

A Technique for Direct Microscopic Observation of Periphyton Assemblages on Aquatic Macrophytes

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ABSTRACT

Periphyton assemblages were examined on two submersed and two emergence macrophyte communities from Orange Lake, Florida, utilizing a technique designed for direct microscopic observation. The submersed species were coontail (*Ceratophyllum demersum* L.) and hydrilla (*Hydrilla verticillata* Royle), and the emergent species were spatterdock [*Nuphar luteum* (L.) Subthrop and Smith] and torpedograss (*Panicum repens* L.). Opaque (emergents) and semitransparent (submersed) plant segments were subjected to bleaching, using Clorox, then stained with

Lugol's preservative. The viewing clarity of periphyton structure was much improved, allowing for better observation, identification and quantification of organisms.

INTRODUCTION

Epiphytic communities are not uniformly distributed and are difficult to separate from the substrate in aquatic situations (3). Sladeckova (12) reported that "the age and growth rate of different parts of a plant, as well as the character of the surface, have an influence upon the quantity and structure of the epiphyton." Artificial substrates, such as suspended glass and plexiglass slides, smooth stones, plastic or wooden dowels, placed in the proximity of macro-

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phytes have been employed to circumvent these problems (1, 2, 7, 12). These techniques, however, are biased relative to characterizing natural communities (6, 13, 15).

Natural substrates pose problems pertaining to sampling design and quantification (15). Due to inherent difficulties associated with the quantification of periphyton, most investigators have used qualitative methods, which consist of removing periphyton from substrates by scraping, then examining them microscopically. The numbers of organisms collected must be related to a specific area in order to be meaningful. These methods, however, are laborious and time-consuming. When the substratum is opaque, the periphyton are usually removed for examination. Stones have been coated with dissolved collodion which is then lifted off the substrate, presumably bearing the periphyton with it (10). Similarly, periphyton have been removed from living leaves as well as plastic (artificial) leaves, using a facial mask (3). However, only the large algae could be examined directly. Plant samples have been sonicated with greater than 90% removal of periphyton (14). This distorts the chloroplasts of larger filamentous forms, but not beyond taxonomic recognition. Alternatively, when substratum is transparent, the periphyton may be viewed directly. This technique allows only observation of larger algal forms, because the clarity and structural characteristics of smaller organisms are not distinguishable from their backgrounds (12).

The objective of the present study was to develop a method for direct quantitative evaluation of naturally occurring periphyton assemblages on both submergent and emergent aquatic macrophytes.

METHODS AND MATERIALS

Four aquatic plants were selected: coontail (*Ceratophyllum demersum* L.), hydrilla (*Hydrilla verticillata* Royle), spatterdock [*Nuphar repens* I., Subtrop and Smith], and torpedograss (*Panicum repens* I.). These hydrophytes were chosen because of differences in their morphologic characters. In general, coontail and hydrilla represent submersed plants whose leaves are basically transparent, whereas spatterdock and torpedograss are emergents possessing opaque stem structure.

All collections were obtained from Orange Lake, Florida. This large, shallow, eutrophic lake, located in Alachua and Marion counties, is 9.2 km in length and 1.9 km in width, with an area of 4912 ha. Maximum lake depth is 4.9 m with a mean depth of 2.9 m. Collecting sites were at a water depth of 1.5 m, shoreward from the torpedograss community. For all plants, a 3.0 cm segment, representative of the zone from 12.0 to 15.0 cm beneath the water surface was removed. This segment was transferred into a 0.1-liter zip-lock bag, filled with distilled water, and stored on ice during transport, and refrigerated at 4 C. Periphyton were analyzed within 72 hours. Two leaves from a common whorl were removed from the midsection of each 3.0-cm hydrilla segment. Twenty filamentous sections of coontail were obtained, and two thin, epidermal surface layers (3.0 x 10 mm) were shaved from the center of each segment of the emergents with a razor blade. Usually there are more

epiphytes on the upper leaf surface than the lower leaf surface (9). Because of this difference, periphyton counts of hydrilla leaves were limited only to the upper leaf surface. Twenty microscopic fields at 400x magnification were counted for each sample, before and after treatment. Treatment consisted of (1) placing the sample in a 10% solution of household Clorox (active ingredient = sodium hypochlorite 5.25%) for 2 to 6 minutes, until the tissue clarified; (2) transferring into a 2% Lugol's solution for 10 to 30 seconds; and (3) placing on a slide for observation using 2% Lugol's as media with coverslip. The Lugol's solution under the coverslip provided a dark background in which to view the periphyton and, except for along the periphery of the plant sections, it did not significantly darken the periphyton on the plant section. In each case, the unbleached, unstained plant segment was observed first. This segment was then clarified, stained and re-examined. Results were expressed as cell numbers per square millimeter.

RESULTS AND DISCUSSION

Differences exist in clarification and staining rates (Table 1). This is probably due to differential absorbance into the cells, inherent to cell structure and thickness. Excessive clarification can distort characters used in the identification of diatoms and chlorophytes, etc., similarly high rates of the staining agent affects the clarity of both the plant tissues and periphyton. The times listed were determined by trial and error to provide the best resolution and staining of the periphyton.

TABLE 1. CLARIFICATION AND STAINING RATES OF PLANT TISSUE SEGMENTS FROM *Hydrilla*, *Ceratophyllum*, *Nuphar* AND *Panicum*.

Plant	10% Chlorox	2% Lugol's
<i>Hydrilla</i>	3 to 4 min	10 to 15 sec
<i>Ceratophyllum</i>	4 to 6 min	10 to 20 sec
<i>Nuphar</i>	2 to 3 min	20 to 30 sec
<i>Panicum</i>	5 to 6 min	20 to 30 sec

The associated densities of periphyton observed on untreated and treated macrophytes is summarized in Table 2. Observations of the semi-transparent plant segments of hydrilla before treatment indicated the presence of eight species at a density of 165.5/mm². After clarification and staining, 14 species were recorded at a density of 236.2/mm². Coontail segments yielded four epiphytic species before treatment at a density of 141.9/mm². Two additional epiphytes were recorded posttreatment and density increased to 210.8/mm². After treatment, the numbers of observed epiphytes increased by 30% on hydrilla and 33% on coontail. This was primarily a result of the increased countability of pennate diatoms (*Fragilaria* and *Cocconeis* spp.). On hydrilla, *Coleochaete nitellarum* (Jost) were found along host plant cell boundaries, and were readily discernible only after treatment. This was especially evident for those cells bearing characteristic sheathed setae.

Observation of the opaque plant segments of spatterdock before treatment indicated the presence of three species at a density of 7.7/mm². After clarification and staining, six

TABLE 2. DENSITIES (CELL NO/MM²) OF PERIPHYTON OBSERVED ON TREATED AND UNTREATED MACRO-PHYTE SEGMENTS OF *Hydrilla*, *Ceratophyllum*, *Nuphar* AND *Panicum*.

PERIPHYTON	<i>Hydrilla</i>		<i>Ceratophyllum</i>		<i>Nuphar</i>		<i>Panicum</i>	
	UT*	T*	UT	T	UT	T	UT	T
PHYLLUM: Chlorophyta								
ORDER: Zygenematales								
<i>Cosmarium</i> sp.	0.6	2.5	0.0	0.0	0.0	0.6	0.0	3.2
<i>Closterium</i> sp.	0.0	0.0	0.0	3.8	0.0	0.0	0.0	0.0
<i>Mougeotia</i> sp.	0.6	1.9	0.0	0.0	0.0	0.0	0.0	0.0
<i>Staurastrum</i> sp.	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0
ORDER: Chaetophorales								
<i>Coleochaeta nit.</i>	0.0	4.4	0.0	0.0	0.0	1.9	0.0	0.0
<i>Coleochaete obic.</i>	0.6	0.6	0.6	0.6	4.1	4.1	1.3	1.3
<i>Stigeoclonium</i> sp.	0.6	1.3	0.0	0.6	1.9	4.4	0.0	631.0
ORDER: Oedogoniales								
<i>Oedogonium</i> sp.	7.0	8.2	0.6	1.9	0.6	0.6	1.3	1.3
PHYLLUM: Cyanophyta								
ORDER: Oscillatoriales								
<i>Lynngbya</i> sp.	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0
<i>Schizothrix</i> sp.	0.0	0.6	0.0	0.0	0.0	0.0	0.0	440.0
PHYLUM: Bacillariophyceae								
ORDER: Pennales								
<i>Amphora</i> sp.	0.0	1.9	0.0	0.0	0.0	0.0	0.0	0.0
<i>Cocconeis</i> sp.	1.9	3.8	1.3	3.8	0.0	37.9	0.0	0.0
<i>Fragilaria</i> sp.	145.6	195.0	139.3	200.0	0.0	0.0	0.0	0.0
<i>Navicula</i> sp.	9.5	13.3	0.0	0.0	0.0	0.0	0.0	0.0
<i>Nitzschia</i> sp.	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0
Totals	165.5	236.2	141.9	210.8	7.7	49.6	2.6	1075.0
Density Increases	—	30%	—	33%	—	500%	—	4130%
Species Observed	8	14	4	6	3	6	2	5

*UT = Untreated; T = Treated.

species were recorded at a density of 49.6/mm². Pretreated torpedograss segments yielded two epiphytic species at a density of 2.6/mm². Three additional epiphytes were recorded posttreatment and the cell numbers of observed epiphytes increased to 1075/mm². The observed increase of periphyton on spatterdock was primarily because of increased countability of pennate diatoms (*Cocconeis*), while treatment of torpedograss segments greatly increased the visibility of *Stigeoclonium* and *Schizothrix*. *Coleochaete nitellarum* was only recorded posttreatment on spatterdock tissue segments and not observed on torpedograss tissue.

Different substrates have posed problems with experimental design and quantification of periphyton. When an untreated leaf section contains many chloroplasts, they mask algal structure and hinder identification and quantification. With this methodology, the most accurate results were obtained where thin-layered and transparent plant segments were observed directly under the microscope. The method described has several other advantages. It not only provides clear and distinct structural characteristics, but also stains the starch present in many cells, thus aiding in algal phylum differentiation. Increased visibility improves the accuracy of direct cell counts. This was especially true in desmids, such as *Cosmarium*, *Closterium* and *Staurastrum* and filamentous algae, such as *Mougeotia* and *Oedogonium*. Very dense periphyton growth has always posed problems with numerical counts without removal from substrates and

requires estimation or subdivision of the fields to be counted. One disadvantage to this technique is that fragile organisms without cell walls, such as the protozoan *Vorticella* sp., should be observed before clarification because Clorox destroys them.

Macrophyte-periphyton communities have been recognized as major contributors to the productivity of aquatic ecosystems (11), as well as providing food for invertebrate grazers (4, 5). Because of the increased interest in periphyton, invertebrate and fish relationships (8), this technique may provide a useful representation of periphyton-macrophyte associations especially when used in conjunction with other methods.

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