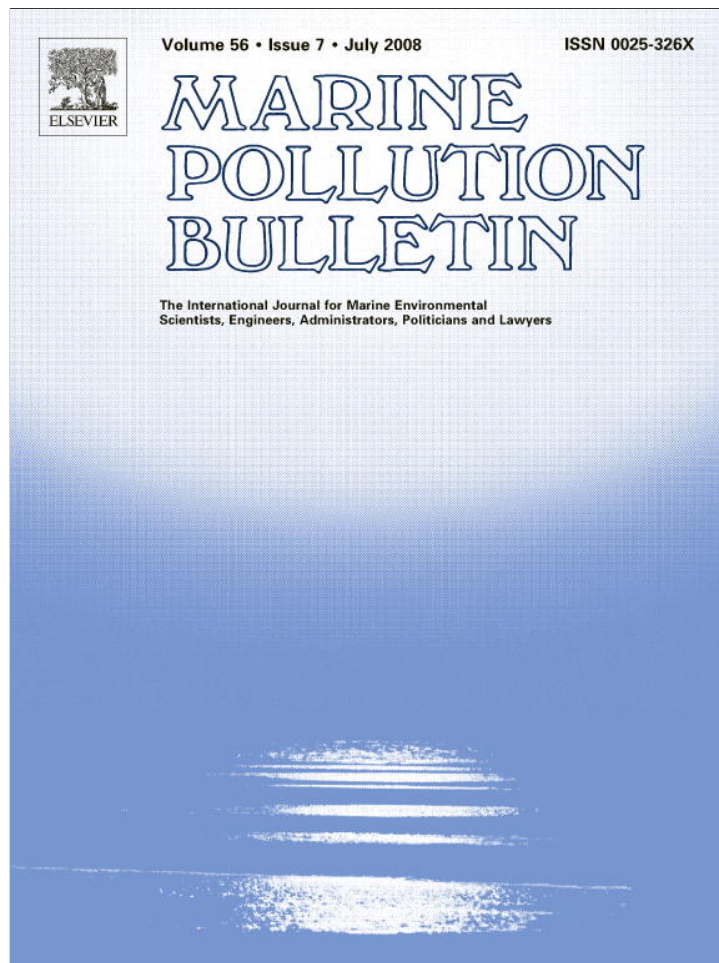


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journal homepage: www.elsevier.com/locate/marpolbulBenthic input rates predict seagrass (*Posidonia oceanica*) fish farm-induced decline

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ABSTRACT

Fish farms represent a growing source of anthropogenic disturbance to benthic communities, and efficient predictors of such impacts are urgently needed. We explored the effects of fish farm benthic organic and nutrient inputs on the population dynamics of a key seagrass species (*Posidonia oceanica*) in four Mediterranean deep meadows adjacent to sea bream and sea bass farms. We performed two annual plant censuses on permanent plots at increasing distance from farms and measured benthic sedimentation rates around plots. High shoot mortality rates were recorded near the cages, up to 20 times greater than at control sites. Recruitment rates increased in variability but could not compensate mortality, leading to rapid seagrass decline within the first 100 m from cages. Seagrass mortality increased with total sedimentation rates ($K = 0.55$, $p < 0.0002$), and with organic matter ($K = 0.50$, $p = 0.001$), total nitrogen ($K = 0.46$, $p = 0.002$) and total phosphorus ($K = 0.56$, $p < 3 \cdot 10^{-5}$) inputs. *P. oceanica* decline accelerated above a phosphorus loading threshold of $50 \text{ mg m}^{-2} \text{ day}^{-1}$. Phosphorus benthic sedimentation rate seems a powerful predictor of seagrass mortality from fish farming. Coupling direct measurements of benthic sedimentation rates with dynamics of key benthic species is proposed as an efficient strategy to predict fish farm impacts to benthic communities.

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1. Introduction

Marine fish farming has developed exponentially across the world, at a rate of $8.8\% \text{ yr}^{-1}$ between 1970 and 2004 (FAO, 2007) and this trend is expected to continue. Around the Mediterranean it has rapidly increased since 1990 and is predicted to grow by 5% annually over the next two decades (UNEP, 2002). In addition, more than half of Mediterranean fish farm production takes place in coastal waters (UNEP, 2002). Fish cages enhance the input of organic matter and nutrients to the water and nearby sediments, mainly through the release of fish faeces and excess feed pellets (e.g. Wu, 1995; Holmer et al., 2003a). Such organic loading immediately affect sediment biogeochemical processes in the vicinity (e.g. La Rosa et al., 2004; Frederiksen et al., 2005), through an increase in oxygen consumption (e.g. Holmer et al., 2002) and thereby promoting anaerobic degradation of organic matter (e.g.

Danovaro et al., 2000; Holmer et al., 2002; 2003b). The depletion of sediment oxygen and excess of reduced toxic products from anaerobic pathways (such as sulphides and ammonium) have an impact on benthic communities (e.g. Terrados et al., 1999; Karakassis et al., 2000; 2002; Ruiz et al., 2001; La Rosa et al., 2001; 2004; Mirto et al., 2002; Vezzulli et al., 2002; Holmer et al., 2003b).

Posidonia oceanica, the dominant and endemic seagrass species in the Mediterranean Sea, extends from 0.3 to 45 m depth in clear waters, which is also the region preferred for fish farm developments. *P. oceanica* is a key species sustaining meadow communities of high diversity (Templado, 1984), which provide important ecosystem functions and services (Hemminga and Duarte, 2000). Such services are being jeopardised by the tendency towards a substantial decline of these ecosystems (e.g. Marbà et al., 2005), faster than the $2\% \text{ yr}^{-1}$ global rate of decline of seagrass ecosystems (Duarte et al., in press).

Posidonia oceanica meadows are highly vulnerable to marine aquaculture activities (Holmer et al., 2003b), as reflected by large-scale losses of *P. oceanica* around shallow and sheltered fish

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farms (e.g. Dimech, 2000; Ruiz et al., 2001), which continue even after farming cessation and water quality recovery (Delgado et al., 1999). In an attempt to minimise the impact to the benthos, new farms have been established in recent years at deeper and more exposed sites. This strategy seems to have succeeded for some benthic communities (Maldonado et al., 2005) but not others (Borg et al., 2006; Lee et al., 2006). In particular, deep *P. oceanica* meadows, growing near their compensation point (Duarte, 1991) may be especially sensitive to fish farm loads (Verneau et al., 1995; Borg et al., 2006).

Posidonia oceanica, with its sparse sexual reproduction (e.g. Díaz-Almela et al., 2006; 2007), is the slowest-growing seagrass species (Marbà and Duarte, 1998), requiring centuries to (re)colonise coastal areas (e.g. Meinesz and Lefevre, 1984; Duarte, 1995; Marbà et al., 2002; Kendrick et al., 2005). Thus, any losses of *P. oceanica* meadows can be considered irreversible at managerial time scales. Moreover, previous investigations suggest that discontinuity of farming operations upon observation of losses in *P. oceanica* cover and density are inefficient remedial measures, as losses continue even after fish farm removal (Delgado et al., 1999). This is probably due to the slow recovery of sediment conditions (Delgado et al., 1999; Karakassis et al., 1999). It is, therefore, essential to develop accurate predictors of aquaculture-derived impacts to *P. oceanica* meadows and to detect early seagrass decline, in order to be able to act before irreversible losses occur. Seagrass cover and density, measured in random quadrats along transects, have been widely used in most monitoring programs as indicators of population disturbance (Short and Coles, 2001) and are good descriptors of fish farm-induced seagrass losses (e.g. Delgado et al., 1997, Ruiz et al., 2001). However, due to the large meadow patchiness in density and cover these techniques detect decline only after significant losses have occurred. Alas, such losses are then difficult to recover for a slow-growing species like *P. oceanica*. Successive measures of seagrass density within permanent plots are more sensitive to detect slow-growing seagrass dynamics, because the noise of meadow density patchiness is eliminated (Heidelbaugh and Nelson, 1996). On the other hand, valuable information on the mechanism of seagrass decline—such as whether it occurs through increased shoot mortality and/or reduced recruitment—may be obtained through shoot tagging (Marbà et al., 2005; Martínez-Daranas et al., 2005).

Detailed individual studies have given much insight on the ways fish farms induce seagrass decline (e.g. Delgado et al., 1997; 1999; Ruiz et al., 2001; Karakassis et al., 2002; Crawford et al., 2003), and common patterns and mechanisms have been identified among sites (Pergent-Martini et al., 2006). However, it is still not easy to quantitatively predict the impacts of any one farm in the surrounding communities, or to define distance or effluent thresholds for sustainable exploitations. Aquaculture effluents are unanimously considered the main drivers of benthic impacts (e.g. Wu, 1995; Dimech et al., 2000; Cancemi et al., 2003). A general quantitative approach, linking fish farm loading rates to benthic impacts on a given ecosystem across sites could provide such predictive tool (Holmer et al., 2003a).

In the present effort we examine the impacts of farming activities on shoot population dynamics of a key seagrass (*P. oceanica*) across four deep (16–28 m) fish farms around the Mediterranean. We aim to establish a general relationship connecting *P. oceanica* population dynamics with benthic organic loading and nutrient input rates from caged fish farms. This relationship could allow us to predict loading thresholds that would significantly increase the impacts of Mediterranean fish farms on *P. oceanica* meadow systems.

2. Materials and methods

We assessed the demography of the seagrass (*P. oceanica*) in deep meadows growing around four fish farms, widely distributed along the Mediterranean, from Cyprus to Spain (Fig. 1). The sediments were fine to coarse grained and carbonate-rich (41–46% in Cyprus, >75% at other sites, Holmer and Frederiksen, 2007) and the water depths varied between 16 and 28 m (Table 1). All the farms initiated their activities in the 1990s (Table 1); they consisted of 20–24 net cages with an annual production of 260–1150 tons (Table 1). The cultured species were gilthead (*Sparus aurata*), sharpnose sea bream (*Diplodus puntazzo*) and sea bass (*Dicentrarchus labrax*), which were fed with dry pellets (more information in Holmer et al., 2007). The farms in Cyprus, Italy and Spain were located on open coasts about 1 km from shores, whereas the farm in Greece was located in a strait about 300 m from shore (Fig. 1). In all the sites, the main currents were parallel to the coast, ranging from 8.59 cm s⁻¹ (Greece) to more than 20 cm s⁻¹ (Italy,

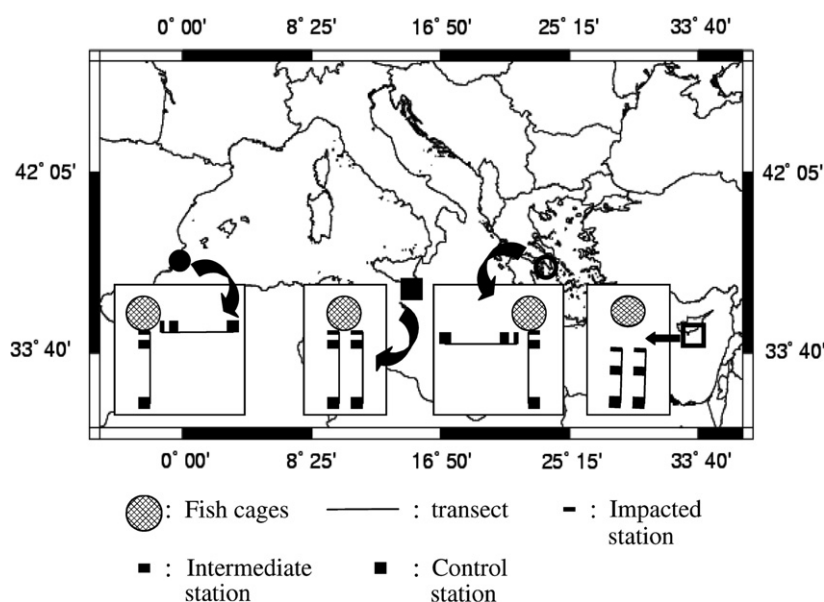


Fig. 1. Locations of the fish farm sites analysed in this study. Filled circle: El Campello (Spain), filled square: Porto Palo (Italy), open circle: Sounion (Greece), open square: Amathous (Cyprus). Outline of the sampling strategy within each site: each station was composed of three permanent plots.

Table 1
Characteristics of the Mediterranean fish farm studied

	Cyprus	Greece	Italy	Spain
Location	Limassol	Sounion	Porto Palo	El Campello
Fish farm initiated in:	1992	1996	1993–94	1995
Annual prod. (Tm)	150 (300 since 2001)	400	1150	260
FCR*	2.2	1.60	2.39	2.00
Mean current speed (cm s ⁻¹)	10–15	8.9	>20 (40% of time)	9.8
Depth (m)	19–20 (fish cages: 39 m)	14–16	21–22	26–28
Initial census date	June 2002	June 2003	September 2002	October 2002
Days between censuses	386	353	307	312
Benthic input sampling	June 2002 July 2003 October 2003	June 2003 June 2004	September 2002 July 2003	September 2003

* FCR, feed conversion ratio (the ratio of food supplied to fish production by weight).

Table 1, Holmer et al., 2007). The existence of a healthy meadow in the presently bare areas, before fish farm initiation has been reported in Sounion (Greece, Karakassis et al., 2000) and in Spain, where the meadow of El Campello is recovering from past intensive otter-trawling (Guillen, 1994; Gonzalez-Correa et al., 2005). Further information on fish farm characteristics and on their environmental effects can be found in Table 1 and elsewhere (Frederiksen, 2005; Pitta et al., 2006; Holmer et al., 2007).

In each site two transects extending from the edge of the meadow closest to the farm to 800 or 1200 m away, were established. In Cyprus and Italy both transects extended parallel, in the direction of the main current, while in the Greek and Spanish sites, in order to explore a wider set of conditions, one transect was extended in the direction of the main current while the other was perpendicular to it, towards the coast (Fig. 1).

At each of these transects, we defined three stations based on knowledge from previous studies (e.g. Pergent et al., 1999; Dimech et al., 2000) and on quantitative (density and cover) and qualitative (leaf length, epiphytes) observations of meadow structure (already affected around fish cages after years of activity). A “disturbed station” was installed in the area vegetated by *P. oceanica* that was closest to the fish farm. This front was located, at the time of the study, 5–15 m away from the net cages across sites, where sparse plants were found. An “intermediate station”, installed at 20–50 m distance from fish cages, where seagrass beds were denser but not yet comparable to those found further away from the farm. Finally, a “control station”, located at 800–1200 m distance to fish cages, where no impacts were evident upon visual inspection. This pattern differed at the Cyprus site, since the fish cages were located over deeper bottoms (40 m depth). Although extremely sparse *P. oceanica* shoots were found close to those cages, it was impractical to census them due to their depth. Moreover, *P. oceanica* formed sparse patches until 300 m away from the cages, towards the shore, forming then a continuous meadow from 20 m depth upwards. Therefore, at this site, the stations were installed 300, 400 and 1000 m away from fish cages, respectively, parallel to the coast (Fig. 1).

2.1. *Posidonia oceanica* demography

Within each station we installed three permanent plots at the bottom, by SCUBA diving, using metal sticks, ropes and buoys, as explained in Marbà et al. (2005). The size of the triplicate quadrats was adjusted to encompass at least 100 shoots per quadrat (from 0.25 m² in “control” stations to 7 m² in Spanish “disturbed” stations). We performed two direct censuses of the shoots present within those permanent plots in each site. Censuses were separated by a period of about one year (from 307 to 386 days, Table 1). During each census, we counted the total number of alive shoots within the plots. As shoot recruitment in *P. oceanica* occurs

by apical branching of vertical and horizontal shoots (the latter called apices), we counted the recently bifurcated vertical shoots and all the apices among the total shoot population within the plot and tagged them 2 cm below the meristems with plastic cable ties (10 cm long). Therefore we modified the procedure described in Marbà et al. (2005) to allow a most efficient use of the limited bottom time of SCUBA diving at those depths. Tagging allowed us to discriminate the new recruits (unmarked bifurcated shoots and apex bifurcations) in the second census, the new apices (produced by transformation of shoots from vertical to a horizontal growth mode or by the entering of a horizontal apex from outside the plot and, thus, lacking marks) and to deduce the total, surviving shoots and apices. We calibrated the counting error by counting two plots by independent observers, yielding an estimated error of ±0.2% and ±3.5% of the total shoot population for recruits and lost shoots, respectively.

The repeated censuses provided direct estimates of specific rates (yr⁻¹) of shoot mortality and recruitment and net population growth, as well as the expected time to lose half of the shoots at each station.

The specific shoot mortality rate (M in year⁻¹, yr⁻¹) was calculated as:

$$M = -\frac{(\ln N_{S1}/N_{t0}) \cdot 365}{t_1 - t_0}, \quad (1)$$

where N_{t0} is the total number of shoots (vertical and horizontal apices) counted in the initial census (t_0 , days) at each plot, N_{S1} the total number of survivor shoots (vertical and apices) at the second census (t_1 , days).

The specific shoot recruitment rate (R , in yr⁻¹) was estimated as:

$$R = \frac{\ln((N_{r1} + N_{S1})/N_{S1}) \cdot 365}{t_1 - t_0}, \quad (2)$$

where N_{r1} is the total number of recruited shoots (i.e. bifurcated vertical shoots and apices) observed at t_1 , and N_{S1} is the number of survivors at t_1 . Specific net population growth rates (μ) were estimated as:

$$\mu = R - M = \frac{\ln(N_{t1}/N_{t0})}{t_1 - t_0}, \quad (3)$$

where N_{t1} is the total number of shoots present at t_1 .

2.2. Sedimentation rates

We measured benthic sedimentation rates at each station and site by deploying benthic sediment traps next to the plots, 1 to 3 times in either summer (the season of maximum production in the farms), for about 48 h periods. The sediment traps were designed after Gacia et al. (1999), and consisted of two replicated

arrays situated 20 cm above the bottom, each supporting five 20 ml cylindrical glass centrifugation tubes with an aspect ratio of 5 (16 mm diameter), in order to minimise internal re-suspension. The contents of 1–3 tubes were combined and collected on a combusted, pre-weighed Whatman GF/F filter. Dry weight of total sediment deposition was obtained after drying the filters at 60 °C to constant weight. Dry weight of organic matter (OM) deposition was measured through combustion of some of the filters. Total phosphorus (TP) was obtained after boiling combusted materials in 1 M HCl for 15 min followed by spectrophotometric determination of phosphate (Koroleff, 1983). We analysed the un-combusted filters for total nitrogen (TN) contents with an elemental analyser (Iso-Analytical Ltd., United Kingdom). Further information on these analyses and spatial patterns of fish farm inputs are shown in Holmer et al. (2007). We estimated total matter, OM, TN and TP sedimentation rates from these measures according to Blomqvist and Håkanson (1981) and Hargrave and Burns (1979), as described in detail in Gacia et al. (1999).

2.3. Statistical analyses

Shoot density changes within stations and between censuses were tested using paired-sample *t*-tests (initial and final shoot density within each plot). Differences in shoot mortality, recruitment and net population growth rates among sites, transects and stations were analysed through full factorial GLM-REML analyses (with JMP for MacOSX®, transect as a random factor and plot as residual). Data were log transformed to meet the requirement of normal distribution. Previous to each analysis, Levene's test for variance homogeneity was performed. When this test was significant, we chose the Dunnett *t*3 test (equal variances not assumed) for post-hoc comparisons between stations, otherwise, the Tukey post-hoc test was used.

Relationships between distance to fish cages, benthic sedimentation rates and shoot population dynamics were also assessed with GLM analyses on station-averaged data. First, the variation of benthic sedimentation rates (total, organic, N or P sedimentation rates) with distance to cages was examined (site as factor, distance as covariate). Secondly, the dependence of demographic data (*m*, *r*, *μ*) on distance or benthic sedimentation rates (total, organic, N or P sedimentation rates, as covariate) was examined. Dependent variables and covariates were log_e transformed when necessary. Initially, the significance of site, the covariate (distance or sedimentation rate) and the interactive effects distance * site (or sedimentation rate * site) were tested. If the site or the interactive effect had a *p* > 0.25 in the model, the analysis was repeated pooling that effect with the residual. This "sometimes pooling" procedure was prosecuted sequentially as far as any effect in the model had a *p* > 0.25. The procedure yielded parameter estimates, their probability and the percentage of the variance explained by the retained model. Finally, we performed bivariate analyses, to obtain the Kendall non-parametric correlation coefficients of demographic parameters with distance and benthic inputs.

3. Results

The meadows varied broadly in shoot density among sites, but a general pattern of density reduction towards cages was observed, except in Cyprus, where the stations closest to the cages were situated 300 m away (Fig. 2). Intensive shoot decline rates were recorded next to the cages in Sounion (Greece) and Porto Palo (Italy). In these sites, the shoot densities at impacted stations (15 and 5 m from cages, respectively) decreased from 102 to 14 shoots m⁻² (90% of reduction, paired-samples *t*-test: *p* < 0.02) and 128 to 22 shoots m⁻² (81% of reduction, *p* < 0.05) in 353 and

307 days, respectively (Fig. 2 and Table 2). In El Campello (Spain), the deepest location, there was a modest density decline in 312 days, which was only significant at the impacted (10 m distance to the cages, paired-samples *t*-test, *p* = 0.03) and intermediate (40 m to the cages, *p* = 0.04) stations of one transect (the one perpendicular to the coast and to the main current, Fig. 2 and Table 2). In Cyprus, shoot density only declined significantly in one of the impacted stations (*p* < 0.04), and this decline was much lower than at the impacted stations from the other sites (Fig. 2 and Table 2). Shoot density in control stations was much more stable. Significant reductions in shoot density were only observed in one control station in Greece (*p* < 0.05, in the transect parallel to the coast) and at the control stations of Italy (only when data from both, parallel transects, were pooled, Fig. 2). Shoot density slightly increased at the control station in one transect of the Spanish farm (*p* = 0.003, Fig. 2 and Table 2). Intermediate stations exhibited intermediate behaviours; except in Greece, where the plots situated perpendicular to the current, experienced a large (30%, *p* < 0.02, Fig. 2 and Table 2) increase in shoot density. In Italy, the shoot densities in the intermediate station at the second visit attained the levels of the shoot densities measured at the impacted station in the initial census (*t*-test, non-significant difference: *p* < 0.98).

Shoot density decline was produced through high shoot mortality rates, reaching 4.19 ± 1.77 (SE) yr⁻¹ at one of the impacted stations in Italy (Fig. 3). Shoot mortality rates were significantly different among sites (*p* < 0.0001, Fig. 3 and Table 3) and stations (*p* < 0.0001), following the general pattern: Impacted > (*p* < 0.01) Intermediate > (*p* < 0.01) Control (Fig. 3 and Table 3). Shoot mortality rates were, on average, 7.5 (Spain) to 19.4 times (Greece) higher at the impacted stations adjacent to fish cages than at control stations (Fig. 3 and Table 2). No significant differences in mortality were found between transects (*p* = 0.08, Table 3).

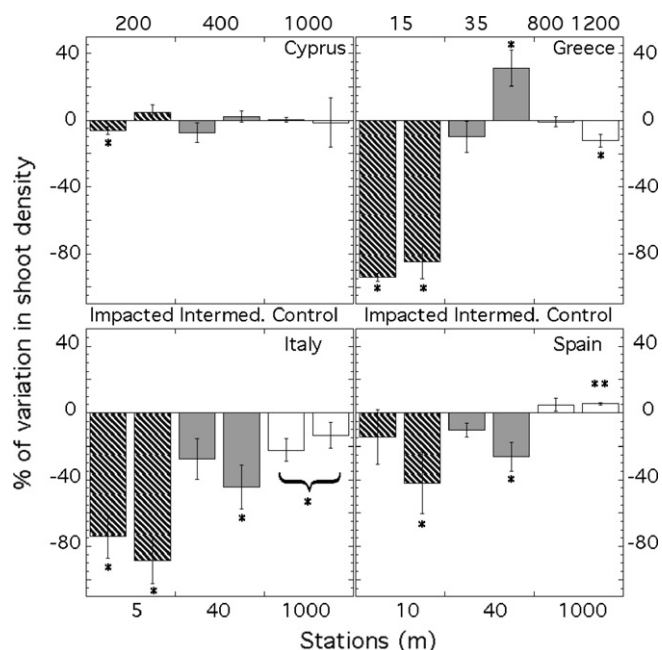


Fig. 2. Average (±standard error) variation (%) in *P. oceanica* shoot density at impacted (lined bars), intermediate (grey bars) and control stations (white bars) in each site, measured between initial and final censuses. Within each station, the left bar corresponds to station from transect 1, and the right one to station from transect 2. *t*-Test significance levels are indicated (*: *p* < 0.05, **: *p* < 0.01), as well as the distances of the stations from fish cages. At the Italian site, density changes at the impacted station are significant only when data from both transects are merged.

Table 2
Initial (N_0) and final (N_1) shoot densities within each station at each site (means \pm standard errors of three plots per station; I_1 : impacted of transect 1, IN_2 : intermediate of transect 2, C_1 : control of transect 1), specific shoot mortality (m), recruitment (r) and net population growth (μ) rates at each station

	$N_0 \pm$ (shoots SE m ⁻²)	$N_1 \pm$ (shoots SE m ⁻²)	$m \pm$ (year SE ⁻¹)	$r \pm$ (year SE ⁻¹)	$\mu \pm$ (year SE ⁻¹)
Cyprus					
I_1	484 \pm 23	455 \pm 32	0.21 \pm 0.09	0.15 \pm 0.07	-0.06 \pm 0.02
I_2	424 \pm 96	437 \pm 83	0.16 \pm 0.08	0.20 \pm 0.12	0.04 \pm 0.04
IN_1	505 \pm 86	473 \pm 111	0.22 \pm 0.05	0.15 \pm 0.01	-0.08 \pm 0.06
IN_2	384 \pm 28	392 \pm 20	0.29 \pm 0.07	0.31 \pm 0.05	0.02 \pm 0.02
C_1	559 \pm 87	560 \pm 86	0.09 \pm 0.04	0.09 \pm 0.04	0.00 \pm 0.01
C_2	423 \pm 37	423 \pm 90	0.30 \pm 0.08	0.27 \pm 0.07	-0.03 \pm 0.15
Greece					
	$I < IN < C$	$I < IN < C$	$I \gg IN = C$	$I < C < IN$	$I < IN = C$
I_1	98 \pm 24	6 \pm 3	3.24 \pm 0.62	0.01 \pm 0.01	-3.23 \pm 0.62
I_2	106 \pm 26	18 \pm 15	2.35 \pm 0.68	0.02 \pm 0.02	-2.33 \pm 0.70
IN_1	287 \pm 14	258 \pm 14	0.29 \pm 0.01	0.15 \pm 0.05	-0.14 \pm 0.07
IN_2	196 \pm 26	254 \pm 23	0.13 \pm 0.07	0.25 \pm 0.03	0.12 \pm 0.09
C_1	372 \pm 47	367 \pm 36	0.07 \pm 0.02	0.06 \pm 0.01	-0.01 \pm 0.03
C_2	357 \pm 24	312 \pm 10	0.21 \pm 0.05	0.07 \pm 0.02	-0.15 \pm 0.05
Italy					
	$I = IN < C$	$I < IN < C$	$C \ll I > IN = C$		$I > IN = C$
I_1	152 \pm 42	36 \pm 21	2.09 \pm 0.80	0.15 \pm 0.11	-1.94 \pm 0.88
I_2	105 \pm 17	10 \pm 12	4.19 \pm 1.77	0.01 \pm 0.02	-4.17 \pm 1.79
IN_1	160 \pm 7	115 \pm 18	0.42 \pm 0.21	0.01 \pm 0.01	-0.41 \pm 0.22
IN_2	253 \pm 61	143 \pm 50	0.84 \pm 0.27	0.09 \pm 0.04	-0.75 \pm 0.31
C_1	357 \pm 62	273 \pm 36	0.33 \pm 0.12	0.02 \pm 0.02	-0.31 \pm 0.10
C_2	432 \pm 27	376 \pm 51	0.22 \pm 0.12	0.05 \pm 0.02	-0.18 \pm 0.10
Spain					
	$I < IN = C$	$I < IN < C$	$I = IN \gg C$		$I = IN \gg C$
I_1	11 \pm 4	9 \pm 3	0.55 \pm 0.23	0.20 \pm 0.05	-0.21 \pm 0.23
I_2	12 \pm 4	8 \pm 4	0.86 \pm 0.35	0.12 \pm 0.09	-0.74 \pm 0.43
IN_1	55 \pm 6	49 \pm 3	0.36 \pm 0.16	0.11 \pm 0.06	-0.13 \pm 0.05
IN_2	58 \pm 4	43 \pm 6	0.45 \pm 0.15	0.08 \pm 0.02	-0.37 \pm 0.14
C_1	60 \pm 6	63 \pm 7	0.06 \pm 0.03	0.11 \pm 0.02	0.05 \pm 0.04
C_2	70 \pm 4	73 \pm 4	0.13 \pm 0.02	0.19 \pm 0.02	0.06 \pm 0.01

In the stations were shoot densities significantly differ between censuses (paired t -tests), the net population growth rates are marked in bold. When significant differences among stations are obtained through ANOVA analyses within each site, patterns of differences among stations (I, IN, C) are obtained from Tukey post-hoc test (equal variances assumed, when Levene's test of homogeneity of variances is not significant), or from Dunnett t_3 post-hoc test (equal variances not assumed, if Levene's test is significant) " \gg " or " \ll ": $p < 0.01$, " $>$ " or " $<$ ": $p < 0.05$.

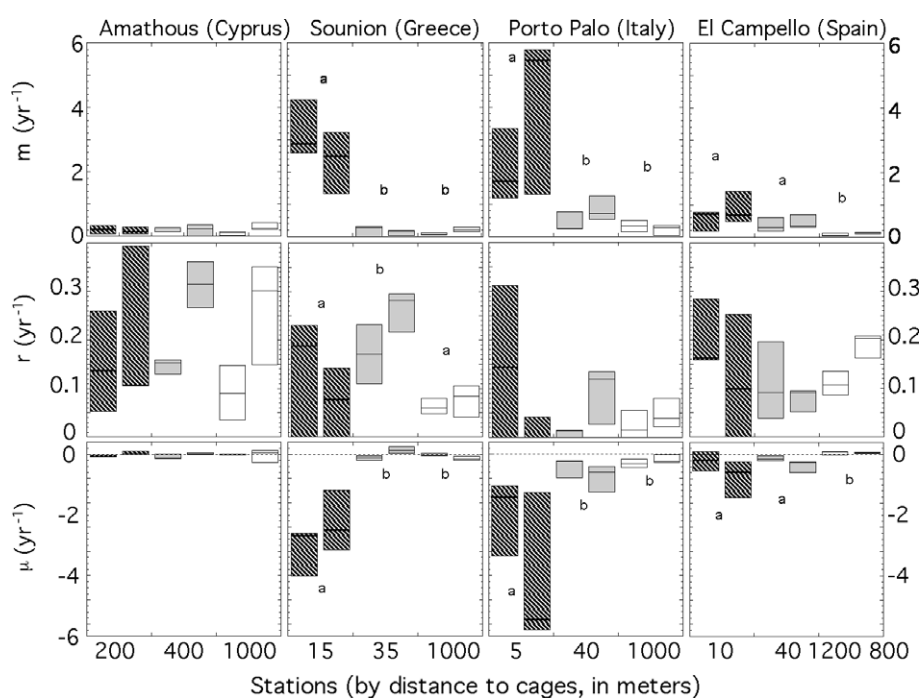


Fig. 3. Distribution of *P. oceanica* shoot demographic parameters (m , specific shoot mortality; r , recruitment and μ , net population growth rates) with distance from the fish cages for the various fish farms examined. The boxes represent the range of shoot mortality, recruitment and net population growth within the station, with upper, middle and lower horizontal lines of the boxes indicating the values recorded in each of the three station plots. Lined bars correspond to impacted stations, grey bars to intermediate stations and white bars to control stations. Within each station, the first bar corresponds to transect 1 and the second bar to transect 2.

Table 3
Results of the full factorial GLM analysis on shoot mortality ($\ln(m)$, raw plot data)

Source	Type III sum of squares	df	Mean square	F	p
Station	45.78	2	22.89	49.66	0.0001
I \gg IN, I \gg C; IN \gg C					
Site	16.82	3	5.61	12.16	0.0001
Italy \gg Cyprus					
Transect	1.53	1	1.53	3.31	0.08
Station * Site	22.53	6	3.76	8.15	0.0001
Station * Transect	1.61	2	0.81	1.75	0.19
Site * Transect	1.40	3	0.47	1.01	0.40
Station * Site * Transect	6.89	6	1.15	2.49	0.04

The fraction of the total variance explained by the model: $R^2 = 0.73$. Dunnett t3 post-hoc comparisons (equal variances not assumed) among stations and sites: " \gg " = $p < 0.01$.

Relative shoot recruitment rates ranged from 0.01 to 0.31 yr^{-1} , among sites and stations (Table 2). No significant structure was detected in shoot recruitment among stations ($p = 0.15$), but there were significant differences among sites ($p < 0.0001$) and between transects ($p = 0.04$). There were also significant interactions between site and station, as well as between station and transect ($p < 0.01$ for both effects). The variability of recruitment rate increased near the cages (Fig. 3). The observed shoot recruitment near fish cages was between 3 and 300 times lower than mortality and therefore could never compensate the losses. This led to high net decline rates of the shoot population in most impacted and intermediate stations, which closely followed mortality rates (Fig. 3 and Table 2).

Shoot mortality, net population decline rates and consequently, shoot half-life decreased as a power-law of the distance to cages ($R^2 = 0.66$, $p < 10^{-4}$; Fig. 4 and Table 4), and no significant effects of site ($p > 0.18$) or site \times distance were detected ($p > 0.87$).

Total benthic sedimentation rate exponentially declined with distance to cages ($R^2 = 0.79$, $p < 0.0001$ Fig. 5). There was also a significant effect of site ($p < 0.004$), but no significant effect of site \times distance was detected ($p = 0.30$). Organic matter (OM) inputs

decreased as a power-law with distance ($R^2 = 0.61$, $p < 0.03$), with a strong site effect ($p < 0.001$, Fig. 5). Total nitrogen (TN) inputs decreased exponentially with distance ($R^2 = 0.63$, $p < 0.02$), but with significant site ($p < 0.01$) and site \times distance ($p < 0.01$) effects (Fig. 5). On the contrary, TP benthic inputs decreased exponentially with distance to the cages ($R^2 = 0.57$, $p < 0.0002$) with no significant effects of site ($p > 0.16$) or site \times distance ($p > 0.30$, Fig. 5).

Specific shoot mortality rate increased as a power-law of TP sedimentation rate ($p < 0.0001$, $R^2 = 0.57$, Fig. 6 and Table 4) and exponentially with TN sedimentation rate ($p < 0.002$, $R^2 = 0.34$, Fig. 6 and Table 4). In both cases no significant effects of site or site \times benthic inputs were detected. Specific mortality rate also increased exponentially with total sediment inputs ($R^2 = 0.61$, $p < 10^{-4}$), with a significant site effect ($p < 0.04$). Finally, mortality also increased exponentially with OM inputs ($R^2 = 0.69$, $p < 0.001$), but there was significant effect of site ($p < 0.002$) and site \times OM inputs interaction ($p < 0.02$), with a steeper effect in Greece (Fig. 6 and Table 4).

Shoot recruitment did not vary with distance ($R^2 = 0$, $p > 0.60$, Table 4), or benthic inputs (Table 4). There was only a significant negative relationship between shoot recruitment and OM inputs ($R^2 = 0.54$, $p < 0.01$, Table 2), with significant site effect ($p < 0.01$) and interaction between site and OM inputs ($p < 0.01$, steeper reduction of recruitment at the Italian farm). But the non-linear Kendall correlation coefficient was non-significant ($K = -0.20$). Net population decline ($-\mu$) showed the same dependence patterns with benthic inputs and distance as those found for mortality rates (Fig. 6 and Table 4).

4. Discussion

The results presented here clearly demonstrate that fish farms affect deep *P. oceanica* meadows growing in open coasts, and that these impacts are still progressing, c.a. 10 years after farm onset. In less than 1 year, the extension of bare sediment and vegetated area with reduced shoot density around cages has sensibly increased in the Greek and the Italian sites. In the latter (the largest

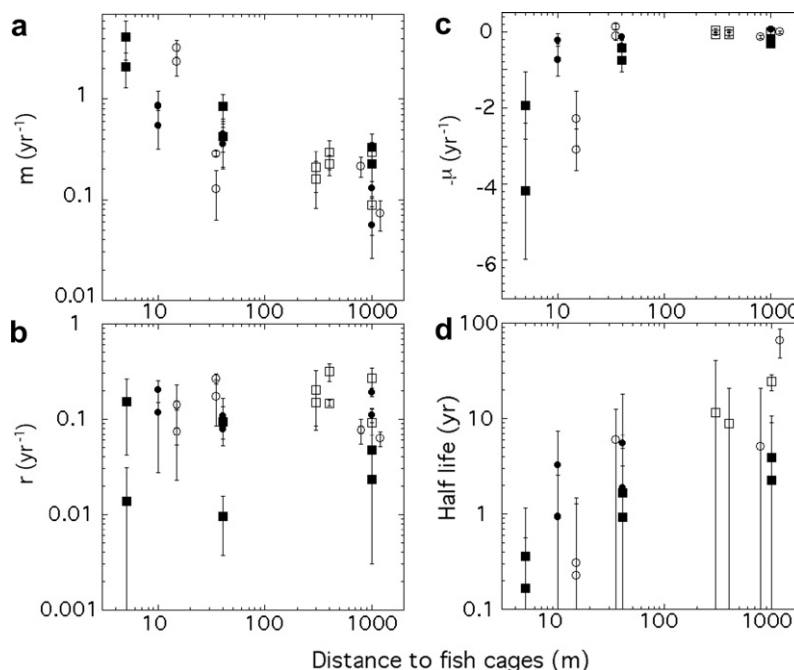


Fig. 4. Variability of *P. oceanica* shoot population dynamics (specific shoot mortality, m ; recruitment, r and net population growth rates, μ , year^{-1}) with distance (m) from the fish cages. All parameters, except μ are represented on a logarithmic scale. Symbols correspond to the sites as represented in Fig. 1.

Table 4
Models describing the significant relationships between *P. oceanica* shoot mortality, shoot recruitment and net declining rates (year⁻¹) with distance from the fish farm, total, organic matter, nitrogen and phosphorus input rate

	Mortality rate (yr ⁻¹)	Recruitment rate (yr ⁻¹)	Net growth rate (yr ⁻¹)
Total sed. rate (g(DW) m ⁻² day ⁻¹) (n = 24)	$R^2 = 0.61^{***}$, $K = 55^{***}$ Sed. rate ^{***} : $b = 0.36 \pm 0.07^{***}$ $a = -3.32 \pm 0.46^{***}$ Site [*] : Greece: $a + 0.91 \pm 0.30^{**}$	$K = -0.08$ ns	$R^2 = 0.63^{***}$, $K = -0.46^{**}$ Sed rate ^{***} : $b = 0.12 \pm 0.04^{**}$ $a = -0.50 \pm 0.22^s$ site \times sed. rate (ns, $p = 0.08$) Site [*] : Greece: $a + 0.60 \pm 0.15^{***}$
OM sed. rate (g(DW) m ⁻² day ⁻¹) (n = 24)	$R^2 = 0.69^{**}$, $K = 50^{***}$ OM sed ^{***} : $b = 0.99 \pm 0.23^{***}$ $a = -2.70 \pm 0.35^{***}$ Site \times OM sed [†] : Greece: $b + 1.75 \pm 0.71^{**}$ Cyprus: $b - 1.39 \pm 0.46^{**}$ Site ^{**} : Greece: $a + 2.84 \pm 0.59^{***}$ Cyprus: $a - 1.22 \pm 0.46^*$	$R^2 = 0.54^{**}$, $K = -0.20$ ns OM sed [†] : $b = -0.66 \pm 0.25^*$ $a = -1.96 \pm 0.37^{***}$ Site \times OM sed [†] : Greece: $b - 1.81 \pm 0.54^{**}$ Italy: $b + 0.82 \pm 0.30^*$ Site ^{**} : Greece: $a - 2.2 \pm 0.63^{**}$ Cyprus: $a - 1.22 \pm 0.46^*$	$R^2 = 0.82^{***}$, $K = -0.49^*$ OM sed [†] : $b = 0.48 \pm 0.08^{***}$ $a = -0.41 \pm 0.12^{**}$ Site \times OM sed [†] : Greece: $b + 0.86 \pm 0.17^{***}$ Cyprus: $b - 0.49 \pm 0.15^{**}$ Site ^{***} : Greece: $a + 1.41 \pm 0.20^{***}$ Cyprus: $a - 0.52 \pm 0.15^{**}$ Italy: $a - 0.44 \pm 0.13^{***}$
N sed. rate (g(DW) m ⁻² day ⁻¹) (n = 24)	$R^2 = 0.34^{**}$, $K = 46^{**}$ N sed. rate ^{**} : $b = 16.25 \pm 4.49^{**}$ $a = -1.82 \pm 0.30^{***}$	$K = -0.3^*$ N sed. rate: ns	$R^2 = 0.30^{**}$, $K = -0.51^{***}$ N sed. rate ^{**} : $b = 6.63 \pm 1.99^{**}$ a (ns, $p > 0.9$)
P sed. rate (mg(DW) m ⁻² day ⁻¹) (n = 23)	$R^2 = 0.57^{***}$, $K = 54^{***}$ P sed. rate [†] : $b = 0.65 \pm 0.12^{***}$ $a = 1.76 \pm 0.51^{**}$	$K = -0.13$ ns	$R^2 = 0.50^{***}$, $K = -0.51^{***}$ P sed. rate [†] : $b = 0.28 \pm 0.06^{***}$ $a = 1.50 \pm 0.25^{***}$
Distance (m) (n = 24)	$R^2 = 0.66^{***}$, $K = -0.59^{***}$ Distance ^{***} : $b = -0.47 \pm 0.08^{***}$ $a = 1.24 \pm 0.40^*$ Site (ns, $p < 0.18$)	$K = 0.004$ ns	$R^2 = 0.53^{***}$, $K = 0.48^*$ Distance ^{***} : $b = -0.17 \pm 0.04^{***}$ $a = 1.12 \pm 0.20^{**}$ Site (ns, $p < 0.16$)

The relationships are exponential ($y = e^{ax^{bx}}$) or power-law ($y = e^{ax^b}$, for variables marked in italics). Parameters a and b are given with standard errors.

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

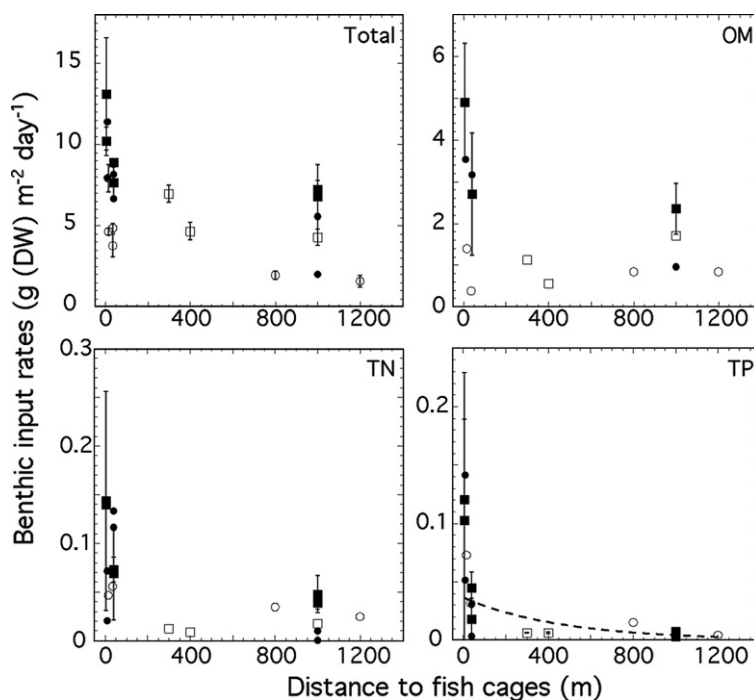


Fig. 5. Benthic sedimentation rates (g (dry weight) m² day⁻¹) in relation to distance to fish cages (m). OM, organic matter; N, nitrogen; P, phosphorus. Lines correspond to the exponential regressions of the simple models (where no significant effects of site or site \times distance where found). Data symbols correspond to the sites as represented in Fig. 1.

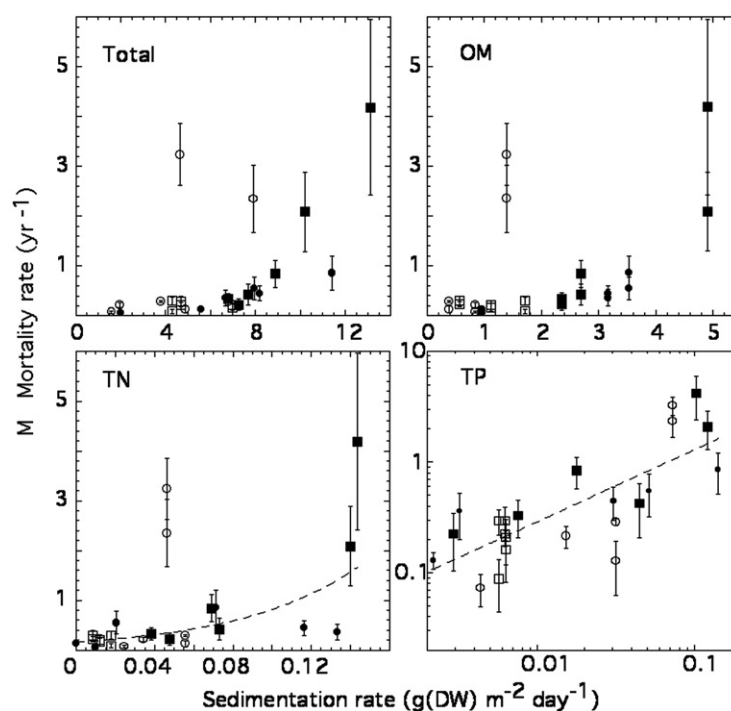


Fig. 6. The relationship between *P. oceanica* specific mortality (m , year⁻¹) and sedimentation rates of total, organic matter (OM), total nitrogen (TN) and total phosphorus (TP). Axes x and y are in logarithmic scale for TP, and linear for the rest. Lines show the fitted regression lines for the simple models (with no significant effects of site or site \times input rate, Table 4). Data symbols correspond to the sites as represented in Fig. 1.

fish farm with the highest production), shoot density at the intermediate station (40 m from cages) reached the levels recorded at impacted stations (5 m) after 1 year. A similar, strong regression of the *P. oceanica* meadow is reported by Delgado et al. (1997, 1999) around a small fish farm on a shallow sheltered site.

Shoot mortality and decline rates rapidly decreased with distance from the farms. The adjusted power-law relationship suggested a reduction by half of those parameters at 80 m distance, when compared to the rates beneath the cages. The seagrass shoot half-life significantly increased within the first 100 m from the cages. Beyond this distance, decline seems much slower. Nevertheless as the curve of mortality did not reach the global (mean \pm SD) recruitment rates (0.13 ± 0.10 year⁻¹) until 400 m from the cages, the complete balance of seagrass dynamics ($R = M$) may be only achieved beyond this distance. This finding is consistent with the observation by Marbà et al. (2006) that the concurrent rhizome vertical growth (which can be considered an early warning indicator of seagrass decline, Marbà et al., 2006) in the same sites was reduced by half after the farm onset at distances as high as 300 m from fish cages and that, in the largest farm (Italy), the growth reduction is still significant 1000 m away (Marbà et al., 2006).

Meadow decline was very fast near the cages. The relationship between shoot half-life and distance from the cages predicts the halving of shoot density in about 3–26 months within the first 15 m from the cages, and in 1–6 years within the first 50 m from the cages. This model also predicts that meadows would be decimated (i.e. density reduced by 90%) after 5–11 years and 11–32 years within the first 15 and 50 m from cages, respectively, at the studied farms. Such predictions are based on the population dynamics registered during only one year and therefore they do not take into account the possible temporal variability of the declining rate. For instance decline could accelerate with the reduction of meadow cover (Duarte, 2002). Nevertheless, the population dynamics approach yielded a prediction of the magnitude and velocity of future decline and gave us some insight on the mechanism i.e. high shoot mortality not coped by recruitment.

Significant net decline was still recorded at the control station closest to the coast in Sounion (Greece), as well as in the control station of the largest fish farm (Italy). However, these declines cannot be exclusively attributed to fish farm influence, as there were other potential sources of impact (e.g. a sewage outfall approximately 2 miles away from the Italian fish cages and 1 mile from the control stations). The decline rates recorded in some control stations are comparable to rates documented for other *P. oceanica* meadows without fish farm influences across the Mediterranean (Marbà et al., 2005).

The extension of seagrass die-off and density reductions observed here are similar to those documented around shallow and sheltered small Mediterranean fish farms producing less than 100 T yr⁻¹ of fish (Delgado et al., 1997; 1999; Pergent et al., 1999; Dimech et al., 2000) and around deep farms of similar production (200 T yr⁻¹, Pergent et al., 1999). The extension of the impact of deep farms would be apparently lower than in shallow and sheltered fish farms of equivalent size. For example, the linear extension of seagrass affected by a fish farm producing 700–800 T yr⁻¹ of sea bream and sea bass in a shallow bay during 8 years is more than 2 times longer (reaching more than 200 m away from fish cages, Ruiz et al., 2001, thus the area affected would be ca 4 to 9 times greater) than observed in the deep farms from this study. As previously hypothesised (e.g. Maldonado et al., 2005) this could be explained by the increased dilution of the waste products and, consequently, lower inputs to the sediments in deep sites. Nevertheless, the extension of fish farm impact on deep meadows was still large, and fairly exceeded the area beneath the cages.

There was, however, significant variability in decline responses among sites and transects, as reflected in the full factorial GLM model as well as in the significant interaction between site and organic sedimentation rate on shoot mortality and net growth rates. This variability likely reflects local environmental particularities, as well as some in-homogeneities of the experimental design across sites. It is interesting to point out that in one intermediate Greek station, which experimented sharp increases in shoot density, the

meadow canopy was heavily covered of dense mucous layers of filamentous algae (ectocarpales and Crysophyceae) during the first census. Such epiphytic algae were absent in the second visit. This raises interesting questions on the capacity of *P. oceanica* meadows to overcome seasonal thick canopy-invasive algae.

Shoot mortality and net population decline increased with enhanced sedimentation rates. Total sediment, organic matter (OM) inputs and total phosphorus (TP) inputs, directly measured on the meadows, proved to be efficient predictors of seagrass dynamics. This could be explained because the intensity of the fish farm benthic loading at any point is mainly a function of the distance to fish cages (Holmer et al., 2007; this work). Distance to cages in this work was as good predictor of seagrass mortality and decline as benthic inputs, but this is probably because the background conditions in the four fish farms studied were more or less similar (deep farms in relatively open waters). Benthic inputs could be more robust predictors, because they would integrate local differences in depth and hydrodynamics or fish farm effluent type and quantity (e.g. Crawford et al., 2003). Moreover, the benthic loading with labile organic matter from fish farms directly fuel the mechanisms inducing seagrass decline (like sediment organic enrichment, Delgado et al., 1999; Cancemi et al., 2000; 2003; bacterial stimulation and shift to anoxic pathways, Danovaro et al., 2000; sediment anoxia, Greve et al., 2003; or pore water sulphide, Halun et al., 2002; as well as increased herbivore pressure, Delgado et al., 1997; Ruiz et al., 2001). Holmer et al. (2007) showed that most sediment organic matter and phosphorus come from fish farm loadings in the meadows of this study. Sulphate reduction rates and acid-volatile sulphides in the sediment, as well as the depth of the oxic front are correlated with sediment input rates (Frederiksen, 2005; Holmer and Frederiksen, 2007). Moreover, the plant sulphur content increases with fish farm inputs and correlations between plant sulphur content and mortality rates have also been detected in the Greek and Italian farms of this study (Frederiksen et al., 2007). Thus, sedimentary inputs would integrate multiple cooperative impacts on seagrass dynamics, thereby allowing the prediction of seagrass decline.

Total phosphorus (TP) inputs were better predictors of seagrass decline than nitrogen and organic matter inputs. This may be due to the fact that Mediterranean coastal systems are usually phosphorus-limited (e.g. Berland et al., 1980), as is often the case in the oligotrophic *P. oceanica* meadows (Alcoverro et al., 1997; Gacia et al., 2002) and especially in their sediment microbial compartment (Lopez et al., 1995; 1998). In pristine meadows, *P. oceanica* exerts a large control on sediment nutrient availability in particular for phosphorus (Lopez et al., 1995). Sediment microbial activity is especially stimulated by the addition of phosphorus to the meadow (Lopez et al., 1998), and this could be observed in the fish farms of this study, where sulphate reduction rates were better correlated with TP inputs and sediment TP content than with organic matter or nitrogen (Frederiksen, 2005; Holmer and Frederiksen, 2007).

In Mediterranean fish farms settled in open and relatively deep coasts, as the ones from this study, most impacts come from particulate labile matter sinking to the bottom (Holmer et al., 2003a). To reduce these impacts to *P. oceanica* meadows or other benthic communities it is essential to reduce such effluents and to make them more refractory to microbial degradation; for example adjusting the phosphorus content of the feed pellets as much as possible. The feeding efficiency in fish farms is directly linked with nutrient and organic loadings (Islam, 2005) and it is usually low. Feed conversion ratios (FCR) range from 1.1 in efficient cultures of Salmon (Nordgarden et al., 2003) to 6.5 in cultures of areolated grouper (Leung et al., 1999). FCR in the sea bream cages from the study had intermediate values, ranging from 1.6 to 2.4 (Holmer et al., 2007). Therefore there is still margin to reduce the FCR ratios

in Mediterranean Sea Bream and Sea Bass farms, and applied research in this direction is still needed. The size of feed pellets has also been shown to affect the sediment quality (Pawar et al., 2002). Finally, there are also experiments to intercept the sinking particles before they reach the sediment. For example, in a sea bream farm in the northern Adriatic sea, D'Agaro and Lanari (2006) deployed benthic artificial barriers under fish cages, attached to the bottom. This method cannot be directly applied to meadow beds, because the barriers would reduce the irradiance to the canopy, but suggests a possible variant strategy, which could consist in hanging such barriers at the bottom of the fish cages. The feasibility of this remains speculative, but in any case we need more research to reduce benthic fish farm inputs.

The relationship between seagrass mortality and sedimentary inputs described here suggests the existence of input thresholds above which seagrass decline is accelerated (Fig. 6). In *P. oceanica*, mortality apparently accelerates and recruitment declines above input rates of 50 mg TP m⁻² day⁻¹, 5 g m⁻² day⁻¹ of total matter or 1.5 g organic matter m⁻² day⁻¹. Such thresholds of inputs for balanced meadow dynamics (which integrate background and fish farm inputs) may provide a powerful tool to set targets to regulate the location and size of new fish farms in the Mediterranean, and to manage existing ones in a sustainable way.

Nevertheless the substantial variability in decline responses to benthic loading among sites suggests that we cannot neglect the importance of other local factors on the response of seagrass meadows to fish farms. For example, in the shallowest (and closest to the coast) meadow of this study (Greece, 15 m depth) the shoot mortality rates were higher than expected from most regression equations (Fig. 6 and Table 4). In this place leaf grazing by sea urchins was much higher than in any other site (J.M. Ruiz, personal communication). Therefore, shallow sites could respond faster to equal levels of benthic organic loading. On the other hand regressions between benthic loading and plant decline have been obtained with sediment inputs measured punctually, 1 to 3 times per site during the study. Benthic input rates based in more frequent measurement could allow us to explain a greater fraction of the variance in shoot mortality and to define more accurate and specific thresholds to fish farm loads. Meanwhile, a cautionary and conservative approach in the use of these thresholds is recommended, that is, in the absence of liable mechanistic models, sedimentation rates should always be kept under the lowest levels found to produce a significant decline at any site, which in this case corresponds to the sedimentation rate thresholds found in Sounion (Greece).

The examination of the seagrass shoot population dynamics within permanent plots (which minimise error), combined with the deployment of benthic sediment traps to measure sediment, organic and/or nutrient inputs to the meadows, could represent an efficient strategy to early detect decline of slow-growing seagrasses near fish farms, allowing us to predict meadow response to fish farm inputs. As a monitoring strategy, it could be simplified (sensibly reducing the costs) by measuring only net shoot density changes within the permanent plots, without marking the shoots, as well as limiting input measurements to total and organic matter, which are simple and cheap to carry out (Gacia et al., 1999), although the best predictor was the phosphorus input.

This study was restricted to sites with sea bream and sea bass fish cages and with an endemic and vulnerable benthic community (*P. oceanica* meadows). However, the findings from studies in other kinds of aquaculture exploitation are consistent with the hypothesis of a strong and quantifiable relationship between benthic fish farm inputs and impact. For example the little or no effect on benthic ecosystems (including seagrasses) under shellfish cages in Tasmania was related by Crawford et al. (2003) to the observation that these cultures of filter feeding organisms did not increase

significantly benthic sedimentation rates with respect to background. The hypothesis is also consistent with the relatively higher impact of similar farming productions on shallow sheltered sites (Ruiz et al., 2001).

Therefore we believe that the approach used here, directly linking input rates to the efficient measure of the population dynamics of a key benthic species, could be extended to other benthic communities and farms (or even pollution sources), as an efficient quantitative way to define accurate effluent thresholds to sustainable activities.

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