Multi-benthic size approach to unveil different environmental conditions in a Mediterranean harbor area (Ancona, Adriatic Sea, Italy) (#81322)

First submission

Guidance from your Editor

Please submit by **27 Jan 2023** for the benefit of the authors (and your token reward) .

Structure and Criteria
Please read the 'Structure and Criteria' page for general guidance.

Raw data check
Review the raw data.

Image check
Check that figures and images have not been inappropriately manipulated.

Privacy reminder: If uploading an annotated PDF, remove identifiable information to remain anonymous.

Files
Download and review all files from the materials page.

10 Figure file(s)
2 Table file(s)

Structure and Criteria

Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

You can also annotate this PDF and upload it as part of your review

When ready submit online.

Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your guidance page.

BASIC REPORTING

- Clear, unambiguous, professional English language used throughout.
- Intro & background to show context. Literature well referenced & relevant.
- Structure conforms to PeerJ standards, discipline norm, or improved for clarity.
- Figures are relevant, high quality, well labelled & described.
- Raw data supplied (see PeerJ policy).

EXPERIMENTAL DESIGN

- Original primary research within Scope of the journal.
- Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
- Rigorous investigation performed to a high technical & ethical standard.
- Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

- Impact and novelty not assessed. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
- All underlying data have been provided; they are robust, statistically sound, & controlled.
- Conclusions are well stated, linked to original research question & limited to supporting results.

Standout reviewing tips

The best reviewers use these techniques

Tip	***Example***
Support criticisms with evidence from the text or from other sources	*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*
Give specific suggestions on how to improve the manuscript	*Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).*
Comment on language and grammar issues	*The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult. I suggest you have a colleague who is proficient in English and familiar with the subject matter review your manuscript, or contact a professional editing service.*
Organize by importance of the issues, and number your points	*1. Your most important issue* *2. The next most important item* *3. …* *4. The least important points*
Please provide constructive criticism, and avoid personal opinions	*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*
Comment on strengths (as well as weaknesses) of the manuscript	*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.*

Multi-benthic size approach to unveil different environmental conditions in a Mediterranean harbor area (Ancona, Adriatic Sea, Italy)

Elisa Baldrighi Corresp., 1, 2 , **Sarah Pizzini** 3, 4 , **Elisa Punzo** 1 , **Angela Santelli** 3 , **Pierluigi Strafella** 3 , **Tommaso Scirocco** 5 , **Elena Manini** 3 , **Daniele Fattorini** 6, 7 , **Claudio Vasapollo** Corresp. 3

1 1Institute for Biological Resources and Marine Biotechnologies—IRBIM, National Research Council—CNR, Italy, ANCONA, MARCHE, Italy
2 2Department of Biology, University of Nevada-Reno, Reno, Nevada, United States of America, Reno, Nevada, USA
3 1Institute for Biological Resources and Marine Biotechnologies—IRBIM, National Research Council—CNR, Italy, Ancona, Italy
4 3Fano Marine Center, The Inter-Institute Center for Research on Marine Biodiversity, Resources and Biotechnologies, Fano, Italy, Fano, Italy
5 1Institute for Biological Resources and Marine Biotechnologies—IRBIM, National Research Council—CNR, Italy, Lesina, Italy
6 4Dipartimento di Scienze della Vita e dell’Ambiente (Disva), Università Politecnica delle Marche (Univpm), Ancona, Italy, Ancona, Italy
7 5Consorzio Nazionale Interuniversitario per le Scienze del Mare (Conisma), Unità di Ricerca di Ancona (Italy)., ANCONA, MARCHE, Italy

Corresponding Authors: Elisa Baldrighi, Claudio Vasapollo
Email address: elisa.baldrighi@irbim.cnr.it, claudio.vasapollo@cnr.it

Ports are hubs of human activity and are subject to the continuous discharge and release of industrial, agricultural, and municipal waste and contaminants. Benthic organisms are largely known to reflect environmental conditions they live in. The meio- and macrofauna are ecologically distinct components of the benthos and as such may not necessarily respond to environmental conditions and/or disturbances in the same way. However, in few field studies the spatial patterns of meio- and macrofauna have been simultaneously compared. In the present study, we hypothesized a different response (*i.e.* abundance, diversity, and distribution patterns) from the two benthic size classes to the different environmental conditions they live in (*i.e.* concentration of selected trace metals and Polycyclic aromatic hydrocarbons (PAHs); organic matter contents into the sediment; grain size and microbial abundance) characterizing the Ancona harbor (Adriatic sea). Meio- and macrofauna provided partially similar and complementary types of information depending on the indices used (univariate measures or community structure/ species composition) and the different ‘response-to-stress’. The community composition of both benthic size components clearly showed differences among sampling stations located from inside to outside the harbor, reflecting the marked environmental heterogeneity and disturbance typically characterizing these systems. Notwithstanding, the univariate measures (*i.e.* meio- and macrofauna total abundance, diversity indices and equitability) didn’t show similar spatial patterns. Meiofauna results were generally statistically more significant than those obtained for macrofauna. Meiofauna were likely to be more sensitive to the effects of environmental features and contaminants than macrofauna. Overall, trace metals and PAHs affected the community composition of the two benthic components, but only the meiofauna abundance and diversity were related to the environmental variables considered (*i.e.* quantity and quality of food sources). Our results pinpoint the importance and the advantage of the complementary use of two sets of faunistic groups which could provide greater insight into the processes affecting the investigated area.

Multi-benthic size approach to unveil different environmental conditions in a Mediterranean harbor area (Ancona, Adriatic Sea, Italy)

Elisa Baldrighi[1,2]*, Sarah Pizzini[1,3], Elisa Punzo[1], Angela Santelli[1], Pierluigi Strafella[1], Tommaso Scirocco[1], Elena Manini[1], Daniele Fattorini[4,5], Claudio Vasapollo[1]*

[1]Institute for Biological Resources and Marine Biotechnologies—IRBIM, National Research Council—CNR, Italy
[2]Department of Biology, University of Nevada-Reno, Reno, Nevada, United States of America
[3]Fano Marine Center, The Inter-Institute Center for Research on Marine Biodiversity, Resources and Biotechnologies, Fano, Italy
[4]Dipartimento di Scienze della Vita e dell'Ambiente (Disva), Università Politecnica delle Marche (Univpm), Ancona, Italy
[5]Consorzio Nazionale Interuniversitario per le Scienze del Mare (Conisma), Unità di Ricerca di Ancona (Italy).

Corresponding Author:
Elisa Baldrighi[1,2]
Largo Fiera della Pesca 2, Ancona, 60125, Italy
Email address: elisa.baldrighi@irbim.cnr.it

Corresponding Author:
Claudio Vasapollo[1]
Largo Fiera della Pesca 2, Ancona, 60125, Italy
Email address: claudio.vasapollo@cnr.it

Abstract

Ports are hubs of human activity and are subject to the continuous discharge and release of industrial, agricultural, and municipal waste and contaminants. Benthic organisms are largely known to reflect environmental conditions they live in. The meio- and macrofauna are ecologically distinct components of the benthos and as such may not necessarily respond to environmental conditions and/or disturbances in the same way. However, in few field studies the spatial patterns of meio- and macrofauna have been simultaneously compared. In the present study, we hypothesized a different response (*i.e.* abundance, diversity, and distribution patterns) from the two benthic size classes to the different environmental conditions they live in (*i.e.* concentration of selected trace metals and Polycyclic aromatic hydrocarbons (PAHs); organic matter contents into the sediment; grain size and microbial abundance) characterizing the Ancona harbor (Adriatic sea).
Meio- and macrofauna provided partially similar and complementary types of information depending on the indices used (univariate measures or community structure/ species

composition) and the different 'response-to-stress'. The community composition of both benthic size components clearly showed differences among sampling stations located from inside to outside the harbor, reflecting the marked environmental heterogeneity and disturbance typically characterizing these systems. Notwithstanding, the univariate measures (*i.e.* meio- and macrofauna total abundance, diversity indices and equitability) didn't show similar spatial patterns. Meiofauna results were generally statistically more significant than those obtained for macrofauna. Meiofauna were likely to be more sensitive to the effects of environmental features and contaminants than macrofauna. Overall, trace metals and PAHs affected the community composition of the two benthic components, but only the meiofauna abundance and diversity were related to the environmental variables considered (*i.e.* quantity and quality of food sources). Our results pinpoint the importance and the advantage of the complementary use of two sets of faunistic groups which could provide greater insight into the processes affecting the investigated area.

Introduction

Coastal waters are widely recognized as marine areas of high ecological and economic value, but also as highly threatened zone, exposed to multiple human activities and their negative impacts (Travizi *et al.*, 2019).

Harbors are enclosed areas and hubs of human activity. They are essential to the economic growth of coastal regions, where maritime traffic, shipping, international trade, and fishing are continuously increasing (Simonini *et al.*, 2005; Franzo *et al.*, 2022). Harbors are usually characterized by high sediment pollution levels due to heavy metals and hydrocarbons produced by intense maritime traffic and huge organic matter loads (Covazzi Harriague *et al.*, 2007; Baldrighi *et al.*, 2019). The high concentrations of contaminants and the relevant inputs of organic matter represent a persistent and ongoing threat, especially for the biota living in the sediment (Moreno *et al.*, 2008a; Veiga *et al.*, 2009). In addition, the exposure of the innermost part of harbors to both wind and waves is limited and may create conditions of reduced water renewal, favoring sedimentation processes, anoxia, and trapping pollutants (Guerra-García & García-Gómez, 2005; Spagnolo *et al.*, 2011). The EU Water Framework Directive (WFD; Directive 2000/60/EC) considers harbors as 'heavily modified water bodies', which cannot meet the common criteria of good ecological quality status. Therefore, their effective management is crucial for the sustainable use of these maritime spaces and for the protection of the adjacent coastal habitats (Chatzinikolaou *et al.*, 2018; Franzo *et al.*, 2022).

Benthic organisms are largely known to reflect environmental conditions they live in. Among benthic components, meio- and macrofauna are widely recognized as good ecological indicators (Schratzberger *et al.*, 2003; Patricio *et al.*, 2012). Benthic meiofaunal (<500 μm) and macrofaunal (>500 μm) communities are regularly utilized in impact assessment, but very few studies are carried out taking into account both communities (Whomersley *et al.*, 2009; Frontalini *et al.*, 2011). Meio- and macrofaunal assemblages do not exist in isolation and therefore are part of an interacting system (Whomersley *et al.*, 2009). However, the meio- and macrofauna are ecologically distinct components of the benthos and as such may not necessarily respond to environmental conditions and/or disturbances in the same way (Patrício *et al.*, 2012; Covazzi Harriague *et al.*, 2013).

As well as being separated on the basis of size, meio- and macrobenthos each have a series of distinctive ecological and evolutionary characteristics. Meiofauna is characterized by small size, high abundance, ubiquitous distribution, rapid generation times, fast metabolic rates, and absence of a planktonic phase, resulting in a short response time and high sensitivity to different environmental conditions and certain types of disturbance. Meiofauna can also suitably reflect the ecological conditions present in a particular system. Due to their ecological characteristics, meiofaunal organisms can act as suitable indicators of changes in environmental conditions over small spatial scales (*e.g.*, Schratzberger & Ingels, 2018; Ridall & Ingels, 2021). Consequently, meiofaunal communities have generated considerable interest as potential indicators of anthropogenic disturbances in aquatic ecosystems (*e.g.*, Balsamo *et al.*, 2012; Semprucci *et al.*, 2015; Semprucci *et al.*, 2022).

Benthic macrofauna is relatively sedentary and thus cannot easily escape natural and anthropogenic disturbances. Moreover, several species are very sensitive to environmental changes (Simboura & Zenetos, 2002). For these reasons, these organisms have been commonly used as bioindicators of local pressure, essential for obtaining information on the state of conservation of marine ecosystems (*e.g.,* Grotti *et al.*, 2016). Macrobenthic invertebrates have been identified by the EU WFD as key biological components to assess the ecological status of aquatic ecosystems, due to their important role in ecosystem functioning and to their involvement in food-web nutrient recycling (Punzo *et al.*, 2017).

Our understanding of benthic systems and how they behave in response to disturbance events may therefore be improved if a more holistic ecosystem approach to disturbance impact studies was taken, simultaneously considering more than a single faunal group (Warwick *et al.*, 2006). The complementary use of two sets of faunistic groups with contrasting ecological characteristics could provide greater insight into the processes affecting such an area.

Distribution and composition of benthic communities is influenced by a wide and complex array of environmental factors (Covazzi Harriague *et al.*, 2013). The physical instability of some environments such as harbors, the morphodynamic features of their sediment texture, presence of pollutants and organic loads contribute to the structure and distribution of benthic assemblages (*e.g.*, Spagnolo *et al.*, 2011; 2019; Losi *et al.*, 2013; Baldrighi *et al.*, 2019). Up to now, in a few field studies in which the spatial patterns of meio- and macrofauna have been simultaneously compared, changes in both assemblages as a response to natural gradients were found to be scattered across habitats (Patrício *et al.*, 2012). These investigations have demonstrated the fundamental advantage of a multi-species approach, with the inclusion of many taxonomic and functional groups that have a broad range of sensitivities to any given environmental regime (*e.g.*, Frontalini *et al.*, 2011).

Adriatic Sea ecosystem is negatively affected by many kinds of biological and ecological threats *e.g.*, eutrophication, pollution, fragmentation of benthic habitats, invasion of alien species (Katsanevakis *et al.*, 2011; Pećarević *et al.*, 2013; Corriero *et al.*, 2016). In the regional perspective the basin is highly positioned on the list of 'Priority issues in the Mediterranean environment' drawn up by the European Environment Agency (EEA), with 20 (15%) out of the 131 hotspot pollution sites identified along the Mediterranean coastline in the frame of the Strategic Action Programme (SAP) of the United Nations Environment Programme (UNEP; EEA, 2006), including many harbors such as the Ancona one (Travizi *et al.*, 2019).
Since previous works published on Ancona harbor have always treated the different benthic components separately (Mirto & Danovaro 2004; Spagnolo *et al.*, 2011; 2019; Baldrighi *et al.*, 2019; Travizi *et al.*, 2019; Franzo *et al.*, 2022), the aim of the present work is to test whether meio- and macrofaunal assemblages could provide a comparable and/or complementary assessment of its ecological conditions. Considering that meio- and macrofauna are ecologically distinct components of the benthos, we hypothesized a different response (*i.e.* abundance, diversity, and distribution patterns) from the two benthic size classes to the different environmental conditions they live in (*i.e.* concentration of selected Trace Elements (TEs) and Polycyclic aromatic hydrocarbons (PAHs); organic matter contents into the sediment; grain size and microbial abundance) characterizing the Ancona harbor.

Materials & Methods

Sampling area and sampling strategy

The Ancona harbor (water depth, 4-15 m) is located in the western coast of central Adriatic Sea (Fig. 1), it has a water sheet of 700,000 m^2 and 5,400 m of docks (Spagnolo *et al.*, 2011). The harbor is one of the most important of the Adriatic Sea, with intense ferryboat and merchant ship activity. More than one million passengers on ferries and cruise ships travel from Ancona to the Adriatic eastern coasts, and both container and oil traffic have also developed in recent years. In the shipyards, all kinds of ships are designed and built, and shipbuilding is the largest entrepreneurial reality in the harbor (Franzo *et al.*, 2022). Previous investigations reported that the area is subjected to organic waste dumping derived from fishing boats and is also affected by a strong industrial pollution due to the presence of shipyards. Consequently, a huge organic matter load and high heavy metal and hydrocarbon concentrations are present inside the harbor area (Mirto & Danovaro, 2004; Bianchelli *et al.*, 2016).
In the present study, sediment samples were collected in winter 2015 from five sampling stations located from inside to outside the Ancona harbor (Fig. 1). The five sampling stations were chosen according to the different environmental features and anthropogenic activities present in the area: MAN station was located in the inner part of the harbor where small fishing boats dock; PORT station was located in a transition area where work ships are berthed; DS and LR stations

were located in a more external position, nearby shipping facilities such as active berths; API station was located outside the harbor where no activity takes place.

At each station, sediment samples for characterizing the benthic fauna (meio- and macrofauna) and environmental features were collected with a box-corer (40×30 cm wide and 50 cm high) in three independent replicates, processed and preserved differently according to the analysis to be performed (see below). At all stations, the temperature and salinity at the sea bottom were measured using CTD (Conductivity, Temperature, and Depth) probe equipped with previously calibrated sensors.

Environmental variables and microbial component

The content of each box-corer was sub-sampled with PVC corers (inner diameter, 4.5 cm) to assess the biochemical composition of the organic matter and grain size. The top 3 cm of sediment from three independent replicates for each parameter were frozen at -20°C, except for the grain size determination, for which samples were kept at *in situ* temperature in single replicate until brought to the laboratory. The biochemical composition of the organic matter (protein, carbohydrate, and lipid concentration) and chloroplastic pigments (chlorophyll-*a* (Chl-*a*) and phaeopigment (Phaeo) concentration) were determined by standard techniques (Danovaro, 2010). Concentrations were calculated using standard curves and normalized to sediment dry weight after desiccation (60°C, 24 h). Biopolymeric organic carbon (BPC) was calculated as the sum of the carbon equivalents of carbohydrates, proteins, and lipids (Fabiano *et al.*, 1995) and was used as a proxy for the available trophic resources. The value of the protein to carbohydrate ratio (PRT/CHO) was utilized as descriptor of the nutritional quality of organic matter in the sediment, with a PRT/CHO ratio (>1) indicating relatively high quality and high food availability (Pusceddu *et al.*, 2010).

For grain size determination, aliquots of fresh sediment were sieved over a 63 µm mesh. The two fractions (>63 µm, sand; <63 µm, silt/mud) were dried in an oven at 60°C and weighed. Data were expressed as a percentage of sediment total dry weight (Pusceddu *et al.*, 2010).

For microbial abundance determination, samples of surface seawater were collected at each sampling station using sterile containers (capacity, 1 L) and preserved in formaldehyde (final concentration, 2%). The Total Prokaryotic Number (TPN) was determined by acridine orange staining technique (Luna *et al.*, 2002), using an Axioskop 2 epifluorescence microscopy (Carl Zeiss AG, Oberkochen, Germany; magnification, 1,000x).

Contaminants into the sediment

Concentration of selected TEs and PAHs was determined in the surface sediment (0-3 cm) collected at each sampling station in triplicate and frozen at -20°C. Analyses were conducted following previous validated methods, fully described in Benedetti *et al.* (2014) and Etiope *et al.* (2014).

In brief, TEs were determined after digestion under pressure with nitric acid and hydrogen peroxide (7:1), using a Mars 6 Microwave Digestion System (CEM Corporation, Charlotte, NC, USA). As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, V, and Zn were analyzed by Atomic Absorption Spectroscopy (AAS), with flame (SpectrAA 220FS Spectrometer, Varian Inc., Palo Alto, CA, USA) and flameless atomization (240Z AA Spectrometer, Agilent Technologies Inc., Santa Clara, CA, USA), while the Hg content was quantified by Cold Vapor Atomic Absorption Spectroscopy (CVAAS; QuickTrace M-6100 Mercury Analyzer, Teledyne CETAC Technologies Ltd., Omaha, NE, USA).

PAHs were determined after KOH-methanol extraction with a Mars 6 Microwave Digestion System (CEM Corporation). Extracts were concentrated using a RC 10.09 Vacuum Concentration System (Jouan SA, Saint-Herblain, France) and purified by J.T.Baker™ BAKERBOND™ Octadecyl (C18) Solid Phase Extraction (SPE) cartridges (Avantor Inc., Radnor, PA, USA). PAHs were analyzed by High-Performance Liquid Chromatography (HPLC) using both Fluorimetric (FLD) and (UV) Diode Array Detection (DAD) (Infinity 1260 Series, Agilent Technologies, Santa Clara, CA, USA).

For both TEs and PAHs, appropriated blank solutions and the Standard Reference Material (SRM) 1944: *New York/New Jersey Waterway Sediment* (NIST – National Institute of Standards and Technology), digested as samples, were used to check for accuracy, precision, and recoveries of the employed analytical methodologies; concentrations obtained from SRM analyses were always within the 95% confidence intervals of the NIST certified values.

The results obtained in this study were compared with threshold values for chemicals specified in the Ministerial Decree 173/2016, the Italian normative that rules the management of dredged sediments and sets out their quality. Only those values exceeding the upper thresholds values (L2) are defined as alerting values (Table 2.5, Ministerial Decree 173/2016).

Meiofauna and macrofauna

For meiofauna samples, the content of each box-corer (three independent replicates) was sub-sampled with PVC corers (inner diameter, 4.5 cm). The top 3 cm of sediment, where meiofaunal organisms are typically more abundant, were preserved in 4% buffered formaldehyde. For meiofaunal extraction, sediment samples were sieved through a 1000 µm mesh; a 32 µm mesh was used to retain the smallest metazoan organisms. The latter fraction was centrifuged 3 times with LUDOX® HS-40 colloidal silica (diluted with water to a final density of 1.18 g cm^{-3}) and stained with Rose Bengal (0.5 g L^{-1}; Heip *et al.*, 1985). Meiofaunal organisms were counted (no. of individuals 10 cm^{-2}) and identified to the higher taxonomic level under a stereomicroscope. For macrofauna samples, the first 20 cm of three independent box-corer deployments were sieved *in situ* using a 500 µm mesh and all organisms retained were preserved in 5% buffered formaldehyde. Macrofauna was sorted in laboratory using a stereomicroscope and a binocular microscope, identified and classified to the lowest possible taxonomic level using standard nomenclature, and quantified (no. of individuals m^{-2}). The data collected was subjected to a control and validation process and organized in a dedicated database. The nomenclature of the species was verified and validated using the web portal https://www.marinespecies.org/ and the 'WoRMS Taxon Match Tool' (World Register of Marine Species Editorial Board, 2022). Taxon richness (*i.e.* higher taxa and species for meio- and macrofauna, respectively; S), total number of individuals per taxon/species (N), Shannon's diversity index (H', based on $\log_2$; Shannon & Weaver, 1949), and Pielou's equitability index (J'; Pielou, 1969) of benthic communities were calculated.

Statistical analysis

To assess differences in benthic communities among the sampling stations a Permutational Multivariate Analysis of Variance (PERMANOVA; 9999, number of random unrestricted permutations of raw data) was used (Anderson, 2001). The design included one factor: the sampling station (five levels, fixed). The analysis was based on Bray-Curtis' similarity of previously fourth root transformed meio- and macrofaunal data. In case of significant differences obtained by the main test, the pairwise test was performed and, as there was a limited number of unique permutations, the *p* values were obtained from Monte Carlo tests (Anderson & Robinson, 2003). Permutational Multivariate Analysis of Dispersion (PERMDISP) test was applied to assess if differences among the sampling stations (between-group) were due to real differences in benthic community composition and not to differences in the multivariate dispersion of replicates (within-group) among their respective centroids. A non-metric Multidimensional Scaling (nMDS) ordination was carried out to visualize similarities among the sampling stations. Similarity Percentages (SIMPER) analysis (cut-off, 90%) was used to identify the meio- and macrofaunal taxa that contributed to the dissimilarity among the sampling stations. These procedures were performed with PRIMER™ and PERMANOVA+ ecological software (Clarke & Gorley, 2006; Anderson *et al.*, 2008). For all tests the significance threshold was set to 0.05. One-way Analysis of Variance (ANOVA) was used to explore differences among the sampling stations for organic matter content and microbial component, total abundance, diversity and equitability indices in meio- and macrofaunal benthic communities. The ANOVA assumptions were tested graphically plotting residuals *vs.* fitted values, normality of residuals and residuals *vs.* covariate (factor station) to assess the variance homogeneity (Zuur *et al.*, 2016). When ANOVA showed significant differences, the Tukey's Honestly Significant Difference (HSD) test was performed to find significant effects between different levels. ANOVA was performed using the free software R (R Core Team, 2018).
The number of environmental and pollutant-related variables were separated in two groups: environmental (comprising TEs, PAHs, and silt/mud percentage) and biotic (comprising bacteria and all the organic compounds of biogenic origin). Since many compounds were linked each other, multicollinearity (cut-off at Spearman's correlation = |0.8|) among variables was assessed to reduce the dataset and avoid problems in the analysis algorithms. In case of multicollinearity, the variables with a more stringent biological or environmental value were retained acting as

proxy for the omitted collinear ones. To further reduce the number of variables (as they were still more numerous than the meio- and macrofaunal ones), both environmental and biotic variables were analyzed (separately) by means of a Principal Component Analysis (PCA) to identify groups with similar variability. Only those principal components showing eigenvalues >1 (Kaiser-Guttman criterion; Zwick & Velicer, 1986) were considered. To obtain a better insight into the output loadings, the orthogonal varimax rotation of extracted PCA components was performed. After a varimax rotation, each original variable tends to be associated with one (or a small number) of PCA axis. By doing so, groups of variables were created each of which represented by a single PCA score, and all the scores obtained were used as covariates in the following analysis to assess the relations between faunal communities and the environmental variables. Spearman's rank correlation analysis was performed to test relationships between meio- and macrofauna total abundance, taxon richness, Shannon's diversity and Pieolu's equitability indices, and the environmental variables considered. Spearman's rank correlation coefficients (ρ, rho) were considered significant at p values <0.05.
In order to verify the existence of a significant relation between the benthos data matrix and the environmental data, the distance-based Linear Modeling (DistLM) procedure was utilized with a backward selection of the variables, and each model assessed by means of the Akaike's Information Criterion corrected for small samples (AICc; Anderson *et al.*, 2008). A distance-based Redundancy Analysis (dbRDA) was then applied to visually investigate the relationship between the community assemblages and the environmental variables (Anderson *et al.*, 2008).

Results

Environmental features and contamination levels in the Ancona harbor

Values of main environmental features and considered pollutant concentrations are reported in Table 1 and Table 2, respectively.
Bottom water temperature reported a mean value of 10.3°C and salinity ranged from a minimum of 31.3 (at LR station) to a maximum of 37.6 PSU (at API station). The sandy fraction (>63 µm) characterized stations located outside the harbor (DS, LR, and API stations), while the silty muddy fraction (<63 µm) was predominant in the inner stations MAN and PORT (Table 1). Analyses of Chl-*a* and organic matter contents into the sediments showed significantly ($F_{4, 10}$ = 12.97, p = 0.001) higher values of 'fresh' material (*i.e.* Chl-*a*) at MAN and DS stations, while the BPC significantly (F = 22.21, 4 d.f., p = 0.001) decreased from inside to outside the harbor (Table 1). The innermost station MAN was also characterized by the highest abundance of prokaryotes (TPN) although not significantly different from the other stations. The significant (F = 12.61, 4 d.f., p = 0.001) highest value in the quality of organic matter (*i.e.* PRT/CHO) was reported at PORT station (Table 1), due to a particularly low concentration of CHO into the sediment of this station (Table S1).

Analyses aimed at determining TE concentrations in the sediment of the Ancona harbor have highlighted a general decrease of contamination values moving from the innermost sampling station (MAN) to those more external (Table 2). MAN station turned out to be affected by pollution levels 3-4 times higher than those detected at API station, reaching even Cu and Zn concentrations 13 and 6 times higher, respectively. Just these two elements, Cu and Zn, were the only exceeding the threshold limits imposed by the Ministerial Decree 173/2016 (46.31 $\mu g\ g^{-1}$ of Cu detected at MAN station; 297.2 and 114.9 $\mu g\ g^{-1}$ of Zn detected at MAN and PORT stations, respectively). In the remaining sampling stations there were no overruns (Table 2).
The concentration levels of Σ_{19} PAHs ranged from 73.38 to 213.4 $\mu g\ g^{-1}$ following, although not linearly, the same pattern highlighted for TEs, with higher values in the innermost sampling station (MAN), decreasing towards outer ones (Table 2). All the sediment samples showed a distinct predominance of Low Molecular Weight PAHs, mainly driven by the Naphthalene concentration (exceeding the legislative thresholds at MAN, DS, and LR stations), which averagely accounted for 37% of the Σ_{19} PAHs, followed by its methylated isomers: 1- and 2-Methylnaphtalene (24 and 22%, respectively). This pronounced prevalence of volatile, easily transportable PAHs, with 2-3 aromatic rings (Fig. 2a) suggest that fuel combustion linked to harbor traffic may be the major source of these organic contaminants in the area (for wind direction and speed on Ancona harbor area during February 2015, please see Fig. S1). Excluding Naphthalene and its related compounds, the PAH residual contamination was mainly ascribable to Phenanthrene and Fluoranthene (Table 2), and the principal PAH diagnostic ratios (Tobiszewski & Namieśnik, 2012), commonly used as a tool to discriminate the analyte origin and sources (Giuliani *et al.*, 2019; Pizzini *et al.*, 2021), would seem to suggest a clear petrogenic origin (Fig. 2b).
The results from PCA plots considering 'biotic' and 'abiotic' environmental variables separately, clearly summarized the differences among the sampling stations. In details, considering the biotic variables (Fig. 3a) MAN and, for a lesser extent, LR were grouped together being characterized by higher values of potential food sources and prokaryotes the former, and by the lowest salinity value the latter. This explained the variability along the first axis, while variability along the second axis was mainly explained by the contrast between the innermost stations (MAN and PORT), characterized by higher quantity and quality of organic matter, and the outer sampling stations. The first two components accounted for 75.7% of the total variability. In regard to abiotic variables (Fig. 3b), MAN station separated from all the other sampling stations due to highest values of pollution (both by inorganic and organic contaminants) and in this case the first two components accounted for 87.3% of the total variability.

Meiofauna assemblages

A total of 12 taxa were identified with Nematoda representing the dominant taxon at all sampling stations, with a percentage ranging from 75 to 95% (Fig. 4a). The second most represented taxon was Copepoda with their *nauplii*; among less represented taxa (*i.e. others*) Bivalvia, Ciliata, Foraminifera, Kinorhyncha, Oligochaeta, Ostracoda, Platyhelminthes, Polychaeta, Sipuncula, and Tardigrada constituted from 3 to 15% of the meiobenthic community (Fig. 4b). Meiofauna abundance and values of its diversity indices are reported in Table S2 and represented in Figure 5. The ANOVA tests detected significant differences among all the sampling stations for N (Table 3), with the highest value reported at DS station (Fig. 5a). The ANOVA tests reported significant differences among the sampling stations also for all diversity and equitability indices (Table 3 and Table S2). Regarding the number of taxa, PORT station showed a significant lower value on average compared to all the other sampling stations (Fig. 5b). Shannon's diversity and Pielou's equitability indices showed both similar patterns, with MAN station presenting the highest value (Fig. 5c and Fig. 5d).

PERMANOVA analysis with pairwise test reported significant differences (Table 4) in meiofaunal community composition among all the sampling stations; PERMDISP test did not show any significant dispersion around centroids, confirming that the differences among the sampling stations were due to a real difference in meiobenthic composition ($F_{2,4}$ = 5.38, P(perm) = 0.088).

The nMDS plot (Fig. 6a) clearly shows the separation among the sampling stations, as well as a low inter-replica variability. In particular, the innermost MAN and PORT stations were separated from to the outermost ones. SIMPER analysis detected a dissimilarity percentage from 15 (LR *vs.* DS stations) to 54% (PORT *vs.* LR stations). The highest values of dissimilarity percentage were always associated with PORT station and mainly due to very low abundances in some of the most represented taxa such as: Copepoda, Foraminifera, Nematoda, and Polychaeta (Table S3). For the other sampling stations, the dissimilarity was mainly due to the presence/absence or differences in the abundances of the taxa *others*: Bivalvia, Ciliata, Kinorhyncha, Oligochaeta, Ostracoda, Platyhelminthes, and Sipuncula (Table S3). Several positive correlations emerged between meiofauna descriptors and quantity of organic matter and TPN; moreover, meiofauna abundance and its diversity were positively correlated to some TEs and to silt/mud content into the sediment (Table S4a). The best model selected by the DistLM analysis, reported in Table 5, comprised only the pollutant compounds (ARC1, 2, 3, and 4). The resulting dbRDA showed that the first two axes explained 91.8% of the fitted model variation and the 83.4% of the variance of meiofaunal community composition, corresponding to the combination of the following environmental factors: V/Ni ratio, Naphthalene, and Benzo[*a*]pyrene (ARC1) and Zn, Fluorene, and percentage of silt/mud (ARC4; Fig. S2a).

Macrofauna assemblages

A total of 93 taxa were identified, these included: 43 Anellida (42 Polychaeta and 1 Oligochaeta), 29 Mollusca (22 Bivalvia, 6 Gastropoda, and 1 Scaphopoda), 10 Crustacea (6 Amphipoda, 2 Cumacea, 1 Isopoda, and 1 Tanaidacea), 5 Nematoda, 2 Bryozoa, 2 Cnidaria (1 Anthozoa and 1 Hydrozoa), 1 Nemertea, and 1 Ophiuroidea (Table S5). Anellida was the most represented group (from 68 to 90% at LR and MAN stations, respectively), followed by

Mollusca (from 4 to 24% at MAN and LR stations, respectively) at all the sampling stations. Other less represented groups such as Cnidaria, Isopoda, Ophiuroidea, and Tanaidacea were found only at one or two sampling stations (Fig. 7). Macrofauna abundance ranged from 610 ± 241 to 3455 ± 425 individuals m^{-2} at MAN and LR stations, respectively; values of its diversity indices are reported in Table S2 and represented in Figure 8. The ANOVA tests reported significant higher abundance value at LR station (Fig. 8a) compared to all the other stations (Table 3). LR station was also characterized by the highest number of taxa (Fig. 8b) but also by lowest values (Fig. 8c and Fig. 8d) of Shannon's diversity and Pielou's equitability indices (Table 3 and Table S2). PERMANOVA analysis with pairwise test reported significant differences (Table 6) in macrofaunal community composition among the majority of the sampling stations; PERMDISP test did not show any significant dispersion around centroids, confirming that the differences among the sampling stations were due to a real difference in macrobenthic composition ($F_{2,4}$ = 0.99, P(perm) = 0.767).

The nMDS plot (Fig. 6b) shows the separation among the sampling stations. In detail, the innermost MAN station and the outermost API station were particularly distinguished from the others. SIMPER analysis reported high percentages of dissimilarity ranging from 63.4 (LR *vs.* DS stations) to 88.3% (MAN *vs.* API stations) between all pairs of sampling stations. The great dissimilarity is mainly due to the presence/absence of species or to a particularly abundant presence of them in one station compared to the others (Table S6). Species like *Spiophanes bombyx, Kurtiella bidentata,* and *Euclymene oerstedii* characterized mainly the outermost API station, while some other species such as *Tubificoides swirencoides, Streblospio* sp.*, Heteromastus filiformis,* and *Chaetozone caputesocis* were found inhabiting the innermost stations (Table S6). Four significant correlations (three out of four were negative) were detected between macrofauna descriptors and environmental variables; only macrofauna species richness was (negatively) correlated to three TEs and to the percentage of finest sediment fraction (Table S4b).

The best model selected by the DistLM analysis, reported in Table 7, comprised only ARC2 (Benzo[*ghi*]perylene, and Anthracene/(Anthracene + Phenanthrene) and Fluoranthene/(Fluoranthene + Pyrene) diagnostic ratios) and ARC4 (Zn, Fluorene, and percentage of silt/mud). The resulting dbRDA showed that the first two axes explained 100% of fitted model variation and the 42.9% of the variance in the macrofaunal community composition (Fig. S2b). This latter low percentage indicates that the residual variance associated to the community was not captured by the graph, and it is likely that an unobserved variable should have had improved the general plot. In any case, along the first axis of the dbRDA there was a clear separation between the innermost stations MAN and PORT, characterized by high values of Zn and percentage of silt/mud compared to the outermost stations LR, DS, and API; while along the second axis LR and DS stations were separated from API, MAN, and PORT stations.

Discussion

Harbors are deeply modified coastal areas to meet human requirements. Pressures associated with harbor ecosystems are becoming increasingly important, causing significant damage to water and sediment quality and, subsequently, to marine life and ecosystems, as well as to human health (Mestres *et al.*, 2010).

Ancona harbor has been the subject of some previous investigations to evaluate the presence and impact of several contaminants on macrobenthic community (Spagnolo *et al.*, 2011) and meiobenthic nematodes (Franzo *et al.*, 2022); to assess the presence of alien species (Non-indigenous species, NIS) introduced by ballast waters (BW) (Spagnolo *et al.*, 2019; Travizi *et al.*, 2019); to investigate the response of meiobenthic communities under BW impact (Baldrighi *et al.*, 2019). All these previous studies, conducted on different benthic size components, have reported that Ancona harbor is a receptor of multiple contaminants, such as toxic compounds, heavy metals, hydrocarbons, and organic matter, as well as a habitat for NIS introduced by BW due to intense maritime traffic and which, in turn, exert consequences on the composition and structure of the benthic communities.

Environmental features of Ancona harbor

In Ancona harbor, the innermost stations were characterized by muddy sediments, while sand dominated the outermost stations. This grain size distribution was clearly due to a reduced exposure to hydrological factors (wind, waves, and currents) which create conditions of poor water renewal inside the harbor, favoring the presence of fine sediments (Spagnolo *et al.*, 2011). The sediment deposition rate can influence sediment organic matter load and pollutant content, with fine-grained components commonly showing a high content in organic matter and pollutants (Papageorgiou *et al.*, 2010). This might have facilitated an overall accumulation of TEs and PAHs inside the harbor. In particular, the high values of Cu and Zn detected at MAN and PORT stations were likely correlated to the shipyard activities present within the harbor, in particular with the use of new generation antifouling paints (Costa *et al.*, 2016; Pereira *et al.*, 2018). Furthermore, the pronounced prevalence of volatile, easily transportable PAHs (*e.g.,* Naphthalene; Fig. 2a) pointed out that fuel combustion linked to maritime traffic was the major source of these organic contaminants in the harbor basin. The petrogenic origin of the PAH residual contamination (*e.g.,* Phenanthrene and Fluoranthene; Fig. 2b) was supported by the detection in the sediment samples of V, Ni, and Pb, commonly considered as tracers of accidental oil spills and/or marine fuels (El Nemr *et al.*, 2006), as well as by the values of the V/Ni ratio, marker of an intense maritime traffic (Viana *et al.*, 2014).

Considering the threshold values for chemicals specified in the Ministerial Decree 173/2016, few values were reported exceeding the established alerting thresholds and always from the innermost stations. Is also true that the Decree does not report threshold values for all TEs and PAHs considered in our study, thus making the sediment quality evaluation based only on Ministerial Decree 173/2016 somewhat weak.

Not only contaminants, but also natural and/or anthropogenic changes in the benthic trophic status (*i.e.* organic matter quantity and sediment biochemical composition) may affect the benthic communities (Pusceddu *et al.*, 2011; Foti *et al.*, 2014). The protein, carbohydrate, lipid, and BPC content in the sediments have been proposed and utilized to assess the benthic trophic status of marine coastal environments, including the Adriatic Sea (Vezzulli & Fabiano, 2006). In Dell'Anno *et al.* (2002) PRT and CHO sedimentary contents were suggested as proxies and threshold values for ranking the trophic status and the environmental quality of coastal marine ecosystems along the Apulia Region. Applying those thresholds to the investigated sediments, the trophic status of Ancona harbor could be ranked as hyper-trophic (PRT >4 mg g^{-1}) with the exception of the API station, ranked as eutrophic (PRT ≈ 4 mg g^{-1}). However, in terms of CHO content, Ancona harbor should be ranked as meso-oligotrophic system (CHO <5 mg g^{-1}). Pusceddu *et al.* (2009; 2011) identified as eutrophic systems those characterized by BPC concentration >3 mgC g^{-1}, as found at MAN, PORT, LR, and DS stations, and as mesotrophic systems those characterized by BPC concentration in the range 1-3 mgC g^{-1}, as in the case of the API station. In Ancona harbor PRT/CHO ratio resulted always >1, indicating a great input of recent production's material and highlighting the good trophic quality of the organic matter (Pusceddu *et al.*, 2009). Concentrations of Chl-*a* here reported were extremely high if compared to those reported in February in a previous study conducted along the Adriatic coasts (0.11-0.23 µg g^{-1}; Bianchelli *et al.*, 2016), indicating the presence of 'fresh' primary organic matter. TPN did not change significantly among the sampling stations, however the trend in the abundance values appeared similar to that of organic matter content.

The PCA on measured environmental variables indicated the presence of a clear spatial heterogeneity among the sampling stations and a separation between innermost stations and outermost ones due to higher organic matter, prokaryote and contaminant loads inside the harbor basin, as previously reported in the same study area (Spagnolo *et al.*, 2011; Baldrighi *et al.*, 2019) and in other enclosed systems (Vezzulli *et al.*, 2003; Losi *et al.*, 2013). This marked environmental variability in harbors is a common feature. Indeed, environmental disturbance within harbors may change rapidly over spatial scales of a few meters, depending on various factors like the localization and magnitude of pollution sources, allochthonous inputs of different nature, tidal regime, water circulation, harbor position, shape, and size (Vassallo *et al.*, 2006).

Meiofaunal response to harbor environmental conditions

Harbor communities are subject to broad spatial and temporal variability of physical-chemical conditions and environmental disturbances, and meiofauna is usually able to respond rapidly to such changes (Vezzulli *et al.*, 2003).

In Ancona harbor, the meiofaunal total abundance, community structure and, for a lesser extent, univariate measures (*i.e.* diversity and equitability indices) reflected the marked spatial heterogeneity showed by the PCA and the clear separation both between inner and outer sampling stations and among the sampling stations themselves (MAN *vs.* PORT *vs.* DS + LR *vs.* API). Meiofaunal abundance was in the range described by Baldrighi *et al.* (2019) for Ancona harbor and for other harbors and coastal areas affected by pollution and/or high organic matter loads (Vezzulli *et al.*, 2003; Veiga *et al.*, 2009; Pusceddu *et al.*, 2011; Dal Zotto *et al.*, 2016; Semprucci *et al.*, 2016). The only exception was represented by the paucity of meiofaunal organisms found at PORT station. Considering that total meiofaunal abundance was positively linked to products derived from primary production (Chl-*a*, Phaeo, and chloroplast pigment equivalents - CPE), its low abundance at PORT station could be partially justified by the lowest detected value of 'fresh' material (Chl-*a*) and/or by a recent physical disturbance due to the position of this sampling station in an area defined of 'transit' within the harbor. Apart from Zn, all TE and PAH values detected at PORT station appeared overall lower if compared to those of the other sampling stations, suggesting a recent resuspension or removal of sediment. Given its small size, low mobility, and lack of dispersive life stages (Giere, 2009), meiofauna is more susceptible to within-habitat physical variability and environmental disturbances than larger, more mobile, and potentially more highly dispersed members of the macrofauna (Schratzberger *et al.*, 2008). This would explain the drop in meiofauna abundance, not reported for the macrofauna, at that sampling station. The meiofauna showed an overall good number of taxa (12) and in four sampling stations out of five the majority of taxa were represented. The measures of diversity (*i.e.* S, H', and J') were comparable to the values reported in harbor areas (*e.g.*, Moreno *et al.*, 2008a; Moreno *et al.*, 2008b) and in enclosed/transitional systems in the Adriatic Sea (*e.g.*, Pusceddu *et al.*, 2007; Pusceddu *et al.*, 2011; Frontalini *et al.*, 2014). However, the strong dominance of Nematoda justified the low values reported for the Pielou's equitability index, particularly at LR and DS stations. The dominance of the most resistant and adaptable group is a peculiarity of more stressed and less stable environments, such as harbors (Semprucci *et al.*, 2015). Abundance and diversity indices were correlated to different proxies of food sources (quantity and quality) into the sediment and to its grain size, confirming the effect of these environmental variables on meiofaunal populations (Balsamo *et al.*, 2010).

As previously mentioned, the structure of the meiofaunal assemblages exhibited a clear spatial variability between inside *vs.* outside the harbor but also among the five sampling stations, consistent with changes detected for environmental features. The dissimilarity among the sampling stations was mainly due to some less abundant and more sensitive taxa, not present in the innermost stations. Usually, organisms that can cope with unfavorable conditions take over (*e.g.,* Nematoda), whereas more sensitive taxa disappear or become rare (Mirto *et al.*, 2014; Zeppilli *et al.*, 2015). In the case of Ancona harbor, Bivalvia, Kinorhyncha, Platyhelminthes, Sipuncula, and Tardigrada were found only at the outermost stations (LR, DS, and API) being identified as less tolerant taxa (Baldrighi *et al.*, 2019 and literature therein). Conversely, the more tolerant and widespread groups of Ciliata, Oligochaeta, and Polychaeta (Pusceddu *et al.*, 2007; Moreno *et al.*, 2008a; Moreno *et al.*, 2008b; Semprucci *et al.*, 2015) characterized the innermost stations, along with an important presence of soft-shelled Foraminifera inhabiting the sediment at all the investigated sampling stations. Soft-shelled monothalamous Foraminifera are an important component living in the sediment and populating the Adriatic Sea (Sabbatini *et al.*, 2013), but most of the time this component is overlooked in meiofauna studies. The high presence of this group has been found to be associate to high values of Chl-*a,* eutrophic conditions, and high variability of environmental parameters (*e.g.*, organic matter loads, salinity, temperature, oxygen content; Sabbatini *et al.*, 2013). Their strong tolerance and positive response to environmental stress (Sabbatini *et al.*, 2010) can perfectly justified their presence in the Ancona harbor. Changes in the community structure were supported by DistLM analysis, which revealed that pollutants and, secondly, the grain size could explain the variability in the meiofaunal composition.

Food sources did not have any effect on the meiobenthic community. According to Dell'Anno *et al.* (2002) and Pusceddu *et al.* (2009), the system of Ancona harbor can be ranked as eutrophic (inside) - mesotrophic (outside) with high quality of organic matter. Thus, food sources did not constitute a limiting factor for the meiofaunal community, as reported instead for oligotrophic systems (*e.g.,* Covazzi Harriague *et al.*, 2013) or in estuary's waters (Patrício *et al.*, 2012). Same results were reported in Franzo *et al.* (2022) analyzing the nematode communities inhabiting different Adriatic harbors, including that of Ancona. Authors showed that the main environmental factor that shaped the nematode assemblages in all harbors were the PAH concentration levels, while food sources and the grain size were much less relevant.

Interestingly, some positive correlations between TEs and meiofaunal abundance and its related univariate measures were reported also in this study. In the study conducted by Cibic *et al.* (2017), authors pinpointed as heavy metal content may influence meiofaunal abundance and its composition. The positive nature of the correlation could be the result of a meiobenthic community well adapted to permanent stress conditions (Cibic *et al.*, 2017).

Macrofaunal response to harbor environmental conditions

Benthic macroinvertebrates are traditionally used as biological indicators of ecosystem health in the marine environment, especially infaunal assemblages associated with soft-bottom habitats (Borja *et al.*, 2003), and they act as integrators of stress over months to years (Weisberg *et al.*, 1997; Paul *et al.*, 2001). Thus, the structure and spatial distribution of benthic communities can be directly linked with pollutant or disturbance exposure (Borja *et al.*, 2003). The macrobenthos in the internal parts of a harbor usually exhibits a low abundance and a numerical importance of pollution-tolerant species (Pearson & Rosenberg, 1978; Callier *et al.*, 2009). Species richness, abundance, biomass, diversity and equitability indices generally show a gradual increase from the interior to the exterior of an harbor, following an internal-external gradient (Callier *et al.*, 2009).

In the present study, total macrofaunal abundance and its measures of diversity (*i.e.* S, H', and J'), fell within the range of values reported by Spagnolo *et al.* (2011) and Travizi *et al.* (2019) for Ancona harbor. Results here reported showed an overall increasing trend in macrofaunal abundance and species richness from inside to outside the study area; however LR station significantly differed from the other sampling stations when univariate measures were considered. Regarding the community structure, species composition was different enough to differentiate between the internal and the external sampling stations.

The macrobenthic community was mostly composed by the dominant groups of Anellida, Mollusca, and Crustacea, as usually reported from enclosed environments impacted by pollutants and characterized by high organic matter loads (Guerra-García & García-Gómez, 2004b; Spagnolo *et al.*, 2011; Travizi *et al.*, 2019). Among the group of Anellida, the Oligochaeta species *T. swirencoides* was identified for the first time in Ancona harbor and it was found to be particularly abundant in all the sampling stations and even dominant at LR station. Only at API station the species was absent. Tubificid oligochaetes, also called sludge worms, are very common in high polluted areas (Brusca & Brusca, 2003) and they are recognized as a pollution-tolerant taxon (Pelletier *et al.*, 2010). Thus, *T. swirencoides* was the species mostly responsible for the difference between the innermost sampling stations and the outermost one. Species composition may be affected by pollutant concentrations and high levels of organic matter, through a decrease in diversity and abundance of sensitive species (Callier *et al.*, 2009). The majority of the species found inhabiting the Ancona harbor sediments belonged to the ecological groups of disturbance-tolerant, second- and first-order opportunistic species (Borja *et al.*, 2000). All these species were typically soft-bottom species with the only exceptions of *Mytilus galloprovincialis* and *Hiatella arctica* found at LR station, as also reported in Spagnolo *et al.* (2011). These two species are commonly from hard-substrates and their origin may have been from the pier close to the LR station. Polychaeta species, along with the tubificid oligochaeta *T. swirencoides,* were the species most represented and diversified. Many Polychaeta species have a high level of tolerance to adverse effects such as pollution and natural perturbations (Borja *et al.*, 2000), and for this reason they usually constitute the majority of benthic organisms living in harbor systems (Guerra-García & García-Gómez, 2004b). Usually, Polychaeta species richness and their diversity inside of harbor areas are low because of high pollution levels and the lack of oxygen in the water column (Estacio *et al.*, 1997; Dhainaut-Courtois *et al.*, 2000). The same trend of increasing Polychaeta diversity moving outside the harbor area was also reported in the present study, with tolerant and opportunistic species such as *Capitella capitata*, *C. caputesocis*, *H. filiformis*, *Sternaspis scutata* and *Streblospio* sp. particularly abundant in the innermost sampling stations. The species *Prionospio cirrifera*, mainly recorded outside the harbor (API station), is traditionally identified as an opportunistic spionid living in silty-clay sediments with high organic content (Borja *et al.*, 2000; Simonini *et al.*, 2004). Spagnolo *et al.* (2011) reported the same finding and authors explained this as a result of a scarce tolerance of *P. cirrifera* to copper, detected at a concentration 9 times higher at the innermost MAN station compared to the average concentration detected at all the other sampling stations. A similar consideration could arise for the high presence of the opportunistic species *S. bombyx* at API station. On the other hand, Polychaeta species ranked as disturbance-sensitive (Borja *et al.*, 2000), such as *Aricidea fragilis, Glycera capitata*, *Jasmineira elegans, Paradoneis armata*, and *Paraonis fulgens* have also been found inhabiting the most impacted sampling stations inside the harbor. Due to their economic and ecological importance, as well as their sedentary life, Mollusca have assumed a major role in monitoring contaminants worldwide (Pizzini *et al.*, 2015; Pizzini *et al.*, 2017; Grotti *et al.*, 2016). *Anadara transversa, Kurtiella bidentata*, *M. galloprovincialis*, and *Nucula* *nitidosa* are defined as disturbance-tolerant species and they tended to dominate the innermost sampling stations in Ancona harbor, with the only exception for *K. bidentata*, particularly abundant at API station. *Abra alba* characterized LR and DS stations, confirming its preference for sandy sediments with medium-high levels of organic matter quantity and quality (Guerra-García & García-Gómez, 2004b). This species has been reported abound in harbors affected by heavy metal pollution (Dhainaut-Courtois *et al.*, 2000).

A large number of crustaceans (Amphipoda, Isopoda, Tanaidacea) have been categorized as pollution-sensitive taxa, especially compared to Polychaeta (Pelletier *et al.*, 2010). Crustacean communities have been considered to be among the most sensitive to changes in environmental variables (Gómez-Gesteira & Dauvin, 2000), and for this reason crustacean species richness and diversity inside harbors are generally considerably low (Estacio *et al.*, 1997; Dhainaut-Courtois *et al.*, 2000). Three abundant species characterized the innermost sampling stations in Ancona harbor: the Amphipoda *Leptocheirus pilosus,* the Caprellida *Phtisica marina*, and the Tanaidacea *Apseudopsis latreillii. P. marina* and *A. latreillii* have been reported in high number in sediments containing less sand and high concentrations of N, P, Cu, and organic matter (Guerra-García & García-Gómez, 2004a). *A. latreillii* belongs to the group of species that may occur under normal conditions, but whose populations are stimulated by organic enrichment; while *P. marina* belongs to the group of species very sensitive to organic enrichment. According to the present study and the results of other previous investigations (Conradi *et al.*, 2000; Guerra-García & García-Gómez, 2001; Guerra-García & García-Gómez, 2004a) this species is able to live even in impacted habitats with moderate-high levels of heavy metals and PAHs. Conversely, the more sensitive Amphipoda *Ampelisca diadema* dominated the crustacean assemblages at API station.

As for the meiofauna, pollutant content and the different sediment texture inside and outside the Ancona harbor affected the macrofaunal composition, as previously reported (Guerra-García and García-Gómez, 2001; Guerra-García & García-Gómez, 2004a; Guerra-García & García-Gómez, 2004b; Spagnolo *et al.*, 2011; Travizi *et al.*, 2019). Univariate descriptors as well as the analysis of species characterizing the benthic communities indicated the presence of modified, but quite diverse and presumably well-established soft-bottom communities in all the investigated sampling stations. This might reflect the successful adaptation of many pollution-tolerant species to the long-term pollution and unstable environmental conditions of Ancona harbor (Travizi *et al.*, 2019). The dominance of opportunistic and tolerant species confirmed the results reported from DistLM analysis, which identified pollution as important driver of macrobenthic assemblage structure.

Conclusions

The benthic community represents a source of information at different food-web levels and can be utilized to investigate and characterize the habitat where the community exists. As meiofauna and macrofauna have different ecological roles in marine ecosystems, they may respond to environmental features and stress conditions at different spatial and temporal scales (Frontalini *et al.*, 2011).

We initially hypothesized a different response from the two benthic size classes to the different environmental conditions characterizing the Ancona harbor. Meio- and macrofauna provided partially similar and complementary types of information depending on the indices used (univariate measures or community structure/ species composition) and the different 'response-to-stress'. The following considerations emerged:

- In Ancona harbor the presence of pollutants and the sediment type determined the meio- and macrofaunal community structure. Moreover, the meiofauna was affected by the quality and quantity of organic matter, suggesting that meiobenthic assemblages were more receptive to within-habitat food variability than macrofauna. Meiofauna results were generally statistically more significant than those obtained for macrofauna. Meiofauna were likely to be more sensitive to the effects of environmental features and contaminants than macrofauna.
- Both invertebrate groups were characterized by distinctive assemblages across the harbor, consistent with changes detected for environmental features. However, for the macrofaunal communities a gradient rather than a clear separation among the sampling stations was reported, while a clear separation among them and moving from inside to outside the harbor area was detected for the meiofaunal communities.
- This investigation confirmed the fundamental advantage of a multi-benthic size approach, with the inclusion of many taxonomic and functional groups. Optimally, both groups should be used in marine pollution monitoring programs included in the EU Marine Strategy Framework Directive (MSFD; Directive 2008/56/EC) in the context of its Descriptor 1 'maintenance of biodiversity' and Descriptor 6 'sea floor integrity'.

Acknowledgements

The first author is very grateful to Dr. Jaques Grall and Vincent Le Garrec (UBO) for the long time spent on the identification of macrofaunal organisms. The authors are grateful to the crews of the boat Tecnopesca that was employed in sampling operations.

References

Anderson MJ. 2001. Permutation tests for univariate or multivariate analysis of variance and regression. Canadian journal of fisheries and aquatic sciences 58(3): 626-639.

Anderson MJ, Robinson J. 2003. Generalized discriminant analysis based on distances. Australian & New Zealand Journal of Statistics 45(3): 301-318.

Anderson M, Gorley R, Clarke K. 2008. PERMANOVA+ for PRIMER: Guide to software and statistical methods. Plymouth, UK: PRIMER-e.

Baldrighi E, Semprucci F, Franzo A, Cvitkovic I, Bogner D, Despalatovic M, Berto D, Malgozata Formalewicz M, Scarpato A, Frapiccini E, Marini M, Grego M. 2019. Meiofaunal communities in four adriatic ports: baseline data for risk assessment in ballast water management. Marine Pollution Bulletin 147: 171–184. https://doi.org/10.1016/j.marpolbul.2018.06.056.

Balsamo M, Albertelli G, Ceccherelli VU, Coccioni R, Colangelo MA, Curini- Galletti M, Danovaro R, D'Addabbo R, De Leonardis C, Fabiano M, Frontalini F, Gallo M, Gambi C, Guidi L, Moreno M, Pusceddu A, Sandulli R, Semprucci F, Todaro MA, Tongiorgi P. 2010. Meiofauna of the Adriatic Sea: current state of knowledge and future perspective. Chemistry and Ecology 26: 45–63.

Balsamo M, Semprucci F, Frontalini F, Coccioni R. 2012. Meiofauna as a tool for marine ecosystem biomonitoring. Marine Ecosystems 4: 77–104.

Barnett BE. 1983. Oligochaetes as indicators of pollution in the humber estuary, with special reference to Tubificoides benedeni, Environmental Pollution Series A, Ecological and Biological, Volume 30, Issue 4, Pages 277-291. https://doi.org/10.1016/0143-1471(83)90055-7.

Benedetti M, Gorbi S, Fattorini D, D'Errico G, Piva F, Pacitti D, Regoli F. 2014. Environmental hazards from natural hydrocarbons seepage: Integrated classification of risk from sediment chemistry, bioavailability and biomarkers responses in sentinel species. Environmental Pollution 185: 116-126. DOI: 10.1016/j.envpol.2013.10.023.

Bianchelli S, Pusceddu A, Buschi E, Danovaro R. 2016. Trophic status and meiofauna biodiversity in the Northern Adriatic Sea: insights for the assessment of good environmental status. Marine Environmental Research 113: 18-30.

Borja A, Franco J, Perez V. 2000. A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. Marine Pollution Bulletin 40: 1100–1114.

Borja A, Muxika I, Franco J. 2003. The application of a marine biotic index to different impact sources affecting soft-bottom benthic communities along European coasts. Marine Pollution Bulletin 46: 835–845.

Brusca RC, Brusca GJ. 2003. Invertebrates. Sinauer Associates, Sunderland, MA, 936 pp.

Callier MD, Fletcher RL, Thorp CH, Fichet D. 2009. Macrofaunal community responses to marina-related pollution on the South coast of England and west coast of France. Journal of the Marine Biological Association of the United Kingdom 89: 19-29.

Chatzinikolaou E, Mandalakis M, Damianidis P, Dailianis T, Gambineri S, Rossano C, Scapini F, Carucci A, Arvanitidis C. 2018. Spatio-temporal benthic biodiversity patterns and pollution pressure in three Mediterranean touristic ports. Science of the Total Environment 624: 648–660. https://doi.org/10.1016/j.scitotenv.2017.12.111.

Cibic T, Franzo A, Nasi F, Auriemma R, Del Negro P. 2017. The port of Trieste (northern Adriatic Sea)— a case study of the “ecosystem approach to management”. Frontiers in Marine Science 4: 336. https://doi.org/10.3389/fmars.2017.00336.

Clarke KR, Gorley RN. 2006. Primer. PRIMER-e, Plymouth, 866.

Conradi M, Lòpez-González PJ, Cervera JL, García-Gómez JC. 2000. Seasonality and spatial distribution of peracarids associated with the bryozoan Bugula neritina in Algeciras Bay, Spain. Journal of Crustacean Biology 20: 126-141.

Corriero G, Pierri C, Accoroni S, Alabiso G, Bavestrello G, Barbone E, Bastianini M, Bazzoni AM, Bernardi Aubry F, Boero F, Buia MC, Cabrini M, Camatti E, Cardone F, Cataletto B, Cattaneo Vietti R, Cecere E, Cibic T, Colangelo P, De Olazabal A, D'onghia G, Finotto S, Fiore N, Fornasaro D, Fraschetti S, Gambi MC, Giangrande A, Gravili C, Guglielmo R, Longo C, Lorenti M, Luglie A, Maiorano P, Mazzocchi MG, Mercurio M, Mastrototaro F, Mistri M, Monti M, Munari C, Musco L, Nonnis-Marzano C, Padedda BM, Patti FP, Petrocelli A, Piraino S, Portacci G, Pugnetti A, Pulina S, Romagnoli T, Rosati I, Sarno D, Satta CT, Sechi N, Schiaparelli S, Scipione B, Sion L, Terlizzi A, Tirelli V, Totti C, Tursi A, Ungaro N, Zingone A, Zupo V, Basset A. 2016. Ecosystem vulnerability to alien and invasive species: a case study on marine habitats along the Italian coast. Aquatic Conservation: Marine and Freshwater Ecosystems 26: 392–409.

Costa LDF, Mirlean N, Wasserman JC, Wallner-Kersanach M. 2016. Variability of labile metals in estuarine sediments in areas under the influence of antifouling paints, southern Brazil. Environmental Earth Sciences 75: 580. https://doi.org/10.1007/s12665-016-5355-5.

Covazzi Harriague A, Misic C, Petrillo M, Albertelli G. 2007. Stressors affecting the macrobenthic community in Rapallo harbour (Ligurian Sea, Italy). Scientia Marina 71: 705-714.

Covazzi Harriague A, Misic C, Valentini I, Polidori E, Albertelli G, Pusceddu A. 2013. Meio- and macrofauna communities in three sandy beaches of the northern Adriatic Sea protected by artificial reefs, Chemistry and Ecology 29:181-195. DOI: 10.1080/02757540.2012.704911.

Dal Zotto M, Santulli A, Simonini R, Todaro MA. 2016. Organic enrichment effects on a marine meiofauna community, with focus on Kinorhyncha. Zoologischer Anzeiger 265: 127–140.

Danovaro R. 2010. Methods for the Study of Deep-sea Sediments, Their Functioning and Biodiversity. CRC Press Taylor & Francis Group.

Dell'Anno A, Mei ML, Pusceddu A, Danovaro R. 2002. Assessing the trophic state and eutrophication of coastal marine systems: a new approach based on the biochemical composition of sediment organic matter. Marine Pollution Bulletin 44 : 611-622.

Dhainaut-Courtois NC, Pruvot A, Baudet EK. 2000. Les peuplements macrozoobenthiques indicateurs des qualités physico-chimiques et chimiques des sédiments portuaires– Exemple du Port de Boulogne-sur-mer (Manche). Bulletin de la Société Zoologique de France, 125: 49-62.

EC, 2000. Directive of the European Parliament and of the Council 2000/60/EC establishing a Framework for Community Action in the Field of Water Policy. Available at: https://eur-lex.europa.eu/resource.html?uri=cellar:5c835afb-2ec6 -4577-bdf8-756d3d694eeb.0004.02/DOC_1&format=PDF.

EEA, UNEP, 2006. Priority Issues in the Mediterranean Environment. European Environment Agency, Copenhagen. https://www.eea.europa.eu/publications/eea_report_2006_4.

El Nemr A, Khaled A, El Sikaily A. 2006. Distribution and Statistical Analysis of Leachable and Total Heavy Metals in the Sediments of the Suez Gulf. Environmental Monitoring and Assessment 118: 89-112. https://doi.org/10.1007/s10661-006-0985-9.

Estacio FJ, García-Adiego EM, Fa DA, García-Gómez JC, Daza JL, Hortas F, Gómez-Ariza JL. 1997. Ecological analysis in a polluted area of Algeciras Bay (southern Spain): external ‘versus’ internal outfalls and environmental implications. Marine Pollution Bulletin 34: 780-793.

Etiope G, Panieri G, Fattorini D, Regoli F, Vannoli P, Italiano F, Locritani M, Carmisciano CA. 2014. Thermogenic hydrocarbon seep in shallow Adriatic Sea (Italy): Gas origin, sediment contamination and benthic foraminifera. Marine and Petroleum Geology 57:283-293. DOI: 10.1016/j.marpetgeo.2014.06.006.

Foti A, Fenzi GA, Di Pippo F, Gravina MF, Magni P. 2014. Testing the saprobity hypothesis in a Mediterranean lagoon: effects of confinement and organic enrichment on benthic communities. Marine Environmental Research 99: 85-94.

Franzo A, Baldrighi E, Grassi E, Grego M, Balsamo M, Basili M, Semprucci F. 2022. Free-living nematodes of Mediterranean ports: A mandatory contribution for their use in ecological quality assessment. Marine Pollution Bulletin 180: 113814. https://doi.org/10.1016/j.marpolbul.2022.113814

Frontalini F, Semprucci F, Coccioni R, Balsamo M, Bittoni P, Covazzi-Harriague A. 2011. On the quantitative distribution and community structure of the meio and macrofaunal communities in the coastal area of the Central Adriatic Sea (Italy). Environmental Monitoring and Assessment 180: 325-344.

Frontalini F, Semprucci F, Armynot du Châtelet E, Francescangeli F, Margaritelli G, Rettori R, Spagnoli F, Balsamo M, Coccioni R. 2014. Biodiversity trends of the meiofauna and foraminifera assemblages of Lake Varano (southern Italy). Proceedings of the Biological Society of Washington 127: 7–22.

Giere O. 2009. Meiobenthology: The Microscopic Motile Fauna of Aquatic Sediments, Second ed. Springer-Verlag (527 pp.).

Giuliani S, Romanelli M, Piazza R, Vecchiato M, Pizzini S, Tranchida G, D’Agostino F, Romano S, Bellucci LG. 2019. When research meets NGOs: The GVC-UCODEP project in the Bắc Giang Province and Cầu River (Northern Vietnam) and its feedback on national monitoring programs. Environmental Science & Policy 101: 279-290. https://doi.org/10.1016/j.envsci.2019.09.004.

Gómez-Gesteira JL, Dauvin JC. 2000. Amphipods are good bioindicators of the impact of oil spills on soft-bottom macrobenthic communities. Marine Pollution Bulletin 40: 1017-1027.

Grotti M, Pizzini S, Abelmoschi ML, Cozzi G, Piazza R, Soggia F. 2016. Retrospective biomonitoring of chemical contamination in the marine coastal environment of Terra Nova Bay (Ross Sea, Antarctica) by environmental specimen banking. Chemosphere 165: 418-426.

Guerra-García JM, García-Gómez JC. 2001. The spatial distribution of Caprellidea (Crustacea: Amphipoda): a stress bioindicator in Ceuta (North Africa, Gibraltar area). Pubbl. Staz. zool. Napoli, (Marine Ecology) 22: 357-367.

Guerra-García JM, García-Gómez JC. 2004a. Polychaete assemblages and sediment pollution in a harbour with two opposing entrances. Helgoland Marine Research 58:183–191. DOI 10.1007/s10152-004-0184-4

Guerra-García JM, García-Gomez JC 2004b. Soft bottom mollusc assemblages and pollution in a harbour with two opposing entrances. Estuarine, Coastal and Shelf Marine Science 60: 273-283.

Heip C, Vincx M, Vranken G. 1985. The ecology of marine nematodes. Oceanography and Marine Biology - An Annual Review 23: 399–489.

Katsanevakis S, Zenetos A, Mačić V, Beqiraj S, Poursanidis D, Kashta L. 2011. Invading the Adriatic: spatial patterns of marine alien species across the Ionian- Adriatic boundary. Aquatic Biology 13: 107–118.

Losi V, Ferrero TJ, Moreno M, Gaozza L, Rovere A, Firpo M, Marques JC, Albertelli G. 2013. The use of nematodes in assessing ecological conditions in shallow waters surrounding a Mediterranean harbour facility. Estuarine, Coastal and Shelf Science 130: 209-221.

Luna GM, Dell'Anno A, Danovaro R. 2006. DNA extraction procedure: a critical issue for bacterial diversity assessment in marine sediments. Environmental Microbiology 8 : 308-320.

Mestres M, Sierra JP, Mösso C, Sánchez-Arcilla A. 2010. Sources of contamination and modelled pollutant trajectories in a Mediterranean harbour (Tarragona, Spain). Marine pollution bulletin 60: 898-907.

Ministerial Decree of the Environment and Protection of the Territory and the Sea of 21 September 2016 n.173 "Regolamento recante modalità e criteri tecnici per l'autorizzazione all'immersione in mare dei materiali di escavo di fondali marini" and related technical attachments. https://www.gazzettaufficiale.it/eli/id/2016/09/06/16G00184/sg

Mirto S, Danovaro R. 2004. Meiofaunal colonisation on artificial substrates: a tool for biomonitoring the environmental quality on coastal marine systems. Marine Pollution Bulletin 48: 919–926.

Mirto S, Arigò C, Genovese L, Pusceddu A, Gambi C, Danovaro R. 2014. Nematode assemblage response to fish-farm impact in vegetated (*Posidonia oceanica*) and non vegetated habitats. Aquaculture Environment Interaction 5: 17–28.

Moreno M, Ferrero TJ, Gallizia I, Vezzulli L, Albertelli G, Fabiano M. 2008a. An assessment of the spatial heterogeneity of environmental disturbance within an enclosed harbour through the analysis of meiofauna and nematode assemblages. Estuarine, Coastal and Shelf Science 77: 565-576.

Moreno M, Vezzulli L, Marin V, Laconi P, Albertelli G, Fabiano M. 2008b. The use of meiofauna diversity as an indicator of pollution in harbours. ICES Journal of Marine Science 65: 1428–1435.

MSFD, 2008. Marine Strategy Framework Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 Establishing a Framework for Community Action in the Field of Marine Environmental Policy. pp. 1–22.

Papageorgiou N, Kalantzi I, Karakassis I. 2010. Effects of fish farming on the biological and geochemical properties of muddy and sandy sediments in the Mediterranean Sea. Marine environmental research 69: 326-336.

Patrício J, Adão H, Neto JM, Alves AS, Traunspurger W, Marques JC. 2012. Do nematode and macrofauna assemblages provide similar ecological assessment information?. Ecological Indicators 14: 124-137.

Paul JF, Scott KJ, Campbell DE, Gentile JH, Strobel CS, Valente RM, Weisberg SB, Holland AF, Ranasinghe JA. 2001. Developing and applying a benthic index of estuarine condition for the Virginian Biogeographic Province. Ecological Indicators 1: 83-99.

Pećarević M, Mikuš J, Bratoš-Cetinić A, Dulčić J, Čalić M. 2013. Introduced marine species in Croatian waters (Eastern Adriatic Sea). Mediterranean Marine Science 14: 224–237.

Pelletier MC, Gold AJ, Heltshe JF, Buffum HW. 2010. A method to identify estuarine macroinvertebrate pollution indicator species in the Virginian Biogeographic Province. Ecological Indicators 10: 1037-1048.

Pereira TL, Wallner-Kersanach M, Costa LDF, Costa DP, Baisch PRM. 2018. Nickel, vanadium, and lead as indicators of sediment contamination of marina, refinery, and shipyard areas. Environmental Science and Pollution Research 25: 1719-1730. https://doi.org/10.1007/s11356-017-0503-3.

Pielou EC. 1969. An Introduction to Mathematical Ecology. Wiley-Interscience, New York (286 pp.).

Pearson TH, Rosenberg R. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanography and Marine Biology 16: 229-311.

Pizzini S, Marchiori E, Piazza R, Cozzi G, Barbante C. 2015. Determination by HRGC/HRMS of PBDE levels in edible Mediterranean bivalves collected from north-western Adriatic coasts. Microchemical Journal 121:184-191. DOI.10.1016/j.microc.2015.03.010

Pizzini S, Sbicego C, Corami F, Grotti M, Magi E, Bonato T, Cozzi G, Barbante C, Piazza R. 2017. 3, 3′-dichlorobiphenyl (non-Aroclor PCB-11) as a marker of non-legacy PCB contamination in marine species: comparison between Antarctic and Mediterranean bivalves. Chemosphere 175: 28 35. DOI.10.1016/j.chemosphere.2017.02.023

Pizzini S, Morabito E, Gregoris E, Vecchiato M, Corami F, Piazza R, Gambaro A. 2021. Occurrence and source apportionment of organic pollutants in deep sediment cores of the Venice Lagoon. Marine Pollution Bulletin 164: 112053. https://doi.org/10.1016/j.marpolbul.2021.112053.

Punzo E, Gomiero A, Tassetti AN, Strafella P, Santelli A, Salvalaggio V, Spagnolo A, Scarcella G, De Biasi AM, Kozinkova L, Fabi G. 2017. Environmental impact of offshore gas activities on the benthic environment: a case study. Environmental management 60: 340-356.

Pusceddu A, Gambi C, Manini E, Danovaro R. 2007. Trophic state, ecosystem efficiency and biodiversity of transitional aquatic ecosystems: analysis of environmental quality based on different benthic indicators. Chemical Ecology 23: 505–515.

Pusceddu A, Dell'Anno A, Fabiano M, Danovaro R. 2009. Quantity and bioavailability of sediment organic matter as signatures of benthic trophic status. Marine Ecology Progress Series 375: 41-52.

Pusceddu A, Bianchelli S, Sanchez Vidal A, Canals M, Durrieu De Madron X, Heussner S, Lykousis V, de Stigter H, Trincardi F, Danovaro R. 2010. Organic matter in sediments of canyons and open slopes of the Portuguese, Catalan, Southern Adriatic and Cretan Sea margins. Deep Sea Research. I 57: 441-457.

Pusceddu A, Bianchelli S, Gambi C, Danovaro R. 2011. Assessment of benthic trophic status of marine coastal ecosystems: significance of meiofaunal rare taxa. Estuarine, Coastal and Shelf Science 93: 420-430.

R Core Team 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing. doi:3-900051-14-3.

Ridall A, Ingels J. 2021. Suitability of free-living marine nematodes as bioindicators: Status and future considerations. Frontiers in Marine Science 8: 685327.

Sabbatini A, Bonatto S, Gooday AJ, Morigi C, Pancotti I, Pucci F, Negri A. 2010. Modern benthic foraminifers at Northern shallow sites of Adriatic Sea and soft-walled, monothalamous taxa: a brief overview. Micropaleontology 359-376.

Sabbatini A, Nardelli MP, Morigi C, Negri A. 2013. Contribution of soft-shelled monothalamous taxa to foraminiferal assemblages in the Adriatic Sea. Acta Protozoologica 52(3).

Schratzberger M, Daniel F, Wall CM, Kilbride R, Macnaughton SJ, Boyd SE, Rees HL, Lee K, Swannell RPJ. 2003. Response of estuarine meio- and macrofauna to in situ bioremediation of oil-contaminated sediment. Marine Pollution Bulletin 46: 430–443.

Schratzberger M, Maxwell TAD, Warr K, Ellis JR, Rogers SI. 2008. Spatial variability of infaunal nematode and polychaete assemblages in two muddy subtidal habitats. Marine Biology 153: 621-642.

Schratzberger M, Ingels J. 2018. Meiofauna matters: the roles of meiofauna in benthic ecosystems. Journal of Experimental Marine Biology and Ecology 502: 12-25.

Semprucci F, Frontalini F, Sbrocca C, Armynot du Châtelet E, Bout-Roumazeilles V, Coccioni R, Balsamo M. 2015. Meiobenthos and free-living nematodes as tools for biomonitoring environments affected by riverine impact. Environmental Monitoring and Assessment 187, 251.

Semprucci F, Balsamo M, Sandulli R. 2016. Assessment of the Ecological quality (EcoQ) of the Venice lagoon using the structure and biodiversity of the meiofaunal assemblages. Ecological Indicators 67: 451–457.

Semprucci F, Grassi E, Balsamo M. 2022. Simple Is the Best: An Alternative Method for the Analysis of Free-Living Nematode Assemblage Structure. Water 14: 1114. https://doi.org/10.3390/w14071114

Shannon C, Weaver W. 1949. The Mathematical Theory of Communication. University of Illinois Press, Urbana, US.

Simboura N, Zenetos A. 2002. Benthic indicators to use in ecological quality classification of Mediterranean soft bottom marine ecosystems, including a new biotic index. Mediterranean Marine Science 3: 77–111.

Simonini R, Ansaloni I, Bonvicini Pagliai AM, Prevedelli D. 2004. Organic enrichment and structure of the macrozoobenthic community in the northern Adriatic Sea in an area facing Adige and Po mouths. ICES Journal of Marine Science 61: 871-881.

Simonini R, Ansaloni I, Cavallini F, Graziosi F, Iotti M, N'siala GM, Mauri M, Montanari G, Preti M, Prevedelli D. 2005. Effects of long-term dumping of harbor-dredged material on macrozoobenthos at four disposal sites along the Emilia-Romagna coast (Northern Adriatic Sea, Italy). Marine Pollution Bulletin 50: 1595-1605. https://doi.org/10.1016/j.marpolbul.2005.06.031

Spagnolo A, Scarcella G, Sarappa A. 2011. Benthic community response to sediment features in Ancona harbour (Northern Adriatic Sea, Italy). Vie Milieu 61: 119–128.

Spagnolo A, Cuicchi C, De Biasi A M, Ferrà C, Montagnini L, Punzo E, Salvalaggio V, Santelli A, Strafella P, Fabi G. 2019. Effects of the installation of offshore pipelines on macrozoobenthic communities (northern and central Adriatic Sea). Marine pollution bulletin 138: 534-544.

Travizi A, Balković I, Bacci T, Bertasi F, Cuicchi C, Flander-Putrle V, Grati F, Grossi L, Jaklin A, Lipej L, Mavrič B, Mikac B, Marusso V, Montagnini L, Nerlović V, Penna P, Salvalaggio V, Santelli A, Scirocco T, Spagnolo A, Trabucco B, Vani D. 2019. Macrozoobenthos in the Adriatic Sea ports: Soft-bottom communities with an overview of non-indigenous species. Marine Pollution Bulletin 147:159–170. https://doi.org/10.1016/j.marpolbul.2019.01.016

Tobiszewski M, Namieśnik J. 2012. PAH diagnostic ratios for the identification of pollution emission sources. Environmental Pollution 162 : 110-119. https://doi.org/10.1016/j.envpol.2011.10.025.

Vassallo P, Fabiano M, Vezzulli L, Sandulli R, Marques JC, Jørgensen SE. 2006. Assessing the health of coastal marine ecosystems: a holistic approach based on sediment micro and meio-benthic measures. Ecological indicators 6: 525-542.

Veiga P, Rubal M, Besteiro C. 2009. Shallow sublittoral meiofauna communities and sediment polycyclic aromatic hydrocarbons (PAHs) content on the Galician coast (NW Spain), six months after the prestige oil spill. Marine Pollution Bulletin 58: 581–588. https://doi.org/10.1016/j.marpolbul.2008.11.002.

Vezzulli L, Marrale D, Moreno M, Fabiano M. 2003. Sediment organic matter and meiofauna community response to long-term fish-farm impact in the Ligurian Sea (Western Mediterranean). Chemistry and Ecology 19: 431-440.

Viana M, Hammingh P, Colette A, Querol X, Degraeuwe B, de Vlieger I, van Aardenne J. 2014. Impact of maritime transport emissions on coastal air quality in Europe. Atmospheric Environment 90: 96-105. https://dx.doi.org/10.1016/j.atmosenv.2014.03.046.

Warwick RM, Dashfield SL, Somerfield PJ. 2006. The integral structure of a benthic infaunal assemblage. Journal of Experimental Marine Biology and Ecology 330: 12-18.

Weisberg SB, Ranasinghe JA, Dauer DM, Schaffner LC, Diaz RJ, Frithsen JB. 1997. An estuarine benthic index of biotic integrity (B-IBI) for Chesapeake Bay. Estuaries 20: 149-158.

Whomersley P, Huxham M, Schratzberger M, Bolam S. 2009. Differential response of meio-and macrofauna to in situ burial. Journal of the Marine Biological Association of the United Kingdom 89: 1091-1098.

WoRMS Editorial Board, 2022. World Register of Marine Species. Available from https://www.marinespecies.org at VLIZ. Accessed 2022. doi:10.14284/170

Zeppilli D, Sarrazin J, Leduc D, Arbizu PM, Fontaneto D, Fontanier C, Gooday AJ, Kristensen RM, Ivanenko VN, Sorensen MV, Vanreusel A, Thébault J, Mea M, Allio N, Andro T, Arvigo A, Castrec J, Danielo M, Foulon V, Fumeron R, Hermabessiere L, Hulot V, James T, Langonne-Augen R, Le Bot T, Long M, Mahabror D, Morel Q, Pantalos M, Pouplard E, Raimondeau L, Rio- Cabello A, Seite S, Traisnel G, Urvoy K, Van Der Stegen T, Weyand M, Fernandes D. 2015. Is the meiofauna a good indicator for climate change and anthropogenic impacts? Marine Biodiversity 45: 505–535.

Zuur AF, Ieno EN. 2016. A protocol for conducting and presenting results of regression-type analyses. Methods in Ecology and Evolution 7: 636–645. doi:10.1111/2041-210X.12577.

Zwick WR, Velicer WF. 1986. Comparison of five rules for determining the number of components to retain. Psychological Bulletin 99: 432–442. https://doi.org/10.1037/0033-2909.99.3.432.

Figure 1

Map of the sampling area (Ancona harbor) and location of the five sampling stations.

The pink rectangle indicates the geographical position of the Ancona harbor, Italy.

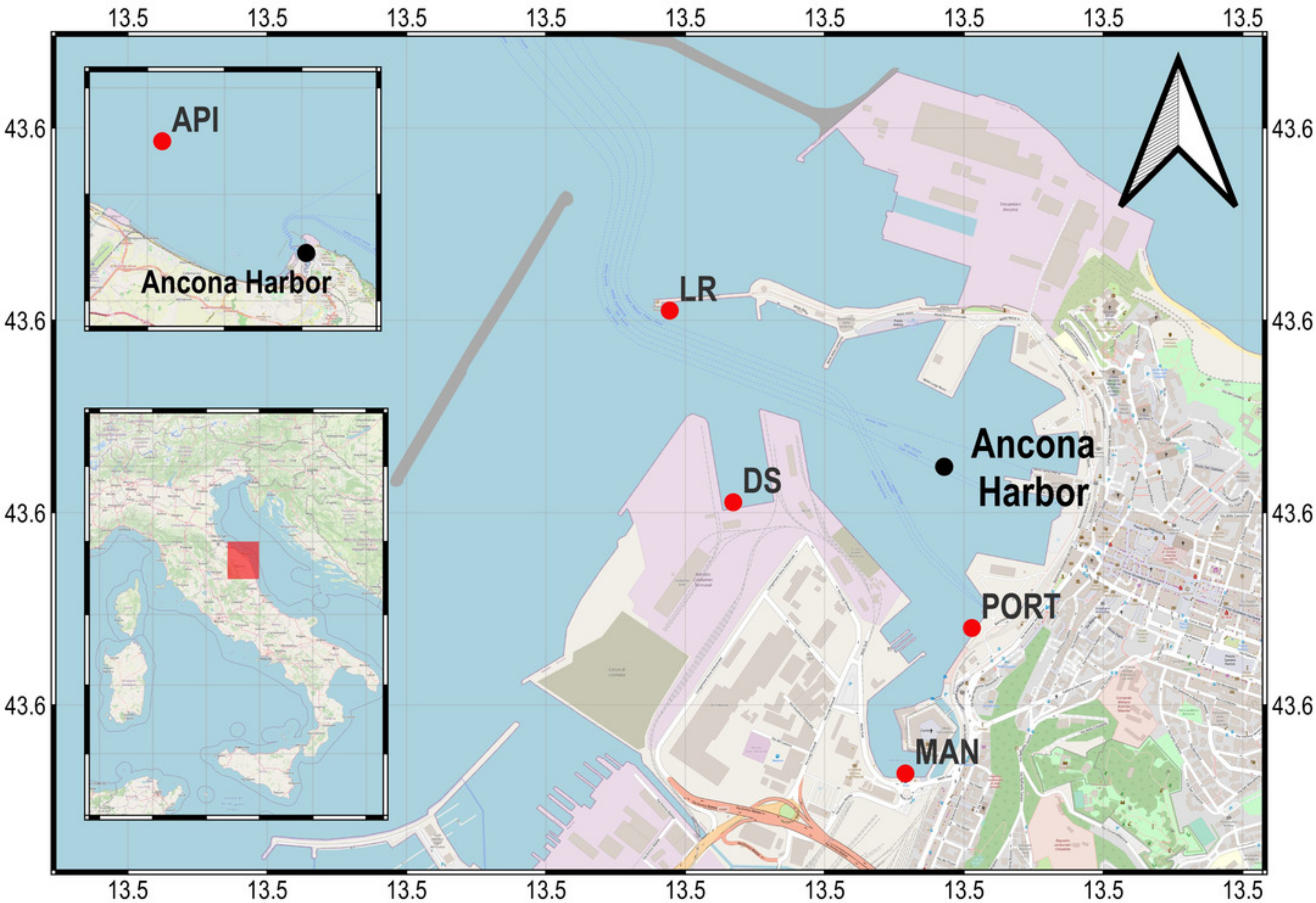


Figure 2

(a) Distribution pattern and (b) origin of Polycyclic aromatic hydrocarbons (PAHs) characterizing the sediment at the investigated sampling stations.

Σ LMW = sum of Low Molecular Weight compounds (Naphthalene, 1-Methylnaphtalene, 2-Methylnaphtalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene); Σ HMW = sum of High Molecular Weight compounds (Fluoranthene, Pyrene, Benz[*a*]anthracene, Chrysene, Benzo[*b*]fluoranthene, Benzo[*k*]fluoranthene, Benzo[*a*]pyrene, 7,12-Dimethylbenz[*a*]anthracene, Benzo[*ghi*]perylene, Indeno[1,2,3-*cd*]pyrene, Dibenz[*a*,*h*]anthracene); Σ COMB = sum of 9 non-alkylated PAHs (Fluoranthene, Pyrene, Benz[*a*]anthracene, Chrysene, Benzo[*b*]fluoranthene, Benzo[*k*]fluoranthene, Benzo[*a*]pyrene, Benzo[*ghi*]perylene, Indeno[1,2,3-*cd*]pyrene).

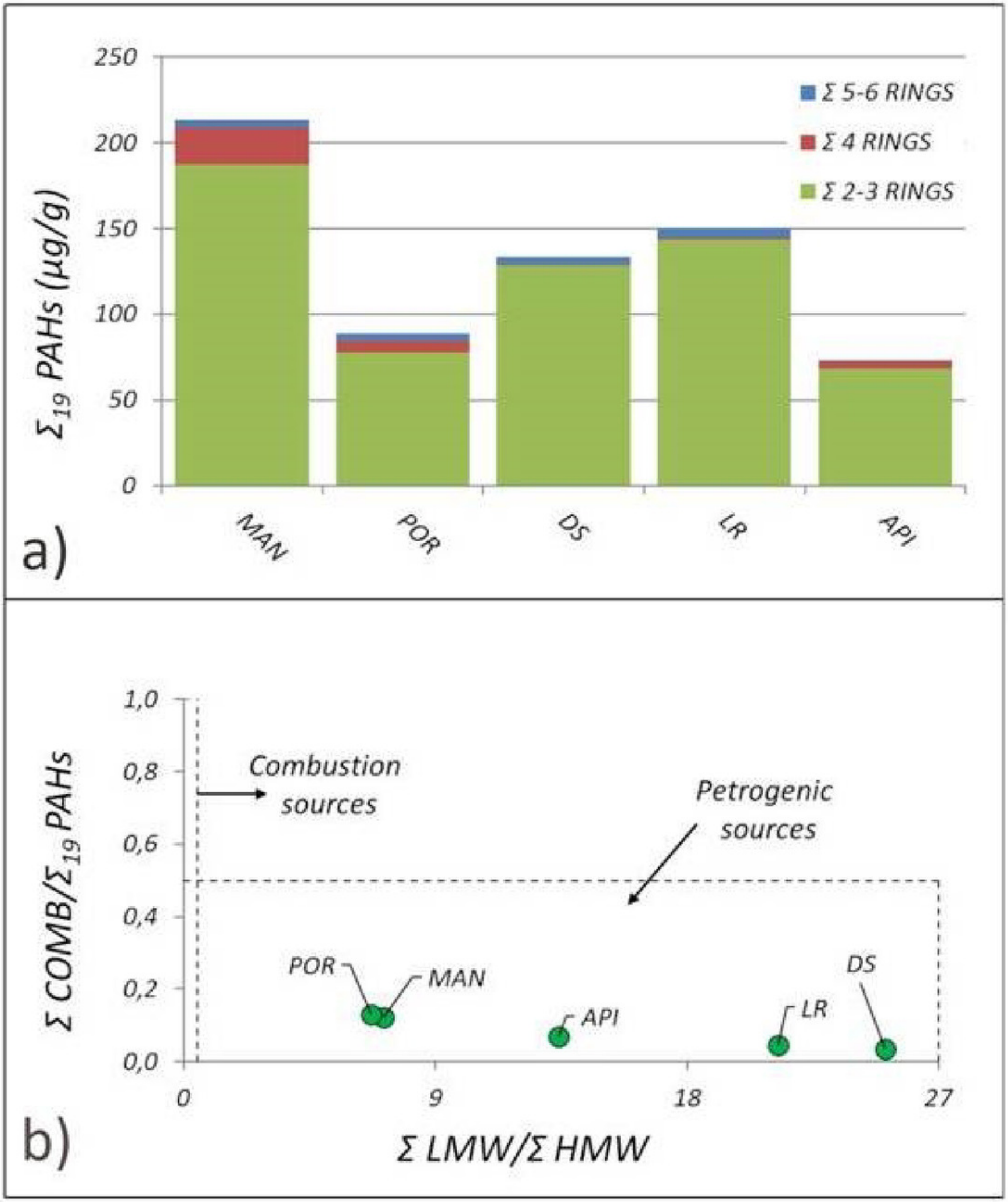
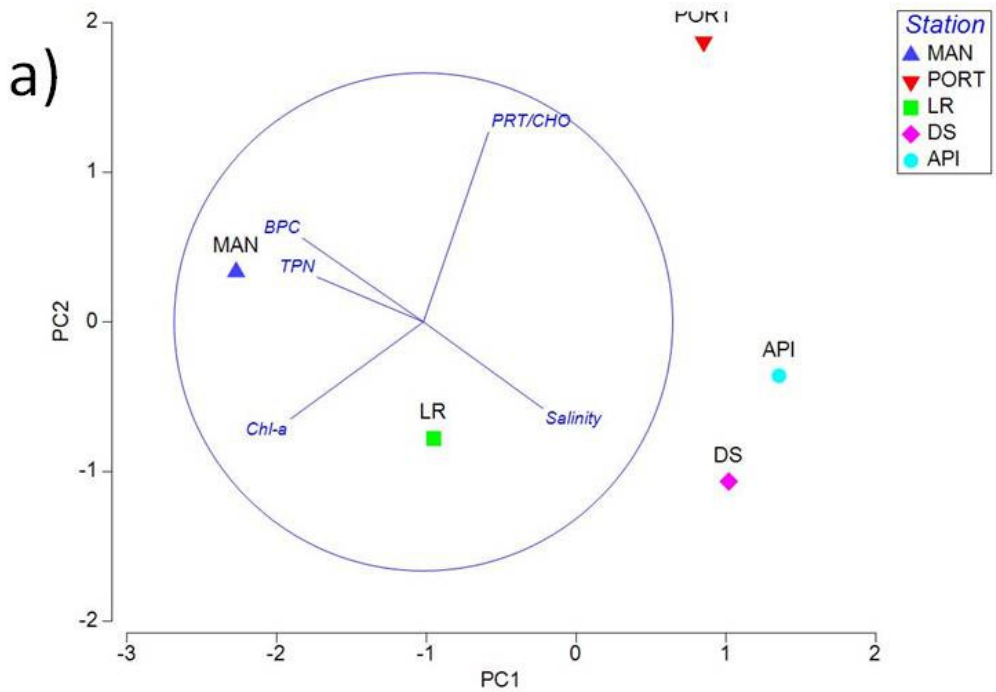

250
200
150
100
50
0
Σ19 PAHs (µg/g)
Σ 5-6 RINGS
Σ 4 RINGS
Σ 2-3 RINGS
MAN
POR
DS
LR
API
a)
1,0
0,8
0,6
0,4
0,2
0,0
Σ COMB/Σ19 PAHs
Combustion sources
Petrogenic sources
POR
MAN
API
LR
DS
0
9
18
27
Σ LMW/Σ HMW
b)

Figure 3

Principal Component Analysis (PCA) with (a) ‘biotic’ and (b) ‘abiotic’ environmental variables.

BPC = Biopolymeric organic carbon; Chl-*a* = Chlorophyll-*a* concentration; PAHs = Polycyclic aromatic hydrocarbons; PRT/CHO = Protein to carbohydrate ratio; TPN = Total Prokaryotic Number.


a)
Station
MAN
PORT
LR
DS
API
PRT/CHO
BPC
TPN
Chl-a
Salinity
MAN
LR
API
DS
PORT
PC1
PC2

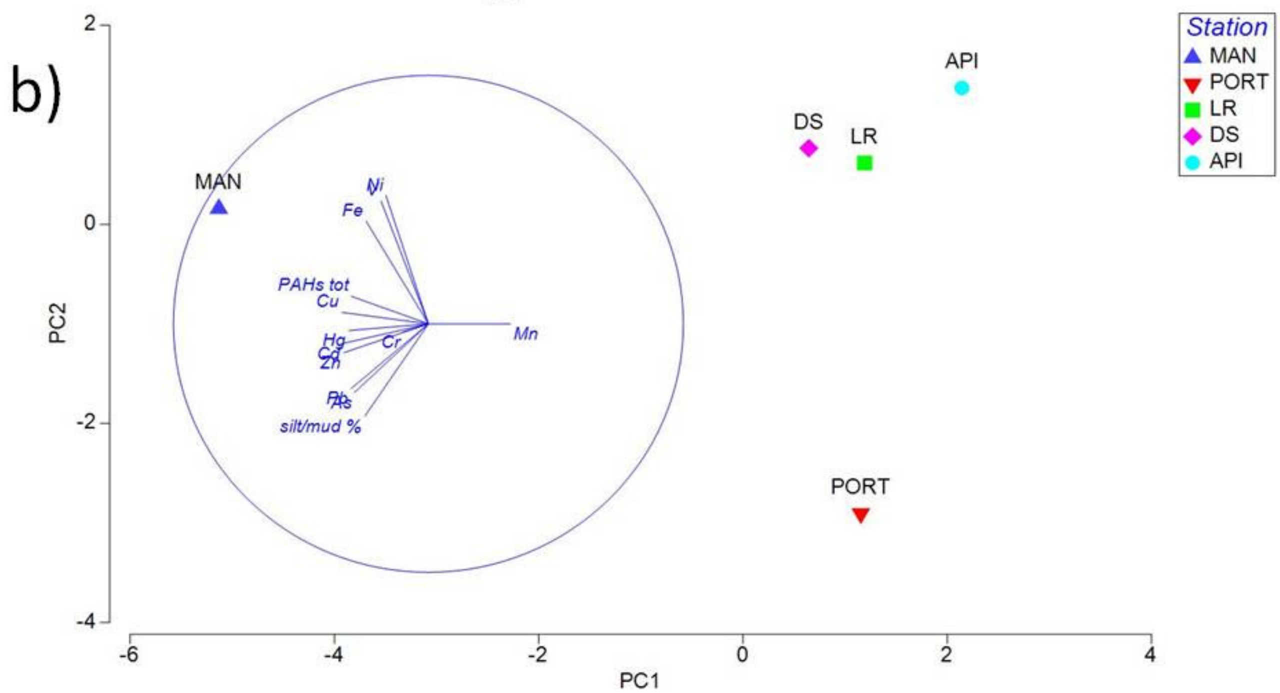

b)
Station
MAN
PORT
LR
DS
API
Ni
Fe
PAHs tot
Cu
Hg
Cd
Zn
Cr
Mn
Pb
As
silt/mud %
MAN
DS
LR
API
PORT
PC1
PC2

Figure 4

(a) Meiofaunal community structure and (b) contribution of taxa *others* at each sampling station.

Mean values are shown.

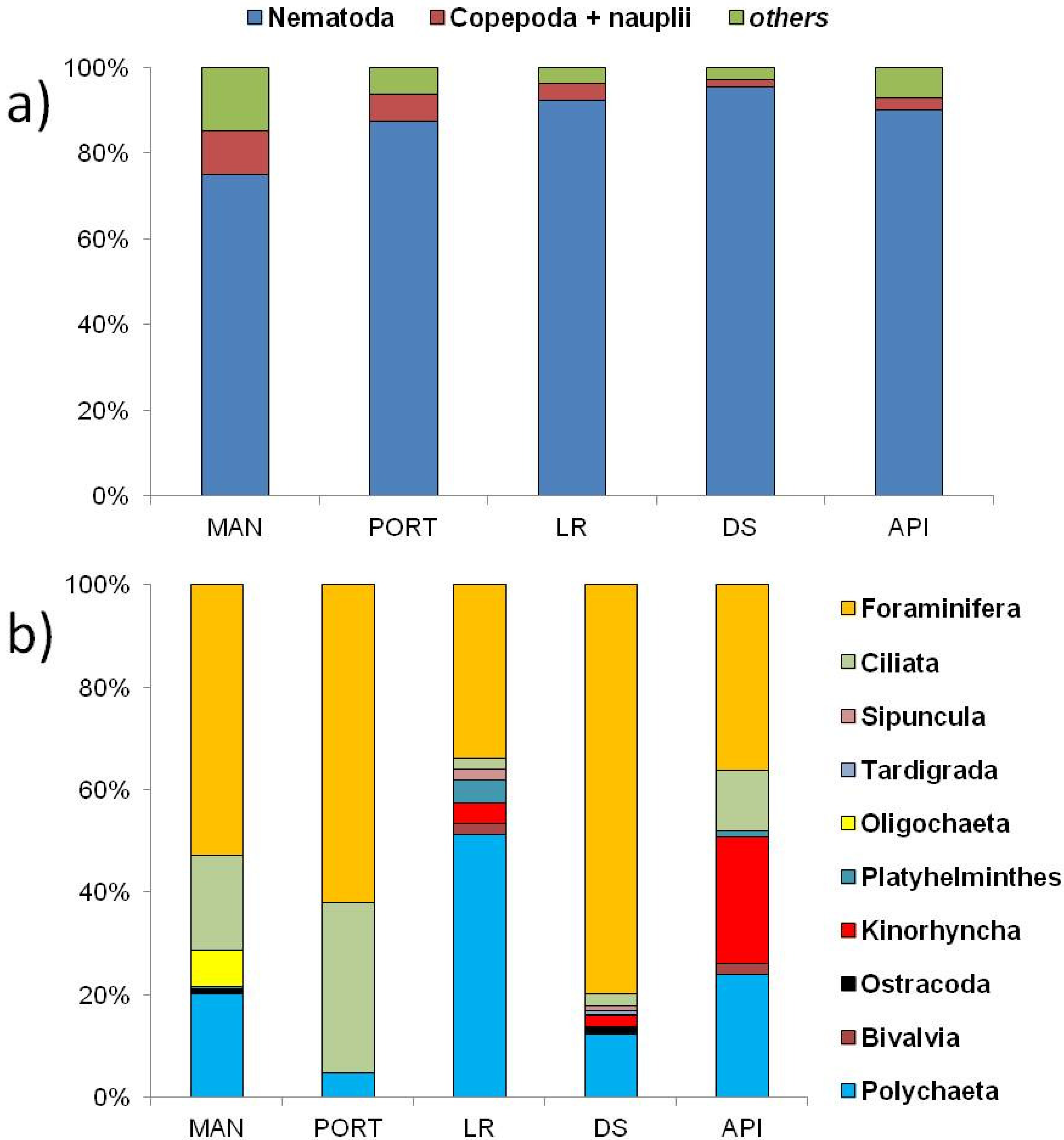

Nematoda
Copepoda + nauplii
others
a)
100%
80%
60%
40%
20%
0%
MAN
PORT
LR
DS
API
b)
100%
80%
60%
40%
20%
0%
MAN
PORT
LR
DS
API
Foraminifera
Ciliata
Sipuncula
Tardigrada
Oligochaeta
Platyhelminthes
Kinorhyncha
Ostracoda
Bivalvia
Polychaeta

Figure 5

Meiofaunal univariate measures.

(a) Meiofauna abundance (N = no. of individuals 10 cm^{-2}) and its diversity indices: (b) meiofauna taxa richness (S), (c) Shannon's diversity index (H', based on log_2), and (d) Pielou's equitability index (J'). Bars represent 95% confidence intervals.

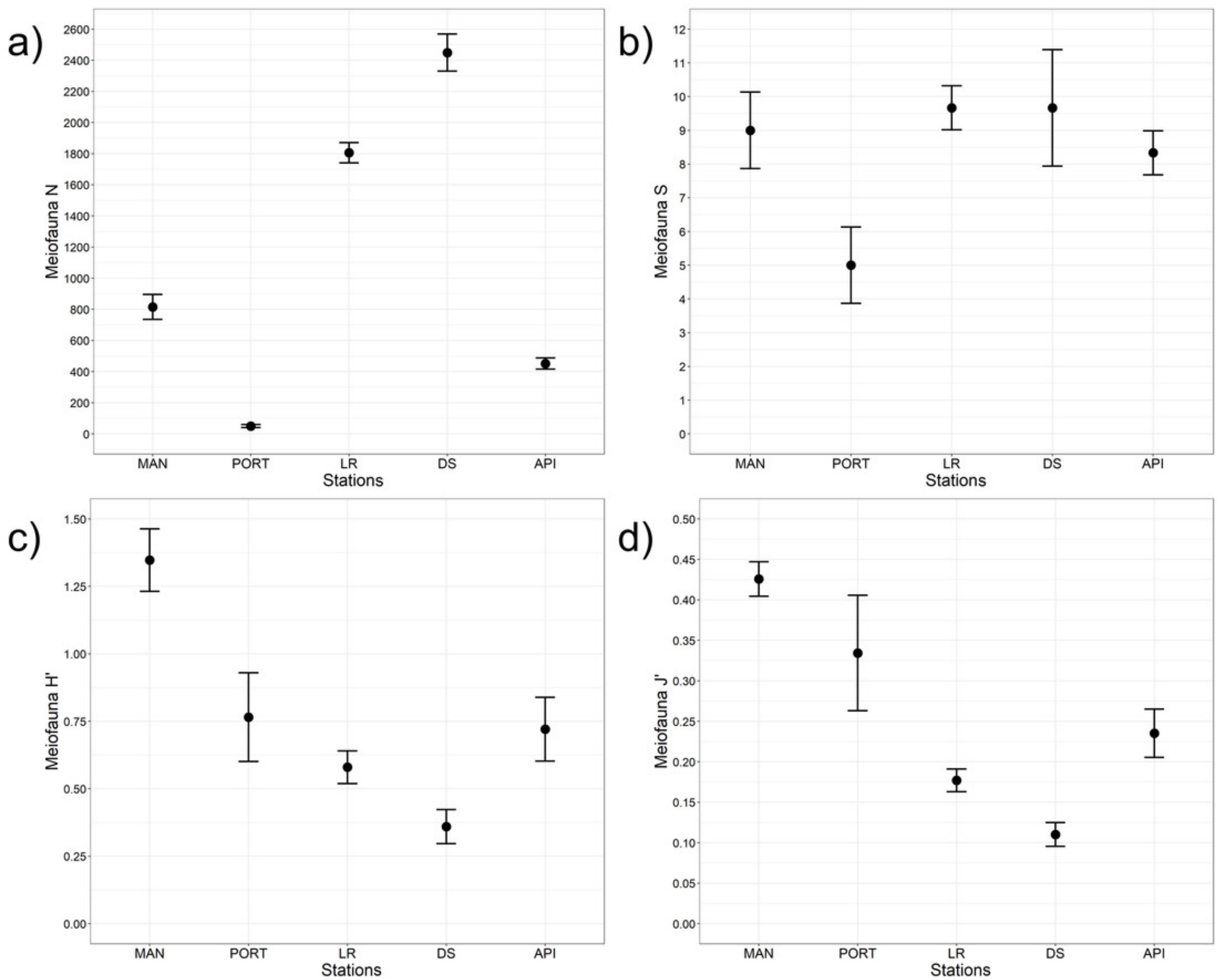


Figure 6

Non-metric Multidimensional Scaling (nMDS) of benthic communities.

Non-metric Multidimensional Scaling (nMDS) plots on (a) meiobenthic and (b) macrobenthic community structures characterizing the sediment at the investigated sampling stations. Green circles represent 40% similarity grouping.

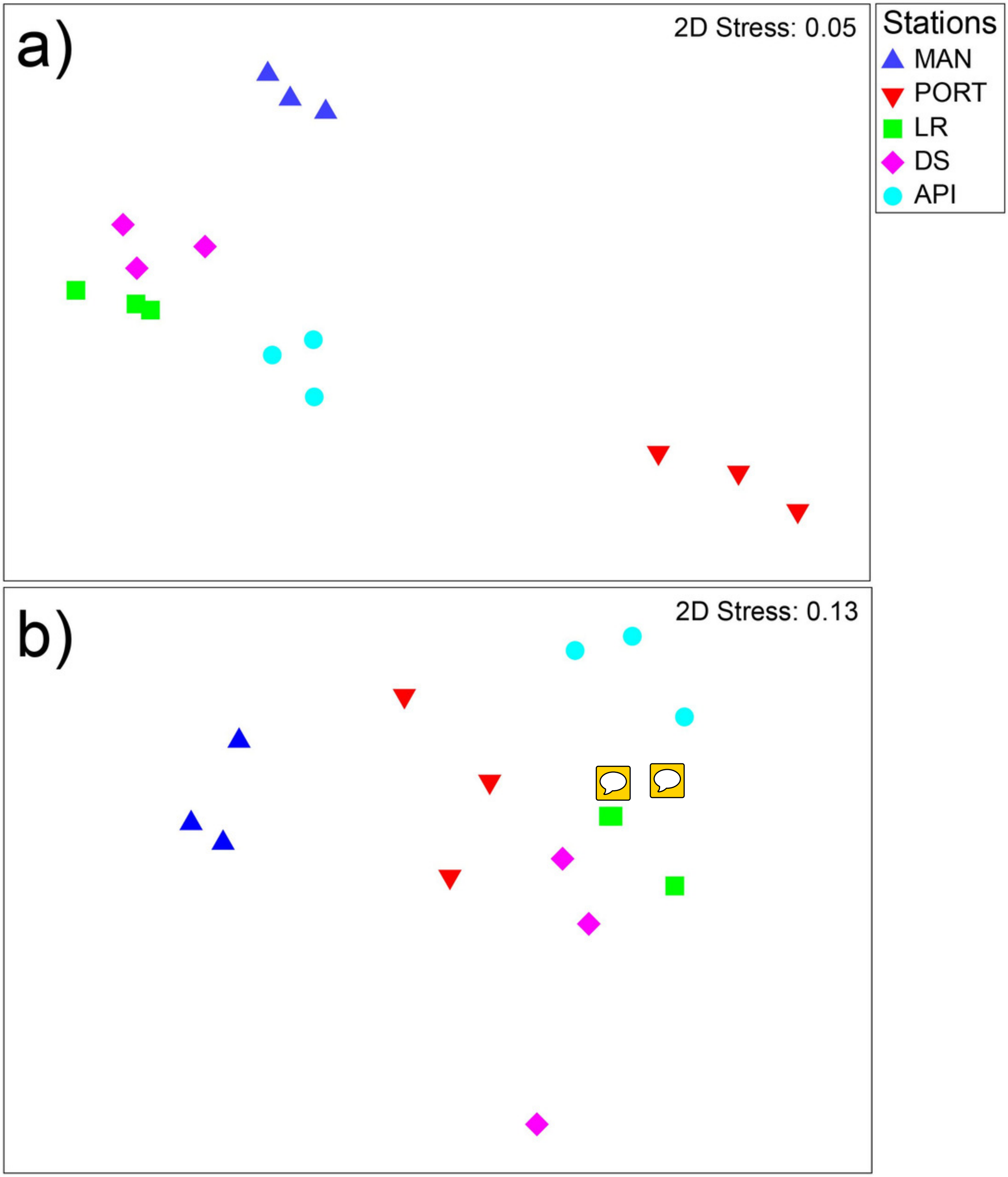

a)
2D Stress: 0.05
Stations
MAN
PORT
LR
DS
API
b)
2D Stress: 0.13

Figure 7

Macrofaunal community structure at each sampling station.

Mean values are shown.

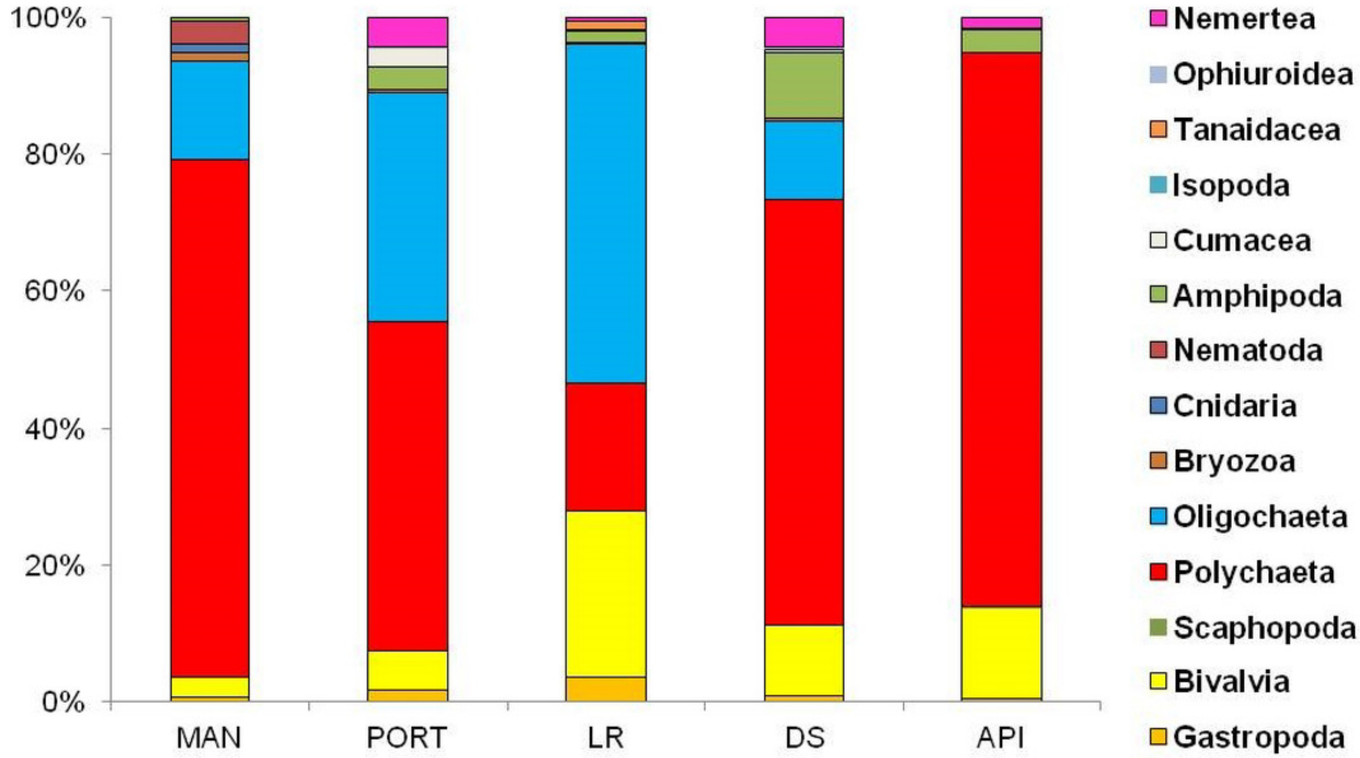


Figure 8

Macrofaunal univariate measures.

(a) Macrofauna abundance (N = no. of individuals m^{-2}) and its diversity indices: (b) macrofauna taxa richness (S), (c) Shannon's diversity index (H', based on log_2), and (d) Pielou's equitability index (J'). Bars represent 95% confidence intervals.

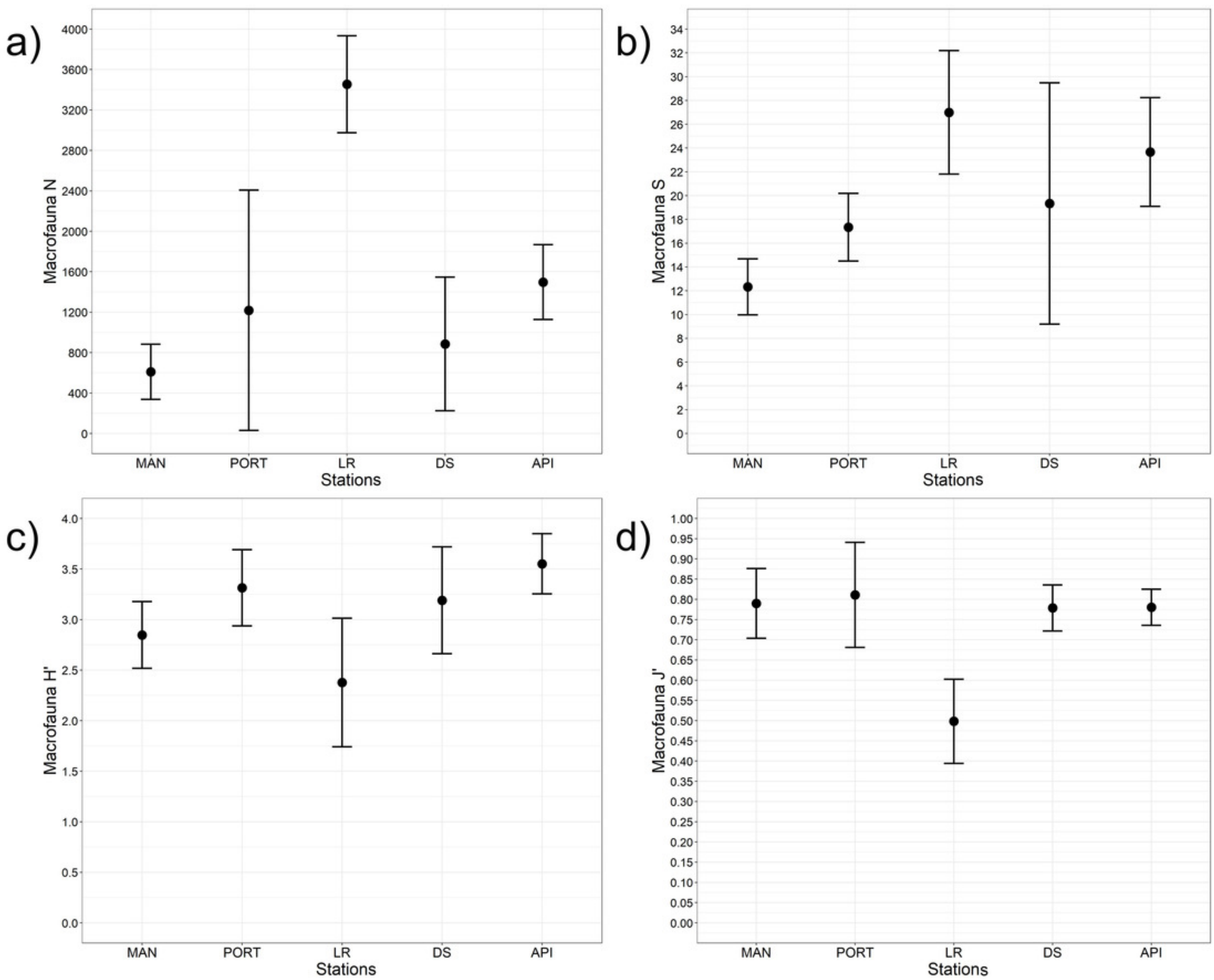


Table 1(on next page)

Table 1. Location of the five sampling stations, environmental variables measured during the sampling, grain size, biochemical composition , and microbial component of the sediment samples.

Chl-*a* = Chlorophyll-*a* concentration; BPC = Biopolymeric organic carbon; PRT/CHO = Protein to carbohydrate ratio; TPN = Total Prokaryotic Number. Mean values ± standard deviation are reported.

Table 1. Location of the five sampling stations, environmental variables measured during the sampling, grain size, biochemical composition, and microbial component of the sediment samples. Chl-*a* = Chlorophyll-*a* concentration; BPC = Biopolymeric organic carbon; PRT/CHO = Protein to carbohydrate ratio; TPN = Total Prokaryotic Number. Mean values ± standard deviation are reported.

Sampling station		**MAN**	**PORT**	**DS**	**LR**	**API**
Latitude N		43°36.793	43°37.020	43°37.130	43°37.309	43°40.297
Longitude E		13°30.174	13°30.317	13°29.482	13°29.400	13°24.344
Water temperature (°C)		10.4	10.2	10.2	10.1	10.7
Salinity (PSU)		32.0	32.5	36.7	31.3	37.6
Depth (m)		3.1	6.2	11.0	5.0	14.0
Grain size	Sand%	2	20	84	84	79
	Silt/mud%	98	80	16	16	21
Chl-*a* ($\mu g\ g^{-1}$)		13.9 ± 5.2	2.3 ± 0.4	17.0 ± 4.6	8.1 ± 1.2	2.9 ± 0.6
BPC ($mgC\ g^{-1}$)		10.2 ± 1.8	5.7 ± 1.5	3.7 ± 0.3	4.5 ± 0.5	2.3 ± 0.5
PRT/CHO		5.5 ± 1.5	40 ± 16.1	5.2 ± 1.4	4.2 ± 1.5	12.9 ± 2.7
TPN (no. of cells mL^{-1})		1.8E+06 ± 4.0E+05	8.4E+05 ± 7.4E+05	1.0E+06 ± 6.1E+05	3.8E+05 ± 3.7E+05	1.3E+06 ± 1.2E+05

Table 2. Concentration (in µg g^{-1}) of selected Trace Elements (TEs) and Polycyclic aromatic hydrocarbons (PAHs) detected in the sediment samples. In bold values exceeding the lower threshold level (L1) specified in the Ministerial Decree 173/2016, in bold and underlined those values exceeding also the upper threshold level (L2).

	Sampling station	**MAN**	**PORT**	**DS**	**LR**	**API**
TEs	Arsenic	3.628	3.193	2.941	2.882	2.386
	Cadmium	0.1915	0.0773	0.0533	0.0523	0.0493
	Chromium	23.07	22.14	26.54	21.17	19.28
	Copper	**46.313**	3.974	7.866	5.331	3.393
	Iron	19119	12332	16709	15666	15937
	Mercury	0.06835	0.02630	0.01865	0.01117	0.02761
	Manganese	355.7	508.0	449.2	507.0	558.4
	Nickel	3.911	2.786	3.555	3.539	3.703
	Lead	19.912	14.835	10.569	11.799	8.348
	Vanadium	28.31	18.99	24.90	24.83	26.09
	Zinc	**297.15**	**114.94**	58.78	67.70	47.03
PAHs	Naphthalene	**64.483**	21.611	**60.266**	**63.414**	30.370
	1-Methylnaphtalene	51.412	21.632	29.975	36.011	17.875
	2-Methylnaphtalene	50.917	24.928	27.438	31.036	13.150
	Acenaphthylene	8.267	<0.5	<0.5	<0.5	<0.5
	Acenaphthene	<0.5	<0.5	<0.5	<0.5	<0.5
	Fluorene	<0.5	1.578	1.280	1.881	1.428
	Phenanthrene	12.184	7.579	9.029	10.800	5.302
	Anthracene	<0.05	0.166	<0.05	0.123	0.163
	Fluoranthene	17.404	6.305	<0.05	0.861	4.022
	Pyrene	4.039	0.787	0.782	0.556	0.172
	Benz[*a*]anthracene	<0.05	<0.05	<0.05	<0.05	<0.05
	Chrysene	<0.05	<0.05	<0.05	<0.05	<0.05
	Benzo[*b*]fluoranthene	3.027	3.363	1.814	3.004	0.475
	Benzo[*k*]fluoranthene	0.308	0.373	1.220	1.114	0.322
	Benzo[*a*]pyrene	1.145	0.332	0.281	0.882	0.099
	7,12-Dimethylbenz[*a*]anthracene	<0.5	<0.5	<0.5	<0.5	<0.5
	Benzo[*ghi*]perylene	0.257	0.376	0.595	0.326	<0.05
	Indeno[1,2,3-*cd*]pyrene	<0.05	<0.05	<0.05	<0.05	<0.05
	Dibenz[*a*,*h*]anthracene	<0.05	<0.05	0.411	<0.05	<0.05
	Σ_8 LMW PAHs	187.26	77.49	127.99	143.27	68.29
	Σ_{11} HMW PAHs	26.18	11.54	5.10	6.74	5.09
	Σ_{19} PAHs	213.44	89.03	133.09	150.01	73.38

LMW = Low Molecular Weight compounds (Naphthalene, 1-Methylnaphtalene, 2-Methylnaphtalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene); HMW = High Molecular Weight compounds

(Fluoranthene, Pyrene, Benz[*a*]anthracene, Chrysene, Benzo[*b*]fluoranthene, Benzo[*k*]fluoranthene, Benzo[*a*]pyrene, 7,12-Dimethylbenz[*a*]anthracene, Benzo[*ghi*]perylene, Indeno[1,2,3-*cd*]pyrene, Dibenz[*a*,*h*]anthracene).

Table 3. One-way Analysis of Variance (ANOVA) results on total meiofauna and macrofauna abundance (N) and their diversity indices. Number of taxa (S), Shannon's diversity index (H', based on log_2), and Pielou's equitability index (J'), characterizing the sediment at the investigated sampling stations.

Meiofauna					
Index		**d.f.**	**F value**	***p* value**	**Tukey's HSD *post hoc* test**
N	Stations	4	714.3	<0.001	DS>LR>MAN>API>PORT
	Residuals	10			
S	Stations	4	11.3	<0.001	PORT<API=DS=LR=MAN
	Residuals	10			
H'	Stations	4	44.1	<0.001	MAN>PORT>API=LR(=DS)>DS
	Residuals	10			
J'	Stations	4	41.6	<0.001	MAN>PORT=API=LR(=DS)>DS
	Residuals	10			
Macrofauna					
Index		**d.f.**	**F value**	***p* value**	**Tukey's HSD *post hoc* test**
N	Stations	4	10.6	0.002	LR>API=DS=MAN=PORT
	Residuals	10			
S	Stations	4	3.8	0.041	MAN<LR
	Residuals	10			
H'	Stations	4	8.2	0.003	LR<API=DS=MAN=PORT
	Residuals	10			
J'	Stations	4	3.9	0.036	LR<API
	Residuals	10			

d.f. = degrees of freedom; HSD = Honestly Significant Difference.

Table 4. Permutational Multivariate Analysis of Variance (PERMANOVA) and pairwise test and results on total meiofaunal community composition. In bold significant values are reported.

PERMANOVA						
Source	**d.f.**	**SS**	**MS**	**Pseudo-F value**	**P(perm)**	**Unique permutations**
Stations	4	7315.8	1829	38.037	**0.0001**	9915
Residuals	10	480.84	48.084			
Total	14	7796.7				
PAIRWISE TEST						
Groups	**t value**	**P(perm)**	**P(MC)**			
MAN, PORT	7.39	0.103	**0.0006**			
MAN, LR	8.2125	0.0993	**0.0006**			
MAN, DS	4.8631	0.0997	**0.0043**			
MAN, API	6.1192	0.0992	**0.0015**			
PORT, LR	8.233	0.0953	**0.0002**			
PORT, DS	6.4351	0.1013	**0.0004**			
PORT, API	5.3464	0.0985	**0.0019**			
LR, DS	2.9523	0.1014	**0.0163**			
LR, API	5.2028	0.1014	**0.0018**			
DS, API	3.9778	0.0985	**0.0054**			

d.f. = degrees of freedom; SS = Sum of Squares; MS = Mean Square; P(perm) = Permutation *p*-value; P(MC) = Monte Carlo *p*-value.

Table 5. Distance-based Linear Modeling (DistLM) analysis on meiofaunal community composition characterizing the sediment at the investigated sampling stations. Variables are coded after Varimax Rotated PCA Axis as follow: ARCx = Abiotic variables associated to the rotated axis (x indicates the number of the axis); BRCx = Biotic variables associated to the rotated axis. For a complete list of the variables refers to the text.

START SOLUTION							
AICc	**R^2**	**RSS**	**No. of variables**	**Selections**			
82.237	0.9374	488.09	6	All			
SEQUENTIAL TESTS							
Variable	**AICc**	**SS**	**Pseudo-F value**	***p* value**	**Prop.**	**Cumul.**	**Res. d.f.**
BRC1	7.390	146.94	2.4083	0.0916	1.88E-02	0.91855	9
BRC2	8.213	78.41	1.1113	0.4061	1.01E-02	0.90849	10
BEST SOLUTION							
AICc	**R^2**	**RSS**	**No. of variables**	**Selections**			
74.597	0.9085	713.44	4	ARC1-ARC4			

AICc = Akaike's Information Criterion corrected for small samples; R^2 = Coefficient of determination; RSS = Residuals Sums of Squares; SS = Sums of Squares; Prop. = Proportion of variation explained by the variable; Cumul. = Cumulative total of Prop; Res. d.f. = Residual degrees of freedom.

Table 6. Permutational Multivariate Analysis of Variance (PERMANOVA) and pairwise test and results on total macrofaunal community composition. In bold significant values are reported.

PERMANOVA						
Source	**d.f.**	**SS**	**MS**	**Pseudo-F value**	**P(perm)**	**Unique permutations**
Stations	4	23534	5883.5	4.5481	**0.0001**	9915
Residuals	10	12936	1293.6			
Total	14	36470				
PAIRWISE TEST						
Groups	**t value**	**P(perm)**	**P(MC)**			
MAN, PORT	2.0289	0.1017	**0.028**			
MAN, LR	2.6607	0.0979	**0.011**			
MAN, DS	2.4096	0.0933	**0.015**			
MAN, API	3.0865	0.1047	**0.007**			
PORT, LR	1.5617	0.1021	0.100			
PORT, DS	1.708	0.1013	0.069			
PORT, API	2.0282	0.1012	**0.031**			
LR, DS	1.5713	0.1040	0.106			
LR, API	2.2314	0.1007	**0.024**			
DS, API	2.034	0.0997	**0.036**			

d.f. = degrees of freedom; SS = Sum of Squares; MS = Mean Square; P(perm) = Permutation *p*-value; P(MC) = Monte Carlo *p*-value.

Table 7. Distance-based Linear Modeling (DistLM) analysis on macrofaunal community composition characterizing the sediment at the investigated sampling stations. Variables are coded after Varimax Rotated PCA Axis as follow: ARCx = Abiotic variables associated to the rotated axis (x indicates the number of the axis); BRCx = Biotic variables associated to the rotated axis. For a complete list of the variables refers to the text.

START SOLUTION							
AICc	**R^2**	**RSS**	**No. of variables**	**Selections**			
130.09	0.6749	11856	6	All			
SEQUENTIAL TESTS							
Variable	**AICc**	**SS**	**Pseudo-F value**	***p* value**	**Prop.**	**Cumul.**	**Res. d.f.**
BRC1	124.52	1625.6	1.0969	0.3775	4.46E-02	0.63033	9
ARC3	121.01	2259.5	1.5084	0.1502	6.20E-02	0.56838	10
BRC2	118.28	2173.9	1.3810	0.1845	5.96E-02	0.50877	11
ARC1	116.72	2906.3	1.7845	0.0484	7.97E-02	0.42908	12
BEST SOLUTION							
AICc	**R^2**	**RSS**	**No. of variables**	**Selections**			
116.72	0.42908	20821	2	ARC2;ARC4			

AICc = Akaike's Information Criterion corrected for small samples; R^2 = Coefficient of determination; RSS = Residuals Sums of Squares; SS = Sums of Squares; Prop. = Proportion of variation explained by the variable; Cumul. = Cumulative total of Prop; Res. d.f. = Residual degrees of freedom.