

Simulated green turtle grazing affects nutrient composition of the seagrass *Thalassia testudinum*

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Abstract Before populations of green turtles (*Chelonia mydas*) were severely reduced by human over-exploitation, the seagrass *Thalassia testudinum* was intensively grazed by green turtles in the Caribbean. To explore how nutrient composition of *T. testudinum* pastures responds to intense grazing pressure, we simulated green turtle grazing in 15 plots (each 3 m × 3 m) for 16 months in the central Bahamas. Comparisons of clipped plots with 15 adjacent control (unclipped) plots revealed that simulated grazing resulted in significantly higher energy, nitrogen, phosphorus, lignin, cutin, and condensed tannin content in blades in clipped plots, but sediment organic content was not affected. By continually re-cropping blades in grazing plots, turtles ingest young, actively growing blade tissue with higher energy, nitrogen, and phosphorus concentrations. Our 16-month clipping trial did not generate the expected decline in nutrient content in *T. testudinum* blades under intensive grazing. However, significant decreases in nitrogen and organic matter reserves in rhizomes, with declines apparent after 16 and 11 months, respectively, indicate that nutrient content of blades and/or blade productivity may decline under continued clipping.

Introduction

Thalassia testudinum is a seagrass that forms dense, extensive pastures in the Greater Caribbean. A robust seagrass with long, strap-like blades, *T. testudinum* has a dense belowground network of roots and rhizomes that serves as a support and storage system for a series of vertical short shoots, each bearing from two to eight leaf blades that grow from a basal meristem.

Green turtles, *Chelonia mydas*, in the Caribbean feed primarily on *T. testudinum* (Bjorndal 1997) and exhibit a foraging pattern in which they select young, actively growing tissue at the base of the blades and allow the upper, older part of the blades to float away (Audubon 1897; Bjorndal 1980). Once the older tissue has been cleared, green turtles can maintain grazing plots ranging from 10 to 100 m² or larger for at least a year by re-cropping blades in those plots (Bjorndal 1980; Ogden et al. 1983; Williams 1988). Grazing turtles lie almost motionless on the substrate and methodically graze every blade of seagrass in an area to within a few centimeters of the substrate, biting off approximately 5-cm-long blades (Bjorndal 1980; Williams 1988). Turtles forage primarily during the morning and afternoon; they rest overnight and often during the middle of the day in hard bottom areas that may be as far as 0.5 km away from their grazing plots (Bjorndal 1980; Ogden et al. 1983). Young blades of *T. testudinum* have been reported to be higher in nitrogen and lower in lignin (Dawes and Lawrence 1979; Bjorndal 1980; Zieman et al. 1984) and usually have fewer epibionts (Zieman 1982) than more mature blades. Therefore, by consuming young blades, either by ingesting the lower parts of the blades in one-time grazing events or by maintaining grazed plots, green turtles have been

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reported to ingest a more digestible diet with higher nitrogen and lower lignin content (Bjorndal 1980) and with low calcified epibiont loads (Mortimer 1981; Zieman et al. 1984).

A suite of responses of *T. testudinum* to intensive grazing by green turtles was proposed by Thayer et al. (1984). Initially, blade growth would increase and blade tissue would have higher nitrogen and lower lignin content than ungrazed blades. Repeated cropping, however, would stress the plant. Blade nutrient quality and width, number of shoots per area, and number of blades per shoot would decrease, and blade production would decline within a year. Reduced blade production would result from depleted rhizome stores as soluble carbohydrates and nitrogen were mobilized for blade growth and from lowered input of nitrogen from detritus. Declining productivity and forage quality in the grazing plots would cause green turtles to abandon the plots and establish new ones elsewhere. These proposed responses in the structure, nutrient composition, and productivity of *T. testudinum* beds to green turtle grazing have been addressed in few studies (Zieman et al. 1984; Williams 1988), and the extent to which the maintenance of grazing plots by green turtles is a sustainable foraging pattern is not known.

Overexploitation by humans has caused a massive decline of green turtles in the Caribbean, perhaps as great as 93–97% (Jackson et al. 2001; Pandolfi et al. 2003). Green turtles, numbering in the tens of millions, may once have consumed much of the primary production of *T. testudinum* (Bjorndal 1982; Jackson 1997; Bjorndal et al. 2000) and certainly affected the structure, nutrient composition, and productivity of seagrass ecosystems in the Caribbean (Bjorndal 1980; Ogden 1980; Thayer et al. 1982, 1984). Understanding the effects of grazing on the structure and function of seagrass ecosystems is critical to evaluating how ecosystem processes have changed since the major herbivore was essentially removed from modern Caribbean seagrass systems. That information is also essential to develop management plans to restore marine ecosystems (Pitcher 2001).

To evaluate the structure, nutrient composition, and productivity of *T. testudinum* pastures grazed by green turtles and compare them with the suite of responses proposed by Thayer et al. (1984), we simulated intensive grazing by green turtles by repeatedly clipping plots of *T. testudinum* in the central Bahamas for 16 months. We evaluated responses to simulated grazing by quantifying—in clipped and unclipped plots—plant structure, above and belowground biomass, nutrient composition of blades and rhizomes, aboveground productivity, and organic content and particle size distri-

bution of the sediment. Simulated grazing reduced the structural complexity, but not the productivity, of *T. testudinum* plots (Moran and Bjorndal 2005). Clipped plots had significantly lower blade biomass, shorter and narrower blades, and thinner detrital layers, but number of blades per shoot, number of shoots per m², and rhizome biomass did not change. Blade mass production (g/m²) was not significantly different in clipped plots and unclipped plots, and blade specific mass growth (productivity to biomass ratio) was significantly elevated in clipped plots. In this paper, we present the changes in nutrient composition of blades and rhizomes and the organic content of sediments during the 16-month clipping regime.

Materials and methods

Study area and experimental plots

The study was conducted from July 1999 through December 2000 in a contiguous monospecific stand of *T. testudinum* at 3 m depth (mean low water level) located approximately 400 m southwest of Lee Stocking Island, Exuma Cays, Bahamas (23.46°N, 76.06°W). Based on eight parameters (temperature, salinity, seagrass structure, and productivity), our study site fell within the range of values for 14 *T. testudinum* sites monitored across the Greater Caribbean (CARI-COMP 1997a, b) except for blade width, which was below the range. The site had a tidal range of approximately 1.5 m, a very low current flow, oligotrophic waters, and carbonate sediments. No green turtles were seen in the area during our study; there is a legal harvest of green turtles in the Bahamas. Herbivorous fishes (*Sparisoma radians*, *Scarus taeniopterus*, *Scarus croicensis*) were present at low levels, herbivorous invertebrates were rare, and epibiont loads on *T. testudinum* blades were very light.

Thirty 3 m × 3 m plots (15 clipped and 15 unclipped) were established in the seagrass bed in July 1999. This plot size represents a realistic size, while allowing sufficient replication for a robust statistical analysis. An additional five clipped plots were established in February 2000 to test for effects of season of clipping initiation (summer vs. winter) on seagrass response. Distances between nearest neighbor plots ranged from 4 to 50 m; clipped and unclipped plots were alternated. Rhizomes around the edges of each clipped plot were severed to a depth of ~35 cm with a flat-bladed shovel every 6–8 weeks to prevent nutrient translocation along rhizomes into the clipped plots from adjacent unclipped areas and to mimic a larger

plot size. Corners of the 3 m × 3 m plot and the central 2 m × 2 m area were marked with PVC pipe, and outer and inner areas were outlined with polypropylene string at sediment height.

The clipping regime mimicked green turtle grazing. All *T. testudinum* blades in each clipped plot were initially clipped with stainless steel scissors just above the blade/sheath junction (~2 cm above the sediment), simulating a green turtle grazing pattern (Bjorndal 1980). All blades in each clipped plot were re-clipped when mean blade length reached 5 cm above the blade/sheath junction, approximating the 5 cm bite size of a foraging green turtle (Ogden et al. 1983; Williams 1988). Clipping interval ranged from 12 to 38 days because growth rates varied with temperature (Moran and Bjorndal 2005). Clipping was conducted during the times of day when green turtles usually forage and by hovering just above the plots to mimic the level of disturbance caused by grazing turtles. Clipping was maintained in the original 15 clipped plots from July 1999 through November 2000 and in the five winter-initiated plots from February 2000 through December 2000. One of us (KLM) logged over 1700 h of SCUBA time conducting this clipping trial.

Sample and data collection

Salinity (‰) and maximum and minimum temperature (°C) were measured weekly at 08:30 h at three sites within the plots. Salinity of water at blade canopy height was determined with a hand-held refractometer calibrated before each use. Maximum–minimum thermometers were anchored to the sediment at blade canopy height and reset each week.

The outer 0.5-m-wide border (5 m² area) of each clipped plot was a buffer zone for any shading/sheltering effects due to adjacent unclipped seagrass (Tomasco and Dawes 1989). Samples of *T. testudinum* and sediments were collected for analyses at the first clipping (prior to any clipping effect for comparison with initial state of the unclipped plots) and 2, 6, 11, and 16 months after initiation of clipping. For nutrient analyses, all blades were collected from the interior 4-m² area of each clipped plot and from three 0.0625 m² (25 cm × 25 cm) quadrats in each unclipped plot. Belowground samples—short shoots, rhizomes, and roots—were collected from one 1,140-cm³ (7.62 cm inner diameter, 25 cm depth) core in each plot except for the five clipped plots established in February. We will refer to aboveground biomass as blades, and belowground biomass as rhizomes. Blade and rhizome samples were rinsed in salt water to remove sediment, dried at 60°C, and stored for subsequent analyses.

At each sampling time, a sediment sample (304 cm³ cores, 5.08 cm inner diameter × 15 cm depth) was collected in each of the 30 original plots for analyses of organic matter content. Sediments were cleaned of invertebrates and vegetation visible to the naked eye and dried at 60°C.

Nutrient and energy analyses

Dried *T. testudinum* blades and rhizomes were ground to pass through a 1 mm screen in a Wiley mill. Rhizomes were analyzed for fewer components than were blades because of the small size of the rhizome samples. Standard procedures were used to determine dry matter, organic matter, energy, nitrogen, phosphorus, cell wall constituents (CWC) (= structural carbohydrates or cellulose, hemicellulose, lignin, and cutin), ligno-cellulose (=cellulose, lignin, and cutin), lignin, cutin, and in vitro organic matter fermentability.

Dry matter and organic matter content were determined by drying a subsample at 105°C for 16 h followed by combustion at 500°C for 3 h in a muffle furnace, a temperature high enough to oxidize organic material without degrading significant quantities of CaCO₃ (Radar and Grimaldi 1961). To ensure that organic matter content of the sediment was not overestimated by combustion of some CaCO₃ or loss of structural water in the clay fraction, organic carbon content of sediments was determined using a chromic acid titration method (Walkley and Black 1934). Energy content was determined with a Parr oxygen bomb calorimeter (Parr Instrument Company 1960). Total nitrogen and phosphorus analyses employed a modification of the aluminum block digestion procedure and semi-automated colorimetry (Gallaher et al. 1975; Hambleton 1977).

Structural compounds were determined from sequential extractions (Goering and Van Soest 1970) with an Ankom²⁰⁰ Fiber Analyzer (Ankom Technology Company 1998, 1999). Blades were exposed to a sequence of extractions with the following solutions: neutral detergent solution (residue = CWC = cellulose, hemicellulose, lignin, and cutin), acid detergent solution [residue = ligno-cellulose fraction (ADF) = cellulose, lignin, and cutin], 72% H₂SO₄ (residue = lignin and cutin), and saturated potassium permanganate solution (residue = cutin). Rhizomes were exposed to a sequence of extractions with neutral detergent, acid detergent, and 72% H₂SO₄ solutions.

In vitro organic matter fermentability (Moore and Mott 1974) is a bioassay that determines the fermentability of a sample with inoculum from the rumen of a fistulated cow. Because microbial fermentations in the

guts of fishes, reptiles, birds, and mammals are similar in composition and process (Stevens and Hume 1995), fermentability of substrates in one vertebrate can be used as a relative indicator of fermentability in other vertebrates.

To take advantage of a visit by T. Arnold to the field site in November 1999, *T. testudinum* blades were collected in November 1999 from clipped and unclipped plots and were analyzed by T. Arnold for total phenolics and condensed tannins. Tissue samples were stored in liquid nitrogen and subsequently cleaned of epiphytes, freeze-dried, homogenized, and extracted (Arnold and Schultz 2002). Condensed tannins were measured as extracted proanthocyanidins using the acid-butanol method (Arnold and Schultz 2002). Total reactive phenolics were measured using a Folin–Denis assay modified from Arnold et al. (1995). Concentrations were determined by comparison with standard curves based on pure *T. testudinum* tannins and purified phenolics from *T. testudinum* blades collected at our site.

Data analyses

All nutrients and energy content were analyzed on both a dry matter and organic matter basis, which yielded similar statistical comparisons for clipped and unclipped plots (Moran 2003); only analyses on a dry matter basis are presented here. For unclipped plots, the mean of the three quadrats was used as the plot value. Percentages were arcsine transformed before analysis to normalize the data. Differences between clipped and unclipped treatments for all parameters over the 16-month clipping trial were compared using a two-factor repeated-measures ANOVA with time as the within-subject factor and treatment (clipped vs. unclipped) as the between-subject factor. If Mauchly's criterion indicated rejection of the compound symmetry assumption, adjusted probability values are presented.

Homogeneity between clipped and unclipped plots at the beginning of the clipping trial was assessed with *t*-tests. To evaluate the effect of season (summer or winter) when clipping was initiated, we controlled for an 11-month clipping interval and, with *t*-tests, compared unclipped plots against summer-initiated clipped plots in May 2000 and unclipped plots against winter-initiated clipped plots in December 2000.

Differences were considered significant at the $\alpha = 0.05$ level. Bonferroni corrections were not applied because of concerns that the probability of making a Type II error would become excessive (Perneger 1998). All analyses were run in SPSS (v. 10.0) or S-PLUS (v. 6.1).

Results

Temperature varied seasonally (Fig. 1). Salinity showed no seasonal cycle, but salinity was reduced in October 1999, when Hurricane Floyd passed over the study area (Fig. 1).

Prior to initiation of clipping, there was no significant difference between clipped and unclipped plots for any nutrient parameter of blades, rhizomes, or sediments (*t*-tests, $0.102 \leq P \leq 0.983$) or any structural parameter [blade length and width, blades per shoot, shoot density, detrital layer thickness, blade biomass, rhizome biomass (Moran and Bjorndal 2005)]. Clipping treatment and time had significant effects on a number of nutrient parameters, but there were no significant time \times treatment interactions (repeated-measures ANOVA, Table 1). Significant time effects were probably the result of seasonal changes in temperature (Fig. 1).

Clipping had a significantly positive effect on all measures of blade nutrient and energy composition except fermentability, which did not change (Table 1). The fact that organic matter was significantly higher in clipped blades could indicate that the differences in the other parameters, all expressed as a percent of dry mass, are merely a result of the higher organic matter content. However, results were the same when values were expressed as a percent of organic matter; clipping had a significant, positive effect on all parameters, except fermentability (Moran 2003). Lignin and cutin are components of both CWC and ADF, and the increases in lignin and cutin account for the majority of the increase in CWC and ADF between unclipped and clipped blades (Table 1).

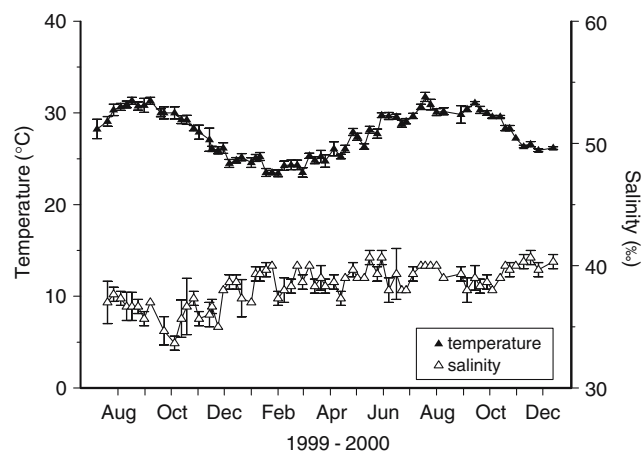


Fig. 1 Mean values (± 1 SD) for each week for water temperature ($^{\circ}\text{C}$; solid triangles) and salinity (‰ ; open triangles) in the study area from July 1999 to December 2000. On x-axis, monthly tick marks represent the first day of each month

Table 1 *Thalassia testudinum*—mean and SD values for nutrient content of blades, rhizomes, and sediments

| Component | Treatment | Time zero | 2 months | 6 months | 11 months | 16 months | P values | |
|---------------|-----------|---------------|---------------|---------------|---------------|---------------|------------------|------------------|
| | | | | | | | Time | Treatment |
| Blades | | | | | | | | |
| OM | Unclip | 64.3 | 64.5 | 70.1 | 70.9 | 71.0 | <0.001 | <0.001 |
| | | 2.4 | 2.9 | 1.3 | 1.0 | 0.8 | | |
| | Clip | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | | |
| | | 63.8 | 68.9 | 73.6 | 72.0 | 73.0 | | |
| Energy | Unclip | 11.04 | 11.21 | 12.72 | 12.73 | 12.78 | <0.001 | <0.001 |
| | | 0.52 | 0.65 | 0.38 | 0.23 | 0.17 | | |
| | Clip | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | | |
| | | 11.07 | 12.45 | 13.96 | 13.39 | 13.73 | | |
| N | Unclip | 1.9 | 2.0 | 2.3 | 2.1 | 2.1 | <0.001 | <0.001 |
| | | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | | |
| | Clip | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | | |
| | | 1.9 | 2.4 | 2.9 | 2.5 | 2.6 | | |
| P | Unclip | 0.19 | 0.17 | 0.19 | 0.21 | 0.20 | <0.001 | <0.001 |
| | | 0.01 | 0.01 | 0.02 | 0.01 | 0.01 | | |
| | Clip | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | | |
| | | 0.19 | 0.21 | 0.20 | 0.23 | 0.22 | | |
| CWC | Unclip | 34.9 | 33.3 | 37.3 | 36.9 | 37.4 | <0.001 | <0.001 |
| | | 1.9 | 2.2 | 1.5 | 1.2 | 1.6 | | |
| | Clip | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | | |
| | | 35.1 | 37.2 | 40.3 | 39.5 | 39.5 | | |
| ADF | Unclip | 26.8 | 25.5 | 27.5 | 26.8 | 28.3 | <0.001 | <0.001 |
| | | 1.4 | 1.9 | 1.4 | 1.0 | 1.1 | | |
| | Clip | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | | |
| | | 26.7 | 28.6 | 31.1 | 30.0 | 30.3 | | |
| Lignin | Unclip | 2.3 | 2.0 | 2.0 | 1.9 | 2.2 | 0.058 | <0.001 |
| | | 0.4 | 0.5 | 0.4 | 0.4 | 0.4 | | |
| | Clip | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | | |
| | | 2.2 | 2.2 | 2.6 | 2.8 | 2.8 | | |
| Cutin | Unclip | 1.5 | 1.7 | 1.5 | 1.2 | 1.6 | 0.062 | <0.001 |
| | | 0.5 | 0.6 | 0.7 | 0.3 | 0.8 | | |
| | Clip | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | | |
| | | 1.5 | 2.3 | 3.2 | 2.5 | 2.8 | | |
| Ferment | Unclip | 25.3 | 29.1 | 30.4 | 34.3 | 32.6 | <0.001 | 0.588 |
| | | 2.3 | 2.9 | 2.6 | 2.3 | 2.6 | | |
| | Clip | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 14 | <i>n</i> = 15 | <i>n</i> = 14 | | |
| | | 26.7 | 29.6 | 30.0 | 31.4 | 34.0 | | |
| | | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 14 | <i>n</i> = 14 | <i>n</i> = 15 | | |
| | | 2.9 | 5.1 | 2.2 | 4.7 | 3.7 | | |
| | | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 14 | <i>n</i> = 14 | <i>n</i> = 15 | | |
| | | | | | | | | |

Rhizomes from clipped plots had significantly lower organic matter content and nitrogen content when expressed as a percent of dry matter, with declines

apparent after 16 and 11 months, respectively (Table 1). However, when nitrogen content was expressed as a percent of organic matter, there was no

Table 1 continued

| Component | Treatment | Time zero | 2 months | 6 months | 11 months | 16 months | <i>P</i> values | |
|------------------|-----------|---------------|---------------|---------------|---------------|---------------|-----------------|--------------|
| | | | | | | | Time | Treatment |
| Rhizomes | | | | | | | | |
| OM | Unclip | 53.6 | 55.2 | 56.1 | 52.6 | 56.1 | 0.010 | 0.001 |
| | | 7.2 | 4.5 | 7.2 | 6.2 | 3.0 | | |
| | | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | | |
| | Clip | 53.6 | 53.5 | 53.2 | 49.2 | 47.4 | | |
| | | 4.6 | 4.4 | 4.3 | 6.1 | 5.6 | | |
| | | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | | |
| N | Unclip | 0.59 | 0.68 | 0.66 | 0.63 | 0.66 | 0.259 | 0.017 |
| | | 0.11 | 0.09 | 0.10 | 0.10 | 0.12 | | |
| | | <i>n</i> = 12 | <i>n</i> = 14 | <i>n</i> = 13 | <i>n</i> = 13 | <i>n</i> = 14 | | |
| | Clip | 0.54 | 0.63 | 0.66 | 0.53 | 0.55 | | |
| | | 0.09 | 0.09 | 0.14 | 0.10 | 0.07 | | |
| | | <i>n</i> = 14 | <i>n</i> = 13 | <i>n</i> = 11 | <i>n</i> = 12 | <i>n</i> = 10 | | |
| N (%OM) | Unclip | 1.12 | 1.22 | 1.14 | 1.17 | 1.17 | 0.751 | 0.242 |
| | | 0.20 | 0.14 | 0.10 | 0.16 | 0.20 | | |
| | | <i>n</i> = 12 | <i>n</i> = 14 | <i>n</i> = 13 | <i>n</i> = 13 | <i>n</i> = 14 | | |
| | Clip | 1.02 | 1.18 | 1.27 | 1.03 | 1.14 | | |
| | | 0.15 | 0.12 | 0.23 | 0.14 | 0.09 | | |
| | | <i>n</i> = 14 | <i>n</i> = 13 | <i>n</i> = 11 | <i>n</i> = 12 | <i>n</i> = 10 | | |
| P | Unclip | 0.11 | 0.15 | 0.13 | 0.12 | 0.12 | 0.077 | 0.852 |
| | | 0.02 | 0.05 | 0.03 | 0.02 | 0.03 | | |
| | | <i>n</i> = 12 | <i>n</i> = 14 | <i>n</i> = 13 | <i>n</i> = 13 | <i>n</i> = 14 | | |
| | Clip | 0.11 | 0.13 | 0.14 | 0.10 | 0.11 | | |
| | | 0.01 | 0.03 | 0.06 | 0.02 | 0.02 | | |
| | | <i>n</i> = 14 | <i>n</i> = 13 | <i>n</i> = 11 | <i>n</i> = 12 | <i>n</i> = 10 | | |
| CWC | Unclip | 22.8 | 23.0 | 25.1 | 23.8 | 23.8 | 0.821 | 0.459 |
| | | 1.8 | 1.9 | 1.3 | 1.5 | 1.9 | | |
| | | <i>n</i> = 12 | <i>n</i> = 14 | <i>n</i> = 13 | <i>n</i> = 12 | <i>n</i> = 12 | | |
| | Clip | 23.2 | 24.0 | 25.1 | 24.3 | 22.0 | | |
| | | 1.9 | 1.7 | 1.9 | 2.6 | 2.4 | | |
| | | <i>n</i> = 14 | <i>n</i> = 13 | <i>n</i> = 13 | <i>n</i> = 10 | <i>n</i> = 12 | | |
| ADF | Unclip | 22.8 | 23.0 | 25.1 | 23.8 | 23.8 | 0.068 | 0.581 |
| | | 1.8 | 1.9 | 1.3 | 1.5 | 1.9 | | |
| | | <i>n</i> = 12 | <i>n</i> = 14 | <i>n</i> = 13 | <i>n</i> = 12 | <i>n</i> = 12 | | |
| | Clip | 23.2 | 24.0 | 25.1 | 24.3 | 22.0 | | |
| | | 1.9 | 1.7 | 1.9 | 2.6 | 2.4 | | |
| | | <i>n</i> = 14 | <i>n</i> = 13 | <i>n</i> = 13 | <i>n</i> = 10 | <i>n</i> = 12 | | |
| Lignin | Unclip | 3.5 | 3.8 | 4.5 | 4.3 | 3.9 | 0.159 | 0.466 |
| | | 0.8 | 0.5 | 0.5 | 0.4 | 0.7 | | |
| | | <i>n</i> = 12 | <i>n</i> = 14 | <i>n</i> = 13 | <i>n</i> = 12 | <i>n</i> = 12 | | |
| | Clip | 3.9 | 4.2 | 4.8 | 4.6 | 3.8 | | |
| | | 0.5 | 0.6 | 0.9 | 0.9 | 0.8 | | |
| | | <i>n</i> = 14 | <i>n</i> = 13 | <i>n</i> = 13 | <i>n</i> = 10 | <i>n</i> = 12 | | |
| Sediments | | | | | | | | |
| OM | Unclip | 4.4 | 4.5 | 4.6 | 4.5 | 4.6 | 0.054 | 0.366 |
| | | 0.4 | 0.4 | 0.4 | 0.5 | 0.4 | | |
| | | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | | |
| | Clip | 4.5 | 4.5 | 4.5 | 4.3 | 4.5 | | |
| | | 0.4 | 0.3 | 0.5 | 0.3 | 0.4 | | |
| | | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | | |
| OC | Unclip | 1.7 | 1.9 | 1.8 | 1.8 | 1.8 | 0.009 | 0.354 |
| | | 0.2 | 0.2 | 0.3 | 0.2 | 0.2 | | |
| | | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | | |
| | Clip | 1.8 | 1.9 | 1.8 | 1.7 | 1.8 | | |
| | | 0.2 | 0.2 | 0.3 | 0.1 | 0.2 | | |
| | | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | <i>n</i> = 15 | | |

All are on % DM basis, except energy is kJ/g DM and rhizome N (%OM) is also presented on organic matter basis. *OM* organic matter, *N* nitrogen, *P* phosphorus, *CWC* cell wall constituents, *ADF* ligno-cellulose-cutin fraction, *ferment* fermentability, *OC* organic carbon. Repeated-measures ANOVA included only those plots with data from all times. There were no significant time × treatment interactions, so *P* values for only time effects and treatment effects are presented here. Significant values are in bold

significant effect of clipping (Table 1). Clipping did not have a significant effect on other rhizome nutrients.

Clipping did not have a significant effect on the organic content of the sediments (Table 1). Total organic matter content tracked organic carbon content closely, indicating that the former technique was an adequate measure.

Based on a single collection of blades in November 1999, approximately 4 months after clipping was initiated, condensed tannins were significantly higher in clipped blades (mean = 6.70% of dry mass, SD = 2.48, $n = 12$) than in unclipped blades (mean = 3.39% of dry mass, SD = 1.09, $n = 9$; t -test, $P < 0.001$). There was a trend toward higher total phenolics in clipped blades (mean = 12.71% of dry mass, SD = 8.30, $n = 12$) than in unclipped blades (mean = 7.38% of dry mass, SD = 3.75, $n = 9$), although the difference was not significant (t -test, $P = 0.066$).

Whether clipping was initiated in summer (July) or winter (February) had little effect on the responses to clipping. The significance and direction of the differences between clipped and unclipped plots were the same at the end of 11 months of clipping in the summer-initiated plots and winter-initiated plots except for lignin. In summer-initiated plots, lignin was significantly higher in blades from clipped plots (t -test, $df = 27$, $P < 0.001$), whereas there was no significant effect of clipping on lignin concentrations in winter-initiated plots (t -test, $df = 18$, $P = 0.317$). Effects of clipping were the same for summer-initiated plots after 11 and 16 months of clipping.

Discussion

Simulation of green turtle grazing

We simulated green turtle grazing as closely as possible at appropriate spatial and temporal scales with re-clipping intervals that incorporated seasonal growth rates and mimicked natural, intense grazing rates. This strength of our study distinguishes it from previous studies that have used pre-determined clipping intervals (e.g., every 2 weeks) that may allow substantially more or less regrowth of seagrass blades than would occur in naturally grazed plots, where grazing organisms adjust their grazing rates in response to changes in vegetation growth.

Nutrient input from green turtle urine and feces was not simulated in the clipped plots but is not a serious concern because turtle feces may not be deposited in grazed plots and turtle urine may not make a substantial contribution to nitrogen budgets in the grazed plots

(Thayer et al. 1984). Turtles often defecate when they first become active at their resting areas before moving to the grazing plots (Bjorndal 1980). Also, gases produced by the hindgut fermentation in green turtles (Bjorndal 1979) are trapped in the feces and cause them to float away from the grazing area (Balazs et al. 1993). Seagrasses absorb most of their nitrogen from sediment pools through roots and rhizomes, not directly from the water column through the blades (Lee and Dunton 1999).

More importantly, because nutrient addition by green turtles was not simulated, our values of *T. testudinum* nutrient quality in clipped plots may be lower than in natural green turtle grazing areas. If we had mimicked feces and urine deposition by green turtles, we could have observed greater increases in blade nutrient content in clipped plots in relation to unclipped plots, and perhaps longer sustainability of the grazing regime (i.e., no decreases in rhizome OM and N contents) through greater nutrient availability. Therefore, our conclusions that simulated grazing increased blade nutrient content and that those elevated contents would last for at least 16 months seem robust.

Effects of grazing on nutrient composition of *T. testudinum* blades

Changes in nutrient composition in response to clipping are important indicators of changes in forage quality (positive or negative) for herbivores and depletion of nutrient stores. Declines in nutrient concentrations can result from an unsustainable grazing regime and may lead to decreased productivity in herbivores. Intensive grazing, in which most of the aboveground biomass is removed, should eventually cause a decline in nutrient composition of *T. testudinum* blades as a result of reduced photosynthetic area, depleted nutrient reserves in rhizomes and sediments, and decreased detritus input (Zieman et al. 1984; Williams 1988; Heck and Valentine 1995). Loss of nitrogen input from detritus may be offset if nitrogen fixation increases in response to nitrogen limitation (Thayer et al. 1984).

All components of *T. testudinum* blades that we measured increased in response to simulated green turtle grazing and remained elevated at the end of the 16-month clipping trial, except fermentability, which did not change. Therefore, by selectively feeding on new blade growth, either by re-cropping grazed plots or ingesting only the lower, younger section of ungrazed blades, green turtles ingest a diet with a higher energy, nitrogen, and phosphorus content. Because productivity in Caribbean green turtles may well be nitrogen lim-

ited (Bjorndal 1982, 1985), the elevated nitrogen levels in blades ingested by green turtles may be the most biologically significant of the three components. To avoid misinterpretation of our results, we emphasize that, as has been stated earlier (Bjorndal 1980, 1985), green turtles feed selectively by ingesting young, actively growing tissues, which results in their ingesting a diet higher in nitrogen. They almost certainly cannot assess the nitrogen content of various forages and select accordingly.

Whether the higher nitrogen level has been the selective force behind the evolution of the grazing habits of green turtles is not known. Feeding on young blades also reduces the intake of epibionts by green turtles (Mortimer 1981), which Zieman et al. (1984) suggested may have been important in the evolution of selective grazing in green turtles. Zieman et al. (1984) proposed two negative results that could accrue to green turtles that ingest calcified epibionts. First, when calcified epibionts reach the acidic stomach of the green turtle, hydrolysis of CaCO_3 could raise the pH of the stomach contents and interfere with digestion. Second, the rapid evolution of CO_2 gas resulting from the hydrolysis of CaCO_3 could result in a rapid absorption of calcium ions, a substantial shift in blood calcium levels, and, perhaps, disruption of cardiac function.

In an earlier study, blades grazed by green turtles had significantly lower levels of lignin than ungrazed blades (Bjorndal 1980). Reduction in lignin substantially improves diet quality for green turtles because the hindgut fermentation in green turtles provides a major portion of their energy requirements from the microbial digestion of CWC in the diet (Bjorndal 1979). Lignin is not only indigestible in vertebrate digestive tracts but also decreases the fermentability of CWC by complexing with cellulose (Van Soest 1982). Clipped blades in our study had significantly higher levels of lignin than unclipped blades in summer-initiated plots after 11 and 16 months of clipping, and lignin content was not significantly different in clipped and unclipped blades after 11 months of clipping in winter-initiated plots. However, lignin content in both clipped and unclipped plots was extremely low (1.9–2.8%) in our study plots compared with values measured in other studies: 4.6% in grazed blades and 9.2% in ungrazed blades in the southern Bahamas (Bjorndal 1980) and 9.1% in ungrazed blades in Puerto Rico (Vicente et al. 1980). These differences may be a result of current flow. Mechanical stimulation can increase lignification in plant tissues (DeJaegher et al. 1985; Cipollini 1997), and current flow at our study site was much lower than that at the study site in the southern

Bahamas (Bjorndal 1980). In our study, the significant, but small, increases in lignin and cutin in clipped blades would probably not have a biologically significant effect on green turtles because fermentability of the clipped blades was not lower than that of unclipped blades.

The significantly higher levels of condensed tannins and the trend for higher total phenolics in clipped blades after 4 months of clipping indicate induction of secondary compounds by *T. testudinum* in response to clipping. The effects of secondary compounds on seagrass herbivory deserve further investigation.

Effects of grazing on nutrient composition of rhizomes and sediments

Prolonged grazing of seagrasses is expected to deplete nutrient stores in rhizomes, as nutrients are mobilized to support blade growth and input of nitrogen from detritus is decreased (Dawes and Lawrence 1979, 1980; Thayer et al. 1984). Nitrogen and phosphorus are the nutrients that most frequently limit growth in seagrasses (Hemminga and Duarte 2000). Carbonate sediments bind substantial quantities of phosphate, so phosphorus often limits growth of seagrasses in carbonate sediments, such as those in our study site (Hemminga and Duarte 2000). Sixteen months of simulated grazing apparently did not cause, or exacerbate any existing, phosphorus limitation because phosphorus levels remained elevated in clipped blades and did not decline in rhizomes in clipped plots, even though higher mass-specific growth was maintained in clipped plots (Moran and Bjorndal 2005).

Of the nutrients that we analyzed in rhizomes, only organic matter and nitrogen (as %DM) responded to clipping, with declines apparent after 16 and 11 months, respectively, presumably as soluble carbohydrates and nitrogen were transported from the rhizomes to the blades to support continued blade growth (Dawes and Lawrence 1979, 1980). However, nitrogen as a percentage of organic matter was not lower in rhizomes from clipped plots, suggesting that carbohydrates and nitrogen were mobilized from the rhizomes in equivalent proportions, so that overall percent of organic matter declined, but the proportion of nitrogen in that organic matter did not change. These durations to initiation of depletion are minimum estimates because enrichment from green turtle feces and urine may postpone depletion in natural grazing plots. Also, substantial mobilization had apparently not occurred by the end of our clipping trial because rhizome biomass was not significantly reduced in clipped plots (Moran and Bjorndal 2005).

Lower levels of total organic matter in sediments sampled at depths of 10–20 cm have been reported in areas with no seagrasses or heavily grazed seagrasses compared with adjacent stands of ungrazed seagrasses (Dawes et al. 1997; Hily and Bouteille 1999; Maciá and Lirman 1999). We expected that simulated grazing would result in a significant decrease in organic content of the sediment as blades were removed instead of being allowed to senesce and decompose in place and as organic content was taken up in support of plant growth. However, organic content did not differ between clipped and unclipped plots, sampled to a depth of 15 cm, by the end of the 16 month clipping trial.

Conclusions

Some of the suite of responses of *T. testudinum* to intensive grazing by green turtles proposed by Thayer et al. (1984) were confirmed by our study and others were not. As Thayer et al. (1984) proposed, regrowth of clipped blades had significantly higher concentrations of nitrogen, and rhizome concentrations of organic matter and nitrogen declined. In contrast to the proposed responses, recropped blades had higher, not lower, lignin content, and the expected decline in blade nitrogen concentration over time did not occur. Combining these results with the results that production of blade biomass and standing crop of rhizome biomass did not decline (Moran and Bjorndal 2005) indicates that re-cropping of *T. testudinum* plots is sustainable for at least 16 months. Although our 16 month clipping trial greatly exceeded the duration of all previous studies, it may not have been long enough to document the expected decline in blade nutrient quality following prolonged, intensive grazing. However, nutrient depletion in rhizomes indicates that blade productivity and/or blade nutrient quality would probably decline under continued clipping. Fecal or urine nutrient inputs from green turtles may increase the duration of sustained nutrient quality and productivity in grazed plots.

This study yielded a greater understanding of how *T. testudinum* responds under intensive grazing, as occurred before the massive decline of green turtles in the Caribbean (Jackson et al. 2001; Pandolfi et al. 2003). These data are being incorporated into mass-balance models to explore the role of green turtles in marine ecosystems and to relate levels of green turtle biomass to levels of productivity in Caribbean seagrass beds (C. Wabnitz, K. Bjorndal, A. Bolten, and D. Pauly, unpublished data).

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