Preliminary DNA Data

Columbia Basin, BC
Wildsight - Creston Valley
September 2023

Photo: ©Melissa Flint

STREAM

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).

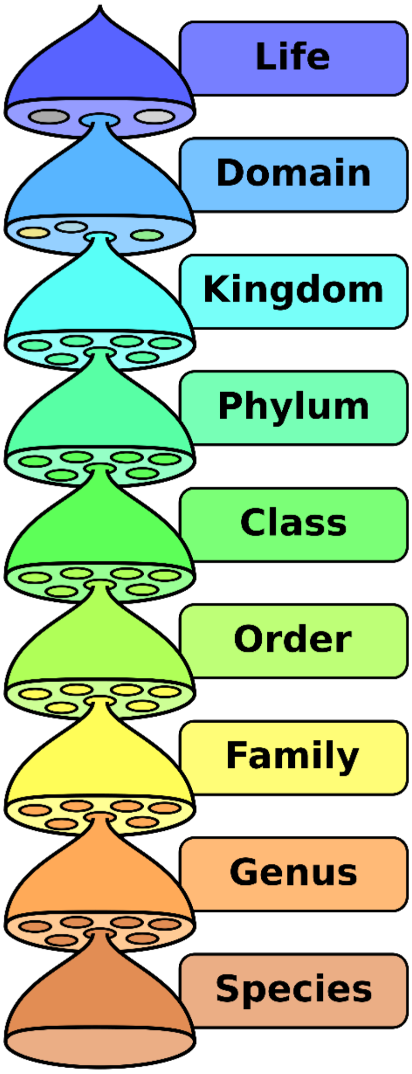

Figure 1. Graphical representation the classification of organisms.

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 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) with World Wildlife Fund Canada as a founding member organization. STREAM employs a standard sampling protocol modified from the Canadian Aquatic Biomonitoring Network (CABIN) programme. Where possible, 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. To date this timeline has not been regularly met, but steps are being taken to further optimize lab processing and reporting to more regularly meet this timeline for the 2023 sampling season.

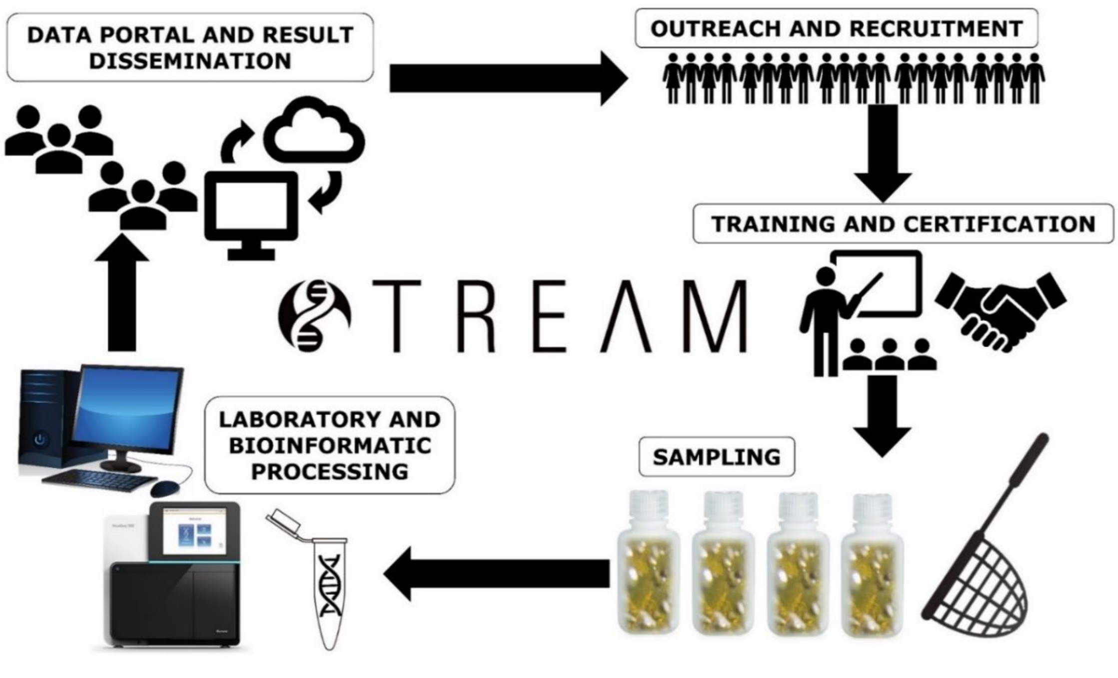

Figure 2. Graphical representation of the STREAM collaborative workflow for DNA biomonitoring of benthic invertebrates.

1.3. Objective of Report

Data and information included in this report is a preliminary examination of results from the Columbia basin (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.

2. METHODOLOGY

2.1. Study Area

In October 2022, this study was conducted at one pre-determined sampling location within the Columbia watershed (BC; Figure 3). Sampling was conducted by Wildsight – Creston Valley for benthic macroinvertebrate monitoring with STREAM.

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

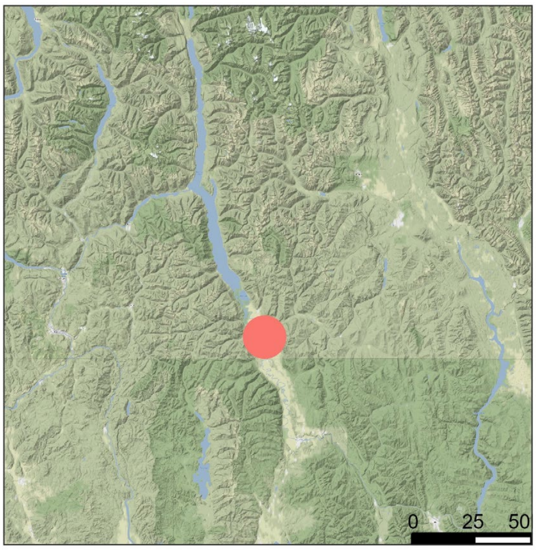

Figure 3. Map of sampling locations within the Columbia basin (BC). Scale bar shown in kilometres.

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 4 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 antifreeze 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 (v1.11.3) 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 results consisted of **64 Orders, 49 Families, 60 Genera, and 55 species of macroinvertebrates**. After normalizing, species richness (number of species present) ranged from 38 in Goat River A to 29 in Goat River C (**Figure 4**). A full taxonomic list identified to the raw genus and species level for macroinvertebrates is included as a separate Excel spreadsheet (STREAM_RP94_Taxonomy.xlsx).

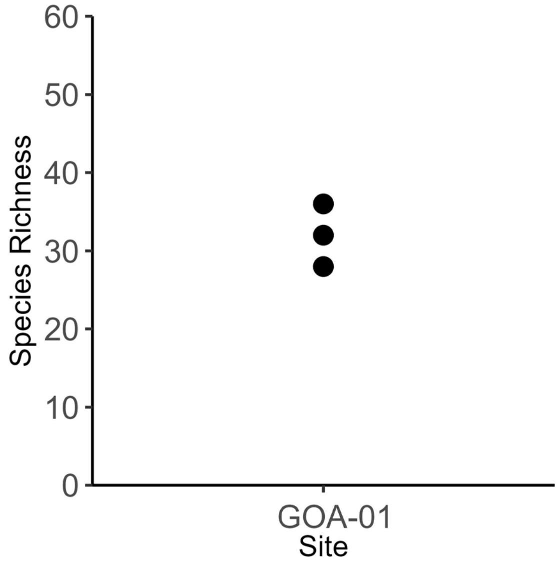

Figure 4. Species richness of each replicate. Only species taxonomically assigned with high confidence (bootstrap support >= 0.70) are included. Based on normalized data.

3.2. Taxonomic Coverage

A range of macroinvertebrate species were detected. Traditional bioindicator EPT species were detected across the 2022 sampling site, including 16 species of Ephemeroptera (mayflies), 12 Plecoptera (stoneflies), and 9 Trichoptera (caddisflies; **Table 1**). Some families of these EPT groups 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).

Please refer to the '**Macroinvertebrate Bioindicator Families Guide v1.2**' attached with your data or visit the corresponding website here for more information on approximate tolerances for the species detected in your sites.

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.

Table 1. List of Ephemeroptera, Plecoptera, and Trichoptera (EPT) occurrences identified to species level. P = present; Grey cells indicate absence. Site name for each column refer to site code (see Appendix A). Only species taxonomically assigned with high confidence (bootstrap support >= 0.70) are included.

Order	Family	Common Name	Species	GOA_1_A	GOA_1_B	GOA_1_C
Ephemeroptera	Ameletidae	Comb-mouthed minnow mayflies	*Ameletus subnotatus*	P	P	P
Ephemeroptera	Baetidae	Small minnow mayflies	*Acentrella insignificans*	P	P	P
Ephemeroptera	Baetidae	Small minnow mayflies	*Acentrella turbida*	P	P	P
Ephemeroptera	Baetidae	Small minnow mayflies	*Baetis phoebus*	P	P	P
Ephemeroptera	Baetidae	Small minnow mayflies	*Baetis tricaudatus*	P		
Ephemeroptera	Baetidae	Small minnow mayflies	*Diphetor hageni*	P	P	
Ephemeroptera	Ephemerellidae	Spiny crawler mayflies	*Attenella margarita*	P		P
Ephemeroptera	Ephemerellidae	Spiny crawler mayflies	*Drunella doddsii*	P	P	P
Ephemeroptera	Ephemerellidae	Spiny crawler mayflies	*Drunella grandis*	P	P	P
Ephemeroptera	Ephemerellidae	Spiny crawler mayflies	*Ephemerella tibialis*	P	P	P
Ephemeroptera	Heptageniidae	Flat-headed mayflies	*Ecdyonurus simplicioides*	P	P	P
Ephemeroptera	Heptageniidae	Flat-headed mayflies	*Epeorus albertae*	P	P	P
Ephemeroptera	Leptohyphidae	Little stout crawler mayflies	*Tricorythodes mosegus*	P		P
Ephemeroptera	Leptophlebiidae	Prong-gilled mayflies	*Paraleptophlebia heteronea*	P	P	
Ephemeroptera	Leptophlebiidae	Prong-gilled mayflies	*Paraleptophlebia memorialis*	P	P	P
Ephemeroptera	Siphlonuridae	Primitive minnow mayflies	*Siphlonurus occidentalis*			P
Plecoptera	Capniidae	Small winter stoneflies	*Eucapnopsis brevicauda*		P	
Plecoptera	Chloroperlidae	Green stoneflies	*Alloperla severa*	P	P	
Plecoptera	Chloroperlidae	Green stoneflies	*Sweltsa coloradensis*	P	P	P
Plecoptera	Nemouridae	Spring stoneflies	*Zapada cinctipes*	P	P	
Plecoptera	Nemouridae	Spring stoneflies	*Zapada columbiana*		P	
Plecoptera	Perlidae	Common stoneflies	*Hesperoperla pacifica*	P	P	
Plecoptera	Perlodidae	Springflies	*Isoperla fulva*	P	P	P
Plecoptera	Perlodidae	Springflies	*Kogotus modestus*	P		
Plecoptera	Perlodidae	Springflies	*Megarcys watertoni*	P		
Plecoptera	Pteronarcyidae	Giant stoneflies	*Pteronarcys princeps*			P
Plecoptera	Taeniopterygidae	Winter stoneflies	*Doddsia occidentalis*	P		
Plecoptera	Taeniopterygidae	Winter stoneflies	*Taenionema pacificum*	P	P	P
Trichoptera	Brachycentridae	Humpless casemaker caddisflies	*Brachycentrus americanus*	P		
Trichoptera	Brachycentridae	Humpless casemaker caddisflies	*Brachycentrus occidentalis*	P	P	
Trichoptera	Hydropsychidae	Net-spinning caddisflies	*Arctopsyche grandis*	P		
Trichoptera	Hydropsychidae	Net-spinning caddisflies	*Ceratopsyche cockerelli*	P	P	P
Trichoptera	Hydropsychidae	Net-spinning caddisflies	*Ceratopsyche oslari*	P	P	P
Trichoptera	Hydropsychidae	Net-spinning caddisflies	*Hydropsyche oslari*	P		
Trichoptera	Lepidostomatidae	Bizarre caddisflies	*Lepidostoma pluviale*		P	P
Trichoptera	Leptoceridae	Long-horned caddisflies	*Ceraclea nepha*	P		
Trichoptera	Limnephilidae	Northern caddisflies	*Dicosmoecus gilvipes*	P	P	

4. 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

5. APPENDICES

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

Site	River	Latitude	Longitude
GOA-01	Goat River	49.08070	-116.51907

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.
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.