**2013 Summer lab protocols**

**Periphyton collection:**

1. **Picked three rocks at random (same size assessed by just feeling around and trying not to be biased with the rocks you picked). Tried to collect from riffles similar in nature.**
2. **Scraped rocks using wire brushes. Slurry was scraped into trays and funneled into 125 ml bottles and put on ice to be analyzed in the lab.**
3. **Trace top-side of rock onto a rite-in-the-rain sheets of paper.**
4. **Cut standard areas of rite-in-the-rain paper (10x10, 100 x 100 etc) and make a standard relationship between weight and area. Cut out our traced paper and weigh applying calculated formula to determine area of rock exposed**

**Splitting Samples:**

1. **Entire slurry poured into beaker with magnetic stirrer and bottle rinsed thoroughly with DI.**
2. **Split samples by collecting subsamples with syringe**
   1. **Either split 50/50 (or close to it)(entire sample) and record overall volume**
   2. **Take subsamples of entire sample (ex. 25ml of 125) and record sample volumes and total volume to calculate proportions**
3. **Filter subsamples using vacuum flasks and 47mm**
   1. **Chl. a: filter and place filters in 20ml scint vials.**
   2. **AFDM: Use pre-ashed and pre-weighed glass filters. Use multiple filters if needed. Place each filter in a pre-labeled aluminum tray (1 filter per tray).**

**Chlorophyll a**

1. **Fill scint vials with 20 ml (or standard amount) xxx% (look up) Acetone and place in freezer to extract chl. a (at least 24 hours).**
2. **Shake and let settle. Remove filters and allow to come to room temperature**
3. **Measure fluorescence using fluorometer on chl. a channel.** 
   1. **Must calibrate fluorometer following instructions on liquid chl. a standards and then using solid standard.**
   2. **Dilute samples if fluorescence is above 1000.**
4. **Record F1 and while vial is still in fluorometer add 2 drops xxx (look up) mol HCL. After 90 seconds, record F2.**
5. **Use equations in Chl. a master sheet**

**AFDM**

1. **Take samples and place in oven at 60c for 24 hours. Record weight of filters (dry weight).**
2. **Place trays in muffle furnace at 500c for 2 hours. Record Ash-free dry weight.**
3. **Follow equations on AFDM master sheet.**

**Forest plots**

1. **Collected 10x10 m forest plots. Alternated side of stream. Plots started at fixed intervals to avoid bias. All plots oriented facing south and then away from stream.**
2. **All trees over 5cm dbh were identified to species and measured (dbh)**
3. **Trees under 5cm dbh were identified and quantified but not measured. (i.e. 10 vine maples under 5 cm)**