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EFFECTS OF INTENSIVE MARICULTURE ON SEDIMENT BIOCHEMISTRY

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Abstract. The exponential growth of off-shore mariculture that has occurred worldwide over the last 10 years has raised concern about the impact of the waste produced by this industry on the ecological integrity of the sea bottom. Investigations into this potential source of impact on the biochemistry of the sea floor have provided contrasting results, and no compelling explanations for these discrepancies have been provided to date. To quantify the impact of fish-farm activities on the biochemistry of sediments, we have investigated the quantity and biochemical composition of sediment organic matter in four different regions in the temperate–warm Mediterranean Sea: Akrotiri Bay (Cyprus), Sounion Bay (Greece), Pachino Bay (Italy), and the Gulf of Alicante (Spain). In these four study regions, the concentrations of phytopigments, proteins, carbohydrates, and lipids in the sediments were measured, comparing locations receiving wastes from fish farms to control locations in two different habitats: seagrass beds and soft nonvegetated substrates. Downward fluxes were also measured in all of the regions, up to 200 m from the fish farms, to assess the potential spatial extent of the impact. In all four regions, with the exception of seagrass sediments in Spain, the biochemistry of the sediments showed significant differences between the control and fish-farm locations. However, the variables explaining the differences observed varied among the regions and between habitats, suggesting idiosyncratic effects of fish-farm waste on the biochemistry of sediments. These are possibly related to differences in the local physico-chemical variables that could explain a significant proportion of the differences seen between the control and fish-farm locations. Biodeposition derived from the fish farms decreased with increasing distance from the fish-farm cages, but with different patterns in the four regions. Our results indicate that quantitative and qualitative changes in the organic loads of the sediments that arise from intensive aquaculture are dependent upon the ecological context and are not predictable only on the basis of fish-farm attributes and hydrodynamic regimes. Therefore, the siting of fish farms should only be allowed after a case-by-case assessment of the ecological context of the region, especially in terms of the organic matter load and its biochemical composition.

Key words: aquaculture; fish-farm wastes; mariculture; Mediterranean Sea regions; Posidonia oceanica; *seagrass; siting fish farms; soft bottoms; trophic state.*

Introduction

Over the last decade, mariculture has experienced an almost exponential development along coastal oceans worldwide. For instance, in the most oligotrophic regions of the Mediterranean Sea, mariculture alone is responsible for up to 7% and 10% of the nitrogen (N) and phosphorous (P) loads, respectively (Pitta et al. 1999). As with other farming activities, the environmental effects of this emerging industry have prompted widespread criticism and have initiated a global effort to develop more sustainable farming techniques (Troell et al. 2003).

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The potential adverse effects of aquaculture discharges are widely referred to, but poorly documented in rigorous scientific studies (Burford et al. 2003). Moreover, most of the descriptors and procedures used to assess environmental impact from aquaculture have rarely been evaluated following proper scientific standards, and the existing publications deal with very local views of the responses of the ecosystem to disturbances (Karakassis et al. 2000, Mirto et al. 2002, Sarà et al. 2004).

Intensive fish farming results in the release of large amounts of dissolved and particulate nutrients to the surrounding environment (Hall et al. 1990, Holmer and Kristensen 1992, Pitta et al. 1999). Previous studies have demonstrated that the most evident consequences of fish farming on the benthic environment are an increase in total organic carbon (C) accumulation in the sediment and a decrease in oxygen availability for the benthos beneath fish cages (Holmer 1991, Holmer and Kristen-

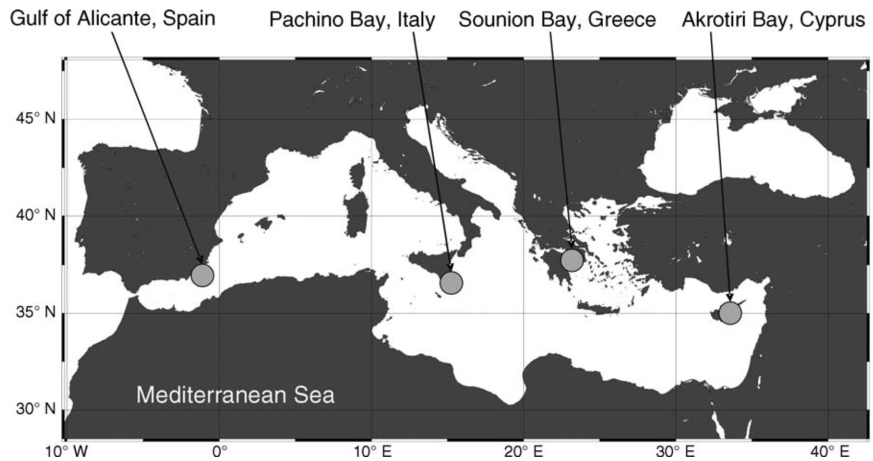

FIG. 1. Location of the four study regions in the Mediterranean Sea.

sen 1992, Karakassis et al. 1998). These changes, in turn, have significant impact on the abundance and biodiversity of micro-, meio- and macrobenthic organisms (Karakassis et al. 2000, Mirto et al. 2002, La Rosa et al. 2004). Other recent studies have demonstrated that fish-farming effluents have effects also on the biochemical composition of the organic matter of sediment. Fish-farm sediments are sometimes enriched in lipid content due to the accumulation of uneaten fish-food pellets on the seafloor (Mirto et al. 2002, Bongiorni et al. 2005), and are characterized by increased microbenthic algal biomass in response to the increased availability of nutrients below the cages (La Rosa et al. 2001). However, the results of these studies are not always consistent across different ecological contexts (Mirto et al. 2002) and have been generally obtained from investigations conducted on small spatial scales (hundreds to thousands of meters; Danovaro et al. 2003). Therefore, it appears of paramount importance to assess whether the effects of organic enrichment caused by fish farms result in changes in the biochemistry of the sediment along different environmental gradients affected by this source of disturbance.

Here, we have quantified the effects and the spatial extent of the benthic eutrophication induced by fish farming, using the quantity and biochemical composition (i.e., protein, carbohydrate, lipid) of sediment organic matter as the relevant variables of the benthic trophic state. Eutrophication is generally assessed through chemical measurements (e.g., inorganic N and P) and/or surrogate measurements of algal biomass in the water column (Stefanou et al. 1999). These proxies, however, may fail to detect the consequences of increased nutrient loads on benthic systems (Cloern 2001). On the other hand, previous studies have demonstrated that changes in the concentrations and relative importance of the different classes of organic compounds reflect relevant changes in the trophic state of the sediment (Dell'Anno et al. 2002, 2003, Pusceddu et al. 2003, 2007). For instance, systems poor in organic-C concentrations (namely, oligotrophic) are generally characterized by a larger carbohydrate fraction, whereas systems with higher organic C concentrations are characterized by a protein dominance, such that increasing protein concentrations in the sediment are typically associated with meso- to eutrophic conditions (Dell'Anno et al. 2002, Pusceddu et al. 2007).

Our study was conducted at a basin scale, to overcome the main weakness of most impact studies, which are often carried out at a local scale (Hewitt et al. 2005). The comparison of the four different regions along the environmental gradient of the east-to-west Mediterranean basin was designed to reveal generalities and/or idiosyncratic responses to the effects of this source of impact on benthic systems. We investigated the quantity and biochemical composition of sediment organic matter 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. In all these four regions, locations with organic matter deposited from fish farms are compared with putatively pristine locations as controls. The potential impact of organic enrichment induced by fish farming was quantified in two different habitats that have often been selected for the siting of aquaculture industries: (a) vegetated substrates, characterized by the presence of meadows of the seagrass *Posidonia oceanica*; and (b) nonvegetated soft sediments, characterized by different structural and functional properties and potentially differently sensitive to this source of impact. Moreover, to assess the significance of the hydrodynamic control on the spatial extent of the fish-farm impact, the downward fluxes of total mass, phytopigments, proteins, carbohydrates and lipids were assessed in the four regions, at increasing distances from the fish farms. The results have been fitted within a parabolic model of particle decay, to discriminate among the different sites in terms of the spatial impact of the fish-farm effluent.

TABLE 1. Characteristics of the fish farms in the four regions in the Mediterranean Sea.

Study area	Sampling location	Bottom temperature (°C)	Bottom current (cm/s)	Sediment type	Chlorophyll *a* (μg/L)	N-NH_4 (μmol/L)
Cyprus	Akrotiri Bay	17–18	20–40	carbonate mud	0.01–0.02	12.5–13.4
Italy	Pachino Bay	17–18	20	carbonate sand	0.03–0.08	52.0–55.8
Greece	Sounion Bay	17–18	6.3	carbonate sand	0.08–0.11	12.1–13.0
Spain	Gulf of Alicante	17–18	4.7	carbonate fine sand	0.07–0.11	11.0–11.8

Note: Data for water-column chlorophyll *a* and nutrient concentrations have been summarized from Karakassis et al. (2005).

METHODS

Study areas

The Mediterranean Sea is a semi-enclosed, temperate–warm, miniature ocean. It is continuously threatened by a combination of spatially extended press-and-pulse anthropogenic disturbances, the detrimental ecological consequences of which are increasingly documented. Due to its high mean temperatures (typically >13°C) and low water-renewal rates (80–100 years), the anthropogenic impact in the Mediterranean Sea is expected to be exacerbated. Wide, shallow regions of the Mediterranean Sea are subjected to strong human exploitation (e.g., fisheries and mariculture) and consequently they are experiencing increasing eutrophication, coastal degradation and habitat fragmentation (Turley 1999). Over the last 10 years, mariculture has increased in the Mediterranean Sea at an almost exponential rate, and it has become a major threat to the integrity of the coastal environment (Karakassis et al. 2000, Danovaro et al. 2003).

Sampling was carried out along an east-to-west longitudinal transect (~3500 km wide) in Akrotiri Bay (Cyprus; 34°39′ N, 34°04′ E; July 2002), Sounion Bay (southern Greece; 37°39′ N, 24°01′ E; July 2003), Pachino Bay (Italy; 36°43′ N, 15°05′ E; September 2002), and the Gulf of Alicante (Spain; 38°24′ N, 0°24′ W; September 2003) (Fig. 1). The sampling areas, located at similar latitudes and depths (between 16 m and 39 m) were selected on the basis of the presence of fish farms, which have been previously characterized in terms of their main environmental features (Table 1). In each of the sampling areas, the effects of the fish farm were investigated in two different habitats: *Posidonia oceanica* meadows, and soft substrates. The Mediterranean seagrass *P. oceanica* is characterized by very slow growth rates (Marbà and Duarte 1998) and it is known to be very sensitive to organic waste introduced by aquaculture activities (Delgado et al. 1999, Pergent et al. 1999, Ruiz et al. 2001).

In each region, a preliminary survey was carried out to ascertain the presence of both the seagrass and the soft substrates, and to characterize the environmental settings of the areas in terms of mean depth and temperature, bottom currents, sediment type and porosity, and chlorophyll *a* and inorganic nutrient concentrations in the water column (Karakassis et al. 2005).

In each habitat the impact was quantified by comparing the fish-farm locations with control locations. The control locations were situated upstream of the main currents, and at least 1000 m from the fish farms. They were characterized by relatively pristine conditions and by environmental features comparable to those found beneath the fish-farm cages. Replicates were selected randomly from the central area of each fish-farm location (i.e., beneath the cages) and in each control location.

Downward fluxes

Sedimentation traps had a 15-cm internal diameter and a 1-m height, corresponding to an aspect ratio of >60:1, a total volume of ~18 L, and a collection surface of ~180 cm^2. Three traps, fastened together and fixed to a cable, were deployed at all of the sites along a gradient downstream of the prevalent water-current direction, at increasing distances from the cages (at 0, 40, and 200 m); they were left operating at 2 m above the sea bottom for 48–72 h, depending on the expected sedimentation regime. The material collected in the three traps was pooled and aliquots (from one quarter to one fifth of the total volume) were stored at −20°C until analysis in the laboratory. For laboratory analysis, the samples were homogenized and filtered onto Whatman GF/F membranes (0.4-μm nominal pore size) and analyzed as described below.

Mass fluxes were determined gravimetrically (after rinsing in distilled water and drying at 60°C for 24 h). Fluxes were estimated using the following formula:

$$F_i = C \times \mathrm{TV} \times \mathrm{TV}_\mathrm{f}^{-1} \times T_\mathrm{d}^{-1} \times S_\mathrm{c}^{-1}$$

where F_i is the flux of the *i*th compound, C is the concentration (in milligrams per liter) of the *i*th compound in the analytical subsample, TV is the total volume of the aliquot (in milliliters), TV_f is the fraction of the total volume collected by the trap (1/4 or 1/5), T_d is the time of trap deployment (in days), and S_c is the collecting surface of the trap (in square meters).

To determine the spatial extent of the impact derived from the fish-farm biodeposition, we used the model of settling particle horizontal displacement proposed by Gowen et al. (1989):

TABLE 1. Extended.

$P\text{-}PO_4$ μmol/L	Began operation	Distance from the shore (m)	Reared species	Annual production (kg)	Food input (kg/yr)
0.9–1.1	1988	1050	Sea bream (*Sparus aurata*); Sea bass (*Dicentrarchus labrax*)	300	660
3.6–4.4	1992	1000	Sea bream (*Sparus aurata*); Sea bass (*Dicentrarchus labrax*); Sharpsnout sea bream (*Diplodus puntazzo*)	1150	2749
0.8–1.0	1996	500	Sea bream (*Sparus aurata*); Sea bass (*Dicentrarchus labrax*)	400	640
0.8–0.9	1996	2600	Sea bream (*Sparus aurata*); Sea bass (*Dicentrarchus labrax*)	260	520

$$d = \frac{D \cdot V_c}{V_s}$$

where d is the particle horizontal dispersion distance, D is the water depth, V_c is the current velocity, and V_s is the settling velocity of waste particles (uneaten food and faeces). This model predicts a parabolic decrease of settling particle velocity with increasing distance from the input. Thus, assuming that the settling velocity is proportional to the downward fluxes, we plotted the downward fluxes of particles released from the fish farm against distance, adopting a best-fit power equation that was obtained using a least-squares method. The exponent of the best-fit power equations were smaller than that (−1) predicted by the Gowen model. This discrepancy is attributable first to the performing of the regression analysis assuming that the water current was constant at all distances from the cage, and second to other environmental forcing factors that were unaccounted for by the Gowen model. Due to the differences in water depths and current velocities at the four regions investigated (Table 1), the models were compared in terms of the distances at which the biopolymeric C fluxes to the sediment were half of the flux beneath the cages, calculated from the best-fit equations. These distances were used as indicators of the spatial extension of the fish-farm impact at three of the four sampling regions (Italy, Greece, and Spain).

Sediment sampling

Sediment samples were collected as three replicates using steel and plexiglass corers, in seagrass and soft-bottom sediments, respectively, in both the control and fish-farm locations. Sediment sampling was carried out by scuba divers using manual coring. Once retrieved, the corers were kept refrigerated (4°C) until further subsampling in the laboratory (within 4 h of collection). Analyses were carried out on the top first centimeter of each core.

Biochemical composition of settling material and sediment organic matter

Chlorophyll *a* and phaeopigment concentrations in the sediment were determined according to the fluorometric method of Yentsch and Menzel (1963). Phytopigments were extracted (90% acetone) overnight in the dark at 4°C, and the phaeopigments were estimated by acidification with 0.1 mol/L HCl.

Protein analyses were carried out according to Hartree (1972), as modified by Rice (1982) to compensate for phenol interference. Carbohydrates were analyzed according to Gerchacov and Hatcher (1972) and expressed as glucose equivalents. Lipids were extracted by direct elution with chloroform–methanol according to Bligh and Dyer (1959), quantified by the method of Marsh and Weinstein (1966), and the concentrations were calculated using standard tripalmitine solutions.

The carbohydrate, protein, and lipid levels were converted into carbon equivalents using 0.40 and 0.49 and 0.75 g C/g as conversion factors, respectively, and their sums are given as biopolymeric carbon (BPC; Fabiano et al. 1995).

Data analyses

A distance-based permutational multivariate analysis of variance (PERMANOVA; Anderson 2001, McArdle and Anderson 2001) was used to test for differences in downward fluxes of the variables investigated, with increasing distance from the cages. The data set comprised 36 observations × 7 variables (total sinking matter, protein, carbohydrate, lipid, biopolymeric C, chlorophyll *a*, and phaeopigment). The design included two factors: region (R, four levels, random) and distance from the cages (D, three levels, fixed and orthogonal), with $n = 3$ for the combination of factors. The analysis was based on Euclidean distances of previously normalized data, using 999 random permutations of the appropriate units (Anderson and ter Braak 2003). The analysis was run using the FORTRAN-written PERMANOVA.exe program (Anderson 2005). The pseudo-multivariate variance components for each term in the model were calculated using direct multivariate analogs to the univariate ANOVA estimators (e.g., Searle et al. 1992). Since the term $R \times D$ was seen to be significant, it was further analyzed through pairwise comparisons. The analysis of this data set also revealed that most of the variability in PERMANOVA was explained by the term region. Principal-components analysis (PCA) was carried out for each region separately, on data previously normalized to visualize the patterns in downward fluxes at the three distances from the cages and to show the importance of the contribution of each variable to the PC axes. PCA plots

TABLE 2. Results of PERMANOVA testing for differences in downward fluxes.

Source of variation	df	MS	F	P	CV†
Region, R	3	43.62			4.9
Distance, D	2	19.98			1.5
$R \times D$	6	9.33	4.21	<0.001	2.4
Residual	24	2.22			
Total	35				

Note: The analysis was based on the distance matrix calculated using Euclidean distances on previously normalized data. Each term was tested using 999 random permutations of the appropriate units.

† Associated multivariate pseudo-variance components, for each term.

were generated with the PRIMER version 6 statistical package (Clarke and Gorley 2001).

PERMANOVA was also used to quantify the effects of the fish farms on the two benthic habitats. The analysis treated the region factor (R, four levels) as random, habitat (H, two levels) as fixed and crossed with R, and impact (I, two levels) as fixed and crossed with R and H. In this case, the data set included 48 observations and 5 variables: chlorophyll *a*, phaeopigments, protein, carbohydrate, and lipid. The interaction term $R \times H \times I$ was significant in the analyses. Pairwise comparisons were used to test differences of I within each level of the factors R and H (using 999 random permutations to obtain P values [Anderson and Robinson 2003]). PCA was carried out on each data set, and a selection of plots were generated with the PRIMER version 6 statistical package (Clarke and Gorley 2001).

A distance-based multivariate multiple regression was carried out with the program DISTLM.exe, to test whether the chosen set of environmental variables (water depth, bottom temperature and current, water content in the sediment, distance from the coast, chlorophyll *a*, ammonia and P in the water column) together explained a significant proportion of the multivariate variation in the biochemical composition of sediment organic matter (Anderson 2004).

RESULTS

Downward fluxes of organic compounds

PERMANOVA provided evidence of a significant region-by-distance ($R \times D$) interaction (Table 2), suggesting that the downward fluxes of the variables investigated varied significantly at increasing distance from the cages, with differences varying from region to region (Table 3). The factor R was also the most relevant component of variation in the analysis. In Cyprus and Italy, pairwise comparisons highlighted appreciable differences in downward fluxes only between 0 m and 200 m from the cages. In Greece, significant differences were detected between all of the distances, while in Spain the fluxes differed between 0 m and 40 m distance, but no difference was seen between 40 m and 200 m from the cage.

Results from the PCA analyses carried out separately for each region showed that in all cases the first two axes (PC1 and PC2) of the ordination accounted for most of the variability (always >90%) in the full matrix. However, the patterns in downward fluxes seen in each region were apparently driven by different environmental variables. For example, in Greece the main contribution to the first axis was from carbohydrate (Fig. 2a), in Italy the vector plot shows that PC1 represents an axis of decreasing total matter (TM) from 0 m to 200 m (Fig. 2b) while in Spain PC1 is a roughly equally weighted combination of most of the investigated variables (Fig. 2c).

The spatial patterns in downward fluxes of biopolymeric C in Cyprus and Italy were clearly different from the Gowen model output (Gowen et al. 1989), whereas in Spain and Greece, they better fitted the model (Fig. 3).

Quantity and biochemical composition of organic matter in the sediment

PERMANOVA showed a significant region-by-habitat-by-impact ($R \times H \times I$) interaction, suggesting that the differences between control and fish-farm locations

TABLE 3. Downward fluxes in the four investigated regions of the Mediterranean Sea, at increasing downstream distances from the fish-farm cages.

Region	Distance from the cage (m)	Total mass ($g \cdot m^{-2} \cdot d^{-1}$)		Protein ($mg \cdot m^{-2} \cdot d^{-1}$)		Carbohydrate ($mg \cdot m^{-2} \cdot d^{-1}$)		Lipid ($mg \cdot m^{-2} \cdot d^{-1}$)		Biopolymeric C ($mg \cdot m^{-2} \cdot d^{-1}$)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Cyprus	0	2.8	0.6	102.6	17.5	118.5	13.9	23.8	1.0	115.6	8.7
	40	4.1	0.2	84.1	17.8	87.2	22.5	18.2	5.8	89.7	16.9
	200	2.3	0.3	39.4	16.0	54.1	11.1	11.9	2.3	49.9	11.8
Italy	0	1.62	0.86	22.65	10.99	26.92	3.55	8.02	4.98	25.48	2.37
	40	0.65	0.39	12.09	6.38	22.92	7.57	4.26	1.53	18.43	2.73
	200	0.35	0.14	4.58	0.49	19.54	1.73	4.98	2.02	13.80	1.71
Greece	0	0.9	0.3	157.9	16.8	557.6	129.5	109.0	57.3	541.4	95.7
	40	1.4	0.5	152.2	67.3	146.5	15.9	112.0	38.0	217.2	61.3
	200	0.6	0.2	53.7	15.3	52.0	7.4	41.0	17.6	77.9	17.8
Spain	0	4.0	0.3	100.7	35.7	271.5	104.9	63.0	23.0	205.2	75.9
	40	3.1	0.7	50.4	8.3	118.1	43.6	25.4	7.1	91.0	26.0
	200	3.5	0.5	47.0	6.3	89.3	18.8	24.2	8.8	76.9	1.6

varied between the two habitats (unvegetated soft substrata and *Posidonia oceanica* meadows) and the four regions (Table 4). Pairwise tests revealed that significant differences between control and fish-farm locations were always detected in nonvegetated sediments in Greece, Italy, and Spain, while the presence of impact on the seagrass was revealed in Italy and Greece. In Cyprus there were no differences between control and fish-farm locations in either habitat (Table 5).

Fig. 4 shows the PCA ordinations of the environmental variables carried out separately for each region and habitat, where significant differences between control and fish-farm samples were revealed. It clearly emerges that different environmental variables contribute to the PCA axes. In particular, in Greece the PC1 axis was explained by the three biochemical classes of organic compounds and chlorophyll *a*, whereas in seagrass sediments carbohydrate, increasing in the fish-farm location, mostly contributed to the first axis (Fig. 4a, b). In Italy the variables explaining the PC1 axis were represented by protein and lipid in nonvegetated sediments and by protein, lipid, and phaeopigment in seagrass sediments (Fig. 4c, d). In Spain, the differences between control and fish-farm locations were observed only in the nonvegetated sediments, where carbohydrate and phaeopigment had decreasing levels in fish-farm locations and contributed mainly to the PC1 axis (Fig. 4e).

Finally, the results from the distance-based multivariate multiple regression showed that the chosen set of environmental variables recorded for the four regions (water depth and temperature, current, sediment water content, and chlorophyll *a* and inorganic nutrients in the water column) together explain a significant proportion of the multivariate variation in the data set (permutation $P = 0.0002$, proportion of variation explained $= 0.42$).

Discussion

The potential spatial impact of fish-farm industries

Downward fluxes derived from fish-farming activities contribute to the accumulation of organic matter in fish-farm sediments and have the potential to change the distribution patterns of benthic assemblages (Cromey et al. 1998, 2000, Danovaro et al. 2003). However, the rates of organic-C accumulation in sediments are less relevant in regions where the current velocity is high, because in these regions biodeposits do not accumulate on the sea floor (Hargrave et al. 1997). The contrary happens in regions with low current velocities (Milligan and Loring 1997). Accordingly, in the present study, downward fluxes were highest in proximity to the cages, and the highest biopolymeric C concentrations were observed in regions with low bottom-current velocities (i.e., Spain and Greece; Fig. 5a).

Another critical issue related to the effects of fish-farm industries on coastal regions is seen in the assessment of the spatial extent of the biodeposition impact. Previous studies based on mathematical models have provided evidence that fish-farm effluents can be used to predict the extent of the potential impact of aquaculture industries on the benthos (Cromey et al. 2002). Other

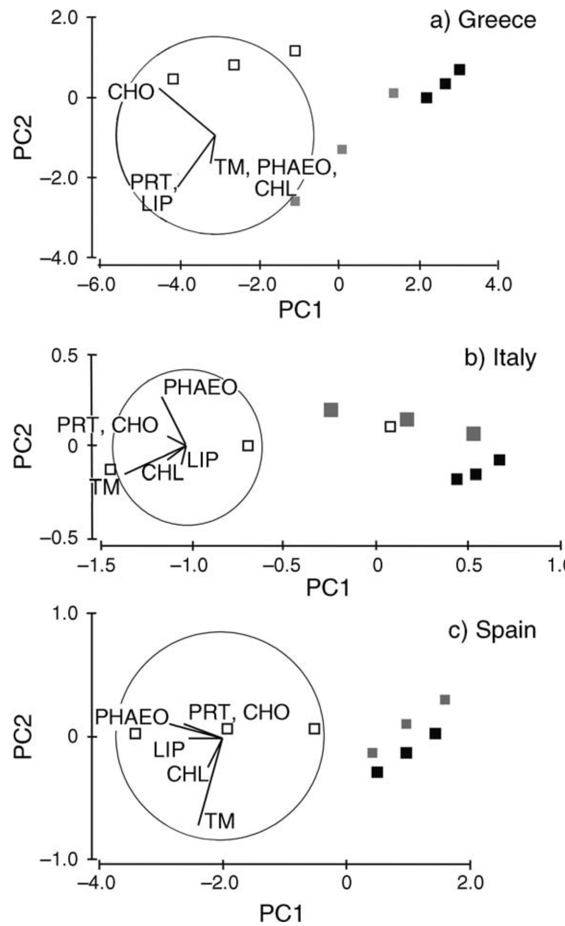

FIG. 2. Two-dimensional PCA ordination of the downward flux of fish-farm waste protein (PRT), carbohydrate (CHO), lipid (LIP), chlorophyll *a* (CHL), phaeopigment (PHAEO), and total matter (TM) in (a) Greece, (b) Italy, and (c) Spain. Downstream distances from the cages: open squares, 0 m; gray squares, 40 m; black squares, 200 m.

TABLE 3. Extended.

Chlorophyll a ($\mu g \cdot m^{-2} \cdot d^{-1}$)		Phaeopigment ($\mu g \cdot m^{-2} \cdot d^{-1}$)	
Mean	SE	Mean	SE
69.4	8.4	277.7	117.2
351.1	26.2	1225.8	414.2
43.9	6.4	254.0	74.3
21.78	18.66	132.57	43.01
9.12	4.48	91.77	39.05
2.78	0.51	17.91	6.96
31.9	1.1	156.9	10.1
52.5	41.8	233.0	140.2
11.3	1.3	89.3	22.8
80.7	25.8	712.0	143.9
61.7	13.3	257.0	40.0
70.7	9.4	292.6	61.2

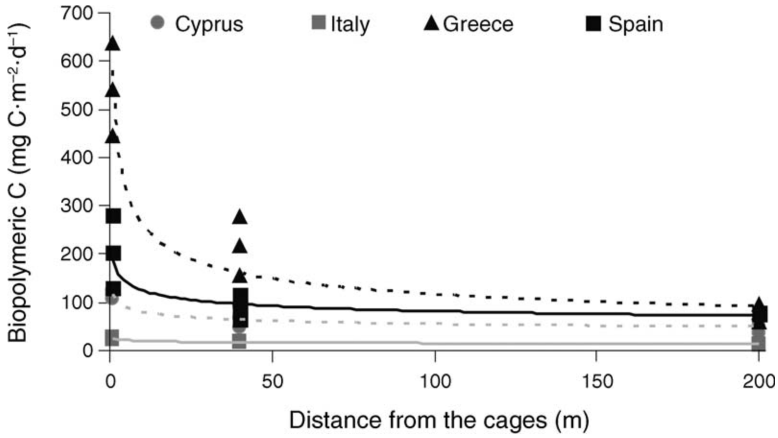

FIG. 3. Downward fluxes of biopolymeric C at increasing downstream distances from the fish-farm cages in the four regions.

studies have used specific compounds, such as fatty acids, digestible protein, sterols, elemental sulphur, stable C/N isotopes (Li-Xun et al. 1991, Johnsen et al. 1993, Findlay et al. 1995, McGhie et al. 2000), and trace elements, such as zinc, to trace fish-feed pellet dispersion patterns (Yeats 2002). Most of these studies have been carried out on small spatial scales (around a particular cage or fish-farm site; i.e., Milewski 2001) and have generally reported that the effective region of impact of these industries on the benthos is <200 m (Doglioli et al. 2004), only exceptionally extending up to 1.2 km from a fish-farm site (Holmer 1991).

The spatial extent of fish-farm impact in the four regions investigated was estimated in terms of the distance at which the fluxes were half those beneath the cages. We estimated that such a distance, on average 100 ± 64 m (mean ± SE; ranging from 5 m in Greece to 209 m in Italy), increases with an increase in the bottom-current velocity (Fig. 5b), but also that the C input to the sea bottom underneath the fish farms was progressively diluted with increasing bottom currents (Fig. 5c). This result indicates that the fish-farm-induced benthic eutrophication will be diluted in those sites characterized by higher bottom currents, but, in turn, that the spatial extent of the potential impact will be spread farther from the cages than in those sites with lower bottom currents.

TABLE 4. Results of PERMANOVA testing for differences in the whole set of sedimentary variables.

Source of variation	df	MS	F	P
Region, R	3	36.56		
Habitat, H	1	5.07		
Impact, I	1	1.84		
$R \times H$	3	8.82		
$R \times I$	3	8.94		
$H \times I$	1	5.67		
$R \times H \times I$	3	8.66	8.27	<0.001
Residual	32	1.04		
Total	47	235		

Note: The analysis was done on the distance matrix calculated using Euclidean distances on previously normalized data. Each term was tested using 999 random permutations of the appropriate units.

The biomass reared in the fish farms had no apparent effects on the downward fluxes. As an example, in Italy, where the biomass production and the food inputs were highest among the fish farms investigated (1150 and 2749 kg/yr, respectively), we saw the lowest sediment C content and downward fluxes. Moreover, other factors, such as the age of the fish farms, had no effects on the sediment organic load below the cages. In Spain and Greece, where the fish farms had been operating since 1996, we recorded organic-C concentrations in fish-farm sediments higher than those in Cyprus and Italy, where the fish farms have been operating since 1988 and 1992, respectively. A more precise assessment of the spatial extent of the impact of fish-farm effluents on the benthos should take into account other factors, such as the spatial and temporal variability of water currents, the relative importance of uneaten food particles and fish faeces in the settling material, and the possible resuspension of sedimented material (Cromey et al. 2002). Nevertheless, the results of this investigation complement those obtained with other experimental approaches (Cromey et al. 2002, Holmer et al. 2006, Marbà et al. 2006) and suggest that relevant changes for benthic assemblages generally occur within 200 m of the cages. This conclusion should result in the application of a precautionary approach, to allow initial siting of fish farms at a good distance from benthic systems such as seagrass beds. According to the results obtained in our present study and adopting the downward fluxes as a proxy of the potential spatial extent of fish-farm impact, such a precautionary distance should be fixed at least at the distance at which the amount of material to the benthos is indistinguishable from the non-affected conditions. In Italy, where the downward fluxes did not change significantly with increasing distance from the cages, this distance is close to 1000 m, so that we would propose 2 km as an acceptable distance between fish farms and any benthic system traditionally consid-

TABLE 5. Biochemical composition of sediment organic matter in the two habitats (seagrass and nonvegetated) of the four regions in (A) control and (B) fish-farm sediments.

Region	Chlorophyll *a* (μg/g)		Phaeopigments (μg/g)		Proteins (mg/g)		Carbohydrates (mg/g)		Lipids (mg/g)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
A) Control locations										
Nonvegetated										
Cyprus	4.00	0.75	20.82	2.25	5.26	1.42	5.43	0.78	1.08	0.30
Greece	0.82	0.97	6.55	1.37	0.22	0.03	0.51	0.12	0.33	0.07
Italy	2.50	0.54	4.90	1.49	0.39	0.06	1.86	0.24	0.11	0.03
Spain	6.48	3.13	38.04	5.31	1.77	0.58	12.74	2.96	0.33	0.07
Seagrass										
Cyprus	2.03	0.86	7.66	1.12	1.51	0.36	2.55	0.63	0.36	0.09
Greece	0.52	0.17	6.21	1.88	1.58	0.58	2.50	0.65	0.44	0.15
Italy	0.84	0.18	3.88	0.33	0.59	0.09	6.56	2.46	0.13	0.01
Spain	12.61	2.90	27.47	2.69	1.99	0.44	7.15	1.46	0.70	0.19
B) Fish-farm locations										
Nonvegetated										
Cyprus	2.75	1.49	16.18	8.82	2.48	0.30	1.91	0.49	0.68	0.22
Greece	0.42	0.17	10.31	1.70	0.95	0.19	4.93	0.10	0.66	0.09
Italy	1.33	0.53	8.62	1.83	1.97	0.29	3.39	0.82	0.38	0.05
Spain	7.67	5.15	12.37	4.49	2.27	0.31	6.07	1.08	0.41	0.07
Seagrass										
Cyprus	2.47	1.21	10.50	3.39	2.92	0.92	5.28	2.46	0.67	0.05
Greece	0.12	0.01	4.81	1.25	0.84	0.08	11.19	1.28	0.40	0.05
Italy	1.61	0.34	10.82	2.53	1.40	0.39	5.22	0.55	0.33	0.13
Spain	18.45	11.28	27.58	6.30	2.10	0.37	7.03	1.34	0.36	0.07

ered as vulnerable. The choice of such a conservative estimate is corroborated by studies conducted synoptically with the present investigation. These studies reported a dramatic reduction in seagrass rhizome vertical growth underneath the fish farms (Marbà et al. 2006) and demonstrated that the seagrass mortality rates, exponentially decreasing with increasing distance from the cages, remained unaltered compared with control locations at an average of 800 m distance from the cages (E. Diaz-Almela et al., *unpublished manuscript*).

Biochemical signatures of fish-farming impact

Recent studies have indicated that the concentration of biopolymeric C in marine sediments is a good proxy for the benthic trophic state, with values typically increasing from oligo- to meso- and hypertrophic conditions (Fabiano et al. 1995, Tselepides et al. 2000, Dell'Anno et al. 2002, Pusceddu et al. 2003).

The analysis of the biopolymeric-C sediment content in control locations more distant from the fish-farm impact revealed decreasing concentrations from Spain to Cyprus (Fig. 6), consistent with the overall trophic gradient that longitudinally characterizes the Mediterranean basin (Danovaro et al. 1999*b*). It is in fact well known that trophic conditions in the Mediterranean Sea range from the ultra-oligotrophy of the South Aegean and Levantine Seas in the Eastern sector to the meso-eutrophic conditions of the Western Basin (with primary production levels in summer ranging from 160–200 to 350–1000 mg $C \cdot m^{-2} \cdot d^{-1}$, respectively; Moutin and Raimbault 1996). This gradient was reflected in the choice of the four regions for our investigation, where the primary productivity (as annual means) went from 215 to 128 to 114 to 106 g $C \cdot m^{-2} \cdot yr^{-1}$ for Spain, Italy, Greece, and Cyprus, respectively (Bosc et al. 2004).

The PERMANOVA analysis indicated a significant region-by-habitat-by-impact ($R \times H \times I$) interaction, which suggests that the effects of fish farming vary across habitats and regions. The quantification of the impact in different habitats separately in each region demonstrated that in both nonvegetated and seagrass habitats the quantity and biochemical composition of sediment organic matter in fish-farm locations did not follow consistent patterns. This indicates that the response of the benthic trophic state to intensive aquaculture is idiosyncratic, and that actual predictions on the potential impact of this industry on the organic loads in the sediments are difficult to make. However, it appears that a significant increase in the organic load can be detected only in those control regions characterized by biopolymeric-C contents typically <2.0 mg C/g, such as in Greece (both habitats), in nonvegetated sediments in Italy, and in seagrass sediments in Cyprus (Fig. 7). Conversely, when biopolymeric-C concentrations exceed 2 mg C/g (as in the case of nonvegetated sediments in Cyprus, and both habitats in Spain), no clear differences in organic-C loads were revealed between control and fish-farm locations.

Sediments characterized by different levels of organic enrichment can have clear differences in the biochemical composition of their sediment organic matter (Dell'An-

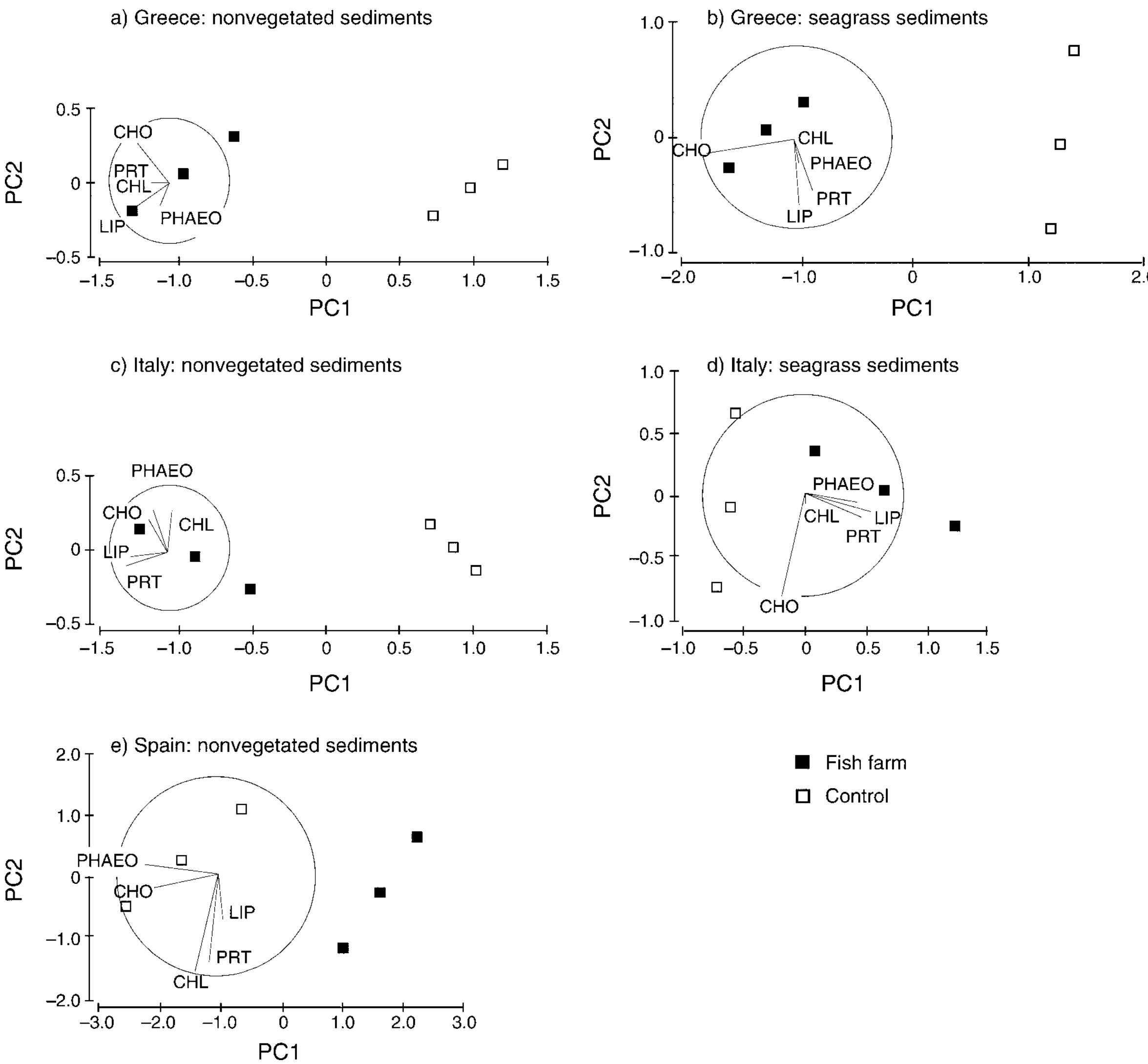

FIG. 4. Two-dimensional PCA ordination of the sedimentary levels of protein (PRT), carbohydrate (CHO), lipid (LIP), chlorophyll *a* (CHL), and phaeopigment (PHAEO), in (a and b) Greece, (c and d) Italy, and (e) Spain, for nonvegetated and seagrass sediments, as indicated.

no et al. 2002). Generally, it appears that systems poor in biopolymeric C are characterized by a larger carbohydrate fraction, whereas systems with higher biopolymeric C concentrations are characterized by the dominance of proteins (Tselepides et al. 2000, Pusceddu et al. 2003). This can be explained by the nutrient limitation of oligotrophic systems, where organic N- and P-rich compounds are rapidly degraded and recycled (Danovaro et al. 1999*a*). In contrast, systems receiving huge inputs of biopolymeric C, such as sediments affected by fish-farm wastes, tend to accumulate N-rich compounds. This trend is generally seen in terms of increasing values of the protein-to-carbohydrate ratio in organically enriched sediments (such as harbor sediments, coastal lagoons, or eutrophic coastal regions; Danovaro et al. 1999*b*, Manini et al. 2004, Pusceddu et al. 2007).

Consistent with this, in the present study the waste released from fish farms altered the biochemical composition of sediment organic matter, but, again, the effects were different across regions and habitats. For instance, sediments of fish farms in regions characterized by background biopolymeric-C concentrations >2.5 mg C/g (e.g., seagrass sediments in Italy, and non-vegetated sediments in Spain and Cyprus) had significantly higher protein-to-carbohydrate ratios than did the sediments of their respective control locations (Fig. 8). The increase in the relative importance of proteinaceous material in fish-farm sediments is likely to be related to the composition of the food pellets provided to the fish being reared. Indeed, at all four

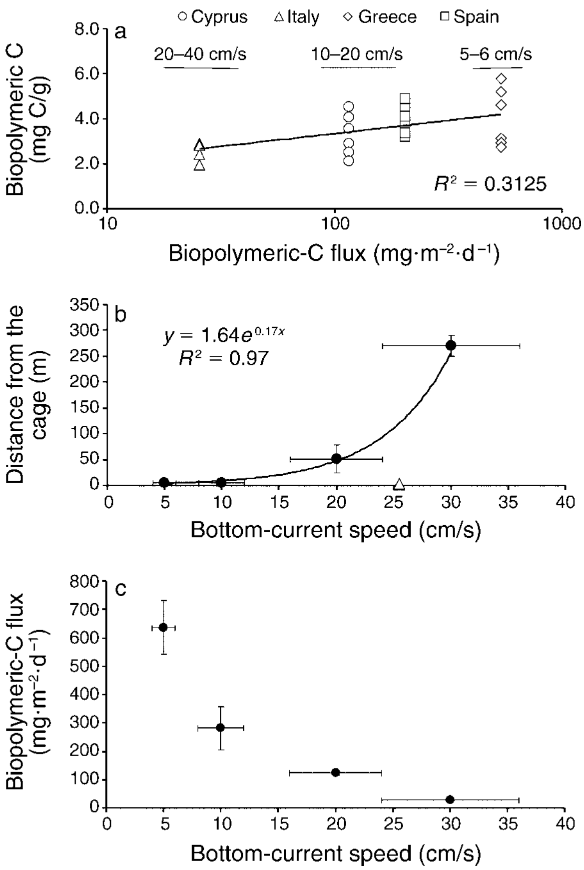

FIG. 5. Relationships among current speed, fish-farm location, and organic effluents and deposition: (a) organic enrichment of fish-farm sediments, downward fluxes of organic mariculture effluents (beneath the cages), and bottom-current speed; (b) the spatial extent of mariculture impact (as the distance at which downward fluxes are half those underneath the cages) and the bottom-current speed; (c) downward fluxes underneath the cages and the bottom-current speed. In panel (b) the spatial extent of mariculture impact was estimated from the best-fit power curves. In (b) and (c) the data are means ± SE.

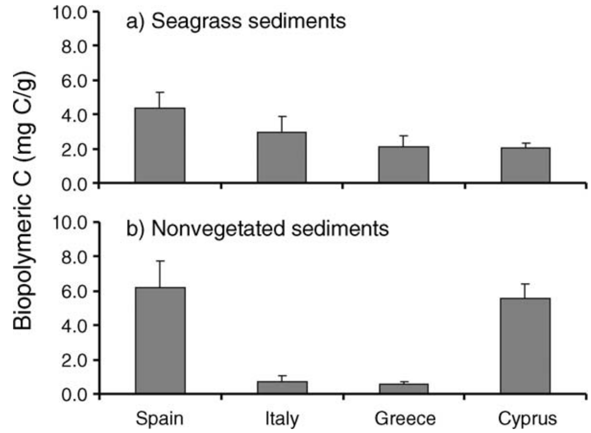

FIG. 6. Biopolymeric-C (BPC) sediment levels in (a) seagrass control sediments and (b) nonvegetated control sediments from the four geographical regions. Data are means + SE. Carbohydrate, protein, and lipid levels were converted into C equivalents and reported together as BPC.

fish farms, although provided by different manufacturers, the food pellets were typically composed of 48–52% protein that, when not consumed by the fish, accumulates in the surface sediments beneath the cages (Mazzola et al. 1999). On the other hand, with fish-farm sediments in both habitats in Greece and in seagrass sediments in Cyprus, which were characterized by lower biopolymeric-C concentrations in control locations, the protein-to-carbohydrate ratios did not differ between control and fish-farm locations. This indicates that the response of the sediment biochemistry to the fish-farm impact also appears to be idiosyncratic, and that no predictions as to these effects can be made here on the basis of the separate determination of organic loads and their biochemical composition.

This study highlights the fact that changes in quantity and biochemical composition of sediment organic matter caused by intensive aquaculture are critical for assessing the presence and levels of impact induced by

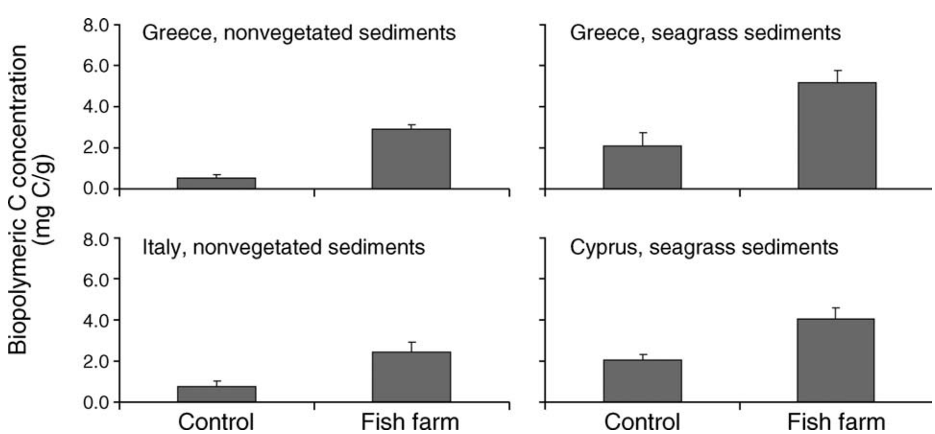

FIG. 7. Biopolymeric-C levels (+SE) in control and fish-farm locations (for systems characterized by control biopolymeric-C levels >2.5 mg C/g).

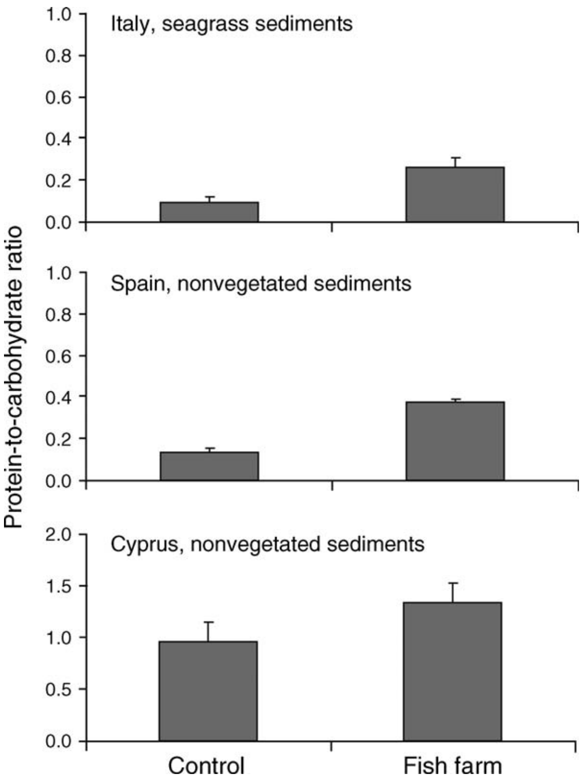

FIG. 8. Protein-to-carbohydrate ratios (+SE) in control and fish-farm locations (for systems characterized by control biopolymeric-C levels >2.5 mg C/g).

fish-farm activities. Although limited by a lack of temporal replication, our results suggest that the impact of aquaculture is site specific. Indeed, the distance-based multivariate multiple regression analysis indicates that environmental variables can explain a significant proportion of the differences seen between control and fish-farm locations, thus confirming the crucial role of local conditions on the possible response to the presence of a fish farm. Therefore, in the perspective of a more environmentally sustainable allocation of marine landscapes to new fish-farming industries, we would stress the need to extend the a priori environmental impact assessment procedures also to the sedimentary organic load, its biochemical composition, and the main physico-chemical characteristics of the region of interest.

The results of the present study confirm that intensive aquaculture can significantly contribute to benthic eutrophication processes, although the extent of the spatial effects of fish-farm effluent is potentially limited. However, even though spatially limited, the impact of the biodeposition derived from fish farms is site specific and can be driven by both physico-chemical and trophic contexts. We have also been able to empirically derive the minimum distance at which the siting of new fish farms should be permitted in the presence of benthic systems traditionally considered vulnerable. To date, since background ecological features on a local scale appear to have the major role in affecting the patterns of fish-farm-induced eutrophication, the future siting of fish farms should include well-designed a priori monitoring programs that are able to describe the whole ecological setting and should be tailored to the basis of the local ecological context.

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