

Temporal changes of the meiofaunal assemblage as a tool for the assessment of the ecological quality status

F. SEMPRUCCI, C. SBROCCA, M. ROCCHI AND M. BALSAMO

Dipartimento di Scienze della Terra, della Vita e dell'Ambiente (DiSTeVA), Università di Urbino, loc. Crocicchia, 61029 Urbino, Italy

The Adriatic Sea, being a semi-closed and shallow basin, is more sensitive to anthropogenic impact than other areas of the Mediterranean Sea. Given the crucial role of meiofauna in the marine ecosystems, temporal series of data on this benthic assemblage are fundamental to give new insights into the health status of this basin. A data set (decade 2002–2012) on the meiofauna of two river mouths (Foglia and Metauro) close to a Natural Park (Monte San Bartolo, Central Adriatic Sea) has been analysed and related to several environmental parameters. In particular, changes in the meiofaunal structure, abundance and diversity have been investigated in order to evaluate possible variations of ecological quality status (EQS), in accordance with the Water Framework Directive. The structure of the meiofaunal assemblage appeared significantly different in the period studied, with a higher abundance of annelids in 2002 and an increase of nematodes in the following sampling campaigns. Among the faunal parameters, the Shannon and Pielou indices suggested a decline of the EQS over time, likely mainly due to the negative effect of chlorophyll-a peaks, which may highlight the influence of eutrophication phenomena and an enhancement of the organic matter supply. The lowering of the EQS of the study area suggests the need to intensify management and conservation efforts in the coastal systems, and supports the use of the meiofaunal assemblage as a useful bioindicator.

Keywords: meiofauna, Adriatic Sea, ecological quality assessment, eutrophication

Submitted 21 May 2014; accepted 4 August 2014; first published online 9 September 2014

INTRODUCTION

On the basis of recent European Directives, the indices which provide information on the functionality and resilience of an ecosystem have to be implemented. In particular, the Water Framework Directive (WFD, 2000/60/EC) has established the concept of ecological quality status (EQS) as a way to assess the biological quality of the waters (see for review Schratzberger, 2012). So far, the ecological status of a habitat has been evaluated by means of macrofauna (e.g. AMBI, BENTHIX, M-AMBI indices) (Borja *et al.*, 2000; Simboura & Zenetos, 2002; Borja *et al.*, 2008). This is because macrofauna are ecologically important, widespread and easily identifiable thanks to numerous taxonomic keys which are the result of a wide availability of data on macrofaunal spatial and temporal distribution (Vanaverbeke *et al.*, 2011). In contrast, few attempts to use meiobenthic assemblages (individual body size 45–500 μm) in EQS assessment have been carried out, in spite of the fact that meiofauna features are good indicators of environmental conditions, and changes in density, diversity, structure and functioning of the meiofauna assemblage may give information on ecosystem health (Moreno *et al.*, 2011; Alves *et al.*, 2013). The response of meiofaunal organisms are, in fact, complementary to those

observed in macrofauna (Semprucci *et al.*, 2013a). Meiofauna, due to their direct benthic development and generation times as short as 1 month, may reveal possible environmental effects over very small spatial and short temporal scales (Kennedy & Jacoby, 1999). Furthermore, meiofaunal assemblages are more sensitive to different types of environmental disturbances than macrofaunal ones are, so allowing their use as bioindicators (Balsamo *et al.*, 2012).

The coastal areas offer numerous resources including food and energy, they help to regulate the climate and they host commercial and recreational activities. However, an increasing number of disturbances (habitat destruction, over-exploitation of natural resources, loss of biodiversity and introduction of alien species) at both global and local scale may compromise the integrity and functioning of these habitats (Danovaro & Pusceddu, 2007).

The Adriatic Sea, due to its particular features, is strongly influenced by meteorological conditions and river run-off (Occhipinti-Ambrogi *et al.*, 2005; Balsamo *et al.*, 2010), and is therefore more sensitive to human impact and climatic variations than other areas of the Mediterranean Sea. This highlights the need for multi-annual data series assessing temporal changes in the meiofaunal assemblages, thus providing information on the EQS of the coastal areas and also a baseline for the effects of stress agents over time.

Data on the meiofaunal assemblages of two rivers (Foglia and Metauro), close to the Natural Park of Monte San Bartolo (Marches, Italy) have been collected by Semprucci *et al.* (2010a, 2013a) since 2002. In particular, changes in

Corresponding author:

F. Semprucci

Email: federica.semprucci@uniurb.it

the meiofaunal structure, abundance and diversity have been analysed in relation to several environmental parameters.

The aims of this study were to evaluate the EQS of the coastal areas in front of the two river mouths and their spatial and temporal variations through the analysis of the meiofauna assemblage, and to verify the importance of meiofauna as a bioindicator. Indeed, meiofauna have not yet been sufficiently considered in the current national and international programs for the monitoring of marine ecosystems despite the fact that they could be of great importance for this purpose.

MATERIALS AND METHODS

Study area and sampling sites

The north-western coast of the Adriatic Sea is a semi-enclosed and very shallow basin with peculiar characteristics: its coastline is extremely long and morphologically complex, so creating a high diversity of hydrodynamic and sedimentary habitats; moreover, it is strongly influenced by the River Po inputs that deeply affect the nutrient load and patterns of the sediment texture of the basin (De Wit & Bendoricchio, 2001; Wang & Pinardi, 2002). However, at a local scale, some small rivers may also have important effects on the coastal areas (Frontalini & Coccioni, 2008). Even if water circulation causes a surprisingly fast water turnover in the Adriatic Sea, bottom sediments store a significant amount of pollutants in some coastal areas, which might be partially released to the overlying waters or affect the benthic fauna (Frasconi *et al.*, 1988).

The investigated area is located in the Central Adriatic Sea, along the coast of the Natural Park of Monte San Bartolo (Marches) (Figure 1).

For this temporal comparison, data obtained from two previous investigations (Semprucci *et al.*, 2010a, 2013a), and

integrated with more recent information, were used. In detail, the periods analysed were: 21 May 2002, 8 June 2004 and 7 June 2012 (Semprucci *et al.*, 2010a, 2013a).

Sampling collection was carried out at the mouth of the two largest rivers of the area: Foglia (FO) and Metauro (ME). At each site, samples were collected from two distinct stations, one at 500 m from the coastline (Station 1, depth 5 m) and the second at 3000 m (Station 2, depth 12 m). During the sampling, depth, temperature, salinity, dissolved oxygen concentration (DO), pH, oxidation-reduction potential (ORP) and chlorophyll-*a* (Chl-*a*) content in the water column were measured using a multi-parametric probe (IDRONAUT). The sediment samples were collected by a modified Van Veen grab (20 l volume). This modified model permits the insertion of a Plexiglas corer from the central part of the grab, enabling portions of almost completely undisturbed sediment to be captured. For the study of meiofauna three replicate cores (surface area 6 cm² each) were taken down to the depth of 9 cm; samples were then treated with 7% MgCl₂ to promote tissue relaxation, and finally fixed with 4% neutralized formalin in seawater and stained with rose Bengal (0.2 g l⁻¹). Two aliquots of sediments were taken for the grain size and total organic matter (TOM) determinations, and were stored at -20°C.

Environmental parameters

The TOM content was determined gravimetrically after loss on ignition (Buchanan & Kain, 1971). The sediment samples were first dried at 60°C for 6 h and weighed in a Scaltec SBC21 (accuracy 0.1 mg) to obtain their dry weight. Then, they were calcined in a muffle furnace (550°C for 4 h) and weighed again to determine the ash weight (inorganic fraction). The organic fraction content was calculated by subtracting the ash weight from the total weight. Grain size analysis was performed by separating the coarse fraction (sand) from the mud fraction by washing. The sandy fraction was then determined by dry-sieving, while a sedigraph (Sedigraph 5200, Micrometric) was used for quantifying the mud fraction (<63 µm).

Meiofauna analysis

In laboratory, the samples were carefully washed through two nested mesh nets (500–45 µm). The first was used to exclude macrofauna, while the second one was essential to retain the meiofaunal organisms. The residual fraction obtained in this way was centrifuged three times with Ludox HS 30 colloidal silica (density 1.18 g cm⁻³) for specimen extraction (McIntyre & Warwick, 1984). Meiofauna were counted and identified at the major taxa level, and their density was standardized as abundance 10 cm⁻². Shannon's diversity (*H'*) and evenness (*J*) indices (both using log-base 2) were calculated to describe the meiofaunal assemblage structure.

Statistical analysis

Non-metric multi-dimensional scaling (nMDS) ordinations derived from Bray–Curtis similarity matrices were used to view differences in the structure of meiofaunal assemblage between stations, sites and years (on fourth root transformed data). A two-way nested ANOSIM (analysis of similarities) was used to assess the statistical significance of any difference

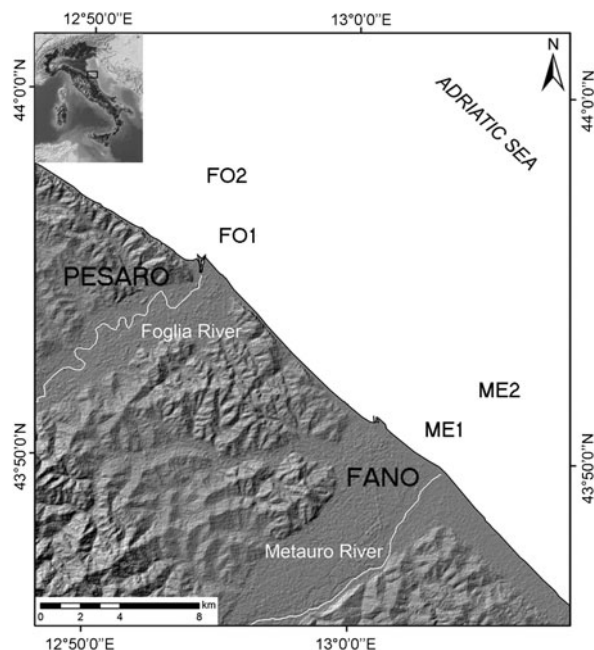


Fig. 1. Geographical map of the study area and location of the sampling stations.

between sites and stations. A two-crossed ANOSIM was used to determinate the possible site \times year and station \times year. The SIMPER test (cut-off of 90%, on fourth root transformed data) was utilized to determine the contribution of each meiofaunal taxon to the total dissimilarity. All of the analyses referred to above were performed using the software package Primer v.5 (Clarke & Gorley, 2001; Clarke & Warwick, 2001).

Analysis of covariance (ANCOVA) models were used in order to evaluate relationships between some abiotic parameters (considered as independent variables) and the basic faunal parameters (i.e. total meiofaunal abundance, S , H' , J , considered as dependent variables, in four different models). nMDS ordination with superimposed circles of increasing size representing the levels of the abiotic variables was used in order to visualize possible relationships between the abiotic variables and the meiofaunal assemblage structure.

RESULTS

Environmental parameters

The environmental parameters measured in the water column are summarized in Table 1. Temperatures showed ranges from 14.2°C (Station ME2, 2002) to 20.8°C (Stations FO1 and ME1, 2004). The salinity ranged from 31.2‰ (Stations FO1 and ME1, 2004) to 38.1‰ (Station ME2, 2012). DO ranged from 101.6% (Station FO2, 2002) to 112.5% (Station FO2, 2004). The pH was alkaline in all the stations, and ranged from 8.2 (Stations FO2 and ME2, 2002) to 8.5 (Stations FO1 and ME1, 2004). ORP showed the lowest value at Station FO2, 2012 (−43.0 mV) and the highest at Station ME2, 2002 (230.3 mV). Chl-*a* ranged from 0.4 $\mu\text{g l}^{-1}$ (Station FO1, 2012) to 6.3 $\mu\text{g l}^{-1}$ (Stations FO1 and ME1, 2004). In the study area the sediments may be classified as medium–fine sands. Gravel showed the highest percentage, 4.7%, at Station ME2, 2004 and the lowest (0%) at the Stations ME1, 2002, FO2, 2004 and FO2, 2012. Sand showed the highest value, 96.4%, at Station FO1, 2002, and the lowest, 10.4%, at Station ME2, 2002. Mud ranged from 3.6% (Station FO1, 2002) to 87.6% (Station FO2, 2002). The TOM content ranged from 17.9 mg g^{-1} (Station ME1, 2002) to 84.8 mg g^{-1} (Station ME2, 2004) (Table 1).

Meiofaunal parameters

Meiofaunal assemblage was overall represented by 16 major taxa (richness, S): nine found in 2002, 12 in 2004 and 13 in 2012. The number of major taxa ranged from four (Station FO2, 2012) to 11 (Station ME1, 2012) (Appendix A, Supplementary material, Figure 2A). An overall lower number of taxa was detected in the 2002 than in the 2004 and 2012, while the meiofauna at ME resulted richer than at FO (Figure 2A).

The J index varied from 0.0 (Station FO2, 2012) to 0.5 (Station ME1, 2002), while the H' index ranged between 0.0 (Station FO2, 2012) and 1.4 (ME1, 2002) (Figure 2B, C). Both indices showed on the whole higher values at ME than at FO, and lower values from 2004 to 2012 (Figure 2B, C).

The average meiofaunal abundances varied between 210.0 \pm 54.9 and 4025.6 \pm 1713.9 ind. 10 cm^{-2} (Station ME1 both in 2002 and 2012) at Station 1, and between 175.5 \pm 108.3 and 6934.7 \pm 1907.4 ind. 10 cm^{-2} (Station ME2, in 2002 and

2012, respectively) at Station 2 (Figure 3). On average, the highest values were recorded offshore (2381.0 \pm 862.7 ind. 10 cm^{-2}); while the lowest ones were recorded at Station 1 (1453.5 \pm 683.8 ind. 10 cm^{-2}). An overall increase in meiofaunal abundance was observed over the 10 years at both depths (Appendix A, Supplementary material).

Nematodes were the dominant taxonomic group at both depths (92.1 and 91.6% of the total meiofauna at Stations 1 and 2, respectively). At Station 1, nematodes were followed in abundance by polychaetes (2.9%), platyhelminthes (1.3%) and oligochaetes (1.1%). Offshore the other dominant taxa were copepods (4.0%), polychaetes (1.2%) and platyhelminthes (1.1%). All of the other taxa of meiofauna (gastrotrichs, halacarida, mollusks, rotifers, ostracods, amphipods, isopods, cumaceans, nemertines, kinorhynchans and archiannelids) counted for less than 1% of the total assemblage (Appendix A, Supplementary material).

Statistical analysis

Two-way nested ANOSIM did not reveal significant differences of the structure of the meiofaunal assemblage in the site \times station interaction ($P > 0.05$). In contrast, significant differences were detected both in the station \times year and site \times year interactions (two-way crossed ANOSIM) (Table 2), a trend partially visible also in the plots obtained by nMDS (Figure 4). However, the highest differences in the meiofaunal structure were detected in the station \times year interaction. In particular, pair-wise comparisons highlighted greater differences in 2002 in comparison with 2004–2012. SIMPER test (cut-off 90%) revealed that the differences in 2002 were due to the higher abundances of annelids (both oligochaetes and polychaetes), and the lower abundances mainly of nematodes and only secondly of copepods (on the base of the SIMPER results copepods represented a quarter of nematode abundances in this temporal range).

The differences between the sites were less significant and mainly related to the higher abundance of all the meiofaunal taxa in the ME river, with the only exception of the oligochaetes, more abundant in the FO river (SIMPER, 90%). Ostracods, oligochaetes, gastrotrichs, platyhelminthes and bivalves were more abundant at the coastal stations, whereas all the others taxa increased offshore (SIMPER, 90%).

Among the abiotic variables considered to explain the structure changes of the meiofaunal assemblage, only the TOM content seemed to show temporal variations (Figure 5f, Supplementary material). An overall higher mud and gravel percentages were detected at ME (Figure 5g, i, Supplementary material), while TOM and sand appeared more abundant at FO (Figure 5f, h, Supplementary material).

In spite of the significance of the ANCOVA model on the total meiofaunal abundance ($F = 10.8$; $P < 0.001$) and S ($F = 6.31$; $P < 0.001$), none of the predictor parameters showed a significant association. Instead, predictor variables explaining the significant differences in the J value ($F = 8.68$; $P < 0.001$) were Chl-*a* ($P < 0.001$), salinity ($P < 0.01$) and the distance from the coast ($P < 0.01$), followed by TOM, gravel percentage, ORP, pH and the year ($P < 0.05$), all parameters negatively related to the J index. The H' index showed a similar trend ($F = 7.68$; $P < 0.001$), but the independent significant variables in this case were only salinity, Chl-*a* and the distance from the coastline ($P < 0.05$), all showing a negative association.

Table 1. Environmental parameters measured in the study area.

Parameters	FO1, 2002	FO2, 2002	ME1, 2002	ME2, 2002	FO1, 2004	FO2, 2004	ME1, 2004	ME2, 2004	FO1, 2012	FO2, 2012	ME1, 2012	ME2, 2012
Temperature (°C)	19.2	14.7	19.2	14.2	20.8	18.5	20.8	18.9	18.3	16.0	18.7	15.3
Salinity (‰)	34.6	36.3	34.8	36.6	31.2	34.3	31.2	33.5	36.5	37.5	36.3	38.1
Dissolved oxygen (%)	102.4	101.6	110.0	111.0	107.5	112.5	107.5	110.8	102.0	105.3	103.5	109.1
pH	8.4	8.2	8.4	8.2	8.5	8.4	8.5	8.4	8.4	8.3	8.4	8.4
Redox potential (mV)	218.4	225.8	220.1	230.3	-19.4	-27.8	-19.4	-27.8	-31.3	-43.0	-28.1	-13.0
Chlorophyll- <i>a</i> (µg l ⁻¹)	1.5	2.9	1.3	3.1	6.3	3.3	6.3	2.6	0.4	1.9	0.5	1.1
Gravel (%)	0.1	0.2	0.0	2.1	0.5	0.0	0.2	4.7	0.5	0.0	1.5	3.9
Sand (%)	96.4	32.6	93.5	10.4	86.2	60.6	89.4	26.3	85.3	12.4	90.9	33.1
Mud (%)	3.6	63.6	6.6	87.5	13.3	39.4	10.4	69.0	14.1	87.6	7.5	63.0
Total organic matter (mg g ⁻¹)	24.6	53.7	17.9	50.3	21.4	58.1	22.2	84.8	22.1	75.3	20.2	38.0

DISCUSSION

The overall biodiversity, especially of macrofauna, of the Adriatic Sea does not seem to have suffered a substantial decrease during recent years. That seems mainly due to the introduction of alien species, which has masked the overall

species loss, but has also altered the balance between the various trophic levels and the functioning of the whole marine ecosystem (e.g. Occhipinti-Ambrogi, 2002, 2007; Giani *et al.*, 2012).

If it is relatively easy to document a change of the macrofaunal assemblage over time, it is more difficult to do the same for the meiofauna. That is because macrofauna organisms have been studied for a long time, so that a greater amount of data on their spatial and temporal variations is available (e.g. Vanaverbeke *et al.*, 2011; Semprucci, 2013). However, given the crucial role of meiofauna in the marine ecosystems, temporal series of data on this benthic component are fundamental and may give new insights on the health status of the Adriatic Sea (Balsamo *et al.*, 2010).

According to the most recent classifications of the ecological quality (Danovaro *et al.*, 2004; Pusceddu *et al.*, 2007; Moreno *et al.*, 2008), the data analysed in the present study suggests from Sufficient (2002) to Good (2004–2012) conditions of the studied ecosystems. However, the richness parameter takes into account only the number of taxa and not their relative abundances, which could be a basic limit for a faunal descriptor. Instead, the diversity indices (namely H' and J) may be more informative and efficient for summarizing the composition and structure of the benthic assemblages, which is really a crucial point for using meiofauna in accordance with WFD. The Directive, in fact, highlights the necessity to create and/or to calibrate community-based indices, which can be more efficiently applied to identify the EQS class of an ecosystem. In this study, the trend of H' and J appeared completely opposite to the richness one, showing a significant change of the assemblage structure over the decade. In detail, the 2002 campaign was characterized by assemblages less dominated by nematodes, as the increase of H' and especially of J values clearly showed. Among the taxa characterizing this period were essentially annelids (namely polychaetes and oligochaetes), a generally not abundant meiofaunal component considered as tolerant (Netto *et al.*, 1999; Vezzulli *et al.*, 2003; Pusceddu *et al.*, 2007; Losi *et al.*, 2012). This last hypothesis was confirmed by a detailed study of the macrofaunal assemblage, dominated in 2002 by *Owenia fusiformis* (Frontalini *et al.*, 2011), a species considered to be resistant to stress and typical of shallow waters with well-sorted sandy substrates (Pères & Picard, 1964; Simboura & Zenetos, 2002). However, due to the highest levels of H' and J , the overall EQS in 2002 can be considered Good.

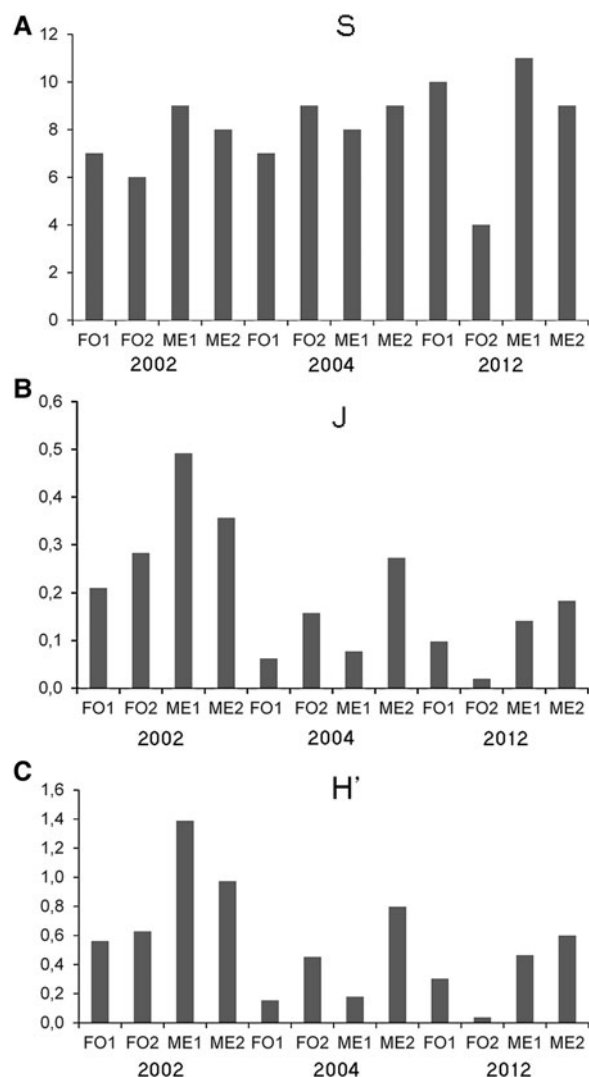


Fig. 2. Richness of taxa (A), Pielou index (B) and Shannon index (C) of the meiofaunal assemblages from the studied locations.

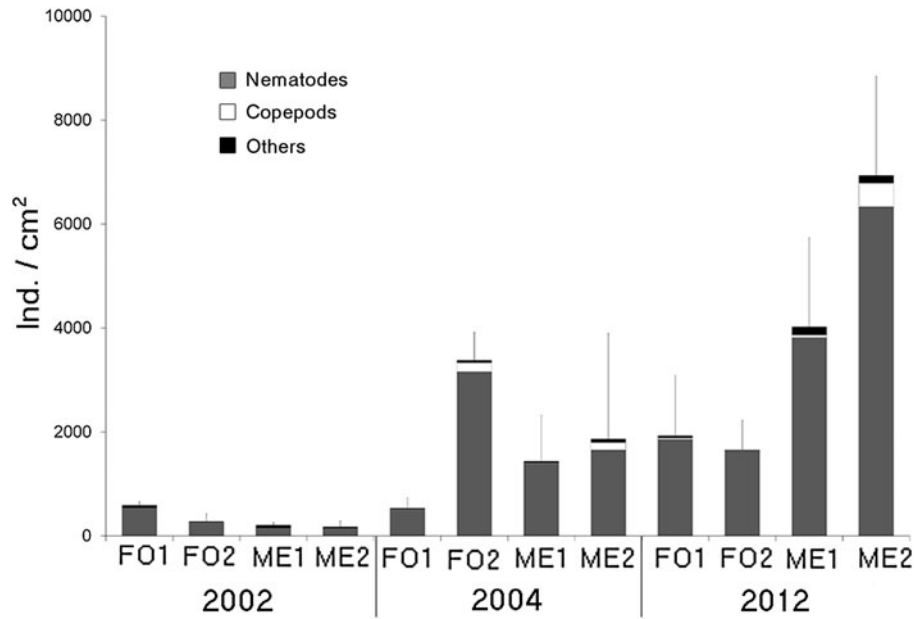


Fig. 3. Meiofaunal abundance values at the study area during the 2002–2012 decade.

On the contrary, the most abundant meiofaunal groups in 2004 and 2012 were nematodes followed by copepods. These two taxa show very different auto-ecological features (Raffaelli & Mason, 1981). Nematodes are well known to increase in terms of abundance with the enhancement of the sediment fine fraction and the organic content (Steyaert *et al.*, 1999, 2003, 2005; Vanaverbeke *et al.*, 2002; Semprucci *et al.*, 2010a, b). Instead, copepods, which generally are more abundant in coarse and well oxygenated sediments (Wetzel *et al.*, 2001; Semprucci *et al.*, 2010b; De Troch *et al.*, 2013), generally show a high sensitivity to environmental perturbations (Raffaelli, 1987; Coull & Chandler, 1992; Lee *et al.*, 2001; McLachlan & Brown, 2006; Ansari *et al.*, 2013). The usefulness of copepods as bioindicators of the human disturbance from riverine inflows has been previously documented by Semprucci *et al.* (2010a). In this respect, also the general increase of copepods offshore, otherwise a less suitable microhabitat for its physical–chemical features, could suggest a greater human impact on these coasts (Semprucci *et al.*, 2010a).

The increasing dominance of nematodes led also to a significant decrease of the overall meiofaunal diversity. The reduction of this faunal descriptor in response to anthropogenic disturbance has already been documented by several authors (e.g. Coull & Chandler, 1992; Kennedy & Jacoby, 1999; Mirto *et al.*, 2000; Vezzulli *et al.*, 2003; Semprucci

et al., 2010a), and accordingly a decline of the EQS of the area may be inferred.

It is often difficult to establish which may be the causes of the temporal variations of the benthic assemblages because changes may be due to both human impact and natural factors. However, among the environmental parameters used as predictor variables (see ANCOVA results), Chl-*a* and TOM appeared to negatively affect the *J* and *H'* indices. In particular, Chl-*a* can be an indicator of a trophic resource of high quality, the sedimentation of which may represent an important resource for benthic assemblages (Boon & Duineveld, 1998). However, the enhancement of nutrients such as phosphorus and nitrogen may also lead to an excessive increase of the microalgal bloom, with a consequent increase of the amount of Chl-*a* to the point of the occurrence of intense eutrophication events, as documented in the study area by Penna *et al.* (2004). Eutrophication phenomena may cause also hypoxia or anoxia in the bottom (Penna *et al.*, 2004), and establish an assemblage mainly represented by opportunistic species (Vanaverbeke *et al.*, 2004a, b; Carriço *et al.*, 2013). In line with this hypothesis there is a significant negative relation with *J* and Chl-*a* and TOM.

Phytoplankton blooms, which are often associated with the discharges of the Adriatic rivers, are usually more significant in the spring and autumn periods (Giani *et al.*, 2012), and have been revealed to have a strong influence on the

Table 2. Results of the ANOSIM carried out on the structure of the meiofaunal assemblage (st., stations; si., sites; ye., years).

	st.	si.	ye.	Pair-wise comparisons
2-way nested ANOSIM st. within si.	$P > 0.05$	$P > 0.05$	–	
2-way crossed ANOSIM st. × ye.	$R = 0.41; P = 0.001$	–	$R = 0.67; P = 0.001$	2002 vs 2004 $R = 0.85; P = 0.001$ 2002 vs 2012 $R = 0.76; P = 0.001$ 2012 vs 2004 $R = 0.47; P = 0.002$
2-way crossed ANOSIM si. × ye.	–	$R = 0.29; P = 0.003$	$R = 0.56; P = 0.001$	2002 vs 2004 $R = 0.64; P = 0.001$ 2002 vs 2012 $R = 0.77; P = 0.001$ 2012 vs 2004 $R = 0.23; P = 0.023$

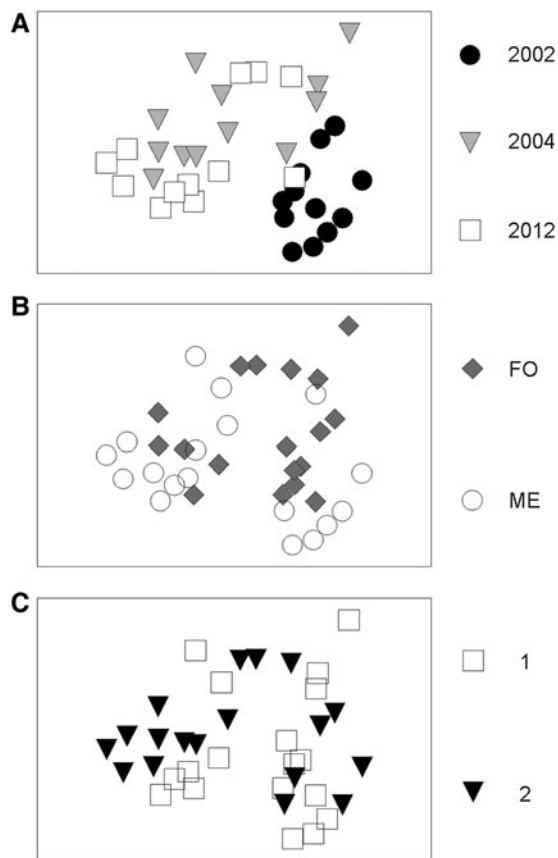


Fig. 4. nMDS plot of meiofaunal assemblages. The labels represent the year (A), site (B) station factors (C).

meiobenthic and nematode assemblages (Semprucci *et al.*, 2010a, 2013b). It is noteworthy that the temporal range analysed by ANCOVA did not reveal a negative impact of the low salinity, and therefore, arguably, of the local riverine inputs, on the composition of meiofaunal biodiversity. However, if the sampling campaigns are considered one by one, it clearly appears that the lowest salinity values in 2004 were associated with the highest peaks of Chl-*a* and very low values of the *J* and *H'* indices (Figure 5e, Supplementary material). Accordingly, even if a direct and unequivocal association between local riverine discharges, eutrophication phenomena, organic supply and decrease of biodiversity cannot be proven beyond all doubt, an important role for these factors in structuring the meiofaunal assemblages may be inferred.

The higher percentage of gravel in the offshore sediments can be explained by the effect of storm events or other intense hydrodynamic conditions that deposit coarse material far from the coastline, and which regular wave action is not able to rework (Semprucci *et al.*, 2010a). The negative association of gravel and meiofaunal diversity was unexpected, because the presence of this large fraction of sediment generally confers a greater heterogeneity in their micro-habitat (Raes & Vanreusel, 2006; Semprucci *et al.*, 2010a). However, the high presence of silt and clay offshore reduced drastically the permeability of sediments, leading to OM accumulation and possible oxygen deficiency in the sediment (Albertelli *et al.*, 1999; Covazzi-Harriague *et al.*, 2006; Frontalini *et al.*, 2011; Semprucci *et al.*, 2013a). The observation of black

spots in the cores taken as well as the increase of TOM at Station 2 could confirm this hypothesis.

The differences between the FO and ME rivers were not strong. However, the overall higher levels of all the faunal descriptors (namely richness, *H'* and *J* indices) at ME suggested a better ecological quality of this river, in agreement with previous studies (Semprucci *et al.*, 2010a, 2013a; Frontalini *et al.*, 2011).

Methods to assess EQS of the coastal ecosystems in accordance with the WFD using meiofauna have already been suggested by Moreno *et al.* (2011), but this classification was based on the use of faunal parameters at genus level (namely nematode genera). The opportunity to obtain a good response of meiofauna, also at a high taxonomic level, to human disturbance, may represent a great advantage because its analysis is not excessively time consuming and does not require taxonomic experts (Kennedy & Jacoby, 1999; Balsamo *et al.*, 2010).

This study confirms that the analysis of meiofauna can give a valuable contribution to the knowledge about coastal ecosystems, and, especially if additional meiofaunal descriptors of EQS are introduced, it may become a basic, integrative tool for the routine protocols currently applied to macrofaunal assemblages for investigating and monitoring marine ecosystems.

Supplementary materials and methods

The supplementary material referred to in this paper can be found online at journals.cambridge.org/mbi.

REFERENCES

- Albertelli G., Covazzi-Harriague A., Danovaro R., Fabiano M. and Fraschetti S. (1999) Differential responses of bacteria, meiofauna and macrofauna in a shelf area (Ligurian Sea, NW Mediterranean): role of food availability. *Journal of Sea Research* 42, 11–26.
- Alves A.S., Adão H., Ferrero T.J., Marques J.C., Costa M.J. and Patrício J. (2013) Benthic meiofauna as indicator of ecological changes in estuarine ecosystems: the use of nematodes in ecological quality assessment. *Ecological Indicators* 24, 462–475.
- Ansari K.G.M.T., Lyla P.S., Ajmal Khan S., Manokaran S. and Raja S. (2013) Community structure of harpacticoid copepods from the southeast continental shelf of India. *Proceedings of the International Academy of Ecology and Environmental Sciences* 3, 87–100.
- Balsamo M., Albertelli G., Ceccherelli V.U., Coccioni R., Colangelo M.A., Curini-Galletti M., Danovaro R., D'Addabbo R., Leonardis C., Fabiano M., Frontalini F., Gallo M., Gambi C., Guidi L., Moreno M., Pusceddu A., Sandulli R., Semprucci F., Todaro M.A. and Tongiorgi P. (2010) Meiofauna of the Adriatic Sea: current state of knowledge and future perspectives. *Chemistry and Ecology* 26, 45–63.
- Balsamo M., Semprucci F., Frontalini F. and Coccioni R. (2012) Meiofauna as a tool for marine ecosystem biomonitoring. In Cruzado A. (ed.) *Marine ecosystems*. Rijeka, Croatia: InTech Publisher, pp. 77–104.
- Boon A.R. and Duineveld G.C.A. (1998) Chlorophyll a as a marker for bioturbation and carbon flux in Southern and Central North Sea sediments. *Marine Ecology Progress Series* 162, 33–43.

- Borja Á., Franco J. and Pérez V.** (2000) A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. *Marine Pollution Bulletin* 12, 1100–1114.
- Borja Á., Mader J., Muxika I., Germán Rodríguez J. and Bald J.** (2008) Using M-AMBI in assessing benthic quality within the Water Framework Directive: some remarks and recommendations. *Marine Pollution Bulletin* 56, 1377–1379.
- Buchanan J.B. and Kain J.M.** (1971) Measurement of the physical and chemical environment. In Holme N.A. and McIntyre A.D. (eds) *Methods for the study of marine benthos*. Oxford: Blackwell Scientific Publication, pp. 30–52.
- Carriço R., Zeppilli D., Quillien N. and Grall J.** (2013) Can meiofauna be a good biological indicator of the impacts of eutrophication caused by green macroalgal blooms? *Anaod-les cahiers naturalistes de l'Observatoire Marin* 2, 9–16.
- Clarke K.R. and Gorley R.N.** (2001) *Primer Version 5*. Plymouth: Primer-E.
- Clarke K.R. and Warwick R.M.** (2001) *Change in marine communities: an approach to statistical analysis and interpretation*. 2nd edition. Plymouth: Primer-E.
- Coull B.C. and Chandler G.T.** (1992) Pollution and meiofauna: field, laboratory and mesocosm studies. *Oceanography and Marine Biology: an Annual Review* 30, 191–271.
- Covazzi-Harriague A., Gaozza L., Montella A. and Misis C.** (2006) Benthic communities on a sandy Ligurian beach (NW Mediterranean). *Hydrobiologia* 571, 383–394.
- Danovaro R., Dell'Anno A. and Pusceddu A.** (2004) Biodiversity response to climate change in a warm deep sea. *Ecology Letters* 7, 821–828.
- Danovaro R. and Pusceddu A.** (2007) Ecomanagement of biodiversity and ecosystem functioning in the Mediterranean Sea: concerns and strategies. *Chemistry and Ecology* 23, 347–360.
- De Troch M., Roelofs M., Riedel B. and Grego M.** (2013) Structural and functional responses of harpacticoid copepods to anoxia in the Northern Adriatic: an experimental approach. *Biogeosciences* 10, 4259–4272.
- De Wit M.J.M. and Bendoricchio G.** (2001) Nutrient fluxes in the Po basin. *Science of the Total Environment* 273, 147–161.
- Frasconi F., Frignani M., Guerzoni S. and Ravaoli M.** (1988) Sediments and pollution in the Northern Adriatic Sea. Living in a chemical world. *Annals of the New York Academy of Sciences* 534, 1000–1020.
- Frontalini F. and Coccioni R.** (2008) Benthic foraminifera for heavy metal pollution monitoring: a case study from the central Adriatic Sea coast of Italy. *Estuarine, Coastal and Shelf Science* 76, 404–417.
- Frontalini F., Semprucci F., Coccioni R., Balsamo M., Bittoni P. and 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.
- Giani M., Djakovac T., Degobbi D., Cozzi S., Solidoro C. and Fonda Umani S.** (2012) Recent changes in the marine ecosystems of the northern Adriatic Sea. *Estuarine, Coastal and Shelf Science* 115, 1–13.
- Kennedy A.D. and Jacoby C.A.** (1999) Biological indicators of marine environmental health: meiofauna a neglected benthic component? *Environmental Monitoring and Assessment* 54, 47–68.
- Lee M.R., Correa J.A. and Castilla J.C.** (2001) An assessment of the potential use of the nematode to copepod ratio in the monitoring of metals pollution. The Chañaral Case. *Marine Pollution Bulletin* 42, 696–701.
- Losi V., Montefalcone M., Moreno M., Giovannetti E., Gaozza L., Grondona M. and Albertelli G.** (2012) Nematodes as indicators of environmental quality in seagrass (*Posidonia oceanica*) meadows of the NW Mediterranean Sea. *Advances in Oceanography and Limnology* 3, 69–91.
- McIntyre A. D. and Warwick R.M.** (1984) Meiofauna techniques. In Holme N.A. and McIntyre A.D. (eds) *Methods for the study of marine benthos*. Oxford: Blackwell, pp. 217–244.
- McLachlan A. and Brown A.C.** (2006) *The ecology of sandy shores*. 2nd edition. Amsterdam: Elsevier Science.
- Mirto S., La Rosa T., Danovaro R. and Mazzola A.** (2000) Microbial and meiofaunal response to intensive Mussel-Farm biodeposition in coastal sediments of the western Mediterranean. *Marine Pollution Bulletin* 40, 244–252.
- Moreno M., Ferrero T.J., Gallizia I., Vezzulli L., Albertelli G. and Fabiano M.** (2008) 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., Semprucci F., Vezzulli L., Balsamo M., Fabiano M. and Albertelli G.** (2011) The use of nematodes in assessing ecological quality status in the Mediterranean coastal ecosystems. *Ecological Indicators* 11, 328–336.
- Netto S.A., Warwick R.M. and Attrill M.J.** (1999) Meiobenthic and macrobenthic community structure in carbonate sediments of 732 Rocas Atoll (North-east, Brazil). *Estuarine, Coastal and Shelf Science* 48, 39–50.
- Occhipinti-Ambrogi A.** (2002) Current status of aquatic introductions in Italy. In Leppakoski E., Gollasch S. and Olenin S. (eds) *Invasive Aquatic species of Europe. Distribution impacts and management*. Dordrecht, Boston, London: Kluwer Academic Publishers, pp. 311–324.
- Occhipinti-Ambrogi A.** (2007) Global change and marine communities: alien species and climate change. *Marine Pollution Bulletin* 55, 342–352.
- Occhipinti-Ambrogi A., Savini D. and Forni G.** (2005) Macrobenthos community structural changes off Cesenatico coast (Emilia Romagna, Northern Adriatic), a six-year monitoring programme. *Science of the Total Environment* 353, 317–328.
- Penna N., Cappellacci S. and Ricci F.** (2004) The influence of the Po River discharge on phytoplankton bloom dynamics along the coastline of Pesaro (Italy) in the Adriatic Sea. *Marine Pollution Bulletin* 48, 321–326.
- Péres J.M. and Picard J.** (1964) Nouveau manuel de bionomie benthique de la Méditerranée. *Recueil des travaux de la Station marine d'Endoume* 31, 1–37.
- Pusceddu A., Gambi C., Manini E. and Danovaro R.** (2007) Trophic state, ecosystem efficiency and biodiversity of transitional aquatic ecosystems: analysis of environmental quality based on different benthic indicators. *Chemistry and Ecology* 23, 505–515.
- Raes M. and Vanreusel A.** (2006) Microhabitat types determine the composition of nematode communities associated with sediment-clogged cold-water coral framework in the Porcupine Seabight (NE Atlantic). *Deep-Sea Research* 53, 1880–1894.
- Raffaelli D.G.** (1987) The behaviour of the nematode/copepod ratio in organic pollution studies. *Marine Environmental Research* 23, 135–152.

- Raffaelli D.G. and Mason D.F.** (1981) Pollution monitoring with meiofauna, using the ratio of nematodes to copepods. *Marine Pollution Bulletin* 12, 158–163.
- Schratzberger M.** (2012) On the relevance of meiobenthic research for policymakers. *Marine Pollution Bulletin* 64, 2639–2644.
- Semprucci F.** (2013) Marine nematodes from the shallow subtidal coast of the Adriatic Sea: species list and distribution. *International Journal of Biodiversity* 1, 1–9.
- Semprucci F., Boi P., Manti A., Covazzi Harriague A., Rocchi M., Colantoni P., Papa S. and Balsamo M.** (2010a) Benthic communities along a littoral of the Central Adriatic Sea (Italy). *Helgoland Marine Research* 64, 101–115.
- Semprucci F., Colantoni P., Baldelli G., Rocchi M. and Balsamo M.** (2010b) The distribution of meiofauna on back-reef sandy platforms in the Maldives (Indian Ocean). *Marine Ecology: an Evolutionary Perspective* 31, 592–607.
- Semprucci F., Frontalini F., Covazzi-Harriague A., Coccioni R. and Balsamo M.** (2013a) Meio- and Macrofauna in the marine area of the Monte St. Bartolo Natural Park (Central Adriatic Sea, Italy). *Scientia Marina* 77, 189–199.
- Semprucci F., Moreno M., Sbrocca S., Rocchi M., Albertelli G. and Balsamo M.** (2013b) The nematode assemblage as a tool for the assessment of marine ecological quality status: a case-study in the Central Adriatic Sea. *Mediterranean Marine Science* 14, 48–57.
- Simboura N. and Zenetos A.** (2002) Benthic indicators to use in ecological quality classification of Mediterranean soft bottoms marine ecosystems, including a new biotic index. *Mediterranean Marine Science* 3/2, 77–111.
- Steyaert M., Garner N., Gansbeke D. and Vincx M.** (1999) Nematode communities from the North Sea: environmental controls on species diversity and vertical distribution within the sediment. *Journal of the Marine Biological Association of the United Kingdom* 79, 253–264.
- Steyaert M., Moodley L., Vanaverbeke J., Vandewiele S. and Vincx M.** (2005) Laboratory experiments on the infaunal activity of intertidal nematodes. *Hydrobiologia* 540, 217–223.
- Steyaert M., Vanaverbeke J., Vanreusel A., Barranguet C., Lucas C. and Vincx M.** (2003) The importance of fine-scale, vertical profiles in characterizing nematode community structure. *Estuarine, Coastal and Shelf Science* 58, 353–366.
- Vanaverbeke J., Gheskiere T., Steyaert M. and Vincx M.** (2002) Nematode assemblages from subtidal sandbanks in the Southern Bight of the North Sea: effect of small sedimentological differences. *Journal of Sea Research* 48, 197–207.
- Vanaverbeke J., Merckx B., Degraer S. and Vincx M.** (2011) Sediment-related distribution patterns of nematodes and macrofauna: two sides of the benthic coin? *Marine Environmental Research* 71, 31–40.
- Vanaverbeke J., Soetaert K. and Vincx M.** (2004a) Changes in morphometric characteristics of nematode communities during a spring phytoplankton bloom deposition. *Marine Ecology Progress Series* 273, 139–146.
- Vanaverbeke J., Steyaert M., Soetaert K., Rousseau V., Van Gansbeke D., Parent J.Y. and Vincx M.** (2004b) Changes in structural and functional diversity of nematode communities during a spring phytoplankton bloom in the southern North Sea. *Journal of Sea Research* 52, 281–292.
- Vezzulli L., Marralle D., Moreno M. and 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.
- Wang X.H. and Pinardi N.** (2002) Modeling the dynamics of sediment transport and resuspension in the northern Adriatic Sea. *Journal of Geophysical Research* 107, 3225.
- and
- Wetzel M.A., Fleeger J.W. and Powers S.P.** (2001) Effects of hypoxia and anoxia on meiofauna: a review with new data from the Gulf of Mexico. In Rabalais N.N. and Turner R.E. (eds) *Coastal hypoxia: consequences for living resources and ecosystems. Coastal and estuarine studies*. Volume 58. Washington, DC: American Geophysical Union, pp. 165–184.

Correspondence should be addressed to:

F. Semprucci
 Dipartimento di Scienze della Terra, della Vita e dell'Ambiente (DiSTeVA), Università di Urbino, loc. Crocicchia, 61029 Urbino, Italy
 email: federica.semprucci@uniurb.it