



Nematode assemblage response to fish-farm impact in vegetated (*Posidonia oceanica*) and non-vegetated habitats

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ABSTRACT: We investigated the impact of fish farming on the benthic biota of 2 habitats (sea-grass meadows of *Posidonia oceanica* and non-vegetated soft bottoms), by examining in detail the nematode assemblages, and the effects of organic enrichment on their abundance, diversity (species richness and trophic diversity), community structure and individual size. All investigated faunal variables differed significantly between impact and control sediments, except for individual nematode biomass. Nematodes displayed reduced biodiversity beneath the investigated fish farms in both vegetated and non-vegetated habitats. The nematode genera *Richtersia*, *Desmoscolex* and *Halalaimus* were highly sensitive to biodeposition and disappeared almost completely in farm sediments, whereas other genera such as *Daptonema* and *Prochromadorella* largely increased their relative abundance. The impact of biodeposition was investigated also in terms of *K*-dominance curves and functional variables (life strategies as maturity index, and functional [i.e. trophic] diversity) which showed that nematodes are very sensitive to this kind of environmental disturbance. Our results indicate that the analysis of nematode assemblage composition, the life traits of the dominant species and the presence/disappearance of certain nematode species represent a reliable tool for monitoring the quality state of marine sediments exposed to fish-farm organic wastes.

KEY WORDS: Aquaculture impact · *Posidonia oceanica* · Nematodes · Life traits · Trophic diversity · Mediterranean Sea

INTRODUCTION

Mediterranean aquaculture production has increased by 77% over the last decade, especially in coastal systems (Rosa et al. 2012). The fast increase in the production of marine carnivorous fish (gilthead seabream *Sparus aurata* and European seabass *Dicentrarchus labrax* in marine areas) is also evident (Neofitou et al. 2010, Rosa et al. 2012). As a consequence, fish-farm biodeposition, by releasing organic and nutrient loads in the surrounding waters, is affecting the marine ecosystem in many ways (Holmer

2010). For instance, the excess fodder used for carnivorous fish, together with their faeces, are responsible for significant changes in sediment chemistry (Karakassis et al. 2002, Holmer et al. 2003) and in benthic flora and fauna (Duplisea & Hargrave 1996, Mazzola et al. 2000, La Rosa et al. 2001, Mirto et al. 2002, 2010, 2012, Grego et al. 2009, Vidovic et al. 2009, Moreno et al. 2011, Martinez-Garcia et al. 2013). Moreover, as fish cages are frequently installed in protected coastal areas, their presence could affect different habitats, and among them, seagrasses can be considered as one of the most susceptible

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ecosystems to fish-farm biodeposition (Holmer et al. 2008, Rountos et al. 2012).

Previous investigations revealed that benthic assemblages can respond to fish-farm activities in terms of: (1) a decrease in abundance, biomass and species richness (Klaoudatos et al. 2006, Tomassetti et al. 2009), due to a selection of a few opportunistic species (Weston 1990); (2) a shift in the relative importance of the different trophic guilds (Pearson & Rosenberg 1978); (3) changes in the size of benthic organisms (Tsujino 1998); and (4) a shift in life strategies, with increasing relevance of opportunistic species (Bongers et al. 1991, Mirto et al. 2002).

Meiofauna, due to their important role in marine benthic food webs (Moens et al. 2005) and their biological traits (small size, high abundance, fast generation times and absence of larval dispersion), are a useful tool to investigate alterations in the sea bottom. In fact, if compared with the use of macrofaunal assemblages, the use of meiofauna in marine sediment monitoring has several operational advantages (Kennedy & Jacoby 1999, Austen & Widdicombe 2006, Patrício et al. 2012), such as their ability to respond more rapidly to a variety of different environmental alterations with clearly detectable changes at different levels of the faunal organisation (i.e. from the higher taxa to the species level). Among the meiofauna, nematodes, which are most often the numerically dominant taxon in marine meiofauna, have been largely utilised as indicators of environmental disturbance because of their ubiquity, high abundance and taxonomic diversity (Bongers & Ferris 1999, Schratzberger et al. 2004, Steyaert et al. 2007, Moreno et al. 2008, 2011, Neher & Darby 2009). For instance, nematodes are sensitive to hydrocarbon contamination (Danovaro et al. 1995) and organic enrichment (Essink & Keidel 1998, Frascchetti et al. 2006, Moreno et al. 2008), including biodeposition from aquaculture activities (Duplisea & Hargrave 1996, Porter et al. 1996, Mazzola et al. 2000, Mirto et al. 2002, Mahmoudi et al. 2008, Vezzulli et al. 2008).

Detailed investigations on the effects of fish-farm biodeposition on nematode assemblages are relatively scant (Mirto et al. 2010), and few, if any, studies have been conducted to assess the effects of aquaculture on nematode assemblages inhabiting *Posidonia oceanica* meadows.

In the present study, we tested the null hypothesis that nematode abundance, species richness, community structure, individual body biomass, maturity index and functional (trophic) diversity do not differ between sediments exposed to fish-farm organic wastes and reference sites. Since in the Mediterran-

ean Sea, meadows of the seagrass *Posidonia oceanica* represent a key habitat and, at the same time, are threatened by multiple stressors (Montefalcone 2009), we conducted our study comparatively in both seagrass and non-vegetated sediments in 3 different regions, each characterised by varying environmental assets. This study completes a previous investigation carried out to ascertain the effects of fish-farm wastes on abundance, biomass and community structure of meiofaunal assemblages (Mirto et al. 2010).

MATERIALS AND METHODS

Study site and sampling

Three regions located at similar latitudes and depths were selected: 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). In each of the 3 sampling regions, the effects of fish farms on nematode assemblages were investigated in 2 different habitats: meadows of the seagrass *Posidonia oceanica*, and soft non-vegetated bottoms. The 2 habitats were >100 m distance from one another. Each fish farm hosted several cages with similar size, volume and fish biomass harvested. In each habitat, the impact of the organic biodeposition from the cages was quantified by contrasting the fish-farm sites just beneath the cages with control sites characterised by pristine conditions and physical characteristics 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. At each site, 3 replicated sediment samples for the analyses of nematode assemblages were

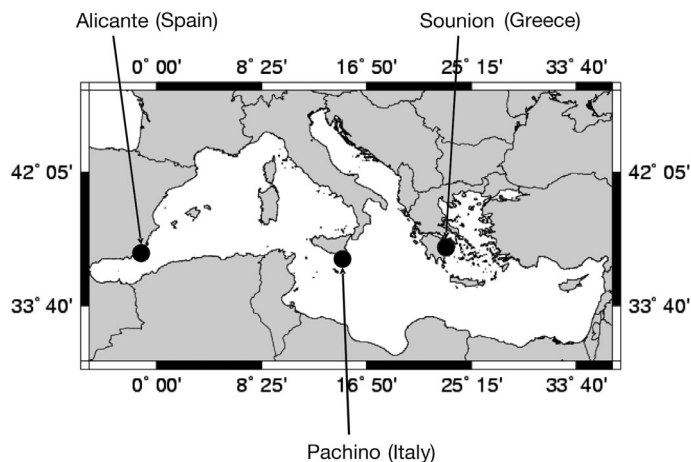


Fig. 1. Sampling areas in the Mediterranean Sea

Table 1. Environmental characteristics and biochemical composition of sedimentary organic matter at all sampling sites in the top 1 cm of the sediment. BPC = biopolymeric carbon, CPE = chloroplastic pigment equivalents

Region	Habitat	Site	Water depth (m)	Sediment type (silt/clay, %)	Grain size (mean diam., mm)	BPC (mg C g ⁻¹)		CPE (µg g ⁻¹)	
						Mean	SD	Mean	SD
Greece	Non-vegetated	Control	16	2.2	1.89	0.57	0.12	7.37	2.34
		Impact	16	6.7	0.38	2.93	0.20	10.73	1.86
	Vegetated	Control	15	13.1	0.76	2.11	0.66	6.73	2.05
		Impact	16	20.2	0.33	5.19	0.59	4.93	1.26
Italy	Non-vegetated	Control	22	3.9	0.84	0.73	0.30	7.40	2.03
		Impact	22	5.7	0.47	2.43	0.47	9.95	1.98
	Vegetated	Control	22	4.8	0.90	2.96	0.94	4.72	0.29
		Impact	22	4.6	0.45	2.84	0.01	12.44	2.50
Spain	Non-vegetated	Control	29	15.6	0.43	6.21	1.53	44.52	8.44
		Impact	29	7.7	0.32	3.84	0.63	20.04	9.64
	Vegetated	Control	29	15.3	0.90	4.36	0.94	40.08	5.58
		Impact	29	16.2	0.40	4.11	0.77	46.03	17.58

randomly collected from 1 station centrally located beneath the cages (impact site) and from 1 station in each control site, by means of manual corers (diam. 3.7 cm, 10.7 cm² surface area, down to a depth of 10 cm) operated by SCUBA divers. All physical-chemical parameters in the top first cm are fully described in Pusceddu et al. (2007) and Mirto et al. (2010) and are summarised in Table 1.

Nematode abundance, biomass and biodiversity

Sediment samples were fixed with 4% buffered formaldehyde in filtered (0.4 µm) seawater solution. The top first cm of each sediment corer was sieved through 1000 and 32 µm mesh nets. The fraction remaining on the 32 µm sieve was centrifuged 3 times with Ludox HS40 (density arranged to 1.18 g cm⁻³), as summarised in Danovaro (2010). 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).

For the analysis of nematode diversity, 100 specimens (or all of the retrieved nematodes if <100 in number) were randomly picked from 3 independent replicates at each site. Nematodes were mounted on slides (following the formalin-ethanol-glycerol technique to prevent dehydration; Seinhorst 1959) and identified to species level. In case of unknown species, these were indicated as sp1, sp2, sp3, etc. according to Platt & Warwick (1983, 1988), Warwick et al. (1998) and the recent literature (NeMys database: Deprez et al. 2005).

In order to investigate the trophic composition of the assemblages, all nematode species were as-

signed to a feeding type according to Wieser (1953): selective (1A) and non-selective (1B) deposit feeders, epigrowth feeders (2A) and predators/omnivores (2B).

Length and width of all nematode specimens were measured using a microscope coupled with image-analyser software (Leica IM 1000).

Total nematode biomass was calculated from bio-volume estimates according to Feller & Warwick (1988), and was determined as the sum of the product of individual body weight (expressed as µg C) and total nematode abundance, and expressed as µg C 10 cm⁻².

Nematode biodiversity (species richness, SR, defined as the total number of species identified at each station; expected number of species, ES51; Margalef index, *D*; Shannon-Wiener diversity index, *H'*), evenness (Pielou's *J*), trophic composition (index of trophic diversity, ITD) and life strategies (maturity index, MI) were calculated in each sampling site for the 2 habitats of each region.

ITD was calculated as follows:

$$ITD = g_1^2 + g_2^2 + g_3^2 \dots + g_n^2 \quad (1)$$

where *g* is the relative contribution of each trophic group to the total number of individuals and *n* is the number of trophic groups (Gambi et al. 2003). For *n* = 4 (as in the present study), ITD ranges from 0.25 to 1.00.

MI was calculated as the weighted mean of the individual taxon scores (Bongers et al. 1991):

$$MI = \sum v(i) \times f(i) \quad (2)$$

where *v* is the colonisers-persisters (c-p) value of taxon *i* (as given in the appendix to Bongers et al. 1991) and *f* is the frequency of that taxon in a sample.

Statistical analyses

To quantify the effects of the fish farms on nematode abundance, body weight, biomass, structural (i.e. species richness and other biodiversity indexes) and functional (i.e. trophic) diversity, and life strategies (maturity index), 3-way permutational analyses of variance (PERANOVA) were carried out separately for each variable, based on Euclidean distances and unrestricted permutations of the residuals. The analysis treated the factor 'region' (R, 3 levels: Greece, Italy and Spain) as random, 'habitat' (H, 2 levels: vegetated and non-vegetated sediments) as fixed and crossed with R, and 'sampling site' (S, 2 levels: impact and control) as fixed and orthogonal to R and H.

The same statistical design was also adopted in the multivariate context (permutational multivariate analysis of variance; PERMANOVA) to quantify the effects of the fish farms on the composition of nematode assemblages. Nematode species abundance data were square-root-transformed to scale down densities of highly abundant species and therefore increase the importance of the less abundant species in the analyses. The test was conducted on a Bray–Curtis similarity matrix and the residuals were permuted under a reduced model, with 999 permutations. Significant differences were examined using *a posteriori* pairwise comparisons for the contrast of interest (i.e. impact vs. control), using 999 permutations under a reduced model.

We also measured turnover diversity to examine the effects of fish-farm biodeposition on nematode species composition in both investigated habitats in each coastal region. Turnover diversity was expressed as percentage of dissimilarity, based on a Bray–Curtis similarity matrix (Gray 2000), calculated using the SIMPER procedure (Clarke 1993). Species abundance data were presence/absence-transformed prior to the analysis (i.e. Jaccard dissimilarity) to search for the most tolerant and sensitive species to fish-farm deposition. The percentage contribution of each species to the dissimilarity between impact and control sites was calculated separately for each region and habitat. To assess whether and how much physical (sediment type and grain size) and trophic (biopolymeric carbon, protein to carbohydrate ratio and chloroplastic pigment equivalents) sedimentary variables explained changes in nematode assemblages, nonparametric multivariate multiple regression analyses, based on Bray–Curtis dissimilarity matrixes, were carried out using the routine DISTLM forward (McArdle & Anderson 2001). The forward selection

of the predictor variables was carried out with tests by permutation. Univariate and multivariate permutational analyses of variance were carried out using the PERMANOVA, SIMPER and DISTLM routines included in the software PRIMER v.6 through the add-on package PERMANOVA+ (Anderson 2001, Anderson et al. 2008).

K-dominance curves were then generated using nematode species identified in control and impact sites synoptically in each habitat and region. The relative abundance of each species was plotted against the decreasing rank of dominating species.

RESULTS

Fish-farm impacts on nematode abundance, biomass and species richness

Nematode abundance in non-vegetated sediments ranged from 367.2 ± 69 to 3406.0 ± 1798.0 ind. 10 cm^{-2} (in impact sediments in Italy and Greece, respectively), and in vegetated sediments ranged from 583.3 ± 261.5 to 3381.0 ± 1625.3 ind. 10 cm^{-2} (in control sediments in Greece and impact sediments in Spain, respectively) (Table 2). PERANOVA analyses revealed a lack of significant interactions among regions, habitats and sites for nematode abundance (pseudo- $F_{2,24} = 2.19$; $p = 0.135$).

Nematode body weight in the non-vegetated sites ranged from 0.47 ± 0.30 to 1.94 ± 1.20 $\mu\text{gC ind.}^{-1}$ (in Spain and Greece, respectively), and was typically higher than in vegetated sediments (range: 0.21 ± 0.02 to 0.65 ± 0.10 $\mu\text{gC ind.}^{-1}$; in Spain and Italy, respectively; Table 2). Total nematode biomass in the non-vegetated sediments ranged from 262.3 ± 59.5 to 7789.8 ± 5989.4 $\mu\text{gC } 10 \text{ cm}^{-2}$ (in Italy and Greece, respectively), while in the vegetated sediments, values ranged from 141.5 ± 86.2 to 648.5 ± 188.5 $\mu\text{gC } 10 \text{ cm}^{-2}$ (in Greece and Spain, respectively). PERANOVA analyses of total nematode biomass revealed significant interactions among regions, habitats and sites (Table 3). The pairwise test for the contrast of interest (i.e. impact vs. control) also showed that total nematode biomass, both in vegetated and non-vegetated sites of Greece, was significantly higher in fish-farm than in control sediments (both $p < 0.02$). In both habitats in Italy and Spain, the differences were not significant.

Overall, approximately 3000 nematode specimens were identified belonging to 3 orders, 37 families, 126 genera and 271 species. In vegetated sediments, 32 families, 97 genera and 157 species were recorded

Table 4. Nematode species richness (SR), expected species richness (ES51), Shannon-Wiener index (H'), Margalef's D , Pielou's J evenness index and trophic diversity index (IDT)

Region	Habitat	Site	SR	ES51		Shannon-Wiener H'		Margalef's D		Pielou's J		IDT	
				Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Greece	Non-vegetated	Control	65	22.44	2.21	2.97	0.14	5.91	0.51	0.94	0.02	0.44	0.12
		Impact	42	14.42	1.10	2.36	0.02	3.98	0.37	0.80	0.02	0.55	0.11
	Vegetated	Control	50	20.69	6.48	2.76	0.54	6.00	2.10	0.85	0.06	0.38	0.02
		Impact	33	13.40	7.56	1.88	0.95	3.79	2.27	0.64	0.20	0.54	0.18
Italy	Non-vegetated	Control	43	18.31	3.89	2.67	0.33	4.82	1.20	0.86	0.03	0.51	0.33
		Impact	26	10.83	5.41	2.14	0.32	3.52	0.90	0.88	0.13	0.38	0.05
	Vegetated	Control	58	21.15	15.59	2.55	1.26	5.69	4.48	0.89	0.07	0.42	0.18
		Impact	54	20.69	2.36	2.77	0.20	5.78	0.79	0.84	0.05	0.45	0.10
Spain	Non-vegetated	Control	59	23.38	2.10	3.08	0.17	6.44	0.66	0.93	0.02	0.40	0.04
		Impact	51	19.51	1.17	2.72	0.15	5.46	0.26	0.84	0.05	0.45	0.06
	Vegetated	Control	58	22.39	4.04	2.91	0.37	6.54	0.94	0.85	0.07	0.37	0.14
		Impact	35	15.97	4.10	2.45	0.36	4.26	1.23	0.83	0.06	0.60	0.12

In non-vegetated sediments, the most common families were Chromadoridae (22.6%, 10 genera and 23 species), Xyalidae (20.5%, 5 genera and 19 species) and Microlaimidae (13.1%, 2 genera and 8 species). At the non-vegetated impact sites, Xyalidae (29.1%), Microlaimidae (18.6%) and Chromadoridae (15.3%) were dominant, while Chromadoridae (32.4%), Desmodoridae (13.4%) and Xyalidae (8.9%) were dominant at the control sites.

In farm sediments of non-vegetated sites, *Daptonema* (21.1%, 12 species), *Prochromadorella* (14.1%, 6 species) and *Microlaimus* (13.0%, 5 species) were the most abundant genera, while at control sites the dominant taxa were *Desmodora* (11.5%, 11 species), *Prochromadorella* (8.2%, 6 species) and *Epsilonema* (6.4%, 1 species).

Species richness, the expected number of species, Shannon-Wiener diversity index, dominance index, evenness index and the trophic diversity index are reported in Table 4. The PERANOVA tests revealed that none of the tested factors or their interactions had significant effects on the different diversity indexes (data not shown), with the exception of the maturity index, which showed a significant $R \times H \times S$ interaction (Table 3). Pairwise comparisons indicated that values of the maturity index decreased significantly in both vegetated ($p = 0.043$) and non-vegetated ($p = 0.009$) impact sites in Italy, Greece ($p = 0.026$ and $p = 0.004$, respectively) and Spain ($p = 0.023$ and $p = 0.002$, respectively) (Fig. 2).

K -dominance curves contrasting impact and control sediments plotted separately for each region and habitat revealed that impact sites were always above those representing assemblages from control sediments (Fig. 3).

Fish-farm effects on nematode assemblages

The results of the PERMANOVA test carried out on the nematode assemblage composition showed a significant $R \times H \times S$ interaction (pseudo- $F_{2,27} = 1.89$; $p = 0.004$). Pairwise comparisons indicated that a significant difference in the nematode assemblages between control and impact sites occurred only in non-vegetated sediments in Greece ($p = 0.03$) and in vegetated ($p = 0.04$) and non-vegetated habitats ($p = 0.03$) in Spain.

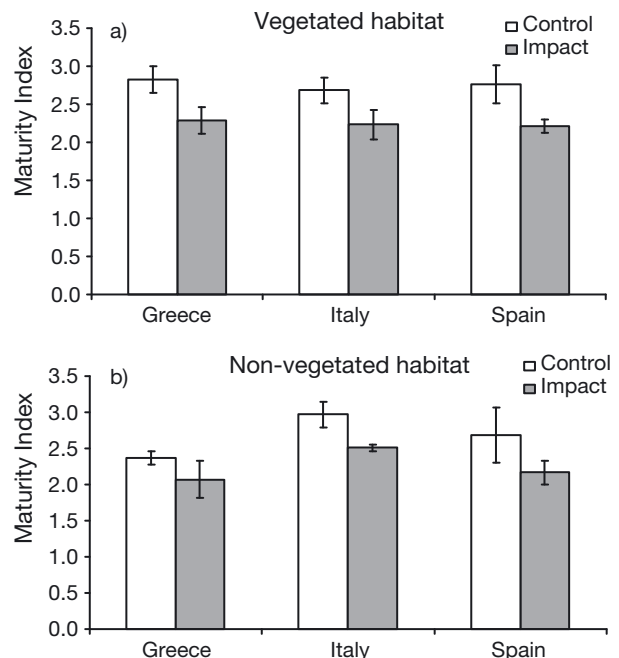


Fig. 2. Maturity index calculated on nematodes in control and impact (fish-farm) sediments in (a) non-vegetated and (b) vegetated habitats. Error bars represent SD

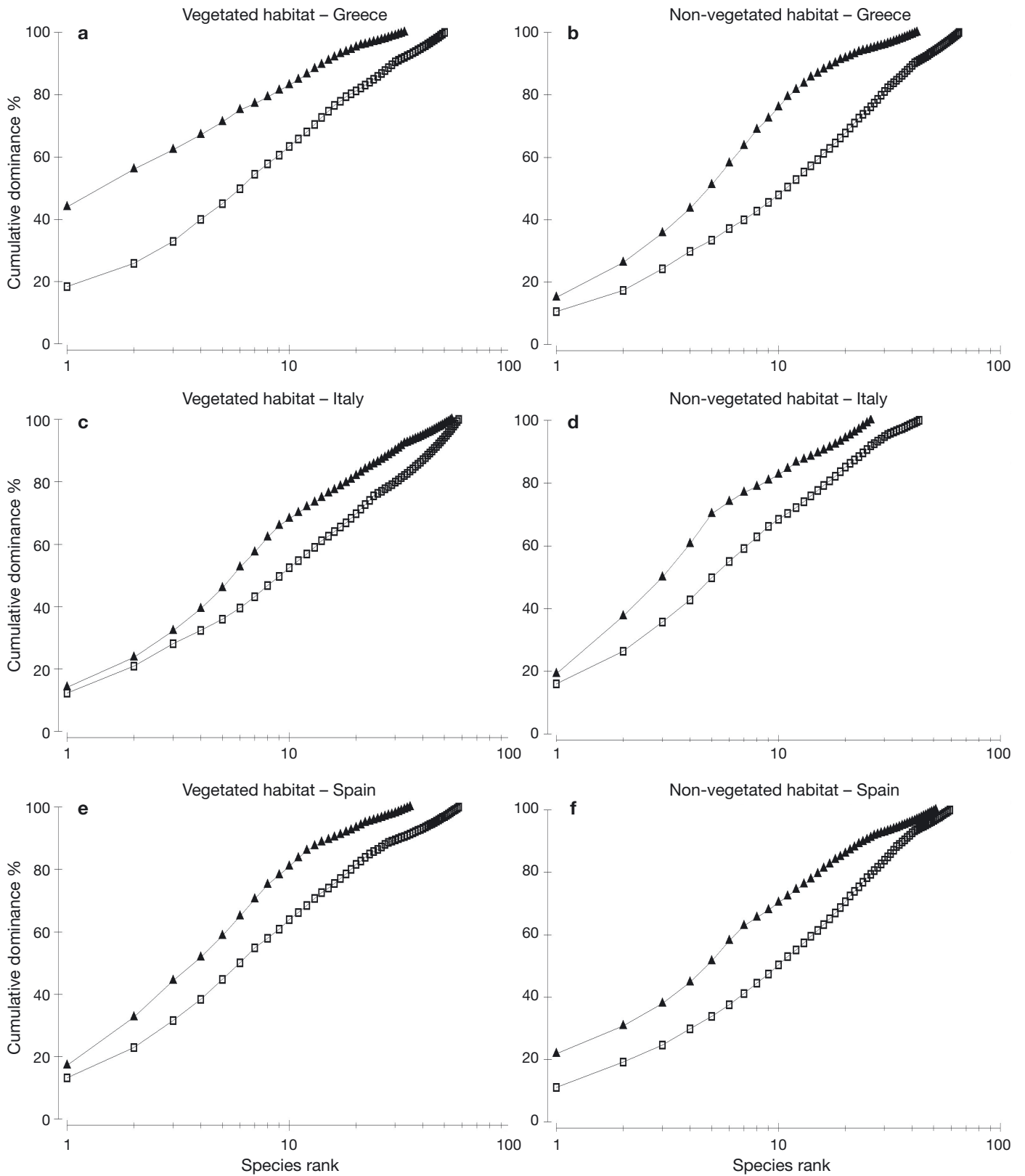


Fig. 3. *K*-dominance curves based on nematode species identified from the 3 investigated regions: (a) vegetated and (b) non-vegetated sediments in Greece, (c) vegetated and (d) non-vegetated sediments in Italy, and (e) vegetated and (f) non-vegetated sediments in Spain. Each curve compares nematode structure in control (□) and impact (▲) sites synoptically

The SIMPER procedure revealed the presence of a high turnover diversity between nematode assemblages inhabiting control and impact sites in both vegetated (ranging from 67 to 81%) and non-vegetated (ranging from 78 to 88%) sediments (Table 5). Detailed analysis of the SIMPER results revealed that the nematode species mostly responsible for the differences between impact and control sediments varied among habitats and regions. Genera recurrently responsible for the observed differences (though

sometimes with different species) included e.g. *Daptonema*, *Richtersia*, *Prochromadorella* and *Desmodora* in vegetated sediments, and *Microlaimus*, *Prochromadorella*, *Desmodora* and *Daptonema* in non-vegetated sediments. Only some of these genera, however, disappeared below fish cages (e.g. *Richtersia*, *Desmoscolex* and *Halalaimus*), whereas others responded positively (e.g. *Daptonema* and *Prochromadorella*).

Considering the entire dataset, 70 species were encountered only in control sediments, and among

Table 5. SIMPER analysis showing nematode species contributing (cut-off 50%) to turnover diversity between control and impact sites in vegetated and non-vegetated habitats in Greece, Italy and Spain. Values are percentage contributions of the *i*th species to turnover diversity. Turnover diversity (in parentheses, per region and habitat) is the dissimilarity in species composition between control and impact sites. Only the contrasts of interest (i.e. impact vs. control in each region and habitat) are shown

Species	Vegetated habitat			Species	Non-vegetated habitat		
	Greece (70.1%)	Italy (81.5%)	Spain (67.1%)		Greece (77.5%)	Italy (88.1%)	Spain (80.1%)
<i>Anticoma acuminata</i>		2.4		<i>Anticoma acuminata</i>	2.53		
<i>Astomonema</i> sp.1	2.38			<i>Axonolaimus similis</i>	1.64		
<i>Calomicrolaimus spirifer</i>		4.26		<i>Calomicrolaimus parahonestus</i>	4.67		
<i>Chromadorita</i> sp.1	3.85			<i>Chromadora</i> sp. 1			1.77
<i>Daptonema normandicus</i>	5.37	3.6	1.97	<i>Cobbia trefusiaeformis</i>		3.10	
<i>Daptonema rusticus</i>	13.16		3	<i>Comesoma arenae</i>	4.87		
<i>Desmoscolex</i> sp.1	3.05			<i>Comesoma</i> sp.1	1.63		
<i>Desmoscolex</i> sp.3			2.97	<i>Cyatholaimus microsetosus</i>		5.35	
<i>Desmodora communis</i>			3.13	<i>Daptonema calceolatus</i>			3.70
<i>Desmodora polychaeta</i>		4.63	3.06	<i>Daptonema fistulatus</i>			2.67
<i>Desmodora pontica</i>		1.89		<i>Daptonema longicaudatus</i>	1.66		
<i>Dichromadora</i> sp.2			2.6	<i>Daptonema xyaliformis</i>			2.00
<i>Dichromadora</i> sp.3			6.51	<i>Daptonema</i> sp.1	3.86		6.14
<i>Draconema</i> sp.1		2.31		<i>Daptonema</i> sp.2	1.53		1.64
<i>Halalaimus</i> sp.1		2.01	2.81	<i>Daptonema</i> sp.3			1.64
<i>Hapalomus</i> sp.1	2.52			<i>Desmodora communis</i>		3.30	1.97
<i>Laimella longicaudata</i>			1.97	<i>Desmodora polychaeta</i>	1.71	2.49	
<i>Linhystera</i> sp.1			2	<i>Desmodora pontica</i>			2.61
<i>Metalinhomoeus effilatus</i>		1.35		<i>Desmodora</i> sp.1			1.72
<i>Metalinhomoeus</i> sp.1		1.98		<i>Enoploilaimus propinquus</i>		3.85	
<i>Microlaimus</i> sp.1	2.95		2.32	<i>Enoplus brevis</i>	1.55		
<i>Neotonchus meeki</i>		1.54		<i>Epsilonema</i> sp.1		6.44	
<i>Paracanthochus macrodon</i>		1.36		<i>Euchromadora gaulica</i>		6.04	
<i>Paracanthochus</i> sp.1		1.62		<i>Halalaimus</i> sp.1			2.30
<i>Paralinhomoeus uniovarium</i>		1.4		<i>Hypodontolaimus balticus</i>			3.47
<i>Prochromadorella attenuata</i>		4.75		<i>Longicyatholaimus longicaudatus</i>		2.92	
<i>Prochromadorella ditlevseni</i>		3.92		<i>Microlaimus</i> sp.1	2.94	2.85	
<i>Prochromadorella septempapillata</i>	3.39	2.38		<i>Microlaimus zosterae</i>	4.52		2.99
<i>Ptycholaimellus ponticus</i>		2		<i>Molgolaimus</i> sp.1		2.90	
<i>Richtersia</i> sp.1	4.9		6.08	<i>Paracanthochus</i> sp.1	2.49		
<i>Sabatieria</i> sp.1		1.53		<i>Paracanthochus</i> sp.2	1.65		
<i>Setopectes</i> sp.1			2.11	<i>Paracomesomea dubium</i>	3.94		
<i>Spiliphora</i> sp.1			3.83	<i>Paramonhystera</i> sp.1		3.14	2.92
<i>Spirinia flagellata</i>	2.82		3.78	<i>Prochromadorella attenuata</i>	2.38		
<i>Terschellingia longicaudata</i>		2.47		<i>Prochromadorella ditlevseni</i>	2.95	3.75	3.33
<i>Tricoma</i> sp.1	2.39			<i>Prochromadorella paramucrodonta</i>	2.07		
<i>Trissonchulus</i> sp.1			2.98	<i>Prochromadorella</i> sp.1	2.08		
<i>Viscosia elegans</i>	4.1	2.88		<i>Spilophorella paradoxa</i>		4.67	1.74
				<i>Steineria simplex</i>			1.95
				<i>Terschellingia longicaudata</i>			2.74
				<i>Viscosia elegans</i>			1.84
				<i>Viscosia glabra</i>			1.77

Table 6. DISTLM-forward analysis carried out to identify the environmental variables responsible for the differences in nematode assemblage composition between impact and control sites in non-vegetated and vegetated sediments. Data from the 3 different regions were pooled. Silt/clay %: sediment type, BPC = biopolymeric carbon content, PRT/CHO = protein-to-carbohydrate ratio, CPE = chloroplastic pigment equivalent content; Prop. % = significant proportion of explained variation; Cumul. % = cumulative proportion of explained variation; SS = sum of squares; *p < 0.05; **p < 0.01; ***p < 0.001. Tests were conducted on Euclidean distance matrices and the residuals were permuted under a reduced model, with 999 permutations

Habitat	Variable	SS (trace)	Pseudo-F	p	Prop. %	Cumul. %
Non-vegetated	BPC	6514.5	1.9602	0.0072***	9.35	9.35
	PRT/CHO	8312.8	2.7289	0.0004***	11.93	21.29
Vegetated	CPE	7739.9	2.9501	0.0002***	15.57	15.57
	BPC	5371.6	2.2011	0.0036**	10.80	26.37
	PRT/CHO	5678.0	2.5702	0.0017**	11.42	37.79
	Silt/clay %	4501.2	2.2142	0.002**	9.05	46.85
	Grain size	3532.7	1.8517	0.0202*	7.11	53.95

these, *Halalaimus* sp., *Spiliphera* sp., *Euchromadora gaulica*, *Cyatholaimus microsetosus*, *Hypodontolaimus balticus*, *Chromadora* sp. and *Draconema* sp. were the most abundant. The number of exclusive species in impact sediments was 119, and among these, *Dichromadora* sp.2, *Enoploilaimus propinquus*, *Daptonema calceolatus*, *Calomicrolaimus spirifer* and *Daptonema fistulatus* were the most abundant.

The results of the multivariate multiple regression analysis (DISTLM) conducted separately for nematode assemblages in the 2 investigated habitat are reported in Table 6. Biopolymeric carbon sedimentary contents and values of the protein-to-carbohydrate ratio were the most important predictor variables explaining variations in nematode assemblages between control and impact non-vegetated sediments (cumulatively, 21.29% of the explained variance). Chloroplastic pigment equivalent and biopolymeric carbon sedimentary contents and values of the protein to carbohydrate ratio were the most important predictor variables explaining variations in nematode assemblages between impact and control vegetated sediments (cumulatively, 37.8% of the explained variance).

DISCUSSION

Ecosystem alterations induced by different typologies of impacts generate shifts in structural and functional variables, including benthic species composition, assemblage structure, biomass and production. Fish farming, by modifying the attributes of the benthic environment beneath the cages, produces modifications in the abundance, biomass, species composition and evenness of meio- and macrofauna (e.g.

Kalantzi & Karakassis 2006, Mirto et al. 2012). In the present study, we focused our attention on the potential effects of fish-farm biodeposition on nematode assemblages. By comparing 2 different habitat types (*Posidonia oceanica* vs. soft bottoms) in 3 different Mediterranean regions, we tried to clarify the importance of this kind of impact in different geographic and trophic conditions.

Available information reports that fish-farm impact affects nematode assemblages

in terms of community structure, trophic composition, individual biomass, maturity index and biodiversity (Mirto et al. 2002, Moreno et al. 2011). However, such changes are often not consistent; for instance, meiofaunal and nematode abundance may either increase or decrease beneath the fish cages, depending on the site or farm characteristics (Mirto et al. 2010).

We report here an idiosyncratic response of nematode assemblages to fish-farm impacts. Our results allowed us to reject the null hypothesis only for nematode species composition and the values of the maturity index. On the other hand, the null hypothesis has to be accepted for nematode abundance, individual and total biomass, and structural and trophic biodiversity. This result is in contrast to previous investigations reporting, for instance, a decrease in nematode abundance in non-vegetated sediments subjected to biodeposition from fish cages (Duplisea & Hargrave 1996, Mirto et al. 2002, 2012, Sutherland et al. 2007), but is in agreement with other studies showing a positive response of nematode abundance as a result of organic enrichment in the sediment beneath the cages (Mirto et al. 2010). Nematode individual biomass is still not a universally accepted variable for detecting organic pollution; previous studies on the effects of biodeposition on nematode individual biomass have provided conflicting results. Our results are consistent with those of Duplisea & Hargrave (1996), who did not find differences in nematode individual biomass comparing fish-farm and control sediments.

Previous studies reported that meiofaunal biomass of organically enriched environments can become increasingly dominated by large specimens when compared to non-enriched sites (Moore & Bett 1989). Mirto et al. (2002) reported the presence of larger

nematodes in fish-farm sediments, while Tsujino (1998) and Porter et al. (1996) found the presence of larger individual biomass in organically impacted sediments. Prein (1988) and Lorenzen et al. (1987) reported that the large Oncholaimidae aggregated in organically polluted fjords. The response of the individual nematode biomass to fish-farm biodeposition is thus variable and apparently site- and habitat-specific. This is consistent with the results of our study, in which the total biomass of nematodes displayed values in impact sediments significantly higher than in control sediments only in Greece (both habitats).

Previous investigations proved that analysis of nematode assemblages to the species level can be efficient for detecting changes in the environmental quality of the sediments beneath the cages (Essink & Keidel 1998, Bongers & Ferris 1999). Our results show that organic wastes from fish farms apparently have no effects on any of the different nematode biodiversity indexes, with the exception of a statistically significant decrease in the maturity index in impact sediments, consistently observed in all habitats and regions. The invariance in species richness is in contrast with the general expectation of a negative response of species richness to organic disturbance. The lack of statistical differences in nematode structural biodiversity between impact and control sediments can be, to a certain extent, explained in terms of a contemporary disappearance of certain intolerant species (Weston 1990), partially offset by an increase in abundance of opportunistic species in fish-farm impact sediments. This mechanism is in fact supported by the significant decrease of the nematode assemblage maturity index at impact sites, indicating an increasing importance of coloniser nematodes (with lower values of the maturity index) and a decreasing importance of persistent species in impact sediments. Thus, the decrease of the nematode assemblage maturity index in fish-farm impact sediments, coupled with the significant variations in the specific composition of the nematode assemblages between impact and control sediments in both habitats and all regions (Table 5), indicates that the nematode maturity index and the turnover diversity are both highly sensitive to fish-farm organic pollution.

At the same time, the K -dominance curves obtained from impact and control sediments plotted at each site and habitat reveal that, along with the nematode maturity index and turnover diversity, the relative importance of certain nematode species (and therefore their taxonomic identity) can provide robust information for the detection of fish-farm impacts. This is also confirmed by the results of the multivariate multiple

regression analysis (Table 6) showing that differences in the composition of the nematode assemblages are clearly and significantly explained by changes in the organic matter accumulation beneath the fish cages, whatever the habit or region considered.

Nematode assemblages in the present study comprised a relatively high number of species, but with a few dominant ones, as observed in other coastal environments, either in non-vegetated (Soetaert et al. 1995) or seagrass sediments (Danovaro & Gambi 2002, Gambi et al. 2008). We report here, moreover, that some species were apparently highly sensitive to the organic pollution derived from fish farms.

In vegetated sediments, species belonging to genera *Richtersia*, *Desmoscolex* and *Halalaimus* strongly decreased or even disappeared in impact sites, where species of *Daptonema* or *Prochromadorella* were instead more abundant than in control sediments. The genus *Daptonema* is known to be a good indicator of stressed conditions (Alves et al. 2013), and it has been found to dominate benthic habitats exposed to domestic sewage (Boyd et al. 2000, Nanajkar & Ingole 2010) or to the biodeposition due to mussel farms (Netto & Valgas 2010). The genus *Richtersia* displayed higher abundances in control sites, and thus our results confirm its high sensitivity to organic biodeposition (Bongers et al. 1991, Danovaro et al. 1995, Vezzulli et al. 2008). Species belonging to the genus *Desmodora* are generally associated with a pristine environmental condition, and indeed, in the present study, they showed higher abundances in control non-vegetated sediments. In contrast, species of *Daptonema*, *Prochromadorella*, and *Microlaimus*, known to thrive in degraded and stressful conditions (Bongers 1990, Palacin et al. 1992, Essink & Romeyn 1994, Gyedu-Ababio et al. 1999, Somerfield et al. 2003, Liu et al. 2008, Vezzulli et al. 2008, Moreno et al. 2011), were mostly encountered in fish-farm-impacted sediments in both habitats and all regions.

Results reported here, on the one hand, indicate that nematode species richness and other structural biodiversity indexes do not respond to the organic pollution associated with aquaculture activities. On the other hand, we show here that the relative importance of coloniser versus persistent nematode species (as measured via the maturity index) and the turnover diversity provide a robust indication of the environmental disturbance associated with organic wastes produced by fish farms in different environmental contexts and regions. Moreover, our analysis has revealed that some nematode genera and species are putative candidates to become biological indicators of fish-farm impact. Since our study was limited to the

Mediterranean Sea context and carried out in a reduced selection of the possible sedimentary habitats affected by fish-farm wastes, we suggest new research to confirm, and possibly enforce, the suitability of the descriptors tested in this study for assessing and monitoring the impacts of aquaculture on soft bottoms.

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