



Sequencing the Rivers for Environmental Assessment and Monitoring

Frequently Asked Questions

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BACKGROUND

1. What does STREAM stand for?

Sequencing The Rivers for Environmental Assessment And Monitoring. This project aims to validate DNA metabarcoding as the mainstream approach to be routinely implemented by ECCC and WWF-Canada/LLC for generating biodiversity data for freshwater benthic macroinvertebrates.

2. Who are the main partner organizations involved in the STREAM project?

The STREAM project is a collaboration between World Wildlife Fund (WWF) Canada, Living Lakes Canada (LLC), Environment and Climate Change Canada (ECCC), and the Hajibabaei Lab in Centre for Biodiversity Genomics (University of Guelph). The project is, in part, funded by Genome Canada.

3. What is DNA metabarcoding and how it is being used in the STREAM project?

For the STREAM project, DNA metabarcoding is being used to characterize the DNA of benthic macroinvertebrates collected from a bulk sample in order to identify taxa present. To analyze, samples are blended together, and DNA is then extracted from the collective biomass, amplified and compared to a known library to identify which taxa of organisms are present.

4. Is special sampling equipment required to collect DNA samples?

No. Benthic macroinvertebrate samples are collected following the [Canadian Aquatic Biomonitoring Network \(CABIN\) Field Manual – Wadeable Streams 2012](#), with modifications to

minimize DNA contamination and preserve the sample. These modifications are outlined in the STREAM Procedure for collecting benthic macroinvertebrate DNA samples in wadeable streams.

5. Has DNA metabarcoding, using benthic macroinvertebrates, been tested or verified?

Yes. DNA metabarcoding has been widely used and verified prior to this project. Please see [Publications](#) page of the STREAM website for examples. This project is investigating the potential for DNA metabarcoding application in routine biological monitoring and assessment, with participants ranging from scientists to community groups.

Yes, DNA metabarcoding is potentially faster and less expensive than morphological taxonomic identification given the capacity to analyze samples in bulk. DNA metabarcoding provides an enhanced picture of benthic macroinvertebrate biodiversity due finer taxonomic resolution; however, this resolution is dependent on the accuracy and completeness of the DNA library. DNA metabarcoding is also unable to provide abundance information; delivering only presence/absence results. At this time, it is not possible to measure benthic macroinvertebrate abundance from a DNA sample. DNA metabarcoding currently provides presence information only. Alternatively, the conventional method based on morphology provides both presence and abundance information for the benthic macroinvertebrate communities.

6. How is DNA metabarcoding different from other eDNA projects?

With DNA metabarcoding, actual samples of organisms are collected while eDNA usually looks at DNA traces present in sampled water, sediment, soil or feces.

For the STREAM project, benthic invertebrate samples are collected, pooled and homogenized to generate a slurry from which DNA is extracted. A small, well-characterized genetic region, known as a “barcode”, is amplified from this DNA and sequenced for comparison against a library of catalogued barcodes. This comparison is used to identify the taxa present in the sample.

Targeted eDNA analysis, by contrast, is a species-specific method used to screen environmental materials, such as water or sediment, for the presence of DNA originating from specific organisms. Given the targeted nature of the analysis, it is best suited for the detection of species at risk or invasive species, rather than biodiversity assessment.

7. What is the benefit of biomonitoring?

Biomonitoring is an effective tool to measure environmental health because it evaluates the condition and composition of living organisms in a given ecosystem. It is based on the idea that living organisms are sensitive to change or environmental stress and, ultimately, indicators of environmental health. While biological indicators are able to provide a signal of environmental stress, detailed investigations are required to determine the cause of the stress.

8. What are the benefits of using benthic macroinvertebrates in biomonitoring?

The use of benthic macroinvertebrates as indicators of aquatic ecosystem health is advantageous because:

- They reflect local impacts due to their tendency to stay in a small area, experiencing constant exposure from local pollutants.
- They reflect cumulative effects due to their relatively long lifespan (1-3 years)
- They are universal
- They are ecologically significant
- They are well-characterized/well-studied

TRAINING

9. How can I get involved in the STREAM project?

Canadian Aquatic Biomonitoring Network (CABIN) Training is a prerequisite to participate in the STREAM project.

Benthic macroinvertebrate collection for STREAM, is based on CABIN methods with some modifications to minimize DNA contamination and preserve the sample. CABIN Training and Certification can be completed through the CABIN program or through one of the STREAM field training courses. For further information related to CABIN Training, which is led by ECCC and offered in association with the Canadian Rivers Institute (CRI), refer to the [CABIN website](#).

Once participants are trained in CABIN methods, The STREAM Procedure for collecting benthic macroinvertebrate DNA samples in wadeable streams is explained in person, during the STREAM field training course.

Training or certification levels will depend on the level of certification required for a given group. Please refer to the CABIN and CRI website for further information about CABIN certification levels.

If you are interested in joining the STREAM project, please send an email outlining your name, location, desired involvement and timeline to Raegan at Living Lakes Canada: raegan@livinglakescanada.ca

10. How much does CABIN Training and Certification cost?

Costs associated with CABIN Training depend on level of training or certification required. For further information please refer to the [CABIN website](#) and [CRI website](#).

There are no additional training costs associated with the STREAM sampling protocols. STREAM Procedure for collecting benthic macroinvertebrate DNA samples in Wadeable streams can be provided after CABIN Training or explained during a CABIN-STREAM field training course.

11. What equipment do I need for STREAM training?

Participants are expected to bring waders, pencils, clipboards and appropriate outerwear for the two-day field course.

12. How long does the CABIN certification last?

CABIN training involves a combination of online modules held by the Canadian Rivers Institute and a 2 day field practicum for the data collection techniques. However, there is also a field assistant level where participants only complete the 2 day field practicum portion. These participants then have 2 years to upgrade their training to either field tech or project manager level.

SAMPLE COLLECTION

13. How does my organization benefit from participating in the STREAM project?

The STREAM project offers support to participating organizations including equipment allocation, field training, sample collection and shipping. Samples contributed to the project will be analyzed at the University of Guelph at no cost. Your organization will also receive a benthic macroinvertebrate data report from the University of Guelph for the samples collected.

14. When is sampling conducted and how much sampling should be done?

Benthic macroinvertebrate samples can be collected for DNA analysis any time of the year, dependent on the safe access and Wadeability of streams. This is different from CABIN protocol where sampling is focused on late summer – early fall.

The collection of three distinct biological replicate samples is recommended at each site. Replicate samples are collected by sampling in a riffle (3 minute kick-net) and then repeating this two more times within the same riffle, with each sample collected upstream of the last. These samples are used for quality assurance and quality control purposes and provide more statistical flexibility for data analysis and interpretation. Refer to our [replicate guide](#) on the STREAM website for more information.

Ensure replicate samples are labeled properly, using the STREAM label format, site name followed by A, B or C.

15. How are benthic macroinvertebrate DNA samples preserved?

Benthic macroinvertebrate DNA samples should be preserved using denatured alcohol at a concentration greater than 90%. This includes rubbing alcohol (ethanol anhydrous, isopropynol etc.) that can be purchased over the counter at drug stores.

DO NOT USE FORMALIN to preserve a benthic macroinvertebrate DNA sample.

16. How do I decontaminate equipment?

Please see the STREAM Procedure for collecting benthic macroinvertebrate DNA samples in wadeable streams for required equipment and instructions on how to properly decontaminate sampling equipment.

17. Why is decontamination of sample equipment so important?

Benthic sampling and processing equipment should be decontaminated between each use to prevent the transfer of DNA-containing material between samples.

18. How do I properly label sample jars?

Each sample jar requires key information to ensure it is properly identified. Both the container and lid require labeling. Key information may be transcribed directly on the jar, on a piece of masking tape or on a STREAM label. Include the following information with a permanent marker:

- Sampling date (MM/DD/YYYY)
- CABIN Code of site
- Sample Preservative
- Sample jar number (e.g. 1 of 3, 2 of 3, 3 of 3)
- Replicate Letter IF replicates were taken (A, B, C)

Please refer to the STREAM Shipping SOP for further detail.

19. How are samples handled in the field and during shipping?

The proper handling and shipping of benthic macroinvertebrate samples is outlined in the STREAM Shipping Standard Operation Procedure (SOP). Please refer to this document for details.

Prior to shipping, notify the University of Guelph (Chloe Robinson, chloer@uguelph.ca) by filling out both a hard and electronic copy of the STREAM Sample Manifest with the required sample information.

Samples must be shipped by a GROUND courier (such as Purolator) following Transportation of Dangerous Goods (TDG) requirements.

The TDG Act and Regulations are designed to promote public safety when goods are handled and/or transported by road, rail, air, or water. The denatured alcohol used to preserve benthic macroinvertebrate DNA samples falls under the TDG Act and Regulations and has special requirement for handling and shipping. Please make yourself aware of the requirements for handling and shipping according to the TDG Act and Regulations: https://www.ccohs.ca/oshanswers/legisl/tdg/tdg_overview.html

ANALYSIS

20. How long does it take to get results (i.e. benthic macroinvertebrate identifications) from DNA sample analysis?

STREAM benthic macroinvertebrate DNA samples are analyzed at the Hajibabaei Lab at the University of Guelph. Typically, sample analysis can take approximately 2 months, however, is dependent on other factors such as lab capacity. Sample analysis may be delayed between September and November due to increased workload from seasonal biomonitoring programs. Consequently, submission of samples immediately following collection is encouraged to reduce sample backlog. We encourage groups to send samples as they are collected to avoid all samples needing to be processed in the fall & winter periods.

21. What can I expect in the results from the DNA sample analysis?

It is possible to identify benthic macroinvertebrates to the species level, however this is dependent on the completeness and accuracy of the DNA library. At this time, there are some DNA sequences that are not yet associated with an identified species.

To ensure accuracy of taxa classification it is also sometimes more appropriate to identify taxa at a higher taxonomic level (e.g. genus as opposed to species). Work is on-going to improve DNA library information.

22. What is taxonomic classification?

Taxonomic classification concerns the level of which organisms (e.g. benthic macroinvertebrates) are classified. For example, the spiny crawler mayfly species, *Drunella coloradensis*, would have the following classification:

- Kingdom: Animalia
- Phylum: Arthropoda
- Class: Insecta
- Order: Ephemeroptera

- Family: Ephemerellidae
- Genus: Drunella
- Species: Drunella coloradensis

23. What is a DNA library?

A DNA library is a collection of DNA sequences from different organisms. These libraries often match morphologically identified specimens with their corresponding DNA sequence.

An example of a DNA library is the Barcode Of Life Datasystem (BOLD): <http://www.boldsystems.org/index.php>.

24. How can I analyze my STREAM DNA results?

There are currently no analytical tools available through the CABIN Database to analyze benthic macroinvertebrate data based on DNA metabarcoding identifications. The results from DNA metabarcoding identification (taxa lists) will eventually be stored in the CABIN database under the STREAM-BERGE project. This project is examining the potential application of DNA metabarcoding in the context of biomonitoring for CABIN, including the development of analytical tools for data analysis.

At the University of Guelph, STREAM samples are analyzed using the latest DNA analysis pipelines and a report is generated with information on total number of phyla, classes, orders, families, genera and species as well as taxa tables highlighting bioindicator species (i.e. species which suggest the water quality status).

25. Is it possible to see a STREAM data report?

An example STREAM data report template is available for distribution. Please [contact us](#) if you would like a copy.

If you have any additional questions not covered in this FAQ, please contact us at raegan@livinglakescanada.ca.