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Protists as Bioindicators of Past  
and Present Environmental Conditions

## Benthic Foraminiferal Assemblages and Biotopes in a Coastal Lake: The Case Study of Lake Varano (Southern Italy)

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**Abstract.** The benthic foraminiferal assemblages in Lake Varano (southern Italy) have been investigated in detail. Statistical analyses enable us to recognize two main biotopes and five sub-biotopes that reflect different ecological and environmental conditions. The assemblages mainly seem to be influenced by the hydrological (namely salinity) and sediment conditions in the lake. These biotopes are characterized by specific sub-assemblages and variations in relative species’ abundances. The Outer Lake Biotope is affected by marine influence and is dominated by the foraminiferal species: *Ammonia beccarii*, *Ammonia parkinsoniana* and *Aubignyna perlucida*, which are more common in open water environments. In contrast, the Inner-Marginal Lake Biotope reflects more restricted conditions where low salinity values and sand are associated with high numbers of miliolids. These two main biotopes are thus subjected to different degrees of confinement and water residence times, both of which are related to the water exchange time of the lake with the Adriatic Sea. In addition five sub-biotopes (Intermediate Marine, Mixing, “Urban”, Marginal, and Innermost) represented by particular foraminiferal assemblages are identified that characterize particular sub-environments. These sub-biotopes are, to some extent, separated by salinity gradients as well as the grain-size and the organic matter of sediments.

**Key words:** Benthic foraminifera, biotopes, sediment, Lake Varano, Italy.

### INTRODUCTION

Coastal lagoons and lakes being located between the sea and land are often economically important transi-

tional environments, particularly for fisheries and aquaculture. They are characterized by a series of gradients that are largely induced by hydrodynamics and play an important role in filtering substances that come from inland areas. Due to their location, transitional environments are extremely dynamic and heterogeneous, with a complex array of processes taking place that give rise to different sedimentological and ecological conditions (Frontalini *et al.* 2011). The great spatial and temporal variability of many environmental, physicochemical

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and geochemical parameters influences and drives the distribution and community structure of benthic organisms. Among these organisms, and due to their short life and reproductive cycles, high turnover rate, great abundance, and diversity, benthic foraminifera are expected to mirror the ecological and environmental conditions occurring in transitional environments (e.g. Debenay *et al.* 2000). As a consequence, they are widely used as pollution bioindicators in monitoring programs (for a review see Armynot du Châtelet and Debenay 2010, Frontalini and Coccioni 2011). Although plenty of investigations have documented the relationships between the distribution and composition of benthic foraminiferal species and numerous water and sediment physicochemical parameters, to our knowledge only a few studies have highlighted how benthic foraminiferal assemblages can be used to recognize distinct sub-environments within a given study area (e.g. Albani and Johnson 1975; Albani *et al.* 1984, 1991; Bernhard 1987; Hayward *et al.* 1996; de Mello e Sousa *et al.* 2006; Abu-Zied *et al.* 2007; Strotz 2012). In particular, by responding to changes in water and sediment parameters, the composition of benthic foraminiferal assemblages may vary in terms of their distribution, leading to the formation of sub-assemblages of different species or variations in their relative abundances, enabling ecologically-similar provinces to be readily identified (Albani *et al.* 1984). These ecological provinces (biotopes) thus include all of the stations that, from a benthic foraminiferal (biofacies) point of view, are similar and may shed light on which abiotic factors, or a combination thereof, drive these distributions. Specifically, on the basis of the benthic foraminiferal similarities, biotopes identify the areas characterized by comparable environmental conditions. A similar approach has been extensively applied in the Venice Lagoon and gulf, and several biotopes have been recognized there (e.g. Albani and Serandrei Barbero 1983; Albani *et al.* 1991, 1998, 2007, 2010; Serandrei Barbero *et al.* 1989, 1997, 1999, 2004). In particular, by applying different ordination and multivariate statistical analyses, researchers have been able to define different biotopes (i.e. marginal urban, marginal urban mixing, enclosed lagoon, inner lagoon, outer lagoon, marsh, near marsh, paleo-deltaic), each of them characterized by specific benthic foraminiferal assemblages and relative species' abundances.

This paper reports for the first time the benthic foraminiferal distribution and species' composition in Lake Varano. In particular, using an advanced statisti-

cal approach, the aim is to identify biotopes and their spatial distribution within the lake. The paper also intends to delineate which ecological and environmental factors contribute to the benthic foraminiferal distributions. The opportunity to identify various biotopes in very complex environments like Lake Varano allows us to evaluate its current environmental and ecological conditions. It also provides a unique opportunity to improve our understanding of transitional marine environments by using benthic foraminifera to develop an environmental management plan in this valuable but poorly known area.

## LAKE VARANO

Lake Varano is a coastal lagoon located on the north side of the Gargano National Park (southeast Italy). It covers a surface area of  $\sim 65 \text{ km}^2$  and the average water depth is  $\sim 4 \text{ m}$  (Spagnoli *et al.* 2002). The lake communicates with the Adriatic Sea via two artificial channels (Capoiale and Varano) that are located at the two ends of an 11 km-long and 1 km-wide baymouth bar with associated coastal dunes (Fig. 1). In addition freshwater is supplied from the catchment area ( $300 \text{ km}^2$ ) by two (Antonino and San Francesco Canals) tributaries and a submarine spring (Beneduce *et al.* 2010, Fabbrocini *et al.* 2010). The low tide excursion and the reduced exchange with the adjacent coastal area are responsible for the long (ca. 1.5 years) water residence time (Specchiulli *et al.* 2008). The surface sediments are very heterogeneous, with the sandy fraction only dominating near the shore (Scardi *et al.* 2008). The lagoon has been intensively used for the farming of mussels (*Mytilus galloprovincialis*) with an annual production of 6–8 kt (Breber and Scirocco 1998), and other aquaculture products (Beneduce *et al.* 2010). Although only a few small towns and no major industrial activities characterize the surrounding area, the relatively high concentration of nitrogen species likely reflects the impact of agricultural activities (Specchiulli *et al.* 2008). Blooms of dark red dinoflagellates have appeared during some summers, forcing the mussel farmers to reduce or remove their stock (Specchiulli *et al.* 2008). Recently, fishing has become the most important activity in the area (Manini *et al.* 2003). Although high nutrient loads and dinoflagellate blooms might suggest eutrophic conditions, Lake Varano has been regarded as an oligo-mesotrophic ecosystem (Belmonte *et al.* 2011).

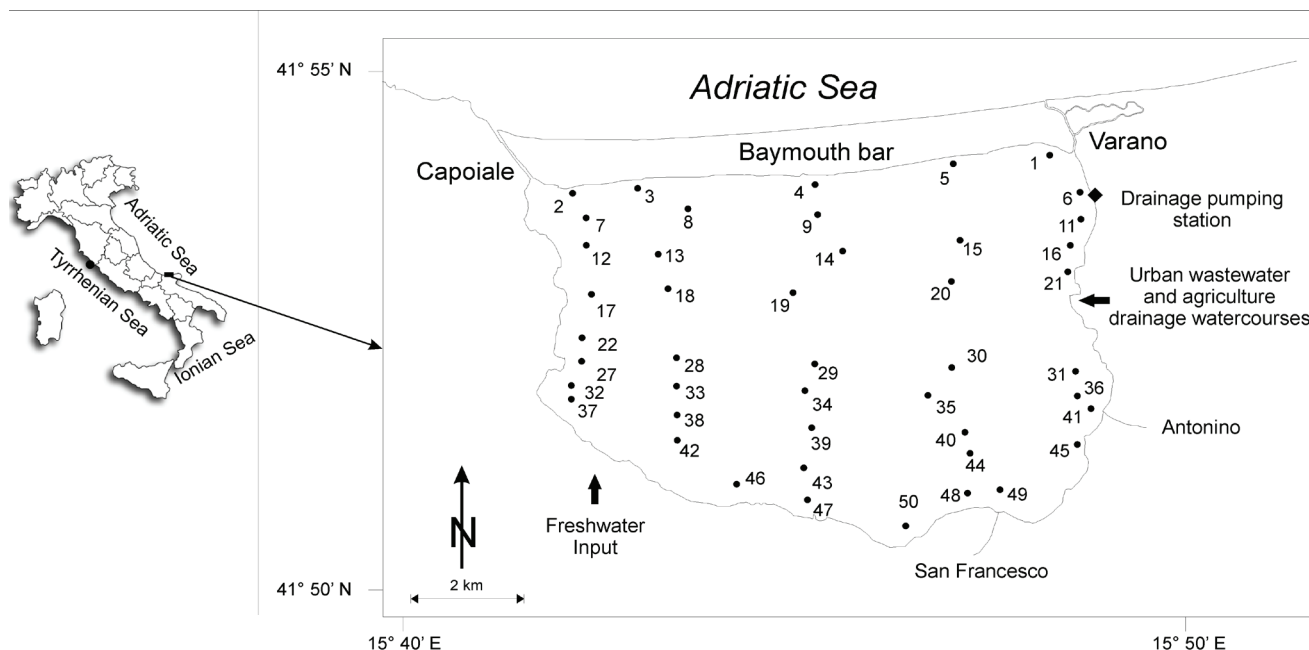


Fig. 1. Location map of the study area with sampling stations.

Only very few faunal investigations (Morigi *et al.* 2002, Manini *et al.* 2005, Florio *et al.* 2008, Specchiulli *et al.* 2010, Belmonte *et al.* 2011) have been carried out in Lake Varano. A paleontological analysis of a sediment core collected in the lake has provided paleoenvironmental information on the reconstruction of shoreline changes to the Gargano coast (Morigi *et al.* 2002). The uppermost part of the core reveals assemblages that are quite well-diversified and mainly dominated by the genera *Ammonia*, *Elphidium*, *Nonion*, and *Aubignyna*, and different species of miliolids and juvenile foraminifera (Morigi *et al.* 2002). The presence of four exotic species (two crustaceans; one gastropod, and one bivalve) in the lake, which is probably linked to accidental releases from aquaculture activities, was reported on by Florio *et al.* (2008). A total of 55 zooplankton categories was recognized in the lake by Belmonte *et al.* (2011) who also documented their maximum and minimum abundance in the eastern and central part of the lake, respectively. The macrobenthic community has been described by Specchiulli *et al.* (2010), who reported that the lowest abundances and diversities were in the central part of the lake. It is suggested that grain-size and the chemistry of the sediment explain much of the macrobenthic spatial variability rather than salinity

(Specchiulli *et al.* 2010). To our knowledge, no spatial investigation of benthic foraminifera has been carried out in Lake Varano.

## MATERIALS AND METHODS

### Sampling

A total of 45 surface (~ 2 cm) sediment samples was collected from Lake Varano in late March 2012. The samples were obtained by means of a Van Veen grab sampling device that collects sediment over a surface area of about 400 cm<sup>2</sup>. At each station, the physico-chemical parameters of the bottom water such as temperature, pH, salinity, ORP (Oxidation Reduction Potential), and DO (Dissolved Oxygen expressed as mg/l) were measured by means of CTD. On board, the grab was immediately and carefully opened in a container, where the sediment was deposited in its initial position. Three aliquots of sediments were subsampled and stored in polyethylene jars. The first aliquot was used for grain-size and organic analyses, and the second and third for investigating the meiofaunal (not included in this study) and the benthic foraminiferal assemblages, respectively.

### Grain-size

Grain-size analyses were conducted using the principle of the diffraction of a monochromatic laser beam on suspended particles (Malvern Mastersizer 2000, red He-Ne laser, 632 and 466 nm wave lengths). The method, following the detailed description in Trente-

saux *et al.* (2001), is based on the near-forward scattering of a laser beam by particles in suspension (Loizeau *et al.* 1994). Measurements can range from 0.02 to 2000  $\mu\text{m}$ , with an obscuration comprised of between 10 and 20%. The sediment was divided into three fractions: clay (< 2  $\mu\text{m}$ ), silt (2 to 63  $\mu\text{m}$ ) and sand (63 to 2000  $\mu\text{m}$ ).

### Organic geochemistry

The quantity and quality of the organic matter (OM) were estimated by analyzing the total C (TC), the total organic carbon (TOC), total sulfur (TS) and total N (TN) content in the sediment following the methodology described by Armynot du Châtelet *et al.* (2013). Measurements were carried out in duplicate on each sample and a mean was calculated after checking that there was no dissimilarity.

### Foraminiferal analysis

Immediately on board, following Walton's technique (1952), an aliquot of each sediment samples was stained with buffered Rose Bengal dye (2 g of Rose Bengal in 1000 ml of ethyl alcohol) for at least 14 days to differentiate between living and dead foraminifera (Schönfeld *et al.* 2012). All of the samples were dried at 50°C and weighed. They were then gently washed with tap water through a 63  $\mu\text{m}$  sieve to remove clay, silt and any excess dye. The residual fraction so obtained was re-dried at 50°C and weighed again. In this investigation the total foraminiferal assemblages were considered to enable us to identify biotopes (de Mello e Sousa *et al.* 2006; Albani *et al.* 2007, 2010; Strotz 2012). Accordingly, the use of the total populations overcomes the need for seasonal sampling, particularly in the case of species reproduction. At least three hundred specimens were, where possible, picked from each sample from the > 125  $\mu\text{m}$  fraction and identified following the generic classifications of Loeblich and Tappan (1987). Although the use of the > 125  $\mu\text{m}$  fraction might have prevented the collection of some species, it is commonly used and proposed even in foraminiferal biomonitoring studies (Schönfeld *et al.* 2012). This fraction was also chosen for consistency in the comparative process with other foraminiferal biotopes (e.g. Albani and Serandrei Barbero 1982; Albani *et al.* 1991, 1998, 2007, 2010; Phipps *et al.* 2010). Several foraminiferal parameters were calculated: species diversity (S, number of species per sample); the Fisher  $\alpha$  index (relationship between the number of species and the number of individuals in an assemblage, Fisher *et al.* 1943); dominance (D); the Shannon-Weaver index or information function (H) (Shannon and Weaver 1963); evenness (J); and equitability (E). These indices were calculated using the PAST – PAleontological STatistics data analysis package (version 1.68, Hammer *et al.* 2001).

### Statistical analysis

In order to reduce background 'noise', only species with a relative abundance greater than 1% in at least one sample were included in the statistical treatment. Prior to statistical analysis, an additive logarithmic transformation  $\log(1 + X)$  was performed to: remove the effects of orders of magnitude difference between variables; normalize the data; and increase the importance of smaller values such as the mid-range species (Frontalini *et al.* 2009). A Q-mode CA was carried out for the ordination of the samples based on the relative abundances of species calculated by adopting the Ward's linkage method and given in terms of the Euclidean distance. A PCA was used to determine the community's relationship to abiotic pa-

rameters and was carried out for the ordination of sample locations based on the matrix constructed using the relative abundances of species. In the PCA, it is possible to compute additional variables (abiotic data) that do not contribute to the results of the analysis.

## RESULTS

### Physicochemical parameters of the bottom water

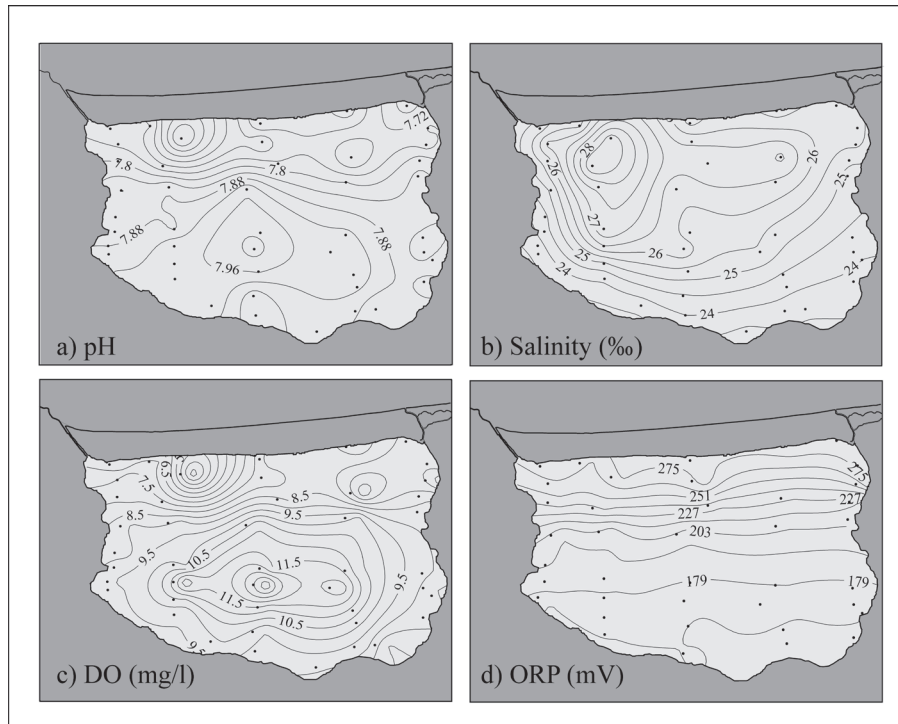
The pH values were quite homogeneous throughout the lake. All of the values fell between 7.4 (V8) and 8.0 (V34), with generally higher pH values in the inner and central parts of the lake (Fig. 2a; Appendix A). Salinity values ranged from 23‰ (V50) to 31.9‰ (V13). Higher salinity values, which never reached normal marine values, were found in the outermost part of the lake, particularly in the vicinity of the Capoiale channel (Fig. 2b; Appendix A). The ORP values ranged between 164.0 (V39, V40, V41, V44, V45) and 286.0 (V1, V5, V6). A clear ORP distribution was found in the lake with the highest values close to the coastal dune and the lowest values located in the central and innermost parts of the lake (Fig. 2c; Appendix A). The DO values varied from 2.7 (V8) to 15.2 (V34). The highest values of DO were found in the central and southwestern parts of the lake, whereas generally lower values were encountered near the two channels (Fig. 2d; Appendix A).

### Grain-size

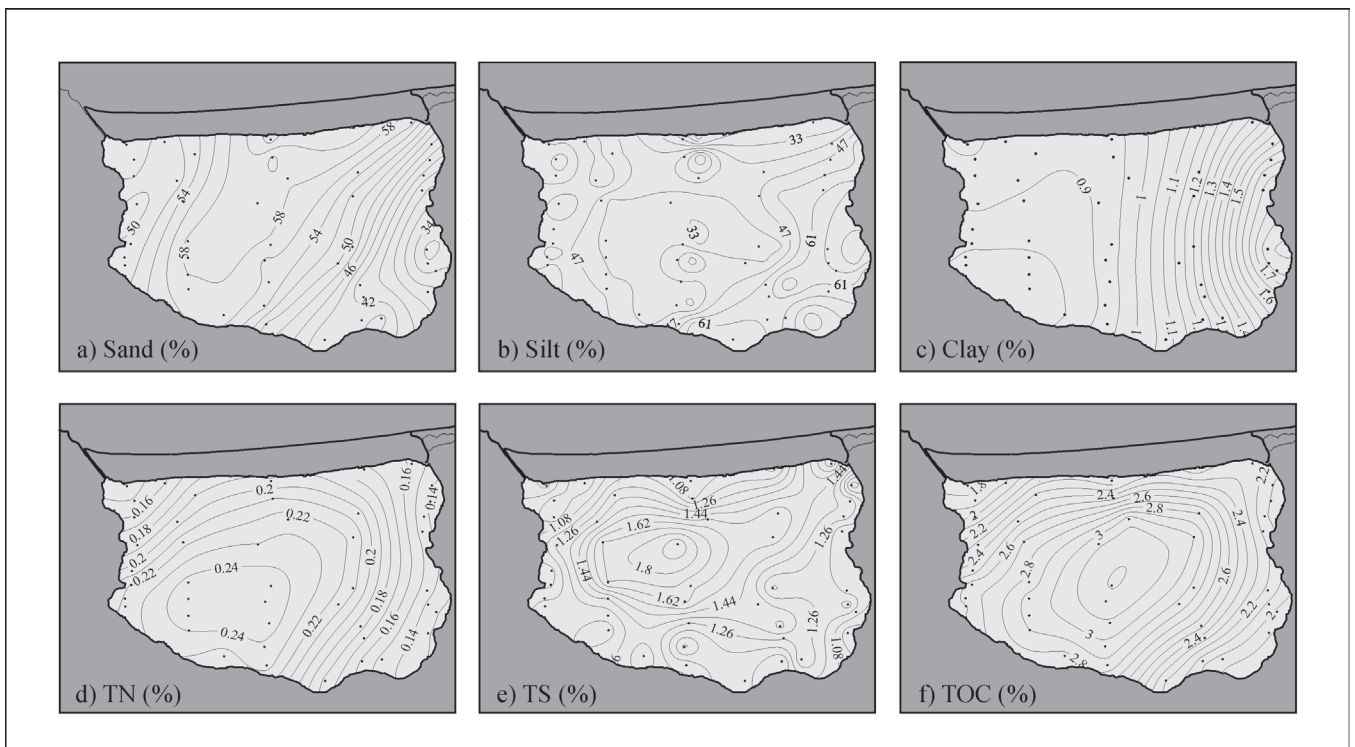
The 45 bottom sediments fell into two principal grain-size type classes: silt-dominated and sand-dominated substrates. The highest percentages of sand were found in the outer, inner south-western and central parts of the lake (Fig. 3a; Appendix A) whereas silt accompanied by clay fractions dominated in the southeastern side of the lake (Fig. 3b, c; Appendix A).

### Geochemistry

TN values were found to be between 0.10% (at several stations) and 0.37% (V27). Higher values were found in the central and southwestern parts of the lake (Fig. 3d; Appendix A). In contrast higher TS values were found in the central and northeastern parts of the lake (Fig. 3e; Appendix A). The TOC ranged from 0.16 (V4) to 5.35 (V14), with higher values in the central part of the lake and slightly higher values in southwestern part of the lake (Fig. 3f; Appendix A). The C/N ratio varied between 1.55 (V4) and 19.42 (V11), with higher values on the eastern periphery of the lake.



**Fig. 2.** Main physicochemical parameters of the bottom water of Lake Varano. The sampling stations are shown here as black dots.



**Fig. 3.** Grain size and geochemistry of sediments from Lake Varano. The sampling stations are shown here as black dots.

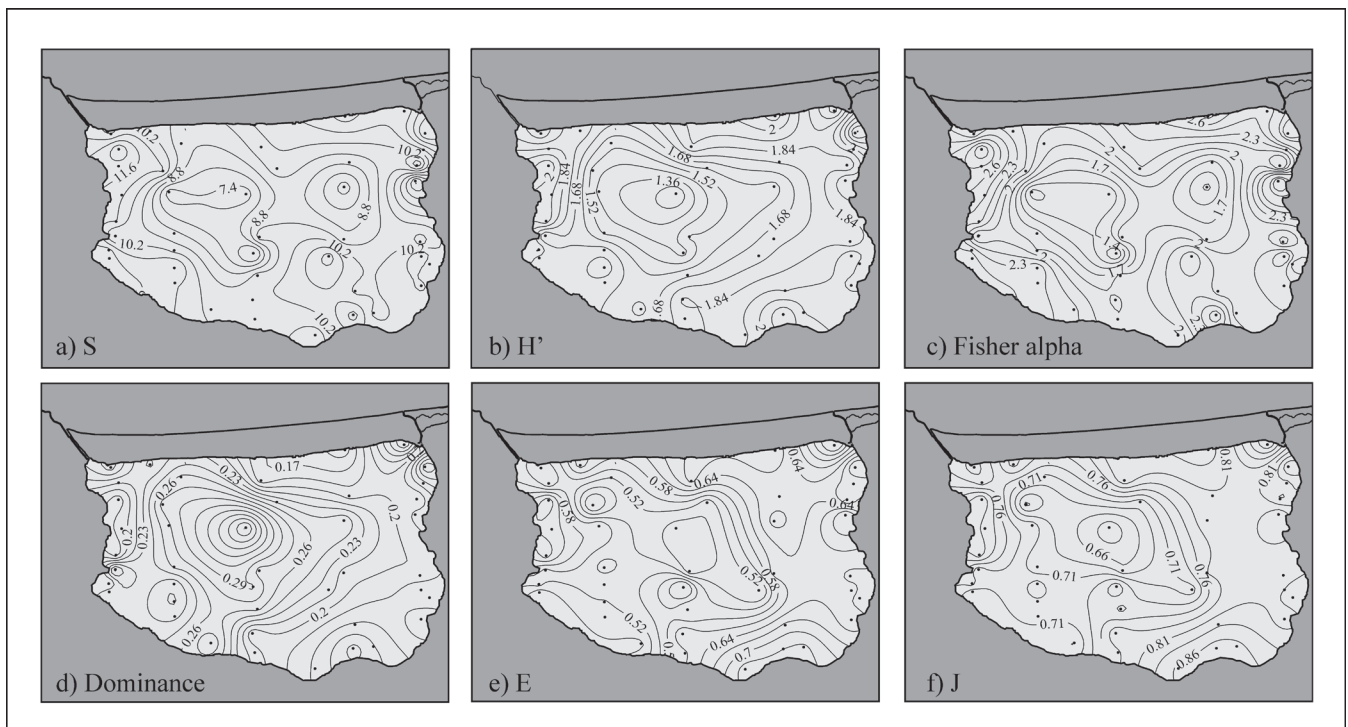
### Foraminiferal analysis

A total of 15 benthic foraminiferal taxa, belonging to eight genera, was identified in the total assemblage. The relative abundance of recognized species varied from station to station, with only 13 taxa having relative abundances of greater than 1% in at least one sample. Two stations (4 and 40) were devoid of foraminifera and were not therefore considered in the statistical analysis. Species diversity (S) varied from 6 (V34) to 14 (V6 and V21). Lower values of S were generally recognized in the central part of the lake (Fig. 4a). Similarly, lower values of the Shannon-Weaver index (Fig. 4b; Appendix B) and the Fisher  $\alpha$  index (Fig. 4c; Appendix B) were also found in this part of the lake. Dominance ranged from 0.11 (V6) to 0.39 (V19), E from 0.41 (V13) to 0.75 (V6), and J from 0.62 (V19) to 0.89 (V6) (Fig. 4d–f; Appendix B). The central part of the lake was characterized by higher values of D and low values of E and J. The assemblages were largely dominated by *Ammonia tepida* (25% on average), *Ammonia beccarii* (22.4% on average), and *Haynesina germanica* (20.4% on average), and subordinately by *Elphidium granosum* (8.9% on average) and *Quin-*

*queloculina seminulum* (3% on average). The highest relative abundances of *Ammonia beccarii* were found close to the two channels and in the central part of the lakes (Fig. 5a; Appendix B), whereas *A. tepida* seemed to dominate in the southwestern and southeastern parts of the lake (Fig. 5b; Appendix B). Although present in relatively low percentages, *Ammonia parkinsoniana* was generally found in the outer part of the lake (Fig. 5c; Appendix B). In contrast higher percentages of *E. granosum* and *E. oceanensis* were found in the eastern and southeastern parts of the lake, respectively (Fig. 5d, e; Appendix B). *Haynesina germanica* had a distinct distribution pattern, with the highest values located in the central part of the lake (Fig. 5f; Appendix B). Miliolids including *Q. seminulum* and *Quinqueloculina pseudobuchiana*, and broken miliolids were found in highest abundance on the periphery of the lake (Fig. 5g–i; Appendix B).

### Statistical analysis

The Q-mode cluster analysis resulted in the grouping of samples into two main clusters (A and B) according to the relative abundances of species (Fig. 6,



**Fig. 4.** Benthic foraminiferal assemblages' parameters from Lake Varano. The sampling stations are shown here as black dots.

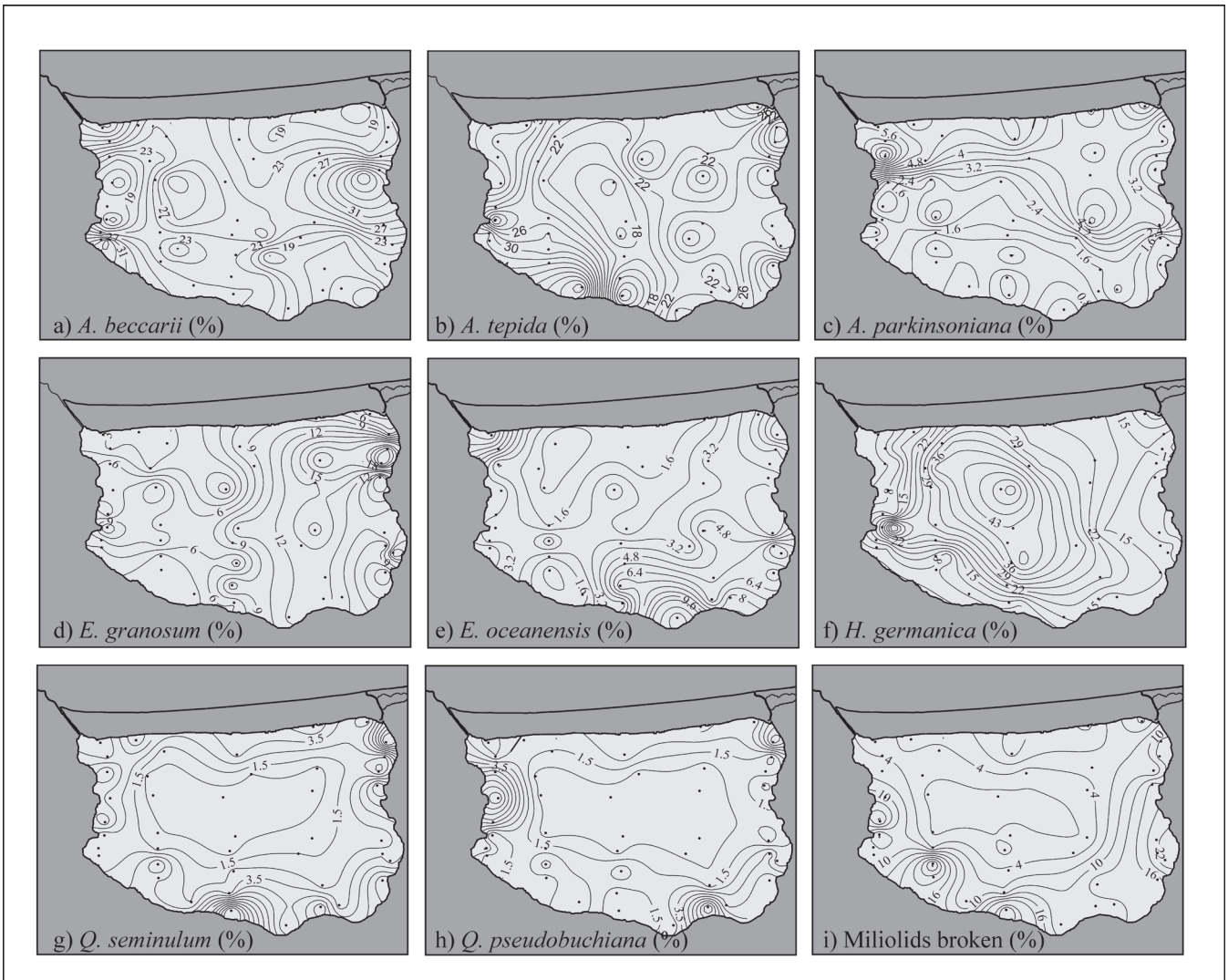


Fig. 5. Distribution of the most abundant species in Lake Varano. The sampling stations are shown here as black dots.

Table 1). Cluster A includes the stations located in correspondence with the two channels or those in the central part of the lake, whereas Cluster B embraces the stations on the margins and in the inner part of the lake. Clusters A and B can be further subdivided into sub-clusters A1, A2a and A2b and B1 and B2, respectively (Fig. 6). Sub-cluster A1 comprises only the stations in correspondence with the two channels, sub-cluster A2a includes the stations located in front of the Capoiale channel, whereas subcluster A2b generally covers the stations in front of the Varano channel. Sub-cluster B1 groups together most of the stations placed on the margins of the lake, whereas sub-cluster B2 comprises

most of the stations in the innermost part of the lake. All of these clusters and sub-clusters reflect the particular environmental characteristics found in Lake Varano.

The PCA revealed that ~ 70% of total variance (inertia) can be explained by the first four principal components (factors). These exhibit eigenvalues greater than one and have therefore been considered. In particular, the eigenvalues of component 1 (~ 34% of inertia), component 2 (15.2% of inertia), component 3 (11.9% of inertia) and component 4 (9% of inertia) were 4.4, 2.0, 1.5 and 1.2, respectively. Relative abundances of *A. beccarii* and miliolids were the predominant elements in the first component, while

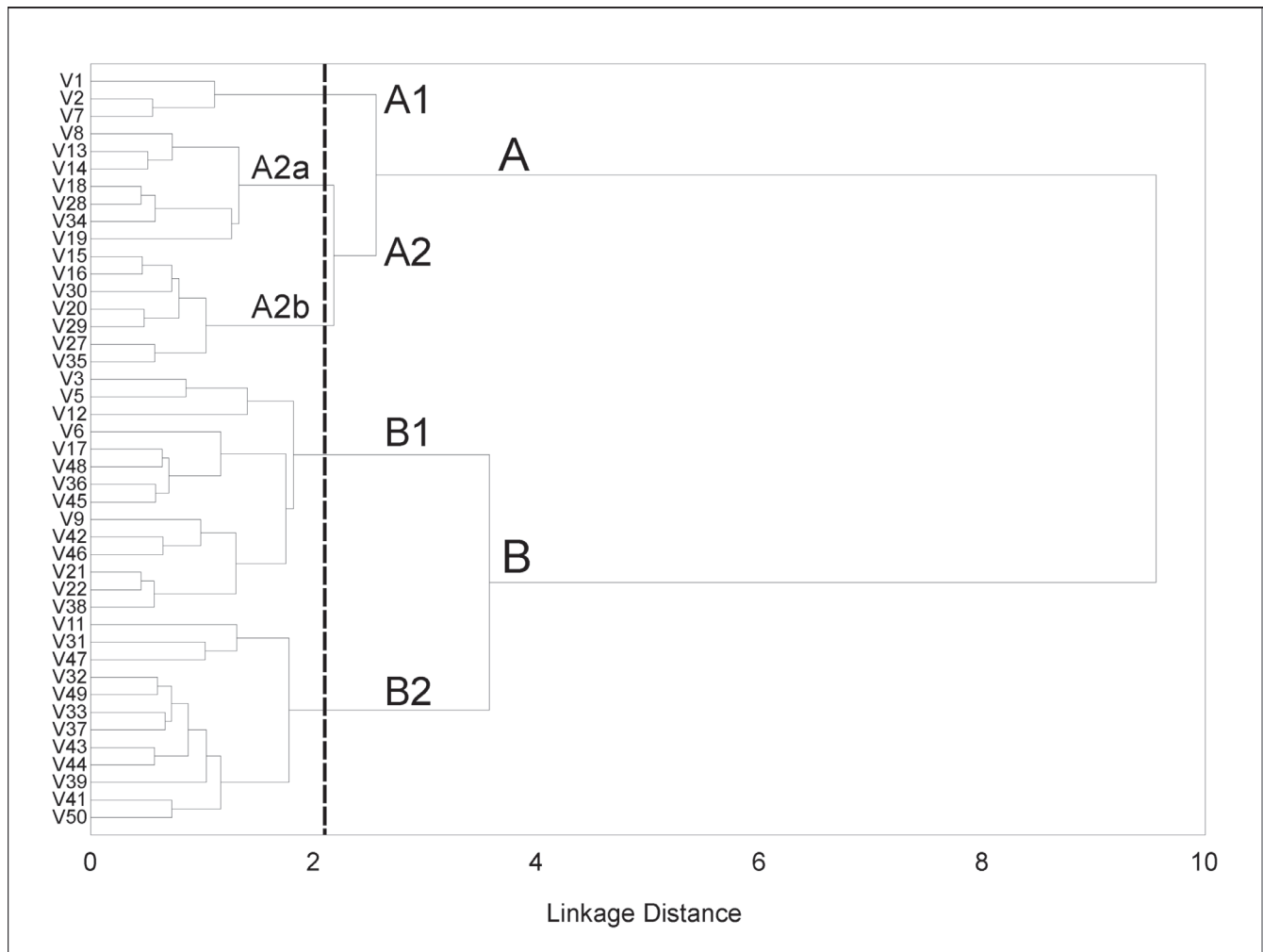


Fig. 6. Dendrogram classification of the stations produced by a Q-mode cluster analysis using the Linkage Distance.

the contributions to the second component were due mainly to *E. granosum*, *E. oceanensis* and *A. tepida* (Fig. 7a). *Ammonia parkinsoniana* and *Aubignyna perlucida* dominated the third component and *Haynesina germanica* the fourth (Fig. 7b). In order to better understand the relationships of biotic and abiotic data, secondary variables (abiotic) were plotted on the factor-planes (Fig. 7a). It is clear that organic matter (TOC, TN, TS) and salinity are linked to the first component, whereas grain-size (silt, clay, sand) and ORP are the main parameters in the second component. The third component is again dominated by ORP and pH and the fourth by clay and depth (Fig. 7b). All of these parameters might have had an influence on the distributions of the benthic foraminiferal assemblages (Fig. 8; Table 1).

## DISCUSSION

Several studies of benthic foraminiferal assemblages' and species' distributions have been carried out in lagoons and coastal lakes (for a review see Frontalini *et al.* 2011). A common pattern of distribution is along a marine-continental gradient, with diverse assemblages, including calcareous forms closest to the ocean, changing landwards into oligospecific-to-monospecific assemblages in both hyposaline and hypersaline environments (Debenay *et al.* 2000, Debenay and Guillou 2002). Numerous studies have suggested that salinity is the major controlling factor for the presence of foraminiferal assemblages in transitional environments. However, their distribution is often more complex, re-



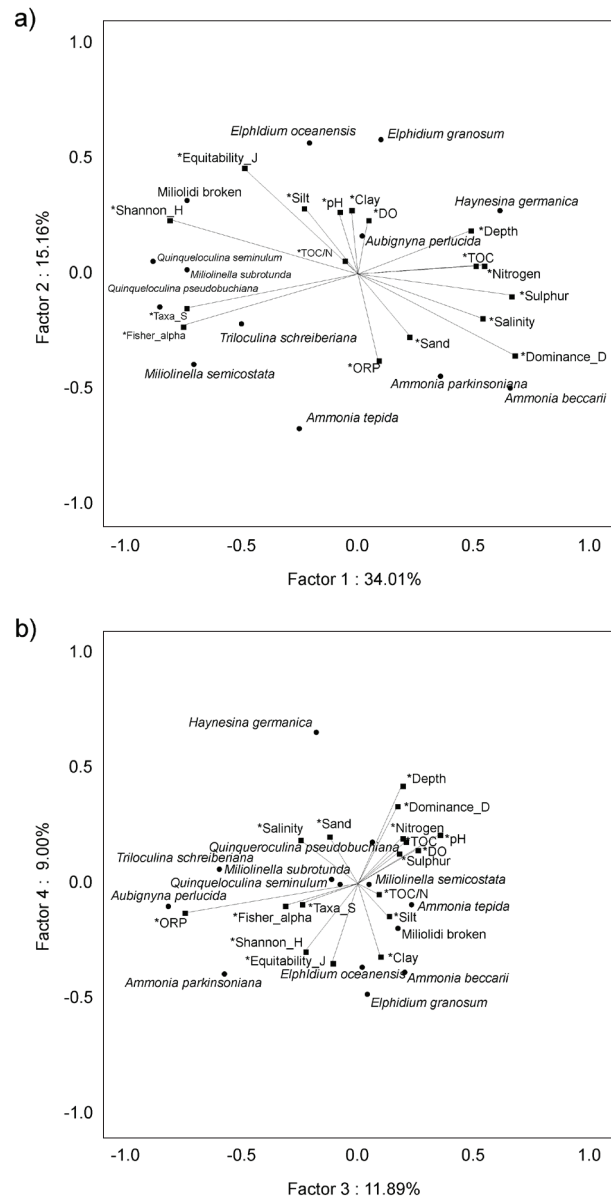
**Table 1.** Mean physicochemical parameters of the bottom water, grain-size and geochemistry of the sediments, benthic foraminiferal assemblages' parameters and relative abundances of selected taxa.

Sub-biotope	A1	A2a	A2b	B1	B2
Depth	1.6	3.9	3.5	2.7	2.9
Salinity	26.4	28.5	25.6	24.3	24.4
DO	7.4	9.0	9.8	8.7	9.3
Silt	36.7	45.1	45.3	52.7	52.1
Sand	62.3	54.0	53.6	46.2	46.4
TN	0.1	0.2	0.3	0.1	0.2
TS	1.3	1.6	1.4	1.1	1.2
TOC	2.1	3.3	3.3	1.8	2.6
<i>Ammonia tepida</i>	32.9	21.9	21.5	29.5	21.7
<i>Ammonia parkinsoniana</i>	5.6	3.1	3.0	2.3	1.8
<i>Ammonia beccarii</i>	35.4	26.6	24.6	17.3	21.4
<i>Elphidium granosum</i>	3.2	5.7	16.2	6.1	11.2
<i>Elphidium oceanensis</i>	6.4	1.6	3.3	3.4	6.1
<i>Haynesina germanica</i>	8.7	38.7	26.5	12.6	18.1
<i>Aubignyna perlucida</i>	1.1	0.3	0.3	0.4	0.5
<i>Quinqueloculina seminulum</i>	1.7	0.3	0.7	5.5	3.2
<i>Quinqueloculina pseudobuchiana</i>	1.1	0.2	0.2	5.3	1.7
<i>Miliolinella subrotunda</i>	0.2	0.1	0.2	0.9	0.5
<i>Miliolinella semicostata</i>	0.5	0.1	0.2	1.5	0.2
<i>Triloculina schreiberiana</i>	0.1	0.0	0.0	1.1	0.0
<i>Miliolids broken</i>	2.2	1.2	3.4	14.1	13.6
Taxa S	11.3	8.4	9.1	11.6	10.3
Shannon H	1.69	1.45	1.67	1.96	1.84
Fisher $\alpha$	2.55	1.63	1.78	2.58	2.03
Dominance D	0.26	0.29	0.23	0.18	0.20

flecting multiple interactions between the distance to the sea, water depth, the nature of the sediment, turnover time, oxygen and nutrient availability in the sediment and so on. Due to its high heterogeneity in terms of water and sediment characteristics Lake Varano is an ideal environment in which to study the distribution of benthic foraminifera, identify the biotopes and understand which parameter(s) determine the distribution of the species and the assemblages.

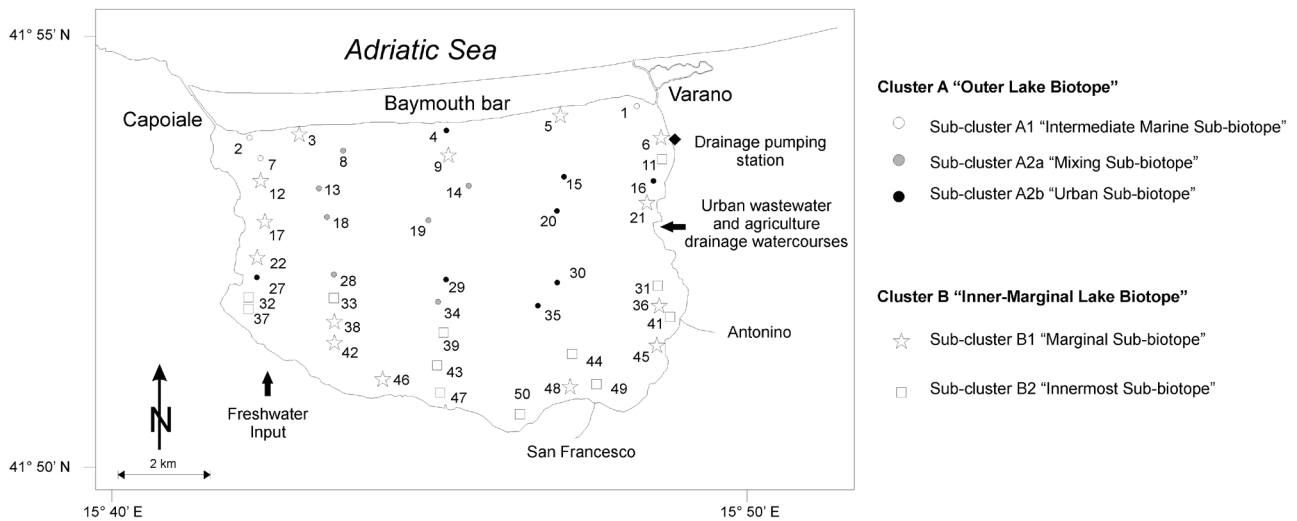
**Foraminiferal assemblages and species**

Benthic foraminifera are generally abundant, but the assemblages are somewhat poorly diversified as testified by the relatively low values of the diversity indi-



**Fig. 7.** PCA ordination diagram by projecting variables on the factor-planes (a: 1 × 2 and b: 3 × 4). The secondary variables are marked with a black square.

ces. The H index exhibits values lower than 2 and can be easily compared with values encountered in other restricted environments. So for instance, H values between 1.0 and 1.9 were found in Lake Qarun (Egypt) by Abu-Zied *et al.* (2007). The assemblages are mainly composed of forms that have different modes of life, and are comparable to the *Ammonia* assemblages with *H. germanica* that are characteristic of lagoons along



**Fig. 8.** Distribution of the five sub-biotopes as identified by Cluster Analysis.

the Mediterranean coast (see Murray 1991, 2006). These species tolerate a wide range of environmental parameters (salinity, temperature, oxygen concentration, sediment substrate) which are typical of transitional marine environments characterized by more or less restricted conditions and a decreased marine influence (Almogi Labin *et al.* 1992; Debenay *et al.* 2000, 2005). The dominance of calcareous hyaline forms might suggest a lower degree of confinement and in turn moderately restricted conditions (Frontalini *et al.* 2011). This inference perfectly matches those based on zooplankton by Belmonte *et al.* (2011) who suggested a not extreme degree of confinement for the lake. The *Ammonia* population of the studied area is represented, in order of decreasing relative abundance, by *A. tepida*, *A. beccarii*, and *A. parkinsoniana*. *Ammonia tepida* is the most abundant species in the lake and has often been recognized in shallow marine environments, lagoons and deltaic zones (Jorissen 1988; Almogi-Labin *et al.* 1992; Frontalini *et al.* 2009, 2010). *Ammonia tepida* is a euryhaline, cosmopolitan species, and is also known for its great tolerance of stress conditions (e.g. Setty and Nigam 1984). The second most abundant *Ammonia* species is *A. beccarii*, which is most plentiful in the Adriatic Sea at a water depths of 15–20 m and in samples with intermediate percentages of organic matter and at least some sand fraction (Jorissen 1988). In contrast *A. parkinsoniana* is only a minor component (< 3%) in the assemblages of the lake. This taxon, which is

typical of relatively clean environments (Seiglie 1975), is common all along the Italian coast between water depths of 10 and 20 m irrespective of substratum type and the percentage of organic matter (Jorissen 1988). It has also been found in the sandy substrates in the northern Adriatic Sea (Jorissen 1988) and dominating in the shallow water depths of the central Adriatic Sea (Frontalini and Coccioni 2008). It is the dominant species of *Ammonia* at shallow depths in the Marmara Sea that are influenced by the brackish outflow water from the Black Sea (Phipps *et al.* 2010). *Haynesina germanica* is the third most abundant species in the investigated area and has been widely considered by Armynot du Châtelet *et al.* (2004) to be a tolerant species and an indicator of stressful environmental conditions. It is normally associated with fine-sediment substrates and has been found in high abundances in several Italian lagoons and coastal lakes like Venice, Orbetello, Santa Gilla and Lesina (for a synthesis, see Frontalini and Coccioni 2011). *Elphidium granosum* is an important component of the benthic foraminiferal assemblages in the lake. This species was found in the clay-belt parallel to the Italian coast and was dominant in clayey substrates with relatively high percentages of organic matter (Jorissen 1988). These assemblages can be compared to those found in the Lesina Lagoon (Gargano promontory), a transitional environment very close to Lake Varano (Frontalini *et al.* 2010). These authors documented oligotipic assemblages, mainly represented by *A. tepida*,

*H. germanica*, bolivinids, *E. oceanensis*, and several species belonging to the genus *Quinqueloculina*. On the basis of the micropaleontological analysis of a sediment core collected from Lake Varano, Morigi *et al.* (2002) documented in the uppermost part of the core assemblages mainly dominated by *Ammonia*, *Elphidium*, *Nonion*, *Aubignyna* and different species of miliolids and juvenile foraminifera. These results can be readily compared with our findings.

### Foraminiferal biotopes and their distribution

The results of the cluster analysis enable us to recognize different assemblages (biofacies) occurring in specific sub-environments (biotopes) in Lake Varano. Cluster A includes the stations located in correspondence of the two channels or in the central part of the lake, whereas Cluster B embraces the stations on the margins and in the inner part of the lake. On the basis of this distribution, Cluster A can be defined as the Outer Lake Biotope whereas Cluster B is the Inner-Marginal Lake Biotope. The former is characterized by the highest values of salinity and sand whereas the latter by more brackish conditions coupled with finer substrates. This well-defined grain-size differentiation can be easily explained by the fact that current dynamics are more active in the northern-outer margins near to the two channels (Specchiulli *et al.* 2010). These different environmental conditions are also reflected by different foraminiferal assemblages. More specifically, these two clusters can be further separated into sub-clusters. The Sub-cluster A1, which includes the stations located in correspondence with the two channels, is characterized by the highest abundance of *A. tepida*, *A. parkinsoniana*, *A. beccarii* and *A. perlucida* and the lowest abundance of *H. germanica*. The distribution of these species clearly reflects the marine influence, namely salinity, and this biotope might be defined as the Intermediate Marine Sub-biotope. The greatest foraminiferal diversity (S, Fisher  $\alpha$  index and H) was found in this biotope. When compared with the others the Intermediate Marine Sub-biotope is characterized by relatively higher values of salinity and includes all of the stations located in the shallowest water depths and with the highest percentages of sand. Sub-cluster A2a, which embraces the stations located in front of the Capoiale channel and in the central-eastern part of the lake, is characterized by the highest abundance of *H. germanica* and, when compared to A1, lower percentages of *Ammonia* assemblages. The stations in this group have the lowest diversity index values and the

highest values of dominance. Remarkably, the low diversity values match those of the macrobenthos documented by Specchiulli *et al.* (2010). This group can be defined as the Mixing Sub-biotope. This biotope exhibits high values of salinity and the highest organic matter content coupled with the lower percentages of sand when compared to Intermediate Marine Sub-biotope. Meanwhile, Sub-cluster A2b groups together the stations in front of the Varano channel and in the central-western part of the lake that are under the direct influence of urban wastewater and agriculture drainage watercourses. Accordingly, this sub-cluster has relatively high values of fine (silt and clay) sediment and the highest values of total nitrogen. The foraminiferal assemblages recognized in this sub-cluster can be well compared to A2a, but it differs due to its higher percentages of *E. granosum* and relatively lower abundance of *H. germanica*. This group of stations can be defined as the "Urban" Sub-biotope. On the basis of foraminiferal assemblages composition, the Outer Lake Biotope can be compared with the Outer-Lagoon Biotope and even more directly with the Marine Sub-biotope identified by Albani *et al.* (1991). On the southern Marmara shelf, *E. granosum* displays maximum abundance at water depths of 20–40 m, in more saline waters just beneath the influence of brackish outflow water from the Black Sea (Phipps *et al.* 2010). Sub-clusters A2a and A2b, when considered together, occupy the central part of the lake and are characterized by the lowest level of diversity, these data perfectly mirror those of macrofauna as documented by Specchiulli *et al.* (2008). These authors suggested that this part of the lake, where clay component prevails and the water exchanged is limited, might experience oxygen depleted conditions. This central part exhibits the highest abundances of *H. germanica* and of total organic carbon. Remarkably, in the organic-rich brackish Lake Saroma (Japan), *Haynesina* sp. was found to dominant in area enriched of total organic carbon (Takata *et al.* 2006). A positive correlation of *H. germanica* and organic matter was also found in five harbors on the Vendée (France) coast by Armynot du Châtelet *et al.* (2004). The same authors suggested that this species might be able to migrate in oxygen-poor but organic-rich environments where other species are excluded. Vidović *et al.* (2009) documented the highest abundance of *H. germanica* in correspondence of cage where the impact of fish farm is the strongest. Sub-cluster B1 comprises most of the stations located on the margins of Lake Varano and is characterized by

the lowest salinity values and the highest silt contents. From a foraminiferal point of view, this sub-cluster is typified by the highest abundance of miliolids, namely *Q. seminulum*, *Q. pseudobuchiana*, *Miliolinella subrotunda*, *Miliolinella semicostata*, and *Triloculina schreiberiana*, and broken unidentified miliolids. It is also characterized by the highest foraminiferal diversity values and the lowest dominance values. The recognized high values of diversity of foraminiferal assemblages are well-mirrored by those of macrofauna (Specchiulli *et al.* 2008). The highest level of total phosphorous, finer particle and decreased organic matter were inferred as the cause of the highest level of macrofaunal diversity indexes (Specchiulli *et al.* 2008). This group of stations can be defined as the Marginal Sub-biotope. Sub-cluster B2 groups together those stations that are mainly located in the innermost part of the lake along with others on the eastern margins. It is characterized by low salinity values and sand like Sub-cluster B1, but with higher values of organic matter content. This group of stations is affected by water discharged from the Antonino and San Francesco canals along with urban and agriculture runoff from the south and urban wastewater and drainage watercourses from the east. The assemblages are again represented by high percentages of porcelanaceous foraminifera and to a lesser extent by a lower abundance of *A. parkinsoniana* and *A. tepida* when compared to Sub-cluster B1. This group reflects the most confined stations in the lake, and the biotope can be defined as the Innermost Sub-biotope. The Inner-Marginal Lake Biotope can be directly compared with the Inner-Lagoon Biotope identified by Albani *et al.* (1991), where there were relatively low values of *A. beccarii* when compared to the Outer Lake Biotope.

## CONCLUSIONS

This study documents for the first time the benthic foraminiferal assemblages present in the transitional environment of Lake Varano. Although the assemblages appear to be poorly diversified, the diversity index values are similar to those generally reported in other transitional environments. The assemblages are composed of forms with different modes of life and are comparable to the *Ammonia* assemblages with *H. germanica* that are characteristic of lagoons along the Mediterranean coast. On the basis of statistical analyses, five foraminiferal assemblages corresponding to

different sub-environments (two main biotopes and five sub-biotopes) are recognized. These biotopes are characterized by specific sub-assemblages and variations in the relative species' abundances. The Outer Lake Biotope is affected by marine influence and is dominated by foraminiferal species that are more common in open water environments. In contrast the Inner-Marginal Lake Biotope reflects more restricted conditions where low values of salinity and sand are associated with high values of miliolids. These two main biotopes are thus subjected to different degrees of confinement and water residence times, both of which are related to the water exchange time of the lake with the Adriatic Sea. In addition five sub-biotopes (Intermediate Marine, Mixing, "Urban", Marginal, and Innermost) represented by particular foraminiferal assemblages have also been identified and their sub-environments characterized. These sub-biotopes are, to some extent, separated by not only the salinity gradient, but also by grain-size and the organic matter of sediments. This study confirms the applicability of benthic foraminifera for improving environmental management plans in the area.

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**Appendix A.** Geographic coordinates of the sampling stations, water depths, physicochemical parameters of the bottom water, grain-size and the geochemistry of sediments.

**Appendix B.** Benthic foraminiferal assemblages' parameters and relative abundances of the recognized taxa.