**Collecting and filtering eDNA samples – BOP Teacher Training**

*General*

\*\*All sampler identification information and other field data will be recorded on the Field Collection Summary sheet.

\*\*Water samples must be collected prior to sediment samples at each site.

\*\*Sampling should proceed in an upstream diagonal direction. The direction traveled for sampling should be recorded on the Field Collection Summary sheet.

\*\* Equipment will be decontaminated between samples by submerging/scrubbing all non-disposable pieces with a solution of water and 10% bleach.

Material: 1L polypropylene Nalgene bottles (wide-mouth are best); filtration apparatus and pump; ziploc bags or falcon tubes for storing filters; 45mm glass fiber filters; falcon tubes (sediment only); scoopulas (sediment only); bleach for sterilization; cooler with ice (if in the field)

*Water*

1. Sample labels should be affixed to the outside of sterile 1L bottles prior to going into the field. Bottles will be labeled with an appropriate ID that does not indicate location (to allow blind processing). We generally use a numbering scheme as follows: YYYYMMDD-SSS, where YYYY is the year (such as 2015), MM is the month (such as 11 for November), DD is the day (such as 13), and SSS is the sample ID number (such as 001, 002, 003, etc). An example for a sampling event scheduled for 11 June 2009 would be: 20090611-001, 20090611-002, etc. The SSS numbers will be consecutive starting from 001 and increasing to the maximum number of samples taken that day (999).

2. When arriving at a sample location, the lead sampler and sampling assistant 1 should put on sterile exam gloves (powderless latex or nitrile). REMINDER – Gloves must be changed before each new site to prevent cross contamination. The same gloves may be worn when collecting duplicate or blank samples in tandem with a regular sample in a transect.

3. Going in consecutive numerical order based on the bottle labels, the lead sampler will remove a labeled 1L sample bottle from the sample cooler. Just prior to collecting the sample, the lead sampler will unscrew and remove the lid from the sample bottle. The lead sampler will then reach over the upstream side with the 1L sample bottle and fill the bottle by skimming the surface of the field water.

4. Once the sample bottle is filled (approximately 1 in. of space should be left within the sample bottle), the lead sampler will screw the lid back on to the bottle until it is tight. The closed bottle will then be returned to the sample cooler.

5. While the lead sampler is collecting the water sample, sampling assistant will take habitat measurements: water temperature, approximate depth, GPS coordinates in Decimal Degrees, time of sample, location (e.g., east bank, center of transect), notes on habitat or other (eg over fine gravel) and record the information on the datasheet next to the appropriate sample ID.

6. Negative control samples should be taken at each site as follows: the sampler will unscrew the lid and remove to expose the bottle contents to the atmosphere for 5 sec, reseal the bottle, fully submerge the bottle in the field water, and return the bottle to the sample cooler from which it was removed. The lead sampler should relay to sampling assistant that the sample was a blank, so that it can be recorded on the data sheet next to the appropriate ID.

7. Once sampling is complete, ice should be added to the sample coolers as soon as possible. Enough ice should be added to each cooler to completely surround each sample bottle and maintain an inside temperature of 4.4°C. If at any time during transport the inside temperature of the cooler(s) rises above 4.4°C, additional ice should be added.

**Filtration:**

\*\* Filtration equipment should be decontaminated between samples by submerging/scrubbing the filter frit, clamp, and open beaker (the three topmost pieces of the setup) with a solution of water and 10% bleach, then rinsing with deionized water. It is not necessary to dry equipment between samples.

\*\*Before beginning, label clean and sterile falcon tubes, Ziploc bags or Powerwater bead tubes with the appropriate sample names.

\*\*For water samples that contain a lot of algae or sediment, it may be necessary to filter 1L water in 2-3 batches in order to move all the sample volume through.

Set-up: Ensure equipment is sterilized via bleach cleaning or UV exposure. Attach the filter frit to the Ehrlenmeyer flask and attach the vacuum pump hose. Using sterile forceps, place a clean 45mm filter (glass fiber) on the filter frit. Check that it is centered properly before placing the beaker on top and then clamp the apparatus together using the metal clamp. Slowly pour the water sample into the open beaker and turn on the vacuum pump. Continue to allow a steady but slow stream of water into the beaker until the sample is done. Wait until the sample has flowed completely through the filter but do not allow the pump to continue running once the water has flowed through.

Use sterile forceps to remove the filter, fold it with the exposed surface on the inside, and place it in a clean and labeled Ziploc bag, falcon tube or a PowerWater bead tube. Clean the equipment and move on to the next sample.

*Sediment*

1. Sediment collections will be taken using either use Wildco sediment corers fitted with sterile, disposable plastic liners or sterile scoops/15-50mL Falcon tubes (depending on availability/depth of soft sediment). Sampler will use sterile gloves. Taking as much care as possible not to disturb surrounding sediment, the sampler will use a sterile 15-mL or 50-mL collection tube to scoop sediment from the top 2 cm of the sediment surface. Tubes should be labeled as above with an additional SED on the end of the sample name.

2. Tubes will be stored in their own sterile Ziploc bags before being placed into the cooler for transport back to the lab.

3. Negative control samples will be taken at each site, following the same protocol listed above for water samples: the sampler will unscrew the lid and remove to expose the bottle contents to the atmosphere for 5 sec, reseal the bottle, fully submerge the bottle in the field water, and return the bottle to the sample cooler from which it was removed. The lead sampler should relay to sampling assistant that the sample was a blank, so that it can be recorded on the data sheet next to the appropriate ID.