Preliminary DNA Data

Liard River, BC

December 2019

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STREAM

www.STREAM-DNA.com

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Environment and Climate Change Canada

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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).

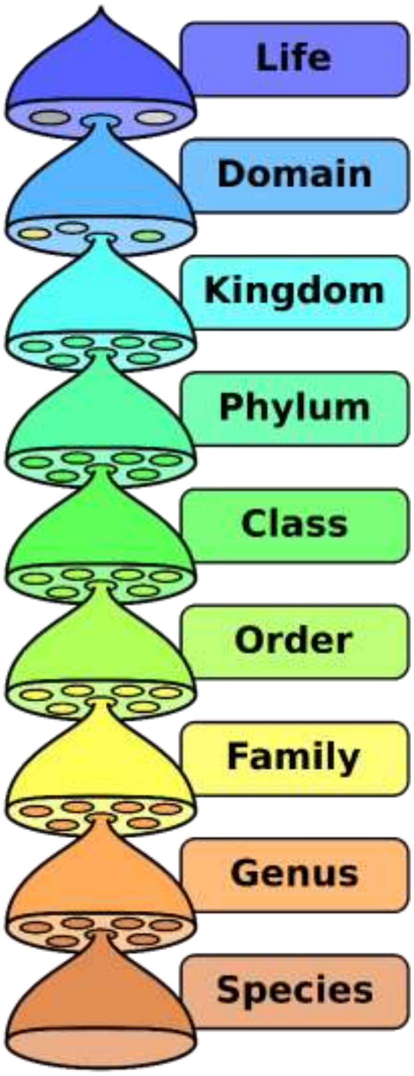

Figure 1. Graphical representation the classification of organisms.

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.

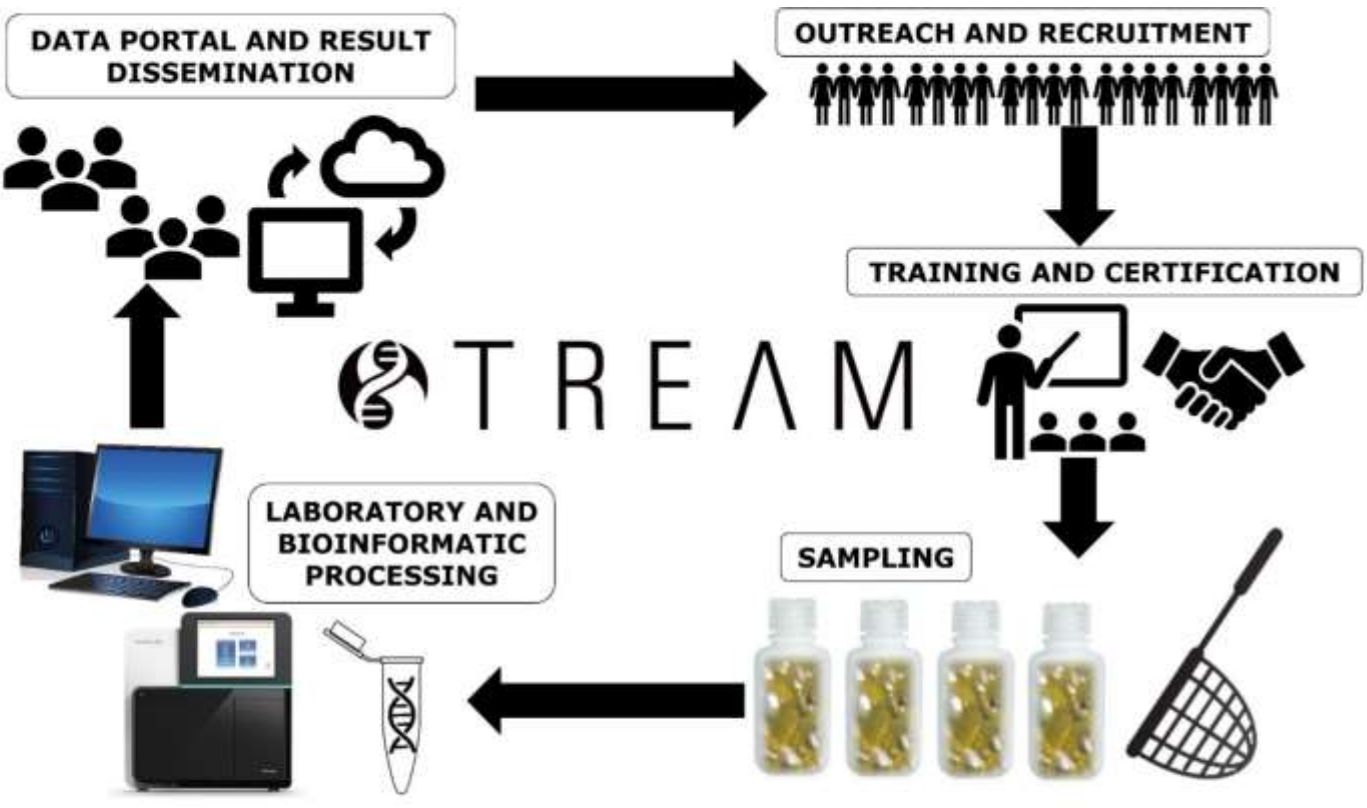

Figure 2. Graphical representation of the STREAM feedback loop for DNA biomonitoring of benthic invertebrates.

1.3. Objective of Report

Data and information included in this report is a first and preliminary examination of results from the Liard River (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

The 'Liard Community-based biomonitoring and eDNA Project' aims to simultaneously build upon the existing Dane Nan Yedah environmental monitoring program to include water quality and benthic invertebrate monitoring, ensuring that there is sufficient baseline data to inform future development decisions. At the same time, this collaboration will further the efforts to create a network of Guardians programs using DNA technology and contributing to a better national understanding of the health of Canada's freshwater ecosystems.

2. METHODOLOGY

2.1. Study Area

In September 2018, this study was conducted across five pre-determined sampling locations within the Liard River Basin (British Columbia; Figure 3). Sampling was conducted by Kaska Land Guardians, for the first year of the annual benthic macroinvertebrate monitoring with STREAM.

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

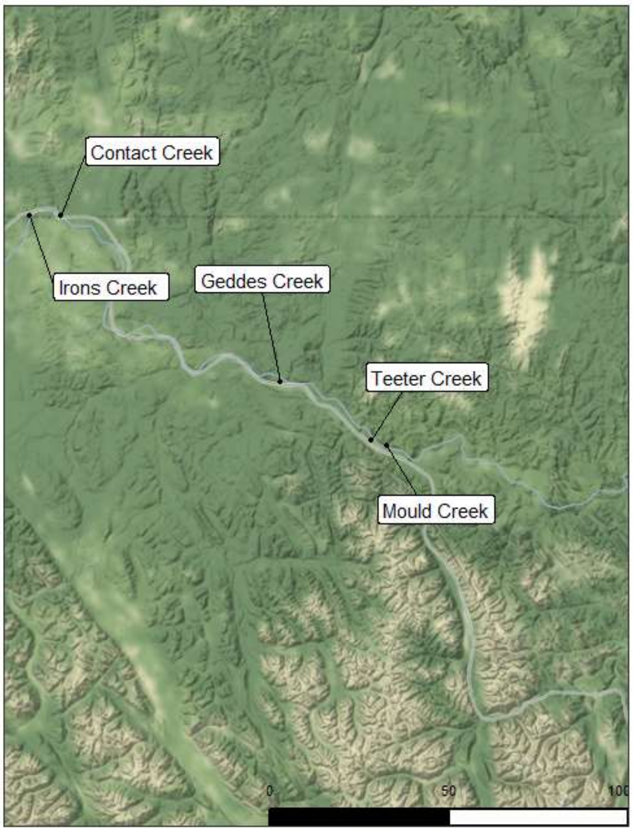

Figure 3. Map of sampling locations within the Liard River Basin, BC.

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 90% Ethanol, 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 90% 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).

3. RESULTS

3.1. Overview

The raw data output from NGS produced sequences for a range of taxa, including vertebrates such as bird and human. 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 21 Orders, 44 Families, 69 Genera, and 84 species of macroinvertebrates. Across all five sites, species richness (number of species present) ranged from 21 in Contact Creek and Geddes Creek to 30 in Irons Creek (**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.

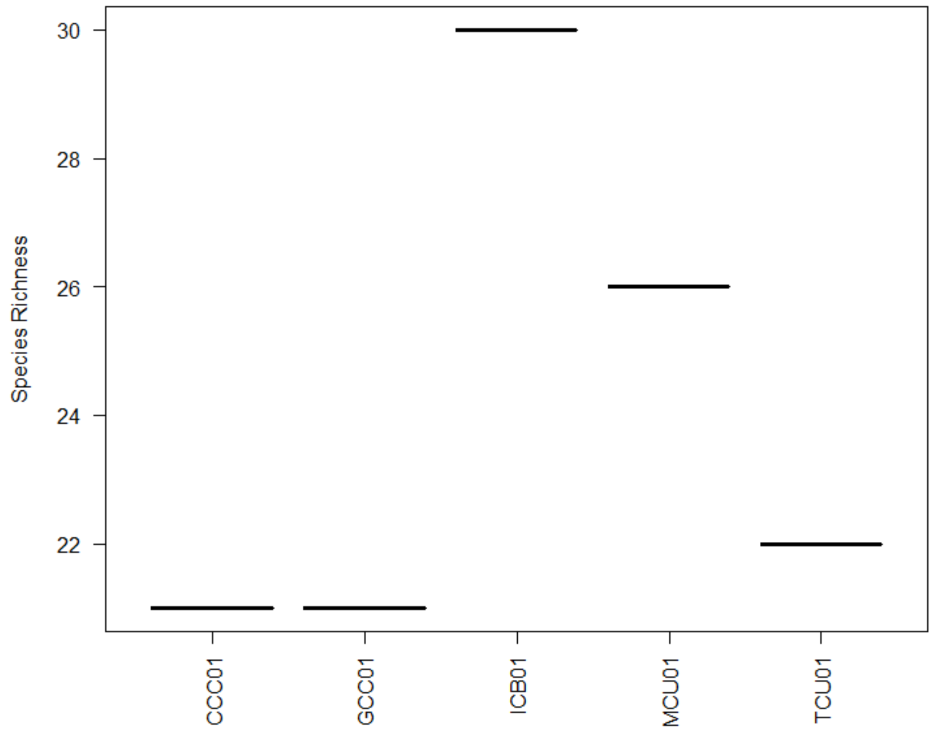

Figure 4. Species richness of each site sampled. Only species taxonomically assigned with high confidence (bootstrap support >= 0.70) are included. Contact Creek (CCC01) = 21 taxa, Geddes Creek (GCC01) = 21 taxa, Irons Creek (ICB01) = 30 taxa, Mould Creek (MCU01) = 26 taxa, Teeter Creek (TCU01) = 22 taxa.

3.2. Taxonomic Coverage

A range of macroinvertebrate species were detected across the five sites. Traditional bioindicator EPT species were detected in all five sites, 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). Within these five sites, some EPT species such as *Baetis bicaudatus* (small minnow mayfly) and *Zapada cinctipes* (common forestfly), were detected across all five sites, whereas *Arctopsyche grandis* (net-spinning caddisfly) and *Paraleptophlebia heteronea* (blue quill mayfly) were only detected in one site.

Table 1. List of macroinvertebrates identified to the species level. P = present. Grey cells indicate absence. Highlighted in blue are the traditional EPT bioindicator orders present. Only species taxonomically assigned with high confidence (bootstrap support >= 0.70) are included. Site 1 = Contact Creek; Site 2 = Geddes Creek; Site 3 = Irons Creek; Site 4 = Mould Creek; Site 5 = Teeter Creek.

Taxa				Sites				
Order	**Species**	**Common name**	**Aquatic?**	**1**	**2**	**3**	**4**	**5**
Coleoptera	*Optioservus ovalis*	Riffle beetles	Yes				P	
Coleoptera	*Stenelmis crenata*	Riffle beetles	Yes	P				P
Coleoptera	*Atheta stercoris*	Rove beetles	No		P			
Diptera	*Eukiefferiella claripennis*	Non-biting midges	Yes			P	P	P
Diptera	*Micropsectra lacustris*	Non-biting midges	Yes			P	P	
Diptera	*Orthocladius dorenus*	Non-biting midges	Yes			P		
Diptera	*Orthocladius mallochi*	Non-biting midges	Yes	P			P	
Diptera	*Orthocladius oliveri*	Non-biting midges	Yes				P	
Diptera	*Pagastia orientalis*	Non-biting midges	Yes	P				
Diptera	*Pagastia orthogonia*	Non-biting midges	Yes				P	
Diptera	*Paratanytarsus laccophilus*	Black flies	Yes			P		
Diptera	*Rheotanytarsus pentapoda*	Black flies	Yes			P		
Diptera	*Helodon pleuralis*	Black flies	Yes			P		
Diptera	*Simulium arcticum*	Black flies	Yes			P		
Diptera	*Simulium argus*	Black flies	Yes					P
Diptera	*Simulium chromatinum*	Black flies	Yes			P		P
Diptera	*Simulium saxosum*	Black flies	Yes			P		
Diptera	*Heringia coxalis*	Hoverflies	No				P	
Ephemeroptera	*Ameletus celer*	Mayflies	Yes				P	
Ephemeroptera	*Baetis bicaudatus*	Mayflies	Yes	P	P	P	P	P
Ephemeroptera	*Baetis flavistriga*	Mayflies	Yes			P		
Ephemeroptera	*Baetis phoebus*	Mayflies	Yes				P	
Ephemeroptera	*Baetis tricaudatus*	Mayflies	Yes	P	P	P		
Ephemeroptera	*Caenis latipennis*	Mayflies	Yes	P		P		
Ephemeroptera	*Drunella coloradensis*	Mayflies	Yes	P	P	P	P	P
Ephemeroptera	*Drunella doddsii*	Mayflies	Yes	P	P	P	P	P
Ephemeroptera	*Drunella grandis*	Mayflies	Yes	P		P		
Ephemeroptera	*Ephemerella dorothea*	Mayflies	Yes	P		P		
Ephemeroptera	*Cinygmula kootenai*	Mayflies	Yes			P		
Ephemeroptera	*Cinygmula mimus*	Mayflies	Yes		P	P		
Ephemeroptera	*Epeorus deceptivus*	Mayflies	Yes				P	P
Ephemeroptera	*Epeorus grandis*	Mayflies	Yes		P		P	P
Ephemeroptera	*Epeorus longimanus*	Mayflies	Yes		P	P		
Ephemeroptera	*Rhithrogena robusta*	Mayflies	Yes	P			P	P
Ephemeroptera	*Paraleptophlebia heteronea*	Mayflies	Yes			P		

Order	Species	Common name	Aquatic?	1	2	3	4	5
Haplotaxida	*Dendrodrilus rubidus*	Worms	No		P			
Haplotaxida	*Eiseniella tetraedra*	Worms	Yes				P	
Haplotaxida	*Chaetogaster diastrophus*	Worms	Yes		P	P		
Haplotaxida	*Nais bretscheri*	Worms	Yes			P		P
Hemiptera	*Chaitophorus populifolii*	Aphids	No				P	
Hemiptera	*Euceraphis gillettei*	True bugs	No				P	P
Hymenoptera	*Vespula alascensis*	Wasps	No				P	
Lepidoptera	*Phyllocnistis populiella*	Aspen leaf miner	No				P	
Plecoptera	*Capnia coloradensis*	Stoneflies	Yes		P	P	P	
Plecoptera	*Capnia petila*	Stoneflies	Yes				P	
Plecoptera	*Eucapnopsis brevicauda*	Stoneflies	Yes			P		
Plecoptera	*Utacapnia columbiana*	Stoneflies	Yes			P	P	
Plecoptera	*Alloperla serrata*	Stoneflies	Yes				P	P
Plecoptera	*Plumiperla diversa*	Stoneflies	Yes			P		P
Plecoptera	*Sweltsa borealis*	Stoneflies	Yes	P		P		
Plecoptera	*Sweltsa coloradensis*	Stoneflies	Yes			P	P	
Plecoptera	*Paraleuctra occidentalis*	Stoneflies	Yes			P		
Plecoptera	*Prostoia besametsa*	Stoneflies	Yes			P		
Plecoptera	*Zapada cinctipes*	Stoneflies	Yes	P	P	P	P	P
Plecoptera	*Zapada columbiana*	Stoneflies	Yes	P	P		P	P
Plecoptera	*Zapada haysi*	Stoneflies	Yes				P	P
Plecoptera	*Zapada oregonensis*	Stoneflies	Yes			P		P
Plecoptera	*Isoperla petersoni*	Stoneflies	Yes		P	P		P
Plecoptera	*Isoperla sobria*	Stoneflies	Yes					P
Plecoptera	*Megarcys signata*	Stoneflies	Yes	P	P	P	P	P
Plecoptera	*Taenionema pacificum*	Stoneflies	Yes		P	P		
Plecoptera	*Taenionema pallidum*	Stoneflies	Yes	P	P	P	P	P
Trichoptera	*Apatania comosa*	Caddisflies	Yes			P		
Trichoptera	*Apatania sorex*	Caddisflies	Yes			P		
Trichoptera	*Brachycentrus americanus*	Caddisflies	Yes		P	P		P
Trichoptera	*Micrasema bactro*	Caddisflies	Yes			P		
Trichoptera	*Glossosoma alascense*	Caddisflies	Yes		P	P		
Trichoptera	*Glossosoma intermedium*	Caddisflies	Yes		P	P	P	
Trichoptera	*Glossosoma verdonum*	Caddisflies	Yes	P			P	
Trichoptera	*Arctopsyche grandis*	Caddisflies	Yes			P		
Trichoptera	*Hydropsyche betteni*	Caddisflies	Yes	P			P	P
Trichoptera	*Parapsyche elsis*	Caddisflies	Yes	P	P		P	P
Trichoptera	*Stactobiella delira*	Caddisflies	Yes			P		
Trichoptera	*Lepidostoma unicolor*	Caddisflies	Yes			P		
Trichoptera	*Chyrandra centralis*	Caddisflies	Yes				P	
Trichoptera	*Ecclisomyia conspersa*	Caddisflies	Yes					P
Trichoptera	*Onocosmoecus unicolor*	Caddisflies	Yes				P	
Trichoptera	*Rhyacophila bifila*	Caddisflies	Yes			P		
Trichoptera	*Rhyacophila brunnea*	Caddisflies	Yes	P		P		P

Order	Species	Common name		1	2	3	4	5
Trichoptera	*Rhyacophila harmstoni*	Caddisflies	Yes				P	P
Trichoptera	*Rhyacophila narvae*	Caddisflies	Yes	P	P			
Trichoptera	*Rhyacophila pellisa*	Caddisflies	Yes			P		
Trichoptera	*Rhyacophila vocala*	Caddisflies	Yes				P	P
Trombidiformes	*Testudacarus minimus*	Mites	No			P		

Figure 5. Some examples of the EPT taxa detected. Top left: *Arctopsyche grandis* - a species of net-spinning caddisfly (Trichoptera) in the family Hydropsychidae. Top right: *Megarcys* - a genus of springflies (Plecoptera) in the family Perlodidae. Bottom left: *Brachycentrus* - a genus of humpless casemaker caddisflies (Trichoptera) in the family Brachycentridae. Bottom right: *Ameletus* - genus of mayfly (Ephemeroptera) in the family Ameletidae. All photos: ©CABIN Taxonomy.

4. FUTURE SUGGESTIONS

As there is currently only one collected sample per site, for future sampling it would be beneficial to sample in triplicate, to ensure results observed are consistent across samples.

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6. APPENDICES

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

Site	Site Name	River	Province	No. Samples Collected	Date of Collection (MM/DD/YYYY)	Latitude	Longitude	CABIN Status
1	Contact Creek	Liard	BC	1	09/26/2018	59.99928	-127.726	Potential Reference
2	Geddes Creek	Liard	BC	1	09/26/2018	59.59694	-126.671	Test
3	Irons Creek	Liard	BC	1	09/24/2018	59.99806	-127.877	Test
4	Mould Creek	Liard	BC	1	09/26/2018	59.44158	-126.156	Potential Reference
5	Teeter Creek	Liard	BC	1	09/26/2018	59.45264	-126.233	Potential Reference

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.