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Periphyton light transmission relationships in Florida Bay and the Florida Keys, USA

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Abstract

Light transmission was measured through intact, submerged periphyton communities on artificial seagrass leaves. The periphyton communities were representative of the communities on *Thalassia testudinum* in subtropical seagrass meadows. The periphyton communities sampled were adhered carbonate sediment, coralline algae, and mixed algal assemblages. Crustose or film-forming periphyton assemblages were best prepared for light transmission measurements using artificial leaves fouled on both sides, while measurements through three-dimensional filamentous algae required the periphyton to be removed from one side. For one-sided samples, light transmission could be measured as the difference between fouled and reference artificial leaf samples. For two-sided samples, the percent periphyton light transmission to the leaf surface was calculated as the square root of the fraction of incident light. Linear, exponential, and hyperbolic equations were evaluated as descriptors of the periphyton dry weight versus light transmission relationship. Hyperbolic and exponential decay models were superior to linear models and exhibited the best fits for the observed relationships. Differences between the coefficients of determination (r^2) of hyperbolic and exponential decay models were statistically insignificant. Constraining these models for 100% light transmission at zero periphyton load did not result in any statistically significant loss in the explanatory capability of the models. In most all cases, increasing model complexity using three-parameter models rather than two-parameter models did not significantly increase the amount of variation explained. Constrained two-parameter hyperbolic or exponential decay models were judged best for describing the periphyton dry weight versus light transmission relationship. On *T. testudinum* in Florida Bay and the Florida Keys, significant differences were not observed in the light

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transmission characteristics of the varying periphyton communities at different study sites. Using pooled data from the study sites, the hyperbolic decay coefficient for periphyton light transmission was estimated to be $4.36 \text{ mg dry wt. cm}^{-2}$. For exponential models, the exponential decay coefficient was estimated to be $0.16 \text{ cm}^2 \text{ mg dry wt.}^{-1}$.

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1. Introduction

The recent decline of seagrasses worldwide is most often attributed to cultural eutrophication of coastal areas resulting in decreased water clarity, macroalgal blooms (Hauxwell et al., 2001; McGlathery, 2001), and higher periphyton loading on the seagrass leaves (Orth and Moore, 1983; Silberstein et al., 1986; Giesen et al., 1990). Periphyton can limit seagrass photosynthesis by reflecting and absorbing available light before it is able to reach leaf surfaces (Bulthuis and Woelkerling, 1983; Orth and Moore, 1983; Neckles et al., 1993). Seagrasses can control the negative effects of periphyton loading by the continuous shedding of the oldest and most epiphytized leaves (Bulthuis and Woelkerling, 1983). When periphyton accumulation rates are high, leaf turnover is no longer an effective mechanism for maintaining low periphyton loads. Seagrass growth models indicate that ambient light and those factors affecting light levels at the leaf surface are the principal factors determining the distribution of seagrasses (Wetzel and Neckles, 1986; Madden and Kemp, 1996). These models are dependent upon accurate determinations of water-column light attenuation and the periphyton loading versus periphyton light transmission relationship.

Despite over two decades of quantifying periphyton light transmission, standard methodologies for measuring this process do not presently exist. A variety of methods have been used to measure light transmission through the periphyton matrix (see Brush and Nixon, 2002, for comprehensive listing). Some studies have removed the periphyton from their surfaces and either resuspended the periphyton material in water (Sand-Jensen and Borum, 1984; Neckles, 1993; Dixon, 2000) or collected the material onto filters prior to measuring light transmission (Cebrian et al., 1999). Recent studies demonstrated that accurate measurements of periphyton light transmission could only be made on intact submerged periphyton communities (Vermaat and Hootsmans, 1994; Brush and Nixon, 2002; Stankelis et al., 2003; Drake et al., 2003). This is especially true for filamentous periphyton, which naturally extend out and away from leaf surfaces (Brush and Nixon, 2002; Stankelis et al., 2003). When filamentous periphyton are collapsed onto a flat surface or restricted within the direct light path, periphyton light transmission is minimized. Coralline algal crusts and diatom films naturally form flat and often continuous surfaces that cannot be duplicated in suspension (Vermaat and Hootsmans, 1994; Drake et al., 2003). Disruption of these periphyton often results in clumping of the periphyton material. These deviations of the natural periphyton orientation can result in both overestimates and underestimates of periphyton light attenuation.

The nature of various periphyton communities may also dictate how periphyton samples are prepared prior to measurement of light transmission. Periphyton light transmission has been measured using natural (Bulthuis and Woelkerling, 1983; Brush and Nixon, 2002; Drake et al., 2003) and artificial seagrass leaves (Glazer, 1999; Stankelis et al., 2003). The nature of the periphyton community may determine the appropriateness of these substrates. Periphyton light transmission has been measured through one-sided (Brush and Nixon, 2002) and two-sided samples (Drake et al., 2003; Stankelis et al., 2003). Light is scattered as it passes through a leaf-periphyton matrix allowing some transmitted light to avoid detection by a light detector (Kirk, 1994; Drake et al., 2003). Drake et al. (2003) states that “uncorrected scattering losses caused epiphyte impacts on light attenuation to be overestimated”. Drake et al. (2003) used an integrating sphere to capture scattered light, though it is not known how much scattered light may be lost if an integrating sphere is not used. Positioning the light detector as close to the leaf-periphyton matrix as possible may intercept much of this reflected light and minimize the amount of transmitted light that escapes measurement by the detector. Only transmitted light that is reflected at very wide angles would escape detection by the sensor (Kirk, 1994). To achieve close positioning of the light detector and maintain the natural periphyton orientation, filamentous periphyton must be scraped from one-side of the natural or artificial seagrass leaf (Brush and Nixon, 2002). Two-sided samples may be more appropriate for delicate diatom films that are easily disrupted by the scraping of one side, and for tightly adhered coralline periphyton that can be resistant to complete removal from seagrass leaves. Stankelis et al. (2003) measured light transmission through diatom periphyton using two-sided samples collected on Mylar™ strips. Brush and Nixon (2002) suggested the use of Mylar™ strips as an alternative to natural seagrass leaves when sampling coralline red algae periphyton.

A variety of periphyton light attenuation equations have been used to describe periphyton load versus light transmission relationships. Glazer (1999) described light transmission by epiphytic bryozoans using a linear equation. Hyperbolic decay equations were used to describe the optical properties of adhered sediment (van Dijk, 1993), microalgal films (Vermaat and Hootsmans, 1994), and filamentous macroalgae (Brush and Nixon, 2002). Exponential decay equations were used for a mixed coralline community (Burt et al., 1995) and diatoms (Stankelis et al., 2003), respectively. Some of these equations were constrained at 100% transmission at zero periphyton load (Vermaat and Hootsmans, 1994; Burt et al., 1995; Brush and Nixon, 2002; Stankelis et al., 2003) and others were not (van Dijk, 1993). Vermaat and Hootsmans (1994) concluded that hyperbolic equations of the Michaelis–Menten variety (constrained two-parameter forms) were superior to linear and exponential forms in describing the periphyton light transmission relationship for cultured periphyton communities. The applicability of a specific type of may be dependent upon the nature of the periphyton community (Brush and Nixon, 2002).

The present investigation measured periphyton light transmissions and associated periphyton loads for the subtropical periphyton communities in Florida Bay and the Florida Keys. Periphyton sampling preparations suitable for different periphyton morphologies and the appropriate methods of calculating periphyton light transmission were evaluated. Various forms of linear, exponential and hyperbolic decay equations were used and compared with each other to describe the periphyton light transmission versus periphyton load relationship.

2. Materials and methods

2.1. Study area

Four study sites were situated in Florida Bay and the upper Florida Keys, FL, USA. Florida Bay is located along the eastern margin of the Gulf of Mexico and is bounded to the north by the Everglades of mainland Florida and along the southeast by the Florida Keys. The bay is open to exchange with the Gulf of Mexico along its western boundary and with the Atlantic Ocean via tidal creeks and passes through the Florida Keys. The Molasses Reef site, located outside of Florida Bay, is characterized by low water-column nutrients and is representative of higher-energy back-reef *Thalassia testudinum* meadows. Relationships between periphyton dry weight and light transmission were determined for the periphyton communities at Duck Key (25°10.59 and 80°29.39), Bob Allen Keys (25°01.48 and 80°40.86), Sprigger Bank (24°54.78 and 80°56.27), and Molasses Reef (25°00.91 and 80°22.42). Previous investigations of the Florida Bay periphyton communities revealed large regional and seasonal variation in periphyton loads (Frankovich and Zieman, 1994). Calcium carbonate from adhered sediment, coralline algae, and the worm tubes of *Spirorbis* sp. often accounts for as much as 70–80% of the periphyton load (Frankovich and Zieman, 1994). Filamentous periphyton was abundant only in the immediate vicinity of bird islands or during winter at westernmost sites (Frankovich and Fourqurean, 1997).

2.2. Determination of periphyton load–light transmission relationships

To determine the periphyton load–light transmission relationships, arrays of Mylar™ artificial seagrass leaves were placed among the seagrass *T. testudinum* at Duck Key, Bob Allen Keys, Sprigger Bank, and Molasses Reef. These arrays were left within the seagrass meadows for 63–75 days to accumulate periphyton. The periphyton composition that developed on the artificial leaves was the same as that observed on the adjacent *T. testudinum* leaves. The arrays consisted of transparent strips (28 cm × 1.6 cm) with styrofoam floats attached at the top. These artificial leaves were secured to the sediment using cable ties attached to wire hardware cloth bases that were pushed into the sediment. These arrays were left within the seagrass meadows for 63–75 days approximating the leaf turnover period measured on *T. testudinum* in Florida Bay (Bricker, 2003). Resulting periphyton loads ranged from near-zero levels at leaf bases to maximum levels at leaf apices. The range in periphyton loads approximated the range of periphyton loads on natural *T. testudinum* leaves in Florida Bay (Armitage et al., 2005). The arrays at Duck Key and Bob Allen Keys were deployed on December 27, 2000 while those at Molasses Reef and Sprigger Bank were deployed on August 8, 2001 and February 26, 2002, respectively. Periphyton light (PAR) transmission was measured in the lab using a modification of the underwater light attenuation measurement apparatus (LAMA) developed by Burt et al. (1995) and refined by Stankelis et al. (2003). Basic configurations of the LAMA are depicted in Fig. 2 of Burt et al. (1995) and Fig. 2 of Stankelis et al. (2003). The LAMA consists of a light source, a water bath, an underwater platform to which the artificial leaves are secured, and a quantum light sensor with datalogger to measure photosynthetically active radiation in the 400–700 nm range. The LAMA

constructed for this study was configured similarly. The light source consisted of a 1500 W halogen lamp positioned on the top of the water bath with a dimmer switch. Clean reference or fouled artificial leaf strips were secured to the underwater platform inside the water bath. A Licor model 192SA quantum sensor was positioned inside a track directly underneath the bottom of a clear polystyrene section of the platform upon which the artificial leaf strips were secured. The track allowed variable positioning of the quantum sensor to measure light transmission at seven locations along the artificial leaves (i.e., 3, 6, 9, 12, 15, 18, and 21 cm) through fouled and clean reference strips (same leaf locations). Light attenuation through reference strips was not detected. The total periphyton loads (mg dry wt. cm⁻² leaf surface) associated with the measured periphyton light transmissions were measured from periphyton removed from 3 cm long sections of the MylarTM leaves surrounding the point of light measurement. Periphyton dry weights were measured by scraping the accumulated material into tared scintillation vials using a razor blade. The samples were then lyophilized (freeze-dried) and weighed.

The morphology of the sampled periphyton communities and the degree to which they were attached to the MylarTM substrate dictated how the periphyton samples were prepared, and how light transmission was calculated from measurements taken through the artificial leaves. Ideally, periphyton material should be removed from one side of the artificial leaf prior to measurement to avoid the assumption of equal periphyton loads on either side of a two-sided sample and to maintain the natural orientation of three-dimensional periphyton assemblages (Brush and Nixon, 2002). This is especially true for three-dimensional filamentous algae that extend away from the leaf surface. Light transmission is greatly affected by the structure of intact periphyton communities (Brush and Nixon, 2002). The periphyton communities sampled in the present study varied from very light accumulations of adhered sediment to flat encrusting coralline red algae to the occasional occurrence of three-dimensional filamentous algae. One-sided sample preparation was not possible for either the light and delicate accumulations of sediment or for large accumulations of the firmly attached coralline algae. The accumulations of coralline algae at Duck Key were sparse and were effectively removed from one-side of the artificial leaves. Conversely, complete removal of large accumulations of firmly attached coralline algae encrustations from one side of the artificial leaves sampled from Sprigger Bank and Molasses Reef was not assured unless both sides were scraped clean. Removal attempts using acidification led to the dissolution of the opposite side of the artificial leaf also. The lightly adhered sediment at Bob Allen Keys was not effectively removed from one-side without causing the removal or disorientation (i.e., clumping) of sediment on the opposite side of the artificial leaf. Consequently, light transmission was measured through both of the periphyton layers for these types of periphyton communities sampled at Bob Allen, Sprigger Bank, and Molasses Reef. For one-sided samples with a transparent substrate (e.g., MylarTM), percent periphyton light transmission [PAR transmission] was calculated as the fraction of incident light passing through the periphyton matrix reaching the artificial leaf [$I_x I_0^{-1}$]:

$$\text{PAR transmission(one-sided samples)} = I_x I_0^{-1} \times 100 \quad (1)$$

For two-sided samples, light must pass through the periphyton layers on each side of the sample. Each periphyton layer acts as a separate in-line filter. Assuming that the periphyton

layers are similar in structure, the relative amount of light reaching the detector [$I_y I_0^{-1}$] is the product of the fractions of light transmitted by each periphyton filter:

$$I_y I_0^{-1} = I_x I_0^{-1} \times I_x I_0^{-1} = I_x^2 I_0^{-2} \quad (2)$$

The percent periphyton light transmission to the leaf surface is the square root of the fraction of incident light (Vermaat and Hootsmans, 1994) measured at the detector:

$$\text{PAR transmission (two-sided samples)} = [I_y I_0^{-1}]^{0.5} \times 100 \quad (3)$$

2.3. Comparison of periphyton light transmission decay models

Linear, exponential and hyperbolic decay models were fit to observed data from the study sites. Both two-parameter and three-parameter forms of the exponential and hyperbolic decay models were evaluated. All models were also evaluated in both constrained (100% light transmission at zero periphyton load) and unconstrained forms. A total of 40 regression equations, representing the combination of different forms of these models and the different study sites, were constructed. Parameter estimates together with their standard errors were calculated using Sigmaplot (2001) software. Equations were evaluated in terms of percent variation captured (i.e., r^2) and how well 100% light transmission was predicted at zero periphyton load (unconstrained equations). Linear, exponential, and hyperbolic decay models were compared against each other to determine the appropriateness of these different types of regression models. A Chi-square test of transformed correlation coefficients and a Tukey-type multiple comparison procedure (Zar, 1996) was used to test for significant differences in the fit of these three different types of regression models in both constrained and unconstrained forms. The suitability of constraining these models was determined by testing for significant differences in the correlation coefficients of constrained and unconstrained forms of these equations using the Z-test (Zar, 1996). The Z-test was also used to compare two-parameter and three-parameter forms of the hyperbolic and exponential models. These models were evaluated to determine if increased model complexity significantly increased the amount of variation explained. Site/periphyton community differences in the light transmission characteristics were determined by testing for significant differences of the decay coefficients using calculated standard errors and t -test (Zar, 1996). Comparisonwise, error rates were adjusted using the Bonferroni adjustment holding the experimentwise Type I error rate to a maximum of 0.05 (Zar, 1996).

3. Results

3.1. Periphyton community composition

The Mylar™ artificial seagrass leaves were effective substrates for sampling the periphyton that was observed occurring naturally on surrounding *T. testudinum* leaves. The

periphyton species composition on the artificial leaves was representative of the periphyton occurring on *T. testudinum* leaves from Florida Bay (personal observation, Frankovich and Zieman, 1994; Armitage et al., 2005). Pigment analyses of the periphyton sampled from both substrates and from similar areas in Florida Bay revealed no differences in relative pigment concentrations between the two (Louda et al., 1999). The sampled macroperiphyton communities were sparse. Filamentous macroalgae were rare and never dominant at the sites and times sampled, though sparse *Polysiphonia binneyi*, *Laurencia intricata*, and *L. poiteaui* occasionally occurred. Periphyton material was mostly adhered carbonate sediment, the coralline red algae *Hydrolithon farinosum* and *Melobesia membranacea* and the ubiquitous diatoms. The periphyton community at Duck Key was sparse corallines, adhered sediment, and rare *P. binneyi*. The periphyton composition at Bob Allen Keys was characteristic of the turbidity observed there and consisted of almost entirely adhered sediment with lesser amounts of the spiral worm tubes of the polychaete *Spirorbis* sp. and occasional *P. binneyi*. The periphyton at Sprigger Bank was dominated by thick encrustations of coralline red algae and adhered sediment. Small thalli of both *Laurencia* species also occurred occasionally and the filamentous red coralline *Amphiroa fragilissima* occurred at the base of some of the artificial leaves. The periphyton community at Molasses Reef was very homogeneous and consisted almost entirely of the crustose coralline red algae.

3.2. Periphyton loads on artificial seagrass leaves

The periphyton loads at Duck Key measured after 73 days along the locations of the artificial leaves were very low. 82% of the periphyton loads were below 2.0 mg dry wt. cm⁻² (Fig. 1A). The periphyton loads at Bob Allen Keys were relatively evenly distributed across the range of values (Fig. 1B) with greater periphyton accumulations observed on the distal portions of the artificial leaves. Converse to Duck Key, 86% of the periphyton loads at Sprigger Bank were above 2.0 mg dry wt. cm⁻² (Fig. 1C) and the entire lengths of the artificial leaves were heavily covered with periphyton. Molasses Reef exhibited the smallest range in periphyton loads (i.e., 0.3–6.0 mg dry wt. cm⁻²) measured along the artificial leaves (Fig. 1D).

3.3. Periphyton light transmission models

The 40 regression models captured 0–87% of the variation in the light transmission versus periphyton load relationships for the periphyton communities at the study sites (Tables 1 and 2). With the exception of the poor fit ($r^2 = 0$) of the constrained linear model for the periphyton community at Sprigger Bank, all other model fits were statistically significant ($P < 0.0001$). Comparison of the correlation coefficients of the unconstrained and constrained regression models revealed that exponential and hyperbolic decay models captured more or statistically equal amounts of the observed variation in the light transmission versus periphyton load relationship than the linear model (Chi-square analysis, $P < 0.05$, comparisonwise Type I error rate = 0.013). $100 \pm 5\%$ light transmission at zero periphyton load was accurately predicted in only 24% of the unconstrained models (Table 3). Analyses of the correlation coefficients of the

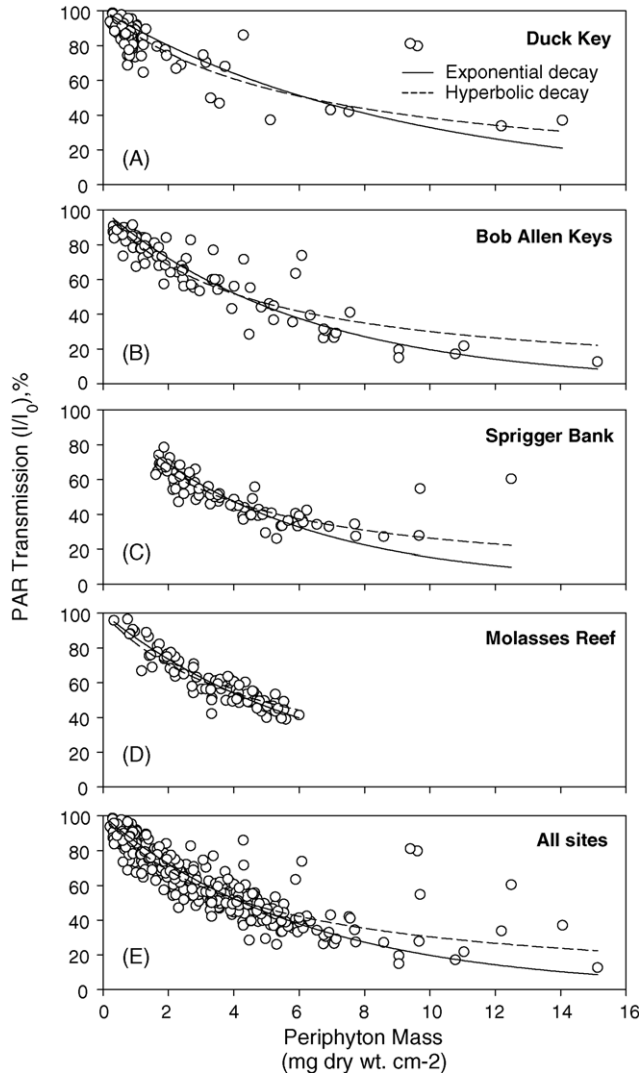


Fig. 1. Exponential and hyperbolic decay relationships between periphyton mass and light transmission for periphyton communities at Duck Key (A), Bob Allen Keys (B), Sprigger Bank (C), Molasses Reef (D), and for pooled data from all sites (E). Regression equations are constrained two-parameter forms and all are statistically significant ($P < 0.001$). Parameter estimates of regression equations are listed in Table 2. $n = 84, 83, 80, 94,$ and 341 for regression equations in panels A–E, respectively.

unconstrained versus constrained forms of the hyperbolic and exponential decay models revealed that differences in the amount of variation explained ranged from 0 to 16% (Tables 1 and 2) and that these differences were statistically insignificant (Z -test, $P > 0.05$, comparisonwise Type I error rate = 0.01). The increased model complexity of three-parameter hyperbolic and exponential equations relative to two-parameter equations

Table 1

Parameter estimates for unconstrained periphyton light extinction equations

	Duck Key	Bob Allen Keys	Sprigger Bank	Molasses Reef
Equations unconstrained				
Linear, $y_x = y_0 + ax$	$y_0 = 89.1$ $a = -4.16$ $r^2 = 0.52$	$y_0 = 85.3$ $a = -6.73$ $r^2 = 0.80$	$y_0 = 67.0$ $a = -4.20$ $r^2 = 0.47$	$y_0 = 91.6$ $a = -9.11$ $r^2 = 0.85$
Exponential decay two-parameter, $y_x = y_0e^{-ax}$	$y_0 = 91.2$ $a = 0.07$ $r^2 = 0.57$	$y_0 = 93.2$ $a = 0.14$ $r^2 = 0.85$	$y_0 = 79.7$ $a = 0.12$ $r^2 = 0.60$	$y_0 = 98.6$ $a = 0.15$ $r^2 = 0.87$
Exponential decay three-parameter, $y_x = y_0 + ae^{-bx}$	$y_0 = 51.1$ $a = 48.3$ $b = 0.41$ $r^2 = 0.64$	$y_0 = -6.5$ $a = 99.1$ $b = 0.13$ $r^2 = 0.85$	$y_0 = 34.6$ $a = 93.2$ $b = 0.56$ $r^2 = 0.76$	$y_0 = 25.8$ $a = 77.9$ $b = 0.26$ $r^2 = 0.87$
Hyperbolic decay two-parameter, $y_x = y_0a(a+x)^{-1}$	$y_0 = 94.0$ $a = 9.00$ $r^2 = 0.60$	$y_0 = 99.1$ $a = 4.39$ $r^2 = 0.83$	$y_0 = 100.4$ $a = 3.52$ $r^2 = 0.68$	$y_0 = 108.3$ $a = 3.92$ $r^2 = 0.87$
Hyperbolic decay three-parameter, $y_x = y_0 + ab(b+x)^{-1}$	$y_0 = 39.4$ $a = 62.4$ $b = 2.29$ $r^2 = 0.64$	$y_0 = -57.2$ $a = 150.5$ $b = 11.15$ $r^2 = 0.85$	$y_0 = 22.8$ $a = 257.7$ $b = 0.39$ $r^2 = 0.73$	$y_0 = -10.8$ $a = 116.2$ $b = 5.00$ $r^2 = 0.87$

y_x = percent light transmission at periphyton load (x , mg dry wt. cm^{-2}) and all regression equations are statistically significant ($P < 0.001$).

resulted in a significant increase in the amount of variation explained (+26%) only for the constrained form of the exponential decay model for the periphyton community at Sprigger Bank (Z -test, $P < 0.05$, comparisonwise Type I error rate = 0.01). For the periphyton communities at all other sites and for the hyperbolic model at Sprigger Bank, increased model complexity of three-parameter equations did not significantly increase the amount of variation captured in the light transmission versus periphyton load relationship (Z -test, $P > 0.05$, comparisonwise Type I error rate = 0.01).

Deducing from the results of these analyses, constrained two-parameter exponential and hyperbolic decay models were most appropriate for describing the light transmission versus periphyton load relationship. These decay models indicate a rapid decline in periphyton light transmission as periphyton starts to accumulate. The decline tapered off at higher periphyton loads when the unoccupied colonization space approached zero. These decay functions were best fit (highest r^2) to observed data when measured periphyton loads were evenly distributed across their range (i.e., Bob Allen, Sprigger, and Molasses) and when the periphyton communities were dominated by a single component (i.e., Bob Allen Keys and Molasses Reef). The best fit was observed for the Molasses Reef periphyton community because of the combination of these factors. The periphyton at Molasses Reef was composed almost entirely of tightly adhering and low profile crustose coralline algae. The high current and wave energy at Molasses Reef did not allow for the periphyton community to accumulate lightly adhering diatoms, sediment or higher profile filamentous algae. The paucity of periphyton loads greater than 2 mg dry wt. cm^{-2} at Duck Key did not

Table 2
Parameter estimates for constrained periphyton light extinction equations

	Duck Key	Bob Allen Keys	Sprigger Bank	Molasses Reef
Linear, $y_x = y_0 + ax$	$y_0 = 100$ $a = -6.13$ $r^2 = 0.14$	$y_0 = 100$ $a = -9.29$ $r^2 = 0.58$	$y_0 = 100$ $a = -10.59$ $r^2 = 0$	$y_0 = 100$ $a = -11.18$ $r^2 = 0.80$
Exponential decay two-parameter, $y_x = y_0 e^{-ax}$	$y_0 = 100$ $a = 0.11$ $r^2 = 0.41$	$y_0 = 100$ $a = 0.16$ $r^2 = 0.83$	$y_0 = 100$ $a = 0.19$ $r^2 = 0.48$	$y_0 = 100$ $a = 0.15$ $r^2 = 0.87$
Exponential decay three-parameter, $y_x = y_0 + ae^{-bx}$	$y_0 = 51.5$ $a = 48.5$ $b = 0.43$ $r^2 = 0.64$	$y_0 = 12.1$ $a = 87.9$ $b = 0.20$ $r^2 = 0.83$	$y_0 = 31.3$ $a = 68.7$ $b = 0.38$ $r^2 = 0.74$	$y_0 = 17.0$ $a = 83.0$ $b = 0.20$ $r^2 = 0.87$
Hyperbolic decay two-parameter, $y_x = y_0 a (a + x)^{-1}$	$y_0 = 100$ $a = 6.19$ $r^2 = 0.56$	$y_0 = 100$ $a = 4.29$ $r^2 = 0.83$	$y_0 = 100$ $a = 3.55$ $r^2 = 0.68$	$y_0 = 100$ $a = 4.73$ $r^2 = 0.86$
Hyperbolic decay three-parameter, $y_x = y_0 + ab (b + x)^{-1}$	$y_0 = 37.4$ $a = 62.7$ $b = 2.69$ $r^2 = 0.64$	$y_0 = -23.2$ $a = 123.2$ $b = 6.22$ $r^2 = 0.84$	$y_0 = 10.7$ $a = 89.3$ $b = 2.76$ $r^2 = 0.69$	$y_0 = -34.7$ $a = 134.7$ $b = 7.71$ $r^2 = 0.87$

y_x = percent light transmission at periphyton load (x , mg dry wt. cm^{-2}). Equations constrained at 100% light transmission at zero periphyton load (i.e., $y_0 = 100$ for two-parameter equations, $y_0 + a = 100$ for three-parameter equations). All regression equations except the linear equation for Sprigger Bank are statistically significant ($P < 0.001$).

adequately represent the decline of periphyton light transmission observed at the higher periphyton loads experienced at the other sites (Fig. 1A–D).

Multiple comparisons of the estimated exponential decay coefficients for each of the four periphyton communities at the study sites revealed that the exponential decay model

Table 3
Predicted light transmission (%) \pm standard error at zero periphyton (y -intercept)

	Duck Key	Bob Allen Keys	Sprigger Bank	Molasses Reef
Equations unconstrained				
Linear	89.1 \pm 1.3	85.3 \pm 1.6	67.0 \pm 2.3	91.6 \pm 1.5
Exponential decay two-parameter	91.2 \pm 1.5	93.2 \pm 1.9	79.7 \pm 3.4	98.6 \pm 1.9
Exponential decay three-parameter	99.4 \pm 1.2	92.6 \pm 1.4	127.8 \pm 1.6	103.7 \pm 1.4
Hyperbolic decay two-parameter	94.0 \pm 1.8	99.1 \pm 2.9	100.4 \pm 7.4	108.3 \pm 2.8
Hyperbolic decay three-parameter	101.8 \pm 1.2	93.3 \pm 1.4	280.6 \pm 1.7	105.4 \pm 1.4
Equations constrained				
Linear	100 \pm 1.8	100 \pm 2.2	100 \pm 4.4	100 \pm 1.8
Exponential decay two-parameter	100 \pm 1.9	100 \pm 2.2	100 \pm 4.9	100 \pm 1.9
Exponential decay three-parameter	100 \pm 1.2	100 \pm 1.5	100 \pm 1.6	100 \pm 1.4
Hyperbolic decay two-parameter	100 \pm 2.2	100 \pm 2.9	100 \pm 3.6	100 \pm 2.7
Hyperbolic decay three-parameter	100 \pm 1.2	100 \pm 1.4	100 \pm 1.8	100 \pm 1.4

y -Intercept of two-parameter equations equals y_0 . y -Intercept of three-parameter equations equals the sum of $y_0 + a$.

for the periphyton community at Duck Key was significantly different from the periphyton communities at the other three sites (*t*-test, $P < 0.05$, comparisonwise error rate = 0.013). No significant differences were evident between the hyperbolic decay coefficients produced from the varying periphyton communities at the study sites (*t*-test, $P < 0.05$, comparisonwise error rate = 0.013). Because of the lack of high periphyton loads at Duck Key and non-significant differences in the exponential and hyperbolic decay coefficients for the modeled relationships at the other sites, all data were pooled and fit to a constrained two-parameter exponential and hyperbolic decay models (Fig. 1E). Both the exponential and hyperbolic decay models were statistically significant ($P < 0.001$) and explained 76.8 and 80.7%, respectively, of the observed variation in the light transmission versus periphyton load relationship. The exponential and hyperbolic decay coefficients were estimated to be $0.16 \text{ cm}^2 \text{ mg dry wt.}^{-1}$ and $4.36 \text{ mg dry wt. cm}^{-2}$, respectively. The difference in the amount of variation explained by the two models was statistically insignificant (*Z*-test, $P = 0.179$).

4. Discussion

The methodology for determination of periphyton light transmission has advanced from measurements of light attenuation through periphyton suspensions (Borum and Wium-Anderson, 1980; Sand-Jensen and Borum, 1984; Twilley et al., 1985; Neckles et al., 1993) to measurements through submerged intact periphyton assemblages (van Dijk, 1993; Vermaat and Hootsmans, 1994; Burt et al., 1995; Brush and Nixon, 2002; Stankelis et al., 2003). The range of periphyton load estimates at 50% light transmission (Table 4) likely results from the wide variety of sample preparations, instrumentation and modelling equations used to produce these estimates.

Previous investigators have used either one-sided or two-sided periphyton-leaf samples to measure light transmission (Brush and Nixon, 2002; Vermaat and Hootsmans, 1994). Periphyton morphology (e.g., two-dimensional algal films or crusts or three-dimensional filamentous algae) determines whether two-sided or one-sided sample preparations are most appropriate. Brush and Nixon (2002) calculated light transmission as the difference between the fraction of incident irradiance transmitted through one periphyton layer and leaf and the fraction of incident irradiance transmitted through just the leaf. Drake et al. (2003) and Stankelis et al. (2003) determined periphyton light transmission similarly but these most recent papers did not distinguish between one-sided periphyton samples and two-sided periphyton samples. Investigators may find that two-sided sample preparations are best for large accumulations of tightly adhering coralline algal periphyton and for delicate films of adhered sediment and diatoms. Two-sided samples are only useful for periphyton assemblages that are two-dimensional on the macroscopic scale. Three-dimensional filamentous algae are not suitable for two-sided preparations because the light sensor cannot be positioned properly without collapsing the algae into a smaller area and therefore exaggerating light attenuation characteristics. One-sided sample preparation is a necessity for these types of periphyton.

As pointed out by Vermaat and Hootsmans (1994) and the present study, the correct calculations for measuring periphyton light transmission are specific to the type of sample

Table 4

Predicted periphyton loads (mg dry wt. cm⁻²) at 50% light transmission for various periphyton compositions

Source transmission	Type of equation	r ²	Periphyton composition	Periphyton load (mg cm ⁻²) at (50)% light
Silberstein et al. (1986)	Three-parameter exponential (constrained)	0.53	Combined measurements from filamentous and coralline communities	3.47
van Dijk (1993)	Two-parameter hyperbolic (unconstrained)	0.99	Siliceous silt	1.06
Burt et al. (1995)	Two-parameter exponential (unconstrained)	0.84	Combined measurements from filamentous and coralline communities	1.36
Brush and Nixon (2002)	Three-parameter hyperbolic (constrained)	0.84	Filamentous chlorophyte	3.61
	Three-parameter hyperbolic (constrained)	0.79	<i>Cladophora</i> sp.	22.12
	Three-parameter hyperbolic (constrained)	0.91	<i>Polysiphonia</i> sp.	2.59
Stankelis et al. (2003)	Three-parameter exponential (constrained)	0.85	Diatoms	0.93
Present study ^a	Two-parameter hyperbolic (constrained)	0.81	Carbonate sediment, corallines,	4.36
	Two-parameter exponential (constrained)	0.77	<i>Polysiphonia binneyi</i>	4.27
			Median (all studies)	3.03

Light transmission equations determined using submerged intact periphyton communities. Constrained equations = 100% light transmission at zero periphyton load.

^a Pooled data, diatoms were minor but ubiquitous component of periphyton communities of present study.

preparation chosen (i.e., one-sided or two-sided). The amount of transmitted light to the leaf surface is overestimated if the periphyton layers on each side of a two-sided periphyton and leaf sample are treated as one light filter (Vermaat and Hootsmans, 1994). Transmissions and absorptances are multiplicative, not additive. Periphyton light transmission cannot be estimated by dividing the total periphyton transmission through both periphyton layers by the total two-sided leaf area (Vermaat and Hootsmans, 1994). Periphyton light transmission for two-sided periphyton and leaf samples should be measured as the square root of the fraction of incident light measured at the detector divided by the total two-sided leaf area (Eq. (3); Vermaat and Hootsmans, 1994).

Once periphyton load and corresponding light transmission data are obtained, investigators have had a choice of many types of equations to model the light transmission versus periphyton load relationship. Previous investigators have employed linear, exponential, and hyperbolic decay models. These models were both unconstrained and constrained at 100% light transmission at zero periphyton. The complexity of these models has varied with some investigators using two-parameter equations while others have used three-parameter equations (Table 4). The analyses and comparisons of 40 different combinations of these regression models using data from the periphyton communities in Florida Bay and the Florida Keys revealed that exponential and hyperbolic decay models captured more of the observed variation in the light transmission versus periphyton load relationship. It was determined that these models could be constrained at 100% light transmission at zero periphyton without a significant loss in the amount of variation explained. It was also concluded that the added model complexity of three-parameter forms of these model equations did not result in significant increases in the amount of variation explained for seven out of eight periphyton communities studied.

Appropriate periphyton light transmission decay models must accurately estimate light transmission across the entire range of periphyton loading (Brush and Nixon, 2002). The four periphyton communities of the present study were best described by both exponential and hyperbolic decay models because they were able to describe the decrease in light attenuation per periphyton density at higher periphyton loads. Linear models may be sufficient at describing periphyton light transmission at lower periphyton loads, but these fail to accurately describe light transmission at higher periphyton loads (Bulthuis and Woelkerling, 1983). At low periphyton loads, periphyton light extinction increases with each increase in periphyton density (Losee and Wetzel, 1983). As periphyton accumulates and unoccupied leaf area approaches zero, light attenuation per periphyton maximizes (Losee and Wetzel, 1983). Beyond this level of accumulation, periphyton layers increase and light attenuation per periphyton density decreases (Losee and Wetzel, 1983) and the slope of the light transmission curve approaches zero (Fig. 1). This asymptotic decrease in light transmission is consistent with the light harvesting model of photosynthetic organisms where light absorption per unit area decreases as absorption layer thickness increases (Agusti et al., 1994).

Theoretical significance is important in selecting the use of constrained or unconstrained equations. Light transmission at zero periphyton load should equal 100%; therefore, unconstrained decay models should accurately produce y-intercepts within a limited range (e.g., $100 \pm 5\%$). Less than half of the unconstrained two-parameter exponential and hyperbolic decay models for the periphyton communities at the four sites

of this study were able to accurately predict $100 \pm 5\%$ light transmission at zero epiphyte load (Table 3). This is likely the result of periphyton heterogeneity. Constraining these model equations to 100% light transmission at zero periphyton did not result in a statistically significant loss in the amount of variation explained.

Some investigators have chosen to use the more complex three-parameter forms of the exponential and hyperbolic decay models (Silberstein et al., 1986; Brush and Nixon, 2002; Stankelis et al., 2003). The results of the present study indicate that the three-parameter models described a statistically significant increase in the amount of variation explained relative to two-parameter models in only one of eight model comparisons. In almost all cases of this study, two-parameter equations were sufficient to describe the periphyton load versus light transmission relationship. Using constrained two-parameter exponential or hyperbolic models enables the periphyton load versus light transmission relationship to be constructed with knowledge of only the exponential or hyperbolic decay coefficient. Much like K_d , the vertical water-column light attenuation coefficient, the exponential or hyperbolic decay coefficients from the constrained two-parameter models have great communicative value because they can be directly compared with one another.

The results of this study indicate that constrained two-parameter forms of the exponential and hyperbolic decay models are preferred when describing the periphyton load versus light transmission relationship. As can be seen from Fig. 1 and the results of the analyses of equation types, the differences in the amount of variation described by the exponential models and that of the hyperbolic models are small and statistically insignificant. The choice of what model to use (exponential or hyperbolic) may depend upon the investigators' focus of interest. Investigators interested in comparing different periphyton compositions may prefer the hyperbolic equation because the hyperbolic decay coefficient of the constrained two-parameter form is also the periphyton load at 50% light transmission. Investigators more interested in total light transmission through the water-column and periphyton matrix may prefer the exponential model. The exponential model of periphyton light transmission is easily combined with the standard exponential model for estimating light penetration through the water-column (Kemp et al., 2000):

$$\text{Total light transmission} = (e^{-K_d z})(e^{-K_{\text{epi}} x}) \quad (4)$$

Differences in the light transmission characteristics of the periphyton communities at the four study sites were determined to be statistically insignificant. The lack of differences in the light transmission characteristics may be due to the predominance of calcium carbonate in all four periphyton communities. The periphyton communities at the four sites consisted of either coralline red algae, adhered carbonate sediment or a mixture of both. Filamentous algal periphyton was rare. The light transmission characteristics of previously investigated periphyton communities vary, but clear patterns according to community type do not presently exist (Table 4). The periphyton load at 50% light transmission exhibits variation across an order of magnitude for the filamentous periphyton (1.36–22.12 mg dry wt. cm^{-2}), while those for adhered sediment periphyton loads exhibited a narrower range (1.06–4.36 mg dry wt. cm^{-2}). Calcareous periphyton of the present study transmitted more light than the siliceous silt periphyton measured by van Dijk (1993). The

light transmission characteristics determined from the periphyton communities on *T. testudinum* of the present study are similar to other studies using submerged intact communities, though median values of periphyton loads at 50% light transmission for all other listed studies are 40% lower than that of the present study. This may reflect statistically insignificant variation, differences in light transmission calculation, or true differences in the light transmission characteristics of different periphyton types. A possible reason for the greater amounts of light transmission observed in the present study is that calcium carbonate crystals absorb less light allowing more to be reflected onto seagrass leaves (Losee and Wetzel, 1983).

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