Light attenuation by periphyton on *Vallisneria americana*

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ABSTRACT

Human activities continue to alter the world's aquatic systems, with increases in nuisance algae being a common problem arising from a suite of anthropogenic activities. In particular, increases in epiphytic algae and associated periphyton on the leaves of submersed macrophytes cause shading that is implicated in the loss of these higher-order plants, along with the refuge, foraging habitat and other ecosystem services they provide. For example, increases in periphytic loads were temporally concordant with losses of important macrophytes, such as *Vallisneria americana*, in the Chassahowitzka River, a spring-fed system in Florida. For these reasons, we explored the relationship between periphytic loads and light attenuation by measuring light transmission through different loads found on *V. americana* leaves. Periphytic loads dominated by filamentous algae ranged from 0.21 mg dry mass (DM) cm^{-2} of leaf to 16.66 mg DM cm^{-2} of leaf, resulting in light being attenuated by 12.8–95.5%. An exponential decay model with two parameters explained over 80% of the variation in data documenting light attenuation at different periphytic loads. The results suggested that relatively low loads of periphyton reduce available light to a level below reported light requirements for a variety of submersed macrophytes. The model predicted a critical threshold of approximately 6 mg DM cm^{-2} of leaf for periphytic loads that will cause detrimental ecological effects on *V. americana*, with this load reducing incident light by 85%. This threshold provides an early warning of degradation, a measure of successful restoration, and a complement to metrics currently used to document the health of Florida's spring-fed systems and other systems where *V. americana* is important.

1. Introduction

Aquatic systems throughout the world have been altered by human activities (Smith, 2003; Dodds et al., 2009). In most coastal and freshwater systems, increased growth of algae is a common change that often leads to shading and loss of rooted macrophytes, along with the refuge, foraging habitat and other ecosystem services they provide (Smith, 2003; Dodds et al., 2009). Such changes are occurring in Florida's spring-fed systems, where records reveal a fundamental shift from dominance by submersed macrophytes to a proliferation of nuisance algae, with this change engendering concern for environmental scientists, managers of natural resources, and all who value these systems (Springs Management Plan, 2016). For example, a survey of 29 Florida springs found that almost all of them harbored macroalgae, and an average of 50% of the substrate was covered by benthic algal mats with an average thickness of 0.5 m and a maximum thickness of 2 m in some springs (Stevenson et al., 2007).

The proliferation of macroalgae has been attributed to chemical, physical, and biological changes in the spring systems, including increased growth stimulated by anthropogenic loads of nutrients, decreased sloughing due to reduced water velocities, and increased survival due to declines in abundances of grazers caused by reduced concentrations of dissolved oxygen (Hauxwell et al., 2004; Hoyer et al., 2004; Heffernan et al., 2010; Liebowitz et al., 2014). As they proliferate, algae overgrow submersed macrophytes, and the resulting reduction in light availability has been considered a major cause of declines in these key primary producers and the subsequent loss of ecosystem services that they provide (Hauxwell et al., 2001; Wynn et al., 2014). Submersed macrophytes covered by macroalgal mats or burdened by heavy epiphytic loads on their leaves may not receive sufficient incident light to support photosynthesis at a rate that permits growth or even meets their metabolic needs (Phillips et al., 1978; Duarte, 1991). Thus, the relationships between light attenuation and both macroalgal mats and epiphytic algal loads on macrophytes

represent key information for successful management of spring-fed systems and other aquatic systems where macrophytes are prevalent.

These types of relationships are used in conceptual models relating loads of nutrients to subsequent eutrophication and detrimental effects on macrophytes (Fong et al., 1997; Buzzelli et al., 1998; Kemp et al., 2000). Additionally, rigorous numerical relationships can help environmental managers identify thresholds for detrimental epiphytic algal loads on macrophytes. In fact, such a threshold has been considered a useful indicator of responses to anthropogenic impacts at the ecosystem level (Wood and Lavery, 2000; U.S. EPA, 2010; Nelson, 2017). Epiphytic algae can be excellent indicators of changes in ecosystems because they respond to environmental changes more rapidly than their host macrophytes (Giovannetti et al., 2010). By providing an early warning of potentially irreversible changes in an ecosystem, appropriately evaluated increases in epiphytic algae could allow managers to take preventative actions. The value of such a capability has increased in recent decades because simply tracking concentrations of nutrients does not appear to be a robust method to assess environmental status (Bricker et al., 2003). Ideally, an indicator based on periphytic loads would complement traditional management of aquatic resources (Gobert et al., 2009; Balata et al., 2010; Giovannetti et al., 2010).

Currently, there is no standardized threshold above which epiphytic algae and associated periphyton are considered problematic, and quantitative information on the effects of periphytic loads on the performance of host plants is extremely rare (Bricker et al., 2003). Additionally, the effects of periphyton on light attenuation have not been investigated in Florida's spring-fed systems, where substantial changes in epiphytic algal loads have been documented (Stevenson et al., 2007; Springs Management Plan, 2016). Given this situation, we measured light transmission through different loads of filamentous, epiphytic algae and associated periphyton that were found on *Vallisneria americana* leaves from the Chassahowitzka River, Florida. The resulting measurements provided the basis for fitting and evaluating exponential and hyperbolic decay models that described the relationship between quantities of periphyton and light attenuation. One of the best numerical models and published light requirements were used to estimate a threshold periphytic load that would detrimentally affect the growth of *V. americana*.

2. Materials and methods

2.1. Study site

Sampling was conducted in the Chassahowitzka River where documented increases in epiphytic algal loads on macrophytes were temporally concordant with losses of important species, such as *V. americana* (Notestein, 2001; Frazer et al., 2006). The river is located along the west coast of peninsular Florida, and it flows for approximately 8 km before discharging into the Gulf of Mexico (Fig. 1). This spring-fed system originates at the Chassahowitzka Spring, which is a first magnitude spring (Yobbi and Knockenmus, 1989). The influence of groundwater keeps water temperatures throughout the river relatively uniform, with an annual average temperature of 24.0 °C. The light regime in the river is good, with depths typically < 1 m of water and light attenuation coefficients (K_d) generally being less than 1.5 m^{-1} (Frazer et al., 2006).

The shallow water, low flow rate and favorable light regime in the river support the growth of submersed aquatic vegetation (SAV), with rooted macrophytes, macroalgae, and periphyton observed throughout most of the river (Notestein, 2001). Historically, *V. americana* was a dominant macrophyte in the Chassahowitzka River (Whitford, 1956), and as recently as 2000, it represented up to 26% of the total vegetative biomass (Frazer et al., 2001). More recent sampling indicates that benthic macroalgae have come to represent approximately 43% of the total biomass of primary producers (Frazer et al., 2006).
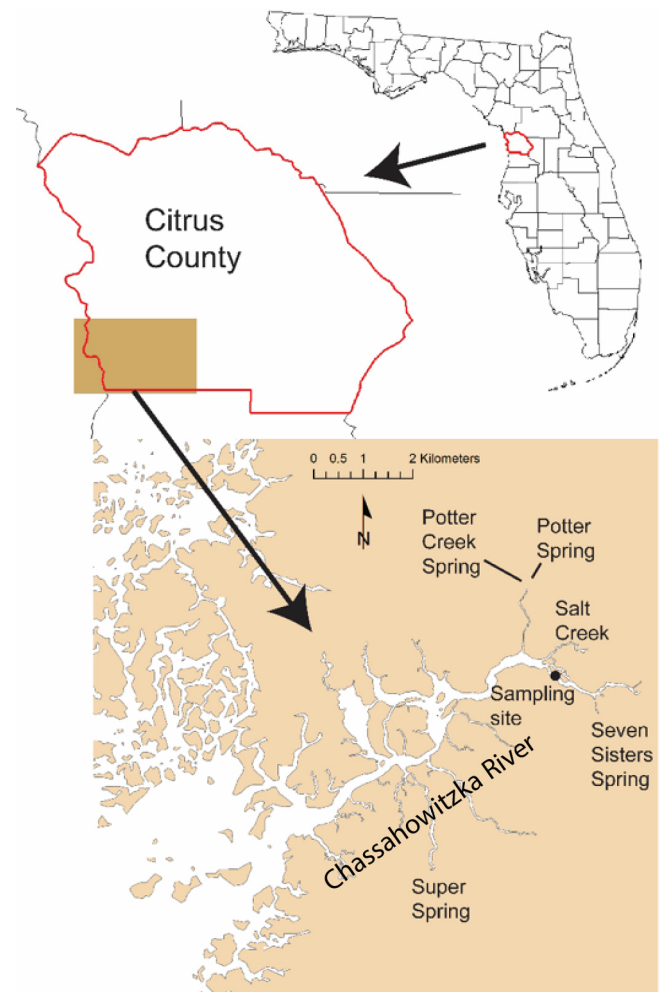

Fig. 1. Location of the sampling site in the Chassahowitzka River, Florida, USA. Red outline in the upper figure of peninsular Florida indicates Citrus County. Brown box in the middle figure indicates the location of the Chassahowitzka River. Black circle in the lower figure indicates the sampling site near Salt Creek, and Potter Creek Spring, Potter Spring, Seven Sisters Spring, and Super Spring are spring vents.

2.2. Measuring light transmission through varying periphytic loads

Between August and October 2015, leaves of *V. americana* with varying amounts of epiphytic algae and associated periphyton were collected from a large, continuous meadow growing in water ≤ 0.5 m deep in the Chassahowitzka River (Latitude 28°43′8″, Longitude 82°35′32″). Four to six leaves were placed in each of ten, wide-mouth, 1-L Nalgene™ jars filled with ambient water, and all jars were stored on ice for transport to the laboratory. In the laboratory, jars were refrigerated, and leaves were processed within 24 h.

Attenuation of natural photosynthetically active radiation (PAR) by periphyton was measured with a LI-1400 datalogger and two, 2-π underwater quantum sensors (LI-192; LI-COR, Lincoln, Nebraska, USA). All measurements were made with the leaves and sensors submersed in a tank filled with groundwater. Measurements were made outdoors, under direct sunlight on clear days when incident light was greater than 1300 μmol quanta m^{-2} s^{-1}, which minimized variation in the quantity and quality of incident light. Due to the heterogeneous distribution of the dominant epiphytic algae along the length of the leaves, each leaf was separated into 6-cm long sections that had relatively homogeneous loads.

The effect of periphyton on light penetration was measured via a two-step process (Fig. 2). Before measuring the penetration of PAR, one
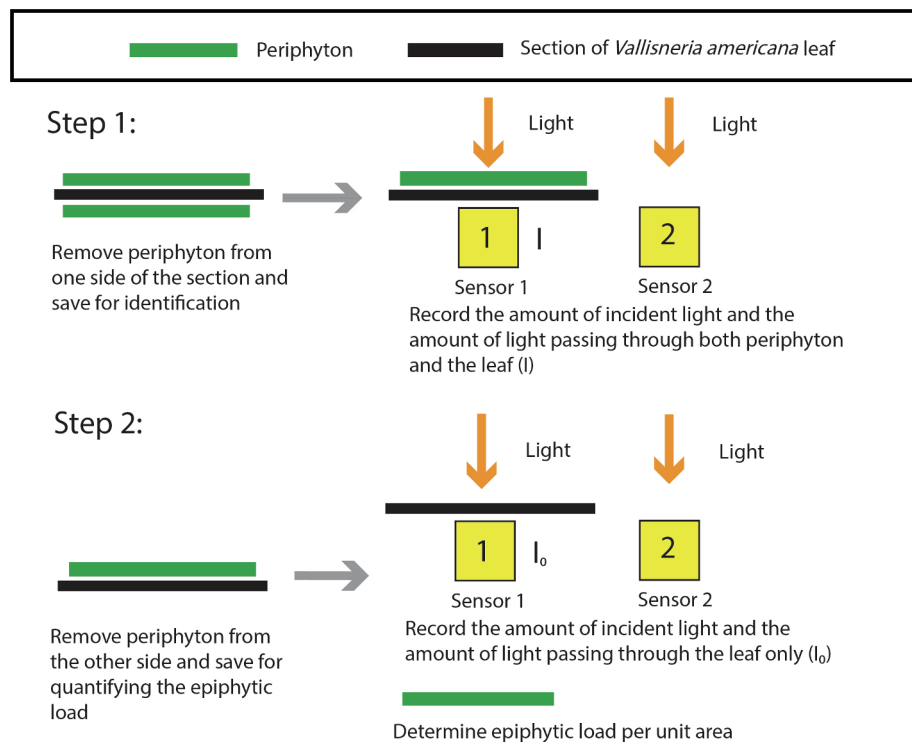
Fig. 2. Diagram illustrating the process to measure light transmission through periphyton. The two-step process also yielded samples for characterizing the dominant epiphytic algae and the total periphytic load. Green bars represent periphyton and black bars represent sections of a *Vallisneria americana* leaf.

side of each section of a leaf was scraped with a scalpel to remove periphyton that were saved for taxonomic identification of the dominant epiphytic algae. Each scraped section was placed on one of the submerged sensors, with the periphyton oriented toward the water's surface. When the uncovered sensor indicated that incident irradiance was stable, the quantities of incident light and light passing through the section of leaf and its attached periphyton were recorded. Subsequently, the remaining periphyton were removed and saved for determination of periphytic biomass per unit area (mg dry mass [DM] cm^{-2} of leaf, mg ash mass [AM] cm^{-2} of leaf, mg ash-free dry mass [AFDM] cm^{-2} of leaf, and µg chlorophyll *a* cm^{-2} of leaf; see Section 2.3). Each of the resulting clean sections was placed over the appropriate submerged sensor, and light passing through it and incident light were measured. In addition, the effects of scraping were assessed by comparing transmission of incident light before and after scraping five sections of leaves that had no visible periphyton. For each section, a change in light transmission due to periphyton (% transmission of PAR) was calculated from the amount of incident light passing through the untreated section (I, µmol quanta $m^{-2} s^{-1}$) and the amount of light passing through the treated section (I$_0$, µmol quanta $m^{-2} s^{-1}$):

$$\% \text{ transmission of PAR} = I/I_0 \times 100 \qquad (1)$$

2.3. Characterizing and quantifying periphytic loads

The periphyton initially removed from sections of leaves were examined under an anatomical lens to distinguish and select representative types of algae based on morphology and color. These representative samples were identified by examining them at magnifications of $100\times$ and $400\times$ and consulting a standard key (Wehr et al., 2015).

The periphyton scraped from the second side of each section were used to determine periphytic and epiphytic algal loads per unit area, as various measures of mass and chlorophyll *a* content. Half of the periphytic material from each sample was placed in a pre-weighed, 20-ml

aluminum tray. Samples in the trays were processed to yield DM, AM, and AFDM. Dry masses were measured after holding samples in a forced-air drying oven at 65 °C for 36–48 h. Ash masses were measured after dried samples had been heated to 450 °C in a muffle furnace for 4 h. Dry mass and AM for each sample were measured to the nearest 0.001 g on a Mettler P 163 balance. Ash-free dry mass was calculated as the difference between DM and AM. The other half of each sample was wrapped in a 47-mm diameter Whatman GF/F glass microfiber filter that was stored in a freezer at −20 °C for no more than 2 d. Chlorophyll *a* was extracted by holding each filter in 90% ethanol heated to 79 °C in a water bath, chlorophyll *a* concentrations were determined spectrophotometrically (Sartory and Grobbelaar, 1984), and concentrations were converted to mass by multiplying by the volume used in the extraction. Using these data, various measures of periphytic and epiphytic algal densities were determined by dividing the DM, AFDM, AM and mass of chlorophyll *a* by the relevant areas for sections of leaves (one side of each section only).

2.4. Modeling light transmission through varying periphytic loads

Data on light transmission and periphytic loads were combined to form the basis for a series of regression models. Regression models were constructed using combinations of exponential and hyperbolic decay equations and four quantitative measures of periphytic loads per unit area. Both two-parameter and three-parameter exponential and hyperbolic decay functions were considered. All models were evaluated in both a constrained and an unconstrained form. Constrained models were forced through 100% light transmission at a periphytic load of zero, and unconstrained models were not forced to pass through this intercept. Parameter estimates, the associated standard errors, and coefficients of determination (R^2) were based on a standard least squares approach and calculated with CurveExpert statistical software (Hyams Development) and Python matplotlib (Hunter, 2007).

All candidate models were evaluated with i) second order Akaike Information Criteria (AICc), which adjusts evaluations for small sample
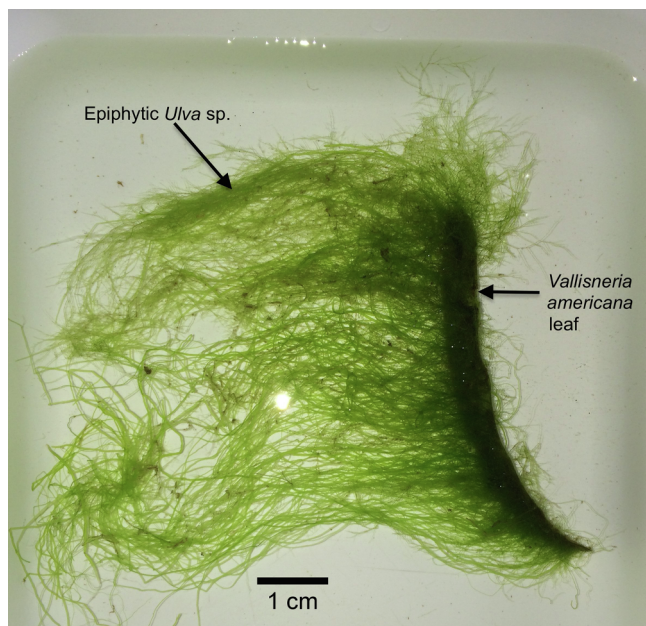
Fig. 3. Photograph of a typical periphytic assemblage on a leaf of *Vallisneria americana*. The assemblage is dominated by *Ulva* sp., and the prevailing current tended to keep the left side of the leaf uppermost.

sizes; ii) differences between each model's AICc and the lowest AICc (Δ_i); iii) Akaike weights (w_i); and iv) evidence ratios calculated as quotients of the relevant w values (Akaike, 1973; Burnham and Anderson, 2001; Anderson, 2008). The best model will have a low AICc, a low Δ_i, a high w_i, and a high evidence ratio.

3. Results

The periphyton on *V. americana* leaves was dominated by a green filamentous macroalgae, *Ulva* sp. (Fig. 3), that was mixed with a lesser amount of *Cladophora* sp. and diatoms. All epiphytes predominantly formed one layer attached directly to the surfaces of the leaves.

Measurements of light attenuated by periphytic material were obtained from 120 sections of leaves. The periphytic and epiphytic algal loads on these sections ranged from 0.21 to 16.66 mg DM cm^{-2} of leaf and 1.05 to 47.35 µg chlorophyll *a* cm^{-2} of leaf. Inorganic materials (represented by AM) comprised 21.9–77.1% of the total DM of periphytic material. Periphytic loads were heavier on older, distal sections of leaves, with minimal or no loads on new, basal sections. In addition, periphytic loads were heavier on the side of leaves that typically faced the sun due to the prevailing current, which was likely due to reduced light levels under the canopy. In fact, leaves without epiphytic algae attenuated 93.5 ± 2.6% of incident light (mean ± standard deviation, n = 60), which was highly likely to reduce the growth of algae under the canopy of leaves.

The transmission of PAR through periphyton ranged from 4.5% to 87.2%, which translated into 95.5–12.8% attenuation of incident light. Scraping did not alter light attenuation substantially, with scraped leaves transmitting approximately 0.5% less incident light. Therefore, measured values were not adjusted prior to fitting explanatory models.

A total of 32 regression models captured 31–83% of the variation in observations of light transmission through different periphytic loads (Table 1). Models based on mg DM cm^{-2} of leaf performed best, with R^2 values as high as 0.83 (Fig. 4). Models based on loads expressed as µg chlorophyll *a* cm^{-2} of leaf yielded inferior fits and a large standard error for one coefficient in the three-parameter, hyperbolic decay model (Table 1). Models based on mg AFDM cm^{-2} and mg AM cm^{-2} yielded moderately good fits. Theoretically, light transmission should be 100% when the periphytic load is zero, but values of R^2 for all models

revealed that the unconstrained models captured as much or more of the variation than the constrained models.

All models were ranked according to AICc values, Δ_i values, and w_i values (Table 2). The top five models had Δ_i values that were < 2, which indicated that a similar amount of variation in the data was explained by these unconstrained, exponential or hyperbolic decay models based on two or three parameters and DM or AFDM. A second set of five models based on DM or AFDM, three unconstrained and two constrained, explained slightly less of the variation in the data (Δ_i = 2.53–4.77). Models based on AM and chlorophyll *a* did not rank higher than fourteenth.

Dividing the Akaike weight (w_i) for the unconstrained, three-parameter exponential decay model based on DM (w_i = 2.28 × 10^{-1}) by the Akaike weight for the unconstrained, two-parameter exponential decay model based on DM (w_i = 1.56 × 10^{-1}) generated an evidence ratio of 1.46, which indicated that both models were suitable. Given this result, the two-parameter exponential decay model was selected because it yielded a periphytic light attenuation coefficient (K_p) that paralleled the coefficient used to calculate light attenuation in the water column (K_d) with the Beer-Lambert model (Kirk, 1994). Light attenuation coefficients characterize how easily light can penetrate through material, like the water column (K_d, per m) or periphyton (K_p, per unit of biomass). Given that *V. americana* leaves grew close to the surface in less than 0.5 m of exceptionally clear water, we assumed that light attenuation by the water was negligible. Thus, the amount of incident light (I_0, µmol quanta m^{-2} s^{-1}) that reached the surface of a leaf (, µmol quanta m^{-2} s^{-1}) after passing through a load of periphyton (B, in the appropriate unit of biomass) was calculated as:

$$I = I_0(e^{-K_p \times B}) \tag{2}$$

This exponential equation can be used to solve for B by setting I to 20 or 10, I_0 to 79.61 (taken from Table 1), and K_p to 0.28 (taken from Table 1), with B being equal to 5.01 mg DM cm^{-2} of leaf or 7.53 mg DM cm^{-2} of leaf, respectively. Previous studies focused on light attenuation in the water column reported that freshwater angiosperms would not survive with less than 13% of incident light reaching the water's surface (Chambers and Kaiff, 1985; Carter et al., 2000; Kemp et al., 2004), and marine macrophytes needed 11% of incident light reaching the water's surface (Duarte, 1991). Based on the results of this study, these minimum light requirements, and an assumption that water column does not attenuate light, the critical periphytic load for *V. americana* was estimated to be 6 mg DM cm^{-2} of leaf, i.e., the load that reduces transmission of PAR to 15% of the available light. This periphytic load is slightly greater than one-third of the maximum load recorded.

4. Discussion

The threshold for detrimental periphytic loads of 6 mg DM cm^{-2} reported here was higher than most previous estimates, which typically were made for seagrasses rather than freshwater macrophytes. For example, Hillman et al. (1991) considered loads > 0.97 mg DM cm^{-2} on Australian *Posidonia* spp. to be excessive. Dennison et al. (1992) stated that loads of *Ulva* sp. would cause mortality for macrophytes in Chesapeake Bay when their dry mass was equal to the dry mass of the host plant (1 g DM g^{-1} DM or 1.93 mg DM cm^{-2}). A study of *Halophila ovalis* in Australia suggested that negative impacts of epiphytic algae occurred at 4.7 mg DM cm^{-2} (Bendell, 2006). In a recent review of available literature, Nelson (2017) characterized epiphytic algal loads that would cause moderate and severe consequences for macrophytes by estimating loads that would reduce incident light by 25% and 50%, respectively, with moderate consequences arising from loads of 0.77–1.35 mg DM cm^{-2} and severe consequences arising from loads of 1.54–2.90 mg DM cm^{-2}. In contrast, Glazer (1999) determined that loads of 20 mg DM cm^{-2} caused some mortality for *Zostera marina*, and loads of 200 mg DM cm^{-2} resulted in nearly 100% mortality. In addition to physiological differences among macrophytes, the variability

Table 1
Results from fitting unconstrained and constrained models of light transmission through periphyton.

Constrained	Load	Parameter	Exponential decay		Hyperbolic decay	
			Two-parameter $y = ae^{-bx}$	Three-parameter $y = c + ae^{-bx}$	Two-parameter $y = a/(1 + x/b)$	Three-parameter $y = a(1 + cx/b)^{-1/c}$
No	DM	a	79.61 (3.35)	74.22 (4.50)	93.31 (5.84)	84.93 (5.83)
		b	0.28 (0.02)	0.36 (0.05)	1.63 (0.23)	2.61 (0.70)
		c	—	8.36 (4.50)	—	0.44 (0.31)
		R^2	0.82	0.83	0.82	0.83
	AFDM	a	73.31 (2.86)	67.53 (4.73)	83.37 (4.45)	78.41 (4.92)
		b	0.43 (0.04)	0.56 (0.12)	1.09 (0.15)	1.57 (0.46)
		c	—	8.01 (5.16)	—	0.56 (0.36)
		R^2	0.82	0.82	0.82	0.82
	AM	a	87.71 (5.42)	81.14 (6.31)	101.05 (8.84)	95.63 (9.44)
		b	0.80 (0.08)	1.19 (0.20)	0.60 (0.11)	0.82 (0.27)
		c	—	13.05 (3.99)	—	0.60 (0.35)
		R^2	0.72	0.33	0.73	0.73
	Chl *a*	a	53.76 (4.09)	63.41 (10.17)	74.09 (8.40)	835.19 (155810.42)
		b	0.03 (0.01)	0.22 (0.10)	9.14 (2.42)	0.005 (2.58)
		c	—	25.77 (2.86)	—	2.70 (0.73)
		R^2	0.31	0.54	0.42	0.51
Yes	DM	a	100.00*	84.26 (4.38)	100.00*	100.00*
		b	0.38 (0.02)	0.59 (0.04)	1.41 (0.09)	1.41 (0.19)
		c	—	15.75 (4.14)	—	1.01 (0.21)
		R^2	0.73	0.79	0.82	0.82
	AFDM	a	100.00*	79.73 (4.42)	100.00*	100.00*
		b	0.69 (0.05)	1.45 (0.03)	0.72 (0.05)	0.52 (0.08)
		c	—	20.25 (4.55)	—	1.53 (0.23)
		R^2	0.60	0.74	0.79	0.81
	AM	a	100.00*	85.97 (5.74)	100.00*	100.00*
		b	0.95 (0.01)	1.32 (0.19)	0.61 (0.09)	0.72 (0.21)
		c	—	14.08 (3.84)	—	0.69 (0.31)
		R^2	0.69	0.75	0.73	0.73
	Chl *a*	a	100.00*	73.28 (7.72)	100.00*	100.00*
		b	0.12 (0.01)	0.26 (0.07)	4.93 (0.38)	2.22 (0.71)
		c	—	26.72 (2.64)	—	2.34 (0.48)
		R^2	0.31	0.53	0.38	0.50

n = 60; y = percent light transmission; x = periphytic load as: DM = mg dry mass cm^{-2} of leaf, AFDM = mg ash-free dry mass cm^{-2} of leaf, AM = mg ash mass cm^{-2} of leaf, Chl *a* = µg chlorophyll *a* cm^{-2} of leaf; a, b, and c = coefficients for the equations; values in parentheses are standard errors for each coefficient; * = coefficient is constrained to be 100 so a standard error is not relevant; R^2 = coefficient of determination; — = coefficient not required.
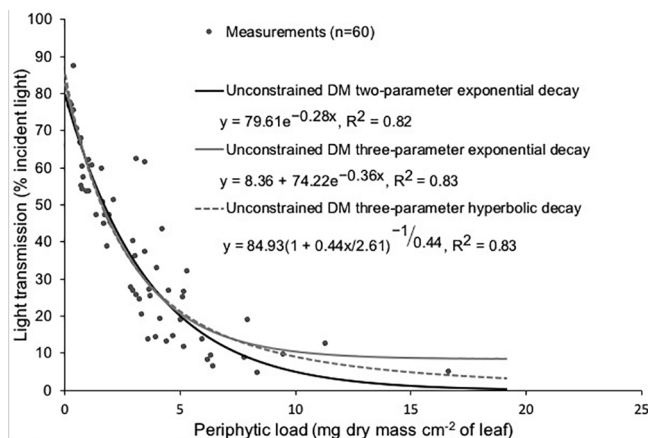
Fig. 4. The top three models fit to the data on light transmission through various periphytic loads. DM = dry mass.

in these estimates may have been due to methodological differences, morphological or physiological differences among epiphytic algae and associated periphyton, differences in the distribution of periphyton, or environmental differences among regions that affect periphytic loads.

One methodological difference among studies was the choice of the model to regress light attenuation on periphytic loads. The reliability of exponential decay models has been noted in many previous studies (Silberstein et al., 1986; Burt et al., 1995; Stankelis et al., 2003; Frankovich and Zieman, 2005), whereas, some studies found that a

hyperbolic decay model was superior (van-Dijk, 1993; Vermaat and Hootsmans, 1994; Brush and Nixon, 2002). In addition, linear regressions were used to describe light attenuation as a function of loads in a few studies with < 10 data points, which meant their range of applicability was likely limited (Bulthuis and Woelkerling, 1983; Agustí et al., 1994; Glazer, 1999). The exponential decay model selected here predicted that incident light would be attenuated by 50% after passing through a periphytic load of 1.66 mg DM cm^{-2}, which fits within predictions of 50% attenuation at 0.8–8.6 mg DM cm^{-2} calculated using similar exponential models (Twilley et al., 1985; Silberstein et al., 1986; Stankelis et al., 1999; Frankovich and Zieman, 2005). Thus, variation in the effects predicted for different periphytic loads was not solely due to the form of the model that was selected.

Other methodological differences among studies were related to the treatment of periphyton. In this study, the green filamentous macroalgae *Ulva* sp. was the dominant species on leaves of *V. americana*, hence, a method that relied on submersing leaves, periphyton and sensors was used to investigate light transmission (Brush and Nixon, 2002). When filamentous epiphytes were underwater, they extended away from the leaves of macrophytes forming a complex three-dimensional structure (Brush and Nixon, 2002). Such complex structures have been demonstrated to be a very important influence on light transmission (Vermaat and Hootsmans, 1994; Brush and Nixon, 2002; Drake et al., 2003; Stankelis et al., 2003). In addition, periphytic loads were markedly different on the two sides of the leaves from our study area; therefore, we removed periphyton from one side of each leaf before measuring light passing through the remaining layer, which eliminates the assumption that both sides of each leaf have the same

Table 2
Ranking of unconstrained and constrained models of light transmission through periphyton. Differences (Δ_i) between each AICc value and the minimum value were calculated. Akaike weights (w_i) represent the normalized relative likelihood values (likelihood value for each model divided by the sum of all likelihood values). The best model will have a low AICc, a low Δ_i, and a large w_i.

Rank	Model	AICc	Δ_i	w_i
1	Unconstrained DM three-parameter exponential decay	268.68	0.00	2.28×10^{-1}
2	Unconstrained DM two-parameter exponential decay	269.44	0.76	1.56×10^{-1}
3	Unconstrained DM three-parameter hyperbolic decay	269.78	1.10	1.32×10^{-1}
4	Unconstrained DM two-parameter hyperbolic decay	270.12	1.44	1.11×10^{-1}
5	Unconstrained AFDM two-parameter hyperbolic decay	270.22	1.54	1.06×10^{-1}
6	Unconstrained AFDM two-parameter exponential decay	271.21	2.53	6.45×10^{-2}
7	Unconstrained AFDM three-parameter hyperbolic decay	271.27	2.59	6.26×10^{-2}
8	Constrained DM two-parameter hyperbolic decay	271.30	2.62	6.17×10^{-2}
9	Unconstrained AFDM three-parameter exponential decay	271.60	2.92	5.31×10^{-2}
10	Constrained DM three-parameter hyperbolic decay	273.45	4.77	2.10×10^{-2}
11	Constrained AFDM three-parameter hyperbolic decay	277.55	8.87	2.71×10^{-3}
12	Constrained AFDM two-parameter hyperbolic decay	280.81	12.13	5.31×10^{-4}
13	Constrained DM three-parameter exponential decay	281.91	13.23	3.06×10^{-4}
14	Unconstrained Chl a three-parameter exponential decay	288.95	20.27	9.06×10^{-6}
15	Constrained Chl a three-parameter exponential decay	289.89	21.21	5.67×10^{-6}
16	Constrained AM three-parameter exponential decay	291.97	23.29	2.00×10^{-6}
17	Unconstrained Chl a three-parameter hyperbolic decay	292.31	23.63	1.69×10^{-6}
18	Constrained Chl a three-parameter hyperbolic decay	293.63	24.95	8.73×10^{-7}
19	Constrained DM two-parameter exponential decay	295.58	26.90	3.29×10^{-7}
20	Constrained AFDM two-parameter exponential decay	295.64	26.96	3.20×10^{-7}
21	Unconstrained AM two-parameter hyperbolic decay	295.84	27.16	2.89×10^{-7}
22	Constrained AM two-parameter hyperbolic decay	295.86	27.18	2.86×10^{-7}
23	Unconstrained AM three-parameter hyperbolic decay	296.56	27.88	2.02×10^{-7}
24	Constrained AM three-parameter hyperbolic decay	296.83	28.15	1.76×10^{-8}
25	Unconstrained AM two-parameter exponential decay	298.06	29.38	9.53×10^{-8}
26	Unconstrained Chl a two-parameter hyperbolic decay	300.00	31.32	3.61×10^{-8}
27	Constrained AM two-parameter exponential decay	302.49	33.81	1.04×10^{-8}
28	Constrained Chl a two-parameter hyperbolic decay	304.63	35.95	3.56×10^{-9}
29	Unconstrained Chl a two-parameter exponential decay	311.10	42.42	1.40×10^{-10}
30	Constrained AFDM two-parameter exponential decay	319.28	50.60	2.35×10^{-12}
31	Constrained Chl a two-parameter exponential decay	337.51	68.83	2.59×10^{-16}
32	Unconstrained AM three-parameter exponential decay	351.88	83.20	1.96×10^{-19}

DM = mg dry mass cm^{-2} of leaf, AFDM = mg ash-free dry mass cm^{-2} of leaf, AM = mg ash mass cm^{-2} of leaf, Chl a = µg chlorophyll a cm^{-2} of leaf.

quantity of periphyton. Some other studies used indirect methods that involved removing periphyton from leaves and measuring light transmission through a suspended slurry or through periphytic biomass captured on a filter (Sand-Jensen and Borum, 1984; Neckles et al., 1993; Cebrián et al., 1999; Dixon, 2000). These methods obviate the complex structural characteristics of the submersed periphytic assemblages, but such approaches may have been suitable for two-dimensional assemblages, such as films or crustose algae (van-Dijk, 1993; Vermaat and Hootsmans, 1994; Burt et al., 1995; Brush and Nixon, 2002; Stankelis et al., 2003). Additional studies measured light passing through the layers of periphyton on both sides of a leaf, which may have been more appropriate for epiphytic diatoms that were disrupted by scraping (Stankelis et al., 2003), crustose coralline algae that needed to be removed with acid (Drake et al., 2003), or other assemblages that formed a thin layer that was difficult to remove (Frankovich and Zieman, 2005).

Two methodological differences that were unlikely to introduce variation among studies were the use of artificial light and artificial substrates rather than natural light and leaves. Drake et al. (2003) found that artificial light sources cannot mimic the quality of natural light accurately, so photosynthesis of aquatic plants will be altered, but such an influence would not have mattered to measurements focused solely on quantities of light. In addition, a literature review suggested there was no substantial difference between studies that used natural and artificial substrates (Nelson, 2017).

Regardless of the methods used, the geometric structure and composition of the periphytic assemblages would have influenced light attenuation. For example, Cebrián et al. (1999) found that a given biomass of encrusting red algae attenuated light more strongly than a similar load of erect, brown algae, because the brown algal blades were buoyant and let additional light pass. Thus, predictions of light attenuation differed for taxa with different relationships between areal cover on leaves and biomass, with longer, floating epiphytes adding biomass without much effect on light attenuation. Furthermore, the efficiency with which a given biomass attenuated light probably differed among periphytic assemblages. For example, about 2 mg DM cm^{-2} of *Ulva* sp. attenuated 50% of incident light in this study, whereas, the same reduction required at least 4 mg DM cm^{-2} of coralline algae and associated carbonate sediment in Florida Bay and the Florida Keys (Frankovich and Zieman, 2005) and approximately 22 mg DM cm^{-2} of *Cladophora* sp. in a study done with *Zostera marina* grown in tanks (Brush and Nixon, 2002). In our study, *Ulva* sp. may have attenuated light more strongly because it is more pigmented and forms long, dense patches on the leaves of *V. americana*.

Variation in environmental conditions at different study sites can influence the amount of light attenuation generated by a given periphytic load by altering the composition of the load. In samples from the Chassahowitzka River, inorganic material (ash mass) represented up to 77% of periphytic loads. Such large amounts of abiotic material may have become trapped in the periphytic matrix when sediments were resuspended by winds or diurnal tidal exchange. In fact, unpigmented components of periphytic loads, such as mucus, frustules, calcium carbonate, and trapped detritus, have been shown to attenuate light (Lin, 1995). Despite the contribution from abiotic matter, chlorophyll a and other pigments in periphyton likely accounted for much of the light attenuation, and pigment contents of periphytic assemblages have been used to represent periphytic loads in past studies (Losee and Wetzel, 1983; Sand-Jensen, 1990; Agustí et al., 1994; Stankelis et al., 1999). In our study, the total dry mass of all material removed from leaves represented an effective and efficient metric for estimating periphytic loads because it predicted light attenuation accurately and it obviated the need to extract chlorophyll a or use a muffle furnace to burn off

organic material.

5. Conclusion

Application of a standard experimental method for estimating light attenuation by periphyton is necessary. The submersion of periphyton and measurement of attenuation through one layer appear to be optimal techniques that are effective for differing periphytic assemblages. Similarly, expressing loads as total DM and generating predictions with an exponential model may be optimal approaches. Along with light requirements drawn from the literature, the exponential model in this study predicted 6 mg DM cm^{-2} as a threshold for detrimental effects on the performance of macrophytes. An accurate threshold would represent a valuable indicator of the health of aquatic systems containing *V. americana* that complements traditional indicators based on water quality. Thus, it will be worthwhile to document the negative influences of periphyton on metrics characterizing the performance of host plants, including accumulation of biomass or growth rate.

Data availability statement

The data that support the findings of this study are available from the corresponding author, Jing Guan, upon reasonable request.

CRediT authorship contribution statement

Jing Guan: Conceptualization, Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **Charles A. Jacoby:** Formal analysis, Writing - original draft, Writing - review & editing. **Thomas K. Frazer:** Funding acquisition, Formal analysis, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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