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Sedimentation of organic matter from fish farms in oligotrophic Mediterranean assessed through bulk and stable isotope (δ^{13}C and δ^{15}N) analyses

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Abstract

Bulk sedimentation and carbon and nitrogen stable isotopes were used to investigate the dispersion of particle waste products from 3 fish farms distributed along the Mediterranean Sea and characterized by the seagrass *Posidonia oceanica* growing in immediate vicinity of the fish cages. The farms were located at sites with rapid water exchange (average current speeds > 5.5 cm s^{-1}) and water depths ranging from 16 to 28 m. Sedimentation traps were deployed along transects from each farm on bare and vegetated sites for 48 h during summer, where the production in the farms is at maximum. The sedimentation under the net cages was 8 to 25 times higher than at control sites located 1 km away. The farm with the largest production showed the highest sedimentation rates. Phosphorus (P) deposition rates were particularly high at all farms, and the underlying sediments were enriched in P. These results indicate that P can be used as a sensitive indicator of farm loadings. The isotopic signals (δ^{13}C and δ^{15}N) of the sediment trap materials at the control sites varied among the 3 study sites (δ^{13}C −14.9‰ to −23.4‰ and δ^{15}N 2.2‰ to 6.2‰), but some general trends were observed with less negative δ^{13}C and more positive δ^{15}N signals under the net cages. These signals were reflected in the underlying sediments, in particular for δ^{15}N, suggesting that N isotopes can be used as indicators of farm waste products in traps and sediments.

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1. Introduction

Marine fish farming has been rapidly increasing in the Mediterranean since 1990 and is expected to rise by 5% annually in the next 2 decades, driven by the increasing demand for marine products in the area (FAO, 2006). The effects of marine fish farming are typically on benthic ecosystems around the cages, where accumulation of organic matter may progressively transform the substrate into a flocculent anoxic surface (Holmer and Kristensen, 1992; Karakassis et al., 1998). The waste

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products from cage farming primarily consist of dissolved and particulate nutrients originating from excretion products from the fish and uneaten feed (Hall et al., 1990, 1992; Holby and Hall, 1991; Lupatsch and Kissil, 1998). Whereas the dissolved compounds are easily dispersed and diluted in the water column, the particulate waste products sink fast to the sea floor (Cromey et al., 2002) where they affect the benthic communities (Karakassis et al., 1998). The organic carbon, nitrogen and phosphorus loads alter sediment characteristics beneath and close to the fish cages (Hall et al., 1990, 1992; Holby and Hall, 1991; Brooks and Mahnken, 2003; Vita et al., 2004). This, in turn, stimulates bacterial activity and may result in benthic oxygen depletion (Holmer and Kristensen, 1992, 1996; Hargrave et al., 1993; Brooks and Mahnken, 2003) causing long-term changes in the structure of the benthic assemblages (reduced density and biodiversity, Weston, 1990; Karakassis et al., 1999, 2000) and seagrass decline (Delgado et al., 1997, 1999; Cancemi et al., 2003; Holmer et al., 2003; Marbá et al., 2006).

Limited information is, however, available on the dispersion and rates of sedimentation of particles near fish farm (Sutherland et al., 2001; Cromey et al., 2002; Cromey and Black, 2005) and in particular in the Mediterranean area (Sarà et al., 2004; Vita et al., 2004). Mediterranean fish farms tend to be located relatively far from the coasts (1–5 km), with exceptions as in Greece where an extensive archipelago allows more sheltered locations. Most studies of Atlantic farms have examined environmental impacts of farms within fjords or close to the shore and in sheltered areas. Because the spatial dispersion of waste products is a function of local hydrodynamics, water depth and the size and production in the farms it is not immediately possible to transfer knowledge from Atlantic to Mediterranean farming (Sarà et al., 2004).

The Mediterranean is characterized by oligotrophic conditions with excellent light climate for benthic vegetation. The seagrass *Posidonia oceanica* is the dominant species covering $2.5–5\times10^{10}$ m^2 of the coastal zone down to 45 m depth (Bethoux and Copin-Móntegu, 1986; Pasqualini et al., 1998). Seagrasses play major ecological roles in the coastal zone (e.g. prevents coastal erosion, increases coastal biodiversity, oxygenates the water and sediments, increases water transparency and accumulates carbon). *P. oceanica* meadows are highly vulnerable to marine aquaculture activities as reflected by the large-scale losses of *P. oceanica* reported nearby fish farms (e.g. Delgado et al., 1997; Ruiz et al., 2001; Cancemi et al., 2003; Holmer et al., 2003; Marbá et al., 2006) even after cessation of farming activities (Delgado et al., 1999). The decline of *P. oceanica* meadows near fish farms has been attributed primarily to the deterioration of sediment quality (Holmer and Nielsen, 1997; Terrados et al., 1999) most likely driven by sedimentation of organic matter (Duarte, 2002; Holmer et al., 2003).

Seagrass meadows are characterized by higher rates of burial of nutrients due to higher rates of sedimentation and lower rates of resuspension (Gacia et al., 2002). Higher rates of sedimentation are considered to derive from reduced water flow over the meadows and sedimentation of organic matter produced by epiphytes and

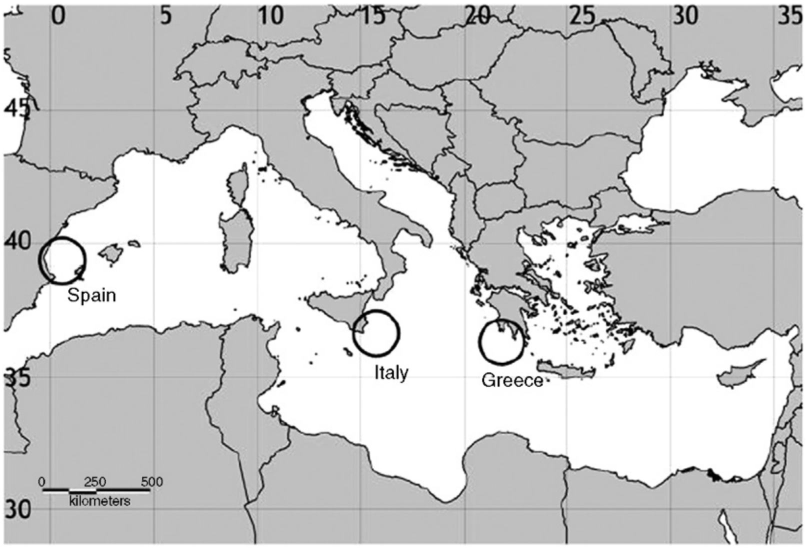

Fig. 1. Location of the 3 study sites in the Mediterranean.

epifauna within the meadows. Resuspension is reduced by the physical setting of the meadow which hampers wave and current velocity within the meadow. Sedimentation in seagrass meadows has not yet been measured near fish farms, but it is likely that the presence of seagrass meadows increases the organic loading of the sediments due to enhanced sedimentation of waste products.

Aquaculture waste products in sediments have been traced to considerable distances (>300 m) around Mediterranean fish farms by application of sensitive tracers such as the stable isotopes δ^{13}C and δ^{15}N (Sarà et al., 2004). In the last two decades, carbon and nitrogen isotopes have been used extensively to trace organic matter such as marine vs. freshwater (Fry and Sherr, 1984) and aquatic vs. terrestrial (Thornton and McManus, 1994). Few studies have applied this technique to trace fish farm waste products. A couple of studies have separated farm products from other sources (Sutherland et al., 2001; Sarà et al., 2004) indicating that bulk sedimentation rates and isotopic signature of the sedimentation materials may be useful for tracing farm wastes at relatively large distances from a source.

In order to ensure sustainable expansion of marine fish farming in the Mediterranean it is essential to quantitatively understand how fish farming affects the sedimentation of waste products in seagrass beds. The objective of this study was to quantify the spatial variation in sedimentation rates around representative Mediterranean fish farms located over *P. oceanica* meadows. The qualitative and quantitative aspects of the sedimentation inside and outside the seagrass meadows were assessed by analysis of stable isotopes (δ^{13}C and δ^{15}N) as tracers of fish farm derived products into the sediment traps and sediments.

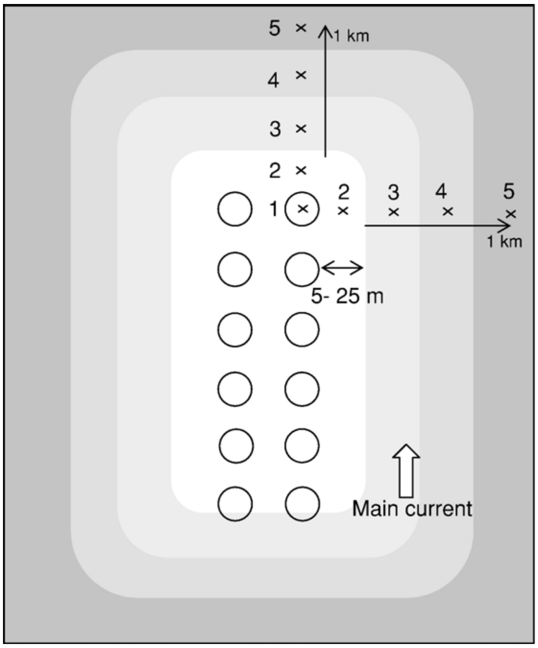

Fig. 2. Conceptual figure of the sampling scheme at the fish farms, where the arrows represent two transects. The white area represents the unvegetated zone under and near the net cages (5–25 m zone), and the shaded areas the *P. oceanica* meadow in different stages of degradation. The dark area represents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

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	Greece	Italy
Annual fish production (tones)	260	400	1150
No. of net cages	24	20	24
Fish species	*Sparus arrata* *Dicentrarchus labrax*	Sparus arrata Dicentrarchus labrax	Sparus arrata Dicentrarchus labrax Diplodus puntazzo
Start of operation	1996	1996	1992
Sampling dates	September 2003 and June 2004	June 2003	September 2002 and June 2003
Water depth (m)	28	16	22
Current speeds (cm s^{-1})	9.7	5.5	>20 (40% of time)*
Feed conversion ratio	2.00	1.60	2.39

*Historical data (Mirto and Danovaro pers. comm.).

2. Materials and methods

2.1. Study area

The study was conducted in three fish farms across the Mediterranean, located on the Spanish east coast (El

Table 2
Presence of *P. oceanica* and sampling distances (m) for the stations along the Transect 1 (along main current) and Transect 2 (perpendicular to main current) measured from the edge of the sampled net cage

Station no.	*P. oceanica*	Spain	Greece	Italy
1	No	Under the cage	Under the cage	Under the cage
2	No	5	10	25
3	Yes	10	15	5
4	Yes	40	35	40
5	Yes	1000	1200	1000

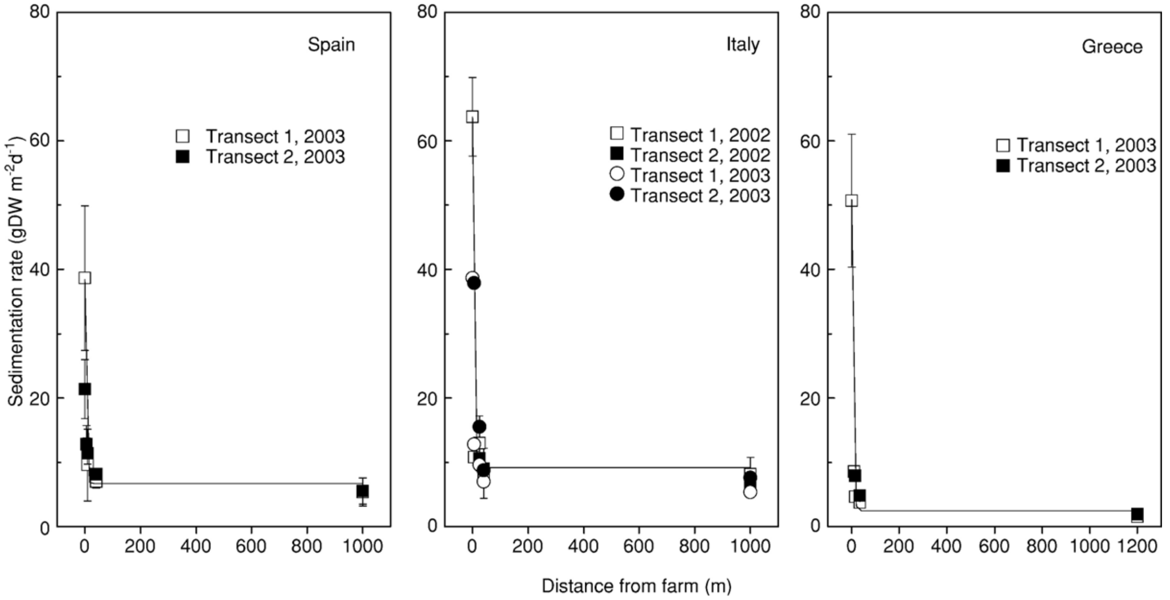

Fig. 3. Bulk sedimentation rates (mg DW m^{-2}d^{-1}) at increasing distances from the farms at the 3 study sites sampled in 2002 and 2003 along transects in main current direction (Transect 1) and perpendicular to this (Transect 2). Each symbol represents average (±SE, $n=3-5$). An exponential function ($y=a\exp(-bx)$, where y = sedimentation, x = distance and a, b are constants) was fitted to each dataset and the slopes ($b\pm$SE) are: Spain 0.324±0.117, Italy 0.699±0.240 and Greece 0.207±0.021.

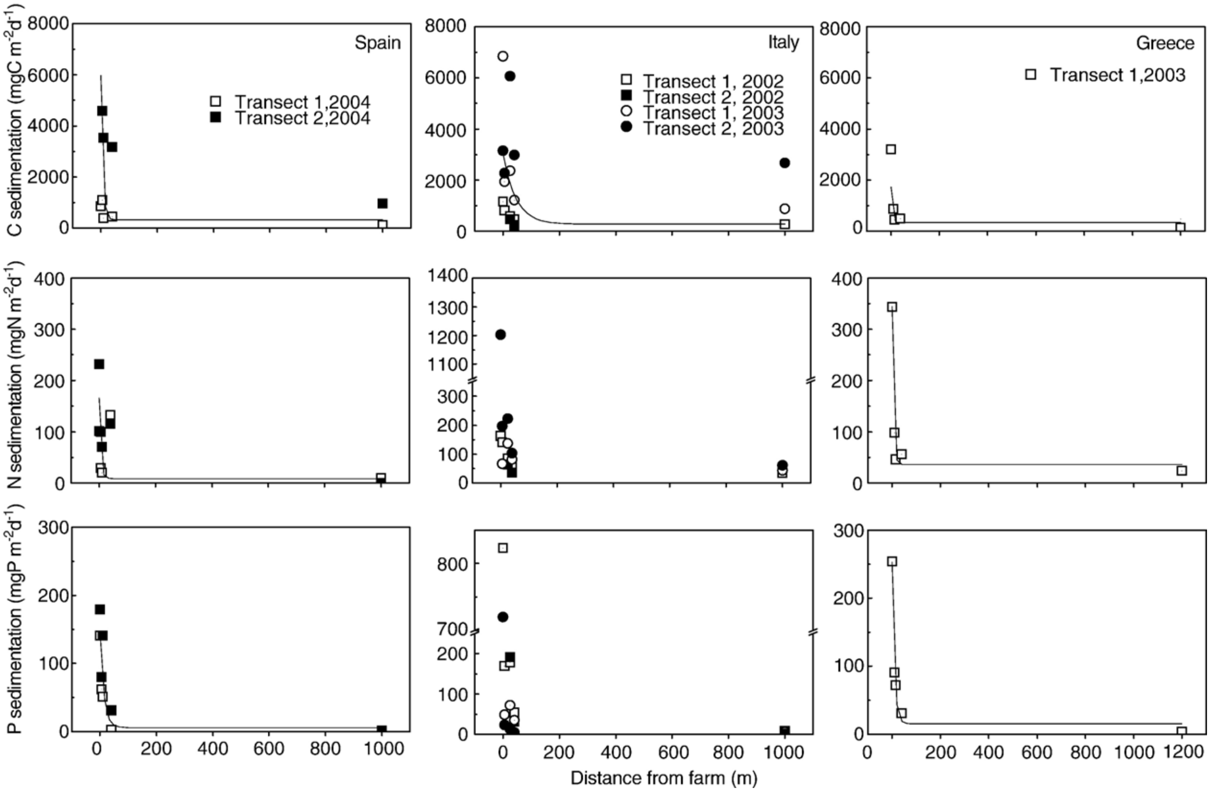

Fig. 4. Sedimentation of nutrients at increasing distance from the farms at the 3 study sites. Upper: POC, middle: PON and lower: TP. Symbols represent single measurements. The slopes of the exponential fits are ($b\pm$SE): Spain POC: 0.14±0.124, PON: 0.18±0.25, TP: 0.07±0.13; Italy POC: 0.02±0.01, PON: no fit, TP: no fit; Greece POC: 10.50±0.25, PON: 0.17±0.04, TP: 0.10±0.02.

Campello) and on the south east coast of Sicily in Italy, both on open coasts about 1–2 km offshore, whereas the farm in Sounio on mainland in Greece was located in a shallow strait about 300 m from shore (Fig. 1). The studied fish farms have been operating since 1996, 1992 and 1996, respectively. Sediments were vegetated with the seagrass *P. oceanica*, except for a bare zone extending 5 to 25 m from the edges of the net cages where the seagrasses are extinct (Fig. 2). The sediments were fine to coarse grained and carbonate-rich (>73%, data not shown), and the water depths varied between 16 and 28 m (Table 1). The farms consisted of 20 to 24 net cages with an annual production of 260 to 1150 tones (Table 1) and the distance between the net cages and the sediments varied between 4–10 m from the shallow to the deepest site. The cultured species were gilthead (*Sparus aurata*), 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).

2.2. Sample collection

The hydrographic regime was measured by deployment of current and CTD meters during sampling in September 2003 in Spain and in June 2003 in Greece, whereas historic data were obtained from Italy. The sedimentary flux was examined along two transects (0 to 1000–1200 m) extending away from the three farms along the direction of the main currents (all farms, Transect 1) and perpendicular to the current (Spain and Greece, Transect 2), where *P. oceanica* was present along parts of the transects (Fig. 2, Table 2). Samples were collected 1–2 times at maximum production in the farms in either June or September (Spain: June 2004, Italy: September 2002 and June 2003 and Greece: June 2003, Table 1). The stations at 1000–1200 m were considered to reflect background conditions at the study sites, and are referred to as control sites. At each station, SCUBA divers 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 to 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 carbon, POC; particulate organic nitrogen, PON; and total phosphorus, TP) and stable carbon and nitrogen isotope composition (δ^{13}C and δ^{15}N) as described below. Feed pellets were collected at the farms and nutrient and isotope analyses were performed.

Sediments were sampled once at each location (Spain: September 2003, Italy: September 2002, Greece: June 2003), where 3 replicate sediment cores were collected by SCUBA divers using plexiglass cores (i.d. = 5 cm). The top 0–5 cm of sediments were sampled and analysed for POC, PON and TP and stable isotope composition (δ^{13}C and δ^{15}N).

2.3. Analysis

Trap material and sediments were analyzed for POC and PON contents, and stable isotopes ratios of organic carbon and nitrogen by elemental analyzer combustion continuous flow isotope ratio mass spectrometry (EA-C-CF-IRMS, Iso-Analytical Ltd. United Kingdom). Prior to analysis, each sample (100 mg) was treated to remove carbonate carbon by acidification in 2 ml 10% HCl for >12 h to allow liberation of CO_2. After treatment, the sample was washed in pure water and dried before homogenization and transfer into tin capsules. Stable carbon isotopes are expressed in the delta notation

Table 3
Molar ratios of organic nutrients (POC, PON and TP) in the sediment traps sampled along transects at the 3 farms

	Station	C:N	C:P	N:P
Spain	1	9.9[a]	15.8[a]	1.6±0.9
	2	44.1[a]	45.3[a]	1.0±1.2
	3	22.3[a]	19.6[a]	0.9±0.2
	4	3.9[a]	361.8[a]	92.3±61.5
	5	13.4[a]	*	*
Italy	1	9.0±4.5	31.7±28.2	2.1±1.7
	2	17.9±14.0	55.3±43.3	2.6±1.5
	3	17.0±9.9	32.0±25.0	2.4±0.6
	4	16.8±6.5	42.2±22.1	3.8±1.2
	5	28.1±15.0	146.9±111.9	11.7±3.6
Greece	1	10.9	32.6	3.0
	2	10.4	24.8	2.4
	3	11.0	15.3	1.4
	4	10.5	40.2	3.8
	5	5.6	72.5	12.9

Values represent mean (±range or SE) of transect 1+2 for 2004 in Spain and for 2002 and 2003 in Italy and single measurements in 2003 for Greece (POC:PON = C:N, POC:TP = C:P and PON:TP = N:P).
*Ratio not calculated as TP was under detection limit.
[a] Transect 2 omitted due to high POC values.

relative to Vienna PDB. The accuracy of the analysis was on average 0.8% and 0.5% for carbon and nitrogen, respectively. The sediment POC and PON contents were measured on dry sediment with a Carlo Erba elemental analyzer 1100EA following Kristensen and Andersen (1987). Total P (TP) on trap material and sediments was obtained after boiling combusted materials in 1 M HCl for 15 min followed by spectrophotometric determination of phosphate (Koroleff, 1983).

3. Results

3.1. Hydrographic regime

The hydrodynamic regime of the cage areas was characterized by dominant currents through the farms with average speeds of 5–20 cm s^{-1} (Table 1). In Spain, current meter deployment indicated that most variation was along an axis 50° counter clock wise from the W–E axis. Range of currents along this axis was −32.6 to 28.3 cm s^{-1} with a mean 4.7 cm s^{-1} (positive towards north east). In Italy the current was in the south–east direction through the farm most of the time (~75%) or to the north–west (~20%). In Greece most of the flow field variance was on an axis 2° counter clock wise from the W–E axis (along isobaths), and currents ranged from −21.0 to 40.7 cm s^{-1} with a mean value of 5.5 cm s^{-1} (positive towards north east). In Spain the water column was fully mixed and the water temperature and salinity were quite similar between stations, 24.9 to 25.7 °C and 37.0 to 37.4 psu. During field sampling in Italy mixed conditions were found at all stations, and the water temperature was around 24.5–25 °C and the salinity ~38.7 psu. In Greece the water column was also mixed at all sites, but there was a larger difference in temperature (17.3 to 27.4 °C) and in salinity (27.4 to 38.6 psu).

3.2. Sedimentation

Sediment deposition rates displayed the same spatial trends at the 3 sites, with high rates right beneath the fish

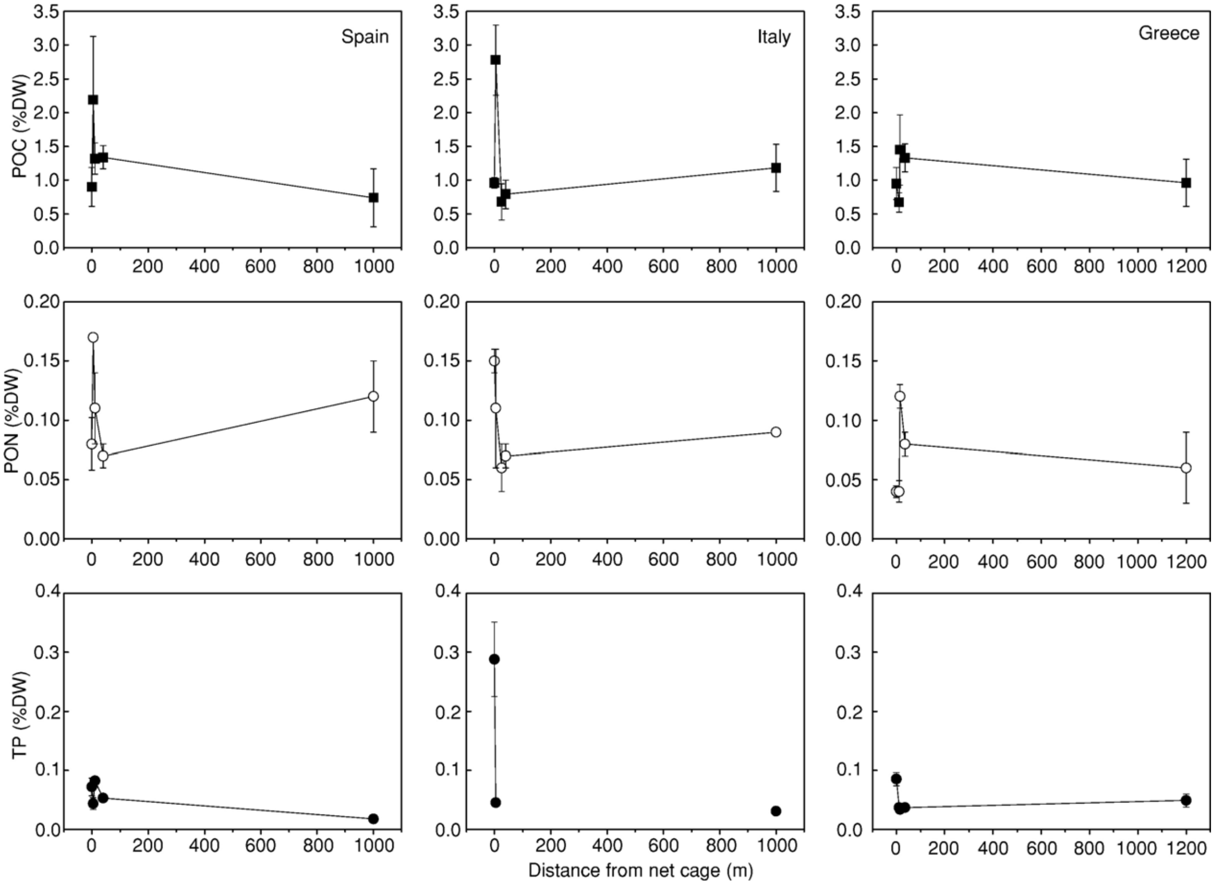

Fig. 5. Surface sediment nutrient content. Values of mean particulate organic carbon (0–2 cm, POC, %DW), organic nitrogen (0–2 cm, PON, %DW) and total phosphorus content (0–5 cm, TP, %DW) are given.

Table 4
Molar ratios of C:N, C:P and N:P in surface sediment along the main current transect (Transect 1) at the 3 fish farms

Location	Station	C:N	C:P	N:P
Spain	1	13.1	31.3	2.4
	2	15.0	124.4	8.3
	3	14.0	40.2	2.9
	4	22.3	63.2	2.8
	5	7.2	102.8	14.3
Italy	1	7.5	8.3	1.1
	2	13.2	154.4	5.2
	3	29.5	n.a	n.a
	4	13.2	n.a	n.a
	5	15.3	95.2	6.2
Greece	1	27.7	27.9	1.0
	2	19.5	45.3	2.3
	3	14.1	106.6	7.6
	4	19.4	89.9	4.6
	5	18.7	49.0	2.6

Values represent mean of 3 replicates as presented in Fig. 5. n.a.=not analysed.

cages (38–64 g DW $m^{-2}d^{-1}$), which rapidly declined with increasing distance from the farms (Fig. 3). The sediment deposition rates were 25 to 98% higher on benthic communities surrounding the Italian farm, the largest farm studied. On the contrary, the sediment deposition rate was lowest on benthic communities near the Spanish farm, which was the smallest farm studied and that located above the greatest water depth. Sedimentation rates were higher throughout both transects in Italy (11–13 g DW $m^{-2}d^{-1}$) as compared to the other sites. This difference was evident even for the control sites (9 g DW $m^{-2}d^{-1}$) for both sampling years (2002 and 2003) indicating the presence of higher background sedimentation rates at the site in Italy. Sedimentation right under the net cages was about 42% lower in 2003 probably due to lower fish biomass in the cages during deployment of traps (pers. obs.). Sedimentation along transects perpendicular to the main current direction in Spain and Greece did not show significant differences (1-way ANOVA, $p>0.05$, Fig. 3) from the main current reflecting the large variance in current direction and speed. The decrease in sediment deposition rates with increasing distance from the cage was fastest for the Spanish compared to the Greek location (Fig. 3) and reflected the lower production at the Spanish farm.

Deposition of organic C, N and P showed the same pattern described for total deposition rates with high rates near the cages, but with some differences between transects (Fig. 4). The P sedimentation was very high in Italy, 91 times higher than at control sites, and in Spain the P sedimentation was below the detection limit at the control site (<1 mg P $m^{-2}d^{-1}$) and the rate under the net cages was thus at least 145 times higher, whereas it was 64 times higher for Greece. POC and PON were also high right under the net cages at all sites, up to 4–14 times higher than control sites. The POC content along the transect in the main current direction in Spain showed rather low nutrient contents compared to the perpendicular transect (88–93% lower), whereas there were no clear differences between the two transects for PON and no major differences between sampling years in Italy (Fig. 4). The molar ratios of C:N, C:P and N:P of the settling particulate organic matter varied along and between the transects and between the study sites (Table 3). Generally, lowest ratios were found under the net cages and increasing with distance, in particular at the control sites. The only exception was a low C:N ratio at the control site in Greece. The variability was very high between transects, and both in Spain and Italy the variability was caused by high POC contents along some of the transects (Transect 2 in Spain, and transect 1+2 in 2003 in Italy).

3.3. Sediments

Sediments near the net cages were at all farms enriched in POC, PON and TP compared to control sites (Fig. 5). It was, however, not always the station right under the net cages, which was enriched the most. In Spain Sta. 2 had the highest organic pools, and this was also the case for POC in Italy. In Greece the two seagrass

Table 5
Isotopic carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) composition of the trap material and surface sediments (0–2 cm) at the 3 study sites

Location	Station	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
		Trap	Trap	Sediment	Sediment
Spain	1	−20.07	5.60	−19.53±0.51	5.26±0.66
	2	−23.53	6.15	−19.18±0.15	6.68±2.10
	3	−22.88	5.26	−16.01	3.71
	4	−22.95	6.42	−14.73	3.30
	5	−23.44	6.19	−18.05	4.65
Italy	1	−21.85	12.41	−16.24	9.34
	2	−20.45	9.55	−19.90	5.50
	3	−20.50	10.12	−20.74	6.67
	4	−17.42	6.95	n.a.	n.a.
	5	−14.93	4.82	−20.09	2.89
Greece	1	−20.11	5.13	−17.21±0.80	3.08±0.28
	2	−21.64	4.82	−16.09±0.20	1.95±0.40
	3	−20.81	4.56	−24.55	2.63
	4	−21.22	5.43	−24.43	2.51
	5	−20.55	2.51	−24.51	2.50

Traps represent single measurements, whereas sediments are mean± SE (n=1–6). n.a.=not analysed.

stations were most enriched in nutrients, except for TP which was high under the net cages. The molar ratios of C:N in the sediments generally showed low ratios under the net cages but no consistent pattern along the transect (Table 4). In Greece the C:N ratio was highest (27.7) under the net cages and less variable along the transect (14.1–19.5). Due to the high TP content under the net cages, C:P ratios were very low at all farms (8–31) and also N:P ratios were low (1.0–2.4) compared to control sites (C:P 49–103; N:P 2.6–14.3).

3.4. Stable isotopes

The stable isotopic composition of the trap material varied along the transects with the most depleted δ^{13}C values under the net cages in Italy, and at Sta. 2 in Spain and Greece and increasing towards the control sites (Table 5). The largest change was found along the transect in Italy (from −21.85 to −14.93), whereas changes were less in Spain (−23.53 to −23.44) and in Greece (−21.64 to −20.55). Also the nitrogen signals showed large variation in Italy with high values under the net cages ($\delta^{15}N=12.41$) and low values at the control site ($\delta^{15}N=4.82$). There were no consistent changes along the transect in Spain, whereas values decreased along the transect in Greece (5.13 to 2.51) with an exception at Sta. 4 with a high value (5.43).

The δ^{13}C values in the sediments were generally less depleted than the trap material (Table 5). In Italy and Greece values decreased along the transects (−16.24 to −20.09 in Italy and −16.09 (at Sta. 2) to −24.51 in Greece). There were no consistent changes in δ^{13}C and δ^{15}N along the transect in Spain. A large decline in δ^{15}N values was found in Italy (9.34 to 2.89) and the same trend, although smaller, was seen in Greece (3.08 to 2.50).

The feed pellets had a carbon, nitrogen and phosphorus content of 47.1–48.1%DW, 7.5–7.8%DW and 1.8–2.1%DW, respectively (Table 6). The isotopic composition of the feed varied between the 3 farms with a low δ^{13}C value in the feed used in Greece (−22.69‰) and highest in Spain (−21.36‰). The nitrogen isotopic signal was highest (7.43‰) in the feed used in Greece and lowest in Spain (6.53‰).

Table 6
Stable isotopic values (δ^{13}C and δ^{15}N) and nutrient contents (POC, PON and TP) of feed pellets used at the 3 farms ($n=1$)

	δ^{13}C	δ^{15}N	POC (%DW)	PON (%DW)	TP (%DW)
Spain	−21.36	6.53	47.12	7.50	1.81
Italy	−21.50	7.30	n.a.	n.a.	n.a.
Greece	−22.69	7.43	48.11	7.77	2.09

n.a. = not analysed.

4. Discussion

The sedimentation of waste particulate products was high under the net cages, exceeding the background sedimentation measured ~1 km away between 8 and 25 times. In contrast to the water column, where nutrients are diluted over large areas (Karakassis et al., 2001; Schendel et al., 2004), sediments are known to accumulate organic matter (Holmer and Kristensen, 1992; Sarà et al., 2004; Schendel et al., 2004; Porrello et al., 2005), and this was also the case at the farms studied. The particle size associated with waste products and pellets from fish farms is much larger than that of ambient seston, resulting in fast sinking rates of the materials derived from fish farms, which settle underneath or close to the cages even at relatively high current velocities, as in the studied farms (Cromey et al., 2002; Sarà et al., 2004; Cromey and Black, 2005). Sedimentation rates decreased rapidly with increasing distance from the farms, diminishing by up to 91% at 5 to 40 m away from the net cages. These rates were, however, still 1.3 to 5.4 times higher than the background sedimentation rates, suggesting that the effect of the farm enrichment was evident at greater distances than examined here. 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. The examination of transects perpendicular to the main current direction at the farms in Spain and Greece showed that sedimentation was high in this direction as well, suggesting that the spreading of waste products is dispersed over a larger area and not only directly along the main current.

Excess sedimentation of particulate organic matter under net cages is driven by loss of waste products from the farms, and in particular, organic carbon and phosphorus are released in particulate form (Hall et al., 1990; Holby and Hall, 1991; Lupatsch and Kissil, 1998; Lupatsch et al., 2003; Schendel et al., 2004; Islam, 2005). Only a limited number of studies of sea bass and sea bream are available, but studies of salmonids have shown that 29 to 71% of carbon (Hall et al., 1990) and 59 to 66% of phosphorus (Holby and Hall, 1991) are released in particulate form of feed and faecal pellets, whereas about 50% of the nitrogen is released in the particulate forms (49 to 51%, Hall et al., 1992). Dissolved nitrogen is available for phytoplankton, epiphyte or seagrass uptake which in turn may settle in the farm surroundings through zooplankton faecal pellets or as phytodetritus (Karakassis et al., 2001). The food conversion ratios for sea bass and sea bream in the examined farms were high (1.6 to 2.4) compared to experimental studies (Vergara et al., 1999) and those for salmonids (Nordgarden et al., 2003). This implies that there is a larger loss of supplied feed in open sea production of sea bream and sea bass compared to salmonids. Direct loss of feed is, however, considered to be low (<1–2% of feed input) and a higher food conversion ratio may be species-specific rather than due to differences in feeding practice. At deep Mediterranean fish farms (>20 m) it is not likely that the bulk of intact pellets reaches the sediment floor, as most farms, and also those observed here, are surrounded by large assemblages of wild fish, which are attracted to the farm installations as to other artificial devices (Dempster et al., 2002, 2005; Machias et al., 2004). Consumption of feed pellets and particulate waste products by wild fish is considered to be an important modifier of the sedimentation regime, e.g. by increasing the area of deposition of farm effluents by slowing down the settling speed of the waste particles (Sarà et al., 2004). Wild fishes consumed about 80% of the lost particulates from a farm located close to our sampling site in Spain (Vita et al., 2004). Also the faecal pellets display varying sinking rates depending upon fish species and size (Cromey and Black, 2005), where faecal pellets of sea bass (*D. labrax*), for instance, sink much faster than pellets from gilt-head sea bream (*S. aurata*, Cromey pers. comm.). Cromey (pers. comm.) found that more than half of the faecal pellets from sea bass were sinking with rates >4 cm s^{-1}, whereas <14% of pellets from sea bream was settling at this rate. Both sea bream and sea bass were cultivated in the farms studied here, and the fast decline in sedimentation rates with distance suggests that faecal pellets contributed to the sedimentation under the cages. The high rates of sedimentation under the cages in Italy, and low rates within short distance from the cages (5 m) are consistent with the large specimens of sea bass held in this particular net cage. The size of the production in the farms may, however, also contribute to the high rates under the cages in Italy. The biomass of fish in the studied net cage was about double than in the other farms, which was reflected in the daily feed input (data not shown). The rates of sedimentation may have been further constrained by local differences in production (feeding practice) during the deployment of the traps. The traps were, however, deployed for 2 days to avoid short term variations in feeding and in June or September, where the biomass of fish was at maximum in all farms. The rates are considered to represent maximum rates of sedimentation during the production season. Resuspension is also considered as an important modifier of dispersion of waste products (Cromey et al., 2002; Sarà et al., 2004), and as the traps were deployed close to the sediment surface, this may have overestimated the measured rates. Recent studies of sedimentation rates around aquaculture facilities in the Mediterranean Sea (rearing sea bass and sea bream) reported sedimentation rates of the same order of magnitude as those reported here (Cromey pers. comm.). Sedimentation rates are, however, at the high end of rates recorded from salmonid farms at similar depths and size of production consistent with the larger FCR for sea bream and sea bass (Sutherland et al., 2001; Cromey et al., 2002).

As the rates of sedimentation declined fast with distance, it was not possible to resolve if the sedimentation rates were higher than expected in the seagrass meadows, as these were located further along the transect compared to unvegetated sites. The sedimentation at the control sites fell in the ranges measured in seagrass beds of *P. oceanica* (Gacia et al., 2002; Holmer et al., 2004), indicating that the locations are representative of oligotrophic Mediterranean and not influenced by the farming activities, despite the exposed conditions and the presence of wild fish at the study sites, which could transport waste products to large distances. Only background sedimentation rate in Italy appeared to be 2 to 5 times higher than that at the Spanish and Greek study sites. The fish production was 2.9 to 4.4 times higher than the other studied farms, and it is possible that the control site was influenced by a sewage outfall from the land-based hatchery belonging to the farm, which may contribute to increased primary productivity and sedimentation in the area (RD pers. observ.).

4.1. Sedimentation of organic nutrients along transects

The sedimentation of the nutrients C, N, P generally showed spatial persistence with high sedimentation confined to the immediate vicinity of the net cages (5–15 m). Other studies have mostly focused on C and N, where C shows spatial persistence, whereas N is dispersed over larger areas (Karakassis et al., 2001; Sarà et al., 2004; Schendel et al., 2004). Phosphorus is considered to follow C sedimentation as P is released in the particulate fraction (Holby and Hall, 1991). The Mediterranean Sea is characterized by low water column and sediment phosphorus concentrations and primary productivity is often P-limited (Duarte et al., 2000). This is consistent with the low sedimentation of P at control stations, whereas P sedimentation rates increased 20 to 1200 times under the net cages. Feed pellets used for production of sea bream and sea bass have high P contents mainly due to the enrichment with proteins and fats, and faecal pellets collected at the farms indicate that the cultured fish did not absorb all the added P, as the excretion products were further enriched (~2 times higher P compared to feed pellets at the Spanish fish farm; data not shown). For sea bream and sea bass, an excretion of 1.3–1.6% of the total feed input has been estimated to be lost in the form of P with about 85% as particles (Karakassis pers. comm.). The high sedimentation of P is not only confined to the net cages, as the stations up to 40 m away had rates 4 to 62 times higher than at the control sites, suggesting that phosphorus deposition can be used as a sensitive indicator of farm waste products in the Mediterranean Sea. Indeed, the underlying sediments, in particular under the net cages, were enriched with P up to 9 times compared with the control sites, which is possibly due to rapid binding of P into carbonates in the carbonate rich sediments (Jensen et al., 1998). The sedimentation of C and N showed rates 4 to 27 times and 2 to 10 times higher, respectively, under the net cages and at stations up to 40 m away. The presence of wild fish may contribute to the relatively lower C and N sedimentation rates in particular directly under the net cages in Spain and Italy (Vita et al., 2004). At these two locations, highest C and N contents in the sediments were found at Sta. 2. This suggests that there was a decoupling of the sedimentation process and accumulation of C and N in the sediments, which could be due to benthic feeding by demersal wild fish and benthic fauna or through enhanced microbial mineralization. As mentioned above, wild fish were attracted to the cages and benthic fauna were present in the sediments (Karakassis pers. comm.). Also mineralization rates were high right under the net cages (Holmer and Frederiksen unpublish.) and may contribute to decomposition of organic matter. The C and N enrichments of the sediments were much lower compared to salmonid farms, and the consumption of waste products by wild fish may be an important factor for less severe benthic impacts found in most Mediterranean farms (Karakassis et al., 2000, 2002; La Rosa et al., 2004) compared to salmon farms at similar water depths and fish production (Hall et al., 1990, 1992; Hargrave et al., 1993). In Greece the seagrass sediments were enriched in C and N compared to the unvegetated sites under and near the net cages, indicating an enhanced burial of nutrients. This farm was located at the shallowest depth of the sites studied, and the settling of particles thus occurs in closer vicinity compared to the deeper farms. Organic enrichment of the sediments has been identified as one of the contributing factors of seagrass decline in fish farm surroundings (Delgado et al., 1997; Marbá et al., 2006).

4.2. Stable isotopes

The sediment deposition rates and organic loads suggest that sediments at a distance of 40 m from the cages or less receive large inputs of waste products from the farming activities. This was confirmed by the stable isotopic signals of the sedimentation trap material and sediments. In Italy the trap material generally had a δ^{13}C signal close to the feed pellets and the signal increased (became less negative) towards the control site indicating farm-derived input under the net cages. This pattern is different from findings by Sarà et al. (2004), as they found small difference between feed material ($\delta^{13}C = -22.8‰$) and control sites (−23.1‰), and could not use δ^{13}C signal for quantification of farm inputs. The nitrogen signal showed a similar pattern to that found by Sarà et al. (2004), with decreasing values towards the control site ($\Delta\delta^{15}N = -9.3‰$), although the difference between farm and control site again was much larger in our study (Sarà et al., 2004, $\Delta\delta^{15}N = -1.5‰$). Bulk sedimentation rates were not reported by Sarà et al. (2004), but their farm was smaller (only 4 cages) and deeper suggesting lower sedimentation in their study. The nitrogen signal in the trap material was higher than in the feed material, and is consistent with an expected increase in δ^{15}N through passage in the trophic chain (Sarà et al., 2004). The sediments did not reflect the sediment traps for δ^{13}C values, as the sediments became more depleted along the transect, whereas the δ^{15}N followed the sediment traps and was thus consistent with observations by Sarà et al. (2004) of declining δ^{15}N values away from fish farms. δ^{15}N appears to be a sensitive indicator of farm inputs to sediments, whereas δ^{13}C values are more difficult due to the discrepancy between trap and sediment values.

Although sediment deposition rates were of the same order of magnitude in Greece, changes in isotopic values along the Greek transect were much less evident and more similar to those observed by Sarà et al. (2004). Large regional variations in isotopic values can be expected depending on the contribution from terrigenous and autochthonous sources. Martinotti et al. (1997) reported values of $\delta^{13}C = -15.0‰$ for terrigenous-continental sources and values of $\delta^{13}C = -19.0‰$ for phytoplankton detritus, and sestonic material around the Balearic Islands have been reported more negative ($\delta^{13}C = -22.1$ to $-22.3‰$, Papadimitriou et al., 2005). The large difference between control stations at the 3 farms may reflect a larger input from terrestrial C4 plant sources in Italy compared to the two other fish-farm sites. The δ^{13}C signals under the cages (−20.1‰) in Greece were similar to the background values, which may be attributed to sedimentation of faecal material, as a shift to less negative values has been found after passage through fishes (Sarà et al., 2004). All other stations at the Greek farm displayed more negative values compared to the control station, suggesting contribution of farm waste products to sedimentation rates in these areas. The δ^{15}N values in the sediment trap also indicated farm derived products in the material, and the sediments right under the net cages were enriched in δ^{15}N value compared to the control site.

There were less clear patterns in Spain representing the deepest farm. The δ^{13}C signal was less negative under the cages compared to the control site, and the stations along the transect showed signals similar to the control sites both for δ^{13}C and δ^{15}N. The feed pellets had signals between the net cage site and the control site suggesting that the material encountered in the traps under the net cages had passed through the fish and was less depleted compared to the feed pellets (Sarà et al., 2004). The δ^{15}N signal was, however, also lower than in feed pellets and did not reflect the expected increase due to fish passage. Unfortunately, there was only one replicate of feed pellets and analytical error may contribute to the inconsistent observations, although Sarà et al. (2004) showed high precision of their analyses of feed material. The changes in δ^{13}C and δ^{15}N in the underlying sediments were also somewhat variable, with the seagrass meadows being different from the patterns observed at the other two farms. These results indicate that more research is needed to fully understanding the variability in stable isotopes in fish farm surroundings.

In conclusion, the difference in isotopic values along the transects offered the possibility to identify waste-farm products in the settling material and in the sediments, and thus emphasizes the potential usefulness of this tool for assessing the spatial extent of farm impacts. Despite the rapid water exchange at the examined farms, sedimentation rates were high in the vicinity of the farms. This altered benthic conditions, increasing organic matter accumulation and causing negative effects on the seagrass communities (Holmer et al., 2003; Diaz-Almela et al., unpublished). In particular, sedimentation of P was high and was, beneath the cages, up to 1200 times higher than at the control site, and up to 62 times higher 40 m away from the net cages. Our results indicate that sedimentation rate of organic P has the potential to act as a sensitive indicator of farm effluents.

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