Standard Operating Procedure
Periphyton biomass in Shark River and Taylor Sloughs
Florida Coastal Everglades LTER

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I. Periphyton Sampling

Equipment and supplies:
$1m^3$ PVC quadrat
Whirlpacs, plastic Ziploc bags
2000 ml perforated plastic graduated cylinder
Cooler with ice, pencil, permanent marker
Meter stick
Waterproof digital camera

Methods (2001 to 07-2005):
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 1 m^2 PVC frame thrown in random direction; sample identification codes are predetermined and unique. This method is repeated in triplicate at each site.
3. Frame is photographed with 3 megapixel waterproof digital camera and percent of the surface covered by periphyton is recorded.
4. Fifteen $9.8cm^2$ cores are removed from the periphyton mat and combined into a single sample and placed in a labeled sample bag.
5. Periphyton samples are transported on ice to the laboratory

Methods (11-07-2005 to present):
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 1 m2 PVC frame thrown in random direction; sample identification codes are predetermined and unique. This method is repeated in triplicate at each site.
3. Frame is photographed with 3 megapixel waterproof digital camera
4. Corresponding environmental data are recorded including predominant periphyton character (calcareous, calcareous and organic, organic) and water depth (3 measures with meter stick)
5. Periphyton cover is estimated according to attachment substrate. Estimates include: percent of sediment/bedrock covered by periphyton; percent of plant stems covered by periphyton and

the identity of the 3 most abundant epiphyte-supporting plants, number of live/dead stems for each of 3 plant species; percent of the water surface covered by periphyton.
6. All periphyton is removed from the quadrat and placed in a perforated 2000 ml graduated cylinder. The total volume is recorded.
7. The periphyton sample is described by substrate type and character (field crew estimates and records the percent of the sample that is benthic calcareous mat, calcareous epiphytes, calcareous floating, green algal and/or flocculent detrital).
8. A 500 ml subsample of the periphyton portion is placed in a labeled bag and transported on ice to the laboratory

References:

Gaiser, E. E., D. L. Childers, R. D. Jones, J. H. Richards, L. J. Scinto and J. C. Trexler. 2006. Periphyton responses to eutrophication in the Florida Everglades: Cross-system patterns of structural and compositional change. *Limnology and Oceanography* 51: 617-630.

II. Periphyton Sample Processing

Equipment and supplies:

200 ml plastic urine cups and caps
250, 500 and 1000 ml beakers
Petrie dishes, forceps
Foil
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. Within 24 hours of sampling
    a. Periphyton samples are weighed wet in the pre-weighed sample bag and recorded
    b. A 120 ml wet subsample is placed in a preweighed plastic cup with water tight lid and weight is recorded
    c. Subsamples are frozen until analysis

    d. Field data is recorded electronically and photographs are downloaded and named by site identifier
3. Frozen periphyton samples are thawed in the dark for <3 hours and transferred to a petrie plate
4. Periphyton is observed using a dissecting microscope and:
    a. Large macrophyte fragments are removed using forceps; fragments are placed in a foil packet and dried to constant weight at 50° C and recorded
    b. Invertebrates are removed and discarded
5. Cleaned periphyton is transferred to a 250 ml beaker and homogenized using a biohomogenizer
6. 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
7. A magnet is dropped into the beaker and the beaker is placed on a stir plate
8. 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
9. A calibrated pipet is used to remove 1 ml for soft algal analysis; subsample is placed into a labeled 2 ml microvial and frozen
10. A calibrated pipet is used to remove 10 ml for diatoms; subsample is placed in labeled 14 ml vial and frozen
11. A total of 40 ml of the homogenized sample is measured in a graduated cylinder and poured into a tared, labeled weigh pan
12. The remaining sample is retained in the plastic cup and are dried to constant weight at 50° C for nutrient analysis
13. Pans and periphyton are dried to constant weight at 50° C and final dry weight is recorded
14. Pans are placed in muffle furnace at 500° C for 1 hour and weighed again to determine ash weight.
15. 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.
16. 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.