Mediterranean Marine Science

Vol 20, No 1 (2019)

Seasonal response of benthic foraminifera to anthropogenic pressure in two stations of the Gulf of Trieste (northern Adriatic Sea, Italy): the marine protected area of Miramare versus the Servola water sewage outfall

ROMANA MELIS, MASSIMO CELIO, VINCENT M.P. BOUCHET, GABRIELLA VARAGONA, MATTEO BAZZARO, MATTEO CROSERA, NEVIO PUGLIESE

doi: 10.12681/mms.16154

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
The journal is available on line at http://www.medit-mar-sc.net
DOI: http://dx.doi.org/10.12681/mms.16154

Seasonal response of benthic foraminifera to anthropogenic pressure in two stations of the Gulf of Trieste (northern Adriatic Sea, Italy): the marine protected area of Miramare versus the Servola water sewage outfall

Romana MELIS[1], Massimo CELIO[2], Vincent M.P. BOUCHET[3], Gabriella VARAGONA[1], Matteo BAZZARO[4,5], Matteo CROSERA[6] and Nevio PUGLIESE[1]

[1] Dipartimento di Matematica e Geoscienze, 34127 Trieste, Italy
[2] ARPA (Regional Environmental Protection Agency), 33057 Palmanova (UD), Italy
[3] Univ. Lille, CNRS, Univ. Littoral Côte d'Opale, UMR 8187, LOG, Laboratoire d'Océanologie et de Géosciences, F 62930 Wimereux, France
[4] OGS (Istituto Nazionale di Oceanografia e di Geofisica Sperimentale), 34010 Sgonico (TS), Italy
[5] Dipartimento di Scienze fisiche, della Terra e dell'ambiente, 53100 Siena, Italy
[6] Dipartimento di Scienze Chimiche e Farmaceutiche, 34127, Trieste, Italy

Corresponding author: melis@units.it

Handling Editor: Ahuva ALMOGI-LABIN

Received: 16 April 2018; Accepted: 17 October 2018; Published on line: 30 April 2019

Abstract

A seasonal survey of living benthic foraminifera was performed in 2013 in the Gulf of Trieste (N Adriatic Sea) to compare two marine coastal sites with different degrees of anthropogenic influence. An assessment of ecological quality statuses showed that the station located near the end of an urban pipeline (**Ser** station), has worse ecological conditions than the site located in a protected marine area (**Res** station) all year around. Stressed conditions at **Ser** station were mainly related to high contents of total organic carbon (TOC) and Zn in the bioavailable fraction, which were a limiting factor for the studied foraminiferal communities. *Ammonia tepida*, *Bolivina* spp., and *Bulimina* spp., which characterised this station, were the most tolerant taxa of the studied assemblage. Conversely, *Elphidium* spp., *Haynesina depressula, Nonionella iridea, Quiqueloculina* spp., *Reophax nana* and *Textularia* spp., could be considered less tolerant species as they benefitted from the less stressful conditions recorded at **Res** station, despite slightly higher concentrations of some potentially toxic elements (PTEs), especially Pb, being recorded in this station in comparison to **Ser** station. Furthermore, foraminiferal assemblages were found to be quite resilient over an annual cycle, being able to recover from a seasonal unbalanced state to a mature one. The beginning of spring and latest summer would be the best period to assess the ecological quality status to avoid any under- or overestimation of the health of the environment.

Keywords: Meiofauna; seasonal biomonitoring; pipeline outfall; marine protected areas; heavy metals; sediments.

Introduction

Coastal marine environments are fragile ecotones at the border between marine and continental ecosystems. Over the last decades, increases in industrial, agricultural, aquacultural and tourism activities have led to pollution in these ecosystems (Bouchet & Sauriau, 2008; Frontalini *et al.*, 2009; Francescangeli *et al.*, 2016) since they serve as receptacle for the majority of coastal terrestrial run-off. The increasing loading of terrestrial nutrients into coastal marine ecosystems induced the expansion of dead zones where hypoxic to anoxic conditions occur (Diaz & Rosenberg, 2008). This pollution has deleterious effects on benthic communities, leading to the loss of sensitive species and the proliferation of opportunistic ones (Lardicci *et al.*, 2001), heavily modifying the functioning of the benthic-ecosystem (Solan *et al.*, 2004).

In order to protect these environments, restoration measures have been taken, e.g. OSPAR, Natura 2000, marine protected areas (MPAs), the European Water Framework Directive (WFD) and the European Marine Strategy Framework Directive (MSFD). Among these measures, the establishment of MPAs (i.e. marine reserves) has proven to be an effective method of conserving marine biodiversity (Guidetti *et al.*, 2008; Edgar *et al.*, 2014). Along the Northern Adriatic coast of Italy, the Gulf of Trieste (GoT, Fig. 1) hosts two contrasting environments: an area strongly affected by anthropogenic activities and a MPA. The GoT hosts two of the largest cargo shipping ports in the Adriatic Sea, Trieste and Koper. This coastal

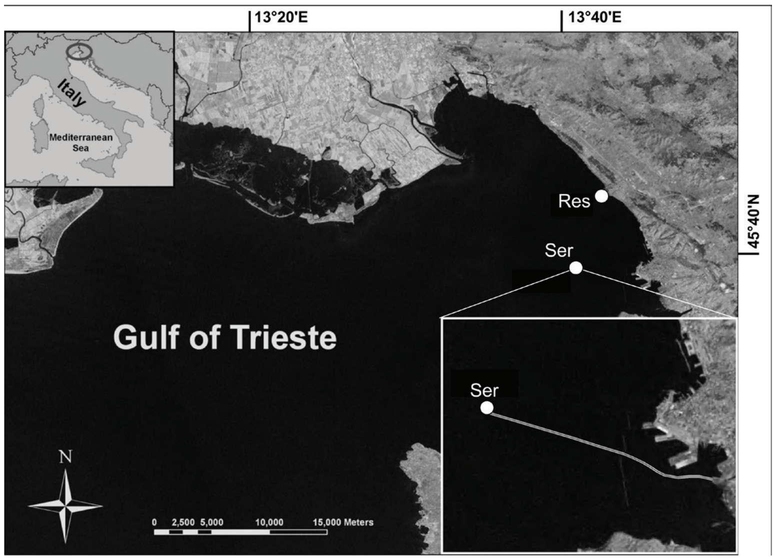


Fig. 1: Study area and location of the **Ser** (Servola) and **Res** (Riserva Naturale Marina di Miramare) stations. The Servola pipeline is evidenced in the enlarged square.

area is also affected by many potential sources of organic and inorganic pollutants, discharged not only by rivers but also by sewers, industries, and activities related to the harbour including an oil-pipeline terminal (Olivotti *et al.*, 1986; Adami *et al.*, 1996, 1998; Cibic *et al.*, 2008). Furthermore, the Servola pipeline rejects the discharges from the main local urban and manufacturing activities in the GoT. To protect the ecosystems of the GoT, the MPA *Riserva Naturale Marina di Miramare* (EUAP 0167) was established in 1986 by decree of the Italian Ministry of the Environment, which was first managed by WWF Italy Onlus Association (D.M. November 12, 1986) and then by WWF Oasis srl.

Although there are numerous MPAs along the coast of Italy, their effectiveness in protecting marine biodiversity has rarely been assessed. Numerous biological groups have been proposed to assess the health of marine systems (e.g., Borja *et al.*, 2000; Krause-Jensen *et al.*, 2005; Coates *et al.*, 2007). The importance of those organisms in characterising environmental status (ES) is highlighted by the MSFD, which aims to achieve Good ES (GES) of the EU's marine waters by 2020. Among these bioindicators, macroalgae, angiosperms, fish and benthic invertebrates are considered particularly relevant. Benthic macro-invertebrates are by far the most popular (e.g., Bouchet & Sauriau, 2008; Lavesque *et al.*, 2009; Munari & Mistri, 2010). Lately, emphasis has been placed on benthic foraminifera (e.g. Bouchet *et al.*, 2012; Barras *et al.*, 2014; Alve *et al.*, 2016; Dimiza *et al.*, 2016; Jorissen *et al.*, 2018).

Benthic foraminifera are single-celled protists with a short reproductive cycle, rapid growth, small size and their tests are well preserved in the sediments allowing for retrospective studies. They are also highly diversified and abundant in comparison with other hard-shelled taxa (e.g. molluscs or ostracods). Moreover, they are easy to collect and are able to provide reliable data for statistical analysis, even with small sample volumes (Schönfeld *et al.*, 2012). Furthermore, a wide range of papers highlights their high potential to serve as relevant bioindicators of anthropogenic pressure in marine ecosystems (e.g. Murray & Alve, 2002; Martínez-Colón *et al.*, 2009; Armynot du Châtelet & Debenay, 2010; Frontalini & Coccioni, 2011; Alve *et al*., 2016; Bouchet *et al*., 2018; Jorissen *et al*., 2018). It has been noted that in stressed and/or polluted environments, diversity and abundances could change, and therefore the structure of the community. It is possible to define a group of species with similar levels of tolerance to contaminant influence (e.g. Alve *et al.*, 2016). In particular, some studies focused on the response of these organisms to the submarine outfall of domestic sewage that could represent significant source of contaminants (Stott *et al.*, 1996; Hyams-Kaphzan *et al.*, 2009; Teodoro *et al.*, 2010; Eichler *et al.;* 2012, Tadir *et al.*, 2017). All this evidence has led to the definition of monitoring methods based on benthic foraminifera (Bouchet *et al.*, 2012; Barras *et al.*, 2014; Schönfeld *et al.*, 2012; Dimiza *et al.*, 2016). Recently, diversity indices based on benthic foraminifera have been proven to be a suitable method to evaluate ecological quality status along the coast of Italy (Bouchet *et al.*, 2018). Hence, benthic foraminifera appear to be a relevant group to compare environmental conditions in two contrasted areas of the Gulf of Trieste: the MPA of Miramare and the area impacted by the Servola sewage outfall.

To date, the GoT has not been carefully studied for living benthic foraminifera. Apart from Hohenegger *et al.* (1989, 1993) and Sabbatini *et al.* (2010), all other studies on foraminifera took into consideration dead assemblages or limited areas close to the Slovenian coast (Langlet *et al.*, 2014). In the present study, a seasonal survey was designed to analyse living benthic foraminifera communities sampled in two stations submitted to different degrees of anthropogenic disturbance located in the GoT (north Adriatic Sea): one station is located close to the domestic sewage outfall from the main submarine pipeline of the municipality and the second station is located in the MPA. The aims of this study are: (1) to describe benthic foraminifera living communities and their response to environmental parameters and seasonal changes at the two sampling sites; (2) to assess the ecological quality status at sampling stations using the diversity index Exp(H'_{bc}) calculated on benthic foraminifera.

Study area

The Adriatic Sea is an elongated NW/SE oriented basin of the central Mediterranean where the depth varies from approximately 35 m in the northern part to a maximum depth of 1250 m in the southern part. In the nortern part, it receives the main contribution of freshwater and sediments from the Isonzo River.

The water circulation of the GoT is driven by the interplay of different forcing: the general circulation of the Adriatic Sea, wind stress (particularly the dominant Bora, N-NE direction) and buoyancy fluxes together with the tides. The surface layer is commonly characterised by a clockwise flow, while the intermediate and bottom layers usually move in a counterclockwise direction. The water column shows a marked seasonal variability. During winter, it is characterised by homogeneous conditions of temperature, salinity and density. Starting from the end of April the onset of the heating process, particularly of the surface layer, causes the beginning of the vertical stratification. Summer is characterised by strong thermal stratification with a temperature gradient of about 10-12°C/20 m in depth. During autumn, the eastern winds blow and cooling processes at the air-sea interface produce the beginning of the convective and mechanical mixing of the water column which continues during the winter months (Malačič & Petelin, 2001; Celio *et al.,* 2006).

The sediment distribution varies from medium to fine sands along the beaches and the delta front to muds in the mid-Gulf and sandy sediments in the western open part of the GoT (Brambati *et al.*, 1983; Ogorelec *et al.*, 1991). Moreover, the site has been recognised as an area where particular conditions related to inputs of fluvial sediments or to a meteo-marine conditions led to important algal productions and blooms, resulting in eutrophication and subsequent hypoxic/anoxic conditions at the bottom at least until the mid-1980s (Giani *et al.*, 2012).

The sediment samples collected for this study come from two sites, one located in the proximity of the Servola pipeline termination and a second used as a reference point for a "pristine" situation in the *Riserva Naturale Marina di Miramare* (hereafter referred to as the Reserve).

The Servola wastewater treatment plant is the largest sewage plant in the city, serving a maximum of 2.5×10^5 equivalent inhabitants. The Servola pipeline began operations in 1992 and disposes of sewage that after chemical treatment, carries the residual waters through a submarine pipeline 7.0 km long of which the last 1.5 km are provided with specific diffusers in order to make the dispersion of wastewaters easier. The delivery capacity of the pipeline varies from 2×10^3 (dry periods) to 6×10^3 l/s (rainy periods). The pipeline reaches a depth of 24 m (Novelli, 1996). The Servola plant was studied by Mattassi *et al.* (2008) and Scroccaro *et al.* (2010) to assess possible risks connected with the microbiological parameter *Escherichia coli*. The authors highlighted that under particular meteo-marine forcing conditions and due to high input concentration values, the submarine discharges may influence the water quality creating several problems for shellfish farming activities located along the coast of Trieste. Starting in 2016, some modernisation works of this plant were undertaken to arm the system with a more effective purification system, such as biological treatment on land. The new plant is expected to be finished by 2018.

The Reserve is located in the GoT at the base of the promontory of Miramare and covers an area of 0.3 km^2 surrounded by a sea area of 0.9 km^2. The area is located along a rocky limestone stretch of marine coast that slopes in boulders, pebbles and muddy formations gradually from the coast to the sea with a maximum depth of 18 m. In June 2011, the Reserve was identified and proposed as a SCI - Site of Community Importance (directives 79/409/EEC and 92/43/EEC). The effectiveness of the application of these directives made it possible to achieve the ecological objectives set before the establishment of the reserve (Guidetti *et al.*, 2008). Since 2009 the regional agency for environmental protection (ARPA-FGV) has been involved in monitoring the GoT following the directive of the *Piano Regionale di Tutela delle Acque*. Monthly monitoring of marine and transition (lagoons and river mouths) waters is performed as per ministerial decree D.lgs. 190/260/10 and 2010. Following the data guide provided by the colleagues from ARPA-FGV, it was decided to operate at **Ser** station, near the pipeline which discharges the urban waste of Servola and at **Res** station (*Riserva Marina di Miramare*) (Fig. 1).

Material and Methods

Two stations **Ser** (45°38.6480 N, 13°40.9620 E; water depth 24 m) and **Res** (45°42.050 N, 13°42.600 E; water depth 17 m) were sampled by the R/V Effevigi (ARPA FVG) for water column features, benthic foraminifera and sediment properties. Sampling was done seasonally for a year: 11/01/2013 - **win13**, 22/05/2013 - **spr13**, 26/08/2013 - **sum13** and 18/11/2013 - **aut13**. The two stations are located off the Servola sewage sludge outfall (**Ser**) and close to the Reserve (**Res**) (Fig. 1) respectively.

Water column vertical profiles of pressure, temperature, conductivity, pH, dissolved oxygen and chlorophyll *a* from the surface to a depth of 22 and 17 m respectively for **Ser** and **Res** stations, were collected seasonally on board using an Idronaut mod. 316 multiparametric probe, which is calibrated following the manufacturer's protocols. The data obtained were processed by Idronaut software in order to verify the quality check. The sediments at each sampling station were sampled three times using a KC Haps bottom corer with a sample area of 0.013 m^2 and an effective depth penetration of 10 cm. The sediment from one box-core was sampled two times using

Perspex pipes (inner Ø=5.4 cm, surface area 22.9 cm^2) for foraminifera and for chemical analyses and sediment texture. Two other sediment samples were taken from the second and third box cores for microorganisms only, for a total of four short cores. The upper two cm from each core were sliced immediately on board into 1 cm thick layers (2 subsamples) with a thin metal plate. Immediately after sampling, the samples for benthic organisms were stored in plastic bottles filled with 23 cc of preservative solution (2 g of Rose Bengal per litre of ethanol at 95%). Sediments for chemical and grain-size analyses were frozen immediately on board. Three replicates were used for microorganism analysis (6 subsamples for each station).

Sixteen samples (2 stations, 0-1 and 1-2 cm, 1 replicate, 4 seasons) were analysed for grain-size analyses. The texture of each sample was determined using a Malvern Mastersizer Hydro2000S Diffraction Laser unit for the < 2 mm size fraction. Sand and mud classes were determined using the Udden-Wentworth (Wentworth, 1922) grain-size classification and the sediment was described following Shepard (1954). Grain-size parameters (Mean Size - Mz - and sorting) were determined using the Folk & Ward formulas (Folk & Ward, 1957). Total organic carbon (TOC) and total nitrogen (TN) content were detected as the mean value of 2 replicates of the same sample using an Elemental Analyser (ECS 4010 CHNSO) and acetanilide as standard for calibration. Prior to analysis, the samples were progressively acidified with HCl 0.1-1.0 N (Hedges & Stern, 1984).

An aliquot of the 16 sampled sediments were treated to determine the content of potentially toxic elements (hereafter PTEs) Cd, Cr, Cu, Ni, Pb and Zn, using the cold diluted HCl leaching technique proposed by Adami *et al.* (2000). This procedure is indicated to determine the "non-residual" metal fraction mainly accumulated in relation to anthropogenic sources (Chester & Voutsinou, 1981; Voutsinou-Taliadouri, 1995; Adami *et al.*, 2000) and corresponds to its bioavailability in agreement with Morse & Luther (1999) and National Research Council (2003). This term defines the rate at which a chemical substance becomes accessible to an organism potentially causing toxic effects (National Research Council, 2003). In order to compare the results with heavy metal content representing a preindustrial condition, two levels of core GT3 (51 and 53 cm below the sea floor) collected in the Gulf of Trieste by R/V OGS Explora in 1997 (Covelli *et al.*, 2006) were treated here in the same manner. All solutions were analysed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) using the Spectroflame Modula E instrument by SPECTRO® of the Department of Chemical and Pharmaceutical Sciences of the University of Trieste. The metal concentrations of Cd, Cr, Cu, Ni, Pb and Zn were evaluated using calibration curves obtained by the dilution of SPECTRASCAN® multi-element standard solutions (for ICP-AES analyses). The limits of detection (LOD) were in the range of 0.010-0.020 mg/L minuscule, that is: mg/l.

Samples for benthic foraminiferal analyses (2 stations, 0-2 cm, 3 replicates, 4 seasons) were stained in Rose Bengal in order to separate living from dead specimens. After 15 days in Rose Bengal solution, the ethanol in the samples was replaced by water and the samples were oven-dried at 50 °C and weighed. The sediment volume was detected subtracting the known preservative volume from the total sediment + preservative volume, as suggested by Schönfeld *et al.* (2012). Each sample was then wet-sieved over a 63 μm mesh. The total sandy sediments were used for picking all live (Rose Bengal stained) benthic foraminifera. Considering the well-known problems in the use of Rose Bengal (Corliss & Emerson, 1990; Bernhard, 2000), only the foraminifera specimens showing a clear pink colour (or red, depending on the species) in all but the last chambers were considered to be living fauna in agreement with Goineau *et al.* (2012) and Barras *et al.* (2014). If necessary, opaque porcellaneous and agglutinated specimens were broken to check for the presence of protoplasm. 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), Fiorini & Vaiani (2001), Milker & Schmiedl (2012). For original descriptions of species, the Ellis & Messina (2017) online catalogue on foraminifera was used (http://www.micropress.org/). The taxa pertaining to genus *Bolivina* (i.e. *Bolivina dilatata*, *B. seminuda*, *B. spathulata* and *B. variabilis*) are linked by numerous intermediate forms (see Barmawidjaja *et al.* 1992) where a consistent division in the different species could be problematic. These species were treated here as group *Bolivina* spp., as also suggested by Duijnstee *et al.* (2003) and Sabbatini *et al.* (2010) in their study of the northern Adriatic Sea. Selected specimens were displayed using a scanning electron microscope (Leica Stereoscan 430i) at the University of Trieste (Fig. S1).

To process the results concerning the foraminifers, we decided to pool the counts from the three replicates (rather than the average) of each sample since the aim of the study was not to assess the local micro-distribution of benthic foraminifera, following the suggestion of Schönfeld & Numberger (2007) and Bouchet *et al*. (2012). For each station, the following faunal parameters were calculated: 1) foraminiferal density (as the number of specimens normalised to 50 cc), 2) species richness, and 3) the respective proportion of the three principal foraminiferal groups (hyaline, porcellaneous and agglutinated foraminifera). To describe the foraminiferal density and to compare it with bottom water column parameters, we used their "complete living assemblage", considering the levels 0-1 and 1-2 cm of each sample as an only assemblage.

From the complete living foraminiferal assemblage, three measures of species diversity were calculated for each station using the PAST (PAlaeontological STatistics) data analysis package (Hammer *et al.*, 2001): 1) species richness (S) as the total number of taxa for each station; 2) Shannon diversity (H') and 3) Dominance (D) indexes. To define the true diversity, bias-corrected Shannon (H'_{bc}) (Chao & Shen, 2003) was converted with the exponential function Exp(H'_{bc}) proposed by Hill (1973), which represents the number of species that would, if each were equally common, produce the same H'_{bc} as the sample (see Bouchet *et al.*, 2012, p. 68 for discussion). Distribution patterns of living foraminiferal abundances are represented by using rank-frequency diagrams (RFD) (Frontier, 1976; Legendre & Legendre, 1984). Ecological interpretation of RFDs was reviewed by Frontier (1985) in a comparison of mathematical models (Mouillot & Lepretre, 2000). It relies on the recognition of 3 ecological succession stages associated with 3 different RFD curve shapes: linear-concave = pioneer assemblage with low species richness (stage 1); convex = intermediate assemblage with increasing species diversity (stage 2) and straight-line = mature assemblage (stage 3) (see Fig. 3 in Frontier, 1976). In some cases, a straight-line may reflect mixtures of different assemblages of intermediate diversity. The method was successfully applied to assess short-term variations in foraminiferal assemblages due to environmental conditions changes (Bouchet *et al.*, 2007). Finally, $Exp(H'_{bc})$ was used to evaluate the ecological quality status (EcoQ) of the studied areas, following the criteria set by Bouchet *et al.* (2012) where ecological quality is expressed as: $Exp(H'_{bc}) < 5$: bad, $5 < Exp(H'_{bc}) < 10$: poor, $10 < Exp(H'_{bc}) < 15$: moderate, $15 < Exp(H'_{bc}) < 20$: good and $Exp(H'_{bc}) > 20$: high.

All statistical analyses were performed considering the species with relative abundance higher than 4% (8 species and 5 genera). Species with a relative abundance < 4.0% were omitted, or gathered into major generic groups (i.e. *Elphidium* spp., *Quinqueloculina* spp., *Textulariina* spp.) in order to reduce background noise due to the infrequent taxa. Taking into consideration the pooled counts from three replicates for each species in all the samples, contingency tables tested by the chi-square test ($\chi 2$) were used to compare the absolute abundance of the most significant foraminifera between the two studied stations and among the seasonal samplings. Two correlations using Spearman's rank correlation were made: a) in the first case the absolute abundance of the major species was used using the 0-2 cm level and the bottom water column parameters and, b) in the second case a matrix with the absolute abundance of the foraminifera was used in the 0-1 cm level with their relative content of heavy metals, TOC, N_{tot} and grain-size. The significance threshold was set at $p < 0.05$ and the analysis was performed using STATISTICA 7.1 software.

Results

Water column

In both stations during the sampling period, the water **temperature** profiles showed a similar pattern during winter, spring and autumn, while they differ slightly during summer, where the **Res** station showed a thermocline close to the bottom. Considering both stations, the maximum thermal stratification was observed during spring. **Salinity** values were obtained from temperature and conductivity. The values were comparable and almost constant around a mean value of 37.3 ± 0.3 along the water column in both stations during summer and autumn. During the winter a particularly anomalous fresh water mass outflowing from the pipe caused a salinity decrease (mean value 29.1 ± 0.1) all over the water column at **Ser** station. During spring, a salinity decrease approximately 33 in the first 5 m of the water column was observed. The **pH** values were quite homogenous along the water column and very similar between the two stations during winter and autumn. They were generally higher than 8 at the surface with a tendency to decrease slightly at the bottom, mainly during spring and summer at **Res** station. Water **dissolved oxygen** during winter, summer and autumn in both stations records values from 90 to 100% of saturation. Values < 90% were registered from 15 m toward the bottom layers of **Ser** station during summer. On the contrary, in spring the water column is oversaturated (> 110% sat.) at all depths except for the bottom layer of both stations which recorded a value of 92.5 and 93.5% sat., for **Ser** and **Res**, respectively. **Chlorophyll *a*** showed the lowest values in autumn in both stations, with mean values of 0.26 ± 0.05 µg/l throughout the water column, a slight increase during winter and summer (around 0.8µg/l) and higher values were seen at both stations during spring. Chlorophyll *a* reached values higher than 2.0 µg/l at the depth interval of 6 to 14 m at both stations, indicating significant phytoplankton activity (mainly due to the diatom *Chaetoceros* spp., ARPA FVG, unpublished data). At **Res** station, the chlorophyll *a* content is higher than that of **Ser** station, reaching the maximum value of 3.2 µg/l at a depth of 7 m (Fig. 2).

Sediment composition

<u>Grain-size distribution</u>

The grain-size analyses indicated that the sediments from **Ser** station varied from clayey silt, sandy silt to silty sand. The sand content varied from 13.3 to 44.8% with a mean value of 21.1 ± 11.1%, the silt from 44.4 to 71.9 with a mean value of 64.8 ± 9.7% and clay (< 4 µm) from 10.8 to 15.9 with a mean value of 14.1 ± 1.7% (Table 1). Sediments were poor to very poorly sorted with a mean size (Mz) varying from 4.8 to 6.0 phi. Altogether, the grain-size were quite similar in two sublevels (0-1 and 1-2 cm) of the same sample and during the seasons except for autumn, where a higher sand content was found in sublevel 1-2 cm.

The sediments from **Res** station were quite homogenous in time and depth. They were prevalently silt, and differ from **Ser** station having lower sand content. The percentage of sand varied from 3.5 to 9.8% with a mean value of 5.3 ± 2.2%, silt from 74.4 to 78.5 with a mean value of 76.5 ± 1.3% and clay (< 4 µm) from 12.5 to 22.1 with a mean value of 18.1 ± 3.0% (Table 1). The sediments were poorly sorted with a mean size (Mz) varying from 5.9 to 6.6 phi.

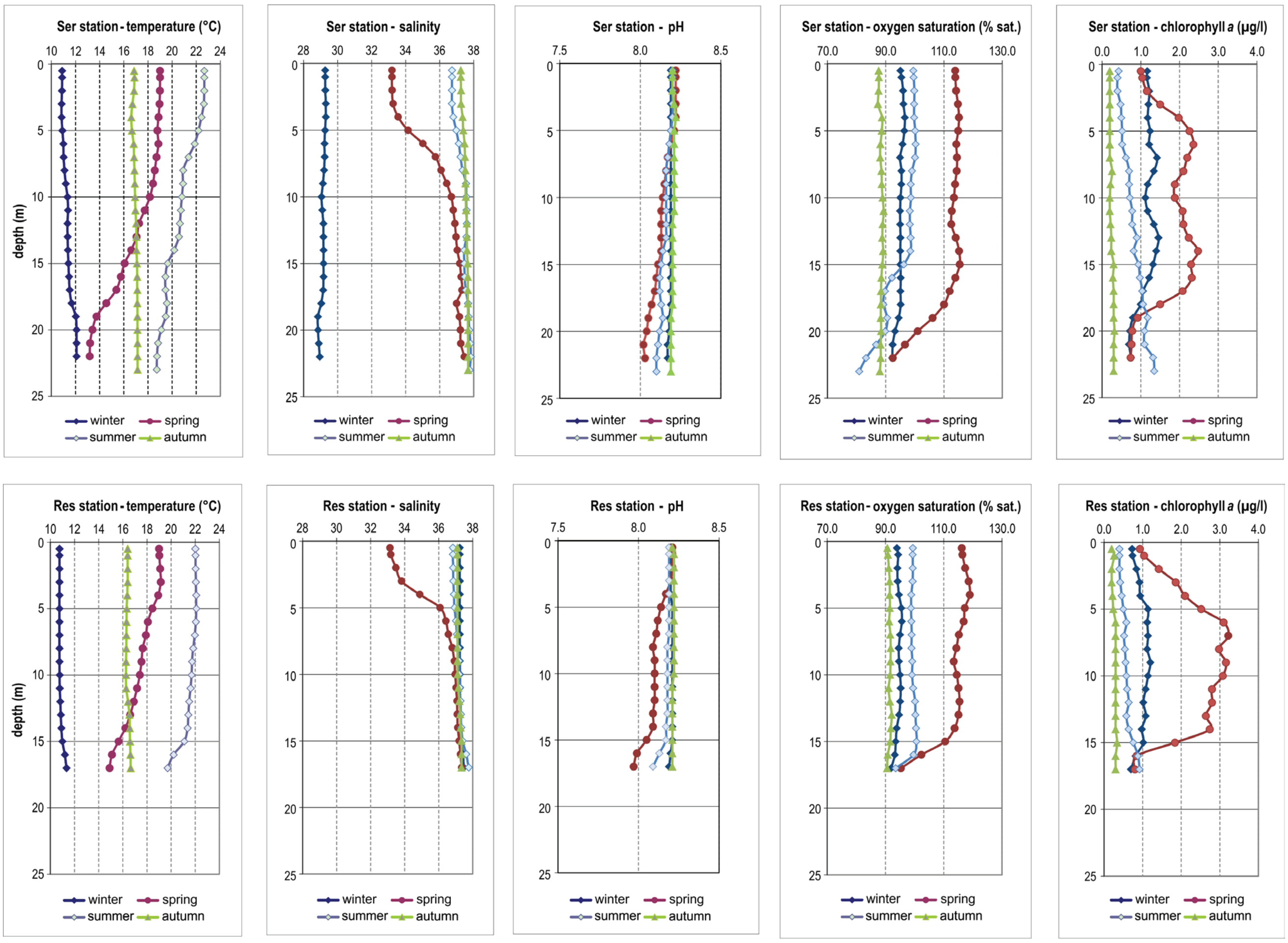


Fig. 2: Vertical profiles of temperature, salinity, pH, oxygen saturation and chlorophyll *a* at **Ser** and **Res** stations during the seasonal sampling.

Organic matter content

At **Ser** station the total organic carbon (TOC) content varied from 1.88 to 3.81% with a mean value of 2.46 ± 0.74 (Table 1). The highest content was recorded during autumn for both the studied levels (0-1 and 1-2 cm). No substantial differences were recorded between the superficial (0-1 cm) and subsurface (1-2 cm) levels of the same sample. The C/N ratio was always > 10.

At **Res** station, TOC was lower than organic carbon content from **Ser**, varying from 1.31 to 1.56% with a mean value of 1.42 ± 0.08 (Table 1). The lowest TOC contents were recorded during summer and the highest during winter at the subsurface level. No substantial differences were recorded between the superficial (0-1 cm) and subsurface (1-2 cm) levels of the same sample. The C/N ratio was always > 10.

Potentially Toxic Element concentration

PTE content detected using HCl extraction are reported in Table 1 and expressed as micrograms per gram. Generally, their concentration was higher at **Res** station than at **Ser**, especially for Cr, Ni and Pb at the superficial levels (0-1 cm). On the contrary, **Ser** station presented values higher than **Res** for Zn, in all the levels and seasons (Fig. 3).

Cd concentration was always below the limit of detection (LOD) value, except for the autumn sample from **Ser** station (1-2 cm), where it reached a value of 0.64 µg/g. Cr concentration ranged from 0.52 to 1.03 and from 0.25 to 7.35 µg/g for **Ser** and **Res**, respectively. Higher values were recorded in the superficial levels of **Res**. Cu content varied from 1.07 to 12.11 and from 0.29 to 15.34 µg/g, for **Ser** and **Res**, respectively. The highest concentrations (15.34 µg/g) were recorded in winter at **Res** (0-1 cm). Ni values ranged from 2.68 to 4.14 and from 1.50 to 8.85 µg/g for **Ser** and **Res**, respectively. The highest concentrations (8.85 µg/g) were recorded in summer at **Res** (0-1 cm). Pb concentration ranged from 0.50 to 11.37 and from 0.50 to 37.89 µg/g for **Ser** and **Res** respectively. The highest concentrations (37.89 µg/g) were always recorded at **Res**in winter (0-1 cm). Zn concentration varied from 70.20 to 195.38 and from 8.43 to 67.41 µg/g for **Ser** and **Res** respectively. This element reached the highest concentrations at **Ser** station, especially during autumn and at the superficial levels of this station.

Living benthic foraminifera

Total standing stocks and diversity

In total, 3868 and 8565 living foraminifera, undetermined specimens included, were counted respectively at

Table 1. Textural data of the studied sediments (% of sand, silt, clay, mean size - Mz, Shepard classification, total organic carbon (TOC), total nitrogen (Ntot), molar ratio TOC/Ntot (C/N) and potentially toxic element (PTE) concentration (expressed micrograms per gram - µg/g) in the **Ser** and **Res** stations. The limits of detection (LOD), the PTE concentration for the reference levels of the core GT3 (Covelli *et al.*, 2006), the literature data of PTE concentration in unpolluted areas (Voutsinou-Taliadouri, 1995) and the PTE concentration in the moderately and polluted sectors of the harbour of Trieste (Adami *et al.*, 2000), were reported.

Sample	sand	silt	clay	Mz	Shepard (1954)	TOC	N tot	C/N	Cd	Cr	Cu	Ni	Pb	Zn
	%	%	%	µm		%	%		µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
station Ser														
win13 (0-1)	17,4	66,7	15,9	16,7	sandy silt	2,19	0,18	14,2	< LOD	1,03	9,36	3,82	11,37	110,09
win13 (1-2)	17,6	67,6	14,7	17,9	sandy silt	2,19	0,18	14,6	< LOD	0,56	2,54	3,46	4,39	108,60
spr13 (0-1)	13,3	71,5	15,2	15,6	clayey silt	1,89	0,18	12,3	< LOD	0,61	3,03	3,58	4,62	77,59
spr13 (1-2)	14,8	71,9	13,3	17,9	sandy silt	2,20	0,19	13,8	< LOD	0,54	1,25	3,41	1,54	70,20
sum13 (0-1)	14,0	71,4	14,6	16,7	clayey silt	2,08	0,16	15,2	< LOD	0,62	4,18	3,93	6,50	93,28
sum13 (1-2)	15,7	68,9	15,4	17,9	sandy silt	1,88	0,15	15,1	< LOD	0,74	6,14	4,14	9,10	84,65
aut13 (0-1)	30,7	56,3	13,0	25,4	sandy silt	3,48	0,28	14,5	< LOD	0,97	12,11	3,77	10,03	195,38
aut13 (1-2)	44,8	44,4	10,8	35,9	silty sand	3,81	0,31	14,6	0,64	0,52	1,07	2,68	0,50	117,59
station Res														
win13 (0-1)	9,8	77,7	12,5	16,7	silt	1,42	0,15	11,4	< LOD	7,35	15,34	8,83	37,89	67,41
win13 (1-2)	7,5	76,3	16,2	13,6	silt	1,56	0,15	12,6	< LOD	0,59	0,73	1,50	0,50	8,43
spr13 (0-1)	4,9	77,4	17,7	11,8	silt	1,48	0,14	12,3	< LOD	3,11	7,01	4,42	25,90	40,40
spr13 (1-2)	3,5	74,4	22,1	10,3	clayey silt	1,41	0,13	13,2	< LOD	0,31	2,08	3,64	17,41	38,69
sum13 (0-1)	4,4	78,5	17,1	11,8	silt	1,31	0,12	13,3	< LOD	2,76	4,81	8,85	18,15	31,48
sum13 (1-2)	3,6	76,8	19,6	10,3	silt	1,37	0,11	14,5	< LOD	0,51	2,02	3,73	13,06	30,76
aut13 (0-1)	4,5	75,5	19,9	11,0	silt	1,43	0,14	12,4	< LOD	4,40	9,50	5,70	33,16	45,85
aut13 (1-2)	4,2	75,7	20,0	11,0	silt	1,35	0,10	16,6	< LOD	0,25	0,29	2,98	6,83	30,85

LOD	**0,12**	**0,25**	**0,25**	**0,25**	**0,500**	**0,25**
GT3-51	< 0.005	0,49	0,52	1,14	< 0.020	< 0.010
GT3-53	< 0.005	0,56	0,54	1,12	< 0.020	< 0.010
Voutsinou-Taliadouri, 1995	n.d.	14-180	3-35	12-207	5-32	17-72
Adami *et al.*, 2000	n.d.	4.1-14.5	27.4-62.3	10.7-26.6	106-234	147-321

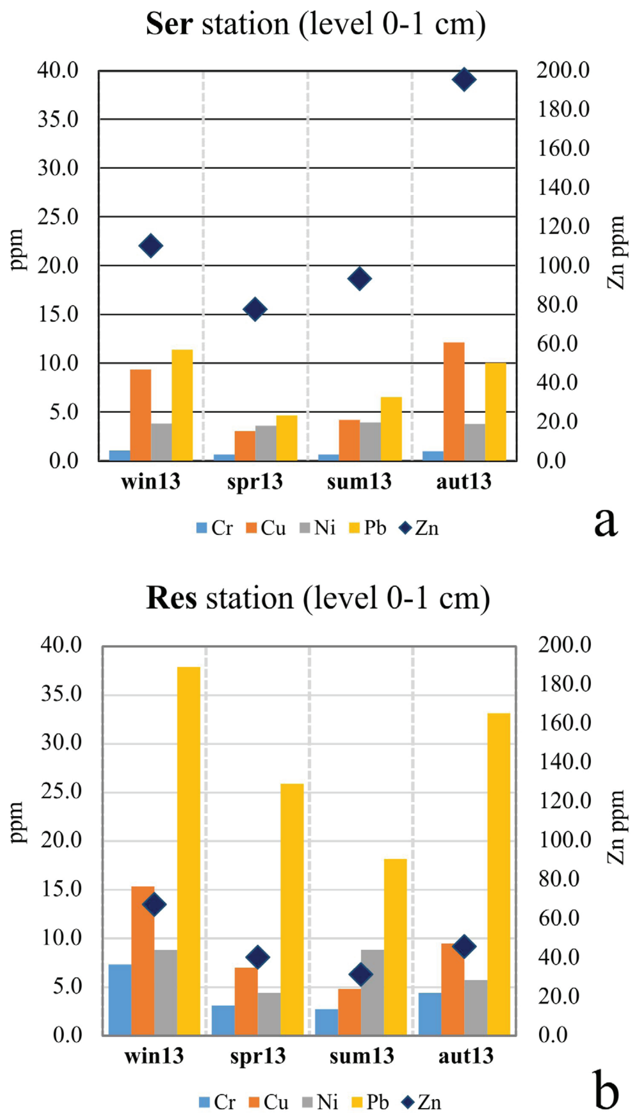


Fig. 3: Potentially toxic elements (PTE) concentration in the **Ser** (a) and **Res** (b) stations calculated for the 0-1 cm level. PTEs are expressed in micrograms per gram (µg/g).

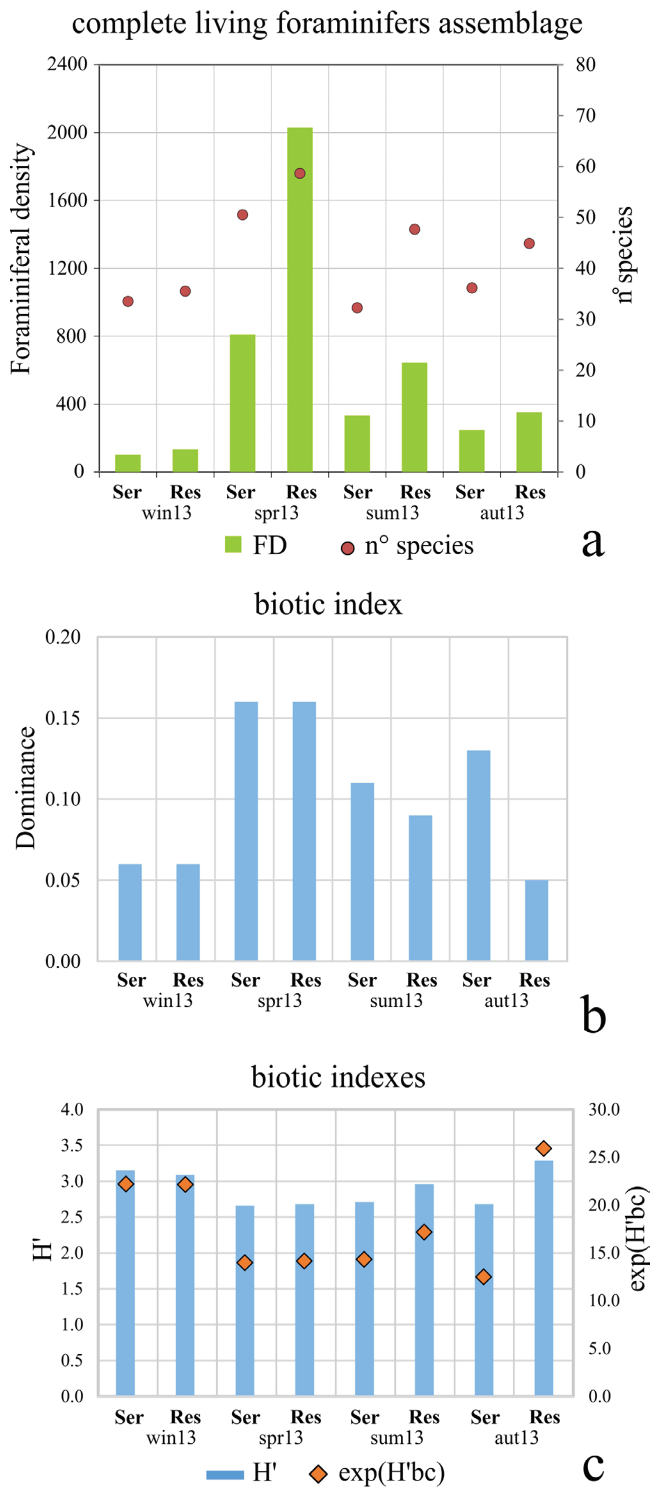


Fig. 4: Foraminiferal data for **Ser** and **Res** stations using the complete living assemblage of the 0-2 cm level: a) Foraminiferal density (FD) as the number of specimens normalised to 50 cc of sediments and species richness; b) Dominance index; c) bias corrected Shannon (H'_{bc}) and the exponential function Exp(H'_{bc}) indexes.

Ser and **Res** stations during the sampling period. For both stations, the foraminiferal density (hereafter FD) showed the lowest values during winter (99 specimen/50cc at **Ser** and 132 specimen/50cc at **Res**), increased considerably during spring reaching the highest values (805 specimen/50cc at **Ser** and 2027 specimens/50cc at **Res**) and decreased during summer and autumn (Fig. 4a). FD values were, however, higher at **Res** station than at **Ser**. More specifically, the FD of **Res** was up to 2.5 times higher than **Ser** during spring and a minimum of 1.3 times higher during winter. The percentage of biocoenosis was mainly concentrated in the 0-1 cm level for all stations and seasons, reaching a mean value of 69.1 ± 11.2% in this level. A particularly high percentage of biocoenosis was recorded in the 0-1 cm level during spring and summer (**Res**) and autumn (**Ser**).

Species richness varied in the studied stations and during the sampled period (Fig. 4a). Winter, summer and autumn at **Ser** and winter at **Res** were characterised by lower richness, while spring presented the highest number of species at both stations. Without considering the genera *Bolivina*, *Fissurina*, *Lagena, Textularia* and Miolids, which were grouped as spp., **Ser** showed a mean richness of 38± 8 species, except for spring, where the richness reached 50 species. **Res** was generally richer than **Ser**, having a mean richness of 46 ± 9 species, except for winter with its lower value of 35 taxa. Spring is richer in species than other seasons with a total of 58 species.

Dominance, H' and Exp(H'$_{bc}$) values are shown in Figs. 4b, c. Dominance values were comparable in the two stations in winter and spring and, conversely, they were lower at **Res** station than **Ser** in summer and autumn. Higher values were recorded in spring (D = 0.16) for both stations (Fig. 4b) while the lowest value pertained to **Res** during autumn (0.05). H' varied from 2.66 (**Ser**, spring) to 3.29 (**Res**, autumn); it was lower at **Ser** during spring and autumn (Fig. 4c). Exp(H'$_{bc}$) values varied from 12.47 (**Ser**, autumn) to 25.92 (**Res**, autumn) with a mean value of 15.74 ± 4.39 and 19.85 ± 5.32 for **Ser** and **Res**, respectively. The lowest values were recorded at **Ser** during autumn and at **Res** during spring (Fig. 4c).

Species composition of benthic foraminiferal communities

A total of 65 species pertaining to 39 genera were recorded at **Ser** station and 68 species pertaining to 37 genera were recorded at **Res** station (Table S1). Of these, about 70% were in common to both stations.

The most common foraminifera were *Ammonia tepida*, *Bolivina* spp., *Bulimina* spp., *Eilohedra vitrea*, *Elphidium* spp., *Haynesina depressula*, *Hopkinsina pacifica* and *Nonionella iridea* among the Rotaliina, *Cribrostomoides kosterensis*, *Eggerelloides scaber*, *Reophax nana* and *Textularia* spp. among the Textulariina. Species pertaining to Miliolina were less abundant and mainly represented by *Miliolinella subrotunda*, *Quinqueloculina* spp. and *Spiroloculina* spp. Genera pertaining to Rotaliina were more abundant at **Ser** station, except for winter where the species of Textulariina dominate. Agglutinated species prevailed at **Res** station during spring and summer; Miliolina were more abundant at **Res**, they were very subordinate at **Ser**, except for winter 2013 (Table S1). The fourteen major taxa (> 4% in at least one sample considering both stations) are shown in Table 2.

Considering their density (Fig. 5), *A. tepida*, *Bolivina* spp., *Bulimina* spp. and *E. vitrea* were more abundant at **Ser**, while *E. scaber*, *Elphidium* spp., *H. depressula*, *M. subrotunda*, *N. iridea*, *Quinqueloculina* spp., *R. nana* and *Textularia* spp. characterised **Res** station. *Miliammina fusca*, agglutinated, was present only once (summer, **Res**) with very high abundance (Table S1). As for the relative abundance, *Bolivina* spp., *E. scaber, Textularia* spp., and

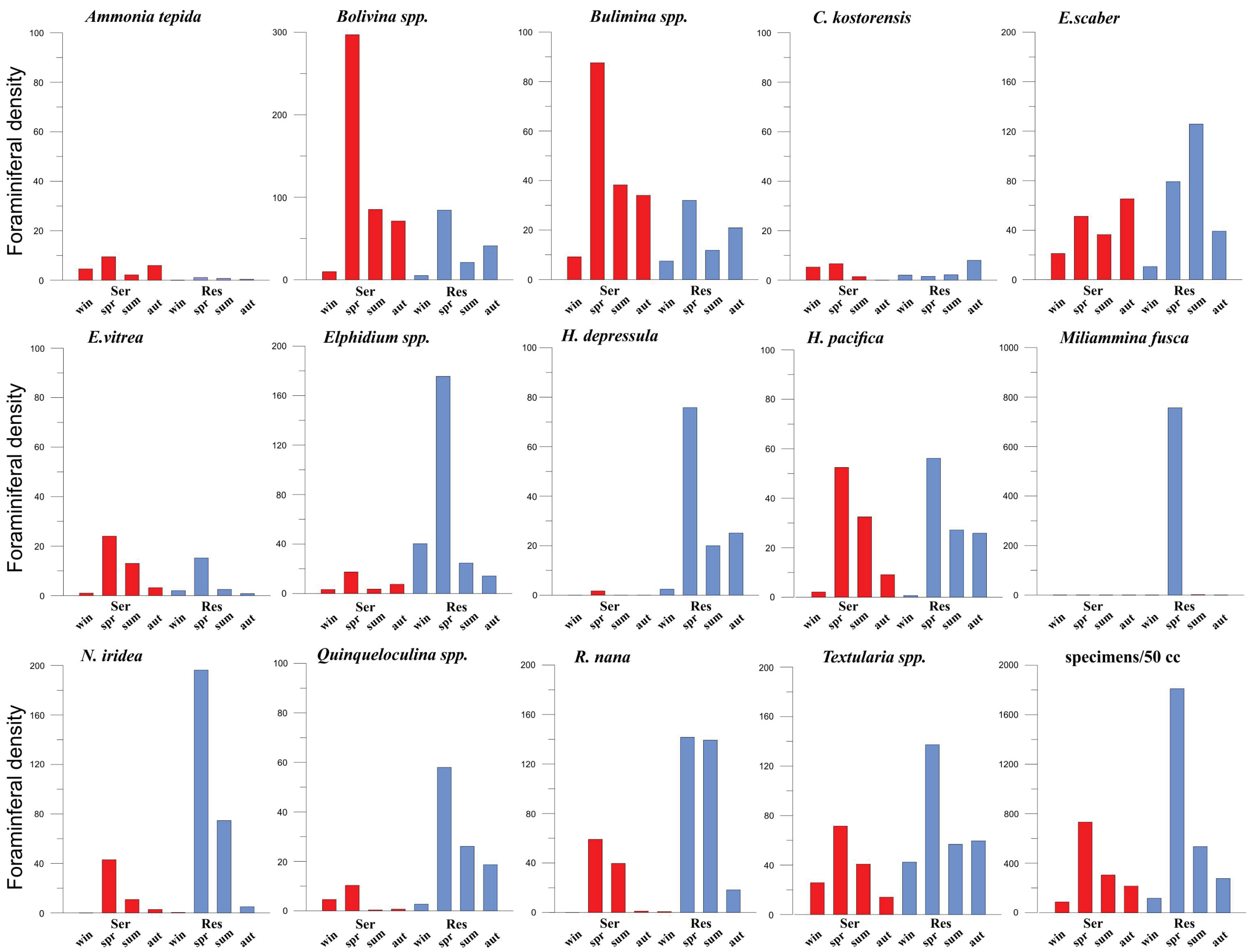


Fig. 5: Distribution of the foraminiferal density of those species and genera comprising at least 4% of the assemblage in at least one sample from the studied stations. Red histogram: **Ser** station; blue histogram: **Res** station. Note that y-axis scale is variable depending on species foraminiferal density.

Table 2. The fourteen most common foraminiferal species and genera (in decreasing order of their relative abundance) found at the two sampling stations summed over 0-2 cm and averaged between seasons. These species were present at least one time (station or season) with a relative abundance > 4%.

station	Ser (winter - autumn 2013)			Res (winter - autumn 2013)	
	min-max %	mean %		min-max %	mean %
species			**species**		
Ammonia tepida	0.6-4.5	2.2	*Ammonia tepida*	0.0-0.1	0.07
Bolivina spp.	9.7-36.7	25.3	*Bolivina* spp.	3.3-12.0	5.81
Bulimina spp.	9.0-13.9	11.3	*Bulimina* spp.	1.6-6.1	3.76
Cribrostomoides kosterensis	0.0-5.2	1.6	*Cribrostomoides kosterensis*	0.1-2.3	1.06
Eggerelloides scaber	6.3-26.6	16.2	*Eggerelloides scaber*	3.9-19.4	10.65
Eilohedra vitrea	1.0-4.0	2.3	*Eilohedra vitrea*	0.2-1.5	0.72
Elphidium spp.	1.1-3.1	2.3	*Elphidium* spp.	3.8-29.9	11.62
Haynesina depressula	0.0-0.2	0.1	*Haynesina depressula*	1.8-7.4	3.99
Hopkinsina pacifica	2.1-9.8	5.5	*Hopkinsina pacifica*	0.5-7.6	3.76
Miliammina fusca	0.0	0.0	*Miliammina fusca*	0.0-37.3	9.39
Nonionella iridea	0.0-5.3	2.4	*Nonionella iridea*	0.2-11.5	5.73
Quinqueloculina spp.	0.1-4.5	1.6	*Quinqueloculina* spp.	2.0-5.5	3.59
Reophax nana	0.0-11.9	4.9	*Reophax nana*	0.5-21.5	8.59
Textularia spp.	5.7-25.3	13.0	*Textularia* spp.	6.8-31.4	16.11

Bulimina spp. exceeded 10% at **Ser** station and *Textularia* spp., *Elphidium* spp., and *E. scaber* exceeded 10% at **Res** station (Table 2).

The Chi-square test (χ^2) indicates an overall significant difference between **Ser** and **Res** stations ($\chi^2 = 2012.4$; df = 12; $p < 0.0001$) in terms of species composition; the main contributions to the χ^2 were given by 5 taxa over 13 species (Table S2). Comparing the two stations and considering all the season, the first and most important contribution in term of difference was due to *Bolivina* spp. (41.3% with respect to the total contribution), followed by *Elphidium* spp. (13.3%), *Bulimina* spp. (12.0%), *H. depressula* (10.1%) and *N. iridea* (10.0%). Even with moderate contributions, *R. nana* and *A. tepida* were also significant (5.3 and 3.6%, respectively). *Bolivina* spp., *Bulimina* spp. and *A. tepida* were more abundant at **Ser** station while *Elphidium* spp., *H. depressula*, *N. iridea* and *R. nana* dominated at **Res** station (Fig. 5). Considering the seasons separately, the difference between **Ser** and **Res** which occurred during winter ($\chi^2 = 140.3$; df = 12; $p < 0.0001$) saw the main contributions of *Elphidium* spp. at **Res** station and *E. scaber*, which was more abundant at **Ser** station. During spring ($\chi^2 = 320.8$; df = 12; $p < 0.0001$) the difference between **Ser** and **Res** had the main contributions of *H. depressula, Textularia* spp., both more abundant at **Res** station (Fig. 5) and *Bolivina* spp., at **Ser** station. In summer ($\chi^2 = 645.3$; df = 12; $p < 0.0001$) the difference was mainly due to *Bolivina* spp. and, subordinately, by *Bulimina* spp., both more abundant at **Ser** station and finally in autumn ($\chi^2 = 1293.4$; df = 12; $p < 0.0001$) to *Bolivina* spp. and *Elphidium* spp., more abundant at **Ser** and **Res** stations, respectively.

<u>Foraminifera vs. textural and geochemical indicators</u>

Spearman's rank order correlations between absolute abundance of living foraminifera of the superficial level (0-1 cm) and the relative geochemical characteristics of the sediments are shown in Table 3, where significant correlations are reported in **bold** ($p < 0.01$) and *italic* ($p < 0.05$). *Haynesina depressula* and *Quinqueloculina* spp. correlated negatively with sand content. *Elphidium* spp., *H. depressula* correlated positively with silt content. Only *Quinqueloculina* spp. correlates with clay content. The other species did not correlate significantly with any textural fraction.

Concerning the other geochemical characteristics, *A. tepida* positively correlated with TOC and N_{tot} contents, while only *H. depressula* negatively correlated with TOC content. *Ammonia tepida* and *Bulimina* spp. were negatively correlated with Cr, Ni and Pb. *Hopkinsina pacifica* was negatively correlated with Cu. *Elphidium* spp., *H. depressula*, *Quinqueloculina* spp. and *Textularia* spp. were negatively correlated with Zn.

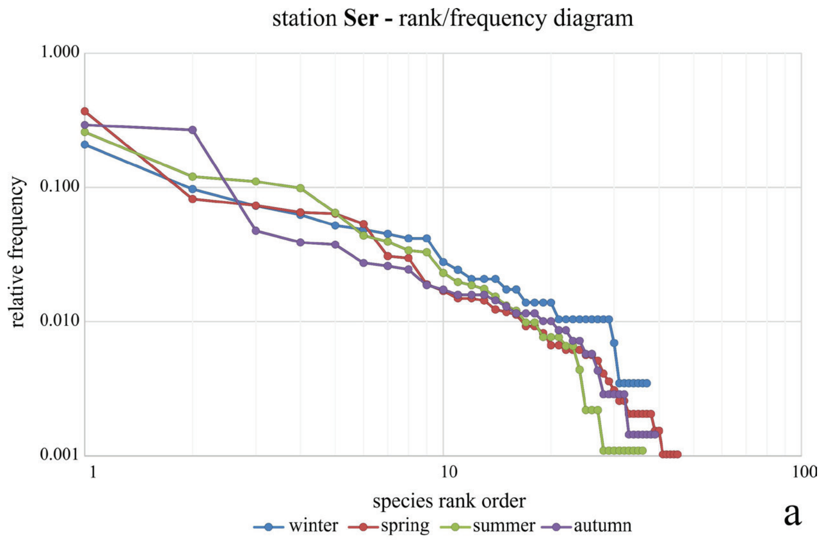
<u>State of foraminiferal communities and EcoQ assessment</u>

At **Ser** station, RFDs appeared as a straight-line in winter, linear-concave in spring and convex during summer and autumn (Fig. 6a). From winter to autumn, foraminiferal assemblages showed an ecological succession from a mature assemblage in winter to an unbalance done in spring, reaching an intermediate assemblage in summer and autumn. At **Res** station, RFDs changed from a straight-line in winter to a linear-concave shape in spring, a convex one in summer, ending up with a straight-line shape during the autumn (Fig. 6b). This highlighted an

Table 3. Spearman's rank correlation using the absolute abundance of the major species at the 0-1 cm level of **Ser** and **Res** stations and the relative content of potentially toxic elements (PTEs), total organic carbon (TOC), total nitrogen (Ntot) and grain-size. The significance threshold p < 0.01 and p < 0.05 were reported in **bold** and *italic*, respectively.

Specie (0-1 cm)	Cr		Cu		Ni		Pb		Zn		TOC		N tot		sand		silt		clay	
	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p	r_s	P
Ammonia tepida	**-0.916**	0.001	-0.470	0.240	**-0.892**	0.003	**-0.916**	0.001	0.651	0.081	*0.747*	0.033	*0.800*	0.017	0.699	0.054	-0.699	0.054	-0.289	0.487
Bolivina spp.	-0.667	0.071	-0.476	0.233	-0.690	0.058	-0.667	0.071	0.214	0.610	0.452	0.260	0.407	0.317	0.262	0.531	-0.405	0.320	0.048	0.911
Bulimina spp.	*-0.762*	0.028	-0.524	0.183	*-0.714*	0.047	*-0.762*	0.028	0.310	0.456	0.500	0.207	0.455	0.257	0.333	0.420	-0.476	0.233	-0.048	0.911
C. kosterensis	0.180	0.670	-0.060	0.888	0.108	0.799	0.180	0.670	-0.156	0.713	-0.275	0.509	-0.241	0.565	-0.299	0.471	0.132	0.756	0.419	0.301
Eggerelloides scaber	-0.071	0.867	-0.143	0.736	0.190	0.651	-0.071	0.867	-0.333	0.420	-0.095	0.823	-0.323	0.435	-0.310	0.456	0.119	0.779	0.405	0.320
Eilohedra vitrea	-0.443	0.272	-0.539	0.168	-0.275	0.509	-0.443	0.272	-0.072	0.866	0.168	0.691	0.114	0.787	0.084	0.844	-0.012	0.978	-0.108	0.799
Elphidium spp.	0.571	0.139	0.143	0.736	0.476	0.233	0.571	0.139	*-0.738*	0.037	-0.690	0.058	-0.515	0.192	-0.643	0.086	*0.786*	0.021	0.190	0.651
Haynesina depressula	0.659	0.076	0.049	0.909	0.610	0.108	0.659	0.076	**-0.903**	0.002	*-0.756*	0.030	*-0.810*	0.015	**-0.878**	0.004	*0.781*	0.022	0.659	0.076
Hopkinsina pacifica	-0.333	0.420	*-0.762*	0.028	-0.095	0.823	-0.333	0.420	-0.500	0.207	-0.167	0.693	-0.299	0.471	-0.405	0.320	0.262	0.531	0.524	0.183
Nonionella iridea	-0.192	0.649	-0.683	0.062	0.120	0.778	0.120	0.778	-0.635	0.091	-0.323	0.435	-0.464	0.247	-0.539	0.168	0.419	0.301	0.527	0.180
Quinqueloculina spp.	0.371	0.365	-0.252	0.548	0.407	0.317	0.371	0.365	**-0.838**	0.009	-0.599	0.117	-0.699	0.054	*-0.790*	0.020	0.647	0.083	*0.826*	0.011
Reophax nana	-0.167	0.693	-0.667	0.071	0.238	0.570	-0.167	0.693	-0.690	0.058	-0.452	0.260	-0.539	0.168	-0.619	0.102	0.524	0.183	0.452	0.260
Textularia spp.	0.024	0.955	-0.619	0.102	0.190	0.651	0.024	0.955	*-0.762*	0.028	-0.524	0.183	-0.563	0.146	-0.690	0.058	0.595	0.120	0.595	0.120
aboundance	0.000	1.000	-0.429	0.289	0.167	0.693	0.000	1.000	-0.690	0.058	-0.405	0.320	-0.491	0.217	-0.619	0.102	0.476	0.233	0.571	0.139
richness	-0.168	0.691	-0.515	0.192	-0.108	0.799	-0.168	0.691	-0.515	0.192	-0.180	0.670	-0.283	0.497	-0.443	0.272	0.252	0.548	0.647	0.083

bold (p < 0.01)
italic (p < 0.05)



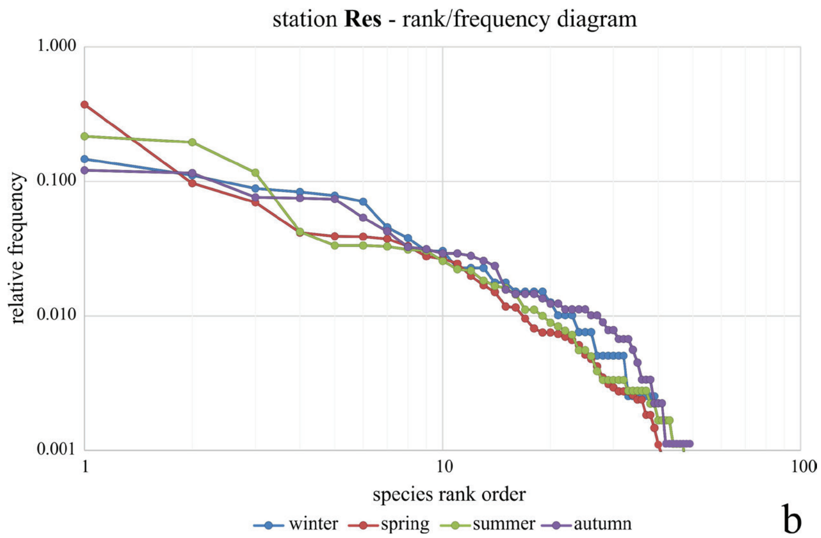


Fig. 6: Rank-frequency diagram for the **Ser**(a) and **Res**(b) stations using the cumulative abundance (as relative frequency) for y-axe and the decreasing rank order for x-axe of each species for each sample. Both axes are on logarithmic scale.

annual ecological succession from a mature assemblage in winter, to an unbalanced one in spring, an intermediate assemblage in summer, ending up with a mature assemblage in autumn. Ecological quality statuses assessed at both stations varied seasonally. Based on $Exp(H'_{bc})$ calculated on benthic foraminifera, the EcoQ is high in winter and autumn, moderate in spring and good in summer at **Res** station. Conversely, the EcoQ is always moderate at **Ser** station except during the winter season, where it is high (Fig. 4c).

Discussion

Environmental conditions

Hydrological features represented typical seasonal variabilities of the GoT (Malačič & Petelin, 2001). Homogenous oceanographic conditions occurred in winter and autumn, while during spring and summer the water column was stratified. Anomalous salinity values observed at **Ser** station during winter indicates that freshwater outflow from the pipeline can occasionally modify the salinity pattern. During spring, the high values of dissolved oxygen indicated the significant activity of primary producers (mainly phytoplankton) occurring in the middle of the water column, as seen by the chlorophyll *a* value higher than 2.0 µg/l from 5 to 17 m of depth at **Ser** station and from 4 to 14 m of depth at **Res** station (Fig. 2).

Environmental parameters at the studied stations showed diverse conditions, both for the stations and for the sampling periods. Grain-size demonstrates that sediments from **Ser** station (from 15 to 45%) were always richer in sand than **Res** station. The relatively high occurrence of sand at **Ser** station differs with the sediment distribution proposed by Brambati *et al.* (1983), Ogorelec *et al.* (1991) and Acquavita *et al.* (2010) for the GoT. These authors indicated high sand content along beaches, in proximity to the Marano and Grado lagoon outlet and in the western open sector of the Gulf and clayey silt for the studied area. This increase in sand at **Ser** station is probably due to the concomitant effect of the greater influence of the solid load of the Isonzo in this area compared to the more strictly coastal one, as well as to the contribution of "organic" residue carried out by the pipeline. The grain-size composition of **Res** station sediments was, on the contrary, in agreement with previous studies and remained comparable over all sampling periods.

TOC content (and N_{tot}) was always higher at **Ser** than at **Res**, especially during autumn, where TOC content > 3.0% was recovered at **Ser** (both levels). These data are in agreement with Cibic *et al.* (2008) which demonstrated a mean TOC content of 2.41 ± 0.4%for June 2004. TOC correlated significantly with sand content ($r_s = 0.919$, $p < 0.001$). This behaviour is not common since TOC is generally associated with finer sediments in relation to adsorption effects (Guerzoni *et al.*, 1984; Faganeli *et al.*, 1991). On the contrary, the positive correlation of TOC with sand which occurred at **Ser** station probably indicates that in this case, the sandy sediments convey the organic residue probably derived from the human activity of the sewage carried out by the pipeline. Mean values of 1.42% at **Res** station were comparable with data from Acquavita *et al.* (2010), but slightly higher than data reported for the Slovenian sector (Faganeli *et al.*, 1991; Covelli & Fontolan, 1997; Acquavita *et al.*, 2010). In all cases, C/N molar ratio was always > 10, indicating that the organic matter is a mixed composition of marine and terrestrial organic compounds (e.g. Meyers, 1994; Twichell *et al.*, 2002; Ramaswamy *et al.*, 2008).

The HCl etching procedure used to determine the heavy metal concentrations allowed us to determine the "non-residual", more labile fraction likely due to recent input, as highlighted by Adami *et al.* (2000). The slightly higher concentration of all the PTEs, except Zn, at **Res** station than at **Ser** (Fig. 3), raises some questions about their origin. The proximity of a touristic harbour and the relative use of different types of paints, including antifouling ones, in an area located at about a quarter of mile to the Reserve might explain this increased concentration, especially as far as Pb is concerned. The presence of elements such as Cd, Cr, Cu, Ni, Sn and Zn has already been observed in sediments contaminated by antifouling paints used for nautical activities (Turner, 2010). A comparison with data obtained by Adami *et al.* (2000) using the same procedure, indicates that the concentrations of Cr, Cu, Ni and Pb obtained in this study were always lower than those found in the moderately polluted and polluted sites of the Trieste harbour.

Zn, on the other hand, was always found with higher values at **Ser** station. Its concentration in this station is comparable with the Zn content found at the moderately polluted sites of the harbour, especially for the autumn sampling and it always exceeded the limit of unpolluted environment defined by Voutsinou-Taliadouri *et al.* (1995). High Zn concentrations are usually related to industrial tailing and wastes (e.g. Donazzolo *et al*., 1984). Furthermore, when precipitation is scarce and the water column is homogeneous metals such as Cu and Zn discharged by the pipeline can reach the surface waters (Cozzi *et al*., 2004). Particularly, in our study Zn was probably transported by the organic residue from the Servola pipeline, as evidenced by its positive correlation ($r_s = 0.916$, $p < 0.001$) with the TOC occurring in the sandy size of the sediments.

Living foraminiferalassemblage

<u>Diversity and foraminiferal density</u>

In this study, species richness was about 30 to 60 species (Fig. 4a) which is relatively higher than the approximately 30 species, without the allogromiids ones, recorded by Sabbatini *et al.* (2010) in their study in the GoT. However, they only carried out one sampling (May 2006) and used a lower volume of sediment than in this work, so their species richness may have been underestimated. A mean of 50 species recorded in this study is comparable with other studies carried out in the northern Adriatic Sea (Jannink, 2001; Duijnstee *et al.*, 2004) and is in agreement with the general mesotrophic-oligotrophic conditions, with episodic eutrophic events that characterise the GoT (Horvat *et al.*, 1999; Turk *et al.*, 2007; Giani *et al.*, 2012). The foraminiferal species found in this study, including the most common ones (*A. tepida*, *Bolivina* spp., *Bulimina* spp., *E. vitrea*, *Elphidium* spp., *H. depressula*, *H. pacifica*, *N. iridea*, *C. kosterensis*, *E. scaber*, *R. nana* and *Textularia* spp.) are characteristic of infra-circalittoral Mediterranean and northern Adriatic Sea environments (Jorissen, 1987, 1988; Hohenegger *et al.*, 1989; Cimerman & Langer, 1991; Barmawidjaja *et al.*, 1992; Sabbatini *et al.*, 2010; Melis & Covelli, 2013). Although approximately 70% of the species are in common between the two studied stations, differences were observed. *Cornuspira involvens*, *Elphidium macellum*, *Fursenkoina complanata*, *F. schreibersiana, Quinqueloculina milletti, Q. oblonga* and *Trochammina ochracea* were found only at **Ser** station. They are, however, rare species. *Ammonia beccarii*, *Cibicides lobatulus*, *Cycloforina rugosa*, *Lagenammina atlantica, Miliammina fusca, Nouria polymophinoides, Quinqueloculina pygmaea, Reophax subfusiformis, Spiroloculina dilatata* and *Triloculina tricarinata* were recorded only at **Res** station (Table S1).

In this study, species richness varied during the seasons in a predictable manner (higher richness during spring and summer and vice versa in winter and autumn). However, species richness was always lower at **Ser** station than at **Res**, while diversity was comparable between the two stations in winter and spring and mainly differed in autumn (Fig. 4c). It suggests that the area around the Servola sewage pipeline (**Ser** station) is more problematic than the Reserve (**Res** station) for the foraminiferal communities. Along the Italian coast lagoons, lakes, and human activities (industries, aquaculture) also induce low diversity in terms of benthic foraminifera (Bouchet *et al.*, 2018). Similarly, in the Saguenay (Canada), benthic foraminiferal diversity was very low due to discharges from a paper mill (Schafer *et al.* 1991). In the Firth of Clyde (Scotland), foraminiferal densities and diversity similarly decreased in the vicinity of a sewage sludge (Mojtahid *et al.*, 2008).

In this study, both number of species and abundance decreased when Zn concentration increased (Table 3). Zn reached higher concentration values in **Ser** during autumn (about 200 µg/g), in the situation of a homogenous water column, and this concentration was comparable with the Zn concentration found in the industrial harbour of the city which is considered highly polluted by Adami *et al.* (2000). Even if Zn is essential for metabolism of the microorganisms (e.g. Venugopal & Luckey, 1975), these high values seem to be a limiting factor for the foraminiferal communities, so confirming the deleterious effects of the trace metals on benthic foraminiferal densities and diversity (e.g. Armynot du Châtelet *et al.*, 2004; Le Cadre & Debenay, 2006; Coccioni *et al.*, 2009; Denoyelle *et al.*, 2012; Nardelli *et al.*, 2013; Francescangeli *et al.*, 2016). As for the other PTEs, no significant correlation was observed.

Foraminiferal densities (FD) of **Res** station was always higher than **Ser** indicating the more favourable environmental conditions of the Reserve. However, FD peaked at both stations during spring when trophic conditions were favourable (Fig. 4a). It is well known that phytoplankton blooms occur during the spring period in the GoT (Cabrini *et al.*, 2012). This suggests that benthic foraminifera would benefit from the phytodetritus accumulation derived from settled phytoplankton (mainly diatoms), as universally recognised (e.g. Gooday, 1988; Boon *et al*., 1998; Pusceddu *et al*., 2003; Sabbatini *et al.*, 2012). The same pattern of the foraminiferal bloom following the natural cycle of chlorophyll *a* was also reported by Hyams-Kaphzan *et al*. (2009) along the coast of Israel. In addition, the bottom water oxygen concentration was likely an important factor in controlling the density variability of some species at **Ser** station during the rest of the year, in agreement with Barmawidjaja *et al.* (1992), Jorissen *et al.* (1992) and Jannink (2001). In fact, during summer and autumn, high FD values of *Bolivina* spp. and *Bulimina* spp., occurred in oxygen stressed bottom water environments of the **Ser** station (% sat. < 90; Fig. 2), confirming their ability to flourish at reduced levels of oxygen concentration (Bernhard, 1986; Barmawidjaja *et al.*, 1992; Jorissen *et al*., 1992; Donnici & Serandrei-Barbero, 2002; Langlet *et al.*, 2014).

<u>Species responses to environmental conditions</u>

Grain-size is a well-known driver of benthic foraminiferal distribution (e.g. Armynot du Châtelet *et al.*, 2009, Frontalini *et al.*, 2013). Considering the correlation with the sediment composition of our study, most of the species prefer to live in silty sediments, except for *Quinqueloculina* spp., which were more abundant in clayey sediments and *A. tepida*,which was more abundant in sandy sediments. This last taxon correlates well with TOC, as previously discussed. Conversely, *Elphidium* spp. and *Haynesina depressula* did not occur at **Ser** station which was the more enriched in TOC, preferring the Miramare MPA.

The correlation between species abundance at the superficial levels (0-1 cm) with PTEs (Table 3), shows significant negative correlations of *A. tepida*, *Bulimina* spp. and *H. pacifica,* with Cr, Ni and Pb concentrations in the bioavailable fraction. This would suggest that these taxa are rather sensitive to these elements and would benefit from the less stressful conditions occurring at **Ser** station especially for lower Cr and Pb content. As already explained in the previous paragraph, the **Res** station showed a greater accumulation of these elements, while remaining within the limits of areas in unpolluted conditions, according to Voutsinou-Taliadouri *et al*. (1995) and Adami *et al*. (2000). Only the concentration of Pb in winter and autumn at **Res** station, exceeds these limits (Table 1). On the contrary, *H. depressula*, *Quinqueloculina* spp., *Textularia* spp. and *Elphidium* spp., in decreasing order, are very sensitive to high Zn content and would benefit from the less stressful conditions of Miramare MPA station (**Res**). Conversely, *A. tepida*, *Bolivina* spp., *Bulimina* spp. and *Eilohedra vitrea* were more abundant at **Ser** station (Fig. 5), where less oxygenated bottom water conditions, coarser sediment texture, higher Zn and TOC contents were highlighted. Species such as *A. tepida* and Bolivinidae are generally known to be good indicators of trace metal pollution (see discussion in Frontalini & Coccioni, 2011). In this study they represent the best tolerance toward the organic matter accumulation and higher Zn content.

In the following, we will discuss the response of the most significant species to the environmental conditions, considering both stations along the studied seasons.

Amongst the calcareous species, *Bolivina* spp. (mainly *B. dilatata, B. striatula* and *B. variabilis*) was the more abundant genus in the sediments and it represents the main contribution in terms of statistical differences between the stations, except for winter (Table S2); their densities reached about 300 specimens/50 cm^3 during spring at **Ser** station, where it was often the dominant genus. *Bolivina* spp. were more abundant in the superficial sediments. These species are considered deep infaunal taxa usually associated with high organic matter flux, low oxygen concentrations and related to finer texture (Bernhard, 1986; Barmawidjaja *et al.*, 1992; Jorissen *et al.*, 1992; Donnici & Serandrei-Barbero, 2002; Langlet *et al.*, 2014). *B. dilatata* is considered able to respond to fresh phytodetritus input (Barmawidjaja *et al.*, 1992; Jorissen *et al.*, 1992; Duchemin *et al.*, 2008; Goineau *et al.*, 2012). The occurrence of *Bolivina* spp., mainly in the superficial studied sediments, could indicate a tendency of these species to colonise shallower sediments especially during autumn when the TOC concentration was higher at deeper levels (Table 1). This finding is in agreement with those of Jorissen *et al.* (1992) and Kaiho (1994). Furthermore, *Bolivina dilatata* and *B. variabilis* have been considered “Indifferent species” to the first stages of organic enrichment in the sediments by Jorissen *et al*. (2018) and this could explain the absence of a significant correlation between *Bolivina* spp. and TOC content (Table 3).

Bulimina spp. (mainly *B. aculeata*, *B. elongata* and *B. marginata*), were, on the contrary, homogenously distributed in the 0-2 cm level, except for autumn at **Ser** station, where their density was higher at 0-1 cm, as already observed for *Bolivina* spp. The genus *Bulimina* prefers environments characterised by the first stages of organic matter enrichment, and can tolerate low oxygen concentrations (e.g. Barmawidjaja *et al*., 1992; Jorissen *et al*., 2018). Furthermore, *Bulimina marginata* (mainly the morphotypes *B. aculeata, B. denudata*) can survive periods of severe anoxic conditions with hydrogen sulphide content (e.g. Pucci *et al*., 2009; Geslin *et al*., 2014; Langlet *et al*., 2014). Nevertheless, in other cases their capacity to migrate into the disoxic superficial sediments to escape conditions of severe oxygen depletion was observed (de Stigter *et al.*, 1998; Barmawidjaja *et al.* 1992; Duijnstee *et al*., 2003). Since an abundance of *Bolivina* and *Bulimina* are associated with oxygen-deficient environments, these conditions in our study can be always inferred in the sediments of **Ser** station, where the black colour of the sediments confirm this situation. However, considering the two genus as a whole, no significant statistical correlation with TOC content was observed (Table 3).

Elphidium spp. (mainly *E. advenum, E. granosum, E. lidoenese* and *E. decipiens*) and *H. depressula* are common in Mediterranean infralittoral settings also in the presence of vegetation cover (Jorissen *et al*., 1988; Albani & Serandrei-Barbero, 1990; Sgarrella & Moncharmont Zei, 1993; Donnici & Serandrei-Barbero, 2002; Murray, 2006; Melis & Protopsalti, 2008). These species were undoubtedly more abundant at **Res** station and in the superficial sediments (0-1 cm). They are all positively correlated with silt content and *H. depressula* is negatively correlated with sand content (Table 3). There is an evident negative correlation between TOC content and *H. depressula* (Table 3), confirming its tendency to disappear in situation of increased organic supplies as evidenced by Jorissen *et al*. (2018). They areal so negatively correlated with Zn. *H. depressula* was nearly absent at **Ser** station and reached a higher abundance during spring in relation to fresh phytodetritus accumulation. This species is frequent in infralittoral fine sands and in shallow deposits near river mouths and in lagoons (Jorissen, 1988; Sgarrella & Moncharmont-Zei, 1993; Bellotti *et al*., 1994; Melis & Covelli, 2013).

Hopkinsina pacifica is negatively correlated to Cu and it too can be considered a sensitive species with regards to this metal. *Hopkinsina pacifica* is considered a species tolerant to organic matter accumulation (Frontalini & Coccioni, 2011; Jorissen *et al*., 2018) and like *N. iridea*, it is able to feed on fresh phytodetritus (Goineau *et al*., 2012). *Nonionella iridea* was very frequent during spring and summer, above all at **Res** station and in the superficial sediments (Fig. 5), however, it did not correlate to any of the studied parameters. *Nonionella iridea* have been recorded in limited numbers by Donnici & Serandrei-Barbero (2002). On the contrary *Nonionella turgida*, often reported in the GoT (Sabbatini *et al*., 2010; Langlet *et al*., 2014), was not present in high densities at either of the studied stations.

Ammonia tepida is recognised worldwide as a brackish-water species able to live in conditions of variable salinity and temperature. It assumes the highest concentrations in confined settings such as lagoons, estuaries, tidal flats, etc. (Jorissen, 1987; Albani & Serandrei Barbero, 1990; Sgarrella & Moncharmont Zei, 1993; Debenay *et al*., 2000; Melis & Violanti, 2006; Melis & Covelli, 2013). Its presence at **Ser** station is mainly concentrated in the upper level studied (0-1 cm) and, even if in moderate frequency, probably indicates more pronounced salinity variations due to the frequent fresh water discharge from the pipeline. Furthermore, the high correlation of this taxon with TOC and N_{tot} content (Table 3) indicates that *A. tepida* could be favoured by an increase in total organic matter used as food resources, as suggested by Armynot du Châtelet *et al.* (2009) and confirms its role of "Second-order opportunist species" underlined by Jorissen *et al*. (2018). It is also negatively correlated with silt content referring to the sandy sediments (Table 3). In particular, it is commonly known that *A. tepida* can live in stressed environments and could be very tolerant to high concentrations of trace elements, including Hg (Yanko *et al.*, 1994; Ferraro *et al.*, 2006; Bergamin *et al.*, 2009; Carboni *et al.*, 2009; Coccioni *et al.*, 2009; Caruso *et al.*, 2011). This study suggests its tolerance versus Zn only.

Eilohedra vitrea was distributed in both levels of the studied sediment. This species is not very frequent in the Adriatic Sea. It is also reported by Duijnstee *et al.* (2004) at the mouth of the Po River delta mainly in relation to low O_2 and temperature values of the bottom waters and by Capotondi *et al.* (2015) in the muddy sediments rich in fresh organic matter of the central Adriatic coasts. In our study it does not correlate to any environmental characters (Table 3). Hence, it is difficult to reach a conclusion regarding the ecological requirements of this species in the GoT.

Regarding porcellaneous species, *Quinqueloculina* was one of the less tolerant genus in comparison to Zn concentration (Table 3). Furthermore, more oxygenated bottom water conditioned their presence ($r_s = 0.857$, $p = 0.006$). Among this group, *Q. parvula*, *Q. pygmaea, Q. seminulum* and *Q. stalkeri* were the dominant species. This genus, and miliolids in general, are considered sensitive to heavy metal pollution (Samir & El-Din 2001; Valenti *et al.* 2008; Ferraro *et al.* 2006), although in some cases, *Q. parvula* was recognised as a pollution-tolerant taxon by Romano *et al.* (2009) and *Q. seminulum* has been considered a stress tolerant species in relation to organic matter accumulation (Mangoni *et al.*, 2016) and capable of surviving in conditions of prolonged anoxic conditions in combination with sulfides (Langlet *et al.*, 2014). This study suggests that *Quinqueloculina* spp. are sensitive species to Zn concentration, only.

As for the agglutinated foraminifera, *Reophax nana*, *Textularia* spp. and *Eggerelloides scaber* do not have the same level of tolerance towards PTE concentration (Table 3). *Reophax nana* (also reported as *R. nanus* and *Aacostata mariae*) is frequent in the northern Adriatic Sea (Barmawidjaja *et al.* 1992; Donnici & Serandrei-Barbero, 2002; Sabbatini *et al.*, 2010; Langlet *et al.*, 2014). It showed a negative correlation with sand, TOC, and N content (Table 3). It was found mostly in the shallowest first centimetres of the sediments, in agreement with Langlet *et al.* (2014). Genus *Textularia* was present with several species, mainly represented by *T. conica, T. pala* and *T. calva* (Table S1). Its density distribution did not vary significantly between the two stations, even if they were more abundant at **Res** where in terms of frequency they are the most representative genus (Fig. 5). *Textularia* spp. were distributed in both superficial and sub superficial levels, preferring oxygenated bottom waters ($r_s = 0.762$, $p = 0.028$) and it was negatively correlated with sand content and Zn concentration (Table 3). *Eggerelloides scaber* was preferably abundant at **Res** especially during spring and summer (Fig. 5) and was more abundant in the surface layer, even if the genus *Eggerelloides* is usually considered a deep infaunal (Duijnstee *et al.*, 2004; Langlet *et al.*, 2014). This taxon is a continental shelf species living in microhabitats with different oxygenation conditions (e.g. Jorissen *et al.*, 1992; Donnici & Serandrei-Barbero, 2002; Duijnstee *et al.*, 2004; Langlet *et al.*, 2014); it is also able to colonise the seaward part of estuaries and lagoons (Albani & Serandrei Barbero, 1990; Murray, 2006; Melis & Covelli, 2013). Although this species could be regarded as a tolerant species, at least in environments with relatively low levels of pollution by Frontalini & Coccioni (2008, 2011), in this study, *Eggerelloides scaber* is not correlated with any of the PTEs (Table 3).

Miliammina fusca was observed in very high densities during spring at **Res** station. It is an epifaunal taxon living mostly in marshes, shallow lagoons (Murray, 2006) or in relict marine areas (Lloyd & Evans, 2002). Its high occurrence practically only during spring (with over 700 specimens/50 cc) and only in this station is not easily explained. This species has never been recorded in the GoT, whereas it has rarely been found in the Marano and Grado lagoon (as a dead specimen) (Melis & Covelli, 2013). The similar environmental conditions of the bottom waters recorded at two stations during spring, except for small differences in bottom water temperature and pH. Particularly, the pH value, which reaches the lowest value at **Res** station during spring (Fig. 2), could justify its presence, as also suggested by Burone *et al*. (2006). In addition, a greater presence of chlorophyll *a* during the spring at this station than at Ser station at a depth closer to the seabed (Fig. 2), may have favoured the *Miliammina* bloom. *Lagenammina atlantica* was observed in high density during summer at **Res** station. This taxon is frequently found in the northeastern Adriatic Sea (Sabbatini *et al.*, 2010) and is considered a passive detritivore organism consuming diatoms and bacteria (Burmistrova *et al.*, 2007). This high density during summer could suggest that this species only found these optimal conditions during this season.

Benthic foraminifera used to assess the ecological status of the studied environment

Following the suggestions of Bouchet *et al.* (2012, 2018), we used the Exp(H'_{bc}) index based on benthic foraminifera to evaluate the ecological status of the studied environments (EcoQs). In this study we decided to define the highest expected Exp(H'_{bc}) to 25 effective species using the complete living assemblage (0-2 cm interval, living foraminifera from cumulative triplicate samples). In fact, these are the same criteria as those designed for Norwegian fjords (Bouchet *et al.*, 2012). Note that in Italian transitional waters (lagoons, coastal lakes), where highly variable environmental conditions occur, Exp(H'bc) calculated on benthic living foraminifera was successfully used to the EcoQs assessment (Bouchet *et al.*, 2018).

In this study, the EcoQs based on benthic foraminifera indexes demonstrated conditions varying from moderate to good, in agreement with the environmental situations occurring at each station. This suggests that criteria developed at higher latitudes (Norwegian fjords) can be used in other environments such as the Gulf of Trieste. This must be further investigated in other open marine coastal habitats. Furthermore, it confirms that although developed along a decreasing bottom-water oxygen gradient, the foraminiferal method used to assess EcoQs works well against organic matter and trace metal pollution.

In general, diversity indices tend to have “natural” seasonal variations, often in relation to the recruitment which occur in spring and summer (Reiss & Kröncke, 2005; Murray, 2006). Such “natural” variations were also observed in this study. It is crucial to make sure that these variations do not hamper the assessment of EcoQs. Hence, our results suggest that the early spring season and latest summer would be the best periods of the year for EcoQ assessment using the foraminiferal method, since assemblages seem to reach equilibrium after an intense period of recruitment.

According to the rank-frequency diagrams (RFDs), benthic foraminiferal assemblages reached different degrees of maturity throughout the year at both stations. Foraminiferal assemblages were found to be quite resilient over the annual cycle under consideration, as they recovered from an unbalanced to a mature state, depending on the season. Seasonal variations in RFDs at both stations are due to changes in diversity and in dominant species. Stressful conditions at **Ser** station, mainly due to TOC accumulation and high Zn concentration, produced a more unbalanced foraminiferal assemblage in comparison with **Res** station. Only winter produces a mature assemblage at **Ser**, despite the low salinity values observed (< 30), which do not seem to have had an effect on the foraminifers. Conversely, the mature assemblage observed at **Res** station suggests that the Miramare MPA is efficient in preserving good environmental conditions in the GoT, despite the fact that in this station there was a larger accumulation of Pb and Cr than **Ser** station. Benthic foraminiferal assemblages responded well to the contrasted conditions occurring at both stations. These results obtained via RFDs support the worse EcoQs obtained at **Ser** station. These findings are in agreement with previous works showing that RFDs are helpful in assessing the health of benthic communities (Sanvicente-Anorve *et al.*, 2002; Bouchet *et al.*, 2007). Furthermore, this confirms that benthic foraminifera are good indicators of organic matter pollution induced by sewage outfalls (Schafer *et al.*, 1991; Mojtahid *et al.*, 2008) and trace metal pollution (Armynot du Châtelet & Debenay 2010; Frontalini & Coccioni 2011).

Conclusions

This study is the first to investigate the effects of MPA on the conservation of benthic foraminifera. The two studied stations (**Ser** and **Res**) showed both similarities and differences among the foraminiferal assemblages in terms of species richness, diversity, and composition during the seasons. Foraminifera bloomed at both stations during spring, corresponding to the peak of chlorophyll *a* recorded in the water column. The taxonomic differences and above all, the diverse density distribution of the major species in two stations were partially driven by the geochemistry of the sediment as well as by the difference in the oxygen concentration of the bottom waters.

Stressed conditions occurred near the sewage outfall (**Ser** station) characterised by coarse sediment texture, high contents of TOC and Zn in the bioavailable fraction, which were a limiting factor for the studied foraminiferal communities. *A. tepida*, *Bolivina* spp. and *Bulimina* spp., which characterised this station, could be considered the most tolerant taxa of the studied assemblage, thus representing good indicators of heavy metal pollution, in agreement with previous studies from the literature. Conversely, *Elphidium* spp., *H. depressula, N. iridea, Quiqueloculina* spp., *R. nana* and *Textularia* spp., could be considered less tolerant species, as they benefitted from the less stressful conditions (lower TOC and Zn contents) recorded at **Res** station. Such benefits occurred despite slightly higher concentrations of some PTEs, especially Pb, was recorded in this station with respect to **Ser** station. However, these PTE values were always within the limits of the unpolluted environments.

As a result, the reported EcoQs near the **Ser** sewage outfall were usually worse than those recorded in the marine protected area of Miramare (**Res** station). This suggests that the marine reserve would be effective at preserving benthic foraminiferal communities. Furthermore, foraminiferal assemblages were found to be quite resilient over an annual cycle, being able to recover from a seasonal unbalanced state to a mature one. We therefore suggest that EcoQ assessment using the foraminiferal method should be applied at the beginning of the spring season, before recruitment starts, or right after the summer when assemblages reach equilibrium after an intense period of recruitment. This study confirms that benthic foraminifera are good bioindicators and encourages further need for testing and assessment in other environments.

Acknowledgements

The authors thank Prof. G. Adami and Prof. S. Covelli for their useful discussions regarding the chemical analyses; M. Bussi and C. Landucci for their help with the sedimentological and geochemical analyses and Dr. S. Sponza for the data analyses. Many thanks to Karolyn Close for the linguistic revision. This work is framed in the project FRA 2012 "Monitoraggio ambientale di siti marini costieri mediterranei attraverso l'utilizzo interdisciplinare di dati abiotici e microfaunistici", University of Trieste.

References

Acquavita, A., Predonzani, S., Mattassi, G. Rossin P., Tamberlich F. *et al*., 2010. Heavy Metal Contents and Distribution in Coastal Sediments of the Gulf of Trieste (Northern Adriatic Sea, Italy). *Water Air Soil Pollution*, 211 (1), 95-111. doi:10.1007/s11270-009-0284-5

Adami, G., Barbieri, P., Campisi, B., Predonzani, S., Reisenhofer, E., 1996. Anthropogenic heavy metal distribution in sediments from an area exposed to industrial pollution (harbour of Trieste, Northern Adriatic Sea). *Bollettino Società Adriatica delle Scienze*, 77, 5-18.

Adami, G., Barbieri, P., Piselli, S., Predonzani, S., Reisenhofer, E., 1998. New data on organic pollutants in surface sediments in the harbour of Trieste. *Annali di Chimica*, 88, 745-754.

Adami, G., Barbieri, P., Reisenhofer, E., 2000. An improved index for monitoring metal pollutants in surface sediments. *Toxicology and Environmental Chemistry,* 77 (3-4), 189-197.

Albani, A.D., Serandrei Barbero, R., 1990. I foraminiferi della laguna e del golfo di Venezia. *Memorie di Scienze Geologiche*, 42, 271-341.

Alve, E., Korsun, S., Schönfeld, J., Dijkstra, N., Golikova, E. *et al*., 2016. Foram-AMBI: a sensitivity index based on benthic foraminiferal faunas from North-East Atlantic and Arctic fjords, continental shelves and slopes. *Marine Micropaleontology*, 122, 1–12.

Armynot du Châtelet, E., Debenay, J.P., Soulard, R., 2004. Foraminiferal proxies for pollution monitoring in moderately polluted harbors. *Environmental Pollution*, 127 (1), 27-40.

Armynot du Châtelet, E., Bout-Roumazeilles, V., Riboulleau, A., Trentesaux, A., 2009. Sediment (grain size and clay mineralogy) and organic matter quality control on living benthic foraminifera. *Revue de Micropaléontologie*, 52, 75-84.

Armynot du Châtelet, E., Debenay, J.P., 2010. Anthropogenic impact on the western French coast as revealed by foraminifera: a review. *Revue de Micropaléontologie*, 53, 129-137.

Barmawidjaja, D.M., Jorissen, F.J., Puskaric, S., Van der Zwaan, G.J., 1992. Microhabitat selection by benthic foraminifera in the northern Adriatic Sea. *Journal of Foraminiferal Research*, 22, 297-317.

Barras, C., Jorissen, F.J., Labrune, C., Andral, B., Boissery, P., 2014. Live benthic foraminiferal faunas from the French mediterranean coast: towards a new biotic index of environmental quality. *Ecological Indicators*, 36, 719-743.

Bellotti, P., Carboni, M.G., Di Bella, L., Palagi, I., 1994. Benthic foraminiferal assemblages in the depositional sequence of the Tiber Delta. p. 29-40. In: *Studies on Ecology and Palaeoecology of Benthic Communities*, Matteucci, R., Carboni, M.G., Pignatti, J.S., *Bollettino della Società Paleon-*

tologica Italiana, Special Publ. 2.

Bergamin, L., Romano, E., Finoia, M.G., Venti, F., Bianchi, J. *et al*., 2009. Benthic foraminifera from the coastal zone of Baia (Naples, Italy): assemblage distribution and modification as tools for environmental characterization. *Marine Pollution Bulletin*, 59, 234–244.

Bernhard, J.M., 1986. Characteristic assemblages and morphologies of benthic foraminifera from anoxic, organic-rich deposits; Jurassic through Holocene. *Journal of Foraminiferal Research*, 16 (3), 207-215.

Bernhard, J.M., 2000. Distinguishing live from dead foraminifera: methods review and proper applications. *Micropaleontology*, 46, 38-46.

Boon, A.R., Duineveld, G.C.A., Berghuis, E.M., Van Der Weele, J.A., 1998. Relationships between benthic activity and the annual phytopigment cycle in near-bottom water and sediments in the southern North Sea. *Estuarine, Coastal and Shelf Science*, 46, 1-13.

Borja, A., Franco, J., Perez, V., 2000. A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. *Marine Pollution Bulletin,* 40, 1100-1114.

Bouchet, V.M.P., Debenay, J.-P., Sauriau, P.-G., Knoery, J. R., Soletchnik, P., 2007. Effects of short-term environmental disturbances on living benthic foraminifera during the Pacific oyster summer mortality in the Marennes-Oléron Bay (France). *Marine Environmental Research*, 64, 358-383. doi: 10.1016/j.marenvres.2007.02.007

Bouchet, V.M.P., Sauriau, P.-G., 2008. Influence of oyster culture practices and environmental conditions on the ecological quality of intertidal mudflats in the Pertuis Charentais (SW France): a multi-index approach. *Marine Pollution Bulletin*, 56, 1892–1912.

Bouchet, V.M.P., Alve, E., Rygg, B., Telford, R.J., 2012. Benthic foraminifera provide a promising tool for ecological quality assessment of marine waters. *Ecological Indicators*, 23, 66–75. doi:10.1016/j.ecolind.2012.03.011

Bouchet, V.M.P., Goberville, E., Frontalini, F., 2018. Benthic foraminifera to assess Ecological Quality Statuses in Italian transitional waters. *Ecological Indicators*, 84, 130-139.

Brambati, A., Ciabatti, M., Fanzutti, G.P., Marabini, F., Marocco, R., 1983. A new sedimentological textural map of the Northern and Central Adriatic Sea. *Bollettino di Oceanologia Teorica e Applicata*, 1, 267-271.

Burmistrova, I.I., Khusid, T.A., Belyaeva, N.V., Chechovskaya M.P., 2007. Agglutinated abyssal foraminifers of the equatorial Pacific. *Oceanology*, 47 (6), 824-832. doi:10.1134/S0001437007060070

Burone, L., Venturini, N., Sprechmann, P., Valente, P., Muniz, P., 2006. Foraminiferal responses to polluted sediments in the Montevideo coastal zone, Uruguay. *Marine Pollution Bulletin*, 52, 61-73.

Cabrini, M., Fornasaro, D., Cossarini, G., Lipizer, M., Virgilio, D., 2012. Phytoplankton temporal changes in a coastal Northern Adriatic site during the last 25 years. *Estuarine and Coastal Shelf Science*, Special Issue: North Adriatic, 113-124. doi: 10.1016/j.ecss.2012.07.007

Capotondi, L., Bergami, C., Orsini, G., Ravaioli, M., Colantoni, P. *et al*., 2015. Benthic foraminifera for environmental monitoring: a case study in the central Adriatic continental shelf. *Environmental Science and Pollution Research*, 22, 6034-6049. doi: 10.1007/s11356-014-3778-7

Carboni, M.G., Succi, M.C., Bergamin, L., Di Bella, L., Frezza, V. *et al*., 2009. Benthic foraminifera from two coastal lakes of southern Latium (Italy). Preliminary evaluation of environmental quality. *Marine Pollution Bulletin*, 59, 268-280.

Caruso, A., Cosentino, C., Tranchina, L., Brai, M., 2011. Response of benthic foraminifera to heavy metal contamination in marine sediments (Sicilian coasts, Mediterranean Sea). *Chemical Ecology*, 27, 9-30.

Celio, M., Malačič, V., Bussani, A., Čeremelj, B., Comici, C. *et al*., 2006. The coastal scale observing system component of ADRICOSM: Gulf of Trieste network, *Acta Adriatica*, 47 (suppl.), 65-79.

Chao, A., Shen, T.-J., 2003. Nonparametric estimation of Shannon's index of diversity when there are unseen species in sample. *Environmental and Ecological Statistics*, 10, 429-443.

Chester, R., Voutsinou, F.G., 1981. The initial assessment of trace metal pollution in coastal sediments. *Marine Pollution Bulletin*, 12, 84-91.

Cibic, T., Acquavita, A., Aleffi, F., Bettoso, N., Blasutto, O. *et al*., 2008. Integrated approach to sediment pollution: A case study in the Gulf of Trieste. *Marine Pollution Bulletin*, 56, 1650-1667.

Cimerman, F., Langer, M.R., 1991. *Mediterranean Foraminifera*. Slovenska Akademija Znanosti in Umetnosti. Ljubljana, 118 pp.

Coates, S., Waugh, A., Anwar, A., Robson, M., 2007. Efficacy of a multi-metric fish index as an analysis tool for the transitional fish component of the Water Framework Directive. *Marine Pollution Bulletin*, 55, 225-240.

Coccioni, R., Frontalini, F., Marsili, A., Mana, D., 2009. Benthic foraminifera and trace element distribution: a case study from the heavily polluted lagoon of Venice (Italy). p. 257-67. In: *Foraminifera and marine pollution*, Romano, E., Bergamin, L., *Marine Pollution Bulletin*, 56.

Corliss, B.H., Emerson, S., 1990. Distribution of Rose Bengal stained deep-sea benthic foraminifera from the Nova Scotia continental margin and Gulf of Maine. *Deep Sea Research*, 37, 381-400.

Covelli, S., Fontolan, G., 1997. Application of a normalization procedure in determining regional geochemical baselines: Gulf of Trieste, Italy. *Environmental Geology*, 30, 34–45.

Covelli, S., Fontolan, G., Faganeli, J., Ogrinc, N., 2006. Anthropogenic markers in the Holocene stratigraphic sequence of the Gulf of Trieste (northern Adriatic Sea). *Marine Geology*, 230, 29-51.

Cozzi, S., Adami, G., Barbieri, P., Cantoni, C., Catalano, G. *et al*., 2004. Matching monitoring and modelling in the Gulf of Trieste. *Marine Pollution Bulletin*, 48, 587-603.

Debenay, J.-P., Guillou, J.-J., Redois, F., Geslin, E., 2000. Distribution trends of foraminiferal assemblages in paralic environments: a base for using foraminifera as bioindicators, p. 39-67. In: *Environmental micropaleontology: The Application of Microfossils to Environmental Geology*, Martin, R.E. (Ed). Kluwer Academic/Plenum Publishers, New York.

Denoyelle, M., Geslin, E., Jorissen, F.J., Cazes, l., Galgani, F., 2012. Innovative use of foraminifera in ecotoxicology: A marine chronic bioassay for testing potential toxicity of drilling muds. *Ecological Indicators*, 12 (1), 17-25.

de Stigter, H.C., Jorissen, F.J., van der Zwaan, G.J., 1998. Bathymetric distribution and microhabitat partitioning of live (Rose Bengal stained) benthic Foraminifera along a shelf to bathyal transect in the southern Adriatic Sea. *Journal of Foraminiferal Research*, 28 (1), 40-65.

Diaz, R. J., Rosenberg, R., 2008. Spreading dead zones and consequences for marine ecosystems. *Science*, 321, 926-929.

Dimiza, M.D., Triantaphyllou, M.V., Koukousioura, O., Hallock, P., 2016. The Foram Stress Index: A new tool for environmental assessment of soft-bottom environments using benthic foraminifera. A case study from the Saronikos Gulf, Greece, Eastern Mediterranean. *Ecological Indicators*, 60, 611-621.

Donazzolo, R., Hieke Merlin, O., Menegazzo Vitturi, L., Pavoni, B., 1984. Heavy metal content and lithological properties of recent sediments in the Northern Adriatic. *Marine Pollution Bulletin*, 15, 93-101.

Donnici, S., Serandrei-Barbero, R., 2002. The benthic foraminiferal communities of the North Adriatic continental shelf. *Marine Micropaleontology*, 44, 93-123.

Duchemin, G., Jorissen, F. J., Le Loc'h, F., Andrieux-Loyer, F., Hily, C., *et al*., 2008. Seasonal variability of living benthic foraminifera from the outer continental shelf of the Bay of Biscay. *Journal of Sea Research*, 59, 297-319.

Duijnstee, I.A.P., Ernst, S.R., Van der Zwaan, G.J., 2003. Effect of anoxia on the vertical migration of benthic foraminifera. *Marine Ecology Progress Series*, 246, 85–94.

Duijnstee, I.A.P., Lugt, I.R., de Vonk Noordegraaf, H., Van der Zwaan, G.J., 2004. Temporal variability of foraminiferal densities in the northern Adriatic Sea. *Marine Micropaleontology*, 50, 125-148. doi:10.1016/S0377-8398(03)00069-0, 2004

Edgar, G.J., Stuart-Smith, R.D., Willis, T.J., Kininmonth, S., Baker, S.C. *et al*., 2014. Global conservation outcomes depend on marine protected areas with five key features. *Nature*, 506, 216–220. doi:10.1038/nature13022

Eichler, P.P.B., Eichler, B.B., Sen Gupta, B., Rodrigues, A.R., 2012. Foraminifera as indicators of marine pollutant contamination on the inner continental shelf of southern Brazil. *Marine Pollution Bulletin*, 64, 22–30. doi:10.1016/j.marpolbul.2011.10.032

Ellis and Messina Catalogues, 2017. *Foraminifera*. http://www.micropress.org/ (Accessed online 2017).

Faganeli, J., Planinic, R., Pedzic, J., Smodis, B., Stegnar, P. *et al*., 1991. Marine geology of the Gulf of Trieste (northern Adriatic): Geochemical aspects. *Marine Geology*, 99, 93-108.

Ferraro, L., Sprovieri, M., Alberico, I., Lirer, F., Prevedello, L. *et al*., 2006. Benthic foraminifera and heavy metals distribution: A case study from the Naples Harbour (Tyrrhenian Sea, Southern Italy). *Environmental Pollution*, 142, 274-287.

Fiorini, F., Vaiani, S.C., 2001. Benthic foraminifers and transgressive-regressive cycles in the Late Quaternary subsurface sediments of the Po Plain near Ravenna (Northern Italy). *Bollettino della Società Paleontologica Italiana*, 40 (3), 357-403.

Folk, R.L., Ward, W.C., 1957. Brazos river bar: a study of significance of grain size parameters. *Journal of Sedimentary Petrology*, 27, 3-26.

Francescangeli, F., Armynot du Chatelet, E., Billon, G., Trentesaux, A., Bouchet, V.M.P., 2016. Palaeo-ecological quality status based on foraminifera of Boulogne-sur-Mer harbour (Pas-de-Calais, Northeastern France) over the last 200 years. *Marine Environmental Research*, 117, 32-43. doi: 10.1016/j.marenvres.2016.04.002

Frontalini, F., Coccioni, R., 2008. Benthic foraminifera for heavy metal pollution monitoring: a case study from the central Adriatic Sea coast of Italy. *Estuarine and Coastal Shelf Science*, 76, 404-417.

Frontalini, F., Coccioni, R., 2011. Benthic foraminifera as bioindicators of pollution: A review of Italian research over the last three decades. *Revue de Micropaléontologie*, 54, 115-127.

Frontalini, F., Buosi, C., Da Pelo, S., Coccioni, R., Cherchi, A. *et al*., C., 2009. Benthic foraminifera as bio-indicators of trace element pollution in the heavily contaminated Santa Gilla lagoon (Cagliari, Italy). *Marine Pollution Bulletin*, 58, 858-877.

Frontalini, F., Margaritelli, G., Francescangeli, F., Rettori, R., Armynot du Chatelet, E. *et al*., 2013. Benthic foraminiferal assemblages and biotopes in a coastal lake: the case study of Lake Varano (Southern Italy). *Acta Protozoologica*, 52, 147-160.

Frontier, S., 1976. Utilisation des diagrammes rang-fréquence dans l'analyse des écosystèmes. *Journal de Recherche Océanographique*, 1, 35-48.

Frontier, S., 1985. Diversity and structure in aquatic ecosystems. *Oceanography and Marine Biology: An Annual Review*, 23, 253-312.

Geslin, E., Barras, C., Langlet, D., Kim, J.-H., Bonnin, J. *et al*., 2014. Survival, reproduction and calcification of three benthic foraminiferal species in response to experimentally induced hypoxia. p. 163-193. In: *Survival, Reproduction and Calcification of Three Benthic Foraminiferal Species in Response to Experimentally induced Hypoxia.* Kitazato H., Bernhard J.M., Förstner U. Salomons W. (Eds). Springer, Berlin.

Giani, M., Djakovac, T., Degobbis, D., Cozzi, S., Solidoro, C. *et al*., 2012. Recent changes in the marine ecosystems of the northern Adriatic Sea. *Estuarine and Coastal Shelf Science*, 115, 1-13. doi:10.1016/j.ecss.2012.08.023.

Gooday, A.J., 1988. A response by benthic foraminifera to the deposition of phytodetritus in the deep sea. *Nature*, 332, 70-73.

Goineau, A., Fontanier, C., Jorissen, F., Buscail, R., Kerhervé, P. *et al*., 2012. Temporal variability of live (stained) benthic foraminiferal faunas in a river-dominated shelf - Faunal response to rapid changes of the river influence (Rhône prodelta, NW Mediterranean). *Biogeosciences*, 9, 1367-1388. doi: 10.5194/bg-9-1367-2012

Guerzoni, S., Frignani, M., Giordani, P., Frascari, F., 1984. Heavy metals in sediments from different environments of a Northern Adriatic Sea area, Italy. *Environmental Geology and Water Sciences*, 6, 111-119.

Guidetti, P., Milazzo, M., Bussotti, S., Molinari, A., Murenu, M., *et al*., 2008. Italian marine reserve effectiveness: Does enforcement matter? *Biological Conservation*, 141, 3, 699-709. doi: 10.1016/j.biocon.2007.12.013.

Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica*, 4 (1), 1-9.

Hedges, J.I., Stern, J.H., 1984. Carbon and nitrogen determinations of carbonate containing solids. *Limnology and Oceanography*, 29, 657-663.

Hill, M., 1973. Diversity and evenness: a unifying notation and its consequences. *Ecology*, 54, 427-432.

Hohenegger, J., Piller, W.E., Baal, C., 1989. Reasons for spatial microdistributions of foraminifers in an intertidal pool (northern Adriatic Sea). *Marine Ecology*, 10 (1), 43-78.

Hohenegger, J., Piller, W.E., Baal, C., 1993. Horizontal and vertical spatial microdistribution of foraminifers in the shallow subtidal Gulf of Trieste, northern Adriatic Sea. *Journal of Foraminiferal Research*, 23, 79-101.

Horvat, M., Covelli, S., Faganeli, J., Logar, M., Mandić, V. *et al*., 1999. Mercury in contaminated coastal environments; a case study: the Gulf of Trieste. *Science of The Total Environment*, 237/238, 43-56.

Hyams-Kaphzan, O., Almogi-Labin, A., Benjamini, C., Herut, B., 2009. Natural oligotrophy vs. pollution-induced eutrophy on the SE Mediterranean shallow shelf (Israel): environmental parameters and benthic foraminifera. *Marine Pollution Bulletin*, 58, 1888-1902. doi:10.1016/j.marpolbul.2009.07.010

Jannink, N.T., 2001. Seasonality, biodiversity and microhabitats in benthic foraminiferal communities. *Geologica Ultraiectina*, 203, 1-190.

Jorissen, F.J., 1987. The distribution of benthic foraminifera in the Adriatic Sea. *Marine Micropaleontology*, 12, 21-48.

Jorissen, F.J., 1988. Benthic foraminifera from the Adriatic Sea: principles of phenotypic variation. *Utrecht Micropaleontological Bulletins*, 37, 1-174.

Jorissen, F. J., Barmawidjaja, D. M., Puskaric, S., van der Zwaan, G. J., 1992. Vertical distribution of benthic foraminifera in the northern Adriatic Sea: The relation with the organic flux. *Marine Micropaleontology*, 19, 131–146. doi: 10.1016/0377-8398(92)90025-F.

Jorissen, F., Nardelli, M.P., Almogi-Labin, A., Barras, C., Bergamin, L. *et al*., 2018. Developing Foram-AMBI for biomonitoring in the Mediterranean: Species assignments to ecological categories. *Marine Micropaleontology*, 140, 33-45. doi: 10.1016/j.marmicro.2017.12.006

Kaiho, K., 1994. Benthic foraminiferal dissolved-oxygen index and dissolved-oxygen levels in the modern ocean. *Geology*, 22, 719-722.

Krause-Jensen, D., Greve, T., Nielsen, K., 2005. Eelgrass as a bioindicator under the European water framework directive. *Water Resources Management*, 19, 63-75.

Langlet, D., Baal, C., Geslin, E., Metzger, E., Zuschin, M. *et al*., 2014. Foraminiferal species responses to in situ, experimentally induced anoxia in the Adriatic Sea. *Biogeosciences*, 11, 1775-1797. doi: 10.5194/bg-11-1775-2014

Lardicci, C., Como, S., Corti, S., Rossi, F., 2001. Recovery of the Macrozoobenthic Community after Severe Dystrophic Crises in a Mediterranean Coastal Lagoon (Orbetello, Italy). *Marine Pollution Bulletin*, 42, 202–214.

Lavesque, N., Blanchet, H., de Montaudouin, X., 2009. Development of a multimetric approach to assess perturbation of benthic macrofauna in *Zostera noltii* beds. *Journal of Experimental Marine Biology and Ecology*, 368, 101-112.

Le Cadre, V., Debenay, J.-P., 2006. Morphological and cytological responses of *Ammonia* (foraminifera) to copper contamination: Implication for the use of foraminifera as bioindicators of pollution. *Environmental Pollution*, 143, 304-317. doi: 10.1016/j.envpol.2005.11.033

Legendre, L., Legendre, P., 1984. *Ecologie numérique. 2nd édition 1. Le traitement multiple des données écologiques.* Masson & les Presses de l'Université du Québec, Paris & Québec , 260pp.

Levy, A., Mathieu, R., Poignant, A., Rosset-Moulinier M., 1992. Foraminifers à arrangement quinqueloculin et triloculin (Miliolacea) de Méditerranée. *Revue de Paléobiologie*, 11, 111-135.

Lloyd, J. M., Evans, J. R. 2002. Contemporary and fossil foraminifera from isolation basins in northwest Scotland. *Journal of Quaternary Science*, 17, 431-443.

Loeblich Jr., A.R., Tappan, H., 1987. *Foraminiferal Genera and their Classification*. Van Reinhold Company, New York, 970 pp.

Malačič, V., Petelin, B., 2001. Gulf of Trieste.p. 167–181. In: *Physical Oceanography of the Adriatic Sea, Past, Present, Future*. Cushman-Roisin, B., Gačić, M., Poulain, P.M., Artegiani, A. (Eds). Kluwer Academics Publisher.

Mangoni, O., Aiello, G., Balbi, S., Barra, D., Bolinesi, F. *et al*., 2016. A multidisciplinary approach for the characterization of the coastal marine ecosystems of Monte Di Procida (Campania, Italy). *Marine Pollution Bulletin*, 112, 443-451.

Martínez-Colón, M., Hallock, P., Green-Ruíz, C., 2009. Strategies for using shallow-water benthic foraminifers as bioindicator of potentially toxic elements: a review. *Journal of Foraminiferal Research*, 39 (4), 278-299.

Mattassi, G., Scroccaro, I., Umgiesser, G., Colugnati, L., Ostoich, M. *et al*., 2008. Dispersion modelling of submarine wastewater discharges in the Northern Adriatic Sea along Friuli Venezia-Giulia and Veneto regions' coasts. 14p. In: *MMWD 2008 - 5th International Conference on Marine Waste Water Disposal and Marine Environment, Dubrovnik, 27-31 October 2008*. MWWD-IEMES, **ISBN**: 9789944556637.

Melis, R., Violanti, D., 2006. Foraminifers biodiversity and Holocene evolution of Phetchaburi coastal area (Thailand Gulf). *Marine Micropaleontology*, 61 (1-3), 94-115.

Melis, R., Protopsalti, I., 2008. Some taphonomic aspects of the benthic foraminifers from Tremiti Islands (Central Adriatic Sea). *Atti Museo Civico Storia Naturale Trieste*, 53, 177-188.

Melis, R., Covelli, S., 2013. Distribution and morphological abnormalities of recent foraminifera in the Marano and Grado Lagoon (North Adriatic Sea, Italy). *Mediterranean Marine Science*, 14/2, 432-450. doi: http://dx.doi.org/10.12681/mms.351

Meyers, P. A., 1994. Preservation of elemental and isotopic source identification of sedimentary organic matter. *Chemical Geology*, 114, 289-302. doi: 10.1016/0009-2541(94)90059-0

Milker, Y., Schmiedl, G., 2012. A taxonomic guide to modern benthic shelf foraminifera of the western Mediterranean Sea. *Palaeontologia Electronica*, 15, 2; 16A, 134p.

Morse, J.W., Luther, G.W. III, 1999. Chemical influences on trace metal-sulphide interactions in anoxic sediments. *Geochimica et Cosmochimica Acta*, 36, 3373-3378.

Mojtahid, M., Jorissen, F., Pearson, T.H., 2008. Comparison of benthic foraminiferal and macrofaunal responses to organic pollution in the Firth of Clyde (Scotland). *Marine Pollution Bulletin*, 56, 42-76.

Mouillot, D., Lepretre, A., 2000. Introduction of relative abundance distribution (RAD) indices, estimated from the rank–frequency diagrams (RFD), to access changes in community diversity. *Environmental Monitoring and Assessment*, 63, 279-295.

Munari, C., Mistri, M., 2010. Towards the application of the Water Framework Directive in Italy: assessing the potential of benthic tools in Adriatic coastal transitional ecosystems. *Marine Pollution Bulletin*, 60, 1040-1050.

Murray, J.W., 2006. *Ecology and Applications of Benthic Foraminifera*. Cambridge University Press, New York, 426 pp.

Murray, J.W., Alve, E., 2002. Benthic foraminifera as indicators of environmental change: estuaries, shelf and upper slope. p. 59-90. In: *Environmental Quaternary Micropalaeontology*. Haslett, S.R. (Ed). London, UK, Hodder Arnold.

National Research Council, 2003. *Bioavailability of contaminants in soils and sediments: processes tools and applications*. National Academy Press, Washington D.C., 416 pp.

Nardelli, M.P., Sabbatini, A., Negri, A., 2013. Experimental Chronic Exposure of the Foraminifer *Pseudotriloculina rotunda* to Zinc. *Acta Protozoologica*, 52, 193-202.

Novelli, G., 1996. Gli scarichi a mare nell`alto Adriatico. *Rassegna tecnica del Friuli Venezia Giulia*, 3, 11-19.

Ogorelec, B., Misic, M., Faganeli, J., 1991. Marine geology of the Gulf of Trieste (northern Adriatic): sedimentological aspects. *Marine Geology*, 99, 79-92.

Olivotti, R., Faganeli, J., Malej, A., 1986. Impact of 'organic' pollutants on coastal waters, Gulf of Trieste. *Water Science and Technology*, 18, 57-68.

Pucci, F., Geslin, E., Barras, C., Morigi, C., Sabbatini, A. *et al*., 2009. Survival of benthic foraminifera under hypoxic conditions: Results of an experimental study using the Cell Tracker Green method. *Marine Pollution Bulletin*, 59, 336-351.

Pusceddu, A., Dell'Anno, A., Danovaro, R., Manini, E., Sarà, G.*et al*., 2003. Enzymatically hydrolyzable protein and carbohydrate sedimentary pools as indicators of the trophic state of 'detritus sink' systems: a case study in a Mediterranean coastal lagoon. *Estuaries*, 26, 641-650.

Ramaswamy, V., Gaye, B., Shirodkar, P. V., Rao, P. S., Chivas, A. R. *et al*., 2008. Distribution and sources of organic carbon, nitrogen and their isotopic signatures in sediments from the Ayeyarwady (Irrawaddy) continental shelf, Northern Andaman Sea. *Marine Chemistry*, 111, 137-150.

Reiss, H., Kröncke, I., 2005. Seasonal variability of benthic indices: An approach to test the applicability of different indices for ecosystem quality assessment. *Marine Pollution Bulletin*, 50, 1490-1499.

Romano, E., Bergamin, L., Ausili, A., Pierfranceschi, G., Maggi, C. *et al*., 2009. The impact of the Bagnoli industrial site (Naples, Italy) on sea-bottom environment. Chemical and textural features of sediments and the related response of benthic foraminifera. p. 245-256. In: *Foraminifera and marine pollution*, Romano, E., Bergamin, L., *Marine Pollution Bulletin*, 59.

Sabbatini, A., Bonatto, S., Gooday, A.J., Morigi, C., Pancotti, I.*et al*., 2010. Modern benthic foraminifers at northern shallow sites of Adriatic Sea and softwalled, monothalamous taxa: a brief overview. *Micropaleontology*, 56, 359-376.

Sabbatini, A., Bonatto, S., Bianchelli S., Pusceddu A., Danovaro R. *et al*., 2012. Foraminiferal assemblages and trophic state in coastal sediments of the Adriatic Sea. *Journal of Marine Systems*, 105, 163-174.

Samir, A.M., El-Din, A.B., 2001. Benthic foraminiferal assemblages and morphological abnormalities as pollution proxies in two Egyptian bays. *Marine Micropaleontology*, 41, 193-227.

Sanvicente-Anorve, L., Leprêtre, A., Davoult, D., 2002. Diversity of benthic macrofauna in the eastern English Channel: comparison among and within communities. *Biodiversity and Conservation*, 11, 265-282.

Schafer, C.T., Collins, E.S., Smith, J.N., 1991. Relationship of foraminifera and thecamoebian distributions sediments contaminated by pulp mill effluent: Saguenay Fiord, Quebec, Canada. *Micropaleontology*, 17, 255-283.

Schönfeld, J., Numberger, L., 2007. The benthic foraminiferal response to the 2004 spring bloom in the western Baltic Sea. *Marine Micropaleontology*, 65, 78-95.

Schönfeld, J., Alve, E., Geslin, E., Jorissen, F., Korsun, S. *et al*., 2012. The FOBIMO (FOraminiferal BIo-MOnitoring) initiative - Towards a standardised protocol for soft-bottom benthic foraminiferal monitoring studies. *Marine Micropaleontology*, 94-95, 1-13.

Scroccaro, I., Ostoich, M., Umgiesser, G., De Pascalis, F., Colugnati, L. *et al*., 2010. Submarine wastewater discharges: dispersion modelling in the Northern Adriatic Sea. *Environmental Science and Pollution Research*, 17, 844-855.

Sgarrella, F., Moncharmont Zei, M., 1993. Benthic foraminifera of the Gulf of Naples (Italy): systematics and autoecology. *Bollettino della Società Paleontologica Italiana*, 32 (2), 145-264.

Shepard, F.P., 1954. Nomenclature based on sand-silt-clay rations. *Journal of Sedimentary Petrology*, 24 (3), 151-158.

Solan, M., Cardinale, B.J., Downing, A.L., Engelhardt, K.A.M., Ruesink, J.L. *et al*., 2004. Extinction and Ecosystem Function in the Marine Benthos. *Science*, 306 (5699), 1177-1180. doi: 10.1126/science.1103960.

Stott, L.D., Hayden, T.P., Griffith, J., 1996. Benthic foraminifera at the Los Angeles County Whites Point outfall revisited. *Journal of Foraminiferal Research*, 26, 357-368.

Tadir, R., Benjamini, C. Almogi-Labin, A., Hyams-Kaphzan, O., 2017. Temporal trends in live foraminiferal assemblages near a pollution outfall on the Levant shelf. *Marine Pollution Bulletin*, 117, 50-60. doi:10.1016/j.marpolbul.2016.12.045

Teodoro, A.C., Duleba, W, Gubitoso, S., Prada, S.M., Lamparelli, C.C. *et al*., 2010. Analysis of foraminifera assemblages and sediment geochemical properties to characterise the environment near Araçá and Saco da Capela domestic sewage submarine outfalls of São Sebastião Channel, São Paulo State, Brazil. *Marine Pollution Bulletin*, 60, 536–553. doi:10.1016/j.marpolbul.2009.11.011

Turner, A., 2010. Marine pollution from antifouling paint particles. *Marine Pollution Bulletin*, 60, 159-171.

Turk, V., Mozetič, P., Malej, A., 2007. Overwiew of eutrophication related events and another irregular episodes in Slovenian Sea (Gulf of Trieste, Adriatic Sea), *Annales Series historia naturalis*, 17, 197-216.

Twichell, S.C., Meyers, P.A., Diester-Haass, L., 2002. Significance of high C/N ratios in organic-carbon-rich Neogene sediments under the Benguela Current upwelling system. *Organic Geochemistry*, 33, 715-722.

Valenti, D., Tranchina, L., Brai, M., Caruso, A., Cosentino, C. *et al*., 2008. Environmental metal pollution considered as noise: Effects on the spatial distribution of benthic foraminifera in two coastal marine areas of Sicily (Southern Italy). *Ecological Modelling*, 213 (3-4), 449-462.

Venugopal, B., Luckey, T.D., 1975. Toxicology of non-radioactive heavy metals and their salts. p. 4–73. In: *Heavy Metals Toxicity Safety and Hormology*, Luckey, T.D., Venugopal, B., Hutchensen, D. (Eds). Thieme, Stuttgart.

Voutsinou-Taliadouri, F., 1995. A weak acid extraction method as a tool for the metal pollution assessment in surface sediments. *Microchimica Acta*, 119(3-4), 243-249.

Wentworth, C.K., 1922. A scale of grade and class terms for clastic sediments. *Journal of Geology*, 30, 377-392.

Yanko, V., Kronfeld, J., Flexer, A., 1994. Response of benthic foraminifera to various pollution sources: implications for pollution monitoring. *Journal of Foraminiferal Research*, 24, 1-17.