Preliminary DNA Data

Bighill Creek (Bow River), AB

December 2019

Photos obtained from: https://bighillcreek.ca/photo-gallery/

STREAM

www.STREAM-DNA.com

Hajibabaei Lab, Centre for Biodiversity Genomics, University of Guelph

WWF Canada

Environment and Climate Change Canada

Table of Contents

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).

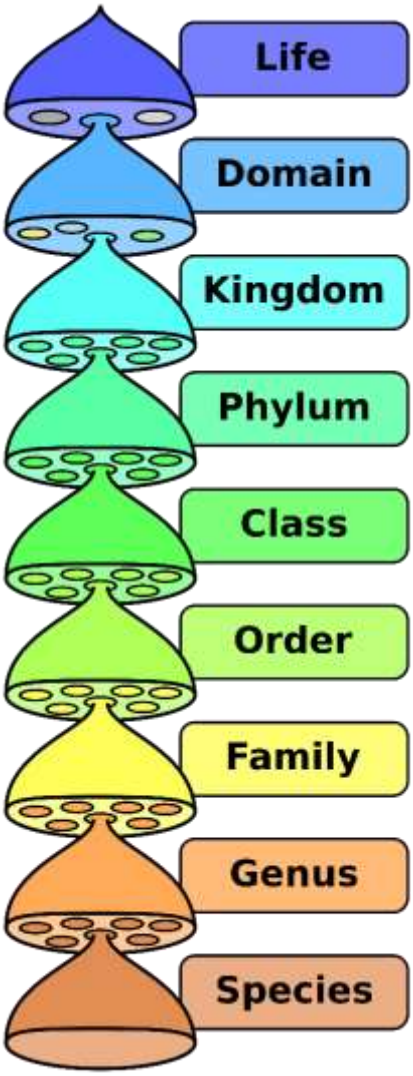

Figure 1. Graphical representation the classification of organisms.

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.

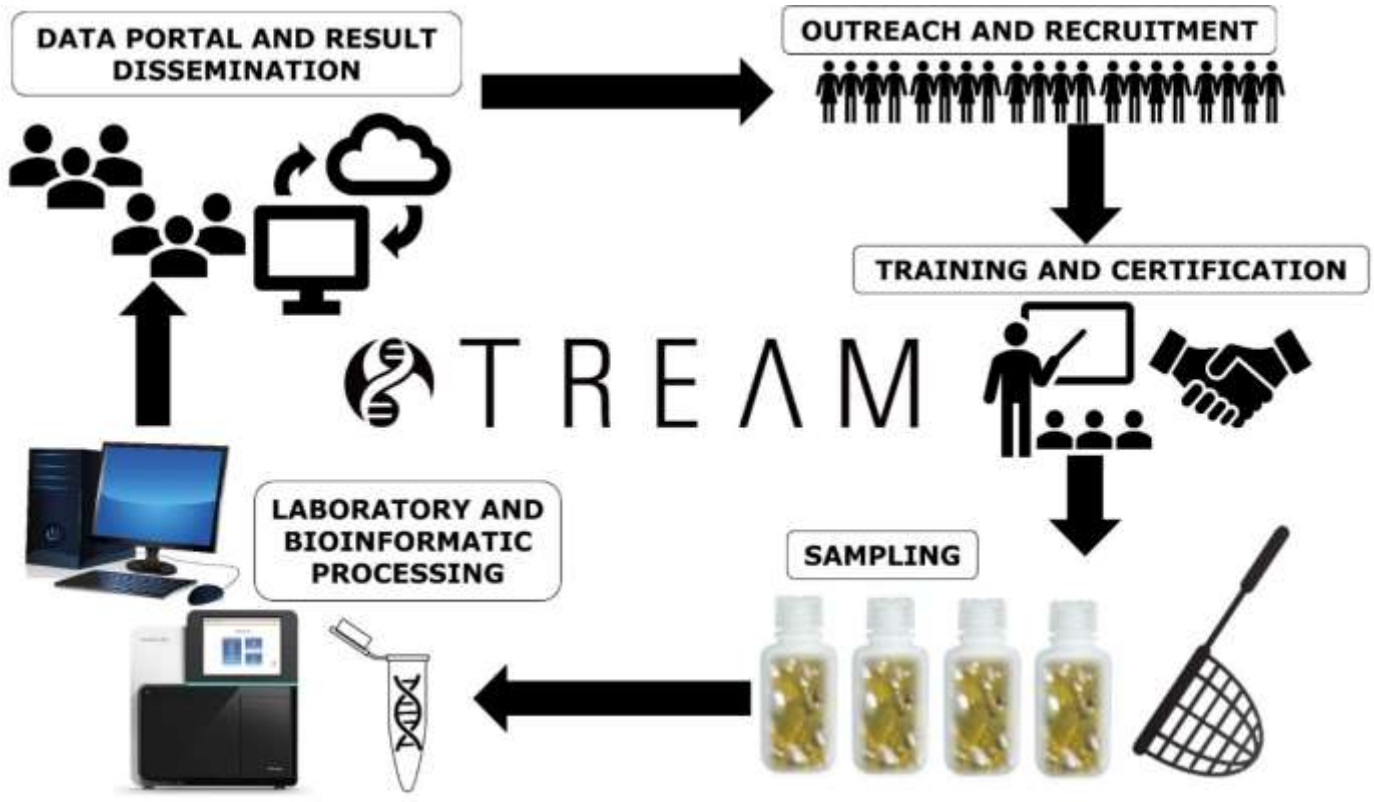

Figure 2. Graphical representation of the STREAM feedback loop for DNA biomonitoring of benthic invertebrates.

1.3. Objective of Report

Data and information included in this report is a first and preliminary examination of results from Bighill Creek (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.

1.4. Study Objective

Bighill Creek Preservation Society is a group of local residents who recognise that unrelenting development and population pressure from the town of Cochrane and surrounding County have the potential to significantly erode the many beneficial attributes of the watershed. The mission of the society is "To ensure the natural and historical values of Bighill Creek Watershed are preserved for this and future generations." This study aims to provide data to facilitate the comparison of macroinvertebrate detection in Bighill Creek using the traditional taxonomic approach with the STREAM DNA metabarcoding approach.

2. METHODOLOGY

2.1. Study Area

In September 2019, this study was conducted across 10 pre-determined sampling locations within the Bow River sub-basin (Alberta; **Figure 3**). Sampling was conducted by Bighill Creek Preservation Society, 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.

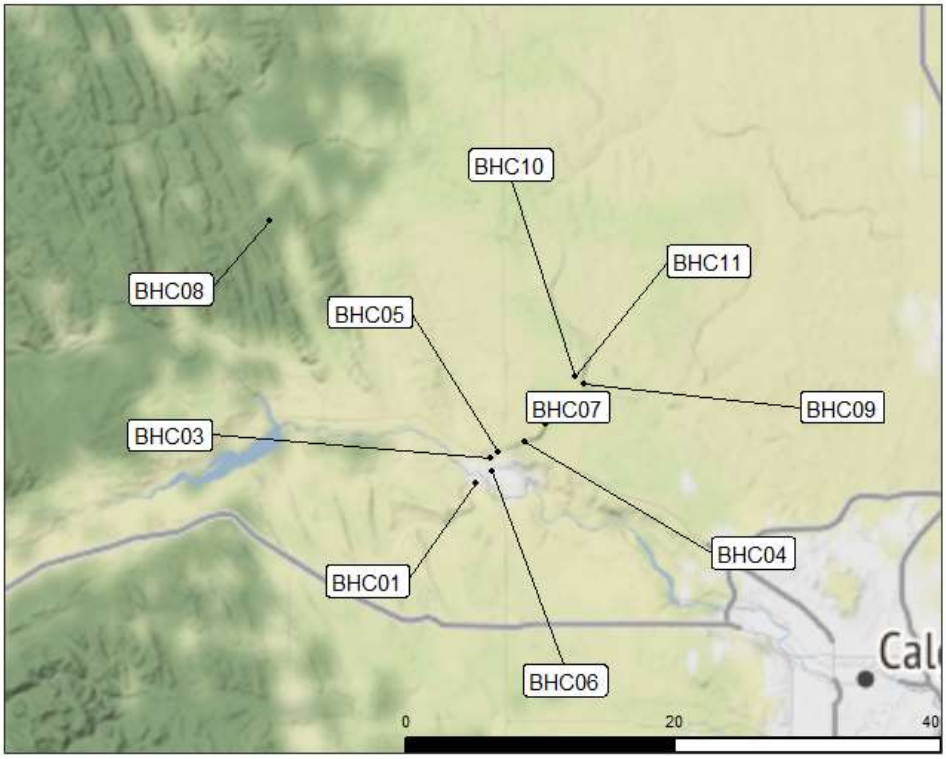

Figure 3. Map of sampling locations within the Bow River sub-basin, AB.

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 56 Orders, 85 Families, 137 Genera, and 148 species of macroinvertebrates. Across all 10 sites, species richness (number of species present) ranged from a mean of 20 in Bighill Creek 10 (BHC10) to 61 in Bighill Creek 9 (BHC09; **Figure 4**). A full taxonomic list identified to the Species and Genus level for macroinvertebrates is included as two separate Excel spreadsheets.

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.

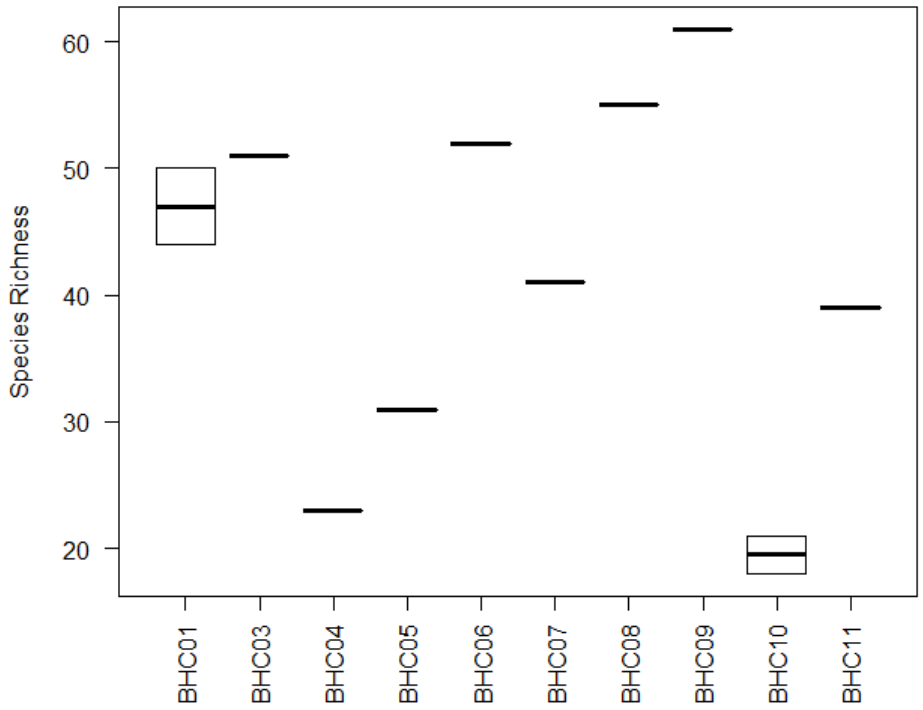

Figure 4. Species richness of each site sampled. Replicates are pooled. Only species taxonomically assigned with high confidence (bootstrap support >= 0.70) are included. Bighill Creek 1 (BHC01) = 20 taxa, Bighill Creek 3 (BHC03) = 51 taxa, Bighill Creek 4 (BHC04) = 23 taxa, Bighill Creek 5 (BHC05) = 31 taxa, Bighill Creek 6 (BHC06) = 52 taxa, Bighill Creek 7 (BHC07) = 41 taxa, Bighill Creek 8 (BHC08) = 55 taxa, Bighill Creek 9 (BHC09) = 61 taxa, Bighill Creek 10 (BHC10) = 20 taxa, Bighill Creek 11 (BHC11) = 39 taxa.

3.2. Taxonomic Coverage

A range of macroinvertebrate species were detected across the 10 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 10 sites, some EPT species such as *Baetis brunneicolor* (small minnow mayfly) and *Brachycentrus americanus* (humpless

casemaker caddisfly), were detected across a majority of sites, whereas *Taenionema pallidum* (willowflies) and *Oecetis disjuncta* (long-horned caddisflies) 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 = Bighill Creek 1; Site 2 = Bighill Creek 3; Site 3 = Bighill Creek 4; Site 4 = Bighill Creek 5; Site 5 = Bighill Creek 6; Site 6 = Bighill Creek 7; Site 7 = Bighill Creek 8; Site 9 = Bighill Creek 10; Site 10 = Bighill Creek 11.

Taxa				Sites									
Order	**Species**	**Common Name**	**Aquatic?**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Amphipoda	*Gammarus kischineffensis*	Freshwater amphipod	Yes	P									
Amphipoda	*Gammarus lacustris*	Freshwater amphipod	Yes	P	P		P	P		P			P
Amphipoda	*Hyalella azteca*	Freshwater amphipod	Yes						P	P	P		P
Arhynchobdellida	*Motobdella montezuma*	Leeches	Yes								P		P
Coleoptera	*Barypeithes pellucidus*	Juniper root weevil	No	P									
Coleoptera	*Liodessus affinis*	Diving beetle	Yes	P	P								
Coleoptera	*Liodessus obscurellus*	Diving beetle	Yes	P	P								
Coleoptera	*Optioservus fastiditus*	Riffle beetle	Yes					P					
Coleoptera	*Optioservus ovalis*	Riffle beetle	Yes	P				P	P	P			
Coleoptera	*Haliplus immaculicollis*	Crawling water beetle	Yes									P	P
Diplostraca	*Pleuroxus varidentatus*	Water fleas	Yes								P		
Diptera	*Conchapelopia pallens*	Non-biting midges	Yes	P		P		P	P	P	P		
Diptera	*Conchapelopia telema*	Non-biting midges	Yes		P			P	P	P	P		P
Diptera	*Corynoneura scutellata*	Non-biting midges	Yes								P		P
Diptera	*Cricotopus bicinctus*	Non-biting midges	Yes	P	P		P	P	P	P	P		P
Diptera	*Cricotopus infuscatus*	Non-biting midges	Yes	P							P		P
Diptera	*Cricotopus sylvestris*	Non-biting midges	Yes								P		
Diptera	*Cricotopus triannulatus*	Non-biting midges	Yes			P		P	P	P			
Diptera	*Cricotopus trifascia*	Non-biting midges	Yes	P	P	P	P	P	P	P	P		P
Diptera	*Dicrotendipes modestus*	Non-biting midges	Yes								P		P
Diptera	*Eukiefferiella claripennis*	Non-biting midges	Yes	P	P	P	P	P	P	P	P		
Diptera	*Heterotrissocladius changi*	Non-biting midges	Yes	P			P						
Diptera	*Micropsectra nigripila*	Non-biting midges	Yes	P	P				P	P	P	P	P
Diptera	*Micropsectra penicillata*	Non-biting midges	Yes	P	P	P	P	P	P	P			
Diptera	*Micropsectra pharetrophora*	Non-biting midges	Yes								P	P	

Order	Species	Common Name	Aquatic?	1	2	3	4	5	6	7	8	9	10
Diptera	*Micropsectra polita*	Non-biting midges	Yes	P	P	P	P	P	P	P	P	P	P
Diptera	*Micropsectra subletteorum*	Non-biting midges	Yes	P	P		P	P	P	P	P	P	P
Diptera	*Microtendipes pedellus*	Non-biting midges	Yes	P		P			P	P	P		
Diptera	*Orthocladius carlatus*	Non-biting midges	Yes							P			
Diptera	*Orthocladius dorenus*	Non-biting midges	Yes	P				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		P	P	P	P	P	P		P
Diptera	*Parachironomus tenuicaudatus*	Non-biting midges	Yes								P		
Diptera	*Paratanytarsus dissimilis*	Non-biting midges	Yes	P	P				P	P	P		P
Diptera	*Polypedilum aviceps*	Non-biting midges	Yes							P			
Diptera	*Procladius denticulatus*	Non-biting midges	Yes								P		
Diptera	*Prodiamesa olivacea*	Non-biting midges	Yes	P	P			P			P		
Diptera	*Stempellinella edwardsi*	Non-biting midges	Yes								P		
Diptera	*Stempellinella fimbriata*	Non-biting midges	Yes		P			P	P				
Diptera	*Synorthocladius semivirens*	Non-biting midges	Yes	P		P			P	P	P		
Diptera	*Tanytarsus buckleyi*	Non-biting midges	Yes								P		
Diptera	*Thienemanniella vittata*	Non-biting midges	Yes					P					
Diptera	*Thienemanniella xena*	Non-biting midges	Yes	P	P	P	P	P		P	P		
Diptera	*Tvetenia paucunca*	Non-biting midges	Yes	P	P	P	P	P	P	P	P		P
Diptera	*Neoplasta scapularis*	Dagger/ balloon flies	Yes	P				P		P			
Diptera	*Hydrellia notata*	Shore flies	Yes										P
Diptera	*Setacera micans*	Shore flies	Yes								P		
Diptera	*Mycetophila lunata*	Fungus gnats	No		P		P		P	P			
Diptera	*Eudorylas subopacus*	Big-headed flies	No		P								
Diptera	*Scatopsciara atomaria*	Dark-winged fungus gnats	No					P					
Diptera	*Simulium apricarium*	Black flies	Yes	P	P								
Diptera	*Simulium arcticum*	Black flies	Yes	P	P					P			
Diptera	*Simulium argus*	Black flies	Yes	P	P		P	P	P	P	P		P
Diptera	*Simulium bracteatum*	Black flies	Yes		P		P				P		P
Diptera	*Simulium carbunculum*	Black flies	Yes									P	
Diptera	*Simulium chromatinum*	Black flies	Yes	P	P					P			
Diptera	*Simulium exulatum*	Black flies	Yes		P		P				P		P
Diptera	*Simulium piperi*	Black flies	Yes			P	P						
Diptera	*Simulium pugetense*	Black flies	Yes									P	
Diptera	*Simulium tuberosum*	Black flies	Yes		P	P	P	P	P	P			
Diptera	*Simulium verecundum*	Black flies	Yes	P	P	P	P	P	P	P	P		P
Diptera	*Simulium vulgare*	Black flies	Yes		P					P			
Diptera	*Chrysops ater*	Horse flies	Yes										P
Diptera	*Diptera 3E-21004-1*	N/A	N/A	P									

Order	Species	Common Name	Aquatic?	1	2	3	4	5	6	7	8	9	10
Entomobryomorpha	*Entomobrya nivalis*	Slender springtails	No	P									
Ephemeroptera	*Baetis brunneicolor*	Small minnow mayfly	Yes	P	P	P	P	P	P	P	P		P
Ephemeroptera	*Baetis phoebus*	Small minnow mayfly	Yes	P					P	P			
Ephemeroptera	*Baetis tricaudatus*	Blue-winged olive mayfly	Yes	P	P	P	P	P	P	P	P	P	
Ephemeroptera	*Diphetor hageni*	Little blue-winged olive mayfly	Yes		P		P	P	P	P			
Ephemeroptera	*Caenis latipennis*	Squaregill mayfly	Yes	P							P		
Ephemeroptera	*Ephemerella excrucians*	Pale morning dun mayfly	Yes	P				P	P	P			
Ephemeroptera	*Cinygmula mimus*	Dark red quill mayfly	Yes				P						
Ephemeroptera	*Ecdyonurus simplicioides*	Flat-headed mayflies	Yes					P					
Ephemeroptera	*Leptophlebia nebulosa*	Black quill mayfly	Yes	P				P			P		
Ephemeroptera	*Paraleptophlebia debilis*	Mahogany dun mayfly	Yes	P				P		P			
Ephemeroptera	*Paraleptophlebia heteronea*	Blue quill mayfly	Yes	P	P	P	P	P	P	P			
Haplotaxida	*Fridericia bulboides*	Potworms	No	P									
Haplotaxida	*Globulidrilus riparius*	Potworms	No		P								
Haplotaxida	*Henlea perpusilla*	Potworms	No									P	
Haplotaxida	*Dendrobaena octaedra*	Earthworms	No									P	
Haplotaxida	*Eiseniella tetraedra*	Earthworms	No	P	P		P	P			P		
Haplotaxida	*Amphichaeta raptisae*	Freshwater worms	Yes									P	
Haplotaxida	*Chaetogaster diaphanus*	Freshwater worms	Yes								P		
Haplotaxida	*Chaetogaster diastrophus*	Freshwater worms	Yes	P				P			P	P	P
Haplotaxida	*Chaetogaster limnaei*	Freshwater worms	Yes										P
Haplotaxida	*Ilyodrilus templetoni*	Freshwater worms	Yes						P	P	P		
Haplotaxida	*Limnodrilus claparedianus*	Freshwater worms	Yes								P		
Haplotaxida	*Limnodrilus hoffmeisteri*	Freshwater worms	Yes	P	P				P		P		
Haplotaxida	*Limnodrilus udekemianus*	Freshwater worms	Yes		P			P		P			
Haplotaxida	*Nais bretscheri*	Freshwater worms	Yes	P				P			P	P	
Haplotaxida	*Nais christinae*	Freshwater worms	Yes							P	P		P
Haplotaxida	*Nais communis*	Freshwater worms	Yes	P			P			P	P	P	
Haplotaxida	*Nais elinguis*	Freshwater worms	Yes		P						P		
Haplotaxida	*Nais stolci*	Freshwater worms	Yes	P	P			P					P
Haplotaxida	*Ophidonais serpentina*	Freshwater worms	Yes	P				P					
Haplotaxida	*Stylaria lacustris*	Freshwater worms	Yes								P		P
Haplotaxida	*Tubifex tubifex*	Freshwater worms	Yes	P	P	P		P	P	P	P	P	
Haplotaxida	*Haplotaxida 21018-4C*	N/A	N/A								P		
Hemiptera	*Aphis fabae*	Black bean aphid	No	P	P	P	P	P	P	P			P
Hemiptera	*Chaitophorus populifolii*	Aphids	No									P	
Hemiptera	*Rhopalosiphum padi*	Bird cherry-oat aphid	No							P		P	
Hemiptera	*Anoscopus flavostriatus*	Leafhoppers	No									P	
Hemiptera	*Athysanus argentarius*	Leafhoppers	No	P									

Order	Species	Common Name	Aquatic?	1	2	3	4	5	6	7	8	9	10
Hemiptera	*Balclutha rhenana*	Leafhoppers	No		P								
Hemiptera	*Doratura stylata*	Leafhoppers	No	P	P								
Hemiptera	*Hecalus major*	Leafhoppers	No		P								
Hemiptera	*Hecalus montanus*	Leafhoppers	No		P								
Hemiptera	*Latalus personatus*	Leafhoppers	No		P								
Hemiptera	*Callicorixa audeni*	Water boatmen	Yes										P
Hemiptera	*Hesperocorixa laevigata*	Water boatmen	Yes										P
Hemiptera	*Sigara mathesoni*	Water boatmen	Yes										P
Hemiptera	*Trichocorixa sexcincta*	Water boatmen	Yes								P		P
Parachela	*Isohypsibius pushkini*	Water bear	Yes									P	
Philodinida	*Philodina flaviceps*	Rotifer	Yes					P				P	
Plecoptera	*Zapada cinctipes*	Common forestfly	Yes		P	P	P		P	P		P	
Plecoptera	*Taenionema pallidum*	Willowflies	Yes									P	
Ploima	*Euchlanis dilatata*	Rotifer	Yes								P		P
Podocopida	*Candona candida*	Ostracod	Yes	P	P			P		P	P		
Podocopida	*Cypridopsis vidua*	Ostracod	Yes								P		
Poduromorpha	*Ceratophysella denticulata*	Springtails	No	P									
Poduromorpha	*Hypogastrura vernalis*	Springtails	No					P					
Stylommatophora	*Discus whitneyi*	Snails/ slugs	No	P									
Symphypleona	*Sminthurinus domesticus*	Collembola	No					P					
Trichoptera	*Amiocentrus aspilus*	Humpless casemaker caddisfly	Yes	P				P		P			
Trichoptera	*Brachycentrus americanus*	Humpless casemaker caddisfly	Yes		P	P	P	P	P	P			
Trichoptera	*Micrasema bactro*	Humpless casemaker caddisfly	Yes		P	P	P	P	P	P			
Trichoptera	*Anagapetus debilis*	Weak saddle-case caddisfly	Yes									P	
Trichoptera	*Ceratopsyche alhedra*	Spotted sedge caddisfly	Yes						P	P			
Trichoptera	*Ceratopsyche slossonae*	Slosson's net=spinning caddisfly	Yes	P	P		P	P	P	P	P		
Trichoptera	*Cheumatopsyche wrighti*	Little sister sedge caddisfly	Yes							P			
Trichoptera	*Hydroptila arctia*	Microcaddisflies	Yes	P									
Trichoptera	*Hydroptila argosa*	Microcaddisflies	Yes							P			
Trichoptera	*Hydroptila consimilis*	Microcaddisflies	Yes						P				P
Trichoptera	*Hydroptila xera*	Microcaddisflies	Yes						P	P			P
Trichoptera	*Lepidostoma unicolor*	Unicoloured bizarre caddisfly	Yes					P					
Trichoptera	*Ceraclea excisa*	Long-horned caddisflies	Yes										P
Trichoptera	*Oecetis disjuncta*	Long-horned caddisflies	Yes	P									
Trichoptera	*Triaenodes tardus*	Long-horned caddisflies	Yes										P
Trichoptera	*Ecclisomyia maculosa*	Case-constructing caddisflies	Yes								P	P	
Trichoptera	*Ptilostomis semifasciata*	Giant casemaker caddisflies	Yes					P			P		
Trichoptera	*Rhyacophila brunnea*	Free-living caddisflies	Yes	P	P		P	P	P	P			
Trichoptera	*Rhyacophila vao*	Free-living caddisflies	Yes								P	P	
Trombidiformes	*Lebertia porosa*	Water mites	Yes	P	P			P		P	P		

Order	Species	Common Name	Aquatic?	1	2	3	4	5	6	7	8	9	10
Trombidiformes	*Limnesia undulata*	Water mites	Yes								P		P
Veneroida	*Pisidium casertanum*	Pea clams	Yes	P									
Veneroida	*Pisidium edlaueri*	Pea clams	Yes						P		P		
Veneroida	*Sphaerium striatinum*	Pea clams	Yes			P							

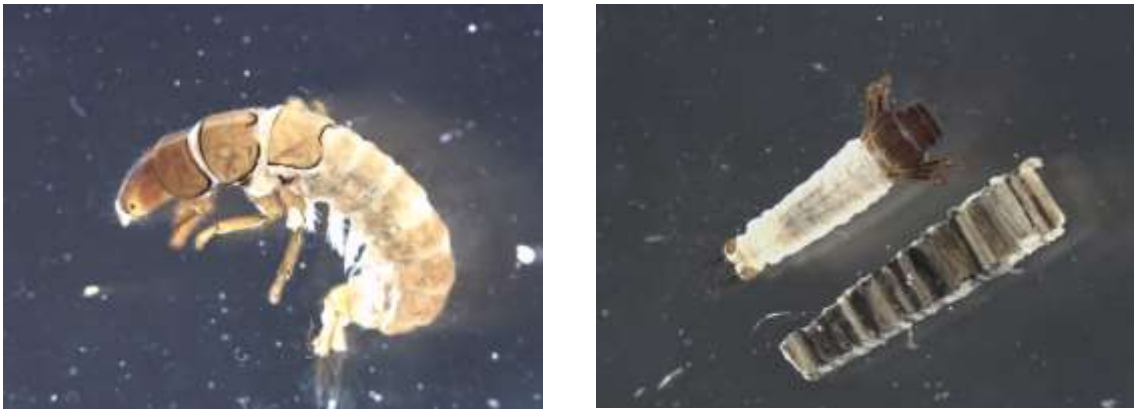

Figure 5. Some examples of the EPT taxa detected in the Liard samples. Left: a species of net-spinning caddisfly (Trichoptera) in the family Hydropsychidae. Right: a species of humpless casemaker caddisflies (Trichoptera) in the family Brachycentridae. All photos: ©CABIN Taxonomy.

4. FUTURE SUGGESTIONS

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

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., Crowl, 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 sites, including site name, date of collection and site coordinates.

Site	CABIN Code	Replicate	Site	Province	Latitude	Longitude	CABIN Status
1	BHC01	A	Bighill_Creek	AB	51.18086	-114.497	Test
1	BHC01	B	Bighill_Creek	AB	51.18086	-114.497	Test
2	BHC03	A	Bighill_Creek	AB	51.19789	-114.482	Test
3	BHC04	A	Bighill_Creek	AB	51.20861	-114.446	Test
4	BHC05	A	Bighill_Creek	AB	51.20131	-114.473	Test
5	BHC06	A	Bighill_Creek	AB	51.18847	-114.481	Test
6	BHC07	A	Bighill_Creek	AB	51.22067	-114.423	Test
7	BHC08	A	Bighill_Creek	AB	51.35617	-114.718	Test
8	BHC09	A	Bighill_Creek	AB	51.24742	-114.382	Test
9	BHC10	A	Bighill_Creek	AB	51.25264	-114.391	Test
9	BHC10	B	Bighill_Creek	AB	51.25264	-114.391	Test
10	BHC11	A	Bighill_Creek	AB	51.25264	-114.391	Test

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.