

## Fish-farming effects on benthic community structure in coastal sediments: analysis of meiofaunal recovery

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To study the impact of organic enrichment and high biodeposition caused by intensive fish farming in coastal sediments, meiofaunal samples were collected on a monthly basis between March and October 1997 at two stations of the Gaeta Gulf: one under the Farmocean cage (2000 m<sup>3</sup>), while the other (the control) was located at about 1 km from an area not impacted by fish farming. The fish farm contained about 120 000 *Dicentrarchus labrax* (density 18 kg m<sup>-3</sup>). The most evident changes in the benthic habitat under the cage were a large accumulation of primary organic material (phytopigment concentrations up to 44 µg g<sup>-1</sup>), changes in sedimentary organic matter composition (increased lipid levels related to the composition of the fish diet), and a strong reduction of redox potential values, which resulted in a significant reduction of meiofaunal penetration depth into the sediments. Organic loads had a clear impact on meiofaunal densities, which were 50% lower under the cage than at the control site (1112 ± 118 and 2160 ± 339 ind. 10 cm<sup>-2</sup>, respectively). Compared with the control, farm sediments also showed an increased importance of copepods, which dominated the meiofauna together with nematodes (both 39% of the total density) and polychaetes (17%). The removal of the fish farm from the site on July 1997 allowed an analysis to be made of the initial short and medium-term recovery of the assemblages. After two months, meiofaunal densities were closer to the control (about 30% recovery). Community structure recovered only partially to the characteristics typical of the study area, with increased importance of nematodes (about 70% of total density) and reduced copepod contribution, but it was still characterized by a lower number of taxa after four months. These data suggest that meiofaunal recovery after fish farm disturbance is rapid, but far from complete after four months.

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### Introduction

The rapid expansion of aquaculture activities in coastal marine areas during the past twenty years has induced a general concern for the impact on critical environmental variables (GESAMP, 1990; Wu, 1995). The most evident effects of fish cages on bottom sediments is the accumulation of organic matter and the progressive transformation of the substrate into a flocculent anoxic environment (Holmer, 1991; Henderson *et al.*, 1997; Karakassis *et al.*, 1998). Such changes in the physical and chemical characteristics of the sediment generally have a strong impact on the structure of the benthic

assemblages (Brown *et al.*, 1987; Pocklington *et al.*, 1994). Despite the evident impact on the sediments under the cages, the results of previous studies carried out at temperate latitudes indicate that environmental impact is restricted to the immediate vicinities of the farm (Wu *et al.*, 1994; Karakassis *et al.*, 1998). The extent of the area affected by fish farms and the speed of recovery of benthic assemblages after their removal is crucial for planning a rational use of the coastal areas for aquaculture activities.

Owing to their small size, high turnover, and lack of larval dispersion, meiofauna are becoming the most popular tool in environmental monitoring (Higgins and

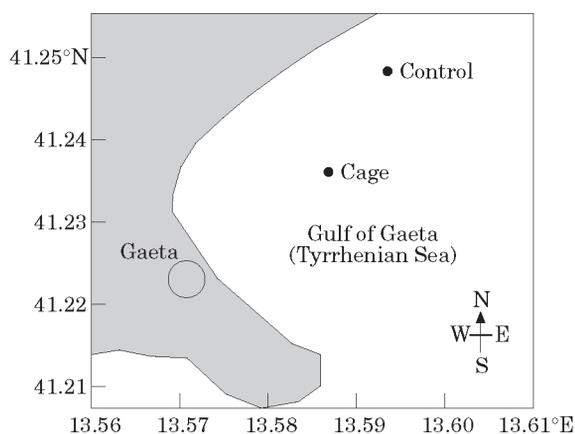


Figure 1. Sampling stations in the Gaeta Gulf, Tyrrhenian Sea (Western Mediterranean).

Thiel, 1988; Sandulli and De Nicola Giudici, 1990; Green and Montagna, 1996; Sarkka, 1996; Austen and McEvoy, 1997). Meiofaunal sensitivity to changes in environmental conditions suggests that this component might provide insights into the spatial and temporal extent of the farm impact (Duplisea and Hargrave, 1996). Moreover, their high turnover rates should provide important information on the recovery of the benthic environment when aquaculture installations are removed. Previous studies on fishfarm impact on meiofaunal assemblages have pointed out that neither biomasses nor size spectra were significantly affected by organic enrichment of the sediment (Duplisea and Hargrave, 1996), but information on the recovery of meiofaunal assemblages after disturbance induced by the presence of farm sediments is practically non-existent (Giere, 1993).

This study deals with the analysis of meiofaunal density and community structure in the sediments beneath a fish farm. The removal of the structure during the investigation provided a unique opportunity to study the recovery of the assemblages. Our aims are: (a) to assess changes in meiofaunal assemblages induced by the presence of a fish farm; (b) to identify the sedimentary parameters among those considered that best describe the impact; (c) to evaluate meiofaunal recovery after removal of the farm; and (d) to investigate differences in response of different taxa during the recovery process.

## Materials and methods

### Study site and sampling

The study was conducted from March to October 1997 in the Gaeta Gulf (Tyrrhenian Sea, NW Mediterranean Sea, Fig. 1). The area is characterized by limited seasonal changes in temperature and by the presence of two major rivers (Garigliano and Volturno), both in the

southern part of the gulf. Dominant currents flow in a SE-NW direction following the cyclonic circulation of the Tyrrhenian Sea. The area is sheltered and characterized by sandy-muddy sediments. *Posidonia oceanica* meadows are present in the northern part, although of limited extent (Ferretti *et al.*, 1989). The fishfarm selected (Farmocean, 2000 m<sup>3</sup>) was known to be scheduled for removal within a few months. It was located in the inner part of the gulf and contained about 120 000 *Dicentrarchus labrax* (final biomass before harvesting, 18 kg m<sup>-3</sup>). Fish were fed using automatic distributors supplying an amount of pellets equivalent to about 3% of the total biomass contained in the cage. Food supplied was composed for 46–51% of DW by proteins, 18–20% carbohydrates, and for 14–17% lipids; the remaining fraction is accounted for by ash, vitamins, and pigments.

Sediment samples were collected manually by scuba divers on a monthly basis. A preliminary survey was carried out to determine the spatial extent of the farm impact and for identifying the control station. Two stations were selected: the cage station and a control (about 1 km to the north of the farm site in an area not affected by aquaculture installations). Both stations were located at a depth of 10 m and displayed a very similar sediment texture (silt-clay fraction accounted for 13.2% at the control and 13.9% at the cage station). In early July 1997, the fish farm was removed, allowing an evaluation of the changes in the benthic environment once input of organic matter from the farm had stopped.

Meiofaunal samples were collected in replicate cores (n=3, inner diameter 3.7 cm, surface area 10.7 cm<sup>2</sup>) down to a depth of 10 cm. The sediment of two additional cores (diameter, 3.7 cm) was sectioned into different layers (0–1, 1–5 and 5–10 cm). Each sediment layer was mixed and frozen at –20°C for the analysis of photosynthetic pigments and organic matter parameters.

### Environmental variables

The redox potential discontinuity (RPD) layer was visually estimated at the depth where sediment colour turns from brown to black. Lipids were extracted from dried sediment samples by direct elution with chloroform and methanol, according to Bligh and Dyer (1959) and Marsh and Weinstein (1966). Protein analyses were carried out according to Hartree (1972). Concentrations are presented as albumin equivalents. Carbohydrates were analysed according to Gerchacov and Hatcher (1972), specifically adapted for carbohydrate determination in sediments. For each analysis about 0.5 g of sediment was used. Carbohydrate, protein, and lipid concentrations were converted to carbon equivalents assuming conversion factors of 0.40, 0.49, and 0.75, respectively. The sum of protein, lipid, and carbohydrate carbon is referred to as the biopolymeric fraction (BPF);

Table 1. Average monthly values ( $\pm$  standard deviations) of Redox Potential Discontinuity layer depth (RPD) and concentrations of proteins (PRT), carbohydrates (CHO), and lipids (LIP) in the top 1-cm sediment layer.

	RPD (cm)		PRT ( $\mu\text{g g}^{-1}$ )		CHO ( $\mu\text{g g}^{-1}$ )		LIP ( $\mu\text{g g}^{-1}$ )	
	Control	Cage	Control	Cage	Control	Cage	Control	Cage
Mar.	1.4 $\pm$ 0.2	1.1 $\pm$ 0.7	1164 $\pm$ 233	997 $\pm$ 157	5257 $\pm$ 106	52 451 $\pm$ 635	489 $\pm$ 112	752 $\pm$ 208
Apr.	2.1 $\pm$ 0.2	0.2 $\pm$ 0.3	2523 $\pm$ 352	2227 $\pm$ 278	1241 $\pm$ 488	1297 $\pm$ 680	1220 $\pm$ 410	1484 $\pm$ 404
May	1.9 $\pm$ 0.4	0.0 $\pm$ 0.0	3898 $\pm$ 1036	2876 $\pm$ 243	1126 $\pm$ 245	2261 $\pm$ 624	368 $\pm$ 151	2151 $\pm$ 1234
June	3.1 $\pm$ 1.0	0.0 $\pm$ 0.0	4053 $\pm$ 527	7069 $\pm$ 1060	1656 $\pm$ 770	5202 $\pm$ 706	1037 $\pm$ 111	6944 $\pm$ 2948
July	2.9 $\pm$ 0.5	2.0 $\pm$ 0.6	2443 $\pm$ 120	1940 $\pm$ 156	2815 $\pm$ 1470	1034 $\pm$ 260	1436 $\pm$ 250	1138 $\pm$ 434
Aug.	1.4 $\pm$ 0.2	1.8 $\pm$ 0.4	2976 $\pm$ 290	1776 $\pm$ 263	1811 $\pm$ 904	1488 $\pm$ 113	1321 $\pm$ 292	1216 $\pm$ 308
Sep.	1.6 $\pm$ 0.4	1.2 $\pm$ 0.4	2568 $\pm$ 273	1848 $\pm$ 341	1583 $\pm$ 409	2211 $\pm$ 503	1010 $\pm$ 244	1717
Oct.	1.7 $\pm$ 0.3	1.1 $\pm$ 0.3	142 $\pm$ 179	1520 $\pm$ 629	821 $\pm$ 182	1890 $\pm$ 567	2096 $\pm$ 332	1049 $\pm$ 49

Fabiano and Danovaro 1994). For each biochemical analysis, blanks were made using previously calcinated sediments (450°C, 2 h) from the same samples. All analyses were carried out in three replicates. Only the results of the top 1 cm layer are reported here. Chlorophyll *a* analysis ( $n=3$ ) was carried out according to Lorenzen and Jeffrey (1980). Chloroplastic pigment equivalents (CPE) are referred as the sum of Chlorophyll *a* (Chl. *a*) and phaeopigment concentrations.

### Meiofaunal analysis

Samples were fixed with 4% (v/v) buffered formaldehyde in 0.4  $\mu\text{m}$  prefiltered seawater solution. Sediments were sieved through 1000 and 37  $\mu\text{m}$  mesh sieves. The fraction remaining on the 37  $\mu\text{m}$  mesh sieve was centrifuged three times with Ludox HS (density 1.18  $\text{g cm}^{-3}$ ) as described by Heip *et al.* (1985). All meiobenthic animals were counted and classified by taxon under a stereomicroscope after staining with Rose Bengal (0.5  $\text{g l}^{-1}$ ).

## Results

### Environmental parameters

The RPD depth (Table 1) was, on average, significantly deeper at the control (range: 1.4 in March to 3.1 cm in June) than at the cage station, (0 in May-June to 1.8 cm in August). After farm removal, RPD depth was not clearly different between the stations.

Chl. *a* concentrations ranged from 4.9 (October) to 21.9  $\mu\text{g g}^{-1}$  (May) at the control station and from 3.7 (September) to 40.9  $\mu\text{g g}^{-1}$  (May) at the cage station (Fig. 2a). Concentrations dropped sharply in farm sediments after cage removal. Similar temporal patterns were displayed by CPE, which decreased by more than 80% between June and July at the cage station (Fig. 2b).

Table 1 provides the biochemical composition of sedimentary organic matter (as protein, lipid, carbohydrate, and biopolymeric carbon concentrations). Proteins were generally the dominant biochemical class of organic compounds with lowest values in March and

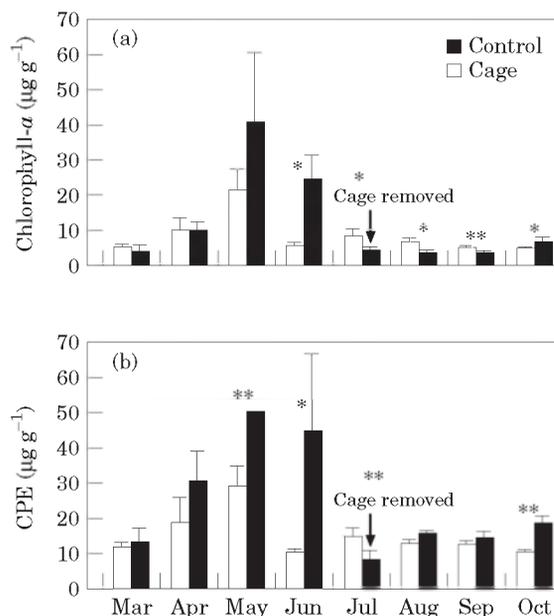


Figure 2. Temporal changes in phytopigment concentrations with standard deviations and indication of significant differences between stations (\*= $p<0.05$ , \*\*= $p<0.01$ ; two-way ANOVA) in the top 1 cm of sediment: (a) Chl. *a* concentrations; (b) chloroplastic pigment equivalent (Chl. *a* plus phaeopigments).

highest ones in June at both stations and no consistent differences between sites. Sedimentary carbohydrates were lowest in October and highest in March at the control site and lowest in July and highest in June at the cage site. Finally, lipids were lowest in May and highest in October at the control and lowest in March and highest in June at the cage station. Their temporal patterns might be summarized by the biopolymeric carbon trend that generally showed no clear differences between the two stations even after farm removal, with the exception of June when farm sediments displayed BPC concentrations about double that of the control.

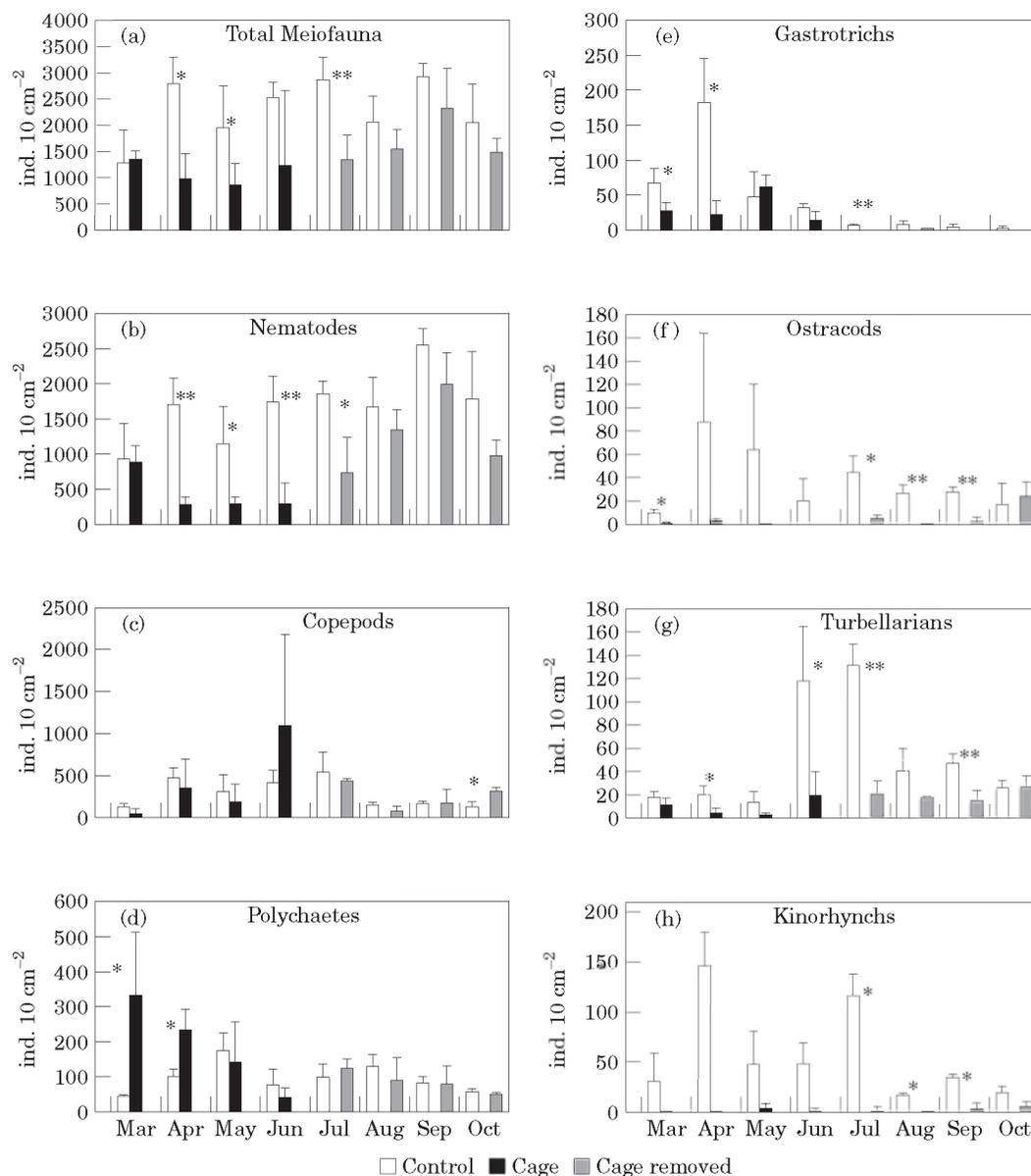


Figure 3. Temporal variations in meiofaunal density (ind./10 cm<sup>2</sup>) with standard deviations and indication of significant differences between stations (\*= $p < 0.05$ , \*\*= $p < 0.01$ ; two-way ANOVA; period of farming and after cage removal indicated): (a) total meiofauna; (b) nematodes; (c) copepods; (d) polychaetes; (e) gastrotrichs; (f) ostracods; (g) turbellarians; (h) kinorhynchs.

### Meiofaunal abundance and community structure

Meiofaunal density in the top 10 cm at the two stations is given by major taxa in Figure 3. The total densities during and after fish farming are compared in Figure 4. During March–June, meiofaunal density at the control was about twice as high as at the cage station (t-test,  $p = 0.02$ ), while after cage removal (July–October) density at the latter was only about 20% lower than at the control. The vertical distribution of the meiofauna in

the sediment during the two periods (Fig. 5) indicated that during the period of farming meiofauna tended to concentrate in the top 1 cm of the sediment (75% of the total density under the cage vs. 64% at the control site), whereas after cage removal, only 42% of the total density was found in the top 1 cm. The community structure displayed clear differences between the stations (Fig. 6). At the control station, nematodes accounted for the largest proportion of the total density, followed by copepods, polychaetes, kinorhynchs, and gastrotrichs.

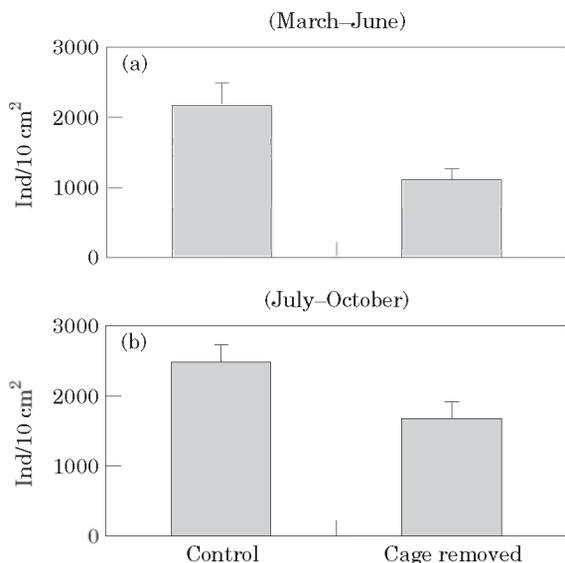


Figure 4. Comparison of mean meiofaunal density and standard error (between-month variability) between control and cage stations: (a) during cage deployment (March–June 1997); and (b) after cage removal (July–October 1997).

By contrast, during the period of farming, harpacticoid copepods were equally abundant as nematodes, both accounting for 38–39% of the total density. Polychaetes were relatively more abundant (17%) than at the control, while kinorhynchs were completely absent. After cage

removal, community structure in farm sediments was characterized by nematode dominance (75%) followed by copepods (15%), polychaetes (5%), and gastrotrichs (0.1%). Kinorhynchs were occasionally encountered and accounted for a small percentage (0.2%).

### Discussion

Between March and June, differences between farm sediments and those at the control were most marked during warmer months just before cage removal. The first expected difference was the reduced oxygen penetration into the sediments under the cage, causing a rise in the RPD layer. Reduced oxygen conditions were observed in May and June when biodeposition increased significantly as a consequence of the increased biomass in the cage. Fishfarm sediments are assumed to represent organic enriched environments (Hargrave *et al.*, 1993; see Danovaro, 1996, and Fabiano *et al.*, 1995, for comparison), but, with the exception of June, consistent differences in biopolymeric carbon concentrations between cage and control station could not be detected. A more detailed analysis of the composition of sedimentary organic matter revealed comparably high concentrations of proteins and carbohydrates at both stations, but lipid concentrations that were four times higher in farm sediments during the first period contrasted with comparable values after cage removal (Table 1). This effect is likely to result from the high lipid content

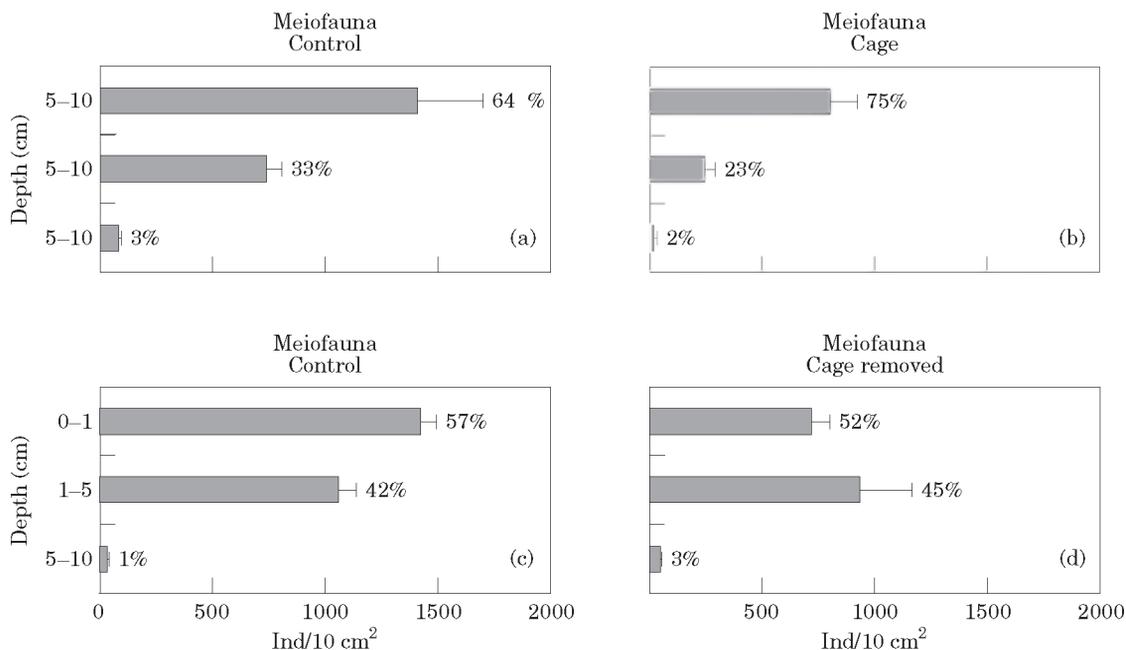


Figure 5. Vertical distribution of the percentage contribution to total meiofaunal density by sediment layer: (a) control station, March–June 1997; (b) cage station, March–June 1996 (during farming); (c) control station, July–October 1997; (d) cage station, July–October 1997 (after farm removal).

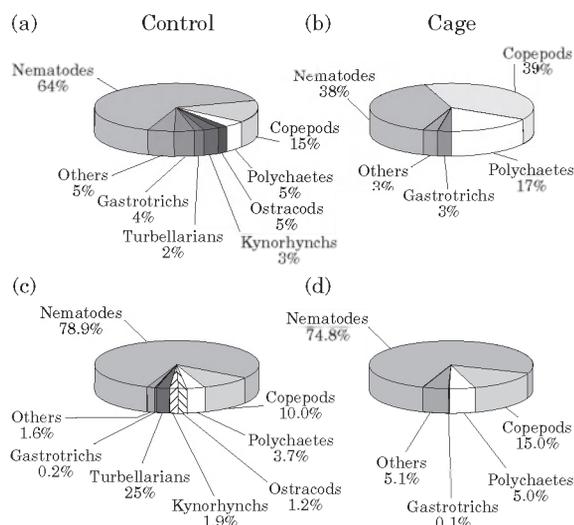


Figure 6. Meiofaunal community structure in terms of percentage contribution by major taxa to total density: (a) control station, March–June 1997; (b) cage station, March–June 1997 (during farming); (c) control station, July–October 1997; (d) cage station, July–October 1997 (after farm removal).

(about 15% of DW) of the fish diet (Henderson *et al.*, 1997). The increased lipid content might have had a significant effect on RPD layer, as it is well known that lipids can reduce the exchange of oxygen at the water–sediment interface. Therefore, our results indicate that farm sediments cannot be identified in terms of gross organic enrichment (Doyle and Garrels, 1985). By contrast, farm impact on the sediments was evident in terms of phytopigment concentrations: during the period of farming, both Chl. *a* and phaeopigment concentrations were about double those in the control. A possible explanation of the increased phytopigment input to the sediment is that bivalves attached to the cage produced large amounts of faeces and pseudofaeces. It is unlikely that the differences between cage and control site can be accounted for by an input of macroalgal debris to the sediments due to cage cleaning, because cleaning occurred almost continuously during rearing. All these elements indicate that organic input from the cage was responsible for the eutrophication (*sensu* Nixon, 1995) of the sediments beneath the farm (Hargrave *et al.*, 1993).

The meiofaunal densities encountered are comparable to those reported by Duplisea and Hargrave (1996) in sediments under a salmon aquaculture farm in the Bay of Fundy (on average  $2307 \pm 573$  ind.  $10\text{ cm}^{-2}$ ). However, these authors did not report significant changes in meiofaunal density due to organic enrichment of the sediment, and concluded that farm impact on meiofauna cannot be identified by examination of the abundance alone. By contrast, we observed a significant reduction (about 50%) of the meiofaunal density at the cage

station during fish farming compared with the control. Meiofauna (particularly nematodes) are assumed to be quite resistant to organic loading of the sediment and the resulting anoxic conditions, and some meiofaunal taxa, defined as thiobios, are permanently found in anoxic sediments (Powell, 1989; Giere, 1993). Nonetheless, our results point to a strong impact of the farm on meiofaunal assemblages, also in terms of abundance. Additional evidence of the impact of aquaculture on the benthic communities is provided by the analysis of the meiofaunal penetration depth in the sediment core. Some 75% of the total number of organisms under the cage were confined in the top 1 cm compared with about 60% at the control. Reducing conditions, particularly evident in May and June, were crucial for the vertical penetration of nematodes, which seems remarkable because nematodes are usually assumed to be more tolerant than other taxa to suboxic/anoxic conditions.

The farm was established at the location described about three years before the initiation of this study, causing semi-permanent reducing conditions under the cage in summer months. This caused a modification of the meiobenthic community structure in comparison with the control with increased contribution of copepods (from 15 to 39% of the total density) and polychaetes (from 5 to 17%) and reduced abundance of nematodes, ostracods, turbellarians, gastrotrichs, and kinorhynchs. The latter two taxa were so strongly affected by farm impact that they had disappeared completely in certain samplings. A similar increase in copepod contribution has been previously reported from shrimp culture areas (Yu and Zhang 1994). The sensitivity of certain taxa, such as ostracods and kinorhynchs, to organic pollution is in agreement with previous studies on the effects of hydrocarbons on meiofaunal assemblages (Danovaro, 2000), but the sensitivity of nematodes to increased organic loads contrasts with the results of most studies in this field (Moore and Bett, 1989). Other taxa profited from the organic enrichment, and among these polychaetes were present in high densities (with monospecific recruitment observed in March), thus displaying opportunistic behaviour. Structural changes were evident also in terms of number of taxa: on average 17.5 at the control station vs. 13.3 at the cage station. Therefore, we conclude that the increasing eutrophication of the farm sediments during the farming period caused clear quantitative and structural changes in meiofaunal assemblages.

Environmental conditions after cage removal (two weeks before the July sampling) changed very rapidly, which further strengthens the importance of the organic loads coming from the cage. Farm sediments showed an immediate increase in the oxidized layer (*sensu* Jørgensen and Revsbech, 1989) from 0 to about 2 cm, thus becoming indistinguishable from the control after a few weeks. Changes in sedimentary characteristics were

evident also in terms of phytopigment concentrations. Chl. *a* decreased from June to July by more than 90% and chloroplastic pigment equivalents by more than 80%, reaching concentrations comparable to or lower than those reported for non-impacted sediments in four weeks.

Meiofaunal organisms are characterized by high turnover rates and consequently have the potential to recover rapidly when disturbance ceases (Higgins and Thiel, 1988). Previous studies carried out in the Ligurian Sea at similar depths (about 10 m) demonstrated that meiofaunal density and community structure recovered rapidly after oil spill disturbances (Danovaro *et al.*, 1995). Our data corroborate these findings, indicating a progressive recovery of meiofaunal density in farm sediments. After two months, meiofauna at the cage site displayed densities only about 20% lower than in the control. The comparison between the periods before and after cage removal indicated that meiofaunal density increased by about 20% in the control and by about 50% in the farm sediments. Therefore, normalizing data from the cage station to the natural seasonal changes, it might be estimated that meiofauna recovered by about 30% within four months after cage removal. Additional insights into meiofaunal recovery are provided by the analysis of community structure (Fig. 6). Although the number of taxa was still reduced when compared with the control (see above), the relative significance of the main taxa (i.e. nematodes, copepods, and polychaetes) was similar to the control.

The best indicator of the meiofaunal recovery, in terms of community structure, was the reduced significance of harpacticoid copepods that apparently showed a decrease, parallel to that observed for phytopigments, their suspected microbial food (Fabiano and Danovaro, 1994). Other taxa such as kinorhynchans reappeared in some replicates after cage removal, although still in low numbers, suggesting that this group could represent an important bioindicator of organic contamination.

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