

In situ quantification of *Tripneustes gratilla* grazing and its effects on three co-occurring tropical seagrass species

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ABSTRACT: We studied sea urchin *Tripneustes gratilla* herbivory in a meadow of co-occurring seagrass species *Thalassia hemprichii* (climax species), *Halodule uninervis* and *Cymodocea rotundata* (colonizing species) to determine effects of sea urchins on seagrass biomass and species composition, to quantify herbivory rate and to determine the role of urchins in the nitrogen (N) cycle. In a 2 mo enclosure experiment in Sulawesi, Indonesia, we increased *T. gratilla* density and measured seagrass shoot density and aboveground biomass. Belowground biomass was determined at the start and end of the experiment and leaf production at the end. Carbon (C) and N concentrations were measured in plant material. Increased *T. gratilla* grazing did not influence seagrass shoot density or leaf production. Herbivory had a negative impact on the biomass per shoot for all species, but only *H. uninervis* and *C. rotundata* aboveground biomass declined significantly. The proportion of aboveground biomass made up by *T. hemprichii* increased significantly from 37 to 68% of the total seagrass biomass, while *C. rotundata* biomass declined significantly from 25 to 11% of the total biomass. No significant decline occurred in *H. uninervis* (from 38 to 21%). Over the experiment, *T. gratilla* grazing significantly reduced the aboveground biomass by 74%, but had no effect on belowground biomass. Grazing caused an increase in leaf N concentration for *H. uninervis* and *C. rotundata*. The calculated mean \pm SE N intake rate was 15.9 ± 1.1 mg N urchin⁻¹ d⁻¹. Total consumption by *T. gratilla* in the meadow at an average urchin density of 1.55 ± 0.07 urchins m⁻² was calculated to be 1.28 g dry weight m⁻² d⁻¹, corresponding to 26% of the net aboveground seagrass production. We conclude that *T. gratilla* consumes a considerable amount of the leaf production and even influences species composition in tropical seagrass meadows. *T. gratilla* grazing may play a role in conserving N within the meadow and create short-circuits in the N cycling of seagrass leaf material.

KEY WORDS: Herbivory · Species composition · Tropical seagrass · Sea urchin · *Tripneustes gratilla* · Indonesia

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INTRODUCTION

Seagrass meadows in the Indo-Pacific often consist of co-occurring seagrass species that maintain a high rate of leaf production year-round (Hemminga & Duarte 2000). Part of this leaf production directly enters the detritus pathway, while another part is consumed by herbivores or exported (Duarte & Cebrian 1996, Cebrian & Duarte 1998). Historically, turtles and

dugongs were the main seagrass herbivores, but their numbers have declined greatly (Jackson et al. 2001). Currently, the most abundant herbivores on seagrasses are fishes, crustaceans and sea urchins (Alcoverro & Mariani 2004, Goecker et al. 2005, Valentine & Duffy 2006).

In the tropical Indo-Pacific region, sea urchins are widely present in seagrass meadows (Klumpp et al. 1993, Alcoverro & Mariani 2002). Sea urchin grazing

affects aboveground biomass, leaf production, shoot density and cover of seagrasses (Keller 1983, Valentine & Heck 1991, Valentine et al. 1997, Alcoverro & Mariani 2004). Leaf herbivory is highly variable among different seagrass species (Cebrian & Duarte 1998); grazing preferences of sea urchins on different seagrass species are poorly understood for the multi-species meadows of the Indo-Pacific (Vaitilingon et al. 2003) where climax and colonizing grass species co-occur. Sea urchin herbivory may play a role in maintaining seagrass biodiversity in these meadows. Other important effects are physical environmental factors (Koch et al. 2006) and nutrients (Hemminga & Duarte 2000).

Few *in situ* studies have been performed to quantify sea urchin grazing in the Indo-Pacific (Valentine & Duffy 2006). The sea urchin *Tripneustes gratilla* occurs widely in shallow waters and grazes predominantly on seagrasses (Klumpp et al. 1993, Lawrence & Agatsuma 2001). By measuring herbivory rate and the nitrogen (N) content of the leaves, N intake rates of *T. gratilla* and the removal of N from the seagrasses by grazing can be estimated (Valentine & Duffy 2006). Not all seagrass material ingested by sea urchins is digested efficiently (Lawrence & Klinger 2001) and finely-fragmented undigested material is voided from the digestive tract (Koike et al. 1987). Sea urchin grazing therefore influences the N cycling of seagrass meadows in different ways.

We propose that sea urchin grazing influences seagrass biomass, density, production and species composition in tropical mixed-species seagrass meadows. We investigated the effects of *Tripneustes gratilla* grazing on above- and belowground biomass, tissue N and carbon (C) concentrations, density, leaf production and species composition of a meadows consisting of the co-occurring seagrass species *Thalassia hemprichii* (climax species), *Halodule uninervis* and *Cymodocea rotundata* (colonizing species). We quantified grazing by *T. gratilla* on the seagrasses and determined the role of sea urchin grazing in N cycling within a tropical oligotrophic seagrass meadow. For this purpose, the density of the sea urchins *T. gratilla* was artificially increased *in situ* in 1×1 m enclosures deployed in the seagrass meadow.

MATERIALS AND METHODS

Study site. The experiments were carried out on Bone Batang ($5^{\circ} 01' S$, $119^{\circ} 19' 30'' E$), an uninhabited coral island in the Spermonde Archipelago, South Sulawesi, Indonesia, located 15 km offshore and 2 km north of Barang Lompo (Fig. 1). The island consists of an intertidal sandbank with a surrounding reef flat and barrier reef. The reef flat consists of coarse carbonate sand and coral rubble and is covered by an extensive seagrass meadow consisting of the co-occurring *Thalassia hemprichii*, *Halodule uninervis* and *Cymodocea rotundata*. *Halophila ovalis* was found in low densities and accounted for <1% of the aboveground biomass.

Urchin densities. Three permanent transects of 15×1 m were pegged out to estimate the densities of *Tripneustes gratilla* in the seagrass meadow. During daytime, the number of urchins present in each of 15 quadrats of 1 m^2 was counted on 11 occasions over the period of 1 yr. On 5 occasions, we measured size of each counted sea urchin.

Grazing experiments. Four replicate enclosures (1×2 m) were constructed at an experimental site in the seagrass bed with a well developed mixture of the 3 co-occurring seagrass species. Each enclosure consisted of two 1×1 m compartments ~40 cm high with different grazing treatments. All visible (i.e. >10 mm) macrobenthos was first removed by hand from the enclosures. The grazing treatments were (1) no grazers added (control) and (2) sea urchins added (*Tripneustes* treatment). Five *T. gratilla* specimens with an average test diameter of 7.1 cm were introduced in the *Tripneustes*

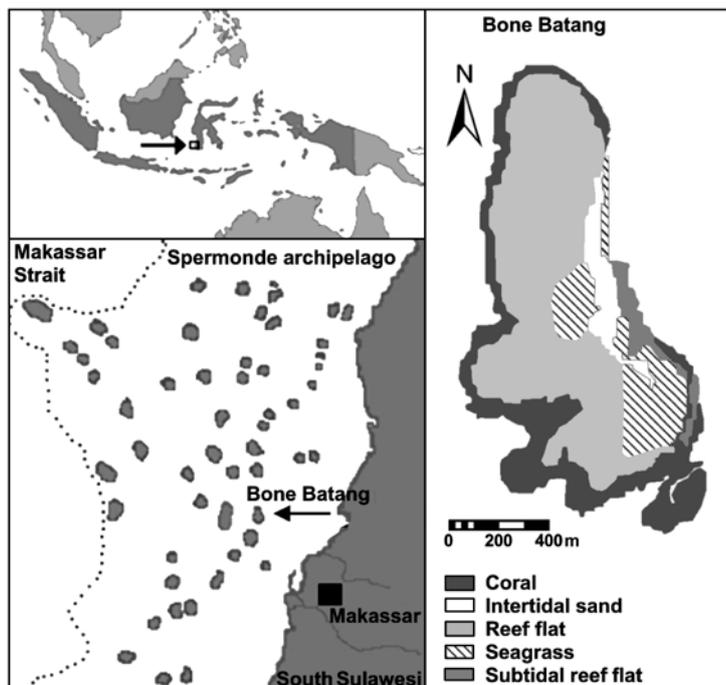


Fig. 1. Study area, showing the location and the islands of the Spermonde Archipelago along the west coast of South Sulawesi (dotted line: shelf edge 200 m), overview of the island Bone Batang ($5^{\circ} 01' 00'' S$, $119^{\circ} 19' 30'' E$) and seagrass meadows

treatment. We used a density of 5 sea urchins m^{-2} (about thrice the average density) deliberately to generate a measurable impact of *T. gratilla* grazing on the seagrass biomass, but made sure that density stayed within the natural range found in this area, which is ≤ 7 urchins m^{-2} .

The enclosures were constructed of zinc screen with 30 mm square mesh. The zinc screen was supported by wooden poles placed on the outside of the enclosures and completely covered the enclosures. The screen was not buried in the sediment (to prevent damage to the rhizomes). The sites chosen for the deployment of enclosures were about 30 cm below extreme low water (ELW). The experiment lasted for 58 d and was performed during the dry season (May to July 2005) with little or no environmental disturbance (e.g. wind, wave action) and stable seagrass growth conditions (Erftemeijer & Herman 1994). Since the enclosures had a wide mesh, were placed at shallow depth in clear off-shore waters and were cleaned of epiphytes every week, we assume light limitation did not influence the experiment.

Sample collection. We used a sample collection method slightly modified from Valentine & Heck (1991). Every 10 d, three 0.01 m^2 square quadrats (sub-sample) within each treatment were sampled according to a predetermined random sampling design applied in the same way to each treatment to assure quadrats were only sampled once during the whole experiment (Fig. 2). The seagrass leaves in the quadrats were cut off at sediment surface level using scraper blades. Total number of shoots per species was counted in each quadrat. Sampled leaves were cleaned of epiphytes using scraper blades, separated by species, and biomass dry weight (DW) was determined after oven drying (70°C, 48 h). Data of the 3

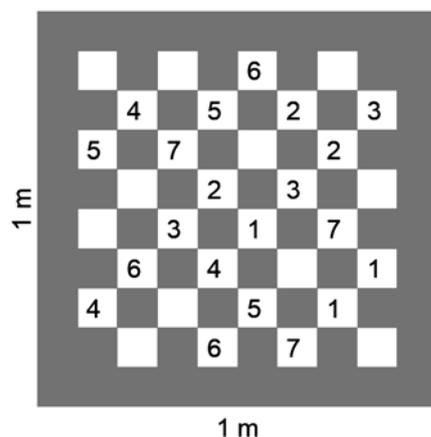


Fig. 2. Schematic overview of the sampling quadrats (0.1 × 0.1 m) harvested in each treatment. Quadrats 1, 2, 3, 4, 5, 6 and 7 were harvested on Days 0, 9, 19, 29, 38, 48 and 58, respectively

quadrats harvested from each treatment at 1 day were pooled together for statistical analysis. Belowground biomasses in the *Tripneustes* treatments were sampled at the start and end of the experiment using 3 cores (diameter 6 cm, depth 15 cm). Belowground plant material was washed free from sediment, divided into roots and rhizomes and treated like the aboveground samples. N and C concentrations (% DW) in aboveground and belowground tissues were determined using a Carlo Erba NA 1500 elemental analyzer.

Leaf production. Leaf production was measured at the end of the experiment. Four replicate samples of 10 shoots for each species were marked in each treatment using the leaf marking technique described by Short & Duarte (2001). The marked shoots were harvested 17 d after leaf marking, divided into newly produced and old material, and treated like aboveground samples. Leaf production ($g\ DW\ shoot^{-1}\ d^{-1}$) was calculated as the amount of newly produced leaf material divided by the product of time (17 d) and the number of shoots (10), resulting in 4 average values per treatment and species.

Leaf loss. We estimated the amount of seagrass production consumed by sea urchins using a mass balance equation that partitions plant production into changes in biomass and leaf loss rate (modified from Valentine & Heck 2001): $L = NAPP - \Delta B$; where L is the leaf loss rate between 2 sampling dates, NAPP is the net aboveground primary production, and ΔB is the change in aboveground biomass. Average NAPP was calculated at each sampling date by multiplying the leaf production per shoot by the measured shoot densities. The leaf loss rate (L) consisted of the sum of sea urchin herbivory rate (H) and the export rate of leaves (E).

Statistical analysis. Before statistical analyses, seagrass density data ($n + 0.5$) were normalized by square root transformation. Shoot and leaf biomass were log-transformed (Sokal & Rohlf 1995). Transformed data were tested for equality of variances using Levene's test. All measured parameters (shoot density, biomass per shoot and aboveground biomass) were analyzed using MANOVA with grazing and time as fixed factors. Significant effect for any of the parameters was followed up by individual independent t -test comparison on these parameters for the start and end of the experiment. The change in percentage of total aboveground biomass per species was compared between Day 0 and Day 58 in the *Tripneustes* treatment. The leaf growth rate, C and N concentrations and belowground biomass were analyzed using independent t -tests. Relative aboveground biomasses per seagrass species at the start and end of the experiment were compared in the *Tripneustes* enclosures. All statistical analyses were performed in SPSS 14.0. Values in the text are given as mean \pm SE and $p < 0.05$ was considered significant.

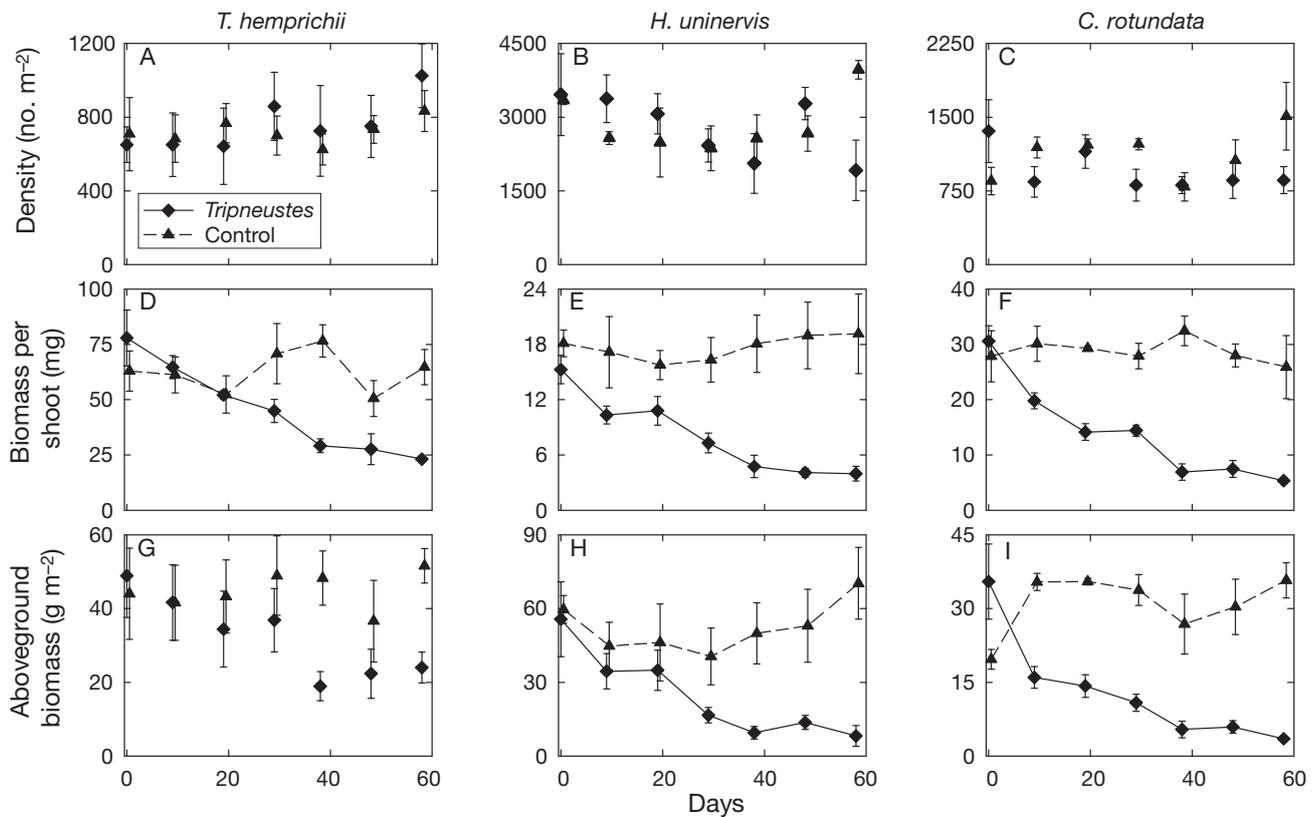


Fig. 3. *Thalassia hemprichii*, *Halodule uninervis*, and *Cymodocea rotundata*. Mean \pm SE (n = 4) seagrass density, shoot biomass and aboveground biomass in the control and *Tripneustes* treatment for the different species during the experiment. Symbols connected by lines indicate significant differences between control and treatment plots (MANOVA, Table 1)

RESULTS

Urchin densities

Tripneustes gratilla density in the seagrass meadow was on average (mean \pm SE) 1.55 ± 0.07 urchins m^{-2} (range 0 to 7 urchins m^{-2}). No trend in urchin density was observed over the year. The average test diameter was 6.8 ± 0.1 cm (range 1.5 to 11.0 cm). All *T. gratilla* individuals were solitary and often covered with seagrass leaves, detritus and other material.

Grazing of seagrass shoots

The responses of seagrass to grazing by *Tripneustes gratilla* during the experiment were measured for shoot density, biomass per shoot and aboveground biomass and tested using MANOVA (Fig. 3, Table 1). No significant changes in shoot density were measured in the treatments during the experiment (Fig. 3A–C). *T. gratilla* grazing resulted in a significant decline of the biomass per shoot for all species over 58 d (all species

$p < 0.01$), while in the control treatment this was stable (Fig. 3D–F). Grazing during 58 d also resulted in a significant decline of aboveground biomass for *Halodule uninervis* ($p = 0.012$) (Fig. 3H), *Cymodocea rotundata* ($p < 0.001$) (Fig. 3I), and total aboveground biomass ($p < 0.001$) (Fig. 4), but not for *Thalassia hemprichii* (Fig. 3G). In the *Tripneustes* treatment, the aboveground biomass of *T. hemprichii* relative to *H. uninervis* and *C. rotundata* increased significantly ($p = 0.042$) from 37 to 68% of the total biomass, *C. rotundata* aboveground biomass declined significantly (from 25 to 11%) relative to the other 2 species ($p = 0.025$), while relative *H. uninervis* biomass did not change significantly (Table 2) due to grazing over 58 d. In the control treatments, no significant change in total aboveground biomass was measured. In the *Tripneustes* treatments, the total aboveground seagrass biomass declined sharply (74%) during the first 38 d and remained constant until the end of the experiment (Fig. 4). At the end of the experiment, the N concentrations in aboveground biomass were significantly higher in the *Tripneustes* grazed treatments than in controls for *H. uninervis* ($t_{14} = -8.81$, $p < 0.001$) and *C.*

Table 1. *Thalassia hemprichii*, *Halodule uninervis*, and *Cymodocea rotundata*. MANOVA (Wilk's lambda) of seagrass responses measured as density (shoots m⁻²), biomass per shoot (mg shoot⁻¹) and aboveground biomass (g m⁻²). Grazing and time were taken as fixed factors (grazing df = 1, time and grazing × time df = 6, error df = 41, and total df = 56). **Bold** values denote significant effects (p < 0.05). Data were transformed before analyses to ensure homogeneity of variances (see 'Materials and methods—Statistical analysis')

Response	R ² (Adj)	Error MS	Grazing		Time		Grazing × Time	
			MS	p	MS	p	MS	p
Density								
<i>T. hemprichii</i>	-0.142	33.307	0.704	0.885	24.773	0.618	9.316	0.943
<i>H. uninervis</i>	0.125	86.632	14.868	0.681	103.224	0.329	195.481	0.056
<i>C. rotundata</i>	0.113	28.193	84.998	0.090	27.868	0.445	51.874	0.114
Biomass per shoot								
<i>T. hemprichii</i>	0.611	0.015	0.411	<0.001	0.088	<0.001	0.097	<0.001
<i>H. uninervis</i>	0.684	0.027	2.164	<0.001	0.111	0.003	0.127	0.001
<i>C. rotundata</i>	0.870	0.010	2.065	<0.001	0.164	<0.001	0.155	<0.001
Aboveground biomass								
<i>T. hemprichii</i>	0.093	0.061	0.447	0.010	0.055	0.497	0.059	0.456
<i>H. uninervis</i>	0.541	0.071	2.556	<0.001	0.214	0.015	0.278	0.003
<i>C. rotundata</i>	0.851	0.020	3.514	<0.001	0.202	<0.001	0.312	<0.001
Total	0.819	0.011	1.440	<0.001	0.098	<0.001	0.137	<0.001

Table 2. *Thalassia hemprichii*, *Halodule uninervis*, and *Cymodocea rotundata*. Mean ± SE percentage of total aboveground biomass per species in the *Tripneustes* treatment at the start and end of the experiment. Significant differences (different superscripts) are **bold** (paired *t*-test, n = 4)

Taxon	% aboveground biomass		Paired <i>t</i> -test	
	t = 0	t = 58	t ₃	p
<i>T. hemprichii</i>	36.6 ^a ± 10.7	67.9 ^b ± 8.5	-3.42	0.042
<i>H. uninervis</i>	37.8 ± 9.1	21.4 ± 8.1	2.50	0.088
<i>C. rotundata</i>	25.5 ^a ± 5.0	10.7 ^b ± 2.5	4.18	0.025

rotundata (t₈ = -7.73, p < 0.001), but not for *T. hemprichii* (Table 3). The aboveground C concentration was equal in treatments and controls for all species.

Belowground

In the *Tripneustes* treatment, total rhizome biomasses were (mean ± SE) 553 ± 48 and 408 ± 42 g DW m⁻² at the start and end of the 2 mo grazing period, respectively (no significant decline). Also, none of the individual seagrass species showed a significant decline in rhizome biomass. Root biomass was 210 ± 15 g DW m⁻² and did not change significantly during the 2 mo grazing experiment. The N concentrations of the rhizomes did not change and were on average 0.84 ± 0.06%, 0.92 ± 0.05% and 0.63 ± 0.04% for *Thalassia hemprichii*, *Halodule uninervis* and *Cymodocea rotundata*, respectively. Over the experimental period, the C concentration in *H. uninervis* rhizomes declined signif-

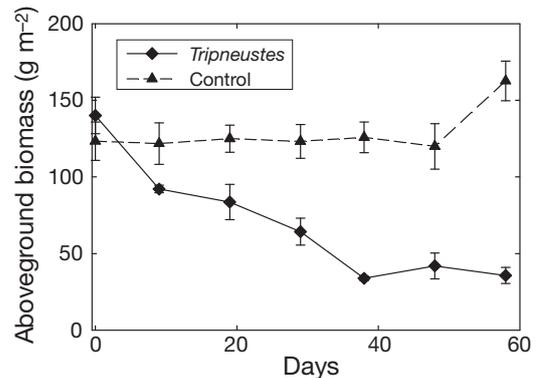


Fig. 4. Mean ± SE (n = 4) aboveground seagrass biomass in the control and *Tripneustes* treatment through 58 d

icantly from 37.5 ± 0.6 to 35.8 ± 0.4% (t₆ = 2.47, p = 0.048), but no significant change was observed for *T. hemprichii* (average 35.7 ± 0.3%) and *C. rotundata* (average 36.8 ± 0.9%).

Leaf production and loss

Leaf production per shoot was highest for *Thalassia hemprichii*, intermediate for *Cymodocea rotundata*, and lowest for *Halodule uninervis*. Leaf production was not significantly different between treatments (Table 4). We combined this production with the measured densities of the different seagrass species to determine NAPP in the different treatments over time. The leaf loss rate (*L*) pattern in the *Tripneustes* treatment was similar to that for the aboveground biomass

Table 3. *Thalassia hemprichii*, *Halodule uninervis*, and *Cymodocea rotundata*. Mean \pm SE nitrogen and carbon concentrations (% dry weight) of the seagrass aboveground biomass measured in the control and *Tripneustes* treatments at the end of the experiment. Significant differences ($p > 0.05$) between treatments are indicated by different superscripts (independent *t*-test; $n = 8$)

Taxon	% N		% C	
	Control	<i>Tripneustes</i>	Control	<i>Tripneustes</i>
<i>T. hemprichii</i>	2.25 \pm 0.05	2.36 \pm 0.11	34.5 \pm 0.2	34.4 \pm 0.3
<i>H. uninervis</i>	1.56 ^a \pm 0.03	2.19 ^b \pm 0.07	35.8 \pm 0.3	35.8 \pm 0.3
<i>C. rotundata</i>	1.89 ^a \pm 0.01	2.37 ^b \pm 0.07	38.5 \pm 0.3	37.8 \pm 0.4

Table 4. *Thalassia hemprichii*, *Halodule uninervis*, and *Cymodocea rotundata*. Mean \pm SE leaf production per shoot ($\text{mg shoot}^{-1} \text{d}^{-1}$) measured for the different seagrass species at the end of the enclosure experiment using the leaf marking technique. There were no significant differences (independent *t*-test; $p > 0.05$) between treatments ($n = 4$, 10 shoots per sample, except for *H. uninervis* $n = 3$)

Taxon	Leaf production	
	Control	<i>Tripneustes</i>
<i>T. hemprichii</i>	2.27 \pm 0.20	2.17 \pm 0.32
<i>H. uninervis</i>	0.68 \pm 0.05	0.43 \pm 0.12
<i>C. rotundata</i>	1.16 \pm 0.22	0.71 \pm 0.16

loss and was also high during the first 38 d (Fig. 5). *L* declined from more than $10 \text{ g m}^{-2} \text{d}^{-1}$ after introduction of *Tripneustes gratilla* to around $4 \text{ g m}^{-2} \text{d}^{-1}$ during the last 20 d of the experiment.

DISCUSSION

Increased *Tripneustes gratilla* grazing significantly influenced seagrass species composition in the enclosures. Aboveground biomass of *Thalassia hemprichii* remained constant, while both *Halodule uninervis* and *Cymodocea rotundata* declined significantly due to the herbivory. The relative aboveground biomass increased significantly for *T. hemprichii* and declined significantly for *C. rotundata* during the grazing experiment. None of the seagrass species studied showed an increase in shoot density, increase in leaf production, or decline in belowground biomass in response to urchin grazing.

The change in aboveground dominance towards *Thalassia hemprichii* in the *Tripneustes* treatment could have been caused by differences in survival of the 3 seagrass species in the enclosures or a *Tripneustes gratilla* feeding preference for the pioneer species *Cymodocea rotundata* and *Halodule uninervis* over the climax species *T. hemprichii*. No decline in

shoot meristem density was measured for any of the seagrasses, indicating that the meristems were not damaged by sea urchin grazing. It is therefore unlikely that the persistence of *T. hemprichii* in the *Tripneustes* treatment resulted from differential exposure of meristems to damage by sea urchin grazing. Overall *T. gratilla* grazing is beneficial for the climax species and may result in a decline of seagrass biodiversity in this meadow. A general pattern of *T. gratilla* grazing on different seagrass species is not clear due to contrasting results from other studies. Koike et al. (1987) reported that *T. gratilla* grazed predominantly on living leaves of the

dominant species *T. hemprichii* rather than on leaves of *Enhalus acoroides* and *Syringodium isoetifolium*; Vaitilingon et al. (2003) showed a *T. gratilla* preference for *S. isoetifolium* and *H. uninervis* in a *C. serulata*-dominated seagrass meadow in which *T. hemprichii* also occurred, while Klumpp et al. (1993) stated that *T. gratilla* grazed to lesser extent on *S. isoetifolium* in a *T. hemprichii*-dominated meadow. N concentration of the seagrass leaf material appears not to influence *T. gratilla* grazing, since reported values for the preferred species are often lower than those of the dominant, non-preferred species.

Increased *Tripneustes gratilla* grazing reduced the aboveground biomass by 74%, but, in terms of total (above- + belowground) biomass, only 17% was removed. No significant effects on the belowground seagrass biomass was discerned, which is in agreement with previous research (Heck & Valentine 1995). In the rhizomes, the plants have stored reserves, which are redistributed to leaves when necessary (Libes & Boudouresque 1987), but these reserves are not readily accessible to most grazers (Valentine & Duffy 2006). At

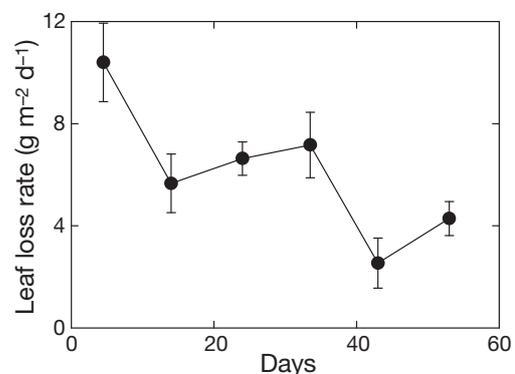


Fig. 5. Mean \pm SE ($n = 4$) leaf loss rate (*L*) in the *Tripneustes* treatment enclosures. The leaf loss rate was calculated as the average leaf production of 2 consecutive measurements minus the change in biomass over the same period and represented the sum of sea urchin herbivory (*H*) rate and export of material (*E*)

the start, 20% of the total seagrass biomass was aboveground and available for herbivory; only 8% remained in the *Tripneustes* treatments at the end of the experiment. Mobilization of stored soluble carbohydrates and nutrients from rhizomes and subsequent translocation of these nutrients among physically integrated ramets (Dawes & Lawrence 1979, Heck & Valentine 1995) may to some extent have contributed to the maintenance of leaf production, as indicated by the significant decline of C concentration in *Halodule uninervis* rhizomes at the end of the experiment.

The fast decline in seagrass aboveground biomass in the *Tripneustes* grazed treatments was comparable to the decline of *Thalassia testudinum* aboveground biomass in *Tripneustes ventricosus* grazed enclosures in the Caribbean (Keller 1983). After 38 d, *Tripneustes gratilla* grazing resulted in short vegetation with a canopy height of about 3 cm. Most leaves showed grazing marks and damaged tips (>90% of leaves damaged), so we were unable to determine whether shoots produced shorter leaves. However, this indicates that a decrease in leaf length (caused by *T. gratilla* grazing) resulted in the decline of total aboveground seagrass biomass during the experiment. Since no old leaves or litter were present in the *Tripneustes* treatments during the last 20 d of the experiment, we assumed that exports (*E*) of detached and old leaf fragments were negligible. In the absence of other grazers, this means that leaf loss rate (*L*) was similar to the urchin herbivory rate (*H*) from day 38 onwards. The herbivory rate of *T. gratilla* was 0.69 ± 0.05 g aboveground biomass urchin⁻¹ d⁻¹. This rate is about twice the average values reported from feeding experiments (Koike et al. 1987, Klumpp et al. 1993), but within the range (0.04 to 1.1 g DW urchin⁻¹ d⁻¹) reported by Koike et al. (1987).

Since *Tripneustes gratilla* is a generalist herbivore (Lawrence & Agatsuma 2001), we assume it has a compensatory feeding strategy as shown for other sea urchins (Vadas 1977, Valentine & Heck 2001). The total biomass intake of *T. gratilla* in the enclosures (0.69 ± 0.05 g DW urchin⁻¹ d⁻¹) was multiplied by the average seagrass leaf N concentration (2.3%) to calculate N intake rate. The value for *T. gratilla* (test diameter 71 mm: 16 ± 1 mg N urchin⁻¹ d⁻¹) in this study was comparable to that found by Koike et al. (1987) for *T. gratilla* (test diameter 72 mm: 13 mg N urchin⁻¹ d⁻¹) grazing on monospecific *Thalassia hemprichii* stands.

We used the principle of compensatory feeding to calculate the *Tripneustes gratilla* herbivory rate under natural conditions. Grazing by *T. gratilla* resulted in an increase in N concentration in the aboveground biomass caused by a relative increase in young leaf tissue, which has higher N concentrations (Stapel & Hemminga 1997). In the natural meadow, the average N

concentration of the aboveground seagrass biomass was 1.93 ± 0.01 % (J. Vonk unpubl. data), comparable to the control. Taking this difference into account, we calculated that for the same N intake rate as in the *Tripneustes* treatments, the grazing rate of *T. gratilla* in the natural meadow would have been 0.82 ± 0.06 g leaf urchin⁻¹ d⁻¹.

The average density of *Tripneustes gratilla* at our research location (1.55 ± 0.07 m⁻²) was comparable with densities found in other Indo-Pacific meadows (Klumpp et al. 1993, Alcoverro & Mariani 2002). Combining the observed *T. gratilla* density at the research location with the estimated grazing rate under natural conditions, we calculated an urchin population consumption rate of 1.28 g DW leaf m⁻² d⁻¹, corresponding to 26% of total aboveground seagrass production in this meadow. The relative amount of seagrass production removed by sea urchin grazing is strongly dependent on sea urchin densities measured. The amount of seagrass leaf material consumed by sea urchins varies between values corresponding to 1% (density 0.06 urchins m⁻²; Koike et al. 1987) and >100% (40 urchins m⁻²; Heck & Valentine 1995) of seagrass aboveground production. A comparable total sea urchin density, consisting of *T. gratilla* and 285 *Salmacis sphaeroides*, grazed 24% of the total aboveground production in the Philippines (Klumpp et al. 1993), comparable to our calculation.

Sea urchin grazing influences N cycling in seagrass meadows in different ways. Tropical meadows have high leaf production, low leaf lifespan and resorption of N from senescing leaves (about 15%; Stapel & Hemminga 1997) in hydrodynamically stressful environments (Koch et al. 2006). Old leaves are still photosynthetically active but become more susceptible to detachment due to mechanical stress and increased epiphyte loading. When these leaves break off from the plant, they are still buoyant and may easily be transported out of the meadow (Hemminga & Duarte 2000). Consumption by *Tripneustes gratilla* prevents this leaf material from floating away.

From the 25 mg N m⁻² d⁻¹ seagrass intake by *Tripneustes gratilla*, it is most likely that only 40% (9.9 mg N m⁻² d⁻¹) is actually removed and assimilated by *T. gratilla* (Koike et al. 1987), corresponding to about 10% of the net leaf N production (87 mg N m⁻² d⁻¹; J. Vonk unpubl. data). Of the remaining leaf N, half is excreted as inorganic N (Koike et al. 1987), mainly ammonium (Dy & Yap 2000, Lawrence & Klinger 2001) and the other part are (pseudo) faeces. The average retention time of seagrass material in the guts of the sea urchin is <2 d, providing a quite rapid process of fragmentation of seagrass leaves as compared to mechanical fragmentation by water movements (Koike et al. 1987, Valentine & Duffy 2006). Since the urchins

are slow moving and the retention time in the gut is low, the faeces with negative buoyancy (authors' unpubl. obs.) are deposited close to the location of grazing.

Herbivory by *Tripneustes gratilla* has a significant influence on species composition of tropical mixed-species meadows. Besides abiotic factors like hydrodynamics, light and nutrient availability, herbivory may be an important agent determining species composition and abundance in tropical meadows. Although the consumption of seagrass leaves by *T. gratilla* corresponds to 26% of the leaf production, probably only 10% is actually assimilated into urchin biomass. The remaining N becomes quickly available again for the meadow. This study provides support for the assumption that *T. gratilla* herbivory preserves N for the meadow and short-circuits and accelerates the N cycling of the seagrass ecosystem.

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