt (gritty, < 0.06 mm particle diameter)
3	Sand (grainy, 0.06 - 2 mm)
4	Gravel (2 - 65 mm)
5	Cobble (65 - 250 mm)
6	Boulder (> 250 mm)
7	Bed Rock

Site Name	Substrate: Dominant			Substrate: 2nd Dominant			Substrate Notes
	Sample 1 (Riffle 1)	Sample 2 (Pool)	Sample 3 (Riffle 2)	Sample 1 (Riffle 1)	Sample 2 (Pool)	Sample 3 (Riffle 2)	
ERCA							
Belle River	2	2	2	1	1	1	Backhoe tracks present
Little River	4	3	4	2	1	2	beer cans and styrofoam; Sulphur pockets
Muddy Creek	1	3	3	2	3	1	NR
Sturgeon Creek	5	3	3	3	4	1	Sulphur pockets
Turkey Creek	3	3	3	2	2	2	NR
West Branch Drain	6	3	4	5	4	3	NR
Wigle Creek	4	3	4	3	4	3	Many boulders present
LTVCA							
Big Creek	1	1	2	2	2	3	Boulders present
Hendry Drain	4	4	4	3	3	3	many large rocks - hard to ponar
McCarson Drain	3	3	3	2	2	2	Soft; duck weed and a lot of macrophyte present
Natural Watercourse (C)	1	1	3	3	3	1	Mucky; sulphur pockets
Natural Watercourse (NE)	1	1	1	3	3	3	Mucky

Site Name	Substrate: Dominant			Substrate: 2nd Dominant			Substrate Notes
	Sample 1 (Riffle 1)	Sample 2 (Pool)	Sample 3 (Riffle 2)	Sample 1 (Riffle 1)	Sample 2 (Pool)	Sample 3 (Riffle 2)	
LTVCA							
Newbiggen	4	4	4	3	3	3	Mucky pool bottoms
Sharon Creek	5	3	5	3	5	3	NR
Sixteen Mile Creek	3	3	3	1	4	4	Pool middle with rocks, ponar done to side
South Dales Creek	1	2	4	3	1	3	NR
Talbot Creek	1	1	1	4	4	4	NR
Two Creeks	3	4	2	2	3	3	Little to no clay; cobble is present
White Ash Creek	7	1	5	6	3	3	NR

Site Name	Organic Matter Areal Coverage: Woody Debris			Organic Matter Areal Coverage: Detritus		
	Sample 1 (Riffle 1)	Sample 2 (Pool)	Sample 3 (Riffle 2)	Sample 1 (Riffle 1)	Sample 2 (Pool)	Sample 3 (Riffle 2)
ERCA						
Belle River	2	2	2	2	2	2
Little River	2	2	2	2	2	2
Muddy Creek	2	2	2	2	2	2
Sturgeon Creek	2	2	2	2	2	2
Turkey Creek	2	2	2	2	2	2
West Branch Drain	1	1	1	2	2	2
Wigle Creek	2	1	2	2	1	1
LTVCA						
Big Creek	2	2	2	2	2	2
Hendry Drain	2	2	2	2	2	2
McCarson Drain	2	2	2	2	2	2
Natural Watercourse (C)	2	2	2	2	2	2
Natural Watercourse (NE)	1	1	1	1	1	1
Newbiggen	2	2	2	3	2	3
Sharon Creek	2	2	2	2	2	2
Sixteen Mile Creek	2	2	2	2	2	2
South Dales Creek	1	1	1	1	1	1
Talbot Creek	2	2	2	2	2	2
Two Creeks	1	1	1	2	2	2
White Ash Creek	2	2	2	2	2	2

Site Name	Riparian Vegetative Community: Left Bank			Riparian Vegetative Community: Right Bank		
	1.5 - 10 m from water's edge	10 - 30 m from water's edge	30 - 100 m from water's edge	1.5 - 10 m from water's edge	10 - 30 m from water's edge	30 - 100 m from water's edge
ERCA						
Belle River	4	2	2	5	2	2
Little River	4	2	2	4	2	2
Muddy Creek	4	2	2	2	2	2
Sturgeon Creek	4	2	2	4	2	2
Turkey Creek	5	4	5	3	2	2
West Branch Drain	6	6	6	6	6	6
Wigle Creek	6	2	2	6	2	2
LTVCA						
Big Creek	4	2	2	4	2	2
Hendry Drain	5	5	5	5	5	5
McCarson Drain	3	2	2	3	2	2
Natural Watercourse (C)	5	5	5	5	5	5
Natural Watercourse (NE)	4	3	3	4	5	5
Newbiggen	5	5	2	5	5	2
Sharon Creek	6	6	6	6	3	3
Sixteen Mile Creek	3	5	5	3	5	2
South Dales Creek	1	1	1	5	1	1
Talbot Creek	3	3	3	6	6	6
Two Creeks	6	6	6	6	6	6
White Ash Creek	4	3	2	4	2	2

Site Name	% Canopy Cover			
	0-24	25-49	50-74	75-100
ERCA				
Belle River	✓			
Little River		✓		
Muddy Creek				✓
Sturgeon Creek	✓			
Turkey Creek	✓			
West Branch Drain				✓
Wigle Creek				✓
LTVCA				
Big Creek	✓			
Hendry Drain		✓		
McCarson Drain	✓			
Natural Watercourse (C)		✓		
Natural Watercourse (NE)	✓			
Newbiggen		✓		
Sharon Creek		✓		
Sixteen Mile Creek	✓			
South Dales Creek			✓	
Talbot Creek	✓			
Two Creeks				✓
White Ash Creek	✓			

Aquatic Macrophytes and Algae:
 1 (Abundant), 2 (Present), 3 (Absent)

Site Name	Macrophytes: Emergent			Macrophytes: Submergent		
	Sample 1 (Riffle 1)	Sample 2 (Pool)	Sample 3 (Riffle 2)	Sample 1 (Riffle 1)	Sample 2 (Pool)	Sample 3 (Riffle 2)
ERCA						
Belle River	3	3	3	3	3	3
Little River	3	3	3	2	2	2
Muddy Creek	3	3	3	3	3	3
Sturgeon Creek	3	3	3	3	3	3
Turkey Creek	3	3	3	2	2	2
West Branch Drain	3	3	3	2	2	2
Wigle Creek	3	3	3	3	3	3
LTVCA						
Big Creek	3	3	2	2	2	2
Hendry Drain	3	3	3	2	2	3
McCarson Drain	1	1	1	1	1	1
Natural Watercourse (C)	3	3	3	3	3	3
Natural Watercourse (NE)	3	3	3	3	3	3
Newbiggen	3	3	3	2	2	2
Sharon Creek	2	3	2	3	3	3
Sixteen Mile Creek	3	3	3	3	2	2
South Dales Creek	3	3	2	3	2	2
Talbot Creek	3	3	3	3	3	3
Two Creeks	3	3	1	2	3	3
White Ash Creek	3	3	3	1	2	2

Aquatic Macrophytes and Algae:
 1 (Abundant), 2 (Present), 3 (Absent)

Site Name	Macrophytes: Rooted Floating			Macrophytes: Free Floating		
	Sample 1 (Riffle 1)	Sample 2 (Pool)	Sample 3 (Riffle 2)	Sample 1 (Riffle 1)	Sample 2 (Pool)	Sample 3 (Riffle 2)
ERCA						
Belle River	3	3	3	3	3	3
Little River	2	2	2	3	3	3
Muddy Creek	3	3	3	3	3	3
Sturgeon Creek	3	2	2	3	3	3
Turkey Creek	2	2	2	3	3	3
West Branch Drain	3	3	3	2	2	2
Wigle Creek	2	2	2	3	3	3
LTVCA						
Big Creek	2	2	3	3	3	3
Hendry Drain	3	3	3	3	3	3
McCarson Drain	1	1	1	2	2	2
Natural Watercourse (C)	3	3	3	3	3	3
Natural Watercourse (NE)	3	3	3	3	3	3
Newbiggen	2	2	2	3	3	3
Sharon Creek	3	3	3	3	3	3
Sixteen Mile Creek	3	3	2	3	3	3
South Dales Creek	3	2	2	2	3	3
Talbot Creek	3	3	3	3	3	3
Two Creeks	3	2	2	3	3	3
White Ash Creek	2	3	3	2	3	3

Aquatic Macrophytes and Algae:

1 (Abundant), 2 (Present), 3 (Absent)

Site Name	Algae: Floating Algae			Algae: Filaments		
	Sample 1 (Riffle 1)	Sample 2 (Pool)	Sample 3 (Riffle 2)	Sample 1 (Riffle 1)	Sample 2 (Pool)	Sample 3 (Riffle 2)
ERCA						
Belle River	3	3	3	3	3	3
Little River	3	3	3	2	2	2
Muddy Creek	3	3	3	3	3	2
Sturgeon Creek	3	3	3	3	3	3
Turkey Creek	3	3	3	2	2	2
West Branch Drain	3	3	3	3	3	3
Wigle Creek	3	3	3	2	2	2
LTVCA						
Big Creek	3	2	2	3	2	1
Hendry Drain	3	3	3	3	3	3
McCarson Drain	3	3	3	3	3	3
Natural Watercourse (C)	3	3	3	2	2	2
Natural Watercourse (NE)	3	3	3	2	2	2
Newbiggen	3	3	3	2	2	2
Sharon Creek	3	3	3	3	3	3
Sixteen Mile Creek	3	3	3	2	3	2
South Dales Creek	3	3	3	3	3	3
Talbot Creek	3	3	3	2	2	2
Two Creeks	2	3	3	2	2	2
White Ash Creek	3	3	3	2	3	2

Aquatic Macrophytes and Algae:
 1 (Abundant), 2 (Present), 3 (Absent)

Site Name	Algae: Attached Algae			Algae: Slimes or Crusts		
	Sample 1 (Riffle 1)	Sample 2 (Pool)	Sample 3 (Riffle 2)	Sample 1 (Riffle 1)	Sample 2 (Pool)	Sample 3 (Riffle 2)
ERCA						
Belle River	2	2	2	2	2	2
Little River	2	2	2	2	2	2
Muddy Creek	3	2	2	2	2	2
Sturgeon Creek	2	2	2	2	2	2
Turkey Creek	2	2	2	2	2	2
West Branch Drain	2	2	2	2	2	2
Wigle Creek	2	2	2	2	2	2
LTVCA						
Big Creek	2	2	3	2	2	3
Hendry Drain	3	3	3	3	3	3
McCarson Drain	2	2	2	3	3	3
Natural Watercourse (C)	2	2	2	3	3	3
Natural Watercourse (NE)	2	2	2	3	3	3
Newbiggen	2	2	2	3	3	3
Sharon Creek	2	2	2	2	2	2
Sixteen Mile Creek	2	2	2	3	3	3
South Dales Creek	2	2	2	3	3	3
Talbot Creek	2	2	2	3	3	3
Two Creeks	1	2	1	3	3	2
White Ash Creek	1	3	2	2	2	2

Site Name	Widths and Depths		
ERCA	Bankfull Width (m)	Wetted Stream Width (m)	Bankfull - Wetted Depth (cm)
Belle River	16.4	14.1	1
Little River	15	N/R	1
Muddy Creek	4.8	1.1	0.56
Sturgeon Creek	12.32	6.48	1.5
Turkey Creek	N/R	N/R	N/R
West Branch Drain	N/R	N/R	0.4
Wigle Creek	9	6.4	2
LTVCA			
Big Creek	4.2	2.7	58.5
Hendry Drain	6.07	5.2	0.55
McCarson Drain	5	4.8	90
Natural Watercourse (C)	7	4.3	1.3
Natural Watercourse (NE)	3.3	2.25	34
Newbiggen	6.52	6.52	2.16
Sharon Creek	7.8	5.71	200
Sixteen Mile Creek	5.5	4.6	1.3
South Dales Creek	5.3	3.6	130
Talbot Creek	13.1	10.4	2
Two Creeks	5.7	N/R	N/R
White Ash Creek	7.5	5.5	7.5

Site Name	Reach Data: Habitat Type				Reach Data: Canopy Coverage				
	Riffle	Rapids	Stright Run	Pool/ Back Eddy	0%	1-25%	26-50%	51-75%	76-100%
ERCA									
Belle River			✓			✓			
Little River			✓				✓		
Muddy Creek			✓						✓
Sturgeon Creek			✓			✓			
Turkey Creek			✓			✓			
West Branch Drain	NR	NR	NR	NR	NR	NR	NR	NR	NR
Wigle Creek	✓		✓						✓
LTVCA									
Big Creek			✓			✓			
Hendry Drain	✓		✓	✓			✓		
McCarson Drain			✓	✓		✓			
Natural Watercourse (C)	✓		✓			✓			
Natural Watercourse (NE)	✓		✓			✓			
Newbiggen	✓						✓		
Sharon Creek	✓			✓			✓		
Sixteen Mile Creek	✓					✓			
South Dales Creek	✓			✓				✓	
Talbot Creek			✓	✓		✓			
Two Creeks	NR	NR	NR	NR	NR	NR	NR	NR	NR
White Ash Creek	✓		✓	✓	NR	NR	NR	NR	NR

Site Name	Reach Data: Macrophyte Coverage					Reach Data: Streamside Vegetation			
	0%	1-25%	26-50%	51-75%	76-100%	Ferns/ grasses	shrubs	deciduous trees	coniferous trees
ERCA									
Belle River		✓				✓		✓	
Little River			✓			✓		✓	
Muddy Creek		✓				✓			
Sturgeon Creek		✓				✓		✓	
Turkey Creek		✓						✓	
West Branch Drain	NR	NR	NR	NR	NR	NR	NR	NR	NR
Wigle Creek		✓						✓	
LTVCA									
Big Creek		✓				✓			
Hendry Drain		✓				✓		✓	
McCarson Drain				✓			✓	✓	
Natural Watercourse (C)		✓				✓	✓	✓	
Natural Watercourse (NE)		✓				✓			
Newbiggen		✓				✓			
Sharon Creek				✓		✓		✓	
Sixteen Mile Creek	✓					✓		✓	✓
South Dales Creek		✓				✓		✓	
Talbot Creek		✓						✓	
Two Creeks	NR	NR	NR	NR	NR	NR	NR	NR	NR
White Ash Creek	NR	NR	NR	NR	NR	NR	NR	NR	NR

Site Name	Reach Data: Dominant Streamside Vegetation				Reach Data: Periphyton Coverage on Substrate				
	Ferns/grasses	shrubs	deciduous trees	coniferous trees	1	2	3	4	5
ERCA									
Belle River	✓					✓			
Little River			✓	✓	✓				
Muddy Creek	✓					✓			
Sturgeon Creek	✓		✓		✓				
Turkey Creek	✓		✓			✓			
West Branch Drain	NR	NR	NR	NR	NR	NR	NR	NR	NR
Wigle Creek			✓				✓		
LTVCA									
Big Creek	✓						✓		
Hendry Drain	✓				✓				
McCarson Drain	✓				✓				
Natural Watercourse (C)	✓					✓			
Natural Watercourse (NE)	✓					✓			
Newbiggen			✓	✓		✓			
Sharon Creek			✓			✓			
Sixteen Mile Creek	✓					✓			
South Dales Creek			✓			✓			
Talbot Creek	✓				✓				
Two Creeks	NR	NR	NR	NR	NR	NR	NR	NR	NR
White Ash Creek	NR	NR	NR	NR	NR	NR	NR	NR	NR

Appendix E: Invertebrate Species List (2017)

List of taxa observed in at least one sample (3-D-nets) in 40 streams sampled in 2017.

<u>LTVCA</u>						10 th Concession Drain	18 & 19 Sideroad Drain	Cameron Drain	Campbell Sideroad Drain	Coleman Drain	Cornwall Drain	David Drain	Government Drain No. 1	Harrison Drain	King Drain
PHYLUM															
SUBPHYLUM															
CLASS															
ORDER															
SUBORDER															
Family															
Subfamily															
Genus															
<i>species</i>															
CNIDARIA															
HYDROZOA															
ANTHOATHECATA															
Hydridae															
Hydra						X					X	X			X
NEMATODA						X	X	X	X	X	X	X	X	X	X
NEMATOMORPHA						X									
ANNELIDA															
CLITELLATA						X									
OLIGOCHAETA						X	X	X	X	X	X	X	X	X	X
ARHYNCHOBDELLIDA															
ERPOBDELLIFORMES															
Erpobdellidae							X			X	X	X	X		
<i>Erpobdella</i>															
<i>punctata</i>						X	X					X	X		
<i>Motobdella</i>											X				
<i>Other</i>															
BRANCHIOBDELLIDA															
RHYNCHOBDELLIDA															

					Glossiphoniidae							X				X	
					<i>Glossiphonia</i>												
					<i>elegans</i>												
					<i>Helobdella</i>		X										
					<i>papillata</i>												
					<i>stagnalis</i>		X	X					X	X	X	X	
					<i>Placobdella</i>												
					<i>montifera</i>												
					<i>Other</i>												
					OTHER												
					MOLLUSCA												
					BIVALVIA												
					SPHAERIIDA												
					Sphaeriidae					X		X	X				
					<i>Sphaerium</i>												
					<i>Musculium</i>												
					<i>Sphaerium/</i>		X	X		X		X					
					<i>Musculium</i>												
					<i>Pisidium</i>			X		X							
					Rissooidea			X		X		X					
					Bithynidae												
					<i>Bithynia</i>												
					<i>Bithynia</i>								X				
					<i>tentaculata</i>												
					GASTROPODA												
					ARCHITAENIOGLOSSA												
					Viviparidae												
					<i>Bellamya</i>												
					<i>Other</i>												
					BASOMMATOPHORA												
					Ancylidae												
					<i>Ferrissia/</i>												
					<i>Laevapex</i>												
					<i>Other</i>												
					Lymnaeidae					X		X	X				
					<i>Lymnaea</i>							X					
					<i>palustris</i>												
					<i>stagnalis</i>							X	X				
					<i>Stagnicola</i>												

							<i>catascopium</i>						X						
							<i>Other</i>												
							Physidae						X						
							<i>Physa</i>		X		X		X	X				X	
							<i>Other</i>												
							Planorbidae						X						
							<i>Biomphalaria</i>												
							<i>Gyraulus</i>												
							<i>Helisoma</i>												
							<i>Lavapex/ Ferrissia</i>												
							<i>Other</i>												
							HETEROSTROPHA												
							Valvatidae												
							<i>Valvata</i>												
							<i>Other</i>	<i>tricarinata</i>											
PLATYHELMINTHES																			
							TURBELLARIA												
							TRICLADIDA		X	X	X			X			X	X	X
							Planariidae												
ARTHROPODA																			
							CHELICERATA												
							ARACHNIDA												
							TROMBIDIFORMES												
							HYDRACARINA						X					X	X
CRUSTACEA																			
							MAXILLOPODA												
							MALACOSTRACA												
							AMPHIPODA				X				X	X			
							Crangonyctidae				X								
							<i>Crangonyx</i>		X	X	X	X	X	X			X		
							Gammaridae		X			X						X	
							<i>Echinogammarus</i>												
							<i>ischnus</i>				X								
							<i>Gammarus</i>			X								X	
							<i>fasciatus</i>												
							<i>lacustris</i>												
							<i>tigrinus</i>											X	

						<i>Other</i>											
						Hyalellidae											
						<i>Hyalella</i>		X					X	X			
						<i>Other</i>											
						DECAPODA											
						Cambaridae					X		X				
						<i>Cambarus</i>											
						<i>Orconectes</i>											
						<i>Other</i>											
						ISOPODA											
						Asellidae						X					
						<i>Caecidotea</i>		X	X	X	X	X	X	X	X	X	X
						<i>Other</i>											
						Stenasellidae											
						OTHER											
						HEXAPODA											
						COLLEMBOLA				X			X				X
						INSECTA											
						EPHEMEROPTERA											
						PISCIFORMA											
						Baetidae						X		X			
						<i>Baetis</i>											
						<i>Callibaetis</i>						X					
						<i>Cloeon</i>											
							<i>dipterum</i>										
						<i>Procloeon</i>											
						<i>Other</i>											
						Heptageniidae											
						<i>Stenacron</i>											
						<i>Stenonema</i>											
							<i>femoratum</i>										
						<i>Other</i>											
						Heptageniinae											
						<i>Macdunnoa</i>											
						Metretopodidae											
						<i>Other</i>											
						Siphonuridae											
						FURCATERGALIA											

				Lestidae																
					<i>Lestes</i>				X											
					<i>Other</i>															
				PLECOPTERA																
					Chloroperlidae															
					Other															
				THYSANOPTERA																
				HEMIPTERA																
				HETEROPTERA																
					Belostomatidae													X		
					Corixidae															X
					Corixinae															
					<i>Callicorixa</i>															
					<i>Corisella</i>															
					<i>Dasycorixa</i>															
					<i>Hesperocorixa</i>															
					<i>Palmocorixa</i>															
					<i>Sigara</i>															
					<i>Trichocorixa</i>															
					<i>Other</i>															
					Hebridae															
					<i>Merragata</i>															
					Gerridae															
					Mesoveliidae															
					<i>Mesovelia</i>						X									
					<i>Other</i>															
					Mesoveliidae/ Veliidae															
					Notonectidae															
					Veliidae															
					<i>Microvelia</i>															
					<i>Rhagovelia</i>															
					<i>Other</i>															
					OTHER															
				MEGALOPTERA																
					Corydalidae															
					<i>Chauliodes</i>															
					Sialidae															
					<i>Sialis</i>															

						<i>Stenelmis</i>				X								X	
						Hydrophilidae													
						Georissidae													
						<i>Georissus</i>													
						Hydrophilinae													
						<i>Berosus</i>													
						<i>Enochrus</i>													X
						<i>Helochares</i>													
						<i>Tropisternus</i>							X						
						Helophorinae													
						<i>Helophorus</i>													
						Lampyridae													
						Scirtidae													
						Staphylinidae													
						TRICHOPTERA													
						ANNULIPALPIA													
						Hydropsychidae													
						Hydropsychinae													
						<i>Cheumatopsyche</i>													
						<i>Hydropsyche</i>													
						<i>Other</i>													
						Macronematinae													
						<i>Smicridea</i>													
						Philopotamidae													
						<i>Chimarra</i>													
						<i>Other</i>													
						Polycentropodidae													
						<i>Polycentropus</i>			X										
						Other													
						INTEGRIPALPIA													
						Hydroptilidae													X
						Hydroptilinae													
						<i>Hydroptila</i>													
						<i>Orthotrichia</i>													
						<i>Oxyethira</i>													
						<i>Other</i>													
						Leptoceridae													
						<i>Oecetis</i>													

					<i>Trianodes</i>													
					<i>Other</i>													
					Helicopsychidae													
					<i>Helicopsyche</i>													
					<i>Other</i>													
					Phryganeidae			X										
					DIPTERA													
					NEMATOCERA													
					Ceratopogonidae			X		X			X	X	X			
					Ceratopogoninae			X										
					<i>Bezzia/ Palpomyia</i>													
					<i>Ceratopogon</i>			X						X				
					<i>Culicoides</i>													
					<i>Serromyia</i>													X
					<i>Stilobezzia</i>													
					<i>Other</i>													
					Chaoboridae													
					<i>Chaoborus</i>													
					<i>Mochlonyx</i>													
					<i>Other</i>													
					Chironomidae			X	X	X	X	X	X	X	X	X	X	X
					Culicidae													
					<i>Aedes</i>													
					<i>Anopheles</i>													
					<i>Culex</i>													
					<i>Other</i>													
					Dixidae													
					<i>Dixa</i>													
					Limoniidae													
					<i>Limonia</i>													
					Limoniinae													
					<i>Antocha</i>													
					<i>Other</i>													
					Simuliidae					X		X						X
					<i>Prosimulium</i>													
					<i>Simulium</i>													
					<i>Other</i>													
					Tipulidae													

					Tipulinae													
					<i>Tipula</i>													
					Psychodidae													
					Psychodinae													
					<i>Pericoma/ Telmatoscopus</i>									X				
					Other													
					BRACHYCERA													
					Athericidae													
					<i>Atherix</i>													
					<i>Other</i>													
					Dolichopodidae									X				
					Ephydriidae				X									
					Ephydrinae													
					<i>Setacera</i>				X									
					Empididae				X					X	X		X	
					Sciomyidae													
					<i>Sepedon</i>										X			
					<i>Other</i>													
					Stratiomyidae										X			
					Stratiomyinae													
					<i>Odontomyia/ Hedriodiscus</i>				X									
					<i>Other</i>													
					Tabanidae				X									
					Chrysopsinae													
					<i>Chrysops</i>													
					<i>Other</i>													

Appendix E: Invertebrate Species List (2017) continued

<u>LTVCA</u>				Lewis Drain	Lundy Drain	McArthur East Drain	Miller Drain	Moore Drain	Nelles Drain	Oullete Drain Branch	Simpson Drain	Two Creeks Drain	Upper Portion Cartmill Drain
PHYLUM													
SUBPHYLUM													
CLASS													
ORDER													
SUBORDER													
Family													
Subfamily													
Genus													
species													
CNIDARIA													
HYDROZOA													
ANTHOATHECATA													
Hydridae													
Hydra					X		X		X	X		X	X
NEMATODA					X	X	X	X		X	X	X	X
NEMATOMORPHA					X		X	X					
ANNELIDA													
CLITELLATA													
OLIGOCHAETA					X	X	X	X	X	X	X	X	X
ARHYNCHOBDELLIDA													
ERPOBDELLIFORMES													
Erpobdellidae						X	X		X	X	X		
<i>Erpobdella</i>										X			
<i>punctata</i>													
<i>Motobdella</i>													
<i>Other</i>													
BRANCHIOBDELLIDA													
RHYNCHOBDELLIDA													
Glossiphoniidae													
<i>Glossiphonia</i>													

							<i>elegans</i>											
							<i>Helobdella</i>											
							<i>papillata</i>											
							<i>stagnalis</i>						X	X				
							<i>Placobdella</i>											
							<i>montifera</i>											
							<i>Other</i>											
						OTHER												
MOLLUSCA																		
						BIVALVIA												
						SPHAERIIDA												
						Sphaeriidae				X	X	X			X	X		
						<i>Sphaerium</i>												
						<i>Musculium</i>									X			
						<i>Sphaerium/</i>												
						<i>Musculium</i>		X	X			X						X
						<i>Pisidium</i>				X	X			X				
						Rissooidea						X	X					
						Bithynidae												
						<i>Bithynia</i>												
							<i>Bithynia</i>											
							<i>tentaculata</i>											
						GASTROPODA												
						ARCHITAENIOGLOSSA												
						Viviparidae												
						<i>Bellamya</i>												
						<i>Cipangopaludina</i>								X				
						<i>Other</i>												
						BASOMMATOPHORA												
						Ancylidae												
						<i>Ferrissia/</i>												
						<i>Laevapex</i>												
						<i>Other</i>												
						Lymnaeidae						X			X			
						<i>Lymnaea</i>						X	X	X				
							<i>palustris</i>											
							<i>stagnalis</i>				X							
						<i>Stagnicola</i>												
							<i>catascopium</i>		X				X		X			

							<i>Pseudosuccinea</i>												
							<i>columella</i>		X				X						
							<i>Other</i>												
							Physidae											X	
							<i>Physa</i>		X	X	X	X	X	X	X				
							<i>Other</i>												
							Planorbidae			X			X						
							<i>Biomphalaria</i>												
							<i>Gyraulus</i>												
							<i>Helisoma</i>							X					
							<i>Lavapex/ Ferrissia</i>												
							<i>Other</i>												
							HETEROSTROPHA												
							Valvatidae												
							<i>Valvata</i>												
							<i>tricarinata</i>												
							<i>Other</i>												
							PLATYHELMINTHES												
							TURBELLARIA		X	X	X	X		X				X	X
							TRICLADIDA												
							Planariidae												
							ARTHROPODA												
							CHELICERATA												
							ARACHNIDA												
							TROMBIDIFORMES												
							HYDRACARINA		X			X							
							CRUSTACEA												
							MAXILLOPODA												
							MALACOSTRACA												
							AMPHIPODA					X							X
							Crangonyctidae												
							<i>Crangonyx</i>		X		X		X	X	X				X
							Gammaridae				X		X						
							<i>Echinogammarus</i>												
							<i>ischnus</i>												
							<i>Gammarus</i>		X		X								
							<i>fasciatus</i>												
							<i>lacustris</i>												

						<i>Nehalennia</i>													
						<i>Other</i>													
						Lestidae													
						<i>Lestes</i>													
						<i>Other</i>													
						PLECOPTERA													
						Chloroperlidae													
						Other													
						THYSANOPTERA													
						HEMIPTERA													
						HETEROPTERA													
						Belostomatidae													
						<i>Belastoma</i>												X	
						Corixidae													
						Corixinae													
						<i>Callicorixa</i>													
						<i>Corisella</i>													
						<i>Dasycorixa</i>													
						<i>Hesperocorixa</i>													
						<i>Palmocorixa</i>													
						<i>Sigara</i>													
						<i>Trichocorixa</i>													
						<i>Other</i>													
						Hebridae													
						<i>Merragata</i>													
						Gerridae													
						Mesoveliidae													
						<i>Mesovelia</i>						X							X
						<i>Other</i>													
						Mesoveliidae/ Veliidae													
						Notonectidae													
						Veliidae													
						<i>Microvelia</i>													
						<i>Rhagovelia</i>													
						<i>Other</i>													
						OTHER													
						MEGALOPTERA													
						Corydalidae													

						<i>Oulimnius</i>													
						<i>Stenelmis</i>													
						Hydrophilidae													X
						Georissidae													
						<i>Georissus</i>													
						Hydrophilinae													
						<i>Berosus</i>													
						<i>Enochrus</i>			X										
						<i>Helochares</i>													
						<i>Tropisternus</i>													
						Helophorinae													
						<i>Helophorus</i>													
						Lampyridae							X						
						Scirtidae												X	
						Staphylinidae													
						TRICHOPTERA													
						ANNULIPALPIA													
						Hydropsychidae													
						Hydropsychinae													
						<i>Cheumatopsyche</i>													
						<i>Hydropsyche</i>													
						<i>Other</i>													
						Macronematinae													
						<i>Smicridea</i>													
						Philopotamidae													
						<i>Chimarra</i>													
						<i>Other</i>													
						Polycentropodidae													
						Other													
						INTEGRIPALPIA													
						Hydroptilidae			X										
						Hydroptilinae													
						<i>Hydroptila</i>			X										
						<i>Orthotrichia</i>												X	
						<i>Oxyethira</i>													
						<i>Other</i>													
						Leptoceridae													
						<i>Oecetis</i>													

						<i>Trianodes</i>												
						<i>Other</i>												
						Helicopsychidae												
						<i>Helicopsyche</i>												
						<i>Other</i>												
						DIPTERA												
						NEMATOCERA												
						Ceratopogonidae			X	X	X							X
						Ceratopogoninae												
						<i>Bezzia/ Palpomyia</i>		X										
						<i>Ceratopogon</i>												
						<i>Culicoides</i>												
						<i>Serromyia</i>												
						<i>Stilobezzia</i>												
						<i>Other</i>												
						Chaoboridae												
						<i>Chaoborus</i>												
						<i>Mochlonyx</i>												
						<i>Other</i>												
						Chironomidae		X	X	X	X	X	X	X	X	X	X	X
						Culicidae												
						<i>Aedes</i>												
						<i>Anopheles</i>												
						<i>Culex</i>												
						<i>Other</i>												
						Dixidae												
						<i>Dixa</i>												
						Limoniidae												
						<i>Limonia</i>												
						Limoniinae												
						<i>Antocha</i>												
						<i>Other</i>												
						Simuliidae		X	X	X	X							
						<i>Prosimulium</i>												X
						<i>Simulium</i>			X		X							
						<i>Other</i>												
						Tipulidae												
						Tipulinae												

						<i>Tipula</i>													
						Psychodidae													
						Psychodinae				X									
						Other													
						BRACHYCERA													X
						Athericidae													
						<i>Atherix</i>													
						Other													
						Dolichopodidae													
						Ephydriidae													
						Ephydrinae													
						<i>Setacera</i>													
						Empididae													
						Sciomyidae													X
						<i>Sepedon</i>													
						Other													
						Stratiomyidae				X									
						Stratiomyinae													
						<i>Odontomyia/</i>													
						<i>Hedriodiscus</i>													
						Other													
						Tabanidae				X									
						Chrysopsinae													
						<i>Chrysops</i>													
						Other													

Appendix E: Invertebrate Species List (2017) continued

<u>ERCA</u>						6 th Concession Drain	9 th Concession Drain	Barlow Drain	Big Creek	CN/Clickener Branch Drain of Renaud Line Drain	Coulson Drain	Desjardin Drain	East Branch of the No.47 Drain	East Townline Road Drain	Hyland & Seymour Drain
PHYLUM															
SUBPHYLUM															
CLASS															
ORDER															
SUBORDER															
Family															
Subfamily															
Genus															
species															
CNIDARIA															
HYDROZOA															
ANTHOATHECATA															
Hydridae															
Hydra						X	X	X	X	X	X	X	X	X	X
NEMATODA						X		X	X	X	X	X	X	X	
NEMATOMORPHA															
ANNELIDA															
CLITELLATA															
OLIGOCHAETA						X	X	X	X	X	X	X	X	X	X
ARHYNCHOBDELLIDA															
ERPOBDELLIFORMES															
Erpobdellidae											X				X
Erpobdella															
punctata														X	
Motobdella															
Other															
BRANCHIOBDELLIDA															

								<i>tigrinus</i>												
								<i>Other</i>												
								Hyalellidae												
								<i>Hyalella</i>				X	X							
								<i>Other</i>												
								DECAPODA												
								Cambaridae								X			X	X
								<i>Cambarus</i>												
								<i>Orconectes</i>												
								<i>Other</i>												
								ISOPODA												
								Asellidae			X		X							
								<i>Caecidotea</i>			X	X	X	X	X			X	X	X
								<i>Lirceus</i>			X									
								<i>Other</i>												
								Stenasellidae												
								OTHER												
								HEXAPODA												
								COLLEMBOLA			X	X								
								INSECTA												
								EPHEMEROPTERA												
								PISCIFORMA												
								Baetidae												
								<i>Baetis</i>												
								<i>Callibaetis</i>												
								<i>Cloeon</i>												
								<i>dipterum</i>												
								<i>Procloeon</i>												
								<i>Other</i>												
								Heptageniidae												
								<i>Stenacron</i>												
								<i>Stenonema</i>												
								<i>femoratum</i>												
								<i>Other</i>												
								Heptageniinae												
								<i>Macdunnoa</i>												
								Metretopodidae												
								<i>Other</i>												

						<i>Nehalennia</i>													
						<i>Other</i>													
						Lestidae													
						<i>Lestes</i>													
						<i>Other</i>													
						PLECOPTERA													
						Chloroperlidae													
						Other													
						THYSANOPTERA			X										
						HEMIPTERA													
						HETEROPTERA													
						Belostomatidae													
						Corixidae				X	X		X					X	
						Corixinae													
						<i>Callicorixa</i>													
						<i>Corisella</i>													
						<i>Dasycorixa</i>													
						<i>Hesperocorixa</i>													
						<i>Palmocorixa</i>													
						<i>Sigara</i>													
						<i>Trichocorixa</i>													
						<i>Other</i>													
						Hebridae													
						<i>Merragata</i>													
						Gerridae													
						Mesoveliidae													
						<i>Mesovelia</i>													
						<i>Other</i>													
						Mesoveliidae/ Veliidae													
						Notonectidae					X								
						Veliidae													
						<i>Microvelia</i>													
						<i>Rhagovelia</i>													
						<i>Other</i>													
						OTHER													
						MEGALOPTERA													
						Corydalidae													
						<i>Chauliodes</i>													

					Sialidae														
						<i>Sialis</i>													
					Other														
					COLEOPTERA														
					ADEPHAGA														
					Carabidae														
					Curculionidae				X	X									
					Dytiscidae				X	X	X								X
					Agabinae														
					<i>Agabus</i>													X	
					<i>Hydrotrupes</i>														
					Hydroporinae							X							X
					<i>Hydroporus</i>														
					Laccophilus														
					Matinae														
					<i>Matus</i>														
					Other														
					Gyrinidae														
					Gyrininae														
					<i>Dineutus</i>														
					<i>Gyrinus</i>														
					Haliplidae														
					<i>Brychius</i>														
					<i>Haliplus</i>														
					<i>Peltodytes</i>							X							
					Other														
					POLYPHAGA														
					Chrysomelidae														
					Dryopidae														
					Elmidae					X									
					Elminae														
					<i>Ancyronyx</i>														
					<i>Dubiraphia</i>														
					<i>Dubiraphia/</i>														
					<i>Narpus</i>														
					<i>Maxronychus</i>														
					<i>Optioservus</i>														
					<i>Ordobrevia</i>														
					<i>Oulimnius</i>														

						<i>Stenelmis</i>														
						Hydrophilidae														
						Georissidae														
						<i>Georissus</i>														
						Hydrophilinae														
						<i>Berosus</i>														X
						<i>Enochrus</i>														
						<i>Helochares</i>														
						<i>Tropisternus</i>														
						Helophorinae														
						<i>Helophorus</i>														
						Lampyridae						X								
						Scirtidae														
						Staphylinidae														
						TRICHOPTERA														
						ANNULIPALPIA														
						Hydropsychidae														
						Hydropsychinae														
						<i>Cheumatopsyche</i>														
						<i>Hydropsyche</i>														
						<i>Other</i>														
						Macronematinae														
						<i>Smicridea</i>														
						Philopotamidae														
						<i>Chimarra</i>														
						<i>Other</i>														
						Polycentropodidae														
						Other														
						INTEGRIPALPIA														
						Hydroptilidae														
						Hydroptilinae														
						<i>Hydroptila</i>														
						<i>Orthotrichia</i>														
						<i>Oxyethira</i>														
						<i>Other</i>														
						Leptoceridae														
						<i>Oecetis</i>														
						<i>Trianodes</i>														

Appendix E: Invertebrate Species List (2017) continued

ERCA				Kerr Drain	McMahon Drain	Mill Creek	Soncrainte Drain	South Townline Drain	Sturgeon Creek	Taylor Drain	Titcombe Road Drain	Washbrook Drain	Wilkinson-Shilson Drain
PHYLUM													
SUBPHYLUM													
CLASS													
ORDER													
SUBORDER													
Family													
Subfamily													
Genus													
species													
CNIDARIA													
HYDROZOA													
ANTHOATHECATA													
Hydridae													
Hydra					X			X		X			X
NEMATODA					X	X		X		X	X	X	X
NEMATOMORPHA													
ANNELIDA													
CLITELLATA													
OLIGOCHAETA					X	X	X	X	X	X	X	X	X
ARHYNCHOBDELLIDA										X			
ERPOBDELLIFORMES													
Erpobdellidae						X				X			X
<i>Erpobdella</i>													
<i>punctata</i>					X			X					
<i>Motobdella</i>													
<i>Other</i>													
BRANCHIOBDELLIDA						X							
RHYNCHOBDELLIDA													
Glossiphoniidae													
<i>Glossiphonia</i>													

							<i>elegans</i>											
							<i>Helobdella</i>											
							<i>papillata</i>											
							<i>stagnalis</i>		X				X			X		
							<i>Placobdella</i>											
							<i>montifera</i>											
							<i>Other</i>											
						OTHER												
						MOLLUSCA												
						BIVALVIA												
						SPHAERIIDA												
						Sphaeriidae				X								
						<i>Sphaerium</i>												
						<i>Musculium</i>												
						<i>Sphaerium/</i>												
						<i>Musculium</i>			X				X		X			
						<i>Pisidium</i>				X			X		X		X	X
						Rissooidea			X									
						Bithynidae												
						<i>Bithynia</i>												
							<i>Bithynia</i>											
							<i>tentaculata</i>											
						GASTROPODA												
						ARCHITAENIOGLOSSA												
						Viviparidae						X						
						<i>Bellamyia</i>												
						<i>Other</i>												
						BASOMMATOPHORA												
						Ancylidae												
						<i>Ferrissia/</i>												
						<i>Laevapex</i>												
						<i>Other</i>												
						Lymnaeidae				X			X					X
						<i>Fossaria</i>												
							<i>Truncatula</i>		X									
							<i>/humilis</i>											
						<i>Lymnaea</i>						X						
							<i>palustris</i>											
							<i>stagnalis</i>											

						<i>Stagnicola</i>													
						<i>catascopium</i>		X											
						<i>Other</i>													
						Physidae													
						<i>Physa</i>	X	X			X					X			
						<i>Other</i>													
						Planorbidae		X			X					X			
						<i>Biomphalaria</i>													
						<i>Gyraulus</i>		X			X								
						<i>Helisoma</i>					X								
						<i>Lavapex/ Ferrissia</i>					X								
						<i>Other</i>													
						HETEROSTROPHA													
						Valvatidae													
						<i>Valvata</i>													
						<i>tricarinata</i>													
						<i>Other</i>													
						PLATYHELMINTHES													
						TURBELLARIA		X	X					X		X		X	
						TRICLADIDA													
						Planariidae													
						ARTHROPODA													
						CHELICERATA													
						ARACHNIDA													
						TROMBIDIFORMES													
						HYDRACARINA			X										X
						CRUSTACEA													
						MAXILLOPODA													
						MALACOSTRACA													
						AMPHIPODA		X			X						X		
						Crangonyctidae	X												
						<i>Crangonyx</i>	X	X		X	X	X			X				
						Gammaridae								X					
						<i>Echinogammarus</i>													
						<i>ischnus</i>													
						<i>Gammarus</i>							X						
						<i>fasciatus</i>													
						<i>lacustris</i>													

								<i>tigrinus</i>										
								<i>Other</i>										
								Hyalellidae										
								<i>Hyalella</i>					X					
								<i>Other</i>										
								DECAPODA										
								Cambaridae		X	X		X	X			X	
								<i>Cambarus</i>										
								<i>Orconectes</i>										
								<i>Other</i>										
								ISOPODA										
								Asellidae			X							
								<i>Caecidotea</i>	X	X	X	X	X	X	X	X	X	X
								<i>Lirceus</i>					X		X			
								<i>Other</i>						X				
								Stenasellidae										
								OTHER										
								HEXAPODA										
								COLLEMBOLA										X
								INSECTA										
								EPHEMEROPTERA										
								PISCIFORMA										
								Baetidae										
								<i>Baetis</i>										
								<i>Callibaetis</i>										
								<i>Cloeon</i>										
								<i>dipterum</i>										
								<i>Procloeon</i>	X									
								<i>Other</i>										
								Heptageniidae										
								<i>Stenacron</i>										
								<i>Stenonema</i>										
								<i>femoratum</i>										
								<i>Other</i>										
								Heptageniinae										
								<i>Macdunnoa</i>										
								Metretopodidae										
								<i>Other</i>										

						<i>Nehalennia</i>													
						<i>Other</i>													
						Lestidae													
						<i>Lestes</i>													
						<i>Other</i>													
						PLECOPTERA													
						Chloroperlidae													
						Other													
						THYSANOPTERA												X	
						HEMIPTERA													
						HETEROPTERA													
						Belostomatidae													
						Corixidae					X	X							
						Corixinae													
						<i>Callicorixa</i>													
						<i>Corisella</i>													
						<i>Dasycorixa</i>													
						<i>Hesperocorixa</i>													
						<i>Palmocorixa</i>													
						<i>Sigara</i>													
						<i>Trichocorixa</i>													
						<i>Other</i>													
						Hebridae													
						<i>Merragata</i>													
						Gerridae													
						Mesoveliidae													
						<i>Mesovelia</i>													X
						<i>Other</i>													
						Mesoveliidae/ Veliidae													
						Notonectidae													
						Veliidae													
						<i>Microvelia</i>													
						<i>Rhagovelia</i>													
						<i>Other</i>													
						OTHER													
						MEGALOPTERA													
						Corydalidae													
						<i>Chauliodes</i>					X								

					Sialidae														
						<i>Sialis</i>													
					Other														
					COLEOPTERA														
					ADEPHAGA														
					Carabidae														
					Curculionidae					X									
					Dytiscidae				X	X			X			X			
					Agabinae														
					<i>Agabus</i>				X									X	
					<i>Hydrotrupes</i>														
					Hydroporinae														X
					<i>Hydroporus</i>														
					Ilybius						X								
					Laccophilus						X								
					Matinae														
					<i>Matus</i>														
					Other														
					Gyrinidae														
					Gyrininae														
					<i>Dineutus</i>														
					<i>Gyrinus</i>														
					Haliplidae														
					<i>Brychius</i>														
					<i>Haliphus</i>						X							X	
					<i>Peltodytes</i>														
					Other														
					POLYPHAGA														
					Chrysomelidae														
					Dryopidae														
					Elmidae														
					Elminae														
					<i>Ancyronyx</i>														
					<i>Dubiraphia</i>														X
					<i>Dubiraphia/</i> <i>Narpus</i>														
					<i>Maxronychus</i>														
					<i>Optioservus</i>														
					<i>Ordobrevia</i>														

						<i>Oulimnius</i>													
						<i>Stenelmis</i>													
						Hydrophilidae													
						Georissidae													
						<i>Georissus</i>													
						Hydrophilinae													
						<i>Berosus</i>													
						<i>Enochrus</i>													
						<i>Helochares</i>													
						<i>Tropisternus</i>													
						Helophorinae													
						<i>Helophorus</i>													
						Lampyridae													
						Scirtidae													X
						Staphylinidae													
						TRICHOPTERA													
						ANNULIPALPIA													
						Hydropsychidae													
						Hydropsychinae													
						<i>Cheumatopsyche</i>													
						<i>Hydropsyche</i>													
						<i>Other</i>													
						Macronematinae													
						<i>Smicridea</i>													
						Philopotamidae													
						<i>Chimarra</i>													
						<i>Other</i>													
						Polycentropodidae													
						<i>Polycentropus</i>													X
						<i>Other</i>													
						Other													
						INTEGRIPALPIA													
						Hydroptilidae													
						Hydroptilinae													
						<i>Hydroptila</i>													
						<i>Orthotrichia</i>													
						<i>Oxyethira</i>													
						Other													

Appendix F: 2017 Field Data:

Field measurements were collected in the field during the 2017 sampling season of 40 sites across southwestern Ontario.

*NR indicates there was no record taken in the field

Site Name ERCA	Water Temperature (°C)	Air Temperature (°C)	DO (mg/l)	Conductivity (uS/cm)	pH	Turbidity (cm)	Time	Elevation (m asl)
6 th Concession Drain	16.1	14.3	9.60	797	8.37	29.8	1:10 PM	177.5
9 th Concession Drain	19.2	24.4	10.65	7.66	8.27	30	2:30 PM	185.7
Barlow Drain	27.2	19.6	19.21	837	10.40	22	4:45 PM	193
Big Creek	17.3	24.9	20.5	435	8.84	45	2:00 PM	4665868
CN/Clickener Branch Drain of Renaud Line Drain	17.2	21.2	7.02	723	8.66	45	1:15 – 3:00 PM	216.4
Coulson Drain	25.8	NR	8.38/%101.2	795	8.47	NR	2:30 PM	169.1
Campbell Sideroad Drain	18.2	14.8	18.39	696	9.03		4:00PM	190.2
Coleman Drain	14.2	16.0	9.71	723	8.24	9	4:24PM	
Cornwall Drain	16.6	21.8	14.55	494	8.61	21	1:35PM	205
Taylor Drain	18.4	23.0	6.77	554	8.74	66	10:30AM	181.2
Titcombe Road Drain	17.2	24.1	6.70	919	8.85	9	1:00PM	181.4
Washbrook Drain	12.5	11.7	9.51	870	7.16		11:15AM	185.7
Wilkinson-Shilson Drain	12.3	13.0	7.57	661	8.65	120	10:30AM	181.3
LTVCA								
10 th Concession Drain	24.0	23.2	13.34	987	8.34	61	2:30PM	184.8
18 & 19 Sideroad Drain	16.0	15.3	8.96	652	8.44	32	1:30PM	195.8
Cameron Drain	13.7	15.8	8.75	618	8.40	4	11:45AM	NR

Simpson Drain	23.0	NR	14.5	603	8.46	NR	3:00PM	NR
Moore Drain	19.6	21.6	8.07	428.4	8.6	2	12:10PM	179.6
Miller Drain	19.1	22.0	7.88	352.4	8.67	4	12:00PM	
Lundy Drain	12.1	10.8	9.66	630	8.78	38	2:30PM	186.4
Lewis Drain	16.0	15.8	8.45	961	8.42	15	3:50PM	185.2
McArthur East Drain	13.8	16.7	9.84	600	8.38	NR	NR	NR
Nelles Drain	14.7	19.7	14.51	791	8.49	105	1:15PM	NR
Two Creeks Drain	19.6	14.4	10.71	755	8.54	77	3:00PM	176.0
Upper Portion Cartmill Drain	13.0	18.8	10.66	663	8.68	40	10:40AM	NR
Ouellette Drain Branch	23.8	21.2	14.4	800	8.5	104	3:50PM	180.0

Site Name	Sample 1: Riffle (Cross-Over)					
	Sampling Distance Covered (m)	Time (min)	Max. Depth (m)	Wetted Width (m)	Max. Hydraulic Head (mm)	# Grabs Pooled per Sample
ERCA						
6 th Concession Drain	2.7	3	0.34	3.9	0.5	0
9 th Concession Drain	2	3	0.124	1.70	NR	NR
Barlow Drain	3	3	0.081	0.58	5	2
Big Creek	1.9	3	0.254	12.4	0	2
CN/Clickener Branch Drain of Renaud Line Drain	NR	3	0.48	3.45	0	2
Coulson Drain	4	3	0.17	1.0	3	2
10 th Concession Drain	2.5	3	0.265	0.60	3	0
18 & 19 Sideroad Drain	1.5	3	0.22	2.3	0	2
Cameron Drain	2	3	54	1.44	40	2
Campbell Sideroad Drain	2	3	16	2	0	0
Coleman Drain	2	3	57	1.3	1	2
Cornwall Drain	1.5	3	0.27	2.15	1	2
Simpson Drain	NR	3	0.15	0.83	0	NR
Moore Drain	1	3	0.28	1.68	0	2
Lundy Drain	2.5	3	0.09	0.76	2	2
Miller Drain	3	3	0.35	1.5	2	0
Taylor Drain	2	3	0.14	1.62	2	2
Titcombe Road Drain	1.5	3	0.21	2.29	0	0
Washbrook Drain	2	3	11	0.88	1	3

Wilkinson-Shilson Drain	2.5	3	23.5	1.40	0	2
Lewis Drain	1,5	3	0.16	0.9	50	NR
McArthur East Drain	2.5	3	32	2.1	3	2
Nelles Drain	2.5	3	0.58	6.11	0	NR
Ouellette Drain Branch	2.5	3	0.75	3.05	0	2
Two Creeks Drain	NR		0.11	2.30	25	2
Upper Portion Cartmill Drain	NR	3	0.14	3.5	2	NR

Site Name	Sample 2: Pool					
	Sampling Distance Covered (m)	Time (min)	Max. Depth (m)	Wetted Width (m)	Max. Hydraulic Head (mm)	# Grabs Pooled per Sample
ERCA						
6 th Concession Drain	3	3	0.54	5.2	1.5	3
9 th Concession Drain		3	20.8	2.09	NR	NR
Barlow Drain	2	3	0.144	1.66	1	3
Big Creek	3	3	0.393	7.2	0	3
CN/Clickener Branch Drain of Renaud Line Drain	2	3	0.48	3.47	0	3
Coulson Drain	2	3	0.25	2.22	1	3
10 th Concession Drain	1.5	3	0.34	2.5	3	0
18 & 19 Sideroad Drain	2	3	0.25	2.61	0	3
Cameron Drain	2	3	49	2.1	30	3
Campbell Sideroad Drain	2	3	20	2.13	0	3
Coleman Drain	2.5	3	57	2.0	1	3
Cornwall Drain	2	3	0.25	2.30	1	3
Simpson Drain	NR	3	0.4	2.5	0	NA
Moore Drain	2	3	0.18	2.22	5	3
Lundy Drain	1.5	3	0.16	1.28	0	3
Miller Drain	2	3	0.49	3.4	3	3
Taylor Drain	3	3	0.245	0.15	4	0
Titcombe Rd Drain	2.25	3	0.19	2.39	0	3
Washbrook Drain	2.5	3	12	1.89	1.5	0

Wilkinson-Shilson Drain	2	3	0.495	1.90	0	3
Lewis Drain	2	3	0.35	2.25	NR	NR
McArthur East Drain	2.5	3	50	3.8	0.5	3
Nelles Drain	2.5	3	0.52	6.1	1	2
Ouellette Drain Branch	2	3	0.14	3.13	0.5	3
Two Creeks Drain	NR	NR	0.505	4.76	2	3
Upper Portion Cartmill Drain	NR	3	0.12	2.1	0	NR

Site Name	Sample 3: Riffle (Cross-Over)					
ERCA	Sampling Distance Covered (m)	Time (min)	Max. Depth (m)	Wetted Width (m)	Max. Hydraulic Head (mm)	# Grabs Pooled per Sample
6 th Concession Drain	2.7	3	0.54	5.0	0.5	2
9 th Concession Drain	3	3	9.8	1.65	NR	NR
Barlow Drain	2	3	0.079	0.84	4	0
Big Creek	2.8	3	0.433	12.0	0	0
CN/Clickener Branch Drain of Renaud Line Drain	2	3	0.38	3.22	0	0
Coulson Drain	3.5	3	0.18	1.7	2	0
10 th Concession Drain	2	3	0.25	1.75	2	2
18 & 19 Sideroad Drain	1.25	3	0.31	2.33	0	0
Cameron Drain	2	3	56	1.85	55	0
Campbell Sideroad Drain	2	3	16	1.64	0	2
Coleman Drain	2	3	57	1.5	2	0
Cornwall Drain	1.5	3	0.255	2.5	1	0
Simpson Drain		3	0.19	1.5	0	NR
Moore Drain	1.5	3	0.16	0.35	3.5	0
Lundy Drain		3	0.09	0.74	1	0
Miller Drain		3	0.46	1.5	2	2
Taylor Drain	1.5	3	0.15	2.4	4	0
Titcombe Rd. Drain	1.5	3	0.13	2.58	1	2
Washbrook Drain	2	3	14	1.85	1.5	0

Wilkinson-Shilson Drain	3	3	19	1.66	0	0
Lewis Drain	2	3	0.30	1.34	0	NA
McArthur East Drain	2	3	21	2.5	7	0
Nelles Drain	2.5	3	0.4	6.3	1	2
Ouellette Drain Branch	1.5	3	0.17	2.5	0	0
Two Creeks Drain	3		0.08	5.65	5	0
Upper Portion Cartmill Drain	2.5	3	0.9	3.2	1	NR

Class	Description
1	Clay (hard pan)
2	Silt (gritty, < 0.06 mm particle diameter)
3	Sand (grainy, 0.06 - 2 mm)
4	Gravel (2 - 65 mm)
5	Cobble (65 - 250 mm)
6	Boulder (> 250 mm)
7	Bed Rock

Site Name	Substrate: Dominant			Substrate: 2nd Dominant		
	Sample 1 (Riffle 1)	Sample 2 (Pool)	Sample 3 (Riffle 2)	Sample 1 (Riffle 1)	Sample 2 (Pool)	Sample 3 (Riffle 2)
ERCA						
6 th Concession Drain	2	2	2	1	1	1
9 th Concession Drain	NR	NR	NR	NR	NR	NR
Barlow Drain	1	1	1	1	1	1
Big Creek	2	2	2	1	1	1
CN/Clickener Branch Drain of Renaud Line Drain	NR	2	NR	NR	3	NR
Coulson Drain	NR	NR	NR	NR	NR	NR
Campbell Sideroad Drain	1	1	1	3	3	3
Coleman Drain	3	3	3	5	5	5
Cornwall Drain	2	2	2	1	1	1
Taylor Drain	3	3	3	2	2	2
Titcombe Rd Drain	2	2	2	3	3	3
Washbrook Drain	1	1	1	1	1	1
Wilkinson-Shilson Drain	3	3	3	2	1	2

Site Name	Substrate: Dominant			Substrate: 2nd Dominant		
	Sample 1 (Riffle 1)	Sample 2 (Pool)	Sample 3 (Riffle 2)	Sample 1 (Riffle 1)	Sample 2 (Pool)	Sample 3 (Riffle 2)
LTVCA						
10 th Concession Drain	3	1	1	4	3	3
18 & 19 Sideroad Drain	1	1	1	1	1	1
Cameron Drain	3	3	3	4	4	4
Simpson Drain	1	1	1	2	2	2
Moore Drain	1	3	1	3	4	4
Lundy Drain	5	4	5	4	3	4
Miller Drain	1	3	3	4	4	4
Lewis Drain	3	3	3	4	1(4)	1
McArthur East Drain	3	1	3	1	2	1
Nelles Drain	1	1	1	2	2	2
Two Creeks Drain	6	3	3	5	6	2
Upper Portion Cartmill Drain	1	1	1	2	2	2
Ouellette Drain Branch	1	1	1	1	1	1

Site Name	Organic Matter Areal Coverage: Woody Debris			Organic Matter Areal Coverage: Detritus		
	Sample 1 (Riffle 1)	Sample 2 (Pool)	Sample 3 (Riffle 2)	Sample 1 (Riffle 1)	Sample 2 (Pool)	Sample 3 (Riffle 2)
ERCA						
6 th Concession Drain	2	2	2	1	1	1
9 th Concession Drain	NR	NR	NR	NR	NR	NR
Barlow Drain	2	2	2	2	2	2
Big Creek	NR	NR	1	NR	NR	1
CN/Clickener Branch Drain of Renaud Line Drain	NR	1	NR	NR	1	NR
Campbell Sideroad Drain	2	2	2	2	2	2
Coleman Drain	3	3	3	2	2	2
Cornwall Drain	3	3	3	2	2	2
Taylor Drain	2	3	1	3	3	2
Titcombe Rd Drain	1	1	1	1	1	1
Washbrook Drain	2	2	2	2	2	2
Wilkinson-Shilson Drain	2	NR	2	2	2	2
LTVCA						
10 th Concession drain	2	2	2	2	2	2
18 & 19 Sideroad Drain	3	3	2	2	2	2
Cameron Drain	1	1	1	1	1	1
Simpson Drain	3	3	3	2	2	2
Moore Drain	2	2	2	2	2	3
Lundy Drain	3	2	2	2	2	2
Miller Drain	3	3	3	2	2	2
Lewis Drain	2	2	2	2	2	2
McArthur East Drain	2	2	2	2	2	2
Nelles Drain	2	2	2	2	2	2

Two Creeks Drain	3	3	2	3	3	2
Upper Portion Cartmill Drain	2	2	2	2	2	2
Ouellette Drain Branch	3	3	3	1	3	3

Site Name	Riparian Vegetative Community: Left Bank			Riparian Vegetative Community: Right Bank		
	1.5 - 10 m from water's edge	10 - 30 m from water's edge	30 - 100 m from water's edge	1.5 - 10 m from water's edge	10 - 30 m from water's edge	30 - 100 m from water's edge
ERCA						
6 th Concession Drain	2	2	2	2	2	2
9 th Concession Drain	NR	NR	NR	NR	NR	NR
Barlow Drain	4	4	4	4	4	4
Big Creek	4	2	2	4	2	2
CN/Clickener Branch Drain of Renaud Line Drain	4	2	2	4	2	St. Clair Lake
Campbell Sideroad Drain	2	2	2	4	2	2
Coleman Drain	3	2	2	3	2	2
Cornwall Drain	2	2	2	2	2	2
Taylor Drain	2	2	2	2	2	2
Titcombe Rd Drain	6	6	6	6	6	6
Washbrook Drain	2	2	2	2	2	2
Wilkinson-Shilson Drain	4	2	2,6	4	2	2,6
LTVCA						
10 th Concession Drain	2	2	2	2	2	2
18 & 19 Sideroad Drain	2	2	2	2	2	2
Cameron Drain	2	2	2	2	2	2
Simpson Drain	2	2	2	2	2	2
Moore Drain	2	2	2	2	2	2
Miller Drain	2	2	2	2	2	2

Lewis Drain	2	2	2	2	2	2
McArthur East Drain	6	2	2	6	2	2
Nelles Drain	2	2	2	2	2	2
Two Creeks Drain	6	6	2,6	6	6	2,6
Upper Portion Cartmill Drain	2	2	2	2	2	2
Ouellette Drain Branch	2	2	2	2	2	2

Site Name	% Canopy Cover			
	0-24	25-49	50-74	75-100
ERCA				
6 th Concession Drain			✓	
9 th Concession Drain				
Barlow Drain	✓			
Big Creek	✓			
CN/Clickener Branch Drain of Renaud Line Drain				✓
Campbell Sideroad Drain	✓			
Coleman Drain	✓			
Cornwall Drain	✓			
Taylor Drain		✓		
Titcombe Rd Drain				✓
Washbrook Drain				✓
Wilkinson-Shilson Drain	✓			
LTVCA				
10 th Concession Drain	✓			
18 & 19 Sideroad Drain	✓			
Cameron Drain	✓			
Simpson Drain	✓			
Moore Drain	✓			
Lundy Drain	✓			
Miller Drain	✓			
Lewis Drain	✓			
McArthur East Drain				✓
Nelles Drain	✓			
Ouellette Drain Branch	✓			
Two Creeks Drain	✓			
Upper Portion Cartmill Drain	✓			

Aquatic Macrophytes and Algae:

1 (Abundant), 2 (Present), 3 (Absent)

Site Name	Macrophytes: Emergent			Macrophytes: Submergent		
	Sample 1 (Riffle 1)	Sample 2 (Pool)	Sample 3 (Riffle 2)	Sample 1 (Riffle 1)	Sample 2 (Pool)	Sample 3 (Riffle 2)
ERCA						
6 th Concession	3	3	3	3	3	3
9 th Concession Drain	NR	NR	NR	NR	NR	NR
Barlow Drain	2	2	2	3	3	3
Big Creek	1	1	1	1	1	1
CN/Clickener Branch Drain of Renaud Line Drain	NR	2	NR	NR	2	NR
Campbell Sideroad Drain	2	2	2	2	2	2
Coleman Drain	2	2	2	3	3	3
Cornwall Drain	1	1	1	2	2	2
Taylor Drain	3	3	3	1	1	2
Titcombe Rd Drain	2	3	3	3	2	3
Washbrook Drain	3	3	3	3	3	3
Wilkinson-Shilson Drain	3	3	3	3	3	3
LTVCA						
10 th Concession Drain	2	2	2	3	3	3
18 & 19 Sideroad Drain	2	3	2	1	1	1
Cameron Drain	2	2	2	3	3	3
Simpson Drain	2	2	1	3	3	3
Moore Drain	3	3	2	3	3	3
Lundy Drain	3	3	3	2	2	2
Miller Drain	2	2	2	3	3	3
Lewis Drain	2	2	2	3	3	3
McArthur East Drain	3	3	3	3	3	3

Nelles Drain	1	1	1	3	3	3
Two Creeks Drain	3	3	3	1	3	2
Upper Portion Cartmill Drain	2	2	2	3	3	3
Ouellette Drain Branch	1	2	2	3	2	2

Aquatic Macrophytes and Algae:
 1 (Abundant), 2 (Present), 3 (Absent)

Site Name	Macrophytes: Rooted Floating			Macrophytes: Free Floating		
	Sample 1 (Riffle 1)	Sample 2 (Pool)	Sample 3 (Riffle 2)	Sample 1 (Riffle 1)	Sample 2 (Pool)	Sample 3 (Riffle 2)
ERCA						
6 th Concession Drain	3	3	3	3	3	3
9 th Concession Drain	NR	NR	NR	NR	NR	NR
Barlow Drain	2	2	2	3	3	3
Big Creek	2	2	2	3	3	3
CN/Clickener Branch Drain of Renaud Line Drain	NR	2	NR	NR	3	NR
Campbell Sideroad Drain	3	3	3	3	3	3
Coleman Drain	3	3	3	3	3	3
Cornwall Drain	3	3	3	3	3	3
Simpson Drain	3	3	3	3	3	3
Taylor Drain	3	3	3	3	3	3
Titcombe Rd Drain	3	3	3	3	3	3
Washbrook Drain	3	3	3	3	3	3
Wilkinson-Shilson Drain	3	3	3	3	3	3
LTVCA						
10 th Concession Drain	3	3	3	3	3	3
18 & 19 Sideroad Drain	13	13	13	3	3	3
Cameron Drain	3	3	3	3	3	3
Moore Drain	3	3	3	3	3	3
Lundy Drain	3	2	3	3	3	3
Miller Drain	3	3	3	3	3	3
Lewis Drain	3	3	3	3	3	3
McArthur East Drain	3	3	3	3	3	3

Nelles Drain	3	3	3	3	3	3
Two Creeks Drain	3	3	3	3	3	3
Upper Portion Cartmill Drain	3	3	3	3	3	3
Ouellette Drain Branch	3	3	3	3	3	3

Aquatic Macrophytes and Algae:

1 (Abundant), 2 (Present), 3 (Absent)

Site Name	Algae: Floating Algae			Algae: Filaments		
	Sample 1 (Riffle 1)	Sample 2 (Pool)	Sample 3 (Riffle 2)	Sample 1 (Riffle 1)	Sample 2 (Pool)	Sample 3 (Riffle 2)
ERCA						
6 th Concession Drain	3	3	3	3	3	3
9 th Concession Drain	NR	NR	NR	NR	NR	NR
Barlow Drain	3	3	3	3	3	3
Big Creek	3	3	3	3	3	3
CN/Clickener Branch Drain of Renaud Line Drain	NR	2	NR	NR	3	NR
Campbell Sideroad Drain	3	3	3	2	2	2
Coleman Drain	3	3	3	3	3	3
Cornwall Drain	1	1	1	3	3	3
Taylor Drain	3	3	3	3	3	3
Titcombe Rd Drain	3	3	3	3	3	3
Washbrook Drain	3	3	3	3	3	3
Wilkinson-Shilson Drain	3	3	3	3	3	3
LTVCA						
10 th Concession Drain	2	2	2	1	1	1
18 & 19 Sideroad Drain	2	2	2	2	2	2
Cameron Drain	3	3	3	3	3	3
Simpson Drain	2	2	2	2	2	2
Moore Drain	3	3	3	3	3	3
Lundy Drain	3	3	3	2	2	2
Miller Drain	3	3	3	3	3	3
Lewis Drain	3	3	3	3	3	3
McArthur East Drain	3	3	3	3	3	3

Nelles Drain	3	3	3	3	3	3
Two Creeks Drain	3	3	3	2	3	3
Upper Portion Cartmill Drain	3	3	3	3	3	3
Ouellette Drain Branch	2	2	2	2	2	2

Aquatic Macrophytes and Algae:
 1 (Abundant), 2 (Present), 3 (Absent)

Site Name	Algae: Attached Algae			Algae: Slimes or Crusts		
	Sample 1 (Riffle 1)	Sample 2 (Pool)	Sample 3 (Riffle 2)	Sample 1 (Riffle 1)	Sample 2 (Pool)	Sample 3 (Riffle 2)
ERCA						
6 th Concession	3	3	3	2	2	2
9 th Concession Drain	NR	NR	NR	NR	NR	NR
Barlow Drain	3	3	3	3	3	3
Big Creek	3	3	3	3	3	3
CN/Clickener Branch Drain of Renaud Line Drain	NR	2	NR	NR	2	NR
Campbell Sideroad Drain	2	2	2	2	2	2
Coleman Drain	2	3	2	3	3	3
Cornwall Drain	3	3	3	3	3	3
Taylor Drain	3	3	3	3	3	3
Titcombe Rd Drain	3	3	3	3	3	3
Washbrook Drain	3	3	3	3	3	3
Wilkinson-Shilson Drain	3	3	3	3	3	3
LTVCA						
10 th Concession Drain	2	2	2	3	3	3
18 & 19 Sideroad Drain	2	2	2	2	2	2
Cameron Drain	3	3	3	3	3	3
Simpson Drain	3	3	3	3	3	3
Moore Drain	3	3	3	3	3	3
Miller Drain	3	3	3	3	3	3
Lundy Drain	2	2	2	2	3	2

Lewis Drain	3	3	3	3		3
McArthur East Drain	3	3	3	3	3	3
Nelles Drain	3	3	3	3	3	3
Two Creeks Drain	1	3	2	1	3	2
Upper Portion Cartmill Drain	3	3	3	3	3	3
Ouellette Drain Branch	2	1	2	3	3	3

Site Name	Candidate Reference Site		
	Yes	No	Comments
6 th Concession Drain			
9 th Concession Drain			
Barlow Drain			
Big Creek		✓	Sediment smells like rotten eggs - sulphur
CN/Clickener Branch Drain of Renaud Line Drain	✓		
10 th Concession Drain	✓		
18 & 19 Sideroad Drain		✓	
Cameron Drain	✓		
Campbell Sideroad Drain		✓	
Coleman Drain		✓	
Cornwall Drain		✓	
Simpson Drain		✓	
Moore Drain		✓	
Miller Drain	✓		
Taylor Drain	✓		
Titcombe Rd Drain	✓		
Washbrook Drain		✓	Turb tube : 98cm, lady who lives next door says it smells, especially when it rains
Wilkinson-Shilson Drain			
Lewis Drain		✓	
Nelles Drain	✓		
Upper Portion Cartmill Drain	✓		

Site Name	Geographical Description: Surrounding Land Use							
	Forest	Field/ Pasture	Agriculture	Residential/ Urban	Logging	Mining	Commercial/ Industrial	Other
6 th Concession Drain								
9 th Concession Drain			✓					
Barlow Drain			✓					
Big Creek	✓	✓	✓	✓				
CN/Clickener Branch Drain of Renaud Line Drain		✓		✓				
10 th Concession Drain		✓	✓	✓				
18 th & 19 th Sideroad Drain		✓	✓	✓				
Cameron Drain	✓	✓	✓					
Campbell Sideroad Drain	✓		✓					
Coleman Drain		✓	✓	✓				
Cornwall Drain		✓	✓	✓				
Simpson Drain	✓	✓	✓					
Moore Drain			✓	✓			✓	
Lundy Drain	✓		✓	✓				
Miller Drain		✓	✓					
Taylor Drain			✓	✓				
Titcombe Rd Drain	✓			✓				
Washbrook Drain		✓	✓					
Wilkinson Shilson Drain		✓	✓	✓				
Lewis Drain			✓	✓				
McArthur East Drain	✓	✓	✓	✓				

Nelles Drain		✓	✓	✓				
Ouellette Drain Branch								
Two Creeks Drain	✓		✓	✓				
Upper Portion Cartmill Drain		✓	✓					

Site Name	Geographical Description: Dominant Surrounding Land Use							
	Forest	Field/ Pasture	Agriculture	Residential/ Urban	Logging	Mining	Commercial/ Industrial	Other
6 th Concession Drain								
9 th Concession Drain			✓					
Barlow Drain			✓					
Big Creek			✓					
CN/Clickener Branch Drain of Renaud Line Drain				✓				
10 th Concession Drain			✓					
18 th and 19 th Sideroad Drain			✓					
Cameron Drain			✓					
Campbell Sideroad Drain			✓					
Coleman Drain			✓					
Cornwall Drain			✓					
Simpson Drain			✓					
Moore Drain			✓					
Lundy Drain			✓					
Miller Drain			✓					
Taylor Drain			✓					
Titcombe Rd Drain	✓							
Washbrook Drain			✓	✓				
Wilkinson-Shilson Drain			✓					
Lewis Drain			✓	✓				
McArthur East Drain			✓					

Nelles Drain				✓				
Ouellette Drain Branch								
Two Creeks Drain	✓							
Upper Portion Cartmill Drain			✓					

Site Name	Widths and Depths		
	Bankfull Width (m)	Wetted Stream Width (m)	Bankfull - Wetted Depth (cm)
ERCA			
6 th Concession	6.4	5.2	88
9 th Concession Drain	3.46	NR	
Barlow Drain	4.25	NR	80
Big Creek	9.1	NR	73
CN/Clickener Branch Drain of Renaud Line Drain	5.66	NR	62
Campbell Sideroad Drain	3.06	NR	33
Coleman Drain	8.2	NR	NA
Cornwall Drain	7.5	NR	1.10
Taylor Drain	4.45	NR	44
Titcombe Rd Drain	5.65	NR	46
Washbrook Drain	4.2	1.89	126
Wilkinson-Shilson Drain	3.17	NR	32
LTVCA			
10 th Concession Drain	4.54	NR	79
18 th and 19 th Sideroad Drain	4.25	NR	62
Cameron Drain	8.2	NR	134
Simpson Drain	4	NR	100
Moore Drain	3.08	NR	40
Lundy Drain	7.8	NR	2.77
Miller Drain	6.51	NR	1.3
Lewis Drain	4.15	7	71
McArthur East Drain	6.9	NR	1.0
Nelles Drain	NA	NR	23

Ouellette Drain Branch	NR	NR	20
Two Creeks Drain	8.77	NR	192
Upper Portion Cartmill Drain	NR	NR	77

Site Name	Reach Data: Habitat Type				Reach Data: Canopy Coverage				
	Riffle	Rapids	Stright Run	Pool/ Back Eddy	0%	1-25%	26-50%	51-75%	76-100%
ERCA									
6 th Concession Drain									
9 th Concession Drain	✓			✓	✓				
Barlow Drain	✓			✓		✓			
Big Creek				✓		✓			
CN/Clickener Branch Drain of Renaud Line Drain			✓						✓
Campbell Sideroad Drain			✓			✓			
Coleman Drain			✓						
Cornwall Drain			✓		✓				
Taylor Drain	✓						✓		
Titcombe Rd Drain			✓						✓
Washbrook Drain			✓						✓
Wilkinson-Shilson Drain			✓			✓			
LTVCA									
10 th Concession Drain			✓			✓			
18 th and 19 th Sideroad Drain			✓			✓			
Cameron Drain			✓		✓				
Simpson Drain			✓		✓				
Moore Drain			✓			✓			
Lundy Drain	✓					✓			
Miller Drain			✓			✓			
Lewis Drain			✓			✓			
McArthur East Drain	✓								✓
Nelles Drain			✓			✓			

Two Creeks Drain	✓								
Upper Portion Cartmill Drain			✓			✓			

Site Name	Reach Data: Macrophyte Coverage					Reach Data: Streamside Vegetation			
	0%	1-25%	26-50%	51-75%	76-100%	Ferns/ grasses	shrubs	deciduous trees	coniferous trees
ERCA									
6 th Concession Drain									
9 th Concession Drain				✓		✓			
Barlow Drain		✓				✓	✓		
Big Creek		✓					✓	✓	
CN/Clickener Branch Drain of Renaud Line Drain		✓				✓	✓	✓	
Campbell Sideroad Drain			✓			✓	✓		
Coleman Drain		✓							
Cornwall Drain									
Taylor Drain				✓		✓	✓	✓	
Titcombe Rd Drain		✓				✓	✓	✓	
Washbrook Drain	✓							✓	
Wilkinson-Shilson Drain	✓					✓	✓	✓	
LTVCA									
10 th Concession Drain					✓	✓	✓		
18 th and 19 th Sideroad Drain					✓	✓	✓		
Cameron Drain		✓				✓			
Cornwall Drain						✓	✓	✓	
Simpson Drain		✓				✓			
Moore Drain		✓				✓	✓	✓	✓
Lundy Drain			✓			✓	✓		
Miller Drain		✓				✓	✓	✓	
Lewis Drain			✓			✓	✓	✓	
McArthur East Drain	✓					✓		✓	

Nelles Drain				✓		✓		✓	
Two Creeks Drain	✓	✓			✓	✓	✓		
Upper Portion Cartmill Drain		✓				✓		✓	

Site Name	Reach Data: Dominant Streamside Vegetation				Reach Data: Periphyton Coverage on Substrate				
	Ferns/grasses	shrubs	deciduous trees	coniferous trees	1	2	3	4	5
ERCA									
6 th Concession Drain									
9 th Concession Drain	✓				✓				
Barlow Drain	✓				✓				
Big Creek			✓			✓			
CN/Clickener Branch Drain of Renaud Line Drain	✓					✓			
Campbell Sideroad Chain	✓					✓			
Coleman Drain	✓				✓				
Cornwall Drain	✓				✓				
Taylor Drain	✓				✓				
Titcombe Drain			✓		✓				
Washbrook Drain			✓		✓				
Wilkinson-Shilson Drain	✓								
LTVCA									
10 th Concession Drain	✓					✓			
18 th and 19 th Sideroad Drain	✓					✓			
Cameron Drain	✓				✓				
Simpson Drain	✓				✓				
Moore Drain	✓								
Lundy Drain	✓					✓			
Miller Drain	✓				✓				
Lewis Drain	✓					✓			
McArthur East Drain	✓				✓				

Nelles Drain	✓				✓				
Two Creeks Drain			✓					✓	
Upper Portion Cartmill Drain	✓				✓				

Appendix G: Sediment Analysis Data (2017 Sampling Year):

Two sediment samples taken at each site (riffle and pool) for the locations listed that were sampled in 2017. LOI is the total loss of ignition and the incremental measurements represent the different sizes of sieves that were used to separate the sediment particles.

ERCA

Site Name	Habit at	LOI	< 63 um	63 um	90 um	125 um	250 um	500 um	1.4 um	2.0 um	Total grams
CN/Clickener Branch	pool	0.89	2.68	2.61	4.24	10.86	9.75	6.35	0.43	0.21	37.13
CN/Clickener Branch	riffle	0.62	0.61	0.42	0.67	1.55	1.19	0.95	0.00	0.00	5.38
Coulson Drain	pool	0.15	1.66	1.77	7.85	25.69	25.31	35.73	8.61	32.71	139.33
Coulson Drain	riffle	0.4	2.72	1.64	4.17	31.43	21.37	21.63	0.00	0.00	82.96
Desjardins Drain	pool	0.22	3.04	0.73	6.19	21.43	11.99	13.95	3.38	5.00	65.71
Desjardins Drain	riffle	0.2	4.78	11.87	21.80	27.51	25.74	14.92	0.00	0.00	106.62
East Branch No.47 Drain	pool	0.12	2.30	6.76	25.76	66.29	54.18	49.81	4.28	14.48	223.86
East Branch No.47 Drain	riffle	0.5	1.16	0.91	5.49	50.66	70.76	47.48	5.05	11.11	192.62
Hyland & Seymour Drain	riffle	0.36	0.96	0.49	2.39	26.90	37.56	24.12	1.57	0.72	94.71
Hyland & Seymour Drain	pool	0.49	1.63	0.44	3.78	8.05	7.63	8.11	0.60	0.31	30.56
Sturgeon Creek	pool	0.1	0.31	0.44	0.81	19.05	43.01	43.29	31.29	105.00	243.20
Sturgeon Creek	riffle	0.09	2.85	2.41	2.64	20.54	20.59	18.97	6.38	51.78	126.16
Taylor Drain	pool	0.09	2.71	2.05	9.96	127.92	7.76	4.09	1.07	0.87	156.43
Taylor Drain	riffle	0.01	3.39	2.07	3.88	138.11	67.29	7.55	0.58	1.81	224.68
Titcombe Road Drain	pool	0.4	0.77	0.74	1.85	15.25	30.60	10.34	0.00	0.00	59.55
Titcombe Road Drain	riffle	0.94	2.08	1.51	2.89	15.54	16.52	11.77	0.00	0.00	50.31
Wilkinson-Shilson Drain	riffle	0.13	4.62	6.60	13.58	35.65	20.19	11.20	1.74	7.25	100.83

LTVCA

Site Name	Habitat	LOI	< 63 um	63 um	90 um	125 um	250 um	500 um	1.4 um	2.0 um	Total grams
10 th Concession Rd Drain	pool	0.33	1.95	2.30	7.52	14.71	10.81	11.28	1.76	2.77	53.10
10 th Concession Road Drain	riffle	0.03	0.22	0.32	0.56	4.92	5.71	7.21	3.43	40.04	62.40
18 & 19 Sideroad Drain	pool	0.27	1.32	0.45	6.34	22.75	14.68	18.31	2.17	2.04	68.06
18 & 19 Sideroad Drain	riffle	0.29	1.58	2.91	6.29	13.04	9.89	10.27	0.94	0.74	45.66
Cameron Drain	pool		1.20	0.63	0.72	4.39	9.04	19.14	6.15	79.93	121.19
Cameron Drain	riffle		1.47	0.55	0.67	2.87	8.59	15.69	3.97	29.79	63.61
Coleman Drain	pool	0.09	0.49	0.32	0.48	2.29	2.99	2.64	0.45	27.04	36.69
Coleman Drain	riffle	0.04	3.02	1.79	1.61	5.79	8.64	15.04	4.08	140.71	180.68
Cornwall Drain	pool	0.18	4.54	3.62	8.85	33.14	12.22	13.20	2.75	7.94	86.26
Cornwall Drain	riffle	0.1	2.17	2.16	4.00	34.28	16.19	21.48	6.64	12.67	99.59
David Drain	pool	0.27	0.88	1.92	7.04	31.43	23.69	21.68	2.45	2.94	92.03
David Drain	riffle	0.33	1.89	1.19	3.47	23.28	14.31	18.08	1.69	1.04	64.95
Government Drain #1	riffle	0.16	4.88	11.38	23.24	40.51	27.94	32.71	5.89	6.49	153.04
Harrison Drain	pool	0.05	1.54	1.40	1.76	5.92	13.22	25.12	7.02	1143.98	1199.96
Mill Drain	pool		2.41	2.02	10.69	52.65	33.93	29.29	4.21	5.33	140.53
Harrison Drain	riffle	0.02	0.00	0.09	0.11	0.24	0.73	5.94	4.47	1112.63	1124.20
Mill Drain	riffle	0.14	8.62	8.43	15.16	48.51	45.88	38.03	3.11	8.19	175.94
King Drain	pool	0.29	3.55	7.00	26.92	42.12	35.41	26.36	0.00	0.00	141.36
King Drain	riffle	0.22	1.46	0.45	2.61	19.63	18.48	16.62	1.31	7.54	68.10
Lewis Drain	pool	0.15	1.52	1.68	5.33	13.07	14.91	19.44	2.36	7.05	65.36
Lewis Drain	riffle		1.12	0.94	3.73	17.52	22.34	37.72	12.60	55.12	151.09
Government Drain #1	pool	0.11	4.28	13.72	18.36	58.02	46.04	18.23	1.46	5.43	165.54
Lundy Drain	pool	0.22	0.64	0.54	3.36	12.51	10.82	8.80	0.69	2.99	40.34
Lundy Drain	riffle	0.09	1.73	2.45	4.61	8.69	10.22	9.52	1.84	60.54	99.60
McArthur East Drain	pool	0.16	4.24	3.63	10.70	20.79	28.05	27.66	1.17	1.30	97.54
McArthur East Drain	riffle	0.16	1.60	1.11	2.71	4.81	6.60	9.06	2.11	5.07	33.07
Miller Drain	pool	0.16	1.43	0.50	3.06	21.92	21.17	24.42	8.91	30.45	111.86
Miller Drain	riffle	0.22	2.80	1.66	1.67	6.40	8.04	17.12	7.97	70.57	116.23
Moore Drain	pool	0.15	1.39	1.54	5.41	19.41	20.84	36.88	12.74	68.90	167.11

Site Name	Habitat	LOI	< 63 um	63 um	90 um	125 um	250 um	500 um	1.4 um	2.0 um	Total grams
Moore Drain	riffle	0.17	2.02	1.71	5.96	16.03	21.96	27.11	6.58	49.23	130.60
Nelles Drain	pool	0.37	1.07	0.47	2.58	13.73	8.51	2.01	0.00	0.00	28.37
Nelles Drain	riffle	0.32	5.09	3.52	10.59	33.75	20.40	22.61	1.02	1.06	98.04
Oullete Drain Branch	pool	0.19	2.39	5.37	19.20	51.35	31.71	14.38	0.00	0.00	124.40
Oullete Drain Branch	riffle	0.22	5.00	5.61	8.73	6.30	5.03	6.00	1.10	0.04	37.81
Simpson Drain	pool	0.15	16.93	24.33	21.05	66.60	57.44	61.56	6.13	12.55	266.59
Simpson Drain	riffle	0.27	3.48	5.91	19.31	38.20	38.05	43.56	2.89	4.75	156.15
Two Creeks Drain	pool	0.02	0.80	0.41	0.39	46.38	312.46	96.33	2.26	3.98	463.01
Two Creeks Drain	riffle		0.00	0.00	0.00	0.00	0.00	0.00	0.00	2000.00	2000.00
Upper Portion Cartmill Drain	pool		0.96	0.69	0.69	3.99	5.41	7.54	1.61	51.28	72.17
Upper Portion Cartmill Drain	riffle		2.19	0.87	1.58	4.52	5.77	5.44	0.00	0.00	20.37
Wilkinson-Shilson Drain	pool		3.21	2.99	9.82	47.01	23.65	9.15	1.21	2.02	99.06

ERCA

Site Name	Habitat	62	63	90	125	250	500	1400	2000	Total percent
CN/Clickener Branch	pool	7.22	7.04	11.42	29.25	26.26	17.11	1.15	0.56	100.00
CN/Clickener Branch	riffle	11.27	7.86	12.39	28.79	22.10	17.59	0.00	0.00	100.00
Coulson Drain	pool	1.19	1.27	5.63	18.44	18.17	25.64	6.18	23.48	100.00
Coulson Drain	riffle	3.28	1.98	5.03	37.89	25.76	26.07	0.00	0.00	100.00
Desjardins Drain	pool	4.63	1.11	9.42	32.61	18.25	21.23	5.14	7.61	100.00
Desjardins Drain	riffle	4.48	11.13	20.45	25.80	24.14	13.99	0.00	0.00	100.00
East Branch No.47 Drain	pool	1.03	3.02	11.51	29.61	24.20	22.25	1.91	6.47	100.00
East Branch No.47 Drain	riffle	0.60	0.47	2.85	26.30	36.74	24.65	2.62	5.77	100.00
Hyland & Seymour Drain	riffle	1.01	0.52	2.52	28.40	39.66	25.47	1.66	0.76	100.00
Hyland & Seymour Drain	pool	5.33	1.44	12.37	26.34	24.97	26.54	1.98	1.02	100.00
Sturgeon Creek	pool	0.13	0.18	0.33	7.83	17.68	17.80	12.87	43.17	100.00
Sturgeon Creek	riffle	2.26	1.91	2.09	16.28	16.32	15.04	5.06	41.04	100.00
Taylor Drain	pool	1.73	1.31	6.37	81.77	4.96	2.61	0.68	0.56	100.00
Taylor Drain	riffle	1.51	0.92	1.73	61.47	29.95	3.36	0.26	0.81	100.00
Titcombe Road Drain	pool	1.29	1.24	3.11	25.61	51.39	17.36	0.00	0.00	100.00
Titcombe Road Drain	riffle	4.13	3.00	5.74	30.89	32.84	23.39	0.00	0.00	100.00
Wilkinson-Shilson Drain	riffle	4.58	6.55	13.47	35.36	20.02	11.11	1.73	7.19	100.00

LTVCA

Site Name	Habitat	62	63	90	125	250	500	1400	2000	Total percent
10 th Concession Rd Drain	pool	3.67	4.33	14.16	27.70	20.36	21.24	3.31	5.22	100.00
10 th Concession Road Drain	riffle	0.34	0.51	0.90	7.88	9.15	11.55	5.50	64.17	100.00
18 & 19 Sideroad Drain	pool	1.94	0.66	9.32	33.43	21.57	26.90	3.19	3.00	100.00
18 & 19 Sideroad Drain	riffle	3.45	6.38	13.78	28.56	21.66	22.49	2.06	1.61	100.00
Cameron Drain	pool	0.99	0.52	0.59	3.62	7.46	15.79	5.07	65.95	100.00
Cameron Drain	riffle	2.31	0.87	1.06	4.51	13.51	24.67	6.24	46.83	100.00
Coleman Drain	pool	1.33	0.86	1.31	6.24	8.15	7.20	1.22	73.70	100.00
Coleman Drain	riffle	1.67	0.99	0.89	3.20	4.78	8.32	2.26	77.88	100.00
Cornwall Drain	pool	5.26	4.20	10.26	38.42	14.17	15.30	3.19	9.20	100.00
Cornwall Drain	riffle	2.18	2.17	4.02	34.42	16.26	21.57	6.67	12.72	100.00
David Drain	pool	0.96	2.09	7.65	34.15	25.74	23.56	2.66	3.19	100.00
David Drain	riffle	2.91	1.83	5.34	35.84	22.03	27.84	2.60	1.60	100.00
Government Drain #1	riffle	3.19	7.44	15.19	26.47	18.26	21.37	3.85	4.24	100.00
Government Drain #1	pool	2.59	8.29	11.09	35.05	27.81	11.01	0.88	3.28	100.00
Harrison Drain	pool	0.13	0.12	0.15	0.49	1.10	2.09	0.59	95.33	100.00
Harrison Drain	riffle	0.00	0.01	0.01	0.02	0.06	0.53	0.40	98.97	100.00
King Drain	pool	2.51	4.95	19.04	29.80	25.05	18.65	0.00	0.00	100.00
King Drain	riffle	2.14	0.66	3.83	28.83	27.14	24.41	1.92	11.07	100.00
Lewis Drain	pool	2.33	2.57	8.15	20.00	22.81	29.74	3.61	10.79	100.00
Lewis Drain	riffle	0.74	0.62	2.47	11.60	14.79	24.97	8.34	36.48	100.00
Lundy Drain	pool	1.57	1.33	8.33	31.01	26.82	21.82	1.71	7.41	100.00
Lundy Drain	riffle	1.74	2.46	4.63	8.72	10.26	9.56	1.85	60.78	100.00
McArthur East Drain	pool	4.35	3.72	10.97	21.31	28.76	28.36	1.20	1.33	100.00
McArthur East Drain	riffle	4.84	3.34	8.20	14.55	19.96	27.40	6.38	15.33	100.00
Mill Drain	pool	1.72	1.44	7.61	37.47	24.14	20.84	3.00	3.79	100.00
Mill Drain	riffle	4.90	4.79	8.62	27.57	26.08	21.62	1.77	4.66	100.00
Miller Drain	pool	1.28	0.45	2.74	19.60	18.93	21.83	7.97	27.22	100.00

Site Name	Habitat	62	63	90	125	250	500	1400	2000	Total percent
Miller Drain	riffle	2.41	1.43	1.44	5.51	6.92	14.73	6.86	60.72	100.00
Moore Drain	pool	0.83	0.92	3.24	11.62	12.47	22.07	7.62	41.23	100.00
Moore Drain	riffle	1.55	1.31	4.56	12.27	16.81	20.76	5.04	37.70	100.00
Nelles Drain	pool	3.77	1.66	9.09	48.40	30.00	7.08	0.00	0.00	100.00
Nelles Drain	riffle	5.19	3.59	10.80	34.42	20.81	23.06	1.04	1.08	100.00
Oullete Drain Branch	pool	1.92	4.32	15.43	41.28	25.49	11.56	0.00	0.00	100.00
Oullete Drain Branch	riffle	13.22	14.85	23.09	16.66	13.29	15.87	2.92	0.12	100.00
Simpson Drain	pool	6.35	9.13	7.90	24.98	21.55	23.09	2.30	4.71	100.00
Simpson Drain	riffle	2.23	3.78	12.37	24.46	24.37	27.90	1.85	3.04	100.00
Two Creeks Drain	pool	0.17	0.09	0.08	10.02	67.48	20.81	0.49	0.86	100.00
Two Creeks Drain	riffle	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	100.00
Upper Portion Cartmill Drain	pool	1.33	0.95	0.96	5.53	7.50	10.45	2.23	71.05	100.00
Upper Portion Cartmill Drain	riffle	10.75	4.27	7.76	22.19	28.33	26.71	0.00	0.00	100.00
Wilkinson-Shilson Drain	pool	3.24	3.02	9.91	47.46	23.87	9.24	1.22	2.04	100.00

ERCA

Site Name	Habitat	4.25	4	3.5	3	2	1	-0.5	-1	D50 (50 th percentile particle diameter)
CN/Clickener Branch	pool	7.22	14.26	25.68	54.93	81.19	98.30	99.44	100	0.118
CN/Clickener Branch	riffle	11.27	19.13	31.52	60.31	82.41	100	100	100	0.111
Coulson Drain	pool	1.19	2.46	8.10	26.53	44.70	70.34	76.52	100	0.289
Coulson Drain	riffle	3.28	5.26	10.28	48.17	73.93	100	100	100	0.131
Desjardins Drain	pool	4.63	5.74	15.16	47.77	66.02	87.25	92.39	100	0.136
Desjardins Drain	riffle	4.48	15.62	36.06	61.86	86.01	100	100	100	0.107
East Branch No.47 Drain	pool	1.03	4.05	15.55	45.17	69.37	91.62	93.53	100	0.144
East Branch No.47 Drain	riffle	0.60	1.07	3.92	30.23	66.96	91.61	94.23	100	0.182
Hyland & Seymour Drain	riffle	1.01	1.53	4.05	32.46	72.11	97.58	99.24	100	0.170
Hyland & Seymour Drain	pool	5.33	6.77	19.14	45.49	70.46	97.00	98.98	100	0.142
Sturgeon Creek	pool	0.13	0.31	0.64	8.48	26.16	43.96	56.83	100	0.811
Sturgeon Creek	riffle	2.26	4.17	6.26	22.54	38.86	53.90	58.96	100	0.418
Taylor Drain	pool	1.73	3.04	9.41	91.18	96.15	98.76	99.44	100	0.106
Taylor Drain	riffle	1.51	2.43	4.16	65.62	95.57	98.93	99.19	100	0.115
Titcombe Road Drain	pool	1.29	2.54	5.64	31.25	82.64	100	100	100	0.161
Titcombe Road Drain	riffle	4.13	7.14	12.88	43.77	76.61	100	100	100	0.143
Wilkinson-Shilson Drain	riffle	4.58	11.13	24.60	59.95	79.98	91.08	92.81	100	0.107

LTVCA

Site Name	Habitat	4.25	4	3.5	3	2	1	-0.5	-1	D50 (50 th percentile particle diameter)
10 th Concession Rd Drain	pool	3.67	8.00	22.17	49.87	70.23	91.47	94.78	100	0.126
10 th Concession Road Drain	riffle	0.34	0.85	1.75	9.63	18.78	30.34	35.83	100	1.515
18 & 19 Sideroad Drain	pool	1.94	2.60	11.91	45.34	66.91	93.81	97.00	100	0.145
18 & 19 Sideroad Drain	riffle	3.45	9.84	23.61	52.17	73.83	96.33	98.39	100	0.122
Cameron Drain	pool	0.99	1.51	2.10	5.72	13.18	28.97	34.05	100	1.526
Cameron Drain	riffle	2.31	3.18	4.24	8.75	22.26	46.93	53.17	100	0.830
Coleman Drain	pool	1.33	2.19	3.50	9.74	17.89	25.09	26.30	100	1.570
Coleman Drain	riffle	1.67	2.66	3.55	6.76	11.54	19.86	22.12	100	1.591
Cornwall Drain	pool	5.26	9.46	19.72	58.14	72.30	87.61	90.80	100	0.117
Cornwall Drain	riffle	2.18	4.35	8.36	42.79	59.04	80.61	87.28	100	0.170
David Drain	pool	0.96	3.04	10.69	44.84	70.59	94.14	96.81	100	0.144
David Drain	riffle	2.91	4.74	10.08	45.93	67.96	95.80	98.40	100	0.142
Government Drain #1	riffle	3.19	10.62	25.81	52.28	70.54	91.91	95.76	100	0.122
Government Drain #1	pool	2.59	10.87	21.96	57.01	84.83	95.84	96.72	100	0.117
Harrison Drain	pool	0.13	0.25	0.39	0.89	1.99	4.08	4.67	100	1.659
Harrison Drain	riffle	0.00	0.01	0.02	0.04	0.10	0.63	1.03	100	1.659
King Drain	pool	2.51	7.46	26.51	56.30	81.35	100	100	100	0.117
King Drain	riffle	2.14	2.80	6.64	35.46	62.60	87.00	88.93	100	0.181
Lewis Drain	pool	2.33	4.90	13.05	33.05	55.86	85.60	89.21	100	0.209
Lewis Drain	riffle	0.74	1.36	3.83	15.43	30.21	55.18	63.52	100	0.433
Lundy Drain	pool	1.57	2.90	11.23	42.24	69.06	90.89	92.59	100	0.153
Lundy Drain	riffle	1.74	4.20	8.83	17.55	27.81	37.37	39.22	100	1.491

Site Name	Habitat	4.25	4	3.5	3	2	1	-0.5	-1	D50 (50 th percentile particle diameter)
McArthur East Drain	pool	4.35	8.07	19.04	40.35	69.11	97.46	98.67	100	0.158
McArthur East Drain	riffle	4.84	8.18	16.38	30.92	50.88	78.29	84.67	100	0.242
Mill Drain	pool	1.72	3.15	10.76	48.23	72.37	93.21	96.21	100	0.132
Mill Drain	riffle	4.90	9.69	18.31	45.88	71.96	93.57	95.34	100	0.139
Miller Drain	pool	1.28	1.73	4.46	24.06	42.98	64.81	72.78	100	0.312
Miller Drain	riffle	2.41	3.84	5.27	10.78	17.70	32.43	39.28	100	1.491
Moore Drain	pool	0.83	1.75	4.99	16.61	29.08	51.15	58.77	100	0.482
Moore Drain	riffle	1.55	2.86	7.42	19.69	36.51	57.27	62.30	100	0.392
Nelles Drain	pool	3.77	5.43	14.52	62.92	92.92	100	100	100	0.115
Nelles Drain	riffle	5.19	8.78	19.58	54.01	74.82	97.88	98.92	100	0.120
Oullete Drain Branch	pool	1.92	6.24	21.67	62.95	88.44	100	100	100	0.113
Oullete Drain Branch	riffle	13.22	28.06	51.15	67.81	81.10	96.97	99.88	100	0.088
Simpson Drain	pool	6.35	15.48	23.37	48.35	69.90	92.99	95.29	100	0.132
Simpson Drain	riffle	2.23	6.01	18.38	42.84	67.21	95.11	96.96	100	0.153
Two Creeks Drain	pool	0.17	0.26	0.34	10.36	77.85	98.65	99.14	100	0.188
Two Creeks Drain	riffle	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100	1.673
Upper Portion Cartmill Drain	pool	1.33	2.29	3.25	8.77	16.27	26.72	28.95	100	1.556
Upper Portion Cartmill Drain	riffle	10.75	15.02	22.78	44.97	73.29	100	100	100	0.141
Wilkinson-Shilson Drain	pool	3.24	6.26	16.17	63.63	87.50	96.74	97.96	100	0.114

Vita Auctoris

NAME	Alyssa A. Frazao
PLACE OF BIRTH	Leamington, Ontario
YEAR OF BIRTH	1992
EDUCATION	Cardinal Carter Catholic Secondary School, Leamington, Ontario, 2006 - 2010 University of Windsor, B.For.Sc – Forensic Science with Biology Specialization, Windsor, Ontario, 2011 – 2016 University of Windsor, M.Sc. in Biology, Windsor, Ontario, 2016 - 2019