

The role of leaf nitrogen content in determining turtlegrass (*Thalassia testudinum*) grazing by a generalized herbivore in the northeastern Gulf of Mexico

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Abstract

In shallow marine environments the variability in grazing on seagrasses has been hypothesized to be controlled, in part, by the nutritive quality (i.e., nitrogen content) of their leaves. The few existing studies of the relationship between leaf nitrogen content and seagrass grazing have all found a positive relationship between leaf nitrogen content and preference by selective vertebrate grazers (i.e., the bucktooth parrotfish, green sea turtles, and dugongs). However, most marine herbivores (both vertebrate and invertebrate) are thought to be extreme generalists with broad diets of variable nutritive quality (e.g., detritus, living plants, and animals), suggesting the currently held view on the role leaf nutrient content in explaining the variability of seagrass grazing is an oversimplification.

In this study, we evaluated how leaf nitrogen content influenced grazing on turtlegrass by a generalist invertebrate herbivore (the pink sea urchin *Lytechinus variegatus*) in the northeastern Gulf of Mexico. Using a short-term laboratory test and a longer-term field experiment, we tested the hypothesis that leaf nitrogen content controls sea-urchin grazing on seagrass leaves. We hypothesized that if poor nutritive value of seagrasses is responsible for reduced rates of feeding, then increasing leaf nitrogen concentrations should lead to increased rates of seagrass consumption by sea urchins.

In the field experiment, we significantly enriched seagrass leaf nitrogen concentrations (some 10–20% depending on month) in experimental plots with a commercial fertilizer and we

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manipulated grazing intensity by enclosing adult sea urchins at densities that bracketed the range of average densities observed in the region (i.e., 0, 10 and 20 individuals/m²). Comparisons of changes in aboveground seagrass production and biomass showed no evidence that sea urchins grazed significantly more in treatments where leaf nitrogen was enriched. Because the statistical power of our test to detect such differences was low and aboveground seagrass production varied significantly among treatments, we also used a mass balance equation to estimate sea urchin consumption of nitrogen-enriched and unenriched leaves. This showed that sea urchins compensated for low nitrogen levels in our unenriched treatments by eating more leaves than in treatments where leaf nitrogen was elevated. Using a laboratory test, we also found that sea urchins ate less nitrogen-enriched seagrass than unenriched seagrass. In combination, these results show that, in contrast to findings reported for vertebrate herbivores, sea urchins feed at higher rates when offered seagrass leaves of lower leaf nitrogen content, and that low levels of leaf nitrogen are not always an effective defense against herbivores. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Plant nutritional quality (often expressed as leaf nitrogen content) has been shown to play a central role in determining herbivore feeding patterns in several terrestrial communities (e.g., Onuf et al., 1977; Slansky and Feeny, 1977; Kraft and Denno, 1982; Coley, 1983). Many insect herbivores preferentially graze on nitrogen-rich leaves (e.g., McNeill and Southwood, 1978; Schroeder, 1986; Athey and Connor, 1989). By feeding on nitrogen-rich plants, herbivorous insects can realize higher survivorship, faster growth, and higher levels of fecundity than grazers feeding on plants with lower leaf nitrogen concentrations (e.g., Feeny, 1970; Scriber and Feeny, 1977; Rausher, 1981; Raupp and Denno, 1983; Schroeder, 1986). These findings have led investigators to hypothesize that low leaf nitrogen concentration represents one form of plant defense against grazing by specialist herbivores (Feeny, 1970; Schroeder, 1986; Augner, 1995).

However, herbivores do not always have the opportunity to choose between plants of varying nitrogen content. Some insect and large vertebrate grazers have adopted foraging strategies that allow them to maximize nitrogen intake while feeding on low quality plants (e.g., McGinnis and Kasting, 1967; Mattson, 1980; Lincoln et al., 1982; Strong et al., 1984; Slansky and Scriber, 1985; Terra et al., 1987; Simpson and Simpson, 1990; Slansky, 1993). These strategies include increasing the length of feeding bouts and/or increasing rates of consumption to compensate for shortages of nitrogen in their forage (Mattson, 1980; Moran and Hamilton, 1980; Price et al., 1980; Clancy and Fenny, 1987). Therefore, low nutritive value, by itself, may not always be an effective defense against grazing (cf. Boyd and Goodyear, 1971; Mattson, 1980; Moran and Hamilton, 1980).

Seagrasses are marine vascular plants that often occur in abundant monocultures along the coasts of every continent except Antarctica. In these settings, herbivore choices are limited to the seagrass leaves themselves, and the epiphytes (both plant and animal) that live on the leaves (Valentine and Heck, 1999). Despite this, seagrasses are reported to experience very low levels of herbivory (Nienhuis and Van Ierland, 1978;

Nienhuis and Groenendijk, 1986; Cebrián et al., 1996). This is thought to be due, at least in part, to the low nitrogen content coupled with the high levels of structural carbohydrates that make the digestion of these leaves inefficient (Thayer et al., 1984; Cebrián and Duarte, 1998). Marine herbivores, however, have morphological adaptations or digestive capabilities that allow them to obtain nutrients and energy from marine vascular plants. Some fishes have low gut pH, allowing them to digest cellulose (e.g., Lobel, 1981; Lobel and Ogden, 1981; Montgomery and Targett, 1992), while other fishes, reptiles, and sirenians (i.e., manatees and dugongs) possess microbial symbionts capable of digesting cellulose in seagrass leaf tissues (Bjorndal, 1979; Thayer et al., 1984; Luczkovich and Stellway, 1993). In addition, many species of marine invertebrates possess cellulase that could allow them to digest seagrass cellulose (cf. Klumpp and Nichols, 1984). These observations have led some to conclude that, as has been suggested for many herbivorous insects and mammals, herbivore feeding intensity on seagrasses is determined by nitrogen availability, rather than by the carbon content of their leaves (e.g., Boyd and Goodyear, 1971; Mann, 1972; Lilly, 1975; Bjorndal, 1980; Zieman et al., 1984; Williams, 1988; McGlathery, 1995; Preen, 1995).

To date, few studies have investigated the relationship between grazing rates and nutrient concentrations in seagrass systems. Of these, each has strongly pointed to a significant, positive relationship between leaf nitrogen content and vertebrate grazing, whether by parrotfishes, sea turtles, or dugongs (Bjorndal, 1980; Zieman et al., 1984; Williams, 1988; McGlathery, 1995; Preen, 1995, but see Cebrián and Duarte, 1998). However, we know of no experimental investigations on the role of seagrass leaf nitrogen in determining invertebrate grazing intensity.

Using a short-term laboratory test and a longer term field experiment, we tested the hypothesis that low nitrogen content limits sea urchin consumption of seagrass leaves. From these tests, we have concluded that the role of leaf nitrogen content in controlling seagrass herbivory is more complex than previously believed and that additional field testing will be required to fully understand the relationship between leaf nitrogen concentration and seagrass grazing intensity.

2. Methods and materials

2.1. Description of the study site

The study site was within the T.H. Stone Memorial Park in St. Joseph Bay, FL, in the northeastern Gulf of Mexico (Fig. 1). We chose St. Joseph Bay because of the excellent water quality, low human impacts, easy access to expansive turtlegrass meadows, and proximity to the second largest continuous seagrass habitat in the continental United States. St. Joseph Bay is a high-salinity (30–36‰), protected shallow coastal embayment (Stewart and Gorsline, 1962; Folger, 1972) that is oligotrophic, with water column dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP) values seldom exceeding 3 and 0.2 μM , respectively (Heck et al., 2000). Phytoplankton abundance is also low, with chl *a* concentrations usually < 5 $\mu\text{g chl a/l}$. Photosyntheti-

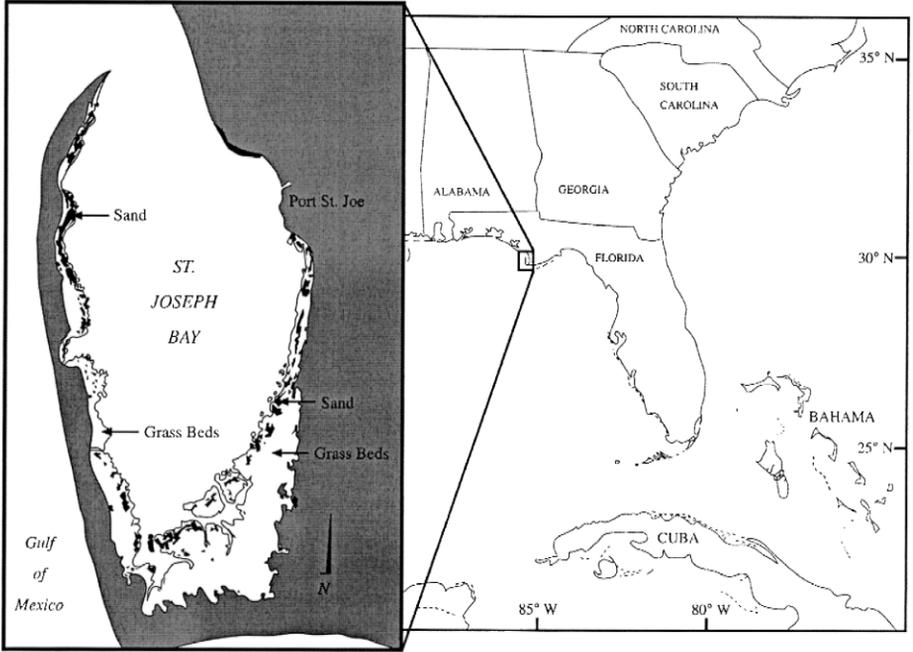


Fig. 1. Geographic setting of the study site in St. Joseph Bay, FL.

cally active radiation (PAR) is high, with approximately 40% of surface irradiance ($200\text{--}400 \mu\text{E}/\text{m}^2/\text{s}$) reaching the seagrass canopy at water depths of 1–3 m. Sediment porewater nutrient concentrations are highly variable, with summer levels of DIN and SRP ranging from $188 \mu\text{M}$ to 1.5 mM and 100 to $150 \mu\text{M}$, respectively (Peterson, 1998). The bay bottom is covered by some 26 km^2 of seagrasses (McNulty et al., 1972; Valentine and Heck, 1993). Seagrass species composition is dominated by turtlegrass (*Thalassia testudinum*), with lesser amounts of manatee grass (*Syringodium filiforme*), and shoal grass (*Halodule wrightii*) (Iverson and Bittaker, 1986). Seagrass production is highly seasonal, with leaf biomass and density peaking near $150 \text{ g Ash Free Dry Mass}/\text{m}^2$ and $3000 \text{ leaves}/\text{m}^2$ from June through August, after which time turtlegrass leaves senesce and biomass decreases rapidly (Iverson and Bittaker, 1986). Only the shallowest portions of the seagrass habitat are exposed during low tides, and wave energy is minimal. For more detailed discussions of this study site see, Iverson and Bittaker (1986), Valentine and Heck (1991, 1993), and Heck and Valentine (1995).

Turtlegrass, the dominant seagrass species in the tropical and subtropical western Atlantic Ocean, is a clonal species possessing multiple vertical branches (called short shoots) connected to a horizontal rhizome that grows beneath the sediment surface. Erect short shoots usually contain 1–5 leaves that extend aboveground from a common rhizome during the growing season. New leaves grow from a centrally located basal meristem and are held together by old leaf sheaths (Tomlinson and Vargo, 1966; Zieman and Zieman, 1989).

The dominant turtlegrass grazer along the west coast of Florida is the pink sea urchin *Lytechinus variegatus*, a generalist herbivore that indiscriminately ingests all types of plant material (Lawrence, 1975; Ogden, 1976; McClintock et al., 1982; Klinger et al., 1994; Beddingfield and McClintock, 1998). This sea urchin is commonly found in high salinity seagrass beds in the western Atlantic Ocean from North Carolina to Brazil (Serafy, 1975).

2.2. Experimental design

2.2.1. Field-based estimates of the importance of leaf nitrogen in determining sea urchin consumption of turtlegrass

We sought to test the impacts of varying leaf nitrogen content on grazing intensity by comparing changes in seagrass growth, and biomass in grazed and ungrazed treatments. The experimental design included manipulations of two main effects, grazing intensity at three levels, and leaf nitrogen content at two levels, and their interactions, yielding six treatments in a 2×3 factorial design. Grazing intensity was manipulated using cages containing 0, 10, and 20 sea urchins/m². These densities are within the range of reported densities of *L. variegatus* in the Gulf of Mexico and Caribbean Sea (e.g., Moore et al., 1963; Greenway, 1976, 1995; Keller, 1983; Thayer et al., 1984; Valentine and Heck, 1991; Heck and Valentine, 1995; Rose et al., 1999).

Leaf nitrogen content was manipulated via the enrichment of sediment pore water nitrogen. Leaf nitrogen treatments were (1) an ambient leaf nitrogen treatment (i.e., no nitrogen addition) and (2) a leaf nitrogen enrichment treatment. In the nitrogen enrichment treatment, leaf nitrogen content was increased by enriching sediment pore waters with slow release Osmocote™ tree stakes (cf. Williams, 1990; McGlathery, 1995). Nutrient stakes were placed in the center of enrichment cages at 2-week intervals and pushed completely into the sediments. Nitrogen was delivered to the sediments primarily as ammonium phosphate.

Each treatment was replicated seven times and each replicate was randomly assigned to 1 of 42 cages. We learned that seven replicates per treatment was adequate to give us sufficient power to detect significant differences ($p < 0.05$) in turtlegrass growth and abundance among our sea-urchin grazing treatments (Valentine and Heck, 1991; Heck and Valentine, 1995). Cages (1 m²) were constructed with 30-mm mesh plastic aquaculture netting, and iron rebar. These cages were placed in a 1×42 array 1 m from a turtlegrass edge to standardize cage location within the seagrass habitat. Cages were scrubbed monthly to reduce the effects of organismal fouling on PAR within the cages.

Sea urchins remained in cages for 3 weeks/month and were removed for 1 week/month. During the week-long grazing hiatus, we estimated aboveground turtlegrass production and biomass using established techniques (cf. Dennison and Alberte, 1982). Grazing impacts on turtlegrass production were measured using a syringe to puncture all the leaves in a shoot at the base of the leaf sheath. Five haphazardly-selected turtlegrass shoots were punctured this way within each cage. After 6 days, all leaves of the marked shoots were clipped at the sediment–water interface, frozen, and returned to the laboratory where primary production and biomass were determined by the analyses

described below. To ensure that previously harvested areas were not reused, the location of the marked short shoots was labeled with engineering tape tied to a wire stake inserted into the sediment. Sea urchins were then restocked in grazing treatments and the process began again. The total length of this experiment was 116 days.

Since turtlegrass leaves grow from a basal meristem, the production by marked shoots can be determined by measuring new growth distal to the hole in punctured leaves, and any new, unpunctured leaves that appear on a marked shoot. Aboveground production was defined as the biomass of all new leaf tissue formed during the 6-day period, dried to a constant mass (± 0.01 mg) at 60°C. Net aboveground primary production (NAPP) was estimated by multiplying average aboveground shoot production by the average shoot density in the cages, which was estimated from 3–0.01 m² clippings of aboveground seagrass material collected at monthly intervals from each cage. Aboveground seagrass biomass was determined from these same quadrats.

To document changes in leaf nitrogen concentrations and leaf C/N ratios due either to nutrient enrichment or grazing as older shoots with higher C/N ratios were replaced by younger shoots with lower C/N ratios (cf. Williams, 1988; McGlathery, 1995), biomass-specific changes in the concentrations of carbon and nitrogen were documented following Fourqurean et al. (1992). Five haphazardly collected shoots from each cage were dried and then ground into powder with a Wiley Intermediate Powder Mill™. The percentages of Carbon and Nitrogen in the samples were determined by combusting preweighed subsamples of ground leaves in a Carlo Erba 1500 CNS Analyzer™. Leaf nitrogen percentages were then compared among treatments by converting the percentages into standardized units (i.e., $\mu\text{g N/mg DW}$ of leaf tissue).

2.2.2. Statistical analyses

To determine the impacts of nutrient additions and sea-urchin grazing on seagrass leaf nitrogen content, leaf C/N ratios, NAPP, shoot density and aboveground biomass, we conducted a separate three way (i.e., time, nutrient treatment, and sea urchin density) Repeated Measures Analysis of Variance (RMANOVA) on each variable, following testing for heteroscedasticity using Levene's test.

RMANOVA analyzes repetitively sampled replicates using both univariate and multivariate approaches. Output is in the form of between-subject effects and within subject effects. Each examines data from a different perspective. Between-subject effects represent a test of the overall significance of the experiment and treatment effects averaged over time. Computationally, the univariate and multivariate analyses of between-subject effects are the same. When differences were detected between subjects, a posteriori comparisons were made on each sample date using the Sheffe's test.

Within subject effects comparisons represent a test of how treatments affect changes in a variable over time. For within-subject effects, the univariate approach is more powerful than the multivariate approach but has more restrictive assumptions (i.e., the circularity of the variance covariance matrix) (von Ende, 1993). To test if the data matrix met circularity, we used Mauchly's test of sphericity. If sphericity is violated the F statistic can be inflated, thus increasing the probability of making a Type I error and adjustments are required to correct the test statistic and the degrees of freedom. When sphericity was violated we made a Huynh–Feldt adjustment by multiplying the numera-

tor and denominator degrees of freedom by epsilon (ϵ) (von Ende, 1993), then F values and probabilities were recalculated (von Ende, 1993). Once the probabilities were recalculated, results were inspected for significant treatment effects. We also used the output of the RMANOVA multivariate testing of within subject effects to interpret the data. While the MANOVA approach does not require that the matrix be circular, it does assume that the variance–covariance matrices across cells are the same. To test this assumption we used a Box M test. In some cases, Box M tests indicated that the covariance matrices were not equal. In those cases, the multivariate analyses were not used in the interpretation of treatment effects on the measured variables.

C/N ratios were transformed using the arc sin square root transformation. Shoot densities were transformed using the square root transformation. When statistically significant differences in main effects were detected, a posteriori comparisons of the between-subject treatment effects were made using Sheffe's Test. Results were considered to be highly significant at $\alpha = 0.05$ and marginally significant at $\alpha = 0.1$. In addition, the power of each comparison to detect significant differences in aboveground biomass among treatments was calculated at the 5% level of significance. All statistical analyses were conducted using the SPSS statistical package for personal computers.

2.2.3. Energy flow from seagrasses to sea urchins

We estimated the amount of seagrass production, both nitrogen enriched and unenriched, consumed by sea urchins using a mass balance equation that partitions plant production into changes in biomass, herbivore consumption, and leaf loss (Krebs, 1985). The formula:

$$\text{NAPP} = \Delta B + C + L$$

was rearranged to read:

$$C = \text{NAPP} - \Delta B - L$$

where C = sea-urchin consumption each month (g DW/m²), NAPP = net aboveground primary production/m² each month (g DW/m²), ΔB = difference in aboveground biomass ($B_{t1} - B_t$) measured between months (g DW/m²) and L = leaf loss each month (g DW/m²).

Using this rearrangement, estimates of the total amount of seagrass biomass ingested by sea urchins were based on changes in the (1) total amount of leaf material produced during this experiment (summed from monthly estimates of seagrass production), and (2) monthly estimates of changes in aboveground seagrass biomass (from observed differences in aboveground biomass measured each month). Since seagrass productivity varied from month to month, monthly production was calculated as the average of daily production measured at the beginning of the month and that measured in the next month's sampling, which was then multiplied by the number of days between sampling intervals. For example, NAPP for June was based on the average daily production measured in June and July multiplied by the number of days between the June and July measurements (29 days). There were two assumptions made when using this approach. First, we assumed that leaf loss did not significantly differ among the treatments.

Therefore, leaf loss was removed from the equation (i.e., $C = \text{NAPP} - \Delta B$). Although sloppy eating of seagrass leaves undoubtedly occurred, we assumed that leaves were totally consumed by sea urchins rather than partially eaten and lost as drifting detritus. We based this assumption on the lack of observed detrital leaves on the bottoms of our cages and the fact that the initial average length of leaves within our cages was greater than the mesh size used on our cages. While it is true that this assumption could lead to an overestimate of the absolute amount of seagrass biomass consumed, it does not compromise our goal of providing a simple estimate of the relative amount of seagrass production consumed in each of our treatments.

2.2.4. Laboratory-based estimates of the role of nitrogen content on sea-urchin grazing

Sea urchins were collected from St. Joseph Bay and returned to the laboratory where they were held unfed in recirculating seawater for 5 days (cf. Lawrence et al., 1989). Turtlegrass leaves were harvested simultaneously, placed on ice and returned to the laboratory where they were stored frozen until the day before the test was to be conducted. On the day before the test, turtlegrass leaves were dried at 60°C. On the day of the test, dried leaves were ground into a fine powder using a Wiley Intermediate Grinding Mill™. This powdered seagrass was then used in two treatments to assess the role of leaf nitrogen content in controlling sea-urchin grazing.

The two treatments consisted of adult sea urchins feeding on either (1) powdered seagrass leaves embedded in agar or (2) powdered seagrass leaves supplemented with

Table 1

Repeated measures ANOVA of the impacts of *L. variegatus* grazing and nutrient additions on turtlegrass leaf nitrogen content in St. Joseph Bay, FL. Bold values indicate significant differences

(A) Between-subjects				
Source	df	MS	F	P
Urchin density	2	0.0000012	0.146	0.864
Nutrients	1	0.000138	16.934	0.000
Nutrients × Urchin density	2	0.000003	0.373	0.691
Error	36	0.0000081		

(B) Within-subjects										
Source	Univariate analysis				Multivariate analysis					
	df	MS	F	H-F	P	H-F	Num df	Den df	Wilks' λ	P
Time	4	0.00027	53.57	0.00	4	33	0.063	0.00		
Time × Urchin density	8	0.000015	2.986	0.01	8	66	0.574	0.01		
Time × Nutrients	4	0.0000195	3.864	0.01	4	33	0.528	0.00		
Nutrient × Urchin density × time	8	0.0000018	0.357	0.89	8	66	0.917	0.93		
Error	144	0.000005								

Data do not show homogeneity of treatment variances (Sphericity test, $P = 0.00$; $df = 9$). Huynh–Feldt corrected probabilities (P H–F) are given for both the univariate within subjects analysis (H–F epsilon = 0.69). Box M test found that observed covariance matrices of dependent variable were equal across groups (Box's $M = 121.8$, $F = 1.01$, $df_1 = 75$, $df_2 = 2179$, $p > 0.464$).

powdered fish meal to increase the protein content embedded in agar. We chose to enrich the protein content of our agar treatment by the addition of “fish meal” (which was 45% protein by mass) rather than using field enriched seagrass leaves, because

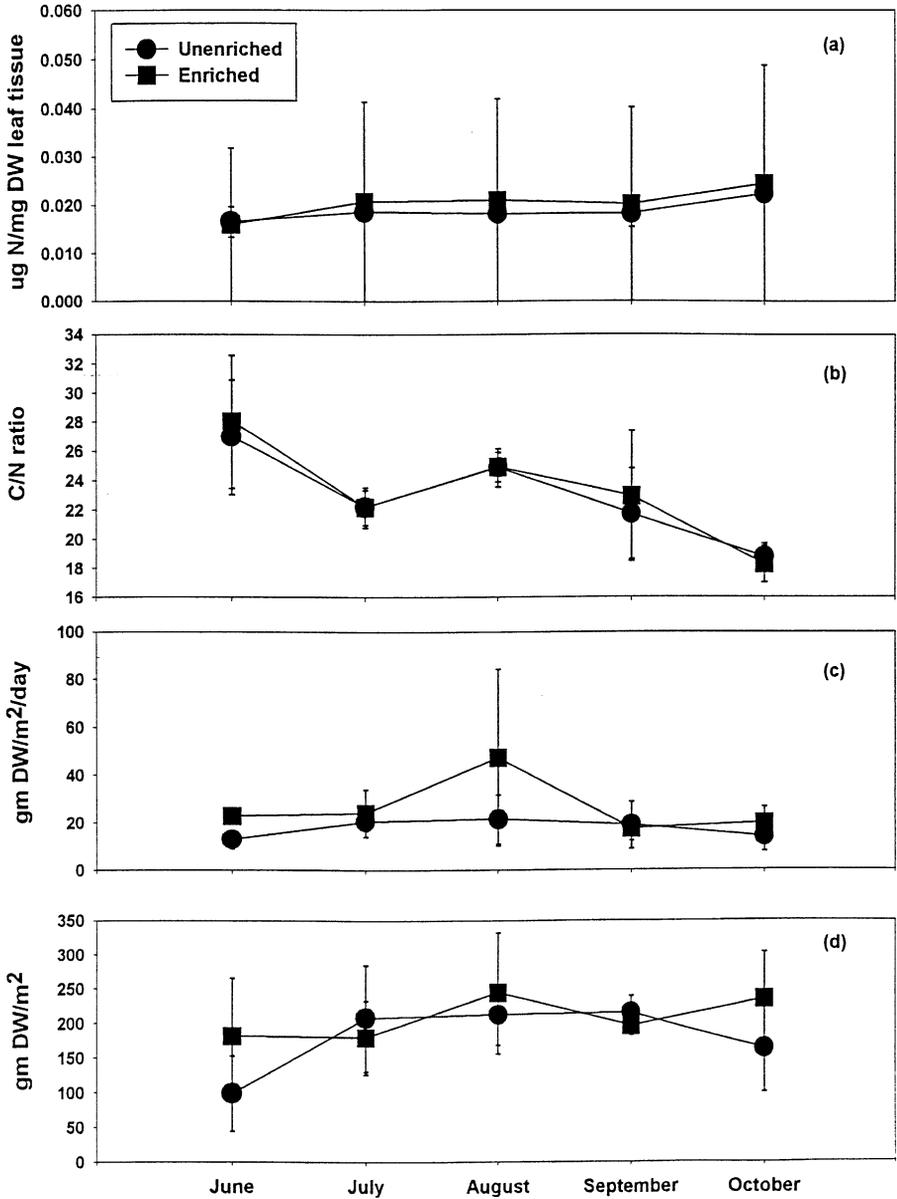


Fig. 2. Enriched vs. unenriched (a) leaf nitrogen content, (b) C/N ratios, (c) aboveground primary production, and (d) aboveground biomass in cages excluding sea urchins (mean \pm 1 S.E., $n = 7$).

seagrass leaves rapidly lose soluble nitrogen when detached from their shoots (e.g., Harrison, 1989). The fish meal protein contained plant, fungal and animal protein. Such diversity of protein content is representative of the diversity of protein sources (both plant and animal) that *L. variegatus* encounters in the natural environment (Lawrence, 1975; Beddingfield and McClintock, 1998). This same approach has been successfully

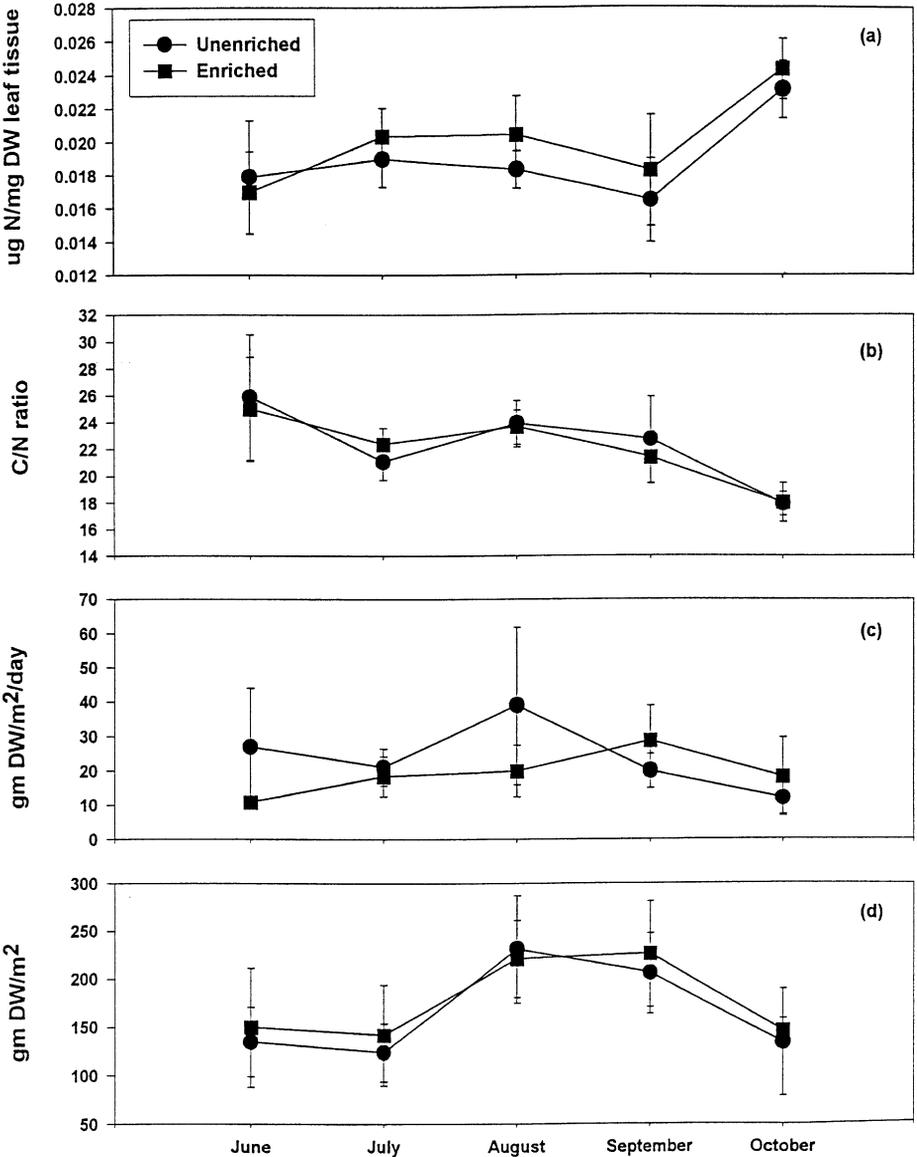


Fig. 3. Enriched vs. unenriched (a) leaf nitrogen content, (b) C/N ratios, (c) aboveground primary production, and (d) aboveground biomass in cages enclosing 10 sea urchins (mean ± 1 S.E., $n = 7$).

used by other investigators to study sea urchin-feeding responses to foods of varying nutritional content (e.g., Klinger, 1982; McClintock et al., 1982; Levin and Naidenko, 1987; Lares and McClintock, 1991; Klinger et al., 1994).

The powdered protein and/or seagrass were thoroughly mixed in boiling 5% agar solutions. Each mixed solution was poured into preweighed plastic containers and

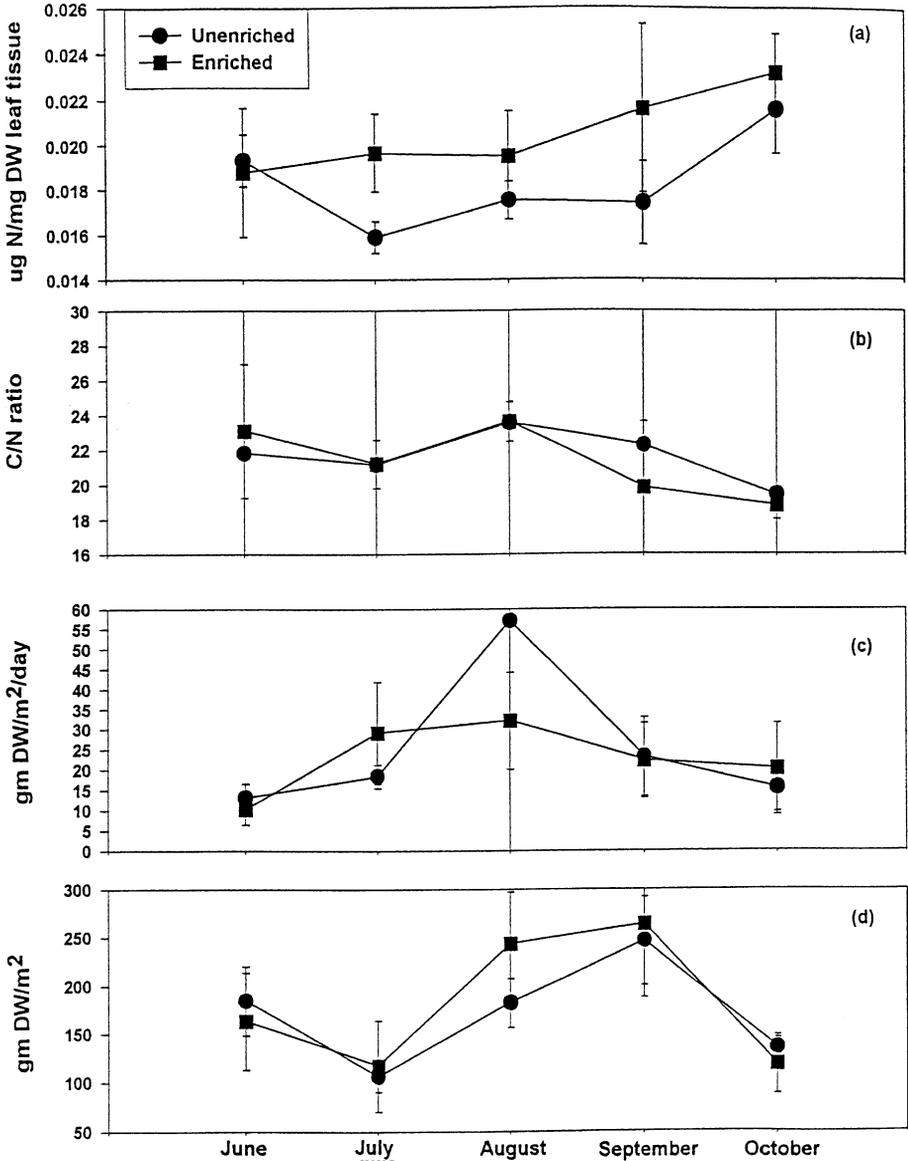


Fig. 4. Enriched vs. unenriched (a) leaf nitrogen content, (b) C/N ratios, (c) aboveground primary production, and (d) aboveground biomass in cages enclosing 20 sea urchins (mean \pm 1 S.E., $n = 7$).

allowed to cool for 2–3 h. Cooled containers were reweighed and one sea urchin (approximately 35 mm horizontal diameter) was placed in each container and covered with a fine mesh top. The containers were then placed in recirculating seawater tanks for 24 h. After 24 h, the containers were removed from the tanks and seawater was decanted. Sea urchins were removed from the containers and returned to the holding tanks. The retained material in the container was air dried for 2 h, then patted dry with tissue paper and weighed. The difference between the initial mass and the final mass was considered the estimate of the ingested agar. Each treatment was replicated 14 times.

The leaf nitrogen content of the powdered seagrass was 2.2% by mass. Seagrass leaf nitrogen content in the powdered leaves was converted to protein content by multiplying leaf nitrogen content by 6.25 (cf. Rice, 1982). The addition of “fish meal” to the seagrass increased protein content from 8% in the agar containing only ground seagrass to 18.8% in the agar containing ground seagrass and fish meal.

Initially we looked for differences between the treatments using a two sample *t*-test. However, violations of normality led us to analyze these data using a Mann Whitney Rank Sum Test. As before, the results of this test were considered to be highly significant if $p < 0.05$ and marginally significant if $p < 0.10$.

3. Results

Between-subject comparisons of our main effects (i.e., manipulations of nutrient additions and sea urchin density) showed that leaf nitrogen concentrations were signifi-

Table 2

Repeated measures ANOVA of the impacts of *L. variegatus* grazing and nutrient additions on turtlegrass leaf C/N ratios in St. Joseph Bay, FL. Bold values indicate significant differences

(A) Between-subjects				
Source	df	MS	F	P
Urchin density	2	0.0025	2.502	0.096
Nutrients	1	0.00019	0.194	0.662
Nutrients × Urchin density	2	0.000319	0.323	0.726
Error	36	0.00099		

(B) Within-subjects								
Source	Univariate analysis				Multivariate analysis			
	df	MS	F H–F	P H–F	Num df	Den df	Wilks' λ	P
Time	4	0.0508	72.08	0.00	4	33	0.048	0.00
Time × Urchin Density	8	0.00085	1.203	0.31	8	66	0.612	0.03
Time × Nutrients	4	0.00046	0.656	0.59	4	33	0.858	0.27
Nutrient × Urchin density × time	8	0.000597	0.847	0.54	8	66	0.877	0.81
Error	144	0.00071						

Data do not show homogeneity of treatment variances (Sphericity test, $P = 0.00$; $df = 9$). Huynh–Feldt corrected probabilities (P H–F) are given for both the univariate within-subjects analysis (H–F epsilon = 0.777). Box *M* test found that observed covariance matrices of dependent variable were equal across groups (Box's $M = 112.58$, $F = 0.931$, $df_1 = 75$, $df_2 = 2179$, $p > 0.64$).

cantly higher in treatments where nutrients were added to the sediment (some 10–20% higher) than in treatments where nutrients were not added (Table 1, between-subject effects) (Figs. 2a, 3a and 4a). Within-subject effects comparisons showed that changes in leaf nitrogen content were also controlled by interactions between time and sea urchin density and between nutrient treatment and time (Table 1). No three-way interactions were noted (Table 1, within-subject effects: time \times sea urchin density \times nutrient). Pairwise comparisons of leaf nitrogen content among sea urchin densities showed that this interaction became significant in October (the last month of the experiment) when leaves in the treatments enclosing sea urchins at a density of 20 individuals/m² had significantly lower nitrogen content than the other treatments. No other significant effects of sea urchin density on leaf nitrogen content were noted. This indicates that sea-urchin grazing did not introduce a significant confounding source of variation into our manipulations of leaf nitrogen content.

Sea urchin density played a marginally significant role in controlling C/N ratios in leaves (Table 2, between-subject effects). A highly significant interaction between grazing density and time showed that sea urchin density also controlled how leaf C/N ratios changed in this experiment (Table 2, within-subjects effects: sea urchin density \times time (multivariate only). Pairwise contrasts of sea urchin density on leaf C/N show that interactions became significant in August when C/N ratios in the ungrazed control treatment were significantly higher than both sea urchin enclosure treatments. No other differences were noted in August. Pairwise differences among grazing treatments were

Table 3

Repeated measures ANOVA of the impacts of *L. variegatus* grazing and nutrient additions on turtlegrass NAPP in St. Joseph Bay, FL. Bold values indicate significant differences

(A) Between-subjects

Source	df	MS	F	P
Urchin density	2	0.00023	0.569	0.571
Nutrients	1	0.00021	0.518	0.476
Nutrients \times Urchin density	2	0.0016	3.88	0.03
Error	36	0.0303		

(B) Within-subjects

Source	Univariate analysis				Multivariate analysis						
	df	MS	F	H–F	P	H–F	Num	Den	df	Wilks' λ	P
Time	4	0.0144	13.17	0.000	4	33	0.239	0.000			
Time \times Urchin density	8	0.0012	1.086	0.363	8	66	0.749	0.269			
Time \times Nutrients	4	0.00066	0.608	0.504	4	33	0.817	0.142			
Nutrient \times Urchin density \times time	8	0.0039	3.58	0.02	8	66	0.511	0.003			
Error	144	0.001									

Data do not show homogeneity of treatment variances (Sphericity test, $P = 0.00$; $df = 9$). Huynh–Feldt corrected probabilities (P H–F) are given for both the univariate within-subjects analysis (H–F epsilon = 0.385). Box M test found that observed covariance matrices of dependent variable were not equal across groups (Box's $M = 223.3$, $F = 1.85$, $df_1 = 75$, $df_2 = 2179$, $p < 0.000$).

noted in October when leaf C/N ratios were significantly lower in the 20 sea urchin treatment than in the 10 sea urchin treatment. No differences were noted between the ungrazed control treatment and either of the sea urchin enclosure treatments. The biological relevance of this interaction is unclear as differences in C/N ratios among the sea-urchin grazing treatments were always small (Figs. 2b, 3b and 4b). The lack of a significant nutrient effect or significant interaction between nutrients and time showed that the nutrient additions had no effect on changes in leaf C/N ratios (Table 2).

A highly significant interaction between nutrient treatments and grazer density controlled NAPP in our experiment (Table 3, between-subject effects). There was a highly significant three-way interaction, indicating that both of the main effects played a significant role in determining how NAPP changed during this experiment (Table 3, within treatment effects: time \times sea urchin density \times nutrient). While nutrient additions did lead to higher NAPP in the ungrazed cages in most months, the pattern was reversed when nutrients were added to cages where sea urchins were present (Table 4). The total estimated amount of NAPP was much higher in grazed cages without nutrient additions (Table 4). In the absence of grazers it seems clear that our nutrient additions led to increased levels of NAPP. However, in treatments where grazers were present, NAPP was much higher than in treatments without grazers.

We found no evidence that the increased levels of NAPP in the treatments where sea urchins were present led to higher levels of aboveground biomass (Table 5, between-

Table 4

Turtlegrass NAPP, aboveground biomass, and sea urchin consumption of enriched and unenriched seagrass leaf production in St. Joseph Bay, FL. The numbers 10, and 20 indicate urchin density. -N indicates unenriched nutrient treatments and +N enriched nutrient treatments

Months	Treatments			
	10/-N	10/+N	20/-N	20/+N
<i>Net aboveground primary production (gm DW / m²)</i>				
June–July	695	423	454	570
July–August	748	477	940	765
August–September	909	751	1240	840
September–October	490	724	595	655
Total Production	2840	2370	3230	2830
<i>Changes in aboveground seagrass biomass (gm DW / m²)</i>				
June–July	-10.9	-7.8	-78.3	-46.3
July–August	107	79.0	76.0	126
August–September	-25.0	5.0	64.0	20.0
September–October	-72.0	-80	-111	-145
Change in biomass	-0.90	-3.8	-49.3	-45.3
<i>Sea urchin consumption (gm DW / m²)</i>				
June–July	705	431	538	617
July–August	641	398	864	639
August–September	934	746	1180	820
September–October	562	804	706	800
Total biomass consumed	2840	2380	3280	2880
Per capita consumption	284	238	164	144

Table 5

Repeated measures ANOVA of the impacts of *L. variegatus* grazing and nutrient additions on turtlegrass aboveground biomass in St. Joseph Bay, FL. Bold values indicate significant differences

(A) Between-subjects

Source	df	MS	F	P
Urchin density	2	0.069	0.767	0.472
Nutrients	1	0.0594	0.661	0.422
Nutrients × Urchin density	2	0.04463	0.497	0.61
Error	36	0.0898		

(B) Within-subjects

Source	Univariate analysis				Multivariate analysis			
	df	MS	F H–F	P H–F	Num df	Den df	Wilks' λ	P
Time	4	0.679	3.795	0.03	4	33	0.211	0.000
Time × Urchin density	8	0.102	0.571	0.69	8	66	0.549	0.008
Time × Nutrients	4	0.063	0.35	0.72	4	33	0.933	0.67
Nutrient × Urchin density × time	8	0.259	1.446	0.23	8	66	0.875	0.797
Error	144	0.179						

Data do not show homogeneity of treatment variances (Sphericity test, $P = 0.00$; $df = 9$). Huynh–Feldt corrected probabilities (P H–F) are given for both the univariate within-subjects analysis (H – F epsilon = 0.52). Box M test found that observed covariance matrices of dependent variable were not equal across groups (Box's $M = 301.4$, $F = 2.5$, $df_1 = 75$, $df_2 = 2179$, $p < 0.000$).

subject effects and within-subject effects: time × sea urchin density interaction; Figs. 2c, 3c and 4c). Aboveground biomass changed little in the 10 sea urchins/m² treatments during the first month, but dropped dramatically in the 20 sea urchin treatments when compared to the changes in the ungrazed control treatment (Table 4, Figs. 2d, 3d and 4d). This suggests that the increased levels of NAPP in our sea urchin enclosure treatments had reached levels that compensated for the material being lost to grazing. In contrast, the nutrient treatments did have a marginally significant effect on how biomass changed (Table 5, within-subject effects: time × nutrients interaction (univariate only).

The power of our test to detect a 50% difference in the impacts of different grazing densities on log transformed aboveground biomass was low at the end of our experiment (0.05 at $\alpha = 0.05$). This low power shows that the probability of making a type II error (failing to reject a false null hypothesis of no significant treatments effects) was very high, suggesting that higher leaf nitrogen content may have, in fact, actually led to the increased consumption by sea urchins.

Because of the low power of this comparison and the fact that sea-urchin grazing higher rates of NAPP in unenriched cages, we estimated sea-urchin grazing using a standard mass balance equation that considered both changes in NAPP and aboveground biomass. In this comparison, NAPP was lower in the 10 sea urchin treatments than in 20 sea urchin treatments (Table 4). We also found that biomass loss was greater in the treatments with higher densities of sea urchins (Table 5). Based on these changes, we estimated that sea urchins ate more of the unenriched aboveground production (either on

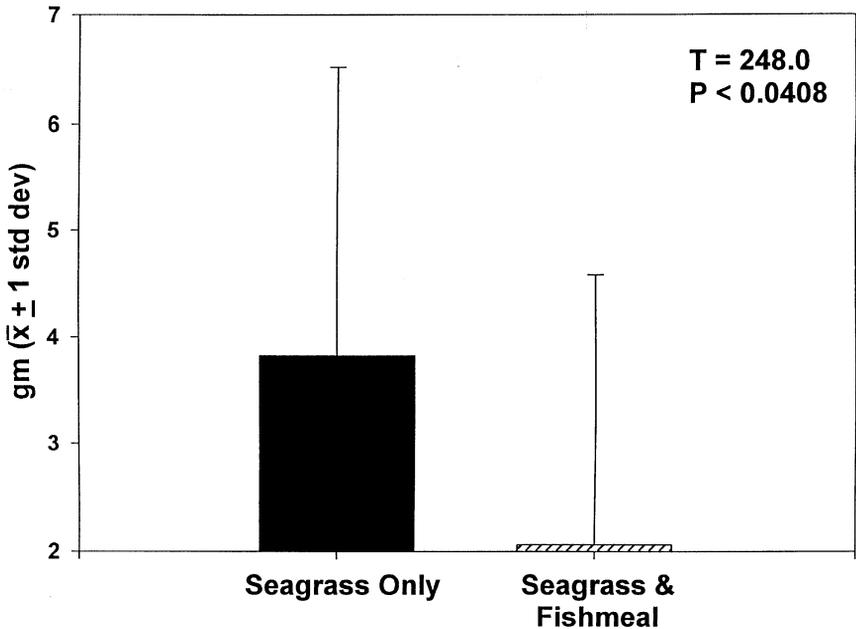


Fig. 5. Sea urchin consumption of nitrogen-enriched and -unenriched seagrass blades embedded in agar (mean \pm 1 S.E., $n = 10$).

a per capita basis or on a treatment basis) than was estimated to have been consumed in enriched treatments (Table 4).

The results of the mass balance estimate of sea urchin consumption of turtlegrass were supported by the laboratory estimates of sea urchin consumption of agar blocks of varying protein content. The statistical comparison of sea urchin consumption of unenriched and protein-enriched agar found that the sea urchins ate significantly more of the unenriched agar ($df = 14$; $T = 248.0$; $p < 0.0408$). In fact, sea urchins ate twice as much of the unenriched agar over a 24-h period (Fig. 5).

4. Discussion

Spatial and temporal variation in herbivory among individual plants is one of the most consistent yet poorly understood aspects of plant–animal interactions (cf. Louda and Collinge, 1992; Hacker and Bertness, 1995, 1996). While many hypotheses have been advanced to explain interplant variability in losses to herbivores, differences in nitrogen availability, which can control growth and affect nutritional content, are considered to be among the most important (cf. Hunter and Price, 1992; Hacker and Bertness, 1995, 1996). In our field experiment, we successfully increased seagrass leaf nitrogen content above background levels recorded either within St. Joseph Bay or elsewhere within natural seagrass beds reported in the literature (Duarte, 1990). How-

ever, we found no evidence in our experiments, that leaf nitrogen content significantly affected sea-urchin grazing rates, despite the fact that differences in leaf nitrogen content among our treatments were similar to differences reported by others studying specialist seagrass herbivores and their forage (Bjorndal, 1980; McGlathery, 1995; Preen, 1995). The very low statistical power of our comparison led us to use a mass balance equation and laboratory ingestion estimates, which showed that sea urchin feeding on seagrasses was affected by leaf N, but not in the expected way. Instead, the sea urchins ate more of the unenriched seagrass leaves, and apparently compensated for the low nitrogen content of their forage by adjusting their intake of nitrogen, as shown previously for other sea urchin species (Vadas, 1977; Lares and McClintock, 1991).

Evidence supporting this can be found by multiplying the monthly estimate of seagrass biomass consumed by *L. variegatus* in each treatment by the average treatment values of leaf nitrogen concentrations between sampling interval estimates (e.g., the average of nitrogen concentrations in a treatment measured in June and July). We then calculate that these sea urchins ingested comparable amounts of nitrogen from unenriched (52.38 and 58.31 mg N/m² in the 10 and 20 individuals/m² treatments, respectively) and enriched treatments (54.45 and 59.08 mg N/m² in the 10 and 20 individuals/m² treatments, respectively) during this experiment. Thus, by eating more seagrass biomass in the treatments with lower leaf nitrogen content, sea urchins received comparable total quantities of nitrogen.

While the mechanisms by which chemical defenses affect plant feeding preferences in marine environments have been frequently reported (summarized Hay and Steinberg, 1992), we do not know how marine herbivores might preferentially select nitrogen-rich foods or avoid plants low in nitrogen. Certainly, such discriminating herbivores would need the ability to detect differences in plant nutritional quality so that they could preferentially feed on more nutritious plants (cf. Westoby, 1974, 1978). To date, we know of no studies that have documented this capability in the marine realm. Indeed, it would be surprising if herbivores selected food based solely on nutritive value, given the lack of specialized feeding by most marine herbivores (Hay, 1991; Hay and Steinberg, 1992). Our test organism, *L. variegatus*, is also a generalist feeder with no evident food preferences (Lawrence, 1975; McClintock et al., 1982; Klinger and Lawrence, 1985; Montague et al., 1991). Because other generalists have been shown to increase their ingestion rates when feeding on marine plants of low nutritional value (Vadas, 1977; Lares and McClintock, 1991, this study), it seems likely that other invertebrate grazers have the ability to compensate for the nutritional quality of their forage as well. Most herbivorous fishes are extreme generalists that feed on vegetation in proportion to its abundance (Ogden, 1980; Lobel and Ogden, 1981; Choate, 1991; Hay and Steinberg, 1992) and have high consumption rates and rapid gut throughput times (Horn, 1989). These fishes can maintain very large populations and exhibit high growth rates on diets low in protein (Russ, 1984; Russ and St. John, 1988). Thus, they must process a great deal of organic material very quickly and could consume significant quantities of seagrass production when herbivore biomass is high.

It is also noteworthy that juveniles and smaller species of parrotfishes feed on seagrasses (Ogden and Zieman, 1977; Handley, 1984; McGlathery, 1995; McAfee and Morgan, 1996). As they grow, many of these fish abandon seagrass habitats for more

structurally complex coral reefs where it has been hypothesized that they find increased protection from large piscivorous fishes (Springer and McErlean, 1962; Ogden and Zieman, 1977; Dubin and Baker, 1982; Handley, 1984; Carpenter, 1986). These observations suggest that the flow of energy from seagrass habitats to coral reefs can be substantial, but quantitative estimates are constrained by the limited amount of information on coral reef dominated food webs (cf. Polunin, 1996). In summary, if these observations are valid, then earlier held views on the role of leaf nitrogen content in limiting grazing on seagrasses (e.g., Bjorndal, 1980; Duarte, 1990; Lalli and Parsons, 1993; McGlathery, 1995; Valiela, 1995) will require reexamination. A first step in resolving the differences between earlier correlative work with herbivorous marine fishes and mammals and our experiments would be to do experiments similar to those reported here with vertebrate grazers. At this time, we do not believe that predictions about when to expect plant nitrogen level to influence grazing rates can accurately be made, and all that can be said is that low levels of nitrogen are not always effective defenses against grazing by marine herbivores.

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