

Posidonia oceanica and *Cymodocea nodosa* seedling tolerance to wave exposure

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Abstract

We studied the role of hydrodynamics in the establishment of sea grass seedlings for two Mediterranean sea grass species, *Posidonia oceanica* and *Cymodocea nodosa*, by combining flume and field studies. Flume measurements under both unidirectional and oscillatory flow showed that *P. oceanica* seedlings experienced higher drag forces than *C. nodosa*, which could be related to the larger total leaf area. Drag coefficients were between 0.01 and 0.1 for Reynolds numbers of 10^3 and 10^5 . As a result, *P. oceanica* seedlings required 40–50% of root length anchored to the sediment before being dislodged, whereas *C. nodosa* required $\approx 20\%$. To validate the flume results, seedling survival in sandy beds was evaluated for two depths (12 and 18 m) at two field locations. To calculate near-bottom orbital velocities at the planting sites, deep-water waves were propagated to shallow water using a numerical model. Our results showed that *P. oceanica* seedlings experienced high losses after the first autumn storms when near-bottom orbital velocities exceeded 18 cm s^{-1} . The loss of *C. nodosa* seedlings was much lower and some seedlings survived velocities as high as 39 cm s^{-1} . Thus, flume and field results are consistent in explaining relative higher losses of *P. oceanica* seedlings than for *C. nodosa*.

Aquatic vegetation like sea grass plays an important role in coastal environments by providing many ecosystem services (Costanza et al. 1997), such as, e.g., preventing shoreline erosion by wave attenuation within sea grass beds (Koch et al. 2009). Sea grass growth and development is, however, highly dependent on physical, chemical, and ecological conditions such as hydrodynamics, light availability, substratum type, organic matter in the sediment, and herbivory (Koch 2001; Short et al. 2002).

Sea grass seedlings are the elements that form new meadows and their survival in nature contributes to sea grass population dynamics. Waves and currents can strongly influence the spatial distribution of meadows (Frederiksen et al. 2004; Infantes et al. 2009), suggesting that hydrodynamics is highly important for seedling establishment. Measurements showing higher seedling survival rates at deeper locations suggest the same (Piazzi et al. 1999). Moreover, it has been shown that hydrodynamics can directly affect the survival of sea grass seedling transplants (van Katwijk and Hermus 2000; Rivers et al. 2011). The effect of waves and currents on seedling establishment is expected to be through sediment movement, as this can cause burial or dislodgment of small sea grass plants.

Sea grass capacity to withstand sediment burial is strongly dependent on size and morphology (Idestam-Almquist and Kautsky 1995; Cabaço et al. 2008). The effect of sediment erosion on survival could be determined by the root capacity to remain anchored to the substratum (Madsen et al. 2001). Hence, seedlings may be expected to be highly vulnerable to sediment movement compared with adult sea grasses, as seedling roots will penetrate less deeply into the sediment. Although the effect of sediment

burial on sea grasses has been reviewed (Cabaço et al. 2008), to our knowledge, little work has been devoted to the effect of sediment dynamics, in particular the effect of sediment erosion and root-anchoring capacity on seedling survival. We address this question for the two most common sea grass species in the Mediterranean Sea (Green and Short 2003) that both strongly declined during the 20th century (Boudouresque et al. 2009): *Posidonia oceanica* and *Cymodocea nodosa*.

Both species have morphological differences that could affect their interaction with hydrodynamics and their survival capacity. *P. oceanica* leaves are longer and wider than *C. nodosa* leaves (Guidetti et al. 2002). These differences will affect the drag force exerted on the leaves and thus the anchoring requirements by the roots. Wicks et al. (2009) suggested that *Zostera marina* seedlings with the same root length but higher leaf area would have less chance to survive by being dislodged from the sediment than seedlings with lower leaf area. As *P. oceanica* has a higher total leaf surface area than *C. nodosa* (Guidetti et al. 2002), the drag experienced on the leaves will be higher in *P. oceanica* than in *C. nodosa*.

The objective of this work was to evaluate the capacity of seedlings of *P. oceanica* and *C. nodosa* to remain anchored to the substrata under different hydrodynamic conditions to get a fundamental understanding of seedling survival in nature. We studied the underlying mechanisms that can affect seedling survival in a biological flume. Drag measurements were carried out to obtain the effective drag forces acting on the seedlings and calculate the drag coefficient under unidirectional and oscillatory flows. Further, the dislodging depths and minimum root lengths necessary to remain anchored to the sediment were measured. Additionally, a replicated short-term field experiment was designed to compare the survival of

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seedlings at two depths (18 m and 12 m) to assess the effect of exposure to hydrodynamics on the survival of *P. oceanica* and *C. nodosa* seedlings.

Methods

Posidonia oceanica and *C. nodosa* seedlings used in the flume and field studies were obtained from fruits collected at sea. *P. oceanica* fruits were collected from drift material on several beaches of Mallorca and Ibiza islands (western Mediterranean Sea) during April–May 2009. Fruits were manually opened and seeds were placed in natural-salinity seawater. Seed germination took place within a week or two after collection. *C. nodosa* seeds were collected during February–April 2009 in a shallow (3 m) meadow in Mallorca Island. Seeds were kept in aquariums until May 2009, the month when *C. nodosa* seeds germinate at sea. Germination of *C. nodosa* was induced by reducing the salinity to 10 (Caye and Meinesz 1986). Both *P. oceanica* and *C. nodosa* germinated seeds were kept in aquariums with natural seawater during early seedling development. Aquariums were in a temperature-controlled room at 20°C with a 14-h photoperiod and a photon irradiance of 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Seedlings were haphazardly distributed into two groups, one for flume experiments and another for field experiments.

Drag forces and drag coefficient—Drag measurements were carried out to obtain the effective drag forces acting on individual seedlings and to calculate the drag coefficients of both species under unidirectional and oscillatory flows. Experiments were carried out in the Netherlands Institute of Ecology racetrack-shaped channel at Yerseke, the Netherlands. The channel is 17.55 m long, 0.6 m wide, and 0.45 m deep. In all experiments, water depth was 0.32 m, water temperature was $20 \pm 0.5^\circ\text{C}$, and salinity 36. The flume allows running both unidirectional and oscillatory flows. Currents in the flume were generated by a conveyor belt. Waves were generated by a vertical wave maker driven by a piston. At the end of the test section waves were dampened by a porous gentle slope. Flow was characterized by measuring vertical profiles of the velocity at 5-cm intervals along the water column using an acoustic Doppler velocimeter (ADV Vectrino, Nortek) at the same cross-section where seedlings were tested. The ADV sampling rate was 25 Hz and measurements were taken for 5 min, sampling volume of 7 mm, and a nominal velocity range of 1 m s^{-1} .

Drag was measured on individual *P. oceanica* and *C. nodosa* seedlings fixed to a force transducer inside the flume. Seedlings were exposed to seven different wave conditions that include wave heights (H) ranging from 1 to 7 cm and periods (T) from 1.5 to 4 s. These waves gave near-bottom orbital velocities between 4 and 16 cm s^{-1} . Seedlings were also exposed to six different current velocities ranging between 5 and 36 cm s^{-1} . Hereafter, u represents the near-bottom orbital velocity (for waves) or the mean velocity (currents). Each wave and current flow condition was repeated for five seedlings. Seedlings were attached to a 2-cm-long metal screw by the stem using a

small cable tie, removing the roots and seed. The metal screw was then fixed into the drag sensor. The drag force of the metal screw with cable ties alone is a constant, and was subtracted from the drag force measured on the seedlings. No breaking of leaves or plant fragments was observed during the drag experiments. Drag forces and wave heights were measured at 20 Hz during 3 min. Drag forces were measured using a drag transducer developed by WL-Delft Hydraulics (Bouma et al. 2005) and calibrated as Stewart (2004). Waves were measured with a pressure sensor (Druck, PT1830). Drag measurements were performed on *P. oceanica* in October 2009 and February 2010, but drag in *C. nodosa* was only measured in October 2009 since seedlings did not survive until February 2010.

The force acting over individual plants measured by the transducer can be expressed as:

$$F_D = \frac{1}{2} \rho C_d A u |u| \quad (1)$$

where ρ is the density of water, A is the total leaf area of the plant, and u the flow velocity (either unidirectional or oscillatory). The drag coefficient, C_d , which depends on the Reynolds number, can be readily obtained from the experiments using Eq. 1. Defining the characteristic length as (Martone and Denny 2008)

$$l_* = \sqrt{A}, \quad (2)$$

the corresponding Reynolds number is defined as

$$\text{Re} = \frac{ul_*}{\nu}, \quad (3)$$

ν being the molecular kinematic viscosity of seawater. Most aquatic vegetation is flexible and becomes streamlined with increasing flow velocities and it is very difficult to measure accurately the frontal area under waves and currents (Sand-Jensen 2003, 2005; but see Boller and Carrington 2007). For this reason, the total surface area was used as the frontal area as it can be accurately measured.

Critical erosion depth and minimum rooting length—Once the drag force was measured for the seedlings the next step was to analyze the capacity of both species to withstand dislodgement by sediment disturbances via root anchoring. Individual *P. oceanica* and *C. nodosa* seedlings were planted in cylindrical pots (height \times diameter: 12 \times 12 cm) over sand with a diameter (d_{50}) of $592 \pm 23 \mu\text{m}$. Pots were placed in the flume under two sets of experiments: periodic waves of $H = 2.5$ cm and $T = 2.6$ s ($u = 5$ cm s^{-1}) and periodic waves of $H = 5.1$ cm and $T = 1.9$ s ($u = 10$ cm s^{-1}) during 15 min (Fig. 1a). Seedlings were repeatedly exposed to 15 min of wave action, while in between wave treatments, sediment erosion was mimicked. This was repeated until we found the critical erosion depth at which seedlings dislodged (Fig. 1b). Sediment erosion was simulated by progressively adding discs of 3-mm thickness underneath the pots, removing carefully the pushed-up top layer of sediment. This process was repeated until the waves dislodged the seedling from the sediment. A total of 18 plants was used for *P. oceanica* and 9 for *C. nodosa*. For

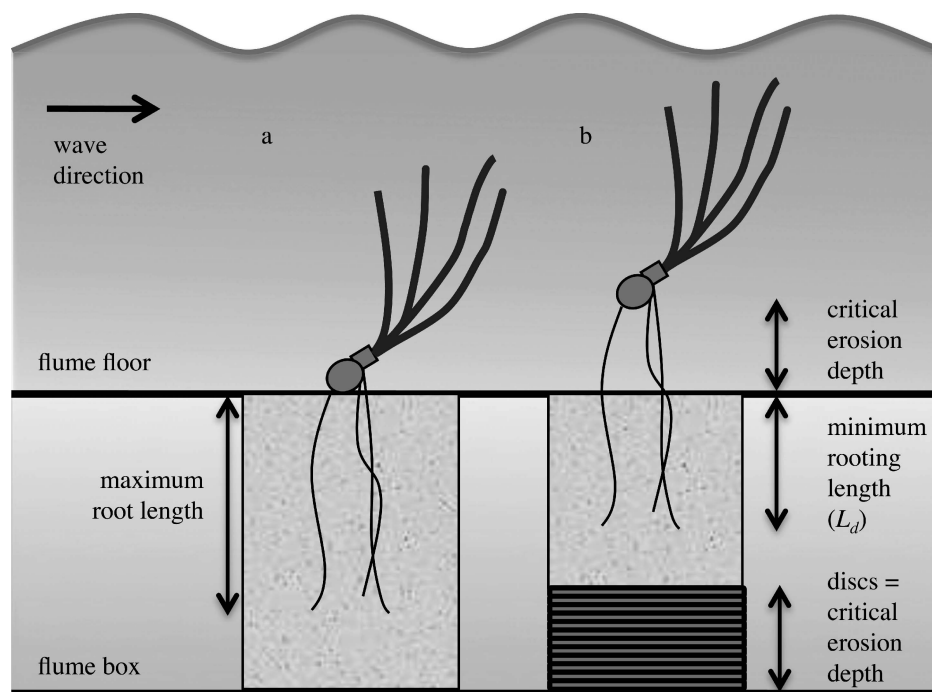


Fig. 1. Sketch of the flume experimental setup showing the critical erosion depth and minimum rooting length (L_d) of seedlings. (a) Seedling in the flume, (b) seedling before dislodging from the sediment after the discs addition. Critical erosion depth is equivalent to the total height of discs added when the seedling is dislodged. Not drawn to scale.

each seedling, root lengths and total leaf area were measured. The critical erosion depth was measured as the thickness of disks added before the plant became dislodged. The minimum rooting length (L_d) or length of the root inside the sediment before the plant became dislodged was obtained by the difference of the maximum root length and the critical erosion depth. The length of the longest root was used for all calculations since in the end, regardless of the number of roots, seedlings remained anchored to the substrata with only one root, even when the rest were dislodged.

Field study—Seedlings were planted at sea to assess their survival and to validate the principles observed in the flume. The field study was performed from August 2009 to February 2010 on Mallorca Island, Spain, western Mediterranean Sea (Fig. 2a,b). In this area, tides are almost negligible, e.g., less than 25 cm. *P. oceanica* and *C. nodosa* seedlings were planted at two locations in the Natural Reserve of Cap de Enderrocat (Fig. 2c). The first location, Cap Enderrocat, is a sandy area between the upper depth limit of *P. oceanica* and the coastline exposed to southwest and southeast waves (triangles in Fig. 2c). The second location, Cala Blava, is located in a large *P. oceanica* meadow with sand gaps (circles in Fig. 2c), which is only exposed to southwest waves. The upper depth limit of *P. oceanica* in Cap Enderrocat is at 19 m, whereas that in Cala Blava is at 12 m. The upper depth limit of *C. nodosa* in Cap Enderrocat and Cala Blava is at 12 m. Within each location two depths were selected, 18 m and 12 m, for the short-term experiment.

Six plots (three for each species) separated by 3-m intervals were established at the four sites. Twelve seedlings were planted on each plot directly on the sediment without any artificial supporting aid, to mimic natural conditions where the roots are the only attachment structure to the substrata. A total of 144 seedlings of each species was planted after measuring the number of roots, root lengths, and root diameter in the laboratory. Additionally, the number of leaves of each seedling and the length and width of each leaf were measured once planted in the field. The number of seedlings in each plot was counted in September, October, and November 2009 and in February 2010. Whenever seedlings were no longer observed in the plots, we determined that the loss was not caused by burial.

Survival rate was related to wave conditions as well as to averaged near-bottom orbital velocities between sampling dates at the four experimental sites. Significant wave height (H_s), peak period (T_p), and direction (θ) were acquired at deep waters from a wave climate database, approximately 15 km from the study sites (Fig. 2b). These wave conditions were propagated to the study sites using a numerical model based on the mild-slope parabolic approximation (Kirby and Dalrymple 1983; Infantes et al. 2011). Wave conditions in the study area ranged between a H_s of 0.5 and 4 m, T_p of 2 and 12 s, and θ of 130 and 270°. Near-bottom orbital velocities at the four locations where *P. oceanica* and *C. nodosa* seedlings were transplanted were computed from the model output between sampling periods using linear wave theory (Dean and Dalrymple 1991).

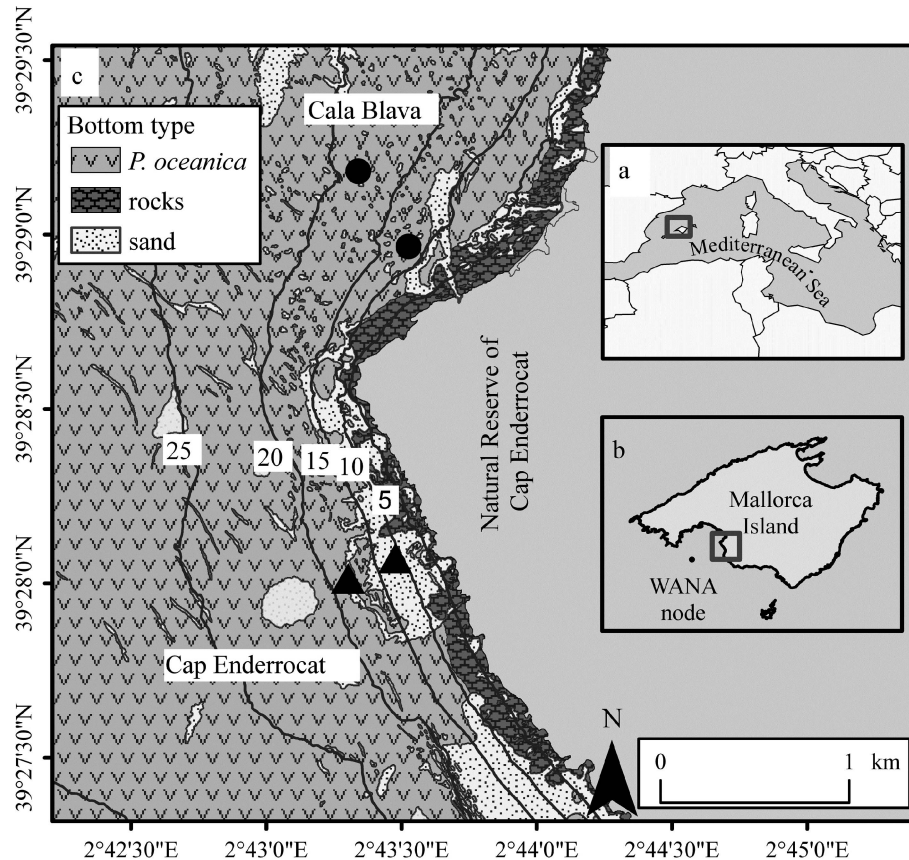


Fig. 2. (a) Location of Mallorca Island in the Mediterranean Sea. (b) Location of study area and deep-water wave climate data (WANA node). (c) Location of the experimental sites, Cap Enderrocat (triangles) and Cala Blava (circles). Bathymetric contours in meters.

Statistical analysis—Differences in drag forces under currents and waves of both species were tested using a Student's *t*-test. Differences among drag coefficients were evaluated using species (*P. oceanica*, *C. nodosa*) and flow (unidirectional, oscillatory) as fixed factors and Reynolds number as the covariate in the analysis of covariance (ANCOVA) model $C_d = \text{species} + \text{flow} + \text{Reynolds} + (\text{species} \times \text{flow}) + (\text{species} \times \text{Reynolds}) + (\text{flow} \times \text{Reynolds}) + (\text{species} \times \text{flow} \times \text{Reynolds})$ (Quinn and Keough 2002). None of the two- or three-way interaction terms was significant in the ANCOVA, so only results for single-factor terms are reported here. Critical erosion depths and minimum rooting lengths in the sediment were evaluated using univariate test of significance, ANOVA. Wave velocity (orbital velocities of 5 cm s^{-1} and 10 cm s^{-1}), species (*P. oceanica* and *C. nodosa*) were the between-subjects factors (fixed). Differences of seedling survivorship in the field experiment were also evaluated using univariate test of significance ANOVA. The test was performed only in October 2009 because we observed differences between sites on that date. After October 2009 no plants were left in most of the plots (see Results). Sites (Cap Enderrocat and Cala Blava), depth (18 m and 12 m), species (*P. oceanica* and *C. nodosa*) were the factors (fixed). A Cochran's *C*-test was used to test for heterogeneity of variances. All data were normally distributed.

Results

Posidonia oceanica and *C. nodosa* have flexible leaves that easily bend with the currents or waves. Drag forces acting on individual seedlings increased with current and wave velocities (see Fig. 3). Drag forces also increased with foliar surface area for both sea grass species (Fig. 4). Drag forces were higher (*t*-test: $t = 7.08$, $df = 14$, $p < 0.01$) for *P. oceanica* ($0.006 \pm 4 \times 10^{-4} \text{ N}$, mean \pm SE) than for *C. nodosa* ($0.003 \pm 3 \times 10^{-4} \text{ N}$) at $u = 16 \text{ cm s}^{-1}$ in unidirectional flow (see Fig. 4). Similarly, drag forces were also higher (*t*-test: $t = 3.2$, $df = 14$, $p < 0.01$) for *P. oceanica* ($0.011 \pm 1 \times 10^{-3} \text{ N}$) than for *C. nodosa* ($0.005 \pm 6 \times 10^{-4} \text{ N}$) at $u = 16 \text{ cm s}^{-1}$ in oscillatory flow (see Fig. 4).

ANCOVA shows that drag coefficient (C_d) depends on Reynolds number (Re), ($F = 298.7$, $df = 1$, $p < 0.001$) and that it is also influenced by the type of flow (C_d currents $>$ C_d waves, $F = 4.8$, $df = 1$, $p < 0.05$), but not by the species ($F = 1.4$, $df = 1$, $p = 0.24$) (Fig. 5). A linear fitting of the drag coefficient for both species under oscillatory motion is:

$$\log_{10} C_d = -0.6653 \cdot \log_{10} Re + 1.1886, (R^2 = 0.77, r = -0.87) \quad (4)$$

and similarly for unidirectional flow:

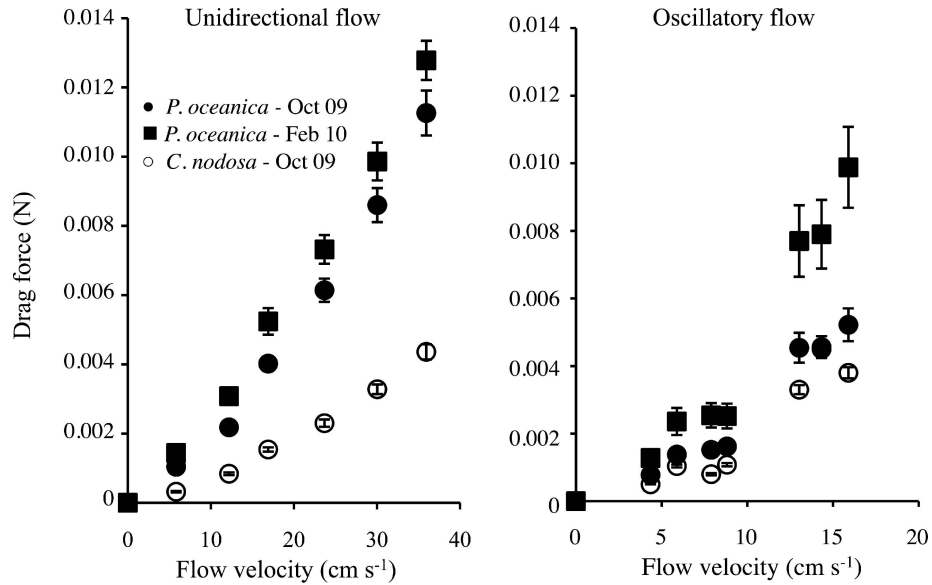


Fig. 3. Drag forces acting on seedlings in unidirectional flow and oscillatory flow (mean, SE, $n = 5$).

$$\log_{10} C_d = -0.7269 \cdot \log_{10} Re + 1.6253, \quad (R^2 = 0.92, r = -0.95). \quad (5)$$

The experiments in the flume have shown that there are significant differences in the minimum rooting length that each species tolerates (Table 1). *P. oceanica* seedlings require 40–50% (2 ± 0.2 cm, mean \pm SE) of root length anchored into the sediment, whereas *C. nodosa* requires only 20% (2.8 ± 0.2 cm, mean \pm SE) of root length anchored into the sediment (Fig. 6). *P. oceanica* seedlings tolerate less sediment erosion (2.7 ± 0.3 cm, mean \pm SE) to become dislodged than *C. nodosa* (5.6 ± 0.4 cm, mean \pm SE) under waves.

The capacity of a plant to remain anchored depends on the force that the roots can manage (which mainly depends on their length L_d) and on the force that the exposed leaves receive from the ambient (which is highly dependent on the total leaf area A). The above suggests that an important dimensionless coefficient in the problem is the ratio L_d/\sqrt{A} . Since other variables could play a role in the problem, the ratio L_d/\sqrt{A} will not be constant in general, and it could depend on other dimensionless groups representing the plant species, flow velocity, type of flow (wave or current), etc.

From the experimental results, only the influence of the species on L_d/\sqrt{A} has been clearly identified as a measure

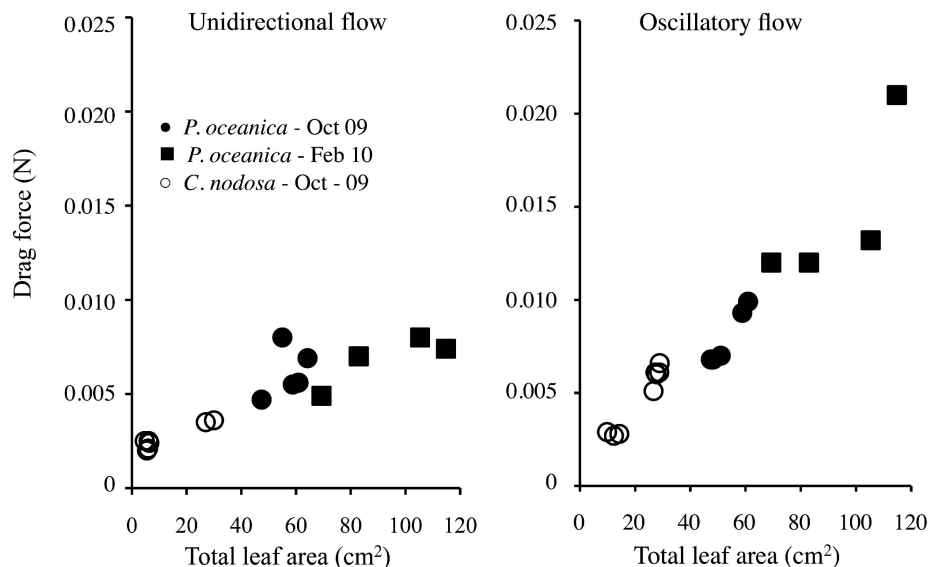


Fig. 4. Drag forces acting on individual seedlings of different surface area in unidirectional flow and oscillatory flow. Flow velocity of 16 cm s^{-1} .

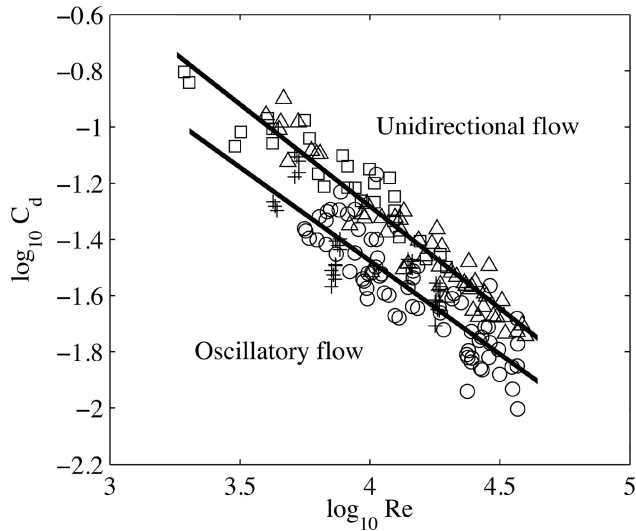


Fig. 5. Drag coefficient vs. Reynolds number for *Posidonia oceanica* and *Cymodocea nodosa* under unidirectional and oscillatory flow. Experimental data under unidirectional flow for *P. oceanica* seedlings (triangles) and for *C. nodosa* (squares). For oscillatory flow *P. oceanica* seedlings (circles) and *C. nodosa* (crosses). Solid lines are the linear fitting for the different flow conditions.

of the dislodgment risk, which can be termed as the dislodgement safety factor. Our results indicate that under periodic flows, seedlings of *P. oceanica* will remain anchored as long as $L_d/\sqrt{A} \approx 0.35$, i.e., if their root lengths are on average 0.35 times the square root of the leaves' area. In the case of *C. nodosa* the condition for the seedlings to remain anchored is $L_d/\sqrt{A} \approx 1.6$.

The foliar surface of *P. oceanica* seedlings at the beginning of the field experiment was four times larger than that of *C. nodosa* seedlings (Table 2). As seedlings develop, the difference in foliar surface increases. In contrast, *C. nodosa* maximum root lengths are higher than those of *P. oceanica*. Root diameters are higher for *P. oceanica* than for *C. nodosa*. Survival was related to wave conditions as well as to averaged near-bottom orbital velocities between sampling dates (Fig. 7; Table 3). No loss of seedlings occurred from August to September 2009. During this period no storms affected the study area and the computed near-bottom orbital velocities were below 5 cm s⁻¹.

Between September and October 2009, a storm with H_s of 1.5 m and T_p of 7 s was measured at deep waters. During this period, the first losses of *P. oceanica* seedlings were observed. Computed u for this period in Cap Enderrocat was 8.6 cm s⁻¹

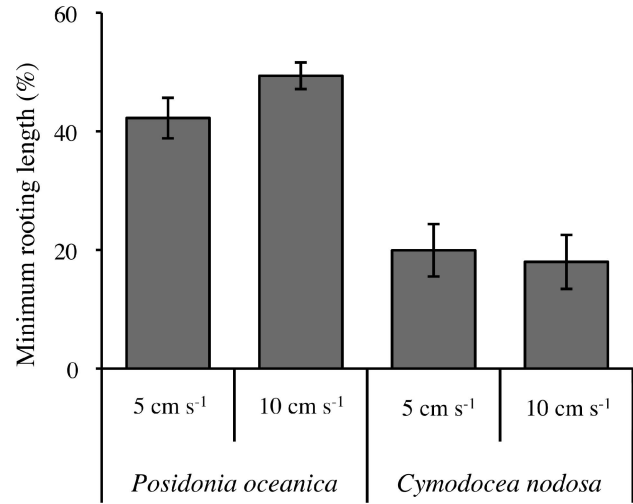


Fig. 6. Minimum rooting length of *Posidonia oceanica* and *Cymodocea nodosa* exposed to two orbital velocities ($u = 5$ and 10 cm s⁻¹).

at 18-m depth and 28 cm s⁻¹ at 12-m depth, whereas in Cala Blava it was 7.3 cm s⁻¹ at 18-m depth and 14.7 cm s⁻¹ at 12-m depth (Table 3). The percentage of *P. oceanica* seedlings surviving in Cala Blava was significantly higher than that in Cap Enderrocat (Fig. 7a; Table 4). Moreover, the percentage was significantly higher in the deeper (18 m) than in the shallow (12 m) sites. The loss for *C. nodosa* was also significant but no differences in the percentage of survivorship among sites and depths was detected.

From October to November 2009, two storms from the southwest with H_s of 4 and 2.5 m and T_p of 10 and 8 s respectively were recorded at deep waters. After these storms, all *P. oceanica* seedlings disappeared in both Cap Enderrocat and Cala Blava sites, whereas *C. nodosa* seedlings persisted in Cala Blava at both depths (Fig. 7b). Computed u at the experimental sites varied between 17 and 34 cm s⁻¹ (Table 3).

From November 2009 to February 2010 several storms were recorded with maximum H_s of 5 m and T_p of 11 s at deep waters (Fig. 7c). Remaining seedlings of *C. nodosa* persisted during this period. Computed u in this site varied between 23 and 39 cm s⁻¹ during that period of time.

Discussion

Present study offers a mechanistic insight into how the combination of drag and erosion may cause seedling

Table 1. Two-way ANOVA testing differences between species and orbital velocities and minimum rooting length. Significant differences are expressed in bold as *** $p < 0.001$, and ns, not significant. Cochran's C-test not significant. O.Velocities is orbital velocities. df, degrees of freedom; MS, mean square.

		df	MS	F	p
Minimum rooting length	Species	1	6828	52.9	***
	O.Velocities	1	63	0.5	ns
	Species × O.Velocities	1	196	1.5	ns
	Error	34	129		

Table 2. Morphological characteristics of seedlings at the beginning of the field experiment (mean \pm SE, $n = 144$).

	<i>Posidonia oceanica</i>	<i>Cymodocea nodosa</i>
Total foliar surface (cm ²)	12.9 \pm 1.6	3.21 \pm 0.4
Number of leaves	6.1 \pm 0.6	2.3 \pm 0.2
Number of roots	5.2 \pm 0.2	4.8 \pm 0.2
Total root length (cm)	15.8 \pm 0.2	26.1 \pm 0.4
Max. root length (cm)	5.5 \pm 0.2	6.8 \pm 0.4
Root diameter (mm)	1.78 \pm 0.1	0.51 \pm 0.03

dislodgement from the substrata. Our results showed that drag forces were higher in *P. oceanica* seedlings than in *C. nodosa* under both current and waves, and that *P. oceanica* seedlings are dislodged earlier than *C. nodosa* seedlings. Thus flume results predict survival of *C. nodosa* seedlings to exceed that of *P. oceanica* seedlings. Field experiments confirmed this, by showing that seedlings were dislodged during storms, and that *C. nodosa* seedlings survived longer than *P. oceanica*.

Measured drag force per unit total leaf surface area is on average (3 ± 1.2 N m⁻² mean \pm SE) for both the *P. oceanica* and *C. nodosa* seedlings at $u = 16$ cm s⁻¹, which

Table 3. Computed near-bottom orbital velocities (cm s⁻¹) at the experimental locations during the sampling periods (mean \pm SD).

	Cap Enderrocat		Cala Blava	
	12 m	18 m	12 m	18 m
Aug–Sep	<5	<5	<5	<5
Sep–Oct	18.0 \pm 1.1	8.6 \pm 0.3	14.7 \pm 0.9	7.3 \pm 0.2
Oct–Nov	34.4 \pm 2.2	18.2 \pm 1.2	29.4 \pm 1.9	17.0 \pm 1.1
Nov–Feb	46.0 \pm 2.3	25.5 \pm 1.2	39.1 \pm 1.5	23.4 \pm 1.1

was in the same order of magnitude as earlier measurements for sea grass model plants with flexible shoots (5.5–7.8 N m⁻²) at 37 cm s⁻¹ (Bouma et al. 2005). For seedling survival, it is, however, the absolute drag force rather than the drag per unit surface area that matters. Present flume experiments show that drag coefficient (C_d) depends on Reynolds number but not on the species and that this relationship is different for current and waves (Fig. 5). The computed drag coefficients for the individual seedlings of *P. oceanica* and *C. nodosa* are between 0.01 and 0.1 for Reynolds numbers between 10³ and 10⁵, which is in accordance with those provided for flexible macrophytes

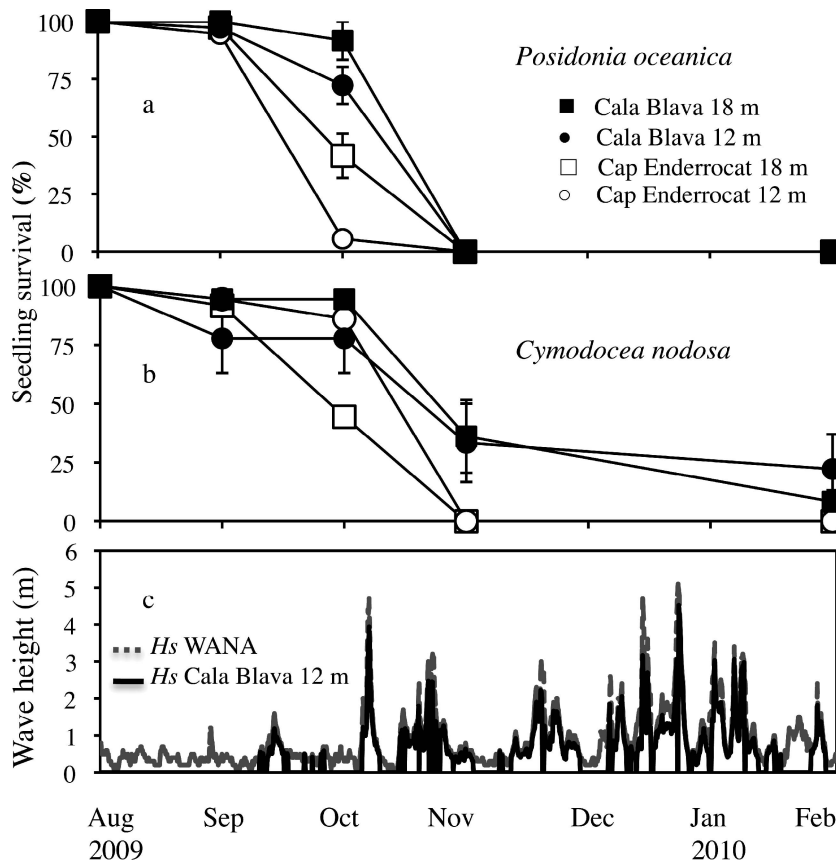


Fig. 7. Seedling survival on the experimental plots in Mallorca, August 2009 to February 2010. (a) *Posidonia oceanica*, (b) *Cymodocea nodosa*, and (c) wave heights in deep water (WANA node) shown in gray line and propagated wave heights in Cala Blava at 12-m depth shown in black line. Gaps in propagated H_s correspond to wave directions other than southwest to southeast not affecting the study area.

Table 4. Results of three-way ANOVA of seedling survival percentage in October 2009. Significant differences are expressed in bold as: ** $p < 0.01$, *** $p < 0.001$, and ns, not significant. Cochran's C -test not significant. df, degrees of freedom; MS, mean square.

	df	MS	F	p
Location	1	9401.04	40.1	***
Depth	1	350.12	1.5	ns
Species	1	3151.04	13.5	**
Location \times depth	1	651.04	2.8	ns
Location \times species	1	2109.37	9.1	**
Depth \times species	1	2433.45	10.3	**
Location \times depth \times species	1	2109.37	9.2	**
Error	16	234.38		

(Sand-Jensen 2003; Martone and Denny 2008). It should be noted that drag coefficients for meadows can depend on many factors such as the height and density of the plants, the distance traveled by waves, etc. and that drag coefficient for meadows thus may differ from the values presented for single plants.

Sediment mobilization is related to hydrodynamics (wave and currents), which can bury or dislodge sea grasses (Madsen et al. 2001; Cabaço et al. 2008). Previous studies have shown that 2 cm of sediment erosion caused 75% of mortality in *C. nodosa* seedlings in stagnant water (Marbà and Duarte 1994), but to our knowledge there are no data about the effect of sediment erosion on *P. oceanica* seedlings. In this study we show that *P. oceanica* seedlings under waves tolerate less sediment erosion (2–3 cm) to become dislodged than *C. nodosa* (5–6 cm). *P. oceanica* seedlings need a root length of 0.35 times the square root of the total leaf area to remain anchored, whereas in *C. nodosa* this condition is increased up to 1.6 times the square root of leaf area. However, seedlings of *P. oceanica* in the flume have a foliar area that is on average 20 times higher than those of *C. nodosa* and therefore the seedling capacity to remain anchored is much more limited for *P. oceanica* than for *C. nodosa*. This suggests that *P. oceanica* seedlings are adapted to grow at ambients less energetic than *C. nodosa*. This may also be inferred from the proportionally higher root biomass and lower leaf biomass that *C. nodosa* allocates compared with *P. oceanica* (Guidetti et al. 2002). Anderson et al. (2006) described the dislodgement of coenocytic green algae from soft sediments by waves measuring the mean force to dislodge the algae (4.9–12.7 N), which increased as the leaf surface area increased.

The seedling survival in the field also showed that *P. oceanica* and *C. nodosa* seedlings behave differently under the same hydrodynamic conditions. *C. nodosa* seedlings survived longer than *P. oceanica* in Cala Blava during the field experiment. *P. oceanica* seedlings survived at a higher proportion in the deep than in the shallow sites at both locations during October 2009. In November 2009, all *P. oceanica* seedlings disappeared in the plots, whereas *C. nodosa* seedlings persisted at both depths in Cala Blava. Two storms took place before we observed the complete

loss of *P. oceanica* seedlings in November 2009. We cannot determine which of these two events caused seedling loss but we can conclude that these storms had a deleterious effect on the survival of *P. oceanica* seedlings. The presence of a large *P. oceanica* meadow from the shallow areas to 30–35-m depths demonstrates that the sea grass is not light limited at the study site (Fig. 2c). Herbivory is not considered to be a cause of seedling loss because bite scars on the leaves were not observed. Different environmental conditions between sites associated with hydrodynamics such as sediment resuspension and turbidity could have an effect on the seedling survival, although these are indirect effects of wave exposure. Some studies have shown high mortality of seedlings in the field after storms. A mortality of 75% to 100% natural and transplanted *P. oceanica* seedlings on pebbles occurred during the first winter after planting, associated with storms (Balestri et al. 1998). Individual seedlings of four different *Posidonia* spp. planted in a blowout area did not succeed after 1 yr (Kirkman 1998).

Water depth had an effect on seedling survival: as wave-induced orbital velocities attenuate with depth, near-bottom orbital velocities will be higher at 12 m than at 18 m. In October 2009, a significantly lower survival was observed for *P. oceanica* at 12 m than at 18 m (Fig. 7a; Table 4). Piazzini et al. (1999) observed lower survival rates of *P. oceanica* seedlings at 2-m depth than at 10-m depth. A correlative study in a *P. oceanica* meadow (Infantes et al. 2009) suggested that at depths between 0 and 5 m near-bottom orbital velocities were too high for *P. oceanica* to be present, whereas at depths greater than 7 m velocities were lower and a dense meadow was present.

Infantes et al. (2009) estimated a threshold in near-bottom orbital velocities of 38–42 cm s⁻¹ for a *P. oceanica* meadow to be present in a shallow bay. In this study, *P. oceanica* seedlings had a 100% survivorship at near-bottom orbital velocities below 5 cm s⁻¹, but for velocities between 7 and 18 cm s⁻¹ the percentage of seedlings surviving decreased and all seedlings disappeared at velocities above 18 cm s⁻¹. Some *C. nodosa* seedlings survived at velocities of 39 cm s⁻¹. Thus, our results suggest that seedlings tolerate lower orbital velocities than mature plants. This is not surprising, because mature plants develop a network of roots and rhizomes that penetrate deeper into the substrata than seedlings. Other studies showed that transplanted *Z. marina* survived at orbital velocities of 40 cm s⁻¹ but not at 60 cm s⁻¹ (van Katwijk and Hermus 2000). For meadows, Cabaço et al. (2010) estimated that the decline of *C. nodosa* occurs at velocities over 60 cm s⁻¹.

Flow velocity is an important factor determining the size of the marine organisms that inhabit benthic ecosystems (Denny et al. 1985). Present results suggest that the higher drag of large species may make small species relatively more successful in the colonization of exposed sites. This agrees with the convention (on the basis of size-related differences of growth rates) that large sea grass species generally occupy low-energy or sheltered habitats, whereas small sea grass species are able to colonize more energetic habitats because the capacity of small species to recover from disturbance through growth is higher than that of

large species (Idestam-Almquist and Kautsky 1995; Blanchette 1997). Our results that seedlings of *C. nodosa* are able to persist under higher wave energies than those of *P. oceanica* are also in agreement with the old but untested assumption that *C. nodosa* is a pioneer species that stabilizes the substrata and facilitates the establishment of *P. oceanica* (Den Hartog 1970).

The findings presented in this paper provide the basis for understanding seedling survivorship of two Mediterranean sea grass species in sandy substratum under different hydrodynamic conditions. We have shown that morphological characteristics of each species such as foliar surface and root length are key factors in colonization processes. Moreover, drag and sediment erosion play an important role in seedling survival, a crucial stage of sea grass life. Habitat degradation and fragmentation caused by anthropogenic effects around the world's coastal zones raises the need to manage and restore sea grass beds (Orth et al. 2006). Seedling plantings could be more effective in sea grass restoration if more quantitative knowledge on the hydrodynamic effects that limit the survival of sea grass seedlings is gathered.

Acknowledgments

We thank Puertos del Estado for the wave climate data. We thank G. Samperio and M. Domínguez for field assistance and M. Luhar and H. Nepf for their comments on drag. Dirección General de Pesca (Govern de les Illes Balears) is thanked for granting permission to perform this study inside the Palma Bay marine reserve, and thanks also to Club Náutico S'Arenal, which kindly made available its harbor facilities to us.

E.I. acknowledges financial support from the Spanish Ministerio de Educación y Ciencia scholarship program (2006-12850). Research funds were provided by the Spanish Ministerio de Ciencia e Innovación (grants CIT-310000-2008-9 and CTM-2010-16915) and Ministerio de Medio Ambiente, Medio Rural y Medio Marino (grant 116/SGTB/2007/1.3).

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Associate editor: Craig L. Stevens

Received: 11 January 2011

Accepted: 03 August 2011

Amended: 09 August 2011