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ARMS in ports: monitoring non-indigenous species through Autonomous Reef Monitoring Structures

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ABSTRACT

Harbors and marinas are considered Non-Indigenous Species (NIS) hotspots, being departure and arrival points for numerous vessels and providing environments with less complex artificial substrates that promote NIS colonization. Monitoring changes in the structure of benthic communities allows to early detect the arrival of new NIS. In this study, we used Autonomous Reef Monitoring Structures, ARMS, 3D standardized passive collectors of benthic organisms, to monitor NIS presence and abundance in ports with different uses, a commercial harbor, and a touristic marina, in the northern Adriatic Sea, over one year. The sessile organisms growing on each ARMS plate were identify by photo analysis while the motile ones were morphologically recognized and counted using stereomicroscope. Benthic communities grown on ARMS were characterized by many taxa typical of coastal urbanized areas and showed high seasonal variability, being richer in spring and summer than in winter. Distinct benthic and NIS communities were found in the harbor and marina, with the marina hosting higher numbers of both native and NIS. This is likely due to the different environmental conditions, port structural complexity, and performed economic activities. Of the 86 macrofaunal taxa detected, nearly 19 % were NIS, mostly belonging to crustaceans, polychaetes, bivalves, and ascidians. Our results indicate that ARMS represent an efficient tool for monitoring NIS and suggest that they should be deployed in the spring season and in environments with different levels of urbanization to capture higher NIS diversity and enable their early detection.

1. Introduction

Shipping is one of the main vectors of the introduction of Non-Indigenous Species (NIS) in the Mediterranean Sea, as it transports organisms and propagules through ballast waters or as fouling organisms on vessel hulls (Galil et al., 2014). This pathway accounts for nearly one-third of the 1006 validated NIS recorded in the region by the end of 2020 (Galanidi et al., 2023). The Adriatic Sea, in particular, is recognized as a hotspot for NIS introductions, primarily due to its intense port activities (Occhipinti-Ambrogi et al., 2011; Spagnolo et al., 2019). A total of 211 validated NIS have been reported in the Adriatic, with shipping transport responsible for up to 49 % of these introduction (Galanidi et al., 2023). However, data on the most common NIS in the Adriatic are limited and dispersed across various local studies, which makes it difficult to provide a comprehensive overview (Spagnolo et al., 2019).

Commercial harbors and touristic marinas, characterized by a large presence of artificial substrates such as docks and floating piers, facilitate NIS colonization (Galil et al., 2018; Ojaveer et al., 2014; Sorte et al., 2010). Nevertheless, the presence and abundance of NIS is significantly influenced by the type and use of the ports (Tempesti et al., 2022b, 2020; Tempesti et al., 2025). Large commercial harbors represent vectors of NIS primary introduction, while their secondary spread is mainly facilitated by small recreational boats (Davidson et al., 2010; Ferrario et al., 2017; Occhipinti-Ambrogi, 2007; Ulman et al., 2019b). For these reasons, major

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efforts in monitoring the distribution of NIS have been conducted in port areas (Ferrario et al., 2020; Lavrador et al., 2024; Spagnolo et al., 2019; Tamburini et al., 2021; Tempesti et al., 2022c; Tempesti et al., 2025). Nevertheless, monitoring should be done using standard protocol for comparing quantitative data as suggested by the European Marine Strategy Framework Directive (MSFD 2008/56/EC). The lack of standardized protocol can lead to the incomparability of obtained data from different geographical areas that can influence the detection and comparison of the benthic diversity patterns. Moreover, as environmental conditions, primary production, and resource availability directly influence the life cycle and abundance of species, including NIS, seasonality must be considered in monitoring studies (Çinar et al., 2006; Coma et al., 2000; Lezzi et al., 2018). Therefore, it becomes essential to improve standardized, innovative, and cost-effective tools and sampling systems that can also generate Findable, Accessible, Interoperable, and Reusable FAIR data (Wilkinson et al., 2016; Yang et al., 2023).

Different non-destructive standardized sampling methods, such as settlement panels and other Artificial Substrates Units ASUs, were developed in the last years with the capability to detect the diversity of the communities and identify NIS (Canning-Clode et al., 2013; Carlton, 2019; Marraffini et al., 2017; Scribano et al., 2021; Tamburini et al., 2021; Mugnai et al., 2023) but they have several constraints. ASUs are more prone to recruit vagile organisms, while settlement panels can capture mainly sessile organisms. Autonomous Reef Monitoring Structures (ARMS, Leray and Knowlton, 2015), 3D structures made of stacked PVC plates that mimic the complex structure of the seafloor, are successful tools to characterize the overall community including sessile and motile organisms (Martaeng et al., 2023; Obst et al., 2020; Santi et al., 2023; Thomasdotter et al., 2023). Although ARMS have already been used worldwide to assess benthic communities, including in the Adriatic Sea (David et al., 2019; Pennesi and Danovaro, 2017; Pieretti et al., 2017), to our knowledge, they have not yet been deployed specifically to assess NIS in port areas.

For the first time, ARMS were employed to: (1) characterize benthic communities in two ports with different uses — a touristic marina and a commercial harbor — focusing specifically on Non-Indigenous Species (NIS) assemblages, and (2) assess the seasonal variability of these communities within one year. The findings will help implement the most effective protocol for applying ARMS to monitor benthic communities in urbanized areas.

Fig. 1. Study area in Ravenna port (Adriatic Sea, Italy). Red stars indicate the two study sites. Map obtained with QGIS 3.22.4-Białowieża. Coordinate system: EPSG 4326 WGS 84. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Materials and methods

2.1. Study area

The sampling area is situated in Ravenna (44°24′58″N, 12°12′06″E), which is the primary coastal city of Emilia-Romagna region, Italy, facing the northern occidental Adriatic Sea. Its port, a key industrial and commercial hub, is shielded by two dams (2.4 Km each) and connects directly to two nearby lagoons: Pialassa Piomboni (3 km^2) and Pialassa Baiona (10 km^2) (Airoldi et al., 2016).

Two sites in the Ravenna port area were selected since they are located at opposite ends of the port's urban gradient, the Candiano Canal (the major 'canal' port extending for 11 km from the center of Ravenna to the tourist seacoast): the Harbor, in Darsena di Città at the inner part of the Candiano Canal, and the Marina, a touristic marina (Circolo Velico Ravennate) located in Marina di Ravenna (Fig. 1). The Harbor functions as a major industrial and commercial port, handling a wide range of cargo and also supporting both passenger and offshore sectors. In contrast, the Marina, the largest international marina in the Adriatic, hosts recreational boats, with over 1150 boat docks (Airoldi et al., 2016). The Harbor being the innermost, is more confined and urbanized, and characterized by higher temperature, salinity, and turbidity, while Marina is the outermost, more flushed periurban site with more stable and marine-influenced condition (Gauff et al., 2024).

2.2. Sampling methods and laboratory analyses

At each site (Harbor and Marina), three Autonomous Reef Monitoring Structures, ARMS (Leray and Knowlton, 2015; Fig. 2a) were randomly deployed 1.5 m below the sea surface (seafloor depth of nearly 2.5 m), several meters apart from each other (Fig. 2b). Original ARMS were modified by adding four iron rings to allow us to attach them to the dock by the ropes.

To assess the seasonal variability of benthic communities in both sites, ARMS were deployed in three different seasons and stayed underwater for about three or six months: "winter" (deployed in October 2021 and retrieved in April 2022; 145 days), "spring" (deployed in April 2022 and retrieved in July 2022; 88 days), "summer" (deployed in July 2022 and retrieved in October 2022; 99 days). Since less recruitment and lower NIS propagule pressure were expected during the winter, it was decided to prolong the deployment duration in that period.

After retrieval, each ARMS was put in a plastic tank and filled with filtered seawater (at 40 μm) to prevent the dehydration of the organisms. Then, the ARMS were disassembled, and each plate was gently shaken in the tank to separate motile fauna to be collected later (during this process few individuals of sessile organisms, such as serpulids, could be detached as well accidentally). To analyze sessile fauna, disassembled plates were first photographed on both sides with a NIKON D5300 camera that was set to a frame, maintaining a constant distance and orientation from the plate, to standardize the captures.

Sessile organisms growing on each plate ("Sessile fraction", 306 plate photos in total) were identified to the lowest possible taxonomic level using photoQuad software (Trygonis and Sini, 2012), by an expert taxonomist. Initially, the plate surface was selected using the function "noded_polygon" (4 nodes) to standardize further analysis processes. Each plate was then divided into 400 cells using the "grid cell count" function, with each cell representing 0.25 % of the surface area. For this analysis, the selected data output was "percent cover", which was determined by manually selecting every cell where an organism was present on the plate surface within the defined quadrat. A "non-colonizable" category was included to account for areas not accessible for organism settlement and recruitment (X-shaped bars and spacers). This method allows multiple taxa, overgrowing each other, to be observed in the same cell, potentially achieving total percentage coverage for the entire plate more than 100 %.

All motile organisms ("motile fauna") that remained in the plastic tank were filtered through 3 stacked sieves (2000 μm, 500 μm, and 106 μm mesh size). Here, according to the ARMS handbook (Version 2.0, Date: 2020-11-09; https://github.com/arms-mbon), only the material retained in the 2000 μm sieve, preserved in 70 % ethanol in falcon tubes and stored at +4 °C, were used for morphological analysis. Organisms were sorted and determined to the lowest possible taxonomic rank and counted using a stereomicroscope and optical light microscope.

Using the most up-to-date literature for the Italian coast (GSA-SIBM, 2024) and integrating it with the most recent pan-Mediterranean scale studies (Galanidi et al., 2023; Ragkousis et al., 2023), each identified species was classified as either native (including cryptogenic species and unresolved taxa, such as juveniles or damaged organisms that could not be identified at the species level) or NIS. This classification helped minimize the effects of NIS on the statistical analysis, as recommended in previous studies (Ferrario et al., 2024, 2017; Tamburini et al., 2021).

2.3. Data analysis

Data were analyzed using multivariate and univariate analyses. The sampling design was a two-way crossed design with Site as a fixed factor

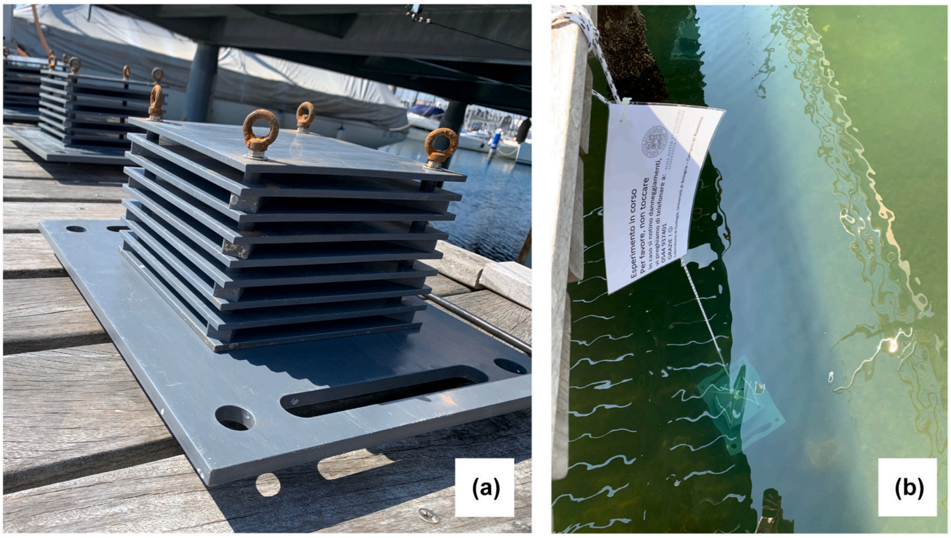

Fig. 2. (a) ARMS ready to be deployed. (b) ARMS deployed underwater and attached with a rope to the dock.

with two levels (Harbor and Marina) and Season as a fixed factor with three levels (winter, spring, summer) and three replicates (ARMS) for each Site x Season interaction.

The collected data were organized into three distinct datasets: ALL_Community, which contains presence/absence data for all identified taxa by combining the sessile and motile fractions; ALL_Sessile, which contains percent cover data of sessile organisms per ARMS; and ALL_Motile, which includes abundance data of motile organisms per ARMS (https://doi.org/10.5281/zenodo.10253209). A subset containing only NIS data was also extracted from each of these datasets (NIS_Community, NIS_Sessile, NIS_Motile).

The community composition of the ALL_Community and NIS_Community datasets was represented using two-dimensional nonmetric multidimensional scaling (nMDS), based on the Sorensen index. The univariate indices – Taxa richness (S), percent cover (for ALL_Sessile and NIS_Sessile), and abundance (N) (for ALL_Motile and NIS_Motile) – were also calculated.

Differences in community composition between Sites and Seasons for both the ALL_Community and NIS_Community datasets were analyzed by permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2017) based on the Sorensen similarity matrix, following the sampling design described above. Pairwise comparisons were done when significant main effects or interactions were detected. All PERMANOVA analyses were performed using unrestricted permutation of raw data and 9999 random permutations. When the number of unique permutations available was less than 100, asymptotic Monte Carlo *p*-values (P(MC)) were considered instead of permutational ones (P (perm)).

Two-way crossed ANOVA was used to assess spatial-temporal differences in univariate indices for all datasets and to evaluate the patterns of the most abundant NIS. The critical value was set to $\alpha < 0.05$, and Student-Newman-Keuls (SNK) tests were used for *post hoc* comparisons when needed. Before ANOVA, homogeneity of variances was tested by the Cochran test and in case of non-homogeneity of variances, the data were appropriately square-root or double square-root transformed. If the transformation was not successful a more conservative critical value ($\alpha < 0.01$) was applied.

To quantify β-diversity (β) and its nestedness (β_{NES}) and turnover (β_{TURN}) components, the approach proposed by Baselga (2012) was applied (Bevilacqua and Terlizzi, 2020). Sorensen diversity index for ALL_Community, and NIS_Community, was calculated in both sites, obtaining six triangular matrices. To evaluate whether seasonal variations in β-diversity differed between the two sites, a distance-based test for homogeneity of multivariate dispersions (PERMDISP; Anderson et al., 2006) was used. In PERMDISP, multivariate dispersion is calculated as the average distance to the group centroid within a resemblance matrix, which itself represents a distance-based measure of β-diversity (Anderson et al., 2011). Six separate PERMDISP tests were performed (9999 permutations were used), one for each dissimilarity matrix. This approach allowed to investigate whether β dissimilarity, together with its nestedness and turnover components, showed consistency between the two studied sites. PERMDISP tests were carried out on the dissimilarity matrices constructed at the site level to assess seasonal variations in β diversity and its components within each site.

All analyses were carried out with R software (R Core Team., 2024), using the packages “vegan” (Oksanen et al., 2024), “GAD” (Sandrini-Neto et al., 2024), “betapart” (Baselga, 2012; Baselga et al., 2023), and “ggplot2” (Wickham, 2016); and PRIMER 7 with PERMANOVA + add-on package (Clarke and Gorley, 2015; Anderson et al., 2008).

3. Results

Overall, 86 benthic taxa belonging to eight phyla were detected (27 Annelida, 23 Arthropoda, 12 Mollusca, 11 Bryozoa, 9 Chordata, 2 Porifera, 1 Echinodermata, 1 Platyhelminthes – all datasets are available in https://doi.org/10.5281/zenodo.10253209), and according to update literature (Galanidi et al., 2023; GSA-SIBM, 2024; Ragkousis et al., 2023), 16 of them are NIS (5 Arthropoda, 4 Annelida, 3 Mollusca, 2 Ascidiacea, 1 Bryozoa and 1 Porifera) (Table 1).

Structures of benthic communities appeared very different between sites and seasons (Fig. 3a) and were significantly influenced by an interaction between Sites x Seasons (pseudo-F = 9.729, p(perm) < 0.001). Pairwise tests revealed significant differences among seasons in each site and between sites in each season (Table SI 2).

Communities structures based only on NIS mirror the results obtained considering the whole communities as shown in the nMDS plot (Fig. 3b; Table SI 3).

The taxa richness of the ALL_Motile was significantly higher in the Marina than in the Harbor ($F = 57.316$, $p < 0.001$). Differences between Seasons were also significant ($F = 10.697$, $p < 0.01$), with spring and summer communities being significantly richer than those in winter (Fig. 4; Table SI 4a). The taxa richness of NIS_Motile followed the same pattern. Significant differences between Sites ($F = 16$, $p < 0.01$) and Seasons ($F = 15.438$, $p < 0.001$) were found, with taxa richness during winter being significantly lower than in spring and summer (Table SI 4b).

Mean abundance of ALL_Motile varied significantly between Sites ($F = 7.195$, $p < 0.05$), Seasons ($F = 24.398$, $p < 0.001$), and interaction Site*Season ($F = 6.334$, $p < 0.05$) (Table SI 5a). The highest abundance was observed in the Harbor during spring, with a mean of 3248 ± 749 individuals for ARMS (due to the low-species recruitment events of peracarids, especially Corophidae), while the lowest abundance was recorded in the Marina during winter, with 122 ± 27 specimens per ARMS (Fig. 5a). The abundance of NIS_Motile was significantly different between Sites ($F = 35.144$, $p < 0.001$), Seasons ($F = 24.100$, $p < 0.001$) and for their interaction Site*Season ($F = 20.707$, $p < 0.001$) (Fig. 5b, Table SI 5b). Overall, a higher abundance of NIS was found in the Marina than in the Harbor where, during winter they were absent. Moreover, in the Marina, a significant decrease of NIS from spring to winter occurred.

Considering ALL_Sessile, patterns of the total taxa richness were like those in the motile fraction (Fig. 6). Cumulative sessile taxa richness was significantly different between Sites ($F = 122.909$, $p < 0.001$) and Seasons ($F = 10.227$, $p < 0.01$) as well as for their interaction Site*Season ($F = 4.136$, $p < 0.05$) (Table SI 6a). The number of sessile taxa was higher in the Marina than in the Harbor across all Seasons ($p < 0.001$). Additionally, in the Marina, the taxa richness of ALL_Sessile was significantly higher during the summer and spring compared to the winter ($p < 0.01$) (Table SI 6a). The richness of NIS_Sessile was significantly different between Sites ($F = 56.333$, $p < 0.001$) and Seasons ($F = 30.333$, $p < 0.001$), and their interaction Site*Season ($F = 30.333$, $p < 0.001$) (Table SI 6b). The SNK pairwise test showed that NIS richness was significantly higher in the Marina than in the Harbor during spring and summer ($p < 0.001$). Moreover, NIS richness in the Marina was significantly higher in spring and summer compared to winter ($p < 0.001$) (Table SI 6b).

The abundance of sessile taxa, expressed as mean percent cover, was significantly different between Seasons ($F = 10.829$, $p < 0.01$) and for the interaction Site*Season ($F = 29.245$, $p < 0.001$) (Fig. 7a, Table SI 7a). Total percent cover was significantly higher in the Marina than in the Harbor during spring ($p < 0.05$) and summer ($p < 0.001$), while the opposite pattern was observed in winter ($p < 0.001$). In the Marina, the percent cover was significantly higher during spring and summer than in winter ($p < 0.001$). ARMS in the Harbor were mostly covered by NIS, whereas the Marina had a higher abundance of native species (Fig. 7). Significant differences in percent cover of NIS were observed between Sites ($F = 71.52$, $p < 0.001$), Seasons ($F = 23.061$, $p < 0.001$), and their interaction ($F = 80.618$, $p < 0.001$) (Fig. 7b, Table SI 7b). During winter and spring, NIS coverage was higher in the Harbor than in the Marina ($p < 0.001$), whereas the opposite was observed in summer ($p < 0.001$) (Table SI 7b). In the Harbor, NIS coverage in winter and spring was significantly higher than in summer ($p < 0.01$; $p < 0.05$), while in the Marina, NIS coverage in summer was significantly higher than in winter

Table 1

NIS found in the Harbor and in the Marina (Ravenna Port, Italy). Year = year of the first finding in the Mediterranean. Country = country of the first finding in the Mediterranean. Origin = native area of the species distribution. Vectors of the first introduction: A – aquaculture, S – ship transport, U – unknown (References in Table SI 1).

Species	Harbor	Marina	Year	Country	Origin	Vector
Annelida						
Ficopomatus enigmaticus (Fauvel, 1923)	X	X	1919	Italy	SW Pacific	S
Hydroides elegans (Haswell, 1883)	X	X	1868	Italy	Indo-Pacific	S
Podarkeopsis capensis (Day, 1963)	X	–	1982	Italy	SW Atlantic	U
Polydora cornuta Bosc, 1802	X	X	1986	Turkey	NW Atlantic	S/A
Arthropoda						
Amphibalanus eburneus (Gould, 1841)	X	–	1864	Italy	NW Atlantic	S/A
Caprella scaura Templeton, 1836	–	X	1994	Italy	W Indian	S
Grandidierella japonica Stephensen, 1938	–	X	2013	Italy	N Pacific	S
Paracerceis sculpta (Holmes, 1904)	–	X	1978	Tunisia	N Pacific	S
Paranthura japonica Richardson, 1909	–	X	2005	Italy	NW Pacific	A
Bryozoa						
Amathia verticillata (delle Chiaje, 1822)	–	X	1822	Italy	N Atlantic	S
Chordata						
Ciona robusta Hoshino & Tokioka, 1967	X	X	1816	Egypt	Uncertain	S
Styela plicata (Lesueur, 1823)	–	X	1877	Italy	NW Pacific	S
Mollusca						
Arcuatula senhousia (Benson, 1842)	X	X	1960	Israel	NW Pacific	S/A
Ruditapes philippinarum (A. Adams & Reeve, 1850)	X	X	1980	France	NW Pacific	A
Xenostrobus securis (Lamarck, 1819)	X	–	1991	Italy	SW Pacific	S/A
Porifera						
Paraleucilla magna Klautau, Monteiro & Borojevic, 2004	–	X	2000	Spain	Atlantic	S/A

and spring ($p < 0.001$) (Table SI 7b).

In the Figs. 8 and 9 are shown the spatial-temporal patterns of the most abundant NIS. The most abundant motile NIS were the arthropods *Paracerceis sculpta* (Holmes, 1904) and *Caprella scaura* Templeton, 1836 (up to 200 specimens each). Arthropods *P. sculpta, C. scaura, Grandidierella japonica* Stephensen, 1938 and *Paranthura japonica* Richardson, 1909 were found only in the Marina. The abundance of *P. sculpta, C. scaura,* and *P. japonica* was significantly higher in spring than in winter and summer, while the abundance of *G. japonica* was significantly higher during summer than during winter and spring (Table SI 8a–d). The polychaeta *Podarkeopsis capensis* (Day, 1963) was found almost exclusively in the Harbor, and its lowest abundance was observed during winter (Table SI 8e). The polychaeta *Polydora cornuta* Bosc, 1802 was found in both Sites, with the highest abundance in the Marina during summer (Table SI 8f).

The most abundant sessile NIS belonged to Serpulidae. Taxa belonging to this family could not be distinguished at the species level during the photo analysis. However, two of the three species later identified under a microscope from specimens detached from the ARMS were non-indigenous: *Ficopomatus enigmaticus* (Fauvel, 1923), and *Hydroides elegans* (Haswell, 1883) both of which were also the most abundant. In contrast, only a few individuals of the native *Hydroides dianthus* (Verrill, 1873) were found. Serpulidae had a maximum coverage of nearly 100 % in the Marina during summer and a minimum (less than 5 %) during winter (Fig. 9; Table SI 9a).

The most abundant non-indigenous ascidian was *Ciona robusta* Hoshino & Tokioka, 1967 which was significantly abundant in the Harbor during winter (Table SI 9b). The other three NIS were found exclusively in the Marina with a mean percent cover of less than 3 %: the bryozoan *Amathia verticillata* (delle Chiaje, 1822), the sponge *Paraleucilla magna* Klautau, Monteiro & Borojevic, 2004, and the ascidian *Styela plicata* (Lesueur, 1823) (Fig. 9; Table SI 9c–e). None of these species was detected during winter.

Regarding β-diversity, PERMDISP did not detect significant variations in compositional dissimilarity (β), nor in its turnover (β_{TURN}) and nestedness components (β_{NES}) between sites (Table SI 10) for both ALL_Community and NIS_Community. For ALL_Community, most of compositional β-diversity derived from species turnover, whereas the contribution of nestedness to the total dissimilarity was very limited (Fig. 10a). In contrast for the NIS_Community, the contribution of the nestedness component was higher than that of turnover r (Fig. 10b). Moreover, the mean values of β-diversity were greater for the ALL_Community than for the NIS_Community.

Turnover was the most significant component of β-diversity in ALL_Community at both the Harbor and Marina sites, with the Harbor exhibiting higher overall β-diversity values (Fig. 11a). Conversely, in NIS_Community, the major contributor to β-diversity was turnover in the Harbor and nestedness in the Marina (Fig. 11b). Detailed results of the PERMDISP tests and pairwise comparisons between seasons within sites are provided in Supplementary Information (Table SI 11).

4. Discussion

For the first time, Autonomous Reef Monitoring Structures (ARMS) have detected significant differences in the early stages of benthic communities between ports with different uses: the Harbor, primarily used by large commercial ships, and the Marina which serves smaller touristic boats. Seasonal differences were also detected and varied depending on the site. Finally, out of the 86 taxa detected, nearly 19 % (16 species) are identified as NIS, updating their list in this area.

The commercial harbor and the touristic marina in Ravenna are characterized by distinct communities in terms of species composition and relative abundance. However, several species are common to both sites, although with different relative abundance. These differences in fouling communities are likely influenced by the structural complexity of the surrounding environment and various environmental factors. Indeed, a significant periurban-urban gradient in key environmental parameters, such as temperature, salinity, turbidity, and nutrient concentration, was

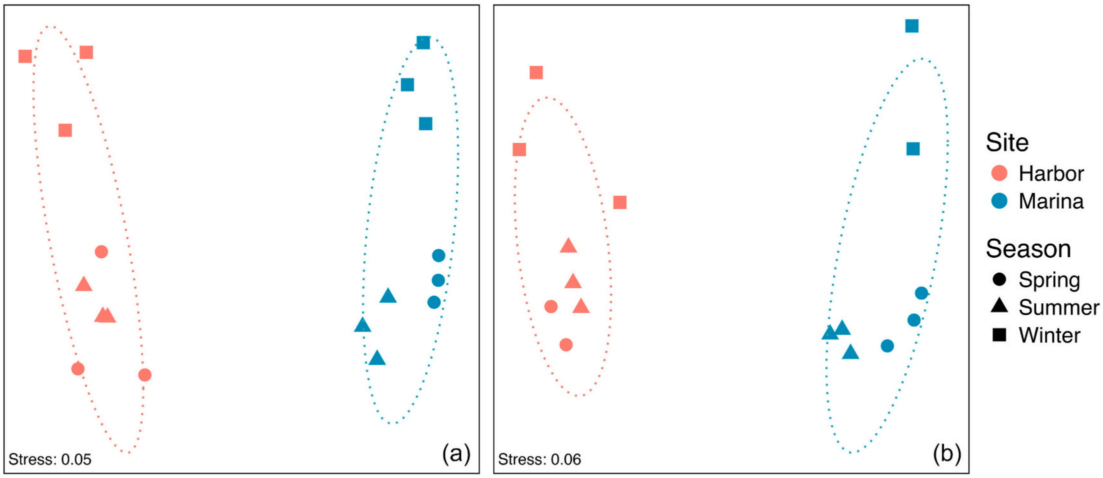

Fig. 3. Non-metric multidimensional scaling (nMDS) showing the differences in the structure of communities considering ALL_Community (a) and considering NIS_Community (b). Based on presence/absence data and Sorensen similarity in the two Sites and three Seasons. Note that the missing points overlap.

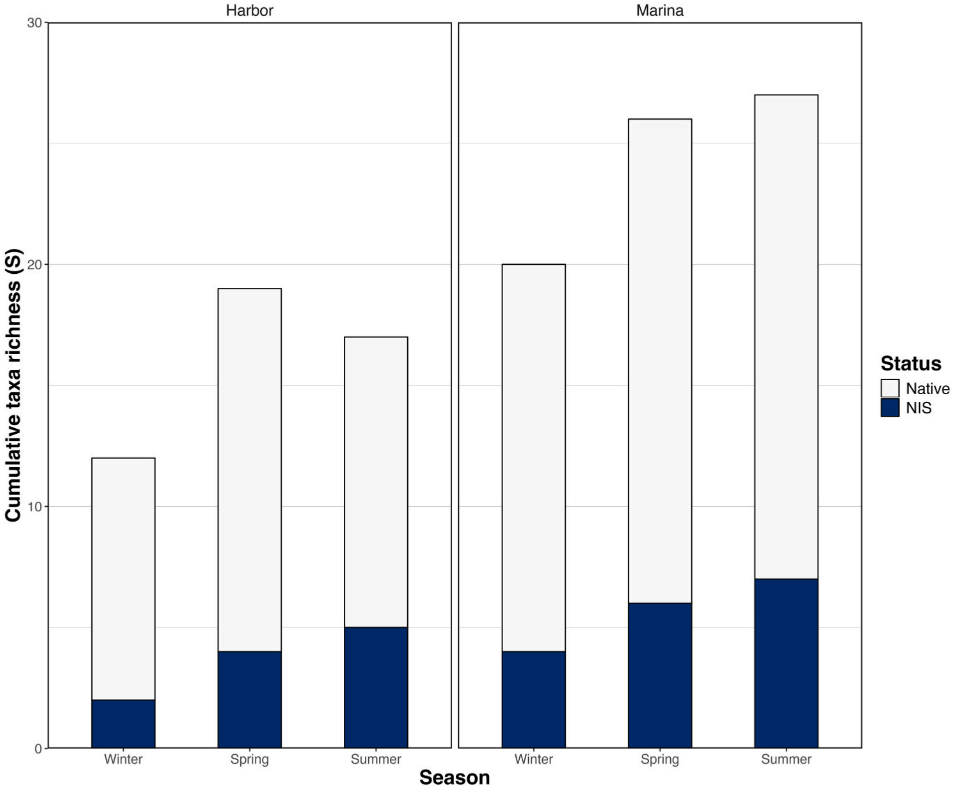

Fig. 4. Cumulative taxa richness (S) of motile fraction for three ARMS at the two Sites for each Season (each bar represents the sum of the taxa identified in the three replicates). Native (including cryptogenic and unresolved taxa); NIS – non-indigenous species.

previously observed, showing consistent dissimilarities between the outermost and innermost parts of Ravenna Port (Gauff et al., 2024). The Marina site, located in the outer part of the channel and separated from the sea by breakwaters, has better water circulation compared to the more confined Harbor site. Touristic marinas often feature more heterogeneous habitats (such as pontoons, docks, pilings, buoys, and ropes) that provide a variety of microhabitats for both native species and NIS to colonize. Moreover, the recreational boating typical of these areas promotes the local spread of NIS between nearby ports (Slišković et al., 2021; Ulman et al., 2019b, 2017). For these reasons, a higher taxa richness and a higher number of NIS were observed in the Marina compared to the Harbor, consistently with previous observations in other Mediterranean regions (Ferrario et al., 2017; Tempesti et al., 2025). In contrast, commercial harbors, typically semi-enclosed and highly polluted, experience frequent disturbances due to regular cleaning, maintenance, and elevated pollution levels. These factors, along with their generally homogeneous infrastructure and low environmental complexity, make harbors less favorable for long-term NIS establishment and spread (Tempesti et al., 2023, 2022b, 2020). Here, the Harbor site is situated at the end of the Candiano Canal, a confined area with limited water circulation, high turbidity, and significant pollutant accumulation from civil and industrial wastewater (Airoldi et al., 2016; Vassallo et al., 2013). This environment is less diverse than the Marina, consisting mainly of vertical concrete walls densely covered by mussels (Gauff et al., 2024). The ARMS in the Harbor

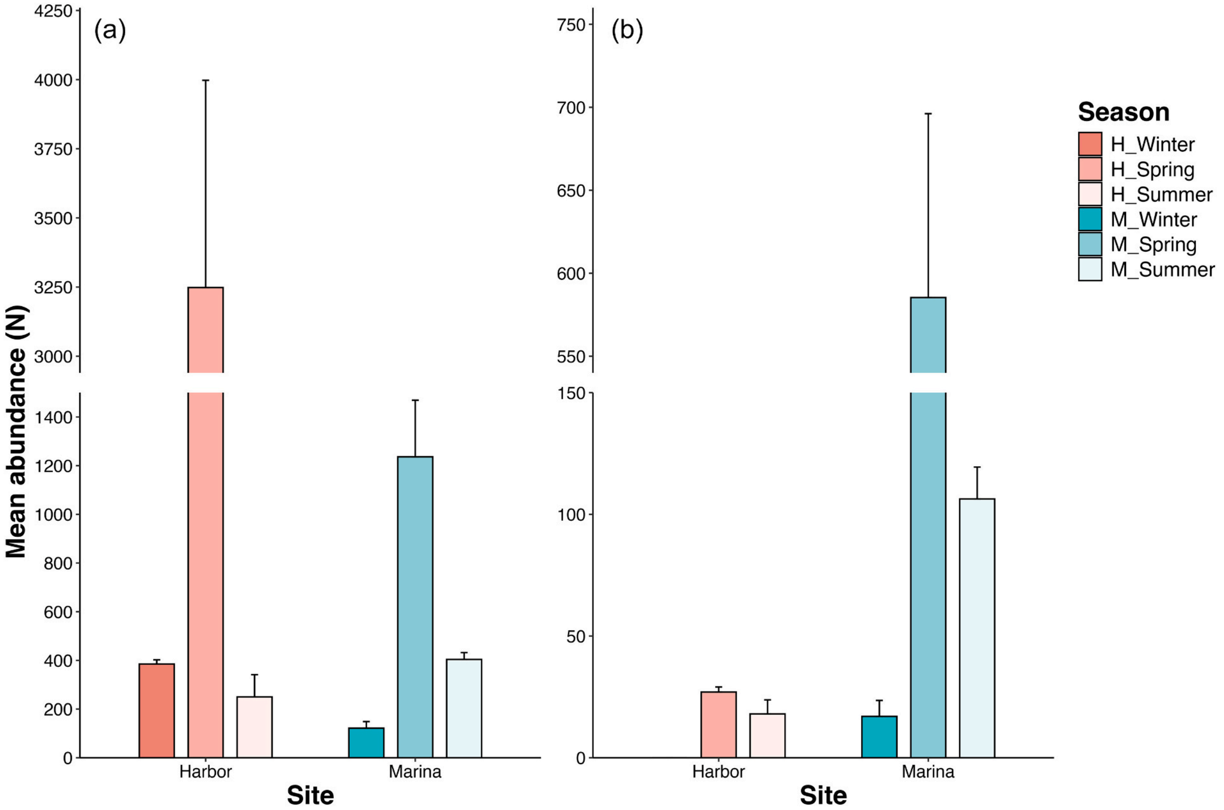

Fig. 5. Mean abundance (+SE, $n = 3$) of the motile fraction for three ARMS at two Sites for each sampling Season. (a) ALL_Motile. (b) NIS_Motile.

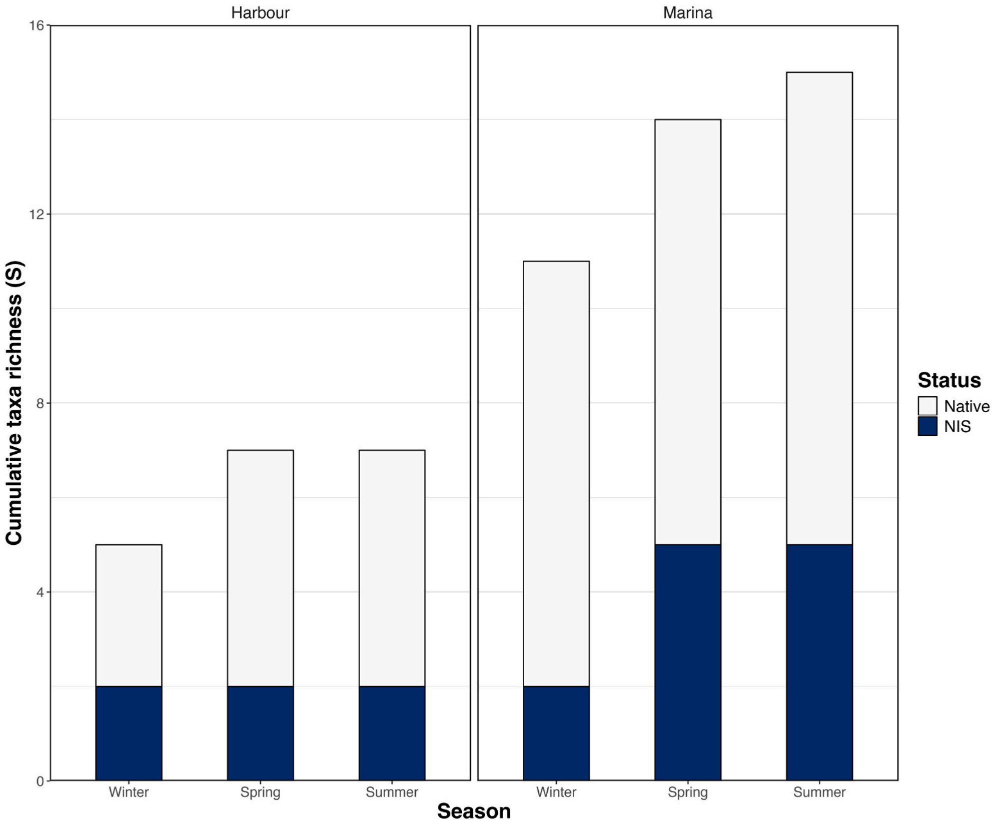

Fig. 6. Cumulative taxa richness of the sessile fraction for three ARMS across two Sites for each Season (each bar represents the total number of taxa identified in the three replicates). Native (including cryptogenic and unresolved taxa); NIS – non-indigenous species.

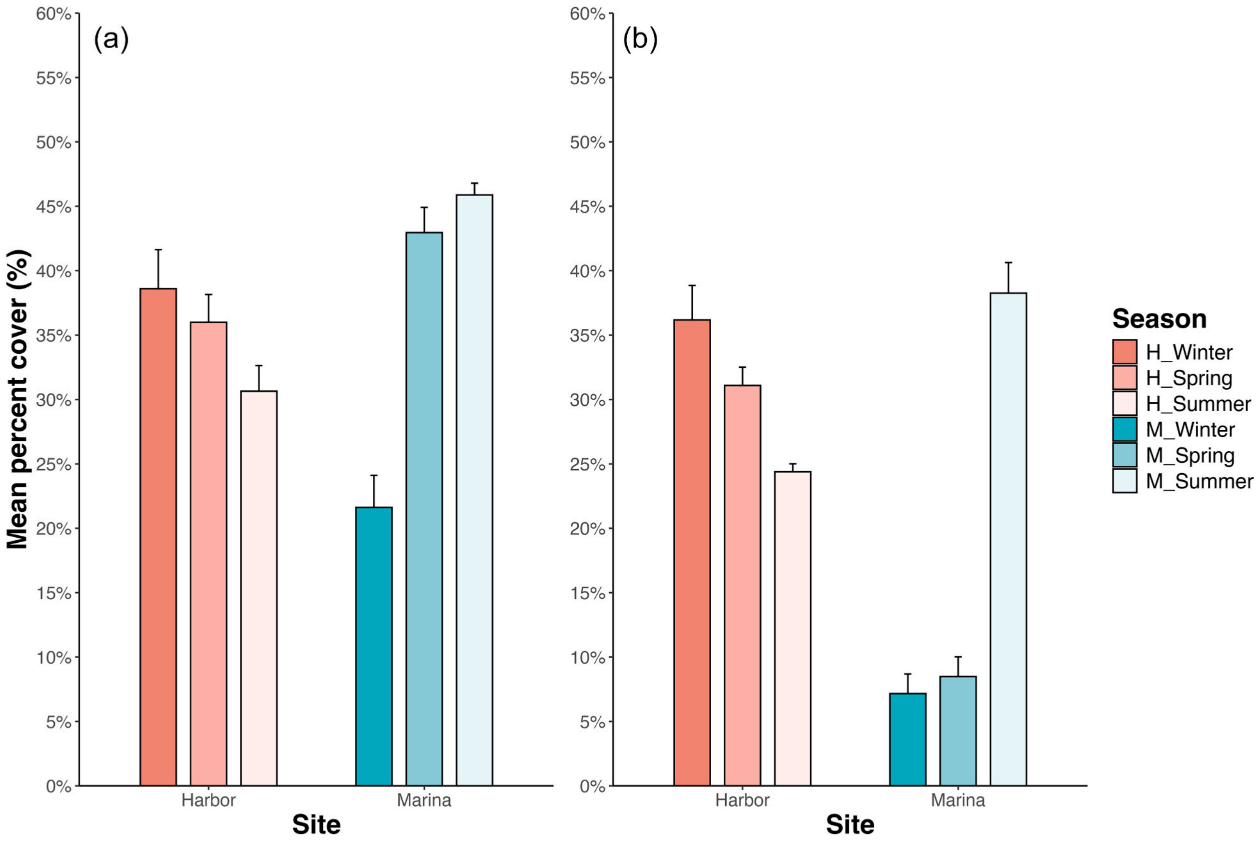

Fig. 7. Mean percent cover (+SE, $n = 3$) of sessile fraction for three ARMS in two Sites for each sampling Season. (a) ALL_Community. (b) NIS_Community.

revealed a relatively poor sessile fraction, dominated year-round by NIS polychaetes and ascidians. Indeed, while the Harbor hosts a greater abundance of motile taxa than the Marina, the taxa richness is lower. This lower environmental complexity tends to favor the proliferation of a few opportunistic species in high abundance (Airoldi and Bulleri, 2011; Olin et al., 2022).

The higher β-turnover compared to β-nestedness observed in the benthic communities indicates that species replacement plays a greater role in driving the overall difference between the Harbor and Marina sites. This suggests that the Harbor's environmental conditions are largely unsuitable for most species found in the Marina. For example, polychaetes and mussels, known for their tolerance to pollution, were richer in Harbor, while bryozoans, peracarids, and sponges, which are less tolerant, were richer in Marina. Conversely, the higher β-nestedness observed in NIS communities suggests that the Marina is richer in alien species than the Harbor. Differences in port usage, availability of empty substrates, and habitat complexity likely contribute to this pattern. Moreover, the β-turnover was the dominant component for benthic communities in both sites across seasons. The seasonal species replacement is likely closely related to the seasonal availability of different larval pools, driven by species phenology. This finding supports the use of ARMS, as they enable the detection of new recruitments each season. The only exception to the previous pattern was observed in the Marina for NIS communities, where nestedness emerged as the major component of β-diversity. This nestedness-resultant component reflects variations in the number of NIS due to species gain or loss across seasons. As before, phenology plays a key role in this pattern. In fact, here we're focusing on a subset of all species – the NIS communities – which are mostly of tropical origin and prosper in warmer conditions. In the Mediterranean, sea temperature increases during spring and summer, enhancing their spread and reproduction during those seasons. Additionally, since recreational boating activity is strongly linked to NIS introduction (Ferrario et al., 2024; Ulman et al., 2019a), and this activity also increases in warmer months, it further contributes to the observed pattern. Notably, the Marina itself hosts over 1150 recreational boats that move across other Mediterranean marinas (Airoldi et al., 2016), potentially introducing new NIS.

The significant seasonal differences in communities' composition observed are primarily related to the massive settlements of several species, mainly amphipods, ascidians, and serpulids following their breeding seasons. Sessile organisms like serpulids and ascidians compete for space and resources after settlement, shaping benthic community dynamics. Serpulids are more abundant in spring and summer, while ascidians, particularly *Ciona robusta* in the Harbor, peak in winter. *C. robusta* follows a two-phase settlement pattern, typically in early summer and fall, although this can vary with temperature and salinity (Bouchemousse et al., 2017; Kim et al., 2019). Dense aggregations of *C. robusta* are known to reduce species richness and may explain the fluctuation in serpulid abundance. *Ficopomatus enigmaticus* settles from April to November (Bianchi and Morri, 1996), overlapping with *C. robusta*, but its abundance is higher in spring and summer due to its faster growth rate. In winter, *C. robusta* becomes more abundant, suggesting that post-settlement competition for space and nutrients may favor ascidians over time (Blum et al., 2007; Carver et al., 2006), as the ARMS were deployed for nearly six months, compared to the three months of spring and summer.

Regarding motile fractions, spring shows the greatest abundance at both sites, with crustaceans and polychaetes as dominant groups. The highest overall abundance is recorded in the Harbor, coinciding with the spawning periods of amphipods and isopods (Delgado et al., 2009). The amphipod genus *Monocorophium* Bousfield & Hoover, 1997 was particularly abundant in the Harbor during spring, forming dense populations due to its preference for suspended sediment and particulate matter in warmer months, especially in polluted areas like harbors and marinas (Minchin, 2007; Nair and Anger, 1979; Rumbold et al., 2016). In the Marina, a higher abundance of *P. sculpta*, *C. scaura*, *P. japonica*, and the

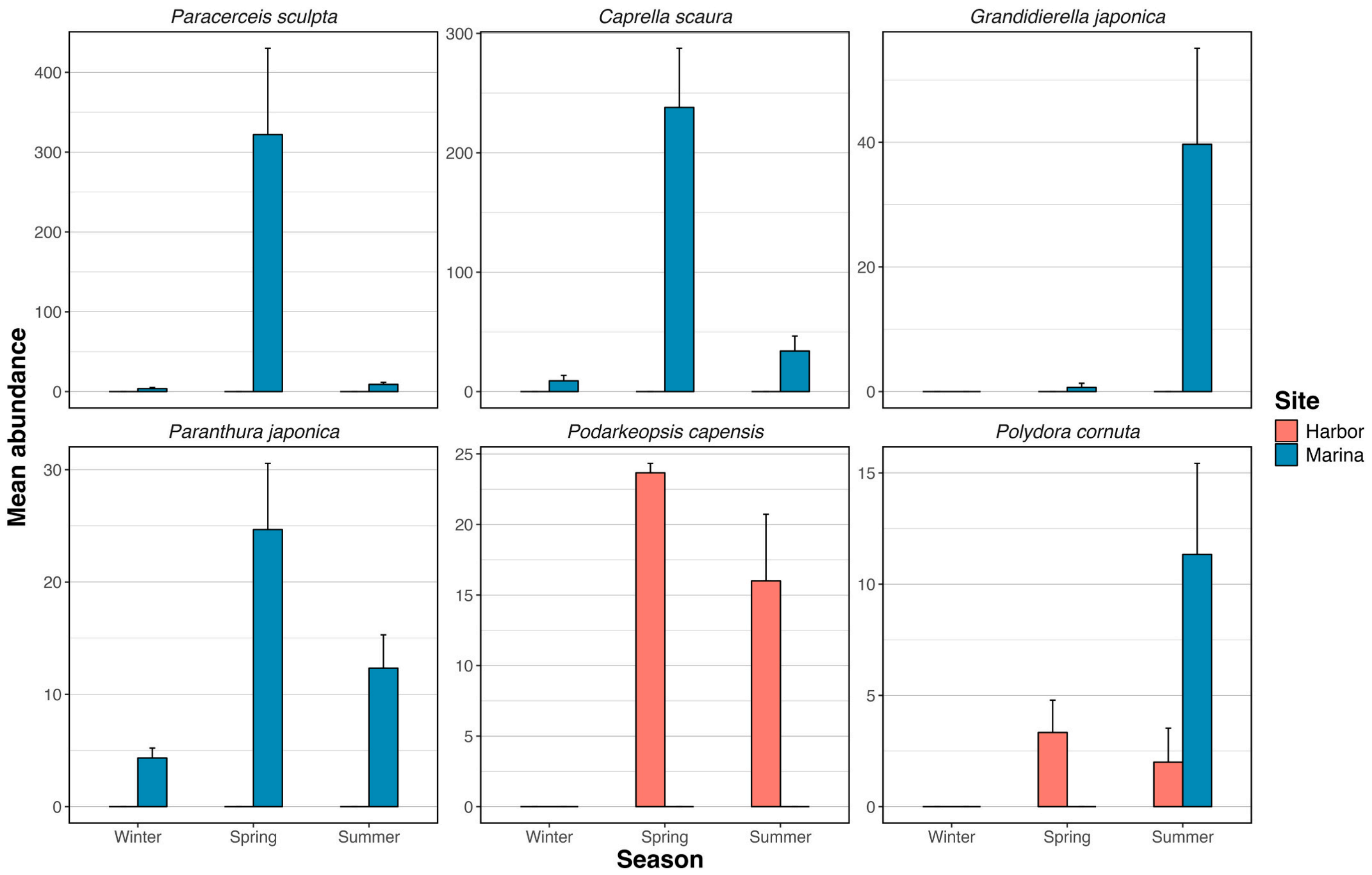

Fig. 8. Mean abundance (+SE, n = 3) of NIS_Motile at two Sites and in three Seasons.

polychaetes *P. capensis* and *P. cornuta* was observed during spring, consistent with their breeding and settlement seasons (Ariyama and Otani, 2004; Scribano et al., 2021). Although the aim of the work was not to evaluate the ecological succession of the communities, the high abundance of these species suggests that similar NIS with comparable characteristics could find suitable conditions for settlement. This observation highlights the need for continuous monitoring and management strategies, as the harbor and marina conditions may promote the settlement and spread of other NIS occupying similar ecological niches.

The NIS richness detected here is higher than that found in other Italian ports using traditional methods like scraping of port infrastructures (Ferrario et al., 2017; Spagnolo et al., 2019; Tempesti et al., 2022c) or others artificial monitoring structures such as settlement panels, Artificial Substrate Units (ASU), and ropes, recently used in the Mediterranean (Tamburini et al., 2021; Tempesti et al., 2023, 2022a). In general, artificial substrates favor non-indigenous and opportunistic species over native ones, resulting in communities that differ significantly from natural habitats (Momota and Hosokawa, 2021; Sanabria-Fernandez et al., 2018). The three-dimensional structure of ARMS, which mimics environmental complexity, allows for a more comprehensive and quantitative assessment of both sessile and motile fractions (Obst et al., 2020), while other methods typically focus on just one (Carlton, 2019; Marraffini et al., 2017). For example, by deploying settlement panels we wouldn't have succeeded to effectively collect or quantify the full range of peracarids that structure our communities. On the other hand, alternative ASUs like bath sponges and ropes would have limited our ability to accurately assess the coverage of sessile organisms, such as ascidians and serpulids, that fully encrust our ARMS during certain seasons. Additionally, the three-month deployment of ARMS, which captures early recruitment, may enhance NIS detection, as these species are known to colonize substrates more quickly and competitively than native species (Airoldi et al., 2015; Glasby et al., 2007). In the future, comparisons with coastal protection structures, such as breakwaters and groins, that are widely distributed in the area, could provide valuable insights on what proportion of the NIS are leaving the port and settling into these areas. The high number of NIS here detected, is in line with the well-documented characteristic of the Adriatic Sea as a hotspot for NIS introductions (Marchini et al., 2015b; Occhipinti-Ambrogi et al., 2011; Spagnolo et al., 2019).

Although all the detected NIS have already been reported in the northern Adriatic Sea, there is very limited research on fouling communities within the port of Ravenna, with existing studies focusing only on a few specific taxa (Gauff et al., 2024). In the Harbor, the most represented NIS were polychaetes, balanids, bivalves, and ascidians, with three species found exclusively therein: *Xenostrobus securis* (Lamarck, 1819), *Amphibalanus eburneus* (Gould, 1841), and *Podarkeopsis capensis.* The latter is considered "debatable/questionable" (Galanidi et al., 2023). These species are considered pioneers, known for their resilience to pollution and their ability to survive under stressful abiotic conditions such as high sea temperatures, elevated salinity, and hypoxia (Dineen and Hines, 1994; Gravina and Giangrande, 1988; Spagnolo et al., 2019).

In contrast, the Marina had a higher presence of NIS peracarids, bryozoan, ascidians, and sponge, with six species found exclusively here: the amphipods *Caprella scaura* and *Grandidieriella japonica*, the isopods *Paranthura japonica* and *Paracerceis sculpta*, the bryozoan *Amathia verticillata*, the sponge *Paraleucilla magna*, and the ascidian *Styela plicata*. Some species, like *A. verticillata*, can facilitate the presence of other NIS by increasing habitat complexity with its soft, bushy structure, providing a substrate and niche for smaller species like *C. scaura*, *P. japonica*, and *P. sculpta* (Marchini et al., 2015a). Additionally, peracarids spread by

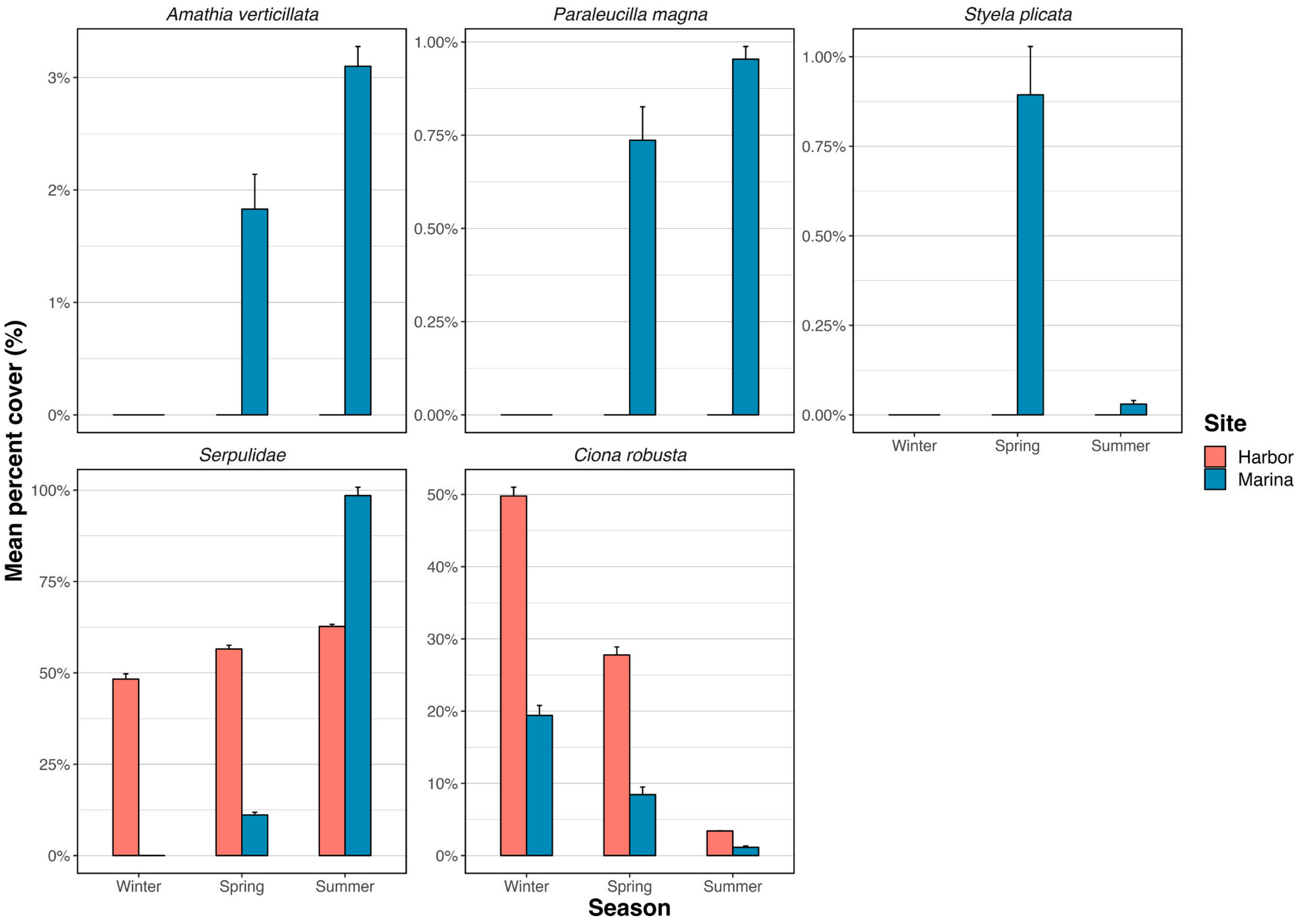

Fig. 9. Mean percent cover (+SE, n = 3) of NIS_Sessile at two Sites and in three Seasons.

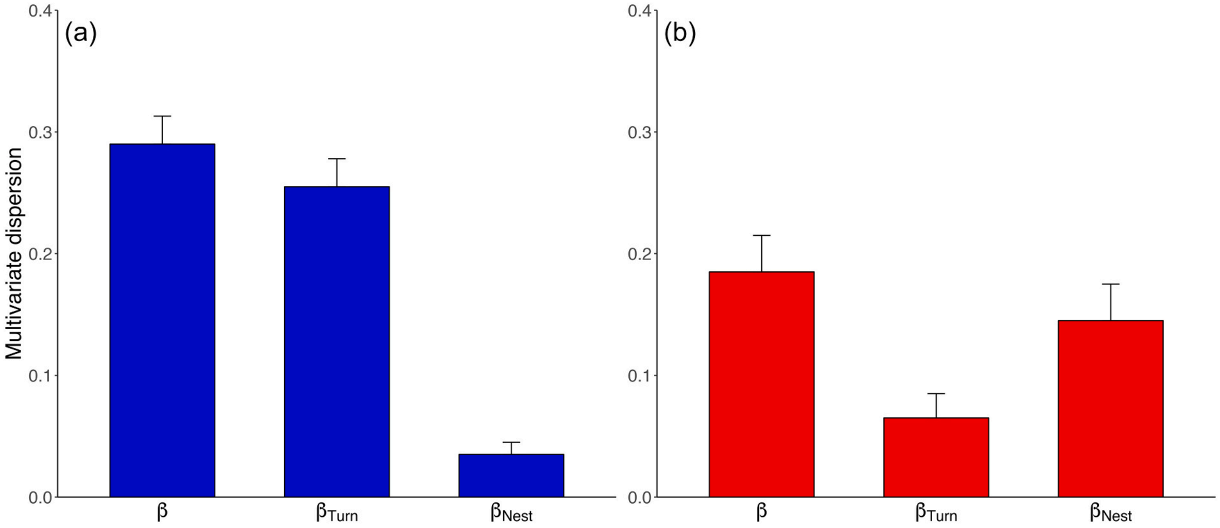

Fig. 10. Average distance (+SE) to the group centroid of benthic communities at sites in the space defined by (a) ALL_Community with blue bars, (b) NIS_Community with red bars, based on Sorensen dissimilarity matrices, and their respective turnover and nestedness-resultant components. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

recreational boats aided by sessile species (macroalgae, serpulids, barnacles, tunicates, bryozoans, and others) which encrust vessel hulls and provide shelter and substrate (Fernandez-Gonzalez and Sanchez-Jerez, 2014; Marchini et al., 2018, 2016, 2014).

The results of this research underlined also the importance to use an integrative approach in the studies of benthic fauna, combining photo analyses and morphological identification. In fact, several NIS of Serpulidae and Balanidae from the sessile fraction were possible to

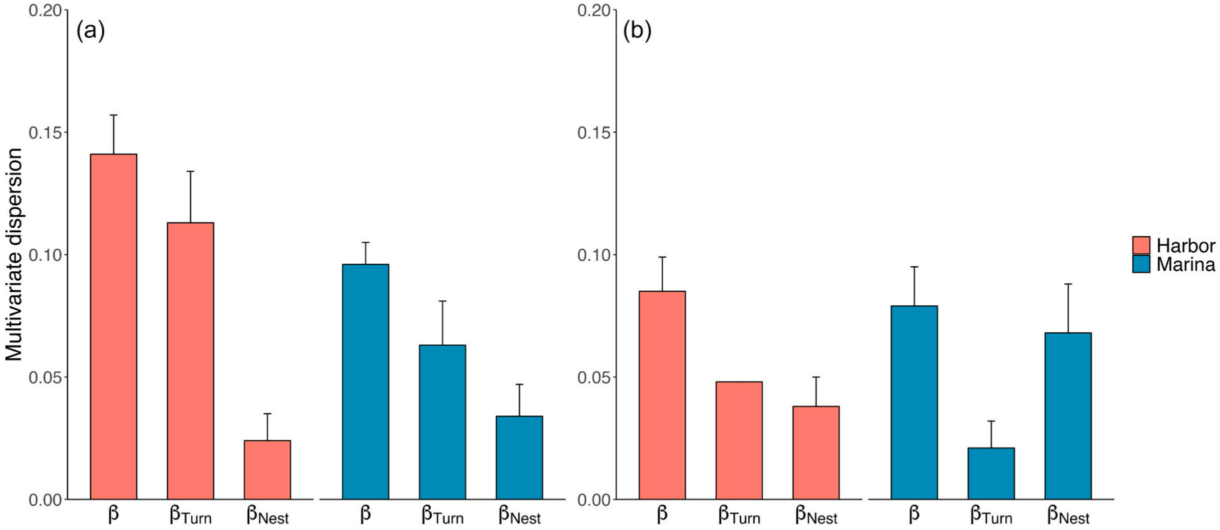

Fig. 11. Average distance (+SE) to the group centroid of benthic communities, between seasons in the space defined by (a) ALL_Community, (b) NIS_Community, based on Sorensen dissimilarity matrices, and their respective turnover and nestedness-resultant components.

determine only thanks to the morphological examination of several detached individuals.

5. Conclusion

In summary, our results demonstrate that ARMS are an effective tool for monitoring benthic communities and detecting NIS in ports. For long-term monitoring programs, deploying ARMS in the spring is ideal, as species richness is higher compared to winter and similar to summer. Furthermore, despite the increasing use of metabarcoding approaches in ARMS (Cecchetto et al., 2024; Martaeng et al., 2023; Obst et al., 2020; Santi et al., 2023; Thomasdotter et al., 2023), also for NIS detection (Pagnier et al., 2025), this study emphasizes the importance of combining photographic and morphological methods to achieve more accurate identification of NIS, methods which are often overlooked. Additionally, all the specimens collected and morphologically identified can contribute to the development of a local barcoding database for the northern Adriatic (Mugnai et al., 2023, 2021) and help prevent false positives (Fonseca et al., 2023), which remain two of the main limitations of complete ARMS processing (DNA metabarcoding of the sessile fauna grown on the ARMS plates).

CRediT authorship contribution statement

Alessandro Piazza: Writing – original draft, Investigation, Formal analysis, Data curation. **Barbara Mikac:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Marina Antonia Colangelo:** Writing – review & editing, Methodology, Formal analysis, Data curation, Conceptualization. **Federica Costantini:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

Authors Alessandro Piazza, Barbara Mikac, Marina Antonia Colangelo and Federica Costantini have nothing to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2025.118545.

Data availability

The datasets generated and/or analyzed during the current study are available in ZENODO: https://doi.org/10.5281/zenodo.10253209.

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