Determination of Chlorophyll-*a* using a Modified Extraction

Protocol Branco Lab updated 09/10/19 by Katlyn Garcia

Phytoplankton biomass can be estimated by the photosynthetic pigment, chlorophyll-*a* found in all phytoplankton cells. This protocol is modified from the Welschmeyer (1994) protocol using acetone as a solvent and a fluorometer measuring chlorophyll-*a* only (avoiding chl *b* interference). Chlorophyll-b is rare in marine waters. If you want to determine the amount of chl-*a* and chl-*b* you will need to acidify samples after measurement (F_0) and read the sample again (F_a). The acid converts all chl-*a* to any phaeopigment that is in the sample (Lorenzen, 1966).

Materials

- Hand-operated vacuum pump with gauge
- Plastic top filtrate holder
- Plastic 500 mL flask
- 25mm Whatman GFF
- 15 mL conical vial
- 100% Methanol
- Sample bottles

In the field

1. Collect the desired volume of sample water in a clean sample bottle.

2. Attach the base stopper to the 500 mL plastic flask, make sure there is a new 25mm Whatman GFF nominal pore size filter pad on the base stopper. Place the filter pad rigid side up on the base stopper with forceps. Attach the filtrate holder to the base stopper and lock in place to prevent sample lost.

3. measure out 60 mL of sample water using the graduated cylinder. Pour the sample into filtrate holder and use the hand pump to push the sample water through the filter.

4. pour a filtered sample of water into a clean sample collection bottle and cap. Keep the water in the cooler if you are analyzing for nutrients in the lab.

5. Remove the filter pad with forceps and place in a prepared 15 mL conical vial with 10 mL of 100% methanol. Wrap the conical vial in foil so it's in complete darkness. Place in a cooler until

you're back in the lab.

In Lab

Day 1:

In all steps, remember to avoid degradation of pigments. Minimize exposure of pigments to light and heat. Put in refrigerator overnight and let soak at least overnight (24 hours). At this point, the samples can be stored for several days.

Day 2:

1. Turn on the fluorometer for 30 minutes before use (make sure it's calibrated-if not you need to record raw absorbance).

2. When samples are ready, vortex for 1 min, remove filter pad then centrifuge for 5 minutes at 3000 rpm to collect particles to the bottom of the vial.

3. Pour methanol into the sample vial (they have a black screw cap) fill up to the top.

4. Place into fluorometer and measure a blank sample. The fluorometer should read less than or equal to 0.0 ug/L.

5. Rinse a clean sample cuvette and carefully pour your field sample. Screw-on cap and wipe off any liquid with a Kimwipe and place in a fluorometer.

6. Record the raw absorbance and the ug/L concentration reading.

Repeat steps 4-6 for all field samples.