**General Aquatic Protocol**
Rev 7/22/16

*Water Quality*

- Use measuring tape to create straight line from rebar at origin to middle of stream
- Note general characteristics of stream reach
    - Note microhabitats present and in what proportion
        - riffle, pool, undercut bank/root mat, sticks/wood, depositional area, edge habitats (from KY)
    - Water condition visual (murky, clear, foamy, etc.)
    - Record weather from previous day
- Record characteristics at origin
    - Water depth (m)
    - Habitat type (riffle, pool, etc.)
    - Water condition visual (murky, clear, foamy, etc.)
    - Canopy (open, closed)
- Collect Water Samples
    - Should be collected midstream at origin
    - Fill each bottle to the neck
        - Nitrate/Nitrite: 250 ml bottle
        - Total Nitrogen: 125 ml bottle
        - E coli: 125 ml bottle
    - Do not rinse bottles
    - Attach label to each bottle and verify that Request for Analytical Services form has been filled out
    - Drop off samples at 8100 Lowry Blvd. within 8 hours of collection; Facility open 8AM-5PM M-F, call Brandon Gambrall 303-692-3666 to let know coming, place in specimen drop off area (faces Lowry Sports Park)
- Record Probe data
    - Temperature (°C)
    - pH
    - Total Dissolved Solids
    - Dissolved Oxygen (mg/L)
    - Electrical conductivity
- Specific Probe Instructions
    - Do not submerge probes past the point where the storage caps seal
    - Swish probe in water to remove air bubbles and allow reading to stabilize before recording
    - DO
        - If probe has not been used in 7 days, requires 3 minutes to polarize…turn probe on, wait 3 minutes before testing
        - Sponge in storage cap must always be moistened (but not soaked) with DI (or RO) water
    - pH

        - pH probe should not be allowed to dry out. Store in pH 4 standard (or storage solution if available)
        - if probe does dry out, must soak in standard or storage solution for 1 hour before use
    - After testing, rinse all probes with RO water and recap
- Flow
    - Flowmeter allows us to calculate velocity (m/s). We want surface velocity at thalweg nearest the transect origin.
    - Sample in section of reach with uniform bottom & uniform flow
        - Should not be too turbulent, should not have dead water, etc.
        - Thus, flowmeter will not necessarily be deployed near origin

*Macroinvertebrates*

- Sampling will vary between transects based on the type and number of microhabitats present in the reach
- Sampling effort will be distributed proportionally between microhabitats present in the reach, so that the composite sample for the transect reflects the diversity and proportionality of microhabitats
- The *sampling effort* will be uniform between transects
    - Starting protocol: every 5 m, 1 sampling unit of kick net, unless water is still and do jab method with d-frame.
    - D-frames: 1 sampling unit = 1 jab
        - Jab: "forcefully thrusting the net into a productive habitat for a linear distance of 0.5m" (Benthic Macroinvertebrate Protocols, 7-7)
    - Kick-nets: 1 sampling unit = 1 minute of kicking/disturbing bottom
        - Kicking: "disturb 1m$^2$ upstream of net, use hell or toe to dislodge the upper layer of cobble/gravel, scrape underlying bed. Pick up larger substrate and rub by hand to remove attached organisms" (Benthic Macroinvertebrate Protocols, 7-4)
    - Is 1 jab equivalent sampling effort to 1 min kicknetting?
        - Seems like much more would be collected in 1 min kicknetting than 0.5m drag of D-net.
        - Maybe 2 jabs=1 min kicknetting?
    - How much sampling effort per transect?
        - Perhaps 10?
            - Maximum of 20 jabs/maximum of 10 minutes kicknetting (if we decide on a 2:1 ratio of sampling units)
                - E.g. 3 min kicknetting + 14 jabs; 8min kicknetting + 4 jabs
    - Define which microhabitats should be sampled with D-net vs. kicknet
        - Kicknet more suitable for rocky/hard bottom
        - D-net useful for undercuts and root mats and still water
- Once sampling effort has been divided between microhabitats, sampling should begin at the most downstream microhabitat and move upstream towards the origin
- Specimens clinging to the outside of nets should be excluded
- Jars for each transect should be no more than 50% full of sample material (CODPHE) so that sufficient ethanol can be added to the jar

*Need to record mesh size of Dnet and Kicknet