

1 Detecting the impacts of harbour construction on a seagrass habitat and its subsequent  
2 recovery

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22 **Abstract:**

23 Managing coastal development requires a set of tools to adequately detect ecosystem  
24 and water column degradation, but it also demands tools to detect any post-disturbance  
25 improvement. Structural seagrass indicators (such as shoot density or cover) are often  
26 used to detect or assess disturbances, but while they may be very sensitive to the impact  
27 itself, it is unclear if those indicators on their own can effectively reflect recovery at  
28 time scales relevant to managers. We used the construction of a harbour affecting a  
29 nearby *Posidonia oceanica* seagrass community to test the ability of a set of indicators  
30 (structural and others) to detect alterations and to evaluate their sensitivity to recovery  
31 of environmental quality after harbour construction was complete and the disturbance  
32 ceased. We used a Beyond Before After Control Impact (BBACI) design to evaluate  
33 effects on one impacted and three control meadows where we used structural,  
34 morphological, community and physiological indicators (26 in total) to assess  
35 disturbance impacts. Additionally, we measured some of the potential environmental  
36 factors that could be altered during and after the construction of the harbour and are  
37 critical to the survival of the seagrass meadow (light, sediment organic matter, sediment  
38 accrual).

39 Harbour construction caused a clear increase in sediment organic matter and in  
40 sediment deposition rates, especially fine sand. Light availability was also reduced due  
41 to suspended sediments. Sediment and light conditions returned to normal levels 5 and  
42 15 months after the construction began. As expected, seagrass structural indicators  
43 responded unequivocally to these environmental changes, with clear reductions in shoot  
44 density. Additionally, reduced light conditions quickly resulted in a decline in  
45 carbohydrate content in affected meadows. Unexpectedly, we also recorded a  
46 significant increase in metal content in plant tissues. No response was detected in the

47 physiological indicators related to eutrophication (e.g. N and P content in tissues) and in  
48 morphological (shoot biomass) and community (epiphyte biomass) indicators. More  
49 than three years after the completion of the harbour, structural indicators did not show  
50 any sign of recovery. In contrast, physiological indicators, mainly heavy metal and  
51 carbohydrates content, were much better in detecting the improvement of the  
52 environmental conditions over the fairly short period of this study. These results  
53 indicate that while structural indicators are critical to evaluate the immediate effect of  
54 disturbances and the recovery on impacted systems, specific physiological indicators  
55 may be much better suited to determining the timing of environmental quality recovery.  
56 The design of impact and monitoring protocols in the wake of coastal developmental  
57 projects need to consider the differential effectiveness and time-response of measured  
58 indicators carefully.

59

60 **Keywords: indicator, disturbance, *P. oceanica*, physiological indicators, time-**  
61 **response.**

62

63 **Introduction:**

64 Coastal zones around the world have been and are still facing intensive  
65 development that includes the construction of marine infrastructures such as harbours  
66 and breakwaters (Short & Wyllie-Ecieverria 1996; Waycott *et al.* 2009). These large,  
67 physical structures modify the interface between the sea and the land, can destroy  
68 valuable marine habitats (Inglis & Lincoln-Smith. 1995) and alter currents and sediment  
69 dynamics (Morales, Borrego & Ballesta 2004). In addition, the process of construction  
70 itself also produces several associated effects that may have extended areas of influence.  
71 Specifically, the construction of harbours has been associated with increases in the fine  
72 sediment fraction and in water turbidity (Erftemeijer & Robin Lewis III 2006) and with  
73 changes in current dynamics that can affect sediment deposition (Morales, Borrego &  
74 Ballesta 2004; Anfuso, Pranzini & Vitale 2011) among other effects. Detecting the  
75 appearance of ecological impacts, assessing their consequences and understanding the  
76 time course for natural conditions to re-establish following cessation of the disturbance  
77 are some of the main challenges for environmental management in the coastal zone.  
78 Indicators are among the most important tools used by managers to detect changes in  
79 ecosystems due to anthropogenic impacts or improvements due to successful  
80 management actions (Heink & Kowarik 2010). Their present-day importance is  
81 reflected by the huge effort devoted to develop a large array of indicators in many  
82 different environments, from forest ecosystems (Brooks *et al.* 1998) to freshwater  
83 (Harig & Bain 1998; Munné & Prat 2009) and coastal water marine systems (Carignan  
84 & Villard 2002; Ballesteros *et al.* 2007; Martínez-Crego *et al.* 2008). According to  
85 Heink and Kowarik (2010), an indicator in ecology and environmental planning is  
86 defined as something used to depict or evaluate environmental conditions or changes or  
87 to set environmental goals, where this *something* can be either a component or a

88 measure of environmentally relevant phenomena. For the present work, we use the term  
89 “indicator” only in the second sense, that is, a measure of environmentally relevant  
90 phenomena.

91 The rate at which the indicators respond to degradation and improvement in physical  
92 environmental conditions is therefore, a key aspect for their use and interpretation, yet it  
93 is often overlooked (Donangelo *et al.* 2010). Indeed, most of them have been validated  
94 only to track trajectories of ecosystem degradation. Few have proven successful in  
95 tracking recovery, since recovery is often more protracted and, in many cases, may  
96 follow complex, non-linear trajectories (Scheffer *et al.* 2001; Carstensen *et al.* 2011).  
97 This is especially true for ecosystems with slow-growing species, where recovery  
98 processes are typically slow, often occurring over significantly longer time periods than  
99 standard monitoring programs are funded for. The failure to detect recovery in these  
100 systems may result in the erroneous conclusion that disturbance has persisted or that  
101 remedial actions were inadequate, both of which may have important consequences for  
102 long-term management.

103         Seagrass meadows are one of the dominant ecosystems in shallow coastal  
104 marine waters over the world with important contributions to their goods and services  
105 (Cullen-Unsworth & Unsworth 2013). Additionally, seagrass ecosystems are extremely  
106 sensitive to changes in water quality and to other human induced disturbances (Short &  
107 Wyllie-Echevarria 1996; Krause-Jensen, Greve & Nielsen 2005; Lopez y Royo *et al.*  
108 2010). As a result, seagrass ecosystems have been used in many monitoring programs  
109 (Marbà *et al.* 2012) to obtain reliable indicators. Among them, structural ones are the  
110 most widely used because of their ease of measurement and their clear links to  
111 ecosystem structure and services. Likewise, morphological parameters have been used  
112 worldwide as a good measure of plant health and stress (Marbà *et al.* 2012). Finally,

113 physiological indicators are increasingly being used in monitoring programs as they are  
114 reported to be efficient tools for early detecting of anthropogenic disturbances  
115 (Martinez-Crego et al. 2008).

116 *Posidonia oceanica* (L.) Delile is the most important and widespread seagrass in  
117 the Mediterranean sea, where it forms extensive meadows from the surface down to 40  
118 m depth (Bouduresque *et al.* 2006). *P. oceanica* is a foundation species (sensu Dayton  
119 & Hessler 1972) that performs important ecological functions in the ecosystem but is  
120 also extremely sensitive to changes in environmental conditions. This makes *P.*  
121 *oceanica* one of the species from which the largest number of indicators have been  
122 described so far (Montefalcone 2009). In particular, with a set of structural,  
123 physiological, morphological and community indicators, this plant has been observed to  
124 effectively detect changes in light availability, sediment characteristics and increases in  
125 organic matter – the most frequent physical changes associated with coastal  
126 development (Ruiz & Romero 2001; Ruiz & Romero 2003; Erftemeijer & Robin Lewis  
127 III 2006; Frederiksen *et al.* 2006; Pérez *et al.* 2007; Serrano, Mateo & Renom 2011). Of  
128 these, physiological indicators are well known to have driver-specific responses and this  
129 specificity has been used as a tool to identify the causal factors behind deterioration in  
130 the ecosystem or in water quality (Martínez-Crego *et al.* 2008). Nevertheless, there is  
131 still little known about the rate of response of these indicators to improvements in the  
132 physical environment once the disturbance has ceased (i.e. how they track recovery). As  
133 already stated, the inability to track the timing and form of response to improved  
134 environmental conditions can lead to erroneous management decisions, with potentially  
135 negative economic, social and ecological consequences. In this context, we examined  
136 the response of a range of indicators within a *P. oceanica* seagrass ecosystem to the  
137 construction of a harbour (discrete disturbance) in NW Catalonia, Spain. Our main

138 objective was to test the ability of 26 commonly used indicators to detect alterations  
139 during the construction of the harbour and their sensitivity to potential recovery in  
140 environmental conditions over three years after the construction had been completed.

## 141 **2. Materials and methods:**

### 142 2.1. Study design

143 The study was designed to detect the impacts of a harbour construction on a nearby  
144 *Posidonia oceanica* meadow and its potential recovery when the construction had been  
145 completed. We employed a Beyond-BACI design (BBACI, Underwood (1992),  
146 measuring responses from a *P. oceanica* meadow close to an expanding harbour  
147 ('impact' location) and at three distant (non-impacted) meadows before, during and  
148 after harbour construction ceased, see (Table 1). At each location we measured 26  
149 commonly used seagrass indicators to test their ability to track the time course of  
150 recovery. In parallel, we also measured the main environmental drivers associated with  
151 the ecological impacts of harbour construction: water transparency, sediment deposition  
152 and sediment grain size, produced during and after the construction (Erftemeijer &  
153 Robin Lewis III 2006).

### 154 **2.2. Study area and harbour construction**

155 The study area is situated in the NE coast of Spain between two localities, Blanes and  
156 Lloret de Mar, both with intense tourism development. Blanes had one of the biggest  
157 harbours in the area, with a mooring capacity for 59 fishing vessels and 684 recreational  
158 boats. In March of 2010 (Table 1) construction began to add a new external breakwater  
159 to the harbour. This meant the occupation of 42.037 m<sup>2</sup> of sea surface, dredging 40,000  
160 m<sup>3</sup> of sediment from the seafloor and using several tonnes of sand and stones to  
161 stabilize the new structure. During this period, which lasted from March to July 2010

162 (Table 1), a superficial and mid water net barrier was placed to limit the spread of fine  
163 sediment released from dredging activities and sand addition to nearby waters. After  
164 this period, some minor works continued, but dredging activities were less intense.

165 The area is characterized by clear coastal waters, and the coastline has numerous *P.*  
166 *oceanica* meadows reported to be in good ecological condition (Romero *et al.* 2010).  
167 We selected a *P. oceanica* meadow situated between 9 and 14 meters depth (density of  
168 290 shoots m<sup>-2</sup>, SE=28) on a rocky substrate and at 160 m (at its closest point) from the  
169 old breakwaters (Fig. 1, 41°40'19"N 2°48'04"E ) as the potentially impacted site. The  
170 foundations of the new breakwaters were built only 20 m from the meadow (at its  
171 closest point). Additionally, three control sites were selected north of the construction  
172 area due to the absence of *P. oceanica* meadows further south. The first control site was  
173 situated close to Mar i Murtra garden in Sa Forcanera beach (41°40'31"N 2°48'19"E ),  
174 the second in Fenals beach (41°41'19"N 2°50'7"E) and the third was situated next to  
175 Cala Canyelles (41°42'4"N, 2°53'21"E, Fig.1).

### 176 **2.3. Sampling design and data acquisition**

177 Driver measurements and seagrass sampling was carried out at the impact and control  
178 meadows before the disturbance started (February 2010), during the disturbance (March  
179 2010 to early August 2010) and after the disturbance (late August 2010 to August 2013;  
180 see Table 1) following a Beyond BACI design. All meadows were sampled between 13  
181 and 15 m depths to minimize bathymetric variability.

#### 182 *2.3.1. Monitoring environmental drivers*

183 To assess light availability, irradiance (photosynthetic active radiation, 400-700 nm)  
184 was measured in situ as photosynthetic photon flux density (PPFD,  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ),  
185 using Apogee PAR QSO-Sun 2.5v light sensors connected to HOBO u12-013 data



186 loggers that recorded at 10-minute intervals. One light sensor was placed in each  
187 meadow just above canopy level for one month when the harbour was being expanded  
188 (April 2010) and 10 months after the disturbance (April 2011). As some fouling  
189 appeared on the sensors at the end of the recording period, and in order to prevent its  
190 shading effects, we only used the first 15 days of data when the sensors were totally  
191 clean.

192 To assess the importance of the sedimentation processes over the meadow, sediment  
193 deposition rates ( $\text{g m}^{-2} \text{ day}^{-1}$ ) were measured using six cylindrical sediment traps (16 cm  
194 height and 4.5 cm diameter) installed in groups of three, in two independent tripods just  
195 above the canopy level at each site, similarly to those used by Gacia, Granata and  
196 Duarte (1999). Sediment traps were installed for one month in all sites with light  
197 sensors only while dredging activities were occurring (in April 2010).

198 To follow changes in sediment granulometry and organic matter content in the seagrass  
199 meadow, three random samples of surface sediments were taken at each site using 50 ml  
200 cups. Samples were taken during the disturbance (April 2010) and at a single time after  
201 construction work ceased (July 2010). Sediment composition was analysed with an  
202 optical particle analyser Mastersizer 2000. Organic matter was determined as the  
203 difference in the weight of the sediments before and after drying in a muffle furnace for  
204 5 hours at 500°C.

### 205 *2.3.2. Monitoring seagrass indicators*

206 We chose 26 seagrass indicators commonly used in ecological assessments (Marbà *et al.*  
207 2012) that have known functional associations with a wide range of coastal  
208 disturbances: morphological and structural indicators (shoot density, cover, number of  
209 leaves, leaf length, leaf area and % of leaf necrotic tissue), physiological indicators

210 related to changes in light availability ( $\delta^{13}\text{C}$ , sucrose, starch and total carbohydrate  
211 content), physiological indicators related to nutrient variations and eutrophic conditions  
212 (C, N, P,  $\delta^{15}\text{N}$ ,  $\delta^{34}\text{S}$ ), physiological indicators linked to metal availability (Fe, Pb, Cd,  
213 Mn, Ni, Cu, Zn) and community indicators (leaf epiphyte biomass). All physiological  
214 parameters were analysed in rhizomes; in addition, we analysed C, N, P,  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$   
215 from the leaves. For most variables, we collected samples before harbour construction  
216 began, during the disturbance and at several intervals after the cessation of dredging  
217 activities, depending on the variable (see Table 1). Sampling consisted of collecting ten  
218 seagrass shoots, randomly chosen in each site and at each sampling period. Seagrass  
219 shoot density was estimated at each site and sampling time in three 40x40 cm fixed  
220 quadrats in each station. Sampling was performed four times (once before the  
221 disturbance, and three times after the disturbance, Table 1). Shoot density counts during  
222 the disturbance (March 2010, April 2010 and July 2010) were not possible due to high  
223 sedimentation values, with underwater visibility close to 0 m during that period.

#### 224 **2.4. Laboratory analysis**

225 From the 10 shoots collected for each station and sampling time, 5 shoots (one replicate  
226 each) were firstly used to measure morphological indicators (number of leaves, leaf  
227 length, leaf area and % of leaf necrotic tissue) and epiphyte biomass. Then, all 10 shoots  
228 were pooled together and randomly grouped in pairs. The first 1-1.5 cm of the rhizomes  
229 and leaf number two (without epiphytes) of each pair were separated, dried and ground  
230 to a fine powder, resulting in 5 replicates per site and time. Laboratory analyses were  
231 performed to measure 14 biochemical or physiological indicators: Carbon and nutrient  
232 content (C, N, P in leaves and rhizomes), stable isotopic composition ( $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  in  
233 leaves and rhizomes,  $\delta^{34}\text{S}$  in rhizomes), metal content (Fe, Pb, Cd, Mn, Ni, Cu, Zn in  
234 rhizomes), sucrose, starch and total carbohydrate content (in rhizomes). Processing and

235 analysis of samples was carried out according to methods detailed in Romero et al.  
236 (2007) and Martínez-Crego *et al.* (2008) at Centre d'Estudis Avançats de Blanes  
237 (CEAB-CSIC), Scientific and Technical Services of the University of Barcelona (SCT-  
238 UB), Servicios de Apoyo a la Investigación (SAI) of University of la Coruña, and  
239 Millbuck labs (United Kingdom).

## 240 **2.5. Data analysis**

241 Asymmetrical analyses of variance were used to examine temporal differences between  
242 the potentially impacted meadow and the average of control meadows. The mechanics  
243 and the logical structure of these analyses are fully explained in Underwood (1991);  
244 (1992; 1993; 1994). Data of the different environmental drivers and indicators were  
245 compared between periods (Before/After or During/After) and meadows  
246 (Impacted/Controls).

247 In order to determine the time of response and recovery of the different indicators  
248 BBACI analyses were carried out comparing values before to each of the sampling  
249 times during and after the disturbance (0-1, 0-2, 0-5, 0-15 months, Table 1, Table 2). If  
250 the BxC test was not significant (Marked with \* in table 2), the impact was tested with  
251 an *F*-ratio Mean Square BxI/Mean Square Residual. Otherwise impact was tested with  
252 an *F*-ratio Mean Square Before vs After x Impact vs Controls (BxI)/ Mean Square  
253 Before vs After x Among Controls (BxC). The same method was used to analyse  
254 changes in shoot density with time (0-8, 0-15 and 0-38 months, Table 1, Table 2).  
255 Differences in sedimentation rates between impacted and control sites were tested using  
256 one-way ANOVA. Changes in light availability, sediment grain composition and  
257 percentage of organic matter in sediments during and after the disturbance were also  
258 tested using an asymmetrical analysis of the variance (Table1).

259 Data were examined for normality with the Shapiro-Wilks test and homogeneity of  
260 variances was tested with Bartlett's test. The assumptions of normality and  
261 homogeneity of variances between samples was not met for all variables and tests.  
262 However, analyses of variance are robust with respect to these problems, particularly in  
263 large designs (Underwood 1981). All statistics were performed in R software (R  
264 Development Core Team 2012).

265

266

### 267 **3. Results:**

#### 268 **3.1. Responses of environmental drivers**

269 We detected an 88.4% reduction in light availability in the impacted meadow relative to  
270 the controls ( $p=0.046$ ; Table S2, Fig. 2). Some seasonal variability not directly related  
271 to the construction was also recorded in light levels at both impacted and control  
272 meadows, and was probably caused by storms during the course of the study (Fig. 2). In  
273 addition, fine materials in suspension increased sedimentation rates over 14 times  
274 compared to controls 2 months after the impact began, from  $33$  to  $480 \text{ g m}^{-2} \text{ day}^{-1}$   
275 ( $p<0.01$ , Table S3, Fig. 3). As a result, the impacted meadow was buried under 5 to 10  
276 cm of fine sediments that produced a significant change in granulometry ( $p<0.01$ , Table  
277 S4, Fig. 4), noticeably decreasing grain size, and a ca. two-fold increase in the organic  
278 matter content of sediments ( $p<0.01$ , Table S5, Fig. 5). Dredging activities and sand  
279 additions were completed 5 months after the works began, and natural hydrodynamics  
280 washed out the fine sediment. Light availability was found to have recovered 15 months  
281 after commencement (Fig.2), but light availability probably recovered sooner, since  
282 granulometry (Fig.4) and organic matter in surface sediments (Fig. 5) recovered to  
283 control levels five months after the construction began.

#### 284 **3.2. Responses of indicators to disturbance:**

285 Shoot density and indicators directly related to light availability (starch, sucrose and  
286 total carbohydrates) and metal pollution (Ni, Fe, Mn, Pb, Cd) responded most to the  
287 disturbance (Table 2). Ni and starch content in the rhizomes showed significant  
288 differences after just one month, being the first indicators to detect the impact of the  
289 harbour construction. After two months, sucrose, starch and total carbohydrates were  
290 significantly lower in the impacted site compared to control sites (Fig. 6A,B), which

291 started to increase their concentrations following the seasonal cycle of carbohydrate  
292 production (Fig. 6-B). Also two months after the start of the disturbance, Fe, Mn and Pb  
293 in the rhizomes showed significant differences from 'before' values (Table 2). Five  
294 months after the start of the disturbance, starch remained significantly lower at the  
295 impact site. Sucrose and total carbohydrates were also lower at the impact site, as seen  
296 in Table 2 and Fig. 6-A, although these differences were not significant due to the high  
297 variability in controls. After five months, the effects of the disturbance were also  
298 evident in Fe, Mn, Pb, (Table 2, Fig. 6-C), which continued to record increasing values  
299 of these metals. Also, five months after the construction began, Cd levels increased for  
300 the first time with respect to the controls. See all results of Beyond BACI variance  
301 analysis of indicators in Table (S6).

302 Shoot density was not measured during the disturbance due to low visibility in the area  
303 (see methods). The first values of shoot density were obtained 8 months later when the  
304 disturbance had already ceased. Shoot density had declined by 50 % at the impacted site  
305 8 months after the disturbance (Fig. 6-E, Table 2, Table S7).

### 306 **3.3. Recovery time of indicators values:**

307 The time taken for the indicators to return to pre-disturbance values varied depending  
308 on the indicators considered. Sucrose and total carbohydrates started recovering right  
309 after the cessation of the disturbance while other physiological indicators (starch, Mn,  
310 Cd, Pb, Fe) recovered fully after 10 months. In contrast, shoot density did not show any  
311 sign of recovery at any sampling time, until our last sampling event, 32 months after the  
312 cessation of the disturbance (Fig. 6, Table 2, Table S7).

313

## 314 **4. Discussion**

315 As expected, harbour works produced a pulsed disturbance that increased water  
316 turbidity, reduced light availability and covered the meadow close to the harbour with  
317 fine sediment for approximately 5 months. After that period, environmental conditions  
318 recovered, i.e. water clarity was restored and the fine sediment that covered the meadow  
319 disappeared, probably washed away by hydrodynamics. The disturbance was  
320 sufficiently intense to halve shoot density in the impacted meadow within 8 months.  
321 Physiological indicators were highly sensitive to the disturbance and changes in  
322 indicators related to light availability (starch, sucrose and total carbohydrates) and  
323 metallic pollution (Ni, Fe, Mn, Pb, Cd) were detected just two months after construction  
324 began. In contrast, morphological indicators (% of leaf necrotic tissue, number, length  
325 and leaf area), epiphyte biomass and nutrient contents and isotopic signatures (%N, %C,  
326  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , %P,  $\delta^{34}\text{S}$ ) did not appear to be affected by the harbour construction.  
327 Physiological indicators began an immediate recovery after the cessation of the work  
328 and 10 months later all these indicators had returned to pre-disturbance levels. In  
329 contrast, the structural indicator (shoot density) did not show any recovery even 38  
330 months after the disturbance.

### 331 **4.1. Drivers of disturbance and impact on the ecosystem**

332 The breakwater construction had important detrimental effects on the surrounding  
333 environment. Sedimentation rates at the impact site during the disturbance period were  
334 extremely high, i.e. 14 times higher than observed at our control sites, where rates  
335 matched natural deposition rates previously observed in the same area by Gacia,  
336 Granata and Duarte (1999). Deposited sediment was mainly composed of fine particles  
337 that spread over the meadow, and, as documented in other instances, the sediment

338 retention net used to prevent the spread of sediment was not fully effective (Erftemeijer  
339 & Robin Lewis III 2006). Sediment in suspension caused a reduction in light  
340 availability comparable to the effect of using an 80-90% shading mesh (Ruiz & Romero  
341 2001; Mackey, Collier & Lavery 2007). Moreover, the content of organic matter in the  
342 sediment of the impacted meadow doubled during the disturbance, probably due to the  
343 effect of the settlement of fine particles. These reductions in light availability may be  
344 the main cause of the recorded decline in shoot density as has been found in previous  
345 studies (Ruiz & Romero 2001; Serrano, Mateo & Renom 2011). However, it is  
346 plausible that sediment deposition, increases in organic matter, or their interactions, also  
347 contributed to the mortality or reduced production of shoots observed in the ecosystem;  
348 each of these three drivers were individually above thresholds known to cause shoot  
349 mortality (Manzanera, Pérez & Romero 1998; Ruiz & Romero 2003; Erftemeijer &  
350 Robin Lewis III 2006; Pérez *et al.* 2007; Serrano, Mateo & Renom 2011). Taken  
351 together, our results clearly indicate that the physical disturbance caused by the  
352 construction of the harbour was too close to the protected *P. oceanica* meadow (Habitat  
353 Directive 92/43/CEE), causing important damage to the ecosystem.

#### 354 **4.2. Responses of indicators to disturbance**

355 Of the 26 seagrass indicators we examined, none of the morphological (number of  
356 leaves, leaf length, leaf area and % of leaf necrotic tissue) or community indicators  
357 (epiphyte biomass) analysed responded to the harbour construction. The physiological  
358 indicators related to nutrients (%N, %P,  $\delta^{15}\text{N}$ , %C,  $\delta^{13}\text{C}$ ) did not show any influence  
359 either. This suggests that sediment dredging and material addition did not spread  
360 nutrients into the system. Additionally, despite the observed increase in organic matter  
361 that has been linked to an increased sulphur production and changes in  $\delta^{34}\text{S}$  (Oakes &  
362 Connolly 2004; Frederiksen *et al.* 2006; Frederiksen *et al.* 2008), we did not detect a



363 response in  $\delta^{34}\text{S}$ . The lack of  $\delta^{34}\text{S}$  response can be due to the high abundance of Fe and  
364 Mn in the environment that can competitively inhibit sulphate reduction processes and,  
365 consequently,  $\text{H}_2\text{S}$  production (Myers & Nealson 1988; Thamdrup, Fossing &  
366 Jørgensen 1994; Frederiksen *et al.* 2006). As pointed out above, shoot density, that  
367 appeared to be mainly responding to a reduction in light availability, halved 8 months  
368 after the harbour works began although, it is highly probable that it had reduced as early  
369 as five months after the disturbance, in accordance with time course of shoot mortality  
370 previously reported (Serrano, Mateo & Renom 2011).

371 Physiological indicators related to carbon storage (starch, sucrose and total  
372 carbohydrates) were the first to respond to the disturbance. It took just one month for  
373 starch, and two months for sucrose and total carbohydrates to respond to light reduction.  
374 The harbour works took place between the end of winter and at the beginning of the  
375 summer season, matching the plants' seasonal peaks in growth, photosynthetic rates and  
376 carbon storage in the rhizomes (Alcoverro, Duarte & Romero 1995; Alcoverro,  
377 Manzanera & Romero 2001; Serrano, Mateo & Renom 2011). This is the period of the  
378 year when light deprivation becomes critical because plants are exhausting their  
379 reserves (Alcoverro, Manzanera & Romero 2001). Therefore, while plants from control  
380 sites were increasing their photosynthetic rates and carbohydrate reserves, plants in the  
381 impacted site could not photosynthesise and consequently could not restore their  
382 carbohydrate reserves after winter. Conversely, no effects were observed in  $\delta^{13}\text{C}$   
383 content in leaves and rhizomes, which contrasts with the results of some shading  
384 experiments where this indicator responded relatively quickly to light reduction  
385 (Serrano, Mateo & Renom 2011).

386 The metal content of tissues also responded quickly to the disturbance, confirming the  
387 response times found in recent studies by Richir *et al.* (2013), and also suggest that

388 metals were remobilized during the harbour construction. Fe and Mn accumulated in the  
389 rhizomes of the plant within 2 months of the commencement of the construction and  
390 continued to increase during the disturbance period. In 5 months, their concentrations  
391 were 3 times greater than observed in the controls and 3 to 4 times higher than observed  
392 at any site of available monitoring programs (Romero *et al.* 2010). Pb and Cd levels  
393 also increased 2 and 5 months after the start of the disturbance. In contrast,  
394 concentrations of Ni in the rhizomes decreased more than the controls during the  
395 disturbance probably due to the antagonistic uptake interactions with Fe, Mn and other  
396 metallic elements (Richir *et al.* 2013).

397

#### 398 **4.3. Recovery time of indicators**

399 More or less ten months after the construction ceased, all physiological indicators  
400 affected by the coastal development returned to pre-disturbance levels. As has been  
401 documented elsewhere, carbohydrate reserves in plant tissues responded positively to  
402 improvements in environmental quality (Longstaff *et al.* 1999; Ruiz & Romero 2001;  
403 Invers *et al.* 2004; Pérez *et al.* 2007; McMahon, Lavery & Mulligan 2011). In contrast,  
404 little is known on the accumulation of metals in seagrasses (but see Richir *et al.* 2013).  
405 Our results show that processes regulating fluxes of metallic elements (Ni, Fe, Mn, Pb,  
406 Cd) in plant rhizomes can be fast and dynamic and can be sensitive indicators to detect  
407 degradation and improvement of water quality conditions. The fact that metallic trace  
408 elements are highly dynamic in plants is especially relevant for managers when  
409 assessing the results of seagrass and water quality monitoring programs. Indeed, it  
410 suggests that the presence of these elements in seagrass rhizomes, at least in the younger  
411 parts of the plant, may reflect the presence of these metals in the surrounding  
412 environment, and is not the consequence of historical accumulation of metals in the

413 plants.  
414 Shoot density did not recover to pre-disturbance levels 38 months after the construction  
415 ceased. Recuperation of *P. oceanica* structural indicators such as shoot density or cover  
416 requires longer time frames to respond due to the very slow rhizome elongation rates for  
417 this species, reported to be only of 2– 4 cm year<sup>-1</sup> (Duarte 1991; Marbà & Duarte 1998)  
418 and the low shoot recruitment observed (Marbà & Duarte 1998). In fact, very few  
419 studies have reported the recuperation of structural indicators except over very long  
420 periods of time (Badalamenti *et al.* (2011). Full recovery, if possible, is thought to  
421 require decades (Duarte 2002; González-Correa *et al.* 2005).

422

## 423 **5. Summary and conclusions**

424 The Blanes harbour breakwater was built with apparently adequate mitigation measures  
425 including sediment retention nets designed to reduce the impacts of dredging and  
426 construction on the threatened *Posidonia oceanica* seagrass meadows. Despite this, the  
427 activity resulted in an intense, pulsed disturbance, significantly reducing water quality  
428 (light levels and sediment deposition), and causing a dramatic structural decline in  
429 adjacent seagrass meadows. Three years after the harbour construction, shoot densities  
430 at meadows 20 m away from the site had still not shown signs of recovery.

431 Unsurprisingly developmental activities of this nature, particularly so close to *P.*  
432 *oceanica* meadows, can be catastrophic for this legally protected ecosystem. From a  
433 management perspective, we observed that not all common seagrass indicators  
434 responded specifically to this type of disturbance, and even from those that responded,  
435 very few were able to detect ecosystem recovery when the disturbance had ceased. Only  
436 some indicators such as shoot density, carbohydrate content and metal-related measures  
437 responded as early indicators to this type of disturbance. These indicators are useful in

438 determining the potential mechanisms of post-disturbance habitat responses, and  
439 suggest that the plant degradation we recorded after harbour construction was linked to  
440 deposition caused by sediment movement, reduced light levels, and metal  
441 contamination. Structural indicators such as shoot density showed no response at  
442 management time scales (3 years), and while this may be linked to the slow growth rate  
443 of the seagrass, it may reflect a potential post-disturbance ecosystem shift. In contrast,  
444 physiological indicators (light and metal-related) were much more sensitive to changes  
445 in environmental quality and returned to a pre-disturbance state within 3 years.  
446 Although improvement of water quality does not represent ecosystem recovery, it can  
447 help evaluate the effectiveness of mitigation and remedial actions, critical for coastal  
448 management and planning.

449

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462

463 **Table footnotes**

464 **Table 1:** Sampling time of environmental drivers and seagrass indicators. Before  
465 (February 2010), During (March, April 2010) and After the disturbance (July 2010,  
466 October 2010, May 2011, July 2013)

467 **Table 2.** Results from BBACI analysis. Time before was compared against 1, 2, 5 and  
468 15 months after the beginning of the disturbance. If BxC was not significant (marked  
469 with \*), impact was tested with the *F*-ratio Mean Square BxI/Mean Square Residual.  
470 Otherwise impact was tested with the *F*-ratio Mean Square Before vs After x Impact vs  
471 Controls (BxI)/ Mean Square Before vs After x Among Controls (BxC). When  
472 significant, arrows indicate increase or decrease of different indicators. Dashes indicate  
473 that no differences were found. Blank spaces mean no measurements taken.

474

475 **Figure footnotes**

476 **Fig.1.** Map of the study area showing the impacted site and the three control sites (C1,  
477 C2, C3).

478 **Fig.2.** Daily maximum irradiance measured at canopy level at the four sites (impact site  
479 with black circles and 3 controls) during the disturbance (upper panel, April 2010) and  
480 after the disturbance (bottom panel, April 2011).

481 **Fig.3.** Average sediment deposition during the disturbance in the impact and control  
482 sites. Due to the low variability we grouped the three control sites together.

483 **Fig.4.** Surface sediment granulometry in the impacted and control sites. Each bar  
484 indicates the % in volume of the different grain size ranges two months after the

485 disturbance (left panel) and 8 months after the disturbance (right panel). Black bars  
486 represent the impacted site and white bars the controls.

487 **Fig.5.** Average organic matter content in surface sediments in the impacted and control  
488 sites. Low variability within controls allowed grouping the three control sites.

489 **Fig.6.** Indicators response in the impacted and control sites. Boxplots of the impacted  
490 site and control sites (grouped) for shoot density, total carbohydrates and Fe indicators  
491 before (time=0) and at different times after the disturbance (time=1, 2, 5, 8, 15, 38). The  
492 grey shadow indicates the duration of the impact.

493

494

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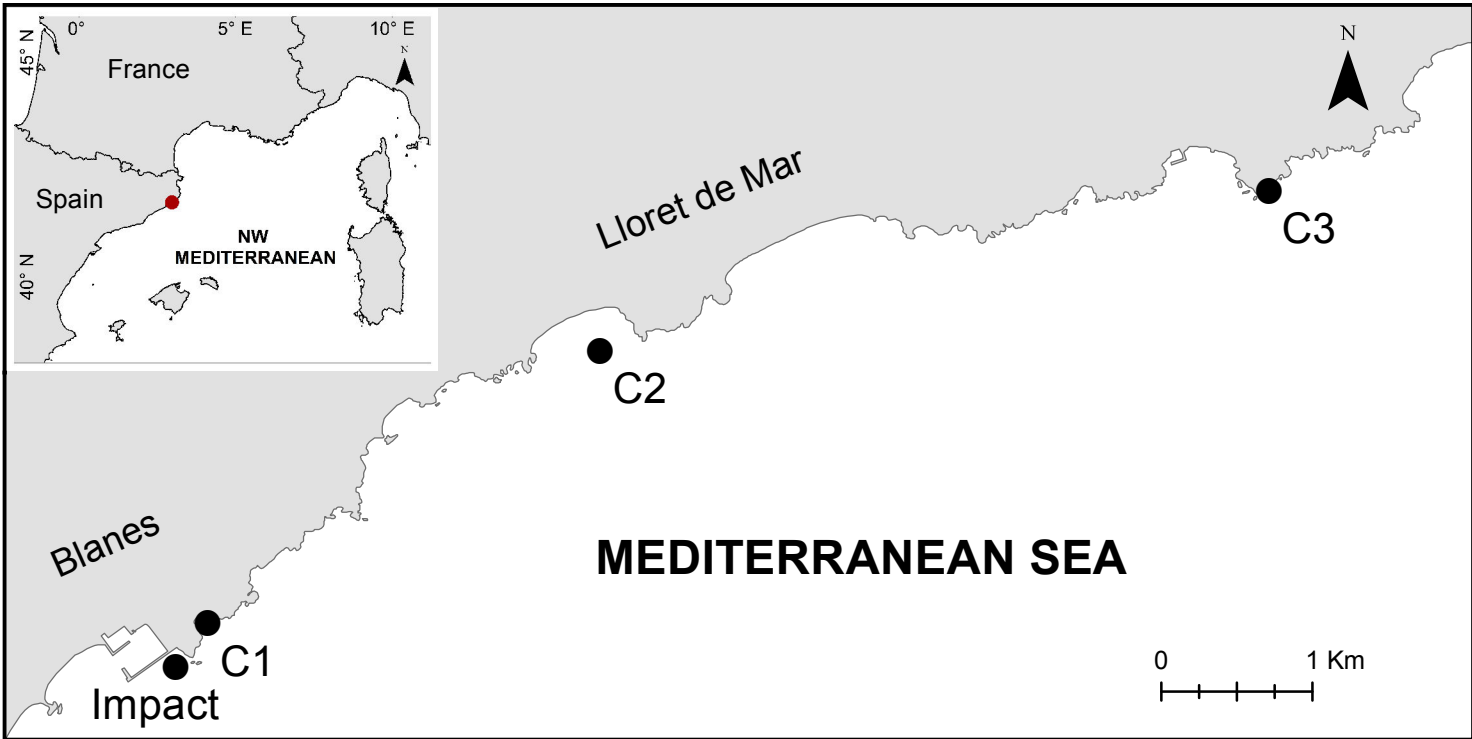
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- 664
- 665

Time from beginning of the works (months)	Before	During				After				
	0	1	2	3	4	5	8	15	38	
<b>Drivers</b>										
Light sensor			x						x	
Sediment traps			x							
Surface sediment		x				x				
<b>Seagrass</b>										
Shoot samples	x	x	x			x			x	
Structure measures	x						x			x

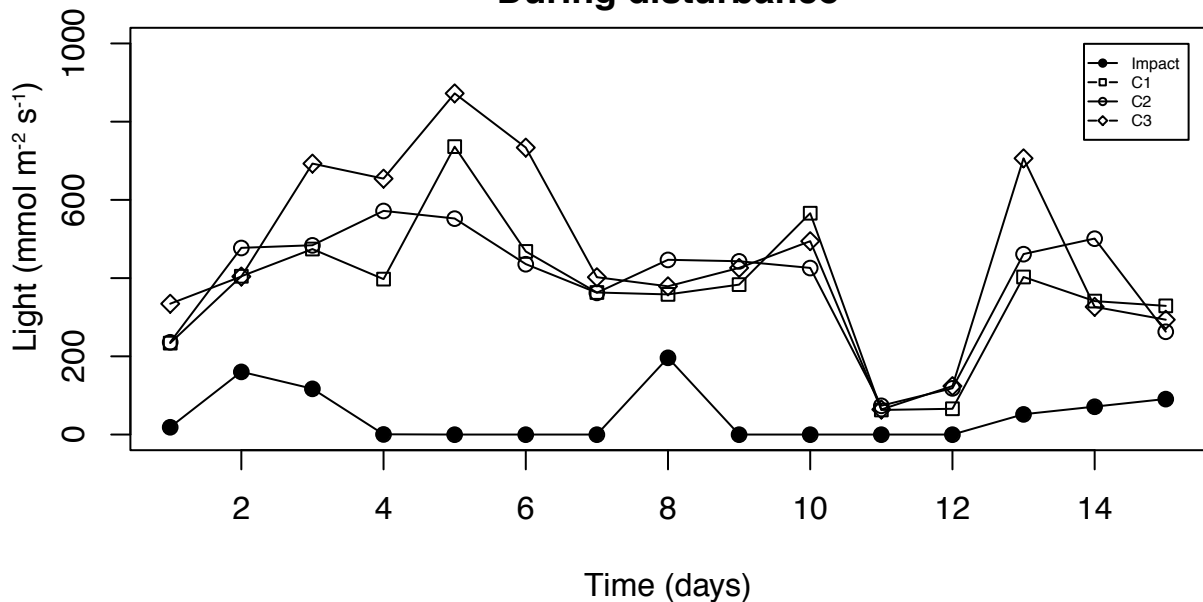
**Table 1:** Sampling time of environmental drivers and seagrass indicators. Before (February 2010), During (March, April 2010) and After the disturbance (July 2010, October 2010, May 2011, July 2013).

Group	Parameters	During disturbance		After disturbance		
		0-1	0-2	0-5	0-15	0-38
Morphology	Leaf length	-	-	-	-	
	Number of leaf	-	-	-	-	
	% Leaf necrosis	-	-	-	-	
	Leaf area	-	-	-	-	
Community	Epiphyte biomass	-	-	-	-	
Physiology	%N	-	-	-	P<0.05*	
	%C	-	-	-	-	
	$\delta^{15}\text{N}$	-	-	-	-	
	$\delta^{13}\text{C}$	-	-	-	-	
	%N Leaf	-	-	P<0.05*	-	
	%C Leaf	-	-	-	-	
	$\delta^{15}\text{N}$ Leaf	-	-	-	-	
	$\delta^{13}\text{C}$ Leaf	-	-	-	-	
	$\delta^{34}\text{S}$	-	-	-	-	
	Fe	-	↑P<0.05	↑P<0.05	-	
	P	-	-	-	-	
	Pb	-	↑P<0.05*	↑P<0.05*	-	
	Cd	-	-	↑P<0.05*	-	
	Mn	-	↑P<0.05	↑P<0.05	-	
	Ni	↑P<0.05	-	-	-	
	Cu	-	-	-	-	
	Zn	-	-	-	-	
	Starch	↓P<0.05*	↓P<0.05	↓P<0.05	-	
	Sucrose	-	↓P<0.05	-	-	
Total Carbohydrates	-	↓P<0.05	-	-		
				0-8	0-15	0-38
Structure	Shoot density			↓P<0.05	↓P<0.05	↓P<0.05

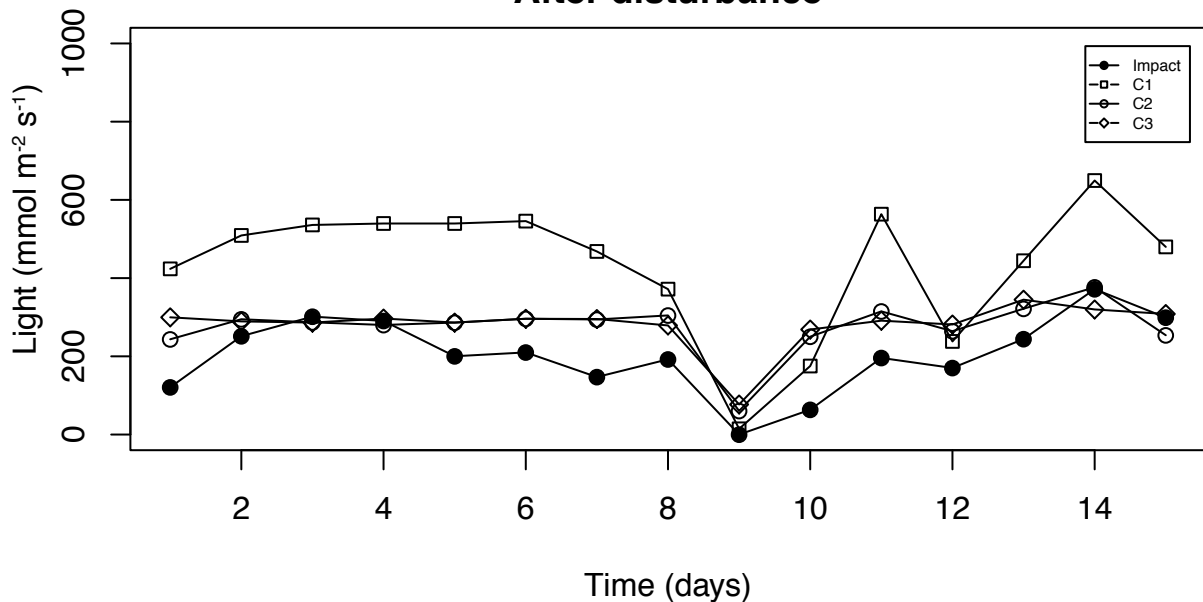
**Table 2.** Results from BACI analysis. Time before was compared against 1, 2, 5 and 15 months after the beginning of the disturbance. If BxC was not significant (marked with \*), impact was tested with the *F*-ratio Mean Square BxI/Mean Square Residual. Otherwise impact was tested with the *F*-ratio Mean Square Before vs After x Impact vs Controls (BxI)/ Mean Square Before vs After x Among Controls (BxC). When significant, arrows indicate increase or decrease of different parameters. Dashes indicate that no differences were found. Blank spaces mean no measurements taken.



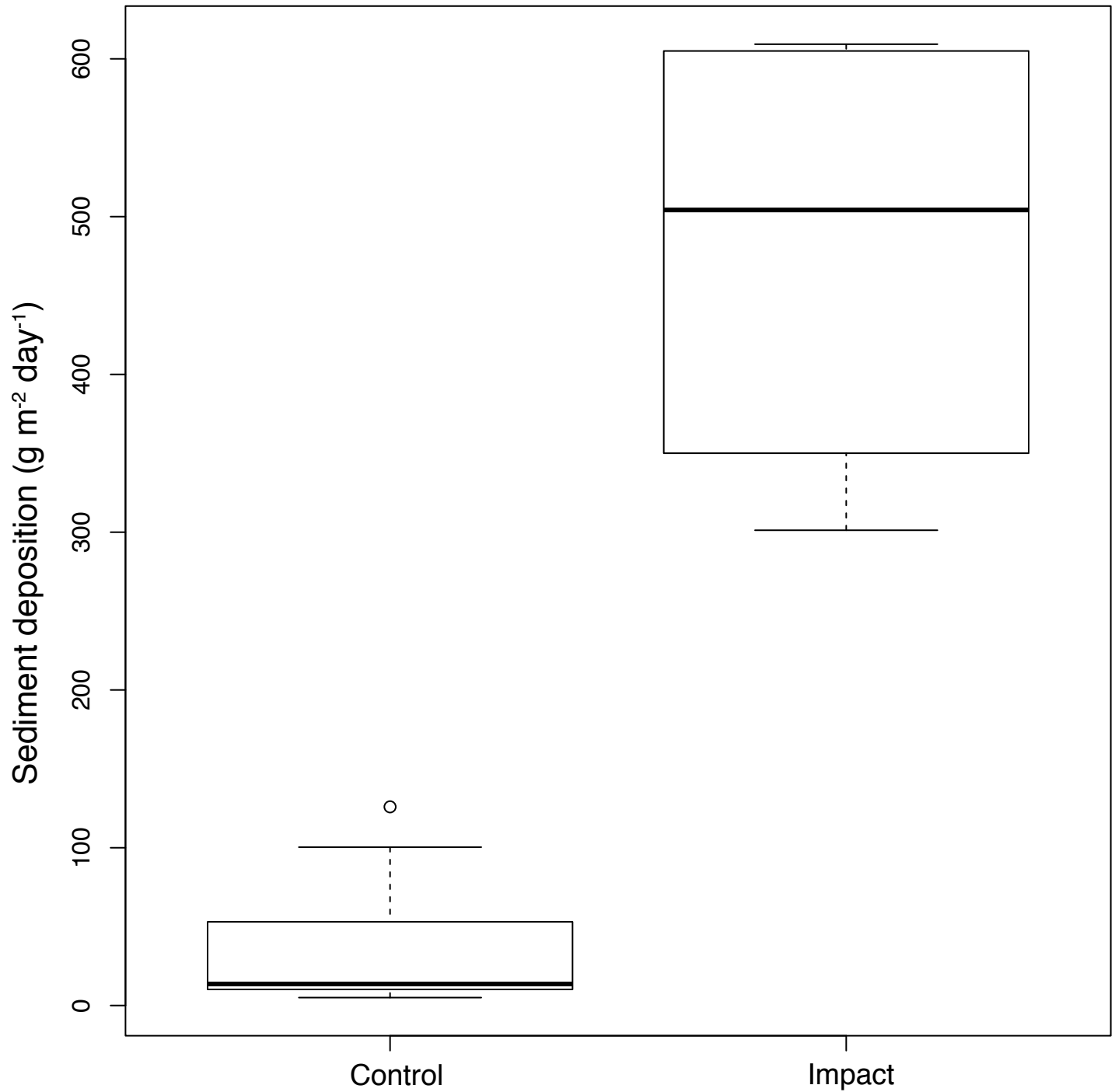
### During disturbance



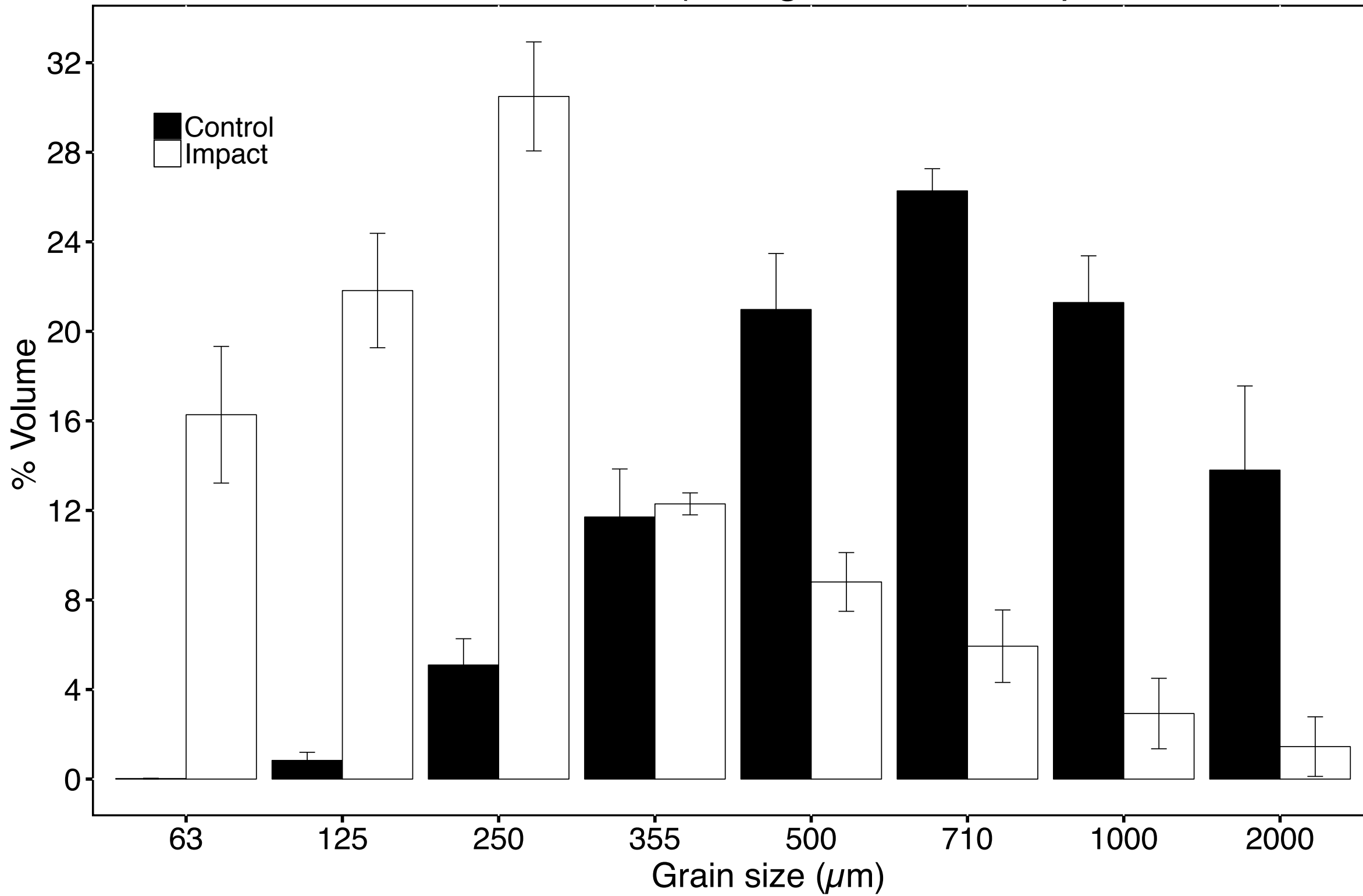
### After disturbance



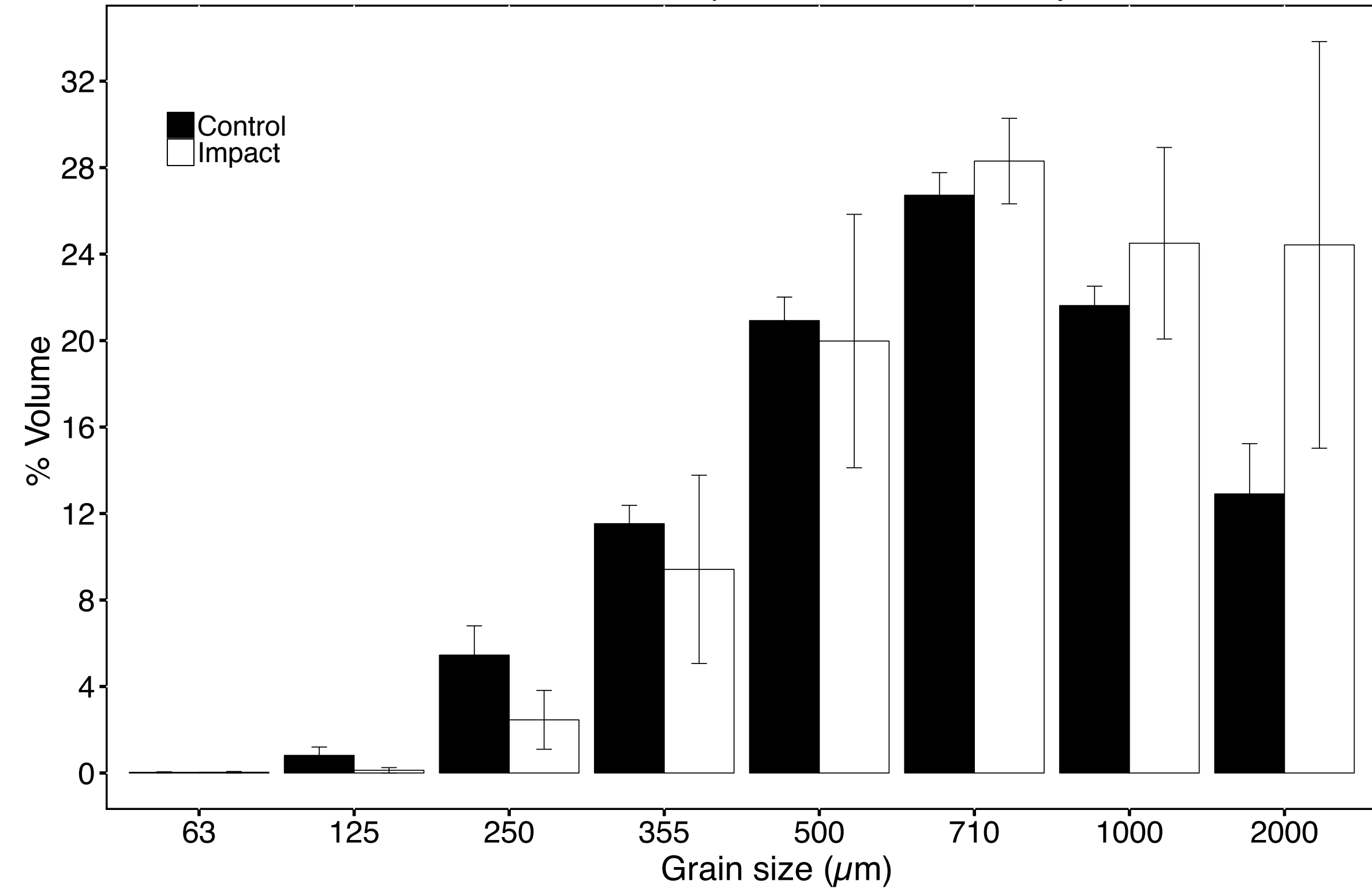
# Sediment traps



**Surface sediment (During the disturbance)**

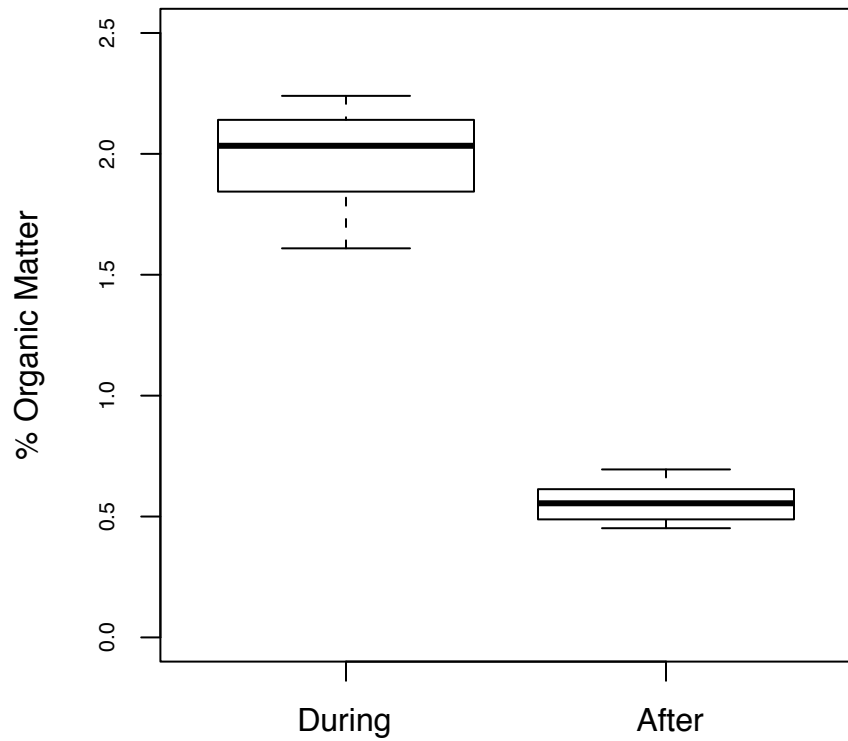


**Surface sediment (After the disturbance)**





## Impact



## Controls

