Preliminary DNA Data

Bow River, AB

December 2019

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STREAM

www.STREAM-DNA.com

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WWF Canada

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).

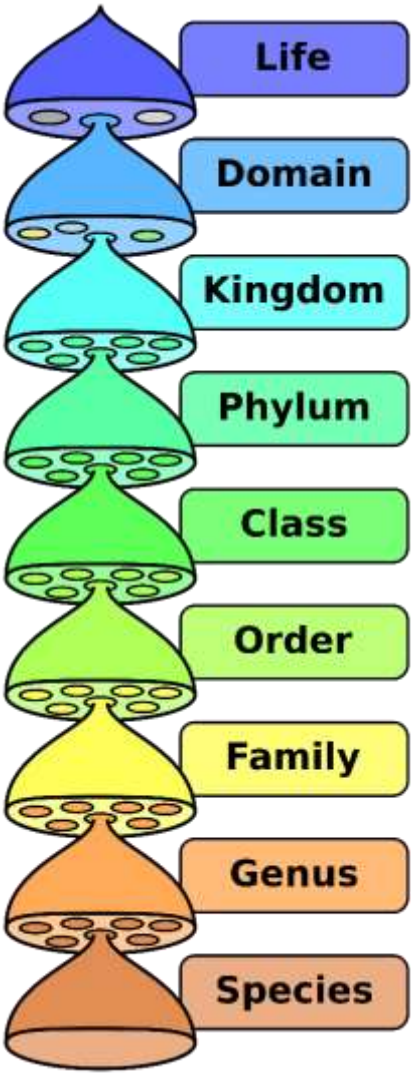

Figure 1. Graphical representation the classification of organisms.

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.

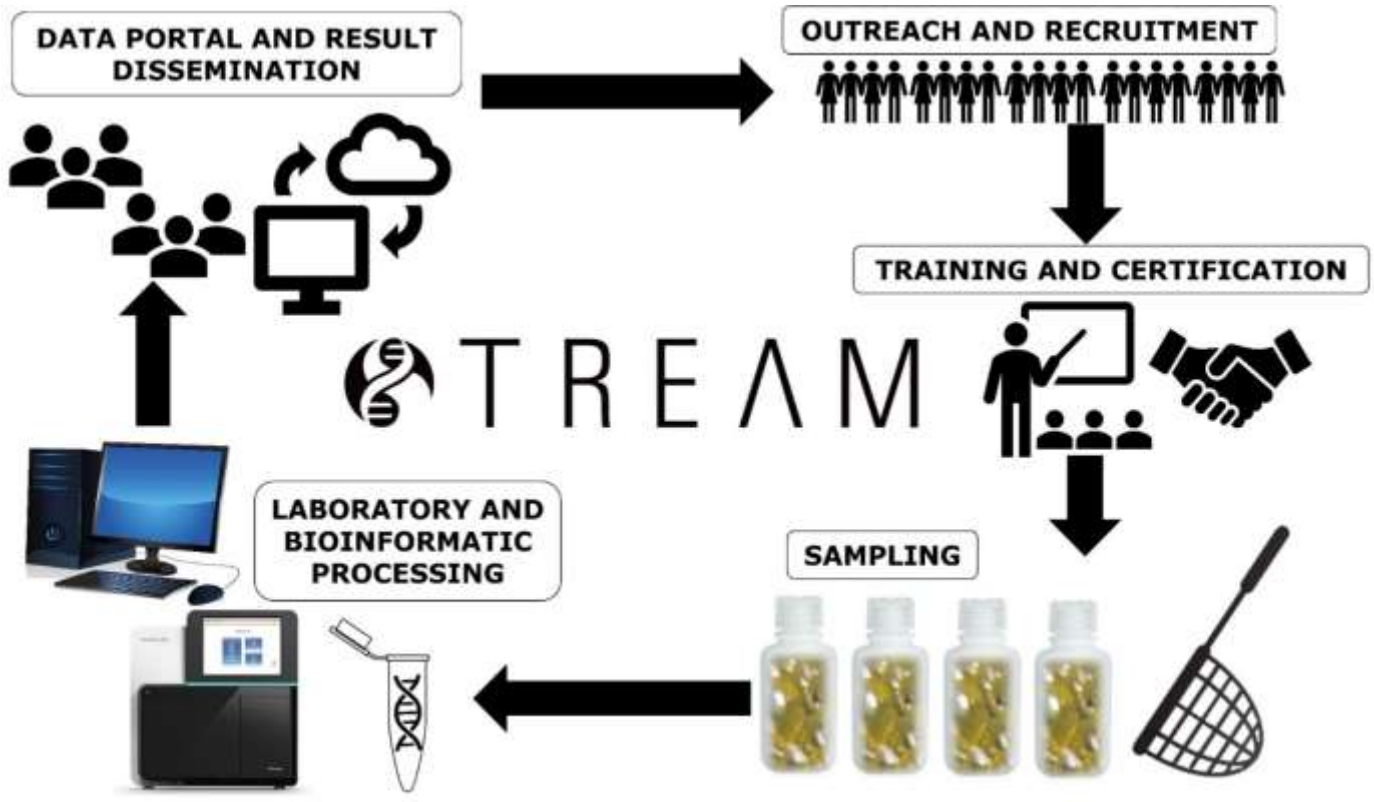

Figure 2. Graphical representation of the STREAM feedback loop for DNA biomonitoring of benthic invertebrates.

1.3. Objective of Report

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

2. METHODOLOGY

2.1. Study Area

In July 2019, this study was conducted across four pre-determined sampling locations within the Bow River Basin (Alberta; **Figure 3**). Sampling was conducted by Ghost Watershed Alliance, Living Lakes Canada and University of Calgary, 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 Bow River, AB.

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 45 Orders, 68 Families, 98 Genera, and 113 species of macroinvertebrates. Across all four sites, species richness (number of species present) ranged from 9 in Bow River (BOW01) to 53 in Bow River A (BOW01A; **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.

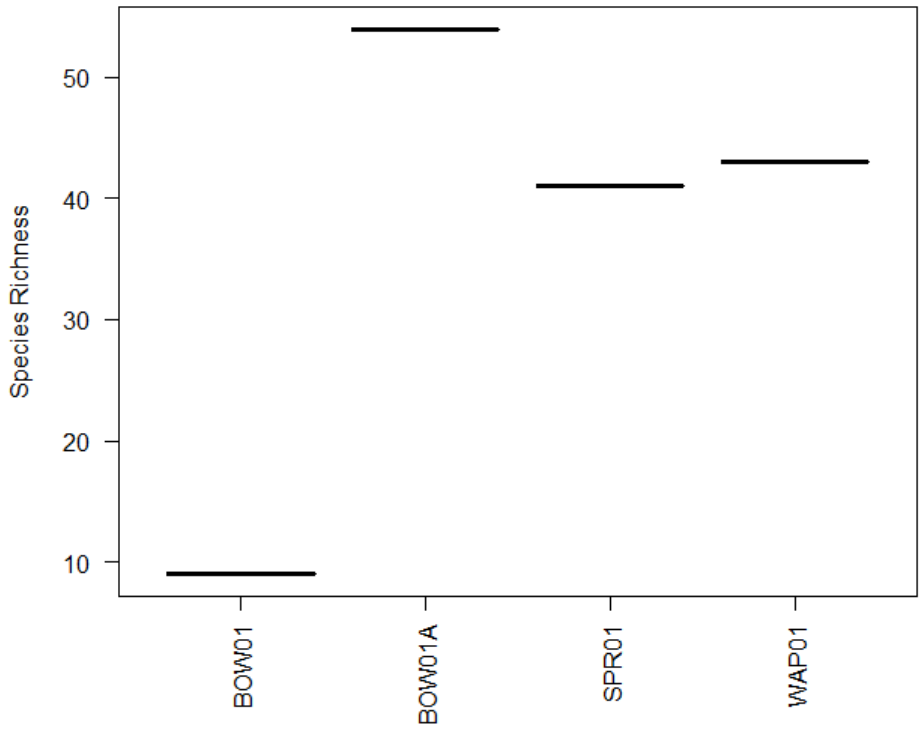

Figure 4. Species richness of each site sampled. Only species taxonomically assigned with high confidence (bootstrap support >= 0.70) are included. Bow River (BOW01) = 9 taxa, Bow River A (BOW01A) = 53 taxa, Spray Basin (SPR01) = 41 taxa, Waiparous Creek (WAP01) = 43 taxa.

3.2. Taxonomic Coverage

A range of macroinvertebrate species were detected across the four sites. Site BOW01A is known to be classified as 'Red' for Whirling Disease, which is a disease caused by *Myxobolus cerebralis*, a microscopic parasite that affects salmonid fish such as trout, salmon and whitefish (Gilbert and Granath, 2003). *M. cerebralis* requires a specific aquatic oligochaete (worm) intermediate host, *Tubifex tubifex* (sludge worm). This species is most commonly associated with poor-quality, eutrophic conditions (Gilbert and Granath, 2003), and the fact this species was

detected exclusively at BOW01A for *T. tubifex* (**Table 1**), further supports both the 'Red' Whirling Disease Status.

Additionally, traditional bioindicator EPT species were detected in three sites (excluding BOW01), 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 three sites, some EPT species such as *Ephemerella tibialis* (spiny crawler mayfly) and *Baetis tricaudatus* (small minnow mayfly), were detected across a majority of sites, whereas *Megarcys signata* (springfly) and *Ceratopsyche cockerelli* (net-spinning caddisfly) 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 = Bow Rover (BOW01); Site 2 = Bow River A (BOW01A); Site 3 = Spray Basin (SPR01); Site 4 = Waiparous Creek (WAP01).

Taxa				Sites			
Order	**Species**	**Common Name**	**Aquatic?**	**1**	**2**	**3**	**4**
Adinetida	*Adineta vaga*	Rotifer	Yes			P	P
Coleoptera	*Dichelotarsus obscurevittatus*	Soldier beetles	No				P
Coleoptera	*Podabrus modestus*	Soldier beetles	No	P			
Coleoptera	*Optioservus ovalis*	Riffle beetles	Yes	P	P		
Decapoda	*Orconectes virilis*	Virile crayfish	Yes			P	
Diptera	*Palpomyia nigripes*	Biting midges	Yes			P	
Diptera	*Chironomidae*	Non-biting midges	Yes			P	
Diptera	*Cladotanytarsus amandus*	Non-biting midges	Yes				P
Diptera	*Conchapelopia pallens*	Non-biting midges	Yes		P		
Diptera	*Cricotopus bicinctus*	Non-biting midges	Yes		P		
Diptera	*Cricotopus infuscatus*	Non-biting midges	Yes		P		
Diptera	*Cricotopus triannulatus*	Non-biting midges	Yes		P		
Diptera	*Cricotopus trifascia*	Non-biting midges	Yes		P		
Diptera	*Cyphomella cornea*	Non-biting midges	Yes		P		
Diptera	*Micropsectra logani*	Non-biting midges	Yes		P		
Diptera	*Micropsectra nigripila*	Non-biting midges	Yes		P		
Diptera	*Micropsectra subletteorum*	Non-biting midges	Yes			P	
Diptera	*Microtendipes pedellus*	Non-biting midges	Yes		P		
Diptera	*Orthocladius carlatus*	Non-biting midges	Yes		P		
Diptera	*Orthocladius fuscimanus*	Non-biting midges	Yes		P		
Diptera	*Orthocladius mallochi*	Non-biting midges	Yes			P	
Diptera	*Orthocladius oblidens*	Non-biting midges	Yes		P		
Diptera	*Orthocladius oliveri*	Non-biting midges	Yes	P	P		
Diptera	*Potthastia gaedii*	Non-biting midges	Yes		P		
Diptera	*Rheopelopia ornata*	Non-biting midges	Yes		P		
Diptera	*Stempellinella fimbriata*	Non-biting midges	Yes		P		
Diptera	*Synorthocladius semivirens*	Non-biting midges	Yes		P		
Diptera	*Tanytarsus buckleyi*	Non-biting midges	Yes		P		
Diptera	*Tanytarsus glabrescens*	Non-biting midges	Yes		P		
Diptera	*Tvetenia paucunca*	Non-biting midges	Yes		P		
Diptera	*Ochlerotatus abserratus*	Mosquito	Yes	P			
Diptera	*Lispe tentaculata*	Houseflies	No		P		
Diptera	*Megaselia eccoptomera*	Hump-backed flies	No				P
Diptera	*Rhagio mystaceus*	Snipe flies	No	P			
Diptera	*Prosimulium exigens*	Black flies	Yes				P
Diptera	*Prosimulium travisi*	Black flies	Yes				P

Order	Species	Common Name	Aquatic?	1	2	3	4
Diptera	*Simulium arcticum*	Black flies	Yes				P
Diptera	*Simulium chromatinum*	Black flies	Yes				P
Diptera	*Simulium defoliarti*	Black flies	Yes				P
Diptera	*Simulium hunteri*	Black flies	Yes			P	
Diptera	*Simulium iriartei*	Black flies	Yes			P	
Diptera	*Simulium negativum*	Black flies	Yes				P
Diptera	*Simulium truncatum*	Black flies	Yes				P
Diptera	*Simulium tuberosum*	Black flies	Yes			P	P
Diptera	*Chrysops vittatus*	Horse-flies	Yes		P		
Entomobryomorpha	*Folsomia nivalis*	Springtails	No	P			
Entomobryomorpha	*Isotoma anglicana*	Springtails	No				P
Entomobryomorpha	*Parisotoma ekmani*	Springtails	No	P			
Ephemeroptera	*Ameletus bellulus*	Mayflies	Yes			P	
Ephemeroptera	*Ameletus celer*	Mayflies	Yes			P	P
Ephemeroptera	*Ameletus cooki*	Mayflies	Yes			P	P
Ephemeroptera	*Ameletus pritchardi*	Mayflies	Yes				P
Ephemeroptera	*Baetis bicaudatus*	Mayflies	Yes			P	P
Ephemeroptera	*Baetis phoebus*	Mayflies	Yes		P	P	
Ephemeroptera	*Baetis tricaudatus*	Mayflies	Yes		P	P	P
Ephemeroptera	*Diphetor hageni*	Mayflies	Yes		P	P	P
Ephemeroptera	*Attenella margarita*	Mayflies	Yes		P		
Ephemeroptera	*Drunella coloradensis*	Mayflies	Yes			P	P
Ephemeroptera	*Drunella doddsii*	Mayflies	Yes		P	P	P
Ephemeroptera	*Drunella grandis*	Mayflies	Yes			P	P
Ephemeroptera	*Ephemerella dorothea*	Mayflies	Yes		P		P
Ephemeroptera	*Ephemerella excrucians*	Mayflies	Yes		P	P	
Ephemeroptera	*Ephemerella tibialis*	Mayflies	Yes		P	P	P
Ephemeroptera	*Cinygmula kootenai*	Mayflies	Yes				P
Ephemeroptera	*Cinygmula mimus*	Mayflies	Yes				P
Ephemeroptera	*Cinygmula subaequalis*	Mayflies	Yes				P
Ephemeroptera	*Ecdyonurus simplicioides*	Mayflies	Yes		P		
Ephemeroptera	*Epeorus deceptivus*	Mayflies	Yes			P	P
Ephemeroptera	*Epeorus longimanus*	Mayflies	Yes			P	P
Ephemeroptera	*Heptagenia pulla*	Mayflies	Yes		P		
Ephemeroptera	*Heptagenia solitaria*	Mayflies	Yes		P		
Ephemeroptera	*Rhithrogena robusta*	Mayflies	Yes				P
Ephemeroptera	*Tricorythodes mosegus*	Mayflies	Yes		P		
Ephemeroptera	*Paraleptophlebia debilis*	Mayflies	Yes		P	P	
Ephemeroptera	*Paraleptophlebia heteronea*	Mayflies	Yes		P	P	P
Haplotaxida	*Enchytraeus buchholzi*	Earthworms	No				P
Haplotaxida	*Dendrobaena octaedra*	Earthworms	No			P	
Haplotaxida	*Eiseniella tetraedra*	Earthworms	No		P	P	
Haplotaxida	*Lumbricus rubellus*	Earthworms	No	P			
Haplotaxida	*Octolasion tyrtaeum*	Earthworms	No		P		

Order	Species	Common Name	Aquatic?	1	2	3	4
Haplotaxida	*Branchiura sowerbyi*	Worms	Yes				P
Haplotaxida	*Chaetogaster diastrophus*	Worms	Yes		P		
Haplotaxida	*Limnodrilus hoffmeisteri*	Worms	Yes		P		
Haplotaxida	*Nais bretscheri*	Worms	Yes		P	P	
Haplotaxida	*Nais variabilis*	Worms	Yes		P		
Haplotaxida	*Tubifex tubifex*	Worms	Yes		P		
Haplotaxida	*Haplotaxida 21018-4C*	Worms	Yes		P		
Hemiptera	*Draeculacephala crassicornis*	Leafhopper	No				P
Isopoda	*Armadillidium nasatum*	Woodlice	No	P			
Lumbriculida	*Rhynchelmis elrodi*	Worms	Yes		P		
Parachela	*Diphascon higginsi*	Waterbear	Yes				P
Plecoptera	*Sweltsa borealis*	Stoneflies	Yes			P	P
Plecoptera	*Sweltsa coloradensis*	Stoneflies	Yes		P		P
Plecoptera	*Paraleuctra occidentalis*	Stoneflies	Yes			P	P
Plecoptera	*Prostoia besametsa*	Stoneflies	Yes			P	P
Plecoptera	*Zapada cinctipes*	Stoneflies	Yes			P	P
Plecoptera	*Zapada columbiana*	Stoneflies	Yes			P	
Plecoptera	*Zapada haysi*	Stoneflies	Yes				P
Plecoptera	*Zapada oregonensis*	Stoneflies	Yes			P	
Plecoptera	*Hesperoperla pacifica*	Stoneflies	Yes		P		P
Plecoptera	*Isoperla petersoni*	Stoneflies	Yes			P	
Plecoptera	*Megarcys signata*	Stoneflies	Yes				P
Plecoptera	*Pteronarcys princeps*	Stoneflies	Yes		P		
Plectida	*Plectus aquatilis*	Roundworms	Yes			P	
Trichoptera	*Arctopsyche inermis*	Caddisflies	Yes		P		
Trichoptera	*Ceratopsyche cockerelli*	Caddisflies	Yes		P		
Trichoptera	*Hydropsyche betteni*	Caddisflies	Yes			P	
Trichoptera	*Parapsyche elsis*	Caddisflies	Yes			P	
Trichoptera	*Hydroptila argosa*	Caddisflies	Yes		P		
Trichoptera	*Lepidostoma pluviale*	Caddisflies	Yes		P		
Trichoptera	*Chimarra obscura*	Caddisflies	Yes			P	
Trichoptera	*Rhyacophila vaccua*	Caddisflies	Yes			P	
Trombidiformes	*Testudacarus minimus*	Mites	No			P	

Figure 5. Some examples of the EPT taxa detected in the Liard samples. Top left: a species of net-spinning caddisfly (Trichoptera) in the family Hydropsychidae. Top right: *Megarcys* - a genus of springflies (Plecoptera) in the family Perlodidae. Bottom middle: *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	CABIN CODE	Site Name	Province	No. Samples Collected	Date of Collection (MM/DD/YYYY)	Latitude	Longitude	CABIN Status	Whirling Disease Status
1	BOW01	Bow River	AB	1	07/16/2019	51.1305	-115.4124	Test	N/A
2	BOW01 A	Bow River A	AB	2	07/23/2019	51.1736	-114.4672	Test	Red
3	SPR01	Spray Basin	AB	1	07/18/2019	50.9199	-115.400	Test	N/A
4	WAP01	Waiparous Creek	AB	1	07/18/2019	51.2837	-114.8381	Test	N/A

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.