

Preliminary DNA Data

Windermere Creek, BC

Columbia Basin Water Quality Monitoring Project - Windermere Creek
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Photo: Clare Suggett.



www.STREAM-DNA.com

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Table of Contents

1. INTRODUCTION	2
1.1 Benthic Macroinvertebrates	2
1.2 Background of STREAM	3
1.3 Objective of Report	5
1.4 Study Objective	5
2. METHODOLOGY	6
2.1 Study Area	6
2.2 DNA Sampling and Processing Methods	7
2.2.1 Measures to Avoid DNA Contamination	7
2.2.2 Benthic Macroinvertebrate Field Sampling Protocol.....	7
2.2.3 Laboratory Methods	7
3. RESULTS	7
3.1 Overview	7
3.2 Taxonomic Coverage	9
4. FUTURE SUGGESTIONS	12
5. REFERENCES	13
6. APPENDICES	14
7. GLOSSARY	15

DISCLAIMER: This report is a preliminary report based on the samples and information provided by the corresponding organisation. Identifications of taxa are based on best available information at time of analysis and reporting.

1. INTRODUCTION

1.1. Benthic Macroinvertebrates

Freshwater benthic macroinvertebrates are typically insect orders, as well as crustaceans (e.g. crayfish), gastropods (e.g. snails), bivalves (e.g. freshwater mussels) and oligochaetes (e.g. worms), which are located on or within the benthic substrate of freshwater systems (i.e. streams, rivers, lakes; (Covich et al., 1999; Schmera et al., 2017)). Benthic macroinvertebrates occupy important roles in the functioning of freshwater ecosystems, namely nutrient cycling within aquatic food webs and also influence numerous processes including microbial production and release of greenhouse gases (Covich et al., 1999; Schmera et al., 2017).

Biological monitoring (biomonitoring), referring to the collection and identification of particular aquatic species is an effective method for measuring the health status of freshwater systems. Currently, macroinvertebrates are routinely used for biomonitoring studies in freshwater habitats because they are relatively sedentary, have high species richness and a range of responses to different environmental stressors and contaminants, including temperature (Curry et al., 2018; Geest et al., 2010; Rosenberg and Resh, 1993; Sidney et al., 2016). Some groups of macroinvertebrates (mayflies, Ephemeroptera; stoneflies, Plecoptera and caddisflies, Trichoptera), commonly referred to as EPT groups, are more sensitive to change in the aquatic environment and are deemed important bioindicator taxa for assessing freshwater quality (Curry et al., 2018; Hajibabaei et al., 2012, 2011).

Traditionally, macroinvertebrates are identified to family level (**Figure 1**) through morphological identification using microscopy, however there has been a shift from this labour-intensive methodology to a DNA-based approach (Curry et al., 2018; Hajibabaei et al., 2012, 2011). ‘Biomonitoring 2.0’ combines bulk-tissue DNA collection (i.e. benthos) with next-generation sequencing (NGS), to produce high-quality data in large quantities and allows identification to a finer resolution than traditional methods (Baird and Hajibabaei, 2012; Hajibabaei et al., 2012).

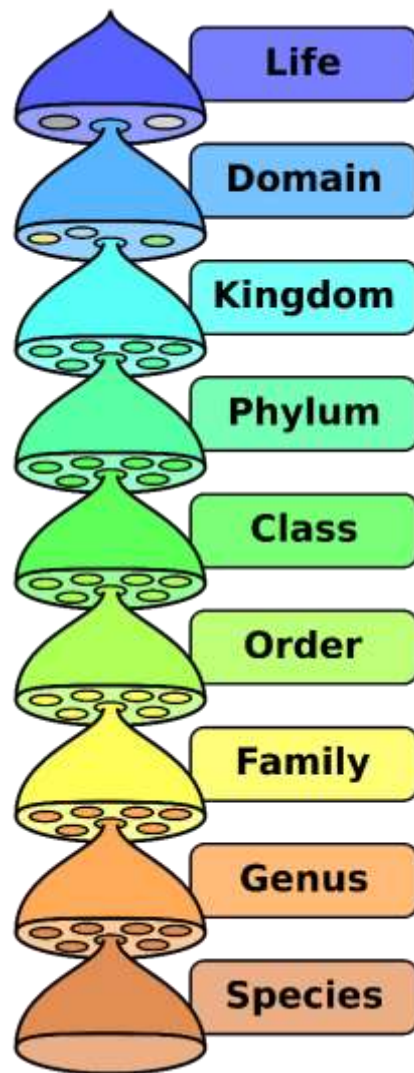


Figure 1. Graphical representation the classification of organisms.

1.2. Background of STREAM

STREAM (Sequencing The Rivers for Environmental Assessment and Monitoring), is a biomonitoring project, which involves the combination of community based monitoring and DNA metabarcoding technologies to assess the benthic macroinvertebrate communities in watersheds across Canada (**Figure 2**). STREAM is a collaboration between World Wildlife Fund (WWF) Canada, Living Lakes Canada

(LLC) and Environmental and Climate Change Canada (ECCC), led by the Hajibabaei Lab at Centre for Biodiversity Genomics (University of Guelph, Canada). STREAM is integrated with the Canadian Aquatic Biomonitoring Network (CABIN) programme, through the implementation of existing nationally standardized protocols for freshwater monitoring. The aquatic biodiversity data generated in STREAM will be added to the existing CABIN database, to improve our understanding of the health of Canadian watersheds.

The main objective of STREAM is to generate baseline benthic macroinvertebrate DNA data from across Canada. To understand the health status of freshwater systems, we first need to understand the natural fluctuations and trends of benthic macroinvertebrates, especially in locations which are data deficient. By building this baseline, in years to come we can investigate the longer-term trends and begin to understand the impact of issues, such as climate change, on freshwater systems. STREAM was established with the main premise of fast-tracking the generation of benthic macroinvertebrate data from 12-18 months to ~2 months, while increasing the taxonomic resolution of the data produced.

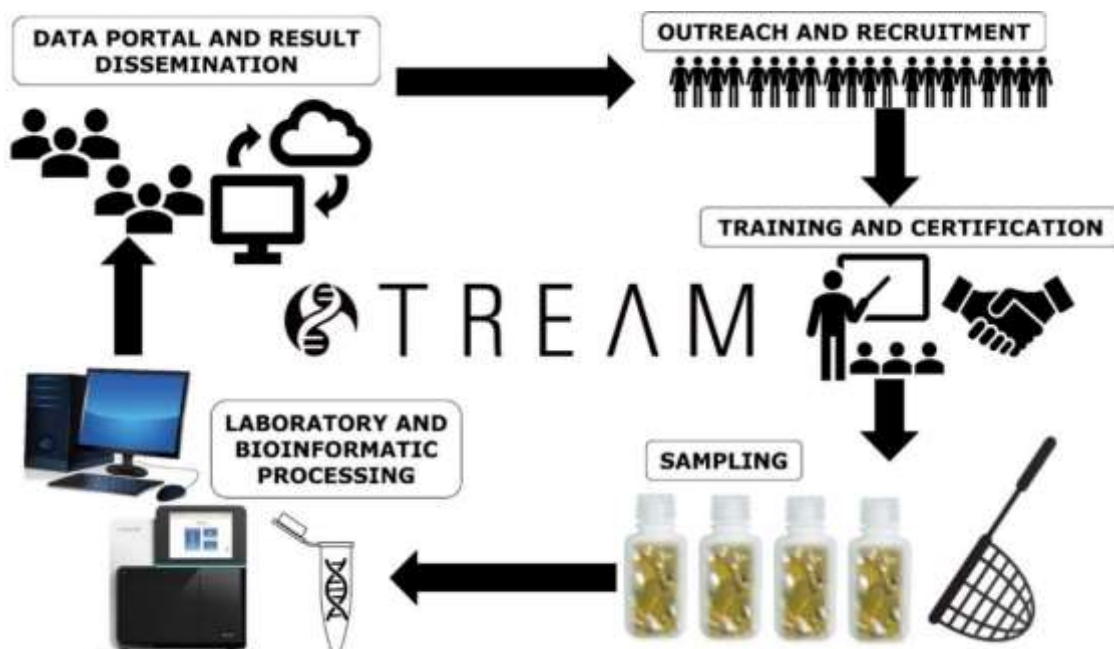


Figure 2. Graphical representation of the STREAM collaborative workflow for DNA biomonitoring of benthic invertebrates.

1.3. Objective of Report

Data and information included in this report is a first and preliminary examination of results from Windermere Creek (BC), which consists of a list of the macroinvertebrate taxa detected within the samples submitted. This report aims to highlight the different macroinvertebrate EPT taxa and provide basic richness metrics as a useful contribution for community groups to assess river health.

1.4. Study Objective

Water Sampling by the Lake Windermere Ambassadors on Windermere Creek began in 2013 with the Columbia Basin Water Quality Monitoring Program. Since then, the Ambassadors have taken over the full operations of this sampling location. We are currently conducting weekly water quality and quantity measurements, monthly lab analysis, and annual CABiN protocol. Adding in monthly DNA sample collection fits well with our program and will provide valuable information to complement our larger monitoring program. Additionally, in 2018 we installed a continuous flow logger on an old Water Survey of Canada station to provide insight into water volume data.

2. METHODOLOGY

2.1. Study Area

Between July and September 2019, this study was conducted at a single pre-determined sampling location within Windermere Creek (BC; Figure 3). Sampling was conducted by Lake Windermere Ambassadors each month for benthic macroinvertebrate monitoring with STREAM.

Additional site information, including coordinates, and number of samples collected is provided in Appendix A.



Figure 3. Map of sampling location within Windermere Creek (BC). Inset map (top left) shows location of sampling area with respect to BC. Scale bar shown in kilometres.

2.2. DNA Sampling and Processing Methods

2.2.1. Measures to Avoid DNA Contamination

Prior to sampling, kick-nets were sanitized in bleach for 45 minutes and kept in clean garbage bags until they were used in the field. Gloves were used when handling all sampling materials to avoid contamination. During the kick-netting, the surveyor in the water wore two pairs of gloves while handling the kick-net. The outer pair of

gloves was removed prior to transferring the contents into sampling containers so that the gloves used when contacting the sample were guaranteed to be clean. Each sampling container was individually sealed in a Ziploc bag prior to placing them in the cooler.

2.2.2. Benthic Macroinvertebrate Field Sampling Protocol

Benthic macroinvertebrate DNA samples were collected following the STREAM Procedure for collecting benthic macroinvertebrate DNA samples in wadeable streams (v1.0 June 2019) and the CABIN Field Manual for Wadeable Streams (2012). The STREAM procedure outlines steps to minimize DNA contamination and preserve DNA samples and was employed in conjunction with sampling steps outlined in the CABIN manual. All samples collected were transported to the University of Guelph Centre for Biodiversity Genomics.

2.2.3. Laboratory Methods

Benthic samples were preserved in 100% ethanol and stored at -20°C until processing. Benthic samples were coarsely homogenized in a sterile blender and DNA was extracted using a DNeasy® PowerSoil® kit (Qiagen, CA) kit. Extracted DNA was then processed following the standard Hajibabaei Lab protocol for Next-Generation Sequencing (NGS). Sequences were then processed through the MetaWorks (v1) pipeline: <https://github.com/terrimporter/MetaWorks>.

3. RESULTS

3.1. Overview

The raw data output from NGS produced sequences for a range of taxa. This taxa list was reduced to only sequences that identified macroinvertebrates associated with freshwater and riparian ecosystems, and that were of high enough quality to match reference sequences. These results consisted of **15 Orders, 34 Families, 46 Genera, and 53 species of macroinvertebrates**. Across the three time points, species richness (number of species present) ranged from 28 in the August sample to 50 in the July sample (**Figure 4**). A full taxonomic list identified to the species level for macroinvertebrates is included as a separate Excel spreadsheet.

Note: The benthic macroinvertebrate kick-net sample procedure often results in collection of both aquatic and terrestrial taxa, however terrestrial taxa are not identified using the traditional taxonomic identification methods. Due to the nature of DNA metabarcoding, both terrestrial and aquatic macroinvertebrates are identified and described using the DNA approach in this report.

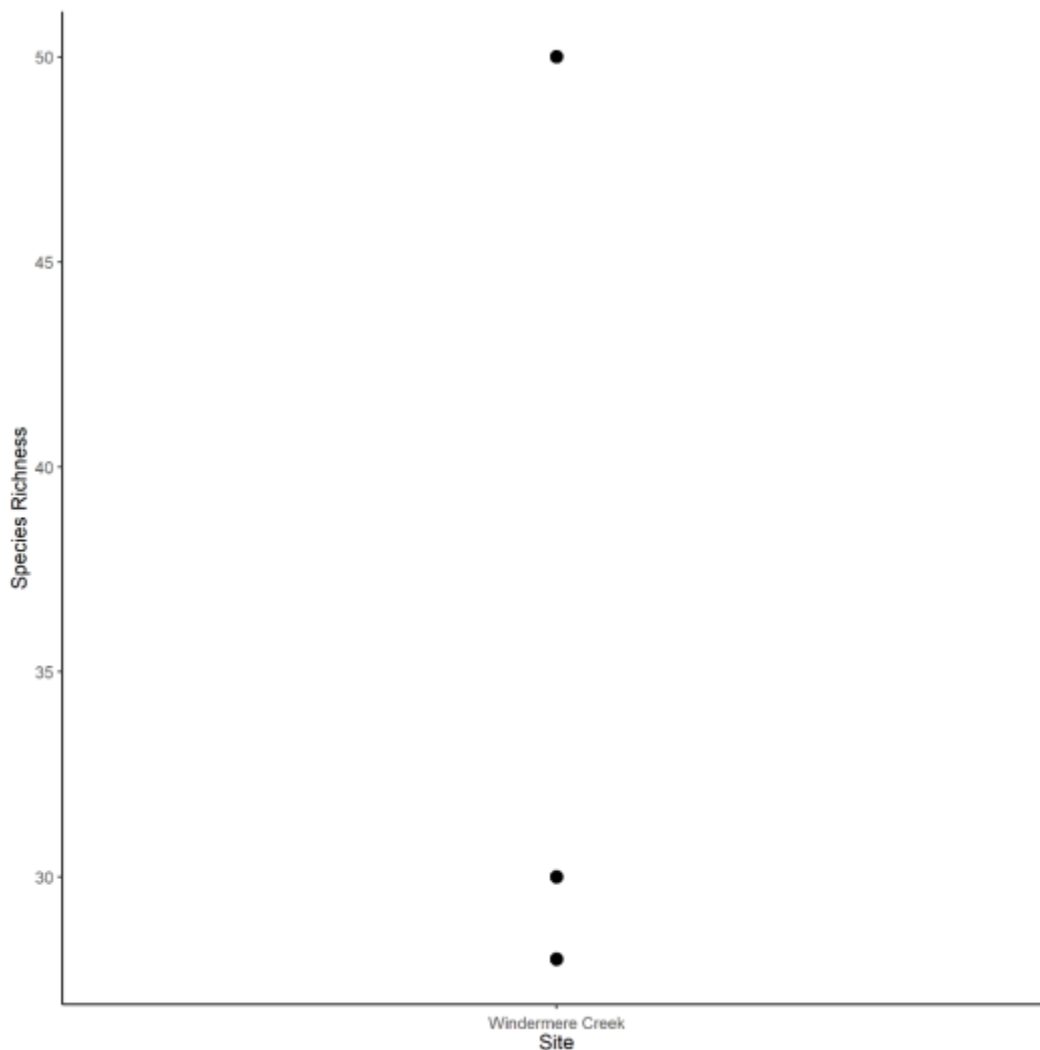


Figure 4. Species richness of each time point sampled for Windermere Creek sample. Top to bottom: July, September, August. Only species taxonomically assigned with high confidence (bootstrap support ≥ 0.70) are included.

3.2. Taxonomic Coverage

A range of macroinvertebrate species were detected. Traditional bioindicator EPT species were detected across the three time points, including Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies; **Table 1**). These EPT species are typically sensitive to many pollutants in the stream environment and are therefore associated with clean water (Gresens et al., 2009; Laini et al., 2019; Loeb and Spacie, 1994). Some picture examples of species detected in this study can be found in Figure 5.

Table 1. List of macroinvertebrates identified to the species level. P = present; (T) = terrestrial. Grey cells indicate absence. Highlighted in blue are the traditional EPT bioindicator orders present. NAWN03_July = Windermere Creek (July sample); NAWN03_Aug = Windermere Creek (August sample), NAWN03_Sep = Windermere Creek (September sample). Only species taxonomically assigned with high confidence (bootstrap support ≥ 0.70) are included.

Order	Family	Family Description	Species	NAWN3_July	NAWN3_Aug	NAWN3_Sep
Coleoptera	Chrysomelidae	Leaf beetles (T)	<i>Donacia clavipes</i>	P		P
Coleoptera	Curculionidae	Weevils (T)	<i>Otiorhynchus raucus</i>	P	P	P
Coleoptera	Curculionidae	Weevils (T)	<i>Otiorhynchus singularis</i>	P		
Coleoptera	Curculionidae	Weevils (T)	<i>Sciaphilus asperatus</i>	P		P
Diptera	Chironomidae	Non-biting midges	<i>Corynoneura kibunelata</i>	P	P	P
Diptera	Chironomidae	Non-biting midges	<i>Eukiefferiella claripennis</i>	P	P	
Diptera	Chironomidae	Non-biting midges	<i>Micropsectra penicillata</i>	P	P	
Diptera	Chironomidae	Non-biting midges	<i>Pagastia orthogonia</i>	P	P	P
Diptera	Chironomidae	Non-biting midges	<i>Paraphaenocladus impensus</i>	P		
Diptera	Empididae	Dagger flies	<i>Oreogeton scopifer</i>	P	P	
Diptera	Simuliidae	Black flies	<i>Simulium arcticum</i>			P
Diptera	Simuliidae	Black flies	<i>Simulium canadense</i>	P	P	P
Diptera	Simuliidae	Black flies	<i>Simulium carbunculum</i>	P		
Diptera	Simuliidae	Black flies	<i>Simulium tuberosum</i>	P	P	
Enchytraeida	Enchytraeidae	Potworms (T)	<i>Henlea nasuta</i>	P		
Ephemeroptera	Ameletidae	Combmouthed minnow mayflies	<i>Ameletus celer</i>	P		
Ephemeroptera	Baetidae	Small minnow mayflies	<i>Baetis tricaudatus</i>	P	P	P
Ephemeroptera	Ephemerellidae	Spiny crawler mayflies	<i>Drunella coloradensis</i>	P	P	P
Ephemeroptera	Ephemerellidae	Spiny crawler mayflies	<i>Drunella grandis</i>	P		
Ephemeroptera	Ephemerellidae	Spiny crawler mayflies	<i>Ephemerella tibialis</i>	P	P	
Ephemeroptera	Heptageniidae	Flat-headed mayflies	<i>Cinygmula spJMW3</i>	P	P	P
Ephemeroptera	Heptageniidae	Flat-headed mayflies	<i>Epeorus deceptivus</i>	P	P	P
Ephemeroptera	Heptageniidae	Flat-headed mayflies	<i>Epeorus grandis</i>	P		P
Ephemeroptera	Heptageniidae	Flat-headed mayflies	<i>Epeorus longimanus</i>	P	P	P
Ephemeroptera	Heptageniidae	Flat-headed mayflies	<i>Rhithrogena robusta</i>	P	P	P
Ephemeroptera	Leptophlebiidae	Prong-gilled mayflies	<i>Paraleptophlebia heteronea</i>	P		
Haplotaxida	Lumbricidae	Earthworms (T)	<i>Dendrodrilus rubidus</i>	P		P
Haplotaxida	Lumbricidae	Earthworms (T)	<i>Eiseniella tetraedra</i>	P	P	
Haplotaxida	Naididae	Detritus worms	<i>Nais bretscheri</i>	P	P	P

Plecoptera	Chloroperlidae	Green stoneflies	<i>Sweltsa borealis</i>	P	P	P
Plecoptera	Chloroperlidae	Green stoneflies	<i>Sweltsa urticae</i>	P		
Plecoptera	Leuctridae	Rolled-winged stoneflies	<i>Paraleuctra occidentalis</i>	P		
Plecoptera	Nemouridae	Spring stoneflies	<i>Visoka cataractae</i>	P		
Plecoptera	Nemouridae	Spring stoneflies	<i>Zapada cinctipes</i>	P	P	P
Plecoptera	Nemouridae	Spring stoneflies	<i>Zapada columbiana</i>	P	P	P
Plecoptera	Nemouridae	Spring stoneflies	<i>Zapada haysi</i>	P		
Plecoptera	Perlidae	Common stoneflies	<i>Hesperoperla pacifica</i>	P	P	P
Plecoptera	Perlodidae	Springflies	<i>Isoperla petersoni</i>	P		
Plecoptera	Taeniopterygidae	Winter stoneflies	<i>Doddsia occidentalis</i>	P	P	P
Podocopida	Candonidae	Freshwater ostracods	<i>Candona candida</i>	P		
Trichoptera	Brachycentridae	Humpless casemaker caddisflies	<i>Brachycentrus americanus</i>	P	P	P
Trichoptera	Glossosomatidae	Saddle-casemaker caddisflies	<i>Glossosoma alascense</i>	P	P	P
Trichoptera	Glossosomatidae	Saddle-casemaker caddisflies	<i>Glossosoma pyroxum</i>	P		P
Trichoptera	Glossosomatidae	Saddle-casemaker caddisflies	<i>Glossosoma wenatchee</i>	P	P	P
Trichoptera	Hydropsychidae	Net-spinning caddisflies	<i>Arctopsyche grandis</i>	P		P
Trichoptera	Hydropsychidae	Net-spinning caddisflies	<i>Parapsyche elsis</i>	P	P	P
Trichoptera	Lepidostomatidae	Bizarre caddisflies	<i>Lepidostoma cascadense</i>	P		
Trichoptera	Lepidostomatidae	Bizarre caddisflies	<i>Lepidostoma roafi</i>	P		
Trichoptera	Lepidostomatidae	Bizarre caddisflies	<i>Lepidostoma unicolor</i>	P		
Trichoptera	Philopotamidae	Finger-net caddisflies	<i>Dolophilodes aequalis</i>	P		P
Trichoptera	Rhyacophilidae	Free-living caddisflies	<i>Rhyacophila hyalinata</i>	P	P	P
Trichoptera	Rhyacophilidae	Free-living caddisflies	<i>Rhyacophila vao</i>	P	P	
Trombidiformes	Torrenticolidae	Torrent mites	<i>Testudacarus minimus</i>	P		P

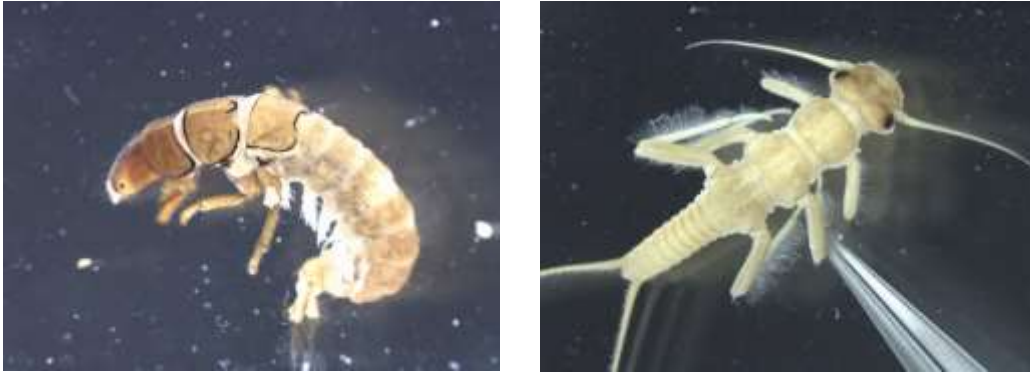


Figure 5. Some examples of the EPT taxa detected. Left: species of net-spinning caddisfly (Trichoptera) in the family Hydropsychidae; Right: species of springflies (Plecoptera) in the family Perlodidae. All photos: ©CABIN Taxonomy.

4. FUTURE SUGGESTIONS

We suggest sampling each site in triplicate, as this is beneficial for representing the true biological diversity of each site and in turn generates more biodiversity data.

5. REFERENCES

- Baird, D.J., Hajibabaei, M., 2012. Biomonitoring 2.0: a new paradigm in ecosystem assessment made possible by next-generation DNA sequencing. *Mol. Ecol.* 21, 2039-2044. <https://doi.org/10.1111/j.1365-294X.2012.05519.x>
- Covich, A.P., Palmer, M.A., Cowl, T.A., 1999. The Role of Benthic Invertebrate Species in Freshwater Ecosystems: Zoobenthic species influence energy flows and nutrient cycling. *BioScience* 49, 119-127. <https://doi.org/10.2307/1313537>
- Curry, C.J., Gibson, J.F., Shokralla, S., Hajibabaei, M., Baird, D.J., 2018. Identifying North American freshwater invertebrates using DNA barcodes: are existing COI sequence libraries fit for purpose? *Freshw. Sci.* 37, 178-189. <https://doi.org/10.1086/696613>
- Geest, J.L.V., Poirier, D.G., Sibley, P.K., Solomon, K.R., 2010. Measuring bioaccumulation of contaminants from field-collected sediment in freshwater organisms: A critical review of laboratory methods. *Environ. Toxicol. Chem.* 29, 2391-2401. <https://doi.org/10.1002/etc.326>
- Gresens, S.E., Smith, R.J., Sutton-Grier, A.E., Kenney, M.A., 2009. Benthic macroinvertebrates as indicators of water quality: The intersection of science and policy. <https://doi.org/10.1163/187498209X12525675906077>
- Hajibabaei, M., Shokralla, S., Zhou, X., Singer, G.A.C., Baird, D.J., 2011. Environmental Barcoding: A Next-Generation Sequencing Approach for Biomonitoring Applications Using River Benthos. *PLOS ONE* 6, e17497. <https://doi.org/10.1371/journal.pone.0017497>
- Hajibabaei, M., Spall, J.L., Shokralla, S., van Konynenburg, S., 2012. Assessing biodiversity of a freshwater benthic macroinvertebrate community through non-destructive environmental barcoding of DNA from preservative ethanol. *BMC Ecol.* 12, 28. <https://doi.org/10.1186/1472-6785-12-28>
- Laini, A., Viaroli, P., Bolpagni, R., Cancellario, T., Racchetti, E., Guareschi, S., 2019. Taxonomic and Functional Responses of Benthic Macroinvertebrate Communities to Hydrological and Water Quality Variations in a Heavily Regulated River. *Water* 11, 1478. <https://doi.org/10.3390/w11071478>
- Loeb, S., L., Spacie, A., 1994. *Biological Monitoring of Aquatic Systems*. CRC Press.
- McQuaid, B., n.d. *Watershed Science Institute* 30.

Rosenberg, D.M., Resh, V.H. (Eds.), 1993. Freshwater Biomonitoring and Benthic Macroinvertebrates. Springer US.

Schmera, D., Heino, J., Podani, J., Erős, T., Dolédec, S., 2017. Functional diversity: a review of methodology and current knowledge in freshwater macroinvertebrate research. *Hydrobiologia* 787, 27-44. <https://doi.org/10.1007/s10750-016-2974-5>

Sidney, L.A., Diepens, N.J., Guo, X., Koelmans, A.A., 2016. Trait-based modelling of bioaccumulation by freshwater benthic invertebrates. *Aquat. Toxicol.* 176, 88-96. <https://doi.org/10.1016/j.aquatox.2016.04.017>

6. APPENDICES

Appendix A. Summary table of sample site, including site name, date of collection and site coordinates.

Site Code	Site Name	Sample Collection	Latitude	Longitude
NAWN03_July	Windermere Creek	July 2019	50.46162	115.98558
NAWN03_Aug	Windermere Creek	August 2019	50.46162	115.98558
NAWN03_Sep	Windermere Creek	September 2019	50.46162	115.98558

7. GLOSSARY

Term	Meaning
Benthic/benthos	The ecological region at the lowest level of a body of water such as an ocean, lake, or stream, including the sediment surface and some sub-surface layers.
Biomonitoring	The science of inferring the ecological condition of an ecosystem (i.e. rivers, lakes, streams, and wetlands) by examining the organisms that live there.
Bootstrap support	Statistical methods used to evaluate and distinguish the confidence of results produced.
Bulk-tissue DNA sample	This refers to the collection and removal of a reasonable quantity of representative material (including organisms such as river bugs) from a location (i.e. river bed).
DNA extraction	Isolation of DNA from either the target organism (i.e. DNA from an insect leg) or from an environmental sample (i.e. DNA from a water or benthos sample).
DNA Metabarcoding	Amplification of DNA using universal barcode primers (e.g. universal for invertebrates) to allow sequencing of DNA from target organisms (e.g. invertebrates) from environmental samples (e.g. river water or benthos).
Environmental DNA (eDNA)	The DNA released into the environment through faeces, urine, gametes, mucus, etc. eDNA can result from the decomposition of dead organisms. eDNA is characterized by a complex mixture of nuclear, mitochondrial or chloroplast DNA, and can be intracellular (from living cells) or extracellular. Environmental DNA: DNA that can be extracted from environmental samples (such as soil, water, or air), without first isolating any target organisms.
EPT groups	The three orders of aquatic insects that are common in the benthic macroinvertebrate community: Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies).
Macroinvertebrate	Organisms that lack a spine and are large enough to be seen with the naked eye. Examples of macro-invertebrates include flatworms, crayfish, snails, clams and insects, such as dragonflies.
Metrics	The method of measuring something, or the results obtained from this.
Next-generation sequencing (NGS)	Use of next-generation sequencers (i.e. Illumina) to millions or billions of DNA strands in parallel.
Richness	The number of species represented in an ecological community, landscape or region. Species richness is simply a count of species, and it does not take into account the abundances of the species or their relative abundance distributions.

Riparian	Relating to or situated on the banks of a river.
Sample homogenization	The process of making an environmental sample (i.e. benthos) uniform. For liquid/benthos samples, this often involves mixing using a blender so that DNA is evenly distributed within the sample.
Taxa	Unit used in the science of biological classification, or taxonomy.