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Fish-farm impact on metazoan meiofauna in the Mediterranean Sea: Analysis of regional vs. habitat effects

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ABSTRACT

The worldwide exponential growth of off-shore mariculture is raising severe concerns about the impacts of this industry on marine habitats and their biodiversity. We investigated the metazoan meiofaunal response to fish-farm impact in four regions of the Mediterranean Sea. Meiofaunal assemblages were investigated in two habitats (seagrass meadows of *Posidonia oceanica* and non-vegetated soft bottoms) comparing sites receiving faeces and uneaten food pellets from fish farms to control sites. We report here that, consistently across different regions, the meiofaunal abundance typically responded positively to fish-farm effluents. Biodeposition caused also significant changes in assemblage structure and the reduction in the richness of higher meiofaunal taxa, but the multivariate analysis of variance revealed that the effects were region- and habitat-specific. In non-vegetated systems, three of the four regions investigated displayed significant effects of the fish farms on richness of meiofaunal taxa. In vegetated habitats, meiofauna did not respond to biodeposition (except in one region), suggesting that seagrass meadows can mask the effects of fish-farm effluents on benthic biodiversity. We conclude that different indicators of fish-farm impact are needed in vegetated and non-vegetated benthic systems.

1. Introduction

Eutrophication is recognized as one of the most important emerging problems of the coastal oceans and, during the past four decades, has increased exponentially in intensity, geographical area and environmental consequences (Cloern, 2001). Eutrophication is typically related to the increase of nutrient and organic matter loads, which cause a progressive decrease of dissolved oxygen concentrations (Dell'Anno et al., 2008). The accumulation of large amounts of organic matter in coastal sediments, associated with eutrophication processes, also induce significant changes in the composition of sediment organic matter, which can have putative effects on the benthos (see Pusceddu et al., 2009 for a review).

Aquaculture is a fast-growing industry which, through the release of organic and inorganic N and P contributes to the progressive eutrophication of coastal areas (see Holmer et al., 2008 and citations therein). Aquaculture activities are now relevant at local and regional scales, and recent estimates indicate that, in Mediterranean coastal areas, the release of nutrients from fish farming contributes for up to 7% and 10% of N and P total discharge, respectively (Pitta et al., 1999).

Aquaculture installations can produce relevant shifts of the whole natural environment (Boyra et al., 2004; Machias et al., 2004), threatening the environmental quality of coastal zones (Gowen and Bradbury, 1987) and generating conflicts between aquaculture and the conservation of marine habitats, including the protection of large benthic primary producers, such as the seagrass *Posidonia oceanica*.

The organic enrichment of the sediments immediately beneath the sea cages is a direct result of the sedimentation of particulate waste products from the fish-farm (Hargrave et al., 1997; Karakassis et al., 1998; Holmer et al., 2008) and a decreasing concentration with the increasing distance from the point source is typically observed (Hargrave et al., 1993; Mazzola et al., 1999; Pusceddu et al., 2007; Holmer et al., 2007). The continuous flow of faeces and food pellets from fish cages alters the quantity and the biochemical composition of sediment organic matter, but with potentially different effects in different regions and/or habitats.

In fact, previous studies, carried out in non-vegetated and seagrass habitats of four Mediterranean regions (Cyrus, Greece, Italy and Spain), have shown that the response of the benthic biochemistry to aquaculture biodeposition is idiosyncratic, and

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significant increase in the organic load can be detected only in those regions characterized by biopolymeric C contents typically, 2 mg C g^{-1} (Pusceddu et al., 2007).

To identify the changes induced by aquaculture activities on benthic ecosystems, we investigated meiofaunal assemblages in different regions of the Mediterranean Sea along a longitudinal gradient of trophic conditions (in terms of primary productivity, Pusceddu et al., 2007). Metazoan meiofauna, for their ecological importance in the benthic ecosystems and the lack of larval dispersion, are considered a sensitive tool for investigating structural and functional changes of natural and anthropogenically-impacted ecosystems (see Vincx and Heip, 1987 for a review).

Here we tested, using a hierarchical sampling strategy under uni- and multivariate contexts, the hypothesis that the presence of fish farms influences significantly the meiofaunal assemblages in terms of abundance, community structure and diversity (i.e. richness and evenness of taxa) and that different patterns can be expected in the four regions according to their different trophic backgrounds.

2. Materials and methods

2.1. Study site and sampling strategy

To cover different environmental conditions characterizing Mediterranean coastal zones along an east-to-west longitudinal transect (ca. 3500 km wide), four regions were selected: 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 Gulf of Alicante (Spain; 38° 24′ N, 0° 24′ W; September, 2003) (Fig. 1). All of these regions, located at similar latitudes and depths were selected on the basis of the presence of the fish farms, which have been previously characterized in terms of their main environmental features (Table 1). In each of the sampling regions, the effects of the fish-farm on the meiofauna were investigated in two different habitats: meadows of the seagrass *P. oceanica*, and soft non-vegetated bottoms. In each region, a preliminary survey was carried out to ascertain the presence of both the seagrass and the soft substrates, and to character-

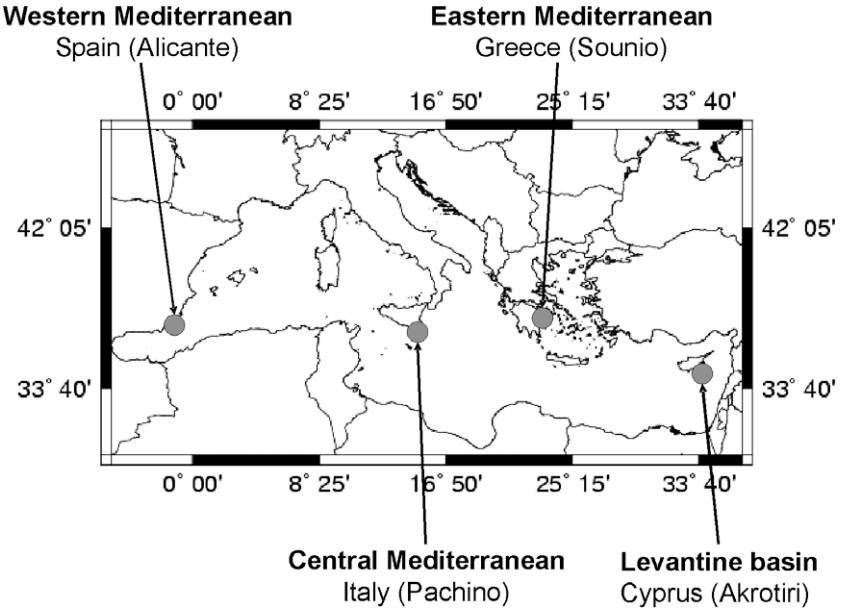

Fig. 1. Sites of the four fish farms investigated in Cyprus, Italy, Greece and Spain in the Mediterranean Sea.

Table 1
Environmental characteristics reported in all sampling sites (distance of the sampling sites from the fish-farm, water depth, current speed, sediment type, porosity, water content and grain size.)

Region	Habitat	Site	Distance from fish-farm (m)	Water depth (m)	Current speed (cm s^{-1})	Sediment type silt/clay (%)	Sediment porosity (%)	Sediment water content (%)	Grain size (mean diameter, mm)
Cyprus	Non-vegetated	Impact	0–20	30	20–40	80.7	1.2	42.9	0.01
		Control	4000 Westward	30	20–40	75.9	1.2	44.9	0.02
	Vegetated	Impact	300 Northward	20	20–40	68.4	1.2	46.4	0.02
		Control	1000 Eastward	20	20–40	2.5	1.1	36.5	0.10
Italy	Non-vegetated	Impact	0	22	20	5.7	1.2	41.6	0.47
		Control	3200 North-east	22	20	3.9	0.8	13.5	0.84
	Vegetated	Impact	5	22	20	4.6	1.2	46.9	0.45
		Control	1000 Northward	22	20	4.8	1.1	33.7	0.90
Greece	Non-vegetated	Impact	0	16	6.3	6.7	1.1	29.5	0.38
		Control	800 Westward	16	6.3	2.2	1.6	18	1.89
	Vegetated	Impact	5	16	6.3	20.2	1.0	27.3	0.33
		Control	1000 Southward	15	6.3	13.1	1.1	34.4	0.76
Spain	Non-vegetated	Impact	0	29	4.7	7.7	1.1	31.7	0.32
		Control	1000 Westward	29	4.7	15.6	1.2	42.3	0.43
	Vegetated	Impact	10	29	4.7	16.2	1.1	32.5	0.40
		Control	1000 Southward	29	4.7	15.3	1.1	32.4	0.9

Table 2
Results of ANOVA testing for differences between impact and control sites in quantity and quality of sediment organic matter in different habitats and regions. *R* = region; *H* = habitat; *I* = impact; SS = sum of squares; DF = degrees of freedom; MS = means square; *F* = *F* value; *P* = probability level; ns = not significant.

	Source	SS	DF	MS	*F*	*P*
Biopolymeric C	*R*	38.74	3	12.91	28.63	***
	H	3.54	1	3.54	0.73	ns
	I	4.40	1	4.39	0.53	ns
	$R \times H$	14.53	3	4.84	10.74	***
	$R \times I$	25.01	3	8.34	18.48	***
	$H \times I$	3.99	1	3.99	1.00	ns
	$R \times H \times I$	11.99	3	3.99	8.86	***
	Residuals	14.43	32	0.45		
	Total	116.64	47			
Protein to carbohydrate ratio	*R*	12.83	3	4.28	285.87	***
	H	1.723	1	1.72	2.65	ns
	I	0.05	1	0.05	0.01	ns
	$R \times H$	1.95	3	0.65	43.42	***
	$R \times I$	10.66	3	3.55	237.45	***
	$H \times I$	1.23	1	1.23	5.12	ns
	$R \times H \times I$	0.72	3	0.24	16.04	***
	Residuals	0.48	32	0.02		
	Total	29.64	47			

*** $P < 0.001$.

ize 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 contrasting the fish-farm sites with control sites characterized by relatively pristine conditions and by environmental features comparable to those found beneath the cages (Table 1). Controls were located upstream of the main currents, and at least 1000 m from the fish farms. It has been demonstrated that this distance avoids any potential effect of fish farms (Holmer et al., 2008) and allows maintaining the spatial variability in background (control) conditions as low as possible.

At each site, three replicates were collected randomly from the central area of each fish-farm site (i.e. beneath the cages) and in each control site, by means of manual corers (diameter 3.7 cm, 10.7 cm^2 surface area, down to a depth of 10 cm) operated by SCUBA divers.

2.2. Sediment biochemistry

Sediment protein, carbohydrate and lipid contents were determined spectrophotometrically and concentrations were converted into carbon equivalents using 0.40 and 0.49 and 0.75 g C g^{-1} conversion factors, respectively, and their sum reported as biopolymeric C (Pusceddu et al., 2009). Data relative to the quantity and biochemical composition (i.e. protein, carbohydrate and lipid contents) of organic matter in the sediments under scrutiny have been detailed elsewhere (Pusceddu et al., 2007). In this study, we focused our attention on the biopolymeric C concentrations and on the values of the protein to carbohydrate ratio as descriptors of the quantity and quality of sediment organic matter, respectively (Pusceddu et al., 2009).

2.3. Meiofaunal analyses

Each sediment sample was fixed with 4% buffered formaldehyde (in filtered seawater solution) and was sieved through 1000 μm sieve (to retain macrobenthos and macroalgae) and 32 μm sieve (to retain smaller meiofauna) in laboratory. The sample fraction retained by a 32 μm mesh net was added to Ludox

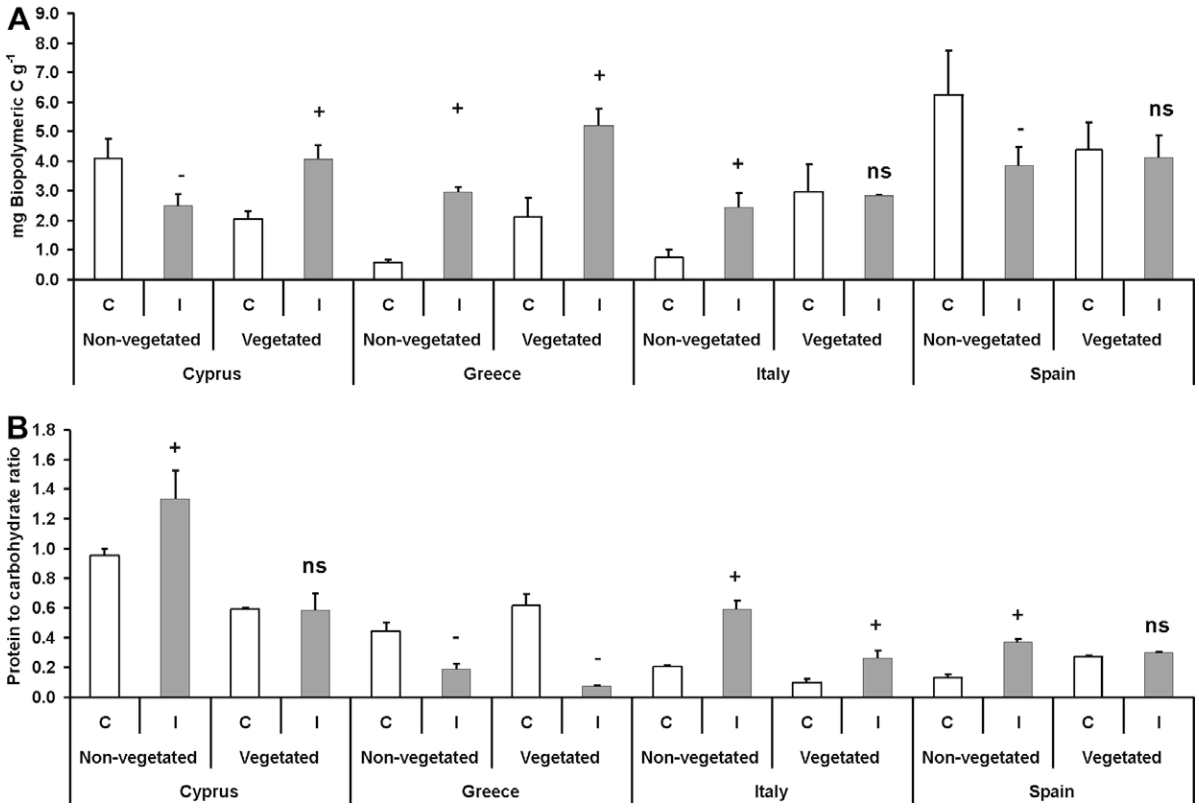

Fig. 2. Biopolymeric C concentrations (A) and values of the protein to carbohydrate ratio (B) in the investigated sediments. According to the SNK tests (at $P < 0.05$), + = increase; − = decrease; ns = not significant. Data are extracted from Pusceddu et al. (2007).

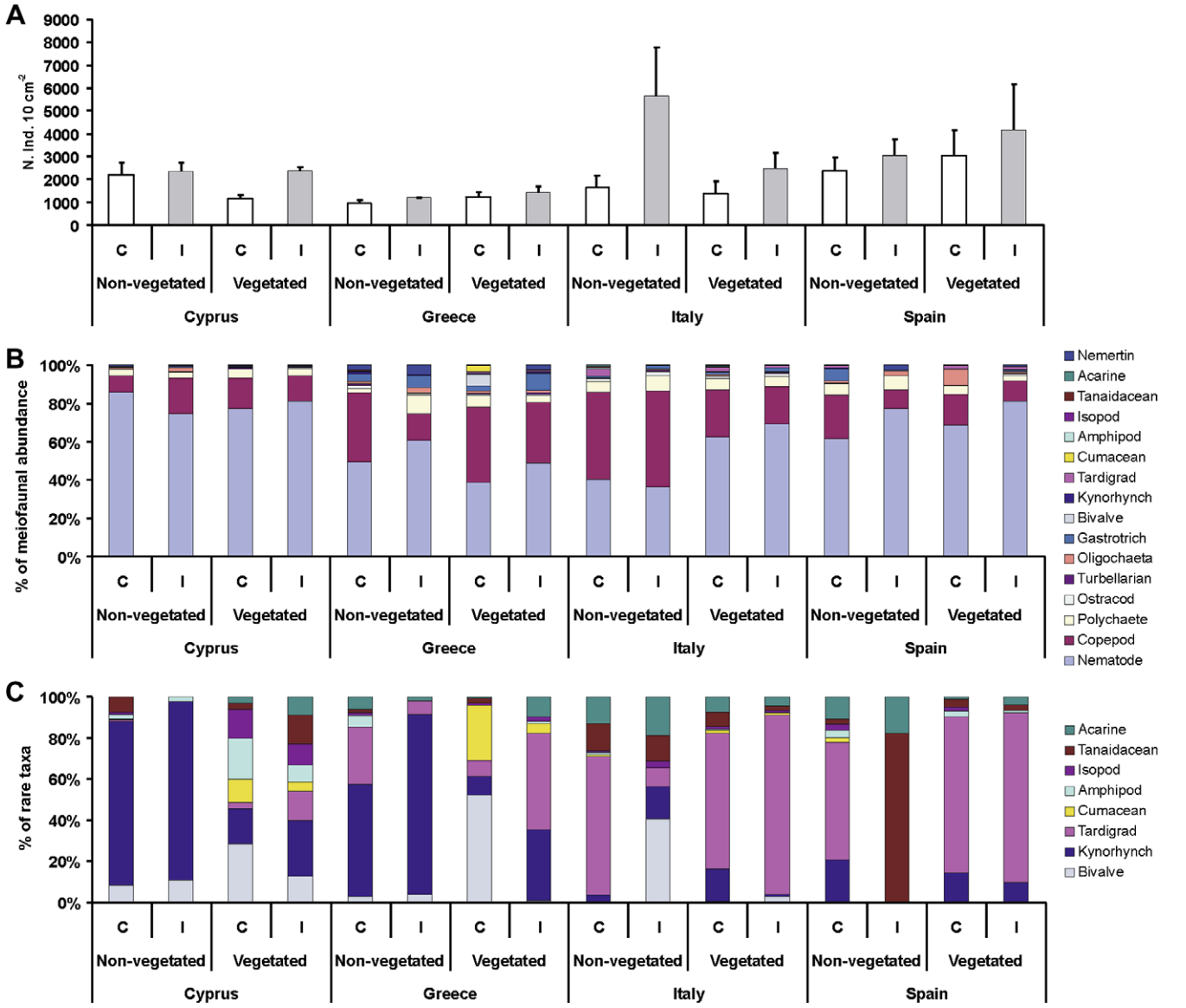

Fig. 3. Total meiofaunal abundance (A), meiofaunal community composition (B) and the relative abundance of rare taxa (C) in Cyprus, Greece, Italy and Spain in non-vegetated and vegetated habitats, in control (*C*) and impact (*I*) sediments.

HS 40 (density arranged to 1.18 g cm^{-3}), for density centrifugation extraction (10 min, 800*g*, for three times) from the sediment (Heip et al., 1985). All metazoan animals were counted and classified per taxon under a stereomicroscope using Delfuss cuvettes, after staining with Rose Bengal (0.5 g L^{-1}). Community evenness was calculated as the Pielou's index using the different meiofaunal taxa as entries, by means of the PRIMER software (Clarke and Gorley, 2003).

2.4. Statistical analyses

Differences between control and impact sediments were assessed for all of the investigated variables using a three-way analysis of variance (ANOVA). The analysis treated the factor region (*R*, 4 levels) as random, habitat (*H*, 2 levels) as fixed and crossed with *R*, and impact (*I*, 2 levels) as fixed and orthogonal to *R* and *H*. When significant differences ($P < 0.05$) were observed, a post-hoc Student–Newman–Kuels' test (SNK) was also performed. ANOVA and SNK tests were carried out using the GMAV software (University of Sidney).

Distance-based permutational multivariate analyses of variance (PERMANOVA, Anderson, 2001) were used to quantify in the two benthic habitats the effects of the fish farms on (i) the organic matter quantity and quality and (ii) the meiofaunal assemblages. The

Table 3
Results of ANOVA testing for differences between impact and control sites in total meiofaunal abundance in different habitats and regions. *R* = region; *H* = habitat; *I* = impact; SS = sum of squares; DF = degrees of freedom; MS = means square; *F* = *F* value; *P* = probability level: $^{*} = P < 0.05$; $^{**} = P < 0.01$; $^{***} = P < 0.001$; ns = not significant.

Source	DF	SS	MS	*F*	*P*
R	3	272,55,106	90,85,035	11.7511	**
H	1	909,986	909,986	0.2431	ns
I	1	139,17,504	139,17,504	4.4563	ns
R × *H*	3	112,28,668	37,42,889	4.8413	**
R × *I*	3	93,69,245	31,23,082	4.0396	*
H × *I*	1	347142.6	347142.6	0.1521	ns
R × *H* × *I*	3	68,48,726	22,82,909	2.9528	*
Residuals	32	247,39,857	773120.5		
Total	47	946,16,234			

Table 4
Meiofaunal taxa abundance (ind 10 cm^{-2}) in fish-farm impacted and control sites across the four regions and the two habitats. Reported are average value ± standard deviation.

Site	Location	Site	Nematoda	Copepoda	Polychaeta	Ostracoda	Turbellaria	Oligochaeta	Gastrotricha	Bivalvia
Cyprus	Non-vegetated	Control	1903 ± 499	192 ± 43	64 ± 14	3 ± 0	9 ± 8	9 ± 6	2 ± 0	1 ± 0
		Impact	1751 ± 365	438 ± 56	69 ± 5	0 ± 0	4 ± 2	53 ± 20	20 ± 17	1 ± 0
	Vegetated	Control	898 ± 157	186 ± 26	52 ± 0	3 ± 2	1 ± 1	8 ± 6	2 ± 2	2 ± 0
		Impact	1919 ± 145	312 ± 65	90 ± 2	5 ± 2	2 ± 2	7 ± 7	9 ± 2	1 ± 0
Greece	Non-vegetated	Control	813 ± 248	595 ± 253	37 ± 13	27 ± 17	16 ± 3	20 ± 6	61 ± 4	1 ± 1
		Impact	3406 ± 1798	757 ± 147	549 ± 217	45 ± 47	23 ± 6	155 ± 73	344 ± 26	1 ± 1
	Vegetated	Control	583 ± 262	582 ± 258	87 ± 53	15 ± 5	7 ± 2	13 ± 12	43 ± 8	86 ± 40
		Impact	1218 ± 201	784 ± 809	87 ± 103	19 ± 27	18 ± 6	35 ± 16	224 ± 80	0 ± 0
Italy	Non-vegetated	Control	367 ± 69	409 ± 48	52 ± 18	14 ± 3	1 ± 0	2 ± 0	5 ± 1	0 ± 0
		Impact	291 ± 7	394 ± 88	65 ± 13	16 ± 0	5 ± 0	6 ± 4	13 ± 4	1 ± 1
	Vegetated	Control	723 ± 95	284 ± 145	69 ± 42	16 ± 10	3 ± 1	5 ± 2	12 ± 8	0 ± 0
		Impact	938 ± 239	255 ± 39	77 ± 36	21 ± 9	2 ± 0	6 ± 1	28 ± 11	0 ± 0
Spain	Non-vegetated	Control	1467 ± 521	539 ± 63	144 ± 32	6 ± 6	3 ± 4	24 ± 3	150 ± 131	0 ± 0
		Impact	2342 ± 737	305 ± 107	222 ± 108	1 ± 2	1 ± 1	72 ± 52	10 ± 12	0 ± 0
	Vegetated	Control	2084 ± 639	488 ± 186	129 ± 65	7 ± 2	2 ± 3	252 ± 102	11 ± 10	0 ± 0
		Impact	3381 ± 406	432 ± 420	125 ± 60	37 ± 12	4 ± 5	33 ± 27	41 ± 47	0 ± 0

Site	Location	Site	Kynorhyncha	Tardigrada	Cumacea	Amphipoda	Isopoda	Tanaidacea	Acarina	Nemertina
Cyprus	Non-vegetated	Control	11 ± 3	0 ± 0	0 ± 0	0 ± 1	0 ± 0	1 ± 0	0 ± 0	20 ± 7
		Impact	9 ± 9	0 ± 0	0 ± 0	0 ± 1	0 ± 0	0 ± 0	0 ± 0	5 ± 3
	Vegetated	Control	1 ± 0	0 ± 0	1 ± 0	1 ± 1	1 ± 0	0 ± 0	0 ± 0	4 ± 4
		Impact	3 ± 0	2 ± 2	0 ± 0	1 ± 1	1 ± 0	2 ± 0	1 ± 1	7 ± 7
Greece	Non-vegetated	Control	19 ± 10	9 ± 9	0 ± 0	2 ± 1	0 ± 0	1 ± 1	2 ± 2	46 ± 34
		Impact	22 ± 7	2 ± 2	0 ± 0	0 ± 1	0 ± 0	0 ± 0	0 ± 0	292 ± 160
	Vegetated	Control	15 ± 5	12 ± 9	44 ± 17	0 ± 1	2 ± 0	5 ± 3	0 ± 0	0 ± 0
		Impact	14 ± 5	20 ± 15	2 ± 1	0 ± 1	1 ± 0	0 ± 0	4 ± 3	62 ± 46
Italy	Non-vegetated	Control	2 ± 2	36 ± 4	0 ± 0	1 ± 1	1 ± 0	7 ± 3	7 ± 0	1 ± 1
		Impact	0 ± 0	0 ± 0	0 ± 0	0 ± 1	0 ± 0	0 ± 0	0 ± 0	1 ± 0
	Vegetated	Control	7 ± 1	27 ± 12	1 ± 1	0 ± 1	1 ± 0	3 ± 2	3 ± 2	5 ± 4
		Impact	0 ± 0	14 ± 8	0 ± 0	0 ± 1	0 ± 0	0 ± 0	1 ± 0	5 ± 1
Spain	Non-vegetated	Control	9 ± 7	26 ± 19	1 ± 1	2 ± 1	1 ± 0	1 ± 0	5 ± 3	4 ± 2
		Impact	0 ± 0	0 ± 0	0 ± 0	0 ± 1	0 ± 0	2 ±	0 ± 0	77 ± 56
	Vegetated	Control	8 ± 0	44 ± 26	0 ± 0	2 ± 1	1 ± 0	2 ± 0	1 ± 1	4 ± 2
		Impact	8 ± 0	66 ± 57	0 ± 0	1 ± 1	0 ± 0	2 ± 0	3 ± 3	30 ± 23

Table 5
Results of ANOVA testing for differences between impact and control sites in abundance of the meiofaunal taxa in different habitats and regions. *R* = region; *H* = habitat; *I* = impact; SS = sum of squares; DF = degrees of freedom; MS = means square; *F* = *F* value; *P* = probability level; ns = not significant.

	Source	SS	DF	MS	*F*	*P*		Source	SS	DF	MS	*F*	*P*
Nematodes	*R*	13.15	3	4.38	38.98	***	Turbellarians	*R*	18.88	3	6.29	12.86	***
	H	0.06	1	0.06	0.04	ns		*H*	2.28	1	2.28	3.69	ns
	I	2.66	1	2.66	4.47	ns		*I*	0.41	1	0.41	1.91	ns
	R × *H*	4.34	3	1.45	12.86	***		*R* × *H*	1.86	3	0.62	1.26	ns
	R × *I*	1.78	3	0.59	5.29	**		*R* × *I*	0.65	3	0.22	0.44	ns
	H × *I*	0.10	1	0.10	0.36	ns		*H* × *I*	0.03	1	0.03	0.05	ns
	R × *H* × *I*	0.82	3	0.27	2.42	ns		*R* × *H* × *I*	1.96	3	0.65	1.33	ns
	Residuals	3.60	32	0.11				Residuals	15.66	32	0.49		
	Total	26.50	47					Total	41.73	47			
Copepods	*R*	1116932.28	3	372310.76	6.23	**	Oligochaetes	*R*	59266.22	3	19755.41	1.81	ns
	H	17542.98	1	17542.98	1.01	ns		*H*	56.14	1	56.14	0.00	ns
	I	30403.35	1	30403.35	0.38	ns		*I*	223.39	1	223.39	0.02	ns
	R × *H*	52331.00	3	17443.67	0.29	ns		*R* × *H*	40553.05	3	13517.68	1.24	ns
	R × *I*	237589.60	3	79196.53	1.33	ns		*R* × *I*	41567.71	3	13855.90	1.27	ns
	H × *I*	1332.47	1	1332.47	0.11	sn		*H* × *I*	34558.65	1	34558.65	3.46	ns
	R × *H* × *I*	35031.09	3	11677.03	0.20	sn		*R* × *H* × *I*	29968.09	3	9989.36	0.91	ns
	Residuals	1912625.14	32	59769.54				Residuals	349658.16	32	10926.82		
	Total	3403787.91	47					Total	555851.41	47			
Polychaetes	*R*	170.08	3	56.69	8.01	***	Gastrotrichs	*R*	49.57	3	16.52	19.58	***
	H	39.79	1	39.79	1.39	ns		*H*	0.66	1	0.66	0.54	ns
	I	96.90	1	96.90	2.63	ns		*I*	5.48	1	5.48	1.82	ns
	R × *H*	85.71	3	28.57	4.04	*		*R* × *H*	3.67	3	1.22	1.45	ns
	R × *I*	110.65	3	36.88	5.21	**		*R* × *I*	9.05	3	3.02	3.57	*
	H × *I*	66.82	1	66.82	1.12	ns		*H* × *I*	1.80	1	1.80	0.44	ns
	R × *H* × *I*	179.26	3	59.75	8.44	***		*R* × *H* × *I*	12.30	3	4.10	4.86	**
	Residuals	226.52	32	7.08				Residuals	27.00	32	0.84		
	Total	975.73	47					Total	109.54	47			

(*continued on next page*)

Table 5 (*continued*)

	Source	SS	DF	MS	*F*	*P*		Source	SS	DF	MS	*F*	*P*
Ostracods	*R*	24.69	3	8.23	16.96	***	Nemertins	*R*	33.06	3	11.02	23.50	***
	H	1.53	1	1.53	0.60	ns		*H*	5.29	1	5.29	0.82	ns
	I	0.01	1	0.01	0.04	ns		*I*	14.88	1	14.88	2.00	ns
	$R \times H$	7.58	3	2.53	5.21	**		$R \times H$	19.28	3	6.43	13.71	***
	$R \times I$	0.40	3	0.13	0.28	ns		$R \times I$	22.38	3	7.46	15.91	***
	$H \times I$	1.75	1	1.75	1.39	ns		$H \times I$	1.25	1	1.25	0.90	ns
	$R \times H \times I$	3.77	3	1.26	2.59	ns		$R \times H \times I$	4.16	3	1.39	2.96	ns
	Residuals	15.53	32	0.49				Residuals	15.00	32	0.47		
	Total	55.26	47					Total	115.29	47			
Acarines	*R*	39.94	3	13.31	6.11	***	Isopods	*R*	1.12	3	0.37	1.77	ns
	H	0.76	1	0.76	0.16	ns		*H*	2.99	1	2.99	3.12	ns
	I	11.47	1	11.47	0.68	ns		*I*	3.89	1	3.89	3.91	ns
	$R \times H$	14.28	3	4.76	2.18	ns		$R \times H$	2.87	3	0.96	4.57	**
	$R \times I$	50.90	3	16.97	7.78	**		$R \times I$	2.99	3	1.00	4.75	**
	$H \times I$	50.51	1	50.51	11.65	*		$H \times I$	0.04	1	0.04	0.65	ns
	$R \times H \times I$	13.01	3	4.34	1.99	ns		$R \times H \times I$	0.17	3	0.06	0.26	ns
	Residuals	69.78	32	2.18				Residuals	6.70	32	0.21		
	Total	250.64	47					Total	20.76	47			
Amphipods	*R*	4.06	3	1.35	2.78	ns	Kinorynchs	*R*	1566.82	3	522.27	20.47	***
	H	0.04	1	0.04	0.03	ns		*H*	40.43	1	40.43	0.42	ns
	I	4.25	1	4.25	8.04	ns		*I*	39.30	1	39.30	1.42	ns
	$R \times H$	4.27	3	1.42	2.92	*		$R \times H$	285.49	3	95.16	3.73	*
	$R \times I$	1.59	3	0.53	1.09	ns		$R \times I$	83.07	3	27.69	1.09	ns
	$H \times I$	3.03	1	3.03	3.46	ns		$H \times I$	2.49	1	2.49	0.08	ns
	$R \times H \times I$	2.62	3	0.87	1.79	ns		$R \times H \times I$	97.20	3	32.40	1.27	ns
	Residuals	15.58	32	0.49				Residuals	816.26	32	25.51		
	Total	35.44	47					Total	2931.04	47			
Bivalves	*R*	15.59	3	5.20	79.49	***	Tanaidaceans	*R*	1.94	3	0.65	5.74	**
	H	2.58	1	2.58	1.16	ns		*H*	0.10	1	0.10	0.25	ns
	I	2.37	1	2.37	0.72	ns		*I*	2.28	1	2.28	1.57	ns
	$R \times H$	6.67	3	2.22	34.01	***		$R \times H$	1.27	3	0.42	3.76	*
	$R \times I$	9.89	3	3.30	50.41	***		$R \times I$	4.35	3	1.45	12.85	***
	H × *I*	3.50	1	3.50	1.22	ns		$H \times I$	0.05	1	0.05	0.07	ns
	$R \times H \times I$	8.61	3	2.87	43.89	***		$R \times H \times I$	2.02	3	0.67	5.96	***
	Residuals	2.09	32	0.07				Residuals	3.61	32	0.11		
	Total	51.30	47					Total	15.64	47			
Cumaceans	*R*	9.05	3	3.02	63.63	***	Tardigrades	*R*	41.73	3	13.91	27.05	***
	H	5.28	1	5.28	1.25	ns		*H*	18.12	1	18.12	11.49	*
	I	2.94	1	2.94	2.79	ns		*I*	9.57	1	9.57	2.81	ns
	$R \times H$	12.65	3	4.22	88.95	***		$R \times H$	4.73	3	1.58	3.07	*
	$R \times I$	3.17	3	1.06	22.28	***		$R \times I$	10.23	3	3.41	6.63	**
	$H \times I$	0.91	1	0.91	0.54	ns		$H \times I$	12.22	1	12.22	12.82	*
	$R \times H \times I$	5.05	3	1.68	35.48	***		$R \times H \times I$	2.86	3	0.95	1.85	ns
	Residuals	1.52	32	0.05				Residuals	16.46	32	0.51		
	Total	40.57	47					Total	115.91	47			

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

analysis treated the factor region (*R*, 4 levels) as random, habitat (*H*, 2 levels) as fixed and crossed with *R*, and impact (*I*, 2 levels) as fixed and orthogonal to *R* and *H*. For organic matter, the data set included 48 observations on concentrations of biopolymeric C and the values of the protein to carbohydrate ratio. For meiofauna, the data set included 48 observations and the abundance of all the taxa. Since the interaction term $R \times H \times I$ was seen to be significant either for organic matter and meiofauna, pairwise comparisons, which also used 499 random permutations to obtain *P*-values, were also carried out to ascertain differences between control and impact sediments in all regions, separately. The *P*-values were calculated using 499 Monte Carlo draws from the asymptotic permutation distribution (Anderson and Robinson, 2003). PERMANOVA tests were carried out (either in the uni- and multivariate contexts) also removing the effects of the main environmental variables (i.e. water depth, current speed, sediment type, porosity and water content, biopolymeric C and protein to carbohydrate ratio) as covariates.

In order to assess whether and how much environmental variables explained changes in meiofaunal abundance, richness and evenness of taxa, a non-parametric multivariate multiple regression analysis that was based on Euclidean distances was carried out using the routine DISTLM forward (McArdle and Anderson, 2001). The forward selection of the predictor variables was carried out with tests by permutation. *P*-values were obtained using 4999 permutations of raw data for the marginal tests (tests of individual variables), while for all of the conditional tests, the routine uses 4999 permutations of residuals under a reduced model.

3. Results

Three-way univariate ANOVA revealed that biopolymeric C concentrations and values of the protein to carbohydrate ratio displayed a significant region (R) × habitat (H) × impact (I) effect (Table 2), with differences between impacted and control sites varying between the two habitats and the four geographical areas. In particular, the SNK tests revealed that biopolymeric C concentrations in farm impacted sediments increased consistently in vegetated sediments in Cyprus, in both habitats in Greece and in

Table 6
Results of PERMANOVA testing for differences in the whole set of meiofaunal taxa. The analysis was done on the distance matrix calculated using Bray–Curtis similarities on untransformed data. Each term was tested using 4999 random permutations of the appropriate units (∗ = $P < 0.05$; ns = not significant). DF = degrees of freedom, MS = mean square, F = F value; P (MC) = Monte Carlo probability level. Reported are also the results of the pairwise comparisons testing differences between impact and control sites in the two habitats separately for the four regions (*$P < 0.02$). R = region; H = habitat; I = impact.

	DF	MS	F	P (MC)
PERMANOVA outputs				
R	3	5835.49	19.49	***
H	1	1111.62	0.76	ns
I	1	3614.80	3.56	ns
$R \times H$	3	1455.63	4.86	**
$R \times I$	3	1016.26	3.39	**
$H \times I$	1	418.29	0.51	ns
$R \times H \times I$	3	818.36	2.73	**
Residuals	32	299.37		
Total	47			
Habitat		Region		P
Pairwise comparisons: impact vs. control sites				
Cyprus		Non-vegetated		**
		Vegetated		ns
Greece		Non-vegetated		**
		Vegetated		ns
Italy		Non-vegetated		ns
		Vegetated		ns
Spain		Non-vegetated		ns
		Vegetated		ns

** $P < 0.01$.
*** $P < 0.001$.

Table 7
Results of ANOVA testing for differences between impact and control sites in richness and evenness of meiofaunal taxa in different habitats and regions. R = region; H = habitat; I = impact; SS = sum of squares; DF = degrees of freedom; MS = means square; F = F value; P = probability level; ns = not significant.

	Source	SS	DF	MS	F	P
Number of taxa	R	47.5	3	15.8	126.67	***
	H	24.1	1	24.1	9.32	ns
	I	48.0	1	48.0	32.00	*
	$R \times H$	7.8	3	2.6	20.67	***
	$R \times I$	4.5	3	1.5	12.00	***
	$H \times I$	30.1	1	30.1	15.70	*
	$R \times H \times I$	5.8	3	1.9	15.33	***
	Residuals	4	32	0.1		
	Total	171.7	47			
Community evenness	R	0.327	3	0.109	27.31	***
	H	0.018	1	0.018	8.59	ns
	I	0.003	1	0.003	0.53	ns
	$R \times H$	0.006	3	0.002	0.53	ns
	$R \times I$	0.015	3	0.005	1.28	ns
	$H \times I$	0.027	1	0.028	4.48	ns
	$R \times H \times I$	0.018	3	0.006	1.54	ns
	Residuals	0.128	32	0.004		
	Total	0.543	47			

* $P < 0.05$.
*** $P < 0.001$.

non-vegetated sediments in Italy (Fig. 2A), whereas values of the protein to carbohydrate ratio increased in impact sites in non-vegetated sediments in Cyprus, in both habitats in Italy and in non-vegetated sediments in Spain and decreased in impact sediments of both habitats in Greece (Fig. 2B).

A general increase of meiofaunal abundance was observed in impacted sediments (Fig. 3A) and the ANOVA revealed significant $R \times H \times I$ variations in total meiofaunal abundance (Table 3). Moreover, the SNK tests indicated that total meiofaunal abundance increased significantly in impact sites in vegetated sediments in Cyprus and non-vegetated sediments in Italy, whereas no signifi-

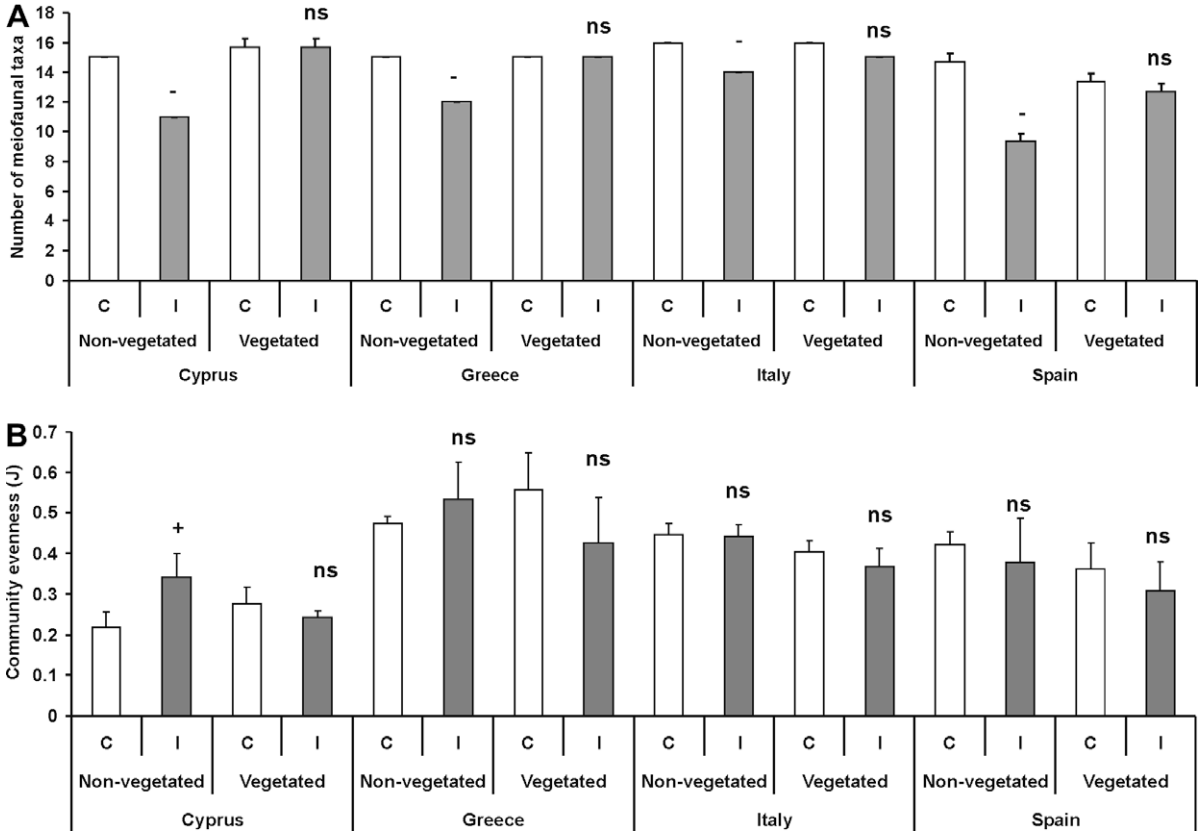

Fig. 4. Taxa richness (A) and community evenness (B) in Cyprus, Greece, Italy and Spain in non-vegetated and vegetated habitats, in control (C) and impact (I) sediments. The statistical significance of the differences among bars (SNK test: + = increase $P < 0.05$, − = decrease $P < 0.05$, ns = not significant) are also reported.

cant differences between impact and control sediments were observed in all other regions and habitats (Fig. 3A).

Abundance of all of the meiofaunal taxa in the sediments are reported in Table 4. ANOVA revealed significant $R \times H \times I$ effects on the abundance of all of the meiofaunal taxa with differences between impact and control sites differing in extent and direction in different regions and habitats and for the different taxa (Table 5). Significant interaction of the $R \times H \times I$ factors, indicating idiosyncratic responses to fish-farm impact in different regions and habitats, was observed for polychaetes, gastrotrichs, bivalves, cumaceans and tanaidaceans. The SNK tests (data not shown) revealed increasing nematode abundance beneath the cages in vegetated sediments in Cyprus and in both habitats in Greece. Copepods increased in both fish-impacted habitats in Cyprus. The abundance of ostracods varied between impacted and control sites only in Spain (in both habitats), whereas oligochaetes, kinorhynchs and amphipods did not display any significant difference between impact and control sediments in any of the investigated region and habitat. Turbellarians' abundance exhibited significant differences only between regions.

The PERMANOVA test applied on the meiofaunal community composition showed a significant $R \times H \times I$ effect, indicating that the differences between control and fish-farm impacted sites varied between the two habitats and the four regions (Table 6, Fig. 3B and C). The analysis carried out separately for all of the four regions and the pairwise comparison tests revealed that differences between impact and control sites in the meiofaunal community composition were significant only in non-vegetated sediments either in Cyprus and Greece.

At all sites nematodes and copepods were the most dominant taxa, followed by polychaetes, ostracods, turbellarians, oligochaetes, gastrotrichs and all other taxa.

The richness of meiofaunal taxa decreased significantly in impacted sites in all non-vegetated sediments, whereas no significant differences between impact and control sites were observed in seagrass sediments (Table 7 and Fig. 4A). On the other hand, significant differences in community evenness were observed between regions and between impact and control sites only in non-vegetated sediments in Cyprus (Table 7, Fig. 4B).

4. Discussion

4.1. Impacts of fish-farm effluents on meiofaunal abundance

Ecosystem alterations induced by different typologies of disturbance, including human impacts, generate variable shifts in benthic species, assemblages and community composition. Fish farming, by modifying the whole attributes of the benthic environment beneath the cages, produces relevant modifications in the abundance, biomass, species composition and evenness of meio- and macrofauna (e.g. Mirto et al., 2002; Kalantzi and Karakassis, 2006).

The responses of metazoan meiofauna to various ecosystem alterations are clearly detected at the highest taxonomic level, with a resolution similar to that provided by the analysis of lower taxonomic levels (Kennedy and Jacoby, 1999; Warwick, 1988), also under the influence of fish farms (Sutherland et al., 2007).

In this study, we analysed the potential effects of fish-farm effluents on the abundance and community composition of meiofauna, by comparing, for the first time, two different habitats in four different regions with different background trophic conditions. The general outcome from the available literature is that fish-farm effluents typically alter meiofaunal abundance, diversity, biomass, and species composition (Duplisea and Hargrave, 1996; Kennedy and Jacoby, 1999; Mirto et al., 2000, 2002; La Rosa et al., 2001; Sutherland et al., 2007). However, changes associated with the presence of fish-farm effluents are often not consistent, as meiofaunal abundance may either increase or decrease beneath the fish cages, depending on the site or the farm characteristics.

In the present study, the idiosyncratic meiofaunal responses to fish-farm impact was confirmed. Although total meiofaunal abundance was generally higher in the fish-farm than in the control sediments, such changes were not always statistically significant. This result is apparently in contrast to previous investigations reporting a decrease of meiofaunal abundance in non-vegetated systems subjected to biodeposition from fish cages (Mirto et al., 2002; Sutherland et al., 2007). The general positive response of meiofaunal abundance to fish-farm biodeposition could be related to the relatively limited organic enrichment in the sediments beneath the cages, as reported in sediments subjected to mussel farm biodeposition, where the organic inputs to the bottom were similarly low (Danovaro et al., 2004).

The results of the PERMANOVA, carried out in the univariate context, revealed that differences in total meiofaunal abundance between impact and control sites were due to the effects of the environmental covariates (Table 8). The effect of organic matter

Table 8
Results of PERMANOVA testing for differences between impact and control sites in total meiofaunal abundance in different habitats and regions, after the removal of the covariate effects. R = region; H = habitat; I = impact; SS = sum of squares; DF = degrees of freedom; MS = means square; $F = F$ value; P = probability level: $^{**} = P < 0.01$; $^{***} = P < 0.001$; ns = not significant. Covariates included: biopolymeric C contents, protein to carbohydrate values, water depth, sediment porosity and water content and current.

Source	DF	SS	MS	F	P
Covariables	6	7.395	1.232	7.45	**
R	3	0.406	0.135	1.28	ns
H	1	0.082	0.081	0.09	ns
I	1	1.128	1.127	−0.78	ns
$R \times H$	3	489.232	163.077	−38.78	ns
$R \times I$	3	−12.148	−4.049	2.22	ns
$H \times I$	1	1.851	1.851	−0.35	ns
$R \times H \times I$	3	0.609	0.203	0.61	ns
Residual	26	8.521	0.327		
Total	47	13.398			

Table 9
Results of the multiple regression analysis of meiofaunal abundance (square root transformed), taxa richness (untransformed) and community evenness over the environmental variables. Prop = proportional; Cu = cumulative.

				Explained variance (%)	
	Variable	Pseudo-F	P	Prop.	Cum.
Meiofaunal abundance	Current	8.17	0.012	15.08	15.08
	Biopolymeric C	5.13	0.032	8.7	23.77
	Protein to carbohydrate ratio	2.64	ns	4.31	28.09
	Water depth	0.13	ns	0.21	28.30
	Sediment porosity	0.07	ns	0.11	28.41
	Sediment water content	0.01	ns	0.01	28.42
Taxa richness	Water depth	11.19	0.004	19.56	19.56
	Current	9.07	0.014	13.49	33.05
	Protein to carbohydrate ratio	12.81	0.002	15.09	48.14
	Sediment porosity	0.58	ns	0.69	48.84
	Biopolymeric C	0.30	ns	0.36	49.20
	Sediment water content	0.87	ns	1.05	50.25
Community evenness	Current	19.71	0.002	29.99	29.99
	Water depth	14.85	0.002	17.37	47.37
	Biopolymeric C	1.15	ns	1.34	48.71
	Sediment porosity	0.90	ns	1.06	49.76
	Protein to carbohydrate ratio	0.85	ns	0.99	50.76
	Sediment water content	0.07	ns	0.09	50.84

enrichment in fish-farm sediments was significant only in oligotrophic regions (i.e. Cyprus and Greece). The multiple regression analysis, indeed, revealed that, among the different environmental variables, only the current speed and the biopolymeric C concentrations explained significant proportions (15% and 9%, respectively) of the total meiofaunal abundance (Table 9). This result supports the expectation that changes in meiofaunal abundance in fish-farm sediments are linked to the organic matter inputs released by the fish-farm, as well as to the local hydrodynamism.

The results of this study suggest that the impact of aquaculture effluents on the meiofaunal abundance is both region- and habitat-specific and also highlight that the direction and intensity of the response of total meiofaunal abundance to aquaculture effluents depend on the environmental conditions.

4.2. Impacts of fish-farm effluents on meiofaunal communities

The results presented here provide evidence that, in all investigated regions, consistent significant effects of fish farming effluents on meiofaunal biodiversity can only be detected in non-vegetated sediments. In all soft bottoms beneath fish farms the richness of higher taxa decreased significantly when compared to control sites (from 13 to 16 in all control sediments to a minimum of 9 taxa in non-vegetated impact sediments in Spain), whereas no significant differences were observed in terms of taxa evenness. The taxa that disappeared beneath the cages changed amongst regions, but always included the rare taxa (i.e. taxa representing <1% of the total meiofaunal abundance; Fig. 3C) of the control sites. Meiofaunal taxa disappeared in fish-farm sediments included kinorhynchs (only in non-vegetated sediments in Spain), cumaceans (in 37.5% of the total cases, i.e., in non-vegetated sediments in Cyprus, Italy and Spain), isopods (37.5%, in non-vegetated sediments in Cyprus and Greece and in vegetated sediments in Spain), thanaidaceans (37.5% in non-vegetated sediments in Cyprus and both habitats in Greece), amphipods (50%, in non-vegetated sediments in Greece, Italy and Spain and in vegetated sediments in Italy), and tardigrades (25% of the total cases: in non-vegetated sediments in Cyprus and Spain). These results do not allow identifying specific taxa as indicators of biodeposition impact.

The analysis of meiofaunal assemblage attributes (meiofaunal abundance, community composition, richness of taxa and community evenness) within seagrass sediments revealed that all of these variables responded idiosyncratically to the presence of fish farming activities. The lack of a clear and consistent meiofaunal response to the fish-farm biodeposition in seagrass sediments could have several possible explanations. *P. oceanica* is a seagrass endemic of the Mediterranean Sea, which covering ca 50.000 km^2, plays a key ecological role for a wide range of organisms and assemblages, preserving their biodiversity and protecting the integrity of the coastal areas (Hemminga and Duarte, 2000). The effects of fish-farm biodeposition on seagrass meadows can be difficult to detect due to the fact that the presence of the seagrass masks the changes in organic matter concentrations and composition (Pusceddu et al., 2007). However, the presence of a large number of filter feeders and detritus feeders associated with the seagrass bed can also act as a buffer for the organic enrichment, by consuming a large fraction of the biodeposits (Balata et al., 2008). Moreover, the presence of a large assemblage of prokaryotes, protozoa and meiofauna within the seagrass sediments makes it difficult to detect the presence of significant changes in these components (Danovaro, 1996; Bongiorni et al., 2005). *P. oceanica* is known to be highly sensitive to anthropogenic impacts, and it has been demonstrated that, in the long term (i.e. years to decades), the increased sedimentation of waste particles and the accumulation of organic matter lead to the deterioration of the seagrass system (Pergent-Martini et al., 2006). In the regions investigated, the seagrass systems displayed a clear impact and several indicators of deterioration of the *Posidonia* meadows were reported (Holmer et al., 2008). However, the effects of biodeposition on meiofaunal variables were not evident, indicating that in seagrass systems the search for meiofaunal indicators of impact is unnecessary as it provides, if any, a slow or late response to the fish-farm impact.

4.3. Conclusions and perspectives

In this study we showed that fish-farm biodeposition in the Mediterranean Sea can provoke changes in meiofaunal abundance, community structure and the biodiversity at high taxonomic levels. However, the hierarchical sampling conducted here demonstrated that the meiofaunal responses varied among regions and habitats. In all non-vegetated sediments the richness of meiofaunal taxa decreased in response to fish-farm biodeposition, and therefore independently from the background environmental conditions. Conversely, in seagrass sediments, the idiosyncratic response did not allow any clear indicator of impacts from fish-farm biodeposition to be identified. The results of this study highlight the importance of using different indicators of fish-farm impact in vegetated and non-vegetated systems.

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