



# Duration of prolonged simulated green turtle grazing affects *Thalassia testudinum* regrowth dynamics following grazing cessation in a subtropical Caribbean ecosystem

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## Abstract

Populations of the green turtle (*Chelonia mydas*), a megaherbivore that maintains distinct areas of seagrass via cultivation grazing, are recovering worldwide. Evaluating seagrass regrowth dynamics in grazed areas following prolonged, known durations of herbivory is challenging in situ, but is critical to understand ecosystem function as meadows return to a natural grazed state. Based on a 27-month study in a subtropical Caribbean seagrass meadow (The Bahamas; 23.46° N, 76.06° W), we evaluate *Thalassia testudinum* regrowth dynamics over 11 months following two durations of simulated green turtle grazing (11 and 16 months; 11clip and 16clip, respectively). By the end of the clipping treatments, simulated grazing had induced significant changes in most *T. testudinum* leaf morphology and nutrient variables in clipped plots compared to reference plots, while belowground biomass and nutrient content were unaffected. However, most leaf variables in clipped plots returned to levels comparable to reference plots by 6.5 months after the cessation of clipping, with the exception of leaf area index (LAI) and leaf width. The effects of grazing duration on regrowth in clipped treatments were evident in 11clip and 16clip plots. In 11clip plots, LAI increased to reference plot levels within 6.5 months after cessation of clipping, while leaves did not widen until 11 months post-clipping. However, LAI in 16clip plots did not reach reference plot levels until 11 months post-clipping, and leaves remained significantly narrow throughout the experiment. These regrowth patterns indicate the capacity of *T. testudinum* to rebound following prolonged, repetitive cropping of leaf biomass by green turtles, and that decreased leaf width and LAI after cessation of grazing may be a lingering effect of shifts in plant growth allocation in grazed systems. This study provides a valuable contribution to understand the effects of cultivation grazing and grazing duration on *T. testudinum* regrowth dynamics and tolerance to herbivory.

**Keywords** Seagrass · Herbivory · Grazing duration · Cultivation grazing · *Chelonia mydas* · Mega-herbivore

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## Introduction

Evaluating disturbance-recovery dynamics is critical to informing the management and restoration of coastal ecosystems (Hewitt et al. 2022). Seagrasses form highly productive meadows in shallow coastal waters (Duarte and Chiscano 1999) and have a remarkable capacity for supporting regrowth following disturbance to leaf biomass (van Tussenbroek et al. 2000; Rasheed 2004; Macreadie et al. 2014), including via natural stressors like herbivory.

Caribbean seagrass ecosystems, dominated by *Thalassia testudinum*, are well adapted to support herbivory (Thayer et al. 1984; Valentine and Heck 1999), having co-evolved under consistent grazing pressure by megaherbivores (i.e., green turtles (*Chelonia mydas*) and large sirenians) (Domning 2001; Jackson et al. 2001; Aragonés et al. 2012). Only

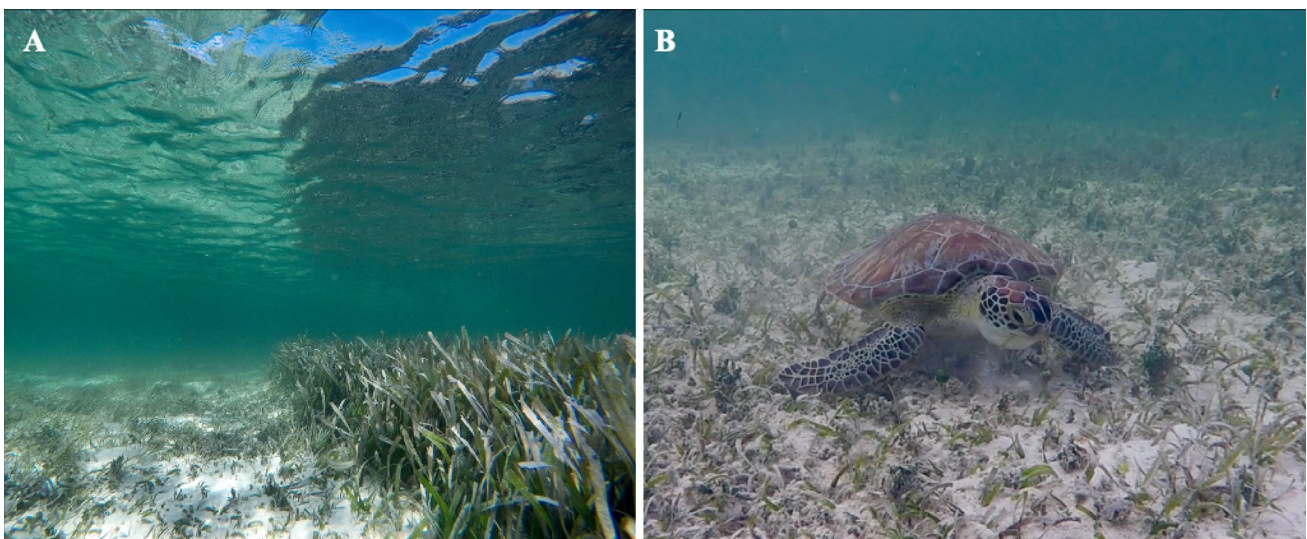
until recently, however, have megaherbivores like green turtles returned to fulfilling their ecological roles as major seagrass consumers in many areas. The recovery of many green turtle populations (Chaloupka et al. 2008; Mazaris et al. 2017; Pritchard et al. 2022), after centuries of low abundance due to overexploitation by humans (Jackson et al. 2001), has important implications for seagrass regrowth dynamics as meadows return to a natural grazed state (Bjorndal and Jackson 2003).

Green turtles often use a cultivation grazing strategy, in which they select distinct areas of seagrass, remove the older leaf matter, and allow it to float away, then repeatedly crop the new leaf growth once it reaches a few centimeters above the substrate (hereafter, referred to as grazed areas; see Fig. 1) (Bjorndal 1980; Zieman et al. 1984; Williams 1988). Green turtles rarely consume *T. testudinum* rhizomes and roots (Gulick et al. 2021a), even in heavily grazed areas like those in Bermuda (Fourqurean et al. 2019). This cultivation grazing strategy stimulates leaf growth and turnover in tropical and subtropical meadows (Moran and Bjorndal 2005; Gulick et al. 2020), creating dense leaf canopies higher in nitrogen content (> 2% dry matter) and low in lignin (< 4.6% dry matter) (Bjorndal 1980; Zieman et al. 1984; Moran and Bjorndal 2007) that maximize digestion rates and nutrient intake (Bjorndal 1980; Gulick et al. 2022). *Thalassia testudinum* meadows support this compensatory growth response to grazing via shifts in plant growth allocation that allow leaf area to be maintained despite the removal of leaf tissue (Gulick et al. 2021b), thereby prioritizing biomass production over photosynthetic surface area (Johnson et al. 2022). Grazing can also result in reduced leaf width (Williams 1988; Fourqurean et al. 2010; Holzer and McGlathery 2016;

Lopez et al. 2019), which is a common stress response of *T. testudinum* to various abiotic and biotic factors (Greenway 1974; Zieman et al. 1984; Lee and Dunton 1997, 2000; Kahn and Durako 2006).

As green turtle populations continue to rebound, several recent studies have provided insight into the effects of green turtle grazing on seagrasses in naturally grazed systems (Fourqurean et al. 2010; Hernández and van Tussenbroek 2014; Christianen et al. 2019; Hearne et al. 2019; Johnson et al. 2020; Rodriguez and Heck 2020; Scott et al. 2021; Smulders et al. 2022). However, a significant limitation to evaluating seagrass responses to grazing pressure in situ is not knowing how long grazed areas have been maintained by turtles. Earlier studies documented grazed areas (of unknown age) to be maintained for under 1 year to at least 3 years (Bjorndal 1980; Ogden et al. 1983; Zieman et al. 1984). However, a recent novel study in known-age grazed areas found that the use of grazed areas is a dynamic process that can be sustainable for much longer periods that previously documented (> 9 years; Constant et al. 2023). The only study of which the authors are aware that mimicked the green turtle grazing pattern and natural re-cropping interval beyond one year is Moran and Bjorndal (2005, 2007). Evaluating seagrass regrowth dynamics following prolonged, known-durations of grazing pressure by green turtles is essential to understand the capacity of seagrasses to support herbivory and the functioning of seagrass ecosystems under natural grazing regimes.

Based on a 27-month study in a subtropical Caribbean seagrass meadow, we evaluate *T. testudinum* regrowth dynamics over 11 months following two durations (11 and 16 months) of simulated green turtle grazing. We address



**Fig. 1** **a** A border of a grazed (left) and ungrazed (right) area in a Caribbean seagrass meadow. **b** A juvenile green turtle (*Chelonia mydas*) forages in a grazed area of *Thalassia testudinum* in a Caribbean seagrass meadow. Photographs: A. Gulick

the following: (1) evaluate grazing effects on *T. testudinum* regrowth dynamics by comparing morphological and nutrient characteristics between clipped and reference plots at the end of the 11- and 16-month clipping treatments, and at 6.5 months and 11 months post-clipping; (2) evaluate the effects of grazing (i.e., clipping) duration on regrowth by comparing variables between plots that had been clipped for 16 months and plots that had been clipped for 11 months, at 6.5 months, and 11 months after cessation of clipping. This study provides a valuable contribution to understand the effects of prolonged cultivation grazing and the duration of grazing pressure on *T. testudinum* regrowth dynamics.

## Methods

### Study site and experimental clipping trial

A simulated grazing experiment was conducted from July 1999 to December 2000, with follow-up sampling to quantify seagrass regrowth in June 2001 and October 2001, at the Caribbean Marine Research Center on Lee Stocking Island, Exuma Cays, The Bahamas (23.46° N, - 76.06° W). The site consisted of a large, monospecific *T. testudinum* meadow (~3 m water depth), with a low current flow (1.5 m tidal range) and calcium carbonate sediment. Water temperature varied seasonally during the study period; weekly temperatures (average of minimum and maximum temperatures) ranged from ~31.5 °C in the late summer to ~23.5 °C in the late winter (Johnson et al. 2022). Salinity ranged from 37.3 psu in July 1999 to 40.2 psu in November 2000, but did not exhibit a seasonal trend (Johnson et al. 2022). No evidence of green turtle grazing was found in the area at the time of this study, largely due to a formerly legal harvest of green turtles in The Bahamas (The harvest was banned in September 2009.)

Thirty 3 × 3 m plots were established in the *T. testudinum* meadow during July 1999, including 15 experimentally clipped plots to simulate green turtle grazing for 16 months (hereafter, 16clip) and 15 unclipped reference plots (hereafter, reference). An additional five clipped plots were established in February 2000 and clipped for 11 months (hereafter, 11clip) to evaluate how clipping duration may affect seagrass responses to grazing via comparisons to 16clip plots. Plots were arranged in a grid with at least 4 m separation between plots and arranged so clipped and unclipped plots were alternated.

Green turtle grazing was simulated in each clipped plot by severing all *T. testudinum* leaves at the leaf-sheath junction with scissors (Moran and Bjorndal 2005), resulting in a shoot height of ~2 cm above the sediment. Leaves in clipped plots were re-clipped when the mean leaf length reached ~5 cm above the leaf-sheath junction (12–37 days

depending on growth conditions), and all leaf mass was removed to mimic the green turtle cultivation grazing pattern (Fig. 1a; Bjorndal 1980). To avoid edge effects from the surrounding ungrazed meadow, all samples in clipped plots were collected from the inner 2 × 2 m area (4 m<sup>2</sup>), leaving a 0.5 m wide buffer zone around the plot edge. Rhizomes were severed around the edge of each clipped plot at the beginning and every 6–8 weeks throughout the experiment, to prevent nutrient translocation (Moran and Bjorndal 2007).

This clipping regime was maintained for 16 months in the 15 clipped plots from July 1999 to November 2000 (16clip) and for 11 months from February 2000 through December 2000 in the 5 February-initiated clipped plots (11clip). A single sampling event in all plots was conducted at 6.5 months (June 2001) and 11 months (October 2001) after the cessation of the clipping treatments, to quantify *T. testudinum* regrowth. In this study, we evaluate the effects of simulated grazing and clipping duration on *T. testudinum* regrowth dynamics using measurements of morphology and nutrient composition at the end of the clipping treatments, and at 6.5 and 11 months post-clipping. Leaf growth rates were not measured post-clipping. The effects of simulated grazing and clipping duration on *T. testudinum* growth, morphology, and nutrient content during the 11- and 16-month clipping treatments are evaluated by previous studies (Moran and Bjorndal 2005, 2007; Johnson et al. 2022). There was no difference in morphological and nutrient variables between plots prior to the initiation of clipping treatments (Moran and Bjorndal 2005, 2007).

### Sample and data collection

*Thalassia testudinum* morphology was quantified in clipped and reference plots every 2 weeks throughout the clipping treatments and at 6.5 and 11 months post-clipping. Shoot density (shoots m<sup>-2</sup>) and the number of leaves per shoot were measured in each plot within three randomly placed 25 × 25 cm quadrats (0.0625 m<sup>2</sup>), and leaf length and width were measured from 30 randomly selected leaves in each plot. A one-sided leaf area index (LAI) was calculated for each plot using these morphological characteristics (Enriquez and Pantoja-Reyes 2005). The growth of *T. testudinum* was not measured after the clipping treatments ended.

Aboveground biomass (g dry mass (DM) m<sup>-2</sup>) was measured in three 25 × 25 cm quadrats in each reference plot at 0, 2, 6, 11, and 16 months during the clipping treatments and at 6.5 and 11 months after cessation of clipping. Aboveground biomass in clipped plots was the DM of all leaves collected from the interior 4 m<sup>2</sup> area of each plot at the time of clipping. Belowground biomass (DM of rhizomes and roots) was collected from a single 1140 cm<sup>3</sup> (7.62 cm inner diameter, 25 cm depth) core in each plot. Belowground biomass was

not collected in 11clip plots at any time or at 6.5 months post-clipping in 16clip plots and reference plots. Above- and belowground biomass samples were rinsed with salt water and dried to a constant mass of 60 °C.

Nutrient and energy composition of dried *T. testudinum* leaves and belowground biomass were determined using standard procedures (see Supplement; Moran and Bjorndal 2007). The following components were quantified on a % DM basis (unless otherwise noted): organic matter, energy (kJ g<sup>-1</sup> DM), nitrogen, phosphorus, cell wall constituents (CWC), acid detergent fiber (ADF), lignin, and cutin. Fewer components were analyzed for belowground biomass because of the small mass of some samples.

### Analytical approach

To evaluate the effect of simulated grazing and grazing duration on *T. testudinum* regrowth, we used two-factor repeated-measures ANOVA with Bonferroni corrections to compare variables between clipped and reference plots at the end of the 11- and 16-month clipping treatments, and at 6.5 and 11 months post-clipping. Time was the within-subject factor, and treatment was the between-subject factor. Separate ANOVAs were performed for 16clip plots ( $n = 14$ ) versus reference plots ( $n = 15$ ) and 11clip plots ( $n = 5$ ) versus reference plots. When necessary, percentage values for nutrient

composition variables were arcsine transformed prior to analysis to normalize the data.

For the variables measured in three quadrats in each plot at each sampling interval (i.e., shoot density and leaves per shoot in all plots; leaf biomass and all nutrient variables in reference plots), the mean of the three quadrats was used as the plot value. Note that one of the 16clip plots was buried by sediment following a hurricane in October 1999, and all data from this plot were excluded from analyses. Analyses were conducted in R.4.2.2 (R Core Team 2022) using dplyr (Wickham et al. 2022). Statistical significance was evaluated at an alpha value of 0.05.

### Results

A summary of *T. testudinum* morphology and nutrient composition in reference ( $n = 15$ ) and clipped plots ( $n = 14$ , 16clip;  $n = 5$ , 11clip) at the end of the clipping treatments and at 6.5 and 11 months post-clipping, is provided in Tables 1 and 2. Test statistics for repeated-measures ANOVA to evaluate treatment and time effects on regrowth variables are reported in Tables S1-S3. Note that the effects of simulated grazing and clipping duration on *T. testudinum* morphology and nutrient content during the 11clip and 16clip treatments are evaluated and interpreted by previous studies (Moran and Bjorndal 2005, 2007).

**Table 1** Summary of *Thalassia testudinum* morphological characteristics (mean  $\pm$  SE) in clipped and unclipped plots at the end of the 11- and 16-month clipping treatments and at 6.5 months and 11 months post-clipping

	End of clipping			6.5 months post-clipping			11 months post-clipping		
	Unclip $n = 15$	16clip $n = 14$	11clip $n = 5$	Unclip $n = 15$	16clip $n = 14$	11clip $n = 5$	Unclip $n = 15$	16clip $n = 14$	11clip $n = 5$
Leaf length (cm)	13.6 $\pm$ 0.4	3.9 $\pm$ 0.3*	3.0 $\pm$ 0.5*	11.0 $\pm$ 0.4	9.8 $\pm$ 0.5	10.7 $\pm$ 1.3	13.8 $\pm$ 0.5	12.4 $\pm$ 0.5	12.0 $\pm$ 0.8
Leaf width (cm)	0.75 $\pm$ 0.01	0.61 $\pm$ 0.01*	0.60 $\pm$ 0.01*	0.72 $\pm$ 0.01	0.65 $\pm$ 0.01*	0.60 $\pm$ 0.03*	0.77 $\pm$ 0.01	0.71 $\pm$ 0.01*	0.67 $\pm$ 0.02
Leaves per shoot	2.7 $\pm$ 0.1	2.8 $\pm$ 0.03	2.6 $\pm$ 0.1	3.2 $\pm$ 0.1	3.3 $\pm$ 0.1	3.0 $\pm$ 0.1	2.8 $\pm$ 0.1	2.9 $\pm$ 0.1	2.5 $\pm$ 0.1
Shoot density (m <sup>-2</sup> )	689.9 $\pm$ 39.9	613.7 $\pm$ 38.7	774.4 $\pm$ 32.5	657.8 $\pm$ 38.9	545.5 $\pm$ 22.6	650.7 $\pm$ 80.0	651.0 $\pm$ 39.6	611.9 $\pm$ 53.5	640.0 $\pm$ 28.3
Leaf biomass (g DM m <sup>-2</sup> )	60.2 $\pm$ 3.8	13.7 $\pm$ 0.5*	14.6 $\pm$ 0.6*	59.7 $\pm$ 5.1	45.6 $\pm$ 2.9	36.6 $\pm$ 2.2	62.9 $\pm$ 4.2	60.1 $\pm$ 3.2	54.8 $\pm$ 3.4
Rhizome biomass (g DM m <sup>-2</sup> )	2.8 $\pm$ 0.3	2.9 $\pm$ 0.6	–	–	–	–	3.6 $\pm$ 0.5	2.9 $\pm$ 0.3	–
Leaf Area Index	1.9 $\pm$ 0.1	0.4 $\pm$ 0.1*	0.4 $\pm$ 0.1*	1.7 $\pm$ 0.1	1.1 $\pm$ 0.1*	1.3 $\pm$ 0.4	1.9 $\pm$ 0.1	1.5 $\pm$ 0.1	1.3 $\pm$ 0.2

Clipped treatments are denoted as 16clip or 11clip for plots clipped for 16 months or 11 months, respectively. Biomass was evaluated on a dry matter (DM) basis. Comparisons between unclipped and clipped plots at each time interval were conducted using separate two-factor repeated-measures ANOVA for 16clip and 11clip plots, respectively (Table S1); \* denotes statistical significance at the 0.01 level ( $P$ -values provided in Table S4); extra significant figures added to leaf width to aid interpretation of statistical significance

**Table 2** Summary of *Thalassia testudinum* nutrient composition (mean ± SE) in clipped and unclipped plots at the end of the 11- and 16-month clipping treatments and at 6.5 months and 11 months post-clipping

	End of clipping				6.5 months post-clipping		11 months post-clipping		
	16clip		11clip		Unclip	11clip	16clip	11clip	
	n=15	n=14	n=14	n=5					n=15
<i>Leaves</i>									
Organic matter	71.0 ± 0.2	73.0 ± 0.1*	73.1 ± 0.1*	–	71.6 ± 0.5	72.8 ± 0.3	72.2 ± 0.8	70.3 ± 0.2	71.0 ± 0.4
Energy (kJ g <sup>-1</sup> DM × 10 <sup>2</sup> )	127.8 ± 0.4	137.3 ± 0.4*	137.7 ± 0.3*	–	131.0 ± 0.1	134.5 ± 0.7*	133.1 ± 1.5	126.7 ± 0.4	124.0 ± 0.6
Nitrogen	2.1 ± 0.02	2.6 ± 0.01*	2.1 ± 0.04	–	2.1 ± 0.03	2.2 ± 0.02*	2.2 ± 0.02	2.0 ± 0.02	2.1 ± 0.04
Phosphorus	0.20 ± 0.002	0.22 ± 0.003*	0.14 ± 0.002*	–	0.17 ± 0.003	0.17 ± 0.003	0.16 ± 0.002*	0.15 ± 0.002	0.14 ± 0.002*
CWC	37.4 ± 0.4	39.5 ± 0.3*	40.4 ± 0.4*	–	38.8 ± 0.5	39.0 ± 0.4	39.2 ± 0.6	37.8 ± 0.4	38.1 ± 0.3
ADF	28.3 ± 0.3	30.4 ± 0.3*	31.3 ± 0.5*	–	29.0 ± 0.4	29.0 ± 0.5	28.8 ± 0.6	28.8 ± 0.2	29.3 ± 0.5
Lignin	2.2 ± 0.1	2.8 ± 0.1*	2.3 ± 0.2	–	2.4 ± 0.1	2.4 ± 0.1	2.0 ± 0.1	2.3 ± 0.1	2.4 ± 0.1
Cutin	1.6 ± 0.2	2.7 ± 0.2*	3.2 ± 0.2*	–	1.7 ± 0.4	1.5 ± 0.2	1.6 ± 0.1	1.6 ± 0.2	2.1 ± 0.3
<i>Rhizomes</i>	<i>n</i> varies	<i>n</i> varies	<i>n</i> varies	–	<i>n</i> varies	<i>n</i> varies	<i>n</i> varies	<i>n</i> varies	<i>n</i> varies
Organic matter	56.1 ± 0.8	48.0 ± 1.5*	–	–	–	–	–	54.7 ± 0.8	53.5 ± 1.7
Nitrogen	0.7 ± 0.03	0.6 ± 0.02	–	–	–	–	–	1.0 ± 0.02	1.0 ± 0.03
Nitrogen (% OM)	1.2 ± 0.1	1.2 ± 0.03	–	–	–	–	–	1.8 ± 0.05	1.9 ± 0.1
Phosphorus	0.1 ± 0.01	0.1 ± 0.01	–	–	–	–	–	0.1 ± 0.01	0.1 ± 0.01
CWC	23.8 ± 0.6	22.1 ± 0.7	–	–	–	–	–	22.2 ± 0.3	22.2 ± 0.6
ADF	18.8 ± 0.6	18.1 ± 0.8	–	–	–	–	–	17.0 ± 0.3	17.7 ± 0.6
Lignin	3.9 ± 0.2	3.8 ± 0.3	–	–	–	–	–	4.1 ± 0.1	4.4 ± 0.2
	<i>n</i> =12	<i>n</i> =11	–	–	–	–	–	<i>n</i> =14	<i>n</i> =12

Clipped treatments are denoted as 16clip or 11clip for plots clipped for 16 months or 11 months, respectively. Sample sizes vary depending on amount of tissue available. All composition values are reported on a dry matter (% DM) basis unless otherwise noted. OM is organic matter; CWC is cell wall constituents; ADF is ligno-cellulose. Comparisons between unclipped and clipped plots at each time interval were conducted using separate two-factor repeated-measures ANOVA for 16clip and 11clip plots, respectively (Tables S2–S3); \* denotes statistical significance at the 0.05 level (*P*-values provided in Table S5); extra significant figures added to aid interpretation of statistical significance

Simulated grazing had significant effects on *T. testudinum* morphology in clipped plots by the end of both the 11- and 16-month clipping treatments (Table 1; *P*-values from between treatment pairwise comparisons in Table S4), with significant decreases in leaf length, leaf width, above-ground biomass, and LAI in all 16clip and 11clip plots relative to reference plots. Shoot density and leaves per shoot were unaffected by the end of both clipping treatments. Most morphological variables returned to levels comparable to reference plots by 6.5 months post-clipping, but the effects of grazing duration on LAI and leaf width were apparent. Leaf area index in 16clip plots was significantly lower than reference plots (ANOVA,  $F = 12.5$ ,  $P < 0.01$ ) at 6.5 months post-clipping, and leaf width was significantly narrower in all 11clip (ANOVA,  $F = 12.5$ ,  $P < 0.01$ ) and 16clip plots (ANOVA,  $F = 22.6$ ,  $P < 0.01$ ). By 11 months post-clipping, there was no longer a difference in LAI between 16clip plots and reference plots, whereas leaf width remained significantly narrower in 16clip plots (ANOVA,  $F = 12.5$ ,  $P < 0.01$ ), but not in 11clip plots.

Simulated grazing had significant effects on the majority of leaf nutrient variables by the end of both clipping treatments, but negligible effects on rhizome nutrient composition throughout the experiment (Table 2; *P*-values from between treatment pairwise comparisons in Table S5). Leaf nutrient quality remained higher in 16clip plots compared to reference plots at 6.5 months post-clipping, in the form of elevated leaf nitrogen (ANOVA,  $F = 11.3$ ,  $P = 0.01$ ) and energy content (ANOVA,  $F = 7.1$ ,  $P = 0.04$ ) (see Bjorndal 1980; Moran and Bjorndal 2007). Leaf phosphorus content was significantly lower in 11clip plots than in reference plots at 6.5 months (ANOVA,  $F = 8.0$ ,  $P = 0.03$ ) and 11 months post-clipping (ANOVA,  $F = 18.3$ ,  $P < 0.01$ ). We believe this trend to be anomalous (Table 2), and not a result of clipping duration, because of the initial increase in phosphorus at the end of the 11clip treatment ( $0.14 \pm 0.002\%$  DM to  $0.16 \pm 0.002\%$  DM at 6.5 months post-clipping), followed by a decrease at 11 months post-clipping to the initial value ( $0.14 \pm 0.002\%$  DM). Note that there was no effect of clipping on the P-content of rhizomes in 11clip or 16clip plots, and that leaf P-content in 16clip plots was significantly higher (not lower) than reference plots only at the end of the clipping treatment (Table 2). All other leaf nutrient variables in clipped plots returned to levels comparable to those of reference plots by 11 months post-clipping.

## Discussion

Following the simulated grazing trial, most *T. testudinum* morphological and nutrient characteristics in clipped plots returned to levels comparable to reference plots within 6.5 months (Tables 1, 2). However, the effects of grazing

duration on LAI and leaf width were still apparent several months after clipping treatments had ended. Six and a half months was sufficient for LAI in 11clip plots to rebound to reference plot levels. However, LAI in 16clip plots and leaf width in 11clip plots did not return to levels comparable to reference plots until 11 months post-clipping, and leaf width in 16clip plots remained significantly narrower for the duration of the experiment. Leaf narrowing has important implications for understanding the physiological changes that occur between grazed and ungrazed states, and the factors that drive *T. testudinum* disturbance-recovery dynamics under natural grazing regimes.

## Regrowth dynamics and mechanisms of leaf narrowing in grazed areas

Leaf narrowing in *T. testudinum* is a stress response caused by a variety of factors, including changes in salinity (Zieman 1975; Kahn and Durako 2006), light availability (Lee and Dunton 1997; Enríquez et al. 2019), nitrogen enrichment (Lee et al. 2007), ammonium additions (Kahn and Durako 2006), and herbivory (Valentine and Heck 1999; Verges et al. 2008). Although decreased leaf width is a well-documented response to green turtle grazing (Moran and Bjorndal 2005; Holzer and McGlathery 2016; Johnson et al. 2017; Lopez et al. 2019) and is reversible after cessation of grazing pressure (Table 1; Williams 1988; Fourqurean et al. 2010), the potential physiological mechanisms behind this response to grazing are less established.

Multiple experiments that simulated grazing by green turtles observed a substantial decrease in leaf width within a few months of initiation of clipping (Moran and Bjorndal 2005; Kuiper-Lindley et al. 2007; Holzer and McGlathery 2016; Johnson et al. 2017; Rodriguez and Heck 2020). During this simulated grazing experiment, leaf width dramatically decreased after the initiation of clipping in July 1999, followed by a plateau that began in November 1999 and was maintained for the remainder of the clipping trial (Moran and Bjorndal 2005). Changes in plant growth allocation after *T. testudinum* is grazed may explain why a decrease in leaf width occurs, and how leaf width increases following cessation of grazing.

*Thalassia testudinum* in tropical/subtropical meadows commonly exhibits compensatory growth responses to herbivory (Valentine et al. 1997; Cebrian et al. 1998; Moran and Bjorndal 2005), including over a broad range of green turtle grazing intensities (Gulick et al. 2020). The plant supports stimulated growth by increasing production of individual shoots in grazed areas, which allows LAI and light-harvesting potential to be maintained, despite the removal of leaf tissue by turtles (Gulick et al. 2021b). This suite of morphological responses is indicative that a shift in plant growth allocation occurs in grazed areas, and a recent analysis of *T.*

*testudinum* growth dynamics during the simulated grazing experiment suggests that a growth tradeoff exists between grazed and ungrazed states (Johnson et al. 2022). The results of that analysis indicate that *T. testudinum* in grazed areas prioritizes increasing biomass production, whereas ungrazed areas prioritizes increasing photosynthetic area (i.e., LAI) (Johnson et al. 2022), and that an inflection point between the two states could be identified following cessation of grazing.

The presence of significantly narrower leaves in clipped plots relative to reference plots at 11 months post-clipping (Table 1), could be a lingering effect of the change in growth allocation toward prioritizing biomass production over photosynthetic area (Johnson et al. 2022). The return of leaf biomass, LAI, and shoot density in clipped plots to ungrazed levels within 6.5 months after the clipping treatments ended demonstrates how quickly a transition back to prioritizing photosynthetic surface area can occur. Because creating wider leaves is indicative of prioritizing photosynthetic leaf area via increased LAI (Enríquez et al. 2019), an increase in leaf width in clipped plots may be the final step in the regrowth process, albeit a slow one, as the plant shifts growth strategies back to that of an ungrazed state. Future studies should evaluate regrowth dynamics at more frequent intervals following the cessation of grazing to identify the potential inflection point at which the priority shifts from biomass production to increasing LAI (Johnson et al. 2022).

### Grazing duration and other drivers of seagrass regrowth

Most morphological and nutrient characteristics in 11clip and 16clip plots returned to levels comparable with reference plots within 6.5 months of cessation of grazing (Tables 1, 2), indicating the capacity of *T. testudinum* to rebound following prolonged cultivation grazing of leaf biomass by turtles (Constant et al. 2023). However, grazing duration clearly affects the process of leaf rewidening after cessation of grazing, as the plant shifts back to prioritizing photosynthetic surface area (i.e., LAI) in an ungrazed state. To our knowledge, the effects of grazing duration by green turtles on seagrass regrowth dynamics have not been previously evaluated in situ or experimentally. Additional data collection on seagrass regrowth trajectories with more frequent measurement intervals (i.e., less than 6.5 months), and under different grazing durations, will provide substantial insight into the effects of grazing duration on the physiological shifts in growth allocation that occur between grazed and ungrazed states (see Johnson et al. 2022). Future studies that evaluate *T. testudinum* regrowth at a cellular level will also benefit our understanding of why leaf rewidening is a slow process and seemingly the final step of the regrowth cycle.

Abiotic factors are also likely drivers of *T. testudinum* regrowth following prolonged grazing by green turtles. Because compensatory growth responses to grazing in tropical/subtropical *T. testudinum* meadows are seasonally dependent (Johnson et al. 2022), water temperature and light availability are undoubtedly important drivers of regrowth once turtles abandon a grazed area. However, we expect that light availability in undisturbed systems would play a lesser role than temperature in regulating regrowth following grazing, until sufficient leaf biomass accumulates and results in leaf self-shading (Gulick et al. 2021a, b). However, if grazed areas experience low light availability combined with high turbidity due to anthropogenic disturbance, we would expect this to result in delayed regrowth and/or potential seagrass loss (Christianen et al. 2014). In contrast to our subtropical study site, the regrowth of leaf width, LAI, and shoot density in naturally grazed temperate *T. testudinum* meadows in Bermuda took over a year to return to ungrazed levels (Fourqurean et al. 2010). This highlights the importance of considering latitudinal gradients in seagrass productivity (van Tussenbroek et al. 2014), driven by seasonal variation in temperature and light availability (Lee et al. 2007), in addition to levels of natural or anthropogenic disturbance, when predicting regrowth trajectories following grazing events.

Nutrient availability should also affect *T. testudinum* regrowth dynamics following grazing, particularly in phosphorus-limited systems with calcium carbonate sediments (Holzer and McGlathery 2016). The results from a simulated grazing experiment conducted in a strongly phosphorus-limited temperate seagrass ecosystem, showed that leaf width was slow to recover in clipping treatments that did not receive P-additions, but rebounded promptly in nutrient enriched clipped plots (Holzer and McGlathery 2016). Tropical *T. testudinum* meadows with higher rates of productivity than those at temperate latitudes (van Tussenbroek et al. 2014), and higher P availability (see Zieman et al. 1984; Moran and Bjorndal 2007; Gulick et al. 2020), may have increased capacity for supporting regrowth following cultivation grazing. These patterns collectively indicate the importance of abiotic factors (Holzer and McGlathery 2016; Johnson et al. 2022), in addition to grazing duration, in driving *T. testudinum* growth allocation and regrowth dynamics in grazed areas.

Our results demonstrate that *T. testudinum* has the capacity to tolerate and rebound following prolonged grazing of leaf biomass by green turtles, and that decreased leaf width after cessation of grazing may be a lingering effect of shifts in plant growth allocation in grazed systems. Evaluating seagrass regrowth trajectories with variation in grazing duration, using more frequent measurement intervals, would benefit our understanding of disturbance-recovery dynamics in grazed systems (Hewitt et al. 2022; Constant et al. 2023). The effects of grazing duration on regrowth dynamics

demonstrated in our study have important implications for the sustainability of seagrass meadows that support green turtle populations.

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**Data availability** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request and in Johnson et al. (2022).

## Declarations

**Conflict of interest** The authors declare no conflicts or competing interests.

**Ethical approval** This study did not require ethics approval.

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