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Morphological and physiological responses of seagrasses (Alismatales) to grazers (Testudines: Cheloniidae) and the role of these responses as grazing patch abandonment cues

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Abstract: Green sea turtles, *Chelonia mydas*, are grazers influencing the distribution of seagrass within shallow coastal ecosystems, yet the drivers behind *C. mydas* patch use within seagrass beds are largely unknown. Current theories center on food quality (nutrient content) as the plant responds to grazing disturbances; however, no study has monitored these parameters in a natural setting without grazer manipulation. To determine the morphological and physiological responses potentially influencing seagrass recovery from grazing disturbances, seagrasses were monitored for one year under three different grazing scenarios (turtle grazed, fish grazed and ungrazed) in a tropical ecosystem in Akumal Bay, Quintana Roo, Mexico. Significantly less soluble carbohydrates and increased nitrogen and phosphorus content in *Thalassia testudinum* were indicative of the stresses placed on seagrasses during herbivory. To determine if these physiological responses were the drivers of the heterogeneous grazing behavior by *C. mydas* recorded in Akumal Bay, patches were mapped and monitored over a six-month interval. The abandoned patches had the lowest standing crop rather than leaf nutrient or rhizome soluble carbohydrate content. This suggests a modified Giving Up Density (GUD) behavior: the critical threshold where cost of continued grazing does not provide minimum nutrients, therefore, new patches must be utilized, explains resource abandonment and mechanism behind *C. mydas* grazing. This study is the first to apply GUD theory, often applied in terrestrial literature, to explain marine herbivore grazing behavior. Rev. Biol. Trop. 62 (4): 1535-1548. Epub 2014 December 01.

Key words: seagrass, *Thalassia testudinum*, patch abandonment, *Chelonia mydas*, herbivory, grazing behavior, giving up density (GUD).

Herbivores are important drivers of ecosystem dynamics (Heck & Valentine, 2007; Valentine & Duffy, 2006) and seagrass productivity (Valentine, Heck, Busby, & Webb, 1997; Zieman, Iverson, & Ogden, 1984), reducing canopy cover and freeing space for competitive interactions between macrophytes (Heck & Valentine, 2006). Grazing by herbivores is not uniform as they selectively feed in certain areas depending on abiotic and biotic factors. This patchy grazing behavior opens space for successional dynamics heterogeneously throughout the seagrass ecosystem, changing overall

ecosystem dynamics. Despite the prevalence of herbivory and its importance in driving seagrass distribution, there is a paucity of literature which explores the physiological mechanisms of seagrasses in driving patch use and abandonment among marine herbivores.

Grazing disturbances by marine herbivores varies in intensity and distribution throughout seagrass beds. Grazing by fishes in patch reefs within seagrass beds typically occurs in areas directly adjacent to the patch reef, creating a halo effect. In order to reduce predation risk, fishes exist within a 'home range' and

repeatedly graze these areas rather than moving throughout the seagrass bed (Randall, 1965). Distribution of grazing patches by fishes is largely determined by this predator avoidance behavior rather than any seagrass physiological driver. In some regions, seagrass halos can be relatively easy to identify as leaves grazed by fishes can be distinguished by unique biting patterns (oval shapes removed laterally), while turtle grazers remove the entire leaf apex (*pers observation*). Green sea turtles (*Chelonia mydas* Linnaeus 1758) grazing behavior differs from fishes in that they repeatedly graze distinct patches of seagrass, which optimizes food quality, as new leaves have higher nutrient content than old leaves (Aragones, Lawler, Foley, & Marsh, 2006; Bjorndal, 1980). The drivers behind the creation of these grazing patches, as well as the cues for herbivores to abandon a grazing patch in favor of a new patch, are not well known. Thayer, Bjorndal, Ogden, Williams, and Zieman (1984) hypothesized that decreases in aboveground nutrient content, and therefore food quality, of repeatedly grazed seagrasses would lead to abandonment of grazed patches. More recent studies indicate that repeated grazing does not lead to a decrease in the food quality of grazed seagrasses (Fourqurean, Manuel, Coates, Kenworthy, & Smith, 2010; Moran & Bjorndal, 2005, 2007). An alternative theory proposed by Fourqurean et al. (2010) as an extension of research by Dawes et al. (1979), suggests that reduced carbon fixation by repeatedly grazed plants, which results in progressive thinning of leaves and decreased storage of soluble carbohydrates in plant rhizomes, may occur prior to seagrass bed abandonment. Based on this theory, decreased soluble carbohydrates may therefore serve as a predictor of herbivore abandonment of particular grazed areas over others. In addition, indices of shoot density, blade width and leaf growth are also used as early indications of chronic stress (Lal, Arthur, Marba, Lill, & Alcoverro, 2010; Lee & Dunton, 1997; Zieman, Iverson, & Ogden, 1984). Decreased food availability for herbivores in a patch may be the cue for grazing patch abandonment.

When the potential harvest rate of thin, sparse, slow-growing seagrasses by sea turtles can no longer balance the associated metabolic costs of foraging, sea turtles should abandon grazing in those patches for areas with higher potential harvest rates, the concept of Giving Up Density (GUD) (Brown, 1988). Giving Up Density theory extends the optimal foraging theory and includes balancing the harvest rate to metabolic costs, predation risk and missed opportunity costs of not engaging in alternative activities. It is likely that GUD, and a modification that considers biomass instead of density, rather than any direct cue that sea turtles could detect from decreased storage of carbohydrates in rhizomes of the grazed plant, serves as the cue for patch abandonment. In order to determine if this theory is applicable, it is necessary to monitor morphology, growth rate, leaf nutrient content and soluble carbohydrate levels in rhizomes, which are all important to seagrass recovery and growth, as they are abandoned by green sea turtles. There is a lack of studies which consider these parameters in natural rather than simulated grazed seagrass beds (Kuiper-Linley, Johnson, & Lanyon, 2007; Moran & Bjorndal, 2005, 2007).

Our study was designed to evaluate the effect of different types of herbivory (fish and turtle) on morphological and physiological characteristics of seagrasses. We also wanted to determine which of the monitored seagrass characteristics may cue grazing patch abandonment by green sea turtles. We expect that grazed patches would have higher nutrient content (nitrogen and phosphorus) in aboveground leaf tissue than ungrazed patches, regardless of grazer identity, as nutrient reserves are mobilized to replace lost tissue. We expect that summer nutrient content of aboveground biomass would be lower than winter, and that summer rhizome soluble carbohydrates would be higher, because high light availability in summer drivers increased carbon fixation (Lee & Dunton, 1997; Perez, Guevara, & Bone, 2006). Owing to the previous finding that nutrient content of seagrass leaves does not decline as a result of repeated grazing (Fourqurean

et al., 2010; Moran & Bjorndal, 2005, 2007), we expect that nutrient content of seagrass leaves would not be an explanation for grazing patch abandonment. If reduced seagrass carbon fixation leads to abandonment by *C. mydas*, we predict that patches abandoned would have significantly lower soluble carbohydrates than patches remaining grazed. However, as the potential harvest rate is determined by the product of seagrass biomass and the specific growth rate, rhizome carbohydrate concentrations may not be a reliable indicator of potential harvest rate. While a paucity of storage in belowground carbohydrate reserves would cause leaf thinning and decreased productivity, herbivores would be unable to determine the amount of sugars within the rhizomes while grazing aboveground tissue. We predict that patches with the lowest biomass, as measured through standing crop biomass, will be abandoned prior to other patches.

MATERIALS AND METHODS

Study site: Akumal ("Place of the Turtles" in Mayan) is located in Quintana Roo, Mexico, in the Yucatan Peninsula along the Caribbean Sea (20°23'45" N, and 87°18'52" W). The region of Akumal experiences seasonal temperatures and precipitation, with a relatively cooler wet season occurring from May to November, and a dry season from December to April. Average air temperatures of the Yucatan range from 21–33°C, water temperatures average 29°C and water salinity 36‰. Akumal Bay is the Northern 400 meter portion of a 2km half moon-shaped lagoon connected to Jade Bay and South Akumal Bay. A barrier reef exists 300m offshore and variable-sized patch reefs are sparsely distributed throughout the entire lagoon. Seagrass herbivores include the green sea turtle *Chelonia mydas* and near the interspersed patch reefs, members of the family *Scaridae* (parrotfish) and *Acanthuridae* (surgeonfish). Seagrass beds consist of *Thalassia testudinum* (Banks ex König), *Halodule wrightii* (Ascherson) and *Syringodium filiforme* (Kuetz), with few intermixed

macroalgae typified by calcareous Chlorophytes (e.g., *Halimeda*, *Udotea*) and calcareous Rhodophytes (e.g., *Neogoniolithon*).

Seagrass response: To determine the species specific response of seagrasses to herbivory, indicators of physiological responses were monitored in turtle and fish grazed and ungrazed patches in the seagrass beds. Distinct patches exist where seagrass beds have been grazed (by either turtle or fish) or not grazed, and within these patches small cages (20cm diameter) were haphazardly placed for six days at a time during two growing seasons (winter and summer) to sample productivity (*T. testudinum* only), leaf morphology, tissue nutrients and soluble carbohydrates of all species present (n=12, 6, 12 in winter for ungrazed, fish grazed and turtle grazed, respectively; n=11, 10, 8 in summer for ungrazed, fish grazed and turtle grazed, respectively). Cages were created with vinyl-coated hardware mesh (ca. 1cm mesh) and four 3mm diameter vinyl-coated metal stakes secured the cage into the substrate. These cages were not intended to be experimental enclosures; rather, they allowed us to measure the production of material for productivity estimates in the short-term without losing biomass to fish or turtle grazers. Cages were deployed on two separate occasions during each season. Areas targeted as fish grazed surrounded a 50m² patch reef adjacent to the same seagrass beds where turtle grazing occurred. Fish herbivory was confirmed by the patterns of bite marks on leaves, which were indicative of parrotfish grazing. Turtle herbivory was confirmed during visual census of the study area.

To measure productivity, morphology and physiological status of seagrass within these cages, all *T. testudinum* blades were punched using a modified leaf marking technique (Fourqurean, Willsie, Rose, & Rutten, 2001; Zieman, 1974). At the end of six days, aboveground and belowground biomass was collected for all seagrass species present from which to measure nitrogen, phosphorus and soluble carbohydrates. Six randomly selected shoots of *T. testudinum* were measured for

length, width and elongation rate (mm/shoot/day), from which elongation area (width x length of new growth at base of leaf) was calculated. All seagrass samples were cleaned of epiphytes, dried to a constant weight at 60°C and ground to a fine powder using a mortar and pestle. Samples were analyzed in duplicate for nitrogen content using a CHN analyzer and for phosphorus using dry-oxidation acid-hydrolysis extraction followed by colorimetric analysis. Dry weight elemental content was calculated as (mass of element/dry weight of sample) x 100%. Rhizome tissue for all species was cleaned of adhering sediments, dried to a constant weight at 60°C and ground to a fine powder using a mortar and pestle. Because of the smaller size and paucity of *H. wrightii* and *S. filiforme* rhizomes, samples were pooled from three cages to provide an adequate amount of sample material. Samples were analyzed for soluble carbohydrates using the adaptation for seagrass tissue (Lee & Dunton, 1997) to the MBTH (3-methyl-2-benzothiazolinone hydrazine hydrochloride) method (Pakulski & Benner, 1992).

In order to test for the hypothesized differences between herbivory patches and seasons on nutrient content, carbohydrate content and growth rates, a randomized complete block ANOVA was used for morphological and productivity data, where shoots within cage were nested within combinations of patch (fish grazed, turtle grazed, ungrazed) x season (winter, summer). Data were analyzed to establish if they conformed to all normality assumptions. Differences among patches for all seagrass species were determined through Least Squares Difference pairwise comparisons with Bonferroni corrected significance levels (0.008). Because of the variability in presence of *H. wrightii* and *S. filiforme* in grazed cages, sample sizes varied between patches and seasons. Therefore, to test for differences between herbivory patches and between seasons, nutrient content and soluble carbohydrate data were analyzed via three-way ANOVA (*T. testudinum*), two-way ANOVA (*S. filiforme*) or via a student's t-test or Mann-Whitney

nonparametric test (*H. wrightii*), depending on how the data met normality assumptions. No *S. filiforme* was present during the winter in the fish grazed patch and no *H. wrightii* was present in the fish (either season) or turtle grazed (winter season) patches.

Patch dynamics: To monitor changes in turtle grazed or ungrazed patch location and seagrass morphological and physiological changes in relation to changes in patch designation over time, patch locations in the seagrass ecosystem were identified and monitored using belt transects. Ten transects were established between pre-existing navigational buoys in Akumal Bay initially (January 2011) and resampled along the same locations six months later (June 2011). The meter wide belt transects differed in length and cardinal ordination, and covered a combined length of 860m within the Northern portion of Akumal Bay where sea turtles are present in higher densities than other portions of the Bay (authors' pers. obs.). Patches were labeled as turtle grazed when leaf apices were incomplete and seagrass blade length was 5cm or less from the blade sheath junction. Ungrazed patches were those with seagrass blade length greater than 5cm and complete apices. A modified Braun-Blanquet (BB) index was used to calculate abundance for each macrophyte present (Fourqurean et al., 2001) within a 0.25m² plot placed haphazardly within each patch. For each macrophyte BB score, 1 indicated coverage less than 5% of the plot, 2 indicated 5-25% cover, 3 indicated 25-50% cover, 4 indicated 50-75% cover; and 5 indicated 75-100% cover. The presence of a different patch along a transect was recorded when grazing status (turtle grazed or ungrazed) or when BB score differed from adjacent seagrass patches along the transect.

Braun-Blanquet scores were converted to abundances using the mean abundance within each score (e.g., 2.5% cover for score 1, 87.5% cover for score 5) and then averaged across similarly designated patches (e.g., all turtle grazed patches). Mean abundances are presented for turtle grazed and ungrazed patches

during both surveys for comparisons between surveys. A Student's t-test was used to test for differences between turtle grazed and ungrazed seagrass abundances as no data were available to determine newly grazed or abandoned status during the initial time period. An ANOVA was used to test for differences in species abundance among patch status (ungrazed, turtle grazed, abandoned, newly grazed) within the second sampling after examination of standardized residuals showed the data conformed to all normality assumptions. Within each patch, grazing status (blade apex complete or incomplete) of *T. testudinum* and seagrass blade length for all species was recorded. In the second survey, patches labeled ungrazed had been ungrazed for six months or more while abandoned patches were recently ungrazed (less than six months since being grazed). An abandoned patch was a patch that was grazed by turtles during the first survey and whose blade lengths had increased and blade apices were intact for the second survey. Following the second survey, a Student's t-test was used to test for differences in proportions of each transect in each status between sampling events. Standing crop was calculated utilizing biomass constants for *T. testudinum* and *S. filiforme* (Fourqurean et al., 2001) and tested for significance using an ANOVA.

At 15 randomly chosen turtle grazed and ungrazed patches along the transects, six randomly selected shoots of *T. testudinum* were collected to determine the change in response variables between sampling intervals as a function of patch status following the methods outlined above. An ANOVA was used to test for hypothesized differences in all *T. testudinum* parameters (width, nitrogen, phosphorus, soluble carbohydrates) among patch status in the second survey (remain ungrazed, remain turtle grazed, abandoned) after examination of standardized residuals showed the data conformed to all normality assumptions. A Student's t-test was used to test for differences in initial survey data between patches designated during the second survey as grazed and abandoned. Means are presented for each of the

three statuses in the initial survey for comparisons between surveys.

RESULTS

Seagrass response: While cages were deployed on two separate occasions during each season, samples were pooled within season after statistical analysis determined there was no significant difference between deployments within each patch (Student's t-test, $p=0.897$). Ungrazed patches had the widest *Thalassia testudinum* blades (ANOVA $F=12.06$, $p<0.001$) with fastest elongation rate (ANOVA $F=16.99$, $p<0.001$) and largest new growth area (ANOVA $F=10.22$, $p=0.002$), when compared to fish and turtle grazed patches (Fig. 1 and 2). The difference between ungrazed and grazed was greater in the summer months and significantly higher in ungrazed patches compared to grazed patches, regardless of grazer identity in both seasons ($p<0.001$ for all three parameters). Fish grazed and turtle grazed patches were significantly different from each other in width during the winter ($p<0.001$), but were not significantly different for other parameters or within the same patch between seasons. Grazed shoots had a maximum of three leaves, while ungrazed shoots had a maximum of six leaves. Fish grazed leaves appeared less pigmented and curled when compared with turtle grazed leaves. All three species of seagrass were present in the majority of ungrazed patches while *T. testudinum* and occasionally *S. filiforme* were present in grazed patches. Ungrazed patches of *T. testudinum* had lower nitrogen and phosphorus content than turtle and fish grazed patches in both seasons (Table 2). Soluble carbohydrates in *T. testudinum* rhizomes were significantly higher in the ungrazed patches for both seasons and were significantly higher in summer compared to winter in all three patches (Table 1, 2).

Similar significant differences to those of *T. testudinum* were found in *Syringodium filiforme*, for which ungrazed patches had significantly lower nitrogen and phosphorus content than turtle grazed patches (Table 1, 3, sample

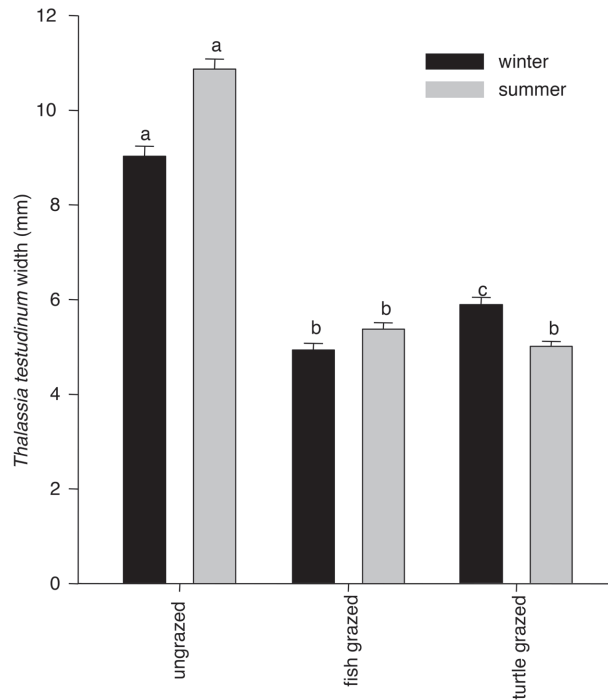


Fig. 1. *Thalassia testudinum* width (\pm SE) in all three grazing patches for both seasons: winter and summer. Superscripted letters indicate significant difference between patches in the same season (ANOVA, $F=12.063$, $p<0.001$).

TABLE 1

Statistical analyses for nitrogen, phosphorus and soluble carbohydrate data comparisons between patch status (Treatment) in same season and within the same patch status between seasons (Season)

			Nitrogen	Phosphorus	Soluble Carbohydrates
Season	<i>Thalassia testudinum</i>	F	25.09	110.41	2.05
		p-value	<0.001	<0.001	<0.001
	<i>Syringodium filiforme</i>	F	11.44	8.42	2.83
		p-value	0.003	0.008	0.106
	<i>Halodule wrightii</i>		Student's t-test		
		p-value	<0.001	<0.001	<0.001
Patch	<i>Thalassia testudinum</i>	F	21.61	74.15	6.17
		p-value	<0.001	<0.001	<0.001
	<i>Syringodium filiforme</i>	F	483.68	22.58	1.01
		p-value	<0.001	<0.001	0.326
	<i>Halodule wrightii</i>		Mann-Whitney		
		p-value	<0.001	<0.001	<0.001

size was too small to test for differences from fish grazed patches). For *Halodule wrightii*, significant differences were found in nitrogen and phosphorus content between ungrazed and turtle grazed patches in the winter only (Table

1, 4). There was significantly higher nitrogen and phosphorus content in winter compared to summer in the ungrazed patches, similar to the trend found in *T. testudinum* and *S. filiforme*. Soluble carbohydrates of rhizomes

TABLE 2
Thalassia testudinum nitrogen, phosphorus and soluble carbohydrate content

		Patch			ANOVA		
		Ungrazed n=12, 11	Fish Grazed n=6, 10	Turtle Grazed n=12, 8	Comparison	F	p
Nitrogen Content (%DW)	Winter	2.32 ± 0.04 ^a	3.29 ± 0.06 ^b	3.40 ± 0.08 ^b	Season	25.09	<0.001
	Summer	2.02 ± 0.04 ^a	2.72 ± 0.06 ^b	3.04 ± 0.05 ^c	Patch	21.61	<0.001
Phosphorus Content (%DW)	Winter	0.182 ± 0.007 ^a	0.228 ± 0.011 ^b	0.257 ± 0.009 ^b	Season	110.41	<0.001
	Summer	0.148 ± 0.006 ^a	0.175 ± 0.010 ^b	0.228 ± 0.009 ^b	Patch	74.15	<0.001
Soluble Carbohydrates (mgC/g rhizome DW)	Winter	102.3 ± 5.8 ^a	54.2 ± 3.1 ^b	51.3 ± 3.8 ^b	Season	2.05	<0.001
	Summer	152.9 ± 12.8 ^a	66.6 ± 7.4 ^b	71.0 ± 10.4 ^b	Patch	6.17	<0.001

DW=dry weight. Values are patch means ± SE. Superscripted letters indicate significant difference among patches in the same season (corresponding to the Patch ANOVA F and p-value).

TABLE 3
Syringodium filiforme nitrogen, phosphorus and soluble carbohydrate content

		Patch			ANOVA		
		Ungrazed n=4, 11	Fish Grazed n=1	Turtle Grazed n=4, 8	Comparison	F	p
Nitrogen Content (%DW)	Winter	1.67 ± 0.03 ^a	—	2.24 ± 0.02 ^b	Season	11.437	0.003
	Summer	1.47 ± 0.02 ^a	2.42	2.23 ± 0.03 ^b	Patch	483.675	<0.001
Phosphorus Content (%DW)	Winter	0.130 ± 0.004 ^a	—	0.145 ± 0.003 ^b	Season	8.417	0.008
	Summer	0.104 ± 0.004 ^a	0.110	0.140 ± 0.005 ^b	Patch	22.575	<0.001
Soluble Carbohydrates (mgC/g rhizome DW)	Winter	57.9 ± 3.3	—	53.5 ± 3.0	Season	2.828	0.106
	Summer	104.5 ± 19.2	76.7	70.8 ± 10.1	Patch	1.006	0.326

DW=dry weight. Values are patch means ± SE (ungrazed and turtle grazed only). Superscripted letters indicate significant difference between patches in the same season (corresponding to the Patch ANOVA F and p-value).

TABLE 4
Halodule wrightii nitrogen, phosphorus and soluble carbohydrate content

		Patch			T-test	
		Ungrazed n=4, 11	Fish Grazed	Turtle Grazed n=3	Comparison	p
Nitrogen Content (%DW)	Winter	2.04 ± 0.03	—	—	Season (t-test)	0.024
	Summer	1.91 ± 0.03	—	3.17 ± 0.15	Patch (M-W)	0.010
Phosphorus Content (%DW)	Winter	0.160 ± 0.006	—	—	Season (t-test)	0.009
	Summer	0.135 ± 0.005	—	0.210 ± 0.010	Patch (M-W)	0.010
Soluble Carbohydrates (mgC/g rhizome DW)	Winter	111.4 ± 6.2	—	—	Season (t-test)	0.509
	Summer	120.8 ± 8.0	—	43.1 ± 4.9	Patch (t-test)	<0.001

DW=dry weight. Values are patch means ± SE (ungrazed and turtle grazed only).

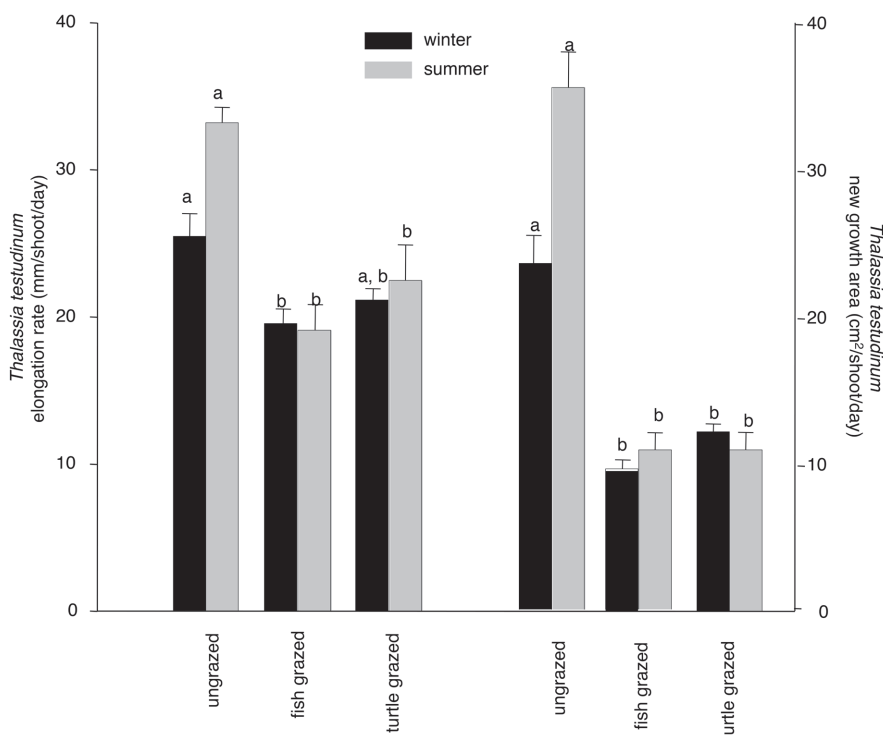


Fig. 2. *Thalassia testudinum* elongation rate (\pm SE) and new growth area (\pm SE) for both seasons: winter and summer. Superscripted letters indicate significant difference among patches in the same season (Elongation rate ANOVA, $F=16.993$, $p<0.001$; New Growth Area ANOVA, $F=10.224$, $p=0.002$).

were significantly higher in ungrazed patches than turtle grazed patches (Table 1), similar to *T. testudinum*. Grazed shoots had a maximum of three leaves while ungrazed shoots had a maximum of seven leaves.

Patch dynamics: The majority of seagrass patches recorded during the initial survey were ungrazed by turtles (63%), while the remaining patches were turtle grazed. The percent of ungrazed patches did not significantly change during the second survey (Student's t-test, $p=0.911$), while significant reductions in turtle grazed patches occurred (Student's t-test, $p<0.003$). From the initial 318m of grazed patches, 27% continued to be grazed, while 73% were abandoned by turtles in the second survey. From the initial 542m of ungrazed patches, 97% remained ungrazed while 3% changed to grazed in the second survey. During

initial surveys, ungrazed patches had significantly higher abundances of all three seagrass species than turtle grazed patches (t-test, $p<0.001$, Table 5).

In the second survey, comparisons could only be made between ungrazed, turtle grazed and abandoned patches, as sample size of newly grazed patches was too small ($n=1$) for statistical comparison. Ungrazed patches had the highest abundance of *T. testudinum* and *S. filiforme* than grazed and abandoned patches (ANOVA, $p=0.015$ and $p=0.037$, respectively), while *H. wrightii* abundance was highest in abandoned patches (ANOVA, $p=0.008$) and increased significantly from initial to second survey (t-test, $p<0.001$). *Syringodium filiforme* and *T. testudinum* abundance did not significantly change for abandoned patches from initial to second survey (t-test, $p=0.912$). Two species of macroalgae not observed in the

TABLE 5
Average percent abundance of seagrass in patches within each grazing designation

	Sampling	Patch Status				Statistic
		Ungrazed n=20	Newly Grazed n=1	Grazed n=6	Abandoned n=11	
<i>Thalassia testudinum</i> abundance	Initial		31%		1%	t-test $p<0.001$
	Second	32% ^a	25%	12% ^b	10% ^b	ANOVA $p=0.015$
<i>Syringodium filiforme</i> abundance	Initial		10%		1%	t-test $p<0.001$
	Second	23% ^a	5%	10% ^b	10% ^b	ANOVA $p=0.037$
<i>Halodule wrightii</i> abundance	Initial		23%		1%	t-test $p<0.001$
	Second	22% ^a	5%	1% ^b	31% ^c	ANOVA $p=0.008$

initial survey occurred within the one abandoned plot (*Halimeda* sp., *Penicillus* sp.). In the one newly grazed patch, there was one less seagrass species present, but the small sample size did not allow statistical comparisons.

Within the fifteen transect patches of *T. testudinum* sampled for nutrients, soluble carbohydrates and morphology during the initial survey, ungrazed patches had significantly lower nitrogen and phosphorus content (Table 6; t-test, $p<0.001$ for both parameters) and significantly higher soluble sugar content (t-test, $p=0.044$) than grazed patches, similar to those results found in the cages. Phosphorus content and soluble carbohydrates were not significantly different between abandoned and ungrazed patches while nitrogen content was not significantly different between abandoned and grazed patches. Therefore, we detected no differences in nitrogen, phosphorus or soluble carbohydrates in *Thalassia testudinum*. Prior

to abandonment, leaves were short and narrow, and a distinct shift in these parameters followed abandonment (Fig. 3). Standing crop biomass indicated abandoned patches exist under a threshold of 42.1g/m², while grazed patches averaged significantly greater at 62.9g/m², and ungrazed patches averaged significantly greater than abandoned and grazed patches at 99.7g/m² (ANOVA, $p<0.001$).

DISCUSSION

Monitoring of patch status provided an opportunity to look at the changes in response variables (nutrient content, morphology, physiology) as a function of patch type. The driver(s) behind changes in patch use are elusive, as monitoring of *Chelonia mydas* patch use through time can be challenging in the field. Contrary to our hypothesis, we did not find that patches with the lowest soluble carbohydrate content

TABLE 6
Thalassia testudinum within the monitored patches

	Sampling	Patch Status			ANOVA	
		Ungrazed	Grazed	Abandoned	F	p-value
Nitrogen Content (%DW)	Initial	2.4467 ± 0.028 ^a	2.7033 ± 0.079 ^{a,b}	2.905 ± 0.154 ^b	Sampling	12.009 <0.001
	Second	1.8933 ± 0.088 ^a	2.7367 ± 0.105 ^b	2.55 ± 0.195 ^b	Patch (Second)	6.218 0.020
Phosphorus Content (%DW)	Initial	0.1917 ± 0.011	0.1957 ± 0.011	0.2215 ± 0.016	Sampling	23.156 <0.001
	Second	0.1195 ± 0.008 ^a	0.1743 ± 0.019 ^b	0.1497 ± 0.013 ^{a,b}	Patch (Second)	3.613 0.042
Soluble Carbohydrates (mgC/g rhizome DW)	Initial	86.833 ± 9.955	65.933 ± 3.882	68.433 ± 15.183	Sampling	8.121 0.009
	Second	136.317 ± 16.603 ^a	80.867 ± 6.217 ^b	100.183 ± 8.187 ^{a,b}	Patch (Second)	4.304 0.025

DW=dry weight. Values are patch means ± SE. Superscripted letters indicate significant difference among patches during the second survey only (corresponding to the Patch ANOVA F and p-value).

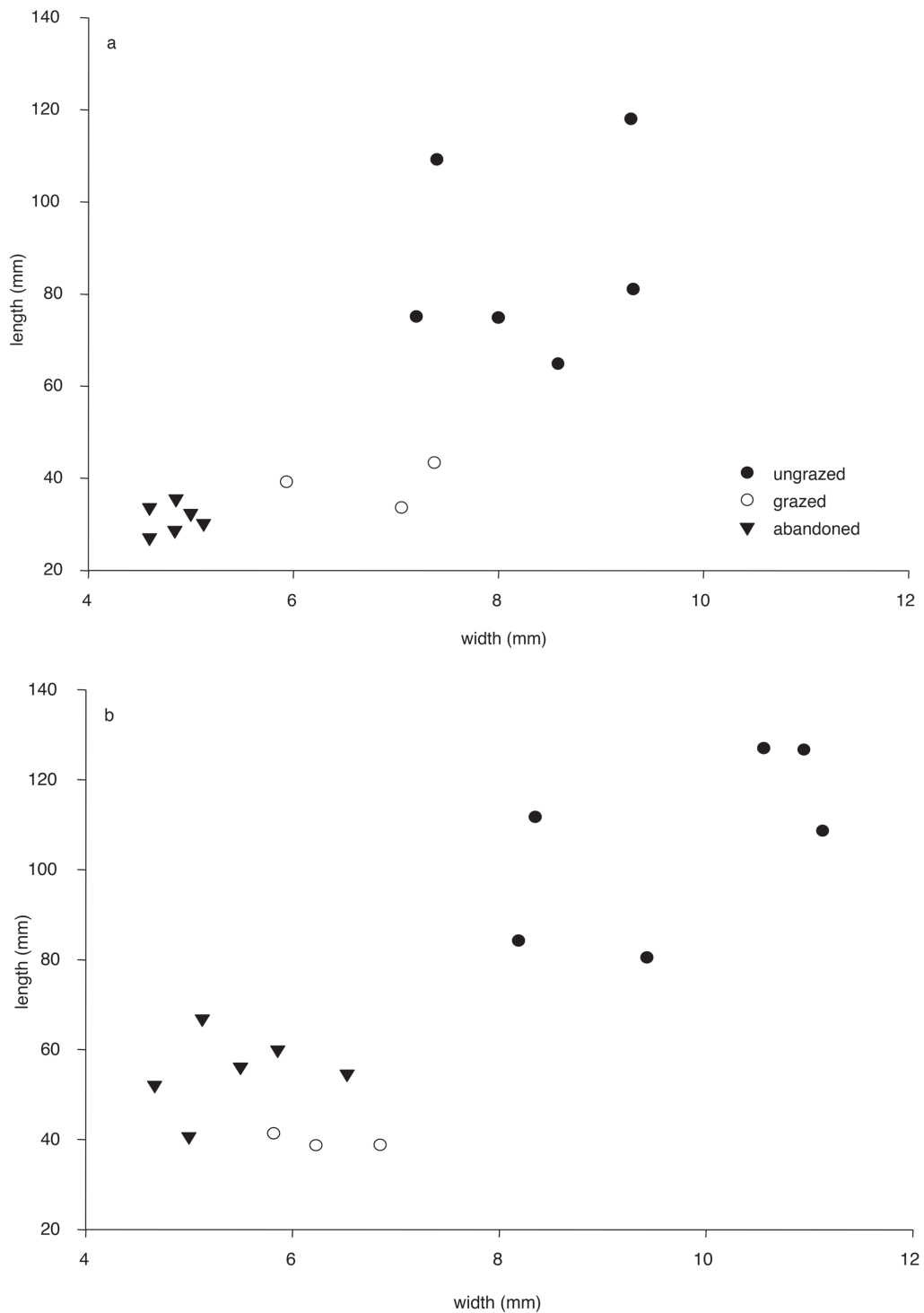


Fig. 3. Scatterplot of *Thalassia testudinum* width by length for initial survey (a) and second survey (b) of patches at three different grazing statuses.

in the seagrass rhizomes were more likely to be abandoned (as suggested by Fourqurean et al., 2010), nor did we find that patches with the lowest nutrient content of seagrass leaves were likely to be abandoned (as suggested by Thayer et al., 1984). However, those patches abandoned had the smallest biomass and therefore density of leaves, indicating that a modified GUD or a 'giving up biomass' may be the mechanism by which patches are abandoned. Further, the sparser grazed patches also had narrower, shorter leaves. When a patch is composed of these much smaller leaves, the effort required by *C. mydas* to consume enough plant biomass to fulfill their nutritional needs could outweigh the nutritional reward, and therefore induce abandonment of the patch by feeding turtles, similar to the mechanism regulating patch use by herbivores reported in terrestrial ecosystems (Brown, 1988).

In our system, patch abandonment was not driven by the quality of seagrass as would be reflected in nutrient content, nor by stored soluble carbohydrates, but cued directly by the low quantity provided by the sparse, thin seagrass blades of the heavily grazed patches. Based on the biomass found in grazed patches during the initial survey that were later abandoned, we propose a threshold of approximately 17g/m², below which patches can be predicted to become abandoned by sea turtle grazers in search of higher quantity yields for their grazing effort. While it may be suggested that the turtles are avoiding highly grazed plots because of the abundance of sandy substrate, should that be the case we would have expected grazed areas to be abandoned uniformly, rather than at a certain leaf biomass as we found. Further work is necessary to spatially and temporally increase the application of this proposed threshold.

The relative physiological (productivity, tissue nutrients and soluble carbohydrates) and morphological effects of grazing on seagrasses were similar to those previously measured from simulated grazing or enclosure experiments (Fourqurean et al., 2010; Moran & Bjorndal, 2005, 2007). Leaf widths of fish

grazed (4.9-5.4mm) and turtle grazed (5.0-5.9mm) patches in this study were within range of other studies on grazing (5.3-6.3mm, Zieman et al., 1984; 5.6-6.3mm, Moran & Bjorndal, 2005; 3.3-4.7mm, Williams, 1988; 1.4-3.0mm, Fourqurean et al., 2010). These differences in leaf widths further emphasize the detrimental effects of intense grazing to seagrasses, which have less photosynthetic leaf tissue, storage of carbohydrates in their rhizomes, and narrower and shorter leaves when subjected to intense grazing. These responses likely decrease the ability of heavily grazed plants to recover from disturbance or survive.

Smaller sample sizes for *Syringodium filiforme* and *Halodule wrightii* limited our determination of significant differences in nutrient and soluble carbohydrate content between patches but their absence in grazed patches may indicate herbivore preference (Armitage & Fourqurean, 2006). The absence of certain species may also indicate a lower tolerance to repeated or prolonged grazing as they are unable to recover sufficiently to remain in the ecosystem (Kuiper-Linley et al., 2007). The lower soluble carbohydrate in the ungrazed *S. filiforme* during the winter may decrease the ability of this species to recover from grazing. Larger species with thicker rhizomes, such as *T. testudinum*, may also be better at translocating their stored carbohydrates longer distances and are therefore better able to compensate for loss than those species with thinner rhizomes, e.g., *H. wrightii* and *S. filiforme* (Marba, Santiago, Diaz-Almela, Alvarez, & Duarte, 2006). Within the timeframe of our study, the reduced productivity and leaf biomass for all three species in grazed patches when compared to ungrazed plants suggest an increased reliance on aboveground biomass and increases the plant's reliance on belowground biomass as a energy reserve (Williams, 1988). As these reserves are depleted to replace leaves lost to grazers, the lower soluble carbohydrate content of the rhizomes reduces the ability of the plant to counter prolonged or future disturbances until the reserves can be replaced (Kuiper-Linley et al., 2007).

Over a six month interval, the location of turtle grazed patches was largely stable, with only a small fraction of area changing status. Our data indicate grazing patches were long-lived enough to affect soluble carbohydrate storage, morphology and nutrient content of seagrass leaves. As expected during the initial survey, turtle herbivory reduced seagrass abundance and canopy complexity (macrophyte species composition and blade length and width). During the second survey, after patches were abandoned, increased leaf lengths, widths and the occurrence of pioneering species (*Halodule wrightii*) increased canopy complexity. The abundance of this species in recently abandoned patches is consistent with successional dynamics reported for other types of seagrass ecosystems, especially in nutrient-replete systems (Duarte, 1995; Fourqurean, Powell, Kenworthy, & Zieman, 1995). Although we cannot statistically test changes in macroalgae as a result of the small sample size, the one patch that had *Udotea* and *Halimeda* during the initial study did have a new genus, *Penicillus*, during the second survey. It was surprising that macroalgae were scarce throughout grazed patches, where canopy cover was decreased and potential competitive interactions increased, or in ungrazed patches, where macroalgae may potentially find refuge within the complex canopy. The absence of macroalgae could be due to limited algal reproductive potential or the result of selective grazing both within grazed and ungrazed seagrass patches, although preferential grazing of the calcareous macroalgae present has not been reported. This is an interesting area for further study on macrophyte dynamics in this unique system.

Impacts of grazers on different seagrass species may also be an important determinant of primary producer distribution, as found in other studies (Armitage & Fourqurean, 2006). Fishes such as members of the family Scaridae preferentially feed on faster-growing, early successional seagrass species such as *Halodule wrightii* over slower-growing, climax species such as *T. testudinum* (Armitage & Fourqurean, 2006; Burkholder, Heithaus, & Fourqurean,

2012), thus impacting the diversity of seagrasses found within these grazed patches. Seagrass preference is less evident for green sea turtles in the Caribbean as their diet largely consists of *T. testudinum* (Mortimer, 1981), which may be an artifact of the lower availability and abundance of *H. wrightii* relative to other species as well as spatial variability between grazing populations and habitat types (Bjorndal, 1997). This is the first study to simultaneously consider the impact of turtle and fish grazing behaviors on morphological and physiological parameters important to seagrass growth and recovery (e.g., productivity, blade width).

Considering the preference of many herbivores for *Halodule wrightii* and *Syringodium filiforme* (Armitage & Fourqurean, 2006; Burkholder, Heithaus, & Fourqurean, 2012), the presence of these species in the ungrazed beds may provide a source for recolonization by these species once patches are abandoned. Similar responses have been reported after other disturbances (Armitage, Frankovich, & Fourqurean, 2011). While *H. wrightii* can replace *T. testudinum* under conditions of high nutrient availability (Fourqurean et al., 1995), if no source for *H. wrightii* propagules exists, the ecosystem may transition to bare sand rather than supporting a macrophyte assemblage. Alternatively, this study suggests that heterogeneous grazing may promote a more species-rich seagrass community in a eutrophic ecosystem.

Our study determined that in Akumal seagrass beds, leaf nutrient content was not a cue to seagrass patch abandonment, as hypothesized by Moran and Bjorndal (2005, 2007) and Fourqurean et al. (2010), but it was surprising that there was no clear signal of lowest soluble carbohydrates in patches being abandoned. Instead, our data suggest that a modified Giving Up Density (GUD) as Giving Up Biomass, similar to the relationship found in terrestrial ecosystems, is the mechanism by which patches are abandoned. Our research is the first to apply a modified GUD theory to the grazing behavior of marine herbivores as we develop this threshold parameter of standing crop biomass.

The application of this theory provides novel insight on the behavior of mesograzers and the crossover between terrestrial and aquatic theories on grazing yield/grazing effort or risk. The application of GUD theory should be examined in other sites experiencing patch abandonment by green sea turtles in order to test this proposed threshold.

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RESUMEN

Respuestas morfológicas y fisiológicas de los pastos marinos (Alismatales) a los herbívoros (Testudines: Cheloniidae) y el papel de estas respuestas como señales de abandono de parches de pastoreo. Las tortugas verdes, *Chelonia mydas*, son herbívoros que influyen la distribución de pastos marinos en sistemas costeros someros, sin embargo los factores que hay detrás del uso de un parche de pastos marinos por *C. mydas* son desconocidos. Teorías actuales se centran en calidad de alimento (contenido de nutrientes en tejido) conforme la planta responde a la alteración causada por el pastoreo; sin embargo no hay estudios que hayan monitoreado estos parámetros en un diseño natural sin manipulación del herbívoro. Para determinar las respuestas morfológicas y fisiológicas (productividad, morfología y almacenamiento de nutrientes) que potencialmente influyen la recuperación de los pastos de la alteración por pastoreo, los pastos fueron monitoreados por un año en tres escenarios diferentes de pastoreo (pastoreo por tortuga, pastoreo por peces, sin pastoreo) en el sistema tropical Bahía de Akumal, Quintana Roo, México. Significativamente menos carbohidratos solubles y mayor contenido de nitrógeno y fósforo fueron indicativos del estrés causado por el pastoreo en los pastos. Para determinar si estas respuestas fisiológicas de los pastos son las causantes del heterogéneo comportamiento de pastoreo de *C.*

mydas, reportado en Akumal, se mapearon y monitorearon parches de pastos en intervalos de seis meses. Los parches abandonados tuvieron menor biomasa, en vez de bajo contenido de nutrientes en hoja, o de carbohidratos en raíces. Estos resultados sugieren un comportamiento modificado de renunciamento por densidad: el umbral crítico donde el costo de pastoreo continuo no provee los nutrimentos mínimos, por lo tanto nuevos parches deberán ser utilizados, explicando así el abandono del recurso y mecanismo detrás del comportamiento de pastoreo por *C. mydas*. Este es el primer estudio en aplicar la teoría de renuncia por densidad, frecuentemente utilizada en la literatura terrestre, para explicar el comportamiento de pastoreo de herbívoros.

Palabras clave: pastos marinos, *Thalassia testudinum*, abandono de parche, *Chelonia mydas*, herbívoro, pastoreo, renunciamento por densidad (GUD).

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