

Response of seagrass epiphyte loading to field manipulations of fertilization, gastropod grazing and leaf turnover rates

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Abstract

This study evaluates the bottom-up and top-down controls on epiphyte loads under low nutrient additions. Nutrients and gastropod grazers were manipulated in a field experiment conducted within a *Thalassia testudinum* meadow in Florida Bay, FL, USA. The effect of seagrass leaf turnover rate on epiphyte loading was also evaluated using novel seagrass short-shoot mimics that “grow,” allowing for the manipulation of leaf turnover rates. During the summer growing season and over the course of one seagrass leaf turnover period, low-level water column nutrient additions increased total epiphyte load, epiphyte chlorophyll *a*, and epiphyte autotrophic index. *T. testudinum* leaf nutrients (N and P) and leaf productivity also increased. Epiphyte loading and *T. testudinum* shoot biomass and productivity did not respond to a 60% mean increase in gastropod abundance. Manipulations of seagrass leaf turnover rates at minimum wintertime and maximum summertime rates resulted in a 20% difference in epiphyte loading. Despite elevated grazer abundances and increased leaf turnover rates, epiphyte loads increased with nutrient addition. These results emphasize the sensitivity of *T. testudinum* and associated epiphytes to low-level nutrient addition in a nutrient-limited environment such as Florida Bay.

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1. Introduction

For decades, there has been much debate whether “bottom-up” (e.g., physico-chemical resources) or “top-down” (e.g., herbivory, predation) effects control community structure. The debate is no longer about the occurrence of top-down or bottom-up control in nature. Rather, the discussion centers on the relative importance of each parameter among various ecosystems and the

elucidation of the temporal and spatial variability or the relative strength of these controls within particular ecosystems (Matson and Hunter, 1992).

Seagrass meadows, and more specifically, the epiphyte communities on seagrass leaves, are ideal for investigating the roles of bottom-up and top-down control mechanisms. Examples of bottom-up controls of epiphyte loading are nutrient availability and seagrass leaf turnover rate, which determine epiphyte loads by limiting the amount of time available for accumulation. Top-down controls of epiphyte loading are achieved by grazing organisms and the higher trophic level activities, which may control the abundance of the epiphytic

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grazers. Consequently, the nature and quantity of epiphyte loads result from the interplay between bottom-up and top-down forces. Optimal stability and productivity of seagrass ecosystems are likely to be highly dependent on a favorable ratio of bottom-up and top-down forces in the control of epiphytes. Studies investigating the process of epiphyte accumulation on seagrass leaves have focused on fertilization (Twilley et al., 1985; Tomasko and Lapointe, 1991; Wear et al., 1999), leaf turnover rate variability (Bulthuis and Woelkerling, 1983; Borum, 1987; Frankovich and Zieman, 1994), and grazing controls (Howard, 1982; Van Montfrans et al., 1982; Hootsmans and Vermaat, 1985; Howard and Short, 1986; Jernakoff and Nielsen, 1997; Nelson, 1997).

Bulthuis and Woelkerling (1983) discussed the importance of leaf productivity as a mechanism ameliorating or exacerbating the effects of epiphyte loading. Higher rates of leaf productivity reduce the mean epiphyte load per short-shoot by producing new epiphyte-free leaf biomass. Older leaf material is subject to greater time periods for epiphyte settlement and growth; therefore, leaf longevity is a contributing factor to epiphyte loading. Due to a lack of direct measurements of leaf longevity, leaf turnover periods (biomass divided by productivity) have been used to infer the amount of time available for epiphyte accumulation (Frankovich and Zieman, 1994).

Observations of greater epiphyte loads in areas of increased nutrient availability (Cambridge and McComb, 1984; Borum, 1985; Tomasko and Lapointe, 1991) suggested significant nutrient control of epiphyte accumulations. This idea was further supported by experimental studies showing increased epiphyte loads under nutrient fertilization (Twilley et al., 1985; Tomasko and Lapointe, 1991). These experimental studies were conducted in artificial ponds, microcosms, and aquaria, and, with the exception of Tomasko and Lapointe (1991), were without grazing organisms. Later fertilization studies indicated that even under highly nutrient-enriched conditions, epiphyte loads did not change when grazers were present at or above mean natural abundances (Williams and Ruckelshaus, 1993; Lin et al., 1996; Wear et al., 1999; Heck et al., 2000). These observed relationships were predicted much earlier in a hypothetical model constructed by Orth and Van Montfrans (1984) that suggested reduced epiphyte loads would occur under the influence of grazing pressure.

Neckles et al. (1993) investigated the interactive effects of nutrient fertilization and grazing pressure on a seagrass community. In microcosm experiments, it was demonstrated that grazer effects on epiphyte loads were stronger than that of fertilization. The experiments

showed also that the strength of these controls, and of the interaction between the two, varied seasonally. These bottom-up and top-down control mechanisms in a seagrass ecosystem were experimentally tested in the field by Heck et al. (2000) who manipulated nutrients and the density of pinfish (a predator of mesograzers) in expectation of finding evidence of a “trophic cascade” (Carpenter et al., 1985). While there were few nutrient effects, pinfish treatments significantly lowered *both* epiphyte biomass and mesograzer density due to their omnivorous feeding and were unable to reduce mesograzers enough to allow epiphyte accumulation (Heck et al., 2000).

The present study investigates the role of gastropod grazers under enriched and ambient nutrient conditions and the effect of seagrass leaf turnover rate on epiphyte loads in a robust seagrass community in Florida Bay. Unique because of its use of seagrass short-shoot mimics to manipulate seagrass “leaf” productivities in an experimental setting, this investigation is the first to manipulate leaf turnover rates. This has not been attempted before because the productivity of natural seagrass short-shoots cannot be manipulated without altering environmental conditions. Utilizing seagrass short-shoot mimics that “grow” allowing for the manipulation of leaf turnover rates, this investigation reports the results of two experiments quantifying the effects of nutrient availability, grazing pressure, and substratum turnover rate manipulations on epiphyte loading and *Thalassia testudinum* productivity.

2. Materials and methods

2.1. Nutrient and grazer manipulative field experiment

The experiments were conducted in eastern Rabbit Key Basin, Florida Bay, Florida U.S.A. (Fig. 1). Seasonal water temperatures and salinities in Florida Bay range from 15–32 °C and 27–43 ppt, respectively (Boyer et al., 1999). The study area is characterized by dense *T. testudinum* beds (mean annual short-shoot density = 1280 ss m⁻², mean above-ground biomass = 100 g m⁻² (Frankovich and Zieman, 1994). The seagrass meadows also contain a robust epiphyte grazing community which includes gastropods (Frankovich and Zieman, 2005b), pinfish *Lagodon rhomboides* (Thayer and Chester, 1989), and caridean and penaeid shrimp (Robblee and Daniels, 2003).

The main effects of grazing pressure (+G) and nutrient manipulation (+N) on *T. testudinum* productivity and its associated epiphyte load were tested using a two-factor (fertilization and grazer addition) factorial design

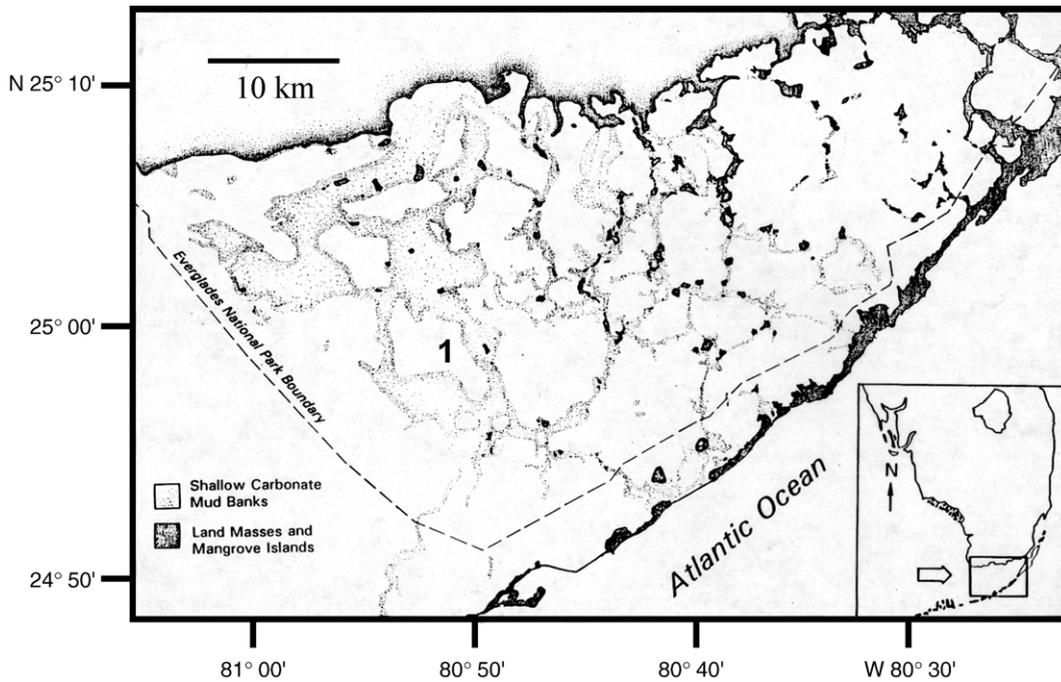


Fig. 1. Location map of Florida Bay, Florida, USA. Number 1 denotes study site.

(Fig. 2). Grazing pressure was manipulated by adding and removing epiphyte-grazing gastropods indigenous to the area, while nutrients were manipulated through water column fertilization. Each treatment (+G+N, +G-N, -G+N, and -G-N) had six replicates within a large continuous seagrass meadow of *T. testudinum* (mean leaf standing crop=99 g m⁻²). The experiment was carried out for 60 days, from June 1 to July 31, 2002, to approximate the summer *T. testudinum* leaf longevity period of 62 days (Bricker, 2003). Individual 0.25 m² experimental plots within the study area were fenced (40 cm high) with plastic 5 mm mesh and were separated from one another by two meter distances. Fertilized and unfertilized treatments were assigned to two separate blocks within the grass bed. Grazer-enhanced and grazer-reduced treatments were assigned randomly within the fertilized and unfertilized blocks. Results and observations from an initial pilot study that manipulated both nutrients and grazers (September–October 2001) were used to determine treatment replication for adequate statistical power.

Osmocote™ 18-6-12 slow release fertilizer pellets [NO₃⁻:NH₄⁺:PO₄³⁻ molar release ratio=7.7:8.5:1 (Heck et al., 2000)] was placed in the center of each of the fertilized treatments (120 g). The fertilizer was contained in nylon stockings placed inside spherical polyethylene dispensers (Wiffle™ balls) with openings evenly dis-

tributed on their bottom half. Attached to PVC stakes, the dispensers were placed at a height of 30 cm at the center of each fertilized plot, with the openings facing downward toward the seagrass bed. Nutrient release was performed in the water column and not on the sediment surface to address the effects of low level increases in watershed nutrient loads. Unfertilized plots had similar dispensers without nutrients. The fertilizer was replaced at day 25 of the experiment. Fertilizer release rates were calculated from the dry weights of the remaining fertilizer inside the 12 fertilized plots at day 25 and at the

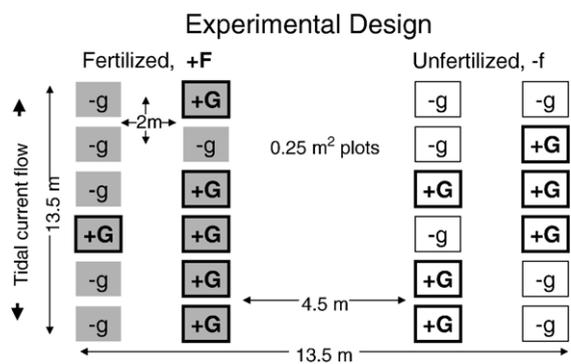


Fig. 2. Randomized split-block experimental design of field enclosures. (+G) denotes gastropod grazer addition plots. (-g) denotes reduced gastropod grazer plots.

completion of the experiment. Nutrient release rates of the Osmocote™ fertilizer were estimated using measurements of fertilizer weight loss and the mean field release rates of this fertilizer as measured by Heck et al. (2000).

Grazing pressure was manipulated by adding the herbivorous gastropods, *Turbo castanea*, *Tegula fasciata*, and *Modulus modulus* to grazer-enhanced (+G) plots at the initiation of the experiment and at days 14, 25, 38, and 51. The collected snail species represent 90% of the relative abundance and 99% of the biomass of the indigenous gastropod grazing community (Frankovich and Zieman, 2005b). At the start of the experiment, all plots were cleared of these species by manual collection. Grazer-enhanced plots were evenly re-stocked, according to number and size, with these species at higher field densities (7.4X–11.3X initial ambient species densities). During the experiment, any snails that were observed in grazer-reduced plots were removed biweekly without disrupting the epiphyte accumulations. Snails observed on the fencing were also removed and placed inside or outside the plots according to the designated treatment. After the experiment was initiated, grazer additions were carried out by collecting the gastropods from six 1 m² arbitrarily-placed quadrats outside but adjacent to the study plots.

At the experiment initiation, days 14, 30, 45, and experiment end, total epiphyte load and epiphyte chlorophyll *a* (chl *a*) load were determined from *T. testudinum* leaf material pooled from two arbitrarily selected short-shoots within each plot (Frankovich and Fourqurean, 1997). Between day 38 and experiment end, *T. testudinum* leaf productivity and biomass within each plot were determined by leaf marking all short-shoots within a 100 cm² quadrat (Zieman, 1974). The nitrogen and phosphorus content of *T. testudinum* leaves was determined at experiment end (Fourqurean et al., 1992a).

A multivariate repeated measures General Linear Model (GLM) was performed on epiphyte loading measurements where the plots were considered the unit of study. As these plots were repeatedly sampled, the different sample dates were not considered independent requiring a repeated measures approach (GLM, repeated measures, SPSS vers.11.0, 2002). Two-way General Linear Model (GLM, multivariate, SPSS vers.11.0, 2002) was performed on *T. testudinum* leaf biomass, productivity and elemental content to detect the main and interactive effects of grazer and nutrient manipulation. A balanced ANOVA design (i.e., equal *n*_i) was employed ensuring statistical robustness with respect to assumptions of homoscedasticity and normality (Zar, 1996).

2.2. Leaf turnover field experiments

This experiment utilized novel devices to mimic the natural growth of *T. testudinum* short-shoots. The devices were designed to manipulate observed natural leaf productivities and turnover rates. Each device consisted of 2–3 Mylar™ seagrass leaf mimics coiled within an opaque 35 mm film canister secured to the seagrass bed by a wire stake and cable ties (Fig. 3). The leaf mimics were cut from transparency film to the dimensions of mature *T. testudinum* leaves from the area. Small styrofoam floats cut from packaging “peanuts” were secured to the leaf tips by silicone glue. These floats and leaf mimics protruded from slits through the side of the film canister. At least weekly, the leaf mimics were manually pulled from the canister by the float at rates approximating *T. testudinum* leaf turnover rates and productivities previously measured in the field. While the mimics did not provide any dissolved organic matter for the heterotrophic component of the epiphytic community, previous studies found that Mylar™ artificial seagrass leaves were effective substrates for sampling the epiphytes that were observed occurring naturally on surrounding *T. testudinum* leaves (Frankovich and

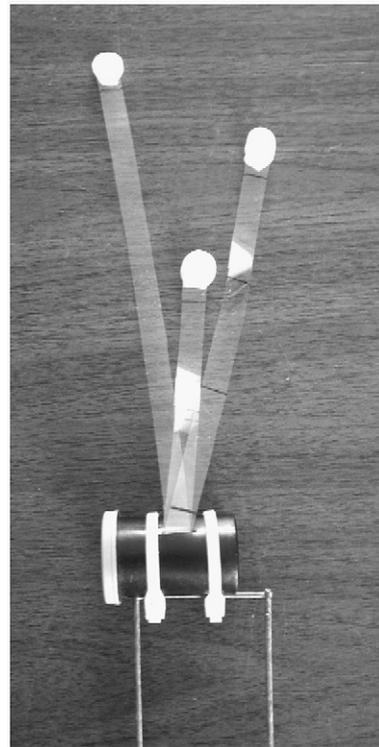


Fig. 3. Photograph of seagrass short-shoot mimic used to simulate varying seagrass leaf turnover rates. Marked lines on mimic leaves mark manipulated growth increments.

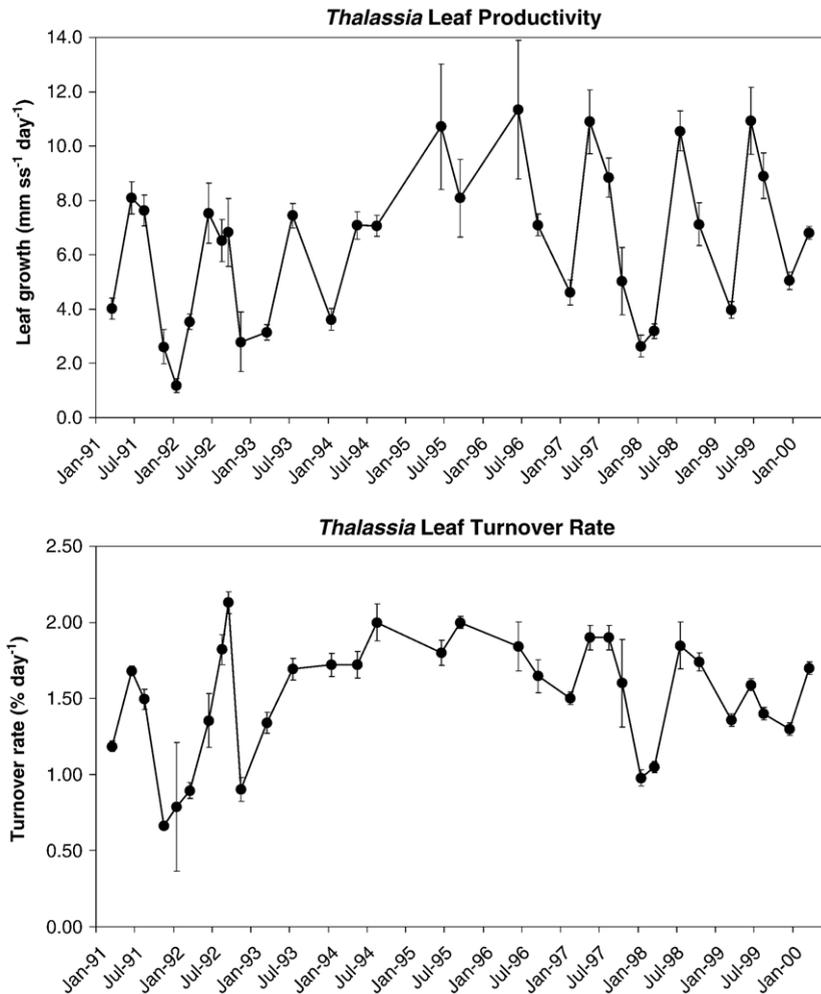


Fig. 4. Historical measurements of seasonal *Thalassia testudinum* leaf productivities and turnover rates from study area.

Zieman, 2005a). The epiphyte species composition on the artificial leaves was representative of the epiphytes occurring on *T. testudinum* leaves from Florida Bay (Frankovich and Zieman, 1994; Armitage et al., 2005) and pigment analyses of the epiphytes sampled from both substrates and from similar areas in Florida Bay revealed no differences in relative pigment concentrations between the two (Louda et al., 1999) suggesting that epiphyte community composition was largely the same in terms of total epiphyte load, epiphyte chl *a* and autotrophic index.

The effect of leaf turnover rates on epiphyte loading was tested using a two-sample *t*-test (SPSS, 2002) to determine differences between mean epiphyte loads measured on seagrass short-shoot mimics “growing” at two different “leaf” productivities and turnover rates. The Levene statistic for homoscedasticity was also applied to the data to ensure that statistical assumptions

were satisfied (SPSS, 2002). The short-shoot mimics (16 per treatment minimum) were used to manipulate “leaf” turnover at rates of 0.9% day⁻¹ and 1.7% day⁻¹, representing winter and summer *T. testudinum* leaf turnover rates, respectively. The 0.9% wintertime value is the mean of 8 measurements taken in the 3 months of Jan–Mar from June 1991–Jan 2001. The summer value of 1.7% was the mean of 13 measurements taken during the 4 months of June–August over the same time period. These were the periods of highest and lowest productivities. The mimics were assigned randomly to equally spaced points within a 2.5 m × 4.0 m area of continuous seagrass bottom, the short-shoot mimics were deployed with one 20 cm “leaf” already exposed, then were allowed to acclimate and accumulate epiphytes for approximately 2 weeks. The epiphytes on the “old” leaves were in direct contact with newly emerging “leaves” at the start of leaf productivity manipulation, providing new

Table 1

Effects of water column fertilization (Osmocote N-P-K) on epiphyte loading and seagrass biomass and productivity

Reference	Fertilization application rate (g m ⁻² day ⁻¹)	Epiphyte load dw bold, chl <i>a</i> (% change)	Seagrass biomass (% change)	Seagrass productivity (% change)	Epiphyte species composition
Wear et al. (1999)	23.4	+38, +209	No effect	+36	+Phaeophyta +Rhodophyta
Heck et al. (2000)	8.0	No effect, NA	No effect	No effect	+Chlorophyta No change
Present study	3.1	+21, +25	No effect	+22	Rhodophyta, diatoms

NA=not available.

epiphyte recruits, just as would have occurred with natural seagrass short-shoots (Borum, 1987). The experiment was run for 36 days from November 6, 2002 until December 12, 2002. Six times throughout the 36 day experiment (November 6, 12, 20, 25, and December 2, 6), 1.5 cm and 5.0 cm “leaf” sections were exposed for the 0.9% day⁻¹ and 1.7% day⁻¹ leaf turnover treatments, respectively. “Leaves” were exposed sequentially. New “leaves” were exposed after the preceding leaf reached 20 cm (maturity). The two leaf growth increments resulted in mean leaf productivity rates over the 36-day period of 2.5 mm short-shoot⁻¹ day⁻¹ and 8.3 mm short-shoot⁻¹ day⁻¹ for the two treatments. These 36-day productivities divided by the standing stock of leaf material (“leaf” growth+20 cm original “standing crop”) produced the desired effects of 0.9% day⁻¹ and 1.7% day⁻¹ “leaf” turnover treatments. Both the “leaf” productivities and the turnover rates approximate those observed at the experimental location during winter and summer seasons (Fig. 4). On day 36, the seagrass short-shoot mimics were harvested and the total epiphyte load was measured for each short-shoot (Frankovich and Fourqurean, 1997).

3. Results

3.1. Nutrient and grazer manipulative field experiment

Study site water temperatures and salinities ranged from 26–30 °C and 34–41 ppt, respectively. Under similar water temperature conditions, Heck et al. (2000) measured mean field release rates to be 0.07 mmol NO₃⁻ g Osmocote⁻¹ day⁻¹, 0.08 mmol NH₄⁺ g Osmocote⁻¹ day⁻¹, and 0.01 mmol PO₄³⁻ g Osmocote⁻¹ day⁻¹. The weight losses of Osmocote fertilizer applied during this experiment averaged 20% or 783 mg day⁻¹ for 2 fertilization periods over the 60-day experiment. Based upon these measurements, the estimated nutrient application rates inside the fertilized treatments were 0.05 mmol NO₃⁻ day⁻¹, 0.06 mmol NH₄⁺ day⁻¹, and 0.01 mmol PO₄³⁻ day⁻¹.

Gastropods increased in all plots throughout the course of the experiment due to new recruitment. The periodic additions of gastropods to grazer-enhanced plots (+G) (Table 1) resulted in a significant 60% increase in total gastropod density at experiment end relative to grazer-reduced plots (Fig. 5, *t*-test, *t*=3.18, *p*<0.005).

Macroscopic epiphyte species composition did not change throughout the course of the experiment or between fertilization and grazer treatments. The observed epiphyte community was typical of that found at the site (Frankovich and Zieman, 1994), dominated by the coralline red algae *Hydrolithon farinosum* and *Melobesia membranacea* and attached diatoms. The only filamentous macroalgae present were occasional small (<1 cm) specimens of *Polysiphonia binneyi*.

Fertilization significantly increased mean total epiphyte loads by an average of 21% relative to unfertilized treatments (Fig. 6, repeated measures ANOVA, *F*=7.189, *p*=0.014). Under experimental conditions, mean total epiphyte loads were highest in fertilized treatments at all times.

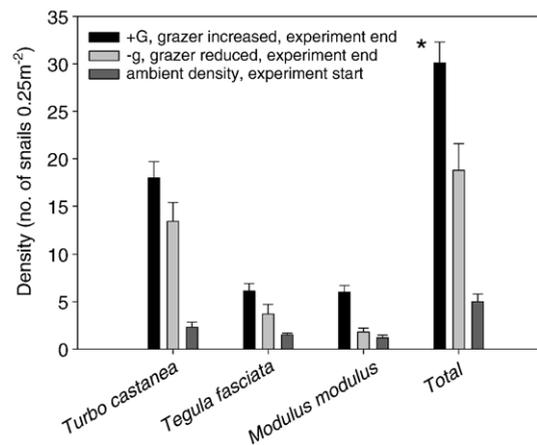


Fig. 5. Mean gastropod plot densities at experiment start and experiment end in gastropod manipulated plots. Bars = means (\pm s.d.) of *n*=6. (*) denotes statistical significance.

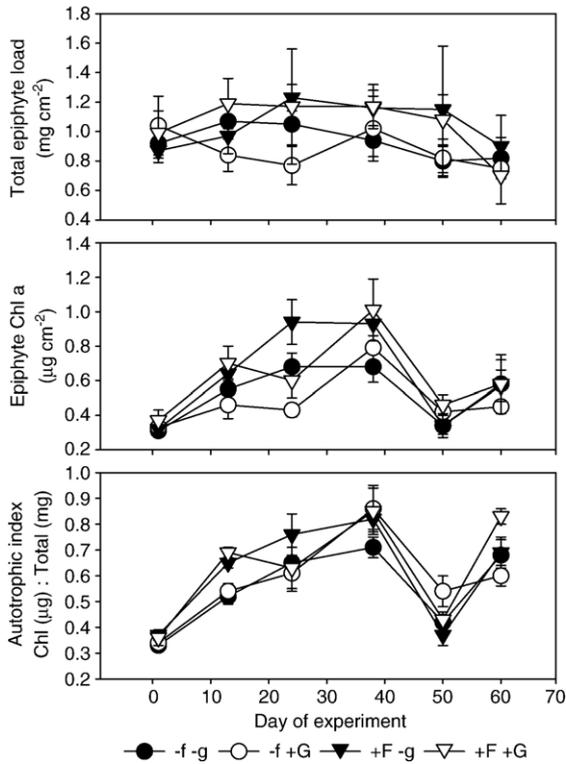


Fig. 6. Mean epiphyte loadings throughout the course of the experiment. (+G) denotes gastropod addition, (-g) denotes gastropod reduction, (+F) denotes nutrient addition, (-f) denotes ambient nutrients. Bars=means (\pm s.d.) of $n=6$.

Epiphyte chl *a* loads in all treatments increased from day 1 to day 38 then decreased by about 50% across all treatments to day 51 (Fig. 6). Fertilization significantly increased mean epiphyte chl *a* loads by an average of 25% relative to unfertilized treatments (repeated measures ANOVA, $F=24.109$, $p<0.001$). Within the experiment, mean epiphyte chl *a* loads were highest in fertilized treatments at all times.

The epiphyte autotrophic index (i.e., ratio between epiphyte chl *a* and total epiphyte load) ranged from 0.33 to 0.86 and due to the stability of total epiphyte loads, followed the general pattern observed in epiphyte chl *a* loads. The autotrophic index can be a useful measure in addition to chl *a* because of the diverse nature of seagrass epiphytes. This index allows the amount of chl *a* relative to their total mass to be assessed. In this experiment, while fertilization significantly increased epiphyte chl *a* by an average of 25% relative to unfertilized treatments, the autotrophic indices increased only 7% suggesting that the increased total epiphyte loads in fertilized treatments was caused by epiphytes with smaller amounts of chl *a* relative to their total mass (e.g., coralline algae). Both epi-

phyte chl *a* and autotrophic index exhibited significant temporal variation (repeated measures ANOVA, $F=10.455$, $p<0.001$ and $F=26.398$, $p<0.001$ respectively). In addition, fertilization significantly increased mean epiphyte autotrophic indices relative to unfertilized treatments (Fig. 6; $F=4.850$, $p=0.04$). Under experimental conditions, mean epiphyte autotrophic indices were highest in fertilized treatments 80% of the time (Fig. 6). Significant grazer main effects or grazer \times fertilization interactions on the measured epiphyte parameters were not observed.

At experiment end, mean *T. testudinum* leaf biomass ranged from 91 to 112 mg short-shoot⁻¹ (Fig. 7). Significant fertilizer or grazer main effects or grazer \times fertilization interactions on *T. testudinum* leaf biomass were not evident. *T. testudinum* leaf productivities ranged from 1.3 to 1.6 mg short-shoot⁻¹ day⁻¹ (Fig. 7) and were significantly increased within the fertilization treatments by 22% (Fig. 7; $F=7.467$, $p=0.013$). Significant grazer main effects or grazer \times fertilization interactions on *T. testudinum* leaf productivity were not evident.

T. testudinum leaf nutrient contents varied across fertilization and grazer treatments. Leaf nitrogen (N) content ranged from 2.2 to 2.5%, while leaf phosphorus (P) content ranged from 0.088 to 0.104%, respectively (Fig. 8). Fertilization significantly increased mean

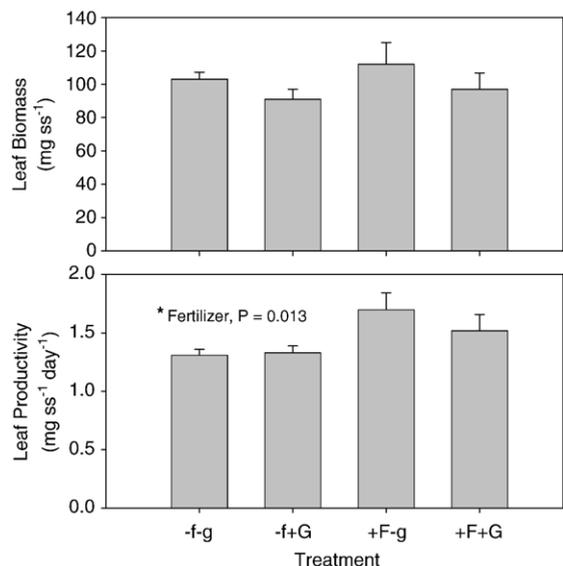


Fig. 7. Mean *Thalassia testudinum* leaf biomass and productivity measured at experiment end. (+G) denotes gastropod addition, (-g) denotes gastropod reduction, (+F) denotes nutrient addition, (-f) denotes ambient nutrients. Bars=means (\pm s.d.) of $n=6$. (*) denotes statistical significance.

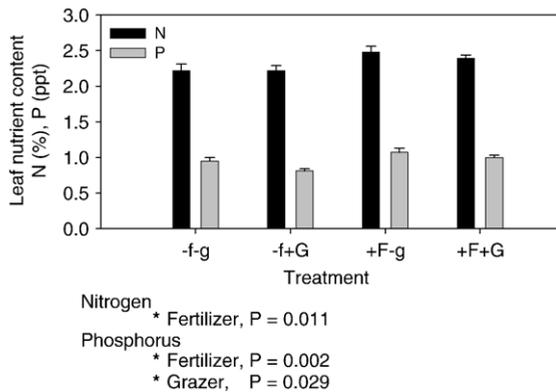


Fig. 8. Mean *Thalassia testudinum* leaf nitrogen and phosphorus content. (+G) denotes gastropod addition, (-g) denotes gastropod reduction, (+F) denotes nutrient addition, (-f) denotes ambient nutrients. Bars=means (\pm s.d.) of $n=6$. (*) denotes statistical significance.

leaf N 9.5% and leaf P 18% relative to unfertilized treatments indicating that the nutrient additions were biologically available to the plants (Fig. 8; $F=7.914$, $p=0.011$ and $F=12.729$, $p=0.002$, respectively). There were no significant grazer main effects or grazer \times fertilization interactions on leaf N. While gastropod additions resulted in a significant 10% decrease in leaf P relative to grazer-reduced treatments (Figure 8; $F=5.568$, $p=0.029$), grazer \times fertilization interactions on leaf P were not observed.

3.2. Leaf turnover field experiments

Manipulating the “leaf” turnover and “leaf” growth rate of *T. testudinum* short-shoot mimics at a summertime leaf turnover rate of $1.7\% \text{ day}^{-1}$ and a wintertime rate of $0.9\% \text{ day}^{-1}$ resulted in significant differences in epiphyte loading (Zar, 1996, t -test, $t=4.5$, $p<0.001$). The short-shoot mimics “growing” at wintertime turnover rates accumulated a 20% higher mean epiphyte load than that on summertime short-shoots. The epiphyte community present on the short-shoot mimics was composed of diatoms and adhered sediment and was indistinguishable from the community on natural *T. testudinum* leaves from the same area and time. Mean total epiphyte loads on the short-shoot mimics ranged from 0.14 to 0.17 mg cm^{-2} , similar to that on natural *T. testudinum* short-shoots from the same area and time (0.16 mg cm^{-2}).

4. Discussion

The results of these field experiments confirm the importance of nutrient availability and leaf turnover

rates in controlling epiphyte loads on *T. testudinum* in Florida Bay. Over the course of one leaf turnover period, water column nutrient enrichment increased epiphyte loads, seagrass leaf nutrient content, and seagrass leaf productivity without decreasing leaf biomass (Figs. 6, 7 and 8). Though the manipulation of gastropod grazer abundances failed to produce any differences in epiphyte loading or seagrass leaf biomass and productivity, increased gastropod densities resulted in decreased seagrass leaf phosphorus (Figs. 7 and 8).

Possible explanations for the observed changes in productivity without concomitant changes in leaf biomass include that leaf productivities as determined using the leaf-mark technique were less variable than biomass measurements. Coefficients of variation of leaf mark productivity were 20–34% lower than the coefficients of variation of the leaf biomass estimates. In addition, changes in leaf biomass per short-shoot are accomplished by changes in both leaf production and leaf senescence and therefore changes in leaf biomass are expected to be more variable if these processes are not tightly correlated. Finally, it is also possible that leaf senescence increased with increased leaf productivity compensating for increases in leaf production, but we do not have estimates of leaf senescence to comment on that possibility.

The accepted model for seagrass decline due to anthropogenic nutrient increase describes a shift from dense seagrasses with low to moderate epiphyte accumulations to sparse seagrasses with high epiphyte loads (Duarte, 1995). The high epiphyte loads cause decreases in the quantity and quality of light at the leaf surface, leading to decreased seagrass production and eventual seagrass loss (Duarte, 1995). However, Neckles et al. (1993) found that grazers, when present at natural field densities, in seagrass microcosms maintained low epiphyte loads under moderate nutrient enrichments. Later studies further indicated that grazer effects vary both regionally and seasonally according to natural abundances and metabolic needs of the grazers (Heck et al., 2000; Lotze et al., 2001; Hillebrand and Kahlert, 2002; Frankovich and Zieman, 2005b) and to the palatability of the algae present (Gacia et al., 1999). The ability of grazers to control epiphytes even under enriched conditions is now accepted. It is now theorized that algal biomass may be controlled in the long-term via a negative feedback loop in which grazers may respond to increased algal biomass of greater palatability by increasing their population, thus increasing grazing control (Hessen et al., 2002; Stelzer and Lamberti, 2002). This was not observed in this field experiment.

The manipulation of grazer densities to 60% above initial ambient field densities failed to produce changes in epiphyte loading (Fig. 6) or in species composition. The present study differs from previous gastropod density manipulations in that the effect of increased gastropod densities were compared to ambient field densities as opposed to the gastropod grazers' complete removal (Table 2). All of the listed previous studies (Van Montfrans et al., 1982; Hootsmans and Vermaat, 1985; Jernakoff and Nielsen, 1997; Nelson, 1997) successfully demonstrated that gastropod grazers consume epiphyte biomass and that higher epiphyte loads would result if these organisms were not present under the experimental conditions (Table 2). According to a scenario of anthropogenic eutrophication, the ability to control epiphyte loading depends on the grazers already present within the ecosystem. Testing the capability of the native grazing communities at or above their natural densities in controlling epiphyte loading is imperative to predicting eutrophication effects. The present study was conducted *in-situ* using fenced field enclosures as opposed to the previous gastropod manipulation studies using either aquaria or enclosed field chambers. Despite repeated stockings of gastropods to grazer-enhanced plots at densities up to 9 times initial density; densities in grazer-enhanced plots were only 60% greater at experiment end. This was due to ambient densities of gastropods increasing over the course of the experiment (Fig. 5). Nonetheless, the results of the gastropod density ma-

nipulations indicate that small increases in gastropod density were unable to produce decreased epiphyte loadings under ambient and nutrient-enriched conditions in the period of this study. This lack of grazer control on increasing epiphytic loads in the fertilizer plots can be understood in view of the autotrophic index that indicated the epiphytic response was primarily from epiphytes that had a low chl *a* content relative to their total biomass and that the observed epiphyte community in the fertilized plots was dominated by the coralline red algae *Hydrolithon farinosum* and *Melobesia membranacea*. The low level fertilization in this experiment while significantly increasing epiphytic loads did not result in a species shift in the epiphytic community. The epiphyte community was composed primarily of grazer resistant epiphytes. This suggests that low levels of nutrient enrichment may not lead to increased algal biomass of greater palatability that the epiphytic grazers respond to as suggested by the current theories of grazer control. The implications of this experiment is that a chronic low nutrient enrichment of the water column may disconnect the epiphyte loads from top-down control by maintaining and elevating grazer resistant epiphytes.

Levels of water column nutrient additions in our experiment were low relative to previous fertilization studies in Gulf of Mexico seagrass beds (Table 1). However, this low level of nutrient addition did result in higher epiphyte loadings (Fig. 6), higher leaf nutrient content (Fig. 8), and increased leaf turnover rate, suggesting that fertilized nutrients were taken up by both the seagrasses and epiphytes. The seagrasses and epiphytic algae in Florida Bay are very sensitive to nutrient inputs due to the nutrient limitation of primary production (Fourqurean et al., 1992a,b; Frankovich and Fourqurean, 1997). Though both nitrogen and phosphorus increased in the leaves of fertilized *T. testudinum* in the present study, the relative increase in phosphorus (18%) was almost double that of nitrogen (9.5%), indicating phosphorus limitation. The coupled positive response of epiphyte loadings and seagrass leaf productivities observed in the present study and that of Wear et al. (1999) may indicate that the applied nutrient loadings were below a threshold at which epiphyte levels become detrimental to seagrasses, at least during periods of rapid growth.

Higher fertilization rates applied in previous investigations produced changes in epiphyte species composition that were not observed in the present study (Table 1). Variations in nutrient availability may not have been large enough to allow species changes to result from the competitive advantages of faster growing algae with higher nutrient uptake rates (Littler and Littler, 1980).

Table 2
Effects of gastropod density manipulation on epiphyte loading

Reference	Gastropod species	Epiphyte biomass	Epiphyte chl <i>a</i>	Density manipulation
		(% change)	(% change)	
Van Montfrans et al. (1982)	<i>Bittium varium</i>	-63 ^a	No effect	30 Individuals leaf ⁻¹ vs zero
Hootsmans and Vermaat (1985)	<i>Littorina littorea</i>	-40 ^b	NA	Ambient field density vs zero
Hootsmans and Vermaat (1985)	<i>Hydrobia ulvae</i>	-U71 ^b	NA	Ambient field density vs zero
Jernakoff and Nielsen (1997)	<i>Thalotia conica</i>	-44 ^b	NA	Ambient field density vs zero
Nelson (1997)	<i>Lacuna variegata</i>	-85 ^a	-93	Highest natural density vs zero
Present study	<i>Turbo castanea</i> <i>Tegula fasciata</i> <i>Modulus modulus</i>	No effect ^a	No effect	60% increase vs ambient field density

NA—not available.

^a Biomass measured as dry weight.

^b Biomass measured as ash-free dry weight.

However, the lack of any species change may also be reflective of the observed low diversity of algal epiphytes in the very nutrient-limited Florida Bay (Frankovich and Zieman, 1994).

Seagrasses have adapted to epiphyte competition for light by shedding old leaves and producing new leaves from a basal meristem (Sand-Jensen, 1977; Bultuis and Woelkerling, 1983) which may allow them to mitigate moderate eutrophication effects by increasing their leaf turnover rate. Increased epiphyte loads have been observed during periods of low seagrass leaf productivity, leading some investigators to speculate that variation in leaf turnover rates could account for some of the observed variation in epiphyte accumulations and species assemblages (Humm, 1964; Frankovich and Zieman, 1994; Kendrick and Burt, 1997). In our experiment, manipulations of seagrass leaf turnover rate demonstrated increased epiphyte loads resulting from slow wintertime growth rates. In previous work, when leaf turnover rates were evaluated with grazing pressure and/or nutrients as controls on epiphyte loading, grazing influences and nutrient availability were better correlated with observed epiphyte loading (Borum, 1985, 1987; Tomasko and Lapointe, 1991; Frankovich and Zieman, 2005b). The determination of the relative importance of leaf turnover rates as an epiphyte loading control has proven difficult because of the co-varying nature of leaf productivities with other environmental variables. During summer months high leaf turnover rates often coincide with high grazer abundances, and during winter, leaf turnover rates decrease as do grazer abundances. The manipulation of leaf turnover rates has also been hindered by the inability to alter leaf productivities without changing environmental conditions (e.g., fertilization to increase the turnover rates of natural short-shoots). This study sought to overcome these obstacles through the use of seagrass short-shoot mimics to manipulate “leaf” turnover rates.

Epiphyte accumulations on the seagrass short-shoot mimics “growing” at slower wintertime leaf turnover rates accumulated 20% more epiphytes than those “growing” at summertime rates. The relatively small differences in epiphyte loading resulting from manipulations of leaf turnover rate under the same environmental conditions indicate that other factors such as changes in water column nutrients or grazer densities are more important than leaf turnover rates. Previous seasonal investigations of leaf turnover rates by Tomasko and Lapointe (1991) found that spatial differences in epiphyte loading on *T. testudinum* correlated more strongly with nutrient availability than with leaf turnover while Borum (1987) found that epiphyte bio-

mass was most strongly influenced by grazer abundance, followed by nutrient availability. Differences in seasonal leaf turnover rates of *Zostera marina* and *T. testudinum* were relatively insignificant in explaining observed epiphyte loading (Borum, 1987; Frankovich and Zieman, 2005b). Epiphyte biomass may be relatively insensitive to changes in leaf turnover rate because the turnover times for epiphytic algal biomass can be 4–5 times faster than the leaf substrates on seagrass short-shoots (Borum, 1987). Our manipulative field experiment on leaf turnover rates support the conclusions of these studies.

4.1. Conclusions

Realistic low-level water column nutrient additions significantly increased both epiphyte loading and seagrass leaf productivity in a natural *T. testudinum* meadow, while moderate increases in gastropod grazer abundances were unable to lower epiphyte accumulations. This experiment suggests that low levels of nutrient enrichment may not lead to increased epiphyte biomass of greater palatability that the epiphytic grazers respond to as suggested by the current theories of grazer control. The implications of this experiment is that a chronic low nutrient enrichment of the water column may disconnect the epiphyte loads from top-down control by maintaining and elevating grazer resistant epiphytes (e.g., coralline algae). Manipulations of “leaf” turnover at minimum wintertime and maximum summertime rates resulted in a significant 20% difference in mean epiphyte loading. The relatively small differences in epiphyte loading resulting from manipulations of leaf turnover rate under the same environmental conditions indicate that leaf turnover alone is not responsible for the dramatic differences in epiphyte loads between winter and summer. As such, these results suggest that epiphytic loading will increase on seagrass leaves even at low nutrient enrichment and that seagrasses are most vulnerable to increasing epiphyte loads during periods of slower leaf turnover rates. Therefore, even low levels of enrichment may be detrimental to seagrasses by increasing loads of grazer resistant epiphytes during the early spring months or late fall when leaf elongation rates are reduced at a time when metabolic demands of the plant are increased. The theory that algal biomass will be controlled in the long-term via a negative feedback loop in which grazers respond to increased algal biomass of greater palatability by increasing their population density, thus increasing grazing control may not be valid in situations of low nutrient enrichment.

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