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Original Articles

Benthic foraminifera in a coastal marine area of the eastern Ligurian Sea (Italy): Response to environmental stress

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ABSTRACT

Benthic foraminiferal assemblages from 19 superficial marine sediment samples of a coastal area in the Ligurian Sea were analysed for benthic foraminifera in order to recognize changes in assemblage composition and structure, decrease of faunal density, increased morphological abnormalities and inclusion of heavy metals in carbonate tests as possible evidences of environmental stress. Also grain size was determined as a main factor conditioning foraminifer's distribution.

The generalized low faunal density was considered as indicative of environmental stress, attributable to the well-known natural Cr and Ni enrichment, which characterizes marine sediments of the region. The cluster analysis highlighted distinct foraminiferal assemblages with decreasing species diversity approaching to the coast. This distribution was interpreted as the result of local source of environmental stress, probably due to the stream contribution. Deformed foraminifera more abundant than background levels of unstressed environments were also recorded in 39% of samples.

Energy Dispersive Spectroscopy (EDS) analyses were carried out on carbonate tests of 55 specimens to investigate the role of the incorporation of heavy metals in the development of morphological abnormalities. Foraminifera showed the inclusion of heavy metals, not present in control specimens, indicating that environmental stress, due to metal sediment enrichment, plays a role in this phenomenon. Although anomalous elements were detected both in normal and deformed specimens and chambers, it was supposed that deformities develop as a result of the toxic effect of heavy metals on cytological activities because, contrarily to the normal specimens, all the deformed ones included anomalous elements. Higher occurrence of deformities in porcelaneous tests, typically enriched in Mg, is associated to the higher number of incorporated elements (Mn, Fe, Cu and Zn).

1. Introduction

Benthic foraminifera have been used since the 1960s as ecological indicators in marine environments (reviews in Nigam et al., 2006; Frontalini and Coccioni, 2011) due to their high density and species diversity, short life cycle and good preservation potential in marine sediments, which make them an ideal tool for characterization and monitoring of marine ecosystems (Gooday, 2003; Jorissen et al., 2007; Schönfeld et al., 2012). The response of benthic foraminifera to environmental stress, both under natural and anthropogenically-impacted conditions, has been deeply studied in the last decades. In particular, the response to trace element enrichment in marine sediments has been highlighted in study areas with different concentration levels; the change of taxonomic structure and composition, reduction of species diversity and abundance, increased development of abnormalities and change of test geochemistry were observed (Alve, 1991; Bergin et al., 2006; Frontalini and Coccioni, 2008; Cherchi et al., 2009; Martins et al., 2013; Li et al., 2014; Youssef, 2015, among the others). Besides, the anomalous inclusion of some trace elements, like as Cu, Fe, Pb and Zn, in the crystal lattice of deformed specimens from contaminated areas was recorded by several authors (Samir and El-Din, 2001; Frontalini et al., 2009; Romano et al., 2009).

Experimental and mesocosm studies considered the effects of a single metal, mainly Cu, Hg and Zn, on foraminiferal assemblages, demonstrating the causal effect of increasing metal concentrations on changes of community structure, lowering of foraminiferal density and

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diversity and increasing of abnormalities (Alve and Olsgard, 1999; Frontalini and Coccioni, 2012; Frontalini et al., 2018). In some cases, studies carried out on single species highlighted apparently contradictory results: *Rosalina leei* showed an increased number of morphological anomalies in treatments under higher Hg concentrations (Saraswat et al., 2004; Nigam et al., 2009), while *Pseudotriloculina rotunda* did not show deformations at the highest Zn concentrations (Nardelli et al., 2013, 2016). On the other hand, analyses of calcareous tests of benthic foraminifera exposed to increasing heavy metal (single or multi-element) concentrations recognized their linear uptake from the culture solutions to the carbonate test, but they did not record the presence of deformed specimens (de Nooijer et al., 2007; Munsel et al., 2010). For these reasons, the relationship between anomalous test geochemistry and development of abnormalities is still matter of debate.

The present study represents the first characterization of living and dead benthic foraminiferal faunas from the coastal zone of the East Ligurian Sea. It considers marine sediments characterized by natural trace metals enrichment, mainly for Cr and Ni, due to the presence in the mainland of ophiolitic sequences (Cosma et al., 1982). The research is finalized to highlight potential evidences of environmental stress in the foraminiferal assemblages attributable to sediment metal enrichment, pointing out the effect of the terrestrial contribution to the marine environment and its effect on biota. Species diversity and foraminiferal density were considered as potential indicators of environmental stress and reliable ecological proxies. Moreover, the investigation of carbonate geochemistry, by means of Energy Dispersive Spectrometry (EDS), was applied to detect the presence of trace metals in the test structure and to recognize their possible role in the development of morphological abnormalities. The improvement of knowledge about the abnormalities affecting foraminifera may be useful in the environmental assessment of trace metal enriched environments.

2. Study area

2.1. Geological setting

The Ligurian region is characterized by a complex geological framework due to the joining, in correspondence of the Sestri-Voltaggio zone, of Alpine (to the West) and Apennine domains (to the East). In the mainland of the study area, belonging to the Apennine domain, Jurassic ophiolitic sequence, covered by thick sedimentary units, outcrops (Elter and Marroni, 1991).

The studied marine area receives sedimentary contribution from the hydrographic basins of Entella and Gromolo streams (Fig. 1). The first one, flowing between Chiavari and Lavagna, drains extremely different lithologies, mainly constituted by flysch, alluvial, colluvial and eluvial debris deposits, ophiolitic lithotypes (peridotites, gabbro, jasper) and carbonate formation (Barsanti et al., 2003 and references therein). The second one drains ophiolitic units (serpentinites, gabbro, basalts), jasper, limestone and pelitic arenaceous turbidites (Dinelli et al., 2001). The diffuse presence of ophiolites has strong influence on the geochemistry of the territory, which is characterized by geochemical anomalies for Cr and Ni, very abundant elements in ultrafemic rocks such as peridotites and serpentinites (Ottonello, 2008). In the mainland facing the study area basin, waste materials produced by an iron/copper mine, which worked from 1864 to 1962, are partially crossed by two tributaries of the Gromolo stream, supplying contaminants in the water and river sediments (Dinelli and Tateo, 2002).

2.2. Marine coastal area

The littoral zone of the study area is mainly fed by sedimentary supply of the Entella stream and, only close to Sestri Levante, of the Gromolo stream. It is influenced by a permanent NW current flowing roughly along the coast and following the narrow shelf, with only short inversion time periods, while a SE gyre is generated by the presence of the headland; moreover, an eastward counter-current is present along the north coast with a resulting drift of the coastal materials from Entella stream to Sestri Levante area (Barsanti et al., 2003; Corradi et al., 2003; Doglioli et al., 2004). Along the coast, between the Entella mouth and Sestri Levante, several authors recognized a sedimentary deficit responsible for to the present erosive trend, mainly due to the building of the Chiavari and Lavagna harbours. A sedimentological study showed that coarse to very coarse sands are confined to the coast, with a seaward gradient, as a result of erosion and suspension by the rip-currents on the seabed, while the finer fractions settle in a more distal position, below 30 m water depth (Corradi et al., 2003). The main mineralogical components of marine sediments are calcite, plagioclase, potassium feldspar, quartz, chlorite, serpentine and mica/illite. The highest concentrations of serpentine were found close to the coast, identifying the transport by traction directed toward south-east, from the mouth of the Entella towards Sestri Levante, in agreement with the direction of the coastal drift (Barsanti et al., 2003).

At a regional scale, natural geochemical anomalies of Cr and Ni in marine sediments were attributed to the outcropping of ophiolitic sequences (Cosma et al., 1979). The geochemical study of marine sediments from sectors close to the studied one revealed natural enrichment mainly for Cr and Ni and, at a lesser extent, Cu and Mn, which is higher close to the mouths of streams crossing areas affected by the presence of ophiolitic lithotypes, as well as for Entella and Gromolo streams (Cosma et al., 1982). In particular, Cr and Ni showed concentrations up to 195 mg kg^{-1} and 154 mg kg^{-1}, respectively, at the mouth of Entella stream, where Bertolotto et al. (2005) recorded Cr concentrations up to 230 mg kg^{-1} in marine sediments. Capello et al. (2016), who investigated part of the study area, considered the high Cr and Ni concentrations (up to 384 mg kg^{-1} and 342 mg kg^{-1}, respectively) in agreement with the bulk chemistry composition of the rocks outcropping in the area.

3. Materials and methods

3.1. Sampling

A total of 19 surface sediment samples were collected in July 2015 by van Veen grab, at depth ranging between 2 and 28 m (Fig. 1; Table 1), because of the textural sediment characteristic. A volume of 50 cm^3, from the upper 2 cm layer of the undisturbed sediment, was collected directly from the upper windows of the grab and immediately stained with ethanol/rose Bengal solution (2 g l^{-1}) for the identification of living benthic foraminifera (Walton, 1952). An aliquot of 50 cm^3 was also collected for the analysis of grain size and the qualitative study of sediment mineralogy.

3.2. Sediment analysis

Samples were pre-treated with a solution of hydrogen peroxide (30%) and distilled water (1:3) to remove salts and organic matter, wet-separated into two fractions using a sieve with a 63 μm mesh and then oven dried at 40 °C. The coarse fraction (> 63 μm) was sieved using ASTM series sieves, with meshes ranging from −1 to +4 ϕ, while 2.5 g of fine fraction (< 63 μm) was analysed by means of laser diffraction granulometer (Sympatec Helos, FKV) after dispersion in a solution of sodium hexametaphosphate (0.05%). The sandy fraction was also observed under stereomicroscope in order to recognize the main components of biotic and abiotic fractions, useful to recognize sedimentary sources (Romano et al., 2009, 2016).

3.3. Benthic foraminiferal analysis

Samples were washed over a 63 μm sieve to remove staining solution and mud particles and then oven dried at 40 °C. Microfaunal

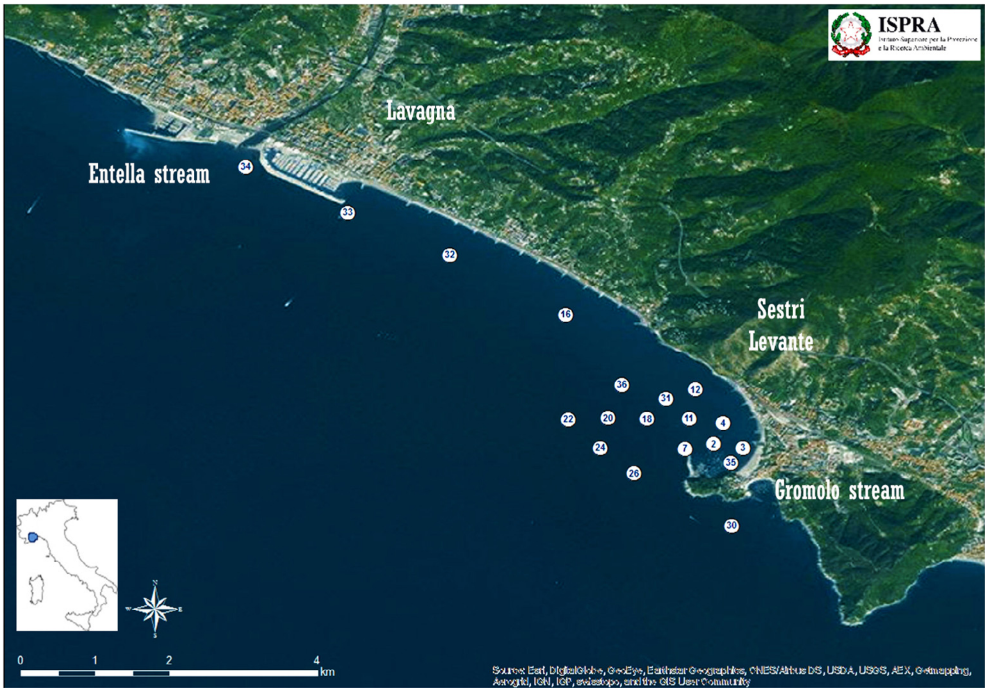


Fig. 1. Location map of the study area and sampling stations.

Table 1
List of samples with geographic coordinates.

Station	Lat °N	Lat 'min	Long °E	Long 'min
GR2	44	16,425	9	23,280
GR3	44	16,399	9	23,489
GR4	44	16,577	9	23,347
GR7	44	16,391	9	23,068
GR11	44	16,611	9	23,103
GR12	44	16,823	9	23,147
GR16	44	17,370	9	22,196
GR18	44	16,608	9	22,794
GR20	44	16,615	9	22,510
GR22	44	16,605	9	22,220
GR24	44	16,396	9	22,449
GR26	44	16,211	9	22,697
GR28	44	15,987	9	22,961
GR30	44	15,830	9	23,407
GR31	44	16,754	9	22,930
GR32	44	17,804	9	21,352
GR33	44	18,110	9	20,611
GR34	44	18,444	9	19,869
GR35	44	16,290	9	23,406

analysis was conducted under a stereomicroscope Leica M165C. The quantitative analysis of benthic foraminifera was separately conducted on living (rose Bengal stained) and dead assemblage and it was based on the count of all specimens present in the whole sample or in representative aliquots, containing at least 300 specimens. For the count of dead specimens, in order to prevent the inclusion of reworked or transported tests, only well-preserved tests, not re-crystallized and free of cracks and abrasions, were picked, counted and classified. The classification at the genus level was made according to the most used taxonomical study on foraminiferal genera (Loeblich and Tappan, 1987), while species were determined according to some important studies on the Mediterranean area (Jorissen, 1988; Cimerman and Langer, 1991; Sgarrella and Moncharmont-Zei, 1993) and to the World Modern Foraminifera Database (Hayward et al., 2011). The foraminiferal density of the total assemblage, represented by the Foraminiferal Number (FN), was calculated as the number of specimens per gram of the > 63 µm dry sediment fraction (Schott, 1935), together with the rate between dead and living specimens (D/L). The species diversity, given by the Shannon index (H) (Shannon, 1948), was calculated by using the statistical package PAlaeontological STatistics-PAST (Hammer et al., 2001; Hammer and Harper, 2006). The relative abundance of deformed specimens (FAI index, i.e. percentage of deformed specimens, according to Coccioni et al., 2005) was also determined. On the whole, 5 types of deformation were considered, most of these already described by previous authors (Table 2).

The sum of living + dead specimens was used to create a matrix for carrying out the Hierarchical Cluster Analysis (HCA), in order to recognize groups of samples with homogeneous foraminiferal content. It included the relative abundance of commonly occurring species (i.e. species > 5% in at least one sample) in only 11 samples, which contained a suitable number of specimens (70–476). For the HCA, the Euclidean distance coefficient to compare samples and the Ward's method of minimum variance to assemble clusters were applied (van

Table 2
Different types of deformation displayed by benthic foraminifera in the study area.

Type	Description of test deformity	Species	Order
1	Aberrant chamber(s) size and/or shape Alve (1991), Yanko et al. (1994, 1998), Frontalini and Coccioni (2008), Frontalini et al. (2009)	*A. beccarii, B. seminuda, E. advenum, E. crispum, E. pulvereum, H. depressula, P. pertusus, Q. parvula, Q. seminulum, T. schreiberiana*	Rotaliida, Miliolida
2	Incomplete development of the last whorl Yanko et al. (1998)	*M. subrotunda, Q. seminulum, T. schreiberiana*	Miliolida
3	Double aperture Alve (1991), Yanko et al. (1998)	*S. aspera*	Miliolida
4	Protuberance on one or more chambers Alve (1991), Yanko et al. (1994)	*S. aspera*	Miliolida
5	Change in coiling axis (This work)	*B. seminuda, B. striatula, E. scaber*	Rotaliida, Textulariida

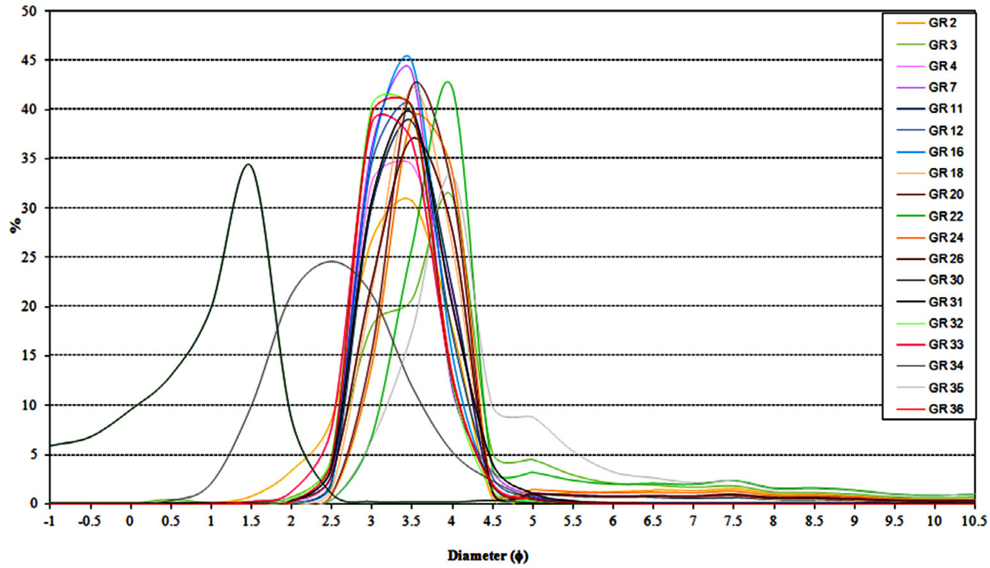


Fig. 2. Frequency curves of grain size distribution. Samples GR30 and GR34 display a coarser mode than the other samples.

Hengstum and Scott, 2011). Also Canonical Correspondence Analysis (CCA), including the relative abundance of commonly occurring species, faunal parameters (H index, FN and FAI) and grain size data, was carried out in order to highlight the effects of sediment grain size on composition and structure of foraminiferal assemblage (Romano et al., 2016).

3.4. SEM imaging and EDS analysis

A total of 55 benthic foraminifera, both normal (38) and deformed (17) were considered for Scanning Electron Microscope (SEM) images and EDS analyses. The deformed ones were selected to be representative of the 5 types of deformation as described in Table 2. As hyaline and porcelaneous carbonate taxa display different structural and compositional features related to different calcification strategies, specimens belonging to the two different groups were analysed, in order to verify if EDS analysis might highlight possible differences. The first one, constituted of hyaline species, includes: *Ammonia beccarii, Bolivina seminuda*, *Bolivina striatula, Buccella granulata, Elphidium advenum*, *Elphidium granosum, Elphidium pulvereum*, and *Haynesina depressula.* The second group is represented by porcelaneous imperforate species like as *Massilina gualteriana, Miliolinella subrotunda, Peneroplis pertusus, Quinqueloculina parvula, Quinqueloculina seminulum,* and *Siphonaperta aspera*. All specimens were collected from GR3, GR4, GR7 and GR35 stations. Normal and deformed specimens of the same species, coming from the same sample, were considered. In order to avoid surface contaminations, all specimens were ultrasonicated in deionised water. Successively, the foraminiferal tests were placed on carbon-coated stubs and, successively, examined and photographed using a SEM (FEI Quanta 400 MK2). Microanalyses were performed with an EDS (EDAX Genesis XM2) detector to check the presence of heavy metals within the deformed and normal foraminiferal tests. Microanalyses for elemental composition were collected for 60 s at 10.4 mm working distance, using an accelerating voltage of 30 kV. The EDS analysis is one of the most utilized methods for the structural and compositional investigations on foraminiferal tests in recent and fossil record, although EDS quantitative results are not totally confident (Samir and El-Din, 2001; Frezza et al., 2013; Mancin et al., 2014; Frontalini et al., 2015).

Considering the lack of a specific EDS investigation strategy, a scheme aimed to collect the needed data optimizing the available resources, was adopted. In this respect, for each deformed specimen, 3 spot microanalyses were performed on different chambers, in order to estimate the compositional variability during the test growth, correlated to the development of deformation: one spot on a deformed chamber (spot A); one spot on the normal chamber before the deformed one (spot B); one spot on the first normal chamber of the last whorl (spot C). Instead, only one spot microanalysis on a chamber of the last whorl was considered in normal specimens. To minimize a potential contamination in the obtained data, a particular care was taken to avoid the edges and pores of specimens (Frontalini et al., 2009). The EDS results were compared with those obtained from control specimens collected in similar, but not impacted environments. The choice of control specimens of different origin with respect to the study area was necessary because a close unimpacted site was not available, due to the regional extension of the geochemical anomaly.

Miliolid specimens come from superficial samples collected at 20 m water depth along the Ponza Island coast (Pontine Archipelago; Frezza et al., 2005). The island, included in a natural reserve, is characterized by a geomorphological setting similar to the one of the study area, with a narrow and steep shelf, on which a mixture of silico-clastic and carbonate sedimentation occurs (Martorelli et al., 2011). The hyaline tests come from a core drilled along the Latium coast (Central Tyrrhenian Sea) and belong to an infralittoral sandy beach (Di Bella et al., 2012).

4. Results

4.1. Sediment analysis

Marine sediments are mainly constituted by sand, which exceeds 95% along the northern coastal belt and in the southernmost sector. All samples, excluding GR30 and GR34 which are coarser, are classified as fine sand, according to Wentworth (1922), and show a mode around 3.5 ϕ (Fig. 2). The only samples with a significant pelitic fraction are GR2, GR3, GR22, GR24 and GR35, located close to the Gromolo mouth, with percentages ranging from 12 to 42% (Fig. 3). The examination under stereo microscope of sandy fraction highlighted the presence of grains of serpentinite, associated with lithic sandstone, dark grey shale, gabbros, and minerals such as quartz, feldspar, serpentine and chlorite (Fig. 4).

4.2. Benthic foraminiferal assemblages

The absolute abundance of species in the dead and living

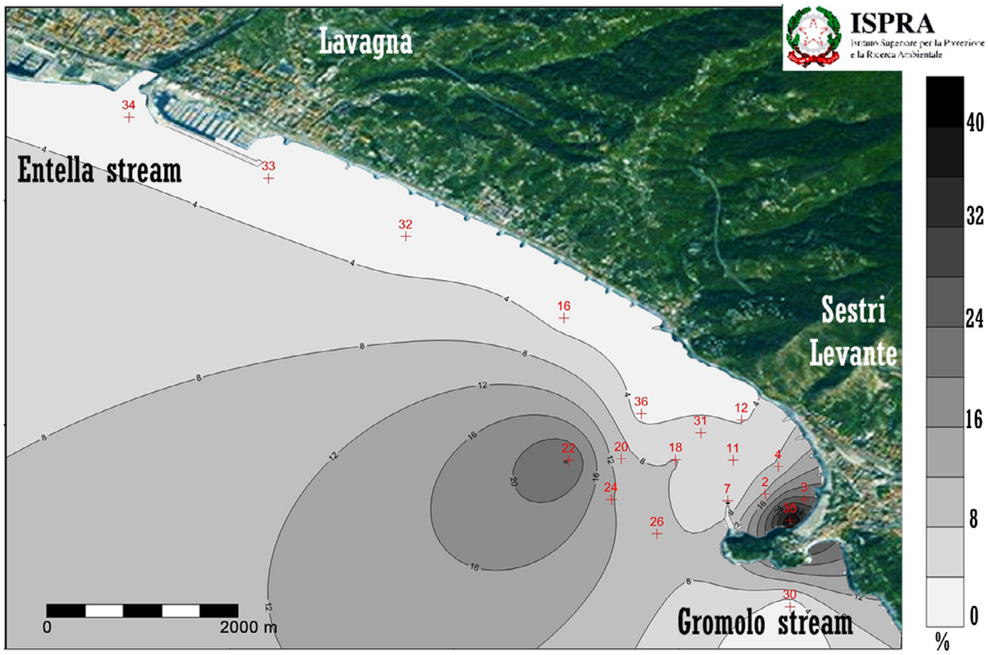


Fig. 3. Spatial distribution of the < 63 µm sediment fraction.

Fig. 4. Sediment fraction > 63 µm under stereo microscope (Sample GR2). Lithic fragments of serpentinite, shales, with quartz and feldspars are visible.

Table 3
Faunal parameters of total foraminiferal assemblages: H-index, Foraminiferal Number (FN), Foraminiferal Abnormality Index (FAI), Dead/Living ratio (D/R).

	H-index	FN	FAI	D/L
GR2	1.4	9	1	4
GR3	1.6	22	4	1
GR4	2.5	3	4	2
GR7	2.9	10	8	4
GR18	2.3	6	1	53
GR20	2.6	6	1	55
GR22	3.3	39	5	21
GR24	2.9	18	6	7
GR26	2.4	5	2	129
GR30	3.1	1	1	1
GR35	2.7	41	7	3

assemblages and the relative abundance of species in the total (living + dead) one are reported in Supplementary material (Tables S1–S3, respectively). Quantitative analysis was conducted on all the collected samples, even if 8 samples (GR11, GR12, GR16, GR31, GR32, GR33, GR34, GR36) contained < 50 specimens, mainly *A. beccarii*, *E. advenum* and *Q. seminulum*, and very rare living specimens. These samples were not considered for the statistical analysis. The remaining 11 samples showed a value of D/L ranging between 1 (GR3 and GR30) and 129 (GR26) (Table 3).

In the total assemblage, the most abundant species were *A. beccarii* (578 specimens), *S. aspera* (299 specimens), *H. depressula* (270 specimens) and *Triloculina schreiberiana* (217 specimens) (Plate 1). Benthic foraminiferal assemblages are generally characterized, in some samples, by very low foraminiferal density (FN: 1–41, median 8.9), low species diversity (H: 1.4–3.3, median 2.6) and relatively high percentages of deformed specimens (FAI: 1–8, median 4). FN shows higher values in the bay and lower values offshore, while H index displays an opposite pattern (Fig. 5); differently, the FAI values display a wide variability, without a definite pattern (Table 3).

In total, 14 species showed morphological abnormalities, divided into 5 types (Table 2). Trocospiral and planispiral foraminifera showed mostly a reduction of one or more chambers of the last whorl (type 1); miliolids had the widest variety of deformations which included aberrant shape of one chamber, incomplete development of the last whorl, double aperture, and chamber protuberance (types 1, 2, 3 and 4, respectively). Finally, biserial or triserial taxa were affected by a change in coiling axis (type 5) (Plate 2).

Cluster analysis highlighted 3 main clusters (Fig. 6). Cluster A includes stations GR2 and GR3, located inside the bay, and it is characterized by high dominance of *A. beccarii* (54.6–63.1%) accompanied, nearly exclusively in GR3, by *H. depressula*. These samples display the lowest species diversity of the study area (H: 1.4–1.6) and a rather variable FN (9–22). Sample GR3 shows the highest FAI (4).

Cluster B includes stations GR4 and GR35 located into the bay, and it is characterized by *H. depressula* as the main species (17.1–23.2%), associated to *A. beccarii* (13–14.3%) and *E. advenum* (8–18.6%). Also *Quinqueloculina* species, mainly *Q. stelligera* (median 10.6%), are rather abundant. These samples show higher species diversity (H: 2.5–2.7) and FAI (4–7) with respect to cluster A, while FN is rather similar (3–41).

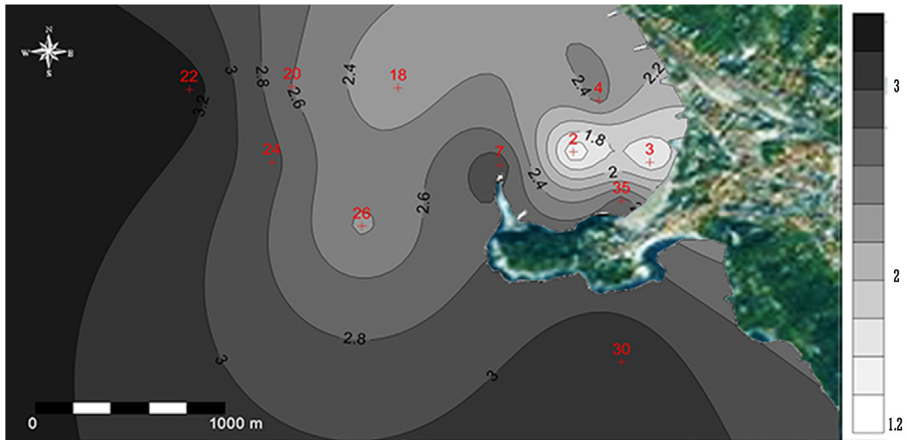


Fig. 5. Spatial distribution of Shannon H index in samples with > 50 specimens.

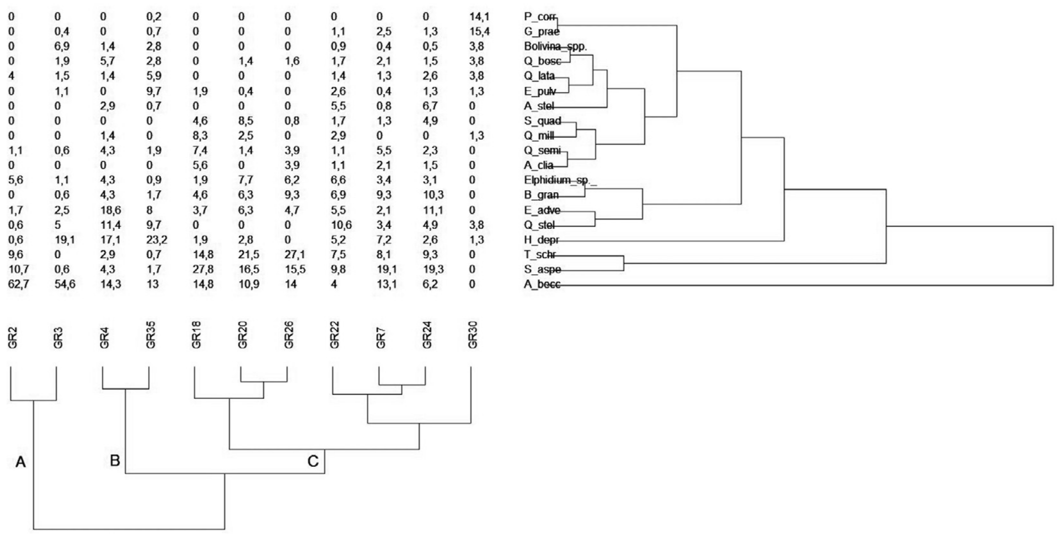


Fig. 6. Two-way (Q-mode and R-mode) Hierarchical Cluster Analysis based on relative abundance of foraminiferal species. Clusters A, B and C may be recognized in the Q-mode output.

Table 4
Summary of the five groups identified by mean of EDS analysis.

Groups	I		II		III		IV		V	
Species	Normal	Deformed	Normal	Deformed	Normal	Deformed	Normal	Deformed	Normal	Deformed
Ammonia beccarii	3 (GR35); 1 (GR3)		3 (GR4); 1(GR3)			1 (GR35)		1 (GR3)	2 (GR3)	
Bolivina seminuda							1 (GR35); 1 (GR3)		1(GR3)	
Bolivina striatula						1 (GR35)	1 (GR3)			
Buccella granulata			2 (GR7)							
Elphidium advenum	2 (GR35); 1(GR3)		2 (GR7)					1 (GR35)		1 (GR3)
Elphidium granosum			1 (GR7)							
Elphidium pulvereum	5 (GR35)						1 (GR35)	2 (GR35)	1 (GR7)	
Haynesina depressula	4 (GR35); 1(GR3)									
Massilina gualteriana									1 (GR7)	
Miliolinella subrotunda										1 (GR7)
Peneroplis pertusus									1 (GR7)	1 (GR7)
Quinqueloculina parvula										1 (GR7)
Quinqueloculina seminulum									1 (GR7)	3 (GR35); 3 (GR7)
Siphonaperta aspera									1 (GR7)	1 (GR7)

Cluster C includes 7 samples located out of the bay, with the highest species diversity (median H: 2.7) and high abundance of *S. aspera* (median 16.8%), associated to *A. beccarii* (median 11.1%) and *T. schreiberiana* (median 9.3%). These samples show FN ranging from 1 to 39, and FAI, from 1 to 8.

4.3. Test geochemistry

The EDS microanalyses, carried out on normal and deformed foraminifera, highlighted the presence of heavy metals in the calcite crystal lattice of all the analysed species. From the multiple analyses,

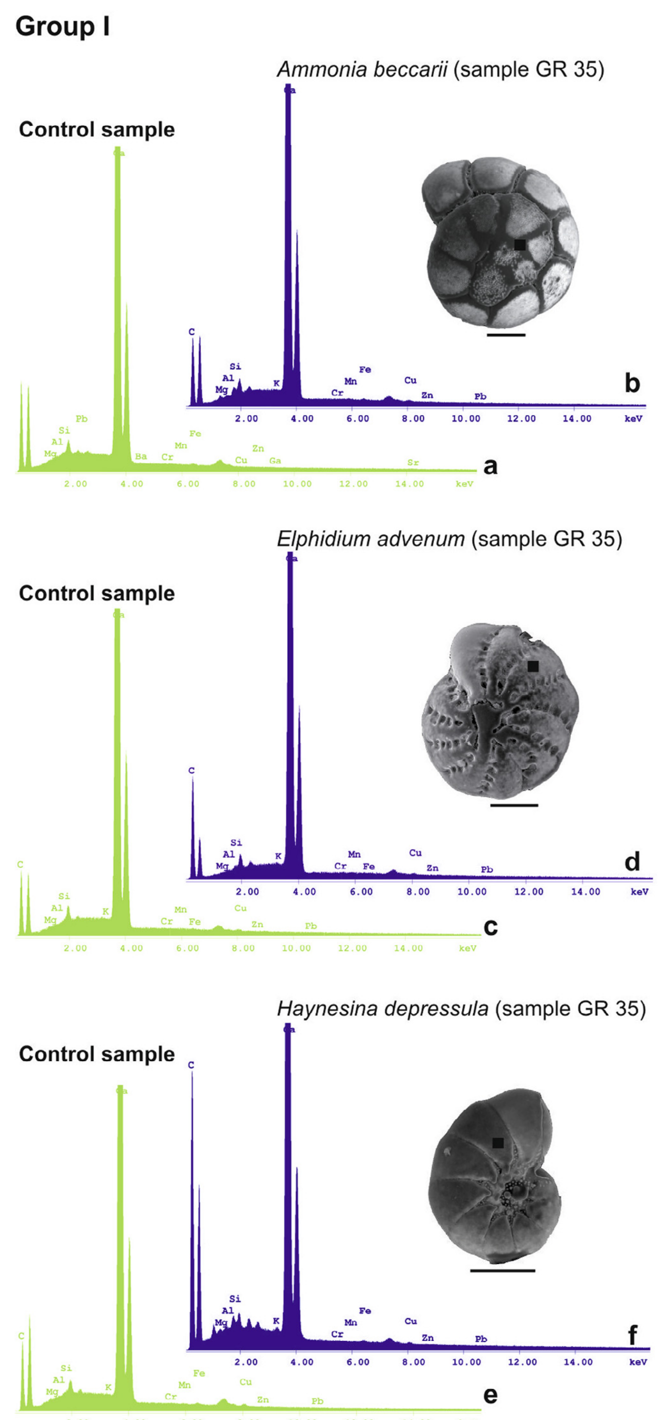


Fig. 7. EDS analysis and SEM micrographs (scale bar = 100 µm) of foraminifera belonging to Group I (only normal specimens). The areas analysed with EDS are indicated by black squares: (a) EDS analysis on *Ammonia beccarii* from a control sample; (b) EDS analysis on *A. beccarii* from sample GR35; (c) EDS analysis on *Elphidium advenum* from a control sample; (d) EDS analysis on *E. advenum* from sample GR35; (e) EDS analysis on *Haynesina depressula* from a control sample; (f) EDS analysis on *H. depressula* from sample GR35.

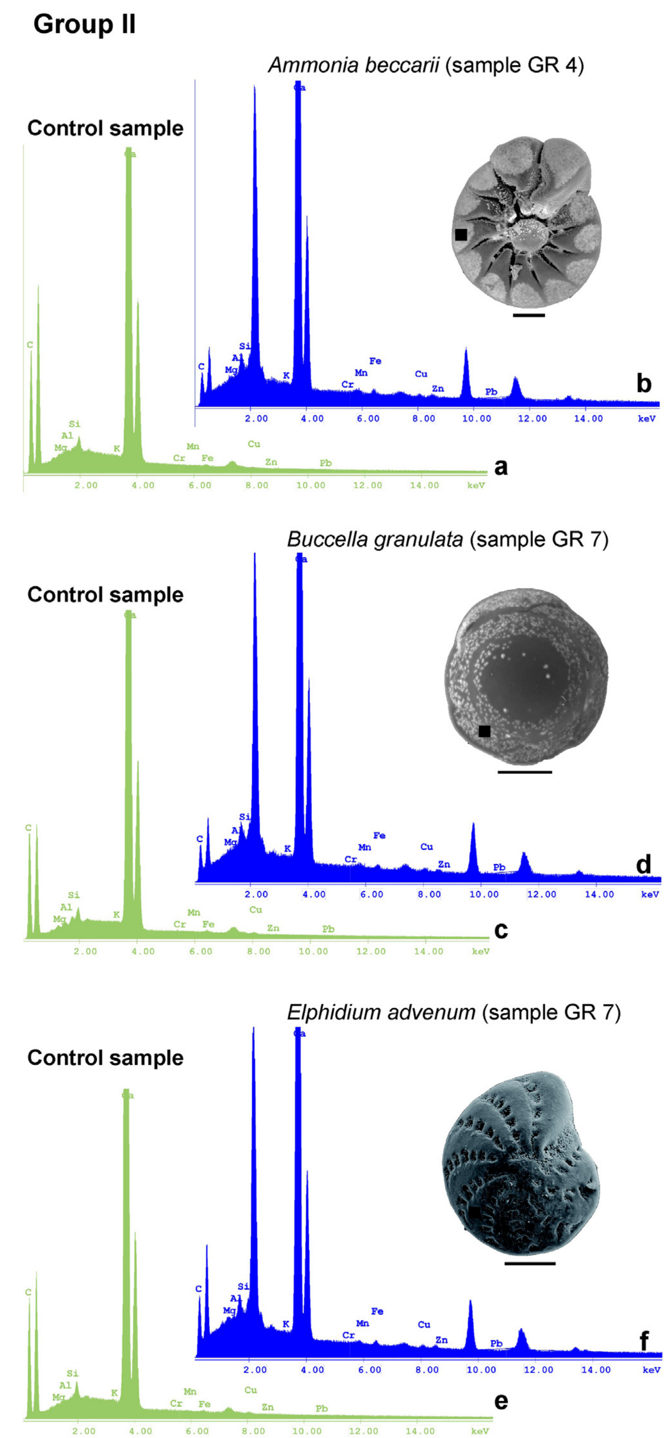


Fig. 8. EDS analysis and SEM micrographs (scale bar = 100 µm) of foraminifera belonging to Group II (only normal specimens). The areas analysed with EDS are indicated by black squares: (a) EDS analysis on *Ammonia beccarii* from a control sample; (b) EDS analysis on *A. beccarii* from sample GR4; (c) EDS analysis on *Buccella granulata* from a control sample; (d) EDS analysis on *B. granulata* from sample GR7; (e) EDS analysis on *Elphidium advenum* from a control sample; (f) EDS analysis on *E. advenum* from sample GR7.

carried out on deformed specimens, it was recognized that heavy metals were present indifferently in deformed and not deformed chambers. The recognized metals were Cu (8.0 keV), Fe (6.4 keV), Zn (8.6 keV), and Mn (5.8 keV), while a peak around 7.6 keV (corresponding to Ni) was probably due to false effect of the instrument, due to the sum-peak of Ca. Sum peaks are produced when the pulse processor cannot distinguish between two X-rays arriving almost simultaneously. In this case, instead of recording two distinct X-rays, a single one, with energy equal to the sum of the energies of the two incoming X-rays, is recorded and plotted in the spectrum. The amplitude of the Ca sum peak is positively correlated to the Ca content. As a result, the false effect is strongly reduced in the imperforate tests, characterized by higher Mg

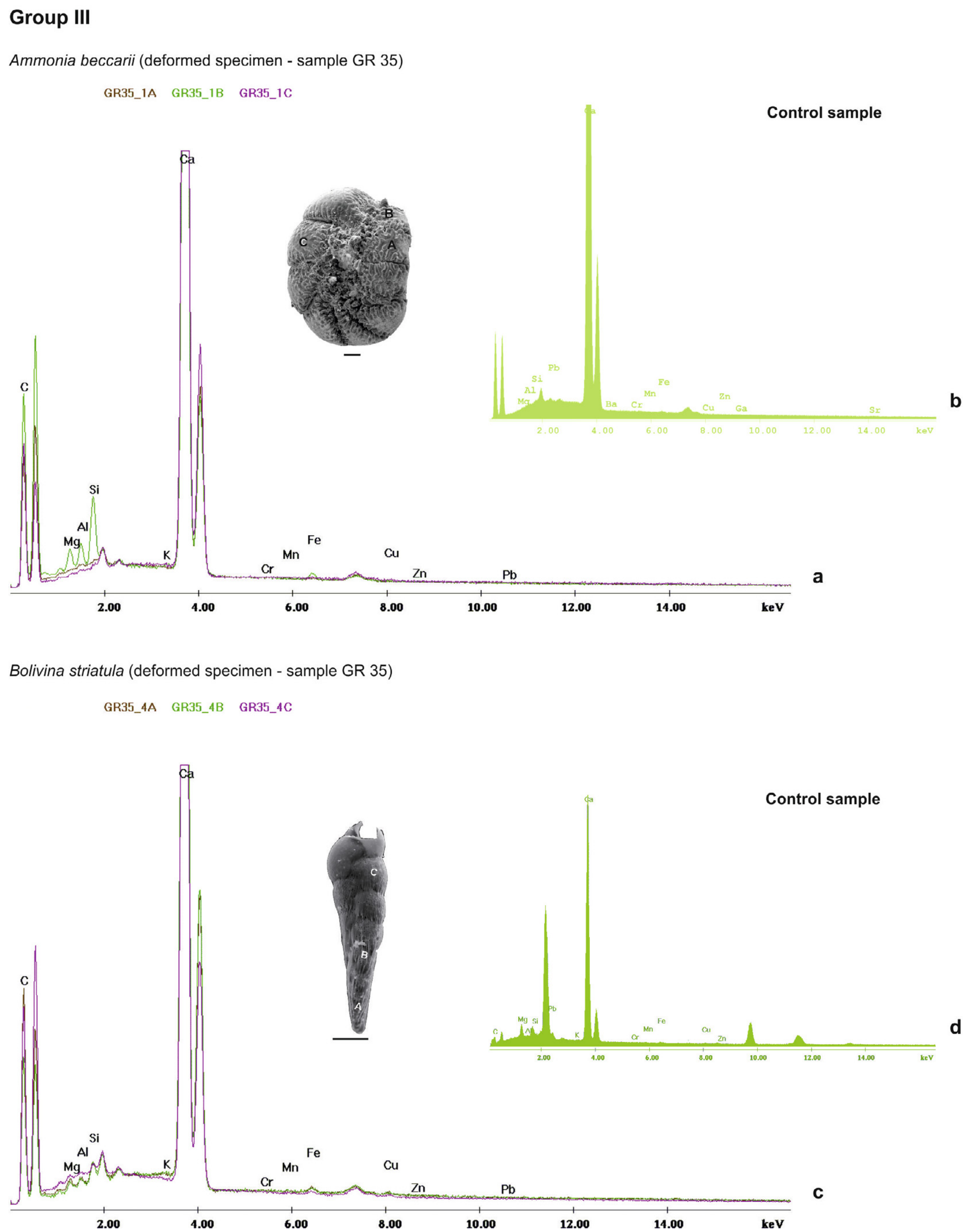
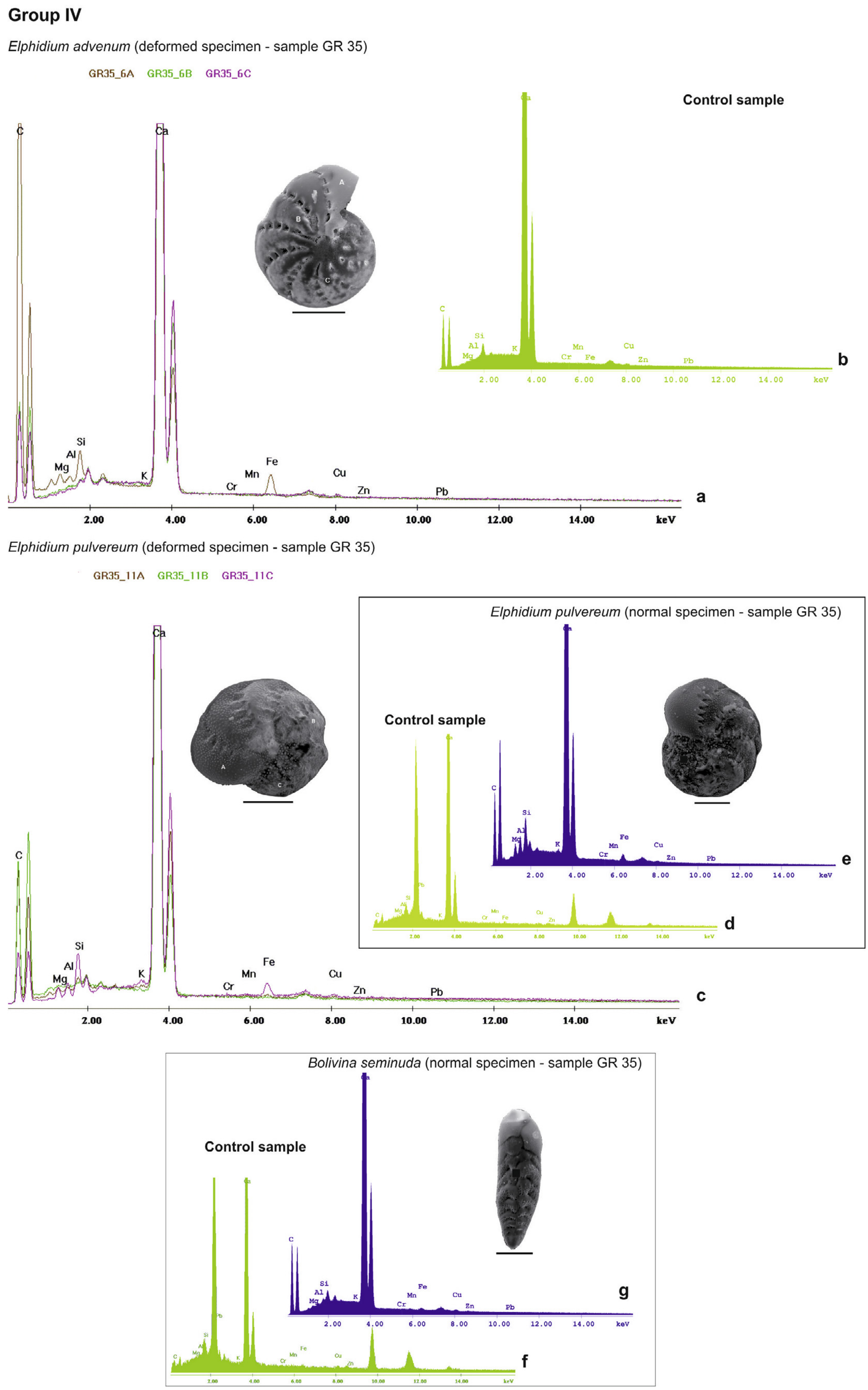


Fig. 9. EDS analysis and SEM micrographs (scale bar = 100 µm) of foraminifera belonging to Group III (only deformed specimens). The areas analysed with EDS are indicated by the letters A, B and C: (a) EDS analysis on *Ammonia beccarii* from sample GR35; (b) EDS analysis on *A. beccarii* from a control sample; (c) EDS analysis on *Bolivina striatula* from sample GR35; (d) EDS analysis on *B. striatula* from a control sample.

and lower Ca content.

Considering the occurrence of the four metals recognized by EDS, five compositional groups were identified (Table 4):

- Group I (17 specimens), composed by normal specimens, is substantially characterized by the absence of heavy metals. It is constituted of *A. beccarii*, *E. advenum*, *E. pulvereum* and *H. depressula* from samples GR3 and GR35. By compositional viewpoint, these tests are similar to those of control specimens as highlighted by EDS diagrams (Fig. 7).
- Group II (9 specimens), represented exclusively by normal specimens, is characterized by the occurrence of the binomial Cu-Zn. These specimens, present in samples GR3, GR4 and GR7 (Fig. 8), are represented by *A. beccarii*, *B. granulata*, *E. advenum* and *E. granosum*.
- Group III includes 2 deformed specimens belonging to *A. beccarii* and *B. striatula* species, both from sample GR35. It is characterized by the only presence of Fe (Fig. 9).
- Group IV (4 normal and 4 deformed specimens) is characterized by the co-occurrence of Fe-Cu. This group includes specimens of *A. beccarii*, *B. seminuda*, *B. striatula*, *E. advenum* and *E. pulvereum* from samples GR3 and GR35 (Fig. 10).
- Group V is constituted by 8 normal and 11 deformed specimens and is characterized by the occurrence of Fe, Cu and Zn. The species are *A. beccarii*, *B. seminuda*, *E. advenum* and *E. pulvereum* from samples



(caption on next page)

Fig. 10. EDS analysis and SEM micrographs (scale bar = 100 μm) of foraminifera belonging to Group IV. The areas analysed with EDS are indicated by the letters A, B and C (deformed specimens) or by black squares (normal specimens): (a) EDS analysis on *Elphidium advenum* (deformed specimen) from sample GR35; (b) EDS analysis on *E. advenum* from a control sample; (c) EDS analysis on *Elphidium pulvereum* (deformed specimen) from sample GR35; (d) EDS analysis on *E. pulvereum* from a control sample; (e) EDS analysis on *E. pulvereum* (normal specimen) from sample GR35; (f) EDS analysis on *Bolivina seminuda* from a control sample; (g) EDS analysis on *B. seminuda* (normal specimen) from sample GR35. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

GR3 and GR7. This group includes also tests with high Mg content, typical of miliolids, characterized by the presence of Mn. The most abundant species are *M. subrotunda, P. pertusus*, *Q. seminulum* and *S. aspera*, from samples GR7 and GR35 (Fig. 11).

5. Discussion

5.1. Significance of dead and living foraminiferal assemblage

The total assemblage was considered in order to obtain reliable calibration proxies between bio and thanatocoenosis (Leorri et al., 2008; Frezza and Carboni, 2009; Fontanier et al., 2014; Jorissen et al., 2018). However, potential post-mortem processes may affect dead foraminiferal assemblages and should be taken into account in order to obtain consistent ecological information. Samples of the present study did not show evidences of significant taphonomical changes such as bad preservation, reworked specimens or uncommonly high densities (Jorissen et al., 2018).

In order to avoid establishing the ecological interpretation on allochtonous taxa, only species with living representative in the study area were included in the matrix used for statistical elaboration. The proportion of dead and living individuals for each of these species in the total assemblage is shown in Fig. 12.

A lower proportion of living specimens is represented mostly by miliolid species such as *T. schreiberiana* and *S. aspera*, at least in part because of the difficulty to detect the rose Bengal stain due to the thick wall. For such reason, these taxa may be underestimated in the living assemblage.

The exclusive, or nearly exclusive, presence of living specimens of small and fragile hyaline species such as *Patellina corrugata* and *B. seminuda* let us suppose the action of post-mortem processes that eliminate part of these specimens from the dead assemblage through selective transport or crushing; dissolution is less probable because carbonate tests do not show evidences of this process. However, their high abundance testifies that sampling methods did not determine significant loss of living taxa.

Larger hyaline taxa, such as *Elphidium* spp., which are represented by living specimens with percentages ranging from 30 to 50%, seem not affected by significant taphonomic processes. For those species, such as *B. granulata* and *Astrononion stelligerum*, which include a smaller part of living specimens, it may be supposed that seasonality of reproductive cycles plays an important role.

5.2. Response of foraminiferal assemblages to the environmental stress

Samples collected in the northern sector are almost barren, probably due to the hydrodynamic features associated to human-induced activities, determining an erosive trend at the seafloor which causes the transport offshore of the pelitic fraction. Then, physical disturbance may be considered as the main stressor in this sector (Badawi and El-Menhawey, 2015). In the southern sector were found living and dead foraminifera, although assemblages are characterized by low diversity, low density and high percentages of deformed specimens, which highlight unfavourable ecological conditions. This area is mainly characterized by fine sands with variable content of pelitic fraction in the inner part of the bay, which testifies lesser hydrodynamism. Although in this study data on salinity and organic matter are not available, the scarcity of euryhaline and eutrophic taxa let us exclude a strong influence of these parameters on the foraminiferal assemblages.

The sediment metal enrichment was considered as a possible environmental stressor for benthic foraminifera because of the general Cr and Ni geochemical anomaly which characterizes marine sediments in the most part of the Ligurian region (Cosma et al., 1979, 1982). Moreover, Capello et al. (2016) considers the localized enrichment of Cu and Zn at the mouth of the Gromolo stream as deriving from the crossing of the watercourse through the Libiola mining area.

This geochemical setting is in good agreement with the features shown by the benthic foraminiferal assemblages identified by cluster analysis. The highest level of environmental stress is recorded close to the Gromolo mouth, where the less diversified assemblage, dominated by *A. beccarii*, was recognized. A more diversified assemblage, characterized by the association of *H. depressula* and *A. beccarii*, is a little further away from the mouth of the stream. Out of the bay species diversity increases and a faunal shift is recognizable with respect to the inner sector of the bay, because the most abundant species are miliolids such as *S. aspera* and *T. schreiberiana*. Nevertheless, the H index values (1.4–3.3) are still low in comparison with other stressed areas such as the Augusta harbour (Sicily, Italy), where it ranged between 3.1 and 3.5 in the contaminated sediment (Romano et al., 2013). Other studies demonstrated a decrease of foraminiferal diversity as a response to sediment contamination due to heavy metals (Burone et al., 2006; Ferraro et al., 2006; Cherchi et al., 2009).

The tolerance to heavy metal enriched sediments mainly of *A. beccarii*, which shows opportunistic behaviour for the high dominance (up to 63%), and also of *H. depressula*, is testified by the presence of prevalent living specimens especially in samples GR2 and GR3. *Ammonia beccarii* is an infaunal oligotrophic taxon, typical of shallow water sandy bottoms, while *H. depressula* is an infaunal species typical of infralittoral fine sands (Murray, 1991, 2006; Sgarrella and Moncharmont-Zei, 1993). Culture studies recognized *Ammonia* species as potential bio indicators of Cu contamination because they survived at high concentrations, although they were affected by low density, dwarfism and increasing abundance of morphological deformations (Le Cadre and Debenay, 2006). The tolerance of both *A. beccarii* and *H. depressula* to polluted environments is also reported by Yanko (1999).

The CCA (Fig. 13) was carried out in order to recognize the effects of sediment fractions on species distribution and faunal parameters, taking into account that sediment texture is a main factor influencing distribution of benthic foraminifera also in contaminated areas (Celia Magno et al., 2012). From the scatter diagram it may be observed that gravel plots on the first axis, with sample GR30, which is the only one containing some gravel (5.6%). This sample, which is also characterized by the coarser sandy fraction (Fig. 2), shows the highest percentages of *Gavelinopsis praegeri* and *P. corrugata*, which are both epifaunal clinging taxa that may live attached to coarse grains (Murray, 2006). The other samples are aligned along the second axis, which corresponds to increasing silt and clay. Foraminiferal species are also distributed along the second axis, to indicate the strong influence of sediment texture on their distribution: the ones along the negative side of the second axis, associated to the highest sand values are *Adelosina cliariensis*, *T. schreiberiana*, *S. aspera* and *S. quadrata*, while the ones along the positive side, associated with highest values of silt and clay are *E. pulvereum*, *H. depressula* and *Bolivina* spp. The position close to the axes origin of some of the most abundant species such as *A. beccarii* and *E. advenum*, points out that sediment texture is not the only factor determining their distribution. The H index and FAI do not appear correlated to sediment

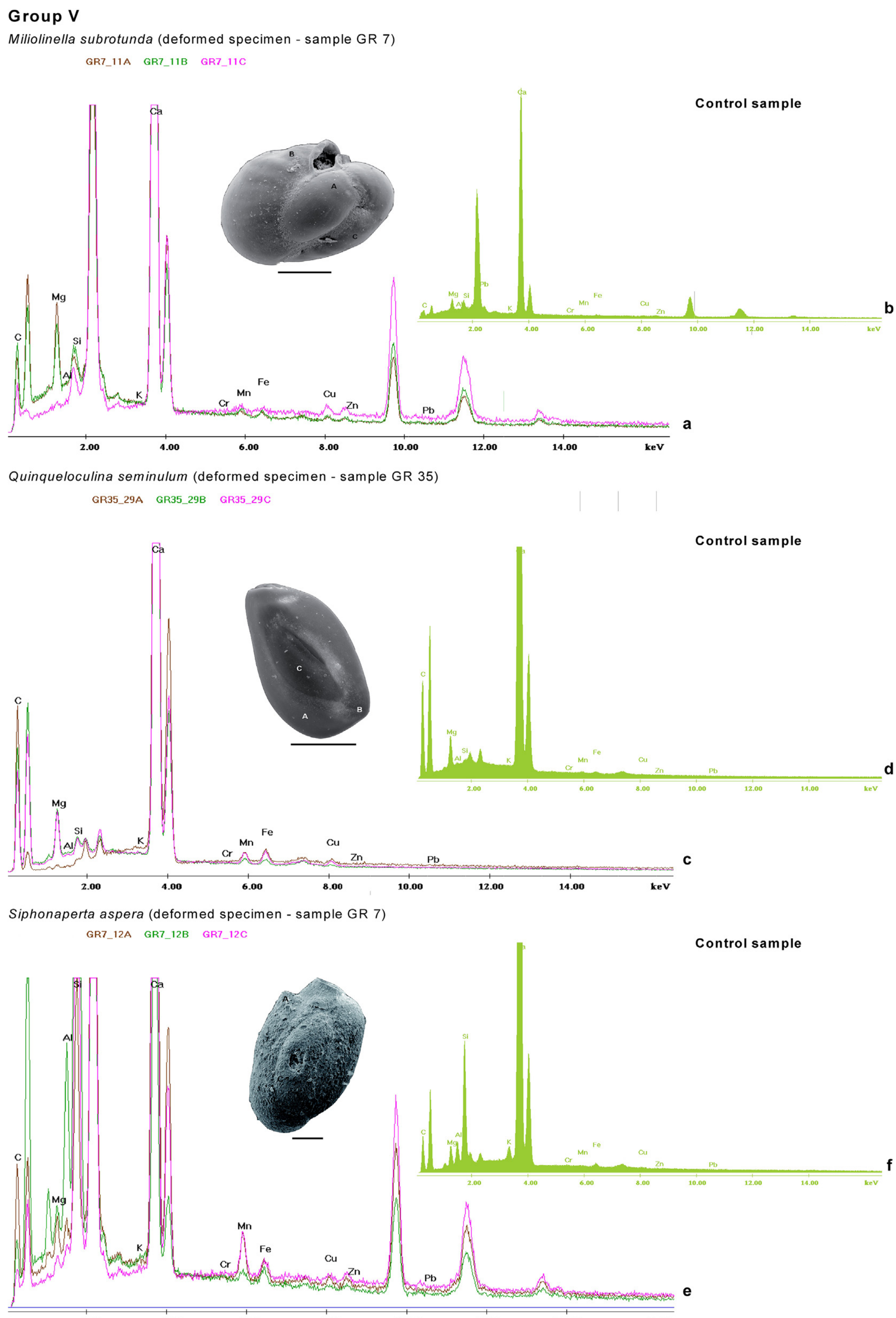


Fig. 11. EDS analysis and SEM micrographs (scale bar = 100 µm) of foraminifera belonging to Group V. The areas analysed with EDS are indicated by the letters A, B and C: (a) EDS analysis on *Miliolinella subrotunda* (deformed specimen) from sample GR7; (b) EDS analysis on *M. subrotunda* from a control sample; (c) EDS analysis on *Quinqueloculina seminulum* (deformed specimen) from sample GR35; (d) EDS analysis on *Q. seminulum* from a control sample; (e) EDS analysis on *Siphonaperta aspera* (deformed specimen) from sample GR7; (f) EDS analysis on *S. aspera* from a control sample.

fractions, while FN is positively correlated to silt and clay.

From the above discussion, it may be deduced that, while species diversity and assemblage composition appear conditioned by environmental stress due to sediment metal enrichment, foraminiferal density is mainly influenced by the percentage of fine sediment fraction. The prevalence of sandy fraction, probably correlated to scarce nutrient availability, explains the generalized low FN in the study area.

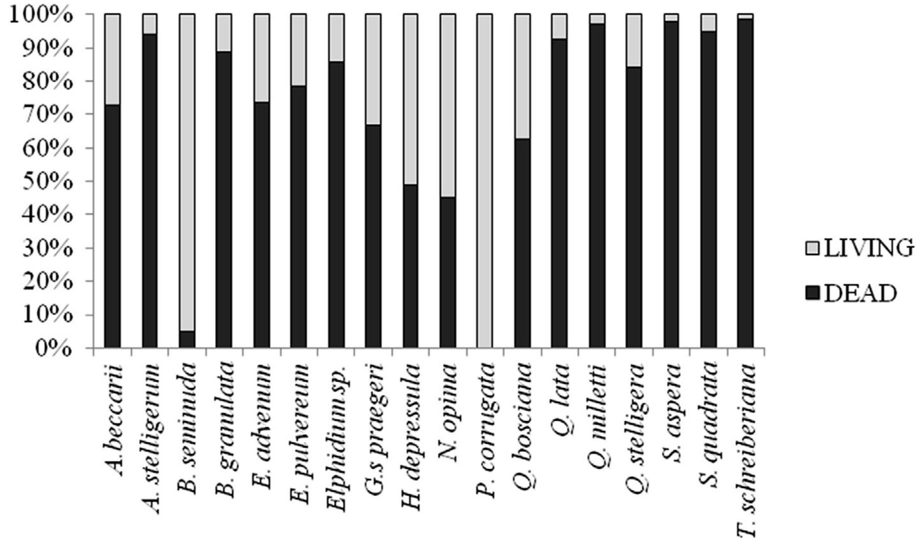


Fig. 12. Proportion between living and dead specimens of commonly occurring species (> 5% in at least one sample).

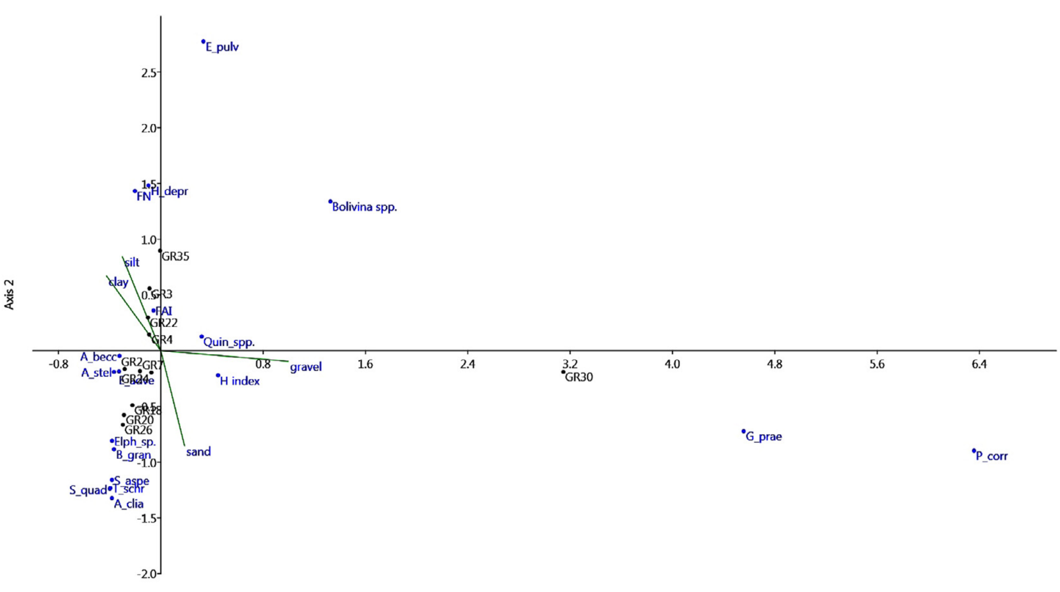


Fig. 13. Canonical Correspondence Analysis (CCA) based on foraminiferal relative abundance, faunal parameters and grain size fractions.

5.3. Foraminiferal proxies for environmental stress: broader implications

The results of this study point out that, among the faunal parameters potentially influenced by environmental stress, only assemblage composition and species diversity are actual stress proxies. Consequently, future biomonitoring surveys in this area could apply a stress index based on the abundance of ecological groups of species with different response to stress, such as the Foram-AMBI (Jorissen et al., 2018), or on the decrease of species diversity with respect to reference conditions, such as the EcoQS (Bouchet et al., 2012). However, the assemblage in the inner bay shows high percentages of *A. beccarii* and *H. depressula* which are considered, according to Jorissen et al. (2018), as indifferent species, not typical of stressed environment, in apparent contradiction with the low diversity of the assemblage, which points out significant environmental stress. This may be due to the fact that the species assignment to ecological categories of the Foram-AMBI index is based on the sediment organic enrichment, while the environmental problem of our study area is due to heavy metals. Consequently, the EcoQS appears the best choice in our case study. Then, although the foraminiferal stress indices are, in general, innovative and powerful tools for environmental assessment, any index supplies only limited information on the ecological status, and the use of indices based on different principles could lead to apparently contradictory results. For this, for an area never studied before, affected by multiple environmental stressors of different origin, it should be recommendable to carry out a preliminary characterization of foraminiferal faunas, in order to recognize the foraminiferal proxies really responding to the environmental stress and, consequently, to apply the suitable stress index for biomonitoring purposes.

5.4. Test geochemistry and deformity

According to Alve (1991), who reported that 1% of abnormal tests can be considered as a natural level in unstressed population, the development of abnormalities exceeded natural threshold in seven stations (GR3, GR4, GR7, GR22, GR24, GR26, GR35), where FAI ranged between 2 and 8 (Table 3).

High FAI values may be attributed to a wide variety of factors, both

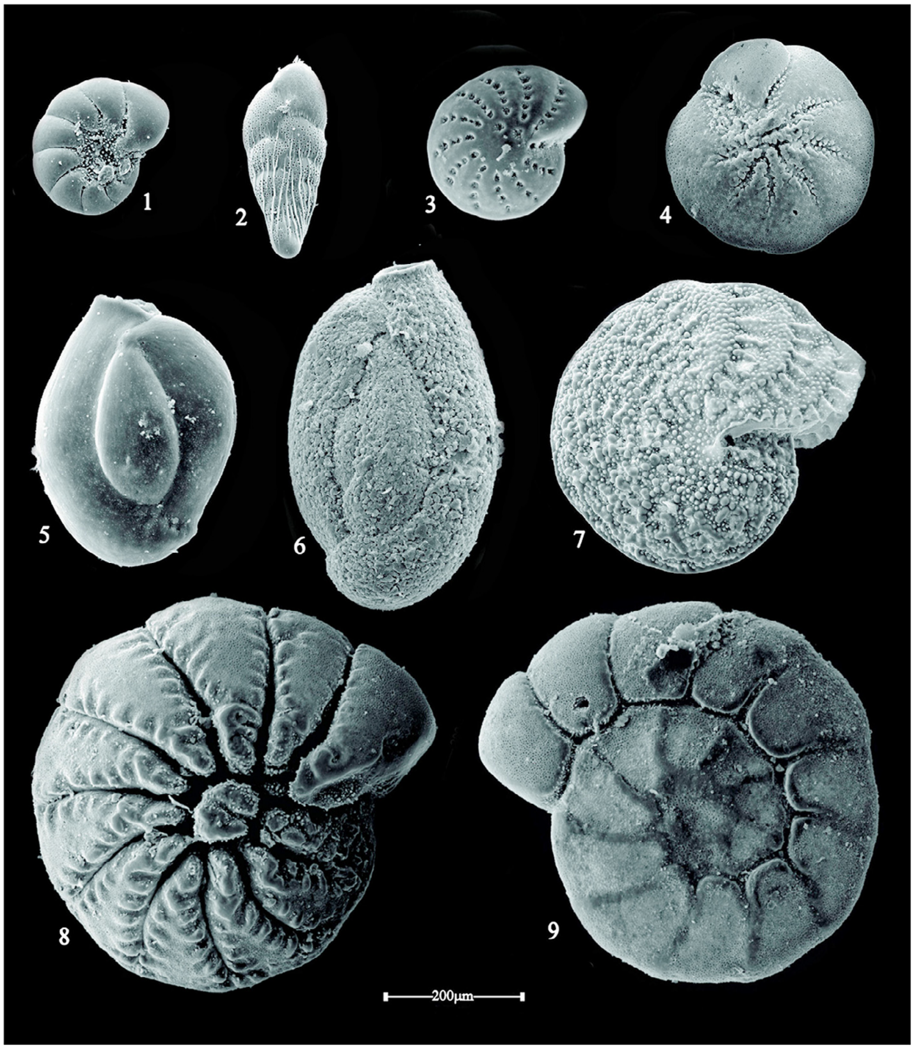


Plate 1. Normal specimens of the most abundant species present in the study area: 1) *Haynesina depressula* side view; 2) *Bolivina striatula*, side view; 3) *Elphidium advenum*, side view; 4) *Buccella granulata*, ventral view; 5) *Triloculina schreiberiana*, side view; 6) *Siphonaperta aspera*, side view; 7) *Elphidium pulvereum*, side view; 8) *Ammonia beccarii*, ventral view, 9) *Ammonia beccarii*, dorsal view.

natural and anthropogenic, all ascribable to environmental stress (Geslin et al., 2000, 2002; Carboni et al., 2009; Romano et al., 2009; Caruso et al., 2011; Lei et al., 2015; Youssef, 2015). Some EDS analyses conducted on both normal and deformed foraminiferal tests from heavy metal polluted areas revealed the anomalous presence of metals only in deformed tests; however, these studies did not carry out a systematic analytic survey on a high number of foraminiferal tests, suitable to exclude the presence of metals at least in a small percentage of the normal specimens (Samir and El-Din, 2001; Romano et al., 2008; Frontalini et al., 2009). On the other hand, several culture studies recognized the development of abnormalities in treatments with high concentrations of heavy metal such as Cu (Le Cadre and Debenay, 2006; Frontalini and Coccioni, 2012) and Hg (Nigam et al., 2009), but did not investigate the actual process determining the morphological abnormality of tests. Then, neither experimental study clarified if the inclusion of anomalous elements in the crystalline framework of carbonate foraminiferal tests is the reason of morphological abnormalities and this hypothesis is still matter of debate (Geslin et al., 1998).

Although FAI values > 1 are patchy distributed in the study area and not associated to a specific sector, this anomaly with respect to a healthy environment is worthy of note. The EDS analyses highlighted the inclusion of heavy metals in the carbonate test of foraminifera from the study area, not present in the control specimens from unimpacted marine areas, and these elements were present both in normal and deformed specimens. Consequently, it may be supposed that environmental stress plays a role in the inclusion of heavy metals in calcite crystal lattice. Probably, the development of abnormalities occurs when heavy metals concentration in the test exceeds well-defined thresholds (Austen et al., 1994; El-Kahawy et al., 2018), even if the present results indicate that it should not be directly linked to the heavy metals ions acquisition during the building of the test. This is confirmed by the presence of abnormalities in the agglutinated foraminiferal tests (e.g. *Eggerelloides scaber*) that are not derived from carbonate production directly by the organism. The development of the morphological abnormalities could be mainly linked to the effect of heavy metals on cytological activities of the cell. Recent studies demonstrated significant cytological changes induced in specimens by exposure to high trace metal concentrations, consisting of thickening of organic lining, proliferation of fibrillar and large lipid vesicles, increased number of residual bodies (Le Cadre and Debenay, 2006; Frontalini et al., 2017) and other cytological damages that lead to the death of the cell (Bresler and Yanko, 1995). Each species has its own threshold of sensitivity to different types and degree of pollution (Sharifi et al., 1991; Madkour and Alì, 2009), over that the toxic effects can induce cytological modifications with different consequences on the organism. This could explain that specimens of the same species belong to different compositional groups (Table 4).

In the present study, the geochemical composition of foraminiferal

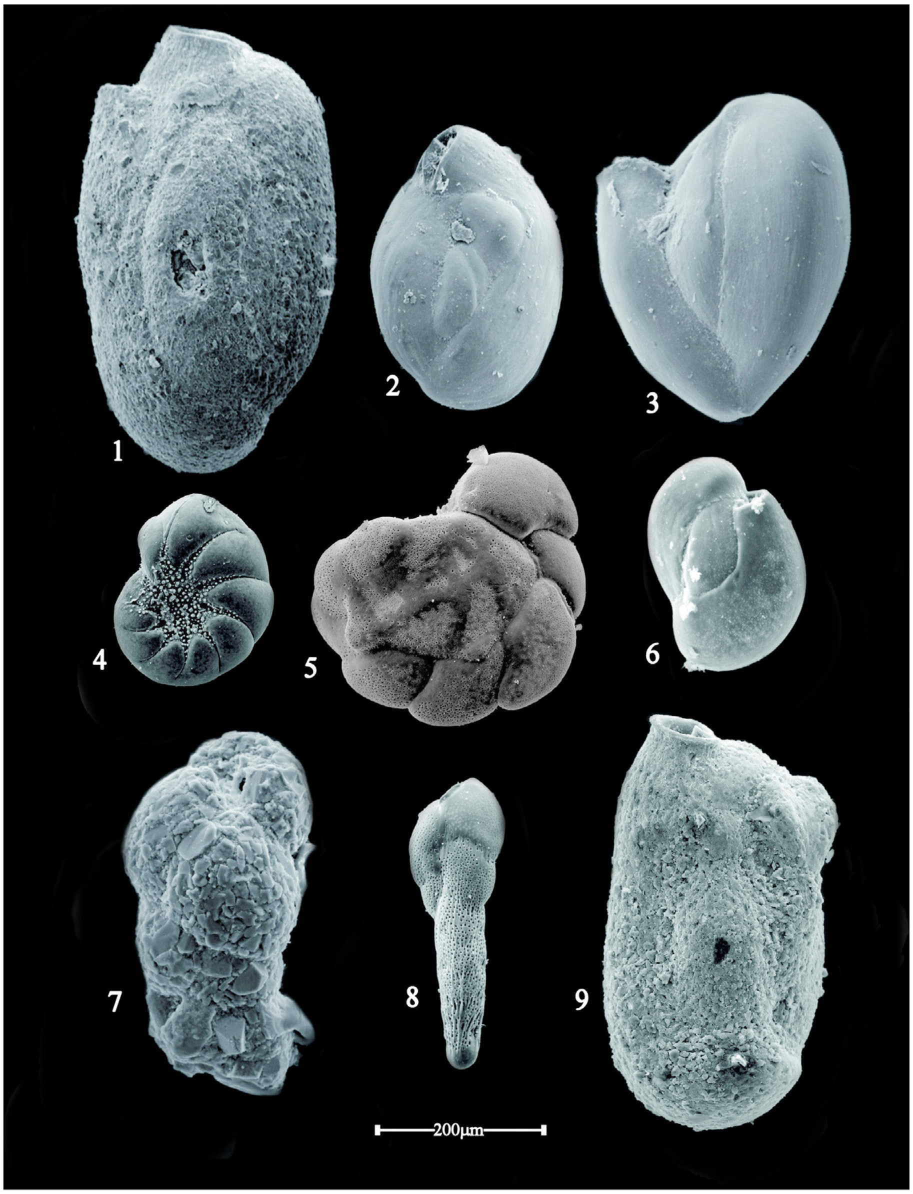


Plate 2. Deformation types according to the categories described in Table 2. 1) *Siphonaperta aspera*, side view, incomplete development of the last whorl and double aperture; 2) *Triloculina schreiberiana*, side view, aberrant chamber; 3) *Triloculina schreiberiana*, side view, incomplete development of the last whorl; 4) *Haynesina depressula*, aberrant chamber; 5) *Ammonia beccarii*, dorsal view, aberrant chambers; 6) *Triloculina schreiberiana*, side view, incomplete development of the last whorl; 7) *Eggerelloides scaber*, side view, change in coiling axis; 8) *Bolivina striatula*, side view, change in coiling axis; 9) *Quinqueloculina parvula*, side view, protuberance on one chamber.

tests displayed a partial correlation with some of the metals found in the sediment. As reported above, in literature data, significant concentrations of Ni, Cr, Cu, Zn and Mn were recognized in the sediments of the study area. The EDS analyses testified that both normal and deformed foraminifera included these heavy metals except for Cr and Ni, which were not recorded by means of EDS analysis. Although Cr is considered, together with Cu and Zn, as more easily absorbed component (Banerji, 1992), the absence of Cr in the test composition of our specimens is probably due to the lack of bioavailability in the study area which is generally related to biological, geochemical and physical environmental factors (Alve and Olsgard, 1999; Armynot du Châtelet et al., 2004; Youssef et al., 2017). As regards Ni, the presence of this element in the tests cannot be excluded because, resulting from EDS analyses, its peak is covered by the sum-peak of Ca.

In the present study, Cu was commonly recorded associated with different elements. It is known that the toxicity of Cu is controlled by the Cu/Zn ratio in the marine ecosystem (Ramadan and Shata, 1993; Youssef et al., 2017). Probably, the presence of the only couple Cu and Zn (Group II), in normal specimens, determines a certain equilibrium inhibiting the mutual toxicity and preventing the development of morphological abnormalities. In the case of other metals (Fe, Mn) joining with Cu in significant concentrations, the toxicity effect could be amplified determining morphological deformities. This could justify the great number of deformed tests in the group V. This group includes many porcelaneous tests resulting the specimens with a higher inclination to acquire external heavy metals (Titelboim et al., 2018). The nature of porcelaneous tests, naturally characterized by high Mg content, could explain the presence of a higher number of components, because Mg ions can be easily replaced by other ions like Mn (Dixon and Webb, 1979; Bresler and Yanko-Hombach, 2000; Bentov and Erez, 2006; Youssef, 2015).

6. Conclusion

In this study, recent shallow water benthic foraminiferal assemblages of the Eastern Ligurian coast were studied, characterized and applied as indicators of ecological stress. Different environmental stressors were recognized to affect foraminiferal assemblages. The generalized low species diversity is considered as indicative of widespread environmental stress associated to the natural Cr and Ni enrichment of sediment, which characterizes the Ligurian coastal zone, due to the outcropping of ophiolitic formations in the mainland.

Additional environmental stress seems associated to local conditions, which characterize the inner bay, close to the Gromolo mouth, and directly influences species diversity and assemblage composition. Species diversity may be considered as an indicator of environmental status. Differently, low foraminiferal abundance, more marked along the coastal belt where samples are nearly barren, is mainly attributable to the environmental instability determined by the erosive trend due to anthropogenic impact. Then, the faunal density was considered to be mainly conditioned by physical disturbance and sediment texture more than by sediment geochemistry. These results point out that, for an area affected by multiple environmental stressors of different nature, a preliminary characterization of foraminiferal faunas is recommendable in order to identify the most reliable stress index for biomonitoring purposes.

The abundance of deformed specimens above natural background levels, although patchy distributed in the study area, could be regarded as another possible evidence of generalized stressed conditions due to the natural heavy metal enrichment characterizing marine sediments. EDS analyses revealed that foraminifera of the study area included in the carbonate test heavy metals, not present in control specimens from unimpacted areas, indicating a possible influence of environmental stress in the biomineralization processes and the role of these elements in the development of abnormalities at cytological level. The common presence of Cu-Zn in normal specimens points out the role of Zn in the control of Cu toxicity effects, while the high Mg content characterizing the porcelaneous tests makes these specimens more suitable to include anomalous elements and to develop morphological deformations.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ecolind.2018.08.050.

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