

## Manuscript Details

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### Abstract

The invasive seaweed *Caulerpa cylindracea* has shown a reduced ability to invade healthy *Posidonia oceanica* meadows by penetrating only meadow margins in early invasion stages in the western Mediterranean Sea. However, the long-term interaction with invasive seaweed could deteriorate the structure of meadows by diminishing their initial resistance to invasion as a result of potential competitive mechanisms between both macrophytes (e.g. allelopathic effects, enhanced sediment anoxia). In this study, populations of both species were monitored over a 10-year period (2007-2016) in invaded and non-invaded sites to assess meadows' resistance evolution to the long-term negative interactions between both macrophytes. The *C. cylindracea* biomass in the seagrass canopy was much lower (from 5- to 60-fold) than the biomass that developed just outside the seagrass meadows at all the invaded sites. The monitored seagrass populations showed stable and/or progressive trends throughout the study period, and no structural differences were observed between invaded and non-invaded meadows. To conclude, our results evidence, for the first time, the absence of a long-term competitive interaction between invasive seaweed and the structure and shoot dynamics of native *P. oceanica* meadows. The long-term presence of persistent gradients of algal biomass from outside to inside meadows supports the existence of highly limiting conditions for algal growth and survival in undisturbed *P. oceanica* canopies, which reinforces the role of healthy meadows acting as ecological barriers against the spread of *C. cylindracea*. This highlights the importance of conserving valuable *P. oceanica* meadows as a way to control bioinvasions in the Mediterranean Sea.

<b>Keywords</b>	Endemic seagrass; Non-native algae; Biotic resistance; Marine bioinvasions; Mediterranean Sea
<b>Taxonomy</b>	Mediterranean Ecosystem, Biological Invasion, Benthic Community Ecology of Marine Habitat, Aquatic Botany, Aquatic Ecology
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Dear Editor,

We send you the manuscript entitled **Evidence for the long-term resistance of *Posidonia oceanica* meadows to *Caulerpa cylindracea* invasion** to be considered for its publication in the international journal **Aquatic Botany**.

This study has been performed by (first author) *Jaime Bernardeau-Esteller* and co-authors: *Lázaro Marín-Guirao, José M. Sandoval-Gil, Rocío García-Muñoz, Aranzazu Ramos-Segura and Juan M. Ruiz*. All authors have made significant contributions to the work, especially in field work, data analysis and interpretation and manuscript preparation. With the submission of this manuscript I would like to state that the above mentioned manuscript has not been published elsewhere, accepted for publication elsewhere or under editorial review for publication elsewhere.

This research was funded by the Servicio de Pesca y Acuicultura (Dirección General de Ganadería y Pesca, Comunidad Autónoma de Murcia). This work represents a relevant contribution to the understanding of the competitive interaction between the invasive alga *Caulerpa cylindracea* and the Mediterranean endemic seagrass *Posidonia oceanica*. Our results evidence, for the first time, the absence of a long-term competitive interaction between invasive seaweed and abundance and dynamic populations of native seagrass.

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**Evidence for the long-term resistance of *Posidonia oceanica* meadows to *Caulerpa cylindracea* invasion**

*Highlights:*

- First long-term study to assess the interaction between *C. cylindracea* and *P. oceanica*
- Poor long-term alga development capacity in seagrass meadows
- Lack of a long-term negative interaction on native seagrasses by invasive seaweed
- Healthy meadows act as ecological barriers against the spread of *C. cylindracea*

1 **Evidence for the long-term resistance of *Posidonia oceanica* meadows to *Caulerpa cylindracea***  
2 **invasion**

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10 **Abstract**

11 The invasive seaweed *Caulerpa cylindracea* has shown a reduced ability to invade healthy *Posidonia*  
12 *oceanica* meadows by penetrating only meadow margins in early invasion stages in the western  
13 Mediterranean Sea. However, the long-term interaction with invasive seaweed could deteriorate the  
14 structure of meadows by diminishing their initial resistance to invasion as a result of potential competitive  
15 mechanisms between both macrophytes (e.g. allelopathic effects, enhanced sediment anoxia). In this  
16 study, populations of both species were monitored over a 10-year period (2007-2016) in invaded and non-  
17 invaded sites to assess meadows' resistance evolution to the long-term negative interactions between both  
18 macrophytes. The *C. cylindracea* biomass in the seagrass canopy was much lower (from 5- to 60-fold)  
19 than the biomass that developed just outside the seagrass meadows at all the invaded sites. The monitored  
20 seagrass populations showed stable and/or progressive trends throughout the study period, and no  
21 structural differences were observed between invaded and non-invaded meadows. To conclude, our  
22 results evidence, for the first time, the absence of a long-term competitive interaction between invasive  
23 seaweed and the structure and shoot dynamics of native *P. oceanica* meadows. The long-term presence of  
24 persistent gradients of algal biomass from outside to inside meadows supports the existence of highly  
25 limiting conditions for algal growth and survival in undisturbed *P. oceanica* canopies, which reinforces  
26 the role of healthy meadows acting as ecological barriers against the spread of *C. cylindracea*. This  
27 highlights the importance of conserving valuable *P. oceanica* meadows as a way to control bioinvasions  
28 in the Mediterranean Sea.

29 **Keywords:**

30 Endemic seagrass; Non-native algae; Biotic resistance; Marine bioinvasions; Mediterranean Sea

## 31 **1. Introduction**

32 The ecological effects of biological invasions on natural communities depend mainly on the  
33 abundance and biological/ecological characteristics of invasive species, and on the resistance of native  
34 communities to invasion (Vitousek, 1990; Lonsdale, 1999; Theodorides and Dukes, 2007). These attributes  
35 are dynamics that can change over time by modulating the interaction between native and exotic species.  
36 Acclimative or evolutionary processes (e.g. hybridisation with endemic species) can improve the growth  
37 and competitive capacity of the introduced species to the detriment of native species (Yamashita et al.,  
38 2000; Ayres et al., 2004). Resistance to the invasion of native communities can also be modified as a  
39 result of the environmental changes induced by the presence of exotic species and the action of natural or  
40 anthropogenic disturbances (Davis et al., 2000). Given the dynamic character of these attributes, long-  
41 term studies need to be conducted under contrasting environmental to understand the potential impact of  
42 exotic species on native communities (Strayer et al., 2006).

43 Exotic macroalgae have been recognised as a potential threat to seagrass habitats in coastal areas  
44 worldwide (Williams, 2007). The replacement of a native seagrass community with exotic macroalgae  
45 can trigger profound ecosystem transformations by generating alternative states characterised by loss of  
46 the valuable ecosystem functions and services that seagrasses provide (McGlathery, 2001; Montefalcone  
47 et al., 2015). Despite this increasing threat, our understanding of the interactions between alien and native  
48 macrophytes, and how these interactions determine the final impact that exotic macroalgae invasion has  
49 on these ecological relevant habitats, is limited (Williams, 2007; Thomsen et al., 2009). To date, most  
50 studies on the effects of exotic macroalgae on seagrass ecosystems are short and have been conducted  
51 more or less indiscriminately during the time course of invasions instead of within the appropriate time  
52 frame.

53 Species of the genus *Caulerpa* (Chlorophyta) are among the most studied invasive macroalgae  
54 and their introduction into new areas has been linked to declining seagrass habitats (Williams and Smith  
55 2007). *C. taxifolia* is the most widely studied species whose proliferation has been correlated with  
56 seagrass habitat loss and degradation in different oceans, including the Mediterranean Sea (Ceccherelli  
57 and Cinelli 1997; Dumay et al., 2002; Williams and Grosholz, 2002; Garcias-Bonet et al., 2008).  
58 However, recent experimental studies have suggested that *C. taxifolia* behaves as a passenger, rather than  
59 as a driver, of decline by responding opportunistically to the degradation of seagrass beds caused by other  
60 disturbances (Glasby 2013). However, *Caulerpa cylindracea* (Sonder) has received much less attention

61 than its congeneric species despite being considered one of the most threatening invasions in the  
62 Mediterranean Sea (Piazzi et al., 2016). Available evidence suggests that the ability of *C. cylindracea* to  
63 proliferate in seagrass habitats is vastly variable, and largely depends on the seagrass species, and also on  
64 its size, growth rate and the complexity of the three-dimensional structures of its meadows. The species is  
65 able to grow and develop dense stands in the seagrass canopies formed by medium-/small-sized species,  
66 such as *Cymodocea nodosa* and *Zostera noltii*, in both the Mediterranean Sea and the Atlantic Ocean  
67 (Raniello et al., 2004, Fabbri et al., 2015). This ability is much more restricted in the more complex leaf  
68 canopies of the larger seagrass species *Posidonia oceanica*, where invader populations were initially  
69 restricted to margins without penetrating inner meadow areas (Katsanevakis et al., 2010; Ceccherelli et  
70 al., 2014; Marín-Guirao et al., 2015). Despite the contrasting resistance observed among seagrasses to  
71 initial *C. cylindracea* invasion, very few works have assessed the existence of biotic interactions among  
72 these macrophytes, and have not explored how these interactions may influence the susceptibility to  
73 invasion of seagrass meadows in the long term. The seagrass *C. nodosa* showed a progressively reduced  
74 shoot density after the initial invasion by *C. cylindracea*, whereas *Z. noltii* displayed an opposite response  
75 as its shoot density increased after invasion (Ceccherelli and Campo, 2002). For *P. oceanica*, no data are  
76 available on the population dynamics following *C. cylindracea* invasion. However, it has been suggested  
77 that *C. cylindracea* secondary metabolites can potentially cause allelopathic stress by negatively affecting  
78 the vegetative development of this seagrass (Dumay et al., 2002). Other authors have suggested that the  
79 increased sediment anoxia promoted by *Caulerpa* species invasion (*C. prolifera* and *C. cylindracea*)  
80 might induce plant mortality and may, therefore, be responsible for the less seagrass abundance observed  
81 in invaded areas (Holmer et al., 2009). Besides the likely existence of interactions between *C. cylindracea*  
82 invasion and seagrasses with potential long-term consequences on the integrity of meadows, no empirical  
83 or experimental evidence exists to date. All the conducted studies are short and based on descriptive or  
84 correlational approaches, which prevents the cause-effect relationships between this invasion and seagrass  
85 decline being established. It is, therefore, of key importance to evaluate and quantify the long-term effects  
86 of *C. cylindracea* invasion on the vitality, structure and function of *P. oceanica* meadows to manage and  
87 conserve this valuable habitat in the future (Boudouresque et al., 2009)..

88 This study is the first quantitative assessment of the evolution of *P. oceanica* meadows'  
89 resistance to *C. cylindracea* invasion over time, which could be influenced by the existence of long-term  
90 interactions between invasive alga and the endemic seagrass. To this end, the population dynamics of

91 both macrophytes and the structure of seagrass meadows were studied over a 10-year period at invaded  
92 and non-invaded sites in the south-western Mediterranean Sea. We hypothesised that if the presence of *C.*  
93 *cylindracea* had negative effects on the initial resistance of *P. oceanica* meadows to this structure over  
94 time, we could expect: (i) *P. oceanica* populations and meadows structure at invaded localities to decline  
95 over time compared to non-invaded sites; (ii) the abundance of the invasive alga in the seagrass canopy to  
96 progressively increase.

## 97 **2. Material and Methods**

### 98 **2.1. Study area and sampling design**

99 The present study was conducted between 2007 and 2016 on the south-eastern coast of Spain,  
100 where the exotic seaweed *C. cylindracea* was observed for the first time in 2005 (Ruiz et al. 2011).  
101 Similarly to other Mediterranean regions, *P. oceanica* is the most abundant and relevant infralitoral  
102 habitat in the study area (Ruiz et al., 2015), where *C. cylindracea* invasion is currently considered one of  
103 the most important potential threats to marine coastal ecosystems (Ruiz et al. 2017). Six sampling sites  
104 were selected in six well-developed *P. oceanica* meadows distributed along a coastline stretching more  
105 than 200 km: three invaded (I1, I2, and I3) by the alga and three not invaded (N1, N2, and N3); the  
106 invaded sites were isla Grosa (I1, -11 m, 0701985-4177946 UTM ETRS89), cabo Tiñoso (I2, -18 m,  
107 0664377-4156507 UTM ETRS89), and Calblanque (I3, -25 m, 0700040-4161890 UTM ETRS89), and  
108 the non-invaded ones were Calabardina (N1, -14 m, 0632933-4142986 UTM ETRS89), Las Palomas (N2,  
109 -17 m, 0673128-4160785 UTM ETRS89) and La Azohia (N3, -20 m, 0661074-4157999). All the selected  
110 meadows spread over biogenic carbonate sediments in oligotrophic waters far from anthropogenic  
111 disturbances (Ruiz et al., 2015). In the three invaded sites, *C. cylindracea* almost completely covered the  
112 sediments outside the meadow, and penetrated only the first few centimetres (up to 50 cm) inside meadow  
113 margins. At each site, a surface area of 450 m<sup>2</sup> was delimited by pushing stainless iron pegs into the  
114 sediment every 15 m. The sampling area was located along the meadow margin and covered a 1-metre  
115 band inside the meadow, and a 1-metre band outside it and adjacent to the meadow edge. This transition  
116 area on meadow margins was selected to assess the existence of the long-term interactions between the  
117 invasive *C. cylindracea* and *P. oceanica* as it represents the contact zone between both macrophytes.  
118 Within each sampling area, six permanent square plots of 1,600 cm<sup>2</sup> were randomly located inside the  
119 meadow following the margin. Each permanent plot consisted in four metal stakes connected by a string  
120 at the sediment level to avoid any effect on seagrass vitality.

## 121 2.2. Sampling procedures

122 Abundance of *C. cylindracea* (standing biomass, g FW m<sup>-2</sup>) was measured at the invaded sites  
123 both within the margins of the *P. oceanica* meadow (from the edge to 1 m inwards; IN) and in the  
124 adjacent substrates outside the meadow edge (OUT). The *C. cylindracea* biomass was measured twice a  
125 year during two seasons, and gave contrasting growth rates during the seaweed's annual cycle in this and  
126 other areas of the western Mediterranean Sea (Bernardeau-Esteller, unpublished data; Ruitton et al., 2005;  
127 Mezgui et al., 2007; Cebrian and Ballesteros, 2009; Enguix et al., 2014): October (autumn) when growth  
128 rates and biomass are still at their highest annual levels; January (winter) when the growth and abundance  
129 of the alga are usually lower. Fronds, stolons and rhizoids of *C. cylindracea* were carefully collected by  
130 hand within 400 cm<sup>2</sup> square frames and placed inside labelled plastic bags. At each site, ten samples were  
131 randomly collected on the seagrass meadow margin (IN) and 10 other samples were obtained in the area  
132 outside and just in front of the meadow edge (OUT). Samples were transported to the laboratory in chilled  
133 containers with seawater. The sediment, debris and fragments of other algal species were gently removed  
134 from each sample to determine the total *C. cylindracea* standing biomass (g FW·m<sup>-2</sup>). Relative alga  
135 abundance was also measured during the autumn sampling in the six permanent plots established within  
136 the meadow margins to characterise the *P. oceanica* shoot density and population dynamics (see below).  
137 This measurement was taken using a square frame of the same surface area in the permanent plot (i.e.  
138 1,600 cm<sup>2</sup>) subdivided into 25-cm<sup>2</sup> subquadrats (Bernardeau-Esteller 2015). *C. cylindracea* abundance  
139 was estimated as the percentage of cover obtained from the number of subquadrats in which the alga was  
140 present in relation to the total number of subquadrats.

141 At each sampling site, the seagrass meadow descriptors shoot density, the percentage of meadow  
142 cover and the net shoot population growth in the permanent plots were measured every year during the  
143 autumn sampling. All these descriptors have been demonstrated to be robust indicators of meadow  
144 structure and vitality, and are the most widely used ones in ecological assessments and long-term  
145 monitoring programmes of this seagrass species (Pergent-Martini et al., 2004; Marbá et al., 2005; Ruiz et  
146 al., 2015). The percentage of meadow cover was estimated along four 10-metre linear transects deployed  
147 from four of the six permanent plots of the sampling station following a fixed compass bearing. Within  
148 each transect, a visual estimation of the percentage of the bottom covered by seagrass patches was  
149 performed inside the 1,600-cm<sup>2</sup> quadrats subdivided into four 20x20 cm squares. The values obtained  
150 along each transect were averaged to represent the cover percentage of the whole transect, which was the



151 replicate (n=4). Shoot density was estimated by counting the number of shoots inside three 400-cm<sup>2</sup>  
152 square frames randomly placed inside the living seagrass patches at the beginning, the middle and the end  
153 of the same transects used for the cover measurements. The average of the three measurements obtained  
154 along each transect was used as an independent replicate (n=4 replicates). The exact number of shoots  
155 was also counted inside the six permanent plots. This shoot census was used to estimate the annual net  
156 shoot population growth (NPG<sub>y</sub>), which is the relative change in shoot numbers that the meadow had  
157 undergone in a per year basis (ln units·year<sup>-1</sup>). This variable was estimated following Marba et al. (2005)  
158 and using the equation:

$$159 \quad NPG_y = \frac{(\ln n_f - \ln n_i) \times 365}{P}$$

160 where n<sub>f</sub> and n<sub>i</sub> are, respectively, the mean value obtained at the end and the beginning of each annual  
161 period, and P is the length of that period in days. The total net population shoot growth (NPG<sub>T</sub>, ln units)  
162 for each permanent plot was calculated by summing all the NPG<sub>y</sub> values obtained throughout the  
163 monitoring period (2007-2016). This variable represents the net balance between recruitments and the  
164 mortality of the shoot population, which take positive values when shoot recruitment exceeds mortality  
165 (population growth), and negative values when mortality exceeds recruitment (population decline).

### 166 **2.3. Statistical analysis**

167 For each season, the spatio-temporal variation of the *C. cylindracea* biomass was analysed by a  
168 three-way ANOVA with Position (two levels: inside the meadow (IN) and outside the meadow (OUT)) as  
169 a fixed factor, and Site (three levels: I1, I2 and I3) and Time (10 levels) as random factors. Multiple  
170 comparisons were made by the Bonferroni test for the *posteriori* analyses.

171 The potential influence of *C. cylindracea* on seagrass descriptors (meadow cover, shoot density  
172 and NPG<sub>y</sub>) with time was explored by a two-way repeated measures ANOVA with Condition (two levels:  
173 invaded and non-invaded) as fixed factor, Site (three levels), a random factor nested within Condition.  
174 Time was included as the repeated measured factor (10 levels corresponding to the 10 successive annual  
175 periods). The analyses of repeated measures ANOVA were computed using Greenhouse-Geisser adjusted  
176 degrees of freedom when the data did not meet the assumption of sphericity (Mauchly's test,  $\alpha = 0.05$ ).  
177 The differences in NPG<sub>T</sub> between the invaded and non-invaded meadows were examined by a two-way  
178 ANOVA with Condition (two levels, invaded, non-invaded) as a fixed factor and Site (three levels) as a  
179 random factor nested within Condition. In all the analyses, depth was introduced as a covariate to control  
180 the possible influence on the measured variables. Prior to carrying out the analyses, data were tested for

181 normality (Kolmogorov–Smirnov test) and equal variances (Levene test), and were transformed whenever  
182 necessary. A significant probability level of 0.05 was regarded, except when data transformation was not  
183 possible. In such cases the level of significance lowered to  $P < 0.01$  to minimise type I errors, and special  
184 care was taken to interpret the results (Underwood, 1997). In order to assess any trends of the time series  
185 on the abundance of both macrophytes (shoot density and meadow cover for *P. oceanica*, and biomass  
186 outside and inside the meadow for *C. cylindracea*), non-parametric Kendall's coefficient of rank  
187 correlation ( $\tau$ ) was used. The relationships between the algal cover percentage and the shoot number of the  
188 seagrass in the permanent plots were also explored with this correlation coefficient. In both cases a  
189 significance value of 0.05 was used. Statistical analyses were performed using version 17.0 of the SPSS  
190 statistical package (SPSS Inc. Chicago, Ill, USA).

### 191 **3. Results**

#### 192 *C. cylindracea* abundance

193 In winter, the complete regression of the *C. cylindracea* populations inside and outside the  
194 meadow were detected over the entire study period at all sites, except for a few cases (I1 in 2011, and I2  
195 and I3 in 2009; Fig. 1). No significant correlations between biomass and time were found for both  
196 positions IN and OUT at all the sites during this season. In autumn, a significant interaction of position,  
197 site and time was observed, which evidenced that the differences in the *C. cylindracea* biomass between  
198 the IN and OUT positions of the meadow depended on both time and site (Table 1). In particular, the  
199 biomass outside the meadow was ~ 5-60-fold higher than it was inside (Fig. 1) at all the sites and for all  
200 the times, except at I1 in 2012, 2014, 2015 and 2016, at I2 in 2010 and 2015, and at I3 in 2015. In these  
201 cases, the biomass recorded outside was very low ( $< 5$  g FW  $m^{-2}$ ), with complete population regression  
202 being recorded in some situations (at I1 in 2014 and at I2 in 2015) (Fig. 1). Vast interannual variations in  
203 the *C. cylindracea* biomass were found in this OUT position, but with different patterns between sites.  
204 The autumn biomass values for the 2007-2010 period were ~10-70-fold higher than in subsequent years at  
205 I1. The maximum biomass values were also recorded at the beginning of the study period for I2 and I3,  
206 but the multiple comparisons did not show any clear temporal pattern at these sites. The autumn values  
207 within the meadow (IN) were generally lower or equalled 5 g FW  $m^{-2}$ , and showed no interannual  
208 differences, except for I2, where the alga abundance in 2007 and 2008 was higher than in later years (Fig.  
209 1). Despite there being no significant interannual differences, the *C. cylindracea* biomass from the IN and  
210 OUT positions showed significant negative temporal trends at I1 and I2, whereas they were stable at the

211 I3 site (Table 2). The *C. cylindracea* cover in the permanent plots correlated highly with the autumn  
212 biomass values recorded inside meadows (I1:  $\tau = 1$ ,  $p < 0.001$ ; I2:  $\tau = 0.975$ ,  $p < 0.001$ ; I3:  $\tau = 1$ ,  $p$   
213  $< 0.001$ ), and ranged from zero for the 2012-2015 period to  $24.7 \pm 4.5$  (2008) at I1, from  $3.8 \pm 1.2$  (2010) to  
214  $74.8 \pm 6.8$  (2008) at I2, and from  $1.3 \pm 0.3$  (2013) to  $11.2 \pm 5.2$  (2011) at I3.

#### 215 *P. oceanica* abundance

216 No differences in meadow cover, shoot density and NPGy were detected between the invaded  
217 and non-invaded *P. oceanica* meadows throughout the study period, but temporal patterns varied  
218 depending on site (Table 3). Considerable interannual fluctuations ( $> 50\%$ ) for these descriptors were  
219 detected in all the meadows, even between consecutive years (Fig. 2). Meadow cover and shoot density  
220 showed positive correlations with time at I2. Positive trends were also observed for meadow cover at I3  
221 and for shoot density at N3. Stable trends (no correlations with time) were found for both descriptors at I1  
222 and N2, and only for meadow cover at N3. Shoot density also displayed stable trends at I3 and N1 (Table  
223 4). The average NPG<sub>T</sub> values were  $0.40 \pm 0.16$  at I1,  $1.02 \pm 0.58$  at I2,  $-0.32 \pm 0.30$  at I3,  $0.60 \pm 0.72$  at N1,  
224  $1.03 \pm 0.38$  at N2 and  $0.24 \pm 0.14$  at N3. No differences between sites were found for this variable (Table 3).  
225 Significant correlations between the seagrass shoot number and the *C. cylindracea* cover values within  
226 permanent plots were found, and were negative at I1 ( $\tau = -0.798$ ,  $p = 0.011$ ) and I2 ( $\tau = -0.683$ ,  $p = 0.029$ ),  
227 and positive at I3 ( $\tau = 0.494$ ,  $p = 0.043$ ).

#### 228 **4. Discussion**

229 This work is the only long-term monitoring study to have been conducted to date to assess the  
230 population dynamics of the native seagrass *P. oceanica* and the invasive seaweed *C. cylindracea*, which  
231 have grown in close contact for more than one decade. The 10-year data series evidenced the absence of  
232 any long-term interactions between both macrophytes. It demonstrated, for the first time, that *P. oceanica*  
233 meadows, in the absence of human perturbations, are able to maintain their initial resistance to *C.*  
234 *cylindracea* invasion.

235 Since the introduction and rapid settlement of the invasive seaweed on the south-eastern coast of  
236 Spain in 2005 (Ruiz et al., 2011), established populations have persisted until the present-day and present  
237 considerable temporal fluctuations in their development level (i.e. biomass and cover). Marked seasonal  
238 fluctuations are common in the *C. cylindracea* populations from the Western Mediterranean, where the  
239 greatest abundance is usually observed in autumn, followed by a drastic winter decline (Buia et al., 2001;  
240 Ruitton et al., 2005; Lenzi et al., 2007; Enguix et al., 2014). The *C. cylindracea* populations have also

241 shown substantial interannual variations, but with different patterns, among study sites, which suggests  
242 the existence of local factors influencing *C. cylindracea* growth and development. Biological invasions in  
243 marine ecosystems are indeed considered highly idiosyncratic and are dependent on local conditions  
244 (Meiners et al., 2004; McQuaid and Arenas, 2009). However, lack of other long-term studies on *C.*  
245 *cylindracea* population dynamics from other Mediterranean regions precludes the identification of such  
246 local factors. Thus further studies are needed.

247         The *P. oceanica* meadows also showed strong interannual fluctuations in their structural  
248 descriptors (cover and density), as previously reported in this and other regions of the western  
249 Mediterranean (Marbà and Duarte, 1997; Gobert 2002; Terrados and Medina-Pons, 2011; Ruiz et al.,  
250 2015). Despite this temporal variability, the monitored meadows followed stable or progressive trends  
251 throughout the studied 10-year period, with no differences observed between the invaded and non-  
252 invaded meadows. Only site I3 showed slightly negative total population growth (NPGt) in the permanent  
253 plots, which could be interpreted as a negative interaction with the invasive seaweed. Nevertheless, both  
254 the invaded and non-invaded meadows showed interannual periods of negative growth (e.g. NPGy  
255 between 2012 and 2014), which suggests that the observed year-to-year changes fall within the natural  
256 demographic fluctuations of *P. oceanica* populations (Guillén et al. 2013; Ruiz et al. 2015). *C.*  
257 *cylindracea* also underwent a net decline at I3 throughout the study period, and this invaded site was the  
258 only one where the abundance of both species correlated positively in the permanent plots. This suggests  
259 the existence of local factors or processes which could act on the meadow edge and similarly affect both  
260 macrophytes. Seagrass meadow margins are indeed very vulnerable to the physical disturbances caused  
261 by sediment dynamics and hydrodynamic forces (Fonseca and Bell, 1998; Infantes et al., 2009). Buried *P.*  
262 *oceanica* shoots in the meadow margins have been frequently observed at this site through the migration  
263 of large sand waves following severe storms. These intense natural events are the likely cause of the  
264 observed declines because *P. oceanica* is particularly sensitive to burial (Manzanera et al., 2014;  
265 Ceccherelli et al., 2018), and *C. cylindracea* is negatively affected by high hydrodynamic forces (Vaselli  
266 et al., 2008; Incera et al., 2010). However, the mechanistic interaction between hydrodynamics and *C.*  
267 *cylindracea* proliferation/abundance is still unknown (Piazzi et al. 2016). Furthermore, only the *P.*  
268 *oceanica* meadow at this site was highly fragmented and formed by scattered patches of tens of meters,  
269 which revealed the existence of a particular natural regime of physical disturbance (Hemminga and  
270 Duarte, 2000). Therefore, the negative population growth shown by both macrophytes in this locality

271 would most likely reflect the effects of extreme storm events rather than a negative interaction between  
272 the native seagrass and the invasive seaweed.

273         Despite the fact that *C. cylindracea* maintained permanent populations with wide temporal  
274 variability in their development level, the abundance of the alga along the edges of the *P. oceanica*  
275 meadows was constantly very much lower than in the adjacent sedimentary bottoms. This supports the  
276 notion of the good resistance of *P. oceanica* meadows to *C. cylindracea* invasion, as reported in previous  
277 studies (Katsanevakis et al., 2010; Ceccherelli et al., 2014; Marín-Guirao et al., 2015), and demonstrates  
278 that this resistance is stable in the long-term, even when the invasive seaweed have been growing within  
279 meadow margins for more than one decade. Several factors have been suggested to contribute to  
280 meadows' initial resistance to invasion, but it is unknown if and how these factors can be modified in the  
281 long-term by invasive seaweed being present. The little light and small substrate surface available for the  
282 alga within meadows have been suggested to significantly contribute to this resistance, as derived from  
283 the fact *C. cylindracea* growth and abundance within meadow margins are facilitated when the seagrass  
284 biomass is manipulatively reduced (Ceccherelli et al., 2014; Tamburello et al., 2014). The marked  
285 reduction in light caused by the dense leaf canopy of meadows has been subsequently demonstrated as a  
286 key factor underlying the initial resistance to *C. cylindracea* invasion (Marín-Guirao et al., 2015;  
287 Bernardeau-Esteller et al., 2015), although this mechanism of resistance could diminish over time given  
288 the potential adverse effects induced by the alga on seagrass productivity (Raniello et al., 2007). This  
289 competitive interaction (i.e. allelopathy) could result in undersized *P. oceanica* plants within meadow  
290 margins (Dumay et al. 2002) by increasing the levels of available light for the alga to grow and favouring  
291 the progressive invasion of meadows. Although we cannot rule out the possibility of phytotoxic-induced  
292 effects on *P. oceanica*, lack of increasing trends in the invasive seaweed abundance within margins  
293 evidences that these potential effects do not impact the meadow's structure. We can, therefore, assume  
294 that the interaction between both macrophytes does not affect the long-term light conditions underneath  
295 the seagrass canopy that confers resistance to invasion. Besides the effects of above-ground processes  
296 (e.g. canopy shading), below-ground processes are also likely to contribute to *P. oceanica* meadows  
297 resisting *C. cylindracea* invasion (Gribben et al. 2018). The particular microbial communities and the  
298 chemical/nutrient properties of sediments populated by both these macrophytes seem to offer positive  
299 feedback for each species to grow. Consequently, the sediment quality and the associated microbial  
300 community within meadows have been recently suggested to play an important role by driving meadows'

301 resistance to *C. cylindracea* invasion. Moreover, the fact that the invasive alga is unable to progressively  
302 develop dense stands within meadows precludes the sediment accumulation of phytotoxic compounds  
303 with potential negative effects on both seagrass performance and mortality (Holmer & Hasler-Sheetal,  
304 2014).

305 In short, our results evidence, for the first time, the absence of a long-term competitive  
306 interaction between invasive seaweed and the structure and shoot dynamics of native *P. oceanica*  
307 meadows. The long-term presence of persistent gradients of algal biomass from outside to inside  
308 meadows supports the existence of highly limiting conditions for algal growth and survival in undisturbed  
309 *P. oceanica* canopies, which reinforces the role of healthy meadows acting as ecological barriers against  
310 the spread of *C. cylindracea*. This highlights the importance of conserving valuable *P. oceanica* meadows  
311 as a way to control bioinvasions in the Mediterranean Sea.

312

### 313 **Author contributions**

314 JBE, LMG, JMSG and JMR planned and designed the study. JBE, LMG, JMSG, RGM, ARS  
315 and JMR conducted fieldwork and analyzed the data. JBE, LM and JMR led the writing of the paper with  
316 contributions from the rest of authors. All Authors reviewed the manuscript.

317

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479 **Tables and Figures**

480

481 **Table 1.** Summary of the three-way ANOVAs performed to assess the effect of Position, Site and Time  
 482 on the *C. cylindracea* biomass in autumn and winter. The Depth factor was included as a covariate.

Source	winter				autumn			
	df	MS	F	p	df	MS	F	p
Depth[covariate]	1	5384311.22	0.54	0.46	1	17.98	0.20	0.65
Position	1	29432112.22	0.98	0.43	1	50131.48	26.83	0.01
Site	2	14515013.49	0.72	0.55	2	660.08	1.27	0.47
Time	8	6514102.40	0.93	0.57	9	3448.16	1.67	0.27
PositionxSite	2	30302985.98	4.20	0.06	2	1006.87	0.80	0.47
PositionxTime	7	7013464.35	0.97	0.49	9	2122.34	1.68	0.17
SitexTime	14	7193845.31	1.00	0.50	18	1199.03	0.95	0.54
PositionxSitexTime	14	7216144.17	0.73	0.74	18	1260.58	14.36	<b>&lt;0.001</b>
Residual	440	9888556.28			535	87.794		

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484 **Table 2.** Relations between *C. cylindracea* abundance (biomass) and time for all the monitored invaded  
 485 sites (I1, I2 and I3) for the two studied seasons (winter, autumn) and the two positions in relation to the  
 486 meadow edge (IN, OUT); □: Kendall's coefficient of rank correlation, p: calculated probability.

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SITE	winter				autumn			
	IN		OUT		IN		OUT	
	□	p	□	p	□	p	□	p
<b>I1</b>	-0.39	0.19	-0.30	0.31	<b>-0.68</b>	<b>0.01</b>	<b>-0.78</b>	<b>&lt;0.01</b>
<b>I2</b>	-0.25	0.17	-0.39	0.19	<b>-0.74</b>	<b>0.02</b>	<b>-0.56</b>	<b>&lt;0.01</b>
<b>I3</b>	-0.35	0.11	-0.33	0.25	-0.24	0.33	-0.20	0.42

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490 **Table 3.** Summary of the three-way Repeated Measured ANOVA performed to assess the effects of  
 491 Condition, Site and Time on Meadow Cover, Shoot Density and NPGy and the two-way ANOVA  
 492 performed to assess the effects of Condition and Site on Total Population Growth (NPGt). The Depth  
 493 factor was included as a covariate. Asterisk indicates the Greenhouse-Geisser-adjusted degrees of  
 494 freedom used when data did not meet the sphericity assumption.

Source	Shoot Density				Meadow Cover				NPGy				NPGt			
	df	MS	F	p	df	MS	F	p	df	MS	F	p	df	MS	F	p
<i>Between-Subjects Effects</i>																
Depth [covariate]	1	10.70	0.26	0.62	1	51.42	0.63	0.44	1	0.002	0.12	0.73	1	0.02	0.16	0.69
Condition	1	27.49	0.66	0.43	1	86.43	1.05	0.32	1	0.002	0.16	0.69	1	0.03	0.21	0.65
Site (Condition)	4	621.11	14.85	<b>&lt;0.001</b>	4	553.62	6.73	<b>0.004</b>	4	0.01	0.58	0.68	4	0.08	0.60	0.67
Residual	17	41.83			13	82.24			28	0.01			28	0.13		
<i>Within-Subjects Effects</i>																
Time	5.38*	10.70	0.73	0.61	4.73*	132.28	2.32	0.06	5.43*	0.12	1.31	0.26				
TimexDepth	5.38*	10.74	0.73	0.61	4.73*	137.71	2.42	0.05	5.43*	0.12	1.31	0.26				
TimexCondition	5.38*	11.28	0.77	0.58	4.73*	131.91	2.32	0.06	5.43*	0.12	1.35	0.24				
TimexSite(Condition)	21.53*	44.21	3.01	<b>&lt;0.001</b>	18.92*	122.30	2.15	<b>0.009</b>	21.72*	0.16	1.76	0.03				

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**Table 4.** Relations between *P. oceanic* abundance (meadow cover and shoot density) and time for the invaded (I1, I2 and I3) and non-invaded sites (N1, N2 and N3);  $\tau$ : Kendall's coefficient of rank correlation, p: calculated probability.

SITE	Meadow Cover		Shoot Density	
	$\tau$	p	$\tau$	p
<b>I1</b>	0.022	0.93	0.27	0.45
<b>I2</b>	<b>0.511</b>	<b>0.04</b>	<b>0.689</b>	<b>0.023</b>
<b>I3</b>	<b>0.82</b>	<b>0.01</b>	0.27	0.45
<b>N1</b>	<b>0.675</b>	<b>0.03</b>	0.156	0.531
<b>N2</b>	0.2	0.421	0.333	0.18
<b>N3</b>	0.111	0.655	<b>0.654</b>	<b>0.04</b>

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527 **Figure 1.** Temporal variation of biomass (g FWm<sup>-2</sup>) of the *C. cylindracea* stands growing inside (IN, full  
528 dots) and outside (OUT, empty dots) the three studied invaded meadows (I1, I2 and I3). Data are  
529 presented as means and standard errors.

530

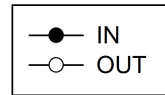
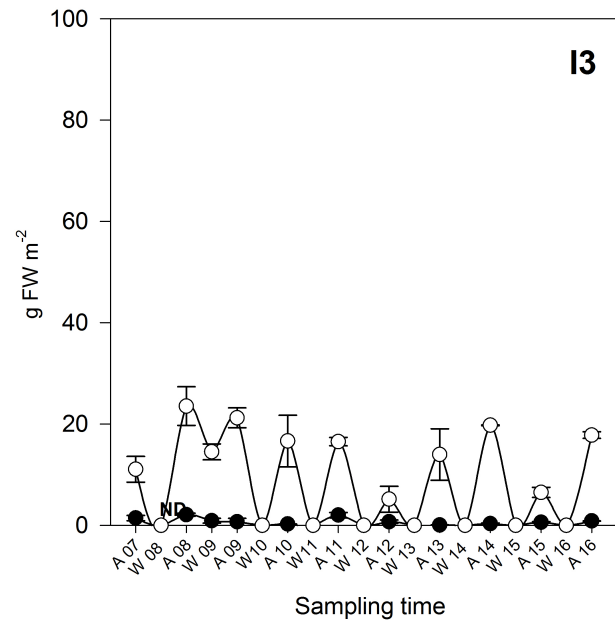
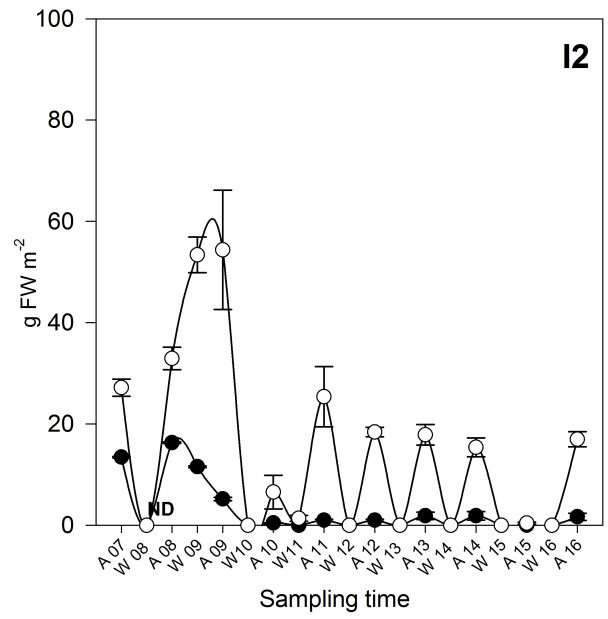
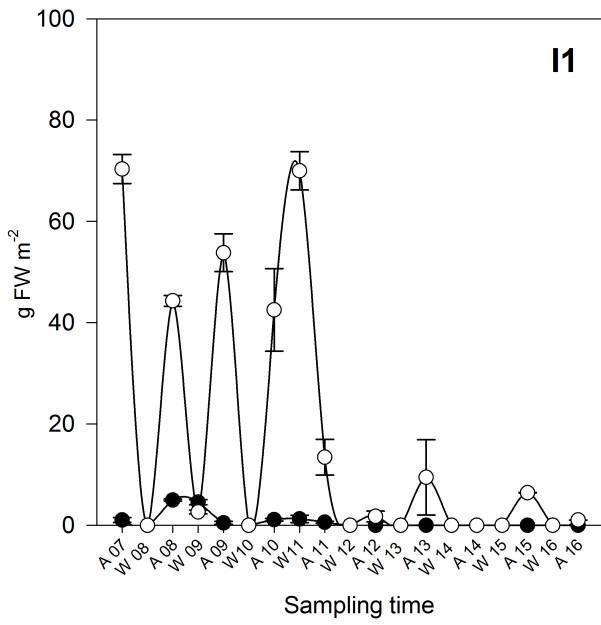
531 **Figure 2.** Temporal variation of the seagrass descriptors (Meadow cover, Shoot density and Annual net  
532 population growth (NPG<sub>y</sub>)) of the and non-invaded (N1, N2 and N3) and invaded (I1, I2 and I3) meadows  
533 from 2007 to 2016. Data are presented as means±standard error.

534

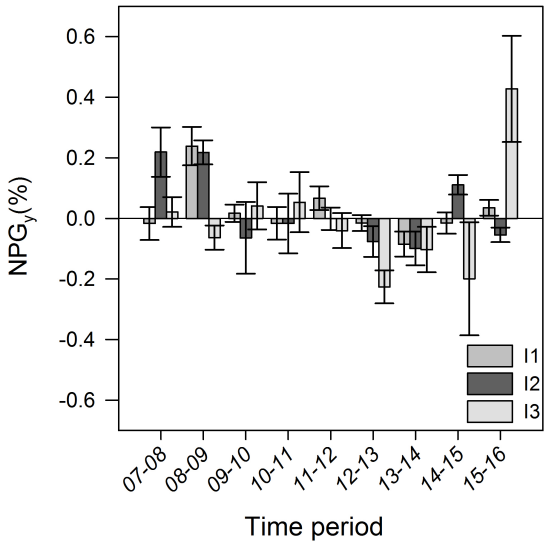
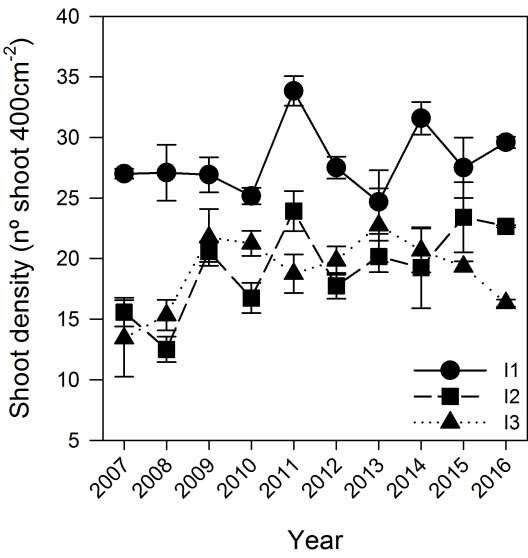
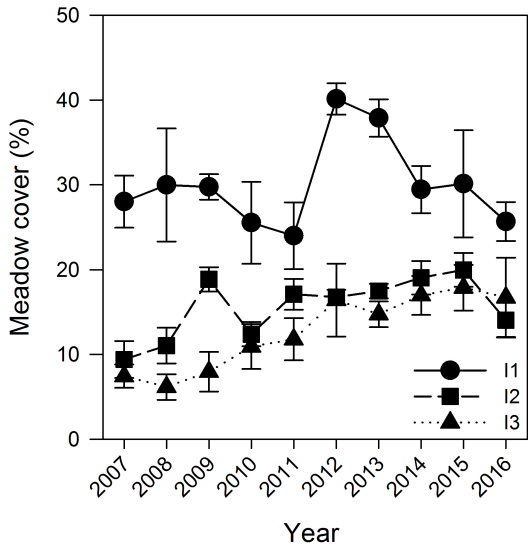
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### Invaded



### Non-invaded

