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ORIGINAL PAPER

Stimulation of sulfate reduction rates in Mediterranean fish farm sediments inhabited by the seagrass *Posidonia oceanica*

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Abstract Sulfate reduction rates and biogeochemical parameters of fish farm sediments across the Mediterranean were investigated in the order to evaluate the potential effects of organic matter inputs on habitat quality for the common seagrass *Posidonia oceanica*. Four study sites were selected in Spain, Italy, Greece and Cyprus to represent the Mediterranean basin. *P. oceanica* was found in immediate vicinity of all the farms, which were located at physically exposed sites about 1 km from the shore lines. Organic matter accumulation, sulfate reduction rates and sulfur pools were measured in depth profiles along transects from the farms in both bare and vegetated sediments. Results show that although the organic matter accumulation was minor at the sites (POC < 2.8% DW), the sulfate reduction rates were high, in particular at the largest farm in Italy (up to 212 mmol m^{-2} d^{-1}), similar to rates found at shallower, temperate fish farm sites, where higher sedimentation rates can be expected. Sulfate reducing bacteria in these low-organic, carbonate-rich Mediterranean sediments respond strongly to organic matter loadings and cause habitat degradation. Sulfate reduction rates measured in the *P. oceanica* sediments were among the highest recorded (7.8–42.0 mmol m^{-2} d^{-1}) similar to rates found in degrading meadows impacted by organic matter loadings. As sulfate reduction rates were correlated with the sedimentation rates along the transects rather than organic matter pools this suggests mineralization processes were controlled by organic matter loading in fish farm sediments. The vegetated sediments near the net cages were more reduced due to accumulation of sulfides compared to control sites, which is a possible contributing factor to the observed seagrass decline in the farm surroundings. It is recommended that Mediterranean fish farms are placed in areas with rapid dispersal of particulate waste products to minimize organic matter loading of the sediments and thereby preserve habitat quality for benthic fauna and flora.

Keywords Fish farm · Sediments · Sulfate reduction · Sulfur pools · *Posidonia oceanica* · Thresholds

Introduction

Marine aquaculture is one of the fastest growing industries in the world and during the past decade the increase in farming of sea bream and sea bass has

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been particularly rapid in the Mediterranean, where the production raised from 6.248 to 165.783 tons from 1990 to 2004 (FAO 2006). In contrast to fish farms in Northern Europe, which are located near the coast or in archipelagos to minimize exposure and operation costs, many of the newly established fish farms in the Mediterranean operate at exposed sites in relatively large distance from the coasts (1–2 km) (Aguado-Gimenez and Garcia-Garcia 2004; Sara et al. 2004). There a several reasons for this selection of sites including minimizing environmental impacts and avoiding conflicts with other users of the coastal zone such as the economically important tourist industry along Mediterranean coasts (McCausland et al. 2006). Due to the exposed character the farms waste products are dispersed over large areas (Sara et al. 2004), and attraction of wild fish to the cages further modify the sedimentation regime by feeding on lost feed pellets and particulate waste products (Dempster et al. 2004; Vita et al. 2004; Dempster 2005). Studies of benthic impacts at these off-shore locations are few, and they generally show less accumulation of organic matter in the sediments and less disturbed benthic communities compared to sheltered and shallower sites (Aguado-Gimenez and Garcia-Garcia 2004).

The Mediterranean is recognized as an oligotrophic basin with ultra-oligotrophic conditions in the Eastern parts (Crispi et al. 2001), and low deposition of natural organic matter to the sediments. In contrast to eutrophic estuarine sediments with high organic loading, mineralization in off-shore sediments is limited by the availability of organic matter, and aerobic respiration dominate the mineralization processes (Stahl et al. 2004). Although there are only few measurements of sediment mineralization processes in the Mediterranean, the rates measured are in the low end compared to organic rich sediments (Barron et al. 2004; Stahl et al. 2004; Jensen et al. 2003). In comparison with undisturbed sediments, fish farm sediments receive large inputs of organic matter several orders of magnitude higher than natural sedimentation (Cromey and Black 2005; Holmer et al. 2007). Such a loading of sediments, even of organic-rich sediments, with waste products from fish farms stimulate the microbial mineralization as also the quality of the organic matter is much higher compared to terrestrial and phytoplanktonic detritus (Holmer and Kristensen 1992, 1996; Holmer et al. 2003b). Oxygen is rapidly exhausted and anaerobic sulfate reduction becomes the most important mineralization process in fish farm sediments (Holmer and Kristensen 1992, 1996; Holmer et al. 2002). Organic enrichment of low-organic sediments enhance carbon mineralization in particular at high loading rates (Buhring et al. 2006) and it can be expected that organic enrichment of the oligotrophic Mediterranean fish farm sediments, even though the enrichment is modified by wild fish attraction, may exhaust the oxygen in the sediments and stimulate the anaerobic microbial processes. Observations of mats of the sulfur oxidizing *Beggiatoa* on the surface sediments around Mediterranean fish farms (Karakassis et al. 2002) suggest presence of high concentrations of sulfide in the sediments and thus high sulfate reduction rates, but sulfate reduction rates have not yet been quantified.

In contrast to Northern Europe, the oligotrophic conditions in the Mediterranean with high water transparency promote benthic vegetation to large water depths. *Posidonia oceanica* is the most common seagrass found in the Mediterranean basin, where it can be found to depths of 30–40 m (Marbá et al. 2005). Even though *P. oceanica* is a protected species under the EU Habitat Directive, some marine fish farms have been sited near or even above seagrass meadows. Surveys show significant seagrass mortality in fish farm surroundings (Delgado et al. 1999; Holmer et al. 2003b; Diaz-Almela et al. submitted) and experimental studies with organic enrichment of *P. oceanica* sediments demonstrate negative effects of sediment loading on the performance of *P. oceanica* with increased mortality and reduced recruitment of new shoots (Perez et al. 2007). Sulfate reduction is an important mineralization process in sediments of *P. oceanica* meadows (Holmer et al. 2003b), and it is likely that the sulfide pressure on the seagrass meadows due to increased sulfate reduction rates near marine fish farms contribute to the observed seagrass mortality.

This study focuses on changes in sediment biogeochemistry induced by sedimentation in fish farm surroundings and their potential for habitat degradation for benthic fauna and flora. Four fish farms have been selected with *P. oceanica* present in close vicinity and the effects of organic enrichment on the sediment microbial processes were investigated along transects from the net cages. Particular focus is on the sulfate reduction rates and the pools of sulfides in the sediments, as anoxia and accumulation of sulfides are considered as important factors for seagrass decline (Frederiksen 2005; Calleja et al., in review; Diaz-Almela et al., submitted; Perez et al. 2007). The iron availability in the sediments is also explored, as iron limitation of seagrasses due to binding of iron into sedimentary sulfides in organic enriched sediments has been suggested as a contributing factor to seagrass decline (Holmer et al. 2005; Marbá et al. 2006).

Materials and methods

Study area

The study was conducted at four fish farms across the Mediterranean, located in Spain, Italy and Cyprus, on open coasts about 1 km offshore, and in Greece where the farm was located about 300 m from shore in a shallow strait open to the sea (Fig. 1). The studied fish farms have been operating since 1996, 1992, 1988 and 1996, respectively (Table 1). Sediments were vegetated with the seagrass *P. oceanica*, except for a bare zone extending 5–10 m from the edge of the net cages, where the seagrasses were extinct (Fig. 2). Due to the large water depth at the farm in Cyprus (39 m) the vegetated stations were located 300 m away from the farm. A total of six stations were established at each farm, with three stations in the bare sediments (B1, B2, B3) and three in the vegetated area (V1, V2, V3) with the distances from the net cages between 0 and 1000 m in the main current direction (Fig. 2). The stations 1 km away (B3 and V3) were considered to be unaffected by farm activities and served as control sites. The sediments were fine to coarse grained and carbonate-rich (>73%), except in Cyprus where the carbonate content was 42–46% (Table 2). The water depths at the farms varied between 16 and 39 m (Table 1), and they consisted of 16–24 net cages with an annual production between 260 and 1150 tons (Table 1). The cultured species were gilthead (*Sparus aurata*) and sharpsnout sea bream (*Diplodus puntazzo*) and sea bass (*Dicentrarchus labrax*), which were fed manually with dry pellets at a feed conversion ratio between 1.6 and 2.4 (Table 1).

Sample collection and rates of sedimentation

Sediment samples were collected at maximum production in the farms in either June or September during 2002–2003 along transects from the farm (Table 1). At each station, SCUBA divers collected 3 replicate sediment cores (i.d. 2.6 cm) for sulfate reduction rates and 3 cores for sediment characteristics using plexiglass cores (i.d. = 5 cm). SCUBA divers also deployed benthic sediment traps for about 48 h as described by Gacia et al. (1999). The traps consisted of 20-ml cylindrical glass centrifugation tubes with an aspect ratio of 5 (16-mm diameter), in order to prevent internal resuspension. Two arrays, each with 5 replicated traps, were deployed at each position along the transects. In the laboratory, the contents of 1–3 tubes were combined and collected on a combusted, pre-weighed Whatman GF/F filter (final replication 2–5). Dry weight of total sediment deposition was obtained after drying the filters at 60°C to constant weight. Sedimentation rates were estimated according to Blomqvist and Håkanson (1981) and Hargrave and Burns (1979) as described in detail in Gacia et al. (1999). The trap material was analyzed for nutrient contents (particulate organic

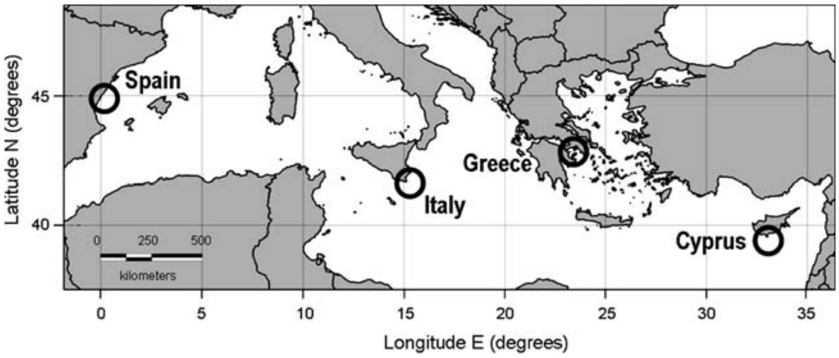


Fig. 1 Location of the four study sites in the Mediterranean

Table 1 Site and fish farm characteristics measured during the sampling period (water depth, current speed) or given as annual values for the year of sampling

	Spain	Italy	Greece	Cyprus
Annual fish production (tones)	260	1150	400	300
No. of net cages	24	24	20	16
Fish species	*Sparus arrata*, *Dicentrarchus labrax*	*Sparus arrata*, *Dicentrarchus labrax*, *Diplodus puntazzo*	*Sparus arrata*, *Dicentrarchus labrax*	*Sparus arrata*, *Dicentrarchus labrax*
Start of operation	1996	1992	1996	1988
Sampling dates	Sept 2003	Sept 2002	June 2003	June 2002
Average water depth (m)	28	22	16	39
Current speeds (cm s^{-1})	9.7	>20 (40% of time)[a]	5.5	>12 (50% of time)
Feed conversion ratio	2.00	2.39	1.60	2.20

[a] Historical data (Mirto and Danovaro, pers. comm.)

carbon, POC; total phosphorus, TP and total iron, TFe) as described below for the sediments.

Sulfate reduction rates and sulfide pools

Sulfate reduction rates (SRR) were determined by the core-injection technique (Jørgensen 1978) where 2 µl of ^{35}S-sulfate (70 kBq) were injected with 1-cm intervals through predrilled silicone-filled holes within 3 hours of collection and incubated for 2 hours in darkness at in situ temperature. The incubation was terminated by sectioning the cores into 2-cm intervals down to 10 cm and 5-cm interval to 15 cm and fixed in 1 M zinc acetate and frozen immediately. Sulfate reduction rates were obtained by the two-step distillation method by Fossing and Jørgensen (1989), which separates sulfides into acid volatile (AVS: porewater H_2S and iron-monosulfides) and chromium reducible sulfides (CRS: elemental sulfur and pyrite) pools. Radioactivity was counted on a Packard TriCarb 2000 scintillation counter and sulfide concentrations were determined spectrophotometrically according to Cline (1969).

Sediment characteristics

The top 0–2 cm of sediments were collected and analyzed for particulate organic carbon (POC) and nitrogen (PON) and total phosphorus (TP) and total iron (TFe), whereas water content, density and porewater were analyzed down to 10–15 cm. Sediment density was obtained by weight of a known volume, and the water content was obtained after drying overnight at 105°C. Porosity was calculated from sediment density and water content. Sediment POC and PON contents were determined by the procedure of Kristensen and Andersen (1987). Sediment carbonate, TP and TFe content were measured on combusted sediment boiled in acid (1 M HCl) for 1 hour. TP was measured after dilution of the supernatant according to Koroleff (1983), and TFe according to Stookey (1970) and Sørensen (1982). The supernatant was evaporated to determine the carbonates by weight of the remaining pellet. Porewater sulfate was obtained by centrifugation of a sediment pellet in double centrifuging tubes (1500 rpm, 5–10 min), and the concentration was determined by a Dionex autosuppressed ion chromatograph equipped with a conductivity detector (ICS-2500).

Results

Sediment deposition rates displayed the same spatial trends at the four sites, with high rates right beneath

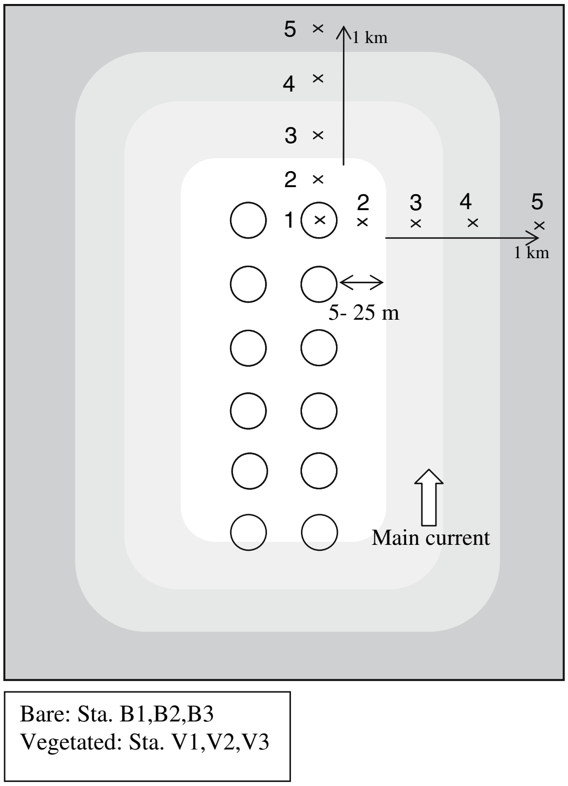


Fig. 2 Conceptual figure of the sampling scheme at the fish farms. The white area represents the unvegetated zone under and near the net cages (5–25 m zone), and the shaded areas the *Posidonia oceanica* meadow in different stages of degradation. The dark area represents reference conditions located 1 km away from the net cages

the fish cages (38–64 g DW m^{-2} d^{-1}), which rapidly declined with increasing distance from the farms (Table 3). The sediment deposition rates were highest at the Italian farm, the largest farm studied. On the contrary, the sediment deposition rate was lowest near the Spanish farm, which was the smallest farm studied. Deposition rates were also low in Cyprus due to the long distance from the farm (>200 m). Deposition of organic C, TP and TFe showed the same pattern described for total deposition rates with high rates near the cages (Table 3).

The sulfate reduction rates were stimulated in the surface layers of both bare and vegetated sediments compared to the control sites (Fig. 3). Rates were up to 16 times higher in the surface layer and in particular in Italy, high rates were found and the stimulation extended down to 8 cm. Highest rates were encountered right under the net cages at all sites, whereas the bare site just outside the net cages already had lower surface rates, and in Cyprus the rates were even lower than at the control site, probably due to a sewage outfall nearby the control site (Argyrou, pers. comm.). At the vegetated sites the depth patterns were less consistent, but highest rates were generally found in the upper 0–5 cm.

The depth-integrated rates were also highest in Italy, where the rates under the net cages were 11 times higher than the control site (Sta. V3 as no bare sediment was found in the area) (Fig. 4). The rates were 3–6 times higher compared to the other fish farms, coinciding with a 3–4 times higher fish production. At the shallow sites (Italy and Greece) the rates declined fast, and at the intermediate station (Sta. B2) accounted for 18–47% of Sta. B1, in contrast to the deeper sites (Spain and Cyprus) were rates at Sta. B2 accounted for 81–88% of Sta. B1. At the deep sites rates were also high at the vegetated site closest to the farm (Sta. V1) with a significant decline at the intermediate seagrass site (Sta. V2). At the more shallow sites there were no significant differences between Sta. V1 and V2, but the rates were up to 1.9 times higher than at the control site. In Cyprus the control site appeared to be affected by organic matter enrichment showing a higher rate at Sta. V3 than at V2.

Both sulfate reduction rates in the AVS and CRS fractions were stimulated by the presence of fish farms (Fig. 4). The sulfate reduction rates in the AVS fraction were generally very low at the control sites (0.9–1.6 mmol m^{-2} d^{-1}) and increased to 8.2–77.1 mmol m^{-2} d^{-1} under the net cages. Sulfate reduction rates in the AVS fraction were highest in Spain and Italy accounting for 36–47% of rates compared to Cyprus and Greece (15–21%). Despite high rates in the AVS fraction the pools were small accounting for <32% of the TRS (Fig. 5). Hence, CRS were the dominating pools and were highest in Cyprus. Here the pools followed the same pattern as the rates with high pools under and near the net cages and a minimum at Sta. V2 and maximum at the bare control site (B3). There were no significant changes in either bare or vegetated sediment along the transects in Spain, in contrast to Italy were pools declined with 53% from Sta. B1 to B2, and surprisingly pools were higher at the vegetated sites. In

Table 2 Surface sediment characteristics (0–2 cm) along transects at the four study sites during sampling campaigns

	Spain	Italy	Greece	Cyprus
POC (% DW)				
Sta. B1	0.98 ± 0.21	0.51 ± 0.08	1.04 ± 0.34	0.84 ± 0.06
Sta. B2	0.95 ± 0.25	1.10 ± 0.27	1.12 ± 0.14	1.26 ± 0.07
Sta. B3	1.42 ± 0.18	n.a.	0.48 ± 0.26	1.51 ± 0.08
Sta. V1	2.78 ± 0.23	1.32 ± 0.48	1.45 ± 0.52	1.30 ± 0.07
Sta. V2	0.79 ± 0.17	1.34 ± 0.23	1.33 ± 0.21	n.a.
Sta. V3	1.60 ± 0.43	0.74 ± 0.41	0.96 ± 0.35	0.37 ± 0.17
PON (% DW)				
Sta. B1	0.12 ± 0.04	0.06 ± 0.01	0.07 ± 0.01	0.09 ± 0.00
Sta. B2	0.11 ± 0.03	0.08 ± 0.02	0.09 ± 0.01	0.20 ± 0.00
Sta. B3	0.15 ± 0.02	n.a.	0.05 ± 0.01	0.28 ± 0.01
Sta. V1	0.11 ± 0.03	0.14 ± 0.05	0.12 ± 0.01	0.21 ± 0.04
Sta. V2	0.07 ± 0.01	0.13 ± 0.01	0.08 ± 0.01	n.a.
Sta. V3	0.12 ± 0.03	n.a.	0.06 ± 0.03	0.13 ± 0.01
TP (% DW)				
Sta. B1	0.057 ± 0.008	0.225 ± 0.018	0.074 ± 0.023	0.090 ± 0.011
Sta. B2	0.034 ± 0.031	0.040 ± 0.001	0.036 ± 0.011	0.040 ± 0.001
Sta. B3	0.016 ± 0.001	n.a.	0.038 ± 0.003	0.030 ± 0.001
Sta. V1	n.a.	n.a.	n.a.	0.040 ± 0.008
Sta. V2	n.a.	n.a.	n.a.	n.a.
Sta. V3	n.a.	n.a.	n.a.	0.010 ± 0.002
TFe (μg g DW^{-1})				
Sta. B1	1949 ± 381	1720 ± 141	1519 ± 74	992 ± 36
Sta. B2	2088 ± 114	2075 ± 16	1425 ± 161	980 ± 134
Sta. B3	1986 ± 140	n.a.	2204 ± 59	n.a.
Sta. V1	2156 ± 138	n.a.	1324 ± 60	2716 ± 252
Sta. V2	1889 ± 146	n.a.	1676 ± 74	n.a.
Sta. V3	2046 ± 149	2228 ± 144	2849 ± 165	932 ± 66
Carbonates (% DW)				
Sta. B1	85.5 ± 3.0	98.3 ± 1.4	91.9 ± 2.6	43.2 ± 0.6
Sta. B2	80.5 ± 1.3	n.a.	93.7 ± 2.2	42.3 ± 0.4
Sta. B3	85.1 ± 2.5	n.a.	72.9 ± 4.3	46.0 ± 0.6
Sta. V1	78.5 ± 0.1	90.6 ± 13.5	93.3 ± 0.8	41.5 ± 1.1
Sta. V2	85.5 ± 0.7	n.a.	88.5 ± 1.3	n.a.
Sta. V3	77.6 ± 0.6	94.2 ± 0.3	79.2 ± 1.4	n.a.
Pyritisation				
Sta. B1	1	1	0.27	1
Sta. B2	1	0.46	0.21	1
Sta. B3	1	n.a.	0.53	1
Sta. V1	1	0.86	0.15	0.57
Sta. V2	1	1	0.17	0.87
Sta. V3	1	0.87	0.26	1

Given are particulate organic carbon (POC) and nitrogen (PON), total phosphorus (TP), total iron (TFe), carbonate content and degree of pyritisation (average). Average of 3 sediment cores (±SE). n.a. = data not available

Table 3 Average rates of benthic sedimentation (n = 2–5) given as total, organic matter (OM), total phosphorus (TP), particulate organic carbon (POC) and total iron (TFe) sedimentation at the sediment sampling stations

Location	Station	Total sedimentation g DW m^{-2} d^{-1}	OM sedimentation g DW m^{-2} d^{-1}	TP sedimentation g P m^{-2} d^{-1}	POC sedimentation g C m^{-2} d^{-1}	TFe sedimentation mg Fe m^{-2} d^{-1}
Spain	Sta. B1	32.26	1.14	0.174	2.56	1264.7
	Sta. B2	9.31	10.28	0.414	1.32	232.4
	Sta. B3	2.01	0.69	0.020	0.10	58.2
	Sta. V1	7.95	7.31	0.507	0.94	222.0
	Sta. V2	6.65	2.86	0.064	0.55	163.3
	Sta. V3	2.01	0	0.019	0.12	59.3
Italy	Sta. B1	63.75	13.28	0.822	1.14	n.a.
	Sta. B2	12.97	5.04	0.178	0.58	n.a.
	Sta. B3	n.a.	n.a.	n.a.	n.a.	n.a.
	Sta. V1	10.83	7.04	0.168	0.81	n.a.
	Sta. V2	8.32	3.73	0.054	0.45	n.a.
	Sta. V3	8.18	2.88	0.008	0.26	n.a.
Greece	Sta. B1	50.96	3.92	0.254	3.21	1064.2
	Sta. B2	8.59	0.32	0.091	0.87	271.1
	Sta. B3	5.04	0	0.011	0.23	164.0
	Sta. V1	4.63	n.a.	n.a.	0.43	n.a.
	Sta. V2	3.77	n.a.	n.a.	0.49	n.a.
	Sta. V3	1.59	n.a.	0.004	0.11	43.7
Cyprus	Sta. B1	n.a.	n.a.	n.a.	n.a.	n.a.
	Sta. B2	5.25	0.57	0.005	0.22	142.3
	Sta. B3	n.a.	n.a.	n.a.	n.a.	n.a.
	Sta. V1	7.63	1.12	0.006	n.a.	51.3
	Sta. V2	5.33	0.56	0.006	n.a.	30.6
	Sta. V3	4.90	1.71	0.006	n.a.	18.7

n.a. = data not available

Greece the highest pools were found at the two control sites showing an opposite trend of the sulfate reduction rates.

Sediment characteristics

Not all of the sediments under the net cages were enriched in organic matter (Table 2). Both in Spain, Italy and Cyprus the pools of POC and PON were higher at the control sites compared to under the net cages, whereas enriched sediments were only found at the Greek farm (2 times higher). In contrast to the bare sediments, the vegetated sites showed higher POC and PON (up to 3.5 times higher) at the stations near the farms compared to the control sites, except for Cyprus were the opposite pattern was found with higher pools at the control site. The POC and PON pools were generally low in all sediments (0.51–1.60% DW and 0.07–0.28% DW, respectively), except for a high value of POC at Sta. V1 in Spain (2.78% DW).

The TP pools, on the other hand, showed clear enrichment at all sites under the net cages with the highest enrichment found under the Italian farm (5.6 times higher than B2). From Cyprus only data from the vegetated sites are available, but here pools were 4 times higher compared to the control site. TFe either showed no change along the transect or an increase with distance as in Greece, where pools almost doubled at the control sites. The highest average pools were found in Spain being 2 times higher than in Cyprus with the lowest levels, except for the one seagrass station where pools

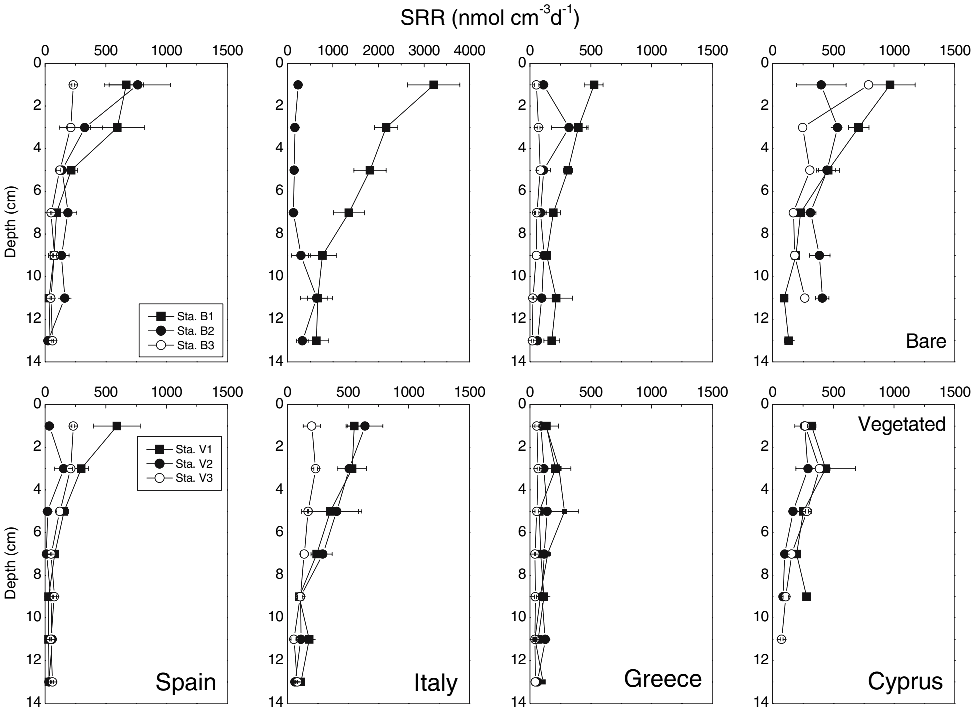
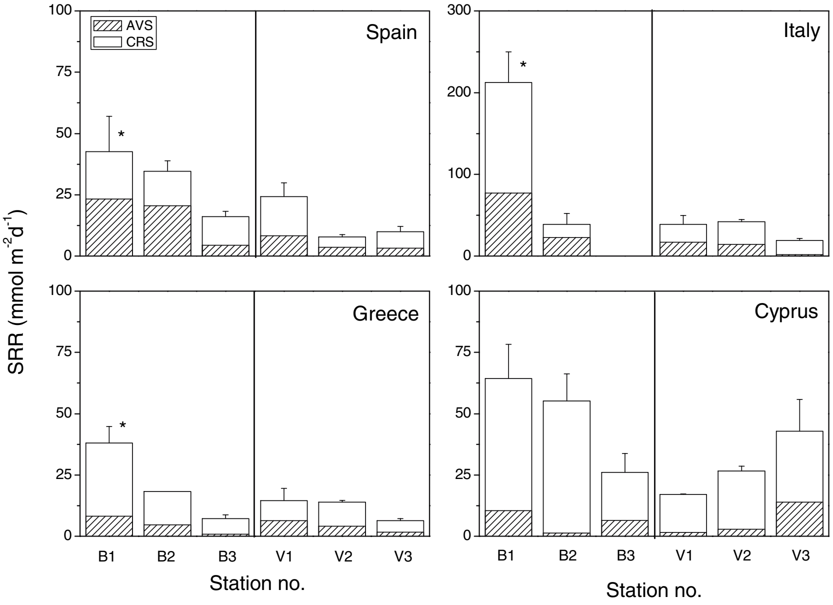


Fig. 3 Sulfate reduction rates with depth at the four study sites. Upper panel show the bare and lower panel the *Posidonia oceanica* vegetated sediments. Symbols represent average (±SE, n = 3). Note the difference in scale for the bare stations in Italy

Fig. 4 Depth integrated sulfate reduction rates at the four study sites. The fraction of AVS and CRS is indicated. Bars represent average (±SE, n = 3). * indicates significant different from control station (p < 0.05). Note the difference in scale in Italy



were 3 times higher indicating an accumulation of Fe at this station (Sta. V1). The porewater sulfate concentrations ranged between 17 and 39 mM across the Mediterranean with a maximum change with depth of 4 mM observed at B1 in Italy (data not shown).

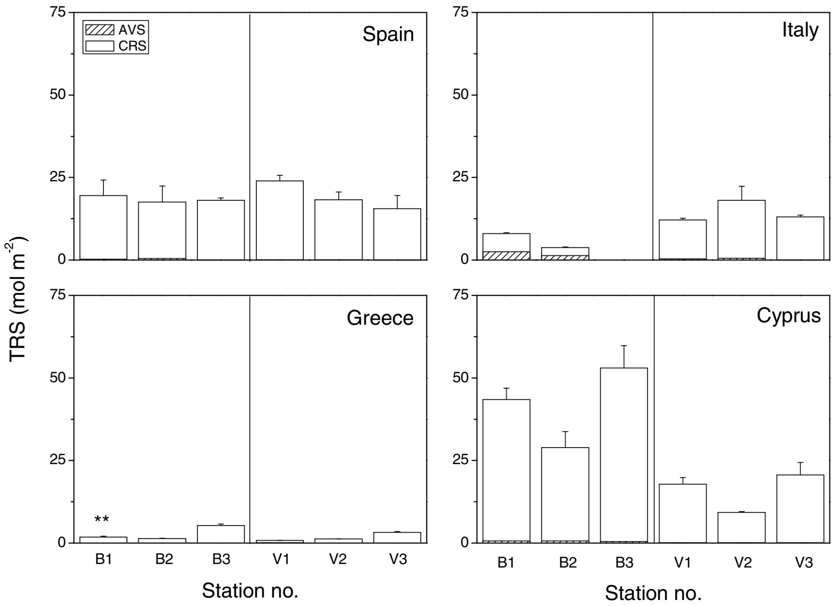


Fig. 5 Depth integrated pools of sulfides at the four study sites. The fraction of AVS and CRS is indicated. Bars represent average (±SE, $n = 3$). ** indicates significant different from control station ($p < 0.001$)

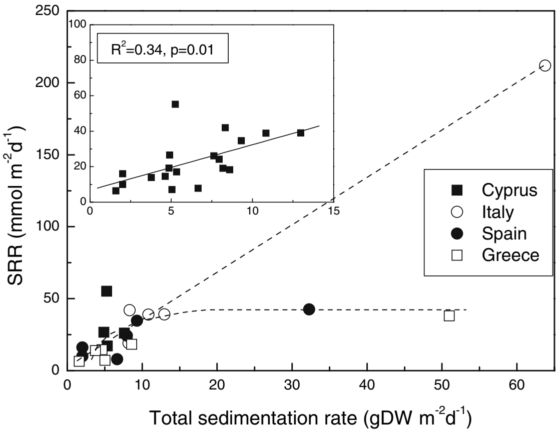


Fig. 6 Depth integrated sulfate reduction rates plotted against total sedimentation rate at the four study sites. Insert shows correlation at the lower sedimentation rates. Dashed lines indicate two different scenarios (see discussion in text)

Discussion

Sediment organic enrichment

The limited burial of organic matter in the sediments underlying the examined fish farms indicates that the benthic impacts were less compared to many other fish farms studied in the past (Hall et al. 1990; Holmer and Kristensen 1992; Vezzulli et al. 2002; Brooks and Mahnken 2003). In a study of a fish farm located 4.8 km off-shore the benthic impacts under the net cages were also minor, but stable isotope analysis of the sedimentation at this farm showed a dispersion of waste products over larger distance (>300 m) indicating the need for studying far field effects (Aguado-Gimenez and Garcia-Garcia 2004; Sara et al. 2004). Analysis of the sedimentation regime at the studied farms showed a similar pattern with higher rates under the net cages due to settling of large particles and a dispersion of finer particles over larger distances (Holmer et al. 2007). The rates of sedimentation were still 50% higher at distances 250 m away compared to the control sites suggesting that the sediments examined here receive considerable amounts of organic matter compared to natural sediments. Although the attraction of wild fish was not quantified in this study, divers observed high numbers of fish around the cages, which was confirmed by the fish farmers (pers. comm.). Attraction of fish to the farm in Spain was examined in a previous study (Dempster et al. 2004) and they found about 20.000 fish near the net cages at this particular farm. Wild fish are considered to modify the sedimentation regime, in particular near the net cages resulting in lower deposition of organic matter on the sediments (Dempster 2005). Also benthic fishes and fauna consume organic matter settling on the sediment surface, and at the fish farm in Cyprus high numbers of the surface living polychaete *Hermodice* *carunculata* may have played an important role in the consumption of waste products (Heilskov et al. 2006). At the farm in Italy and Greece high numbers of the sea urchin *Paracentrotus lividus* were observed (up to 60 m^{-2}), and they also feed on waste products and thereby reduce the amount of organic matter buried in the sediments (Ruiz et al. 2001).

Sulfate reduction rates

Despite high sedimentation rates the burial of organic matter was limited and the microbial processes were significantly enhanced at all the farms. The sulfate reduction rates were stimulated 3–11 times compared to control sites, and in particular the large farm in Italy (1150 tons $year^{-1}$) showed high rates, similar to rates found at a farm of comparable size with rainbow trout but at much shallower depth (5 m) in the Baltic Sea (Holmer and Kristensen 1992, 1996). Similar rates have been measured inside net pens (1 m) in milk fish farms in the tropics, where loading rates were almost 10 times higher than measured here (up to 493 g DW m^{-2} d^{-1}, Holmer et al. 2003a). This suggests that the microbial processes in Mediterranean sediments are quite sensitive to organic matter inputs from fish farms. At the other farms rates were also in the high range compared to previous studies of fish farm sediments (Table 4) and the sulfate reducing bacteria in the Mediterranean thus appear to react strongly to organic matter inputs.

As sulfate reduction rates showed a positive correlation with the sedimentation rates rather than the pools of organic matter in the sediments (Fig. 6, Table 5) this suggests that the input rather than the pools of organic matter plays a role in stimulating the sulfate reduction rates in fish farm sediments. The relationship is best at the low sedimentation rates, whereas the high sedimentation rates experienced under the net cages resulted in two different scenarios: (1). At the farm in Italy the rates were stimulated following the linear relationship indicating a direct coupling between loading and sulfate reduction rates; (2). Whereas at the farms in Spain, Greece and Cyprus sulfate reduction rates showed a saturation level independent of the sedimentation. In Italy rates were stimulated to large depths (8–10 cm), whereas at the other sites sulfate reduction rates were only stimulated in the surface layer. Enhanced sulfate reduction rates in deeper layers have been found in bioturbated sediments, where the fauna redistribute the organic matter and stimulate deeper sulfate reduction rates (Aller and Yingst 1978; Heilskov and Holmer 2001, 2003), but this was not the case in Italy where the sediment was devoid of large fauna (Danovaro, pers. comm.). It is also possible that dissolved organic matter produced during decomposition of waste products at the surface can diffuse to deeper layers, which has been observed in fish farm sediments devoid of benthic fauna (Holmer and Kristensen 1996). A possible explanation for the lack of stimulation in deeper parts of the sediment at the three farms is that consumption of waste products by benthic fishes and fauna is off-setting the relationship with sedimentation rates as discussed above.

It is surprising that sulfate reduction attained such high rates as observed here similar to fish farms located at shallower depth (Holmer and Kristensen 1992, 1996; Holmer et al. 2002). Due to the low sediment organic content at the study sites, it was not expected that sulfate reduction, but other more oxidized mineralization processes such as aerobic mineralization, denitrification and iron reduction were stimulated (Jensen et al. 2003). Oxygen penetration depth is, however, small (<10 mm) in *P. oceanica* sediments (Holmer et al. 2003b) and in bare sediments in the Mediterranean (Rabouille et al. 2003), limiting the potential for aerobic respiration. Oxygen is consumed during reoxidation of reduced compounds due to low buffer capacity in the iron-poor carbonate sediments (Chambers et al. 2001; Azzoni et al. 2005). Sediment oxygen uptake was measured at the bare sites at the four farms in this study, and rates were generally low (12–48 mmol m^{-2} d^{-1}) indicating that the oxygen diffusing from the water column was consumed in the reoxidation process rather than used for aerobic respiration (Dalsgaard, unpublish.). Also nitrate concentrations were low both in the water column and in the sediments at all study sites (Dalsgaard and Krause-Jensen 2006) and denitrification played a minor role in the consumption of organic matter due to the limited availability of nitrate and low rates of nitrification (Dalsgaard, unpublish.). Finally oxidized iron pools were low (data not shown), and as microbial iron reduction is controlled by the availability of oxidized iron, its importance for organic matter mineralization is considered to be limited (Thamdrup 2000; Jensen et al. 2003). On the other

Table 4 Sulfate reduction rates (SRR) and sulfur pools measured around marine fish farms and from unvegetated sites in this study

Location/cultured species	Distance from net cage m	SRR mmol m^{-2} d^{-1}	% AVS	S – pools mol S m^{-2}	% AVS	Reference
Denmark – Rainbow trout	0	10–310[a]	47–91[a]	–	12–36[a]	Holmer and Kristensen (1992, 1996)
	30	4–19[a]	30–56[a]	–	2–6[a]	
Germany – Rainbow trout	0	51[b]	–	–	–	Krost et al. (1994)
	Edge	0–14[b]	–	–	–	
Philippines – Milk fish	0	14–185[c]		5–17[c]	–	Holmer et al. (2003a)
Philippines – Milk fish	0	14–36[d]	–	3–6[d]	–	Holmer, unpublish.
	15	14–26[d]	–	3–7[d]	–	
	100	2–24[d]	–	1–5[d]	–	
Spain – Sea bream/Sea bass	0	42.6	55	19.6	1.6	This study
	5	34.6	59	17.6	2.3	
	1000	16.1	28	18.1	0.1	
Italy – Sea bream/Sea bass	0	212.4	36	8.0	32	This study
	10	39.0	58	3.8	35	
Greece – Sea bream/Sea bass	0	38.0	22	1.8	1.6	This study
	25	18.2	26	1.3	2.2	
	1000	7.2	12	5.3	0.2	
Cyprus – Sea bream/Sea bass	0	64.4	16	43.5	1.5	This study
	20	55.2	2.4	28.9	2.2	
	1000	42.8	32	53.1	0.8	

The percent contribution of the acid volatile pool to the SRR and the sulfur pool is given when available

[a] Seasonal variation over one year of sampling

[b] Flux of H_2S estimated from porewater profiles of H_2S

[c] Range of 12 fish farms

[d] Range of 3 fish farms

Table 5 Correlation coefficients (R^2) and significance levels (p) for linear correlations between farm related parameters and sulfate reduction rates (SRR)

Parameter	R^2	p
Distance	0.01	0.57
Farm production[a]	0.94	0.03
Total sedimentation	0.61	<0.001
OM sedimentation	0.50	<0.001
POC sedimentation	0.06	0.34
TP sedimentation	0.60	<0.001
TFe sedimentation	0.26	0.05
Sediment OM	0.03	0.43
Sediment POC	0.07	0.24
Sediment TP	0.86	<0.001
Sediment Fe	0.07	0.25

[a] For Sta. B1

hand, sulfate concentrations are high in the Mediterranean due to the high salinities (>35 psu), and sulfate reduction thus has the potential as an important mineralization process when organic matter loadings increase. Similarly sulfate reduction dominates in other fish farm sediments (Holmer and Kristensen 1992, 1996; Holmer et al. 2003b; Heilskov and Holmer 2001, 2003; Heilskov et al. 2006).

Sulfate reduction rates in vegetated sediments

The sulfate reduction rates measured within the seagrass meadows were the highest recorded so far for *P. oceanica* sediments (Table 6). Rates were particularly high at the Italian farm with the largest fish production, but also in Spain and Cyprus high rates were found, similar to rates measured in degrading *P. oceanica* meadows in Mallorca

(Holmer et al. 2003b; Holmer et al. 2005). This degrading meadow is impacted by increased sedimentation of phytoplanktonic detritus and raw sewage and high sulfate reduction rates, high pools of sulfides and iron limitation contribute to the observed seagrass decline. The seagrass meadows were fast degrading at all the four fish farms ($t_{½}$ = 3–26 months), both in impacted and intermediate sediments, and particularly near the net cages in Italy and Greece (Diaz-Almela et al. submitted). The rates of degradation were correlated with sedimentation suggesting that the input of organic matter negatively affected seagrass growth (Diaz-Almela et al. submitted). Sulfide invasion into the seagrasses due to the high sulfate reduction rates has been suggested as a contributing factor to the observed seagrass decline (Frederiksen 2005; Frederiksen et al., in press).

Sulfur pools

The impacted sediments were more reduced with a larger AVS fraction in the sulfate reduction rates compared to control sediments. Similar high AVS production rates have been found in other fish farm sediments (Table 4) and are due to high production of H_2S and FeS compared to pyrite (FeS_2, Thode-Andersen and Jørgensen 1989). One particular feature of the examined sediments was the low iron contents due to the carbonate nature of these sediments. Binding of sulfides into FeS and pyrite is iron limited in carbonate sediments (Berner 1984; Holmer et al. 2005), and calculations of the degree of pyritisation at the study sites based on the pools of AVS and CRS and the TFe content ((AVS + 0.5*CRS)/TFe, Table 2) support iron limited sulfide burial. The iron was all bound with sulfides in the sediments in Spain and at the stations near the net cages in Italy and Cyprus, whereas excess iron was only found at the Greek farm. The pools of AVS and CRS were generally low at all sites compared to the temperate fish farm sediments most likely limited by the iron availability (Table 4). A consequence of low sulfide burial capacity is a higher reoxidation of sulfides in carbonate sediments compared to mineral sediments (Berner 1984), and in particular at the studied farms where the sulfate reduction rates were as high as found in mineral fish farm sediments with much higher iron availability and higher burial of sulfides (Table 4). High rates of reoxidation implies high

Table 6 Sulfate reduction rates (SRR) and sulfur pools in *Posidonia oceanica* meadows including this study

Location	SRR mmol m^{-2} d^{-1}	% AVS	Pools mol S m^{-2}	% AVS	Reference
Mallorca Spain	3–12	–	0.2–3.9	–	Holmer et al. (2003)
Mallorca Spain	6–23	–	0.3–2.9	–	Holmer et al. (2005)
Mallorca Spain			0.05–1.8	–	Calleja et al., in review
Mallorca Spain	11–14	2–25	0.88–3.4	<0.01–2	Holmer et al., in prep
Spain – Sta. V1 Impacted	24.2	34	24.0	0.5	This study
Spain – Sta. V2 Intermediate	7.8	46	18.3	0.7	This study
Spain – Sta. V3 Control	10.0	32	15.6	0.2	This study
Italy – Sta. V1 Impacted	38.9	43	12.1	3.3	This study
Italy – Sta. V2 Intermediate	42.0	34	18.1	3.2	This study
Italy – Sta. V3 Control	19.1	8	13.1	<0.01	This study
Greece – Sta. V1 Impacted	14.5	44	0.77	9.0	This study
Greece – Sta. V2 Intermediate	13.9	29	1.2	3.3	This study
Greece – Sta. V3 Control	6.4	27	3.3	1.2	This study
Cyprus – Sta. V1 Impacted	26.1	25	17.8	2.6	This study
Cyprus – Sta. V2 Intermediate	17.0	9	9.3	7.0	This study
Cyprus – Sta. V3 Control	26.6	11	20.7	3.3	This study

The percent contribution of the acid volatile pool to the SRR and the sulfur pool is given when available oxygen consumption rate, which imposes a risk of oxygen depletion in the sediments and overlying water column, and is consistent with observations of dissolved sulfide in the surface layers and high sediment oxygen uptake (Dalsgaard unpublish.). The AVS contribution at the Italian farm was one of the highest observed in fish farm sediments (Table 4) and was consistent with high porewater pools of H_2S in the sediments at this farm (Dalsgaard, unpublish.).

Sulfur pools in vegetated sediments

Also the AVS and CRS pools in the seagrass meadows were the highest observed so far in *P. oceanica* sediments (Table 6). Pools were particularly high in Spain, Italy and Cyprus, and characteristic for these sites was the high degree of pyritisation and thus a saturation of the iron with sulfides. The pools of AVS and CRS along the transects followed the sulfate reduction rates with higher pools near the cages compared to the intermediate station in Spain and Cyprus, whereas there was no clear pattern for the Italian and the Greek sites. Sulfide invasion into the seagrasses near the net cages was evident as lower values of stable sulfur isotope ratios in the seagrass tissues (Frederiksen 2005; Frederiksen et al., in press) and H_2S was detected by sulfide sticks in the rhizosphere sediments near the net cages compared to no and much deeper H_2S pools at control sites (Frederiksen 2005). Whereas porewater H_2S may reflect the measured sulfate reduction rates, the pools of FeS and pyrite are considered to represent the history of the sediments rather than the actual processes occurring, as up to 90% of the sulfides produced are reoxidized and only 10% buried permanently (Thode-Andersen and Jørgensen 1989). The observed increase in the pools of sulfides in the seagrass sediments thus suggests that sulfate reduction rates have been enhanced for a longer period of time reflecting the fish production at the sites, which had taken place between 7 and 14 years at the studied farms.

Sedimentation of phosphorus and iron

The correlations between sulfate reduction rates and TP sedimentation and for AVS pools and TP sedimentation suggest TP as a sensitive indicator of fish farm waste products (Tables 5, 7). In contrast to both POC and PON, where limited burial was found, TP accumulated along the transects with high pools under and near the net cages compared to the control site. TP is primarily released from fish farms as particulates together with carbon (Holby and Hall 1991; Holmer et al. 2003a; Islam 2005), whereas nitrogen is released to the water column in dissolved form and dispersed over larger areas (Hall et al. 1992). One possible explanation for the burial of phosphorus in the fish farm sediments is binding of P to calcium in carbonate sediments (Jensen et al. 1998). No or only small efflux of P could be detected from the sediments receiving high inputs of TP indicating that the remineralized P was retained in the sediments rather than released back to the water column (Dalsgaard unpublish; Heilskov et al. 2006). TP sedimentation and accumulation in the sediments may be useful as indicators of benthic impacts in Mediterranean fish farm sediments and for managing fish farms at sustainable levels.

The sulfate reduction rates under the net cages were correlated with the fish production in the farms (Table 5), but as the correlation was based on only four farms and was driven by the largest farm, the correlation was not significant ($p > 0.05$). More farms have to be examined to strengthen the relationship and future studies should focus on measuring the sedimentation regime around the farms, as this is crucial for the benthic impacts.

Table 7 Correlations coefficients (R^2) and significance levels (p) for linear correlations between farm related parameters and AVS pools

Parameter	R^2	p
SRR	0.84	<0.001
Distance	0.02	0.54
Total sedimentation	0.41	0.001
OM sedimentation	0.50	<0.001
POC sedimentation	0.01	0.75
TP sedimentation	0.50	<0.001
TFe sedimentation	0.03	0.53
Sediment OM	0.04	0.37
Sediment POC	0.06	0.29
Sediment TP	0.66	0.002
Sediment Fe	0.06	0.31

Conclusion

Sulfate reduction, which is an important process for controlling the biogeochemical conditions of marine sediments and for habitat quality of benthic fauna and flora, is directly correlated with the sedimentation of waste products from the examined fish farms. These Mediterranean sediments appear to be highly sensitive to increased sedimentation and attain high sulfate reduction rates despite the farms location at large water depths and at relatively exposed sites compared to previous studies of Atlantic fish farms. Due to low iron availability in the sediments, iron pools were saturated with sulfides and had a limited capacity for sulfide burial. Sulfides were accumulating in the sediments resulting in higher pools and more reduced conditions at stations near the farm compared to control sites. The loss of the seagrass *P. oceanica* under and near the net cages and the high mortality rates at distance from the farms is a clear indicator of benthic habitat degradation, which appears to be correlated with the input of organic matter from the fish farms. It is thus recommended to.place fish farms at locations with rapid dispersal of waste products to minimize the sedimentation of waste products over *P. oceanica* meadows. At the same time it is important to monitor far field effects of farm production, as the sulfate reduction rates were stimulated beyond the area, where accumulation of organic matter in the sediments was found. Sedimentation and accumulation of phosphorus is a much more sensitive indicator of benthic impacts compared to organic matter pools, which until know has been the most commonly used indicator in monitoring programs.

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