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Effects of epiphyte load on optical properties and photosynthetic potential of the seagrasses *Thalassia testudinum* Banks ex König and *Zostera marina* L.

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Abstract

The biomass and optical properties of seagrass leaf epiphytes were measured to evaluate their potential impact on the photosynthetic performance of the seagrasses *Thalassia testudinum* Banks ex König (turtlegrass) and *Zostera marina* L. (eelgrass). Turtlegrass was obtained from oligotrophic waters near Lee Stocking Island, Bahamas; eelgrass was collected from a eutrophic environment in Monterey Bay, California. Leaf–epiphyte loads were characterized visually and quantified using measurements of their phospholipid biomass. Light absorption and reflectance of the intact epiphyte layer were determined spectrophotometrically. Turtlegrass epiphytes from the oligotrophic site absorbed a maximum of 36% of incident light in peak chlorophyll absorption bands, whereas higher epiphyte loads on eelgrass from the more eutrophic Monterey Bay absorbed 60% of incident light in peak chlorophyll absorption bands. The combination of intact epiphyte–leaf complexes and spectral measurements enabled us to construct a quantitative relationship between epiphyte biomass and light attenuation, and, by extension, between epiphyte biomass and seagrass photosynthesis. The model yielded a robust, positive relationship between epiphyte biomass and the absorption of photons in photosynthetically important wavelengths, and it generated a strong negative relationship between epiphyte biomass and spectral photosynthesis of their seagrass hosts. Furthermore, the calculations of photosynthesis highlighted the significant differences between PAR and spectral models of photosynthesis, illustrating that the spectral quality of the incident flux must be considered when evaluating the effects of epiphyte load on seagrass leaf photosynthesis. Verification of the model—using direct measurements of photosynthesis and a variety of epiphyte and macrophyte combinations from different locations—is warranted.

Seagrass leaves are colonized by a diverse array of epiphytic microorganisms, macroalgae, and metazoans. The epiphyte complex consists of (i) all organisms that grow attached to or crawl over the leaf surface, (ii) the associated extracellular matrix deposited on the leaves by these organisms, and (iii) mineral and organic particles embedded in the organic matrix. This complex provides a significant fraction of the overall productivity of seagrass ecosystems (e.g., Penhale 1977; Mazzella and Alberte 1986; Klumpp et al. 1992), as well as refuge and food for an assemblage of invertebrates and fish (e.g., Orth and van Montfrans 1984; van Montfrans et al. 1984; Neckles et al. 1994; Short et al. 2001). A modest epiphyte layer may benefit seagrasses by preventing damage from ultraviolet radiation (Trocine et al. 1981) or repelling potential leaf grazers (Karez et al. 2000). It has been argued that epiphytes composed of nonchlorophyte algae and cyanobacteria can accumulate to high densities without affecting seagrass photosynthesis because they preferentially absorb green light, which is an inefficient driver of seagrass photosynthesis (Mazzella and Alberte 1986). Nonetheless, epiphytes may also have negative effects on their seagrass hosts—creating physical barriers to light absorption (Losee and Wetzel 1983; Dalla Via et al. 1998; Brush and Nixon 2002), nutrient uptake, gas exchange, or a combination of these factors (Sand-Jensen 1977; van Montfrans et al. 1984; Sand-Jensen et al. 1985).

Eutrophication is a common feature of estuarine environments throughout the world, creating blooms of nuisance phytoplankton and increased epiphyte biomass that may have dramatic impacts on seagrass distribution, density, and productivity (e.g., Sand-Jensen and Søndergaard 1981; Orth and Moore 1983; Cambridge and McComb 1984). Epiphyte growth on seagrass leaves can be stimulated by eutrophication (e.g., Borum 1985; Twilley et al. 1985; Tomasko and Lapointe 1991; Coleman and Burkholder 1994), removal of epiphyte grazers (e.g., Caine 1980), and a combination of both factors (e.g., Neckles et al. 1993; Williams and Ruckelshaus 1993).

A variety of methods have been employed to develop quantitative relationships between epiphyte load on seagrass productivity. Biomass on intact leaves or artificial substrates has been quantified using chlorophyll *a* concentration, mass (dry weight or ash-free dry weight), or carbon and expressed as a function of leaf mass or leaf area (summarized in Kemp et al. 2000). Likewise, various methods have been used to

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measure epiphytes' effects on light attenuation. Although local correlations were generally reported for individual studies, methodological differences often make intercomparisons difficult. These analyses include disrupting the epiphyte–leaf complex by suspending epiphytes in solution and measuring either broadband light transmission (Borum and Wium-Andersen 1980; Twilley et al. 1985; Kemp et al. 1988) or spectral attenuation (Sand-Jensen and Borum 1984); or isolating representative components of periphyton (algae, bacteria, or calcium carbonate) and measuring the spectral attenuation of each component in Petri dishes (correcting for scattering using a variation of the opal glass technique) (Losee and Wetzel 1983). Intact epiphyte–leaf complexes were evaluated by measuring the transmission of broadband light through scraped and unscraped leaves (Bulthuis and Woelkerling 1983; Brush and Nixon 2002). Finally, laboratory, field, and modeling data were combined to evaluate epiphyte effects on light attenuation (Kemp et al. 2000).

Although some studies employed spectral measures (with a relatively broad resolution of 10 nm: Losee and Wetzel 1983; Sand-Jensen and Borum 1984) and at least two examined intact leaf–epiphyte complexes (Bulthuis and Woelkerling 1983; Brush and Nixon 2002), no study has measured spectral absorption of intact epiphyte–leaf complexes with optical instruments that fully account for forward scattering through turbid media. Photosynthesis is not driven uniformly by photosynthetically available radiation (PAR) but is instead heavily weighted in absorption bands; thus, spectral measurements and spectral calculations of photosynthesis will yield the most accurate estimates of photosynthetic capability.

Here, we measured the spectral absorption properties of intact epiphyte–leaf complexes collected from two different environments and calculated their effects on light-limited photosynthesis of seagrass leaves. The objectives of this study were to develop quantitative relationships predicting (1) the impact of epiphyte loading on light transmission to the leaves and (2) the subsequent effect on the potential rate of leaf photosynthesis. Generality of this relationship was tested by comparing optical impacts of epiphytes growing on *Thalassia testudinum* Banks ex König (turtlegrass) from oligotrophic sites in the Exuma Islands, Bahamas with epiphytes growing on *Zostera marina* L. (eelgrass) in Elkhorn Slough, a eutrophic environment in Monterey Bay, California.

Methods

Study sites—Turtlegrass shoots were collected in May 2000 at two sites near Lee Stocking Island, Exuma Islands, Bahamas (23°46.2′N, 076°06.8′W). Shoots with extremely low epiphyte loads were collected near the Caribbean Marine Research Center, from a dense turtlegrass meadow (Channel Marker) at 3.3–5.0 m depth. Shoots with more developed epiphyte loads were collected from another dense meadow (Rainbow South) growing at 10 m depth just north of Norman's Pond Cay. The waters of Exuma Sound and Bahamas Banks are extremely oligotrophic; PAR attenuation coefficients are on the order of 0.1 m^{-1}, and chlorophyll *a* concentration in the water column is less than 0.2 mg m^{-3} (Zimmerman unpubl. data). Seagrasses can grow at depths as great as 20 m in this region.

Eelgrass shoots were collected in April 2000 from a meadow growing at 2 m depth in Elkhorn Slough, California (36°48.9′N, 121°46.2′W). The estuary is tidally flushed by the nutrient-rich water of Monterey Bay and receives runoff from the intensively cultivated upland watershed (Bricker et al. 1999). Water column turbidity is high and variable, and eelgrass distributions are limited to depths shallower than 2 m (Zimmerman et al. 1994).

In situ flux—Downwelling spectral fluxes were measured at the top of each seagrass canopy using a HydroRad (HOBI Labs) three-channel spectroradiometer mounted in a portable, diver-operated configuration called the diver-operated benthic biooptical spectrometer (DOBBS). All three input channels were fitted with cosine-corrected irradiance collectors and calibrated for wavelength precision and radiometric flux by the HOBI Labs calibration facility using National Institute of Standards and Technology-traceable lamps. The data represent the average of 10 measurements from each site taken at high tide, at noon, on one day. Fluxes at the top of the seagrass canopy were measured at the Lee Stocking Island, Bahamas sites (Channel Marker and Rainbow South) in May 2000 and in August 2000 at the site in Elkhorn Slough, California.

Relative age of seagrass leaves—Leaves were assigned to different age classes for analysis of epiphyte load based on their relative position within the shoot. The innermost leaf was assigned to the youngest age class (1). Sequentially older leaves (determined by leaf length and condition) on alternating sides of the shoot bundle were assigned to sequentially older leaf classes (2, 3, and 4). Leaf class 5, when present, was generally senescent (chlorotic or nearly dead) and was not included in the analysis. Although absolute leaf ages were not determined, growth rate measurements performed using the leaf marking technique (Zieman and Wetzel 1980) indicate that the plastochron interval was about 2 weeks for each age class.

Epiphyte biomass on seagrass leaves—Epiphyte biomass was determined by quantifying phospholipid phosphate in cellular membranes (Dobbs and Findlay 1993). Four- to eight-centimeter long sections were cut from the seagrass leaves, and the area of each section was determined from measurements of length and width. Epiphytes were removed from both sides of each leaf section by gentle scraping with a razor blade. The scraped epiphytes from ten leaf sections (representing ten plants) were pooled within each leaf class (1 to 4) to obtain samples for lipid analysis. Three or four replicate samples of pooled scrapings were prepared for each age class at each site. Lipids were extracted from the scraped epiphytes in methanol–chloroform buffer (Bligh and Dyer 1959; White et al. 1979) and analyzed for their phospholipid phosphate content (Dobbs and Findlay 1993). Values reported here were converted to carbon equivalents assuming 100 μmol P g C^{-1} (Dobbs and Findlay 1993), then normalized to leaf area scraped. This method, which measures only

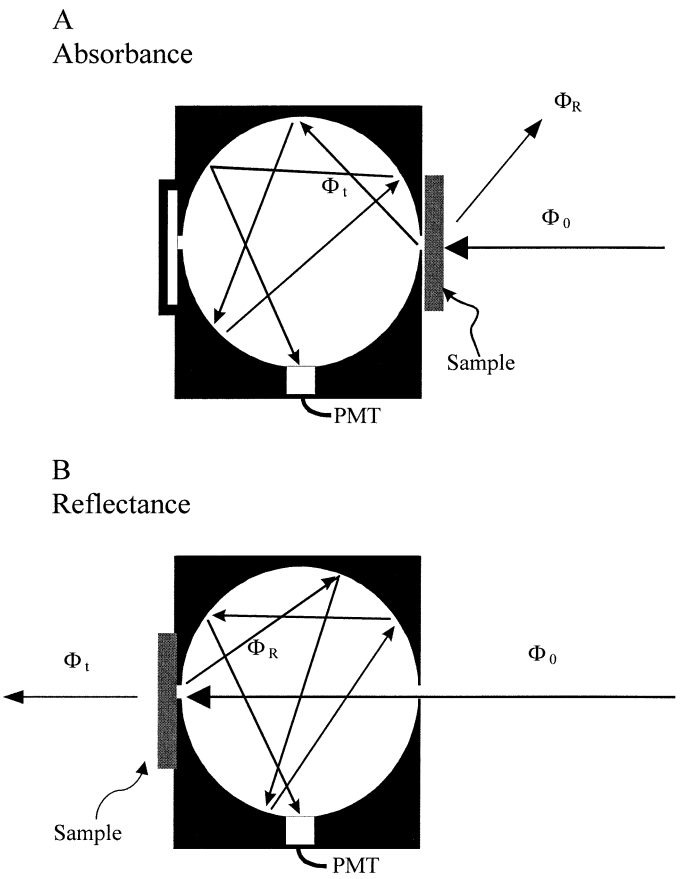

Fig. 1. Orientation of leaf samples containing intact epiphyte communities with respect to the integrating sphere and incident beam (Φ_0) for measurement of (A) leaf absorbance, *D*, and (B) reflectance, *R*. PMT = photomultiplier tube.

epiphyte cellular biomass, underestimates the total mass of noncellular organic and inorganic material that constitutes the epiphyte matrix or biofilm.

Leaf and epiphyte optical properties—Optical properties were determined using a Shimadzu UV2101 scanning spectrophotometer fitted with an integrating sphere that accurately measures absorbance and reflectance properties of turbid samples (e.g., leaves). Fresh leaves were gently patted with a tissue to remove water immediately before being placed into the optical path of the spectrophotometer. Absorbances and reflectances of fouled leaves were measured between 350 and 750 nm at 0.5-nm resolution using a 2-nm slit. Absorbance was measured by placing a leaf sample at the front of the integrating sphere to capture all light transmitted through the sample (Fig. 1A). Reflectance was measured by placing a leaf sample at the back of the integrating sphere to capture all light reflected from the incident surface (Fig. 1B). Leaf absorbance and reflectance measures were repeated after gently scraping the epiphytes from each leaf with a razor blade. Five or ten replicate leaves were scanned for each leaf class from each population. The epiphyte absorbance measured on leaf 1 samples was negligible, and there were no detectable changes in leaf optical properties after scraping. Thus epiphyte absorbances were zero on the youngest leaves.

Calculation of epiphyte and leaf absorptance—The spectral photon flux transmitted through the fouled leaf–epiphyte complex [$\Phi_t(\lambda)$] was calculated as a function of the incident flux [$\Phi_0(\lambda)$] measured in situ

$$\Phi_t(\lambda) = \Phi_0(\lambda)[1 - A_{\text{fouled}}(\lambda)] \quad (1)$$

where the fraction of incident flux lost by absorption, or absorptance, [$A_{\text{fouled}}(\lambda)$] was calculated from the spectrophotometrically measured absorbance [$D_{\text{fouled}}(\lambda)$] according to Kirk (1994):

$$A_{\text{fouled}}(\lambda) = 1 - 10^{-[D_{\text{fouled}}(\lambda)]} \quad (2)$$

Absorptance of the epiphyte layer was calculated as the difference between absorptances of the clean and fouled leaves:

$$A_{\text{epi}}(\lambda) = A_{\text{fouled}}(\lambda) - A_{\text{clean}}(\lambda) \quad (3)$$

The photon flux absorbed by the epiphyte layer was calculated as

$$\Phi_{\text{epi}}(\lambda) = \Phi_0(\lambda)A_{\text{epi}}(\lambda) \quad (4)$$

The photon flux reaching the surface of the clean leaf after being filtered through the epiphyte layer was calculated by subtracting the flux absorbed by the epiphyte layer:

$$\Phi_{\text{leaf}}(\lambda) = \Phi_0(\lambda) - \Phi_{\text{epi}}(\lambda) \quad (5)$$

Thus, the flux reaching the leaf surface was inversely proportional to epiphyte absorptance. The true leaf absorptance was corrected for reflectance [$R_{\text{clean}}(\lambda)$]

$$A_{\text{true}}(\lambda) = A_{\text{clean}}(\lambda) - R_{\text{clean}}(\lambda) \quad (6)$$

and photosynthetic absorptance [$A_p(\lambda)$] was calculated by subtracting nonspecific absorptance [$A_{\text{true}}(750)$]:

$$A_p(\lambda) = A_{\text{true}}(\lambda) - A_{\text{true}}(750) \quad (7)$$

$\bar{A}_p$ was calculated as the spectral average of $A_p(\lambda)$.

Leaf photosynthesis—The photosynthetically absorbed flux was then calculated as the product of the photon flux incident on the leaf and the photosynthetic absorptance:

$$\Phi_p(\lambda) = \Phi_{\text{leaf}}(\lambda)A_p(\lambda) \quad (8)$$

Finally, instantaneous spectral photosynthesis (P_λ) was expressed using a one-hit Poisson function (Falkowski and Raven 1997) in which both P_λ and the photosynthetic light use efficiency (ϕ_p) were scaled to the maximum rate of light-saturated, biomass-specific gross photosynthesis ($P_m = 1$) to provide a general context for evaluating the effects of leaf and biofilm optical properties on the potential for photosynthetic light use. In normalizing photosynthesis to P_m, the light use efficiency (ϕ_p) became an aggregate term with units of (cm^2 leaf s)/quanta absorbed and P_λ became a dimensionless factor that ranged from 0 to 1:

$$P_\lambda = \left[1 - \exp\left(-\phi_p \sum_{\lambda=400}^{700} \Phi_p(\lambda)\right)\right] \quad (9)$$

Spectral sensitivity was removed for the calculation of

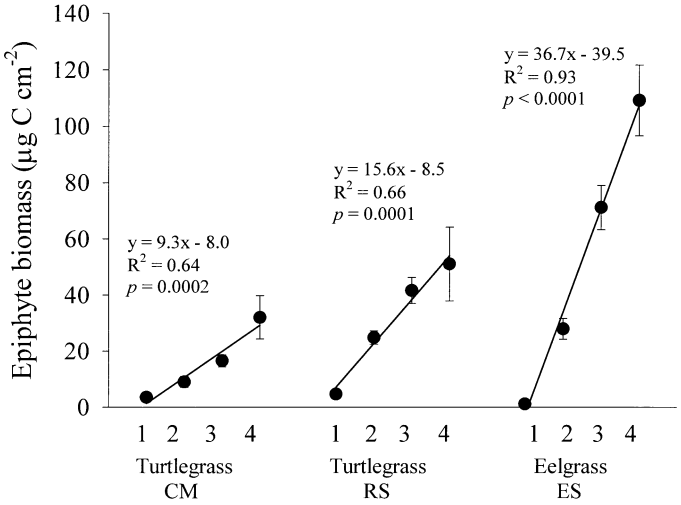

Fig. 2. Epiphyte biomass (μg carbon cm^{-2}) versus leaf age class for turtlegrass and eelgrass leaves. Locations of shoot collection are designated as CM = Channel Marker, RS = Rainbow South, and ES = Elkhorn Slough. Data are mean values (n = 3 or 4) $\pm$ 1 SE.

Table 1. Analysis of covariance (ANCOVA) for differences in slope among treatments.

Effect	df	Mean square	*F* ratio	*P*
Effect of location on epiphyte biomass (Fig. 2)				
Location	2	4,249	13.3	<0.0001
Error	40	319		
Effect of location (epiphyte composition) on epiphyte absorptance at 440 nm (Fig. 4)				
Location	2	20.0	0.41	0.68
Error	8	49.0		
Effect of location (epiphyte composition) on epiphyte absorptance at 550 nm (Fig. 4)				
Location	2	11.7	0.54	0.60
Error	8	21.5		
Effect of location (epiphyte composition) on leaf spectral photosynthesis (Fig. 6)				
Location	2	0.008	1.97	0.20
Error	8	0.004		

broadband, PAR-based photosynthesis by defining Φ_p (PAR) as the product of the spectrally averaged photosynthetic absorptance and the integrated spectral flux:

$$\Phi_p(\text{PAR}) = \bar{A}_p \sum_{\lambda=400}^{700} \Phi_{\text{leaf}}(\lambda) \tag{10}$$

and substituting it into the exponential photosynthesis function:

$$P_{\text{PAR}} = \{1 - \exp[-\phi_p \Phi_p(\text{PAR})]\} \tag{11}$$

As with P_λ, P_{PAR} represents a dimensionless coefficient ranging from 0 to 1 (P_m = 1).

Results

Epiphyte community characteristics—Other than a few small, black tunicates on turtlegrass shoots collected at Rainbow South, there were no visible epiphytes using a low-magnification dissecting microscope on the class 1 leaves from all three populations. In contrast, the epiphyte assemblages on class 4 leaves were more luxuriant and more diverse. Turtlegrass class 4 leaves collected from Lee Stocking Island were dominated by calcareous organisms that included spirorbid polychaetes and encrusting coralline algae. The epiphytes on eelgrass from Elkhorn Slough were dominated by diatoms, filamentous algae, and fine particles trapped in mucilaginous biofilm.

Epiphyte biomass—Biomass increased 15-fold with leaf class on turtlegrass at channel marker and Rainbow South (from 3.5 to 51 μg C cm^{-2}) and 99-fold on eelgrass at Elkhorn Slough (from 1.1 to 109 μg C cm^{-2}) (Fig. 2). Regression of epiphyte biomass against leaf class resulted in epiphyte accumulation rates of 9.3 μg C cm^{-2} leaf class^{-1} for Channel Marker (R^2 = 0.64, p = 0.0002), 15.6 μg C cm^{-2} leaf class^{-1} for Rainbow South (R^2 = 0.66, p = 0.0001), and 36.7 μg C cm^{-2} leaf class^{-1} for Elkhorn Slough (R^2 = 0.93, $p < 0.0001$) (Fig. 2). The rate of epiphyte accumulation was significantly higher on eelgrass from the eutrophic Elkhorn Slough than on turtlegrass from the oligotrophic sites in the Bahamas (Table 1; post hoc least significant difference (LSD) $p < 0.05$). Epiphyte accumulation rates were statistically identical at Channel Marker and Rainbow South (LSD post hoc $p > 0.05$).

Leaf and epiphyte absorptance—Absorptance spectra of clean (epiphytes removed) class 1 leaves from all three sites were typical of chlorophytes (Fig. 3A–C). Leaves were highly absorbent (80 to 93%) in the Soret band (400 to 500 nm), with a strong shoulder at 490 nm and a narrow peak at 680 nm. The transmission window in the green region absorbed about 50% of the incident photon flux. Absorptances of class 1 leaves were nearly identical for turtlegrass from the two Bahamian sites despite as much as a threefold difference in depth between Rainbow South (10 m deep) and Channel Marker (3.3 m deep) (Fig. 3A–B). Additionally, leaf class 1 absorptance of eelgrass collected from a depth of 1 m in Elkhorn Slough was only 7% higher across the visible spectrum (Fig. 3C).

As with biomass, epiphyte absorptances increased with leaf age class at all three sites (Fig. 3A–C). The absorptance spectra of turtlegrass epiphytes from the Bahamas were considerably flatter than spectra of clean class 1 leaves. As much as 74% of the wavelength-specific absorptance of clean class 1 leaves could be attributed to photosynthetic pigments (subtract A(750 nm) from $A(\lambda)$). However, photosynthetic pigments accounted for only 8–33% of the total absorptance by turtlegrass epiphytes. This result is likely due to a large fraction of nonphotosynthetic material, especially carbonate salts, in the epiphyte layers. In contrast, the absorptance spectra of eelgrass epiphytes from Elkhorn Slough revealed stronger peaks in the chlorophyll *a* bands, accounting for a maximum of 63% of the total absorptance. This result indicates that the eelgrass epiphytes consisted of a higher pro-

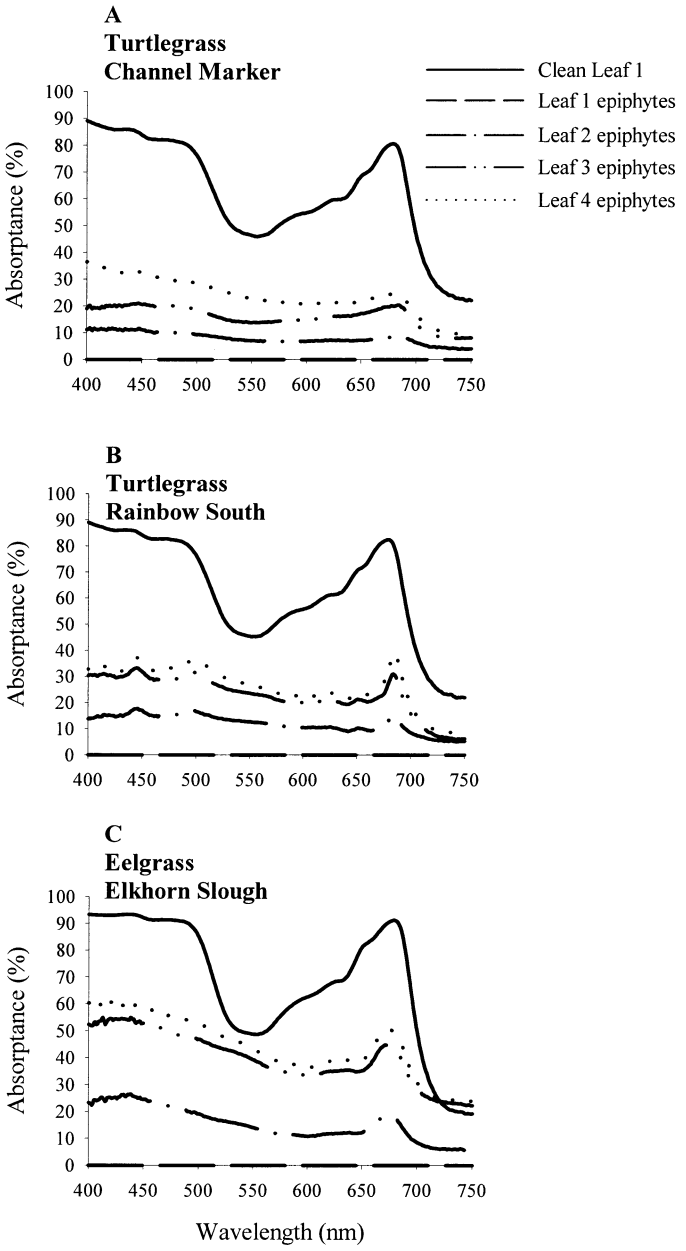

Fig. 3. Absorptance of leaf class 1 and epiphytes from leaf classes 1–4 from all sites (Eqs. 6 and 3, respectively). Data are mean values (n = 5 or 10).

portion of photosynthetic organisms than the turtlegrass epiphytes.

Relationship between epiphyte biomass and epiphyte absorptance—Epiphyte absorptance increased linearly with epiphyte biomass, and there was no significant difference among sites with regard to the slope of this relationship (Fig. 4, Table 1). Ninety percent of the epiphyte absorptance could be explained simply by biomass when wavelength-specific data were combined for all sites. The presence of photosynthetic pigments in the epiphyte layers produced higher slopes at 440 nm and 680 nm than 550 nm (A_{440} = 0.67(biomass), R^2_{440} = 0.87, $p_{440} < 0.0001$; A_{680} = 0.55(biomass), R^2_{680} = 0.87, p_{680}

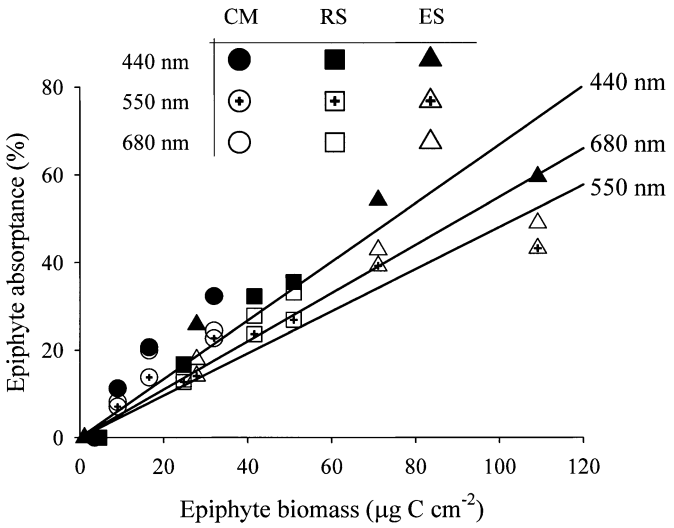

Fig. 4. Epiphyte absorptance at 440, 550, and 680 nm versus epiphyte biomass (Eq. 3). Data represent measurements from leaf classes 1–4 at each site. CM = Channel Marker, RS = Rainbow South, and ES = Elkhorn Slough.

< 0.0001; A_{550} = 0.48(biomass), R^2_{550} = 0.90, $p_{550} < 0.0001$, Fig. 4). The highest epiphyte loads observed in this study (on the oldest eelgrass leaves) absorbed as much as 60% of incident photons in the photosynthetically important Soret region (400 to 500 nm) (Fig. 4).

Light transmission through the epiphyte layer—Downwelling flux at the oligotrophic turtlegrass sites had similar spectral shapes (Fig. 5A). The differences in depth, however, resulted in a higher incident flux and more red light at the shallow Channel Marker site (3.3 m depth) than the deeper Rainbow South site (10 m depth). Although the Bahamian sites were 2.3 m and 8.5 m deeper than Elkhorn Slough, the midday photon flux to the turtlegrass canopies was 2–5 fold higher than to the shallow eelgrass canopy in Elkhorn Slough (1.5 m depth). Epiphytes on class 4 leaves removed an average of 28–49% of the downwelling flux (Fig. 5A) across the visible spectrum (Fig. 5B). Consequently, the flux to the underlying class 4 leaves was an average of 51–72% of the downwelling flux (Fig. 5A) and was relatively enriched in green light (500 to 600 nm, Fig. 5C).

Class 4 leaves below the epiphyte layer (Fig. 5D) absorbed an average of 25–31% of the downwelling flux incident on the seagrass canopy (Fig. 5A) and an average of 38–53% of the flux transmitted to the leaf surface (Fig. 5C). Class 4 leaves of turtlegrass exhibited absorption spectra typical of chlorophytes (Fig. 5D), but the class 4 leaves of eelgrass showed less absorption in the Soret band than the other sites (Fig. 5D) due to higher absorptance by epiphytes in that region (Figs. 3C, 5B).

In contrast, class 1 leaves absorbed an average of 43–52% of the downwelling flux across the visible spectrum (Fig. 5A,E). Absorption spectra from all three sites were typical of chlorophytes, and the spectra differed from the downwelling flux (Fig. 5A), which had a broad, relatively flat peak from 475 nm to 600 nm.

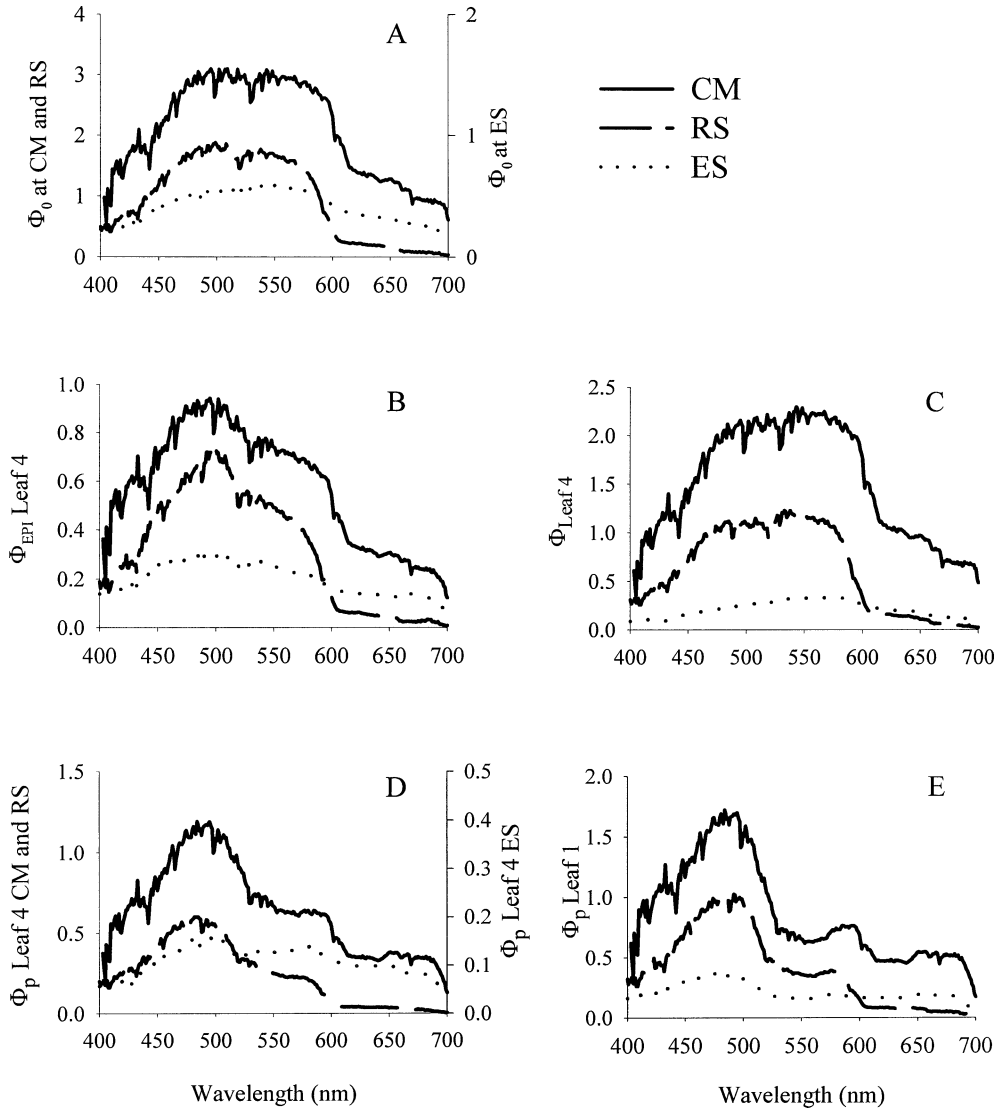

Fig. 5. Spectral fluxes. (A) Downwelling flux (Φ_0) in the water column; (B) flux absorbed by epiphytes (Φ_{epi}) on leaf class 4 (Eq. 4); (C) flux transmitted to the leaf class 4 surface (underneath the epiphyte layer) ($\Phi_{Leaf\ 4}$, Eq. 5); (D) flux absorbed by leaf class 4 (Φ_p, Eq. 8); and (E) flux absorbed by leaf class 1 (Φ_p, Eq. 8). All units are μmol quanta m^{-2} s^{-1} nm^{-1}. Data in plot A were collected from the following depths: 10 m (Channel Marker), 3.3 m (Rainbow South), and 1.5 m (Elkhorn Slough). CM = Channel Marker, RS = Rainbow South, and ES = Elkhorn Slough. Unless noted, axes on plots represent data from all three sites. Data are mean values (n = 5 or 10).

Biofilm effect on modeled leaf photosynthesis—Spectrally integrated leaf photosynthesis (normalized to P_m), declined linearly as epiphyte biomass increased ($P_\lambda = -0.0055$(biomass) + 1.0; $R^2 = 0.83$; $p < 0.0001$; Fig. 6). The greatest amount of epiphyte fouling observed here (109 μg C cm^{-2}) reduced the modeled estimate of photosynthesis by 49%. There was, however, no significant effect of location (i.e., epiphyte composition) on the relationship between epiphyte biomass and spectral photosynthesis, despite the spectral differences noted in the epiphyte optical properties among these populations (Table 1).

Calculations of PAR photosynthesis (normalized to P_m), which was not weighted for the changes in spectral bias of the incident flux or the light absorbed, decreased less than modeled spectral photosynthesis with increasing biomass load ($P_{PAR} = -0.0025$(biomass) + 1.0; $R^2 = 0.87$; $p < 0.0001$; Fig. 6). PAR photosynthesis relative to P_m was reduced by 30% for the oldest, most heavily fouled leaves of eelgrass.

Discussion

This study developed a simple optical model relating epiphyte load to the reduction of spectral photosynthesis of seagrass leaves. The model's formulation begins with our observation that epiphyte biomass increases with leaf class, a classic pattern of age-dependent increase documented by other investigators (e.g., Bulthuis and Woelkerling 1983; Törnblom and Søndergaard 1999). In measuring epiphyte

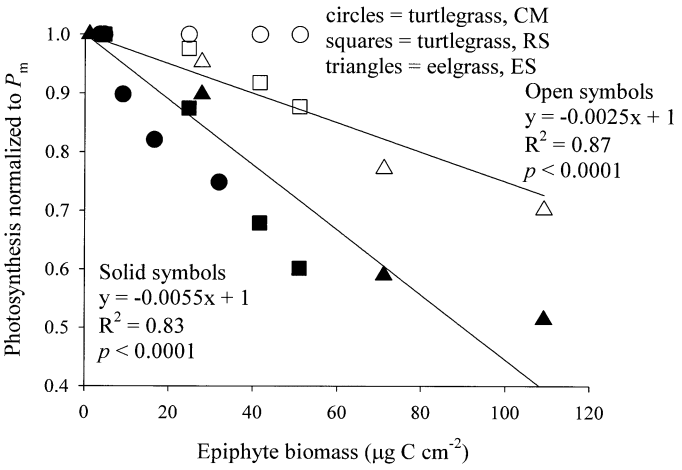

Fig. 6. Modeled spectral and PAR photosynthesis normalized to P_m versus epiphyte biomass (Eqs. 9 and 11, respectively). Data are values from leaf age classes 1–4 at each site. Solid symbols represent spectral photosynthesis; open symbols represent PAR photosynthesis. CM = Channel Marker, RS = Rainbow South, and ES = Elkhorn Slough.

effects on leaf optical properties, previous investigators were unable to measure spectroscopically accurate optical properties of intact epiphyte–leaf complexes. Consequently, uncorrected scattering losses caused epiphyte impacts on light attenuation to be overestimated, and nonspectral measures underestimated the impact of spectral shifts in the transmitted flux on seagrass photosynthesis. Thus, epiphyte biomass measurements must be coupled with spectral attenuation of light by intact biofilms (corrected for forward scattering, using a spectrophotometer with an integrating sphere) to accurately assess epiphyte effects on light attenuation, and, by extension, leaf photosynthesis.

Our results show that epiphytes did not act merely as neutral-density filters situated above the seagrass leaves but exhibited varying degrees of chlorophyll-like absorbance spectra. The epiphytes preferentially absorbed light in the blue and red, thus competing for photons with the underlying leaves. This result is not unexpected. Laboratory experiments measuring absorbance spectra of algal components of periphyton (Losee and Wetzel 1983) support this finding, and absorbance curves generated in that study are similarly shaped to spectra presented here.

Incorporating our absorptance data into a model of spectral photosynthesis shows that the light attenuated by epiphyte loads observed in this study was capable of reducing seagrass leaf photosynthesis as much as 49%. Furthermore, our spectral calculation yielded greater effects of epiphytes than the PAR model, which showed smaller effects on photosynthesis on turtlegrass and a 30% reduction of photosynthesis by epiphytes on class 4 leaves of eelgrass. This result highlights the necessity of making spectral measurements, since broadband measures may seriously underestimate the detrimental effects of epiphytes on seagrass photosynthesis. It is important to note that these calculations did not consider the effects of epiphytes on gas exchange, and CO_2 uptake in particular, which may have additional, detrimental effects on leaf photosynthesis (Durako 1993; Zimmerman et al. 1995; Invers et al. 2001).

The slope of the epiphyte biomass versus absorptance curve measured here was more than four times greater than that reported by Bulthuis and Woelkerling (1983) (assuming that their epiphyte dry weight was composed of 30% carbon). This discrepancy is likely due to methodological differences in both the measurement of light attenuation and epiphyte biomass. First, our measurements accounted for the spectral nature of the incident flux on the leaves and the subsequent effects on photosynthesis, whereas nonspectral measurements tend to underestimate photosynthesis. Our use of an integrating sphere accounted for losses due to scattering, whereas measurements without one tend to overestimate epiphyte effects on photosynthesis. Second, our measurements of epiphyte biomass, based on phospholipid fatty acids and converted to carbon equivalents, did not account for noncellular organic and inorganic material in the epiphyte matrix. Thus, our biomass-normalized absorbances tend to overestimate the effect of the epiphytes when mucilaginous polysaccharides, carbonate sediment, or siliceous sediment are embedded in the epiphyte matrix. Finally, it is worth noting that the calculations presented here assumed that the leaf surfaces were oriented normal to a collimated beam. Evaluation of epiphytic effects on in situ production of seagrass meadows, however, must include a more realistic formulation that accounts for (i) the age structure of the seagrass population and its effect on epiphyte loading, (ii) the geometric orientation of seagrass leaves with respect to the diffuse irradiance field of natural waters, and (iii) the architecture of the seagrass canopy (e.g., Zimmerman 2003). The predictive understanding derived from such a biophysical approach offers to dramatically improve our ability to evaluate how future changes in the environment will affect the distribution, productivity, and structure of seagrass communities.

References

BLIGH, E. G., AND W. M. DYER. 1959. A rapid method of lipid extraction and purification. Can. J. Biochem. Physiol. **35:** 911–917.

BORUM, J. 1985. Development of epiphytic communities on eelgrass (*Zostera marina*) along a nutrient gradient in a Danish estuary. Mar. Biol. **87:** 211–218.

———, AND S. WIUM-ANDERSEN. 1980. Biomass and production of epiphytes on eelgrass (*Zostera marina* L.) in the Øresund, Denmark. Ophelia Suppl. **1:** 57–64.

BRICKER, S., C. CLEMENT, D. PIRHALLA, S. ORLANDO, AND D. FARROW. 1999. National estuarine eutrophication assessment: Effects of nutrients on the nation's estuaries. NOAA, National Ocean Service, Special Projects Office and the National Centers for Coastal Ocean Science.

BRUSH, M. J., AND S. W. NIXON. 2002. Direct measurements of light attenuation by epiphytes on eelgrass *Zostera marina*. Mar. Ecol. Prog. Ser. **238:** 73–79.

BULTHUIS, D. A., AND W. J. WOELKERLING. 1983. Biomass accumulation and shading effects of epiphytes on leaves of the seagrass, *Heterozostera tasmanica*, in Victoria, Australia. Aquat. Bot. **16:** 137–148.

CAINE, E. A. 1980. Ecology of two littoral species of Caprellid amphipods (Crustacea) from Washington, USA. Mar. Biol. **56:** 327–335.

CAMBRIDGE, M. L., AND A. J. MCCOMB. 1984. The loss of sea-

grasses in Cockburn Sound, Western Australia. I. The time course and magnitude of seagrass decline in relation to industrial development. Aquat. Bot. **20:** 229–243.

COLEMAN, V. L., AND J. A. BURKHOLDER. 1994. Community structure and productivity of epiphytic microalgae on eelgrass (*Zostera marina* L.) under water-column nitrate enrichment. J. Exp. Mar. Biol. Ecol. **179:** 29–48.

DALLA VIA, J., C. STURMBAUER, G. SCHÖNWEGER, E. SÖTZ, S. MATHEKOWITSCH, M. STIFTER, AND R. REIGER. 1998. Light gradients and meadow structure in *Posidonia oceanica:* Ecomorphological and functional correlates. Mar. Ecol. Prog. Ser. **163:** 267–278.

DOBBS, F. C., AND R. H. FINDLAY. 1993. Analysis of microbial lipids to determine biomass and detect the response of sedimentary microbes to disturbance, p. 347–358. *In* P. F. Kemp, B. Sherr, E. Sherr, and J. J. Cole [eds.], Handbook of methods in aquatic microbial ecology. Lewis.

DURAKO, M. J. 1993. Photosynthetic utilization of CO_2(aq) and HCO_3^- in *Thalassia testudinum* (Hydrocharitacae). Mar. Biol. **115:** 373–380.

FALKOWSKI, P. G., AND J. RAVEN. 1997. Aquatic photosynthesis. Blackwell.

INVERS, O., R. ZIMMERMAN, R. ALBERTE, M. PEREZ, AND J. ROMERO. 2001. Inorganic carbon sources for seagrass photosynthesis: An experimental evaluation for bicarbonate use in temperate species. J. Exp. Mar. Biol. Ecol. **265:** 203–217.

KAREZ, R., S. ENGELBERT, AND U. SOMMER. 2000. 'Co-consumption' and 'protective coating': two new proposed effects of epiphytes on their macroalgal hosts in mesograzer-epiphyte-host interactions. Mar. Ecol. Prog. Ser. **205:** 85–93.

KEMP, W. M., R. BARTLESON, AND L. MURRAY. 2000. Epiphyte contributions to light attenuation at the leaf surface, p. 55–69. *In* Chesapeake Bay submerged aquatic vegetation water quality and habitat-based requirements and restoration targets: A second technical synthesis. U.S. Environmental Protection Agency.

———, W. R. BOYNTON, L. MURRAY, C. J. MADDEN, R. L. WETZEL, AND F. VERA HERRERA. 1988. Light relations for the seagrass *Thalassia testudinum,* and its epiphytic algae in a tropical estuarine environment, p. 193–206. *In* A. Yáñez-Arancibia and J. W. Day, Jr. [eds.], Ecology of coastal ecosystems in the Southern Gulf of Mexico: The Terminos Lagoon region. Inst. Cienc. del Mar y Limnol. UNAM, Coast. Ecol. Inst. LSU. Editorial Universitaria, Mexico City.

KIRK, J. T. O. 1994. Light and photosynthesis in aquatic ecosystems. Cambridge Univ. Press.

KLUMPP, D. W., J. S. SALITA-ESPINOSA, AND M. D. FORTES. 1992. The role of epiphytic periphyton and macroinvertebrate grazers in the trophic flux of a tropical seagrass community. Aquat. Bot. **43:** 327–349.

LOSEE, R. F., AND R. G. WETZEL. 1983. Selective light attenuation by the periphyton complex, p. 89–96. *In* R. G. Wetzel [ed.] Periphyton of freshwater ecosystems. Dr. W. Junk.

MAZZELLA, L., AND R. S. ALBERTE. 1986. Light adaptation and the role of autotrophic epiphytes in primary production of the temperate seagrass, *Zostera marina* L. J. Exp. Mar. Biol. Ecol. **100:** 165–180.

NECKLES, H. A., E. T. KOEPFLER, L. W. HAAS, R. L. WETZEL, AND R. J. ORTH. 1994. Dynamics of epiphytic photoautotrophs and heterotrophs in *Zostera marina* (eelgrass) microcosms: Responses to nutrient enrichment and grazing. Estuaries **17:** 597–605.

———, R. L. WETZEL, AND R. J. ORTH. 1993. Relative effects of nutrient enrichment and grazing on epiphyte-macrophyte (*Zostera marina* L.) dynamics. Oecologia **93:** 285–295.

ORTH, R., AND K. MOORE. 1983. Chesapeake Bay: an unprecedented decline in submerged aquatic vegetation. Science **222:** 51–53.

———, AND J. VAN MONTFRANS. 1984. Epiphyte-seagrass relationships with an emphasis on the role of micrograzing: A review. Aquat. Bot. **18:** 43–69.

PENHALE, P. A. 1977. Macrophyte-epiphyte biomass and productivity in an eelgrass (*Zostera marina* L.) community. J. Exp. Mar. Biol. Ecol. **26:** 211–224.

SAND-JENSEN, K. 1977. Effect of epiphytes on eelgrass photosynthesis. Aquat. Bot. **3:** 55–63.

———, AND J. BORUM. 1984. Epiphyte shading and its effect on photosynthesis and diel metabolism of *Lobelia dortmanna* L. during the spring bloom in a Danish lake. Aquat. Bot. **20:** 109–119.

———, N. P. REVSBECH, AND B. B. JØRGENSEN. 1985. Microprofiles of oxygen in epiphyte communities on submerged macrophytes. Mar. Biol. **89:** 55–62.

———, AND M. SØNDERGAARD. 1981. Phytoplankton and epiphyte development and their shading effect on submerged macrophytes in lakes of different nutrient status. Int. Rev. Gesamten Hydrobiol. **66:** 529–552.

SHORT, F. T., K. MATSO, H. M. HOVEN, J. WHITTEN, D. M. BURDICK, AND C. A. SHORT. 2001. Lobster use of eelgrass habitat in the Piscataqua River on the New Hampshire/Maine border, USA. Estuaries **24:** 277–284.

TOMASKO, D. A., AND B. E. LAPOINTE. 1991. Productivity and biomass of *Thalassia testudinum* as related to water column nutrient availability and epiphyte levels: Field observations and experimental studies. Mar. Ecol. Prog. Ser. **75:** 9–17.

TÖRNBLOM, E., AND M. SØNDERGAARD. 1999. Seasonal dynamics of bacterial biomass and production on eelgrass *Zostera marina* leaves. Mar. Ecol. Prog. Ser. **179:** 231–240.

TROCINE, R., J. RICE, AND G. WELLS. 1981. Inhibition of seagrass photosynthesis by ultraviolet-B radiation. Plant Physiol. **68:** 74–81.

TWILLEY, R. R., W. M. KEMP, K. W. STAVER, J. C. STEVENSON, AND W. R. BOYNTON. 1985. Nutrient enrichment of estuarine submersed vascular plant communities. 1. Algal growth and effects on production of plants and associated communities. Mar. Ecol. Prog. Ser. **23:** 179–191.

VAN MONTFRANS, J., R. L. WETZEL, AND R. J. ORTH. 1984. Epiphyte-grazer relationships in seagrass meadows: Consequences for seagrass growth and production. Estuaries **7:** 289–309.

WHITE, D. C., W. M. DAVIS, J. S. NICHOLS, J. D. KING, AND R. J. BOBBIE. 1979. Determination of sedimentary microbial biomass by extractable lipid phosphate. Oecologia **40:** 51–62.

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

ZIEMAN, J. C., AND R. G. WETZEL. 1980. Methods and rates of productivity in seagrasses, p. 87–116. *In* R. C. Phillips and C. P. McRoy [eds.] Handbook of seagrass biology. Garland STMP Press.

ZIMMERMAN, R. C. 2003. A biooptical model of irradiance distribution and photosynthesis in seagrass canopies. Limnol. Oceanogr. **48:** 568–585.

———, A. CABELLO-PASINI, AND R. S. ALBERTE. 1994. Modeling daily production of aquatic macrophytes from irradiance measurements: A comparative analysis. Mar. Ecol. Prog. Ser. **114:** 185–196.

———, D. G. KOHRS, D. L. STELLER, AND R. ALBERTE. 1995. Sucrose partitioning in *Zostera marina* L. in relation to photosynthesis and the daily light-dark cycle. Plant Physiol. **108:** 1665–1671.

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