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2   **Title: Propagules are not all equal: traits of vegetative fragments and disturbance**

3   **regulate invasion success.**

4   Running title: Propagules, disturbance and establishment.

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ABSTRACT

Invasion success is regulated by multiple factors. While the roles of disturbance and propagule pressure in regulating the establishment of non-native species are widely acknowledged, that of propagule morphology (a proxy for quality) is poorly known. By means of a multi-factorial field experiment, we tested how the number (5 versus 10) and quality (intact, without fronds or without rhizoids) of fragments of the clonal invasive seaweed, *Caulerpa cylindracea*, influenced its ability to establish in patches of the native seagrass, *Posidonia oceanica*, exposed to different intensities of disturbance (0, 50 or 100 % reduction in canopy cover). We hypothesized that the ability of fragments to establish would be greater for intact fragments (high quality) and reduced more by frond removal (low quality) than rhizoid removal (intermediate quality). At low propagule pressure or quality, fragment establishment was predicted to increase with increasing disturbance, whereas, at high propagule pressure or quality, it was predicted to be high regardless of disturbance intensity. Disturbance intensity, fragment number and quality had independent effects on *C. cylindracea* establishment success. Disturbance always facilitated fragment establishment. However, fragments retaining fronds, either intact or deprived of rhizoids, had higher establishment success than fragments deprived of fronds. Increasing propagule number had weak effects on the cover of *C. cylindracea*. Our results demonstrate that propagule traits enabling the acquisition of resources made available by disturbance can be more important than propagule number in determining the establishment and spread of clonal non-native plants. More generally, our study suggests that propagule quality is a key, yet underexplored, determinant of invasion success.

Keywords: biological invasion; biotic resistance; disturbance; propagule pressure; propagule quality; clonal seaweeds; *Caulerpa cylindracea*

1. INTRODUCTION

Understanding the mechanisms that underlie biological invasions, one of the major causes of biodiversity loss worldwide (Mack et al. 2000, Dextrase and Mandrak 2006), is key to predicting habitats vulnerable to future invasions. Many factors, such as features of the recipient native community, disturbance regimes, the biology and ecology of the invasive species and its associated propagule pressure, can regulate the success of invasive species (D'Antonio and Vitousek 1992, Davis et al. 2000, Stachowicz et al. 2002, Lockwood et al. 2005). However, manipulative experimental studies have generally focused on only one of these factors, despite compelling evidence indicating that invasion success is likely dependent on interactions among them (Leung and Mandrak 2007, Britton-Simmons and Abbott 2008, Clark and Johnston 2009, Eschtruth and Battles 2009).

Disturbance to native communities is often a key determinant of invasion success (Davis et al. 2000). For example, disturbance to native canopies can release non-native plants from biotic resistance by providing access to limited resources such as light and space (Elton 1958, Hobbs and Huenneke 1992, Stachowicz et al. 2002, Corbin and D'Antonio 2004, Clark and Johnston 2005, Bulleri et al. 2010, Byun et al. 2017). However, the effects of increased resource availability (Davis et al. 2000) can interact with invader propagule pressure to determine invasion success (D'Antonio et al. 2001, Thomsen et al. 2006, Britton-Simmons and Abbott 2008, Byun et al. 2015). For instance, disturbance to native fouling communities fostered the establishment of the invasive bryozoan *Watersipora suborquata* only when it occurred in association with high propagule pressure (Clark and Johnston 2009). Likewise, in a Californian coastal grassland, decreased biotic resistance due to higher springtime water availability amplified the positive effects of increased propagule pressure on the establishment of the European perennial grass *Holcus lanatus* (Thomsen et al. 2006). On the other hand, resistance to invasion can be overwhelmed by high invader propagule pressure,

71   irrespective of disturbance levels (Hollebone and Hay 2007, 2008). For example, high

72   propagule pressure of the Japanese stiltgrass, *Microstegium vimineum*, overwhelmed the

73   resistance to invasion of a deciduous forest ecosystem (Warren et al. 2012). Thus, disturbance

74   might be essential for invader establishment when propagule pressure is low, but have weaker

75   effects when propagule pressure is high.

76   An overlooked factor influencing the success of invasive species is propagule quality

77   (here referred to as the physical condition, such as the morphology or biomass of a

78   propagule). For example, the settlement, metamorphosis and performance of the larvae of

79   some invasive marine invertebrates increase with their body size (Marshall et al. 2003,

80   Marshall and Keough 2003, Marshall et al. 2006). Likewise, the number and size of

81   internodes on rhizome fragments of invasive clonal plants with vegetative reproduction

82   significantly affect their settlement and expansion dynamics (Quinn and Holt 2008, Estrada et

83   al. 2016). Production of these propagules by biotic or abiotic disturbance results in fragments

84   of differing size/biomass or containing different morphological components (e.g.

85   combinations of roots/shoots/leaves) that are essential for acquiring different resources

86   (leaves or fronds for photosynthesis; roots for nutrient uptake). Thus, the presence/absence of

87   different morphological components - which may generate fragments of different quality -

88   could be as important as propagule number in regulating individual fragment success (Estrada

89   et al. 2016). Disturbance is, in fact, unlikely to foster the establishment of plant fragments

90   lacking those functional structures necessary for an efficient uptake of freed resources.

91   Despite asexually reproducing plants being some of the most invasive species globally

92   (Kaiser 2000, Allendorf and Lundquist 2003, Williams and Smith 2007), we know very little

93   about how variations in the morphological characteristics of vegetative fragments interact

94   with disturbance regimes to determine invasion success.

The clonal seaweed, *Caulerpa cylindracea* Sonder, (previously *Caulerpa racemosa* var. *cylindracea*), is among the most widespread non-native species in the Mediterranean Sea (Renoncourt and Meinesz 2002, Piazzi and Balata 2009). *C. cylindracea* colonizes a variety of habitats, including dead rhizomes of the native seagrass *Posidonia oceanica* (Linnaeus) Delile (Bulleri et al. 2011). Although intact seagrass meadows appear resistant to *C. cylindracea*, canopy removal can promote the establishment of *C. cylindracea* at their margins, suggesting a key role of disturbance in facilitating its establishment (Ceccherelli et al. 2014). *C. cylindracea* can reproduce sexually but mostly spreads through drifting asexual fragments generated by abiotic (i.e. wave surge and currents) or biotic disturbance (i.e. herbivore grazing) (Ceccherelli et al. 2002, Klein and Verlaque 2008, Bulleri et al. 2009). Fragments vary not only in number but also in morphology since they can be formed by prostrate stolons carrying both fronds and rhizoids or lacking either one or both structures (authors' personal observation). In Caulerpales, rhizoids provide firm attachment to the substratum and allow nutrient uptake, while fronds are deputed to light harvesting (Komatsu et al. 1997, Chisholm and Moulin 2003). Therefore, the presence/absence of either rhizoids or fronds likely determines the response of *C. cylindracea* to disturbances increasing the availability of different resources. Importantly, however, enhanced uptake of nutrients may not foster establishment if photosynthetic efficiency is impaired by the absence or reduced density of fronds. Moreover, rhizoids, representing a smaller proportion of fragment biomass (Capiomont et al. 2005), can be re-generated more rapidly compared to fronds and their loss has less impact on fragment establishment than frond loss (Bulleri et al. unpublished data). Thus, fragments lacking rhizoids may generally be of higher quality than fragments lacking fronds.

Here, we experimentally evaluated how the quality and number of *C. cylindracea* fragments influenced its ability to establish in patches of *P. oceanica* exposed to disturbances

120 of varying intensity (i.e. amount of canopy removal). We predicted that: (1) the ability of

121 fragments to establish would be greater for intact fragments (high quality) and that it would

122 be reduced more by frond removal (low quality) than rhizoid removal (intermediate quality);

123 (2) at either low propagule density or low fragment quality, fragment establishment would

124 increase with increasing disturbance; (3) disturbance would have weaker effects on

125 establishment success at either high propagule density or high fragment quality; (4) propagule

126 quality would have stronger effects on fragment success compared to propagule number at

127 any disturbance level.

128 **2. MATERIALS AND METHODS**

129 *Study system*

130   *Caulerpa cylindracea* is a clonal green alga considered one of the 100 most invasive

131 species in the Mediterranean (Streftaris and Zenetos 2006). It was first recorded in Libya in

132 1990 and has now spread throughout the Mediterranean (see Klein and Verlaque 2008 for

133 review). *C. cylindracea* occurs on a variety of habitats, from rocky shores to soft-sediments,

134 and across a broad depth range, from the intertidal to 70 m (Klein and Verlaque 2008, Bulleri

135 et al. 2011).

136   *Posidonia oceanica* is one of the most important habitat-forming seagrass species in

137 the Mediterranean, occurring across a broad depth range on sandy bottoms (Bethoux and

138 Copinmontegut 1986). *P. oceanica* has leaves up to 70 cm long and forms large, dense beds

139 that support high biodiversity and important fisheries (Ott 1980, Marbà et al. 1996, Guidetti

140 2000).

141   This study was conducted about 10 km south of Livorno (Antignano, 43º29'N,

142 10º19'E; NW Mediterranean), in a dense subtidal *P. oceanica* seagrass meadow (1 ha x 1.5

143   ha, mean ± SE, 316.4 ±11.5 shoots per m^2; Uyà unpublished data), occurring at 4-8 m water

144   depth and surrounded by a matrix of boulder and sandy substrata. *C. cylindracea* is abundant

145   at this site, often occurring along the margins of the seagrass meadow.

146   **Effects of disturbance, propagule quality and pressure on *C. cylindracea* establishment.**

147   We experimentally tested the effects of disturbance intensity (3 levels; canopy intact,

148   50 % canopy reduction and 100 % canopy reduction), propagule pressure (2 levels; 5 and 10

149   fragments) and propagule quality (3 levels; intact fragments carrying both rhizoids and

150   fronds, fragments lacking either rhizoids or fronds) on the establishment of *C. cylindracea*.

151   Disturbance intensity treatments were created just before the peak in abundance of *C.*

152   *cylindracea*, in mid-July (summer) (Ruitton et al. 2005). We randomly established seventy-

153   two 0.5 x 0.5 m plots at the margin of the *P. oceanica* meadow, at a depth of ~6 m. Plots had

154   an initial seagrass cover of 100 % and were scattered along a 170 m stretch of the meadow.

155   Twenty four plots were then randomly assigned to either 0, 50 or 100% *P. oceanica* cover

156   reduction (Fig. 1). In order to allow the seagrass to recover, reductions in canopy cover were

157   achieved by cutting the leaves whilst leaving the rhizomes intact. Seagrass canopy cover in

158   plots assigned to different disturbance intensity levels were maintained every three weeks

159   throughout the duration of the experiment (10 weeks), by cutting re-grown leaves without

160   disturbing *C. cylindracea* fragments.

161   Four plots for each level of disturbance were then randomly allocated to each of the

162   six combinations of propagule pressure and quality (Fig. 1). Fragments of *C. cylindracea* (10

163   cm stolon length) were collected two days after the implementation of disturbance treatments

164   from a nearby area (~ 100 m away from the experiment location) characterized by dead

165   seagrass rhizomes. Either fronds or rhizoids were removed from fragments in the field, soon

166   after their collection, using scissors. Either five or ten fragments were fixed with U-shaped

167   metal staples within the central 20 x 20 cm area of each plot, to avoid edge effects (n = 540

168   fragments in total). Plots were thoroughly searched for the presence of *C. cylindracea* before

169   the experimental transplantation and, when present, it was removed. Both fronds and rhizoids

170   represent a small proportion of the total biomass of the invasive seaweed (up to 12x less

171   biomass than the stolon) (Capiomont et al. 2005), thus we controlled for initial propagule

172   length, and not biomass, as differences between propagule morphologies in biomass were

173   considered negligible. The attachment of all fragments was checked two days after they were

174   transplanted to ensure all fragments had remained in place.

Sampling and data analysis

176   The percentage cover of *C. cylindracea* in each plot was visually estimated after three

177   (August), six (September) and ten weeks (October) from the start of the experiment, using a

178   20 x 20 cm plastic frame subdivided into 25 sub-quadrats. A score from 0 (absence) to 4

179   (completely covered) was given to each sub-quadrat and the percentage cover was obtained

180   by summing over the entire set of sub-quadrats (Dethier et al. 1993). At the end of the

181   experiment, *C. cylindracea* fragments were retrieved from the central 20 x 20 cm area of each

182   plot and brought to the lab for analysis. The total number of fronds, mean length of three

183   randomly selected fronds, total stolon length and the number of rhizoids per quadrat were

184   measured. Finally, total fragment biomass per quadrat was estimated as dry weight (g $\cdot$ m^2)

185   after drying the material at 60 ºC for 48 hours.

186   The effects of canopy disturbance, propagule number and quality on the percentage

187   cover of *C. cylindracea* were analyzed using a linear mixed model. Seagrass disturbance

188   intensity, propagule number and quality were considered as fixed effects. Time of sampling

189   was considered as a random effect to take into account temporal auto-correlation in the data

190   generated by repeatedly sampling the same quadrats. The baseline for the linear mixed model

191   was set *a priori* as the, supposedly, most invasible scenario: the combination of 100 %

192   disturbance inoculated with 10 intact fragments. Chi-Square likelihood ratio tests were used

193   to detect significant differences amongst treatments. The analysis was performed in R

194   (version 3.3.2) using the lmer function within the lme4 package and the anova function with

195   Chisq test (Bates et al. 2015). When significant main effects were detected, multiple *post-hoc*

196   TukeyHSD tests were used to determine differences among the levels of disturbance intensity

197   and propagule quality using the glht function within the multcomp package (Hothorn et al.

198   2013). Assumptions of linearity and variance homogeneity were checked by plotting the

199   standardized residuals against fitted values (Zuur et al. 2009).

200   The effects of experimental conditions on the density and length of fronds, total stolon

201   length, density of rhizoids and biomass of *C. cylindracea,* sampled at the end of the

202   experiment, were analyzed by means of three-way ANOVA. The model included canopy

203   disturbance, propagule number and quality as fixed, crossed factors. Data were square root

204   transformed when Cochran's test indicated significant heterogeneity of variances

205   (Underwood 1997). TukeyHSD tests were used for *post-hoc* comparison of the means. One

206   plot assigned to the 100 % seagrass cover reduction and transplanted with 10 intact fragments

207   of *C. cylindracea* was lost at the start of the experiment. In order to maintain a balanced

208   design, the missing value was replaced with the mean of the remaining replicates for this

209   treatment and residual degrees of freedom were adjusted accordingly (Underwood 1997). All

210   ANOVA tests were performed in R (version 3.3.2) using the lm function within the GAD

211   package (Sandrini-Neto and Camargo 2012).

212   **3. RESULTS**

213   Disturbance, propagule pressure and quality had significant effects on the cover of *C.*

214   *cylindracea* in experimental plots (Fig 2, Table 1A). Across the study, the cover of the

215   seaweed increased significantly with increasing disturbance intensity. In particular, the

216   percent cover of the seaweed was, on average, 30 and 10 times higher in 100 % and 50 %

217   canopy removals compared to control treatments, respectively, on the last sampling date (Fig.

218   2, Table 1B).

219   The cover of *C. cylindracea* was significantly higher in plots inoculated with intact

220   and no-rhizoid fragments than no-frond fragments (Fig. 2, Table 1) and increased with

221   increasing propagule pressure (Fig. 2, Table 1A). When transplanted at high density (i.e. 10

222   fragments/plot), some intact and no-rhizoid fragments were also able to persist under intact

223   canopies throughout the experiment. The interaction between disturbance and either

224   propagule pressure or quality was non-significant, but only marginally so ($P \leq 0.06$, Table

225   1A).

226   At the end of the experiment, the density of fronds and rhizoids, total stolon length

227   and biomass increased with increasing intensity of disturbance (Fig. 3-4, Table 2A and

228   Appendix S1: Fig. S1-S3). Moreover, total stolon length increased significantly with the

229   number of fragments inoculated (Table 2A, Appendix S1: Fig S3). There was a significant

230   effect of the interaction Disturbance x Propagule quality on the mean length of fronds. The

231   *post-hoc* test indicated that fronds grew longer from intact and no-rhizoid fragments than no-

232   frond fragments in 50 % canopy reduction plots, while no differences occurred for the other

233   disturbance levels (Table 2B and Appendix S1: Fig S1).

234   The biomass of *C. cylindracea* was up to 50x and 80x higher in the 50 % and 100 %

235   canopy cover reduction treatments, respectively, when compared to intact *P. oceanica.* In

236   addition, in 100 % canopy reduction plots inoculated with 5 fragments, final seaweed

237   biomass was about 2.4x higher for intact than no-fronds (Fig. 4, Table 2A). Biomass was also

238 significantly higher in plots inoculated with intact and no-rhizoid fragments than plots

239 inoculated with no-frond fragments (Fig. 4, Table 2B).

240 **4. DISCUSSION**

241   While there is increasing acknowledgement that invasion success is regulated by

242 multiple factors, experimental tests including combinations of factors are still relatively

243 uncommon (but see Thomsen et al. 2006, Clark and Johnston 2009, Estrada et al. 2016, Byun

244 et al. 2017). Here, we show that disturbance to native canopies, propagule quality and number

245 influence invasion success. As predicted, disturbance had the strongest effect on invasion

246 success, with seaweed cover, biomass, total stolon length and frond density per plot

247 increasing with growing intensity of disturbance to seagrass canopies. In addition, propagule

248 quality had a stronger influence on fragment biomass and traits compared to propagule

249 number. The effects of the individual factors were independent and did not interact to

250 determine fragment establishment.

251   Increasing reductions in the cover of the seagrass *Posidonia oceanica* enhanced the

252 establishment of *C. cylindracea*. This supports previous studies showing positive effects of

253 disturbance to native assemblages on invasion success via increased resource availability

254 (Burke and Grime 1996, Davis et al. 2000, Bulleri et al. 2010, Ceccherelli et al. 2014).

255 Although we did not investigate how disturbance of *P. oceanica* canopy promoted the

256 establishment of *C. cylindracea,* the main mechanism was likely an increase in access to light

257 (Marin-Guirao et al. 2015). Light levels can be reduced by 60-89 % in intact seagrass beds

258 compared to outside, potentially limiting the growth of *C. cylindracea* (Marin-Guirao et al.

259 2015). In fact, in our study, a 50 % canopy reduction was sufficient to increase the cover,

260 biomass and frond density of *C. cylindracea* to values observed in the 100 % canopy removal

261 treatment. Thus, invasion of native marine macrophytes may happen at lower levels of

262   disturbance than often considered, highlighting the limitations of marine studies that have

263   typically considered removal of the entire native canopy vs. non-removal (Valentine and

264   Johnson 2003, Britton-Simmons and Abbott 2008, Bulleri et al. 2010). Understanding critical

265   levels, or thresholds, at which disturbance facilitates invasion is clearly an important avenue

266   for future research. This is a pressing issue for *C. cylindracea* given the current declining

267   trends of *P. oceanica* seagrass meadows in the Mediterranean Sea (Marbà et al. 2014).

268   General theory and empirical research provide compelling evidence of the role of

269   species' traits in regulating establishment success (Stearns 1992, Marshall et al. 2006).

270   Although rarely studied in an invasion context, fragment quality had strong consequences for

271   cover and total biomass measured at the end of the experiment. Importantly, fragments

272   without fronds consistently performed worst when compared to fragments with fronds (both

273   intact and without rhizoids). This was somewhat surprising given that the rhizoids of other

274   invasive *Caulerpa* spp. perform vital functions such as fixing N_2, which in turns promotes

275   organic matter turnover and nutrient acquisition (Chisholm and Moulin 2003). The strong

276   effect of the presence/absence of fronds suggests that light was, again, the likely limiting

277   resource for *C. cylindracea.*

278   The stolon and fronds of *C. cylindracea* contain photosynthetic pigments such as

279   chlorophyll *a*, siphonoxanthin and siphonein (Raniello et al. 2004). *C. cylindracea* fragments

280   initially deprived of fronds had lower total stolon length and biomass, but, by the end of the

281   experiment, they were able to re-grow fronds to densities matching those found in other types

282   of fragments. Since this seaweed is coenocytic, the removal of fronds could result in a rapid

283   reallocation of energy to increase their re-growth at expense of the lateral expansion, as found

284   for several other *Caulerpa* species (Collado-Vides and Robledo 1999). The lack of

285   differences in rhizoid density between fragments that had their rhizoids removed and intact

286   ones at the end of the experiment suggests that there is also a reallocation of energy to rhizoid

287 re-growth. Because of the small loss of biomass associated with rhizoid removal (Capiomont

288 et al. 2005), new rhizoids may be quickly generated without major consequences on final

289 biomass and total stolon length.

290   Increasing fragment number, although having weaker effects than disturbance and

291 fragment quality on seaweed establishment, increased the final percent cover of *C.*

292 *cylindracea*. However, a doubling from 5 to 10 fragments/quadrat did not result in a doubling

293 of percent cover or biomass of fragments in plots. This might be a consequence of

294 intraspecific competition among fragments at the highest fragment number treatment.

295 Competition for light among fragments is unlikely to explain this pattern, as *C cylindracea*

296 can be found up to 70 m deep at high densities (Klein and Verlaque 2008). At higher

297 densities, fragments may be competing for other resources such as nutrient supply. Intra-

298 specific competition as a consequence of nutrient limitation among early growth stages has

299 been documented in invasive terrestrial plants (Blank 2010) and marine macroalgae (Steen

300 2003, Steen and Scrosati 2004). We suggest that intraspecific competition among invasive

301 propagules may have important, but currently underestimated, consequences for the

302 establishment and demography of non-native plants.

303   Previous studies have clearly demonstrated that different factors (e.g. disturbance and

304 propagule pressure) interact to determine invasion success (D'Antonio et al. 2001, Thomsen

305 et al. 2006, Britton-Simmons and Abbott 2008). In our experiment, the effects of canopy

306 disturbance, fragment quality and number were largely independent. Our prediction that the

307 success of low quality fragments (fragments without fronds) would increase with increasing

308 disturbance levels was supported, as no-frond fragments could establish only in total canopy

309 removal plots. In contrast, the prediction that high quality fragments (i.e. intact) would be

310 successful across all disturbance regimes, independently of their number was not supported;

311 in fact, high and intermediate quality fragments persisted under intact seagrass canopy only

312   when transplanted in large numbers. This suggests subtle interactions among disturbance,

313   propagule quality and number may occur, but were not detected in our analyses because of

314   the low values of cover and biomass achieved by *C. cylindracea* under full canopies. *P*-

315   values close to significance for interactions between disturbance and either propagule quality

316   or propagule pressure on seaweed cover (see Table 1) support the proposition that the cover

317   of *C. cylindracea,* at the peak of the growing season (when our experiment was conducted),

318   may be dependent upon the characteristics of both propagules and the recipient habitat.

319   In summary, our results suggest a stronger role of propagule quality compared to

320   propagule number in the establishment of *C. cylindracea*. For fast-growing, asexually

321   reproducing invasive species, propagule traits enhancing the ability to acquire the specific

322   resources freed by disturbance - in our case light - appear key for their successful

323   establishment and spread. Under these circumstances, the traits of propagules conferring

324   greater establishment ability likely vary among disturbances that free different resources (i.e.

325   beneficial traits may be disturbance-specific). Our results may also reconcile contrasting

326   results of the effects of disturbance on invasion success (Moles et al. 2012): disturbance may

327   facilitate non-native establishment only when propagules possess the traits necessary to

328   acquire freed resources. By contrast, weak effects of resource release or input can be

329   expected when propagules are unable to exploit them, independently of their abundance.

330   Under these circumstances, assessing the matching between the quality or type of resource

331   made available through disturbance and the characteristics of non-native propagules may

332   greatly enhance our ability to forecast invasion success in environments increasingly exposed

333   to human perturbations. More broadly, a functional approach, based on resource-exploitation

334   traits of propagules (i.e. fragments, larvae, spores or adults), might allow identifying non-

335   native species or morpho-types within species more likely to benefit from a specific

336   disturbance regime.

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347

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519   **Table 1.** (A) Chi-Square (X^2) likelihood ratio test and (B) *post-hoc* Tukey HSD tests of the

520   linear mixed model on the effects of disturbance intensity (D), propagule pressure (P) and

521   propagule quality (Q) on the percentage cover of *Caulerpa cylindracea*. The baseline for the

522   model is the supposedly most invasible scenario: 100% disturbance inoculated with 10 intact

523   fragments.

A) Chi-Square (X^2) likelihood ratio test

Source of Variation	df	MS	F-value
Disturbance intensity (D)	2	**10419.5**	**68.801*****
Propagule pressure (P)	1	**1583.3**	**10.455****
Propagule quality (Q)	2	**1556.8**	**10.280*****
DxP	2	436.1	2.880 ·
DxQ	4	327.3	2.161 ·
PxQ	2	268.2	1.77
DxPxQ	4	89.9	0.594

Significance codes: ·p≤0.06;*p<0.05; ** p<0.01; *** p<0.001.

B) *post-hoc* Tukey HSD tests

D	100 % > 50 % > Control
Q	Intact = no rhizoids > no fronds

Table 2. (A) Source of Variation and (B) *post-hoc* Tukey HSD tests on the effects of disturbance intensity (D), propagule pressure (P) and propagule quality (Q) on frond density, mean frond length, total stolon length, rhizoid density and biomass of *Caulerpa cylindracea*.

A) Source of Variation

Source of Variation	df	Frond density MS	F	Mean frond length MS	F	Total stolon length MS	F	Rhizoid density MS	F	Biomass MS	F
D	2	**16368.7**	**27.871***	**12.125**	**47.925***	**104601**	**21.086***	**35212.0**	**16.638***	**1.746**	**39.682***
P	1	1250	2.128	0.001	0.004	**23875**	**4.181***	5425.0	2.563	0.071	1.614
Q	2	1320.9	2.247	**1.794**	**7.091****	13461	2.714	4150.0	1.961	**0.191**	**4.341***
DxP	2	339.5	0.578	0.030	0.119	6323	1,275	1803.0	0.852	0.009	0.205
DxQ	4	384.9	0.655	**0.884**	**3.494***	3956	0.797	1303.0	0.616	0.058	1.312
PxQ	2	174.9	0.300	0.188	0.743	2874	0.579	216.0	0.102	0.046	1.045
DxPxQ	4	148.9	0.254	0.414	1.636	2828	0.570	682.0	0.322	0.044	1
Residual	53	587.3		0.253		4960.6		2116.3		0.044	

Transformation	none	none	none	none	Square root
Cochran's test	p<0.05	p=0.120	p<0.05	p<0.05	p=0.120

Significance codes: *p<0.05; ** p<0.01; *** p<0.001.

B) *post-hoc* **Tukey HSD tests**

Frond density	D	100 % > 50 % > Control
Mean frond length	DxQ	Control: Intact = no rhizoids = no fronds 50 %: Intact = no rhizoids > no fronds 100 %: Intact = no rhizoids = no fronds
Total stolon length	D	100 % > 50 % > Control
Rhizoid density	D	100 % > 50 % ; 100 % > Control
Biomass	D	100 % > 50 % > Control
	Q	Intact = no rhizoids > no fronds

Figure 1. Schematic diagram of the fully factorial experiment conducted in a *Posidonia oceanica* seagrass meadow investigating how disturbance intensity, propagule pressure and propagule quality regulate the establishment of *Caulerpa cylindracea* fragments. Four plots were assigned to each combination of disturbance intensity (3 levels), propagule pressure (2 levels) and propagule quality (3 levels), resulting in a total of 72 manipulated plots.

Figure 2. Temporal trend of the percentage cover of *Caulerpa cylindracea* under different combinations of disturbance intensity, propagule pressure and propagule quality. Data are means ± Standard Error. Continuous lines represent 5 fragment treatments whilst dashed lines indicate 10 fragment treatments. Differences in y-axes scale should be considered when comparing disturbance intensity levels.

Figure 3. Density of fronds of *Caulerpa cylindracea* (number/m^2) under different combinations of disturbance intensity, propagule pressure and propagule quality. Data are means ± Standard Error. Differences in y-axes scale should be considered when comparing disturbance intensity levels.
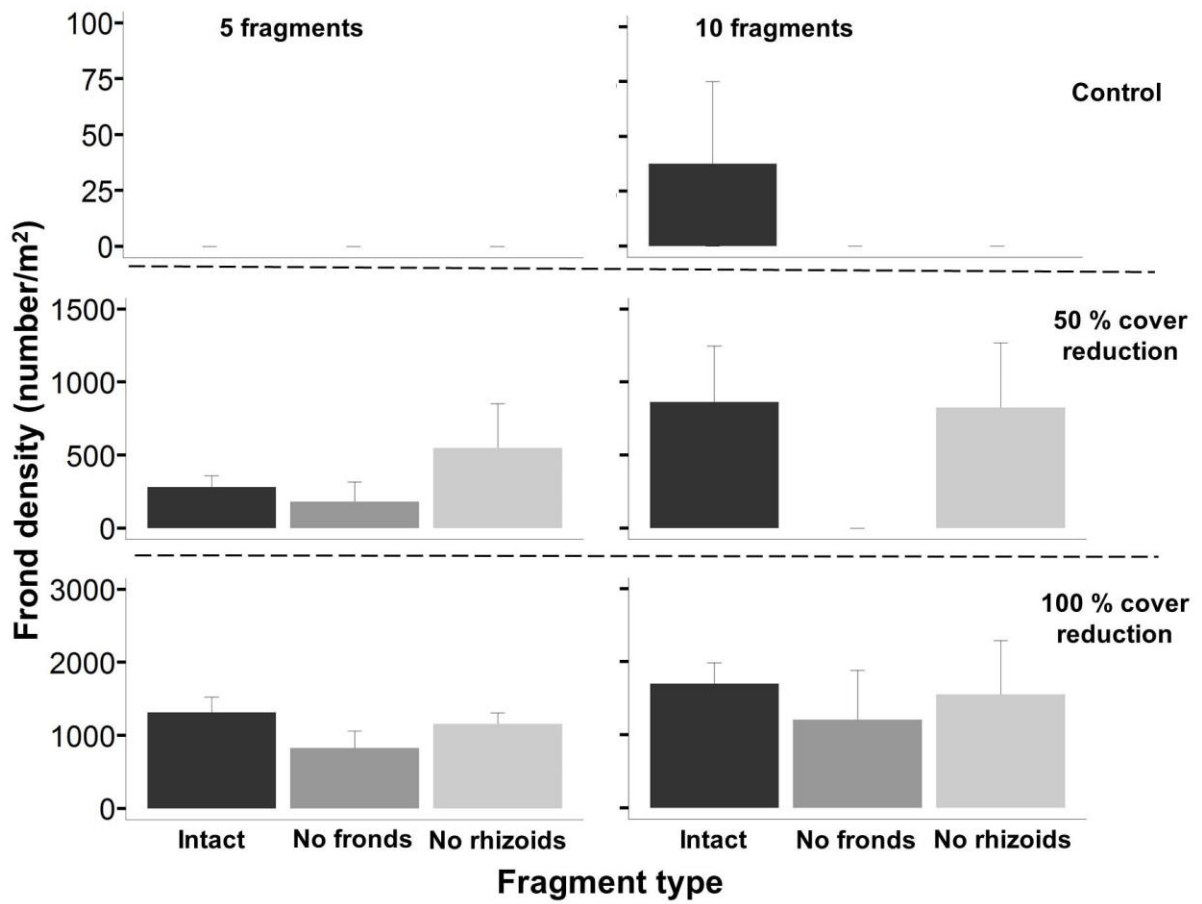
Figure 4. Biomass of *Caulerpa cylindracea* (g/m^2) under different combinations of disturbance intensity, propagule pressure and propagule quality. Data are means ± Standard Error. Differences in y-axes scale should be considered when comparing disturbance intensity levels.
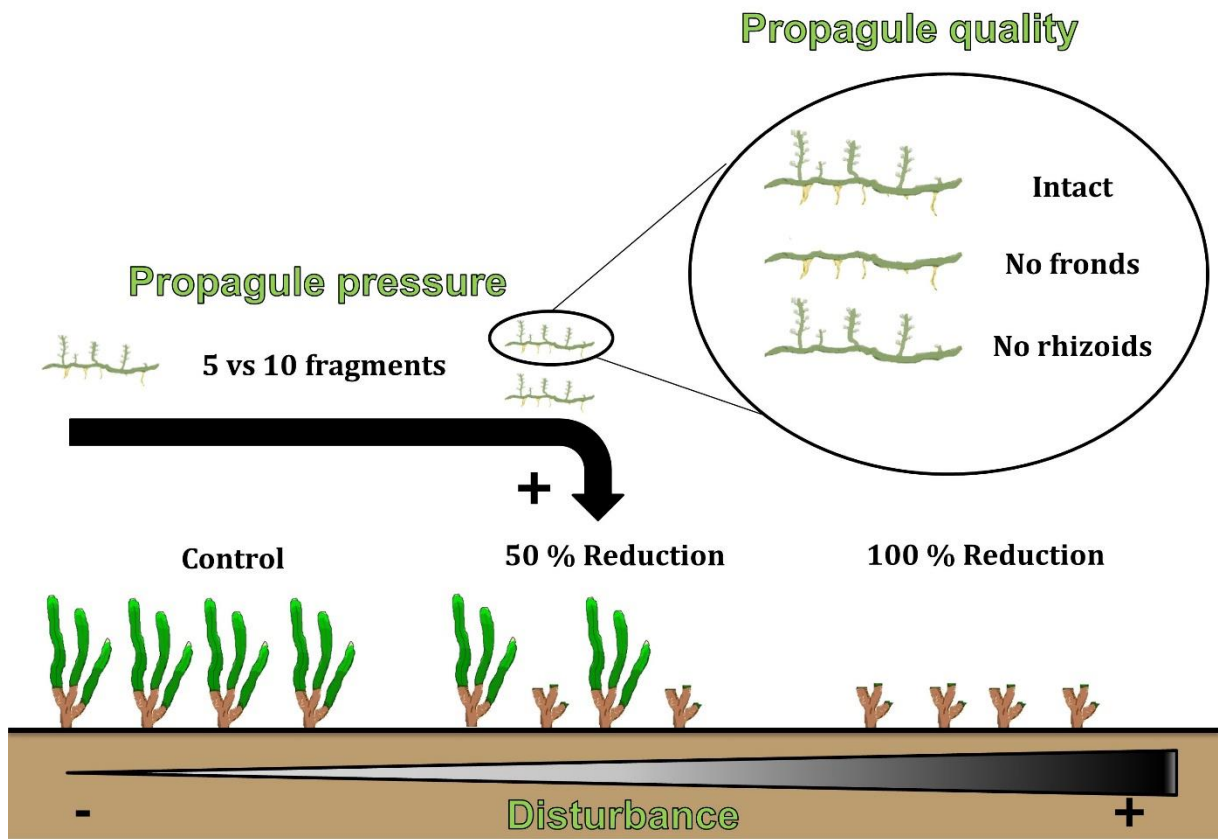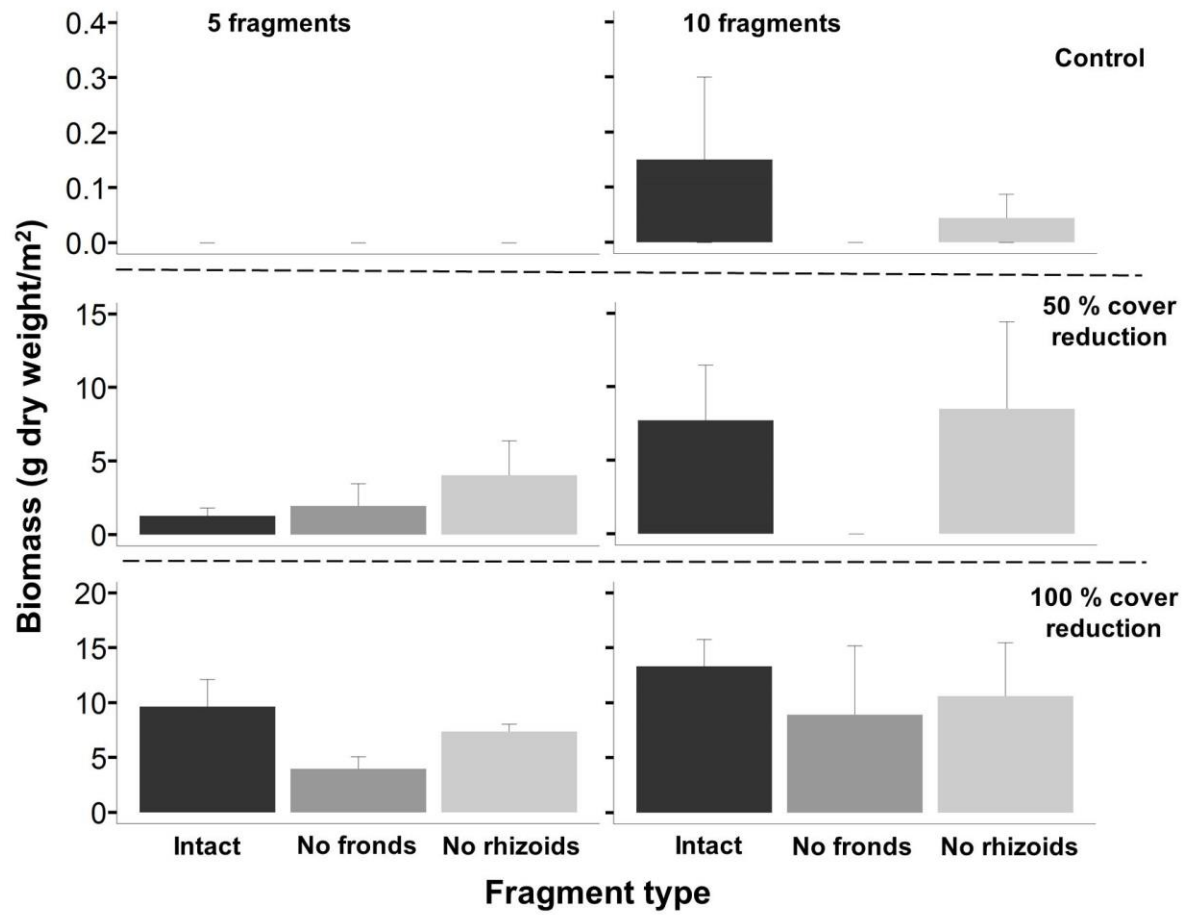
Uyà et al. Figure 1
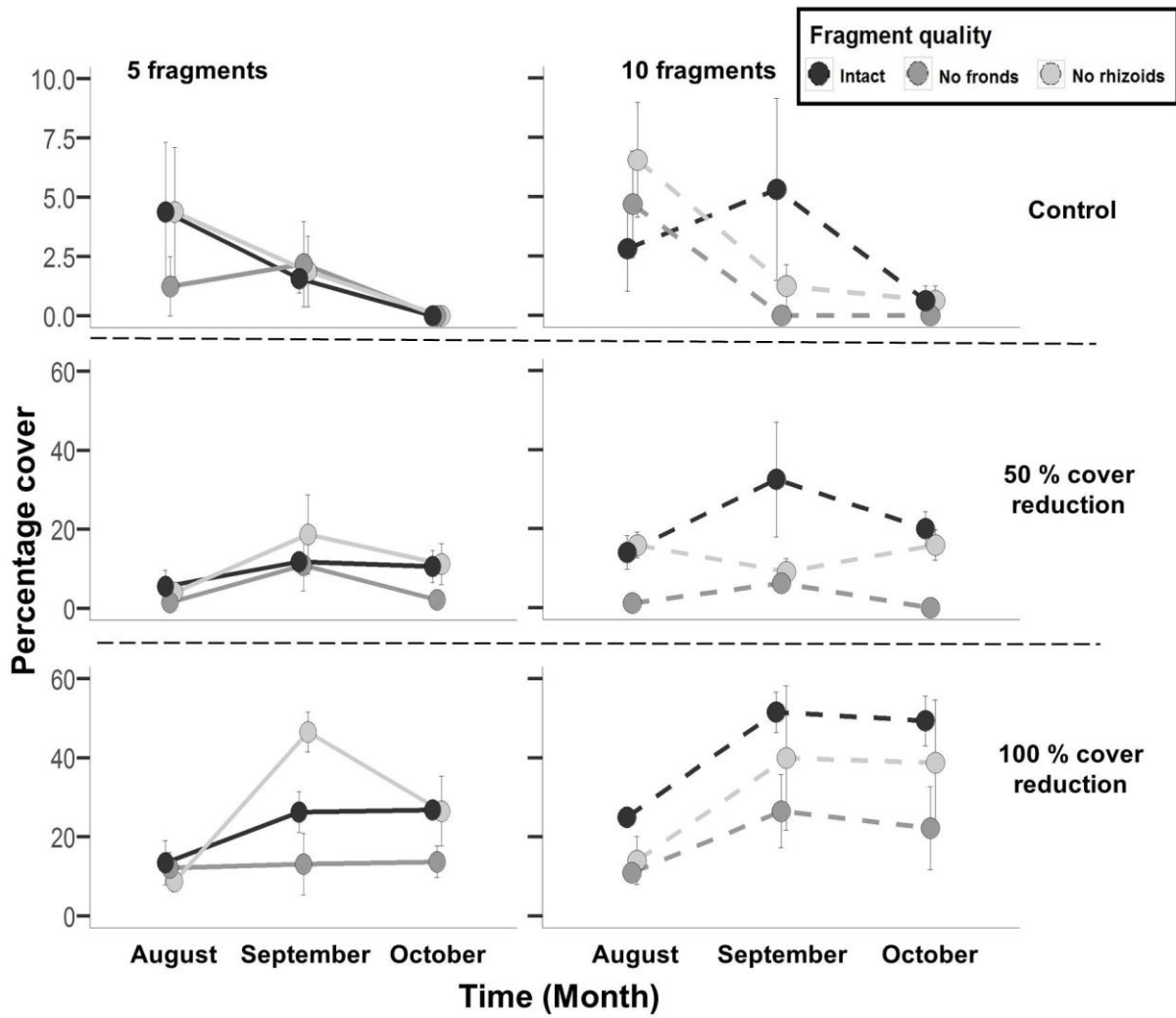
Uyà et al. Figure 2

Uyà et al. Figure 3

Uyà et al. Figure 4