Revised 09/30/15

BIOLOGICAL ASSESSMENT 2015/2016
PROTOCOL & VOCABULARY

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INTRODUCTION: WHAT IS BIO ASSESSMENT?

Biological Assessment collects and analyzes aquatic macroinvertebrates in the creek. The quantity and variety of macroinvertebrates indicate the overall health of the creek, dependent on the different toxicities of the water.

VOCABULARY

Even before you go out to the creek, it's important to use the correct terminology for the materials and methods that we use in Bio Assessment.

- **Benthic layer -** the topmost layer (6 in) of sediment in the creek bed
- **EPT taxa -** the number of taxa in only the following three families: Ephemeroptera (mayflies), Plecoptera (stone flies), and Trichoptera (caddis flies)
- **EPT index -** percentage of EPT macroinvertebrates found in riffle samples; add the number of organisms in the caddisfly, mayfly, and stonefly orders; divide by the total number of organisms; multiply by 100%
- **Functional feeding group composition -** percentage of macroinvertebrates in a particular feeding group found in riffle samples; add the total number of with similar designation; divide each group by the total number of organisms; multiply by 100%
- **Indicator species -** a biological species whose presence or absence indicates the general health of its environment, or changes in its environment; macroinvertebrates that indicate the health of the creek, since different macroinvertebrates are able to survive at different toxicity levels in the creek
- **Macroinvertebrate -** invertebrate (organism without a backbone) that can be seen with the naked eye; lives in the benthic layer of the creek; species indicates the health of the creek
- **Point sampling -** gathering all samples from the same riffle
- **Non-point sampling -** gathering all samples from different riffles
- **QAQC -** Quality Assurance, Quality Control; a technique practiced every semester after riffle analysis to verify the exact number and taxa of each macroinvertebrate found (see QAQC protocol)
- **Relative abundance -** estimation of the total number of macroinvertebrates in a riffle sample. Add the actual number of organisms found in the fully processed grids, divide that number by the number of grids used to collect the organisms, multiply by the total number of grids in the pan

- **Riffle -** disturbance in the water caused by an obstruction of water flow (i.e, rocks, sticks, etc.); water churns over the object, creating an air pocket
- **Riparian corridor -** from an aerial view, the green along the banks of the creek; indicates existence of water
- **Taxa richness -** total number of taxa identified
- **Tolerant taxa index (value) -** the mean tolerance value of all macroinvertebrates found in riffle samples; multiply the t value (0 -10) for each taxon of organisms found in the sample by the number of organisms in that taxon; add these values together; divide by the total number of insects. (NOTE: Highly intolerant organisms are indicated by a tolerance value of 0, 1, or 2; highly tolerant organisms are indicated by a tolerance value of 8, 9, or 10)

PROCEDURES AT THE CREEK

Equipment for Creek Sampling

- D-shaped Kick Net (0.5 mm mesh)
- Hand Rake
- 3 Wide-Mouth Plastic Sampling Jars
- 95% Ethanol
- Masking Tape
- Marker
- Waders (or boots, depending on rainfall and level of creek water)

STANDARD PROTOCOL TO COLLECT RIFFLE SAMPLES

1. Before going to the creek, label each sampling jar (using the masking tape and marker) with the following: site (A-G), sample # (1-3), and month and year of collected riffle (i.e., May 2016).
2. At the creek, indicate the transect # from which the samples will be taken on each sampling jar.
3. Starting downstream, place the D-shaped kick net into the creek (preferably along the side margins or the center of the stream), with the current flowing into the net. (NOTE: Always approach riffles from downstream, so the riffle is not disturbed during the sampling process.)
4. In a 1' x 2' (0.3 m x 0.6 m) space in front of the kick net, use the hand rake to agitate the top 6" of the creek's benthic layer upstream (to loosen the macroinvertebrates inhabiting the benthic layer).
5. Pick up and discard any large rocks and/or other obstructions from inside the kick net, washing off any large organic material and inspecting for clinging organisms (to make sure all macroinvertebrates are included in the collected riffle sample).

6. Pour the loose sediment inside the kick net into one of the sampling jars, filling approximately 3/4 of the jar. Filter water through the kick net to make sure all loose sediment on the sides of the net is collected and placed into the sampling jar as well.
7. Fill the rest of the sampling jar with 95% ethanol and close the lid.
8. Repeat this process for the other two riffle samples.

SMALL AREAS OF STAGNANT WATER (SASW) PROTOCOL

If the creek has no running water but does have small areas of stagnant water, proceed with the SASW protocol.

1. Mark SASW along with the site (A-G), sample # (1-3), and date of collected riffle (month and year) on the label of each sampling jar.
2. Locate suitable stagnant pools of water.
3. Use the wide-mouth jars to scoop up the benthic layer. Fill ¾ of the jar with samples from the benthic layer.
4. If possible, strain the sediment first using the D-shaped kicknet. If you use the kicknet, filter water through the kicknet to get as much sediment off of the sides as possible.
5. If there is more than one pool of stagnant water, then apply non-point sampling.
6. If only one pool of stagnant water is present, then apply point sampling.
7. Fill the rest of the jar up with 95% ethanol.
8. Repeat this process for the other two samples.

PROCEDURES IN THE LAB

Equipment for Riffle Analysis

- 10x dissecting microscope
- Gridded White Enamel Pan
- Forceps
- 95% Ethanol
- Plastic Wrap
- Plastic Petri Dishes
- Plastic White-Topped Vials
- Macroinvertebrates Reference Books
- Putty knife
- Write-in-Rain Paper
- Pencil (NOT PEN)
- Wash Bottles

RIFFLE ANALYSIS PROTOCOL

1. Evenly distribute the contents of one riffle sample into the white enamel pan, rinsing the sample with 95% ethanol.
2. Choose grid numbers of the white pan to be examined using a random number generator chart (or die). Examine the corresponding grid for any macroinvertebrates as detailed in the following steps.
3. Use a putty knife to scoop a minute portion of the sample into a clean Petri dish; fill with water.
4. Place the Petri dish under the dissecting microscope. Using the reference books when needed, find macroinvertebrates by poking around the sample with forceps.
5. Continue this process until 100 macroinvertebrates have been collected, or the entire riffle sample has been analyzed. If no macroinvertebrates have been found after analyzing 10 squares, then empty the tray and begin on the next riffle sample.
6. Store all macroinvertebrates in a plastic jar as you find them; make sure all macroinvertebrates are fully immersed in ethanol. Label the jar with masking tape according to the month and year, site, and riffle number.
7. When all macroinvertebrates have been found, proceed with classification. To classify, compare macroinvertebrates with the families in the reference books; once the macroinvertebrates are identified, store each identified family of macroinvertebrates in a glass vial. Write the following on the write-in-rain paper (WITH PENCIL) on both sides: family name, site, riffle #, and month and year sampled. The number of organisms from each family should be continuously recorded by the group on a separate sheet of paper.
8. Calculate taxonomic information using appropriate equations and paperwork.
9. Any uncompleted riffle samples should be filled with ethanol and covered with plastic wrap to prevent contamination.

QUALITY ASSURANCE QUALITY CONTROL (QAQC) PROTOCOL

1. After all the riffles samples have been analyzed, exchange identified macroinvertebrates with another site.
2. After exchanging, each site should use the keying manuals to ensure that the quantity and identification of the macroinvertebrates are correct. (NOTE: Do not tell the other site what your findings are. They are supposed to identify the macroinvertebrates independently.)
3. Record findings and compare with the results found by the original site members.
4. If a discrepancy occurs, QAQC with another site.
5. Sort the macroinvertebrates into correctly labeled vials. If the labels were correct in the first evaluation, the return the macroinvertebrates to those vials. Fill new vials and make new labels for identified macroinvertebrates if necessary.

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BIOLOGICAL ASSESSMENT 2015/2016
HESTER-DENDY PROTOCOL

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The following list of materials and protocol is credited to the California Department of Pesticide Regulation. (http://www.cdpr.ca.gov/docs/emon/pubs/sops/eqwa006.00.pdf)*
**The following protocol needs to be and will be adjusted to fit the Arcade Creek. The following is to give a general overview of how the HD Samplers are used.*

MATERIALS

- Square Hester-Dendy (Fourteen 3" (7.5 cm) square plates and 1" diameter spacers. Total sampling area is 16 cm.)
- 3 to 5 metres of paracord
- S-binder
- Flagging tape or markers
- Wide-mouth plastic sampling jars
- 95% ethanol
- Waders
- D-shaped kick net

LOCATION SELECTION PROCEDURE

1. Choose collection sites as stated in study protocol.
2. Within the sites chosen, decide on a location to place the Hester-Dendy (H-D) sampler.
3. The H-D sampler should be placed in an area in which low watermark will not expose sampler. If possible place the H-D near vegetation.

H-D DEPLOYMENT PROCEDURE

1. Attach S-binder to H-D.
2. Tie a "surgeon's end loop"* with paracord to S-binder.
3. While keeping a firm hold on the end of the paracord, throw (or place) the H-D in the water so that:
    a. If the water level falls the H-D will not become stranded on the shore
    b. It is not in the middle of the stream to block navigation or become entangled with debris
    c. Will remain submerged the entire duration of deployment (14 days)
    d. Will be exposed to flow velocity of at least 0.2 feet per second for the duration of deployment
    e. Will be in a location that will be accessible should depth in the stream rise

f. It will not be easily visible from bridges, known fishing locations, trails, etc. so as to minimize the chance of disturbance and or vandalism

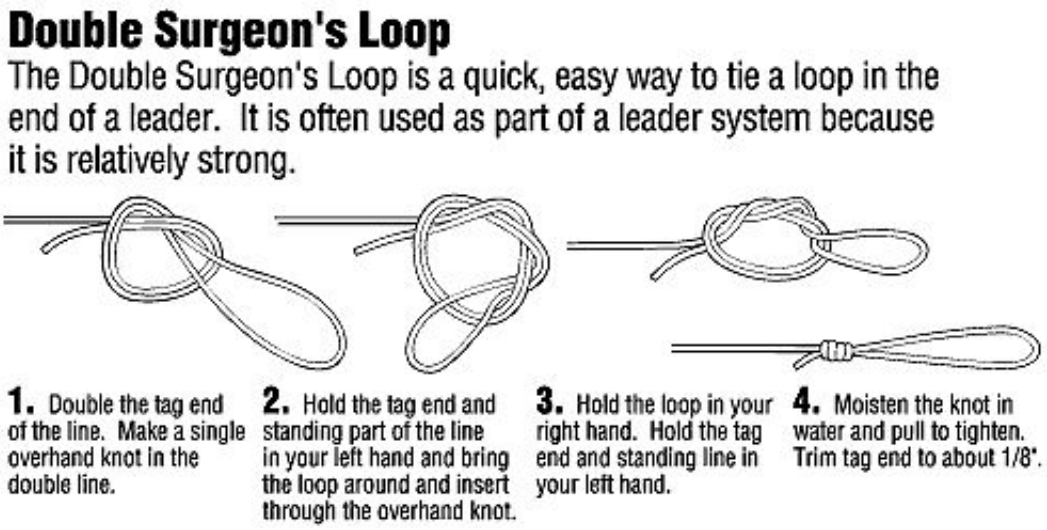
4. Tie the other end of the paracord around a tree or bush, or if not available, anything heavy/large enough to hold the H-D in place, should the water level rise and flow velocity increase.
5. Place flagging tape or marker along the bank of stream to identify the location of the sampler for later retrieval.

RETRIEVAL PROCEDURE

Retrieval of H-D should occur exactly 14 days after deployment.

1. Enter the stream while being sure not to disturb areas around the H-D samplers.
2. Place the D-shaped kick net downstream of the H-D sampler to catch any organisms that may detach during removal of the H-D.
3. Carefully detach the H-D sampler from S-binder and paracord.
4. Place the H-D sampler and any material from the net into a labeled wide-mouth plastic sampling jar (label should specify site (A-G), date (day-month-year), and H-D sample #) .
5. Fill sampling jar with 95% ethanol and seal.

* How to tie a surgeon's end loop:



PROCEDURES IN THE LAB

Equipment for H-D Analysis

- 10x dissecting microscope
- White Enamel Pan
- Forceps
- 95% Ethanol

- Plastic Wrap
- Plastic Petri Dishes
- Plastic White-Topped Vials
- Macroinvertebrates Reference Books
- Spoon
- Write-in-Rain Paper
- Pencil (NOT PEN)
- Wash Bottles

H-D ANALYSIS PROTOCOL

1. Remove H-D from the plastic collection jar.
2. Unscrew wing nut at bottom of H-D and remove spacers and Masonite plates from eyebolts.
3. Evenly distribute the contents of H-D sample (including material in left in collection jar) into the white enamel pan, rinsing each part of the H-D (spacers and Masonite plates) with 95% ethanol.
4. Scoop the sample into petri dishes with a spoon. Fill a plastic petri dish halfway.
5. For analysis, follow the riffle sample protocol.
6. Repeat this process until the entire H-D sample has been analyzed.

QUALITY ASSURANCE QUALITY CONTROL (QAQC) PROTOCOL

QAQC for H-D samples should follow Riffle QAQC Protocol