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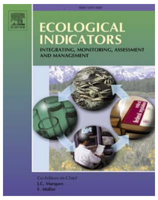

Original Articles

Benthic foraminifera for the ecological status assessment of tourist marinas

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ABSTRACT

Living benthic foraminifera were investigated in three tourist marinas of the Central Adriatic in order to test the suitability of these organisms for a quick and cost-effective assessment of the ecological quality status (EcoQS). Local high concentrations of biocidal metals (Cu, Zn, Cr, Sn, Ni and Pb) were measured in the sediments nearby the boathouse areas, i.e. where the careening activity takes place, suggesting that the accumulation of current and past antifouling residues is the main source of pollution in these particular maritime spaces. Factors such as the capacity of the marina, its shape as well as the location of the boathouse area concur to the degree of antifouling residues accumulation. Foraminifera responded to the different environmental conditions and to metal contamination in terms of abundance and species composition. At the boathouse stations these organisms were scarcely numerous and no living specimen was observed in the most polluted sediments. Three categories of indices were tested based on: (1) the percentages of abnormal tests, (2) biodiversity and (3) the sensitive-tolerant species occurrence (i.e. Foram-AMBI). The most diverse assemblage was observed at intermediate metal levels and seemed to be influenced by other environmental factors such as the presence of submerged vegetation that likely provides additional resources and increased heterogeneity. Unexpectedly, the station with the lowest metal levels was characterized by the least biodiverse assemblage. Notwithstanding, among the few species observed, at this site the dominant one was the sole species encountered in the present study that is recognized as sensitive, i.e. *Ammonia parkinsoniana*. Consequently, while the diversity indices provided very low values, Foram-AMBI outputs indicated the presence of an assemblage not exclusively dominated by opportunistic taxa. This result suggests a complementarity of these two categories of indices that should be taken into account for an accurate EcoQS assessment of tourist marinas.

1. Introduction

The Mediterranean Sea is one of the most popular nautical tourist destinations worldwide (Venturini et al., 2016). The leisure boating sector generates several economic benefits (jobs, investments and revenue) and is one of the most important incomes for coastal and insular economies. In EU, this sector creates up to 234,000 jobs and generates 28 billion euros annually, with 59 % of its economic outputs coming from the Mediterranean and in particular from its northern areas (European Commission, 2017). EU coasts are dotted with several facilities for the mooring, the manufacturing, the refitting, and the repair of leisure boats (Cappato, 2011). There are around 400,000 berths in the Mediterranean, distributed in 940 marinas (Billé and Lowezanin, 2010; Cappato, 2011), especially in Italy, France and Spain, where marina port capacities can reach very high numbers (up to 100 moorings per kilometre of coastline, Carreño and Lloret, 2021).

To date, different detrimental impacts of leisure boating on the marine environments have been identified. In a comprehensive review, Carreño and Lloret (2021) identified the antifouling paints and their toxicity on marine biota among the most important ones. These products contain compounds that act as biocides against the biological colonization of the boat hulls. For a long time, they were based on tributyltin (TBT) but the European legislation banned them since 2003 because TBT and its degradation derivates (monobutyltin, dibutyltin and triphenyltin) have been recognized as harmful to a wide range of organisms such as invertebrates (Matthiessen, 2019), fishes (de Araújo et al., 2018), mammals (Tanabe, 1999) and humans (Antizar-Ladislao, 2008). Although TBT-free, even the currently used antifouling paints arise concerns about their detrimental effects on the marine environment (Turner, 2010). These products, in fact, contain heavy metals that may bioaccumulate and biomagnify through the food chain, reaching the human beings through the seafood consumption (Moreau, 2009; Steiner

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and Feral, 2016; Ytreberg et al., 2016; Egardt et al., 2018). Among them, zinc, copper and lead are known for their toxic effects on the biota. For instance, zinc alters the absorption of calcium and impairs gill-based processes such as oxygen uptake and ion regulation, leading to fish death (McRae et al., 2016). On the other hand, copper can affect the olfactory system of fishes, altering their ability to sense predators and preys (Baldwin et al., 2011; Scholz et al., 2012), while lead is known for negatively affecting the osmoregulation and the survival of many organisms such as crustaceans (Usman et al., 1931).

Although with an ecological footprint surely less profound than that ascribable to large commercial or industrial ports, the capillary distribution of tourist marinas and the expanding of the leisure boating sector call for an effective management of these facilities that passes through their monitoring as the first mandatory step. Among the monitoring tools, the two main EU frameworks on marine environmental policy, i.e. the Water Framework Directive (WFD, 2000/60/EC) and the Marine Strategy Framework Directive (MSFD, 2008/56/EC), recommend the use of the Biological Quality Elements (BQEs). BQEs are biological communities recognised as fundamental constituents of the ecosystem that are able to respond to the environmental degradation in terms of altered abundance and taxonomic composition (WFD, 2000/60/EC). Over time, the use of the BQEs has been implemented by the development of a multitude of indices that have the advantage of summarising the response of a community in a synthetic output (a number). This single result can be inserted in the 5-point scale of thresholds that delimit the field of the Ecological Quality Status (EcoQS) from Bad to High, i.e. in a quality judgement easily understandable even by non-experts and that can support governmental bodies' legislative decision-making processes.

Since the benthic macro-invertebrates are by far the most popular BQEs, several indices have been developed for this community, such as BENTIX (Simboura and Zenetos, 2002), BITS (Mistri and Munari, 2008), AMBI (Borja et al., 2000), FINE (Mistri et al., 2008) and BOPA (Dauvin and Ruellet, 2007). Among the possible BQEs, benthic foraminifera have demonstrated to be potentially a good alternative to macrozoobenthos because of the promising results obtained in different environmental contexts such as coastal areas (Frontalini and Coccioni, 2008), transitional environments (Coccioni et al., 2009; Frontalini et al., 2009; Bouchet et al., 2018) and ports (Debenay et al., 2001; Armynot du Châtelet et al., 2011; Schintu et al., 2016). These organisms are single-celled protists with a short reproductive cycle, rapid growth, small body size and high abundance. In particular, their short life spans (3 months to 2 years; Murray, 1994) make their response to anthropogenic disturbance quicker than that exhibited by the macrofauna, providing an early warning about recent or ongoing environmental stress (Kennedy and Jacoby, 1999). On the other hand, their relatively high abundance and small body size allow a limited sampling effort because small sample volumes are sufficient for building datasets reliable for statistical analysis (Schönfeld et al., 2012). Finally, due to the high biodiversity of the phylum (>10,000 modern taxa, Sen Gupta, 1999), foraminifera species span from those very sensitive to those tolerant to different kinds of environmental stress such as organic enrichment (Alve, 1995; Mojtahid et al., 2008; Parent et al., 2021), oxygen deficiency (Sen Gupta and Machain-Castillo, 1993; Bernhard and Sen Gupta, 1999; Pucci et al., 2009) and heavy metals (Coccioni et al., 2009; Martins et al., 2013; Frontalini et al., 2018). In stress conditions, the assemblage responds in terms of lowered abundances, biodiversity loss, dwarfism and increased abnormalities of the tests (for review, see Frontalini and Coccioni, 2011). Both classical diversity indices (e.g. the Shannon H' index, Shannon and Weaver, 1949) and dedicated metrics concur to the assessment of foraminifera response to the environmental conditions and consequently to the EcoQS of a given area as exhaustively reviewed by O'Brien et al. (2021). The dedicated indices comprise the Foraminiferal Abnormalities Index (FAI, Coccioni et al., 2009), which is based on the occurrence of abnormal tests, and the Foraminiferal AZTI Marine Biotic Index (Foram-AMBI, Alve et al., 2016; Jorissen et al. 2018), which is based on the presence of sensitive-tolerant species.

In order to support small tourist marinas in designing and applying the better environmental strategies aimed at a sustainable management of their maritime space, the international European project ECOMAP (ECOsustainable management of MArine and tourist Ports) has been established. Within this framework, benthic foraminifera were investigated in three Adriatic tourist ports in order to evaluate the suitability of these organisms as BQEs for the EcoQS assessment of these particular coastal areas. The abundance, taxonomic composition and different kinds of indices – FAI, H′, ES(100), Exp(H'_{bc}), Foram-AMBI- were related to metal pollution associated to careening activities. The following questions were then addressed: 1. Are benthic foraminifera suitable BQEs for a reliable EcoQs assessment of tourist marinas? 2. Do the tested indices provide different responses to metal contamination?

2. Material and methods

2.1. Study area

The Adriatic Sea is characterized by a cyclonic (counterclockwise) thermohaline circulation that forces river water (and sediments) to flow southward along the western coast (Artegiani et al., 1997). The water exchange between the Adriatic and the Mediterranean is characterized by an outflow along the western coast and an inflow along the eastern one. The outflowing water portion consists of surface Adriatic water and in deep cold and dense water that formed in the northern part of the basin. The inflowing water is oligotrophic, saltier and warmer and comes from the Eastern Mediterranean.

Belonging to the Mediterranean climatological region, the Central Adriatic is characterized by hot summers, mild winters and long periods of low rainfall. Although sediment distribution along the eastern coast is still poorly known, in the Northern and Central Adriatic the shallower seabed is characterized by relict sand (Cosovic et al., 2011 and reference therein).

According to bathymetry, the Adriatic can be subdivided into 3 sub-basins: the Northern, the Central and the Southern Adriatic (Artegiani et al., 1997). The three marinas of the present study are located along both sides of the Central Adriatic, two in Croatia and one in Italy (Fig. 1).

The two Croatian marinas, i.e. Podstrana and Špinut, are located respectively in the northern and in the south-eastern areas of Split, one of the two cities with more than 200,000 inhabitants along the Croatian coastline (Fig. 1). Špinut is located 8 km eastward from the port of Split and is influenced by the inputs of the karstic Žrnovnica River.

The shape of the two marinas is quite different. Špinut marina is characterized by a single and relatively narrow entrance and has an elongated shape with at least 12 piers arranged as the teeth of a comb. Differently, Strožanac (Podstrana) marina is composed of three main basins, each with its own entrance, in which the boats are mainly located along the perimeter with the exception of the central basin where there is also a single pier with berths. Both marinas offer the hull cleaning service that is carried out in dedicated areas. Counting the piers from the port entrance, in Špinut this area is located at the end of the first two piers and in front of the last one. In Podstrana, there is a single boathouse area located nearby the entrance of the third basin (Fig. 1, Table S1). With more than 700 berths, Špinut has a higher reception capacity than Podstrana (circa 300 berths).

Marina Dorica is one of the widest marinas in Italy. Located less than 2 km southwards from the commercial port of Ancona, this marina has more than 1300 berths distributed along 20 piers. The basin is almost square with a single narrow entrance. The boathouse area is exactly at the opposite side of the port entrance (Fig. 1, Table S1).

2.2. Sampling

In all marinas, five stations were sampled along a confinement

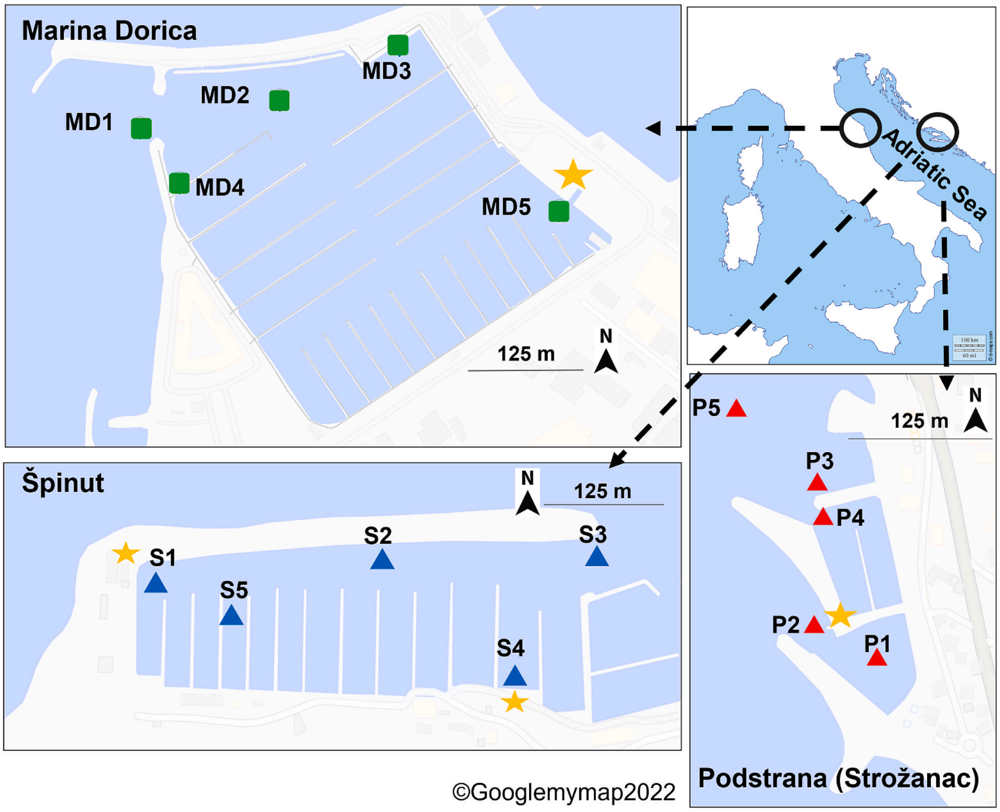


Fig. 1. The three investigated marinas and the location of the sampling sites inside each marina. The stars indicate the boathouse areas.

gradient and/or nearby the careening areas (Fig. 1). Špinut and Podstrana were sampled twice, i.e. in May 2019 and April 2021, while Marina Dorica was sampled in September 2020.

During each campaign, depth, bottom sea temperature and salinity were measured using a multiparametric probe (YSI ECO2 EXP 7 2001). At each station, the sediments for both foraminifera and chemical analyses were collected by means of a Van Veen grab (sampling area of 0.1 m^2), i.e. 3 grabs per sampling site.

From each of the three grabs, the top 4 cm of sediments for foraminifera were sampled with one cut-off plastic syringe (internal diameter: 2.7 cm, surface area: 5.72 cm^2), resulting therefore in 3 actual replicates per station. The remaining surface sediment (top 0–1 cm layer) from each grab was collected and homogeneized for the analyses of environmental variables such as the sediment grain-size, Total Organic

Table 1

Main environmental variables in the three marinas. P = Podstrana; S = Špinut; MD = Marina Dorica; 1 = May 2019; 2 = April 2021.

Station	Sand	Silt	Clay	TOC	As	Cd	Cr	Cu	Pb	Ni	Zn	Sn
	%				mg kg^{-1}							
P1_1	12.3	63.2	24.5	3.29	9.71	0.21	94.12	64.44	18.31	54.93	72.53	3.90
P2_1	38.5	52.0	9.5	1.57	10.71	0.15	53.66	19.30	8.25	28.47	30.89	1.49
P3_1	10.3	68.0	21.7	1.66	10.33	0.25	89.78	45.48	15.20	48.97	71.25	2.84
P4_1	16.6	56.2	27.2	1.56	9.74	0.16	68.32	33.01	11.09	42.75	46.59	2.20
P5_1	21.3	59.0	19.7	2.03	9.64	0.21	73.57	25.48	11.23	43.27	40.81	1.64
S1_1	14.0	61.8	24.2	1.96	12.50	0.20	76.07	253.41	28.64	49.20	146.23	8.90
S2_1	38.2	46.0	15.8	1.82	13.09	0.22	62.96	92.07	23.44	42.39	105.68	5.73
S3_1	98.8	0.7	0.5	0.69	22.71	0.08	12.48	35.78	10.29	7.26	26.54	4.89
S4_1	47.2	36.4	16.4	3.58	10.35	0.50	84.71	8987.06	1597.27	39.31	4046.15	206.49
S5_1	12.0	57.6	30.4	3.03	14.78	0.21	94.64	173.00	34.51	54.48	145.02	8.93
P1_2	10.5	64.1	25.4	0.83	7.51	0.29	89.33	67.28	21.85	65.16	89.94	1.89
P2_2	38.0	53.1	8.9	0.45	6.88	0.23	46.42	22.90	6.79	29.35	39.06	0.74
P3_2	20.5	59.2	20.3	1.07	7.70	0.32	87.90	55.78	14.14	58.92	93.43	1.74
P4_2	54.5	35.8	9.7	0.76	5.91	0.23	56.33	19.25	7.06	32.69	38.28	0.66
P5_2	42.4	48.0	9.6	0.85	7.69	0.25	69.54	52.15	9.58	37.40	56.59	2.47
S1_2	59.7	25.6	14.7	2.83	13.02	0.58	107.56	4714.59	97.02	62.69	3244.05	51.11
S2_2	71.6	20.7	7.7	2.21	18.31	0.20	38.59	64.64	12.85	28.42	80.79	2.48
S3_2	96.9	2.1	1.0	3.50	17.61	0.15	14.96	17.97	6.08	7.59	36.11	1.33
S4_2	38.2	43.9	17.9	1.71	12.17	0.57	98.78	5548.33	228.20	52.84	3462.43	133.56
S5_2	39.3	46.8	13.9	0.16	11.32	0.33	70.92	174.62	23.05	50.96	146.38	3.66
MD1	76.8	17.8	5.4	3.72	17.42	0.16	80.65	58.14	15.21	46.63	97.72	2.71
MD2	63.0	27.3	9.7	7.28	14.22	0.24	71.51	77.13	15.60	42.56	113.91	3.04
MD3	21.3	49.8	28.9	4.61	11.05	0.21	93.82	202.36	17.56	48.64	177.07	2.52
MD4	74.6	18.4	7.0	3.29	14.93	0.18	67.42	58.81	14.27	40.73	93.58	2.04
MD5	28.2	41.5	30.3	0.48	13.47	0.26	107.53	493.26	30.86	54.20	313.19	5.85

Carbon (TOC) and 8 metals (Table 1). The choice to sample the 0–1 cm sediment layer for the latter variables instead of the top 4 cm was dictated by the project requirements. However, considering the high resuspension inside the marinas caused by boat engines and the major macrofaunal bioturbation activity within the upper 5 cm-sediment layer resulting in active sediment mixing (data not shown), we did not expect to find remarkable differences in TOC and metal concentrations between the 0–1 cm and 0–4 cm sediment layers, in line with similar studies (Franzo et al. 2016; Franzo et al., 2019; Cibic et al. 2017).

2.3. Sediment grain-size, total organic carbon (TOC) and metals

Sediment grain-size analyses were performed with a BECKMAN COULTER LS 13 320 Laser Diffraction Particle Size Analyzer, measuring the 0.017–2000 µm fraction. The samples were pre-treated with 10 % v/v hydrogen peroxide, dispersed with 5 % m/v sodium-hexametaphosphate and sonicated for 1 min before measurement. The results were expressed as percentages of sand, silt, and clay according to the Udden-Wentworth classification (Wentworth, 1922).

For Total Organic Carbon (TOC) and metals analyses, sediment samples were powdered in an agate mill. TOC concentrations were estimated using an Elementar SoliTOC cube, i.e. an elemental analyser that works in temperature ramp mode and that shows the release of carbon from a solid matrix, such as soils or sediments, at increasing temperatures. This release is related to the thermal destabilisation of organic and inorganic phases. The investigated samples were analysed according to the DIN 19,539 standard. The fractions are referred to as TOC = TOC400 + ROC600, where TOC400 is the thermally labile organic carbon stripped out at temperatures below 400 °C while ROC600 is the residual oxidisable carbon obtained at temperatures between 400 and 600 °C. Data are expressed as TOC%.

A total of 8 metals (Table 1) were investigated in the sediments of the three marinas using the inductively coupled plasma mass spectrometry (ICP-MS). For these analyses, powders (0.15 g) were totally digested with suprapure grade HF and HNO_3 (Merck, Darmstadt, Germany) on a hot plate. Dissolved samples were dried out and then re-dissolved in ultrapure water obtained from a Milli-Q purifier system (Millipore Corp., Bedford, MA, USA). The analyses were carried out using an X Series Thermo-Scientific spectrometer. Data are expressed as mg kg^{-1} dry weight.

2.4. Foraminifera

From each replicate, the top 4 cm of sediments were immediately extruded and soaked in >70 % ethanol (Murray, 2006; Schönfeld et al., 2012). Once in laboratory, each sample was transferred to a container with a solution of >70 % ethanol and Rose Bengal (2 g/L) in order to distinguish living from dead foraminifera. After at least 14 days (the minimum time accepted for a sufficient staining of the organisms with Rose Bengal, Schönfeld et al., 2012), all samples were washed through a 63 µm sieve and dried at 50 °C. Considering the well-known problems in the use of Rose Bengal (Corliss and 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). All the tests with these characteristics were counted under a stereomicroscope Leica S6 D (up to 50× of magnification) and identified at the species level following Loeblich and Tappan (1987) and Milker and Schmiedl (2012).

In accordance to Bouchet et al. (2012) and Melis et al. (2019), 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. For each station, therefore, the abundance of each species was expressed as the number of specimens normalised to 50 cc.

2.4.1. Indices

The foraminifera indices considered in the present study can be subdivided in three categories: (1) indices based on morphological alterations; (2) indices based on diversity; (3) indices based on the occurrence of ‘sensitive-tolerant species’. To the first category belongs the FAI index that was calculated as the percentage of abnormal tests over the whole assemblage (Coccioni et al., 2009). Among the diversity indices, we considered both those universally accepted in monitoring studies (total species s; richness d, Margalef, 1986; diversity H′, Shannon and Weaver, 1949) and those that demonstrated to be particularly suitable for the study of benthic foraminifera, i.e. ES(100) (Hurlbert, 1971) and Exp(H'_{bc}) (Chao and Shen, 2003). Intended as the expected number of species from a subsample of 100 individuals taken from a population, ES(100) is appropriate for studying the foraminifera in under-sampling conditions because it is able to catch the actual diversity of the assemblage even if rare species are unobserved because not contained in the subsample. With regards to Exp(H′ bc), Chao and Shen (2003) introduced a bias-corrected version of the Shannon’s diversity H′ (i.e. H′bc) because also this latter is biased when there are unobserved species in the community (Beck and Schwanghart, 2010). Furthermore, H′ is an entropy rather than a diversity. The entropy gives the average uncertainty of the identity of an individual picked from the community, not the number of species in the community (e.g. Hayek and Buzas, 1997; Jost, 2006). It can be converted to true diversity, the effective number of species, by means of its exponential function (Exp(H′bc), Hill, 1973). Exp(H′bc) gives the number of species that would produce the same H′bc if each were equally common.

Being an adaptation of the AZTI Marine Biotic Index developed for the macrozoobenthos (Borja et al., 2000), the Foram-AMBI is calculated by assigning each foraminifera species to an Ecological Group (EG) according to its degree of tolerance/sensitivity to environmental stress. The EGs are the following: ‘sensitive’ species (EGI), ‘indifferent’ species (EGII), ‘tolerant’ or ‘third order opportunistic’ species (EGIII), ‘second-order opportunistic’ species (EGVI) and ‘first-order opportunistic’ species (EGV). Since the present study was carried out in three Mediterranean marinas, the species assignment proposed by Jorissen et al. (2018) was used because developed for this particular sea. The Foram-AMBI was then calculated according to the following formula:

$$\text{Foram-AMBI} = (0*\text{EGI}) + (1{,}5*\text{EGII}) + (3*\text{EGIII}) + (4{,}5*\text{EGIV}) + (6*\text{EGV})$$

where each Ecological Group is expressed as Relative Abundance.

2.5. Statistical analysis

Using STATISTICA v. 7 computer program, a Principal Component Analysis (PCA) was carried out on TOC, sand% and all the measured metals (Table 1) in order to explore the trends of these abiotic variables at all stations.

Since S4, one of the two stations located nearby the boathouse areas of Špinut, was characterized by the absence of living foraminifera, this station was not included in any statistical analysis performed on biological data. Univariate and multivariate analyses on foraminifera data were performed using the PRIMER v7 software package (Clarke and Warwick, 2001) with the PERMANOVA add-on package (Anderson et al., 2008) and STATISTICA v. 7. To test for spatial differences in the composition of foraminifera a data matrix based on the abundance of species at each station was constructed by applying the Bray-Curtis dissimilarity after a square root transformation of data. A one-way PERMANOVA test was conducted on the matrix using “marina” as a fixed factor (Špinut-S, Podstrana-P and Marina Dorica-MD) and the unrestricted permutation of raw data was performed (9999 permutations). The null hypothesis (i.e. no significant difference among sampling sites) was rejected when p was < 0.05. The Monte Carlo permutation p was used when the number of permutations was lower than 150. If significant differences were detected, *a posteriori* pair-wise comparisons were performed (9999 permutations). Since the Croatian marinas were sampled twice, the temporal variability of foraminifera species in Špinut and Podstrana was investigated by means of a one-way PERMANOVA test with the same design described above except for the fixed factor that in this case was the "sampling campaign". To check for spatial differences of total foraminifera abundance (N), diversity (s, d, ES(100), H′log2, Exp(H'_{bc})), FAI and Foram-AMBI, a Kruskall-Wallis ANOVA analysis was applied, using "marina" as a fixed factor (Špinut-S, Podstrana-P and Marina Dorica-MD). The post hoc comparisons of all pairs of independent groups (marinas) were subsequently computed. Since the Croatian marinas were sampled twice, the temporal variability of indices in Špinut and Podstrana was investigated by means of a Mann-Whitney test. The Spearman rank correlation was carried out in order to investigate how the abiotic variables (Table 1) and the measured indices were correlated.

In order to determine whether foraminifera were influenced by the main environmental variables (Table 1), a distance-based linear model (DISTLM, McArdle and Anderson, 2001) routine was carried out on species composition dataset. The all specified selection procedure and the adjusted R^2 were used as a selection criterion to enable the fitting of the best explanatory environmental variables in the model (Anderson et al., 2008). Prior to analysis, the environmental variables were normalized.

Redundancy analysis (RDA) was used as constrained ordination analysis to look for relationship between the foraminifera metrics (Table 3) and the abiotic variables (Table 1) in each location independently. The response variables were square root transformed. RDA ordination was performed using the Vegan package in the R program ver. 4.1.0, (Oksanen et al., 2007; https://www.R-project.org/).

3. Results

The three marinas showed comparable depth values since ranging between 3.5 and 6 m in Špinut, between 3 and 6 m in Podstrana, 2.5 and 6 m in Marina Dorica. The deepest stations were those located nearby the port entrance while some variability characterized the inner sampling sites (Table S1). The main environmental parameters of the three marinas are reported in Table 1. The sediment grain-size was rather variable among stations and marinas. Overall, the percentage of sand prevailed at the stations located nearby the port entrance, such as S3 (>96 %), MD1 (76.8 %) and MD4 (74.6 %), and a gradual increase of fine-grained particles corresponded to the increasing confinement. The latter was less evident in Podstrana, likely due to the shape of this marina which is characterized by wide connections with the open sea. Similarly, also the TOC content displayed a broad variability. The values varied between 0.45 e 3.29 % in Podstrana, between 0.16 and 3.58 % in Špinut, and between 0.48 and 7.28 % in Marina Dorica.

Regarding the analysed metals (Table 1), Špinut was the most contaminated marina, especially at its boathouse stations (S1 and S4) where the concentrations of Cu, Pb, Zn and Sn were up to 2 orders of magnitude higher than those at the other sampling sites. Although to a lesser extent, also the boathouse station of Marina Dorica (MD5) showed overall higher metal levels.

In the PCA performed considering all stations, two principal components (factors) (eigenvalue > 1) together explained 75.36 % of the total variance, whereas the first and the second factors explained 47.29 % and 28.07 % of the total variance, respectively (Fig. S1). The major contributors of the first factor were Cd, Cu, Sn and Zn, while Sand%, As and Ni were the predominant elements of the second factor. The boathouse sites of Špinut, S4 and S1, were well separated from the other stations due to their higher concentrations of heavy metals, especially Cu, Pb and Zn. On the other hand, the station located nearby Špinut port entrance (S3) was influenced more than all the other sampling sites by higher levels of As and Sand%. The differences among the other stations were less accentuated because partially masked by higher concentrations of metals at S1 and S4.

Foraminifera abundances and composition of the main species are reported in Fig. 2. During both sampling campaigns, the sediments of the most contaminated station (S4) were completely azoic, i.e. characterized by the absence of living tests. Overall, the Croatian marinas showed higher abundances than Marina Dorica but only Podstrana showed a significantly more numerous assemblage (Table 2). There was not a significant temporal difference for Špinut and Podstrana in terms of total abundance if comparing the two sampling campaigns (Mann-Whitney Z = 0.577, p = 0.564 for Špinut; Mann-Whitney Z = 0.104, p = 0.917 for Podstrana).

Regarding the taxonomic composition of the assemblage, a total of 34 species were observed (Table S2). Among them, 12 were found only in the sediments of Podstrana, 3 were collected exclusively in Špinut (*Quinqueloculina laevigata, Spiroloculina communis* and *Treptophalus bulloides*) while specimens of *Nonionella auris* were observed only in Marina Dorica. The assemblages inhabiting the three marinas were significantly different in terms of species composition (PERMANOVA Pseudo-F = 5.794, P(perm) = 0.0001) while Špinut and Podstrana did not display a significant temporal variability when comparing the two sampling campaigns (PERMANOVA Pseudo-F = 0.359, P(perm) = 0.974). Špinut stations were dominated by *Ammonia parkinsoniana* and *Elphidium crispum*, especially at S2 and S3. On the contrary, Podstrana samples were characterized by a more structured and diverse assemblage with higher abundances of *Haynesina depressula, Cribroelphidium poeyanum* and, to a lesser extent, *Eggerelloides scaber* (Fig. 2). Comparable numbers of *Ammonia tepida* were found in Podstrana and Marina Dorica while slightly higher abundances of *Rosalina bradyi* were observed in the sediments of the Italian marina.

Overall, tests with abnormalities showed very low abundances at all sampling stations and belonged to few species that were also the most abundant: *A. parkinsoniana*, *A. tepida*, *H. tepida* and *C. poeyanum* (Table S2). Furthermore, abnormal tests were observed at those stations where the foraminifera were more numerous (S2, S3, P2 and P4).

The results of foraminifera indices are reported in Table 3. For what concerns the Croatian marinas, the comparison between the two sampling campaigns indicated that none of the tested indices showed a significant temporal variability (data not shown).

Overall, FAI values were very low in the three marinas since the percentage of abnormal tests varied between 0 and 1.6 % in Špinut, between 0 and 3.0 % in Podstrana and between 0 and 2.6 % in Marina Dorica. Significant spatial differences were not detected among the three tourist ports (Table 2).

Ranging between 6 and 27, the number of species (s) was overall higher in Podstrana than in the other two marinas, although this difference was significant only when compared to Špinut (Table 2). Similarly, richness (d) values were statistically more elevated in Podstrana, especially at P2 (>3 during both sampling campaigns). Notwithstanding, the Kruskall-Wallis ANOVA outputs pointed out that this index was significantly higher in Podstrana only when compared to Špinut (Table 2).

In Špinut ES(100) varied between 2.9 and 10.8 and corresponded to a Bad or Poor EcoQS during both campaigns. On the contrary, in Podstrana this index ranged from 6.0 and 17.8 resulting in a Good EcoQS in 50 % of the observations. Similarly to Špinut, in Marina Dorica the values varied between 3.0 and 11.1, corresponding mainly to a Bad EcoQS. As for s and d, ES(100) were significantly higher in Podstrana than in Špinut (Table 2).

Although the Kruskall-Wallis outputs for H′ and Exp(H'_{bc}) did not highlight any significant difference among the three marinas (Table 2), Podstrana was characterized by a more biodiverse assemblage (Table 3). Varying between 2.2 and 3.6, H′ values in this marina were in fact higher than in Špinut (range 1.1–2.9) and in Marina Dorica (range 1.3–3.1). The EcoQS obtained according to this metrics were maintained over time in the two Croatian marinas and ranged from Moderate (P1 and P5) to High (P2) in Podstrana and from Bad/Poor (S2, S3) to Good (S1, S5) in

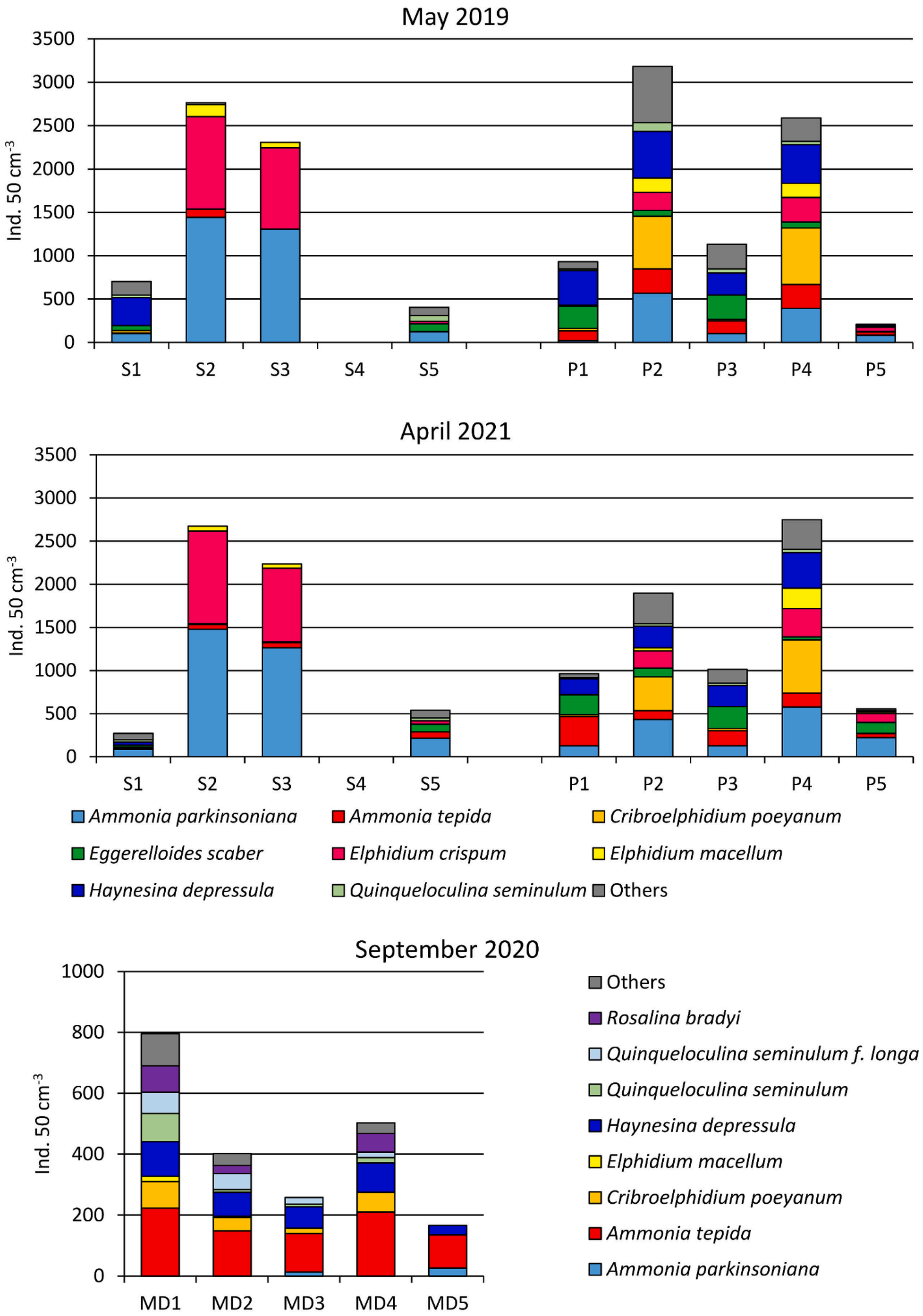


Fig. 2. Total abundance (Ind. 50 cm^{-3}) of the dominant foraminifera species at all stations and during both campaigns. The scale of the y-axis is different for Marina Dorica.

Špinut. In Marina Dorica, the EcoQS varied from Poor to Good, with the lowest score obtained at the boathouse station (MD5). Similarly to H′, Exp(H′$_{bc}$) showed higher values in Podstrana (4.9–12.2) than in Špinut and Marina Dorica (2.2–7.4 and 2.4–8.4, respectively). The corresponding EcoQS were overall lower than those obtained according to H′ (Table 3): from Bad to Moderate for Podstrana and from Bad to Poor for the other two ports.

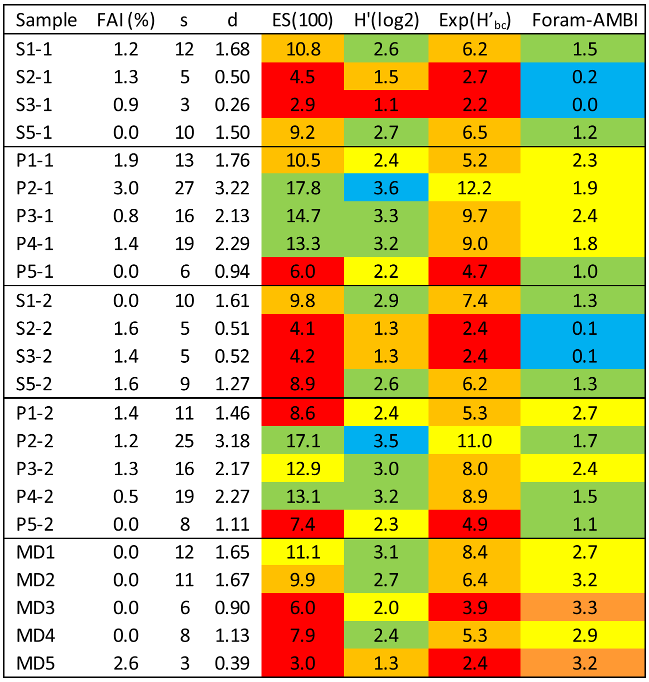
The values of Foram-AMBI were calculated according to the species assignment to the EG groups proposed by Jorissen et al. (2018). Although for 10 species the EG assignment was lacking, these taxa represented a minor fraction of the whole assemblage at all stations (<7.6 %) with the exception of S1 where higher percentages of unassigned species were reached (13.7 % and 19.4 % in May 2019 and April 2021, respectively). Anyway, Foram-AMBI results were significantly higher in Marina Dorica (range 2.7–3.3) than in the other two marinas (ranges 0.0–1.5 and 1.0–2.7 for Špinut and Podstrana, respectively). Since higher Foram-AMBI values correspond to lower EcoQS, the Italian marina was characterized by a score that ranged from Poor to Moderate, i.e. worse than the EcoQS obtained in Podstrana (Moderate/Good) and in particular in Špinut (Good/High).

The Spearman correlation outputs between the abiotic variables and the tested indices are reported in Table 4. Total abundance of foraminifera resulted significantly and negatively correlated with most of the trace metals. In other words, the least numerous assemblages were

Table 2
Outputs of the Kruskall-Wallis ANOVA performed on foraminifera total abundance and indices. MD = Marina Dorica; P = Podstrana; S = Špinut; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

	H	Post hoc comparisons
N	6.207*	P > MD
FAI	2.322	n.s.
s	9.224**	P > S
d	8.235*	P > S
ES(100)	7.257*	P > S
H′(log2)	5.226	n.s.
Exp(Hb,c)	5.226	n.s.
Foram-AMBI	12.707**	MD > S, P

Table 3
Values of the tested indices calculated at the sampling sites. The colours correspond to the EcoQS (Red = Bad; Orange = Poor; Yellow = Moderate; Green = Good; Blue = High) according to the most accepted thresholds (for details see the review by O'Brien et al. (2021)).



Sample	FAI (%)	s	d	ES(100)	H'(log2)	$\text{Exp}(H'_{bc})$	Foram-AMBI
S1-1	1.2	12	1.68	10.8	2.6	6.2	1.5
S2-1	1.3	5	0.50	4.5	1.5	2.7	0.2
S3-1	0.9	3	0.26	2.9	1.1	2.2	0.0
S5-1	0.0	10	1.50	9.2	2.7	6.5	1.2
P1-1	1.9	13	1.76	10.5	2.4	5.2	2.3
P2-1	3.0	27	3.22	17.8	3.6	12.2	1.9
P3-1	0.8	16	2.13	14.7	3.3	9.7	2.4
P4-1	1.4	19	2.29	13.3	3.2	9.0	1.8
P5-1	0.0	6	0.94	6.0	2.2	4.7	1.0
S1-2	0.0	10	1.61	9.8	2.9	7.4	1.3
S2-2	1.6	5	0.51	4.1	1.3	2.4	0.1
S3-2	1.4	5	0.52	4.2	1.3	2.4	0.1
S5-2	1.6	9	1.27	8.9	2.6	6.2	1.3
P1-2	1.4	11	1.46	8.6	2.4	5.3	2.7
P2-2	1.2	25	3.18	17.1	3.5	11.0	1.7
P3-2	1.3	16	2.17	12.9	3.0	8.0	2.4
P4-2	0.5	19	2.27	13.1	3.2	8.9	1.5
P5-2	0.0	8	1.11	7.4	2.3	4.9	1.1
MD1	0.0	12	1.65	11.1	3.1	8.4	2.7
MD2	0.0	11	1.67	9.9	2.7	6.4	3.2
MD3	0.0	6	0.90	6.0	2.0	3.9	3.3
MD4	0.0	8	1.13	7.9	2.4	5.3	2.9
MD5	2.6	3	0.39	3.0	1.3	2.4	3.2

observed in the sediments characterized by higher concentrations of Cr, Cu, Pb, Ni and Zn. The occurrence of tests with abnormalities, indicated by FAI index, was not correlated with any of the tested abiotic variables. On the contrary, the negative and significant correlations between As and all the indices based on biodiversity indicated that the most biodiverse assemblages inhabited the sediments with low levels of this particular metal. Finally, higher values of Foram-AMBI, which correspond to assemblages dominated by opportunistic species, co-occurred with higher concentrations of Cr and Zn.

The DISTLM routine performed on foraminifera species indicated that sand, As, Pb and in particular Cr and Ni were the abiotic variables that significantly shaped the assemblage (Table 5).

In the RDA performed on foraminifera indices, two principal components were identified and together explained 55.5 % of the total variance (Fig. 3). The percentage of sand and As concentrations were the predominant elements of the first factor (RDA1 loading = 0.57 and 0.82, respectively), whereas the major contributors of the second factor were Cr and Ni (RDA2 loading = −0.70 and −0.56, respectively). In this constrained analysis, Podstrana stations (except P5) were grouped together nearby all the diversity indices due to the higher diversity that characterized these stations. Conversely, P5 differed from the other sites of this marina because of its lower total abundance during both campaigns. Marina Dorica stations, S1 and S5 were located nearby most of the metals due to the more elevated concentrations at these stations. The vicinity of Foram-AMBI is in accordance with the fact that higher values of this index correspond to an assemblage dominated by opportunistic species rather than by sensitive ones. Finally, S2 and S3 were gathered together away from the other stations due to the higher percentages of sand and the concomitant lower values of both Foram-AMBI and diversity indices.

4. Discussion

In the present study the suitability of benthic foraminifera for the monitoring of small marinas was investigated in three tourist ports located on the two opposite sides of the Adriatic Sea: Špinut and Podstrana in Croatia, Marina Dorica in Italy. Five stations were sampled in each marina according to the following criteria: at least one station was located nearby the boathouse area (S1, S4, P2, MD5), one nearby the main port entrance (S3, P5 and MD1) while the remaining sampling sites were positioned as much as possible along a confinement gradient.

The analysis of 8 metals as well as of TOC and grain-size provided a comprehensive screening of the environmental conditions in each

Table 5
DistLM outputs carried out on foraminifera assemblage structure. P = significant value; SS = sum of square. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; n.s. = not significant.

Variable	SS(trace)	Pseudo-F	P
Sand	5149.6	2.8577	*
TOC	4087.5	2.2064	n.s.
As	4616.3	2.5262	*
Cd	3832.6	2.0554	n.s.
Cr	11,344	7.5273	***
Cu	2426.2	1.256	n.s.
Pb	4029.6	2.1719	*
Ni	10,210	6.5408	***
Sn	2780	1.4518	n.s.
Zn	2382.8	1.2322	n.s.

Table 4
Spearman correlation outputs between the environmental variables and the tested indices (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; n.s. = not significant).

	N	FAI	s	d	ES(100)	H′(log2)	Exp(H′bc)	Foram-AMBI
Sand	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
TOC	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
As	n.s.	n.s.	**−0.604****	**−0.569****	**−0.532****	**−0.459***	**−0.458***	n.s.
Cd	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Cr	**−0.685*****	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**0.554****
Cu	**−0.588****	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Pb	**−0.544****	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Ni	**−0.536****	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Sn	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Zn	**−0.660*****	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**0.451***

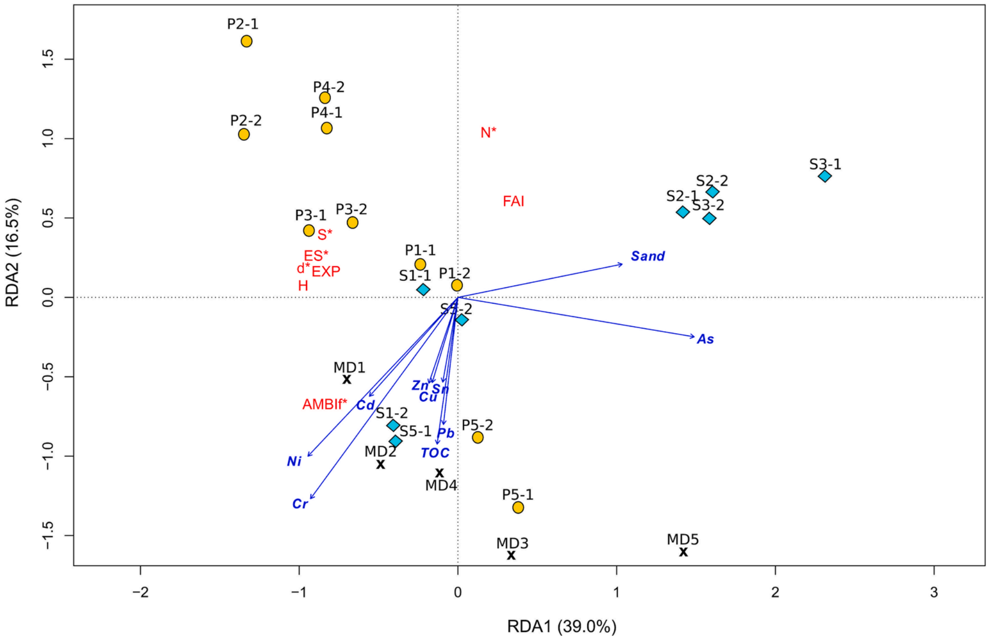


Fig. 3. Redundancy analysis (RDA) performed on foraminifera indices in relation to the environmental variables (Table 1) at all stations. The asterisks indicate the response variables that significantly differed among marinas (Kruskall-Wallis ANOVA, see Table 2). P = Podstrana; S = Špinut; MD = Marina Dorica; 1 = May 2019; 2 = April 2021.

marina. The high metal levels at the boathouse stations S1, MD5 and in particular S4 indicate the nearby careening activity as the most important impact in marinas. At these sampling sites, in fact, the concentrations of Cu, Pb, Zn and Sn were even two orders of magnitude higher than at the other stations (Table 1). The careening activity of leisure boats has been already reported as a source of metals for the marine environment (see Turner, 2010 for a review). Among the most used metals in current paintings, Cu and Zn tend to be dispersed in the environment as demonstrated by the composition of contemporary composites collected in a variety of EU locations, which is characterized by concentrations of up to 35 % and 15 % of these metals by weight. Also Ni and Pb have been observed in discarded antifouling residues in amounts well above those of ambient dusts and sediments, although the manufacturers are not obliged to specify the presence or function of these metals in their products when their concentrations are below 1 % by weight (Sandberg et al., 2007). Besides the residues of current antifouling products, composites with high levels of Sn derive from the removal of historic antifouling paints which formulations were enriched with triorganotin (Turner, 2010). Considering that these Sn-containing products were banned since 2003 in Europe (and since 2013 in Croatia), the high concentrations of Sn observed in the present study at S1, S4 and MD5 highlight the persistent nature of this kind of contamination.

The metal pollution caused by antifouling residues has been already reported in the Eastern Adriatic Sea (Popadić et al., 2013; Valković et al., 2007). The concentrations of the biocidal elements Cu, Zn, As and Pb (Valković et al., 2007) measured in Špinut, Podstrana and Marina Dorica were overall comparable or higher than those reported by Popadić et al. (2013) in the Bay of Bakar, although this latter semi-enclosed area is likely characterized by more severe anthropogenic pressures than the simple careening activity of leisure boats (i.e. bulk cargo terminal, an oil refinery plant, a dismissed coke industry). The confined position of the boathouse stations of Špinut and Marina Dorica as well as the sheltered shapes of these two marinas might favor the formation of localized metals hotspots as already described by Valković et al. (2007) in one of the oldest small marinas of Croatia, i.e. the Punat shipyard (Kravner Bay, Primorsko-goranska County). Although lower than the values measured at S1, S4 and MD5, the authors reported Cu and Zn concentrations at least one order of magnitude higher at the boathouse station of Punat marina (1934 and 2706 mg kg^{-1}, respectively) than at the other sampling sites, further confirming that the careening activities might cause severe local metal pollution.

Regarding the effects of these metals on the biota, at the boathouse stations the concentrations of Cu, Pb, Ni and Zn were above their respective Probable Effects Levels (PEL), i.e. the thresholds above which adverse effects on the biota are frequently expected (Sediment Quality Guidelines-SQGs, Buchman, 2008). Such adverse effects were indicated by the absence of living foraminifera at S4 during both campaigns. At this station the levels of metals were so elevated and persisted over time that the foraminifera were not able to survive. Similarly, Bergamin et al. (2003, 2005) reported barren samples in the Bagnoli Bay, a decommissioned industrial site characterized by high levels of contamination (Gulf of Naples, Tyrrhenian Sea). The authors ascribed the presence of barren sediments to the inability of foraminifera to cope with the detrimental effects exerted by Cu concentrations which were anyway lower than those observed at S4 (<850 mg kg^{-1}). For what concerns the other sampling sites of the present study, significantly lower abundances of foraminifera were observed where most of the metals showed higher concentrations (Table 4), in accordance with previous studies (Le Cadre and Debenay, 2006; Frontalini and Coccioni, 2008; Frontalini et al., 2009). Even the foraminifera species composition seemed to be affected by metals as shown by DISTLM outputs (Table 5). This routine indicated As, Pb and in particular Ni and Cr among the variables that significantly shaped the composition of the assemblage.

The three marinas were significantly different in terms of species composition as testified by the PERMANOVA outputs. Since the same dominant species were observed in all ports (Fig. 2), such difference relies mainly on their abundances that varied, even considerably, from marina to marina. In accordance with Frontalini and Coccioni (2008), overall, the observed biocenosis was largely dominated by infaunal taxa such as *A. tepida, A. parkinsoniana* and *E. scaber*. Recognized worldwide as a brackish-water species able to live in conditions of variable salinity and high concentrations of total organic matter (Melis et al., 2019 and references therein), in the present study, *A. tepida* specimens were more numerous mainly in Podstrana and Marina Dorica, i.e. the former influenced by the karstic Žrnovnica River and the latter characterized by the highest TOC values. In a culture study, Le Cadre and Debenay (2006) demonstrated the degree to which this species can tolerate metal pollution since specimens were able to survive at very high levels of Cu concentrations (up to 400 μg/L) for a long time (1 year), confirming the reputation of *A. tepida* as a species tolerant to this kind of contamination (Bergamin et al., 2009; Coccioni et al., 2009; Caruso et al., 2011). On the other hand, *A. parkinsoniana* was the dominant species at S3, the station nearby the entrance of Špinut, characterized by the overall lowest levels of metal contamination. This result is in accordance with previous findings since, as reviewed by Frontalini and Coccioni (2011), this organism prefers clean to moderately polluted environments and is very sensitive to trace metals, irrespective of the substratum type and percentage of organic matter.

In Podstrana, the presence of a significantly more structured and diverse foraminifera assemblage (Table 2) might be favored by the vegetation that characterizes the bottom of this marina as testified by the presence of submerged plants or algae in the grab during the sampling activities. The vegetation might contribute to increase the microhabitat complexity of Podstrana, resulting in additional resources and substrata suitable for species otherwise not observed in unvegetated sediments (Langer 1993; Mateu-Vicens et al. 2014).

Focusing on foraminifera indices, in the present study FAI was not particularly informative. Although higher percentages of abnormal tests have been clearly observed in foraminifera exposed to high concentrations of trace metals such as Cu (e.g. Le Cadre and Debenay, 2006; Frontalini and Coccioni, 2012), in the three marinas low FAI values were calculated at all stations (<3%) and no significant difference was detected among marinas. According to Geslin (1999), foraminifera are subjected to conditions of environmental stress only if the abnormal tests represent more than 3 % of the whole assemblage. In the present study this threshold was reached only at P2 during the first sampling campaign (Table 3) while at stations characterized by high levels of metal pollution (e.g. S1 and most of Marina Dorica sampling sites), no abnormal tests were observed. The significant positive correlation between FAI and foraminifera total abundance ($R = 0.464$, $p < 0.05$) suggests that in the present study the abnormalities were mainly associated to the higher probability to occur in a numerous population. Le Cadre and Debenay (2006) documented that one of the main responses of foraminifera to metal pollution is an increased delay in the reproduction rates that leads consequently to scarcely numerous assemblages. In Marina Dorica, the high concentrations of Ni, Cu and Zn likely limit foraminifera proliferation, resulting in a poorly numerous assemblage, in which the juveniles with abnormalities have a low probability to develop and survive. In accordance with Le Cadre and Debenay (2006), a cautious interpretation of FAI results is therefore recommended. On the other hand, this metrics demonstrated to be useful in catching the response of sensitive species to the detrimental effects of chemical pollution. More than 60 % of the abnormal tests, in fact, belonged to the species *A. parkinsoniana*, confirming the sensitivity of this taxon to environmental stress.

The diversity indices considered in the present study -d, ES(100), H' and Exp(H'_{bc})- provided similar results to those emerged from the RDA output (Fig. 3), although significant differences among marinas were not detected for all indices (Table 2). A more diverse and structured assemblage characterized Podstrana, likely due to the concomitant presence of intermediate metal levels and of the submerged vegetation that represents an additional substratum for further foraminifera species as discussed above. The higher values of Foram-AMBI, which indicate an assemblage dominated by opportunistic species, were calculated at the most polluted sites, in particular to those characterized by higher concentrations of Cr and Zn. Unexpectedly, the station with the lowest metal levels (S3) was characterized also by the least biodiverse assemblage. Notwithstanding, among the few observed species, at this site the dominant one was the sole species encountered in the present study that is recognized as sensitive, i.e. *A. parkinsoniana*. Consequently, while the diversity indices provided very low values, Foram-AMBI outputs indicated the presence of an assemblage not exclusively composed of opportunistic taxa. These results suggest a complementarity among diversity indices and Foram-AMBI that should be taken into account for an accurate EcoQS assessment of tourist marinas.

5. Conclusions

The careening activity of leisure boats can be an important source of chemical contamination in tourist marinas because the residues of current and past antifouling products enter the marine environment over time determining local hotspots of metals. The capacity of the marina, its shape as well as the location of the boathouse area concur to the degree of antifouling residues accumulation. Foraminifera demonstrated to be suitable bioindicators for an effective monitoring of tourist marinas because they respond to metal contamination in terms of variable species composition and abundance. Azoic sediments were observed with very high levels of metals, indicating that the foraminifera were not able to cope with the detrimental effects exerted by these major contamination levels. The comparison between diversity indices and Foram-AMBI revealed a complementarity between these two categories of metrics in contributing to the achievement of a reliable EcoQS assessment. In light of the main results obtained in the present study, some simple recommendations are provided for the monitoring of tourist marinas:

- Sampling stations should comprise the boathouse area, the port entrance, and a sufficient number of sites in between
- The analysis of metals contained in current and past antifouling products is sufficient to detect the contamination ascribable to the careening activity (i.e. Sn, Cr, Zn, Ni, Pb, Cu, Cd, As)
- Both the Foram-AMBI and one diversity index should be calculated because of their complementarity of the EcoQS assessment. Among the diversity indices we suggest to apply Exp(H'_{bc}) because it is more suitable for small samples and because it tends to provide slightly lower EcoQS scores in accordance with the precautionary attitude that should be maintained in environmental monitoring.

CRediT authorship contribution statement

A. Franzo: Writing – original draft, Formal analysis. **M. Caffau:** Investigation, Resources, Data curation. **F. Nasi:** Investigation, Formal analysis. **E. Marrocchino:** Investigation, Resources, Data curation. **M.G. Paletta:** Investigation, Resources, Data curation. **M. Bazzaro:** Investigation, Resources, Data curation. **T. Cibic:** Investigation, Methodology, Validation, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2023.110006.

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