

CBP Benthic Macroinvertebrate Sub-Sampling Protocol

The following protocol is meant to support a special study project that will contribute to the ongoing assessment of Chesapeake Bay Stream Health. This protocol is intended to follow the CBP Benthic Sample Collection protocol, in order to pick the sample before sending it to the Wheeling laboratory for analysis. The goal is to get a clean sample of 110 bugs (+/-10%) that are subsampled (a representative portion of the sample). This will most likely occur once or twice a year during picking days, where CMC team staff will work with volunteers to pick all the samples collected during the sampling period. The CMC team will then coordinate sample drop off with the Wheeling Lab annually.

Equipment:

- 500µm Sieve (Standard #30)
- Gridded sub-sampling bins (x4)
- Round die cutters (x8)
- Plastic spoons/pipettes
- Picking Trays (x6)
- 2 Buckets
- Fresh water (either bottles or collected from a stream)
- Wash/Spray bottles
- Forceps
- Counter
- Sample Vials
- Waterproof labels for sample jars
- Waterproof pen/pencil/marker
- Magnifiers/Glasses
- Field microscope
- Folding table (x2)
- Binder with random numbers list, protocol and sample log
- Safety gear such as gloves, sun tan lotion, bug spray, and first aid kit
- Paper towels
- Strong Flashlight (helpful not necessary)

Picking Process:

Set up the folding table making sure it is on level ground and place the sub-sampling bin on top. Prepare two buckets, one empty, for ethanol and one with freshwater water high enough that the sieve bottom can be filled with water.

Rinse the Sample:

Choose your sample jar, and pour the sample through the sieve with the empty bucket underneath to capture the ethanol. Try to only have ethanol in this bucket, so it can be reused in future sample collection. Save the sample label from inside the jar and move to the picking label, so all information can remain with the sample.

Rinse the sample in the sieve with freshwater (can use freshwater from a nearby stream or bring tap water in jugs) in the second bucket.

- Dip the base of the sieve into a bucket with freshwater and a second person can swirl contents and use a spray bottle to spray down leaves and debris to clean the sample.
- Submerge the sieve and contents just enough to cover the debris, but not enough that the sample flows out over the top of the sieve. This will remove the fine silt from the sample.
- Remove large debris after inspecting for clinging organisms.



Dump the sample from the sieve into the gridded pan. Add some freshwater and evenly distribute over the tray, making sure that the sample is not clumped up in one or two areas of the tray. Place on a flat surface (can use the folding tables) that can sit undisturbed for the entire picking process.

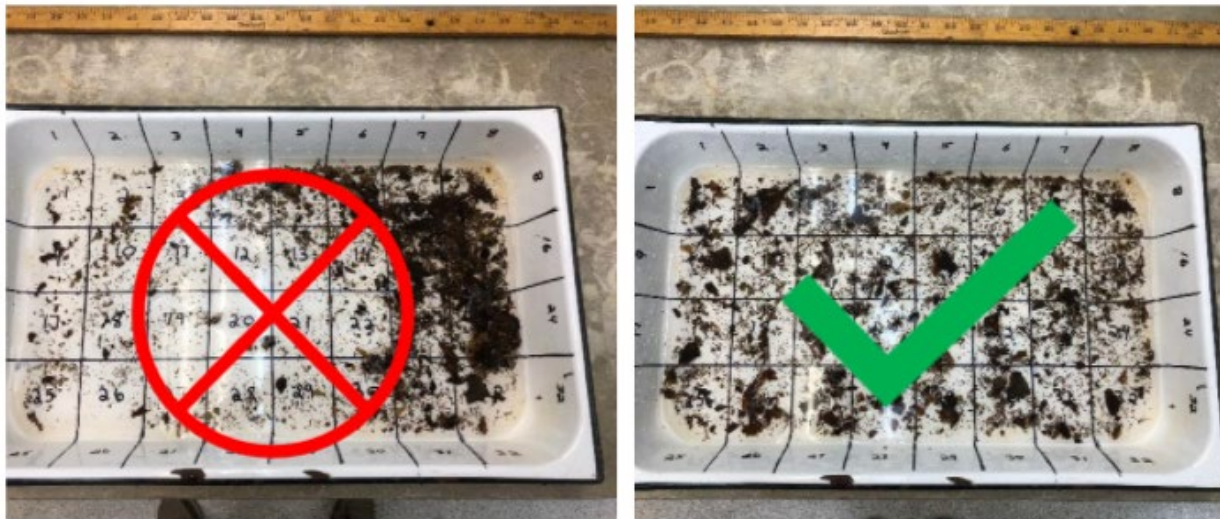


Image: From Kelly's presentation

Labeling the sample vial:

Fill the small sample container (vial) with ethanol (can reuse the ethanol from the sample jar) and the label. If the label from the original jar is too big or damaged to transfer to the vial, fill out a new label. Label should include:

- Station ID
- Stream Name
- Location (Lat/Long Coordinates)
- Sample No. - labeled the sampling date with XX number attached to it for all the samples collected that day (ie. 09262101, 09262102, etc.)

Pull random grids to pick out the bugs:

- Start at the top of the random number list, select the grid that corresponds to the first number and cross off the number from the list.
- Place the circular die cutter in the selected grid. Using the forceps gently scrap around the outside of the pipe to cut through any debris stuck underneath. Using the spoon, pipette and forceps, remove all of the contents from inside of the pipe area and place it into the picking tray (water, debris, bugs, etc). Inspect the empty grid for any remaining organisms.



- Add more water to the picking tray– enough so bugs and debris can float. (Would be good to add a picture of the sample in the picking tray)

Picking the sample:

The goal is to reach at least 110 bugs in the vial. Pull one grid at a time into the picking tray and pick completely before pulling another grid. If a sample seems of low abundance, more than one grid can be pulled from the sub-sampling bin at a time. These pulls should remain in separate picking trays and picked by different people, but can speed up the process. Refer to the flyer for what to include and what not to include.

1. Place the uncapped vial with ethanol into the vial holding rack.
2. Start picking the sample and putting the bugs into the sample vial. Use the counter to keep track of the number of bugs in the container. Ideally grab large, obvious, and floating bugs first. Then search the tray, going in thirds lengthwise to sift through the debris for smaller bugs. (Kelly powerpoint has great photos for this)
3. After the first pass, gently mix the sample to redistribute the sample and repeat the search. Spraying with water can help redistribute the sample or show a bug where you didn't see one before.
4. If you are unsure if something is a bug, check it under the microscope to determine if it should be added to the sample.
5. Once you haven't found a bug for 5 mins, swap with another person and have them do a second check for more bugs. A CMC team member should do the last check to see if there are any remaining bugs in the sample tray. Once there are no more bugs to pick, that grid is completed.
6. Dump the remaining debris and water out of the picking tray and rinse so it is ready to be used again. If you have not reached 110 bugs in the sample, pull another grid from the sub-sampling bin. Use the next number on the random number generator to choose the next grid and repeat steps 2-5.
 - a. Continue down the random numbers list and pick more grids as needed until you have reached at least 110 bugs in the sample. If you hit a number on the random number list that you have already picked, skip that number.
 - b. If you reach 110 bugs in the middle of picking a grid, finish what is on your picking tray.
7. Close the vial and record the number of grids sampled (ie. 6 out of 35 in the sample picking log book and in pencil on the vial cap.



The sub-sample vials will remain with the CMC service provider and be stored in a cool dry place away from direct sunlight or flammable materials. Vials should be transferred to the Wheeling Lab or designated drop off location and include a copy of the log for the samples included in that batch.

Clean up:

- Dump all remaining sample in the sub-sampling bins and picking trays and rinse with freshwater. *Do not dump directly into a stream or waterbody*
- Dump the bucket of freshwater and rinse off the sieves.
- Dump the used ethanol into an empty ethanol container to reuse for other samples.

Example Log:

Sample Collection					Sample Picking			
Station ID	Stream Name	Latitude	Longitude	Collection date	Sample No.	Final count	# of grids	Picking date and Initials

Chain of Custody to Wheeling Lab

Date Transferred: _____ Location: _____

CMC Team Signature: _____

Wheeling Lab Signature: _____