**Plateau Methods**

Equipment Required:

* Pump and tubing
* Battery
* Conductivity meter
* Cooler half-filled with ice
* 1 L graduated cylinder
* 1 mL pipette
* Large carboy
* Acid washed 60 mL syringe and new filter for each: -10m, 0m, 30m, 60m, 90m, 120m (6 total)
* At least 26 acid washed centrifuge tubes (plus an additional tube for measuring pump rate)

\*Do downstream reach before doing upstream reach

1. Using graduated cylinder, measure out proper amount of stream water into carboy; add in the salt and nutrients, then shake until everything is dissolved
   1. Don’t get any salt or nutrients into the stream
2. Assemble pump setup – lean pump so that it is angled with the battery connection side lower than the rate adjustment side
3. Take background sample at -10m, also measuring conductivity (spc) and writing the time
   1. Note: always fill and empty syringe three times with stream water, then push one syringe worth of water through the filter; then, rinse centrifuge tube and cap three times with filtered water before filling to between 10mL and 12mL
   2. Note: label centrifuge tubes with location in reach (-10m), site (W-100\_DS), date, and which sample and repetition (Bkg. R1, R3)
   3. Note: immediately place samples into cooler
4. Work towards downstream end of reach, taking one background sample at 30m, 60m, and 90m and three background samples at 0m and 120m; measure conductivity and write the time at each location
5. At each location, leave three labeled tubes for post-injection sampling (label R1, R2, and R3) along with syringe and filter used at that location
6. Leave cooler at downstream end and return to -10m
7. Using stream water, measure the pump rate by pumping water for 10 seconds into a centrifuge tube, then multiplying mL by 6 to get mL/min; adjust until desired rate is reached
8. Fill the graduated cylinder to 1L; measure the conductivity in the stream, and then in the cylinder
9. Add 1mL of solution in the carboy to the graduated cylinder using the pipette, mix, and measure conductivity again
10. Dump cylinder away from stream
11. Put pump intake into carboy, and set output to drip directly into the stream; write down start time
12. Take conductivity meter to downstream end (120m) and set up meter in water
13. Wait for conductivity to plateau
14. When plateau is reached, begin sampling at 120m; take three samples (rinsing the syringe and filter again) and measure conductivity and time
    1. Place used syringes into cooler with samples
15. Repeat sampling and measurement at 90m, 60m, 30m, and 0m
16. Take one -10m sample and measure conductivity above pump setup
17. Repeat the cylinder conductivity measurement with stream water from above the pump setup and 1mL of the carboy solution

**Gas Sampling**

Equipment Required:

* Gas bag with proper amount of gas and water, well mixed
* Pump and tubing
* Battery
* Glass vials, rubber stoppers, metal caps
* Cap crimper
* Vial labels

1. Take five background samples at -10m
2. Set up pump as in plateau sampling, measuring and adjusting the pump rate
3. Hang gas bag over box filled with stream water, so that connection port is under water
4. Attach pump tubing to connection port, and place output tubing in stream
5. Turn on pump to start injection and write down time
6. Prelabel bottles with location in reach (-10m), site (W-100\_DS), date, and which sample and repetition (Bkg. R1, R3)
7. Take samples at the same time as plateau samples are taken
8. To take sample, rinse bottle three times, then fill underwater; keep in water
9. Put rubber cap under water
10. Ensure there are NO bubbles, even tiny ones, on either bottle or cap
11. Cap bottle, place metal cap over stopper, then crimp cap, all while holding under water
12. Take bottle out of stream and tap it against metal part of crimper to ensure there are no bubbles in the sample
13. Repeat for a total of three samples at each distance in reach (0m, 30m, …, 120m)

**Fluorescein**

Prepare before:

1. Dilute fluorescein to proper concentration, fill vials, and save a few for standards
2. Make flags with numbers written on them
3. Attach three vials of fluorescein to each flag with zipties
   1. Have a few flags with a control – a fourth vial covered in foil
   2. Make sure there are no repeat numbers in a set
4. Right before leaving, measure standards

Procedure:

1. Place one flag every five meters, between and including 0m and 90m
2. Write down start time and end time of flag placement
3. Try to place in thalweg, with flag stem oriented to point downstream
4. Hold flag in place by placing a rock on flag
5. Write down number on flag and location in reach
6. Leave for 24 hours; try to set very early in the morning or late in the evening
7. Pick up the flags in the same pattern they were deployed, and write down start and end time
8. Keep the flags in the dark when transporting – use a black trashbag, in a box or backpack

Measuring:

1. Wait for vials to reach room temperature
2. Keep room dark – cover windows and turn off lights
3. Take out flags one at a time
4. Remove the vials and pour each into a cuvette
5. Measure with meter
6. Write down all three readings (four if there is a control) in a spreadsheet with the flag number and location

**Tiles**

1. Leave tiles (3 per location) in stream
2. Remove tiles from stream – place in a single layer in a bucket filled with enough stream water to cover the tiles
3. Using a wire brush, scrub tiles one at a time, rinsing with DI water; collect all water from brush and tile and filter through 0.7 micron filter
4. Randomly choose one of the three at each location to be tested for chlorophyll a; keep the filter and place in freezer for 24 hours
5. For the other two tiles, measure the ash free dry mass

*Chl a*

1. Place filter in 20mL scintillation vial with 15mL of acetone
2. Leave for 2-4 hours in the dark at room temperature, shaking at least once
3. Calibrate fluorimeter if required
4. Take 3mL of the extracted fluid and add to a cuvette
5. Take reading; if it is good, record as Fo in workbook
   1. If above standard, dilute sample, 1mL sample to 2mL acetone
   2. Mix in cuvette
   3. Read again
   4. Dilute until value is below standard value
6. Add 0.26mL HCl solution to 3mL of sample
7. Mix solution thoroughly with pipette
8. Wait 90 seconds, then measure in fluorometer again; record value as Fa