STREAM Procedure for collecting benthic macroinvertebrate DNA samples in wadeable streams

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Objective

To outline a preliminary procedure for benthic macroinvertebrate DNA sample collection following CABIN Field Manual Wadeable Streams 2012, with several modifications to minimize DNA contamination and ensure proper preservation of DNA material in a sample.

Modifications between CABIN morphological sample and DNA sample collection include:

- Nitrile gloves are worn during sampling and sample processing, a new pair of gloves are required for each new DNA sample collected;
- Sampling and processing equipment are decontaminated before each DNA sample;
- New or otherwise decontaminated benthic jars are used;
- Sample material are transferred to the sample jar using denatured ethanol in a spray-style bottle;
- DNA samples are preserved in denatured ethanol and kept cold once complete and during transfer to a sequencing facility.

> ***Practitioners must be aware of and follow all required Workplace Hazardous Material Information System (WHMIS 2015), Transportation of Dangerous Goods (TDG) and other health and safety considerations, requirements and/or regulations when using or transporting bleach, denatured ethanol, samples containing denatured ethanol and rinse wastewaters.***

Required Sampling Equipment and Materials

In addition to the CABIN standard field sampling equipment for aquatic biomonitoring (https://www.canada.ca/en/environment-climate-change/services/canadian-aquatic-biomonitoring-network/resources/standard-field-sampling-equipment.html) the following equipment and materials are required for DNA sampling:

1. Unscented household bleach
2. Prepared bleach solution* (see preparation below)
3. Mist-spray bottle (bleach solution)* (see preparation below)
4. Safety glasses (with side shield)
5. Cleaning brush
6. Nitrile gloves
7. Heavy rubber gloves (for decontamination using bleach)
8. Denatured ethanol (95%)*
9. Spray-style bottle (denatured ethanol)*
10. Waste water collection tray or bucket (sealable)
11. Cooler for sample jars*
12. Cooler for denatured ethanol (labelled according to TDG requirements)*

*Note: labelled according to Workplace Hazardous Materials Information System (WHMIS) and Transportation of Dangerous Goods (TDG) requirements and Material Safety Data Sheets (MSDS) are readily available

Equipment for benthic macroinvertebrate sample collection

- standard CABIN sampling gear (refer to CABIN Field Manual Wadeable Streams 2012),
- denatured ethanol*
- spray-style bottles, and
- nitrile gloves (multiple)
- safety glasses (when using ethanol)

Equipment for decontamination

- unscented household bleach*
- prepared bleach solution* (see preparation below)
- 1L mist-spray bottle for bleach solution*
- spray-style bottle for rinse water
- cleaning brush
- heavier rubber gloves, and
- large bucket (swirl bucket can be substituted)
- safety glasses

Denatured ethanol is a chemical used in DNA sample preservation.

Denatured ethanol includes additional ingredients (or denaturants) which are added to the ethanol to that make it inconsumable.

For properties and precautions for using denatured ethanol refer to the MSDS.

Bleach is a chemical used to decontaminate sample equipment for DNA sampling.

For properties and precautions for using bleach refer to the MSDS.

DNA Sample Collection Considerations

If collecting samples for morphological and DNA identification sampling options for different site scenarios are outlined below:

a) *Multiple suitable sample riffles or large sample riffle in reach*[1]
1 DNA sample, 1 morphological sample and 1 kick net: collect the DNA sample first in the more downstream location. This eliminates need to decontaminate between sample kicks.

b) Single riffle in reach[1]
1 DNA sample, 1 morphological sample and 2 kick nets: sample collection can occur simultaneously with two individuals kicking. Using two nets and starting on opposite sides of the same riffle (zig-zagging across the riffle towards the center) (Figure X). Kicking individuals should avoid being in the "disturbance shadow" of the other kicker.

If multiple DNA samples are to be collected at a single site all collection and processing equipment must be decontaminated between DNA samples, using the procedure outlined below. Options to make the sample collection and processing more efficient and minimize contamination include using separate decontaminated nets and sample processing equipment.

[1]a sample reach is defined as six times bankfull width

Sample Equipment Decontamination and Sample Collection

Bleach solution preparation

1. Prepare a 1 in 10 dilution bleach solution by mixing 1 part unscented household bleach (minimum concentration of 5.25%)) with 9 parts water in a labelled mist-spray bottle. This results in a minimum 0.525% final concentration of sodium hypochlorite.

Decontamination of sampling and sample processing equipment

2. At the site and outside of the riparian zone, Inspect sampling equipment to ensure it is free of organic debris, clean as necessary.

3. While wearing decontaminated rubber gloves, and over a collection bucket/tray, use spray-mist bottle to spray diluted bleach solution to cover all sampling and sample processing equipment including: waders, boots, kicknet, sieve, swirl bucket, forceps, spray-style bottle, and any other equipment that is used to collect or process the benthic samples. Let rest for 2 to 5 minutes.

4. Thoroughly rinse all bleached equipment with water[2] into bucket (or swirling bucket). Rinse water is to be collected and disposed of in a municipal water flow in accordance to any required precautions or regulations.

[2]Note: clean potable water is preferred but likely impractical in the field. Clear stream water, from the stream site in spray-style bottles can be used.

Sample collection and processing

5. Wearing a new pair of nitrile gloves, proceed to collect kick sample and complete sample processing by bucket swirling according to CABIN Field Manual Wadeable Streams 2012. The following additional steps or exceptions should be noted:

> A new pair of nitrile gloves should be worn for each DNA sample. Gloves should always be worn when handling samples.

a) When transferring an invertebrate sample to a decontaminated sample jar and,

- where vegetation is minimal, rinse and inspect leaves and other vegetation from sieve with stream water from the spray bottle, over top of sieve.

  OR

- where large amounts of aquatic plants (e.g. Myriophyllum or coon tail) that can trap invertebrates during bucket-swirling is present, plant material should be rinsed by dunking in a bucket of clear stream water. Rinse water is strained through the sieve once rinsing is complete.

Discard vegetation in order to minimize the amount of non-invertebrate organic material in the sample jar. Keep rotting sticks if they appear inhabited by invertebrates. Continue with the bucket swirling of the rocks and sediment.

b) Rinse and remove rocks and small pebbles from sieve as these will damage equipment during the homogenization of the sample. A small amount of sand is desirable to help with the blending process (e.g. 1-2 cm covering bottom of sample jar).

c) If a sample jar is more than half full it is best to split the sample into two or more jars. This is important to ensure that final **concentration of denatured ethanol is 50% or higher** in the sample for storage purposes.

d) Use the spray-type bottle of denatured ethanol preservative to wash the last bit of sample in the sieve into the sample jar(s).

> Denatured ethanol is used in sample preservation.
>
> Decontaminated sample jars should remain unopened until needed.

e) Add additional denatured ethanol to sample jar to ensure preservation of sample, a final **concentration of denatured ethanol is 50% or higher.**

f) Label lid and side of sample jar accordingly (e.g. a wax pen may be used as the label will not be dissolved by the ethanol). **A site code label should not be placed inside the jar to minimize potential of DNA contamination**.

g) To prevent leaks and cross-contamination during transportation, use parafilm on top of the jars.

6. Samples should immediately be placed on ice in a cooler or in a portable freezer. Samples should be placed in a freezer until they are shipped to a sequencing facility.

Any shipment of denatured ethanol containing material must follow Transportation of Dangerous Goods (TDG) regulations.

7. Repeat decontamination of sampling and processing equipment before next DNA sample is collected.