PROTOCOLS FOR CPOM AND FPOM SAMPLING (Coarse and Fine Particulate Organic Matter)

Place D-Net firmly onto stream substrate at ¼ distance from left shore. Dislodge any organic matter trapped among the substrates by kicking and shuffling among rocks and debris for 20-30 seconds. Disturb an area that creates a square, using the D-Net as a guide (30x30 cm). Repeat for ½and ¾distance from left shore, retaining all material collected in net except large rocks which should be washed in net and discarded. A glove (rubber or neoprene) may make it easier to vigorously sample. This is one of three replicates taken at the bottom, middle, and top of the 5 random riffle transects also selected for macroinvertebrate sampling.

Invert contents of D-Net into 1/4 full bucket, being sure to get all detritus off the net. Invertebrates that are clinging to the net can be ignored. Wash and remove large stones from the bucket, being sure to remove detritus from the rocks. Elutriate into 2nd bucket by swirling bucket and pouring off lighter material while leaving behind gravel and sand. Repeat 2-3 times. Discard the cleaned rocks and sand left behind in the rinsing bucket.

Place 1 mm mesh screen over empty bucket, and secure with cord lock. It helps to create a small depression in the mesh. Pour contents of 2nd bucket onto mesh screen. Use this as another elutriation- when close to the end, swirl bucket and stop pouring when only sand is left.

Pick invertebrates from 1mm mesh screen for 3 minutes. Use constant effort for all samples. The biggest and heaviest bugs should be removed first. Squirt the mesh with a rinse bottle so that all particles <1mm wash through (=FPOM fraction of 0.25 to 1.0 mm). Carefully remove the cord-locked mesh from the top of the bucket and fold corners of the 1 mm mesh screen to create a bag. Shake bag gently to remove excess water. Wrap mesh in towel to remove any remaining water.

Weigh the 1mm mesh bag using the appropriate Pesola scale. If the scale has a hook instead of a clip, secure the mesh bag using a rubber band, then hook the band. Record weight on FPOM vial label, then rinse bag. Shake excess water from mesh and dry in towel, then obtain a tare weight of the mesh bag. If a rubber band was used, include this in the tare. Record tare weight T.
[Ex. label for a 40.3 gram gross weight: CPOM = 40.3 - 26.5T]

From bucket containing FPOM, remove small mayflies or other insects swimming or floating (plastic pipette works well). Do this for no more than a few minutes and be careful not to remove FPOM. Strain FPOM through 100 µm handnet (using empty bucket as support). This can act as an elutriation step as well. Use BioQuip forceps to remove FPOM from net and place into scintillation vial. Use wash bottle to gather FPOM in bottom of net to facilitate removal. Add water to vial to ½full and add 1-2 mL of formaldehyde to preserve. Store in field ice chest. This FPOM fraction is for the size range 250 - 1000 µm since particles less than 250 will have passed through the sampling D-net.

Vial Label: stream, site, date (day- month (roman numeral)- year), and replicate # (1-3)

Example:

FPOM #1
Convict Creek
lower SNARL
6 VII 96
CPOM = 40.3 - 26.5T

Materials Needed:

D-Net
1 mm mesh square (1.5 x 1.5 feet)
2 buckets, 100 µm handnet
BioQuip forceps, label tape, sharpies
500 mL washbottle
Pesola scales 50, 100, 500 g
Hand towel (to dry mesh)
Cord (w/ cord loc to tighten around bucket)
Scintillation vials, rubber bands
Formalin (buffered over $MgCO_3$)
Plastic pipettes for small insect removal

ALGAE SAMPLING PROTOCOL

Select cobble size substrate (5-25 cm range) from center of transect line (upper, middle and lower random transects for 3 replicates total). Lift cobble from bed into submerged collecting tray. Pay attention to rock orientation to assist with measurements. Pour off all but a small volume of water from the collecting tray.

Remove algae from substrate using wire or nylon brush until all algae is visibly removed (brush down, into water in the sample container for 3-5 minutes). Rinse substrate, brush, and fingers into collection container (w/ about 50-100 mL).

Measure exposed surfaces of substrate using plastic ruler to record length, width, and height along the mid-lines of the rock. Only the height of the exposed upper surface should be recorded (or half the height if the exposed surface is not obvious). Measure the circumference (=the longest perimeter) using the tape measure. Record measurements on sample collection bag for the filter (see below). [For example as 14L, 8W, 4H, 34LP]. Only now should you discard the sampled rock.

Measure total volume of sample by pouring through fine net into graduated cylinder. This will remove sand/rock/bugs but if filamentous or mat algae are present, they should be gently elutriated, leaving behind sand/rock. Rinse collection container and pour sample back into it. Record total volume on sample bag.

Homogenize sample using repeated suction-expulsion with a 60 mL syringe. Use scissors to cut large pieces of algae if necessary and use syringe with cut-off tip to prevent clogging of needle-connector. Filamentous algae may be set aside to dry in the sun for about 5 minutes and can then be crushed/crumbled with fingers into the processing water. Use syringe to measure 20 mL of sample into 25 mL scintillation vial. Add 2 mL formaldehyde and 0.5 ml Lugols soln. Store in ice chest. Label bottle with date, stream, site, and sample # (1,2,3). This is for later algal ID and biomass (AFDM), or direct cell counts and sizes as an index of algal food resource density.

Re-homogenize sample and fill syringe with 50 mL of sample (or use 10 mL syringe for dense algal samples). Place 25 mm glass fiber filter on metal screen filter holder (use BioQuip forceps), place O-ring and gently secure syringe adapter. Holding onto filter adapter pointed down, expel sample until resistance to filtration occurs (do not force further volume through). Use an empty syring to push air through filter assembly to expel any remaining water. Record mL of sample expelled as well as the total volume on sample bag.*
Remove filter, fold in half, place on aluminum foil square, fold foil and place in sample bag. Label with date, site, and sample #. Place bag in field ice chest. This filtered sample is for later chlorophyll analysis or AFDM burning. If for AFDM analysis in the lab, each filter should be pre-tared and a drop of buffered formalin placed on filter to preserve (drop on chl filter also). Discard remaining sample. Rinse materials thoroughly, proceed to next sample.

*Completed sample bag should be labeled with date, stream, site, sample #, substrate dimensions, and volume measurements (the 20 mL vial only with date, stream, site and replicate #):
Example Label for filter storage bag:

Convict Creek	Algae #1
lower SNARL	14L, 8W, 4H, 34LP
6 VII 96	8 filt. / 175 vol.

Materials Needed for Algae Sampling:

Sample collection box (tupperware type)	Metric ruler
Wire brush	Metric measure tape
250 mL rinse bottle	Formalin (over $MgCO_3$) and dropper pipette
250 mL graduated cylinder	Labeling tape and sharpie pen
60 mL syringes (cut/uncut tips)	Scintillation vials
25 mm GF/A filters	Plastic storage bags
Filter holder assembly	Aluminum foil squares
Fine mesh net (100 µm)	Small scissors
Small ice chest (w/blue ice)	BioQuip forceps