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RESEARCH ARTICLE

Benthic foraminifera for environmental monitoring: a case study in the central Adriatic continental shelf

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Abstract A study of benthic foraminifera was carried out in sediment samples collected from the central Adriatic coast of Italy, near the Ancona harbour and the Falconara Marittima oil refinery, in order to validate and support their use as bioindicators of ecosystem quality. On the basis of a principal component analysis (PCA), three biotopes (following the bathymetric gradient) have been documented, showing that the distribution pattern of benthic foraminifera is principally related to riverine inputs, organic matter contents at the seafloor, and sediment grain size. We observed higher abundances of opportunistic, low-oxygen tolerant taxa along the coastline, thus being representative of polluted environmental conditions. Near the Falconara Marittima oil refinery, the microfaunal assemblages is characterized by the absence of living specimens and by a low diversity associated with the dominance of opportunistic species. At this site, aberrant tests were also found. The data point out that *Ammonia parkinsoniana* and Quinqueloculina seem to be the most sensitive taxa and can be considered as good bioindicators of environmental stress in this area. This study confirms that faunal composition and morphology of benthic foraminifera respond to human-induced environmental perturbations, making their study potentially useful for biomonitoring in coastal-marine areas.

Keywords Adriatic Sea · Benthic foraminifera · Trace elements · Marine pollution · Aberrant tests · Water Framework Directive

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Introduction

In 2000, the EU's Member States, Norway, and the European Commission agreed on the Water Framework Directive (WFD 2000) with the aims to better manage, preserve, and protect European water environments. This directive established a framework for long-term protection of all water resources (i.e., rivers, lakes, and coastal waters) and was later complemented by the Marine Strategy Framework Directive (MSFD 2008).

In the framework of these directives, the scientific community is involved on monitoring the status of coastal-marine environments, and in particular, on describing the impact of pollutants on living organisms by selecting sensitive key groups or species (Borja et al. 2009) and by utilizing biological indicators (bioindicators). Benthic foraminifera (protist), marine unicellular organisms with a calcareous or agglutinated shell, are among the most abundant microorganisms found in the surface sediments in shallow and marginal-marine environments; they are very sensitive to changing environmental conditions, thus potentially providing information on the quality of the ecosystem where they live (Schönfeld et al. 2012). Recently, Barras et al. (2014) developed a foraminiferal index of ecosystem quality for the coastal Mediterranean Sea, based on different faunal parameters, to be tested at other stations in the contest of the MSFD.

Previous work largely demonstrates that foraminifera provide one of the most sensitive and inexpensive bioindicator of the deterioration of coastal environment. They present several advantages in comparison to the more commonly used macrofaunal organisms (e.g., Alve 1995; Mojtahid et al. 2006; Bouchet et al. 2007; Alve et al. 2009; Jorissen et al. 2009). They can be easily collected in great numbers, providing a highly reliable database for statistical analysis, even when small sample volumes are available. In addition, they are characterized by fast turnover rates; different species require species-specific habitats and can be maintained in culture, which allows determining the responses of individual taxa to selected pollutants or stresses (e.g., de Nooijer et al. 2007; Munsel et al. 2010).

Benthic foraminifera have been successfully used as proxies for studying the impact of different kinds of pollutants such as heavy metals and hydrocarbons, in a wide range of marine and transitional marine environments (for reviews, see Alve 1991, 1995; Yanko et al. 1994, 1999; Martin 2000; Martínez-Colón et al. 2009; Armynot du Châtelet and Debenay 2010; Frontalini and Coccioni 2011). These investigations documented that they generally respond to adverse ecological conditions by adopting different strategies: decrease of faunal density and diversity, local disappearance, increase of opportunistic taxa, development of test abnormalities, among others (e.g., Alve 1991; Yanko et al. 1998; Geslin et al. 1998; Ferraro et al. 2006; Di Leonardo et al. 2007; Cherchi et al. 2009; Martins et al. 2010; Armynot du Châtelet and Debenay 2010; Frontalini and Coccioni 2011). Furthermore, polluted areas are often naturally stressed as well, thus making it difficult to separate the respective influence of natural and anthropogenic stresses, since they are both reflected in assemblage composition and faunal parameters.

Using an integrated approach (micropaleontological and geochemical analyses), this study aims at investigating the relationship between benthic foraminiferal assemblages and environmental conditions in the central Adriatic Sea. Previous studies conducted near this area (Frontalini and Coccioni 2008, 2011; Coccioni et al. 2003, 2005) provided information on the possible influence of heavy metals on the taxonomic composition of the benthic foraminiferal microfauna. Here, we focus on (i) assemblages and biotopes and their spatial distribution within the study area and (ii) the relationship between environmental factors and pollution and benthic foraminiferal distribution and characteristics.

Study area

Geographical and environmental setting

The investigated area is located in the central-northern part of the Marche region (Italy), and extends from Falconara to the Musone river estuary (south of Ancona) along the Adriatic Sea coast (Fig. 1).

The Adriatic Sea is an elongated NW/SE-oriented basin, with a low axial topographic gradient in the north and a narrower steeper shelf further south. The late Quaternary sedimentary setting is the result of significant and recurring changes in response to global sea level excursions driven by glacial-interglacial cycles (Cattaneo et al. 2007).

Sedimentation along the central Adriatic coastline of Italy results from a series of small, distributed fluvial sources (i.e., a line source), draining the Apennine Mountains. These rivers deliver ~3×10^7 t/year of sediment, contributing to the formation of a shore-parallel shelf clinoform that has developed throughout the Holocene (Palinkas and Nittrouer 2006).

Previous estimates of sediment accumulation in this area have indicated that rates increase southward along-shelf, reaching a maximum >1 cm/year near the Gargano Peninsula (Cattaneo et al. 2003; Frignani et al. 2005). Across-shelf rates are generally highest on the foreset of the clinoform but can vary depending on local morphologic features (e.g., seafloor crenulations, where accumulation rates range from 0.4 to 1.6 cm/year on the dipping and flat surfaces, respectively; Correggiari et al. 2001). Sediment deposition is affected by the circulation patterns characterized by a cyclonic gyre, driven by thermohaline circulation, and by a high seasonal variability (Artegiani et al. 1997).

The fine materials carried to the sea by the rivers accumulate along the coast in belts that are hydraulically sorted in grain size, in accordance with the classic model of modern sedimentation on continental shelves: coastal sands, mud, and shelf relict sand further offshore (Frignani et al. 2005 and references therein).

The sampled stations mainly consist of silty sands in the coastal area and a mud belt moving seawards (Colantoni et al. 2003); the sediment accumulation rates in this area range between 0.01 and 0.62 cm/year (Orsini 2006).

Although the area is characterized by low primary productivity (Zavatarelli et al. 2000), the local contribution of Apennine rivers and nutrient-rich shore current coming from the northern basin sustain nutrient availability (Artegiani et al. 1997). The organic carbon content in surface sediments spans from 0.5 wt.% in coastal area to 0.8 wt.% in the offshore stations (Tesi et al. 2013).

The main contaminant sources are the Ancona harbour and the Falconara Marittima oil refinery. The Ancona harbour is one of the most important in the Adriatic Sea for touristic, commercial, and fishing activities, and it had its major expansion after World War II. The Falconara Marittima refinery was founded in 1939 as a fuel repository to become active only by the end of 1950, with a significant expansion since the mid-eighties. The refinery area is one of the "contaminated sites of national relevance" as defined by the Italian Ministry of the

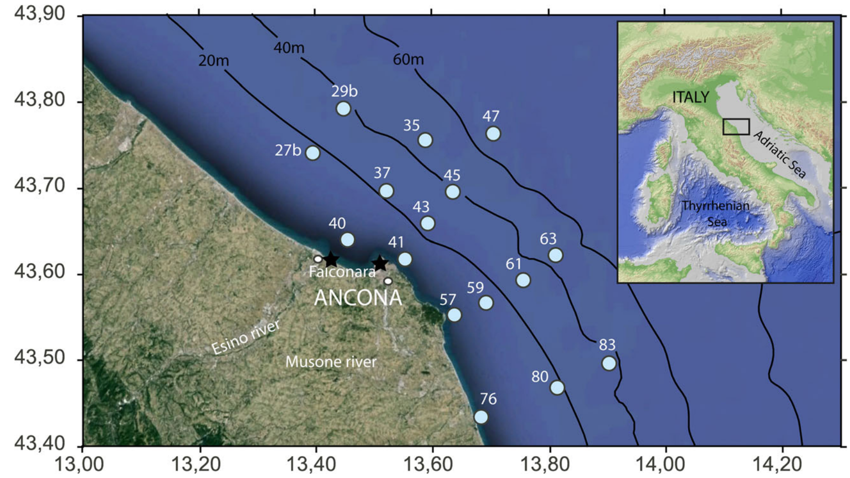

Fig. 1 Location map of the study area with sampling stations (map of lands retrieved from Google Maps). *Stars* indicate the location of the main contaminant sources

Environment and Protection of Land and Sea. Other pollutant sources may be related to several other anthropogenic activities such as tourism, which peaks in the summer, and sewage outfalls.

Hydrological features

The mean sea temperature in the study area, during the sampling period (26 February–3 March 1997), ranged between 11 and 12 °C, while salinity at the bottom varied from 37 to 38 psu (Artegiani et al. 2003). The pH of the sediments varied from 7.01 to 7.63 (Colantoni et al. 2003).

Materials and methods

Sampling and sample preparation

Sixteen box cores were collected on February 1997 in the central Adriatic Sea on board the oceanographic vessel Thetis in the frame of the PRISMA II Project. The samples were subdivided into five bathymetric transects, covering the area between 43° 26–43° 47 N latitudes and 13° 24–13° 55 E longitudes at a water depths ranging between 11 and 64 m (Fig. 1, Table 1).

On board, box core samples were subsampled using a tube (8-cm diameter), and the obtained cores were divided into 1-cm-thick slice.

Sediment samples were stored in buffered ethanol stained with rose Bengal (1 g of rose Bengal in 1000 ml of alcohol) immediately after sampling for at least 48 h, following the procedure of von Daniels (1970) in order to distinguish biocenosis from tanatocenosis.

Foraminiferal analysis

Samples were dried at 50 °C and weighed. They were then gently washed with water through a 63-μm sieve following the method described by Donnici and Serandrei Barbero (2002) and Alve and Murray (2001). Accordingly, quantitative analyses on living and dead foraminifera were performed on the size fraction >63 μm in order to consider also small species such as taxa tolerant to low-oxygen contents (Thomas et al. 2000). In agreement with Schröder et al. (1987), the lower size limit of 63 μm provides a more reliable statistical basis for paleoenvironmental studies.

Only specimens containing dense, brightly red-stained protoplasm were considered as living at the moment of sampling,

Table 1 Geographical position and water depth of the sampling stations

Station	Latitude	Longitude	Water depth (m)	% mud
27b	43°44,846'	13°24,311'	20.0	97.5
29b	43°46,789'	13°26,005'	28.0	98.8
35	43°45,588'	13°35,303'	48.0	99.2
37	43°41,934'	13°31,273'	21.0	97.1
40	43°38,346'	13°27,354'	12.0	33.9
41	43°36,900'	13°33,314'	12.0	9.7
43	43°39,433'	13°35,581'	20.5	95.7
45	43°41,848'	13°38,301'	36.0	99.2
47	43°45,763'	13°42,267'	64.0	96.4
57	43°33,275'	13°38,432'	13.0	49.5
59	43°34,484'	13°41,776'	18.0	75.4
61	43°35,925'	13°45,551'	29.0	99.0
63	43°37,681'	13°48,936'	50.0	99.6
76	43°26,810'	13°41,348'	11.0	41.3
80	43°28,526'	13°48,506'	19.0	98.2
83	43°30,141'	13°54,172'	43.0	99.6

Mud contents (%) of the bottom sediments are from Colantoni et al. (2003)

in agreement with the literature (Barmawidjaja et al. 1992; De Stigter et al. 1998; Bernhard 2000; Duijnstee et al. 2004).

Our foraminiferal analyses were performed prior to the establishment of the methodological recommendations of the FOBIMO group (Schönfeld et al. 2012); however, in our study, we considered the 0–1-cm interval for the recent benthic fauna distribution, as suggested by the authors. We analyzed the top centimeter of the sediment since Colantoni et al. (2003) documented, in this area, negative values of the redox potential from the water-sediment interface down to 2 cm within the sediment, which typically cause the migration of infaunal specimens toward the better oxygenated sediment-water interface (Jorissen et al. 1995; De Stigter et al. 1998). Moreover, some authors consider that more than >80 % of the standing stock of benthic foraminifers lives in the top centimeter of the sediment (inter alias, Van der Zwaan et al. 1999; Frontalini and Coccioni 2008; Armynot du Châtelet et al. 2009), so their study is sufficient to obtain relevant information for biomonitoring purposes (e.g., Barras et al. 2014). Furthermore, the sedimentation rate in the entire surveyed area is no lower than 0.1 cm/year, with the exception of station 76 where ^{210}Pb values suggest a sedimentation rate of 0.01 cm/year (Orsini 2006). On this basis, the entire first centimeter at virtually all stations corresponds to a time interval that goes back no longer than 10 years preceding the sampling, which largely falls in the time interval when the refinery has been active.

Where possible, 300 specimens were picked from each subsample, divided by using an Otto microsplitter, and identified largely following AGIP Atlas (1982), Cimerman and Langer (1991), Loeblich and Tappan (1987), and Sgarrella and Moncharmont Zei (1993).

For the genus Ammonia, we referred to Debenay et al. (1998), Buzas-Stephens et al. (2002), and Hayward et al. (2004), while for Textularia and Elphidium genera, we adopted the taxonomy proposed by Fiorini and Vaiani (2001). As *Elphidium* gr., we considered together *Elphidium advenum*, *Elphidium crispum*, *Elphidium decipiens*, *Elphidium granosum*, *Elphidium poyeanum*, and *Elphidium jenseni*.

Eggerella scabra and *Eggerella advena* are counted together as *Eggerella* spp. because of their similar ecological requirements, while *Bolivina seminuda*, *Bolivina spathulata*, and *Bolivina dilatata* are grouped in *Bolivina* spp. in agreement with Duijnstee et al. (2003). *Quinqueloculina longirostra* and *Quinqueloculina padana* are grouped as *Quinqueloculina* gr. 1 while *Quinqueloculina seminulum* and *Quinqueloculina oblonga* as *Quinqueloculina* gr. 2. *Bulimina* spp. includes *Bulimina fusiformis*, *Bulimina elongata*, *Bulimina elegans*, and *Bulimina inflata*.

Nonionella turgida and *Nonionella opima* are considered together as *Nonionella* spp.

In each sample, deformed benthic foraminifera were counted to verify whether a relationship between pollutants and morphological deformations in foraminiferal assemblages exists.

Deformed specimens belonging to *Ammonia parkinsoniana*, *E. crispum*, and *Quinqueloculina* were recognized, separately counted, and reported as total deformed foraminifera (TDF, Appendix A).

Foraminiferal data were reported as relative abundance (%). Shannon-Weaver index or information function (H) (Shannon and Weaver 1963) was calculated using the Paleontological Statistics Data Analysis (PAST) software (Hammer et al. 2001) (Appendix A).

Statistical analysis

All statistical analyses were performed on the relative abundances of benthic foraminiferal species.

A principal component analysis (PCA) was performed using the MVSP program (Kovach 1993). In order to reduce the background noise, only species with abundance greater than 2 % in at least one sample were considered for statistical treatment. Prior to statistical analysis, an additive logarithmic transformation to base 10 was performed on abundances of individual taxa to remove the effects of orders of magnitude difference between variables. The PCA was then used to determine the community's relationship to individual sampling stations. PCA reduces large data matrices composed of several variables to a smaller number of components representing the main modes of variation and helps interpreting large volume of data.

Additionally, a Q-mode cluster analysis was carried out for ordering the sampling stations based on the relative abundance of species calculated by adopting the Ward's linkage method and given in terms of the Euclidean distance. Maps were created with RockWorks.

Trace metals analysis on foraminiferal shells

Trace metals content was investigated in the most abundant taxa (*Quinqueloculina*, *A. parkinsoniana*, and *Hopkinsina pacifica*) of all coastal stations 40, 41, 57, and 76, and of offshore stations 37 and 29b, which are near to the main contaminant sources. About 0.2 to 0.5 mg of foraminifera were picked from the top centimeter considering both biocenosis and tanatocenosis (Table 3). The foraminiferal tests were next cleaned using a multistep trace metal protocol including reductive cleaning with buffered hydrazine (Boyle and Keigwin 1985). Chemical analyses were performed by ICP-AES and ICP-MS at the geochemistry laboratory of IAMC-CNR of Naples (Italy). Particularly, simultaneous analyses of V, Cu, Co, Zn, Mn, Cd, and Pb concentrations were carried out using a Varian ICP-MS, whereas Ca was determined by a

Varian Vista MPXICP-AES. In detail, the tests were gently crushed and then cleaned following procedures modified from Lea and Boyle (1993). Samples were ultrasonically cleaned four times with ultrapure water (>18 MΩ) and twice with methanol. Metal oxide coatings were reduced in a solution consisting of anhydrous-hydrazine, citric acid, and ammonium hydroxide, and organic matter was oxidized in a solution of hydrogen peroxide and sodium hydroxide. All the water samples were treated under a laminar air flow clean bench to minimize contamination risks, and the sampling materials were cleaned with high purity grade reagents. The remaining tests material was then dissolved in 0.1 N nitric acid and simultaneously analyzed for magnesium with the Varian ICP-MS inductively coupled plasma-mass spectrometer. A multi-element standard was prepared with ICP-MS grade High-Purity Standards. Based on repeated analyses of the standard and samples over several runs, on different days, the 2σ error in the ICP analyses is estimated at ±5 %. Replicate analyses on five samples yielded an average external precision (1σ) of about 5 %. Metal to calcium ratios (Me/Ca) were determined from intensity ratios with an external matrix-matched standard using the method developed by Rosenthal et al. (1999).

Moreover, some specimens of blackened Miliolids were observed with a scanning electron microscope (SEM, LEO 1530 FEG) to qualitatively characterize the occurrence of trace element nanoparticles or foreign elements within the foraminiferal test. The SEM, coupled with an energy dispersive spectrometer (EDS), is used to assess the chemical composition of particles within the foraminiferal test.

Results

Benthic foraminifera

For this study, we consider the biocenosis and tanatocenosis together. All the studied samples contain well-preserved benthic foraminifera. Living specimens represent a reduced amount of the total assemblage with proportions ranging from 0 % (station 40) to 19.1 % (station 35), and their abundances are particularly low in stations close to the coast (Table 2).

A total of 63 species were identified, dominated by calcareous shells. The most common benthic foraminifera are Elphidium (*E. decipiens*, *E. granosum*, *E. poyeanum*, and *E. advenum*), Ammonia (*A. parkinsoniana*, *Ammonia perlucida*, and *Ammonia tepida*), Nonionella (*N. turgida* and *N. opima*), *Bolivina* spp. and *Bulimina* spp.

Among agglutinants, Textularia and Eggerella are the most significant taxa. The Shannon-Weaver index varies between 1.6 (station 41) and 2.6 (station 61) (Fig. 2, Appendix A). The highest values of diversity are commonly found in station located between 10 and 20 m water depth, then the values decrease at 40-m water depth, suggesting a nonlinear relationship between diversity and water depth.

In Fig. 3, the map of distribution of the most abundant taxa in the top centimeter is shown.

Statistics

The data matrix, consisting of 16 stations and 29 taxa/groups (more abundant than 2 %), provided the basis for the cluster analysis.

The PCA allowed us to distinguish two principal components that together account for 56.8 % of the total data variance. Axis 1, which accounts for 41.9 % of the total variance, is negatively correlated with depth. Axis 2, accounting for only 14.9 % of the total variance, reflects lateral variations probably related to the proximity of river outputs or circulation patterns. A plot of the first two PCA axes scores (Fig. 4) allows distinguishing three groups of sampling stations, basically based on scores on axis 1, thus reflecting a bathymetric arrangement. The first group shows negative score on axis 1 due to its shallow depth, while the group IIa is positively correlated with axis 1; group IIb is in an intermediate position between groups I and IIa. The Q-mode cluster analysis confirms the results of the PCA by clearly separating two major branches at an index value of ~9.7 (Fig. 5). The first group consists of six stations corresponding to group I identified by the PCA. Within the second larger cluster, the cluster analysis isolates two subgroups of samples corresponding to groups IIa and IIb, discriminated by the cluster analysis.

Trace metals contents in foraminifera shells

Me/Ca ratios measured in foraminifera shells and expressed as micromole per mole Ca are reported in Table 3.

Anomalously, high Mn/Ca ratios were not considered, as attributed to an inadequate cleaning procedure of the benthic foraminifera. V/Ca ratios measured in *Quinqueloculina* shells span from 0.2 to 27, and the highest values were found in stations 40 and 76. Co/Ca ratios show values ranging from 1.6 μmol/mol (St. 76, *A. parkinsoniana*) to 7.1 μmol/mol (St. 57, living specimens of *A. parkinsoniana*). Cu/Ca ratios presented high values in all stations, ranging between 0.9 μmol/mol (*A. parkinsoniana* from St. 41) and 771 μmol/mol (living specimens of *H. pacifica* from St. 29b). Zn/Ca ratios are high in station 40 in both *A. parkinsoniana* (284.5 μmol/mol) and Quinqueloculina (14.3 and 45.0 μmol/mol), whereas Pb/Ca ratios ranged between 1.4 μmol/mol (St. 76, *A. parkinsoniana*) and 17.9 μmol/mol (St. 40, *Quinqueloculina* gr. 2). Cadmium was detected only in *A. parkinsoniana* shells from St. 40 (0.39 μmol/mol).

Energy dispersal X-ray analyses of not-blackened test of *Q. oblonga* collected in the study area (station 40) show that

Table 2 Relative abundances of living benthic foraminifera and the most representative taxa

Station	Living benthic foraminifera (% of total assemblage)	Main taxa
27b	5.7	*A. perlucida*, *Bolivina* spp.
29b	12.7	*E. advenum*, *Bulimina* spp.
35	19.1	*Bulimina* spp.
37	4.9	*A. perlucida*
40	0.0	*A. parkinsoniana*
41	4.0	*A. parkinsoniana*
43	17.6	*E. advenum*, *Bolivina* spp., *H. pacifica*
45	5.7	*Bolivina* spp., *Bulimina* spp., *E. vitrea*
47	4.8	*Bulimina* spp., *E. vitrea*
57	14.5	*A. tepida*, *A. parkinsoniana*
59	3.6	*A. perlucida*, *Bolivina* spp.
61	15.7	*Bolivina* spp., *Bulimina* spp.
63	12.7	*Bolivina* spp., *Bulimina* spp., *E. vitrea*, *H. pacifica*
76	6.4	*A. parkinsoniana*
80	12.8	*A. perlucida*, *Bolivina* spp.
83	9.2	*Bolivina* spp., *Bulimina* spp., *H. pacifica*

the test is made of calcite (Fig. 6a). In the blackened suture, the test exhibits the occurrence of foreign elements including Fe, Al, Si, and S (Fig. 6b).

Benthic foraminiferal abnormalities

Some benthic foraminifera taxa exhibit morphological deformities (Appendix A and Fig. 7). The deformities were restricted mainly to the species *E. crispum*, *A. parkinsoniana*, and Quinqueloculina. Following Yanko et al. (1998) classification, these abnormalities are indentified as aberrant chamber shape, abnormal growth of last chamber, and compressed tests. In several cases, some specimens presented more than one type of deformation. Few foraminifera, belonging in particular to the genus Quinqueloculina, were characterized by a blackened test. The black material was primarily evident on test surface or along suture lines of the porcelaneous shells. Both morphological abnormalities and blackened tests were mainly observed in samples from station 40 where the deformed taxa represent the 3.1 % of the total assemblage.

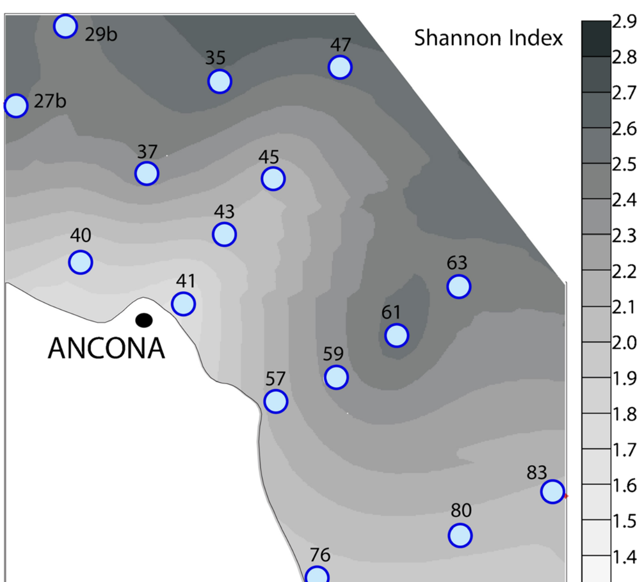

Fig. 2 Diversity (Shannon Index) of benthic foraminiferal faunas in the investigated stations

Discussion

Foraminiferal distribution

With increasing worldwide consciousness of environmental problems, ways to detect and monitor marine pollution over time are the subjects of active research. The studied coastal area is characterized by quite stable physicochemical conditions and a low to high degree of heavy metal pollution, due principally to the activity of the Ancona harbour and the API refinery of Falconara Marittima.

Benthic foraminiferal assemblages are somewhat poorly diversified as testified by relatively low values of the diversity index. The H index exhibits values lower than 2 in coastal areas and lower than 3 in offshore stations. The lowest values are found in the north-western part of the study area, at St. 41

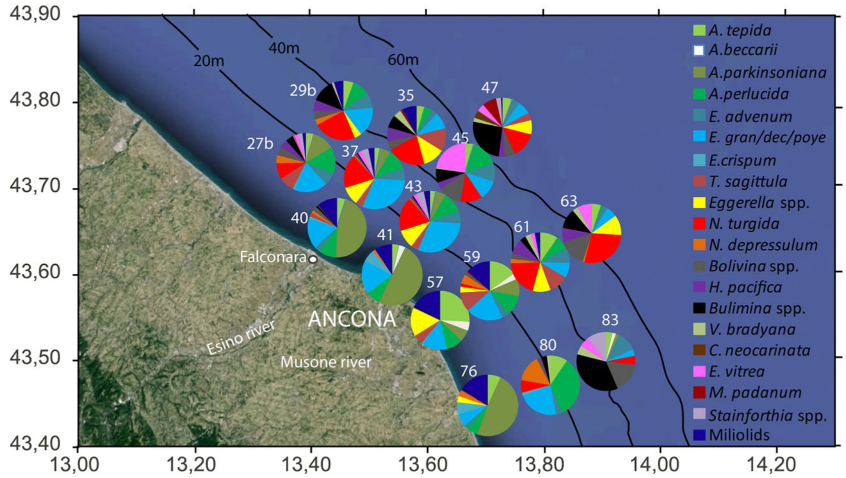

Fig. 3 Pie charts showing the distribution of the most abundant taxa of benthic foraminifera in the first centimeter of sediment for each station. *E. gran/dec/poye*, *Elphidium granosum/decipiens/poyeanum*

and St. 40 (Fig. 2). Living specimens range from 0.7 (station 40) to 16.1 % (station 35) of the total population, and their abundances are particularly low in the stations closest to the coast (Table 2).

At stations located in the area between Ancona and Falconara Marittima (St. 40 and St. 41), evident changes in the abundance and composition of benthic foraminiferal association, both in terms of number of specimens and species, were observed. This area is subject to local sources of pollution due to an intense industrial activity and is considered at high risk for environmental crisis (data from the Region Marche). The association is characterized by the almost total lack of living specimens and is dominated by species sensitive to oxygen changes and typical of areas subject to high human impacts.

We can therefore presume a correlation between pollution derived from the intense industrial activity in this part of the coast (harbour and refinery) and the distribution of benthic life.

The results of the cluster analysis enable us to recognize three biotopes in the study area (Fig. 8) representing different environments.

Biotope 1 corresponds to the near-shore stations. The foraminiferal assemblage is dominated by *A. parkinsoniana* with the presence of Miliolids (Quinqueloculina, *Triloculina tricarinata*, *Pyrgo depressa*), *Elphidium* gr., and *A. tepida*

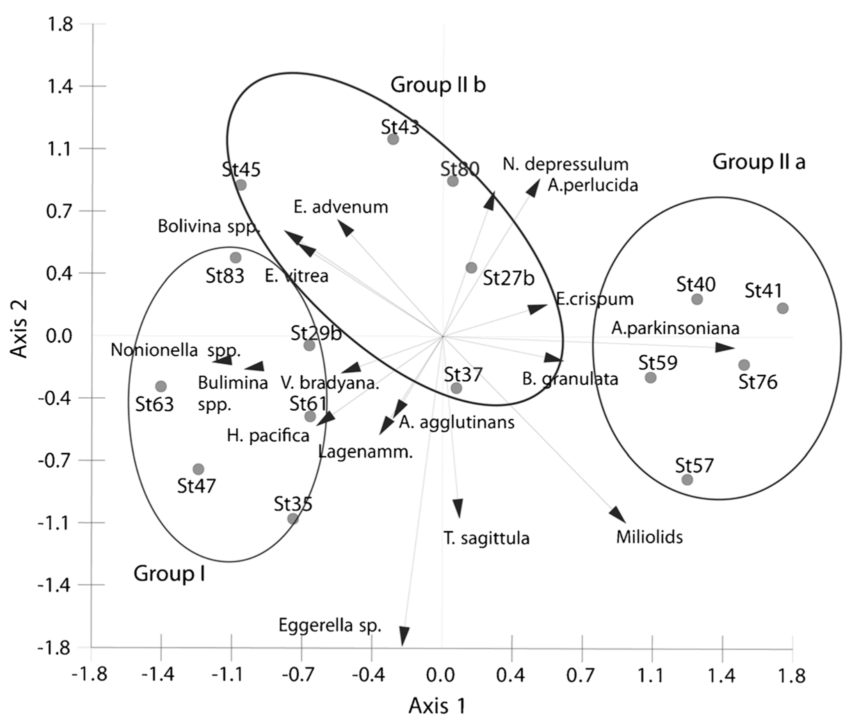

Fig. 4 PCA ordination diagram plotting sampling stations and variables

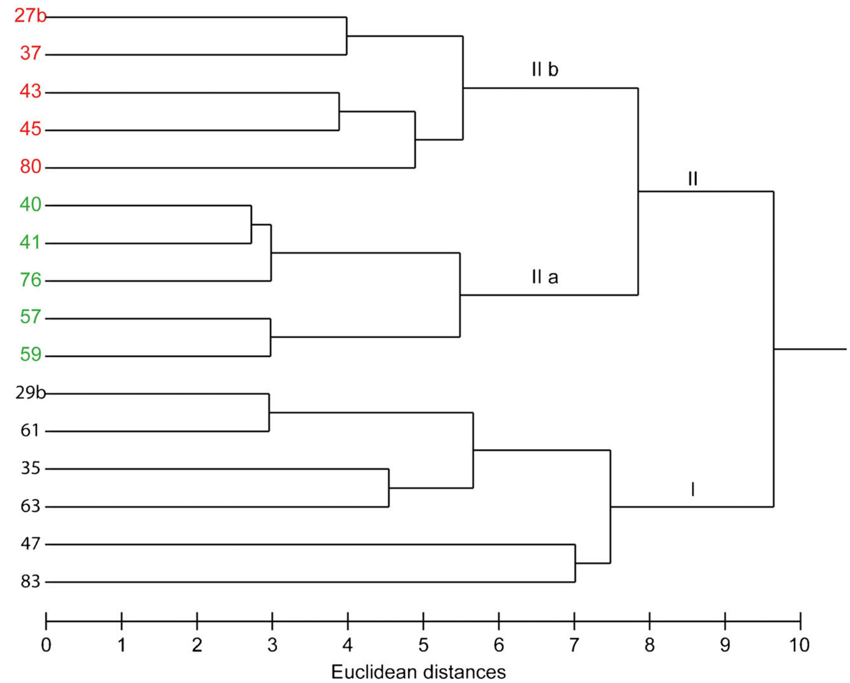

Fig. 5 Dendrogram classification of stations produced by Q-mode cluster analysis using Euclidean distance. *Numbers* and *letters* indicate the recognized clusters

(Table 4). These taxa, very tolerant to large salinity variations, are typical of environments characterized by sandy substrate and high hydrodynamic energy, and they are also related to the riverine inputs (Jorissen 1988; Van der Zwaan and Jorissen 1991; Barmawidjaja et al. 1995).

Biotope 2 includes stations characterized by high frequencies of *A. perlucida*, *Elphidium* gr., *Nonionella* spp., and *A. tepida* (Table 4). In the study area, this microfauna occurred in environments characterized by sandy to muddy sediments and high content of organic matter. A number of changes in the hydrological and sedimentological features occurred at the boundary between biotope 1 and 2, which marks the transition between the coastal area, characterized by a sandy substrate of Apennine provenance and high hydrodynamic energy, and the offshore area dominated by a mud belt feed by the Po river and deposited parallel to the coastline (Colantoni et al. 1979; Frignani et al. 2005).

Table 3 Me/Ca ratios measured on carbonate shells of benthic foraminifera from stations 29b, 37, 40, 41, 57, and 76

St.	Species#	V/Ca (μmol/mol)	Mn/Ca (μmol/mol)	Co/Ca (μmol/mol)	Cu/Ca (μmol/mol)	Zn/Ca (μmol/mol)	Cd/Ca (μmol/mol)	Pb/Ca (μmol/mol)
29b	A. park	<dl	167.7	3.7	581.8	<dl	<dl	<dl
29b	H. paci°	<dl	<dl	3.2	771.0	<dl	<dl	<dl
37	A. park	<dl	201.4	6.6	68.5	0.0	<dl	<dl
40	Quinq.1	5.8	0.0	5.0	11.1	14.3	<dl	11.5
40	A. park	<dl	266.3	1.7	6.7	284.5	0.39	2.7
41	A. park	<dl	232.7	1.9	0.9	<dl	<dl	<dl
57	A. park°	<dl	210.9	7.1	<dl	<dl	<dl	<dl
76	Quinq. 1	0.2	0.0	2.9	37.7	<dl	<dl	10.5
76	A. park	<dl	270.5	1.6	12.9	<dl	<dl	1.4
76	A. park°	13.5	202.1	1.7	<dl	<dl	<dl	<dl
Mean	Benthic foram	0.112	1–500	n.a.	0.2	1.5–6	0.2–0.25	n.a.

Abbreviations are A. park., Ammonia parkinsoniana; H. paci., Hopkinsina pacifica; Quinq. 1, Quinqueloculinagr. 1

° Living specimens

In the last row, mean value of Me/Ca ratios of benthic foraminifera living in unpolluted marine environments from Lea (1999) are shown. <dl indicates Me/Ca ratios below the detectable levels

n.a. Not available

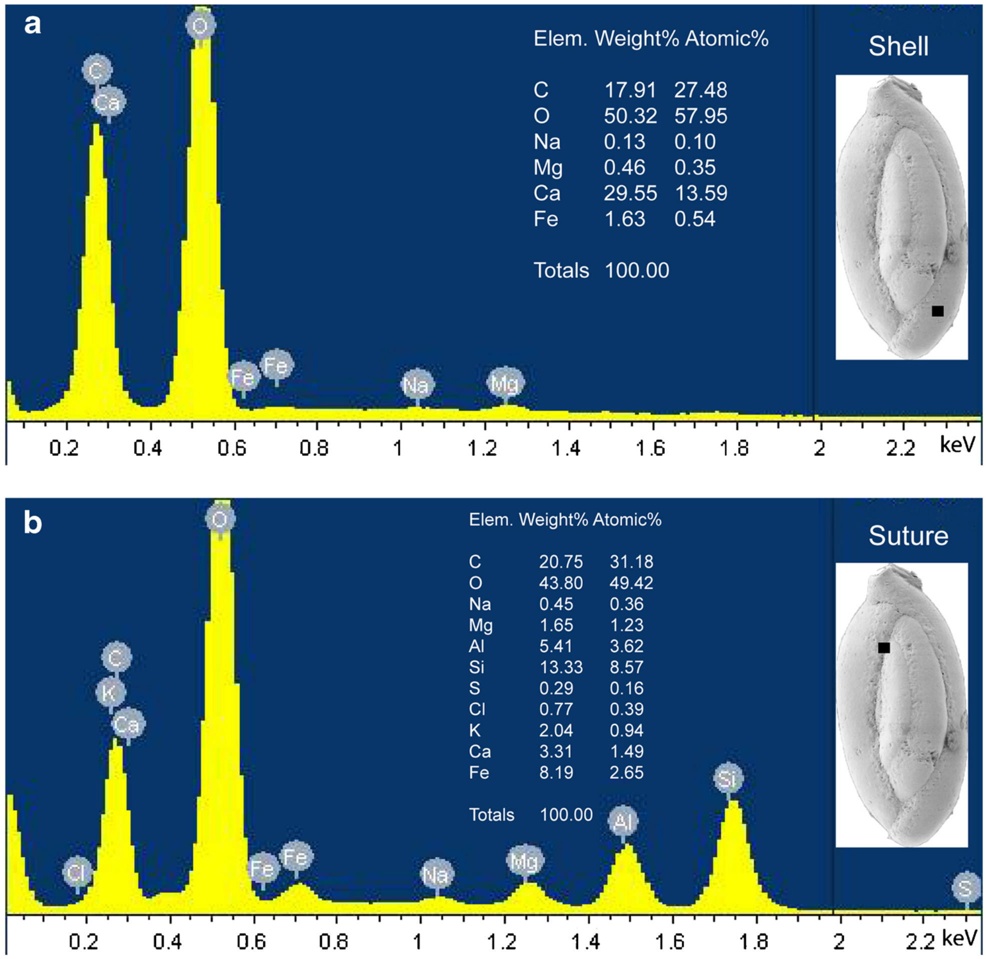

Fig. 6 Energy dispersal X-ray analyses show the elemental composition as measured in not-blackened test (**a**) and blackened sutures (**b**) of a specimen of *Q. oblonga*

Biotope 3 represents the offshore stations (Fig. 8). The foraminiferal microfauna differs from the one typical of the biotope 2 for the higher percentage of *Nonionella* spp. and infaunal species such as *Bulimina* spp. and *Bolivina* spp. (Table 4). These two latter genera have very low oxygen requirements and, in dysoxic conditions, are able to actively migrate to the top centimeter of the sediments becoming competitive next to epifaunal species, which are more negatively affected by low oxygen concentrations (De Stigter et al. 1998; Barmawidjaja et al. 1992; Duijnstee et al. 2003).

In this area, we also observed several specimens of *N. turgida* and *Epistominella vitrea*, epifaunal species typical of environments characterized by high concentrations of organic matter, and *H. pacifica. Nonionella turgida* is described by several authors as an opportunistic species well adapted to live in low oxygen and high organic matter conditions (Barmawidjaja et al. 1995; Van der Zwaan et al. 1999), whereas *E. vitrea* is considered to be an indicator of high concentrations of fresh organic matter (Gooday 1994). These species profit from an organic matter pulse, also at a seasonal level, as documented by Saraswat et al. (2005) for *E. vitrea*, because they possess the ability to reproduce rapidly and even immaturely, so that they reach extremely high numbers in a short time, the so-called opportunistic model (Van der Zwaan et al. 1999). The same strategy in the Adriatic Sea is used by *H. pacifica* that reaches extremely high abundances immediately following anoxic periods (Jorissen et al. 1992). The presence of infaunal and opportunistic taxa in the mud belt documents that this area is rich in organic matter both fresh (*E. vitrea*) or degraded (*N. turgida*), and has a reduced dissolved oxygen content (*H. pacifica*). These species are characteristic of the late Holocene mud deposits in the Adriatic Sea and other continental shelves where sedimentation and faunal distribution are affected by the strong predominance of fluvial deposits and their deposition along the coast (Van der Zwaan et al. 1999).

The adaptability of these species is also evident in the characteristics of the biocenosis: the highest percentages of living specimens were found in the stations characterized by muddy sediments, where the association is dominated by opportunistic species (*Bulimina* spp., *Bolivina* spp., *E. vitrea*, *H. pacifica*) (Table 2) with a high reproductive rate. In the stations close to the coast, the presence of living specimens is lower and *A. parkinsoniana* dominates the biocenosis, showing the highest degree of opportunism among the species that constitute the coastal association.

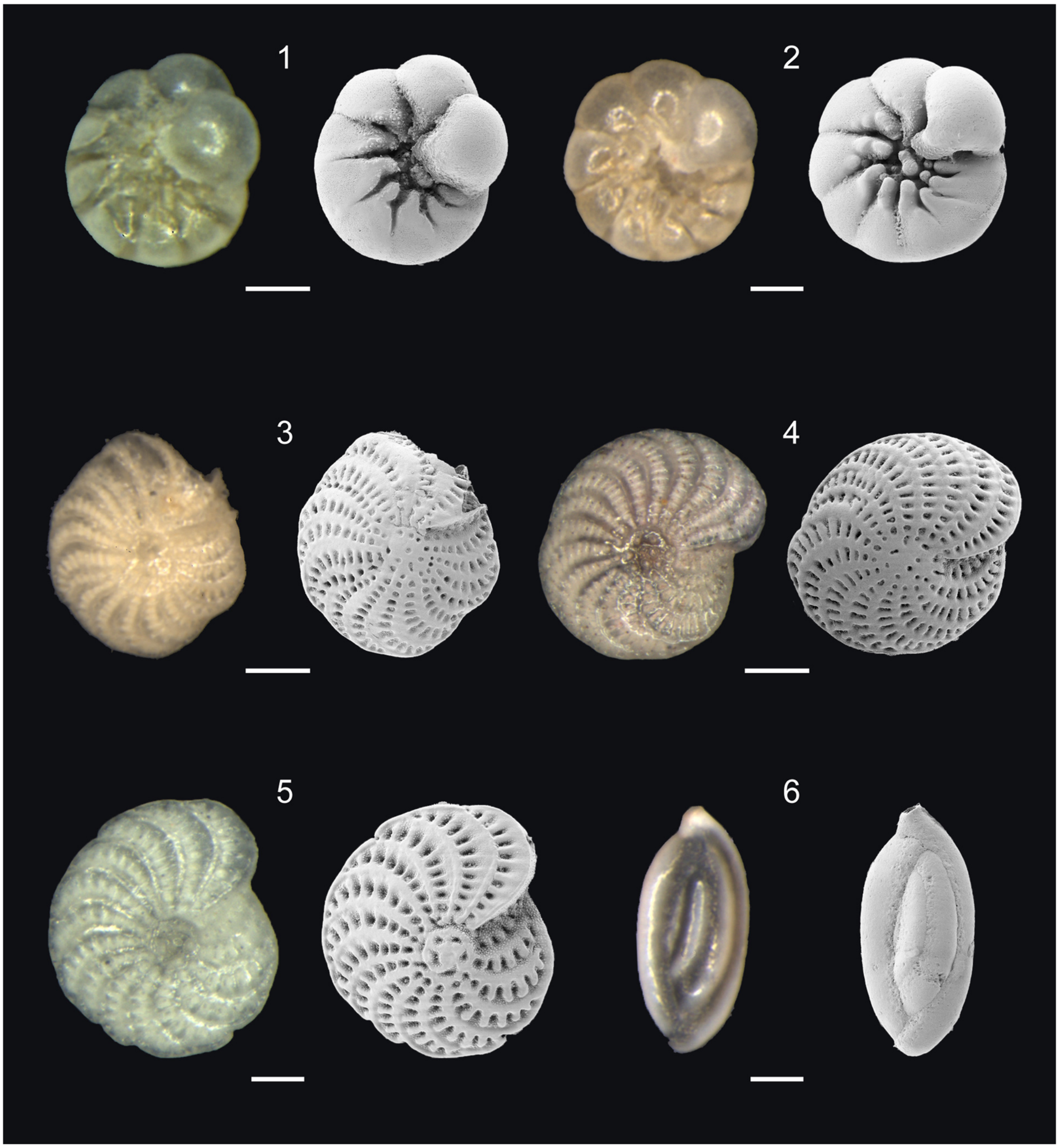

Fig. 7 Light microscope (*left*) and SEM (*right*) images of some foraminiferal specimens bearing different morphological abnormalities: (*1*, *2*) *Ammonia parkinsoniana*, abnormal growth of last chamber; (*3–5*) *Elphidium crispum*, aberrant chamber shape; (*6*) *Quinqueloculina oblonga*, shell blackened. *Scale bar*=100 μm; (*3–4*) *scale bar*=200 μm

The distribution of the biotopes, following the bathymetric gradient, reflects the distribution of nutrients and organic matter in the area, which, in turn, is strictly correlated to the distribution of the finest sediments (Colantoni et al. 2003; Tesi et al. 2013). Our data are consistent with the cluster distribution of benthic foraminifera in bottom sediments from the Adriatic Sea previously reported by Jorissen (1987, 1988) and confirm that the benthic microfauna in this area is strongly influenced by inputs of large amounts of nutrients from river runoff and by significant variations in sediment grain size.

Based on ecological requirements, we grouped some of the most abundant species in oxygen/salinity sensitive (Fig. 9a), opportunistic/river input (Fig. 9b), or indicative of polluted areas (Fig. 9c), in order to evidence the relationship between the distribution of meiofauna and environmental conditions.

According to the literature, *A. parkinsoniana*, *Quinqueloculina* spp., and *Elphidium* gr. were grouped and considered as sensitive to oxygen and/or salinity changes (Fig. 9a). On the basis of observations made on culture experiments, Moodley and Hess (1992) reported that some species are able to tolerate anoxic conditions for long periods. In

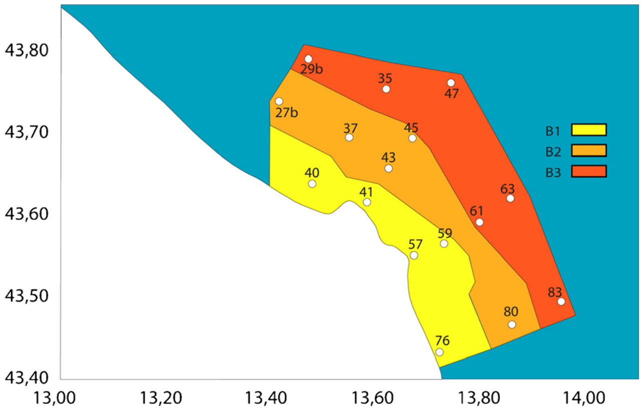

Fig. 8 Benthic foraminiferal biotopes (*B1*, *B2*, and *B3*) identified on the base of station scores on the first axis of the PCA and cluster analysis

particular, *Ammonia beccarii*, *Q. seminulum*, and *Elphidium excavatum* (the latter two typical of the coastal area in our study) show signs of activity even after 24 h without oxygen. In addition, this group tolerates rapid salinity variations (Sgarrella and Moncharmont Zei 1993; Jorissen 1988; Murray 2006 and reference therein). Several authors have reported that these three taxa are also sensitive to contamination by heavy metals and can, therefore, be considered in biomonitoring studies (e.g., Ferraro et al. 2006). Consequently, the association of these three taxa can be used as an index of sediments oxygenation: their relative abundance varies considerably within the studied area and the highest values are documented in St. 41 (81.8 %) and St. 40 (75.8 %). Moreover, the occurrence at these stations of blackened tests, rich in finely particulate iron sulphides (pyrite) (see paragraph 5.2 for details), supports the presence of low oxygen conditions. This data reveals that the northern area is in general more subject to oxygen deficit; this could be due either to its proximity to the Po river delta and the resulting increased frequency of anoxic events, or to the presence of industrial activity such as Ancona

Table 4 Biotopes and relative abundances of the most representative taxa

Biotopes	Representative taxa	Relative abundances (% of the total assemblages)
Biotope 1	*Ammonia parkinsoniana*	30.6
	Miliolids	14
	Elphidium gr.	12.5
	Ammonia tepida	11.3
Biotope 2	*Ammonia perlucida*	17.1
	Elphidium gr.	17.0
	Nonionella spp.	12.2
	Ammonia tepida	10.0
Biotope 3	*Bulimina* spp.	18.8
	Nonionella spp.	17.5
	Bolivina spp.	11.7

harbour and Falconara refinery, or both. Moreover, the coastal area where these taxa peak show largely changing values of salinity particularly because of seasonal changes in riverine input.

Nonionella turgida, *Bulimina marginata*, and *Valvulineria bradyana* were considered as opportunistic taxa tolerant to low oxygen and high nutrients concentrations (Van der Zwaan and Jorissen 1991; Fig. 9b). Their presence is therefore strongly linked to the influence of river runoff (Van der Zwaan and Jorissen 1991). This association can be correlated with the extent and distribution of the nutrient derived from the Po river. These species constitute the 44.3 % of the association in St. 83 where the hydrological condition favor the accumulation of the fine material and nutrients derived from the Po river, whereas they are completely absent in St. 41.

The distribution of *Elphidium* gr., *Buccella granulata*, *E. advena*, and *A. parkinsoniana*—considered among the most tolerant taxa to pollution/environmental stress (Yanko et al. 1999; Fig. 9c)—shows high abundances of these species along the coast. A similar association (*E. excavatum clavatum*, *B. frigida*, *E. advena*, and *A. beccarii*), typical of marginal-marine environments (Murray 1991), dominates also the meiofauna of the Long Island Sound (LIS), a large bay located in front of New York city and studied by Thomas et al. (2000). This area is subject to strong inputs of nutrients, which cause the development of episodic anoxia, and emissions of both organic pollutants and heavy metals.

Effects of heavy metals on foraminiferal tests

The occurrence and abundance of deformed tests of benthic foraminifera can be considered significant and inexpensive indicators of the presence of different type of pollutants including heavy metals (Alve 1991; Yanko et al. 1998; Coccioni 2000; Samir and El-Din 2001; Di Leonardo et al. 2007; Frontalini and Coccioni 2008; Rumolo et al. 2009; Caruso et al. 2011).

On the other hand, morphological deformation of benthic foraminiferal tests is a common feature occurring independently from latitude, taxonomy, or feeding strategy (Samir and El-Din 2001). It can also be related to natural environmental stresses such as rapidly changing salinity, pH, and organic matter, among other controlling factors (Alve 1991; Almogi-Labin et al. 1992; Geslin et al. 2000; Debenay et al. 2001; Scott et al. 2005; Luciani 2007; Melis and Covelli 2013). In moderately polluted areas, it is not easy to distinguish the influence on environmental quality of natural factors from anthropogenic ones (Thomas et al. 2000; Buzas-Stephens and Buzas 2005).

For several decades, the Adriatic Sea has been subject to large inputs of nutrients and organic matter, discharge from urban, agricultural, and industrial activities located inland, that

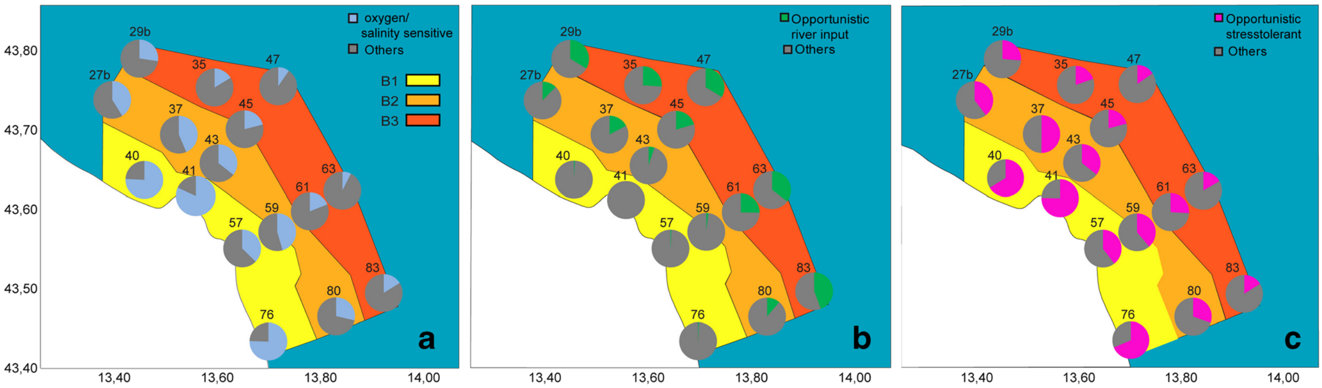

Fig. 9 Pie charts showing the distribution of taxa oxygen/salinity sensitive (**a**), opportunistic/river input influenced taxa (**b**), and opportunistic/stress tolerant taxa (**c**) for each station

led to periodic episodes of anoxia (Frignani and Turci 1981; Justic 1987). Industrial settlements along the coast (harbour and refinery) contribute to increase anthropogenic pressure over the studied area.

The investigated area is characterized by higher concentration of heavy metal (Fe, Mn, Cu, Cd, and Pb) in coastal stations (for details, see Table 5.4 in Frache et al. 2003), although other authors defined the study area as a quite stable environment, unpolluted to moderately polluted by heavy metals (Frontalini and Coccioni 2008).

According to the literature (Alve 1995; Frontalini and Coccioni 2008), we considered deformed tests as indicators of stressed environmental conditions when their percentages represent more than 1 % of the total fauna. We noticed an increasing number of deformed tests (more than 3 %) and the presence of some blackened ones in station 40 (Fig. 7, Appendix A), located near the Falconara refinery. This peculiar feature supported our hypothesis that perturbed environmental conditions, caused by discharges from coastal refineries, may influence benthic foraminiferal morphologies, although it is impossible to correlate the specific foraminiferal variations to a specific type of pollutant.

Although the influence of high concentrations of hydrocarbons on the foraminiferal fauna is not yet fully understood, many authors documented alterations in the distribution of species, such as low species diversity and density (Buckley et al. 1974; Armynot du Châtelet et al. 2004) or high percentages of deformed tests (Vénec-Peyré 1984; Yanko et al. 1998; Samir 2000; Di Leonardo et al. 2007) in areas contaminated by hydrocarbons. Similar variations were observed also in areas characterized by high concentrations of heavy metals (Ellison et al. 1986; Alve 1991; Yanko et al. 1994; Cosentino et al. 2013).

The blackening observed in some porcelaneous specimens is due to the presence of finely particulate iron sulphides (pyrite) as documented by energy dispersal X-ray analyses on foraminiferal test (Fig. 6). Similar features were reported also by Romano et al. (2008) who found inclusions of Fe ions in the crystalline reticulum of deformed specimens of *Miliolinella subrotunda* from the Bagnoli industrial area. Also, Madkour and Ali (2008) documented blackened foraminiferal tests due to selective iron absorption from some coastal lagoons located along the Egyptian Red Sea coast and strongly influenced both by natural inputs and anthropogenic activities.

Despite several investigations on pollution and its effects on benthic microfauna (Yanko et al. 1998; Ferraro et al. 2006; Di Leonardo et al. 2007; Romano et al. 2008; Cherchi et al. 2009; Caruso et al. 2011; Cosentino et al. 2013), little is known about the response of benthic foraminifera to different types and concentrations of pollutants. The relationship between pollution and the characteristics of the foraminiferal fauna is rather difficult to understand, not only because of the multiplicity of pollutants discharged into an environment, but also because their effects can be species-specific. Since studies concerning trace metal contents in foraminiferal tests as proxies to monitor short-term changes on the marine environment are promising (Morel and Hering 1993; Elderfield et al. 1996; Samir and El-Din 2001; Madkour and Ali 2008; Rumolo et al. 2009), we performed some geochemical analyses in order to investigate trace metal ratios in benthic foraminiferal shells. It is known that foraminifera incorporate into their shells a quantity of some trace elements proportionally to their concentration in ambient sea water (Lea and Boyle 1989), even if the mechanisms of biogenic incorporation of these elements are not yet completely understood (Rumolo et al. 2009 and references therein).

Our results document a high variability of trace metal contents in foraminiferal tests from the investigated area (Table 3). Highest values of Me/Ca ratios are documented in the coastal area, in particular from stations 40 and 41 (in front of the Falconara refinery and the Ancona harbour). This area can be considered as a receptor of industrial and domestic discharges and is strongly influenced by the Ancona harbour activities. In particular, Quinqueloculina seems to be most susceptible to high concentrations of V and Pb. These metals are characteristic of areas affected by different anthropogenic activities and also by the presence of hydrocarbons (Metwally et al. 1997). It is noteworthy that the metal contents in Quinqueloculina from station 40 are generally higher than those recorded by the same group from station 76 suggesting that the major sources of pollution are linked to the coastal sites. Specimens of *A. parkinsoniana* show Zn, Co, and Cu bioaccumulation in their shells. This species is described in literature as a very sensitive bioindicator of pollution being strongly affected by heavy metal content even at low concentrations (Frontalini and Coccioni 2008).

H. pacifica seems to be very sensitive to Cu concentrations (Table 3). The effects of high levels of Cu on foraminiferal communities' structure have been documented by several authors (Ellison et al. 1986; Samir and El-Din 2001; Hallock et al. 2003; Armynot du Châtelet et al. 2004; Ruiz et al. 2004) and also evidenced by culturing experiments (Reichart et al. 2003; de Nooijer et al. 2007). Our data show that *H. pacifica* tends to accumulate this metal and can be considered as a good indicator of Cu pollution over the studied area.

The restricted number of analyzed samples and taxa in this study (Table 3) do not allow to precisely quantify the impact of pollutants, specifically hydrocarbons, on the distribution of benthic both dead and living foraminifera. However, our data suggest that industrial and human activities affect the geochemistry of benthic microfauna, and we are persuaded that this kind of investigations can provide important information about environmental pollutions due to anthropogenic pressure.

Conclusion

Benthic assemblages were studied in 16 box cores along 5 transects perpendicular to the coast at a water depth ranging between 11 and 64 m off Ancona (central Adriatic sea). PCA allowed us to identify three biotopes following a bathymetric gradient. The biotopes distribution documented that the microfaunal characteristics in this area cannot be strictly correlated only with depth, but are also influenced by riverine inputs (Po, Esino, and Musone rivers), organic matter contents at the seafloor, and sediment grain size.

Higher abundances of opportunistic, low-oxygen tolerant taxa and the almost total absence of living specimens were observed along the coastline. This can be explained with the influence of human activities along the coast in this part of the Adriatic Sea.

A striking feature is the distribution and morphology of the microfauna collected at station 40 (close to the Falconara refinery). In this area, we observed the almost total absence of living benthic foraminifera and a very low diversity accompanied by increasing proportions of opportunistic taxa. In addition, at these stations, some specimens of Quinqueloculina, Ammonia, and Elphidium show different kinds of morphological abnormalities, which are likely imputable to the presence of heavy metals and/or hydrocarbons in the sediments. This feature may support the correlation between quantitative and qualitative characteristics of the benthic microfauna and the different types of anthropogenic activities.

A. parkinsoniana and Quinqueloculina seem to be the most sensitive taxa and can be considered as good bioindicators of environmental stresses in this area, while *H. pacifica* tends to accumulate Cu, which makes it a good indicator of pollution derived from the presence of this metal in the sediments.

Our investigation provide additional information in the frame of a sustainable coastal management, documenting that benthic foraminifera represent a good tool for biomonitoring the state of the marine environments.

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