



# Environmental DNA metabarcoding reveals the effects of seafloor litter and trawling on marine biodiversity

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## ABSTRACT

Environmental DNA (eDNA) techniques are emerging as promising tools for monitoring marine communities. However, they have not been applied to study the integrated effects of anthropogenic pressures on marine biodiversity. This study examined the relationships between demersal community species composition, key environmental features, and anthropogenic impacts such as fishing effort and seafloor litter using eDNA data in the central Tyrrhenian Sea. The results indicated that both fishing effort and seafloor litter influenced species composition and diversity. The adaptive traits of marine species played a critical role in their response to debris accumulation and fishing. Mobile species appeared to use relocation strategies, while sessile species showed flexibility in the face of disturbance. Epibiotic species relied on passive transport. The use of eDNA-based methods is a valuable resource for monitoring anthropogenic impacts during scientific surveys, enhancing our ability to monitor marine ecosystems and more effectively assess the effects of pollution.

## 1. Introduction

Marine biodiversity plays a key role in maintaining the health and balance of marine ecosystems (Gouletquer et al., 2014). The seafloor hosts many marine species, encompassing a wide range of organisms that collectively contribute to the complex structural arrangement of life in the depths of the sea. However, this priceless biodiversity faces significant challenges from direct and/or indirect anthropogenic impacts, including fishing, and pollutants (Halpern et al., 2008).

Commercial trawling is recognized as a major anthropogenic disturbance to seafloor ecosystems, leading to significant changes in benthic community composition, in terms of abundance and spatial distribution (Beauchard et al., 2021; Eigaard et al., 2017; Oberle et al., 2016; Clark et al., 2016; Pusceddu et al., 2014). The effects of trawling on benthic communities vary depending on the sensitivity of the animals and the resilience of the population (Hiddink et al., 2017; Kaiser et al., 2006; Tiano et al., 2020). Fishing activity has direct and indirect impacts on the environment. Direct effects include substrate scraping, sediment resuspension, and benthos destruction. Indirect effects include

post-fishing mortality and long-term changes in the community. Additionally, trawling can alter the availability of food and habitats, affecting species differently, depending on their feeding strategy (Johnson et al., 2015; Mangano et al., 2017). Indeed, it could increase food availability for opportunistic species (Hiddink et al., 2008) or remove certain prey for more selective organisms (Hiddink et al., 2017). Previous studies (Bozzano and Sardaà, 2002; Groenewold et al., 2000; Kaiser and Spencer, 1994; Lejeune et al., 2023) showed that scavengers may benefit from the discards produced by trawling, while more vulnerable species, such as sessile filter feeders, were removed.

Nowadays, marine litter represents one of the most predominant and persistent pollutants (Deudero and Alomar, 2015). Litter waste mainly originates from the mainland and is transported to the sea by surface runoff and rivers (Boucher et al., 2020). Once enters the ocean, litter can be trapped on the seafloor and persist for extended periods ranging from decades to even centuries (Canals et al., 2021). Seafloor litter pollution has emerged as a significant environmental concern, posing potential threats to marine biodiversity and ecosystem functioning (Kühn et al., 2015). The seafloor is a key habitat for numerous marine species, and

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the accumulation of litter disrupts the fundamental equilibrium and functioning of many ecological systems (Angiolillo and Fortibuoni, 2020; de Carvalho-Souza et al., 2018; Galgani et al., 2018). Indeed, persistent pollution not only inflicts physical harm upon marine organisms through entanglement and ingestion but also disrupts seafloor ecosystems by altering nutrient availability and by modifying the physical and chemical properties of the environment (Angiolillo and Fortibuoni, 2020; Sbrana et al., 2022). Finally, a relevant consequence of litter accumulation consists of the phenomenon known as biofouling: various benthic organisms colonize and establish communities on submerged surfaces, including litter (Mancini et al., 2021; Pinochet et al., 2020; Póvoa et al., 2021; Rech et al., 2018). Biofilm formation on plastic, the so-called Plastisphere (Zettler et al., 2013), could alter the physical, chemical, and biological characteristics of plastic in aquatic environments, causing an increase in plastic density and sinking. Moreover, benthic plastic debris substrates may have the potential to change the biodiversity and structure of benthic communities (Subías-Barata et al., 2022).

In the last decade, various monitoring programs and modelling approaches have been applied to assess the accumulation of litter at sea and the importance of its drivers. Recently, (Cau et al., 2024) used a machine learning approach (i.e., Random Forests) to model and investigate pathways from a large available dataset of marine litter distribution in the western and central Mediterranean Sea, producing suitable results.

Understanding the risk posed by anthropogenic pressures on the seafloor, such as trawling activities and marine litter, is crucial for developing effective conservation and management strategies. This could benefit greatly from a multidisciplinary framework that includes sources, distribution, and environmental impacts. (Hardesty et al., 2019). The assessment of seafloor impacts is particularly urgent in the Mediterranean Sea, which is known to be a biodiversity hotspot at high risk of degradation, due to its semi-enclosed and highly anthropised basin (Boucher and Billard, 2020; Coll et al., 2012; Cózar et al., 2015; Deudero and Alomar, 2015). Nevertheless, knowledge about the impacts on the seafloors remains limited, probably due to the difficulties involved in sampling the diversity and understanding ecosystem processes and ecological interactions in these environments (Sandra et al., 2023). Many studies focused on the impact of marine litter and trawling on indicative species of Vulnerable Marine Ecosystems (Consoli et al., 2020; Downie et al., 2021; Horton and Barnes, 2020; Mačić et al., 2022) but there is a lack of studies that address the problem from a broader ecological perspective (i.e., at the scale of populations or communities) (Hardesty et al., 2019).

Ecological investigations involve the examination of species occurrence patterns as a fundamental task. Traditionally, assessing biodiversity in marine ecosystems has relied on scientific surveys, traps, or fishery-dependent data (Angiolillo et al., 2023; Kipson et al., 2011; Mallet and Pelletier, 2014; Pennino et al., 2016). However, these methods have various limitations, such as their high costs, logistical challenges, and potential disturbance to the fragile ecosystems under investigation (Brandt et al., 2016). As a result, there is a need to explore alternative approaches able to overcome these limitations while providing valuable insights into the effects of anthropogenic pressures on marine communities. Environmental DNA (eDNA) techniques have gained increasing recognition and usage in biodiversity studies, offering promising opportunities for monitoring the impacts of different human-induced stressors (Yoccoz, 2018). By analysing genetic material shed by organisms into the surrounding environment, eDNA sequencing allows for a non-invasive and efficient assessment of species presence and diversity (Bohmann et al., 2014; Thomsen and Willerslev, 2015). Recently, it has also been shown that eDNA can be collected efficiently and inexpensively during normal fishing activities of the commercial fleet (Maiello et al., 2022, 2023). eDNA has been successfully applied in various contexts, including the detection of rare or elusive species, monitoring invasive species, and assessing community dynamics (Cicala

et al., 2024; Albonetti et al., 2023; Fonseca et al., 2023; Juhel et al., 2022). However, the potential application of eDNA metabarcoding techniques to investigate the impact of seafloor litter pollution on biodiversity has not yet been implemented.

The aim of this study is therefore to examine the impact of seafloor litter pollution and trawling activities on marine communities. The study uses opportunistically collected eDNA data to identify species occurrence and relate them to the various levels of fishing effort and litter pollution. The metabarcoding approach overcomes the limitations of traditional methods in assessing marine communities, promoting the study of the effects of anthropogenic pressures on organisms under investigation. The association of species with marine debris and fishing activities could provide insight into possible biological interactions and the broader consequences of these pressures on marine biodiversity.

This research would represent a significant step towards understanding the complex relationship between anthropogenic pressures and marine communities. In particular, it could help expand knowledge on marine litter, with potential implications for the development of targeted mitigation and management strategies.

## 2. Material and methods

### 2.1. Area of study

Environmental DNA samples were gathered during summer 2020 from 24 sampling locations aboard a commercial bottom-trawler (Fig. 1). The study area is located in the central Tyrrhenian Sea (FAO Geographical Sub Area 9 – Western Mediterranean Sea) and covers a bathymetric range spanning from the continental shelf edge (average depth ~70 m) to the deep slope (~820 m), with a distance from the coast going from 4 to 24 km.

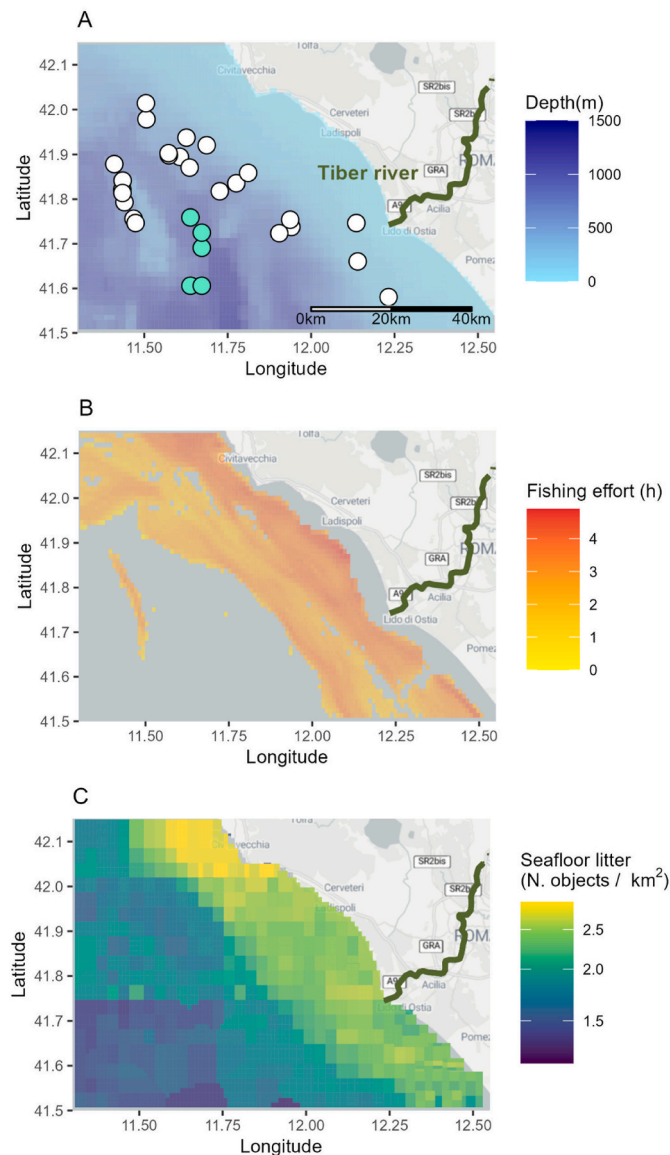
This area has a very narrow continental shelf, characterised by fine sands and muddy bottoms (Ardizzone et al., 2018). The coastal area is heavily populated with large urban centres, industrial settlements, and important ports, being close to the city of Rome. The Tiber River run-off significantly affects the chemical-physical traits of the area, bringing contaminants such as marine debris (Cesarini et al., 2023; Inghilesi et al., 2008). During winter, the river Tiber discharge is carried along the coast and dispersed offshore by the strong Tyrrhenian Sea currents from the northwest. In contrast, during summer, weak sea breezes cause downwelling conditions that limit the river's fresh waters near the mouth (Inghilesi et al., 2008).

### 2.2. Species distribution from eDNA samples

The analysis performed in this study integrated and developed the ones in Maiello et al. (2023), and as a result, the initial stage of the operational procedure, which includes the data acquisition from the eDNA sample, is the same in both studies. The data acquisition process from eDNA will be briefly described.

Two approaches were adopted to sample eDNA aboard the commercial trawler: *slush* water and the *metaprobe* rolls of gauze. The *metaprobe* is a hollow-perforated reusable plastic ball (the 3D project is freely available at: <https://github.com/GiuliaMaiello/Metaprobe-2.0>), into which three rolls of sterile gauze were placed to passively absorb DNA from the surrounding environment. To account for possible contamination linked with the fishing boat background, field blanks were collected along with environmental samples.

Samples were processed in high containment rooms to avoid cross-contamination. After eDNA extraction, two taxonomically informative mitochondrial regions were PCR amplified from eDNA samples: a ~167 bp fragment of the 12 S gene and a ~313 bp fragment of the COI gene. The first fragment was amplified using the fish-specific Tele02 primers, while the second fragment was amplified using highly degenerated universal metazoan primers. A positive PCR control (*Sebastes mentella*, a subarctic species absent in the Mediterranean Sea) and a negative



**Fig. 1.** A: Map of the 24 eDNA sampling sites (white circles) and outgroup hauls (blue circles); B: Map of fishing effort (hours of bottom trawl fishing activity, log-transformed); C: Map of seafloor litter (number of objects  $\bullet$  km $^{-2}$ , log-transformed) in the central Tyrrhenian Sea (FAO Geographical Sub Area, GSA 9–western Mediterranean Sea).

control were included, to monitor the degree of contamination associated with laboratory procedures.

Bioinformatic analysis followed the OBITOOLS pipeline (Boyer et al., 2016). Sequences were clustered into Molecular Operational Taxonomic Units (MOTU) using SWARM (Mahé et al., 2015), setting a clustering threshold at  $d = 3$  for TeleO2 12 S and  $d = 13$  for the universal COI (Kemp et al., 2019; Siegenthaler et al., 2019; Wangenstein et al., 2018). The taxonomic assignment was then performed with ECOTAG, followed by the examination of ambiguous and poorly resolved MOTUs/taxa (e.g., non-Mediterranean taxa) using BLASTn search in the NCBI system. Finally, the data were filtered to retain only sequences showing identity matches  $>98\%$  and removing potential contamination noise using blanks and negative controls with the decontam package in R (Davis et al., 2018), using the prevalence method with a threshold of 0.5.

### 2.3. Anthropogenic pressures and environmental features

In this study, we aimed to investigate the structure of demersal

communities, with a focus on the primary edaphic factors and key sources of anthropogenic disturbance such as pollution and fishing effort, using information obtained through eDNA metabarcoding. To achieve this goal, we quantified various descriptors at each sampling site, integrating them into the statistical analyses described in the next sections.

- Seafloor litter data were extrapolated from a Random Forests (RF) model on the whole western Mediterranean Sea (<https://doi.org/10.17632/r2b6svy7h7.1>; Cau et al., 2024). Input data used for the RF model were collected by the MEDiterranean International bottom Trawl Survey (MEDITS) from 2013 to 2020, according to the standardized official protocol (MEDITS working group, 2012). A long series of datasets has provided a more accurate representation of litter presence on the seabed and its potential impact on species with multi-year life cycles. The MEDITS protocol for monitoring marine litter (in agreement with the requirements of the Marine Strategy Directive Framework (Directive, 2008/56/EC)) is based on a stratified random sampling design, providing reliable estimates for the area between 0 and 1000 m in depth, and the total number of objects collected is then standardized according to the swept area, expressed in number of objects  $\bullet$  km $^{-2}$ .
- The Mean Fishing effort (bottom trawling) was calculated as the mean annual hours of trawling, over the last 5 years before the sampling (2016–2020), in the cell of a 1 km $^2$  grid defined for the area of study, using the Vessel Monitoring System data provided by the Italian Ministry of Agriculture, Food Sovereignty and Forests and applying the procedures described in (Russo et al., 2014, 2016). Gridded fishing effort data were used to assign an effort value to each sampling site by matching the sites with their respective grid cells.
- Cumulative human impacts: we integrated data from various sources to identify anthropogenic descriptors that affect marine communities: Mean Distance from Shipping Lanes (<https://knb.ecoinformatics.org/view/doi:10.5063/F1S180FS>); Impact of Port Areas (<https://msi.nga.mil/Publications/WPI>); Impact of Urban Areas (<https://www.efrainmaps.es/english-version/free-downloads/eur-ope/>).
- The distance (in Km) from the Tiber River mouth, assuming that rivers are the main carriers of nutrients and pollutants for the marine communities.
- The Sea bottom Depth (m) estimated from the NOAA ETOPO1 Global Relief Model using the R package *marmap* (Pante and Bouhet, 2015), assuming that depth is a major forcer of demersal community structure (Péres and Picard, 1964).
- The Distance from the coast (Km), computed using the *dist2Line* function of the R package *geosphere* (Hijmans, 2019).

### 2.4. Data analysis

The final dataset comprised, for each of the 24 sampling locations: species presence as detected by eDNA metabarcoding, number of seafloor litter, fishing effort, environmental features, and anthropogenic pressures.

Taxonomic composition of marine species, resulting from eDNA data analysis, was examined in presence-absence format. Only species directly related to seafloor litter were maintained in the analysis (i.e., individuals of benthic and necto-benthic species, identified from the FishBase database for fishes (<http://www.fishbase.org>) or SeaLifeBase (<http://www.sealifebase.org>) for invertebrates), thus excluding those embracing strictly pelagic behaviours. Furthermore, we excluded rare species from the analysis, defined as those found in less than 25% of the sampling sites.

The total amount of marine litter (number of objects  $\bullet$  km $^{-2}$ ) and the Mean Fishing effort (total annual hours of bottom trawling) were used to perform, in parallel, a hierarchical cluster analysis (using a Manhattan distance and the “ward.D2” agglomeration method as provided by the

*hclust* function of R). We used an additional set of five hauls, corresponding to areas with low fishing effort and low litter levels (out-groups), to provide a comparative framework for evaluating the groups resulting from the cluster analysis. Then, we categorized the sampling sites based on a combined scale of litter (low/high) and fishing pressure (low/high).

We performed a redundancy analysis (RDA) using the *vegan* package in R (Oksanen, 2022) to analyse the community structure, under the influence of environmental features and anthropogenic pressures. RDA is a multivariate technique used to explore the relationship between a

set of response variables and a set of explanatory variables (Israels, 1984). RDA maximize the amount of the dependent variables' variance that can be explained by a linear combination of the explanatory factors. In this study, the presence/absence of the different species (response variable) was modelled with respect to the environmental and anthropogenic features (explanatory variables).

Marine community composition, extrapolated from eDNA data, has been set on the family level, so to gain into the ecological roles and patterns within broader taxonomic groups.

The association between biodiversity patterns and combinations of

**Table 1**

List of taxa selected for this study, identified through eDNA metabarcoding (COI and 12 S) detection, to investigate relationship within the species community in the central Tyrrhenian Sea (Lazio region).). Code numbers refer to the family level.

Class	Family	Species	Code	Class	Family	Species	Code
Anthozoa	Alcyoniidae	<i>Alcyonium acaule</i>	1	Malacostraca	Sergestidae	<i>Robustosergia robusta</i>	
	Epizoanthidae	<i>Epizoanthus arenaceus</i>	2	Polychaeta	Acoetidae	<i>Panthalis oerstedii</i>	63
	Funiculinidae	<i>Funiculina quadrangularis</i>	3		Onuphidae	<i>Paradiopatra calliopae</i>	64
	Sagartiidae	<i>Sagartiogeton</i> spp.	4	Teleostei	Spionidae	<i>Laonice cirrata</i>	65
Ascidacea	Asciidiidae	<i>Ascidia</i> spp.	5		Argentinidae	<i>Argentina sphyræna</i>	66
Asteroidea	Astropectinidae	<i>Astropecten irregularis</i>	6		Bothidae	<i>Glossanodon leioglossus</i>	
	Luidiidae	<i>Luidia sarsi</i>	7			<i>Arnoglossus laterna</i>	68
Bivalvia	Teredinidae	<i>Psiloterodo megotara</i>	8		Callionymidae	<i>Arnoglossus rueppellii</i>	
Cephalopoda	Eledonidae	<i>Eledone cirrhosa</i>	9			<i>Synchiropus phaeton</i>	70
	Enoploteuthidae	<i>Abralia veranyi</i>	10		Caproidae	<i>Capros aper</i>	71
	Histioteluthidae	<i>Histioteluthis bonnellii</i>	11		Centrolophidae	<i>Centrolophus niger</i>	72
		<i>Histioteluthis reversa</i>			Cepolidae	<i>Cepola macrophthalma</i>	73
	Loliginidae	<i>Alloteuthis media</i>	13		Chlorophthalmidae	<i>Chlorophthalmus agassizi</i>	74
		<i>Alloteuthis subulata</i>			Citharidae	<i>Citharus linguatula</i>	75
	Octopodidae	<i>Octopus vulgaris</i>	15		Congridae	<i>Conger conger</i>	76
		<i>Pteroctopus tetracirrhus</i>			Epigonidae	<i>Gnathopis mystax</i>	
	Ommastrephidae	<i>Scaevargus unicolor</i>				<i>Epigonus denticulatus</i>	78
		<i>Illex coindetii</i>	18	Gadidae		<i>Epigonus telescopus</i>	
	Onychoteuthidae	<i>Todarodes sagittatus</i>				<i>Gadidulus argenteus</i>	80
		<i>Todaropsis eblanae</i>		Gobiidae		<i>Micromesistius pouassou</i>	
	Sepiidae	<i>Onychoteuthis banksii</i>				<i>Trisopterus capelanus</i>	
		<i>Sepia elegans</i>	22	Gonostomatidae		<i>Lesueurigobius friesii</i>	83
	Sepiolidae	<i>Heteroteuthis dispar</i>	23			<i>Cyclothone braueri</i>	84
		<i>Neorossia caroli</i>		Lophiidae		<i>Gonostoma denudatum</i>	
		<i>Rondeletiola minor</i>				<i>Lophius budegassa</i>	86
		<i>Rossia macrosoma</i>		Lotidae		<i>Lophius piscatorius</i>	
		<i>Sepietta oweniana</i>				<i>Molva molva</i>	88
		<i>Stoloteuthis leucoptera</i>		Macrouridae		<i>Coelorrhinus caelorrhinus</i>	89
Crinoidea	Antedonidae	<i>Antedon mediterranea</i>	29			<i>Hymenoccephalus italicus</i>	
Echinoidea	Brissidae	<i>Leptometra phalangium</i>		Merlucciidae		<i>Nezumia sclerorhynchus</i>	
		<i>Brissopsis lyrifera</i>	31			<i>Merluccius merluccius</i>	92
Elasmobranchii	Echinidae	<i>Echinus acutus</i>	32	Moridae		<i>Mora moro</i>	93
	Dalatiidae	<i>Dalatias licha</i>	33			<i>Mullus barbatus</i>	94
	Etmopteridae	<i>Etmopterus spinax</i>	34	Myctophidae		<i>Ceratoscopelus maderensis</i>	95
	Hexanchidae	<i>Hexanchus griseus</i>	35			<i>Diaphus holti</i>	
	Pentanchidae	<i>Galeus melastomus</i>	36			<i>Diaphus metopoclampus</i>	
	Rajidae	<i>Dipturus oxyrinchus</i>	37			<i>Electrona risso</i>	
		<i>Leucoraja circularis</i>				<i>Hygophum benoiti</i>	
		<i>Raja clavata</i>				<i>Lampyriscus crocodilus</i>	
	Scyliorhinidae	<i>Scyliorhinus canicula</i>	40			<i>Lobianchia dofleini</i>	
Gastropoda		<i>Coccoligya</i> spp.	41			<i>Myctophum punctatum</i>	
Holocephali	Chimaeridae	<i>Chimaera monstrosa</i>	42			<i>Notoscopelus elongatus</i>	
Hydrozoa	Aglaopheniidae	<i>Lytocarpia myriophyllum</i>	43	Nemichthyidae		<i>Nemichthys scolopaceus</i>	104
	Bougainvillidae	<i>Bougainvillia muscus</i>	44			<i>Facciolella oxyrhyncha</i>	105
	Campanulariidae	<i>Clytia hemisphaerica</i>	45	Notacanthidae		<i>Nettastoma melanurum</i>	
	Plumulariidae	<i>Nemertesia ramosa</i>	46			<i>Notacanthus bonaparte</i>	107
Malacostraca	Aristidae	<i>Aristeus antennatus</i>	47	Peristediidae		<i>Peristedion cataphractum</i>	108
	Cirolanidae	<i>Natatanolana borealis</i>	49			<i>Phycis blennoides</i>	109
	Goneplacidae	<i>Goneplax rhomboides</i>	50	Scophthalmidae		<i>Lepidorhombus boschii</i>	110
	Nephropidae	<i>Nephrops norvegicus</i>	51			<i>Lepidorhombus whiffiagonis</i>	
	Pandalidae	<i>Plesionika acanthonotus</i>	52	Sternoptychidae		<i>Argyropelecus hemigymnus</i>	112
		<i>Plesionika edwardsii</i>				<i>Maurollicus muelleri</i>	
		<i>Plesionika heterocarpus</i>		Stomiidae		<i>Chauliodus sloani</i>	114
		<i>Plesionika martia</i>				<i>Stomias boa</i>	
	Pasiphaeidae	<i>Pasiphaea multidentata</i>	56	Trachichthyidae		<i>Hoplostethus mediterraneus</i>	116
		<i>Pasiphaea sivado</i>				<i>Trachyrincus scabrus</i>	117
	Penaeidae	<i>Parapenaeus longirostris</i>	58	Trichiuridae		<i>Lepidopus caudatus</i>	118
	Polychelidae	<i>Polycheles typhlops</i>	59			<i>Chelidonichthys cuculus</i>	119
	Sergestidae	<i>Eusegestes arcticus</i>	60	Triglidae		<i>Lepidotrigla cavillone</i>	
		<i>Parasergestes vigilax</i>				<i>Trigla lyra</i>	



site groups was studied using a multi-level pattern analysis (*indicspecies* package in R; De Cáceres et al., 2010). This function generates various combinations of the input clusters and evaluates each combination against the species in the input matrix. It then selects the combination with the highest association value for each species and assesses the statistical significance of this relationship through a permutation test.

### 3. Results

A total of 305 species (193 families) were identified through eDNA metabarcoding (Supplementary Table 1), and among them, 60 pelagic families were excluded from the analysis. Finally, after removing species present in less than 25% of the sampling sites, a total of 122 species (79 families) were retained for the study (Table 1).

The random forest model yielded a total estimation of 46.37 litter objects per square kilometre, with a mean value of 1.93 n. objects·km<sup>-2</sup> ( $\pm 0.25$  n. objects·km<sup>-2</sup>) in each sampling site. Total fishing effort was 54.47 h, with a mean of 2.27 ( $\pm 0.28$ ) hours. The cluster analysis separated sampling locations into Low litter (N = 17), High litter (N = 7), Low effort (N = 7) and High effort (N = 17) subgroups for seafloor litter and fishing effort respectively (Fig. 2A). The locations were thus split into Low litter - Low effort (N = 7), Low litter - High effort (N = 10), and High litter - High effort (N = 7) by combining the obtained classifications (Fig. 2B).

The redundancy analysis (RDA) was performed to evaluate the relationship of taxonomic group distribution with environmental features, anthropic pressures, and sampling sites. According to the cluster analysis, sites were classified into three groups, with arrows indicating the correlation between the parameters. Sites and organisms were exposed to different stressors (Fig. 3). In particular, variables related to river and seafloor litter are connected with the I quadrant. The distance from the coast is related to the II quadrant, while depth, urban areas, shipping, and harbours are linked to the III quadrant. Finally, the IV quadrant is related to fishing effort pressures. Sampling sites were spread across all four quadrants, with the low litter-low effort sites being concentrated exclusively in quadrant II. In contrast, the remaining two clusters appear to be distributed across multiple quadrants to some extent. The RDA species representation revealed a clear separation of ecological groups along a left-to-right gradient of the x-axis (RDA1). The bathypelagic and bathydemersal individuals were positioned on the left

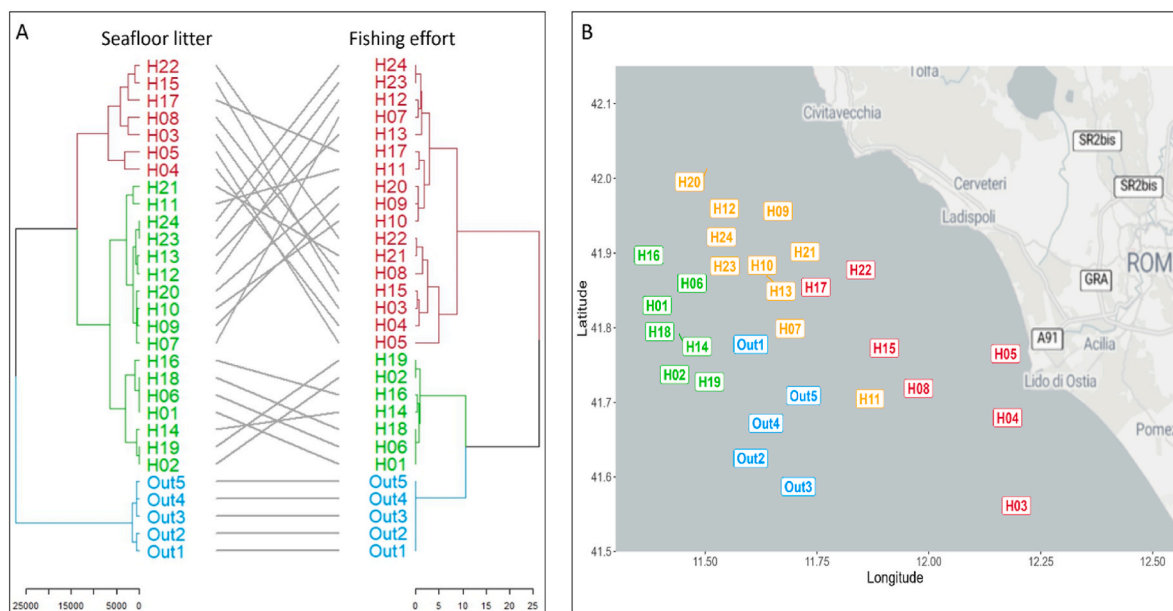
of this axis, while the sessile individuals were on the right side. In contrast, the distribution of benthic, demersal, and benthopelagic species was widespread.

According to the results of the indicator species analysis (Table 2), Moridae and Sergestidae are significantly related with low litter and low effort sampling sites, Epizoanthidae, Peristediidae, and Funiculinidae are strongly related with low litter and high effort sampling sites, and Ascidiidae and Luidiidae are related with high litter and high effort sampling sites.

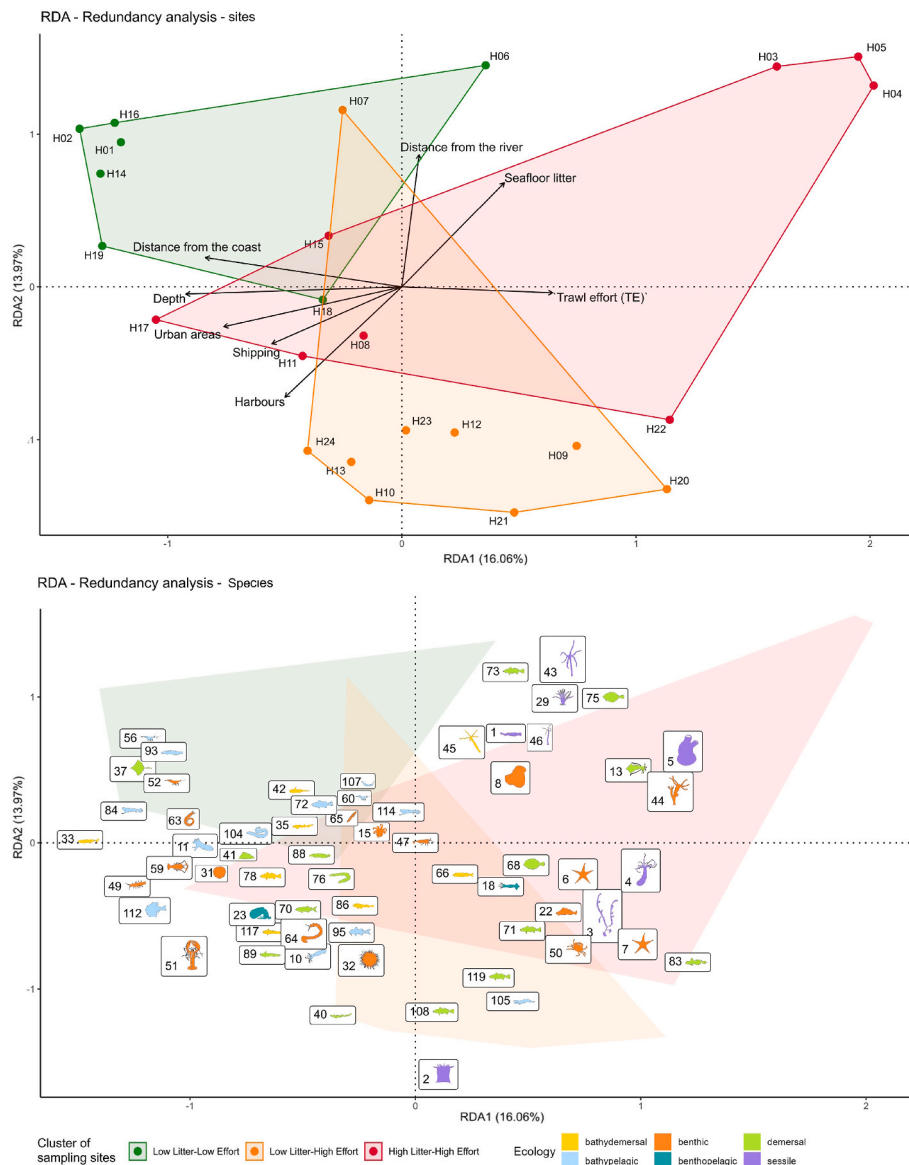
### 4. Discussion

The results provided in this study offer a thorough examination of the spatial distribution and prevalence of marine organisms with various anthropogenic pressures in the central Tyrrhenian Sea. These findings hold critical significance for the enhancement of our comprehension of anthropogenic stressors' repercussions on marine ecosystems and the complex interconnections between environmental characteristics and the fauna inhabiting this area. In this study, eDNA data are used for the first time to relate the spatial distribution of species composition with the accumulation of seafloor litter and fishing activities at different sites. Using this method, it was possible to identify consistent differences in the faunal community composition under various levels of pressure.

The study included a comprehensive selection of 120 species identified in 24 sampling sites, which covered a wide spectrum of organisms, from sharks, rays, and bony fish to invertebrates, such as cnidaria hydroids, polychaetes, crustaceans, and echinoderms. Sampling sites presented a clear separation in terms of both litter abundance and fishing effort. The cluster analysis facilitated the discrimination of areas characterised by distinct levels of accumulation, which included both low and high-impact litter, and represented a fundamental starting point for understanding the spatial distribution patterns of marine litter. As observed in the map, high-impact sampling sites were near the Tiber river mouth. This is also supported by the RDA analysis, which showed a strong relationship between river and seafloor litter. Rivers are important sources of waste. Their runoff and discharged debris significantly influence seas and the surrounding seabed (Atwood et al., 2019; Campanale et al., 2020; Crosti et al., 2018; Noce et al., 2013; Poeta et al., 2016; Rech et al., 2014; Sbrana et al., 2020). The fishing effort followed a decreasing gradient from coast to offshore depending on both



**Fig. 2.** Results of the cluster analyses, which grouped eDNA sampling sites according to fishing effort (h) and seafloor litter (number of objects · km<sup>-2</sup>) (A). Map of the combined classifications (B). *blue*: outgroup sites (control); *green*: low pressures; *red*: high pressures; *orange*: low litter – high effort.



**Fig. 3.** Results of the redundancy analysis showing the associations between environmental characteristics (distance from the coast, depth, distance from the river), anthropogenic pressures (urban areas, shipping, harbours, seafloor litter, and trawl effort), sampling sites (RDA - sites) and the distribution of the taxonomic groups found (RDA - species) in the central Tyrrhenian Sea. The sampling sites are enclosed in coloured polygons that belong to their respective cluster group. Centroids represent species based on their respective families (Refer to Table 1 for a list of their code numbers).








bathymetry and distance from the coast. The analysis indicated that most fishing activities took place close to the coast, while only a small number of vessels operated in deep waters off the coast influenced by the presence of specific habitats occupied by target species, e.g., the bathyal muddy sediments hosting the red prawns *Aristeomorpha foliacea* and *Aristeus antennatus* (Ardizzone et al., 2018). Indeed, coastal location facilitated cheaper and more accessible fishing resources, and legal restrictions limited access to deeper areas (i.e., Regulation (EU) 2019/1241 of the European Parliament and of the Council of June 20, 2019 on the conservation of fisheries resources and the protection of marine ecosystems through technical measures).

The combination of seafloor litter and fishing effort clusters led to the identification of three different groups (i.e., low litter - low effort, low litter - high effort, and high litter - high effort), which were influenced by the synergistic interplay between the hydrodynamic conditions and the Tiber river discharges. Notably, heavily impacted sampling sites (high litter - high effort) are constrained between the river mouth and the southward-descending current from the northern coast (Iacono

et al., 2021), while low litter - high effort sampling sites are influenced primarily by the fishing activity itself.

The Redundancy Analysis (RDA) results connected multiple environmental features and anthropogenic disturbances that mainly influenced the distribution of fauna on the bathyal seabed. Seafloor litter accumulation was found to be predominantly associated with invertebrates, such as hydroids, alcyonidiids, crinoids, and Lolinidae cephalopods, and with bony fishes (i.e., Cepolidae and Citharidae). These organisms differ considerably in motility modes: the first two being sessile, the others slow-moving, and the latter two are typically nektonic demersal animals. Based on the constraints of the animal-substrate relationships, we hypothesise that these organisms benefit from the availability of additional substrates and increased habitat complexity provided by the accumulated litter, which promotes spatial heterogeneity and offers new settlement opportunities, particularly for sessile organisms (e.g., hydroids and alcyonidiids). This interpretation was documented by the numerous findings of such animals as a peculiar component of fouling, mainly in shallow waters (Angiolillo et al., 2015;

**Table 2**  
Significant species resulting from Indicator Species Analysis for each cluster of sampling sites. Silhouettes are coloured based on ecological groups: bathypelagic (light blue), sessile (purple), demersal (green), and benthic (orange). Significant codes: \*\*: p-value <0.01; \*: p-value <0.05

Low Litter - Low Effort			stat	p-value
Moridae	<i>Mora moro</i>		0.727	0.018*
Sergestidae	<i>Eusergestes arcticus</i>		0.715	0.039*
	<i>Parasergestes vigilax</i>			
	<i>Robustosergia robusta</i>			
Low Litter - High Effort				
Epizoanthidae	<i>Epizoanthus arenaceus</i>		0.754	0.008**
Peristediidae	<i>Peristedion cataphractum</i>		0.714	0.018*
Funiculinidae	<i>Funiculina quadrangularis</i>		0.678	0.013*
High Litter - High Effort				
Asciidiidae	<i>Ascidia</i> spp.		0.728	0.009**
Luidiidae	<i>Luidia sarsi</i>		0.678	0.003**

Giangrande et al., 2020; Mancini et al., 2021) and was confirmed in the present study for deep substrates.

The other stressors, such as depth, urban areas, shipping, harbours, and distance from the coast, mainly affected fish and cephalopods. In particular, these included bathypelagic fishes (e.g., Mictophyidae, Sternoptychidae), and the cephalopods of the family Enoploteuthidae and Histioteluthidae, with a high capacity for movement, as well as bathydemersal fish (e.g. some Chondrichthyes such as Dalatiidae, Lophidae and Trachirincidae), which inhabit deep seabeds and are fairly mobile. The distribution of these organisms is primarily influenced by depth and strongly associated with the distance from the coast. This is especially true for deeper species, which are a significant part of the assemblages of the bathyal plain of the Mediterranean Sea (Carlucci et al., 2018; Follesa et al., 2011).

Finally, the indicator species analysis highlighted a significant correlation between the offshore sampling sites subjected to low litter – low effort and two bathypelagic families of Moridae and Sergestidae, which are highly motile swimming in deep water. These species are typical inhabitants of the deeper muddy seabed and so their observed distribution can be explained by both depth and distance from the coast.

These results showed that environmental features and anthropogenic disturbances have different effects in shaping marine community structure, depending on the life strategy traits and adaptability of the species. In addition, they suggest important implications for the assessment of trawling impacts. The stressors connected with the intensity of fishing effort were proved to play a pivotal role in shaping the faunal assemblage, which was mainly composed of typical demersal species, widely distributed over the seabed, from the edge of the continental shelf to the middle-bathyal slope. Many of these species belonged to fish families, like Bothidae, Caproidae, Triglididae, and Gobidae, that swim near the bottom to hunt their prey (Colloca et al., 2003; Relini et al., 2011). Specifically, the Peristediidae *Peristedion cataphractum* was identified as an indicator of low litter-high effort. This species uses its distinctive morphological feature, the rostrum, to dig in the mud and extract prey, and probably they benefit from the

resuspension of sediments by trawling to find their prey. Others were properly benthic species, such as the asteroids of the families Luidiidae and Astropectinidae, which exhibited their positive response to trawling disturbance. We hypothesise that trawling supplies them with the discarded bycatch or bait from the fishing gear, which perfectly matches their scavenger habit and carnivorous diet (Groenewold et al., 2000; Juan et al., 2007). Moreover, some sessile species, such as the anthozoan, Epizoanthidae and Funiculinidae, were significantly associated with the sites subjected to low litter – high effort. This can be explained by the characteristic species-specific response that *Funiculina quadrangularis* (Funiculinidae) has to withstand the fishing pressures (Pierdomenico et al., 2018). This species has a bulb, i.e., peduncle, that enters the sediment up to about 50 cm, allowing the colony to strongly anchor to the seafloor, but maintaining it to bend and lie flat temporarily when fishing gear passes over it (Lauria et al., 2017). As regards the other species *Epizoanthus arenaceus* (Epizoanthidae), we assume that it withstands the pressures of fishing effort due to its epibiotic habit on motile invertebrates (Reimer, 2023), like molluscs and hermit crabs, which allow it to move away from the impact along with its host species.

Two species were significantly associated with high impacts of seafloor litter and fishing effort: *Ascidia* spp. (Asciidiidae) and *Luidia sarsi* (Luidiidae). These species were typically found on the seafloor with high litter accumulation and high fishing effort due to their ecological preferences and behaviour. Ascidia species are frequently found in the biofouling of seabed litter (Ramalhosa et al., 2021; Subías-Barata et al., 2022). As filter-feeding invertebrates, they tend to inhabit areas with high concentrations of organic matter and detritus, which are often accompanied by litter accumulation. *Luidia sarsi*, a species of starfish, is commonly found in seabeds with a high abundance of food (Koukouras and Kitsos 2010; Lejeune et al., 2023). Due to the abundance of potential food sources, including waste and decaying material, these species could be attracted to areas with high levels of littering and fishing activity. Based on the idea that non-random distributions of species co-occurrence are the fundamental components of ecological communities (Tulloch et al., 2018), the presence of different species in a given area may be influenced by both environmental requirements and biological interactions, such as competition, mutualism and predation. As a result, certain taxa coexist more frequently than others, while some coexist less frequently than expected by chance. Our results reveal significant non-random associations, indicating potential ecological relationships between taxonomic groups, particularly in varying environmental conditions. The indicator species analysis supports the hypothesis that most species exhibit specific adaptations to litter and trawling effort, based on their substrate interaction and motility patterns.

5. Conclusions

The bathyal zone in the central Tyrrhenian Sea off the Latium coast was affected by multiple environmental features and anthropogenic pressures. Our results showed that the species composition may be related to fishing effort and seafloor litter. The adaptive traits of species have played a significant role in explaining faunal assemblages, primarily related to their environment, mobility, and feeding habits. The accumulation of litter on the seafloor and fishing activity forces these species to exhibit shared and effective responses to disturbances. These responses typically involve the relocation of mobile species, the resistance of some sessile species, or the passive transport of epibiotic species. Conversely, some species thrive in the most affected areas, taking advantage of available food sources.

In conclusion, the results provide a comprehensive overview of the distribution of seafloor litter and fishing pressures, analysing their impacts on marine organisms in the central Tyrrhenian Sea. The methodologies employed, including metabarcoding, statistical analyses, and indicator species analysis, converge to understanding the complex relationships between environmental variables, anthropogenic factors,

and community composition. This study not only improves our understanding of the response of marine ecosystems to litter accumulation and fishing pressures but also provides essential information for designing effective conservation and management strategies to mitigate their impact on marine organisms.

### CRediT authorship contribution statement

**Alice Sbrana:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. **Giulia Maiello:** Data curation, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Maria Flavia Gravina:** Investigation, Resources, Writing – original draft. **Davide Cicala:** Investigation. **Simone Galli:** Investigation, Resources, Validation, Writing – original draft. **Matteo Stefani:** Investigation, Methodology. **Tommaso Russo:** Conceptualization, Formal analysis, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing.

### Declaration of competing interest

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

### Data availability

Data will be made available on request.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marenvres.2024.106415>.

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