Canadian Aquatic Biomonitoring Network

APPLICATION OF DNA METABARCODING TO BIOMONITORING

Is DNA metabarcoding the future for CABIN?

Advances in DNA sequencing, such as DNA metabarcoding, can be used to better understand aquatic environments including the identification of aquatic benthic macroinvertebrates. In Canada, DNA metabarcoding of benthic macroinvertebrate samples has been used by Environment and Climate Change Canada (ECCC) researchers in Atlantic Canada and the Peace-Athabasca Delta over the last decade (e.g. Parks et al., 2013; Gibson et al., 2015).

The identification of benthic macroinvertebrates through DNA metabarcoding has the potential to provide a rapid, inexpensive and informative approach to aquatic biomonitoring (Baird and Hajibabaei, 2012). ECCC, through the Canadian Aquatic Biomonitoring Network (CABIN) is currently collaborating with the University of Guelph, World Wildlife Fund-Canada and Living Lakes Canada to collect bulk benthic macroinvertebrate samples for DNA metabarcoding, through community based monitoring efforts across Canada (see the project announcement). As part of the Sequencing the River for Environmental Assessment and Monitoring (STREAM) project, ECCC scientists will explore the potential for nationally standardized protocols and analysis using DNA metabarcoding as an additional tool in the assessment of aquatic ecosystem health.

Deoxyribonucleic acid (DNA) encodes the structure, function and metabolism information for an organism.

The process of identifying the nucleic acid base pairs that make up a DNA strand, also known as a **gene,** is called **sequencing**.

Cytochrome oxidase c subunit 1, or CO1 is a gene, or barcode sequence, that is several hundred base pairs long and possessed by all invertebrates. CO1 is useful in their identification (Herbert et al., 2003).

DNA metabarcoding is a combination of DNA identification and automated DNA sequencing for multiple species (Taberlet et al., 2012a).

How do you collect benthic macroinvertebrate DNA samples?

It is possible to collect benthic macroinvertebrate DNA samples, or bulk tissue samples for DNA, following the procedures outlined in the *Benthic Macroinvertebrates Sample Collection* section of the CABIN Field Manual Wadeable Stream 2012 and the CABIN Wetland Macroinvertebrate Protocol, with a few adjustments. The most important consideration is to **minimize DNA contamination** among samples. Steps to minimize DNA contamination, such as cleaning collection equipment with an appropriate disinfectant, such as a bleach solution or Eliminase®,

between samples, and wearing nitrile gloves, are currently outlined in the CABIN Wetland Macroinvertebrate Protocol, Appendix F.

It is important to note the difference between a bulk tissue DNA sample and environmental DNA (eDNA). Bulk tissue samples include actual organisms; in this case invertebrates present at a site as the DNA source. In eDNA sampling, DNA is extracted from an environmental sample (such as soil or water), and is composed of DNA fragments from mucus, feces, or other organismal cells rather than directly from the organisms (Taberlet et al., 2012b).


Photo credit: ECCC CABIN Team

How are DNA samples analyzed?

Invertebrate samples are submitted to a genomics laboratory with specialized experience in handling bulk tissue samples. In preparation for DNA analysis the invertebrate sample is homogenized and a small amount of sample is extracted. DNA metabarcoding analysis targets a single gene set of base pairs, also known as a barcode, which identifies the taxa for further sequencing. This process relies on high throughput sequencing where many fragments of DNA from different organisms are sequenced simultaneously.


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Resulting DNA sequences are processed through a series of complex computational procedures known as a bioinformatics workflow. As part of this process, sequences are compared to publically available DNA reference barcode libraries to determine which taxa are present. As a result, taxa identified are dependent on the completeness of the DNA reference barcode library. This process is known as DNA metabarcoding.

What is the difference between conventional taxonomic data and taxonomic information from a DNA sample?

CABIN presently uses conventional taxonomic identification where invertebrate samples are sorted and taxa are morphologically identified and counted by a certified taxonomist using a microscope. Morphological identification generates a taxa list where benthic macroinvertebrates are typically identified to family level, sometimes genus and/or species. These results, alongside abundance information, are based on subsampling of the collected sample.

With DNA analysis, it is possible to identify all taxa present in a sample to a higher taxonomic resolution; often to species level depending on the completeness of the DNA library. DNA analysis provides a presence-absence dataset (i.e. taxa list only). At this time, it is not possible to obtain a reliable measure of taxon abundance from DNA sequencing.


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Will CABIN adopt the use of DNA for identification of benthic macroinvertebrates?

The use of DNA metabarcoding for the identification of benthic macroinvertebrates is being evaluated for use in CABIN aquatic health assessments. The adoption of this approach involves many considerations including modifications to the current sampling protocol, quality assurance, data analysis and interpretation, and establishing standardized laboratory procedures.


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As CABIN collaborates with researchers to investigate the potential application of DNA metabarcoding to aquatic health assessment, comparable morphological samples will be collected to better understand the differences between the two approaches and the modifications that may be needed for CABIN application.

Community groups or other organizations interested in incorporating DNA taxonomy information into their biomonitoring program and taking part in the STREAM project should contact their regional CABIN lead for more information. Refer to the CABIN news webpage for updates as this project progresses.

CANADIAN AQUATIC BIOMONITORING NETWORK

References

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