Preliminary DNA Data

Upper Athabasca River, AB

Athabasca Watershed Council

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Photo: Erik Lizee - McLeod River, Alberta Public Domain

www.STREAM-DNA.com

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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).

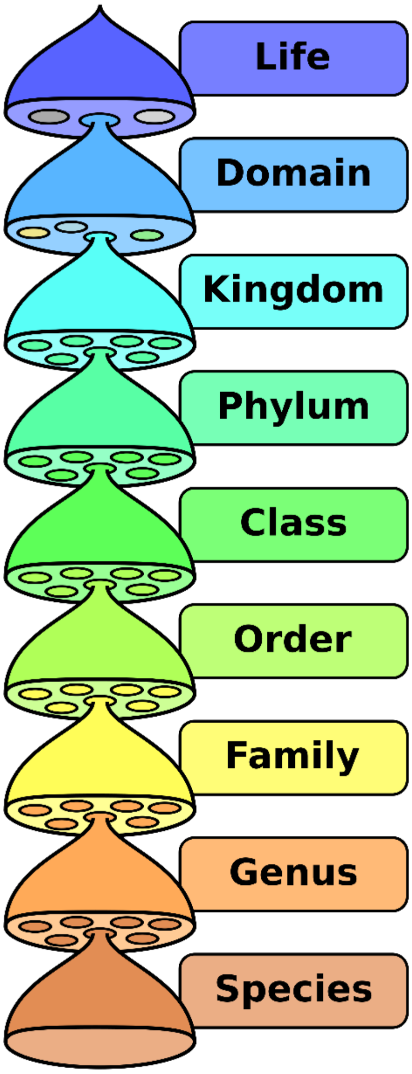

Figure 1. Graphical representation the classification of organisms.

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.

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.

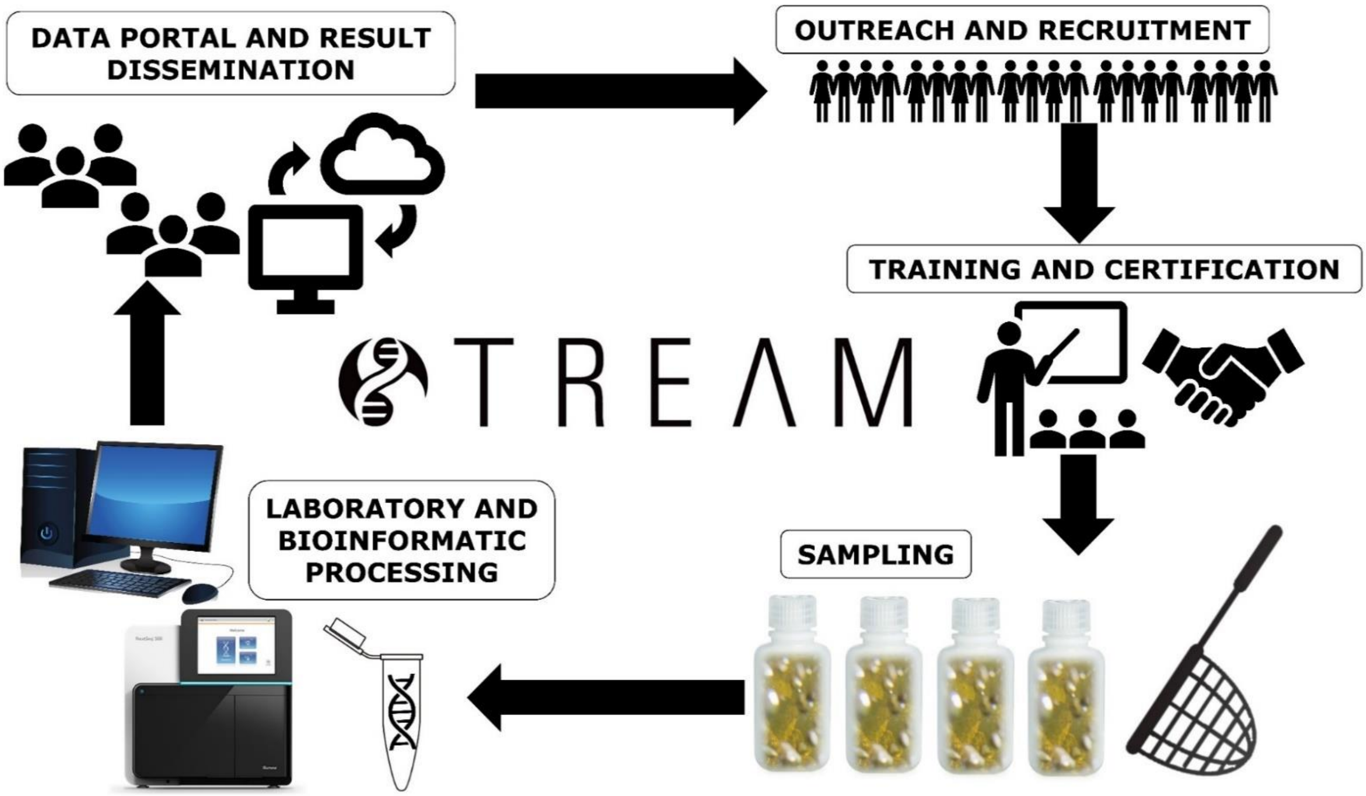

Figure 2. Graphical representation of the STREAM feedback loop for DNA biomonitoring of benthic invertebrates.

1.3. Objective of Report

Data and information included in this report is a first and preliminary examination of results from the Upper Athabasca, AB, 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

The AWC and its partners aim to understand and respond to shifts in benthic macroinvertebrate communities in the Upper Athabasca Watershed.

2. METHODOLOGY

2.1. Study Area

In October 2021 sampling was conducted in triplicate at four locations within the Upper Athabasca watershed (Alberta; refer to map). Sampling for benthic macroinvertebrate monitoring with STREAM was lead by members of the Athabasca Watershed Council.

Additional site information, including coordinates is provided in Appendix A.

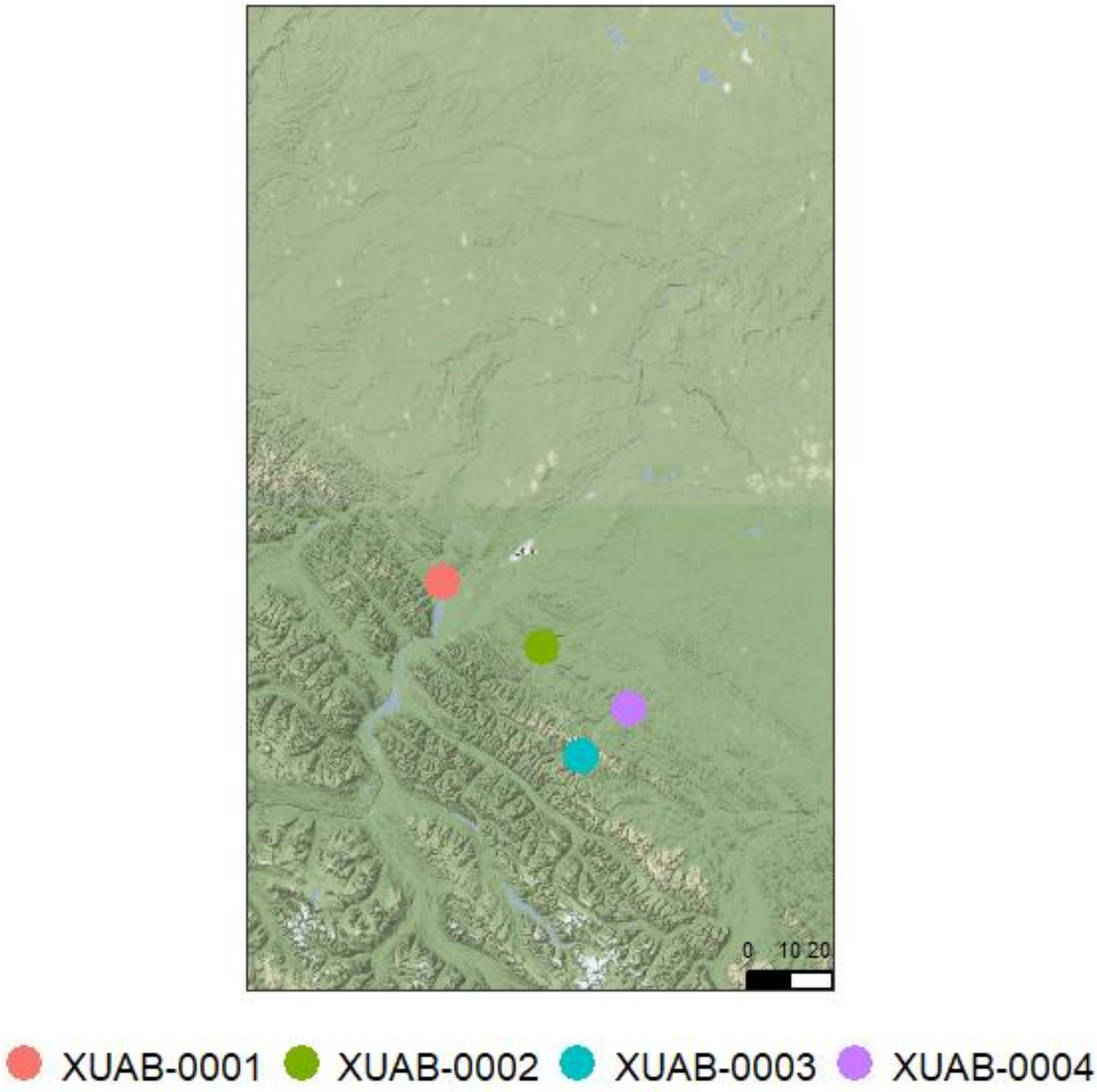

Figure 3. Map of sampling location within the Upper Athabasca watershed, Alberta.

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, preserved in Absolut Zero antifreeze (Propylene Glycol), and stored in freezers at -20°C in the lab until they could be processed.

2.2.3. Laboratory Methods

Benthic samples were preserved in propylene glycol and stored at -20°C until processing. Benthic samples were coarsely homogenized in a sterile blender and DNA was extracted using a DNeasy® PowerSoil Pro® 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 (v1.9.6) pipeline: https://github.com/terrimporter/MetaWorks.

3. RESULTS

3.1. Overview

The raw data output from NGS produced sequences for a range of invertebrate taxa. This taxa list was reduced to only sequences that identified as macroinvertebrates associated with freshwater and riparian ecosystems, and that were of high enough quality to match reference sequences. These results consisted of 105 Orders, 80 Families, 117 Genera, and 127 Species of macroinvertebrates. Across all 4 sites, species richness (number of species present) ranged from 29 (UAB-03A) to 51 (UAB-01A) in (**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.

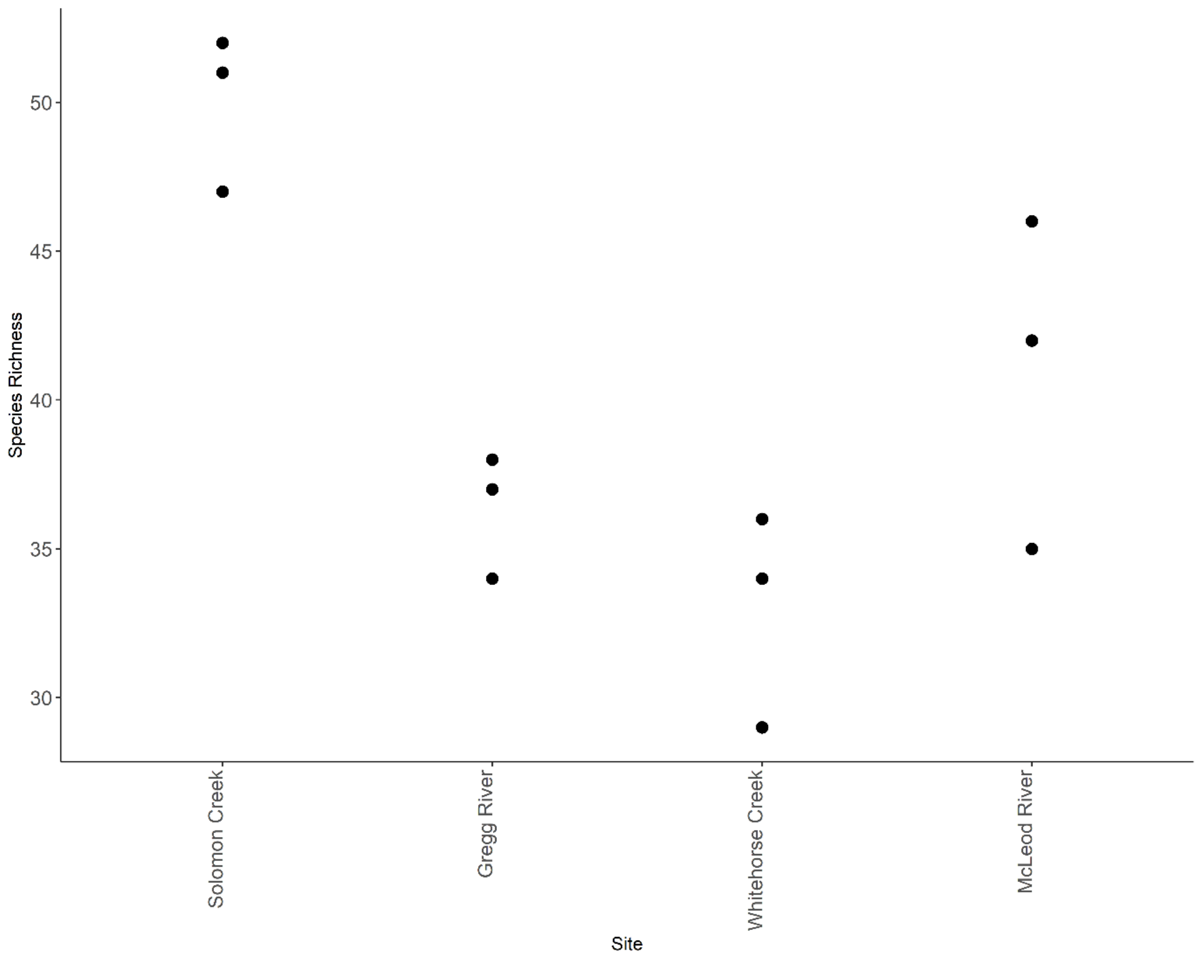

Figure 4. Species richness of each site sampled. Only species taxonomically assigned with high confidence (bootstrap support >= 0.70) are included. Species richness was determined from normalized sequence data (see glossary).

3.2. Taxonomic Coverage

A range of macroinvertebrate species were detected across the sites. Traditional bioindicator EPT species were detected in all sites, including 18 species of

Ephemeroptera (mayflies), 32 Plecoptera (stoneflies) and 16 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).

Table 1 (next page). List of EPT taxa identified to the species level. P = present. Grey cells indicate absence. Only species taxonomically assigned with high confidence (bootstrap support >= 0.70) are included. Replicates pooled.

Order	Family	Common Name	Species	UAB-1	UAB-2	UAB-3	UAB-4
Ephemeroptera	Ameletidae	Comb-mouthed minnow mayflies	*Ameletus celer*	P	P	P	P
Ephemeroptera	Baetidae	Small minnow mayflies	*Acentrella turbida*	P		P	P
Ephemeroptera	Baetidae	Small minnow mayflies	*Baetis bicaudatus*	P	P	P	P
Ephemeroptera	Baetidae	Small minnow mayflies	*Baetis tricaudatus*	P	P	P	P
Ephemeroptera	Baetidae	Small minnow mayflies	*Diphetor hageni*	P	P		
Ephemeroptera	Caenidae	Small square-gilled mayflies	*Caenis amica*		P		
Ephemeroptera	Caenidae	Small square-gilled mayflies	*Caenis diminuta*	P			
Ephemeroptera	Ephemerellidae	Spiny crawler mayflies	*Drunella coloradensis*	P	P	P	P
Ephemeroptera	Ephemerellidae	Spiny crawler mayflies	*Drunella doddsii*	P	P	P	P
Ephemeroptera	Ephemerellidae	Spiny crawler mayflies	*Drunella grandis*	P	P		P
Ephemeroptera	Ephemerellidae	Spiny crawler mayflies	*Ephemerella tibialis*	P			
Ephemeroptera	Heptageniidae	Flat-headed mayflies	*Cinygmula spJMW3*		P	P	P
Ephemeroptera	Heptageniidae	Flat-headed mayflies	*Cinygmula subaequalis*	P			
Ephemeroptera	Heptageniidae	Flat-headed mayflies	*Epeorus deceptivus*		P	P	P
Ephemeroptera	Heptageniidae	Flat-headed mayflies	*Epeorus grandis*			P	
Ephemeroptera	Heptageniidae	Flat-headed mayflies	*Epeorus longimanus*		P		P
Ephemeroptera	Heptageniidae	Flat-headed mayflies	*Rhithrogena robusta*	P	P	P	P
Ephemeroptera	Leptophlebiidae	Prong-gilled mayflies	*Paraleptophlebia heteronea*	P	P		P
Plecoptera	Capniidae	Small winter stoneflies	*Capnia coloradensis*		P		P
Plecoptera	Capniidae	Small winter stoneflies	*Capnia gracilaria*	P	P		
Plecoptera	Capniidae	Small winter stoneflies	*Capnia petila*	P		P	
Plecoptera	Capniidae	Small winter stoneflies	*Eucapnopsis brevicauda*		P		P
Plecoptera	Capniidae	Small winter stoneflies	*Utacapnia columbiana*	P			
Plecoptera	Capniidae	Small winter stoneflies	*Utacapnia logana*		P		
Plecoptera	Chloroperlidae	Green stoneflies	*Alloperla serrata*			P	P
Plecoptera	Chloroperlidae	Green stoneflies	*Alloperla severa*				P
Plecoptera	Chloroperlidae	Green stoneflies	*Paraperla frontalis*				P
Plecoptera	Chloroperlidae	Green stoneflies	*Plumiperla diversa*	P		P	P
Plecoptera	Chloroperlidae	Green stoneflies	*Suwallia teleckojensis*				P
Plecoptera	Chloroperlidae	Green stoneflies	*Sweltsa borealis*	P	P		P
Plecoptera	Chloroperlidae	Green stoneflies	*Sweltsa coloradensis*	P	P	P	P
Plecoptera	Chloroperlidae	Green stoneflies	*Triznaka signata*				P
Plecoptera	Leuctridae	Rolled-winged stoneflies	*Paraleuctra occidentalis*		P		P
Plecoptera	Nemouridae	Spring stoneflies	*Amphinemura linda*	P			
Plecoptera	Nemouridae	Spring stoneflies	*Podmosta delicatula*				P
Plecoptera	Nemouridae	Spring stoneflies	*Prostoia besametsa*	P	P	P	P
Plecoptera	Nemouridae	Spring stoneflies	*Visoka cataractae*		P		P
Plecoptera	Nemouridae	Spring stoneflies	*Zapada cinctipes*	P	P	P	P
Plecoptera	Nemouridae	Spring stoneflies	*Zapada columbiana*	P	P	P	P
Plecoptera	Nemouridae	Spring stoneflies	*Zapada haysi*	P	P	P	P
Plecoptera	Perlidae	Common stoneflies	*Hesperoperla pacifica*	P	P	P	P
Plecoptera	Perlodidae	Springflies	*Isoperla petersoni*	P	P		P
Plecoptera	Perlodidae	Springflies	*Isoperla sobria*	P	P	P	P
Plecoptera	Perlodidae	Springflies	*Kogotus modestus*	P	P	P	P
Plecoptera	Perlodidae	Springflies	*Megarcys signata*			P	P
Plecoptera	Perlodidae	Springflies	*Megarcys subtruncata*			P	P
Plecoptera	Perlodidae	Springflies	*Megarcys watertoni*			P	
Plecoptera	Taeniopterygidae	Winter stoneflies	*Doddsia occidentalis*	P	P	P	P
Plecoptera	Taeniopterygidae	Winter stoneflies	*Taenionema pacificum*	P			
Plecoptera	Taeniopterygidae	Winter stoneflies	*Taenionema pallidum*	P	P	P	P
Trichoptera	Brachycentridae	Humpless casemaker caddisflies	*Brachycentrus americanus*	P	P		P
Trichoptera	Brachycentridae	Humpless casemaker caddisflies	*Micrasema bactro*				P
Trichoptera	Hydropsychidae	Net-spinning caddisflies	*Arctopsyche grandis*		P		P
Trichoptera	Hydropsychidae	Net-spinning caddisflies	*Arctopsyche inermis*		P		
Trichoptera	Hydropsychidae	Net-spinning caddisflies	*Ceratopsyche cockerelli*	P			
Trichoptera	Hydropsychidae	Net-spinning caddisflies	*Cheumatopsyche analis*			P	
Trichoptera	Hydropsychidae	Net-spinning caddisflies	*Parapsyche elsis*	P		P	
Trichoptera	Lepidostomatidae	Bizarre caddisflies	*Lepidostoma cascadense*		P		
Trichoptera	Lepidostomatidae	Bizarre caddisflies	*Lepidostoma pluviale*				P
Trichoptera	Lepidostomatidae	Bizarre caddisflies	*Lepidostoma roafi*				P
Trichoptera	Limnephilidae	Northern caddisflies	*Onocosmoecus unicolor*	P			
Trichoptera	Limnephilidae	Northern caddisflies	*Psychoglypha alascensis*	P			
Trichoptera	Limnephilidae	Northern caddisflies	*Psychoglypha subborealis*	P			
Trichoptera	Rhyacophilidae	Free-living caddisflies	*Rhyacophila brunnea*	P	P		P
Trichoptera	Rhyacophilidae	Free-living caddisflies	*Rhyacophila hyalinata*			P	
Trichoptera	Rhyacophilidae	Free-living caddisflies	*Rhyacophila vaccua*			P	

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5. APPENDICES

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

Site	River	Latitude	Longitude
UAB-0001	Solomon Creek	53.340625	-117.836024
UAB-0002	Gregg River	53.206458	-117.493302
UAB-0003	Whitehorse Creek	52.984855	-117.360807
UAB-0004	McLeod River	53.080206	-117.198929

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 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.