

Preliminary DNA Data

Peace Watershed, BC
Blueberry River First Nations
February 2021



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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).

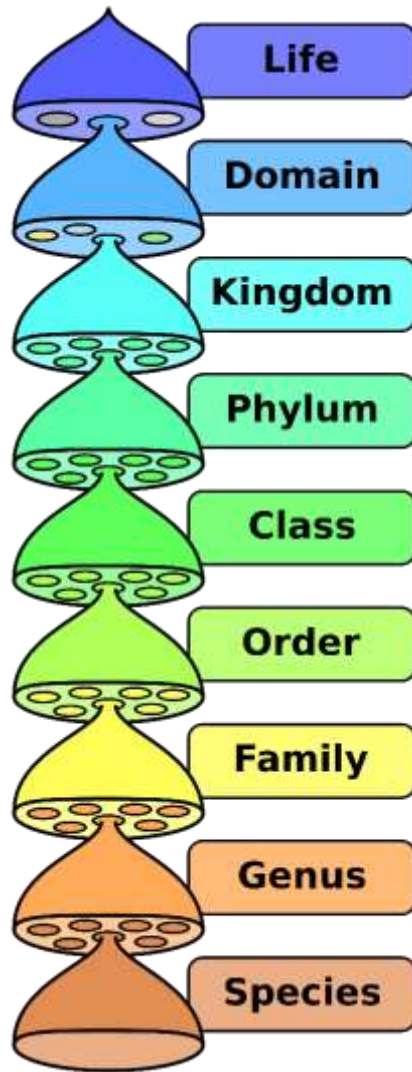


Figure 1. Graphical representation the classification of organisms.

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.

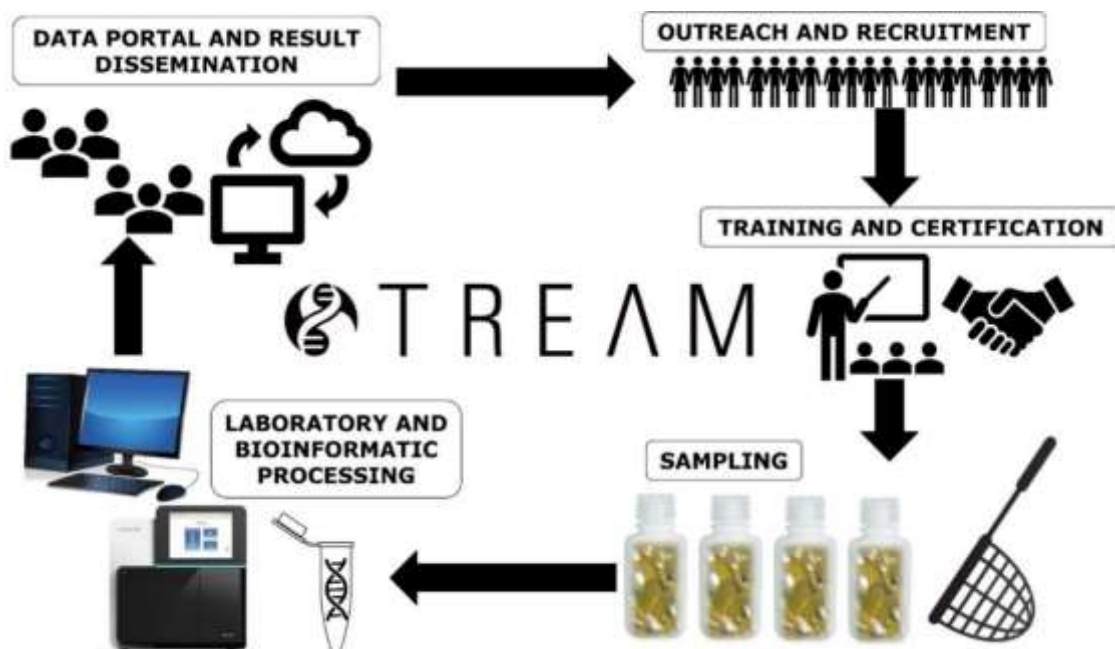


Figure 2. Graphical representation of the STREAM collaborative workflow for DNA biomonitoring of benthic invertebrates.

1.3. Objective of Report

Data and information included in this report is an examination of results from the Peace Watershed (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/Background

This project with STREAM is designed to increase local capacity in order to scope development of a water quality monitoring program in the watersheds around the Blueberry River First Nations (BRFN) reserve. Monitoring will allow BRFN to contribute to baseline information and will help inform research questions about the scope and breadth of impacts on aquatic systems in BRFN territory.

2. METHODOLOGY

2.1. Study Area

In September 2020, this study was conducted at a pre-determined sampling location within the Peace Watershed (**Figure 3**). The focal area of the Peace Watershed for the overall study is located in northeast BC. Sampling was conducted by BRFN for benthic macroinvertebrate monitoring with STREAM.

DNA data from Blueberry River (BLU01) collected in 2019 has been re-analyzed and is included in this report for a 2-year comparison.

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



Figure 3. Map of sampling location within the Peace Watershed (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 isopropyl alcohol 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 (v1.3.4) 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 raw results consisted of **55 orders, 43 families, 73 genera, and 74 species of macroinvertebrates**. After normalizing, species richness (number of species present) for each replicate ranged from 9 in BLU01C (2019) to 34 in BLU01A and BLU01B (2020; **Figure 4**). A full taxonomic list identified to the raw family, genus and species level for macroinvertebrates is included as a separate Excel spreadsheet (RP41_Taxonomy).

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.

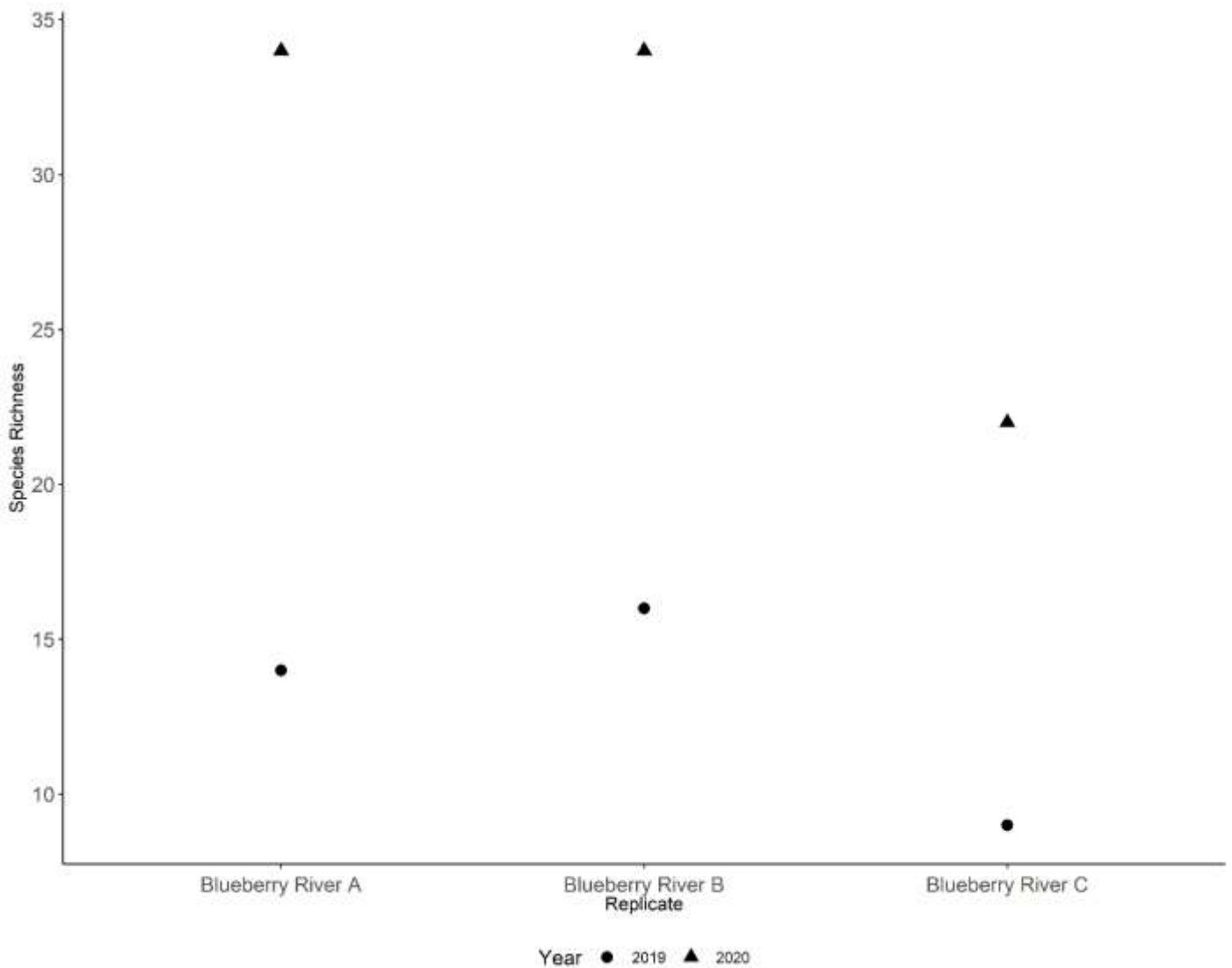


Figure 4. Species richness of each replicate. 2019 (circle) and 2020 (triangle) samples are plotted. Only species taxonomically assigned with high confidence (bootstrap support ≥ 0.70) are included. Based on normalized data.

3.2. Taxonomic Coverage

For all replicates, species richness was greater in 2020 compared to 2019 (Figure 4). A range of macroinvertebrate species were detected at the site. Traditional bioindicator EPT species were detected, including Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies; **Table 1**). These EPT species in rivers 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).

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. BLU01_2019 = Blueberry River 2019; BLU01_2020 = Blueberry River 2020. All replicates combined.

Order	Family	Family Description	Species	BLU01_2019	BLU01_2020
Coleoptera	Curculionidae	True weevils (T)	<i>Isochnus flagellum</i>		P
Diptera	Bibionidae	March flies (T)	<i>Biblio longipes</i>		P
Diptera	Chironomidae	Non-biting midges	<i>Conchapelopia telema</i>		P
Diptera	Chironomidae	Non-biting midges	<i>Heterotrissocladius changi</i>	P	P
Diptera	Chironomidae	Non-biting midges	<i>Krenosmittia halvorseni</i>	P	
Diptera	Chironomidae	Non-biting midges	<i>Limnophyes pumilio</i>		P
Diptera	Chironomidae	Non-biting midges	<i>Micropsectra logani</i>	P	P
Diptera	Chironomidae	Non-biting midges	<i>Micropsectra penicillata</i>		P
Diptera	Chironomidae	Non-biting midges	<i>Micropsectra polita</i>		P
Diptera	Chironomidae	Non-biting midges	<i>Micropsectra subletteorum</i>		P
Diptera	Chironomidae	Non-biting midges	<i>Paratanytarsus austriacus</i>		P
Diptera	Chironomidae	Non-biting midges	<i>Paratanytarsus dissimilis</i>		P
Diptera	Chironomidae	Non-biting midges	<i>Polypedilum aviceps</i>	P	
Diptera	Chironomidae	Non-biting midges	<i>Tanytarsus anderseni</i>		P
Diptera	Limoniidae	Limoniid crane flies	<i>Dicranomyia halterella</i>		P
Diptera	Limoniidae	Limoniid crane flies	<i>Symplecta cana</i>		P
Diptera	Pipunculidae	Big-headed flies (T)	<i>Cephalops mainensis</i>		P
Diptera	Simuliidae	Black flies	<i>Simulium tuberosum</i>	P	
Diptera	Tipulidae	Crane flies	<i>Tipula abdominalis</i>		P
Enchytraeida	Enchytraeidae	Potworms (T)	<i>Enchytraeus buchholzi</i>	P	
Ephemeroptera	Ameletidae	Combmouthed minnow mayflies	<i>Ameletus subnotatus</i>	P	P
Ephemeroptera	Baetidae	Small minnow mayflies	<i>Baetis bundyae</i>		P

Ephemeroptera	Baetidae	Small minnow mayflies	<i>Baetis phoebus</i>	P	
Ephemeroptera	Baetidae	Small minnow mayflies	<i>Baetis tricaudatus</i>		P
Ephemeroptera	Baetidae	Small minnow mayflies	<i>Baetis vernus</i>	P	
Ephemeroptera	Baetidae	Small minnow mayflies	<i>Callibaetis ferrugineus</i>		P
Ephemeroptera	Baetidae	Small minnow mayflies	<i>Centroptilum bifurcatum</i>		P
Ephemeroptera	Baetidae	Small minnow mayflies	<i>Proclleon pennulatum</i>	P	P
Ephemeroptera	Heptageniidae	Flat-headed mayflies	<i>Ecdyonurus criddlei</i>		P
Ephemeroptera	Heptageniidae	Flat-headed mayflies	<i>Epeorus grandis</i>		P
Ephemeroptera	Heptageniidae	Flat-headed mayflies	<i>Heptagenia pulla</i>	P	P
Ephemeroptera	Heptageniidae	Flat-headed mayflies	<i>Leucrocuta minerva</i>	P	P
Ephemeroptera	Heptageniidae	Flat-headed mayflies	<i>Rhithrogena impersonata</i>		P
Ephemeroptera	Leptophlebiidae	Prong-gilled mayflies	<i>Leptophlebia cupida</i>		P
Ephemeroptera	Leptophlebiidae	Prong-gilled mayflies	<i>Leptophlebia nebulosa</i>	P	P
Ephemeroptera	Leptophlebiidae	Prong-gilled mayflies	<i>Paraleptophlebia heteronea</i>	P	P
Ephemeroptera	Leptophlebiidae	Prong-gilled mayflies	<i>Paraleptophlebia memorialis</i>		P
Ephemeroptera	Metropodidae	Cleftfooted minnow mayflies	<i>Siphloplecton basale</i>	P	P
Haplotaenida	Lumbricidae	Earthworms (T)	<i>Dendrobaena octaedra</i>	P	
Haplotaenida	Naididae	Oligochaete worms	<i>Limnodrilus claparedianus</i>		P
Haplotaenida	Naididae	Oligochaete worms	<i>Nais communis</i>		P
Haplotaenida	Naididae	Oligochaete worms	<i>Nais variabilis</i>	P	P
Hemiptera	Corixidae	Water boatmen	<i>Callicorixa alaskensis</i>		P
Hemiptera	Corixidae	Water boatmen	<i>Callicorixa audeni</i>		P
Hemiptera	Corixidae	Water boatmen	<i>Hesperocorixa atopodonta</i>		P
Hemiptera	Corixidae	Water boatmen	<i>Sigara bicoloripennis</i>		P
Hemiptera	Corixidae	Water boatmen	<i>Sigara solensis</i>		P
Hemiptera	Delphacidae	Plant hoppers (T)	<i>Delphacodes kilmani</i>		P
Hemiptera	Nabidae	Damsel bugs (T)	<i>Nabis americanoferus</i>		P

Hymenoptera	Formicidae	Ants (T)	<i>Formica subaenescens</i>		P
Odonata	Corduliidae	Emerald dragonflies	<i>Somatochlora minor</i>		P
Plecoptera	Capniidae	Small winter stoneflies	<i>Capnia confusa</i>		P
Plecoptera	Capniidae	Small winter stoneflies	<i>Utacapnia columbiana</i>	P	P
Plecoptera	Capniidae	Small winter stoneflies	<i>Utacapnia logana</i>	P	P
Plecoptera	Capniidae	Small winter stoneflies	<i>Utacapnia trava</i>		P
Plecoptera	Chloroperlidae	Green stoneflies	<i>Haploperla brevis</i>		P
Plecoptera	Chloroperlidae	Green stoneflies	<i>Triznaka signata</i>		P
Plecoptera	Nemouridae	Spring stoneflies	<i>Zapada columbiana</i>		P
Plecoptera	Pteronarcyidae	Giant stoneflies	<i>Pteronarcys dorsata</i>		P
Plecoptera	Pteronarcyidae	Giant stoneflies	<i>Pteronarcys princeps</i>		P
Plectida	Plectidae	Worms	<i>Plectus aquatilis</i>		P
Podocopida	Candonidae	Freshwater ostracods	<i>Candona candida</i>		P
Thysanoptera	Phlaeothripidae	Thrips (T)	<i>Haplothrips leucanthemi</i>		P
Trichoptera	Brachycentridae	Humpless casemaker caddisflies	<i>Brachycentrus americanus</i>		P
Trichoptera	Hydropsychidae	Net-spinning caddisflies	<i>Arctopsyche ladogensis</i>	P	
Trichoptera	Hydropsychidae	Net-spinning caddisflies	<i>Ceratopsyche cockerelli</i>		P
Trichoptera	Hydropsychidae	Net-spinning caddisflies	<i>Cheumatopsyche wrighti</i>	P	
Trichoptera	Hydropsychidae	Net-spinning caddisflies	<i>Hydropsyche bronta</i>	P	
Trichoptera	Limnephilidae	Tube-case caddisflies	<i>Hesperophylax designatus</i>		P
Trichoptera	Limnephilidae	Tube-case caddisflies	<i>Onocosmoecus unicolor</i>		P
Trichoptera	Limnephilidae	Tube-case caddisflies	<i>Pycnopsyche guttifer</i>		P
Trichoptera	Phryganeidae	Giant casemaker caddisflies	<i>Agrypnia improba</i>		P
Trichoptera	Phryganeidae	Giant casemaker caddisflies	<i>Ptilostomis semifasciata</i>	P	P
Gastropoda	Lymnaeidae	Pond snails	<i>Galba schirazensis</i>	P	

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5. APPENDICES

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

Site	No. Replicates	River/Tributary	Year collected	Latitude	Longitude
BLU01	3	Blueberry River (1)	2019	56.749	-121.714
BLU01	3	Blueberry River (1)	2020	56.749	-121.714

6. 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 macroinvertebrates 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.
Normalizing	The process of rarefying samples down to the smallest library size - a common practice in DNA metabarcoding methods.
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.