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CICLO XXXVI

FROM THE MAINLAND TO SEA AND BACK: PLASTIC'S PATH IN DEMERSAL COMMUNITIES EXPLOITED BY FISHING

Ph.D. Thesis

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Abstract

Seafloor litter pollution has emerged as a significant environmental concern, posing potential threats to marine biodiversity and ecosystem functioning. The seafloor is a key habitat for numerous marine species, and the accumulation of litter disturbs the essential balance and function of this ecological system. This pollution may affect organisms at both the individual level, through ingestion or entanglement, and the community level by altering nutrient availability and modifying the physical and chemical properties of the environment. Additionally, seafloor litter pollution has been observed to have a negative impact on marine fisheries worldwide, affecting them both directly and indirectly. For instance, the presence of marine litter in fishing nets can reduce their catch efficiency, cause damage, reduce the time available for fishing, inflate repair expenses, obstruct equipment, and necessitate increased fuel consumption. Moreover, fisheries' target species, along with their prey, are at risk of both lethal and sub-lethal harm due to plastic pollution and that could reduce the fishing quality. In particular, many studies have correlated seafloor plastic pollution with reduced reproductive success, growth limitations, and reduced body mass growth in exposed organisms, which could reduce the economic value of a species and thus have a negative impact on system production.

Despite the growing awareness and concern about the effects of bottom trawling and seafloor litter, there are no studies on the effects of litter pollution on fisheries and stock management, and closing the knowledge gap would be a critical task. For these reasons, the main objective of this PhD project is to evaluate the potential effects of seafloor litter within individual, community, and fishery contexts in the central Tyrrhenian Sea, with a particular emphasis on the following aspects: reconstructing the spatial distribution of seafloor litter throughout the Western Mediterranean Sea (background); evaluating the feeding habits and plastic consumption of blackmouth catsharks (individual level); investigating the impact of seafloor debris on marine communities through eDNA metabarcoding (community level); and assessing the risk

of fishing grounds through an overlap analysis of seafloor plastic hotspots with commercially important species.

The findings of this research indicate significant levels of seafloor litter in the western Mediterranean Sea, with multiple hotspots that could adversely affect ecosystems, specifically harming certain species. Indeed, the generalist-opportunistic feeding behavior of the blackmouth catshark leads to the incidental ingestion of plastic particles, which can be indirectly associated with the presence of macroplastics on the seafloor. Additionally, eDNA metabarcoding was utilized to examine the impact of trawling and litter on the marine community. The study revealed the presence of certain species closely associated with litter on the seafloor, while others were linked to trawling. Furthermore, it was demonstrated that seafloor plastic hotspots coincide with fishing grounds of economically significant species, resulting in adverse effects on fishermen's economic productivity. In this work, it was also possible to quantify the probability of exposure and impact of litter for each of the key trawling species.

This PhD thesis provides a thorough investigation of the distribution and effects of seafloor debris on marine life in the central Tyrrhenian Sea. The methods used converge to understand the complex relationships between environmental variables, anthropogenic factors, and community composition. This study enhances our comprehension of the responses of marine ecosystems to litter accumulation and also provides essential information for designing effective conservation and management strategies to mitigate the impact of litter on marine ecosystems.

Riassunto

L'inquinamento da rifiuti nei fondali marini è emerso come un problema ambientale significativo, che pone potenziali minacce alla biodiversità marina e al funzionamento dell'ecosistema. I fondali marini sono un habitat fondamentale per numerose specie marine e l'accumulo di rifiuti altera l'equilibrio e la funzione essenziale di questo sistema ecologico. Questo inquinamento può influenzare gli organismi sia a livello individuale, attraverso l'ingestione o l'impigliamento, sia a livello di comunità, alterando la disponibilità di nutrienti e modificando le proprietà fisiche e chimiche dell'ambiente. Inoltre, è stato osservato che l'inquinamento dei rifiuti marini ha un impatto negativo sulla pesca marina in tutto il mondo, influenzandola sia direttamente che indirettamente. Ad esempio, la presenza di rifiuti marini nelle reti da pesca può ridurre l'efficienza delle catture, causare danni meccanici, ridurre il tempo disponibile per la pesca, aumentare le spese di riparazione, ostruire le attrezzature e richiedere un maggiore consumo di carburante. Inoltre, le specie bersaglio della pesca, insieme alle loro prede, sono a rischio di danni letali e sub letali a causa dell'inquinamento da plastica, che potrebbero ridurre la qualità del pescato. In particolare, molti studi hanno messo in relazione l'inquinamento da plastica dei fondali marini con la riduzione del successo riproduttivo, le limitazioni della crescita e la riduzione della crescita della massa corporea negli organismi esposti, che potrebbero ridurre il valore economico di una specie e quindi avere un impatto negativo sul sistema produttivo.

Nonostante la crescente consapevolezza e preoccupazione per gli effetti della pesca a strascico e dei rifiuti sui fondali marini, non esistono studi sugli effetti di questi inquinanti sulla pesca e sulla gestione degli stock. Per questi motivi, l'obiettivo principale del mio progetto di dottorato è quello di valutare i potenziali effetti dei rifiuti dei fondali marini a livello individuale, comunitario e di pesca nel Mar Tirreno Centrale, con particolare attenzione ai seguenti aspetti: ricostruire la distribuzione spaziale dei rifiuti del fondale marino nel Mediterraneo occidentale (*background*); valutare le abitudini alimentari e il consumo di plastica degli squali gattucci boccanera (livello individuale); indagare l'impatto dei detriti del fondale marino sulle comunità

marine attraverso il DNA ambientale (livello di comunità); valutare il rischio delle zone di pesca attraverso un'analisi di sovrapposizione degli *hotspot* di plastica del fondale marino con le specie commercialmente importanti.

I risultati di questa ricerca indicano livelli significativi di rifiuti sul fondale marino nel Mar Mediterraneo occidentale, con molteplici *hotspot* che potrebbero influire negativamente sugli ecosistemi, danneggiando in particolare alcune specie. Infatti, il comportamento alimentare generalista-opportunista dello squalo boccanera porta all'ingestione accidentale di particelle di plastica, che possono essere indirettamente associate alla presenza di macroplastiche sul fondale marino. Dopodiché, l'utilizzo del DNA ambientale per esaminare l'impatto della pesca a strascico e dei rifiuti sulla comunità marina ha rivelato la presenza di alcune specie strettamente associate ai rifiuti sul fondo marino, mentre altre erano strettamente legate a zone ad alto impatto di pesca a strascico. Inoltre, è stato dimostrato che gli *hotspot* di plastica sul fondale marino coincidono con le zone di pesca di specie economicamente importanti, con conseguenti effetti negativi sui profitti dei pescatori. In questo lavoro è stato anche possibile quantificare la probabilità di esposizione e l'impatto dei rifiuti per ciascuna delle principali specie di pesca a strascico.

La mia tesi di dottorato fornisce un'indagine approfondita sulla distribuzione e sugli effetti dei rifiuti sul fondale marino sulla vita marina nel Mar Tirreno centrale. I metodi utilizzati convergono nel comprendere le complesse relazioni tra variabili ambientali, fattori antropici e composizione delle comunità. Questo studio migliora la nostra comprensione delle risposte degli ecosistemi marini all'accumulo di rifiuti e fornisce anche informazioni essenziali per la progettazione di strategie di conservazione e gestione efficaci per mitigare l'impatto dei rifiuti sugli ecosistemi marini.

General introduction

Marine litter – Sources and distribution

According to the definition by UNEP 1995, marine litter refers to "*any solid material that is persistent, manufactured, or processed, and is left behind, disposed of, or abandoned in the marine and coastal areas*". In recent years, the presence of these materials has increased significantly on the sea surface, beaches and seabed (Beaumont et al., 2019). First noted in marine environments during the 1960s, marine litter is now a widespread phenomenon across all oceans (Law, 2017). The increasing production of waste by humans has led to a continuous global increase in the overall quantities. Plastics, owing to their versatility, durability, malleability, and cost-effectiveness, have emerged as the primary constituent of this litter (Geyer et al., 2017). They have become part of everyday life and delivered social benefits (Boucher et al., 2020). Consequently, the development and diffusion of plastic materials have resulted in obvious repercussions, becoming a topic of discussion in recent years (GESAMP, 2019; Markic et al., 2020; Thompson et al., 2009). These materials have become ubiquitous, accounting for as much as 95% of the waste accumulating on shorelines, the sea surface, and the seafloor. The most frequently encountered items are plastic bags, fishing equipment, and food and beverage containers, constituting over 80% of the litter found on beaches (Thiel et al., 2013). A total of 35% of plastic leakage is attributed to the cities and towns located directly on the coast, while the remaining 65% is generated from the inland and comes into seawater by surface run-off (Boucher & Billard, 2020). An important contribution of plastics also comes from rivers, with the highest leakage rates from catchment areas (basins) hosting large rivers (Hurley et al., 2018).

Due to its physio-chemical properties, plastics are widely recognized as having a high resistance to degradation, and they can persist in marine environments for thousands of years (Chamas et al., 2020a). Extensive research has documented the degradation of plastic litter on ocean surfaces and beaches through processes like photo-oxidation,

thermal stress, mechanical breakdown, and biodegradation (Corcoran, 2015; Masry et al., 2021). The deep sea, characterized by its distinct environmental conditions, including lower temperatures, absence of UV light, and oxygen depletion (Chamas et al., 2020), remains an uncharted zone in terms of plastic degradation, and recent studies assumed that plastics required about 292 years to be totally degraded (Zhang & Peng, 2022). In the deep sea, the conditions significantly hinder thermal and photo-oxidative degradation (Nakajima et al., 2021), leading to the assumption that biodegradation by microorganisms may emerge as the predominant mode of plastic degradation.

The presence and dispersal of human-made debris exhibit significant spatial differences. Several factors, including ocean current patterns, climate conditions, tides, proximity to urban, industrial, and recreational areas, shipping routes, and fishing zones, influence the types and quantities of litter found in open waters or along coastlines. These factors can also influence the rates at which these materials accumulate (Galgani et al., 2015). In enclosed seas, such as the Mediterranean or Black Sea, the seafloor can host some of the highest concentrations of marine litter, with densities exceeding 100,000 items per square kilometre, as reported by (Galgani et al., 2000). In surface waters, the issue of plastic fragments has become more pronounced over the past few decades. However, there is limited knowledge regarding the long-term trends in debris accumulation in the deep sea.

Understanding the composition of marine litter is crucial because it yields essential insights into individual litter items, most of which can be traced back to their origins. The sources of marine litter could be either land-based or ocean-based, depending on the point at which the litter enters the ocean (Browne et al., 2011). Some items can be attributed to specific sources, such as fishing equipment, debris linked to sewage, and litter from tourists. Land-based sources primarily encompass recreational activities along the coastline, litter from the public, industrial waste, materials from harbours, and unprotected landfills (Galgani et al., 2015). Additionally, sewage overflows, accidental losses, and extreme events contribute to land-based sources. The transport

of marine litter to the sea can occur through rivers industrial discharges, run-offs, or even wind-driven transport into the marine environment (Rech et al., 2014; Woodall et al., 2014). Ocean-based sources of marine litter encompass commercial shipping, ferries, cruise liners, both commercial and recreational fishing vessels, and offshore platforms or aquaculture sites.

Seafloor litter - Impacts

Despite a lack of information, it is becoming increasingly apparent that the seabed and particularly the deep seafloor is a major sink for marine litter (Lebreton et al., 2019). Recent studies have shown that the abundance of litter in some areas is numerically higher than that of the macro biota (Angiolillo & Fortibuoni, 2020; Pierdomenico et al., 2016) and in other areas, trawl surveys have shown that the megafaunal biomass is similar to the total weight of waste (Cau et al., 2019). After reaching the sea, most waste can rapidly spread over the coastal and offshore regions, but it is bound to sink to the bottom of the sea finally (Canals et al., 2021). The type of object, composition, density, and hydrodynamic properties are important for the dispersion and accumulation of macrolitter. For example, heavy objects, such as fishing gear, tyres, tanks, or high-density plastic polymers, will remain on the ground where they have been stranded, lost, or discarded, with a rare subsequent mobilisation. In the case of light litter items, these can remain suspended for an uncertain period, sinking by aggregation, biological action, ballasting, or biofouling (Amaral-Zettler et al., 2021; Enrichetti et al., 2021; Kühn et al., 2015; Saldanha et al., 2003; Subías-Baratau et al., 2022; Zettler et al., 2013). On the seabed, litter is subject to a secondary dispersion, due to the remobilisation by varied human activities, such as dredging or bottom trawling (Canals et al., 2021) This may result in further degradation and fragmentation of litter. In addition, these activities lead to the resuspension of sediment flows and the redistribution of sediment particles, which could also result in the burial of waste (Tubau et al., 2015).

Seafloor litter pollution has emerged as a significant environmental concern, posing potential threats to marine biodiversity and ecosystem functioning. Larger pieces of

marine debris, such as ghost nets and bulky objects, can entangle and harm sessile and motile individuals on the seafloor, such as sponges, corals, and benthopelagic species (Colmenero et al., 2017; de Carvalho-Souza et al., 2018; Galgani et al., 2018). Fishing gear that is abandoned, lost, or discarded can damage species and habitats that are important for conservation and ecosystem structure (Canals et al., 2021). Corals can get abrasions from rubbing against tangled or moving gears, which can increase their risk of infection and death(Angiolillo et al., 2014; Consoli et al., 2020; Enrichetti et al., 2021).

Seafloor litter modifies soft-bottom habitats by introducing artificial surfaces. In fact, litter increases habitat heterogeneity by providing new hard habitats for encrusting and sessile organisms, known as biofouling, and vagile fauna in environments that are otherwise dominated by soft sediments (Angiolillo et al., 2023; Bergmann et al., 2015). While these substrata may enhance local biodiversity, they interfere with seafloor organisms and community structure. Indeed, litter objects attract a variety of organisms, potentially promoting the establishment of non-native species. Additionally, marine litter disrupts crucial functions such as filter-feeding in organisms, contributing to ecological imbalances (Canals et al., 2021).

Furthermore, litter objects can be mistakenly ingested by animals, and this can cause blockages or internal bleeding in the intestines of the species, for large objects, while microparticles could have indirect consequences (Camedda et al., 2019; Domènech et al., 2019; Provencher et al., 2017; Van Franeker et al., 2011). The presence of micro-litter is of particular concern because these tiny particles can be ingested by marine life, entering the food chain and posing potential threats to both marine ecosystems and human health. In recent years, many studies have focused on micro-litter, in particular microplastic, ingestion by marine biota (Auta et al., 2017; Bray et al., 2019; Fossi et al., 2018; Güven et al., 2017; Kühn et al., 2015; Markic et al., 2020). Ingestion events can take place directly (primary ingestion) or indirectly, by ingesting food which contains microplastics (secondary ingestion). The wide range of negative effects on marine organisms includes decrease of mobility, reduction of feeding and

growth, and reduced body condition (Critchell & Hoogenboom, 2018; de Sá et al., 2015). Another concerning factor is that persistent organic pollutants such as pesticides, fertilizers and industrial chemicals readily adhere to microplastic surfaces once introduced into marine environment (Hermsen et al., 2018; Napper et al., 2015; Rochman et al., 2015), posing potentially physiological alterations, including endocrine disruption once ingested (Rochman et al., 2014).

Several studies have sought to understand the relationship between ingestion of plastic and environmental contamination, with very promising results. A study conducted in coastal areas of the Western Mediterranean Sea found a correlation between the amount of plastic in the digestive tract of the demersal to semi-pelagic species, *Boops boops*, and the level of pollution in the environment, using an index based on several factors, including population density, river inputs, distance from the coast, and shipping routes (Sbrana et al., 2020). More recent studies revealed that proximity to hotspots of macroplastic accumulation significantly correlates with microplastic ingestion by *Nephrops norvegicus* (Linnaeus, 1758) (Cau et al., 2019; Franceschini et al., 2021). It is worth noting that there is a growing trend of evidence that marine plastics pollution contaminates key stocks for fisheries, with potential negative consequences to human health, resources status, and socio-economic sectors (Lusher, Welden, et al., 2017).

Mediterranean Sea – Monitoring litter in a European context

The Mediterranean Sea, considered one of the most threatened environments in the world, is also subject to this ubiquitous pollutant (Cózar et al., 2015; Eriksen et al., 2014; Liubartseva et al., 2018; Suaria et al., 2016; Tsiaras et al., 2021; Zambianchi et al., 2017). The total litter accumulated in the Mediterranean Sea is estimated at 1,178,000 tonnes, with an annual marine leakage of 229,000 tonnes. Based on these data, Italy (and Rome locality) appears to be one of the three top contributors of plastic leakage in the Mediterranean Sea (Boucher & Billard, 2020). The spread and potential impacts of plastic waste in the marine environment are a global emergency that is pushing the scientific community to make a huge effort to understand and help stem

these phenomena (GESAMP, 2019; Santos et al., 2021; Thompson et al., 2009) As plastic is under- going, even in the marine environment, a phenomenon of miniaturization and degradation that makes it increasingly less traceable (Chamas et al., 2020), it is crucial to enhance the monitoring of macroplastics and their role in the process of environmental contamination. At the same time, plastic can impact biota through accidental ingestion (Savoca et al., 2016). Therefore, it is paramount to understand whether and how the accumulation of plastic waste on the seafloor can lead to increased ingestion events. However, given that marine pollution has also reached very high levels in the Mediterranean and larger areas, it is very complicated and costly to make spatial and temporal assessments of the different levels of contamination (Galgani et al., 2015). To effectively address the issue of marine litter, intensive efforts must be directed towards the mitigation and prevention of both micro and macro litter, encompassing strategies to reduce their production, enhance waste management practices, and establish mechanisms for the removal and deterrence of marine debris at various scales.

The European Commission, through the Descriptor 10 of the Marine Strategy Framework Directive (EU 2008/56/EC - Marine Litter, Criterion D10C3), has established criteria to assess pollutants in marine waters owned by Member States, including seafloor litter. These criteria encompass monitoring the quantity, composition, and potential hazards posed by these pollutants. Despite the diligent efforts of the EC technical group (MSFD TG-ML) and the significance of this issue, our understanding of the consequences of litter on organisms remains incomplete. This knowledge gap persists due to the absence of agreed-upon, standardized methods, resulting in often incomparable and lower-quality data (Hermsen et al., 2018a; Wesch et al., 2016). Consequently, it is needed to employ precise analyses and utilize the most appropriate tools to accurately predict the effects and repercussions of this pollutant in the marine ecosystem.

Fisheries productivity and profitability - Implication and management strategy

Further research must focus on the critical task of examining the connection between litter pollution and the management of fisheries and stocks. Globally, seafood is the primary source of animal protein, constituting over 20% of the dietary intake by weight for approximately 1.4 billion people (19% of the global population), as highlighted in the study by Golden et al. (2016). Litter pollution has been observed to have a negative impact on marine fisheries worldwide, affecting them both directly and indirectly. For instance, the presence of marine litter in fishing nets can reduce their catch efficiency, cause damage, reduce the time available for fishing, inflate repair expenses, obstruct equipment, and necessitate increased fuel consumption (Beaumont et al., 2019; Ivar Do Sul & Costa, 2014; Mghili et al., 2023) Moreover, as mentioned before, fisheries target species, along with their prey, are at risk of both lethal and sub-lethal harm due to plastic pollution, including reduced reproductive success and growth limitations, with the possibility of broader impacts at the population level (Galloway & Lewis, 2017). When combined with other major concerns like overfishing and climate change, marine plastic pollution could have a profoundly negative impact on the productivity, sustainability, profitability, and safety of the fishing and aquaculture industries (Beaumont et al., 2019). Given the high reliance on seafood for nutrition, a significant percentage of the world's population is particularly vulnerable to changes in the quality, quantity, and safety of this vital food supply (Golden et al., 2016). The shortcomings in our comprehension of this crucial issue could be resolved by combining the goals of the fishing industry with those aimed at reducing sea pollution.

The assessment and evaluation of management strategy for plastic pollution require the development of a conceptual framework which comprises the probability of exposure and its impact (Hardesty et al., 2019). For these reasons is important to elucidate the sources, distribution, and impacts within the environment from a systemic view (Hardesty et al., 2019). Understanding the risk posed by marine litter could greatly benefit from this framework (Schuyler et al., 2016). The application of risk assessment can clarify the species most vulnerable to risk and identify areas of

highest concern. Risk assessments frequently represent the first stage in the development of pollution regulations, improved resource management, and policies designed to preserve the environment and public health. To date, various research and studies have addressed the topic of risk assessment for plastic impact using a combination of numerical models, ingestion studies and species distribution ranges (Compa et al., 2019, 2022), but very often these data are incomplete or fragmented. A functional approach to the development of a risk assessment at sea could include the integration of fishing data, which can give us a lot of information on the distribution of species of commercial interest, but also on aggregation areas, which very often overlap with feeding habits of the species.

Aims of the project

This Ph.D. project aims to contribute to the understanding of the impact of marine litter on the seafloor of the central Tyrrhenian Sea through a comprehensive investigation at different ecological scales, focusing on individual organisms, marine communities, and economically important species. The project would like to fill a gap in our knowledge of a current topic, using advanced techniques and innovative approaches, and could provide a basis for mitigation and monitoring actions. The objectives include a detailed plan to address specific aspects of this broader area.

Specifically, the aims of my dissertation are: a) To study the interaction between the feeding habits of fish individuals and plastic consumption by analysing the diets of individuals living in areas with different levels of seafloor litter impact. b) To understand whether macro debris on the seafloor can affect the distribution of necto-benthic species by extrapolating marine community information using a molecular approach (eDNA metabarcoding). c) To assess the impact of seafloor litter on the fishing grounds of commercial species measured as the risk of exposure of individual species and the potential impact on fishermen's profits in terms of gross value added.

The Ph.D. thesis is divided into four main chapters, including two published and two submitted papers. **Chapter 1** presents an extensive analysis of the distribution of seafloor litter in the western and central Mediterranean macro-region using a machine learning (i.e., Random Forest; RF - Breiman, 2001) method to model the temporal and spatial distribution of seafloor macro-litter. **Chapter 2** examines the effects of seafloor litter, particularly plastic, on 164 blackmouth catshark (*G. melastomus*) individuals. The study will investigate the ingestion of plastics by the species and its correlation with environmental contamination levels (i.e., distribution and abundance of macro debris on the seafloor). **Chapter 3** uses eDNA data to inspect the complex relationships between demersal community species composition, key environmental features, and anthropogenic impacts such as fishing efforts and seafloor litter. Finally, **Chapter 4** evaluates the effects of seafloor litter on commercially important species. Specifically, the study examines the economic performance of fisheries, measured by

Gross Value Added, and the risk of species exposure as the amount of plastic on the seafloor increases.

This PhD thesis would represent a significant step towards understanding the complex relationship between anthropogenic pressures and marine communities. Specifically, it could contribute significantly to the growing body of knowledge surrounding the ecological consequences of marine litter, with potential implications for developing targeted mitigation and management strategies.

Chapter 1 - What, where and when: spatial-temporal distribution of macro-litter on the seafloor of the western and central Mediterranean Sea

Abstract

The progressive increase of marine macro-litter on the bottom of the Mediterranean Sea is an urgent problem that needs accurate information and guidance to identify those areas most at risk of accumulation. In the absence of dedicated monitoring programs, an important source of opportunistic data are fishery-independent monitoring campaigns of demersal resources. These data have long been used but not yet extensively. In this paper, MEDiterranean International Trawl Survey (MEDITS) data was supplemented with 18 layers of information related to major environmental (e.g. depth, sea water and wind velocity, sea waves) and anthropogenic (e.g. river inputs, shipping lanes, urban areas and ports, fishing effort) forcings that influence seafloor macro-litter distribution. The Random Forest (RF), a machine learning approach, was applied to: i) model the distribution of several litter categories at a high spatial resolution (i.e. 1 km^2); ii) identify major accumulation hot spots and their temporal trends. Results indicate that RF is a very effective approach to model the distribution of marine macro-litter and provides a consistent picture of the heterogeneous distribution of different macro-litter categories. The most critical situation in the study area was observed in the north-eastern part of the western basin. In addition, the combined analysis of weight and density data identified a tendency for lighter items to accumulate in areas (such as the northern part of the Tyrrhenian Sea) with more stagnant currents. This approach, based on georeferenced information widely available in public databases, seems a natural candidate to be applied in other basins as a support and complement tool to field monitoring activities and strategies for protection and remediation of the most impacted areas.

Keywords: Marine waste; Seafloor macro-litter hotspots; Waste management; Mitigation strategies; Litter removal.

Introduction

The mass of human-made materials on our planet has recently overweight the total living biomass (Elhacham et al., 2020) and one of the consequences is that human-generated waste is consistently being dispersed in the environment, with oceans ahead (Jambeck et al., 2015). Global waste doubled every ~20 years over the last century and it is forecasted to reach 53 million tons year^{-1} in 2030, for plastic alone (Borrelle et al., 2020), that numerically accounts for ca. 60% of the whole amount of litter dispersed in the marine environment (https://litterbase.awi.de/litter_graph; Bergmann et al., 2017). Marine macro-litter accumulates on the seafloor, which is regarded as the ultimate sink for macro-litter dispersed in the environment (Woodall et al., 2014).

According to recent scientific literature, it is widely recognized that litter-free seas represent a *utopia*; however, more realistic targets (set to tackle plastic contamination but still applicable to all macro-litter) suggest the use of multiple mitigation measures, that should act synergically to meet ambitious goals (Borrelle et al., 2020; Lau et al., 2020). One of these measures is the removal of the fraction of macro-litter already accumulated in the ocean, which should be coupled with a cap in production of ecologically impacting materials like plastic (Bergmann et al., 2022; Rochman, 2016; Rochman et al., 2013). Consequently, understanding distribution patterns and monitoring of accumulation hotspots becomes crucial steps to drive future remedial actions.

A proper assessment of the distribution and effects of macro-litter on the seafloor is primarily challenged by reduced data availability and comparability, especially in deep-sea environments. However, data is accumulating and studies dealing with spatio-temporal variability of macro-litter on the seabed are becoming available (Buhl-Mortensen et al., 2022; Canals et al., 2021; Galgani et al., 2021; Parga Martínez et al., 2020), thus providing useful information to identify distribution and accumulation patterns and hotspots (Cau et al., 2022; Garofalo et al., 2020; Tubau et al., 2015). This is particularly relevant within the Mediterranean basin, which is a globally recognized as a litter hotspot due to its features of semi-enclosed and highly

anthropized basin (Canals et al., 2021; Galgani et al., 2000; Pierdomenico et al., 2019); however, only few studies developed models to identify and predict possible locations where macro-litter might accumulate (e.g., Cau et al., 2022; Franceschini et al., 2019; Spedicato et al., 2019).

From a technical point of view, detection and characterization of macro-litter on the seafloor relies mainly on different approaches, including litter collection with bottom trawlers (Melli et al., 2016; Mifsud et al., 2013; Strafella et al., 2015) and optical and acoustic mapping of the seafloor (Angiolillo et al., 2015; Cau et al., 2017; Madricardo et al., 2020). Due to their high costs, these latter approaches can often be performed over a limited spatial and temporal scale. Considering the few and scattered data available and that the distribution of waste is essentially determined by its release and passive transport (unlike nekton organisms that move autonomously), machine learning techniques can be a useful tool to profitably use the already available, yet limited, information collected in the field to possibly infer about macro-litter distribution over large areas for which direct observations are not available.

In this study, we used a machine learning method (i.e., Random Forest; RF - Breiman, 2001), to model the temporal and spatial distribution of seafloor macro-litter in the western and central Mediterranean macro-region. A series of spatial layers, corresponding to the main sources and/or drivers of waste contamination at sea, were gathered from various sources, including the Copernicus Marine Service and the MEDiterranean International Trawl Surveys (MEDITS), across six General Fisheries Commission for the Mediterranean (GFCM) Geographical Sub-Areas (GSA; GFCM, 2007). These layers were used to train a set of RF models with very high spatial resolution (i.e. 1 km square grid), devised to predict abundance of each typology of macro-litter. Our results confirmed the power of machine learning techniques in capturing the relationships between predictors and the spatio-temporal distribution of different types of marine litter. Furthermore, it is possible to distinguish some important differences within the vast study area examined and to highlight the importance of some anthropogenic forcings.

Materials and Methods

Study Area

The study area (Figure 1) belongs to the western and central Mediterranean Sea, covering a total surface of 125,000 km^2 and incorporates FAO GSA 7 (southern France), 8 (Corsica), 9 (Ligurian and northern Tyrrhenian), 10 (south and central Tyrrhenian), 11 (Sardinian Seas, considering GSA 11.1 and GSA 11.2 as a single unit) and 16 (south Sicily). This area encompasses different environments of the western Mediterranean basin such as the Sardinia channel, the strait of Sicily, the Gulf of Lyon, and the Tyrrhenian Sea. Whose geological morphologies and local circulation feature cumulatively affect water masses circulation within the basin (Millot, 1999), thus representing a relevant and interesting case study to investigate seafloor macro-litter distribution and accumulation patterns. The detailed description of each GSA is available in a dedicated section of Supplementary materials.

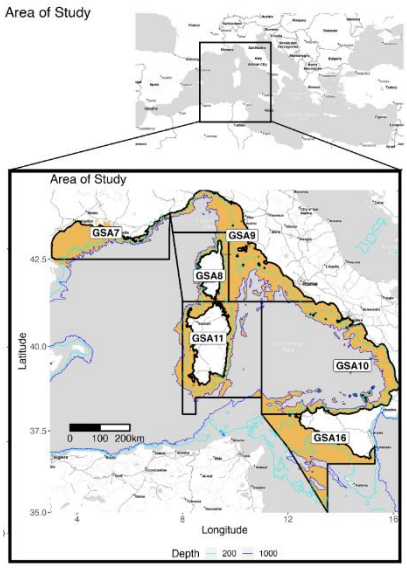


Figure 1. Area of study (western and central Mediterranean Sea) in which the borders of the six Geographical Sub Areas are shown together with the portion (in orange) of the sea bottom from the coastline to the 1,000 m depth isobath.

Data collection

Data used in the present study were collected in the framework of the MEDITS survey conducted from 2013 to 2019, in the above-mentioned GSAs. The MEDITS is the main bottom trawling survey conducted in the whole Mediterranean Sea, aiming at collecting data on demersal resources and, since 2013, it has also become a valuable source of information about seafloor macro-litter (MEDITS working group, 2012).

The data series used in this study was built taking advantage of 495 hauls performed yearly across the six GSAs considered, for a total of 3,465 hauls covering 7 years (Supplementary Table 1). In the MEDITS protocol, hauls are located according to a depth-stratified random design with the following strata: A [0-50m); B [50-100m); C [100-200m); D [200-500m) and E [500-800m). For the implementation of the RF model, MEDITS hauls were assigned to cells of a 1 × 1 Km square grid. The cells of the grid were then considered as statistical units of the applied model. Given that, in five of the six GSAs considered and throughout the temporal period inspected, dozens of hauls per year were associated with cells belonging to the [800-1000m) stratum (the depth of each cell was computed as the average of the NOAA ETOPO1 records - see Supplementary Materials), this additional stratum was considered in the model.

Onboard operations include the separation of seafloor macro-litter from the catch and its classification according to nine major categories (i.e., L1: Plastic; L2: Rubber; L3: Metal; L4: Glass/Concrete; L5: Cloth; L6: Processed wood; L7: Paper and cardboard; L8: Other; L9: Unspecified) and relative sub-categories, according to the MEDITS handbook (MEDITS working group, 2012). Within each category, items were counted, and wet weight was measured; in case of containers, water and sediment contents were washed/removed prior to weighting. The total weight and the number of items collected per each sub-category was standardized according to the swept area, expressed as number of items and weight km^{-2}. For the purposes of this paper, five out of the nine categories of macro-litter were used in the analysis (L1, L2, L3, L4, L5). Plastic litter (L1) was further divided in two sub-categories, distinguishing

objects that are ‘related’ to fishing activities (i.e., fishing nets, lines, ropes, etc.) and ‘non-related’ (i.e., plastic bags, bottles, wraps, etc.; Table 1).

Table 1. Classification scheme of marine litter categories and sub-categories applied in the present study.

Category	Sub-category	Type	Source	Groups
L1		Plastics	Mixed	
	L1a	Bags	Non Fishing-related	L1-Non Fishing-related
	L1b	Bottles	Non Fishing-related	L1-Non Fishing-related
	L1c	Food wrappers	Non Fishing-related	L1-Non Fishing-related
	L1d	Sheets	Non Fishing-related	L1-Non Fishing-related
	L1e	Hard objects	Non Fishing-related	L1-Non Fishing-related
	L1f	Fishing nets	Fishing-related	L1-Fishing-related
	L1g	Fishing lines	Fishing-related	L1-Fishing-related
	L1h	Other fishing-related	Fishing-related	L1-Fishing-related
	L1i	Synthetic ropes	Fishing-related	L1-Fishing-related
	L1j	Others	Others	
L2		Rubber	Others	L2
L3		Metal	Others	L3
L4		Glass/Ceramic/Concrete	Others	L4
L5		Cloth(textile)/Natural fibres	Others	L5

Several environmental and anthropogenic variables were considered and used as predictors in the models (Table 2). These variables were considered reliable proxies for the main sources and drivers of the distribution of marine macro-litter. Sea bottom depth was estimated, for each cell of the grid, querying the NOAA ETOPO1 Global Relief Model using the R package “marmap” (Pante and Bouhet, 2022). Distance from coastline was computed for each cell of the grid, using the ‘dist2Line’ function of the R package “geosphere” (Hijmans et al., 2021). Rivers’ mouth positions and catchment area (in km^2) were obtained from the European Environmental Agency https://www.eea.europa.eu/data-and-maps/data/european-river-catchments-1) and used to compute, for each cell of the grid, an index of the Impact of River Basins

defined as the average of catchment areas weighted by the distance of cell centre from the river mouths. The positions of the main shipping lanes were download from (Halpern et al., 2015) (https://knb.ecoinformatics.org/view/doi:10.5063/F1S180FS) and used to compute, for each cell of the grid, the Mean Distance from Shipping Lanes. The positions and the surface (in km^2) of the Urban areas were download from the Efrain Maps Website (https://www.efrainmaps.es/english-version/free-downloads/europe/) and used to compute an index of the Impact of Urban Areas defined as the average of urban areas weighted by the inverse of their distance from the cell centre. Positions and size class of the port areas were downloaded from the National Geospatial-Intelligence Agency website (https://msi.nga.mil/Publications/WPI) and used to compute an index of the Impact of Port Areas, defined as the average of port classes weighted by the inverse of their distance from the cell centre. The Northward and Eastward Sea water velocities in m-s, the Northward and Eastward Sea surface wave stokes drift in m-s the Mean Sea Level in m, the Northward and Eastward wind speed in m-s and the Mean Sea Wave Height in m were downloaded from the Copernicus Marine Service (https://marine.copernicus.eu/) for the period of interest and average to obtain single values for the cells of the grid. Rugosity (RUG) (i.e. the roughness of the seafloor), an indicator of occurrence of hard-bottom habitat, was derived from the bathymetry layer using the Benthic Terrain Modeller tool in ArcGIS 10.1. RUG is quantified as the likelihood of hard-bottom habitat presence and ranges from zero to one. Finally, three different variables were generated from the analysis of Vessel Monitoring System (VMS) data, according to the procedures described in (Russo et al., 2014) and Russo et al (2016). The spatial representation of considered environmental and anthropogenic variables is reported in Supplementary Figures S1 and S2a-b.

Table 2. Environmental and anthropogenic factors collected and processed in order to train the random forest model for the distribution of seafloor litter in the western Mediterranean Sea.

Environmental variable	Anthropogenic variable
– Sea bottom depth	– Impact of River Basins
– Distance from the coast	– Mean Distance from Shipping Lanes
– Northward sea water velocity	– Impact of Urban Areas
– Eastward sea water velocity	– Impact of Port Areas
– Northward Sea surface wave stokes drift	– Mean Fishing effort (bottom otter trawling)
– Eastward Sea surface wave stokes drift	– Average effort in neighboring cells
– Mean Sea Level	– Delta effort inside/outside
– Eastward wind velocity	
– Northward wind velocity	
– Mean Sea Wave Height	
– Rugosity	

Random Forest

Classification (or Decision) Trees (CTs) are a non-parametric supervised learning method based on a model that predicts the value of a target variable (which can be qualitative or quantitative) by inferring simple decision rules from the input data. When an ensemble of Classification Trees is combined (into a "Forest") and their predictions are averaged (if the target variable is quantitative) or used to establish the most voted class (if the target variable is qualitative), we are dealing with a RF (Breiman, 2001). The procedure begins by choosing a bootstrap sample from a subset of the training data for each tree in the forest (Breiman 2001). Out-Of-Bag (OOB) records are those that were not included in the current bootstrap sample of the data. Each tree is then automatically built to its maximum depth and left unpruned for each bootstrap sample. Only a randomly chosen (hence the term "Random") subset of q predictive variables, where p is the total number of predictors, are accessible for binary partitioning at each split in the tree (Breiman 2001). Usually, the number of Classification Trees in the forest is represented by the number ntree, which is how many times this method is repeated. The final step is to average the results of all the

trees for regression applications (Breiman, 2004; Cutler et al., 2007; Gislason et al., 2006; Liaw and Wiener, 2002). The performance of RF can be modified using a number of its parameters. The primary variables that significantly impacted the accuracy of this method were the total number of trees in the forest (ntree), the number of randomly chosen predictors available at each split for the binary partitioning, known as mtry, as well as the minimum number of records contained in each leaf to stop the splitting procedure (ndsize) (Cutler et al., 2012; Scornet, 2017). All 3 of these parameters are tuned by means of an iterative procedure in which a wide range of values is explored for each of them. The final values obtained from the tuning procedure and used in this study were: ntree = 1000, mtry = 7, ndsize = 5.

In this study, the RF approach provided in the R package "RandomForest" (Liaw and Wiener, 2002) was applied to predict the spatial distribution of each of the categories and sub-categories, and groups listed in Table 1. All variables described above as well as the MEDITS data about litter density and weight, per each category/sub-category, were standardized over the set of 11,341 cells covering the portion of GSAs from the coastline to the 1,000 m isobath. The number of cells in which at least one MEDITS sampling occurred in the period considered was 1048 (around 10% of the total domain of 11,341 cells). This set of 1048 was used to train and test the RF models, as described in the next section.

The applied procedure can be summarized as follows:

- For each of these litter typologies, the set of 1048 cells containing the MEDITS records was split into two subsets: the training set and the test set, including 70 % and 30%, respectively, of the total cells. This splitting procedure, repeated 100 times, was pseudo-random as we forced the sampling to guarantee that the 70/30% proportion occurred for each of the bathymetric strata of the MEDITS (see Section 2.2).
- 10 RFs models were trained and tested for each of these pairs of training and test sets. Namely, the adjusted R2 and the root-Mean-Square Error (rMSE) indexes were used to compare the observed vs predicted values of litter categories and sub-

categories in number of objects and weights by cell. For each RF model, the relative importance of each predictor was internally assessed by the randomforest function in R, using the approach described in (Breiman, 2001). This approach was devised to assess how much removing or noising each variable reduces the accuracy of the model prediction (on the training dataset). Finally, the trained RFs were used to predict the amount of litter over the whole domain (11,341 cells), to obtain a series of spatial maps of litter categories and sub-categories in the study area.

Relationship between number and weight of objects: Generalized Additive Models

The visual inspection of the results (see next section) suggested a further analysis of the relationship between number and weight of objects in the original MEDITS data (which are actual observations). This was done by fitting Generalised Additive Model (GAM - Hastie and Tibshirani, 1990) model in which:

$$Log_{10}(W_{Year,cell}) = s(Log_{10}(N_{Year,cell})) + Category + GSA + \epsilon$$

Where WYear,cell is the weight of objects (kg km^{-2}) in a given cell in a given year, N is the corresponding number of objects km^{-2}, and category is one of the six groups represented in Table 1. GAMs are non-parametric regression models allowing to model the associations between variables without defining the exact shape of the underlying regression function. Compared to parametric (including linear) forms of models, GAMs provide more flexibility when smooth functions are used as regressors. GAMs were applied using the R package "mgcv" (Wood, 2023).

This "naive" modelling approach was devised to assess whether the average weight of waste is significantly different across different GSAs and litter categories. In particular, the Davies' approach (Davies, 1987) was applied to test for statistical difference between regression parameters related to the GSAs, eventually supporting the existence of statistical differences between the slopes of the regression between GSAs (different mean weight of litter categories in different areas). All the analyses describe above were carried out in the R environment (R Core Team, 2023).

Results and discussion

Data exploration, areas of accumulation and hotspots

The mean distribution of seafloor macro-litter collected from 3,465 hauls in the period 2013-2019 is shown in Figure 2, for both number (2A) and weight (2 B) of objects. In all GSAs, the main group of litter is non-fishing related plastics, followed by fishing related plastics, whereas the other groups contribute with lower, often marginal, percentages to the total. The northern part of the west Mediterranean, which includes northern Tyrrhenian Sea (Italy; GSA9), southern Tyrrhenian Sea (GSA10), and the Gulf of Lion (France, GSA7) consistently appeared to be the most impacted region, with mean densities that sometimes reaches ~ 105 items km^{-2} in the case of fishing-related or non-fishing-related plastic, being three orders of magnitude (102 to 105) more impacted than e.g. the strait of Sicily (Figure 2). The pattern described above is consistent with previous findings that reported an accumulation of macro-litter along the eastern coast of Corsica (Gerigny et al., 2019). Moreover, this section of the Western Mediterranean hosts important active fisheries and it is close to significant commercial routes. Further environmental drivers are local wind and water velocity that cumulatively could provide clues on the observed patterns, as already documented by Spedicato et al. (2019), that documented a similar pattern of plastic accumulation in this region and attributed this phenomenon to the peculiar local circulation pattern.

When considering the total amount of litter in terms of weight of the objects is more balanced (Figure 2B). Rubber (e.g. tires) is present in large quantities in all GSAs, as for fishery-related plastics and metals. Only GSA07 shows high values of weight for non-fishery plastics. The kernel densities (Supplementary Figure S3) report unimodal distributions for almost all the categories and sub-categories across the GSAs. The distributions show skewness near to zero, but in the GSA16, they are often platykurtic, which means that litter items are, on average, heavier than in other areas. Results indicate that rubber accounted for a small portion in terms of the number of items; however, it was among the most abundant in terms of weight, being mostly composed

by dumped car tires (i.e., fewer but heavier objects). Car tires and rubber in general do not decompose easily and can remain on the seafloor for a long time (Kole et al. 2017). Car tires, for instance, slowly degrade into micro-sized rubber fragments. As they break down over time, they release harmful chemicals and pollutants into the marine environment, including heavy metals and toxic compounds (Halsband et al 2020). Leachates from different plastics and car tire rubber contain a variety of metals and organic additives that cumulatively can affect fish behaviour, gamete fertilization, embryonic development, larvae motility and survival of different species (Capolupo et al. 2021, Halsband et al 2020; Gorule et al., in press).

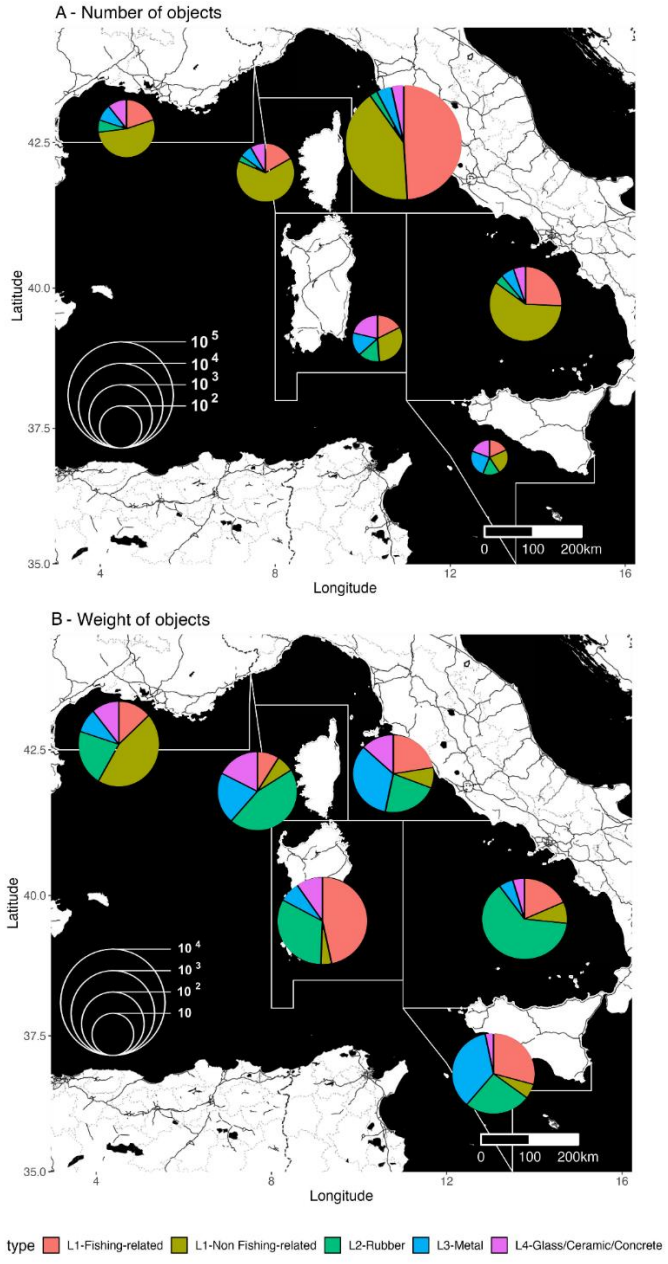


Figure 2 Maps with pie charts showing the mean total amount (radius of the pie) and the mean composition (with respect to the main categories) of marine litter, by GSA, as number of objects km^{-2} (A) and weight of objects kg km^{-2}; (B).

Random Forest performance and main drivers of litter accumulation

The results of the application of RF (Supplementary Figure S4) indicate that the corresponding models largely have a value of median R2 on the test sets higher than 0.8. The exceptions, represented by L1-Plastics, L1e- Hard objects, L1j-Others and L6-Others, when quantified as weight, have median R2 values around 0.25, which indicate a low predictive ability of the trained RF models. The median rMSE is always below 0.15 and often below 0.1. Overall, the RF models showed great efficacies for all categories of macro-litter, and particularly when the number of items per category was used as response variable rather than weight. Considering also how the model output showed low values for rMSE, the lower predicting power of the model for those categories could be ascribed to the fact that some relevant categories of predictive factors were not included in the model.

With respect to the relative importance of predictors (Supplementary Figure S5a and S5b), it is possible to notice that, for most of the categories/sub-categories, only one or a few predictors tower above the others. Interestingly, trawl effort influences the distribution of fishing related categories (i.e., hard objects [L1e] and other fishing related [L1h]), while distance from the coast (dCoast) is associated with fishing lines (L1g). In contrast, a large set of predictors influence the amount of litter in terms of weight of the objects (Supplementary Figure S5b). It is interesting to notice that single use plastics (i.e., bags [L1a], bottles [L1b] and food wrappers [L1c]) are mainly influenced by their relative position with respect to urban areas, and by the distance from the coast and shipping, while fishing lines (L1g) are associated with trawling. This resulted in scattered accumulation hotspots for the two different categories, with the former being more abundant in areas closer to the coastline and, likely, to their source point, while the latter was more concentrated on fishing grounds far from the coastline (Figure 3a).

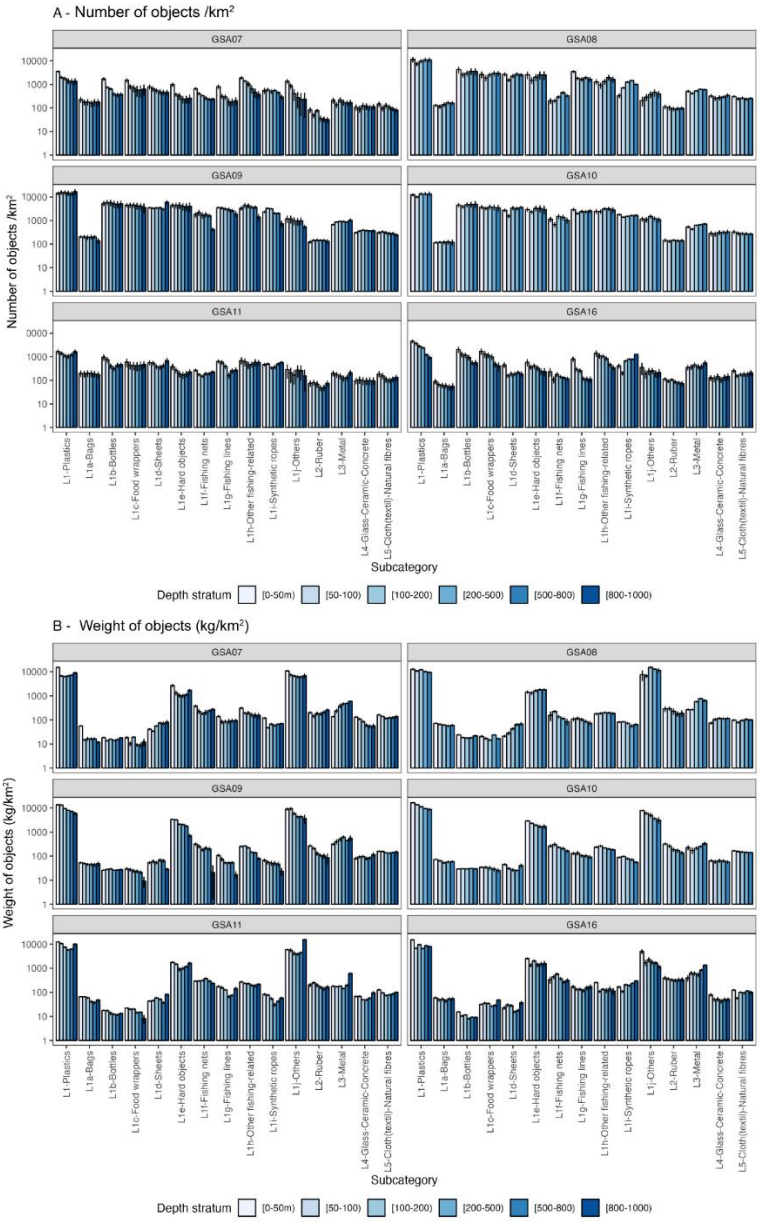


Figure 3a Barplots of the predicted seafloor litter abundance in the western Mediterranean Sea, by depth stratum, and Geographical Sub Areas, for all the sub-categories, as (A) mean number of objects km^{-2} and (B) mean weight of objects per (kg km^{-2}). Bars represent the mean value over the period (years 2013-2019) considered, and the standard deviation is represented by the error bars.

However, the Year is by far the most important variable when modelling all the categories and sub-categories in terms of number of objects. Temporal pattern of the absolute amount of seafloor macro-litter in the study area shows interannual fluctuating trends (Figure 4); still, an overall increase is detectable in most of the GSAs. Observed trends become more fluctuating when the weight of objects is considered. Nonetheless, our results further highlight how the standing stock of marine macro-litter is highly unstable due to variations in inter-annual dynamics of both natural events (e.g., flooding, heavy rain, storms) or human activities that modulate macro-litter leak into aquatic environments. On top of these, the fraction of macro-litter already accumulated in the environments can possibly get dislocated by trawling activities (Franceschini et al., 2019), resuspended in case of lighter objects, or even buried in case of proximity peculiar hydrological and/or geomorphological settings (e.g., Pierdomenico et al., 2023). These documented but still poorly understood patterns could play a role in explaining the sharp increase or decrease (i.e., in the order of ~30-50%) that was observed for some categories of macro-litter such as glass (L4) or Rubber (L2; Figure 4) over certain years.

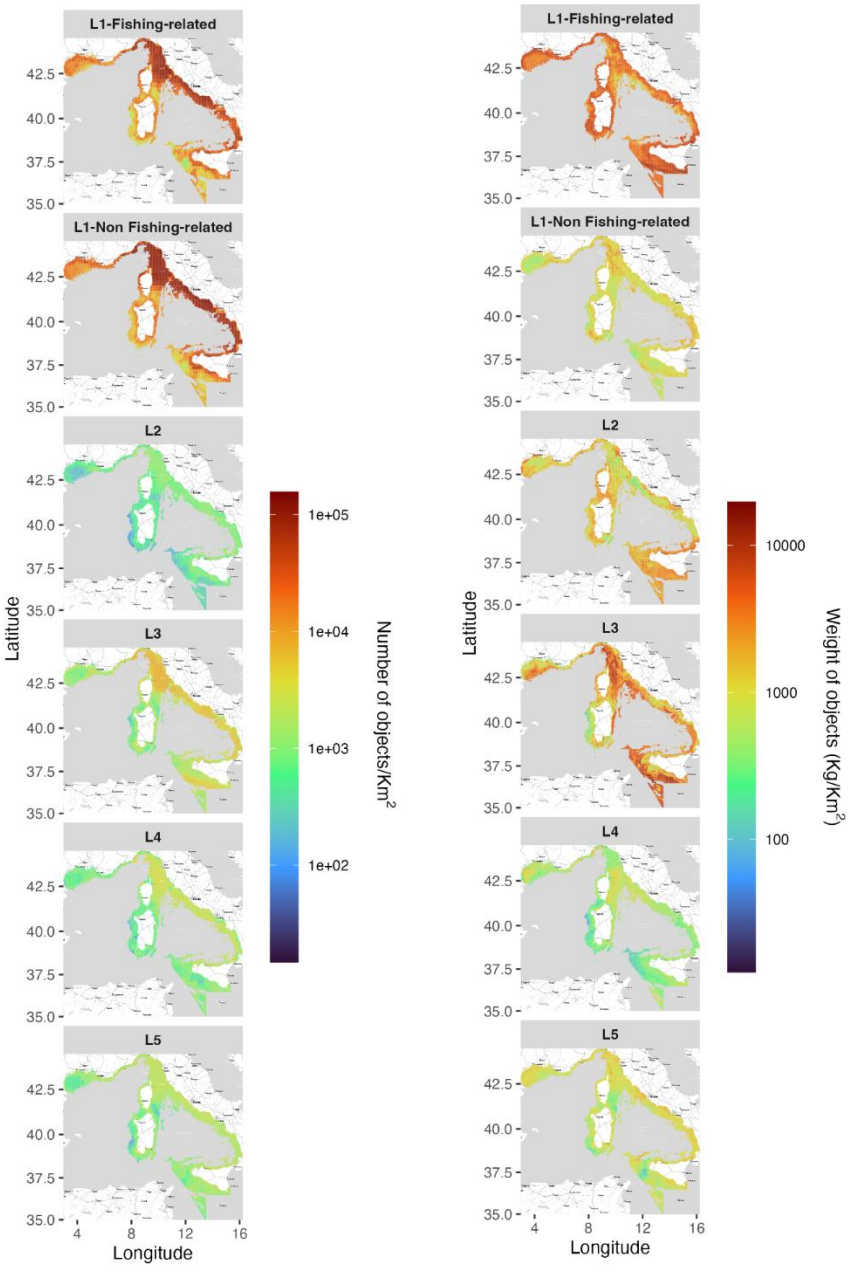


Figure 3b. Maps showing the average amount of marine litter categories over the period 2013-2019, as total number and weight of objects km^{-2}.

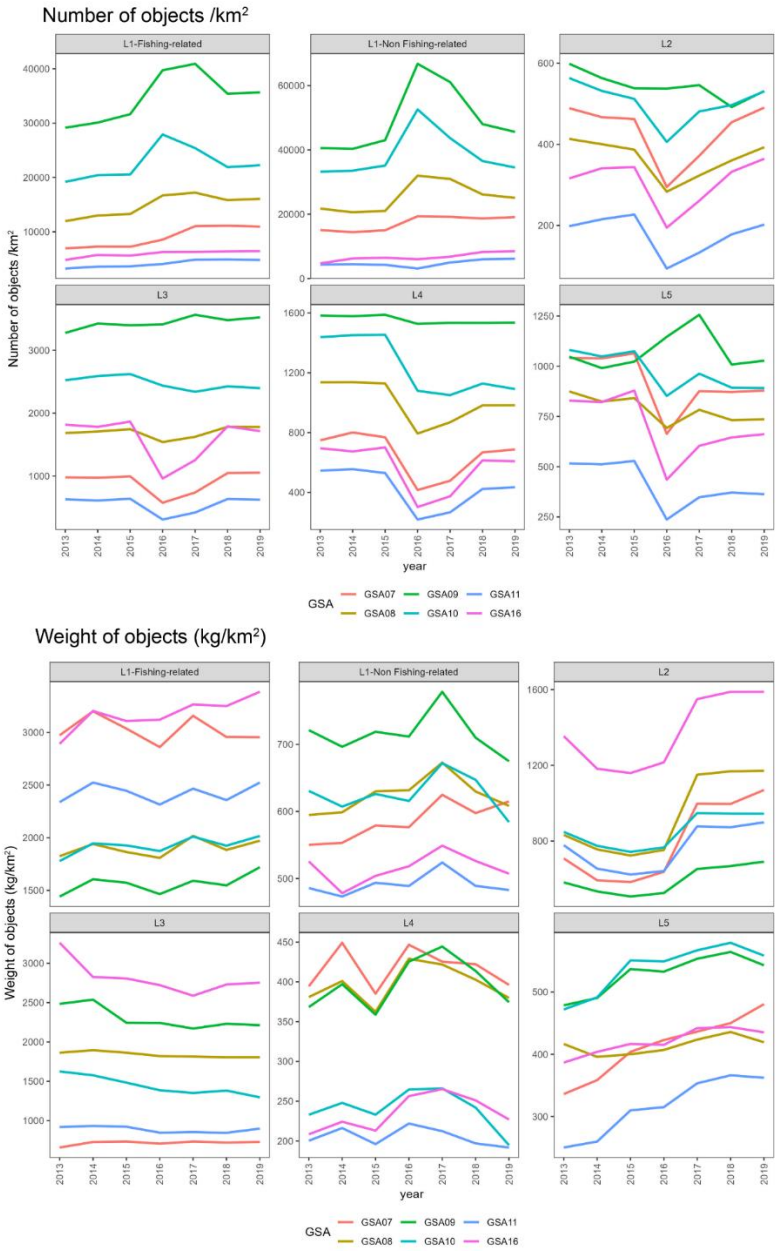


Figure 4. Temporal trends (2013-2019) for the main litter groups across different GSAs, expressed both as n. of items and kg of objects km^{-2}.

Predicted spatio-temporal distribution of seafloor macro-litter

Trained RF was used to forecast the amount of litter for each category and sub-category within the spatial domain considered in the study (11,341 cells), on a yearly basis from 2013 to 2019. The averaged spatio-temporal patterns are proposed in Figures 3a and 3b. Figure 3a provides clues on the predicted density and abundance of macro-litter towards the 6 bathymetric strata depth gradient. It appears that single use items, or more generally lightweight objects, tend to be consistently more abundant in the shallower depth range and consistently decreases with depth across all GSAs (Figure 3a_A). Indeed, a significant proportion of land-based litter is composed of single use objects. When litter originates from the mainland or areas close to the coast, most of them are found to be stranded quickly and a significant portion remains in coastal waters near their point of origin. (Critchell 2016).

On the contrary, heavier objects are predicted to be more abundant in the deepest strata or at least as abundant as in shallower ones. This is the case of plastic subcategories such as L1h - Other fishing-related objects, L1i - Synthetic ropes, L1j - Others, or other heavier categories such as L2 Rubber and L3 Metal. These items are expected to increase with depth in GSA 07, 08, 11, and 16. The increase of small or light objects on the seafloor at high depths relies essentially on two mechanisms: i)

temporal changes in the weight of floating litter due to biofouling (Amaral-Zettler et al., 2021) and/or ii) local features of water circulation and geomorphologies such as submarine canyons, which are known to funnel huge quantities of macro-litter to the deep ocean (Hernandez et al., 2022). On the other hand, heavy objects are likely to be dropped directly into the sea and proximity to major trade routes may have been a contributing factor. The "other" category of L1j is a subcategory that is especially important in the deepest depths. Overall, any object which does not fall within a specific category or subcategory in the classification list is to be classified under the 'other' category: e.g. as regards its size or quantity of material. Therefore, it is not easy to know which type of objects are covered by the particular category and any speculation on those patterns cannot be substantiated. Unfortunately, this aspect does

not vary from one classification scheme to the other and is becomes even more important for protocols such as the MEDITS survey that proposes a rather limited number of subcategories. In this view, we emphasize the need for waste monitoring protocols to propose a workflow for the correct use of the "other" category.

The main spatial results were simplified by focusing on the different degrees of accumulation of the various types of macro-litter across various areas. To achieve this, a trade-off was made between the heterogeneity of the litter, its origin in relation to human activities, and the heterogeneity of the spatial distribution.

Spatial patterns for macro-litter sub-categories are represented in Figure 3b. This is because spatial patterns are of particular interest since they allow the identification of the main hotspots, which are the most relevant areas to be identified and, eventually, where mitigation actions should be prioritized. Moreover, the temporal persistence of accumulation areas can be a further diagnostic tool to give relevance to a certain hotspot. This aspect has never been tested so far and the present study provides the first insights on the temporal pattern, based on a yearly basis, of accumulation hotspots.

Regions with moderate to low accumulations of seafloor macro-litter include the coasts of Sardinia (GSA11) and Sicily (GSA16), the two largest Italian islands. In comparison to the accumulation observed along northern Italian coastline, non-fishing-related plastic items showed to be very scarce. This pattern changes a bit when considering weight of the objects (kg per km^{-2}) as response variable and Sicily and Sardinia islands (GSA16 and GSA11, respectively), showed very large values for the weight of L1 - Fishing-related items and L3 - Metal. More in general, those areas in these two GSAs that appear numerically less contaminated with waste, do become among the most contaminated if the weight of waste is considered.

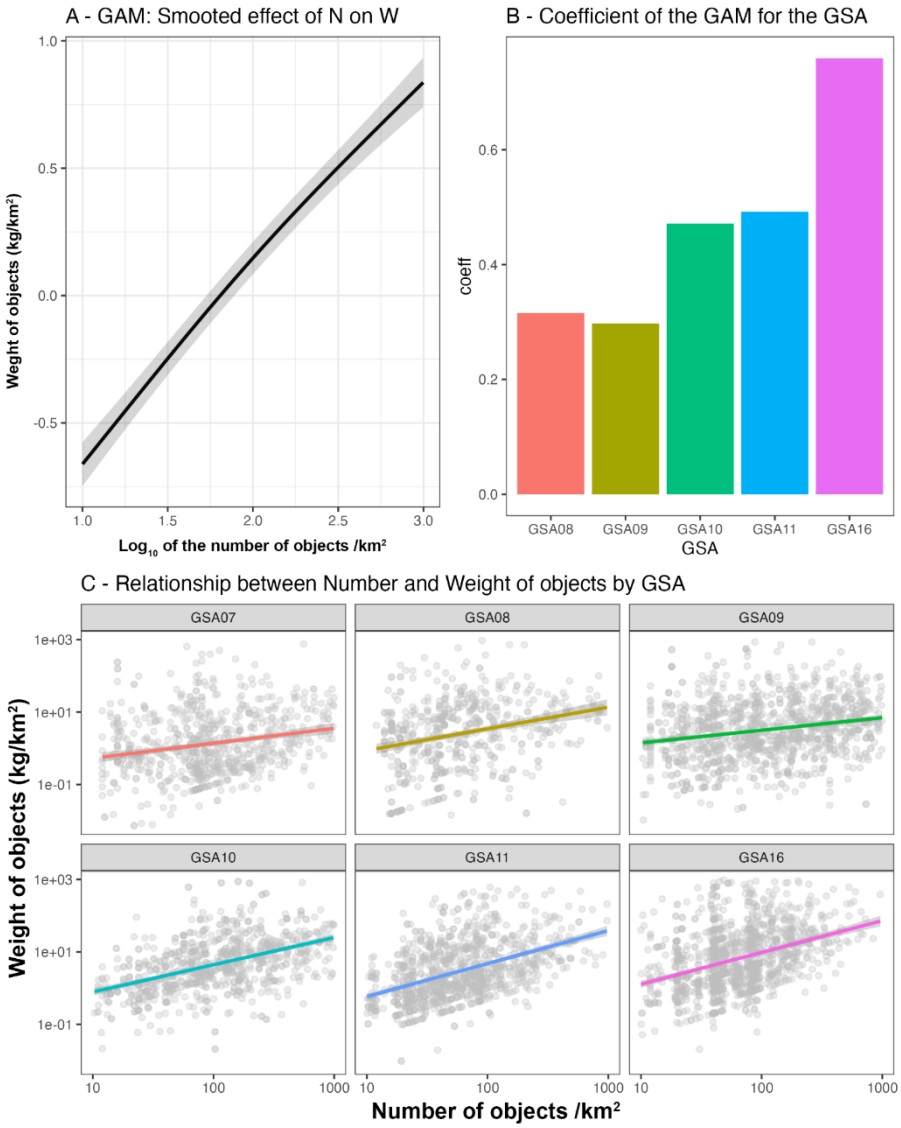


Figure 5. A) General relationship (GAM smoothed effect of the number of objects on the weight of objects, irrespectively of the GSA; **B**) Barplot of the GSA-specific coefficient for the weight of objects; C - GSA-specific relationship between weight and number of object in which the linear trend is represented.

Table 3. GAM coefficients and main statistics for the relationship between number of objects km^{-2} and weight of objects (kg km^{-2}). The GAM model was fitted on the dataset of 5869 records corresponding to the values of marine litter abundance in 1,073 cells (corresponding to the MEDITS sampling sites) monitored over seven years. Asterisks mark significant values (* P-value <0.05, ** P-value <0.01, *** P-values < 0.001), which are also highlighted in bold.

Term	**Coefficient**
GSA08	**0.32*****
GSA09	0.3***
GSA10	0.47***
GSA11	0.49 ***
GSA16	0.76***
L1-Fishing-related	0.05
L1-Non Fishing-related	-0.11***
L2-Rubber	0.75***
L3-Metal	0.08*
L4-Glass/Ceramic/Concrete	0.57***
Deviance explained of the GAM	47.5%

Number versus weight

Monitoring litter by number or weight can lead to different results, as explained by other authors (Smith & Turrell, 2021). The efficacy of monitoring based on number is influenced by factors such as the minimum detectable fragment size, the age of the debris, and environmental forcings that can increase the fragmentation processes (Smith & Turrell, 2021). Given that the single presence or absence of mega-litter may constitute strong bias in weight-based monitoring (Smith & Turrell, 2021), it is important to combine data on the number and weight of objects in order to generate reliable information. The effect of the number of objects on the respective value of

weight of the object is, as expected, close to linear (Figure 5A). In addition, GAM detected a significant effect of the GSA, with a pattern of the coefficients (Figure 5B) in which the GSA16 has the highest value and the GSAs 09 and 08 the smallest ones (Table 3). Davie's test also allowed to reject the null hypothesis that coefficients (slopes) of the number/weight relationship for the different GSAs belong to the same distribution. This demonstrates that, in the Strait of Sicily (GSA16), macro-litter is heavier than in other areas, as observed in the steeper slope of the area (Figure 5C). The difference between number and weight patterns, represented in Figure 3a and 3b, could be justified by the fact that along the southern coast of Sicily, stronger currents prevent the deposition on the seafloor of lighter items. In contrast, the ribs of GSA09 and GSA10 are characterised by greater stagnation, which allows even lighter items to be deposited on the bottom. Indeed, (Collignon et al., 2014) demonstrated that the mean weight of particles in the northwestern Mediterranean Sea is smaller than in other areas, in agreement with the results of this study. Northwestern Mediterranean coasts were also characterised with the presence of high accumulation zones in the near-shore region (Pedrotti et al., 2016). In essence, it seems reasonable to assume that the part north of the Tyrrhenian Sea is the one (within the six GSAs considered in this study) with the highest number of plastic items but with a lower average weight than other areas (such as the southern coasts of Sicily), and this is due to the synergistic effect of two factors: the presence of major rivers (the Tiber, the Arno, the Rhone), and a hydrodynamic regime that favours stagnation and accumulation.

Results here presented are based over a broader geographical scale compared to available studies conducted in single GSAs within the study area (e.g., Alvito et al., 2018; Franceschini et al., 2019; Garofalo et al., 2020); which eventually allowed us to put into a broader perspective some of the results obtained locally; this is the case of GSA11, that appeared less critical once put into a larger spatial scale and perspective, not showing any relevant accumulation hotspot compared to the whole western Mediterranean pattern. This, however, should not divert attention from local peculiarities, since potential mitigation actions would mostly act locally, based on the effort of local fisheries (e.g., fishing for litter initiatives). Still, present results could

provide useful insights to define sub-basin spots that would deserve priority for broader, coordinated efforts.

Replicability and limitations

One of the strengths of the approach proposed in this paper is that it is based on widely and easily accessible information through online portals such as those of NOAA and Copernicus. This could make it possible to expand predictions to other areas of the Mediterranean or, more generally, of European seas and world oceans (considering that initiatives similar to Copernicus exist in other areas of the globe). In addition, future applications of this kind of predictive models could allow for the refinement of predictions in already investigated areas, since machine learning techniques (such as RF) are born precisely to exploit the mass of information (big data) that is progressively accumulated. On the other hand, it will be necessary to supplement direct information, like that from MEDITS but also other forms of data collection such as image-based analysis, on the amount of macro-litter present on the seabed. With this respect, a number of limitations of this work emerge: 1) it is a data-demanding approach; 2) it has so far only been applied to macro-litter, as there is no geo-referenced information for litter of smaller size classes; 3) we do not know whether this kind of approach can also work in 3D (for litter in the water column) and for surface litter. These aspects, however, open the prospect for new work and stimulate the integration of observations from different sources in order to explore the limits of the method itself. Marine litter threat is, indeed, a trans-boundary issue and the best strategy to tackle it requires close cooperation across geographically close and distant countries.

Conclusions, implications and future perspectives

The ecological modelling exercise presented in this study aims to try to answer the question in the title: what, where, and when (and, potentially, why) does litter accumulation occur on the seafloor? From a compositional point of view, the category of non-fishing related objects represents the numerically most abundant component, especially along the continental coasts. By weight, however, the situation is more

heterogeneous. Islands (Sicily, Sardinia and Corsica) are the least contaminated areas, while considering the temporal pattern of hotspots, as anticipated, results here put emphasis on the temporal consistency of hotspots, an overlooked that would deserve consideration. This is of crucial importance since it is well documented how macro-litter can be displaced or buried and a proper identification of hotspots could be compromised. In case of consistent and spatially stable hotspots, stakeholders would have the tool to identify areas where the litter standing stock is stable across time and where: i) mitigation can be prioritised and ii) test for the efficacy of broad transnational litter reduction policies. Indeed, beside few environmental factors, input from land, in all its forms, represents the main contributor and driver of macro-litter distribution. Our approach, which likely does not allow us to assess local measures' effectiveness could, on the contrary, be very effective in detecting the temporal effects of binding targets foreseen within transnational initiatives such as the plastic treaty of the United Nation (Bergmann et al., 2022).

Mapping the distribution of marine macro-litter is also a key element to assess the exposure of marine organisms to potential plastic pollution. In the case of plastic ingestion by marine species or just entanglement in ghost fishing gear, the risk assessment must indicate where and when harm may occur. These risks are largely defined by the potential encounter of marine organisms with litter, but also by litter' nature and form. Risk assessment has been used recently to study areas where species may be harmed by the presence of litter and, more specifically, to predict areas where the risk of ingestion is high (e.g., ecological threats to marine biota at the population level are often unclear, as is the geographic extent of impacts). Modelling the likelihood of litter ingestion by cross mapping the distribution of both litter and sea turtles or cetaceans has been proposed as a tool to define areas at risk, in terms of exposure (Darmon et al., 2017; Fossi et al., 2018). This tool was then used to investigate the possible overlap between plastic accumulation maps and microplastics in bioindicator species and ichthyofauna in Mediterranean Marine Protected Areas (Compa et al., 2023, 2022). As the mapping of demersal fish abundance has been recently described in the Mediterranean Sea (Colloca et al., 2015), the same approach

could be used, taking advantage of the results of the modelled macro-litter distribution to predict areas where the demersal fish population may be affected, environmentally, but also with possible consequences for the quality of fish as seafood.

Within mitigation initiatives, a good example is "Fishing for litter" initiatives (https://fishingforlitter.org) (García-Hermosa and Woodall, 2023). Indeed, trawls cover the largest total swept area (Haarr et al., 2022) and, most importantly, remain the only available option for reaching greater depths, during the regular working routine of fisheries. However, trawls come at great environmental costs due to their disturbance (and possible destruction) of substrate and biota (Canals et al., 2021; Pusceddu et al., 2014). Scientific evidence informs on how trawl-based mitigation action could prioritize macro-litter hotspots to be more effective and how cumulative maps of hotspots could be spatially misleading (Cau et al., 2022); we stress here that the temporal factor can play a crucial role as well. This latter information was missing from available literature and clearly shows how hotspots identified during a survey conducted in a specific year may not be consistent in the following years. The reasons that can explain such temporal changes are ascribable, as anticipated above, to remobilization or burial as major causes, but also to differential riverine input driven by major atmospheric events across years (Laverre et al., 2023), or fluctuations in seasonal anthropic activities such as coastal tourism (Ronchi et al., 2019).

Initiatives that involve fishermen to collect macro-litter are relatively low-cost and efficient, at least considering exclusively fishing grounds, even though several difficulties arise in their execution and implementation (Cho, 2009; Ronchi et al., 2019; Viejo et al., 2023). The effective reduction of marine litter' contamination should begin with a cap on the production, especially of hazardous materials like plastic (Bergmann et al., 2022). However, for that fraction of macro-litter already dispersed in sea bottoms worldwide, mitigation initiatives represent the most realistic and feasible strategy to pursue, still considering the enormous limitations that arise when working at high depths in the marine environment (Canals et al., 2021). Our approach could also provide a benchmark for monitoring the effectiveness of

numerous directives that are put in place at both national and international level. Since macro-litter can be identified and divided into subcategories (i.e. plastics, rubbers, metals, glass, and clothes), the temporal trends of each of these categories could be informative of the actual effectiveness of specific measures, such as waste management policies, bans of certain products or introduction of taxes and charges (Chen 2015).

Supplementary material

Environmental and anthropogenic variables used for random forest machine learning technique:

The sea bottom depth was estimated, for each cell of the grid, querying the NOAA ETOPO1 Global Relief Model using the R package 'marmap' (Pante & Bouhet, 2022). The distance from the coast was computed, for each cell of the grid, using the 'dist2Line' function of the R package geosphere (Hijmans et al., 2021). Rivers' mouth positions and catchment area (in km^2) were obtained from the European Environmental Agency https://www.eea.europa.eu/data-and-maps/data/european-river-catchments-1) and used to compute, for each cell of the grid, an index of the Impact of River Basins defined as the average of catchments areas weighted by the distance of cell centre from the river mouths. The positions of the main shipping lanes were download from (Halpern et al., 2015) (https://knb.ecoinformatics.org/view/doi:10.5063/F1S180FS) and used to compute, for each cell of the grid, the Mean Distance from Shipping Lanes. The positions and the size (in km^2) of the Urban areas were download from the Efrain Maps Website (https://www.efrainmaps.es/english-version/free-downloads/europe/) and used to compute an index of the Impact of Urban Areas defined as the average of urban areas weighted by the inverse of their distance from the cell centre. Positions and size class of the port areas were downloaded from the National Geospatial-Intelligence Agency website (https://msi.nga.mil/Publications/WPI) and used to compute an index of the Impact of Port Areas, defined as the average of port classes weighted by the inverse of their distance from the cell centre. The Northward and Eastward sea water velocities in m^{-s} (7 and 8), the Northward and Eastward Sea surface wave stokes drift in m^{-s} (9 and 10) the Mean Sea Level in m (11), the Northward and Eastward wind speed in m^{-s} (12 and 13) and the Mean Sea Wave Height in m (14) were downloaded from the Copernicus Marine Service (https://marine.copernicus.eu/) for the period of interest and average to obtain single values for the cells of the grid.

Rugosity (RUG) (i.e. the roughness of the seafloor), an indicator of hard-bottom habitat, was derived from the bathymetry layer using the Benthic Terrain Modeller tool in ArcGIS 10.1. RUG is quantified as the likelihood of hard-bottom habitat presence and ranges from zero to one.

Finally, three different variables were generated from the analysis of Vessel Monitoring System (VMS) data, according to the procedures described in (Russo et al., 2014a) and Russo et al (2016).

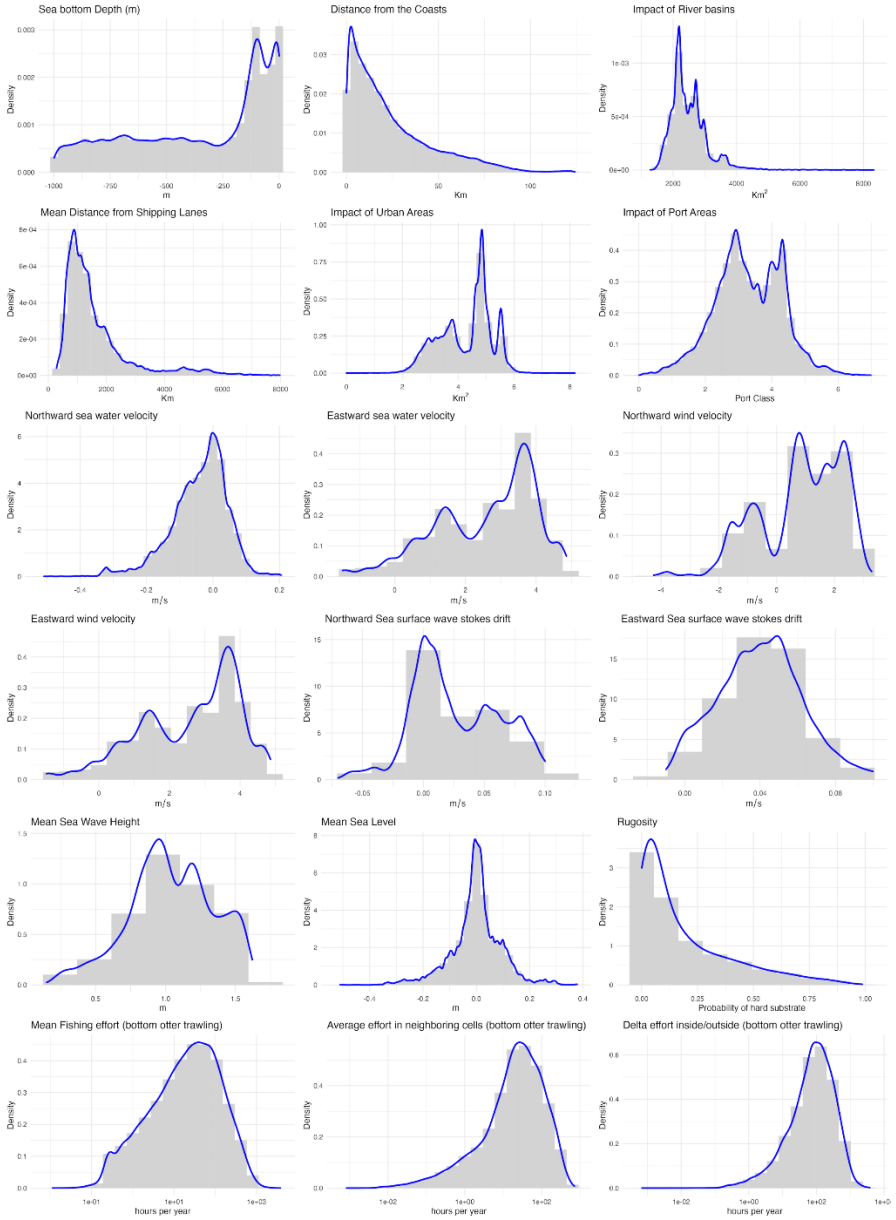


Figure S1 Density plot of environmental and anthropogenic variables collected and processed to train and develop the random forest model to predict seafloor litter in the western Mediterranean Sea.

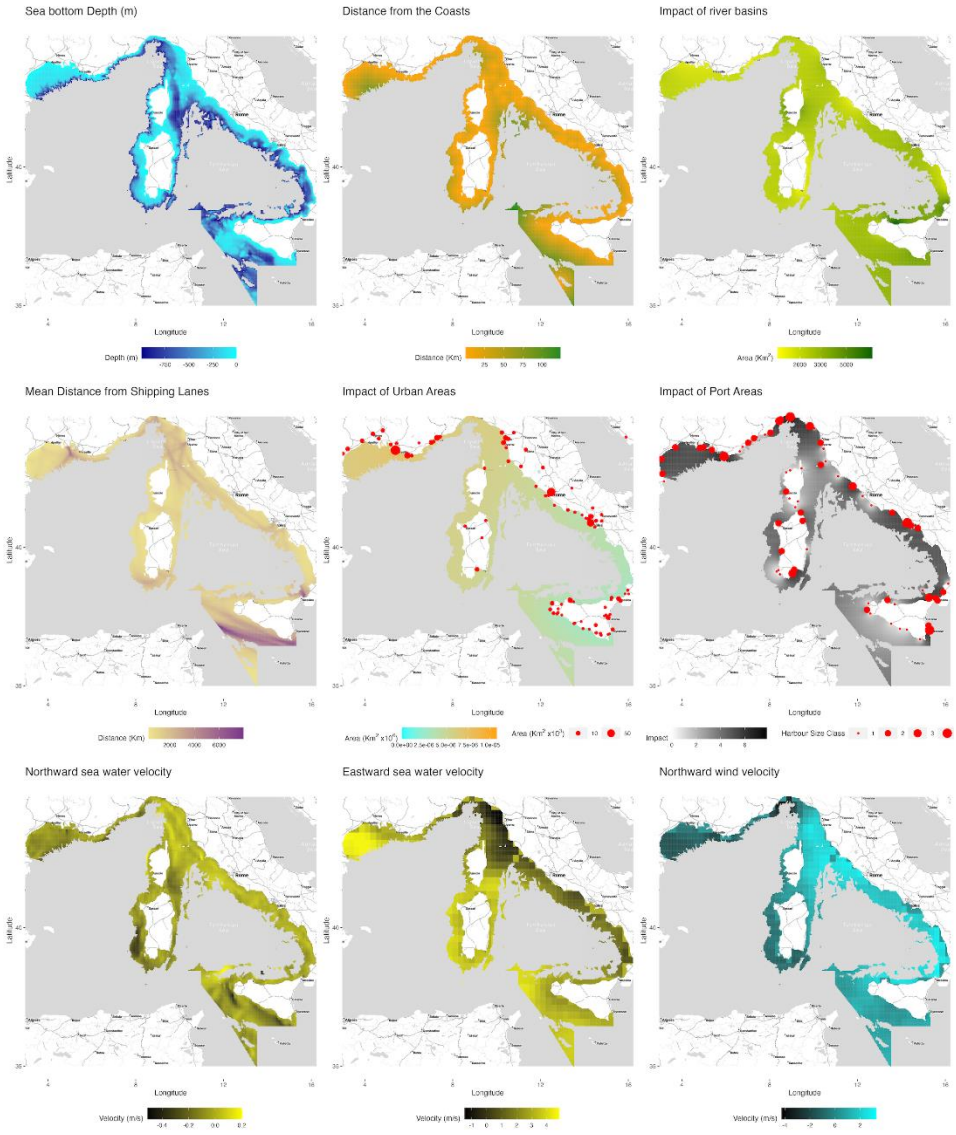


Figure S2a. Maps of environmental and anthropogenic variables collected and processed to train and develop the random forest model to predict seafloor litter in the western Mediterranean Sea.

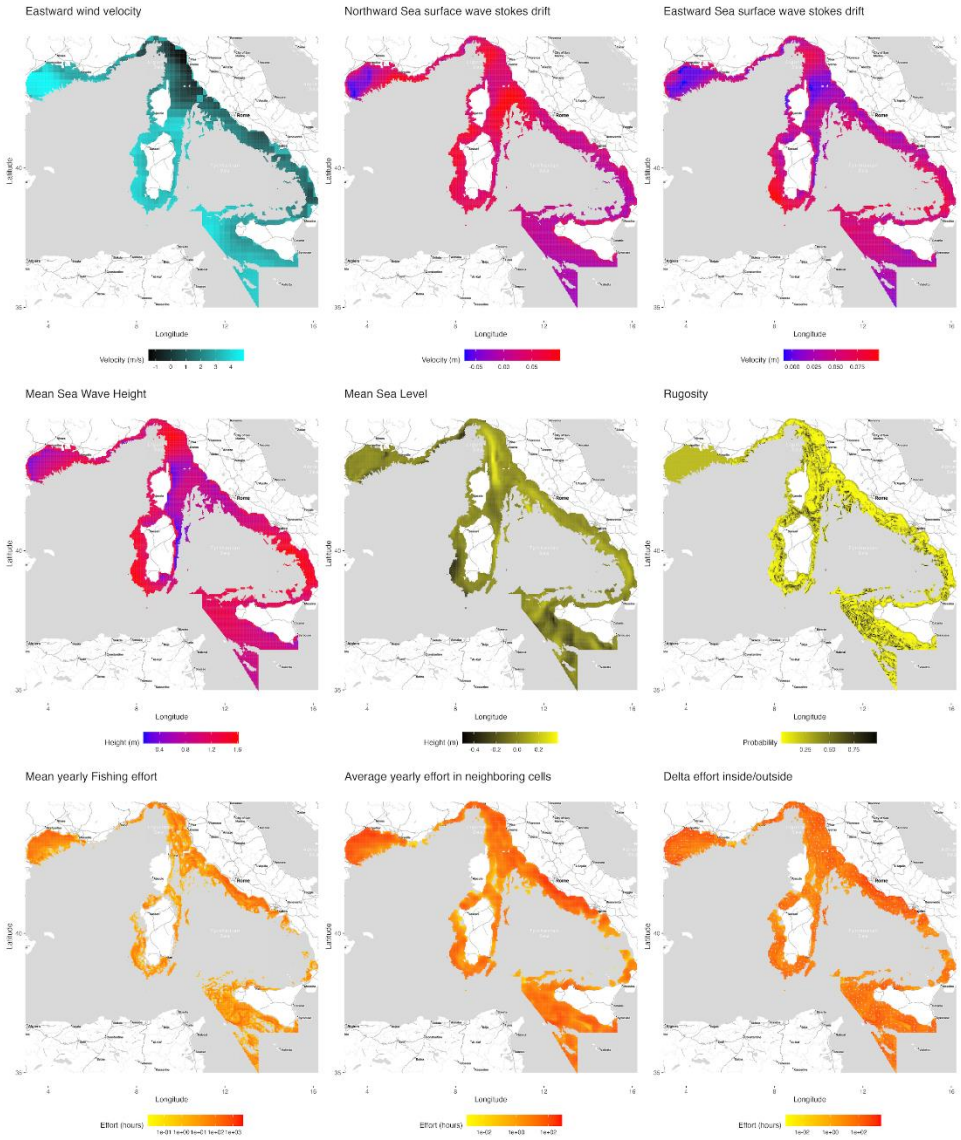


Figure S1b. Maps of environmental and anthropogenic variables collected and processed to train and develop the random forest model to predict seafloor litter in the western Mediterranean Sea.

GSA7 shows a total surface of ~13,870 km^2 and comprises the Gulf of Lion and the neighbouring French coasts towards Italian waters. Much of the coast has deep-water bottoms near the shore, where the continental shelve is narrow, with the exception of the Gulf of Lyon, that shows a wide continental shelf (up to 72 km wide) and a well-defined shelf break at 100–200m depth and a complex network of adjacent submarine canyons. Due to differences in shelf width along the continental margin, some of these canyons can be found relatively close to the shore (e.g. Cap de Creus canyon), while others appear relatively far offshore [Grand and Petit-Rhône canyons (UNEP-MAP-RAC/SPA, 2013)]. As a general feature, submarine canyons in this area are more closely spaced, more dentritic, shorter and steeper than canyons found in other regions of the world (Fabri et al., 2014). In the Gulf of Lion, the Liguro-Provencal Current runs from east to west and shows high seasonal variability and possible anti-cyclonic eddies at the limits of the continental slope; this current results from the convergence of the Eastern and Western Corsican Currents (Rubio et al., 2009). Anti-cyclonic circulation schemes also occur in both the northern and western parts of the Gulf of Lion and the mouth of the Rhône river also drives circulation, bringing also debris at sea (Fabri et al., 2014; Hu et al., 2011). The Gulf of Lion is an area subject to strong anthropogenic impact, with the presence of numerous activities: industries, tourism, harbours, fisheries, aquaculture, maritime traffic and large cities, including the city of Marseille (France's second largest city) which has a commercial port, an industrial zone and strong seasonal touristic activity (Galgani et al., 2000).

GSA 8 is the smallest area investigated in the present study (ca. 4,600 km^2 of extension). Circulation around Corsica is driven by East and West Corsica Current (Millot, 2013). The eastern coast of Corsica is affected by the Tyrrhenian Sea current, which runs along the Italian coast and passes through the Corsica channel. Off the coast of the town of Bastia, on the eastern coast, the presence of eddies divides the current into two branches, the first oriented northward and the second oriented southward (Angeletti et al., 2020). On the west coast, the current flows northward, merging with the eastern current at Cape Corsica and the currents ascending the west coasts of Italy from the Tyrrhenian Sea to generate the Ligurian current, flowing

westward along the Italian and French coasts. The continental shelves also in GSA8 is narrow and incised by closely spaced and steep submarine canyons; however, their number is limited on the eastern part of Corsica with limited depths in the Corsican channel.

GSA 9 has an extension of ~42,000 km^2 and is the largest GSA considered in the present study. It includes the Ligurian Sea and Northern Tyrrhenian Sea, from Liguria moving southward along Italian coasts until Lazio (Fig. 1). It is a heterogeneous area in terms of morphological and ecological features. The continental shelf is very narrow in the Ligurian Sea and widens southward. The southernmost part of GSA9, in front the central area of the Lazio shelf is significantly affected by the Tiber River. River supplies determine the physical, chemical, and trophic characteristics of this area. In addition, being close to Rome city and surrounded by large urban centres, industrial settlements, and important ports, the Tiber River runoff also brings contaminants such as plastic debris (Crosti et al., 2018a).

GSA 10, the central-southern Tyrrhenian (extension: 20,255 km^2) exhibits more complex morphology, geophysics, and water circulation than other Italian seas. Its bottom morphology and topography, which affect the circulation of the water masses, are similar to those of the oceans, with a well-developed continental platform, slope, abyssal plain, and seamounts. Along the north margins of Sicilian coasts and along Calabria and Basilicata coasts, the continental platform (~200 m depth) is less developed compared to the coasts of Campania and Lazio. There is a relatively homogeneous oceanographic and ecological environment along the northern Sicilian coast and the southern Tyrrhenian Italian coast (Cataudella and Spagnolo, 2011).

GSA 11 covers waters around Sardinia Island (27,000 km^2). Sea bottoms are not homogenous in terms of extension, geomorphology, and associated biodiversity, with the eastern coast showing a continental shelf and slope connected to inland orographic structures and river basins through a very narrow and complex continental shelf incised by profound submarine canyons (Cau et al., 2017; Moccia et al., 2019). On the contrary, western side shows a wider continental shelf that can extend for hundreds of

kilometres from the coast, especially on the southwestern side of the island. The main hydrological features are the western Sardinian Current, which reaches its maximum intensity in the south-west corner of the island and the Levantine Intermediate Water that flows along the entire eastern coast of the island. GSA 11 also includes the Sardinia Channel that is a crucial area for the control of water mass exchange between the Eastern and the western Mediterranean basin (Millot, 2013; Moccia et al., 2021; Olita et al., 2013).

GSA 16 is located off the southern coast of Sicily (Strait of Sicily), covers about 31,400 km^2 and is characterized by both complex bathymetry and important hydrodynamic processes. The shelf is characterized by two wide and shallow banks in the western (Adventure Bank) and eastern (Malta Bank) side, with a narrow shelf in the middle. The surface currents in Strait of Sicily are mainly formed by the Modified Atlantic Water flowing eastward, and the Levantine Intermediate Water (flowing westward along the Sicilian slope between 200 and 500 m. When entering the Strait of Sicily, the MAW bifurcates in two paths, one directed toward the Tyrrhenian Sea, and the other directed toward the Strait. The latter splits in the Atlantic Ionian Stream flowing along the south Sicilian coast and the Atlantic Tunisian Current moving on the Tunisian continental slope. Along this path, the Atlantic Ionian Stream circulates around two well-known large semi-permanent cyclonic vortices; the first one lying over the Adventure Bank and the second over the Malta shelf. The area is subject to intense and varied human pressure including many important commercial and tourist ports, agricultural and industrial activities, aquaculture plants, oil re-fineries and offshore platforms (Buhl-Mortensen et al., 2017) . In addition, the Strait of Sicily is one of the most important fishing areas in the Mediterranean, with a large fishing fleet of mostly trawlers, based in Mazara del Vallo, engaged in deep-sea fishing (Milisenda et al., 2017; Russo, D'Andrea, et al., 2019). Here, the bottom trawl fishing fleet grew over the past decades due to the high fishing productivity and biodiversity due to the wide extension of the continental shelf and quite stable seasonal upwelling processes (Di Lorenzo et al., 2018).

Finally, due to its central position connecting the eastern and western Mediterranean basins, the Strait of Sicily represents an important crossroads for Mediterranean trade and touristic routes including the most important oil traffic lane (e.g. to have an idea of this intense traffic it is estimated that a total of 170,000 vessels per year passed through the Strait of Sicily in one year between 2016 and 2017 (Deidun et al., 2018).

Kernel densities (Figure S3) evidence, in most of the case, multimodal distributions with peaks in the range 10-102 items km^{-2}. In three of the five GSAs considered (namely GSA07, GSA11 and GSA16) the distributions are symmetric (with low values of the skewness), whereas, for the GSA09 and the GSA10, the distributions are asymmetric, with a long right tail (positive skewness) and platykurtic. GSA08 belongs to the first group in some cases (e.g. L2-rubber) and to the second group for (SU-single use).

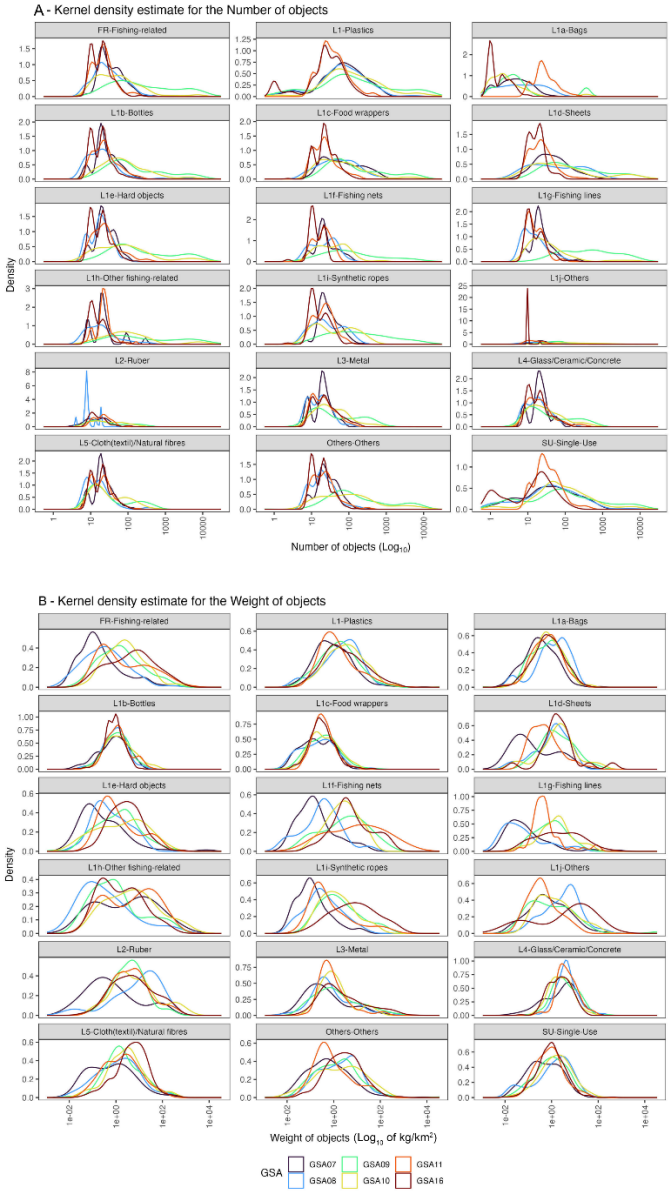


Figure S3 Kernel density estimates of the distributions of the different categories and sub-categories, as number of objects km^{-2} (A) and weight of objects kg km^{-2} (B). GSAs are in different colours.

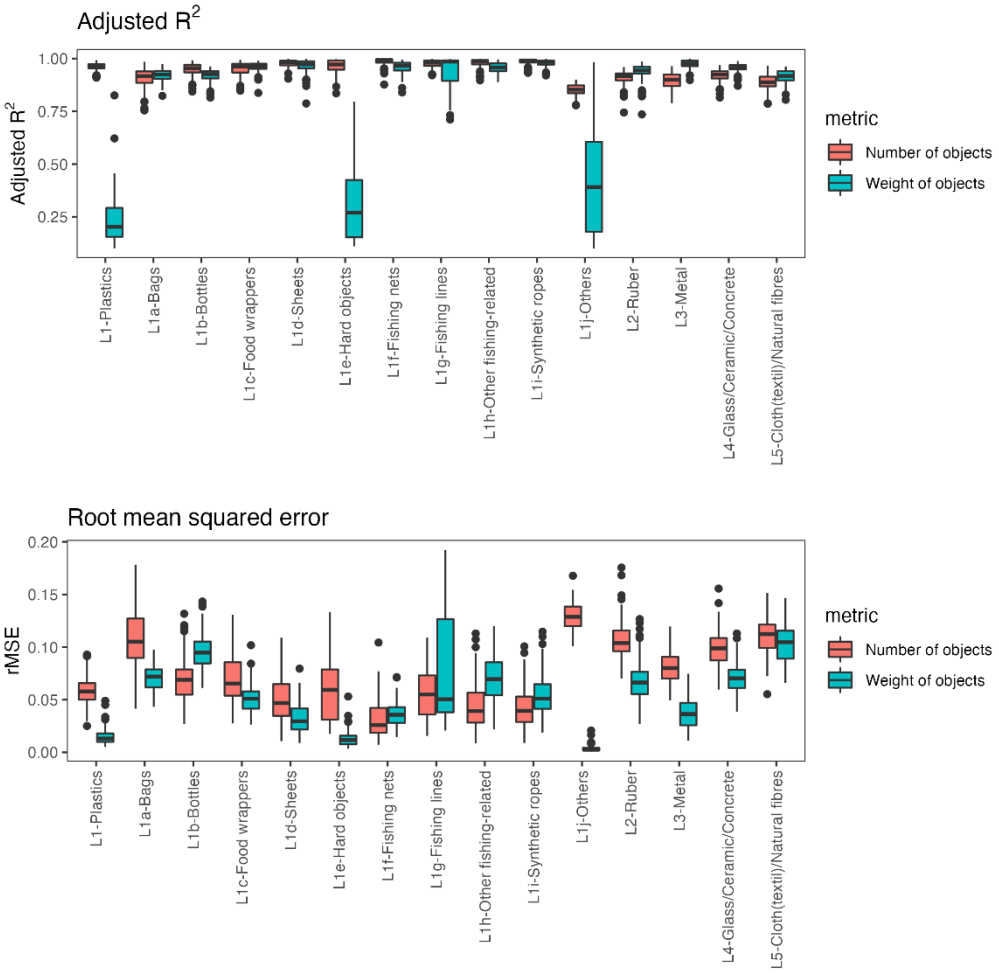


Figure S4. Histogram of adjusted square root (R^2) and root Mean Squared Error (rMSE) for the response variables considered in the model (i.e., number and weight of objects)

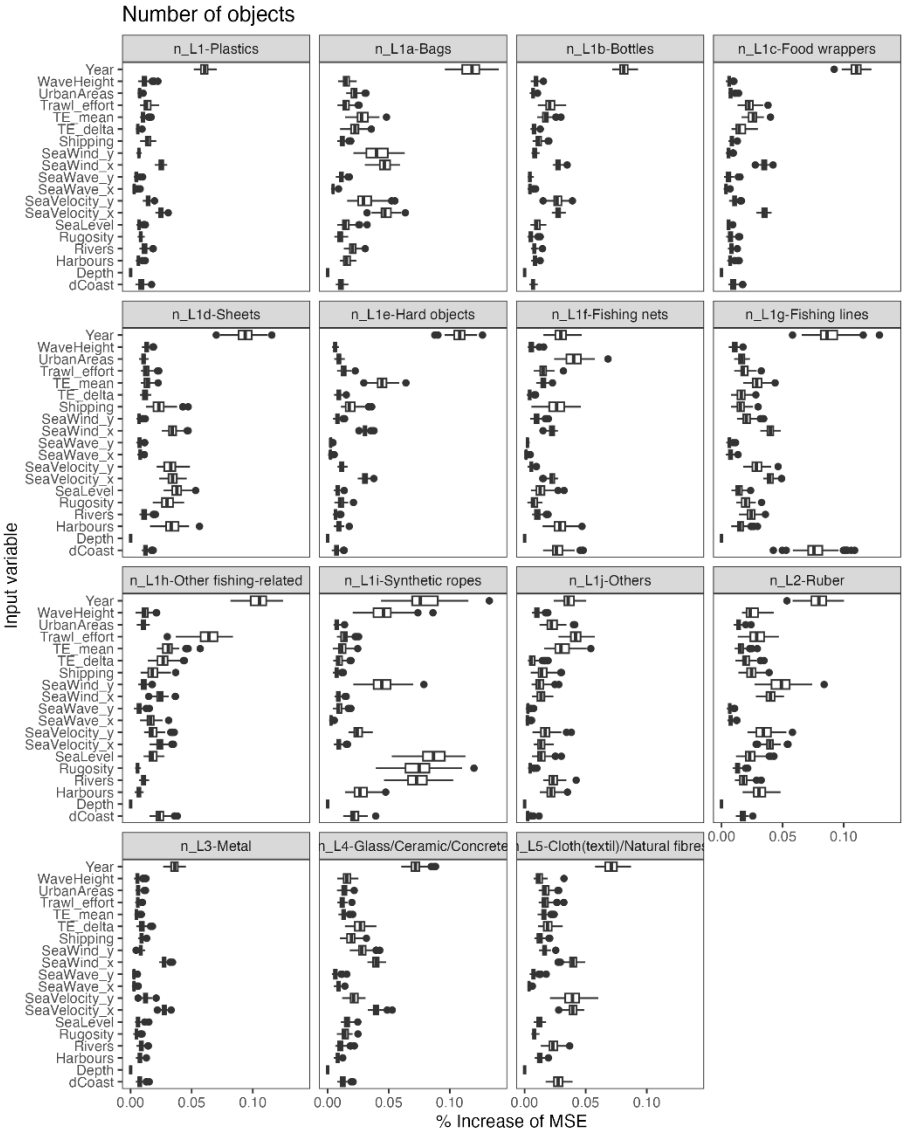


Figure S5a. Assessment of the relative importance of the input variables used to train the RF models. The distributions of the % of increase of MSE are represented for each set of 100 RF models trained for each category and sub-category of marine litter, quantified as number of objects km^{-2}.

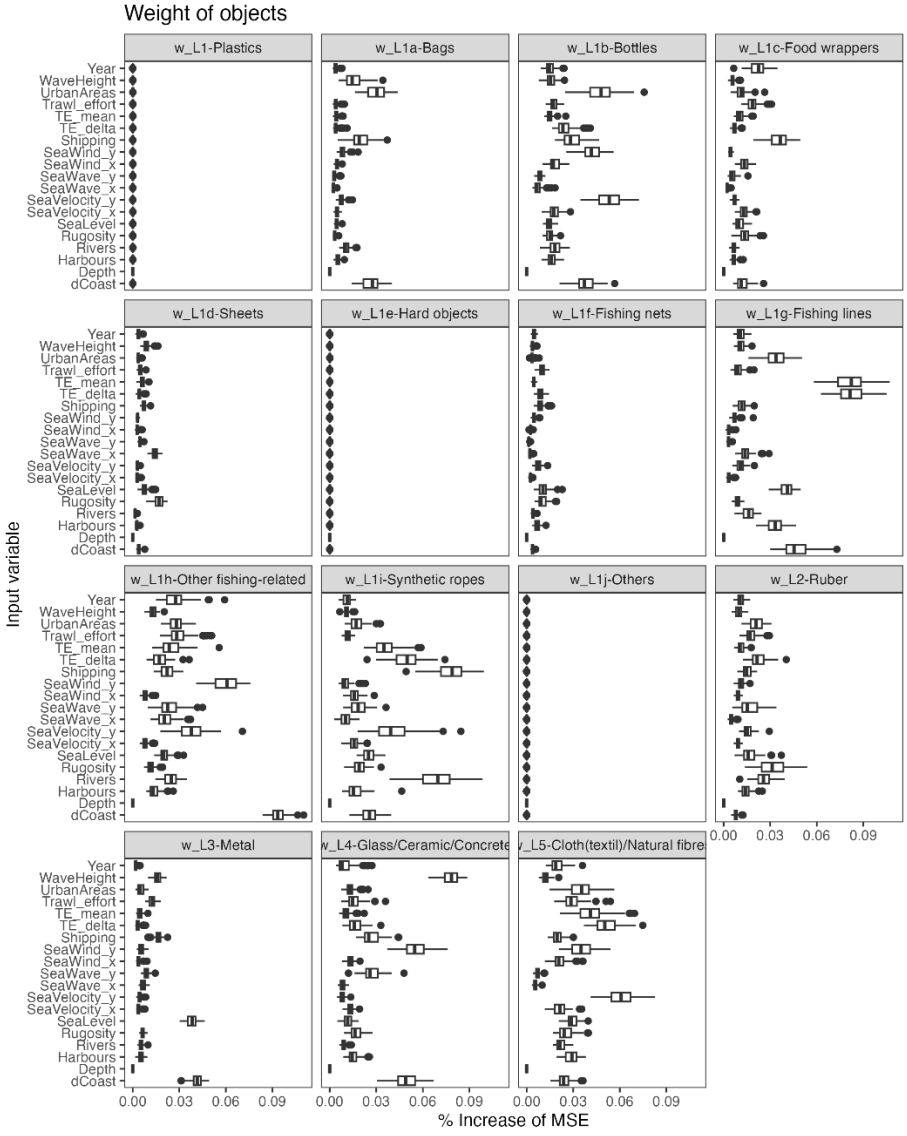


Figure S5b. Assessment of the relative importance of the input variables used to train the RF models. The distributions of the % of increase of MSE are represented for each set of 100 RF models trained for each category and sub-category of marine litter, quantified as weight of objects km^{-2}.

Table S1. Summary of the extension (km^2), total number of hauls and years of surveys for all Geographical Sub Areas (GSA) included in the present study.

GSA	Area (km^2)	n. of hauls $year^{-1}$	years of survey
7	13,870	64	2013-2019
8	4,600	22	2013-2019
9	42,400	120	2013-2019
10	20,600	70	2013-2019
11	27,000	99	2013-2019
16	31,400	120	2013-2019

Chapter 2 - Ask the shark: Blackmouth catshark (*Galeus melastomus*) as another sentinel of plastic waste on the seabed

Abstract

The presence of plastic waste in the marine environment has driven the scientific community to make significant efforts to study and mitigate its possible effects. One of the critical aspects is to determine if and how an increase in ingestion events may occur as a result of the accumulation of plastic waste on the seabed. In this study, *G. melastomus* is examined for its ability to indirectly provide information on the amount of macroplastics accumulating on the seafloor. Plastic ingestion is explored by describing the feeding habits of the species, which have the potential to provide very useful information regarding biological or ecological issues. The diet of *G. melastomus* mainly consisted of cephalopods, bathypelagic fishes, and decapod crustaceans, increasing in diversity during growth. The generalist-opportunistic feeding behaviour of this species leads to the incidental ingestion of plastic particles (N= 47, with a mean (± SD) of 1.47 ± 0.28 per specimen) which can be indirectly associated to the presence of macroplastics on the seafloor. Indeed, our results indicate a significant relationship between the amount of macroplastics present on the seabed and the frequency of ingestion of plastic particles by blackmouth catshark. We propose *G. melastomus* as an excellent candidate for developing a valid monitoring strategy for the presence of plastics on the seabed, as requested by the EU Marine Strategy Framework Directive.

Keywords: Plastic pollution; Mediterranean Sea; Biomonitoring; *Galeus melastomus*; Diet

Introduction

Over the last century, plastic production has grown exponentially as a result of its versatility, durability, malleability, and low price, becoming part of everyday life and delivering social benefits (Geyer et al. 2017; Boucher et al. 2020). The development and diffusion of plastic materials have led to inevitable consequences with repercussions on waste management, becoming a topic of discussion in recent years (GESAMP, 2019b; Markic et al., 2020b; Thompson et al., 2009b). The release of plastic wastes affects both terrestrial and aquatic ecosystems, contributing to environmental pollution (Kumar et al., 2021). Irrational production, inappropriate disposal at landfills, and insufficient recycling lead to plastic becoming waste. And the waste, sooner or later, comes to sea (Eriksen et al., 2014b). In the case of improper discharge, the plastic enters waterways and into the oceans, causing marine plastic leaks (Boucher et al., 2020b). A total of 35% of plastic leakage is attributed to the cities and towns located directly on the coast (Boucher & Billard, 2020b). The remaining 65% is generated from the inland and comes into sea water by surface run-off (Boucher & Billard, 2020b). An important contribution of plastics also comes from rivers, with the highest leakage rates from catchment areas (basins) hosting large rivers (Hurley et al., 2018b).

The abundance, persistence, and ubiquity of plastic in seawaters have made this pollutant one of the most controversial topics in recent years, representing a serious threat to marine organisms. A wide range of animals can ingest plastic particles once they reach the sea (Capone et al., 2020; Cau et al., 2019b; Markic et al., 2020b; Santos et al., 2021b), either intentionally - by mistaking plastic particles for natural prey, or accidentally - by ingesting items already consumed by their prey (Bergmann et al., 2015b). Most of the plastic seems to be accumulated on the seafloor in form of microplastics (MiP < 5mm), or macroplastics (MaP 25 - 1000 mm), or mesoplastics (MeP 5 - 25 mm) (GESAMP, 2019b) with very heterogeneous and complex patterns (Franceschini et al., 2019; Reisser et al., 2015; Tsiaras et al., 2021b). These accumulation areas increase the risk of coming into contact with the pollutant,

especially for species that live or feed near the seafloor (Alomar et al., 2020; Franceschini et al., 2021b).

Plastic ingestion is associated with a variety of negative health effects. Macroplastics may provoke blockages or internal bleeding in the intestines of the species (Camedda et al., 2014; Domènech et al., 2019b; Provencher et al., 2017b; Van Franeker et al., 2011b). The smaller pieces of plastic (MiP or MeP), on the other hand, can have indirect effects such as decrease of mobility, reduction of feeding and growth, and worsened body condition (Markic et al., 2020b; Sbrana, Valente, Scacco, Bianchi, Silvestri, Palazzo, Andrea, et al., 2020b; Valente et al., 2019). In addition, plastic additives may induce physiological alteration, such as endocrine functions (Flint et al., 2012; Rochman et al., 2014b). Several studies have sought to understand the relationship between ingestion of plastic and environmental contamination, with very promising results. A study conducted in coastal areas of the Western Mediterranean Sea found a correlation between the amount of plastic in the digestive tract of the demersal to semi-pelagic species, *Boops boops*, and the level of pollution in the environment, using an index based on several factors, including population density, river inputs, distance from the coast, and shipping routes (Sbrana, Valente, Scacco, Bianchi, Silvestri, Palazzo, Andrea, et al., 2020b). More recent studies revealed that proximity to hotspots of macroplastics accumulation significantly correlates with microplastic ingestion by *Nephrops norvegicus* (Linnaeus, 1758) (Cau et al., 2019b, 2020; Franceschini et al., 2021b). It is worth noting that there is a growing trend of evidence that marine plastics pollution contaminates key stocks for fisheries, with potential negative consequences to human health, resources status, and socio-economic sectors (Lusher, Hollman, et al., 2017; Rochman et al., 2015b). Numerous studies have suggested also that plastics in the marine environment can adversely affect sharks (Alomar & Deudero, 2017; Anastasopoulou et al., 2013; Bernardini et al., 2018; Cartes et al., 2016; Valente et al., 2019). In marine food webs, elasmobranchs play an important role as apex predators and seem more frequently affected by plastic ingestion than bony fishes within deep-water habitats (López-López et al., 2018; Valente et al., 2020). Therefore, sharks and rays could be

considered as sentinel species for marine pollution biomonitoring studies (Alves et al., 2016; Fossi et al., 2018b).

The Mediterranean Sea, considered as one of the most threatened environments in the world, is also subject to this ubiquitous pollutant (Cózar et al., 2015b; Eriksen et al., 2014b; Liubartseva et al., 2018b; Suaria et al., 2016b; Tsiaras et al., 2021b; Zambianchi et al., 2017b). The total plastic accumulated in the Mediterranean Sea is estimated at 1,178,000 tonnes, with an annual marine leakage of 229,000 tonnes. Based on these data, Italy (and Rome locality) appears to be one of the three top contributors of plastic leakage in the Mediterranean Sea (Boucher & Billard, 2020b).

The spread and potential impacts of plastic waste in the marine environment are a global emergency that is pushing the scientific community to make a huge effort to understand and help stem these phenomena (GESAMP, 2019b; Santos et al., 2021b; Thompson et al., 2009b). As plastic is undergoing, even in the marine environment, a phenomenon of miniaturization and degradation that makes it increasingly less traceable (Chamas et al., 2020b), it is crucial to enhance the monitoring of macroplastics and their role in the process of environmental contamination. At the same time, plastic can impact biota through accidental ingestion (S. Savoca et al., 2019). Therefore, it is paramount to understand whether and how the accumulation of plastic waste on the seafloor can lead to increased ingestion events. However, given that marine pollution has also reached very high levels in the Mediterranean and larger areas, it is very complicated and costly to make spatial and temporal assessments of the different levels of contamination (Galgani, 2015).

The blackmouth catshark *Galeus melastomus* (Rafinesque, 1810) is a demersal bottom dwelling species, inhabiting continental shelf breaks, and slope habitats (common depth range: 150 - 1200 m). As a generalist meso predator, it plays an important role in the deep-water food web in providing a link between the upper and lower trophic levels (D'iglio et al., 2021). *G. melastomus* is the most abundant species of Selacii in the Mediterranean Sea (Bradai et al., 2012). The species has a considerable part of its population living in the Mediterranean beyond the deepest boundary trawled by

vessels (Peristeraki et al., 2020). Indeed, the species and especially its reproductors, can live up to 1600 m, being abundant still at 1400 m (Carrassón et al. 1992; Cartes et al. 2016). In this way, the deepest part of its spatial distribution is outside the range of activity of bottom trawlers and protected by direct fishing exploitation, contributing to the stability of the whole stock. This species is therefore an ideal candidate for monitoring plastics distribution on different fishing grounds (Fossi et al. 2018).

In this context, this study aims to: I) examine the stomach contents of *G. melastomus* to reconstruct its feeding ecology; II) evaluate and characterize the plastics ingested by the species, in order to detect sources of plastic pollution; III) relate plastics on the seabed and the ingestion of plastics to determine whether it can provide useful information about the amount of macroplastics accumulating on the seafloor.

Material and methods

Study area and collection of samples

Specimens of *G. melastomus* were collected in the central Tyrrhenian Sea (FAO Geographical Sub Area 9 – Western Mediterranean Sea), in summer 2020-2021. This area has a very narrow continental shelf, characterized by fine sands and muddy bottoms (Ardizzone et al., 2018a). The coastal area is heavily populated with large urban centres, industrial settlements, and important ports, being close to Rome city. The Tiber River runoff significantly affects the chemical-physical traits of the area, also bringing contaminants such as plastic debris (Crosti et al., 2018b). Samples were collected both on board of a commercial bottom trawl fishing vessel and during the MEDITS (MEDiterranean International bottom Trawl Survey) experimental trawl surveys. Based on a stratified random sampling design, 29 fishing hauls were collected over 29 sampling locations (Fig. 1), covering the major fishing grounds in the study area. In this way, sampling sites mirror the fishing footprint of the Italian fleet operating in the GSA9 (Russo, Franceschini, et al., 2019). The specimens of *G. melastomus* collected at each sampling site were immediately separated during the

sorting of catch, frozen, and stored at -20°C on board of the vessel and, successively, transferred to the laboratory for analyses.

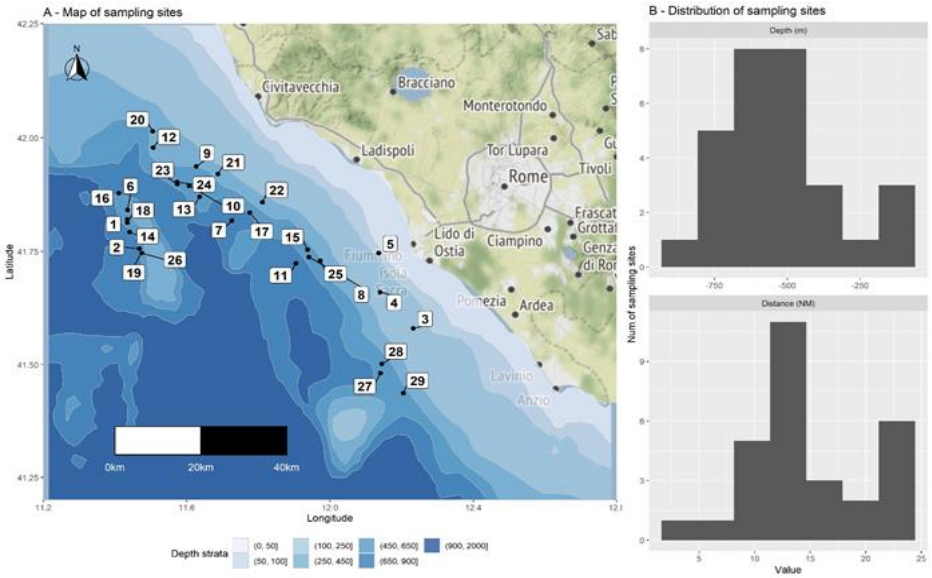


Fig. 1 Map of sampling sites of Galeus melastomus (A), histogram of haul distribution by depth (B) and haul distance from the coast (C) in the central Tyrrhenian Sea

Laboratory analyses

Once thawed to room temperature, basic measurements were recorded for each fish, including total length (TL, mm), body wet weight (TW, g), gastrointestinal wet weight (GIW, g), sex (F: Female and M: Male) and maturity stage (1: immature virgin, 2: maturing, 3a: mature, 3b: mature/extruding) assigned according to (Follesa & Carbonara, 2019). Gastrointestinal tracts (stomach and intestine with their contents and wall) were removed by dissection from each fish and stored individually in glass beakers. The total volume of stomach contents was recorded for each individual. Preys within the stomach were identified at the lowest possible taxonomic level (decapod crustaceans, fish, and cephalopods were usually identified to species) using specific taxonomic literature (e.g. (Clarke 1986; Riedl and Abel 1991; Falciai and Minervini 1992; Tuset et al. 2008). For each *G. melastomus*, the following diet data were recorded: the percentage of stomach volume, the number, and the weight of prey

items. Finally, plastics were identified and classified in shape: fragment, film, sphere, and including the distinction between fibre and filament, as reported by (Matiddi et al. 2021); colours, and subdivided into three size classes (330 µm – 1 mm; 1- 5 mm; 5-25 mm). Plastics identifications were performed considering: i) the resistance of the particles to the contact with tweezers; ii) the absence of biological structures; iii) either typical skewed shapes and crooked edges or uniform thickness; iv) distinctive colours. Furthermore, we considered plastic items those that were showing a dark sticky mark when touched with a hot needle (Hermsen et al., 2018b).

During sample processing within the laboratory, best practice contamination control measures were applied to reduce the risk of contamination. In all laboratory analyses, a 100% cotton lab coat was worn at all times, and all equipment and surfaces were thoroughly washed with 70% ethanol and/or rinsed with water (GESAMP, 2019b). Plastics found in the samples were compared with equipment used during all procedural steps, based on their characteristics (polymer, colour, and type). Additionally, fibres detected in samples were excluded from the analysis due to the inability to prevent efficiently their background contamination (Torre et al., 2016).

After that, polymer detection was obtained using Nicolet iS10 Fourier Transform Infrared Spectroscopy with Attenuated Total Reflection (FT-IR ATR, Thermo Fisher Scientific, Madison, WI, USA). FT-IR was used to identify objects greater than 1 mm in size in 10% or more of the particles collected in this study, according to guidelines developed by the MSFD technical group on marine litter (Galgani et al., 2013). The identification was based on the comparison among the analysed IR spectra and known polymer spectra libraries ("HR Spectra Polymers and Plasticizers by ATR", "HR Hummel Polymer and Additives", "HR Polymer Additives and Plasticizers"), provided with software OMNIC 9.8.286 (Thermo Fisher Scientific Inc.). The level of certainty of the comparison between the sample spectrum and the polymer library database for all analyses was set up to a minimum of 70%.

Seafloor macroplastics were collected through MEDITS trawl campaign (143 sampling sites from 2013 to 2019). The survey follows a stratified random sampling

design, proportional to bathymetric strata (i.e. 10- 50, 51-100, 101-200, 201-500, and 501-800 m). Information on the plastic litter was recorded in terms of total weight, the total number following the procedural protocol by (Fiorentino et al. 2013). The amount of marine litter occurring at each sampling site was defined according to MEDITS protocol as the distance-weighted mean of the amount of litter in the sampling sites of the MEDITS campaign. We adopted this approach considering that: 1) the MEDITS protocol for litter assessment represents an acknowledged and powerful source of information for the distribution of MaP litter in the Mediterranean Sea; 2) the distribution of the MEDITS sampling sites always allowed to use from 3 to 14 MEDITS sites to estimate the amount of litter in the sampling sites of this study.

Data and statistical analyses

The feeding incidence (%FI = individuals with identifiable prey remains/total number of fishes × 100) was used to evaluate the rate of feeding activity, whereas the degree of stomach fullness was estimated by the fullness index = GIW/TW, being GIW the gastrointestinal wet weight and TW the body wet weight. To assess the importance of prey items in the diet of *G. melastomus*, the following dietary indices were calculated: relative abundance (%N = number of individuals of prey i/total number of prey × 100), weight percentage (%W = weight of prey i/total weight of all prey × 100), volume percentage (%Vol = volume of prey i/ total volume of all prey × 100), and frequency of occurrence (%FO = number of stomachs containing prey i/ total number of stomachs containing prey × 100). Based on the Costello graphical method (Costello, 1990) modified by (Amundsen et al. 1996), the feeding behaviour of *G. melastomus* at different length classes (size) was determined by plotting prey specific abundance (%Vol) against the frequency of occurrence (%FO) on a two-dimensional graph. The position of prey types in the two-dimensional plot can be used to infer precise information regarding prey importance, feeding strategy, and niche size contribution, according to Amundsen et al. (1996). Correspondence analysis (CA) was used to graphically inspect the variation in diet (preys volume abundance) and relate the preference towards different food items according to depth, size, and sex. Also, non-parametric multivariate PERmutational ANalysis of VAriance (PERMANOVA) was

applied to test for differences in *G. melastomus* diet according to different dwelling depths, size, and sex. To do so preys volume abundance was used as the response variable, using depth, size, and sex as explanatory factors.

For each prey species a trophic level (TrL) was assigned using data from the FishBase database for fishes (http://www.fishbase.org) or SeaLifeBase (http://www.sealifebase.org) for invertebrates. To estimate individual TrL of *G. melastomus*, the mean TrL of prey items was proportionally weighted based on their contribution to the diet (weighted mean of TrL by the volume occupied by each prey), aiming at estimating the instantaneous Trophic level of each "meal" (Cortés, 1999), a snapshot of the short-term diet of *G. melastomus* individuals. Trophic levels of each prey were listed in Table 2.

Microplastic abundance was expressed as the number of microplastic items found in every single gut. The frequency of occurrence (FO%) of ingested microplastics was computed as the percentage of individuals examined containing at least one microplastic upon the total sample.

Generalized Additive Models (GAMs), a non-parametric extension of GLMs that includes smooth functions (a piecewise polynomial curve) of explanatory variables (Leathwick et al., 2006), were used to evaluate whether different factors including fish biometric parameters, the weighted mean of the trophic level of each prey, characteristics of the sampling sites, and the amount of MaP on the seafloor explain variations in the frequency of plastics ingested by *G. melastomus.* The R packages mgcv (Wood, 2017) was used to fit a series of models in which the number of plastic items ingested by each specimen of *G. melastomus* represented the dependent variable and the following predictors:

- Fish length
- Fish sex
- Depth of the sampling site

- Distance from the coast of the sampling site
- Trophic Level
- The stomach fullness
- Amount of MaP as the number of items per Km^2;
- Amount of MaP as the weight of items per Km^2

All these potential predictors were evaluated using a model selection on all the possible combinations of them. The Quasi-Poisson distribution was used to model the distribution of the dependent variable (number of plastic items ingested). The Quasi-Poisson distribution is a generalization of the Poisson distribution and is commonly used to model overdispersal count (discrete) variables when the variance is greater than the mean (Zeileis et al., 2015). Model selections were based on the information-theoretic approach (Burnham & Anderson, 2007) by comparing models AICs (Akaike's Information Criterion; (Akaike 1974).

Results

A total of 164 *G. melastomus* were collected in the central Tyrrhenian Sea: 31 stomachs were empty or regurgitated (19%) and 133 contained food (%FI = 81%). Overall, stomach fullness ranged between 0.01 and 0.36 with a median value of 0.06. The analysis of the length-frequency distribution for this species indicated that the samples contained individuals of three distinct length classes, defined according to the natural breaks in the observed distribution (15-25cm, 26-35cm, 36-55cm). Details on biometrics and stomach contents are presented in Table 1, where: the number of individuals, sex, fullness, and frequency of empty-full stomachs are summarized for each length class.

Table 2 presents the prey list and dietary index values (%Vol, %N, %FO, and TrL) for each food item. A total of 28 prey taxa were found in the stomach contents. Most of the diet consisted of Cephalopoda (Histioteuthidae and Sepiolidae), fishes (both

bathypelagic species, as Myctophidae and Stomiiformes benthic species, such as Pleuronectiformes and demersal species, such as Gadiformes), and Crustacea (Decapoda and Euphausiacea). In general, the number of preys was lower in the smaller individuals (length class: 15 – 25 cm) than in the other length classes (pie charts of Fig 2). The largest individuals (length class: 36 – 55 cm) consume a broad range of different prey items.

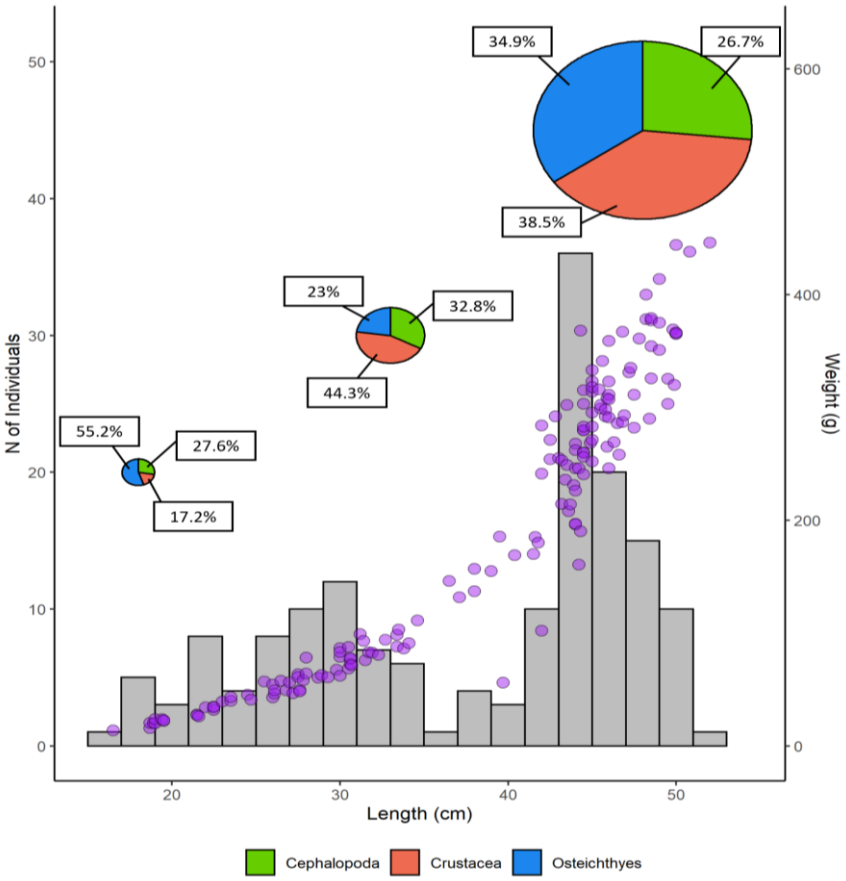
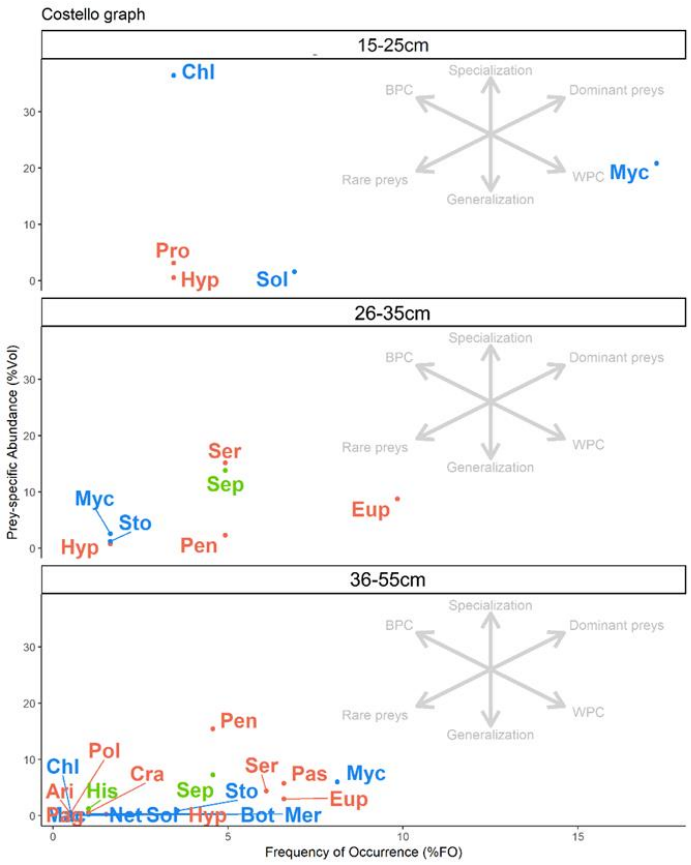


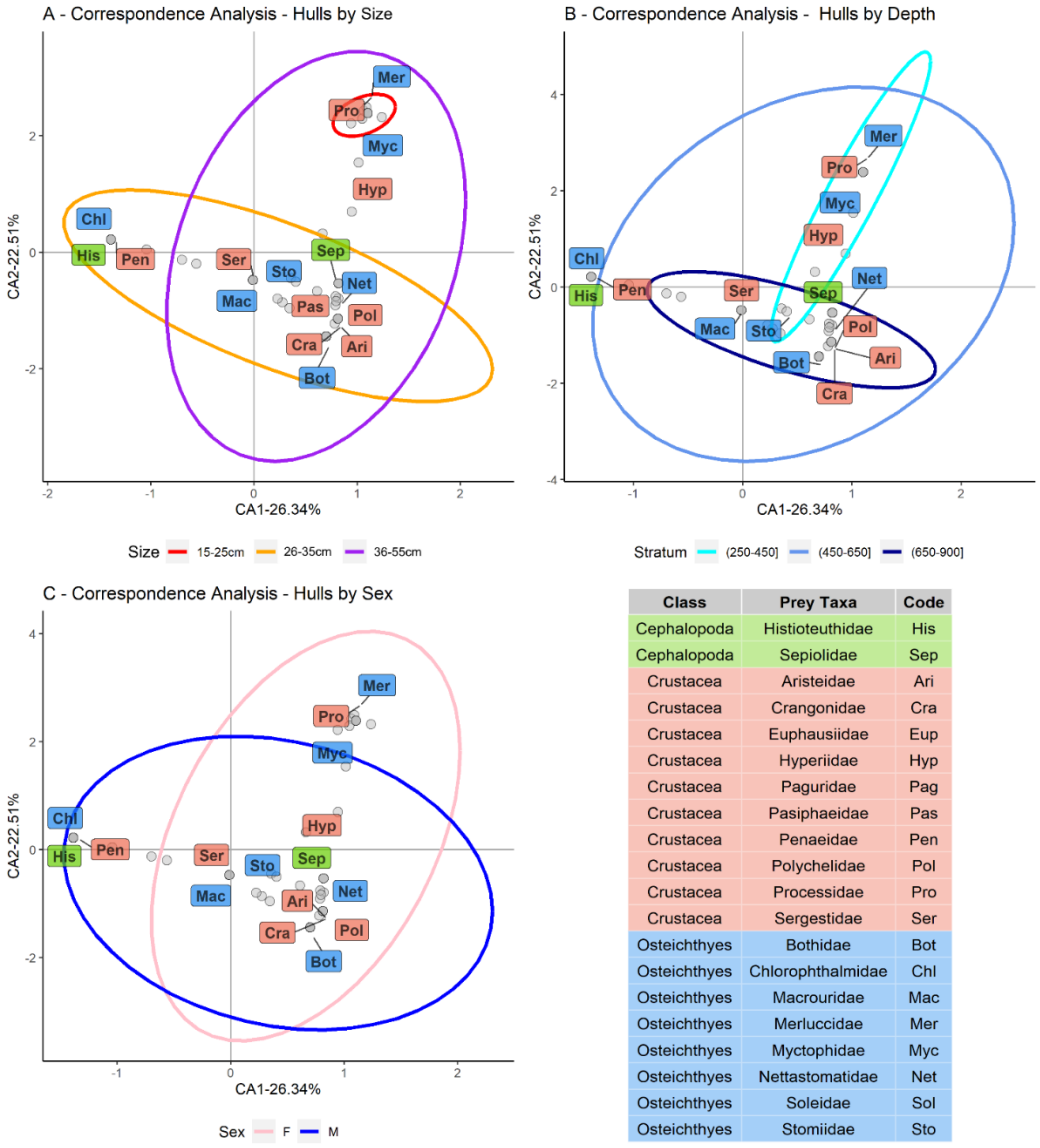
Fig. 2 Frequency distribution (grey) and length-weight relationship (purple) of *Galeus melastomus* collected in the central Tyrrhenian Sea. Pie charts indicate the percentage of prey species (%FO) present in individuals' stomach contents, and they are sized based on the number of prey species present in each length class (15-25cm, 26-35cm, 36-55cm).

In Fig. 3, the frequency of occurrence (%FO) is plotted against prey-specific abundance (%Vol) and subdivided into length classes (15-25cm, 26-35cm, 36-55cm), based on the Costello graphical method (Costello, 1990) modified by Amundsen et al. (1996). The majority of the prey points are depicted at the lower-left corner of the diagrams or near the y-axis, indicating that many preys were consumed at low frequency and abundance. Five taxa of prey have been identified in the smallest size class; two out of these 5 groups (Myctophidae and Chlorophthalmidae) occupy the upper portion of the y-axis. As shown in Fig. 3, the first group (Myctophidae) consists of prey with a high specific abundance and occurrence, while the second group (Chlorophthalmidae) includes prey with a low occurrence and high abundance (upper left). A total of seven groups of prey are found in the size class 26-35 cm, which occupied the lower-middle part of the x and y axes. Prey groups raise to nineteen in the largest size class, located at the lower portion of the axes.



Class	Prey Taxa	Code
Cephalopoda	Histioteuthidae	His
Cephalopoda	Sepiolidae	Sep
Crustacea	Aristeidae	Ari
Crustacea	Crangonidae	Cra
Crustacea	Euphausiidae	Eup
Crustacea	Hyperiidae	Hyp
Crustacea	Paguridae	Pag
Crustacea	Pasiphaeidae	Pas
Crustacea	Penaeidae	Pen
Crustacea	Polychelidae	Pol
Crustacea	Processidae	Pro
Crustacea	Sergestidae	Ser
Osteichthyes	Bothidae	Bot
Osteichthyes	Chlorophthalmidae	Chl
Osteichthyes	Macrouridae	Mac
Osteichthyes	Merluccidae	Mer
Osteichthyes	Myctophidae	Myc
Osteichthyes	Nettastomatidae	Net
Osteichthyes	Soleidae	Sol
Osteichthyes	Stomiidae	Sto

Fig. 3 *Galeus melastomus* collected in the central Tyrrhenian Sea: frequency of occurrence (%FO) was plotted against prey-specific abundance (%Vol) and subdivided into length classes (15-25cm, 26-35cm, 36-55cm), based on the Costello graphical method (Costello, 1990) modified by Amundsen et al. (1996) (BPC: Between Phenotype Component; WPC: Within Phenotype Component). Preys are subdivided into three groups (Cephalopoda, Crustacea, and Osteichthyes).



Class	Prey Taxa	Code
Cephalopoda	Histioteuthidae	His
Cephalopoda	Sepiolidae	Sep
Crustacea	Aristeidae	Ari
Crustacea	Crangonidae	Cra
Crustacea	Euphausiidae	Eup
Crustacea	Hyperiidae	Hyp
Crustacea	Paguridae	Pag
Crustacea	Pasiphaeidae	Pas
Crustacea	Penaeidae	Pen
Crustacea	Polychelidae	Pol
Crustacea	Processidae	Pro
Crustacea	Sergestidae	Ser
Osteichthyes	Bothidae	Bot
Osteichthyes	Chlorophthalmidae	Chl
Osteichthyes	Macrouridae	Mac
Osteichthyes	Merluccidae	Mer
Osteichthyes	Myctophidae	Myc
Osteichthyes	Nettastomatidae	Net
Osteichthyes	Soleidae	Sol
Osteichthyes	Stomiidae	Sto

Fig. 4 *Galeus melastomus* collected in the central Tyrrhenian Sea: Correspondence analysis (CA) graphically inspect the variation in diet and relate the preference for different food items based on size (15-25cm, 26-35cm, 36-55cm) (A), depth (B), and sex (Female: F; Male: M) (C). Preys are subdivided into three groups (Cephalopoda, Crustacea, and Osteichthyes).

In the CA plot, the first two axes accounted for 48.85% of the variance (Fig. 4). The positive portion of axis I and II (I quadrant) condensed only a small portion of prey, including two groups of crustaceans (Processidae and Hyperiidae), and two fish (Merlucciidae and Myctophidae). A large number of individuals and prey were located in IV quadrant. The groups Histioteuthidae, Penaeidae, and Chlorophthalmidae occupied the regions between the II and III quadrants. Convex hulls were plotted and coloured according to depth, size, and sex. Preys in the stomach contents differed significantly according to length class (pseudo-F= 4.64; $p < 0.01$) and depth distribution (pseudo-F = 2.73; $p < 0.02$). The results of the PERMANOVA did not reveal differences between sexes (Table 3).

Concerning plastics, the FT-IR analysis revealed the polymeric nature of all isolated plastic particles (20 particles), corresponding to Polyethylene (PE: 50%), Polypropylene (PP: 25%), Polyamide (PA: 12.5%), and Polystyrene (PS: 12.5%). Plastics were found in the stomach contents of 33 individuals, with a percentage of occurrence of about 20%. Individuals of *G. melastomus* ingested 45 plastics in total, ranging from 1 to 11 items, with a mean of 1.47 ± 0.28 (± SD) (Fig. 5). Plastic size class between 1 mm and 5 mm was the most abundant (44.7%) and included all shape categories. The most abundant plastic shape category was filaments (53.2%), followed by fragments (25.5%), films (17%), and spheres (4.3%) (Fig. 5). Most plastic items were transparent (59.6%), then black (17%), white (14.9%), green (4.3%), blue (2.1%), and red (2.1%).

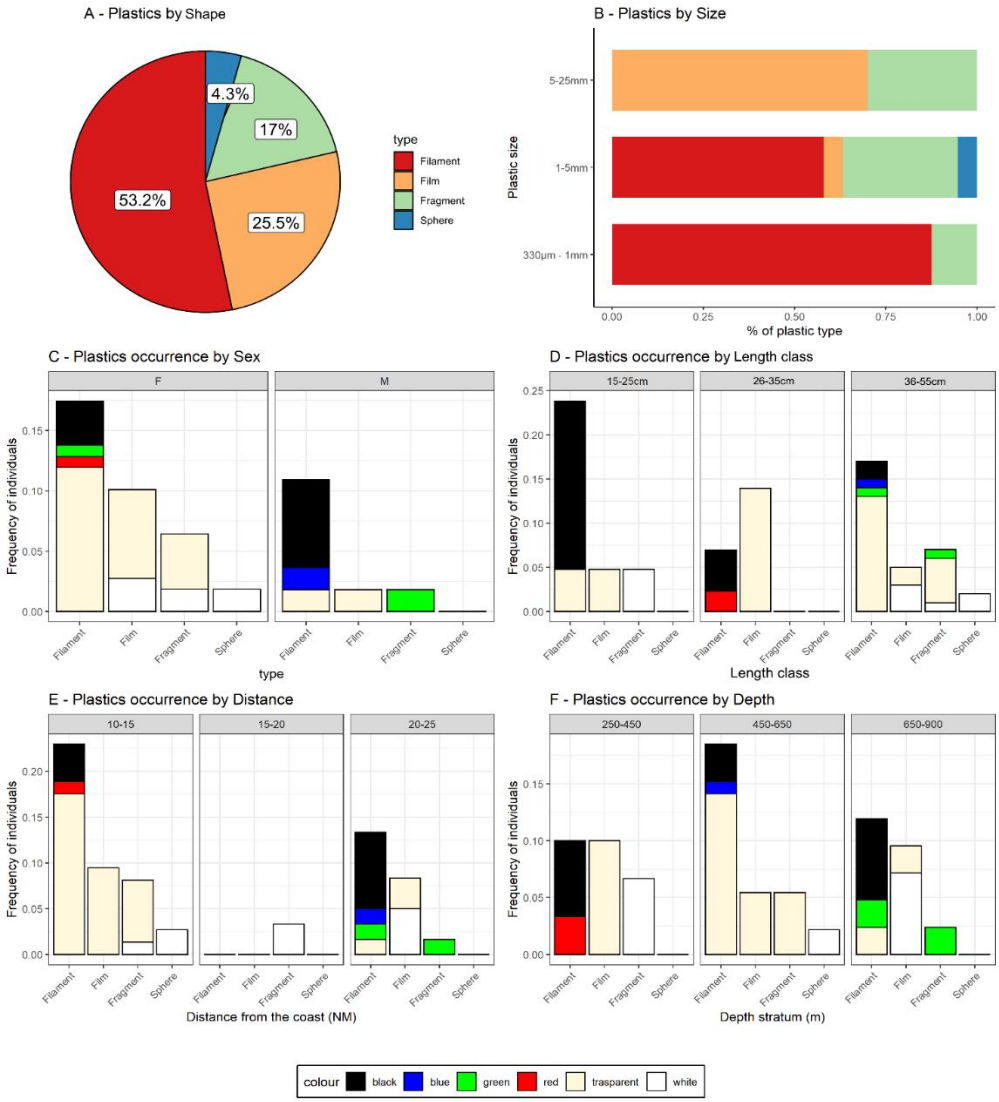


Fig. 5 Characterization of plastics found in *G. melastomus* stomach contents in the central Tyrrhenian Sea. A) Pie chart by plastic-shape; B) histograms by plastic size; C) plastic occurrence by sex (Female: F; Male: M); D) by length class (15-25cm, 26-35cm, 36-55cm); E) by distance from the coast; F) and depth strata, subdivided into colours.

The analysis of the frequency of occurrence of plastic items by shape, size, and according to sex, size, distance from the coast, and depth reveals that transparent filament is the most common ingested item, (N= 14), followed by transparent films (N= 9), black filament (N= 8), transparent fragments (N= 5), and white spheres (N= 2). The largest amount of plastic was found in fish that were caught within the buffer of 10-15 nautical miles from the coast, in the depth stratum between 450-650 meters, in the length class of 36-55 cm, and in female (Fig. 5).

The GAM model selection returned as best model the following relationship:

Num of Items ~ s(Distance) + s(TrophicLevel) + s(Fullness) + s(L1n) + s(L1w)

where Distance is the distance from the coast of the haul, Trophic level is the weighted mean of the trophic level of each prey (Table 2). Fullness is the degree of stomach fullness, which could be important to consider that empty stomachs could contain neither food nor plastic, and L1n and L1w are the amounts of MaP in number of items (n) and weight (w).

This model allowed explaining as much as 53.7% of the total variance in the data, and it fits adequately the pattern in the input data (Fig. 6). Distance from the coast and amount of MaP litter as weight was significantly related to the dependent variable, but with opposite effects (Figure 6B-D-E). In particular, the former was associated with a decreasing and monotonic (almost linear) effect on the number of ingested plastics, while the latter was associated with an increasing and monotonic effect. The other predictors, although retained in the model, were not significant.

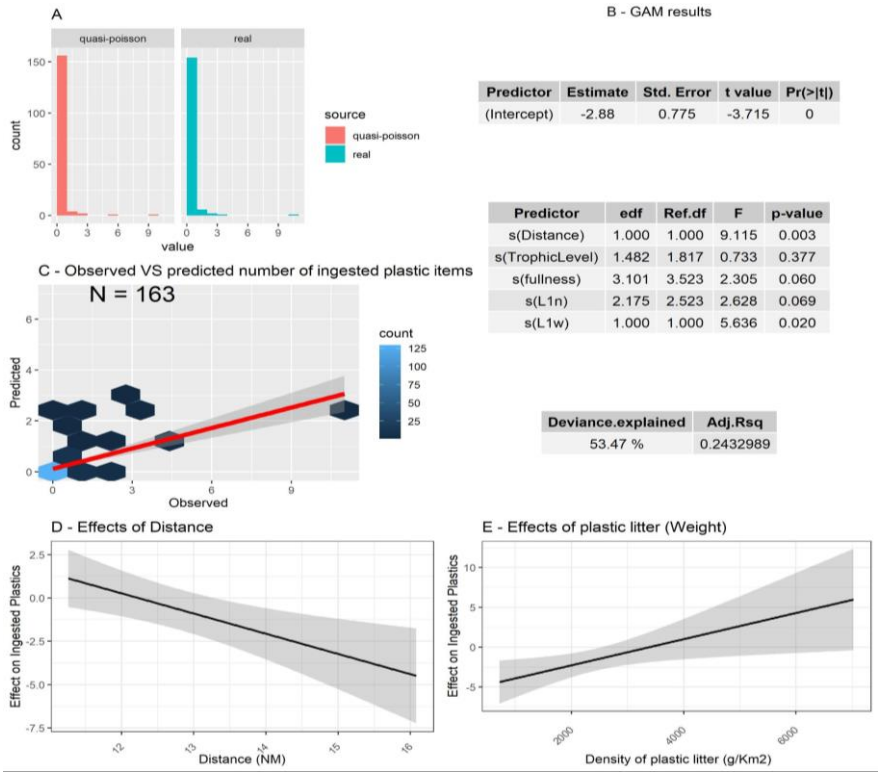


Fig. 6 Summary of results of the best-fit Generalized Additive Model (GAM) used to evaluate whether different factors including fish biometric parameters (fullness), the weighted mean of the trophic level of each prey, characteristics of the sampling sites (depth and distance from the coast) and the amount of macroplastics on the seafloor (number and weight of plastic items) explain variations in the frequency of plastics ingested by *G. melastomus* collected in the central Tyrrhenian Sea. A - Comparison between theoretical (Quasi-Poisson distribution) and observed distribution of plastic ingestion (Number of items per individual of *G. melastomus*); B - Tables of GAM results; C - Observed versus GAM predicted values of plastic ingestion (Number of items per individual of *G. melastomus*); D, E - The effects of the significant predictors, independent of the other predictors represented as response variable shape. The degree of smoothing is indicated in the y-axis label. Confidence intervals (95%) around the response curve are represented in grey.

Discussion

The diet analysis of *G. melastomus* confirmed that several mesopelagic and bathypelagic species belonging to fish, cephalopods and crustaceans, provide the primary food source for this species, and contributed to filling the knowledge gap

highlighted by D'Iglio et al. (2021) for the central Tyrrhenian Sea. Food habits of blackmouth catshark revealed changes during its ontogenetic development, with an increasing diversity along size. Due to this, the diet of small and young individuals mainly consisted of fish, whereas the diet of intermediate-sized individuals (26-35 cm) mostly included decapod crustaceans. The diet of large individuals resulted in a more balanced composition with similar proportions of crustaceans, cephalopods, and fish.

The diet of *G. melastomus* exhibited a high diversity in species composition, since it mainly included bathypelagic (Myctophidae) and demersal (Chlorophthalmidae) fish in the smaller individuals. Prey diversity increased in the individual of intermediate size, and additionally included demersal crustaceans (Penaeidae) and cephalopods (Sepiolidae), together with pelagic Euphausiidae and Sergestidae. The most diversified diet was observed in adult individuals, and comprised demersal crustaceans (e.g. Penaeidae, Aristeidae, Polychelidae), as well as pelagic crustaceans (e.g. Euphausiidae, Pasiphaeidae, Sergestidae and Hyperiidae amphipods), which may have been captured during their daily migrations in mid-water (Elder & Seibel, 2014; Onsrud & Kaartvedt, 1998). Moreover, adult individuals prey on many bathypelagic (Stomiidae, Myctophidae) and demersal fish (e.g. Merlucciidae, Macrouridae, Soleidae, Bothidae). Finally, cephalopods included both bathypelagic (*Histioteuthis bonnellii*) and demersal species (*Rossia macrosoma* and *Heterotheutis dispar*), as well as they are an important food source for other bathyal selachians (Bello, 1997; Darna et al., 2019; D'Iglio et al., 2021; Fanelli et al., 2009; Rey et al., 2005; Valls et al., 2011).

The presence of typically benthic preys, such as fish of the families Bothidae and Soleidae, as well as the crustaceans of the family Paguridae, indicate that *G. melastomus* is a benthic feeder and scavenger, as reported by other authors (Anastasopoulou et al., 2013; Barría et al., 2018; Taleb Bendiab et al., 2016). In addition, the presence of bathypelagic species highlighted that *G. melastomus* behaves as a supra-benthic predator capable of moving from the bottom to catch prey in the

bathypelagic environment, confirming the behaviour previously reported for *G. melastomus* in other areas of the Tyrrhenian Sea (D'Iglio et al. 2021; Ricci et al. 2021).

Small individuals were the only ones that displayed preys with a high specific abundance and occurrence, as the Costello model revealed, and such evidence is an indication of their specialization. In fact, preys with a low specific abundance and presence were only occasionally consumed by a few individuals. In contrast, individuals of intermediate and large size did not display a close trophic relationship with specific prey species, since no dominant species were found in their diet. This result suggests that, for most of its life cycle, blackmouth catshark has a generalist behaviour and, accordingly to the prey ecological habits, the results confirmed that it actively catches preys on the bottom, as well as in the supra-benthic layer, feeding on all available preys, as also reported by other authors (Anastasopoulou et al. 2013; Barria et al. 2018; D'Iglio et al. 2021).

Further differences in diet related to depth distribution and size are also revealed by the CA analysis and were consistent with the behaviour of both preys and predator. Based on the prey composition of the stomach contents, the differences among the size groups were confirmed and could be explained by the vertical movement of blackmouth catshark. Indeed, the dynamics of the species suggest that younger individuals move through the water column towards surface waters. Specifically, the smaller blackmouth catshark are more active near the continental plateau (250-450 m depth), while the adult individuals are found at depths between 450 and 900 m. Our results are also in agreement with other studies which highlighted variations in the blackmouth catshark habits and composition of their preys during their growth in relation to the availability of the resources (Carrassón et al., 1992; Fanelli et al., 2009; Olaso et al., 2005).

Our study allowed to show that blackmouth catshark ingest a wide variety of plastics in terms of shape, colour, and size, which are in a clearly higher proportion than in the study of Carrassón et al. (1992). Particularly, there was a high proportion of microplastics (1-5 mm), and transparent filaments and films were the most abundant

particles found in the samples. Films and filaments are the most abundant plastic particles in seawater and sediments (Gago et al. 2018). In areas densely populated such as near Rome, the sources of filaments might be more abundant, also for the presence of Tiber River mouth, which discharge in the Tyrrhenian Sea pollutant like plastic litter (Crosti et al. 2018). The transport of filaments can also accumulate in deep areas, as a consequence of their greater sinking capacity if compared to spheres and fragments (Chubarenko et al. 2016). Conversely, low-density particles (polystyrene spheres found in the samples) tend to float (Hidalgo-Ruz et al., 2012), but they can even sink after biofouling. Therefore, this can change the weight of the specific particle, making it easier to detect them within deep-sea sediments (Alomar & Deudero, 2017; Eriksen et al., 2014b; Valente et al., 2019). Finally, several polymers of polyamide have been found in the stomach contents of *G. melastomus*. As this polymer is the main component of the nylon used in the ropes and fishing nets (Deopura and Padaki 2015), the wastes from fishing gears appeared a considerable cause of plastic pollution in the Tyrrhenian Sea, in line with other studies in deep-sea areas (Güven et al., 2017b; Lusher et al., 2013; Murray & Cowie, 2011; Neves et al., 2015; Pruter, 1987a; Welden & Cowie, 2017).

In summary, significant sources of plastic pollution were identified through the GAM model, which was used to disentangle the role of different factors in the ingestion of plastic by *G. melastomus*. Based on this, two predictors were identified as major contributors affecting the number of plastic items ingested. Indeed, out of all the potential sources of variability considered (i.e. fish size, sex, depth of capture, distance from the coast, short-term trophic level, stomach fullness, and amount of MaP in terms of the number of items and total weight), only the distance from the coast and the total weight of MaP on the seafloor significantly affect the number of plastic items ingested by *G. melastomus*. The decreasing amount of plastics associated with the distance from the coast could be interpreted as the move away from the source of pollution. This variable was one of the major factors that negatively affect the amount of waste on the seabed along the coast (Coll et al., 2012; Franceschini et al., 2021b; Sbrana, Valente, Scacco, Bianchi, Silvestri, Palazzo, Andrea, et al., 2020b; Steer et al., 2017).

Accordingly, the coast of the Tyrrhenian Sea (GSA9) hosts a series of fishing grounds along a narrowed shelf that receives plastic waste through some important sources including the rivers Tiber and Arno (Crosti et al., 2018b; Inghilesi et al., 2008a; Montreuil & Ludwig, 2013; Montuori et al., 2016).

Similarly, the ingestion by *G. melastomus* is positively correlated with the amount of MaP present on the bottom This second factor deserves an additional reflection since, in this study, the amount of MaP was associated with the ingestion of smaller particles and not directly associated to the MaP. It is well known that plastic waste at sea is undergoing a process of fragmentation and miniaturization, so it is logical to expect that large quantities of MaP can generate corresponding quantities of MeP and MiP (Chamas et al., 2020b; Crawford & Quinn, 2017). Similar studies have already identified positive relationships between MiP ingestion and MaP hotspots at the sea bottom (Alomar et al., 2020; Franceschini et al., 2021b). Particles, however, increase their ability to be transported as they become smaller (Zhang, 2017), and thus oceanographic factors can greatly affect the spread and accumulation of different sizes of plastic particles. In addition, waste is stored in deposition centres (low-hydrodynamic areas). Finally, trawling contributes to the resuspension of litter on the bottom, slowing down plastic deposition. Leaving aside the oceanographic dynamics of plastic pollutants, the biomonitoring of their abundance and distribution as revealed by the analysis of stomach contents in non-commercial species, such as the blackmouth catshark, could represent an important approach to assessing the risk of contamination of fisheries landings of commercial species. The results of this analysis did not reveal any adverse effects on shark health, although we did not check the volume occupied by plastics in the stomach contents. However, it is still difficult to determine what are the real threats to a species that ingest plastic. In this way, it would be useful to further research using an ecotoxicological perspective. Moreover, a future study could examine whether the pattern returned by the blackmouth catshark could serve as a proxy for those of other species, including key resources for fisheries, given that the samples collected during this study were taken over important fishing grounds (Russo et al. 2018).

In conclusion, this study provides a contribution to knowledge on trophic ecology and on the contamination of the diet by plastic. Several novel insights have been gained into the feeding ecology of *G. melastomus* in the central Tyrrhenian Sea, which is an area where this predator is very abundant and commonly caught as a by catch in bottom trawl fisheries, but adequate information is lacking. The dietary approach, which begins with a description of the organism's feeding habits, allows further exploration of plastic ingestion with great potential and versatility for most biological or ecological studies (Baker et al., 2014; Mahesh et al., 2019). Measurement of instantaneous trophic levels should be further investigated, even though the results are not significant. In our study the estimates for the trophic level were assigned based on FishBase or SeaLifeBase. These estimates were derived from a number of food items using a randomized resampling routine. However, if we consider some relevant dataset on Stable Isotopes Analysis (SIA) for deep-sea species in the Mediterranean Sea, the trophic levels of benthos feeders adopted in this study are likely to be underestimated. We are aware that stable isotope allows us to estimate trophic levels more accurately and would be fundamental to deepen this topic. Unfortunately, the isotopic values (especially $\delta^{15}N$) for some species are not recorded, while for other species the values are not available for the Central Tyrrhenian Sea. The opportunistic feeding behaviour of *G. melastomus* affects the incidental ingestions of numerous plastic particles likely confused for other preys, or indirectly ingested by feeding, in line with the results of previous research (Alomar & Deudero, 2017; Anastasopoulou et al., 2013; Cartes et al., 2016; Valente et al., 2019).

It is widely acknowledged that the use of a single bioindicator species for monitoring plastic ingestion cannot be used to all European marine waters, even in areas with very different characteristics and levels of plastic pollution. Instead, it is recommended to used specific bioindicators based on habitat type and spatial distribution (Fossi et al. 2018). For instance, some crustaceans such *as Nephrops norvegicus* (in the Sea of Sardinia) and *Aristeus antennatus* (around the Balearic Islands) are considered effective indicators of the presence and impact of microlitter (mainly microplastics) in the Mediterranean deep sea (Carreras-Colom et al. 2018;

Cau et al. 2019). Additionally, individuals of different sizes could be exposed to particles of different sizes (Worm et al. 2017). In this way, bioindicator species should be carefully selected according to the spatial domain being studied. Given this, and considering the generalist behaviour of blackmouth catshark, its abundance and distribution, and interaction with plastic hotspots, we consider, in line with Fossi et al. (2018), this species as a suitable candidate for developing a monitoring programme for the presence of plastics on the seabed, as requested in the Marine Strategy Framework Directive for European waters.

Chapter 3 - Environmental DNA metabarcoding reveals the effects of seafloor litter and trawling on marine biodiversity

Abstract

Anthropogenic pressures like seafloor litter and trawling activities have significant implications for marine biodiversity and seafloor ecosystem health, by altering nutrient availability and changing environmental properties. Traditional biodiversity assessment methods are often costly, time-consuming, and have limitations at sea. Environmental DNA (eDNA) techniques are emerging as promising tools for monitoring anthropogenic impacts on biodiversity, yet they have not been applied to study the integrated effects of seafloor litter and trawling on marine biodiversity. This study uses eDNA metabarcoding to measure these combining pressures on the seafloor. Our research revealed that both have an impact on the composition and diversity of species. Certain species are indicators of litter pollution, while others signal of the influence of trawling activities. The employment of eDNA-based methods represents a valuable resource for monitoring anthropogenic impacts in data collection, particularly during scientific surveys, thus enhancing our ability to monitor marine ecosystems and assess pollution effects more effectively.

Keywords: environmental DNA, anthropogenic impacts, seafloor litter, community composition, ecosystem disturbance, trawling

Introduction

Marine biodiversity plays a key role in maintaining the well-being and balance of marine ecosystems (Goulletquer et al., 2014). The seafloor hosts many marine species, encompassing a wide spectrum of organisms, collectively contributing to the complex structural arrangement of life within the depths of the sea. Nevertheless, this priceless biodiversity encounters substantial challenges arising from direct and/or indirect anthropogenic activities, including fisheries, and pollutants (Halpern et al., 2008).

Commercial trawling is recognized as a major human-induced influence on seafloor ecosystems, leading to substantial alterations in benthic community composition (Beauchard et al., 2021; Eigaard et al., 2017; Oberle et al., 2016). As a result, the distribution of species is expected to be significantly affected by the spatial distribution of fishing activities (Clark et al., 2016; Pusceddu et al., 2014). Due to the direct disturbance of benthic species, the primary effects of trawling on benthic communities vary depending on the animals' susceptibility to fishing activity and the populations' resilience (Hiddink et al., 2017; Kaiser et al., 2006; Tiano et al., 2020). Trawling may also have an indirect impact on the community by altering the availability of food and habitats (Johnson et al., 2015; Mangano et al., 2017). Changes in prey availability and community composition may have different impacts on species with different feeding strategies. As a result, the effects of trawling may be positive by increasing food availability for opportunistic species (Hiddink et al., 2008), or negative by removing certain prey for more selective organisms (Hiddink et al., 2017). Previous research (Bozzano and Sardà, 2002; Groenewold et al., 2000; Kaiser and Spencer, 1994; Lejeune et al., 2023) have shown that scavengers possibly take advantage of the discard produced by trawling, while more susceptible species, like sessile filter feeders, are 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 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 on the bottom of the ocean 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).

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., n.d.) used a machine learning approach (i.e., Random Forests) to model and investigate pathways from the largest available dataset of marine litter distribution in the Western and Central Mediterranean Sea. The results showed that the methods used to quantify some litter categories, such as plastic, have reached an advanced stage of development. However, the resulting impacts of litter on marine communities remain largely unknown.

Understanding the risk posed by anthropogenic pressures on the seafloor, such as trawling activities and marine litter, is crucial for devising effective conservation and management strategies. This could greatly benefit from a multidisciplinary framework, which includes the sources, distribution, and impacts within the environment from a systemic point of view (Hardesty et al., 2019). The assessment of environmental impacts on the seafloor is particularly urgent in the Mediterranean Sea,

which is known to be a global hotspot, being a semi-enclosed and highly anthropized basin (Boucher and Billard, 2020; Coll et al., 2012; Cózar et al., 2015; Deudero and Alomar, 2015). However, knowledge about the impacts on the seafloors remain 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). There are many studies focused on the impact of marine litter and trawling on species indicative 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). Moreover, fishing nets are selective tools that provides an inaccurate description of marine communities. 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., 2023, 2022). eDNA has been successfully applied in various contexts, including the detection of rare or elusive species, monitoring invasive species, and assessing community dynamics (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 remains unexplored.

The aim of this study is therefore to examine the impact of seafloor litter pollution and trawling activities on marine communities by using opportunistically collected eDNA data to identify species occurrence and relate them to the various levels of fishing effort and litter pollution. The use of eDNA metabarcoding approach overtakes traditional methods limitations on marine community assessment, promoting the investigation of the ecological effects of anthropogenic pressures on the examined communities. The identification of species relationship with marine debris and fishing activities has the potential to shed light on 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. Particularly, it could thus drastically contribute to the growing body of knowledge surrounding the ecological consequences of marine litter, with potential implications for the development of targeted mitigation and management strategies.

Material and Methods

Area of study

Environmental DNA samples were gathered during summer 2020 from 24 sampling locations aboard a commercial bottom-trawler (Figure 1), using an opportunistic sampling strategy. 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., 2018b). 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, also bringing contaminants such as marine debris (Cesarini et al., 2023; Inghilesi et al., 2008b).

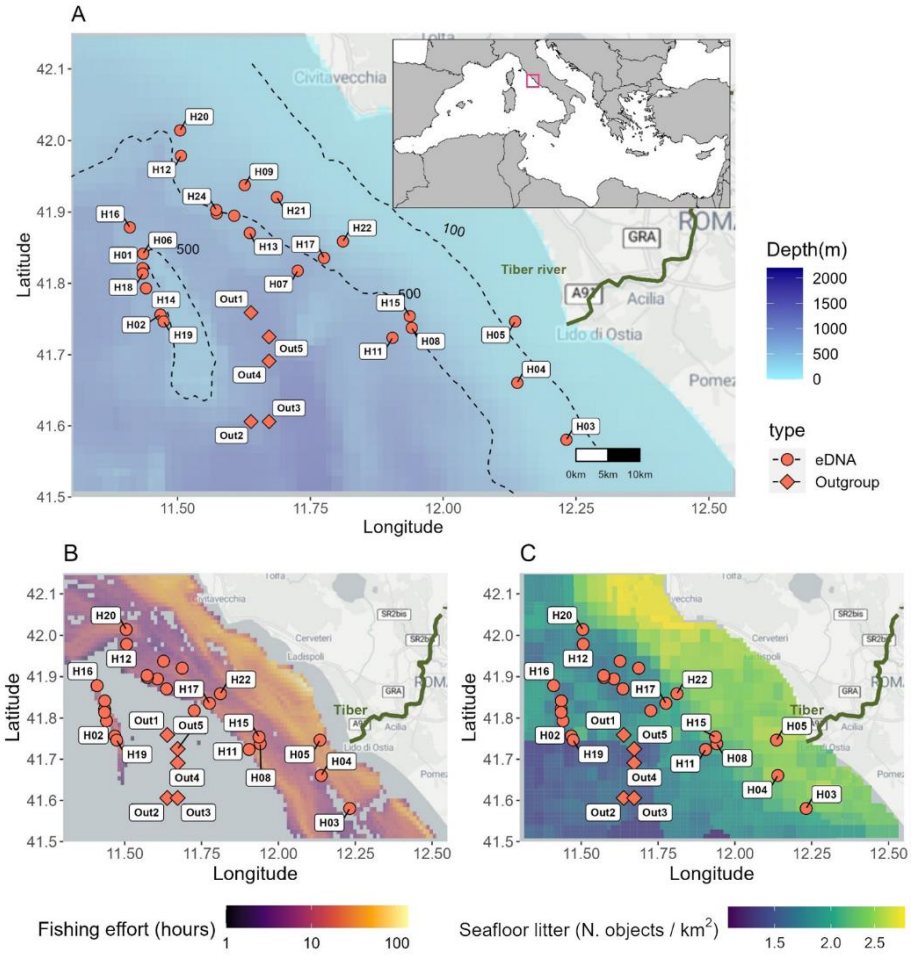


Figure 1 Map of the 24 sampling locations in the central Tyrrhenian Sea (FAO Geographical Sub Area, GSA 9–western Mediterranean Sea) (A), with related fishing effort (B), and seafloor litter (C).

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 and 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 inserted to passively absorb DNA from the surrounding environment.

Two taxonomically informative mitochondrial regions were PCR amplified from the eDNA samples: a 167 bp fragment of the 12S gene and a 313 bp fragment of the COI gene. The first fragment was amplified using Tele02 primers specific to fish, while the second fragment was amplified using highly degenerate universal metazoan primers. To account for possible contamination, a positive PCR control (*Sebastes mentella*, a subarctic species absent in the Mediterranean Sea) and a negative control were included.

Bioinformatic analysis followed the OBITOOLS pipeline (Boyer et al., 2016) and the taxonomic assignment was 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%.

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 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 (doi:10.17632/r2b6svy7h7.1;Cau et al., in press) to provide reliable estimates for the area, between 0 and 1000 meters in depth. 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 (Fiorentino et al., 2013in MEDITS-Handbook). 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. The total number of objects collected is then standardized according to the swept area, expressed in number of objects · km^{-2}. We consider this approach to be most appropriate due to the extensive temporal scope of MEDITS data, spanning seven years. This extended timeframe provides a more accurate representation of litter presence, as well as its potential impact on species with multi-year life cycles. Furthermore, the incorporation of the MEDITS data offers the additional advantage of encompassing regions with minimal or no litter, serving as valuable outgroups in low-impact areas.
- The Mean Fishing effort (bottom otter trawling) was calculated as the mean annual total effort, in 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., 2016, 2014).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 obtained from different resources for some anthropogenic descriptors recognized for altering marine communities, such as: 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/europe/) .

- The distance (in Km) from the Tiber River mouth, assuming that rivers are the main carriers of nutrients 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 (Peres and Picard 1964).
- The Distance from the coast (Km), computed using the *dist2Line* function of the R package geosphere (Hijmans, 2019).

Data analysis

The final dataset comprised, for each of the 24 sampling locations, species presence as detected by eDNA metabarcoding, number of seafloor plastic, 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 from 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 only in the lowest quartile (25%) of the occurrence distribution at the sampling sites.

The total amount of marine litter (number of objects · km^{-2}) and the Mean Fishing effort (total annual hours of bottom otter 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

(outgroups) as a reference point in our cluster analyses to provide a comparative framework for evaluating groupings within the dataset. Then, we categorized the sampling sites based on a combined binary scale representing the presence of litter (low/high) and a corresponding binary scale indicating the level of fishing pressure (low/high).

We performed a redundancy analysis (RDA) using the *vegan* package in R (Oksanen J et al., 2007) 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 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.

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).

Table 1 List of taxa selected for this study, identified through eDNA metabarcoding (COI and 12S) 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	*Alcyonium acaule*	1	Malacostraca	Sergestidae	*Robustosergia robusta*	
	Epizoanthidae	*Epizoanthus arenaceus*	2	Polychaeta	Acoetidae	*Panthalis oerstedi*	63
	Funiculinidae	*Funiculina quadrangularis*	3		Onuphidae	*Paradiopatra calliopae*	64
	Sagartiidae	*Sagartiogeton spp.*	4		Spionidae	*Laonice cirrata*	65
Ascidiacea	Ascidiidae	*Ascidia spp.*	5	Teleostei	Argentinidae	*Argentina sphyraena*	66
Asteroidea	Astropectinidae	*Astropecten irregularis*	6			*Glossanodon leioglossus*	
	Luidiidae	*Luidia sarsi*	7		Bothidae	*Arnoglossus laterna*	68
Bivalvia	Teredinidae	*Psiloteredo megotara*	8			*Arnoglossus rueppelii*	
Cephalopoda	Eledonidae	*Eledone cirrhosa*	9		Callionymidae	*Synchiropus phaeton*	70
	Enoploteuthidae	*Abralia veranyi*	10		Caproidae	*Capros aper*	71
	Histioteuthidae	*Histioteuthis bonnellii*	11		Centrolophidae	*Centrolophus niger*	72
		Histioteuthis reversa			Cepolidae	*Cepola macrophthalma*	73
	Loliginidae	*Alloteuthis media*	13		Chlorophthalmidae	*Chlorophthalmus agassizi*	74
		Alloteuthis subulata			Citharidae	*Citharus linguatula*	75
	Octopodidae	*Octopus vulgaris*	15		Congridae	*Conger conger*	76
		Pteroctopus tetracirrhus				*Gnathophis mystax*	
		Scaeurgus unicirrhus			Epigonidae	*Epigonus denticulatus*	78
	Ommastrephidae	*Illex coindetii*	18			*Epigonus telescopus*	
		Todarodes sagittatus			Gadidae	*Gadiculus argenteus*	80
		Todaropsis eblanae				*Micromesistius poutassou*	
	Onychoteuthidae	*Onychoteuthis banksii*				*Trisopterus capelanus*	
	Sepiidae	*Sepia elegans*	22		Gobiidae	*Lesueurigobius friesii*	83
	Sepiolidae	*Heteroteuthis dispar*	23		Gonostomatidae	*Cyclothone braueri*	84
		Neorossia caroli				*Gonostoma denudatum*	
		Rondeletiola minor			Lophiidae	*Lophius budegassa*	86

		Rossia macrosoma				*Lophius piscatorius*	
		Sepietta oweniana			Lotidae	*Molva molva*	88
		Stoloteuthis leucoptera			Macrouridae	*Coelorinchus caelorhincus*	89
Crinoidea	Antedonidae	*Antedon mediterranea*	29			*Hymenocephalus italicus*	
		Leptometra phalangium				*Nezumia sclerorhynchus*	
Echinoidea	Brissidae	*Brissopsis lyrifera*	31		Merlucciidae	*Merluccius merluccius*	92
	Echinidae	*Echinus acutus*	32		Moridae	*Mora moro*	93
Elasmobranchii	Dalatiidae	*Dalatias licha*	33		Mullidae	*Mullus barbatus*	94
	Etmopteridae	*Etmopterus spinax*	34		Myctophidae	*Ceratoscopelus maderensis*	95
	Hexanchidae	*Hexanchus griseus*	35			*Diaphus holti*	
	Pentanchidae	*Galeus melastomus*	36			*Diaphus metopoclampus*	
	Rajidae	*Dipturus oxyrinchus*	37			*Electrona risso*	
		Leucoraja circularis				*Hygophum benoiti*	
		Raja clavata				*Lampanyctus crocodilus*	
	Scyliorhinidae	*Scyliorhinus canicula*	40			*Lobianchia dofleini*	
Gastropoda	Cocculinidae	*Coccopigya spp.*	41			*Myctophum punctatum*	
Holocephali	Chimaeridae	*Chimaera monstrosa*	42			*Notoscopelus elongatus*	
Hydrozoa	Aglaopheniidae	*Lytocarpia myriophyllum*	43		Nemichthyidae	*Nemichthys scolopaceus*	104
	Bougainvilliidae	*Bougainvillia muscus*	44		Nettastomatidae	*Facciolella oxyrhyncha*	105
	Campanulariidae	*Clytia hemisphaerica*	45			*Nettastoma melanurum*	
	Plumulariidae	*Nemertesia ramosa*	46		Notacanthidae	*Notacanthus bonaparte*	107
Malacostraca	Aristeidae	*Aristeus antennatus*	47		Peristediidae	*Peristedion cataphractum*	108
	Cirolanidae	*Natatolana borealis*	49		Phycidae	*Phycis blennoides*	109
	Goneplacidae	*Goneplax rhomboides*	50		Scophthalmidae	*Lepidorhombus boscii*	110
	Nephropidae	*Nephrops norvegicus*	51			*Lepidorhombus whiffiagonis*	
	Pandalidae	*Plesionika acanthonotus*	52		Sternoptychidae	*Argyropelecus hemigymnus*	112
		Plesionika edwardsii				*Maurolicus muelleri*	
		Plesionika heterocarpus			Stomiidae	*Chauliodus sloani*	114
		Plesionika martia				*Stomias boa*	

	Pasiphaeidae	*Pasiphaea multidentata*	56		Trachichthyidae	*Hoplostethus mediterraneus*	116
		Pasiphaea sivado			Trachyrincidae	*Trachyrincus scabrus*	117
	Penaeidae	*Parapenaeus longirostris*	58		Trichiuridae	*Lepidopus caudatus*	118
	Polychelidae	*Polycheles typhlops*	59		Triglidae	*Chelidonichthys cuculus*	119
	Sergestidae	*Eusergestes arcticus*	60			*Lepidotrigla cavillone*	
		Parasergestes vigilax				*Trigla lyra*	

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^{-2} (± 0.25 n. objects·km^{-2}) in each sampling site. Total fishing effort was 54.47 hours, with a mean of 2.27 (± 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 (Figure 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 (Figure 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 (Figure 3).

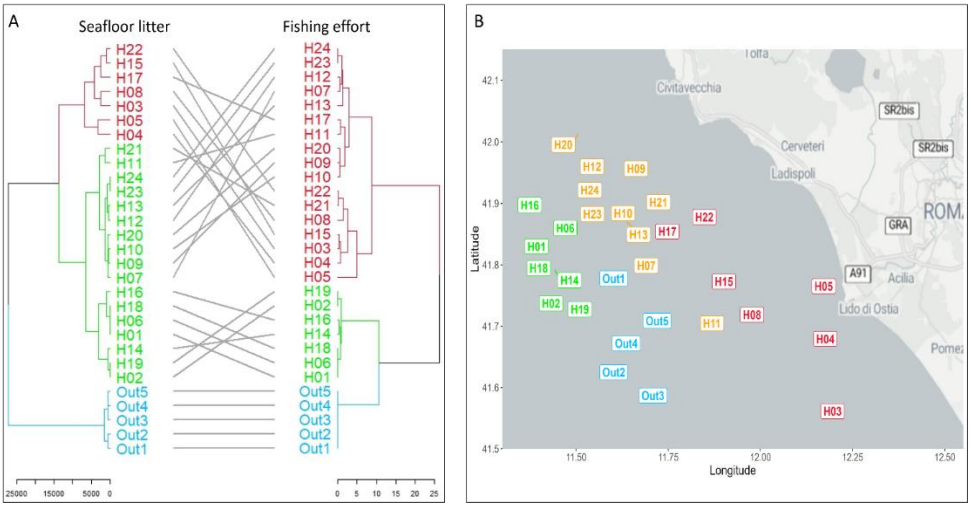


Figure 2 Results of the cluster analyses, which categorized sampling locations according to fishing effort (h) and seafloor litter (Number of objects · km^{-2}) (A). Map of the combined classifications (B). *blue:* outgroup sites; *green:* low pressures; *red:* high pressures; *orange:* low litter – high effort.

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 to the cluster of sampling sites with low litter and low effort, Epizoanthidae, Peristediidae, and Funiculinidae are strongly related to the sampling sites with low litter and high effort, and Ascidiidae and Luidiidae are related to the group of sampling sites with high litter and high effort.

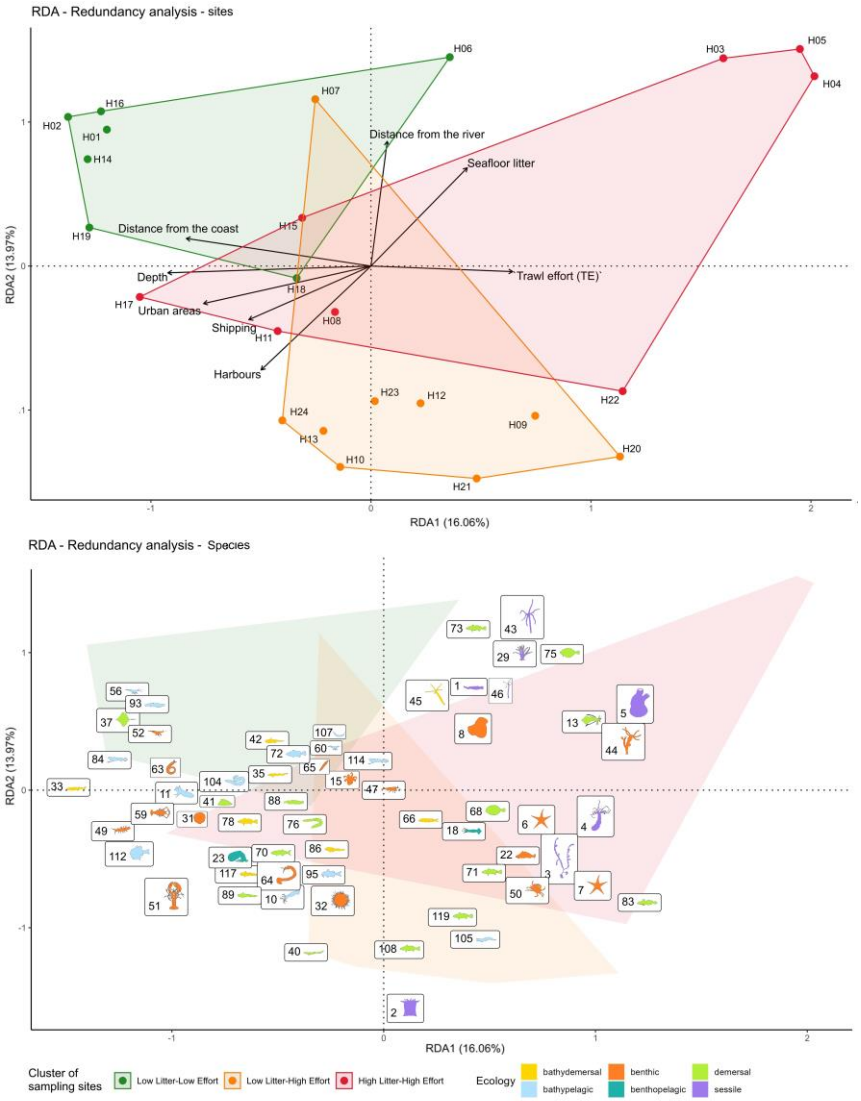


Figure 3 Redundancy Analysis result indicating the associations between environmental features and anthropogenic pressures, sampling sites, and the distribution of the taxonomic groups found in the central Tyrrhenian Sea. The coloured polygons enclose the sites belonging to the respective cluster group. The species are represented as centroids according to the respective families (Table 1 provides a list of their code numbers).

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	*Mora moro*		0.727	0.018*
Segestidae	*Eusergestes arcticus* *Parasergestes vigilax* *Robustosergia robusta*		0.715	0.039*
Low Litter - High Effort				
Epizoanthidae	*Epizoanthus arenaceus*		0.754	0.008**
Peristediidae	*Peristedion cataphractum*		0.714	0.018*
Funiculinidae	*Funiculina quadrangularis*		0.678	0.013*
High Litter - High Effort				
Ascidiidae	*Ascidia spp.*		0.728	0.009**
Luidiidae	*Luidia sarsi*		0.678	0.003**

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, encompassing a wide spectrum of organisms, ranging 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. Seafloor litter clusters reflect expected trends of litter deposits along the Italian coast (Scotti et al., 2021). This classification, which included both low and high impact of litter, represents a fundamental starting point for understanding the spatial distribution patterns of marine litter and thus facilitates the discrimination of areas characterised by distinct levels of accumulation. As observed in the map, high-impact sampling sites were in front of the Tiber River mouth. This is also supported by the RDA analysis, which showed a strong relation between the variables representing river and seafloor litter. Rivers are one of the main sources of litter and outflows and discharged debris significantly affects the water mass and the facing seabed (Atwood et al., 2019; Campanale et al., 2020; Crosti et al., 2018c; Noce et al., 2013; Poeta et al., 2016; Rech et al., 2014; Sbrana, Valente, Scacco, Bianchi, Silvestri, Palazzo, de Lucia, et al., 2020). Fishing effort followed a decreasing gradient from coast to offshore depending on both bathymetry and distance from the coast. Our analysis evidenced that the bulk of fishing activities are located close to the coast, while only a few vessels went offshore to deep water, realistically due to several factors. First, the proximity to the coast may provide easier and cheaper access to fishery resources, or it could be the result of legal or regulatory restrictions that limit access to more remote areas (i.e., Regulation (EU) 2019/1241 of the European Parliament and of the Council of 20 June 2019 on the conservation of fisheries resources and the protection of marine ecosystems through technical measures). Furthermore, the fishing effort may be 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).

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 discharge from the Tiber River. 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 showed the connection between 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 Loliginidae 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; 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, primarily influenced the faunal groups that were characterized by differentiated connections with the substrate and motility modes. They were bathypelagic fishes (e.g., Mictophydae, Sternoptychidae), and the cephalopods of the family Enoploteuthidae

and Histioteuthidae, which are swimming with high movement capacity and also bathydemersal fishes (e.g., some Chondrichthyes such as Dalatiidae, Lophidae, and Trachirincidae), which inhabit deep-seabed and are discretely motile. Being a remarkable part of the assemblages of the bathyal plain of the Mediterranean Sea (Carlucci et al., 2018; Follesa et al., 2011), the distribution of these organisms was mainly affected by the depth and greatly associated with the distance from the coast especially in the case of deeper species. Finally, confirming the Redundancy Analysis, the Indicator Species Analysis highlighted a significant correlation between the offshore sampling sites subjected to low litter – low effort and two bathypelagic family 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 impact of trawling assessment. 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, which are 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, Triglidae and Gobidae, which 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. They probably 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., s 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.

Regarding the physical disturbance by litter on the seafloor associated with fishing pressures (high litter – high effort), two species were significantly associated with it: *Ascidia spp.* (Ascidiidae) and *Luidia sarsi* (Luidiidae), which were typically found on the seafloor with high litter accumulation and high fishing effort due to their ecological preferences and behaviour. *Ascidia spp.* is often associated with biofouling of seafloor litter (Ramalhosa et al., 2021; Subías-Baratau et al., 2022),being filter-feeding invertebrates that commonly thrive in areas with high concentrations of organic matter and detritus, which often accompany the accumulation of litter. The sea star *Luidia sarsi* is known to inhabit the seafloor where there is a rich supply of food, including discarded and decaying matter, which is abundant in the presence of litter accumulation (Koukouras and Kitsos 2010). Additionally, the sites subjected to high litter - high effort may attract these species because they offer abundant potential food items as well as by-catch provided by fishing (Lejeune et al., 2023). Based on the idea that non-random distributions of species co-occurrence constitute the fundamental components of ecological communities (Tulloch et al., 2018), the presence of different species in the same area depends on both their environmental requirements and biological interactions, such as competition, mutualism and predation. Therefore, some taxa coexist more often and others less often than expected by chance. Our results, provide the identification of significant non-random

associations as evidence of potential ecological relationships between taxonomic groups, especially in the context of different environmental conditions, such as various levels and types of disturbance. The evidence from the indicator species analysis in the present study supports the hypothesis that most of the species showed special adaptations to litter and trawling effort, according to their specific relation with the substratum and motility mode.

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, depending on two major pressures: fishing effort and the seafloor litter, a different degree of variation resulted in the species composition of the assemblages of the investigated sites. The species' adaptive life traits have played a significant role in elucidating the fauna's reactions, primarily associated with their relationship with the 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 mobile species relocating when needed, sessile species with flexible stalks bending without breaking, or epibiotic species relying on passive transport to move. Conversely, certain species thrive in the most affected regions, capitalizing on the favourable conditions and consistent food sources available in these areas.

In conclusion, the provided results offer a comprehensive overview of the distribution and impact of seafloor litter and fishing pressures 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.

Supplementary material

Table 1 List of total taxa identified through eDNA metabarcoding (COI and 12S) detection, to investigate the relationship within the species community in the central Tyrrhenian Sea (Lazio region).

Class	Family	Species	Class	Family	Species
Anthozoa	Alcyonidiidae	*Alcyonium acaule*	Cephalopoda	Sepiolidae	*Rossia macrosoma*
Anthozoa	Epizoanthidae	*Epizoanthus arenaceus*	Cephalopoda	Sepiolidae	*Sepietta oweniana*
Anthozoa	Funiculinidae	*Funiculina quadrangularis*	Cephalopoda	Sepiolidae	*Stoloteuthis leucoptera*
Anthozoa	Gonactiniidae	*Gonactinia prolifera*	Chondrichthyes	Centrophoridae	*Centrophorus granulosus*
Anthozoa	Pennatulidae	*Pennatula phosphorea*	Chondrichthyes	Chimaeridae	*Chimaera monstrosa*
Anthozoa	Sagartiidae	*Sagartiogeton spp.*	Chondrichthyes	Dalatiidae	*Dalatias licha*
Ascidiacea	Ascidiidae	*Ascidia spp.*	Chondrichthyes	Dasyatidae	*Pteroplatytrygon violacea*
Asteroidea	Astropectinidae	*Astropecten irregularis*	Chondrichthyes	Etmopteridae	*Etmopterus spinax*
Asteroidea	Luidiidae	*Luidia sarsi*	Chondrichthyes	Hexanchidae	*Hexanchus griseus*
Bacillariophyceae	Bacillariaceae	*Cylindrotheca closterium*	Chondrichthyes	Rajidae	*Dipturus oxyrinchus*
Bacillariophyceae	Pleurosigmataceae	*Pleurosigma sp.*	Chondrichthyes	Rajidae	*Leucoraja circularis*
Bivalvia	Pectinidae	*Pseudamussium clavatum*	Chondrichthyes	Rajidae	*Raja asterias*
Bivalvia	Teredinidae	*Psiloteredo megotara*	Chondrichthyes	Rajidae	*Raja clavata*
Bivalvia	Veneridae	*Chamelea gallina*	Chondrichthyes	Scyliorhinidae	*Galeus melastomus*
Branchiopoda	Podonidae	*Evadne spinifera*	Chondrichthyes	Scyliorhinidae	*Scyliorhinus canicula*
Branchiopoda	Sididae	*Penilia avirostris*	Chondrichthyes	Scyliorhinidae	*Scyliorhinus stellaris*
Cephalopoda	Argonautidae	*Argonauta argo*	Coscinodiscophyceae	Chaetocerotaceae	*Chaetoceros socialis*
Cephalopoda	Brachioteuthidae	*Brachioteuthis riisei*	Coscinodiscophyceae	Skeletonemataceae	*Skeletonema menzelii*
Cephalopoda	Chtenopterygidae	*Chtenopteryx sicula*	Coscinodiscophyceae	Stephanodiscaceae	*Cyclotella cryptica*
Cephalopoda	Enoploteuthidae	*Abralia veranyi*	Crinoidea	Antedonidae	*Antedon mediterranea*
Cephalopoda	Enoploteuthidae	*Abraliopsis morisii*	Crinoidea	Antedonidae	*Leptometra phalangium*

Cephalopoda	Histioteuthidae	*Histioteuthis bonnellii*	Demospongiae	Chalinidae	*Haliclona sp.*
Cephalopoda	Histioteuthidae	*Histioteuthis reversa*	Echinoidea	Brissidae	*Brissopsis lyrifera*
Cephalopoda	Loliginidae	*Alloteuthis media*	Echinoidea	Echinidae	*Echinus acutus*
Cephalopoda	Loliginidae	*Alloteuthis subulata*	Echinoidea	Spatangidae	*Spatangus purpureus*
Cephalopoda	Loliginidae	*Loligo forbesii*	Gastropoda	Cavoliniidae	*Clio pyramidata*
Cephalopoda	Loliginidae	*Loligo vulgaris*	Gastropoda	Cocculinidae	*Coccopigya spp.*
Cephalopoda	Octopodidae	*Eledone cirrhosa*	Gastropoda	Creseidae	*Creseis acicula*
Cephalopoda	Octopodidae	*Octopus vulgaris*	Gastropoda	Creseidae	*Creseis virgula*
Cephalopoda	Octopodidae	*Pteroctopus tetracirrhus*	Gastropoda	Glaucidae	*Dondice banyulensis*
Cephalopoda	Octopodidae	*Scaeurgus unicirrhus*	Gastropoda	Scaphandridae	*Scaphander lignarius*
Cephalopoda	Ommastrephidae	*Illex coindetii*	Gymnolaemata	Bugulidae	*Bugula neritina*
Cephalopoda	Ommastrephidae	*Ommastrephes bartramii*	Hexanauplia	Calocalanidae	*Calocalanus contractus*
Cephalopoda	Ommastrephidae	*Todarodes sagittatus*	Hexanauplia	Canthocamptidae	*Mesochra pygmaea*
Cephalopoda	Ommastrephidae	*Todaropsis eblanae*	Hexanauplia	Centropagidae	*Isias clavipes*
Cephalopoda	Onychoteuthidae	*Onychoteuthis banksii*	Hexanauplia	Clausocalanidae	*Clausocalanus arcuicornis*
Cephalopoda	Sepiidae	*Sepia elegans*	Hexanauplia	Clausocalanidae	*Clausocalanus furcatus*
Cephalopoda	Sepiolidae	*Heteroteuthis dispar*	Hexanauplia	Clausocalanidae	*Clausocalanus mastigophorus*
Cephalopoda	Sepiolidae	*Neorossia caroli*	Hexanauplia	Clausocalanidae	*Clausocalanus paululus*
Cephalopoda	Sepiolidae	*Rondeletiola minor*	Hexanauplia	Clausocalanidae	*Clausocalanus pergens*
Hexanauplia	Heterorhabdidae	*Heterorhabdus papilliger*	Hydrozoa	Tubulariidae	*Ectopleura dumortierii*
Hexanauplia	Metridinidae	*Pleuromamma gracilis*	Malacostraca	Alpheidae	*Alpheus glaber*
Hexanauplia	Oncaeidae	*Oncaea scottodicarloi*	Malacostraca	Aristeidae	*Aristeus antennatus*
Hexanauplia	Paracalanidae	*Paracalanus spp.*	Malacostraca	Aristeidae	*Aristeus virilis*
Hexanauplia	Scalpellidae	*Scalpellum scalpellum*	Malacostraca	Benthesicymidae	*Gennadas elegans*
Hexanauplia	Temoridae	*Temora stylifera*	Malacostraca	Brachyscelidae	*Brachyscelus sp.*

Holothuroidea	Synallactidae	*Mesothuria intestinalis*	Malacostraca	Cirolanidae	*Natatolana borealis*
Hydrozoa	Abylidae	*Abylopsis tetragona*	Malacostraca	Diogenidae	*Dardanus arrosor*
Hydrozoa	Aequoreidae	*Aequorea forskalea*	Malacostraca	Dorippidae	*Medorippe lanata*
Hydrozoa	Agalmatidae	*Halistemma rubrum*	Malacostraca	Euphausiidae	*Euphausia hemigibba*
Hydrozoa	Aglaopheniidae	*Lytocarpia myriophyllum*	Malacostraca	Euphausiidae	*Euphausia krohni*
Hydrozoa	Bougainvilliidae	*Bougainvillia muscus*	Malacostraca	Euphausiidae	*Meganyctiphanes norvegica*
Hydrozoa	Bougainvilliidae	*Lizzia fulgurans*	Malacostraca	Euphausiidae	*Nematoscelis megalops*
Hydrozoa	Campanulariidae	*Eucheilota maculata*	Malacostraca	Euphausiidae	*Nyctiphanes couchi*
Hydrozoa	Clytiidae	*Clytia gracilis*	Malacostraca	Euphausiidae	*Thysanopoda aequalis*
Hydrozoa	Clytiidae	*Clytia hemisphaerica*	Malacostraca	Geryonidae	*Geryon longipes*
Hydrozoa	Cuninidae	*Solmissus marshalli*	Malacostraca	Goneplacidae	*Goneplax rhomboides*
Hydrozoa	Diphyidae	*Chelophyes appendiculata*	Malacostraca	Inachidae	*Inachus dorsettensis*
Hydrozoa	Diphyidae	*Lensia achilles*	Malacostraca	Latreilliidae	*Latreillia elegans*
Hydrozoa	Eirenidae	*Helgicirrha sp.*	Malacostraca	Leucosiidae	*Ebalia nux*
Hydrozoa	Forskaliidae	*Forskalia asymmetrica*	Malacostraca	Munididae	*Munida intermedia*
Hydrozoa	Forskaliidae	*Forskalia edwardsii*	Malacostraca	Munididae	*Munida speciosa*
Hydrozoa	Geryoniidae	*Geryonia proboscidalis*	Malacostraca	Nephropidae	*Nephrops norvegicus*
Hydrozoa	Geryoniidae	*Liriope tetraphylla*	Malacostraca	Oplophoridae	*Acanthephyra pelagica*
Hydrozoa	Laodiceidae	*Laodicea undulata*	Malacostraca	Paguridae	*Pagurus alatus*
Hydrozoa	Mitrocomidae	*Halopsis ocellata*	Malacostraca	Pandalidae	*Chlorotocus crassicornis*
Hydrozoa	Obeliidae	*Obelia sp.*	Malacostraca	Pandalidae	*Plesionika acanthonotus*
Hydrozoa	Oceaniidae	*Oceania armata*	Malacostraca	Pandalidae	*Plesionika antigai*
Hydrozoa	Pandeidae	*Leuckartiara octona*	Malacostraca	Pandalidae	*Plesionika edwardsii*
Hydrozoa	Plumulariidae	*Nemertesia ramosa*	Malacostraca	Pandalidae	*Plesionika heterocarpus*
Hydrozoa	Porpitidae	*Porpita porpita*	Malacostraca	Pandalidae	*Plesionika martia*

Hydrozoa	Prayidae	*Rosacea sp.*	Malacostraca	Parthenopidae	*Spinolambrus spp.*
Hydrozoa	Rathkeidae	*Podocorynoides minima*	Malacostraca	Pasiphaeidae	*Pasiphaea multidentata*
Hydrozoa	Rhizophysidae	*Rhizophysa filiformis*	Malacostraca	Pasiphaeidae	*Pasiphaea sivado*
Hydrozoa	Rhopalonematidae	*Aglaura hemistoma*	Malacostraca	Penaeidae	*Parapenaeus longirostris*
Hydrozoa	Rhopalonematidae	*Rhopalonema velatum*	Malacostraca	Phronimidae	*Phronima sedentaria*
Hydrozoa	Sphaeronectidae	*Sphaeronectes koellikeri*	Malacostraca	Phrosinidae	*Phrosina semilunata*
Hydrozoa	Tetraplatiidae	*Tetraplatia volitans*	Malacostraca	Polybiidae	*Liocarcinus vernalis*
Malacostraca	Polychelidae	*Polycheles typhlops*	Teleostei	Argentinidae	*Glossanodon leioglossus*
Malacostraca	Portunidae	*Macropipus tuberculatus*	Teleostei	Aulopidae	*Aulopus filamentosus*
Malacostraca	Processidae	*Processa nouveli*	Teleostei	Blenniidae	*Blennius ocellaris*
Malacostraca	Scyllaridae	*Scyllarus arctus*	Teleostei	Bothidae	*Arnoglossus laterna*
Malacostraca	Scyllaridae	*Scyllarus pygmaeus*	Teleostei	Bothidae	*Arnoglossus rueppelii*
Malacostraca	Sergestidae	*Allosergestes sargassi*	Teleostei	Callionymidae	*Callionymus maculatus*
Malacostraca	Sergestidae	*Eusergestes arcticus*	Teleostei	Callionymidae	*Synchiropus phaeton*
Malacostraca	Sergestidae	*Parasergestes vigilax*	Teleostei	Caproidae	*Capros aper*
Malacostraca	Sergestidae	*Robustosergia robusta*	Teleostei	Carangidae	*Trachurus mediterraneus*
Malacostraca	Solenoceridae	*Solenocera membranacea*	Teleostei	Carangidae	*Trachurus trachurus*
Malacostraca	Squillidae	*Squilla mantis*	Teleostei	Centracanthidae	*Spicara maena*
Malacostraca	Xanthidae	*Monodaeus couchii*	Teleostei	Centracanthidae	*Spicara smaris*
Mammalia	Delphinidae	*Delphinus delphis*	Teleostei	Centriscidae	*Macrorhamphosus scolopax*
Mammalia	Delphinidae	*Stenella coeruleoalba*	Teleostei	Centrolophidae	*Centrolophus niger*
Ophiuroidea	Ophiuridae	*Ophiura ophiura*	Teleostei	Cepolidae	*Cepola macrophthalma*
Ostracoda	Halocyprididae	*Porroecia spinirostris*	Teleostei	Chlorophthalmidae	*Chlorophthalmus agassizi*
Ostracoda	Halocyprididae	*Proceroecia procera*	Teleostei	Citharidae	*Citharus linguatula*

Palaeonemertea	Cephalothricidae	*Cephalothrix linearis*	Teleostei	Clupeidae	*Alosa fallax*
Phaeophyceae	Ectocarpaceae	*Ectocarpus sp.*	Teleostei	Clupeidae	*Sardina pilchardus*
Polychaeta	Acoetidae	*Panthalis oerstedi*	Teleostei	Clupeidae	*Sardinella aurita*
Polychaeta	Ampharetidae	*Sosane sulcata*	Teleostei	Congridae	*Ariosoma balearicum*
Polychaeta	Dorvilleidae	*Ophryotrocha spp.*	Teleostei	Congridae	*Conger conger*
Polychaeta	Nephtyidae	*Aglaophamus rebellus*	Teleostei	Congridae	*Gnathophis mystax*
Polychaeta	Nephtyidae	*Nephtys hystricis*	Teleostei	Engraulidae	*Engraulis encrasicolus*
Polychaeta	Onuphidae	*Paradiopatra calliopae*	Teleostei	Epigonidae	*Epigonus denticulatus*
Polychaeta	Sigalionidae	*Labioleanira yhleni*	Teleostei	Epigonidae	*Epigonus telescopus*
Polychaeta	Sphaerodoridae	*Ephesiella cantonei*	Teleostei	Evermannellidae	*Evermannella balbo*
Polychaeta	Spionidae	*Laonice cirrata*	Teleostei	Exocoetidae	*Cheilopogon heterurus*
Polychaeta	Spionidae	*Prionospio spp.*	Teleostei	Gadidae	*Gadiculus argenteus*
Polychaeta	Sternaspidae	*Sternaspis scutata*	Teleostei	Gadidae	*Micromesistius poutassou*
Polychaeta	Terebellidae	*Pista spp.*	Teleostei	Gadidae	*Trisopterus capelanus*
Polychaeta	Trichobranchidae	*Trichobranchus glacialis*	Teleostei	Gobiidae	*Crystallogobius linearis*
Sagittoidea	Sagittidae	*Pseudosagitta lyra*	Teleostei	Gobiidae	*Lesueurigobius friesii*
Scaphopoda	Entalinidae	*Entalina tetragona*	Teleostei	Gobiidae	*Lesueurigobius suerii*
Scyphozoa	Nausithoidae	*Nausithoe spp.*	Teleostei	Gobiidae	*Pomatoschistus minutus*
Scyphozoa	Pelagiidae	*Pelagia noctiluca*	Teleostei	Gonostomatidae	*Cyclothone braueri*
Teleostei	Alepisauridae	*Arctozenus risso*	Teleostei	Gonostomatidae	*Cyclothone microdon*
Teleostei	Argentinidae	*Argentina sphyraena*	Teleostei	Gonostomatidae	*Gonostoma denudatum*
Teleostei	Labridae	*Acantholabrus palloni*	Teleostei	Phosichthyidae	*Vinciguerria poweriae*
Teleostei	Lophiidae	*Lophius budegassa*	Teleostei	Phycidae	*Phycis blennoides*
Teleostei	Lophiidae	*Lophius piscatorius*	Teleostei	Phycidae	*Phycis phycis*
Teleostei	Lotidae	*Molva molva*	Teleostei	Scombridae	*Auxis rochei*
Teleostei	Macrouridae	*Coelorinchus caelorhincus*	Teleostei	Scombridae	*Scomber colias*

Teleostei	Macrouridae	*Hymenocephalus italicus*	Teleostei	Scombridae	*Scomber scombrus*
Teleostei	Macrouridae	*Nezumia sclerorhynchus*	Teleostei	Scombridae	*Thunnus thynnus*
Teleostei	Macrouridae	*Trachyrincus scabrus*	Teleostei	Scophthalmidae	*Lepidorhombus boscii*
Teleostei	Merlucciidae	*Merluccius merluccius*	Teleostei	Scophthalmidae	*Lepidorhombus whiffiagonis*
Teleostei	Molidae	*Mola mola*	Teleostei	Scorpaenidae	*Scorpaena notata*
Teleostei	Moridae	*Mora moro*	Teleostei	Scorpaenidae	*Scorpaena scrofa*
Teleostei	Mullidae	*Mullus barbatus*	Teleostei	Sebastidae	*Helicolenus dactylopterus*
Teleostei	Myctophidae	*Benthosema glaciale*	Teleostei	Serranidae	*Serranus cabrilla*
Teleostei	Myctophidae	*Ceratoscopelus maderensis*	Teleostei	Serranidae	*Serranus hepatus*
Teleostei	Myctophidae	*Diaphus holti*	Teleostei	Sparidae	*Boops boops*
Teleostei	Myctophidae	*Diaphus metopoclampus*	Teleostei	Sparidae	*Diplodus annularis*
Teleostei	Myctophidae	*Diaphus rafinesquii*	Teleostei	Sparidae	*Pagellus acarne*
Teleostei	Myctophidae	*Electrona risso*	Teleostei	Sparidae	*Pagellus bogaraveo*
Teleostei	Myctophidae	*Hygophum benoiti*	Teleostei	Sparidae	*Pagellus erythrinus*
Teleostei	Myctophidae	*Hygophum hygomii*	Teleostei	Sparidae	*Pagrus pagrus*
Teleostei	Myctophidae	*Lampanyctus crocodilus*	Teleostei	Sternoptychidae	*Argyropelecus hemigymnus*
Teleostei	Myctophidae	*Lobianchia dofleini*	Teleostei	Sternoptychidae	*Maurolicus muelleri*
Teleostei	Myctophidae	*Myctophum punctatum*	Teleostei	Stomiidae	*Chauliodus sloani*
Teleostei	Myctophidae	*Notoscopelus elongatus*	Teleostei	Stomiidae	*Stomias boa*
Teleostei	Nemichthyidae	*Nemichthys scolopaceus*	Teleostei	Syngnathidae	*Syngnathus spp.*
Teleostei	Nettastomatidae	*Facciolella oxyrhyncha*	Teleostei	Trachichthyidae	*Hoplostethus mediterraneus*
Teleostei	Nettastomatidae	*Nettastoma melanurum*	Teleostei	Trichiuridae	*Lepidopus caudatus*
Teleostei	Notacanthidae	*Notacanthus bonaparte*	Teleostei	Triglidae	*Chelidonichthys cuculus*
Teleostei	Ophichthidae	*Dalophis imberbis*	Teleostei	Triglidae	*Chelidonichthys lucerna*

Teleostei	Ophichthidae	*Echelus myrus*	Teleostei	Triglidae	*Lepidotrigla cavillone*
Teleostei	Paralepididae	*Paralepis coregonoides*	Teleostei	Triglidae	*Lepidotrigla dieuzeidei*
Teleostei	Paralepididae	*Sudis hyalina*	Teleostei	Triglidae	*Trigla lyra*
Teleostei	Peristediidae	*Peristedion cataphractum*	Teleostei	Xiphiidae	*Xiphias gladius*
Teleostei	Phosichthyidae	*Ichthyococcus ovatus*	Teleostei	Zeidae	*Zeus faber*
Teleostei	Phosichthyidae	*Ichthyococcus ovatus*	Tentaculata	Leucotheidae	*Leucothea multicornis*
Teleostei	Phosichthyidae	*Vinciguerria attenuata*			
Teleostei	Phosichthyidae	*Vinciguerria attenuata*			
Teleostei	Phosichthyidae	*Vinciguerria poweriae*			

Chapter 4 - The cost of waste: overlap analysis of plastic concentrations on the seafloor and commercially important species reveals potential loss of economic productivity due to marine litter accumulation.

Abstract

Seabed plastic pollution represents a serious threat to marine biodiversity and ecosystem functioning by damaging marine organisms and disrupting ecosystems. Litter accumulation zones may overlap with fishing grounds for commercially important species, reducing productivity and yield. As no studies have been conducted on this topic, there is an urgent need to fill the knowledge gap on the impact of plastic pollution on fisheries and stock management. For these reasons, the aim of this study was to analyze the impact of seafloor plastic on fishing economic performance (as Gross Values Added) and on commercially important species by mapping trawl areas and identifying litter hotspots on the seafloor. A model based on landing and Vessel Monitoring System data was employed to estimate the fishing grounds of the species, and a random forest machine-learning technique was used to identify seafloor litter hotspots. The findings demonstrate that seafloor plastic hotspots overlap with the fishing grounds, thus having a negative impact on economic productivity. The implications of this problem pose a significant threat of exposure and impact to certain species. Our findings indicate that seabed plastic pollution should be recognized as a factor affecting fisheries administration and conservation approaches.

Keywords: Seafloor litter; Fishing ground; Risk assessment; Fisheries; Gross Value Added

Introduction

Seafloor biodiversity is the diversity of life forms that inhabit the bottom of the oceans, from the shallow coastal areas to the deep abyssal plains, and supports several ecosystem services including the production of seafood through fishing activities. In this way, seafloor biodiversity plays a crucial role in maintaining the health and functioning of marine ecosystems, as well as providing valuable services and resources for human well-being (Beauchard et al., 2023). However, seafloor biodiversity is directly or indirectly threatened by various human activities, such as fishing, pollution, and climate change. Among these, bottom trawling is one of the most widespread and destructive practices, as it involves dragging heavy nets or gears along the seafloor, scraping and plowing the sediment, and removing or damaging the benthic organisms and habitats (Pusceddu et al., 2014). Bottom trawling not only reduces the biomass and diversity of seafloor communities but also alters the physical and chemical properties of the sediment, affecting the biogeochemical cycles and the sequestration of carbon and nutrients (Bradshaw et al., 2021). Moreover, bottom trawling contributes to the accumulation of litter on the seafloor, as fishing gears often lose or discard plastic and metal items, such as nets, ropes, hooks, cans, and bottles (Canals et al., 2021). Seafloor litter pollution, which is determined by different human activities including waste dispersal and fishing, has been observed to have a negative impact on marine fisheries worldwide, affecting them both directly and indirectly. For instance, the presence of marine litter in fishing nets can reduce their catch efficiency, cause damage, reduce the time available for fishing, inflate repair expenses, obstruct equipment, and necessitate increased fuel (Beaumont et al., 2019; Ivar Do Sul & Costa, 2014; Mghili et al., 2023). Additionally, fisheries target species, along with their prey, are at risk of both lethal and sub-lethal harm due to plastic pollution, including reduced reproductive success and growth limitations, with the possibility of broader impacts at the population level (Galloway & Lewis, 2017). Finally, trawling can also redistribute the litter across different depths and habitats, increasing its exposure to marine organisms (Franceschini et al., 2019). When combined with other major concerns like overfishing and climate change, marine plastic pollution could

have a profoundly negative impact on the productivity, sustainability, profitability, and safety of the fishing and aquaculture industries (Beaumont et al., 2019). Despite the growing awareness and concern about the effects of bottom trawling and seafloor litter, there are no studies on the effects of litter pollution on stocks exploited by fishing, and closing the knowledge gap would be a critical task. Although nowadays many modeling approaches to perform spatial and temporal analyses of fisheries dynamics are available and, in the meantime, the direct effects of plastics presence (e.g. ingestion and reduction of growth rates) have been documented, no study addressed the potential effects of marine litter accumulation on fishing ground and on the bio-economic performances of related fisheries. Furthermore, there is a need to develop and implement effective and innovative solutions to prevent, reduce, and manage the impacts of bottom trawling and seafloor litter on biodiversity, involving multiple stakeholders, such as fishers, managers, policy makers, scientists, and civil society. The assessment and evaluation of management strategy for plastic pollution require the development of a conceptual framework that comprises the probability of exposure and its impact and aims to balance the environmental, social, and economic aspects of the marine sector (Hardesty et al., 2019). For these reasons, it is important to elucidate the sources, distribution, and impacts in the environment from a systems perspective (Hardesty et al., 2019) to understand the risks posed by marine debris (Hardesty & Wilcox, 2017). The application of risk assessment can clarify the species most vulnerable to risk and identify areas of highest concern. Risk assessments frequently represent the first stage in the development of pollution regulations, improved resource management, and policies designed to preserve the environment and public health. To date, various research and studies have addressed the topic of risk assessment for plastic impact using a combination of numerical models, ingestion studies, and species distribution ranges (Compa et al., 2019), but very often these data are incomplete or fragmented. A functional approach to the development of a risk assessment at sea could include the integration of fishing data, which can give us a lot of information on the distribution of species of commercial interest, but also on aggregation areas, which very often overlap with the feeding habits of the species.

For these reasons, the main objectives of this work were to 1) identify the effects (if any) of plastic debris accumulation on demersal fishing grounds, and 2) assess the risk of exposure of demersal stocks to plastic debris accumulation using a spatial approach. The issues are addressed through a statistical analysis of the distribution of seafloor litter together with fisheries-related metrics (landings per unit of effort. fishing effort, landings, costs, and related Gross Value Added - GVA). GVA is an important figure for fisheries policy and decision-makers (Carvalho et al., 2020). It shows the return of the fishing vessel operations to the economy, as the revenue left to compensate for labor and capital. Assuming that GVA is a good proxy of the economic performance of a fishery and using the obtained model of the relationship between GVA and the amount of plastic litter, a risk assessment spatial analysis is carried out to inform fishers, managers, and researchers about the demersal resources mainly hampered by plastic litter accumulation and to indicate possible future investigations on the field.

Material and methods

The research area is situated in the central Tyrrhenian Sea (FAO Geographical Sub Area 9 – Western Mediterranean Sea), along the coast of the Lazio region. The Tiber River has a significant impact on the continental shelf of this area (Inghilesi et al., 2008). Indeed, the physical, chemical, and trophic characteristics of this area are largely determined by the river supply (Noce et al., 2013). In addition, being close to Rome city and surrounded by large urban centers, industrial settlements, and important ports, the Tiber River runoff also brings contaminants such as plastic debris (Cesarini et al., 2023).

The study area was divided into individual spatial units using a grid of 1 square km, for a total of 3785 cells (Fig. 1). This grid was used to assess the distribution of marine litter on the seafloor and a set of biological and economic features related to the activity of the fishing fleet operating with bottom otter trawling in the area. Then, these features were cross-analyzed to identify a spatial relationship between the productivity of fishing areas and the accumulation of litter on the sea bottom and whether this relationship impacts the fishery from a bio-economic point of view.

Seafloor plastic data

The number of seafloor plastics (marine litter) occurring in each cell of the grid was retrieved using the model described in (Cau et al., in press), an application of Random Forests (RF) on the western and central Mediterranean Sea. These data available as shape files through the Mendeley Data platform (https://doi.org/10.17632/r2b6svy7h7.1), were used to estimate the aggregated spatial distribution of marine litter, as the number of objects · km^{-2}. 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 (Fiorentino et al., 2013). 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. The total number of objects collected per category (plastics, wood, metals, glass, rubber, clothing, and paper) and sub-category is then standardized according to the swept area.

Modelling of Fishing ground

The identification of fishing grounds for individual species was carried out through the SMART model (D'Andrea et al., 2020), a tool to reconstruct the spatial and temporal fluxes of landings coming from well-defined areas (the fishing grounds) and times to harbors to which they are delivered for sale (Russo et al., 2014, 2018). The spatial modeling of fishing effort was based on the analysis of Vessel Monitoring System (VMS) data, a remote tracking device that is mandatory for fishing vessels with length overall (LOA) ≥ 12 m in European waters (EC, 2011). The VMS data were combined with the corresponding information about landings acquired from fishing logbooks, where information on landings by species and harbors is stored by fishers (Gerritsen & Lordan, 2011). All these data have been collected within the Data Collection Framework for Fisheries (https://dcf.ec.europa.eu/index_en) and were provided by the Italian "Minister of Agriculture, Food Sovereignty and Forests". The processing of VMS followed these steps: at first, the VMS dataset of 558,278 records describing the position, speed, and routes of 97 bottom otter trawling (OTB) operating in the central Tyrrhenian Sea, between 2018 and 2020, was analyzed to assess fishing

effort based on vessel, cell, and time. VMS data were interpolated, and each fishing trip was reconstructed according to the procedures described in Russo et al., (2011a; b). The fishing set positions within each fishing trip were also identified and separated from other behaviors (steaming, resting) (Russo et al., 2011a; b). Subsequently, landings data were combined with the fishing set position of each vessel, at a monthly scale, to determine the spatial and temporal productivity of each cell (defined as the mean value for four distinct seasons: 1= January – March; 2= April – June; 3= July – September; 4= October – December), according to the modeling approach described in Russo et al., 2018 in which the productivity is defined as Landing per Unit of Effort (Kg of landing /m of length of the vessel/hours fishing).

The cross-analysis of VMS and Landings (Logbook) data ultimately allowed us to estimate the seasonal $LPUE_{c,t}$ for each cell *c* of the grid and for each season *t* for nine target species. These nine species (specifically: 3 crustaceans, 3 fishes, and 3 cephalopods), representing the most exploited ones and accounting for (on average) the Y % of the monthly landing of each vessel, were considered.

Bio-economic modelling

The SMART platform (D'Andrea et al., 2020), allows to estimate, for each fishing vessel and for each spatial unit and time:

1) The expected landings (L) in Kg per species, vessel, time and species as:

$$L_{c,t,s,v} = LPUE_{c,t,s} \times Effort_{c,t,v}$$

where $LPUE_{c,t,s}$ is the LPUE in cell *c* in season *t* of species *s* and $Effort_{c,t,v}$ is the fishing effort of vessel *v* in cell *c* in season *t*.

2) The expected landing value (LV) in Euros per species:

$$LV_{c,t,s,v} = L_{c,t,s,v} \times Price_s$$

where $Price_s$ is the price, in Euros per Kg, of species *s*;

3) The expected Fuel cost (FC in Euros) obtained as the sum of steaming and fishing times of vessels operating in the cell *c* and season *t* considered, and obtained as:

$$FC_{c,t} = \sum_{v=1}^{V} Cost\ Effort_{v,c,t} + Cost\ Steaming_{v,c,t}$$

where $Cost\ Effort_{v,c,t}$ is the product of the Fuel consumption of the vessel during its fishing activity by the price of fuel (in Euros/liter) and $Cost\ Steamng_{v,c,t}$ is the product of the Fuel consumption of the vessel during its steaming activity by the price of fuel (in Euros/liter). The parameters for fuel consumption (during steaming and fishing phases, respectively) of Italian trawlers targeting demersal species are available in Sala et al., 2022.

4) The expected Additional cost (AC in Euros), defined as:

$$AC_{c,t} = \sum_{v=1}^{V} Fixed\ cost_{v,c,t} + Variable\ Cost_{v,c,t}$$

where $Fixed\ cost_{v,c,t}$ are the maintenance of vessel and gear and insurance for cell *c* in season *t*; while $Variable\ Cost_{v,c,t}$ are smaller items summarized as miscellaneous other costs for cell *c* in season *t.*

5) Data obtained were used to calculate the Gross Value Added (GVA in Euros) in cell *c*, defined as:

$$GVA_c = LV_{c,t,s,v} - \left(FC_{c,t} + AC_{c,t}\right)$$

The goodness-of-fit of the bioeconomic model returned by SMART was tested by comparing the seasonal landings values predicted by the model for each vessel/species with those observed (goodness-of-fit). The values returned by the SMART application for economic parameters (landing values, costs, GVA) were compared with the official values available for the fishing segments in the Annual Economic Report of the European Commission.

Generalized Additive Models (GAM)

A Generalized Additive Models (GAMs) with a Gaussian distribution was used to investigate the correlation between fishing economic performance (GVA) (response variable) as a function of seafloor litter hotspots, fishing effort and seasonality (explanatory variables). GAMs are non-parametric regression techniques that allow to model the relationships between variables without specifying any particular form for the underlying regression function. The use of smooth functions as regressors gives GAMs greater flexibility over linear (or other parametric) types of models

(Hastie & Tibshirani, 1986). GAMs were chosen among other statistical techniques because they represent the best compromise between the model's predictive ability and its interpretability.

The results of the application of SMART as spatial/temporal LPUE, Fishing effort, Landings, Costs and GVA were graphically inspected. Then, the GAMs were used to fit a model in which the Seasonal GVA of the fleet are a function of fishing effort, LPUE for the species, amount of Seafloor litter and the Season. The model, in which the cells/season represent the statistical units, is:

$$P_{c,t} = \sum_{s=1}^{S} s\left(LPUE_{c,s,t}\right) + s(FE_{c,t}) + s(PL_c) + Season$$

where *S* are the species, $P_{c,t}$ is the GVA in cell *c* in season *t*, $LPUE_{c,s,t}$ is the LPUE of species *s* in the cell *c* during the season *t*, PLc is the amount of Plastic Litter in cell *c* as Number of objects. The function *s()* indicates the smoothed terms. With this model formulation, we wanted to investigate how the productivity of different fishing grounds (LPUE), their degree of exploitation (FE), and the degree of litter contamination (PL) interact in determining the GVA (i.e., economic performance) of the fleet. The reasoning behind this model is that GVA are the result of the combination of environmental productivity (captured by LPUEs, which are a proxy for the characteristics of each fishing ground), the distribution of fishing effort (i.e., the exploitation strategy applied by fishers), and the amount of plastic waste on the bottom. These three aspects, of course, are intertwined because LPUEs depend on fishing effort and pollution level but, in turn, fishing effort adjusts to the productivity of different fishing grounds and the distribution of litter also depends on fishing effort.

Risk assessment

Assuming that plastic on the seabed has a negative impact on demersal species' productivity, estimating potential damage risk requires comparing their productivity (LPUE) in different fishing grounds (particularly those significant for fishing that species) with hot spots of marine litter accumulation. Here, potential damage is

defined objectively as all negative interactions, whether direct (e.g. ingestion) or indirect (due to reduced resource availability), that may result from the presence of plastic litter on the seafloor.

Therefore, the LPUE distributions for each species and seafloor litter were analyzed to evaluate the potential exposure risk to plastic items on a scale of 0 (low risk) to 1 (high risk) for each species.

The potential exposure was assessed by multiplying the LPUE that was rescaled (i.e. within the range of 0-1) and the rescaled plastic debris field in a given cell, utilizing the subsequent formula:

$$\text{Risk of exposure} = LPUE \cdot Seafloor\ litter$$

These values were used to produce an Impact Score (IS) as an indicator of the probability that a species could be affected by the accumulation of waste on the seabed, as a proxy for impact risk. Therefore, the risk of exposure was divided by the number of total cells where the species was present (LPUE values > 0), as follows:

$$\text{IS} = \frac{Risk\ of\ exposure}{Total\ number\ of\ cells}$$

All the analyses were performed using R version 4.3.

Results

The spatial distribution map of seafloor litter by season highlighted the presence of plastic accumulation hotspots on the seabed, with mean values of 15659 number of objects · km^{-2}, and did not change among seasons. These are located mainly in the coastal area and have the highest values in the north of the Tiber River while decreasing offshore (Figure 1A). GVA had seasonal variations with a maximum peak of 2999.73 Euros in the autumn season (4; October - December) and in the winter season (1; January - March), mainly in the northern portion of the study area and offshore. While negative GVA values were recorded (up to -1212.55 Euros) for coastal cells in summer (3; July - September). Constant low productivity values (near

zero) were evident in all seasons in the southern part of the examined area (Figure 1B). Data used in the present study were summarized in Figure 1C-D and Table 1; we also reported fishing hours for the entire area (mean values: 3.35 h).

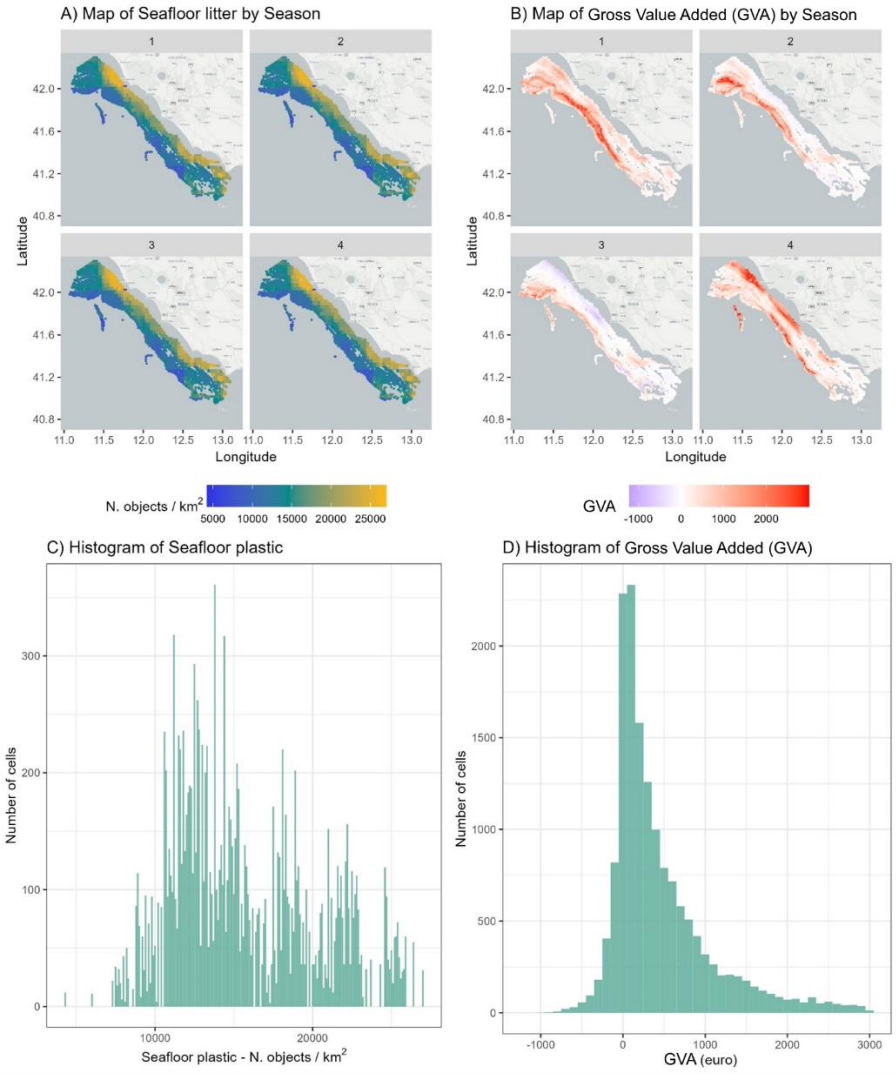


Figure 1 Map of seafloor litter (A) and Gross Value Added (GVA) (B) with the corresponding histograms (C-D) in the central Tyrrhenian Sea (GSA 9).

Table 1 Summary results of the spatial distribution of Gross Value Added (GVA) (Euros), seafloor litter (n · km^{-2}), and fishing hours (h) in the central Tyrrhenian Sea (GSA 9).

	GVA (Euros)	Seafloor plastic (n · km^{-2})	Fishing hours (h)
Min	-1212.55	4253	0.16
Mean	443.45	15659	3.35
Max	2999.73	27026	20.12

The LPUE for the most key commercial species of the selected area is represented in Figure 2. DPS had the highest values of LPUE with a mean abundance of 1.54 kg for cells. LPUE followed a very similar trend to GVA and is associated with high values in coastal areas for most of the species, while for shrimp species (ARA, ARS, and NEP) LPUE were highest in the deeper zone.

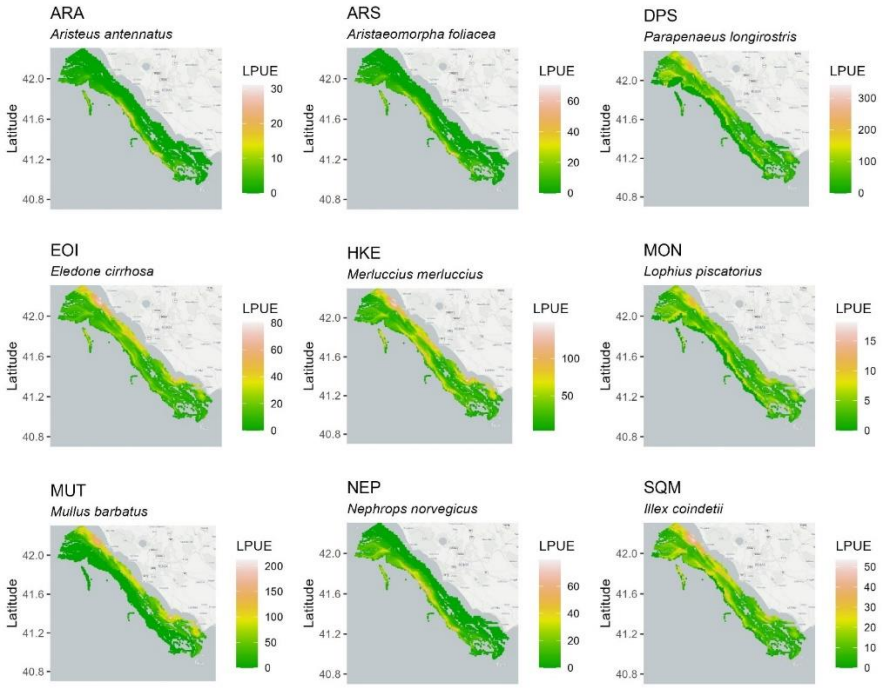


Figure 2 Map of landing (kg) per unit effort (LPUE) for 9 species that are crucial for trawling in the central Tyrrhenian Sea (GSA 9).

Table 2 Landing Per Unit of Effort (LPUE) recorded for 9 species that are crucial for trawling in the central Tyrrhenian Sea (GSA 9). LPUEs are expressed in kg.

Key commercial species	Mean	Std. Dev.	Min	Max
ARA - *Aristeus antennatus*	0.05	0.08	0.00	0.57
ARS - *Aristaeomorpha foliacea*	0.12	0.17	0.00	1.30
DPS - *Parapenaeus longirostris*	1.54	1.37	0.00	4.56
EOI – *Eledone cirrhosa*	0.38	0.17	0	0.71
HKE - *Merluccius merluccius*	0.94	0.42	0.26	1.67
MON - *Lophius piscatorius*	0.01	0.06	0.00	0.27
MUT - *Mullus barbatus*	0.54	0.59	0.00	1.86
NEP - *Nephrops norvegicus*	0.23	0.28	0.00	0.97
SQM – Illex coindetii	0.28	0.13	0	0.50

Table 2 Generalized Additive Model results used to identify the correlation between trawling Gross Value Added (GVA) (response variable) and seafloor litter hotspots (explanatory variable). The model incorporated fishing hours, species-specific LPUE (Landings Per Unit of Effort), and seasons as significant factors affecting economic productivity.

Component	Term	Estimate	Std Error	t-value	p-value	
A. parametric coefficients	GVA (Intercept)	7.270	0.004	1,684.140	0.0000	***
	Season	0.047	0.002	29.669	0.0000	***
Component	**Term**	**edf**	**Ref. df**	**F-value**	**p-value**	
B. smooth terms	s(Seafloor litter)	1.987	2.000	71.122	0.0000	***
	s(Fishing hours)	1.999	2.000	12,718.030	0.0000	***
	s(LPUE_ARA)	1.000	1.000	85.075	0.0000	***
	s(LPUE_ARS)	1.992	2.000	39.608	0.0000	***
	s(LPUE_BOG)	1.982	2.000	57.686	0.0000	***
	s(LPUE_DPS)	1.993	2.000	1,316.509	0.0000	***
	s(LPUE_HKE)	1.295	1.503	742.508	0.0000	***
	s(LPUE_MON)	1.998	2.000	249.106	0.0000	***
	s(LPUE_MTS)	1.996	2.000	1,152.179	0.0000	***
	s(LPUE_MUT)	1.999	2.000	524.409	0.0000	***
	s(LPUE_NEP)	1.994	2.000	2,701.895	0.0000	***

Signif. codes: 0 <= *** < 0.001 < ** < 0.01 < * < 0.05

Adjusted R-squared: 0.78, Deviance explained 0.78

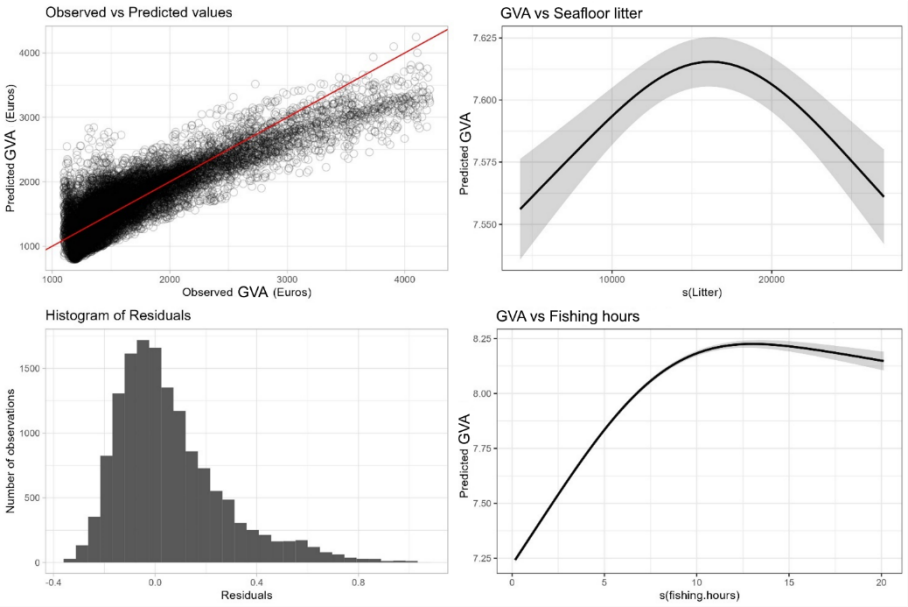


Figure 3 Summary of the Generalized Additive Model (GAM) results used to identify the correlation between trawling Gross Value Added (GVA) (response variable) and seafloor litter hotspots (explanatory variable), and incorporating fishing hours, species-specific LPUE (Landings Per Unit of Effort), and seasons as significant factors affecting economic productivity. The results comprise a comparison of the observed and GAM-predicted values of trawling Gross Value Added (GVA) in Euros, a histogram depicting the model residuals, and an analysis of the effects of fishing hours and seafloor litter on Gross Value Added (GVA). Confidence intervals (95%) around the response curve are represented in grey.

The GAM model explained 78% of the total variance, and it fits adequately the pattern of the observed data (Table 2). All the explanatory variables significantly contributed ($p < 0.05$) to determining the economic performance (GVA) associated with the studied area (Table 2). In particular, GVA values increased with increasing fishing hours (Figure 3) and with most of the species LPUEs (Supplementary Figure 1) until reached a plateau. With respect to seafloor litter, it was observed that GVA increased with an increase in the amount of debris up to a certain threshold (approximately 15,000 objects per km^{-2}), and then GVA decreased rapidly once the amount of debris exceeded this threshold (Figure 3).

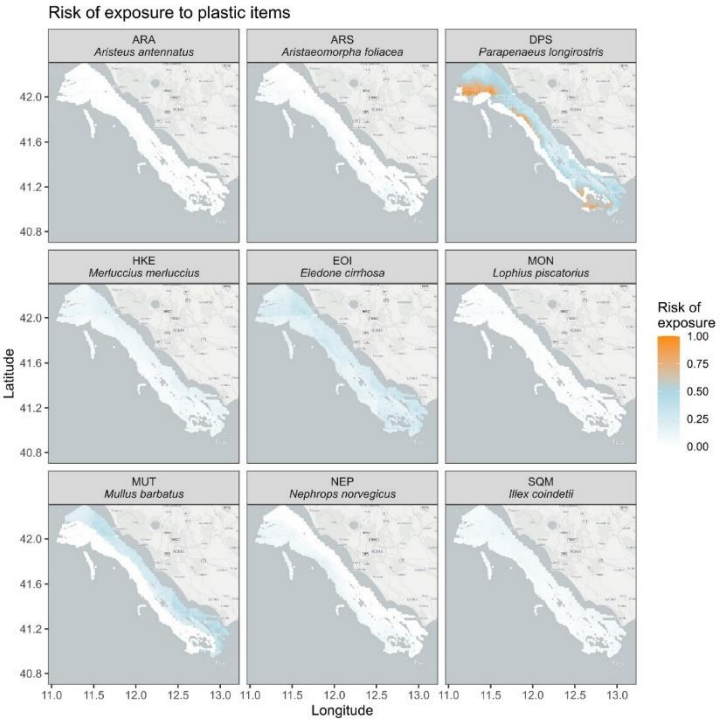


Figure 4 Maps of risk of exposure to seafloor plastic for 9 species that are crucial for trawling in the central Tyrrhenian Sea (GSA 9). This was achieved by multiplying the LPUE of each species and the plastic litter field. The resulting maps were then rescaled from 0 to 1.

The risk of exposure in this study refers to the likelihood of a certain species encountering seafloor plastic. Figure 4 highlights several areas with significant concentrations of plastic debris and high values of species abundance (LPUE). High species abundances of DPS, EOI, and MUT were found in coastal zones, together with significant litter concentrations. In particular, DPS showed the highest risk of exposure throughout the entire coastal area, with high peaks in the northern and southern parts of the Latium zone. EOI had intermediate risk or exposure for the entire studied area, while MUT had high risk or exposure in all the coastal zones. Figure 5 shows the impact scores (IS) for the key commercial species, based on the risk of exposure. The figure revealed that *Parapenaeus longirostris* (DPS) had the highest IS, followed by *Mullus barbatus* (MUT), *Eledone cirrhosa* (EOI), *Merluccius*

merluccius (HKE), *Nephrops norvegicus* (NEP), *Illex coindetii* (SQM), *Aristaeomorpha foliacea* (ARS), *Lophius piscatorius* (MON), *Aristeus antennatus* (ARA).

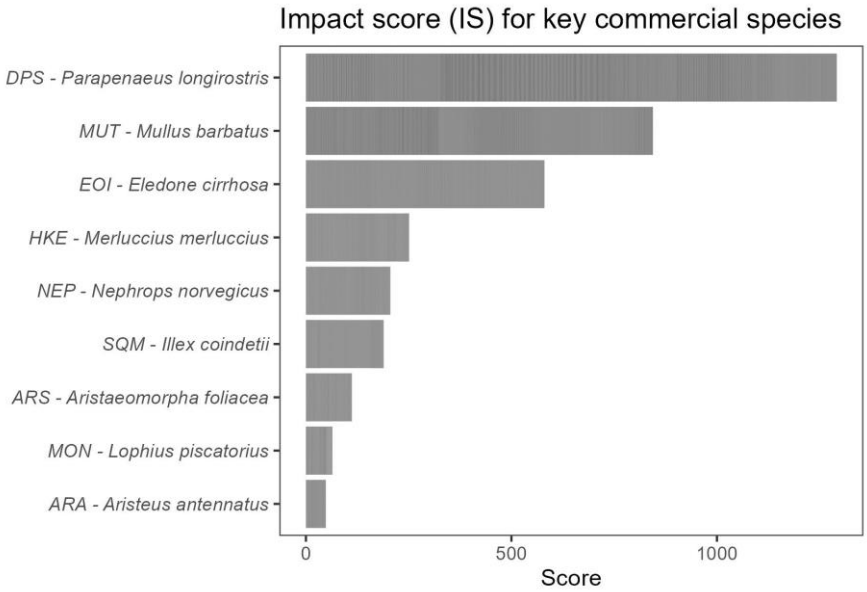


Figure 5 Impact score (IS) results. IS was considered an indicator of the probability that a species could be affected by the accumulation of waste on the seabed for 9 species that are crucial for trawling in the central Tyrrhenian Sea (GSA 9).

Discussion

The result of this study revealed a detectable effect of seafloor litter accumulation on productivity and, moreover, on the economic performance (GVA) of bottom otter trawl fishing in the Latium region of the central Tyrrhenian Sea. The results showed that seafloor litter had a negative impact on GVA when litter density exceeded a certain threshold.

The distribution of plastic litter on the seafloor in our study was consistent with predicted patterns of litter accumulation along the Italian coast. (Scotti et al., 2021). Indeed, consistently to previous studies, high-impact zones were located mainly in

coastal areas and decreased offshore (Alomar et al., 2016; Critchell & Lambrechts, 2016; Passarello, 2017; Poeta et al., 2016; Scotti et al., 2021; Thiel et al., 2013). The results suggest that the debris on the seafloor is more abundant and more persistent in the vicinity of the Tiber River, where urban and industrial practices may be contributing to the introduction of plastic waste into the sea (Cesarini et al., 2023; Crosti et al., 2018; Inghilesi et al., 2008; Noce et al., 2013). The source of marine litter is primarily derived from the mainland, depending on population density, river inputs, industries, and harbors (Browne et al., 2011; Campanale et al., 2020; Pruter, 1987; Rech et al., 2014; Veiga et al., 2016). The proximity to the city of Rome (population density 2231.5 inhabits·km^{-2}; ISTAT, 2018) and the presence of significant local sources of pollution, such as the Tiber River, give the Latium coastal zone one of the most polluted areas in the Tyrrhenian Sea (Poeta et al., 2016).

The application of SMART on the VMS and logbook data allowed us to model the spatial and temporal dynamics of this fishery. The LPUE for the main nine species, at a seasonal scale, was estimated from VMS and logbook data together with associated costs. Landings were then used to compute landing value and, subtracting the full list of costs, the GVA was obtained for each cell and season. Finally, the spatial and temporal GVA were regressed (using GAM) against the corresponding values of LPUE by species, fishing effort, and amount of plastic litter on the seafloor.

The results of the GAM model supported the hypothesis that seafloor litter reduced the GVA of commercial fishing. The GAM model displayed a pattern analogous to that of the intermediate disturbance theory, with a peak in species diversity occurring at an intermediate level of disturbance (Osman, 2015). Similarly, GVA increased with increasing litter up to a maximum, after which GVA decreased with increasing litter.

The nonlinear effect of plastic litter abundance on the GVA deserves further explanation. First of all, it is important to recall that plastic litter is partially generated by fishing effort, and in addition, bottom otter trawling displace the seafloor litter (Franceschini et al., 2019). This implies that, in areas exploited by bottom otter trawling, a certain level of litter abundance is expected, and that is unreasonable to

expect high LPUE in areas with absence of seafloor litter (at least in areas historically exploited). A low-medium presence of marine litter on the seafloor could be instead present in highly fished areas were bottom otter trawling acts also a “cleaning” force. But, when the amount of seafloor litter becomes high (and this can happen in the peripheral areas of fishing grounds), it can negatively impact the results of the fishing activity.

Since we were not directly assessing species diversity in relation to the amount of disturbance, LPUEs were considered a proxy for the availability of fisheries resources in the environment. This may indicate that low or intermediate levels of litter were acceptable, and did not interfere with resource availability, and GVA; while excessive litter caused resources to decline. Moreover, it must be considered that this decline is not only due to direct impacts on the resource itself but also to litter disturbance to fisheries and secondary effects on the species (Nguyen & Brouwer, 2022). For instance, the presence of marine litter in fishing nets can reduce their catch efficiency, cause damage, reduce the time available for fishing, inflate repair expenses, obstruct equipment, and necessitate increased fuel consumption (Beaumont et al., 2019; Ivar Do Sul & Costa, 2014; Mghili et al., 2023). Moreover, plastic that accumulates on the seafloor can interfere with animals' activities on the seafloor, such as foraging for food, with secondary effects on the growth and survival of individuals.

The findings indicated varying levels of risk of exposure and impact of seafloor litter across different species and their abundances. Within the scope of this research, seafloor plastic data and LPUE (as a proxy of habitat suitability for each species examined) were used for the first time to map fishing grounds according to levels of anthropogenic contamination. The investigated study area covered about 34% of the trawl fleet of the GSA 9 (97 boats out of 279 total) (Cataudella S. & Spagnolo M., 2011), and included three of the most important trawl fleets of the whole GSA 9 (Civitavecchia, Fiumicino, and Anzio). These trends were perfectly reflected in the cumulative GVA in the different seasons, and the highest GVA were attributable to areas where we found the highest values of catches. The results also show that GVA

had seasonal variations, with higher values in autumn and winter, possibly due to changes in species composition, relative abundances, or length. Notably, in two seasons (spring and summer) there were negative values of incomes. This is almost certainly due to the presence of the seasonal closure, which from 2018 to 2020 was between September 9 and October 9, while in 2021 it was from June 12 to July 11. Closure means a complete cessation of fishing activity for a certain period, which results in a reduction of annual effort (Regulation (EC) 1967/2006).

The catch trend resulting from this study follows the general pattern of Lazio, indeed in this region, the trawling effort is evenly distributed both on the platform and on the continental slope. Smaller boats normally operate on the platform and target hake (*Merluccius merluccius*), red mullet (*Mullus barbatus*), Shortfin squid (*Illex coindetii*), and curled octopus (*Eledone cirrhosa*), while larger boats mainly operate on the slope, with an activity aimed at pink shrimp (*Parapenaeus longirostris*), red shrimp (*Aristaeomorpha foliacea*), blue shrimps (*Aristeus antennatus*) and Norway lobster (*Nephrops norvegicus*) (Cataudella S. & Spagnolo M., 2011). Therefore, this study applied a risk framework to assess the exposure of commercially important species to plastic litter on the seafloor. Other studies have attempted to identify plastic exposure hotspots using numerical models of floating plastic, species distribution maps, and literature reviews of plastic ingestion (Compa et al., 2019, 2022). One novelty of our research was the use of a spatial distribution model of plastic on the seafloor, which, unlike floating plastic, has limited mobility and therefore maintains a stronger connection to the areas where it is deposited, providing a more accurate predictive model. In addition, we assigned a probability of impact (IS) to each species based on its habitat preference and distribution, in relation to the accumulation of seafloor litter. For example, the highest probability of exposure and impact from seafloor debris was found for *Parapenaeus longirostris* (DPS). This crustacean is currently one of the most significant commercial species in the Mediterranean Sea trawl fisheries. Due to a rapid growth in the size of the stock, landings of this species in the North Tyrrhenian-Ligurian Sea have been steadily increasing in recent years (Colloca et al., 2014). Indeed, in Lazio, the pink shrimp is a major trawl target, and

seabed litter impacts can have significant consequences for the fishing industry. Proximity to plastic hotspots has been shown to be a proxy for the probability of plastic exposure. This effect is amplified by feeding strategies that either increase or decrease a species' likelihood of ingesting plastic. DPS are highly susceptible to ingesting plastic as they shift from a hunting phase in which they prey on benthopelagic species (e.g. crustaceans, cephalopods, small fish) to a scavenging phase in which they burrow into the mud in search of food such as polychaetes, echinoderms, and bivalves (Kapiris Kostas, 2003). In fact, up to 76 percent of individuals (from a sample of n = 50) have been documented to have ingested plastic in the southwestern Ionian Sea (D'Iglio et al., 2022), and even up to 100 percent of individuals (n = 46) in the northeastern Mediterranean (Yücel, 2023). In contrast, blue shrimp, *Aristeus antennatus*, was found to be the species with the lowest risk of impact. This species is an important component of the deep-sea food web, and inhabits the muddy bottoms of the continental slope, with a great abundance in the 1000m depth (Sardà et al., 2004). The low-impact risk of the species is likely due to its distribution; indeed, we observed a higher concentration of plastic in the coastal zone, while it was present in lower concentrations in the deeper areas.

A distinct separation between plastic debris and fishing activities was identified in the study area. Such categorization simplifies the recognition of areas characterized by different levels of accumulation and serves as a primary reference for understanding the spatial distribution of marine debris and fishing activities. The results of this study had important implications for the management and conservation of marine resources and ecosystems in the Latium region. Seafloor litter was found to seriously endanger the sustainability and profitability of commercial fishing, along with the health and well-being of the species. Our research has identified areas and species that are at high risk of exposure and impact but cannot demonstrate the relative consequences. The goal of the study was to encourage new research to focus sampling and monitoring efforts in targeted areas where the risk of impact has been assessed. Future research could investigate whether more direct or indirect contamination is occurring for these species, affecting their economic productivity. From the European perspective, the

assessment and monitoring of impacts caused by marine litter is a prerogative (MSFD descriptor 10). In this context, the results of our research could be a valuable starting point to integrate the aforementioned impacts with the ones caused by the fishing industry itself. It is well known that fishing contributes significantly to the production of marine litter (discarded fishing gear) (Canals et al., 2021) and recognizing that the presence of waste is a potential cause of decreased economic productivity could encourage the participation of the fisher's community to remove and monitor seafloor litter (Forleo & Romagnoli, 2023). Moreover, our results could be useful to establish a threshold level of seafloor litter density that should not be exceeded to ensure the sustainability and profitability of commercial fishing.

Supplementary materials

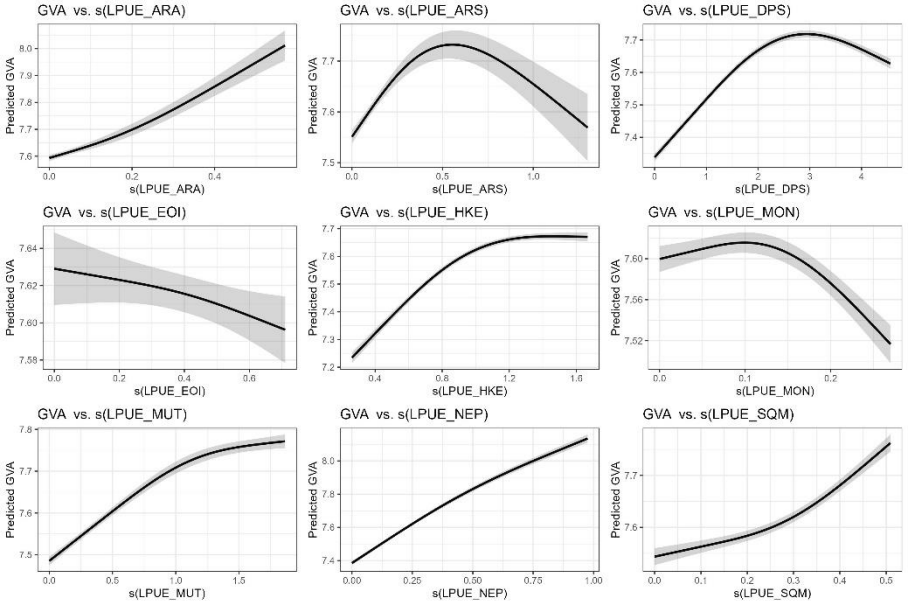


Figure S1 Summary of the Generalized Additive Model (GAM) results used to identify the correlation between trawling Gross Value Added (GVA) (response variable) and a set of explanatory variables. The results comprised an analysis of the effects of LPUE for 9 commercial important species on Gross Value Added (GVA). Confidence intervals (95%) around the response curve are represented in grey.

General conclusion

This work aimed to contribute to a better understanding of the responses of marine ecosystems to seafloor litter accumulation in the central Tyrrhenian Sea. This was achieved through a comprehensive investigation at different ecological scales, focusing on individual organisms, marine communities, and economically important fishery species. The novels of the study were the employment of updated procedures for data analysis and innovative techniques for the investigation of species, such as machine learning and environmental DNA, providing a holistic basis for mitigation and monitoring actions.

The four papers produced for my Ph.D. project provide collective insights that offer a detailed and comprehensive picture of the complex challenges posed by seafloor litter in the central Tyrrhenian Sea. The distribution maps of marine macro-litter (**Chapter 1**) have emerged as a key tool in: understanding the exposure of the sentinel shark species *Galeus melastomus* to plastic pollution (**Chapter 2**), understanding the impact of litter hotspots on the marine community (**Chapter 3**), and developing tools to assess the risk of exposure of species of commercial interest in areas of high accumulation (**Chapter 4**).

As shown in the first chapter of this thesis, waste on the seabed is widely distributed throughout the Western Mediterranean Sea. It included a large number of non-fishing related objects and has a highly heterogeneous distribution. Consequently, the abundance of seafloor litter has ecological repercussions and can impact organisms at all levels of ecosystem organization, from individuals to communities. *Galeus melastomus*, which is a species with opportunistic feeding habits, accidentally ingested a significant amount of plastic when encountering macro debris hotspots, mistaking them for prey, or accidentally ingesting them during feeding. Additionally, the marine community species composition was strongly influenced by anthropic pressures, particularly seafloor debris and fishing efforts. The adaptive life traits of marine species played a critical role in their response to debris accumulation and fishing: mobile species appeared to use relocation strategies, sessile species showed

flexibility in the face of disturbance, and epibiotic species relied on passive transport. Finally, this research has shown that seafloor debris poses a serious threat to the sustainability and profitability of commercial fisheries, as well as to the health and well-being of species, identifying areas and species at high risk of exposure and impact.

The study used innovative techniques and ecological models to provide essential fragments to the complex understanding of the impacts of marine debris at different ecological levels. First, the results indicate that the Random Forest machine learning approach is a very effective tool for modeling the distribution of marine macro-litter, representing a step forward in the search for intricate patterns of seafloor litter distribution. Several novel insights have been gained into the feeding ecology of *G. melastomus* in the central Tyrrhenian Sea, which was first studied in this area and proved to be a key-species predator in the benthic-pelagic community of the bathyal ecosystem. Moreover, eDNA metabarcoding data provided the ability to relate the spatial distribution of species composition with the accumulation of seafloor litter and fishing activities at different sites. Then, a spatial modelling approach was used to emphasize the significance of overlapping seafloor plastic and trawling activity maps. This provides valuable insights into areas with varying pressure levels in the marine environment.

This PhD research can provide essential information for designing effective conservation and management strategies to mitigate the impact of litter on marine ecosystems. The proposed mapping of litter hotspots and the identification of the blackmouth catshark as a potential bioindicator species provide valuable tools for ongoing monitoring efforts, in line with European directives, and highlight the need for adapted approaches based on habitat type and spatial distribution. This study sheds light on the responses of marine ecosystems to litter accumulation and fishing pressures. It provides practical insights for effective conservation measures and enhances our ecological understanding. The implications of these findings extend beyond scientific inquiry and reach into the field of resource management in the Lazio

region. Recognizing waste as a potential threat to economic productivity may encourage fishing community participation in waste monitoring and removal efforts. Additionally, the study recommends establishing a threshold level of seafloor litter density as a practical approach to ensuring the sustainability and profitability of commercial fishing operations in the face of evolving environmental challenges.

This PhD project, exploring the complexity of marine pollution in the central Tyrrhenian Sea, not only enhanced our scientific understanding but also provided practical insights for policymakers, stakeholders, and the fishing community. The need for integrated, multidisciplinary approaches emerged throughout, highlighting the importance of adaptive conservation strategies that take into account the dynamic nature of marine ecosystems and the ongoing challenges posed by marine debris. This dissertation provided a framework for further research, collaboration, and innovation to protect the delicate balance of the Central Tyrrhenian Sea's marine environment.

List of publications based on this thesis

- Cau, A., **Sbrana, A.**, Franceschini, S., Fiorentino, F., Follesa, M. C., Galgani, F., Garofalo, G., Gerigny, O., Profeta, A., Rinelli, P., Sbrana, M., & Russo, T. (2024). What, where, and when: Spatial-temporal distribution of macro-litter on the seafloor of the western and central Mediterranean Sea. Environmental Pollution, 342. https://doi.org/10.1016/j.envpol.2023.123028

- **Sbrana, A.**, Cau, A., Cicala, D., Franceschini, S., Giarrizzo, T., Gravina, M. F., Ligas A., Maiello G., Matiddi M., Parisi A., Sartor P., Sbrana M., Scacco U., Valente T., Viva C., Russo, T. (2022). Ask the shark: Blackmouth catshark (*Galeus melastomus*) as a sentinel of plastic waste on the seabed. Marine Biology, 169(7), 98. https://doi.org/10.1007/s00227-022-04084-1

- **Sbrana, A.**, Maiello, G., Gravina, M.F., Cicala, D., Galli, S., Stefani, M., Russo, T. (*Under Review*). Environmental DNA metabarcoding reveals the effects of seafloor litter and trawling on marine biodiversity. Marine Environmental Research.

- **Sbrana, A.**, Galli, S., Stefani, M., Russo, T. (*In preparation*). The cost of waste: overlap analysis of plastic concentrations on the seafloor and commercially important species reveals potential loss of economic productivity due to marine litter accumulation. Fisheries Research.

Related published papers

Valente, T., Costantini, M. L., Careddu, G., Berto, D., Piermarini, R., Rampazzo, F., **Sbrana, A**., Silvestri, C., Ventura, D., & Matiddi, M. (2023). Tracing the route: Using stable isotope analysis to understand microplastic pathways through the pelagic-neritic food web of the Tyrrhenian Sea (Western Mediterranean). Science of The Total Environment, 885, 163875. https://doi.org/10.1016/J.SCITOTENV.2023.163875

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