Seagrass response to burial and breakage of expanding horizontal rhizomes: implications for clone spread

Elena Balestri*, Claudio Lardicci

Department of Biology, University of Pisa, Via Derna 1, Pisa 56126, Italy

ABSTRACT: In seagrasses, elongation of horizontal rhizomes, and hence clone expansion, is supported by physiological integration. However, horizontal rhizomes are subjected to breakage by physical disturbances and to burial, often simultaneously. Little is known about the combined impact of such factors on the growth of apical rhizome regions. We factorially manipulated connections on runners of *Cymodocea nodosa* and sediment height for 1 mo at 2 sites within a meadow to test the hypothesis that breakage of interconnections close (10 cm) to the rhizome apex causing premature loss of physiological integration would negatively affect the performance of runners and reduce their ability to tolerate burial (60% of shoot height). Shoot survival, length of vertical internodes and horizontal rhizome network, biomass production, partitioning, and costs were unaffected by the investigated factors. Rhizome breakage interacted with burial for some characteristics. Breakage reduced branch length and rhizome diameter only in unburied runners, while burial reduced branch length only in intact runners. Moreover, burial stimulated leaf sheath elongation and greater branching, irrespective of the status of connections. These findings indicate that breakage only delays lateral spread and increases the risk of fragmentation by subsequent disturbances, and, contrary to our hypothesis, has no substantial influence on the capacity of runners to withstand transitory and moderate burial. The findings also reveal that runners may escape from burial not only vertically, but also horizontally, changing growth pattern and direction from predominantly unidirectional to multidirectional. This additional strategy could allow buried runners to accelerate space occupation even if disconnected from clones.

KEY WORDS: Clonal growth · *Cymodocea nodosa* · Physical disturbance · Physiological integration · Sediment · Runner

INTRODUCTION

Seagrasses are a dominant component of communities in shallow coastal habitats, where they experience a variety of physical disturbances, often simultaneously (Short et al. 2007). Burial by sediment is one of the most severe types of disturbance for seagrasses (Cabaço et al. 2008, Ooi et al. 2011). Studies have demonstrated that burial may have detrimental effects on the performance of seagrasses due to both direct and indirect effects, including reduced light availability, and physical and chemical modifications of the sediment–plant micro-environment (Marbà & Duarte 1995, Manzanera et al. 1998, Mills & Fonseca 2003, Cabaço et al. 2008, 2010). Some seagrasses, however, such as *Cymodocea nodosa* (Ucria) Ascherson and *Thalassia testudinum* Banks ex König, may withstand burial events of moderate intensity by morphological adjustments such as increased elongation of vertical structures (internodes, leaves and leaf sheaths) in surviving shoots (Marbà & Duarte 1994, 1995, Marbà et al. 1994, Duarte et al. 1997,

Cabaço et al. 2010, Ooi et al. 2011, Balestri & Lardicci 2013) in a fashion analogous to that of coastal sand dune plants (Maun 1998, Yu et al. 2001, 2004, Gilbert et al. 2008, Chen et al. 2010, Frosini et al. 2012, Balestri & Lardicci 2014). More recent studies have shown that source–sink transport of resources (such as water, carbohydrates, and nutrients) between connected ramets through physiological integration may buffer the adverse effects of burial in some species, thereby enhancing their capacity to survive in stressful habitats (Ooi et al. 2011, Tuya et al. 2013a). They have also provided evidence that greater investment in vertical growth to escape from burial occurs only when resource translocation through connecting rhizomes is physically prevented, at least in some species (Ooi et al. 2011, Tuya et al. 2013a). In those studies, however, the role of physiological integration in mediating the effect of burial was investigated by severing rhizome connections inside established patches consisting of adult ramets that are potentially able to survive and grow after isolation from the rest of clone. Yet no study has explicitly addressed the possible impact of severing horizontal rhizomes extending outside patches on the performance of young ramets subjected to burial. Such information is critical, as it may provide new insights into the implications of environmentally induced alterations for the occupation of the space by a clone.

Clone expansion depends upon the production of new ramets by the apical meristem of horizontal rhizomes and branching (Tomlinson 1974, Duarte & Sand-Jensen 1990, Marbà & Duarte 1998, Hemminga & Duarte 2000). In many species, both the production of young ramets and their growth is supported by acropetal translocation of resources, i.e. internal transport of assimilates within the clone predominantly directed towards the rhizome apex (Libes & Boudouresque 1987, Tomasko & Dawes 1989, Terrados et al. 1997a,b, Hemminga & Duarte 2000, Marbà et al. 2002, Schwarzschild & Zieman 2008a,b). The dependence declines as ramets mature and acquire physiological independence, although this development stage is species-specific and may be influenced by local environmental conditions (Marbà et al. 2002, 2006). Physical disturbances by currents, waves, herbivores, bioturbation, or human activities may frequently break horizontal rhizomes at the edge of patches (Sargent et al. 1995, Burdick & Short 1999, Ramage & Schiel 1999, Hemminga & Duarte 2000, Lefebvre et al. 2000, Kenworthy et al. 2002, Cabaço et al. 2005) and cause the disconnection of rhizome portions consisting of an apical meristem and a set of interconnected ramets from the parent clone. Young

ramets on apical disconnected rhizome portions are expected to suffer from reduced survival and/or growth rates due to premature loss of physiological integration, and hence have lower capacity to cope with burial than disconnected adult ramets inside patches, with potential consequences for the spread rate and extent of site exploitation.

In this study, we evaluated the effect of transitory burial and rhizome breakage, both alone and in combination, on the performance (in terms of survival, growth, biomass allocation, and production costs) of horizontal rhizomes extending over the edge of established patches (i.e. runners) of the seagrass *C. nodosa.* This species was chosen, as increasing attempts have recently been made to restore damaged rhizomes by transplantation of rhizome fragments and seedlings (Zarranz et al. 2010, Balestri & Lardicci 2012, 2014). Previous studies have shown that the growth of the apical region of runners (0.50–1 m from the apex or the first 11 shoots on the rhizome) is strongly supported by translocation of resources from older connected ramets (Marbà et al. 2002), and the inhibitory control of the apical meristem on the development of branches is effective on lateral meristems located within 6 to 8 internodes (Terrados et al. 1997a,b). In addition, recent studies (Tuya et al. 2013a,b) have revealed that physiological integration may help adult shoots to tolerate moderate and high burial events (8–27 cm, corresponding respectively to 25–75% of the mean leaf height). We hypothesized that (1) the performance of apical regions of runners would be reduced following the breakage of the rhizome connection in proximity to the apex (i.e. 10 cm) due to premature loss of physiological integration; and (2) moderate burial would affect the performance of apical regions when the rhizome connection is broken, while it would have little or no effect when the connection is left intact due to the mediating effect of physiological integration (non-additive effect).

MATERIALS AND METHODS

Experiment

The experiment was conducted within a shallow (0.40–1 m) *Cymodocea nodosa* meadow (43° 19′ 01.75″ N, 10° 25′ 52.76″ E) located at Rosignano Solvay (Italy). The substrate is highly heterogeneous, composed of rock intermingled with patches of carbonate sand. The tide amplitude is typically low (10–30 cm). Sea surface water tem-

perature varied from 12°C in winter to 27°C in summer, and salinity ranged from 37.5 to 38. Here, the species shows a unimodal annual growth cycle, with a peak during June and July and a cessation of rhizome growth from October to January, as observed in other Mediterranean areas (Caye & Meinesz 1985). Runners with severed apical portions due to physical disturbances have often been observed at the edge of meadow patches (E. Balestri pers. obs.).

The experiment was set up in 2 sites separated by several hundred meters and selected at random within the meadow to test for consistency in space of plant responses. This was done because microscale processes and local factors may result in substantial differences in plant morphology within seagrass meadows (Perez et al. 1994, Balestri et al. 2003, Balestri 2004, Mascaró et al. 2009, Cabaço et al. 2010), which could mask the effects of experimental treatments. In late June 2011, 12 runners with undamaged apical meristems and unbranched ramets located at the edge of meadow patches were selected at each site, giving a total of 24 runners (1 runner per patch). At each site, runners were randomly assigned to 2 treatments, rhizome connection (2 levels, severed and intact) and burial (2 levels, burial and no burial), in a full factorial design. There were 3 replicates for each combination of treatments at each site. At each site, 6 runners assigned to the rhizome connection treatment were severed with a sharp blade at 10 cm from the rhizome apex so that each fragment consisted of up to 10 successive ramets connected to the apical meristem, while 6 other runners were left intact. This distance was chosen in an attempt to standardize conditions and because previous studies suggest that all shoots present in this portion would depend on resources translocated from old parts of the clone in *C. nodosa* (Terrados et al. 1997b). All selected runner portions were marked with a painted plastic cable tie label placed on the horizontal internode at 10 cm from the apex, and the number of living shoots, the number of leaves per shoot, and the length of the longest leaf were measured. Our preliminary observations indicated that the apical meristem and the 3−4 youngest shoots of runners were recruited just before the start of the experiment (late May 2011). On the basis of the maximum number (6) of internodes present in the oldest short vertical shoot before the start of the experiment, we estimated that the remaining shoots on the tagged runners were recruited during the previous growing season (June−August 2010). Runners assigned to the burial treatment were then totally covered with a layer of sediment, while the others were left unmanipulated. Before sediment addition, the leaf meristem on the runners was located 0.2–0.4 cm below the sediment surface, and the mean length of the longest leaf was 6.5 cm. As sediment addition deflected leaves, a layer of sediment 4 cm in height was sufficient to fully cover all shoots. This burial level nominally corresponded to about 60% of the main leaf length of unburied plants and was thus close to the burial level (24 cm, equivalent to 75% of leaf length) considered 'high' for this species by Tuya et al. (2013a). No structure was used to maintain the experimental sediment level to realistically simulate natural burial events which are usually followed by sediment resuspension due to currents, to avoid any possible artefact effect. However, runners were monitored at weekly intervals until the end of the experiment (August 2011), and at each census a ruler was gently pushed into the sediment up to the rhizome stratum to measure deviations in height from the assigned burial levels. At each census, the sediment was removed or re-added when necessary to re-establish the original burial levels. In total, 7 sediment additions that simulated repeated and moderate burial events were made during the experiment. The sediment used for burial treatments was collected in the study location and sieved (through a 1 mm mesh) to remove seeds and extraneous material prior to use.

At the end of the experiment, all runner portions were carefully excavated from the sediment and transported to the laboratory for measurements of morphological and growth-related characteristics. In each runner portion, the number of dead and living shoots, the number and length of any new horizontal branch produced on the main axis, the number of standing leaves per shoot, and the length of the longest leaf and leaf sheath were recorded. The mean rhizome diameter of the main horizontal rhizome axis of each runner portion was estimated using a mechanical dual thickness gauge placed at the middle of each horizontal internode on the respective portion. Total length of the horizontal rhizome network of each runner portion was calculated as the sum of the length of the main horizontal rhizome axis and the length of all new horizontal branches of the main rhizome axis. One older shoot was randomly chosen on each runner, and the length of the 3 youngest, vertical short internodes (i.e. nearer to the leaf meristem) of that shoot was measured after removing all standing leaves. Shoots, roots, and rhizomes of each runner portion were then separated and dried at 60°C until they reached con-

stant weight to determine the respective biomasses (g dry weight). The root-to-shoot ratio was calculated by dividing root by the shoot biomass. The specific rhizome length, estimated as the ratio between total length (cm) and dry weight (g) of rhizome, and the specific root length, determined as the ratio between total length (cm) and dry weight (g) of roots, which are considered an indicator of the dry mass cost of producing tissue (Ryser 1998, Gilbert & Ripley 2008), were calculated. In addition, shoot mortality was calculated as percentage of dead shoots relative to the total number of shoots (already present plus those produced during the experimental period) in each runner portion. Finally, the loss of sediment cover due to resuspension by currents was estimated for each monitoring date and expressed as percentage relative to the initial imposed burial height.

Data analysis

Morphological variables measured at the beginning of the experiment were analyzed by ANOVA to check for homogeneity in morphology of runners assigned to the different treatments at the 2 sites. The experimental design included the orthogonal factors, site (2 levels, random), burial (2 levels, fixed), and rhizome connection (2 levels, fixed). Final data on morphological and growth-related variables were analyzed through multivariate analysis of variance by permutation (PERMANOVA) (Anderson et al. 2008) according to the model described above. Since PERMANOVA showed significant effects of the study factors, separate analyses (ANOVAs) were performed. Separate ANOVAs were also conducted on total plant biomass, root:shoot ratio, specific rhizome and root lengths, and shoot mortality. Finally, relative loss in burial height at the 2 sites over the experimental period was analyzed through repeated-measures ANOVA.

Prior to PERMANOVA, data were normalized and dissimilarities calculated as Euclidean distances. Significance levels were calculated from 9999 permutations of the residuals under the reduced model. Whenever possible, post hoc pooling of mixed terms of the model was performed to increase analysis power (Underwood 1997, Anderson et al. 2008). When a significant effect was found, pair-wise comparisons (PERMANOVA t-statistic and 999 permutations) were used to distinguish between means. For some terms, there were not enough permutable units to obtain a reasonable test by permutation, so p-values were obtained using a Monte Carlo random sample from the

asymptotic permutation distribution (Anderson et al. 2008). Statistically significant ($\alpha = 0.05$) terms were checked for differences in multivariate group dispersion with the permutational analysis of multivariate dispersions (PERMDISP; Anderson 2006), and pairwise comparisons of multivariate dispersion were performed between all pairs of levels of the factor(s) identified as significant in the PERMANOVA (see Table 2b). Homogeneity of variances was checked using Cochran's C-test. For some variables (leaf sheath length and root:shoot ratio), data were log-transformed to meet the assumption of the ANOVA. When significant effects were detected in the ANOVA, Student-Newman-Keuls (SNK) tests were used for *a posteriori* comparisons of the means (Underwood 1997), and post hoc pooling of the mixed interaction term was applied whenever possible. For repeated-measures ANOVA, the assumption of sphericity was tested with Mauchly's test. PERMANOVA and PERMDISP were run in PRIMER version 6 (PRIMER-E, Plymouth) with PERMANOVA add-on software (Clarke & Gorley 2006), while statistical software R version 2.12.2 (R Development Core Team 2011) and R packages 'GAD' (Sandrini-Neto & Camargo 2011) and 'car' (Fox & Weisberg 2011) were used for ANOVAs and repeated-measures ANOVA, respectively.

RESULTS

Before the start of the experiment, there was no difference in size or morphological characteristics of the apical portion of runners grown at the 2 sites (Table 1) between burial levels ($F_{1,16} = 0.06$, p = 0.84 for number of shoots; $F_{1,16} = 1$, p = 0.50 for number of leaves per shoot; and $F_{1,16} = 0.02$, p = 0.91 for leaf length), rhizome connection treatments ($F_{1,16} = 0.04$, p = 0.87 for number of shoots; $F_{1,16} = 1$, p = 0.50 for number of leaves per shoot; and $F_{1,16} = 0.46$, p = 0.62 for leaf length), sites ($F_{1,16} = 0.90$, p = 0.73 for number of shoots; $F_{1,16} = 1$, p = 0.66 for number of leaves per shoot; and $F_{1,16} = 0.10$, p = 0.81 for leaf length), or their interactions.

Morphological and growth-related characteristics at the end of the experiment are shown in Figs. 1–3. All runners survived to the end of the experiment. Runners had produced at least 1 new shoot, and the length of the horizontal rhizome network increased up to 2.5 times, indicating that they were in an active growth phase (Fig. 1, Table 1). In addition, the majority of runners (80%) produced at least 1 lateral branch by the horizontal rhizome during the experimental period independently of the experimental

Table 1. *Cymodocea nodosa*. Size and morphological characteristics of apical runner portions assigned to the different treatments recorded before the start of the experiment at each of the 2 sites. Data are means ± SE

Treatment	Rhizome connection intact		Rhizome connection severed	
	Burial	No burial	Burial	No burial
Site 1				
Horizontal rhizome length (cm)	10	10	10	10
No. of living shoots	7.3 ± 0.6	6.3 ± 1.2	5.6 ±1.8	10 ± 2
No. of leaves per shoot	2.6 ± 0.3	3.3 ± 0.3	3 ± 0.5	3
Length of longest leaf (cm)	5.8 ± 0.2	7.5 ± 1	6 ± 0.4	7 ± 0.1
No. of branches	0	0	0	0
Site 2				
Horizontal rhizome length (cm)	10	10	10	10
No. of living shoots	7.3 ± 0.3	6 ± 1.1	6.3 ± 0.8	5.6 ± 1.8
No. of leaves per shoot	2.6 ± 0.6	2.6 ± 0.3	3 ± 0.5	3
Length of longest leaf (cm)	5.7 ± 0.6	6 ± 1.1	7.7 ± 0.9	5.4 ± 0.7
No. of branches	0	0	0	0

treatment applied. The interaction between rhizome connection and burial significantly affected whole runner growth (Table 2). Overall, buried and unburied runners were significantly different only when rhizome connection was severed. Since no differences in multivariate dispersion were detected between the groups (PERMDISP test for clonal integration × burial interaction term; $F_{3,20} = 4.38$, p = 0.07), the observed effect might be effectively ascribed to the interaction factor.

The results of separate ANOVAs detected a significant effect of burial on 2 out of the 12 investigated morphological and growth-related variables, number of rhizome branches, and leaf sheath length (Table 3). Regardless of the rhizome connection, buried plants produced more branches (Fig. 1g,h) and longer leaf sheath than unburied ones (Fig. 2c,d). However, a significant interaction between rhizome connection and burial was detected for mean branch length and rhizome diameter (Table 3). Branches on intact runners were longer than on severed ones only under unburied conditions (Fig. 1i,j). On the other hand, branches were on average 5 times shorter under buried conditions than under unburied ones only when the rhizome connection was left intact (Fig. 1i,j). Rhizome diameter of intact runners was larger than that of severed ones only under unburied conditions (Fig. 1e,f). On the other hand, the rhizome diameter of unburied plants was smaller than that of buried ones when the rhizome connection was severed (Fig. 1e,f). A significant interaction between rhizome connection and site was also detected for this variable (Table 3). When severed, the rhizome diameter of runners at one site (hereinafter referred to as Site 1)

was smaller than that at the other site (hereinafter referred to as Site 2), and at this latter site the diameter of intact runners was about 15% larger (under unburied conditions) than that of severed ones (Fig. 1e,f). A significant 3-way interaction between integration, burial, and site was recorded for leaf length (Table 3). At Site 2, intact runners produced longer leaves than severed ones when under unburied conditions, and also had longer leaves than those subjected to burial. Moreover, intact runners had longer leaves at Site 2 than at Site 1 under unburied conditions (Fig. 2a,b). For the remaining variables (number of living shoots, total length of horizontal rhizome, vertical internode length, number of leaves per shoot, and biomass of roots, shoots, and rhizomes), no significant effect of the investigated factors, alone or in combination, was observed (Table 3).

Total plant biomass (Fig. 3a,b) and specific root length (Fig. 3g,h) were also not affected by any of the investigated factors (Table 4), while root:shoot ratio (Fig. 3c,d) significantly differed between the 2 sites (Table 4). Again, specific rhizome length differed significantly between the 2 sites, but only for intact and unburied runners (Fig. 3e,f, Table 4). The percentage of dead shoots per runner varied among treatments (from 6.3 to 51.6%; Fig. 4), but ANOVA did not detect significant differences in shoot mortality among treatments or sites (Table 4). Finally, there was consistent variation of sediment height (%) relative to the imposed burial level (Fig. 5) among dates ($F_{5,50} = 9.64$, p < 0.0001), but not among sites ($F_{1,10} = 0.75$, p > 0.05). On average, 23% of the imposed sediment height (about 1 cm of sediment) was lost weekly due to resuspension by currents, reducing the burial level to about 53% of shoot height.

DISCUSSION

Breakage of interconnections on runners of *Cymodocea nodosa* at a distance of 10 cm from the rhizome apex did not affect survival and production in terms of total length of horizontal rhizome, number of shoots, and biomass allocation, irrespective of burial treatment. However, when not experimentally buried, severed runners produced shorter branches and thinner internodes on horizontal rhizomes compared to intact ones. These findings suggest that the growth
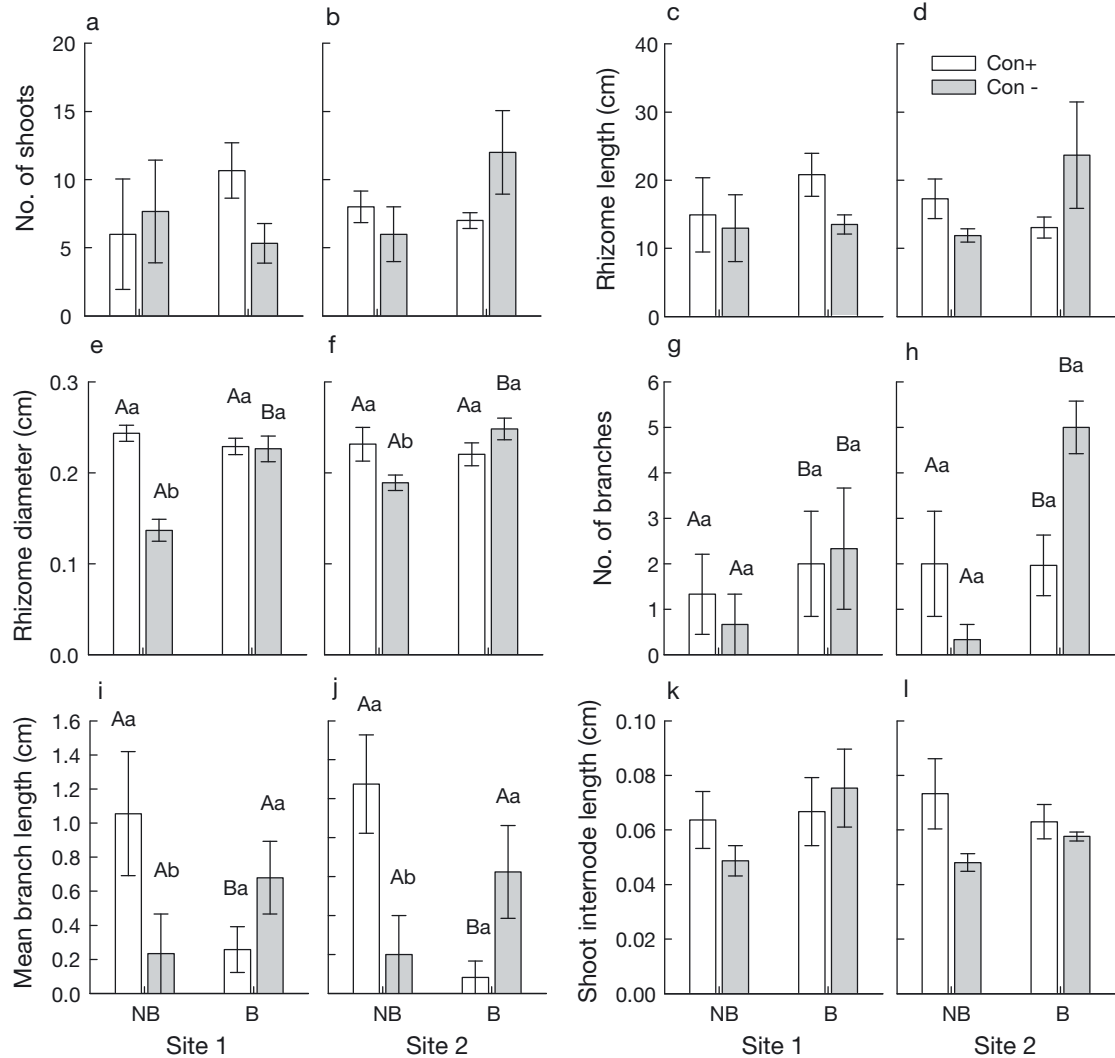
Fig. 1. *Cymodocea nodosa.* (a,b) Total number of living shoots, (c,d) total length of the horizontal rhizome network, (e,f) horizontal rhizome diameter, (g,h) total number of branches, (i,j) branch length, and (k,l) shoot internode length of runners at the 2 study sites. Different letters above columns indicate significant differences (α = 0.05) between burial levels (uppercase letters) for each of 2 rhizome connection levels and between rhizome connection levels (lowercase letters) for each of 2 burial levels. NB: no burial; B: burial; Con+: rhizome connection intact; Con−: rhizome connection severed. Data are means ± SE

of an apical runner region was initially supported by assimilates from ramets already present or by reserves stored in the rhizome once isolated from the rest of clone. In some rhizomatous/stoloniferous herbs, indeed, internodes contain storage materials that ramets can remobilize to survive after clonal fragmentation (Stuefer & Huber 1999, Dong et al. 2010, 2011). In our study, however, the support was insufficient to sustain the growth of newly produced branches at a rate similar to that of an equivalent region on intact runners of *C. nodosa*. These results therefore do not totally support our first hypothesis that apical growth of runners was strongly dependent on physiological integration, and are in disagree-

ment with a previous study which showed that in this species, severing the rhizome connection to up to 50–100 cm from the apical meristem suppressed branching, decreased the production of horizontal internodes and shoots, and reduced the cost of rhizome biomass production in the apical isolated region (Terrados et al. 1997a). A possible explanation for the discrepancy in results may be the lower colonization capacity of the runners selected in the present study than those examined by Terrados et al. (1997a). This difference possibly reflected differences in the growing environment (sandy platform in an estuarine embayment at the Spanish site investigated by Terrados et al. (1997a), rock intermingled
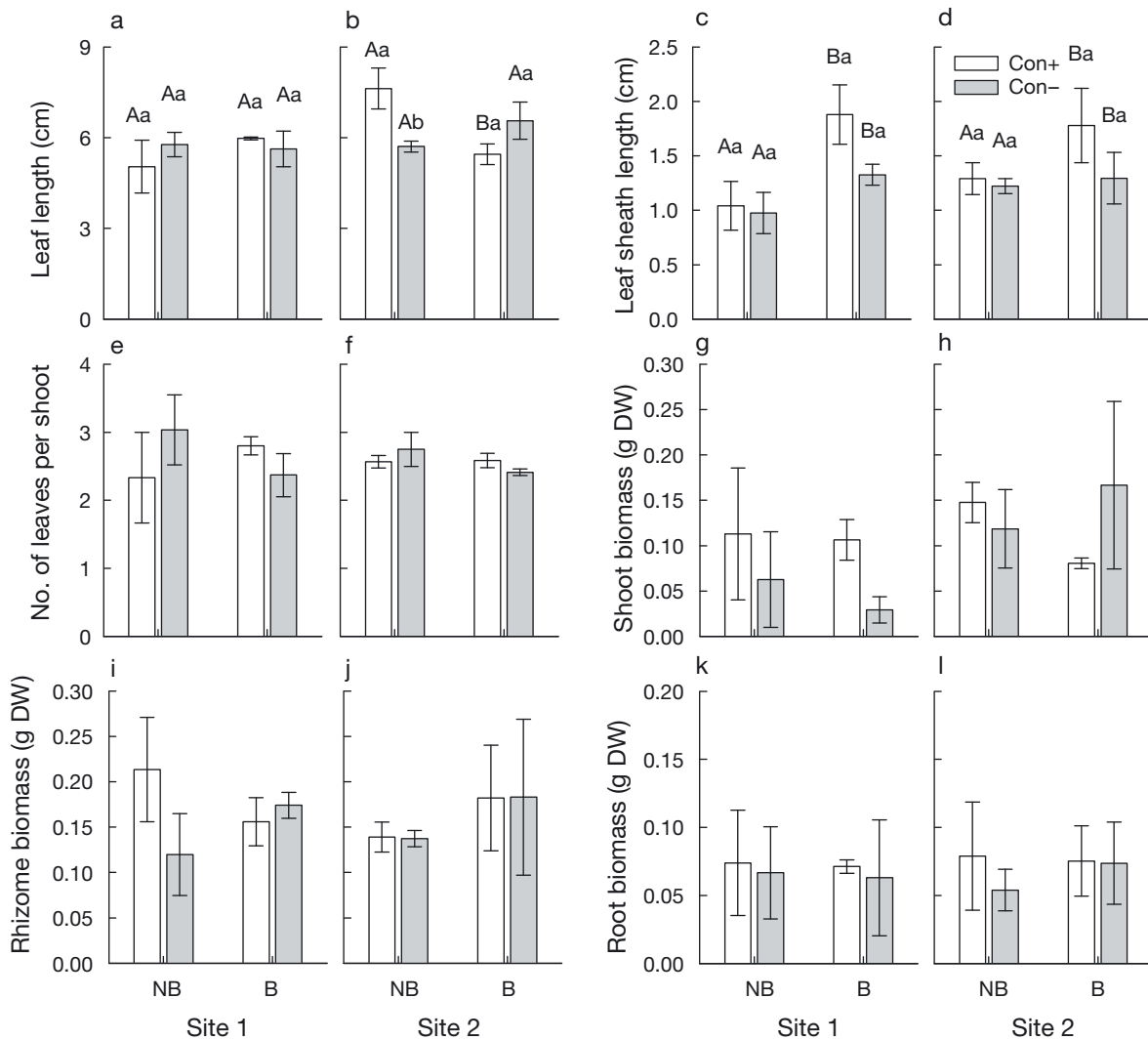
Fig. 2. *Cymodocea nodosa*. (a,b) Length of the longest leaf, (c,d) leaf sheath length, (e,f) number of leaves per shoot, (g,h) biomass of shoots, (i,j) biomass of rhizomes, and (k,l) biomass of roots of runners at the 2 study sites. Different letters above columns indicate significant differences ($\alpha = 0.05$) between burial levels (uppercase letters) for each of 2 rhizome connection levels and between rhizome connection levels (lowercase letters) for each of 2 burial levels. NB: no burial; B: burial; Con+: rhizome connection intact; Con−: rhizome connection severed. Data are means ± SE

with sandy patches in open sea in the present study). Additionally, ramets on the apical portions were probably in a more advanced ontogenetic stage than those situated in equivalent positions on the runners selected by Terrados et al. (1997a), and thus were less dependent on physiological integration. There is evidence that in some terrestrial species, clonal integration maximizes the growth of the sink ramets under heterogeneous resource conditions but has no effect in homogeneous resource conditions where resource patches and ramet spacing are on similar scales (Caraco & Kelly 1991, Evans 1992, Wijesinghe & Handel 1994, Alpert 1999, Dong & Alaten 1999, Xiao et al. 2007). Recent studies have revealed that

growth response of *C. nodosa* to nutrient availability is dependent on clonal integration (Tuya et al. 2013b), and that the pattern of growth of young patches may be influenced by the pattern of nutrient distribution (homogeneous vs. heterogeneous) in the sediment (Balestri et al. 2010). As nutrients are often distributed heterogeneously in coastal habitats, the influence of resource patchiness on the response of seagrasses to the persistence (or loss) of physiological integration therefore deserves more attention in the future. Since severed runners of *C. nodosa* continued to grow and produce new branches at a similar rate to intact ones, the impact of physical interruption of connections in terms of occupation of space is proba-
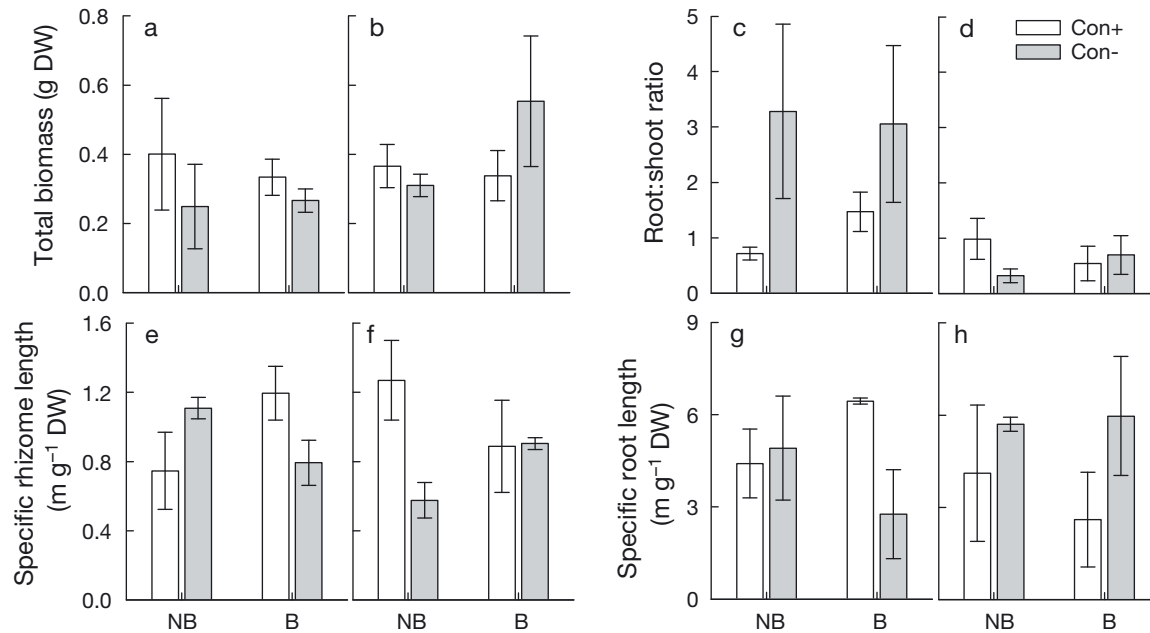
Fig. 3. *Cymodocea nodosa*. (a,b) Total biomass, (c,d) root:shoot ratio, (e,f) specific rhizome length, and (g,h) specific root height of runners at the 2 study sites. NB: no burial; B: burial; Con+: rhizome connection intact; Con−: rhizome connection severed. Data are means ± SE

Table 2. *Cymodocea nodosa*. (a) PERMANOVA results for responses of morphological and growth-related variables (number of living shoots, length of horizontal rhizome, rhizome diameter, number of branches, mean branch length, shoot internode length, length of longest leaf, leaf sheath length, number of leaves per shoot, and biomass of shoots, rhizome, and roots) of runners assigned to the experimental treatments. (b) Results of *a posteriori* pair-wise comparisons for the significant rhizome connection × burial interaction term. NB: no burial; B: burial; Con+: rhizome connection intact; Con−: rhizome connection severed

(a) Source	df	SS	MS	Pseudo-F	p
Rhizome connection = R	1	5.85	5.85	0.61	0.750
Burial = B	1	14.13	14.13	1.47	0.167
Site = S	1	6.68	6.68	0.70	0.669
R × S	1	8.76	8.76[a]		
R × B	1	21.34	21.34	2.23	0.045
B × S	1	5.02	5.02[a]		
R × B × S	1	14.45	14.45[a]		
Residual	16	153.77	9.61[a]		

(b) Pair-wise comparisons for rhizome connection × burial	Contrast	t	p
Con+	NB vs. B	1.06	0.337
Con−	NB vs. B	1.70	0.026
NB	Con+ vs. Con−	1.12	0.245
B	Con+ vs. Con−	1.28	0.121

[a]Denotes post hoc pooling (p > 0.25): all pseudo-F values are given for those tested against the pooled term

bly not substantial for a clone, at least under the study conditions, even though the smaller diameter of rhizomes could make rhizomes potentially more vulnerable to subsequent disturbances. Moreover, a previous experimental study has reported increased branching frequency on the runner portion still connected to the parent clone after the loss of the apical region caused by release from apical dominance (Terrados et al. 1997b). Thus, any eventual reduction in growth of runners following physical disturbance damage may be expected to be totally or partially compensated by the activation of a lateral meristem close to the severing point.

Exposure to intermittent and moderate burial had no detrimental effects on survival, production of shoots, total rhizome length, vertical internode length or biomass of runners, but it resulted in a decrease in branch length in runners only when the connection was left intact (interactive effect burial and connection). In this respect, the effect of burial was similar to that of severing rhizome connection. However, the number of newly produced branches and the length of leaf sheaths greatly increased when runners were buried, independently of the status of the connection. Plastic adjustments in clonal architecture, not only at ramet level (increased leaf sheath length) but also at fragment level (higher number of branches), allowed intact runners to counterbalance the reduction in branch length, as the mean length of their horizontal

Table 3. *Cymodocea nodosa*. ANOVA results for responses of morphological variables of runners assigned to the experimental treatments. Results of Student-Newman-Keuls (SNK) tests are also reported. NB: no burial; B: burial; Con+: rhizome connection intact; Con−: rhizome connection severed; S1: Site 1; S2: Site 2. *p < 0.05, **p < 0.01

Source	df	No. of living shoots MS	F	Horizontal rhizome length MS	F	Rhizome diameter MS	F
Rhizome connection = R	1	1.042	0.054	6.000	0.077	<0.001	0.104
Burial = B	1	15.042	0.785	73.500	1.503	<0.001	0.850
Site = S	1	7.042	0.367	5.230	0.107	0.002	3.185
R × B	1	0.375	0.004	42.130	0.247	0.003	5.57*
R × S	1	22.04[a]		78.480	1.605	0.004	8.61**
B × S	1	5.04[a]		0.483[a]		0.001[a]	
R × B × S	1	84.375	4.401	170.670	3.490	0.001[a]	
Residual	16	19.88[a]		830.87[a]		<0.001[a]	
SNK						Con−: S2>S1, S1: Con+>Con− Con−: NB<B, NB: Con+>Con−	

Source	df	No. of branches MS	F	Mean branch length MS	F	Shoot internode length MS	F
Rhizome connection = R	1	2.042	0.853	0.203	0.341	0.005	1.997
Burial = B	1	12.042	5.03*	0.316	0.533	0.003	1.229
Site = S	1	1.042	0.435	0.023	0.038	0.001	0.227
R × B	1	18.375	1.960	2.652	4.47*	0.007	2.740
R × S	1	3.38[a]		0.002[a]		0.002[a]	
B × S	1	0.38[a]		0.018[a]		0.003[a]	
R × B × S	1	9.375	3.917	0.01[a]		<0.001[a]	
Residual	16	2.46[a]		0.703[a]		0.003[a]	
SNK		B>NB		Con+: B<NB, NB: Con+>Con−			

Source	df	Length of the longest leaf MS	F	Length of leaf sheath MS	F	No. of leaves per shoot MS	F
Rhizome connection = R	1	0.001	0.001	0.514	4.153	0.030	0.099
Burial = B	1	0.001	0.005	1.150	9.28**	0.100	0.334
Site = S	1	0.092	3.413	0.050	0.405	0.019	0.063
R × B	1	0.019	0.105	0.306	2.467	0.829	2.754
R × S	1	0.01[a]		<0.002[a]		0.03[a]	
B × S	1	0.052	1.939	0.15[a]		<0.001[a]	
R × B × S	1	0.184	6.87*	<0.002[a]		0.23[a]	
Residual	16	0.03[a]		0.14[a]		0.34[a]	
SNK		Con+, NB: S2>S1 S2, Con+: NB >B S2, NB: Con+>Con−		B>NB			

Source	df	Shoot biomass MS	F	Rhizome biomass MS	F	Root biomass MS	F
Rhizome connection = R	1	0.001	0.002	<0.001	0.002	0.007	0.268
Burial = B	1	0.001	<0.001	0.009	1.410	<0.001	0.014
Site = S	1	0.026	3.640	0.001	0.136	<0.001	0.007
R × B	1	0.008	0.541	0.013	1.977	0.002	0.074
R × S	1	0.022	3.157	0.008[a]		<0.001[a]	
B × S	1	0.002[a]		0.010[a]		0.002[a]	
R × B × S	1	0.015	2.151	0.001[a]		0.002[a]	
Residual	16	0.007[a]		0.007[a]		0.029[a]	

[a]Denotes post hoc pooling (p > 0.25); all *F*-values are given for those tested against the pooled term
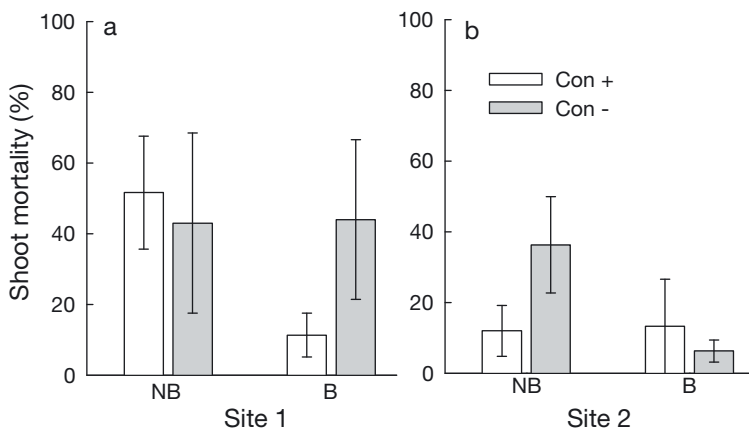
Table 4. *Cymodocea nodosa*. ANOVA results for responses of total biomass, root:shoot ratio, dry mass production cost, and shoot mortality of runners assigned to the experimental treatments. Results of Student-Newman-Keuls (SNK) tests are also reported. NB: no burial; B: burial; Con+: rhizome connection intact; Con−: rhizome connection severed; S1: Site 1; S2: Site 2. *p < 0.05

Source	df	Total biomass		Root:shoot ratio	
		MS	*F*	MS	*F*
Rhizome connection = R	1	0.001	0.02	0.94	0.51
Burial = B	1	0.01	0.32	0.20	0.29
Site = S	1	0.04	1.17	5.44	7.82*
R × B	1	0.05	1.46	0.10	0.14
R × S	1	0.05	1.66	1.85	2.66
B × S	1	0.03[a]		0.08[a]	
R × B × S	1	0.01[a]		1.91[a]	
Residual	16	0.03[a]		0.66[a]	
SNK				S1 > S2	

Source	df	Specific rhizome length		Specific root length	
		MS	*F*	MS	*F*
Rhizome connection = R	1	0.07	0.78	1.19	0.51
Burial = B	1	0.02	0.22	0.69	0.29
Site = S	1	0.00	0.04	0.01	7.82
R × S	1	0.04[a]		24.85	2.66
B × S	1	0.09[a]		0.47[a]	
R × B	1	0.05	0.09	2.18	0.14
R × B × S	1	0.52	6.14*	13.28[a]	
Residual	16	0.09[a]		6.49[a]	
SNK		Con+, NB: S2 > S1			

Source	df	Shoot mortality (%)	
		MS	*F*
Rhizome connection = R	1	642.73	37.00
Burial = B	1	1737.40	40.72
Site = S	1	2521.50	3.60
R × S	1	17.34	0.02
B × S	1	42.66	0.06
R × B	1	38.00	0.02
R × B × S	1	1980.16	2.83
Residual	16	699.65	

[a]Denotes post hoc pooling, p > 0.25; all *F*-values are given for those tested against the pooled term

Fig. 4. *Cymodocea nodosa*. Percent shoot mortality relative to total number of shoots (those already present plus those produced in runners) over the experimental period at (a) Site 1 and (b) Site 2. NB: no burial; B: burial; Con+: rhizome connection intact; Con−: rhizome connection severed. Data are means ± SE

rhizome network was similar to that of unburied runners.

This suggests that the growth of intact buried runners was initially supported by transfer of resources from old unburied connected ramets, but with time the resource demand from surviving ramets subjected to repeated burial had probably forced supporting ramets to abandon connected buried ramets to allocate all available resources to their own needs. In fact, there may be a cost of integration to a supporting ramet, and some studies have reported that chronic or severe stresses may result in lower fitness, so mother ramets may cease to support dependent daughter ramets (Chesson & Peterson 2002, Xiao et al. 2007). Compensatory growth responses (increased branching and leaf sheath growth) to burial had presumably occurred in *C. nodosa* runners after functional interruption of integration imposed by burial. This would explain the delay in the development of lateral branches and thus reduced branch length. Instead, in severed runners, the loss of integration might have promptly induced compensatory responses to alleviate burial stress. The additional resources needed for their growth were possibly obtained by enhancing the efficiency of the photosynthetic apparatus of surviving ramets rather than from stored resources, as suggested by the fact that the diameter of the main rhizome axis was not reduced as in unburied severed runners. In addition, above- and belowground biomass production, partitioning and dry mass production costs did not change in response to burial and connection status. These results do
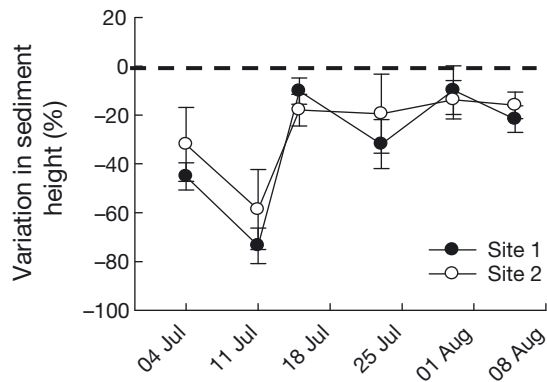
Fig. 5. *Cymodocea nodosa*. Weekly variation (%) in sediment height relative to that experimentally imposed (4 cm, dashed line) to runners over the study period in each of the 2 study sites. Data are means ± SE

not support, therefore, our second hypothesis that breakage reduced the ability of young ramets on isolated runners to cope with moderate burial and to expand further. A recent study has shown that the effects of prolonged exposure (15 wk) to burial heights of 8 and 24 cm, corresponding respectively to 25 and 75 % of the mean leaf height of adult shoots, on shoot density, number of leaves per shoot, leaf length, and above-ground biomass of adult *C. nodosa* plants depended on whether physiological integration was maintained or severed (Tuya et al. 2013a). In addition, increased vertical internode elongation in buried shoots was found to be greater when the rhizome connections were severed than when they were left connected to the rest of clone, leading the latter authors to conclude that physiological integration ameliorated the stress induced by small-scale burial (Tuya et al. 2013a). In the present study, the lack of major effects of burial on the performance of severed runners might be due to the shorter duration of the experiment (4 wk) and fluctuations in the magnitude of the imposed burial level caused by sediment resuspension. Other studies on the effect of burial on *C. nodosa* reported 50 % of shoot mortality in seedlings after 35 d of exposure at a burial height (4 cm), thus equal to that imposed here, and increased elongation of vertical rhizome internodes and leaf sheaths in surviving shoots only at lower burial levels; however, seedlings are probably less resilient to burial than adult plants because of their smaller size (Marbà & Duarte 1994). Increased vertical internodes elongation and branching frequency of adult shoots at burial levels of 4–8 cm have also been observed in other seagrasses (Marbà & Duarte 1994, Duarte et al. 1997, Cabaço et al. 2008). These growth responses are considered to be an adaptive strategy to relocate the shoot meristem

closer to the sediment surface and reduce the proportion of photosynthetic tissue that remains buried (Terrados 1997). The stimulatory effect of burial on branching of horizontal rhizomes observed here is in itself a novel finding for seagrasses. This 'proliferative branching' response might reflect decreased apical dominance, a phenomenon previously observed in this species as well as in other clonal species in response to the excision of the apical meristem or high nutrient levels (Tomlinson 1974, Callaghan et al. 1986, Sutherland & Stillman 1988, Terrados et al. 1997b). It can be hypothesized that deterioration of the micro-habitat associated with repeated burial might have disrupted apical dominance, but a positive effect of increased nutrient supply due to addition of fresh sediments on branching rate cannot be excluded. Interestingly, increased branching promoted a shift in the direction of growth, from predominantly unidirectional to multidirectional. This could represent an additional strategy evolved in *C. nodosa* to promote escape from small-sized burial disturbances, including bioturbation, in a horizontal plane. In fact, a high investment of resources exclusively in vertical internodes on the expanding younger buried region of clones would not be useful for 'guerrilla' species like *C. nodosa* that usually produce long runners to maximize horizontal spread. Instead, the production of multiple new lateral growth axes would be more profitable in the long term, promoting the colonization of proximal disturbance areas and thereby increasing the probability that new ramets encounter more favorable patches and import resources from them.

Finally, our results showed that the effect of rhizome connection and burial treatments depended on the site examined for 3 variables: rhizome diameter, leaf length, and specific rhizome length. Since the loss of sediment relative to that initially imposed on plants did not differ between sites, such dependence might not be related to variations in the burial intensity experienced by runners. Investigating the specific reasons underlying this site dependence was beyond the objective of this study. However, water depth in Site 1 was on average lower than in Site 2 (40 vs. 55 cm). This, in addition to variations in substrate topography, might explain the different response of plants to treatments. Different availability of nutrients in the sediment at the sites might also have played a role, since nutrient concentration is likely to mediate the responses of runners to burial for coastal dune plants (Frosini et al. 2012).

In conclusion, the present study provides new and interesting experimental results on the combined effect of small-sized burial and breakage of runners

by physical disturbances on clonal expansion of *C. nodosa*. The short duration of the experiment, the intermittent and moderate imposed burial level, and the limited growth potential of plants used here did not allow us to draw general conclusions on the possible consequences of such disturbances for the structure and dynamics of seagrass populations. Nonetheless, the results suggest that the drivers of seagrasses' responses to such disturbances may be more complex than those assumed to date. Therefore, further research should examine the spatial extension or temporal persistence of physiological integration in seagrass populations experiencing different environmental conditions, as well as determine the abiotic factors that can affect plant responses to burial and interruption of physiological integration.

LITERATURE CITED

Alpert P (1999) Clonal integration in *Fragaria chiloensis* differs between populations: ramets from grassland are selfsh. Oecologia 120:69–76

Anderson MJ (2006) Distance-based tests for homogeneity of multivariate dispersions. Biometrics 62:245–253

Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E, Plymouth

Balestri E (2004) Flowering of the seagrass *Posidonia oceanica* in a north-western Mediterranean coastal area: temporal and spatial variations. Mar Biol 145:61–68

Balestri E, Lardicci C (2012) Nursery-propagated plants from seed: a novel tool to improve the effectiveness and sustainability of seagrass restoration. J Appl Ecol 49:1426–1435

Balestri E, Lardicci C (2013) The impact of physical disturbance and increased sand burial on clonal growth and spatial colonization of *Sporobolus virginicus* in a coastal dune system. PLoS ONE 8:e72598

Balestri E, Lardicci C (2014) Effects of sediment fertilization and burial on *Cymodocea nodosa* transplants; implications for seagrass restoration under a changing climate. Restor Ecol 22:240–247

Balestri E, Cinelli F, Lardicci C (2003) Spatial variation in *Posidonia oceanica* structural, morphological and dynamic features in a northwestern Mediterranean coastal area: a multi-scale analysis. Mar Ecol Prog Ser 250:51–60

Balestri E, Vallerini F, Lardicci C (2010) Effect of seed density and sediment nutrient heterogeneity on recruitment and early patch growth in the seagrass *Cymodocea nodosa*. Mar Ecol Prog Ser 417:63–72

Burdick DM, Short FT (1999) The effects of boat docks on eelgrass beds in coastal waters of Massachusetts. Environ Manage 23:231–240

Cabaço S, Alexandre A, Santos R (2005) Population-level effects of clam harvesting on the seagrass *Zostera noltii*. Mar Ecol Prog Ser 298:123–129

Cabaço S, Santos R, Duarte CM (2008) The impact of sediment burial and erosion on seagrasses: a review. Estuar Coast Shelf Sci 79:354–366

Cabaço S, Ferreira Ó, Santos R (2010) Population dynamics of the seagrass *Cymodocea nodosa* in Ria Formosa lagoon following inlet artificial relocation. Estuar Coast Shelf Sci 87:510–516

Callaghan TV, Headley AD, Svensson BM, Lixian L, Lee JA, Lindley DK (1986) Modular growth and function in the vascular cryptogam *Lycopodium annotinum*. Proc R Soc Lond B 228:195–206

Caraco T, Kelly CK (1991) On the adaptive value of physiological integration in clonal plants. Ecology 72:81–93

Caye G, Meinesz A (1985) Observations on the vegetative development, flowering and seeding of *Cymodocea nodosa* (Ucria) Ascherson, on the Mediterranean coast of France. Aquat Bot 22:277–289

Chen JS, Lei NF, Dong M (2010) Clonal integration improves the tolerance of *Carex praeclara* to sand burial by compensatory response. Acta Oecol 36:23–28

Chesson P, Peterson AG (2002) The quantitative assessment of the benefits of physiological integration in clonal plants. Evol Ecol Res 4:1153–1176

Clarke KR, Gorley RN (2006) PRIMER v6: user manual/tutorial. PRIMER-E, Plymouth

Dong M, Alaten B (1999) Clonal plasticity in response to rhizome severing and heterogeneous resources supply in the rhizomatous grass *Psammochloa villosa* in an Inner Mongolia dune, China. Plant Ecol 141:53–58

Dong BC, Yu GL, Guo W, Zhang MX, Dong M, Yu FH (2010) How internode length, position and presence of leaves affect survival and growth of *Alternanthera philoxeroides* after fragmentation? Evol Ecol 24:1447–1461

Dong BC, Liu RH, Zhang Q, Li HL, Zhang MX, Lei CG, Yu FH (2011) Burial depth and stolon internode length independently affect survival of small clonal fragments. PLoS ONE 6:e23942

Duarte CM, Sand-Jensen K (1990) Seagrass colonization: patch formation and patch growth in *Cymodocea nodosa*. Mar Ecol Prog Ser 65:193–200

Duarte CM, Terrados J, Agawin NSR, Fortes MD, Bach S, Kenworthy WJ (1997) Response of a mixed Philippine seagrass meadow to experimental burial. Mar Ecol Prog Ser 147:285–294

Evans JP (1992) The effect of local resource availability and clonal integration on ramet functional morphology in *Hydrocotyle bonariensis*. Oecologia 89:265–274

Fox J, Weisberg S (2011) An R companion to applied regression, 2nd edn. R Foundation for Statistical Computing, Vienna. http://cran.r-project.org/

Frosini S, Lardicci C, Balestri E (2012) Global change and response of coastal dune plants to the combined effects of increased sand accretion (burial) and nutrient availability. PLoS ONE 7:e47561

Gilbert ME, Ripley BS (2008) Biomass reallocation and the mobilization of leaf resources support dune plant growth after sand burial. Physiol Plant 134:464–472

Gilbert M, Pammenter N, Ripley B (2008) The growth responses of coastal dune species are determined by nutrient limitation and sand burial. Oecologia 156:169–178

Hemminga MA, Duarte CM (2000) Seagrass ecology. Cambridge University Press, London

Kenworthy WJ, Fonseca M, Whitfield PE, Hammerstrom KK (2002) Analysis of seagrass recovery in experimental excavations and propeller-scar disturbances in the Florida Keys National Marine Sanctuary. J Coast Res 37: 75–85

Lefebvre LW, Reid JP, Kenworthy WJ, Powell JA (2000) Characterizing manatee habitat use and seagrass grazing in Florida and Puerto Rico: implications for conservation and management. Pac Conserv Biol 5:289–298

Libes M, Boudouresque CF (1987) Uptake and long-distance transport of carbon in the marine phanerogam *Posidonia oceanica.* Mar Ecol Prog Ser 38:177–186

Manzanera M, Perez M, Romero J (1998) Seagrass mortality due to over-sedimentation: an experimental approach. J Coast Conserv 4:67–70

Marbà N, Duarte CM (1994) Growth response of the seagrass *Cymodocea nodosa* to experimental burial and erosion. Mar Ecol Prog Ser 107:307–311

Marbà N, Duarte CM (1995) Coupling of seagrass (*Cymodocea nodosa*) patch dynamics to subaqueous dune migration. J Ecol 83:381–389

Marbà N, Duarte CM (1998) Rhizome elongation and seagrass clonal growth. Mar Ecol Prog Ser 174:269–280

Marbà N, Cebrian J, Enriquez S, Duarte CM (1994) Migration of large-scale subaqueous bedforms measured with seagrasses (*Cymodocea nodosa*) as tracers. Limnol Oceanogr 39:126–133

Marbà N, Hemminga MA, Mateo MA, Duarte CM, Mass YEM, Terrados J, Gacia E (2002) Carbon and nitrogen translocation between seagrass ramets. Mar Ecol Prog Ser 226:287–300

Marbà N, Hemminga MA, Duarte CM (2006) Resource translocation within seagrass clones: allometric scaling to plant size and productivity. Oecologia 150:362–372

Mascaró O, Oliva S, Pérez M, Romero J (2009) Spatial variability in ecological attributes of the seagrass *Cymodocea nodosa.* Bot Mar 52:429–438

Maun MA (1998) Adaptations of plants to burial in coastal sand dunes. Can J Bot 76:713–738

Mills KE, Fonseca MS (2003) Mortality and productivity of eelgrass *Zostera marina* under conditions of experimental burial with two sediment types. Mar Ecol Prog Ser 255:127–134

Ooi JLS, Kendrick GA, van Nielb KP (2011) Effects of sediment burial on tropical ruderal seagrasses are moderated by clonal integration. Cont Shelf Res 31:1945–1954

Perez M, Duarte CM, Romero J, Sand-Jensen K, Alcoverro T (1994) Growth plasticity in *Cymodocea nodosa* stands: the importance of nutrient availability. Aquat Bot 47:249–264

R Development Core Team (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. www.r-project.org

Ramage DL, Schiel DR (1999) Patch dynamics and response to disturbance of the seagrass *Zostera novazelandica* on intertidal platforms in southern New Zealand. Mar Ecol Prog Ser 189:275–288

Ryser P (1998) Intra- and interspecific variation in root length, root turnover and the underlying parameters. In: Lambers H, Poorter H, Van Vuuren MMI (eds) Variation in plant growth. Physiological mechanisms and ecological consequences. Backhuys Publishers, Leiden, p 441–465

Sandrini-Neto L, Camargo MG (2011) GAD: an R package for ANOVA designs from general principles. R Foundation for Statistical Computing, Vienna. http://cran.r-project.org/

Sargent FJ, Leary TJ, Crewz DW, Kruer CR (1995) Scarring of Florida's seagrasses: assessment and management options. FMRI Tech Rep TR-1. Florida Marine Research Institute, St. Petersburg, FL

Schwarzschild AC, Zieman JC (2008a) Apical dominance and the importance of clonal integration to apical growth in the seagrass *Syringodium filiforme.* Mar Ecol Prog Ser 360:37–46

Schwarzschild AC, Zieman JC (2008b) Effects of physiological integration on the survival and growth of ramets and clonal fragments in the seagrass *Syringodium filiforme.* Mar Ecol Prog Ser 372:97–104

Short F, Carruthers T, Dennison W, Waycott M (2007) Global seagrass distribution and diversity: a bioregional model. J Exp Mar Biol Ecol 350:3–20

Stuefer JF, Huber H (1999) The role of stolon internodes for ramet survival after clonal fragmentation in *Potentilla anserina.* Ecol Lett 2:135–139

Sutherland WJ, Stillman RA (1988) The foraging tactics of plants. Oikos 52:239–244

Terrados J (1997) Is light involved in the vertical growth response of seagrasses when buried by sand? Mar Ecol Prog Ser 152:295–299

Terrados J, Duarte CM, Kenworthy WJ (1997a) Is the apical growth of *Cymodocea nodosa* dependent on clonal integration? Mar Ecol Prog Ser 158:103–110

Terrados J, Duarte CM, Kenworthy WJ (1997b) Experimental evidence for apical dominance in the seagrass *Cymodocea nodosa.* Mar Ecol Prog Ser 148:263–268

Tomasko DA, Dawes CJ (1989) Evidence for physiological integration between shaded and unshaded short shoots of *Thalassia testudinum.* Mar Ecol Prog Ser 54:299–305

Tomlinson PB (1974) Vegetative morphology and meristem dependence — the foundation of productivity in seagrasses. Aquaculture 4:107–130

Tuya F, Espino F, Terrados J (2013a) Preservation of seagrass clonal integration buffers against burial stress. J Exp Mar Biol Ecol 439:42–46

Tuya F, Viera-Rodríguez MA, Guedes R, Espino F, Haroun R, Terrados J (2013b) Seagrass responses to nutrient enrichment depend on clonal integration, but not flow-on effects on associated biota. Mar Ecol Prog Ser 490: 23–25

Underwood AJ (1997) Experiments in ecology: their logical design and interpretation using analysis of variance. Cambridge University Press, Cambridge

Wijesinghe DK, Handel ST (1994) Advantages of clonal growth in heterogeneous habitats: an experiment with *Potentilla simplex.* J Ecol 82:495–502

Xiao KY, Yu D, Xu XW, Xiong W (2007) Benefits of clonal integration between interconnected ramets of *Vallisneria spiralis* in heterogeneous light environments. Aquat Bot 86:76–82

Yu FH, Chen YF, Dong M (2001) Clonal integration enhances survival and performance of *Potentilla anserina*, suffering from partial sand burial on Ordos plateau, China. Evol Ecol 15:303–318

Yu FH, Dong M, Krusi B (2004) Clonal integration helps *Psammochloa villosa* survive sand burial in an inland dune. New Phytol 162:697–704

Zarranz ME, González-Henríquez N, García-Jiménez P, Robaina RR (2010) Restoration of *Cymodocea nodosa* (Uchria) Ascherson seagrass meadows through seed propagation. Germination in vitro, seedlings culture and field transplants. Bot Mar 53:173–181