GEODES Chlorophyll measurement protocol

*Based on NTL-LTER protocols at lter.limnology.wisc.edu*

Sample Collection

1. Save one liter of unfiltered water from integrated epilimnion sample. Make sure the collection bottle is wrapped in tin foil.
2. Back inside, filter 250 uL\* of water through a 0.3 micron nitrocellulose filter using a peristaltic pump.
3. Fold the filter with tweezers and place in a 2mL cryogenic vial. Drop the vial in liquid nitrogen.
4. Repeat with 2 more replicate filters.
5. Store filters in -80C freezer until extraction.

\*Actual volume filtered varied due to filter clogging

Extraction

1. Work in dim light conditions. Transfer filters to well-labeled 15mL Falcon tubes and add 10 mL of methanol (good quality methanol, not the stuff used for washes)\*
2. Invert to mix and incubate tubes with methanol for 20 minutes.
3. Homogenize each sample with physical disruption by inserting the tip of homogenizer into the sample and moving up and down until the filter is completely destroyed. This should take about 30 seconds to 1 minute.
4. Wash the tip of the homogenizer twice between samples using 10mL of methanol in empty Falcon tubes. Never run the homogenizer when the tip is not in liquid!
5. Incubate samples at 4C for 24 hours\*\*

\*Steps 1-5 were performed on batches of 20 samples

\*\*Actual incubation times ranged from 18 – 20 hours due to the large number of samples and time constraints

Measurement

Before starting, turn the fluorimeter on at least half an hour before the first measurement. Make sure the D lamp is in the fluorimeter, and check the calibration using both the low and high solid standards.

1. Spin the samples at 12,000 rpm for 15 minutes at room temperature.
2. Add 4 mL of each sample’s supernatant to glass vials for insertion into the fluorimeter.\*
3. Insert the vial into the fluorimeter. Record the reading (in ug/L) and the dilution factor.\*\*
4. Add 120 uL of 0.1 N HCL to the vial. Gently shake the vial and let it sit for 90 seconds before reading. Record the reading and the dilution factor.

\*This step is done in batches of 10 samples. Keep the vials next to the labelled Falcon tubes, as the glass vials cannot be labelled.

\*\* We aimed for readings between 10 – 100 ug/L. We found that samples from Sparkling Lake needed no dilution, samples from Lake Mendota needed a 1:4 dilution (1mL sample + 3 mL methanol) and Trout Bog samples needed either a 1:2 or a 1:4 dilution.

Calculations

Uncorrected chlorophyll A = Dilution factor \* reading \* (mL extraction volume/mL filtered volume)

Phycocyanin = Dilution factor \* (reading after acid – reading before acid) \* (mL extraction volume/mL filtered volume)