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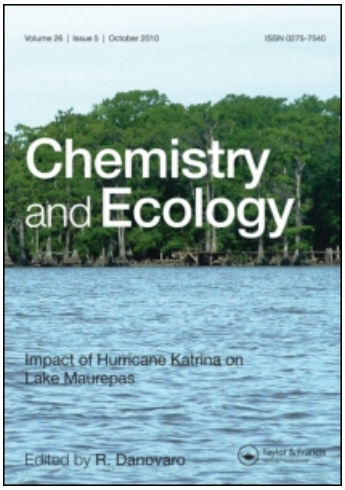

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SEDIMENT ORGANIC MATTER AND MEIOFAUNA COMMUNITY RESPONSE TO LONG-TERM FISH-FARM IMPACT IN THE LIGURIAN SEA (WESTERN MEDITERRANEAN)

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Quantitative and qualitative changes in meiofauna community structure were investigated to assess the impact of a fish farm, which was operating continuously for 15 years (La Spezia Gulf, W Mediterranean). Sediment samples were collected in June, July, September, October 2000 and February 2001 for the analysis of phytopigments (chlorophyll-*a* and phaeopigments), the biochemical composition of organic matter (proteins, carbohydrates and lipids) and related to meiofaunal parameters.

Sediment organic matter reached extremely high concentrations beneath the fish cages when compared to the control. Particularly lipids, carbohydrates and chlorophyll-*a* were significantly higher in fish-farm sediments. On a long-term basis meiofauna displayed adaptations in sediments beneath the cages resulting in an increase of density. Organic impact on meiofaunal community structure was evident in terms of an increase of the nematodes to copepods (Ne/Co) and nauplius to copepods (Na/Co) ratios in fish farm sediments. Cumaceans and kinorhynchs were encountered in control sediments, but disappeared in fish-farm samples. These data suggest that meiofauna is a sensitive tool for evaluating the effects of organic enrichment in fish farm impacted areas.

Keywords: Meiofauna; Organic matter; Fish farm; Long-term impact; Organic enrichment

1 INTRODUCTION

In recent years, aquaculture production and intensive fish-farm activities has rapidly increased worldwide, causing increasing concern for their impact on the environment. This appears to be of particular importance in semi-enclosed systems such as the Mediterranean Sea (GESAMP, 1990).

The most evident effects of the fish cages on bottom sediments are the accumulation of organic matter (OM) and the progressive transformation of the substrate into a flocculent anoxic environment (Holmer, 1991). Previous studies demonstrated that increasing organic loads determined by fish farming in coastal areas have an impact on the community structure and biodiversity of the benthic assemblages (Warwick, 1986; Mazzola *et al.*, 1999;

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Mirto *et al.*, 1999; 2000) and, in some extreme cases, might result in azoic sediments (Weston, 1990). Meiofaunal assemblages – due to their small size, high turnover rates and the lack of larval dispersion – respond rapidly to changes in environmental conditions, thus becoming one of the most popular tools in environmental monitoring (Warwick *et al.*, 1990; Warwick, 1993; Danovaro, 2000; Danovaro *et al.*, 2000). Recent investigation on meiofaunal changes in fish-farm impacted areas provide new insights on the spatial and temporal extent of the organic enrichment (Duplisea and Hargrave, 1996) and demonstrate that increasing organic loads caused disturbance beneath the cages determining a strong reduction of the meiofaunal biomass and richness (Mazzola *et al.*, 1999; Mirto *et al.*, 1999; 2000). However, all these studies investigated the initial impact of fish farms on meiofauna and to our knowledge no information is available on fish farms operating continuously for several years in the same location without being stopped. This information is of primary importance when planning the management of fish-farm plants.

We investigated the impact of organic loads due to the biodeposition of a fifteen-years established fish farm on the meiofaunal assemblages. Meiofaunal density and community structure in farm sediments were compared to a control station. Our aims were:

(i) to assess changes in meiofaunal density and community structure due to the presence of the fish farm and the establishment of reducing conditions in the upper sediment layer.
(ii) to investigate the relationships between the organic matter enrichment and meiofauna after a long-term impact.
(iii) to identify the parameters, among those considered, which better describe the long-term impact.

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2 MATERIAL AND METHODS

2.1 Study Site and Sampling Strategy

This study was carried out in the Gulf of La Spezia (Ligurian Sea, NW Mediterranean Sea). The fish-farm under investigation is located in a semi-enclosed bay and, at the time of study, contained a biomass of 16 tonnes of fish (12 Kg m^3). Up to the day of sampling in June 2000 the site has been used for approximately 15 years for the commercial rearing of the gilthead sea bream (*Sparus aurata*). The farm consisted of a total of 4 floating-wharves aligned from the coast to the open sea, grouping 22 cages each for a total of 88 cages (each 36 m^3). Grouped cages were arranged in two rows and equipped with automatic feeders. Supplied food was composed of proteins (46–51% of dry weight), carbohydrates (18–20%), lipids (14–17%) and the remaining fraction is made up of ash, vitamins and pigments.

The area (water depth 10 m) is sheltered and dominant current flows in the SE–NW direction, following the cyclonic circulation of the NW Mediterranean Sea. A preliminary survey was conducted to determine the spatial extent of the farm impact and for identifying the control station. Three stations were selected: two cage stations located along a transect crossing the farming area running from the coast towards the open sea (station 2 and 1 respectively) and a control site (station 3, sampled on June, September and February) located about 200 m distance from the fish farm (Fig. 1). Sediment samples were collected manually by SCUBA divers in June, July, September, and October 2000 when the effect of biodeposition was expected to be highest and on February 2001 (station 2 was not sampled on June 2000). At each sampling, a total of 10 sediment cores was collected by pushing Plexiglas tubes into the sediments. Once brought to the surface, the sediment core was cooled in ice and immediately transported to the laboratory.

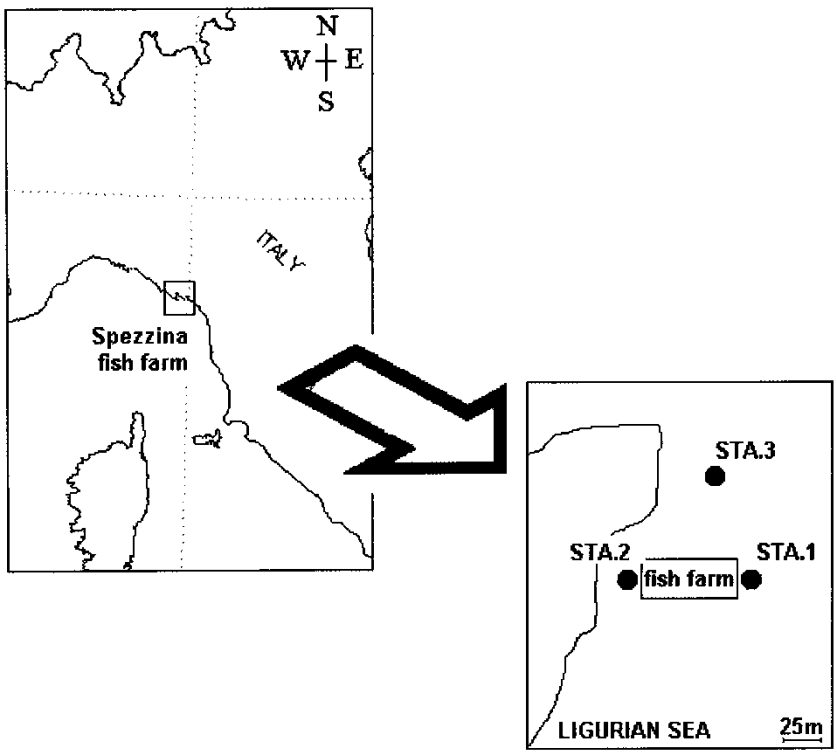

FIGURE 1 Sampling area and station locations (Gulf of La Spezia, Western Mediterranean).

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Meiofaunal samples were collected in replicate cores ($n = 3$, inner diameter 3.9 cm, surface area 11 cm^2) and each core was vertically sectioned into different layers. After a preliminary assessment of the percentage of recovery (PR) of total meiofaunal density in the top 10 cm layer (data not shown) only the top 3 cm (0–1 and 1–3 cm depth) were analyzed (PR > 90%). For the analysis of phytopigments and sedimentary organic matter, each sediment layer was mixed and frozen at $-20\,^{\circ}C$.

2.2 Environmental Parameters

Vertical profiles of redox potential (*Eh*) were determined immediately after collection using an *Eh*-meter (HI-9025), previously calibrated using a conductivity solution HI7030L (11.67 mS/cm at 20 °C), down to 3 cm depth. Chlorophyll-*a* (Chl-*a*) and phaeopigment (Phaeo) concentrations were determined according to Lorenzen and Jeffrey (1980). Chloroplastic pigments were extracted in 90% acetone (overnight, 4 °C). After centrifugation the supernatant was used to determine Chl-*a* content, and then acidified with 0.1 N HCl to estimate phaeopigments. Chloroplastic pigment equivalent (CPE) were calculated as sum of chlorophyll-*a* and phaeopigments.

Proteins (PRT) were determined according to Hartree (1972). Bovine albumin solutions were used as standard. Carbohydrates (CHO) were analyzed according to Dubois *et al.* (1956) and D(+) glucose solutions were used as standard. Lipids (LIP) were extracted according to Bligh and Dyer (1959) and measured following Marsh and Weinstein (1966). Tripalmitine solutions were used as standard. Biopolymeric carbon concentrations (BPC)

were calculated according to Fabiano and Danovaro (1994) as sum of lipid, protein and carbohydrate carbon.

2.3 Meiofaunal Analysis

For meiofaunal analysis, sediment samples were passed through 1000 and 37 μm mesh sieves to retain the smallest meiofaunal organisms (Danovaro *et al.*, 2000). The fraction remaining on the 37 μm sieve was centrifuged three times with Ludox HS 40 (density 1.24 g cm^{-3}) as described by Heip *et al.* (1985). All meiobenthic animals were counted and classified per taxon under a stereomicroscope after staining with Rose Bengal (0.5 g L^{-1}).

2.4 Statistical Analyses

Two way Factorial analysis of variance (ANOVA) and Tukey-Post hoc test were used to detect differences in environmental parameters among sediment layers and sampling stations. The Kruskal–Wallis test was used to compare meiofauna abundance among sites. When necessary square root transformed data were used to avoid heteroscedasticity and/or correct residuals. Spearman rank correlations were performed between meiofaunal density and environmental variables. All statistical tests and correlation analyses were performed using the statistics toolbox, R12, of MATLAB.

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3 RESULTS

3.1 Environmental Parameters

The investigated environmental parameters are reported in Table I and the results of ANOVA and Tukey-Post hoc test are reported in Table II. Reducing conditions were observed in the top 1 cm sediments layer beneath the fish cages ($Eh < -50$ mV) while positive values were observed at the control station. Chloroplastic pigments equivalent (CPE) concentrations were higher in farm sediments ranging from 24.6 to 256.4 μg g^{-1} in the top 3 cm, while lower values (range 8.9–35.6 μg g^{-1}) were observed at the control station. The BPC was significantly higher beneath the fish cages (sta 2, mean 5.54 ± 1.29 mgC g^{-1}) than at station 3 (mean 2.70 ± 1.32 mgC g^{-1}) in the top 3 cm. All biochemical components displayed highest values in the top 1 cm layer (ANOVA, $p < 0.05$). Proteins ranged from 0.75 to 6.20 mg g^{-1} in the top 3 cm, but did not display significant differences between fish farm and control stations. Carbohydrates were the dominant biochemical class of organic compounds and ranged in the top 3 cm from 0.39 in June to 6.58 mg g^{-1} in October. Carbohydrate concentrations were significantly higher beneath fish cages in the top 1 cm of the sediment (range 1.83–6.58 mg g^{-1}) than at the control site (range from 0.71 to 0.85 mg g^{-1}). No significant differences were found comparing station 1 and 2 for either protein or carbohydrate concentrations.

Lipid concentrations ranged from 0.28 to 5.02 mg g^{-1} in the top 3 cm and displayed spatial pattern similar to that observed for carbohydrates. Their concentrations were higher in sediments beneath the fish cages and ranged from 0.65 to 4.97 mg g^{-1} and from 0.71 to 2.93 mg g^{-1} in the top 1 cm in fish farm and control stations, respectively. A difference in lipid concentration occurred between fish-farm stations, with the highest values recorded at station 2 in September (4.97 mg g^{-1}).

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TABLE I Environmental Parameters (Average and Standard Deviations) in the Sediment of the Three Sampling Stations During the Study Periods.

Date	*Station*	*Layer (cm)*	*Eh (mv)*	*BPC* $mgC\,g^{-1}$	*PRT* $mg\,g^{-1}$	*Std*	*CHO* $mg\,g^{-1}$	*Std*	*LIP* $mg\,g^{-1}$	*Std*	*CPE* $\mu g\,g^{-1}$	*Chl-a* $\mu g\,g^{-1}$	*Std*	*Phaeo* $\mu g\,g^{-1}$	*Std*
23-Jun-2000	1	0–1	−98	4.71	2.99	0.3	3.78	1.0	2.31	0.6	256.4	37.7	4.0	218.7	9.5
		1–3	−105	3.24	1.95	0.1	1.54	0.2	2.23	0.9	90.3	8.2	0.0	82.1	30.7
	3	0–1	−52	1.86	2.07	0.2	0.78	0.0	0.71	0.2	35.6	3.7	0.1	31.9	12.7
		1–3	−116	1.57	1.68	0.2	0.39	0.1	0.79	0.2	na	na	na	na	na
18-Jul-2000	1	0–1	na	2.06	0.75	0.1	3.00	1.4	0.65	0.0	138.0	15.6	0.5	122.4	34.6
		1–3	na	1.09	0.98	0.1	1.00	0.4	0.28	0.0	63.4	6.7	0.2	56.7	15.1
	2	0–1	na	5.90	3.49	0.7	3.09	0.4	3.94	1.7	88.7	10.5	0.0	78.2	2.7
		1–3	na	4.80	2.72	0.3	3.18	0.7	2.93	1.4	87.8	7.3	0.1	80.4	36.2
14-Sep-2000	1	0–1	−150	3.19	1.72	0.3	3.36	1.0	1.34	0.4	177.2	25.9	3.7	151.4	10.2
		1–3	−200	3.78	2.06	0.1	2.53	0.9	2.34	0.2	104.4	8.3	0.1	96.1	12.1
	2	0–1	−144	5.10	1.08	0.2	2.11	0.5	4.97	0.8	61.0	5.5	0.3	55.5	4.0
		1–3	−220	3.44	1.00	0.2	1.12	0.1	3.33	0.9	53.4	2.9	0.2	50.5	4.9
	3	0–1	77	2.55	2.18	0.2	0.85	0.0	1.52	0.3	21.5	1.2	0.0	20.4	7.5
		1–3	−49	1.55	1.05	0.2	0.60	0.0	1.06	0.3	8.9	0.6	0.0	8.3	0.0
27-Oct-2000	1	0–1	−180	4.48	2.22	0.7	3.74	1.0	2.53	0.5	157.8	19.6	1.7	138.2	27.3
		1–3	−200	4.71	3.25	0.8	2.78	0.8	2.68	1.2	78.2	6.0	0.1	72.2	4.8
	2	0–1	−84	6.07	2.31	0.2	6.58	0.2	3.07	0.7	126.9	11.0	0.1	115.9	16.2
		1–3	−110	4.71	1.88	0.1	3.21	0.2	3.34	0.9	31.4	3.4	0.1	28.0	14.5
2-Feb-2001	1	0–1	−220	7.44	6.20	0.9	1.83	0.0	4.90	0.6	36.2	8.1	5.0	28.1	2.0
		1–3	−292	5.95	6.17	2.0	1.77	0.1	2.96	1.0	54.6	7.7	0.9	46.9	0.2
	2	0–1	−62	7.05	4.79	1.6	2.90	0.4	4.72	1.8	24.6	2.7	0.3	21.9	2.1
		1–3	−172	7.29	4.19	1.2	3.69	0.6	5.02	1.7	31.2	3.3	0.8	27.9	5.8
	3	0–1	45	4.43	3.97	0.4	0.71	0.0	2.93	1.6	10.7	1.0	0.3	9.7	2.0
		1–3	−72	4.26	3.34	0.4	0.94	0.0	3.00	0.3	13.6	1.0	0.3	12.6	2.6

Note: Eh, redox potential values; BPC, biopolymeric carbon; PRT, proteins; CHO, carbohydrates; LIP, lipids; CPE, chloroplastic pigment equivalent; Chl-*a*, chlorophyll-*a*; Phaeo, phaeopigments; na, not available.

TABLE II Results of the ANOVA Test and Multiple Comparison of Means for the Investigated Environmental Parameters.

	ANOVA	*Tukey-Post hoc*		
	Sta1–sta2–sta3 p-level	*Sta1–sta2 p-level*	*Sta1–sta3 p-level*	*Sta2–sta3 p-level*
BPC	0.001	ns	ns	s
PRT	ns	ns	ns	ns
CHO	0.001	ns	s	s
LIP	0.002	s	ns	s
CPE	0.01	ns	s	ns

Note: BPC, biopolymeric carbon; PRT, proteins; CHO, carbohydrates; LIP, lipids; CPE, chloroplastic pigment equivalent; s, significant, ns, not significant, sta, station.

3.2 Meiofaunal Density and Community Structure

Total meiofauna densities and community structure are shown in Figures 2 and 3 respectively.

Meiofauna abundance ranged from 1627 to 8076 ind./10 cm^2 in the top 3 cm of the sediment. Highest densities were always found in fish-farm stations (mean 3959 ± 1713 and 5261 ± 1896 ind./10 cm^2 at stations 1 and 2, respectively) and the lowest densities were measured at the control site (mean 2099 ± 646 ind./10 cm^2 at station 3). Both total meiofauna and the nematode density (range 1054–6771 ind./10 cm^2 in the top 3 cm) displayed significant differences among stations (Kruskal–Wallis, $p < 0.05$) and did not show seasonal variability (ANOVA, ns).

Nematodes comprised on average more than 80% of the total meiofaunal abundance and in particular showed the highest percentage in sediment beneath the fish cages (up to 92% at station 1 in June). Nauplii and harpacticoid copepods together accounted for 10% and polychaetes accounted for 1%. The relative abundance of copepods increased considerably at the control and reached a mean percentage of 26% in the top 3 cm layer. The increase of copepods was mainly confined to the upper 1 cm of the sediment where over 80% of the individuals were found. Other minor taxa accounted for a very small percentage (less than 1%) of total meiofaunal density and did not display variation either between sites or sediment layers. The group 'others' includes tanaidaceans, isopods and amphipods and though in a small percentage (1%) were mostly detected at station 3 in the top 1 cm. Other minor taxa such as cumaceans and kynorhynchs were only found in control sediments.

4 DISCUSSION

The results of the present study clearly indicate an accumulation of organic matter in sediments underneath fish cages. Reducing conditions were also observed in the top 1 cm layer of farm sediments during the entire sampling period. Clear differences between fish farm and control sediments were measured in terms of chloroplastic pigments equivalent and biopolymeric carbon concentrations that were significantly higher in sediment beneath fish cages. Also the biochemical composition of sediment organic matter in farm sediments and in the control was different. First, the contribution of the carbohydrates and lipids to the biopolymeric carbon fraction was significantly higher at the fish-farm stations (Tab. II). The high lipid concentrations observed in fish-farm sediments is likely the result of the high lipid content (about average 15% of DW) of the fish diet.

An enrichment in terms of sedimentary organic carbon has been reported in several studies dealing with fish-farm impact (Castel *et al.*, 1989; Gilbert *et al.*, 1997; Grenz *et al.*, 1990), and particularly of the biopolymeric carbon fraction (Mazzola *et al.*, 1999).

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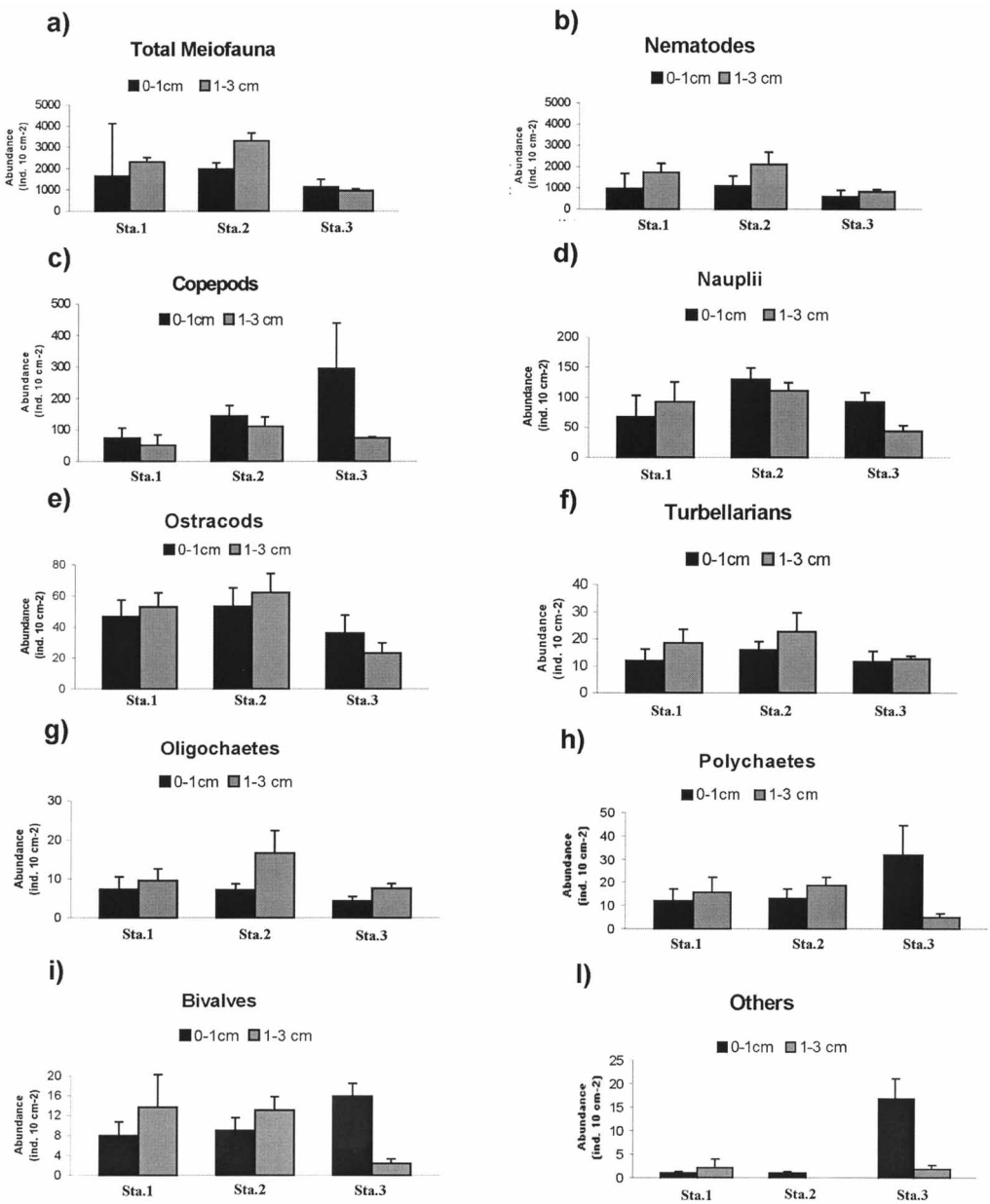

FIGURE 2 Meiofaunal density for the top 3 cm (layers 0–1 cm and 1–3 cm) of the sediment at the three sampling stations. Reported are: (a) total meiofauna density; (b) nematodes; (c) copepods; (d) nauplii; (e) ostracods; (f) turbellarians; (g) oligochaetes; (h) polychaetes; (i) bivalves; (j) others (see main text). Data are expressed as ind. $10\,cm^{-2}$ ($\pm$SD).

Meiofaunal densities at the control station were much higher than values reported at similar depth in the Ligurian Sea (Danovaro, 1996), but comparable to those reported in rich/eutrophic coastal areas, such as the Adriatic Sea (Danovaro *et al.*, 2000) and in other fish-farm sediments of the Mediterranean Sea (Mazzola *et al.*, 1999; Mirto *et al.*, 2000). Previous studies in fish-farm areas have shown that organic enrichment might determine

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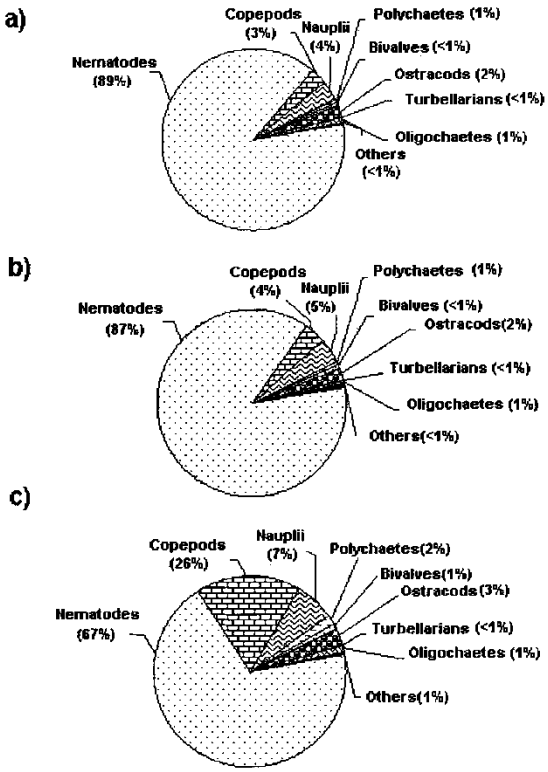

FIGURE 3 Meiofaunal community structure (as mean percentage during the sampling period) for the top 3 cm of the sediment at the control and cage stations. (a) Station 1; (b) station 2; (c) station 3.

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an increase of meiofaunal density due to the higher organic matter availability (Castel *et al.*, 1989; Dinet *et al.*, 1990; Geulorget *et al.*, 1994). Generally, the initially positive response of meiofaunal density to organic enrichment is followed by a decrease in meiofaunal abundance, due to changes in the sedimentary characteristics (*i.e.* reduced oxygen fluxes at water-sediments interface). Mazzola *et al.* (2000) reported a significant reduction (50%) of the meiofaunal density in a Mediterranean fish-farm as a consequence of the disturbance caused by the establishment of the farm activity. In contrast, in our study, meiofauna densities in the fish-farm sediments were significantly higher than in the control. Therefore, in the long term, meiofauna apparently adapted to the modified sedimentary conditions, displaying an increased density.

Meiofaunal abundance did not change markedly with season and was not correlated with phytopigments and bacterial densities (data not shown), which generally represent the main food sources for meiofauna (Danovaro, 1996). Conversely, both total meiofauna and nematode abundance were significantly related with biopolymeric carbon ($n = 10$, $p < 0.05$) and lipids ($n = 10$, $p < 0.05$). The strong relationship between meiofaunal density and lipid concentrations is particular intriguing since lipids have been recently recognized as a potential biochemical marker of fish-farm activity in coastal sediments (Vezzulli *et al.*, 2002) and may alter sediment characteristics (*e.g.* oxygen exchange at the water-sediment interface).

Generally, nematodes are considered a taxon resistant to low oxygen concentrations and exposure to sulphide, while copepods could be more sensitive (Murrel and Fleeger, 1989; Hendelberg and Jensen, 1993). Organic impact on meiofaunal community structure was evident in terms of increased ratio of nematodes to copepods (Ne/Co), in fish farm sediments.

Also the nauplius to copepod abundance ratio (Na/Co) was found to be four times higher in sediments beneath the cages. This interesting result may be addressed and verified in future studies aimed at the investigation of the fish-farm impact on meiofaunal recruitment. The high Na/Co ratio found in fish-farm sediments may indicate an increase of the recruitment rates of resistant copepods in organic-rich sediments. A plausible alternative explanation for such an increase may be the reduction in adults copepods combined with unchanging abundances of nauplius.

The long-term effect (*i.e.* 15 years) of organic enrichment and the establishment of reducing condition in fish-farm sediments caused a clear change in the structure of meiofaunal assemblages. Polychaetes, ostracods, turbellarians, bivalves and oligochaetes did not display significant changes among sites, whereas other taxa appeared particularly sensitive to organic enrichment and have been largely reduced in abundance (amphipods and isopods) or disappeared (cumaceans and kinorhynchs) in fish-farm sediments.

We conclude that short and long term impact of fish farm on the meiofauna might have completely different ecological consequences. On a short term basis, as pointed out by several authors, meiofaunal density decreases (Mazzola *et al.*, 1999; Mazzola *et al.*, 2000; Mirto *et al.*, 2000; La Rosa *et al.*, 2001), whereas in the long-term meiofauna displayed adaptations in the farm sediments resulting in an increase of density and changes of the assemblages structure.

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