

# Effects of nutrient enrichment and grazing on shoalgrass *Halodule wrightii* and its epiphytes: results of a field experiment

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**ABSTRACT:** We assessed the individual and combined effects of removing large predators and enriching water column nutrients on shoalgrass *Halodule wrightii* meadows in Big Lagoon, Florida, USA. To simulate the first-order effects of large predator reductions, we stocked 2.0 m<sup>2</sup> enclosures with elevated (~3 to 4× ambient) densities of the omnivorous pinfish *Lagodon rhomboides*, the dominant fish in local seagrass habitats, and we supplemented N and P in the water column to nearly 3× ambient levels. Monthly determinations of water column nutrients and chlorophyll *a* (chl *a*), coupled with bimonthly measurements of leaf epiphyte biomass, seagrass growth and biomass, and beginning and ending comparisons of mesograzers abundance, were used to evaluate the effects of increasing nutrient supply and changing food web structure. Results showed significant predator and nutrient effects, although there were fewer consumer effects and more negative nutrient effects on seagrasses than in our previous experiments, which had shown that mesograzers ameliorated the harmful effects of elevated nutrients on seagrasses. Epiphyte proliferation in enrichment treatments did not occur; thus, algal overgrowth could not explain the negative effects of nutrient loading on seagrass biomass. Instead, nutrient loading resulted in nitrogen-rich shoalgrass, and it appears that this high-quality food stimulated pinfish herbivory. Elevated pinfish consumption of the enriched shoalgrass then resulted in the decline of seagrass biomass in enrichment enclosures. These results add additional complexity to understanding and predicting the effects of eutrophication in coastal waters.

**KEY WORDS:** Seagrass · Nutrients · Herbivory · Trophic cascades · Pinfish

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## INTRODUCTION

Global human fixation of nitrogen has increased 2- to 3-fold since 1960 and global riverine discharges of nitrogen have almost doubled over the past 2 centuries (NAS 2000). This increasing input of nutrients to the coastal ocean has been closely linked to many negative impacts on biological resources (NSF 2000), and a variety of recent summaries argue that eutrophication is the most serious of human impacts on nearshore waters (Howarth et al. 2000, NAS 2000, NSF 2000). The most obvious consequences of coastal eutrophication include increasing occurrences of hypoxic and anoxic events,

and elevated frequencies and extents of harmful algal blooms (HABs), both of which are often associated with extensive fish kills and the overgrowth of extraordinarily productive seagrass meadows and coral reefs by rapidly growing macroalgae (Howarth et al. 2000).

Certain key characteristics appear to determine responses to coastal eutrophication. They include biotic factors such as the composition and relative abundance of the primary producers and consumers present, physical factors such as estuarine geomorphology, stratification, and nutrient loading rate, as well as hydrodynamic factors such as water residence time and flushing characteristics (NAS 2000).

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Among these factors, the types and abundances of herbivores present may be seriously underrated determinants of the severity of the effects of eutrophication in coastal systems (Duffy et al. 2003). Evidence supporting this hypothesis comes from a variety of locations and types of herbivores. For example, experimental studies of nutrient enrichment have indicated that small benthic herbivores such as amphipods and gastropods, which can consume their body weight or more in algae daily (for a review see Jernakoff et al. 1996), can offset the effects of nutrient enrichment on seagrass meadows (Neckles et al. 1993, Williams & Ruckelshaus 1993, Heck et al. 2000). Very similar results have recently been reported for coral reefs, where the effects of experimental nutrient enrichment were negligible when herbivorous fishes were present (Larkum & Koop 1997, Miller et al. 1999), and for aquatic microalgae growing on ceramic tile substrates, where the effects of nutrient addition on algal growth were balanced by amphipod and gastropod consumption (Lotze & Worm 2002).

An increasing number of well-documented studies implicate fishing as both a direct and indirect cause of shifts in herbivore numbers and species compositions, often with dramatic effects on algal biomass (for a review see Steneck & Carlton 2001). Two especially well-known cases discussed by Steneck & Carlton (2001) involve sea urchin herbivores from cool temperate shores of both the east and west coasts of the United States. On the west coast, after their sea otter predators were overharvested, sea urchin populations increased dramatically, and the feeding activities of the larger urchin populations led to dramatic decreases in the size of kelp forests. On the east coast, significant reductions in the density of finfish predators also led to increased urchin abundance, causing similar large-scale kelp disappearances. Recent intense harvesting of sea urchins for the export market during the 1990s has dramatically reduced their numbers, resulting in predicted large increases in kelp abundance.

Studies have also shown that herbivores in seagrass meadows (as well as in mangroves and on coral reefs; Boyer et al. 2004) can differentiate between nutrient-enriched and unenriched plants and strongly prefer to feed on nutrient-enriched tissues. Williams (1987) was one of the first to document that nutrient enrichment of turtlegrass sediments led to consumption of virtually all leaf tissue by small parrotfish within 1 wk. Subsequently, McGlathery (1995) reported nearly total removal of aboveground turtlegrass biomass only 9 d after enrichment, and Goecker et al. (2005) found similar results for turtlegrass leaves from eutrophic waters. In their study, near total consumption of enriched leaves occurred and was 5 to 15 times greater than that of leaves from oligotrophic waters. These studies

clearly show that seagrasses can accumulate nitrogen from the water column, and that herbivores will feed selectively on nitrogen-rich seagrass leaves. This could then result in reduced algal accumulation in nutrient-enriched seagrass meadows as leaves are consistently cropped, thereby eliminating settlement sites for epiphytic algal spores.

Here we report the results of a test of the hypothesis that consumption by small herbivores can effectively buffer seagrasses from the negative effects of algal overgrowth in eutrophic waters. To do this we conducted field studies at small spatial scales that allowed us to easily manipulate consumer density and elevate nutrient concentrations to levels that mimic those of highly eutrophic coastal waters. Our focus is on seagrass-dominated ecosystems because they are well-known as essential nursery habitats for a broad variety of economically valuable finfish and shellfish (for a review see Heck et al. 2003), and because, as noted above, eutrophication is strongly implicated in their disappearance (e.g. Howarth et al. 2000).

The species we manipulated, the pinfish *Lagodon rhomboides*, dominates the small fish fauna in northern Gulf of Mexico seagrass meadows from spring to fall (Huh 1984, Stoner & Livingston 1984). Many investigators have recognized the ability of pinfish to control invertebrate and epiphyte abundance (Stoner & Livingston 1984, Luczkovich & Stellwag 1993, Heck et al. 2000). Stomach content analyses show that distinctive feeding stages exist, which vary depending on the area sampled and the time of year (Stoner & Livingston 1984, Luczkovich & Stellwag 1993). In both Florida and North Carolina pinfish are omnivorous when small (approximately 26 to 40 mm SL) and feed heavily on polychaetes, small crustaceans, and epiphytes. As they grow (from 40 to 120 mm SL) algae become increasingly more common in their diet. Finally (>120 mm SL), pinfish feed almost entirely on both algae and seagrasses (cf. Luczkovich & Stellwag 1993).

**Study site.** Big Lagoon, Florida, located in the Perdido Bay system in the north central Gulf of Mexico (30° 23' N, 87° 24' W), is a semi-enclosed lagoon characterized by low energy regimes. In the shallows (<2 m) it contains seagrass meadows dominated by turtlegrass *Thalassia testudinum* and shoalgrass *Halodule wrightii*, along with unvegetated sand flats. Salinity generally ranges from mesohaline to polyhaline (13 to 30 psu annually; Spitzer et al. 2000), temperature varies seasonally from approximately 7 to 30°C (authors' pers. obs.) and the mean tidal range is 0.5 m (Spitzer et al. 2000). Our previously unpublished measurements have shown relatively low summer nutrient levels, ranging from 0.01 to 2.73  $\mu\text{M}$  nitrate, 0.3 to 2.6  $\mu\text{M}$  ammonium, 0.0 to 15.35  $\mu\text{M}$  silicate and 0.0 to 0.14  $\mu\text{M}$  phosphate. We have also observed that sum-

mer water column chl *a* values are low, ranging from 0.17 to 6.16  $\mu\text{g l}^{-1}$  (unpubl. data).

## MATERIALS AND METHODS

**Experimental design.** To test the combined effects of nutrient enrichment and increased pinfish density on mesograzers abundance and species composition, epiphyte abundance, and seagrass biomass and growth, 24 approx. 2 m<sup>2</sup> (1.4 × 1.4 m) enclosures were installed in a dense stand of shoalgrass, parallel to shore at depths of approx. 1 m. Each enclosure was made of plastic net (1.2 × 1.6 cm mesh) held in place by a PVC frame. Bird netting tops (1.9 cm mesh) maximized light passage and prevented fish from entering or leaving enclosures.

The experiment's two main effects, pinfish density manipulation (ambient density pinfish = 1× F, and elevated pinfish density = 10× F) and nutrient enrichment (ambient = -N and elevated nutrient concentrations = +N), were tested in a factorial design. Each treatment had 6 replicates and treatments were randomly assigned. Cross-contamination was avoided by increasing the spacing between the nutrient and nonnutrient cages. To establish baseline conditions, pre-experiment sampling (Time 0, T0) was conducted on May 22, 2000, and all treatments were in place by the end of May 2000. The experiment was run for 139 d with midterm sampling (T1) of selected parameters in July and the final sampling (T2) during the second week of October 2000.

**Nutrient additions.** In each nutrient enclosure, PVC tubes (11 cm diameter × 30 cm length with twenty 2 cm holes) containing 500 g dry wt (DW) of Osmocote™ slow release fertilizer (N:P molar ratio = 16:3) were attached to each cage leg at a distance of 10 cm above the substrate. One additional tube was suspended in the center of the cage within the seagrass canopy. Nutrients were replaced at approximately 4 wk intervals, as previous studies (see below) have shown that the nutrients are initially released in a 'burst' with subsequent slow release over a number of weeks. Replacing the Osmocote™ every 4 wk should have limited the amount of time between 'bursts' and resulted in high overall nutrient loading. The exteriors of nutrient tubes were cleaned with wire brushes where required, and hosed down during each replacement of Osmocote™.

To characterize the dissolution rates of Osmocote™, laboratory experiments (reported in Heck et al. 2000) were conducted at temperatures commonly encountered during the seagrass growing season (15, 20, 25, and 30°C). Individual PVC tubes containing 500 g DW of Osmocote™ were submerged in 30 psu seawater in

19 l buckets that were mixed with magnetic stirrers. Five replicates were used for each temperature treatment. Samples were collected on Days 0 and 1, and subsequently at approximately 3 d intervals over a 10 to 15 d period. These experiments showed that fertilizer release occurred in an initial burst phase followed by a constant release rate until the nutrients were exhausted (cf. Fig. 2 in Heck et al. 2000). These laboratory data allowed us to estimate nutrient loading rates during our field experiments. During each nutrient tube replacement, 25 tubes (20 attached to support poles and 5 free hanging tubes) were collected and dried to a constant weight. Osmocote™ loss rates ( $\text{g tube}^{-1} \text{d}^{-1}$ ) were calculated based on the change in Osmocote™ weight over time and N and P delivery rates ( $\text{mmol tube}^{-1} \text{d}^{-1}$ ), and loading rates ( $\text{mmol m}^{-2} \text{d}^{-1}$ ) were calculated (Fig. 1; Table 2).

**Fish manipulations.** Previous studies have shown that such enclosures effectively excluded large fish predators (e.g. sharks, red drum, spotted sea trout, and jacks) while allowing smaller invertebrates (e.g. grass, shrimp) and fish smaller than the cage mesh (e.g. young-of-the-year pinfish) access and egress (cf. Heck et al. 2000).

Young-of-the-year pinfish *Lagodon rhomboides* were captured by trawling and used to stock 1× F and 10× F cages at densities of 6 ind. cage<sup>-1</sup> (or 3 m<sup>-2</sup>) and 60 ind. cage<sup>-1</sup> (or 30 m<sup>-2</sup>), respectively. The majority of fish ranged in size from 73 to 100 mm in total length (TL), as pinfish >73 mm TL could be retained by the mesh of the enclosures. Prior to pinfish additions, all cages were seined to remove fish larger than the cage mesh.

Our small predator treatments were initially estimated to approximate ambient pinfish densities (3 fish m<sup>-2</sup>) and 10× ambient 'natural' densities (30 fish m<sup>-2</sup>)

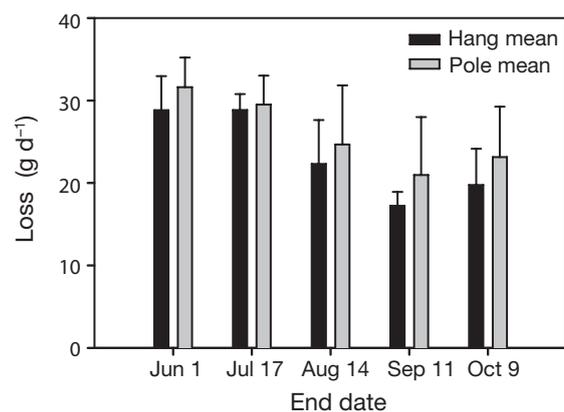


Fig. 1. Dissolution rates of Osmocote™ held in 5 cm diameter PVC tubes containing 500 g DW premeasured Osmocote™. Tubes were either hung on rebar stakes (noted as hang mean) or attached to the poles supporting the field enclosures (pole mean)

in May. This ambient estimate was based on the few semi-quantitative studies of pinfish densities found in the literature. For example, mean pinfish density in Redfish Bay was estimated to be  $2.3 \text{ m}^{-2}$  (Huh 1984), and in St. Joseph Bay, Florida, density ranged from around  $0.14$  to  $4.6 \text{ m}^{-2}$  during the spring to fall months (Thompson 2000). However, because sampling of juvenile fish abundance using seines and trawls typically underestimates fish density by 30 to 70% (Kjelson & Johnson 1978), we concluded that our initial stocking density was probably substantially lower than the nominal  $10\times$  'natural' pinfish densities. For example, if our estimate of  $3 \text{ fish m}^{-2}$  were only 30% of actual densities, the actual abundances would be around  $10 \text{ m}^{-2}$ . If they represented 70%, actual densities would be around  $4.3 \text{ m}^{-2}$ . Therefore, we estimate that our nominal  $10\times$  densities actually ranged from around  $1.9$  to  $4.4\times$  'natural densities.' These 'enhanced' pinfish densities were used to simulate what might happen if large piscivorous predators were removed from the system by overharvesting, and pinfish populations responded by increasing their densities.

**Sampling regime.** All experimental plots were sampled monthly for water column nutrients and chl *a* concentrations from May to October in 2000, the period of greatest seagrass growth in the northern Gulf of Mexico (Valentine & Heck 1991). During each nutrient sampling, duplicate water samples were collected from each enclosure to document the magnitude of our nutrient enrichment and its impact on water-column chl *a* concentrations. To minimize disturbance within the enclosures, water samples were collected at the canopy height from outside each enclosure. To do this, a 2 m long aluminum pipe containing Tygon tubing was inserted through the cage mesh and samples were collected using acid-washed 60 ml syringes. Water samples were placed on ice until (<2 h) they could be filtered through Whatman<sup>TM</sup> GF/C filters and frozen in 60 ml plastic bottles. Nutrient analyses were carried out on samples using standard wet chemical techniques (Skalar Manual Publication # 0101022 A.US) adapted for use on an SAN<sup>plus</sup> Autoanalyzer. Chl *a* concentrations were determined using a Turner Designs Model TD-700 Fluorometer following the acidification method of Lorenzen (detailed in Strickland & Parsons 1972).

Bimonthly estimates of epiphyte chl *a* concentration and seagrass biomass were determined from shoots collected in 1 haphazardly placed  $0.06 \text{ m}^2$  core per enclosure. Samples were placed in pre-labeled plastic bags, frozen and stored for later analyses. In the lab, 5 to 10 randomly selected shoots from each sample were used to measure leaf length and width (not reported separately here) and to quantify epibiota on the leaves.

All measured leaves were then scraped to remove epibionts, and the epibionts were then filtered onto a Whatman<sup>TM</sup> GF/F filter for chl *a* analysis. We used this chlorophyll measure as a surrogate for the biomass of epiphytic algae.

Shoalgrass growth was estimated at the midpoint and end of the experiment using a modified clipping technique. One 10 cm diameter ring was placed in each cage and all plant material above the sheath was removed by cutting with scissors. Samples were collected within 10 to 14 d after clipping by inserting a corer into the ring and removing all above and below ground material. Any aboveground material within the core was considered new growth and was separated from the sample. All new leaves were dried to a constant weight at  $80^\circ\text{C}$  and ashed at  $500^\circ\text{C}$  for 3 h to estimate net aboveground primary production ( $\text{g ash-free dry wt [AFDW] m}^{-2} \text{ d}^{-1}$ ). This method measures re-growth of seagrass leaves and is known to underestimate net above ground primary production by 38 to 56% (Hauxwell et al. 2001).

Invertebrate mesograzers were collected at the beginning and end of the experiment from each enclosure using a  $0.07 \text{ m}^2$  plastic cylinder whose lower edge was embedded in the sediment. The contents of the cylinder were sampled by a gasoline-powered suction pump (cf. Orth & Van Montfrans 1987) and all material was passed through a 0.5 mm collecting bag where larger motile epibiota were retained. Following collection, samples were sieved on a 0.5 mm mesh screen to remove additional material, placed on ice, and frozen. Animals were identified only to the extent necessary to classify them trophically (Table 1), according to published information (cf. Neckles et al. 1993, Williams & Ruckelshaus 1993). Free-living amphipods, caridean shrimp, and gastropods constituted the majority of mesograzers we collected.

Seagrass samples were taken at the beginning and the end of the experiment and used to determine C:N:P ratios of the leaves as a means of documenting the effectiveness of delivery of nutrients by the Osmo-

Table 1. Mesograzer categories used in classification of mobile epibiota

Gastropoda	Chitons
<i>Turbo</i>	Isopods
<i>Rissoina</i>	Amphipods
<i>Modulus</i>	Penaeid shrimp
<i>Bittiolium</i>	Caridean shrimp (minus alpheids)
<i>Cerithium</i>	Hermit crabs
<i>Crepidula</i>	Spider crabs
<i>Mitrella</i>	
<i>Anachis</i>	
<i>Dentimargo</i>	

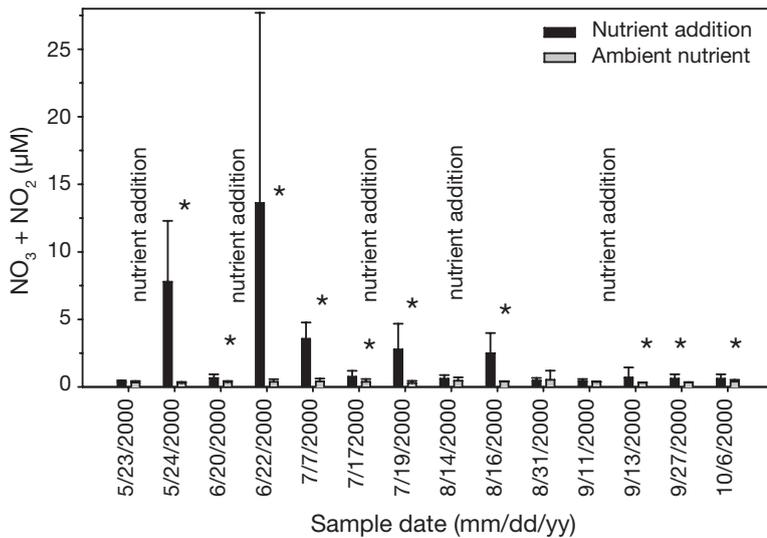


Fig. 2. Pooled nitrate and nitrite concentrations (+1 SE) by date during 2000. Nutrient additions are indicated, and significant differences in water column concentrations following nutrient additions are noted by an asterisk

cote™ in the enrichment treatments. An additional core was taken from each cage and haphazardly selected leaves were then removed from the sample and scraped of epibionts. The seagrass portion was dried at 60°C to a constant weight and then ground to a powder with a mortar and pestle. The dried samples were then analyzed for percent carbon and percent nitrogen in the Carlo Erba NA1500 CNS analyzer following a standard protocol (Carlo-Erba NA 1500 operating manual). Percent particulate organic phosphorus was determined using a standard wet chemical technique (Solorzano & Sharp 1980).

Cage inspection and repair was conducted biweekly to ensure enclosure integrity. After the final sampling in October, all fish in each cage were collected by seining and were identified, enumerated and measured for TL and SL in mm.

**Statistical analyses.** Two-way analysis of variance (ANOVA) was used to analyze the effects of small predator manipulation and nutrient enrichment on measurements of seagrass biomass and growth, mesograzers abundance, epibiont abundance as estimated by chl *a*, inorganic nutrients, and water column chl *a*. Final fish counts were also analyzed using 2-way ANOVA. When data failed the homogeneity of variance assumption of ANOVA (according to Levene's test), data were log-transformed and retested to ensure that this assumption was met. Results were considered to be significant when  $p < 0.05$ , unless otherwise noted.

## RESULTS

### Water column nutrients and chl *a*

Water samples collected in May before the start of the experiment were not significantly different in nitrate-nitrite, ammonium, or phosphate levels among treatments. While nutrient concentrations varied during the experiment as a result of natural input and wind-mixing, very large amounts of Osmocote™ were dissolved in the +N treatments, and nitrogen concentrations were often 3× greater than those in the -N plots (Figs. 2 & 3). Nitrogen levels decreased over time, but generally remained elevated in the +N treatments throughout the experiment (Figs. 2 & 3), and high concentrations were consistently observed following the replacement of fertilizer tubes (Figs. 2 & 3). Across all months, nitrite-nitrate concentrations were greatest immediately after nutrient addition, with levels ranging from 0.37 µM in August and September to around 14.0 µM in June (Fig. 2). Excluding the initial sampling date and the August 31, 2000 sampling, nitrite-nitrate concentrations were always higher in the +N treatments than in the -N treatments. The lowest ammonium levels in the -N treatments were in July (0.21 µM) and the highest during August (2.17 µM; Figure 3). Ammonium concentrations in nutrient enriched treatments ranged from 0.37 µM in September to over 21.0 µM

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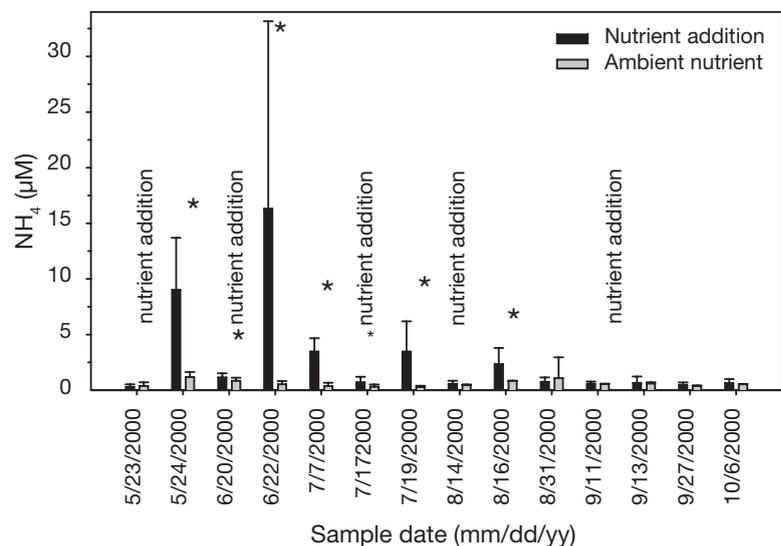


Fig. 3. Ammonium concentrations (+1 SE) by date during 2000. Nutrient additions are indicated, and significant differences in water column concentrations following nutrient additions are noted by an asterisk

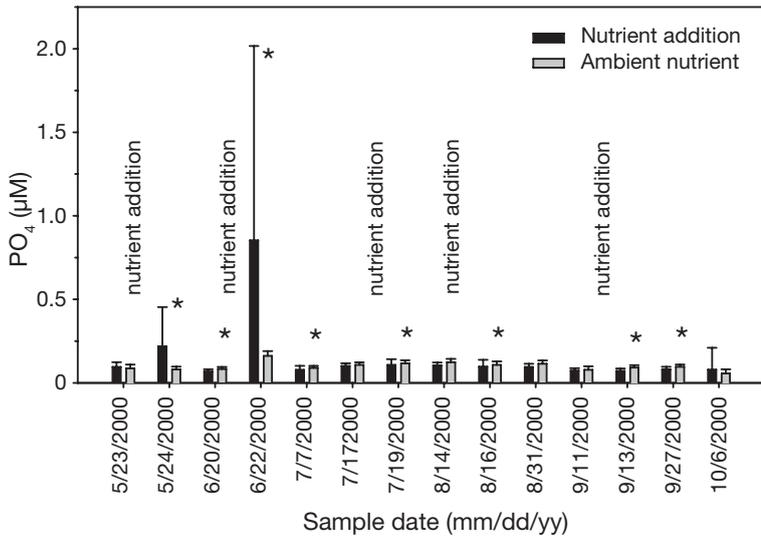


Fig. 4. Phosphate concentrations (+1 SE) by date during 2000. Nutrient additions are indicated, and significant differences in water column concentrations following nutrient additions are noted by an asterisk

in June. Significant enrichment effects occurred during May, June, July, and the second sampling date in August (Fig. 3), but no significant effects were detected later in the year.

Phosphate concentrations ranged from 0.046 µM (October) to 0.18 µM (June) in the -N treatments (Fig. 4), and were highest in the +N treatments in June (nearly 1.0 µM) and lowest in September (0.062 µM). Only twice was there a clear signal of elevated phosphate concentration (Fig. 4).

Even though water column concentrations were seldom measured at extremely high levels, nutrient loading rates were very high when compared to loading rates measured in prior studies (Table 2). Only the study by Harlin & Thorne-Miller (1982) delivered nutrients at higher rates than we did, with loading rates in their experiment of approximately 10× that of our earlier experiments in turtlegrass meadows in St. Joseph Bay, Florida.

Water column chl *a* concentrations were variable (from 2 to nearly 8 µg l<sup>-1</sup>) and showed few treatment effects except in July and the final sampling in October (Fig. 5).

**Mesograzers**

There was substantial, albeit nonsignificant, heterogeneity in mesograzer abundance among treatment plots at the begin-

ning of the experiment owing to natural spatial variability (Fig. 6). By the end of the experiment, although mesograzer densities declined by more than 50% within each treatment during the experiment (Fig. 6), there were no significant treatment effects ( $p > 0.05$ ). Unexpectedly, however, there were more, not fewer, mesograzers in the elevated pinfish treatments at the end of the experiment. However, this was primarily due to an abundance of the small gastropod *Bittium varium*, which is infrequently fed upon by juvenile pinfish (Luczkovich & Stellweg 1993). When *B. varium* was removed from consideration and only crustacean mesograzers were analyzed, there were fewer grazers in the high density fish treatment, as expected, although differences were again not significant (Fig. 7).

**Epiphytes**

There were no significant differences in epiphyte chl *a* concentrations among treatments at the start of the experiment, although there was a significant fish effect on epiphyte chl *a* at the midpoint of the experiment (Fig. 8; T1,  $F_{1,28} = 5.36$ ;  $p < 0.03$ ), with less chl *a* in high fish density treatments. By the end of the experiment, there were both significant nutrient and fish effects (Fig. 8;  $F_{1,28} = 6.4$ ,  $p < 0.02$ ), with significantly more chl *a* on the seagrass leaves in the high nutrient

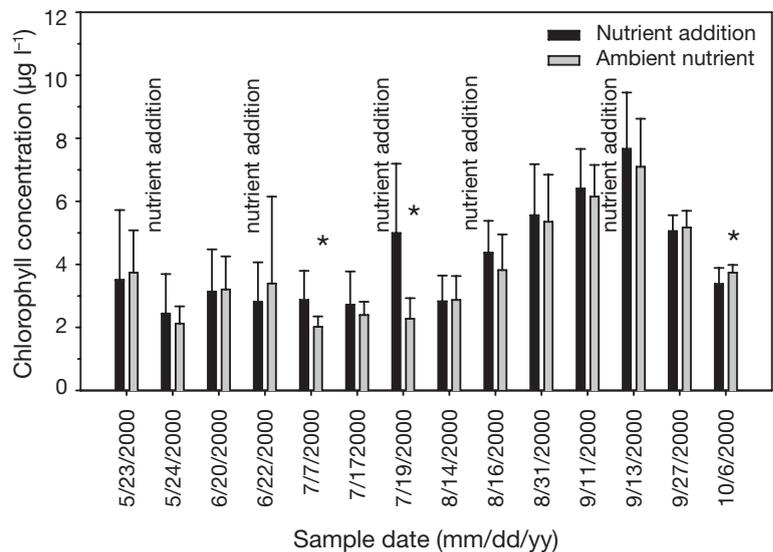


Fig. 5. Chl *a* concentrations (+1 SE) by date and treatment during 2000. Nutrient additions are indicated, and significant differences in water column concentrations following nutrient additions are noted by an asterisk

Table 2. Estimated loading rates and measured nitrogen and phosphorus concentrations in the present study compared with those of previous experimental manipulations in seagrass systems and selected estuaries

	N-loading (mmol m <sup>-2</sup> d <sup>-1</sup> )	P-loading (mmol m <sup>-2</sup> d <sup>-1</sup> )	Ambient [N] (μM)	Treatment [N] (μM)	Ambient [P] (μM)	Treatment [P] (μM)
<b>Field manipulations</b>						
Present study <sup>a</sup>	576–886	35–54	0.3–0.5	0.4–13.6	0.06–0.2	0.07–0.85
Heck et al. (2000) <sup>a</sup>	77–123	5–7	0.3–5.3	0.5–7.0	0.0–0.14	0.1–0.4
Harlin & Thorne-Miller (1982) <sup>a</sup>	1971–3857	257–429	0.4–2.3	1.0–21.6	0.0–1.0	0.1–5.6
Neckles et al. (1993) <sup>b</sup>			4.0–10.8	10.6–37.8	10.7–1.6	1.8–3.4
Williams & Ruckelshaus (1993) <sup>b</sup>	476–510		3.6–45.0	3.6–45.0		
McGlathery (1995)	300	34.5	1.0–2.0	4.0–4.5	<0.05	0.10
<b>Selected estuaries<sup>c</sup></b>						
Apalachicola Bay			5.4		0.2	
Chesapeake Bay	1.9	0.11	20		0.5	
Delaware Bay	19.2	1.6	110		1.2	
Narragansett Bay	2.7	0.22	8.1		1.9	
Thames Estuary, UK	87.7	8.7				

<sup>a</sup>Dissolved inorganic nitrogen (DIN) added as NO<sub>3</sub> and NH<sub>4</sub>, <sup>b</sup>DIN added as NH<sub>4</sub> only, <sup>c</sup>Estimated from Nixon et al. (1986)

treatments and in the ambient density fish treatments. However, mean epiphyte chl *a* concentrations were lower at the end than at the beginning of the experiment in all treatments except the nutrient enrichment-ambient fish density treatment.

### Seagrass biomass and growth

Seagrass biomass was significantly lower in the nutrient enrichment enclosures at the start of the experiment in May (T0) due to existing spatial heterogeneity and remained lower (although not significantly so) at the midpoint of the experiment in July (T1). At the end of the experiment in October (T2), biomasses were once again significantly lower in the nutrient enrichment treatments (Fig. 9;  $F_{1,28} = 12.36$ ;  $p < 0.01$ ). No significant fish effects were observed at the end of the experiment. Even though the biomass in the elevated nutrient treatment was significantly lower than the ambient treatment at T0, all treatments decreased in biomass by the end of the experiment. The observed decreases were proportionally greater in nutrient than in fish treatments; thus, there was greater seagrass biomass decline in the nutrient addition enclosures.

Although no seagrass growth measurements were made in individual enclosures at the beginning of the experiment (to minimize disturbance), at the midpoint there were no significant differences among treatments. By the end of the experiment in October, however, there were significant nutrient effects (Fig. 10;  $F_{1,28} = 7.02$ ;  $p < 0.02$ ); seagrass in treatments with added nutrients grew significantly slower than treatments with ambient nutrient concentrations.

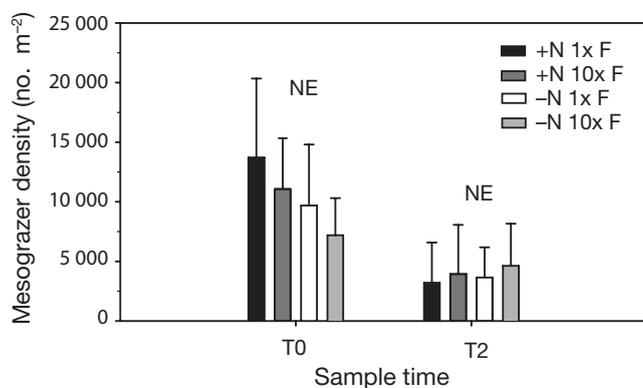


Fig. 6. Grazer density by sample time (T0, T2) and treatment during 2000. Treatments represented by: -N = no nutrient additions; +N = nutrient additions; 1x F = ambient pinfish; and 10x F = pinfish additions. NE = no significant effects. Error bars +1 SE

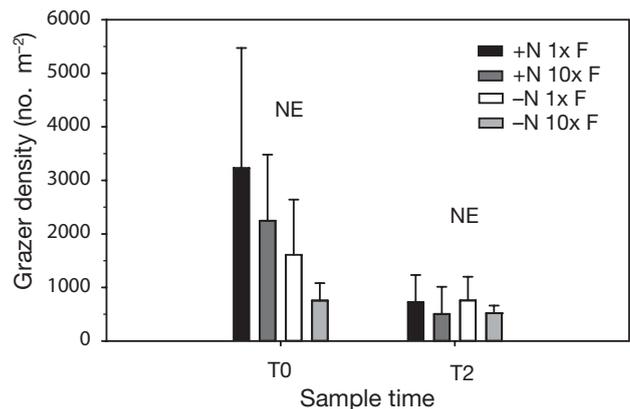


Fig. 7. Grazer density without *Bittium varium* (no. m<sup>-2</sup>) by month and treatment during 2000. Abbreviations as in Fig. 6. Error bars +1 SE

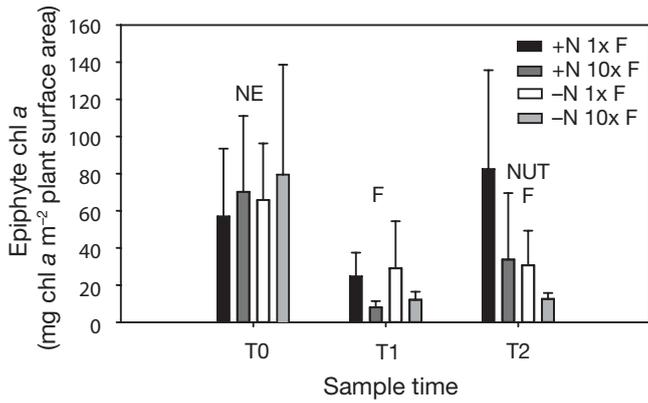


Fig. 8. Epiphyte chl a by month and treatment during 2000. NE: no significant effects; F: significant pinfish effects; NUT: significant nutrient effects. Abbreviations as in Fig. 6. Error bars +1 SE

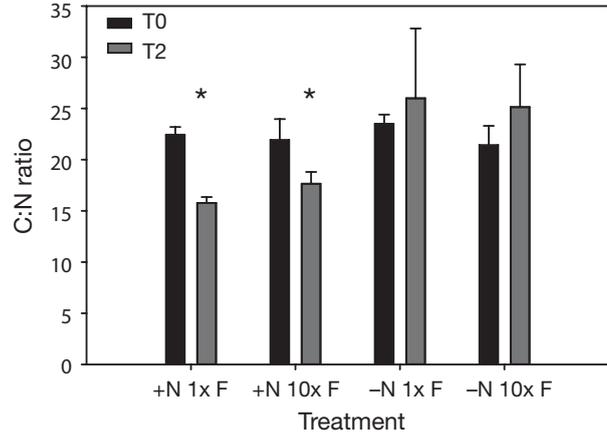


Fig. 11. C:N ratios by sample time (T0, T2) and treatment during 2000. Abbreviations as in Fig. 6. Error bars +1 SE. \*Significant differences ( $p < 0.01$ )

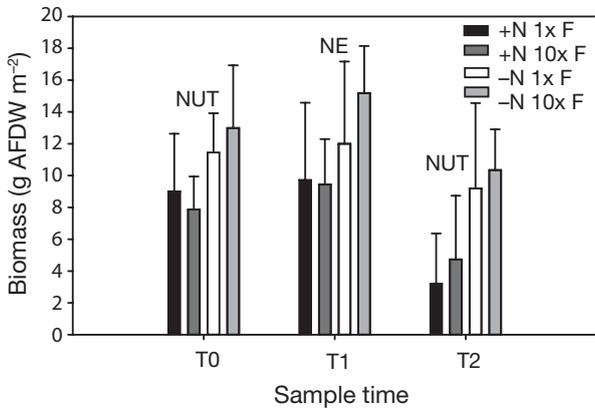


Fig. 9. Aboveground plant biomass by month and treatment during 2000. AFDW: ash-free dry wt. Other abbreviations as in Figs. 6 & 8. Error bars +1 SE

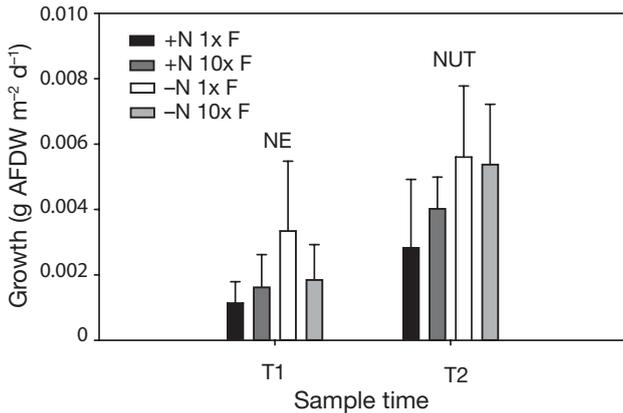


Fig. 10. Mean leaf growth by sample time and treatment. Abbreviations as in Figs. 6 & 8. Error bars +1 SE

### Effectiveness and maintenance of treatments during the experiment

At the time of the final sampling in October, additional seagrass leaves were collected to compare C:N and C:P ratios between nutrient treatments. Results clearly indicated that leaf C:N ratios were significantly reduced in nutrient addition enclosures (Fig. 11). Although there were no significant reductions in C:P ratios, all treatments did show reductions in C:P ratios by the end of the experiment (Fig. 12). In addition, it is noteworthy that results for the -N treatments show no evidence that the presence of fish or their excretory products were taken up by the seagrass leaves.

Initially, *Lagodon rhomboides* was stocked at 6 (1×F treatments) and 60 fish per cage (10×F treatments). Six mo later pinfish numbers had declined in all enclosures (presumably because of escapes or mortality), so that the 10×F treatments contained approximately 7 to 8× the density of ambient density enclosures (Fig. 13). Nevertheless, this difference was highly significant ( $F_{1,34} = 186.95$ ;  $p < 0.0001$ ). Additional immigrant fish species removed from cages included small pigfish, toadfish, pipefish, and gobies. These additional species never exceeded a mean density of  $>0.2$  fish cage<sup>-1</sup>. Pinfish in the enclosures ranged from 50 to 135 mm TL, with the majority in the 70 to 100 mm TL range (Fig. 14). Only about 10 to 15% of these fish were smaller than the size ( $<72$  mm SL) that was able to enter the enclosures, and they therefore represent fish that colonized the enclosures during the experiment. In addition, nearly 10% of the fish in the enclosures grew to sizes greater than 100 mm SL, and these fish were of the size that frequently consumes seagrass leaves (Luczkovich & Stellwag 1993).

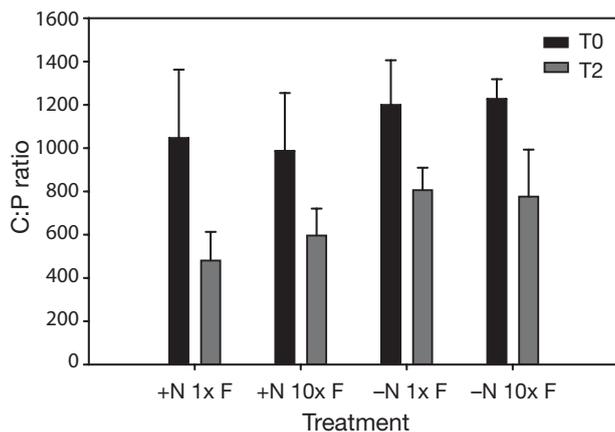


Fig. 12. C:P ratios by sample time (T0, T2) and treatment during 2000. Abbreviations as in Fig. 6. Error bars +1 SE

## DISCUSSION

The conventional wisdom regarding the effects of nutrient enrichment on submerged aquatic vegetation (SAV) has been that increasing nutrient supplies lead to the overgrowth of seagrasses by fast-growing algae, resulting in the eventual disappearance of seagrasses from eutrophic systems (Duarte 1995, Howarth et al. 2000, NAS 2000, Hauxwell et al. 2001). This is the explanation most often proposed to account for the loss of seagrasses in North America (Short et al. 1995), Europe (Giesen et al. 1990), and Australia (Cambridge & McComb 1984). However, as noted previously (Heck et al. 2000), experimental studies that included manipulations of mesograzers (small crustacean, gastropod, or fish grazers) in their design have consistently found that grazing effects on epiphytic algal biomass were more significant than those of nutrient enrichment. In our previous experiments in turtlegrass meadows (Heck et al. 2000) we, too, found few significant nutrient effects. In contrast, manipulation of pinfish densities resulted in significant effects on mesograzer density, epiphyte biomass, and the production, leaf length, and shoot density of *Thalassia testudinum*. In aggregate, the results from these studies, many of which have been reviewed previously by Jernakoff et al. (1996), clearly showed that the stimulatory effects of increased nutrient loading on epiphyte abundance were greatly reduced when grazers were included in the design of experiments. More recently, a meta-analysis (Hughes et al. 2004) of all of the existing studies, including those cited above, that have compared the relative effects of nutrients and grazers on the epiphytic biomass support our qualitative conclusion that grazers are a key determinant of the extent to which epiphytes can overgrow living seagrass leaves. As they state: 'The positive ef-

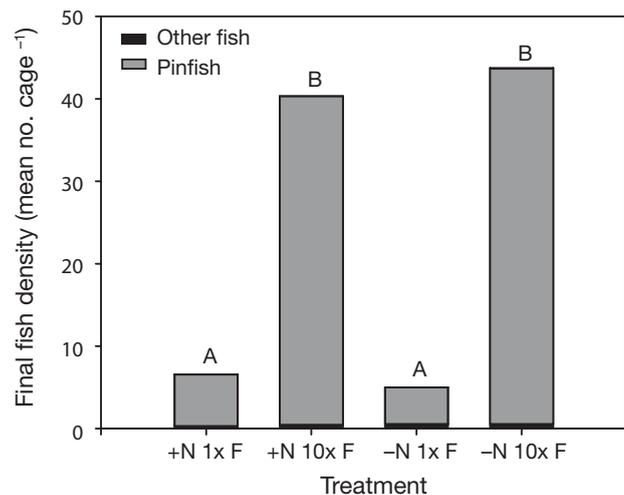


Fig. 13. Mean number of pinfish *Lagodon rhomboides* and other fish species by treatment seined from cages in October 2000. Treatments with different letters above them are significantly different ( $p < 0.05$ ). Abbreviations as in Fig. 6. Error bars +1 SE

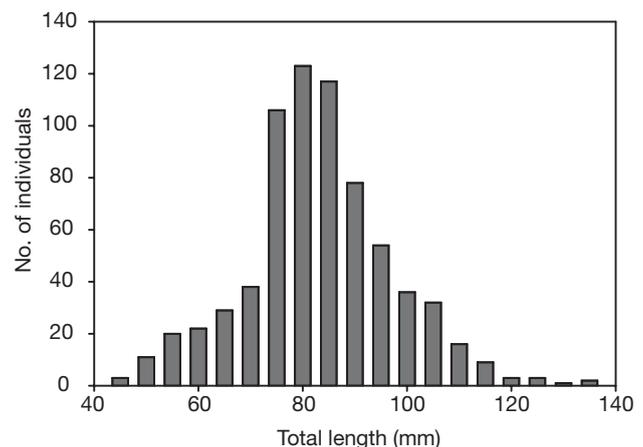


Fig. 14. *Lagodon rhomboides*. Size frequency distribution of pinfish seined from cages in October 2000

fects of epiphyte grazers were comparable in magnitude to the negative impacts of water column nutrient enrichments, suggesting that the 2 factors should not be considered in isolation of each other.' Thus, mesograzers usually control the abundance of epiphytes, even in enriched conditions, a result inconsistent with the simple paradigm of nutrient-enrichment caused seagrass decline summarized by Duarte (1995) and a number of more recent publications (Howarth et al. 2000, NAS 2000, Hauxwell et al. 2001).

In attempting to interpret our current results, it was important to establish the level of nutrient enrichment and consumer abundances actually achieved in our treatments. We estimate (based on temperature dependent nutrient release rates shown in Fig. 2 of

Heck et al. 2000) that we delivered between 576 and 886 mmol dissolved inorganic nitrogen (DIN)  $\text{m}^{-2} \text{d}^{-1}$  and 35 and 54 mmol P  $\text{m}^{-2} \text{d}^{-1}$  to the +N treatments. These daily rates translate to annual loadings of around 210 to 323 mol DIN  $\text{m}^{-2} \text{yr}^{-1}$  and 12.8 to 19.7 mol  $\text{PO}_4 \text{m}^{-2} \text{yr}^{-1}$  and are much higher than those estimated for most of the major estuaries of the world, as well as those achieved in other seagrass nutrient enrichment studies, including our own (Table 2). While advection of nutrients from our experimental cages undoubtedly occurred, we believe that we were effective in delivering nutrients to our enrichment plots for the following reasons: (1) Big Lagoon is in a low energy environment with limited potential for water flow to remove nutrients from the enclosures (cf. Gorsline 1966); (2) placing the nutrient delivery tubes within the seagrass canopy ensured that nutrient release occurred in an area of very low flow (Koch 2001), and immediately adjacent to algal epiphytes; (3) water column nutrient concentrations were measurably increased; and (4) there was a substantial increase in nitrogen concentrations in *Halodule wrightii* leaves in +N treatments (Fig. 11). In addition, we also successfully maintained the pinfish treatments: Fig. 13 shows that densities in the pinfish addition treatment were significantly higher than those in the ambient density treatment, and only a small percentage were smaller than the mesh size of the enclosures. Thus, we did maintain the treatments as intended.

Although we did not observe simple trophic cascades, presumably because pinfish feed at several different trophic levels, the elevated pinfish density treatment did produce a number of consumer effects. For example, reductions in mean crustacean mesograzer densities were observed in the elevated pinfish treatment at the end of the experiment (Fig. 7), even though these differences were not significant. In addition, the high density fish enclosures showed reduced epiphyte chl *a* (Fig. 8). We believe that two factors account for this. First, omnivorous pinfish consume increasing amounts of plants, including epiphytes, as they grow (Stoner & Livingston 1984) and were likely to have been consuming substantial amounts of epiphytes during the latter part of the experiment. Second, pinfish also consume seagrass leaves once they grow beyond 100 mm SL (Luczkovich & Stellwag 1993), and a number of fish in our enclosures were large enough to be consuming shoalgrass at the end of the experiment (Fig. 14). Thus, pinfish could have consumed many of the older and more heavily epiphytized leaves, resulting in an increased relative abundance of young, unepiphytized leaves. And, as we observed, by feeding on leaves they probably also reduced the total number of leaves and biomass of shoalgrass in the +N enclosures by the end of the experiment (Fig. 9; see below).

With regard to nutrient effects on epiphytes, even though there were significantly more epiphytes (as indicated by chl *a* concentrations) in the nutrient enrichment treatments at the end of the experiments (due primarily to the +N 1×F treatment; Fig. 8), the mean epiphyte abundance in the enriched treatments was actually lower at the end than it was at the beginning of the experiments. Thus, even though epiphyte chl *a* was elevated substantially compared to the start and middle of the experiment in the +N 1×F treatment, there was a large overall decrease in this treatment from the beginning to the end of the experiment. For this reason, it is difficult to see how epiphyte shading could have been solely responsible for the shoalgrass losses and decreased growth rates in the +N treatments, since there was no mean accumulation of epiphyte chl *a* by the end of the experiment, and values were at very low levels during the midpoint (Fig. 8).

As to why the nutrient enrichment enclosures contained lower shoalgrass biomasses than the unenriched enclosures if epiphyte shading is primarily not to blame, we offer two possibilities. One is that the nutrient additions themselves could have produced harmful effects on shoalgrass. This seems unlikely however, as Burkholder et al. (1994) showed that shoalgrass was quite tolerant of very high rates of water column nitrate loading, in contrast to eelgrass, whose growth was negatively affected by nitrate enrichment.

A second explanation, which we favor, is that pinfish and other small grazers such as amphipods fed at high rates on leaves with elevated nitrogen content, just as other fish, turtles, marine mammals, and small crustaceans have been shown to do (Williams 1987, Williams & Ruckelshaus 1993, McGlathery 1995, Preen 1995, Boyer et al. 2004, Goecker et al. 2005). The prevalence of preferential feeding on nutrient rich foods is well documented (see review by Cebrian 1999), and the discipline of ecological stoichiometry has generated many new and testable hypotheses about the manifold impacts that shifts in nutrient balance can have for consumers (Sterner & Elser 2002). In this study we loaded nutrients at much higher rates than in our previous experiments (Heck et al. 2000) and thereby increased leaf nitrogen content to very high levels. By doing this it seems that we were unintentionally inducing pinfish and small crustaceans to feed on the enriched seagrass leaves, which resulted in a different outcome than we obtained previously with lower leaf nitrogen concentrations. Thus we believe that the low shoalgrass biomass found in the enriched plots at the conclusion of the experiment resulted from preferential feeding on the nitrogen-rich leaves by the larger pinfish and the small crustaceans and not primarily as an indirect consequence of epiphytic algal overgrowth.

We conclude by noting that the role of grazers in mediating the effects of eutrophication in seagrass meadows can vary greatly, especially for organisms like the pinfish, which switch from carnivory to herbivory during ontogeny. The complexities in the relationships among nutrients, algal and seagrass food quality, and grazers that we have observed indicate that there is still much to learn about the interacting effects of nutrient supplies and consumer effects in seagrass meadows. In particular, the occasions when enriching food items makes them more palatable to consumers injects additional complexity into understanding and predicting the effects of eutrophication in coastal waters.

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