

## Assessment of substratum effect on the distribution of two invasive *Caulerpa* (Chlorophyta) species

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### ARTICLE INFO

#### Article history:

Received 22 February 2010

Accepted 28 November 2010

Available online 3 December 2010

#### Keywords:

*Caulerpa racemosa* var. *cylindracea*

*Caulerpa taxifolia*

*Posidonia oceanica*

substratum

invasive macroalgae

### ABSTRACT

Two-year monitoring of the invasive marine Chlorophyta *Caulerpa taxifolia* and *Caulerpa racemosa* var. *cylindracea* shows the great influence of substratum on their spatial distribution. The cover of *C. taxifolia* and *C. racemosa* was measured in shallow (<8 m) areas indicating that these species are more abundant in rocks with photophilic algae and in the dead matte of the seagrass *Posidonia oceanica* than in sand or inside the *P. oceanica* meadow. A short-term experiment comparing the persistence of *C. taxifolia* and *C. racemosa* planted either in a model of dead matte of *P. oceanica* or in sand shows that the persistence of these species was higher in the dead matte model than in sand. Correlative evidence suggests that *C. taxifolia* and *C. racemosa* tolerate near-bottom orbital velocities below  $15 \text{ cm s}^{-1}$  and that *C. taxifolia* cover declines at velocities above that value. These results contribute to understand the process of invasion of these *Caulerpa* species predicting which substrata would be more susceptible to be invaded and to the adoption of appropriate management strategies.

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### 1. Introduction

Light and temperature are identified as the main determinants for the spatial distribution of marine macrophytes but substratum type and hydrodynamic conditions are also key factors limiting the distribution of marine vegetation (Shepherd and Womersley, 1981; Fonseca and Kenworthy, 1987; Riis and Hawes, 2003; Frederiksen et al., 2004; Koch et al., 2006). Consolidated substrata (i.e., rocks) offer a stable, non-mobile surface where macroalgae and some seagrass species attach effectively. Unconsolidated substrata (i.e., sand, mud) are unstable, mobile and only seagrasses and macroalgae with root-like structures can colonize them. Waves and currents can mobilize sediment particles producing sediment erosion and accretion that may affect seagrasses negatively through uprooting and burial (Fonseca and Kenworthy, 1987; Williams, 1988; Terrados, 1997; Cabaço et al., 2008). Thresholds of maximum wave energy tolerated by some seagrasses have been determined (Fonseca and Bell, 1998; van Katwijk and Hermus, 2000; Koch, 2001; Infantes et al., 2009) as well as the relationship between wave exposure and temporal variability of seagrass coverage in shallow sands (Fonseca et al., 1983; Fonseca and Bell, 1998; Frederiksen et al., 2004). By contrast, information about the wave energy levels that macroalgae tolerate is limited (D'Amours and Scheibling, 2007; Scheibling and Melady, 2008), especially in unconsolidated substrata.

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Most marine macroalgae grow over consolidated substrata such as rocks or over other macrophytes but a few Chlorophyta species of the order Caulerpales are also able to grow on unconsolidated substrata (Taylor, 1960). The thallus of *Caulerpa* species is composed of a creeping portion, the stolon, that it is attached to the substrata by root-like structures, the rhizoids, and an erect portion, the fronds that have different shapes depending on the species (Taylor, 1960; Bold and Wynne, 1978). The rhizoids of *Caulerpa* are able to bind sediment particles (Chisholm and Moulin, 2003) and anchor the plant in sandy sediments or other unconsolidated substrata, and to attach to rocks and other macrophytes (Taylor, 1960; Meinesz and Hesse, 1991; Klein and Verlaque, 2008). Hence *Caulerpa* species may be present both in wave exposed, rocky bottoms and sheltered, sandy-muddy sediments (Thibaut et al., 2004).

*Caulerpa taxifolia* (Vahl) C. Agardh and *Caulerpa racemosa* var. *cylindracea* (Sonder) Verlaque, Heisman et Boudouresque (hereafter *C. racemosa*) have been introduced in the Mediterranean Sea within the last 25 years showing an invasive behaviour (Meinesz and Hesse, 1991; Boudouresque and Verlaque, 2002; Klein and Verlaque, 2008). Understanding the factors that influence the establishment and spread of these introduced *Caulerpa* species is essential to identify benthic communities susceptible of being invaded and to have scientific tools to predict future invasions. Initial observations of *C. taxifolia* invasion suggested that rocks and dead matte of *Posidonia oceanica* were appropriate for the establishment of this species but sandy or muddy sediments in sheltered conditions were also colonized by this species (Hill et al., 1998). A short-term experiment

showed that algal turfs were more favourable for the establishment of *C. taxifolia* and *C. racemosa* than other macroalgal communities on rocky bottoms (Ceccherelli et al., 2002). Both *Caulerpa* species seem to tolerate a certain level of sediment deposition and burial (Glasby et al., 2005; Piazzini et al., 2005) a feature that likely facilitates their development in sandy sediments.

In this study we evaluate the effect of substratum on the persistence of two invasive *Caulerpa* species. First, we quantify the presence of *Caulerpa taxifolia* and *Caulerpa racemosa* in different types of substrata during two consecutive summers and winters testing if their presence was independent of the type of substratum. Then, we perform a short-term experiment where fragments of *C. taxifolia* and *C. racemosa* are established in a model of dead matte of *Posidonia oceanica* and in sand and their development followed from summer to winter. The cover of both *Caulerpa* species is correlated to near-bottom orbital velocities and friction coefficients over sand and the model dead matte are computed.

## 2. Material and methods

### 2.1. Study sites

The study was carried out in four shallow (depth less than 8 m) locations on the South coast of Mallorca Island, Western Mediterranean sea (Fig. 1a). Cala D'Or was chosen for this study because it is the only location in the Balearic Islands where *Caulerpa taxifolia* is present. *Caulerpa racemosa* is mainly found in the South of Mallorca, and the locations of Sant Elm, Cala Estancia and Portals Vells were chosen (Fig. 1b). Sand patches, rocky reefs covered by an erect stratum of photophilous macroalgae and meadows of the seagrass *Posidonia oceanica* are the main components of the undersea landscape at the study sites.

### 2.2. Presence of *Caulerpa* over different substrata

The cover of *Caulerpa taxifolia* in Cala D'Or and that of *Caulerpa racemosa* in Cala Estancia and Portals Vells was measured during two consecutive summers and winters (years 2007 and 2008) which represent the periods of maximum and minimum vegetative development of these species in the Western Mediterranean (Piazzini and Cinelli, 1999). Four to eight 20-m long transects were laid haphazardly at each location and the percentage of substratum covered by *Caulerpa* was estimated at 1-m intervals along the

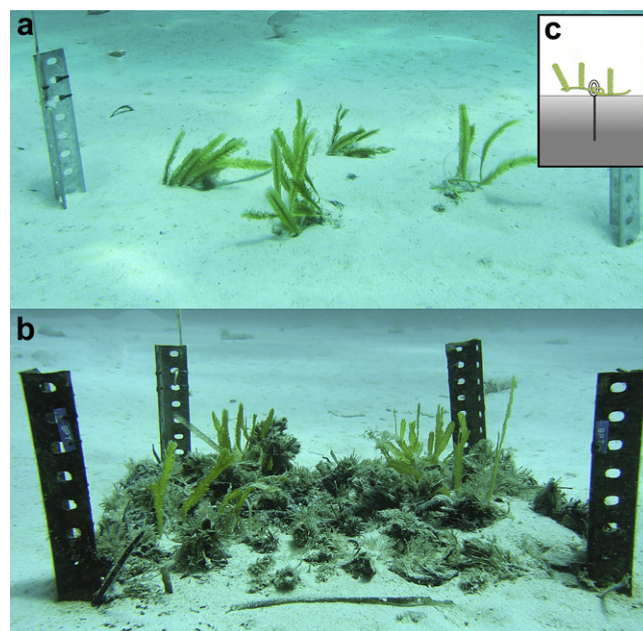


Fig. 2. Photographs of the experimental set up for *Caulerpa taxifolia* in a) natural sandy bottom and b) the model of dead matte of the seagrass *Posidonia oceanica*. c) Illustration of *Caulerpa* fragments fixed to the plots using pickets.

transects by placing two 20 cm × 20 cm quadrats divided in 25 cells of 4 cm<sup>2</sup>. The number of cells corresponding to each type of substratum in each quadrat was counted as well as the number of cells of each substratum type colonized by *Caulerpa*. Substrata were classified either as sand, rocks (always covered by photophilic algae), *Posidonia oceanica* meadow or dead matte of *P. oceanica*. The matte of *P. oceanica* is formed by the accumulation of sediment, rhizomes and/or roots of this seagrass species over time, building a terraced structure that retains the sediment even after seagrass death and that may last hundreds of years (Mateo et al., 1997).

### 2.3. Short-term experiment evaluating substratum effect on *Caulerpa* persistence

The experiment evaluated the persistence of *Caulerpa* fragments planted in sand plots (Fig. 2a) and in plots of a model of *Posidonia oceanica* dead matte (Fig. 2b). The experiment was performed in two

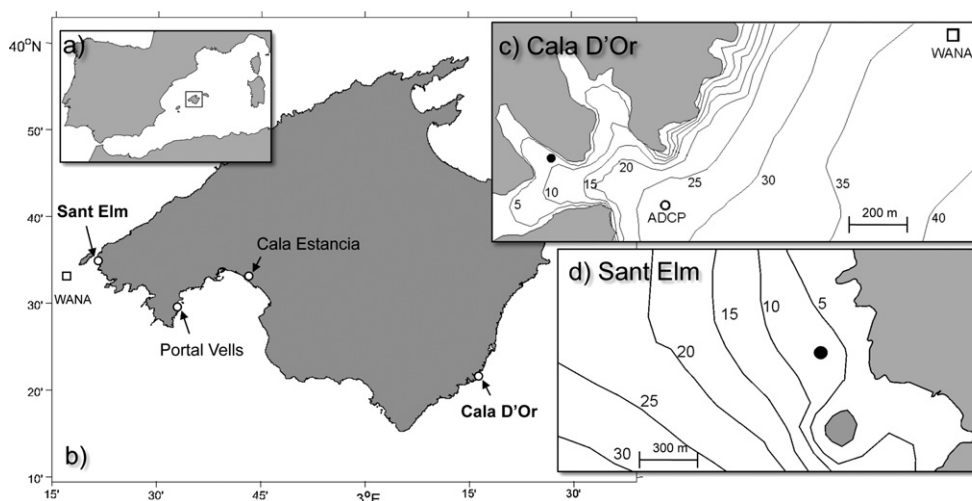


Fig. 1. Location of the study areas. (a) Mallorca Island in the Mediterranean Sea. (b) Location of the four study areas and deep water wave data (WANA nodes). (c) Bathymetry of Cala D'Or with location of WANA node. (d) Bathymetry of Sant Elm. ● Experimental plots, □ WANA nodes and ○ ADCP location in Cala D'Or.

locations: Cala D'Or for *Caulerpa taxifolia* and Sant Elm for *Caulerpa racemosa* (Fig. 1b). We did so because *C. racemosa* is not present in Cala D'Or, which is the only place where *C. taxifolia* is present in the Balearic Islands, and we did not consider appropriate to contribute to the dispersion of these invasive species by transplanting them to other locations. Besides, we did not perform the *C. racemosa* experiment in Cala Estancia or Portals Vells because these sites are highly visited by swimmers and snorkelers and the risk of disturbance to experimental plots was high. A sand patch large enough to hold all the experimental plots at a depth of 7–8 m was selected at each location. These sand patches were surrounded by *P. oceanica* meadows.

Six 50 cm × 50 cm plots were established in each location separated every 5 m. Plots were delimited at the corners with galvanized iron bars. The model of dead matte of *Posidonia oceanica* was constructed by attaching fragments of dead *P. oceanica* rhizomes collected from fresh beach-cast plants after a storm to 50 cm × 50 cm squares of plastic gardening mesh, using plastic cable ties (Fig. 2b). A density of around 500 vertical rhizomes per m<sup>2</sup> was used as this is a typical *P. oceanica* shoot density found at 8 m depth in clear Mediterranean waters (Marbà et al., 2002; Gobert et al., 2003; Terrados and Medina-Pons, 2008). A model of dead matte of *P. oceanica* was used instead of natural occurring dead matte to standardize the methodology having the same substratum types in both locations. The dead matte models were attached to the bars delimiting the randomly assigned plots ( $n = 3$ ) with cable ties and using four pickets to anchor it firmly to the sediment (Fig. 2b). The sand plots ( $n = 3$ ) were made of the naturally occurring sand and placement of the galvanized iron bars delimiting plot corners was the only manipulation performed.

Fragments of *Caulerpa* including at least one stolon apex and 3–4 fronds each were manually collected from the same area and depth where the experimental plots were located and held in aerated seawater at sea surface until planting. Sixteen *Caulerpa* fragments were fixed to each plot within 2 h after collection by threading them in groups of four into four cable-tie loops anchored in each plot using galvanized iron pickets (see Fig. 2c). The experiment started in July of 2007 as previous studies on biomass seasonality of *Caulerpa taxifolia* in shallow waters (6–10 m) have shown that maximum biomass occurs in summer and autumn (Thibaut et al., 2004). Monitoring of *Caulerpa* cover in each plot was carried out approximately every 30 days for *C. taxifolia* and 15 days for *Caulerpa racemosa* as the latter species has a higher growth rate (Piazzi et al., 2001) using underwater digital photography. Photographic sampling techniques have been previously used to measure *C. racemosa* cover (Piazzi et al., 2003). In this study, underwater photographs were taken to evaluate the persistence of *Caulerpa* in the plots by analyzing the area covered by the algae over time. This method allowed taking many samples in the field as diving time is limited. Each plot was divided on 4 sub-plots of 25 × 25 cm to improve the delineation of the algae on the photographic images. As algae can swing with wave orbital movements that could affect the area facing up on the images, three images of each sub-plot were taken on calm days. A scale was placed on each sub-plot next to the algae when taking the underwater photographs. Open software (ImageJ, NIH) was used to measure the area of *Caulerpa* on each photograph. To determine the absolute area on the images a relationship between image pixels and real dimensions ratio was established on each image using the scale. In order to test the method, photographs were taken in the laboratory and in the sea before the experiment began. A set of figures of known areas was used to test the output of the image processing analysis, providing an error around 6%.

#### 2.4. Wave climate and modelling at the short-term experimental sites

The propagation of the deep water mean wave climate between sampling dates was performed using a numerical model which

allows the calculation of the wave induced near-bottom orbital velocity at the study sites. Significant wave height ( $H_s$ ), peak period ( $T_p$ ) and direction ( $\theta$ ) were acquired from July 2007 to March 2008 at deep water which corresponds to the duration of the short-term experiment (see Fig. 1b–c for the location of these points). The deep water data have been obtained from the WANA system, a reanalysis with real data of a third generation spectral WAM model and was provided by the Spanish harbour authority operational system. These deep water wave conditions were propagated to the study sites using a numerical model based on the mild slope parabolic approximation (Kirby and Dalrymple, 1983) and near-bottom orbital velocities ( $U_b$ ) at the experimental locations were obtained from the model outputs using linear wave theory (Infantes et al., 2009). These models are widely used in engineering and scientific approaches in order to obtain the accurate wave field in coastal areas from open sea conditions since refraction and diffraction are well captured (Alvarez-Ellacuria et al., 2010).

#### 2.5. Field wave measurements and velocity profiles

To test the results of deep water wave propagations, field measurements were performed on the 8th of November of 2007 in Cala D'Or measuring wave conditions ( $H_s$  and  $T_p$ ) at deep and shallow water as well as near-bottom velocities in shallow water on both substrata. An Acoustic Doppler Current Profiler (ADCP) RDI, Sentinel 600 kHz, was deployed at approximately 400 m from the experimental site at a depth of 26 m. The ADCP was set to record 20 min burst intervals every 60 min with a 2 Hz frequency. It was mounted on a tripod at 0.5 m above the bed.  $H_s$  obtained by propagating the deep water conditions to shallow waters, are within a ~15% error to those measured in situ at deep and shallow waters, showing the accuracy of modelled wave conditions. Table 1 shows the data provided by the numerical model and those measured by the ADCP at deep water (26 m) and with the ADV at shallow water (7.5 m) on the 8th of November of 2007.

Additionally, vertical profiles of velocity were measured on the *Posidonia oceanica* dead matte model and on sand using Acoustic Doppler Velocimeters (ADV), Vector, Nortek. Simultaneous measurements on both substrata were performed using two ADVs at seven positions (2.5, 5, 10, 20, 40, 60 and 100 cm above the bottom). The instruments were set to measure during 25 min with a frequency of 32 Hz, a nominal velocity range of 0.1 m/s and a sampling volume of 11.8 mm. The ADVs were mounted in a down-looking position on stainless steel bottom fixed structures. Stability of the equipment was verified with the compass, tilt and roll sensors. Raw data were processed by first filtering for low beam correlation (<70%). Velocity data were then filtered with a low pass filter to remove high frequency Doppler noise (Lane et al., 1998). Horizontal velocity was calculated as the root mean square (rms). From the velocity profiles, friction coefficients  $C_f$  over sand and dead matte substrata were computed following Simarro et al. (2008).

$$C_f = \frac{\tau_b}{\rho u_c^2} \quad (1)$$

where  $\tau_b$  is the bottom shear stress,  $\rho$  the density of seawater and  $u_c$  a characteristic velocity outside the boundary layer. The bed shear stress can be found from,

**Table 1**

Wave heights from model outputs and field measurements in Cala D'Or (means ± 95% CL).

		Model outputs	Field measurements
Hs	Deep water (26 m)	0.53 ± 0.02 m	0.47 ± 0.03 m
	Shallow water (7.5 m)	0.26 ± 0.02 m	0.21 ± 0.02 m



$$\tau_b = \rho \left( \nu_T \frac{du}{dz} \right)_{z=\delta} \quad (2)$$

where  $\nu_T$  is the eddy viscosity and  $du/dz$  the vertical variation of the velocity evaluated at the outside of the boundary layer  $\delta$ .

### 2.6. Water temperature and light at the short-term experimental sites

Temperature HOBO StowAway Tidbit® loggers were moored next to the plots in Cala D'Or and Sant Elm during the experiment. Temperature was recorded every 2 h and averages of water temperature between sampling dates were calculated. The numbers of daylight hours were obtained from the US Naval Observatory (<http://aa.usno.navy.mil/>) for the study areas and averages of daylight hours between sampling dates were also calculated.

### 2.7. Statistical analysis

A Chi-squared test ( $\chi^2$ ) was used to evaluate if the colonization of benthic communities by *Caulerpa* species depends on the type of substratum, that is, if some substrata are more favourable/unfavourable than others for the colonization of these species. The null hypothesis of the test assumes substratum-independent, random colonization and allows the calculation of expected cover of *Caulerpa* species in the different substrata that is compared with the cover observed. Changes in cover of *Caulerpa* species through time in the short-term experiments were evaluated using univariate repeated-measures ANOVA. "Substratum" (sand versus model of dead matte of *Posidonia oceanica*) was the between-subjects factors (fixed) and "Time" was the within-subjects factor (random) for both *Caulerpa* experiments. A significant Time  $\times$  Substratum interaction was to be expected if the type of substratum has an effect on the persistence of *Caulerpa* in the plots. A Cochran's C-test was used to test for heterogeneity of variances.

## 3. Results

*Caulerpa taxifolia* was more abundant in the dead matte of *Posidonia oceanica* than in the rest of substratum types available in Cala D'Or (Fig. 3a). *Caulerpa racemosa* was also more abundant in

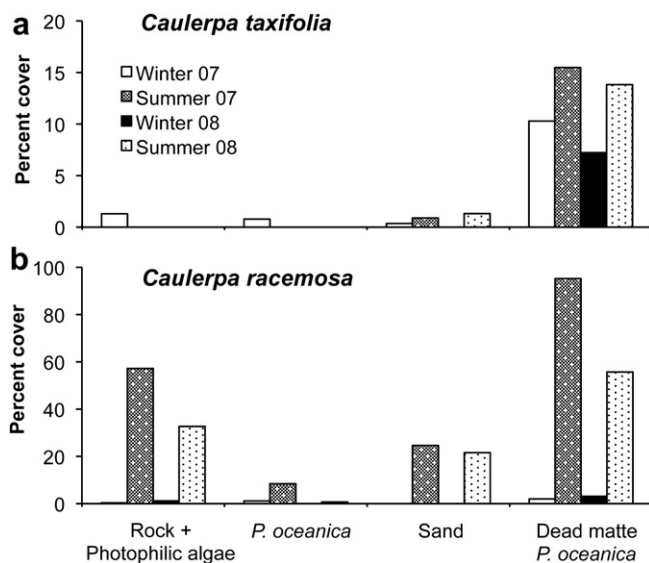


Fig. 3. Percent cover of (a) *Caulerpa taxifolia* and (b) *Caulerpa racemosa* in the different types of substratum during two consecutive years.

the dead matte of *P. oceanica* but it additionally colonized other substrata such as rocks with photophilic algae and sand (Fig. 3b). The percent cover of *C. taxifolia* over dead matte showed small changes between summer and winter (Fig. 3a). In contrast, the percent cover of *C. racemosa* was highly seasonal since large differences between summer and winter were observed (Fig. 3b).

The relative frequencies of the different types of substratum in the total surface of sea bottom surveyed and of the presence of *Caulerpa* on each substratum are shown in Fig. 4 for each sampling event. Different distribution of frequencies indicates that *Caulerpa* species do not distribute randomly in the substrata (Table 2). Results from the  $\chi^2$  test show that both *Caulerpa* species are found in the dead matte of *Posidonia oceanica* and in rock on a higher proportion than expected in relation to the amount of substratum type available at the study sites. In contrast, *Caulerpa* species are found in a lower proportion than expected in sand and inside the *P. oceanica* meadow. Experimental  $\chi^2$  were largely above critical values (Table 2).

The significant "Time  $\times$  Substratum" interaction in both species (Table 3) indicates that substratum type affected the persistence of the two *Caulerpa* species. The temporal evolution of *Caulerpa* cover in the plots (Figs. 5a and 6a) shows that the persistence of *Caulerpa* was higher in the model of dead matte of *Posidonia oceanica* than in sand. *Caulerpa taxifolia* cover on dead matte increased until the 28th of September while *C. taxifolia* cover on sand did not change during the same period of time (Fig. 5a). After the 28th of September, *C. taxifolia* cover decreased with time in both substrata and by the end of winter no *C. taxifolia* was present in the plots. The cover of *Caulerpa racemosa* in the model of dead matte increased during the first five weeks of the experiment while on sand was roughly maintained (Fig. 6a). After August 30th, the *C. racemosa* experiment was disturbed by a storm that buried the plots with a 30–70 cm thick layer of drifting macroalgae and *P. oceanica* leaves causing the death of all *C. racemosa* fragments in the plots.

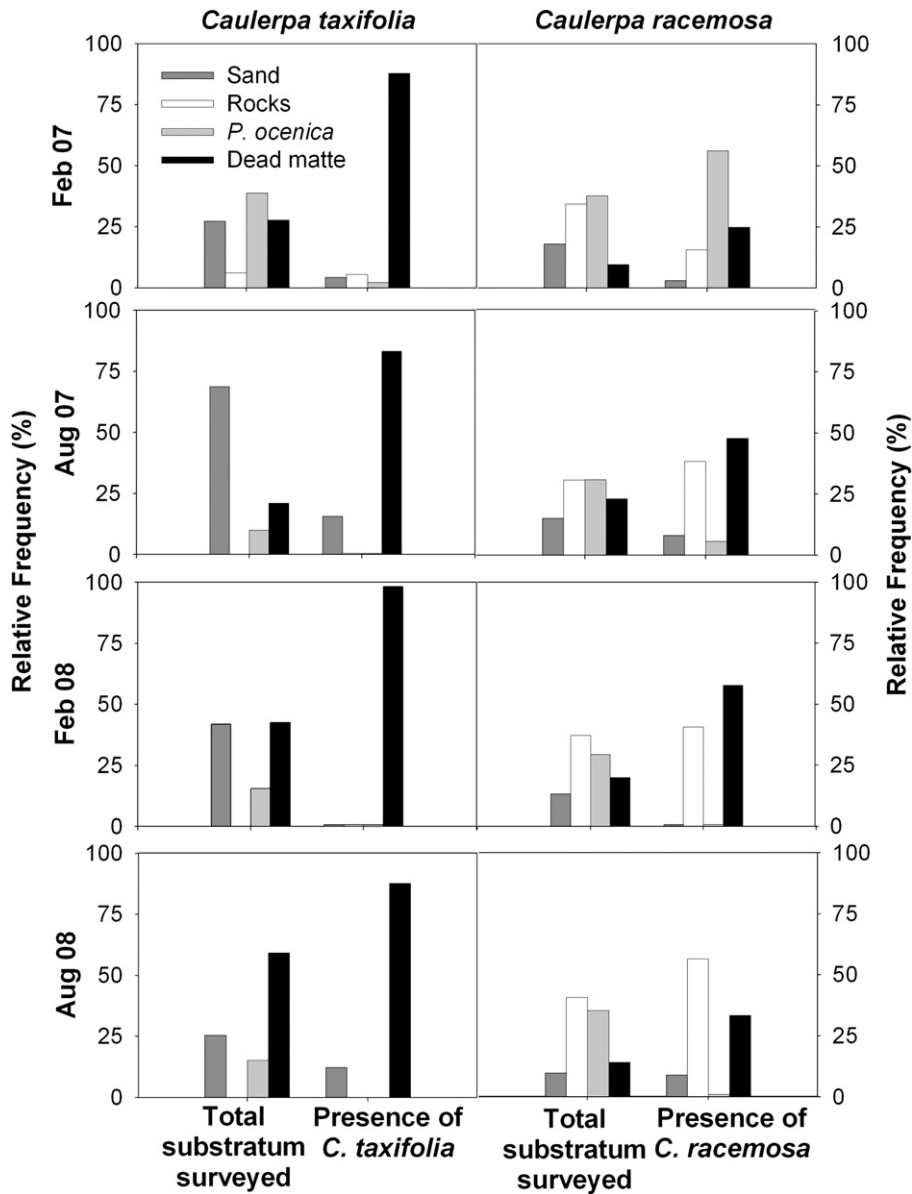
Seawater temperature in Cala D'Or (*Caulerpa taxifolia* site) changed from 26 °C in July to 14 °C in March, while the number of daylight hours was reduced from 14.5 h to 9 h (Fig. 5b). Conditions in Sant Elm (*Caulerpa racemosa* site) during the experiment were rather constant with a seawater temperature of 26 °C and the number of daylight hours between 14 and 13 h (Fig. 6b).

*Caulerpa taxifolia* cover was maintained or increased with estimated near-bottom orbital velocities below 15 cm s<sup>-1</sup> and declined above that value (Fig. 5b). *Caulerpa racemosa* was also able to maintain or increase its cover at the same velocity values (Fig. 6b). The vertical profiles of velocity indicate that the width of the boundary layer is around 10 cm (Fig. 7) showing also a significantly higher reduction of the horizontal rms velocity in the model of dead matte of *Posidonia oceanica* (1.5 ± 0.2 cm s<sup>-1</sup>, mean ± 95% CL) than in sand (2.2 ± 0.2 cm s<sup>-1</sup>, mean ± 95% CL), (*t*-test: *t* = 115, *df* = 103598, *p* < 0.01).

The friction coefficient computed for the dead matte model was higher (1.04 E-01) than for sand (2.33 E-02). Increased friction by the dead matte model would reduce the magnitude of the velocities compared to sand. Wave data from the ADCP showed that wave conditions did not change during the ADV profile measurements, with *H*<sub>s</sub> of 0.5 ± 0.1 m, *T*<sub>p</sub> of 9.5 ± 0.5 s and South East direction.

## 4. Discussion

The results of this study indicate that substratum plays an important role in the distribution of these invasive *Caulerpa* species. Field measurements show that the presence of *Caulerpa taxifolia* and *Caulerpa racemosa* on the substrata is not at random.



**Fig. 4.** Relative frequency of total substratum surveyed and of the presence of *Caulerpa taxifolia* and *Caulerpa racemosa* on each substratum. Low presence of rocks was recorded at the *C. taxifolia* site. Rocky bottom with photophilic algae is indicated as Rocks.

Dead matte of the seagrass *Posidonia oceanica* and rock covered with photophilic algae are more favourable than sand to the presence of *Caulerpa* species. Experimental results show that substratum has a significant effect on the persistence of both

*Caulerpa* species and confirm that is higher in the model of dead matte of *P. oceanica* than in sand. Hence, the results of both analyses are consistent to each other and suggest that the type of substratum influences the abundance of these invasive *Caulerpa* species.

**Table 2**  
Chi-square ( $\chi^2$ ) test of observed *Caulerpa* cover on different substrata. *Caulerpa taxifolia* has a critical  $\chi^2_{p=0.001***, 2df} = 13.81$  and *Caulerpa racemosa* has a critical  $\chi^2_{p=0.001***, 3df} = 16.26$ . *C. taxifolia* was not found on rock substratum in enough quantities for the test. The type of contribution was classified as more than expected (M) or less than expected (L).

		Rock	<i>P. oceanica</i>	Sand	Dead matte <i>P. oceanica</i>	$\chi^2$ Experimental
<i>C. taxifolia</i>	Winter 07	–	64 L	36 L	240 M	340***
	Summer 07	–	20 L	79 L	366 M	465***
	Winter 08	–	23 L	65 L	121 M	209***
	Summer 08	–	77 L	32 L	69 M	178***
<i>C. racemosa</i>	Winter 07	3 -	3 -	7 M	7 M	20***
	Summer 07	80 M	897 L	142 L	1153 M	2272***
	Winter 08	1 M	34 L	16 L	91 M	142***
	Summer 08	146 M	796 L	2 L	629 M	1573***

**Table 3**

Results of repeated measures of ANOVA performed to evaluate if the cover of *Caulerpa taxifolia* and *Caulerpa racemosa* was different between a model of dead matte of *Posidonia oceanica* and a sandy substratum (Subst.). \* $p < 0.05$ , \*\*\* $p < 0.001$ , ns = not significant. Cochran tests were not significant.

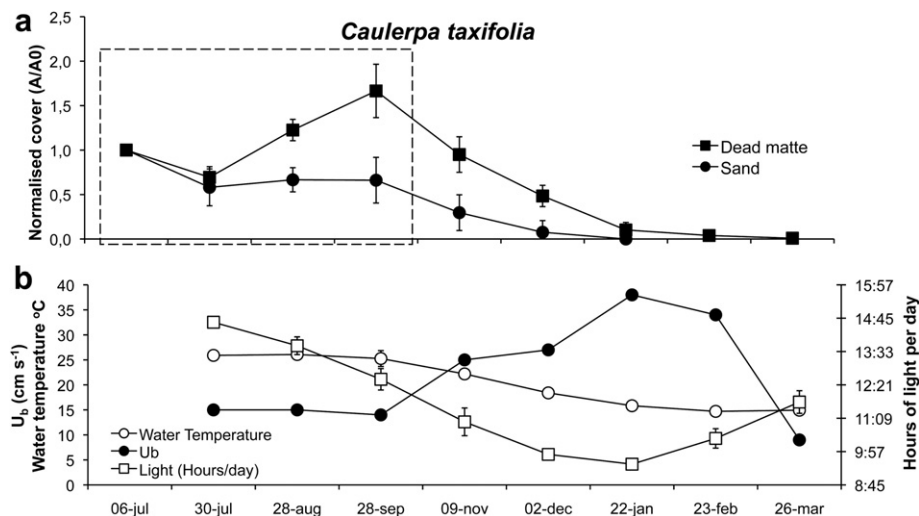
	df	MS	F	p
<i>Caulerpa taxifolia</i>				
Intercept	1	469997.4	293.6	0.0000***
Subst.	1	17685.9	11.0	0.0292 ns
Error	4	1600.7		
Time	3	5580.2	2.9	0.0749 ns
Time × Subst.	3	7086.1	3.7	0.0410*
Residual	12	1884.1		
<i>Caulerpa racemosa</i>				
Intercept	1	41205.3	36.5	0.0037***
Subst.	1	8321.6	7.4	0.0532 ns
Error	4	1128.9		
Time	3	1790.3	4.6	0.0231*
Time × Subst.	3	1929.1	4.9	0.0184*
Residual	12	390.2		

Understanding the factors that regulate the establishment and spread of introduced *Caulerpa* species is important to predict future pathways of invasion as well as the susceptible areas to be invaded (Carlton and Geller, 1993; Bax et al., 2003; Cebrian and Ballesteros, 2009). Previous studies mainly based on direct observation indicated that favourable substrata for the establishment of *Caulerpa* spp. are dead matte of *Posidonia oceanica* (Piazzi and Cinelli, 1999; Piazzi et al., 2001; Ruitton et al., 2005) and rocky bottoms covered by turf algae (Ceccherelli et al., 2002; Piazzi et al., 2003; Bulleri and Benedetti-Cecchi, 2008). A propagation model of *Caulerpa taxifolia* assumed that the settlement probabilities of this species were high for dead matte, harbour mud, and rocks with photophilic algae and low for *P. oceanica* and unstable sand (Hill et al., 1998) but no explanation of how these probabilities were set is given. Our study provides the first quantitative evaluation of previous descriptions of dead matte of *P. oceanica* as being a favourable substratum for the colonization of invasive *Caulerpa* species. It also provides quantitative evidence that rocks covered with photophilic algae are also a favourable substratum for *Caulerpa racemosa* colonization. Sand and *P. oceanica* meadows are less favourable than the dead matte of *P. oceanica* and rocks covered with photophilic algae for the colonization of *C. taxifolia* and *C. racemosa*.

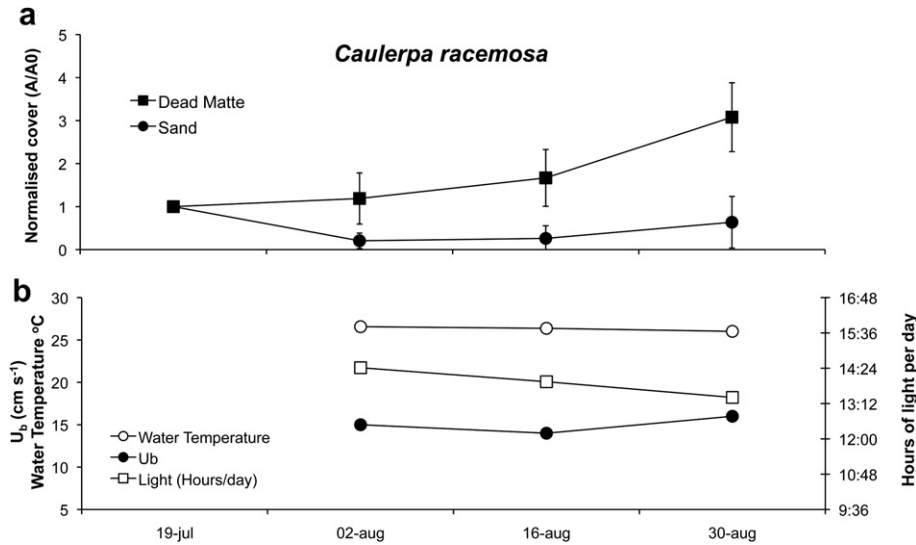
The positive association between consolidated substrata (rocks and dead dead matte) and the presence of invasive *Caulerpa* species suggests that near-bottom orbital velocity may be an important determinant of the spatial distribution of these species at shallow depths. The capacity of *Caulerpa* to acquire nutrients from the sediment through the rhizoids (Williams, 1984; Chisholm et al., 1996) might be invoked to explain the preferential distribution of invasive *Caulerpa* on dead matte of *Posidonia oceanica* but not over rocky substrata where the amount of sediment is much lower than in dead matte. The structural complexity of the substratum facilitates the retention of *Caulerpa taxifolia* fragments (Davis et al., 2009) and could be a factor to explain why rocky substrata covered by an erect layer of photophilous macroalgae and the *P. oceanica* dead matte are favourable substrata for the establishment of *Caulerpa*. The calculated friction coefficients for the dead matte and sand indicate that the surface of the dead matte is structurally more complex than sand and that *Caulerpa* fragments established on it will experience lower flow velocities than in sand.

*Caulerpa taxifolia* and *Caulerpa racemosa* were rarely observed inside the *Posidonia oceanica* meadow in this study and the  $\chi^2$  test shows that *P. oceanica* is not a favourable substratum for the establishment of the introduced *Caulerpa* species even though the matte of the *P. oceanica* meadow is a favourable substratum. Previous studies have also described that dense *P. oceanica* meadows are not invaded by *C. taxifolia* and *C. racemosa* in the short-term (Ceccherelli and Cinelli, 1999; Ceccherelli et al., 2000). Dense *P. oceanica* meadows appear to resist the invasion of *C. taxifolia* for more than eight years (Jaubert et al., 1999). Our results suggest that degraded, low-density *P. oceanica* meadows would be suitable locations for the settlement and growth of invasive *Caulerpa* species if shading by the leaf canopy was the main constraint of *Caulerpa* invasion (Ceccherelli and Cinelli, 1999; Ceccherelli et al., 2000), and thus protecting existing meadows would likely reduce the spread of these species in the Mediterranean Sea.

There has been little effort to correlate wave and current energy with the spatial distribution and growth of invasive algae. The effects of wave exposure, morphology and drag forces of the invasive *Codium fragile* were studied under field and flume conditions by D'Amours and Scheibling (2007). These authors suggest that the *C. fragile* became dislodged at flow velocities exceeding  $50 \text{ cm s}^{-1}$ . Moreover, Scheibling and Melady (2008) found that a sheltered location was a more suitable habitat than an exposed location for this species. The effect of wave exposure on *Caulerpa*



**Fig. 5.** (a) *Caulerpa taxifolia* cover (normalised means  $\pm$  SE,  $n = 3$ ). (b) Water temperature at the experimental site, hours of light per day and near-bottom orbital velocities ( $U_b$ ) (means  $\pm$  SD). Cover area on each sampling date (A) in  $\text{cm}^2$  normalised by initial cover area ( $A_0$ ). Dotted square indicates the period used for the statistical analysis ANOVA, Table 3.



**Fig. 6.** (a) *Caulerpa racemosa* cover (normalised means  $\pm$  SE,  $n = 3$ ). (b) Water temperature at the experimental site, hours of light per day and near-bottom orbital velocities ( $U_b$ ) (means  $\pm$  SD). Cover area on each sampling date (A) in  $\text{cm}^2$  normalised by initial cover area (A0).

*racemosa* its not clear since it can be found in both exposed and sheltered locations (Thibaut et al., 2004; Klein and Verlaque, 2008). To date, there are no available data on near-bottom orbital velocities that allow the establishment and development of invasive *Caulerpa* species. Our results show that *Caulerpa taxifolia* and *C. racemosa* do only occur at near-bottom orbital velocities below  $15 \text{ cm s}^{-1}$  and that *C. taxifolia* cover could decline at velocities above that value. This value, however, should not be considered as

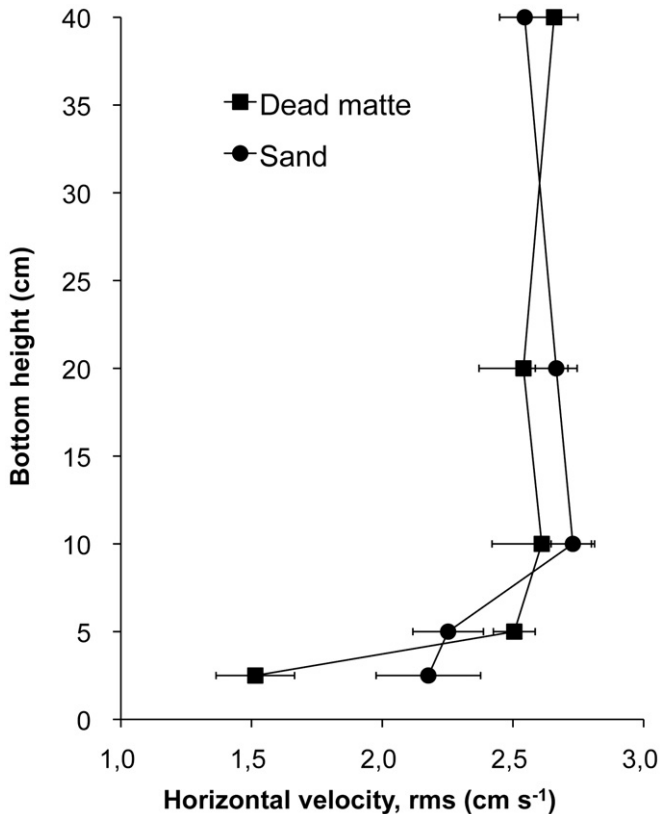
a velocity threshold for the persistence of *C. taxifolia* because the evidence provided by our study is correlative only and other factors such as seawater temperature and light availability declined as velocities increased in the experimental site (see Figs. 5b and 6b).

We observed that *Caulerpa racemosa* was able to colonize sand in summer but disappeared in winter, a season with higher wave energy. One of the potential problems for aquatic macrophytes growing on unconsolidated substrata is sediment movement and deposition that causes uprooting and burial (Fonseca and Kenworthy, 1987; Williams, 1988; Cabaço et al., 2008). Previous works described that *C. racemosa* was not colonizing fine sand with large ripple marks (Ruitton et al., 2005). Sand with large ripples indicates that orbital velocities are or have been high enough to mobilize the sediment suggesting that only those macrophytes with high anchoring capacity could survive in that location.

Our results have provided novel quantitative evidence highlighting the importance of substratum for the distribution of invasive *Caulerpa* species at shallow depths. We have shown that the two introduced species of *Caulerpa* in the Mediterranean Sea are more abundant in dead matte of *Posidonia oceanica* or rock than inside the *P. oceanica* meadow or sand. Our results also suggest the quantitative hydrodynamic conditions that shape colonization as their cover started to decline at near-bottom orbital velocities over  $15 \text{ cm s}^{-1}$ . Further studies should be done to elucidate the validity of this velocity threshold of the colonization of introduced *Caulerpa*.

#### Acknowledgments

E. Infantes would like to thank financial support from the Spanish Ministerio de Educación y Ciencia (MEC) FPI scholarship program (BES-2006-12850). Research funds were provided by MEC grants CTM2005-01434/MAR, CTM2006-12072/MAR and by the Govern of the Balearic Islands, project Integrated Coastal Zone Management (UGIZC). The authors thank Puertos del Estado for WANA wave climate data and the Marina of Cala D'Or (Santanyi) which kindly made available its harbour facilities for executing this study. We thank Drs. G. Zarruk and G. Simarro for their advice in the velocimeter data. Thanks to B. Casas, F. Medina-Pons, I. Castejón and T. Box for field assistance.



**Fig. 7.** Vertical profiles of velocity (means  $\pm$  95% CL). For convenience only the 40 cm above the bottom is shown.



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