(Chlorphyll2007.doc)

CHLOROPHYLL *a* ANALYSIS

EQUIPMENT: Film canisters
Turner 450 Fluorometer fitted with:
1. Quartz-halogen lamp
2. Emission filter -SC665
3. Excitation filter -NB440

47mm Whatman GF/F filters
12 x 75 mm disposable glass culture cuvettes (**Do not reuse cuvettes!**)
1-5 mL Oxford pipettor
Finnpipette Stepper Pipetter with 5 mL tip

NOTE
-**Change filters with fluorometer off!** (Remember that chlorophyll analysis filters are different from APA analysis filters.)
-**Make sure Fluorometer has been calibrated for chlorophyll a** (see **Fluorometer Calibration for Chlorophyll a Analysis**).

REAGENTS: 100% Methanol, spectrophotometric grade
CAUTION - wear gloves whenever you use methanol.
0.1 N HCL
Ethidium Bromide Stock 3 standard (40μM solution)

PROCEDURE:

A. **Filter water samples from each of the 6 light-depths onto a 47 mm GF/F filter.**
1. Filters have a grid side and a "smooth" side. Place filter smooth side up.
2. Shake sample bottle well before filtering (do this ***after*** the DIC sample has been taken from the same bottle.)
3. For each depth, filter enough water so there is a faint color on the filter. For our lakes this ranges between 100-300ml. Record the volume filtered. Make sure you filer at less than 200 mm Hg pressure.
4. Rinse filter towers and filters with DI water, place filters in labeled film canisters and place in freezer. Labels should include lake, date, and depth ID.
5. If measuring edible chlorophyll as well, repeat steps 1-4 above, but first filter the sample through 35 μm mesh. (This has not been done since 2001, inclusive.)

B. **Extraction - DO IN DIM LIGHT and WEAR GLOVES!!**

1. Remove one tray of film canisters from the freezer. Extract chlorophyll by adding 25 mL 100% MeOH to each film canister. If using re-pipettor, verify dispensed volume. (Record extraction volume if different from 25 mL.) Note the extraction time for each group of samples.
2. Re-cap and place canisters in refrigerator to extract for exactly 24 hours (in the dark).
3 Repeat steps 1 and 2 for all trays that have been in the freezer more than 24 hours.

C. **Fluorometry**

Calibration of the fluorometer using a chlorophyll standard is typically performed at the beginning of the field season, or when a bulb is changed. Calibration using Ethidium Bromide is done at the beginning of each sample set.

1. Insert correct filters in fluorometer while fluorometer is off. (Emission filter -SC665, Excitation filter -NB440), and warm it up for 1 hour .
2. **TURN LIGHTS OUT**. Chlorophylls must be read in low light and samples must be kept cool. Do not remove film canisters from the refrigerator until you are ready to process the samples.
3. Place clean cuvettes into a labeled rack (12 cuvettes per rack). Remove one lake-day of film canisters from the refrigerator.
4. Place Ethidium Bromide Stock 3 standard into fluorometer and record reading on datasheet. Then, turn the span knob until the reading is **908**. Record this on the datasheet.
5. **Shake film canister**, remove the lid, and rinse the pipette tip with 2.5 mL of the sample. Then remove 2.5 mL of sample and place in cuvette.* Repeat for all film canisters.
6. Pipette 2.5 mL of 100% methanol into a cuvette for the blank and use it to zero the fluorometer. Choose a gain and turn the zero knob until the fluorometer reads 000. **You must zero the machine every time you change gains.**
7. Remove the first sample cuvette from the rack, wipe with a Kimwipe, and place in fluorometer. Record the gain and the fluorescence before acidification, $\mathbf{F_b}$. Repeat for all 12 cuvettes in the rack. Readings should be between about 200 and 1000. If not, adjust the gain and re-zero.
8. Acidify each cuvette with 100 µL 0.0773 N HCl using the repeating pipetter and mix (hold the top of the cuvette securely, then "thump" the bottom several times). Check for condensation on the outside of the cuvettes, and wipe with a Kimwipe if necessary. Wait about 1 min from the acidification of the first cuvette.
9. Record the fluorescence after acidification for all 12 cuvettes. VERY IMPORTANT: **Make sure you read the $\mathbf{F_b}$ and $\mathbf{F_a}$ values for each sample on the same gain**.

10. Remove a new lake-day batch of film canisters from the refrigerator and repeat steps 3-9.

* if particulate matter is present, centrifuge sample for 10 min. and use supernatant.

D. **Clean Up**: **DO THIS UNDER THE HOOD!**

1. Dump methanol solution from cuvettes and film canisters into a metal tray. Place the film canisters and lids in a separate tray. Position them in one layer on the tray with their openings facing up. Leave the trays under the hood overnight to evaporate the methanol.

CALCULATIONS:

Chl *a* (μgL^{-1}) = $(\mathbf{F_b}-\mathbf{F_a}) * \mathbf{Q}$

Pheoph. (μgL^{-1}) = $((\mathbf{R} * \mathbf{F_a}) - \mathbf{F_b}) * \mathbf{Q}$

Where:

$$\mathbf{Q} = \mathbf{m} * \frac{\mathbf{R}}{\mathbf{(R-1)}} * \frac{\text{extraction volume}}{\text{filter volume}}$$

m = scale factor (slope)
R = acid ratio
(see **Fluorometer Calibration for Chl. *a*)**

$\mathbf{F_b}$ = fluorescence before acidification

$\mathbf{F_a}$ = fluorescence after acidification

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