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Macroplastic colonization by macroinvertebrates in a Mediterranean wetland: A biodiversity enrichment opportunity

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ABSTRACT

Coastal wetlands are sensitive ecological systems that provide crucial ecosystem services, but often affected by anthropogenic pollutants. Plastics, in particular, represent a threat to the survival and fitness of many aquatic species. In fact, once plastics are released into freshwater environments, they can result in critical threats for fitness and survival of many aquatic organisms. Among these, macroinvertebrates represent a sensitive bioindicator for evaluating the environmental impacts of plastics. In this context, we investigated the colonization of virgin macroplastic substrates composed of two different polymers and located at two different depths in a protected wetland in Central Italy over a period of 10 months. The results show the tendency of macroinvertebrates to colonize plastic substrates artificially placed in water. Our findings highlight that macroinvertebrates mainly colonize polystyrene substrates over than polyethylene terephthalate ones. Moreover, floating substrates show a greater number of taxa found than dipped ones, highlighting that depth is also an important factor to discriminate the colonization of macroinvertebrates on plastic substrates. Furthermore, an ecologically diversified community emerged, in which there are mostly univoltine organisms, with dimensions between 5 and 20 mm, predators, choppers and scrapers that feed on plant organisms and animals. Consequently, plastic substrates might increase biodiversity in polluted waters by offering new surfaces for colonization. Overall, further studies are needed to determine whether the presence of plastic litter could also support the establishment of a macroinvertebrate community comprising taxa that exploit different ecological niches.

1. Introduction

Plastic is a material used daily worldwide, mostly because it is easy to produce and has a low cost of production (Pan et al., 2020). The use of plastics has increased in the last decades and the resulting pollution is considered one of the greatest risks for human health and ecosystems, as well as climate change (Cera et al., 2020; Napper and Thompson, 2020; Cesarini et al., 2021). To date, it is estimated that several million tonnes of plastic ended up in natural environments (So et al., 2022); for this reason, the “Anthropocene” era has been also called “Plasticene” era (Khan et al., 2020). It has been proven that the accumulation of plastics follows a specific gradient, being transported from continental waters to marine environments and in transitional areas such as wetlands (Ribeiro-Brasil et al., 2022). Additionally, plastics require millions of years to completely degrade (Chamas et al., 2020)and floating plastics can be subject to degradation phenomena in smaller items, causing secondary microplastics (< 5 mm), mainly due to mechanical degradation, UV degradation and photodegradation (van Emmerik and Schwarz, 2019; Andrady et al., 2022). However, the large part of plastic found in aquatic ecosystems (Cesarini et al., 2022) is mainly represented by macroplastics, defined by van Emmerik and Schwarz (2019) as plastic items > 5 cm., Macroplastics, characterized by large exposed surface (Cesarini et al., 2023), can be easily colonized by bacteria and other microorganisms that form a biofilm (Gui et al., 2021). Amaral-Zettler et al. (2020) coined the term "Plastisphere", referring to the niche, composed of one or more microbial communities, that forms

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above the plastic surface. Plastisphere is a unique microbial biofilm community colonizing plastic debris and microplastics (MPs) in aquatic environments: here it is possible find microbial photoautotrophs, heterotrophs, protistan grazers and decomposers (Delacuvellerie et al., 2019; Bocci et al., 2024; Yu et al., 2023). In fact, once in water, plastic debris provides hard surfaces for rapid microbial colonization and a new pelagic habitat for benthonic species. These organisms represent the first stages of plastic colonization. Plastisphere is not limited to microorganism but also includes other organisms, like macroinvertebrates, affected by the presence of plastics (Awuor et al., 2020; Tang, 2024), which represent the secondary colonizers of those items, attracted by food availability (Bocci et al., 2024; Gallitelli et al., 2023). Therefore, plastics can act as carriers for the transport of viruses, pathogens, and environmental contaminants (Bowley et al., 2022; Delacuvellerie et al., 2022). To date, several research have been published regarding the impacts of plastics in marine and freshwater ecosystems, while the research on plastics in wetlands is in its first stages (Qian et al., 2021; Taurozzi et al., 2022). Furthermore, biofilm on plastics is very similar to food sources of most aquatic biota in shape, smell, and colour (Du et al., 2022).

A significant portion of Plastisphere is often composed of bacterial, cyanobacterial, and fungal cells (Reisser et al., 2014); moreover, invertebrates represent an important part of Plastisphere, even if overlooked and little-studied (Amaral-Zettler et al., 2021). Most of studies focused on the microbial unicellular component of the plastisphere, omitting the secondary pluricellular colonizers of Plastisphere. Macroinvertebrates are one of the animal groups most used for the bioindication, and their use is frequently inserted in ecosystem monitoring projects and legislation around the world (De la Torre et al., 2021). Inland-water macroinvertebrates are animals that live, at least part of their life, on available substrates, using adaptation mechanisms capable of resisting the current (Buffagni, 2021). Macroinvertebrate communities are often characterized by different taxa that show varying degrees of sensitivity to pollution (Sumudumali and Jayawardana, 2021) and the community structure is influenced by numerous physical and chemical factors like the land use, altered catchment hydrology and inputs of organic nutrients (Dou et al., 2022). The characteristics of sensitivity to pollutants, high ecological relevance, long life cycles, easy sampling and identification, low mobility, make macroinvertebrates excellent bioindicators exploited in biomonitoring activities (de Almeida Pinto et al., 2021; Sumudumali and Jayawardana, 2021; Mezgebu, 2022). Since macroinvertebrates are benthic organisms, they can be impacted by plastic dispersed in aquatic environments (Ribeiro-Brasil et al., 2022). Plastic effects on biota are largely studied in literature. They mainly include ingestion and entanglement, which represent a widespread threat for biota (Staffieri et al., 2019; Poeta et al., 2017). Poeta et al. (2015) described the phenomenon of trap effect also on invertebrates by macroplastics: they demonstrated that the presence of the discarded bottles in dune systems may affect the structure and dynamic in macroinvertebrates populations and, as a consequence, could have a negative impact also on trophic chains. Similar results were highlighted by Gallitelli et al. (2023), where macroinvertebrates entrapment was evinced also in riverine habitats.

While several studies have examined the interactions between plastic litter and biota in marine environments (Aliani and Molcard, 2003; Astudillo et al., 2009; Goldstein et al., 2014) and freshwater environments (Wilson et al., 2021), there is a noticeable research gap concerning the specific effects and interactions of plastics on biota in transitional waters, particularly in coastal wetlands (Blettler and Mitchell, 2021). In Mediterranean wetlands, knowledge about the macroinvertebrate community associated with plastic surfaces remains limited. Besides, wetlands are considered as an aquatic-terrestrial ecotone, so it is important to evaluate the contribution of terrestrial and aquatic macroinvertebrate communities simultaneously and to date there are very few studies that have addressed this problem (Holmquist et al., 2018). Aquatic macroinvertebrates show different ecological niches and adaptations to the diverse ecological conditions of aquatic systems (Colas et al., 2014). In a coastal wetland, ecological conditions can be very different between the surface and the benthic (Perillo et al., 2018). While in the former there is a direct contact with atmosphere, which can lead in an enrichment of O_2 concentration, in the latter the O_2 concentration is generally lower. For this reason, we want to evaluate the colonization of macroinvertebrates community on plastic substrates in a wetland ecosystem. This study represents the first attempt to investigate the possible relationships between plastics and macroinvertebrates in a Mediterranean wetland (Ribeiro-Brasil et al., 2022). Here, two different plastic polymers, polystyrene (PS) and polyethylene terephthalate (PET), were selected to investigate whether the macroinvertebrate community showed preferences regarding the type of substrate to colonize in relation to the different depths of the substrates. PS is one of the six major polymers most produced and consumed, contributing to environmental pollution (Kwon et al., 2015). PS has been widely used as a packaging material for food and non-food applications, as a building insulator, on the manufacture of household items, on aquaculture, and on the electric, electronic and automobile industries (De la Torre et al., 2020). PET is used in daily life, and it is extensively used in the industries of plastic films and fibres (Dhaka et al., 2022). PS was demonstrated to reduce survival rate of organisms (Hsieh et al., 2023), to generate alterations in genetic material as well as the production of ROS (Nugnes et al., 2022) and swimming behaviour alterations (Gambardella et al., 2018). PET is believed to have transgenerational epigenetic effects on organisms, that is, an altered phenotype is expressed in the absence of mutational change (Heindler et al., 2017), ROS generation (Pencik et al., 2023) and reduced egg production (Shen et al., 2021).

In this sense, this research aims to evaluate the colonization patterns of macroinvertebrates on macroplastics artificially posed in a Mediterranean wetland. To do that, we also provided a complete overview about macroinvertebrates community patterns inhabiting artificial plastic substrates. To date, little is still known about the colonization patterns of macroinvertebrates on macroplastic litter, despite the large distribution of these contaminants. Moreover, while a certain importance is given to monitoring the “plastisphere” of marine litter, low attention is given to the biotic compounds of the coastal wetlands. To the best of our knowledge, this is the first research exploring macroinvertebrates richness, ecological characteristics and distribution on macroplastics in a Mediterranean coastal wetland.

We hypothesize that:

a. macroinvertebrates colonize plastic substrates
b. macroinvertebrates show a preference of polystyrene due to the presence of interstitial spaces which can be used as refuge.;
c. substrate depth does not influence macroinvertebrates colonization;
d. macroinvertebrates show ecologically diverse community assemblages.

2. Material and methods

2.1. Study area

The study area is the “Torre Flavia Wetland”, a protected area (40 ha) that is part of the municipality of Ladispoli and Cerveteri (Latium, Italy), located along the Tyrrhenian coast of Mediterranean Sea. The “Torre Flavia Wetland” consists of a sandy area open to the public adjacent to the sea and a system of marshy canals behind the dunes (Battisti et al., 2020a) (Fig. 1a). At a landscape scale, this area represents a remnant fragment of wetland inside an agricultural and urbanized matrix (Battisti et al., 2024). From the analysis of the land use extrapolated from Corine Land Cover (CORINE Land Cover, 2018), this area is described as “Complex cultivation patterns” in its entire extension; moreover, considering a 10 km^2 buffer area, 40 % of the area is described as “complex cultivation patterns”, 30 % as “non irrigated

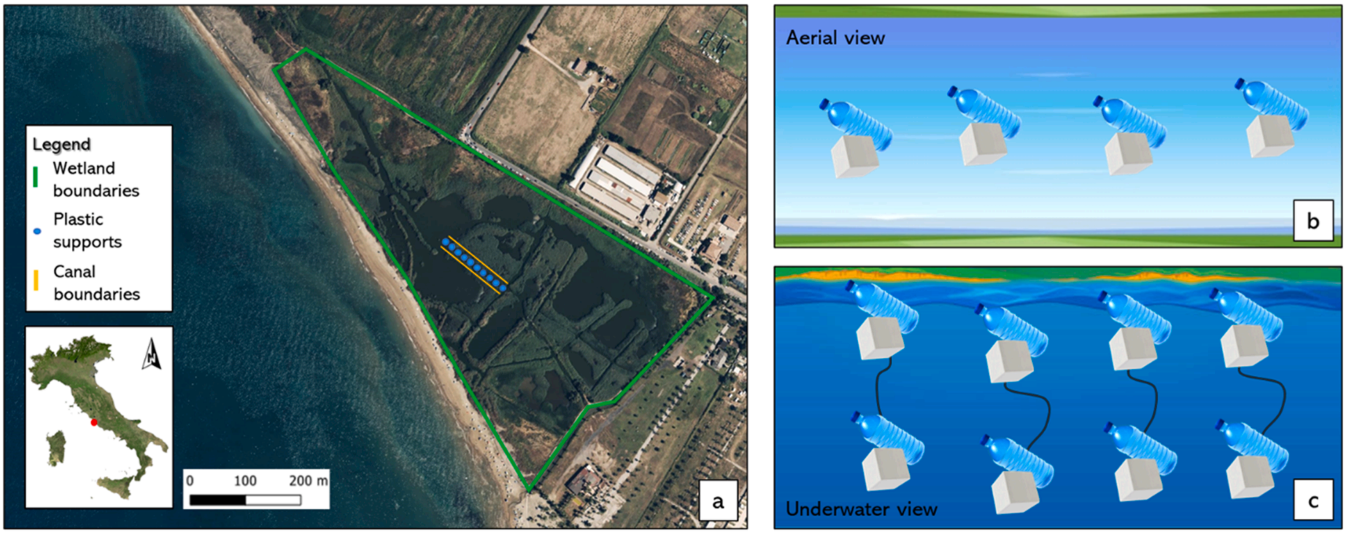

Fig. 1. Experiment location and design. a) Geographical location of the study site (the red dot indicates the sampling site located in Central Italy); b) diagram of the positioning of the substrates in the canal viewed from above; c) diagram of the position of the substrates in the water column.

arable lands" and 30 % as "discontinuous urban fabric" (Fig. S1). Given the absence of hydrologic dynamics and atmospheric data in this area, it is challenging to address the real threat for plastic pollution. The wetland *per se* is considered a well preserved ecosystem; however, sea spray (Harb et al., 2023), atmospheric deposition (Taurozzi et al., 2024) and direct pollution should be considered and analysed in further studies as sources of plastics in this area. This coastal wetland represents a hotspot of biodiversity for plants and animals (Battisti et al., 2021), where the presence of species such as *Eryngium maritimum* (Linnaeus, 1753), *Elymus farctus* (Runemark ex Melderis, 1978) is documented as well as many animal species such as *Mugil cephalus* (Linnaeus, 1758), *Anguilla anguilla* (Linnaeus, 1758), *Pseudopidalea viridis* (Laurenti, 1768), *Natrix natrix* (Linnaeus, 1758), *Procambarus clarkii* (Girard, 1852). Several studies were performed about invertebrates for this wetland: Romiti et al. (2021) investigated the impact of anthropogenic litter on sand-dwelling beetles; Battisti et al. (2023) highlighted the occurrence of the alien invasive crab *Callinectes sapidus*; Amori and Battisti (2007) and Scalici et al. (2010) provided an account of alien species richness and composition, including several invertebrates like *Procambarus clarkii, Stenopelmus rufinasus*; Iannilli et al. (2018) described evidences of microplastics in *Talitrus saltator*. Here, for the first time, we investigated macroplastic colonization by macroinvertebrates.

2.2. Experimental design

Ten substrates were inserted within one of the swamp canals with an approximate distance of about 3 m from each other and 1 m from the banks of the canal to investigate the macroinvertebrate colonization (Fig. 1). Entrance to the investigated canal is forbidden to the public.

The substrates were created in the laboratory and then transported to the field. Each substrate was made of 4 units: two polystyrene (PS) cubes (20 cm on a side) and two polyethylene terephthalate (PET) bottles (32.6 ×8.2 cm), two of the most common used plastic polymers (van Emmerik and Schwarz, 2019). PET is the largest tonnage plastics polymer (Ronca, 2017) and the highest volume global plastic available today (Patel, 2016). PS is one of the most important and widely used plastic; it is heat resistant, lighter in weight and, exhibits good strength and durability that make this polymer fit for a variety of applications (Maafa, 2021) Indeed, PET and PS were recorded as one of the most abundant plastic polymer in the sampling area (Gallitelli et al., 2022; Cresta and Battisti, 2021).

The PS cube and the PET bottle were connected by a rope and one of the two bottles was filled with sand to make possible to sink on the bottom and act as ballast. Therefore, four typologies of epiplastic microhabitats were evaluated: floating and dipped polystyrene (fPS and dPS, respectively) and floating and dipped polyethylene (fPE and dPE, respectively). Samplings were conducted every 20 days, during 10 months from November 2019 to August 2020, with an interruption of sampling for 3 months from March to May due to the COVID-19 pandemic. Depth was around 1.5 m and clarity was low because of eutrophication.

During each sampling, the substrates were recovered handling only the rope which linked the surface substrate with the bottom ones to avoid macroinvertebrate individuals' loss: only one of the colonization substrates (floating and dipped) was recovered without replacement. Then, substrates were stored into plastic trays. The analysis of each plastic unit in the field was performed into white sorting boxes for the better identification of macroinvertebrates, and to avoid macroinvertebrates losses. The surface of each plastic unit was analysed in field for preliminary research of macroinvertebrates: the organisms were collected with metal tweezers and immersed in a 50 ml falcon containing ethanol (70 %). After that, the substrates, inserted into different containers to avoid contamination of material between one unit and the others, were transported to the laboratory for a more accurate search of macroinvertebrates, especially within the PS substrates due to the porosity. Considering the possible plastic loss and the consequent wetland pollution, the substrates were weighted before the deposition and after the withdrawal from the canal. The substrates weight results the same before and after the deposition (the weight of organic matter entrapped during the experiments, i.e., macroinvertebrates and algal depositions, was subtract after the weigh). So, no plastic losses occurred.

2.3. Data analysis

The analysis on macroinvertebrates were carried out using a stereomicroscope (Nikon C-LEDS with 4.0x objective) for detailed identification of samples. The morphological identification of the taxa was carried out using specific atlases for the recognition of macroinvertebrates (Ghetti, 2001; Campaioli et al., 1994; Sansoni, 1998; Chinery, 2010), considering the possible presence of species belonging to terrestrial taxa and not necessarily related to the aquatic environment. Using dichotomous keys, it was possible to identify each individual at least at the level of the family they belong to. All samples were preserved in falcon with an ethanol solution (80 % v/v) divided by taxon, date, plastic substrate, and position of the substrates (floating or dipped).

Normality, homogeneity of variance and independence were tested before performing statistical analysis. Given all the assumptions checked, we performed both parametric and no-parametric tests in our analysis.

Alpha diversity was calculated through diversity indexes. Diversity indexes were calculated to test differences in the species community assemblages. Specifically, the diversity indexes calculated were: a) Shannon diversity index (H); b) Simpson index (D); c) Pielou's evenness; d) Number of species.

Analysis of Variance (ANOVA) test is a statistical method that simultaneously compares means across several groups to determine if observed differences are due to chance or reflect genuine distinctions; to evaluate differences considering both water depth and plastic polymers as categorical variables and their interaction on macroinvertebrates communities, a two-way ANOVA was performed. Permutational Multivariate Analysis of Variance (PERMANOVA) is a non-parametric test of significant difference between two or more groups, based on any distance measure: here, a two-way PERMANOVA with Bray-Curtis dissimilarity index was performed to compare the community structure between the two substrates and the depths.

Given the non-normal distribution of the data and differences in the homogeneity of error variance, Kruskal-Wallis tests were performed to evidence differences on the number of taxa and individuals found in the four substrates. Then a Dunn's post hoc test was applied.

The values of the alfa diversity indices were transformed using log $(x + 1)$ and temporal trends in the indices were search through the application of linear bivariate models. The analysis of temporal trends could provide important insights about turnover in species traits and ecological community stabilization.

Seven ecological traits were considered for macroinvertebrates, including biological information on life cycle, ability of resistance and resilience and behavioural aspects of nutrition (Tachet et al., 2010; Usseglio-Polatera et al., 2000): 1) Maximal size, 2) Life cycle duration, 3) Potential number of reproduction cycles per year, 4) Dispersal, 5) Resistance form, 6) Food, 7) Feeding habits. Traits were coded using a 'fuzzy' approach, in which a value given to each trait category indicates if the taxon has no (0), weak (1), moderate (2), strong (3) very strong (4) affinity with the category (subtrait or modality) (Tab. S6) (Tachet et al., 2010). Affinities were determined based on expert opinions that fell within each category for each trait. Fuzzy coding can incorporate intra-taxon variability when trait profiles differ among e.g. species within a genus, early and late instars of one species, or individuals of one species in different environments. Most traits were coded at family level, because of their complex taxonomy, identification difficulties and the scarcity of reliable information about their traits.

Bivariate linear models were applied to relate the number of species belonging to a specific trait found in each of the four cases with time, in order to search for any significant temporal trends. ANOVA tests were performed for comparing the distribution of individuals among traits' subclasses between different plastic supports.

All the statistical analysis were carried out using the software "Paleontological Statistics" (PAST) (Version 4.02), considering significative p-values those with values lower than 0.05.

3. Results

3.1. Macroinvertebrate community on plastic substrates

Overall, 36 families within the 39 samples (one PS cube has been lost) were found. Macroinvertebrates were found in each sample on the fPS and dPS substrates, while, regarding dPE and fPE substrates, macroinvertebrates were found in 30 % of the samples for both the categories. Both aquatic and terrestrial species were found.

All identified families belong to 13 classes, orders and/or suborders: Diptera, Coleoptera, Ephemeral, Heteroptera, Hymenoptera, Annelid, Arachnid, Colembola, Oligochaeta, Trichoptera, Tisanoptera, Gastropod and Crustacean. The classes most represented (i.e., those with a number of taxa found in at least 5 of the 10 total samplings) were Arachnids (22 %), Coleoptera (22 %), Diptera (20 %) (Fig. 2).

On fPS substrates we identified individuals belonging to 30 families; on dPS substrates we identified individuals belonging to 5 families; on fPE substrates we identified individuals belonging to only 1 family; on dPE substrates we identified individuals belonging to 3 families (Fig. 3).

The taxa found with higher frequencies (i.e., those taxa found in at least 5 of the 10 total samplings) on fPS substrates respect to the total number of taxa found were: Chironomidae (10 %), Staphilinidae (9 %), Hydrenidae (7 %), Naididae (7 %), Ceratopogonidae (6 %), Phlaeotripidae (6 %), Formicidae (6 %), Trachelidae (5 %). The only taxon found in all the four cases was Chironomidae (Table S1).

The two-way ANOVA test performed on the number of taxa found showed significant differences only for the "polymer" variable ($p < 0.05$), while "depth" and the interaction of these two variables didn't show significant differences (Tab. S4, Fig. 4).

PERMANOVA test was performed to compare the community structure between the two substrates and the depths. "Polymer" and "interaction" showed significant differences ($P < 0.05$) between community structures, while "depth" was not significant (Tab. S5).

Kruskal-Wallis test performed on the number of taxa found in the four substrates highlighted that there was a significant difference between sample medians ($H = 27.62$, $p < 0.05$). Dunn's post hoc test highlighted significative differences ($p < 0.05$) between fPS and dPS, fPS and fPE, fPS and dPE, dPS and fPE, dPS and dPE.

Overall, 802 individuals belonging to 36 taxa were identified (Fig. 5).

Overall, the largest number of individuals (> 75 %) was found in Chironomidae, Naididae and Phlaeothripidae. On floating substrates, the largest number of individuals was found within the Hydrenidae, Phlaeothripidae, Staphilinidae, Chironomidae, Naididae, Formicidae. On dipped substrates, the largest number of individuals was found within the Chironomidae and Naididae. Sampling VII (after 216 days) was the only one in which individuals were found in all four analysed samples.

In particular, we identified 6 families with an abundance higher than 2 % respect to the total number of individuals (Table 1).

Dunn's post hoc test highlighted significative differences ($p < 0.05$) between fPS and fPE, fPS and dPE, fPE and dPS, dPS and fPE, dPS and dPE. (Fig. 6).

3.2. Diversity indexes

Four diversity indices were calculated for each substrate: Shannon index (H'), Simpson index (λ), Evenness (J), number of species (No. species) (Table S2, Table S3, Fig. 7). Individuals were found in the PE substrates in only 3 samples out of 10, therefore the analysis of the indices focused only on the PS substrates.

The values of the indices were transformed using log $(x + 1)$ and

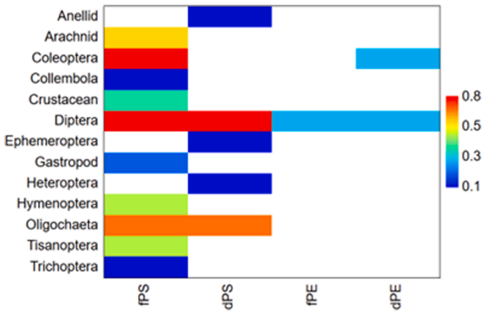

Fig. 2. Frequencies of the taxa found respect the total number of substrates on floating (fPS) and dipped polystyrene (dPS), and on floating (fPE) and dipped polyethylene (dPE).

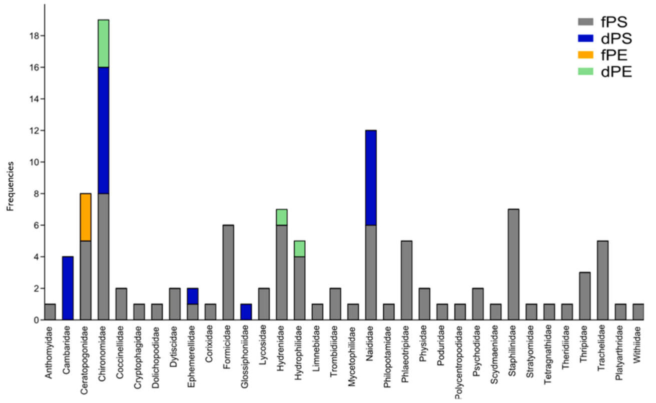

Fig. 3. Frequencies of the macroinvertebrate families found on the four substrates: floating polystyrene (fPS), floating polyethylene (fPE), dipped polystyrene (dPS) and dipped polyethylene (dPE).

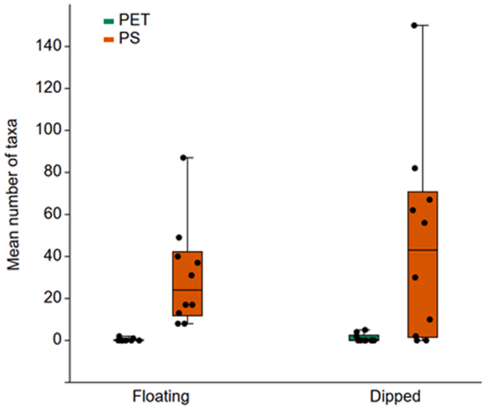

Fig. 4. Estimated taxa means in function of depth (floating and dipped) and plastic polymer (polystyrene (PS) and (PET)). Mean number of individuals for PS (orange) are much higher than for PET (green). Means for floating substrates are slightly lower than the means for dipped substrates.

temporal trends in the indices were search through the application of linear bivariate models.

The results obtained show a significant correlation with time regarding the Shannon index on dPS ($p = 0.02$, $R^2 = 0.55$) and the Evenness index on fPS ($p = 0.03$, $R^2 = 0.45$), while there are no significant correlations for the Simpson indices and for the number of species, in addition to the Shannon index on fPS and the Evenness index on dPS (Fig. 8).

3.3. *Functional traits*

Macroinvertebrates biological functional traits showed large heterogeneity regarding body measures, feeding habits and dispersion, while a certain homogeneity emerged for life duration, reproduction strategies and resistance forms: as regards the “Maximal size” trait, 72 % of individuals ranged from 10 and 20 mm, 25 % between 2.5 and 5 mm, 2 % between 5 and 10 mm and 1 % between 20 and 40 mm. As regards the “Life cycle duration” trait, emerged that 64 % of taxa and 73 % of individuals had a life duration less or equal than one year. As regards the “Potential number of reproduction cycles per year” trait, a 78 % of polyvoltine individuals and 58 % of univoltine taxa emerged. As regards the “Dispersal” trait, there is a uniform distribution in the four subclasses, with animal taxa characterized by active, passive, aquatic and aerial dispersion; however, most of individuals (67 %) show passive anemochory, followed by active anemochory (27 %), aquatic passive (5 %) and active dispersion (1 %). As regards the “Resistance form” trait, animal taxa without any form of resistance are predominant (61 %; 99 % of individuals). As regards the “Food” trait, emerge that most animal taxa (21 %) and individuals (68 %) feed on detritus smaller than a millimetre, like live macrophytes (25 %) and live microinvertebrates (5 %). As regards the “Feeding habits” trait, 74 % of animal taxa were choppers, scrapers and predators were found. For all the traits considered the analysis did not show significant temporal trends (MS: $R^2 = 0.02$, $p > 0.05$; RF: $R^2 = 0.2$, $p > 0.05$; F: $R^2 = 0.004$, $p > 0.05$; FH: $R^2 = 0.003$, $p > 0.05$). Moreover, plastic substrates did not show significant differences in the distribution of individuals among traits’ subclasses for “Maximal size” ($F = 0.73$, $p > 0.05$), “Life cycle duration” ($F = 0.79$, $p > 0.05$), “Potential number of reproduction cycles per year” ($F = 0.73$, $p > 0.05$), “Dispersal” ($F = 0.74$, $p > 0.05$), “Resistance form” ($F = 0.68$, $p > 0.05$), “Food” ($F = 0.73$, $p > 0.05$) and “Feeding habits” ($F = 0.74$, $p > 0.05$).

4. Discussion

There is a lack of knowledge about the impacts of plastic pollution in Mediterranean wetlands (Taylor et al., 2021). Studies regarding the colonization of macroinvertebrates on plastic litter (Wilson et al., 2020; De la Torre et al., 2021; Kallenbach et al., 2022) and their possible interaction are lacking, many of which focusing on marine ecosistems. The results obtained in this study show a direct relationship between

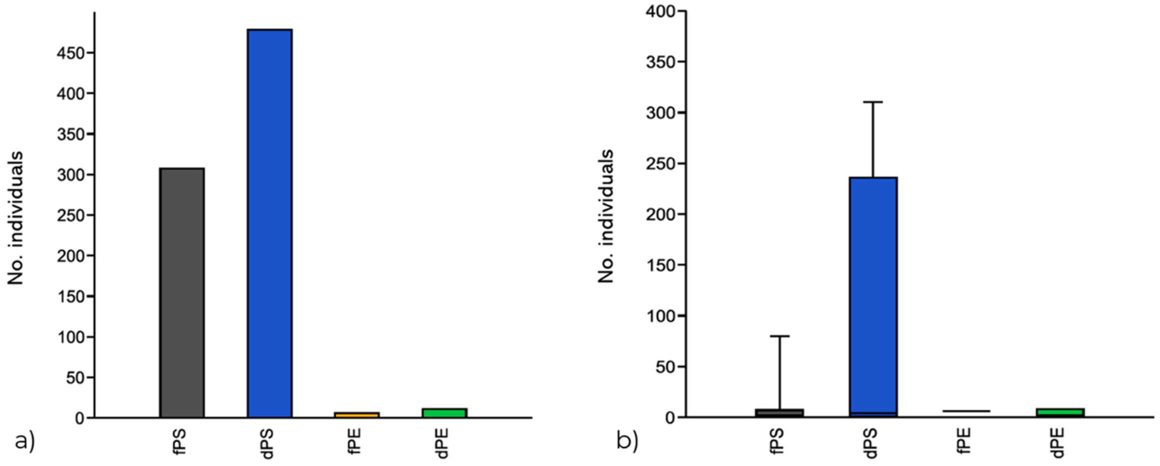

Fig. 5. a) Number of individuals found and b) distribution of individuals among samplings on floating (fPS) and dipped polystyrene substrates (dPS), and on floating (fPE) and dipped polyethylene substrates (dPE) (No. individuals= number of individuals).

Table 1
Values of minimum (Min), median and maximum (Max) of the families with the higher number of individuals (> 2 %) respect to the total number of individuals.

Macroinvertebrates families	fPS			dPS			fPE			dPE		
	Min	Median	Max	Min	Median	Max	Min	Median	Max	Min	Median	Max
Chironomidae	0	2	4	0	7	63	1	2	3	2	2	4
Formicidae	0	1	13	-	-	-	-	-	-	-	-	-
Hydrenidae	0	7	20	-	-	-	-	-	-	-	-	-
Naididae	0	1	21	0	12	147	-	-	-	-	-	-
Phlaeothripidae	0	2	40	-	-	-	-	-	-	-	-	-
Staphilinidae	0	2	8	-	-	-	-	-	-	-	-	-

Kruskal-Wallis test performed on the number of individuals found in the four substrates highlighted that there was a significant difference between sample medians ($H = 24.52$, $p < 0.05$).

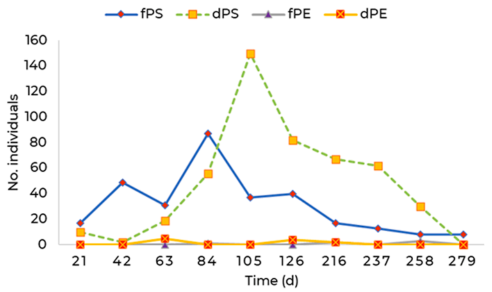

Fig. 6. Number of individuals found on floating polystyrene (fPS), floating polyethylene (fPE), dipped polystyrene (dPS) and dipped polyethylene (dPE) substrates from the first sample to the last one (time is expressed in days) (No. individuals = number of individuals).

macroinvertebrates and macroplastics in a coastal wetland: macroinvertebrates tend to colonize plastic substrates placed in wetlands. In particular, the results obtained show a greater trend in the taxa colonization in PS substrates than in PET ones. Redondo-Hasselerharm et al. (2020) demonstrated the reduction of abundance of macroinvertebrates after exposure to different PS particles but there are no studies that investigates the preference of macroinvertebrates for settlement on PS than other plastic polymers. Precedent studies also focused on the importance of two parameters, rugosity and the refuges offered both by macrophytes, for the settlement of macroinvertebrates (do Nascimento Filho et al., 2021). One reason for the different colonization on plastic substrates can be found in the intrinsic structure of the substrates used: PS is made up of small, rounded units interspersed with interstices, while PET is a homogeneous substrate (Yang et al., 2009). The importance to find shelter could favour the choice of PS over PET, because numerous animal taxa can find refuge within, avoiding stresses from natural phenomena such as wind or rain and predation phenomena (Wilson et al., 2020). There are several factors which can enhance the settlement of macroinvertebrates communities, in particular the type of material substrate (Molokwu et al., 2014; Gustafsson et al., 2013; Schmude et al., 1998), the roughness of substrates (Natsumeda et al., 2019; Barnes et al., 2013), the substrate colour (Gallitelli et al., 2023) and the presence of a biofilm on the surface (Devakie and Ali, 2002). The importance of substratum to macroinvertebrate communities has been recognized for a long time. Substratum surfaces provide attachment sites and shelter from predators, and allow the development of an epilithic layer, which serves as food for many species, especially scrapers (Nicola et al., 2010). Substrate roughness was proved to be an important discriminant in macroinvertebrates settlement (Downes et al., 2000), which prefer this kind of substrates rather than smoother ones (Way et al., 1995). Moreover, the high percentage of macroinvertebrates larvae found on our substrates are in accordance with the observations from Pinochet et al. (2020), where larvae show a preference for settlement on plastic surfaces. Evidence for different plastic polymers preferences for settlement was confirmed by Pinochet et al. (2020): when macroinvertebrates larvae had the chance to select the substrate, PS surfaces were preferred over the other materials, with PET being selected the least.

Macroinvertebrates plastic colonization could influence food webs and promote colonization by other species: predator–prey relationships

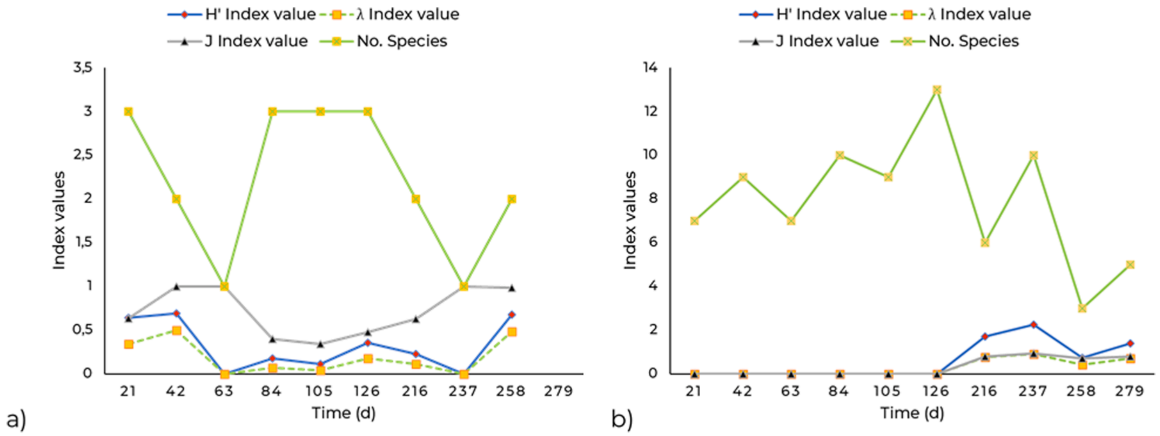

Fig. 7. Temporal trends of the 4 diversity indices (H' = Shannon, λ = Simpson, J = evenness, No. species = number of species), calculated for each sampling. a) fPS = floating polystyrene; b) dPS= dipped polystyrene (time is expressed in days).

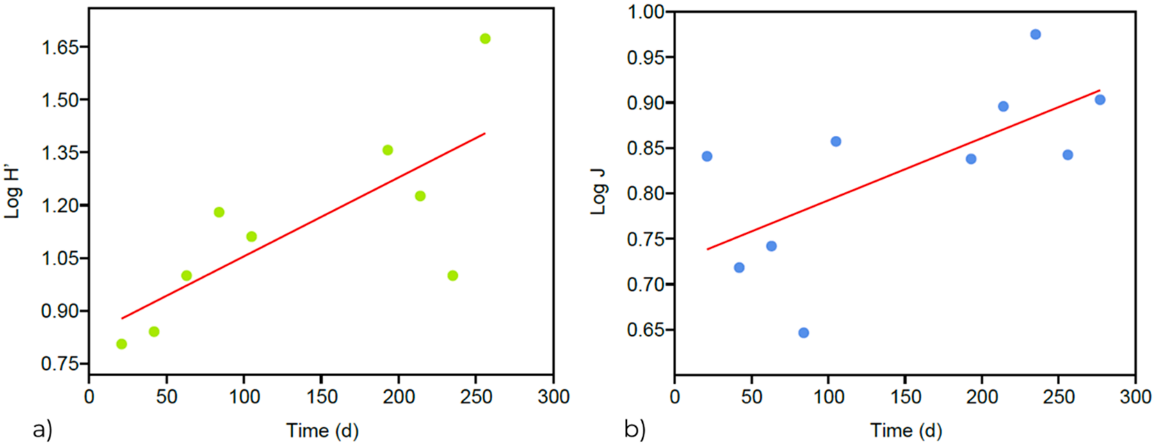

Fig. 8. Plot of log transformed data showing the regression line as regards a) the Shannon index (H') calculated for the dipped polystyrene substrates and b) the Evenness index (J) calculated for the floating polystyrene substrates (time is expressed in days).

between fish and aquatic macroinvertebrates are crucial in structuring freshwater communities and well known by scientific literature (Nieoczym et al., 2023). Moreover, macroinvertebrates are food for a variety of crustacean, birds, and other organisms. They contribute significantly to the food chain for higher animal taxa by transmitting energy and matter from phytoplankton, zooplankton, and macrophytes to fish, amphibians, reptiles, birds, and mammals as they serve as key food sources for them (Jadhav et al., 2022). However, as described by O'Connor et al. (2022), benthic macroinvertebrates could ingest microplastics (Rodríguez et al., 2024) and transfer them in freshwater food webs: this effect could be enhanced since, in our study, macroinvertebrates live close to plastics. Furthermore, although the ingestion of microplastics by macroinvertebrates may not be lethal (Redondo-Hasselerharm et al., 2018), it can affect the way organisms feed and assimilate their food: additives and colorants could have ecotoxicological consequences on all the organisms of the food chain (D'Souza et al., 2020).

Although the first evidence of PS and PET colonization were reported by Pinochet et al. (2020), our study represents the first evidences of the use of this two polymers substrates as microhabitats for macroinvertebrates colonization in a protected Mediterranean coastal wetland. The observed difference in the number of taxa found on floating substrates compared to the dipped ones could be related to the ecological features characterising the bottom of a marshy canal, which makes survival difficult for most animal taxa (Cortés-Guzmán et al., 2021). These results show that the small-scale distribution of macroinvertebrate communities depends both on the physical and chemical characteristics of the substrates used (Wilson et al., 2020). The taxa found on dPE substrates are only three: Chironomidae, Hydrenidae, Hydrophilidae. Regarding the latter two taxa, they are adult Coleoptera found in a single sampling and their presence on dipped substrates can be explained as a casual finding during the collection. Regarding Chironomidae, their presence, in the form of the larval stage, can be explained because they are the only insects to have haemoglobin in the blood, so they can colonize almost completely anoxic environments (de Melo et al., 2022). The great adaptability of Chironomidae is also evident from their constant presence in all four cases examined, the only taxa among those investigated that showed this tendency. On the other hand, the presence of species such as *Procambarus clarkii* and individuals belonging to the Naididae family, as well as Helobdella and Ephemerella, on dPS substrates confirm the tendency of individuals to colonize plastic substrates placed on the bottom, provided they have a space for refuge (Bolotov et al., 2022; Luiza-Andrade et al., 2022; Yünlü et al., 2022). The absence of *Procambarus clarkii* on fPS substrates highlights how their presence on the bottom depends both on the availability of a shelter and on the ecological characteristics of the species (Gao et al., 2022). The construction of the den, made up of underground tunnels, is fundamental for this species both to defend itself from predators in critical moments of the life cycle and in dry conditions or in case of extreme temperatures (Barbaresi, 2002). Instead, its presence, found in the central hole (made for the passage of the rope that held the two substrates fixed to each other) of the dPS substrates in four samples, is an example of the great adaptability of this species in the use of artificial substrates (Curti et al., 2021). This is in contrast with De la Torre et al. (2021) according to which larger substrates present highest abundance of organisms than the smaller ones, as the PS cubes used in this study had smaller dimensions than the PE bottles. Moreover, also the presence of terrestrial taxa on the surface substrates seems to be closely related to the availability of spaces where take refuge. Furthermore, a predatory relationship between terrestrial and aquatic taxa can be hypothesized. Essentially, all aquatic and many terrestrial invertebrates are omnivorous (Merritt et al., 2017). Among these, there are many taxa which are carnivorous, e.g. hemiptera, odonatan and plecopteran (Merritt et al., 2017). Spiders represent a diverse, widespread and abundant group of carnivores (Wilder, 2011). Referring to predator-prey relationship, body size is considered a key trait for understanding this ecological connection (Klecka and Boukal, 2013). Our results show that the individuals of spiders recovered (Araneae) were all found from the sixth sampling (except one individual in the first and third sampling) and vice-versa families such as Formicidae and Hydrenidae, abundant from the first sampling, were no longer traced, respectively, from the fourth and seventh sampling. Nilsson et al. (2008) highlighted that diversity of predatory macroinvertebrates decreased with increasing predator species richness. Moreover, feeding mode modifies predator–prey size allometry and can potentially alter the impact of predators on the prey assemblage (Wirtz et al., 2012). For these reasons, a competitive relationship can be hypotesized between spiders as predators and some Hymenoptera and Coleoptera as prey.

It's also important to highlight that, despite the analysis of the indices focused only on the PS substrates because of the scarce presence of macroinvertebrates on PET substrates, the role of PET substrates should not be underrated. Plastic items present in water could favour the transport of biota through long distances; they can act as substrates that, under natural phenomena, can connect distant places and allow the colonization of different ecosystems by non-indigenous species (García-Gómez et al., 2021; Gunaalan et al., 2020). This carrying effect has already been demonstrated by Battisti et al. (2020b), highlighting the carrying capacity of PS as medium that carries soil opportunistically used as a substrate for dispersing native plants along drainage channels embedded in a cultivated landscape. Here we introduce the term "trunk effect" to describe, to the best of our knowledge for the first time, the potential carrying ability of macroinvertebrates by plastic waste in waters, from a place to another, referring to the "Island Biogeography Theory" (MacArthur and Wilson, 2016) and considering the plastic waste as an ecological corridor.

Analysis on ecological traits highlighted that, for each trait, the various modalities are equally represented starting from the first sampling up to the last. These results can be explained both (i) considering the large surface of the plastic substrates compared to macroinvertebrates body dimensions, which could provide the availability of a surface with physical characteristics particularly suitable for colonization and (ii) the colonizing capacity of different taxa. In particular, the distribution of taxa within the traits used shows the presence of an ecologically diversified community, in which there are mostly univoltine organisms, with dimensions between 5 and 20 mm, predators, choppers and scrapers that feed on plant organisms and animals. Feeding habits highlighted the capacity of different taxa to share the same plastic substrates for settlement. The presence of macroinvertebrates with different ecological characteristics can be indicative of a large number of ecological niches that can be exploited within an artificial support (Gallitelli et al., 2023). Moreover, the similar distribution of individuals representing different subtraits between plastic supports highlight the low influence of ecological conditions on colonization patterns: the capacity of macroinvertebrates, characterized by different functional ecology (food resources, body measures, dispersion strategies), to reach and live on different substrates independently from light and turbidity conditions, represents their great ecological plasticity. Adaptation and ecological acclimatization are the fundamental features for expand macroinvertebrates colonization also on plastic substrates. However, no ecological patterns emerged, suggesting that community ecological stabilization could be reached in larger times. For this reason, further studies are necessary, considering longer sampling periods, to investigate the ecological patterns and community stabilization phenomena of macroinvertebrates colonizing macroplastics.

The lack of a trend over time in the number of macroinvertebrate species can be seen in a quantitative rather than qualitative key. Indeed, the number of taxa has remained constant over time, while the macroinvertebrates composition changed, such as previously highlighted in the case of the Araneae. The absence of a temporal trend in the Simpson index highlights a lack of dominance among the taxa found. This result highlighted the coexistence of several taxa with different ecological niches (McArtor et al., 2021), suggesting the existence of unconsidered variables influencing macroinvertebrates settlement Further studies are therefore necessary to identify the main environmental drivers of macroinvertebrates plastic colonization The significance of the Evenness values for floating PS can be explained considering that individuals are equally divided within their respective taxa. The increase over time of this index could refer to a trend towards achieving stabilization of the macroinvertebrate community. On the contrary, on dPS substrates, the taxa of the Chironomidae and Naididae contain most of the individuals found and don't show a significant temporal trend. The higher abundance of individuals of these two taxa could also be explained by the physical characteristics of the substrates; unlike *Procambarus clarkii*, the larvae of Chironomidae and Naididae have relatively smaller body dimensions, which provides them greater space to colonize than the crustacean.

5. Conclusions

Literature offers many studies about plastic pollution in marine waters, less in freshwaters and there are only few studies regarding the relationship between plastics and macroinvertebrates in wetlands. This study represents the first evidence of macroinvertebrates colonization on plastic substrates (PS and PET) artificially settled in a Mediterranean wetland. The present work has contributed to expand the research on the increasing effects of plastic debris on macroinvertebrates communities regarding the rate of colonization and the biodiversity enrichment of plastic-impacted wetlands.

In summary, this study shows the tendency of macroinvertebrates to colonize plastic substrates artificially placed in a wetland. The use of macroinvertebrates in this study made it possible to hypothesize the main reasons driving the colonization of artificial substrates: availability of food and shelters provided by the material. In fact, most of individuals found feed on detritus and vegetal fragments, supporting the hypothesis of food resource as main environmental driver for macroinvertebrates colonization. We also highlight that our research was performed to evaluate the colonization of macroinvertebrates on plastic substrates. This study presents the first evidence that macroinvertebrates prefer rough PS substrates for the colonization in coastal wetlands. However, given the uniformity of distribution of individuals in the four "dispersion" modalities (active, passive, aquatic and aerial dispersion), we cannot consider the substrate type as main environmental driver for macroinvertebrates colonization. Instead, we hypothesise that PS can favour the deposition and adsorption of food resources due to its rough surface, making an attractive effect for macroinvertebrates. Further studies are therefore necessary to investigate: (i) differences in community pattern and colonization strategies between natural and artificial substrates; (ii) the possible predatory relationship between different taxa; (iii) the possibility that the plastics used contained additives that may have influenced our results (iv) a possible biodiversity enrichment in plastic-impacted wetlands.

On the other hand, plastic pollution in wetlands represent an important topic and its management could be challenging. In fact, while an enrichment in macroinvertebrates diversity was demonstrated, it is clear that the main aim of scientific research in this sense is to remove and prevent plastic pollution. Moreover, the presence of macroinvertebrate taxa on plastic waste could also favour the colonization of long-distance ecosystems by alien species, creating an important impact to the colonized habitats. The "Torre Flavia" wetland represents an open environment, where plastic inputs can reach freshwaters following different ways (sea spray, atmospheric transport, direct contamination). Identification of plastic sources and items removal could be difficult, particularly regarding micro- and nanoplastics. However, taking some precautions and actions, such as improving the control of plastic dumping through the use of security cameras, removing macro and mesoplastics with the help of volunteers and citizens, educating people (especially children) and raising awareness about the threats of plastic could help in reducing and mitigating plastic pollution.

Ethics approval

Not applicable

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CRediT authorship contribution statement

Scalici Massimiliano: Writing – review & editing, Validation, Supervision, Resources, Conceptualization. **Cesarini Giulia:** Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation. **Taurozzi Davide:** Writing – review & editing, Writing – original draft, Visualization, Investigation.

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.

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Consent to participate

Not applicable.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ancene.2025.100461.

Data availability

Data will be made available on request.

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