Seasonal dynamics of *Posidonia oceanica* in Magalluf Bay (Mallorca, Spain): Temperature effects on seagrass mortality

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Abstract

We recorded seasonal and interannual population dynamics of the endemic Mediterranean seagrass *Posidonia oceanica* in a shallow meadow, Magalluf Bay, Spain (39°30.25′N, 2°32.59′E), performing direct monthly–bimonthly censuses from June 2001 to November 2004, in 12 permanent plots. Initially, there were 568 ± 54 (mean $\pm$ SE) shoots m^{-2}. An average decline of -63 ± 10 SE shoots m^{-2} yr^{-1} was detected. Shoot mortality (median: 1.2% month^{-1}; range: 0% to 12.1% month^{-1}) increased in summer, enhanced by seawater temperature. There was also substantial interannual variability characterized by very high mortality in summer–autumn 2001 and in autumn–winter 2003–2004, coinciding with hot summers and meadow flowering in autumn (2001: $2.0\% \pm 1.4\%$ of shoots; 2003: $53.8\% \pm 10.4\%$ of shoots), but fruiting and seedling recruitment were only observed in 2004. Annual vegetative recruitment (0.034 ± 0.007 yr^{-1}) was 53 times higher than annual sexual recruitment (0.0006 ± 0.0007 yr^{-1}), and total recruitment (vegetative + sexual) was on average 4.5 times lower than mortality. Specific net population growth was always negative during the study. No seasonality was detected in shoot recruitment (median: 0.002 month^{-1}; range: 0 to 0.021 month^{-1}), which did not correlate with solar radiation or temperature. Shoots recruited during the study had a steeper exponential depletion rate ($r_r = -0.34 \pm 0.05$ SE yr^{-1}) than the global population ($r_{N0} = -0.16 \pm 0.01$ SE yr^{-1}). Shoot probability to survive to the next age class (p_x) increased with shoot age. Our results suggest that the general decline of *P. oceanica* meadows could be enhanced by the decadal Mediterranean warming trend.

Seagrass meadows form extensive coastal ecosystems of high diversity worldwide (Hemminga and Duarte 2000). In the Mediterranean coasts, the endemic *Posidonia oceanica* is the dominant seagrass, covering between 2.5×10^{10} m^2 and 5×10^{10} m^2 in the shallow (0.3 to 45 m depth) fringe of the continental platform (Pasqualini et al. 1998). Like other seagrass species, *P. oceanica* meadows provide important ecosystem services, such as carbon sequestration (Gacia et al. 2002; Mateo et al. 2006), sustenance of secondary production (Cebrián and Duarte 2001), sediment stabilization (Terrados and Duarte 2000) and sediment production (Canals and Ballesteros 1997), water oxygenation (Gazeau et al. 2005), or provision of habitat and shelter for a diverse and exploited community (Hemminga and Duarte 2000).

Seagrass ecosystems are declining worldwide, at rates of about 2% yr^{-1} (Orth et al. 2006). In the case of *P. oceanica* meadows, the reported rates of decline across the Western Mediterranean are even faster (5–10% yr^{-1}; Marbà et al. 2005), and widespread meadow regression at the decadal scale has also been reported in the Eastern Mediterranean (Zavodnik and Jaklin 1990). Such decline cannot always be directly attributed to specific human perturbations (Marbà et al. 1996*b*, 2005), suggesting that a background decline trend may exist as a consequence of sea climate change (Duarte et al. 1999).

The stability of seagrass meadows depends on the balance between shoot mortality and recruitment (Hemminga and Duarte 2000). As clonal plants, seagrass recruitment can proceed through both clonal and sexual processes. Sexual reproduction has variable importance among seagrasses (Hemminga and Duarte 2000), but in

most cases, meadow development relies largely on vegetative recruitment. In *P. oceanica* meadows, seedlings are observed episodically, and the relative importance of sexual reproduction is considered to be low (Buia and Mazzella 1991), although it has not been fully quantified. Vegetative recruitment in seagrasses is produced through branching of the apical shoot meristems, which produce leaf-bearing shoots and elongate the rhizomes (Hemminga and Duarte 2000). *P. oceanica* has two types of shoots, both of which bear leaves: vertical shoots, the most common ones, which are responsible for meadow vertical (also called orthotropic) growth to compensate for sediment accumulation, and horizontal shoots, or apices, which are less frequent, but which are responsible for horizontal spread (also called plagiotropic growth) and which show faster elongation rates (1–6 cm yr^{-1}) than vertical shoots (0.1–4 cm yr^{-1}; Hemminga and Duarte 2000).

The physiological mechanisms of individual shoot death, often preceded by the death of their apical meristems (Hemminga and Duarte 2000), are still poorly understood. Several kinds of disturbances have been shown to induce seagrass shoot death (Neverauskas 1988; Marbà and Duarte 1995; Frederiksen et al. 2007), but the causes of shoot mortality in apparently undisturbed meadows remain unknown. Negative relationships between maximum shoot densities and species shoot size indicate the existence of density-dependent regulation mechanisms on mortality and/or shoot recruitment (Duarte and Kalff 1987) through allometric and feedback regulations of their clonal growth (Marbà and Duarte 2003), but little is known about the patterns of shoot survival with shoot age. Seasonality in shoot population dynamics has been shown to occur among temperate seagrass species, and it has been related

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to seasonal variations in solar radiation (Olesen and Sand-Jensen 1994; Marbà et al. 1996*a*) and adverse winter conditions (Bigley and Harrison 1986; Harrison 1993). Among tropical seagrasses, seasonality seems to be lower (Gallegos et al. 1993; Martínez-Daranas et al. 2005), but shoot population changes at monthly scale have been linked to meteorological events (Martínez-Daranas et al. 2005).

In *P. oceanica*, the seasonal patterns of photosynthesis (Drew 1978; Masini and Manning 1997), leaf production, or module (leaves, rhizomes, roots) elongation rates are well established (Caye and Rossignol 1983; Alcoverro et al. 1995; Marbà et al. 1996*a*). Such patterns are attributable to seasonal climate forcing (Alcoverro et al. 1995) and to internal biorhythms (Ott 1979). However, little is known on the seasonality of *P. oceanica* shoot population dynamics. As in other seagrass species, apex branching follow peaks of leaf production, being maximal in spring and, to a lesser extent, in autumn (Caye and Rossignol 1983; Marbà et al. 1996*a*). However, the contribution of vertical branching to recruitment and the overall shoot recruitment seasonality are unknown. Meadows of *P. oceanica* do not experience the wide seasonal variations in shoot density observed in other temperate seagrasses (Hemminga and Duarte 2000), and there is no available information on the seasonal variability of shoot mortality. Knowledge on the seasonal dynamics of shoot recruitment and mortality would likely reveal possible drivers of these processes and, particularly, help us to detect links between climatic factors and *P. oceanica* population dynamics.

Here, we report spatial, seasonal, and interannual variability of *P. oceanica* shoot population dynamics in a shallow Mediterranean meadow, measured during ~4 yr (spring 2001 to autumn 2004) through direct censuses. We explore the links between this temporal variability and potential climatic factors, including seawater temperature, solar radiation, and wave regime. We also quantify the contribution of sexual recruitment vs. vegetative recruitment to shoot population within the study period and explore the relationship between shoot survival and shoot age. This effort represents a challenge because *P. oceanica* is the slowest growing seagrass and possibly one of the slowest growing plants on Earth. Resolving shoot demography at monthly timescales requires large sample sizes because the frequency of demographic events (shoot death, birth, and sexual recruitment) is very low.

Methods

The study was conducted in the bay of Magalluf (Mallorca Island, Spain), at 7 m depth, next to the islet of Sa Porrassa (39°30.25′N, 2°32.59′E; Fig. 1). This *P. oceanica* meadow starts at 3 m depth (the continuous meadow starts at a median distance of 76 m offshore; Sánchez 2003) and grows deeper than 25 m, covering approximately 1.2 km² or 89% of the bay surface (Fornes et al. 2006). It is exposed to eastern and southern winds (Basterretxea et al. 2004). The meadow grows in waters with high light penetration on biogenic carbonate sediments (98%; Holmer et al. 2003), but the seagrass leaf

C : N : P ratios suggest no phosphorus limitation (Fourqurean et al. 2007).

Direct censuses of *Posidonia oceanica* shoots were performed on 12 0.25 m² plots. The perimeter of each plot was delimited with a rope running around four metal poles inserted into the sediment. All of the shoots within each plot (vertical shoots and horizontal apices) were counted and tagged by attaching a plastic cable tie around the rhizome, 2 cm below the leaf meristems, as described in Marbà et al. (2005). For subsequent censuses, plots were grouped in four clusters of three plots each. Monthly to bimonthly visits to the site were performed from March 2001 to October 2004, and one cluster was recensused per visit, following a rotary scheme. Therefore, each particular plot group was censused every 4 to 8 months. The rotary strategy was adopted to cope with the extremely slow growth rate of the species (Marbà and Duarte 1998). It was also adopted to be able to resolve dynamics of *P. oceanica* shoots at monthly to bimonthly timescales, while reducing error and potential effects of the census procedure at any particular plot. In each new census, total shoot density within the plot cluster was counted again, as was the number of horizontal shoots (apices). The new, untagged shoots, formed from the branching of apices and vertical shoots, as well as the new apices entering the plot through horizontal growth were counted separately as new recruits in the area and tagged with a cable tie of a different color than that used in the previous census. By doing so, the fate of the new cohorts within the plot could be followed and differentiated from that of the initial shoot population. The mortality and recruitment of vertical shoots and of apices could be estimated as well. When vegetative fragments or seedlings appeared in the plot, they were tagged but were computed as recruits only if they were rooted during at least two censuses. The shoot deaths that took place between two censuses, in the initial shoot population and within each cohort, was the difference between the shoots initially tagged with a specific color and the number of survivors tagged with the same color in the following census. During the whole study, in total, 1991 shoots were tagged, and their fate was followed; the large sample size allowed us to resolve demographic events, despite their low frequency in this species.

Shoot and seedling density outside the plots was measured four times during the study along transects of 35 to 60 m by counting 21 to 62 quadrats of 0.063 m² per transect. Shoot counting error was calibrated by counting three census plots repeatedly by three independent divers, yielding a standard error of 15 dead shoots m^{-2} and 1 new recruit m^{-2} per census.

The evolution of shoot and apex densities was examined within each plot through linear regression (type I, both with the data not transformed and log-transformed), and globally by performing a general linear model (GLM)–repeated measures analysis (subject: plot, repeated measure: census), with SPSS 11.04 for MacOsX© (GLM–repeated measures procedure): the effects of plot, census, and census × plot were tested. The marginal means of shoot density at each census were also calculated in this procedure. The slopes of the regression models and of the
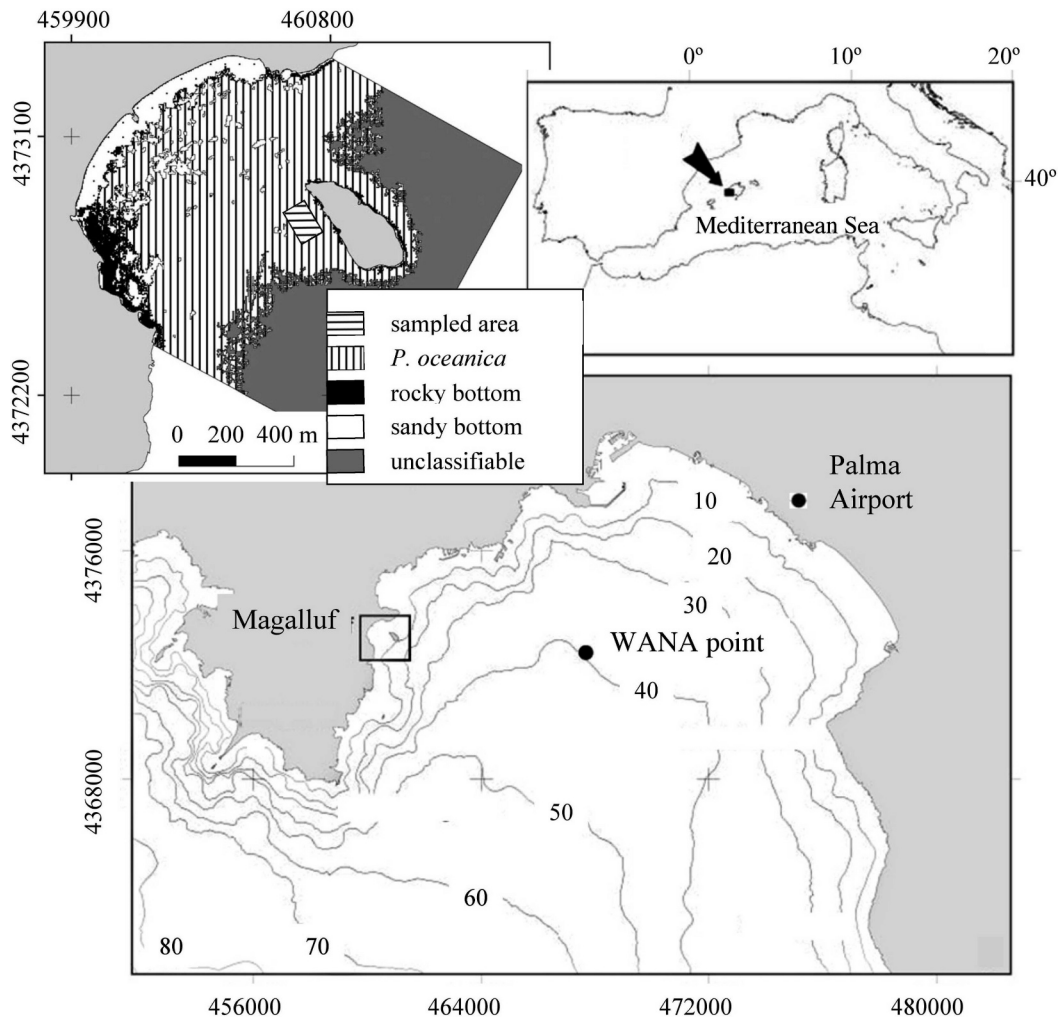
Fig. 1. Location of the study site in Magalluf Bay, the airport, and the point WANA2069036 in the Bay of Palma (Mallorca Island, Spain). Meadow map was extracted from Fornes et al. (2006) with permission of the authors.

(GLM)–repeated measures model were taken as estimators of mean net population growth within each plot and in the meadow, respectively, in the whole study period. Relationships between plot shoot density and apex fraction or apex density were explored through linear regression (type II) and nonparametric (Kendall's tau-b) bivariate correlation analysis.

Meadow shoot density along transects was compared to the marginal means of meadow shoot density in the dates closest to transect sampling, estimated after the GLM–repeated measures model.

The new, tagged shoots were grouped in two cohorts per year: one group included all shoots born from March to August (both months included), and the second group was made up of the recruits between September and February of the following year. We chose this grouping frequency because it provided the maximum possible precision to allocate the shoot birth timings throughout the study given the frequency of visits to each plot group. Had it been possible, a shorter grouping would have yielded too few shoots for the analyses. The semester grouping provided 8 to 31 shoots within each cohort. This recruitment grouping

also has biological significance for *P. oceanica*, because the recruitment of horizontal shoots seems to present two maxima during the year: one in spring and a smaller one in autumn (Caye and Rossignol 1983; Marbà et al. 1996*a*).

We estimated the depletion curves of the shoot population initially marked (i.e., shoots present at the onset of the study, N_0) and of semester cohorts (N_1, N_2,…N_i) through regression analysis (type I), fitting a linear and an exponential model. The exponential model of decline fitted the data slightly better (higher R and smaller probability of type-I error than the linear model). Therefore, we adopted it. The 95% confidence intervals of the regression coefficients were calculated in order to look for significant differences between depletion slopes of newly recruited shoots and of the general population of shoots initially marked.

We analyzed shoot population dynamics between two censuses within each plot. In order to estimate shoot-specific net population growth, and shoot-specific recruitment and mortality rates, we assumed a simple and continuous model of unlimited population growth, of the exponential form (Ebert 1999)

$$N(t) = N(0) \cdot e^{(\bar{b} - \bar{m})t} \qquad (1)$$

where $N(t)$ is the total number of shoots per unit area (shoots m^{-2}) at time t (in months), $N(0)$ is the total number of shoots per unit area at $t = 0$, $\bar{b}$ is the average shoot-specific recruitment rate over the time interval 0 to t (month^{-1}), and $\bar{m}$ is the average shoot-specific mortality rate over the same time interval.

Taking logarithms on both sides of Eq. 1, for any time interval we obtain

$$\mu(t) = b(t) - m(t) \approx \ln\left(\frac{N(t)}{N(t - \Delta t)}\right) \Big/ \Delta t \qquad (2)$$

where $\mu(t)$, the shoot-specific net population growth between $t - \Delta t$ and t, is equal to the difference between shoot-specific recruitment and shoot-specific mortality in the time interval Δt; specific net population growth approximates to the term in the right side of Eq. 2 (Ebert 1999) and is estimated from it; and where $N(t)$ is the total number of shoots, per unit area, measured in the plot at time t, and $N(t - \Delta t)$ is the total number of shoots, per unit area, counted in the plot at time $t - \Delta t$.

The average shoot-specific recruitment and mortality rates in the interval Δt are defined such that Eq. 2 holds true. Therefore, we estimated the shoot-specific recruitment rate (also called intrinsic population recruitment rate) as

$$b(t) \approx \frac{\ln\left((N(t)/N_s(t)\right)}{\Delta t} \qquad (3)$$

where $N_s(t)$ is the number of survivors at time t (calculated as $N[t] - N_{rec}[t]$, where $N_{rec}[t]$ is the number of new shoots, per unit area, recruited during the time interval Δt, counted at time t). For the estimation of shoot recruitment rates, we related the number of shoot recruits against the number of shoot survivors, instead of the most common strategy of comparing the number of recruits to the initial number of individuals at time $t - \Delta t$. Taking into account the seagrass growth mode, this strategy has more biological sense because in seagrasses, shoots are produced by bifurcation of the living, apical meristems; it is rare for a dying shoot, with a decayed meristematic activity, to undergo branching. Moreover, even if this improbable event occurred, we would be unable to detect it given our sampling scheme. Specific vertical (b_v) and apex (b_a) branching rates were also estimated separately using Eq. 3, but in the case of vertical or apex branching rates, $N_{rec}(t)$ refers only to shoots produced by vertical shoots or apices, respectively, at time t, while $N_s(t)$ refers to the number of alive vertical shoots or apices that remain within the plot until time t. Specific sexual recruitment rate was also calculated with Eq. 3, but $N(t)$ was equal to the number of survivor shoots within the plot plus the number of seedlings recruited at time t. Moreover, as seedling recruitment is usually observed only some months after fruit dehiscence (usually from April to October), we estimated sexual recruitment on an annual basis. To do that, we pooled together all of the survivor shoots from all plots at the end of each year, as well as all the seedlings rooted in any plot at the end of that year.

Annual sexual specific recruitment was then calculated. In order to compare sexual recruitment with vegetative recruitment, we also calculated specific vegetative recruitment rate on an annual basis, that is, we pooled together all surviving shoots and all recruited shoots produced each year within all the plots, as if the censuses had been annual.

Specific mortality rate (also called intrinsic mortality rate) within each plot was estimated as

$$m(t) \approx \frac{\ln\left(N(t - \Delta t)/N_s(t)\right)}{\Delta t} \qquad (4)$$

The mean shoot survival probability (p_s) was estimated as $p_s = 1 - m$, where m is the mean specific mortality rate across plots during the whole period of study.

Absolute shoot recruitment rate ($B[t]$, in shoots m^{-2} month^{-1}) between censuses was calculated for each of the plots, assuming a simple and discrete geometric model of unlimited population growth (Ebert 1999), as

$$B(t) \approx \frac{N_{rec}(t)}{\Delta t} \qquad (5)$$

In addition to total absolute recruitment rate, we estimated absolute vertical and apex branching rates, as well as absolute sexual recruitment rates within plots, using Eq. 5, but in each case, $N_{rec}(t)$ corresponded to the number of new shoots recruited from each shoot kind (i.e., new shoots branched from vertical shoots, and new shoots branched from horizontal apices within each plot, respectively). Absolute seedling recruitment was estimated in an annual basis as the total number of seedlings rooted in any plot per unit area during that year. We compared absolute sexual recruitment rate with absolute vegetative recruitment rate calculated also on that annual basis. We can see that $b(t) \approx B(t)/N_s(t)$, when $B(t)/N_s(t) \ll 1$, as is the case in our study.

Absolute shoot mortality rates ($M[t]$, in shoots m^{-2} month^{-1}) between censuses were calculated for each plot assuming the same growth model, as

$$M(t) \approx \frac{(N(t - \Delta t) - N_s(t))}{\Delta t} \qquad (6)$$

We can also see that $m(t) \approx M(t)/N(t - \Delta t)$ if $M(t)/N(t - \Delta t) \ll 1$, as is the case in our study.

Finally, we estimated the absolute net population growth $\lambda(t)$ within each plot between two censuses, as

$$\lambda(t) \approx \frac{N(t) - N(t - \Delta t)}{\Delta t} = B(t) - M(t) \qquad (7)$$

We estimated global shoot recruitment, mortality, and net population growth rates (both, specific and absolute) as the mean and median of those parameters measured in every single plot between all consecutive censuses.

In order to analyze the seasonal variability of population dynamics, we averaged plot mortality and recruitment rates for each plot cluster (group of three plots censused in the same date). Then, specific mortality and recruitment rates between two visits to the meadow (that is, monthly to trimester shoot mortality and recruitment rates) were estimated as the running averages of those parameters,

recorded in the plot clusters that were visited during the observational windows (four to eight months) including the time interval of interest.

A temperature data logger (TidBit, Onset™) was anchored within the meadow canopy. The seawater temperature was measured every 2 h from 10 August 2001 until the end of the study. Daily data of solar radiation (W m^{-2}) at the Palma Airport, 15 km from the study site (Fig. 1), were provided by the Spanish National Institute of Meteorology (INM) for the whole study period. Data on wave height, direction, and period at the center of the Bay of Palma (point WANA2069036 of the National Network of Spanish Harbours and the INM; frequency: 2 h; Fig. 1) were also obtained. We extracted the parameters of height and period for the waves coming from the east-south sector, to which the Magalluf meadow is exposed. We calculated the average water temperature, wave height, and wave period for the same monthly time periods as for the monthly estimates of shoot mortality and recruitment, and we explored the relationships between seasonal shoot dynamics and climate parameters (seawater temperature, wave height and period, and solar radiation) using linear regression analysis (type II).

When flowering was observed, the flowering intensity (i.e., fraction of shoots bearing an inflorescence, FI) was measured inside the plots.

The probability of shoots to survive to the next age class (p_x; where x is the age class: {0, 6, 12, 18, 24, 30, 36 months}) was estimated for shoot recruits as $p_x = 1 - m_x$, where m_x is the semester mortality of shoots passing from age class x to age class $x + 1$, calculated as in Eq. 8:

$$m_x = \ln\left(\frac{N_{(x)}}{N_{s(x+1)}}\right) \tag{8}$$

where $N(x)$ is the initial number of shoots in the age class x, and $N_{s(x+1)}$ is the number of shoots that have survived to the age class $x + 1$ (in this case, 1 semester more). In order to increase the power of the analysis, we performed this calculation pooling together all the shoot cohorts, as if they were born at the same time. Next we performed a simple regression analysis (type I) of shoot survival probability with age. In order to check the robustness of this result, we performed the same analysis considering annual cohorts and annual age classes, instead of semester.

Results

The initial shoot density within the plots was 568 ± 54 shoots m^{-2} (first marginal mean $\pm$ SE from ANOVA repeated measures analysis). Initial plot density was not significantly (t-test, $t = -0.801$, $p = 0.44$, df = 12) different from meadow shoot density estimated outside the plots (614 ± 22 shoots m^{-2}). During the 4 yr of the study, shoot density decreased continuously within every plot, although at different rates (coefficients of determination range: $R^2 = 0.72$–0.97; $0.005 < p < 10^{-6}$; df = 7 to 9; absolute net growth rate λ: -23 to -119 shoots m^{-2} yr^{-1}; mean $\lambda = -63.3 \pm 9.7$ [SE] shoots m^{-2} yr^{-1}; specific net growth rate μ: -5.5% to -26.1% yr^{-1}; mean $\mu = -13.5\% \pm 2.1\%$ [SE]

yr^{-1}; Fig. 2). The GLM–repeated measures analysis showed that, altogether, 74% of the variance in shoot density was attributable to spatial heterogeneity (plot effect: $p < 0.001$, df = 11; Fig. 2); only 19% of variance corresponded to temporal changes ($p < 0.01$, df = 9; Fig. 3A), and 7% of variance was attributable to census $\times$ plot interactions, and thus to differences in decline intensity among plots ($p < 0.02$, df = 11; Fig. 2). The sustained meadow decline was not evident using shoot density estimates from random transects (Table 1) because they confounded temporal and spatial variability, and standard errors were often in the same order of the decline detected within plots (Fig. 3A). Nevertheless, shoot densities within and outside the plots did not differ significantly during the study (Table 1). At the end of the study, meadow shoot density within the plots was of 436 ± 44 (SE) shoots m^{-2} (last marginal mean in the ANOVA repeated measures model; Fig. 3A).

Contrary to the significant decline of global shoot density, the abundance of horizontal apices within the plots did not significantly decrease with time. Moreover, apex density was not significantly correlated with total ($K = 0.11$, $p = 0.19$, $n = 89$) or vertical ($K = 0.07$, $p = 0.34$, $n = 89$) shoot densities. The apex fraction in the shoot population did not significantly increase with time; however, around the middle of the study, apex density and apex fraction started to fluctuate within most of the plots (Fig. 3B).

The number of survivors of the shoot population initially marked (N_0) and those of the new cohorts decreased exponentially with time ($R^2 = 0.97$, $p < 10^{-4}$; $R^2 = 0.65$, $p < 10^{-5}$, respectively; Fig. 4), but the rate of decline of the shoots recruited during the study ($r_r = -0.34 \pm 0.05$ SE yr^{-1}) was twice as high as the rate of decline of the initial shoot population, of mixed ages ($r_{N0} = -0.16 \pm 0.01$ SE yr^{-1}; $p < 0.05$). Shoot depletion curves indicate that shoot population half-life was 4.33 ± 0.29 yr for a population of shoots of mixed ages and only 2.04 ± 0.35 yr for newly recruited shoots.

The shoot probability to survive to the next semester age class (p_x) increased with age (Fig. 5), as described by the equation fitted on the pooled cohorts (Eq. 9):

$$p_x = 0.008 \pm 0.002\text{SE} \times x + 0.677 \pm 0.046\text{SE};$$
$$x = \text{age(months)} \tag{9}$$

where the 95% confidence interval for the slope = (0.002; 0.013); $R^2 = 0.73$; $p = 0.018$; and $n = 6$.

The same pattern of increased survival probability with age was observed when annual cohorts, instead of semester cohorts, were considered.

The mean absolute shoot recruitment rate was $B = 1.4 \pm 0.2$ SE shoots m^{-2} month^{-1}, and the specific recruitment rate averaged $b = 0.0030 \pm 0.0005$ SE month^{-1} (Table 2). Despite the fact that the specific apex branching rate ($b_a = 0.071 \pm 0.014$ SE month^{-1}) was 35 times higher (Welch approximate t-test, $t = 5.12$, df = 44, $p < 0.001$) than the specific branching rate of vertical shoots ($b_v = 0.002 \pm 0.000$ SE month^{-1}), the latter provided most (69.4% $\pm$ 13.1% SE, on average) of the vegetative recruits in absolute terms due to the higher abundance of vertical shoots.
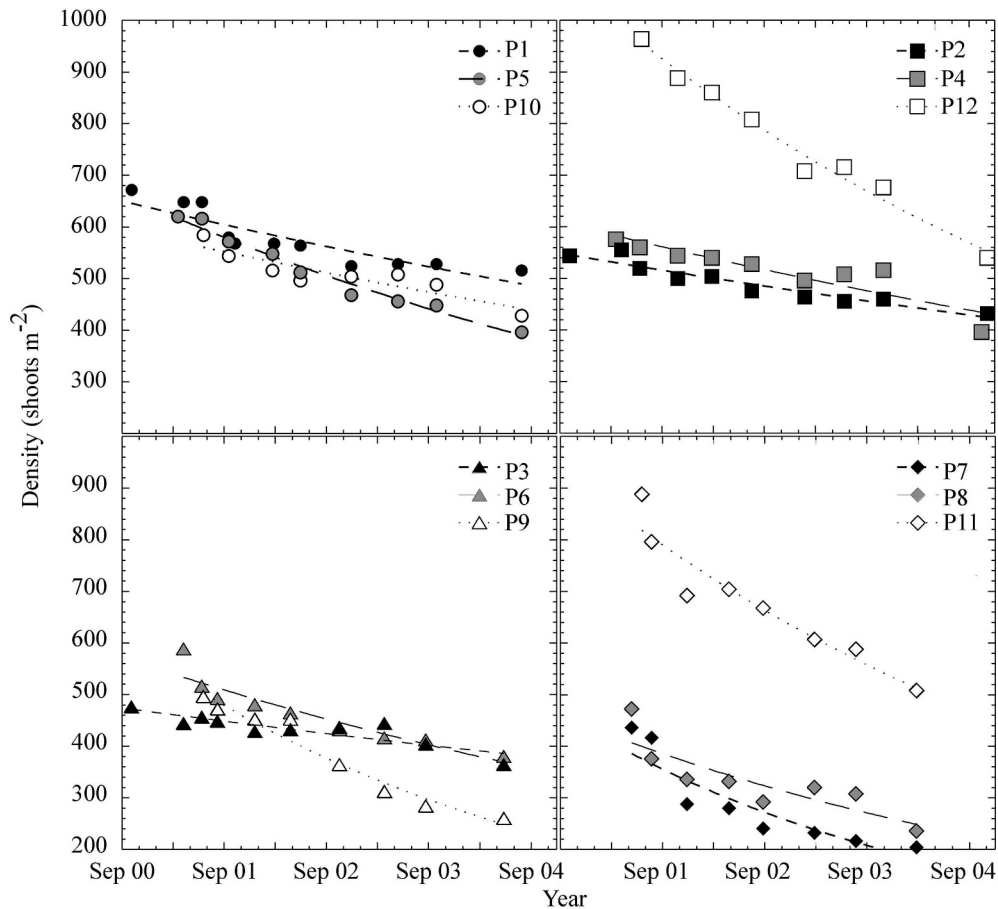
Fig. 2. Changes during the study of shoot density within the 12 permanent census plots installed in the Magalluf meadow at 7-m depth. The three plots belonging to each plot cluster are represented in the same graph. The dashed lines represent the model of exponential decline adjusted to the data of each plot.

From 2001 to 2004, the meadow flowered twice. The first flowering event was observed in autumn 2001, when 2.0% $\pm$ 1.4% SE of shoots within the plots produced inflorescences, but fructification failed, and no sexual recruitment was recorded the following spring and summer. In autumn 2003, an intensive flowering occurred within the meadow, when 53.8% $\pm$ 10.4% of the shoots within the plots flowered. Between March and June 2004, massive fructification occurred within the meadow (156 $\pm$ 63 mature fruits m^{-2}), and in July 2004, three rooted seedlings were observed in one of the plots (1 rooted seedling m^{-2}). Those three seedlings were still alive in May 2007, but none had branched. The sexual recruitment rate inside or outside the plots was never observed during the whole study, with the exception of the periods between May and July 2004 (0.043 yr^{-1}) and between July and October 2004 (0.013 yr^{-1}). Seedling establishment was a minimal component of shoot recruitment (b_s: 0.0006 $\pm$ 0.0007 SE yr^{-1}, nearly 2% of global annual recruitment, on average, b = 0.035 $\pm$ 0.007 yr^{-1}). During the entire study, vegetative fragments rooted only once within the permanent plots, but these survived for less than 138 d.

Direct apex death was never observed because it was always preceded by apical transformation in a vertical shoot. Apex losses during the 4 yr of the study (1.9 apices m^{-2} yr^{-1}) were due to rhizome apices growing outside the boundaries of the plots (37%) or their transformation in vertical shoots (63%), but only a few of these transformed shoots eventually died. Apex casualties were nearly balanced by apex recruitment (1.6 apices m^{-2} yr^{-1}) derived from migration to the plots of growing external apices (31%) and transformation of vertical shoots to horizontal apices (69%). Therefore, the overall density of apices did not change significantly across the study.

Absolute and specific shoot mortality rates averaged M = 8.4 $\pm$ 1.2 SE shoots m^{-2} month^{-1} and m = 0.017 $\pm$ 0.002 SE month^{-1}, respectively, throughout the study (Table 2) and were six times higher than absolute and specific mean recruitment.

Specific shoot mortality varied substantially (V^* = 114%; Table 2) across the study. The monthly estimates of shoot mortality had high interannual and seasonal variability, and these increased in summer months and decreased in winter 2001–2002 and winter 2002–2003 (Fig. 6). Moreover, the monthly estimates of specific shoot mortality increased linearly with increasing mean seawater temperature during the corresponding period (Fig. 7), as described by the fitted regression equation
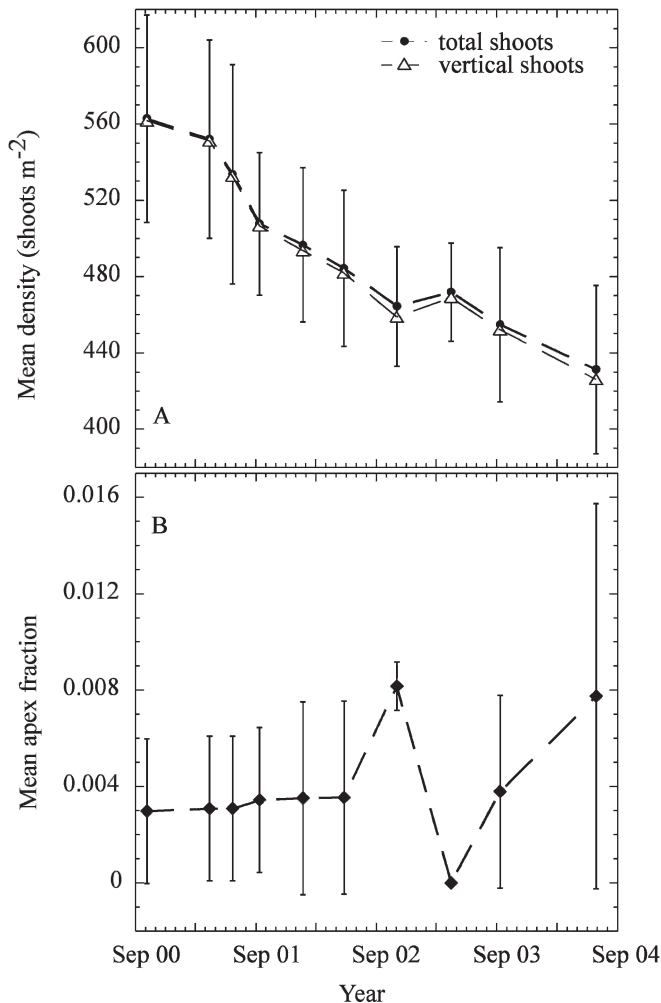
Fig. 3. (A) The evolution of the estimated census marginal means of total (black circles) and vertical (triangles) shoot densities across censuses in the fixed plots. (B) Variation of the estimated census marginal means of plot apex fraction. The vertical bars represent standard errors of marginal means.

(Eq. 10),

$$m(\text{month}^{-1}) = 6.0 \times 10^{-4} \pm 1.8 \times 10^{-4}\text{SE} \times T \,(°\text{C}) \quad (10)$$

where the 95% confidence interval for the slope = (2.3 × 10^{-4}; 9.8 × 10^{-4}); R^2 = 0.31; p < 0.003; and n = 24.

The highest winter mortalities occurred in winter 2003–2004 (Fig. 6), following the highest summer temperatures registered in the meadow during the study (August 2003 mean temperature 28.7°C, 1.4°C above the mean August temperature).

The specific shoot mortality rates were independent of mean solar radiation and wave height (mean or maximum, p > 0.05, n = 28).

Mean mortality rates showed a peak from June to November 2001. In this period, we observed large mortalities in most of the plots, coinciding with their second or third census. Mortalities of the same or greater magnitude were observed within all plots in other censuses, but not with the same consistency among plots.

Specific shoot recruitment was highly variable (V^* = 147%; Table 2) among censuses and plots, but the monthly estimates did not show a seasonal pattern (Fig. 6). No significant correlation was observed between specific shoot recruitment estimates and mean solar radiation, seawater temperature, wave height, or wave frequency during the same periods, and no lagged response was detected. The mean annual vegetative recruitment increased throughout the study, with a sharp increase in 2004 (Fig. 6), but it was always well below the mean annual mortality. Indeed, the net rate of population growth increased from year 2001 (μ = −0.182 yr^{-1}) to 2004 (μ = −0.127 yr^{-1}) although, on average, it remained negative throughout the study (Fig. 8A) and was determined by mortality rate. The fraction between mean annual recruitment and mortality increased steadily from year to year during the study (Fig. 8B).

Monthly estimates of net population growth significantly decreased with increasing mean seawater temperature during the corresponding period, as described by the fitted regression equation (Eq. 11),

$$\mu(\text{month}^{-1}) = -6.1 \times 10^{-4} \pm 1.8 \times 10^{-4}\text{SE} \times T \,(°\text{C}) \quad (11)$$

where the 95% confidence interval for the slope = (2.4 × 10^{-4}; 9.9 × 10^{-4}); R^2 = 0.32; p < 0.002; and n = 24.

Mean annual population growth calculated from monthly estimates (μ = −0.157 ± 0.020 [SE] yr^{-1}) was similar to mean annual population growth estimated as if the censuses were annual within the pooled plots (μ = −0.125 ± 0.024 [SE] yr^{-1}). It was also similar to mean annual net population growth estimated from the slope of the regression of shoot decline with time (shown before). This reinforced the reliability of the monthly estimates of population dynamics.

Discussion

The seagrass mortality and recruitment rates recorded within permanent plots during the 4 yr of the study confirmed that the *Posidonia oceanica* meadow in Magalluf Bay has experienced a steady decline. If the decline rates observed during 4 yr were to continue in the future, shoot density would be reduced to 10% of present shoot densities within 12 to 18 yr. Our results also demonstrate that even such a relatively fast decline remained unresolved by random shoot density counts, which represent the basis for most monitoring programs, because shoot density decline is masked by the patchiness of the meadow. This observation is consistent with results of Heidelbaugh and Nelson (1996) regarding the low power of random quadrat counts to detect changes in perennial, slow-growing seagrasses (Fourqurean et al. 2003).

The recruitment and losses of rhizome apices were nearly balanced, and apex density did not significantly decrease with time. Given the larger growth and branching rates of apices, their higher persistence in the shoot population could facilitate meadow recovery if the environmental conditions improved. Our results also suggest that shoot survival probability increased with shoot age. However,

Table 1. Mean shoot density at Magalluf estimated within transects and within permanent plots (marginal means of the GLM–repeated measures model) on different dates.

	Transects			Plots	
Date	Shoot density (shoots m^{-2})	SE	Date	Shoot density (shoots m^{-2})	SE
20 Mar 01	614	12	22 Apr 01	557	52
07 Nov 01	427	52	25 Sep 01	511	37
23 Feb 02	534	67	01 Feb 02	500	40
22 Dec 03	527	29	17 Sep 03	459	40

this effect was not reflected in the shoot depletion curves, which were fitted better by an exponential decay, as expected for an age-independent death probability. Nevertheless, the loss of shoots recruited during the study doubled that of preexisting shoots, suggesting a higher vulnerability of young shoots to adverse environmental conditions. This finding is in agreement with results in another *P. oceanica* meadow at the Balearic Islands (Marbà et al. 2007), where shoots recruited during the study showed a stronger response to remedial actions than older shoots. An increased shoot survival with age in *P. oceanica* would contrast with the tropical seagrass *Thalassia testudinum* (van Tussenbroek 2002), in which young shoots have been reported to exhibit lower mortality rates than old ones; as well as with *Cymodocea nodosa*, where the shoot mortality is independent of age (Duarte and Sand-Jensen 1990). However, the age dependence observed here may apply only under adverse conditions.

Posidonia oceanica shoot demography exhibited some seasonality, despite the slow growth and shoot turnover rates of this species, which show moderate seasonal signals (Alcoverro et al. 1995; Marbá et al. 1996*a*). The seasonal shoot dynamics of *P. oceanica* observed here were driven by shoot mortality, which increased during summer months. The only winter in which we observed high mortality was in winter 2003–2004, following the extreme summer temperature of 2003. However, this also coincided with a reduction in the frequency of visits to the meadows (Fig. 6): the demographic estimates of the last year of the study covered trimesters instead of months, and they were also based on plot censuses encompassing longer periods (8 months). This lower census frequency did not allow us to resolve a seasonal pattern for the last year, and it only allows us to say that shoot mortality in the year subsequent to the summer temperature peak of 2003 was high. On the other hand, the extreme shoot mortalities registered in the first months of the study, could be attributed to (1) a deleterious effect of plot deployment at the beginning of the study and/or (2) the combination of a secondary peak in summer water temperature with extreme storms registered in early autumn 2001. This pattern of higher summer mortality coincides with reports for some *Zostera marina* meadows (Olesen and Sand-Jensen 1994), but it contrasts with seasonal variations found in other temperate seagrasses growing at higher latitudes, like *Zostera japonica* and *Ruppia maritima*. In these species, shoot mortality concentrates in autumn and winter, coinciding with low temperatures and solar radiation (Bigley and Harrison 1986; *Zostera noltii* and *Z. marina*, Harrison 1993). The resources stored in the thick rhizomes and roots of *P.*

Table 2. Mean, median, standard error (SE), unbiased coefficient of variation (V^*, percentage) in space and time, minimum and maximum values of shoot population parameters in the permanent plots of: shoot and apex density per m², apex fraction (percentage). Specific mortality rate (m), specific recruitment rates (global: b, through apex branching: b_a, through branching of vertical shoots: b_v, or through seedlings: b_s), and specific net population growth rate (μ); all parameters have units of month^{-1}, except specific seedling recruitment rate, which is expressed in yr^{-1}. Absolute mortality rate (M), absolute recruitment rates (global: B, through apex branching: B_a, through branching of vertical shoots: B_v, or through seedlings: B_s), and absolute net population growth rate (λ); all parameters appear in shoots m^{-2} month^{-1} units, except for sexual recruitment rates, which have time units in years.

|	Mean	Median	SE	V^* (%)	Min	Max
Shoot density (m^{-2})	494.7	484.0	14.8	30	204.0	1012.0
Apex density	4.9	4.0	0.6	118	0	20.0
% apices	1.0	0.6	0.1	121	0	4.4
m (month^{-1})	0.017	0.011	0.002	114	0	0.108
b	0.0030	0.002	0.0005	147	0	0.021
b_a	0.071	0.022	0.014	141	0	0.348
b_v	0.002	0.000	0.000	130	0	0.021
b_s (yr^{-1})	0.0006	0	0.0007	213	0	0.0026
μ (month^{-1})	−0.014	0.009	0.002	182	−0.099	0.015
M (shoots m^{-2} month^{-1})	8.4	5.261	1.2	131	0	80.0
B	1.4	0.733	0.2	143	0	9.4
B_a	0.6	0.000	0.1	160	0	5.1
B_v	1.0	0.000	0.2	161	0	9.4
B_s (shoots m^{-2} yr^{-1})	0.49	0	0.57	212	0	1.97
λ (shoots m^{-2} month^{-1})	−7.0	−3.905	1.2	178	−80.0	5.6
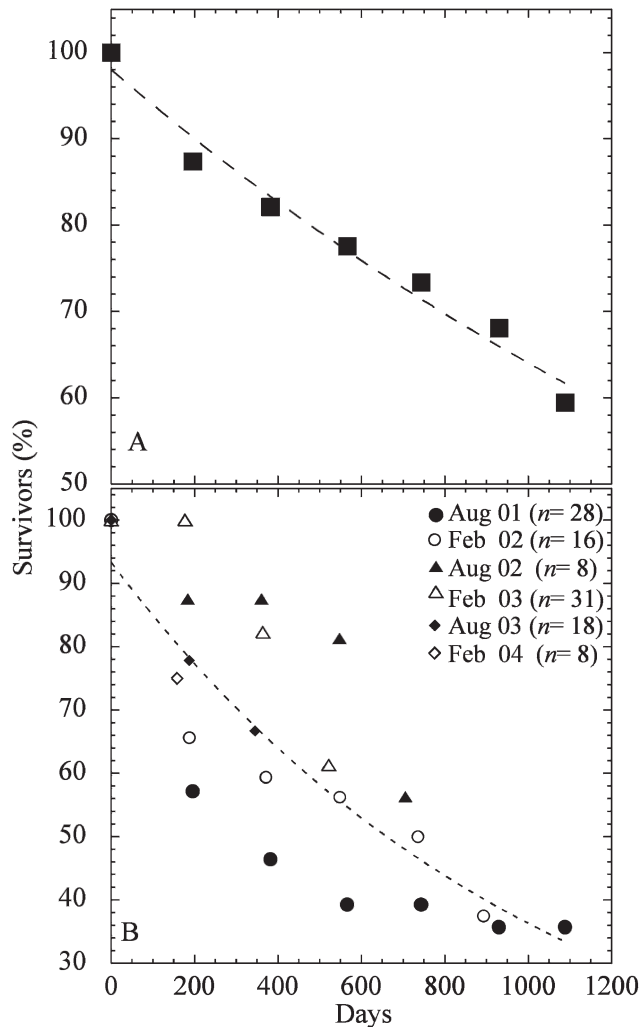
Fig. 4. (A) Depletion curves of the shoot population initially marked, formed of a mixture of shoots of different ages, and (B) depletion curve for the shoots recruited during the study, grouped in two semester cohorts per year.
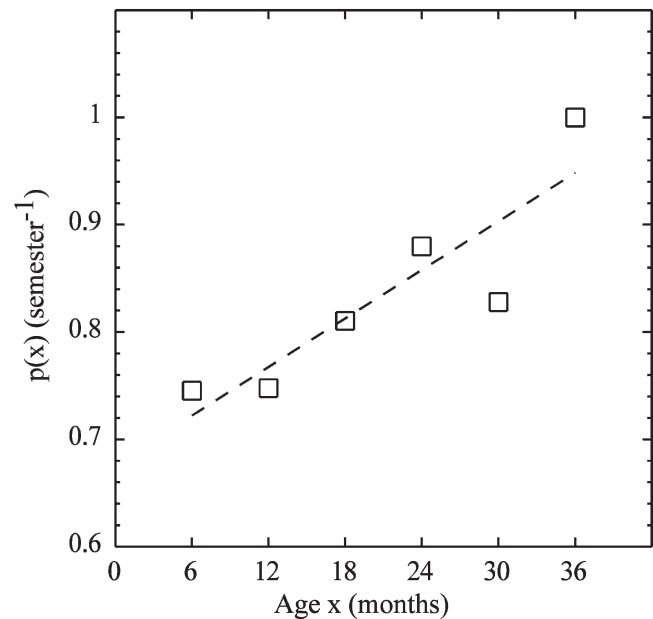

Fig. 5. The linear relationship between shoot probability to survive to the next age class and age (in semesters).

oceanica during summer (Alcoverro et al. 2001) and the milder winter temperature and solar regime of the Mediterranean Sea may facilitate shoot survival during the cold season.

The observed increase in shoot mortality with increasing seawater temperature points to this factor as the main driver of seasonal mortality for *P. oceanica*. These results are in agreement with previous reports identifying warm temperatures as a potential stressor for seagrasses (Thorhaug et al. 1978; Koch and Erskine 2001) and for *P. oceanica* growth in particular (Mayot et al. 2005). High temperature may stress plants through a combination of internal and external factors. Seagrass respiration increases with temperature faster that photosynthesis, thereby generating imbalances in the carbon budget of the plants (Masini and Manning 1997; Greve et al. 2003). Indeed, the oxygen demand of the Magalluf meadow is maximal during summer months, and it increases significantly with temperature (Barrón et al. 2006).

Moreover, high temperatures enhance benthic microbial activity (López et al. 1995; Holmer et al. 2003), stimulating anaerobic metabolic pathways, such as sulfate reduction. A prolonged exposure to sulfides reduces *P. oceanica* growth and survival (Frederiksen et al. 2007; Marbà et al. 2007), as shown also for the tropical seagrass *Thalassia testudinum* (Koch and Erskine 2001), which experiences sudden die-offs during prolonged calm and warm summer periods (Borum et al. 2005). *P. oceanica* at Magalluf may, occasionally, experience sulfide intrusion, as indicated by their leaf sulfur isotopic ratios (19.37‰ during summer 2004; N. Marbà, M. Holmer, C. M. Duarte unpubl. results). Nevertheless, measured benthic sulfate reduction rates and sulfide concentrations are usually low in Magalluf (Holmer et al. 2003; Calleja et al. 2007).

Summer is the only season when *P. oceanica* shoots have a positive carbon balance, allowing them to last through winter and to support growth in the subsequent spring (Alcoverro et al. 2001). Temperature stress during summer could reduce shoot storage of carbohydrate reserves, reducing their performance to overcome the next winter and spring seasons. The high shoot mortalities recorded during winter 2003–2004, when compared with winter mortality rates observed in the previous two years, could be the outcome of such an imbalance following the heat wave of summer 2003, when mean temperatures throughout Europe reached the highest values registered over the past 500 yr (Luterbacher et al. 2004). This heat wave generated anomalously high sea-surface temperature across the Mediterranean Sea (Díaz-Almela et al. 2007) and also led to a remarkable flowering event of *P. oceanica* in Magalluf and elsewhere in autumn 2003 (Díaz-Almela et al. 2007), and the highest flowering densities ever registered in that meadow (Díaz-Almela et al. 2006). Indeed, the flowering prevalence and intensity of *P. oceanica* increase with
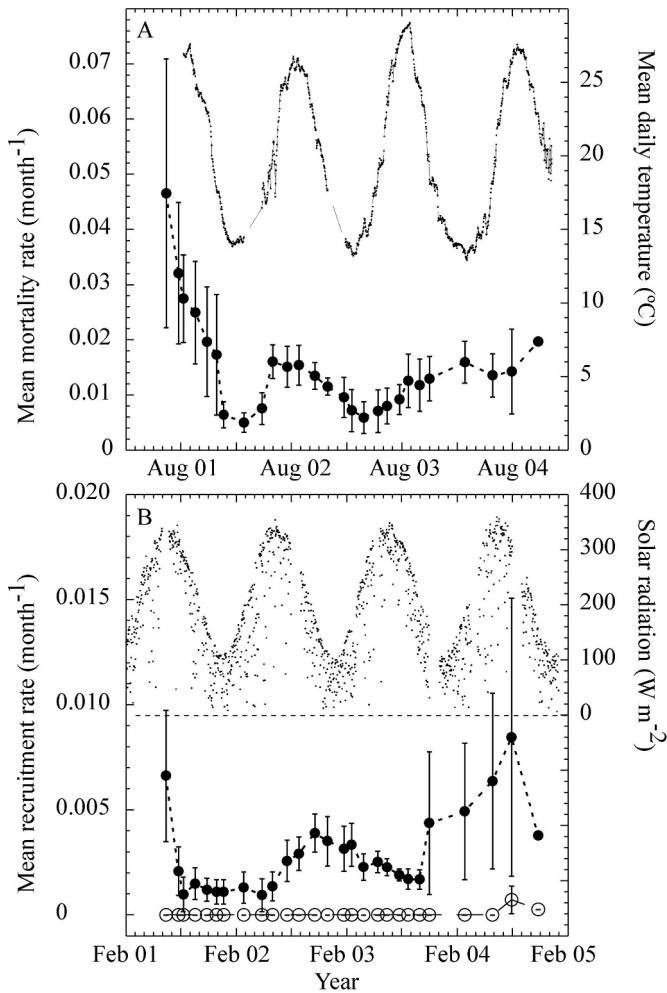
Fig. 6. (A) Temporal variability of the mean (±SE) monthly estimates of specific shoot mortality (black circles), and daily seawater temperatures (small dots, °C) in the meadow from August 2001 to December 2004. (B) Temporal variability of the mean (±SE) monthly estimates of specific shoot recruitment rates (black circles) and of seedling recruitment rates (open circles) and the daily solar radiation in the Palma Bay (small dots, INM data, in 10 kJ m^{-2} d^{-1}). Vertical bars represent standard errors.
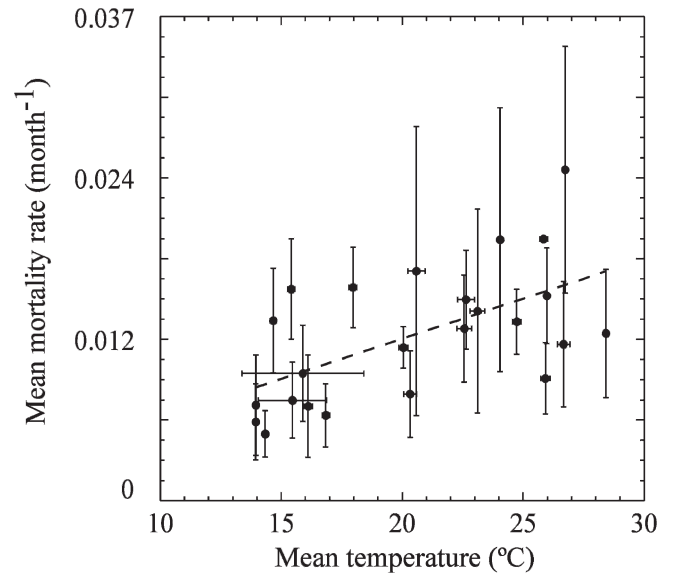

Fig. 7. The linear relationships between mean specific mortality rate and mean seawater temperature in consecutive visits to the meadow. The dashed line represents the fitted least squares regression equation. Horizontal and vertical bars represent standard errors of temperature and mortality, respectively.

positive summer temperature anomalies (Díaz-Almela et al. 2007). We observed, in winter 2004, many shoots that had a dead apical meristem (no leaves) but were still bearing developing, green infrutescences. This suggests that flowering in *P. oceanica* could constitute a response to stress generated by high temperature, as is the case in other organisms (Ofir and Kigel 2003; Nedelcu et al. 2004).

We did not detect any seasonal pattern in shoot recruitment. Vegetative recruitment appeared to occur continuously throughout the year. This is in contrast with previous results based on *P. oceanica* reconstructive growth (Caye and Rossignol 1983; Marbà et al. 1996a), which show that apex branching is maximal between February and May and has a second, lower maximum, between October and November. The failure to detect such a seasonal pattern in this study may arise from the extended periods in between censuses of the same plot group, the

rotary sampling, and the low frequency of shoot recruitment events (often one or no new shoots per plot per visit). The lack of correlation between vegetative recruitment rates and solar radiation coincides with observations from Marbà et al. (1996a) on apex branching rates, and is in contrast with observations in other seagrasses like *C. nodosa* and *Z. marina* (Marbà et al. 1996a). Sexual recruitment is strongly seasonal, leading to recruitment in early summer to early autumn (Buia and Mazzella 1991); however, sexual recruitment was so sparse that its seasonal contribution could not be observed in the trends in total recruitment.

The ratio of shoot recruitment to mortality increased from 2001 (0.08) to 2004 (0.37; Fig. 8B); this tendency and the enhanced recruitment in years of high mortality (2001 and 2004) suggest that, in *P. oceanica*, shoot recruitment can respond to stress and/or shoot mortality. Seagrass branching is stimulated by incident light on shoot meristems (Terrados 1997), which increases as shoot density declines (Duarte and Kalff 1987). Nevertheless, the shoot recruitment rates during the study did not suffice to balance mortality. Even the enhanced recruitment observed during the year 2004, when vegetative recruitment was one order of magnitude higher than that in previous years and sexual recruitment occurred for the first time in the study, was far from compensating for the increased mortality rates. Thus, positive temperature anomalies seem to affect *P. oceanica* population dynamics, enhancing recruitment through increased sexual reproduction (Díaz-Almela et al. 2007), and increasing shoot mortality. The results presented here suggest that the latter, negative effect prevails.

The annual maximal sea-surface temperature in the Bay of Palma has increased 1°C to 2°C from the period 1988–
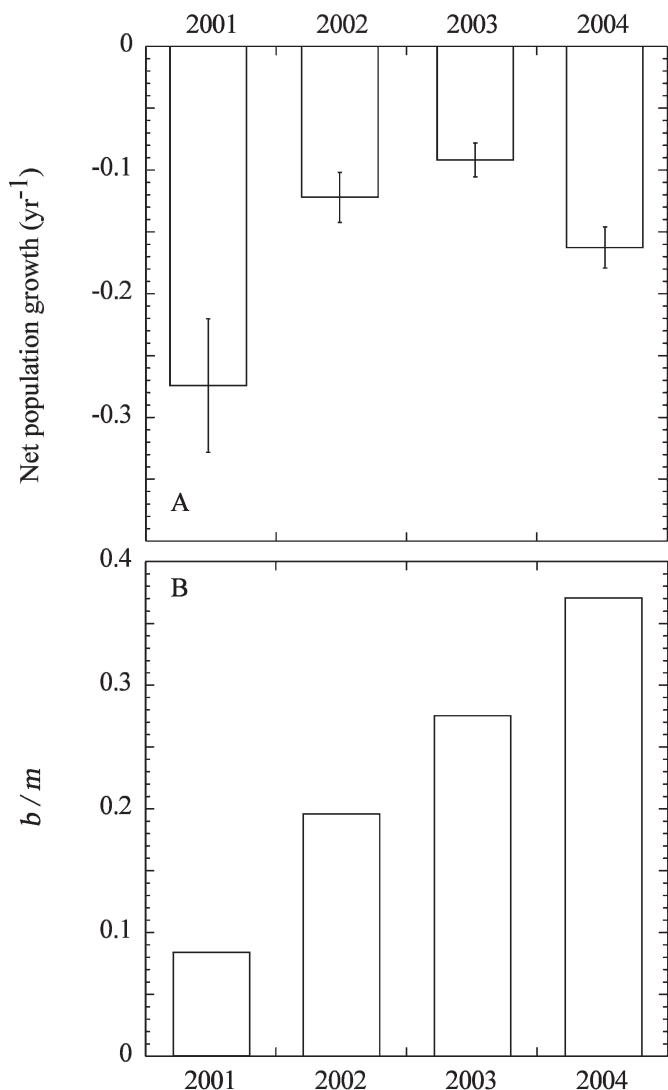
Fig. 8. (A) Mean annual net population growth rates, and standard errors, calculated from monthly estimates. (B) Ratio of mean annual recruitment to mean annual mortality for each year of the study.

1991 (26.4 ± 0.1°C; Jansá et al. 1994) to the period of this study (2001–2004: 28.1 ± 0.4°C; this study). This warming could account, at least partially, for the meadow decline observed in Magalluf. Given the proximity of the meadow to the tourist town of Magalluf, other sources of anthropogenic pressure cannot be excluded. From 1956 to 2001, parallel to the development of tourism in the area, the meadow of Magalluf experienced a slight areal loss of 3.8%, mainly affecting its shallow limit, and the median distance of the continuous meadow to the beach increased from 18 to 76 m (Sánchez 2003). However, our study site is 800 m from the beach, next to the desert islet of Sa Porrassa (Fig. 1). There are no evident signals of nutrient inputs or organic pollution in this area in the N isotopic ratios of the plants (Fourqurean et al. 2007). Slight organic pollution may occur for particular years, as leaf sulfur isotope abundance is occasionally lower than 20–21‰ (N.

Marbà, M. Holmer, C. M. Duarte unpubl. results), indicative of sulfide intrusions (Marbà et al. 2007). However, the sediments are undisturbed and have some of the lowest organic matter content, sulfide concentrations, and sulfate reduction rates measured in the region (Holmer et al. 2003; Calleja et al. 2007). Moreover, a retrospective analysis of shoot dynamics in this area of Magalluf, performed in year 2000, indicates that this part of the meadow has been in dynamic equilibrium during the past two decades (Holmer et al. 2003).

The decline observed in Magalluf is consistent with observations of a general decline of *P. oceanica* meadows in the Mediterranean (Marbà et al. 1996b, 2005), partially attributed to changes in temperature and water transparency (Duarte et al. 1999). Indeed, from the relationship of decline with water temperature reported here, we could expect that a 4°C increase, as predicted for the Mediterranean during the present century (IPCC 2001 *in* Cubash et al. 2001) would lead to steeper rates of decline, i.e., increased by a further 2.0% to 3.8% yr^{-1}. Taking into account the present average net decline of this species in the Western Mediterranean, which is around 5–10% yr^{-1} (Marbà et al. 2005), if such temperature-driven increase was general, it would represent a steep acceleration of decline of *P. oceanica* meadows. This is in agreement with the severe damage observed on the subtropical seagrass *Thalassia* spp. meadows, which were exposed to a local temperature increase of 4°C by heated effluents from power plants (Thorhaug et al. 1978). The increasing rates of decline with increasing temperature should be tested for other *P. oceanica* meadows to assess the effects of warming at the basin scale.

A sustained net *P. oceanica* decline has been demonstrated in this study along with an enhancement of vegetative and sexual recruitment following intensive flowering and shoot mortalities in autumn 2003–winter 2004. This suggests that sexual reproduction in *P. oceanica* may be a stress-mediated response. Our results identify global warming as a driver of the decline of *P. oceanica* meadows and raise concern about the future of these important ecosystems as Mediterranean warming proceeds further.

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