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14. ABSTRACT
Lipid-based microbial biomass varied greater than 10-fold among sediment archetypes collected at LSI during field campaigns. Seagrass epiphyte loads, determined by measuring their lipid biomass, increased non-linearly with leaf age. The highest epiphyte loads, on eelgrass from Monterey Bay, absorbed 60% of incident light in peak chlorophyll absorption bands and reduced modeled photosynthesis by 49%. In the course of this research, we have found large, refractive, rhomboidal crystals in turtle grass from the Bahamas, Florida, and Texas. At all collection sites, crystals were present in leaves of all ages and in most, but not all, epidermal cells.

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Influence Of Sedimentary And Seagrass Microbial Communities On Shallow-Water Benthic Optical Properties—Data Mining

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LONG-TERM GOALS

An overall goal of the CoBOP program was to produce a working radiative-transfer model for selected sub-littoral environments. From a microbiological context, it is important to investigate the entire community of microorganisms associated with the benthic environments of focus, i.e., sediments and seagrass. Light must pass through a "microbial gateway", both before it reaches the sediment or seagrass and prior to its return to the water column. To understand time-and-space variations in optical parameters, we must understand the microbial milieu in which they exist.

OBJECTIVES

Determine the interannual variation in the biomass and composition of sedimentary microorganisms at field sites nearby Lee Stocking Island, Bahamas, and in Monterey Bay, California.

Similarly, determine the biomass, composition, and temporal variation of microorganisms epiphytic on seagrass blades at Lee Stocking Island (turtle grass, *Thalassia testudinum*) and in Monterey Bay (eel grass, *Zostera marina*).

Assess how the microbial community affects the flux of photons to and from the sediments and seagrass blades and how temporal changes in the microbiological community influence temporal changes in benthic optical characteristics.

APPROACH

Our approach was to combine extensive field sampling of sediments and seagrasses with biochemical determination (membrane lipids—Dobbs and Drake) and microscopic examination (light and scanning electron microscopy—Drake and Dobbs) of their microbiological constituents. These fundamental observations then serve as the basis for comparison with the sediments' and seagrasses' optical properties.

WORK COMPLETED

Between September 1997 and June 2000, we participated in five expeditions at Lee Stocking Island (LSI) in the Bahamas as well as six sampling trips to Monterey Bay, California; in all cases, a suite of samples was collected for analysis of microorganisms associated with sediments or seagrass. Fiscal year 2001 served as the "out-year" of this grant and was dedicated to sample work-up, data analysis, and preparation of manuscripts. Fiscal years 2002 and 2003 are the "data-mining" portion of this project. Here we summarize our progress on several research fronts. Highlights of our results are presented in the following section.

1) We wrote a manuscript, authored by Drake, Dobbs, and Zimmerman, and entitled "Effects of epiphyte load on optical properties and photosynthetic potential of the seagrasses *Thalassia testudinum* Banks ex König and *Zostera marina* L.". The manuscript was published in the special optics issue of Limnology and Oceanography, "Light in Shallow Waters" (January 2003).

2) We have given four presentations describing this research at national and international conferences (listed in the References section).

3) We have completed lipid analysis on more than 550 samples of surficial sediment and seagrass epiphytes collected from LSI and Monterey Bay. There are two types of lipid analyses we have performed. The first yields a microbial biomass value (Dobbs and Findlay, 1993) and the second, more involved analysis, yields a profile of the microbial community based on its membrane lipid, fatty-acid signatures (Findlay and Dobbs, 1993).

4) In the course of this research, we have developed our intriguing observation of large, refractive crystals in *T. testudinum* from LSI. We have pursued this serendipitous discovery, and at the Estuarine Research Federation meeting in 2001, we presented a poster by Dobbs, Drake, and Zimmerman entitled, "Crystalline inclusions in epidermal cells of turtlegrass, *Thalassia testudinum*: What is their significance?" We have a manuscript regarding this work in review at Aquatic Botany.

5) Finally, as required by Program Manager Steve Ackleson, we submitted our data in a timely fashion. A summary of all data we collected throughout the CoBOP field program was submitted to Dr. Charlie Mazel on 1 February 2001. The synopsis, located on the CoBOP website (http://www.psicorp.com/lsi/dobbs_drake_data.htm), includes general descriptions of each data type, the analytical procedures used, data format, and data storage location.

RESULTS

1) Leaf epiphyte loads were determined quantitatively, by removing epiphytes and measuring their lipid biomass, and qualitatively, using light microscopy to characterize the colonizing organisms. Light absorption and backscattering of the intact epiphyte layer were determined spectrophotometrically. Epiphyte biomass increased non-linearly with leaf age. *T. testudinum* epiphytes from LSI absorbed a maximum of 36% of incident light in peak chlorophyll absorption bands and reduced modeled photosynthesis by 40%. Higher epiphyte loads on *Z. marina* from Monterey Bay absorbed 60% of incident light in peak chlorophyll absorption bands and reduced

modeled photosynthesis by 49%. Data were incorporated into a model that predicts the biomass-dependent light attenuation of the epiphyte community (Fig. 1). These results are useful for determining quantitative and qualitative impacts of epiphyte loading on the photosynthetic performance of seagrass leaves. (Drake et al., 1999a,b; Drake et al. 2001; Drake et al., 2003)

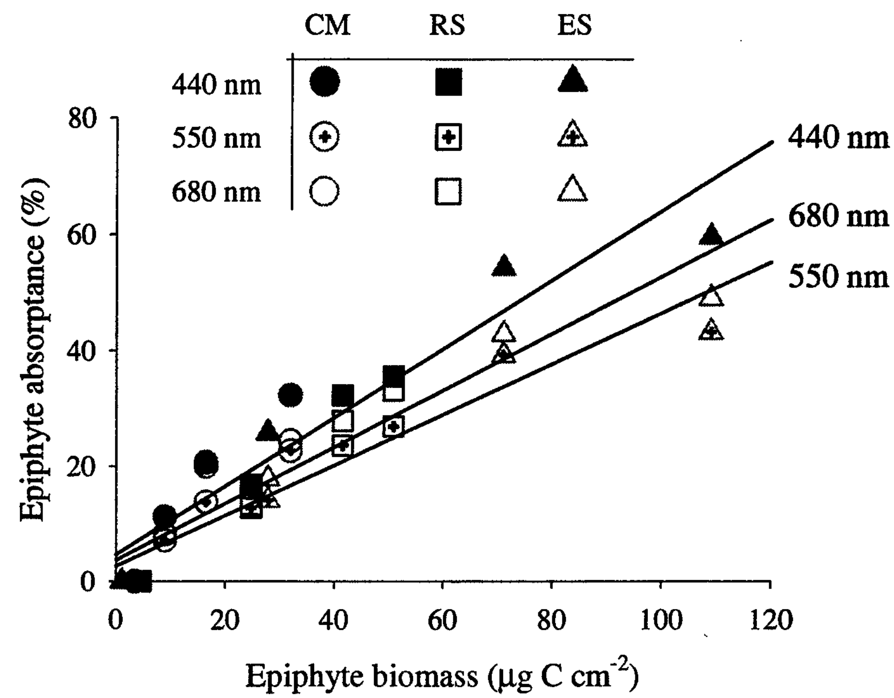

Figure 1. Epiphyte absorptance vs. epiphyte biomass at 440, 550, and 680 nm. Data represent measurements from seagrass leaves from each of three sites: circles = Channel Marker, LSI, Bahamas; squares = Rainbow South, LSI, Bahamas; triangles = Elkhorn Slough, Monterey Bay, California. Dashed lines represent 95% confidence intervals (Drake et al., 2003). [graph: epiphyte absorptance increases with increasing epiphyte biomass at all sites for all wavelengths]

2) In an example of lipid-based microbial biomass shown here (Figs. 2, 3), there was more than a 10-fold difference in sedimentary biomass among sediment archetypes collected at LSI during the 2000 campaign. We are now assembling the very large data set of microbial biomass and fatty-acid profiles we generated for LSI and Monterey Bay sediments. Our intent is to assess interannual variations in those sedimentary microbial communities. (Dobbs and Drake, in preparation)

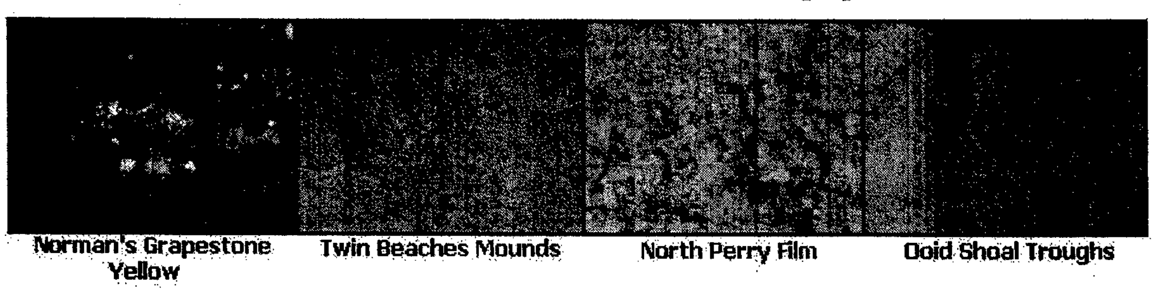

Figure 2. Sediment archetypes collected nearby Lee Stocking Island, Bahamas. Original magnification = 7x. [sediment archetypes range from darkly pigmented sediment at Norman's Grapestone Yellow to white, clean-looking sediment at Ooid Shoal Troughs]

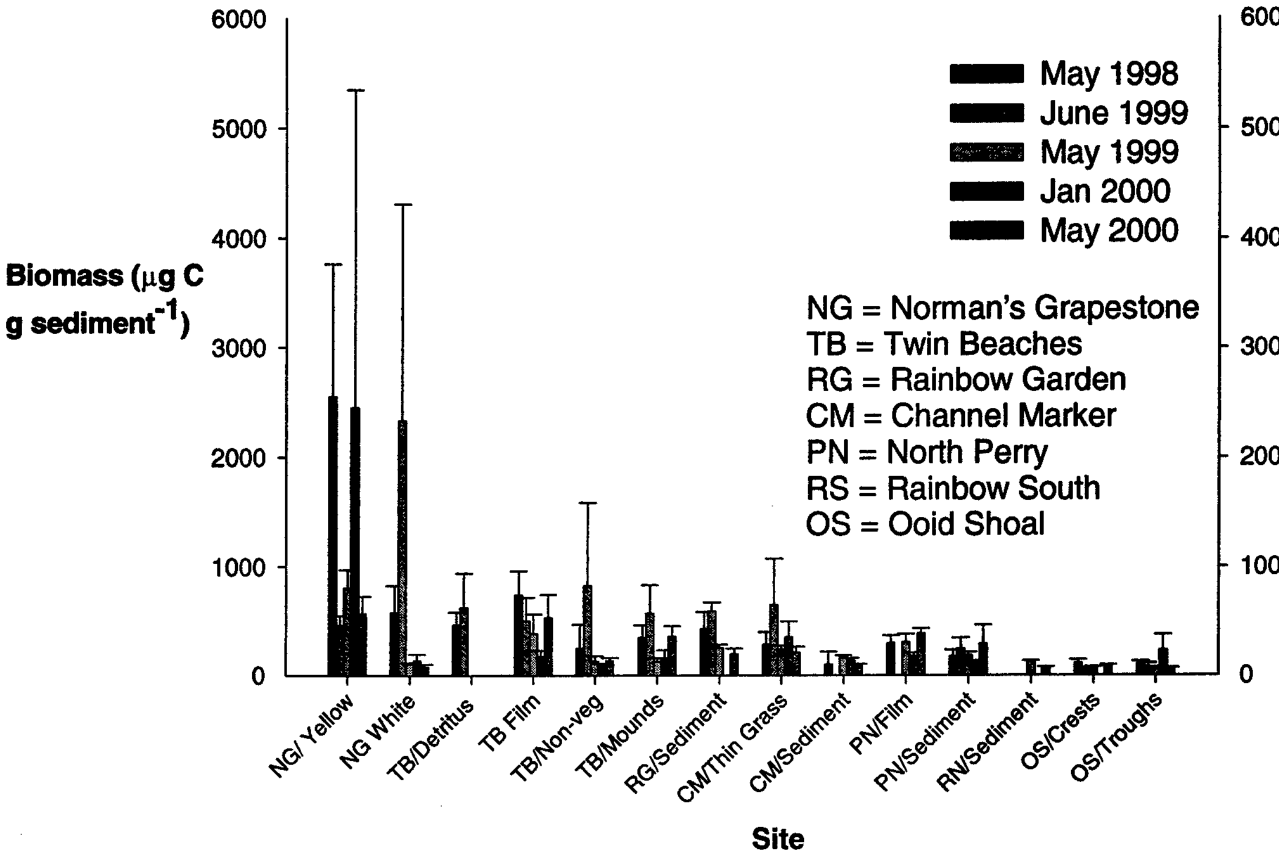

Figure 3. Microbial biomass in surficial sediments nearby Lee Stocking Island, Bahamas. Samples collected from May 1998 - May 2000; error bars represent one standard deviation. [graph: biomass is variable among sites and over time. It is highest at NG Yellow (2500 μg C g sediment^{-1}) and lowest at the OS sites (55 μg C g sediment^{-1})]

3) Intracellular crystals have been reported from a variety of vascular plants and macroalgae, but their biological significance remains enigmatic. In the course of this research, we have found large, refractive, rhomboidal crystals (Fig. 4) in *Thalassia testudinum* from the Bahamas (Lee Stocking Island), Florida (Key Largo and Key West), and Texas (Laguna Madre). At all four collection sites, crystals were present in leaves of all ages and in most, but not all, epidermal cells. The presence of a surrounding membrane has not been detected with light microscopy, but absolute determination will require electron microscopy. Epidermal cells generally contained a single, monolithic, rhomboidal crystal, but cells from Key West frequently contained two or more conjoined crystals. Crystals were stable in acid (pH 2.5) but were partially dissolved in base (pH 14). The size, morphology, and distribution of *T. testudinum* crystals are remarkably similar to proteinaceous crystals described in red and brown algae, but are very different from calcium salt crystals found in idioblasts of terrestrial plants. We are further investigating these unusual and previously unrecorded structures. (Dobbs et al., submitted.)

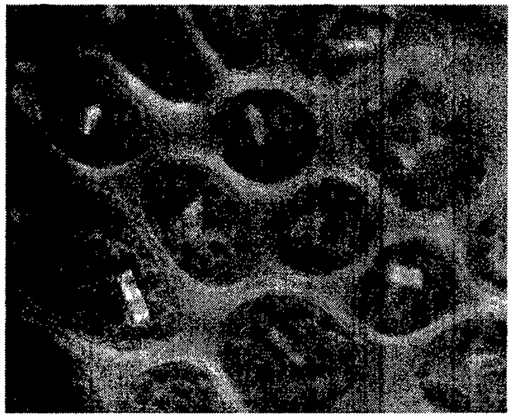

Figure 4. Intracellular crystals within epidermal cells of turtle grass, Thalassia testudinum, collected nearby Lee Stocking Island, Bahamas. Original magnification = 400x. [each epidermal cell contains many green chloroplasts and one rhomboidal, golden crystal]

IMPACT/APPLICATIONS

Any surface in an aquatic environment is covered to some degree with a microbial menagerie that may affect the quantity and spectral quality of light for at least several different reasons. Thus, before incoming light reaches the sediment or seagrass blades and before any light returns to the water column, it must pass through a microbial "gateway" that may affect its quantity and quality. The present incorporation of microbiology into environmental optics research sets the stage for future investigations, in which not only closure is a goal, but a more precise understanding of the interactions between light and organisms.

TRANSITIONS

See collaborations with other CoBOP researchers listed in "Related Projects".

RELATED PROJECTS

We continue our collaboration with CoBOP researcher Dr. Dick Zimmerman. In May 2002, Drake visited Lee Stocking Island to continue epiphyte research with Zimmerman, who was conducting fieldwork on Lee Stocking Island as part of an NSF-funded project with fellow CoBOP PI Dr. David Burdige. The epiphyte data will supplement the photophysiological information obtained by Zimmerman in his development of a canopy production model for sea grasses.

We have incorporated the CoBOP research into outreach efforts. In the summers of 1999, 2000, and 2001, 'site visits' were made to Old Dominion University by high-school girls participating in an NSF-funded internship program conducted by Dr. Elizabeth Canuel at the Virginia Institute of Marine Science. These visits, co-coordinated by Drake, consisted of presentations by researchers and a luncheon with women faculty and staff. During the 'show-and-tell' lunch, Drake and Research Technician Leslie Kampschmidt (previously funded by ONR) described their CoBOP research experiences. Also, Drake developed a hands-on laboratory for high-school students in which they investigate epiphyte load on seagrasses collected from Chesapeake Bay. She delivered a lecture then presented the laboratory to two AP/IB biology classes at Granby High School, Norfolk, Virginia in October 2003.

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