12.1 PROTOCOL FOR BIOFILM SAMPLING

Overview

Biofilm can be defined as the largely biotic community attached to rocks and cobbles of a stream or lake, largely made up of periphyton with associated invertebrates, zooplankton, and usually some abiotic material. This material is indicative of the productivity of a stream, and due to its integrative capacity (high organic carbon) can be used to measure the degree of organic contamination. Qualitative and quantitative sampling methods for biofilm exist. In rivers and streams, benthic algal communities typically account for most primary productivity. Benthic algal communities living on substrate surfaces are collectively referred to as periphyton. Sampling protocols in this section are concerned with the quantitative assessment of two kinds of periphyton; epilithic periphyton that are attached to the surfaces of rocks or other objects projecting above stream bottom, and episammic periphyton that are associated with sand. There are two main components to this type of sampling:

- location of the sampling points along a transect in the stream or river, and
- collection of periphyton from the substrata.

Sources

Environment Canada (1999), British Columbia MWLAP (2003), Alberta Environment (2006 a)

Special safety concerns

Don't attempt to lift rocks that are too heavy and don't enter water that may pose a threat to your safety. Watch your footing while returning to shore with the rock.

At a glance

Qualitative method

1 Collect a number of submerged rocks from the river cross-section, and scrape the biofilm with a pre-cleaned (soap and water, acetone then hexane) knife or spatula into appropriate sample containers (disposable gloves should be worn).

minimal to substantial effort

2 Collect appropriate ample volumes according to the study design. The amount of effort to collect 40 mL of material can vary from minimal (5-8 rocks) to substantial (20 rocks) depending upon the productivity of the stream, time of year, sunlight/shading, etc.

3 Cool or freeze the sample (depending on the analytical requirement).

Quantitative method

method to select rocks

1 Randomly select rocks to represent the variation in biofilm growth present at the sample location (i.e. they should not be chosen to maximize the sample size as in the case of the qualitative sample). A method to select rocks is to collect the rock closest to the left foot after a predetermined number of paces are taken through the cross-section, and to continue this

process until all necessary rocks are collected.

2 Collect samples by placing a 5 cm diameter template over a randomly selected submerged rock, scribing around the circumference of the template, and scraping the biofilm material from within the scribed area into a sample container (usually a Whirl-pak bag). Repeat this process for three randomly selected rocks to produce a single sample, which is immersed in a small quantity of river water. Triplicate the process.

Chlorophyll-a sampling

1 Rocks to be sampled for periphyton should be sampled across a transect extending the width of the watercourse unless the river is too deep. This can either be an imaginary transect or a defined transect. A transect can be defined in smaller watercourses by:

- selecting a reference point in the middle of the site and driving a peg into the ground on one of the banks,
- attaching a tape measure to the peg and laying it out taught across the watercourse. Anchor the far end with the second peg (other bank), and
- divide the stream width into equally spaced intervals according to the number of rocks that are to be sampled (consult with the project manager).

random rock selection

2 It is important to be random in the rock selection. Wade along the imaginary transect out from shore (a rope can be stretched across the river). Taking 2 steps select a rock (minimum size of 5 cm diameter) from approximately a 40 cm depth. Long arm gloves can be worn for this. For the defined transect, wade out to the first marked point and without looking, pick up a stone. If the stone is <5 cm diameter or a sandy, silty areas between cobbles is touched, then take the nearest stone that is > 5 cm diameter.

3 All the stones may be collected at once or individually.. Place the stone(s) on a white tray with a small amount of stream water and return to the stream bank.

4 If the river becomes too deep, head upstream repeating the above steps until all rocks are collected.

thickness of algae

5 Orient each rock as it was in the river, and place the 4 cm^2 template over the area (chosen randomly) to be scraped. Only take a 2 cm^2 scrape per rock when the algae is really thick (instead of a 4 cm^2 scrape per rock). Remember to note this on the field sheets and labels. If the algae is extremely thick, a diagonal section of the template can be scraped, ensure the area is recorded.

6 Using a scalpel, completely scrape off the algae found inside the appropriate template.

7 The number of rocks and number of replicates collected will depend on the river and project (consult the project manager). For example, scrapes from three rocks have generally been

consult project manager

combined onto one filter. Three filters can be submitted per site. This should be pre-defined by the project manager prior to the sampling trip.

8 Alternatively, pour a small amount of de-ionized water over the freshly scraped algae (on the rock), this will form a slurry. Remove the slurry from the stone using a disposable pipette or a turkey baster, and transfer it to a 250 mL dark Nalgene bottle. Rinse the scalpel with de-ionized water into the Nalgene bottle to ensure transfer of any residual algae. When all the rocks in a sample are scraped, add 10-15 mg powdered $MgCO_3$ to the bottle. Add double distilled/deionized water for an approximate total volume of 25 mL. Label the bottles with the total area of scrape contained in the sample, site, date, and "epilithic chloro". Store the Nalgene container at 4°C and transport to the laboratory within 24 hours.

transport to laboratory within 24 hours

9 If the alternate method was not used on #8 above, place the algae from the scalpel directly onto a GF/C filter.

10 Apply a light sprinkling of powdered $MgCO_3$ to the material on the filter once all necessary rock scrapes for the replicate are completed.

11 Wrap the filter in the aluminum foil in a way that the analyst can easily unwrap it to get at the filter and so that material doesn't come off on the foil.

12 Label the wrapper with site, date, "epilithic chlorophyll", and the total area of scrape that it contains in cm^2 (e.g., three rocks x 4 cm^2=12 cm^2).

13 Repeat the process for the other groups of rocks.

14 Put the samples in a Whirl-pac or Ziploc bag and store on regular or dry ice (at -4°C).

15 Place the samples in the laboratory freezer when you return from the field.

16 Ship frozen samples to the laboratory every week for extraction.

Air-dried weight and species identification sampling

1 Follow the procedures outlined for chlorophyll-a for collection of samples. For example, pool scrapes from three rocks into a small jar for analysis of air-dried weight. Take species identification from the same number and sub-set of stones (e.g., pool scrapes from two into scintillation vial containing 10 mL of de-ionized/RO water).

2 Add 2 mL of Lugol's solution to each vial.

3 Line the scintillation vial cap with Parafilm prior to sealing the vials.

4 Label each vial with site, location, date, area of scrape, and sampler's initials.

5 Store vial in the dark.

Sampling clean-looking but slimy rocks

1. Follow the rock sampling instructions described above for the template sampling technique for chlorophyll-a (steps 1-4).
2. Collect the number of rocks and number of replicates according to study design.
3. Select a rock and affix the collar over an area of rock that was oriented upward in the stream.
4. Use an artist's brush to physically rub the area of rock within the collar area, to dislodge the slime.
5. Use a small amount of water to produce a slurry within the collar that can be transferred using a turkey baster or poured into a 1 L dark Nalgene bottle. Use a funnel to avoid spillage during pouring. Use a squirt bottle to rinse the slurry thoroughly from the collar, baster, and brush into the dark bottle. Try and use a minimal amount of water.

filter within 24 hours

6. Repeat this process for three rocks ensuring enough slurry is obtained. Shake some $MgCO_3$ into the bottle (optional), store bottle (properly labeled with site, date, area sampled by collar multiplied by the number of rocks) in cooler until such time as sample can be filtered (not more than 24 hours later), set up the filter apparatus with GF-C filter, rinse filter, and proceed to filter the slurry through the apparatus. Rinse bottle adequately and filter to ensure all slurry is obtained. Cover the filter with powdered $MgCO_3$ (optional), fold the filter in quarters, place in aluminum foil, and label with date, location, site number, total area of rock sampled, and sampler's initials.
7. If too much slurry is obtained, a sub-sampling procedure may be used by mixing the slurry in a shallow graduated cylinder, and then draw up 10 mL of well-mixed slurry into the syringe (5 mL if the slurry is extremely thick).
8. Filter the slurry through a GF/C filter. Rinse the syringe with a small amount of distilled water and filter this through the same filter. Cover the filter with powdered $MgCO_3$ (optional), fold the filter in quarters, place in aluminum foil, and label with date, location, site number, total volume of slurry (using a graduated cylinder), volume of slurry filtered, and sampler's initials.
9. Put samples on ice, or freeze with dry ice and deliver to the laboratory.

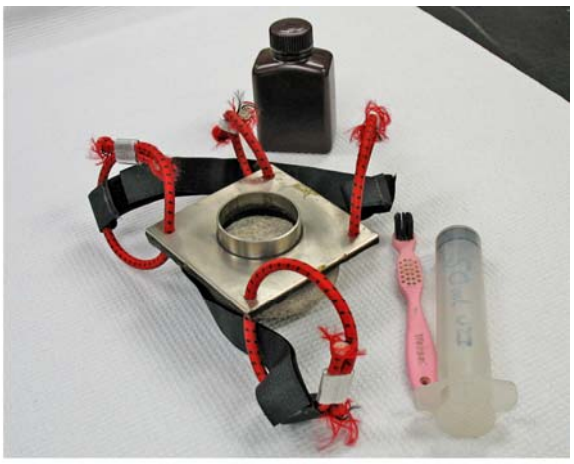

Photo 23 (left). Example of flexible plastic disc (Source: Alberta Environment (2006))

Photo 24 (right). Epicollar (Source: Alberta Environment (2006))