**Chlorophyll *a*: Methanol Extraction**

3 May 2011

**Chemicals**

Methanol

Sodium hydroxide (NaOH)

**Reagents**

0.5M Sodium hydroxide: dissolve 4g NaOH in 200ml of Q water

Basic Methanol: add 2 mL of 0.5M NaOH to 1 L methanol.

**Extraction**

1. Freeze the glass crucible covers or filters for 24 hours to lyse cells.
2. After 24 hours, place the crucible covers in a film canister and add 5-15 mL of basic methanol (volume depends on concentration of chl *a* in sample and standards used with fluorometer). Cover and let sit for 24 hours in the dark.

**Reading samples**

Turn on fluorometer

            Switch in back

            Flip again if you get squares

            Warm up for 10 min

Calibrate

            Insert tube 2 (in drawer)

            Hit enter

            Select calibration (hit 2, enter)

            Set sample to 800 (hit 1)

            Should read sensitivity factor = 25

            Should read sample = 800

            Hit enter

            Select Automatically Subtract Blank (hit 1)

            Insert tube 0

            Hit enter

            Press 0 when stable

                        Wait (will say Printing Calibration Report)

            Don’t have to hit any more buttons after calibrated

Read samples

            Analyze chlorophyll in each replicate (2) sample

                        Insert tube

                        Reading may fluctuate

                        Record value in notebook, round decimal place up

                        Dilute to 10% if reading is over max

                                    Remove filters

                                    Fill clean tube with 4.5 mL methanol

                                    Pour sample into a beaker

                                    Pipette 0.5 mL of sample to clean tube

                                    Rinse pipette and beaker with fresh methanol

                                                if additional dilutions are required

                                    Discard remaining sample and waste

                                    Mark in notebook as 10%

                                    Multiply value by ten when adding to spreadsheet

            Analyze phaeopigments in each replicate (2) sample

                        Remove filters

                        Discard caps

                        Add 50 L of 0.3 N HCL to each tube

                                    (32 mL nanno H2O + 1 mL HCL)

                        Keep in the dark for 30 – 60 min

Read tubes

                        Subtract from Chlorophyll to calculate phaeopigment dissolved by acid