ELSEVIER

Journal of Experimental Marine Biology and Ecology, 221 (1998) 59–70

JOURNAL OF EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

Microalgae on seagrass mimics: Does epiphyte community structure differ from live seagrasses?

James L. Pinckney*, Fiorenza Micheli

University of North Carolina at Chapel Hill, Institute of Marine Sciences, 3431 Arendell Street, Morehead City, NC 28557, USA

Received 2 December 1996; received in revised form 17 March 1997; accepted 16 April 1997

Abstract

The role of seagrasses in regulating epiphytes is a question of central importance for understanding structuring processes in seagrass communities. This study tests the hypothesis that seagrasses are not simply bare substrata for microalgal attachment, but rather influence community composition by altering competitive interactions between different microalgal groups. The effects of wave exposure, depth, seagrass species and seagrass type (mimic vs. live blades) on eelgrass (*Zostera marina* L.) and shoalgrass (*Halodule wrightii* Ascher) epiphyte community structure were examined using manipulative field experiments. Relative abundances of major microalgal groups were determined using high-performance liquid chromatographic measurements of diagnostic photopigments. Exposure to wave energy, depth, and seagrass species did not affect epiphyte total biomass. However, epiphyte biomass was significantly greater on live than mimic blades. Diatom biomass was higher under conditions of low wave energy, in deep habitats and on mimic blades. Cyanobacterial biomass was higher in high energy habitats and on live seagrass blades. Although diatoms had a significantly higher biomass on mimic blades, their biomass contribution relative to cyanobacteria was higher on live seagrass blades. The differences in epiphyte community structure on live vs. mimic seagrass blades suggest that competitive interactions between seagrass and epiphytes may result in selection against cyanobacteria or for diatoms. Another possibility is that seagrasses modify the microenvironment on blade surfaces in a way that alters the outcome of competitive interactions between major algal groups (i.e., diatoms and cyanobacteria). © 1998 Elsevier Science B.V.

Keywords: Community; Epiphyte; *Halodule*; HPLC; Seagrass; *Zostera*

*Corresponding author. Tel.: + 1 919 726-6841; fax: + 1 919 726-2426; e-mail: J_Pinckney@unc.edu

PII S0022-0981(97)00115-9

1. Introduction

Epiphytes inhabiting the exposed surfaces of seagrass blades are highly productive, constitute a valuable food source for herbivores and play an important role in the trophodynamics of seagrass communities. In seagrass beds, epiphytes may provide as much as 46% of the total autotrophic production (Daehnick et al., 1992). Several studies have shown that microalgae support most herbivores inhabiting seagrass patches (Fry, 1984; Kitting et al., 1984; Nichols et al., 1985; Gleason, 1986; Dauby, 1989). The abundance and species composition of epiphytes also influences meiofaunal abundance on seagrass blades (Hall and Bell, 1993). However, little is known about the ecological factors that regulate epiphytic microalgal community composition in marine habitats. A question of central importance is whether or not the seagrass itself plays a role (via direct or indirect factors) in structuring epiphytic communities. This study was a test of the hypothesis that seagrasses are not simply bare substrata for microalgal attachment, but rather influence community composition by altering competitive interactions between different microalgal groups. Moreover, the possible effects of seagrass species on epiphyte community structure were examined for both live and artificial blades.

Two macrophyte species dominate seagrass communities of the Atlantic coast of North America. The eelgrass, *Zostera marina* L., is found from Nova Scotia to North Carolina. The shoal grass, *Halodule wrightii* Ascher, ranges from North Carolina to the Gulf of Mexico and the Caribbean. The distributions of the two species overlap in the Albemarle–Pamlico Sound region of North Carolina. The overlapping ranges of these two seagrass species provides the opportunity to examine and compare the epiphyte communities of two morphologically distinct seagrasses under similar environmental conditions.

High-performance liquid chromatography (HPLC), which provides rapid and accurate quantification of chlorophylls and carotenoids, has become the preferred method for pigment-based chemosystematic characterization of microalgae (e.g., Gieskes and Kraay, 1986; Bidigare et al., 1990; Wilhelm and Manns, 1991; Millie et al., 1993; Tester et al., 1995). Photopigments may be used as indicators of the relative abundance of major microalgal taxonomic groups and offer a means for assessing changes or differences in the relative abundance of phototrophic functional groups in mixed assemblages (Pinckney et al., 1995). The photopigment approach provides measurements of the quantitative changes in relative abundance of major microalgal groups under similar environmental conditions and habitats. For seagrasses in North Carolina, diatoms and cyanobacteria are the dominant (in both number and biomass) epiphytic microalgal groups (Coleman and Burkholder, 1994, 1995). Diagnostic photopigments for these groups consist of chlorophyll *a* (all microalgal oxygenic phototrophs) and the carotenoids, fucoxanthin (diatoms) and zeaxanthin (cyanobacteria) (Rowan, 1989). Removal and analysis of epiphytes can provide estimates of the relative abundance of diatoms and cyanobacteria on seagrass blades. Differences in the molar ratios of fucoxanthin to zeaxanthin can also be used as an indirect indicator of epiphyte community composition (Pinckney et al., 1995). Using manipulative experiments, treatment effects on epiphyte community composition can be quantitatively measured based on differences in photopigment concentrations. The purpose of this study was to quantify the differences

in epiphytic microalgal community structure on seagrass blades from two different species and on mimic blades in four different habitat types. These results were then used to examine the potential for seagrasses to influence epiphyte community composition.

2. Materials and methods

The Bogue Sound–Back Sound region at the southern end of Pamlico Sound contains extensive seagrass beds consisting of *Halodule wrightii* in intertidal and shallow subtidal waters and *Zostera marina* in deeper (< 1 m at low tide) waters. The Middle Marsh is located in Back Sound and within the Rachel Carson National Estuarine Research Reserve (Fig. 1). Mimic seagrass patches of *Z. marina* and *H. wrightii* were deployed at two Middle Marsh sites on March 22, 1993. One site (MM1) was protected from wave exposure (low energy site) and the other was exposed to frequent periods of wind-induced wave activity (high energy site). The two sites were subjectively assigned the attributes of high and low energy, based on long-term exposure to prevailing winds, wave activity and tidal currents. At each site, four *Zostera* and four *Halodule* mimic grass patches were deployed near existing live seagrass beds at each of two depths; subtidal sand flats (deep, i.e., always covered by at least 10 cm of water) and low-intertidal sand flats (shallow, i.e., exposed to air at the lowest spring tides but covered by a few cm of water at most low tides). The seagrass beds in the shallow habitats were composed primarily of *Halodule*, but *Zostera* shoots were also present (Table 1). Live *Zostera* blades were not sampled in the shallow habitats. Mimic seagrass blades were constructed from green plastic ribbon cut to the desired length and width

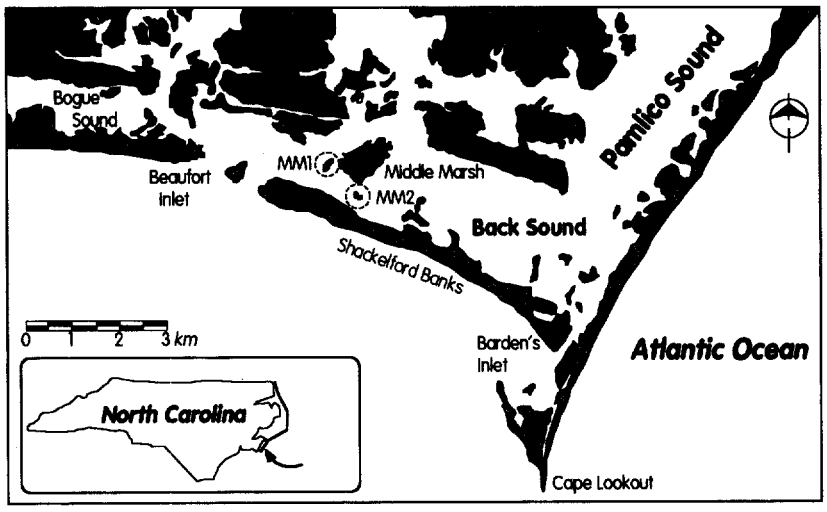

Fig. 1. Location map showing study area (34.7°N, 76.4°W) experimental sites (MM1 and MM2). MM2 is exposed to waves generated by periodic northeasterly winds and was designated as the high wave energy site. MM1 is protected from wind and waves and represents the low wave energy site.

Table 1
Characteristics of the natural and mimic seagrass patches

Parameter	Patch type			
	Halodule	*Halodule* mimic	*Zostera* mimic	*Zostera*
Number of *Halodule* shoots (m^{-2})	1558±330	3000	0	0
Number of *Zostera* shoots (m^{-2})	917±172	0	1416±33	1000
Halodule shoot length (cm)	9.1±0.6	15.0	–	–
Zostera shoot length (cm)	14.9±0.8	–	21.7±1.0	15.0
Halodule blade width (cm)	0.1±0.0	0.2	–	–
Zostera blade width (cm)	0.3±0.0	–	0.4±0.0	0.5

Two replicate cores (9.8 cm diameter) were collected from four patches of each type at both Middle Marsh sites.
Values are the mean±1 standard error.

and attached to a 1-m^2 base of 1.5 cm polypropylene mesh. The plastic mesh was sewn to a circular frame and anchored to the bottom with staples. The *Zostera* and *Halodule* mimics were similar in density and blade size to naturally occurring seagrass patches (Table 1). The experimental design consisted of nested treatments for location (high and low energy), depth (deep and shallow), species (*Halodule* and *Zostera*) and type (live seagrass and mimic blades). After 60 days (21 May 1993), 4 blades of mimics and living plants from each treatment (48 blades) were harvested, placed in individual plastic bags, transported to the laboratory on ice (in a darkened cooler) and were stored frozen (−20°C) until analysis.

Epiphytes were removed from seagrass blades and mimics using a soft silicon stopper (Burkholder et al., 1990), rinsed with filtered (0.2 μm) seawater, and collected by vacuum filtration of the suspension onto a 2.5-cm Whatman GF/F glass fiber filter. The plastic bags containing samples were also rinsed to collect epiphytes dislodged during handling and freezing. After epiphyte removal, blades and mimics were patted dry (between paper towels) and weighed to obtain a relative measure of blade area for normalizing epiphyte biomass (chlorophyll *a* only) across treatments.

Epiphyte photopigments were extracted from filters using a methanol (45%), acetone (45%), deionized water (10%) solvent mixture (Bowles et al., 1985; Pinckney et al., 1995). Filters were placed in disposable polypropylene plastic centrifuge tubes (10 ml), 2 ml of solvent were added and the mixture was sonicated (Fisher sonic dismembrator, model 300, with microtip) for 30–60 s in an ice slurry to reduce heating. Tubes were wrapped in aluminum foil, placed in a freezer (−20°C), and extracted overnight (ca. 12 h). After extraction, samples were centrifuged (5 min @ 4500 rpm) and the supernatant was filtered through a 0.45-μm PTFE filter (Gelman Acrodisc). Extracts were then dispensed into amber glass autosampler vials (2.0 ml), sealed with PTFE caps and placed in an autosampler for analysis.

Photopigments (chlorophylls and carotenoids) were identified and quantified using HPLC. Extracts (200 μl injection volume) were separated using a Rainin Microsorb-MV C_{18} column (100 × 4.6 mm, 3 μm particle size) and peak areas were quantified at 440 nm using a Shimadzu (SPD-M6a) photodiode array spectrophotometer (PDAS). The

protocol for solvents and gradients is provided in Wright et al. (1991). Photopigment peaks were identified by comparing retention times and absorbance spectra (380–670 nm) with pure (crystalline) pigment standards using the methods outlined in Wright et al. (1991). The most common diagnostic photopigments of primary interest for this study were chlorophyll *a* (Chl *a*), fucoxanthin and zeaxanthin.

Data were analysed using a multifactor analysis of variance with 4 factor levels (all fixed effects) consisting of location (2 levels; high and low energy), depth (2 levels; deep and shallow), species (2 levels; *Zostera* and *Halodule*) and type (2 levels; live and mimic blades, with 4 replicates for each level). Because of the natural depth distribution patterns of *Halodule* and *Zostera*, specimens of live, healthy *Halodule* blades from deep habitats or of *Zostera* blades from shallow habitats were not available. Therefore, the analysis of live seagrass blades was limited to the habitats in which they occurred naturally. For statistical analyses, these differences in natural distribution patterns of live seagrasses resulted in some cases where missing data prevented the calculation of interaction terms involving live blades and depth. Unique sums of squares were calculated assuming the redundant effects (caused by missing interaction terms) were actually null. Seagrass mimics for both species, however, were obtained for all factor levels. Data were checked for normality, homogeneity of variances (Cochran's C test, $\alpha = 0.05$), independence of error terms, and transformed ($\log_{10}$), when necessary, before statistical testing.

3. Results

Total epiphyte biomass (as Chl *a* normalized to relative blade weight) was not different between the low (MM1) and high (MM2) wave energy sites, deep and shallow habitats, or species (*Zostera* and *Halodule*) (Table 2, Fig. 2). However, live blades supported a significantly higher epiphyte biomass than mimic blades ($F = 37.18$, $df = 1,36$; $p < 0.001$). The mean normalized epiphyte biomass (nmol Chl a g^{-1}) for live seagrass blades (660.4±103.9 SE) was more than three times the mean for mimic blades (187.0±17.1 SE). No significant factor interactions were detected (Table 2).

Fucoxanthin, a carotenoid photopigment characteristic of diatoms, was used as an indicator of relative epiphytic diatom biomass on seagrass and mimic blades. When normalized to Chl *a* concentration [nmol fucoxanthin (nmol Chl $a)^{-1}$], the ratio is a relative measure of the proportion of the epiphyte community that consists of diatoms (Fig. 3). In the experiment, the low wave energy site had a higher mean diatom relative biomass (0.641±0.060 SE) than the high wave energy site (0.413±0.045 SE) ($F = 14.45$, $df = 1,36$; $p < 0.01$) (Table 2). Deep habitats showed a higher mean diatom relative abundance (0.618±0.059) than shallow habitats (0.436±0.050) ($F = 7.96$, $df = 1,36$; $p < 0.05$). Diatom relative abundance on mimic blades (0.633±0.046) was more than twice that on live seagrass (0.315±0.049) ($F = 29.33$, $df = 1,36$; $p < 0.001$). However, there was no significant difference in diatom abundance on *Halodule* and *Zostera* (both live and mimic) blades. No significant factor interactions were detected (Table 2).

Table 2
Summary results from ANOVA.

Factors	Variables			
	Total epiphyte biomass chlorophyll *a*	Diatom biomass fucoxanthin	Cyanobacteria biomass zeaxanthin	Diatoms to cyanobacteria ratio fucoxanthin/zeaxanthin
Main				
A. Location	ns	[b]	[b]	[c]
B. Depth	ns	[a]	ns	ns
C. Species	ns	ns	ns	ns
D. Type	[c]	[c]	[a]	[c]
Interactions				
A × B	ns	ns	ns	ns
A × C	ns	ns	ns	ns
A × D	ns	ns	[a]	[b]
B × C	ns	ns	ns	ns
B × D	ns	ns	ns	[a]
C × D	NC	NC	NC	NC
A × B × C	ns	ns	ns	ns
A × B × D	ns	ns	ns	ns
A × C × D	NC	NC	NC	NC
B × C × D	NC	NC	NC	NC
A × B × C × D	NC	NC	NC	NC

The symbols [a], [b] and [c] denote $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.
The abbreviation ns indicates a non-significant F-value. Because of the absence of some live seagrass species in deep and shallow habitats, interaction terms could not be calculated for these cases (symbolized by NC) (see text).

The relative contribution of cyanobacteria (blue–green algae) to total epiphyte biomass was measured using the molar ratio of the diagnostic carotenoid photopigment, zeaxanthin, to Chl *a* (Fig. 4). In contrast to diatoms, the cyanobacterial component of the epiphyte community showed a higher relative abundance (0.040 ± 0.012) at the high wave energy site than at the low site (0.011 ± 0.002) ($F = 6.26$, $df = 1,36$; $p < 0.01$). There was also a trend towards a higher cyanobacterial contribution on live seagrass (0.0420 ± 0.018) than on mimic blades (0.017 ± 0.002) ($F = 3.88$, $df = 1,36$; $p < 0.05$). Location and type factors showed a weak but significant interaction effect ($F = 7.48$, $df = 1,36$; $p < 0.05$) that was more pronounced at the high energy site than the low energy site (Fig. 4). There was no significant difference in cyanobacterial relative abundance in deep vs. shallow habitats or with respect to *Halodule* vs. *Zostera* species (Table 2).

The molar ratio of fucoxanthin to zeaxanthin provides a measure of the contribution of diatoms relative to cyanobacterial epiphytes. Under experimental conditions, this ratio can be used to infer differences in epiphyte community composition (i.e., community structure) between treatments. An increase in this ratio reflects a shift in community composition towards an increased relative abundance of diatoms. Alternatively, a decrease shows an increase in the relative contribution of cyanobacteria to total epiphyte

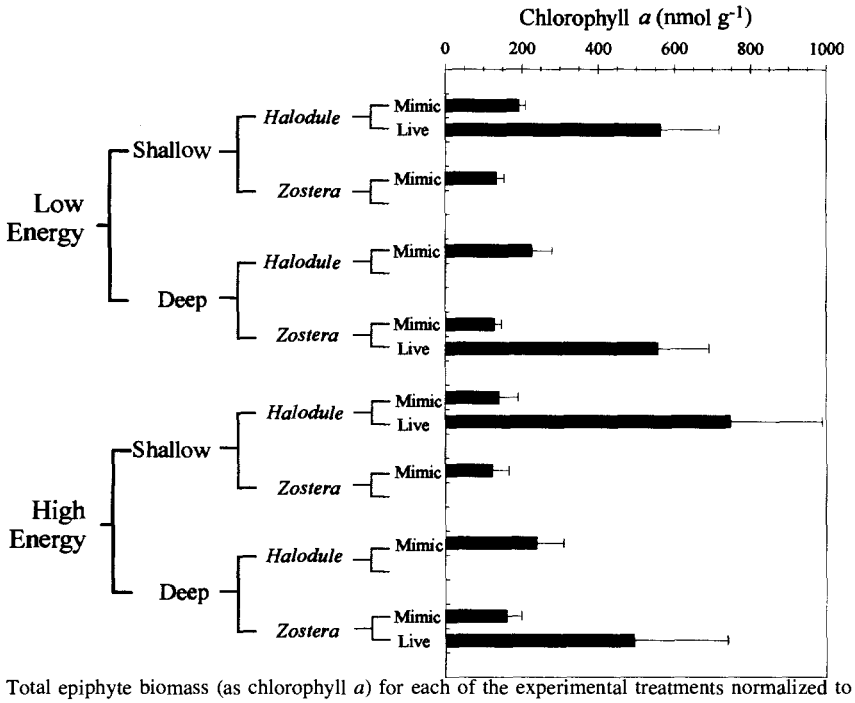

Fig. 2. Total epiphyte biomass (as chlorophyll *a*) for each of the experimental treatments normalized to blade weight (g) for each sample. The mean blade weight (g) for all types (live and mimics) was 0.164±0.017 SE. Graph values are the mean ($n = 4$)±1 standard error. Blank labels on the y-axis show that no specimens were obtained.

biomass (Fig. 5). The fucoxanthin to zeaxanthin ratio was calculated for each replicate (blade) and was $\log_{10}$ transformed for statistical analysis. Because of low zeaxanthin concentrations in some samples, the ratio data within replicates was highly variable. For this reason, the data shown in Fig. 5 do not reflect a simple division of the fucoxanthin and zeaxanthin concentrations shown in Figs. 3 and 4. The low wave energy site (MM1) showed a tendency for increased mean diatom abundance relative to cyanobacteria (175.1±38.4) when compared to the high energy (MM2) site (49.7±14.7) ($F = 11.86$, $df = 1,36$; $p < 0.001$) (Table 2). Similarly, diatoms exhibited a higher abundance relative to cyanobacteria on live seagrass (210.1±56.4) than on mimic blades (63.5±11.4) ($F = 14.40$, $df = 1,36$; $p < 0.001$). However, this effect was more pronounced at the low rather than the high energy site (Fig. 5). With respect to diatoms and cyanobacteria, epiphyte community structure was not significantly different in deep vs. shallow habitats or on *Halodule* vs. *Zostera* blades. Significant interactions were detected for location × type and depth × type (Table 2).

4. Discussion

The experimental results suggest that live seagrasses support a higher epiphyte

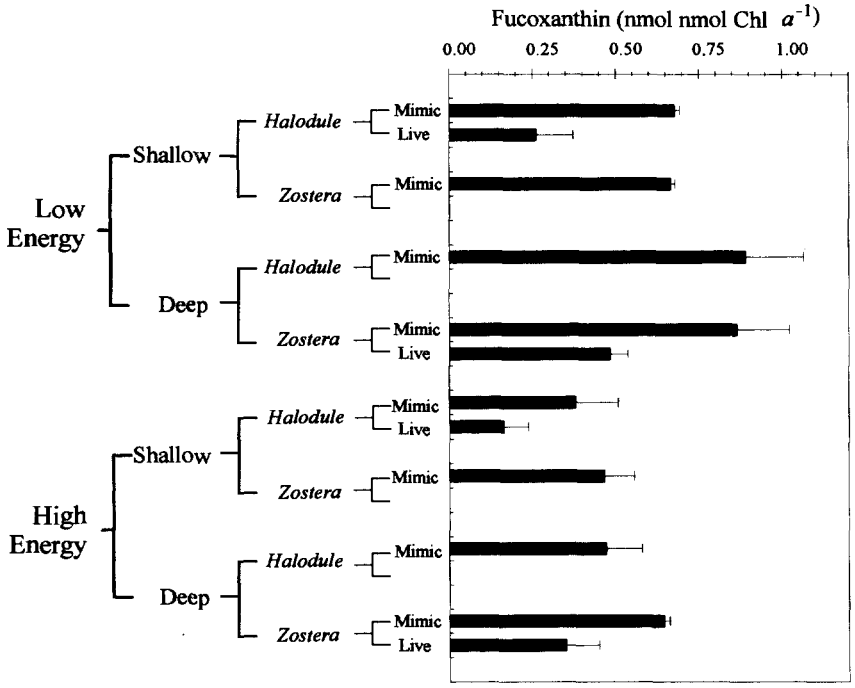

Fig. 3. Total diatom biomass (as fucoxanthin) for each treatment normalized to total epiphyte biomass (as chlorophyll *a*). Graph values are the mean ($n = 4$) ± 1 standard error. Blank labels on the y-axis show that no specimens were obtained.

biomass than mimic blades. Although the relatively short deployment time (60 days) may not have been sufficient for the development of a "climax" epiphyte community, the generation times of most of the microalgal species ranges from one to three days (Coleman and Burkholder, 1994). The period of mimic deployment should have been sufficient to allow adequate colonization and growth. Longer deployments (over at least one annual cycle) would be needed to determine if epiphyte biomass on mimics would eventually equal (or surpass) that on living seagrass blades. Exposure to wave energy, depth and seagrass species had no measurable effect on epiphyte total biomass.

Diatom biomass was higher under conditions of low wave energy, in deep habitats, and on mimic blades. However, there was no difference in diatom biomass on live *Zostera* or *Halodule* blades. High wave energy may have a negative impact on diatoms through abrasion and removal of stalked species attached to blades. The difference between deep and shallow habitats could be due to several factors, including photoacclimation responses by diatoms to less light, reduced herbivory, abrasion at shallower depths, etc. Blade mimics consistently showed higher relative abundances of diatoms than living seagrasses, regardless of habitat type or seagrass species. These results collectively suggest that some factor (either direct or indirect) reduces the relative abundance of diatoms on blades of living seagrasses compared to blade mimics.

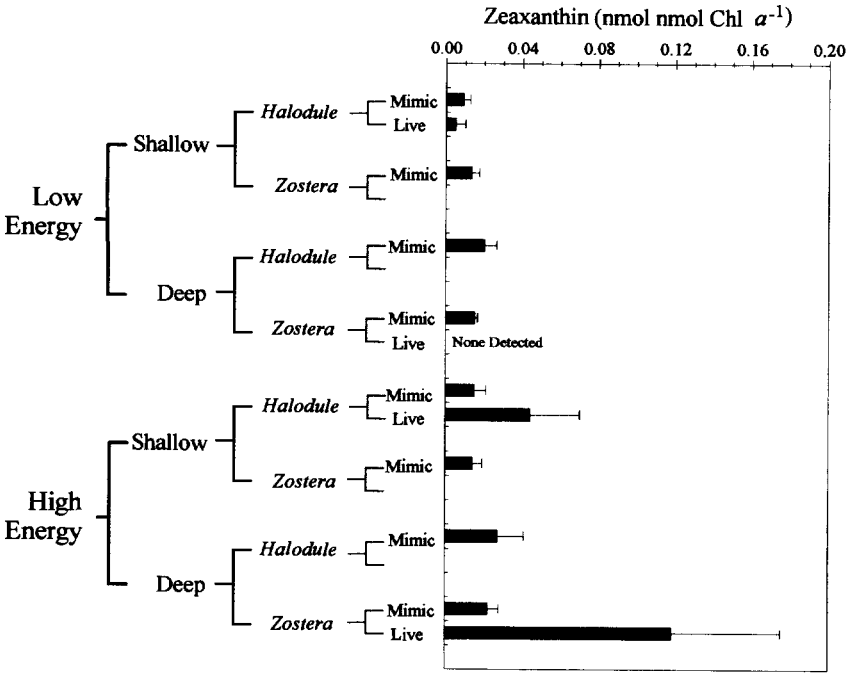

Fig. 4. Total cyanobacteria biomass (as zeaxanthin) for each of the treatments normalized to total epiphyte biomass (as chlorophyll *a*). Values are the mean ($n = 4$)±1 standard error. Blank labels on the y-axis indicate that no specimens were obtained.

The relative abundance of cyanobacteria was higher in high energy habitats and on live seagrass blades. Seagrass species and water depth had no effects on cyanobacterial abundance. Cyanobacteria, which are typically smaller that diatoms and more firmly attached to the substratum, are much less susceptible to abrasion induced by blade movement. Mimic blades also support a lower cyanobacterial biomass than live seagrasses. Although the reason for this difference is not clear, one possible explanation is that competitive interactions with high diatom biomass on mimic blades may reduce cyanobacterial fitness. However, it is also possible that live seagrass produces a microhabitat that favors cyanobacterial growth.

Comparisons of faunal composition within natural seagrass patches and seagrass mimics conducted in a separate study indicated that the grazer community differed between live and mimic grass patches. In particular, small crustaceans were significantly more abundant on live than on mimic blades in all habitat types. Different grazing intensity or selective grazing of different microalgal groups may also explain the effects of seagrass type (live vs. mimics) on epiphyte community structure. Alternatively, differences in the microalgal communities on live and mimic seagrasses may explain the effects of seagrass type on faunal composition of the seagrass patches, suggesting that microalgal community composition may play a role in structuring seagrass faunal communities.

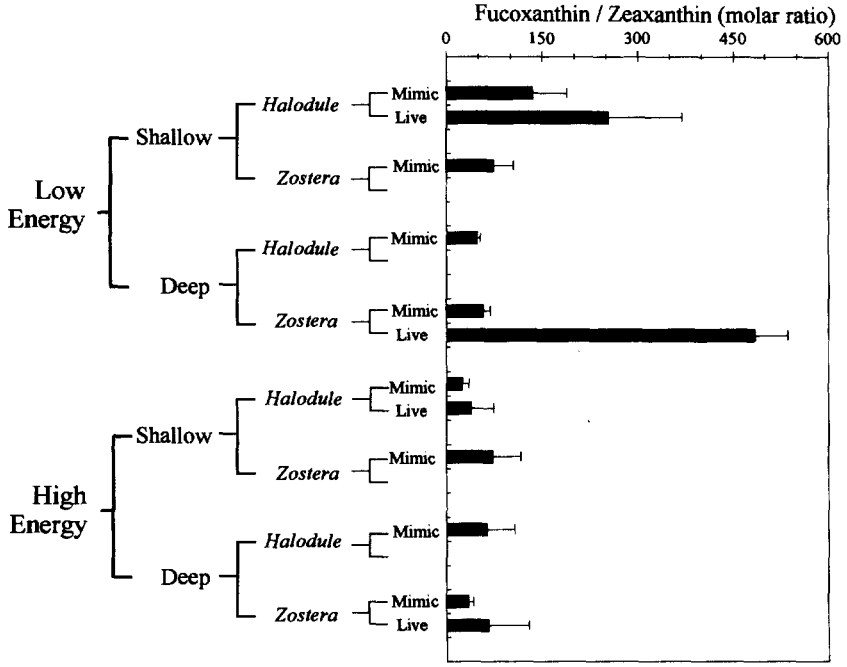

Fig. 5. Relative contributions of diatoms and cyanobacteria to epiphyte community composition, expressed as the molar ratios of fucoxanthin to zeaxanthin. Values are the mean ($n = 4$)±1 standard error. Blank labels on the y-axis indicate that no specimens were obtained.

The dominant microalgal groups in the epiphyte community were diatoms and cyanobacteria. The primary goal of this experiment was to determine if substratum type affects epiphyte community structure over a range of habitat types. In our experiments, wave exposure (energy) and live vs. mimic blades showed significant differences in epiphyte communities. Low energy habitats seemed to favor diatoms, while high wave energy habitats were conducive for cyanobacteria. Abrasion may be a primary factor that structures epiphyte communities. Although diatoms had a significantly higher biomass on mimic blades, their contribution relative to cyanobacteria was higher on live seagrass blades than on the mimics. These data further support our hypothesis that seagrasses are not simply a substratum for attachment, but may play a role in regulating epiphyte community structure.

Collectively, the photopigment data illustrate the spatial variability in epiphyte communities. Community structure and biomass are clearly influenced by degree of wave exposure, depth and seagrass type (live vs. mimic). Our experiments were designed to provide some preliminary evidence of the factors that need to be considered in future studies of epiphyte–seagrass interactions. The results show that all of these factors are potentially important in structuring epiphytic communities. Although photoacclimation (i.e., alteration of photopigment per cell) by microalgae to different light environments (Falkowski and LaRoche, 1991) is a complicating factor when using

photopigments to assess epiphyte communities, comparisons of communities exposed to similar light environments are reliable measures of microalgal group-specific biomass (Pinckney et al., 1995). In our experiments, the differences in diatom communities in deep and shallow habitats could be explained by photoacclimation (i.e., increased concentration of fucoxanthin per cell at lower light levels) because of the different light environments. However, all other treatment comparisons (i.e., within a depth stratum) should reflect real differences in diatom biomass. In contrast, the diagnostic photopigment of cyanobacteria (zeaxanthin) is not used for photoacclimation and is a reliable indicator of biomass (Rowan, 1989).

There are several conceivable mechanisms through which seagrasses could influence the structure of epiphyte communities. Metabolite (dissolved organic carbon, nutrients, etc.) exchange, carbon limitation of photosynthesis in microzones on blade surfaces and nutrient recycling are all important factors known to operate in biofilms (Wetzel, 1993; Paerl and Pinckney, 1995). Seagrasses are in direct competition with epiphytes for several important resources, such as light, nutrients and dissolved carbon (Wetzel, 1993; Williams and Ruckelshaus, 1993; Coleman and Burkholder, 1994, 1995). The differences in epiphyte community structure on live vs. mimic seagrass blades suggest that competitive interactions between seagrass and epiphytes may result in the selection against cyanobacteria (or for diatoms). Another possibility is that seagrasses modify the microenvironment on blade surfaces in a way that alters the outcome of competitive interactions between major algal groups (i.e., diatoms and cyanobacteria). In summary, we have provided preliminary evidence for the existence of epiphyte community structuring processes on the surfaces of seagrass blades. Discerning and documenting the nature of these processes is essential for understanding the ecology and trophodynamics of temperate zone seagrass communities.

Acknowledgements

We thank two anonymous reviewers for their helpful comments. Funding for this study was provided by NSF Project OCE 94-15985 and a NOAA-NEERS grant to C.H. Peterson. Partial support for J.L.P. was provided by an appointment to the Global Change Distinguished Postdoctoral Fellowships, sponsored by the US Department of Energy, Office of Health and Environmental Research, and administered by the Oak Ridge Institute for Science and Education. We are grateful to W.L. Falls for field assistance and H.S. Lenihan, B.A. Orlando, E.A. Irlandi, M.L. Kellog, A.N. Hendrix, P. Wyrick, M. Go and S.B. Joye for constructing seagrass mimics.

References

Bidigare, R.R., Marra, J., Dickey, T.D., Iturriaga, R., Baker, K.S., Smith, R.C., Pak, H., 1990. Evidence for phytoplankton succession and chromatic adaptation in the Sargasso Sea during spring 1985. Mar. Ecol. Prog. Ser. 60, 113–122.

Bowles, H., Paerl, H., Tucker, J., 1985. Effective solvents and extraction periods employed in phytoplankton carotenoid and chlorophyll determinations. Can J. Fish. Aquat. Sci. 42, 1127–1131.

Burkholder, J.M., Wetzel, R.G., Klomparens, K.L., 1990. Direct comparison of phosphate uptake by adnate and loosely attached microalgae within an intact biofilm matrix. Environ. Microbiol. 56, 2882–2890.

Coleman, V.L., Burkholder, J.M., 1994. Community structure and productivity of epiphyte microalgae on eelgrass (*Zostera marina* L.) under water-column nitrate enrichment. J. Exp. Mar. Ecol. 179, 29–48.

Coleman, V.L., Burkholder, J.M., 1995. Response of microalgal epiphyte communities to nitrate enrichment in an eelgrass (*Zostera marina*) meadow. J. Phycol. 31, 36–43.

Daehnick, A.E., Sullivan, M.J., Moncreiff, C.A., 1992. Primary production of the sand microflora in seagrass beds of Mississippi Sound. Bot. Mar. 35, 131–139.

Dauby, P., 1989. The stable carbon isotope ratios of benthic food webs of the Gulf of Calvi. Corsica Cont. Shelf Res. 9, 181–195.

Falkowski, P., LaRoche, J., 1991. Acclimation to spectral irradiance in algae. J. Phycol. 27, 8–14.

Fry, B., 1984. $^{13}C/^{12}C$ ratios and the trophic importance of algae in Florida *Syringodium filiforme* seagrass meadows. Mar. Biol. 79, 11–19.

Gieskes, W.W.C., Kraay, G.W., 1986. Floristic and physiological differences between the shallow and deep nanophytoplankton community in the eutrophic zone of the open tropical Atlantic revealed by HPLC analysis of pigments. Mar. Biol. 91, 567–576.

Gleason, B.F., 1986. Utilization of salt marsh plants by postlarval brown shrimp: carbon assimilation rates and food preferences. Mar. Ecol. Prog. Ser. 31, 151–158.

Hall, M.O., Bell, S.S., 1993. Meiofauna on the seagrass *Thalassia testudinum*: population characteristics of harpacticoid copepods and associations with algal epiphytes. Mar. Biol. 116, 137–146.

Kitting, C.L., Fry, B., Morgan, M.D., 1984. Detection of inconspicuous epiphytic algae supporting food webs seagrass meadows. Oecologia 62, 145–149.

Millie, D.F., Paerl, H.W., Hurley, J.P., 1993. Microalgal pigment assessments using high-performance liquid chromatography: A synopsis of organismal and ecological applications. Can. J. Fish. Aquat. Sci. 50, 2513–2527.

Nichols, P.D., Klump, D.W., Johns, R.B., 1985. A study of food chains in seagrass communities. III. Stable carbon isotope ratios. Aust. J. Mar. Freshwater Res. 36, 683–690.

Paerl, H.W., Pinckney, J., 1995. Microbial consortia: their roles in aquatic production and biogeochemical cycling. Microb. Ecol. 31, 225–247.

Pinckney, J., Paerl, H.W., Fitzpatrick, M., 1995. Impacts of seasonality and nutrients on microbial mat community structure and function. Mar. Ecol Prog. Ser. 123, 207–216.

Rowan, K.-S., 1989. Photosynthetic Pigments of Algae, Cambridge University Press, New York, 334 pp.

Tester, P.A., Geesey, M.E., Guo, C., Paerl, H.W., Millie, D.F., 1995. Evaluating phytoplankton dynamics in the Newport River estuary (North Carolina, USA) by HPLC-derived pigment profiles. Mar. Ecol. Prog. Ser. 124, 237–245.

Wetzel, R.G., 1993. Microcommunities and microgradients: linking nutrient regeneration, microbial mutualism, and high sustained aquatic primary production. Neth. J. Aquat. Ecol. 27, 3–9.

Wilhelm, C., Manns, L., 1991. Changes in pigmentation of phytoplankton species during growth in stationary phase—Consequences for reliability of pigment-based methods for biomass determinations. J. Appl. Phycol. 3, 305–310.

Williams, S.L., Ruckelshaus, M.H., 1993. Effects of nitrogen availability and herbivory on eelgrass (*Zostera marina*) and epiphytes. Ecology 74, 904–918.

Wright, S., Jeffrey, S., Mantoura, R., Llewellyn, C., Bjørnland, T., Repeta, D., Welschmeyer, N., 1991. An improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. Mar. Ecol. Prog. Ser. 77, 183–196.