



# Effects of Fish Farm Loadings on Seagrass (*Posidonia oceanica*) Distribution, Growth and Photosynthesis

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The spatial extent and timing of the impact of fish farms on the distribution and performance of a *Posidonia oceanica* meadow were examined in an embayment of the south-eastern coast of Spain (Hornillo Bay, Murcia). Changes in seagrass distribution were determined using available seagrass mapping (from 1988, i.e., before the onset of aquaculture activities and 1998) and by successive sampling in 1994 and 1998. Environmental variables (light attenuation coefficient, water-column dissolved nutrients and organic content of sediments) together with plant performance (shoot biomass, leaf growth rate, photosynthetic activity, carbohydrate reserves, the number of leaves per shoot, epiphyte loads and herbivore pressure) were measured in plants affected by organic discharges, and were compared with those found in reference healthy plants over an annual growth cycle. Since the onset of fish farm activity, 11.29 ha of *P. oceanica* meadow has been completely lost and 9.86 ha significantly degraded, thus resulting in a total affected area which accounts for about 53% of the former meadow, or 7-fold the fish farming area. Unequal propagation of seagrass die-off or degradation reflects the relevance of local factors such as depth and hydrodynamism on the true extent of fish farm impact. Water transparency decreases and dissolved nutrient and organic content of sediments increases in the vicinity of cages compared to distant reference stations, thus supporting the notion of environmental gradients caused by the organic release from cages, which spreads outwards. Shoot size, leaf growth rate and the number of leaves per shoot in plants close to the fish farm decreased. Moreover, low leaf growth and low rhizome carbohydrate

concentration (always relative to that found in an undisturbed area) indicated carbon budget imbalances. Since light reduction in the affected area was only modest (31% of light reaching the sea surface, while at the same depth this figure was 39% at the reference site), and light availability was well above the minimum requirement estimated for this species, neither this factor nor epiphyte overgrowth (epiphyte load was lower in the affected area) seem to explain such carbon imbalances or the observed meadow regression. Alternatively, the high herbivore pressure found in the affected zone suggests that overgrazing is one of the main causes of decreasing shoot sizes and hence of carbon imbalance, reduced growth and shoot mortality. The impact of fish farms on seagrasses, therefore, seems to be highly variable and depends on complex interactions between a large number of processes. © 2001 Published by Elsevier Science Ltd.

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The worldwide decline in fisheries has favoured the development of marine aquacultural activities in many coastal areas (Barg, 1994). Such activities bring about changes in the environment, and potentially influence coastal marine ecosystems and, in particular, benthic communities (Beveridge, 1996; GESAMP, 1996; Barg, 1994). The severity and the extent of the impact depend on the interplay of a large number of factors, such as the kind of aquacultural activity, stocking density, the physical/chemical features of the water body and sediments, the local hydrodynamism and the local communities at risk (e.g. Pillay, 1991; Wu, 1995).

In the generally oligotrophic Mediterranean, fish farming in suspended cages appears to be one of the most promising aquacultural strategies. In this kind of practice, growing fishes (sparidae and other species) are fed with dry fodder. The excess fodder, together with

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fish faeces are released into the environment, and account for a high input of organic matter which, in many cases, reaches the bottom and disturbs the benthic fauna and/or flora (Iwama, 1991; Rönberg *et al.*, 1992; Everett *et al.*, 1995; Casabianca *et al.*, 1997; Karakasis *et al.*, 1999). As cages are frequently installed in sheltered areas, such as bays or coves, and in relatively shallow waters, seagrasses are one of the most susceptible communities.

Mediterranean seagrass meadows, like others worldwide (see Short and Wyllie-Echeverria, 1996), are highly sensitive to human impact and their extensive decline has become a long-standing matter of concern (e.g. Pérès, 1984). The regression of *P. oceanica* meadows has been historically attributed to a large array of causes, such as bottom-trawling fisheries (Sánchez-Lizaso *et al.*, 1990; Ruiz *et al.*, 1999), coastal constructions (Blanc and Jeudy de Grissac, 1989; Ruiz *et al.*, 1993) and urban and industrial waste (Bourcier, 1989; Pergent-Martini and Pergent, 1995) and, more recently, to competitive exclusion by invasive species (*Caulerpa taxifolia*, UNEP, 1999), among others. The recent increase in fish farming represents an additional anthropic disturbance on these already endangered seagrass community, and the immediate need of adequate scientific knowledge for the correct and environmentally safe management of such activity is evident.

All the available information on the effects of fish farming on Mediterranean seagrasses (Delgado *et al.*, 1997; Delgado *et al.*, 1999; Pergent *et al.*, 1999) shows that *P. oceanica* disappears under fish cages, while surrounding areas are significantly degraded. Several suggestions have been offered to explain this decline, including both changes in the water column (e.g. increasing nutrients and decreasing light) and in sediment properties (e.g. anoxia caused by high organic matter content). However, to our knowledge, all the available information to date has been obtained from two sites, and little attention has been paid to key aspects such as changes in plant performance in response to organic loading or a proper assessment of changes in environmental conditions (e.g. light, nutrient concentration). Moreover, little is known about the time-course of the effects, and the spatial variability of the response (e.g. Delgado *et al.*, 1997).

To further understanding of seagrass vulnerability, we studied the impact of a large fish farm on a *P. oceanica* meadow in an embayment on the south-eastern coast of Spain (Hornillo Bay, Murcia). We first examined in detail the spatial distribution of both seagrass die-off (i.e., total mortality) and seagrass decline (i.e., partial mortality); we then assessed the time-course of the former over a 10-year period by combining existing cartographies with our own data. Finally, to improve our knowledge of the process of decline, the performance (photosynthesis and growth) of plants affected by organic discharge was studied and compared with that of healthy plants.

## Material and Methods

### Study site

The study was conducted in Hornillo Bay, a Mediterranean embayment (ca 50 ha) in the locality of Águilas (Murcia, south-eastern Spain; Fig. 1(a)). The bay is partially exposed to prevailing N-NE and W winds, and the water temperature varies seasonally from 13°C to 26°C (Ruiz *et al.*, 1993). Benthic communities are dominated by seagrasses, *P. oceanica* being the most abundant species, while *Cymodocea nodosa*, is present only in shallow (2–6 m depth) sandy bottoms near Fraile Island. The distribution of the *P. oceanica* meadow in Hornillo Bay before the deployment of fish cages was described by Calvín *et al.* (1989), who observed a continuous meadow from 3 to 25 m depth, with a surface area of ca 40 ha.

Fish farming began in 1989 in the western part of the bay (Fig. 1). Initially, there was a relatively small production (ca 50 metric tons per year) of amber jack, *Seriola dumerili* (Risso) L. One year later, this cultured species was replaced by gilthead seabream, *Sparus auratus* (L.) and sea-bass, *Dicentrarchus labrax* (L.) and annual production increased to 700–800 metric tons. At this time, 30 fish cages, 20 m in diameter, were deployed over an area of 7 ha. The same situation was observed at the time of the present study.

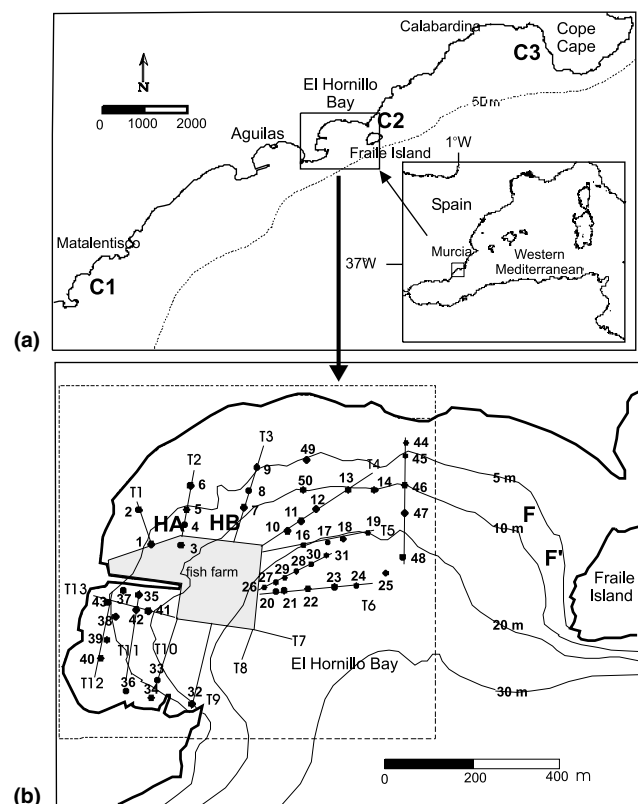


Fig. 1 (a) Location of the study site, sampling points in Hornillo Bay and control meadows (reference sites C1, C2 and C3). (b) Location of sampling points for meadow density and cover measurements (black points) and sampling stations (HA, HB and F) selected for environmental and plant performance measurements.

### Seagrass distribution and status

The study was initiated in spring 1994, five years after the onset of fish farming. The spatial impact of this activity was assessed by estimating shoot density and meadow cover in 13 transects situated around the perimeter of the fish farm (Fig. 1(b)); within this perimeter, no living seagrass was observed during the field work. In each transect, shoot density and cover were measured at several points which were 25–50 m apart. The total number of sampling points per transect depended on its total length and the position of the meadow limit. Measurements were also performed in additional points (i.e., sampling points 26–31 and 44–50 shown in Fig. 1(b)) to obtain a homogeneous density of sampling points in the study area. This sampling design gave a total of 50 sampling points in a 200–400 m radius around the fish farm perimeter (Fig. 1(b)). At each sampling point, shoot density ( $d$ ) was measured by counting all the shoots in a randomly selected 400 cm<sup>2</sup> quadrat of seagrass (4–5 replicates per sampling point) and cover was estimated as the proportion of living patches in a 20 m transect (placed perpendicular to the main transect, 3 random replicates per sampling point), following Sánchez-Lizaso (1996). The precise limit of the meadow in each transect was recorded and used to draw a contour map of the 1994 situation. The position of each transect was marked by pushing/driving iron pegs into the substrate. In summer 1998, the area was revisited, the transects were placed at the same positions using the iron pegs as references and the new meadow limits recorded for a new contour map as in the previous visit.

For comparison, shoot density and meadow cover were also measured in three undisturbed reference sites (Matalentisco, C1; Fraile Island, C2; Calabardina, C3; Fig. 1(a)) located in nearby areas which were not affected by human activity and where no changes in *P. oceanica* meadows had been recorded during the previous decade (Calvín *et al.* (1999)). At each site, replicate measures ( $n = 3$ ) of density and cover were performed in each of the following depth ranges: < 5, 6–10, 11–15, 16–20, 21–25 m. Based on the relative homogeneity of seagrass characteristics (shoot density, plant cover, productivity, etc.) between pristine meadows (Alcoverro, 1995; Sánchez-Lizaso, 1996) we assume that seagrass features at those reference sites are representative of natural variability in the study area and, hence, they should be similar to those originally found in Hornillo Bay before the onset of fish culture. For each range, the mean values obtained in the three control sites were used as independent replicates for comparisons with replicate measures from the sampling points in Hornillo Bay at the same depth.

### Plant measurements

Leaf growth, shoot size (as biomass), number of leaves per shoot, epiphyte load and herbivore pressure were measured at three stations; two of which were in

the disturbed area (Hornillo Bay), *ca* 40 m from the fish cages (stations HA and HB, respectively), and the third was located in an undisturbed reference area (Fraile Island, station F). All three stations were located at 8 m depth (Fig. 1(b)).

Leaf growth was evaluated using a modified Zieman's method (Zieman, 1974; Romero, 1989). At each station, three random square (900 cm<sup>2</sup>) plots (5–10 m apart) were delimited with iron pegs. During each visit, 10 shoots per plot were marked by punching all the leaves together with a hypodermic needle, just above the ligula of the outermost, oldest leaf and the 10 shoots marked in the previous visit were collected. In the laboratory, epiphytes were removed from the harvested shoots with a razor blade and weighed. All the leaves on the shoot were measured both leaf length (to the nearest cm) and leaf width (to the nearest cm) were obtained. Epiphyte load was expressed as the epiphyte weight per leaf surface unit (mg DW cm<sup>-2</sup>). Leaf tissue was further sorted into 'old' (i.e., that present before punching, situated above the holes) and 'new' (below the holes), and leaf growth rate (mg DW shoot<sup>-1</sup> d<sup>-1</sup>) expressed as the weight of new tissue divided by the number of days elapsed between the two sampling events. Shoot size was estimated as the weight of 'old' plus 'new' tissues (g DW shoot<sup>-1</sup>). Leaves bearing marks of herbivore attack were counted and the marks were identified (Boudouresque and Meinesz, 1982; Alcoverro *et al.*, 1997). Herbivore pressure was expressed as the frequency of tips eaten by each macrograzer (the fish *Sarpa salpa* and the sea urchin *Paracentrotus lividus*). All weights mentioned here are dry weights (i.e., dried for at least 12 h at 100°C). Measurements were made in eight visits from 31 January 1995 to 11 November 1995 (mean periodicity of 40 ± 19.6 days).

### Photosynthetic rates and carbon reserves

Photosynthesis vs irradiance curves were established for leaves from stations HA, HB and F in May 1995 and August 1995. Thirty shoots were randomly collected at each sampling station in the morning (8–9 h a.m.) and transported in aerated and refrigerated seawater to the laboratory within 2 h of collection. There, 10–15 cm leaf segments of the second youngest leaf were separated from each shoot (Alcoverro *et al.*, 1998) and gently cleaned of epiphytes using a razor blade, which has been shown to be an effective method to remove most macroscopic and microscopic epiphytes (Kirchman *et al.*, 1984). Each leaf segment was placed in a 250 ml glass bottle filled with seawater and put in an incubator at the same temperature recorded in the field at the time of collection. Light was provided by halogen lamps, and several irradiances (0, 25, 50, 75, 150, 250, 600 and 800 μmol quanta m<sup>-2</sup> s<sup>-1</sup>) were obtained by inserting neutral filters between the light source and the incubation chambers. All incubations were performed in triplicate. Bottle contents were mixed using multisite magnetic stirrers (*ca* 200 rpm).

Dissolved oxygen concentration inside the glass bottles was measured after 2 h using an oxygen probe (Orbisphere 27141), and was then compared with that obtained in control (without plants) bottles ( $n = 4$ ). The coefficient of variation between control bottles ranged between 0% and 1.6%. After incubation, plant material was dried at 100°C for 24 h and subsequently weighed. Data were expressed as mg of oxygen released per gram of dry leaf weight and unit time ( $\text{mg O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$ ). This method has proved to give reliable estimates of photosynthetic activity (Alcoverro *et al.*, 1998).

To estimate the  $P-I$  curve parameters using non-linear regression methods, the data were fitted to the equation of Edwards and Walker (1983) which was modified by Pérez and Romero (1992)

$$P = P_{\max}^*(I - I_c)/(I_k + (I - I_c)), \quad (1)$$

where  $P$  is the net oxygen release ( $\text{mg O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$ ) at irradiance  $I$  ( $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ );  $P_{\max}$ , the net oxygen release at saturating irradiance ( $\text{mg O}_2 \text{ g}^{-1} \text{ DW h}^{-1}$ );  $I_c$ , the compensation irradiance ( $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ ) and  $I_k$  the saturating irradiance ( $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ ), as classically defined by most authors (Pirc, 1986). Quantum photosynthetic efficiency ( $\alpha$ ,  $\text{mg O}_2 \text{ g DW}^{-1} \text{ h}^{-1} \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ ) was estimated as  $P_{\max}$  divided by  $I_k$ .

To further assess possible light limitation during the growth season, carbohydrate reserves (starch) in rhizomes were determined at each station during August photosynthesis incubations. Starch was analyzed in ground dried (100°C for 24 h) rhizome fragments (4–6 cm from apex) which were separated from 10 randomly collected shoots. Starch was extracted from the ethanol-insoluble residue obtained after the sample had remained overnight in 1 N KOH, and was determined spectrophotometrically using an anthrone assay which was standardized to sucrose (Jensen, 1962).

#### Water and sediment characteristics

Irradiance photosynthetically active radiation (PAR) was measured *in situ* as photosynthetic photon flux density (PPFD), using a  $2\pi$ , cosine-corrected underwater PAR sensor (LiCor LI-192SA). At each station, 5 min integrated PPFD measurements were performed on the sea surface ( $I_s$ ) and immediately above the seagrass leaf canopy ( $I_z$ ) at noon (1200–1400 h) to minimize the apparent effects of the solar zenith angle (Moore *et al.*, 1997). Water-column turbidity was estimated as the coefficient of light attenuation ( $k, \text{m}^{-1}$ ) from the Beer-Lambert expression ( $I_z = I_s * e^{-kz}$ ), where  $z$  represents depth (m). PPFD measurements to calculate turbidity were performed in 30 randomly selected days between January and November 1995.

Dissolved nutrient concentration (nitrate, nitrite, ammonia and phosphate) was determined using standard methods (Parsons, 1984) for the same stations as for plant measurements (HA, HB and F). In the reference station F, an additional site 100 m away (therein-

after station F', Fig. 1) was sampled to assess the variability of these variables within the reference area. At each sampling (i.e., a single day), five replicate water samples were collected by SCUBA divers within each sampling station just above the meadow leaf canopy. This was repeated several times in February 1995 (3 days) and in July 1995 (5 days). Sediment samples were taken in March 1995 and September 1995 with PVC cores (3 replicates) of 10 cm depth at HA and F for fine fraction ( $<50 \mu\text{m}$  diameter) and organic matter content (% organic carbon) determinations, following the methods described by Buchanan (1984).

#### Data processing and statistics

From the density and cover values obtained at each sampling point (as a mean of the different within-point replicates), contour maps were obtained by kriging (Olea, 1974) using the SURFER © v.6 computer package; this technique has already been successfully used to obtain shoot density maps of *P. oceanica* (e.g. Scardi *et al.*, 1989). To discriminate changes in meadow structure (density and cover) caused by the proximity to fish cages from those due to depth (Romero, 1989; Sánchez-Lizaso, 1996), one-way ANOVA tests were applied separately for sampling points within a given depth range. (i.e.,  $< 5$ , 6–10, 11–15, 16–20, 21–25 m, i.e., as for the controls). When differences between these sampling points were significant, *a posteriori* pairwise comparison of means was performed using the Student-Newman-Keuls test (SNK,  $p = 0.05$ , Zar, 1984) and disturbed points were considered as those having a significantly lower mean density and/or cover than the mean value of the reference sites. The significantly disturbed meadow areas (i.e., areas with reduced density or cover) were those delimited by disturbed points and their surface area was estimated using the Atlas-GIS © v.2.1 computer package.

For each shoot variable (leaf growth rate, biomass, number of leaves, epiphyte load) the mean of each plot was estimated and used as an individual, independent replicate within a given sampling station (i.e.,  $n = 3$ ). A two-way ANOVA was used to assess the significance of differences in selected shoot variables and to partition the observed variance into distinct components (Underwood, 1997): between stations (spatial variance), between sampling times (large-scale seasonal variance) and time-trends between stations (small-scale seasonal variance). A two-way ANOVA was used to assess significant differences in the phosphate concentrations and sediment characteristics between stations and sampling times. Data on dissolved forms of nitrogen were only available at one sampling time, therefore a one-way ANOVA was used. In both cases, stations within locations were considered as a random factor nested into the sampling area (i.e., fish farm and reference areas). A one-way ANOVA was also used to test the differences in the annual means of the light attenuation coefficient between stations.

## Results

### Water and sediment conditions

Attenuation coefficients averaged over the study at the stations closest to the fish farm (HA,  $k = 0.171 \text{ m}^{-1}$ ; HB,  $k = 0.170 \text{ m}^{-1}$ ) were not significantly different between them, but were significantly higher than at the reference site (F,  $0.134 \text{ m}^{-1}$ , SNK,  $p < 0.05$ ; Table 1). This resulted in significant but mild differences between stations in the light reaching the canopy, which was about 30% of surface irradiance in the two fish farm stations and slightly higher in the reference station (F, 39.08%; Table 1).

Dissolved nutrient concentration showed marked seasonality and considerable differences between the fish farm area and the reference meadow (Table 1). In summer, dissolved inorganic phosphorus and ammonium showed significantly higher (4.1–7.2-fold and 7–13-fold, respectively) concentrations in stations HA and HB (disturbed) than in the reference station F. Dissolved inorganic phosphorus showed the same pattern in winter although the differences between the fish farm area and the reference meadow were smaller. Nitrate and nitrite water concentrations were higher in winter than in summer, when these nutrients were not detected in water (except nitrite in HA and HB). Organic matter concentration in sediments was significantly higher in stations HA and HB (disturbed) than in the reference station F at both sampling times, the differences being higher in March (4-fold) than in September (2-fold). The mean values of stations HA and HB did not show significant differences (SNK,  $p < 0.05$ ).

### Changes in meadow limits

Dramatic changes took place in the surface cover of the *P. oceanica* meadow in Hornillo Bay from 1988 to

1998 (Fig. 2, Table 2). Available maps (Calvín *et al.*, 1989) indicate the presence of a continuous and healthy *P. oceanica* meadow which extended to a maximum depth of 25 m throughout the bay in 1988 and had a total area of ca 40 ha (Fig. 2(a), Table 2). In 1994, 5 years after the onset of fish farming, 9.35 ha (i.e., 23.3% of the former surface) of *P. oceanica* had disappeared from the bay (Fig. 2(b), Table 2). This denuded area comprised mostly the sea bottom located beneath the fish farm and an extensive area towards the south; here, most of the meadow decline occurred near the former depth limit, which moved from close to the 25-m isobath to between the 8 and 10 m isobaths. Between 1994 and 1998, further meadow die-off, affecting 1.94 ha, was observed to the north of the fish farm, mainly near transect 4, where the meadow limit receded 100 m from its original position in 1994 (Fig. 2(c), Table 2). A total area of 11.29 ha (i.e., 28.2% of the 1988 surface area) of *P. oceanica* meadow completely disappeared in the 10-year period (Table 2).

### Meadow spatial structure

In 1994, shoot density and meadow cover showed a common pattern, decreasing markedly from the vicinity of the fish farm (Fig. 3(a) and (b)) outwards; this pattern overrode the known effect of depth on these variables (note the density and cover isopleths crossing the isobaths in Fig. 3(a) and (b)). Except in some isolated cases, density and cover varied simultaneously, thus leading to similar spatial patterns of variation. Within Hornillo Bay, maximum values were found in the most distant sampling points, and did not show significant differences to those from the reference sites (Table 3). Significantly lower values of density and/or cover than those at the reference sites (Table 4) were detected around the fish farming area; the meadow zone including these points

TABLE 1

Mean  $\pm$  S.E. values of water nutrient concentrations (i.e., dissolved inorganic nitrogen and phosphorus), light (i.e., light attenuation coefficient ( $k$ ,  $\text{m}^{-1}$ ) and percentage of surface irradiance) and organic matter content in sediments.

Period	Nutrient	Reference stations		Fish farm stations		<i>p</i> -value
		F	F'	HA	HB	
February	$\text{PO}_4^{3-}$ <sup>a</sup>	$0.123 \pm 0.03$	$0.114 \pm 0.03$	$0.24 \pm 0.04$	$0.31 \pm 0.11$	*
	$\text{NO}_3^-$	$4.18 \pm 0.39$	$4.55 \pm 0.67$	$5.94 \pm 2.9$	$5.04 \pm 0.48$	ns
	$\text{NO}_2^-$	$0.809 \pm 0.23$	$0.553 \pm 0.18$	$0.49 \pm 0.15$	$0.70 \pm 0.46$	ns <sup>b</sup>
July	$\text{PO}_4^{3-}$ <sup>a</sup>	$0.115 \pm 0.06$	$0.084 \pm 0.02$	$0.61 \pm 0.05$	$0.48 \pm 0.17$	*
	$\text{NO}_3^-$	nd <sup>c</sup>	nd	nd	nd	
	$\text{NO}_2^-$	nd	nd	$1.38 \pm 0.14$	$0.58 \pm 0.26$	
	$\text{NH}_4^+$ <sup>d</sup>	$4.176 \pm 5.52$	$3.154 \pm 3.9$	$41 \pm 22.1$	$30.5 \pm 10.5$	*
K ( $\text{m}^{-1}$ )		$0.134 \pm 0.01$		$0.171 \pm 0.007$	$0.170 \pm 0.006$	*
% surface irradiance		$39.08 \pm 1.7$		$30.12 \pm 1.54$	$30.07 \pm 1.41$	*
Sediment organic matter (%)						
March		$0.51 \pm 0.06$		$2.4 \pm 0.01$	$2 \pm 0.1$	*
September		$0.87 \pm 0.07$		$1.91 \pm 0.02$	$1.84 \pm 0.09$	*

<sup>a</sup>  $a = (x + 1)$  transformed data.

<sup>b</sup> ns: not significant.

<sup>c</sup> nd: not detected.

<sup>d</sup>  $b = \sqrt{(x + 1)}$  transformed data.

\* The significance of between-station variability is indicated. ANOVA.  $p < 0.05$ .

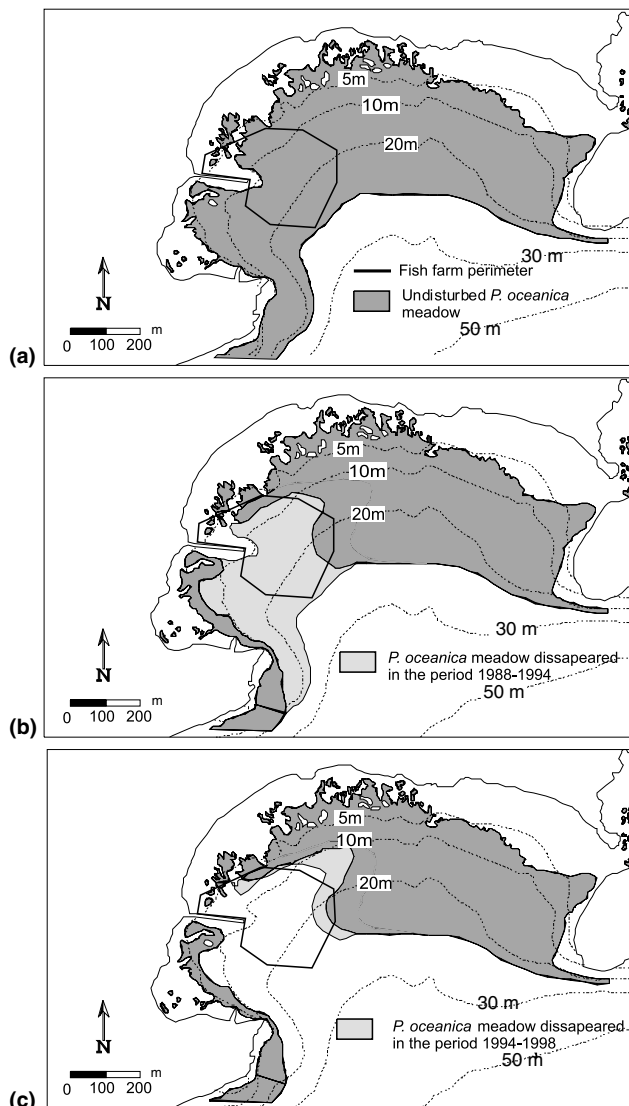


Fig. 2 Distribution of the *P. oceanica* meadow in: (a) 1988, (b) 1994 and (c) 1998. Dark area corresponds to the living *P. oceanica* meadow and the grey area to the meadow die-off since the previous measurement.

was considered 'disturbed', and comprised 11.8 ha (Fig. 3(c)). Meadow losses recorded in the period 1994–1998 (i.e., 1.94 ha) occurred within this disturbed meadow area. Shallower areas of the *P. oceanica* meadow (<5 m depth) at the northern side of the fish farm were less

disturbed than those to the south, even in parts where fish farm limits were very close to some of the cages (<50 m, e.g. transects 1 and 2).

#### Plant variables

Significant differences in shoot biomass, leaf growth, number of leaves per shoot, epiphyte loading and herbivore pressure were observed between sampling times and sampling stations. However, since no significant differences were found in these variables between stations HA and HB at any sampling time (SNK test), the reported variability can be attributed to differences between the fish farm area and the reference meadow. In the fish farm stations (HA and HB), shoot size and leaf growth were substantially lower than in the reference station F (Fig. 4 and Table 5). For both variables, these differences peaked in May (i.e., 60–75% lower in HA and HB than in F) and minimum (i.e., 0–33% lower) in autumn (leaf growth) and winter (shoot size). The number of leaves per shoot showed the opposite pattern, with minimum differences in summer and maximum during the rest of the year. The epiphytic load in the reference meadow was between 2- and 7-fold higher than that of the fish farm stations throughout the year except in winter (January and February), when no significant differences were detected between stations. Herbivore pressure near the fish farm (stations HA and HB) was higher (60–90%) than in the reference meadow (station F, 20–50%) throughout the study period.

Variability in the results of all plant variables was mostly due to variance between sampling stations (56.4–97.4%, Table 5), while the contribution of small-scale (i.e., stations  $\times$  times) seasonal variance was considerably lower (0.81–9.8%). The contribution of large-scale (i.e., between times) seasonal variance was also low (<9%), except for shoot biomass and number of leaves per shoot, where it was clearly higher (25–32%).

#### Photosynthetic activity

All photosynthetic parameters ( $P_{\max}$ ,  $I_c$ ,  $I_k$  and  $\alpha$ ) increased from spring to summer (Fig. 5, Table 6), following the previously described seasonal pattern of *P. oceanica* (Alcoverro *et al.*, 1995). Photosynthetic parameters showed considerable differences between stations, which were maintained in the two sampling events

TABLE 2

Changes in the *P. oceanica* meadow in Hornillo Bay from the onset of fish farming activity (1988) to 1998.<sup>a</sup>

Meadow categories	Net changes in meadow areas recorded in the periods				
	Initial area (ha)	1988–1994 (ha)	1994–1998 (ha)	Final area (ha)	% of the former meadow area
Undisturbed	40	–21.15	0	18.85	47.12
Disturbed	0	+11.8	–1.94	9.86	24.65
Bare	0	+9.35	+1.94	11.29	28.22
Total affected meadow				21.15	53

<sup>a</sup> Positive values indicate an increase in the area occupied by the meadow category and negative values indicate a decrease.

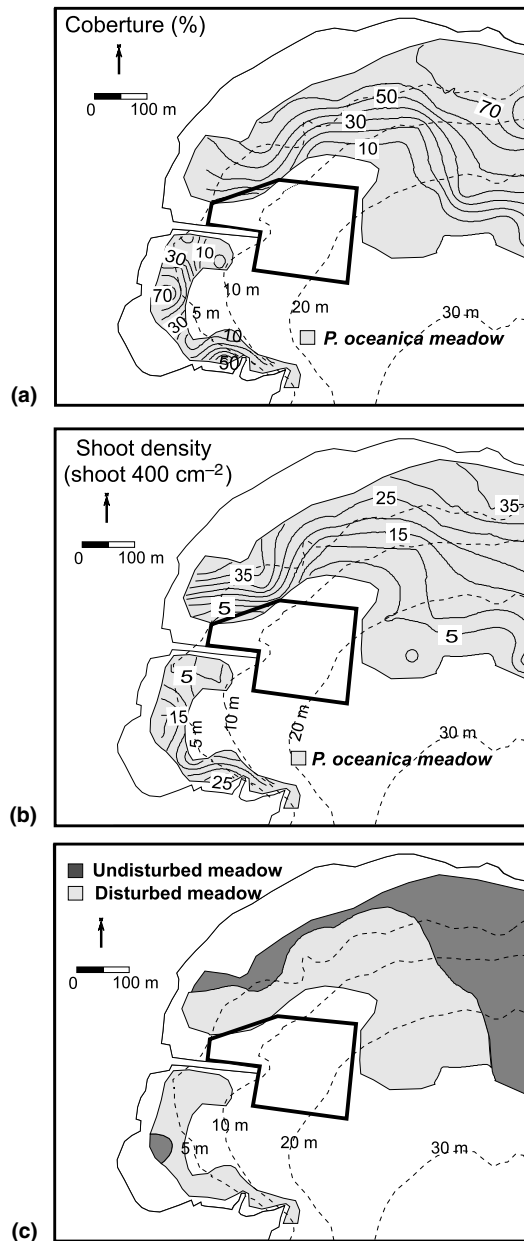


Fig. 3 Isoline maps of: (a) patch density (shoots 400 cm<sup>-2</sup>), (b) meadow cover (%) and (c) disturbed (grey) and undisturbed (dark) surface of *P. oceanica* meadow.

TABLE 3

Averaged values of shoot density (shoots 400 cm<sup>-2</sup>) and meadow cover (%) for each depth range, measured in three selected control meadows near the study area (i.e., reference sites, see Fig. 1).

Depth ranges (m)	Shoot density (shoots 400 cm <sup>-2</sup> )	Meadow cover (%)
<5	37.9 ± 2.7	76.8 ± 5.5
6-10	21 ± 2.1	72.9 ± 8.7
11-15	18.3 ± 1.5	62.8 ± 4
16-20	17.6 ± 3.2	59.7 ± 6.5
21-25	11.9 ± 2.6	36.7 ± 9.3

(Table 6, Fig. 5). In all cases, the photosynthetic parameters of the two fish farm stations (HA and HB) differed from the reference station F, but did not differ

from each other. In the fish farm stations,  $P_{max}$  increased and dark respiration rates decreased in relation to plants from the reference station (one-way ANOVA,  $p < 0.05$ ). This resulted in  $P_{max}:R$  ratios which were 2-5-fold higher in fish farm stations than in the reference meadow.  $I_{sat}$  and  $I_c$  presented similar values in all sampling stations, while photosynthetic efficiency ( $\alpha$ ) showed clearly higher values (1.5-2.5-fold) in the fish farm stations than in the reference station. The rhizome starch concentration in fish farm stations showed a significant reduction (ca 40%) in comparison with the values measured in the reference meadow (one-way ANOVA,  $p < 0.0001$ , Table 6).

### Discussion

The results presented in this study indicate that the seagrass meadow studied underwent great changes between 1988 and 1998. These alterations include the loss of a large vegetated area (11.29 ha, 28.2% of total) and the degradation (i.e., significant decrease in shoot density) of another part of the meadow (9.86 ha, 24.65% of total; Table 2), resulting in a total affected area of 21.15 ha, i.e., 53% of the former *P. oceanica* meadow. It is unlikely that such a considerable regression could be explained by factors other than those associated with fish farming activity. A comparison of data in Calvin *et al.* (1989) and Calvin *et al.* (1999), which include extensive, repeated seagrass mapping over a 224 km coastline over a 10-year interval, failed to detect any similar dramatic decline except in zones affected by clearly recognizable human impact (e.g. close to the depth limit) due to trawler fishing. Moreover no other source of anthropic disturbance is present in Hornillo Bay. Therefore, we conclude that fish farming has caused the extensive decline observed in this study, thus confirming the very large potential impact of such an activity on *P. oceanica* meadows (Delgado *et al.*, 1997; Delgado *et al.*, 1999; Pergent *et al.*, 1999). This is by no means surprising, since fish farming implies a heavy release of organic detritus. Based on food conversion rates for these fish species (1:2.5) and on nutrient composition of fodder and fish tissues (Pillay, 1991; Ackefors and Enell, 1994; Enell, 1995), we estimate that the fish farming in Hornillo Bay supplies yearly about 24 kg of phosphorus, 162 kg of nitrogen and 330 kg of carbon per ton of fish produced (these values do not include direct losses i.e., uneaten fodder). These values approximate others reported for the same species (Jambrina and Caparro, 1997) and are near the ranges estimated for salmonids (Gowen and Bradbury, 1987; Pillay, 1991). The magnitude of these loads has been compared with small sludge sewage and they are likely to cause significant local changes in water and sediment quality (Pillay, 1991; Wu *et al.*, 1994; Alonso-Rodríguez *et al.*, 2000). A considerable amount of this organic matter, both in dissolved and particulate forms, is dispersed by waves and currents thereby creating a gradient from the cages

TABLE 4

Summary of one-way ANOVA performed to test the significance of differences in patch density (shoots 400 m<sup>-2</sup>) and meadow cover (%) between sampling points within each depth range.<sup>a</sup>

Depth	Main effect	df	MS	F	p	Disturbed points	
Patch shoot density (shoot 400 cm <sup>-2</sup> )	≤5 m	Points	9	590.6	24.9	*	34 9 39 36 8
		Residual	42	23.7			
	6–10 m	Points	13	661.6	64.6	*	32 38 37 35 41 43 42 37
		Residual	58	10.33			
	11–15 m	Points	3	0.477	29.8	*	12 33
		Residual	18	0.016			
16–20 m	Points	6	243.2	34.1	*	19 18 11 16 17 10	
	Residual	30	7.12				
≥21 m	Points	6	140.5	33.6	*	22 20 27 30 31	
	Residual	30	4.17				
Meadow cover (%)	≤5 m	Points	9	1608.4	36.03	*	5 2 8 36
		Residual	24	44.6			
	6–10 m	Points	13	2651.1	58.8	*	38 4 37 1 3 32 43 41 7 42 35
		Residual	32	45.01			
	11–15 m	Points	3	4496.8	65.2	*	12 33
		Residual	12	68.94			
	16–20 m	Points	6	3568.2	33.4	*	17 11 18 10 16
		Residual	18	106.7			
	≥21 m	Points	6	1811.5	12.01	*	20 22 30 31 27
		Residual	18	150.7			

<sup>a</sup> Sampling points (Fig. 1(b)) with mean values significantly lower than those of reference meadows, as detected by the *post hoc* SNK multiple mean comparison test, are indicated as 'disturbed'.

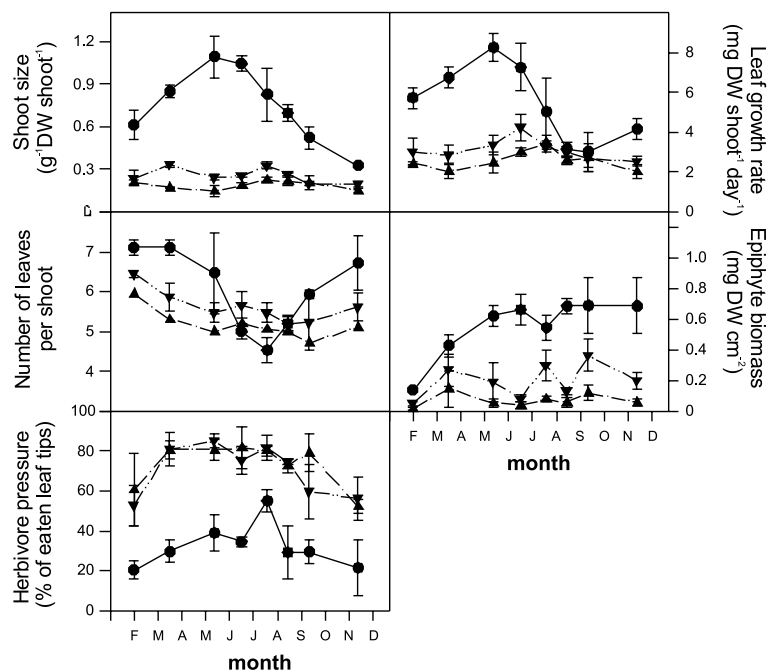


Fig. 4 Shoot variables in fish farm stations (HA, triangle, and HB, inverted triangles) and in the reference station F (circles).

to the surrounding areas in a number of environmental variables (GESAMP, 1996; Pergent *et al.*, 1999; Wu *et al.*, 1994). The low water transparency, high dissolved nutrient concentrations and high organic sediment content reported in the vicinity of cages (stations HA and HB) compared to distant areas (station F), confirm the presence of such a gradient in our study area.

In the meadow studied, degradation was maximum under the cages, where total seagrass die-off was observed, and it decreased towards the surrounding areas, where shoot density and meadow cover were significantly reduced within a radius of *ca* 300 m from the fish farm perimeter (Fig. 3). Similar spatial patterns of *P. oceanica* regression have been described in previous



**TABLE 5**  
Results of the two-way ANOVA performed on plant variables.

Main effects:	Station		Time		Station × time		Error
	2		7		14		48
<i>df</i> <sup>a</sup>							
Plant variables:	<i>p</i> <sup>b</sup>	% var <sup>c</sup>	<i>p</i>	% var	<i>p</i>	% var	% var
Shoot size <sup>d</sup> (g DW shoot <sup>-1</sup> )	*	60.5	*	26	*	12.1	1.3
Leaf growth rate (mg DW shoot <sup>-1</sup> d <sup>-1</sup> )	*	85	*	8.8	*	5.4	0.63
Number of leaves per shoot	*	56.4	*	31.4	*	9.8	2.3
Epiphyte biomass <sup>d</sup> (mg DW cm <sup>-2</sup> )	*	92.4	*	5	*	2	0.37
Herbivore pressure (%)	*	91.5	*	7.1	ns <sup>e</sup>	0.81	0.52

<sup>a</sup> *df*: degrees of freedom.

<sup>b</sup> *p* < 0.001.

<sup>c</sup> % var.: percentage of explained variance.

<sup>d</sup> log (*x* + 1) transformed data.

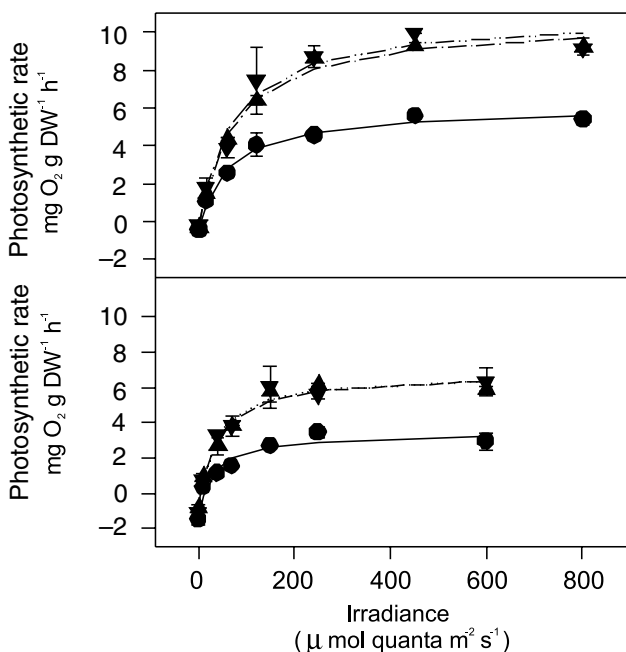
<sup>e</sup> ns: not significant.

studies related to similar fish farm activities in other Mediterranean localities (e.g. Fornells Bay, Menorca, Delgado *et al.*, 1997). The estimated extent of the meadow affected was >20-fold greater than that directly occupied by cages or 6.8-fold higher than the surface area of the farming activity as shown in Fig. 3; this ratio was 28.5-fold higher in Fornells Bay, a very shallow (average depth 5.5 m) and semi-enclosed bay, where annual fish yields (between 0.5 and 0.8 metric tons per year, Delgado *et al.*, 1997) were substantially lower than those sustained at Hornillo Bay (more than 500 metric tons per year). Therefore it is evident that the effective impact derived from fish farming is highly variable, and depends on both water exchange and, probably, on other features such as depth and aquacultural practices (fish feeding strategy, fish yield, etc.). The contribution of local hydrodynamism and farming conditions in de-

termining the environmental impact of fish farms has been showed in other comparative studies (e.g. Wu *et al.*, 1994). Spatial heterogeneity in hydrodynamic and bathymetric conditions probably explain the differential propagation of fish farm loading effects on the *P. oceanica* meadow in our case study. Thus, shallow and deep *P. oceanica* meadow located to the south of the fish farm was more extensively degraded than to the north, where shallow areas were less affected, probably because the southern area is more sheltered from prevailing winds, which restrict the dispersion of organic detritus.

Seagrass degradation has been attributed to a direct reduction in light availability by the shading effect of the cages themselves and to high concentrations of organic matter in sediments caused by the settling of particulate material (uneaten food and faeces: Wu *et al.*, 1994; Everett *et al.*, 1995; Pergent *et al.*, 1999). Both factors are known to be critical for seagrass survival (Dennison and Alberte, 1982; Fitzpatrick and Kirkman, 1995; Goodman *et al.*, 1995; Terrados *et al.*, 1999) and their combined effects could explain, in our case, the complete degradation of the *P. oceanica* meadow immediately below the cages; however, this accounts for only about 20% of the total degraded meadow area.

The propagation of meadow regression outwards from the cage zone can only be accounted for by the dispersion of dissolved nutrients and detritus from the farming activity. Light availability measured in the vicinity of cages (30–31% of surface irradiance) did not show a dramatic reduction in relation to control areas (39% of surface irradiance, Table 1) and was both well above the minimum light requirement estimated for *P. oceanica* in this area (*ca* 10% of surface irradiance), and above irradiance levels needed to cause significant shoot mortality in shading experiments (*ca* 20%, Ruiz and Romero, in press). Hence, light limitation caused by increased turbidity does not appear to be able to account for seagrass decline in the surrounding areas of the fish farm. This is consistent with other studies that failed to detect a consistent effect of turbidity on light availability in *P. oceanica* meadows close to fish farms (Pergent *et al.*, 1999).



**Fig. 5** *P* – *I* curves in: (a) May 1995 and (b) August 1995 for stations F (circles), HA (triangles) and HB (inverted triangles).

TABLE 6

Photosynthetic parameters (mean  $\pm$  S.E.) estimated from  $P$  vs  $I$  curves obtained in May and August 1995 for the reference (F) and fish farm stations (HA and HB).<sup>a</sup>

Period	Photosynthetic parameters	Reference station			Fish farm stations		
		F	HA	HB			
May 1995	$P_{\max}$	3.42 $\pm$ 0.43	6.84 $\pm$ 0.44	6.76 $\pm$ 0.39			
	$I_{\text{sat}}$	44.2 $\pm$ 17.4	44.5 $\pm$ 10.8	38.6 $\pm$ 8.56			
	$I_c$	12.01 $\pm$ 3.8	4.3 $\pm$ 1.9	5.38 $\pm$ 1.56			
	$\alpha$	0.07 $\pm$ 0.003	0.15 $\pm$ 0.001	0.17 $\pm$ 0.02			
	$R$	-1.65 $\pm$ 0.38	-0.81 $\pm$ 0.33	-1.09 $\pm$ 0.32			
	$P_{\max}:R$	2.07	8.44	6.2			
August 1995	$P_{\max}$	6.02 $\pm$ 0.26	10.5 $\pm$ 0.41	10.8 $\pm$ 0.84			
	$I_{\text{sat}}$	66.4 $\pm$ 11.8	74.3 $\pm$ 11.3	72.2 $\pm$ 22.6			
	$I_c$	3.88 $\pm$ 2.17	2.57 $\pm$ 2.18	2.03 $\pm$ 4.32			
	$\alpha$	0.09 $\pm$ 0.001	0.14 $\pm$ 0.002	0.15 $\pm$ 0.01			
	$R$	-0.44 $\pm$ 0.15	-0.29 $\pm$ 0.16	-0.22 $\pm$ 0.1			
	$P_{\max}:R$	13.68	36.5	49.45			
Rhizome starch concentration (mg g DW <sup>-1</sup> )		60.52 $\pm$ 4.71	36.85 $\pm$ 9.94	34.74 $\pm$ 8.12			

<sup>a</sup> Rhizome starch concentrations determined in August 1995 are also indicated.

Elevated dissolved nutrient concentration, like that found close to the fish farm, can stimulate epiphyte overgrowth on seagrass leaves (Harlin and Thorne-Miller, 1981; Silverstein *et al.*, 1986; Neverauskas, 1987; Tomasko and Lapointe, 1991; Neckles *et al.*, 1993; Williams and Ruckelshaus, 1993; Sullivan and Wear, 1998), which eventually reduces the light reaching the leaf surface and affects the carbon budget of the plant (e.g. Alcoverro *et al.*, in press). However, this epiphyte increase was not found in this study; on the contrary, epiphyte loading was much smaller in the disturbed area than in the reference site (Fig. 4). This is explained by the exceptionally high herbivore pressure found close to the fish farm (stations HA and HB), which was much higher than that found in the reference meadow (station F) and higher than the values reported for undisturbed *P. oceanica* meadows in other localities (Alcoverro *et al.*, 1997). As seagrass grazers feed mainly on leaf tips, where the highest epiphyte biomass occurs, herbivore activity seems to account not only for the smaller shoot sizes found at the disturbed stations but also for the lower epiphyte loading. Similarly, high herbivore pressure has been also reported in *P. oceanica* in the vicinity of other fish farm facilities (Delgado *et al.*, 1997; Pergent *et al.*, 1999) and can be related not only to the observed increase in sea urchin density in zones with high organic matter concentration (Kirkman and Young, 1981) but also to changes in the palatability of epiphyte tissues induced by increases in water-column nutrient concentrations (Ruiz and Romeo, in progress). Macrograzer-exclusion experiments conducted in fish farm stations in Hornillo Bay have provided consistent evidence that overgrazing is the main cause of the reduced shoot sizes observed in this area (Ruiz *et al.*, submitted). Such an increase in grazing rates could explain seagrass mortality outside (and even inside) the affected area; in effect, a reduction in the photosynthetic biomass (with no reduction in the high belowground, respiratory biomass:

see Fourqurean and Zieman, 1991) could lead to a more negative carbon balance, and thus to seagrass mortality (Ruiz *et al.*, submitted; Alcoverro *et al.*, in press). The plant seems to react to such adverse conditions, and an adjustment of the photosynthetic parameters (e.g. higher  $P_{\max}$  values, Table 6) partially counteracts the photosynthetic biomass tissue. However, the observations of reduced leaf growth rates and the low rhizome starch concentration in plants from the affected zone (relative to those from the reference site) support the notion of a carbon budget imbalance. Such imbalances can cause the mortality of belowground organs both by the incapacity to supply organic substances for root/rhizome metabolism and by the decrease in the oxygen flux to these parts, which is necessary to maintain adequate aeration of the tissues and their rhizosphere, considered crucial for seagrass survival (Smith *et al.*, 1988; Czerny and Dunton, 1995).

In addition, damage to seagrasses can be caused by the deposition of particulate carbon released by the fish farm, which accumulates in the sediment and explains the high organic matter content measured in the vicinity of the fish farm (stations HA and HB) compared to the reference station (F). In effect, sediment anoxia has been shown to enhance seagrass mortality through the direct toxic effects of reduced compounds (Carlson *et al.*, 1994) or through increasing the photosynthetic oxygen demand of belowground tissues (Penhale and Wetzel, 1983; Smith *et al.*, 1988). Organic deposition has been used to explain continued seagrass die-off long after the cessation of fish farming activities (Delgado *et al.*, 1999). These authors also hypothesized that once a former seagrass area has been denuded, enriched sediments are easily resuspended, thus increasing dissolved nutrient availability and favouring further seagrass decline. Thus, since changes in water quality may not only be a direct consequence of fish farming, but also an indirect effect of a relatively complicated process we suggest that

the prediction of the timing and spatial propagation of seagrass regression is more complex than previously thought.

As shown, fish farms are a potential cause of seagrass decline, since they can affect light reduction, epiphyte overgrowth, overgrazing, sediment resuspension and sediment redox changes. Although we are unable to elucidate which factor (or which subset or interactions) accounts for the observed seagrass degradation in our study, it is clear that this seagrass species is highly sensitive to the effects of fish farming. To understand the response of this seagrass species to fish farm loading, questions such as the sharp increase in the grazing pressure in the fish farm vicinity or the tolerance of *P. oceanica* to anoxic conditions should be further assessed through additional experimental work. This understanding is crucial to prevent or minimize losses of this key ecosystem, which, in addition to being highly sensitive has very limited colonizing ability (Meinesz and Lefevre, 1984), thus making the recovery of denuded areas a very slow process (from decades to centuries). Therefore, extreme caution should be taken regarding future aquacultural activities.

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