

Six-year study of meiofaunal dynamics in fish farms in Tenerife (Canary Islands, NE Atlantic Ocean)

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Abstract In our six-year study, we investigated the dynamics of the meiofaunal community directly under the influence of a fish farm, in adjacent areas and in control areas outside the fish farm influence. Our data showed spatial, seasonal and annual variation in the meiofaunal community under the influence of the fish farm; however, no clear trend is discernible. Copepods are positively associated with mud and very fine sands, which seem to increase with time under the fish farm and adjacent areas, particularly in summer. As shown throughout the study period, copepods could soon take over other components of the community, resulting in a community shift. Our approach to investigate the dynamics of a community with a quick response to environmental changes proves useful to detect early-stage deviations from non-affected areas, which could be critical to distinguish environmental impacts before they cause major shifts in the environment.

Keywords Tenerife · Canary Islands · Meiofauna · Nematodes · Copepods · Fish farming · Impact · Seasonal

Introduction

Fish farming is now considered as a potential source of pollution in the marine environment (Ruíz et al. 2001), and this industrial activity interacts with the environment on different spatial and temporal scales (Karakassis 2005). Intensive aquaculture production systems produce considerable amounts of nutrients in dissolved (ammonia and urea) and solid forms (faeces and uneaten food). Vergara et al. (2005) showed a waste of 120 kg of nitrogen and 17 kg of phosphorus for each ton of cultured fish (seabass and gilthead seabream). A small percentage of food supplied to fish is retrieved through harvest, whereas a considerable proportion reaches the seabed as unconsumed food pellets or as fish faeces (Kutti et al. 2007). Seabed organic enrichment is the most widely encountered impact of cage fish farming (Iwama 1991). In salmon farms, it has been studied that 29% of carbon supplied through fish food is lost in particulate form and deposited on the seabed (Hall et al. 1990). These waste products are dispersed in the surrounding environment, and the input of organic matter produces a consequent nutritional enrichment of the seabed (Vezzulli et al. 2003) and affects the local benthic flora and fauna (Karakassis et al. 1999; Mazzola et al. 2000; Mirto et al. 2000; Ruíz et al. 2001). Effects of this particular type of organic enrichment have been assessed for sediment geochemistry (Karakassis et al. 1998) and macrobenthic communities (Karakassis et al. 1999 and references therein), showing significant differences in

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seabeds located beneath to fish cages compared to control stations. Benthic assemblages are particularly sensitive to changes in shallow marine sediments subjected to major nutrient inputs related to anthropogenic activity (Danovaro et al. 2000 and references therein). Among benthic components, meiofauna proved to be a sensitive tool for detecting biodeposition impact (Albertelli et al. 1999; Danovaro et al. 2000; Mazzola et al. 2000; Lamparidou et al. 2005), with a series of advantages compared to the macrofauna. The advantages are as follows: (a) their small size (0.5–0.063 mm long), (b) short generation time and (c) lack of larval dispersion (Higgins and Thiel 1988; Kennedy and Jacoby 1999). Moreover, they are also reported to have two potential roles in marine systems: (a) as a food for higher trophic levels (e.g. macrofauna) and (b) as energy cyclers (Schratzberger et al. 2002). Meiofauna sensitivity to changes in environmental conditions suggests that this component might provide insights into the spatio-temporal monitoring studies.

Environmental studies on fish cages using meiofaunal assemblages have shown confusing results. Duplisea and Hargrave (1996) found no clear trend in total meiofaunal abundance with changes in organic matter content of the sediment, so the farm impact could not be identified by the study of meiofaunal assemblages. However, Mazzola et al. (1999) showed that changes in the sediment characteristics beneath cage farms determined a significant reduction in the overall meiofaunal abundance. Lamparidou et al. (2005) found important variations (abundance increase of 150–300%) in impacted stations located at a distance of 25 m from the cages, and they concluded that meiofaunal assemblages seem to reflect more readily even subtle changes in the sediments, and this faunal community could be considered a reliable tool for detecting early signs of environmental change. Grego et al. (2009) showed an effect on the meiofauna in the sediment below, and in the vicinity of the fish cages (20 m), with significant lower abundance under the cage during summer period.

Several authors used the meiofaunal component in seasonal studies (1–2 years) and, in general, observed significant variations in the main meiofaunal groups (nematodes and copepods) (Mazzola et al. 1999; La Rosa et al. 2001). However, Vezzulli et al. (2003) analysed meiofauna on a long-term basis (15 years) and its adaptation to the organic matter enrichment that resulted in the increase in meiofaunal abundance.

In this study, we analysed separately three different factors: Year (2003–2008), Season (winter–summer) and Farm (impact, influence and control). The variations of meiofauna assemblages among the former factors permit to carry out a tendency analysis in order to separate the effects of the exposed factors and their relative importance in the meiofaunal community structure.

This work deals with interannual variability of meiofauna, and no former information of these mid-term studies was available before in the Canarian archipelago. Riera et al. (submitted) studied the monthly variations of meiofauna assemblages during one-year basis in an intertidal beach of Tenerife. The results of the present study are part of the environmental monitoring assessment carried out in two fish farms on the east coast of Tenerife (Canary Islands, Spain). This monitoring study aims at developing and testing several qualitative and quantitative indicators of the aquaculture effects on the environment, with special emphasis on seabed assemblages. The sampling strategy and effort of this study fulfilled the requirements of the Viceconsejería de Pesca (Canarian Government) required to monitoring studies of fish cages in the Canary Islands.

Our main aim is to identify relationships between the presence of fish farms and meiofaunal assemblages located beneath as well as to study the effects of farms on the interannual (2003–2008) variability of meiofaunal abundance. Meiofauna were determined to major meiofauna taxa in order to test their use as a tool for environmental assessment studies. In this context, the present paper provides information that could improve our understanding of processes related to organic enrichment from aquaculture. In particular, subtle changes in abiotic parameters (granulometry and organic matter) were studied and their consequences in meiofauna composition.

Materials and methods

Study area

This study was conducted in Iguete de San Andrés Bay, a locality on the north-east coast of Tenerife (Canary Islands, NE Atlantic Ocean) (Fig. 1). This bay is characterized by the absence of noticeable human settlements, with the exception of a small

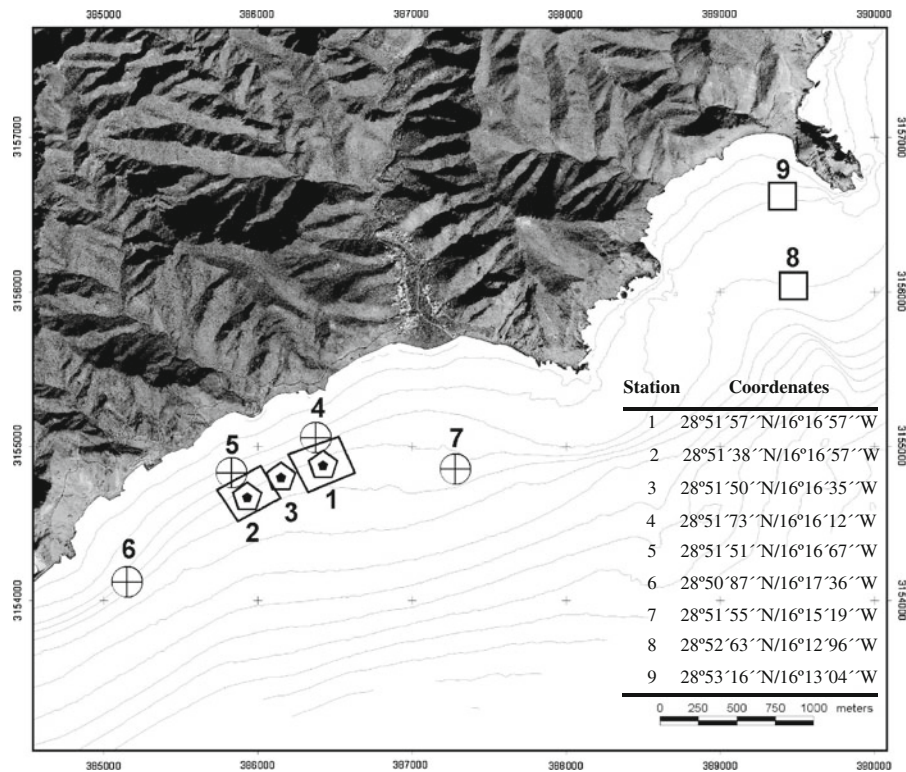


Fig. 1 Map of study area showing sampling stations (Impact: 1, 2 and 3; Influence: 4, 5, 6 and 7; Control: 8 and 9). Fish cage concessions are represented by *squares*

village (Iguete de San Andrés) located northwards in the bay and several kilometres far from cages. Species cultured in studied fish cages were gilt-head seabream (*Sparus aurata*) (200 Tm) and seabass (*Dicentratus labrax*) (150 Tm). Fishes were fed both by hand and automatically using commercial, pelleted and extruded diets with average nutrient content (on a dry weight basis): 49% protein, 18.5% fat, 22% carbohydrate, 7.9% nitrogen and 1.08% phosphorus. Pellet size ranged from 2 to 7 mm, and fish were fed at an average rate of 2% (% biomass/day), with resulting food conversion ratio of ~ 1.5 –1.6. Continuous currents throughout the year are present, with north-west (34%) and east (30%) directions (Riera, *unpubl. data*) and a mean speed of 12–15 cm/s. Former information about macrofaunal soft-bottom assemblages of the studied area was published by Monterroso et al. (2004).

The study zone is characterized by the presence of extensive sandy areas (fine and medium sands), with a scarce content of silt/clay. Rocky substrates are scarce in the subtidal, but dominate the coastline.

No extensive seagrass meadows are found in the study area, and only small patches of the seagrasses *Cymodocea nodosa* and *Halophila decipiens* were found.

Sampling strategy

Samples were collected during 6 years, from April 2003 to December 2008 along 12 sampling campaigns (April 2003 (1st), August 2003 (2nd)), August 2004 (3rd), December 2004 (4th), April 2005 (5th), August 2005 (6th), April 2006 (7th), December 2006 (8th), April 2007 (9th), December 2007 (10th), April 2008 (11th) and December 2008 (12th)). April and August are considered as summer months, and December as a winter month. Nine sampling points were sampled, three located beneath cage farms (impact group, depth 28–30 m), four on the surroundings of the farms (influence group, 50–200 m from cages, depth 18–32 m) and two in another bay (Antequera bay) northwards (control group, >1 km from cages, depth 15–25 m) (Fig. 1).

Sediment cores (3.6 cm inner diameter, area: 10 cm²) were pushed into the sediment to a depth of 30 cm. In each sampling station, five replicates were collected for faunistic analysis and one for analysis of abiotic factors (organic matter and granulometry). The whole sediment layer was analysed for both analysis (meiofauna and abiotic variables). However, organic matter data were not used in statistical analysis since no differences were observed among impact, influence and control groups. All sampling stations are characterized by low organic matter content (<0.5%).

To assess granulometry composition of the analysed sediment, 100 g sediment from each sampling station was oven-dried at 105°C, passed through a graded series (2, 1, 0.5, 0.25, 0.125 and 0.063 mm) of sieves and then weighed (Buchanan 1984). These sieves characterized seven different sedimentary types (gravels, very coarse sands, coarse sands, medium sands, fine sands, very fine sands and silt/clay). The method of Walkley and Black (1934) was used to determine the organic matter content in the sediment.

Samples were preserved in 10% seawater formaldehyde solution and sieved through a 0.5- and a 0.063-mm-mesh sieve (Sommerfield and Warwick 1996). The fraction remaining on the 0.063-mm-mesh sieve was separated into different taxonomical groups under a binocular microscope and preserved in 70% ethanol. All meiobenthic animals were counted and classified per taxon (Nematoda, Copepoda, Polychaeta, Tanaidacea, Oligochaeta, Turbellaria, Acari, Ostracoda, Amphipoda, Nemertea, Isopoda and Cumacea).

We used several statistical methods available in PRIMER 6 software (Clarke and Warwick 2001) and Systat 12 (SPSS 1999) to analyse meiofaunal and granulometric data as a function of Farm (farm impact, farm influence and control), Month (April, August and December) and Year (2003 to 2008). We calculated Bray–Curtis similarity on square-root-transformed meiofaunal abundance and used permutational multivariate analyses of variance (PERMANOVA) to test for differences in meiofaunal abundance across factors. We ran the same analysis with the granulometric data since both biotic and abiotic data consisted of multiple variables.

Because of the large number of granulometric types quantified in this study, we used factor analysis (FA) to look for coherent granulometric groups of variables that were correlated with one another within groups but

largely independent between groups (Tabachnick and Fidell 2001). These groups of correlated variables or factors help interpret the underlying mechanisms that have created the relationship between variables. Specifically, we used a principal component analysis extraction (PCA) with a minimum eigenvalue of one to estimate number of factors. To facilitate interpretation, we used varimax rotation since it minimizes the number of variables that load highly on a factor and maximizes the loading variance across factors. The resulting independent factors were used as variables in a canonical correlation analysis (CCA) to test whether the granulometric factors were correlated with the four meiofaunal variables. To further facilitate interpretation, we used correlation analysis between those granulometric variables loading high in the factors and their associated meiofaunal groups. We also used analysis of covariance (ANCOVA) from SYSTAT 12 software to test for differences in temporal trends between the sites directly under the fish farm, the influence area and the control zones.

Results

Environmental variables

Granulometric fractions showed differences in silt/clay and very fine sands in impacted stations, where they are well represented (12.80% silt/clay and 53.43% very fine sands). In influence and control groups, silt and clay content was scarce (1.50% in control group and 7.85% in influence group). Control group was dominated by fine (50.36%) and medium sands (25.34%), whilst influence group was dominated by very fine (42.13%) and fine sands (30.43%). Coarse sedimentary types (coarse sands, very coarse sands and gravels) were scarce in all groups (<10%) (Table 1).

Organic matter content remained constant throughout the three sampling groups (impact, control and influence), with low percentages (<0.5%) (Table 1). Thus, no eutrophication episodes have occurred in sediments of the study area throughout the study period.

Meiofauna

A total of 54,005 meiofaunal specimens were collected along 12 sampling campaigns. The

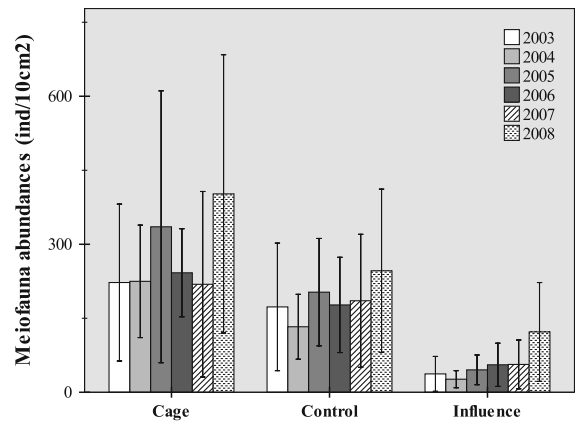
Table 1 Percentage (\pm STD, $n = 36$) of sedimentary types and organic matter content in sediments of sampling stations throughout the study period

| | Impact | Control | Influence |
|-------------------|-------------------|-------------------|-------------------|
| Gravels | 0.08 \pm 0.06 | 0.03 \pm 0.02 | 0.02 \pm 0.02 |
| Very coarse sands | 2.45 \pm 1.87 | 0.78 \pm 0.50 | 1.01 \pm 0.34 |
| Coarse sands | 6.70 \pm 3.78 | 2.34 \pm 1.12 | 5.46 \pm 4.35 |
| Medium sands | 11.45 \pm 8.79 | 25.34 \pm 12.98 | 10.34 \pm 8.39 |
| Fine sands | 21.23 \pm 14.56 | 50.36 \pm 34.32 | 30.43 \pm 24.59 |
| Very fine sands | 53.43 \pm 13.45 | 18.45 \pm 13.42 | 42.13 \pm 24.56 |
| Silt/clay | 12.80 \pm 6.78 | 1.50 \pm 1.23 | 7.85 \pm 5.64 |
| Organic matter | 0.45 \pm 0.34 | 0.38 \pm 0.34 | 0.29 \pm 0.26 |

dominant taxonomic groups were nematodes (73.78%), copepods (10.36%), tanaids (6.11%) and polychaetes (4.67%). The remaining groups (Oligochaeta, Turbellaria, Acari, Ostracoda, Amphipoda, Nemertea Isopoda and Cumacea) represented 5.07% of the overall meiofaunal abundance. Meiofauna obtained their maximum abundance in sampling stations located under the farms, mainly due to high abundance of nematodes. In this impacted group, meiofaunal abundance ranged from 405 ± 85 (2008) to 218 ± 54 ind. 10 cm^{-2} (2007). Control stations are characterized by intermediate abundance, ranging from 245 ± 52 (2008) to 129 ± 32 ind. 10 cm^{-2} (2004). Influence stations obtained the lowest meiofaunal abundance throughout the study period, ranging from 102 ± 26 (2008) to 27 ± 3 ind. 10 cm^{-2} (2004). Significant differences were found among the three groups (impact, influence and control (pseudo- $F = 35.21$; $P = 0.001$) (Fig. 2).

Meiofaunal abundance fluctuated along the 12 sampling campaigns during the 6-year study period, ranging from 110 ± 21 (August 2004) to 281 ± 46 ind. 10 cm^{-2} (May 2008), with a significant increase during the last sampling year (2008) (Fig. 3). This increase is mainly due to the highest abundance of harpacticoid copepods in impacted stations throughout 2008, reaching abundance of 280 ind 10 cm^{-2} (Fig. 4).

Significant variations were found in meiofaunal assemblage structure considering the three factors, Month (pseudo- $F = 3.77$; $P = 0.024$), Year (pseudo- $F = 2.66$; $P = 0.007$) and Farm (pseudo- $F = 35.23$;

**Fig. 2** Meiofaunal abundance (\pm STD, $n = 9$) in sampling station groups (cage, control and influence) throughout the studied period

$P = 0.0001$); however, no differences were observed among interactions of the former factors (Table 2). The former analysis was run with granulometric data, and significant differences were observed between three single factors (Month; pseudo- $F = 3.44$; $P = 0.043$; Year; pseudo- $F = 20.18$, $P = 0.001$; Farm; pseudo- $F = 41.542$, $P = 0.001$) as well as the interaction between Month and Year (pseudo- $F = 3.31$, $P = 0.007$) and Year and Farm (pseudo- $F = 2.01$, $P = 0.022$) (Table 2).

A factor analysis (FA) was used with granulometric variables, and two factors were identified. Factor 1 comprises fine sedimentary types (muds, very fine sands, fine sands and medium sands), and Factor 2 includes coarse sediments (Coarse sands, Very coarse sands and gravels) (Table 3). The percentage of variance explained for each factor is 41.77% (axe 1) and 32.68% (axe 2), with a total of 74.45%.

The correlation between factor 1 and copepods showed significant similarities ($R = 0.254$, $P = 0.016$). A detailed analysis revealed significant differences with muds ($R = 0.251$, $P = 0.017$), very fine sands ($R = 0.249$, $P = 0.018$) and fine sands ($R = -0.322$, $P = 0.002$). Tanaids also showed significant differences with factor 1 ($R = 0.152$, $P = 0.036$); however, no significant differences were found in fine sedimentary types (muds, very fine sands, fine sands and medium sands), so the combination of these four variables establish significant global differences and no single variable is responsible.

Nematodes and tanaids registered significant differences with factor 2 (nematodes, $P = 0.039$;

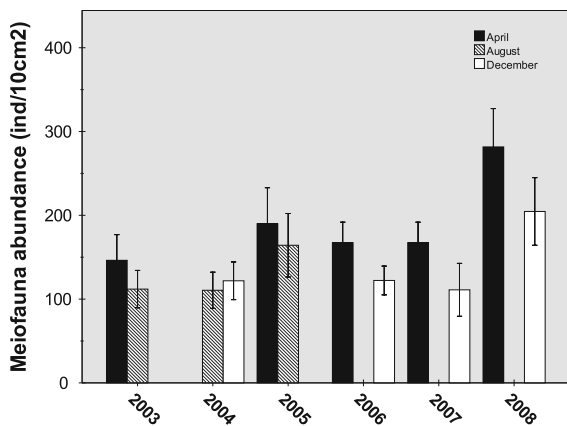


Fig. 3 Overall meiofauna abundance (\pm STD, $n = 27$) throughout sampling years

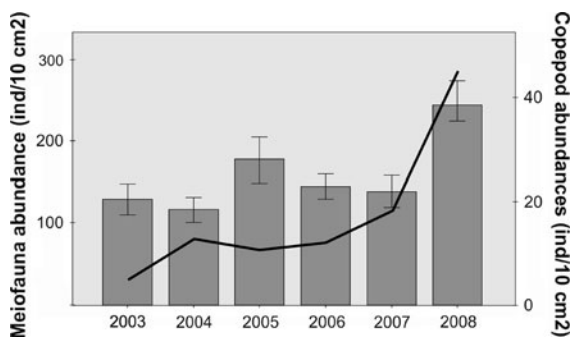


Fig. 4 Comparison of meiofaunal abundance (\pm STD, $n = 54$) (bars) and harpacticoid copepod abundance (line) throughout the study period

Table 2 Results of PERMANOVA testing the effects of Month (random factor), Year (fixed factor) and Farm (fixed factor) on the entire meiofaunal assemblage structure

| Source of variation | df | SS | MS | Pseudo- F | P |
|-----------------------------------|----|--------|--------|-------------|--------------|
| Month | 2 | 1452.2 | 1452.2 | 3.77 | 0.024 |
| Year | 5 | 5124.9 | 1025 | 2.66 | 0.007 |
| Farm | 2 | 27075 | 13538 | 35.23 | 0.001 |
| Month \times Year | 10 | 3428.6 | 685.73 | 1.78 | 0.062 |
| Month \times Farm | 4 | 1263.2 | 631.61 | 1.64 | 0.133 |
| Year \times Farm | 10 | 3837.5 | 383.75 | 0.99 | 0.469 |
| Month \times Year \times Farm | 20 | 2327.1 | 232.71 | 0.6 | 0.937 |
| Residual | 36 | 27669 | 384.3 | | |
| Total | 54 | 74451 | | | |

Significant differentials in bold

Table 3 Factor analysis with granulometric variables

| | Factor 1 | Factor 2 |
|-------------------|---------------|---------------|
| Very fine sands | -0.958 | 0.030 |
| Fine sands | 0.873 | 0.036 |
| Muds | -0.872 | 0.146 |
| Medium sands | 0.682 | 0.012 |
| Very coarse sands | -0.038 | -0.958 |
| Coarse sands | 0.130 | -0.926 |
| Gravels | 0.001 | -0.699 |

Significant differentials in bold

tanaiids, $P = 0.002$). Nematode abundance varied significantly with coarse sands ($R = 0.284$; $P = 0.007$) and very coarse sands ($R = 0.276$, $P = 0.009$). Tanaid abundance fluctuated significantly with coarse sands ($R = 0.348$, $P = 0.001$) and very coarse sands ($R = 0.396$, $P < 0.001$).

A three-way analysis of covariance (ANCOVA) was made with two selected factors (Year and Farm) nested in factor "Month". In winter, Factor 1 (fine sediments) outcomes highly significant values for factor Farm ($F = 11,396$; $P < 0.0001$) and no significant results for factor Year ($F = 3.046$; $P = 0.091$). In summer, highly significant differences were found for both factors (Year, $F = 10.156$; $P = 0.002$; Farm, $F = 22.112$; $P < 0.0001$).

In winter, Factor 2 (coarse sediments) outcomes highly significant values for factor Farm ($F = 10.158$; $P < 0.0001$) and no significant results for factor Year ($F = 0.624$, $P < 0.436$). In summer, Factor 2 obtained highly significant results for both factors (Year; $F = 17.629$, $P < 0.001$; Farm; $F = 9.123$, $P < 0.0001$).

Copepoda

Copepod abundance showed a clear pattern in relation to the three factors studied (Year, Farm and Month). As done formerly, a three-way ANCOVA was made with two selected factors (Year and Farm) nested in factor "Month". In winter, copepods showed highly significant differences for the factor Year ($F = 8.984$; $P = 0.004$) and Farm ($F = 10.073$; $P < 0.0001$) as well as the interaction Year \times Farm ($F = 10.092$; $P < 0.0001$). In summer, Copepods obtained highly significant differences for the factor Year ($F = 15.084$; $P < 0.0001$) and Farm ($F = 6.580$; $P = 0.003$) and the interaction Year \times

Farm ($F = 6.591$; $P = 0.003$). Copepods varied significantly due to the presence of cage farms, and throughout the study period, with an increase in abundance with a marked evidence in the last two campaigns (April 2008 and December 2008) (Fig. 5).

Discussion

The results of this study indicate that cage farms have a significant effect on the meiofaunal community located beneath as well as sediment properties (granulometry). Meiofaunal abundance increases in sediments underneath cages and fine sediments (muds, very fine and fine sands) dominated overwhelmingly the granulometric fractions, due to biodeposition processes. In terms of taxonomic groups, nematodes were the most abundant group in impacted stations with high abundance and the remaining groups were accessory. Influence and control stations were characterized by intermediate abundance of the two most abundant taxonomic groups in the study area (nematodes and harpacticoid copepods).

In short, in the present study, there is no clear trend of an increase in meiofaunal abundance related to the effect of fish cages. However, the second most abundant taxonomic group (copepods) showed an increase in their abundance along the studied period

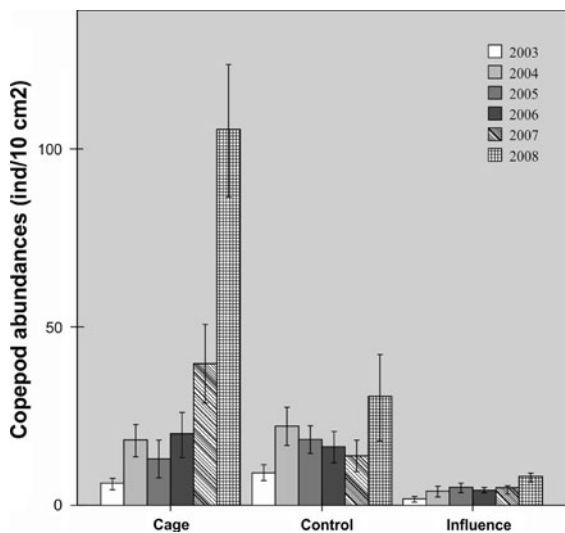


Fig. 5 Copepod abundance (\pm STD, $n = 18$) in sampling station groups (cage, control and influence) throughout the study period

due to the presence of fish farms. This result could be partially explained by the dominance of grazer forms of copepods in the meiofaunal community structure; however, a detailed taxonomic study should be undertaken to determine harpacticoid copepods to species level. These copepod species feed on diatom biofilms, well established in fine sediments (Decho 2000; Herlory et al. 2004).

In former studies, one of the most evident effects of the presence of cage farms is the accumulation of organic matter in sediments underneath cages (Gilbert et al. 1997; Vezzulli et al. 2003). This organic enrichment determines an increase in meiofaunal density due to the higher organic matter availability (Geulorget et al. 1994). However, the initial response of meiofauna to organic enrichment is followed by a decrease in abundance due to changes in sediment characteristics (Mazzola et al. 1999), with a high proportion of silt and clay where deposition is greater than resuspension and consolidation processes (Sutherland et al. 2007). In the present study, no significant changes in organic matter content of the sediment were found in terms of spatial (impacted stations, influence and control sampling points) and temporal (along the study period) variability, mainly due to continuous currents.

Vezzulli et al. (2003) studied the long-term effects (i.e. 15 years) of sediment organic matter from fish cages on meiofaunal community and found a significant change in the structure of meiofaunal assemblages. Certain taxonomic groups (polychaetes, ostracods, turbellarians, bivalves and oligochaetes) remained constant along the studied period, and others have been greatly reduced in density (amphipods and isopods) or disappear completely (cumaceans and kinorhynchs) in cage sediments. In short, they concluded that on short-term basis, meiofaunal density decreases, as pointed out by other authors (Mazzola et al. 1999; Mazzola et al. 2000; Mirto et al. 2000; La Rosa et al. 2001), whereas in a long-term basis, meiofauna changes resulting in an increase in meiofaunal abundance in sediments beneath cages (Vezzulli et al. 2003). Those results partially supported meiofaunal patterns observed in the present study; however, significant meiofaunal seasonality was supported by only one taxonomic group (harpacticoid copepods), with no significant trends in the remaining meiofaunal taxa (nematodes, polychaetes and tanaids). This effect has been previously observed

in Mediterranean fish cages, with an increase in copepod abundance in farm sediments that posteriorly decreased after cage removal (Mazzola et al. 2000). However, Grego et al. (2009) observed that harpacticoid copepods showed a drastic reduction in the proximity of the cage but not seasonal environmental changes.

To summarize, Month (April–August–December) and Year factors (2003–2008) do not mask the effects of fish cages on meiofaunal assemblages. This effect can be detected by an increase in harpacticoid copepod abundance throughout the study period, mainly due to the substitution of farm sediments in finer granulometric fractions (mainly very fine sands and silt/clay), due to the biodeposition of faeces and uneaten pellets. Therefore, interannual meiofaunal studies could be considered to be suitable as bioindicators in order to detect environmental disturbances from fish cages.

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