



Sediment and bottom water eDNA metabarcoding to support coastal management

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ABSTRACT

Ocean sprawl and climate change exacerbate coastal erosion and flooding, resulting in habitat loss and decreasing biodiversity. To counteract these threats, different coastal defence tools have been developed, with an increasing emphasis on nature-based solutions. However, tracking the impacts of these interventions on marine benthic organisms requires appropriate sampling designs and timely investigation methods due to the dynamic nature of coastal environments. Environmental DNA metabarcoding is a promising, non-invasive, and quick technique to monitor community changes. Here, environmental DNA COI-based metabarcoding data from sediment and bottom water samples were used to characterize benthic communities at three sites along the Emilia-Romagna coast differing in the topology of coastal defence actions (from no defences to groynes and low-crested barriers) and to evaluate the effectiveness of the two sampling matrices in detecting local biodiversity. The findings revealed significant differences in the structure of the benthic communities depending on site, sample type (i.e., sediment versus bottom water), and their interaction. The three sites differ in abiotic characteristic affecting the community composition. Lido di Dante and Riccione showed higher species diversity due to the new type of substrata provided by the hard defence structure, while Foce del Bevano showed the presence of species typical of low impacted areas. Bottom water, hosting more traces of pelagic and nektonic species, showed significantly different species composition compared to sediment samples, suggesting the need to consider both matrices in coastal monitoring.

1. Introduction

Coastal urbanization is one of the most visible consequences of human activities related to the direct and indirect use of marine resources. This urban sprawl has a significant impact on coastal ecosystems by increasing habitat fragmentation and loss, as well as decreasing biodiversity, which has negative effects on ecosystem functions and services (Firth et al., 2020). This exacerbates the effect of coastal erosion and flooding caused by climate change (Van Rijn, 2011), raising the need to adopt artificial hard (e.g., breakwaters, seawalls), soft (e.g., beach nourishment) and nature-based (wetland and seagrass bed restoration) solutions for coastal defence (Firth et al., 2020;

Temmerman et al., 2013).

Emilia-Romagna region (Italy) is a perfect example of this: its rapid coastal urbanisation has resulted in an increase in economic sectors and touristic activities, enhancing the value of the area (Perini et al., 2017). With the aim to preserve this crucial socio-economic value, a variety of structures designed to contrast the effect of erosion and subsidence and to stabilise the beach were implemented (Bacchiocchi and Airoidi, 2003). A variety of hard coastal defence structures have been built since the 1950s (e.g., submerged or semi-submerged breakwaters, continuous or semicontinuous structures parallel to the shoreline, seawalls, and groynes; Liberatore, 1992) and now cover 75 out of 130 km of regional coastline (Vecchi et al., 2020). These artificial structures have an impact

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on the native environment and on benthic communities (Bertasi et al., 2007; D'Alessandro et al., 2021). Hard structures are colonised by few dominant species, such as the mussels *Mytilus galloprovincialis* Lamarck 1819, and facilitate non-indigenous species, like the Pacific oysters *Magallana gigas* (Thunberg, 1793), the ascidian *Styela plicata* (Lesueur, 1823) and several invasive seaweeds (Airoldi et al., 2015). Moreover, they result in low genetic diversity compared to natural rocky bottoms (Fauvelot et al., 2009). To avoid the negative impacts caused by hard coastal defence structures and to foster more sustainable governance, Emilia-Romagna's coastal management adopted soft solutions, such as beach nourishment (Colosio et al., 2007; Vecchi et al., 2020). However, also these interventions affect the benthic communities (GESAMP/UNESCO 1994; Speybroeck et al., 2006; Targusi et al., 2019). They, indeed, may cause a change in the communities structure (in terms of decreasing species richness and abundance), leading to dominance of few bivalves such as *Chamelea gallina* (Linnaeus, 1758), *Donax semistriatus* Poli, 1795 and *Lentidium mediterraneum* (O. G. Costa, 1830; Colosio et al., 2007).

Species identification in the field or laboratory has traditionally been used to characterize the structure of benthic communities to assess environmental disturbances (Dauer et al., 2000; Ponti et al., 2012). These methods are time-consuming and requires skilled taxonomic knowledge. In recent years, environmental DNA (eDNA; Ficetola et al., 2008) has emerged as a promising tool for environmental quality assessment (Compson et al., 2020). This technique relies on DNA extraction from various matrices (e.g., air, water, sediments and soil, faeces, and stomach contents). eDNA analysis combined with metabarcoding can provide a snapshot of the species richness in a sample (Taberlet et al., 2018). However, despite the rapid growth of eDNA metabarcoding studies, no consistent approaches or standardised methodologies for characterizing benthic communities exist (Holman et al., 2019; Pawlowski et al., 2020). The use of diverse environmental matrices (such as bottom water and sediment) may affect the outcomes of the analyses, and few recent studies have combined data from the various environmental matrices (Holman et al., 2019; Koziol et al., 2019). Bottom water, for example, maybe a better choice than sediments for assessing benthic communities because it allows for a simpler and less damaging sample process, but DNA in water degrades faster than in sediments (Torti et al., 2015). On the other hand, bottom water samples may contain more traces of pelagic and nektonic species (Koziol et al., 2019) giving complementary results to the sediment matrix.

In this work, a mitochondrial cytochrome oxidase subunit 1 (COI) metabarcoding technique based on eDNA from bottom water and sediments was used to characterize the eukaryotic communities in three sites along the Emilia-Romagna coast. The aims are to demonstrate the applicability of eDNA metabarcoding to analyse the differences in community composition and structure among study sites characterised by different coastal defence interventions, and the effectiveness of combining the results of the two types of eDNA matrices to better describe the diversity of coastal benthic communities. Finally, we parsed the eDNA metabarcoding dataset to identify species with high commercial and ecological value in the area, whose fate is usually given more consideration in coastal management policies. Moreover, these eDNA data provide the first marine baseline metabarcoding reference dataset for the Emilia-Romagna coasts, which allows to monitor changes in community structure over space and time and to support better coastal management.

2. Materials and methods

2.1. Study area

The Emilia-Romagna coast (Italy) stretches for over 130 km along the Adriatic Sea from Po di Goro (province of Ferrara) to Cattolica (province of Rimini). Except for artificial habitats, the coastline is characterised by sandy, gently sloping beaches (Airoldi et al., 2016). The

coast is almost totally urbanised, supporting a wide range of economic activities such as methane gas extraction, industries (flour, ceramics, animal feed, fertilizer), tourist and commercial ports, mussels farms, and aquaculture facilities (Airoldi et al., 2016; Martinelli et al., 2011). In a social context, the so-called "Riviera Romagnola" is a valuable, economic asset (it has one of Europe's largest tourism businesses) as well as a social gathering place due to the region's recreational activities (Rodella et al., 2020).

Three sampling study sites were chosen along the coast: Riccione, Foce del Bevano, and Lido di Dante. The three sites were chosen due to their varying levels of impact and coastal defence structures (SM.1). The most natural site is Foce del Bevano, a protected coastal area surrounding the Bevano River mouth, without urbanization and with strictly limited access, while Riccione is the most urbanised one surrounded by hotels and bathing establishments, subject for many years to beach nourishment and installation of coastal defence works, including a submerged sandbag barrier, periodical recharged (Martinelli et al., 2011), and, more recently, experimental submerged concrete walls with horizontal openings (Romagnoli et al., 2021). Lido di Dante represents intermediate urbanisation conditions between the other two sites, however it is affected by high subsidence, also due to the extraction of natural gas, which is why the beach in front of the village and camping has been protected since the 1980s by groynes and low-crested barriers, recently extend southward (Zanutigh et al., 2005; Stanghellini et al., 2022).

The surveys were conducted in the summer of 2020 because some benthic organisms are typically abundant in communities up to 2 m deep and are often found in sediments even during periods following spring beach nourishment (Targusi et al., 2019). The sampling was carried out from the coastline down to 5 m depth in a pre-determined area with different dimensions for each site according to local bathymetry: 173,000 m² in Riccione, 980,000 m² in Foce del Bevano, and 168,000 m² in Lido di Dante (SM.1, Figure SM.1). The sampling areas correspond to the "active coastal zone" of the beach (Archetti et al., 2016), and they are mainly characterized by fine to very fine sand with medium particle size reduction towards the open sea and variable mud content depending by local sedimentary regimes. QGIS software (QGIS Association, 2020) was used to plan and randomly distribute a different number of sampling points to allow an adequate mapping of each area: 10 points were sampled at Riccione, 25 at Foce del Bevano, and 20 at Lido di Dante. At each sampling point, the distance from the coast and depth were measured (SM.1, Table SM.1).

2.2. Environmental DNA sampling

At each site, two types of samples, bottom water and sediment, were manually taken by scuba divers. The only exception was in Foce del Bevano, where bottom water was sampled at only 15 of 25 sediment sampling points (SM.1, Table SM.1). Bottom water samples were gathered using 500 mL Nalgene bottles at the sediment-water interface. Sediment samples were collected immediately after water samples from the first centimetre of the sea bottom using 10 mL falcon tubes. To avoid contamination, bottles and tubes were previously sterilised with 70% ethanol alcohol (EtOH) and filled with deionised water. Subsequently, both types of samples were maintained for no more than 6 h in a field mini fridge full of ice (dark and low-temperature conditions) to avoid eDNA denaturation and algae growth (Laramie et al., 2015).

Bottom water samples were held in a 4 °C dark room immediately after each sampling day, while sediment samples were stored at -20 °C.

After less than 12 h, bottom water samples were filtered using 0.22 µm sterile cellulose filters (one for each sample), collected in β-ray sterilised tubes, and frozen at -20 °C until the eDNA extraction procedure (Hinlo et al., 2017).

2.3. Environmental DNA extraction

All laboratory workflow was performed in sterile conditions thanks to self-protection instruments (lab coat, mask, and gloves) and a sterilised work area (70% EtOH and 10% bleach).

Bottom water DNA extraction was performed through an overnight digest in Proteinase K and lysis buffer at 56 °C in a shaking incubator. After digestion, samples were processed using the DNneasy® Blood & Tissue kit (Qiagen, Hilden, Germany) following the manufacturer's protocol and according to Spens et al. (2017) (SM.2.1).

Sediment extraction was performed on 500 mg of each sediment sample using the NucleoSpin® Soil Kit (Macherey-Nagel, Germany) according to the manufacturer's protocol.

DNA concentrations were quantified using the dsDNA BR Assay Kit (ThermoFisher Scientific, Massachusetts, USA) and a Qubit 2.0 Fluorometer (Invitrogen, ThermoFisher Scientific Inc, USA). Samples with low DNA yield were concentrated using the NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel, Germany) according to the manufacturer's protocol in a final elution volume of 30 µl.

2.4. Primer selection and library preparation

Custom metabarcoding primers, adapted from Leray et al. (2013), were used to amplify a 313 bp portion of the cytochrome *c* oxidase subunit I gene's standard DNA barcoding region following the metabarcoding protocol described in Thomasdotter et al., (2023) and Mugnai et al. (2023b) (primer sequences available upon request). Sequencing libraries were prepared using a two-step PCR protocol outlined in Corse et al. (2017) and described in SM.2.2.

The target amplicon was normalised according to its fluorescence using the Qubit dsDNA Broad-range Assay Kit. These normalised samples were pooled at an equimolar concentration and sent to Genomix4life (Salerno, Italy) for quantification using KAPA Library Quantification Kit and sequencing on the Illumina MiSeq platform (Illumina, CA, USA) using v2 chemistry (2x250 bp paired-end).

Negative and positive controls were added to the sequencing library. The negative control consisted of RNase-free water; while the positive control was DNA extracted from a ready "mock community" (available upon request to Dubut V., but see also Thomasdotter et al., 2023 and Mugnai et al. (2023b) containing a set of well-characterised sequences of continental species.

2.5. Bioinformatic analysis

FastQC (Andrews, 2010) was used to assess reads quality after Illumina sequencing. The reads were then processed using programs implemented in the OBITools software (Boyer et al., 2016). The "illumina-pairedend" was used to assemble forward and reverse reads and trim those with quality Phred values of 30 or less. The "Ngsfilter" script was used to remove primer sequences during the demultiplexed step and "Obigrep" was used to remove any sequences that were longer than 320 bp and shorter than 300 bp. "Obiuniq" allowed to dereplicate into unique sequences, and the "uchime_denovo" algorithm implemented in VSEARCH (Rognes et al., 2016) was used to trim all sequences that had one or multiple "N" as base pairs, chimeras and/or sequences that appeared less than 10 times in the dataset. Because COI is prone to sequencing errors (i.e., random points) which can result in a high proportion of singletons, we chose to cluster the dataset rather than denoising it to avoid an excessively reduced dataset (Wangenstein et al., 2018).

The SWARM algorithm (Mahé et al., 2014) clustered sequences at 97% similarity and a Molecular Operational Taxonomic Unit (MOTU) count table was generated.

The taxonomic assignment was done using the Ribosomal Database Project (RDP) naïve Bayesian Classifier (Wang et al., 2007) with two different COI reference databases: the COI classifier Database (Porter

and Hajibabaei, 2018) and the Barcode of Life project public database (iBOL).

To find the best fitting hit, different thresholds (98%: species level, 95%: genus level, 90%: family level, 85%: order level, <85%: class level) were applied for each taxonomic level.

Bacteria, Archaea, and any other organisms of terrestrial origin were removed from the resulting MOTU table. The open-source R (RStudio Team, 2020) package "decontam" was used to remove MOTUs related to positive and negative controls (Davis et al., 2018).

Finally, the dataset was refined by removing MOTUs that had not been assigned to marine eukaryotes and had fewer than 4 reads across all samples.

2.6. Statistical analysis

MOTU count data were processed and graphically represented using R V4.1.0 (RStudio Team, 2020). Rarefaction to 1,000 reads (the minimum number of reads per sample) (McKnight et al., 2019; Weiss et al., 2017) was chosen as the normalization method, using the function Rarefy in the Vegan R package (Dixon, 2003). Sediment sample LD-03, with less than 1,000 reads, was excluded from the following analysis.

A differential abundance analysis was performed using the DESeq2 R package (Love et al., 2014) with MOTUs collapsed to the main taxa level (Phylum and Class) ($P = 0.05$), to have an overview of the different distribution among samples.

All the following statistical analyses were performed at the MOTU level. To test possible differences in alpha diversity (MOTUs richness, \log_e Shannon and Pielou's indexes) between sediment and bottom water samples and among sites, univariate permutational analysis of variance (PERMANOVA, $\alpha = 0.05$; Anderson et al., 2008) was performed using Euclidean distance matrices on untransformed data and following a two-way design that included two fixed factors: Site (Si) with three levels (Foce del Bevano, Riccione, and Lido di Dante) and Sample Type (Sa) with two levels (bottom water and sediment).

Two-way crossed multivariate PERMANOVA, based on the Bray-Curtis similarity matrix on 4th root transformed data, was used to test significant differences in community structure ($\alpha = 0.05$, 9999 permutations) among Sites, between Sample Type, and their interactions.

Principal Coordinates Analysis (PCO) was used to visualize the changes in eukaryotic communities.

Post-hoc pair-wise tests were performed for all significant PERMANOVA results.

A similarity percentage analysis (SIMPER, Clarke, 1993) was used to find the MOTUs that contribute the most to the differentiation between Sites, Sample Type, and their interactions. A shade plot to visualize the results was also generated.

Univariate and multivariate analyses were performed using PRIMER v7 with the PERMANOVA + add-on package (Clarke and Gorley, 2015).

Finally, we compared the distribution and abundance of several ecologically and commercially important species, chosen from the MOTUs assigned to species level.

3. Results

3.1. Data exploration

Illumina sequencing produced a total of 8,730,993 paired-end reads that considerably decreased after their merging and quality filtering. Merged reads were 8,710,989; after size selection, 2,665,818 reads remained, distributed across 14,000 MOTUs. A total of 1,091,732 reads, equal to 6,866 MOTUs, were erased from the dataset after the taxonomic assignment. The R "decontam" package left 1,539,462 reads, equal to 7,086 MOTUs. 2,920 MOTUs with fewer than four reads were removed from the final dataset, corresponding to 7,850 reads. The final MOTUs table consisted of 1,531,612 reads per 4,166 MOTUs (Table SM.3.1). The MOTU table is available at Zenodo (<https://doi.org/10.5281/zenodo>

o.8077927).

We got fewer reads in the bottom water samples (682,248) than in the sediment samples (849,364) but a higher number of MOTUs (3,857 vs 3,012) and reads per sample ($19,412.80 \pm 147.94$ s.d. vs. $18,874.76 \pm 231.76$ s.d.). Riccione had the lowest number of MOTUs (4,307), but Lido di Dante had the lowest number of reads (221,445, Table SM.3.2).

The comparison of taxonomic compositions of the samples (Deseq analysis), in terms of number of reads among the different MOTUs (Fig. 1), showed that the relative abundance of 18 of the 29 main taxa found in the dataset changed significantly across Sample Type (Table SM.4.1, Figure SM.4.1). Annelida, Bryozoa, Chlorophyta, Chordata, Echinodermata, Haptophyta, Ochrophyta, Porifera, Rhodophyta,

and Rotifera were more present in bottom water samples than in sediment samples ($P < 0.05$); on the other hand, in the sediment samples Amoebozoa, Arthropoda, Bacillariophyta, Gastrotricha, Heterokontophyta, Nematoda, Pyrrophycophyta, and Phoronida had a higher abundance in terms of reads ($P < 0.05$). The abundance of Bryophyta, Chaetognatha, Cnidaria, Ctenophora, Cryptophyta, Magnoliophyta, Mollusca, Nemertea, Platyhelminthes, Sipuncula and Zygomycota did not differ by Sample Type ($P > 0.05$).

The main taxa were also differently distributed across Site (Fig. 1). Bacillariophyta, Cryptophyta, Echinodermata, and Rotifera dominated Foce del Bevano; while Bryozoa, Chordata, Porifera and Rodophyta were abundant in Riccione and Heterokontophyta in Lido di Dante. The

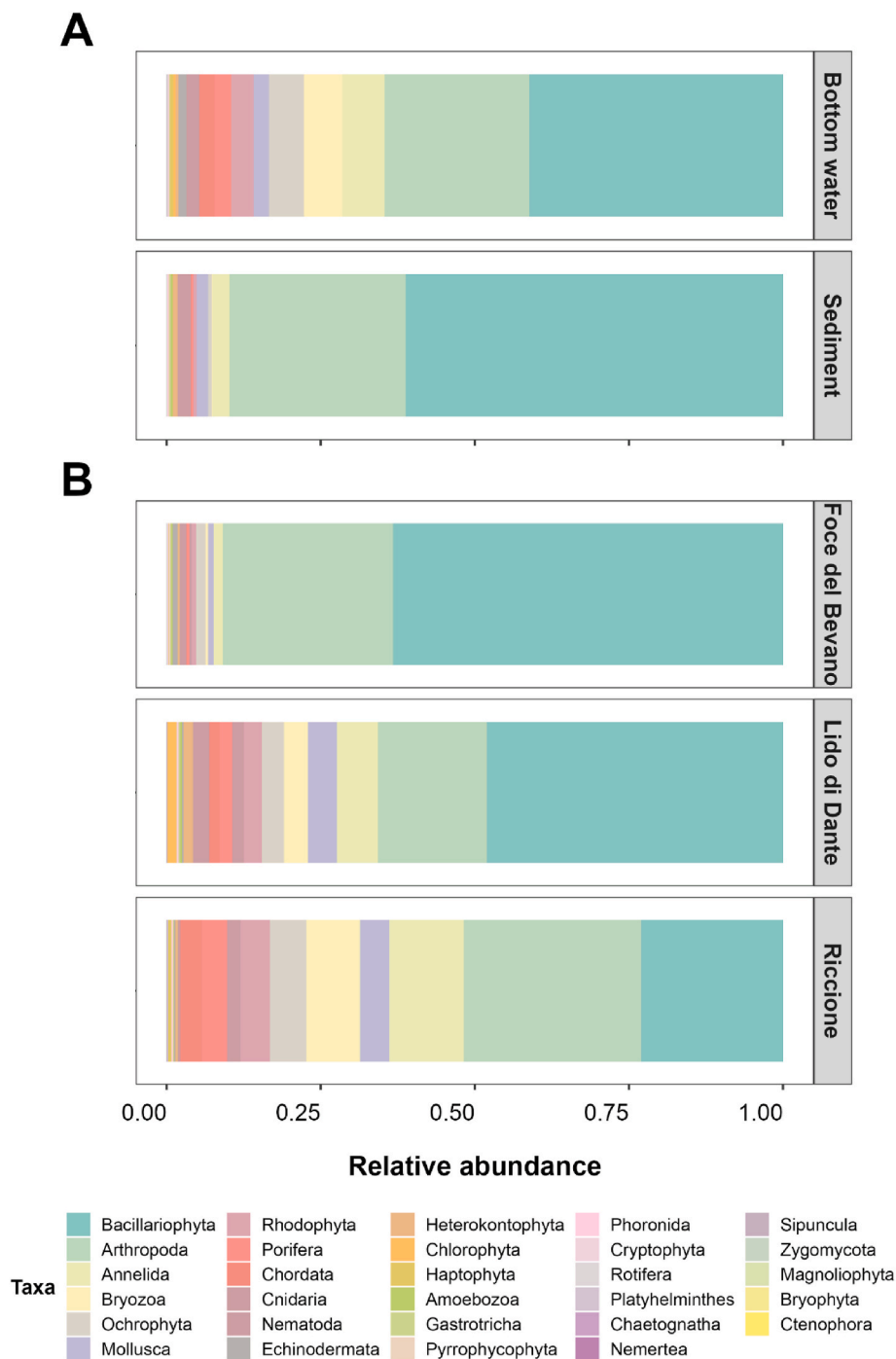


Fig. 1. MOTU relative abundances collapsed at the main taxon level (Phylum and Class), as detected in bottom water and sediment samples (A) and Foce del Bevano, Lido di Dante and Riccione samples (B) by COI metabarcoding.

relative abundance of Annelida, Cnidaria, and Mollusca was higher in Lido di Dante and Riccione, while Gastrotricha was most abundant in Lido di Dante and Foce del Bevano ($P < 0.05$, Table SM.4.2, Figure SM.4.2). Amoebozoa, Arthropoda, Chaetognatha, Chlorophyta, Haptophyta, Magnoliophyta, Nematoda, Nemertea, Ochrophyta, Phoronida, Pyrrophytophyta, Platyhelminthes, Sipuncula, and Zygomycota abundance did not differ among sites ($P > 0.05$, Table SM.4.2, Figure SM.4.2).

Furthermore, whereas some MOTUs were present in both matrices, others were exclusive to sediment or bottom water samples (Fig. 2).

3.2. MOTUs richness and community structure

The total number of MOTUs varied by Site and Sample Type. Bottom water samples from Riccione had the highest number of MOTUs (1,032) followed by sediment samples from Lido di Dante (987), while sediment samples from Riccione had the lowest number of MOTUs (608).

The two-way PERMANOVAs analyses detected, for all the α -diversity metrics, a significant effect of the interaction between Site and Sample Type (Table SM.5.1). Pairwise test for the interaction ($Si \times Sa$) showed higher significant values of MOTUs richness, Shannon and Pielou's indices in bottom water samples at Foce del Bevano and Riccione than in sediment samples, conversely at Lido di Dante the MOTUs values for all the indexes were significantly higher in sediment samples than in bottom water ones (Table SM.5.1, Fig. 3). In the sediment samples, all the indices at Lido di Dante were significantly higher than at Riccione and Foce del Bevano. Conversely, in the bottom water sample, all the indexes were higher at Riccione than in the other two sites (Table SM.5.1, Fig. 3).

The two-way multivariate PERMANOVAs analyses evidenced that the structure of the communities was significantly affected by Site, Sample Type, and their interaction (Table 1).

The PCO plot of the benthic community structure supported the PERMANOVA results revealed a clear separation between Sites and Sample Types, as well as their interaction (Fig. 4).

Owing to the significant differences in Site \times Sample Type interaction, SIMPER analysis at the main taxa level was performed between Sample Types at each Site and between Sites in each Sample Type. SIMPER analysis revealed a high similarity within both Sample Types at each Site (Average similarity_{water} > 84%, Average similarity_{sediment} > 86%). SIMPER analysis revealed that the average dissimilarity from

bottom water and sediment samples (Sample Type group) across the three sites was higher in Riccione (27.26%) caused mostly by Annelida (13.32%), Rhodopyta (13.32%) and Chordata (12.41%). The highest dissimilarity was indeed observed between Foce del Bevano and Lido di Dante in sediment samples (28.65%) mostly caused by Nematoda (14.45%) followed by Mollusca (13.03%). In bottom water samples, the highest dissimilarity was between Foce del Bevano, and Riccione (22.3%) caused first of all by Annelida (14.48%) (Fig. 5, Table SM.6.1).

3.3. Focus on species of ecological and commercial interest

Among the MOTUs that were possible to taxonomically assigned at the species level, some warrant special consideration because of their ecological role or commercial value. Each consideration was done on the total amount of reads found in the dataset (SM.7).

The wedge clam *Donax trunculus* Linnaeus, 1758 and the polychaeta *Owenia fusiformis* Delle Chiaje, 1844 were observed in high abundance in the samples of Foce del Bevano. *O. fusiformis* was found mostly in sediment samples (63 reads compared to the 4 found in bottom water) while the number of reads belonging to *D. trunculus* was higher in bottom water samples (13) than in the sediment ones (9) (SM.7).

The mussel *Mytilus galloprovincialis* Lamarck, 1819 and the barnacle *Balanus trigonus* Darwin, 1854 were dominant in Riccione bottom water samples (43 and 46 reads respectively), such as the ascidian *Styela plicata* (Lesueur, 1823) with 65 reads. *S. plicata* was also abundant (46 reads) at Lido di Dante and, with a lower abundance (4 reads), at Foce del Bevano. The Pacific oyster *Magallana gigas* (Thunberg, 1793) was not recorded at Riccione.

The striped venus clam *Chamelea gallina* (Linnaeus, 1758) was abundant in Foce del Bevano and it was also found in Lido di Dante and Riccione, though not in great abundance.

Finally, the ctenophore *Mnemiopsis* sp. (presumably as the invasive *Mnemiopsis leidyi* A. Agassiz, 1865) was only found in the bottom water samples at Foce del Bevano; while the marine turtle *Caretta caretta* (Linnaeus, 1758) was found in high abundance at Riccione, especially in the bottom water samples (2627 reads), followed by Foce del Bevano (605 reads which 592 belonging to bottom water samples) and with low abundance in Lido di Dante (15 reads).

A high abundance of fishes was observed in bottom water samples and as expected, most of the detected species were benthonic species such as the Actinopterygii *Chelon labrosus* (Risso, 1827), *Mora* (Risso,

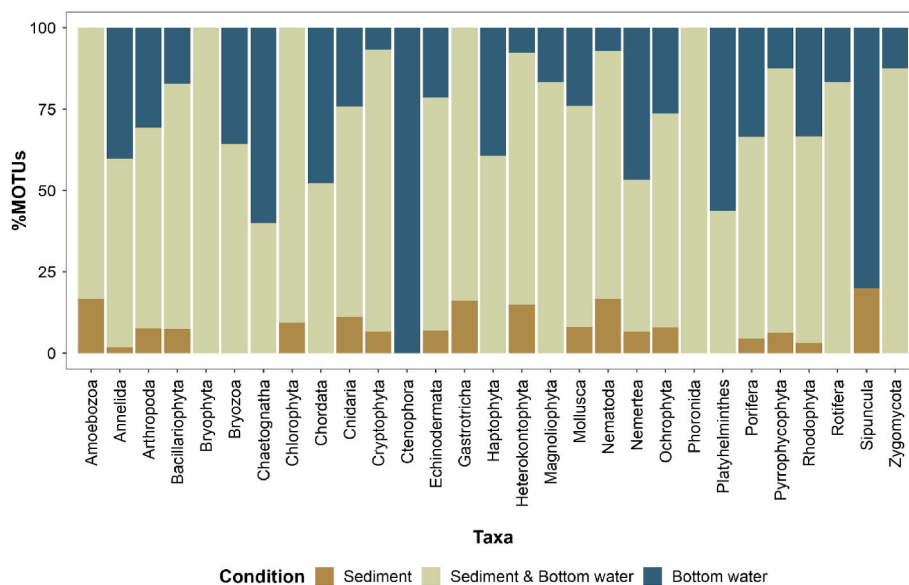


Fig. 2. Stacked bar chart representing the proportion of MOTUs, across the 29 main taxa, detected exclusively in sediment (brown), bottom water (blue) or both Sample Types (light green).

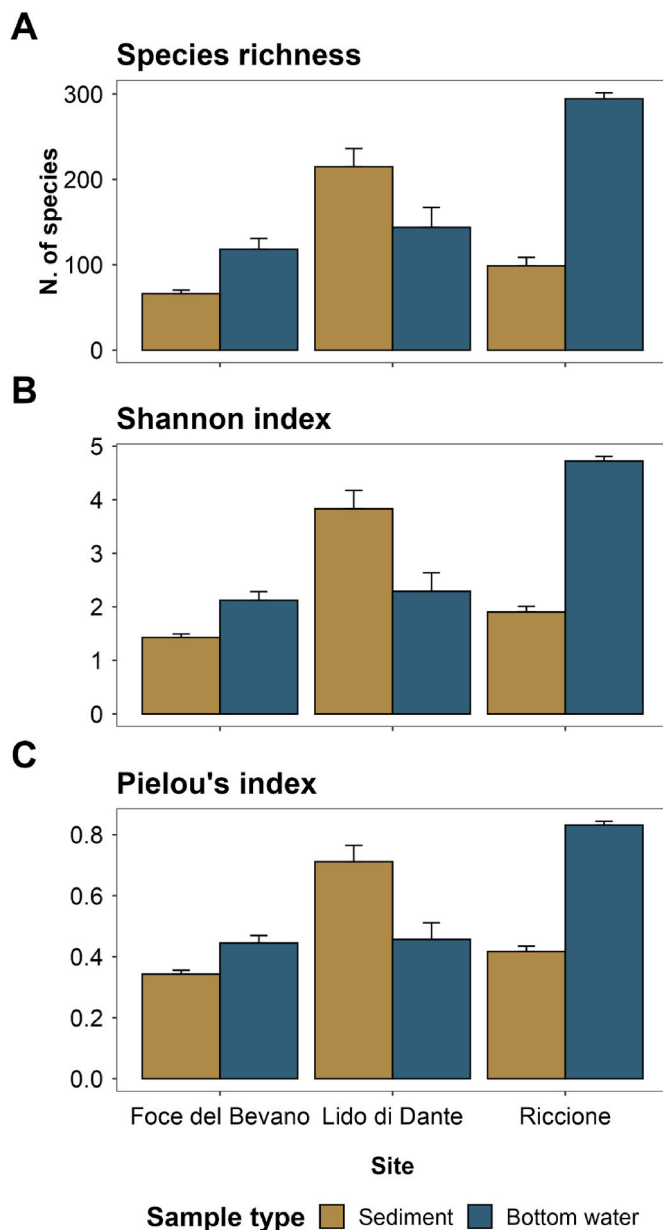


Fig. 3. Mean + standard error of A) Species richness B) Shannon index, and C) Pielou's index of the benthic communities in the two Sample types (Sediment sample in brown and bottom water sample in blue) at the three study sites.

1810), and *Mullus barbatus* Linnaeus, 1758, but pelagic species, such as *Engraulis encrasicolus* (Linnaeus, 1758) were also found.

4. Discussion

Our results confirm that combing environmental DNA and COI metabarcoding represents a quick and low-cost method for characterizing the macrobenthic communities. Moreover, as Holman et al. (2019) and Antich et al. (2021) observed, the use of different environmental sample types can affect the composition of taxa within communities. In fact, we found a significant effect of the Site-Sample Type interaction, suggesting that the differences in benthic communities observed between the two sampled matrices are site-dependent.

Differences observed at the local scale, enabling to assess differences among sites some kilometres away differing in environmental conditions and anthropogenic pressures (Holman et al., 2019; Jeunen et al., 2019), can support better coastal management, as suggested by Pawlowski et al.

Table 1

Results of the permutational analysis of variance (PERMANOVA) of 4th-root transformed abundance data and pair-wise analysis testing for differences in community structure of benthic communities between Sample Type and Sites. Df = degrees of freedom; MS = mean squares; P (perm) = permutational P values; LD = Lido di Dante; RI= Riccione; BE= Foce del Bevano; W = bottom water sample; S = sediment sample. Significance level: ***P < 0.001.

Source	df	MS	Pseudo-F	P (perm)	Unique perms
Site (Si)	2	15771	6.50	0.0001	*** 9820
Sample Type (Sa)	1	28982	11.94	0.0001	*** 9865
Si x Sa	2	11058	4.56	0.0001	*** 9804
Residual	73	2426.9			
Total	78				

Pair-wise test, Si x Sa:

Sediment: BE ≠ LD ≠ RI
Bottom water: BE ≠ LD ≠ RI
Foce del Bevano: S ≠ W
Lido di Dante: S ≠ W
Riccione: S ≠ W

(2020). The observed differences in the benthic communities among sites may be due to the abiotic characteristics of the three sites, which are differently affected by river inputs, urbanization, coastal defence structures, beach nourishment, and seaside tourism (Abbiati et al., 2019; Colosio et al., 2007; Menegon et al., 2023; Vecchi et al., 2020; Zanuttigh et al., 2005). The greater number of coastal defence elements present in Lido di Dante and the presence of concrete experimental structures at Riccione, which brought a new type of substrate, can increase the species diversity at the local scale (Martinelli et al., 2011; Sherrard et al., 2016) providing substrate for sessile species (e.g. the mussels *Mytilus galloprovincialis*) and allow the establishment of hard-bottom non-native species, like *Styela plicata* and *Balanus trigonus* that were highly abundant at these sites (Airoldi et al., 2015, 2016; Martinelli et al., 2011). On the contrary, Foce del Bevano protected area, the most pristine site, has shown the lowest taxa diversity but with the presence of some species of ecological importance that are typical of low-impacted areas.

The Pacific oyster *Magallana gigas* was found at Lido di Dante and Foce del Bevano and was already observed on nearby breakwaters and on the muddy bottoms inside the Bevano river mouth (Abbiati et al., 2019). Nevertheless, it was present with a very low number of reads, despite it forms extensive beds in both these sites (Zanuttigh et al., 2005).

Furthermore, our study has identified some benthic species of high commercial and ecological interest that were abundant in single sites, evidencing the need for detailed studies on single species. In particular, the wedge clam *Donax trunculus* and the polychaeta *Owenia fusiformis*, usually associated with the striped venus clam *Chamelea gallina* (Grazioli et al., 2022), were observed in a high abundance at Foce del Bevano. These species have already been identified quantitatively in the area through morphological analysis (Abbiati et al., 2019) supporting the idea that this area is suitable for their settlement and reproduction.

Noteworthy was the case of the striped venus clam *Chamelea gallina*, an important commercial species that nowadays is in decline all along the Mediterranean Sea due to the overexploitation (Grazioli et al., 2022). The species was detected both at Foce del Bevano and Riccione. A study focusing on *C. gallina* could be useful to depict the distribution of this species and improve the effectiveness of its conservation.

The spatial variability in the community structure and species distribution observed in this work suggest the adoption of site-specific coastal defence interventions because species can be impacted and respond differently to management interventions (Colosio et al., 2007; Bulleri and Chapman, 2010).

Overall, we found significantly more MOTUs in bottom water samples than in sediment samples, and the proportion of unique MOTUs (MOTUs found only in that sample type) detected in bottom water was higher than in the sediments. Benthic and pelagic fishes are better

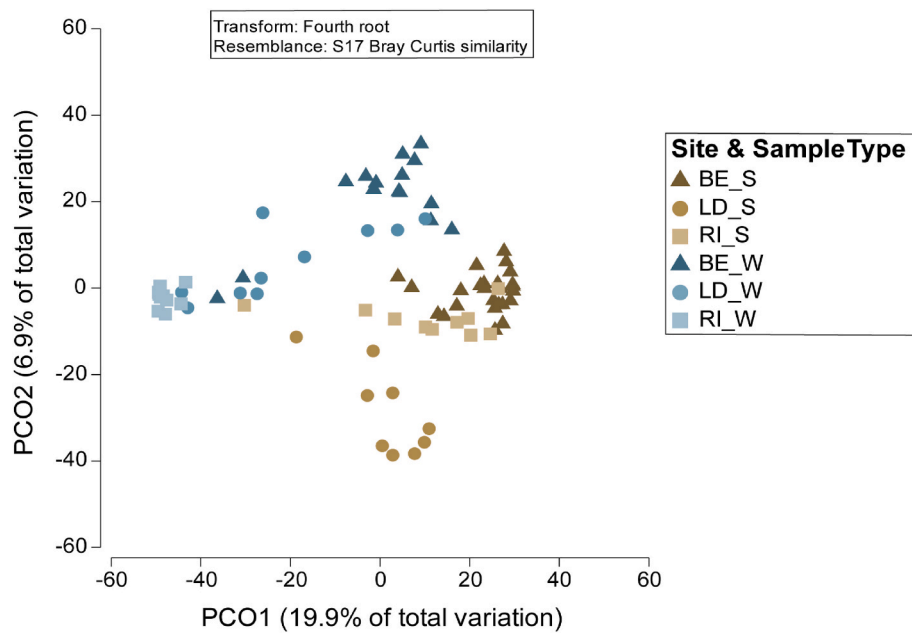


Fig. 4. Principal Coordinate Analysis (PCO) plot comparing the structure of benthic communities between samples among Sample Type and Site. S = sediment samples; W = bottom water samples; BE= Foce del Bevano; LD = Lido di Dante; RI= Riccione.

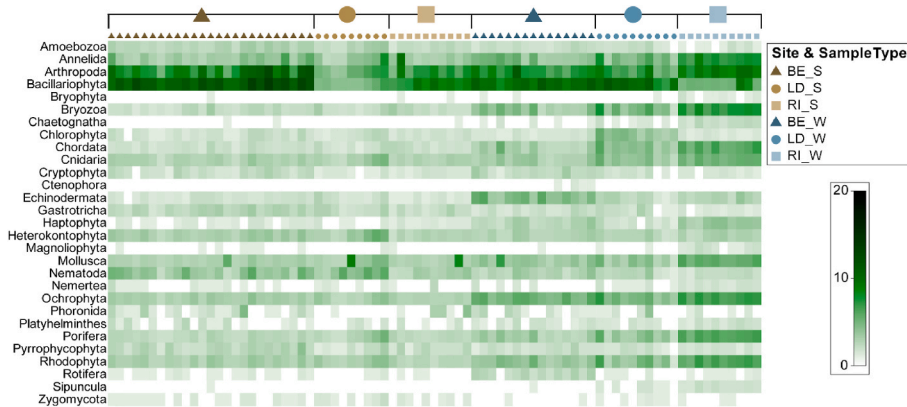


Fig. 5. Shade plot of 4th-root transformed abundance data of main taxa (rows) × sample replicates (columns) matrix of abundances at each Site and Sample Type. S = sediment sample; W = bottom water sample; BE= Foce del Bevano; LD = Lido di Dante; RI= Riccione.

represented in bottom water than in sediment samples, probably because their vagile nature and their capability to move across a wider area (Holman et al., 2019). Bottom water samples also contained a higher number of MOTUs of the Phylum Porifera, suggesting that 1) wave action and the high human presence may have broken these organisms into fragments that were suspended in the water column; and/or 2) they are present as oocytes and larvae due to their reproductive period that occurred in summer, when the sampling was performed (Di Camillo et al., 2012). The phylum Ctenophora was found only in bottom water samples, as *Mnemiopsis* sp., likely *M. leidyi*, one of the 100 World’s worst invasive alien species according to the International Union for Conservation of Nature (Lowe et al., 2000) and recently widespread in the northern Adriatic Sea (Malej et al., 2017). The prevalence of certain taxa such as *M. leidyi* or Bacillariophyta, can be linked to their strong seasonality, primarily which is primarily influenced by variations in environmental factors, such as temperature, sunlight, and nutrient availability, that profoundly influence the growth and reproductive cycles of various organisms (Coma et al., 2000). To have a more detailed picture of the benthic communities that characterize the studied areas a sampling during each season should be performed.

Our results are in accordance with Holman et al. (2019) who, in order to use metabarcoding to characterize the overall communities in an area, emphasized the need for an integration of different eDNA sample types. However, to perform routinely spatial and temporal monitoring to evaluate changes in the benthic communities caused by coastal interventions, environmental DNA sampling from sediments can be sufficient, reducing the likelihood of detecting species that do not truly belong to the sea bottom and that, due to their large size (i.e., *Caretta caretta*), can mask the detection of species more sensitive to anthropogenic impacts (Holman et al., 2019). The presence of *C. caretta* is not surprising because the Adriatic Sea represent a well-known foraging area for this species (Bertuccio et al., 2019). However, the high abundance of sequences related to *C. caretta* in Riccione samples compared to the other two sites can be attributed to the nearby presence of the Fondazione Cetacea, a turtle recovery centre that during the sampling period freed previously recovered specimens.

5. Conclusion

The congruence of the metabarcoding results with those carried out

by morphological identification of the organisms strengthens the call for this kind of molecular approach in the assessment and monitoring of benthic communities also considering the need to use different environmental DNA sample type to have an overall understanding of the diversity and structure of the benthic communities and the species in the surrounding areas. Nevertheless, taxonomic approaches are still very important to implement the barcoding databases and create local ones (Mugnai et al., 2023a).

In conclusion, this is the first molecular assessment of the marine biotic assemblages of three sites along the Emilia-Romagna coastline that were and are nowadays affected by coastal erosion and human disturbances. Being fast and effective in distinguishing local communities, even using only one type of sample, this approach could be included as integrative approach in the monitoring plans for integrated coastal zone management, as well as to survey the spread of non-indigenous species and the possible effects of climate change (Toimil et al., 2020).

Authors' contributions

FC, MA and MP conceived the ideas and designed the methodology. AT, MP and ET performed the field activities. AT performed the molecular analysis and analysed the data. KM contributed to the bioinformatic analysis, while ET to the graphical and statistical analysis. FC and AT led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Federica Costantini reports financial support was provided by European Regional Development Fund.

Data availability

The MOTU table is available at Zenodo (doi: 10.5281/zenodo.8077927).

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Appendix A. Supplementary data

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