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Author: Matteo Dal Zotto Andrea Santulli Roberto Simonini M. Antonio Todaro

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Organic enrichment effects on a marine meiofauna community, with focus on Kinorhyncha

Matteo Dal Zotto[1,2], Andrea Santulli[3], Roberto Simonini[2], M. Antonio Todaro[2]

[1]*Consortium for the Interuniversity Center of Marine Biology and Applied Ecology, Livorno, Italy*

[2]*Department of Life Sciences, University of Modena and Reggio Emilia, via Campi, 213/d, 41125 Modena, Italy*

[3]*Marine Biology Institute, Consorzio Universitario della Provincia di Trapani, via Barlotta 4, 91100, Trapani, Italy*

Corresponding author: Matteo Dal Zotto, dalzotto.matteo@yahoo.com

Highlights:

Organic enrichment effects on meiofauna and kinorhynchs

Abstract

Within the framework of a programme aimed at monitoring the impact of fish farming on the marine biota, we have had the opportunity to study the effect of the organic enrichment caused by the fish farm on meiofauna abundances and Kinorhyncha communities' structure over two farming cycles. Up to now, studies on kinorhynchs have focussed mostly on the taxonomy, biogeography, and the ultrastructure, and, more recently, on the phylogenetic aspects of the taxon. Only few studies have dealt with the ecology of these creatures and studies focusing on the response of these animals to disturbances of anthropogenic origin are rare. The study took place in the Western Mediterranean and fauna was investigated based on three replicate cores collected from eight sites: one beneath the farm, four along a transect with increasing distances from the farm, and three control sites. Density data from beneath the cage and the three control sites was analysed within a beyond-B.A.C.I. (Before-After, Control-Impact) with asymmetrical sampling design, while a Before-After approach was used to analyse data from other sites. The latter approach was applied also to investigate the environmental variables from all the surveyed sites. Overall, 21 major meiofaunal groups were found in the area, with total densities ranging from 595 to 6818 ind/10 cm^2. We recorded a variation of the densities of several taxa after each cycle. In particular, we observed a significant increase of the total meiofauna and nematodes abundances, and a marked decrease of kinorhynchs diversity and density at the sites beneath and near the farming 'Cage'. Conversely, kinorhynch density increased at sites far from the farm. Kinorhynchs were present with ten species, including a representative of the rare genus *Condyloderes*, and densities up to more than 245 ind./10 cm^2. Analyses indicate that kinorhynchs are particularly sensitive to sulphides. Accumulation of organic matter and high concentration of sulphides caused a marked reduction or even the disappearance of kinorhynchs. If confirmed by additional studies, the nematodes/kinorhynchs ratio could be used as simple and useful tool for the assessment of organic enrichment in marine environments, especially in muddy bottoms.

Keywords: benthos, bioindicators, fish farming, sulfides, pollution

1. Introduction

Meiofauna is a highly diverse biocoenosis made-up by the microscopic benthic invertebrates inhabiting the aquatic ecosystem of the world (Artois et al., 2011; Curini-Galletti et al., 2012; Giere, 2009). Kinorhynchs are marine micrometazoans usually representing 1–8% of the total meiofauna, but able to reach occasionally 15–33% of the entire assemblage, thus representing an important component of this community (de Bovee and Soyer 1974; Cibic et al. 2009; Mazzola et al. 2000). To date, studies on kinorhynchs have focussed principally on the taxonomy (e.g. Altenburger et al., 2015; Dal Zotto, 2015; Sánchez et al., 2014; Yamasaki, 2015), biogeography (Sánchez et al., 2012), ultrastructure (Neuhaus and Higgins, 2002) and, more recently, on the phylogenetic aspects of the group (e.g. Dal Zotto et al., 2013; Sørensen et al., 2015). Relatively few studies have dealt with the ecology of these organisms and studies focusing on the response of these animals to disturbances of anthropogenic origin appear to be quite rare (e.g. Mirto et al., 2012). Recently, in an authoritative review of the phylum by Birger Neuhaus, it is clearly highlighted how the ecological information on kinorhynchs often suffers from the lack of identification beyond phylum level and that autecological data at species or at genus level is scanty (Neuhaus, 2013).

Within a programme aimed at monitoring the impact of a fish farm on the marine biota, we have had the opportunity to study the effects of the organic enrichment caused by the farm on meiofauna and Kinorhyncha communities. Aquaculture is probably the fastest growing animal food-producing sector and, to outpace human population growth, with per capita supply from aquaculture increasing from 0.7 kg in 1970 to almost 8 kg in 2006, an average annual growth rate of 6.9% is expected. The total production, which was less than 1 million tonnes per year in the early 1950s, was reported to be 52 million tonnes in 2006, with a value of almost 80 billion US$, representing an annual growth rate of nearly 7% (FAO, 2008).

The progressive expansion of intensive marine aquaculture (FAO, 2008; GESAMP, 1996) promoted a general concern for possible effects on some environmental variables. The most evident effect of the farms appears to be the large organic matter loading on bottom sediments (e.g. Gowen et al., 1991; Holmer, 1991). As a matter of fact, most of the food used for farming reaches the seabed, both unconsumed and as faeces. The effects of this organic enrichment may cause an alteration of the sediment geochemistry - originating an increase of the anoxic conditions (e.g. Newell, 2004) that, in turn, may determine alteration - and of the local biota, in particular, variations in density and structure of the benthic communities (e.g. Brown et al., 1987; Karakassis et al., 1999; Vita and Marín, 2007). Included among the good practices for a sustainable development of the mariculture is the monitoring of the potential impact on the surrounding natural biota. In virtue of its small size, high densities, benthic, sedentary habits, and short life-cycles,

meiofauna is considered one of the most important tools for biomonitoring the marine ecosystems (e.g. Balsamo et al., 2010; Danovaro et al., 2003; Fraschetti et al., 2006; Kennedy and Jacoby, 1999; Lampadariou et al., 2005a; Semprucci et al., 2015; Todaro et al., 2001; Zeppilli et al., 2015). Several surveys concerning a meiofauna response to organic enrichment have been conducted in the past (e.g. Coull and Chandler, 1992; De-Ming et al., 2014), including studies concerning enrichment by fish farming (e.g., Duplisea and Hargrave, 1996; Sutherland et al., 2007), a number of which run in the Mediterranean Sea (e.g. Grego et al., 2009; Lampadariou et al., 2005b; La Rosa et al., 2001; Mazzola et al., 1999; Mirto et al., 2014; Vezzulli et al., 2008; Vidović et al., 2014). Unfortunately, none of these have reported the effects on kinorhynchs beyond the group level.

The present study analyses the effect of the organic enrichment caused by a fish farm on meiofaunal abundances and the Kinorhyncha community structure over two farming cycles.

2. Material and methods

2.1 Farm features

The surveyed farm is used for tuna fish fattening and is located at Castellammare del Golfo (Sicily, Italy) at about 650m east of the town's harbour, where the seabed reaches a depth of 40-50m. The total utilized area of the farm amounts to 18,000m^2, subdivided in six round cages, 50m in diameter (Fig. 1). Each cage is constituted by a high density polyethylene ring from which a mesh is hung. The structure is secured to the bottom through 36 anchors and 24 buoys, which ensure floating. The farm has been in place since 2001 and yields about 700 metric tonnes of tunas per cycle. At the beginning of each cycle (July), Atlantic bluefin tunas (*Thunnus thynnus* Linnaeus, 1758), captured locally through purse seines, are stocked in off-shore cages and fed daily with frozen or fresh fish (*Clupea* spp. and/or *Scomber* spp.). In November, fattened tunas are captured and marketed.

2.2 Sediment analysis

Analyses were performed on sediment cores, in three replicates, collected from the same stations and at the same time of samples collected for meiofauna analysis (see below). Soon after, the collection samples were stored at -80 ˚C. Subsequently, parameters were determined respectively according to: Parker (1983) for TOM (total organic matter); Wildish et al. (1999) for

sulfides, nitrogen, and proteins; Dubois et al. (1956) for carbohydrates; ORION (1997) for redox potential. For each analysis, approximately 0.5 g of sediment was used, and blanks were made using the same previously calcinated sediments (500 °C, 5 h). The granulometry analysis was carried out on dried sediment using the method of sieving through 2, 1, 0.5, 0.250, 0.125, and 0.063 mm mesh size sieves (Folk, 1958). The finer fraction (<0.063 mm) was suspended in a 0,05% Sodium hexametaphosphate solution and analysed through a laser granulometer (Sympatec Helos), after being exposed to ultrasounds for 10 seconds. The mean grain size, sorting coefficient, kurtosis, and skewness were calculated on the basis of the formulae proposed by Folk and Ward (1957).

2.3 Meiofauna sampling and analysis

Samplings took place in June ('Before' phase) and December ('After' phase) in 2006 and 2007. Four sampling sites/stations placed along a transect following the dominant current were identified (Fig. 1): 'NW100', placed at 100m north-west of the farm; 'Cage', located beneath the farming cages; 'SE50' and 'SE100' located south-east of the farm at 50m and 100m from the farm. In addition, the sites to be used as control sites were identified: 'CT1' at about 1 km north-west from the farm, 'CT2'and 'CT3' at about 0.5 km and 1 km respectively south-east of the farm. Locations of the stations were identified during a preliminary study carried out by Santulli et al. (2003). In the 2007 cycle, an additional site, 'SE25', was identified at 25m south-east of the farming cages (Fig. 1). The stations were placed at a depth of 35-45m, where the bottom is composed of very fine sand to coarse silt. At each site, samples in three replicates were collected, coring the sediment with a hand-held piston corer (3.4 cm i.d. x 5 cm h). The fauna was narcotised using a 7% magnesium chloride solution, fixed on site with a 10% buffered formalin solution, pre-stained with rose bengal and stored for later analyses (Todaro et al., 2006). In the laboratory, each sample was filtered using two sieves, 1 and 0.045 mm, respectively, laid one upon the other and fauna extracted thrice from the finer fraction using the silica gel gradient centrifugation technique (LUDOX AM, d = 1.210; Pfannkuche and Thiel 1988; Todaro et al., 2001). The analysis of the remaining sediment (pellet) indicated an extraction efficiency near 100%. The extracted fraction of each replicate was subdivided in aliquots, transferred to a counting chamber, and all metazoans and ciliates were identified to major groups and counted under a Wild M8 stereomicroscope. For the identification of several organisms, a compound microscope was used. When encountered, kinorhynchs were removed with a micropipette, mounted on HS slides, observed with Nomarski optics using a Nikon, Eclipse 90i microscope, and photographed using a DS-5M digital camera. Some specimens were

prepared for observation under scanning electron microscopy. Detailed information regarding kinorhynchs preparation, analyses, and identification are reported in Dal Zotto and Todaro (2016).

2.4 Statistical analysis

To evaluate the potential effects of the farming activity on the local biota, meiofauna data from the 'Cage' and the three control sites ('CT1', 'CT2', and 'CT3') was analysed within a beyond-B.A.C.I .with asymmetrical sampling design (null hypothesis: no effect) (Underwood, 1992). We considered three factors: (1) B, Before-After (fixed, two levels: 'Before' and 'After'); (2) Y, Year (random, two levels: 'year 1' and 'year 2', nested in Time); (3) L, Locations (fixed, four levels: 'Impact', 'CT1', 'CT2', and 'CT3'). Differences between meiofaunal densities at 'Cage' and control sites were evaluated by permutational non-parametric multivariate analysis of variance (PERMANOVA; Anderson, 2001). Data analysis was performed on log(x+1) transformed abundance matrices. Similarity matrices were calculated using the Bray–Curtis similarity index (Bray and Curtis, 1957). PERMANOVA was carried out to test the effects of B x L and Y(B) x L factors. Test of permutational multivariate dispersion (PERMDISP) (Anderson et al., 2005) was performed for testing homogeneity of dispersion among the investigated sample groups. Single meiofaunal groups abundances were analysed according to the rules suggested by Underwood (1992). Density data from the other sites were analysed within a Before-After approach by a two way analysis of variance (ANOVA). We considered two factors: (1) B, Before-After, with two levels, and (2) L, Locations, with eight levels ('CT1', 'CT2', 'CT3', 'NW100', 'Cage', 'SE25', 'SE50', 'SE100'). The same approach was also used for environmental variables from all the investigated sites. The pairwise Tukey-test was used if significant differences using the ANOVA were found ($p < 0.05$). Data normal distribution and homogeneity of variance were tested using Cochrans C test (Winer, 1971) and data were log(x+1) transformed as necessary. If the transformation resulted unsuccessful, non-parametric analyses were adopted (ANOVA on Ranks and Mann-Whitney Rank Sum Test). Among the significant interactions, we only analysed the trend of factor L with factor B. Abundances of meiofaunal taxa and environmental variables in the samples were additionally compared using multivariate procedures. Cluster Analysis, non-metric Multidimensional Scaling (nMDS), Analysis of similarity (ANOSIM), and Principal Component Analysis (PCA) were performed on a similarity matrix constructed using the Bray-Curtis measure of similarity on log(x+1) (biotic) or square root (abiotic) transformed data. Similarity Percentage analysis (SIMPER) was calculated to investigate which taxa were responsible for the differences. Biotic and abiotic data matrixes were correlated with BIOENV analysis (Biota and/or Environment Matching; Clarke and Ainsworth, 1993).

PERMANOVA was executed with PRIMER 6.0 (PRIMER-E Ltd, Plymouth, U. K; Clarke and Gorley, 2006; Clarke and Warwick, 2001) with PERMANOVA+add-on (Anderson et al., 2008). ANOVA and linear regression were performed with SigmaStat-SigmaPlot 9.0 (Systat software, California, USA). SIMPER, Cluster Analysis, nMDS, ANOSIM, PCA, and BIOENV were executed with PRIMER 6.0.

3. Results

3.1 Sediment parameters

Of the measured parameters, only some of them i.e., granulometry, TOM, sulfides, and redox potential, exhibited significant variations and/or were in correlation with the densities of meiofaunal taxa. Considering the intent of the present paper, here we only report data for these parameters (see Table 1).

The sediment of the investigated sites was composed of very fine sand to coarse silt, moderately sorted, and which displayed a similar sediment texture as the silt-clay fraction, accounting for 70-80% at the control sites and 60-80% at the potentially influenced sites. The remaining fraction was characterized by very fine sand.

PCA indicates a variation of environmental parameters (mainly TOM and sulfides) along a gradient related to distance from the farm, apparently not detectable further than 100m (Fig. 2). The first two components of the graph account for approx. 89% of the variability. Sulfide concentrations positively correlated to PC1 (variability: 63.5%), while TOM is correlated to PC2 (variability: 25.2%). The investigated sites are separated along a distance gradient, related to sulfides and TOM concentrations. The former exhibit the highest values beneath and, secondly, at 25-50m from the cages, the latter shows the highest values at Control 1 (‘CT1’), even though with a minimal variation among sites (average values: 3-5%; ‘Cage’ and ‘CT1’: 6-7% and 7-8%, respectively). The sulfide concentrations seem to rise globally from the ‘Before’ to ‘After’ phase, even though the major variation is related only to the ‘Cage’ site. Values decrease according to a gradient following the distance from farm. At 100m distance (‘SE100’), the effects of farming activities are not detectable. Beneath the cages (‘Cage’), sulfide concentrations vary significantly (1st year survey: 1740 to 2260 μM; 2nd year: 616 to 2100 μM). In the ‘After’ phase, the sediment organic enrichment level corresponds to the hypoxic B reported by Sutherland et al. (2007). Values are high also during the 1st year survey ‘Before’ phase in the ‘Cage’ and ‘SE50’ sites (hypoxic A status).

3.2 Meiofauna

During the first year survey the meiobenthic community of the investigated area (all the sites considered), reveals an average density of 1688.6 ± 790.3 ind./10 cm^2 in the 'Before' phase (range, 595.3 – 2406.2 ind./10 cm^2), and a comparable values during the 'After' phase: 1750.4 ± 826.1 ind./10 cm^2 (range, 697.2 – 3225.5 ind./10 cm^2). Nematodes are the dominant taxon ('Before': 1469.1 ± 598.6; 'After': 1369.7 ± 787.5 ind./10 cm^2), followed by harpacticoid copepods ('Before': 177.3 ± 142.1; 'After': 212.0 ± 152.8 ind./10 cm^2) and, in the 'After' phase, by kinorhynchs ('Before': 24.4 ± 17.8;'After': 41.0 ± 31.0 ind./10 cm^2).

In the second year survey, the total meiofauna of the area reaches an average density of 2562.7 ± 781.5 ind./10 cm^2 in the 'Before' phase (range, 1465.7 – 3623.7 ind./10 cm^2), and 2736.0 ± 1922.7 ind./10 cm^2 in the 'After' phase, showing a much ample numerical range among sites (1188.3 – 6818.4 ind./10 cm^2). Nematodes are always the dominant taxon ('Before': 1790.3 ± 614.1 'After': 2148.0 ± 1972.5 ind./10 cm^2), followed by harpacticoid copepods ('Before': 329.4 ± 134.0 'After': 357.1 ± 168.4 ind./10 cm^2), by their larval stage in June (224.5 ± 99.8 ind./10 cm^2) and by kinorhynchs in December (105.9 ± 92.9 ind./10 cm^2).

Ample variations in the densities of total meiofauna, nematodes, kinorhynchs, and polychaetes have been registered in the 'Cage' site over the two farming cycles. Nematodes almost double or triplicate their abundance after the use of the farm, while kinorhynchs and polychaetes tend to decrease, the former almost disappearing at this site. By contrast, the three control sites have revealed lower density fluctuations during the surveyed cycles (Tables 2, 3). PERMANOVA reveals significant effects of the interaction Before-After x Locations (B x L) on meiofauna abundances (Table 4; Pseudo-F = 19.30; $p < 0.05$), while the interaction Year (Before-After) x Locations (Y(B) x L) is not significant. This indicates that the fish farm activity determined changes in the spatial heterogeneity.

PERMDISP analysis reveals a significant difference between 'Cage' and 'Controls' (L factor; $p < 0.001$), whilst the differences between the levels of factors B and Y are not significant. Pairwise tests show that the main difference is between 'Cage' and 'CT2' sites ($p < 0.01$), followed by 'Cage' and 'CT3', and 'Cage' and 'CT1' ($p < 0.05$). The analysis performed on the single meiofaunal groups indicate an impact of the farming activity on nematodes, kinorhynchs, polychaetes, and total meiofauna as the effects of B x L interaction were detected while the interaction of Y(B) x L was not significant (Table 4).

Before-After analyses revealed a statistically significant reduction of kinorhynchs, nauplii, and polychaetes density at the 'SE25' site. By contrast, a significant increase of kinorhynchs during both cycles occurs at the 'SE50' site, where nematodes and total meiofauna decrease their densities

in the first year survey. At the 'SE100' site, nauplii densities diminish in the first cycle while, during the second year, the abundances of kinorhynchs and copepods increase significantly.

Results indicated a general congruence between the data obtained in the two investigated farming cycles, e.g., density variation of selected taxa in the 'After' phase at some selected sites as a response to fish farming.

Faunistic differentiations at the 'Cage', 'SE25', and 'SE50' sites are clearly visible in the graph resulting from the nMDS analysis (Fig. 3), and the ANOSIM confirms these results: the pairwise permutation test indicates that the 'Cage' site is significantly different from all the other sites, except for 'SE25' ($0.65 < R < 0.71$; $p = 0.001$; data not shown). SIMPER analysis underlines a high similarity (81-87%) among the fauna of the surveyed sites, grouped both according to the distance from the farm and the farming phase. Nematodes and copepods are the taxa that give main contribution to the similarity (26-43% and 18-24%, respectively), whilst kinorhynchs and nauplii are the groups mainly influencing the dissimilarity among sites (10 to 22%; data not shown).

BIOENV analysis globally shows an intermediate correspondence value between biotic and abiotic data. The best combination links biotic data with sulfide concentrations, carbohydrates, and sediment sorting ($\rho = 0.545$; $p = 0.01$). The results deriving from each of the two surveys are more relevant. The best combination for the first year data relates biotic data to sulfide concentrations, total organic matter, and sediment sorting ($\rho = 0.700$; $p = 0.02$); while in the second year cycle, the matching is between sulfide concentrations, total organic matter, and redox potential ($\rho = 0.616$; $p = 0.01$; data not shown).

3.3 Kinorhyncha

The kinorhynch fauna of the area is made up of ten species (Tables 5, 6), five of which belonging to the class Allomalorhagida (two families and three genera), and the other five to the class Cyclorhagida (three families and three genera). Among Allomalorhagida were: *Paracentrophyes quadridentatus* Zelinka, 1928 (Neocentrophyidae), *Kinorhynchus giganteus* Zelinka, 1928; *Pycnophyes carinatus* Zelinka, 1928, *P. communis* Zelinka, 1928, *P. robustus* Zelinka, 1928 (Pycnophyidae). Among the Cyclorhagida were: *Echinoderes capitatus* (Zelinka, 1928), *E. gerardi* Higgins, 1978, *E. ferrugineus* Zelinka, 1928 (Echinoderidae), *Semnoderes armiger* Zelinka, 1928 (Semnoderidae). We also found what appears to be an undescribed species of the rare genus *Condyloderes* (Centroderidae). Some morphological details regarding *E. ferrugineus* and *P. carinatus* need to be further investigated, since they differ from the original description by Zelinka (1928; see Dal Zotto and Todaro, 2016).

Echinoderes capitatus is the most abundant cyclorhagidan species while *Pycnophyes carinatus* is the allomalorhagidan displaying the highest density (Fig. 4). *E. capitatus* is the dominant taxon of the area and, at the majority of the investigated sites (Fig. 5), often exceeding densities of 50 ind./10 cm^2, with a peak of 184 ind./10 cm^2 (station 'SE100', 'After' phase, 2nd year survey). These statistics should be considered conservative, since they only include the adult stages. In fact, as based on morphology, it is very difficult, and sometimes almost impossible, to allocate, to a given species, the juvenile stages of *Echinoderes* (the same applies to *Pycnophyes*). The numerous juveniles found, and belonging to these genera, are not affiliated to any known species but are reported as *Pycnophyes* spp. juv. and *Echinoderes* spp. juv. instead (Tables 5, 6).

Pycnophyes carinatus, Kinorhynchus giganteus and *Echinoderes ferrugineus* result to be the subdominant taxa at specific sites (Tables 5, 6) The other six species are much less abundant, with densities generally lower than 10 ind./10 cm^2. The two most abundant taxa (*E. capitatus* and *P. carinatus*) exhibit also the widest distribution in the investigated area, being present in all the investigated sites. By contrast, and despite its name, *Pycnophyes communis* appears to be the least common species, since it was found at only 1 out of 8 sites ('SE100'); rare or uncommon are also *Echinoderes gerardi*, and *Pycnophyes robustus*, present only in half of the investigated sites (4/8). Nine out of ten species were found over the two year period; *Echinoderes gerardi* was found only in the second year of the study, yet, an increased number of juveniles of *Echinoderes* spp. further characterizes the 2007 survey with respect to the previous farming cycle.

While three sites ('NW100', 'SE100' and 'CT2') appear to host all of the 10 species found in the area, the highest species richness (9 spp) was recorded for the 'NW100' and 'SE100' sites during the 'After' phase of the 2007 survey (Tables 5, 6).

The site exhibiting the highest kinorhynch density was 'SE100' ('After' 2007; mean abundance: 245 ind./10 cm^2, with 304 ind./10 cm^2 found in a single core), followed by the 'SE50' site ('After' 2007, 208 ind./10 cm^2). The lowest densities were reported for the 'Cage' site during the 'After' phase of both farming cycles: 1.1±1.1 and 0.7±1.3 ind./10 cm^2, respectively (Tables 5, 6; Figures 6, 7).

Density values above 100 ind./10 cm^2 were quite common. At more than 50% of the surveyed sites, Kinorhyncha represented the third meiofaunal taxon in terms of abundances, following nematodes and harpacticoid copepods. Population densities did not show significant variations through seasons (summer vs. winter), as indicated by data collected at the control sites.

Based on nMDS analysis (Fig. 8), 'CT1' and 'Cage' host kinorhynch assemblages clearly different from each other and from the other sites. The 'Cage' site is characterized by a few species: the only two reported in the 'After' phase were *Echinoderes capitatus* and *Pycnophyes carinatus*,

even though with very low abundances (0.36 ± 0.63 ind./10 cm^2 for both species). Other taxa totally disappeared beneath the cages. In 'CT1', seven species were found, even if with rather low densities compared to other sites. This site is near a *Posidonia* meadow, hence it is possible that the local fauna is affected by the presence of this seagrass, as reported for a variety of meiobenthic organisms, including kinorhynchs, by Mirto et al. (2010). The significant increase registered at the 'SE50' and 'SE100' sites is mainly due to the more abundant taxa: *E. capitatus*, *P. carinatus*, and *K. giganteus* (ANOVA; $p < 0.05$).

SIMPER analysis stresses that the main contribution to the similarity among samples grouped on the basis of the distance from the cages derives from these taxa (17-44%), while *E. ferrugineus* and juvenile stages of *Echinoderes* spp. and *Pycnophyes* spp. provided the main contribution to the dissimilarity among samples (13-25%). Grouping the samples on the basis of the farming phase ('Before'-'After'), the main contribution to the dissimilarity among samples derives from juveniles *Echinoderes* spp. BIOENV analysis points out a good correspondence between the variations of kinorhynch taxa and the modification of sulfides, total organic matter and, secondarily, carbohydrates and nitrogen content in the sediment ($\rho = 0.637$; $p = 0.01$). Again, the main dissimilarities among samples regarded the noticeable decrease in the juvenile stages of the two most abundant genera (*Echinoderes* and *Pycnophyes*) at the mostly impacted sites.

4. Discussion

4.1 Environmental parameters

Previous surveys in the area of the farm (Santulli et al., 2003) showed that water chemico-physical parameters were well within the range of values registered for the entire Gulf of Castellammare (e.g. Favaloro et al., 1996), showing typical characters of coastal highly hydrodynamic oligotrophic waters. Observed variations were due to seasonality and not to the presence of the farm (Santulli et al., 2003). Organic matter build-up along a dominant current influenced gradient was detected on the sea beds beneath and in the proximity of the farm after every farming cycle. The accumulation was highest immediately beneath the cages ('Cage' station), and it exhibited a gradual reduction moving away from them. At a distance of 250m from the cages, the values were comparable to those observed before the start of fish farming (Santulli et al., 2003).

In our surveys, TOM at the investigated sites did not vary significantly from the 'Before' to the 'After' phase. By contrast, we did find a statistical significant increase in sulfide concentration at the 'Cage' and 'SE25', 'SE50' 'SE100' sites in the 'After' phase. According to Sutherland et al.

(2007), the increase of sulfide is an indication of organic enrichment. Consequently, in our case, the recorded general increase of this compound (Fig. 8) is likely due to organic enrichment caused by the farm activity, although the unexpected data on TOM would suggest otherwise.

A surprising high TOM value (both in 'Before' and 'After' phases) was also found at 'CT1'. A working hypothesis links the high TOM content at this site to the presence of a *Posidonia* meadow in its vicinity. A recent study revealed the influence of *Posidonia* meadows on the seabed, reporting effects substantially comparable to those of a fish farm (Mirto et al., 2010). Another feature that may influence the organic load of sediment is its granulometry: low organic matter contents are found in sandy sediments, while the high values are reported in pelitic bottoms (Papageorgiou et al., 2010). In 'CT1', the sediment contains a slightly higher fraction of silt-clay compared to other sites, and this may account for the high TOM recorded at the site. All in all, the proteins and carbohydrates signatures, and the low sulfide concentration found in 'CT1', suggest an origin that is not linked to the fish farming activity for this organic load.

The effects of farming activities on the seabed are similar to those reported in other studies, showing an organic matter accumulation deriving from unconsumed food and feces, which is the cause of high sulfide concentrations and hypoxic conditions (Gowen and Brandbury, 1987; Gowen et al., 1991; Holmer, 1991; Holmer et al., 2005; Iwama, 1991).

4.2 Meiofauna

The main faunistic variations detected during two farming cycles concern the sites located closer to the farm, specifically 'Cage' and 'SE50'. During both surveyed cycles, nematodes and total meiofauna exhibit a strong population increase while kinorhynchs decrease at the 'Cage' site. Conversely, kinorhynchs increase at 'SE50' in both surveys. Additional statistical significant variations were site and/or cycle specific and, in general, were indicating a decrease of the abundance, with some noticeable exceptions (see Tables 2, 3). Regarding the latter, the increase of the kinorhynchs at the 'SE100' site during the second cycle survey is worth mentioning for the context.

It has been shown that the change in the concentration of a variety of environmental parameters has direct effects on the alteration of meiofaunal densities (Giere, 2009). A previous analysis on macrofauna conducted in this same farm using a similar experimental design (Santulli et al., 2003), reported that organic matter distribution gradient influenced the macrobenthic fauna in agreement with the Pearson and Rosenberg (1978) model. More specifically, at the 'Cage' site, the macrobenthos tended to be impoverished or absent, while it increased at the 'SE100' site (100m far

from the farm), according to the fertilization effect described by Pearson and Rosenberg (1978). At a distance of 250m from the cages, the biota did not show any change, suggesting that the fertilization effect due to fish farming activity was nil at this distance.

With regard to kinorhynchs, our data seem to follow this model. On the other hand, nematodes appear to have an opposite response to organic enrichment, as this taxon increases at the 'Cage' site. Significant increase in meiofaunal and nematodes abundances, due to the organic enrichment deriving from fish farming, have been reported, e.g., by Lampadariou et al. (2005b) for three Greek areas, while a decrease of kinorhynchs has been recorded e.g. by Mirto et al. (2012).

It has been shown that a high sulfide concentration has deleterious effects on macrobenthic communities. For instance, a general decrease of the abundances of all taxa is expected when the sulfide concentrations are comprised between 2000 and 10000 μM, with a total defaunation above these values (Brooks and Mahnken, 2003; Wildish and Pohle, 2005).

Once again, with regard to kinorhynchs our data seem to agree with previous observations on macrobenthos, as their densities are inversely related to the concentration of sulfides (cf. Figures 6, 7 with Figure 9), with a near complete defaunation at site 'Cage' where the sulfide concentration is above 2000 μM.

Meiobenthic taxa can either be sulfide tolerant or sulfide sensitive (Sutherland et al., 2007); high concentration of nematodes at the 'Cage' site can account for sulfide-tolerant species, on the other hand the kinorhynch decrease reported for the same site underlines the general sulfide sensitiveness of the species of this taxon. Many studies underline that the effects of the organic enrichment on a given meiobenthic community depends mainly on the number of sulfides-sensitive and sulfides-tolerant taxa that make up the assemblage, together with the ratio between epi- and endobenthic taxa, and the duration and level of the hypoxic conditions (Mirto et al., 2002; Raffaelli, 1987; Warwick, 1981). The decrease of kinorhynchs and polychaetes at the 'Cage' and 'SE25' sites, suggests that these taxa are mainly represented by sulfides-sensitive species.

Based on SIMPER analysis, kinorhynchs are the main taxon contributing to the dissimilarity among sites grouped on the basis of the distances from the farm, and the use of the structure ('Before'-'After'). The ANOSIM analysis underlines a clear differentiation between the meiofaunal community at the 'Cage' site and the other ones. BIOENV analysis relates the variation observed in the biocoenosis to the accumulation of organic matter and sulfide concentrations in bottom sediment, together with redox potential measured in the first 2 cm of the sediment (analysis conducted only during the second farming cycle). Concordantly, PCA run on abiotic data reveals that the variation of some parameters, particularly sulfides and total organic matter, depends on the distance from the

farm. Apparently, at a distance of 100m or more, the effect of the fish farming activities was undetectable.

Based on literature data, the substantial effect of fish farming on the sediment was predictable; unconsumed food and feces caused hypoxic conditions and an increase in sulfidic compounds, which influenced the meiofauna community.

Globally, the utilization of the analysed farm has shown pronounced effects on the meiofauna community beneath or at close distance from the nets (0-25m), which tend to reduce and disappear together with the increase of the distance from the farm. Similar patterns have been reported for this farm by Santulli et al. (2003) relatively to the macrobenthic community. The marked effects reported for both the analysed cycles seem to exclude the adaptation of most of the meiofaunal groups to the stressing events.

The sensitivity of the kinorhynchs toward the sulfidic compounds derived from the degradation of the organic material produced by the fish farm suggests that these animals could be used as bioindicators. Their response, that contrasts with those of the nematodes, further suggests that the nematodes/kinorhynchs ratio (Ne/Ki) could be utilized as a useful tool for the assessment of anthropogenic organic enrichment in marine environments, as proposed by Mirto et al. (2012). In general, the highest values of Ne/Ki ratio correspond to a high organic enrichment (e.g., Ne/Ki ratio: 130-180 at impacted sites, 20-50 at not impacted; Mirto et al., 2012). Our data are in line with Mirto and co-workers' findings and support the reliability of the Ne/Ki ratio in detecting organic enrichment, and also in being a better indicator for this type of pollution than nematodes/copepods ratio (Ne/Co), at least for fine bottom sediments. The highest values calculated for this index (>1000) refer to the most impacted sites in the investigated area (beneath and in the proximity of the cages). Conversely, the Ne/Co ratio exhibits scarcely marked variations in the area (<30), even though its trend is globally similar to the variation of the Ne/Ki ratio (Fig. 10). The Ne/Ki ratio, not requiring high taxonomic expertise, is confirmed as an easy and reliable tool for detecting organic enrichment. This index could be considered an additional indicator to those proposed by Borja et al. (2009) for the monitoring of the benthic communities exposed to organic enrichment. However, as remarked also by Mirto et al. (2012), the Ne/Ki ratio needs further testing under a variety of environmental conditions, before it can routinely be adopted in the marine ecosystems monitoring practices.

4.3 Kinorhyncha

The results of our study stimulate some general considerations on the response of kinorhynchs to the organic enrichment. Beneath the cages and at 25m distance from them, due to hypoxic or anoxic conditions, mainly deriving from the accumulation of unconsumed food from the farming activity, kinorhynchs died or partially migrated to proximate sites, searching for an oxygenated sediment. Beneath the cages, some months after the stressing event ('Before' phase), subsequently to the re-oxygenation of the sediment, the kinorhynchs re-colonized the seabed. Only epibenthic organisms are known to avoid the sediment change to hypoxic or anoxic conditions (Raffaelli, 1987). Some authors stress that kinorhynchs inhabiting fine sediments are strictly epibenthic taxa, living in the first few millimeters of the seabed, and rarely occurring below 1-2 cm in depth (Kristensen and Higgins, 1991; Neuhaus, 2013; Neuhaus and Higgins, 2002). The area investigated in the present study is characterized by very fine sand to coarse silt, and hence the species found can reasonably be considered epibenthic.

Kinorhynchs are thought to feed on the bacteria involved in the decomposition of the phytodetritus and the particulate organic matter (Nomaki et al., 2008). We speculate that a slight organic enrichment may have favoured the proliferation of food sources (bacteria and microalgae) causing the increase of kinorhynchs abundance in sites SE50 and SE100. A similar effect on kinorhynch densities has been reported for a Brazilian shrimp farm (de Paula et al., 2006).

The two years monitoring of the kinorhynch community from the Gulf of Castellammare allowed the acquisition of information on the synecology of these animals. In the Sicilian Gulf, kinorhynchs densities above 100 ind./10 cm^2 were quite common, with a peak of 245.7 ind./10 cm^2 (2007, 'SE100', 'After', Table 3), accounting for some of the highest values ever recorded (Neuhaus, 2013). Higher values were recorded during the second farming cycles and, in general, during the 'After' phase, with the exclusion of the sites closer to the farm ('Cage' and 'SE25'), where a decrease was noticed.

Ten species were found in total, equally subdivided between the two currently recognized orders, Allomalorhagida and Cyclorhagida (see Sørensen et al., 2015). For the context, it is worth mentioning the presence of a putative new species belonging to the genus *Condyloderes* that, thus far, was unreported for the Mediterranean Sea (see also Dal Zotto et al., 2008; Dal Zotto and Todaro, 2010; 2016). All but one species were recorded during both the farming cycles. *Echinoderes gerardi* was recorded, in low numbers, only during the second farming cycle, especially during the 'Before' phase (Tables 5, 6). Similarly, *Pycnophyes communis* found only at the 'SE100' site during the first cycles, was quite common in the 'Before' phase of the second farming cycle. *Echinoderes capitatus*

and *Pycnophyes carinatus* turned out as the dominant taxa of the area and in most of the sites. Consequently, the recorded variation in densities at the phylum level was mostly due to the abundance variation of these two taxa. While the analysis led at a specific level did not allow to identify taxa, resulting more sensitive than others to the organic matter enrichment, the recorded presence/absence and/or the numerical variation of certain species in selected sites/phase must be further investigated e.g. in conjunction with their relation to life habits (epibenthic, endobentic life style) and the potential variation of their food source (microalgae and bacteria). Movements of kinorhynchs have been registered in relation to the seasonal amount of nutrients (Shimanaga et al., 2000). Even though in the mentioned study only vertical movements were observed, it is likely that kinorhynchs are able to shift horizontally. Some studies indicate that kinorhynchs prefer the best oxygenated sediment layer (top sediment), which contains the highest density of microorganisms eaten by these metazoans, and tend to diminish exponentially with the increase of sediment depth (Meadows et al., 1994; Vidaković, 1984).

The nMDS chart (Fig. 8) shows the 'Cage' site and, to the same extent, the 'CT1's quite separated from the others because of their peculiar fauna, which appear quite different from that of the other sites. Low species richness and low abundances characterize the 'Cage' site, while low abundances characterize the 'CT1' site. While high sulfide concentration accounts for the low biodiversity in the 'Cage' site (see above), the faunistic differentiation of the 'CT1' site appears to have other causes. As previously indicated, this control site was characterized by the proximity of a *Posidonia* meadow. Mirto et al. (2010), suggest the effects of this seagrass on meiofaunal taxa and, more specifically, on kinorhynchs to be similar to those originated by a fish farm. Our study indicated that the high organic load caused by the *Posidonia* meadow is not translated into an increase in sulfides (Fig. 9). Consequently, the recorded reduction of species richness and/or abundances occurs for other causes, which at the moment appear elusive.

SIMPER analysis underlines that the main contribution to the similarity among samples grouped on the basis of the distance from the cages derives from *E. capitatus* (17-44%), while *E. ferrugineus* and juvenile stages of *Echinoderes* spp. and *Pycnophyes* spp. provided the main contribution to the dissimilarity among samples (13-25%). Grouping the samples on the basis of the farming phase ('Before'-'After'), the main contribution to the dissimilarity among samples derives from juveniles *Echinoderes* spp. BIOENV analysis points out a good correspondence between the variations of kinorhynch taxa and the modification of sulfides, total organic matter, and, secondarily, carbohydrates and nitrogen content in the sediment ($\rho = 0.637$; $p = 0.01$). Again, the main dissimilarities among samples regarded the conspicuous diminishing of the juvenile stages of the two most abundant genera (*Echinoderes* and *Pycnophyes*) at the mostly impacted sites. These results

could indicate that fish farming and, more in general, organic enrichment with consequential increase of sulfide concentration, has a potential impact on the reproduction of kinorhynchs. Absence of numerical variations of the juvenile stages of the two most abundant genera at the control sites seems to exclude the effect of seasonality as a possible source of variability. Other studies report a possible decrease of reproduction under hypoxic conditions (Murrell and Fleeger, 1989; Sergeeva et al., 2012).

5.1 Conclusive remarks

Our data indicate an impact of the fish farm on meiofaunal taxa. However, the most relevant effects are on biota inhabiting the sediment beneath the farming cages, where density and structure of the meiobenthic community result altered after the plant has been used ('Before' vs 'After' phases). Effects decrease with the increasing distance from the farm. At 50m from the farm, consistent effects over the two farming cycles are recorded only on kinorhynchs, while at 100m and over, effects on the investigated groups were not detected or were not consistent over the two farming cycles. Likely, this mild impact on meiobenthos has much to do with the physiography, hydrodynamics, and water quality of the area that allow for a rapid dispersion and/or degradation of the organic material released by the farm.

Kinorhynchs result to be the meiobenthic group that, more than others, has been affected by the farming plant (e.g., Table 6). Their density decreases dramatically at the sites positioned under the cages and at 25m from them, but increases at 50m and 100m. While the high sulfide concentrations detected in the 'Cage' and 'SE25' sites can be accounted for the decline of the kinorhynch population at these sites, the reasons of the growth at the 'SE50' and 'SE100' sites remain unknown.

The sensitivity of the kinorhynchs toward the sulfidic compounds derived from the degradation of the organic material produced by the fish farm suggests that these animals could be used as indicators in the programmes of biomonitoring of these industrial plants. Their response, that appears opposite to that of the nematodes, suggests that the nematodes/kinorhynchs ratio could also be utilized as a useful tool for the assessment of anthropogenic organic enrichment in marine environments, as proposed by Mirto et al. (2012). However, additional studies are needed to support or disprove the usefulness of the kinorhynchs as bioindicators.

Kinorhynchs are among the least studied animals. Information on their distribution and basic ecology are particularly scanty. The report of ten species, among which one belonging to a genus that is new for the Mediterranean sea, along with information on their distribution and abundance,

make the results of the present study of a relevance that goes beyond the ecological monitoring of a fish farm, and paves the way for new and interesting investigations.

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Fig. 1. Location of the fish farm in the Gulf of Castellammare (Sicily, Italy) and the sampling sites.

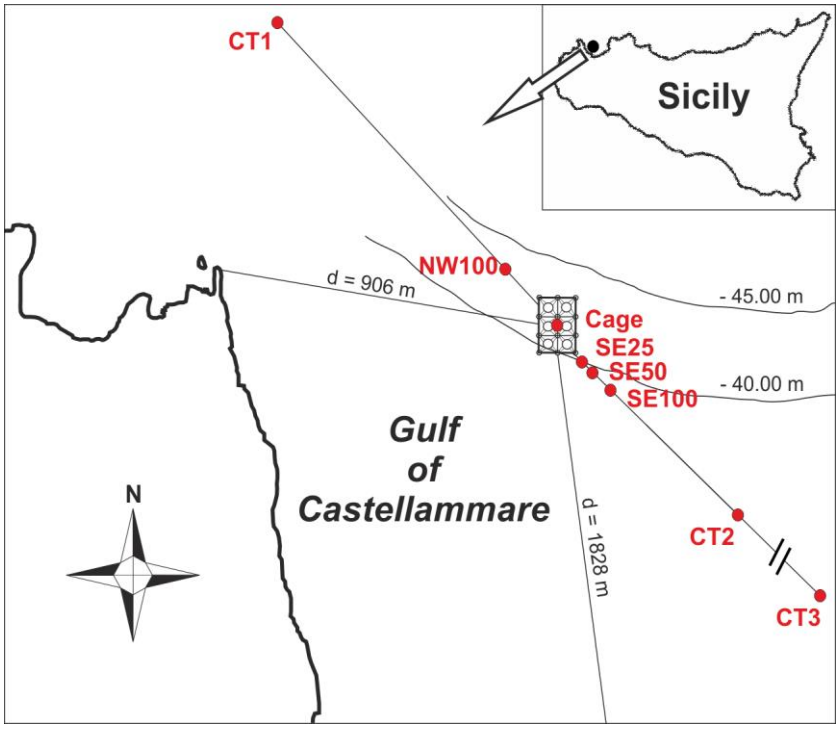

Fig. 2. Principal Component Analysis (PCA) ordination of samples from the Gulf of Castellammare, based on the square root transformed and normalized environmental data (TOM, sulfides, sediment mean size and sorting).

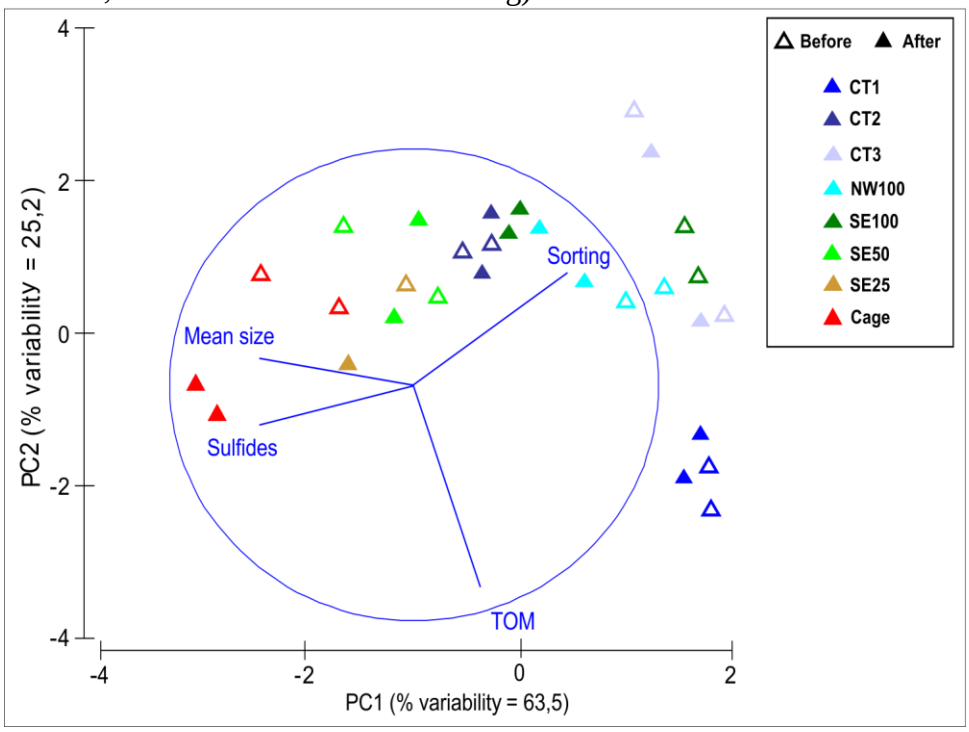

Fig. 3. nMDS plot based on meiofaunal abundances from eight sites (3 replicates) sampled over the two farming cycles. The samples ordination was based on Bray–Curtis similarity matrix calculated on log(x+1) transformed data.

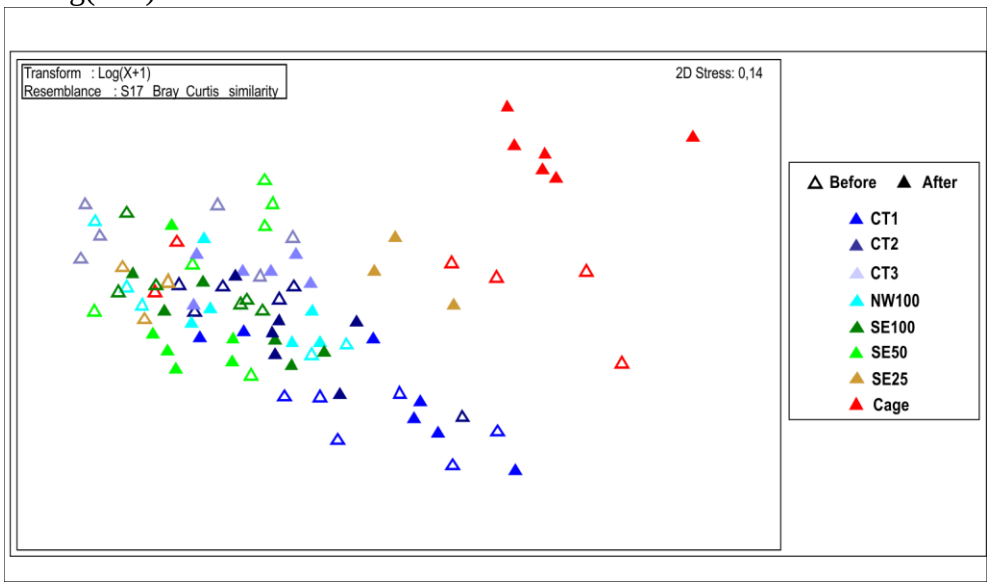

Fig. 4. Kinorhynchs from the Gulf of Castellammare. The two most abundant species found at the eight sites over the two sampling cycles. DIC photomicrographs. (A, C) *Echinoderes capitatus*; (A) adult, ventral view; (C) close up of the final segments; (B, D) *Pycnophyes carinatus*; (B) adult, ventral view; (D) close up of the first segment.

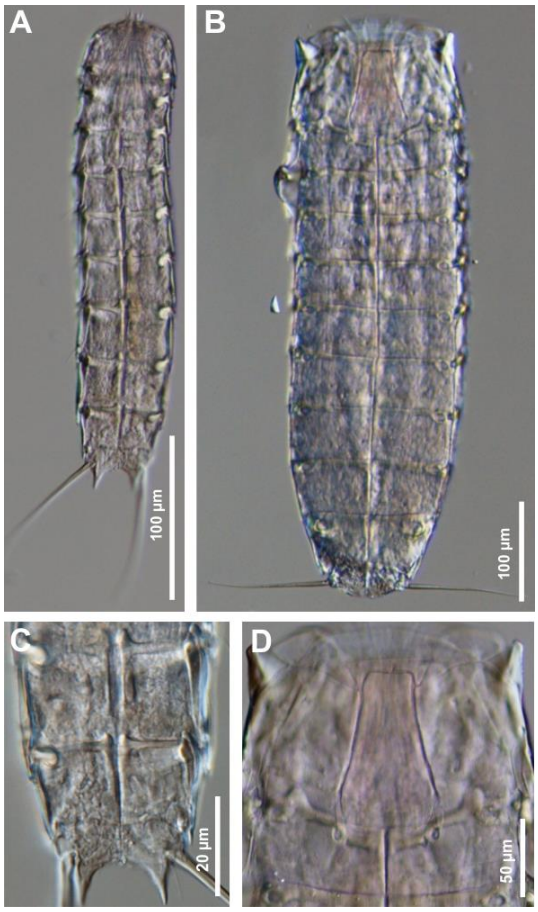

Fig. 5. Kinorhynchs from the Gulf of Castellammare. Community species composition (%) at the eight sampling sites over the two farming cycles.

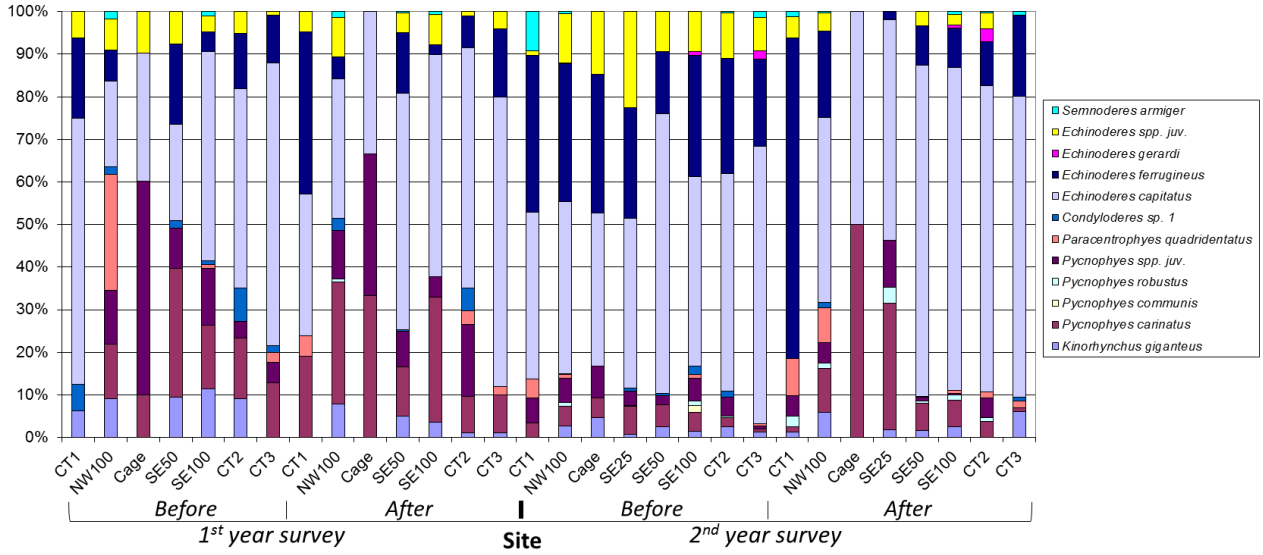

Fig. 6. Kinorhynchs from the Gulf of Castellammare. Average densities found at the seven investigated sites over the 2006 farming cycle. Error bars represent ± 1 standard deviations.

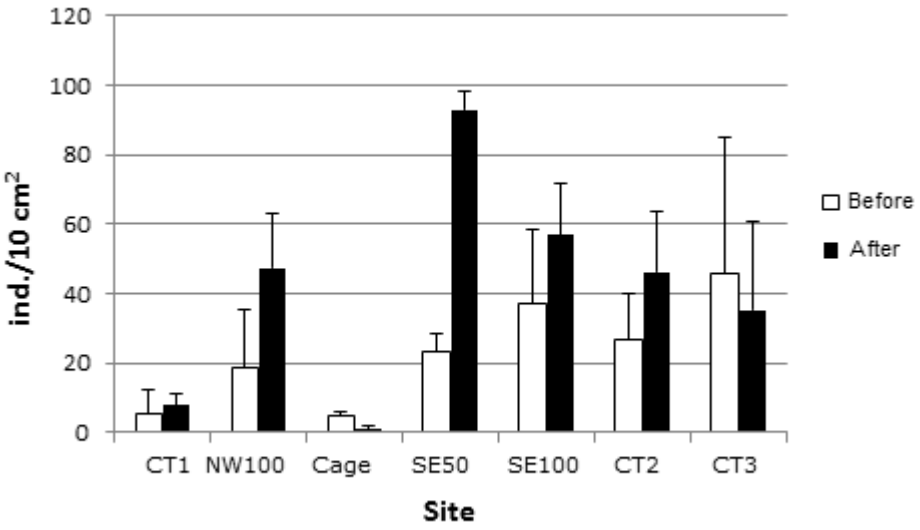

Fig. 7. Kinorhynchs from the Gulf of Castellammare. Average densities found at the eight investigated sites over the 2007 farming cycle. Error bars represent ± 1 standard deviations.

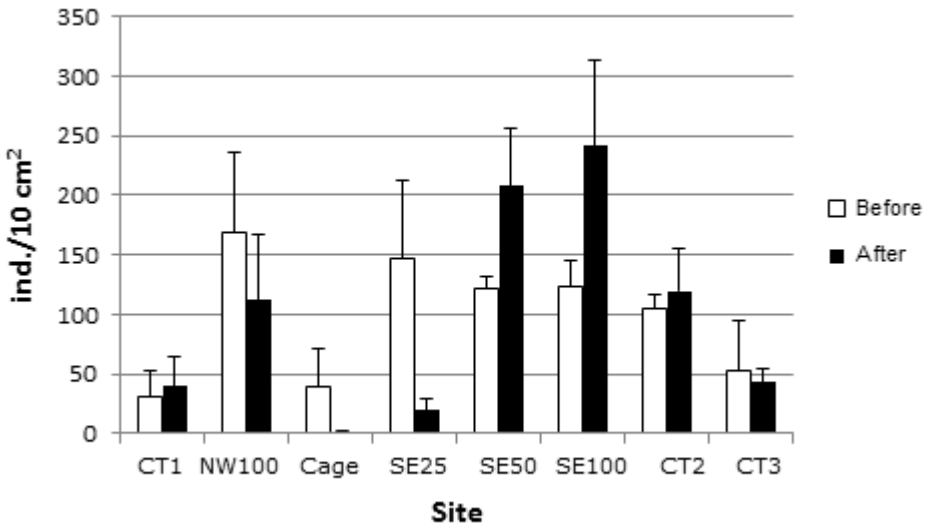

Fig. 8. nMDS plot based on the mean abundances of the kinorhynch species from eight sites sampled over the two farming cycles. The samples ordination was based o on Bray–Curtis similarity matrix calculated on square root transformed data.

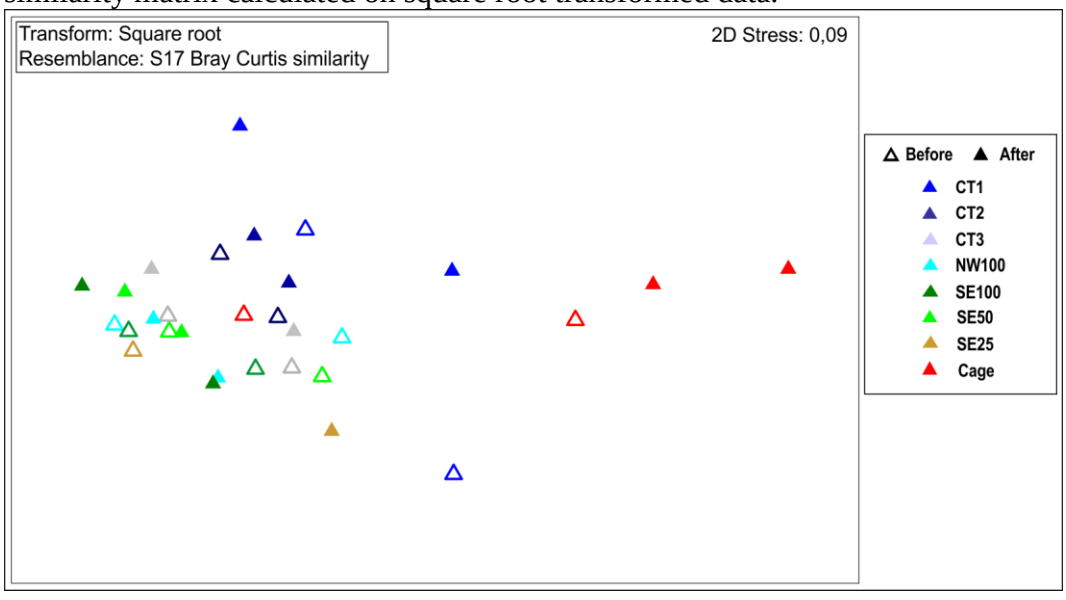

Fig. 9. Sulfide concentrations (μM) at the investigated sites in the Gulf of Castellammare during the two farming cycles.

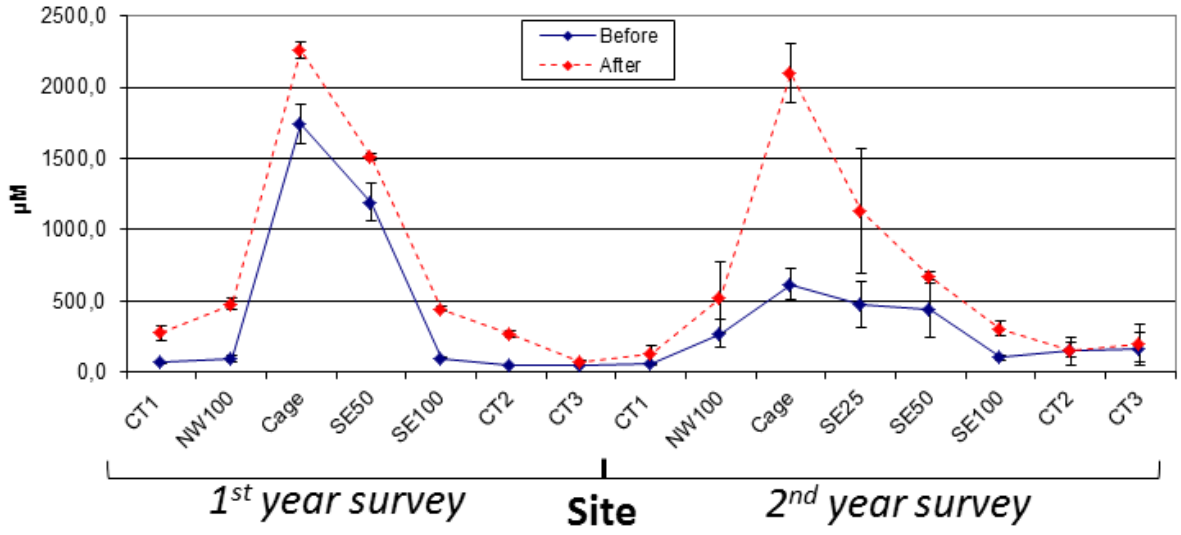

Fig. 10. Comparison among nematodes/kinorhynchs ratio, nematodes/copepods ratio, and sulfide concentration (μM) at the surveyed sites in the Gulf of Castellammare.

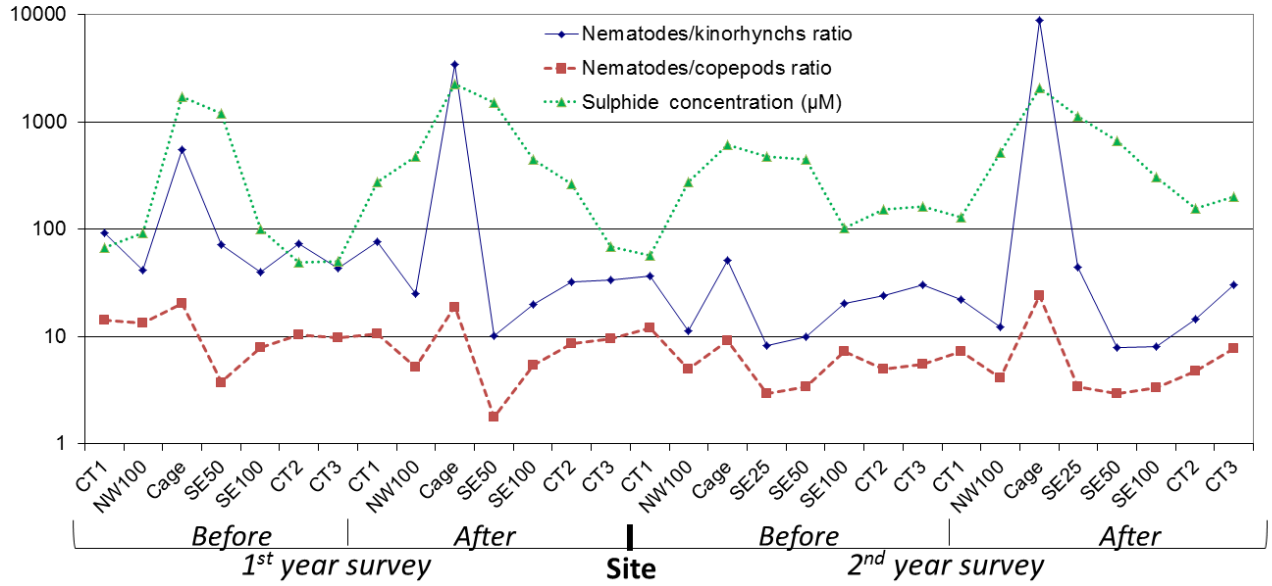

Table 1 Mean density ± standard deviation of some environmental variables (sulphides (μM), TOM (%), sediment mean size (mm) and sorting) from the eight sampling sites in the Gulf of Castellammare during the 'Before' (B) and 'After' (A) phases, 2006 and 2007 surveys.

Site	Phase	Sulfides	TOM	Mean size	Sorting
Year 2006					
CT1	B	68.10 ± 2.12	8.17 ± 0.23	0.05	0.59
	A	277.0 ± 53.70	7.47 ± 0.29	0.05	0.60
NW100	B	92.30 ± 23.62	4.71 ± 0.21	0.06	0.61
	A	480.5 ± 41.70	4.57 ± 0.09	0.06	0.60
Cage	B	1740.00 ± 141.42	3.48 ± 0.32	0.06	0.53
	A	2260.0 ± 56.60	4.67 ± 0.72	0.06	0.50
SE50	B	1195.00 ± 134.35	3.52 ± 0.02	0.07	0.58
	A	1515.0 ± 21.20	4.52 ± 0.89	0.06	0.58
SE100	B	101.95 ± 2.90	3.76 ± 0.14	0.06	0.62
	A	445.0 ± 14.10	4.15 ± 0.45	0.06	0.60
CT2	B	48.95 ± 0.49	4.29 ± 0.42	0.06	0.59
	A	264.0 ± 22.60	4.15 ± 1.28	0.06	0.58
CT3	B	50.75 ± 0.49	5.16 ± 0.16	0.06	0.63
	A	68.7 ± 13.30	4.99 ± 0.51	0.05	0.63
Year 2007					
CT1	B	56.80 ± 7.69	7.35 ± 0.25	0.05	0.57
	A	128.70 ± 64.33	6.71 ± 0.27	0.05	0.60
NW100	B	274.67 ± 100.25	4.97 ± 0.80	0.06	0.60
	A	525.00 ± 246.02	3.96 ± 0.92	0.06	0.62
Cage	B	616.67 ± 107.94	3.94 ± 0.54	0.06	0.52
	A	2100.67 ± 205.51	4.19 ± 0.72	0.06	0.50
SE25	B	476.67 ± 165.02	4.14 ± 1.35	0.06	0.56
	A	1132.33 ± 439.94	4.88 ± 1.23	0.06	0.54
SE50	B	447.00 ± 202.90	4.63 ± 0.82	0.07	0.58
	B	668.33 ± 42.72	3.49 ± 0.28	0.06	0.59
SE100	A	102.57 ± 22.43	4.55 ± 0.42	0.06	0.61
	B	309.33 ± 53.46	3.94 ± 0.51	0.06	0.60
CT2	A	153.37 ± 98.34	4.25 ± 0.12	0.05	0.58
	B	158.67 ± 53.15	4.41 ± 0.34	0.06	0.57
CT3	A	165.10 ± 119.51	2.44 ± 0.36	0.06	0.63
	B	203.13 ± 130.59	2.72 ± 0.13	0.06	0.61

Table 2 Meiofauna of the Gulf of Castellammare, 2006 survey. Mean density ± standard deviation (ind./10 cm^2) of major taxa and total meiofauna found in the seven sampling sites during the 'Before' (B) and 'After' (A) phases.

Taxon	Phase	Site						
		CT1	NW100	Cage	SE50	SE100	CT2	CT3
Nematoda	B	511.3±40.3	790.1±358.5	1430.6±127.5*****	1662.0±27.0*****	1956.2±432.5	1964.7±428.6	1968.7±584.9
	A	591.7±93.4	1189.7±208.8	3041.2±462.6*****	946.5±57.3*****	1128.0±30.6	1504.1±72.7	1186.7±425.7
Harpacticoida	B	36.0±10.6	58.8±32.3*****	69.4±38.2	441.9±47.9	246.8±27.5	187.7±52.5	200.9±66.9
	A	55.4±24.3	228.1±117.5*****	160.2±74.5	533.3±199.1	209.4±44.9	174.5±23.7	123.0±69.2
Nauplii	B	13.6±7.8	16.5±10.6	7.9±8.7	70.9±12.7	65.0±23.1*****	62.8±17.3	58.4±46.9
	A	7.4±3.0	29.0±23.6	3.5±2.9	85.2±61.7	17.6±6.9*****	33.4±11.6	13.2±7.6
Kinorhyncha	B	7.7±5.0	20.2±16.5	4.4±2.2*	19.5±10.5*	40.0±19.9	28.3±13.3	45.9±39.2
	A	12.8±4.2	51.4±15.2	0.7±0.6*	97.3±5.2*	62.4±14.2	34.5±27.4	36.7±27.3
Polychaeta	B	19.5±6.7	29.4±12.4	34.5±14.9*****	105.0±9.4	56.2±8.8	85.9±10.9	112.4±36.8
	A	30.0±15.9	60.2±34.4	11.7±3.9*****	40.0±10.5	36.4±12.7	66.1±11.0	78.2±10.5
Others	B	8.4±0.6	9.9±3.8	12.7±7.4	22.8±12.1	12.5±3.4	17.3±10.8	18.0±5.6
	A	5.0±1.1	17.6±7.7	7.9±4.8	14.0±3.9	9.2±3.5	13.6±6.1	13.6±2.3
Total meiofauna	B	595.4±58.6	923.4±418.0	1557.7±123.3*	2325.7±79.4*	2386.3±487.0	2345.6±487.1	2406.2±741.7
	A	697.2±115.0	1572.0±386.4	3225.5±438.2*	1712.0±286.9*	1457.4±41.7	1838.0±2.9	1450.1±460.2

*, Statistically significant differences.

Table 3 Meiofauna of the Gulf of Castellammare, 2007 survey. Mean density ± standard deviation (ind./10 cm^2) of major taxa and total meiofauna found in the eight sampling sites during the 'Before' (B) and 'After' (A) phases.

Taxon	Phase	Site							
		CT1	NW100	Cage	SE25	SE50	SE100	CT2	CT3
Nematoda	B	1131.4±160.8	1914.5±163.7	2041.6±614.0*	1203.8±304.5	1203.4±233.9	2504.8±431.1	2532.4±504.9	1599.4±987.5
	A	889.9±175.8	1392.2±447.6	6520.5±1178.8*	874.3±467.4	1642.7±563.7	1972.6±234.4	1744.1±414.6	1305.1±51.2
Harpacticoida	B	93.7±70.4	379.8±74.6	224.1±173.2	406.6±137.7	348.6±83.9	344.9±64.6*	508.4±54.6	289.5±224.9
	A	122.3±40.8	337.9±143.4	267.4±215.6	256.0±111.7	560.9±138.6	591.4±70.0*	364.0±112.3	167.1±50.7
Nauplii	B	110.2±114.4	354.1±89.6*	117.9±95.4	250.2±88.4*	158.7±115.9	240.6±78.7	339.8±95.9*	144.4±129.2
	A	53.4±6.1	98.8±64.4*	11.4±16.9	15.1±2.3*	69.8±15.8	75.3±40.9	56.2±22.6*	42.6±32.0
Kinorhyncha	B	32.0±22.4	167.1±68.1	39.7±31.7*	146.2±64.8*	120.9±11.0*	126.0±21.6*	104.3±12.5	55.8±45.0
	A	29.8±29.8	113.5±56.7	0.7±1.3*	19.8±9.0*	207.9±48.6*	245.7±74.4*	119.8±36.3	42.6±12.5
Polychaeta	B	56.9±6.7	73.1±26.1	68.3±54.4	115.3 ± 24.8*	76.0 ± 26.4	115.3±10.2	98.8±27.4	59.1±43.5
	A	84.3±47.9	52.5±21.7	14.0±8.4	16.9 ± 9.2*	47.0 ± 30.2	100.6±13.1	87.8±16.9	51.1±31.3
Others	B	43.0±28.2	32.3±6.3	14.0±11.1	18.7 ± 5.0	20.9 ± 15.3	22.8±4.5	40.0±5.0*	15.4±6.7
	A	12.1±7.7	10.7±3.9	4.4±2.9	6.2 ± 5.2	22.8 ± 5.2	22.4±7.3	12.5±7.1*	9.2±4.6
Total meiofauna	B	1465.7±340.3	2922.1±415.1	2505.6±976.0*	2141.6 ± 609.9	1928.5 ± 448.3	3351.9±591.0	3623.7±589.2	2160.7±1409.9
	A	1201.7±302.5	2004.5±683.6	6818.4±1297.5*	1188.3 ± 595.7	2551.1 ± 652.2	3003.7±282.0	2384.4±594.5	1617.7±171.3

*, Statistically significant differences.

Table 4 Testing for differences between impact and control sites in meiofaunal community abundances and single taxa densities (only significant results are shown) within the Gulf of Castellammare. PERMANOVA tests were conducted on Bray-Curtis similarity matrices and the residuals were permutated under a reduced model, with 999 permutations. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Source	df	Meiofauna		Nematoda		Kinorhyncha		Polychaeta	
		MS	#F	MS	F	MS	F	MS	F
Before vs. After = B	1	151.70	0.73	0.11	0.22	1.15	0.27	0.92	5.75
Locations = L	3	571.64	12.08**	3.60	15.00**	16.24	29.39***	4.35	5.88*
Impact vs. Control = I	1	1045.60	55.66	5.60	32.94*	39.36	57.93***	9.90	990.00***
Among controls = C	2	334.65	5.44	2.6	32.5***	4.68	8.83***	1.57	4.24*
Years = Y(B)	2	206.90	5.60**	0.50	6.25**	4.30	8.16**	0.16	0.43
B x L	3	134.03	2.83*	1.05	13.12***	3.79	7.15***	1.32	3.57*
B x I	1	362.55	19.30*	3.01	37.62***	11.06	20.87***	3.38	9.13**
B x C	2	19.76	0.32	0.07	0.26	0.15	0.31	0.29	0.26
Y(B) x L	6	47.30	1.28	0.24	3.00	0.55	1.04	0.74	2.00
Y(B) x I	2	18.78	0.35	0.17	2.12	0.68	1.28	0.01	0.03
Y(B) x C	4	61.56	1.66	0.27	3.37	0.49	0.92	1.11	3.00
Residuals	32	36.97		0.08		0.53		0.37	
Total	47								

Table 5 Kinorhyncha of the Gulf of Castellammare, 2006 survey. Mean density ± standard deviation (ind./10 cm^2) of the species found in the seven sampling sites during the 'Before' (B) and 'After' (A) phases.

Taxon	Phase	Site						
		CT1	NW100	Cage	SE50	SE100	CT2	CT3
Condyloderes sp. 1	B	0.4±0.6	0.4±0.6	-	0.4±0.6	0.4±0.6	2.2±1.1	0.7±0.6
	A	-	1.5±2.5	-	0.4±0.6	-	1.8±1.3	-
Echinoderes capitatus	B	3.7±5.4	4.0±1.7	1.1±1.1	4.4±2.9	19.1±9.0	13.2±4.0	30.5±29.0
	A	2.6±1.3	16.9±10.2	0.4±0.6	54.0±22.0	32.0±14.8	19.5±20.7	25.0±16.4
Echinoderes ferrugineus	B	1.1±1.1	1.5±0.6	-	3.7±2.3	1.8±2.3	3.7±1.7	5.1±5.1
	A	2.9±2.3	2.6±2.5	-	14.0±9.4	1.5±1.7	2.6±2.3	5.9±6.1
Echinoderes spp. juv.	B	0.4±0.6	1.5±1.7	0.4±0.6	1.5±1.7	1.5±1.7	1.5±1.7	0.4±0.6
	A	0.4±0.6	4.8±6.5	-	4.4±2.9	4.4±2.9	0.4±0.6	1.5±1.7
Kinorhynchus giganteus	B	0.4±0.6	1.8±3.2	-	1.8±3.2	4.4±4.0	2.6±2.5	-
	A	-	4.0±3.5	-	4.8±1.3	2.2±0.0	0.4±0.6	0.4±0.6
Paracentrophyes. quadridentatus	B	-	5.5±4.8	-	-	0.4±0.6	-	1.1±1.1
	A	0.4±0.6	-	-	-	-	1.1±1.1	0.7±1.3
Pycnophyes carinatus	B	-	2.6±4.5	0.4±0.6	5.9±3.4	5.9±4.2	4.0±5.2	5.9±2.8
	A	1.5±1.3	14.7±4.2	0.4±0.6	11.4±1.7	18.0±6.1	2.9±2.8	3.3±2.9
Pycnophyes communis	B	-	-	-	-	1.1±1.1	-	-
	A	-	-	-	-	1.1±1.1	-	-
Pycnophyes robustus	B	-	-	-	-	-	-	-
	A	-	0.4±0.6	-	-	-	-	-
Pycnophyes spp. juv.	B	-	2.6±1.7	1.8±1.3	1.8±0.6	5.1±3.9	1.1±1.1	2.2±1.9
	A	-	5.9±2.5	0.4±0.6	8.1±8.9	2.9±1.3	5.9±1.7	-
Semnoderes armiger	B	-	0.4±0.6	-	-	0.4±0.6	-	-
	A	-	0.7±0.6	-	0.4±0.6	0.4±0.6	-	-
Total	B	7.7±5.0	20.2±16.5	4.4±2.2	19.5±10.5	40.0±19.9	28.3±13.3	45.9±39.2
	A	12.8±4.2	51.4±15.2	0.7±0.6	97.3±5.2	62.4±14.2	34.5±27.4	36.7±27.3

Table 6 Kinorhyncha of the of the Gulf of Castellammare. 2007 survey. Mean density ± standard deviation (ind./10 cm^2) of the species found in the eight sampling sites during the 'Before' (B) and 'After' (A) phases.

Taxon	Phase	Site							
		CT1	**NW100**	**Cage**	**SE50**	**SE50**	**SE100**	**CT2**	**CT3**
Condyloderes sp. 1	B	-	0.4±0.6	-	1.1±1.1	0.7±1.3	2.6±0.6	1.5±0.6	-
	A	-	1.5±1.7	-	-	-	-	-	0.4±0.6
Echinoderes capitatus	B	12.5±13.3	67.6±21.9	14.3±10.1	58.4±29.8	79.3±26.1	55.8±19.3	53.3±2.3	36.4±28.2
	A	-	49.2±30.3	0.4±0.6	10.3±4.5	162.0±38.0	184.8±60.9	86.0±34.7	30.1±7.3
Echinoderes ferrugineus	B	11.8±10.2	54.0±32.1	12.9±11.3	37.8±16.0	17.6±4.8	36.0±4.2	28.3±6.7	11.4±8.9
	A	22.4±25.7	23.1±9.0	-	0.4±0.6	19.1±7.2	22.4±7.5	12.5±2.8	8.1±3.4
Echinoderes gerardi	B	-	0.4±0.6	-	-	-	1.1±1.1	-	1.1±1.1
	A	-	-	-	-	-	1.8±1.7	3.7±1.3	-
Echinoderes spp. juv.	B	0.4±0.6	19.5±13.1	5.9±5.5	33.1±10.9	11.4±9.6	11.8±7.1	11.0±4.0	4.4±7.6
	A	1.5±1.7	4.8±6.5	-	-	7.0±2.8	6.2±4.6	4.4±2.9	0.0±.0
Kinorhynchus giganteus	B	-	4.4±3.8	1.8±1.7	1.1±1.1	2.9±2.3	1.8±1.3	2.6±1.3	0.7±1.3
	A	0.4±0.6	6.6±4.8	-	0.4±0.6	3.3±2.9	6.2±5.1	-	2.6±2.3
Paracentrophyes quadridentatus	B	1.5±2.5	1.5±1.3	-	-	-	1.1±1.1	-	0.4±0.6
	A	2.6±2.5	9.2±3.4	-	-	0.4±0.6	1.8±1.3	1.8±0.6	0.7±0.6
Pycnophyes carinatus	B	-	-	-	-	-	2.2±1.1	-	-
	A	0.4±0.6	11.8±5.7	0.4±0.6	5.9±3.4	13.2±5.0	15.1±4.2	4.4±1.9	0.4±0.6
Pycnophyes communis	B	1.1±1.1	7.7±4.8	1.8±1.7	9.6±2.8	6.2±1.7	5.5±1.9	2.2±1.1	0.4±0.6
	A	-	-	-	-	-	2.2±1.1	-	-
Pycnophyes robustus	B	-	1.5±2.5	-	0.4±0.6	-	1.5±0.6	0.4±0.6	-
	A	0.7±1.3	1.5±1.7	-	0.7±0.6	1.1±1.1	2.9±2.8	1.1±0.0	-
Pycnophyes spp. juv.	B	1.8±2.3	9.6±9.0	2.9±2.5	4.8±5.4	2.6±1.7	6.6±6.1	4.8±1.7	0.4±0.6
	A	1.5±2.5	5.5±2.2	-	2.2±2.2	1.8±0.6	0.7±0.6	5.5±2.2	-
Semnoderes armiger	B	2.9±5.1	0.7±1.3	-	-	-	-	0.4±0.6	0.7±1.3
	A	0.4±0.6	0.4±0.6	-	-	-	1.5±0.6	0.4±0.6	0.4±0.6
Total	B	32.0±22.4	167.1±68.1	39.7±31.7	146.2±64.8	120.9±11.0	126.0±21.6	104.3±12.5	55.8±45.0
	A	29.8±29.8	113.5±56.7	0.7±1.3	19.8±9.0	207.9±48.6	245.7±74.4	119.8±36.3	42.6±12.5