

1 **Different kelp life stages modulated by herbivory: compensatory growth versus**
2 **population damage**

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27 **Abstract**

28 Partitioning the effects of herbivory on the different life stages of primary producers is
29 key to **understanding** the population-wide consequences of herbivory. We assessed the
30 performance of microscopic (MiS<1 mm) juveniles, macroscopic (MaS) juveniles and
31 adult kelp (*Laminaria ochroleuca*) under contrasting herbivory regimes through a
32 herbivore exclusion field experiment. The abundance of MiS and the survival of MaS
33 decreased by 67% and 63%, respectively, when herbivorous fishes and sea urchins were
34 present. **Blade growth (linear and area)** of adult kelp displayed contrasting patterns
35 under herbivore pressure: a 60% increase and a 46% decrease, respectively. These
36 results indicate that while herbivory severely reduces juvenile survival, it induces
37 compensatory growth (**measured as linear growth**) in adult kelp. In summary, we have
38 here demonstrated how herbivory affects all sporophyte life stages of the kelp *L.*
39 *ochroleuca*. This is likely to have important implications for all situations where
40 historical patterns of herbivore presence and herbivory are changing, such as in many
41 temperate to tropical transition zones around the world.

42

43 **Keywords:** Atlantic Ocean; climate; exclusion; grazing; kelp forests; *Laminaria*
44 *ochroleuca*; Portugal; plant-herbivore interaction; range limit; southern Europe

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50 **Introduction**

51 Plant-herbivore interactions play a critical role in the dynamics of populations and
52 assemblages across different habitats (Burkepile 2013) and productivity gradients
53 (Proulx and Mazumder 1998). Green food webs, where herbivore–plant interactions
54 play a significant role in energy transfer, are ubiquitous (e.g. Gaines & Lubchenco
55 1982, Duffy & Hay 2000, Moles et al. 2011) and the strength of such interactions
56 shapes the patterns of distribution and abundance of many species involved (Maron &
57 Crone 2006). Consequently, changes in the direction, frequency, and intensity of
58 herbivory can ultimately lead to substantial shifts in the structure of whole ecosystems
59 (Pace et al. 1999; Bruno and O’Connor 2005; Vergés et al. 2014; Hanley and La Pierre
60 2015). This is of particular concern since ongoing global pressures, including climate
61 change, may impair biotic interactions through direct effects on both primary producers
62 (e.g. reducing net productivity and/or diversity) and herbivores (e.g. changing behavior
63 and/or diversity).

64 In producer-based food webs, understanding plant-herbivore interactions is a
65 challenging requirement for addressing a number of relevant questions, from predicting
66 ecological dynamics to managing ecosystems under natural and anthropogenic
67 pressures. In this context, it is important to understand the impact of herbivory over the
68 lifetime of marine plants, which can be exposed to different levels of herbivory as they
69 develop through different life stages. As a result, the impact of herbivores on plant
70 resistance traits can vary with plant ontogeny, promoting changes in the amount and
71 type of resistance traits during the development of the plant to minimize the impacts of
72 herbivory (Boege and Marquis 2005). These changes can also be shaped by
73 demographic priorities such as establishment, growth, or reproduction of the individuals
74 (Boege and Marquis 2005). Intraspecific variation in resistance to herbivory, including

75 the different life stages of primary producers, should be tackled as an important element
76 of plant–herbivore interactions. However, the vast majority of studies currently focus on
77 effects on a single species and at a single ontogenetic stage; very few studies examine
78 effects on multiple life stages of habitat-forming species, leading to population-level
79 extrapolations that might be misleading or incomplete (Boege and Marquis 2005;
80 Vergés et al. 2008).

81 Kelp are foundation species (Dayton 1972) in many near-shore temperate areas,
82 where there is wide evidence that their patterns of abundance, distribution and
83 functioning may be critically shaped by herbivores (Zarco-perello et al.; Franco et al.
84 2015a). **Kelp** possess natural defences against herbivores through a range of
85 mechanisms, e.g. physiological adaptation, physical and associative traits, chemical
86 defence and compensatory growth (Targett and Coen 1992; Gagnon et al. 2003; Cerda
87 et al. 2009; Biskup et al. 2014). However, ‘extreme’ changes in herbivory levels may
88 compromise the resilience of whole communities supported by these seaweeds (Steneck
89 et al. 2002; Bennett et al. 2015a; Vergés et al. 2016). Notable examples of this include
90 the effects of sea-urchins which, through their intense grazing activity, may lead to the
91 eradication of erect algae and their replacement with ‘barren’ areas (Ling et al. 2010).
92 Negative effects of herbivory on kelp are also reported for tropical fishes currently
93 expanding into temperate regions (Zarco-perello et al.; Vergés et al. 2014, 2016;
94 Bennett et al. 2015a).

95 Declines in abundance and induced herbivore- and/or temperature-driven phase
96 shifts of kelp at regional or local **scales** have been extensively documented (e.g. Filbee-
97 Dexter and Scheibling 2014; Ling et al. 2014). In Europe, the causes for changes in kelp
98 abundance are generally reported as being species-dependent and regionally variable
99 (Araújo et al. 2016). For example, declines in northern and central Europe have mainly

100 been attributed to human overharvesting of kelp and/or overgrazing by sea urchins
101 (Sivertsen 2006; Raybaud et al. 2013). In southern Europe, increasing ocean
102 temperature is a likely driver of declining kelp forests (Fernandez 2011; Voerman et al.
103 2013; Assis et al. 2016). In this context, Franco et al. (2015) recently reported that kelp
104 from warmer, southern, locations along the Portuguese coast are exposed to more
105 intense herbivory pressure than kelp from colder, northern, locations, suggesting
106 indirect effects of temperature through herbivory could also play a key role in southern
107 Europe.

108 The Portuguese coast spans more than 800 kilometres and is recognized as an
109 important transitional zone between north-eastern Atlantic warm-temperate and cold-
110 temperate species, which makes this coast an area of great sensitivity to the effects of
111 climate change (Tuya et al. 2012; Teixeira et al. 2014). A large number of cold- and
112 warm-water species have their southern, or northern, distributional range edges along
113 the west coast of the Iberian Peninsula. These include the cool-water kelp *Laminaria*
114 *hyperborea* and *Saccharina latissima* (Lima et al. 2007). The number of records of fish
115 and algal species with sub-tropical affinities extending northwards, relative to their
116 usual **distributional** range or increasing in abundance, have become more frequent in
117 the last decade (Lima et al. 2007; Bañón and Mucientes 2009; Rodrigues et al. 2012;
118 Piñeiro-Corbeira et al. 2016).

119 The present study aimed at examining the influence of herbivory on the ‘golden
120 kelp’, *Laminaria ochroleuca*, in central Portugal. This warm-water species is currently
121 expanding its **distributional** range northwards, locally competing with the native species
122 *Laminaria hyperborea* (Smale et al. 2015). We excluded macro-herbivores (fishes and
123 sea urchins) from experimental plots and, subsequently, estimated the abundance,
124 survival and growth of *L. ochroleuca* compared to control plots under herbivory. The

125 responses of *L. ochroleuca* were examined for different development stages, including
126 the abundance of microscopic (<1 mm) juveniles, the survival of macroscopic (between
127 3.5 and 4.5 cm, lamina length) juveniles and the growth of adult (> 25 cm, lamina
128 length) individuals. This allowed partitioning the effects of herbivory on three life
129 stages of a kelp. Specifically, we have tested the hypothesis that herbivory would exert
130 a negative impact on *L. ochroleuca* at different life stages, but that such an effect could
131 be, at least in part, counteracted by compensatory growth of adult individuals.

132

133 **Materials and Methods**

134 **Study region and abiotic characterization**

135 The study was carried out at Peniche, central Portugal (39.2° N, 9.2° W). This is
136 considered a warm-temperate region on the Portuguese west coast (Tuya et al. 2012),
137 with recent evidence of intense herbivore pressure on kelp (Franco et al. 2015).
138 Macroalgal assemblages in the study region include perennial kelp species, which were
139 once abundant and now show a sparse distribution, such as *Laminaria ochroleuca*, or
140 annual species, such as *Phyllariopsis* sp. (Assis et al. 2009; Tuya et al. 2012; Pinho et
141 al. 2015). To the abiotic characterization of the study area a set of data loggers were
142 deployed throughout the course of the experiment to measure sea water temperature
143 (°C) and illuminance (Lux) (HOBO data-loggers Pendant Temp-Light, Onset Computer
144 Corporation), and water movement (m s^{-2}) using gravitational data loggers (HOBO
145 Pendant G, Onset Computer Corporation), following (Bennett et al. 2015b). The loggers
146 were set to record the temperature and illuminance at 15 min intervals, and acceleration
147 at 6 min intervals. For light, loggers were cleaned on a weekly basis to avoid fouling
148 that would interfere measurements. Mean levels of illuminance between 08:00 to 17:00
149 (n =1,878 measurements) were extracted. For water movement (WM), all values

150 (n=26,160 measurements) were extracted. Water samples were collected, at six
151 occasions (two randomly dates within every 30 days), water samples (n = 4),
152 approximately 1 m above the bottom, and immediately filtered and stored on ice until
153 return to land. Samples were then frozen (-20°C) until chemical analysis for NO_3 and
154 PO_4 , which was performed through a colorimetric auto-analyzer (Skalar® SAN Plus
155 Segmented Flow Analyser). For representativeness of the region, this study was
156 replicated in two haphazardly chosen sites, ~ 6 km apart at 5-7 m depth, with similar
157 rocky substrate in terms of bottoms topography. The inclusion of two sites added the
158 natural variation in herbivory associated with inter-reef variation in the abundances of
159 kelp herbivores in the system (Franco et al. 2015).

160

161 **Macro-herbivores abundance in the study region**

162 Macro-herbivore abundances were visually assessed in July 2014 and July 2015 at five
163 rocky reefs chosen at random within the study region. On each reef, all adult and sub-
164 adult fishes were counted along five, randomly oriented, 25 x 4 m belt transects (Tuya
165 et al. 2012). Fishes were categorized according to their trophic affinities (according to
166 Henriques et al. 2013; fishbase.org). Herbivorous fishes were considered as those able
167 to consume algae, thus also including omnivorous species (Franco et al. 2015a). The
168 number of *Paracentrotus lividus*, the most common sea-urchin in continental Portugal
169 (Jacinto et al. 2013), was counted along five 25 x 2 m transects.

170

171 **Experimental design**

172 A caging experiment was set up to evaluate the exclusion of macro-herbivores on the
173 survival and growth of *L. ochroleuca* at two sites within the study region. At each site,
174 steel cages (50 x 50 x 50 cm) were attached to the bottom through stainless steel

175 eyebolts (Fig. S1). At each site, six cages were randomly allocated to each of three
176 experimental treatments, for a total of 36 cages: exclusion of macro-herbivores (full
177 cage: F), procedural control (partial cage with a roof and two half-sides, thus allowing
178 access to herbivores: P), and unmanipulated control (50 x 50 cm plots just marked at
179 corners with eyebolts: C). The cages (plots) were covered by polyethylene net (2 cm
180 mesh size). Cages were randomly assigned to plots to ensure any small-scale effects of
181 illuminance and water movement did not confound herbivore exclusion. Moreover, both
182 illuminance (HOBO data-loggers Pendant Temp-Light, Onset Computer Corporation)
183 and water movement (HOBO Pendant G, Onset Computer Corporation) were compared
184 between a randomly chosen experimental replicate allocated to the F, P and C
185 treatments. Measurements of illuminance and water movement across all treatments
186 showed no cage artefacts (Fig. S2a, S2b, S3a, S3b, methodological details and data in
187 supplementary).

188

189 **Set-up and sampling of *L. ochroleuca* abundance, survival and growth**

190 Three different life stages of *L. ochroleuca* were placed in each experimental plot
191 (n=6, Fig. S1): (a) two adult individuals (total length >25cm), fastened by the holdfast
192 to a 16 x 4 cm PVC tile using a rubber-protected cable tie that allowed the natural
193 movement of each kelp; (b) four epoxy surface discs (total disk area = 2,460 mm²)
194 screwed to a PVC tile with microscopic (< 1 mm) sporophytes (MiS) (440 ± 50, mean
195 ± SE, n=144 discs); (c) 10 macroscopic (lamina length between 3.5 and 4.5 cm) juvenile
196 sporophytes (MaS) distributed on a 15 cm long cotton rope attached to a PVC tile.

197 All adult individuals were collected at Mindelo (41.1°N, 8.74° W) and
198 individually tagged with a numbered cable tie around the stipe. The blade area of each
199 individual was measured, before the start of the experiment, by taking a picture over a

200 blank scaled surface where the blade was stretched by an acrylic transparent board. The
201 same procedure was repeated at the end of the experiment. Using Image J (Muth 2012),
202 the contrast analyses (before vs. after) of kelp laminas provided, for each individual, the
203 growth during the course of the experiment. The linear growth of adult *L. ochroleuca*
204 was estimated using the hole-punch method (Parke 1948). At the deployment day, two
205 holes were punched in the centre of the lamina, at 2 and 4 cm above the main meristem
206 at the junction between the stipe and the main blade. Thallus extension was assessed *in*
207 *situ* every month after deployment (July, August and September 2014) by measuring the
208 distance between the two holes and the meristem and subtracting the initial 4 cm
209 distance. The total linear growth was calculated as the sum of all months.

210 Four weeks before the experiment started, gametophytes of *L. ochroleuca* from
211 adults collected at Mindelo were cultivated in the laboratory (adapted from guidelines
212 described in Pereira et al. 2011). The gametophyte solution resulting from the
213 cultivation was then left in contact with sterile epoxy discs to allow them to become
214 homogenized, fixed to the disk and **develop into** to MiS. **The number of individuals**
215 **growing on the seeded discs were counted *in situ* at the end of the experiment and final**
216 **differences in MiS abundance between treatments result from varying herbivory**
217 **pressure rather than from initial differences in MiS abundances between treatments.** The
218 same procedure was performed to obtain MaS, but cultivation started earlier to allow the
219 growth until the desired size; a cotton rope was used instead of epoxy discs. The
220 number of living individuals were counted *in situ* at the end of the experiment. All the
221 biological material was kept in an aerated 500 L outdoor tank at 15 °C until deployment.
222 The experiment ran from the 14 June to the 30 September 2014, including weekly visits
223 for cleaning, maintaining and opening cages when needed for measurements.

224

225 **Statistical analyses**

226 The total linear growth, the total blade area growth, the abundance of MiS and the
227 survival of MaS, at the end of the experiment, were analysed by 2-way ANOVA,
228 including the crossed factors 'Site' (2 levels, random) and 'Herbivore exclusion' (3
229 levels: F, P and C, fixed). The same model was used to analyse differences in the linear
230 growth of *L. ochroleuca* separately for each of three sampling times. Before each
231 ANOVA, the assumption of homogeneity of variances was checked with Cochran's *C*
232 test. **Percentage of survival** of MaS were Arcsin transformed to achieve homogeneous
233 variances. When relevant, post-hoc comparisons of significant means were carried out
234 with Student-Newman-Keuls (SNK) tests.

235

236 **Results**

237 **Abiotic characterization and abundance of macro-herbivores**

238 During the experiment (July to September 2014), the water temperature ranged between
239 14.4 and 21.5 °C (Fig. 1a), the mean illuminance at the bottom between 1,296 and
240 13,395 Lux (Fig. 1b) and the water movement between 0.16 and 1.82 m s⁻² (Fig.1c).
241 The concentration of nitrate varied from 1.82 to 1.38 μmol L⁻¹, while that of phosphate
242 between 1.69 and 1.80 μmol L⁻¹, respectively (Fig.1d).

243 Abundance of herbivorous fishes was not different between reefs or among
244 surveys (Table 1, Fig. 2a), while abundance of sea urchins varied significantly between
245 reefs but not among surveys (Table 1, Fig 2b). Macro-herbivores including omnivorous
246 species able to consume algae, were dominated by four fish species: *Sarpa salpa*,
247 *Oblada melanura*, *Diplodus vulgaris* and *Diplodus sargus* (out of 13 recorded fish
248 species; Table S1 in the supplementary material) and one sea urchin, *Paracentrotus*
249 *lividus* (Fig. 2b).

250

251 **Abundance of MiS and survival of MaS**

252 The abundance of MiS was, **on average**, 60 and 73% times greater within full cages
253 compared to procedural and the unmanipulated controls (Table 1, Fig. 3a), respectively.

254 The survival of MaS was, **on average**, 68 and 72% higher where herbivores were
255 excluded relative to procedural and unmanipulated controls, respectively (Table 1, Fig.
256 3b).

257

258 **Blade area and linear growth**

259 Total blade area growth in the herbivory-excluded treatment was larger compared to
260 both procedural and unmanipulated controls, with, respectively, an increase by $101.7 \pm$
261 26.2% and a decrease by $32.5 \pm 9.3\%$ and $58.5 \pm 8.7\%$ relative to the initial blade area
262 (Table 1, Fig. 4). Conversely, the total linear growth, at the end of the experiment, **was**
263 significantly reduced within full cages compared to procedural and unmanipulated
264 controls (59.6 ± 1.2 and $60.7 \pm 1.1\%$, respectively, Table 1, Fig. 4). The mean monthly
265 linear growth was, along the three measured times, also significantly reduced within full
266 cages compared to procedural and unmanipulated controls (60.3 ± 1.1 and $61.0 \pm 1.6\%$,
267 respectively, Table 1, Fig. 5).

268

269 **Discussion**

270 We have demonstrated that the survival of both microscopic and macroscopic juveniles,
271 and lamina extension of adult *L. ochroleuca* were considerably increased when released
272 from herbivory by sea urchins and fishes. Consequently, this study showed clear effects
273 of herbivory for different life stages of this kelp. Our findings are consistent with a
274 previous study from the study region, indicating that high intensity of herbivory could

275 be contributing to limiting kelp distribution and abundance in southern Europe (Franco
276 et al. 2015).

277 Herbivory is often selective and effects frequently vary according to algal life-
278 stages. Indeed, a change of herbivores food preferences can occur as algae mature; a
279 species that is a preferred food for juveniles is not necessarily a preferred food for
280 adults. Differences between juvenile and adult algae in a number of physical and
281 chemical properties, e.g. texture, deterrent and nitrogen concentration, are within the
282 causes that may affect herbivore food preferences (Van Alstyne et al. 1999). Even
283 though we were not able to quantify the variation of herbivory among different kelp life
284 stages, our study demonstrated large herbivory pressure in each of the studied life-
285 stages of *L. ochroleuca*, as previous studies with other kelp species have demonstrated
286 (Van Alstyne et al. 2001). Herbivorous sea urchins and fishes may reduce the
287 population-level biomass of *L. ochroleuca* through both the consumption of blades of
288 adult individuals and the elimination of small-sized sporophytes. Such biological
289 disturbance is likely to compromise the future of this habitat-forming species which,
290 once apparently abundant and widespread across Portugal, is already sparse in central
291 and southern Portugal (Assis et al. 2009; Tuya et al. 2012; Pinho et al. 2015). It has
292 been reported that damage induced by herbivorous fishes and sea urchins on adult kelp
293 may lead to a critical reduction in their physiological performance and/or extent of
294 reproductive tissue, which can ultimately cause death (Davenport and Anderson 2007).
295 In extreme situations, overgrazing can provoke the collapse of entire kelp forests
296 (Filbee-Dexter and Scheibling 2014; Ling et al. 2014; Vergés et al. 2016). At the same
297 time, the consumption of kelp recruits can prevent the establishment of new adult
298 populations (or recovery of old populations lost to other processes, Bennett et al.
299 (2015)), although such an adverse effect can be mitigated by the occurrence of natural

300 refuges, e.g. crevices in the substratum, providing protection to juvenile kelp (Franco et
301 al. 2015). Moreover, the effects of large herbivores can be further exacerbated by
302 mesoherbivores, such as gastropods, amphipods and isopods, which were not examined
303 in this study. In some cases, it has been shown that mesoherbivores may
304 disproportionately compromise the individual fitness and the structure of whole
305 populations of kelp (Poore et al. 2014).

306 The evidence for strong kelp-herbivore interactions is especially relevant in
307 transition zones such as the study region in Portugal, where temperature increments can
308 have profound implications for local marine communities (Lima et al. 2006; Wernberg
309 et al. 2016). Warming temperatures can strengthen plant-herbivore interactions
310 (O'Connor 2009), particularly at the tropical-temperate boundary (Zarco-perello et al.;
311 Vergés et al. 2014). For example, a complete shift from a temperate kelp forest to a
312 system dominated by tropical and sub-tropical species was described in western
313 Australia following a marine heatwave (Wernberg et al. 2016). **Sea-water temperature**
314 **has recently been described as an important driver for the distributional patterns and**
315 **growth of *L. ochroleuca* (Franco et al. *in press*). In addition, climate-driven expansion**
316 **of herbivorous fishes, and increasing abundance of existing herbivores, have been**
317 **reported along the coast of Portugal (Franco et al. 2015). Despite sea-water temperature**
318 **was within the optimal range (< 20 °C, Franco et al, *in press*) throughout the**
319 **experiment, our results likely reflect the cumulative effects of a range of biotic and**
320 **abiotic influences eroding kelp forests.**

321 Kelp have natural responses against herbivores, including physical and/or
322 chemical defences (reviewed by Bartsch et al. 2008). Compensatory growth is
323 considered an alternative strategy, which has been widely reported for terrestrial plants,
324 but only in a few cases demonstrated for macroalgae (Hay et al. 2011), **including kelp**

325 (Cerda et al. 2009, Gao et al. 2013). In this experiment, adult kelp exposed to herbivores
326 displayed higher elongation rates of the meristematic tissue (i.e. linear growth). To our
327 knowledge, this ability has never been shown for *L. ochroleuca* and, as far as we are
328 aware, has only been described for two kelp species, *Macrocystis pyrifera* (Cerda et al.
329 2009) and *Undaria pinnatifida* (Gao et al. 2013). The development of reproductive
330 structures (sori) in *L. ochroleuca* occurs through the transition from the vegetative to the
331 reproductive stage (Bartsch et al. 2008) and this mechanism may allow to compensate
332 the loss of blade tissue. The same was reported for *Lessonia nigrescens*, a kelp species
333 with similar reproductive structures as *L. ochroleuca* (Pansch et al. 2008). However, the
334 effectiveness of this compensation mechanism may vary through the year. Gao et al.
335 (2013) demonstrated increased growth of *Undaria pinnatifida*, compensating artificial
336 excision during the growing season, but not during the maturation season encompassed
337 within the annual life cycle. In this experiment, *L. ochroleuca* showed a reduction of
338 linear growth over the experimental period, which started in June and finished in
339 September, corresponding to its growing and early maturation season, respectively. It is
340 worth noting, however, that our experimental procedures could only control for the
341 exclusion of herbivory, but not for its intensity. Therefore, it cannot be excluded that,
342 analogously to other Laminariales, *L. ochroleuca* is unable to compensate extreme
343 losses of blade biomass at very high herbivory pressure (Cerda et al. 2009).

344 In conclusion, the present study showed compensatory growth of adult *L.*
345 *ochroleuca* in response to herbivory. However, this ability was counterbalanced by
346 negative effects on juvenile kelp, including their micro and macroscopic phases.
347 Negative effects of simultaneous herbivory pressure at different life stages are likely
348 expected to be more evident in sparse kelp populations and/or in regions where patterns
349 of herbivory are more prone to change (e.g. central Portugal). In conjunction with

350 environmental perturbations, such as changes in seawater temperature this herbivore
351 pressure might contribute to the loss of these important habitat formers and,
352 consequently, the loss of associated ecological and economic goods and services.

353

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366

367 **Conflict of interest**

368 The authors declare that they have no conflict of interest.

369

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541 consumer pressure on habitat-forming seaweeds.

542

543 **Table 1. Results of ANOVAs on the abundance of herbivorous fishes and sea urchins**
 544 **according to years (2014 vs. 2015) and reefs, and on effect of exclusion of herbivores at**
 545 **two reef sites on the abundance of microscopic sporophytes (MiS), the survival of**
 546 **macroscopic sporophytes (MaS), the total blade area growth, the total linear growth and**
 547 **the monthly linear growth of *Laminaria ochroleuca*.**

	Transformation	Cochran's	df	MS	F	P
Abundance of grazing fishes	none	C = 0.119 (Ns)				
Year			1	69.62	0.55	0.479
Reef (Year)			8	126.22	0.76	0.639
Residual			40	165.90		
Abundance of sea-urchin	Ln(X+1)	C = 0.291 (Ns)				
Year			1	11.4317	0.85	0.3832
Reef (Year)			8	13.4276	11.11	<0.001
Residual			40	1.2091		
Abundance of MiS	none	C = 0.330 (Ns)				
Site			1	2500.00	1.45	0.238
Protection			2	56319.44	38.62	0.025
Site × Protection			2	1458.33	0.85	0.439
Residual			30	1722.22		
Survival of MaS	ArcSin %	C = 0.302 (Ns)				
Site			1	51.32	3.15	0.0861
Protection			2	494.16	70.7	0.013
Site × Protection			2	6.99	0.43	0.655
Residual			30	16.29		
Total blade area growth	none	C = 0.413 (P < 0.01)				
Site			1	7545.04	0.17	0.680
Protection			2	1540214.40	112.28	0.009
Site × Protection			2	13717.62	0.31	0.733
Residual			66	43957.98		
Total linear growth	none	C = 0.2193 (Ns)				
Site			1	690.68	2.11	0.152
Protection			2	239936.10	752.12	0.001
Site × Protection			2	319.01	0.97	0.384
Residual			66	328.02		
July: Linear growth	none	C = 0.214 (Ns)				
Site			1	2	0.01	0.9275
Protection			2	68065.71	83.34	0.011
Site × Protection			2	816.69	3.41	0.0389
Residual			66	239.42		
August: Linear growth	none	C = 0.286 (Ns)				
Site			1	288	3.93	0.5165
Protection			2	51411.88	455.2	0.002
Site × Protection			2	112.94	1.54	0.2217
Residual			66	73.27		
September: Linear growth	none	C = 0.283 (Ns)				
Site			1	34.72	0.73	0.3968
Protection			2	43874.06	12523	<0.001
Site × Protection			2	3.5	0.07	0.9293
Residual			66	47.73		

549 **Figure Captions**

550

551 Figure 1. Abiotic characterization of the study region. (a) Temperature daily mean
552 values, (b) mean illuminance between 8:00-17:00h, (c) water movement every 6
553 minutes, during the experimental period, and (d) mean nutrient concentration at three
554 random times through the course of the experiment.

555

556 Fig. 2. (a) Abundance of grazing fishes and (b) sea urchins in 2014 and 2015 (mean +
557 SE, n = 5).

558

559 Fig 3. (a) Abundance of microscopic sporophytes and (b) survival of macroscopic
560 sporophytes (mean + SE, n=6 and n=6, respectively) for each experimental treatment at
561 the end of the study. Different letters above bars indicate significant differences.

562

563 Fig 4. Total blade area and linear growth of adult sporophytes (mean + SE, n=6) for
564 each experimental treatment at the end of the study. Different letters above/below bars
565 and dots indicate significant differences.

566

567 Fig 5. Monthly linear growth of adult sporophytes (mean + SE, n=6) for each
568 experimental treatment. For each month, different letters above bars indicate significant
569 differences.

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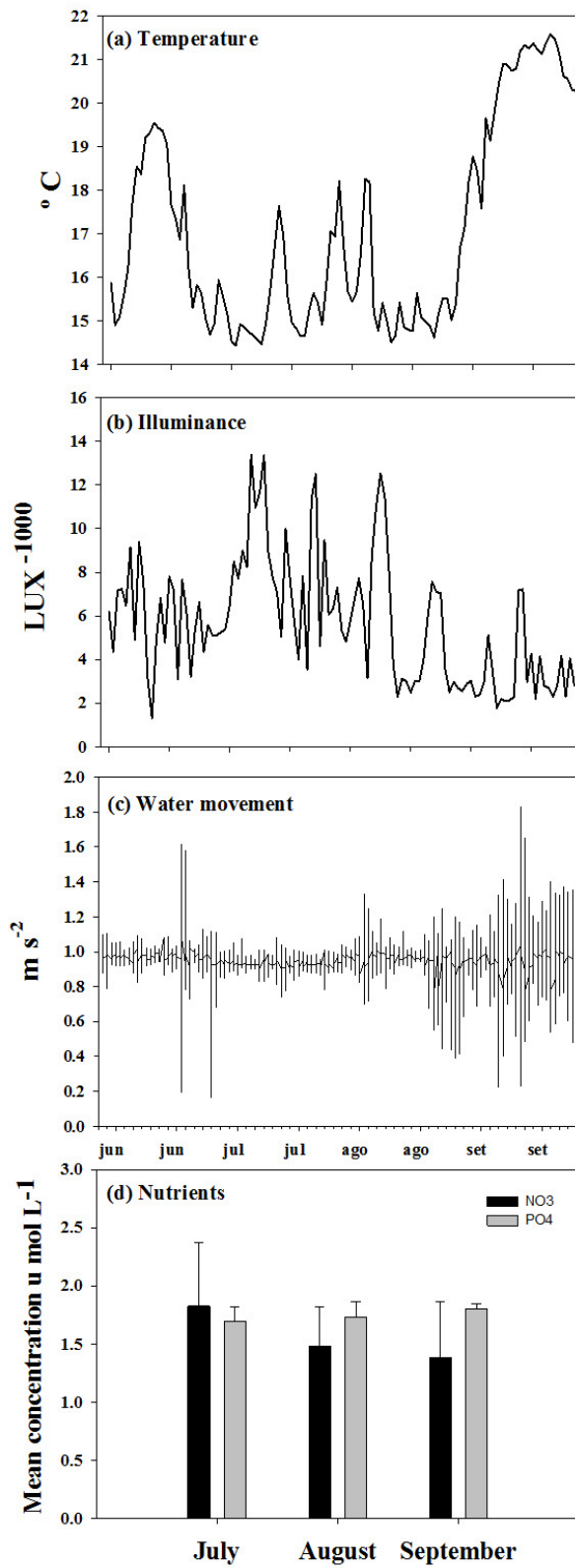
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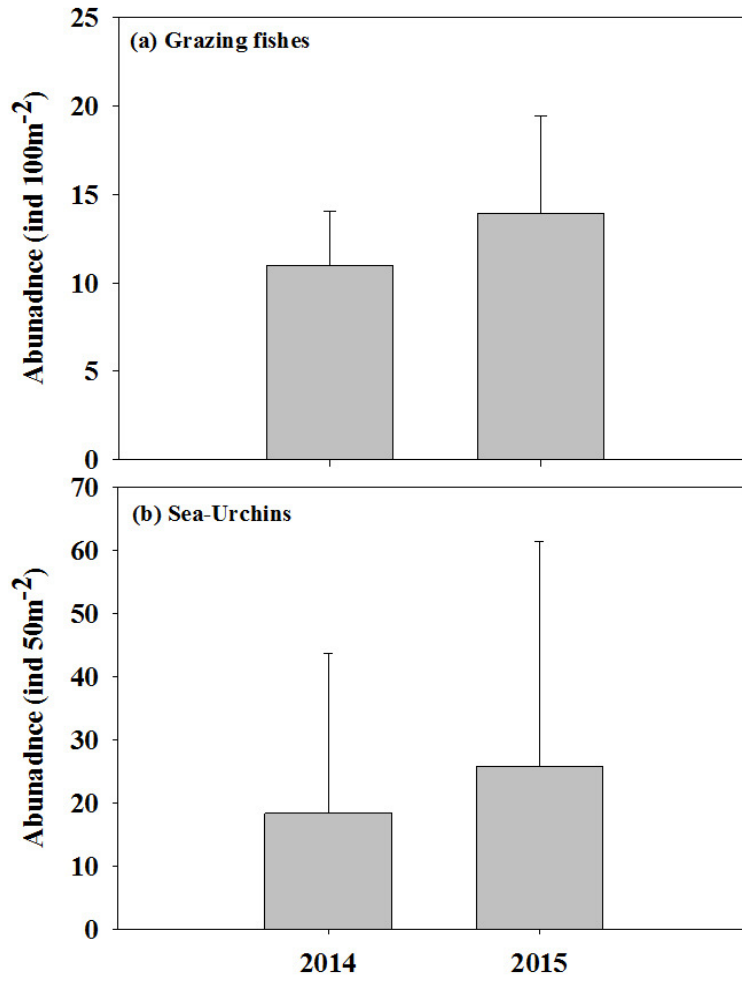
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584 **Figure 2**

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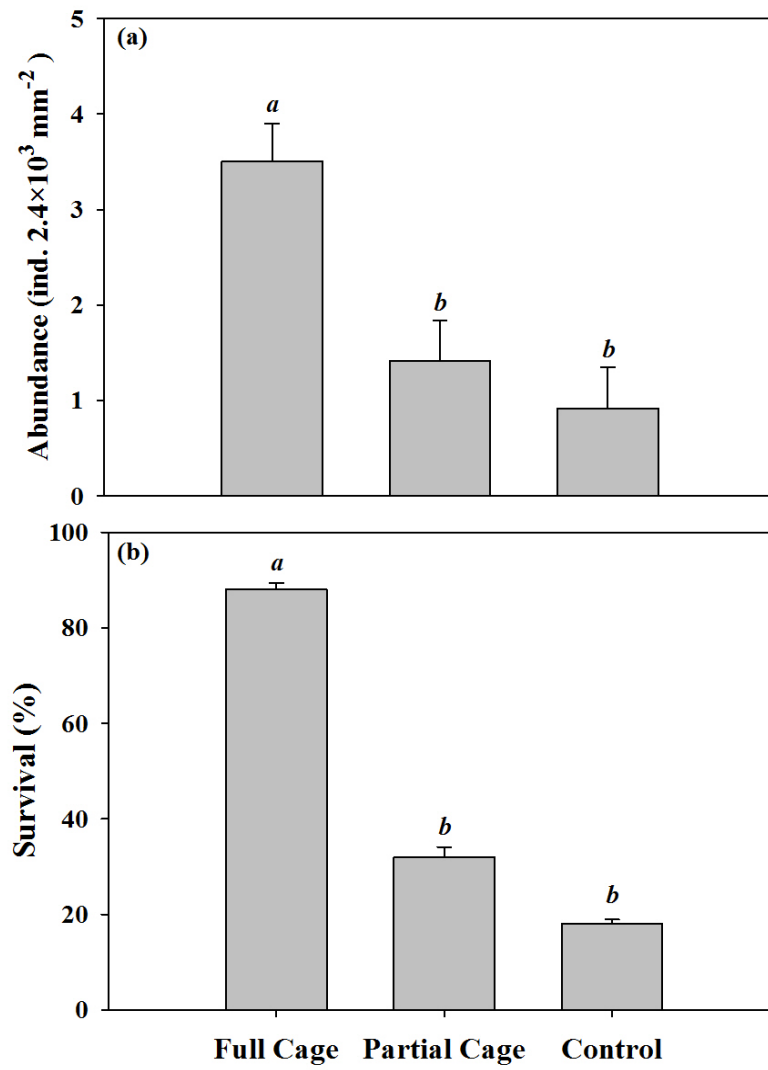
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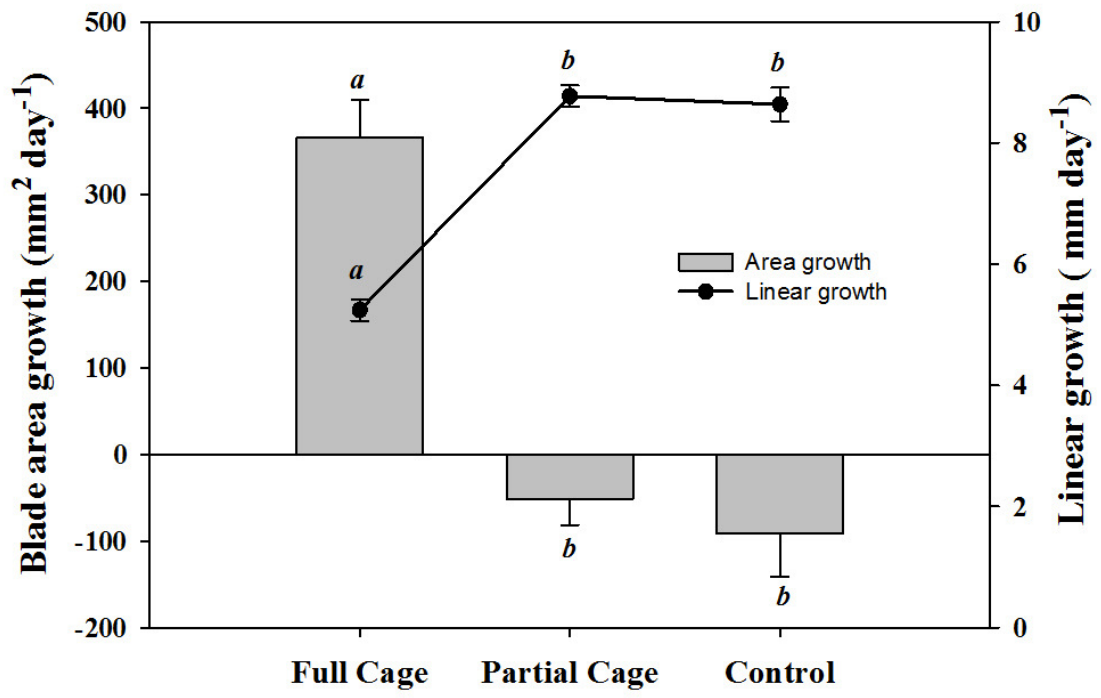
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609 **Figure 4**

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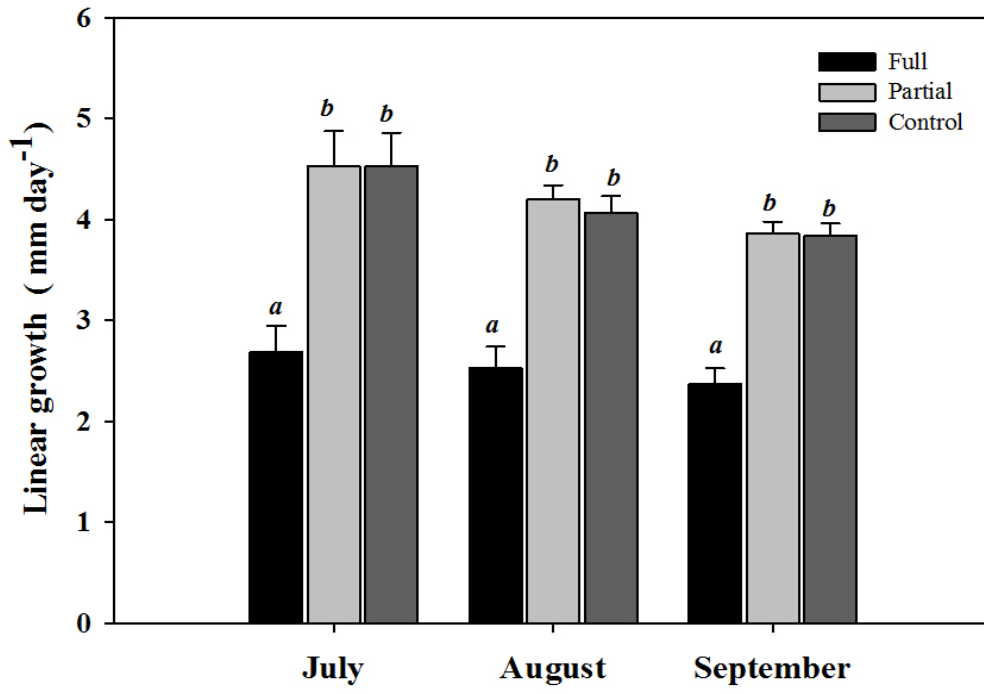
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626 **Figure 5**

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