MULTIPLE HUMAN PRESSURES IN COASTAL HABITATS: VARIATION OF MEIOFAUNAL ASSEMBLAGES ASSOCIATED WITH SEWAGE DISCHARGE IN A POST-INDUSTRIAL AREA

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ABSTRACT

Marine ecosystems are globally threatened by human activities, but some areas, such as those affected by abandoned industrial plants, show an overlap of acute and chronic impacts, which determine a considerable deterioration of their health status. Here we report the results of a research conducted on coastal sewers that discharge their loads in the highly contaminated area of Bagnoli-Coroglio (Tyrrhenian Sea, Western Mediterranean). The sampling area is characterised by heavy industrial activities (a steel plant using coal, iron and limestone) started in 1905 and ceased in 1990, which left widespread heavy metals and hydrocarbon contamination. After taking into account the potential influence of sediment grain size ranges through their inclusion as covariates in the analysis, we tested the potential impact of sewage discharge on the total abundance and multivariate structure of meiofaunal assemblages, as well as on the abundance of single taxa. The organic matter was analysed in terms of total phytopigment and biopolymeric carbon concentrations. Nematoda, Copepoda (including their nauplii), and Tardigrada were the most abundant meiofaunal taxa at all sites, but nematodes did not show a consistent pattern relative to the sewage outfalls. However, the sewer located in the historically most contaminated area showed a minimal abundance of all taxa, including nematodes, while copepods were relatively less abundant at the two southernmost sewers. Comparing the north *vs.* south site of the sewers, higher meiofaunal abundances were observed in the southward part, likely as a result of the local circulation. The results of this study indicate the general adaptation of meiofauna to multiple stressors (sewage discharge, superimposed to chronic industrial contamination) and its likely modulation by other local processes. They also provide relevant baseline information for future restoration interventions that would take into account the spatial variation of target organisms as needed.

Keywords: Anthropogenic disturbance – Brownfield – Contamination – Environmental impact – Sediment – Subtidal

1. Introduction

Marine ecosystems are globally threatened by human activities that have the potential to directly and indirectly alter their biodiversity and environmental quality, jeopardise valuable goods and services and ultimately harm human health (Myers and Worm, 2003; Lotze et al., 2006; Halpern et al., 2008). Increased urban and industrial development and the discharge of contaminants, nutrients and sediments due to growing human pressure are responsible for habitat alteration, especially in coastal areas (Vitousek et al., 1997; Agardy et al., 2005; Spalding et al., 2014; Neumann et al., 2015). The impacts of such activities on all biological components and on the physical and chemical environmental conditions can drastically vary depending on their intensity, spatial and temporal extent, and the complex interactions among different stressors (Halpern et al., 2007). The Mediterranean Sea, for example, is one of the historically most impacted marine regions of the world (Costello et al., 2010), and current impacts are exacerbated by climate change (Airoldi and Beck, 2007; Claudet and Fraschetti, 2010; Lejeusne et al., 2010; Coll et al., 2012; Micheli et al., 2013).

A notable result of the past and current human use of coastal areas is the inheritance of derelict post-industrial plants and sites (Krinke, 2001; Loures, 2015), in most cases associated with a major accumulation of a range of xenobiotic compounds of environmental and social concern. Restoring impacted marine habitats and returning healthy marine landscapes to the local community is a major challenge from an ecological and societal point of view. The achievement of such objectives is complicated by the complexity of the habitat degradation, the degree of contamination by xenobiotics, and the difficulty of reconciling multiple, consolidated and contrasting uses (McGrath, 2000; Amekudzi, 2004; Alberini et al., 2005; Kaufman and Cloutier, 2006). The first step of any attempt towards ecological restoration in post-industrial areas is to assess the presence of chronic conditions of crucial concerns from both the environmental and the societal point of view, and their interaction with other stressors. The ultimate goal is to transform degraded landscapes into new development opportunities (Meyer, 2000; ECCREDI, 2001). In this context, the environmental inheritance of former industrial activities may be superimposed by the potential impact of sewage discharge, a common source of anthropogenic disturbance in coastal marine systems (e.g., Bishop et al., 2002; Fraschetti et al., 2006). Gradients of decreasing contamination occur typically along increasing distance from point-source emissions (Keough and Black, 1996; Raimondi and Reed, 1996), depending on the dispersal of contaminants by waves and their dilution with seawater (Foe and Knight, 1987; Chapman et al., 1995; Pinto and Bemvenuti, 2006). Sewage discharge, in particular, may determine gradients in water temperature, salinity, concentration of nutrients, heavy metals and organic pollutants, the effects of which have been reported on a wide range of habitats and organisms. These include seagrasses and macroalgae, sessile and mobile animal species, including macro-, megafauna and fish either inhabiting hard substrates or soft bottoms, pathogens and bacteria, although with largely variable responses in terms of abundance, functional diversity, and spatial and temporal patterns of distribution (Fairweather, 1990; Smith et al., 1999; Otway et al., 1996; Guidetti et al., 2003; Balestri et al., 2004; Terlizzi et al., 2005a, 2005b; Ballesteros et al., 2007; Korajkic et al., 2010). Concomitantly, sewage discharge affects the organic content and biogeochemistry of sediments (Cotano and Villate, 2006) and alters the biochemical composition of suspended particulate organic matter in the water column (Oviatt et al., 1987). It is known, however, that the inverse gradient, with the highest contamination occurring at relatively large distance from the source, may occur due to the decrease of solubility of organic compounds driven by the increase of salinity. Such a ‘salting out’ phenomenon can occur when the sewage is mixed with seawater, leading to increased precipitation of contaminants at increasing distance from the outlet (Lu and Pignatello, 2004; Qi et al., 2008).

Meiofauna are widely used for environmental impact assessments due to their life-traits, namely small size, high turnover and lack of larval dispersal, that make them sensitive to a range of extant environmental alterations (reviewed by Zeppilli et al., 2015) and experimental manipulations of contaminants (e.g., Gallucci et al., 2015; Santos et al., 2018). In addition, meiofauna are one of the most diversified groups in the marine realm (Heip et al., 1985; Giere, 2009), are widely distributed in all habitats (e.g., Danovaro and Fraschetti, 2002; Mirto and Danovaro, 2004; Danovaro et al., 2009; Riera et al., 2013; Dupuy et al., 2015) and play a key role in ecosystem functioning, including the transfer of organic matter to higher trophic levels and the flux of energy through food webs (Woodward, 2010; Balsamo et al., 2012). Meiofauna are found on all types of substrates, from intertidal and subtidal habitats to hadal sediments, with patterns of abundance and composition typically variable depending on the grain size and the availability of food (Sandulli and Pinckney, 1999; Balsamo et al., 2010; Semprucci et al., 2011).

In this study, we examined the potential effect of sewage discharge on meiofaunal assemblages inhabiting sediments historically affected by abandoned industrial plants in the Bagnoli-Coroglio area (Tyrrhenian Sea, southern Italy), which were built for the production of chemicals, steel, concrete and asbestos during most of the 20th century, until their complete cessation in the mid-‘90s. The available information indicates that the sediments are characterized by high concentrations of heavy metals and polycyclic aromatic hydrocarbons (Sharp and Nardi, 1987; Romano et al., 2004, 2009; Arienzo et al., 2017; Trifuoggi et al., 2017). Due to its high contamination, the Bagnoli-Coroglio area has been classified as a Site of National Interest (SIN). The potential impact of sewage discharge overlaps the one due to industrial chemical contamination, further complicating any possible effort towards decontamination and restoration.

Specifically, we tested the hypotheses that, independently of the specific underlying processes and mechanisms that could not be univocally tested by the present descriptive study, the abundance and taxa composition of meiofaunal assemblages from shallow-water sediments (i) differed at increasing distance from coastal sewers spread along the coast in the Bagnoli-

Coroglio area, (ii) differed between the northern and the southern side of the sewage outfall, and (iii) these patterns were consistent among a set of sewers in spite of their putative differences in location, nature and amount of effluents.

2. Materials and methods

2.1. Study area

The study area is located within the Gulf of Naples (southern Tyrrhenian Sea) at the south-eastern portion of the Pozzuoli Bay, about 10 km west of the city of Naples (Fig. 1). As part of the Campi Flegrei volcanic district, it is characterised by an intense and persistent volcanic activity, with underwater gas emissions and bradyseism. This, combined with anthropogenic interventions and urban sprawl, has severely altered the coastline and the circulation of water and sediments. With the exception of the rocky shore around Nisida Island, the coast is generally low and sandy, although often protected by artificial rocky reefs. The predominant water circulation rotates clockwise in the inner part of the Gulf of Naples and flows northward offshore during the winter. In summer, the inner circulation turns into anticlockwise, while the offshore current flows southward (Pennetta et al., 1998). The marine sediments are mainly represented by coarse sand and sandy silt on the littoral shelf, fine sand at the margin of the gulf (Cocco et al., 1998) and silty and silty-clay particles in the central basin (De Pippo et al., 1988). The geological and oceanographic characteristics of the study area were illustrated in detail by Romano et al. (2004) and Arienzo et al. (2015).

The industrial activities in the Bagnoli-Coroglio area began in 1905 and were mainly represented by a steel plant using coal, iron ores and limestone which were carried by ships to the coast as raw materials, transported by a conveyor belt to the plant and then processed. The iron production was increased until its interruption due to the Second World War in 1943. By this date, the plant was also enlarged, including the construction in 1930 of a northern long pier for large ships delivering raw materials, and a southern pier where the final products were loaded onto outgoing ships. The steel and iron production restarted in 1946 and lasted until 1990, when all activities ceased. Between 1962 and 1964, the marine area between the two piers was partially filled up with contaminated soil from the plant to obtain new space for enlargement. A comprehensive reclamation project has been recently developed, including a multidisciplinary assessment aimed at evaluating the ecological impacts of past industrial activities with reference to the natural and anthropogenic characteristics of the local environment (ABBaCo project, see http://www.gazzettaufficiale.it/eli/id/2017/03/08/17A01736/sg%20).

A previous study reported high concentrations of heavy metals (Cu, Fe, Hg, Mn, Pb and Zn), polychlorinated biphenyls (PCB), polycyclic aromatic hydrocarbons (PAH) and dichlorine diphenyl trichlorethane (DDT) in the study area (Damiani et al., 1987). Similarly, anomalous high concentrations of Ag, As, Cd, Co, Cr, Cu, Hg, Ni, Pb and Zn were found in sediments between the two long piers (Sharp and Nardi, 1987) with contamination by Cd, Pb, Zn and Mn mainly restricted to the southern side of the plant, and contamination by Fe and Mn widespread over the entire area (Romano et al., 2004). Arienzo et al. (2017) also found PAH concentrations in the sediments three to four orders of magnitude higher than those reported from several marine benthic ecosystems worldwide.

A total of ten sewers have been mapped in the study area, including eight located at ground level along the coast and two located offshore at ~30 m depth (Fig. 1). The chemical composition, amount and temporal variability of the effluents are not known in detail for each sewer, but they are generally mixed, including domestic sewage and terrestrial runoff. For instance, the Coroglio physical treatment plant collects urban sewage from the western part of Naples, with the primary aim of reducing its solid load. Then, flow rates up to 2.2 m/s are pre-treated there and then sent to the Cuma treatment plant, while higher rates, up to 6.2 m/s are pre-treated and then delivered to the sea through two submarine pipes about 1600 m long. Exceeding flow rates by-pass the plant and are directly delivered to the sea through a sewer tunnel located under the Posillipo Promontory and opening along the shore south of the Island of Nisida (Messina and Iacone, 2011). The sewer located close to the Dazio lift station in the city of Bagnoli collects, throughout the year, excess wastewater from the plants belonging to the *Consorzio di Bonifica Conca di Agnano e dei Bacini Flegrei* (http://www.bonificagnanoeflegrei.it/), which is released directly to the sea.

2.2. Sampling design and collection of sediment samples

The study was conducted in April 2017, focusing on the sewers located along about 3 km of coast in the Pozzuoli Bay. Out of total eight sewers mapped, four (Dazio, Conca di Agnano, Canale Bianchettaro, and Coroglio tunnel) were selected at random provided they were interspersed across the entire study area (Fig. 1). Since the exact characteristics of each sewer in terms of chemical composition, amount and periodicity of the effluents were unknown, such four sewers were intended as representative of the variability in sewage discharge spanned by all eight coastal sewers. The sampling was carried out on shallow (1-3 m depth) soft-bottom at two distances (10 m and 50 m) and on opposite sides (north and south) relative to the outlet of each sewer. In each of these conditions, three sediment samples were randomly collected by SCUBA divers using Plexiglas corers (3.6 cm inner diameter, 25 cm length, tens cm apart) and immediately stored at -20°C until their analysis in the laboratory. All sewers were sampled during the same day.

2.3. Grain size and biochemical composition of organic matter

Sediment grain size was determined by the sieving technique (e.g., Danovaro et al., 2008; Danovaro, 2010). Samples were treated with a 10% H_2O_2 solution to remove organic matter, and the total and non-biogenic grain size distribution determined. Data of the grain size were analysed with GradiStat software (Blott and Pye, 2001).

Phytopigments (chlorophyll-a and phaeopigments) were analysed fluorometrically according to Lorenzen and Jeffrey (1980). Pigments were extracted with 90% acetone (24 h in the dark at 4 C). After centrifugation (800 × g), the supernatant was used to determine the functional chlorophyll-a and acidified with 0.1 N HCl to estimate the amount of phaeopigments. Total phytopigment concentrations were defined as the sum of chlorophyll-a and phaeopigment concentrations (Pusceddu et al. 2009).

Protein, carbohydrate and lipid concentrations in surface sediments were determined spectrophotometrically, following the protocols detailed in Danovaro (2010), and expressed as bovine serum albumin, glucose and tripalmitine equivalents, respectively. Carbohydrate, protein and lipid concentrations were converted into carbon equivalents using the conversion factors of 0.40, 0.49 and 0.75 µgC µg^{-1}, respectively, and their sum defined as biopolymeric carbon (BPC) (Dell'Anno et al., 2002).

2.4. Meiofaunal abundance and diversity

Meiofauna were extracted from the sediment by decantation (Danovaro et al., 2004a). Sediments were rinsed with filtered seawater and sieved onto a 500 µm mesh sieve. All the material passing the sieve was collected in a beaker. Sediments were mixed and resuspended in filtered seawater and, after a few seconds (to let the coarse fraction to settle), the water was filtered onto a 30 µm mesh sieve. This procedure was repeated 10 times to ensure an extraction efficiency of meiofaunal organisms of 100%. Such organisms included a fraction of temporary meiofauna (animals that are of meiofaunal size during their early life stages, but become macrofauna when they grow). In ecological studies, temporary and permanent meiofauna are normally considered together as their size is crucial for determining their response to changing environmental conditions (e.g., Danovaro et al., 2004b; Fraschetti et al., 2006; Moreno et al., 2008; Bianchelli et al., 2010; Kalogeropoulou et al., 2010; Mirto et al., 2010). All sediment residuals were checked and no organisms were found. The material collected onto the filter was then fixed with 4% buffered formalin solution and stained with Bengal Rose. All specimens from three independent replicates per station were counted and sorted by taxa, under a stereomicroscope.

2.5. Statistical analyses

Multivariate analysis of variance based on permutations (PERMANOVA, Anderson, 2001) based on Bray-Curtis untransformed dissimilarities was used to test for differences in the composition of meiofaunal assemblages depending on the distance and the side relatively to each examined sewer. The analysis was based on a three-way model including the three crossed factors ‘Sewer’ (random, four levels), ‘Distance’ (fixed, two levels: 10 m *vs.* 50 m) and ‘Side’ (fixed, two levels: north *vs.* south), with replicates provided by the three cores sampled in each combination of levels of these factors. The sediment grain size data corresponding to the ‘very fine gravel’ and the ‘coarse sand’ were included as covariates in PERMANOVA, after the removal of the other grain size categories which were found to be significantly correlated with them (see Supplement A for details). When relevant, post-hoc comparisons were made by means of pair-wise *t* tests for levels of factors involved in significant interactions. A multivariate multiple regression analysis (DISTLM forward) was carried out, using the routine included in the PRIMER 6+ software (Clarke and Gorley, 2006) to quantify the percentage of variability explained by each relevant grain size category. This analysis was based on Bray-Curtis dissimilarities with 4999 permutations of residuals.

Multivariate patterns of ‘average’ assemblages in each core for each combination of sewer × distance and sewer × side were visualized by non-metric multidimensional scaling (nMDS) based on Bray-Curtis untransformed dissimilarities.

Differences in the total abundance of meiofaunal individuals and the abundance of single meiofaunal taxa were tested with analysis of variance (ANOVA), based on the same model as PERMANOVA. Before each ANOVA, homogeneity of variances was checked with Cochran’s

C test. When relevant, data were square root- or log-transformed to remove the heterogeneity of variances. When this was not possible, untransformed data were analysed and the results were considered robust if not significant (at $p>0.05$) or significant at $p<0.01$ to compensate for increased probability of type I error (Underwood, 1997).

3. Results

3.1. Sediment grain size

Fine and medium sands (0.25 mm to 0.5 mm) were the dominant components (from ~78% to ~94%) of the sediments sampled at each distance and side to the outlet of the two northernmost sewers (Dazio and Conca Agnano, Fig. 2 A and B, respectively). Sediments were generally coarser at the two southernmost sewers, i.e., the closest to the former industrial plant. Specifically, more than 90% of the sediment was characterized by particles larger than 0.5 mm, between 0.25 mm and 2 mm, larger than 0.125 mm (with >53% provided by very fine gravel) and between 0.125 and 1 mm 10 m northward, 10 m southward, 50 m northward and 50 m southward, respectively, to the outlet of Canale Bianchettaro (Fig. 2 C). At Coroglio tunnel, more than 90% of sediment particles were larger than 0.25 mm at both northward distances and larger than 1 mm (with ~47% provided by very fine gravel) 10 m southward to the outlet (Fig. 2 D).

The calculation of Pearson’s r coefficients identified a number of significant correlations between paired grain size categories (details in Supplement A). The proportion of very fine gravel, in particular, was positively correlated with that of very coarse sand and silt-clay and negatively correlated with that of medium and fine sand. The two other grain size ranges, i.e., coarse and fine sand, were negatively correlated each other. Therefore, only the sediment proportions of very fine gravel and coarse sand were retained and included as covariates in the PERMANOVA model to test for differences in meiofaunal assemblage composition once the possible influence of such variables was taken into account.

The organic matter content of the sediments changed considerably among sewers, distances and sides (Fig. 3). On average, however, the concentration of total phytopigments, proteins, carbohydrates, lipids and biopolymeric organic C was higher for the two sewers close to the industrial plant compared to the other sewers (Fig. 3 A, B, C, D and E, respectively). The most prominent specific patterns included (i) relatively higher concentration of phytopigments (Fig. 3 A), lipids (Fig. 3 D) and biopolymeric organic C (Fig. 3 E) in sediments located 50 m northward to Canale Bianchettaro; (ii) higher concentration of proteins in sediments 10 m southward and 50 m northward to Canale Bianchettaro, and 10 m southward to Coroglio tunnel compared to the other distances and sides relatively to the same sewers (Fig. 3 B); (iii) higher concentration of carbohydrates in sediments 50 m northward and southward compared to 10 m on both sides relatively to the Canale Bianchettaro outlet (Fig. 3 C).

3.2. Meiofaunal assemblage composition and total abundance

We initially took into consideration the possible influence of the grain size by including the very fine gravel and the coarse sand components in the PERMANOVA model, but such co-varying variables were not significant. Then, we tested how the composition (combining the identity of taxa and their relative abundance) of meiofaunal assemblages varied between distances and between sides depending on the sewer. Significant differences, in particular, were found between the 10 m and the 50 m distance from both the Canale Bianchettaro and the Coroglio tunnel sewers, and between the northern and the southern side of the Conca Agnano sewer, but not for all other comparisons (Table 1 and Fig. 4, A, B). The two grain sizes, however, collectively explained 44.5% of the total variability of meiofaunal assemblages, with 14.7% provided by very fine gravel and 29.8% provided by coarse sand (DISTLM results).

A significant ‘Sewer × Distance × Side’ interaction resulted for the total abundance (Table 2). This variable was larger at 10 m compared to 50 m northward to Conca Agnano, and on the southern compared to the northern side at 10 m and 50 m from Dazio and Conca Agnano, respectively (Table 3 and Fig. 5 A).

3.3. Meiofaunal higher taxa

Nematoda, Copepoda (including their nauplii), and Tardigrada were the most abundant meiofaunal taxa (Fig. 5 B, C and D) and showed the same pattern reported for the total meiofaunal abundance, varying in combinations of sewer, distance and side (Table 2 and Table 4). Specifically, the abundance of copepods was higher in sediments at 10 m than 50 m northward and 50 m than 10 m southward to Conca Agnano and Coroglio tunnel, respectively, and on the southern compared to the northern side at both distances from Dazio and 50 m northward to Conca Agnano (Table 4 and Fig. 5 B). Copepod nauplii were more abundant only at 10 m than 50 m northward to Dazio (Table 4 and Fig. 5 C). Nematoda were the most abundant taxon and showed similar patterns of differences as those of Copepoda, with the only exception represented by the lack of a significant difference between sides 50 m northward to Dazio (Table 4 and Fig. 5 D). Significant differences in Tardigrada were found only at Dazio, where their abundance was larger at 10 m compared to 50 m southwards, and on the southern than on the northern side 10 m away (Table 4 and Fig. 5 E).

Distance- and side-related differences were shown by Acarina (Table 3). This taxon was more abundant at 10 m than at 50 m and southwards compared to northwards relatively to Dazio, while such patterns were reversed at Coroglio tunnel (Table 4 and Fig. 5 F). No significant differences were found for Oligochaeta, although these annelids tended to be relatively more abundant 10 m northward to Coroglio tunnel (Table 3 and Fig. 5 G). Ostracods, instead, varied in abundance among sewers, independently of both distance and side (Table 3 and Fig. 5 H). The remaining 4 taxa, namely Polychaeta, Gastropoda, Bivalvia and Amphipoda were not formally analysed as either completely absent in most combinations of sewer, distance and side, or comparably abundant when present in more than one of the other conditions, thus showing obvious patterns. In particular, Polychaeta occurred only 50 m northward to Dazio, 50 m southward to Canale Bianchettaro and 10 m and 50 m northward to Coroglio tunnel (Fig. 5 I). Gastropoda were found only 10 m southward to Conca Agnano, 10 m both northward and southward to Canale Bianchettaro, 10 m northward and 50 m both northward and southward to Coroglio tunnel (Fig. 5 J). Bivalvia occurred only 10 m northward to Dazio, 50 m southward to Canale Bianchettaro, 10 m southward and 50 m northward to Coroglio tunnel (Fig. 5 K). Finally, Amphipoda were found only in sediments sampled 50 m northward to Canale Bianchettaro (Fig. 5 L).

4. Discussion

Meiofaunal responses to global and local human activities have been widely reported (Sandulli and De Nicola-Giudici, 1991; Austen et al., 1994; Danovaro et al., 1995; Mirto et al., 2000, 2002; Fraschetti et al., 2006; Pusceddu et al., 2007; Riera et al., 2013). Changes in meiofaunal abundance, diversity, biomass and distribution are among the widely described effects of anthropogenic disturbance, although the specific responses and their direction may be largely variable (reviewed by Coull and Chandler, 1992; Zeppilli et al., 2015). Such an idiosyncrasy was highlighted by the present study, where a number of potential responses of meiofauna to sewage discharge have been documented, but with variable patterns depending on the sewer. Therefore, our initial hypotheses were partially supported in terms of multivariate and univariate differences associated with the distance and side relative to the outlets, but not in terms of their consistency among multiple sewers.

As far as sewage discharge is specifically concerned, meiofaunal responses may depend on a range of abiotic and biotic factors and their complex interactions. For instance, sewage-related eutrophic conditions can indirectly affect meiofauna as a consequence of blooms of algae or cyanobacteria. Eutrophic conditions, when not associated with oxygen limitation, may enhance microbial activity and eventually increase meiofaunal abundance and diversity (Giere, 2009). On the contrary, oxygen depletion in sediments can cause the selection of opportunistic taxa tolerant to hypoxic conditions (Rabalais et al., 2001) or drastic decreases in meiofaunal abundance and diversity, as reported in the case of blooms of filamentous cyanobacteria (García and Johnstone, 2006) and green tides of macroalgae (Villano and Warwick, 1995; Neira and Rackemann, 1996; Teichberg et al., 2010; Shi et al., 2015). Such mechanisms, however, are unlikely to have occurred in the present system since phytopigment concentrations of the sediments were typically low and no algal blooms were observed in the study area even where the sewage was released directly to the sea, such as by the Dazio sewer. Similarly, the concentrations of the main biochemical components of organic matter, namely proteins, lipids and carbohydrates, were relatively low and within the range of Mediterranean coastal systems at the same depths (e.g., Pusceddu et al., 2009). The only notable exception was represented by the peak of lipid concentration north of sewer 3 (Canale Bianchettaro). Such a peculiar condition could be responsible for the minimal total abundance and the loss of several sensitive taxa of meiofauna at this site. Finally, we found no evidence of dominance by nitrophilic macroalgae in association with the examined sewers, which clearly suggests the lack of major eutrophication effects. Such a factor, combined with its possible modification of the complexity of the substrate, was typically considered the main responsible for changes in meiofaunal assemblages along gradients of pollution from sewage discharge on hard substrates (Gee and Warwick, 1994; Danovaro and Fraschetti, 2002; Terlizzi et al., 2002; Fraschetti et al., 2006).

There is evidence that sewage discharge can alter the composition of meiofaunal assemblages towards an increase in the abundance of opportunistic nematodes (Vidakovic, 1983; Armenteros et al., 2010). Such responses have been ascribed to the high resistance of nematodes to osmotic stress (Forster, 1998), their ability to use the enriched organic content of sediments as potential food source (Arthington et al., 1986; Sandulli and De Nicola-Giudici, 1990, 1991; Bongers and Ferris, 1999), and their general capability to colonize all marine systems also under extreme conditions (Lambshead, 2004; Fraschetti et al., 2006; Danovaro et al., 2008; Wilson and Kakouli-Duarte, 2009; Gambi et al., 2009). In the present study, nematodes were, in general, the most abundant meiofaunal taxon. Therefore, it is not surprising that their variation in abundance matched with the total meiofaunal abundance. Nevertheless, nematodes did not show any consistent pattern of distribution associated with the distance and/or side relative to the examined sewage outfalls. The widely reported positive relationship between nematodes and sewage discharge may have led to predict a relatively higher abundance of this taxon closer to the outlet of each sewer, but this occurred at one sewer only (i.e., Conca Agnano). Interestingly, such a pattern was also not documented by a previous study on shallow subtidal sediments from another Mediterranean location, where nematode abundance was negatively affected by sewage (Fraschetti et al., 2006). The same response, however, was never found associated with any of the present sewers, although the actual drivers of the observed patterns require, to be elucidated, the combination of larger data sets and experimental manipulations and can be just hypothesized due to the descriptive nature of the study. For example, a considerable input of heavy metals to the marine environment is caused by industrial activities and may impact meiofaunal assemblages in terms of driven disappearance of some species/taxa and dominance of others (Hack et al., 2007; but see also Swartz et al., 1986). Nematodes, in particular, can accumulate relatively high levels of Cu, Cd, Zn and Pb (e.g., van der Wurff et al., 2007), but their tolerance to metal pollutants is widely variable among species (Moens et al., 2014). The *Monhystera* and *Theristus* genera may become dominant in sites severely impacted by heavy metals (Gyedu-Ababio et al., 1999). Similarly, species such as *Diplolaimella dievengatensis* and *Halomonhystera disjuncta* can tolerate high concentrations of heavy metals, while species such as *Enoplus communis* are much more sensitive to these pollutants (Howell, 1983; Vranken et al., 1991; Gyedu-Ababio and Baird, 2006). The coarse taxonomic resolution of the present study did not allow assessing such possible differential responses, but it is worth noting that the analyses conducted by

Fraschetti et al. (2006) at multiple resolutions identified sewage impacts in terms of number of nematode individuals only, but not in terms of genera or higher taxonomic composition. Although an actual impact of a range of disturbances on meiofauna was previously documented at fine taxonomic resolution (i.e., genera or species, as reviewed by Moore and Bett, 1989; Balsamo et al., 2012; Zeppilli et al., 2015), other studies detected significant responses of meiofauna to anthropogenic pressures and variations of environmental conditions using coarse taxonomic categories similar to those reported here (Guerrini et al., 1998; Lee and Correa, 2005; Moreno et al., 2006; Veiga et al., 2009; Bianchelli et al., 2016a, 2016b; Ape et al., 2018). For example, Somerfield et al. (1994) reported different responses of phyla such as nematodes and copepods to heavy metal contamination. Indeed, we observed a general reduction of nematode abundance in association with the two sewers closer to the former industrial plant, i.e., an area that previous investigations revealed as considerably subject to heavy metal contamination (Damiani et al., 1987; Sharp and Nardi, 1987; Romano et al., 2004). A reduction in fecundity and development rates could explain, at least in part, such a response (Vranken et al., 1991). At the same time, the negative effect of heavy metals could have balanced the potentially positive influence of the available organic matter, which tended to be on average higher at the same two sewers compared to the northernmost ones.

Copepods, which ranked second in terms of the overall number of individuals, also tended to be less abundant at the two southernmost sewers, especially Canale Bianchettaro. Copepods are generally considered amongst the most sensitive taxa to sewage-related conditions, including hypoxia and anoxia (e.g., De Troch et al., 2013; Sergeeva and Zaika, 2013), increased concentrations of anthropogenic chemicals, such as insecticides (Dahl and Breitholtz, 2008), and reduced salinity (Richmond et al., 2007). A response of this taxon was thus expected, although it was in contrast with the positive association with sewage discharge typically observed due to the selection of opportunistic and tolerant copepod species (Sandulli and De Nicola-Giudici, 1990, 1991). Other studies also revealed that some copepod species can tolerate high levels of heavy metals (Burton et al., 2001), which may have contributed to the present findings.

Changes in patterns of meiofauna, especially nematodes, copepods and ostracods, are widely used as proxies for environmental health and indicators of a range of impacts (Ahnert and Schriever, 2001; Mirto et al., 2002; Pusceddu et al., 2007; Danovaro et al., 2009; Gambi et al., 2009; Goodsell et al., 2009; Moreno et al., 2011). In this context, the present results did not allow the identification of multivariate or univariate patterns of meiofauna that could overwhelm the local variability likely driven by factors operating at one distance or the other, northwards or southwards, relative to the outlet of single sewers. Actually, a large spatial variability, including at small scale, is a common feature of many natural systems even independently of pollution (Lardicci et al., 1999; Terlizzi et al., 2002; Piazzi et al., 2004; Fraschetti et al., 2006, 2016; Bertocci et al., 2017). For instance, a multi-scale analysis of the spatial distribution of richness, turnover and functional diversity of nematodes across deep-sea habitats in the Mediterranean Sea revealed a relatively high variability at the scale of tens of metres (Gambi et al., 2014), i.e., a scale comparable to the areal extent around the sewers examined in the present study. A similar result has been reported in the deep Arctic Sea (Gallucci et al., 2009). In agreement with the evidence on terrestrial ecosystems (Huston, 1999), small-scale variation of nematodes could be due to the separate or combined effects of biological interactions and habitat heterogeneity. The underlying drivers could include small-scale variation in the quality and composition of food sources (Gambi et al., 2014) and the sediment grain size (Rodríguez et al., 2003). Specifically, Rodríguez and colleagues (2003) reported a linear increase of the number of major meiofaunal taxa with the mean grain size, consistently with earlier findings of McLachlan et al. (1981). This trend was explained with the direct relationship between greater grain size and larger oxygenated interstitial space (McLachlan, 1989). The concentration of interstitial oxygen is a key physical factor that drives the presence and abundance of meiofauna (e.g., Berninger and Epstein, 1995; Moodley et al., 1997). Such a combination of factors could explain, at least in part, the higher total abundance and abundance of the main meiofaunal taxa (Nematoda and Copepoda) observed at our northernmost sewers that were characterized by a relatively larger proportion of fine sediments. Once again, the actual role of these processes cannot be unambiguously ascertained by this study. It is worth noting, however, that sediment grain sizes were included as covariates in our analysis. This allowed testing for patterns of variation of meiofaunal assemblages associated with the examined factors once any potential influence, possibly indicated by the relatively large proportion of total variance provided by such covariables in the multiple regression approach, was taken into account and removed.

Similarly to what was observed for the distance-related comparisons, no consistent north-to-south gradients were found in the distribution of meiofauna in the examined area. Interestingly, however, in the cases where significant north *vs.* south differences resulted, these were always in the direction of a larger abundance of a meiofaunal taxon southward to a particular sewer. The main water circulation patterns in the Gulf of Naples could be responsible, at least in part, for this finding, although with detailed mechanisms difficult to hypothesize based on present data and the oceanographic complexity of the system involving interactions between the prevalent offshore Tyrrhenian currents and smaller-scale varying wind-driven currents (Cianelli et al., 2011). In fact, while, in winter, dominant winds blowing from NNE-NE produce an offshore directed jet, in spring (when the present study was carried out), summer and fall alternating NE and SW winds drive the formation of cyclonic and anticyclonic structures, varying on smaller spatial and temporal scales (Gravili et al., 2001; Grieco et al., 2005; Menna et al., 2007; Uttieri et al., 2001). Such circulation patterns could have exerted some effects on the spatial distribution of meiofauna, but the large variability among sewers suggests that these were likely modulated by other local processes.

This study contributes to characterise biologically the area of Bagnoli with reference to current sources of anthropogenic disturbance that are superimposed to the potential impact of the former industry. In this respect, it was found that local processes might play a prominent role in shaping spatial patterns of distribution of meiofaunal abundance and composition besides the possible effects of the expected gradients related to the proximity/orientation to the origin of sewage discharge and the possible chronic influence of environmental contamination from industrial activities. Although the exact nature of such processes and their potential interactions cannot be univocally elucidated by the descriptive approach and limited temporal scale of the present study, the reported findings provide relevant baseline information for future restoration interventions that would take into account the spatial variation of target organisms as needed (e.g., Chapman et al., 1995; Bishop et al., 2002; Benedetti-Cecchi et al., 2003; Terlizzi et al., 2005a, b; Fraschetti et al., 2006). In a broader perspective, the present findings highlight the potentially misleading conclusions that could be drawn by studies not including proper spatial replication when assessing the impacts of anthropogenic disturbance or environmental gradients. Examples of such a limitation can be found among studies on meiofaunal assemblages subject to a range of disturbances, despite their wide use as indicators of environmental impacts (e.g., Albertelli et al., 1999; Mazzola et al., 1999, 2000). Eventually, our results also strengthen the concept that, in case studies such as that of the Bagnoli area, environmental impact assessments involving, as traditionally done, a single control would easily lead to confound the putative impact(s) with other concomitant sources of variability (Benedetti-Cecchi, 2001).

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Table 1. Permutational multivariate analysis of variance (PERMANOVA) on meiofaunal assemblage composition at two distances (10 m *vs.* 50 m) and two sides (north *vs.* south) relative to four sewers in the post-industrial area of Bagnoli-Coroglio. Significant effects are indicated in bold.

Source	df	MS	pseudo-*F*	p	# unique permutations
Covariates	2	392.5	0.6	0.766	999
Sewer = Se	3	7410.8	10.5	**0.001**	999
Distance = D	1	805.8	0.3	0.964	998
Side = Si	1	879.9	0.4	0.909	999
Se x D	3	2759.5	3.9	**0.003**	999
Se x Si	3	2389.5	3.4	**0.004**	999
D x Si	1	2056.0	1.1	0.411	997
Se x D x Si	3	1476.6	2.1	0.052	998
Residual	30	706.8			

Se x D pair-wise comparisons:

Sewer	1 (Dazio)	2 (Conca Agnano)	3 (Canale Bianchettaro)	4 (Coroglio tunnel)
	10=50	10≠50	10=50	10≠50
	t=0.45, p=0.986	t=2.75, p=**0.017**	t=1.59, p=0.053	t=2.69, p=**0.006**

Se x Si pair-wise comparisons:

Sewer	1 (Dazio)	2 (Conca Agnano)	3 (Canale Bianchettaro)	4 (Coroglio tunnel)
	north=south	north≠south	north=south	north=south
	t=1.20, p=0.269	t=3.34, p=**0.002**	t=1.34, p=0.140	t=1.02, p=0.365

Table 2. ANOVA on the total meiofaunal abundance and the abundance of single taxa at two distances (10 m *vs.* 50 m) and two sides (north *vs.* south) relative to four sewers in the post-industrial area of Bagnoli-Coroglio. * $p<0.05$; ** $p<0.01$, *** $p<0.001$, ns = not significant ($p>0.05$).

			Total abundance		**Copepoda**		**Nauplii**		**Nematoda**		
Source		df	MS	*F*	MS	*F*	MS	*F*	MS	*F*	Denominator for *F*
Sewer	= Se	3	2934506.0	35.78***	28.1	5.39**	14.82	20.98***	1562.6	66.46***	Residual
Distance	= D	1	35928.1	0.28	2.4	0.08	0.01	0.02	7.9	0.09	Se x D
Side	= Si	1	351360.7	3.00	9.5	0.20	0.03	0.17	161.5	2.02	Se x Si
Se x D		3	128843.3	1.57	30.3	5.82**	0.81	1.15	89.2	3.79 *	Residual
Se x Si		3	117173.1	1.43	47.7	9.15***	0.15	0.22	80.1	3.41 *	Residual
D x Si		1	12498.8	0.04	13.3	0.52	1.26	0.48	36.1	0.39	Se x D x Si
Se x D x Si		3	307708.3	3.75 *	25.5	4.90**	2.60	3.68 *	114.4	4.87**	Residual
Residual		32	82013.6		5.2		0.71	23.5	23.5		
Cochran's test			*C* = 0.325, ns		*C* = 0.278, ns		*C* = 0.214, ns		*C* = 0.247, ns		
Transformation			None		Square root		Ln(x+1)		Square root		

Table 3. ANOVA on the abundance of single meiofaunal taxa at two distances (10 m *vs.* 50 m) and two sides (north *vs.* south) relative to four sewers in the post-industrial area of Bagnoli-Coroglio. Symbols as in Table 2.

		Tardigrada		**Acarina**		**Oligochaeta**		**Ostracoda**		
Source	df	MS	*F*	MS	*F*	MS	*F*	MS	*F*	Denominator for *F*
Sewer = Se	3	15.1	6.95**	6.0	48.10***	0.87	1.99	3.42	13.24***	Residual
Distance = D	1	0.3	0.34	0.2	0.13	0.08	0.28	0.04	0.08	Se x D
Side = Si	1	0.7	2.45	1.5	0.87	0.42	0.89	0.21	0.78	Se x Si
Se x D	3	1.0	0.45	1.6	12.51***	0.29	0.66	0.51	1.98	Residual
Se x Si	3	0.3	0.14	1.7	13.62***	0.47	1.08	0.27	1.03	Residual
D x Si	1	5.2	0.73	0.3	1.74	1.63	4.46	0.12	0.62	Se x D x Si
Se x D x Si	3	7.1	3.26**	0.2	1.51	0.37	0.84	0.19	0.75	Residual
Residual	32	2.2		0.1		0.44		0.26		
Cochran's test		*C* = 0.704**		*C* = 0.287, ns		*C* = 0.263, ns		*C* = 0.329, ns		
Transformation		None		Square root		Ln(x+1)		Ln(x+1)		

Table 4. Results of SNK tests for post-hoc relevant comparisons on the total number of individuals and the abundance of single meiofaunal taxa. Abbreviations indicate levels of sewer (1, 2, 3, 4 = Dazio, Conca Agnano, Canale Bianchettaro, Coroglio tunnel, respectively), distance (10 = 10 m, 50 = 50 m) and side (N = north, S = south). Significant differences are in bold.

Total abundance

Sewer x Distance x Side, Standard Error for comparison = 165.34

	N side	S side	10 m	50 m
Sewer 1	50=10	10=50	**S>N**	N=S
Sewer 2	**10>50**	50=10	N=S	**S>N**
Sewer 3	50=10	50=10	N=S	N=S
Sewer 4	50=10	50=10	N=S	S=N

Copepoda

Sewer x Distance x Side, Standard Error for comparison = 1.32

	N side	S side	10 m	50 m
Sewer 1	50=10	10=50	**S>N**	**S>N**
Sewer 2	**10>50**	50=10	S=N	**S>N**
Sewer 3	50=10	50=10	N=S	N=S
Sewer 4	50=10	**50>10**	N=S	N=S

Nauplii

Sewer x Distance x Side, Standard Error for comparison = 0.49

	N side	S side	10 m	50 m
Sewer 1	50=10	10=50	S=N	N=S

Sewer 2	**10>50**	50=10	N=S	S=N
Sewer 3	10=50	50=10	N=S	S=N
Sewer 4	50=10	50=10	S=N	S=N

Nematoda

Sewer x Distance x Side, Standard Error for comparison = 0.80

	N side	S side	10 m	50 m
Sewer 1	50=10	10=50	**S>N**	N=S
Sewer 2	**10>50**	50=10	S=N	**S>N**
Sewer 3	50=10	50=10	N=S	N=S
Sewer 4	50=10	**50>10**	N=S	S=N

Tardigrada

Sewer x Distance x Side, Standard Error for comparison = 0.85

	N side	S side	10 m	50 m
Sewer 1	50=10	**10>50**	**S>N**	N=S
Sewer 2	10=50	50=10	N=S	S=N
Sewer 3	10=50	10=50	N=S	N=S
Sewer 4	10=50	10=50	N=S	N=S

Acarina

Sewer x Distance, Sewer x Side, Standard Error for comparison = 0.14

Sewer 1	**10>50**	**S>N**
Sewer 2	10=50	N=S
Sewer 3	10=50	N=S

Sewer 4	**50>10**	**N>S**

FIGURE CAPTIONS

Figure 1. Map of the Bagnoli-Coroglio study area, with sewers represented with black dots. Numbers from 1 to 4 indicate the four sampled sewers (1 = Dazio, 2 = Conca Agnano, 3 = Canale Bianchettaro, 4 = Coroglio tunnel).

Figure 2. Mean (+SE, n=3) percentage of particles of seven size ranges (very fine gravel, >2 mm = 1; very coarse sand, 1 to 2 mm = 2; coarse sand, 0.5 to 1 mm = 3; medium sand, 0.25 to 0.5 mm = 4; fine sand, 0.125 to 0.25 mm = 5; very fine sand, 0.063 to 0.125 mm = 6; silt-clay, <0.063 mm = 7) in the sediment at two distances (10 *vs.* 50 m) and two sides (north *vs.* south) relative to four sewers in the Bagnoli-Coroglio area.

Figure 3. Biochemical composition of organic matter (mean + SE) of the sediment at two distances (10 *vs.* 50 m) and two sides (north *vs.* south) relative to four sewers (1, 2, 3, 4 = Dazio, Conca Agnano, Canale Bianchettaro, Coroglio tunnel, respectively) in the Bagnoli-Coroglio area. Data averaged over three cores; na = not available.

Figure 4. nMDS ordination of ‘average’ meiofaunal assemblages from sediment cores in each combination of (A) sewer (1, 2, 3 and 4 = Dazio, Conca di Agnano, Canale Bianchettaro, and Coroglio tunnel, respectively) and distance, and (B) sewer and side.

Figure 5. Mean (+SE) total abundance and number of individuals (x 10 cm^{-2}) of single meiofaunal taxa in each combination of sewer, side and distance. Data averaged over three replicate cores. Abbreviations as in Figure 2.

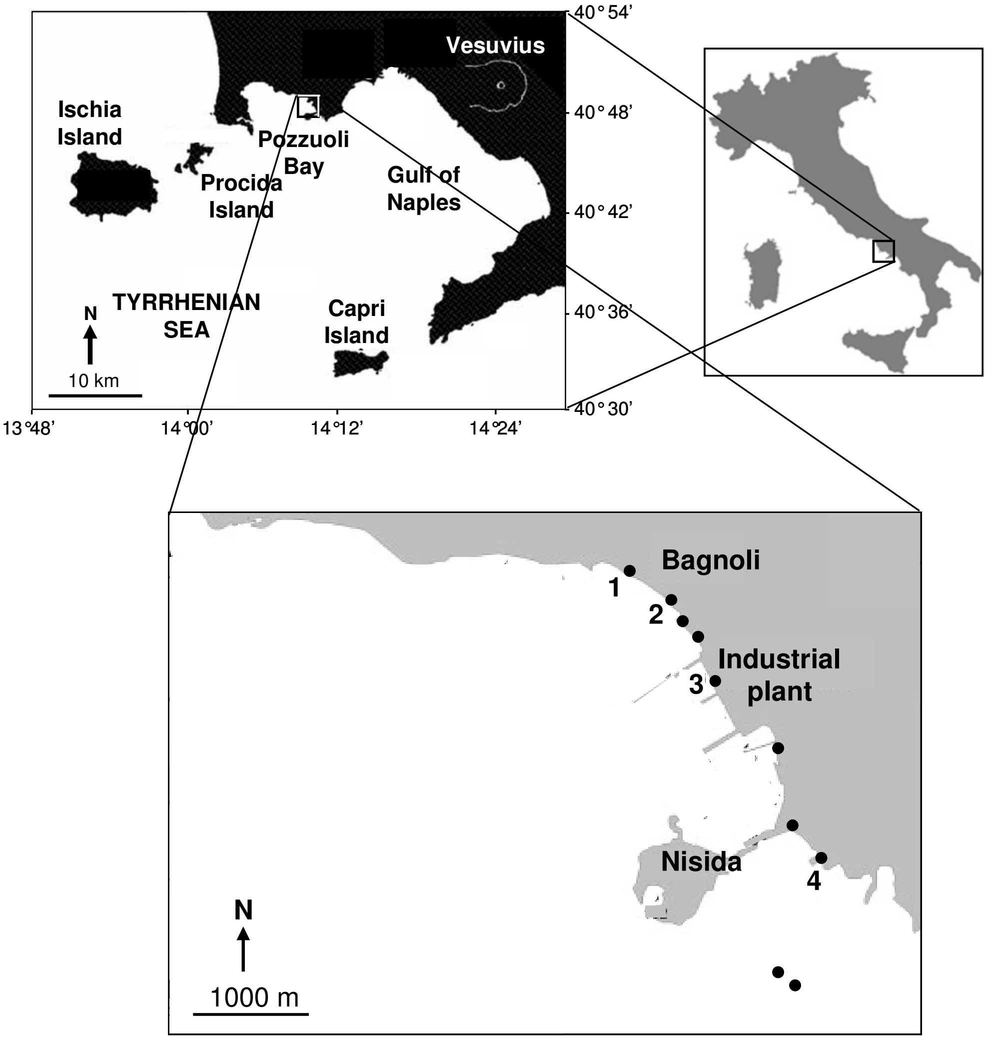
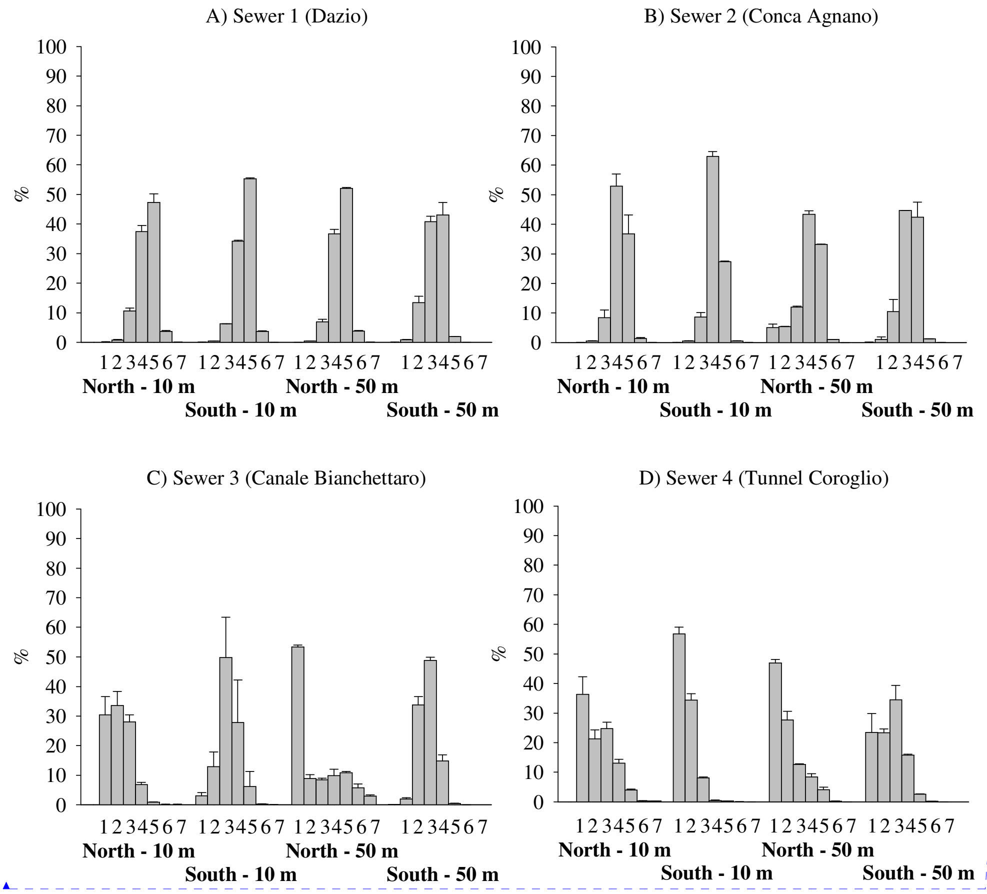

40° 54'
Vesuvius
Ischia Island
40° 48'
Pozzuoli Bay
Procida Island
Gulf of Naples
40° 42'
N
TYRRHENIAN SEA
Capri Island
40° 36'
10 km
40° 30'
13°48'
14°00'
14°12'
14°24'
Bagnoli
1
2
Industrial plant
3
Nisida
4
N
1000 m

Figure 1



Formattato: Inglese (Regno Unito)

Figure 2

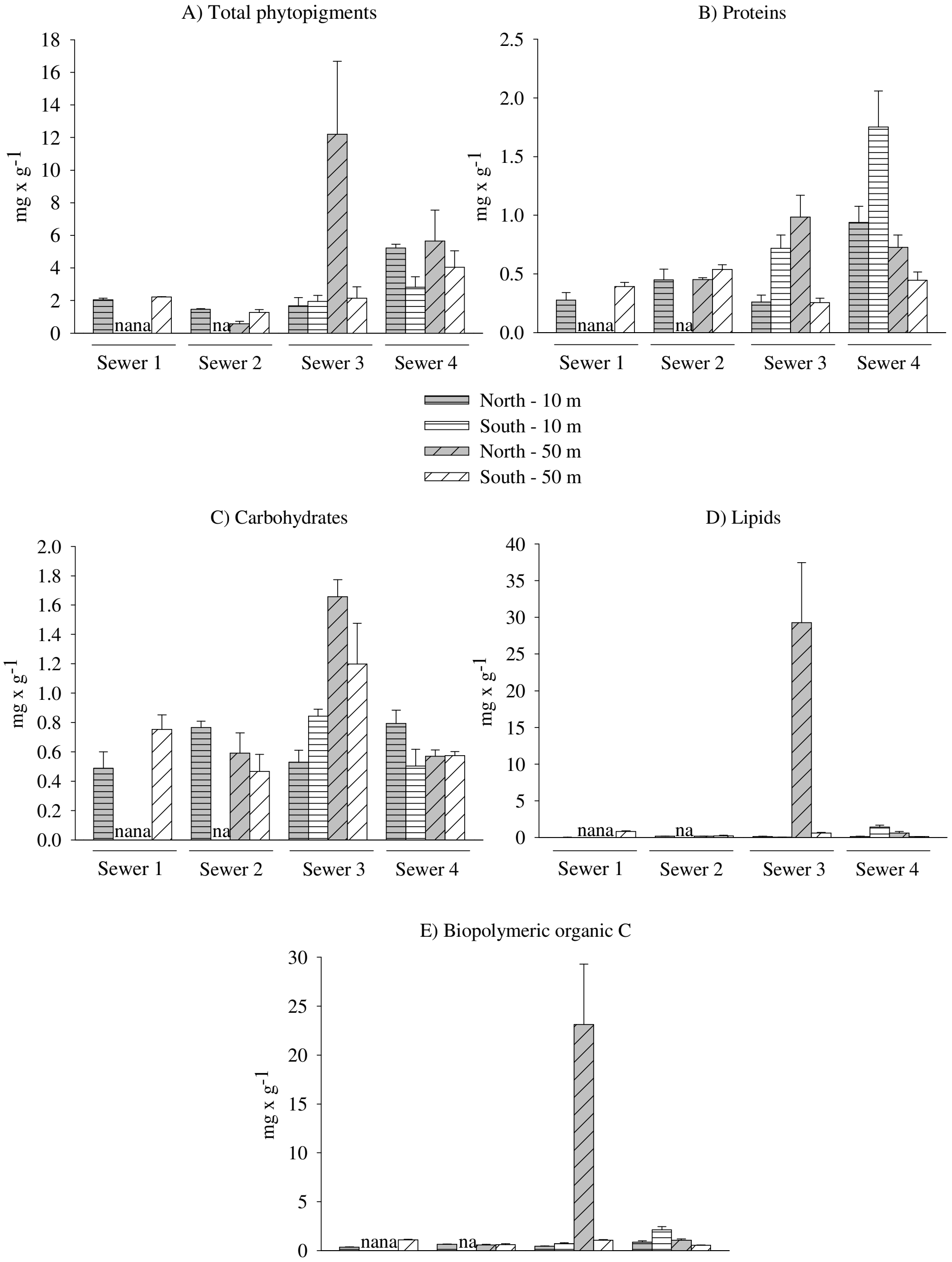

A) Total phytopigments
B) Proteins
C) Carbohydrates
D) Lipids
E) Biopolymeric organic C
mg x g^{-1}
0
2
4
6
8
10
12
14
16
18
0.0
0.5
1.0
1.5
2.0
2.5
0.2
0.4
0.6
0.8
1.2
1.4
1.6
1.8
5
15
20
25
30
35
40
na
Sewer 1
Sewer 2
Sewer 3
Sewer 4
North - 10 m
South - 10 m
North - 50 m
South - 50 m

Figure 3

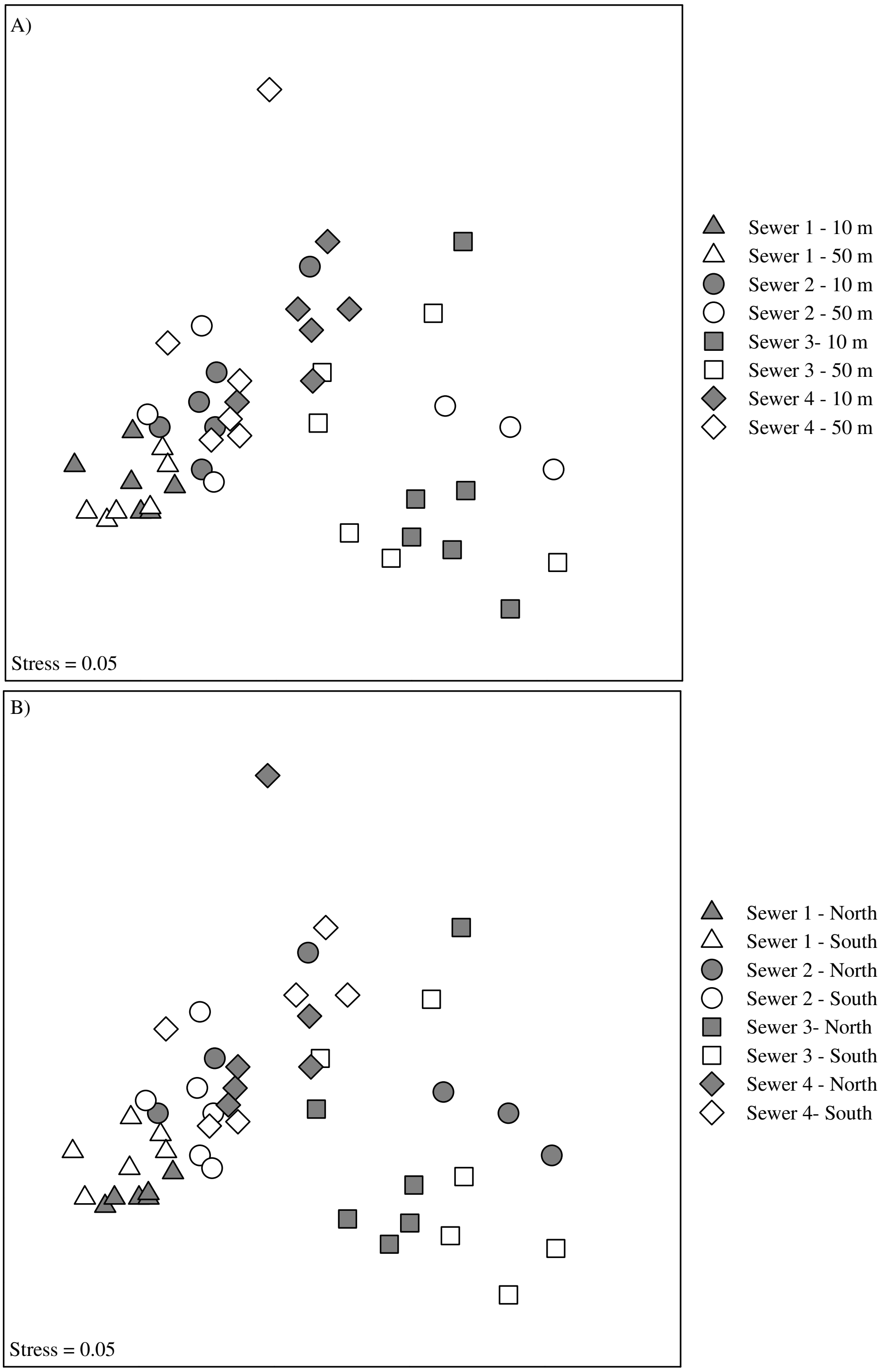

A)
Sewer 1 - 10 m
Sewer 1 - 50 m
Sewer 2 - 10 m
Sewer 2 - 50 m
Sewer 3- 10 m
Sewer 3 - 50 m
Sewer 4 - 10 m
Sewer 4 - 50 m
Stress = 0.05
B)
Sewer 1 - North
Sewer 1 - South
Sewer 2 - North
Sewer 2 - South
Sewer 3- North
Sewer 3 - South
Sewer 4 - North
Sewer 4- South
Stress = 0.05

Figure 4

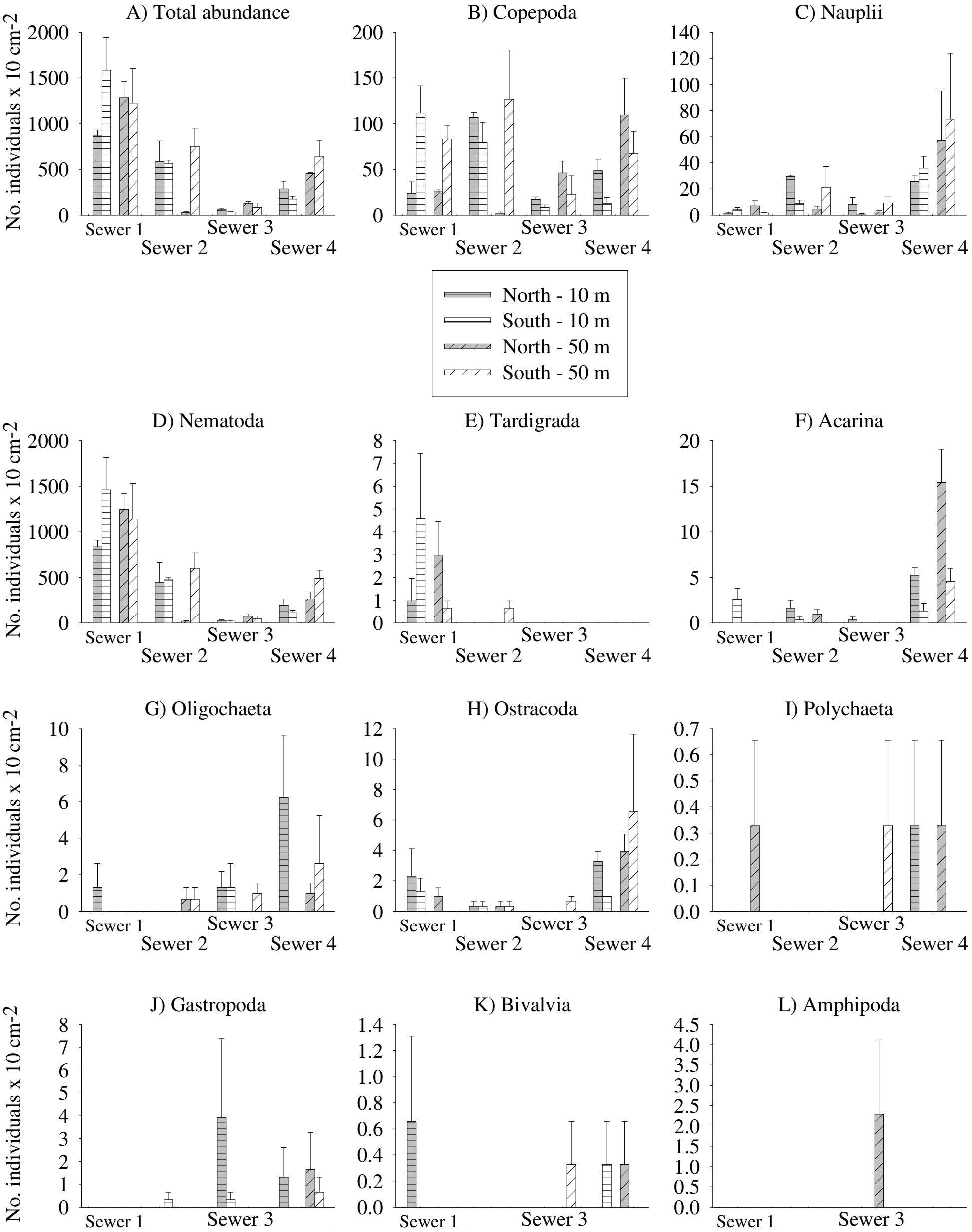

A) Total abundance
B) Copepoda
C) Nauplii
D) Nematoda
E) Tardigrada
F) Acarina
G) Oligochaeta
H) Ostracoda
I) Polychaeta
J) Gastropoda
K) Bivalvia
L) Amphipoda
No. individuals x 10 cm-2
Sewer 1
Sewer 2
Sewer 3
Sewer 4
North - 10 m
South - 10 m
North - 50 m
South - 50 m

Figure 5