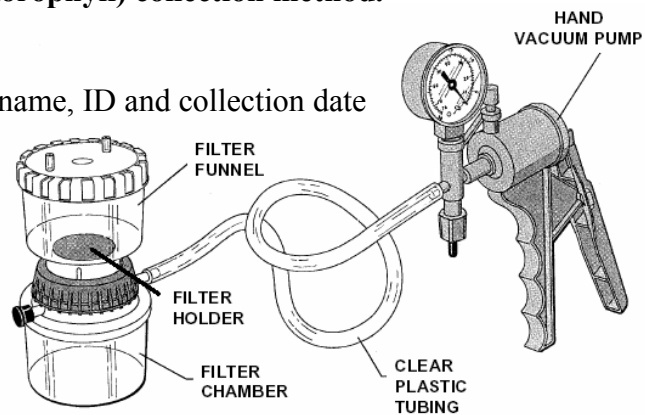


Appendix A. Phytoplankton (suspended chlorophyll) collection method.

Equipment

20-mL borosilicate glass vial labeled with site name, ID and collection date
50-mL graduated cylinder
Hand vacuum pump or mechanized pump
Forceps (2)
Whatman glass-fiber filters
Distilled or deionized water (DI water)



Phytoplankton (suspended chlorophyll) is collected at the same location where you collect the USEPA lab water samples and measure chemistry with the U-10 Horiba water quality checker. Because chlorophyll can degrade rapidly when exposed to bright light, prepare sample in shade (or subdued light) and filter as quickly as possible after collection to minimize degradation. Avoid touching the glass-fiber filter with your fingers. Rinse the filtration apparatus with deionized (or distilled) water each day before use at the base site and then seal in a plastic bag until use at the stream. Keep the glass-fiber filters in a dispenser inside a sealed plastic bag until use.

1. Using clean forceps, place a glass-fiber filter on the filter holder.
2. Use a small amount of DI water from a wash bottle to help seat the filter properly. Attach the filter funnel to the filter holder and filter chamber, then attach the hand vacuum pump to the chamber.
3. Rinse the sides of the filter funnel and the filter with a small volume of DI water.
4. Rinse the graduated cylinder in the stream 3 times.
5. Collect 50 mL of stream water to be filtered. Note: If different volume of water is filtered, record the volume filtered on the 20-mL sample vial.
6. Pour the water sample into the filter funnel, replace the filter cap, and pump the sample through the filter using the hand pump or mechanized pump. NOTE: Vacuum pressure from the pump should not exceed 7 pounds per square inch (psi) to avoid rupture of fragile algal cells.
7. Remove both plugs from the filtration chamber and pour out the filtered water in the chamber.
8. Remove the filter with clean forceps, fold the filter in quarters, and place the folded filter into a 20-mL glass vial. Do not touch the filter with your fingers!
9. Seal the phytoplankton sample in a zip-lock plastic bag, and store that on dry ice or between two sealed plastic bags of ice in a cooler. Keep the samples out of the light!
10. Rinse the filter funnel, filter holder, filter chamber, and graduated cylinder thoroughly with DI water.

Appendix B. CPCB periphyton collection method.

PART I. Sample collection

Equipment

PVC delimiter of 3.2 cm diameter

Aspirator with rubber stopper and two tubes

Distilled or deionized water (DI water)

Brush with short stiff bristles

Five 40-mL borosilicate glass vials, labeled 1 – 5, with the site name, site ID and collection date

- Periphyton is collected at sampling point away from the river bank, but in shallow (< 1 m) water on the same bank as the macroinvertebrates.
- Periphyton is collected from every other transect, each transect into its own vial.
- If you cannot collect at a transect, skip it and collect from the very next transect.
- Determine if the dominant substrate in the sampling area is coarse (i.e., an erosional [riffle] habitat) or fine (i.e., a slack water [pool] habitat).
- For each vial, on the CPCB field sheet (Table 4) record bank, transect, and substrate. It is very important that each vial be associated with the substrate.

Solid Substrate (rock, leaf, wood, etc.) – Use the brush and DI water to aspirate the entire surface within the delimiter.

1. If necessary, gently remove the substrate from the water so as not to disturb the layer of periphyton.
2. Place the delimiter firmly on the substrate surface so that a seal is formed between the surface and the foam collar.
3. Using the stiff brush, vigorously loosen the periphyton within the well formed by the foam collar and squeeze a small amount of DI water into the well.
4. With the aspirator fitted into a 40-mL vial, suction off the material in the well.
5. Vigorously brush the substrate surface a second time; squeeze some water into the well; and suction off the material again. Repeat a third time.
6. Rinse off the brush and delimiter into the sample vial using a little DI water.
7. Rinse off the aspirator's tube into the vial and cap the vial.
8. Return the substrate to the stream.

Soft Substrate (sand, silt, etc.) – Use the aspirator to collect the top 0.5 cm of substrate within the delimiter.

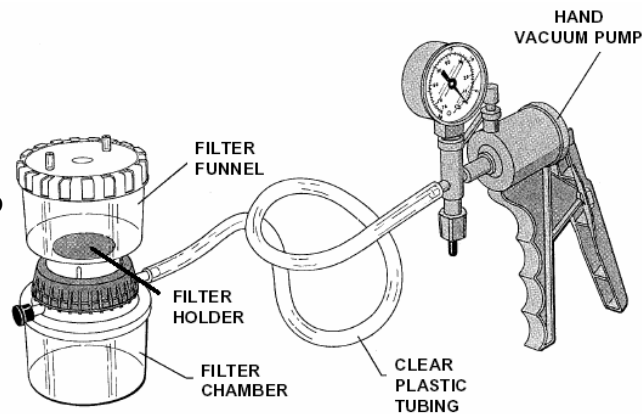
1. Place the delimiter firmly on the substrate surface so that a seal is formed between the surface and the foam collar.
2. With the aspirator fitted into a 40-mL vial, suction off the top 0.5 cm of substrate.
3. Avoid aspirating excessive amounts of overlying stream water (< 10 mL).
4. Rinse off the delimiter and aspirator's tube into the vial and cap the vial.

Sample vials should be stored on ice and in the dark for up to 4 hours, and then the samples must be filtered (Part II, you can do this back at the vehicle).

PART II. Sample filtration

Equipment

The 5 periphyton samples
Hand vacuum pump or mechanized pump
Forceps (2)
Whatman glass-fiber filters
Distilled or deionized water (DI water)



Because chlorophyll can degrade rapidly when exposed to bright light, prepare the sample in shade (or subdued light) and filter as quickly as possible after collection to minimize degradation. Avoid touching the filter with your fingers. Rinse the filtration apparatus with deionized (or distilled) water each day before use at the base site and then seal in a plastic bag until use at the stream. Keep the glass-fiber filters in a dispenser inside a sealed plastic bag until use.

1. Using clean forceps, place a glass-fiber filter on the filter holder.
2. Use a small amount of DI water from a wash bottle to help seat the filter properly. Attach the filter funnel to the filter holder and filter chamber, then attach the hand vacuum pump to the chamber.
3. Rinse the sides of the filter funnel and the filter with a small volume of DI water.
4. Select the first sample to be filtered, and gently shake the vial to thoroughly mix the contents.
5. Pour the sample into the filter funnel; replace the filter cap; and pump the sample through the filter using the hand pump or mechanized pump. NOTE: Vacuum pressure from the pump should not exceed 7 pounds per square inch (psi) to avoid rupture of fragile algal cells.
6. If filtration slows down before you have filtered the entire sample, pour unfiltered sample back to the sample vial carefully and then place a new filter on the filter holder. Proceed with steps 7 and 8 until the entire sample is filtered. NOTE: Filter a small portion of sample at a time will help eliminate filter clogging issue.
7. Remove both plugs from the filtration chamber and pour out the filtered water in the chamber.
8. Remove the filter with clean forceps, fold the filter in quarters, and place the folded filter back into the same sample vial. Do not touch the filter with your fingers!
9. Repeat steps 1 – 8 for the remaining 4 samples.
10. Seal the samples in a zip-lock plastic bag and store that on dry ice, or between two sealed plastic bags of ice in a cooler. Keep the samples out of the light!
11. Rinse the filter funnel, filter holder, and filter chamber thoroughly with DI water.