

**Standard Operating Procedure  
Periphyton production on artificial blades in Florida Bay  
Florida Coastal Everglades LTER**

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**I. Periphyton Sampling**

***Equipment and supplies:***

Mylar transparency sheets  
Chicken wire frames  
Plastic ties  
Stainless steel wire cutter  
Whirl packs, plastic sample bags  
Cooler with ice  
Pencil, permanent marker  
Meter stick  
Waterproof digital camera

***Methods:***

1. Field records are maintained in pencil on waterproof paper in a field notebook and entered electronically upon return to the laboratory
2. Each sampling unit is a box made of chicken wire containing 12 mylar strips of pre-determined size. The strips are attached at one end to the chicken wire with a tie and the other end has a small piece of Styrofoam to ensure that the strip floats. The wire boxes are anchored into the mud and strips float upward into the water column to mimic seagrass blades. Triplicate sets of mylar are placed at each site.
3. Strips are “incubated” *in situ* for about two months. The time period in days is recorded.
4. The strips are removed from the chicken wire with a wire cutter, rolled with all associated periphyton and put into a labeled plastic sample bag and then placed in a cooler with ice for transport to the laboratory.

***References:***

Frankovich, T. A. and J. W. Fourqurean. 1997. Seagrass epiphyte loads along a nutrient availability gradient, Florida Bay, USA. *Marine Ecology Progress Series* 159:37-50.

## II. Periphyton Sample Processing

### *Equipment and supplies:*

250, 500 and 1000 ml beakers  
Stir plate, magnet  
0-1 ml calibrated micropipets and tips  
5-10 ml calibrated pipets and tips  
Biohomogenizer  
50, 100, 500 ml graduated cylinders  
2 ml plastic microvials and racks  
14 ml plastic freezer vials and racks  
Weigh pans  
Acetone  
10 ml glass vials and lids  
Fluorometer  
Distilled/deionized/filtered water  
Fume hood and chemical gloves, apron, goggles  
Hot plate  
Razor blades

### *Methods for subsampling and biomass determination:*

1. Laboratory records are maintained in pencil on waterproof paper, entered electronically and later printed for archiving.
2. Frozen periphyton samples are thawed in the dark for <3 hours and transferred to a 250 ml beaker
3. Periphyton is scraped from all sides of each transparency strip into the beaker; the number of strips recovered are recorded
4. Periphyton is homogenized using a biohomogenizer
5. Homogenized sample is poured into a graduated cylinder (using micropore filtered water to clean all surfaces) and volume is diluted to the nearest 10 ml (maximum dilution is 300 mL) and the final volume is recorded.
6. A magnet is dropped into the beaker and the beaker is placed on a stir plate
7. A calibrated pipet is used to remove 1 ml of subsample for chlorophyll a, which is filtered through a 25 mm GFF filter, rolled into a cone and placed into a 2 ml microvial and frozen
8. A calibrated pipet is used to remove 1 ml for soft algal analysis; subsample is placed into a labeled 2 ml microvial and frozen
9. A calibrated pipet is used to remove 10 ml for diatoms; subsample is placed in labeled 14 ml vial and frozen
10. A total of 40 ml of the homogenized sample is measured in a graduated cylinder and poured into a tared, labeled weigh pan
11. The remaining sample is retained in the plastic cup and are dried to constant weight at 50° C for nutrient analysis
12. Pans and periphyton are dried to constant weight at 50° C and final dry weight is recorded
13. Pans are placed in muffle furnace at 500° C for 1 hour and weighed again to determine ash weight.

14. Ash-free dry mass (AFDM) is calculated as the difference between the dried and ash weight. Percent organic content is the ratio of the AFDM to dry mass.
15. Dried samples for nutrients are scraped into labeled scintillation vial and are later ground and analyzed for total phosphorus.

### ***Methods for Chlorophyll a analysis***

#### Extraction:

1. 1.5 ml of 90% buffered acetone is added to each frozen microvial, recapped and inverted 3 times. Extraction volume is recorded on datasheet
2. Samples are covered and returned to freezer.

#### Measurement (performed in dim light within 24 hr of extraction):

1. Fluorometer is warmed up for 1 hr before analysis
2. Microvials are centrifuged on high speed for 3 min.
3. Fluorometer excitation is set at 435 nm and emission to 667 nm
4. Fluorometer is calibrated with clean, empty cuvette; set at high voltage to 700 with 4 sec. response time
5. Cuvette is filled with 90% buffered acetone blank and checked to read at 0.0. Blank is re-analyzed every 20-30 samples.
6. Chl a standards are analyzed first; using 1 ml micropipetter, 0.75 ml of sample is transferred into cuvette; 2.25 ml acetone is added. Samples are read, recorded and/or printed through the computer.
7. Dilution factors, if necessary, are recorded.

#### ***References:***

Welschmeyer, N.A. 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. *Limnol. Oceanogr.* 39(8): 1985-1992.