



Effects of fish farm waste on *Posidonia oceanica* meadows: Synthesis and provision of monitoring and management tools

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ABSTRACT

This paper provides a synthesis of the EU project MedVeg addressing the fate of nutrients released from fish farming in the Mediterranean with particular focus on the endemic seagrass *Posidonia oceanica* habitat. The objectives were to identify the main drivers of seagrass decline linked to fish farming and to provide sensitive indicators of environmental change, which can be used for monitoring purposes. The sedimentation of waste particles in the farm vicinities emerges as the main driver of benthic deterioration, such as accumulation of organic matter, sediment anoxia as well as seagrass decline. The effects of fish farming on *P. oceanica* meadows are diverse and complex and detected through various metrics and indicators. A safety distance of 400 m is suggested for management of *P. oceanica* near fish farms followed by establishment of permanent seagrass plots revisited annually for monitoring the health of the meadows.

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1. Introduction

The rapid expansion of aquaculture in the coastal zones of the Mediterranean Sea has increased the risk for degradation of sensitive marine habitats such as rocky reefs, macroalgal beds, seagrass meadows and rhodolith communities (Holmer et al., 2003; Cancemi et al., 2006; Wilson et al., 2004). The meadows of *Posidonia oceanica*, an endemic seagrass widely spread along the coastlines of the Mediterranean Sea, play a critical role for the structural and functional attributes of the coastal ecosystem (Gobert et al., 2006). Seagrasses indeed represent an important food source for benthic consumers, offer refuge from predation to several benthic species, are nursery areas for commercial fish juveniles and have an important structuring role, enhancing habitat complexity, supporting epiphytes and epifauna, modifying sediment texture and hydrodynamic regime (Duarte, 2002).

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During the last 30 years, mostly because of anthropogenic disturbances, meadows of *P. oceanica* are progressively decreasing in density and extension (Marbà et al., 2005; Pergent-Martini et al., 2006). *P. oceanica* meadow recovery may require several centuries due to the extremely slow growth and low reproductive effort (Marbà et al., 2002; Kendrick et al., 2005; Gobert et al., 2006). Losses of *P. oceanica* should, therefore, be considered as irreversible. For these reasons, *P. oceanica* meadows are protected habitats under various international conventions, agreements and other legal documents (e.g. EU Habitat Directive, national laws, Bern Convention).

Many environmental requirements for coastal fish farming (e.g. good water quality and adequate water renewal) are, unfortunately, almost identical to the habitat preferences of *P. oceanica*. As a consequence, the weak enforcement of regulations of fish farm siting and the unequal efficiency in environmental monitoring or impact assessment procedures among Mediterranean countries or even regions has allowed that a considerable number of fish farms are placed over or very near *P. oceanica* meadows (Per-

gent-Martini et al., 2006). Therefore, the forecasted expansion of fish farming may accelerate the decline of this important marine habitat. The detrimental effect of fish farming on *P. oceanica* meadows has been demonstrated in various cases (Delgado et al., 1997, 1999; Pergent et al., 1999; Ruiz et al., 2001; Pergent-Martini et al., 2006). The main impacts on *P. oceanica* derive from the release of dissolved and particulate nutrients, and to a certain extent from the direct shading of the meadows by the net cages. Generally, the seagrasses disappear in a still increasing circle around the net cages, with an immediate high mortality just beneath the net cages (Delgado et al., 1997, 1999). Over the years, the seagrass front progressively moves away from the net cages, even after fish farming has ceased (Delgado et al., 1999). So far, it has, however, not been possible to identify all of the drivers derived from fish farming activities of seagrass decline, nor the thresholds of decline. The lack of this information prevents the provision of precise and appropriate advice for management and monitoring of *P. oceanica* meadows in fish farm vicinities.

In contrast to the difficulties encountered for detecting fish farm-induced environmental effects using water quality response parameters, benthic impacts are reported worldwide (Holmer et al., 2005; Kalantzi and Karakassis, 2006). A suite of different response parameters extending from simple measures of organic matter pools in the sediment to more sophisticated measures such as sediment microbial processes and complex macrofauna indices have been applied in fish farm surroundings with various performances (Kalantzi and Karakassis, 2006). Benthic responses to Mediterranean fish farming, in particular in *P. oceanica* habitats, can be expected to differ significantly compared to existing literature as the sediments generally are more advective, coarser grained with high carbonate contents and low organic matter pools compared to Atlantic fish farm sediments (Holmer et al., 2005; Marbá et al., 2006). Within the MedVeg project a number of different benthic measures were used to assess impacts on the benthic communities

and to explore their applicability as sensitive indicators of fish farm benthic impacts.

From 2001 to 2004 the EU-funded research project MedVeg (effects of nutrient release from Mediterranean fish farms on benthic vegetation in coastal ecosystems) has investigated the effects of fish farming on *P. oceanica* habitats by combining traditional monitoring methods and new sampling and experimental approaches in order (i) to identify the main drivers of seagrass decline linked to fish farming; (ii) to provide sensitive indicators of environmental change, which can be used for monitoring purposes and (iii) to provide estimates of “safety distances” between fish farms and *P. oceanica* meadows for management purposes.

To achieve these objectives we investigated the effects of sea bream/sea bass farming on the *P. oceanica* habitats in four regions of the temperate–warm Mediterranean Sea: Akrotiri Bay in Cyprus, Sounion Bay in Greece, Pachino Bay in Italy and the Gulf of Alicante in Spain. Our study was conducted at basin scale to overcome the main weakness of most fish farm impact studies, which have been carried out mostly at a local scale. To cope with this issue, while the four selected sites covered the entire range of environmental coastal conditions most commonly encountered in the Mediterranean Sea, the farming conditions were as similar as possible between the sites (Table 1). In the four sites, study locations with variable amounts of organic matter deposited from fish farms were compared with putatively pristine locations, used as controls. Focus of this study was the fate of dissolved and particulate nutrients released from the farms and their potential impact on the water quality, sedimentation rates, organic pools and microbial activity in the sediments as well as on the biological, ecological and physiological responses of the *P. oceanica* seagrass community to farming activities.

Results of this project have been published or submitted in various papers addressing separately specific aspects such as effects on the water column (Dalsgaard and Krause-Jensen, 2006; Pitta

Table 1
Main characteristics of the four fish farms and study sites

| Site | | Bottom temperature (°C) | Bottom current (cm s ⁻¹) | Sediment type | Chl. <i>a</i> (µg L ⁻¹) | N-NH ₄ (µM) | P-PO ₄ (µM) | In activity since | Distance from the shore (m) | Reared species | Farm area (m ²) | Annual production (tonne) | Food input (tonne yr ⁻¹) |
|--------|--------------|-------------------------|--------------------------------------|---------------------|-------------------------------------|------------------------|------------------------|-------------------|-----------------------------|---|-----------------------------|---------------------------|--------------------------------------|
| Cyprus | Akrotiri Bay | 17–18 | 10–15 | Carbonate mud | 0.01–0.02 | 12.5–13.4 | 0.9–1.1 | 1988 | 1050 | Sea bream (<i>Sparus aurata</i>) Sea bass (<i>Dicentrarchus labrax</i>) | 20.000 | 300 | 660 |
| Italy | Pachino Bay | 17–18 | 20 | Carbonate sand | 0.03–0.08 | 52.0–55.8 | 3.6–4.4 | 1992 | 1000 | Sea bream (<i>Sparus aurata</i>) Sea bass (<i>Dicentrarchus labrax</i>) Sharpsnout sea bream (<i>Diplodus puntazzo</i>) | 77.000 | 1150 | 2749 |
| Greece | Sounion | 17–18 | 6.3 | Carbonate sand | 0.08–0.11 | 12.1–13.0 | 0.8–1.0 | 1996 | 500 | Sea bream (<i>Sparus aurata</i>) Sea bass (<i>Dicentrarchus labrax</i>) | 27.000 | 400 | 640 |
| Spain | Alicante | 17–18 | 4.7 | Carbonate fine sand | 0.07–0.11 | 11.0–11.8 | 0.8–0.9 | 1996 | 2600 | Sea bream (<i>Sparus aurata</i>) Sea bass (<i>Dicentrarchus labrax</i>) | 17.000 | 260 | 520 |

Given are the water depth (m), bottom temperature (°C) and bottom current (cm s⁻¹), sediment type, chlorophyll *a* (chl. *a*, µg L⁻¹) and nutrient contents (ammonium (N-NH₄) and phosphate (P-PO₄) in µM) in the water column during sampling, as well as information on the fish farm production provided by the fish farmers for the year of sampling.

et al., 2006), sedimentation rates (Holmer et al., 2007), bio(geo)chemistry of the sediments (Holmer and Frederiksen, 2007; Pusceddu et al., 2007), benthic fauna (Heilskov et al., 2006; Apostolaki et al., 2007; Mirto et al., 2007) and *P. oceanica* (Marbá et al., 2006; Frederiksen et al., 2007; Pérez et al., 2007; Diaz-Almela et al., in press).

The aim of this paper is to provide a synthesis of the results and a comparative analysis of the performance of the indicators tested in this study in order to assist the scientists, managers and decision and policy makers to select the most relevant variables, descriptors and indicators for monitoring and management programs and, specially to provide suggestions for siting new farms.

2. Materials and methods

2.1. Study sites and sampling

Sampling was carried out along a Mediterranean east-to-west longitudinal transect (3500 km wide) encompassing Akrotiri Bay (Cyprus; July 2002), Sounion Bay (southern Greece; July 2003), Pachino Bay (Italy; September 2002) and the Gulf of Alicante (Spain; September 2003) (Fig. 1).

The sampling sites, located at similar latitudes and at depths ranging between 16 and 39 m, were selected on the basis of the presence of fish farms and characterized in terms of their main environmental features (e.g. start of activities, distance from the shore, species reared, annual production and feed input; Table 1).

In each site, a preliminary survey was carried out to characterize the environmental settings of the sites in terms of mean depth and temperature, bottom currents, sediment type and porosity, and chlorophyll *a* and inorganic nutrient concentrations in the water column (Table 1). At each site, six stations were established in bare sediments (three stations) and in *P. oceanica* meadows (three stations), two highly impacted (HI, 0–15 m from net cages), two less impacted (LI, 15–50 m from net cages) and two control situated upstream of the main currents, and at least 1000 m from the fish farms (Fig. 2). Furthermore, impacts on *P. oceanica* were also measured on three additional stations along a second transect, perpendicular to the main current direction in Greece and Spain (Fig. 2), and along it in Cyprus and Italy. Controls were characterized by relatively pristine conditions and by environmental fea-

tures comparable to those found beneath the fish-farm cages (one vegetated and one bare, Fig. 2). At each station we quantified the organic loads and their composition (rates of sedimentation of nutrients and biopolymeric fraction; Pusceddu et al., 2007), meio-faunal abundance, macrobenthic biomass and diversity, sediment biogeochemical conditions (sediment nutrient pools, sulfate reduction rates, sulfide pools), *P. oceanica* community (population dynamics, shoot density, shoot size, epiphyte cover and grazing impact), growth (vertical rhizome growth) and physiological response variables (nutrient and carbohydrate content, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$; see details in Table 2). Details on sampling techniques and analytical procedures used for the different variables are reported elsewhere (see references in Table 2). The performance of the different indicators is tested through correlation analysis between the identified drivers and the response parameters.

3. Results and discussion

3.1. Loss of waste products on annual cycle

In order to assess the amounts of nutrients released from the studied farms, estimations of dissolved and particulate nutrient losses were obtained by using previously reported mass balance models (Lupatsch and Kissil, 1998; Tzapakis et al., 2006) and from the feed inputs at the four studied farms, the annual release of nutrients were estimated (Table 3). The fish farm in Italy had the highest annual loss due to its large size and high feed conversion ratio. The other three farms attained similar annual nutrient losses, corresponding to about 25% of those estimated for the farm in Italy. In all the investigated regions, the dominant form of waste products was ammonium (11.0–55.8 tonne yr^{-1}), followed by fine particulate nitrogen (2.0–13.5 tonne yr^{-1}). Regarding phosphorus, the highest release was of fine particulates (2.6–12.1 tonne yr^{-1}) and less as dissolved phosphate (0.8–4.4 tonne yr^{-1}). Due to higher FCRs (1.6–2.4, Table 1), the release of N and P from sea bream and sea bass farming is about 1.5 times higher compared to salmon farming (Divanach, pers. comm.; Islam, 2005) and more severe environmental impacts can thus be expected from sea bream and sea bass farming.

Most of the Mediterranean fish farms operate in remote places characterised by the absence of significant point sources of nutri-

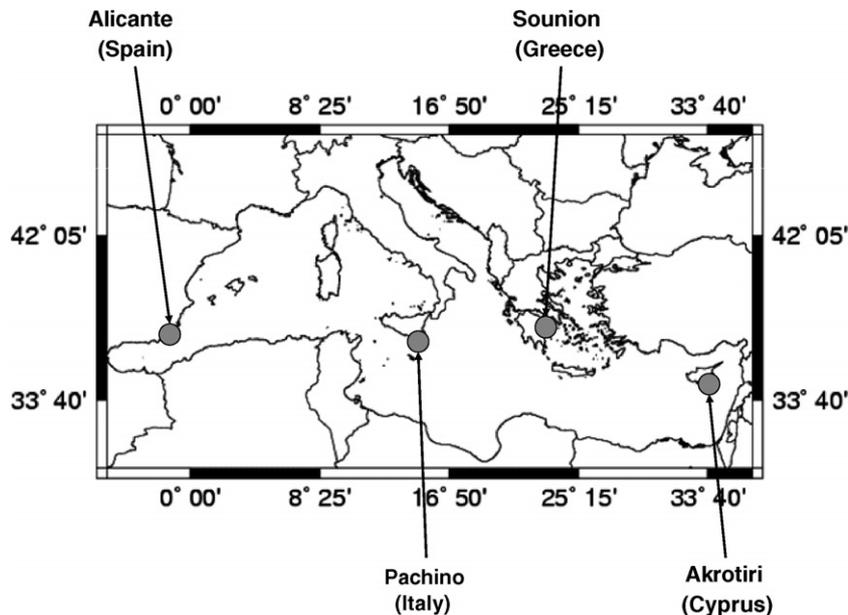


Fig. 1. Location of the four study sites in the Mediterranean.

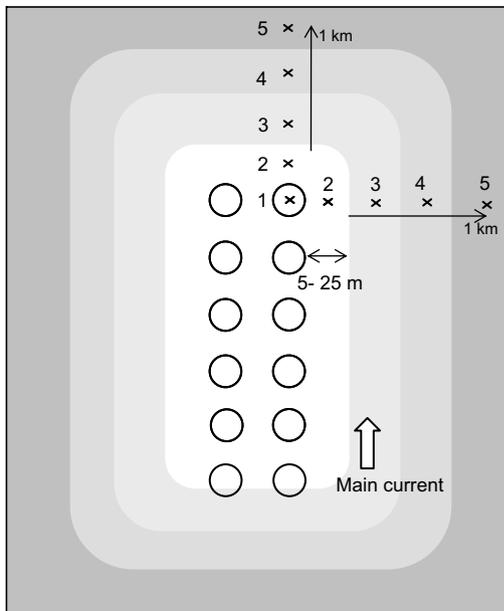


Fig. 2. Sampling design in the MedVeg project. Shaded areas represent *P. oceanica* meadow in different stages (stage 1, bare under the net cages; stage 2, bare outside the net cages; stage 3, most impacted *P. oceanica*; stage 4, low impacted *P. oceanica* and stage 5, control = pristine *P. oceanica*). For most parameters only the transect in the main current direction was examined. Station 6 was located in the vicinity of station 5, but in an area without presence of *P. oceanica*.

ent pollution (e.g. rivers and municipal waste treatment plants). Therefore, in these sites, and specifically in the more oligotrophic areas of this basin, the atmospheric deposition of nutrients may be of major importance (Markaki et al., 2003; Bergametti et al., 1992; Migon and Bethoux, 2001; Ridame and Guieu, 2002). The values of annual atmospheric deposition of dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) in the Eastern Mediterranean (including wet and dry inputs) are 31.3 mmol m⁻² yr⁻¹ and 193.4 μmol m⁻² yr⁻¹ for DIN and DIP, respec-

Table 3

Fish production, feed input and calculation of the annual waste productions of the four fish farms (range, tonne yr⁻¹, see text for explanations) and the atmospheric deposition corresponding to the farm area (kg yr⁻¹)

| Site | Fish production (tonne yr ⁻¹) | Feed input (tonne yr ⁻¹) | NH ₄ min–max (tonne yr ⁻¹) | PO ₄ min–max (tonne yr ⁻¹) | PON min–max (tonne yr ⁻¹) | POP average (tonne yr ⁻¹) | Atm. DIP ^a (kg yr ⁻¹) | Atm. DIN ^a (kg yr ⁻¹) |
|--------|---|--------------------------------------|---|---|---------------------------------------|---------------------------------------|--|--|
| Cyprus | 300 | 660 | 12.5–13.4 | 0.9–1.1 | 2.3–3.2 | 2.9 | 0.1 | 8.8 |
| Greece | 400 | 640 | 12.1–13.0 | 0.8–1.0 | 2.2–3.1 | 2.8 | 0.2 | 11.7 |
| Italy | 1150 | 2749 | 52.0–55.8 | 3.6–4.4 | 9.6–13.5 | 12.1 | 0.5 | 33.6 |
| Spain | 260 | 520 | 11.0–11.8 | 0.8–0.9 | 2.0–2.8 | 2.6 | 0.1 | 7.6 |

^a Annual atmospheric deposition (kg yr⁻¹) of dissolved inorganic phosphorus (DIP) and nitrogen (DIN) was estimated using the deposition fluxes published by Markaki et al. (2003) multiplied with the corresponding area of each fish farm (see Table 1).

tively (Markaki et al., 2003). The estimated annual atmospheric input of DIN in an area corresponding to the farms ranges from 7.6 to 33.6 kg yr⁻¹ in Spain and Italy farm areas, respectively, and for DIP from 0.5 kg yr⁻¹ (Italy) to 0.1 kg yr⁻¹ (Spain, Cyprus, Table 3). We estimated that the fish farm loads of ammonium alone correspond to the DIN atmospheric inputs on a total of 26 km² (Spain) and 123 km² (Italy). Significantly larger areas (140–665 km²) would be needed in order to accumulate the same amount of DIP from the atmosphere as that released from fish farms. These calculations indicate that, in the Mediterranean Sea, the inputs from fish farms can be considered as significant point sources of nutrient pollution.

3.2. Effects of nutrient release on water quality

Despite the high fish farm loads of nitrogen and phosphorus to the marine environment, insignificant deterioration of the water quality near fish farms has been observed worldwide and in the

Table 2

Sampling of variables along transects at the four fish farms

| Water column | Sedimentation | Sediments | Seagrasses | Benthic fauna ^b |
|--|---|---|---|---|
| ^{a,c} Inorganic nutrients (NO ₃ ⁻ , NO ₂ ⁻ , NH ₄ ⁺ and PO ₄ ³⁻) | ^e Pelagic sedimentation (TOM, POC, PON and TP) | ^{e,f} Organic matter (TOM, POC, PON, TP, bio-polymeric fraction and protein) | ⁱ Recruitment | ^e Meiofauna abundance |
| ^a Organic nutrients (DOC and DON) | ^f Benthic sedimentation (TOM, POC, PON and TP) | Density, porosity and grain size | ^j Rhizome growth | ^f Macrofauna diversity and biomass |
| ^c Chlorophyll <i>a</i> | | Fluxes (oxygen and inorganic nutrients) | ⁱ Shoot density | |
| ^d Bio-assays (phytoplankton and macroalgae) | | Porewater (nutrients and ^h sulfides) | Global density | |
| | | ^{g,h} Sulfate reduction rates | Shoot size | |
| | | ^{g,h} Sulfur pools | Epiphyte cover | |
| | | ^h Sulfide front | Grazing impact | |
| | | | ^k Nutrient content (N, P) | |
| | | | ^k Carbohydrate and amino acid content | |
| | | | ^{h,k} Stable isotope (¹⁵ N, ³⁴ S) | |

Original references are given for published results.

^a Integrated sample over entire water column.

^b Only high impacted and control were compared.

^c Pitta et al. (accepted for publication).

^d Dalsgaard and Krause-Jensen (2006).

^e Pusceddu et al. (2007), sampled only in Greece and Spain.

^f Holmer et al. (2007).

^g Holmer and Frederiksen (2007).

^h Frederiksen et al. (2007).

ⁱ Diaz-Almela et al. (in press).

^j Marbá et al. (2006).

^k Pérez et al. (in press).

^l Apostolaki et al. (2007).

Mediterranean (La Rosa et al., 2002; Soto and Norambuena, 2004; Pitta et al., 1999, 2006; Sara, 2007). Increased nutrient availability was, however, documented at the four fish farms by applying a novel technique using microalgae and macroalgae bioassays for assessment of nutrient availability in fish farm surroundings (Dalsgaard and Krause-Jensen, 2006). The bioassays showed dramatic increases in primary productivity induced by the fish farms, with up to 6.7 times higher phytoplankton biomass in the assay at the edge of the fish farms than in the control after 6 days of deployment. The bioassays detected enhancement of primary production up to at least 150 m downstream in the dominant current direction, which is further than previous findings (Karakassis et al., 2001; La Rosa et al., 2002; Pitta et al., 2006). In our study, the stimulation of algal productivity induced by nutrients released from fish farms may be attributed to the general oligotrophic conditions of the Mediterranean, where it can be expected that even a small input of nutrients initiate a rapid algal growth followed by intensive grazing (Zohary et al., 2005). Soto and Norambuena (2004) suggested that increased nutrient concentrations are not usually found in the vicinity of fish farms not only because of dilution processes but also because they pass through the food web very rapidly. The rapid transfer of nutrients in the food web is consistent with recent results by Machias et al. (2004, 2005) who found an increased abundance and biomass of wild fish in response to the presence of fish farming zones, stimulated by increased primary production and transfer of this organic matter to higher trophic levels. Fast dispersal of phytoplankton due to water movement as well as efficient grazing could, to some extent, explain the uniform distribution of chlorophyll *a* in the vicinity of aquaculture installations (Table 1, Pitta et al., accepted for publication.).

3.3. Effects of nutrient release on sedimentation rates

Our study also pointed out that, in all investigated sites, the sedimentation of particulate organic matter was enhanced near the net cages, and that the sedimentation patterns were similar at the four farms. The sedimentation rates were consistent with the mass balance model above, showing high particulate P sedimentation (Holmer et al., 2007). The size of the area affected by particulate waste products from fish farms in Greece and Spain was estimated by deploying pelagic sediment traps. Sedimentation of P around the cages was used to develop a sedimentation model, and the rates decreased rapidly with distance from the net cages and were comparable between the two sites, despite differences in water depth, farm production and current regime (Fig. 3). The model showed that the sedimentation rates at 10 m distance from the cages were reduced to 24–35% when compared with the rates just beneath the cages, and progressively decreased down to 5–10% at 100 m and to 2–5% at 250 m, in Spain and Greece, respectively (Fig. 3). On the other hand, at 250 and 500 m from the cages the sedimentation rates were approximately 100% and 40% higher than that measured at the control stations. Sarà et al. (2004) found, on the basis of isotopic studies, that the dispersion of waste products from a fish farm at a site with hydrodynamic characteristics similar to those reported here reach beyond 300 m. Furthermore, benthic sedimentation rates showed similar patterns with distance from the farms as the pelagic traps with high sedimentation right under the net cages and exponential decline with distance (Holmer et al., 2007). Sedimentation rates appeared to be important drivers of the benthic impacts, as will be discussed below in detail. The benthic rates were measured at all fish farms and are used in the analysis of the results (Table 4). As observed for the nutrient loading of the water column, fish farms in the oligotrophic Mediterranean Sea contribute significantly and at long distances to the nutrient input to the benthic ecosystem.

Since the benthic response to organic enrichment depends more upon the quality of the inputs than to their absolute values (Albertelli et al., 1999), the biochemical composition of the sedimentation was investigated to assess the lability of the organic matter depositing in fish farm areas. At all study sites, the sedimentation of biopolymeric C (as the sum of protein, carbohydrate and lipid C equivalents, Fabiano et al., 1995) varied significantly at increasing distance from the cages, suggesting that the lability of the organic matter deposited in the sediments is high near the cages and decreases with distance from the farm (Pusceddu et al., 2007).

3.4. Quantity and biochemical composition of sediment organic matter

The high sedimentation rates of P was reflected in an accumulation of P in the sediments, and as for sedimentation of P, sediment P was a useful indicator of fish farm waste loading, in contrast to the accumulation of organic C and N, which were lower and more variable (Table 4, Holmer et al., 2007). C and N pools themselves are often applied successfully for fish farms located over bare sediments (Kalantzi and Karakassis, 2006), but they appear weak descriptors of impact at farms surrounded by *P. oceanica* habitats. P, on the other hand, has also been proposed as an indicator of fish farm impact in *P. oceanica* habitats by Pergent-Martini et al. (2006). Although the different sedimentary pools of P were not investigated in this study, P is considered to bind strongly to the carbonates in these carbonate rich sediments (Jensen et al., 1998), and may as such be buried in the sediments and thereby reflect fish farm waste loss over time. The biopolymeric C concentration can also be considered a sensitive descriptor of the farm impact, although the quantitative changes are site-specific (Pusceddu et al., 2007). In the more eutrophic waters along the Spanish and Italian coasts, sediments were not characterized by increased biopolymeric C concentrations. In these areas, however, the values of the protein to carbohydrate ratio, used as an indicator of the shift in the biochemical composition of the sediment, displayed the highest values below the cages (Pusceddu et al., 2007). In oligotrophic sites (i.e. with biopolymeric C concentrations in controls stations below 2.5 mg C g⁻¹, as in Cyprus and Greece, sensu Pusceddu et al., 2007), the correlation analysis revealed no significant relationships between the C, N and P sedimentation rates and the biopolymeric C concentration in the sediments, and showed negative relationships between C, N and P sedimentation with the values of the protein to carbohydrate ratio (Table 4). On the other hand, in eutrophic areas (i.e. with biopolymeric C concentrations

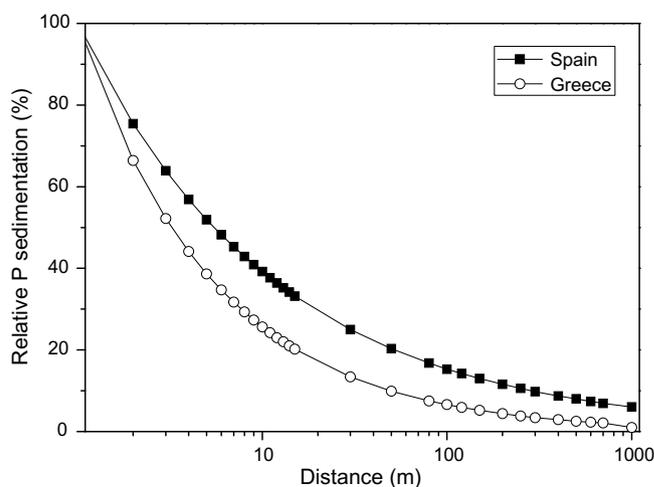


Fig. 3. Relative rates of sedimentation of phosphorus along the transects from the farms in Greece and Spain. See text for more information.

Table 4

Variables investigated and their ability to show environmental changes near fish farms as a function of the rates of benthic sedimentation (total mass, OM, TN, TC or TP sedR, data reported in Holmer et al., 2007)

| Variable | Drivers | | | | |
|---|------------------------|------------------------|------------------------|------------------------|------------------------|
| | Total mass sedR | Total OM sedR | Total N sedR | Total C sedR | Total P sedR |
| <i>Sediment</i> | | | | | |
| TP | $R = 0.82; p = 0.010$ | $R = 0.96; p < 0.001$ | + | + | $R = 0.97; p < 0.001$ |
| POC | + | + | + | + | + |
| PON | + | + | + | + | + |
| C:N | + | + | + | $R = 0.66; p = 0.05$ | + |
| C:P | $R = 0.78; p = 0.01$ | + | + | + | $R = 0.68; p = 0.04$ |
| Biopolymeric C in oligotrophic sites | + | + | + | + | + |
| Biopolymeric C in eutrophic sites | $R = -0.58; p = 0.011$ | + | $R = -0.72; p < 0.001$ | $R = -0.61; p = 0.004$ | + |
| Protein to carbohydrate in oligotrophic sites | – | – | $R = -0.78; p = 0.003$ | $R = -0.78; p = 0.003$ | $R = -0.80; p < 0.001$ |
| Protein to carbohydrate in eutrophic sites | $R = 0.63; p = 0.005$ | $R = 0.95; p < 0.001$ | $R = 0.50; p = 0.035$ | $R = 0.78; p < 0.001$ | $R = 0.65; p < 0.005$ |
| SRR | $R = 0.75; p < 0.001$ | $R = 0.85; p < 0.001$ | $R = 0.35; p = 0.028$ | + | $R = 0.91; p < 0.001$ |
| <i>Seagrass</i> | | | | | |
| Shoot density | + | + | $R = -0.62; p = 0.030$ | + | $R = -0.71; p = 0.014$ |
| Shoot size | + | + | + | + | $R = -0.73; p = 0.017$ |
| Vertical rhizome growth | + | + | + | + | + |
| Shoot mortality | $R = 0.78; p < 0.001$ | $R = 0.83; p < 0.01$ | $R = 0.58; p < 0.01$ | + | $R = 0.75; p < 0.001$ |
| Shoot recruitment | – | – | – | – | – |
| Net population decline | $R = 0.79; p < 0.001$ | $R = 0.91; p < 0.001$ | $R = 0.55; p < 0.01$ | + | $R = 0.71; p < 0.001$ |
| $\delta^{34}\text{S}$ in roots | + | + | $R = -0.75; p = 0.019$ | $R = -0.93; p = 0.008$ | $R = -0.74; p = 0.037$ |
| <i>Fauna</i> | | | | | |
| Meiofaunal abundance | + | + | $R = 0.776; p < 0.001$ | $R = 0.844; p < 0.001$ | + |
| Number of meiofaunal taxa | $R = -0.37; p = 0.027$ | $R = -0.55; p = 0.003$ | + | $R = -0.38; p = 0.038$ | + |
| Nanobenthos abundance in Italy only | + | + | + | + | + |

Strength of the indicator is defined as the correlation coefficient between drivers and the investigated variables: – = not significant and levels of the variable in the impacted site not significantly distinguishable from the control; + = not significant, but levels of the variable in the impacted site significantly different from the control. When significant, the Spearman rank correlation coefficients (R) and the p level are reported.

in the controls higher than 2.5 mg C g^{-1}) weak correlations between sedimentation rates and the biopolymeric C concentrations were observed, whereas positive correlations were revealed between C, N, and P sedimentation and the values of the protein to carbohydrate ratio. These results highlight that changes in quantity and biochemical composition of sediment organic matter induced by fish farming are idiosyncratic, site-specific and depend upon the background trophic conditions. Therefore, their determination appears to be critical for assessing the trajectories of change induced by fish farming activities.

3.5. Effects on sediment microbial activity and sulfur cycling

One possible reason for low accumulation of organic matter at the four fish farms could be a rapid mineralization of organic matter in the sediments. The microbial activity in low-organic sediments, in particular those of the oligotrophic Mediterranean Sea, are considered to be limited by inputs of labile organic matter (Danovaro et al., 2000). When sediments are loaded with labile organic matter, the microbial electron acceptor chain is usually shifted towards the anaerobic processes, and in the case of oligotrophic carbonate-rich sediments with low nitrate and iron contents as at the four study sites, sulfate reduction is expected to become an important process for the decomposition of organic matter (Holmer et al., 2005). This is consistent with findings of high sulfate reduction rates (SRR) observed under the net cage at the largest farm in Italy (up to $212 \text{ mmol m}^{-2} \text{ d}^{-1}$), where SRR were stimulated deep into the sediment (Holmer and Frederiksen, 2007). The SRR at the four farms were similar to rates found at shallower, temperate fish farms (Holmer and Kristensen, 1992), where higher sedimentation rates can be expected, indicating that the sulfate reducing bacteria in these low-organic sediments respond strongly to organic matter loadings. Sulfate reduction rates in the *P. oceanica* sediments were among the highest recorded so far in the literature, similar to rates found in degrading *P. oceanica* meadows impacted by organic matter loadings derived from phy-

toplankton, raw sewage or detritus of macroalgae (Holmer et al., 2004, 2005). The significant correlation between sulfate reduction rates and the sedimentation rates (Table 4) suggests that the sediment organic matter mineralization processes under the cages were controlled by inputs of labile organic matter from the fish farm. This supports that the sedimentation of farm effluents is an important driver of changes in sediment biogeochemical conditions in fish farm sediments. The higher SRR in the impacted sediments resulted in increased pools of sulfides accumulating in the sediments (Holmer and Frederiksen, 2007) and high concentrations ($1\text{--}125 \text{ }\mu\text{M}$) of sulfides in the pore waters were detected near the net cages (Frederiksen et al., 2007; Dalsgaard, pers. comm.). Further, the nutrient fluxes of nitrogen and phosphate across the sediment–water interface were affected by the loading of the sediments underneath the fish cages. The sediments changed from being autotrophic, with oxygen production and nutrient uptake in the presence of light and microphytobenthos at the sediment surface, into being net heterotrophic with release of ammonium and phosphate to the water column and enhanced pore water pools of both nutrients (Dalsgaard, pers. comm.). Impoverished sediment conditions, such as increased anoxia and presence of sulfides, may negatively affect the benthic flora and fauna (Pearson and Rosenberg, 1978; Holmer et al., 2005; Calleja et al., 2007; Frederiksen et al., 2007).

The presence of burrowing macrofauna plays an important role for the decomposition of organic matter in fish farm sediments, as the fauna increase the rates of decomposition by digesting the deposited organic matter as well as by ventilating the sediments, which, in turn, stimulate the microbial decomposition of settled organic matter (Heilskov and Holmer, 2001). The effect of macrofauna on the sediment biogeochemical conditions was studied at the farm in Cyprus in an experimental setup with the surface-living opportunistic polychaete (*Hermodice carunculata*) and the two burrowing climax polychaetes (*Glycera rousii* and *Naineris laevigata*, Heilskov et al., 2006). The decomposition and nutrient fluxes across the sediment–water interface were affected by irrigation

activity, in particular by the burrowing polychaetes. These organisms ventilate their burrows and thus increased the rate of nutrient cycling relatively more than *H. carunculata*. The nutrient retention was, however, much higher in these oligotrophic sediments than under temperate eutrophic conditions, and the nutrient release to the water column was thus relatively lower than that found in more organic rich fish farm sediments (Holmer and Kristensen, 1996; Heilskov and Holmer, 2001). This is probably due to a number of reasons, such as, for both N and P, a build-up of bacterial biomass incorporating nutrients and for P in particular, the binding to sedimentary carbonates. This study thus emphasizes the importance of maintaining a healthy and diverse benthic fauna community also in oligotrophic fish farm sediments to stimulate decomposition of depositing waste products and thereby minimize accumulation of organic matter and prevent development of anoxia in the water column and sulfidic sediments (Heilskov et al., 2006).

3.6. Impacts on seagrasses: community structure

Fish farms effluents had major negative effects on the structure and functioning of *P. oceanica* meadows near the cages compared to controls at almost all of the investigated sites, similar to that found in others studies (see review in Pergent-Martini et al., 2006). Meadow cover, assessed by the visual estimation of seagrass abundance (as percentage of bottom cover by seagrass shoots) in 40 × 40 cm squares situated along a 10 m transects, was found to decrease by 63–85% in most impacted stations (station 3 in Fig. 2) and by 34–45% in low impacted stations (station 4 in Fig. 2) relative to control stations whereas shoot density (Fig. 4) was from 70% to 90% lower in the most impacted stations and from 30% to 60% in the low impacted stations relative to control stations. These changes could be attributed to a series of direct and indirect mechanisms driven by the increase in sedimentation rates of organic matter due to fish farm effluents, as shoot densities were negatively correlated with N and P sedimentation rates (Table 4).

Other consistent finding was the increase of the abundance sea urchins (*Paracentrotus lividus*) in surroundings of fish farms in Italy and Greece. Sea urchin density (15 and 50 individuals m⁻² in Italy and Greece, respectively) around the fish farms was up to 3-folds higher than in control stations, and are among the highest values presently observed in *P. oceanica* meadows in the entire Mediterranean Sea (2–14 individuals m⁻², Prado et al., in press). Although increases of sea urchin density have been associated to predators' release (overfishing of sea urchins predators, cascading effects, Sala et al., 1998) the contribution of increased nutrient loading should not be ignored (bottom-up effects, McGlathery, 1995). Nutrient enrichment can enhance epiphyte abundance and plant tissue palatability (Tewfik et al., 2005) which can explain the observed high abundance of sea urchins around fish farms. The high sea urchin abundance can affect the seagrass performance as found by the reduced shoot size in the most impacted stations (from 40% to 70% relative to controls). But as a reduction in shoot size also occurred in a fish farm with no sea urchins (Spain) other direct or indirect factors besides grazing must also be implicated in the reduction of plant performance.

3.7. Impacts on seagrasses: vertical rhizome growth

The decrease in shoot size at impacted stations when compared to that at control ones may also reflect a decrease of plant growth in response to fish farming activity. *P. oceanica* growth, assessed on vertical rhizomes, is sensitive to disturbance and stress, such as those derived from increased sediment burial and erosion (Marbá and Duarte, 1997; Guidetti, 2001) and fish farming activities (Delgado et al., 1999). Vertical *P. oceanica* rhizomes grew faster prior (from 2.7 ± 0.38 to 5.9 ± 0.58 mm yr⁻¹) than after (from 1.9 ± 0.16 to 4.8 ± 0.45 mm yr⁻¹) the onset of fish farm activity, with rhizomes in Cyprus growing at the fastest and in Spain at the slowest rates (Marbá et al., 2006). Impacts of fish farm activity on *P. oceanica* vertical growth were detected up to 400 m distance from the cages (Marbá et al., 2006), with the largest decrease in vertical growth observed in the vicinity of the cages. Vertical rhizome

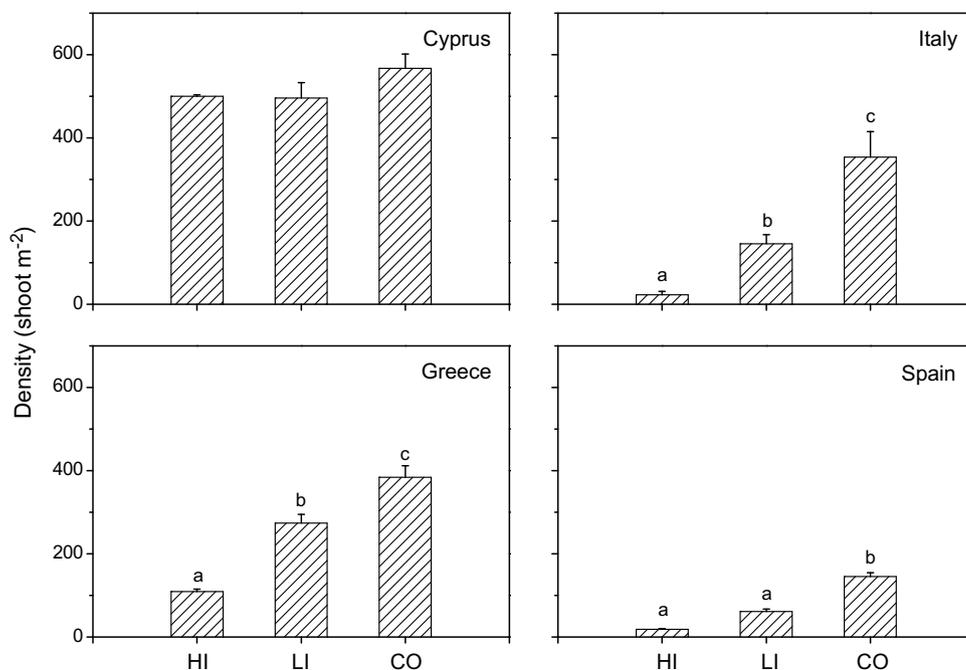


Fig. 4. Shoot density of *P. oceanica* meadows at increasing distances from the fish farms in the four sites studied. HI = high impact station (station 3), LI = low impact station (station 4) and CO = control station (station 5). Bars indicate standard error of the mean. Stations significantly different are indicated with different letters (ANOVA, $p < 0.001$).

growth rapidly responded to fish farm activities, since the time course of vertical growth for individual rhizomes near the cages showed a decrease in growth within at most 1 year after the onset of fish farm activities (Marbà et al., 2006). Variability of vertical rhizome growth was independent from sedimentary inputs (Table 4), revealing that fish farm activities impact plant growth through multiple stressors. The high sensitivity, including the fast response, of *P. oceanica* vertical growth to fish farm activities demonstrate the value of reductions in vertical rhizome growth as an early warning indicator of stress and fish farm impacts to *P. oceanica* meadows.

3.8. Impacts on seagrasses: population dynamics

The changes observed in density and cover of *P. oceanica* beds in the studied sites reflected the strong impacts of fish farm activities on shoot population dynamics. Fish farm operations dramatically increased shoot mortality, the rate of mortality decreasing exponentially with increasing distance from the cages, mostly due to the decrease of sedimentary inputs derived from fish farm operations (Table 4, Diaz-Almela et al., in press). The relationship between mortality rate and sedimentary inputs revealed the existence of thresholds of nutrient inputs ($50 \text{ mg P m}^{-2} \text{ day}^{-1}$ or $1.5 \text{ g organic matter m}^{-2} \text{ day}^{-1}$) above which shoot mortality accelerated. Shoot recruitment in the studied sites was low, ranging from negligible 0.01 to 0.31 yr^{-1} , and similar to shoot recruitment estimates reported for other *P. oceanica* meadows (Marbà et al., 2005). Shoot recruitment did not vary with variability in sedimentary inputs (Table 4), nor with distance from the cages (Diaz-Almela et al., in press). The effect of fish farm operations on shoot mortality, however, resulted in shoot populations nearby the cages to decline at annual rates ranging between $-0.74 (\pm 0.43)$ and $-4.17 (\pm 1.79) \text{ yr}^{-1}$. Decline rates exponentially decreased with increasing distance from the cages, and they were reduced to half of those observed in the vicinity of the cages at 80 m distance from the farms (Diaz-Almela et al., in press). However, regression curve of shoot mortality with distance did not reach global shoot recruitment rates ($0.13 + 0.10 \text{ yr}^{-1}$) until 400 m away from the cages, demonstrating that fish farm impacts on meadow stability were still evident within this distance from the farms (Diaz-Almela et al., in press). Beyond this distance, shoot populations were in steady state or exhibited declining rates similar to those observed in other *P. oceanica* meadows not impacted by fish farming activities (Marbà et al., 2005; Diaz-Almela et al., in press). The decline rates quantified in this study predicted shoot density to be reduced by half in about 3–26 months and in about 1–6 years, respectively, within the 15 and 50 m distance from the cages (Diaz-Almela et al., in press). Similarly, meadows at 15 and 50 m from the farms were predicted to be lost (i.e. shoot density decrease $>90\%$) within 5–11 years and 11–32 years (Diaz-Almela et al., in press).

The results of this study clearly demonstrate that *P. oceanica* demography is highly impacted by fish farm activities. Assessment of shoot demography in permanent plots, as conducted in this study, allows detection of fish farm impacts before they are evident in meadow cover or in shoot density quantified using random quadrates (Heidelbaugh and Nelson, 1996; Marbà et al., 2005). Therefore, shoot demography can be used as early indicator of fish farm impacts on *P. oceanica* beds. Quantification of net shoot population growth in permanent quadrates can easily be incorporated in fish farm monitoring programs. The magnitude of fish farm impacts on *P. oceanica* population may be predicted from nutrient input rate to vegetated sediments. The power of nutrient inputs to predict fish farm impacts on *P. oceanica* demography relies on the fact that it drives impacts on sediment biogeochemistry (e.g. anoxia, sulfate reduction rates and sulfide concentration, Table 4), grazing

pressure and epiphyte load which all have deleterious effects on seagrasses (Delgado et al., 1997; Ruiz et al., 2001; Greve et al., 2003; Marba et al., 2007).

3.9. Impacts on seagrasses: seagrass physiology

Fish farms effluents had a significant effect on *P. oceanica* physiology due to both nutrient increase and sediment organic matter enrichment. The most consistent responses to nutrient enrichment were the increase in total nitrogen content and the nitrogen isotopic ratio ($\delta^{15}\text{N}$) in the epiphytes, in total phosphorus content in epiphytes and plant rhizomes and a decrease in total non-structural carbohydrates in plant rhizomes (Pérez et al., in press). Besides shoot density, which is considered as a general response following human impacts and can be considered as a robust and integrative indicator of meadow health (Pergent-Martini et al., 2005; Romero et al., 2007), these descriptors can be recommended as monitoring tools in aquaculture management.

Furthermore, sulfide concentrations as low as $10 \mu\text{M}$ in the pore waters have been found to cause net decline of *P. oceanica* (Calleja et al., 2007), probably due to sulfide invasion into the plants (Frederiksen et al., 2007). Due to the increased microbial production of sulfide in the sediments (Holmer and Frederiksen, 2007), there is a risk of sulfide invasion into *P. oceanica* growing close to the net cages (Frederiksen et al., 2007). An invasion of sulfides will show up as lower $\delta^{34}\text{S}$ signals in the affected plants tissues (Rennenberg, 1984; Frederiksen et al., 2006), and lower $\delta^{34}\text{S}$ values, in particular in *P. oceanica* root and rhizomes, were found consistently near all the farms, and the contribution of sediment sulfides to the sulfur content of the plants was up to 37% of the sulfur in the plant (Frederiksen et al., 2007). The sulfide invasion in the roots correlated with the depth of the sulfide front (i.e. the sediment depth where sulfide is present, $R^2 = 0.61$, $p = 0.01$), and the $\delta^{34}\text{S}$ in plant roots was negatively correlated with the sedimentation rates of C, N and P (Table 4). This suggests that $\delta^{34}\text{S}$ signals in the roots along with the sulfide front and the rates of sedimentation can be used as indicator of the sulfide pressure on the plants. The mortality of *P. oceanica* has been found negatively correlated to the $\delta^{34}\text{S}$ of the plants indicating higher plant mortality with increasing sulfide invasion (Marbà, pers. comm.), but it was not possible to confirm this observation at the fish farm sites due to the few data available.

3.10. Impacts on benthic fauna: macrofauna

Past studies in the Mediterranean have shown that the effects of fish farming on macrofauna were significant up to 25 m from the edge of the cages (Karakassis et al., 2000; Lampadariou et al., 2005) which is comparable with what is known for the effects on fine sediments globally (Kalantzi and Karakassis, 2006). However, in the study of macrofauna communities at the four fish farms in the coarse sediments of the *P. oceanica* habitat, the species number and abundance showed almost no variability between the impacted and the control stations at all sites (Apostolaki et al., 2007). Furthermore, all diversity indices used were found to be quite high at all stations, implying that the macrofauna at the specific sites were not under severe stress due to fish farm activities. Although macrofauna is an established method for monitoring environmental impacts in the marine environment (Gray, 1981) and has been highly recommended for the monitoring of fish farms (GESAMP, 1996), it is not necessarily an absolute measure for the health of the system and our results suggest that it cannot be recommended for use in *P. oceanica* habitats. Apostolaki et al. (2007) suggested that the additional organic material supplied by the fish farm effluents, in combination with oxic conditions induced by intense currents and high advection in the coarse sediments, allows

the existence of diverse communities of high abundance and biomass.

3.11. Impacts on benthic fauna: meiofaunal assemblages

Meiofauna are, for their ecological importance and the lack of larval dispersion, becoming a popular tool for investigating structural and functional changes of natural and anthropogenic impacted ecosystems (Mirto and Danovaro, 2004). However, the direction of such changes is not easily predictable since the differences between the impacted assemblages and those in the controls are often not consistent (e.g. meiofaunal abundance may either increase or decrease beneath the fish cages, depending on the site, reared species, etc., Fig. 5). The general pattern observed in the *P. oceanica* sediments at the four fish farms was an increase of meiofaunal abundance in impacted sediments when compared with controls, although these differences were significant only in Cyprus and Greece. These results are in contrast with previous studies that reported a decrease of meiofaunal abundance in systems subjected to high organic loads (Mirto et al., 2002), but are in good agreement with the results reported in studies dealing with the impact of mussel farm biodeposition (Danovaro et al., 2004). This partially unexpected effect could be related with the fact that the organic enrichment in the sediments beneath the cages was relatively limited when compared with other fish farm studies (Holmer and Kristensen, 1996; Kalantzi and Karakassis, 2006). At all investigated farms the meiofaunal taxon richness decreased significantly beneath the cages, due to the disappearance of the more sensitive taxa. The taxa that disappeared beneath the cages were, however, different at the four farms, suggesting that meiofaunal community response to biodeposition can be site-specific. The changes in taxa number were driven by the sedimentation as significant negative correlations between taxa number and sedimentation rates, in terms of total sedimentation rate, total organic matter and organic carbon sedimentation, were revealed (Table 4). These results indicate that the inputs of organic matter, released from the cages to

the benthic environment, induce changes in the sediment characteristics, as well as in the structure of the meiofaunal assemblages. The meiofauna abundance and taxa number thus appear rather sensitive to organic matter inputs and may be useful as indicators of fish farm waste inputs in *P. oceanica* habitats.

4. Perspectives and recommendations

The effects of fish farming on *P. oceanica* meadows are diverse and complex and were detected through various metrics and indicators. However, it is worth noting that the monitoring of these issues needs to take into account the peculiarities of this particular ecosystem and not to adopt the standard monitoring protocols used elsewhere. The results of our study clearly show that the most important process affecting *P. oceanica* is the sedimentation of organic material. This material has relatively low or even insignificant effects on benthic macrofauna, but it interferes with various biogeochemical processes in the sediments thereby modifying microbial activity, nutrient regeneration and increases the sulfide concentrations in the sediments and sulfide invasion into the seagrasses, which may be one of the factors contributing to the observed decline of *P. oceanica* at the four study sites. The seagrass community is also affected by increased epiphytic loading and in some locations increased grazing by sea urchins leading in general to reduced shoot density and shoot size. All meadows experienced increased mortality rates and reduced recruitment, revealing that, if current environmental conditions persist, shoot density would decrease by half within 3–26 months and the meadow would be lost within 5–11 years close to the cages.

Sedimentation is considered the main driver of the negative environmental changes in the *P. oceanica* meadows and appears as a strong indicator of fish farming impacts with a threshold value of $50 \text{ mg P m}^{-2} \text{ d}^{-1}$ or $1.5 \text{ g organic matter m}^{-2} \text{ d}^{-1}$ (Diaz-Almela et al., in press). Monitoring of sedimentation rates is thus a strong tool for the management of seagrasses near fish farms. They, in

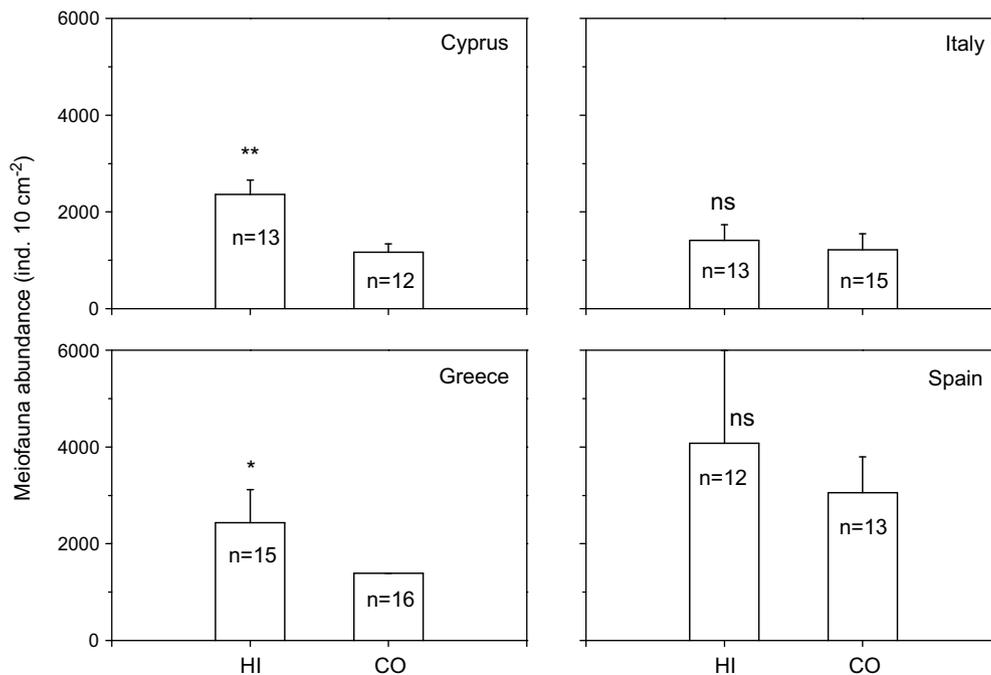


Fig. 5. Total meiofaunal abundance and meiofaunal taxon richness (n = mean value of total taxa), in Cyprus, Italy, Greece and Spain in vegetated (station 3, HI = high impact station and station 5 CO = control station) sites. Reported is also the statistical significance of the differences among bars (t -test: $p < 0.05$, $^* p < 0.01$, no indications is reported when difference is not significant).

fact, will allow the manager to decide if the fish farm is operating in a sustainable manner or if the sedimentation rates exceed the threshold value, thus providing advice on a reduction of the production or a suggestion for moving the farm to another location. Assessments of benthic sedimentation can be done by deployment of benthic traps as described by Hargrave and Burns (1979) and modified by Gacia and Duarte (2001). The traps are typically deployed for 2 days and can be done by SCUBA divers after a brief introduction to the method. The processing of the trap material is straightforward and can be done in almost any laboratory.

We observed that sediments of *P. oceanica* meadows contained typically natural but highly variable levels of organic C even at a very short spatial scale (Bongiorni et al., 2005; Pusceddu et al., 2007). Therefore, the mere comparison of organic C between impacted and control stations does not appear a reliable descriptor of fish farm-induced changes of *P. oceanica* meadows sediments. Among the variables tested in this study those more consistently reflecting the area of impact were: accumulation of P and biopolymeric C in the sediments, C:P ratios in the sediments, sulfate reduction rates, meiofaunal population structure, seagrass shoot density and shoot size, seagrass net population growth and vertical rhizome growth. Where some of these measures require specific knowledge and intensive work (such as identification of meiofauna) and certified laboratories (such as sulfate reduction rates), the measures of sediment nutrient pools and seagrass response parameters are straightforward. It is recommended that sampling is done by SCUBA divers, as surface operated sampling of sediments and seagrasses is not recommended within seagrass meadows.

The optimal indicator of seagrass decline should integrate the impacts over time rather than reflecting short term changes such as a seasonal change in nutrient conditions. The net population change of the meadows provides the ultimate measure of the population dynamics and can be considered as the community response to fish farming activities. The most sensitive method to monitor the population change is by installing permanent seagrass plots, where shoots are annually censused by SCUBA divers and changes in shoot density within the plots are followed (Short and Duarte, 2001). The shoot density will either stay the same, increase or decrease reflecting a meadow in steady state, in expansion or in decline.

The data obtained in this project considering the size of the fish farms (260–1000 tonne yr⁻¹), sampling depths (16–39 m) and hydrological regime (5–40 cm s⁻¹) at the study locations imply that the effects on *P. oceanica* could reach a distance of 400 m from the edge of the cages, which is larger than the distance of 200 m recently suggested by Pergent-Martini et al. (2006) in a review of existing data and less sensitive indicators of particularly seagrass performance (Diaz-Almela et al., in press). We thus recommend a safety distance of 400 m between a fish farm and a *P. oceanica* meadow during the initial siting of a new farm. During the operation of the farm, it is important to monitor the seagrass meadow located at the safety distance to ensure that the meadow remains unaffected by the farming activities along with a control site unimpacted by farming to account for natural variability. It is recommended that the meadow is monitored by installing permanent plots as described above to be able to detect changes in population dynamics with the largest precision during one annual visit and that the rates of sedimentation are measured during the maximum production in the farm also once per year. The farm area close to the net cages should be subject to a controlled monitoring of the water column (e.g. by bio-assays) and sediment conditions (e.g. labile organic matter pools, microbial activity, nutrient fluxes and meiofauna) to ensure that the impact does not reach beyond the objectives set for the particular area.

Further research is needed in order to follow possible dynamics of seagrass mortality and recruitment near fish farms. In this study the seagrass community was only studied for one year, and mortality and recruitment are subject to change, e.g. if thresholds of sediment deterioration are exceeded. The strength of the different indicators suggested should be tested at many more farms expressing a range of farm sizes, water depths and hydrological regimes.

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