Ecological Indicators 119 (2020) 106775

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

In-situ assessment of the effects of periphyton on the growth of *Vallisneria americana*

Jing Guan[a,b,*], Charles A. Jacoby[c], Thomas K. Frazer[b,d]

[a] South Florida Water Management District, West Palm Beach, FL, USA
[b] School of Natural Resources and Environment, University of Florida, Gainesville, FL, USA
[c] Soil and Water Sciences Department, University of Florida, Gainesville, FL, USA
[d] Fisheries and Aquatic Sciences Program, University of Florida, Gainesville, FL, USA

ARTICLE INFO

Keywords:
Macrophytes
Spring-fed system
Bioindicator

ABSTRACT

Proliferation of nuisance algae in aquatic ecosystems is an increasingly common, global problem that can be attributed to a broad suite of anthropogenic activities. One particular concern is that algae can shade rooted vascular plants, decrease their productivity, and, in extreme cases, cause mortality and a loss of the ecological services they provide. The loss of macrophytes in Florida's iconic spring-fed systems is especially alarming. In the Chassahowitzka River, for example, increases in epiphytic algae correlated with reductions in the abundance of important macrophytes such as *Vallisneria americana*. However, the relationships between loads of epiphytic algae and associated periphyton and the growth of vascular plants have not been characterized adequately. Here, we quantified the effect of epiphytic algae and associated periphyton using direct, *in-situ* measurements of growth rates for *V. americana*. Periphytic loads ranged from 11 to 15,211 mg DM per shoot (0.08 to 32.37 mg DM cm^{-2} of leaf), and weekly growth rates ranged from 0 to 197 mg DM or 0 to 87 cm^2 per shoot (0 to 94 mg DM or 0 to 39 cm^2 per leaf). Data indicated that changes in the growth rates of *V. americana* were the consequences of combined stresses from environmental factors (e.g., light at the water's surface and periphytic loads) and physiological factors (e.g., leaf age). Life spans were shorter and turnover rates were faster only when shoots were exposed to higher periphytic loads throughout their lives. Boundary analyses of data for growth of leaves identified a threshold of 4–5 mg DM cm^{-2} of leaf in full sunlight, with heavier periphytic loads considered detrimental, and that threshold indicated that 20%–26% of incident, full sunlight was the minimum light requirement for *V. americana* in the Chassahowitzka River. A threshold for periphytic loads represents a valuable indicator that complements data on water quality, and the method to calculate a threshold provides a valuable tool that can improve management of all aquatic systems where the effects of periphyton on macrophytes are of concern.

1. Introduction

Submersed macrophytes are critical components of many aquatic systems, because they stabilize sediments and reduce turbidity, assimilate and store nutrients, sequester carbon, and provide refuge and foraging habitat for higher-level organisms (Gregg and Rose, 1985; van Donk and van de Bund, 2002; O'Hare et al., 2017; Ruiz-Frau et al., 2017). However, reductions in the abundance of submersed macrophytes have been reported globally over the past decades, with human activities in the affected watersheds commonly viewed as causes. Increased loads of macronutrients (nitrogen and phosphorus) represent key changes, but other important stressors include reductions in water velocities due to water withdrawals and declines in abundances of grazers (Seddon et al., 2000; Smith, 2003; Dodds et al., 2009; Waycott et al., 2009; Liebowitz, et al., 2014; Reaver et al., 2019).

In combination, these stressors can lead to eutrophication of aquatic systems that manifests as phytoplankton blooms or increased loads of epiphytic algae that have detrimental effects on rooted macrophytes (Silberstein et al., 1986; Lauridsen et al., 1994; Duarte, 1995; Dodds et al., 2009). In particular, epiphytic algae and associated periphyton inhibit performance of macrophytes by shading leaves (Taylor and Lewis, 1970; Bulthuis and Woelkerling, 1983; Kurtz et al., 2003; Guan et al., 2020), increasing the boundary layer around leaves leading to slower exchange of carbon dioxide and oxygen (Sand-Jensen, 1977; Sand-Jensen et al., 1985), competing for nutrients (Silberstein et al., 1986; Borowitzka et al., 2007), and causing physical damage (Howard-

* Corresponding author at: South Florida Water Management District, 3301 Gun Club Rd, West Palm Beach, FL 33406, USA.
E-mail addresses: jguan@sfwmd.gov (J. Guan), cajacoby@ufl.edu (C.A. Jacoby), frazer@ufl.edu (T.K. Frazer).

https://doi.org/10.1016/j.ecolind.2020.106775
Received 16 June 2019; Received in revised form 3 June 2020; Accepted 27 July 2020

Table 1
Trends in key water quality parameters in the Chassahowitzka River from 2000 to 2016.

Parameter	Anderson-Darling test	Levene's test	Regression equation	df	Significance	R^2
Total nitrogen (TN, μg L^{-1})	$p > 0.05$	$p > 0.01$	TN = 12.35 × year + 360.61	1, 15	$p < 0.001$	0.71
Total phosphorus (TP, μg L^{-1})	$p > 0.05$	$p > 0.05$	TP = 0.05 × year + 20.65	1, 15	$p > 0.05$	0.02
Color (Col, Pt-Co units)	$p > 0.05$	$p > 0.05$	Col = 0.26 × year + 12.24	1, 15	$p > 0.05$	0.10
Chlorophyll-*a* (Chl, μg L^{-1})	$p > 0.05$	$p > 0.05$	Chl = 0.15 × year + 7.02	1, 15	$p > 0.05$	0.14
Light attenuation coefficient (K_d, m^{-1})	$p > 0.05$	$p > 0.05$	K_d = -0.005 × year + 1.508	1, 15	$p > 0.05$	0.02

Williams et al., 1978; Kiørbe, 1980; Fong et al., 2000). Among these effects, heavy periphytic loads that shade the leaves of submersed macrophytes have been shown to be a major cause of reduced photosynthetic rates (Orth and Van Montfrans, 1984; Neckles et al., 1993; Drake et al., 2003; Guan et al., 2020). Without sufficient photosynthetic production, growth of submersed macrophytes declines, and, ultimately, these key primary producers die, which leads to loss of the ecosystem services they provide (Phillips et al., 1978; Duarte, 1991; Wynn et al., 2014). Thus, an understanding of the influence of epiphytic algae and associated periphyton on growth of submerged macrophytes represents a valuable guide for successful management of aquatic systems.

Although interactions between periphyton and macrophytes have been studied for decades, most work has focused on estuarine or nearshore marine environments. A few studies related to freshwater systems have been conducted in lakes (Phillips et al., 1978; Sand-Jensen and Søndergaard, 1981; Cattaneo et al., 1998) or in the laboratory (Asaeda et al., 2004). In particular, there is little quantitative information on the effects of periphytic loads on the performance of host plants in lotic systems. For example, the effects of periphyton on the growth of macrophytes has not been investigated in Florida's spring-fed systems, even though substantial ecological changes in periphytic loads and macrophytes have been documented (Stevenson et al., 2007; Spring Management Plan, 2016). The Chassahowitzka River represents a good example of this situation, with documentation of a fundamental shift from dominance by submersed macrophytes, especially *Vallisneria americana*, to a proliferation of epiphytic algae and associate periphyton (Frazer, 2000; Frazer et al., 2001; Frazer et al., 2006).

Given this situation, we quantified the effect of periphytic loads on the growth of *V. americana* through *in-situ* measurements in the Chassahowitzka River, Florida. The resulting measurements provided a basis for identifying a threshold for problematic loads of periphyton, and such a threshold represents an indicator of the health of the system and a useful guide for management actions, e.g., establishing safe loads of nutrients or safe levels of flow that can remove excess periphyton.

2. Materials and methods

2.1. Study sites

The study was conducted in the Chassahowitzka River, a spring-fed system along the west coast of peninsular Florida. The river originates at the Chassahowitzka Spring, a first magnitude spring, and it flows into the Gulf of Mexico (Yobbi and Knockenmus, 1989). The river is influenced by approximately 1 m tides, and a supply of groundwater maintains relatively uniform water temperatures that average about 24.0 °C across 12 months throughout the river (Frazer et al., 2006).

Conditions in the river are suitable for the growth of submersed aquatic vegetation (SAV), with macrophytes, macroalgae, and epiphytic algae observed throughout much of its length (Hoyer et al., 2004). Based on visual estimates, *Vallisneria americana* was a dominant macrophyte, comprising up to 26% of the total vegetative biomass (Frazer et al., 2001). However, more recent sampling documents losses of *V. americana* and increases in loads of epiphytic algae (Frazer et al., 2006). Compared to surveys from 1998 to 2005 (Frazer, 2000; Frazer et al., 2001, 2006), surveys in 2016 (J. Guan unpubl) recorded aboveground areal biomass of *V. americana* that was close to the historical low or 10% of the historic average (mean ± standard deviation = 1,295.53 ± 189.68 g DM m^{-2}); mean epiphytic loads on leaves of *V. americana* that were approximately twice the historic average of 2.45 ± 1.48 μg chlorophyll-*a* g^{-1} DM host plant; and maximum loads that were about four times higher than the historic maximum of 6.53 μg chlorophyll-*a* g^{-1} DM host plant.

The increase in epiphytic loads correlates with increasing concentrations of total nitrogen but not with changes in other key parameters. From 2000 to 2016, the average annual concentrations of total nitrogen increased approximately 12-fold per year (Table 1). In contrast, there were no statistically significant changes in the average annual concentrations of total phosphorus, color, concentrations of chlorophyll-*a*, or light attenuation coefficients (Table 1). Thus, the Chassahowitzka River receives increased loads of nitrogen that are not accompanied by substantial decreases in light attenuation in the water column due to increasing color or concentrations of chlorophyll-*a*. These conditions make the river a suitable place to examine the influence of epiphytic algae and associated periphyton on the growth of *V. americana* as an indicator of eutrophication resulting from increased loads of a key macronutrient.

During the months of June, July, and August in 2016, investigations to elucidate the effects of periphyton on growth of *V. americana* were conducted at two sites in the Chassahowitzka River: Salt Creek (28°43′18″ N, 82°35′30″ W) and Small Creek (28°43′13″ N, 82°35′29″ W). The two sites were characterized by similar environmental conditions, including mean summer water temperatures of 25.9 °C; mean salinities of 3.55; coefficients of light attenuation in the water column (K_d) of 3.27 m^{-1}; concentrations of dissolved oxygen at 8.84 mg L^{-1}; mean pH values of 7.89; and meadows of *V. americana*, with varying periphytic loads on their leaves and little drift macroalgae. In contrast, there were substantial differences in water depths and surface irradiances between the two sites. The mean water depth in Small Creek (0.41 m) was approximately half that in Salt Creek (0.74 m). In addition, shade from riparian vegetation in Small Creek lowers its mean surface irradiance to 26% of Salt Creek, which receives full sunlight or 1,560 to 1,904 μE m^{-2} s^{-1}.

2.2. Characterizing the two meadows of Vallisneria americana

Due to the significant differences in incident light at the water's surface in Salt Creek and Small Creek and consequent effects on the morphology of *V. americana*, samples were collected and processed separately for the two meadows. A snorkeler counted the number of shoots in five, haphazardly tossed, quadrats (25 cm × 25 cm) before collecting six shoots for estimates of biomass of *V. americana* and periphyton. Samples were stored in labeled plastic bags on ice during transport to the laboratory where they were frozen until processing.

In the laboratory, the number of leaves per shoot were counted, and the heights and maximum widths of leaves were measured using a transparent sheet of plastic marked with a 1-cm grid. Leaves were gently scraped to deposit periphyton into individual plastic weighing boats that had been pre-weighed and labeled. The clean leaves and the periphyton were dried at 60 °C to a constant mass before being weighed to the nearest 0.001 with an electronic balance. Areal biomasses (g DM

m^{-2}) for *V. americana* and its associated periphyton were calculated for each site by multiplying the mean dry weight of *V. americana* shoots or the mean dry weight of periphyton on shoots by the mean density of shoots. Analyses of areal biomass, periphytic loads, and morphological characteristics of *V. americana* (including dry mass per shoot, number of leaves per shoot, leaf area, and new leaves recruited) were performed with Levene's test that assesses the equality of variances and Student's *t*-test (assuming equal variances or unequal variances as appropriate).

2.3. Measuring growth rates for Vallisneria americana and quantifying periphytic loads

During the June to August 2016 growing season for *V. americana* (Hauxwell et al., 2007), the negative influences of periphyton on growth rates were investigated in Salt Creek and Small Creek. Growth of *V. americana* was measured via a modified leaf-marking technique that has been applied for SAV with wide leaves and a basal meristem (Odum, 1957; Zieman, 1968; Zieman and Wetzel, 1980; Hauxwell et al., 2007). During each sampling event, a quadrat (25 cm × 25 cm) was tossed haphazardly five times within each meadow, and six shoots of *V. americana* in each quadrat were identified by placing a pink flag on a stake immediately adjacent to each shoot. All leaves comprising the selected shoots were marked by carefully punching two holes with a syringe needle approximately 3 cm above the rhizome (Hauxwell et al., 2007). Each of the aforementioned shoots was retrieved one week after punching, at which time new shoots were identified and punched. This procedure provided 7 consecutive sets of measurements. At the appropriate times, shoots and their associated belowground tissue were collected by hand, rinsed in ambient water, and placed in each of 30 pre-labeled plastic bags. All samples were stored on ice for transport to the laboratory and stored frozen until they were processed.

Processing involved multiple steps that yielded information on growth of leaves, and the amount and composition of periphytic material. Leaves comprising each shoot were separated and ranked by age from senescent to new. For a given shoot, senescent leaves were located at the outside of the bundle, and they bore holes that were at the same height as when they were initially punched because these leaves did not grow. These holes served as reference points for measuring the growth of younger leaves. New growth for younger leaves was identified as the material between their holes and the reference point. All leaves without holes were considered new growth. Sections of new growth were separated from old leaf tissues with a scalpel for subsequent measures of dry mass, and the area of each section was estimated with a reference grid. The rate of leaf growth was calculated by dividing the dry mass or area of the new growth by the number of days of growth (one week). The absolute leaf age in days was estimated from the rank of each leaf by multiplying the plastochrone index by the plastochrone interval (Erickson and Michelini, 1957; Lamoreaux et al., 1978). Life span for each shoot was estimated as the product of total number of leaves per shoot and the plastochrone interval, and turnover rate for each shoot was calculated as the inverse of life span expressed as a percentage change per day (Bulthuis and Woelkerling, 1983). The periphyton on each leaf was gently removed with a scalpel and saved for determination of load per unit area. Representative samples of periphyton were examined with the aid of a microscope to identify the dominant epiphytic algae using 100 × and 400 × magnifications and a standard key (Wehr et al., 2015).

To yield dry mass (DM) as a standard measure of periphytic loads, new growth, and biomass of old leaf tissues, the appropriate materials were placed in preweighed weighing boats. The weighing boats were held in a forced-air drying oven at 60 °C for 36–48 h before dry masses were measured to the nearest 0.001 g on a Mettler P 163 balance. Using these data, weekly growth rate was determined for *V. americana* as an increase in either area (cm^2) or dry mass (mg DM), and periphytic densities were determined by dividing the dry mass of periphyton by the surface area of one side of the relevant section of a leaf (mg DM cm^{-2}). Periphytic loads for leaves were summed to yield periphytic loads per shoot.

In addition, the heterogeneous distribution of periphytic loads along the leaves of *V. americana* was assessed from another set of 30 shoots taken from Salt Creek. From base to tip, each leaf was separated into consecutive, 5-cm sections that had relatively homogeneous periphytic loads. The distance to the base of the leaf, area, and periphytic loads were measured and recorded for each section.

As one approach to estimating a threshold for detrimental loads of periphyton, a logarithmic curve was fit to growth rates recorded for leaves with different periphytic loads. The x-intercept, where no growth was recorded, represented one estimate of the threshold for detrimental effects.

Boundary analyses also were employed to identify periphytic loads that impeded the growth of *V. americana* (Ludwig and Tongway, 1995). The analyses involved (1) ranking growth rates from those associated with the highest periphytic loads to those associated with the lowest loads, (2) bracketing contiguous sets of growth rates in a window of preassigned width (w = 4, 6 or 8), (3) splitting each window into two equal groups ($\frac{1}{2}w$ = 2, 3 or 4), (4) averaging the growth rates in the two groups (H_1 and H_2), (5) computing a dissimilarity index as squared Euclidean distance (SED) = $[(Mean\ H_1) - (Mean\ H_2)]^2$, (6) moving the window one position further along the ordered series of growth rates, (7) computing another dissimilarity index, (8) repeating this process for the entire dataset, and (9) examining the dissimilarity indices versus the appropriate loads of periphyton. A threshold for the effect of periphytic loads in each creek was identified as the load associated with the first large increase in a dissimilarity index. To confirm the thresholds, growth rates of all leaves equal to and below the thresholds were compared to growth rates of leaves with periphytic loads above the thresholds with an appropriate Student's *t*-test selected using the results of Levene's tests for homoscedasticity.

Life spans and turnover rates for shoots were evaluated to determine if they varied with periphytic loads. Differences in life spans and turnover rates between the two creeks were evaluated with Levene's test to assess the equality of variances and the appropriate Student's *t*-test.

3. Results

Analyses examining the influence of light regimes on the two meadows of seagrass identified significant differences in some morphological characteristics of *V. americana*. Dry mass per shoot was significantly higher in Salt Creek than in Small Creek (*t*-test assuming unequal variances, df = 109, $p < 0.05$), with a mean dry mass per shoot of 0.8 g DM in Salt Creek and 0.5 g DM in Small Creek. In contrast, the number of leaves per shoot was significantly higher in Small Creek compared to Salt Creek, with means of 11 leaves per shoot and 8 leaves per shoot, respectively (*t*-test assuming equal variances, df = 131, $p < 0.05$). Finally, mean leaf areas per shoot differed by < 2 cm^2, and they were not significantly different (256.5 cm^2 for Small Creek and 258.4 cm^2 for Salt Creek; *t*-test assuming equal variances, df = 131, $p > 0.10$).

Measurements of the growth rates of *V. americana* with varying periphytic loads were obtained from 134 shoots and 1,236 leaves from the two sites. The weekly growth rates of *V. americana* ranged from 0 to 205.0 mg DM per shoot and 0 to 87.0 cm^2 per shoot. The mean growth rates of *V. americana* in the shaded Small Creek were higher than rates in Salt Creek, with more new leaves recruiting per shoot per week and the addition of more dry mass and surface area per shoot per week. Shoots in Small Creek gained an average of 2 leaves per week, while shoots in Salt Creek averaged 1 new leaf per week (*t*-test assuming unequal variances, df = 97, $p < 0.05$). The mean dry mass of weekly new growth per shoot was 98.5 mg DM in Small Creek and 61.4 mg DM in Salt Creek (*t*-test assuming equal variances, df = 83, $p < 0.05$), and the mean surface area added per week was 34.9 cm^{-2} per week in

Small Creek and 29.3 cm^{-2} per week in Salt Creek (*t*-test assuming equal variances, df = 129, $p < 0.05$).

Furthermore, areal productivity of *V. americana* and periphytic loads were significantly different between the two creeks. The mean areal biomass (aboveground) of *V. americana* in Salt Creek was 143 g DM m^{-2} as estimated by multiplying mean dry mass per shoot by mean shoot density (177 shoots m^{-2}); whereas the mean areal biomass in Small Creek was 37 g DM m^{-2}, with a mean shoot density of 70 shoots m^{-2}. At both sites, the periphyton on *V. americana* leaves was dominated by *Ulva* sp., a green filamentous macroalgae. The mean periphytic loads of 2.88 g DM per shoot in Salt Creek were significantly higher than the mean loads of 0.17 g DM per shoot in Small Creek (*t*-test assuming unequal variances, df = 76, $p < 0.05$). Thus, Salt Creek, with higher incident light held more biomass of both *V. americana* and periphyton.

Distributions of leaf ages were different between Small Creek and Salt Creek, which likely arose due to the difference in growth rates of *V. americana* at the two sites. Estimated leaf ages ranged from 3 to 81 days, with new leaves produced about every 5 days. Leaves were grouped into three age classes: new leaves (< 10 days old), maturing leaves (11 to 30 days old), and senescing leaves (> 30 days old). Of the total number of leaves sampled in Small and Salt creeks respectively, new leaves represented 22% and 17%, maturing leaves represented 37% and 27%, and senescing leaves represented 41% and 56%.

The growth rates of leaves were affected by leaf age. Growth rates increased with leaf age until they reached a maximum value for leaves that were 15 days old. The maximum growth rates were 84.3 mg DM per leaf per week and 38.6 cm^{-2} per leaf per week in Small Creek and 94.4 mg DM per leaf per week and 30.2 cm^{-2} per leaf per week in Salt Creek. Subsequently, growth rates decreased to essentially zero in the few leaves that were older than 40 days.

Periphytic loads on the sampled shoots varied greatly, ranging from 11 to 15,211 mg DM per shoot (or 0.08 to 32.37 mg DM cm^{-2} of leaf). The mean periphytic load ± one standard deviation (n = 134) was 1,735 ± 2,556 mg DM per shoot or 6.66 ± 7.26 mg DM cm^{-2} of leaf. Although total periphytic loads per shoot varied widely, the mean periphytic loads on different sections of leaves increased linearly along most of the length of the leaves of *V. americana* (Table 2). That is, periphytic loads were heavier on the older, distal sections of leaves, with no or minimal periphyton visible on newer, basal sections. In addition, periphytic loads increased with leaf age (Table 3). Although the distribution of periphytic loads on leaves of different ages was variable, the mean reached a maximum of 6.86 mg DM cm^{-2} on leaves that were 39 days old. There was no or minimal periphyton visible on leaves younger than 3 days. Up to 39 days, the rate that periphyton accumulated apparently exceeded the rate of losses from grazing or sloughing. Eventually, declines of periphytic loads were likely due to loss of heavily loaded leaf tissue because leaves became shorter (mean length ± SE for leaves 10–50 d old = 28.2 ± 0.5 cm and older than 50 d = 26.2 ± 0.7 cm, *t*-test assuming unequal variances, df = 522, $p < 0.05$), and an overload of periphyton can reduce the flexibility of leaves and lead to mortality or sloughing of sections of leaves (Howard-Williams et al., 1978; Fong et al., 2000).

Of the 1,236 leaves that were processed to determine growth rates, 8 were excluded due to damage, 684 were senescent and displayed no growth, and 407 had no epiphytes. Separate logarithmic curves fit to the remaining data for Salt and Small creeks explained 43% and 24% of the variation in the relationship between additions of surface area (cm^{-2} per leaf per week) and epiphytic loads (mg DM cm^{-2}), respectively (Fig. 1). The x-intercepts or thresholds for no growth were 6.7 mg DM cm^{-2} (95% confidence limits of 3.5 mg DM cm^{-2} and 21.7 mg DM cm^{-2}) and 0.8 mg DM cm^{-2} (95% confidence limits of 0.2 mg DM cm^{-2} and 1.4 mg DM cm^{-2}) for Salt and Small creeks, respectively. Based on previous measures of light attenuation by periphyton on *V. americana* in the Chassahowitzka River (Guan, et al., 2020), periphytic loads of 6.7 mg DM cm^{-2} of leaf in Salt Creek would attenuate ~ 88% of incident light, and in Small Creek, with an average surface irradiance of 26% of full sunlight, periphytic loads of 0.8 mg DM cm^{-2} would equate to ~ 84% attenuation of full sunlight.

Boundary analyses also identified thresholds for periphytic loads that inhibited but did not preclude the growth of *V. americana* leaves, with lack of periphyton on most new leaves or lack of growth for most senescing leaves restricting these analyses to maturing leaves. For Small Creek, boundary analyses using windows of four, six or eight datapoints indicated thresholds of ~ 0.4 mg DM cm^{-2}, and for Salt Creek, boundary analyses using the three different windows indicated thresholds of 5.1–5.3 mg DM cm^{-2}. Levene's tests and appropriate Student's t-tests confirmed consistent differences in the growth rates of *V. americana* when periphytic loads were less than or greater than or equal to the thresholds ($p < 0.05$ in all cases; Fig. 2). Overall, plants appeared to be more vulnerable to periphytic loads in low light, which was found in Small Creek. Based on previous measures of light attenuation by periphyton on *V. americana* in the Chassahowitzka River (Guan, et al., 2020), periphytic loads of 4.0–5.3 mg DM cm^{-2} of leaf in Salt Creek would attenuate 74%–82% incident light, and in Small Creek, with an average surface irradiance of 26% of full sunlight, periphytic loads of 0.4–0.8 mg DM cm cm^{-2} would equate to 82%–83% light attenuation. Thus, growth of *V. americana* was measurably affected when incident light was attenuated by 74%–83%, which is equivalent to less than 17%–26% of incident light reaching the surfaces of leaves.

Life spans and turnover rates for shoots varied between the two creeks, but these metrics did not vary consistently with periphytic loads (Fig. 3). Periphytic loads per shoot ranged from 11 mg to 15,211 mg, life spans ranged from 30 to 84 d, and turnover rates per shoot ranged from 1.2% d^{-1} to 3.3% d^{-1}. Life spans were 1.4 × longer in Small Creek, and as expected, turnover rates were 1.4 × faster in Salt Creek (Fig. 3). It appears that leaves were replaced more rapidly when shoots were exposed to higher periphytic loads for periods greater than their life spans, but responses to differing periphytic loads over shorter intervals were not consistent.

4. Discussion

This study identified a threshold for periphyton on *V. americana* leaves of 4–7 mg DM cm^{-2} derived from measuring realized detrimental effects of periphytic loads at a site receiving full sunlight. This result is in line with an estimate of 6 mg DM cm^{-2} of leaf for the Chassahowitzka River derived from measurements of light transmitted through periphyton and previous reports of minimum light

Table 2
Distribution of periphytic loads along the leaves of *Vallisneria americana*.

Value	Distance from leaf base (cm)															
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75
Mean periphytic load (mg DM cm^{-2})	0.00	0.00	0.05	0.17	0.57	0.69	2.86	2.57	3.38	3.36	4.25	5.84	6.05	8.95	8.61	8.23
Standard deviation	0.00	0.00	0.16	0.41	1.11	1.19	1.62	1.51	2.63	3.24	3.28	4.64	3.52	3.46	4.00	2.90
Equation and R^2 of trendline	$y = 0.14x - 1.83$, $R^2 = 0.94$															

DM = dry mass, y = periphytic load (mg DM cm^{-2}), x = distance from the leaf base (cm)

Table 3
Distribution of periphytic loads on leaves of *Vallisneria americana* at different ages.

Value	Leaf age (days)														
	0	3	9	15	21	27	33	39	45	51	57	63	69	75	81
Mean of periphytic loads (mg DM cm^{-2})	0.00	0.00	0.14	0.94	3.64	5.39	6.81	6.86	6.36	5.54	3.88	3.36	2.05	2.97	1.79
Standard deviation	0.00	0.00	0.78	3.18	7.33	8.07	8.79	10.39	10.26	8.94	6.34	4.27	2.85	4.32	0.59

DM = dry mass

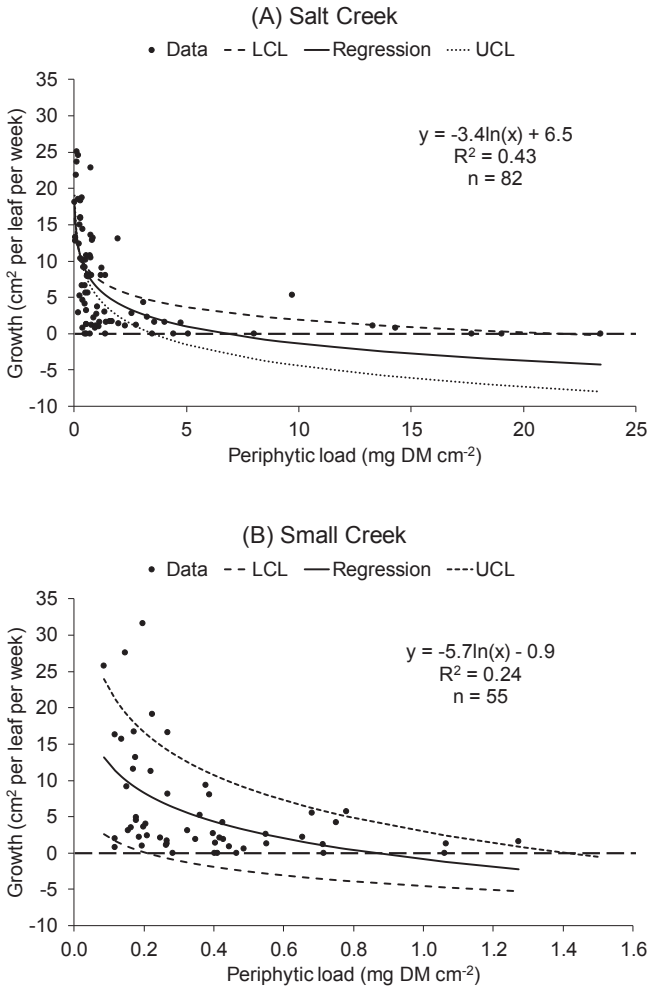

Fig. 1. Logarithmic curves fit to data on growth versus periphytic load for *Vallisneria americana* from (A) Salt Creek and (B) Small Creek. Data = measured values; LCL and UCL = 95% lower and upper confidence limits, respectively; Regression = curves described by the equations; dashed line = x-axis or zero growth; DM = dry mass.

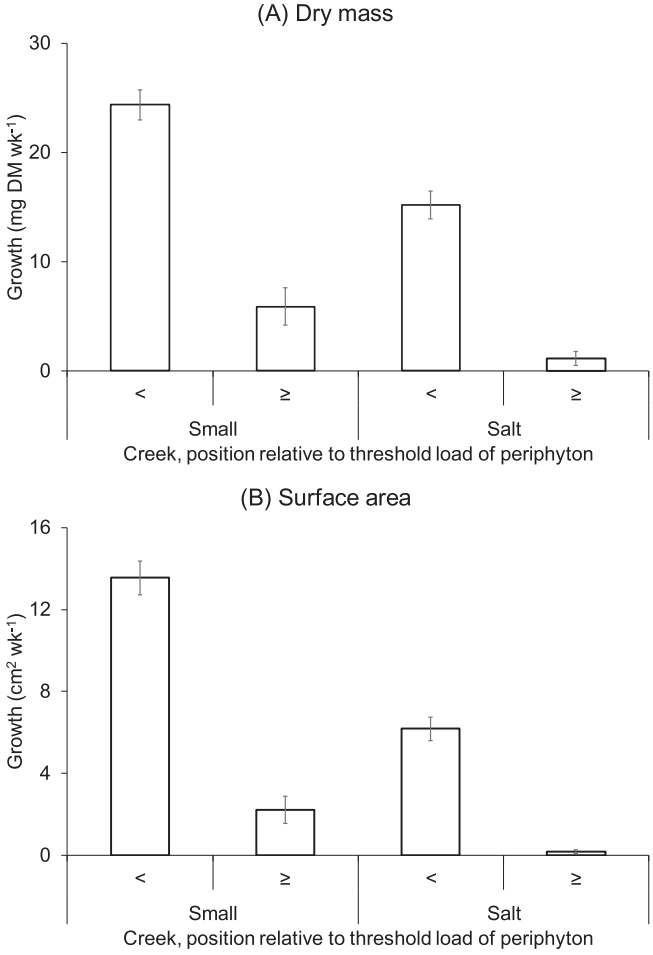

Fig. 2. Growth of *Vallisneria americana* leaves with periphytic loads less than or greater than or equal to a threshold expressed as change in (A) dry mass and (B) surface area. In each creek, growth rates were significantly higher (Student's *t*-test; $p < 0.05$) for leaves with periphytic loads below the threshold. DM = dry mass; < = periphytic load was below the threshold; ≥ = periphytic load was equal to or above the threshold.

requirements for submersed focused on light attenuation in the water column (Guan, et al., 2020). Unlike this estimate of potential effects, the direct, *in-situ* measurements of growth accounted for additional environmental factors other than periphyton, for example, light attenuation in the water column. In fact, light attenuation by water in the Chassahowitzka River is affected significantly by tide and freshwater runoff from the adjacent watershed, with K_d values ranging from 0.43 m^{-1} to 3.27 m^{-1} during our study. Moreover, the direct measurements accounted for any mechanical and biological effects of periphyton on tissues of *V. americana* beyond the effects of light attenuation (Sand-Jensen, 1977; Howard-Williams et al., 1978; Kiørbe, 1980; Sand-Jensen et al., 1985; Silberstein et al., 1986; Fong et al., 2000; Borowitzka et al., 2007). Finally, the higher thresholds derived from curves fit to relevant data represent estimates of periphytic loads that will preclude growth, whereas the thresholds derived from boundary analyses represent estimates of periphytic loads that reduce growth rates substantially and indicate the presence of stress.

The threshold for detrimental periphytic loads of 4–7 mg DM cm^{-2} was higher than most previous estimates, which primarily focused on seagrass in brackish or marine environments. In a review of available literature, Nelson (2017) proposed four categories for periphytic loads that would cause increasingly severe consequences, with the categories based on loads that would cause < 25%, 25%–50%, 51%–75%, and > 75% reductions in responses of macrophytes. Converted upper boundaries for the first three classes were 0.77 mg DM cm^{-2}, 1.35 mg DM cm^{-2}, and 2.90 mg DM cm^{-2}. Hillman et al. (1991) suggested that periphytic loads > 0.97 mg DM cm^{-2} were excessive for Australian *Posidonia* spp., and Dennison et al. (1992) stated that when the dry mass of periphyton was equal to the dry mass of the host plant (1 g DM g^{-1} DM or 1.93 mg DM cm^{-2}) periphytic loads of *Ulva* sp. would cause

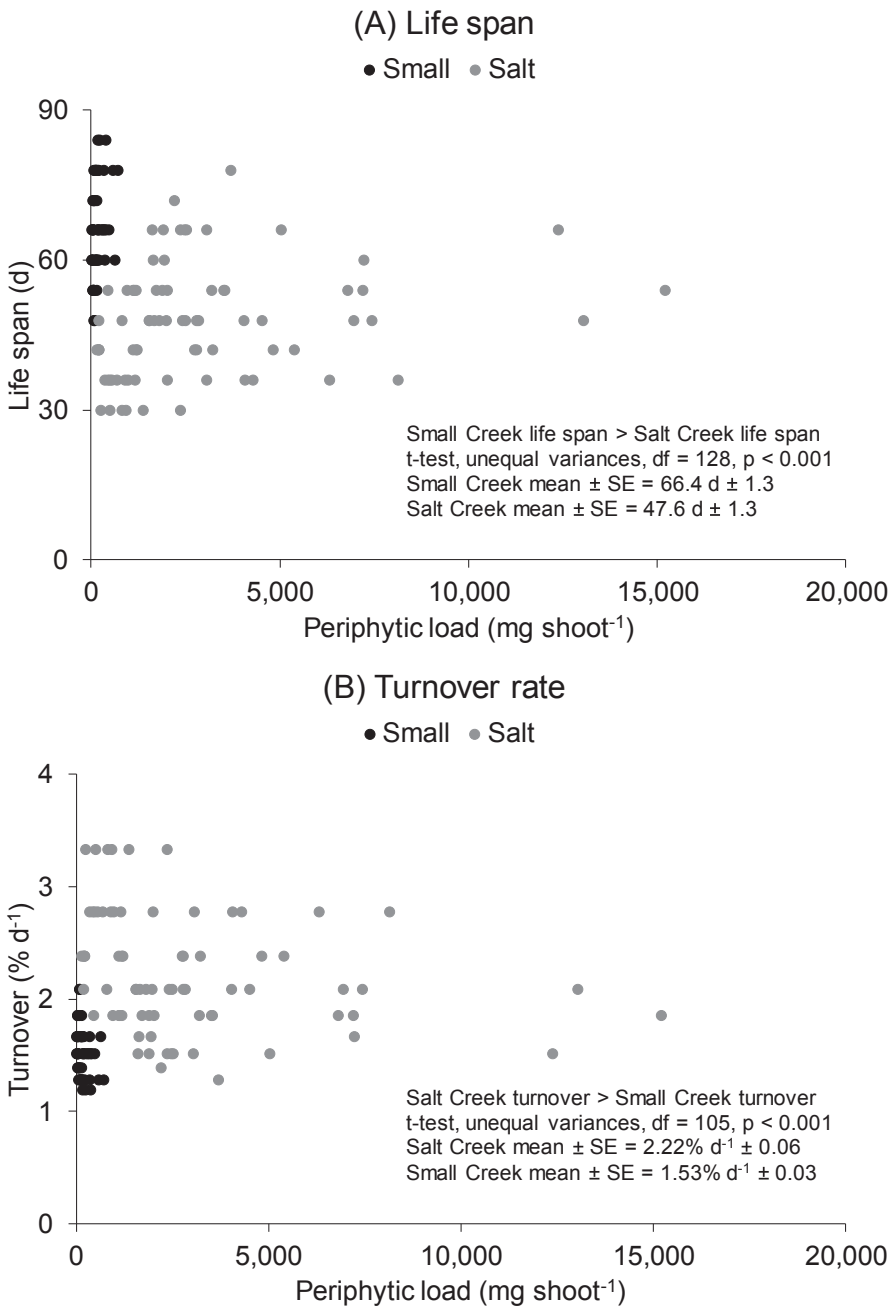

Fig. 3. Life spans (A) and turnover rates per *Vallisneria americana* shoot (B) versus periphytic loads per shoot in Small (black dots) and Salt (gray dots) creeks.

mortality of macrophytes in the Chesapeake Bay. In another study, Glazer (1999) determined that periphytic 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. However, Bendell (2006)'s study of *Halophila ovalis* in Australia suggested that negative impacts of periphyton occurred at 4.7 mg DM cm^{-2}. The differences among these estimates likely are due to varying morphologies among species of macrophytes and periphyton, methodological differences in measurement of macrophytic responses and periphytic loads, or environmental differences among the systems.

The periphytic thresholds derived from boundary analyses suggested that 20%–26% of incident, full sunlight may be the minimum light requirement for *V. americana* in the Chassahowitzka River. This result was consistent with the report of Choice et al. (2014) indicating that 18%–25% of incident light was required by most prominent estuarine macrophytes in this area. A similar result was reported for Lake Onalaska, where survival of *V. americana* seedlings was significantly greater when they received at least 25% of incident light (Kimber et al., 1995). In addition, other studies have reported that some seagrasses have similar light requirements for long-term survival. For example, in Tampa Bay, Florida the minimum light requirement for *Thalassia testudinum* was determined to be 20%–25% of incident light (Dixon and Leverone, 1997), and Kenworthy and Haunert (1991) concluded that at least 20% of incident light was required to maintain a seagrass community. These observations provide support for the critical thresholds calculated for *V. americana* in the Chassahowitzka River.

In this study, periphytic loads influenced the growth rates of macrophytes more than overall light regime. In Salt Creek, where more light was available, heavier periphytic loads suppressed the growth of plants more strongly, shortened the life span of shoots, and led to more rapid turnover rates compared to Small Creek, where leaves had lower periphytic loads. Similar results can be found in earlier work on a

variety of freshwater macrophytes (Sand-Jensen, 1977; Phillips et al., 1978; Bulthuis and Woelkerling, 1983; Twilley et al., 1985; Asaeda et al., 2004).

Besides periphytic loads, aging has been shown to lead to changes in photosynthesis (Mazzella and Alberte, 1986), synthesis of proteins (Thayer et al., 1984; Zieman et al., 1984), and other physiological processes (Cebrián and Duarte, 1994) that influence growth rates of macrophytes. In addition, the increased biomass of periphyton that accumulated on older leaves created a more detrimental influence (Bulthuis and Woelkerling, 1983; Borum, 1987; Asaeda et al., 2004). The variation in growth documented for *V. americana* leaves of different ages confirms and extends these results.

The ambient light regime is another important influence on interactions between *V. americana* and periphyton. In this study, the areal biomass of *V. americana* and the mean periphytic loads per shoot in Salt Creek were higher than values recorded in Small Creek, which had 74% less incident light. These differences in biomass demonstrated the strong positive effect of incident light on productivity of both macrophytes and periphyton (Burnell et al., 2014; Asaeda et al., 2004). Similar effects on the growth of *V. americana* were reported by Blanch et al. (1998) where available light in the River Murray in South Australia was lowered by turbidity, and by Kurtz et al. (2003) who conducted a shading experiment in Perdido Bay, at the border between Alabama and Florida. In addition, higher light availability generated a greater abundance and diversity of macrophytes in eight coastal systems along the Gulf coast of peninsular Florida (Choice et al., 2014). Furthermore, the higher levels of incident light and periphytic loads in Salt Creek were accompanied by shorter life spans and faster turnover rates, which confirms previous findings that overall productivity responds to light regimes over the longer term because there was not a clear relationship between life spans or turnover rates and periphytic loads within either creek (Bulthuis and Woelkerling, 1983; Ruesink, 2016).

Potential morphological adaptations to low light and shallow water were observed for *V. americana* in Small Creek, with short and narrow leaves yielding reductions in mean masses of shoots and areas of leaves. Similarly, *V. americana* was found to have small leaves at other locations with low light availability (Blanch et al., 1998; Kurtz et al., 2003). Furthermore, Asaeda et al. (2004) recorded decreases in leaf area for *Potamogeton perfoliatus* growing in areas with low light. Although plants growing in low light environments within our study area had small leaves, they also tended to have more leaves per shoot resulting in no significant difference in total leaf area per shoot as compared to plants in higher light. These findings apparently contradicted earlier studies that reported an increased total leaf area per shoot in low light conditions to optimize exposure to light (Tomasko and Dawes, 1988; Abal et al., 1994; Dalla Via et al., 1998; Carlson et al., 2003). This discrepancy likely is due to lower periphytic loads in Small Creek that offset the suppression of growth due to lower light availability. In this study, numbers of new leaves that recruited to each shoot each week, growth rates per shoot, and total leaves per shoot were higher in the low light environment that characterized Small Creek. These values may be due to enhanced production of leaves in light conditions where they are protected from direct sunlight (Muenscher, 1936; Kimber et al., 1995).

Information about the health of *V. americana* is valuable because it is a very hardy macrophyte that is native to Florida, and historically, it was distributed extensively throughout fresh and brackish systems (Kraemer et al., 1999). As a major factor causing the decline of this species, the over-proliferation of periphytic algae should engender concern for all who value these aquatic systems. A threshold for detrimental periphytic loads on macrophytes represents a valuable indicator of an aquatic system's response to anthropogenic impacts (Wood and Lavery, 2000; Bricker et al, 2003; Gobert et al., 2009; U.S. EPA, 2010; Sutula et al., 2011; Nelson, 2017). Periphyton can respond to environmental changes more rapidly than their host macrophytes (Giovannetti et al., 2010); therefore, they provide an early warning of the need for management actions. Additionally, a threshold for periphytic loads can be considered complementary to traditional indicators based on water quality parameters (Wood and Lavery, 2000; Gobert et al., 2009; Balata et al., 2010; Giovannetti et al., 2010), especially given that tracking concentrations of nutrients has not always yielded a robust assessment of environmental status given the variety of factors that influence the effects of increased nutrient loads or concentrations (Bricker et al, 2003).

5. Conclusions

Since the performance of macrophytes is a consequence of combined stresses from environmental factors (e.g., light availability and loads of epiphytic algae and associated periphyton) and physiological factors (e.g., leaf age), it is essential to apply a standard experimental method for estimating thresholds for periphytic loads that have detrimental impacts on macrophytes. Direct, *in-situ* measurement of growth via a leaf-marking technique appears to be an optimal approach that is effective for quantifying detrimental changes due to periphytic loads on *V. americana*. Applying boundary analyses to growth data identified a threshold of 4–5 mg DM cm^{-2}, which translated into a minimum light requirement of 20%–26% of incident, full sunlight for *V. americana* in the Chassahowitzka River. As an indicator that complements traditional indicators based on water quality parameters, this threshold can guide improved management of aquatic systems containing this important macrophyte. For example, if there is a level of flow in a lotic system that maintains periphytic loads below the threshold that causes detrimental effects, then management actions should safeguard that amount of flow. In addition, the methods used here can be transferred to other systems where the effects of epiphytes on macrophytes are of concern.

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.

References

Abal, E.G., Loneragan, N.R., Bowen, P., Perry, C.J., Udy, J.W., Dennison, W.C., 1994. Physiological and morphological responses of the seagrass *Zostera capricorni* Aschers, to light intensity. J. Exp. Mar. Biol. Ecol. 178 (1), 113–129. https://doi.org/10.1016/0022-0981(94)90228-3.

Asaeda, T., Sultana, M., Manatunge, J., Fujino, T., 2004. The effect of periphytic algae on the growth and production of *Potamogeton perfoliatus* L. in two light conditions. Environ. Exp. Bot. 52 (3), 225–238. https://doi.org/10.1016/j.envexpbot.2004.02.001.

Balata, D., Piazzi, L., Nesti, U., Bulleri, F., Bertocci, I., 2010. Effects of enhanced loads of nutrients on periphyton on leaves and rhizomes of *Posidonia oceanica*. J. Sea Res. 63 (3–4), 173–179. https://doi.org/10.1016/j.seares.2009.12.001.

Bendell, B.E., 2006. Interactions amongst invertebrates, periphyton, and seagrasses in tropical intertidal meadows. Ph.D. dissertation. James Cook University.

Blanch, S.J., Ganf, G.G., Walker, K.F., 1998. Growth and recruitment in *Vallisneria americana* as related to average irradiance in the water column. Aquat. Bot. 61 (3), 181–205. https://doi.org/10.1016/S0304-3770(98)00065-5.

Borowitzka, M.A., Lavery, P.S., van Keulen, M., 2007. Periphyton of seagrasses. In: Larkum, A., Orth, R.J., Duarte, C. (Eds.), Seagrasses: biology, ecology and conservation. Springer, Dordrecht, pp. 441–461.

Borum, J., 1987. Dynamics of epiphyton on eelgrass (*Zostera marina* L.) leaves: relative roles of algal growth, herbivory, and substratum turnover. Limnol. Oceanogr. 32 (4), 986–992. https://doi.org/10.4319/lo.1987.32.4.0986.

Bricker, S.B., Ferreira, J.G., Simas, T., 2003. An integrated methodology for assessment of

estuarine trophic status. Ecol. Model. 169 (1), 39–60. https://doi.org/10.1016/S0304-3800(03)00199-6.
Bulthuis, D.A., Woelkerling, W.J., 1983. Biomass accumulation and shading effects of periphyton on leaves of the seagrass, *Heterozostera tasmanica*, in Victoria. Australia. Aquat. Bot. 16 (2), 137–148. https://doi.org/10.1016/0304-3770(83)90089-X.
Burnell, O.W., Russell, B.D., Irving, A.D., Connell, S.D., 2014. Seagrass response to CO_2 contingent on periphytic algae: indirect effects can overwhelm direct effects. Oecologia 176 (3), 871–882. https://doi.org/10.1007/s00442-014-3054-z.
Carlson Jr., P.R., Yarbro, L.A., Madley, K., Arnold, H., Merello, M., Vanderbloemen, L., McRae, G., Durako, M.J., 2003. Effect of El Niño on demographic, morphological, and chemical parameters in turtle-grass (*Thalassia testudinum*): an unexpected test of indicators. Environ. Monit. Assess. 81 (1–3), 393–408. https://doi.org/10.1023/A:1021322301725.
Cattaneo, A., Galanti, G., Gentinetta, S., Susana, A., 1998. Periphytic algae and macroinvertebrates on submerged and floating leaved macrophytes in an Italian lake. Freshwater Biol. 39 (4), 725–740. https://doi.org/10.1046/j.1365-2427.1998.00325.x.
Cebrián, J., Duarte, C.M., 1994. The dependence of herbivory on growth rate in natural plant communities. Funct. Ecol. 8 (4), 518–525. https://doi.org/10.2307/2390077.
Choice, Z.D., Frazer, T.K., Jacoby, C.A., 2014. Mar. Pollut. Bull. 81 (1), 94–102. https://doi.org/10.1016/j.marpolbul.2014.02.015.
Dalla Via, J., Sturmbauer, C., Schönweger, G., Sötz, E., Mathekowitsch, S., Stifter, M., Rieger, R., 1998. Light gradients and meadow structure in *Posidonia oceanica*: ecomorphological and functional correlates. Mar. Ecol. Prog. Ser. 163, 267–278. https://doi.org/10.3354/meps163267.
Dennison, W.C., Moore, K.A., Stevenson, J.C., 1992. SAV habitat requirements development. In: Batiuk, R.A., Orth, R.J., Moore, K.A., Dennison, W.C., Stevenson, J.C., Staver, L.W., Carter, V., Rybicki, N.B., Hickman, R.E., Kollar, S., Bieber, S., Heasly, P. (Eds.), Chesapeake Bay submerged aquatic vegetation habitat requirements and restoration targets: a technical synthesis. U.S, Environmental Protection Agency, Chesapeake Bay Program, Annapolis, pp. 13–26.
Dixon, L.K., Leverone, J., 1997. Annual light regime of light limited *Thalassia testudinum* in Tampa Bay, Florida. In: Treat, S. (Ed.), Proceedings, Tampa Bay Area Scientific Information Symposium 3: Applying Our Knowledge. Tampa Bay Regional Planning Council, St. Petersburg, pp. 171–181.
Dodds, W.K., Bouska, W.W., Eitzmann, J.L., Pilger, T.J., Pitts, K.L., Riley, A.J., Schloesser, J.T., Thornbrugh, D.J., 2009. Eutrophication of U.S. freshwaters: analysis of potential economic damages. Environ. Sci. Technol. 43 (1), 12–19. https://doi.org/10.1021/es801217q.
Drake, L.A., Dobbs, F.C., Zimmerman, R.C., 2003. Effects of epiphyte load on optical properties and photosynthetic potential of the seagrasses *Thalassia testudinum* Banks ex König and *Zostera marina* L. Limnol. Oceanogr. 48 (1, part 2), 456–463. https://doi.org/10.4319/lo.2003.48.1_part_2.0456.
Duarte, C.M., 1991. Seagrass depth limits. Aquat. Bot. 40 (4), 363–377. https://doi.org/10.1016/0304-3770(91)90081-F.
Duarte, C.M., 1995. Submerged aquatic vegetation in relation to different nutrient regimes. Ophelia 41 (1), 87–112. https://doi.org/10.1080/00785236.1995.10422039.
Erickson, R.O., Michelini, F.J., 1957. The plastochron index. Am. J. Bot. 44, 297–305. https://doi.org/10.1002/j.1537-2197.1957.tb10544.x.
Fong, C.W., Lee, S.Y., Wu, R.S., 2000. The effects of periphytic algae and their grazers on the intertidal seagrass *Zostera japonica*. Aquat. Bot. 67 (4), 251–261. https://doi.org/10.1016/S0304-3770(00)00101-7.
Frazer, T.K., 2000. Coastal nitrate assessment: nutrient assimilation capacity of five Gulf coast rivers. Second annual project summary, Southwest Florida Water Management District, Tampa.
Frazer, T.K., Hoyer, M.V., Notestein, S.K., Hale, J.A., Canfield, D.E., 2001. Physical, chemical and vegetative characteristics of five Gulf coast rivers. Final report, Southwest Florida Water Management District, Tampa.
Frazer, K.T., Notestein, S.K., Pine Jr., W.E., 2006. Changes in the physical, chemical and vegetative characteristics of the Homosassa. Chassahowitzka and Weeki Wachee Rivers, Final report, Southwest Florida Water Management District, Tampa.
Giovannetti, E., Montefalcone, M., Morri, C., Bianchi, C.N., Albertelli, G., 2010. Early warning response of *Posidonia oceanica* epiphyte community to environmental alterations (Ligurian Sea, NW Mediterranean). Mar. Pollut. Bull. 60 (7), 1031–1039. https://doi.org/10.1016/j.marpolbul.2010.01.024.
Glazer, B.T., 1999. Analysis of physical, chemical, and biological factors inhibiting growth and restoration of submerged vascular plants in Delaware's Indian River and Rehoboth Bays. M.S. thesis. University of Delaware.
Gobert, S., Sartoretto, S., Rico-Raimondino, V., Andral, B., Chery, A., Lejeune, P., Boissery, P., 2009. Assessment of the ecological status of Mediterranean French coastal waters as required by the Water Framework Directive using the *Posidonia oceanica* Rapid Easy Index: PREI. Mar. Pollut. Bull. 58 (11), 1727–1733. https://doi.org/10.1016/j.marpolbul.2009.06.012.
Gregg, W.W., Rose, F.L., 1985. Influences of aquatic macrophytes on invertebrate community structure, guild structure, and microdistribution in streams. Hydrobiologia 128 (1), 45–56. https://doi.org/10.1007/BF00008939.
Guan, J., Jacoby, C.A., Frazer, T.K., 2020. Light attenuation by periphyton on *Vallisneria americana*. Ecol. Indic. https://doi.org/10.1016/j.ecolind.2020.106498.
Hauxwell, J.A., Frazer, T.K., Osenberg, C.W., 2007. An annual cycle of biomass and productivity of *Vallisneria americana* in a subtropical spring-fed estuary. Aquat. Bot. 87 (1), 61–68. https://doi.org/10.1016/j.aquabot.2007.03.003.
Hillman, K., Lukatelich, R.J., Bastyan, G., McComb. A.J., (1991. Water quality and seagrass biomass, productivity and epiphyte load in Princess Royal Harbour, Oyster Harbour and King George Sound. Technical Series 39, Environmental Protection Authority, Perth.
Howard-Williams, C., Davies, B.R., Cross, R.H.M., 1978. The influence of periphyton on the surface structure of a *Potamogeton pectinatus* L. leaf (an hypothesis). Aquat. Bot. 5, 87–91. https://doi.org/10.1016/0304-3770(78)90049-9.
Hoyer, M.V., Frazer, T.K., Notestein, S.K., Canfield, D.E., 2004. Vegetative characteristics of three low-lying Florida coastal rivers in relation to flow, light, salinity and nutrients. Hydrobiologia 528, 31–43.
Kenworthy. W.J., Haunert, D.E., 1991. The light requirements of seagrasses: proceedings of a workshop to examine the capability of water quality criteria, standards and monitoring programs to protect seagrasses. NOAA Technical Memorandum NMFS-SEFC-287. Washington, D.C.
Kimber, A., Owens, J.L., Crumpton, W.G., 1995. Light availability and growth of wild celery (*Vallisneria americana*) in upper Mississippi River backwaters. Regul. River. 11 (2), 167–174. https://doi.org/10.1002/rrr.3450110206.
Kiørbe, T., 1980. Production of *Ruppia cirrhosa* (petagna) grande in mixed beds in Ringkøbing Fjord (Denmark). Aquat. Bot. 9, 135–143. https://doi.org/10.1016/0304-3770(80)90014-5.
Kraemer, G.P., Chamberlain, R.H., Doering, P.H., Steinman, A.D., Hanisak, M.D., 1999. Physiological responses of transplants of the freshwater angiosperm *Vallisneria americana* along a salinity gradient in the Caloosahatchee Estuary (Southwestern Florida). Estuaries 22 (1), 138–148. https://doi.org/10.2307/1352934.
Kurtz, J.C., Yates, D.F., Macauley, J.M., Quarles, R.L., Genthner, F.J., Chancy, C.A., Devereux, R., 2003. Effects of light reduction on growth of the submerged macrophyte *Vallisneria americana* and the community of root-associated heterotrophic bacteria. J. Exp. Mar. Biol. Ecol. 291 (2), 199–218. https://doi.org/10.1016/S0022-0981(03)00120-5.
Lamoreaux, R.J., Chaney, W.R., Brown, K.M., 1978. The plastochron index: a review after two decades of use. Am. J. Bot. 65 (5), 586–593. https://doi.org/10.2307/2442595.
Lauridsen, T.L., Jeppesen, E., Søndergaard, M., 1994. Colonization and succession of submerged macrophytes in shallow Lake Vaeng during the first five years following fish manipulation. Hydrobiologia 275 (1), 233–242. https://doi.org/10.1007/BF00026714.
Liebowitz, D.M., Cohen, M.J., Heffernan, J.B., Korhnak, L.V., Frazer, T.K., 2014. Environmentally-mediated consumer control of algal proliferation in Florida springs. Freshwater Biol. 59 (10), 2009–2023. https://doi.org/10.1111/fwb.12403.
Ludwig, J.A., Tongway, D.J., 1995. Spatial organization of landscapes and its function in semi-arid woodlands. Australia. Landscape Ecol. 10 (1), 51–63. https://doi.org/10.1007/BF00158553.
Mazzella, L., Alberte, R.S., 1986. Light adaptation and the role of autotrophic periphyton in primary production of the temperate seagrass, *Zostera marina* L. J. Exp. Mar. Biol. Ecol. 100 (1–3), 165–180. https://doi.org/10.1016/0022-0981(86)90161-9.
Muenscher, W.C., 1936. The germination of seeds of *Potamogeton*. Ann. Bot.-London 50 (4), 805–821. https://doi.org/10.1093/oxfordjournals.aob.a090618.
Neckles, H.A., Wetzel, R.L., Orth, R.J., 1993. Relative effects of nutrient enrichment and grazing on epiphyte macrophyte (*Zostera marina* L) dynamics. Oecologia 93 (2), 285–295. https://doi.org/10.1007/BF00317683.
Nelson, W.G., 2017. Development of an epiphyte indicator of nutrient enrichment: threshold values for seagrass epiphyte load. Ecol. Indic. 74, 343–356. https://doi.org/10.1016/j.ecolind.2016.11.035.
Odum, H.T., 1957. Trophic structure and productivity of Silver Springs. Florida. Ecol. Monogr. 27 (1), 55–112. https://doi.org/10.2307/1948571.
O'Hare, M.T., Aguiar, F.C., Asaeda, T., Bakker, E.S., Chambers, P.A., Clayton, J.S., Elger, A., Ferreira, T.M., Gros, E.M., Gunn, I.D.M., Gurnell, A.M., Hellsten, S., Hofstra, D.E., Li, W., Mohr, S., Puijalon, S., Szoszkiewicz, K., Willby, N.J., Wood, K.A., 2017. Plants in aquatic ecosystems: current trends and future directions. Hydrobiologia 812 (1), 1–11. https://doi.org/10.1007/s10750-017-3190-7.
Orth, R.J., Van Montfrans, J., 1984. Periphyton seagrass relationships with an emphasis on the role of micrograzing: a review. Aquat Bot. 18 (1–2), 43–69. https://doi.org/10.1016/0304-3770(84)90080-9.
Phillips, G.L., Eminson, D., Moss, B., 1978. A mechanism to account for macrophyte decline in progressively eutrophicated freshwaters. Aquat. Bot. 4, 103–126. https://doi.org/10.1016/0304-3770(78)90012-8.
Reaver, N.G.F., Kaplan, D.A., Mattson, R.A., Carter, E., Sucsy, P.V., Frazer, T.K., 2019. Hydrodynamic controls on primary producer communities in spring-fed rivers. Geophys. Res. Lett. 46 (9), 4715–4725. https://doi.org/10.1029/2019GL082571.
Ruesink, J.L., 2016. Epiphyte load and seagrass performance are decoupled in an estuary with low eutrophication risk. J. Exp. Mar. Biol. Ecol. 178 (1), 113–129. https://doi.org/10.1016/j.jembe.2016.03.022.
Ruiz-Frau, A., Gelcich, S., Hendriks, I.E., Duarte, C.M., Marbà, N., 2017. Current state of seagrass ecosystem services: research and policy integration. Ocean Coast. Manage. 149, 107–115. https://doi.org/10.1016/j.ocecoaman.2017.10.004.
Sand-Jensen, K.A.J., 1977. Effect of periphyton on eelgrass photosynthesis. Aquat. Bot. 3, 55–63. https://doi.org/10.1016/0304-3770(77)90004-3.
Sand-Jensen, K., Revsbech, N.P., Jörgensen, B.B., 1985. Microprofiles of oxygen in epiphyte communities on submerged macrophytes. Mar. Biol. 89 (1), 55–62. https://doi.org/10.1007/BF00392877.
Sand-Jensen, K., Søndergaard, M., 1981. Phytoplankton and epiphyte development and their shading effect on submerged macrophytes in lakes of different nutrient status. Int. Rev. Ges. Hydrobio. 66 (4), 529–552. https://doi.org/10.1002/iroh.19810660406.
Seddon, S., Connolly, R.M., Edyvane, K.S., 2000. Large-scale seagrass dieback in northern Spencer Gulf. South Australia. Aquat. Bot. 66 (4), 297–310. https://doi.org/10.1016/S0304-3770(99)00080-7.
Silberstein, K., Chiffings, A.W., McComb, A.J., 1986. The loss of seagrass in Cockburn Sound, Western Australia. III. The effect of periphyton on productivity of *Posidonia australis* Hook. f. Aquat. Bot. 24 (4), 355–371. https://doi.org/10.1016/0304-3770(86)90102-6.
Smith, V.H., 2003. Eutrophication of freshwater and coastal marine ecosystems a global

problem. Environ. Sci. Pollut. R. 10 (2), 126–139. https://doi.org/10.1065/espr2002.12.142.
Springs Management Plan., 2016. Southwest Florida Water Management District, Tampa.
Stevenson, R.J., Pinowska, A., Albertin, A., Sickman, J.O., 2007. Ecological condition of algae and nutrients in Florida springs: the synthesis report. Florida Department of Environmental Protection, Tallahassee.
Sutula, M., Fong, P., Kaldy, J., McLaughlin, K., Gillett, D., Howard, M., Ranasinghe, A., Fong, P., Green, L., Kennison, R., Kaldy, J., Madden, C., Beck, N., 2011. Review of indicators for development of nutrient numeric endpoints in California estuaries. Southern California Coastal Water Research Project Technical Report 646. California State Water Board, Sacramento.
Taylor, J.D., Lewis, M.S., 1970. The flora, fauna and sediments of the marine grass beds of Mahé. Seychelles. J. Nat. Hist. 4 (2), 199–220. https://doi.org/10.1080/00222937000770201.
Thayer, G.W., Kenworthy, W.J., Fonseca, M.S., 1984. Ecology of eelgrass meadows of the Atlantic coast: a community profile (No. FWS/OBS-84/02). National Marine Fisheries Service, U.S. Department of the Interior, Washington D.C.
Tomasko, D.A., Dawes, C.J., 1988. Depth distribution of *Thalassia testudinum* in two meadows on the west coast of Florida: a difference in effect of light availability. Mar. Ecol. 9 (2), 123–130. https://doi.org/10.1111/j.1439-0485.1988.tb00203.x.
Twilley, R., Kemp, W., Staver, K., Stevenson, J., Boynton, W., 1985. Nutrient enrichment of estuarine submersed vascular plant communities. I. Algal growth and effects on production of plants and associated communities. Mar. Ecol. Prog. Ser. 23, 179–191. https://doi.org/10.3354/meps023179.
U.S. EPA., 2010. Methods and approaches for deriving numeric criteria for nitrogen/phosphorus pollution in Florida's estuaries, coastal waters, and southern inland flowing waters. U.S. Environmental Protection Agency, Washington D.C.
van Donk, E., van de Bund, W.J., 2002. Impact of submerged macrophytes including charophytes on phyto-and zooplankton communities: allelopathy versus other mechanisms. Aquat. Bot. 72 (3–4), 261–274. https://doi.org/10.1016/S0304-3770(01)00205-4.
Waycott, M., Duarte, C.M., Carruthers, T.J., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck Jr., K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Short, F.T., Williams, S.L., 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. P. Natl. Acad. Sci. 106 (30), 12377–12381. https://doi.org/10.1073/pnas.0905620106.
Wehr, J., Sheath, R., Kociolek, J.P., 2015. Freshwater algae of North America (2nd ed.). Academic Press, Boston.
Wood, N., Lavery, P., 2000. Monitoring seagrass ecosystem health–the role of perception in defining health and indicators. Ecosyst. Health 6 (2), 134–148. https://doi.org/10.1046/j.1526-0992.2000.00015.x.
Wynn, S., Borisova, T., Hodges, A., 2014. Economic value of the services provided by Florida springs and other water bodies: a summary of existing studies. University of Florida. http://edis.ifas.ufl.edu/fe959, accessed 22 July 2018.
Yobbi, D.K., Knockenmus, L.A., 1989. Salinity and flow relations and effects of reduced flow in Chassahowitzka River and Homosassa River estuaries, Southwest Florida. Water-Resources Investigations Report, 88, 4044, U.S. Geological Survey, Tallahassee.
Zieman, J.C., 1968. A study of the growth and decomposition of the seagrass: *Thalassia testudinum*. M.S. University of Miami, Florida.
Zieman, J.C., Wetzel, R.G., 1980. Productivity in seagrasses: methods and rates, in: Phillips, R.C., McRoy, C.P. (Eds.), Handbook of seagrass biology: an ecosystem perspective. Garland STPM Press, pp. 87–116. https://doi.org/10.1002/iroh.19810660125.
Zieman, J.C., Macko, S.A., Mills, A.L., 1984. Role of seagrasses and mangroves in estuarine food webs: temporal and spatial changes in stable isotope composition and amino acid content during decomposition. B. Mar. Sci. 35 (3), 380–392.