

## Effects of microalgal communities on reflectance spectra of carbonate sediments in subtidal optically shallow marine environments

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### *Abstract*

This study was conducted in subtidal areas around Lee Stocking Island, Bahamas, to investigate how microalgal biomass and community structure affect hyperspectral reflectance of sediments. Hyperspectral reflectance was measured on the surfaces of sediment cores collected from several types of carbonate sediments and habitats. Subsequently, photosynthetic and photoprotective pigments within the microalgae colonizing the top 5 mm of the sediment cores were quantified by high-performance liquid chromatography (HPLC). Results of pigment analyses indicate that both microalgal biomass and community structure varied within and among sampling sites. Examination of spectral reflectance revealed differences both in the magnitude of overall reflectance between 400 and 710 nm and in the magnitude of absorption features. Second derivative analysis of reflectance spectra was used to identify nine narrow wavebands that correspond to wavelengths most affected by *in vivo* absorption by specific pigments. Results of linear regression analyses of the ratio of second derivatives at 676 nm to reflectance at 676 nm versus chlorophyll *a* plus chlorophyllide *a* indicate that total (living plus senescent or dead) microalgal biomass can be estimated from measurements of hyperspectral reflectance. Estimates of microalgal biomass can also be made based on the ratios of second derivatives at 444 nm to reflectance at 444 nm. Concentrations of other pigment groups can be estimated from second derivatives at 492 and 540 nm. These relationships between hyperspectral reflectance of sediments and benthic microalgal pigments suggest that remote sensing reflectance might be useful for distinguishing major differences among benthic habitats in some optically shallow areas.

Focus on nearshore marine environments is increasing because of the importance of coastal areas for fisheries and recreation and because of the intensification of anthropogenic changes in these areas. Benthic microalgae are an integral part of shallow marine ecosystems at depths within the photic zone, contributing significantly to primary production of the ecosystem as a whole, oxygenation of sediments, nutrient cycling, and sediment stabilization (Paterson 1994; Yallop et al. 1994; MacIntyre et al. 1996; Nelson et al. 1999). Because benthic microalgae form a significant part of the base of the food web in optically shallow waters, changes in biomass and community structure could provide insight into the overall health of nearshore ecosystems.

A number of studies designed to characterize microalgal communities near the sediment surface in intertidal habitats have been conducted (Pinckney and Zingmark 1993; Yallop et al. 1994; Brotas et al. 1995; Barranguet et al. 1998; Paterson et al. 1998). Other studies have described vertical dis-

tributions of microalgae in terms of biomass, major taxonomic groups, or their effects on the spectral composition of light at different depths within the top few millimeters of the sediment (Jørgensen and Des Marais 1988; Lassen et al. 1992; Ploug et al. 1993; Kühl et al. 1994; MacIntyre and Cullen 1995). Paterson et al. (1998) described relationships between spectral reflectance at the sediment surface of an exposed intertidal mudflat and different microalgal communities.

Although numerous studies describe microalgal communities in intertidal areas, there is a paucity of information available concerning microalgal communities inhabiting subtidal sediments. Furthermore, most studies conducted in either intertidal or subtidal habitats have been focused in small localized areas. Paterson et al. (1998) pointed out the need for more comprehensive coverage of major types of ecosystems than can be accomplished using ground-level monitoring and suggested that analysis of remote sensing images could be key to defining the nature of marine sediments within large geographical areas. Before remote sensing can be used effectively as a tool for characterizing large areas of nearshore sediments, a better understanding is needed about how light interacts with different types of sediments and with major constituents of sediments. In particular, a more detailed understanding is needed about how photosynthetic and photoprotective pigments produced by different taxonomic groups of benthic microalgae affect hyperspectral

### *Acknowledgments*

We thank the staff of the Caribbean Marine Research Center (CMRC) at Lee Stocking Island, Bahamas, for support and the use of their facilities for conducting our field studies. This work was a part of the Coastal Benthic Optical Properties (CoBOP) project funded by the Environmental Optics Program of the Office of Naval Research (ONR) (Steven Ackleson, Program Officer), ONR grants N000149910130, N000149710010, and N0001498C0002.

reflectance. The effects of organic substances, especially extracellular polysaccharide secretions (EPS) that are produced by both microalgae and bacteria (Taylor and Paterson 1998; Decho et al. 2003), and within which microalgae are frequently embedded, also must be defined.

In this paper, we present the results of a study that focused on the effects of benthic microalgal pigments on hyperspectral reflectance of the seafloor in a variety of optically shallow (water transparency such that light reflected from the seafloor is detectable from the sea surface) tropical subtidal habitats (calm lagoon, areas with high tidal flow, near and within seagrass beds, near a coral reef, and in biologically disturbed areas) and sediment types (oolitic, peloidal, grapestone) in the vicinity of Lee Stocking Island (LSI), Bahamas. Our main objectives were to determine how microalgal biomass affects the magnitude of reflectance spectra (400–710 nm), to determine how microalgal community structure affects the shape of reflectance spectra, and to investigate the usefulness of hyperspectral reflectance signatures of sediments for estimating benthic microalgal biomass and for characterizing community structure. Implications of our findings for the use of remote sensing reflectance to characterize and map benthic microalgal communities in optically shallow environments are discussed.

### Study area

Shallow marine environments in the vicinity of LSI are characterized by clear water ( $\sim 0.1$  mg chlorophyll *a* [Chl *a*]  $m^{-3}$ ) and sediment that is composed primarily of carbonate sand. Sediments are commonly colonized by microalgae and, in some areas, by seagrass (mainly *Thalassia*) as well. Final site selection was based on visual observations during numerous diving excursions conducted at potential sampling sites that were identified using information from a mapping study conducted around LSI by Dill (1991) and published diver observations (Gonzalez and Eberli 1997). The objective was to select sites with different sediment types and sites within different types of environments in order to understand the relationships between sediment reflectance and pigments produced by different microalgal communities growing on a variety of sediment types. Eighteen sites near LSI, where water depths ranged from 0.5 to 16.5 m, (Fig. 1; Table 1) were studied. These sites included areas with diverse sediment types, including oolitic (coated ovoid grains), grapestone (clusters of cemented sand grains), peloid (micritic fecal pellets and other microcrystalline grains), skeletal, and foraminiferal. Habitat types included areas near a coral reef, in and around seagrass beds, a calm lagoon, areas with strong tidal flows, and biologically disturbed areas.

### Methods

**Sampling**—This study was conducted in May 1999 and May 2000. Two sampling designs were used to collect sediment cores around LSI. Multiple sediment cores (22 mm diameter) were collected by divers either within a 0.25-m<sup>2</sup> grid or along transects sampled at 0.5-m intervals over a distance of 5 m (Table 1).

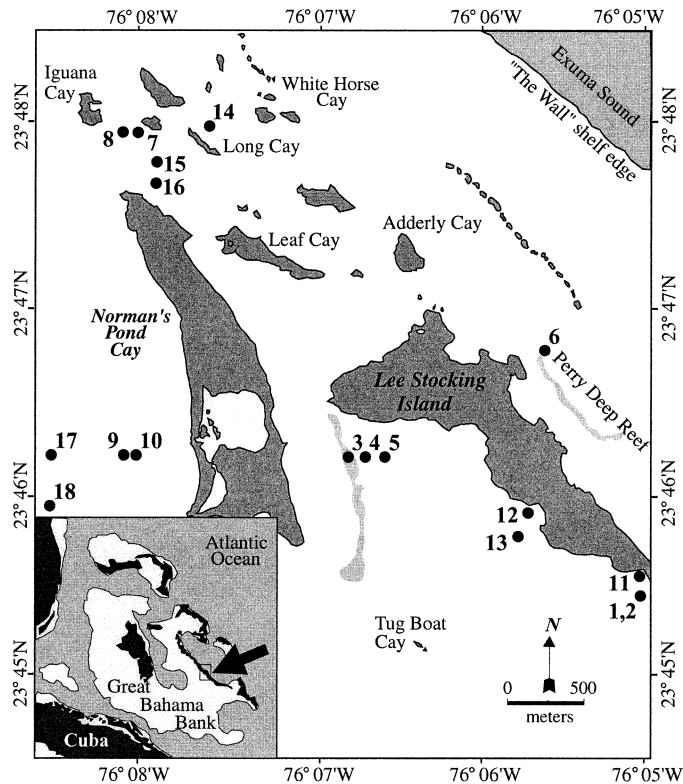


Fig. 1. Map (after Dill 1991) of study area showing sampling sites. Sites numbers are identified in Table 1.

On completion of sampling in 1999 (sites 1–10), sediment cores were immediately taken to the laboratory at LSI where reflectance measurements were made on 5–20 samples from each site. Samples collected in 2000 (sites 11–18) remained on the boat for several hours, while other work was conducted, before being taken to the laboratory for reflectance measurements. Samples were kept in subdued light in the laboratory until reflectance measurements could be completed (2–3 h from the time they first arrived in the laboratory). On completion of the reflectance measurements, the upper 5 mm of the cores was removed and frozen in liquid nitrogen for microalgal pigment analysis. Additional cores were used for determination of grain size and type.

**Grain type**—The top 1.0 cm of sediment cored was removed and impregnated with Epotek 301 epoxy resin. Thin sections of the fixed sediments were examined with an Olympus BH-2 petrographic microscope and photographed with an Olympus DP10 digital camera. Photographs were digitally overlaid with a 100- $\mu$ m grid for point counting (Mcmanus 1983). Grains larger than 63  $\mu$ m that fell on a grid intersection were categorized by their structure and origin (Milliman 1974).

**Grain size**—The top 1.0 cm of sediment cores were removed and freeze-dried then sieved to remove grains greater than 2 mm diameter. A Coulter LS-200 was used to measure the grain size distribution for grains <2 mm diameter. Grain size from 2.00 to 4.75 mm was measured by sieving (Mcmanus 1983).

Table 1. Sampling sites. Asterisk indicates samples collected in 0.25-m<sup>2</sup> grid. At other sites samples were collected along 50m transects.

Site no.	Location	Year	Site description	Water depth (m)
1*	Twin Beaches	1999	Peloidal sand on surface of shrimp mound	1.5
2*	Twin Beaches	1999	Peloidal and oolitic sand with biofilm surrounding shrimp mound	1.5
3*	Channel marker	1999	Migrating oolitic sand adjacent to a tidal channel	5.0
4*	East of channel marker	1999	Oolitic and peloidal sand in sparse <i>Thalassia</i> bed	5.0
5*	East of channel marker	1999	Oolitic, peloidal, and foraminiferal sand in dense <i>Thalassia</i> bed	5.0
6*	North Perry Reef	1999	Oolitic, peloidal, and skeletal sand covered by dense biofilm	16.5
7*	Rainbow Gardens	1999	Peloidal and skeletal sand adjacent to <i>Thalassia</i> bed	6.5
8*	Rainbow Gardens	1999	Peloidal, skeletal, and foraminiferal sand in sparse <i>Thalassia</i> bed	6.5
9*	West of Norman's Pond Cay	1999	Grapestone with thin biofilm	2.0
10*	West of Norman's Pond Cay	1999	Grapestone with dense biofilm	2.0
11	Twin Beaches	2000	Peloidal and oolitic sand with green film underneath sediment surface	0.5
12*	Coconut Beach	2000	Peloidal sand with surrounding shrimp mounds	1.5
13*	Coconut Beach	2000	Peloidal sand with biofilm in area without shrimp mounds	1.5
14	Long Cay	2000	Peloidal sand in sparse <i>Thalassia</i> bed	4.0
15	Rainbow South	2000	Migrating oolitic sand	5.0
16	Rainbow South	2000	Oolitic, peloidal, and foraminiferal sand in sparse <i>Thalassia</i> bed	5.0
17	West of Norman's Pond Cay	2000	Peloidal, foraminiferal, and grapestone sand with thin biofilm	2.0
18	West of Norman's Pond Cay	2000	Peloidal, foraminiferal, and grapestone sand in sparse <i>Thalassia</i> bed	2.0

**Microscopy**—Sediment surfaces were examined using an Olympus SZX-12 stereo microscope. Photomicrographs were made to document grain characteristics, microalgal communities, and EPS at the sediment surface.

**Pigments**—Photosynthetic and photoprotective pigments were extracted from the sediment samples with 100% acetone ( $-10^{\circ}\text{C}$ ) by sonication in an ice bath for 1 min in 15-s bursts. Pigment extraction was allowed to continue for 24 h in a freezer at  $-10^{\circ}\text{C}$ , after which the samples were vigorously agitated with a vortex mixer and allowed to settle for at least 1 h in the freezer before analysis. Pigment analysis was conducted with a Hewlett Packard 1100 series high-performance liquid chromatograph equipped with a diode array detector. The method used was similar to that described by Van Heukelem et al. (1994) with columns kept at  $35^{\circ}\text{C}$ . However, as suggested by Pinckney et al. (1996), three C18 columns were used in series to enhance pigment separation. The first column was a Rainin monomeric Microsorb-MV column,  $0.46 \times 10$  cm,  $3 \mu\text{m}$  packing, followed by two polymeric Vydac 201TP columns,  $0.46 \times 25$  cm,  $5 \mu\text{m}$  packing. A binary solvent system was used with solvent A (80:20 methanol:ammonium acetate [0.5 M]) and solvent B (80:20 methanol:acetone)

to separate pigments. Identification and quantification of pigments was made by comparing retention times and peak areas (peak areas measured at 405, 430, 445, 458, and 475 nm) with pigment standards obtained from VKI Water Quality Institute. Pigment standards used for calibration of the high-performance liquid chromatography (HPLC) system were as follows: Chl *a* and *b*, fucoxanthin, peridinin, zeaxanthin, lutein,  $\alpha$ - and  $\beta$ -carotene, diadinoxanthin, alloxanthin, 19'-hexanoyloxyfucoxanthin, and 19'-butanoyloxyfucoxanthin. These standards were chosen because of their importance as taxonomic markers. Chlorophyllide *a* standard was not available, so concentrations of chlorophyllide *a* were estimated using the same conversion factors used for determining Chl *a* concentration from peak area and then multiplied by the ratio of the specific extinction coefficients of Chl *a* ( $87.7 \text{ g}^{-1} \text{ cm}^{-1}$ ; Jeffrey and Humphrey 1975) to chlorophyllide *a* ( $127 \text{ g}^{-1} \text{ cm}^{-1}$ ; Jeffrey et al. 1997) at 664 nm in 90% acetone. Pigment peak identifications and purity were confirmed with the diode array detector. On the completion of pigment analyses, extracted sediment was rinsed with distilled water and dried in a  $90^{\circ}\text{C}$  oven for dry weight determination. Individual pigment concentrations were calculated relative to dry weight of each sample.

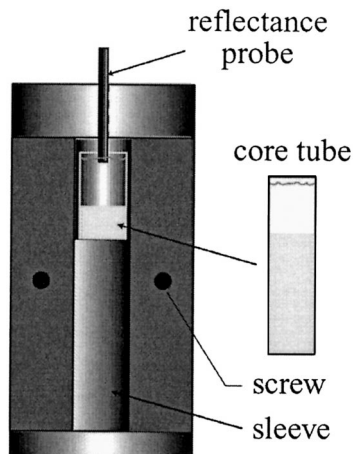


Fig. 2. Sediment core and reflectance probe holder.

**Hyperspectral reflectance**—The methodology used to measure spectral reflectance of sediment surfaces was a newly developed core technique. The sediment core tube, containing the sediment and overlying seawater, was placed in a custom-built polyvinyl carbonate (PVC) core holder designed to exclude ambient light (Fig. 2). A reflectance probe (Ocean Optics RP200-7) was inserted into the top of the core holder so that the probe tip was under water, 25 mm from and orientated normal to the sediment surface. An Ocean Optics RP2000 UV-VIS spectrometer (grating #2), with 2,048 channels (dispersion,  $0.32 \text{ nm pixel}^{-1}$ ; optical resolution, 2.06 nm) was connected to the RP200-7 probe by a 200- $\mu\text{m}$  fiber optic cable and cojoined to an Ocean Optics LS-1 tungsten halogen light source. Light emitted from six fibers around the periphery of the reflectance probe was reflected from the sediment surface and measured from 190–890 nm by a central fiber as the number of spectrometer counts. Likewise, spectrometer counts of a diffuse-reflectance WS-1 Spectralon (Labsphere) standard (99% reflective) were measured 10 times in a PVC cylinder filled with filtered seawater that was similar in design to the one used for the core measurements. Spectrometer counts were also measured with the lamp off and were used to correct for the dark current noise of the instrument. Measurements of spectrometer counts of samples under illumination and in darkness were made three times each. Each core was rotated a third of a turn for each of the three measurements made under illumination. Integration times over which the measurements were made were varied to maximize the signal to noise ratio without saturating the spectrometer. Reflectance ( $R$ ) was calculated as the ratio of the mean spectrometer counts measured on the sample divided by the mean spectrometer counts per integration time of the calibrated Spectralon diffuse reflectance standard, multiplied by a calibration factor of 0.99 for the 99% Spectralon reflectance standard as follows:

$$R = 0.99 \left( \frac{CS_L - CS_D}{CR_L - CR_D} \right) \left( \frac{int_R}{int_S} \right)$$

$CS_L$  is the mean spectrometer counts for the sample,  $CS_D$  is the mean dark current counts for the sample,  $CR_L$  is the

mean spectrometer counts for the reflectance standard,  $CR_D$  is the mean dark current counts for the reflectance standard,  $int_R$  is the integration time for the reference standard, and  $int_S$  is the integration time for the sample.

**Derivative analysis**—Reflectance spectra were smoothed using a cubic spline interpolation with 60 nodes at equally spaced intervals and 2,048 data points. The use of 60 nodes was the best compromise between reducing noise and retaining resolution. Second derivatives of the smoothed reflectance spectra, which represent the acceleration of the change (slope of the slope) of reflectance over  $\sim 7\text{-nm}$  wavebands (21 data points), were calculated and used for resolving overlapping spectral features. These second derivative spectra, along with published *in vivo* absorption peaks for individual pigments (Jørgensen and DesMarais 1988; Johnsen et al. 1994; Smith and Alberte 1994; Bricaud et al. 1995) were used to identify narrow wavebands in the reflectance spectra that were most affected by specific pigments.

**Derivatives and pigment concentrations**—To explore quantitative relationships between pigments and reflectance, the ratio of the second derivative to reflectance at the wavelength most affected by each pigment or group of pigments was plotted against the concentration of the corresponding pigment or group of pigments. The ratio of the second derivative to reflectance was used to correct for spectral flattening, which could be caused by EPS and layering of microalgae near the sediment surface. For Chl *a* and chlorophyllide *a*, which have almost identical absorption spectra, the ratio of the second derivative at 444 nm to reflectance at 444 nm was plotted against the sum of Chl *a* and chlorophyllide *a*. The absorption spectra of Chl *a* and chlorophyllide *a* are characterized by having two major absorption peaks: one at 444 nm and one at 678 nm. For comparison with results of the analysis at 444 nm, the relationship between the ratio of the second derivative to reflectance at 678 nm and the sum of Chl *a* and chlorophyllide *a* was investigated in the same manner. To explore the relationship between zeaxanthin,  $\beta$ -carotene, lutein, and diadinoxanthin (which have similar absorption spectra to one another), the ratio of the second derivative at 492 nm to reflectance at 492 nm was plotted against the sum of these four pigments. Similarly, the relationship between the sum of fucoxanthin, peridinin, and 19'-butanoyloxyfucoxanthin (which have similar absorption spectra) and the ratio of the second derivative to reflectance at 540 nm was investigated.

## Results

**Sediment characteristics**—Grain size of sediments varied at different sites near LSI. Sites with mean grain size ranging from 500 to 750  $\mu\text{m}$  include 6, 7, 9, 10, 17, and 18. Mean grain size of sediments at the other sites ranged from 200 to 500  $\mu\text{m}$ . Several types of carbonate sediments were found at the various sampling sites, including oolitic, peloidal, skeletal, foraminiferal, and grapestone (Table 1).

One of the most striking characteristics of sediments around LSI is the presence of varying amounts EPS on the sediment surface, within which microalgae are embedded.

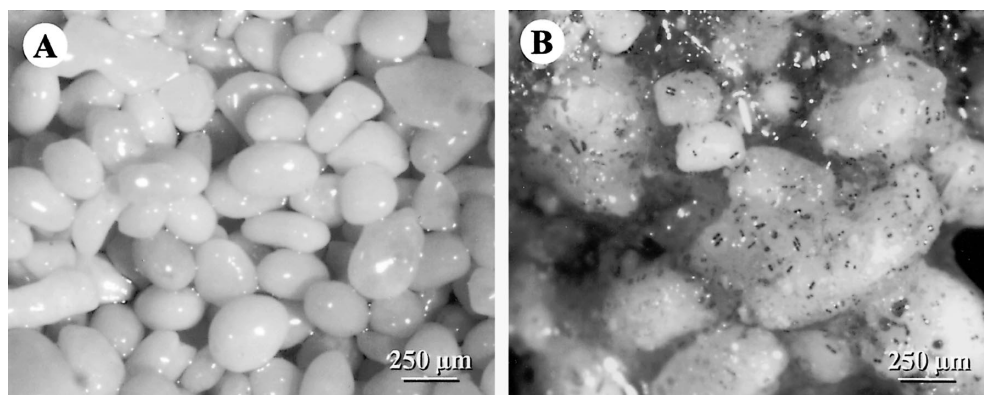


Fig. 3. Photomicrographs of sediment surface. (A) Surface of sediment that has little EPS film and few microalgae (site 3); (B) surface of sediment that has thick EPS film within which numerous microalgae are embedded (site 6).

Microscopic examination of the surfaces of sediment cores indicated that samples taken at site 3 (Fig. 3A) had the least EPS film and embedded microalgae and that samples taken at site 6 (Fig. 3B) had the most EPS film and embedded microalgae.

**Pigments and microalgae**—Microalgal pigments in the top 5 mm of sediment cores collected within grids in 1999 are listed in Table 2. Living microalgal biomass (Chl *a*) varied greatly among sampling sites. In 1999, mean Chl *a* concentration in the sediments ranged from  $<1 \mu\text{g g}^{-1}$  atop a

burrowing shrimp mound at site 1, which had little EPS, to  $14.2 \mu\text{g g}^{-1}$  at site 6, where numerous microalgae were embedded in a thick EPS film (Fig. 3B). In addition to among-site differences in microalgal biomass, Chl *a* concentrations measured on numerous samples collected within 0.25-m<sup>2</sup> grids indicate that biomass was patchy within sites on scales of centimeters (Table 2).

Chl *a* concentrations measured in many of the samples collected in 2000, particularly at sites 13–17, were unrealistically low relative the concentrations of other pigments measured in the same samples. We believe this is an artifact

Table 2. Microalgal pigments in the top 5 mm of sediments. (A) May 1999, (B) May 2000. Means  $\pm$  standard deviations of Chlorophyll *a* (Chl *a*) and Chlorophyllide *a* (Chllide *a*) in  $\mu\text{g g}^{-1}$ . Means  $\pm$  standard deviations of ratios of taxonomically significant pigments to Chlorophyll *a* (Chl *b*, Chlorophyll *b*; Fuc, Fucoxanthin; Per, Peridinin; Zea, Zeaxanthin; Lut, Lutein; But, 19'-Butanoloxfucoxanthin). Not detected, ND. Pigment ranges in  $\mu\text{g g}^{-1}$ .

Site no.	Pigment ( $\mu\text{g g}^{-1}$ )							
	Chl <i>a</i>	Chllide <i>a</i>	Chl <i>b</i> : Chl <i>a</i>	Fuc: Chl <i>a</i>	Per: Chl <i>a</i>	Zea: Chl <i>a</i>	Lut: Chl <i>a</i>	But: Chl <i>a</i>
<b>(A) May 1999</b>								
1	0.84 $\pm$ 0.43	0.96 $\pm$ 0.54	ND	0.58 $\pm$ 0.14	0.06 $\pm$ 0.02	0.09 $\pm$ 0.04	0.02 $\pm$ 0.02	ND
2	3.96 $\pm$ 1.65	4.68 $\pm$ 1.93	ND	0.48 $\pm$ 0.05	0.02 $\pm$ 0.01	0.04 $\pm$ 0.01	0.01 $\pm$ <0.01	ND
3	1.55 $\pm$ 1.36	ND	ND	0.01 $\pm$ 0.01	ND	0.20 $\pm$ 0.04	ND	ND
4	1.33 $\pm$ 0.47	0.12 $\pm$ 0.10	ND	0.26 $\pm$ 0.14	0.04 $\pm$ 0.03	0.11 $\pm$ 0.04	ND	ND
5	3.67 $\pm$ 2.48	1.04 $\pm$ 1.00	0.02 $\pm$ 0.02	0.72 $\pm$ 0.45	0.02 $\pm$ 0.01	0.12 $\pm$ 0.10	0.07 $\pm$ 0.06	ND
6	14.22 $\pm$ 5.13	3.46 $\pm$ 2.77	0.01 $\pm$ 0.01	0.52 $\pm$ 0.11	<0.01	0.04 $\pm$ 0.01	0.01 $\pm$ 0.01	<0.01
7	1.28 $\pm$ 0.49	0.16 $\pm$ 0.07	0.03 $\pm$ 0.01	0.55 $\pm$ 0.23	0.08 $\pm$ 0.04	0.11 $\pm$ 0.04	0.02 $\pm$ 0.01	ND
8	4.25 $\pm$ 3.10	0.70 $\pm$ 0.60	0.01 $\pm$ 0.01	0.31 $\pm$ 0.10	0.01 $\pm$ 0.01	0.09 $\pm$ 0.04	0.01 $\pm$ <0.01	<0.01
9	1.76 $\pm$ 0.84	0.25 $\pm$ 0.16	ND	0.71 $\pm$ 0.16	0.03 $\pm$ 0.02	0.03 $\pm$ 0.01	0.02 $\pm$ 0.01	<0.01
10	10.17 $\pm$ 1.89	2.39 $\pm$ 0.94	0.01 $\pm$ 0.01	0.47 $\pm$ 0.06	<0.01	0.04 $\pm$ 0.01	0.01 $\pm$ <0.01	0.01 $\pm$ 0.01
<b>(B) May 2000</b>								
11	0.55–9.44	0.44–1.15	ND–0.08	1.89–2.85	0.35–0.56	0.42–1.01	ND–0.07	ND
12	0.67–5.41	0.07–3.46	ND	0.79–4.49	0.02–0.53	0.03–0.33	ND	ND–0.02
13	2.78–24.43	0.94–15.71	ND	3.23–9.46	0.06–0.95	0.09–0.71	ND	ND–0.85
14	0.01–0.98	0.43–0.066	ND	0.47–0.66	<0.01–0.03	0.15–0.30	<0.01–0.03	<0.01–0.10
15	0.06–2.62	<0.01–0.61	ND	0.07–1.38	ND–0.18	0.06–0.74	ND	ND
16	0.02–0.07	0.35–0.80	ND	1.34–1.39	0.02–0.03	0.13–0.18	0.01–0.02	<0.01–0.01
17	<0.01	0.09–0.10	ND	0.42–0.67	<0.01–0.01	0.01–0.02	0.01–0.01	<0.01
18	20.80–78.23	0.62–3.19	ND	11.99–30.24	ND	0.21–0.53	ND	0.44–1.40

Table 3. Major groups of microalgae identified by HPLC pigment analyses in sediments collected in 1999 and 2000 in the vicinity of LSI. The ? indicates that cyanobacteria might be present, but their presence cannot be confirmed by HPLC alone.

Site no.	Cyanobacteria	Diatoms	Dinoflagellates	Chlorophytes	Chrysophytes or Prymnesiophytes
1	?	Dominant	Present	Present	Not detected
2	?	Dominant	Present	Present	Not detected
3	Dominant	Present	Not detected	Not detected	Not detected
4	Present	Dominant	Present	Not detected	Not detected
5	?	Dominant	Present	Present	Not detected
6	?	Dominant	Present	Present	Present
7	?	Dominant	Present	Present	Not detected
8	?	Dominant	Present	Present	Present
9	?	Dominant	Present	Present	Present
10	?	Dominant	Present	Present	Present
11	?	Dominant	Present	Present	Not detected
12	Present	Dominant	Present	Not detected	Present
13	Present	Dominant	Present	Not detected	Present
14	?	Dominant	Present	Present	Present
15	Present	Dominant	Present	Not detected	Not detected
16	?	Dominant	Present	Present	Present
17	?	Dominant	Present	Present	Present
18	Present	Dominant	Not detected	Not detected	Present

caused by sample handling. These samples were not immediately taken to the laboratory for analysis after collection, as was the case with the 1999 samples. Instead, the samples remained on the boat for several hours while other work was completed. We believe that the high temperature may have caused cells to rupture so that chlorophyllase was released, causing the destruction of chlorophylls. For this reason, these data collected in 2000 are excluded from our discussions except for mention of taxonomically significant accessory pigments, which seemed less affected.

Chlorophyllide *a*, a Chl *a* degradation product that occurs in senescent cells and in fecal pellets, was present in varying amounts at all sites except site 3. The ratio of chlorophyllide *a*:Chl *a* was highest (approximately 1:1) atop a burrowing shrimp (*Callinassa*) mound (site 1) and around the mound (site 2, Table 2). This corresponds with observations of numerous white and pink fecal pellets on the surfaces of sediment cores collected from these sites.

Microalgal community structure varied among sites, but one site (3) stands out as being unique (Tables 2, 3). This site is characterized by very strong tidal currents and shoaling oolitic sand. Microscopic examination revealed little EPS and few microalgae on the sediment surface. Only two taxonomically significant pigments—zeaxanthin and fucoxanthin—were identified by HPLC analysis. The ratio of zeaxanthin:Chl *a* was more than an order of magnitude higher than the ratio of fucoxanthin:Chl *a*, indicating that, while some diatoms were present, cyanobacteria dominated the microalgal community at site 3.

Community structure at all of the other study sites was more diverse (Table 3) than the community structure at site 3. Based on the amounts of taxonomically significant pigments, it appears that diatoms dominated the communities at all sites except site 3. The presence of peridinin at all sites except sites 3 and 18 indicates that dinoflagellates were

widely distributed. Peridinin:Chl *a* ratios were highest on a shrimp mound (site 1) and in a sandy area adjacent to a seagrass bed (site 7). Zeaxanthin, a pigment found in cyanobacteria and chlorophytes, was also present in the sediments at all sites. The apparent absence of lutein, Chl *b*, or both, which are found in chlorophytes but not in cyanobacteria, indicates that at some sites (3, 4, 12, 13, 15, and 18) cyanobacteria, rather than chlorophytes, were present. At the other sites, small amounts of lutein, Chl *b*, or both were measured, indicating that at least some of the zeaxanthin containing microalgae were chlorophytes. One other pigment—19'-butanoyloxyfucoxanthin—was present in small amounts at sites 6, 8–10, 12–14, and 16–18, suggesting the presence of small numbers of chrysophytes, prymnesiophytes, or both. With the exception of site 18, it appears that community structure was most diverse at the sites at which 19'-butanoyloxyfucoxanthin was identified.

*Reflectance*—The magnitude of the reflectance spectra varied both among and within sites studied in 1999 (Fig. 4). The overall magnitude of reflectance was greatest on top of the burrowing shrimp mound (site 1), on the surface of the shoaling oolitic sand (site 3), and in a sparse seagrass bed (site 4). In contrast, the magnitude of reflectance was least at site 6, which had the largest microalgal biomass and the most EPS. Standard deviations of the reflectance measured on cores taken within a 0.25-m<sup>2</sup> grid indicate that the magnitude of reflectance across the spectrum was quite uniform on small scales (centimeters) within some sites (1, 2), but more variable within others. The magnitude of reflectance was most variable at sites 6, 8, and 9.

The shape of reflectance spectra varied among sampling sites (Fig. 4). Subtle differences in the shapes of the reflectance spectra were enhanced using second derivative analysis. Peaks of the derivative spectra identify 10 narrow (ap-

proximately 7 nm) bands within the reflectance spectra between 400 and 710 nm that are most affected by absorbing substances. Nine of these peaks correspond to regions in the spectrum at which maximum *in vivo* absorption by specific microalgal pigments are known to occur (Jørgensen and DesMarais 1988; Johnsen et al. 1994; Smith and Alberte 1994; Bricaud et al. 1995; Fig. 4). Derivative peaks 1, 2, and 9 (at 422, 444, and 676 nm) correspond to Chl *a* and chlorophyllide *a*; peak 3 (468 nm) corresponds to Chl *b* and *c*; peak 4 (492 nm) corresponds to zeaxanthin, lutein,  $\beta$ -carotene, and diadinoxanthin; peak 5 (538 nm) corresponds to fucoxanthin, peridinin, and 19'-butanoyloxyfucoxanthin; peak 6 (572 nm) corresponds to phycoerythrin; peak 7 (620 nm) corresponds to phycocyanin; and peak 8 (643 nm) corresponds to Chl *a* and *c* and chlorophyllide *a*.

Second derivative analysis of hyperspectral reflectance measured at the sediment surface was also used to explore the relationship between the amounts of specific microalgal pigments and reflectance at specific wavelengths. Results of linear regression analysis of the ratio of the second derivative at 678 nm to reflectance at 678 nm plotted against the sum of Chl *a* and chlorophyllide *a* indicate that this method can be used to obtain coarse estimates of the sum of these two pigments (Fig. 5A). However, it should be noted that the correlations between pigment concentrations and reflectance are rather poor in the lower concentration ranges. Similar results were obtained by linear regression analysis when the ratio of the second derivative at 444 nm to reflectance at 444 nm was plotted against the sum of Chl *a* and chlorophyllide *a* (Fig. 5B). It must be pointed out, however, that it is not possible to separately estimate Chl *a* and its degradation product, chlorophyllide *a*, because they have almost identical absorption spectra. Thus, it is not possible to determine the percentage of live versus senescent or dead microalgal biomass in sediments. Concentrations of other groups of taxonomically significant pigments (Fig. 5C,D) might also be similarly estimated. These results were obtained using the 1999 data.

Correlations between Chl *a* and reflectance measured in 2000 is not as good (Fig. 5E). As mentioned before, these samples were left on the boat while other studies were being conducted and exhibit signs of Chl *a* degradation.

## Discussion

*Microalgal distributions*—Types and amounts of microalgal pigments measured within 0.25-m<sup>2</sup> grids in 1999 near LSI indicate that microalgal biomass and community structure were variable both within and among different environments and sediment types. Water depth within the observed range between 0.5 and 16.5 m (Table 1) did not appear to be a dominant factor in affecting either amounts or types of microalgae (Table 2A) inhabiting the different benthic environments included in this study. Although we do not have data on bottom turbulence, some patterns in the distribution of Chl *a* with respect to our field observations are notable. Site 3 is located near a tidal channel, and except for brief periods during slack tide, currents are very strong. This site is characterized by low benthic microalgal biomass (Chl *a*)

and low species diversity. Pigment analyses indicated that only two types of microalgae—cyanobacteria and diatoms—were present at this site. Moving away from the tidal channel toward LSI, site 4 is located in a sparse seagrass bed and site 5 is located in a dense seagrass bed. Microalgal biomass increased, and community structure became more complex with increasing distance from the tidal channel, even though water depth at all three sites was the same (5 m). The highest biomass measured was at the deepest (16.5 m) site (6), and the second highest biomass measured was at a shallow (2 m) site (10). These combined observations suggest that bottom turbulence, especially tidal currents, might play a more important role than water depth in determining microalgal biomass at some sites near LSI.

Other factors, such as biological disturbance, affect the biomass of microalgae on the sediment surface. For example, the lowest microalgal biomass measured was on the surface of a burrowing shrimp mound (site 1) located in a calm shallow (1.5 m) lagoon. In contrast to this low biomass on the surface of the mound ( $\sim 1 \mu\text{g [g Chl } a^{-1}]$ ), biomass around the periphery of the mound (site 2) was much higher ( $\sim 4 \mu\text{g [g Chl } a^{-1}]$ ).

*Hyperspectral reflectance*—The differences in microalgal biomass and community structure among various sampling sites at LSI provided an opportunity to study the effects of microalgal pigments on reflectance spectra. An understanding of the relationships between pigments produced by different microalgal communities and hyperspectral reflectance signatures of different types of sediments and habitats is needed to identify narrow wavebands that are most valuable for using hyperspectral imagery for characterizing and mapping benthic habitats (see Louchard et al. 2003).

Our first objective was to determine how the overall magnitudes of reflectance spectra (400–710 nm) are affected by microalgal biomass. Reflectance across the spectrum (400–710 nm) was highest at sites 1, 3, and 4 (Fig. 4), where biomass was low (Chl *a* plus chlorophyllide *a*  $< 2 \mu\text{g g}^{-1}$ ). In contrast, reflectance was lowest at site 6 where the biomass was almost an order of magnitude higher (Table 2). However, at site 9, biomass was similar to that at sites 1, 3, and 4, but the overall reflectance was much lower. These observations indicate that, although microalgal biomass could be one factor in determining the overall magnitude of reflectance, factors other than microalgal pigments might be equally or even more important. For example, the presence of varying amounts of EPS produced by microalgae and bacteria might have significantly affected the magnitude of reflectance spectra measured at different sites. Results of studies conducted by Decho et al. (2003) indicate that EPS can be responsible for as much as a 25% decrease in reflectance across the visible spectrum (400–700 nm). EPS could decrease reflectance in two ways (Decho et al. 2003). First, EPS increases the size of the spaces between sand grains so that photons penetrate deeper into the sediment, increasing the likelihood that the photons will be absorbed by microalgae and other components of the sediments. Second, a coating of EPS around sediment grains reduces the index of refraction of the grains, compared with grains in seawater, so that forward scattering is increased relative to backscatter-

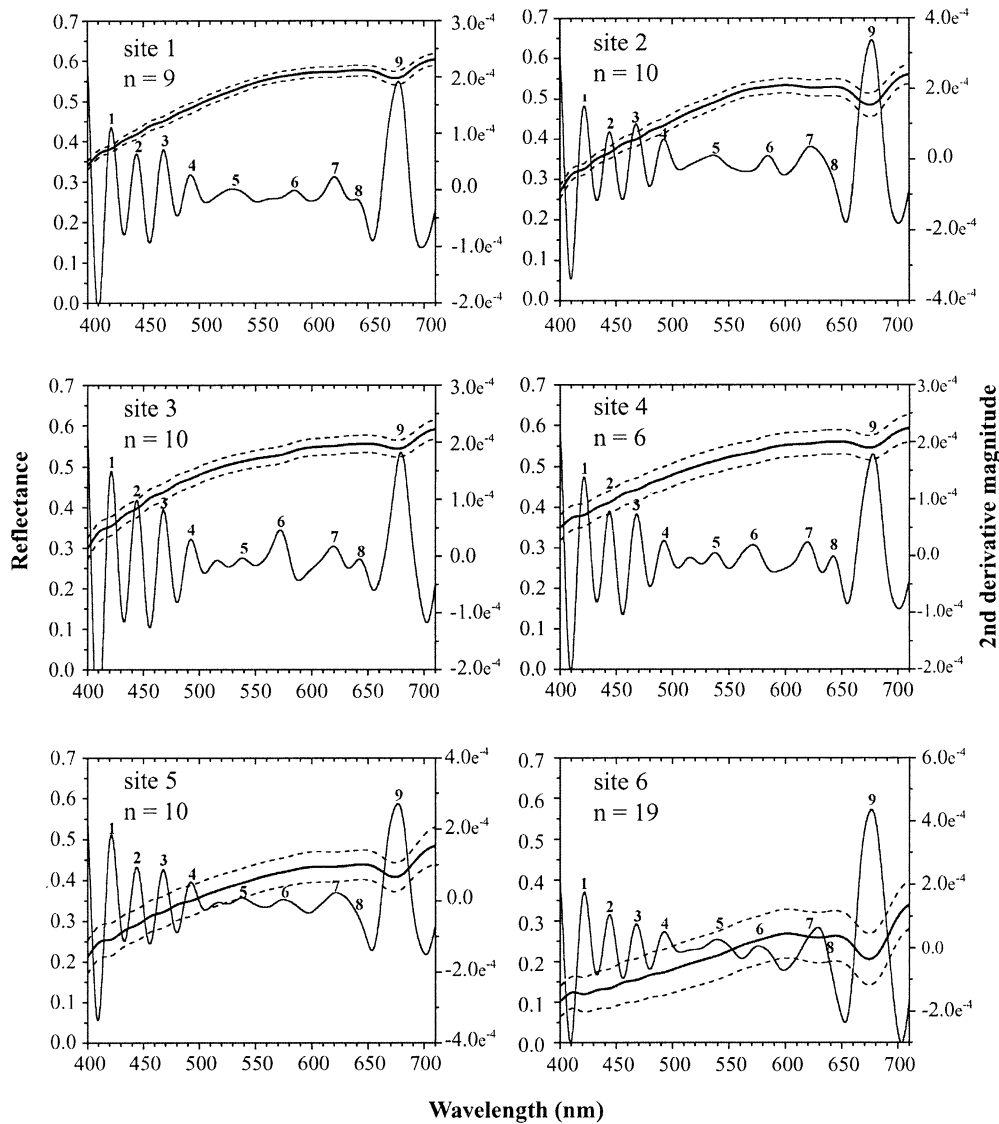


Fig. 4. Hyperspectral reflectance. Dark solid line, mean of smoothed reflectance spectra measured on multiple cores collected within a 0.5-m<sup>2</sup> grid; dashed lines, standard deviation of reflectance measurements made on multiple cores; thin solid line, magnitude of second derivatives of the smoothed mean reflectance spectra. Numbers on second derivative spectra indicate pigment peak identifications as follows: 1, 2, 9, Chl *a*; 3, Chl *b*, *c*; 4, zeaxanthin, lutein,  $\beta$ -carotene, diadinoxanthin; 5, fucoxanthin, peridinin, 19'-butanoyloxyfucoxanthin; 6, phycoerythrin; 7, phycocyanin; 8, Chl *a*, *c*.

ing. This increase in forward scattering enhances the likelihood that photons will penetrate deeper into the sediment and be absorbed. Reflectance is also affected by grain size (Clark and Roush 1984; Hiroi and Pieters 1992), with larger grains reflecting less light than smaller grains. Sediments at site 9 are composed mainly of cemented carbonate grains (grapestone), and the mean grain size is larger than the mean grain size at sites 1, 3, and 4. Thus, grain size might have been responsible for some of the difference in the magnitude of reflectance between site 9 and sites 1, 3, and 4.

Our second objective was to determine how pigments within microalgae growing on and near the surface of sediments affect the shape of reflectance spectra. The shape of

the reflectance spectrum (400–710 nm) of washed and bleached ooid sand is very similar to the reflectance spectrum of unbleached ooid sand collected from site 3, except that the small dips in reflectance at narrow wavebands that might be attributed to absorption by microalgal pigments are not apparent (*see* Decho et al. 2003).

Second derivative analysis proved to be an effective method for enhancing subtle effects of microalgal pigments on the shape of reflectance spectra so that narrow wavebands within reflectance spectra most affected by specific pigments or groups of pigments could be identified (Fig. 4). Ten dips within the reflectance spectra between 400 and 710 nm were identified with this technique, nine of which correspond to

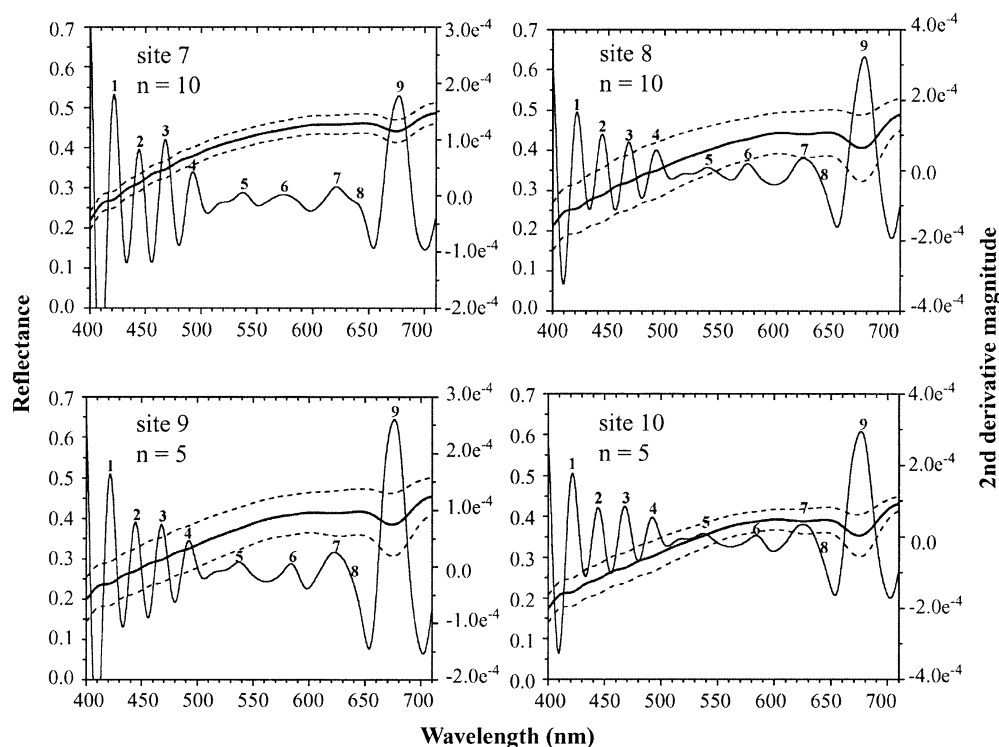


Fig. 4. Continued.

regions in the spectrum at which maximum *in vivo* absorption by specific microalgal pigments are known to occur. However, because some pigments have very similar absorption spectra, it is not possible to separate the effects of all individual pigments. For example, at site 3, HPLC pigment analyses indicated that the microalgal community was composed predominantly of cyanobacteria (Table 3). Peak number 4 of the second derivative spectrum at this site (Fig. 4) corresponds to the waveband where zeaxanthin,  $\beta$ -carotene, lutein, and diadinoxanthin absorb. From this information alone it is not possible to conclude whether cyanobacteria, chlorophytes, or both are present in the community because zeaxanthin and  $\beta$ -carotene are known to occur in both groups and lutein is produced by chlorophytes but not cyanobacteria. However, the presence of distinct second derivative peaks at about 585 nm (peak 6, phycoerythrin) and 615 nm (peak 7, phycocyanin) suggests that cyanobacteria were part of the community. Still, the presence or absence of chlorophytes cannot be confirmed because absorption by lutein could not be separated from absorption by the other pigments with similar absorption maxima. Likewise, the absorption spectra for fucoxanthin (diagnostic for diatoms), peridinin (diagnostic for dinoflagellates), and 19'-butanoyloxyfucoxanthin (found in chrysophytes, some prymnesiophytes, and a few dinoflagellates) are so similar that it was not possible to separate these three pigments using second derivative analysis (peak 5). Thus, one can only conclude from the presence of peak 5 of the derivative spectrum that one or more of these three types of microalgae was present. Based on these findings, it is concluded that second derivative analysis of reflectance spectra is of limited value for determining the exact composition of complex benthic mi-

croalgal communities. However, it might be possible to use reflectance data for distinguishing between areas dominated by cyanobacteria (e.g., site 3) and areas dominated by other types of microalgae (e.g., all other sites studied at LSI) and for detecting large changes in the ratio of cyanobacteria to other microalgae over time.

Our third objective was to explore the possibility of extracting quantitative information about microalgal pigments from reflectance spectra. Linear regression analysis was used to explore quantitative relationships between specific microalgal pigments or groups of pigments and the magnitudes of second derivatives at specific wavelengths. Results of linear regression analysis of the ratio of the second derivative at a specific wavelength to reflectance at that wavelength plotted against the amount of a specific pigment or group of pigments that absorb at that wavelength was used to determine whether pigment concentrations could be estimated from reflectance spectra (Fig. 5A–D). In this study, it appears that microalgal pigments were within a concentration range low enough that the relationship between pigment concentration and second derivatives of reflectance spectra did not approach an asymptotic level. In different environments with extremely dense microalgal communities, this method might be less useful. The accuracy of estimates of Chl *a* plus chlorophyllide *a* obtained using second derivatives at two wavelengths—678 nm and 444 nm—is approximately the same even though absorption bands of carotenoids overlap with Chl *a* and chlorophyllide *a* at 444 nm. The results of Chl *a* plus chlorophyllide *a* estimation at 444 nm are encouraging for application to remote sensing of water-leaving radiance where radiance at wavelengths greater than 600 nm is attenuated through water absorption. It should also be not-

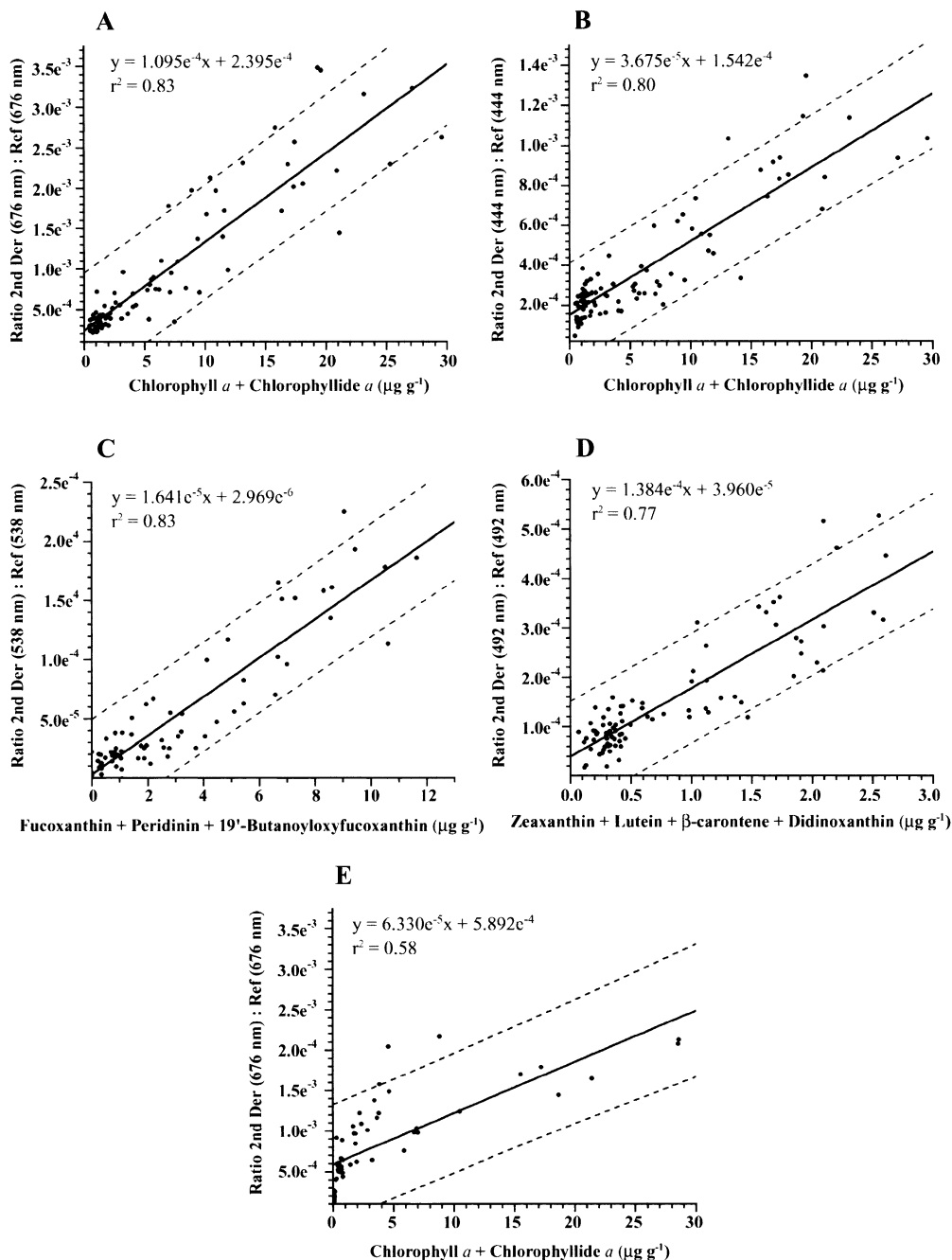


Fig. 5. (A–D) Sites sampled in 1999. (E) Sites sampled in 2000. Solid line, regression line; dashed lines, 95% prediction interval. (A) Linear regression of the ratio of the second derivative to reflectance at 676 nm versus the sum of Chl *a* and chlorophyllide *a*. (B) Linear regression of the ratio of the second derivative to reflectance at 444 nm versus the sum of Chl *a* and chlorophyllide *a*. (C) Linear regression of the ratio of the second derivative to reflectance at 538 nm versus the sum of fucoxanthin, peridinin, and 19'-butanoyloxyfucoxanthin. (D) Linear regression of the ratio of the second derivative to reflectance at 492 nm versus the sum of zeaxanthin,  $\beta$ -carotene, lutein, and diadinoxanthin. (E) Linear regression of the ratio of the second derivative to reflectance at 676 nm versus the sum of Chl *a* and chlorophyllide *a*.

ed that phycocyanin, which is present in cyanobacteria and absorbs maximally around 615 nm, might not be detected by remote sensing of water-leaving radiance. Therefore, it is likely that only those benthic cyanobacteria that contain phy-

coerythrin (maximal absorption at about 585 nm) can be detected. At LSI,  $\alpha$ -carotene was not detected, indicating that cryptophytes were either absent or present in minute quantities. In other geographical areas, some confusion might

arise if cryptophytes are present, because they contain phycoobiliproteins that have absorption spectra similar to phycoerythrin produced by many cyanobacteria. Although we know of no reports of large populations of cryptophytes in sediments, the possibility that cryptophytes might confound interpretations about the origin of a dip in reflectance near 585 nm should be investigated before it is stated unequivocally that phycoerythrin-containing cyanobacteria can be distinguished.

It is possible that refinement of techniques used in this study could improve the correlations between pigment concentrations and second derivatives of reflectance spectra. Two factors that might have affected the results of this study should be considered when designing future studies. First, there does not seem to be one optimal core sectioning protocol. Determination of the optimal depth to which a sediment core should be sectioned for pigment analysis is difficult because the vertical distribution of microalgae within sediments varies among different types of sediments and environments. Also, based upon measurements of downwelling irradiance within sediments at several sites around LSI, it is now known that the depth to which 10% of incident light penetrates into the sediments is influenced by the amount of film (EPS and embedded microalgae) on and near the sediment surfaces (Decho et al. 2003). The depth to which 10% of incident light penetrated sediments at several sites around LSI ranged from 1.4 to 1.6 mm at 500 nm in sediments with little film and increased by approximately 80% in sediments with thick film (site 6). It might, therefore, be possible to improve the correlations between pigment concentrations and reflectance by measuring the pigments in the top 2 mm of the sediment, rather than in the top 5 mm as was done in this study. Second, it is possible that vertical migration by some microalgae occurred between the time that the reflectance measurements were made and the time the cores were sectioned and frozen for pigment analysis because we first measured reflectance on from 10 to 20 cores before removing and freezing the top sections of the cores for pigment analysis. This potential problem might be avoided by removing and freezing the top section of each core immediately after each reflectance measurement is made. Furthermore, it is essential that reflectance be measured quickly after cores are collected. The importance of this is illustrated by the poor correlation between reflectance and Chl *a* obtained for samples that remained on the boat for several hours before being taken to the laboratory in 2000 (Fig. 5E).

There is, however, one problem that cannot be resolved: Chl *a* and one of its degradation forms (chlorophyllide *a*) have almost identical absorption spectra, making it impossible to differentiate between healthy cells that contain only Chl *a* and senescent cells or fecal pellets that contain chlorophyllide *a*. Thus, accurate estimates of photosynthetically active microalgal biomass in sediments based on spectral reflectance measurements will always be problematic. The same problem exists when using remote sensing reflectance to estimate planktonic microalgal biomass. However, chlorophyllide *a* is generally found in low concentrations in phytoplankton (2–5% of Chl *a*, Trees et al. 2000). It is plausible that chlorophyllide *a* might be high in some sediments because senescent phytoplankton sink to the bottom, senescent

benthic microalgae accumulate within EPS matrices, and numerous fecal pellets are produced and deposited by bottom feeders. Despite the fact that the components of estimated benthic microalgal biomass cannot be separated into photosynthetically active, senescent, or dead (e.g., remains of microalgae in fecal pellets), estimates of the total of these components could be useful for monitoring large changes in shallow water benthic biomass over time.

*Summary and conclusions*—Pigment analyses indicate that microalgal biomass and community structure were variable within and among sites around LSI. In 1999, microalgal biomass (Chl *a*) ranged from <1.0 to 14.2  $\mu\text{g g}^{-1}$  among sites. Concentrations of chlorophyllide *a*, a degradation product of Chl *a*, were also variable within and among sites. The highest ratios of chlorophyllide *a*:Chl *a* were measured in sediments on and around a burrowing shrimp (*Callinassa*) mound (sites 1, 2), where numerous white and pink fecal pellets were observed.

The magnitudes of reflectance spectra varied both within and among sampling sites. Variations in the shapes of reflectance spectra were most obvious among sites. Microalgae colonizing the sediment surfaces affect reflectance properties of the sediments in two ways. First, microalgae cause a decrease in the magnitude of reflectance across the visible spectrum (400–700 nm) because of the combined absorptive properties of their photosynthetic and photoprotective pigments. Second, microalgal community structure affects the shape of reflectance spectra because different pigments that are characteristic of specific microalgal groups have unique absorptive properties. Other factors, such as the amount of EPS produced by microalgae and bacteria, and sediment grain type and size, also play significant roles in determining reflectance properties of sediments.

Second derivative analyses of reflectance spectra measured at LSI indicate that subtle differences in the shape of reflectance spectra can be enhanced so that the narrow wavebands most affected by a specific pigment or group of pigments can be identified. This methodology is, however, subject to some limitations. Specifically, when the absorption spectra are the same or very similar for two or more pigments (e.g., Chl *a* and chlorophyllide *a* or fucoxanthin, peridinin, and 19'-butanoyloxyfucoxanthin), it is not possible to access relative contributions of the individual pigments.

Results of linear regression analyses of the ratio of second derivatives at 676 or 444 nm to reflectance at 676 or 444 nm plotted against concentrations of Chl *a* plus chlorophyllide *a* indicate that hyperspectral reflectance of sediment surfaces can be used to estimate total (physiologically active, senescent, and dead) benthic microalgal biomass. Concentrations of other groups of pigments can be estimated in a similar manner. Correlations between reflectance and pigment concentrations might be improved by measuring pigments in the top 2 mm of sediments, rather than the top 5 mm of sediments. The possibilities for using second derivative analysis of high-resolution reflectance spectra for distinguishing major habitat types and for monitoring changes over time in benthic microalgal communities is promising and warrants further investigation.

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Received: 2 October 2001

Accepted: 13 September 2002

Amended: 23 September 2002