A new technique for examining the physical structure of Everglades floating periphyton mat

by

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With 4 figures and 1 table


Abstract: The embedding and thin sectioning technique we describe allows for the routine examination of the distribution and numerical abundance of algae, cyanobacteria, bacteria, and calcareous deposits within intact Everglades floating periphyton mats (FPM's) using fluorescent microscopy of cryosections. The dominant species in FPM's was a calcareous filamentous cyanobacterium, Phormidium sp., that was found throughout the whole mat but which, together with Scytonema hofmannii, had a higher abundance in the bottom layer. Small coccoid cyanobacteria, characterized the upper surface and interior regions of the mat, which was also inhabited by diatoms. Of all diatoms, Mastagloia smithii var. smithii was the most abundant. Floating periphyton mat structure is characterized by high porosity and numerous void spaces.

Introduction

Fresh water ecosystems of the Florida Everglades contain a form of periphyton mat that is an assemblage of algae, cyanobacteria, bacteria, aquatic plants, protozoa, and micro-invertebrates, all coexisting within a matrix of mucilage, calcium carbonate precipitate, and detritus (Vymazal & Richardson 1995). The relative abundance and species composition of algae found within floating periphyton mats (FPM's) has been investigated (Raschke 1993, Vymazal & Richardson 1995, McCormick et al.

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1996), and many researchers have observed the importance of filamentous blue-green algae in the maintenance of periphyton mat structure (Gleason & Spackman 1974, Van Meter-Kasanof 1973, Swift & Nicholas 1987). Maintenance of floating periphyton mat structure may be dependent on complex interactions involving the metabolic activity of algae, heterotrophs, and the physical-chemical environment surrounding the mat. High rates of photosynthesis and depletion of carbon dioxide by algae from Everglades water accelerates the formation of calcite encrusted mucilaginous sheaths of the blue-green algae Scytomena hofmannii and Schizothrix calcicola (Gleason & Spackman 1974). Over time, dense layers of calcium carbonate precipitate are deposited on internal and external surfaces of the mat, creating microhabitats for other algae, bacteria and invertebrates.

Disintegration of periphyton mats in response to anthropogenic phosphorus enrichment has been documented along phosphorus gradients within water conservation areas of the Everglades (McCormick & O’Dell 1996, Raschke 1993), in the Shark River Slough (Gaiser, unpublished data), and in experimental treatment cells (Pan et al. 2000). Elevated P levels can inhibit the precipitation of calcium in Everglades water (Veithiyathan et al. 1997), potentially disrupting the formation of calcite deposits on mucilaginous sheaths of periphyton mat algae. The relative abundance of calcareous cyanobacteria declines in response to phosphorus enrichment (McCormick et al. 1996), and is followed by a succession of algae that includes an increase in filamentous green algae and diatoms (McCormick et al. 1996, Pan et al. 2000). Changes in the taxonomic composition of FPM’S and their disintegration in response to phosphorus enrichment have been documented by several authors (McCormick & O’Dell 1996, McCormick et al. 1996, Pan et al. 2000). The disintegration of FPM’S and replacement with filamentous green algae in response to P enrichment is an indication of the interrelation of biotic and abiotic elements in the maintenance of periphyton mat structure.

Seasonal development of periphyton is regulated by physical factors including irradiance, temperature, hydroperiod, water depth, nutrient concentrations, and substratum availability (McCormick et al. 1996). A pulse of FPM growth and biomass production generally occurs in spring (Vymazal & Richardson 1995), concurrent with increased rainfall and growth of Utricularia purpurea. Floating periphyton mat growth occurs primarily in areas of flowing water above 60 cm in depth (Gleason & Spackman 1974). Maximum biomass and percent coverage of FPM’S occurs in late summer (McCormick & O’Dell 1996). Fully mature FPM’S can be found persisting in long hydroperiod areas of the Everglades until late fall (Childers et al. 2002).

Photosynthetic output and biomass production by floating periphyton mats forms a base of the Everglades food web and shapes the biogeochemical character of terrestrial ecosystems of the Florida Everglades (Browder et al. 1994). For instance, periphyton controls concentration of dissolved oxygen, carbon dioxide, calcium and influences the formation of marl soils (Veithiyathan et al. 1997). Human-induced changes in the hydrology and nutrient concentrations in surface waters have a significant impact on the overall ecology of the Everglades. It has been suggested that periphyton mats can be used as a useful indicator of water chemistry for the purpose of wetland assessment (Browder et al. 1994, McCormick & Stevenson 1998). Measurements
such as percent floating mat coverage, abundance and taxonomic composition of algae and periphyton mat chemistry can provide a means of evaluating the effectiveness of management practice and restoration efforts. However, micro-scale changes in the biotic/abiotic structure and metabolic activity of FPM’s may result in ecosystem scale changes over time. For this reason, periphyton mats have the potential to reveal diagnostic measurements at the scale of organisms that are informative and applicable to processes occurring at the ecosystem scale. The abundance and spatial relationships of different groups of algae and heterotrophs, and presence of physical/chemical gradients within FPM’s, are measurements that can be obtained by spatial analysis of periphyton mat elements combined with a detailed characterization of FPM’s metabolic activity.

The profound importance of FPM’s within the Everglades ecosystem merits an investigation of the structure, function, and metabolic activity of this biotic assemblage. The goal of this research is to devise a technique that preserves the physical structure, distribution and fluorescence signatures of periphyton mat elements, (algae, cyanobacteria, mucilaginous matrix, and calcite) and will permit examination of spatial and numerical relationships within different layers of an intact floating periphyton mat. Various methods have been developed to examine community structure within periphyton mats, including freeze fracturing of biofilms and SEM analysis (Greenwood et al. 1999), embedding in Spurr’s resin (Johnson et al. 1997), and track autoradiography (Hamilton & Duthie 1984). As a prelude to studies of metabolic activity and species interactions within periphyton mats, it is necessary to develop an embedding and sectioning technique that will preserve the cellular structures and fluorescence signatures of periphyton mat elements while retaining the physical structure and spatial relationships of those elements.

Materials and methods
Floating periphyton mats were collected from the Shark Valley Slough of the Everglades National Park from February to August 2000. The material was stored in a refrigerator overnight and processed the next day. Structural organization of FPM’s was studied by examining thin sections (~10-40 μm) obtained in several ways including embedding in paraffin wax, embedding in mixtures of glycerin and gelatin, and cryotechniques. Paraaffin embedding of FPM’s followed the technique described by Berlyn & Miksche (1976). Floating periphyton mats were dehydrated in a graded series of ethyl alcohol (40%, 60%, 80%, 95%, 100% EtOH), then placed in an embedding mold partially filled with 37°C liquid paraffin. The embedding mold was then completely filled with paraffin and placed in a vacuum oven (37°C, ~ 5-7 lbs) for 5 min. Thin sections (10-45 μm) of paraffin embedded FPM’s were cut on a Reichert™ ultramicrotome. Gelatin and glycerol embedding followed the technique described by Pearse (1968). A formula series of gelatin (5, 10, 15 gm) was added to 15 ml of glycerin and 70 ml dH₂O to produce a semi solid embedding medium. Thin sections (10-45 μm) of gelatin and glycerol embedded FPM’s were cut on a Reichert™ ultramicrotome.

Cryotechniques included rapid freezing of intact FPM’s, and embedding FPM’s in TBS ™ Tissue Freezing Medium (Triangle Biomedical Sciences, Durham, NC), a mixture of water-soluble glycols and resins. Rapid freezing was accomplished by immersing FPM’s in liquid Nitrogen at – 60°C, followed by sectioning with a Bright ™ cryostat. Specimens processed with TBS ™ Tissue Freezing Medium were placed in labeled Coplin jars that were filled with pre-warmed embedding media (37°C for ~ 30-60 min). Coplin jars were then placed in a vacuum oven (37°C, ~ 5-7 lbs) for 5 min to improve the infiltration of embedding media. Jars were removed from the vacuum oven, covered, and placed in a water-bath at 37°C for 60 min. After removing the Coplin jars from the water bath, plastic
embedding molds were labeled and partially filled with warm embedding medium. Periphyton mat samples were carefully placed into embedding molds with a spatula and the orientation of the specimens recorded. The embedding medium was allowed to set for ~60 min, then wrapped in plastic and placed on the rapid-freeze bar of a Cryostat and allowed to freeze completely (~1-4 hours). The embedded and frozen periphyton mat was sectioned by using a Bright cryostat. Sections between 35-45 μm in thickness were found to be optimal in terms of handling characteristics and distribution of material on microscope slides. Sections were placed on gel-subbed slides and stored in a desiccant protected slide box. Five sections were collected from each block and sections were taken at 700 nm intervals. Three slides were selected at random from each block for analysis. Slides were kept frozen until viewed with a microscope.

Enumeration

All slides were viewed using an epi-illumination fluorescent microscope (Leitz™) with a narrow band-pass, primary excitation filter of 546 nm and a triple band pass emission filter at 400x magnification (Fig. 1). Algae and other mat structures were first observed using bright field microscopy (Fig. 2). The orientation of the specimen was confirmed at low magnification. A start point was selected at random just above the top surface of the FPM’s. The enumeration of cyanobacteria, algae, and calcite coverage was determined by using image analysis software (Image-Pro ©). Image-Pro software was used to capture and store images taken along a transect line, the coordinates of which were recorded. Each image represented a quadrant of a known area (19,000 μm²) and thickness (40 μm) at known mat depth. A total of 307 quadrants were quantified from 27 transects from FPM’s collected in August 2000.

Fig. 1. Natural fluorescence of floating periphyton mat in the 40 μm thick cryosection. Scale bar represents 10 μm.
To visualize bacteria, the sections were stained with SYBR Green I®. One ml of a working solution (1/1000 SYBR Green dH₂O) was placed on the periphyton mat section and allowed to stain for 15 min in darkness. Stain was removed with distilled H₂O and one drop of Gel-mount® anti-fade compound was placed on the section and covered with a coverslip. Stained sections were observed with a narrow band-pass, primary excitation filter of 460 nm and a triple band pass emission filter at 1000x magnification.

Results

Embedding techniques

Hundreds of thin sections of Everglades FPM's were examined over the course of this study, providing images of periphyton mat internal structures and biotic components. Evaluation of the four embedding techniques is based on the visual properties of thin sections produced, the methodology needed to produce thin sections and the physical characteristics and storage potential of thin sections. With regard to physical appearance of thin sections, preserving the fluorescent signatures of FPM elements, intact cellular structures and intact colonial algae were given the highest priority. Preserving the position of FPM elements within the organic matrix and the ability to optically dissect FPM thin sections were also desirable visual properties of thin sections. Figure 2a-d, illustrates thin sections produced by the four embedding techniques.

Embedding of FPM's in TBS Tissue Freezing Medium consistently produced sections that preserved the natural fluorescence of algae and maintained the physical structure and position of periphyton mat elements (Fig. 2a). The resins and water-soluble glycols contained in TBS completely and consistently infiltrated FPM's. Thin sections produced by this embedding technique were easily manipulated and still retain the natural fluorescence of cyanobacteria and algae after two years of storage. TBS Tissue Freezing Medium is more expensive than paraffin and glycerin and gelatin mixture, it is less expensive than cryotechniques and it is the most time efficient means of producing high quality thin sections for quantitative analysis and taxonomic identification of algae.

Paraffin embedding is the most inexpensive technique and most efficient at producing large numbers of thin sections of varying thickness (Fig. 2b). Paraffin is best for preserving the structure and spatial orientation of FPM elements. Thin sections produced by this method are well suited for long term storage, however, fixing and dehydration of FPM's resulted in a total loss of photosynthetic pigments and natural fluorescence of FPM elements.

Embedding in mixtures of glycerin and gelatin (Pearse 1968) resulted in some loss of natural fluorescence and infiltration of these mixtures into FPM void spaces was inconsistent (Fig. 2c). Thin sections produced with glycerin and gelatin mixtures were difficult to manipulate though relatively inexpensive to produce.

Rapid freezing of un-embedded FPMs (immersion in −60°C liquid nitrogen, Fig. 2d) was best at preserving the natural fluorescence of algae and fluorescent signatures of FPM elements were visible one year after preparation when slides are stored at -
Fig. 2a-d. Thin sections of floating periphyton mat viewed using brightfield microscopy. Figure 2 shows a thin section produced with TBS embedding medium (a), paraffin wax (b), mixtures of glycerin and gelatin (c), and rapid-freeze cryosection (d). Scale bar represents 10 μm.
20°C in a desiccant protected slide box. The rapid freezing technique lacks an
intervening medium that fills the numerous void spaces that are found in FPM’s,
because of this distortion of the FPM’s internal structure is unavoidable during the
sectioning process. Furthermore, thin sections produced by this method are extremely
fragile and very difficult to manipulate.

Algae and cyanobacteria

Identification of cyanobacterial and algal species in the sections revealed the presence
of 12 species of cyanobacteria, 2 desmids, and 5 diatoms (Table 1). The total number
of algal taxa encountered in this study is much lower than in other studies [Vymazal
& Richardson 1995 (69 algal taxa), McCormick et al. 1996 (73 taxa), McCormick &
O’Dell 1996 (210 taxa)]. Cryosections contain relatively small volumes of FPM’s,
capturing only a limited region of the FPM’s in each section. In terms of absolute
abundance filamentous cyanobacterium *Phormidium* sp. (Fig. 3) was the dominant
organism throughout the whole mat. The next most abundant organisms were small
coccosid cyanobacteria (Fig. 3) and *Scytonema* sp. Coccosid cyanobacteria (Fig. 3)
were more abundant in surface quadrants than in other regions of the mat. Absolute
and relative abundances of the filamentous cyanobacterium *Scytonema hofmannii*
showed little variation throughout the mat. The colonial cyanobacterium *Aphanathece*
sp. was encountered sporadically throughout the periphyton mats.

<table>
<thead>
<tr>
<th>Table 1. Species composition of cyanobacteria and algae observed in cryosections.</th>
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<tr>
<td><strong>Cyanobacteria</strong></td>
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<td><em>Aphanathece</em> sp.</td>
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<tr>
<td><em>Chroococcus minutus</em> (Kützing) Nägeli</td>
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<tr>
<td><em>Chroococcus turgidus</em> (Kützing) Nägeli</td>
</tr>
<tr>
<td><em>Fischerella muscicola</em> (Bornt et Flahault) Gomont</td>
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<tr>
<td><em>Gloeocapsa</em> sp.</td>
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<tr>
<td><em>Johannesbeaktisia pellicida</em> (Dickie) Taylor et Drouet in Drouet</td>
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<tr>
<td><em>Leptolyngbya</em> sp.</td>
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<tr>
<td><em>Merismopedia elegans</em> A. Braun in Kützing</td>
</tr>
<tr>
<td><em>Oscillatoria</em> sp.</td>
</tr>
<tr>
<td><em>Rhodobacter lineare</em> Schmidle et Lauterborn</td>
</tr>
<tr>
<td><em>Phormidium</em> sp.</td>
</tr>
<tr>
<td><em>Scytonema hofmannii</em> Agardh ex Bornt et Flahault</td>
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<tr>
<td>Coccosid cyanobacteria</td>
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| **Desmidiaceae**                                             |
| *Staurastrum* sp.                                            |
| *Cosmarium* sp.                                               |

| **Diatoms**                                                  |
| *Nitzschia* serpentinaphe Lange-Bertalot                      |
| *Mastogloia smithii* Thwaites ex W. Smith var. smithii        |
| *Fragilaria synegrotasca* Lange-Bertalot                      |
| *Encyonema* evergladianum Krammer                             |
| *Gomphonema* sp.                                              |
Fig. 3. Mean abundance of cyanobacteria and algae in the cryosections of floating periphyton mat.

Filamentous and solitary desmids were observed infrequently. Desmids had no apparent preference for a particular region of the periphyton mat and the distribution of both filamentous and solitary forms appeared random and widely dispersed.

*Mastogloia smithii* var. *smithii*, a mobile, epipelic, and alkaliophilous diatom (Van Dam et al. 1994), was the most abundant diatom in absolute abundance in all quadrants examined. *Enchyonea evergladianum*, a sessile epiphytic diatom, was the next most abundant diatom. *Nitzschia seriata* and *Fragilaria synegetesca* were two other diatoms observed in interior regions of FPM’s.

**Bacteria**

A thin, densely populated film of bacteria was observed on the upper surface and lower quadrants of all transects observed. Occasionally, very dense colonies of bacteria were detected at various depths within the FPM’s.

**Calcite**

Percent calcite coverage of quadrants ranged from 0 to 95% and averaged 31% over all quadrants. Surface quadrants contained the highest percentage of calcite coverage and contained a greater amount of densely compacted calcite. The lowermost quadrants in all blocks of periphyton analyzed contained a calcite precipitate that was mostly associated with the mucilaginous sheaths of *Scytonema hofmannii*. 

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Discussion

Floating periphyton mat structure

The taxonomic composition of the Everglades periphyton has been discussed in several papers (Raschke 1993, Vymazal & Richardson 1995, McCormick et al. 1996, McCormick et al. 1998), however, to our knowledge this is the first report on the spatial distribution of algae, cyanobacteria, and calcite within an intact floating periphyton mat. Thin sections produced by embedding FPM's in TBS tissue freezing medium followed by cryosectioning proved to be an efficient method of quantifying mat components.

Our observation that the cyanobacteria are the dominant species in the mat sections is in accordance with earlier reports on periphyton mat taxonomic composition (Browder et al. 1994, McCormick et al. 1998, Vymazal & Richardson 1995). Cyanobacteria were observed throughout the whole mat, although certain representatives were more numerous in particular layers. The top layer of the mat is characterized by calcite deposits (Fig. 4a). The middle section contains mostly coccolid cyanobacteria and diatoms (Fig. 4b). The bottom layer of FPM's contain mostly filamentous cyanobacteria (Fig. 4c).

The importance of filamentous cyanobacteria such as Scytosema hofmannii and Leptolyngbya spp. (the latter often reported as Schizothrix calcicola Agardh ex Gomont) in the maintenance of periphyton mat structure has been observed by Gleason & Spackman (1974), Van Meter-Kasanof (1973), and Swift & Nicholas (1987). The cohesiveness of the mat elements was associated with the mucilaginous sheaths of filamentous cyanobacteria (Van Meter-Kasanof 1973, Swift & Nicholas 1987). Our sections show that the filamentous cyanobacterium Phormidium sp. is not only the most abundant species in the mat but is also the most significant contributor to mucilage production.

Calcaceous periphyton mats are characteristic of the Everglades and their accelerated formation has been attributed to the filamentous cyanobacteria Scytosema hofmannii and Schizothrix calcicola (Gleason & Spackman 1974). In our mat sections (cryosections) we routinely observed a dense layer of compacted CaCO_3 crystals at the periphyton mat surface/water interface, the thickness of which varied greatly. However, cluster analysis identified a group of quadrants that contained numerous internal surfaces covered with CaCO_3 precipitate that were located deeper in the mat at a mean depth of 1165 μm. These internal surfaces were observed adjacent to void spaces within the FPM’s interior. One possible explanation is that internal regions of the FPM’s are very fluid and that the biotic/abiotic elements within the mat are not rigidly fixed in place and may be occasionally subjected to contortion and folding. Attenuation of radiant energy with depth in FPM’s is possibly a gradient that produces the distinctive upper surface region and a transitional area between the surface and interior regions. This top calcaceous layer was formed by filamentous cyanobacteria that produced thick mucilaginous sheaths encrusted with calcite. Below the surface layer, the quality and quantity of radiant energy diminishes, void spaces are more numerous, and diatoms are more abundant. Homogenizing a quantity of periphyton mat or using periphyton collected from periphytometers, will produce a greater number of algal taxa.
Fig. 4. Thin sections of floating periphyton mat prepared with TBS embedding medium and viewed using brightfield microscopy, showing the top calcified layer (a), middle coccoid cyanobacteria and diatom layer (b) and bottom filamentous cyanobacterial layer.
Johnson et al. (1997) observed that some araphid diatoms and diatoms of the genus *Nitzschia* prefer microhabitats in interior regions of benthic mats. Geddes (1999) also described the presence of diatom pockets within the Everglades FPM's. We observed *Fragilaria synegrotesca*, an attached araphid diatom, and *Nitzschia serpentinophrone* more frequently in interior regions of FPM's, particularly in areas of increased void space. *Mastogloia smithii* was observed throughout the FPM's, which can be attributed to its ability to migrate between microhabitats within the mat.

Resource gradients, diffusion gradients, and boundary layer effects within periphyton mats have been extensively documented (Wetzel 1996, Stevenson 1996, Burkholder et al. 1990, Carlton & Wetzel 1987, Jones et al. 2000). The floating periphyton mats observed in this study contained a high percentage of vacant space in lowermost and internal regions. This high degree of porosity, which is absent in the structure of benthic periphyton mats (our unpublished data), likely facilitates the movement of nutrients through internal regions of FPM's. Some vacant spaces contain water, others may represent gas bubbles. Jorgensen & Revsbech (1983) pointed out that gas bubbles in cyanobacterial mats may act as reservoirs for oxygen during diurnal light cycling. Although the close aggregation of algal and heterotrophic microbial components has been attributed to the efficiency of nutrient recycling within the periphyton mat (Wetzel 1996), we did not observe pronounced bacterial colonization on or in close vicinity to cyanobacterial or algal cells. Future studies can utilize the embedding and quantification methods described in this study to determine the effects of P concentrations on structural integrity and species composition within periphyton mats, to examine the sequence of benthic and FPM's development on submerged surfaces, and to observe the effects of changing hydroperiod and the re-activation of dormant periphyton.

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References


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