

1 **Hotspots of predation persist outside marine reserves in the historically fished Mediterranean**
2 **Sea**

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27

28

29 **Abstract**

30

31 The Mediterranean Sea has sustained historically high levels of fishing since pre-Roman times.
32 This once-abundant sea has witnessed major declines in apex predators, now largely restricted
33 to isolated pockets within marine reserves. This depletion could critically impact macrophyte
34 communities that are strongly structured by top-down processes. We evaluated rates of
35 predation on the sea urchin *Paracentrotus lividus*, a key herbivore of macroalgal and *Posidonia*
36 *oceanica* seagrass seascapes, across a large stretch of the Western Mediterranean coastline. Fish
37 predation was generally higher inside reserves, but was equally high at several locations outside
38 these boundaries. Although critically low at some locations compared to reserves, predation was
39 functionally ubiquitous in most habitats, seasons and sites. Fish were still primarily responsible
40 for this predation with no clear evidence of meso-predator release. Macroalgal habitats were
41 consistently subject to higher predation than in seagrass meadows, functionally critical given
42 the vulnerability of macroalgal systems to overgrazing. Predation hotspots were clearly
43 associated with high fish predator numbers and low refuge availability. Taken together, these
44 results suggest that long-term overfishing may not necessarily reflect a complete loss of trophic
45 function. Pockets of fish predation may still persist, linked to habitat complexity, predator
46 behavioural adaptations and landscape-level features. Given the essential role top-down control
47 plays in macroalgal communities, regulating fishing at these predation hotspots is vital to
48 effectively conserving habitats from future hysteretic shifts. Even historically fished seas may
49 retain areas where trophic function persists; identifying these areas is critical to preserving the
50 remaining ecological integrity of these coastlines.

51

52 **Introduction**

53 One of the clearest signatures of the increasing human imprint on the biosphere is the gradual
54 weakening of trophic processes as top predators decline from natural ecosystems under the combined
55 onslaught of direct extraction and habitat loss (Ripple et al., 2014). Predation is a critical agent of
56 community structuring (Hairston et al., 1960); the depletion of key predators leave both terrestrial
57 and marine ecosystems increasingly prone to catastrophic and often hysteretic collapses from which
58 recovery can be protracted. Marine macrophyte communities are particularly susceptible;
59 uncontrolled by predation, marine herbivores can undergo major population explosions, overgrazing
60 macrophyte-dominated ecosystems (Kempf, 1962). In a classic example, otters have been identified
61 as principal structuring agents of kelp communities in the Eastern Pacific by regulating urchin
62 populations (Tegner and Dayton, 2000). Similarly, the structuring of Western Mediterranean
63 macrophytes appears to be strongly mediated by top-down control of urchins by fish predators
64 (Pinnegar et al., 2000).

65 Marine ecosystem managers have long recognized the importance of conserving higher trophic
66 functions, and regulating fishing of top predators has been the instrument of choice in managing
67 nearshore ecosystems (Estes et al., 2011). There has been a growing call to expand networks of
68 marine reserves and impose fishing restrictions to protect key predators and enhance the natural
69 resilience of the ecosystems they structure (Pinnegar et al., 2000). This is predicated on the
70 assumption that fish predator numbers link well with rates of predation, and that healthy predator
71 populations will ensure their functional roles within the ecosystem (Clemente et al., 2010). There is
72 growing evidence demonstrating that marine reserves have been largely effective in reversing the
73 direct and indirect effects of trophic decline (Shears and Babcock, 2002), and they clearly enhance
74 ecosystem functioning. However, it is becoming increasingly clear that predation is an inherently
75 dynamic process, and predator-prey interactions can vary considerably across the seascape. The
76 distribution and densities of predators and prey within the mosaic may be influenced by recruit
77 supply, which may, in turn, be mediated by habitat differences (Hereu et al., 2004). Independent of
78 numbers, predator-prey interactions may be strongly driven by how both predators and their prey use

79 these habitats (Farina et al., 2014). These habitat-specific factors may also interact in complex ways
80 making predator-prey interactions often difficult to predict. Both fish predators and their prey may
81 modify their behaviours in relation to each other's presence, the abundance of conspecifics, the
82 availability of refugia and the configuration of the habitat within the larger seascape. For instance,
83 habitat structural complexity, by modifying the presence of prey refugia is fundamental in
84 determining predation rates and, in turn, prey population structures (Farina et al., 2009; Hereu et al.,
85 2005). Moreover, predators may also be implicated in complex indirect interactions in macrophyte
86 communities; fish herbivores, by reducing the leaf canopy of macrophyte communities, can enhance
87 fish predation on urchin herbivores by reducing refuge availability (Pagès et al., 2012). Further, a
88 reduction of top predators can sometimes lead to the competitive release of benthic meso-predators
89 that may potentially compensate rates of functional predation experienced by the system (Levi and
90 Wilmers, 2012). This can also be highly habitat dependent since every system could be host to a
91 very different suite of predators. Finally, both predators and prey may move between habitats in the
92 mosaic, and predation may be strongly influenced by patterns of habitat connectivity or isolation
93 within the larger seascape (Hitt et al., 2011).

94 Two macrophyte habitats dominate the North Western Mediterranean: *Posidonia oceanica* seagrass
95 meadows and shallow macroalgae-dominated rocky habitats, both potentially structured by top-down
96 control of the herbivorous sea urchin *Paracentrotus lividus* (Fig. 1) (Verlaque, 1987). The
97 Mediterranean has been seriously overfished for millennia (Sala et al., 2012), and determining if
98 predation still plays a functional role is essential to planning conservation actions across the region
99 (e.g. creations of marine reserves, management of coastal development, etc.). While it is well
100 established that predation intensity is relatively high inside existing protected areas (Sala and Zabala,
101 1996) it is unclear to what extent this function is conserved beyond their boundaries, although it is
102 generally assumed to be low because of this historically sustained fishing pressure (Guidetti et al.,
103 2010). However, there is little information available on the factors that influence predation in
104 different macrophyte habitats. The decline of fish predators could have triggered a functional
105 substitution by other benthic predators. In addition, given that reserves are principally established to

106 enhance predator numbers, understanding how predation activity is linked to fish predator abundance
107 is critical. To answer these questions, we measured relative rates of sea urchin predation by fish and
108 benthic predators at eight representative locations across a large stretch of the NW Mediterranean
109 coast in both algal communities and seagrass meadows in different seasons. In addition, we
110 attempted to identify if predator habitat use or habitat-specific factors (presence of refuges) can drive
111 functional rates of predation in these dominant macrophyte habitats.

112

113

114 **Materials and Methods**

115 *Study system*

116 The shallow seascape of the Western Mediterranean is dominated by rocky macroalgal communities
117 and *P. oceanica* seagrass meadows. Although the sea urchin *P. lividus* is a key herbivore in both
118 habitats, they may differ considerably in their susceptibility to urchin herbivory (Boudouresque and
119 Verlaque, 2001). In macroalgal systems, urchin overgrazing can cause ecosystem barrens from
120 which recovery is often protracted (Pinnegar et al., 2000). Predators likely play a vital role in
121 regulating sea urchin populations (Supplementary, A1), preventing these ecosystem shifts (Guidetti,
122 2004; Sala, 1997). While *P. oceanica* meadows may experience very similar rates of urchin
123 herbivory, they may cope better with this offtake because of their inherent evolutionary adaptations
124 (Vergés et al., 2008). However, heavy eutrophication could make meadows susceptible to
125 overgrazing (Ruiz et al., 2009). Several fish species prey on *P. lividus*, and many of these are
126 important commercial and recreational fishery targets (Guidetti, 2006). Additionally, benthic
127 predators including starfish and some gastropods may also be important contributors to sea urchin
128 predation (Boudouresque and Verlaque, 2001).

129

130 *Study site and Sampling design*

131 The study was conducted along the NW Mediterranean (~600km). Eight sites were selected along
132 the coast, characterized by shallow seagrass *P. oceanica* habitats and photophilic macroalgae on
133 rocky substrates (Fig. 2). Sites were not randomly selected since all sites required both habitats to be
134 present and at least one unfished reserve was required for the study objectives. Fishing is permitted
135 at all sites except the Medes Island Marine Protected Area, which has been a marine reserve since
136 1990, and partially controlled in Portlligat since 2006 as part of the Cap de Creus Natural Park but
137 with low fishing regulation. The reserve is characterized by a high abundance and biomass of
138 predatory fish (Garcia-Rubies et al., 2013). In each habitat we assessed predation on the sea urchin
139 *P. lividus*, the most important key herbivore in NW Mediterranean macrophyte habitats (Harmelin et
140 al., 1980). We evaluated predation impact by fish and benthic predators (see below) in each of the
141 selected sites in summer and winter. In addition, we estimated the habitat use by the most important

142 urchin predators, and evaluated habitat characteristics that could constitute an effective predation
143 refuge for the urchin (i.e. canopy height in both habitats, crevices in rocky substrates and bare root-
144 rhizome layer in seagrass meadows) (Orth et al., 1984). All measurements were recorded within a
145 depth range of 3 to 8 meters for both habitats.

146

147 *Predation impact*

148 Predation impact was measured using tethering techniques (Boada et al., 2015; Aronson and Heck,
149 1995) on adult urchins. This comparative method has been used extensively to assess predation in
150 Mediterranean and other temperate ecosystems (Farina et al., 2009; Guidetti and Sala, 2007) and
151 coral reefs (McClanahan and Muthiga, 1989; Pederson and Johnson, 2006). The urchin was
152 harnessed by threading a thin nylon line through its test. The harness was then attached to a brick or
153 metal stake, which were deployed in rocky reefs or meadows respectively. The harness left the
154 urchin a 50 cm radius space to move from its point of attachment. Within this radius, the urchin
155 could actively seek available shelters as it would in natural conditions. To assess tethering-related
156 mortality before the experiment we tracked the survival of caged urchins (to exclude predators);
157 none of the 14 tethered urchins died in the 12 days of this assessment. Twenty urchins were placed in
158 groups of 5 at each site and habitat (total of 4 replicated groups) in two different seasons (i.e.
159 summer and winter). Predation impact was measured 15 days after the start of the experiment.
160 Predation impact was calculated for each group of urchins using the percentage of dead urchins with
161 respect to the initial number (0, 20, 40, 60, 80 and 100% predation). Fish or benthic predators leave
162 clearly distinguishable bites/marks on sea urchin carcasses (Shears and Babcock, 2002) and we
163 carefully examined dead urchin tests to assign predation impact to their respective consumers; fish
164 predator impact (FPI) and benthic predator impact (BPI).

165

166 *Predator abundances and habitat use*

167 We measured the habitat use of the most common identified benthic predators of *P. lividus* within
168 the two selected habitats, *Hexaplex trunculus* and *Marthasterias glacialis* (Boudouresque and
169 Verlaque, 2001). We frequently found these benthic predators still attached to the prey. In addition

170 we recorded a few predation events by the starfish *Coscinasterias tenuispina* (S. Farina pers. obs.)
171 and added this species to our sampling. Benthic predator habitat use was estimated through
172 accounting the abundance of benthic predators at each site and habitat along 4 underwater visual belt
173 transects (10 m x 2 m). These were used to calculate benthic predator densities per square meter for
174 each site.

175

176 We also investigated the habitat use by fish predators during daylight hours, when predators are most
177 active (Savy, 1987). We used underwater video cameras in each habitat and season (i.e. GoPro Hero
178 2, 10Mp) (Harasti et al., 2014) to record 4 fixed videos of 20 minutes within each site. We used
179 underwater buoys to mark a 5 x 5 m area in front of the camera and counted the number of
180 individuals of the principal predators and scavengers of medium and large sea urchins (*Diplodus*
181 *sargus*, *D. vulgaris*, *Sparus aurata* and *Labrus merula*) (Guidetti, 2004; Sala, 1997) and two more
182 potential predator or scavenger species of the genus *Diplodus* (*D. puntazzo* and *D. cervinus*) seen
183 traveling through the marked area. This was then multiplied by the total time each species spent
184 within the zone and divided by the total observed area. Habitat use (U) was calculated as follows:

185

186 •
$$U = \frac{\sum(T_i)}{T * A}$$

187

188 where i is the number of predators observed during the sampling interval, T_i is the total time each
189 predator species spent in the area, A is the total area observed in each video and T is the video
190 recording time (approx. 20 minutes each).

191

192 *Habitat structural parameters*

193 To test the influence of habitat structural parameters on predation rates, we measured habitat
194 complexity (canopy height and number of refuges) in both habitats. We measured canopy height
195 with a measuring tape at 20 random locations within the macroalgal and *P. oceanica* canopy at each
196 site and for each season. In addition, we measured the depth of the total unburied rhizome layer

197 (Prado et al., 2009) in *P. oceanica* meadows since this is often used as an important refuge by sea
198 urchins in seagrass meadows (Orth et al., 1984). This was done at 20 random points at each meadow
199 with a measuring stick inserted into the unburied matrix. In rocky macroalgal systems we counted
200 the number of potential shelters (crevices and niches that were estimated to harbor an urchin of at
201 least 4 cm diameter) within a 50 cm diameter range at 20 random points per site and season.

202

203 *Statistical analyses*

204 3-way ANOVA's tests were performed to establish the effect of habitat, season and site on the
205 following dependent variables: total predation impact, fish predator impact (FPI), bottom predator
206 impact (BPI), sea urchin predator fish habitat use (U) and canopy height. The factors considered
207 were 'site' (8 levels, fixed factor), 'habitat' (2 levels; *P. oceanica* meadows and macroalgal habitats,
208 fixed factor) and 'season' (2 levels; summer and winter, fixed factor). Prior to the analyses we tested
209 for normality (Shapiro-Wilk test) and homogeneity of the variance (Bartlett's test). When
210 assumptions were not met, we set the significance level to $p < 0.01$ as the F statistic is robust despite
211 violation of these assumptions when the sampling size is large enough (Underwood, 1981).
212 Significant differences between sites were further explored with Tukey HSD *post hoc* tests.

213

214 A continuous approach (GLM) was used to test the significance of the explanatory variables
215 related to fish habitat use (for the three main predators) and habitat structural parameters
216 (presence of habitat-specific refuges, see below) to explain the observed patterns of fish
217 predation (FPI) within each habitat. We could not test this model for bottom predation impact
218 (BPI) as the number of predation events observed was too low to reliably establish any causal
219 link. A General Linear Model (GLM) with a Binomial distribution (and a logarithmic link
220 function) was fitted to test significance. To describe the response of FPI within each habitat a
221 specific analysis was performed according to habitat-specific explanatory variables. For the *P.*
222 *oceanica* habitats the specific variables included in the model were canopy height (canopy),
223 depth of the dead matte rhizome layer (matte) and the habitat use of fish of the *Diplodus* genus
224 as the main *P. lividus* predators. For the macroalgal dominated rocky habitats the variables

225 included were canopy height (canopy), number of refuges (shelters) and the habitat use of fish
226 of the *Diplodus* genus as the main *P. lividus* predators. We used mean values of habitat use
227 from the 8 replicates (summer and winter together) to better investigate the use in each location
228 and habitat. We started with a full model considering all predator and habitat-associated
229 variables for model selection. We then chose the best model by dropping each effect
230 sequentially and using Akaike's Information Criterion (AIC) and likelihood ratio tests (Zuur et
231 al., 2009). All the statistical analyses were performed using 'lme4' package (Bates et al. 2014)
232 in the open source software R (Bates et al., 2014; R Development Core Team, 2013).

233 **Results**

234 *Spatio-temporal variation in benthic and fish predation rates: habitat, season and site.*

235 Predation impact varied substantially between habitats, sites and seasons (Table 1, Fig. 3). Predation
236 impact in rocky habitats was at least double of that measured in *P. oceanica* habitats, while at some
237 sites this difference was even more marked (Table 1, Fig. 3). On average, the predation impact in
238 rocky reef communities was 54.9% ($\pm 9.2\%$), compared with a predation impact of 17.8% ($\pm 8.6\%$)
239 in *P. oceanica* meadows (Fig. 4). The highest predation impact for both habitats was found inside the
240 Medes Islands Marine Reserve in which fishing had been restricted for more than 2 decades.
241 Interestingly though, other locations along the coast, outside any NTA, experienced similar predation
242 impact both in macroalgal communities (*post hoc* Site A = B = C < D = F > E = G = H) and in *P.*
243 *oceanica* meadows (*post hoc* Site A = B = E = F = G = H < C = D) (Fig. 3). In contrast, predation
244 impact in other sites was very low in both habitats especially in a particular season (e.g. Site A or
245 Site B, Fig. 3). While predation was generally higher in summer than in winter, this was only true for
246 some sites (Table 1, Fig. 3). At sites where predation was very high (i.e. Site D or Site F), the
247 difference between seasons was almost absent; where predation was low, these differences were
248 considerably more marked (Fig. 3). Even outside the marine reserves, fish predators continued to be
249 responsible for the bulk of predation (Fig. 3). In macroalgal habitats the few observed cases of
250 benthic predation occurred outside the reserve. In contrast, in *P. oceanica* habitats predation by
251 benthic predators was more prevalent, although still considerably lower than fish predation. A high
252 peak of benthic predation was observed in the seagrass habitat in winter in Medes Islands as a result
253 of an observed increase in the abundance of the predator starfish *C. tenuispina* (a few tethered sea
254 urchins still had the star attached to the carcass). An interesting pattern is that season was significant
255 when both fish and benthic predation were considered separately, but not together (Table 1, Table 2)
256 since both predators seem to prefer distinct seasons particularly in determinate sites. Predation by
257 fish was significantly higher in summer ($41.5\% \pm 9.7\%$, Table 2), than in winter ($32.3\% \pm 8.1\%$)
258 while benthic predation was significantly lower in summer ($1.0\% \pm 0.7\%$) than in winter ($3.5\% \pm$
259 2.2%).

260

261 *Variation in predator habitat use and canopy height between habitats, sites and season.*

262 Predator habitat use also showed clear differences between habitats (Fig. 4) and sites for certain
263 seasons (Table 2, Fig. A2). The index of predator habitat use was more than 10 times higher in rocky
264 habitats than in seagrass habitats, a difference not reflected in the magnitude of predation impact
265 (Fig. 4). This suggests that although predators may use the habitat much less, predation rates
266 continue to be relatively high in seagrass meadows.

267

268 As expected, canopy height varied considerably between macrophyte habitats; *P. oceanica* canopies
269 were almost an order of magnitude taller (~ 35 cm long) than macroalgal dominated habitats (~ 6 cm
270 height, Fig. 4, Table 2). Canopy height also varied between sites and seasons, although in the same
271 direction for both habitats (Table 2, interaction between site, habitat and season, Fig. A3). Within
272 each habitat, canopy height varied considerably between seasons, with summer canopies consistently
273 taller than winter canopies (average values summer *P. oceanica* > average winter *P. oceanica* >
274 average macroalgal summer and winter), a trend that was more pronounced at some sites (Table 2,
275 interaction between site and season).

276

277 Habitat-specific refugia also varied considerably between sites (Fig. A2). In macroalgal habitats the
278 number of refugia differed considerably between sites with a mean maximum value of 9.2 refugia
279 per sampling and a mean minimum of 2 (ANOVA p-value < 0.001). In seagrass habitats as well, the
280 depth of the dead matte rhizome layer could differ by an order of magnitude between sites (mean
281 max. 14.4 cm and min. 0.8 cm; ANOVA p-value < 0.001).

282

283 *Determinants of predation rates in rocky and seagrass habitats*

284 The GLM results showed that in rocky habitats, predation rates were best predicted by the number of
285 available shelters (p < 0.05) together with the abundance of fish predator-species of the genus
286 *Diplodus* (p < 0.05, Table 3). The same pattern was found in *P. oceanica* meadows where the depth
287 of the dead matte rhizome layer (p < 0.05) together with habitat use by fish species in the genus

288 *Diplodus* were key determinants of predation impact ($p < 0.05$, Table 3).

289 **Discussion**

290 Despite a long history of commercial, artisanal and recreational fishing, fish predation continues to
291 be a ubiquitous process along the North Western Mediterranean coast, albeit with considerable site-
292 level variation in intensity. While the Medes Islands marine reserve (with over two decades of
293 fishing prohibition) unsurprisingly received the highest level of predation in both habitats, locations
294 with no such restrictions also received comparable levels of functional predation. Fish predators
295 continue to be the principal agent of predation in these waters with benthic predators apparently
296 playing a relatively minor role. Perhaps most strikingly, there were strong differences in the intensity
297 of predation between macrophyte habitats, even when separated by just a few meters. Predation
298 impact in macroalgal habitats were at least twice as high as in *P. oceanica* seagrass meadows, even
299 when these habitats were very closely connected, a difference that appears clearly linked to the
300 greater abundance of predators in rocky systems. These predator-prey interactions appeared to be
301 controlled by the same agents in both habitats, the number of available refugia as well as predator
302 habitat use.

303 The now well-documented specter of trophic downgrading across the world's oceans has raised
304 serious questions of the continued functional resilience of important coastal ecosystems (Estes et al.,
305 2011). On coastlines that have been dominated by heavy human extractive use for as long as the
306 Mediterranean has, it is difficult to conceive of fish predatory functions still being ubiquitous and
307 relevant outside the most strictly protected reserves. However, this assumption has rarely been tested
308 at regional scales; our results are an encouraging indication that, despite the considerable trophic
309 downgrading the Mediterranean has experienced with centuries of human use (Pauly et al., 1998),
310 predation continues to be a relevant trophic process in its macrophyte communities. To be sure,
311 predation was highest in the only marine reserve we studied (Medes Islands), where predatory fish
312 guilds have increased notably in the last decades (Garcia-Rubies et al., 2013). However, a few sites
313 along the coast without the benefit of this careful management (i.e. site F, site C) showed rates very

314 similar to Medes, indicating that locations across the NW Mediterranean were still able to maintain
315 intact higher trophic functions. These sites were characterised by subtidal rocky extensions and
316 outcrops that may attract predators (personal observation); these contingent factors may help
317 determine how predator-prey processes vary across the coastline. Each of these processes is likely
318 governed by a complex suite of factors acting together to determine the relative importance of
319 predatory functions, supply side processes and bottom-up drivers in structuring macrophyte
320 communities. For instance, while predation may be an important agent of population control, urchin
321 populations can themselves be highly dependent on recruitment (Prado et al., 2012) and bottom-up
322 processes controlling algal growth (Menge, 2000); these factors likely interact in complex ways to
323 determine the capacity of predators to control the system.

324

325 What is clear, however, is that benthic predators do not substitute fish as the top trophic agents along
326 this coast, even in sites where fish predation is very low (Site A, Fig. 2). The only observation that
327 could potentially indicate a certain level of competitive release is seen at a seasonal level. The fish
328 predatory guild is less active during winter, and bottom predators become more active during these
329 months, especially in seagrass meadows. This increased winter activity may represent a competitive
330 exclusion between predatory groups. Chemotactic benthic predators may take much longer to locate
331 their prey than visual fish predators, and since winter temperatures constrain fish metabolic activity,
332 benthic invertebrates may derive seasonal benefits from this reduced movement (Bonaviri et al.,
333 2009) but see also (Farina et al., 2014). This competitive exclusion was not observed in sites where
334 fish predation was high throughout the year and the impact of benthic predators was low (i.e. in
335 rocky habitats from Site D and Site F). Clearly though, these appear to represent merely seasonal
336 changes in behavior rather than any real change in community composition, and the overall
337 predominance of fish predators indicates that these systems are not witnessing either a release of
338 benthic meso-predators due to overfishing or competitive exclusion. This contrasts with macrophyte
339 habitats in other fished regions (like Australia) where benthic predators dominate top trophic roles,
340 with fish predators virtually absent (Farina et al., 2014). In other coastal ecosystems, notably

341 Caribbean coral reefs, the removal of large predatory fish triggers a distinct meso-predator release
342 (Burkepile and Hay, 2007). The apparently low functional replaceability of the predator guild in the
343 Mediterranean highlights the centrality of fish to predation pathways in these systems. This further
344 emphasizes the importance of managing predatory fish against fishing overexploitation if this crucial
345 process is to be preserved.

346

347 The intensity of predation we recorded was clearly habitat dependent, even when the seascape was a
348 patchy mosaic dominated by both habitats. Macroalgal habitats received rates of predation at least
349 twice as high as seagrass meadows. This can, in part, be explained by higher habitat use of predators
350 in rocky systems and the taller canopies characteristic of *P. oceanica* meadows. Surprisingly,
351 predation impact in seagrass meadows were disproportionately high compared to predator use of
352 these habitats. This uncoupling between predator numbers and predation impact between habitats
353 indicates that predator-prey interactions may be highly dependent on habitat-specific traits in relation
354 to refuge availability, predator efficiency, prey abundance, and other factors. It is well known that
355 predatory fish abundance and habitat attributes plays a critical and often complex role in determining
356 predation impact in marine ecosystems (Canion and Heck, 2009; Farina et al., 2009; Hereu et al.,
357 2005). These habitat-specific differences in predation have important implications given what we
358 know of the functioning of these ecosystems. Rocky macroalgal communities along the
359 Mediterranean (as in several regions) appear inherently vulnerable to urchin overgrazing and may be
360 subject to functional discontinuities once they switch to urchin barrens (Ling et al., 2014). A series of
361 feedbacks – continued scraping by urchins preventing recolonisation by algae, urchin bioerosion of
362 rocks creating their own refugia, etc – may make recovery very difficult past this threshold (Guidetti
363 et al., 2003). The generally high levels of predation experienced here may be critical in ensuring that
364 these points of discontinuity are not breached. In contrast, *P. oceanica* meadows may be less
365 “dependent” on top-down control as a process in maintaining ecosystem function (Prado et al., 2011;
366 Vergés et al., 2008). This may be due to the inherent ability of this seagrass to resist herbivory with
367 its suite of coping mechanisms (Ruiz et al., 2009) unless urchin density is particularly high.

368 Perhaps most interestingly, predation was clearly predictable in both seagrass systems and
369 macroalgal dominated rocky systems. Predation impact was a clear function of refuge availability
370 and fish predators. *D. sargus* has been previously described as the main sea urchin consumer inside
371 marine reserves (Guidetti, 2004; Sala, 1997); our results confirm that it may be a key agent of top-
372 down control in Mediterranean macroalgal habitats even outside these protected areas. Unfortunately
373 it also underscores the low functional redundancy these systems have, since no species appears to
374 substitute *D. sargus* when its numbers decline. *D. sargus* may be one of few extant species in the
375 Mediterranean capable of breaking the urchin carapace after they reach adulthood although several
376 other fish predators can be important secondary consumers (Guidetti, 2004; Sala, 1997). This
377 coupling of number and function validates the effectiveness of fishing restrictions in ensuring high
378 predation in areas that require conservation. It is important however, to account for the structural
379 complexity of these sites since, as this study and others indicate, refuge availability can critically
380 mediate predatory-prey interactions in rocky macroalgal systems (Clemente et al., 2012; Hereu et al.,
381 2005) and in seagrass meadows.

382 Our results show that fish interactions with their sea urchin prey are still prevalent across the
383 Mediterranean coast despite centuries of human use and are highly dependent on site specificities
384 and habitat characteristics. Given that the release from predatory functions can have cascading
385 effects (e.g. creation of urchin barrens in rocky bottoms), it is critical to maintain and protect
386 these higher trophic functions, particularly where they continue to be important. Identifying these
387 hotspots of functional predation may be an essential first step when establishing new marine reserves
388 to conserve macrophyte communities in temperate seas vulnerable to overgrazing events. This is
389 particularly important given that meso-predators do not appear able to replace fish, even when their
390 numbers decline, as the principal predator in coastal habitats. Our results indicate that, despite a long
391 history of fishing, conserving functional predation may still be achievable outside marine reserves
392 and is critical to ensure the resilience of ecosystems where top down processes still control the
393 structuring of ecological communities.

394

395 **References**

- 396 Aronson, R.B., Heck, K.L., Jr, 1995. Tethering experiments and hypothesis testing in ecology.
397 Mar. Ecol. Prog. Ser. 121, 307–309.
- 398 Bates, D., Maechler, M., Ben Bolker, Walker, S., 2014. Linear mixed-effects models using
399 Eigen and S4.
- 400 Boada, J., Sanmartí, N., Selden, R. L., Lucas, A., Pérez, M., Alcoverro, T., Romero, J., 2015.
401 Evaluating potential artifacts of tethering techniques to estimate predation on sea urchins. J.
402 Exp. Mar. Biol. Ecol. 471, 17–22. doi:10.1016/j.jembe.2015.05.011
- 403 Bonaviri, C., Vega Fernández, T., Badalamenti, F., Gianguzza, P., Di Lorenzo, M., Riggio, S.,
404 2009. Fish versus starfish predation in controlling sea urchin populations in Mediterranean
405 rocky shores. Mar. Ecol. Prog. Ser. 382, 129–138. doi:10.3354/meps07976
- 406 Boudouresque, C.-F., Verlaque, M., 2001. Ecology of *Paracentrotus lividus*. Elsevier.
407 doi:10.1016/S0167-9309(01)80013-2
- 408 Burkepile, D.E., Hay, M.E., 2007. Predator release of the gastropod *Cyphoma gibbosum*
409 increases predation on gorgonian corals. Oecologia 154, 167–173. doi:10.1007/s00442-
410 007-0801-4
- 411 Canion, C., Heck, K.L., Jr, 2009. Effect of habitat complexity on predation success: re-
412 evaluating the current paradigm in seagrass beds. Mar. Ecol. Prog. Ser. 393, 37–46.
413 doi:10.3354/meps08272
- 414 Clemente, S., Hernandez, J.C., Montaña-Moctezuma, G., Russell, M.P., Ebert, T.A., 2012.
415 Predators of juvenile sea urchins and the effect of habitat refuges. Mar Biol 160, 579–590.
416 doi:10.1007/s00227-012-2114-3
- 417 Clemente, S., Hernandez, J.C., Rodríguez, A., Brito, A., 2010. Identifying keystone predators
418 and the importance of preserving functional diversity in sublittoral rocky-bottom areas. Mar.
419 Ecol. Prog. Ser. 413, 55–67. doi:10.3354/meps08700
- 420 Estes, J.A., Terborgh, J., Brashares, J.S., Power, M.E., Berger, J., Bond, W.J., Carpenter, S.,
421 Essington, T.E., Holt, R.D., Jackson, J.B.C., Marquis, R.J., Oksanen, L., Oksanen, T., Paine,
422 R.T., Pickett, E.K., Ripple, W.J., Sandin, S.A., Scheffer, M., Schoener, T.W., Shurin, J.B.,

423 Sinclair, A.R.E., Soule, M.E., Virtanen, R., Wardle, D.A., 2011. Trophic Downgrading of
424 Planet Earth. *Science* 333, 301–306. doi:10.1126/science.1205106

425 Farina, S., Arthur, R., Pagès, J.F., Prado, P., Romero, J., Vergés, A., Hyndes, G., Heck, K.L.,
426 Glenos, S., Alcoverro, T., 2014. Differences in predator composition alter the direction of
427 structure-mediated predation risk in macrophyte communities. *Oikos* 123, 1311–1322.
428 doi:10.1111/oik.01382

429 Farina, S., Tomas, F., Prado, P., Romero, J., Alcoverro, T., 2009. Seagrass meadow structure
430 alters interactions between the sea urchin *Paracentrotus lividus* and its predators. *Mar. Ecol.*
431 *Prog. Ser.* 377, 131–137. doi:10.3354/meps07692

432 Garcia-Rubies, A., Hereu, B., Zabala, M., 2013. Long-Term Recovery Patterns and Limited
433 Spillover of Large Predatory Fish in a Mediterranean MPA. *PLoS ONE* 8, e73922.
434 doi:10.1371/journal.pone.0073922.s001

435 Guidetti, P., 2004. Consumers of sea urchins, *Paracentrotus lividus* and *Arbacia lixula*, in
436 shallow Mediterranean rocky reefs. *Helgol Mar Res* 58, 110–116. doi:10.1007/s10152-004-
437 0176-4

438 Guidetti, P., 2006. Marine reserves reestablish lost predatory interactions and cause community
439 changes in rocky reefs. *Ecological Applications* 16, 963–976.

440 Guidetti, P., Fraschetti, S., Terlizzi, A., Boero, F., 2003. Distribution patterns of sea urchins and
441 barrens in shallow Mediterranean rocky reefs impacted by the illegal fishery of the rock-
442 boring mollusc *Lithophaga lithophaga*. *Mar Biol* 143, 1135–1142. doi:10.1007/s00227-
443 003-1163-z

444 Guidetti, P., Sala, E., 2007. Community-wide effects of marine reserves in the Mediterranean
445 Sea. *Mar. Ecol. Prog. Ser.* 335.

446 Guidetti, P., Sala, E., Ballesteros, E., Di Franco, A., Hereu, B., Macpherson, E., Micheli, F.,
447 Pais, A., Panzalis, P., Rosenberg, A., Zabala, M., 2010. Fish assemblages across the
448 Mediterranean sea and the effects of protection from fishing. *Biol. Mar. Mediterr.* 17, 39-42.

449 Hairston, N.G., Smith, F.E., Slobodkin, L.B., 1960. Community Structure, Population Control,
450 and Competition. *The American Naturalist* 94, 421–425.

451 Harasti, D., Gallen, C., Malcolm, H., Tegart, P., Hughes, B., 2014. Where are the little ones:
452 distribution and abundance of the threatened serranid *Epinephelus daemeli*(Günther, 1876)
453 in intertidal habitats in New South Wales, Australia. *J. Appl. Ichthyol.* n/a–n/a.
454 doi:10.1111/jai.12446

455 Harmelin, J.G., Bouchon, C., Duval, C., Hong, J.S., 1980. Les échinodermes des substrats durs
456 de l'île de Port-Cros, Parc National (Méditerranée Nord-occidentale). ... *Trav Sci Parc*
457 *National de Port-Cros.*

458 Hereu, B., Zabala, M., Linares, C., Sala, E., 2004. Temporal and spatial variability in settlement
459 of the sea urchin *Paracentrotus lividus* in the NW Mediterranean. *Mar Biol* 144, 1011–1018.
460 doi:10.1007/s00227-003-1266-6

461 Hereu, B., Zabala, M., Linares, C., Sala, E., 2005. The effects of predator abundance and habitat
462 structural complexity on survival of juvenile sea urchins. *Mar Biol* 146, 293–299.
463 doi:10.1007/s00227-004-1439-y

464 Hitt, S., Pittman, S.J., Nemeth, R.S., 2011. Diel movements of fishes linked to benthic seascape
465 structure in a Caribbean coral reef ecosystem. *Mar. Ecol. Prog. Ser.* 427, 275–291.
466 doi:10.3354/meps09093

467 Kempf, M., 1962. Recherches d'écologie comparée sur *Paracentrotus lividus* (Lmk.) et *Arbacia*
468 *lixula* (L.) . *Rec Trav Stat Mar Endoume* 25, 47–116.

469 Levi, T., Wilmers, C.C., 2012. Wolves-coyotes-foxes: a cascade among carnivores. *Ecology* 93,
470 921–929.

471 Ling, S.D., Scheibling, R.E., Rassweiler, A., Johnson, C.R., Shears, N., Connell, S.D., Salomon,
472 A.K., Norderhaug, K.M., Perez-Matus, A., Hernandez, J.C., Clemente, S., Blamey, L.K.,
473 Hereu, B., Ballesteros, E., Sala, E., Garrabou, J., Cebrian, E., Zabala, M., Fujita, D.,
474 Johnson, L.E., 2014. Global regime shift dynamics of catastrophic sea urchin overgrazing.
475 *Philosophical Transactions of the Royal Society B: Biological Sciences* 370, 20130269–
476 20130269. doi:10.1098/rstb.2013.0269

477 McClanahan, T.R., Muthiga, N.A., 1989. Patterns of predation on a sea urchin, *Echinometra*
478 *mathaei* (de Blainville), on Kenyan coral reefs. *Journal of Experimental Marine Biology*

479 and Ecology 126, 77–94. doi:doi: 10.1016/0022-0981(89)90125-1

480 Menge, B.A., 2000. Top-down and bottom-up community regulation in marine rocky intertidal
481 habitats. *Journal of Experimental Marine Biology and Ecology* 250, 257–289.
482 doi:10.1016/S0022-0981(00)00200-8

483 Orth, R.J., Kenneth L Heck, S.J.S., van Montfrans, J., 1984. Faunal Communities in Seagrass
484 Beds: A Review of the Influence of Plant Structure and Prey Characteristics on Predator:
485 Prey Relationships. *Estuaries* 7, 339–350. doi:10.2307/1351618?ref=search-
486 gateway:be94717c1c97851b853d538e14b1c0c3

487 Pagès, J.F., Farina, S., Gera, A., Arthur, R., Romero, J., Alcoverro, T., 2012. Indirect
488 interactions in seagrasses: fish herbivores increase predation risk to sea urchins by
489 modifying plant traits. *Funct Ecol* 26, 1015–1023. doi:10.1111/j.1365-2435.2012.02038.x

490 Pauly, D., Christensen, V., Dalsgaard, J., Froese, R., Torres, F., Jr, 1998. Fishing Down Marine
491 Food Webs. *Science* 279, 860–863. doi:10.1126/science.279.5352.860

492 Pederson, H.G., Johnson, C.R., 2006. Predation of the sea urchin *Heliocidaris erythrogramma*
493 by rock lobsters (*Jasus edwardsii*) in no-take marine reserves. *Journal of Experimental*
494 *Marine Biology and Ecology* 336, 120–134. doi:10.1016/j.jembe.2006.04.010

495 Pinnegar, J.K., POLUNIN, N., Francour, P., Badalamenti, F., CHEMELLO, R., HARMELIN-
496 VIVIEN, M.-L., Hereu, B., Milazzo, M., Zabala, M., DANNA, G., 2000. Trophic cascades
497 in benthic marine ecosystems: lessons for fisheries and protected-area management.
498 *Environmental Conservation* 27, 179–200.

499 Prado, P., Collier, C.J., Romero, J., Alcoverro, T., 2011. Distinctive types of leaf tissue damage
500 influence nutrient supply to growing tissues within seagrass shoots. *Mar Biol* 158, 1473–
501 1482. doi:10.1007/s00227-011-1664-0

502 Prado, P., Romero, J., Alcoverro, T., 2009. Welcome mats? The role of seagrass meadow
503 structure in controlling post-settlement survival in a keystone sea-urchin species. *Estuarine,*
504 *Coastal and Shelf Science* 85, 472–478. doi:10.1016/j.ecss.2009.09.012

505 Prado, P., Tomas, F., Pinna, S., Farina, S., Roca, G., Ceccherelli, G., Romero, J., Alcoverro, T.,
506 2012. Habitat and Scale Shape the Demographic Fate of the Keystone Sea Urchin

507 Paracentrotus lividus in Mediterranean Macrophyte Communities. PLoS ONE 7, e35170.
508 doi:10.1371/journal.pone.0035170.t002

509 R Development Core Team, 2013. R: A language and environment for statistical computing. R
510 Foundation for Statistical computing, Vienna, Austria. <http://www.R-project.org/>.

511 Ripple, W.J., Estes, J.A., Beschta, R.L., Wilmers, C.C., Ritchie, E.G., Hebblewhite, M., Berger,
512 J., Elmhagen, B., Letnic, M., Nelson, M.P., Schmitz, O.J., Smith, D.W., Wallach, A.D.,
513 Wirsing, A.J., 2014. Status and Ecological Effects of the World's Largest Carnivores.
514 Science 343, 151–1162. doi:10.1126/science.1241484

515 Ruiz, J.M., Pérez, M., Romero, J., Tomas, F., 2009. The importance of herbivory in the decline
516 of a seagrass (*Posidonia oceanica*) meadow near a fish farm: An experimental approach.
517 Botanica Marina 52, 449–458. doi:10.1515/BOT.2009.053

518 Sala, E., 1997. Fish predators and scavengers of the sea urchin *Paracentrotus lividus* in
519 protected areas of the north-west Mediterranean Sea. Mar Biol.

520 Sala, E., Ballesteros, E., Dendrinis, P., Di Franco, A., Ferretti, F., Foley, D., Fraschetti, S.,
521 Friedlander, A., Garrabou, J., Güçlüsoy, H., Guidetti, P., Halpern, B.S., Hereu, B.,
522 Karamanlidis, A.A., Kizilkaya, Z., Macpherson, E., Mangialajo, L., Mariani, S., Micheli, F.,
523 Pais, A., Riser, K., Rosenberg, A.A., Sales, M., Selkoe, K.A., Starr, R., Tomas, F., Zabala,
524 M., 2012. The Structure of Mediterranean Rocky Reef Ecosystems across Environmental
525 and Human Gradients, and Conservation Implications. PLoS ONE 7, e32742.
526 doi:10.1371/journal.pone.0032742.t004

527 Sala, E., Zabala, M., 1996. Fish predation and the structure of the sea urchin *Paracentrotus*
528 *lividus* populations in the NW Mediterranean. Mar. Ecol. Prog. Ser. 140, 71–81.
529 doi:10.3354/meps140071

530 Savy, S., 1987. Les predateurs de *Paracentrotus lividus* (Echinodermata). Colloque International
531 sur *Paracentrotus lividus* et les oursins comestibles. GIS Posidonie, Marseilles, 37-57.

532 Shears, N., Babcock, R., 2002. Marine reserves demonstrate top-down control of community
533 structure on temperate reefs. Oecologia 132, 131–142. doi:10.1007/s00442-002-0920-x

534 Tegner, M.J., Dayton, P.K., 2000. Ecosystem effects of fishing in kelp forest communities.

535 ICES Journal of Marine Science: Journal du Conseil 57, 579–589.
536 doi:10.1006/jmsc.2000.0715

537 Underwood, A.J., 1981. Techniques of Analysis of Variance in Experimental Marine Biology
538 and Ecology.

539 Vergés, A., Pérez, M., Alcoverro, T., Romero, J., 2008. Compensation and resistance to
540 herbivory in seagrasses: induced responses to simulated consumption by fish. *Oecologia*
541 155, 751–760. doi:10.1007/s00442-007-0943-4

542 Verlaque, M., 1987. Relations entre *Paracentrotus lividus* (Lamarck) et le phytobenthos de
543 Méditerranée occidentale.

544 Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A., Smith, G.M., 2009. Mixed effects models and
545 extensions in ecology with R. Springer.

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547

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555

556 Table 1. Three-way ANOVAs for total and specific predation impact. p-values correspond to F-
 557 test results. d.f. = degrees of freedom.

Variable	Source of variation	d.f.	p-value
Total Predation	Site	7	< 0.001
	Habitat	1	< 0.001
	Season	1	0.062
	Site x Habitat	7	< 0.001
	Site x Season	7	< 0.001
	Habitat x Season	1	0.117
	Site x Habitat x Season	7	0.523
Predation Fish	Site	7	< 0.001
	Habitat	1	< 0.001
	Season	1	0.009
	Site x Habitat	7	< 0.001
	Site x Season	7	0.005
	Habitat x Season	1	0.202
	Site x Habitat x Season	7	0.494
Predation Benthic	Site	7	0.033
	Habitat	1	0.016
	Season	1	0.017
	Site x Habitat	7	0.092
	Site x Season	7	0.003
	Habitat x Season	1	0.601
	Site x Habitat x Season	7	0.003

558

559 Table 2. ANOVA analyses for predator habitat use U (see text) and the canopy height. p-values
 560 correspond to those provided by an F-test. d.f., degrees of freedom.

Variable	Source of variation	d.f.	p-value
Predators	Site	7	< 0.001
	Habitat	1	< 0.001
	Season	1	0.018
	Site x Habitat	7	< 0.001
	Site x Season	7	0.005
	Habitat x Season	1	0.236
	Site x Habitat x Season	7	0.493
	Canopy	Site	7
Habitat		1	< 0.001
Season		1	< 0.001
Site x Habitat		7	< 0.001
Site x Season		7	< 0.001
Habitat x Season		1	< 0.001
Site x Habitat x Season		6	< 0.001

561

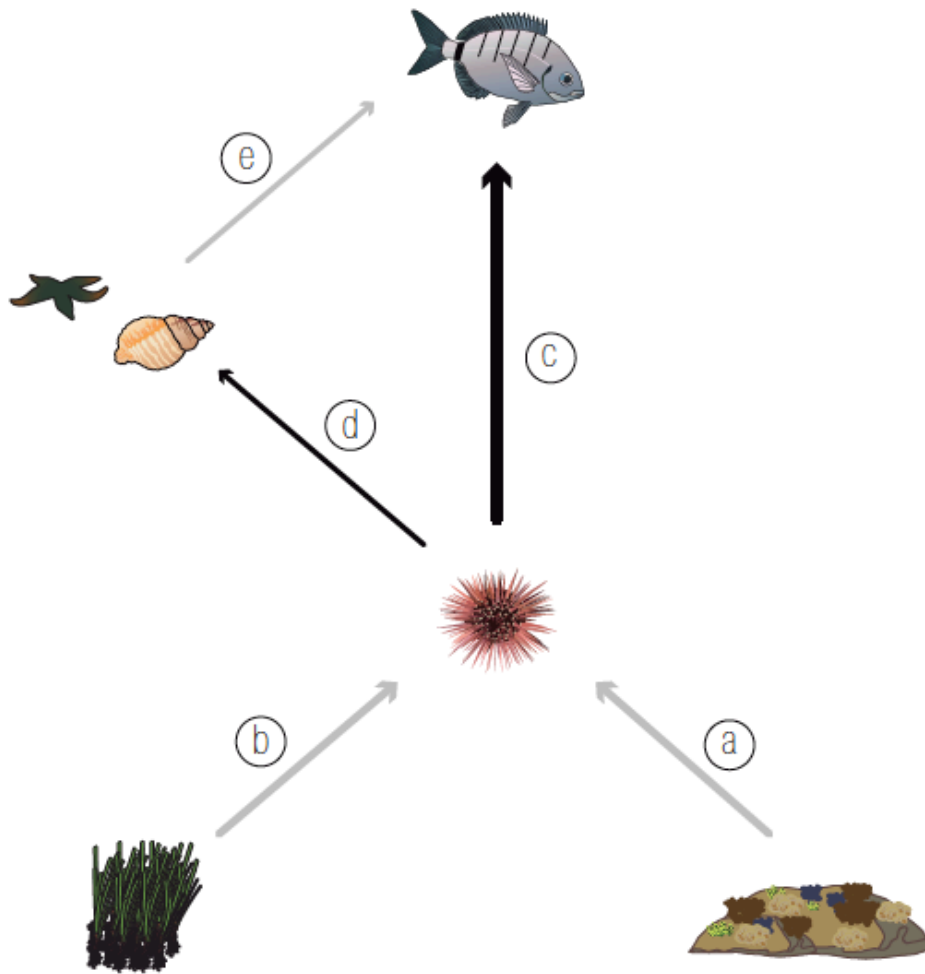
562

563 Table 3. Model fitting using GLM for fish predator impact (FPI) inside rocky habitats and
 564 *Posidonia oceanica* meadows as a response variable dependent on predator species use of
 565 habitat (U) and specific structural parameters of each habitat.
 566

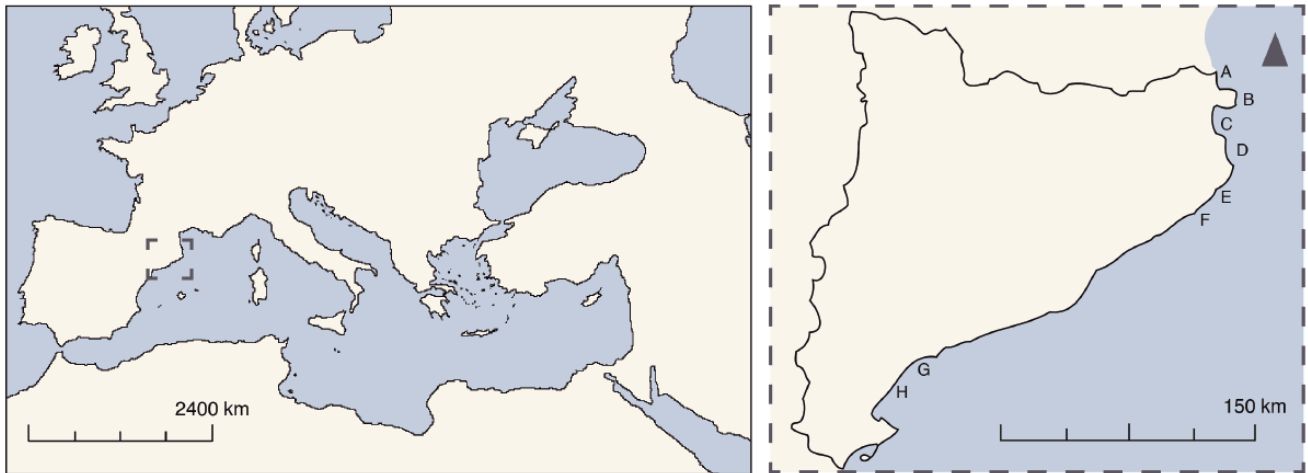
Habitat	Response variable	Full model	Selected model	Effects	df	p-value
Rocky habitat	Predation Fish	~Canopy + Shelters + <i>Diplodus spp</i> + <i>S. aurata</i> + <i>L. merula</i>	~Shelters + <i>Diplodus spp</i>	Shelters	1	0.009
				<i>Diplodus spp</i>	1	0.008
	Predation Benthic	Insufficient data				
Posidonia	Predation Fish	~Canopy + Matte + <i>Diplodus spp</i> + <i>S. aurata</i> + <i>L. merula</i>	~Matte + <i>Diplodus spp</i>	Matte	1	0.012
				<i>Diplodus spp</i>	1	0.001
	Predation Benthic	Insufficient data				

567

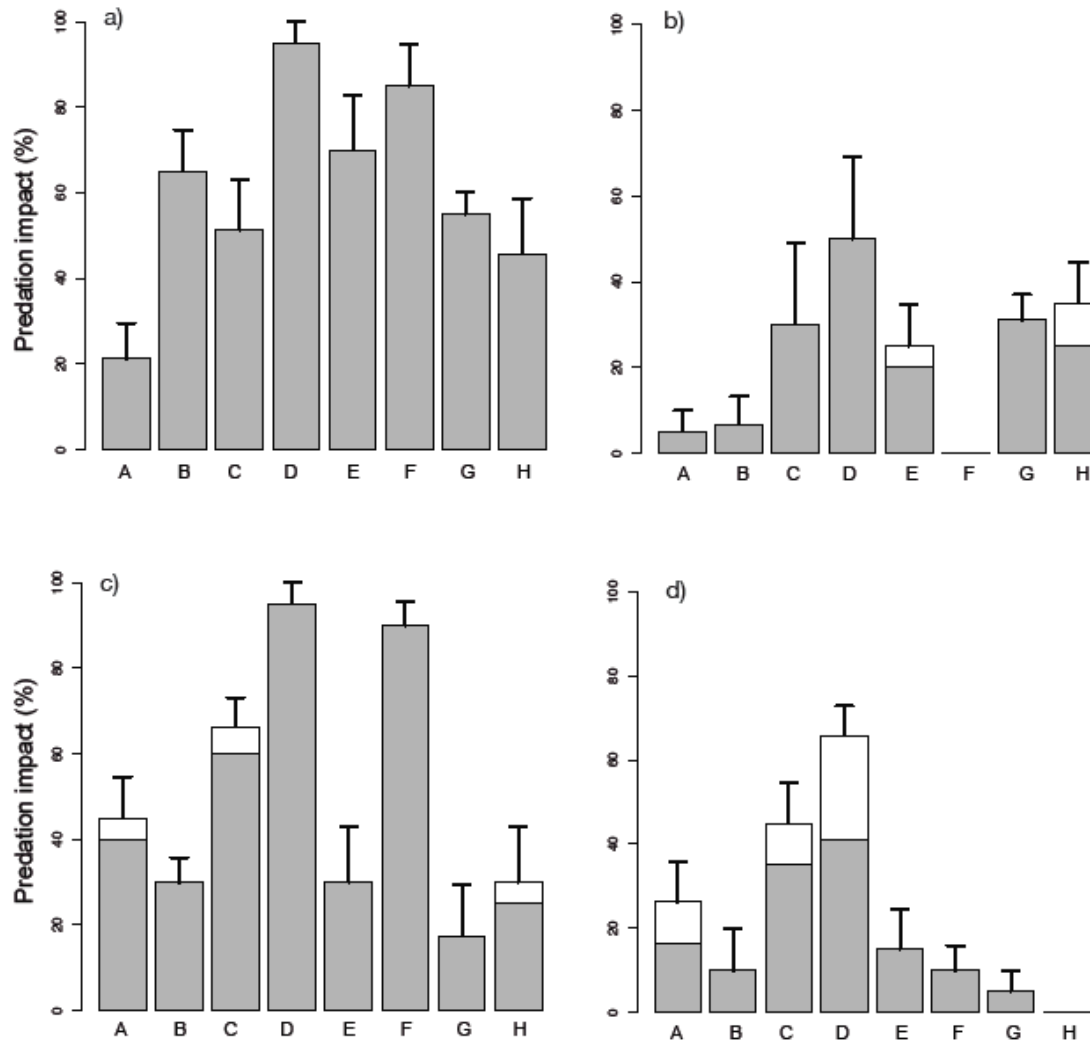
568 Figure 1. Principal interactions in Mediterranean macrophyte communities. The sea urchin
569 *Paracentrotus lividus* lives in both seagrass meadows of *Posidonia oceanica* and macroalgal
570 dominated rocky habitats in the Mediterranean. Letters represent trophic interactions; herbivory
571 (a) and (b), and predation (c), (d) and (e). Black arrows show the predator-prey interactions
572 studied in the present work.
573



574 Figure 2. Map of the Mediterranean Sea showing the study locations within the Catalan Coast.
575 Llançà (A), Portlligat (B), Montgó (C), Medes Islands (D), Giverola (E), Fenals (F), Hospitalet
576 (G) and Ametlla de Mar (H).
577



578 Figure 3. Mean predation impact on *P. lividus* (%) + SE in a) rocky habitats in summer, b) *P.*
 579 *oceanica* meadows in summer, c) rocky habitats in winter and d) *P. oceanica* meadows in
 580 winter. Filled bars represent fish predator impact (FPI) and clear bars represent benthic predator
 581 impact (BPI). Total absence of predation (0%) in sites F and H in b) and a) respectively.
 582



583 Figure 4. Percentage of urchins eaten (a), index of habitat use by sea urchin fish predator guild
584 (b) and length of the canopy height (c) in both habitats. Mean values of two seasons \pm SE, n=64.
585 Asterisk indicates significant differences based on ANOVA test.
586

