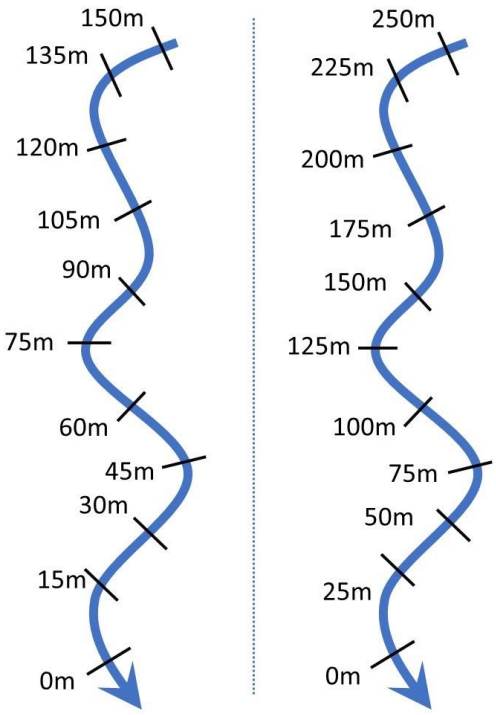
# StreamCLIMES Benthic Macroinvertebrate Sampling Protocol

**Reach-wide benthic (RWB) samples**

1. Determine site length: for streams <10m wide on average, use a 150 meter reach length and for streams >10m wide, use a 250m reach length. Take ~10 width measurements when you first get to the site.

2. For 150m reach length, benthic macroinvertebrates will be sampled every 15 meters (starting at 0m and continuing to 150m) as described below. For 250m reach length, benthic macroinvertebrates will be sampled every 25 meters (starting at 0m and continuing to 250m). Individual samples will be composited across the reach as described below.




3. Sampling is based on the reach-wide-benthic design of Ode et al. (2016). Briefly, this method creates a composite sample from 11 'kicks' (0.09 $m^2$ each, using 500 µm Surber sampler or D-net) in various habitats (e.g. riffles, runs, pools) in proportion to their occurrence across the reach, for a total area sampled of 1.08 $m^2$ per reach. Starting at the downstream end of the reach (0m), the position of each of the 11 kicks or subsamples alternates between left, center, and right portions of the channel, as one proceeds upstream. These left, center, and right subsampling locations are defined as the points at 25% of the (wetted) width from the left bank ("left"), halfway across the channel ("center") and 75% from the left bank ("right") at each transect. If you cannot collect a sample at the designated point because of deep water, obstacles, or unsafe conditions, adjust the sampling spot while keeping the point as close as possible to the designated position.

4. At each of these 11 subsampling positions, place the Surber or D-net so that its mouth facing into the flow of the water. If flow volume is sufficient to move material into the net with nothing more than the current, then either a Surber or D-net can be used to collect the subsample. In still water, we recommend using a D-net so that the net can be swept through the water (as described below).

5. Once the Suber sampler or D-net is in position, visually define a square shape (a "sampling plot") on the stream bottom upstream of the net opening, approximately one net-width wide and one net-width long (for a Surber sampler, this will be defined for you by the sampling device). Sampling protocol from Ode et al. 2016:

Because standard D-nets are 12 inches wide, the area within this plot is 1 sq. ft. (0.09 sq. m.). Restrict sampling to within that area. Pick up and clean all of the rocks larger than a golf ball within the sampling plot such that all the organisms attached to them are washed downstream into the net. Set these rocks outside the sampling plot after they have been cleaned. If the substrate is consolidated, bedrock, or comprised of large, heavy rocks, run your hands or feet along the substrate to displace BMIs into the net. While disturbing the plot, let the water current carry all loosened material into the net. Once the coarser substrates have been removed from the sampling plot, dig through the remaining underlying material with fingers to a depth of about 10 cm (less in sandy streams), where gravels and finer particles are often dominant. Thoroughly manipulate the substrates in the plot to encourage flow to dislodge any resistant organisms. Note: the sampler may spend as much time as necessary to inspect and clean larger substrates, but should take a standard time of 30 seconds for the digging portion of this step. To the extent practical, reduce the amount of sand particles in the net, as they damage organisms and degrade taxonomic data quality.

For slack-water habitats, vigorously sweep the surface of the subsampling area with your hands or feet while dragging the net through the disturbed area just above the bottom. Keep moving the net so that the organisms trapped in the net will not escape. Continue kicking the substrate and moving the net for 30 seconds. For vegetation-choked sampling points, sweep the net through the vegetation within a 1sq. ft. (0.09 sq. m.) plot for 30 seconds. After 30 seconds, remove the net from the water with a quick, upward motion to wash the organisms to the bottom of the net.

6. Clean the sample of excess fine silt by rising the net in the water repeatedly (without submerging the lip of the net which would allow organisms to escape), until the water running out of the bottom of the net is clear. Also inspect and remove any rocks larger than very fine gravel, as these rocks will smash organisms in preserved samples. Invert the net into a bucket of water for each subsample, until all subsamples are placed in the composite sample in the bucket.

7. Once the full composite sample is in the bucket, process the sampled material to remove coarse organic debris, sand and gravel, and excess filamentous algae. Samples can be passed through a coarse sieve, but sample elutriation works best to preserve the sample in good condition. Using a series of three or so buckets, grab coarse organic debris, larger gravel pieces, and excess filamentous algae one handful at a time, and rinse it in subsequent buckets, with the goal of removing most organisms clinging to the material. Then place the material in a tray for visual inspection and hand removal. Once all larger debris has been rinsed and inspected, elutriate the remaining material by swirling the water in the bucket so that invertebrates and fine debris are suspended, but sand and small gravel remains at the bottom of the bucket. Then pour the water and suspended material through a fine-mesh aquarium net, and invert the aquarium net into a 500mL wide-mouth Nalgene container half filled with 95% or 100% ethanol. Repeat as needed (using additional stream water) until all fine organic material and invertebrates have been washed out of the bucket, and then visually inspect the remaining sand and gravel for invertebrates (especially bivalves, gastropods, and stone-cased caddisflies). Repeat for each of the three rinse buckets until 100% of the sample is processed and preserved. After all of sample is added to container, top off with ethanol.

**Substrate visual estimation for RWB samples**

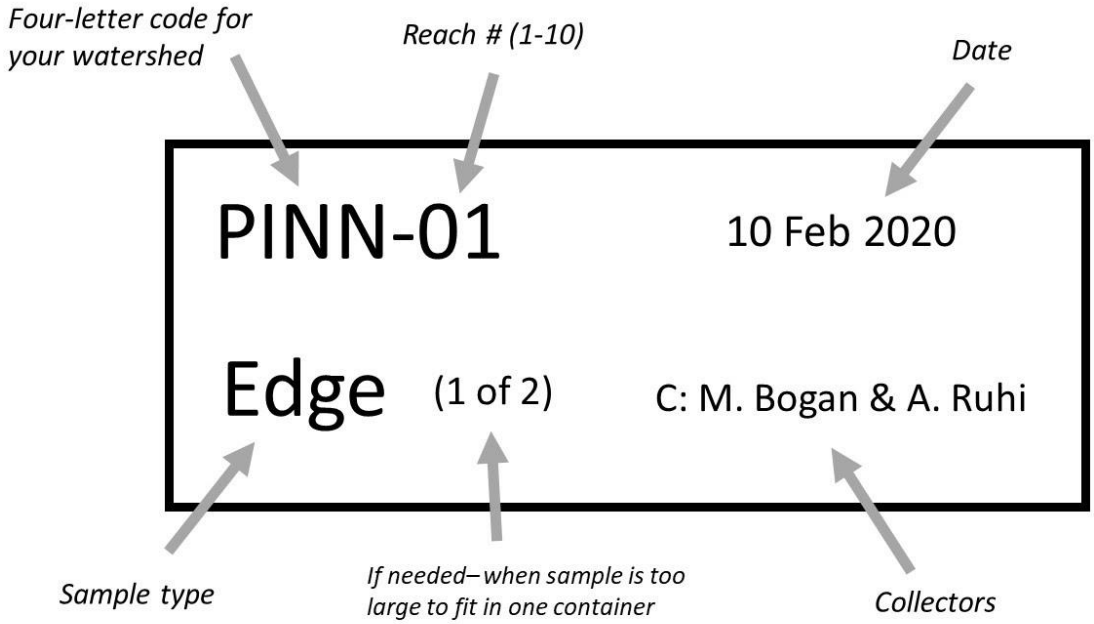
While collecting the 11 subsamples to be composited into the RWB sample, please make note of the substrate sizes and percent cover that are present. For the entire composite sampled area (1.08 sq. m.), make a rough estimate of the relative cover percentages of sediment on the Wentworth scale (i.e. silt, sand, gravel, pebble, cobble, boulder, bedrock).

**Edge samples**

1. Edge samples are comprised of a composite of five sweeps (covering ~0.33 $m^2$ each) through submerged vegetation, roots, or rocky stream margins along the banks, using a D-net with 0.246mm mesh size. The five sweeps should be spaced roughly equally along the 150 m or 250m reach, with a goal of targeting the variety of edge types present in the reach (e.g. root masses, emergent aquatic vegetation, overhanging banks). Edge samples are qualitative in nature and will be used to detect taxa that may be missed in the reach-wide benthic samples. All five sweeps can be placed together in one bucket and processed and preserved in ethanol as described above for RWB

samples. Make sure to label the Nalgene wide-mouth sample containers externally and with internal paper labels to avoid confusion between edge and RWB samples at each study reach.

**Sample labels**
Please include the site code (stream name and reach number), the sampling date, the sample type, and the sample collectors on an internal paper label (in pencil) as well as writing it externally on the Nalgene wide-mouth lid (in Sharpie). If samples are too large to fit into one 500mL Nalgene wide-mouth sample containers, also include the sample container number (e.g. 1 of 2, 2 of 2). An example label would be:




**Sample preservation in lab**
Top off container with ethanol in the field. Within 24 hours of RWB and edge sample collection, visually inspect each filled sample container. If the ethanol is dark green or brown, the organic material has used up most of the original ethanol in the container. Carefully pour the sample into a fine-mesh aquarium net, then invert the contents of the net back into the container and add fresh ethanol. Repeat as needed every 24 hours until the sample is not darkly colored and 'smells' like ethanol rather than rotting organic material.

**Sample Timing:**
Preferably, we want to sample streams when the intermittent reaches have been flowing long enough for communities to develop (30-60 days). We also want to sample streams when flow conditions will promote good sample collection, i.e. not immediately after floods or during high flow periods. Suggest min of 10-14 days after a flood, potentially longer if needed. In general the sample timing will vary from region to region. As an example, if flow is seasonal with a wetter period and a drier period then we would want to sample during the descending limb of the hydrograph during the wet season, assuming that this is a time period when intermittent reaches would still be wetted.

**Photos:** At each reach during each sampling, take a photo looking upstream from the study reach from the same location (preferably the most downstream point, 0 m, assuming that a good stretch of the reach is visible).

**Field Notes:** During each sampling event, take detailed notes describing the sampling in a field notebook. Be sure to include: watershed and reach ID, collectors, date, number of samples (‘kicks’) contributing to the reach wide

sample, recordings of the wetted widths measured, sediment relative cover percentages. Take photos of field notes and upload to cloud service (dropbox etc), daily in the field if possible and internet access is available.

**References**

Ode, P. R., A. E., Fetscher, and L. B. Busse. 2016. *Standard operating procedures for the collection of field data for bioassessments of California wadeable streams: Benthic macroinvertebrates, algae, and physical habitat*. California State Water Resources Control Board Surface Water Ambient Monitoring Program: Sacramento, USA. https://www.waterboards.ca.gov/water_issues/programs/swamp/bioassessment/sops.html

*Links to relevant materials*

Easy Catch fine mesh aquarium net:
https://www.amazon.com/Blue-Ribbon-Products-ABLEC4-4-Inch/dp/B002DZI3F4/ref=sr_1_9?keywords=easy+catch+aquarium+net&qid=1582133118&sr=8-9

16 oz./500mL Nalgene™ Polypropylene Jar with 120mm Cap:
https://www.usplastic.com/catalog/item.aspx?itemid=26935&catid=

Ward's D-frame net:
https://www.wardsci.com/store/product/20456704/ward-s-aquatic-d-frame-net

Regular Surber sampler (select 500 micron mesh size):
https://www.forestry-suppliers.com/product_pages/products.php?mi=50421&itemnum=77247&redir=Y

Mini-Surber sampler (500 micron mesh size):
https://www.forestry-suppliers.com/product_pages/products.php?mi=25150&itemnum=77456&redir=Y