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## Distribution and morphological abnormalities of recent foraminifera in the Marano and Grado Lagoon (North Adriatic Sea, Italy)

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### Abstract

The Marano and Grado Lagoon, is a northern Adriatic wetland system of relevant naturalistic and economic value that is constantly under quality control in accordance with the current environmental directives. The benthic foraminifer community with its morphological abnormalities was investigated in the recent sediments (about 10 years old) of 21 stations; the samples were collected within the framework of the “MIRACLE” Project, which aimed at testing the coexistence of clam farming with high Hg contamination. Euryhaline foraminifers, well known in Mediterranean brackish-waters, mainly characterize the total assemblage. *Ammonia tepida* dominates in areas characterized by low salinity, high clay and organic carbon content, but is also subjected to anthropogenic pressure. *Elphidium gunteri* and *Haynesina germanica* are recorded in the western sector of the lagoon, which is more affected by salinity variations and agricultural activities. Slightly higher values of assemblage diversity appear in less restricted areas of the lagoon or, at least, where physical parameters such as temperature and salinity are less variable. The test abnormalities, carried out on total assemblage, show that the FAI (Foraminiferal Abnormality Index) values always exceed 1% of the total assemblage, with clear decreasing gradients from inland to the sea (from N to S) and from W to E in the studied area.

**Keywords:** Foraminifers, Marano and Grado Lagoon, Recent, paralic assemblage, test abnormality.

### Introduction

Lagoons are peculiar naturalistic environments, characterized by an extremely delicate biological balance, that host a large variety of living organisms. Together with fjords, deltas, and estuaries, they represent the classic paralic environment, where the variation of salinity, oxygenation, nutrients, etc. creates a complex degree of confinement, which represents the time necessary for the renewal of marine waters (Guelorget & Perthuisot, 1983, 1992). In the recent decades, lagoons, like other coastal marine environments, have been subjected to intensive, human-related uses with severe consequences on the aquatic ecosystem. These human actions produce different biological effects, both in animal and plant species, whose study allows us to identify biological indicators (also known as bio-indicators) that are able to signal changes caused by human activity. A component of the meiofauna, benthic foraminifera (protists), have a well-established record as environmental bio-indicators in coastal and transitional waters, and many studies have focused on the response of the foraminifers to various forms of pollutants, such as trace elements, hydrocarbons, thermal pollution etc. (see reviews by Martinez-Colon *et al.*, 2009; Arminot du Châtelet & Debenay, 2010; Frontalini & Coccioni, 2011).

It has been noted that in stressed and/or polluted environments, these protozoa may react through a series of changes that affect not only variations in abundance and species richness (and, therefore, the structure of the community), but also the development of morphological abnormalities of the test. These abnormalities, which are found mostly in calcareous species, can affect the shape, size or disposition of one or more chambers of the test. This may be due to a diverse crystalline organization or the development of cavities between the inner and outer lamellae of the chambers, or regeneration after damage to the test (termed ‘deformation’) (Geslin *et al.*, 1998; Stouff *et al.*, 1999). Abnormal tests can be presented in a variety of ways and several classifications of these abnormalities have been proposed (e.g. Alve, 1991; Yanko *et al.*, 1994, 1998; Geslin *et al.*, 1998). Foraminifera test abnormalities are frequently observed in naturally stressed marine environments – possibly induced by a deviation from optimum chemical and physical parameters, such as temperature, salinity, dissolved oxygen, organic matter etc. (Boltovskoy *et al.*, 1991; Almogi-Labin *et al.*, 1992; Yanko *et al.*, 1998; Geslin *et al.*, 2000) – as well as in environments affected by relevant anthropogenic pollution.

Based on these results, the main efforts are now focused on including these organisms in monitoring protocols (Frontalini & Coccioni, 2011; Bouchet *et al.*, 2012; Schönfeld *et al.*, 2012).

This paper investigates the distribution of the recent foraminifera in the Marano and Grado Lagoon, where data are scarce until now (Masoli *et al.*, 1995). Unpublished preliminary data regarding their distribution and the occurrence of morphological anomalies of foraminifer tests on recent and Holocene sediments are provided by Bugarski (2011).

The foraminifera of the Marano and Grado Lagoon were studied in order to address the following goals:

1- To increase and update the knowledge on recent benthic foraminifera in this lagoon environment.

2- To investigate the relationships between the distribution of foraminifera and a number of sedimentary features, such as grain-size and organic matter content.

3- To describe the morphological abnormalities of tests, which can be initially attributed to certain adverse local environmental conditions such as, for instance, the high content of mercury in the sediments.

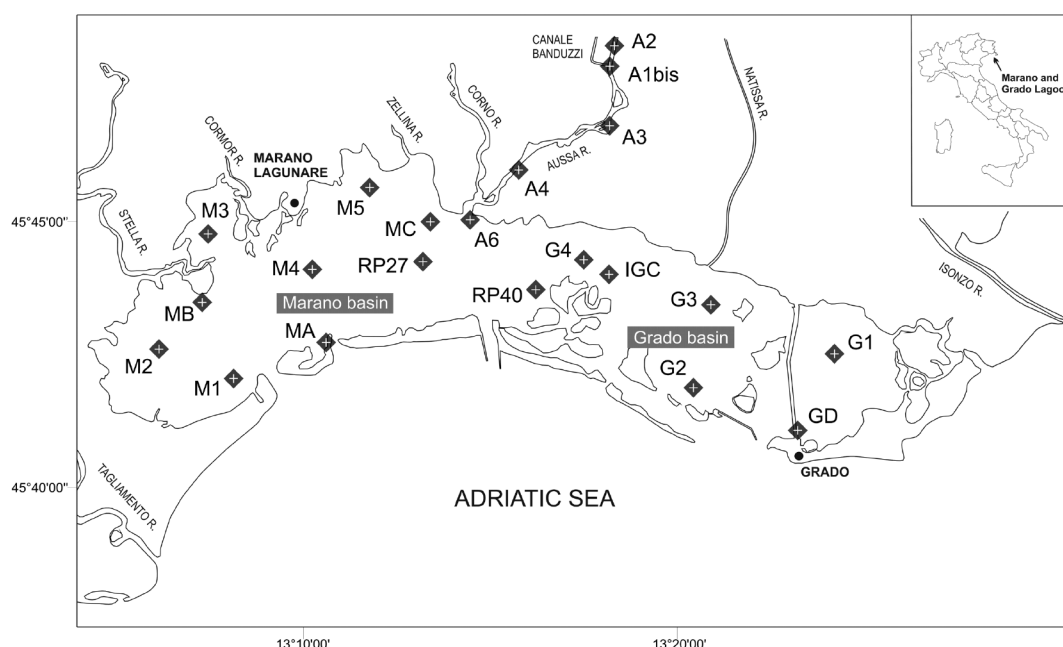
## Study area

Marano and Grado Lagoon is a coastal shallow water system located along the northern Adriatic coast between the Tagliamento and Isonzo River deltas, covering an area of 160 km<sup>2</sup> (Fig. 1). Two major sub-basins, with different geomorphological and geological evolution, characterize the study area. The Marano Lagoon (90 km<sup>2</sup>) is the western semi-enclosed tidal basin, with few marshes and islands above mean sea level. It is mainly affected by the terrigenous inputs of the Tagliamento River, but it also receives freshwater directly from several other spring rivers (from west to east: the Stella, Cormor, Zेलina and Aussa-Corno rivers). Conversely, in the east-

ern part, the Grado Lagoon (70 km<sup>2</sup>) is characterized by several reliefs (islands) and tidal marshes, where the only direct freshwater contribution is from the Natissa River (Marocco, 1995). However, sediment supply in the Grado Lagoon has been largely provided by the Isonzo River inputs which enter the lagoon through the tidal inlets. Both lagoon basins reach a maximum depth of 1–2 m, with the exception of the main channels, which reach depths of up to 6 m (Triches *et al.*, 2011). Sedimentological, micropaleontological and radiometric data indicate that the eastern sector of the lagoon (Grado) is geologically younger than the western part (Marano). In fact, according to Marocco (1991), the Marano Lagoon has existed in the present shape for approximately 5500 years, following a marine transgression on the previous flood plain. On the contrary, the Grado Lagoon was created in post-Roman times, about 1200 years ago.

The lagoon is characterized by semi-diurnal tidal fluxes, averaging 65 cm in general and 105 cm for the spring tidal range (Gatto & Marocco, 1993). Salinity is, on average, very low (2–7) in the western areas close to the river mouths, increasing towards the tidal inlets and along a NW–SE gradient. Temperature and dissolved oxygen values show increasing trends along the same gradient, due to the greater contribution of fluvial waters in the Marano area compared to Grado (ARPA, 2008; Falace *et al.*, 2009; Ferrarin *et al.*, 2010).

Rivers flowing into the lagoon contribute fine suspended matter resulting from material washed out from the groundwater table (Brambati, 1972). However, a more relevant source of sediment is the sea, arriving through six tidal inlets as a contribution of the two river deltas (build of silty and clayey particles) and from erosion of the barrier islands (sands) (Brambati, 1970).



**Fig. 1:** Map of the Marano and Grado Lagoon showing the location of the sampling stations.

This lagoon is generally considered as a well-preserved area where conservation of the natural environment must coexist with several human activities such as fishing, clam harvesting, shipping and industries. In addition, the lagoon has been recognized as a Special Protection Area (Zona di Protezione Speciale) and Site of Community Importance (Sito di Interesse Comunitario) under the EU “Habitats” (92/43/EEC) and “Birds” (2009/147/EC) Directives.

In spite of the level of protection, part of the Lagoon was also declared a “polluted site of national interest” (Sito d’Interesse Nazionale; Italian Ministerial Decree 468/01) in 2001 because of its high level of potential environmental risk. The Lagoon has historically been affected by high levels of Hg accumulation, originating from both natural and anthropogenic sources (Piani *et al.*, 2005; Covelli *et al.*, 2009; Covelli, 2012). For this reason, the regional agency for environmental protection (ARPA, FVG) and the University of Trieste have been involved in monitoring this lagoon by means of several bioindicators (including the edible mollusc *Tapes philippinarum*) and chemical-physical parameters during the last years.

## Materials and Methods

A total of twenty-one grab samples were collected in summer 2008 during the MIRACLE (Mercury Interdisciplinary Research for Appropriate Clam farming in La-

goon Environment) Project (Fig. 1; Table 1). The project was financially supported by the Commissario Delegato for the Marano and Grado Lagoon in order to understand the Hg biogeochemical cycling and test the coexistence of clam (*Tapes philippinarum*) farming with Hg contamination in the sediments (Covelli, 2012).

Surficial sediments were carefully collected by scraping the upper cm of the samples collected using a 5 l capacity Van Veen grab. The recovered samples were preserved in a refrigerator. Considering an average tidal range of 64 cm, most of the samples belong to the intertidal zone, except for samples A1bis, A2, A3, A4 and A6, which were taken from the Aussa River channel at water depths between 4 and 6 m.

A small part of each sample (about 50 ml) was washed using a 62.5 µm sieve and dried at 50°C. The > 62.5 µm fraction was observed under a binocular stereoscopic microscope (maximum lens magnification 50x). Total assemblages of benthic foraminifera were considered in this work owing to the fact that the aim of this study is to provide integrated information about the general conditions over a long period of time. Although the use of living specimens is strongly recommended for bio-monitoring studies (Murray 2000, 2006; Schönfeld *et al.*, 2012), other authors consider that data from the total assemblage are mostly comparable to assemblages in core samples and provide a perspective for environmental stratigraphy studies (Alve & Nagy, 1986; Morvan *et al.*, 2006; Debenay *et al.*, 2006, 2009; Martinez-Colon *et al.*, 2009).

**Table 1.** Geographic coordinates, grain-size values, organic C and N<sub>tot</sub> percentage, and C<sub>org</sub>/N<sub>tot</sub> (molar) value of sampling stations.

Station	Latitude N	Longitude E	Sand (%)	Silt (%)	Clay (%)	C <sub>org</sub> (%)	N <sub>tot</sub> (%)	C <sub>org</sub> /N <sub>tot</sub>
M1	45°42'.211	13°08'.065	48.9	44.4	6.6	0.80	0.12	7.7
M2	45°42'.765	13°06'.061	10.9	80.1	9.0	1.82	0.28	7.6
M3	45°44'.930	13°07'.381	6.6	87.0	6.4	1.21	0.19	7.6
M4	45°44'.265	13°10'.180	22.3	70.7	6.9	1.04	0.17	7.1
M5	45°45'.802	13°11'.718	8.9	84.6	6.5	1.78	0.29	7.3
MA	45°42'.890	13°10'.550	55.4	40.2	4.5	0.88	0.10	10.7
MB	45°43'.645	13°07'.221	40.9	54.0	5.1	0.71	0.12	7.2
MC	45°45'.160	13°13'.348	20.9	73.6	5.5	1.07	0.14	9.2
RP27	45°44'.410	13°13'.149	46.4	49.1	4.5	1.38	0.19	8.4
RP40	45°43'.884	13°16'.180	11.9	81.6	6.5	2.07	0.24	10.1
G1	45°42'.677	13°24'.199	13.0	78.8	8.2	1.52	0.24	7.5
G2	45°42'.038	13°20'.412	52.8	42.1	5.1	0.56	0.07	9.3
G3	45°43'.598	13°20'.882	8.4	81.3	10.4	1.83	0.21	10.1
G4	45°44'.445	13°17'.469	13.2	78.6	8.3	1.30	0.18	8.6
GD	45°41'.234	13°23'.212	49.6	45.4	5.0	0.51	0.05	11.9
IGC	45°44'.171	13°18'.145	47.3	46.3	6.4	0.68	0.09	9.3
A1bis	45°48'.082	13°18'.165	1.8	84.9	13.3	3.39	0.32	12.3
A2	45°48'.468	13°18'.289	1.2	84.4	14.5	4.04	0.49	9.6
A3	45°46'.964	13°18'.162	3.1	70.0	26.9	2.96	0.33	10.6
A4	45°46'.134	13°15'.722	2.7	80.5	16.8	2.30	0.29	9.2
A6	45°45'.195	13°14'.415	59.2	32.7	8.1	0.53	0.11	5.6

The determination of  $^{210}\text{Pb}$  content in core samples taken during a previous study at stations MB, M4, M5 and G3 showed an average recent sedimentation rate of about 1 mm per year (Covelli *et al.*, 2012). This means that the first centimetre of sediment deposition represented at least the last 10 years and consequently the total assemblage was used to obtain integrated information over this period of time.

Moreover, the possible influence of taphonomic processes was taken into account. Only well-preserved tests, without dissolution evidence, breakage or abrasion due to transport were counted and used for statistical analyses. However, the preservation state of the tests was always noted in order to highlight particular environmental conditions. Since foraminifera were always abundant, samples were subdivided using a dry splitter until an aliquot containing approximately 300 specimens was reached. After initial qualitative analysis, species counts were performed and recorded as the number of specimens of each taxon; normal and abnormal forms for each species were classified separately. Successively, these data were reported as relative abundance (%). Faunal density (FD) was calculated as number of specimens per gram of dry sediment (specimens  $\text{g}^{-1}$ ).

Selected specimens were displayed using a scanning electron microscope (Leica Stereoscan 430i) at the University of Trieste.

From the total assemblage data, three measures of species diversity were calculated for each station using the PAST (version 2.09) (PALaeontological STATistics) data analysis package (Hammer *et al.*, 2001): 1) species richness (S), as the total number of taxa for each station; 2) Shannon-Weaver (H) and 3) Dominance (D) indexes.

In order to check anthropogenic and/or natural disturbances in the study area, the Foraminiferal Abnormality Index (FAI), corresponding to the total percentage of abnormal foraminifera, was calculated for each sample (Coccioni *et al.*, 2005; Frontalini & Coccioni, 2008). The types of test abnormalities were recognized following Alve (1991), Yanko *et al.* (1994) and Geslin *et al.* (1998).

The generic taxonomy of foraminifera was assessed in line with Loeblich & Tappan (1987), and species classification followed the Mediterranean systematic studies of Jorissen (1987, 1988), Albani & Serandrei Barbero (1990), Cimerman & Langer (1991), Levy *et al.* (1992), Sgarrella & Moncharmont Zei (1993), and Fiorini & Viani (2001). For original descriptions of species, the Ellis & Messina (2013) online catalogue on foraminifera was used (<http://www.micropress.org/>).

For hierarchical analysis, a matrix containing the frequency percentage of those foraminifera with a higher than 3% abundance (fifteen species) in at least one sample was considered. Species with relative abundance < 3.0% were omitted, or gathered into major generic groups (i.e. *Eggerelloides* spp.). Two *formae* of *Criboelphidium poeyanum* were pooled as unique species.

Q-mode hierarchical cluster analysis (HCA), using an unweighted pair-group average (UPGMA) algorithm and the Euclidean distance as a similarity measurement, was performed to recognize a natural group of samples based on their similar foraminiferal content.

Abiotic data used in this paper, such as grain-size and geochemical parameters, come from a previous paper (Acquavita *et al.*, 2012), where the methodologies have been described exhaustively.

## Results

### *Distribution and diversity of foraminifera*

The foraminifera found in the 21 surface samples comprised 47 species, pertaining to 28 genera, including 14 agglutinated, 24 hyaline and 9 porcelaneous species (listed in Table 2 as percentage). Some species representative of this environment are reported in Plate I.

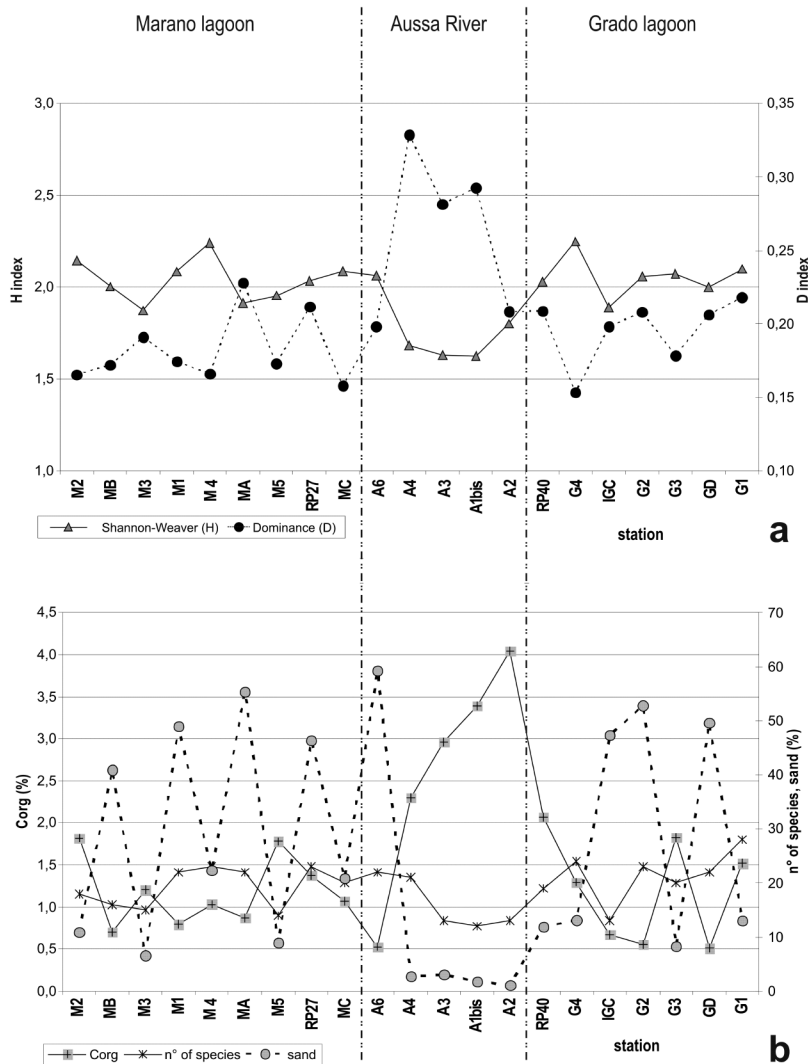
The high morphological variability led to some difficulties in the taxonomic attribution of *Ammonia* species. In the lagoon area, two main forms existed: one with a typical lobate outline, 6-7 inflated chambers in the last whorl and open umbilicus with very deep sutures, defined as *Ammonia tepida* (Plate I, Figs. 14 and 15); and a second one showing more chambers than the previous form, with a rounded outline and reduced umbilical area with less depressed sutures, where a size-variable knob in the umbilicus could be present (Plate I, Figs. 13, 16-18). This taxon has been reported here as *A. parkinsoniana*, in agreement with Jorissen (1988). Concerning *Criboelphidium poeyanum*, two forms were evidenced (*C. poeyanum* forma *decipiens*, Plate I, Fig. 26 and *C. poeyanum* forma *poeyanum*, Plate I, Fig. 27), also in agreement with Jorissen (1988).

*Ammonia*, *Elphidium* and *Haynesina* were the most frequent genera. Five taxa were always present (*Ammonia parkinsoniana*, *A. tepida*, *Aubignyna perlucida*, *Elphidium gunteri* and *Haynesina germanica*) and two species occurred at almost 80% of the studied stations but usually in small quantities (*Elphidium excavatum* forma *selseyensis*, and *Haynesina depressula*). Other species, such as *Ammobaculites exiguus*, *Ammoscalaria runiana*, *Criboelphidium poeyanum* forma *poeyanum*, *Haplophragmoides australensis*, *H. subglobosum* and *Quinqueloculina seminulum* were also very common, but appeared in much smaller quantities.

The number of species found in the lagoon ranged from a minimum of 12 (A1bis; Aussa River) to a maximum of 28 (G1; Grado Lagoon) and, generally, increased eastwards (Fig. 2b). The Shannon-Weaver index value (H) varied from a minimum of 1.62 (A1bis) to a maximum of 2.24 (M4; Marano sector). Lower H values corresponded to a higher Dominance index (D) (Fig. 2a). Comparing these indexes with the geographic location of the samples, it was possible to observe that lower H and higher D indexes corresponded to the stations located along the Aussa River channel (Fig. 2a), where muddy sediments prevailed (Fig. 2b).

**Table 2.** Relative abundance of the foraminifera as a percentage of each species with respect to the total assemblage present in each sample. Taxa are listed in alphabetical order. The asterisk marks the species affected by some kind of morphological abnormalities. Total count, foraminifera density (specimens/g) and FAI = Foraminiferal Abnormality Index are reported.

Species	M2	MB	M3	M1	M4	MA	M5	RP27	MC	A6	A4	A3	A1bis	A2	RP40	G4	IGC	G2	G3	GD	G1	
<i>Adelosina carinata-striata</i> * Wiesner	0.7		0.3		0.3			0.8				0.3					0.9				2.0	
<i>Adelosina</i> sp.																						
<i>Ammonaculites exiguus</i> Cushman & Bronnimann	1.6	0.9		0.6	3.7		0.7	1.1	0.5					2.9	1.4		1.5	0.3	0.3	0.3	0.3	
<i>Ammonia beccarii</i> (Linnaeus)																						
<i>Ammonia parkinsoniana</i> * (d'Orbigny)	13.3	11.3	7.4	23.6	19.3	37.3	9.0	28.4	14.2	22.5	16.2	12.0	9.7	7.5	22.4	16.7	19.7	29.6	25.1	33.8	26.3	
<i>Ammonia tepida</i> * (Cushman)	26.8	22.8	24.9	26.1	31.9	25.4	22.7	33.9	23.9	34.2	53.8	47.3	48.8	24.9	36.9	27.6	32.5	32.4	27.7	26.0	36.8	
<i>Ammonoscalaria runiana</i> (Heron-Allen and Earland)	4.8	2.0		6.4	11.5	1.4	0.7	4.1	2.2	4.2				11.3	4.1	1.4	3.1	5.1	3.9	2.3		
<i>Asterigerinata mamilla</i> (Williamson)	0.5				0.4					0.3												
<i>Aubignyna perlucida</i> * (Heron-Allen and Earland)	3.4	3.5	6.6	2.7	3.7	5.6	7.3	7.4	7.2	7.5	1.5	12.9	0.7	6.9	1.2	10.7	7.5	7.4	15.4	4.2	4.2	
<i>Bigennerina nodosaria</i> d'Orbigny								0.3						0.3								
<i>Brizalina catanensis</i> Seguenza								0.3			1.2	0.6			0.5	0.3					0.3	
<i>Brizalina striatula</i> (Cushman)				0.6		0.4															0.3	
<i>Buccella granulata</i> (Di Napoli) Alliatia						0.4															0.3	
<i>Cassidulina crassa</i> d'Orbigny						0.4															0.6	
<i>Cassidulina</i> sp.																						
<i>Criboelphidium poeyanum</i> (d'Orbigny) forma <i>decipiens</i> * (Costa)	2.7								0.2						1.2	0.3						
<i>Criboelphidium poeyanum</i> (d'Orbigny) forma <i>poeyanum</i> * (d'Orbigny)	0.9		1.3	0.3	0.3	1.8	1.2	1.9	0.7	1.4	0.2			0.6	1.9		3.4	1.6	0.3	0.8		
<i>Cycloforina schlumbergeri</i> (Wiesner)						0.4		0.3		1.4	0.5											
<i>Cycloforina</i> sp.																						
<i>Discaminina compressa</i> (Goess)				0.3	1.1				0.2					1.5							0.3	
<i>Discorinopsis aguayoi</i> (Bermudez)						0.4																
<i>Eggerelloides advenus</i> (Cushman)				0.3						0.6	1.0			4.9	0.3		0.6			2.3		
<i>Eggerelloides scaber</i> (Williamson)														5.8	0.8		4.0			10.9	2.0	
<i>Elphidium aculeatum</i> (d'Orbigny)																						
<i>Elphidium advenum</i> (Cushman)				1.2	0.6			0.3	0.2	1.7				0.3		0.3	1.2	0.3	0.3	0.3	0.3	
<i>Elphidium excavatum</i> (Terquem) forma <i>selseyensis</i> * (Heron-Allen and Earland)	5.5	9.8	8.9	0.3	3.4		15.4	1.6	8.4	2.8	0.5	0.9	2.0	3.3		4.9	7.5	0.3	1.6	0.3	2.0	
<i>Elphidium gerthi</i> van Voorhuyzen	1.1			0.6		1.8				0.8											0.3	
<i>Elphidium granosum</i> (d'Orbigny) forma <i>libdense</i> Cushman				0.6	0.3	0.4				0.8											0.6	
<i>Elphidium gunteri</i> * Cole	24.3	19.4	25.4	18.8	6.3	4.9	25.4	6.0	18.1	1.9	4.9	14.2	15.4	23.7	1.2	17.2	19.7	3.7	9.0	1.9	8.5	
<i>Elphidium incertum</i> (Williamson)					1.1	1.1		0.5		0.6						0.5	1.4	0.3	0.6	0.6	1.1	
<i>Elphidium pulverum</i> * Todd				0.3			0.2														1.7	
<i>Glomospira charoides</i> (Heron-Allen and Earland)													0.3									
<i>Haplophragmoides australensis</i> Albani	1.4	1.2	0.3	3.6	4.3	0.4	0.5	0.8	1.0	0.3					2.6	2.7	0.6	0.6	0.6		0.8	
<i>Haplophragmoides subglobosum</i> Cushman	2.7			1.8	3.7	0.4		1.9	1.0	1.4	0.2				2.3	1.6	1.5	1.3	1.6	0.3		
<i>Haynesina depressula</i> (Walker & Jacob)	0.5	2.0	1.3	0.6	0.9	1.4	0.9	1.6	1.9	0.8	0.2			0.6	0.3	1.6	2.6	0.6	0.3	0.6	1.4	
<i>Haynesina germanica</i> * (Ehrenberg)	9.2	24.0	21.4	9.1	4.6	13.0	13.7	6.0	18.6	14.4	7.4	7.6	13.4	27.9	2.3	4.6	4.9	6.2	4.8	8.7	3.4	
<i>Hopkinsina pacifica</i> Cushman											1.7											
<i>Jadammina macrescens</i> (Brady)		0.3		0.3	0.4										0.9		0.9	0.9	0.6		0.8	
<i>Jadammina fusca</i> (Brady)				0.3	0.9				0.3	0.2												
<i>Milutinella subrotunda</i> (Montagu)	0.2																					
<i>Nonion</i> sp.*																						
<i>Quinqueloculina laevigata</i> d'Orbigny		0.3			0.3	0.4	1.7	0.8	0.5	1.4				0.9	0.3	0.3	0.6	0.6			0.3	
<i>Quinqueloculina lata</i> Terquem		0.6	0.5	0.3				0.5	0.2		0.5		0.7								0.3	
<i>Quinqueloculina milleleti</i> (Wiesner)		0.3	0.3	0.9				0.5		0.8	0.5	0.6	1.3	0.6		0.8					0.3	
<i>Quinqueloculina seminulum</i> * (Linnaeus)		0.3	0.3	0.9				0.5		0.8	1.5	0.9	0.7	1.5		0.3					0.8	
<i>Reophax nana</i> Rhumbler	0.5	0.9	1.0	1.5	0.3	0.4	0.5	0.5	0.2	0.6	6.1	1.6	3.7	1.5			0.6	0.6		1.0	1.1	
<i>Spirolectinella earlandi</i> (Parker)									0.5		1.0						0.3	0.6			0.3	
<i>Triloculina rotunda</i> * d'Orbigny					0.3				0.5		0.5						0.3					
<i>Trochammina inflata</i> (Montagu)										0.3			3.3	0.3	1.2	0.3	0.6	0.6	3.2			
Number of specimens	437	346	393	330	348	284	422	366	415	360	407	317	299	333	344	366	345	324	311	311	353	
specimens/g	11205	1182	10208	701	3442	663	23977	3128	13175	2089	13658	19329	7119	3165	3094	6203	475	1171	5447	487	4738	
foraminiferal abnormality index (%)	16.5	19.1	18.1	12.7	11.5	8.1	16.1	9.3	14.5	13.1	9.1	12.0	13.7	14.1	4.7	10.7	12.2	5.2	8.7	3.5	6.2	



**Fig. 2:** Distribution of Shannon-Weaver (H) and Dominance (D) indexes in the Marano and Grado Lagoon (a), and distribution of sand (%), organic carbon (%) and number of species in the same samples (b). Sampling stations are arranged from West (left) to East (right).

Areas with higher D values were characterized by *A. tepida* at frequencies > of 45.5% with respect to total assemblage.

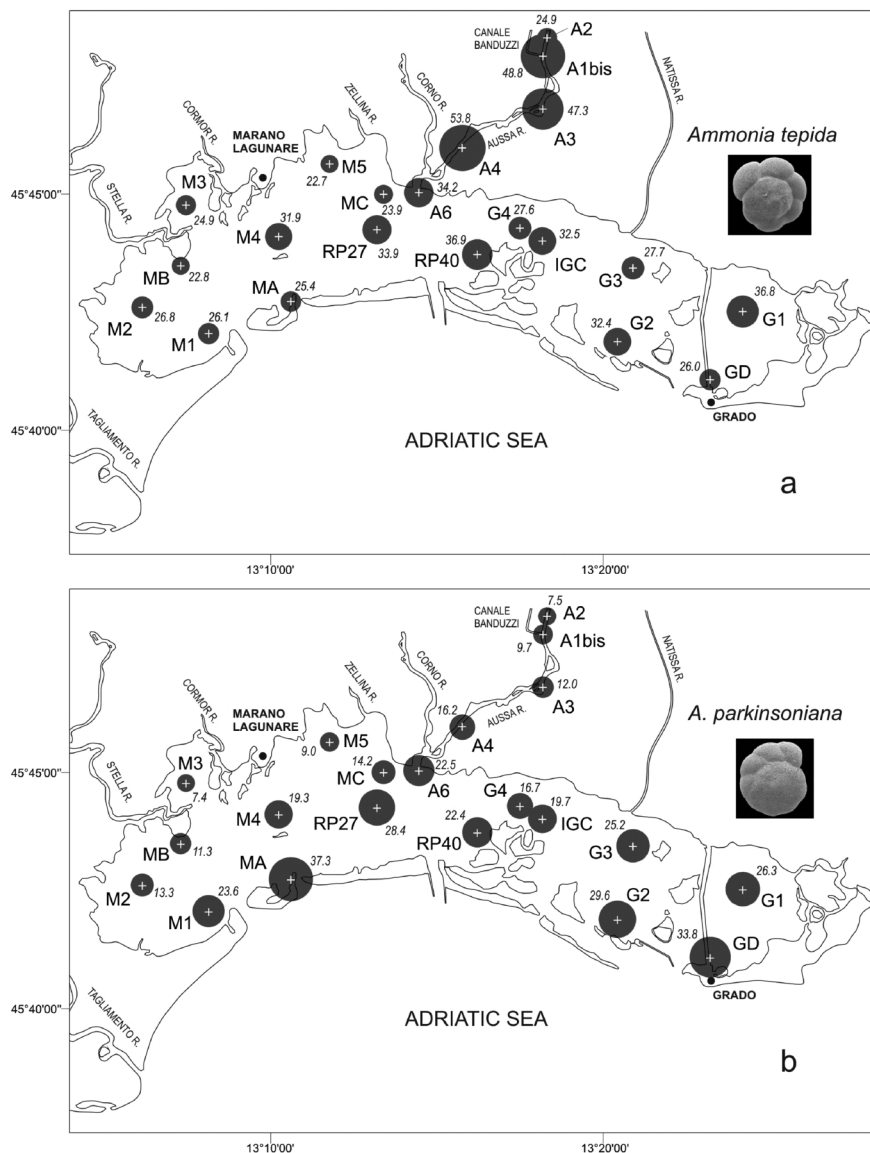
*Ammonia tepida* varied from 22.7% (M5) to 53.8% (A4), and was more frequent in the central-eastern part of the lagoon and along the Aussa River channel, where it reached its maximum frequency (A4) (Fig. 3a). *Ammonia parkinsoniana* varied from a minimum frequency of 7.4% (M3) to 37.3% (MA), increasing eastwards and reaching higher values at the stations located near the tidal inlets (MA and GD) (Fig. 3b). In particular, the specimens of this taxon recovered along the Aussa River channel were often found to reach larger pore sizes (diameter about 2.2  $\mu\text{m}$ ) at greater densities than their counterparts found in the lagoon. *Haynesina germanica* ranged from 2.3 (RP40) to 27.9% (A2), decreasing from west to east across the lagoon and from north to south along the Aussa River channel (Fig. 4a). *Elphidium gunteri* varied from 1.2 (RP40) to 25.4% (M3 and M5), its distribution being partially similar to that of *H.*

*germanica* (Fig. 4b). Faunal density (specimens/g) varied from 474.9 (IGC, Grado Lagoon) to 23977.3 (M5, Marano Lagoon) (Table 2), with generally higher values in those stations where sediments are muddy.

The shells were generally well preserved, except at those stations closest to the lagoon inlets (MA and GD), where broken and worn tests were frequently observed. At almost all the stations, calcareous tests in partial dissolution were found, but in quantities less than 5% of the total number of individuals per station.

### Multivariate analysis

The data matrix, consisting of 19 stations and 15 species (more abundant than 3%) provided the basis for the cluster analysis. Four main groups of stations (A–D), each characterized by a different benthic foraminiferal content, were clearly distinguished (Fig. 5). The dominant traits of the four clusters are listed below and reported in Table 3. Stations GD and MA were excluded from



**Fig. 3:** Relative abundance of *Ammonia tepida* (a) and *Ammonia parkinsoniana* (b) in the Marano and Grado Lagoon; the size of the black circles is proportional to the percentage frequency, which is reported near the symbol.

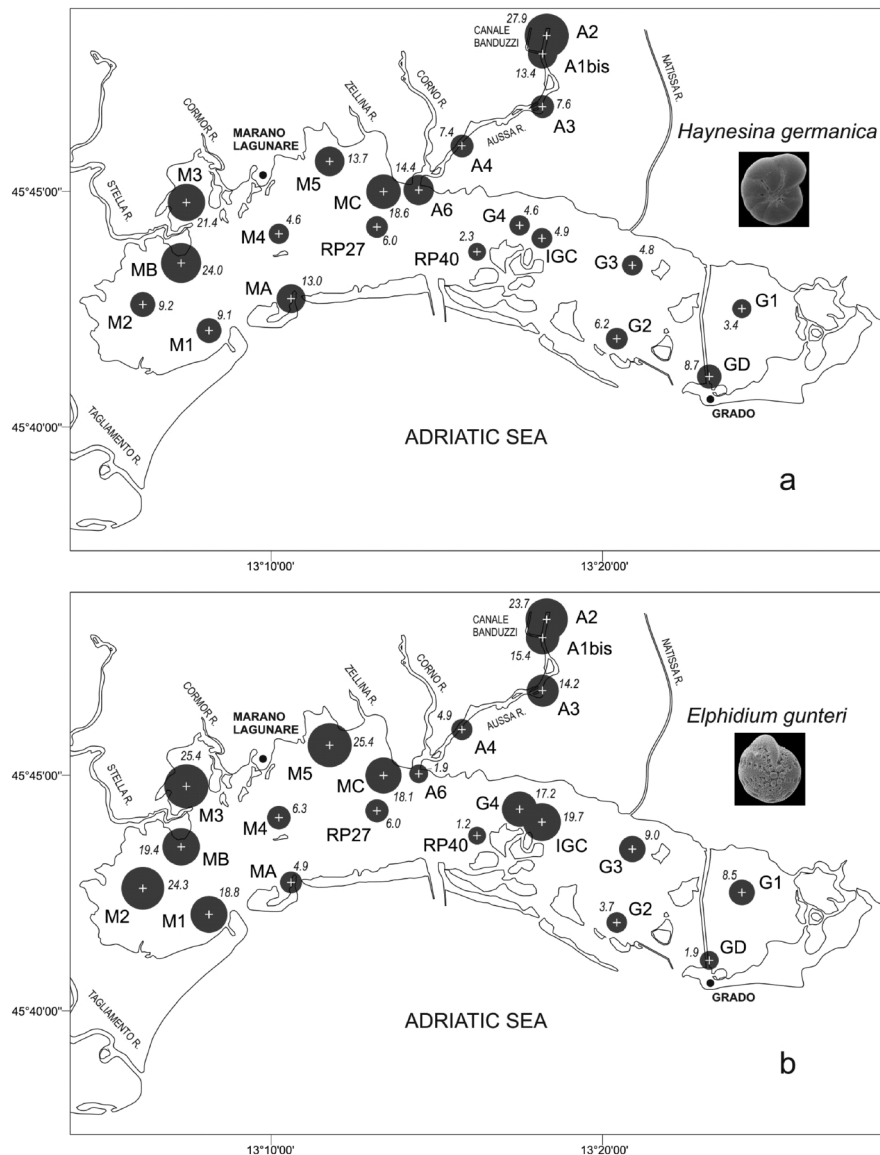
this analysis since the foraminifers were characterized by poor preservation of the shell material, possibly, affected by transport processes. Since useful for the palaeoenvironmental reconstruction, the foraminiferal content of these two stations and the relationships with the abiotic characters were discussed anyway and reported in Table 3.

**Cluster A** included three stations (A4, A3, A1bis) located along the Aussa River, defined by the dominance of *A. tepida* (47.3 - 53.8%), low values of *A. parkinsoniana* (9.7-16.2%), *E. gunteri* (4.9-15.4%) and *H. germanica* (7.4-13.4), and by the lowest value or absence of the agglutinated species. It also included a significant occurrence of miliolids, represented by *Q. seminulum*, and *Triloculina rotunda*. On average, the lowest H index values were recorded for these stations, along with higher Dominance values. An average value of 13369 specimens/g was evidenced. The sediments contained the highest values of clay (13.3–26.9%) and the lowest of sand

(1.8–3.1%) (Fig. 2b), with the organic C content reaching its highest mean value (2.9%) and the  $C_{org}/N_{tot}$  ratio > 10.

**Cluster B** included seven stations (M4, RP40, G3, A6, G1, RP27, G2), a group corresponding to those locations in proximity to the Aussa river mouth in the Marano basin, and partially in the Grado basin. Compared to the other clusters, foraminiferal diversity was defined by high and comparable proportions of *A. parkinsoniana* (19.3-29.6%) and *A. tepida* (27.7-36.9%), along with a significant occurrence of *A. perlucida* (1.2-15.4%), and by far a lower representation of *E. gunteri* and *H. germanica*. The agglutinated species in Cluster B were well represented, both in terms of frequency and number of species. In these assemblages, high H index values were recorded. An average value of 3301 specimens/g was evidenced. In terms of sediments, this cluster corresponded to a higher and variable sand content (8.4–59.2%), with





**Fig. 4:** Relative abundance of *Haynesina germanica* (a) and *Elphidium gunteri* (b) in the Marano and Grado Lagoon; the size of the black circles is proportional to the percentage frequency, which is reported near the symbol.

variable organic C content (0.5–2.1%) and mean  $C_{org}/N_{tot}$  ratio values < 10.

**Cluster C** included five stations (M5, MB, MC, M3, A2) located along the inner coastline of the Marano basin, except for A2, which was located in the Aussa River. This cluster was characterized by a high abundance of *E. gunteri* (18.1–25.4%), *H. germanica* (13.7–27.9%) and *Elphidium excavatum selseyensis* (3.3–15.4%), and lower occurrence of *Ammonia* species compared to the previous cluster. The benthic fauna of station A2 also includes a great abundance of Thecamoebians, freshwater protozoans. In these assemblages, the H index showed a value of < 2 on average. An average value of 10341 specimens/g was evidenced. The sediments were rich in silt (54.0–87.0) and organic C (0.7–4.0%), with low  $C_{org}/N_{tot}$  ratios.

**Cluster D** included four stations (M2, G4, IGC, M1) located in both Marano and Grado Lagoon. The foraminifera

were represented mainly by *A. tepida* (26.1–32.5%), *E. gunteri* (17.2–24.3%), *A. parkinsoniana* (13.3–23.6%) and, subordinately, by *A. perlucida* and *H. germanica*, along with a reasonable occurrence of agglutinated taxa (above all *A. rutiliana* and *H. australensis*). In this assemblage, a mean value of the H index > 2, together with lower D values were recorded. An average value of 4646 specimens/g was evidenced. The sediments in this cluster comprised high sand content (10.9–48.9%), with low organic C content (0.7–1.8%) (Fig. 2b), and a  $C_{org}/N_{tot}$  ratio < 10, similar to those of cluster C.

#### Test abnormalities

All of the studied samples contained a certain number of foraminifera exhibiting abnormal characteristics. The FAI index varied from a minimum of 3.5 (GD) to a maximum of 19.1% (MB), and clearly decreased in two directions: one from the inner part of the Marano Lagoon

**Table 3.** Foraminiferal, grain-size, geochemical data: minimum, maximum and mean values of parameters calculated in each cluster identified by cluster analysis; n = number of cases.

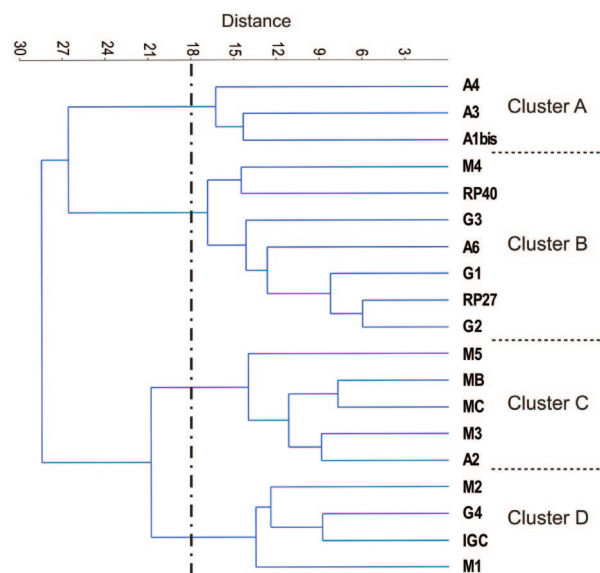
	cluster A (n = 3)			cluster B (n = 7)			cluster C (n = 5)			cluster D (n = 4)		
	min	max	mean	min	max	mean	min	max	mean	min	max	mean
<i>A. exiguus</i>	0.0	0.0	<b>0.0</b>	0.0	3.7	<b>1.4</b>	0.0	0.9	<b>0.4</b>	0.0	1.6	<b>0.9</b>
<i>A. parkinsoniana</i>	9.7	16.2	<b>12.6</b>	19.3	29.6	<b>24.8</b>	7.4	14.2	<b>9.9</b>	13.3	23.6	<b>18.3</b>
<i>A. tepida</i>	47.3	53.8	<b>50.0</b>	27.7	36.9	<b>33.4</b>	22.7	24.9	<b>23.9</b>	26.1	32.5	<b>28.2</b>
<i>A. runiana</i>	0.0	0.0	<b>0.0</b>	2.3	11.5	<b>5.9</b>	0.0	2.2	<b>1.0</b>	1.4	6.4	<b>4.2</b>
<i>A. perlucida</i>	0.7	12.9	<b>5.0</b>	1.2	15.4	<b>6.7</b>	3.5	7.3	<b>6.3</b>	2.7	10.7	<b>6.1</b>
<i>C. poeyanum</i>	0.0	0.2	<b>0.1</b>	0.3	3.4	<b>1.6</b>	0.0	1.3	<b>0.7</b>	0.0	3.7	<b>1.5</b>
<i>Eggerelloides</i> spp.	0.0	1.2	<b>0.4</b>	0.0	10.8	<b>2.6</b>	0.0	0.0	<b>0.0</b>	0.0	1.1	<b>0.3</b>
<i>E. excav. sels.</i>	0.5	2.0	<b>1.1</b>	0.0	3.4	<b>1.7</b>	3.3	15.4	<b>9.2</b>	0.3	7.5	<b>4.6</b>
<i>E. gunteri</i>	4.9	15.4	<b>11.5</b>	1.2	9.0	<b>5.2</b>	18.1	25.4	<b>22.4</b>	17.2	24.3	<b>20.0</b>
<i>H. australensis</i>	0.0	0.0	<b>0.0</b>	0.3	4.3	<b>1.4</b>	0.0	1.2	<b>0.6</b>	0.6	3.6	<b>2.1</b>
<i>H. subglobosum</i>	0.0	0.2	<b>0.1</b>	0.3	3.7	<b>1.8</b>	0.0	1.0	<b>0.2</b>	0.0	2.7	<b>1.6</b>
<i>H. germanica</i>	7.4	13.4	<b>9.4</b>	2.3	14.4	<b>6.0</b>	13.7	27.9	<b>21.1</b>	4.6	9.2	<b>7.0</b>
<i>Q. seminulum</i>	1.6	6.1	<b>3.8</b>	0.0	1.1	<b>0.4</b>	0.0	1.5	<b>0.8</b>	0.0	1.5	<b>0.5</b>
<i>T. rotunda</i>	0.5	3.3	<b>1.5</b>	0.0	0.0	<b>0.0</b>	0.0	0.3	<b>0.1</b>	0.0	0.3	<b>0.1</b>
<i>Tr. inflata</i>	0.0	0.0	<b>0.0</b>	0.0	3.2	<b>0.8</b>	0.0	0.0	<b>0.0</b>	0.0	0.3	<b>0.1</b>
sand (%)	1.8	3.1	<b>2.5</b>	8.4	59.2	<b>30.6</b>	1.2	40.9	<b>15.7</b>	10.9	48.9	<b>30.1</b>
silt (%)	70.0	84.9	<b>78.5</b>	32.7	81.6	<b>62.3</b>	54.0	87.0	<b>76.7</b>	44.5	80.1	<b>62.3</b>
clay (%)	13.3	26.9	<b>19.0</b>	4.5	10.4	<b>7.1</b>	5.1	14.5	<b>7.6</b>	6.4	9.0	<b>7.6</b>
Corg	2.3	3.4	<b>2.9</b>	0.5	2.1	<b>1.3</b>	0.7	4.0	<b>1.8</b>	0.7	1.8	<b>1.1</b>
C <sub>org</sub> /N <sub>tot</sub>	9.2	12.3	<b>10.7</b>	5.6	10.1	<b>7.9</b>	7.2	9.6	<b>8.2</b>	7.6	9.3	<b>8.3</b>
FAI (%)	9.1	13.7	<b>11.6</b>	4.7	13.1	<b>8.4</b>	14.1	19.1	<b>16.4</b>	10.7	16.5	<b>13.0</b>
Shannon H	1.62	1.68	<b>1.64</b>	2.03	2.24	<b>2.08</b>	1.80	2.09	<b>1.94</b>	1.89	2.25	<b>2.09</b>
Dominance <sub>D</sub>	0.28	0.33	<b>0.30</b>	0.17	0.22	<b>0.20</b>	0.16	0.21	<b>0.18</b>	0.15	0.20	<b>0.17</b>
FD (spec./g)	7119	19329	<b>13369</b>	1171	5447	<b>3301</b>	1182	23977	<b>10341</b>	475	11205	<b>4646</b>
Taxa S	12	21	<b>15</b>	19	28	<b>23</b>	13	20	<b>16</b>	13	24	<b>19</b>

eastward, and the other one along the Aussa River, from north to south (Fig. 6).

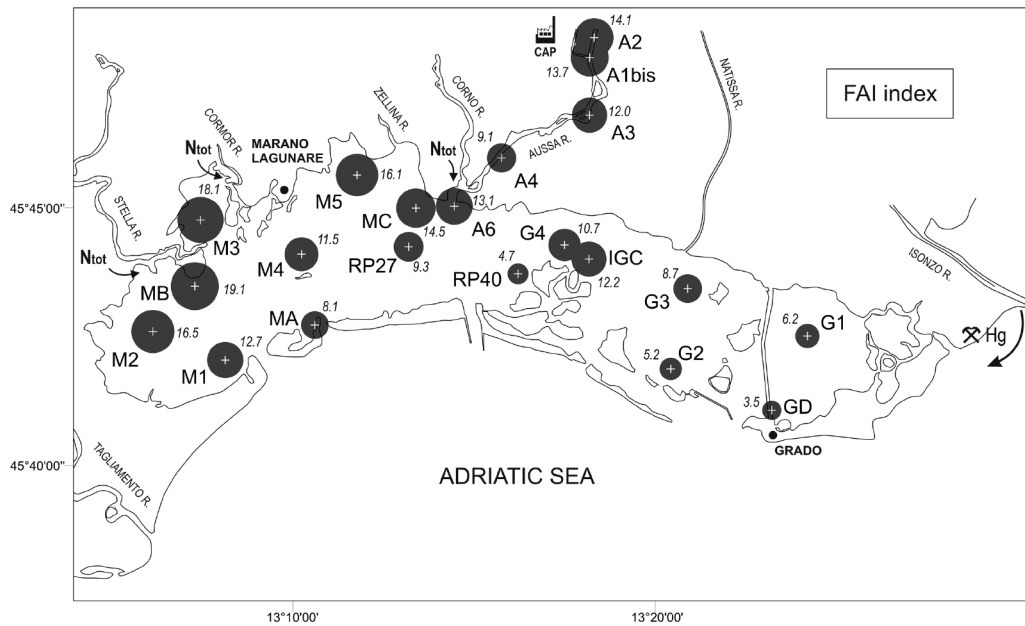
Among those recognised in the literature, several types of test abnormalities were noted: abnormal addition

of a chamber; abnormally protruding chamber; reduced chamber size; distorted chamber arrangement; twisted tests; and complex forms (Plate II).

Thirteen species (including two *formae*) among the



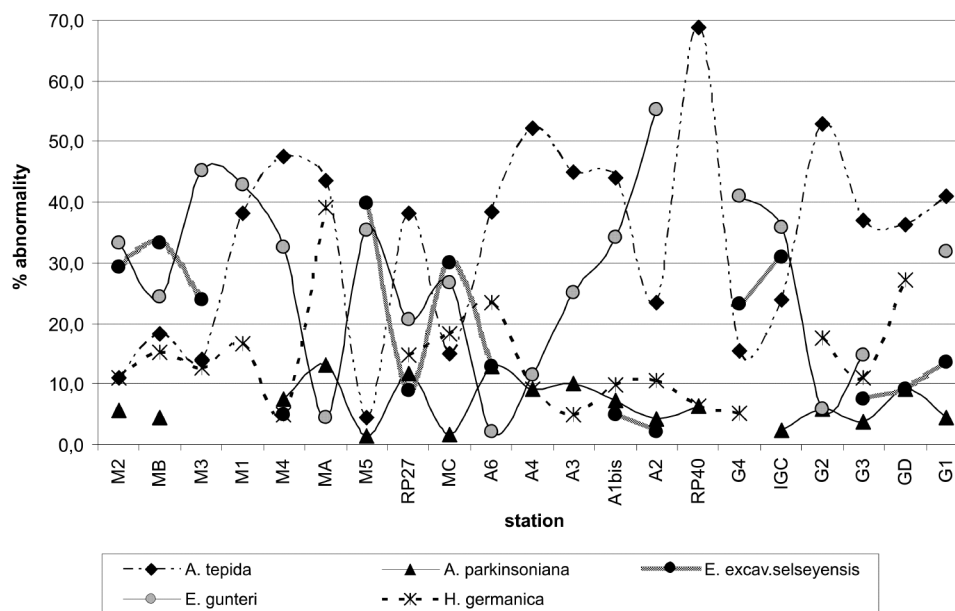
**Fig. 5:** Dendrogram classification of the sampling stations produced by Q-mode cluster analysis, using an unweighted pair-group average (UPGMA) algorithm and the Euclidean distance.



**Fig. 6:** Variability of FAI (Foraminiferal Abnormality Index), corresponding to the total percentage of abnormal foraminifera in the Marano and Grado Lagoon; the size of the black circles is proportional to the percentage values, which are reported near the symbol. The main sources of anthropogenic inputs (nutrients and Hg) are shown.

total assemblage exhibited some kind of test abnormality (Table 2). Some species, such as *A. tepida*, *A. perlucida*, *E. excavatum selseyensis*, *E. gunteri*, and *H. germanica* displayed different types of test abnormality, while other species, such as *E. poeyanum*, *Quinqueloculina seminulum* and *Triloculina rotunda* showed only one. Among them, *A. tepida* was the species most frequently malformed (mean abnormality percentage:

33.7), followed by *E. gunteri* (27.5%), *E. excavatum selseyensis* (18.3%), *H. germanica* (14.3%), *A. parkinsoniana* (6.7%) and *A. perlucida* (6.2%), in decreasing order *Ammonia tepida* was more deformed in the Grado Lagoon and along the Ausa River (except at stations G4 and A2), whereas *E. gunteri* showed a higher frequency of morphological abnormalities in the central-western sector (Fig. 7).



**Fig. 7:** Distribution of the abnormality, as a percentage, for the species most frequently malformed in the Marano and Grado Lagoon. Sampling stations are arranged from West (left) to East (right).

## Discussion

### Distribution

The foraminiferal species found in the Marano and Grado Lagoon are distinctive euryhaline taxa, well known in Mediterranean lagoons (Carbonel & Pujos, 1981; Zaninetti, 1982; Zampi & D'Onofrio, 1984, 1987; Vismara Schilling & Ferretti, 1987; Albani & Serandrei Barbero, 1990; Masoli *et al.*, 1995; Albani *et al.*, 1998; Carboni *et al.*, 2009; Coccioni *et al.*, 2009). These taxa are associated with more thalassic species, especially in areas close to lagoon inlets. The low diversity and richness of the assemblage is consistent with the characteristics of the lagoon environment (Murray, 2006), where conditions vary depending on the degree of confinement from the sea (*sensu* Guelorget & Perthuisot, 1983).

As already discussed in Materials and Methods, using the thanathocoenosis, the top 1 cm of the sediments studied probably reflects the last decade of time-averaged test accumulation. The limited occurrence of calcareous tests with dissolution and the generally good level of preservation of the tests, except for some stations (see below), seems to indicate a low influence of the post-mortem processes. Foraminiferal density appears to increase in relation to higher mud content in the sediments, possibly due to a relatively high nutrients availability associated to the fine fraction.

Q-mode cluster analysis was used to separate the sampling stations into four groups (Fig. 5), each of them characterized by a well-defined set of taxa (Table 2), and interpreted as reflecting different environmental/paleoenvironmental conditions. The areal distribution of these assemblages was reported in Figure 8.

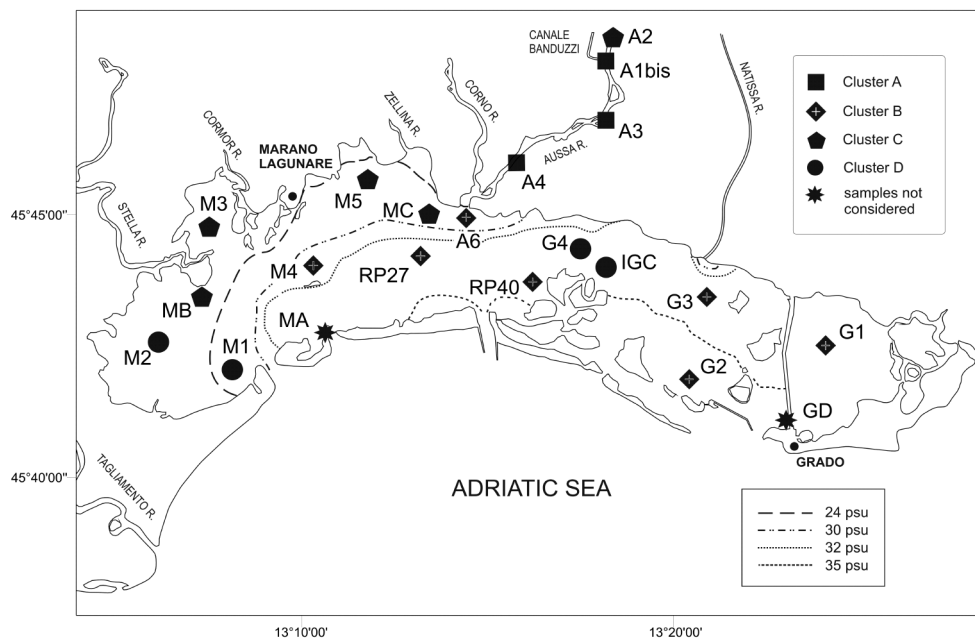
### *Ammonia tepida* assemblage (Cluster A; stations A4, A3, A1bis)

**Additional common species:** *E. gunteri*, *H. germanica* and a significant occurrence of *Quinqueloculina* spp.

This assemblage represented the greatest occurrence of *A. tepida* (47.3-53.8%). The sampling stations were located along the Aussa River, where a clear thermohaline stratification of the water column has been recognized on the basis of CTD profiles by Covelli *et al.* (2009). The results reported by these authors show that the residence time of the saline bottom water (28–30 psu) was practically permanent throughout the year. These environmental conditions at the river bottom allow foraminifera to colonize this area, far upstream from the river mouth.

At these sites, where sediments show greater quantities of clay and organic matter of continental origin ( $C_{org}/N_{tot}$  ratio > 10, see Pocklington & Leonard, 1979; Gofñi *et al.*, 2003), the specimens of *A. parkinsoniana* exhibited larger pore sizes at greater densities than their counterparts found in the lagoon. A similar situation was described by Bernhard & Sen Gupta (1999) for forms living in oxygen-depleted environments. *Ammonia tepida* showed high levels of similarity to *A. parkinsoniana* forma *tepida* mph. 1-3, linked to lower salinities and the Po river runoff system (Jorissen, 1988).

The occurrence of *Ammonia* spp. along a river channel disagrees with the model proposed by Debenay *et al.* (2000). The fluvial environment is usually more confined compared to the lagoon and other species such as *Haynesina germanica* or agglutinated taxa should be found, whereas *A. tepida* generally represents the outer paralic



**Fig. 8:** Geographical distribution of the foraminiferal assemblages in surface sediments of the Marano and Grado Lagoon. Assemblages are determined by cluster analysis of quantitative data. The simplified annual average salinity distribution in the Marano and Grado Lagoon (modified and adapted from Ferrarin *et al.*, 2010) is also reported.

systems. The thermohaline stratification, muddy composition of the sediments and high content of organic matter could justify the occurrence of this species. In this regard, the relative abundance of *A. tepida* is typically favoured by an increase of total organic matter used as food resources (Armynot du Châtelet *et al.*, 2009).

Compared to the rest of the lagoon, a greater presence of *Quinqueloculina seminulum* and *T. rotunda* was found at these stations. The good level of test preservation (see Pl. I) and the high mud content in these sediments allow excluding transport by bed load currents. *Quinqueloculina seminulum* is widely distributed and frequent from lagoons to infralittoral environments (Jorissen, 1988; Albani & Serandrei Barbero, 1990; Sgarrella & Monchatmont Zei, 1993). *Triloculina rotunda* is common in the lagoons of S. Gilla (Sardinia, Italy) and Orbetello (Tuscany, Italy) (Foresi *et al.*, 2006), where it can achieve a high degree of morphological variability within the population in relation to the lagoon's confinement. Moreover, we cannot exclude the possibility that these species have colonized this very confined part of the lagoon in relation to the permanent occurrence of saline bottom waters.

Several specimens of *Spiroloculina lucida* have been reported exclusively at this station and not in the rest of the lagoon; as these species usually characterize less confined areas of lagoons (Albani & Serandrei Barbero, 1990), they were considered allochthonous and not incorporated in the table of frequency.

Low diversity index values (< 1.70) characterized the Aussa River, where the dominance of *A. tepida*, and subordinately *H. germanica*, could be related to environmental conditions, such as low salinity, high clay and C<sub>org</sub> content etc. (Fig. 2b), but also to anthropogenic pressure occurring in this area. In fact, the fluvial environment has been affected by the activity of a chlor-alkali plant (CAP) used for the production of pulp, which was in operation until 1992 (Covelli *et al.*, 2009). Although the levels of Hg<sub>tot</sub> and methyl-Hg (CH<sub>3</sub>Hg) in riverine sediments (mean value of 2.96 µg/g and 2.95 ng/g, respectively) collected for the MIRACLE Project (Acquavita *et al.*, 2012) were lower than those determined by Covelli *et al.* (2009) (Hg<sub>tot</sub> mean value of 3.19 µg/g), regular and persistent contamination of Hg still exists in the area related to fine particles transported by freshwater to the Marano Lagoon (Piani *et al.*, 2005).

In the Lagoon of Venice biocoenosis, and in other Mediterranean lagoons and coastal areas, *A. tepida* and *H. germanica* are reported to be very tolerant to high concentrations of trace elements, including Hg (Yanko *et al.*, 1994; Coccioni *et al.*, 2005; Bergamin *et al.*, 2009; Carboni *et al.*, 2009; Coccioni *et al.*, 2009; Caruso *et al.*, 2011), and consequently their dominance in the Aussa River could be partially indicative of a polluted environment.

### ***Ammonia tepida* and *Ammonia parkinsoniana* assemblage (Cluster B; stations M4, RP40, G3, A6, G1, RP27, G2)**

**Additional common species:** *A. perlucida* and *A. runiana*

This assemblage corresponded to the highest co-occurrence of *A. parkinsoniana* and *A. tepida* (Tab. 3). It represented intertidal and subtidal areas of the outer lagoon with medium-low levels of hydrodynamics, as suggested by the sand content of its sediment, which varied from 8.4 to 59.2%.

The higher presence of *A. perlucida* in this assemblage confirms water circulation, as also suggested by Vismara Schilling & Ferretti (1987) and Albani & Serandrei Barbero (1990) in Mediterranean lagoons. *Ammonia parkinsoniana* is reported as a coastal taxon by Jorissen (1988), as *A. parkinsoniana* forma *parkinsoniana*.

This assemblage also included agglutinated taxa, such as *Ammobaculites exiguus*, *A. runiana*, *Eggerelloides scaber*, *H. australensis* and *H. subglobosum*, representing areas with limited confinement and almost marine-level salinity. *Ammobaculites exiguus* is often associated with plant fibres, thus indicating a vegetated lagoon bottom, as also reported by Debenay *et al.* (2000) and Duchemin *et al.* (2005). *Ammoscalaria runiana* is actually present in the mud-flat estuaries, fjords and lakes of northern Europe (Murray, 2006), but up to now is scarcely reported in the modern sediments of the northern Adriatic Sea.

*Eggerelloides scaber* is a continental shelf species that lives in microhabitats with different oxygenation conditions (e.g. Jorissen *et al.*, 1992; Donnici & Serandrei Barbero, 2002; Duijnsteet *et al.*, 2004); it is also able to colonize the seaward part of estuaries and lagoons (Albani & Serandrei Barbero, 1990; Murray, 2006). *Haplophragmoides australensis* is present in the intertidal areas of the Lagoon of Venice (Albani & Serandrei Barbero, 1990); *H. subglobosum* is generally reported as occurring from depths of around 10 m, but it is more frequent at depths greater than 100 m (Sgarrella & Moncharmont-Zei, 1993). Yet, the areas represented by this assemblage correspond to those "marine areas", with mesotrophic conditions, prevalently colonized by *Cymodocea nodosa* and *Zoostera marina*, reported for the Marano and Grado Lagoon by Falace *et al.* (2009). Slightly higher H and Fisher- $\alpha$  index values in this sector of the lagoon indicate that physical parameters, such as temperature and salinity, are less variable than in the previous sector.

### ***Elphidium gunteri* and *Haynesina germanica* assemblage (Cluster C; stations M5, MB, MC, M3 and A2)**

**Additional common species:** *A. tepida* and *E. excavatum* forma *selseyensis*

This assemblage was distributed along the intertidal coastal area of the western lagoon (Marano basin) and at station A2, located landward in the Aussa River, where some

of the lowest values of salinity with high standard deviations have been recorded (Ferrarin *et al.*, 2010) (Fig. 8).

Although *A. tepida* remained well represented (an average of 23.9%), the lowest proportion of *A. parkinsoniana* (an average of 9.9%), together with a greater presence of *E. gunteri* with *H. germanica* (an average of 22.4 and 21.1%, respectively), was noted in this association. *Elphidium gunteri* is generally recorded in intertidal environments of the northern Adriatic Sea, which Hohenegger *et al.* (1989) related to the high presence of blue-green algae - food dependency. It is common in the Goro (Po river delta, Italy) and S. Gilla lagoons (Coccioni, 2000; Buosi *et al.*, 2010), but has not been reported in the Venice Lagoon, where it might be recognized as *E. granosum*, an iposaline taxon characteristic of low salinity areas (Albani & Serandrei Barbero, 1990). The relative dominance of *E. gunteri* in this area confirms the preference of this taxon for intertidal environments, but it could also be related to the greater variability in salinity and/or anthropogenic conditions. *Haynesina germanica*, a species abundant in some sectors of the study area, has been considered by Armynot du Châtelet *et al.* (2004) and Carboni *et al.* (2009) as tolerant to trace element pollution. In the Venice Lagoon, this taxon (as *Nonion pauciloculum* in Albani *et al.*, 2007) identifies the “Marginal Urban Biotope”, the inner areas of the lagoon, subjected to lower water exchanges and, consequently, high accumulation of pollutants. However, within the same lagoon, its distribution could even be controlled by the degree of confinement from the sea (Coccioni *et al.*, 2009).

In this assemblage, the significant occurrence of *E. excavatum* forma *selseyensis* was noted. *Elphidium excavatum* is a eutrophic species described in different coastal marine ecosystems by Murray (1991, 2006). It is also capable of developing several ecophenotypic variations and can be present in polluted environments (Feyling-Hanssen, 1972; Yanko *et al.*, 1999).

The results from station A2 seem anomalous if compared with the *A. tepida* assemblage (Cluster A): unlike the other stations along the Aussa River characterized by the dominance of *A. tepida* (Cluster A), A2 showed the highest rate of *H. germanica*. At this station, saline waters only occasionally occur at the bottom, according to the tidal range and the levels of freshwater discharge. Moreover, the presence at this station of well-preserved tests of Thecamoebians, freshwater protozoans which occasionally occur in brackish waters (salinity < 5) (Scott *et al.*, 2001), is indicative of the decreased influence of the salt wedge. This situation represents a greater degree of confinement from the sea, in agreement with the model proposed by Debenay *et al.* (2000) for the temperate meso- to macrotidal estuarine systems of the French Atlantic coast.

The areas represented by this assemblage corresponded to eutrophic areas with relatively high nutrient concentrations (both phosphorus and nitrogen) linked to freshwater outfalls (Falace *et al.*, 2009). A mean H index

value of 1.94 suggests moderately stressed or variable environmental conditions.

#### ***Ammonia tepida* and *Elphidium gunteri* assemblage (Cluster D; stations M2, G4, IGC and M1)**

**Additional common species:** *A. parkinsoniana*

This assemblage was characterized by a comparable frequency of *A. tepida* and *E. gunteri* (Tab. 3). Although this cluster consisted of sandy sediments, it differed from Cluster C by its lower presence of *H. germanica* (an average of 7.0%). The sampling stations in this group were located in the western sector of the lagoon (Stella river mouth) and in the Grado Basin (IGC), with salinity almost at marine levels (Fig. 8).

The significant presence of *E. gunteri* could indicate the tolerance of this taxon to high concentrations of nutrients, which characterize the area near the outflows of the Stella and Natissa Rivers (ARPA, 2008; Falace *et al.*, 2009). Slightly higher H index values in this sector indicate that physical parameters, such as temperature and salinity, are less variable (or more constant).

#### ***Taphonomically controlled assemblage (stations MA and GD)***

Two stations (MA, GD) represented subtidal areas located in proximity to the lagoon inlets, with marine salinity and elevated water circulation, where tidal currents reach their maximum velocities both during flood and ebb phases (Ferrarin *et al.*, 2010). The assemblage was characterized by the highest occurrence of *A. parkinsoniana* (mean of 35.5%) along with a low occurrence of *A. tepida* (mean of 25.7%). Although the counting was done only on the well-preserved forms, these data were not included in the cluster analysis, since the diagnostic characteristics of this assemblage were poor preservation of the shell material, with the specimens being somewhat abraded and broken. Here, the foraminiferal density reaches its lowest value (575 specimens/g on average), probably due to the high sand percentage in these sediments, which determines a decreasing concentration of tests.

The sediments were characterized by the highest value of sand content (mean of 52.5%) and the lowest of clay (mean of 4.8%), with the lowest organic C content (mean of 0.7%) (Fig. 2b) and  $C_{org}/N_{tot} > 10$ .

#### ***Test abnormalities and environmental/anthropogenic relationships***

Considering the different types of test abnormality recorded, the percentage of abnormalities in test morphology (FAI) always exceeded 1% of the total foraminifera. Since this value is considered the threshold in a non-stressed population, as suggested by Alve (1991), Geslin *et al.* (2002), and Frontalini & Coccioni (2008), the presence of abnormal tests in the studied lagoon

could suggest the adverse effects of recent environmental conditions and/or anthropogenic pollutants influencing foraminiferal fauna, in agreement with these authors.

Two clearly decreasing FAI gradients in the lagoon environment were detected; one from North to South and the other from West to East (Fig. 6). Lower FAI values corresponded to those areas in the eastern sector of the lagoon with the highest  $Hg_{tot}$  concentrations in sediments ( $Hg_{tot} > 4.0 \mu\text{g/g}$ ) and where the presence of the metal is mainly due to microcrystalline cinnabar (see figs. 4 and 5a in Acquavita *et al.*, 2012). This mineral has been transported into the lagoon by the suspended matter delivered by the Isonzo River, which drains the cinnabar deposits of the world's second largest Hg mine in Idrija (Slovenia), in operation for 500 years.

This could indicate that Hg, a chalcophilic element showing low affinity with carbonates (Garrett, 2005), probably has not interfered with the processes of crystallization of the tests responsible of the morphological abnormalities, as conversely reported for other elements like Cu and Zn (Sharifi *et al.*, 1991; Geslin *et al.*, 2000).

Furthermore, the western part of the lagoon, where a greater number of abnormalities were recorded, coincides with the sector with lower and more variable values of salinity, temperature and dissolved oxygen (ARPA, 2008; Ferrarin *et al.*, 2010). Furthermore, Falace *et al.* (2009), studying the distribution of benthic macrophytes in the Marano and Grado Lagoon, indicated that the most eutrophic conditions are found in this sector of the lagoon, which is characterized by the worst ecological evaluation index (EEI). This situation arose because of the high concentration of nutrients, mainly as  $\text{NO}_3^-$  and subordinately as  $\text{NH}_4^+$  and  $\text{NO}_2^-$ , flowing in with the waters of the Stella, Cormor and Aussa-Corno rivers (Fig. 6). Considering the  $N_{tot}$  concentration in lagoon waters during the period 2003-2006, medium values of 1916.2  $\mu\text{g/l}$  and 854.9  $\mu\text{g/l}$  were recorded for the Marano and Grado sectors, respectively (ARPA, 2008). Along the Aussa-Corno river, high FAI values also seem to reflect the environment conditions related to the activity of the chlor-alkali plant (CAP) used for the production of pulp, which was in operation until 1992.

Among the taxa, the results allow to define a degree of sensitivity to environmental/anthropogenic stress. In descending order, *A. tepida* was the species found to deform most frequently, followed by *E. gunteri*, *E. excavatum* forma *selseyensis*, *H. germanica*, *A. parkinsoniana* and *A. perlucida* (Fig. 7). This confirms the high degree of sensitivity of these opportunistic species, as recorded in other paralic settings. In particular *A. tepida* seemed to be more sensitive to organic matter content in the Aussa River, whereas the other species were found to be deformed more frequently in relation to the trophic conditions of the lagoon, exhibiting greater numbers in the Marano basin.

## Conclusions

The data presented in this study have shown that the recent foraminifera of the Marano and Grado Lagoon are characterized by a group of common euryhaline Mediterranean taxa. The study of the total assemblage, carried out on the first centimetre of the sediments, represented at least the last 10 years of foraminifera generations. The foraminifera found in the 21 surface samples comprised 47 species, pertaining to 28 genera, including 14 agglutinated, 24 hyaline and 9 porcelaneous species. *Ammonia parkinsoniana*, *A. tepida*, *A. perlucida*, *E. excavatum* forma *selseyensis*, *E. gunteri* and *H. germanica* characterize this environment.

The geographic distribution of the four foraminiferal assemblages, defined on the basis of cluster analysis, indicated that the north-western lagoon sector (Marano basin) and the Aussa River are affected by stressed environmental conditions (lower diversity indexes values). In the former case, strong fluctuations in salinity and, probably, the input of organic pollutants such as nutrients, could be the controlling factors for the distribution of foraminifera. In the latter case, the strong thermohaline stratification of the water column and the high organic matter and clay content in the sediment are the main environmental factors affecting the biotic communities. The sector less confined to the lagoon, located near the tidal inlets, demonstrated a greater presence of agglutinated foraminifera, such as *A. runiana*, *Haplophragmoides* spp. and *E. scaber*, together with *A. parkinsoniana*.

The observation of deformities shows that the foraminifera in the lagoon are prone to developing a series of morphological abnormalities that represent at least 10 years of stressed environments. Using the total assemblage, it is not possible to explain the mechanisms inducing these abnormalities; however, it was possible to observe that high percentages of abnormalities were found for *E. gunteri* and *H. germanica* in the most eutrophic areas of the western basin (Marano), where not only the highest concentrations of nutrients, but also the highest variations in salinity, are reported. Conversely, the FAI index decreased eastwards to the Grado Lagoon, where the highest concentrations of  $Hg_{tot}$  in sediments are historically recorded. Since a large proportion of Hg in sediments occurs in the form of sulphides, we can therefore underline that  $Hg_{tot}$  concentration has no apparent influence on the overall development of foraminiferal abnormalities in this lagoon. However, further research is needed to explore the possible relationships between these organisms and other types of contaminants, including other anthropogenic heavy metals (Cr, Cu, Zn, Ni, As).

Based on the rate of deformity occurrence, it can be affirmed that *A. tepida*, *E. gunteri*, *E. excavatum* forma *selseyensis*, *H. germanica*, *A. parkinsoniana* and *A. perlucida*, in descending order, are the forms that are particularly sensitive to environmental and anthropogenic factors in this lagoon.

In conclusion, these results provide original data on

recent benthic foraminiferal distribution in an area not previously investigated through use of these biomarkers, and emphasize the presence of important morphological abnormalities; further investigations related to living foraminifera will be necessary to confirm this behaviour. Moreover, these preliminary results will be useful for future research on the environmental stratigraphy of the sediment cores collected within the MIRACLE Project.

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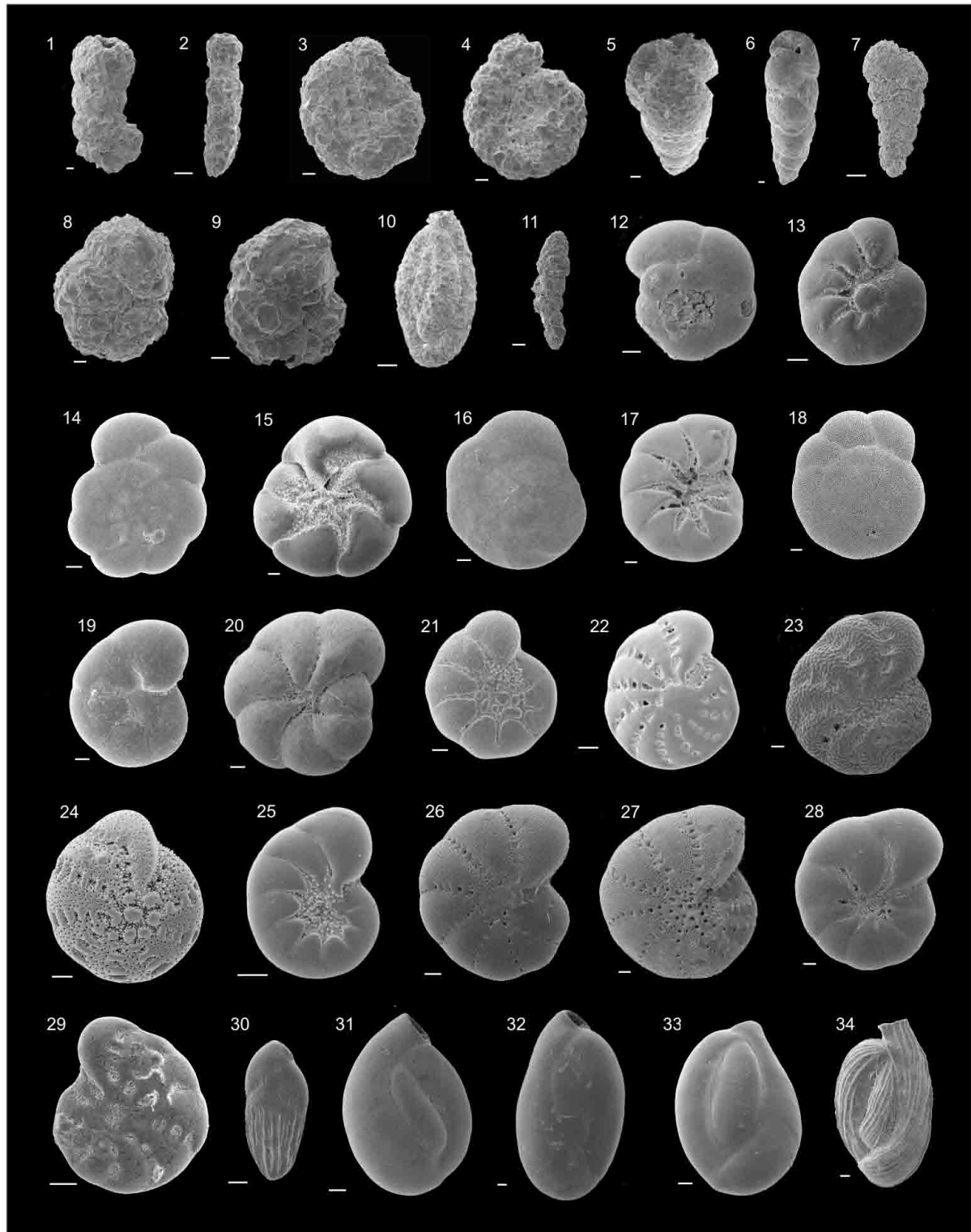
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**Plate I** - SEM photomicrographs of some foraminifer species representative of the environments recorded in the samples (magnification: bar = 30  $\mu$ m): 1 - *Ammobaculites exiguus*, side view; 2 - *Bigenerina nodosaria*, side view; 3 - *Ammoscalaria runiana*, side view; 4 - *Discammina compressa*, side view; 5 - *Eggerelloides scaber*, side view; 6 - *E. advenus*, side view; 7 - *Spiroplectinella earlandi*, side view; 8 - *Haplophragmoides australensis*, side view; 9 - *H. subglobosum*, side view; 10 - *Miliammina fusca*, chamber view; 11 - *Reophax nana*, side view; 12 - *Trochammina inflata*, spiral side; 13 and 17 - *Ammonia parkinsoniana*, umbilical side; 14 - *A. tepida*, spiral side; 15 - *A. tepida*, umbilical side; 16 and 18 - *A. parkinsoniana*, spiral side; 19 - *Aubignyna perlucida*, spiral side; 20 - *Nonion* sp., side view; 21 - *Elphidium granosum* forma *lidoense*, side view; 22 - *E. gerthi*, side view; 23 - *Elphidium pulvereum*, side view; 24 - *E. gunteri*, side view; 25 - *Haynesina depressula*, side view; 26 - *Criboelphidium poeyanum* forma *decepiens*, side view; 27 - *C. poeyanum* forma *poeyanum*, side view; 28 - *Haynesina germanica*, side view; 29 - *E. incertum*, side view; 30 - *Brizalina striatula*, side view; 31 - *Quinqueloculina milletti*, side view; 32 - *Q. seminulum*, side view; 33 - *Triloculina rotunda*, side view; 34 - *Adelosina carinata-striata*, side view.



**Plate II** – SEM photomicrographs of the most abundant abnormal benthic foraminifera grouped in six (1 to 6) different typologies (magnification: bar = 30  $\mu\text{m}$ ): a - *Ammonia parkinsoniana*, spiral side; b - *A. parkinsoniana*, umbilical side; c - *Triloculina rotunda*; d, o, q and u - *Elphidium gunteri*; e, i and l - *Haynesina germanica*; f and s - *Elphidium excavatum* forma *selseyensis*; g - *Ammonia tepida*, spiral side; h and m - *Aubignyna perlucida*, spiral side; n - *Nonion* sp.; p - *A. tepida*, lateral side; r, t and v - undetermined taxa.

