CBP BENTHIC MACROINVERTEBRATE SAMPLE COLLECTION PROTOCOL

CMC
Chesapeake Monitoring Cooperative

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Background

The following protocol is meant to support a special study project that will contribute to the ongoing assessment of Chesapeake Bay Stream Health. The current watershed-wide assessment utilized a 2006–2017 Index of Biotic Integrity (IBI) dataset and identified many areas of the watershed that have little or no data. The purpose of this project is to fill these data gaps by targeting the areas of the watershed with little or no data for macroinvertebrate sample collection.

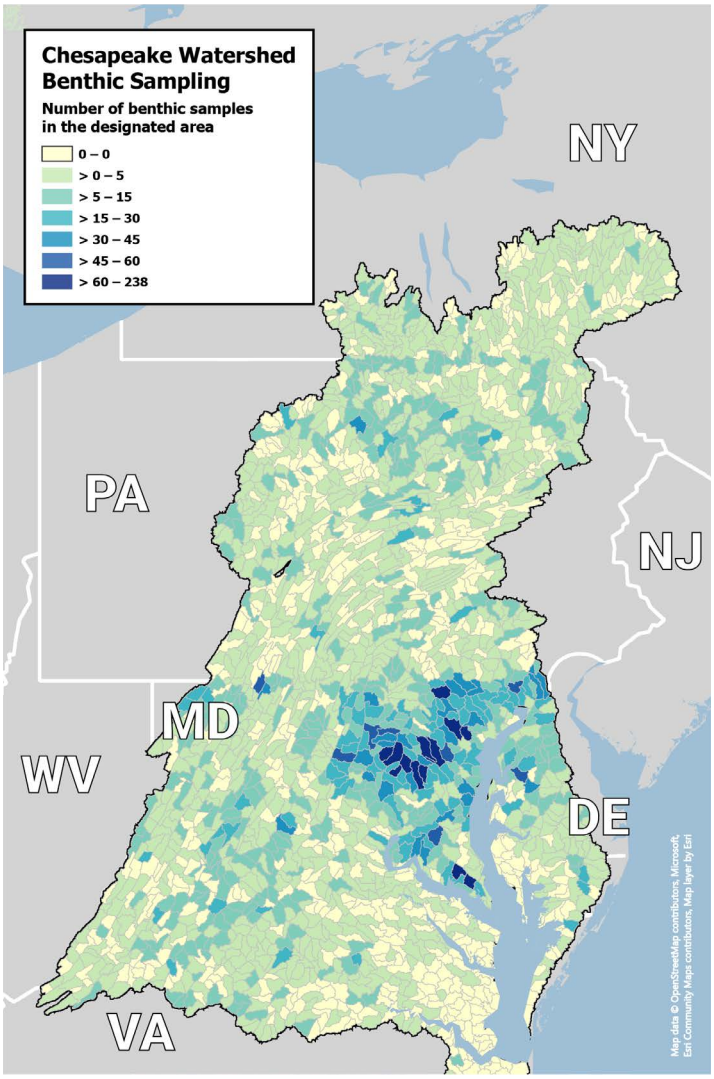
Your sample collection and the results produced from this special study on macroinvertebrate diversity will help extend the Chesapeake Bay watershed area coverage of the Stream Health assessment. Sampling at single sites in target locations is of interest, however, multiple sites in these catchments (two or more) would be welcome. Adopting one station to sample for enhanced diversity assessment each year would provide added insights on trends in the response of our watershed to changing management and climate conditions over time.

The sampling process requires the collection and retention of the macroinvertebrates (organisms) in the sample. The entire sample (not picked) will be preserved for later processing in a lab. Importantly, the organisms in the sample will be identified and classified down to the family level (at a minimum, with some bugs identified to genus or species level) in order to be included in the IBI stream health assessment used by the Chesapeake Bay Program partnership. The family-level identification results from your sample will be shared with you within a year for each site. The final data will become part of the history documented in the CMC Chesapeake Data Explorer database.

Background

SITE SELECTION

This project is collecting samples from historically un-sampled or undersampled watersheds according to the Chesapeake Bay Program Stream Health Benthic Sampling Map, sampling first to fourth order headwater streams throughout the Chesapeake Bay watershed. This project will monitor approximately 20 sites annually, with a goal of reaching 100 sites by 2027. All monitoring sites for a given year will be determined by the CMC team in coordination with a local monitoring group prior to training and sample collection. Most sites will be monitored once throughout the six year period, however, some sites may be selected for additional sampling.



This project is targeting unsampled or undersampled watersheds throughout the Chesapeake Bay watersheds (White or Light Blue watersheds).

SAMPLE TIMEFRAME

Monitoring for this project will occur from Spring 2022 through Fall 2027. Samples are collected in the spring (March-May) and fall (September-November) of each year. Samples can be collected on any day of the week or month as determined by the sampling team. Be sure the sampling date is at least 48 hours after a rain event. Each sampling team will pick up all equipment from their regional CMC service provider prior to sampling, and return all equipment with their collected sample after sample collection.

SAMPLE PROCESS

During this process you are going to collect six sub-samples that will be combined to yield one sample. The six sub-samples should be collected within a 100 meter reach of the selected stream and should capture a variety of riffle-run habitats.

Supplies

EQUIPMENT LIST

- D-net with 500 µm mesh
- Sieve bucket with 500 µm mesh or DIY equivalent
- Collection container (to store preserved sample)
- 95% Ethanol preservative
- Spray or squirt Bottle (for cleaning the D-net into the jar)
- Forceps
- Waterproof/Rite in the Rain labels for sample jars
- Waterproof pen/pencil/marker
- Boots (no felt soles!) to get into the stream
- Tape/gallon plastic bags (secondary containment, to make sure the jars don't leak)
- Safety gear such as gloves, hand sanitizer, and first aid kit
- Flagging tape
- Measuring tape
- Thermometer
- Data sheet
- Sampling manual

All of the equipment that will be included in your monitoring kit provided by your CMC service provider.

Sampling Method

STEP 1: IDENTIFY YOUR STREAM REACH

Upon arrival at a sampling site, first you will determine a 100 meter reach from which you will be collecting your sample and evaluate the reach for suitable riffle-run habitat. The selected location should be wadeable with riffles and at least 30 meter (100 feet) upstream from the nearest bridge or culvert.

Walk along the bank to measure out 100 meters to determine your reach. Mark or note the starting (downstream) and end (upstream) points, a transition spot between riffle-run habitat and a pool can be a good starting point.

Picture A is bad because the water is too high, moving too fast and there is no accessible bank. Picture B is good because the water is low, there are plenty of riffles, and a safe bank for access.

STEP 2: IDENTIFY SIX SUB-SAMPLING LOCATIONS

Use the following considerations to find your six sub-sampling locations within that reach:

- Make sure there are at least two riffles where you will be able to rub cobbles with your hands and disturb the substrate beneath the cobbles (ie. does not have bedrock).
- Look for a variety of riffle-run habitats including different velocities and stone sizes as these microhabitats are important to different species. Kicks on the 100 meter reach should be distributed and representative of the riffle-run habitat (slow flowing shallow riffles and fast-flowing deeper riffles).
- Look for locations throughout the width of the stream to include left-descending, middle and right-descending areas.

Sampling Method

Example Stream Reach: On this map, you will see the beginning and ending markers of the 100 meter stream reach. The sub sampling locations are not evenly distributed, some are clumped together, and some are further apart. Within the 100 meter stream reach, there will be riffles (the rocky, shallow sections with rippling and bubbling over the surface, where we do our sampling) and runs and pools, where the water runs fast and slow, but has less available habitat for benthic macroinvertebrates.

Riffles can be identified by bubbling or rippling water, going over the surface of cobbles and rocks, like the spots highlighted in these pictures. Samples should be collected from riffles large and small, faster and slower, and in the left, middle, and right sections of the stream. As you can see from the highlighted riffles in these example photos, there are often many more riffles to choose from than needed for this protocol. Use your best judgement to capture a variety of riffles in your sample.

STEP 3: TAKE THE WATER TEMPERATURE MEASUREMENT

Assuming there are no significant issues with the stream reach, head to the most downstream point of your reach to start collecting your sample.

Start in a pool just below your first riffle and take your water temperature reading using the digital thermometer in your sampling kit (only take one temperature reading at the downstream end of your reach).

1. Remove the black plastic covering from your thermometer.
2. Hold the thermometer by the white plastic piece and dip the metal into the stream. Be sure to take the reading from a flowing section of the stream and avoid any standing water or eddies.
3. Wait for the thermometer to stabilize (reading doesn't change for approximately 10 seconds, this may take 1-2 minutes) and record the reading in celsius on your field datasheet.

Take the water temperature in the middle of the channel.

Sampling Method

STEP 4: COLLECT YOUR FIRST SUB-SAMPLE

Start at the downstream end of your reach and work upstream subsampling the riffle-run habitat when collecting your sample. Check your net and bucket to make sure there are no holes or tears in the mesh.

The collection process used is referred to as a “kick”. A kick is done to disturb the sample area 2–4 inches down into the substrate. The sample area for a single kick is one width of the net and two net widths upstream. One kick is done for 60 seconds total. Spend 40 seconds rubbing by hand any large sticks and/or stones from the disturbed area to dislodge any tightly clinging organisms, place all rubbed rocks off to the side of the sample area. Then use a flat rock to disturb lower rocks and substrate for 20 seconds so that the water runs brown. DO NOT use your feet, since people often grind down, instead of kick up—a flat rock is easier to use correctly. To complete a double wide kick, repeat this process directly adjacent to the first spot.

Start your kick at the downstream location, it is helpful to have three people, one to hold the net, one to do the kick, and one timekeeper.

Sampling Method

1. Properly place the D-net—one partner should rest the D-net along the bottom of the stream facing upstream while the other stands directly in front of the net. Ensure that there are no gaps underneath the net where the sample could be lost.
2. When everyone is set, start the timer for 60 seconds and start the kick process.

Make sure the D-net is flush against the bottom of the stream bed and facing upstream to perform your kick.

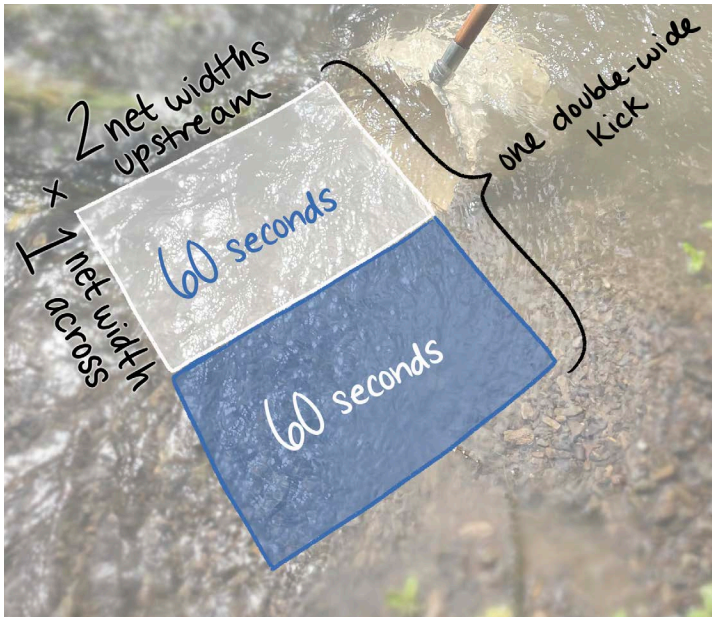
Kick Process

For the first 40 seconds, use your hands to pick up and rub rocks in the sampling area to dislodge macros clinging to them. The time keeper will indicate when 40 seconds is up.

For the last 20 seconds, disturb the substrate in front of the net down 2-4 inches using a stone or handheld shovel/ rake, trying to get the water to run a muddy brown.

Sampling Method

3. The targeted area should be the width of the net across, and two net widths upstream.
4. Once the 60 seconds are over, move the net directly beside where you just sampled and repeat the process—these combined two samples constitute the “double-wide” procedure.
5. Carefully lift the D-net out of the water, ensuring that the opening in the net is facing upwards.



The targeted kick area should be the width of the net across, and two net widths upstream.

To watch all of the steps, please refer to the CBP Benthic Macroinvertebrate Collection Protocol Video.

After your kicks, the contents of the net should have debris, leaves, and bugs.

Sampling Method

STEP 5: EMPTY THE NET INTO THE BUCKET

Empty contents of the net into the bucket after every double-wide kick. If there isn't a lot of debris in the sample, you may be able to empty the contents after two or three kicks.

1. Sit the sieve bucket into the stream so that it is about half full of water. You can use a pool in between the riffles, just be sure to go to a riffle downstream of you if you have not finished your kicks within the current riffle.
2. Carefully dump the contents of the net into the sieve bucket.
3. Carefully turn the net inside out and rinse any missed part of the collection into the bucket.
4. With a squirt bottle filled with stream water, rinse the net into the sieve bucket to get any last clinging part of the sample.
5. Double check the net to make sure you didn't miss any organisms, hand pick or rinse any additional organisms into the bucket.

Once the net is cleaned, repeat this process five more times moving upstream with each kick. Remember, these kicks should be done in at least two different riffles within your reach and should cover the left, middle, and right of the stream, as well as slow and fast moving riffles. Keep your bucket with you and continue to empty the contents of the net into the bucket as you move along.

Sit the sieve bucket into the stream so that it is about half full of stream water.

Dump the contents of the D-net into the bucket, turn the net inside out and rinse into the bucket. You can use a squirt bottle filled with stream water to help rinse.

Use the spray bottle filled with stream water to rinse off the net into the bucket. Visually check the net to make sure you didn't miss any bugs clinging to the net.

Sampling Method

STEP 6: FINAL RINSE AND BUCKET CHECK

Once all of your kicks have been completed, do a final rinse of your net into the bucket and check the sample bucket for large debris and species that should not be included in the sample.

If you remove anything from the sample, be sure to inspect it closely for macros. Rinse their surfaces with stream water over the bucket, and then remove them from the sample.

Remove: fish, mussels, salamanders, turtles, large sticks, stones over 3 cm, and any trash.	**Keep:** Crayfish, all leaf litter, and small sticks and pebbles.
	

Once you have your final sample, gently wash the sides of the sieve bucket with stream water to get everything to the bottom of the bucket.

Dump the remaining water out of your spray/squirt bottles, take all your equipment and your bucket and move out of the stream.

NOTE

If you have collected crayfish, check on the bucket periodically to ensure they are not climbing out.

Sampling Method

STEP 7: TRANSFER YOUR SAMPLE

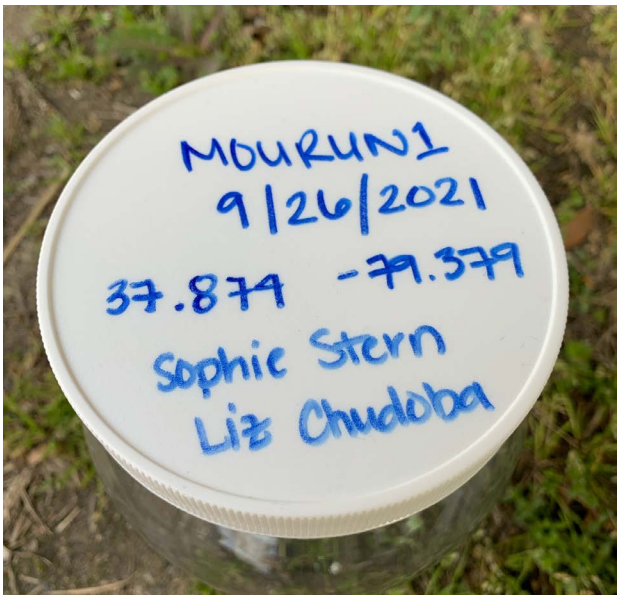
Move to an easily accessible place, such as the shore of the stream or to a parking lot area, in order to transfer the sample to the sample jar.

Before any organisms are placed into the collection container, fill out and insert the waterproof label into the collection container to avoid harming the sample later on. Using a pencil, fill out the label provided to you with your sampling kit (should be a small piece of rite-in-the-rain paper) with the following information.

Station ID	Date (m/d/yyyy)
MOURUN1	9/26/2021
Latitude (decimal degrees)	**Longitude (decimal degrees)**
37.874	-79.379
Certified Monitor(s)	
Sophie Stern and Liz Chudoba	

Example Label: Everything on the label should match your datasheet exactly. The Station ID is provided by your CMC service provider.

Using a sharpie, write the same information on the lid of the sample jar.



Fill your spray or squirt bottle with ethanol and put on your nitrile gloves. Empty all contents of bucket into the sample jar provided to you using one of the following methods.

Sampling Method

From the Sieve Bucket:

1. Use your hand (with gloves) to carefully transfer the bulk of the materials from your bucket into the sample jar. Get as much of the large pieces into the jar as you can.
2. Use the spray bottle filled with ethanol to wash the sides and bottom of the bucket to get the remaining smaller debris and bugs into the jar. **All rinsing during the transfer process should be done with ethanol; using stream water will dilute the preservative.** This will take some time and you may need to spray from multiple angles to get everything into the jar. Make sure to spray from the backside through the bottom of the bucket to get any bugs that may have gotten caught in the bottom of the bucket. You can use the forceps or your fingers to pick up individual bugs and transfer them to the jar.
3. Keep checking the bucket until you don't see any more moving bugs, there may still be some minimal debris left in the bucket.

Using your hands, carefully move the bulk of the materials from your bucket into the sample jar.

Using the spray bottle filled with ethanol, wash the sides and bottom of the bucket to get the remaining smaller debris and bugs into the jar.

Sampling Method

From the DIY Bucket:

1. Remove the mesh bag from the bucket.
2. Gently grab the bag just above the debris and roll the sides of the bag down. Turn upside down into the sample jar and push the bag and the contents into the jar.
3. Gently pull the mesh bag out as you pour ethanol through the bag into the sample jar to wash the debris and bugs off.
4. Once the bag is completely out of the sample jar, inspect for bugs. Either pull them off with your fingers or forceps, or place the mesh over the opening of the jar and use the ethanol squirt bottle to gently wash any remaining contents into the jar. Repeat the process until all the bugs are in the jar.

Gently grab the mesh bag just above the debris.

Roll down the sides of the bag over your hand. Turn upside down and gently push the bag into the sample jar.

Gently pull the mesh bag out of the sample jar and pour ethanol through the bag to wash the bugs into the jar.

Sampling Method

Troubleshooting:

Getting all of the contents out of either type of bucket can be difficult; using a white bin and sieve can help get the last contents of the bucket or mesh bag.

1. Holding the DIY mesh bag or sieve bucket upside down over a white bin, use a bottle of water to rinse the contents of the bag or bucket into the bin—this can be done several times until the bag or bucket are totally clean.
2. The bin, now a mix of water and the sample, can be tipped and run through a 500 micron sieve. It is easier if the sieve is held at an angle so the sample is collected in a corner that can easily be transferred into the sample jar with ethanol.
3. Water can be run back and forth between the bin and the sieve several times to get all remaining contents available for the sample jar. Contents in the sieve should be transferred into the sample jar with ethanol.

A white bin and a bottle of water can make removing all debris and organisms from the net easier.

Pouring the contents from the white bin through the sieve concentrates the debris and organisms in the sample.

Once all of the contents of the bin are on one edge of the sieve, they can be more easily transferred to the sample jar.

Sampling Method

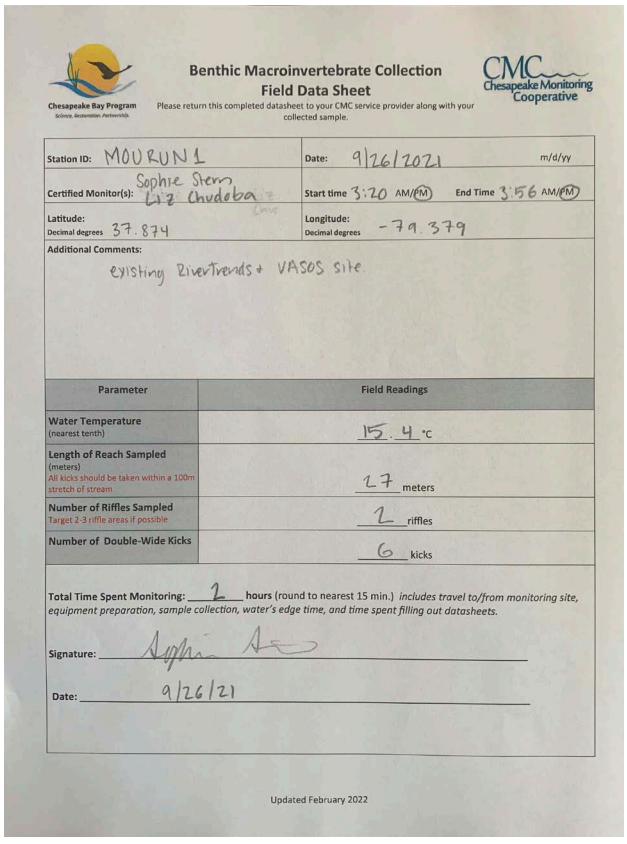
Final Steps:

1. Check to make sure that the label is in the container, add more ethanol until you have doubled the volume of the solids alone.
2. Close the lid well. If needed for transport, secure the jar with duck tape and place it in a plastic bag.

Your completed sample!

STEP 8: FILL OUT YOUR DATASHEET

Fill out the front and back of your data sheet before you leave your location. On the front fill out all of the information about your sampling day.



Chesapeake Bay Program

Benthic Macroinvertebrate Collection Field Data Sheet

Please return this completed datasheet to your CMC service provider along with your collected sample.

CMC Chesapeake Monitoring Cooperative

Station ID: MOURUN1	Date: 9/26/2021 m/d/yy
Certified Monitor(s): Sophie Stern, Liz Chudoba	Start time 3:20 AM/PM End Time 3:56 AM/PM
Latitude: Decimal degrees 37.874	Longitude: Decimal degrees -79.379

Additional Comments: existing RiverTrends & VASOS site

Parameter	Field Readings
Water Temperature (nearest tenth)	15.4 °c
Length of Reach Sampled (meters) All kicks should be taken within a 100m stretch of stream	27 meters
Number of Riffles Sampled Target 2-3 riffle areas if possible	2 riffles
Number of Double-Wide Kicks	6 kicks

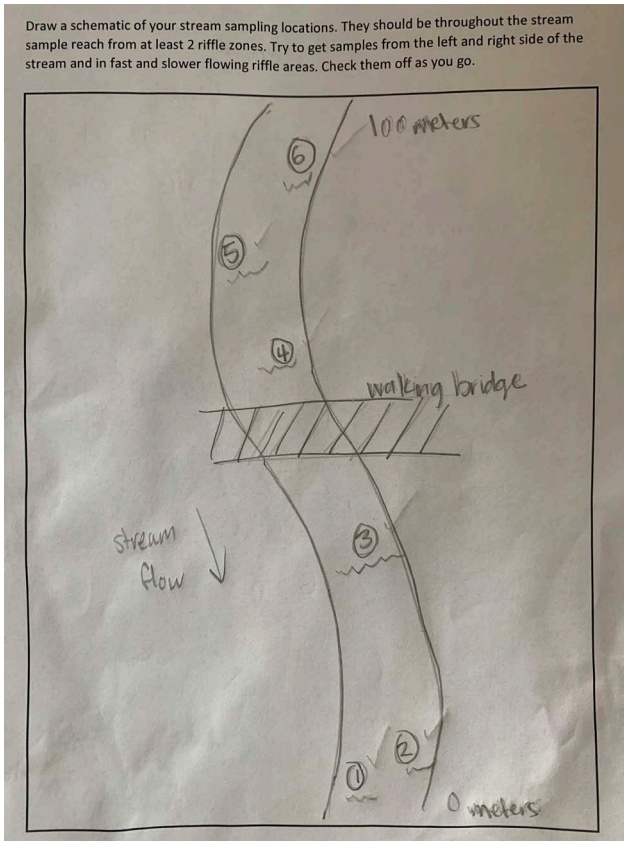
Total Time Spent Monitoring: 2 hours (round to nearest 15 min.) *includes travel to/from monitoring site, equipment preparation, sample collection, water's edge time, and time spent filling out datasheets.*

Signature: Sophie Stern

Date: 9/26/21

Updated February 2022

On the back of the datasheet include a stream diagram. Be sure to label the riffles, each kick location, and the length of stream reached.



STEP 9: CLEAN UP

Gather all of your supplies and equipment. Clean all equipment—nets, bucket, boots, etc.—by washing them off in the stream. If you're not going to another stream that day, your equipment and boots just need to be dry for 48+ hours prior to another sampling event. This drying ideally happens in the sun which can act as an excellent disinfectant.

If you're going to another stream that same day, you must decontaminate your equipment before leaving the site using either a 10% bleach solution (nine parts water to one part bleach in a spray bottle) or Alconox (a biodegradable soap product dissolved in water in a spray bottle). Spray down boots, nets, and all other gear at least 50 feet away from the stream, and scrub with a bristled scrub brush. Dry gear with a towel before entering the next stream site.

STEP 10: STORAGE AND RETURN

Please keep samples stored in a cool dry place away from direct sunlight or flammable materials. Bring your sample(s) and all equipment to a collection event or coordinate pick-up with a CMC Service Provider within six months of sample collection.

If you are not going to another stream today, wash your boots by scrubbing in the stream water and let air dry.

Wash all buckets, net, and mesh bag in the stream water before leaving the site.