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RESEARCH ARTICLE

Experimental Evaluation of the Restoration Capacity of a Fish-Farm Impacted Area with *Posidonia oceanica* (L.) Delile Seedlings

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Abstract

Marine aquaculture is an activity that has induced severe local losses of seagrass meadows along the coastal areas. The purpose of this study was to evaluate the capacity of an area degraded by fish-farm activities to support *Posidonia oceanica* seedlings. In the study site, a bay in the southeast coast of Spain where part of a meadow disappeared by fish-farm activities, seedlings inside mesh-pots were planted in three areas. Two plots were established in each area, one in *P. oceanica* dead matte and another inside a *P. oceanica* meadow. To evaluate if sediment conditions were adequate for the life of the seedlings, half of them were planted in direct contact with the sediment and the other half were planted above the surface of the sediment in each plot. Monitoring during 1 year showed that there were large differences in seedling survival between the dead matte and the *P. oceanica* meadow. While seedlings planted in dead matte had a high survivorship after 1 year (75%), seedlings planted in *P. oceanica* progressively died (survivorship of 20% after 1 year). The average leaf length of the seedlings surviving in the two substrata was not different, but the leaf area per seedling was lower in the seedlings growing inside the *P. oceanica* meadow during most part of the year. Seedling survivorship and vegetative development were not affected by the level of planting and suggest that the sediment conditions are adequate for the life of *P. oceanica* seedlings.

Key words: **fish-farm, planting level, *Posidonia oceanica*, seedlings, substratum type, survival, vegetative development.**

Introduction

Seagrasses are key components of shallow coastal ecosystems for their contribution to biological productivity and the maintenance of biodiversity, the control of water quality, and the protection of the shoreline (Hemminga & Duarte 2000). Seagrass populations have decreased due to the impact caused by human activities such as coastal development, pollution, and trawling (Boudouresque et al. 2009). The increasing demand for marine products by human population has promoted the growth of marine aquaculture, which is the fastest growing animal food-producing sector (FAO 2009). Coastal fish aquaculture has contributed to the decline of seagrass populations (Holmer et al. 2003*a*). For this reason, there is a growing concern about the environmental impact of marine aquaculture (Naylor et al. 2000) and as a result severe environmental impacts have been documented regarding net cage production (Hall et al. 1990, 1992; Holmer & Kristensen 1992; Karakassis et al. 2000; Holmer et al. 2002, 2003*a*).

The loading of organic matter coming from fish feces and uneaten fish food is considered the main driver of habitat degradation (Pergent-Martini et al. 2006; Holmer et al. 2008). Fish-farm-derived organic matter inputs to the sediment increase sulfate reduction rates (Holmer & Frederiksen 2007) and promote the invasion of the rhizomes and roots by sulfide which is associated with reduced growth and increased mortality of seagrasses (Calleja et al. 2007; Frederiksen et al. 2007, 2008). The nutrients released by the mineralization of organic matter may also cause local nutrient enrichments and the increase of the epiphytes (Pérez et al. 2008) and, likely, of their palatability to herbivores. Shading by epiphytic overgrowth has been considered another process involved in seagrass losses in fish-farm impacted areas (Pergent-Martini et al. 2006).

Posidonia oceanica (L.) Delile forms extensive meadows in the Mediterranean Sea but its surface has declined by 5–20% during the last century. Coastal development, pollution, and trawling are considered the main causes of this loss (Boudouresque et al. 2009). Coastal fish aquaculture

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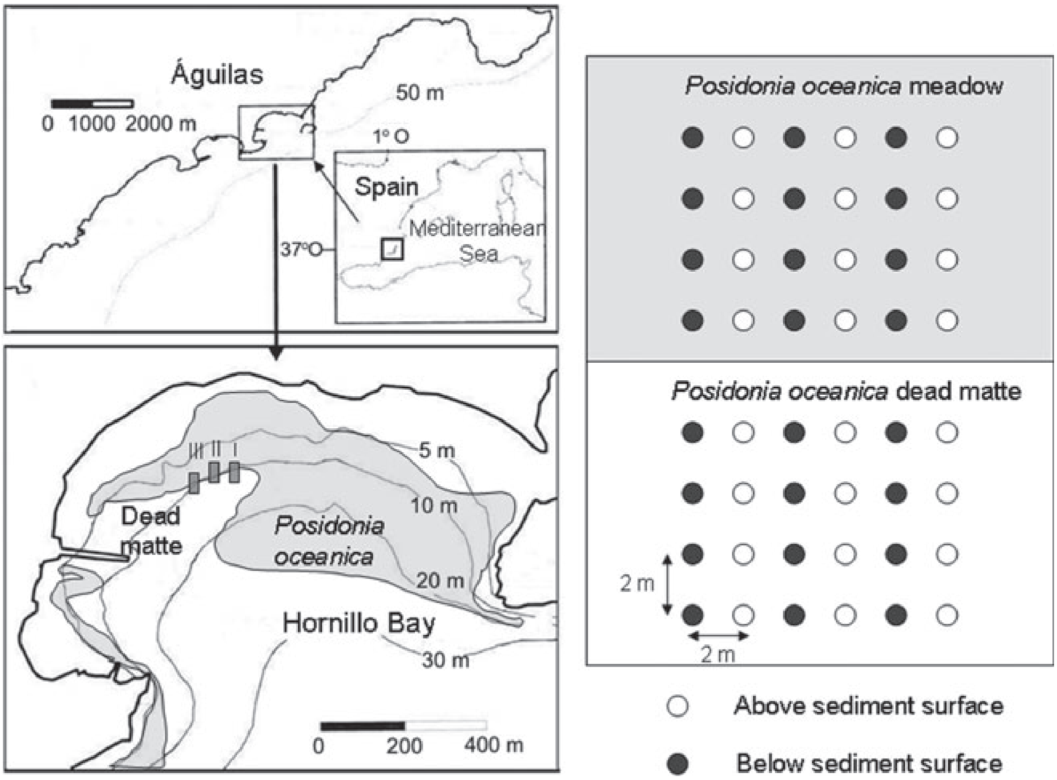

Figure 1. Hornillo Bay, Águilas, Spain. Present distribution of the *Posidonia oceanica* meadow is represented by light gray. The three study areas are represented by dark gray rectangles. Spatial arrangement of the seedlings planted in each area with indication of planting level.

is a fast growing activity in the Mediterranean that also threats *P. oceanica* (Holmer et al. 2003*b*). Indeed, reports of *P. oceanica* losses associated to fish farming are numerous (Delgado et al. 1997; Pergent et al. 1999; Ruiz et al. 2001; Cancemi et al. 2003; Pergent-Martini et al. 2006; Díaz-Almela et al. 2008) and may be observed within months of the initiation of fish-farm activities (Marbà et al. 2006; Pérez et al. 2007).

The changes in sediment biogeochemistry may persist for some years even if organic inputs are stopped and sustain further declines of *P. oceanica* (Delgado et al. 1999; Holmer et al. 2003*a*). The recovery of *P. oceanica* is also limited by its slow growth (Marbà & Duarte 1998; Kendrick et al. 2005), low flowering (Díaz-Almela et al. 2006), and high rates of fruit abortion and predation (Balestri & Cinelli 2003).

Various studies have revealed the great importance that careful habitat selection has for seagrass transplantation (van Katwijk 2009) and pilot studies have been carried out to develop effective restoration methods (Kirkman 1998). The aim of this study was to evaluate the capacity of one area impacted by fish-farming activities to support the life of seagrasses under the current environmental conditions. To that end, we planted in the impacted area *P. oceanica* seedlings cultured in the laboratory from fruits collected in nearby beaches. The seedlings were planted inside pots anchored to substratum and their vegetative development was followed for a year.

Methods

Study Site

The study was performed in Hornillo Bay, Águilas, Spain (Fig. 1), a small bay that once supported a continuous *Posidonia oceanica* meadow between the depths of 3–5 and 25 m (Calvín et al. 1989). Fish farming (*Seriola dumerili* (Risso) L., *Sparus auratus* (L.), and *Dicentrarchus labrax* (L.)) started in 1989 on the west side of the bay and by 1998, 11 ha (28% of total surface) of the *P. oceanica* meadow had been completely lost while another 10 ha (25% of total surface) were degraded (Ruiz et al. 2001). Fish farming caused a 10% reduction in light availability and increases in dissolved inorganic phosphorus (from 0.1 to 0.2–0.6 μM) and ammonium (from 3–4 to 30–40 μM), and also of the organic matter content of the sediment (from 0.5–0.9% DW to 1.8–2.4% DW). The fish-farm was progressively dismantled in three phases between 2000 and 2003. Two years after the stopping of fish farming, the concentration of organic matter and acid-volatile reduced sulfur compounds was higher in the impacted area than in a non-impacted, control site (Ramos et al. 2003; Sanz-Lázaro and Marín 2006). Where *P. oceanica* survived, the size and leaf growth rate of the shoots were up to 75% lower than those of shoots in non-impacted control sites while the herbivore pressure inferred from grazing marks on the leaves was 20% higher at least (Ruiz et al. 2001). An in situ herbivore exclusion experiment indicates that grazing by sea urchins that were

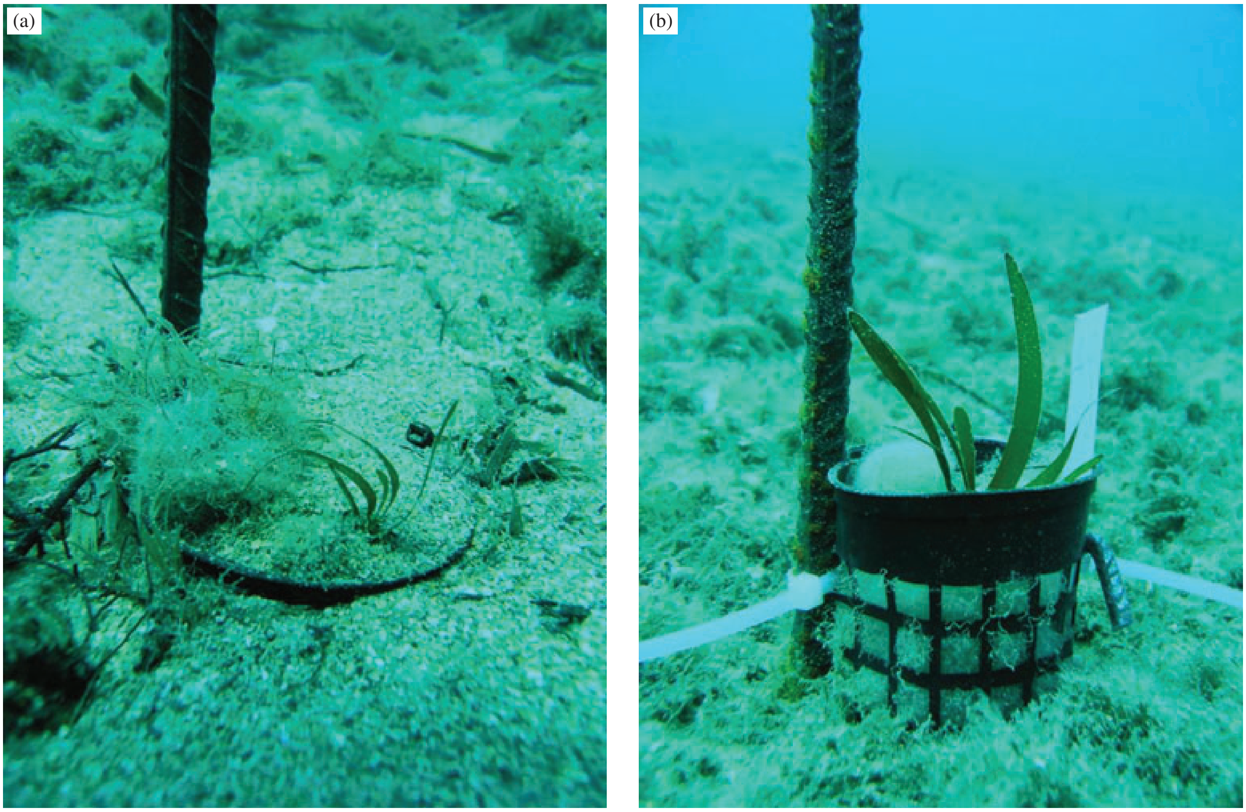

Figure 2. (a) Photograph of mesh-pot with seedling inside, totally buried in sediment of a dead matte plot. (b) Photograph of mesh-pot with seedling inside planted above the surface of the sediment in a dead matte plot.

5–59 times more abundant than previously reported for other non-impacted *P. oceanica* meadows was the major contributor to the loss of *P. oceanica* (Ruiz et al. 2009). Sea urchins have disappeared since then and no *P. oceanica* recruitment has been observed in the impacted area where the substratum is made of a dead matte, the characteristic structure that the rhizomes and roots of *P. oceanica* form.

Experimental Design

Posidonia oceanica seedlings were used to evaluate the capacity of the fish-farming impacted area to be restored under current environmental conditions. Seedlings were chosen for several reasons: (1) they are physiologically independent planting units of small size and this facilitates handling; (2) given the distance to the nearest meadows and the slow growth of the rhizomes, the recruitment of *P. oceanica* into the impacted area should proceed through seed/seedling recruitment; (3) the use of seedlings makes the estimation of plant mortality straightforward; (4) avoids the damage caused to donor meadows if fragments of adult plants were used; and also (5) the interference that the disturbance caused to the fragments after extraction might exert on the response of the planting units. The seedlings were cultured in the laboratory from fruits collected in nearby beaches that were maintained in aquaria where they germinated and produced five to six leaves and one to three roots within 2–3 months. To assure the persistence of the seedlings at the experimental site and minimize damage, to roots specially, during handling and planting each seedling was grown inside a plastic mesh-pot filled with fiberglass wool (Fig. 2). Each pot was anchored to the substratum by a 60-cm corrugated iron bar and a 30-cm galvanized iron spike fixed in opposite sides.

Two factors were evaluated: substratum type (dead matte *versus P. oceanica*) and planting level (above vs. below sediment surface). We wanted to compare the responses of seedlings when planted in a substratum where fish farming caused the complete loss of *P. oceanica* and no recruitment has occurred since then to those planted in a substratum where *P. oceanica* persisted and currently supports a well-developed, 100% cover meadow. As sediment conditions, particularly its state of reduction, may affect seagrass development (Calleja et al. 2007; Frederiksen et al. 2007, 2008), we also wanted to compare the performance of seedlings planted in direct contact with the sediment to that of seedlings planted above the surface of the sediment and consequently in a more oxic substratum. We planted the seedlings following a two-way factorial design with three replicates of each combination of factor levels. To that end, we selected three experimental areas (I, II, and III) along the current edge between the dead matte and the *P. oceanica* meadow (37°24.6′N, 1°33.4′W), and we established a "dead matte" plot and a "*P. oceanica*" plot at the two sides of the edge in each of the three areas (Fig. 1). Distance between the consecutive areas was 25 m and water depth changed from 12.1 to 8.3 m. Each plot was made up of four parallel lines of six seedlings: the distance between consecutive seedlings in a line was 2 m and the distance between lines was also 2 m. Thus a total of 24 seedlings were planted in each plot and the planting level (either below or above the sediment surface) was assigned alternatively to the seedlings along the lines, that is, there were 12 seedlings per planting level. Planting of the seedlings was performed on 28–29 July 2008.

The seedlings were haphazardly assigned to each combination of factor levels and the vegetative development of the seedlings at the time of planting was evaluated in the seedlings assigned to area I by counting the number of leaves of each seedling and the length and width of all the leaves. These measurements provide an estimation of the initial vegetative development of the seedlings in the experiment.

The cover of *P. oceanica* in each of the three "*P. oceanica*" plots was 90–100%. The density of shoots was estimated from counts of the number of shoots present in ten 30 × 30 cm quadrats placed haphazardly in each plot.

Six samples of the sediment were collected in each plot to characterize the sediment types as well as to estimate the content of organic matter. Sediments were sampled using clear acrylic tubes (length, 50 cm; internal diameter, 3.6 cm) that were inserted 40 cm into the sediment by hand. The upper 10-cm sediment layer was retained for analysis. Three core samples were used to determine the size distribution of sediment particles and were analyzed by first removing all organic matter by washing with hydrogen peroxide and deaggregating with sodium hexametaphosphate. Samples were oven-dried at 60°C for 48 hours and at 100°C for 24 hours. Samples were then sieved (mesh sizes 2, 1, 0.5, 0.25, 0.125, and 0.063 mm). Sediment type and D_{50} were calculated using the grain size analysis program GRADISTAT v4.0 (Blott & Pye 2001). The other three core samples were used to determine the organic matter content as percentage of sediment dry weight and it was assessed by loss on ignition (450°C for 6 hours) on a sediment subsample.

The vertical attenuation coefficient of light in the water column (K_d: Kirk 1983) and the shading provided by the *P. oceanica* leaf canopy were estimated by measuring PAR photon flux density immediately below the sea surface and above the canopy, and inside the canopy just above the sediment using a LiCor LI-193SA spherical quantum sensor. Photon flux density was measured at noon under clear skies and at three haphazardly chosen places within the meadow. Shoot density counts, photon flux density measurements, and sediment sampling and analysis were performed at the initiation of the experiment (July 2008).

The plots were monitored every 3 months (October 2008, January 2009, May 2009, and July 2009) since the initiation of the experiment to assess seedling survival and vegetative development. Seedling survival at each time was expressed as the percentage of seedlings surviving relative to the initial number of seedlings. The number of leaves of each seedling was counted and the length and width of each leaf of each seedling were measured to quantify the vegetative development achieved by the seedlings at each time.

Statistical Analysis

One-way ANOVA was used to evaluate the significance of the differences of *P. oceanica* shoot density between areas while differences in sediment properties (organic matter content, D_{50}) between areas and substratum type (dead matte, *P. oceanica*) were evaluated using two-way ANOVA. If ANOVA was significant, multiple comparisons were performed using Tukey HSD test. Data transformation was applied if Cochran's test indicated that variances were not homogeneous.

One-way ANOVA was also used to evaluate if the seedlings haphazardly assigned to each combination of factor levels had similar vegetative development. To that end, we compared the number of leaves, and the average leaf length and leaf area per seedling, of the seedlings ($n = 12$) assigned to each combination of factor levels in area I. Two-way factorial ANOVA was used to evaluate the significance of the effect of substratum type, planting level and the interaction of both factors on seedling survival and vegetative development at each time. Cochran's test indicated that most data variances were homogeneous. The variances of leaf length of the seedlings measured in July 2009 were heterogeneous but data transformation (logarithm and square root) did not homogenize them. Significance level was set to 0.05.

Results

Environmental Characterization

The density of *Posidonia oceanica* shoots varied between 124 and 187 shoots m^{-2} (Table 1) and was not different between the areas. The content of organic matter of the sediment varied between 2.75 and 3.95% in *P. oceanica* and between 2.2 and 3.48% of sediment dry weight in the dead matte (Table 1). There were significant differences in the content of organic matter between dead matte and *P. oceanica* and the content of organic matter in the area III was significantly lower than that in areas I and II. The average grain size of the sediment was similar in areas I and II (predominance of medium-fine sand), whereas sediment of area III was coarser. Regarding the grain size, statistical analysis (Tukey HSD test) shows that dead matte plot in area III was significantly different from the other plots. The average grain size of the sediment was also different between substratum types (*P. oceanica* vs. dead matte) being smaller in *P. oceanica.*

PAR photon flux density at the top of the *P. oceanica* leaf canopy varied between 490 and 540 μmol m^{-2} s^{-1}. The light reaching the canopy was 30.3 ± 0.9% of that at the sea surface and K_d was 0.109 ± 0.003 m^{-1}. PAR photon flux density at the surface of the sediment inside the *P. oceanica* meadow

Table 1. Depth, shoot density, organic matter content of the sediment and size distribution of sediment particles in each of the experimental plots.

	Area I		*Area II*		*Area III*		
Variables Measured (July 2008)	P. oceanica	*Dead matte*	P. oceanica	*Dead matte*	P. oceanica	*Dead matte*	*Results of ANOVA*
Depth (m)	11.4	12.1	9.6	11	8.3	9.5	
Shoot density (number of shoots m^{-2})	124 ± 14	—	187 ± 16	—	151 ± 32	—	F: 1.963, p: 0.1599
Organic matter in sediment (% DW)	3.9 ± 0.2	3.1 ± 0.2	3.5 ± 0.4	3.5 ± 0.0	2.75 ± 0.2	2.2 ± 0.1	Area: F: 13.309, p: **0.0009** Substrate: F: 5.407, p: **0.0384** Area × substrate: F: 1.821, p: 0.2038
D_{50} (μm)	253.9 ± 38.9	254.6 ± 16.8	242.2 ± 39.9	224.4 ± 7.2	369.8 ± 10.1	576.2 ± 26.4	Area: F: 49.866, p: **0.0000** Substrate: F: 8.450, p: **0.0132** Area × Substrate: F: 10.966, p: **0.0002**

Mean ± 1 SE. Result of one-way (depth) and two-way ANOVA (organic matter in sediment and D_{50}). Bold type indicates statistical significance at $p < 0.05$.

varied from 14 to 60 μmol m^{-2} s^{-1}. The shading provided by the leaf canopy was high, for only 5.8 ± 2.4% of the light at the top of the canopy reached the sediment surface inside the meadow.

Seedling Performance

During the first few months of the experiment, from July 2008 to October 2008, the seedlings experienced low mortality (Fig. 3). An effect of the substratum type on the survival of the seedlings became evident by May 2009 and confirmed by two-way factorial ANOVA. The percentage of seedlings surviving in the dead matte was higher than that in *P. oceanica* both in May 2009 ($F = 26.036$, $p = 0.0009$) and in July 2009 ($F = 68.762$, $p < 0.0001$) (Fig. 3). The percentage of seedlings surviving in dead matte was 75 ± 4.3% in May 2009 and 75 ± 4.8% in July 2009 but only 37.5 ± 6.7% and 22.2 ± 4.1%, respectively, in *P. oceanica*. Seedling survival was not affected by the planting level.

No differences of vegetative development of the seedlings assigned to each combination of factor levels in area I were detected (one-way ANOVA). The average number of leaves per seedling in this area at the initiation of the experiment was 8.4 ± 0.3 leaves, the average leaf length was 4.3 ± 0.1 cm and the average leaf area per seedling was 17.9 ± 0.8 cm^2.

The number of leaves per seedling decreased after 3 months in all combinations of factor levels and this reduction was maintained during the rest of the study (Fig. 4a). The number of leaves per seedling in dead matte after October 2008 was from 6.3 ± 0.22 to 5.6 ± 0.16, always higher than in *P. oceanica* (from 4.3 ± 0.18 to 3.4 ± 0.39) (October 2008: $F = 34.377$, $p = 0.0004$; January 2009: $F = 24.340$, $p = 0.0011$; May 2009: $F = 50.629$, $p = 0.0001$; July 2009: $F = 53.545$, $p = 0.0001$). The number of leaves per seedling was not affected by the level of planting of the seedlings.

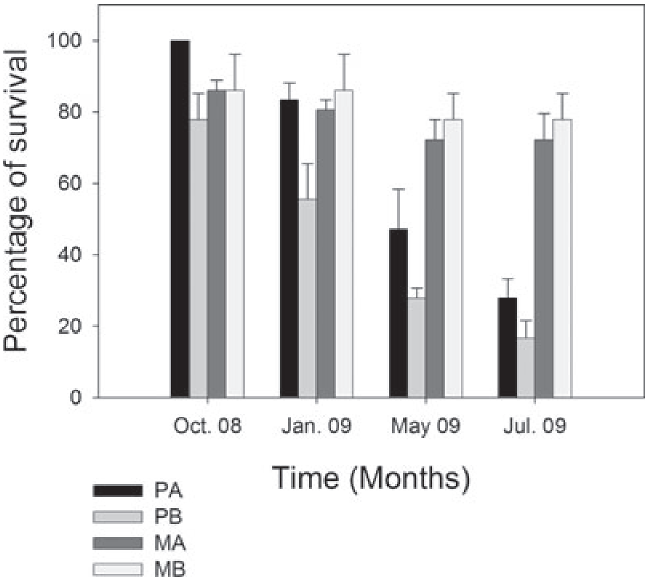

Figure 3. Survival percentage of *Posidonia oceanica* seedlings for each combination of factor levels. PA, *Posidonia oceanica*—above sediment surface; PB, *Posidonia oceanica*—below sediment surface; MA, Dead matte—above sediment surface; MB, Dead matte—below sediment surface.

The average length of the leaves increased after plantings in all combinations of factor levels (Fig. 4b) reaching a maximum of 13.0 ± 0.5 cm in May 2009. No effect of substratum type and planting level was detected. In July 2009, the average leaf length tended to decrease in the seedlings planted in dead matte but the difference with those planted in *P. oceanica* was not significant ($F = 4.244$, $p = 0.0733$).

The leaf area per seedling increased progressively in all combinations of factor levels since the initiation of the experiment, reaching the maximum surface in May 2009 (Fig. 4c). The seedlings planted in dead matte had a higher leaf area than those planted in *P. oceanica* in October 2008 ($F = 13.266$, $p = 0.0066$) and May 2009 ($F = 6.294$, $p = 0.0364$) (Fig. 4). The reduction of leaf area of the seedlings planted in dead matte from May 2009 to July 2009 turned the effect of substratum to not significant in July 2009. The level of planting had no effect on the leaf area of the seedlings at any of the measurement dates.

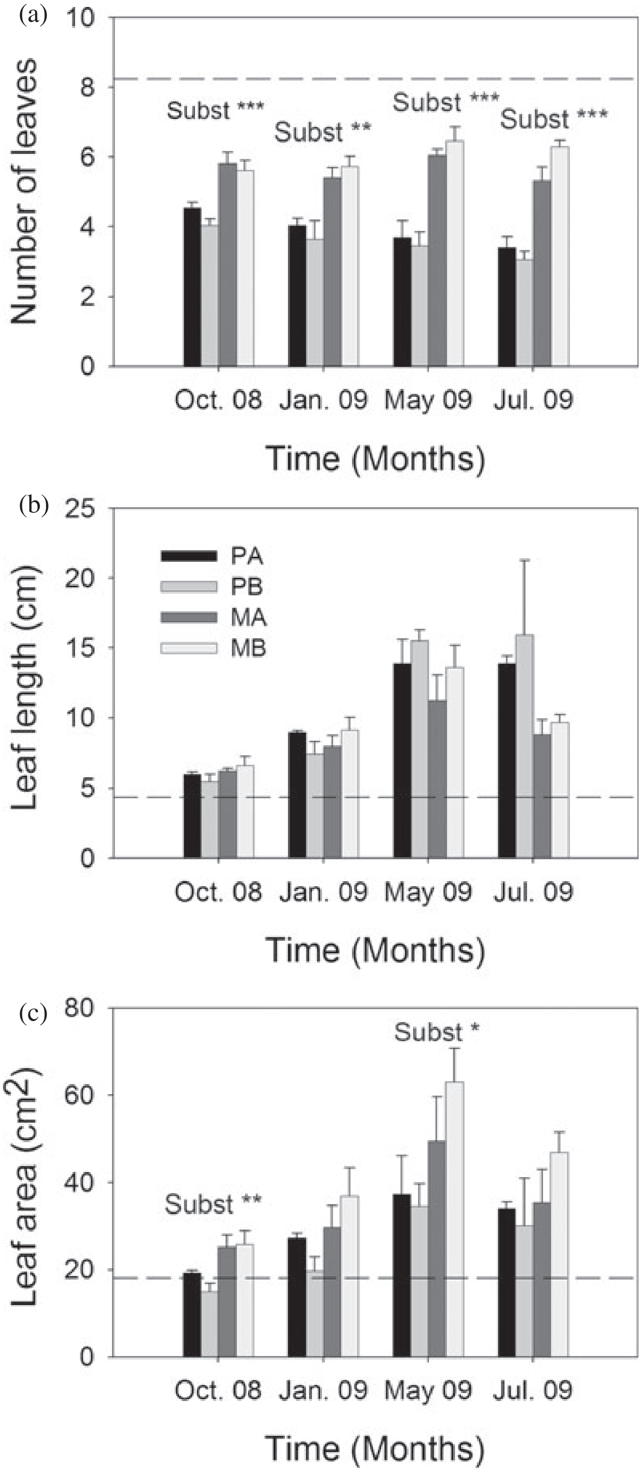

Figure 4. Vegetative development of *Posidonia oceanica* seedlings for each combination of factor levels. (a) Number of leaves per seedling, (b) average leaf length per seedling, (c) average leaf area per seedling. Initial value (M) of the seedlings measured in July 2008 are represented by the horizontal dashed line. The significance of factors is indicated by asterisks: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. PA, *Posidonia oceanica*—above sediment surface; PB, *Posidonia oceanica*—below sediment surface; MA, Dead matte—above sediment surface; MB, Dead matte—below sediment surface.

Discussion

Our results show that seedlings of *Posidonia oceanica* produced in the laboratory from beach-cast fruits and planted in a fish-farm impacted area were able to survive for 1 year. This suggests that the current environmental conditions in the impacted area make the restoration of *P. oceanica* feasible using this type of planting unit.

We found that dead matte in this area is the most adequate substratum for the development of *P. oceanica* seedlings, at least during their first year of life. After an initial mortality during the first 3 months, the percentage of seedlings surviving in dead matte remained stable for the rest of the experiment yielding a 75% survival after 1 year. On the contrary, the seedlings planted inside the *P. oceanica* meadow died progressively during the experiment so that the percentage of seedlings surviving at the end of experiment was only about 20%. The shading imposed by the leaf canopy is a possible reason to explain the low survivorship of the seedlings planted inside the *P. oceanica* meadow. Our measurements of the percentage of light reaching the top of the leaf canopy that is absorbed by it (around 95%) indicate that there is not much light available for a small seedling growing inside the leaf canopy. PAR photon flux density inside the meadow was lower than 60 $\mu m^{-2}\ s^{-1}$ and this light level is known to limit the photosynthetic rate of this species (Alcoverro et al. 1998). In the studied meadow, only 5% of the light reaching the top of the leaf canopy is available at the lower level which means that less than 1% of surface irradiance reaches the bottom. The shading we measured might be considered as maximal because it is in summer when the *P. oceanica* leaf canopy reaches the maximum development (Alcoverro et al. 1995). Shading will likely be less in other seasons what might explain why the mortality of the planted seedlings in *P. oceanica* has not been completed.

The use of early life stages in restoration is currently an important focus of interest in many parts of the world where seagrass populations have been damaged (Kirkman 1998; Reed et al. 1998; Holbrook et al. 2002; Balestri & Bertini 2003; Bull et al. 2004; Bos & van Katwijk 2007). One of the main problems of using early life stages for transplanting is the low capacity to anchor in the substratum during the first months, and consequently the high loss of seedlings (Cooper 1979; Balestri et al. 1998; Balestri & Bertini 2003). Methods have been developed for facilitating the settlement of seagrass propagules, either enhancing early root growth (Reed et al.

1998) or using artificial structures (Kirkman 1998; Harwell & Orth 1999; Holbrook et al. 2002; Bull et al. 2004). In Australia, seedlings of *Posidonia* and sprigs of *Amphibolis* were used in a pilot experiment to discover the ability of some propagules to grow after being planted (Kirkman 1998). This study showed that after about 1 year, plantings of single seedlings of *Posidonia* were not successful in either of the artificial media used (Jiffy pots and Growool blocks) because the rhizomes do not spread quickly. The planting system we used facilitated the establishment of the seedlings and assured their persistence during 1 year. It seems feasible, therefore, to consider the restoration of the meadow through the planting of seedlings inside the mesh-pots. Furthermore, this structure permits the direct contact of the roots with the sediment, allowing to evaluate if sediment conditions are adequate for seedling development.

Our results show that sediment conditions in the fish-farm impacted area do not seem detrimental for the development of *P. oceanica* seedlings because (1) seedling survival after 1 year was similar to that in other locations (Balestri et al. 1998; Piazzi et al. 1999) and (2) there was no effect of the planting level and, therefore, of the degree of contact of the seedlings with the sediment on seedling survival.

The content of organic matter of the sediment increased as a result of fish farming from less than 1% of sediment dry weight to 1.8–2.4% after 5 years of farming activities (from 1989 to 1994, Ruiz et al. 2001). The organic matter content of the sediment at the initiation of our study varied from 2.2 to 3.9% in July 2008 which indicates that the organic matter content of the sediment 5 years after the cease of farming activities has not been reduced to values considered as reference, indicative of non-impacted conditions in the area (<1–2% of sediment dry weight (Ruiz et al. 2001; Ramos et al. 2003)). In spite of this, the survival and vegetative development of the seedlings did not seem to be affected negatively by these contents of organic matter. This is not surprising as the negative effects of high levels of organic matter in the sediment on seagrass performance have been related more to the accumulation of reduced sulfur in the sediment as a consequence of increased sulfate reduction rates than to high organic matter levels per se (Holmer & Frederiksen 2007).

The few planted seedlings surviving inside the *P. oceanica* meadow tended to have longer leaf lengths in July 2009 than those planted in dead matte. The observation of bite marks on the leaves of the seedlings growing in the dead matte but not in those living inside the *P. oceanica* meadow suggests that this reduction of leaf length in summer might be due to fish herbivory. The seedlings living in dead matte might be more easily located by herbivore fish than those inside the leaf canopy and probably support more intense pressure from herbivores. Although sea urchin herbivory was the main driver of *P. oceanica* loss in the fish-farm impacted area in Hornillo Bay (Ruiz et al. 2009) our results indicate that herbivore pressure is not an important process in the early life of *P. oceanica* seedlings but this conclusion requires further evaluation.

Implications for Practice

- Seedlings of seagrass may be used successfully in experiments to evaluate if sediment and environmental conditions in disturbed areas are appropriate for meadow restoration.
- Plastic mesh-pots filled with fiberglass wool and firmly anchored to the substratum provide a reliable planting structure, allowing the persistence of seedlings for at least a year.
- Dead matte is a more adequate substratum for the development of planted seedlings than inside of a well-developed *P. oceanica* meadow.

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