



Amphipod control of epiphyte load and its concomitant effects on shoalgrass *Halodule wrightii* biomass

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ABSTRACT: We investigated the interaction between epiphyte-grazer abundance and eutrophication to assess the relative importance of top-down and bottom-up effects in subtropical seagrass meadows. In field experiments using a cageless technique to control amphipod abundance, we measured the effects of grazing and nutrient supply on the growth and productivity of shoalgrass *Halodule wrightii* and its epiphytes at both protected and wave exposed sites. Amphipod removal at the protected site resulted in 70% greater epiphyte loads on shoalgrass leaves and a 36% reduction in leaf biomass after 10 wk. At the wave-exposed site, where amphipod abundance was consistently low, we found no significant effects of grazer presence or nutrients on epiphyte load or leaf biomass. Average leaf length, however, was significantly reduced in nitrogen-enriched plots. Our results indicate that natural densities of amphipods can reduce seagrass loss by controlling epiphytes.

KEY WORDS: Herbivory · Amphipods · Epiphytes · Seagrass · Eutrophication · Field exclusion

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INTRODUCTION

Seagrasses provide important services to near-shore communities by serving as predation refuges for commercially and recreationally important fishes, stabilizing shorelines against erosion and through the production and transfer of energy to support higher trophic levels (Orth et al. 2006). Unfortunately, seagrasses are declining at an alarming rate worldwide (Short & Wyllie-Echeverria 1995, Orth et al. 2006, Waycott et al. 2009), and coastal areas are losing the suite of services that they provide. This decline is believed to be largely due to increases in coastal eutrophication (Orth et al. 2006, Short et al. 2006, Waycott et al. 2009), which are produced by the 40% of Earth's population that now lives within 150 km of the coastline (Cohen et al. 1997). Excess nutrients in the water column can be utilized by opportunistic primary producers, such as epiphytic

algae and phytoplankton, which reduce water clarity, overgrow seagrass leaves, and compete for water column nutrients (Cloern 2001, Hughes et al. 2004, Morris et al. 2007). As a result, seagrasses could suffer from reduced growth and the inability to recover from the stress induced by limited light and nutrient availability (van Montfrans et al. 1984, Dennison et al. 1993).

Herbivory is a primary determinant of the abundance and composition of marine primary producers, including seagrass epiphytes (Valentine & Heck 1999, Moncreiff & Sullivan 2001, Wressnig & Booth 2007), and epiphyte-grazers such as amphipods and isopods have the potential to relieve seagrasses from stress induced by increased eutrophication (Hughes et al. 2004, Heck & Valentine 2007). However, accurately measuring the effects of mesograzer herbivory has proven to be complicated, which has led to conflicting reports on

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its significance. For example, some researchers have found that amphipods produce large impacts on primary producers (Neckles et al. 1993, Duffy & Hay 2000, Graham 2002, Hughes et al. 2004, Heck & Valentine 2006, Heck et al. 2006, Davenport & Anderson 2007, Moksnes et al. 2008, Baggett et al. 2010), while others have found that amphipods and other mesograzers have little or no effect on epiphyte or seagrass growth (Keuskamp 2004, Jaschinski & Sommer 2008, Poore et al. 2009). This discrepancy may be partially explained by differences in the biology and life history of amphipod species, but it is also likely results from differences in the experimental conditions of grazing studies (Hughes et al. 2004).

The difficulty of controlling mesograzer abundances in the field due to their small size may contribute to differing conclusions on the importance of amphipod herbivory. Previous studies have relied on a number of different means of controlling mesograzer abundance, including the use of mesocosms (Howard & Short 1986, Duffy & Hay 2000, Duffy & Harvilicz 2001, Jaschinski & Sommer 2008), predators (Heck et al. 2000, Heck et al. 2006, Davenport & Anderson 2007, Moksnes et al. 2008, Andersson et al. 2009), and caging (Korpinen et al. 2007, Baggett et al. 2010). However, the experimental artifacts introduced by these methods may confound results, making it difficult to accurately measure the effects of grazing.

Fortunately, a novel technique developed by Poore et al. (2009) allows for the direct exclusion of amphipod mesograzers *in situ* without the use of cages. This method can simulate the effects of natural amphipod reductions, allowing a more direct investigation of the role of amphipod mesograzers in controlling epiphytic growth on macrophytes. Initially, Poore et al. (2009) found that natural densities of amphipods were unable to control the growth of the brown alga *Sargassum linearifolium* or its epiphytes. More recent work in *Posidonia sinuosa* seagrass meadows showed that amphipods were capable of limiting epiphyte growth on seagrass leaves, although concomitant effects on seagrass biomass were not observed (Cook et al. 2011).

Utilizing direct amphipod exclusion, we investigated the separate and interactive effects of amphipod abundance and increased nutrient loading on primary producers within shoalgrass *Halodule wrightii* meadows of Big Lagoon, Florida, USA. Our goal was to determine how grazing and nutrient supply impacted epiphyte abundance on shoalgrass leaves and the growth and productivity of *H. wrightii*.

MATERIALS AND METHODS

Study area

The study was conducted in Big Lagoon, Florida, within the Perdido Bay system of the north central Gulf of Mexico (30° 18.300' N, 87° 25.250' W and 30° 18.570' N, 87° 24.330' W). Salinity in Big Lagoon ranges from 13 to 30 psu annually (Spitzer et al. 2000), temperature varies seasonally from ~7 to 30°C (Heck et al. 2006), and the mean tidal range is 0.5 m (Spitzer et al. 2000). Summer water column chlorophyll *a* values are low, ranging from 0.17 to 6.16 µg l⁻¹ (Heck et al. 2006). Nutrient levels are also relatively low in summer, ranging from 0.01 to 2.73 µM nitrate, 0.3 to 2.6 µM ammonium, 0.0 to 15.35 µM silicate and 0.0 to 0.14 µM phosphate (Heck et al. 2006).

Two sites containing large monotypic stands of shoalgrass *Halodule wrightii* were chosen to establish experimental plots. One site received relatively low wave energy due to its position behind a small island (fetch <200 m), while the second was more exposed and received greater wave energy (fetch 900 m). These protected and exposed sites were spaced ~1 km apart.

Experimental design and treatments

A 3 × 3 factorial ANOVA design was employed in which we manipulated the abundance of amphipods (Present, Suppressed, and Control) and nutrient level (Enriched, Ambient, and Control). The 9 treatments were replicated 10 times each within 90 plots of *Halodule wrightii*, and plots were evenly divided among the wave-protected and -exposed sites. Plots were spaced ~1.5 m apart at a depth of 1 m. The experiment ran for 10 wk from March 24 to June 2, 2010, to ensure elevated season amphipod densities. Amphipod abundances within the northern Gulf of Mexico peak in late-spring/early-summer but then experience yearly lows in late-summer coinciding with a peak in the abundance of predatory juvenile pinfish (Stoner 1980).

Amphipod suppression

The presence of amphipods was controlled through the use of commercially available Sevin, a rapidly degrading carbaryl pesticide capable of directly excluding amphipods from plots. This was accomplished by dissolving 189 g of wettable car-

baryl powder (1-naphthyl-*N*-methylcarbamate) into 1050 ml of cold water and then incorporating this solution into a slow-release matrix of 1800 g of dental plaster (sensu Poore et al. 2009). This mixture was poured into 100 ml molds and dried to create ~350 g of carbaryl-plaster blocks. The use of carbaryl pesticide has been previously shown to target amphipod mesograzers, while having no measurable effect on isopods, gastropods, or the growth of algae (Carpenter 1986, Poore et al. 2009). In treatments where amphipods were to be suppressed, 3 carbaryl-plaster blocks were placed 30 cm equidistant from one another in a triangle to create an area of amphipod exclusion $>0.33 \text{ m}^2$. To control for any possible effect that the presence of a plaster block might have within plots, a control treatment consisted of plots containing blocks made entirely of dental plaster with no carbaryl pesticide. In the field, these blocks lost ~40% of their mass after 1 wk of exposure, so all blocks were replaced after 7 d. Blocks were dried and weighed after being removed from the field to ensure that the amount of carbaryl loading was equal among treatments over the 7 d period. In plots where amphipods were present at ambient densities, no plaster blocks were added. To measure the effectiveness of the carbaryl-plaster blocks, 20 cm diameter cores were taken at the end of the experiment from a subsample of plots from which mesograzer composition and abundance was estimated. Cores were passed over a 500 μm sieve and frozen until fauna could be sorted and enumerated in the lab.

Nutrient addition

The effect of nutrient enrichment was assessed by increasing water column nutrient load using Osmocote™ pellet fertilizer (19:6:12, C:N:P). Nylon stockings were filled with 400 g of fertilizer and then inserted into a 22 cm PVC tube perforated with 1 cm holes. A single PVC fertilizer tube was suspended above the sediment surface within each plot of the enriched treatments. Water advection through the holes in the tube dissolved the pellet fertilizer and enriched the water surrounding the plots. Since the additional structure of the PVC tube could incidentally promote increased amphipod abundance within plots, a control treatment contained an empty PVC tube. All tubes were replaced after 5 wk to ensure continued enrichment and to minimize fouling on the PVC. The ambient-nutrient treatments had no PVC tubes or fertilizer added to plots. To

ensure significant enrichment, total percent carbon and nitrogen were determined from a subsample of seagrass leaves collected from each treatment. The leaf samples were dried at 70°C and then ground to a powder using a mortar and pestle before processing in an automated CHN analyzer (Carlo Erba NA 1500).

Sample collection and processing

Sampling occurred midway through the experiment and again after 10 wk. Samples were taken using a modified 'clip and harvest' method as described in Hauxwell et al. (2001). A thin 8 cm diameter PVC ring was secured to the sediment surface within each plot, and all above-ground leaf material within the ring was clipped at the sediment–water interface and collected. In the lab, this above-ground material (a total of $>10\,000$ seagrass leaves) was processed to measure leaf density, and average length was determined by measuring a subsample of 36 haphazardly selected leaves from the collections at each plot ($>33\%$ of total leaves). Epiphytes consisted primarily of filamentous red and green macroalgae and were scraped from all seagrass leaves using a glass slide. Both leaves and epiphytes were then separately dried to a constant weight at 70°C. Leaf biomass was calculated by dividing leaf dry weight by the area of the PVC ring (50.26 cm^2), and epiphyte load was calculated by dividing the dry weight of epiphytes by the dry weight of seagrass leaves for each collection. Once biomass was calculated, the dried samples of seagrass leaves were used to determine total percent carbon and nitrogen for each nutrient treatment using an automated CHN analyzer (Carlo Erba NA 1500).

The shortened patches of seagrass created by clipping within the PVC rings were then given time to allow for leaf regrowth. After 2 wk, an 8 cm core was taken from within the PVC ring to harvest all above-ground and below-ground material. This material was then separated and dried at 70°C to obtain individual dry weights for above-ground and below-ground material. The above-ground dry weights were used to estimate net primary production within plots by dividing leaf weight by the length of time given for regrowth (2 wk) and the area of the core (46.83 cm^2). This 'clip and harvest' method has been shown to underestimate leaf production by 38–56% (Hauxwell et al. 2001); however, it is a useful metric to compare relative changes in production rates among treatments. The dry weights for the below-

ground roots and rhizomes were calculated to determine if any changes occurred due to increased stress of physical disturbance and reduced light availability.

Statistical analyses

The loss of experimental replicates from the exposed study site (discussed later in the 'Results') prevented the use of a 3-way ANOVA using amphipod presence, nutrient enrichment, and site as factors due to issues of orthogonality. Instead, independent 2-way ANOVAs were performed with amphipod presence and nutrient enrichment as factors for the protected and exposed sites to determine if changes occurred in epiphyte loading, seagrass leaf biomass, leaf length, density, production, or below-ground biomass. Before statistical analyses were carried out, all data were tested for homoscedasticity with Cochran's *C*-test, and a square-root transformation was used to homogenize variance when necessary. A Shapiro-Wilk test was used to assess normality of data. If no interaction was found between the main factors, Tukey's post hoc test was used to make pairwise comparisons between treatments when a significant response was found ($p < 0.05$).

RESULTS

Higher wave energy at the exposed site led to greater weekly dissolution of carbaryl-plaster blocks when compared to the weight loss at the protected site (40–75% less weight loss at protected site; Student's $t_{(18)} = 6.48$, $p < 0.001$; Fig. 1). Total percent nitrogen in seagrass leaf material was significantly greater in fertilized treatments when compared to the levels observed in ambient and control treatments ($F_{2,45} = 69.70$, $p < 0.001$; Table 1). On average, nitrogen content was 17.5% greater for fertilized than ambient seagrass. While there was also a significant increase in percent carbon content in fertilized treatments ($F_{2,45} = 25.60$, $p < 0.001$; Table 1), this difference was small, and C:N ratios remained significantly different among enriched and ambient nutrient treatments ($F_{2,45} = 62.42$, $p < 0.001$; Table 1). The use of pesticide did not significantly alter nitrogen content in seagrass leaves ($F_{2,45} = 0.92$, $p > 0.313$). Likewise, the difference in wave exposure between study sites had no effect on leaf enrichment ($F_{1,48} = 0.37$, $p > 0.546$).

Mesograzer composition

Mesograzer composition at both sites was comprised primarily of gammarid amphipods and cymothoid isopods (32 and 62% of total mesograzer abundance, respectively). At the wave-protected site, there were significant pesticide effects on amphipod abundance among treatments ($F_{2,9} = 38.64$, $p < 0.001$; Fig. 2A), and carbaryl pesticide reduced amphipod abundance by 92%. It was anticipated that the additional structure provided by plaster control blocks might promote increased amphipod densities. Instead, the opposite occurred, as amphipod densities in control treatments ($1305 \pm$

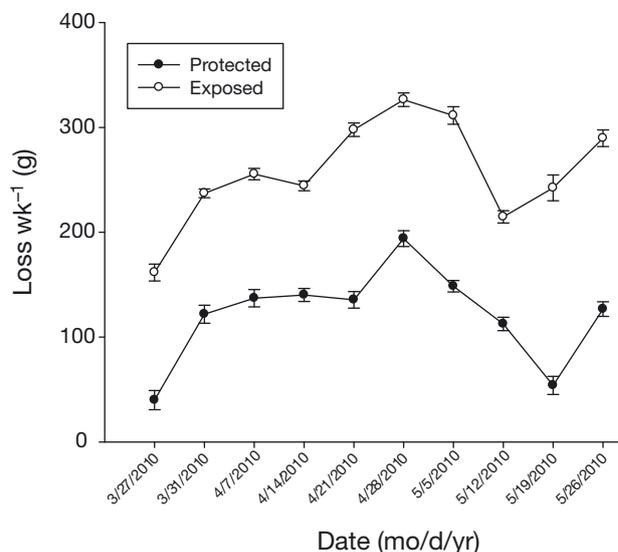


Fig. 1. Mean (\pm 1SE) weekly weight loss of carbaryl-plaster blocks at the protected and exposed sites

Table 1. *Halodule wrightii*. Nitrogen and carbon content (g g^{-1} ash-free weight [AFDW] shoalgrass $\times 10^{-5}$) in enriched and ambient shoalgrass leaves at protected and wave exposed sites (mean \pm 1SE). The results of Tukey's (HSD) post hoc analyses are reported for each 2-way ANOVA between site and nutrient enrichment performed for nitrogen, carbon and carbon:nitrogen ratios. Different superscripts indicate significantly different values at $p < 0.05$

Treatment	Nitrogen	Carbon	C:N
Wave-protected			
Enriched	2.64 \pm 0.02 ^a	42.66 \pm 0.08 ^c	16.19 \pm 0.12 ^e
Ambient	2.22 \pm 0.02 ^b	42.11 \pm 0.12 ^d	18.94 \pm 0.16 ^f
Control	2.20 \pm 0.03 ^b	42.17 \pm 0.05 ^d	19.17 \pm 0.24 ^f
Wave-exposed			
Enriched	2.57 \pm 0.06 ^a	42.72 \pm 0.10 ^c	16.52 \pm 0.33 ^e
Ambient	2.24 \pm 0.03 ^b	42.24 \pm 0.08 ^d	18.91 \pm 0.26 ^f
Control	2.29 \pm 0.05 ^b	42.24 \pm 0.07 ^d	18.54 \pm 0.37 ^f

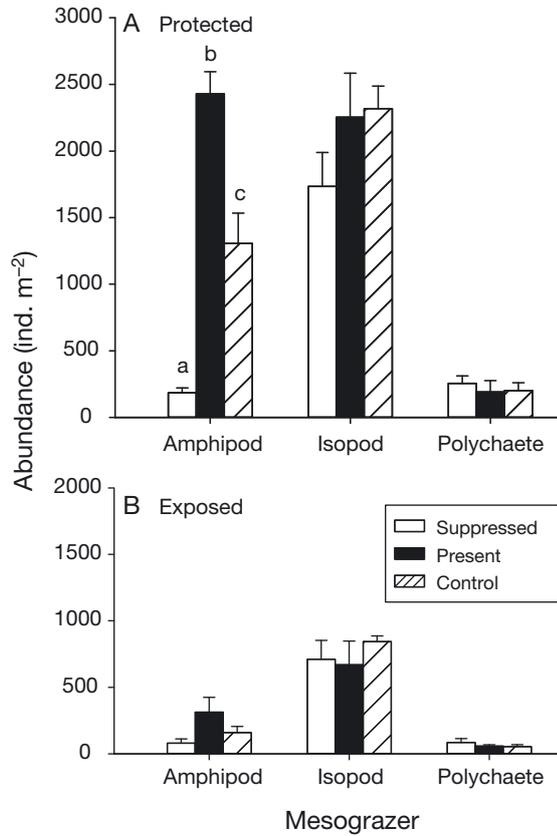


Fig. 2. Mean (+1SE) mesograzzer composition at (A) protected and (B) exposed sites. Amphipod removal (white bars), amphipod presence (black bars), and control (hatched bars). Letters above bars group treatments with significantly different means

228 ind. m⁻²) were significantly less than densities from treatments containing no blocks (2430 ± 165 ind. m⁻²). The use of carbaryl pesticide did not significantly reduce isopod and polychaete abundances ($F_{2,9} = 1.86$, $p > 0.21$ and $F_{2,9} = 0.343$, $p > 0.719$, respectively; Fig. 2A).

At the wave-exposed site, the carbaryl pesticide had no significant effect on the abundances of amphipods, isopods, or polychaetes ($F_{2,9} = 2.04$, $p > 0.186$; $F_{2,9} = 0.49$, $p > 0.628$; and $F_{2,9} = 0.61$, $p > 0.565$, respectively). In separate 2-way ANOVAs between site and pesticide treatment, a significant interaction was found only for amphipod abundance ($F_{2,30} = 21.12$, $p < 0.001$). However, the abundances of all mesograzers (amphipods, isopods, and polychaetes) were significantly lower at the exposed site compared to the protected site ($F_{1,30} = 118.13$, $p < 0.001$; $F_{1,30} = 65.37$, $p < 0.001$; and $F_{1,30} = 13.41$, $p < 0.001$, respectively) and were consistently reduced across all pesticide treatments (Fig. 2B).

Protected site

At the mid-point of the experiment, epiphyte loads on *Halodule wrightii* were not significantly different among treatments ($F_{3,36} = 0.80$, $p > 0.600$). However, after 10 wk epiphyte loads showed a significant interaction between amphipod presence and nutrient enrichment ($F_{4,36} = 2.89$, $p < 0.036$; Fig. 3A, Tables 2 & 3). On average, epiphyte loads on shoalgrass were nearly 70% greater when amphipods were absent (359 ± 46 mg g⁻¹) than when they were present (210 ± 15 mg g⁻¹). The treatment which combined low amphipod abundances with high nutrient loading produced the greatest values for epiphyte load (489 ± 103 mg g⁻¹; Fig. 3A).

Shoalgrass leaf biomass also showed a significant interaction between amphipod abundance and nutrient enrichment ($F_{4,36} = 2.66$, $p < 0.048$; Fig. 3B, Tables 2 & 3). In plots where epiphyte loads were significantly reduced by amphipods, shoalgrass leaf

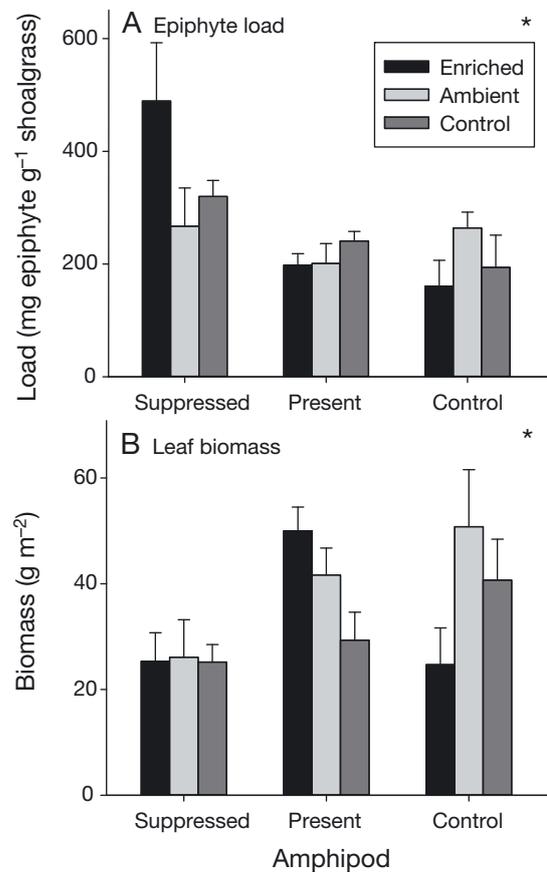


Fig. 3. Mean (+1SE) response of amphipod presence and nutrient addition on (A) epiphyte load and (B) *Halodule wrightii* leaf biomass at the protected site after 10 wk. Load and biomass are dry weights. Asterisks signify significant interaction (see text)

Table 2. Results of Tukey's (HSD) post hoc analysis for epiphyte load and *Halodule wrightii* leaf biomass at the protected site after 10 wk. q: studentized range. Significant values ($p < 0.05$) are in **bold**. For all tests, $df = 2$

Contrast	Mean difference	q	p
Epiphyte load			
Amphipod presence			
Suppressed vs. Present	0.145	4.850	0.004
Suppressed vs. Control	0.152	5.083	0.003
Present vs. Control	0.007	0.233	0.985
Nutrient level			
Enriched vs. Ambient	0.039	1.289	0.637
Enriched vs. Control	0.031	1.035	0.747
Ambient vs. Control	-0.008	0.254	0.982
Leaf biomass			
Amphipod presence			
Suppressed vs. Present	-0.067	3.894	0.024
Suppressed vs. Control	-0.060	3.473	0.049
Present vs. Control	0.007	0.421	0.952
Nutrient level			
Enriched vs. Ambient	-0.035	2.045	0.329
Enriched vs. Control	0.007	0.424	0.952
Ambient vs. Control	0.028	1.621	0.493

biomass averaged 54 % greater ($39.5 \pm 3.2 \text{ g m}^{-2}$ compared to $25.5 \pm 2.9 \text{ g m}^{-2}$); however, no significant differences were found in leaf density, average leaf length, net leaf production, or below-ground biomass (Table 3).

Exposed site

Large blooms of ephemeral drift macroalgae were found in plots at the wave-exposed site. Also, the shoalgrass within some plots was displaced by turtlegrass *Thalassia testudinum* during the course of the experiment. These factors resulted in the loss of a large number of experimental plots from the exposed site. For analysis, plots that contained fewer than 5 shoalgrass shoots were considered to be unacceptably altered by drift algae or *T. testudinum* and were not used in analyses (20 of 45 plots). Of the plots that remained, the enriched nutrient treatment consisted of 3 replicates of each amphipod level (suppressed, present, and control); the ambient nutrient treatment consisted of 3 replicates of the suppressed and present amphipod levels, and 2 replicates of amphipod control, and the nutrient control treatment consisted of 2 replicates of the suppressed and present amphipod levels, and 4 replicates of amphipod control. As the remaining replicates were relatively balanced across treatment levels, it was determined that the

use of a 2-way ANOVA between amphipod presence and nutrient enrichment was appropriate for further analysis of the exposed study site.

At the mid-point of the experiment, epiphyte loads on shoalgrass were not significantly different among treatments ($F_{8,36} = 0.52$, $p > 0.832$). After 10 wk, there were still no significant treatment effects on epiphyte loads among treatments ($F_{8,16} = 0.85$, $p > 0.575$). However, there was a significant nutrient effect on leaf length among treatments at the end of the experiment ($F_{2,16} = 4.55$, $p < 0.027$; Fig. 4, Table 4). Average leaf length was 25 % less in enriched treatments ($69.2 \pm 6.6 \text{ mm}$) compared to ambient and control treatments ($94.5 \pm 6.2 \text{ mm}$ and $94.9 \pm 7.3 \text{ mm}$, respectively), although there were no significant changes in leaf biomass, density, rate of production, or below-ground biomass (Table 4).

DISCUSSION

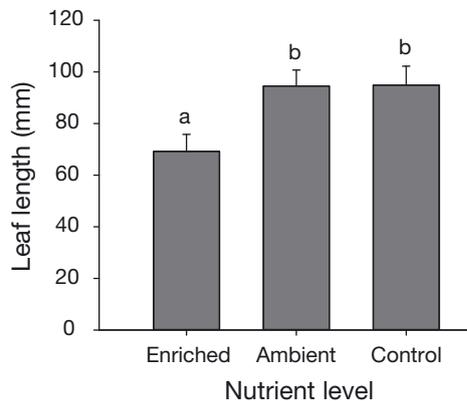
The cageless amphipod exclusion technique described in Poore et al. (2009) proved to be highly effective at suppressing amphipod presence in field conditions, making it a viable method for evaluating the impacts of amphipod grazers in Gulf of Mexico shoalgrass meadows. However, as seen in our study, the utility of this method is dependent on amphipods being present in sufficiently high initial densities. Similar to adding additional nutrients to an already eutrophic system, reducing the abundance of amphipods from already low initial densities is likely to produce a weak response. Unfortunately, we cannot report on the identity of amphipod species comprised within our study and are therefore unable to draw conclusions based on specific amphipod diets or feeding strategies. However, we found that the reduction in total amphipod abundance achieved in our study reflects values recorded in previous cageless exclusion studies. At our wave-protected site, we measured 92 % reduced amphipod densities in plots with carbaryl, compared to 94 and 81 % reductions recorded by Poore et al. (2009) and Cook et al. (2011), respectively. However, initial amphipod densities for our study (334 ind. g^{-1} seagrass) were substantially greater than those of Poore et al. (2009) (12 ind. g^{-1} alga) and Cook et al. (2011) ($20\text{--}65 \text{ ind. g}^{-1}$ seagrass). It has been previously suggested that a threshold of amphipod abundance must be surpassed in order to control epiphyte growth on some benthic producers (Andersson et al. 2009). Poore et al. (2009) found no evidence for the control of epiphyte growth on brown macroalgae when average natural amphipods densi-

Table 3. Results of 2-way ANOVAs for protected site after 10 wk. DW: dry weight. Significant values ($p < 0.05$) are in **bold**

Source of variation	df	MS	F	p
Epiphyte load (g epiphyte g⁻¹ seagrass)				
Nutrients	2	0.006	0.466	0.631
Amphipods	2	0.111	8.236	0.001
Nutrients × Amphipods	4	0.039	2.889	0.036
Residuals	36	0.013		
Total	44			
Leaf mass (g DW)				
Nutrients	2	0.005	1.165	0.323
Amphipods	2	0.021	4.566	0.017
Nutrients × Amphipods	4	0.012	2.663	0.048
Residuals	36	0.005		
Total	44			
Leaf length (mm)				
Nutrients	2	893.407	1.118	0.338
Amphipods	2	603.803	0.755	0.477
Nutrients × Amphipods	4	720.947	0.902	0.473
Residuals	36	799.291		
Total	44			
Leaf number (no. of leaves)				
Nutrients	2	185.267	0.071	0.932
Amphipods	2	806.467	0.309	0.736
Nutrients × Amphipods	4	2605.333	0.998	0.421
Residuals	36	2610.233		
Total	44			
Below-ground biomass (g DW)				
Nutrients	2	0.009	0.257	0.775
Amphipods	2	0.027	0.724	0.494
Nutrients × Amphipods	4	0.051	1.373	0.270
Residuals	36	0.027		
Total	44			
Leaf production (g DW d⁻¹ m⁻²)				
Nutrients	2	0.001	0.130	0.878
Amphipods	2	0.000	0.004	0.996
Nutrients × Amphipods	4	0.009	0.916	0.470
Residuals	36	0.010		
Total	44			

Table 4. Results of 2-way ANOVAs for exposed site after 10 wk. DW: dry weight. Significant values ($p < 0.05$) are in **bold**

Source of variation	df	MS	F	p
Epiphyte load (g epiphyte g⁻¹ seagrass)				
Nutrients	2	0.010	0.828	0.455
Amphipods	2	0.002	0.130	0.879
Nutrients × Amphipods	4	0.015	1.222	0.340
Residuals	16	0.008		
Total	24			
Leaf mass (g DW)				
Nutrients	2	0.005	1.007	0.387
Amphipods	2	0.011	2.003	0.167
Nutrients × Amphipods	4	0.009	1.585	0.226
Residuals	16	0.005		
Total	24			
Leaf length (mm)				
Nutrients	2	1548.334	4.550	0.027
Amphipods	2	545.086	1.602	0.232
Nutrients × Amphipods	4	434.564	1.277	0.320
Residuals	16	340.280		
Total	24			
Leaf number (no. of leaves)				
Nutrients	2	2868.315	1.530	0.247
Amphipods	2	2695.770	1.438	0.266
Nutrients × Amphipods	4	2385.860	1.273	0.322
Residuals	16	1874.615		
Total	24			
Below-ground biomass (g DW)				
Nutrients	2	0.101	0.601	0.564
Amphipods	2	0.030	0.179	0.839
Nutrients × Amphipods	4	0.136	0.807	0.544
Residuals	16	0.168		
Total	24			
Leaf production (g DW d⁻¹ m⁻²)				
Nutrients	2	0.011	1.682	0.227
Amphipods	2	0.001	0.157	0.856
Nutrients × Amphipods	4	0.001	0.143	0.963
Residuals	16	0.007		
Total	24			

Fig. 4. *Halodule wrightii*. Mean (+1SE) leaf length at the exposed site after 10 wk. Letters above bars group treatments with significantly different means

ties were 12 ind. g⁻¹ alga. This value is less than the densities recorded in our study from treatments where amphipods were suppressed (45 ind. g⁻¹ seagrass) or where initial densities were low at the wave-exposed site (34 ind. g⁻¹). While our study reports significant grazing impacts under high amphipod densities, Cook et al. (2011) found that amphipods only affected epiphyte loads in *Posidonia sinuosa* meadows, despite densities being greater in *Amphibolis* spp. meadows (19.62 vs. 65.23 ind. g⁻¹ seagrass, respectively). As such, it is likely that the magnitude of epiphyte herbivory will be influenced by a number of factors in addition to the density of amphipods.

While epiphyte loads at our wave-protected site were significantly affected by the manipulation of

grazer density and nutrient supply, similar changes in epiphyte load were not seen at the wave-exposed site. We believe that this was primarily a result of the low initial amphipod densities at the exposed site; however, an alternative explanation for the difference between sites could be the high variability and reduced number of replicates at the exposed site. A large area of the exposed site was displaced by drift macroalgae and turtlegrass *Thalassia testudinum*, and many plots contained no *Halodule wrightii* by the end of the experiment. To determine if low treatment replication and high variability resulted in no significant epiphyte changes, we used equations for calculating Cohen's *d* for epiphyte loads between protected and exposed sites. Cohen's *d* can be used to estimate the appropriate sample size required to achieve an effect size at our exposed site similar to what was measured for epiphyte load at our protected site (Cohen 1992). With a large effect size ranging from 0.803 to 0.962 for the change in epiphyte load measured at the protected site, our reduced sample size at the wave-exposed site ($N = 25$) lies within the range indicated by Cohen's *d* ($N = 19$ – 26). This suggests that reduced replication was not responsible for the non-significant results found for changes in epiphyte loads among treatments at the exposed site. Instead, this is likely the result of other factors, such as the low initial amphipod abundances recorded at the exposed site (Fig. 2B).

In contrast to the frequently stated notion that increased nutrient loading leads to the decline of seagrasses through increased epiphyte growth (Short & Neckles 1999, Burkholder et al. 2007), our study found that nutrient enrichment alone did not explain the changes in epiphyte load in shoalgrass meadows of Big Lagoon, Florida. Rather, the removal of amphipod mesograzers had the greatest impact on epiphytes and seagrasses, with increased epiphyte loads resulting in decreased seagrass leaf biomass when amphipods were removed. This supports a growing body of literature that suggests that bottom-up controls on nearshore benthic primary producers can be outweighed by the impacts of top-down grazing effects (Neckles et al. 1993, Heck & Valentine 2007, Moksnes et al. 2008, Baden et al. 2010, Baggett et al. 2010). For example, nearly 20 yr ago Neckles et al. (1993) found that enriching ambient nitrogen and phosphate concentrations to 3 times greater levels did not increase epiphyte growth sufficiently to overcome grazing pressure by mesograzers in a 2 mo microcosm experiment. The role of nutrients in driving epiphyte growth on seagrasses, resulting in seagrass decline, has been shown repeatedly to depend

on a number of other factors, most importantly including the structure and abundance of grazers (Heck & Valentine 2007, Moksnes et al. 2008, Baden et al. 2010).

It is important to better understand the factors that influence where and when mesograzer function is removed from benthic communities. Mesograzers face multiple threats from biological, chemical, and physical disturbance to benthic habitats. We found that increased wave energy at our exposed site may have led to reduced amphipod abundances (but see Fenwick 1976). Our study also demonstrates the threat of coastal pesticide addition to functionally remove amphipods from shoalgrass meadows, resulting in a 36% decrease in seagrass biomass. The impacts to seagrasses may be further compounded if the addition of pesticides is also coupled with fertilizer input from non-point sources, as our study found an interactive effect of nutrient enrichment and amphipod removal on epiphyte loading. At our wave-exposed site, average leaf lengths were significantly shorter for shoalgrass exposed to increased nutrient loading (Fig. 4). As such, it may be beneficial to focus restoration efforts towards protecting epiphyte-grazer communities by controlling pesticide use, in addition to improving water quality. Many farmers are likely practicing risk aversion by over-using pesticides and fertilizer in order to avoid the risk of lower crop yield on profits (Horowitz & Lichtenberg 1994). It may be possible to reduce agricultural pesticide and fertilizer input without significantly reducing farmers' yield through better public awareness and education.

Increased predation pressure is an additional threat which can lead to the localized and seasonal removal of amphipods from benthic communities. In the northern Gulf of Mexico, amphipod abundances exhibit seasonal highs and lows which are dictated by the yearly peak in the effects of predatory juvenile pinfish *Lagodon rhomboides* in late summer (Stoner 1980). Trophic cascades caused by the removal of large commercially important fishes can also lead to increased predation pressure on mesograzers by intermediate consumers (Heck et al. 2000). For example, along the west coast of Sweden, where amphipod abundances are often low throughout the year, reducing the predation pressure on mesograzers can result in the rapid recovery of amphipod densities to values greater than 4000 ind. m^{-2} (Andersson et al. 2009). By restoring epiphyte-grazer communities, it may be possible to influence seagrass growth and productivity by controlling the overgrowth of ephemeral algae.

While amphipods were found to have substantial influence on shoalgrass and epiphytes, amphipods were not the most abundant mesograzers within our shoalgrass sites. Regardless of pesticide treatment, isopods contributed the largest proportion to total mesograzers composition at both our exposed and protected sites (75 and 58% of total mesograzers abundance, respectively). However, it has been shown that isopod diet is species-specific, as some species in the northern Gulf of Mexico prefer seagrass tissue over epiphytes (Boström & Mattila 2005). As such, natural densities of isopods in treatments where amphipods had been removed were not sufficient to control the growth of epiphytes on shoalgrass leaves in our study. This suggests that the contribution of isopods to controlling epiphyte growth is substantially weaker than that of amphipods and may be confounded by direct consumption of seagrass material. It also supports the findings of Boström & Mattila (2005), who estimated that isopods at moderate densities (100 ind. m⁻²) could take as long as 8 wk to remove the average standing crop of epiphytes found on *Thalassia testudinum* in the northern Gulf of Mexico. The presence of amphipods, however, reduced epiphyte loads in our study by 40% (from 359 to 210 mg algae g⁻¹ seagrass), reflecting similar values for median epiphyte load (376 mg g⁻¹) recorded in previous studies (Frankovich & Fourqurean 1997).

Increased shading stress caused by greater epiphyte loads is expected to cause seagrasses to rely more heavily on nutrient stores from their roots and rhizomes, leading to a decrease in the biomass of these below-ground materials. Shoalgrass *Halodule wrightii* is characterized as having a shallow root system with little rhizome storage capacity; however, it was anticipated that changes in below-ground biomass would take longer to manifest than the changes to leaf and epiphyte responses. A previous study found that the response to above-ground disturbance can take a full year to manifest itself in the below-ground biomass of some seagrasses (Di Carlo & Kenworthy 2008). In our 10 wk study, increased nutrient load and the reduction of epiphyte grazer abundances resulted in no significant change to shoalgrass below-ground biomass. In addition, results indicated no significant treatment effects on our estimates of leaf production. This suggests that shoalgrass below-ground stores were able to compensate for the increased stress over the 10 wk of the experiment. Moreover, rhizome stores were sufficient to support the regrowth capabilities of *H. wrightii* leaves during the 2 wk following an acute disturbance (i.e. the removal of >80% of the above-ground

material). While chronic exposure to elevated nutrient levels and the absence of epiphyte grazers may lead to declines in shoalgrass health, our results show no immediate reduction of below-ground biomass and leaf production in the presence of this stress.

Future research should consider the appropriate timescale when evaluating the interactions among seagrasses, epiphytes, and mesograzers, as the duration of disturbance could drastically affect the outcome. In our study, we found little change in our system within the first 5 wk of monitoring, but after 10 wk both epiphytes and seagrass showed significant response to the removal of mesograzers and addition of nutrients. Similar results were found in a 3 mo study by Howard & Short (1986), where cultures of epiphytized *Halodule wrightii* were grown in tanks either with or without a suite of mesograzers. They found an immediate reduction in epiphyte loading after 3 wk of grazer presence but did not observe measurable changes to the growth and morphology of seagrass until nearly 2 mo of sustained disturbance had occurred. This suggests that the sustained removal of epiphyte grazers (1.5–2 mo) may be required for a response to manifest in seagrasses. Further investigations into the dynamics among mesograzers, epiphytes, and seagrasses should take care to ensure that improper experimental timescales do not result in significant changes going unobserved.

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