Exploring the underwater life in transitional environments: Benthic foraminifera, ostracods, and dinoflagellate cysts – Biotic trends and EcoQS assessment in the Mar Piccolo of Taranto (Ionian sea, southern Italy)

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A B S T R A C T

Coastal areas have historically thrived as centers of human activities due to their resources, economic opportunities, and natural allure. The rapid growth of coastal populations has however brought forth a multitude of challenges to tackle, with pollution emerging as a significant and far-reaching issue. Our study focuses on the Mar Piccolo of Taranto (Ionian Sea, Southern Italy), a lagoon-like coastal basin (separated in two sub-basins) that, since decades, has been heavily affected by human activities and aquaculture, leading to environmental deterioration. Although past studies have looked at environmental conditions in the Mar Piccolo from a chemical perspective, the biological component (e.g., biological indicators) has been mostly neglected. In this study, we firstly aim to examine the distribution and diversity of foraminifera, ostracods, and dinoflagellate cysts in December 2016 and compare our findings with data collected in December 2011. Foraminiferal and ostracod communities exhibit similar patterns in the two sampling campaigns, while the communities of encysted dinoflagellates show differences concerning both densities and diversity. Then, we evaluate the Ecological Quality Status (EcoQS) using ecological indices. While the indices in the inner basin appear to reflect an actual ecological degradation, they yield conflicting results in the outer basin. In the outer basin, indeed, the indices overestimate the EcoQS. This study highlights the potential of these indices for characterizing the EcoQS but emphasizes the need for improvements in their reliability. This research also contributes to a more holistic understanding of environmental condition in the Mar Piccolo and underscores the importance of integrating biological quality elements into ecosystem management and remediation strategies.

1. Introduction

Coastal areas have been historically served as centers of human activities and host large populations due to their abundant resources, economic opportunities, and natural attractions. However, the rapid demographic increase in coastal regions has brought about a multitude of challenges, the most impactful of which is chemical pollution (e.g., metal and hydrocarbons pollution, municipal waste, plastic pollution) (e.g., Chen and Liao, 2006; Thompson et al., 2004; Wang et al., 2013) together with biological pollution (e.g., Elliott, 2003). In both cases, pollution in coastal areas has led to devastating cascade effects on marine life, including the destruction of habitats, decline in biodiversity, and harmful algal blooms among others (e.g., Barrenechea Angeles et al., 2023; Diaz and Rosenberg, 2008; Sellner et al., 2003). Toxic contaminants can be accumulated in coastal sediments and pose a severe ecological threat to marine biota. In addition, the bioaccumulation of

substances by tiny marine organisms, at the base of the food chain, represents a potential risk to human health (e.g., Pandion et al., 2022; Smith et al., 2018; Zhao et al., 2018). Therefore, the complex interplay between population growth, industrialization, and pollution requires immediate attention and concerted efforts from government agencies, local communities, and individuals. Addressing these challenges requires a comprehensive and multi-disciplinary approach (i.e., sedimentological, chemical and biological) that has proven to be effective in identifying environmental features in coastal areas (e.g., Armynot du Châtelet et al., 2016; Ferraro et al., 2017; Francescangeli et al., 2020; Giordano et al., 2019).

Before planning remediation strategies of marine ecosystems, baseline surveys and monitoring studies are required to assess the ecological conditions. For this purpose, marine legislations (e.g., the Water Framework Directive - WFD in Europe) over the world have highlighted the importance of including biological quality elements (BQE) such as

zooplankton, benthic invertebrates, seagrasses, fishes, corals and marine mammals to assess the Ecological Quality Status (EcoQS) of marine ecosystems (e.g., Cabral et al., 2012; Krause-Jensen et al., 2005; Muxika et al., 2005; Williams et al., 2023). Amongst these groups, benthic macrofauna (i.e., organisms retained by a 1 mm-mesh sieve) is the most widely used BQE to detect the EcoQS (e.g., Blanchet et al., 2008; Bouchet and Sauriau, 2008; Dauvin, 2018). However, meiofauna (i.e., organisms <1 mm), such as foraminifera and ostracods together with merobenthos, i.e., the dormant stages (cysts) of planktonic organisms (Marcus and Boero, 1998), may represent a promising complementary-to-alternative tool to evaluate and monitor the environmental quality of coastal environments (e.g., Al-Enezi et al., 2022; Cavaliere et al., 2021; Liu et al., 2012; Ruiz et al., 2006).

Benthic foraminifera are protozoa with a widespread distribution from transitional to deep-sea environments and have been widely used as bioindicators of human-derived activities (e.g., Francescangeli et al., 2016; Martins et al., 2016). Their distribution is controlled by a wide range of variables and ecological factors (e.g., Bunzel et al., 2023; Francescangeli et al., 2021; Martins et al., 2015; Rostami et al., 2023). More recently, a set of ecological indices based on benthic foraminifera (e.g., Foram-AMBI, expH'$_{bc}$, Foraminiferal Stress Index) has been successfully developed and applied for the EcoQS evaluation (Bouchet et al., 2012, 2021; Dimiza et al., 2016; Jorissen et al., 2018).

Ostracods are aquatic microcrustaceans very sensitive to changes of environmental conditions (Cronin and Vann, 2003; Montenegro and Pugliese, 1996; Pascual et al., 2008; Ruiz et al., 2004), and have been used as environmental bioindicators to natural or anthropogenic stressors (e.g., Bodergat et al., 1998; Frenzel and Boomer, 2005; Ruiz et al., 2004). The analysis of the ostracod fauna is useful for environmental characterization and for estimating anthropic impact of aquatic coastal systems and is, therefore, a helpful indicator of water quality (Ruiz et al., 2005). Stressed conditions, such as significant pollution, are unfavorable factors for the development of ostracod fauna (Ruiz et al., 2004). The response of ostracod to natural or anthropogenic environmental stress can be recorded through biotic indices (e.g., species richness, the index of Shannon, total abundance), in the composition of ostracod assemblages and in the chemical composition of their carapace (Ruiz et al., 2005). These organisms have a bivalve calcified carapace that can be easily fossilized and used, therefore, in paleoenvironmental reconstructions and changes (e.g., Anadón et al., 2002; Marco-Barba et al., 2013; Ruiz et al., 2004).

In shallow confined environments, the resting stages produced by planktonic organisms tend to accumulate in bottom sediments, so that the structure of encysted communities' mirrors that of the active/motile ones in the water column (e.g., Belmonte and Rubino, 2019), at least for the species that produce resting stages. Cyst assemblages, built up in the sediments through time, can respond to anthropogenic changes of the water quality in such environments, related to chemical pollution and eutrophication (e.g., Dale, 2009; Pospelova et al., 2002).

The Mar Piccolo (MP) of Taranto (Southern Italy) is a lagoon-like coastal basin in connection to the Mar Grande through two narrow channels. Many anthropogenic impacts, including the presence of a historically important naval base, shipyards and mussel cultures, fishing aquaculture plants and, not at least, the urban settlements along the banks, have been responsible for environmental damages (Cardellicchio et al., 2016a). Consequently, this area has been enlisted by the Italian Ministry of the Environment as Site of National Interest for environmental remediation and restoration (National Law n. 426, 1998). On the other side, due to the huge biodiversity and its unique ecological features, the MP is one of the Long-Term Ecosystem Research sites (Morabito et al., 2018). The long-lasting discharge of industrial and urban wastes and toxic substances has produced significant environmental issues in the MP. For instance, the historical production of mussels, *Mytilus galloprovincialis*, in the area has been lowered in the recent years, reducing the spatial distribution of the plants and regulating the rearing of the different life stages of the mussels between the two sub-basins of

the MP (Di Leo et al., 2014; Giordano et al., 2019). Over the last two decades, many studies have been carried out on the MP to investigate the environmental conditions, both under a geochemical and ecological perspective (for reviews Cardellicchio et al., 2016a; Cardellicchio et al., 2016b). The composition and distribution of benthic communities including active and resting microphyto-benthos (Rubino et al., 2016), and macrofauna (Franzo et al., 2016) have been previously reported in the MP. Furthermore, Ferraro et al. (2017) investigated the holobenthic (foraminifera and ostracods) and merobenthic (dinoflagellate cysts) assemblages identifying the complexity of ecological patterns in the two sub-basins of the MP. Nevertheless, only Franzo et al. (2018) have applied biological approaches to evaluate the *in situ* EcoQS, using nematode-based indices.

In continuity to the work of Ferraro et al. (2017), the present study, carried out in December 2016, aims to: 1) investigate the distribution and diversity of foraminifera, ostracods and dinoflagellate cysts in the MP and document the changes after the first survey in 2011; 2) evaluate the EcoQS by using biological-based proxies.

2. Study area

The MP is a shallow, nearly enclosed marine basin located north of the town of Taranto in southern Italy (Fig. 1A). It is divided by a promontory into two sub-basins, an outer basin (MPI) and an inner one (MPII), with maximum depths of 13 and 10 m and a surface area of 8.28 km² and 12.43 km², respectively (Fig. 1B). The MPI is directly connected with the larger embayment of the Mar Grande, whereas the MPII represents the most confined part of a marine system comprising also the Mar Grande and the open area of the Gulf of Taranto (Belmonte et al., 2013).

The MP is characterized by a scarce hydrodynamic regime and low water exchange since it is connected to the Mar Grande by only two narrow channels in the MPI. The resultant hydrodynamics exhibit low intensity, leading to pronounced water stratification (Petronio et al., 2012). Despite this, density gradients are significant enough to induce a persistent baroclinic estuarine circulation. On an average yearly timescale, this circulation involves the inflow of cold, salty water at the bottom layer, while less salty and warmer water moves outward along the surface (Pascalis et al., 2016). Water renewal times of 25 and 36 days have been measured in the MPI and MPII, respectively (Umgiesser et al., 2014).

Water salinity and temperature in the two sub-basins are also influenced by seasonal freshwater flows from small surface watercourses and submarine springs (locally called *citri*), located in the north of the two basins. The sediment load of these basins arises mainly from land runoff, several submarine springs, small streams, sewage outfalls and industrial discharges rather than that provided by marine currents (Cibic et al., 2016).

Since 1960s, Taranto and its coastline have been subjected to an intense industrialization process that has triggered deep environmental changes. The industrial zone hosts the largest steelworks in Europe and navy arsenal in Italy, a major oil refinery, shipbuilding and other human activities. As a consequence, the MP is one of the most polluted areas in the Mediterranean Sea (Cardellicchio et al., 2016b; Spada et al., 2012). In the MPI, the main pollutants are trace metals (e.g., Cd, Cu, Pb, Hg and Zn) and organic pollutants (e.g., polycyclic aromatic hydrocarbons - PAHs, polychlorinated biphenyls - PCBs, polychlorinated dibenzo-p-dioxins) with concentrations commonly exceeding permissible thresholds (e.g., Annicchiarico et al., 2007; Calace et al., 2008; Cardellicchio et al., 2007; Costa et al., 2016; Franzo et al., 2018; Spada et al., 2012). In MPII, the presence of contaminants is less severe. Indeed, Bellucci et al. (2016) documented the highest levels of Cu, Hg and Pb in the MPI compared to MPII. Copper had maximum values around 230 (mg kg^{-1}) in MPI compared to values of about 60 (mg kg^{-1}) in the MPII. Lead showed maximum values of around 130 (mg kg^{-1}) in MPI and 40 (mg kg^{-1}) in MPII. The same trend applies to Hg, with
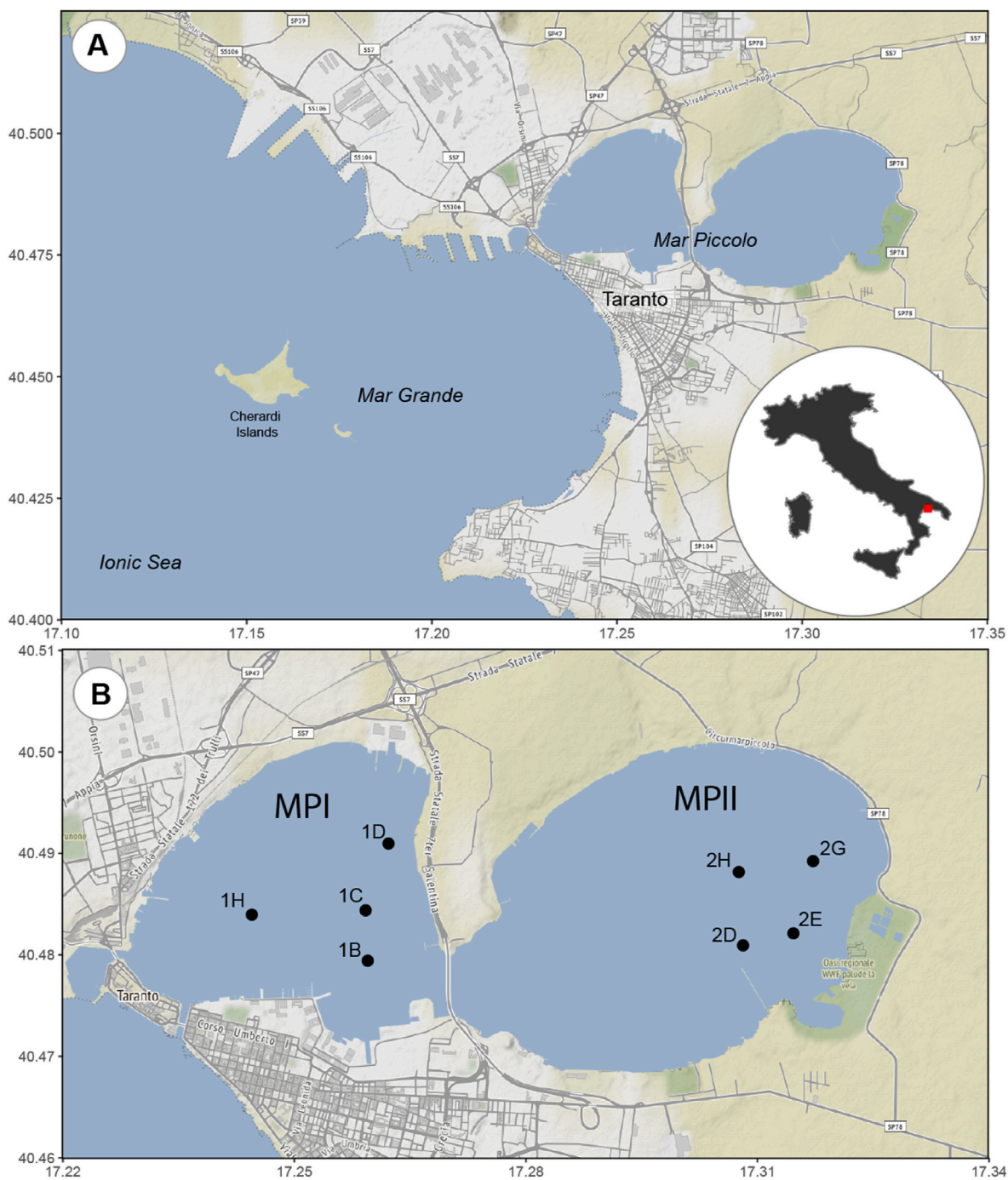
Fig. 1. A) Position of Taranto marine area; B) Localization of the sampling stations within the Mar Piccolo sub-basins (MPI and MPII). The maps outline was drawn by using the R software ("ggmap" package). Geographic coordinates are referred to World Geodetic System 1984 (WGS-84).

maximum values of 9 (mg kg^{-1}) in MPI compared to values of 3 (mg kg^{-1}) in MPII. Cibic et al. (2016) also reported higher PCBs values (Max = 1159 ng g^{-1}) in MPI compared to maximum values of 46 (ng g^{-1}) in MPII.

The MP is also one of the most important mussel farming in Italy, (ca. 30,000 t year−1) over a total legal farm area of ca. 10 km², which is mostly localized in the MPII (Bellucci et al., 2016; Caroppo et al., 2012). The high organic load deriving by the presence of mussels, together with the large amount of sewage conveyed by small rivers and channels from the nearby small towns, produces a generalized impact, such that the MPII suffers from dystrophic events, mainly in late summer when the surface temperature regularly exceeds 30 °C.

3. Material and methods

3.1. Sampling

Surface sediment samples were collected on 10th December 2016 aboard the m/b "Attilio Cerruti" operated by CNR-IRSA of Taranto at eight stations, selected from the dataset of the 1st December 2011 (Ferraro et al., 2017) as representative of the two sub-basins of the MP (Fig. 1B).

At each station, the main physic-chemical variables of the seawater (temperature, salinity, dissolved oxygen, pH and photosynthetically active radiation - PAR) were measured along the water column by means

of a multiparametric probe (Idromar IP050D, Genoa, Italy) at the bottom and at the surface of the water column.

For biotic analyses, three replicated samples were collected by SCUBA divers for each biological groups, namely benthic foraminifera, ostracods, and cysts, and stored in an icebox. For the study of benthic foraminifera and ostracods, samples were immediately fixed with Rose Bengal (ethyl alcohol solution, 2 g in 1000 ml of ethyl alcohol), to differentiate between living and dead specimens, according to Walton (1952). For the study of cysts, three replicates of the uppermost layers (top 2 cm) of undisturbed sediment were collected at each sampling site and stored at 4 °C without preservatives.

3.2. Benthic foraminifera and ostracods

Once in the laboratory, the sediment samples for benthic foraminifera and ostracods were dried at 50 °C, weighted, gently washed with tap water on a 63 μm sieve to remove clay, silt, and the dye excess. This residual fraction was then re-dried at 50 °C and weighed. Quantitative analyses of benthic foraminifera and ostracods were carried out on the fraction >63 μm. When possible, 300 specimens, were picked for each sample. For benthic foraminiferal analysis, the methodological recommendations of the FOBIMO protocol were followed (Schönfeld et al., 2012). Moreover, as living, or recently died specimens of ostracods, were considered only those intact, stained or individuals with soft parts.

The taxonomical identification followed the generic assignment of Loeblich and Tappan (1987), and at the species level, the contributions of Cimerman and Langer (1991), and Sgarrella and Sgarrella and Monchamont Zei, 1993 for benthic foraminifera. Ostracod species have been identified according to scientific literature (e.g., Bonaduce et al., 1976; Meisch, 2000). For benthic foraminifera, the different morphotypes of *Ammonia tepida* were lumped into *Ammonia tepida* group.

3.3. Dinoflagellate cysts

For the analysis of cysts, the sieving method described in Montresor et al. (2010) was adopted on subsamples of 2–3 ml of wet sediment for each replicate. These wet amounts were weighed and screened through a 20-μm mesh (Endecotts Limited steel sieves, ISO3310-1, London, England) using natural filtered (0.45 μm) seawater. The obtained fraction was ultrasonicated (Branson-Emerson CP102, Danbury, CT, USA) for 1 min and screened again through a sieve battery (200, 75 and 20 μm mesh sizes). The resulting fine-grained fraction (20–75 μm) contained mainly protistan cysts, while the 75–200 μm fraction contained also resting stages of larger taxa (e.g. *Polykrikos*) together with spiny (e.g., *Lingulodinium*) cysts that could remain stuck in the larger mesh. Both fractions were suspended in a small amount of filtered seawater and stored at 4 °C in the dark until observation. The material retained by the 200-μm sieve was discarded. No chemicals were used to dissolve sediment particles to preserve calcareous and siliceous cyst walls.

Qualitative and quantitative analyses were carried out under a light microscopy inverted microscope (Zeiss Axiovert 100 – Carl Zeiss, Oberkochen, Germany) equipped with a Leica MIC 170HD (Leica microsystems, Wetzlar, Germany) digital camera, at × 200 (75–200 μm fraction) and × 320–400 (fine-grained fraction) final magnifications. Only full cysts with cytoplasmic content (i.e., presumably viable) were enumerated to obtain a data set more easily comparable to that of 2011; for the fine-grained fraction 200 cysts were counted at least for each replicate to obtain abundance values as homogeneous as possible and to evaluate rare species too. The 75–200 μm fractions were entirely examined. An aliquot of each sample (≈5 ml of wet sediment) was weighed and dried overnight at 70 °C to estimate the water content of the sediments, so, quantitative data are presented as cysts g^{-1} of dry sediment (cysts g^{-1}) (average ± SD).

The abundance of cysts (A) in each replicate was calculated as follows:

$$A = (N * F) / DW$$

N: sum of the cysts counted in the aliquots; F: fraction of the sub-sample observed to obtain a count of 200 full cysts at least; DW: dry weight of the subsample in grams.

The resting stage morphotypes were identified based on the following keys/published descriptions: Mudie et al. (2017) – Appendix B and, Modern Dinocyst Key Website (https://www.marum.de/en/Karin-Zonneveld/Modern-Dinocyst-Key.html; Zonneveld et al., 2007) and previous germination experiments (Rubino et al., 2017). Identification was performed to the species level when possible. As a rule, the modern biological names were used. For dinoflagellate cyst morphotypes whose motile stage is unknown, the paleontological (fossil) names were also considered.

3.4. Data analyses

Before analyses, due to the low densities of foraminifera and ostracods, the data from three replicates were pooled as number of living specimens. Differently, replicates for cysts were averaged. At each sample and for each group (i.e., benthic foraminifera, ostracods, and cysts), the density (individuals g^{-1}), the species richness (S), the index of Shannon biased-corrected H'$_{bc}$ (Chao and Shen, 2003) and relative abundance were calculated. Additionally, a non-metric multidimensional scaling (NMDS) was performed on relative abundance's matrix. A Krustal-Wallis non-parametric test was used to assess significant differences amongst the faunal parameters between MPI and MPII. The NMDS was meant to compare the distributions of benthic foraminifera, ostracods, and cysts in 2016 with the ones in 2011 (Ferraro et al., 2017). We used NMDS with two dimensions and Bray-Curtis distance as similarity measure. The stress was minimized using a monotone regression. Due to the low number of individuals, the samples 2D, 2H and 2G for ostracods and 2G for foraminifera, from 2011 dataset, were removed from the analysis.

The R software (R-Core-Team, 2014) and the R package "dplyr" was used to perform the Kruskal-Wallis test (Wickham et al., 2016). R packages "*entropy*" (Hausser and Strimmer, 2014) and "*vegan*" (Oksanen et al., 2016) were used for diversity measures and NMDS analysis, respectively. Chords diagrams were used to visualize the taxonomic compositions of foraminifera, ostracods and dinoflagellate cyst data using the package "*circlize*" (Gu et al., 2014).

3.5. Biotic indices

To evaluate the EcoQS, the Foram-AMBI and the exp(H'$_{bc}$) were calculated on 2011 and 2016 data. The Foram-AMBI is an adaptation to benthic foraminifera of the macrofauna-based AMBI (Borja et al., 2000), where foraminiferal species are classified into five ecological groups (EGs) in relation to their response to organic matter enrichment. The EGI contains "Sensitive species" to organic enrichment, and mainly occur in natural, oligotrophic, unpolluted ecosystems. The EGII concerns "Indifferent species" that are indifferent to the first stages of organic enrichment but disappear in case of strongly increased of organic loads. The EGII is made up of "Third-order opportunists", which are species that are initially found at reference site(s) under natural conditions. These species can tolerate the early stages of organic enrichment and tend to thrive in such conditions. The EGIV contains "Second-order opportunists" that are rarely seen (less than 2%) or absent in natural conditions at reference site(s). They become more abundant closer to sites with high organic enrichment. The EGV consists of "First-order opportunists". These species are not commonly found (less than 2%) at the reference site(s). Their numbers increase significantly closer to the source of organic enrichment. However, their highest abundance is closer to the site(s) with the most enrichment, unlike species in Group IV.

So far, three species lists with the species-specific ecological behaviors, have been published for different eco-regions: North-East Atlantic and Arctic fjords (Alve et al., 2016), Mediterranean coastal environments (Jorissen et al., 2018) and European intertidal areas and transitional waters (Bouchet et al., 2021). As the two sub-basins of the Mar Piccolo have different characteristics, we have distinctly used the species list of Jorissen et al. (2018) (F-AMBI$_{Jor}$) and Bouchet et al. (2021) (F-AMBI$_{Bou}$) for the MPI and MPII, respectively. The class boundaries for the EcoQS (i.e., high, good, moderate, poor, and bad) were based on Parent et al. (2021a) (Table 1).

The H'$_{bc}$ is based on foraminifera, ostracods and dinocysts Shannon diversity biased-correct (Chao and Shen, 2003). The Shannon entropy was then exponentially transformed to the true diversity (details in Bouchet et al., 2012). For exp(H'$_{bc}$)$_{For}$ (benthic foraminifera), the class boundaries for the EcoQS were based on Bouchet et al. (2018) for Italian transitional waters (Table 1). Unfortunately, class boundaries for ostracods exp(H'$_{bc}$)$_{Ost}$ (ostracods) and exp(H'$_{bc}$)$_{cyst}$ (dinocysts) do not exist yet. A Spearman's correlation was computed on all biotic indices to verify whether they provided similar ecological quality trends, by using the R package "*PerformanceAnalytics*" (Peterson et al., 2014).

4. Results

4.1. Physico-chemical variables

Physico-chemical variables of water are presented in Table 2. The temperature ranged from 12.9 at the surface to 15.7 °C at the sea bottom (13.9–14.6 °C on average, respectively). The salinity varied from 37.3 at the surface to 39.6 at the sea bottom (37.4–38.6 on average, respectively). The dissolved oxygen ranged from 100.9 at the surface to 87.2% at the sea bottom (100.1–91.9 % on average, respectively). The pH did not vary along the column water and spanned between 8.8 and 8.9. The PAR varied from 5990 at surface and 50.8 μmol phot m^{-2} s^{-1} at the sea bottom (4411 - 205 μmol phot m^{-2} s^{-1} on average, respectively). The bottom values of PAR were on average higher in MPII (81.65 μmol phot m^{-2} s^{-1}) than in MPI (328 μmol phot m^{-2} s^{-1}).

4.2. Benthic foraminifera

Sixty-two living benthic foraminiferal species belonging to 39 genera were identified in the two sub-basins (Table 1S). Although all stations recorded living benthic foraminifera after pooling the three replicates, a very low density (<2 individuals g^{-1}) was recorded at 1H. Overall, much higher densities (104 individuals g^{-1}, on average) were recorded in MPII than in MPI (6 individuals g^{-1}, on average) (Fig. 2A). The S was higher in MPI (39-18; 28.3 on average) than in the MPII (23 - 11; 15.5 on average) (Fig. 2B) and the same trend was observed for H'$_{bc}$ with an average value of 3.07 for MPI and 1.67 for MPII (Fig. 2C). The density, S and H'$_{bc}$ showed significant differences between the sub-basins (Kruskal-Wallis $p < 0.05$).

The living assemblages were mainly represented (>5%) by *Aubignyna perlucida* (14.4% of total abundance), *Ammonia tepida* group (13.1%), *Asterigerinata mamilla* (8.7%), *Ammonia parkinsoniana* (8.1%), *Bolivina striatula* (6.7%), *Haynesina germanica* (5.8%), and *Hopkinsina pacifica* (5.2%) (Fig. 3). These species were, however, unevenly

distributed between the two sub-basins. A higher relative abundance of *A. perlucida* (3.2 *vs.* 25.4%), *A. tepida* (4.5 *vs.* 21.8%), and *A. parkinsoniana* (1.9 *vs.* 14.4%) was found in the MPII. On the other hand, *A. mamilla* (17.4 *vs.* 0.0%), *B. striatula* (7.6 *vs.* 6.3%), *Textularia agglutinans* (5.6 vs 0.0%), and *Planorbulina mediterranensis* (5.2 vs 0.0%) were more abundant in the MPI.

4.3. Ostracods

A total of 21 ostracod species belonging to 16 genera were identified (Table S1). Although all stations recorded living ostracods after pooling the three replicates, a very low density (<2 valves g^{-1}) was recorded at 2H. The density was quite variable (1.6–117 valves g^{-1}) and resulted higher in MPII (79 valves g^{-1}, on average) than in MPI (26 valves g^{-1}, on average) (Fig. 2D). The S was higher in MPI (13-9; 12 on average) than in MPII (6-1; 3.75 on average) (Fig. 2E) as well as the diversity (H'$_{bc}$): 1.91-1.24; 1.49 on average in MPI and 1.23 - 0; 0.63 on average in MPII (Fig. 2F). The density did not show any significant variations (Kruskal-Wallis $p > 0.05$), while S and H'$_{bc}$ showed a significant variation between the sub-basins (Kruskal-Wallis $p < 0.05$).

The living ostracod fauna was mainly represented (>5%) by *Propontocypris pirifera* (43,4%), *Cyprideis torosa* (13.9%), *Leptocythere bacescoi* (10.9%), *Basslerites berchoni* (9.1%), and *Loxoconcha rhomboidea* (8.9%) (Fig. 3). The abundance of these species varied in the two sub-basins. *Propontocypris pirifera* was the most abundant species in both basins, even though this taxon showed lower values in MPII (52.1 *vs.* 32.4%). A higher relative abundance of *C. torosa* (0.6 *vs.* 27.2%), *L. bacescoi* (0.0 *vs.* 21.8%), and *B. berchoni* (0 *vs.* 18.2%) was found in the MPII. On the contrary, *L. rhomboidei* (17.8 *vs.* 0.1%), and *Semicytherura rarecostata* (5.1% *vs.* 0) showed higher abundance in MPI.

4.4. Cysts

The analysis revealed a high diversity with a total of 55 cyst morphotypes identified, corresponding to 51 taxa, due to the attribution of more types to *Pentapharsodinium tyrrhenicum* (2 types) and *Scrippsiella acuminata* (4 types) (Table S1). Most of the cyst morphotypes were identified at the genus level at least; one was identified as produced by species of the family Thoracospaeraceae and two remained as Dinophyta (Table S1). Dinocysts were found at all the sampling stations. Total densities of full (i.e., presumably viable) cysts ranged from 460 ± 57 cysts g^{-1} at station 2E to 1198 ± 171 cysts g^{-1} at station 1C (Fig. 2G). The values of S were similar in the two sub-basins, even though in the inner one higher variability was registered (MPI 23.2 ± 2.8 vs MPII 24.5 ± 4.8) (Fig. 2H), as well as S. In fact, the values of H'$_{bc}$ were 1.5 ± 0.09 in MPI and 1.4 ± 0.15 in MPII (Fig. 2I). The density, S and H'$_{bc}$ did not show significant differences between the sub-basins (Kruskal-Wallis p > 0.05).

The community of dinoflagellates encysted in the sediments was by far dominated by *S. acuminata* in both the sub-basins (Fig. 3). Four different cyst types were ascribed to this taxon, that were equally distributed in the whole study area. Its relative abundance of full cysts was comparable (63.7% and 65.5%) between the MPI and MPII. Other abundant species were *Scrippsiella erinaceus*, *Scrippsiella* sp. 1, and *P. tyrrhenicum*.

4.5. Ecological Quality Status

The evaluation of the EcoQS in the Mar Piccolo for 2011 and 2016 is reported in Table 3 and in Table S1. In 2011, the F-AMBI$_{Jor}$, ranged from 1.6 to 3.4. The assigned species were, on average, 85% for MPI and 98% for MPII. In MPI, 61% of assigned species belongs to EGIII, 28% to EGI, 8% EGII and 4% to EGIV. In MPII, 56% of assigned species belongs to EGIII, 24% to EGIV, 13% to EGI and 7% to EGII. The F-AMBI$_{Jor}$ indicated good EcoQS in MPI and moderate-poor EcoQS in MPII. The F-AMBI$_{Bou}$ ranged from 1.1 to 6.0. The assigned species were, on average, 71% for

Table 1
Class boundaries and relative EcoQS for Foram-AMBI (F-AMBI) based on Parent et al., (2021) and Exp(H'$_{bc}$) based on Bouchet et al. (2018).

F-AMBI	Eco-QS	Exp(H'$_{bc}$)	Eco-QS
0-1,4	High	>15	High
1,4-2,4	Good	15–11	Good
2,4-3,4	Moderate	11–7	Moderate
3,4-4,4	Poor	3–7	Poor
>4,4	Bad	<3	Bad

Table 2

Geographic position, water depth and physico-chemical features (temperature, salinity, dissolved oxygen, pH and photosyntetically active radiation - PAR), measured at the surface (s) and over the bottom (b) at the 8 stations investigated in the Mar Piccolo of Taranto.

| Station	Lat N	Long E	Depth (m)	T (°C)		Sal (psu)		O₂ %		pH		PAR (µmol phot m⁻² s⁻¹)	
				s	b	s	b	s	b	s	b	s	b
1B	40°28.782′	17°15.593′	12.7	13.9	15.6	37.7	39.6	100.2	87.2	8.8	8.8	4370	50.8
1C	40°29.054′	17°15.588′	13.9	15.3	15.6	37.4	39.5	100.3	95.5	8.8	8.8	5710	92.4
1D	40°29.501′	17°15.751′	8.6	14.8	15.7	37.6	39.1	100.4	91.1	8.8	8.8	4190	91.1
1H	40°29.069′	17°14.783′	12.9	14.4	15.4	37.3	39.4	100.9	92.3	8.8	8.9	4890	92.3
2D	40°28.863′	17°18.444′	7.5	12.9	13.8	37.6	37.9	99.6	89.4	8.9	8.8	5480	199
2E	40°28.893′	17°18.830′	3.9	13.1	12.9	37.7	37.9	100.1	94.8	8.9	8.9	5600	604
2G	40°29.400′	17°18.987′	6.2	13.5	13.4	37.3	37.7	99.6	91.3	8.8	8.9	3430	204
2H	40°29.328′	17°18.492′	7.9	13.5	14.0	37.5	38.2	99.4	93.9	8.9	8.9	5990	305
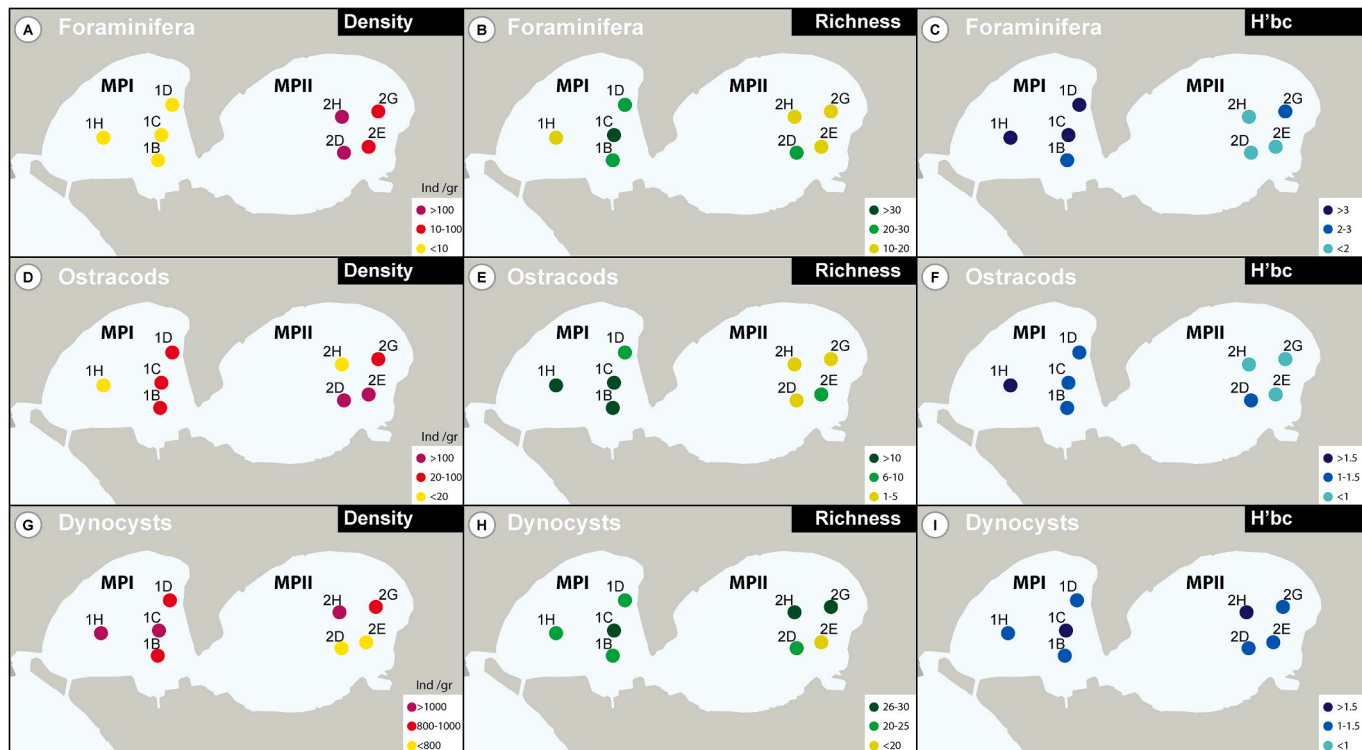
Fig. 2. Density (individuals/gr), richness, and diversity (H'bc) of benthic foraminifera (a–c), ostracods (d–f) and dinocysts (g–i) respectively in the Mar Piccolo sub-basins.

MPI and 85% for MPII. In MPI, 34% of assigned species belongs to EGI, 26% to EGII, 25% EGIII and 7% to EGV. In MPII, 70% of assigned species belongs to EGIII, 42% to EGV, 17% to EGI and 9% to EGII. The F-AMBI$_{Bou}$ indicated high-good EcoQS in MPI and moderate-bad EcoQS in MPII. The exp(H'$_{bc}$)$_{For}$ ranged from 5 to 23 providing High EcoQS in MPI and moderate-poor EcoQS in MPII. The exp(H'$_{bc}$)$_{Ost}$ ranged from 2 to 7 and exp(H'$_{bc}$)$_{cyst}$ from 6 to 19 (Table S1). For exp(H'$_{bc}$)$_{Ost}$ and exp(H'$_{bc}$)$_{cyst}$, values were higher in MPI.

In 2016, the F-AMBI$_{Jor}$, ranged from 1.5 to 3.0. The assigned species were, on average, 89% for MPI and 96% for MPII. In MPI, 48% of assigned species belongs to EGIII, 42% to EGI, 5% EGIV and 4% to EGII. In MPII, 54% of assigned species belongs to EGIII, 23% to EGIV, 16% to EGI and 7% to EGII. The F-AMBI$_{Jor}$ indicated good EcoQS in MPI and moderate EcoQS in MPII. The F-AMBI$_{Bou}$ ranged from 0.6 to 7.9. The assigned species were, on average, 76% for MPI and 92% for MPII. In MPI, 55% of assigned species belongs to EGI, 22% to EGIII, 11% EGV and 9% to EGII. In MPII, 68% of assigned species belongs to EGIII, 38% to EGV, 26% to EGI and 4% to EGII. The F-AMBI$_{Bou}$ indicated high-moderate EcoQs in MPI and moderate-bad EcoQS in MPII. The exp

(H'$_{bc}$)$_{For}$ ranged from 2 to 26 providing high-good EcoQS in MPI and moderate-bad EcoQS in MPII. The exp(H'$_{bc}$)$_{Ost}$ ranged from 1 to 7 and exp(H'$_{bc}$)$_{cyst}$ from 9 to 14 (Table S1). For exp(H'$_{bc}$)$_{Ost}$ and exp(H'$_{bc}$)$_{cyst}$, values were higher in MPI.

4.6. Statistical analysis

The Spearman's correlation among the ecological indices ranged between −0.15 and 0.88. All the indices showed a significant correlation between themselves (p-value <0.05) except for exp(H'$_{bc}$)$_{cyst}$ that did not show any significant correlation (Fig. 4).

For the three communities, the NMDS showing the comparison between sampling stations in 2011 and 2016 had stress values < 0.1. Two distinct clusters resulted in the NMDS plot for benthic foraminifera (stress value = 0.092), corresponding to samples collected from MPI (located on the left side of NMDS1) and samples collected from MPII (situated on the right side of NMDS1), apart from sample 2G collected in 2016 (situated on the right side of NMDS1) (Fig. 5A). A similar pattern was evident for ostracod communities (stress value = 0.097), where
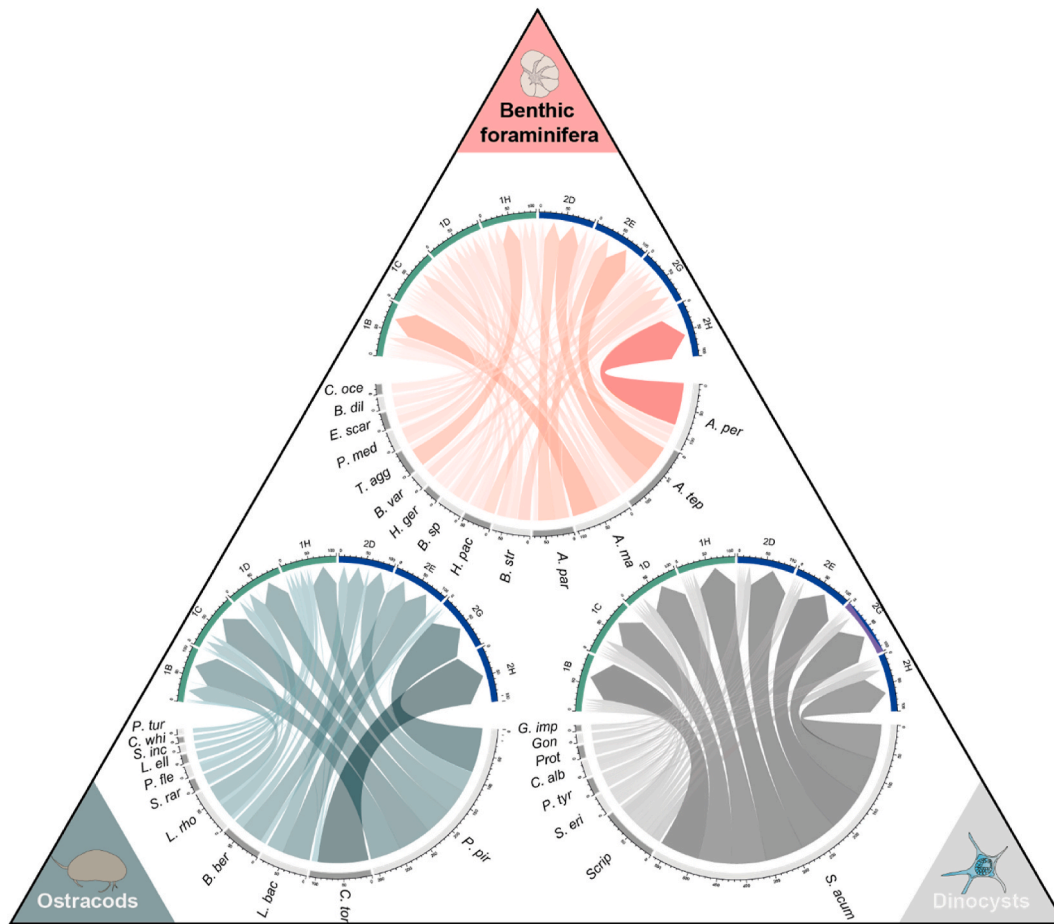
Fig. 3. Chord diagrams illustrating the main species distribution of benthic foraminifera, ostracods, and dinocysts in the Mar Piccolo sub-basins (MPI = 1B–H; MPII = 2D-H). The list of species' abbreviations is reported in Table 1S.

Table 3
EcoQS foraminiferal-based indices: F-AMBI$_{Jor}$ (Jorissen et al., 2018), F-AMBI$_{Bou}$ (Bouchet et al., 2021) and exp(H'$_{bc}$) (Bouchet et al., 2012). The EcoQS have been calculated at the 8 stations in Mar Piccolo for 2011 (Ferraro et al., 2017; in 2G missing values due to low foraminiferal abundances) and 2016 (present study).

2011				2016			
	F-AMBI$_{Jor}$	F-AMBI$_{Bou}$	exp(H'$_{bc}$)		F-AMBI$_{Jor}$	F-AMBI$_{Bou}$	exp(H'$_{bc}$)
1B	Good	Good	High	**1B**	Good	High	Good
1C	Good	Good	High	**1C**	Good	Moderate	High
1D	Good	High	High	**1D**	Good	Good	High
1H	Moderate	Good	High	**1H**	Good	High	High
2D	Moderate	Bad	Poor	**2D**	Moderate	Moderate	Poor
2E	Poor	Bad	Poor	**2E**	Moderate	Moderate	Moderate
2G				**2G**	Moderate	Poor	Moderate
2H	Moderate	Moderate	Moderate	**2H**	Moderate	Bad	Bad

samples collected from MPI were located on the left side of NMDS1, while samples from MPII fell on the right side of NMDS1 (Fig. 5B). For dinoflagellate cysts (stress value = 0.059), samples collected in 2016 clustered closely on the left side of NMDS1, while samples from 2011 were predominantly situated on the right side (Fig. 5C). However, samples from 2011 exhibited a more dispersed distribution in the ordination plot.

5. Discussion

5.1. Assemblages' comparison between 2011 and 2016

The comparison between benthic foraminifera and ostracod communities from 2011 to 2016 reveals similar trends in the two sub-basins in terms of faunal distribution and turnover, diversity, and densities. For both benthic foraminifera and ostracods, the NMDS shows dissimilarities and a clear separation between the faunal assemblages from the two sub-basins of the MP. As previously reported by several authors (Ferraro et al., 2017, and references thein), this separation is due to the different environmental conditions (e.g.., degree of confinement, hydrology, level of pollution) that constrains and differently shapes foraminiferal and ostracod communities in the two sub-basins. In fact, in MPII, foraminiferal species are typical of transitional environments with restricted circulation (such as *A. perlucida* and *Ammonia tepida* group) (e.g., Armynot du Châtelet et al., 2016). The euryhaline (following for the salinity classification the Venice Symposium System, 1958) ostracod species *C. torosa*, typical of transitional and aquatic continental environments (Mazzini et al., 2017) is well represented in MPII. On the
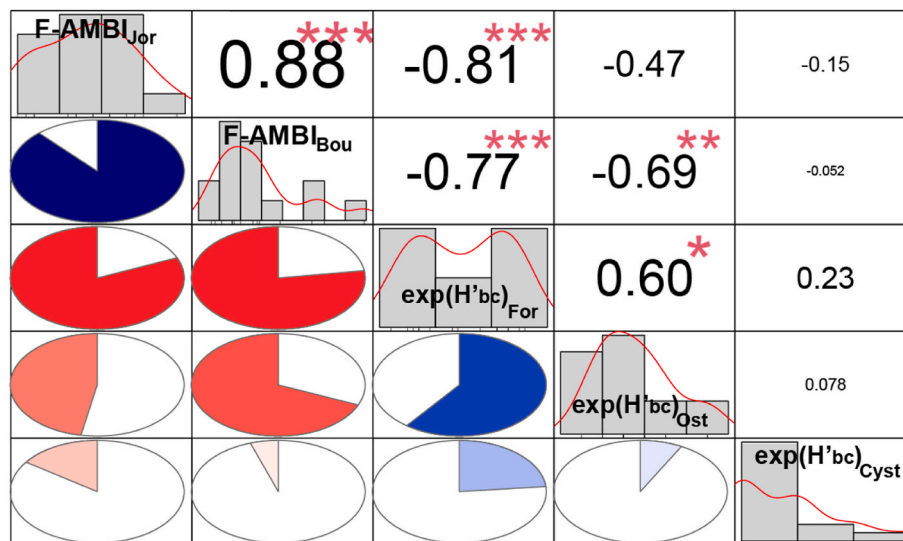
Fig. 4. Spearman's correlation between the biotic indices based on values from 2011 (Ferraro et al., 2017) and 2016 (present study). Significance levels are reported as stars: 0.001, 0.01, 0.05 <> "***", "**", "*". Blue = positive correlation; Red = negative correlation.

contrary, in MPI, foraminiferal and ostracod assemblages are dominated by taxa with a greater affinity to open marine conditions (such as *A. mamilla, T. agglutinans, P. mediterranensis, N. longevaginata, C. whitei,* and *L. rhomboidea*).

Although there may be differences in the composition of foraminifera and ostracods between 2011 and 2016, the NMDS analysis does not indicate any significant turnover in fauna between the two sampling years. On the same way, the diversity trends (i.e., higher values in MPI compared to MPII) and value ranges are quite similar for the two communities during the sampling periods (Table S1). The densities show an opposite trend (i.e., higher values in MPII and lower ones in MPI) and value ranges for the foraminiferal and ostracod assemblages from 2011 to 2016, although foraminiferal densities are, on overall, lower in 2016 (Table S1). Among the most abundant and frequent species of foraminifera, *T. agglutinans* and *H. germanica* have been found in higher abundance compared to Ferraro et al. (2017). *Textularia agglutinans* is a common shelf species able to span from very shallow habitats to few hundred meters (Arieli et al., 2011; Armynot du Châtelet et al., 2013; Merkado et al., 2015) and is found only in MPI. *Haynesina germanica* is widespread in marginal marine environments (Armynot du Châtelet et al., 2018b; Francescangeli et al., 2017) and is found in higher abundance in MPII. It is also worth mentioning that the abundance of *B. spathulata* significantly decreases in 2016 in MPI. Compared to the study of Ferraro et al. (2017), five additional ostracod species (*B. berchoni, L. elliptica, N. longevaginata, P. flexuosa* and *P. mediterraneum*) are found in low percentages. The marine species *N. longevaginata* and *P. flexuosa* are found exclusively in the MPI. The oligo-polyhaline species *L. elliptica* and euhaline *P. mediterraneum,* that also live in marine coastal environment associated with algae (Frenzel et al., 2010; Uzun, 2022), are recorded in the MPI. Instead, the poly-euhaline species *L. bacescoi,* characteristic of marine coastal settings (Mazzini et al., 2017) and *B. berchoni,* that lives in mixo–euhaline shallow water also with vegetated bottoms (Pugliese and Stanley, 1991), are uniquely observed in the MPII. In addition, compared to the first sampling, the euhaline brackish species *Leptocythere castanea* (Ruiz et al., 2006) and the euryhaline species *L. lagunae* (Montenegro and Pugliese, 1996; Ruiz et al., 2006) collected in the MPII, are not identified.

Concerning the community structure of dinocysts, the comparison of the two sampling (2011 and 2016) results clearly different, but in 2011 a higher segregation is apparent between the two sub-basins. This pattern is evident also considering the values of densities and diversity. In 2016,

the overall density results higher than in 2011 (873.4 $\pm$ 224.8 vs 513.9 $\pm$ 273.0 cysts g^{-1}), while the diversity (H') follows an opposite trend, i. e., was higher in 2011 (1.9 $\pm$ 0.3 vs 1.5 $\pm$ 0.1) (Table S1). These results are mainly due to a very low abundance of dinocysts in MPII in 2011 compared to 2016 (290.6 $\pm$ 0.3 vs 753.7 $\pm$ 0.2 cysts g^{-1}) with a higher diversity (H': 1.9 $\pm$ 0.3 in 2016 vs 1.4 $\pm$ 0.2 in 2016), confirmed by a higher value of equitability in 2011 (J' = 0.6 $\pm$ 0.1). As cysts are produced in the water column, the structure of their communities in the sediments might be potentially influenced by seasonality, different encystment rates in the two years, linked for instance to chemical contamination, but many other factors could play a major role, like sedimentation rates, horizontal transport, bioturbation, reworking of the sediments and degradation induced by the oxidative conditions in the sediments (Zonneveld et al., 2007). Most of them contribute to differentiate MPI from MPII; the hydrodynamic regime is very reduced in MPII and, consequently, the sedimentation rate is higher (more than 1 mm per year – ARPA, 2014) and the oxygen penetration in the sediment layers is very limited (Rubino et al., 2016), but other factors may be important, particularly in winter, when the sampling was carried out both in 2011 and 2016. The influence of the seasonality of planktonic communities in the water column is more difficult to evaluate because the sediments represent an archive of the cyst production through time. However, the studies of the phytoplanktonic communities in the Mar Piccolo reveal differences in the total abundance among seasons, but not significative differences between the two sub-basins (e.g., Caroppo et al., 2012), actually discovered through the analysis of encysted communities in the sediments.

5.2. Ecological quality assessment in the inner and outer basins of the Mar piccolo

The F-AMBI and the exp(H'$_{bc}$) are respectively sensitive- and diversity-based indices that have been successfully applied to evaluate the EcoQS in transitional and fully marine environments (e.g., Al-Enezi et al., 2022; Bouchet et al., 2018; Cavaliere et al., 2021; Nunes et al., 2023; Parent et al., 2021b). In the MP, these biotic indices show similar ecological outcomes and trends in 2011 and 2016. Both F-AMBI and the exp(H'$_{bc}$), overall indicate a different EcoQS in the two sub-basins, going from good in MPI to moderate up to bad in MPII. The index based on ostracod diversity is also well correlated with the EcoQS indices of foraminifera. Taking into consideration the previous studies in the study area, primarily relying on geochemical data, our findings align with
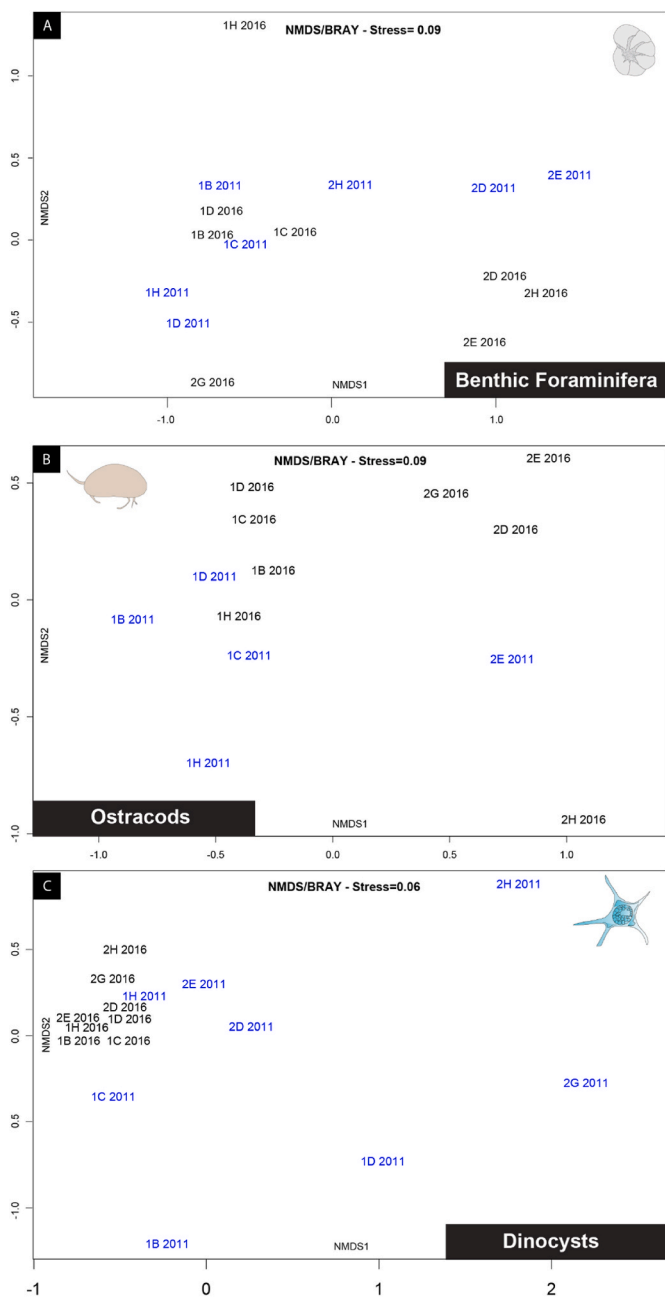
Fig. 5. Non metrical multidimensional scale (NMDS) based on the relative abundance of benthic foraminifera, ostracods and dinocysts in the Mar Piccolo sub-basins (MPI = 1B–H; MPII = 2D-H) considering assemblages from 2011 (Ferraro et al., 2017; blue) and 2016 (present study; black).

sediment and water quality in MPII, while present a notable contrast in MPI (for reviews Cardellicchio et al., 2016a; Cardellicchio et al., 2016b; Rizzo et al., 2022). The MPI is a severely contaminated ecosystem where toxic metals and PCB levels commonly exceed permissible thresholds (Calace et al., 2008; Cardellicchio et al., 2006, 2007; Franzo et al., 2018). Although the response of organisms to pollutants is not always straightforward, it must be acknowledged that our results tend to overestimate the EcoQS in MPI. This is particularly significant considering that MPI is contaminated, yet our results indicate a good EcoQS. In contrast, in MPII, the EcoQS derived from foraminifera accurately reflects degraded conditions. This is primarily due to the presence of extensive aquaculture facilities, which introduce a significant organic load.

At this point, it is reasonable to question the factors contributing to

the variability in our results, leading to occasional controversies and inconsistencies. The reasons behind this could be manifold. First and foremost, it should be considered that the indices and class boundaries have been developed and calibrated for specific environments and regions. In this study, we used for the exp(H'$_{bc}$) class boundaries from Italian transitional waters (Bouchet et al., 2018). However, despite the two basins of the MP are considered as part of a transitional system, their hydrological characteristics as well as their pollutant sources are completely different. The MPI shows more affinity with marine systems due to its direct connection to the open sea through the Mar Grande. The MPII is a confined environment with a lower yearly averaged water renewal time (Pascalis et al., 2016). Hence, it appears that the class boundaries are well-suited for MPII, given that the diversity range aligns with the characteristics typical of the European transitional environments (e.g., Armynot du Châtelet et al., 2018a; Francescangeli et al., 2021). Conversely, these boundaries do not seem to be appropriate for MPI, because the increase of diversity is primarily associated with a shift towards more marine conditions rather than being directly indicative of better EcoQS. It is known, indeed, that marine environments have generally higher diversities than transitional ones (Murray, 2006; Rostami et al., 2023). At the moment, however, class boundaries for exp(H'$_{bc}$) have not been calibrated yet for open marine environments.

For the F-AMBI, we have used two different species lists resulting from intertidal and transitional waters (Bouchet et al., 2021) and Mediterranean open waters (Jorissen et al., 2018). The F-AMBI$_{Bou}$ was not reliable for MPI as the number of assigned species to ecological groups was always below 76%. Indeed, when the percentage of assigned species is less than 80%, this index is not considered reliable (Borja et al., 2000; Muxika et al., 2005). On the contrary, the F-AMBI$_{Jor}$ should be reliable for the MPI, as the number of assigned species exceeds 90%. However, it seems quite unlikely that in a contaminated environment like this, only an average of 4% of the species (considering both 2011 and 2016) belongs to second-order opportunistic (EGIV). Moreover, not a single species falls under first-order opportunistic (EGV). It is evident, therefore, that there is a need to enhance our understanding of the ecological behavior of certain species concerning their tolerance and sensitivity to specific contaminants. For instance, it is possible that the most abundant species in MPI, *A. mamilla*, may not be as sensitive to pollution as it has traditionally been described. Another possible explanation might be related to the low reliability of Foram-AMBI in tracking the EcoQS in severely non-organic polluted environments. Accordingly, the AMBI and its derivatives indices (e.g., Foram-AMBI) have been conceived, developed, and tested on organic enrichment gradient (i.e., total organic carbon or total organic matter). As previously highlighted, the two sub-basins are affected by two different sources of stress. The MPI is substantially interested by inorganic pollutants (i.e., heavy metals), PCBs and dioxins, whereas the MPII is strongly conditioned by high organic matter load leading to dystrophic conditions. Based on these, it can be explained the well-performance and reliability of EcoQS evaluation on Foams-AMBI in MPII compared to MPI.

It is a curious coincidence, however, that in a recent study conducted in the MP by Franzo et al. (2018), the authors obtained similar results. In that study, the authors applied indices based on nematodes (meiofauna) to assess the EcoQS. In MPI, the nematodes metrics provided EcoQS ranging from moderate to good, which is not consistent, as in our results, with the degraded conditions in MPI. The authors indicated, indeed, that the values of Hg and PCBs provide moderate to bad conditions. We similarly agree with them on the fact that studies like this one, which contain controversial results, deserve to be critically treated, hopefully opening a critical debate in the scientific community. Like in our case, the authors questioned the correct attribution of certain genera to sensitive/tolerant ecological groups. Despite the considerable attention given to bioindication in both research and legislative contexts in recent decades, we require a greater number of studies to calibrate and improve current biotic indices, particularly for groups belonging to meiofauna,

which have been less studied compared to, for example, macrofauna. Indeed, there is a need for more studies to provide us with a clearer understanding of the ecological response of certain species to their sensitivity or tolerance to specific pollutants, and studies like this one strongly advocates for that.

In the present research, we also attempted for the first time to use an index (i.e., exp(H'$_{bc}$)) based on the diversity of ostracods and cysts to characterize EcoQS. For ostracods, the results appear to be quite promising. Indeed, the ecological trend aligns with that provided by foraminifera. More quantitative studies should be carried out to define suitable class boundaries applicable to specific environments. As for cysts, however, diversity does not seem to be, at least in the case of the MP, an ideal metric for assessing EcoQS. Nevertheless, it might be worth considering the use of sensitivity metrics (which have not yet been developed) for monitoring a site over time. Despite the sampling strategy has foreseen to spatially cover the entire MP and systematically used three replicates for each biological group, it is also worth to mention that the present study has been based on a limited number of samples (eight in 2016 and twenty in 2011) that might not necessarily capture all the environmental variability in the MP, across the two-sub-basins (i.e., MPI and MPII) and between the sampling events (i.e., 2011 and 2016).

6. Conclusions

The Mar Piccolo of Taranto is a unique transitional environment and previous studies on hydrological conditions and sources of pollution have shown differences between the outer and inner basins (MPI and MPII). Our results corroborate this statement, in fact, the comparison of two sampling campaigns conducted in December 2011 and December 2016, focusing on both benthic communities (foraminifera and ostracods) and dinoflagellate cysts communities, has revealed significant differences in the two sub-basins (MPI and MPII), despite their geographic proximity. Although benthic communities display consistent patterns over the years (in terms of assemblage composition), notable differences are observed in dinoflagellate cyst communities between 2011 and 2016. The biotic indices used to assess the ecological quality in the Mar Piccolo appear to reflect ecological degradation in MPII. However, they yield conflicting results in MPI, considering that MPI is contaminated, yet our results indicate a good EcoQS. This study highlights the potential of these indices for characterizing EcoQS but also emphasizes the need for improvements in their reliability. Nevertheless, these studies are crucial in driving new approaches and enhancing the applicability of existing ones. More research is essential to test the suitability of biotic-based indices, particularly in coastal areas where anthropogenic stress commonly combines with natural stressors.

CRediT authorship contribution statement

F. Francescangeli: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **L. Ferraro:** Writing – review & editing, Writing – original draft, Validation, Methodology, Funding acquisition, Formal analysis, Data curation. **F. Frontalini:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **S. Da Prato:** Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation. **F. Rubino:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Funding acquisition, Formal analysis, Data curation.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2024.106545.

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