

Combined effects of fragmentation and herbivory on *Posidonia oceanica* seagrass ecosystems

Alessandro Gera^{1*}, Jordi F. Pagès¹, Javier Romero² and Teresa Alcoverro^{1,3}

¹Centre d'Estudis Avançats de Blanes CEAB-CSIC, C/Acc. Cala St. Francesc 14, 17300 Blanes, Girona, Spain;

²Departamento de Ecología, Facultad de Biología, Universidad de Barcelona, Av. Diagonal 645, 08028 Barcelona, Spain; and ³Nature Conservation Foundation, 3076/5, 4th Cross, Gokulam Park, 570 002 Mysore, Karnataka, India

Summary

1. Fragmentation is a major agent for seagrass meadow decline, yet little is known about how it interacts with processes like herbivory, an important functional driver of seagrass meadows. The interaction with external stressors like fragmentation could exacerbate the effects of internal ecosystem drivers like herbivory, with distinct implications for ecosystem management.

2. We used manipulative field experiments to assess these interactive effects in two *Posidonia oceanica* seagrass meadows. We monitored replicated plots in small and large patches in two meadows suffering fragmentation with and without herbivores (using exclusion cages) to test whether fragment size and herbivory could act together to alter ecosystem functioning. We measured changes in defoliation rates, primary production, canopy height and nutrient content in all plots after 4 months of herbivore exclusion.

3. Our results show that herbivores increased defoliation rates resulting in reduced primary production, nutrient content and canopy structure (canopy height). Patch size (fragment) on its own also reduced primary production, nutrient content and canopy structure. We also observed significant additive interactions between herbivores and fragmentation on canopy structure and production responses. In addition, small patches showed nutrient limitation but were able to accumulate more carbohydrate reserves, probably due to a higher light availability. This may explain why small patches can persist under significant herbivore pressure.

4. *Synthesis.* While fragmentation has already been identified as an important external agent of seagrass decline, the combination of fragmentation and herbivory can seriously exacerbate structural losses and affect primary production, profoundly compromising the role of seagrasses as habitat-forming ecosystems. These interactions between external stressors and internal drivers may result in large unexpected consequences that may flow on to the rest of the ecosystem.

Key-words: Mediterranean, *Paracentrotus lividus*, patch selection, patch size, plant responses, plant–herbivore interactions, *Posidonia oceanica*, *Sarpa salpa*, seagrass

Introduction

Ecosystems are often affected by multiple stressors, which, although not individually catastrophic, may collectively result in dramatic shifts in ecosystem function associated with the removal of habitat-specific species or functionally important attributes (Thrush *et al.* 2008). Predicting the joint impacts of co-occurring drivers is a major concern for conservation ecology because they can act independently when they influence unrelated ecosystem functions or interact if they simultaneously affect the same ecosystem functions (Sala *et al.* 2000; Didham *et al.* 2007; Brook, Sodhi & Bradshaw 2008;

Crain, Kroeker & Halpern 2008; Darling & Côté 2008; Graham *et al.* 2011). In the instance that multiple stressors (or drivers) act independently from each other, a simple additive model may be adequate to predict ecosystem responses to their co-occurrence (Sala *et al.* 2000; Halpern *et al.* 2008). If, however, multiple stressors have interactive effects, alternative (i.e. synergistic or antagonistic) models are required. The lack of knowledge about how stressors and/or drivers behave when co-occurring represents a major source of uncertainty for projections of biodiversity and ecosystem resilience (Sala *et al.* 2000; Folke *et al.* 2004). To date, there have been few studies explicitly exploring the joint effects of external stressors such as fragmentation with internal drivers such as herbivory on ecosystem functioning.

*Correspondence author. E-mail: agera@ceab.csic.es

Anthropogenic habitat fragmentation has been recognized as a major threat to plant communities and can cause significant impacts on ecosystem functioning by altering major ecosystem processes (Lienert 2004). Fragmentation changes patterns of connectivity, potentially isolating populations and communities and limiting them to suboptimal habitats (Kohn & Walsh 1994; Thrush *et al.* 2008), leading to a higher risk of local extinction due to stochastic events (Stoll *et al.* 2006). Even where local populations are highly interconnected, or connected to a mainland, if too many patches of habitat degrade, the remaining healthy ones can catastrophically collapse once a critical threshold is passed (Hughes *et al.* 2005). More perniciously, fragmentation is known to influence crucial ecosystem processes such as primary production, nutrient dynamics, pollination, plant–pathogen and intraspecific interactions in ways that may affect plant performance or population fitness (Kunin 1997; Lienert & Fischer 2003). However, few studies have explicitly examined how fragmentation interacts with herbivory (Vásquez *et al.* 2007), often one of the most significant ecosystem drivers of plant-dominated communities that is sometimes capable of triggering major shifts in ecosystem state (Heck & Valentine 2006; del-Val *et al.* 2007; Veblen & Young 2010; Veblen 2012). The evidence is equivocal; as the impact of grazing has been observed to be either offset or exacerbated in small habitat fragments, depending on the choices, herbivores make in relation to individual patches and the type of processes they affect (Simonet *et al.* 2007). From an optimal foraging perspective, mobile herbivores should be expected to abandon/selectively avoid smaller, less nutritious, putatively less profitable patches, thereby reducing herbivore pressure on these small fragments (Belisle 2005; Baguette & Van Dyck 2007; del-Val *et al.* 2007; Fáveri, Vasconcelos & Dirzo 2008; Kolb 2008; Haynes & Crist 2009). Overall, for terrestrial ecosystems, the most frequent outcome of forest fragmentation is a lowered level of herbivory (Kéry, Matthies & Fischer 2001; Simonetti *et al.* 2007; Fáveri, Vasconcelos & Dirzo 2008; Ruiz-Guerra *et al.* 2010) although the opposite has also been observed (Lienert, Diemer & Schmid 2002; Terborgh *et al.* 2006; Kolb 2008; Haynes & Crist 2009). Even if herbivores do not make selective choices between fragments, herbivory and fragmentation may still have interactive effects on ecosystem responses as fragmentation reduces a patch's ability to withstand the impacts of herbivory (Crain, Kroeker & Halpern 2008).

In marine systems, seagrass meadows are the most important structural habitat on sandy bottoms, making major contributions to coastal primary production and nutrient dynamics, and are a critical ecosystem for several associated species as well as serving an important nursery role for surrounding ecosystems as well (Hemminga, Harrison & Van Lent 1991; Hemminga & Duarte 2000; Boström, Jackson & Simenstad 2006). A raft of recent research has established herbivory as one of the most important drivers of seagrass communities (Heck & Valentine 2006; Unsworth *et al.* 2007; Planes *et al.* 2011; White, Westera & Kendrick 2011; Christianen *et al.* 2012). Fragmentation *per se* is also an important external

stressor in seagrass ecosystems, and much of the existing literature focuses on how seagrass-associated species respond to this process (Eggleston *et al.* 1999; Frost, Rowden & Attrill 1999; Hovel & Lipcius 2001; Vega Fernández *et al.* 2005; Johnson & Heck 2006; Reed & Hovel 2006; Hirst & Attrill 2008; Mills & Berkenbusch 2009; Borg *et al.* 2010; Boström *et al.* 2011; Arponen & Boström 2012). In addition, authors have discussed the seascape-level impacts of meadow fragmentation on gap dynamics and patch shape (Vidondo *et al.* 1997; Bell, Robbins & Jensen 1999; Ramage & Schiel 1999; Borg *et al.* 2005; Diaz-Almela *et al.* 2008). However, as these ecosystems become increasingly vulnerable to multiple anthropogenic stressors, including eutrophication, coastal development and fish trawling among others (Duarte & Sand-Jensen 1990; Cebrián *et al.* 1996; Duarte 2002; Sleeman *et al.* 2005; Reed & Hovel 2006; Diaz-Almela *et al.* 2008; Prado *et al.* 2008b; Planes *et al.* 2011), a high percentage of shallow seagrass meadows, at least in the N-W Mediterranean, are becoming increasingly patchy (Alcoverro *et al.* 2012), making fragmentation one of the biggest challenges for extant seagrass ecosystems (Frederiksen *et al.* 2004; Sleeman *et al.* 2005).

This study was designed to determine the combined effects of seagrass fragmentation and herbivory. We took advantage of two *Posidonia oceanica* seagrass meadows suffering a sustained reduction in patch size (Alcoverro *et al.* 2012). Together with increased patch isolation, edge effect and within-patch habitat degradation, patch size reduction is one of the most important effects of fragmentation. We used cage-controlled experiments in those two meadows to first evaluate how fish herbivores select patch size and then to examine plant responses (canopy structure, primary production and nutrient content) to herbivory in small and large patches.

Materials and methods

STUDY SITE AND EXPERIMENTAL DESIGN

This study was designed to test whether patch size, herbivory and their combined effects can trigger changes in seagrass functional responses. We first evaluated whether herbivores showed any size-based patch selection by measuring the impact of herbivory on different-sized seagrass fragments (Objective 1). In addition, we evaluated the impacts of increasing fragmentation (addressing specifically the effects of reduction in fragment size), herbivory and their joint effects on essential seagrass functional responses: primary production, nutrient dynamics and seagrass meadow structure (Objective 2). We tested both objectives with a mixed-effects experiment conducted in small and large seagrass patches, with and without (using exclusion cages) herbivores. To increase the generality of our results, we reproduced this protocol in two distinct seagrass meadows (Site 1: Fenals 41°41.3' N, 002°49.7' E; and Site 2: Giverola 41°44.2' N, 002°57.3' E Spain, NW Mediterranean) that have been subjected to major storm-related fragmentation events in the last decade (Alcoverro *et al.* 2012). Both sites were selected to represent the most common distinct fragmented meadows in that area. Fenals (Site 1) is a meadow, which is fully exposed to most storms and winds; Giverola (Site 2) is inside a small bay mainly protected from predominant winds. At each site,

we identified 10 small (area $\leq 1 \text{ m}^2$) and 10 large (area $\geq 5 \text{ m}^2$) seagrass fragments. We followed this sampling strategy to capture as much spatial variability between fragments as possible, since within-patch variability is considerably lower than between-fragment variation (Short & Duarte 2001). Both meadows are dominated by the seagrass *P. oceanica*, the most important benthic primary producer in the Mediterranean (Cebrián *et al.* 1996). The two main herbivores of this seagrass system are the sparid fish *Sarpa salpa* and the sea urchin *Paracentrotus lividus* (Boudouresque & Verlaque 2001), although the pressure of the former is predominant, consuming on average more than 40% of the primary production (Prado *et al.* 2007). Experimental fieldwork was conducted within the depth range and season (i.e. 5–8 m depth, from June to September 2010) of maximum herbivore activity for both species (Boudouresque & Verlaque 2001; Prado *et al.* 2007).

At each site, five small fragments and five large meadow fragments were randomly selected and subjected to natural grazing intensities, representing a fragmentation and herbivory effect (Small patch + Herbivores) and an herbivory effect alone (Large patch + Herbivores), respectively. Herbivores were excluded from the remaining fragments to represent a fragmentation (patch size) effect (Small patch + No Herbivores) and a control effect (Large patch + No Herbivores). At these fragments, we first extracted all sea urchins and then set up exclusion cages from June to the end of September to restrict herbivore access. The cages measured $1.5 \times 1.5 \text{ m}$ in area and 1 m high and were marked with 1.5 m high iron bars at each corner to which green plastic mesh fences (2-cm mesh size) were attached. The roofs consisted of fine transparent fishing net (3-cm mesh size). The caged patches were visually checked to ensure that no fish or sea urchins entered below the fences. Every 2 weeks, SCUBA divers cleaned the cages with brushes in order to reduce potential cage-induced light-reduction artefacts. To test for possible cage effects, we compared above canopy light availability inside and outside the cages. Four HOBOware pro light sensors (Onset Computer Corporation, Cape Cod, Massachusetts, USA: Apogee, Amplified Quantum Sensor, model SQ-200) with a 10-min reading interval were deployed for 2 weeks at both sites (Site 1 and Site 2) inside and outside the cages. The results showed no significant differences between treatments (*t*-test, caged vs. uncaged light values, $P = 0.5238$). Additionally to avoid edge effects caused by the cage, we restricted all sampling to the central area of the plot (Planes *et al.* 2011).

We measured the following variables at each fragment: herbivore pressure and type of herbivore mark (Objective 1) and the response variables of the system: leaf primary production, rhizome nitrogen and carbohydrate content, shoot density and canopy height (Objective 2).

Herbivore pressure

We assessed shoot defoliation and herbivore type (fish or sea urchin) in July when herbivory is at its seasonal maximum (Prado *et al.* 2007). At the beginning of July, SCUBA divers marked 20 shoots per treatment (two shoots per plot, five plots, two sites, 2×2 treatments) for which we counted the initial number of leaves, the initial length and the state of the apical part of each leaf (broken, eaten by fish, eaten by sea urchin or intact). Both herbivores leave bite marks of distinctly identifiable shapes (Boudouresque & Meinesz 1982; Prado *et al.* 2007). Leaf defoliation (shoot herbivory, $\text{cm shoot}^{-1} \text{ day}^{-1}$) was estimated for each of the collected shoots after 15 days by adding leaf elongation to the initial length and subtracting this total from the final leaf length (Prado *et al.* 2007), finally divided by the number of days elapsed since marking. Only leaves

that had clear herbivore marks were assigned to herbivory, and the rest discarded to avoid herbivory overestimates (Prado *et al.* 2007). Obviously, the measurement of herbivore pressure only makes sense in uncaged plots, although we measured it in all plots to confirm the absence of herbivore activity in caged plots.

Seagrass canopy structure

Canopy height (cm) was also estimated in each plot during the period of maximum herbivore activity (July) by measuring the height of the canopy (vertical distance between the substrate and the top of the standing leaves) in two different areas of the plot. Shoot density (shoots m^{-2}) was measured at the end of the experimental period (September) using two haphazardly placed quadrats ($40 \times 40 \text{ cm}$) for each fragment.

Primary production

Leaf primary production was measured at the end of the experiment (September) to account for the integration of herbivore pressure throughout the treatment. At the beginning of September, SCUBA divers marked two shoots per plot. In each shoot, we marked the base of the leaves (piercing the leaf with a syringe needle) to measure leaf elongation rates, using a modified method from Zieman (1974). 15 days later, we collected all marked shoots and measured for each leaf the new leaf tissue produced (between the pierced mark and the ligula) per shoot. Leaf elongation rate ($\text{cm}^2 \text{ shoot}^{-1} \text{ day}^{-1}$) of pierced shoots was determined by dividing the area of new tissue produced by the number of days elapsed since marking.

Nitrogen and carbohydrate content

Rhizome nitrogen (%N) and total nonstructural carbohydrates (%NSC) were analysed at the end of the experiment (September). To obtain adequate rhizome biomass for those analyses, we pooled 2 shoots per plot. The top 2 cm of each rhizome was dried at 70°C for 48 h and ground to a fine powder. Rhizome nitrogen concentration was measured using an Elemental Analyzer FlashEA1112 (Thermo-Finnigan Unidade de Técnicas Instrumentais de Análise, Universidade de Coruña). Total nonstructural carbohydrates (NSC, sucrose and starch) were also analysed for the same dried rhizomes and replicates using a modified method from Alcoverro, Manzanera & Romero (2001). Ground rhizomes were dissolved in 96% (v/v) ethanol and heated at 80°C for 15 min to extract sucrose; this process was repeated three times. Starch was extracted from the ethanol-insoluble remnant pellet by dissolving it in 0.1 NaOH and incubating it for 24 h at room temperature. Sucrose and starch content were determined spectrophotometrically using an anthrone assay with sucrose as standard (Alcoverro, Manzanera & Romero 2001). Sucrose and starch content were combined as nonstructural carbohydrate content (NSC).

STATISTICAL ANALYSIS

To test for 'patch size' (F), 'herbivory' (H) (hereinafter called drivers for the sake of simplicity) and their combined effects on response variables, we used a 3-way mixed-effects ANOVA design. We considered 'site' (St; two levels: Site 1, Site 2), 'patch size' (F; two levels: Large, Small), 'herbivory' (H; two levels: Herbivores, No Herbivores) and their interactions ($\text{St} \times \text{F}$, $\text{St} \times \text{H}$, $\text{F} \times \text{H}$ and $\text{St} \times \text{F} \times \text{H}$) as fixed factors for %N and %NSC response variables. For the other

response variables (i.e. herbivore pressure – only the plots exposed to herbivores – canopy height, primary production and shoot density), an additional random factor ‘plot’, nested within the interaction of the others ($St \times F \times H$), was added. Site was considered a fixed factor because both meadows displayed distinct features and were deliberately chosen to represent the most common typologies of fragmented meadows within the area. Whenever an ANOVA was significant, a multiple range contrast test was applied (Tukey’s HSD [honestly significant difference]) to determine differences among treatments. All the ANOVA analyses were performed in Statistica 8 software (StatSoft, Inc. Tulsa, OK, USA). Assumptions of normality (K-S & Lilliefors and Shapiro–Wilk’s tests) and homogeneity of variances (Cochran’s test) necessary for the ANOVA were not always met despite transformation. Nonetheless, Underwood (Underwood 1981) has indicated that the ANOVA F statistic is robust despite these violations of assumptions, provided that the working sample size is large enough. When such assumptions were not met, we set the significance level to $P < 0.01$ to minimize the risk of making a type I error.

The relationship between rhizome nutrient content and nonstructural carbohydrates with patch area was further explored using a linear regression model in R (R Development Core Team 2010).

Results

HERBIVORE PRESSURE

Herbivore pressure was not significantly different between differently sized patches, that is, we found similar defoliation rates independently of patch size (Fig. 1a and Table 1). The fish *S. salpa* accounted for 62% of the total grazing pressure across both sites, with the sea urchin *P. lividus* accounting for the rest.

CANOPY HEIGHT

The canopy height of plots exposed to both drivers (Small patch + Herbivores: 39 ± 3 cm, Fig. 1b) was 39%, significantly lower (see the interaction $St \times F \times H$ in Table 1) than

the plots without drivers (Large patch + No Herbivores: 64 ± 3 cm, Fig. 1b), suggesting additive effects between them on this meadow attribute. Both herbivory and fragmentation on their own had also significant effects (Table 1) in reducing canopy height relative to plots without drivers (Large patch + No Herbivores, Fig. 1b). Site and plot also showed significant effects for this variable (Table 1).

PRIMARY PRODUCTION

Primary production responded significantly to herbivory and fragmentation effects (Table 1, Fig. 1c). Herbivory alone caused a 32% decrease (comparing production on Large patch + Herbivores: 0.39 ± 0.04 cm² shoot⁻¹ day⁻¹ relative to Large patch + No Herbivores: 0.57 ± 0.04 cm² shoot⁻¹ day⁻¹), and fragmentation caused a 42% reduction in seagrass primary production (comparing production on Small patch + No Herbivores: 0.33 ± 0.04 cm² shoot⁻¹ day⁻¹ relative to Large patch + No Herbivores). Both drivers together (Small patch + Herbivores: 0.23 ± 0.04 cm² shoot⁻¹ day⁻¹) showed an additive effect that resulted in a 60% decrease in production relative to controls (Large patch + No Herbivores, Fig. 1c). Plot also showed significant effects for this variable (Table 1).

NUTRIENT CONTENT

Nitrogen in the rhizomes responded significantly to fragmentation alone (Small patch + No Herbivores: $1.05 \pm 0.14\%$) with a 43% decrease relative to control plots (Large patch + No Herbivores: 1.85 ± 0.14 %N) but not to herbivory (Fig. 1d and Table 1), evidencing a nutrient limitation of the small fragments. Herbivory and fragmentation acting together (i.e. in Small patch + Herbivores) did not produce an additive effect on this variable, since the N content decrease observed in grazed patches was similar to that found in patches with fragmentation alone (Small patch + No Herbivores). Site also showed significant effects for this variable (Table 1).

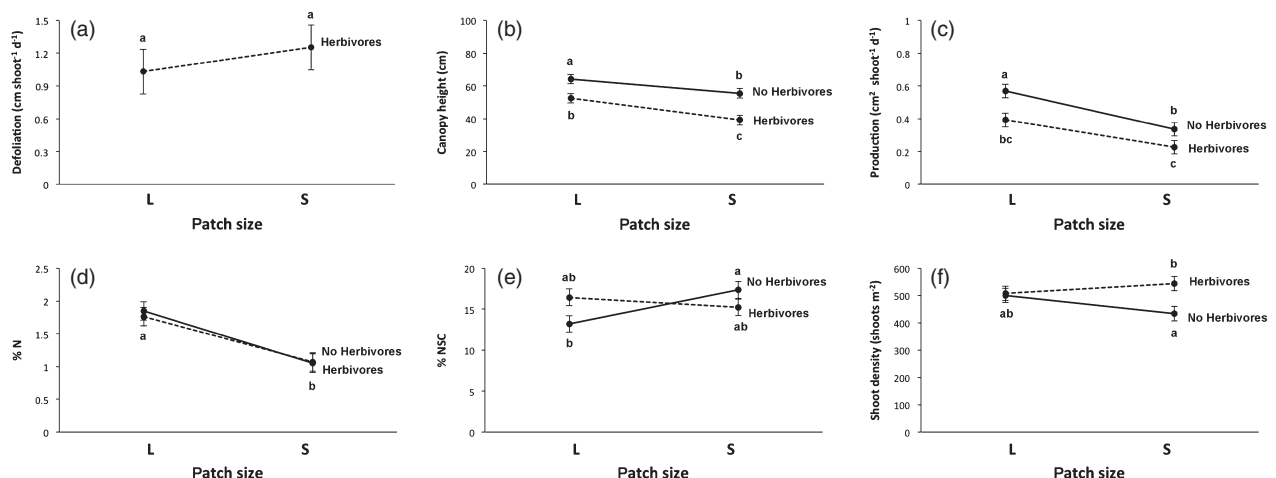


Fig. 1. Individual and combined effects of patch size (two levels: large (L) and small (S)) and herbivory (two levels: herbivores present (dotted line) and no herbivores (caged plots, continuous line)) for each of the response variables (mean \pm SE): herbivore pressure (a), canopy height (b), primary production (c), %nitrogen (d), % total nonstructural carbohydrates (NSC) (e) and shoot density (f). Values labelled with the same lower case letter do not differ significantly according to Tukey’s HSD *post hoc* test.

Table 1. Summary of the different anova analyses performed. *P*-values correspond to those provided by an *F*-test. For the physiological response variables (%N and %NSC), the effects of site (St), patch size (F), herbivory (H) and their interactions were tested. For the other response variables (herbivore pressure, canopy height, primary production and shoot density) in addition to the aforesaid factors, plot was considered a random factor nested within (St \times F \times H). Primary production was square root transformed to meet anova assumptions, but for herbivore pressure, the assumptions were not met after transformations, and we set the significance level to $P < 0.01$ to minimize the risk of making a type I error

Herbivory pressure					%Nitrogen				
Source of variation	d.f.	Sum of square	F	<i>P</i>	Source of variation	d.f.	Sum of square	F	<i>P</i>
St	1	2.204	2.000	0.173	St	1	5.162	25.783	< 0.001
F	1	0.491	0.445	0.512	F	1	5.558	27.757	< 0.001
St \times F	1	0.491	0.445	0.512	H	1	0.011	0.056	0.814
Plot (St \times F)	16	2.663	2.416	0.032	St \times F	1	0.367	1.832	0.185
Error	20	22.041	—	—	St \times H	1	0.131	0.655	0.424
					F \times H	1	0.028	0.138	0.713
					St \times F \times H	1	0.068	0.340	0.564
					Error	32	6.407	—	—

Canopy					%NSC				
Source of variation	d.f.	Sum of square	F	<i>P</i>	Source of variation	d.f.	Sum of square	F	<i>P</i>
St	1	9926.273	225.030	< 0.001	St	1	19.853	1.926	0.175
F	1	2303.290	52.216	< 0.001	F	1	21.025	2.040	0.163
H	1	3485.934	79.027	< 0.001	H	1	3.181	0.309	0.582
St \times F	1	104.497	2.369	0.131	St \times F	1	45.156	4.382	0.044
St \times H	1	32.633	0.740	0.395	St \times H	1	4.422	0.429	0.517
F \times H	1	146.446	3.320	0.076	F \times H	1	70.756	6.866	0.013
St \times F \times H	1	805.120	18.252	< 0.001	St \times F \times H	1	43.723	4.243	0.048
Plot (St \times F \times H)	32	413.724	9.379	< 0.001	Error	32	329.768	—	—
—	—	—	—	—	—	—	—	—	—
Error	40	1852.7	—	—	—	—	—	—	—

Production					Shoot density				
Source of variation	d.f.	Sum of square	F	<i>P</i>	Source of variation	d.f.	Sum of square	F	<i>P</i>
St	1	0.067	3.530	0.068	St	1	43 950	8.768	0.005
F	1	0.792	41.554	< 0.001	F	1	6127	1.222	0.276
H	1	0.409	21.458	< 0.001	H	1	64 690	12.905	0.001
St \times F	1	0.043	2.269	0.140	St \times F	1	45 716	9.120	0.004
St \times H	1	0.001	0.044	0.834	St \times H	1	9301	1.855	0.181
F \times H	1	0.024	1.249	0.270	F \times H	1	57 117	11.394	0.002
St \times F \times H	1	0.058	3.060	0.088	St \times F \times H	1	5697	1.137	0.293
Plot (St \times F \times H)	32	0.051	2.663	0.002	Plot (St \times F \times H)	32	28 710	5.727	< 0.001
Error	40	0.762	—	—	Error	40	200 508	—	—

d.f., degree of freedom; NSC, nonstructural carbohydrates. Bold values indicate $P < 0.05$.

TOTAL NONSTRUCTURAL CARBOHYDRATES

When both herbivory and fragmentation were acting together, total nonstructural carbohydrates in rhizomes (%NSC) increased (Small patch + Herbivores: $15.2 \pm 1.0\%$, Fig. 1e) compared with the control plots (Large patch + No Herbivores: $13.2 \pm 1.0\%$), showing a significant interaction (Table 1). This was mainly due to the important increase caused by fragmentation (Small patch + No Herbivores: $17.3 \pm 1.0\%$). The interaction between Site and fragmentation was also significant (see St \times F in Table 1).

SHOOT DENSITY

Shoot density increased when both drivers were acting together (Small patch + Herbivores: 544 ± 26 shoots m^{-2} , see Fig. 1f), mainly due to their significant interaction (F \times H, see Table 1). In contrast, shoot density decreased significantly when fragmentation acted without herbivory (Small patch + No Herbivores: 433 ± 26 shoots m^{-2}), but there were no significant differences between the plots without drivers (Large patch + No Herbivores) and all the other treatments (Fig. 1f). Site and plot also showed significant effects for this variable (Table 1).

RELATIONSHIP BETWEEN VARIABLES

Patch area and percentage of nitrogen in the rhizomes showed a significant ($P < 0.01$) linear regression ($n = 40$, $R^2 = 0.6738$), suggesting a possible plant limitation by nitrogen availability with decreasing patch size (Fig. 2). Additionally, %N and %NSC showed a significant ($P < 0.01$) negative linear relationship ($n = 40$, $R^2 = 0.219$, Fig. 3).

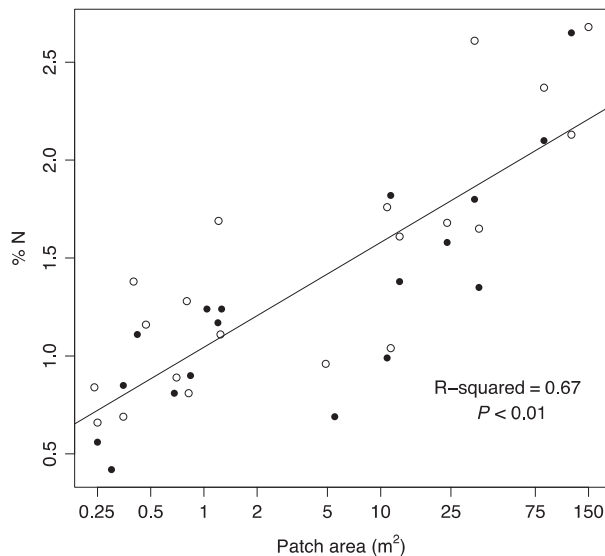


Fig. 2. Linear regression showing a significant relationship between patch size (log transformed) and the nitrogen content (%N) of *Posidonia oceanica* rhizomes taken at the end of the experiment ($n = 40$). Full circles (●) indicate plots where herbivores were present, while empty circles (○) indicate caged plots without herbivores.

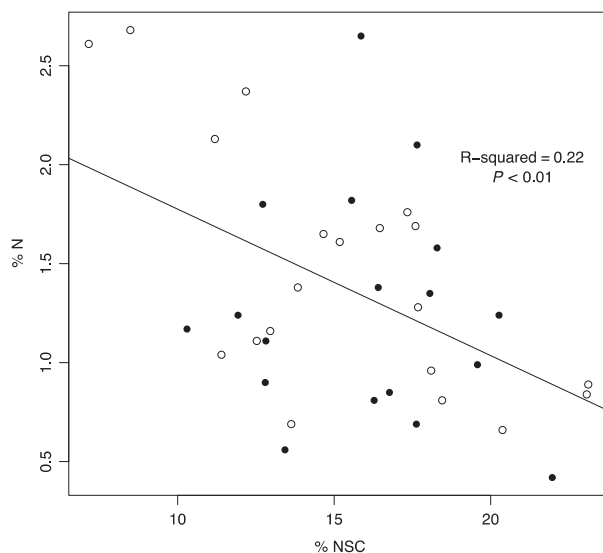


Fig. 3. Linear regression showing a significant relationship between the nitrogen content (%N) and the total nonstructural carbohydrates (% NSC) of *Posidonia oceanica* rhizomes taken at the end of the experiment ($n = 40$). Full circles (●) indicate plots where herbivores were present, while empty circles (○) indicate caged plots without herbivores.

Discussion

As expected, herbivory alone increased seagrass defoliation rates that were, interestingly, sufficient to cause reduced primary production and canopy height in seagrass meadows. Patch size did not change herbivore pressure, but resulted in very similar trends, also reducing primary production, canopy height and nutrient content. What our results suggest, however, is that when meadows are exposed to both drivers together, the effects on some meadow attributes are even larger than in isolation. In particular, although patch size does not appear to modify the way herbivores graze across the landscape, the joint effects of herbivory and fragmentation result in significantly reduced canopies in smaller patches. Seagrasses in small fragments showed reduced primary production and nutrient content, probably the cause of reduced canopies in the absence of herbivores. Despite this deterioration in plant conditions, shoot density did not reflect these changes, which may even increase under herbivory because of reduced self-shading (Vergés *et al.* 2008; Planes *et al.* 2011). However, the severe (39%) structural reduction in canopy height of small patches might compromise their functional role. The prevalence of joint effects between fragmentation and herbivory strongly suggests that predictions based exclusively on a single driver will underestimate their ecological impact on seagrass meadows.

The way consumers interact with their landscape for resource acquisition can have major implications for ecosystem function, particularly when the resource is also a major structural element of the ecosystem. Habitat fragmentation results in increasing patchiness and can seriously modify the way consumers use the landscape mosaic. Optimal foraging theory assumes that natural selection will favour the development of feeding preferences that will maximize the net caloric intake per individual and unit time (Emlen 1966; MacArthur & Pianka 1966). Adult sea urchins may have a limited ability to move between patches, but even without accounting for movement, differential herbivore consumption rates may still arise as a function of differences in nutrient content between different-sized patches. Other studies have demonstrated that sea urchins resort to compensatory feeding in less nutritious patches, increasing their consumption of leaves to meet their nutritional requirements (Valentine & Heck 2001). Our results indicate, in contrast, that *P. lividus* did not show any compensatory feeding and had similar rates of herbivory in small and large fragments despite the different nitrogen content these patches had. In the case of mobile herbivores, ecological theory predicts that they should abandon smaller, putatively less nutritious and unprofitable patches (Kolb 2008); this behaviour has been repeatedly observed in several terrestrial systems where, for instance, insects tend to select larger patches for feeding (Lienert, Diemer & Schmid 2002; Kolb 2008; Haynes & Crist 2009). The mobile marine herbivores in our study system, however, appeared indifferent to patch size despite the higher nitrogen content and nutritional value of larger fragments (Fig. 2). The mechanisms underlying the feeding preferences of *S. salpa*, the only herbivore that is able

to move between patches, are not yet completely understood although it has been observed that *S. salpa* counter-intuitively prefer less nutritive, more chemically defended plant tissues (Vergés *et al.* 2008). Where food selection as a function of plant nutrient content has been observed, this trend has more to do with epiphyte composition rather than the properties of the leaf tissue itself (Prado, Alcoverro & Romero 2010). Moreover, habitat-generalist species such as *S. salpa* (Prado *et al.* 2007) are potentially less likely to display positive density–area relationships than habitat-specialists (Hambäck *et al.* 2007). Additionally, this fish travels in schools that can often reach hundreds of individuals feeding in the same area (Raventos, Ferrari & Planes 2009); even if these feeding schools showed no patch selection across the mosaic, herbivory impacts could accrue more dramatically in the smallest seagrass patches compared with larger fragments.

Despite the lack of patch selection by herbivores, the fact that fragments were subjected to similar herbivory rates independent of their size resulted in major habitat-level consequences. Herbivory on small patches resulted in rates of primary production 44% lower than in larger patches exposed to the same levels of herbivory. Herbivory by the sparid fish *S. salpa* can be very intense in seagrass ecosystems with rates as high as 40% of primary production on an annual basis (Prado *et al.* 2007), becoming even higher in Marine Protected Areas (Prado *et al.* 2008b), where it can seriously compromise sexual reproduction in *P. oceanica* (Planes *et al.* 2011). Fish herbivory does not merely affect plant primary production but can also substantially alter canopy structure, as observed in this study, with important flow-on consequences for the rest of the ecosystem. The impact of herbivores on canopy height has been observed to mediate predator–prey interactions by decreasing refuges, thus increasing predation rates which can ultimately cause the disappearance of important functional species (Pagès *et al.* 2012). The joint effect of herbivory and fragmentation observed in this study could further exacerbate these effects with unknown consequences for the rest of the meadow community.

Our results show that reduction in fragment size altered primary production in small fragments. This is probably related to nitrogen limitation, as observed by the lower concentration of nitrogen in smaller patches (Fig. 2). The underlying mechanism may be linked to a higher export of autochthonous primary production in small fragments compared with larger ones, or an enhanced clonal integration in larger fragments (Prado, Collier & Lavery 2008a). Conversely, small patches accumulate more carbon reserves in rhizomes than larger ones, a mechanism that has already been observed under conditions of nitrogen limitation (Invers *et al.* 2004). The higher percentage of carbohydrates observed in these smaller fragments could be the result of less self-shading and a consequent increase in light availability (Burke, Dennison & Moore 1996; Hamilton *et al.* 2001). In fact, the correlation between carbohydrates and nitrogen (Fig. 3) also points to a possible nutrient limitation given the low nutrient content observed in this work when compared with plants under nitrogen limitation (Duarte 1990). Similar effects have been detected in

terrestrial ecosystems when habitat fragmentation imposes nutrient limitations and poor physical conditions in small patches, affecting the survival of nonmobile herbivores due to the change in abundance of food and the risk of predation (Villafuerte, Litvaitis & Smith 1997).

Despite the profound effects that herbivores have on small patches by indirectly reducing patch biomass, the plant appears to be able not merely to resist but also to partially compensate for these combined stressors. In effect, *P. oceanica* shoot density in small fragments subjected to herbivory was maintained at values similar to the controls indicating that the clonal growth was not limited by the biomass lost to herbivores and fragmentation effects, even though the ecosystem itself accrues significant impacts (see previous paragraph). However, the effects of herbivory and reduction in patch size on such a conservative structural parameter (i.e. shoot density) of a particularly slow-growing species may not be visible in the short term (4 months). These results add to a growing body of evidence showing that *P. oceanica* apparently has evolved several mechanisms to compensate for herbivore pressure including compensatory growth, increased clonal growth and increased nutrient translocation from senescent leaves (Vergés *et al.* 2008; Planes *et al.* 2011). This high tolerance to herbivory is probably the result of the co-evolution of the plant with important and even more damaging herbivores in the past (Planes *et al.* 2011). It is, in fact, well recognized that seagrasses, like their terrestrial counterparts, resist high herbivory with a series of adaptations such as inaccessible basal meristems, branching rhizomes that enhance resistance to grazing and investment in below-ground reserves (Valentine *et al.* 1997; Valentine & Heck 1999). In practical terms, the fact that *P. oceanica*, an important ecosystem engineer, responds to fragmentation (specifically, to reduction in patch size) and herbivory with a smaller change than expected in primary production, nutrient content and population dynamics indicates that these combined drivers may be much less damaging at least in terms of plant functional survival. This response may explain why very small patches can continue to survive for several decades (unpublished personal observations and Alcoverro *et al.* 2012).

The interaction of drivers can make ecosystems more vulnerable to change (Folke *et al.* 2004). Our results point to the importance of understanding how environmental stressors modify key internal ecosystem processes since they may interact in potentially surprising ways, not entirely predictable by merely knowing how the system responds to each individually (Crain, Kroeker & Halpern 2008). Unlike internal processes, external stressors like anthropogenic fragmentation are not ecosystem dependent. While external stressors may on their own modify just a few key attributes of the system, their ability to modify internal ecosystem processes may set in motion major functional changes to the system that the disturbance alone may not directly cause. In the example of the seagrass meadows, the plant seems to cope adequately with internal drivers like herbivore pressure thanks to their evolutionary adaptations. However, the introduction of external stressors like fragmentation into the system can have far