

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

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4 Running headline: Patch size and herbivory effects

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7 **Summary**

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

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

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

- 29 4. *Synthesis*. While fragmentation has already been identified as an important
30 external agent of seagrass decline, the combination of fragmentation and
31 herbivory can seriously exacerbate structural losses and affect primary
32 production, profoundly compromise the role of seagrasses as habitat-forming
33 ecosystems. These interactions between external stressors and internal

34 drivers may result in large unexpected consequences that may flow on to the
35 rest of the ecosystem.

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

40

41 **Introduction**

42

43 Ecosystems are often affected by multiple stressors, which, although not
44 individually catastrophic, may collectively result in dramatic shifts in ecosystem
45 function associated with the removal of habitat-specific species or functionally
46 important attributes (Thrush *et al.* 2008). Predicting the joint impacts of co-occurring
47 drivers is a major concern for conservation ecology because they can act
48 independently when they influence unrelated ecosystem functions, or interact if they
49 simultaneously affect the same ecosystem functions (Sala *et al.* 2000; Didham *et al.*
50 2007; Brook, Sodhi & Bradshaw 2008; Crain, Kroeker & Halpern 2008; Darling &
51 Côté 2008; Graham *et al.* 2011). In the instance that multiple stressors (or drivers)
52 act independently from each other, a simple additive model may be adequate to
53 predict ecosystem responses to their co-occurrence (Sala *et al.* 2000; Halpern *et al.*
54 2008). If, however, multiple stressors have interactive effects, alternative (i.e.
55 synergistic or antagonistic) models are required. The lack of knowledge about how
56 stressors and/or drivers behave when co-occurring represents a major source of
57 uncertainty for projections of biodiversity and ecosystem resilience (Sala *et al.* 2000;
58 Folke *et al.* 2004). To date, there have been few studies explicitly exploring the joint
59 effects of external stressors such as fragmentation with internal drivers such as
60 herbivory on ecosystem functioning.

61 Anthropogenic habitat fragmentation has been recognized as a major threat to
62 plant communities and can cause significant impacts on ecosystem functioning by
63 altering major ecosystem processes (Lienert 2004). Fragmentation changes patterns
64 of connectivity, potentially isolating populations and communities and limiting them to
65 suboptimal habitats (Kohn & Walsh 1994; Thrush *et al.* 2008), leading to a higher risk
66 of local extinction due to stochastic events (Stoll *et al.* 2006). Even where local
67 populations are highly interconnected, or connected to a mainland, if too many
68 patches of habitat degrade, the remaining healthy ones can catastrophically collapse

69 once a critical threshold is passed (Hughes *et al.* 2005). More perniciously,
70 fragmentation is known to influence crucial ecosystem processes such as primary
71 production, nutrient dynamics, pollination, plant–pathogen and intraspecific
72 interactions in ways that may affect plant performance or population fitness (Kunin
73 1997; Lienert & Fischer 2003). However, few studies have explicitly examined how
74 fragmentation interacts with herbivory (Vásquez *et al.* 2007), often one of the most
75 significant ecosystem drivers of plant-dominated communities that is sometimes
76 capable of triggering major shifts in ecosystem state (Heck & Valentine 2006; del-Val
77 *et al.* 2007; Veblen & Young 2010; Veblen 2012). The evidence is equivocal; as the
78 impact of grazing has been observed to be either offset or exacerbated in small
79 habitat fragments, depending on the choices herbivores make in relation to individual
80 patches and the type of processes they affect (Simonetti *et al.* 2007). From an
81 optimal foraging perspective, mobile herbivores should be expected to
82 abandon/selectively-avoid smaller, less nutritious, putatively less profitable patches,
83 thereby reducing herbivore pressure on these small fragments (Belisle 2005;
84 Baguette & Van Dyck 2007; del-Val *et al.* 2007; Fáveri, Vasconcelos & Dirzo 2008;
85 Kolb 2008; Haynes & Crist 2009). Overall, for terrestrial ecosystems, the most
86 frequent outcome of forest fragmentation is a lowered level of herbivory (Kéry,
87 Matthies & Fischer 2001; Simonetti *et al.* 2007; Fáveri, Vasconcelos & Dirzo 2008;
88 Ruiz-Guerra *et al.* 2010) although the opposite has also been observed (Lienert,
89 Diemer & Schmid 2002; Terborgh *et al.* 2006; Kolb 2008; Haynes & Crist 2009).
90 Even if herbivores do not make selective choices between fragments, herbivory and
91 fragmentation may still have interactive effects on ecosystem responses as
92 fragmentation reduces a patch’s ability to withstand the impacts of herbivory (Crain,
93 Kroeker & Halpern 2008).

94 In marine systems, seagrass meadows are the most important structural habitat
95 on sandy bottoms, making major contributions to coastal primary production and
96 nutrient dynamics and are a critical ecosystem for several associated species as well

97 as serving an important nursery role for surrounding ecosystems as well (Hemminga,
98 Harrison & Van Lent 1991; Hemminga & Duarte 2000; Boström, Jackson &
99 Simenstad 2006). A raft of recent research has established herbivory as one of the
100 most important drivers of seagrass communities (Heck & Valentine 2006; Unsworth
101 *et al.* 2007; Planes *et al.* 2011; White, Westera & Kendrick 2011; Christianen *et al.*
102 2012). Fragmentation per se is also an important external stressor in seagrass
103 ecosystems and much of the existing literature focuses on how seagrass-associated
104 species respond to this process (Eggleston *et al.* 1999; Frost, Rowden & Attrill 1999;
105 Hovel & Lipcius 2001; Vega Fernández *et al.* 2005; Johnson & Heck 2006; Reed &
106 Hovel 2006; Hirst & Attrill 2008; Mills & Berkenbusch 2009; Borg *et al.* 2010; Boström
107 *et al.* 2011; Arponen & Boström 2012). In addition, authors have discussed the
108 seascape-level impacts of meadow fragmentation on gap dynamics and patch shape
109 (Vidondo *et al.* 1997; Bell, Robbins & Jensen 1999; Ramage & Schiel 1999; Borg *et al.*
110 *et al.* 2005; Diaz-Almela *et al.* 2008). However, as these ecosystems become
111 increasingly vulnerable to multiple anthropogenic stressors, including eutrophication,
112 coastal development and fish trawling among others (Duarte & Sand-Jensen 1990;
113 Cebrián *et al.* 1996; Duarte 2002; Sleeman *et al.* 2005; Reed & Hovel 2006; Diaz-
114 Almela *et al.* 2008; Prado *et al.* 2008; Planes *et al.* 2011), a high percentage of
115 shallow seagrass meadows, at least in the N-W Mediterranean, are becoming
116 increasingly patchy (Alcoverro *et al.* 2012), making fragmentation one of the biggest
117 challenges for extant seagrass ecosystems (Frederiksen *et al.* 2004; Sleeman *et al.*
118 2005).

119 This study was designed to determine the combined effects of seagrass
120 fragmentation and herbivory. We took advantage of two *Posidonia oceanica*
121 seagrass meadows suffering a sustained reduction in patch size (Alcoverro *et al.*
122 2012). Together with increased patch isolation, edge effect and within-patch habitat
123 degradation, patch size reduction is one of the most important effects of
124 fragmentation. We used cage-controlled experiments in those two meadows to firstly

125 evaluate how fish herbivores select patch size and then to examine plant responses
126 (canopy structure, primary production and nutrient content) to herbivory in small and
127 large patches.

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130 **Materials and methods**

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132 **Study site and experimental design**

133 This study was designed to test if patch size, herbivory and their combined effects
134 can trigger changes in seagrass functional responses. We first evaluated if
135 herbivores showed any size-based patch selection by measuring the impact of
136 herbivory on different sized seagrass fragments (Objective 1). In addition, we
137 evaluated the impacts of increasing fragmentation (addressing specifically the effects
138 of reduction in fragment size), herbivory and their joint effects on essential seagrass
139 functional responses: primary production, nutrient dynamics and seagrass meadow
140 structure (Objective 2). We tested both objectives with a mixed-effects experiment
141 conducted in small and large seagrass patches, with and without (using exclusion
142 cages) herbivores. To increase the generality of our results, we reproduced this
143 protocol in two distinct seagrass meadows (Site 1: Fenals 41°41.3" N, 002°49.7' E;
144 Site 2: Giverola 41°44.2' N, 002°57.3' E Spain, NW Mediterranean) that have been
145 subjected to major storm-related fragmentation events in the last decade (Alcoverro
146 *et al.* 2012). Both sites were selected to represent the most common distinct
147 fragmented meadows in that area. Fenals (Site 1) is a meadow, which is fully
148 exposed to most storms and winds; Giverola (Site 2) is inside a small bay mainly
149 protected from predominant winds. At each site, we identified 10 small ($\text{area} \leq 1\text{m}^2$)
150 and 10 large ($\text{area} \geq 5\text{m}^2$) seagrass fragments. We followed this sampling strategy to
151 capture as much spatial variability between fragments as possible, since within-patch

152 variability is considerably lower than between-fragment variation (Short & Duarte
153 2001). Both meadows are dominated by the seagrass *Posidonia oceanica*, the most
154 important benthic primary producer in the Mediterranean (Cebrián *et al.* 1996). The
155 two main herbivores of this seagrass system are the sparid fish *Sarpa salpa* and the
156 sea urchin *Paracentrotus lividus* (Boudouresque & Verlaque 2001), although the
157 pressure of the former is predominant, consuming on average more than 40% of the
158 primary production (Prado *et al.* 2007). Experimental fieldwork was conducted within
159 the depth range and season (i.e. 5 to 8 m depth, from June to September 2010) of
160 maximum herbivore activity for both species (Boudouresque & Verlaque 2001; Prado
161 *et al.* 2007).

162 At each site, five small fragments and five large meadow fragments were
163 randomly selected and subjected to natural grazing intensities, representing a
164 Fragmentation and Herbivory effect (Small patch + Herbivores) and an Herbivory
165 effect alone (Large patch + Herbivores) respectively. Herbivores were excluded from
166 the remaining fragments to represent a Fragmentation (patch size) effect (Small
167 patch + No Herbivores) and a Control effect (Large patch + No Herbivores). At these
168 fragments, we first extracted all sea urchins and then set up exclusion cages from
169 June to the end of September to restrict herbivore access. The cages measured
170 1.5m x 1.5m in area and 1m high, and were marked with 1.5 m high iron bars at each
171 corner to which green plastic mesh fences (2 cm mesh size) were attached. The
172 roofs consisted of fine transparent fishing net (3 cm mesh size). The caged patches
173 were visually checked to ensure that no fish or sea urchins entered below the fences.
174 Every two weeks SCUBA divers cleaned the cages with brushes in order to reduce
175 potential cage-induced light-reduction artefacts. To test for possible cage effects we
176 compared above canopy light availability inside and outside the cages. Four
177 HOBOware pro light sensors (Onset Computer Corporation: Apogee, Amplified
178 Quantum Sensor, model SQ-200) with a 10 min reading interval were deployed for 2
179 weeks at both sites (*Site 1* and *Site 2*) inside and outside the cages. The results

180 showed no significant differences between treatments (t-test, caged versus uncaged
181 light values, $P = 0.5238$). Additionally to avoid edge effects caused by the cage, we
182 restricted all sampling to the central area of the plot (Planes *et al.* 2011).

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

187 **Herbivore pressure:** We assessed shoot defoliation and herbivore type (fish or
188 sea urchin) in July when herbivory is at its seasonal maximum (Prado *et al.* 2007). At
189 the beginning of July, SCUBA divers marked 20 shoots per treatment (2 shoots per
190 plot, 5 plots, 2 sites, 2x2 treatments) for which we counted the initial number of
191 leaves, the initial length and the state of the apical part of each leaf (broken, eaten by
192 fish, eaten by sea urchin or intact). Both herbivores leave bite marks of distinctly
193 identifiable shapes (Boudouresque & Meinesz 1982; Prado *et al.* 2007). Leaf
194 defoliation (shoot herbivory, $\text{cm shoot}^{-1} \text{d}^{-1}$) was estimated for each of the collected
195 shoots after 15 days by adding leaf elongation to the initial length and subtracting this
196 total from the final leaf length (Prado *et al.* 2007), finally divided by the number of
197 days elapsed since marking. Only leaves that had clear herbivore marks were
198 assigned to herbivory and the rest discarded to avoid herbivory overestimates (Prado
199 *et al.* 2007). Obviously, the measurement of herbivore pressure only makes sense in
200 uncaged plots, although we measured it in all plots to confirm the absence of
201 herbivore activity in caged plots.

202 **Seagrass canopy structure:** Canopy height (cm) was also estimated in each plot
203 during the period of maximum herbivore activity (July) by measuring the height of the
204 canopy (vertical distance between the substrate and the top of the standing leaves)
205 in two different areas of the plot. Shoot density (shoots m^{-2}) was measured at the end
206 of the experimental period (September) using 2 haphazardly placed quadrats (40x40
207 cm) for each fragment.

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

218 **Nitrogen and carbohydrate content:** Rhizome nitrogen (% N) and total non-
219 structural carbohydrates (% NSC) were analysed at the end of the experiment
220 (September). To obtain adequate rhizome biomass for those analyses we pooled 2
221 shoots per plot. The top 2 cm of each rhizome were dried at 70°C for 48 hours and
222 ground to a fine powder. Rhizome nitrogen concentration was measured using an
223 Elemental Analyzer FlashEA1112 (ThermoFinnigan Unidade de Técnicas
224 Instrumentais de Análise, Universidade de Coruña). Total non-structural
225 carbohydrates (NSC, sucrose and starch) were also analysed for the same dried
226 rhizomes and replicates using a modified method from Alcoverro, Manzanera and
227 Romero (2001). Ground rhizomes were dissolved in 96% (v/v) ethanol, heated at
228 80°C for 15 minutes to extract sucrose; this process was repeated 3 times. Starch
229 was extracted from the ethanol-insoluble remnant pellet by dissolving it in 0.1 NaOH
230 and incubating it for 24 hours at room temperature. Sucrose and starch content were
231 determined spectrophotometrically using an anthrone assay with sucrose as
232 standard (Alcoverro, Manzanera & Romero 2001). Sucrose and starch content were
233 combined as non-structural carbohydrate content (NSC).

234

235 **Statistical analysis**

236 To test for 'patch size' (F), 'herbivory' (H) (hereinafter called drivers for the sake of
237 simplicity) and their combined effects on response variables we used a 3-way mixed
238 effects ANOVA design. We considered 'site' (St; 2 levels: Site 1, Site 2), 'patch size'
239 (F; 2 levels: Large, Small), 'herbivory' (H; 2 levels: Herbivores, No Herbivores) and
240 their interactions (St x F, St x H, F x H and St x F x H) as fixed factors for %N and
241 %NSC response variables. For the other response variables (i.e. herbivore pressure
242 -only the plots exposed to herbivores-, canopy height, primary production and shoot
243 density) an additional random factor 'plot', nested within the interaction of the others
244 (St x F x H) was added. Site was considered a fixed factor because both meadows
245 displayed distinct features, and were deliberately chosen to represent the most
246 common typologies of fragmented meadows within the area. Whenever an ANOVA
247 was significant, a multiple range contrast test was applied (Tukey's HSD) to
248 determine differences among treatments. All the ANOVA analyses were performed in
249 Statistica 8 software. Assumptions of normality (K-S & Lilliefors and Shapiro-Wilk's
250 tests) and homogeneity of variances (Cochran's test) necessary for the ANOVA were
251 not always met despite transformation. Nonetheless, Underwood (Underwood 1981)
252 has indicated that the ANOVA F statistic is robust despite these violations of
253 assumptions, provided that the working sample size is large enough. When such
254 assumptions were not met, we set the significance level to $P < 0.01$ to minimise the
255 risk of making a type I error.

256 The relationship between rhizome nutrient content and non-structural
257 carbohydrates with patch area were further explored using a linear regression model
258 in R (RDevelopmentCoreTeam 2010).

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262 **Results**

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264 **Herbivore pressure**

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

269 **Canopy height**

270 The canopy height of plots exposed to both drivers (Small patch + Herbivores:
271 39 ± 3 cm, Fig. 1b) was 39%, significantly lower (see the interaction $St \times F \times H$ in Table
272 1) than the plots without drivers (Large patch + No Herbivores: 64 ± 3 cm, Fig. 1b),
273 suggesting additive effects between them on this meadow attribute. Both herbivory
274 and fragmentation on their own had also significant effects (Table 1) in reducing
275 canopy height relative to plots without drivers (Large patch + No Herbivores, Fig. 1b).
276 Site and plot also showed significant effects for this variable (Table 1).

277 **Primary production**

278 Primary production responded significantly to herbivory and fragmentation effects
279 (Table 1, Fig. 1c). Herbivory alone caused a 32% decrease (comparing production on
280 Large patch + Herbivores: 0.39 ± 0.04 cm² shoot⁻¹ d⁻¹ relative to Large patch + No
281 Herbivores: 0.57 ± 0.04 cm² shoot⁻¹ d⁻¹) and fragmentation caused a 42% reduction in
282 seagrass primary production (comparing production on Small patch + No Herbivores:
283 0.33 ± 0.04 cm² shoot⁻¹ d⁻¹ relative to Large patch + No Herbivores). Both drivers
284 together (Small patch + Herbivores: 0.23 ± 0.04 cm² shoot⁻¹ d⁻¹), showed an additive
285 effect that resulted in a 60% decrease in production relative to controls (Large patch
286 + No Herbivores, Fig. 1c). Plot also showed significant effects for this variable (Table
287 1).

288 **Nutrient content**

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

297 **Total non-structural carbohydrates**

298 When both herbivory and fragmentation were acting together, Total non-structural
299 carbohydrates in rhizomes (% NSC) increased (Small patch + Herbivores: 15.2 ± 1.0
300 %, Fig. 1e) compared to the control plots (Large patch + No Herbivores: 13.2 ± 1.0 %),
301 showing a significant interaction (Table 1). This was mainly due to the important
302 increase caused by fragmentation (Small patch + No Herbivores: 17.3 ± 1.0 %). The
303 interaction between Site and fragmentation was also significant (see St \times F in Table
304 1).

305 **Shoot density**

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

313

314 **Relationship between variables**

315 Patch area and percentage of nitrogen in the rhizomes showed a significant
316 ($P<0.01$) linear regression ($n=40$, $R^2=0.6738$), suggesting a possible plant limitation
317 by nitrogen availability with decreasing patch size (Fig. 2). Additionally %N and
318 %NSC showed a significant ($P<0.01$) negative linear relationship ($n=40$, $R^2=0.219$,
319 Fig. 3).

320

321

322 Discussion

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324 As expected, herbivory alone increased seagrass defoliation rates that were,
325 interestingly, sufficient to cause reduced primary production and canopy height in
326 seagrass meadows. Patch size did not change herbivore pressure, but resulted in
327 very similar trends, also reducing primary production, canopy height and nutrient
328 content. What our results suggest, however, is that, when exposed to both drivers
329 together, the effects on some meadow attributes are even larger than in isolation. In
330 particular, although patch size does not appear to modify the way herbivores graze
331 across the landscape, the joint effects of herbivory and fragmentation result in
332 significantly reduced canopies in smaller patches. Seagrasses in small fragments
333 showed reduced primary production and nutrient content, probably the cause of
334 reduced canopies in the absence of herbivores. Despite this deterioration in plant
335 conditions shoot density did not reflect these changes, which may even increase
336 under herbivory because of reduced self-shading (Vergés *et al.* 2008; Planes *et al.*
337 2011). However the severe (39%) structural reduction in canopy height of small
338 patches might compromise their functional role. The prevalence of joint effects
339 between fragmentation and herbivory strongly suggests that predictions based
340 exclusively on a single driver will underestimate their ecological impact on seagrass
341 meadows.

342 The way consumers interact with their landscape for resource acquisition can

343 have major implications for ecosystem function, particularly when the resource is also
344 a major structural element of the ecosystem. Habitat fragmentation results in
345 increasing patchiness, and can seriously modify the way consumers use the
346 landscape mosaic. Optimal foraging theory assumes that natural selection will favour
347 the development of feeding preferences that will maximize the net caloric intake per
348 individual and unit time (Emlen 1966; MacArthur & Pianka 1966). Adult sea urchins
349 may have a limited ability to move between patches, but even without accounting for
350 movement, differential herbivore consumption rates may still arise as a function of
351 differences in nutrient content between different-sized patches. Other studies have
352 demonstrated that sea urchins resort to compensatory feeding in less nutritious
353 patches, increasing their consumption of leaves to meet their nutritional requirements
354 (Valentine & Heck Jr 2001). Our results indicate, in contrast, that *P. lividus* did not
355 show any compensatory feeding, and had similar rates of herbivory in small and
356 large fragments despite the different nitrogen content these patches had. In the case
357 of mobile herbivores, ecological theory predicts that they should abandon smaller,
358 putatively less nutritious and unprofitable patches (Kolb 2008); this behaviour has
359 been repeatedly observed in several terrestrial systems where, for instance, insects
360 tend to select larger patches for feeding (Lienert, Diemer & Schmid 2002; Kolb 2008;
361 Haynes & Crist 2009). The mobile marine herbivores in our study system however
362 appeared indifferent to patch size despite the higher nitrogen content and nutritional
363 value of larger fragments (Fig. 2). The mechanisms underlying the feeding
364 preferences of *Sarpa salpa*, the only herbivore that is able to move between patches,
365 are not yet completely understood although it has been observed that *S. salpa*
366 counter-intuitively prefer less nutritive, more chemically defended plant tissues
367 (Vergés *et al.* 2008). Where food selection as a function of plant nutrient content has
368 been observed, this trend has more to do with epiphyte composition rather than the
369 properties of the leaf tissue itself (Prado, Alcoverro & Romero 2010). Moreover,
370 habitat-generalist species such as *S. salpa* (Prado *et al.* 2007), are potentially less

371 likely to display positive density-area relationships than habitat-specialists (Hambäck
372 *et al.* 2007). Additionally, this fish travels in schools that can often reach hundreds of
373 individuals feeding in the same area (Raventos, Ferrari & Planes 2009); even if these
374 feeding schools showed no patch selection across the mosaic, herbivory impacts
375 could accrue more dramatically in the smallest seagrass patches compared with
376 larger fragments.

377 Despite the lack of patch selection by herbivores, the fact that fragments were
378 subjected to similar herbivory rates independent of their size resulted in major
379 habitat-level consequences. Herbivory on small patches resulted in rates of primary
380 production 44% lower than in larger patches exposed to the same levels of herbivory.
381 Herbivory by the sparid fish *S. salpa* can be very intense in seagrass ecosystems
382 with rates as high as 40% of primary production on an annual basis (Prado *et al.*
383 2007), becoming, even higher in Marine Protected Areas (Prado *et al.* 2008), where it
384 can seriously compromise sexual reproduction in *P. oceanica* (Planes *et al.* 2011).
385 Fish herbivory does not merely affect plant primary production but can also
386 substantially alter canopy structure, as observed in this study, with important flow-on
387 consequences for the rest of the ecosystem. The impact of herbivores on canopy
388 height has been observed to mediate predator-prey interactions by decreasing
389 refuges, thus increasing predation rates which can ultimately cause the
390 disappearance of important functional species (Pagès *et al.* 2012). The joint effect of
391 herbivory and fragmentation observed in this study could further exacerbate these
392 effects with unknown consequences for the rest of the meadow community.

393 Our results show that reduction in fragment size altered primary production in
394 small fragments. This is probably related to nitrogen limitation, as observed by the
395 lower concentration of nitrogen in smaller patches (Fig. 2). The underlying
396 mechanism may be linked to a higher export of autochthonous primary production in
397 small fragments compared to larger ones, or an enhanced clonal integration in larger
398 fragments (Prado, Collier & Lavery 2008). Conversely, small patches accumulate

399 more carbon reserves in rhizomes than larger ones, a mechanism that has already
400 been observed under conditions of nitrogen limitation (Invers *et al.* 2004). The higher
401 percentage of carbohydrates observed in these smaller fragments could be the result
402 of less self-shading and a consequent increase in light availability (Burke, Dennison
403 & Moore 1996; Hamilton *et al.* 2001). In fact, the correlation between carbohydrates
404 and nitrogen (Fig. 3) also points to a possible nutrient limitation given the low nutrient
405 content observed in this work when compared with plants under nitrogen limitation
406 (Duarte 1990). Similar effects have been detected in terrestrial ecosystems when
407 habitat fragmentation imposes nutrient limitations and poor physical conditions in
408 small patches, affecting the survival of non-mobile herbivores due to the change in
409 abundance of food and the risk of predation (Villafuerte, Litvaitis & Smith 1997).

410 Despite the profound effects that herbivores have on small patches by indirectly
411 reducing patch biomass, the plant appears to be able not merely to resist but also to
412 partially compensate for these combined stressors. In effect, *P. oceanica* shoot
413 density in small fragments subjected to herbivory was maintained at values similar to
414 the controls indicating that the clonal growth was not limited by the biomass lost to
415 herbivores and fragmentation effects, even though the ecosystem itself accrues
416 significant impacts (see previous paragraph). However, the effects of herbivory and
417 reduction in patch size on such a conservative structural parameter (i.e. shoot
418 density) of a particularly slow growing species may not be visible in the short term
419 (four months). These results add to a growing body of evidence showing that *P.*
420 *oceanica* apparently has evolved several mechanisms to compensate for herbivore
421 pressure including compensatory growth, increased clonal growth and increased
422 nutrient translocation from senescent leaves (Vergés *et al.* 2008; Planes *et al.* 2011).
423 This high tolerance to herbivory is probably the result of the coevolution of the plant
424 with important and even more damaging herbivores in the past (Planes *et al.* 2011). It
425 is, in fact, well recognised that seagrasses, like their terrestrial counterparts, resist
426 high herbivory with a series of adaptations such as inaccessible basal meristems,

427 branching rhizomes that enhance resistance to grazing and investment in
428 belowground reserves (Valentine *et al.* 1997; Valentine & Heck Jr 1999). In practical
429 terms, the fact that *P. oceanica*, an important ecosystem engineer, responds to
430 fragmentation (specifically, to reduction in patch size) and herbivory with a smaller
431 change than expected in primary production, nutrient content and population
432 dynamics indicates that these combined drivers may be much less damaging at least
433 in terms of plant functional survival. This response may explain why very small
434 patches can continue to survive for several decades (unpublished personal
435 observations and Alcoverro *et al.* 2012).

436 The interaction of drivers can make ecosystems more vulnerable to change
437 (Folke *et al.* 2004). Our results point to the importance of understanding how
438 environmental stressors modify key internal ecosystem processes since they may
439 interact in potentially surprising ways, not entirely predictable by merely knowing how
440 the system responds to each individually (Crain, Kroeker & Halpern 2008). Unlike
441 internal processes, external stressors like anthropogenic fragmentation are not
442 ecosystem dependent. While external stressors may on their own modify just a few
443 key attributes of the system, their ability to modify internal ecosystem processes may
444 set in motion major functional changes to the system that the disturbance alone may
445 not directly cause. In the example of the seagrass meadows, the plant seems to cope
446 adequately with internal drivers like herbivore pressure thanks to their evolutionary
447 adaptations. However, the introduction of external stressors like fragmentation into
448 the system can have far larger effects than expected, particularly on the structure of
449 these systems. While fragmentation has already been recognised for its ability to
450 impact a suite of ecosystem parameters, the fact that it interacts with herbivory can
451 exacerbate these losses and seriously compromise the role of seagrasses as habitat-
452 forming ecosystems.

453

454

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699
700

Source of variation	Herbivory pressure				Source of variation	%Nitrogen			
	Df	Sum of Square	F	P		Df	Sum of Square	F	P
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 × F	1	0.491	0.445	0.512	H	1	0.011	0.056	0.814
PLOT [St × F]	16	2.663	2.416	0.032	St × F	1	0.367	1.832	0.185
ERROR	20	22.041			St × H	1	0.131	0.655	0.424
					F × H	1	0.028	0.138	0.713
					St × F × H	1	0.068	0.340	0.564
					ERROR	32	6.407		

Source of variation	Canopy				Source of variation	% NSC			
	Df	Sum of Square	F	P		Df	Sum of Square	F	P
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 × F	1	104.497	2.369	0.131	St × F	1	45.156	4.382	0.044
St × H	1	32.633	0.740	0.395	St × H	1	4.422	0.429	0.517
F × H	1	146.446	3.320	0.076	F × H	1	70.756	6.866	0.013
St × F × H	1	805.120	18.252	<0.001	St × F × H	1	43.723	4.243	0.048

PLOT [St × F × H]	32	413.724	9.379	<0.001	ERROR	32	329.768
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ERROR	40	1852.7
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Source of variation	Production				Source of variation	Shoot density			
	Df	Sum of Square	F	P		Df	Sum of Square	F	P
St	1	0.067	3.530	0.068	St	1	43950	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	64690	12.905	0.001
St × F	1	0.043	2.269	0.140	St × F	1	45716	9.120	0.004
St × H	1	0.001	0.044	0.834	St × H	1	9301	1.855	0.181
F × H	1	0.024	1.249	0.270	F × H	1	57117	11.394	0.002
St × F × H	1	0.058	3.060	0.088	St × F × H	1	5697	1.137	0.293
PLOT [St × F × H]	32	0.051	2.663	0.002	PLOT [St × F × H]	32	28710	5.727	<0.001
ERROR	40	0.762			ERROR	40	200508		

701

702

703 Table 1: Summary of the different ANOVA analyses performed. *P*-values correspond

704 to those provided by an F-test. For the physiological response variables (%N and

705 %NSC) the effects of site (St), patch size (F), herbivory (H) and their interactions

706 were tested. For the other response variables (herbivore pressure, canopy height,

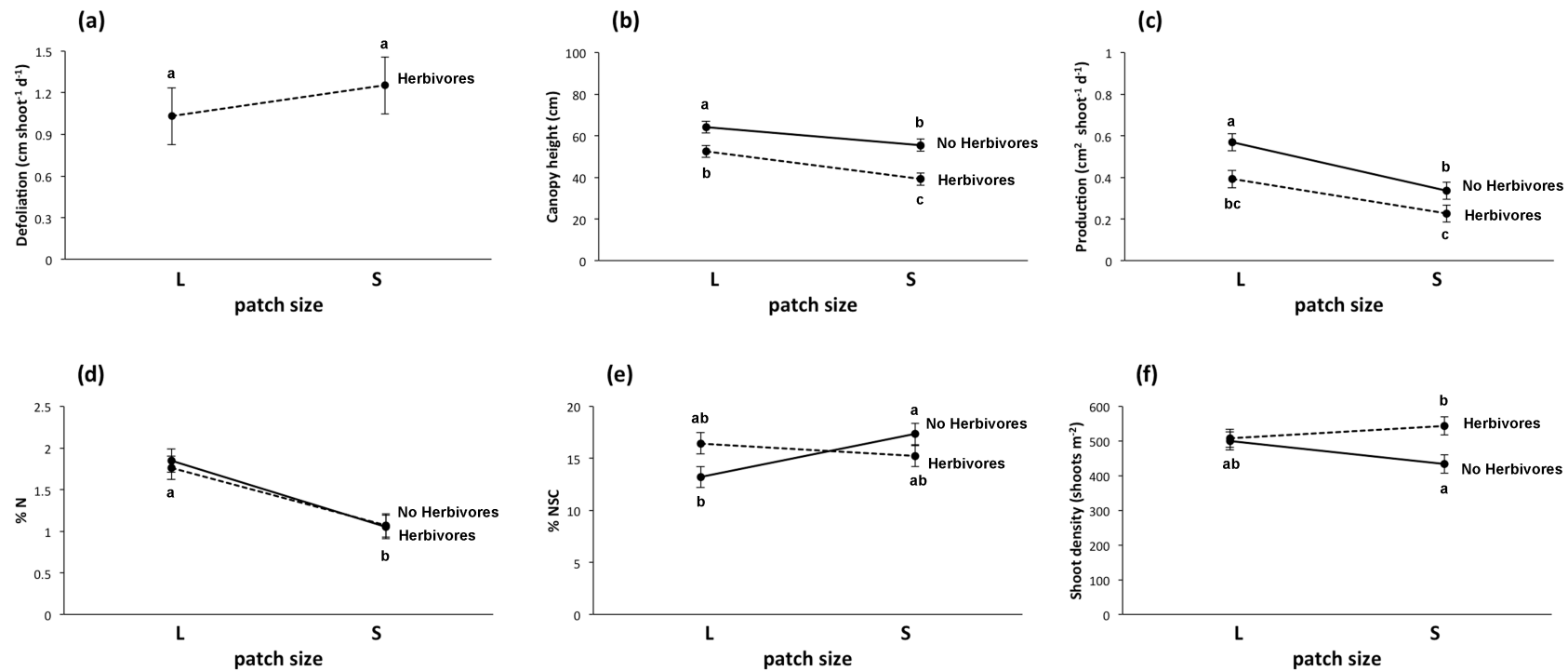
707 primary production and shoot density) in addition to the aforesaid factors, plot was

708 considered a random factor nested within (St × F × H). Primary production was

709 square root transformed to meet ANOVA assumptions, but for herbivore pressure the

710 assumptions were not met after transformations and we set the significance level to
711 $P < 0.01$ to minimise the risk of making a type I error. Df, degree of freedom
712

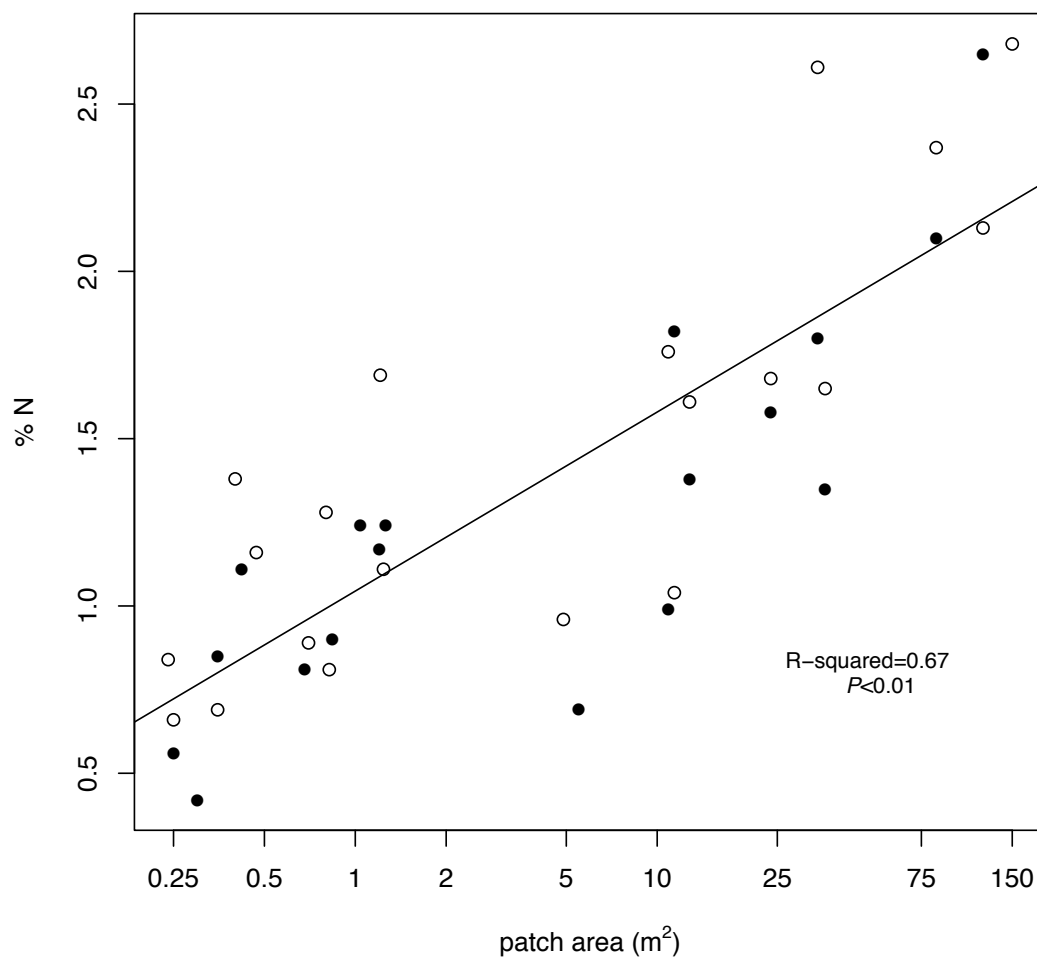
713 Fig 1. Individual and combined effects of patch size (2 levels: large (L) and small (S)) and Herbivory (2 levels: Herbivores present [dotted line]
 714 and No Herbivores [caged plots, continuous line]) for each of the response variables (mean±SE): herbivore pressure (a), canopy height (b),
 715 primary production (c), % nitrogen (d), % total non-structural carbohydrates (NSC) (e) and shoot density (f). Values labelled with the same
 716 lower case letter do not differ significantly according to Tukey's HSD *post hoc* test.



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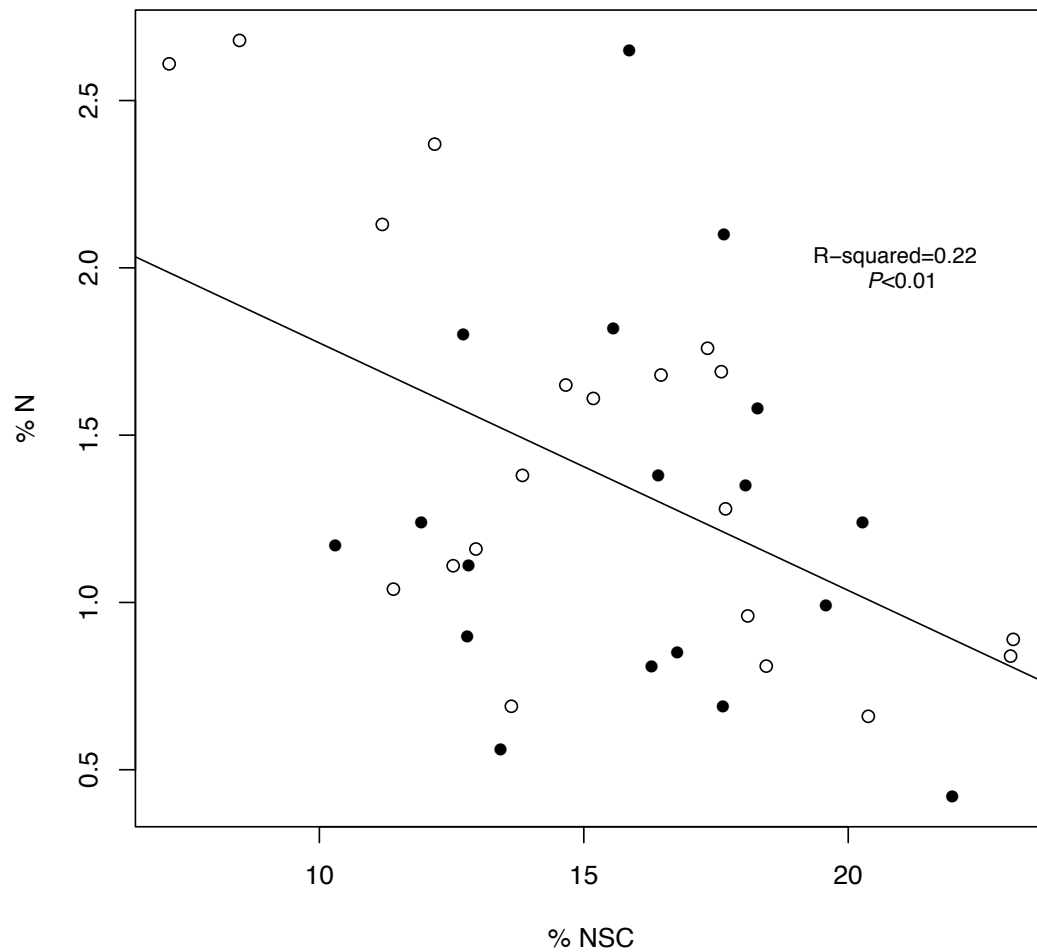
718

719 Fig 2. Linear regression showing a significant relationship between patch size (log
720 transformed) and the nitrogen content (% N) of *Posidonia oceanica* rhizomes taken
721 at the end of the experiment (n=40). Full circles (●) indicate plots where herbivores
722 were present, while empty circles (○) indicate caged plots without herbivores.
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728 Fig 3. Linear regression showing a significant relationship between the nitrogen
729 content (% N) and the total Non-Structural Carbohydrates (% NSC) of *Posidonia*
730 *oceanica* rhizomes taken at the end of the experiment (n=40). Full circles (●) indicate
731 plots where herbivores were present, while empty circles (○) indicate caged plots
732 without herbivores.
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