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Development, harmonizing and application of innovative methodologies for the study of the presence and effects of marine litter on organisms in Mediterranean marine protected areas within the Plastic Busters MPAs project

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..Prof. Arronax: Voi amate il mare, capitano?

Nemo: Sì! L'amo! Il mare è tutto. Copre i sette decimi del globo terrestre. Il suo respiro è puro e sano. È l'immenso deserto dove l'uomo non è mai solo, poiché sente fremere la vita accanto a sé. Il mare non è altro che il veicolo di un'esistenza soprannaturale e prodigiosa; non è che movimento e amore, è l'infinito vivente, come ha detto uno dei vostri poeti. Infatti, professore, la natura vi si manifesta con i suoi tre regni: minerale, vegetale, animale“.

Ventimila leghe sotto i mari. Jules Verne (1870).

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ABSTRACT

The irreversibility and global ubiquity of marine litter pollution and plastic, in particular, make this material a potential planetary boundary threat. Although the growing attention from the scientific community and the increasing number of peer-reviewed papers, the occurrence and distribution of plastic litter in the Mediterranean Marine Protected Areas (MPAs) and its impacts and effects on marine wildlife remain still poorly investigated. Within the Plastic Busters MPAs project, this PhD thesis provided a comprehensive assessment of marine litter pollution in the sea surface waters and beaches of the Pelagos Sanctuary and the Tuscan Archipelago National Park and the potential physical and chemical impacts related to plastic ingestion on several Mediterannean bioindicators. The experimental designs planned *ad-hoc* for the selected study areas (Chapter 3), harmonised and implemented the current methods for sampling marine litter in the different environments and defined a new simultaneous multilevel approach reflecting the strong pressure that marine litter, and in particular plastics, exert on organisms inhabiting the protected areas. A total of 273 monitoring transects of floating macrolitter, 141 manta trawl and 14 beaches were sampled and monitored evaluating the occurrence, abundances and composition of marine litter according to the characterization protocols implementing the Marine Strategy Framework Directive (MSFD) (Chapter 4). Particular attention was applied to investigate the potential influences of environmental and anthropic variables affecting the litter distribution and to identify potential hotspot accumulation areas representing a major hazard for marine organisms. Several species were collected, starting from invertebrates to cetaceans, to evaluate the frequency of ingestion and confirm/validate their potential role as marine litter bioindicators (Chapter 5). For the first time, an exhaustive analysis of phthalate acid esters (PAEs) presence was assessed on different organisms and biological tissues through the GC-MS analysis (Chapter 5). Strong litter inputs were identified to originate from the mainland and accumulate in coastal waters within about 10-15 nautical miles. Harbours and riverine outfalls may contribute significantly to plastic pollution representing the main sources of inputs as well as areas with warmer waters and weak oceanographic features could facilitate the accumulation of litter. The high concentrations of plastics floating on the sea surface (399 items/km^2 for macrolitter and 259,490 items/km^2 for MPs) and stranded on beaches (up to 1,033 ± 915 items/100m) indicate a potentially threatening trend of particle accumulation that may pose a serious risk to organisms living in the Pelagos Sanctuary. The twofold monitoring approach, simultaneously investigating plastic and MP ingestion in several species and concentrations of plasticizers has allowed gaining information on the direct link between synthetic particle ingestion and its additive substances release. Microplastic ingestion was assessed for the first time in the Mediterranean Sea in *Velella velella* organisms (0.71 items/ind), filter-feeding organisms such as the *Mobula mobular* (23 items/ind.) and *Balaenoptera physalus* (35 items/ind.), as well as in poorly investigated species i.e. seabirds, lanternfishes and odontocete cetaceans. Phthalate acid ester loads (mainly DIBP, DBP and DEHP compound) and their pattern of accumulation were evaluated in several species and different biological tissues respectively, according to their feeding behaviour, long life span and spatial distribution. Finally, the spatial risk assessment (Chapter 6) indicated the Gulf of La Spezia and the National Park of the Tuscan Archipelago as the most affected by the accumulation of plastic waste and at higher risk of exposure to organisms as well as the Genova canyon and the seamount area. The results obtained here provide further indications for dealing with plastic pollution in MPAs and could facilitate future recommendations for the management and use of the marine and coastal environment of these protected areas.

RIASSUNTO

La presenza ubiquitaria di rifiuti marini ed in particolare della plastica, rende questo materiale una potenziale minaccia planetaria. Nonostante la crescente attenzione da parte della comunità scientifica, gli impatti e gli effetti dei rifiuti marini nelle aree marine protette del Mar Mediterraneo (AMP) rimangono ancora poco indagati. Questo studio, inserito nell'ambito del progetto Plastic Busters MPAs, rappresenta una valutazione ad ampio spettro della presenza di rifiuti marini galleggianti e accumulati lungo le spiagge del Santuario Pelagos e del Parco Nazionale dell'Arcipelago Toscano e dei potenziali impatti fisici e chimici legati all'ingestione di plastica in diverse specie bioindicatrici. I disegni sperimentali, pianificati *ad-hoc* per le aree di studio selezionate (Capitolo 3), hanno armonizzato e implementato i più utilizzati metodi di campionamento dei rifiuti marini nei diversi comparti ambientali. Un nuovo approccio simultaneo multilivello è stato sviluppato e testato al fine di valutare i potenziali impatti che questi materiali possono provocare sugli organismi che abitano le aree protette. Un totale di 273 transetti per il monitoraggio dei macroggetti galleggianti, 141 campioni neustonici volti all'isolamento di microplastiche (MP) e 14 spiagge è stato campionato per valutare la presenza, l'abbondanza e la composizione dei rifiuti marini secondo i protocolli di caratterizzazione implementati all'interno della Direttiva Quadro sulla Strategia Marina (Capitolo 4). Particolare attenzione è stata posta nel considerare le influenze di diversi fattori ambientali e antropici sulla distribuzione dei rifiuti al fine di identificare potenziali aree di accumulo in grado di costituire un maggiore pericolo per gli organismi marini. Diverse specie, dagli invertebrati ai mammiferi marini, sono state campionate per valutare la potenziale ingestione di plastica e confermare/validare così il loro potenziale ruolo come bioindicatori (Capitolo 5). Per la prima volta, è stata effettuata un'esaustiva analisi della presenza degli ftalati (composti additivi della plastica) in diversi organismi e tessuti biologici (Capitolo 5). Gli apporti di rifiuti marini provenienti dai porti commerciali e dai fiumi, sono stati individuati come le principali cause di inquinamento, determinando un accumulo di questi materiali entro 10-15 miglia nautiche dalla costa ed in aree caratterizzate da acque calde e caratteristiche oceanografiche stabili. Le alte concentrazioni di plastica ritrovate sia in superficie (399 oggetti/km^2 per i macroggetti e 259490 particelle/km^2 per le MP) che lungo le spiagge (fino a 1033 ± 915 oggetti/100m) delle aree marine protette indagate suggeriscono un graduale incremento di questi rifiuti in grado di rappresentare un grave rischio per gli organismi marini che vivono nel Santuario Pelagos. La valutazione simultanea dell'ingestione di plastica e MP e delle concentrazioni di ftalati nelle diverse specie, ha permesso di ottenere informazioni sul legame tra l'ingestione di queste particelle e il rilascio dei loro principali additivi. L'ingestione di MP è stata valutata per la prima volta nel Mar Mediterraneo in *Velella velella* (0,71 pezzi/ind), negli organismi filtratori *Mobula mobular* (23 pezzi/ind.) e *Balaenoptera physalus* (35 pezzi/ind.), così come in specie poco studiate di uccelli marini, pesci lanterna e cetacei odontoceti. Considerando il comportamento alimentare, la lunga durata di vita e la distribuzione spaziale delle specie indagate, la presenza di ftalati (principalmente DIBP, DBP e DEHP) ed i loro profili di accumulo in diversi tessuti biologici, sono stati analizzati. Il Golfo di La Spezia e il Parco Nazionale dell'Arcipelago Toscano, così come il canyon di Genova, sono state evidenziate come la aree maggiormente interessate dall'accumulo dei rifiuti marini e a maggior rischio di esposizione per gli organismi dall'analisi di rischio spaziale effettuata sulla base di tutti i dati raccolti. I risultati ottenuti rappresentano una valutazione completa ed accurata del grado di inquinamento da rifiuti marini nelle AMP investigate, e possono costituire le basi per un aggiornamento delle raccomandazioni per la loro gestione e l'utilizzo dell'ambiente marino e costiero.

LIST OF ABBREVIATIONS AND ACRONYMS

AC 1	Upper accumulation zone
AC 2	Lower accumulation zone
ARPAT	Environmental Protection Agency of Tuscany Region
BBzP	Butyl benzyl phthalate
BDL	Below detection limit
BSH	German Federal Maritime and Hydrographic Agency
Bp	Balaenoptera physalus
CD	Scopoli's shearwater
CI	Common Indicator
CIMA	Centro Internazionale in Monitoraggio Ambientale
DAP	Diallyl phthalate
DBP	Dibutyl phthalate
DcHP	Dicyclohexyl phthalate
DDTs	Dichloro-diphenyl-trichloroethane
DE	Digestion efficiency
DEEPD	Deep divers
DEP	Diethyl phthalate
DEHP	Di(2-ethylhexyl) phthalate
DIBP	Diisobutyl phthalate
DIDP	Diisodecyl
DiNP	Diisononyl phthalate
DnPP	Di-n-pentyl phthalate
DnOP	Di-n-octyl phthalate
DMP	Dimethyl phthalate
DPrP	Dipropyl phthalate
EA/NALG	Environment Agency National Aquatic Litter Group
EEA	European Environment Agency
EDs	Endocrine disruptors
ER	Encounter rate
FTIR	Fourier Transform Infrared Spectroscopy
GAMs	Generalized Additive Models
GC-MS	Gas Chromatography-Mass Spectrometry
GIS	Geographic Information System
GES	Good Environmental Status
GESAMP	Group of Experts on Scientific Aspects of Marine Environmental Protection
GITs	Gastrointestinal tracts
GPS	Geographical Positioning Systems
H_2O_2	Hydrogen peroxide
IA	Audouin's gull
ICC	International Coastal Cleanup

IMAP	Integrated Monitoring and Assessment Programme
IMO	International Maritime Organization
ISPRA	Italian National Institute for Environmental Protection and Research
IUCN	International Union for Conservation of Nature
JELLYF	Jellyfish
KDE	Kernel density estimation
KOH	Potassium hydroxide
LAMMA	Laboratorio di Meteorologia Modellistica Ambientale
LMPs	Large microparticles
LODs	Limits of detections
MARPOL	International Convention for the Prevention of Pollution from Ships
MB	Mobula mobula
MC	Membrane clogging
MLD	Mixed layer depth
MM	Mola mola
MMOs	Marine Mammal Observers
MPs	Microplastics
MPAs	Marine Protected Areas
MSFD	Marine Strategy Framework Directive
MSFD TG 10	Marine Strategy Framework Directive Tasking Group 10
Mt	Million tons
NaCl	Sodium chloride
NLS	Noxious Liquid Substances
OAC	Off accumulation zone
OEC/UAC	Office de l'Environment de la Corse
OECMs	Other Effective area-based Conservation Measures
OSPAR	Belgium, Denmark, Finland, France, Germany, Iceland, Ireland, Luxembourg, The Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and United Kingdom joint governments
PAEs	Phthalate acid esters
PAHs	Polycyclic aromatic hydrocarbons
PBDEs	Polybrominated diphenyl ethers
PCA	Principal component analysis
PCBs	Polychlorinated biphenyls
PE	Polyethylene
PET	Polyethylene terephthalate
POPs	Persistent organic pollutants
PP	Polypropylene
PS	Polystyrene
PU	Polyurethane
PY	Yelkouan shearwater
PVC	Polyvinyl chloride

R_2	correlation coefficient
Re	Remote beaches
RE	Recovery rates
RSD	Relative standard deviation
R/V	Research vessel
Ru	Rural beaches
SC	Setenella coeruleoalba
SEAB	Seabirds
SEACleaner	Sea Cleaner beach litter protocol
SIM	Single ion monitoring
SMPs	Small microparticles
SPAMI	Special Protected Area of Mediterranean Importance
SSH	Sea surface Height
SST	Sea Surface Temperature
SUPs	Single-Use Plastics
TT	Tursiops truncatus
U	Urban beaches
UNEP	United Nations Environment Program
UNEP/IOC	United Nations Environment Program Intergovernmental Oceanographic Commission

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indicate the 25th and 75th percentiles, the whiskers above and below the boxes the 95th and 5th percentiles. The horizontal line inside the boxplots denotes the median value. Outliers are indicated by black dots. The black line shows the reference value for mean MPs concentration in Northwestern Mediterranean Sea, while the dashed line represents the reference value for the standard deviation of MPs concentration in Northwestern Mediterranean Sea.* Indicates difference statistically significative ($p < 0.05$).

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CHAPTER 1: GENERAL INTRODUCTION

1.1 The Plastic Age

First produced in the early XX century, plastic has become a widely used material due to its versatility, durability, inexpensive production, and lightweight. Since then, the plastics industry has experienced rapid growth worldwide, now directly employing more than 1.56 million people in Europe and generating a turnover of more than €350 billion in 2019, with undeniable societal benefits (PlasticsEurope, 2020). By far the largest end-use markets in Europe are packaging and building and construction, followed by automotive (PlasticsEurope, 2020) (Fig. 1). It is therefore evident that plastic products play an essential role in modern society and are an indispensable part of our daily lives. With a slight downward trend, European plastic production reached almost 58 million tonnes (Mt) in 2019 (PlasticsEurope, 2020) (Fig.1), with synthetic fibres accounting for 65 Mt worldwide (Fogh Mortensen et al., 2021). Overall, thermoplastic polymers are the most commonly produced polymers, with polyolefins (polyethylene, PE, and polypropylene, PP) mainly used for food packaging, reusable bags, trays and containers, accounting for almost 50% of total plastics demand in Europe (Fig. 1) (PlasticsEurope, 2020). Thermoplastics, which include polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET) and polyurethane (PU), in addition to PE and PP (SAPEA, 2019), were among the most common types of plastics polluting the aquatic and terrestrial environments (Erni-Cassola et al., 2019; Llorca et al., 2021; Piehl et al., 2019; Suaria et al., 2016; Ter Halle et al., 2017).

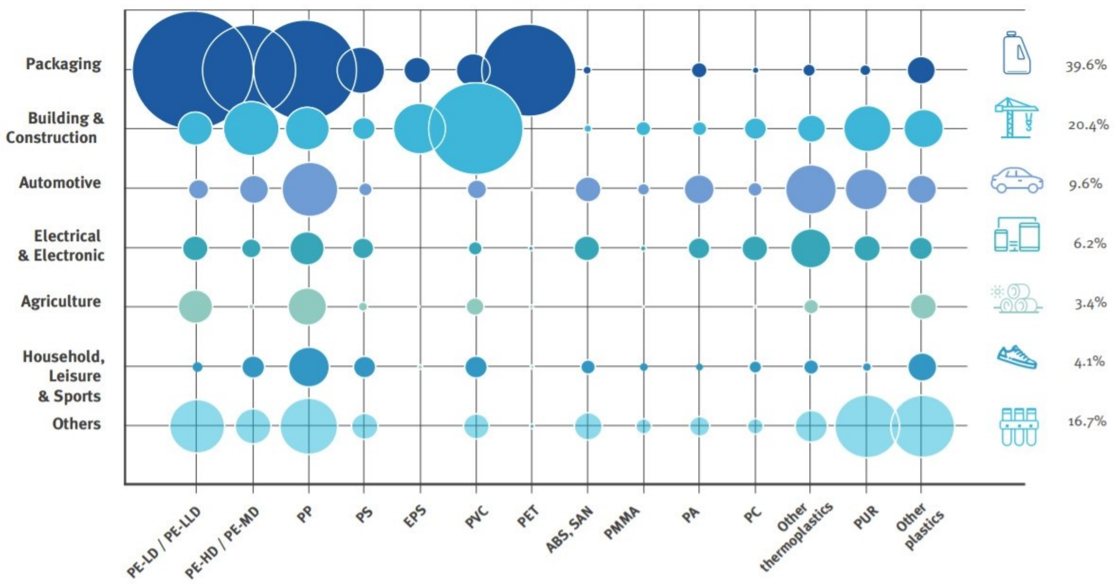


Fig:1. Plastic demand by segment and polymer type in 2019 in Europe. Source: PlasticsEurope Market Research Group (PEMRG) and Conversio Market & Strategy GmbH.

While much attention has been paid in recent years to promoting a more circular and sustainable plastics system in terms of reuse, recycling, proper waste management and the use of renewable raw materials, there are still environmental and climate challenges associated with the production, use and disposal of the different types of plastics (Fogh Mortensen et al., 2021; Lau et al., 2020; PlasticsEurope, 2020). Recently, it has been suggested that our ability to create new materials such as plastic and its ubiquitous presence in the environment may be one of the key factors in stratigraphically marking the new geological epoch of the 'Anthropocene' (Waters et al., 2016; Zalasiewicz et al., 2016). The evidence for this is the growth of new geological formations, such as plastiglomerates, due to the interaction of melted plastic waste and the environment (De-la-Torre et al., 2021; Ehlers and Ellrich, 2020), proving the irreversible changes that humans have been subjected our planet to. The oceans are not exempt from this threat. They represent one of the most important sinks in which anthropogenic materials, including plastic, tend to accumulate. In light of these considerations, the term "plastic age" is increasingly used in the literature (Yarsley and Couzens, 1941) and scientific works (Thompson et al., 2009; Villarrubia-Gómez et al., 2018) to define the last decades starting from the 1950s.

1.2 Marine litter and plastic fate in the environment

Information on quantities, trends, sources and impacts (including human health and socio-economic impacts) of marine litter is incomplete worldwide. However, it is widely accepted that both the amount of litter in the sea and the amount of input to the oceans are increasing. As defined by the United Nations Environment Program (UNEP), as "any persistent, manufactured or processed solid material discarded, disposed or abandoned in the marine and coastal environment", the marine litter consists of 80% of plastic (Galgani et al., 2015). Sources of pollution can come from land (e.g., recreational activities and industry, transport in urban areas through rivers, sewage or landfills), marine activities (e.g., commercial shipping, ferries, commercial and recreational fishing vessels) and offshore infrastructure (e.g., platforms, oil rigs and aquaculture facilities) (Galgani et al., 2015; Veiga et al., 2016:). It can be transported by ocean currents over long distances from its origin and can be found in all marine environments, even in remote areas such as uninhabited islands in the open ocean or the deep sea (Budziak et al., 2016). Nowadays, the irreversibility and global ubiquity of marine litter pollution, and plastic, in particular, make this material a potential threat to planetary boundaries (Nash et al., 2017; Rockström et al., 2009; Villarrubia-Gómez et al., 2018) (Fig. 2).

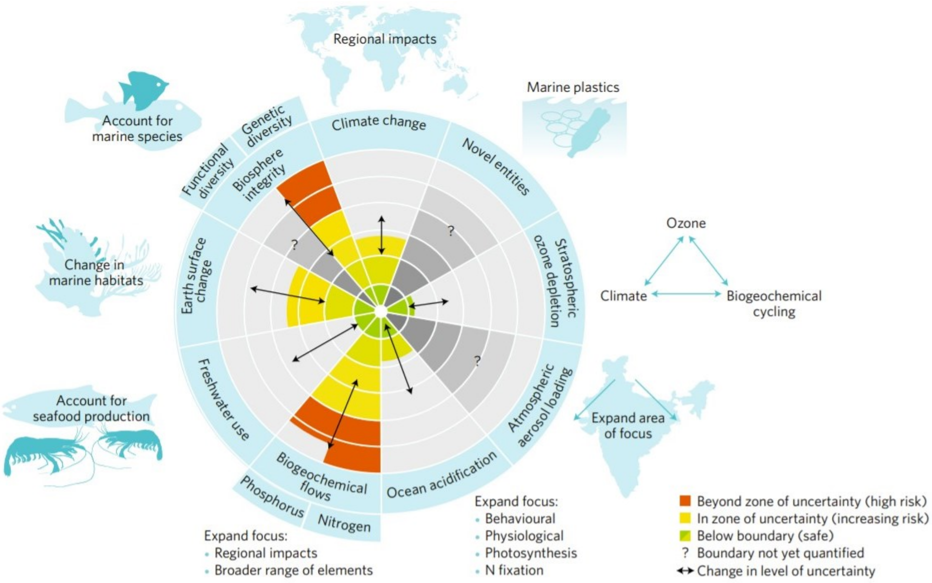


Fig:2. Planetary boundaries in the marine ecosystems: uncertainty and risks associated. Source: Nash et al., 2017; https://doi.org/10.1038/s41559-017-0319-z.

Their highly desirable properties, i.e., durability, flexibility, and degradability, make these materials extremely threatening from an environmental perspective. Based on size classes, plastics can be classified into nano- (< 1 um), micro- (1 um - 5 mm), meso- (5 mm - 2.5 cm) and macroplastics (> 2.5 cm) (GESAMP, 2019). Microplastics (MPs) are composed of a series of polymers modified by varying amounts of additives and sorbed pollutants, and exhibit a range of morphologies, sizes and visual properties (Lusher et al., 2020). They are classified into primary particles, which are specifically designed to be microscopic such as pellets (Andrady et al., 2011) or for direct use as abrasives in personal care products (Cole et al., 2011). Secondary microplastics result from the breakdown of larger plastic debris, both marine and terrestrial, through mechanical abrasion, moisture, elevated temperature, UV radiation, or microbial activity (Brown et al., 2007; Thompson et al., 2004). It is estimated that losses of primary microplastics to the natural environment globally are in the order of 1.8 - 5.0 million tonnes per year, with 0.8 - 2.5 million tonnes entering the oceans (Boucher and Friot, 2017). MPs have been observed in many different areas of the marine system (Erni-Cassola et al., 2019), including at the sea surface (Fossi et al., 2017; Suaria et al., 2016) and in the underlying water column (Baini et al., 2018; Egger et al., 2020; Kooi et al., 2016). It has also been described to accumulate in deep-sea sediments (Sanchez-Vidal et al., 2018; Taylor et al., 2016; Van Cauwenberghe et al., 2013; Woodall et al., 2014), coastal sandy beaches (Vlachogianni et al., 2020), seafloor (Angiolillo et al., 2015; Consoli et al., 2020; Munari et al., 2017) and sea ice (Bergmann et al., 2019; Kelly et al., 2020) in both the Arctic and Antarctic. Their behaviour in seawater could be strongly influenced by their chemical composition. Due to different densities, polyolefins (polyethylene, PE, and polypropylene, PP) tend to float in the upper layers of the water column, while polyvinyl chloride (PVC), polyethylene terephthalate (PET), and polyurethane (PU) tend to experience negative buoyancy and can sink and reach the seafloor (Erni-Cassola et al., 2019; Suaria et al., 2016; Zeri et al., 2018).

1.3 Marine litter and plastic impacts on marine organisms

Social and scientific awareness of marine litter pollution has developed over the past decade as research on the issue boomed and policy makers recognized it as a primary environmental concern (Rochman et al., 2013). In particular, the persistence and extent of marine pollution have attracted the attention of the scientific community, so much so that plastics have recently been identified as a potential threat to planetary boundaries (Villarrubia-Gómez et al., 2018). There is a growing body of peer-reviewed literature addressing the question of whether plastics, and MPs in particular, cause toxicity to organisms and, if so, what the main causes of this toxicity are. To date, over 900 marine species have been observed interacting with plastic pollution, ranging from marine megafauna to fish and invertebrates (Gall and Thompson, 2015; Kühn and van Franeker, 2020) (Fig. 3).

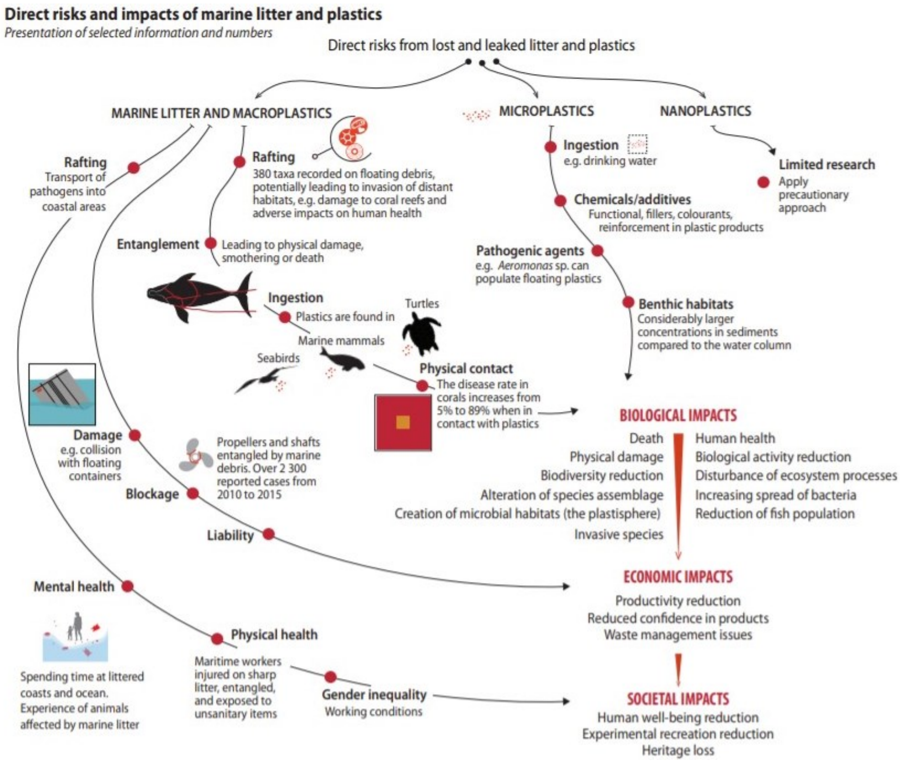


Fig.3: Direct risks and impacts of marine litter and plastics. Source: UNEP, 2021.

Marine organisms can eventually suffer lethal and sublethal damage, including drowning, starvation, physical injury, reduced mobility, and physiological stress due to entanglement or ingestion (Browne et al., 2015; Senko et al., 2020). Under laboratory conditions, MPs caused mortality (Gray and Weinstein, 2017; Jemec et al., 2016), decreased food intake (Cole et al., 2019, 2015), and growth (Redondo-Hasselerharm et al., 2018), behavioural and histopathological changes (Brun et al., 2019; Limonta et al., 2019), and impaired reproduction (Sussarellu et al., 2016; Zhang et al., 2020).

In addition, plastics can adsorb, concentrate and release chemical pollutants from the marine environment (Teuten et al. 2009). Plastic polymers have a high sorption capacity for toxins due to their polymeric chain structure and increased surface area (Rochman et al. 2013), which is reflected in a high capacity to absorb metals and persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), dichloro-diphenyl-trichloroethane (DDTs), and polycyclic aromatic hydrocarbons (PAHs) (Gewert et al., 2015; Rochman et al., 2014). This capacity increases with degradation and a corresponding increase in surface area, resulting in the plastic becoming more hazardous the longer it remains in the marine environment (Andrady 2011). Furthermore, chemicals such as bisphenol A, phthalates, nonylphenol and polybrominated diphenyl esters are added to plastic polymers during the manufacturing process (Rochman et al., 2014) to improve the properties of the final product (Teuten et al., 2009) and make them more stable, durable and resistant to degradation (Gewert et al., 2015). Since plastics are expected to remain in the environment for hundreds or even thousands of years (Barnes et al., 2009), hydrophobic monomers and plastic additives can accumulate on their surface over time and cause toxicity, carcinogenesis, endocrine disruption, and physical damage (Barrick et al., 2021; Hermabessiere et al., 2017), as well as bioaccumulation of persistent toxic substances in organisms and the trophic web (Koelmans et al., 2014).

Although there is ample evidence of the effects of plastic debris at organismic and sub-organismic levels, ecological risk assessments remain challenging because the pathway and spatial and temporal patterns of exposure of organisms and habitats in the marine environment are poorly understood (Browne et al., 2015; Koelmans et al., 2017; Rochman et al., 2016). The available preliminary risk assessments do not clearly state immediate risk to the marine environment from MPs (Beiras and Schoemann 2020; Everaert et al., 2018). Nevertheless, the authors point out that negative environmental impacts are to be expected in heavily polluted areas and when considering future scenarios with increasing MP concentrations and call for further research and improved analytical methods to quantify MPs in marine ecosystems. In this sense, scientific data on plastic ingestion in marine species as well as physiological effects upon species is needed to understand at a first stage, plastic effects at the species level to move further on to investigate implications at a population level and lastly consider the whole ecosystem scale.

1.4 Marine litter in the Mediterranean Sea

The Mediterranean Sea is a biodiversity hotspot with unique geological, biogeographical, physical and ecological features (Coll et al., 2010). It has 1231 MPAs and OECMs (Other Effective area-based Conservation Measures) covering an area of 179,798 km2, for a total area of 7% that is legally designated. Nevertheless, it is also highly vulnerable to human impacts due to its geographical and political location. This sea is highly anthropized and hosts more than 25% of the world's tourism, 7% of the world's coastal population and 30% of the world's maritime traffic passing through its waters (UNEP, 2009). All this leads to coastal development, habitat loss and degradation, overfishing, resource depletion, eutrophication and pollution (Coll et al., 2010). Moreover, high concentrations of marine debris have been detected in this basin in recent decades (Fig. 4), which is confirmed by several predictive distribution models (Fossi et al., 2017; Liubartseva et al., 2018; Mansui et al., 2015; Ourmieres et al., 2018; Zambianchi et al., 2017).

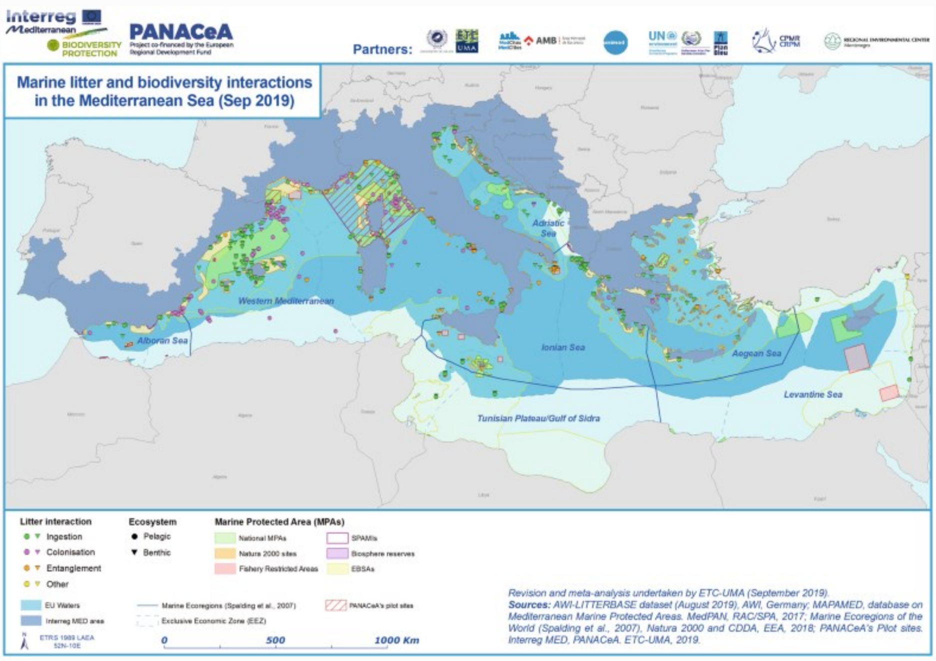


Fig. 4: Spatial distribution of marine litter in the Mediterranean Sea and biodiversity interactions in four categories (ingestion, entanglement, colonization, and others); Source: Guitart et al. 2019.

Zambianchi et al. (2017) predicted that litter and plastic pollution accumulates in the southeastern part of the Levantine Basin and along the southern Mediterranean coasts. This is consistent with the observations of Cózar et al. (2015), who showed that there is a clear zonation of litter in the Mediterranean Sea with a maximum in the southern part of the basin, both in the western and eastern basins and with the observations of Mansui et al. (2015), who identified the southern coastal strip of the eastern sector as an accumulation area or preferred stranding destination. Floating debris also occurs in the Algero-Provençal Basin, the Sardinia Channel and south of the Balearic Islands, with other areas of high concentration in the north associated with the Northern Current or with the northward propagation of Algerian eddies (Cózar et al. 2015). Observations in the Tyrrhenian Sea suggest a greater occurrence in the southern part of this sub-basin, characterized by very slow, basically stagnant dynamics (Guerranti et al. 2017), and in the Corsica Channel, the bottleneck for the transition from the Tyrrhenian to the Ligurian Sea. Observations of the distribution of litter on the seabed indicate that it is largely influenced by the vertical details of the Mediterranean circulation, with a long-term presence of litter in the southern Algerian basin and southeast of Crete, as well as in canyons and other areas influenced by strong sinking patterns, such as the Gulf of Lyon (Zambianchi et al., 2017). For this reason, in 2013, the Parties to the Barcelona Convention (all Mediterranean coastal states and the EU) agreed on a Regional Plan for the Management of Marine Litter in the Mediterranean Sea. The Regional Plan, which is the first legally binding instrument at the regional seas level, aims to minimize the presence and impact of marine litter in the Mediterranean Sea. Moreover, at a European level, the Marine Strategy Framework Directive (MSFD) (2008/56/ EC) and a Mediterranean level, the Integrated Monitoring and Assessment Programme (IMAP) and Related Assessment Criteria have been developed to protect the marine environment and ensure its sustainable use. Based on several descriptors, the ultimate objective of these rules is the achievement of good environmental status (GES) for marine waters by the Member States. In particular, the provisions of descriptor 10 of the MSFD and common indicator 24 of the IMAP aim to protect marine ecosystems from harm caused by the emerging problem of marine litter, through the definition of indicators of plastic particles ingestion. The generation of data on the amount and distribution of marine litter at the sea surface and in other ecological compartments, as well as baseline data on the uptake of plastic particles by indicator species, is a priority and a real need for the conservation measures of marine ecosystems in this region.

1.4.1 Sea surface floating litter: state of the art

The concentration of marine litter in the surface layers of the Mediterranean Sea is strongly influenced both by the various anthropogenic pressures and by the geomorphological and hydrodynamic factors that characterise this basin locally. The semi-enclosed nature with a low water exchange with other seas and oceans (Cózar et al., 2015; Danovaro et al., 2020) favours the temporary formation of convergence areas for litter (Fossi et al., 2017; Suaria et al., 2016) and it is estimated that 62 million macrolitter objects float on the sea surface of the entire basin (Compa et al., 2019; Suaria and Aliani, 2014). Differences in vessel types used, ranging from small or medium-sized boats (Di-Méglio and Campana, 2017; Fossi et al., 2014; Zeri et al., 2018;) to large vessels (Aliani et al., 2003; Campanale et al., 2018; Ryan, 2013), including ferries and cargo ships (Arcangeli et al., 2018), observation conditions (i.e., observation height and width), sea and wind conditions (Kukulka et al., 2012), survey methods used and technical equipment (e.g., Manta net, Neuston net, WP2) (Collignon et al., 2014; Compa et al., 2020; Fossi et al., 2017; Pedrotti et al., 2016) can also strongly influence marine debris monitoring and collection. Their presence was first highlighted in the 1980s by two studies conducted by Morris and McCoy in the central and eastern parts of the Mediterranean Sea, which found concentrations of 1,300 and 2,000 items per km^2, respectively (McCoy, 1988; Morris, 1980). Since that time, the attention of the scientific community to this issue has increased considerably, as shown by the approximately 80 scientific articles published in the literature to date (Tabs. 1 and 2).

Concentrations of floating macrolitter are very variable in the different sub-regions considered according to the MSFD classification, and in all cases exceed the median threshold proposed by UNEP/MAP for 2020 (5 items/km^2) (Tab.1). The highest abundances were reported in the Adriatic Sea (avg. 212.4 ± 336.1 items/km^2), followed by the Ionian and Central Mediterranean Sea (avg. 118 ± 250.7 items/km^2) and the Aegean Levantine Sea (avg. 117.1 ± 162.6 items/km^2). The lowest concentrations were found in the western sector of this basin with an average of 29.7 ± 46.8 items/km^2.

Floating microliter and MPs particularly in the Mediterranean Sea sub-region (Tab. 2) showed an average concentration of 573,703 ± 1,331,658 items/km^2. As mentioned above, the highest average values were reported for the Aegean-Levantine basin (1,964,488 ± 3,224,132 items/km^2) and the Adriatic Sea (498,813 ± 456,768 items/km^2). These two sub-regions showed values exceeding the mean baseline of 340,000 items/km^2 proposed by UNEP/MAP in 2017. In contrast, the western sector (216,399 ± 284,360 items/km^2) and the Ionian and Central Mediterranean Sea (avg. 197,739 ± 204,808 items/km^2) showed mean concentrations below the proposed threshold.

The heterogeneity of the data distribution reported in the Mediterranean Sea could be due to the variability of sea surface circulation patterns, as well as to the location of survey sites (i.e. coastal waters, open sea) and proximity to potential sources of marine debris (i.e. urban and tourist centres, shipping lanes, fishing areas) and transmission pathways (i.e. rivers, sewage treatment plants). In particular, in the Adriatic Sea, land-based sources have been considered as an important input of marine litter (UNEP/MAP, 2015; Vlachogianni et al., 2017, Zeri et al., 2018), with fisheries and aquaculture being important contributors. In addition, freshwater inputs, mainly from the Pò River, are assumed to contribute 46.3 litter items (>3.2 mm) per second to the Adriatic Sea, which has been extrapolated to 120 tonnes per year (Van der Wall et al., 2015). In the eastern part of the Mediterranean Sea, the Lagrangian circulation model worked by Mansui et al. (2015) shows how hydrological conditions make this area more prone to the accumulation of significant amounts of marine litter, as suggested by the high concentration of floating objects (max. 136,514 items/km^2) reported by Tata et al. (2020) and MPs (7,699,716 items/km^2) (Gündoğdu et al., 2018). The western and central sectors of the Mediterranean appear to be less affected by floating litter accumulation, except for the highest levels reported in the waters around the islands of Malta and Gozo (average 681 pieces/km^2) (Curmi and Axiak, 2021). These basins seem to be more affected by currents and hydrological features that promote particle circulation rather than accumulation (Mansui et al., 2015). Nevertheless, some coastal accumulation areas may occur along the Ligurian coast (Fossi et al., 2012, 2017; Panti et al., 2015), between Corsica and the

Capraia Islands (Collignon et al., 2014; Fossi et al., 2017) and in the waters off the Balearic Islands (Compa et al., 2020; Ruiz-Orejón et al., 2018).

Tab. 1: Current status of peer-reviewed papers published on floating macrolitter abundance (items/km^2) in the Mediterranean Sea Sub Regions proposed by MSFD. Detailed sampling information (sampling site and year, vessel speed, observer height and width and distance travelled) and the minimum detectable size of the object considered are also reported. UNEP/MAP threshold value for the Mediterranean Sea is reported in red. Studies entirely or partially performed in the MPAs are reported in bold. n.a. is used when no information is available.

Mediterranean Sea sub-region	Sampling_area (MPAs)	Sampling year	Vessel speed (kt)	Observer height (m)	Observed width (m)	Distance travelled (km)	Density items/km^2	Min. detected size (cm)	References
Mediterranean Sea	**-**	**-**	**-**	**-**	**-**	**-**	**5**	**-**	**UNEP/MAP, 2020**
Western Mediterranean Sea	Catalan Sea	2018-2019	n.a.	2.5	10	85	19.7 ± 25.8	n.a.	Garcia-garin et al., 2020
Western Mediterranean Sea	Balearic basin	2005-2015	2	0.5	Nets mouth: 0.8×0.6 m.	613,112	116.6 ± 254.3 kg/km2	2.5	Compa et al., 2019
Western Mediterranean Sea	**Ligurian Sea (Pelagos Sanctuary)**	**2013-2016**	**19-25**	**17-25**	**n.a.**	**3724**	**1.8 ± 0.2**	**20**	**Arcangeli et al., 2018**
Western Mediterranean Sea	Sardinian-Balearic basin	2013-2016	19-25	17-25	n.a.	5098	2.5 ± 0.3	20	Arcangeli et al., 2018
Western Mediterranean Sea	**Bonifacio Strait (Bouches de Bonifacio)**	**2013-2016**	**19-25**	**17-25**	**n.a.**	**2303**	**2.4 ± 0.4**	**20**	**Arcangeli et al., 2018**
Western Mediterranean Sea	Central Tyrrhenian Sea	2013-2016	19-25	17-25	n.a.	2488	2.1 ± 0.4	20	Arcangeli et al., 2018
Western Mediterranean Sea	Sicilian-Sardinian Channel	2013-2016	19-25	17-25	n.a.	4500	2.8 ± 0.5	20	Arcangeli et al., 2018
Western Mediterranean Sea	Balearic Sea, Bonifacio Strait and Tyrrhenian Sea	2013-2016	19–25	17–25	100	18,113	2.3 ± 0.4	20	Campana et al., 2018
Western Mediterranean Sea	**Ligurian Sea (Pelagos Sanctuary)**	**2006–2015**	**6**	**3**	**n.a.**	**5171.57**	**15 ± 23**	**1**	**Di-Méglio and Campana, 2017**
Western Mediterranean Sea	**Ligurian Sea (Pelagos Sanctuary)**	**2014**	**n.a.**	**n.a.**	**20**	**125.53**	**175.2**	**2.5**	**Fossi et al., 2017**
Western Mediterranean Sea	**Ligurian Sea (Pelagos Sanctuary)**	**1996**	**3.2–11.5**	**top deck**	**50**	**176**	**15 –25** (range)	**n.a.**	**Aliani et al., 2003**
Western Mediterranean Sea	**Ligurian Sea (Pelagos Sanctuary)**	**2000**	**6**	**top deck**	**n.a.**	**252**	**1.5 - 3.0** (range)	**n.a.**	**Aliani et al., 2003**
Western Mediterranean Sea	**Ligurian Sea (Pelagos Sanctuary)**	**2013**	**10**	**5**	**30**	**1538**	**24.9 ± 2.5**	**2**	**Suaria and Aliani, 2014**
Western Mediterranean Sea	**Corsica Channel (Pelagos Sanctuary)**	**2013**	**10**	**5**	**n.a.**	**73.1**	**24.7**	**2**	**Suaria and Aliani, 2014**
Western Mediterranean Sea	Strait of Sicily	2013	10	5	n.a.	37.4	10.4	2	Suaria and Aliani, 2014
Western Mediterranean Sea	Central Tyrrhenian Sea	2013	10	5	n.a.	70.1	4.9	2	Suaria and Aliani, 2014
Western Mediterranean Sea	South Tyrrhenian Sea	2013	10	5	n.a.	110.8	24.1	2	Suaria and Aliani, 2014

Western Mediterranean Sea	Sea of Sardinia	2013	10	5	n.a.	103.8	19.3	2	Suaria and Aliani, 2014
Western Mediterranean Sea	Balearic Sea	2013	10	5	n.a.	141.8	30.7	2	Suaria and Aliani, 2014
Western Mediterranean Sea	Algerian Basin	2013	10	5	n.a.	187.4	52.9	2	Suaria and Aliani, 2014
Western Mediterranean Sea	Sardinia Channel	2013	10	5	n.a.	342.8	10.9	2	Suaria and Aliani, 2014
Western Mediterranean Sea	**Corsica Channel (Pelagos Sanctuary)**	**2016**	**7**	**9**	**10**	**n.a.**	**165**	**2.5**	**Campanale et al., 2019**
Western Mediterranean Sea	Sardinian Sea	2016	7	9	10	n.a.	47	2.5	Campanale et al., 2019
Western Mediterranean Sea	Central-southern Tyrrhenian Sea	2016	7	9	10	n.a.	16	2.5	Campanale et al., 2019
Western Mediterranean Sea	Strait of Messina	2016	7	9	10	n.a.	4.8	2.5	Campanale et al., 2019
Western Mediterranean Sea	n.a.	2013	n.a.	n.a.	n.a.	n.a.	40.5	n.a.	Galgani et al., 2013
Western Mediterranean Sea	n.a.	2006-2008	n.a.	n.a.	n.a.	n.a.	3.1	n.a.	UNEP/MAP, 2015
Adriatic Sea	**Croatia (Archipelago of Zadar)**	**2015**	**2–3**	**2.2**	**7**	**36.6**	**175 ± 181**	**2.5**	**Palatinus et al., 2019**
Adriatic Sea	Italy, Slovenia, Croatia, and Montenegro	2014-2015	2–3	1–3	10	415	260 ± 596	2.5–5	Zeri et al., 2018
Adriatic Sea	Italy, Slovenia, Croatia, and Montenegro	2014-2015	26	2_3	100	9.062	4 ± 3	20	Vlachogianni et al., 2017
Adriatic Sea	Italy, Slovenia, Croatia, and Montenegro	2014-2015	25	1_3	8	415	332 ± 749	2.5	Vlachogianni et al., 2017
Adriatic Sea	Slovenia	2008	n.a.	n.a.	n.a.	n.a.	5.7	n.a.	UNEP/MAP, 2015
Adriatic Sea	Slovenia	2011	n.a.	n.a.	n.a.	n.a.	2	n.a.	UNEP/MAP, 2015
Adriatic Sea	Central sector	2013	10	5	n.a.	28.4	54.6	n.a.	Suaria and Aliani, 2014
Adriatic Sea	Southwestern sector	2013	10	5	n.a.	101.8	52.1	n.a.	Suaria and Aliani, 2014
Adriatic Sea	Southeaster sector	2013	10	5	n.a.	47.2	25.8	n.a.	Suaria and Aliani, 2014
Adriatic Sea	Central southern sector	2013-2016	19-25	17-25	n.a.	6733	4.7 ± 0.5	20	Arcangeli et al., 2018

Adriatic Sea	Northern sector	2016	7	9	10	n.a.	414	2.5	Campanale et al., 2019
Adriatic Sea	Central sector	2016	7	9	10	n.a.	535	2.5	Campanale et al., 2019
Adriatic Sea	Southern sector	2016	7	9	10	n.a.	1,313	2.5	Campanale et al., 2019
Adriatic Sea	Central sector.	2013	n.a.	n.a.	31	922.2	31.5	2.5	Carlson et al., 2017
Adriatic Sea	Southern sector	2015	n.a.	n.a.	23.6	922.2	114.7	2.5	Carlson et al., 2017
Adriatic Sea	Northern central sector	2015	n.a.	n.a.	10	922.2	74.8	2.5	Carlson et al., 2017
Ionian Sea and the Central Mediterranean Sea	Ionian Sea	2016	7	9	10	n.a.	100	2.5	Campanale et al., 2019
Ionian Sea and the Central Mediterranean Sea	Ionian Sea	2013-2016	19-25	17-25	n.a.	4565	1.9 ± 0.2	20	Arcangeli et al., 2018
Ionian Sea and the Central Mediterranean Sea	Strait of Otranto	2013	10	5	n.a.	100.2	12.9	2	Suaria and Aliani, 2014
Ionian Sea and the Central Mediterranean Sea	North-western Ionian Sea	2013	10	5	n.a.	61.8	21.6	2	Suaria and Aliani, 2014
Ionian Sea and the Central Mediterranean Sea	Sicilian Sea	2013	10	5	n.a.	26.2	6.3	n.a.	Suaria and Aliani, 2014
Ionian Sea and the Central Mediterranean Sea	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	2.1	n.a.	UNEP/MAP, 2015
Ionian Sea and the Central Mediterranean Sea	**Malta and Gozo mpa**	**2018-2019**	**n.a.**	**2**	**6**	**n.a.**	**681 ± 1,004**	**2.5**	**Curmi and Axiak, 2021**
Ionian Sea and the Central Mediterranean Sea	**Malta and Gozo mpa**	**2018-2019**	**n.a.**	**2**	**6**	**n.a.**	**933 ± 1,594**	**2.5**	**Curmi and Axiak, 2021**
Ionian Sea and the Central Mediterranean Sea	**Malta and Gozo mpa**	**2018-2019**	**n.a.**	**2**	**6**	**n.a.**	**1,272 ± 4,403**	**2.5**	**Curmi and Axiak, 2021**
Ionian Sea and the Central Mediterranean Sea	**Malta and Gozo mpa**	**2018-2019**	**n.a.**	**2**	**6**	**n.a.**	**2,392 ± 7,477**	**2.5**	**Curmi and Axiak, 2021**
Aegean-Levantine Sea	Algeria	2017-2018	n.a.	n.a.	n.a.	n.a.	136,514 max items	2.5	Tata et al., 2020
Aegean-Levantine Sea	n.a.	2017	n.a.	27	50	1784	232 ± 325	2.5 - 50	Constantino et al., 2019
Aegean-Levantine Sea	n.a.	2008	n.a.	n.a.	n.a.	n.a.	2.1	n.a.	UNEP, 2011

Black Sea	n.a.	2017	n.a.	10	50	n.a.	41.5 ± 30.1	2.5	Berov and Klayn, 2021
Mediterranean Sea	n.a.	1979	n.a.	12	10	n.a.	19.7 ± 25.8	1.5	Morris, 1980

Tab. 2: Current status of peer-reviewed papers published on floating microlitter abundance (items/km^2 and corresponding value expressed as items/m^3 "in parentheses") in the Mediterranean Sea Sub Regions proposed by MSFD. Detailed sampling information (sampling site and year, number of samples, sampling nets and mesh) are reported. UNEP/MAP mean baseline value for the Mediterranean Sea is reported in red. Studies entirely or partially performed in the MPAs are reported in bold. n.a. is used when no information is available.

Mediterranean Sea sub-region	Sampling area (MPAs)	Sampling year	N°. Samples	Sampling nets	Net mesh (µm)	Abundance items/km^2 (items/m^3)	References
Mediterranean Sea	**-**	**-**	**-**	**-**	**-**	**340.000**	**UNEP/MAP, 2017**
Western Mediterranean Sea	**Ligurian Sea (Pelagos Sanctuary)**	**2018-2019**	**11**	**Manta trawl**	**300**	**59,388 ± 107,913**	**Tesán Onrubia et al., 2021**
Western Mediterranean Sea	Balearic sea, Mallorca	2017	63	Manta trawl	335	858,029 ± 4,082,964	Compa et al., 2020
Western Mediterranean Sea	**Ligurian Sea (Pelagos Sanctuary)**	**2019**	20	**Manta trawl**	**330**	**255,865 ± 841,221**	**Caldwell et al., 2020**
Western Mediterranean Sea	**Balearic sea, (Menorca channel)**	**2014-2015**	**48**	**Manta trawl**	**333**	**224,294**	**Ruiz-Orejón et al., 2019**
Western Mediterranean Sea	**Ligurian Sea (Pelagos Sanctuary)**	**2018**	**34**	**Manta trawl**	**330**	**28,376 ± 28,917**	**Caldwell et al., 2019**
Western Mediterranean Sea	Alboran and Catalan-Balearic Sea	2015	21	Manta trawl	330	108,000 ± 90,000	de Haan et al., 2019
Western Mediterranean Sea	Gulf of Lion	2014-2016	43	Manta trawl	780	112,000	Schmidt et al., 2018
Western Mediterranean Sea	Balearic sea	2014	20	Manta trawl	333	900,324 ± 1,171,738 (3.28 ± 4.05)	Ruiz-Orejón et al., 2018
Western Mediterranean Sea	**Ligurian Sea (Pelagos Sanctuary)**	**2014**	**21**	**High-speed manta trawl**	**330**	**82,000 ± 79,000**	**Fossi et al., 2017**
Western Mediterranean Sea	**Ligurian Sea (Pelagos Sanctuary)**	**2013**	**33**	**Neuston net**	**200**	**125,930 ± 132,485**	**Pedrotti et al., 2016**
Western Mediterranean Sea	Gulf of Lion, Balearic Islands, Sardinia and Corsica	2012	41	Manta trawl	330	129,682	Faure et al., 2015

Western Mediterranean Sea	**Ligurian Sea (Pelagos Sanctuary)**	**2011-2012**	**38**	**WP2**	**200**	**115,000**	**Collignon et al., 2014**
Western Mediterranean Sea	**Gulf of Lion, Ligurian Sea (Pelagos Sanctuary) and Tyrrhenian Sea**	**2010**	**40**	**Manta trawl**	**333**	**116,000**	**Collignon et al., 2012**
Western Mediterranean Sea	**Tyrrhenian Sea (Pelagos Sanctuary)**	**2013-2014**	**24**	**Manta trawl**	**330**	**69,161 ± 83,244 (0.26 ± 0.33)**	**Baini et al., 2018**
Western Mediterranean Sea	Balearic island and Tyrrhenian Sea	2011	26	Manta trawl	333	101,408 ± 148,114	Ruiz-Orejón et al., 2016
Western Mediterranean Sea	**Balearic sea (Cabrera national park)**	**2019**	n.a.	**Manta trawl**	**330**	**n.a. (3.52 ± 8.81)**	**Fagiano et al., 2022**
Western Mediterranean Sea	Algerian coast	2018	n.a.	n.a.	n.a.	n.a. (0.86 ± 0.35)	Setiti et al., 2021
Western Mediterranean Sea	**Bay of Marseille (Calanque National Park)**	**2017-2018**	n.a.	**Manta trawl**	**150**	**n.a. (0.05)**	**Schmidt et al., 2021**
Western Mediterranean Sea	Gulf of Lion	2015	17	WP2 plankton net	200	n.a. (0.23 ± 0.20)	Lefebvre et al., 2019
Western Mediterranean Sea		2013	74	Neuston net	200	n.a. (1.00 ± 1.84)	Suaria et al., 2016
Western Mediterranean Sea	**Ligurian sea (Asinara National Park and Pelagos Sanctuary)**	**2012**	**70**	**WP2**	**200**	**n.a. (0.31 ± 1.17)**	**Fossi et al., 2016**
Western Mediterranean Sea	**Ligurian sea (Asinara National Park and Pelagos Sanctuary)**	**2012-2013**	**27**	**WP2**	**200**	**n.a. (0.17 ± 0.32)**	**Panti et al., 2015**
Western Mediterranean Sea	Sardinian Sea	2013	30	Manta trawl	500	n.a. (0.15 ± 0.11)	de Lucia et al., 2014
Western Mediterranean Sea	**Ligurian Sea (Pelagos Sanctuary) and Sardinian Sea**	**2011**	**23**	**WP2**	**200**	**n.a. (0.62 ± 2.00)**	**Fossi et al., 2012**
Western Mediterranean Sea	Têt river	2016	13	Manta trawl	333	n.a. (0.18)	Constant et al., 2018
Western Mediterranean Sea	Rhône river	2016	18	Manta trawl	333	n.a. (0.19)	Constant et al., 2018
Western Mediterranean Sea	Tyrrhenian Sea, Eolie islands	2015		Manta trawl and WP2	333	n.a. (0.27 ± 0.08)	de Lucia et al., 2018

Western Mediterranean Sea	**Tyrrhenian Sea, (Ischia, Regno di Nettuno MPA)**	**2015**		**Manta trawl and WP2**	**333**	**n.a. (0.49 ± 0.14)**	**de Lucia et al., 2018**
Western Mediterranean Sea	**Tyrrhenian Sea, (Ventotene MPA)**	**2015**		**Manta trawl and WP2**	**333**	**n.a. (0.20 ± 0.09)**	**de Lucia et al., 2018**
Western Mediterranean Sea	Tyrrhenian Sea	2015		Manta trawl and WP2	333	n.a. (0.23 ± 0.06)	de Lucia et al., 2018
Western Mediterranean Sea	**Ligurian sea (Asinara National Park)**	**2015**		**Manta trawl and WP2**	**333**	**n.a. (0.12 ± 0.04)**	**de Lucia et al., 2018**
Western Mediterranean Sea	Tyrrhenian Sea	2015		Manta trawl and WP2	333	n.a. (0.57 ± 0.16)	de Lucia et al., 2018
Ionian Sea and the Central Mediterranean Sea	Tunisian waters	2017	8	Manta trawl	200	63,739	Zayen et al., 2020
Ionian Sea and the Central Mediterranean Sea	Italian and Greek waters	n.a.	43	n.a.	n.a.	1,300	Morris, 1980
Ionian Sea and the Central Mediterranean Sea	Otranto Strait, North Ionian waters, and Kerkyraikos Gulf	2014-2015	30	Manta trawl	200	410,000	Digka et al., 2018
Ionian Sea and the Central Mediterranean Sea	n.a.	2011	n.a.	Manta trawl	333	181,918 ± 242,799	Ruiz-Orejón et al., 2016
Aegean-Levantine Sea	Iskenderun Bay	2017	14	Manta trawl	333	1,067,120	Gündoğdu et al., 2017
Aegean-Levantine Sea	Iskenderun Bay and Mersin Bay	2016-2017	8	Manta trawl	333	539,189	Gündoğdu et al., 2018
Aegean-Levantine Sea	Iskenderun Bay and Mersin Bay	2016-2017	8	Manta trawl	333	7,699,716	Gündoğdu et al., 2018
Aegean-Levantine Sea	Turkish waters	2015	17	Manta trawl	330	140,418 ± 120,671	Güven et al., 2017
Aegean-Levantine Sea	Iskenderun Bay and Mersin Bay	2016	7	Manta trawl	333	376,000 (2.73)	Gündoğdu and Çevik, 2017
Aegean-Levantine Sea	n.a.	2013-2015	108	Manta trawl	333	n.a. (7.68 ± 2.38)	van der Hal et al., 2017
Aegean-Levantine Sea	Lebanese waters	2018	n.a.	Manta trawl	52	n.a. (4.3)	Kazour et al., 2019

Aegean-Levantine Sea	Turkish waters	n.a.	17	Manta trawl	333	n.a. (0.7)	Güven et al. 2017
Aegean-Levantine Sea	Iskenderun Bay	n.a.	n.a.	Manta trawl	333	n.a. (7.26)	Gündoğdu 2017
Adriatic Sea	Northern sector	2014	8	Manta trawl	330	1,200,861 ± 2,683,014	Vianello et al., 2018
Adriatic Sea	Northern sector	2014	17	Neuston net	300	472,000 ± 201,000	Gajšt et al., 2016
Adriatic Sea	n.a.	2014-2015	2	Manta trawl	308	228,046 ± 30,060	UNEP/MAP, 2015
Adriatic Sea	n.a.	2014-2015	4	Manta trawl	308	287,924 ± 52,979.5	UNEP/MAP, 2015
Adriatic Sea	n.a.	2014	11	n.a.	n.a.	63,175	UNEP/MAP, 2015
Adriatic Sea	**Northern central sector (Archipelago of Zadar)**	**2015**	**26**	**n.a.**	**308**	**127,135 ± 294,847 (0.9 ± 1.9)**	**Palatinus et al., 2019**
Adriatic Sea	Northern central sector	2014-2015	65	Manta trawl	330	315,009 ± 568,578	Zeri et al., 2018
Adriatic Sea	Northern sector	2014-2015	n.a.	n.a.	308	259,310 ±57,096	Kovač Viršek et al., 2017
Adriatic Sea	Northern sector	2014-2015	n.a.	Manta trawl	308	1,304,811 ± 609,426	Kovač Viršek et al., 2017
Adriatic Sea	Northern sector	2011	11	Manta trawl	333	178,676 ± 292,753	Ruiz-Orejón et al., 2016
Adriatic Sea	Central sector	2018	7	Manta trawl	300	n.a. (0.8)	Capriotti et al., 2021
Adriatic Sea	Southern sector, Tremiti islands	2015	n.a.	Manta trawl and WP2	333	n.a. (0.16 ± 0.04)	de Lucia et al., 2018
Adriatic Sea	Northern sector, Po' river	2015	n.a.	Manta trawl and WP2	333	n.a. (0.64 ± 0.23)	de Lucia et al., 2018
Adriatic Sea	Northern sector, Po' river	2016	n.a.	Manta trawl	300	n.a. (1 – 84 range)	Atwood et al., 2019

Black Sea	Marmara Sea	2017	18	n.a.	333	12,626,775	Tuncer et al., 2018
Black Sea	South-western sector	2017	10	Manta trawl	300	46,200	Berov and Klayn, 2020
Black Sea	Southern sector	2015-2016	n.a.	Manta trawl	300	656,000	Oztekin and Bat, 2017
Black Sea	Marmara Sea	2017	18	Manta trawl	333	n.a. (12.6)	Tuncer et al., 2018
Black Sea	Romanian waters	2018	12	Neuston net	200	n.a. (7)	Pojar et al., 2021
Black Sea	n.a.	2014-2015	12	Neuston net	200	n.a. (11,000)	Aytan et al., 2016
Black Sea	North-western sector	n.a.	12	Neuston net	200	n.a. (9)	Pojar and Stock 2019
Black Sea	Southern sector	2015-2016	n.a.	Neuston net	300	n.a. (2.67 ± 2.33)	Oztekin and Bat, 2017
Whole Mediterranean	n.a.	2013	39	Neuston net	200	243,853	Cózar et al., 2015

1.4.2 Beach litter: state of the art

Beaches can be defined as a highly dynamic ecosystem in which the presence and distribution of debris are influenced by several factors. The geological setting, the complex hydrodynamics regulated by currents, wave action and tidal excursions that characterize the intertidal zone, and atmospheric events such as rain and wind can combine to promote the presence and distribution of litter (GESAMP, 2019). In this complex scenario, anthropogenic influences (e.g., tourism and recreational activities) and missed or improper recycling and storage processes of waste materials can strongly influence the accumulation of waste. The presence of macro waste, with particular attention to macro and microplastics, has been extensively studied on the beaches of different Mediterranean countries (Tabs. 3 and 4). Due to the different protocols (e.g., OSPAR, MSFD TG 10, EA /NALG 2000, UNEP list, SEACleaner protocol, ICC and UNEP/IOC), frequency and timing, sampling unit monitored (e.g., 50-m transect, 100-m transect, no fixed transect), heterogeneity of the target sampled (macro objects or microscopic particles), and different units of measure used to report results (items/m, items/m^2, items/m^3 and items/kg dry sediment), it is not always possible to adequately compare available data. For this reason, the MSFD Technical Group on Marine Litter (MSFD TG ML) has produced the Joint List (JL), which allows a comparable monitoring of marine litter in the European regional seas and the different marine compartments. It was based on the "Master List" published in 2013 by MSFD TG ML (Galgani et al., 2013) and combines litter types from different monitoring lists (OSPAR, ICES, UNEP, etc.) to provide an updated, refined and fine-tuned list of litter occurring in the coastal and marine environment. Based on a hierarchical system with different levels of detail in the characterization of the collected objects, starting with the classes of materials they are composed of, the JL aims to facilitate the harmonized collection of litter items and to allow the linking of marine litter monitoring data with the potential sources of pollution to better define the mitigation and prevention actions to be taken (Fleet et al., 2021). Considering the abundance of macrolitter on beaches, the MSFD TG ML has set a threshold of 20 litter/100 m of coastline (Van Loon et al., 2020). Few studies report litter concentrations as suggested by the MSFD, and in every case, these levels exceed the threshold (Fortibuoni et al., 2021; Gjyli et al., 2020; Maziane et al., 2018; Nachite et al., 2019; Vlachogianni et al., 2020). Vlachogianni et al. (2020), who evaluated macrolitter densities on beaches in different sectors of the Mediterranean Sea, reported a mean concentration of 714 items/100m (0.61 items/m^2), with sites in the Adriatic, Ionian and Central Mediterranean Sectors identified as the most polluted. The highest concentrations were also identified in the Aegean and Levantine basins and on the Turkish coast (Gündogdu and Çevik 2019, Gündogdu et al., 2019). This area, already identified as particularly prone to the accumulation of scattered floating marine debris, also appears to be characterized by high flows of stranded objects (Mansui et al., 2015) and MPs, as confirmed by Lots et al. (2017). The presence and distribution of MPs on the beaches of the Mediterranean Sea are highly variable (Tab. 4), with some local pollution hotspots on the coasts of Slovenia (Adriatic Sea) (UNEP/MAP, 2015), the island of Malta (Ionian and Central Mediterranean Sea) (Turner and Holmes, 2011), Algeria (Western Mediterranean) (Tata et al., 2020), the Tyrrhenian Sea (Cesarini et al., 2021), Turkey and Israel (Aegean Sea) (Lots et al., 2017).

Tab. 3: Current status of peer-reviewed papers published on beach macrolitter abundance (items/100m, items/m^2 or specified) in the Mediterranean Sea Sub Regions proposed by MSFD. Detailed sampling information (sampling site and year, n.° of beaches monitored, and protocol adopted) and the minimum detectable size of the object considered are also reported. EU MSFD TG 10 and UNEP/MAP 2020 threshold values for the Mediterranean beaches are reported in red. Studies entirely or partially performed in the MPAs are reported in bold. n.a. is used when no information is available.

Mediterranean Sea sub-region	Sampling area (MPAs)	Sampling year	N°. beach	Beach litter Protocol	Min. detected size (mm)	N°. Items	Density	Reference
Mediterranean Sea	**-**	**-**	**-**	**-**	**25**	**-**	**20 items/100m** **59 items/100m**	**Van Loon et al., 2020** **UNEP/MAP, 2020**
Western Mediterranean Sea	Spain	2018	56	EA/NALG (2000)	n.a.	10,101	0.12 items/m^2	Asensio-Montesinos et al., 2019a
Western Mediterranean Sea	Morocco	2015-2017	14	UNEP/MAP (2016) and EU MSFD TG10	n.a.	n.a.	0.054 ± 0.036 items/m^2 (390.8 ± 255.3 items/100m)	Nachite et al., 2019
Western Mediterranean Sea	**Tyrrhenian Sea (Torre Flavia Wetland)**	**2018**	**1**	n.a.	n.a.	**276**	**n.a.**	**Battisti et al., 2019**
Western Mediterranean Sea	**Ligurian Sea (Pelagos Sanctuary)**	**2014-2015**	**11**	**SEACleaner Protocol:**	**25**	**34,027**	**1.06 items/m^2**	**Giovacchini et al., 2018**
Western Mediterranean Sea	**Ligurian Sea (Pelagos Sanctuary)**	**2014-2016**	**6**	**OSPAR**	**5**	**500**	**0.72 ± 0.43 items/m^2 (plastic objects)**	**Merlino et al., 2018**
Western Mediterranean Sea	Morocco	2015	n.a.	UNEP/MAP 2016	25	8,021	494 items/100m	Maziane et al., 2018
Western Mediterranean Sea	**Tyrrhenian Sea (Torre Flavia Wetland)**	**n.a.**	**17**	**n.a.**	**n.a.**	**6,700**	**n.a.**	**Battisti et al., 2016**
Western Mediterranean Sea	**Sardinean Sea (Pelagos Sanctuary)**	**2013-2016**	**7**	**EU MSFD TG10**	**5**	**39,972**	**n.a.**	**Camedda et al., 2021**
Western Mediterranean Sea	Spain	2013-2014	12	n.a.	n.a.	n.a.	11-2,263 items/100m (range)	UNEP/MAP 2015
Western Mediterranean Sea	Spain	2013-2014	27	n.a.	n.a.	n.a.	11-2,137 items/100m (range)	UNEP/MAP 2015
Western Mediterranean Sea	Spain	2012	1	n.a.	n.a.	n.a.	2,245 items/m^2	UNEP/MAP 2015
Western Mediterranean Sea	Tyrrhenian Sea	2012	153	n.a.	n.a.	n.a.	n.a.	Poeta et al., 2014
Western Mediterranean Sea	France	2011	15	n.a.	5	n.a.	2,920 items/m^2	Klosterman et al., 2012

Western Mediterranean Sea	Balearic Islands	2015	32	n.a.	n.a.	11,321	36 items/m	Martinez et al., 2007
Western Mediterranean Sea	Spain	1984	18	n.a.	n.a.	n.a.	n.a.	Shiber 1987
Western Mediterranean Sea	Spain	1980-1981	13	n.a.	n.a.	n.a.	n.a.	Shiber 1982
Ionian and Central Mediterranean Sea	Corfù	2014-2015	4	EU MSFD TG10	25	41,617	0.08 - 0.91 items/m^2 (range)	Prevenios et al., 2018
Ionian and Central Mediterranean Sea	Greece	2014-2015	6	n.a.	n.a.	n.a.	0.715 items/m^2	UNEP/MAP 2015
Ionian and Central Mediterranean Sea	Greece	2012	12	n.a.	n.a.	n.a.	10 – 1218 items/m^2 (range)	UNEP/MAP 2015
Ionian and Central Mediterranean Sea	Greece	2006-2007	80	n.a.	n.a.	110,423	n.a.	Kordella et al., 2013
Aegean-Levantine Sea	Turkey	2018	39	n.a.	25	n.a.	19.5 ± 1.2 items/m^2	Gundogu et al., 2019
Aegean-Levantine Sea	Turkey	2018	13	n.a.	5	1424	12.2 ± 3.5 items/m^2	Gündogdu and Çevik 2019
Aegean-Levantine Sea	Israel	2012-2015	9	ICC and UNEP/IOC	25	69,122	0.12 items/m^2	Pasternak et al., 2018
Aegean-Levantine Sea	Israel	2017	3	EU MSFD TG10	25	n.a.	5.1 items/m^2	Portman et al., 2017
Aegean-Levantine Sea	Turkey	2014	13	EU MSFD TG10	25	n.a.	0.92 ± 0.36 items/m^2	Aydın et al., 2016
Aegean-Levantine Sea	Greece, Lesvos Island	2015	2	n.a.	n.a.	810	n.a.	Katsanevakis et al., 2015
Aegean-Levantine Sea	Turkey	2003	1	n.a.	n.a.	n.a.	21,915 kg	Özdilek et al., 2006
Aegean-Levantine Sea	Israel	1988-1989	6	n.a.	20	17,355	n.a.	Golik 1992
Aegean-Levantine Sea	Lebanon	1979	2	n.a.	n.a.	n.a.	n.a.	Shiber 1979

Adriatic Sea	**Southern sector (Torre Guaceto)**	**2021**	n.a.	**EU MSFD TG10**	**20**	**47**	**0.47 items/m^2**	**Rizzo et al., 2021**
Adriatic Sea	Albania	2018	10	EU MSFD TG10	25	n.a.	0.14 items/m^2 (333 items/100m)	Gjyli et al., 2020
Adriatic Sea	Slovenia	2017	9	n.a.	n.a.	231	7.2 ± 1.9 - 10.9 ± 6.0 items/kg (range)	Korez et al., 2019
Adriatic Sea	Central sector	2014-2015		OSPAR	20	2,040	n.a.	de Francesco et al., 2018
Adriatic Sea	Southern sector, Montenegro	2017	121	EU MSFD TG10	n.a.	585	n.a.	Šilc et al., 2018
Adriatic Sea	Northern sector, Po' river	2015	5	UNEP/IOC	20	2,502	0.2 items/m^2 282– 1,143 items/100m (range)	Munari et al., 2016
Adriatic Sea	Northern sector, Slovenia	2014-2015	3	n.a.	20	n.a.	(3.95 items/m)	UNEP/MAP 2015
Adriatic Sea	Northern sector, Slovenia	2014	27	n.a.	n.a.	n.a.	3-80 items/100g	Bajt et al., 2015
Adriatic Sea	Northern sector, Slovenia	2007-2013	6	n.a.	20	n.a.	1.9 items/m^2	UNEP/MAP 2015
Adriatic Sea	Northern sector	2015	2	n.a.	n.a.	n.a.	1.13 items/m^2	UNEP/MAP 2015
Adriatic Sea	Northern sector, Slovenia	2007	3	n.a.	n.a.	n.a.	12,158 items/km	UNEP/MAP 2015
Adriatic and Ionian and Central Mediterranean Sea	Albania, Bosnia and Herzegovina, Croatia, Greece, Italy, Montenegro, and Slovenia	2014-2016	31	EU MSFD TG10	25	70,581	0.67 items/m^2	Vlachogianni et al., 2018
Mediterranean Sea	Italy peninsula	2015-2018	64	EU MSFD TG10	25	317,745	477 items/100m	Fortibuoni et al., 2021
Mediterranean Sea	Croatia, Cyprus, France, Greece and Italy	2018	62	EU MSFD TG10	25	37,991	0.61 items/m^2 (714 items/100m)	Vlachogianni et al., 2020
Mediterranean Sea	Mallorca, Sicily, Rab, Malta, Crete, Rhodes and Mikonos and Cyprus	2017	147	EU MSFD TG10		162,320	526.9 ± 794.2 items/100m	Grelaud and Zivieri, 2020
Mediterranean Sea	Spain, Italy (Sicily), Turkey, Cyprus and Israel	1988-1989	13	n.a.	10	n.a.	32.4 items/m	Gabrielides et al., 1991

Tab. 4: Current status of peer-reviewed papers published on beach microlitter abundance (items/m^2, items/kg or specified) in the Mediterranean Sea Sub Regions proposed by MSFD. Detailed sampling information (sampling site and year, n.° of beaches monitored, and protocol adopted) and the minimum detectable size of the object considered are also reported. Studies entirely or partially performed in the MPAs are reported in bold. n.a. is used when no information is available.

Mediterranean Sea sub-region	Sampling area (MPAs)	Sampling year	N°. beach	Beach litter Protocol	Min. detected size (mm)	N°. Items	Density	Reference
Western Mediterranean Sea	**Ligurian Sea (Pelagos Sanctuary)**	**2015-2017**	**1**	**DeFishGear Protocols**	**1.2 µm**	**n.a.**	**207 ± 30 items/kg dry sediment**	**Scopetani et al., 2021**
Western Mediterranean Sea	**Sardinean Sea (Pelagos Sanctuary)**	**2013-2016**	**7**	**EU MSFD TG10**	**5**	**72,922**	**n.a.**	**Camedda et al., 2021**
Western Mediterranean Sea	**Tyrrhenian Sea (Torre Flavia Wetland)**	**2019**	**1**	**EU MSFD TG10**	**2.5 - 5**	**n.a.**	**140 items/m^2**	**Cesarini et al., 2021**
Western Mediterranean Sea	Algeria	2017-2018	4	EU MSFD TG10 and OSPAR	n.a.	1619	182.66 ± 27.32 - 649, 33 ± 184,02 items/kg dry sediment (range)	Tata et al., 2020
Western Mediterranean Sea	**Ligurian Sea (Pelagos Sanctuary)**	**2016-2017**	**3**	**EU MSFD TG10**	**1**	**26,486**	**535.13 ± 389 items/m^2**	**Merlino et al., 2020**
Western Mediterranean Sea	France, Tet River	2016	2	n.a.	0.7 µm	7,049	166 ± 205 - 58 ± 53 items/kg dry sediment (range)	Constant et al., 2019
Western Mediterranean Sea	**Spain, Murcia (Mar Menor Lagoon)**	**2017-2018**	**17**	**n.a.**	**0.45 µm**	**742**	**105.4 ± 9.2 items/kg dry sediment**	**Bayo et al., 2019**
Ionian and Central Mediterranean Sea	**(Malta MPA)**	**2018**	**4**	n.a.	**1**	**10,975**	**n.a.**	**Gauci et al., 2019**
Ionian and Central Mediterranean Sea	**(Malta MPA)**	**2010**	**8**	**n.a.**	**< 5**	**n.a.**	**14.2 items/m^2**	**Turner and Holmes. 2011**
Ionian and Central Mediterranean Sea	Greece, Salamina island	2016	2	n.a.	0.5 - 5	98	n.a.	Tziourrou et al., 2019
Aegean-Levantine Sea	Cyprus	2016	17	n.a.	1	n.a.	45,497 ± 11,456 items/m^3	Duncan et al., 2018
Adriatic Sea	Northern sector	2016	3	n.a.	1	3395	2.92 ± 4.86 items/kg dry sediment	Piehl et al., 2019
Adriatic Sea	Northern sector, Slovenia	2014-2015	1	n.a.	< 5	n.a.	MPs large: 516 ± 224 items/kg dry sediment MPs small: 616 ± 325 items/kg dry sediment	UNEP/MAP 2015
Adriatic Sea	Northern sector, Slovenia	2012	6	Cheshire et al. (2009).	< 5	5870	1.25 items/m^2 (MPs: 1.51 items/m^2)	Laglbauer et al., 2014

Mediterranean Sea	Western and eastern sectors	2015-2017	23	www.lucmicroplastic.wordpress.com	0.3	n.a.	291 ± 62 items/kg dry sediment	Lots et al.,2017

1.5 Plastic ingestion in marine biota: a major threat to the Mediterranean Sea

Ingestion of plastic litter, although less visible than entanglement, is one of the most threatening impacts on marine organisms. However, it is difficult to determine and quantify the causal links between mortality and ingestion of plastic particles. There is a growing number of researches to better understand the origin of pollution and causes of death (Unger et al., 2016). Plastic ingestion has been found in a variety of organisms, from invertebrates to vertebrates, including endangered species (Duncan et al., 2017; Fossi et al., 2018a; Kühn et al., 2015, 2020; Laist et al., 1997). Globally, the total number of organisms affected by this phenomenon has increased from 143 in 1997 (Laist, 1997) and 233 in 2015 (Kühn et al., 2015) to at least 714 species currently (Kühn et al., 2020). Marine organisms may ingest litter intentionally because it resembles prey (Campani et al., 2013; Cole et al., 2011; Romeo et al., 2016; Wright et al., 2013) or incidentally during feeding (Battaglia et al., 2016; Fossi et al., 2014; Romeo et al., 2015) or as a result of secondary ingestion. Depending on litter size and species, plastic particles may be excreted or accumulated in the gastrointestinal tract and cause physical and mechanical damage such as abrasion, inflammation, clogging of food appendages or filters, gastrointestinal tract obstruction (Cole et al., 2011; Li et al., 2016; Peda et al., 2016; Wright et al., 2013), or pseudo-satiation resulting in reduced food intake (Kuhn et al., 2015). Ingestion of plastics by organisms in the Mediterranean Sea has been reported since 1988, with a significant increase in the number of scientific papers in recent years. In this basin, plastic ingestion has been documented for 127 species from different taxonomic groups (Annexe 1). In particular, plastic ingestion by fish species has gained particular interest over the last decade, related to concerns about their value as fisheries resources. The first information came from studies on the feeding ecology of Mediterranean species (Carrason et al., 1992; Deudero, 1998; Madurell, 2003; Massuti et al., 1998), but in recent years the detection of debris in the gastrointestinal tract has been the main objective of most studies in this field. About 70% of fish species belonging to 17 orders are reported to ingest plastic debris (Annexe 1).

All Mediterranean turtles (*Caretta caretta, Chelonia mydas* and *Dermochelys coriacea*) and some marine mammals (*Physeter macrocephalus*, *Balaenoptera physalus*, *Grampus griseus*, *Ziphius cavirostris*, *Tursiops truncatus*, *Stenella coerulealba*, *Delphinus delphis* and *Phocoena phocoena*) have been affected by debris ingestion (Annexe 1). Most studies on these endangered species dealt with stranded individuals. Plastic ingestion by seabirds is a well-documented phenomenon worldwide, first reported by Laist (1997) and deeply investigated by Kühn et al. (2020), whereas in the Mediterranean basin only one study by Codina-Garcia et al. (2013) investigated the presence of marine litter in bird species belonging to the order Procellariiformes, Suliformes and Charadriiformes (Annexe 1). The few available studies on plastic ingestion by marine invertebrates (Alomar et al., 2016; Cristo and Cartes, 1998; Digka et al., 2018; Fossi et al., 2014; Gusmao et al., 2016; Remy et al., 2015; Vandermeersch et al., 2015) investigated several species belonging to the annelids, crustaceans, echinoderms, cnidarians and molluscs (Annex 1). Although the number of studies has increased in recent years, information on the interactions between marine debris and Mediterranean biota remains very scarce and inconsistent. As suggested by Deudero and Alomar (2015), this is partly due to the lack of standardized methods and protocols for monitoring and sampling procedures. In addition, most studies only consider the occurrence of macro and mesolitter in marine organisms, which may lead to underestimation of the impact of microparticles.

1.6 Plastic particles chemical hazard

In addition to the physical harm associated with marine litter, recently concern is growing regarding the chemical hazard related to the ingestion of marine litter. Plastic additives (e.g., PBDEs and PAEs) could be directly leached from plastic debris, leading to the accumulation within marine organisms of chemicals such as persistent, bioaccumulating and toxic substances that are absorbed and transported by marine debris.

PAEs presence has recently attracted the attention of the scientific community. This is a group of chemicals widely used as additives to make plastics more flexible and harder to break, and their content can be up to 10 − 60% by weight (Earls et al., 2003). Industrial formulations of phthalate esters include a large number of compounds, which vary in alkyl chain length, branching and molecular weight with a total European consumption accounting for approximately 1 million tons (Net et al., 2015; Mackintosh et al., 2004). Not chemically but only physically bound to the polymeric matrix, PAEs can easily be released into the environment directly and/or indirectly, during manufacture, use, and disposal (Net et al., 2015). Numerous studies have confirmed their presence in the air, soil, water and animal and human body fluids (Net et al., 2014; Staples et al., 1997; Xie et al., 2007). However, as most of the plastic additives exhibit high K_{ow} (octanol−water partitioning), higher concentrations are expected in sediment and marine organisms (Hermabessiere et al., 2017). PAEs can have various noxious toxic effects on organisms. In particular, they can act as endocrine disruptors (EDs) even at very low concentrations. EDs are chemicals with molecular features that are similar to those of hormones secreted by the endocrine system. EDs can interact with hormone synthesis and alter reproduction or other physiological and metabolic functions (e.g., causing oxidative stress, immunotoxicity) of organisms (Mathieu-Denoncourt et al., 2015; Talsness et al., 2009). For that reason, the following eight phthalates: dibutyl phthalate (DBP), diisobutyl phthalate (DIBP), butyl benzyl phthalate (BBP), di-n-pentyl phthalate (DnPP), di(2-ethylhexyl) phthalate (DEHP), di-n-octyl phthalate (DnOP), diisononyl phthalate (DINP), and diisodecyl phthalate (DIDP) are listed as priority pollutants by the Environmental Protection Agency and European Union taking into account their toxicity, prevalence in the environment and widespread use (Savoca et al., 2021).

Some studies have shown that plastic additives, especially PAEs and PBDEs, are quantifiable in MPs found in sediments or marine waters, suggesting that leaching of additives into the environment is occurring. This is of great concern as microplastics have a high propensity to reach all trophic levels due to their small size and ubiquity in the marine environment, and as leaching into the digestive tract of organisms can also occur when MPs are ingested (Alkan et al., 2021; Faure et al., 2015; Paluselli et al., 2018; 2019; Rochman et al., 2014). The possible link between the chemical effects of plastic ingestion and the risk of bioaccumulation along the trophic web has been evaluated by Fossi et al. (2014), Fossi et al. (2016), and Baini et al. (2017). In assessing the levels of phthalates and organochlorines in samples of *Euphausia krohnii*, *Cetorhinus maximus*, and four cetaceans species (*Balaenoptera physalus, Tursiops truncatus, Grampus griseus, and Stenella* *coeruleoalba*), the presence of various toxic compounds was used as a possible indicator of exposure to plastic ingestion. PAEs levels in Mediterranean species have been evaluated also in two species of sea turtles (*Caretta caretta* and *Dermochelys coriacea*) (Savoca et al., 2018; 2021) several fish species (Gugliandolo et al., 2020; Guerranti et al., 2016; Fourgous et al., 2016) and invertebrates (Lo Brutto et al., 2021; Schmidt et al., 2021). When these chemicals become bioavailable, they can enter cells and chemically interact with biologically important molecules. This can lead to adverse effects at various levels of biological organisation, from the molecular level to the tissue level, including liver toxicity (Avio et al., 2015; Rochman et al., 2013), changes in gene expression (Karami et al., 2017; Sleight et al., 2017), genotoxic effects (Avio et al., 2015), endocrine disruption (Rochman et al., 2014; Teuten et al., 2009), and histological changes (Peda et al., 2016). However, most of these effects have been demonstrated in laboratory studies. Very few data are available from field studies, especially in Mediterranean organisms.

1.7 The Plastic Busters MPAs – Interreg MED – Project

The Plastic Busters MPAs project is a concrete example of a Mediterranean partnership that consolidates the fight against marine litter in Mediterranean MPAs and bridges the gap between science, policy and society (https://plasticbustersmpas.interreg-med.eu/). It was awarded in 2016 under the Union for the Mediterranean and received political support from 43 European Mediterranean countries. This project uses the multidisciplinary strategy and common framework of activities developed under the Plastic Busters initiative led by the University of Siena, the flagship project of the UN Mediterranean Sustainable Development Solutions Network.

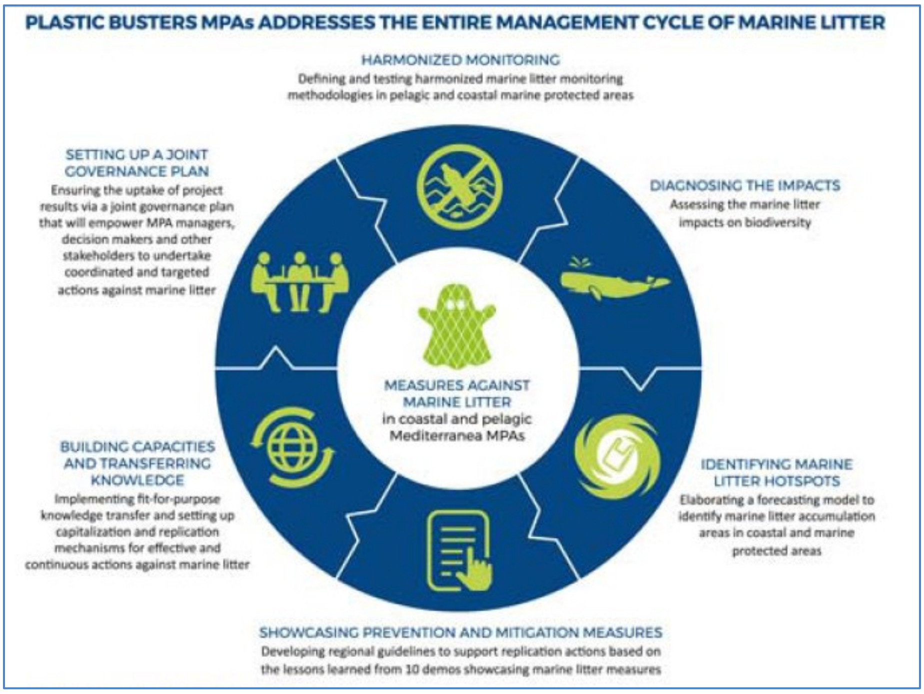


Fig. 5. PB MPAs Interreg Med Projects goals.

The Plastic Busters MPAs project is a 4-year Interreg Mediterranean funded project that aims to contribute to the conservation of biodiversity and natural ecosystems in pelagic and coastal MPAs and to strengthen connectivity between these areas to address the full cycle of marine litter management (Fig. 5). A total of 15 partners and 17 associated partners are involved in this project, which focuses on four marine protected areas in the Mediterranean Sea: the Pelagos Sanctuary Protected Area, the Tuscan Archipelago National Park, the Cabrera Archipelago National Park and the Zakynthos National Marine Park. An *ad hoc* experimental design will be carried out in each protected area, taking into account their extent, habitat diversity, and potential litter sources, by harmonising and implementing the current sampling methodologies of marine litter in the different environments. A new simultaneous multi-level approach, integrating the existing knowledge, will be defined and tested to evaluate the marine litter potential impacts. Reliable information on the distribution of marine litter at the sea surface, on the seafloor and beaches, will be collected to assess the potential physical and chemical effects of plastic litter on various selected Mediterranean bioindicators. Finally, a comprehensive risk assessment will be carried out to identify critical areas and provide the basis for the development of effective protection and mitigation measures to be taken forward. The gaps identified through this project and the selected sentinel species could provide a useful improvement for the implementation of descriptor 10 (D10) in the MSFD (Galgani et al. 2013), the Integrated Monitoring and Assessment Programme (IMAP) indicators and the implementation of mitigation measures for habitats and species affected by marine litter ingestion in the Mediterranean Sea (RAC/SPA 2017).

CHAPTER 2: AIM OF THE STUDY

Effective measures to tackle marine litter concerns in the Mediterranean Sea are limited by the lack of clarity of its induced effects at the ecological level. The pathways and spatial and temporal patterns of exposure of organisms and habitats remain poorly understood due to the lack of harmonized monitoring approaches, which still represent a challenging issue to be addressed to make data on marine litter reliable and comparable. Despite the growing attention of the scientific community and the increasing number of peer-reviewed publications, there is still little research on the occurrence and distribution of plastic litter in marine protected areas and its impact on marine life. To provide further information for addressing plastic pollution in MPAs and recommendations for management, this study, within the PB MPAs Project, aims to gain a better understanding of the problem of plastic litter in the Pelagos Sanctuary Special Protected Area of Mediterranean Interest (SPAMI), a pelagic area with high biodiversity, and in the Tuscan Archipelago National Park, a coastal area with high ecological value. Due to the different oceanographical features, habitat and extent of these MPAs, a wide range of marine organisms could be sampled, from invertebrates to cetaceans and from coastal to pelagic species, which could provide a comprehensive and integrated framework of the current situation.

The main objectives of the study are:

a) To harmonise and implement the current sampling methodologies of marine litter in the different environments, defining and testing a new simultaneous multi-level experimental design based on the characteristics of the protected areas to be sampled (Chapter 3).

b) To provide a comprehensive assessment of the quantities and composition of floating marine litter in pelagic and coastal MPAs (Chapter 4).

   Specifically:

   (1) *Ad hoc* sampling campaigns based on both the oceanographical characteristics and the extent of the monitored MPAs were conducted to simultaneously collect data on the abundance, distribution and characterization of macro and microlitter.

   (2) Conduct a hotspot analysis/mapping of areas of marine debris accumulation to support the implementation of targeted marine debris management actions at the most impacted locations.

c) To perform a comprehensive assessment of the quantities and composition of beach litter at various sites monitored along the Tuscan coast, in the Tuscan Archipelago National Park and the southern part of Corsica (Chapter 4).

   Specifically:

   (1) Potential seasonal variations in the quantity, distribution and characterization of macro and microlitter were assessed.

   (2) Analysis of potential sources of pollution and associated property uses were examined to help determine specific mitigation measures.

d) To evaluate the physical and chemical impacts of marine litter on biota, according to trophic level and feeding habits (Chapter 5).

   Specifically:

(1) Ingestion of plastics and characterization of isolated particles were evaluated in known (*Mytilus galloprovincialis* and *Mullus surmuletus*) and potentially novel (*Myctophum punctatum* and *Velella velella*) species of bioindicators of plastic ingestion.
(2) Plastic ingestion and characterization of isolated particles were studied in stranded endangered species and sentinel species: seabirds, sea turtles and cetaceans.
(3) The levels of PAE compounds associated with plastic particle ingestion in various biological tissues of the above mentioned marine organisms were evaluated.

e) To allow a concurrent assessment of the richness of biodiversity in the MPAs studied, a preliminary risk assessment was carried out to show the harm caused to marine fauna by floating plastics (Chapter 6).

Specifically:

(1) Spatial distribution of marine megafauna and associated species was monitored simultaneously during the sampling campaigns.
(2) The density of marine floating debris was overlaid with the distribution of the most commonly sighted species to perform a spatial risk assessment.

The final results of this project obtained through the development and application of a new simultaneous multi-level approach, which integrates the existing sampling methodologies, will provide indications on the marine litter potential threats in the Pelagos Sanctuary and Tuscan Archipelago National Park. The main anthropogenic factors and oceanographic features influencing the spatial distribution of marine litter in pelagic and coastal zones will be investigated, as well as the potential physical and chemical impacts of plastic, and in particular MPs, on various selected Mediterranean bioindicators. Finally, a marine litter spatial risk assessment will be carried out to identify critical areas and provide the basis for the development of effective protection and mitigation measures to be taken forward for the implementation of descriptors indicated by the MSFD and the Integrated Monitoring and Assessment Programme (IMAP).

CHAPTER 3: THE EXPERIMENTAL DESIGN

The management of marine litter in the Mediterranean Sea falls within the framework of two main regional drivers: the Regional Plan on Marine Litter Management in the Mediterranean (UN Environmental Programme/Mediterranean Action Plan), and the Marine Strategy Framework Directive (MSFD; 2008/56/EC, Descriptor 10) for the European marine waters. In this context, the Interreg MED Project PB MPAs was conceived to address marine litter impacts in specific areas of the Mediterranean basin described as key zones of biological and ecological importance to be potentially harmed by marine litter pollution where appropriate conservation measures should be adopted and recognized. Among the areas selected within the project (see Section 1.7), this thesis focuses on two protected areas located in the northwestern sector of the Mediterranean Sea: the Pelagos Sanctuary for Mediterranean Marine Mammals and the Tuscan Archipelago National Park.

The Pelagos Sanctuary for Mediterranean Marine Mammals, hereafter Pelagos Sanctuary, is a Special Protection Area of Mediterranean Importance (SPAMI) established in 1999 by an international agreement between Italy, France and the Principality of Monaco, representing the most extended protected area of the whole basin. It covers an area of 87,500 km^2, characterized by a very high primary productivity and different habitats suitable for the breeding and feeding activities of all cetacean species regularly found in the Mediterranean Sea. The Sanctuary was established to protect marine mammals and their habitat and to better assess actual and potential threats to the cetacean populations (e.g., intense shipping traffic, fishing, whale-watching activities, chemical pollution, coastal development, military exercises, seismic prospecting, and global climate change) (Mackelworth, 2016; Notarbartolo-di-Sciara et al., 2008; Notarbartolo-di-Sciara and Birkun, 2010; Panigada et al., 2017; Pennino et al., 2017). Recently, it has been recognized as an area particularly affected by high concentrations of microplastics and plastic additives, which may constitute an additional threat to the endangered species inhabiting the area (baleen whales, sea turtles, filter-feeding sharks) (Baini et al. 2017; Fossi et al. 2012, 2014, 2016, 2017, 2018a; Germanov et al. 2018) and to the overall biodiversity of the Mediterranean Sea (Compa et al. 2019; Deudero and Alomar 2015; Galgani et al. 2014; Romeo et al. 2015;).

Located in the eastern sector of the Pelagos Sanctuary between the island of Corsica (France) and the Tuscan coast, the Tuscan Archipelago National Park is the largest marine park in the Mediterranean as well as being classified as a biosphere reserve (Angeletti and Ceregato, 2010). It consists of 7 main islands: Gorgona, Capraia, Elba, Pianosa, Montecristo, Giglio and Giannutri, intermingled with countless smaller islands managed according to different levels of protection (Fratini et al., 2013). Protection Zone 1 or integral reserve refers to adjacent strips of water up to 1 km offshore, where recreational fishing and diving, as well as overnight mooring and circumnavigation, are prohibited. This level of protection, which also regulates scientific activities, fully affects the islands of Montecristo and Pianosa and partially the islands of Gorgona, Capraia and Giannutri, which fall under protection zone 2 (simple protection) (Renzi et al., 2010). No restrictions concern the islands of Elba and Giglio. The Tuscan Archipelago is an interesting study area for the central Tyrrhenian Sea due to its geographical position, geomorphological structure and high biological value given by the presence of several fish nursery areas (Renzi et al., 2010; Sbrana et al., 2016; Serena et al., 1998). Nevertheless, the intense maritime traffic, the pressure of tourist activities and the presence of several local pollution sources (e.g., maritime and commercial ports, river inputs, agricultural land and industrial activities) (Renzi et al., 2010) make this area highly anthropized and prone to the accumulation of floating plastic. This is confirmed by the temporary formation of a well-known retention area near the island of Capraia, where floating debris may accumulate (Fossi et al., 2017; Suaria et al., 2016).

According to the main aims of the PB MPAs project, a multidisciplinary approach was carried out to assess marine litter impacts on the selected areas. The quantification of plastic items in the environment was integrated with environmental and oceanographic features data and with the information obtained from endangered and bioindicator species to gather knowledge not only on the occurrence of marine litter within species and their environment but also to figure out the threat posed to the organisms and the potential related biological effects (Fossi et al., 2018b). Therefore, widely recognized distinct methodologies already adopted by other European projects (e.g. DeFishGear, MEDSEALITTER and INDICIT) were harmonised and tested to define a new simultaneous approach at multiple scales, to create a standardized protocol providing comparable and reliable data. These data could be useful for the improvement of descriptor 10 (D10) in the MSFD (Galgani et al. 2013) and the Integrated Monitoring and Assessment Programme (IMAP) indicators.

Information on the occurrence and abundance of floating marine macro and microlitter in pelagic and coastal surface waters, as well as on beach litter accumulation in the Pelagos Sanctuary and Tuscan Archipelago National Park were obtained, taking into account their physical characteristics (e.g., extent, habitat, and species diversity), potential litter sources (e.g., ports, estuaries, urban and tourism coastal areas), and levels of protection of the considered areas (e.g., SPAMI, national park, areas located inside and outside the protection zones). Sampling campaigns for floating litter were planned *a priori* based on the marine litter distribution prediction model developed by the LaMMA consortium, daily updated according to the currents and winds prevailing in the study areas during the sampling period (Fig. 6).

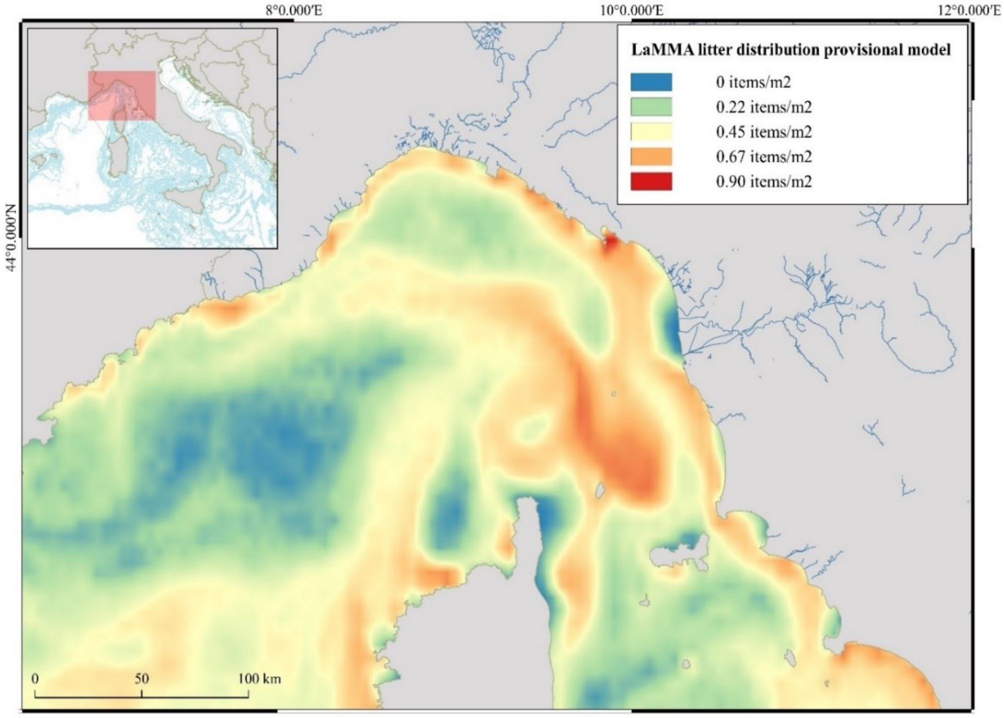


Fig. 6. Example of litter distribution provisional model map developed by Consorzio LaMMA used to plan floating litter sampling campaigns.

The purpose of using this model was to validate the predicted distribution and concentration of marine litter with field data to verify the strength and usefulness of litter prediction and as a result to identify potential accumulation hotspots in the investigated region. The *ad hoc* sampling strategies for simultaneous monitoring of floating macro and microlitter are shown in Fig. 7 A and B. In the Pelagos Sanctuary SPAMI, monitoring transects were performed starting from one nautical mile (nm) offshore (Step 1, Fig. 7 A) and every 10 nm in pelagic areas (Steps 5 and 8; Fig. 7 A). Additional macrolitter transects (Steps 4 and 7; Fig. 7 A) were conducted before the simultaneous sampling described above. In the Tuscan Archipelago National Park, a star-shaped experimental design was adopted on the coastal waters off the 7 main islands to assess potential differences in marine litter distribution as a function of different levels of protection (monitoring zones inside and outside the protected areas) and distance from the coast (Fig. 7 B). Simultaneous transects were started one nautical mile offshore and repeated at 3 nm. Macrolitter objects monitoring was carried out throughout the circumnavigation of each island (Fig. 7 B). All the sampling transects for the evaluation of the abundances and distribution of floating litter items were displayed in Fig. 7 C.

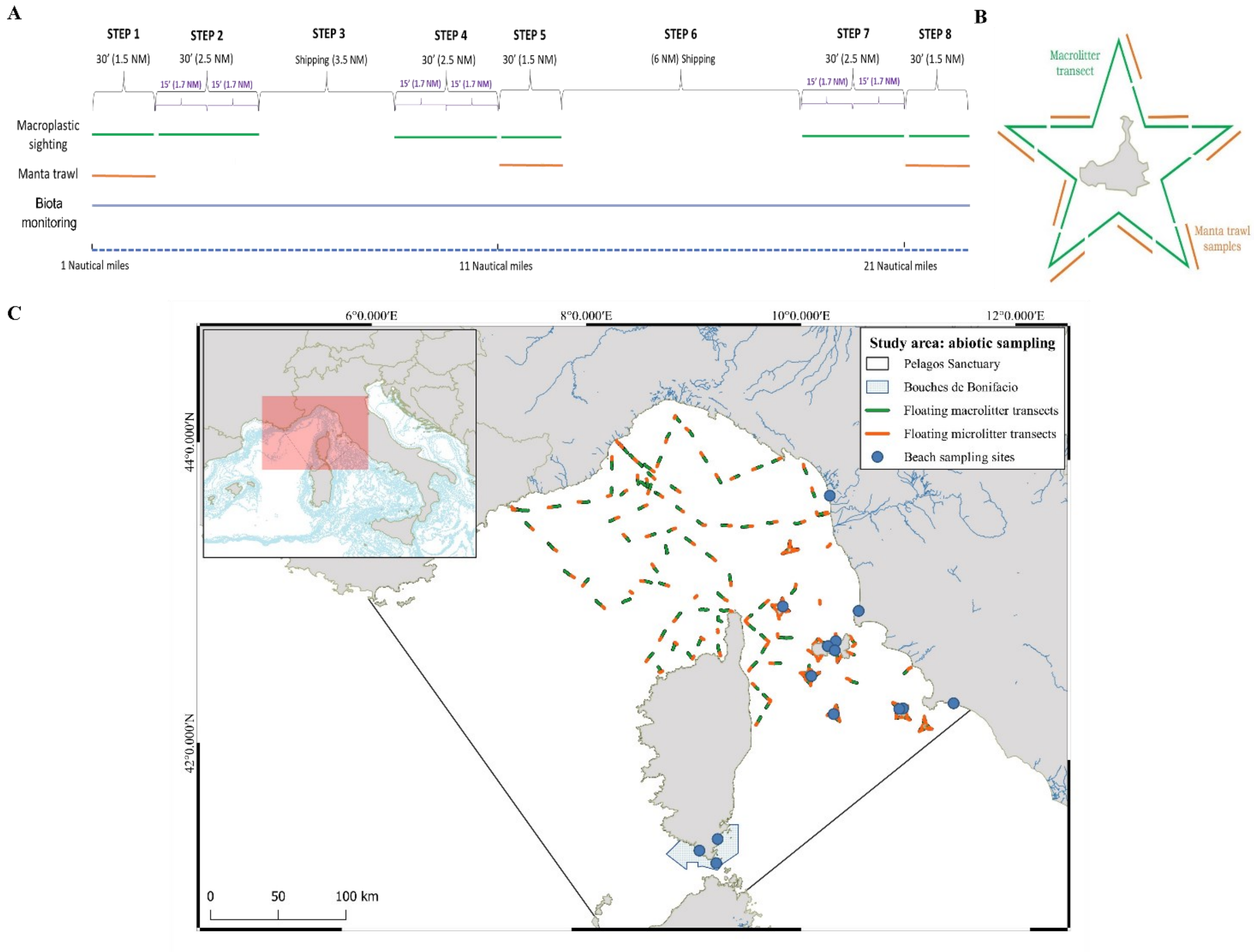


Fig. 7. Experimental designs carried out during the Pelagos Sanctuary (A) and Tuscan Archipelago National Park (B) sampling campaigns. Macro (green) and microlitter transects (orange) were performed simultaneously starting one nautical mile from the coast and repeated every 3 and 10 nautical miles in the Tuscan Archipelago National Park and Pelagos Sanctuary, respectively. Biota (cetaceans and associated species) monitoring were performed during all the sampling activities. Map of the sampling activities carried out in the monitored areas (C).

Beach litter monitoring was conducted in several sites along the Tuscan and Corsican coasts in the Pelagos Sanctuary and five islands of the Tuscan Archipelago National Park (Fig. 8) evaluating potential differences in the accumulation of litter according to seasonal variations and potential accumulation zones. Sampling sites were selected considering specific physical characteristics of beaches (e.g., total length of 100 m, moderate slope, easy accessibility and ideally no cleaning activities) and potential human impacts and were classified according to Ariza et al. (2008) and Giovacchini et al. (2018) in:

- Urban (U): beaches in residential and tourist areas that are regularly cleaned, especially during the tourist season, and as a public service.
- Rural (Ru): beaches located near urban centres and can be visited by tourists but are less frequented and less regularly cleaned compared to the aforementioned beaches.
- Remote (Re): beaches with low human impact and located in MPAs. At these beaches, marine debris is transported by ocean currents, rivers, waves and wind. Cleaning activities are usually not carried out.

A detailed description of the sampling campaigns and methodological protocols adopted to gain data on litter abundances and distribution on the sea surface and several beaches is presented in Chapter 4.

Concurrently to the monitoring activities of floating marine litter distribution, well-known and potentially new bioindicator species of plastic ingestion were collected to assess the potential physical and chemical impacts of plastics and their additive compounds (i.e., PAEs), as well as the presence of marine megafauna and associated species, was monitored to evaluate the biodiversity richness in the investigated areas.

Bioindicators are important tools for detecting changes in the environment and have the potential for assessing the health of the ecosystem before the functionality is compromised by providing biological responses that can guide policymakers and environmental managers (Bonanno and Orlando-Bonaca, 2018). The biomonitoring of plastic pollution should be considered an additional tool to assess the state of the marine environment as underlined by the MSFD D10 C3 and IMAP C24 indicators. Thus, the selection of bioindicator species is a crucial procedure to monitor the impact of marine litter on Mediterranean fauna. Bioindicators species must meet specific criteria: background information (including biological and ecological characteristics of the species), habitat information (allow monitoring at different spatial scales), trophic position and feeding behaviour (potentially strongly influence marine litter ingestion), commercial importance and conservation status. In addition to that, species must reflect the features and the pollution of the marine habitats monitored, from coastal to offshore areas, from benthic to pelagic environments and being susceptible to plastic ingestion (Fossi et al., 2018b).

According to the data available on the interaction between marine litter, particularly MPs, and Mediterranean marine organisms (Annexe 1) and taking into account the above-indicated criteria, well-known sentinel species (sea turtles: *Caretta caretta* and Mollusca: *Mytilus galloprovincialis*) and potentially new bioindicators of plastic pollution impacts (cetacean odontocetes: *Physeter macrocephalus*; *Ziphius cavirostris*, *Stenella coeruleoalba,* and *Tursiops truncatus;* cetacean mysticetes: *Balaenoptera physalus*; seabirds: *Calionectris diomedea* and *Puffinus yelkouan*; sea turtles: *Chelonia mydas;* elasmobranchs: *Mobula mobular*; teleosts: *Myctophum punctatum* and *Mullus surmuletus*; hydrozoans: *Velella velella*) have been selected (Tab. 5). The selection of organisms was carried out considering the investigated habitats (pelagic, mesopelagic, benthopelagic, neritic, demersal, benthic) in the Pelagos Sanctuary and Tuscan Archipelago National Park, in order to provide integrated information on plastic pollution impacts at different spatial scales.

Tab. 5. Bioindicator species proposed in relation to habitat and home range modified by Fossi et al. (2018b).

	Pelagic	**Mesopelagic**	**Benthopelagic**	**Neritic**	**Demersal**	**Benthic**
Pelagos Sanctuary and Tuscan Archipelago National Park **(Whole area)**	*Balaenoptera physalus* *Physeter macrocephalus*		*Caretta caretta* *Chelonia mydas*	*Calionectris diomedea* *Puffinus yelkouan* *Mobula mobular*		
Pelagos Sanctuary and Tuscan Archipelago National Park	*Ziphius cavirostris* *Stenella. Coeruleoalba*			*Tursiops truncatus*		

(Medium scale)	*Velella velella*					
Pelagos Sanctuary and Tuscan Archipelago National Park **(Small and local scale)**		*Myctophum punctatum*			*Mullus surmuletus*	*Mytilus galloprovincialis*

The approach adopted in sample collection was previously described by Fossi and coworkers (2018) as a threefold approach, which combines the occurrence of plastic ingestion by organisms, with the evaluation of plastic additives levels in tissues and the related toxicological effects. In this perspective, both commercial and protected species were investigated for plastic ingestion and PAEs accumulation levels. As illustrated in Fig. 8, samples were collected both from living organisms during marine litter monitoring campaigns, as well as from stranded organisms (cetaceans, seabirds, sea turtles and rays) found dead along the Tuscan coast. A detailed description of bioindicators species, consolidated and innovative analysis protocols adopted in the dissection of the organisms, digestion of GITs and plastic extraction are thoroughly described in Chapter 5.

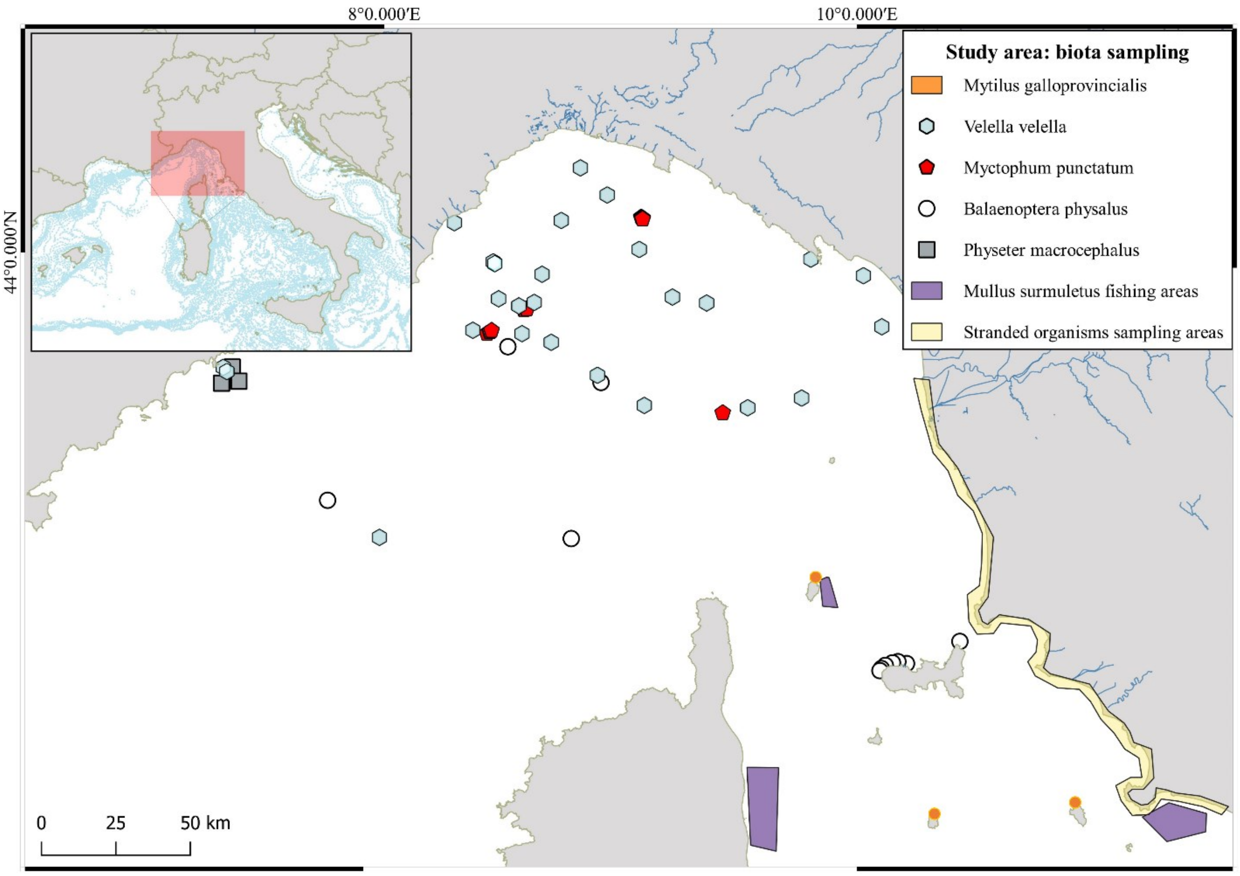


Fig. 8. Sampling site and areas of the species collected within the Pelagos Sanctuary and the Tuscan Archipelago National Park.

All the data gained made it possible to carry out a comprehensive risk assessment analysis described in Chapter 6 to identify critical areas and provide the basis for the development of effective protection and mitigation measures to be taken forward.

CHAPTER 4: PRESENCE, DISTRIBUTION, AND COMPOSITION OF MARNE LITTER IN THE SPAMI – PELAGOS SANCTUARY AND IN THE TUSCAN ARCHIPELAGO NATIONAL PARK

This chapter presents the presence, distribution and composition of marine surface floating and stranded litter at several beaches in the SPAMI - Pelagos Sanctuary and the Tuscan Archipelago Natural Park. Potential hotspots for litter accumulation are investigated, with particular attention paid to potential sources of pollution, to support the implementation of targeted marine litter management measures in the MPAs investigated.

4.1 Introduction

4.1.1 Sea surface floating litter and beach litter in the MPAs

Although there are several scientific studies on floating litter and beach debris in the Mediterranean Sea covering different areas, slightly more than 30% have focused on marine protected areas (Tabs. 1 - 4). The Langrangian modelling analysis of plastic fluxes on six selected coasts of marine protected areas in the Mediterranean proposed by Liubartseva et al. (2019) represents one of the first attempts to predict marine litter distribution and potential impacts in MPAs. It showed that the input of litter was relatively low (0.4-3.6 kg (km/day) compared to the average flux of 6.2 ± 0.8 kg (km/day) calculated for the Mediterranean Sea in 2013-2017 (Liubartseva et al. 2019), assessing the synergistic role of anthropogenic factors emitting plastic and hydrodynamic transport bringing pollution into MPAs. A different approach was proposed by Fossi et al. (2017) in the SPAMI Pelagos Sanctuary, where simulated and *in situ* MP concentrations were evaluated to verify the accuracy and strength of the predictive plastic distribution model and to highlight the potential risk associated with ingestion by fin whales in this important ecological MPA. This area, in particular, is one of the most investigated in the Mediterranean Sea (Tabs. 1 and 2), 70% of the studies conducted in MPAs are carried out here, with an average abundance of floating objects and MPs of 0.73 ± 82.3 items/km^2 and 85,122 ± 35,726 items/km^2 (0.30 ± 0.23 items/m^3), respectively. This MPA also seems to be affected by the temporary formation of the marine litter convergence area between the islands of Corsica and Capraia, where a high concentration of plastic has been reported (Baini et al., 2018; Fossi et al., 2017, Suaria et al., 2016). Giovacchini et al. (2018) and Merlino et al. (2018) found relatively large amounts of stranded macrolitter objects on the Tuscan and Ligurian coasts, with an average abundance of 1.06 objects/m^2 and 0.72 ± 0.43 objects/m^2, respectively, and on the Sardinian coast with about 40,000 characterised trash objects (Camedda et al., 2017). The presence of MP was recently reported by Merlino et al. (2020) and Scopetani et al. (2021), focusing on the beaches of the Migliarino San Rossore Massaciuccoli Natural Park. In the western part of the Mediterranean, the presence and distribution of marine litter in other MPAs have been studied: in the Menorca Channel (Ruiz-Orejon et al., 2019), in the Cabrera National Park (Fagiano et al., 2021), Calanque National Park (Schmidt et al., 2021), Ischia and Ventotene Marine Protected Area (de Lucia et al., 2018) and Torre Flavia wetland (Battisti et al., 2019, 2016; Cesarini et al., 2021).

Exceptionally high concentrations of floating macrolitter were detected in surface waters of the MPAs of Gozo and Malta (Ionian Sea and Central Mediterranean sub-regions). This study highlights the potential influence of seasonal variation and distance from the coast on the distribution and accumulation of litter, showing the highest levels during the winter season (2,392 ± 7,477 items/km^2) and in coastal areas (6,371 ± 11,968 items/km^2) (Curmi and Axiak, 2021). The high pollution level of these MPAs is also confirmed by the presence of MP on beaches, as shown by Gauci et al. (2019) and Turner and Holmes (2011). Finally, in the Adriatic Sea, Palatinus et al. (2019) conducted simultaneous surveys of floating macro and microlitter in the Zadar archipelago, including the Kornati Islands National Park and Telascica Nature Park. The potential relationship between the distributions of floating objects (175 items/km^2) and microparticles (127,000 items/km^2) was evaluated, but no statistical correlation was demonstrated. In the southern sector of this sub-region, the presence of macrolitter on the beach in the Torre Guaceto MPA was assessed with an average concentration of 0.47 items/m^2 (Rizzo et al., 2021). In summary, the data reveal a significant data gap regarding the amount, distribution, composition and sources of marine litter on the sea surface and beaches in nearshore and pelagic marine protected areas.

4.2 Materials and methods

4.2.1 Sampling campaigns

The sea surface floating litter sampling campaign in the Pelagos Sanctuary was carried out in both pelagic and neritic areas during three different periods:

- From 30 May to 5 June 2019 and from 16 June to 21 June 2019, focusing on the northern sector (including the coasts of Liguria and Tuscany) and the central-western sector (including the coasts of Liguria and France) of the Ligurian Sea, which represents the summer feeding area of the fin whale and is characterised by upwelling areas. This campaign was carried out taking into account the different depths and slopes of the study area, focusing on the submarine canyons of Genova and Imperia, which have an important influence on the distribution of cetaceans (Moulins et al., 2007, 2008; Würtz, 2012). In addition, sampling was also carried out near potential sources of marine litter pollution, such as the port of Livorno and Marina di Pisa (along the Tuscan coast) and La Spezia, Genova and Loano (along the Ligurian coast), as well as the river discharges of Arno, Serchio and Magra.
- From 20 to 23 July 2019, focusing on the northeast coast of the island of Corsica, identified as a potential area for plastic accumulation due to the temporary formation of convergence currents (Fossi et al., 2017).
- From 14 to 19 September 2019, along the northwest coast of the island of Corsica and the canyon of Saint-Florent, considered a special feeding area for cetaceans and fin whales in particular (Würtz, 2012).

The first and third parts of the sampling campaign were conducted onboard the 54-foot sailing catamaran "Headwind" in collaboration with the CIMA Research Foundation. The catamaran was specially equipped for marine debris monitoring and sampling, as well as sightings of large pelagic animals and marine mammals. The July campaign was carried out in collaboration with ISPRA (Italian National Institute for Environmental Protection and Research) onboard the oceanographic vessel “ASTREA” equipped with multibeam echo sounder to obtain bathymetric data and the main marine sampling instruments (sediment, water and biota). A total of 1568 nautical miles were navigated, with 168 floating macrolitter monitoring transects and 84 manta trawls conducted to assess floating microlitter (Fig. 9).

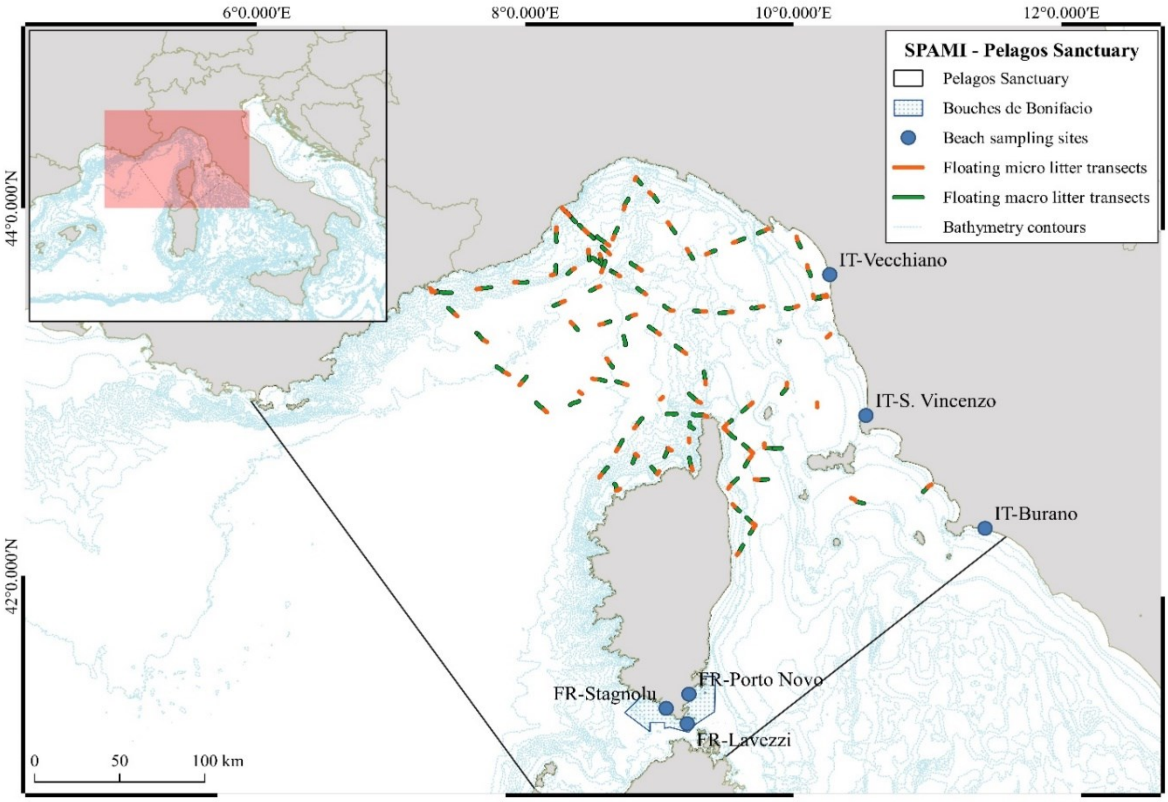


Fig. 9. Sampling activities carried out during the Pelagos Sanctuary sampling campaign. Macro (green lines) and microlitter transects (orange lines) and beach litter sites were reported (blue dots).

Beach litter monitoring activities in the Pelagos Sanctuary were carried out along the Tuscan coast and the southernmost sector of the Corsica Islands. The Tuscan coastline is a microtidal environment, about 380 km long, mainly characterized by sandy beaches (215 km), with few sections of mixed sand and gravel sediments (Pranzini et al., 2020). Monitoring activities in this areas were carried out during the four seasons (autumn, winter, spring and summer) of 2019 at three beach sites (Fig. 9):

- Vecchiano (Rural): the northern sampling area belongs to the Migliarino - San Rossore - Massaciuccoli Regional Natural Park, established in 1979 (Bertacchi et al., 2017). The beach is located near the mouth of the Serchio River, but it could also receive waste from the mouth of the Arno River, which is only 15 km to the south. The Arno is one of the most important rivers in Italy and flows through a highly urbanized city as well as agricultural land and industrial areas (Cincinelli et al., 2001; Cortecci et al., 2002; Scopetani et al., 2021). Moreover, the beach is located between Viareggio, a town subject to strong anthropogenic pressure due to its high population density and the conspicuous presence of tourists, and the commercial port of Livorno, one of the largest in Italy. The presence of visitors and tourists is allowed.
- San Vincenzo (Rural): is located in the Regional Natural Coastal Park of Rimigliano and includes a coastal strip of about 6 kilometres with a natural sandy beach open to tourists (Santilli and Bagliacca, 2010). The area is exposed to anthropogenic pressure mainly from tourist and recreational activities and may be affected by the effluents of the Cecina River, described as potentially rich in nutrients and pollutants from inland agriculture and urban centres (Blašković et al., 2017; Renzi et al., 2010).

- Burano (Remote): is the southernmost monitored beach, characterized by a sandy beach and located in the National Nature Reserve of Lake Burano, proclaimed in 1980. According to the Ramsar Convention, the reserve has been designated as a Wetland of International Importance and is one of the Sites of Community Importance and Special Protection Areas (Colombini and Chelazzi, 2010). The large influx of tourists during the summer months may have a significant impact on the environment. As well as the potential contribution of the Fiora river located 20 km to the south (Cannas et al., 2017).

Mechanical cleaning of these coastal areas is limited to the summer season only and affects only a portion of the monitored sites. All beaches present a fine grain with mobile and fixed dunes and typical Mediterranean flora.

Monitoring of beach litter in the southernmost sector of the island of Corsica was carried out in collaboration with the Office de l'Environment de la Corse (OEC/UAC) in the Bouches de Bonifacio Nature Reserve (Fig. 9), which according to the litter dispersion model of Liubartseva et al. (2019) is considered to be slightly affected by the accumulation of marine litter, mainly caused by fishing and tourist activities. The following beaches were monitored during different seasonal periods in 2019, 2020 and 2021 (Tab. 5):

- Stagnolu (Rural): Stagnolu Bay is located in the south of the Gulf of Ventilegne and is characterized by shallow waters and sandy beaches (about 300 m) surrounded by an extensive wetland (Cibecchini et al., 2006).
- Portu Novu (Rural): Portu Novu Bay is located at the end of the Gulf of Portu Novu and consists of a fine sandy beach about 350 m long on a rocky bay. It is sheltered from the wind and is characterized by the presence of natural dunes and a wetland in the hinterland.
- Cala di U Lioni (Remote): Located in the south-western sector of the island of Lavezzi, this beach is characterized by fine-grained sand with solid dune landscapes and typical Mediterranean flora. Visits are restricted and managed by the OEC/UAC.

The Tuscan Archipelago National Park sampling campaign was conducted from 8 to 19 July 2019 aboard the oceanographic vessel (ASTREA), focussing on the coastal waters off the 7 main islands of the archipelago. A total of 585 nautical miles were covered, with 105 transects monitoring floating macrolitter and 57 manta trawls assessing floating microlitter (Fig. 10).

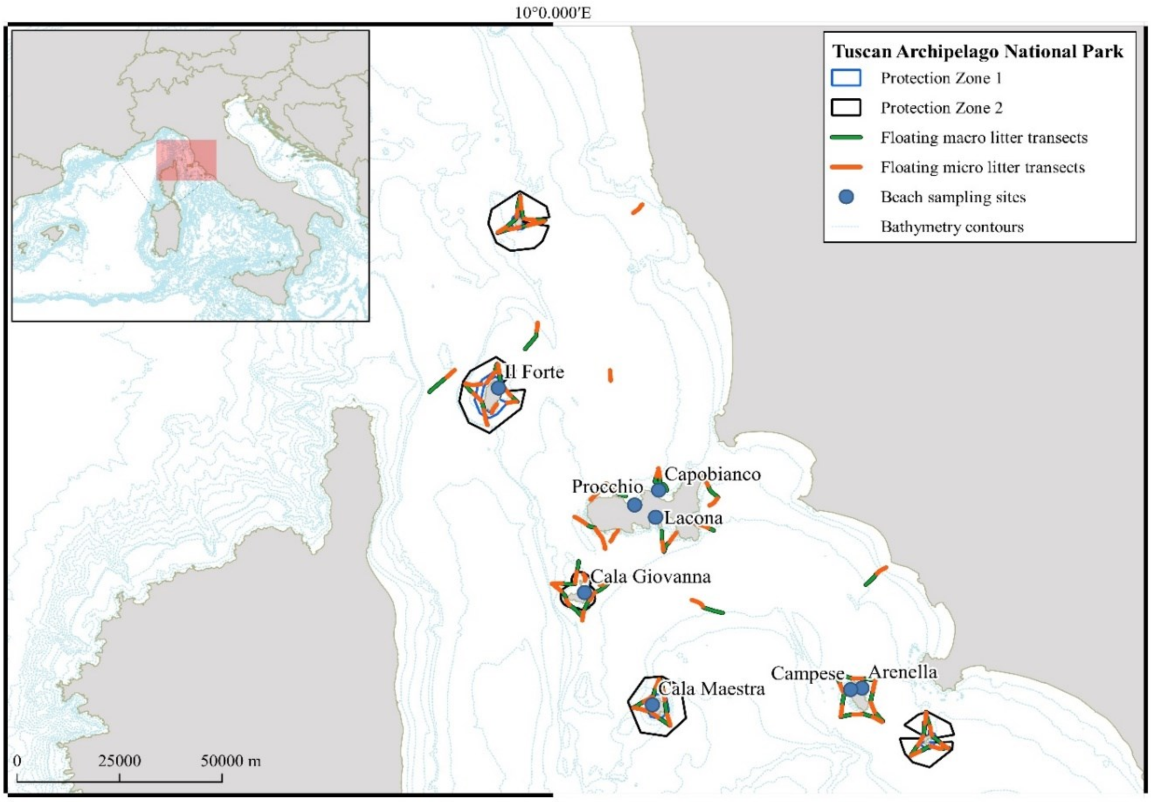


Fig. 10. Sampling activities carried out during the Tuscan Archipelago National Park sampling campaign. Macro (green lines) and microlitter transects (orange lines) and beach litter sites were reported (blue dots).

Off the Tuscan coast, 5 islands (Capraia, Elba, Montecristo, Pianosa and Giglio) and 8 different beaches were monitored in collaboration with the Tuscan Archipelago National Park (Fig. 10) during different seasons between 2019 and 2020 (Tab. 5). The potential inputs from the land from the Tuscan coast, as well as the intense maritime traffic and fishing, are the main sources of pollution that can affect this area. In addition, due to the numerous tourist and recreational resorts, this area is highly anthropized and prone to marine litter accumulation, especially during the summer season.

The following beaches were monitored:

- Il Frate (Urban). The beach is located near the port of Capraia Island, in an unprotected area and is characterized by the presence of volcanic pebbles. It is one of the few beaches on the island, and the strong tourist presence during the summer season can affect the distribution of garbage.
- Capobianco (Rural), Lacona (Rural), and Procchio (Urban). The geology of the island of Elba is various, with both igneous and sedimentary and calcareous rocks (Bowman et al., 2014). The beach of Capobianco is composed of small, grey-green felsite pebbles, while Lacona and Procchio have golden and quartzitic sand. The area is very touristic and exposed to high anthropogenic pressure, especially in the summer months.
- Cala Maestra (Remote). Located in Montecristo Island, it has a sandy beach of granodiorite with large orthoclase crystals. It is located in a nature reserve that encompasses the entire island, and very few visitors (2000/year) are allowed.

- Cala Giovanna (Remote). This beach, located in Pianosa Island, is mainly composed of sedimentary rocks (Graciotti et al., 2008). Since 1997 it has been part of a Marine Protected Area of National Interest, which regulates access to the island and activities in the offshore waters up to 1 nm from the coast. Visits are limited to a maximum of 250 people per day.

- Arenella (Rural) and Campese (Urban). The Giglio island has a rocky coastline composed mainly of granite. The beach of Campese is the largest beach of Giglio. It has dark red, coarse sand, while the sand of Arenella is characterized by coarse-grained, light golden sand. The area is very touristy and exposed to high anthropogenic pressure, especially in the summer months.

Only the beaches on the islands of Elba and Giglio are completely mechanically cleaned during the summer season.

4.2.2 Quantification and characterization of sea surface floating litter

Sea surface floating macrolitter

As recommended in the monitoring guidelines developed by the EU MSFD Technical Subgroup on Marine Litter (Galgani et al., 2013) and the MEDSEALITTER project, the distribution, abundance and composition of floating macrolitter (> 2.5 cm) were assessed using the fixed-width strip transect method. The strip transect method allows counting the number of objects detected within a fixed width strip. This fixed limit should be representative of the visibility conditions during the survey and depends mainly on the speed of the vessel and the height of the observer above sea level. All observations were made with the naked eye from the bow of the ship (3 m above sea level) at a speed of 4 knots for 30 minutes. Due to the characteristics of our observation set up, a relatively narrow strip of 7 m was monitored, following the recommendation of Galgani et al. (2013) (Fig. 11).

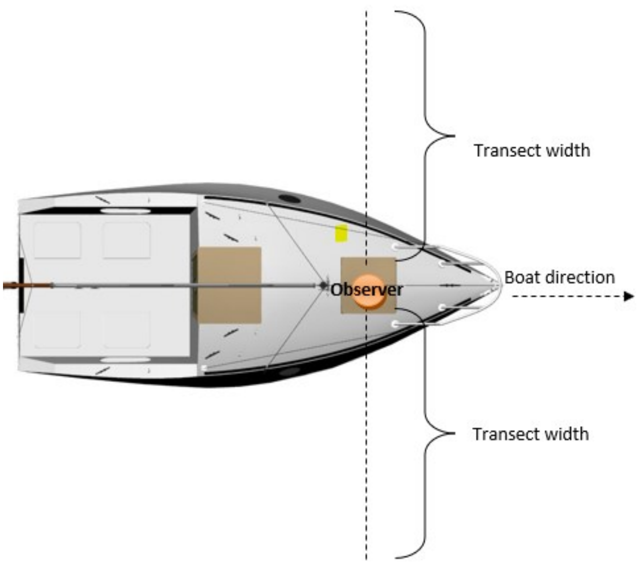


Fig. 11. Fixed-width strip transect method: schematic representation of observation position and transect width on a vessel during floating macrolitter monitoring transect. Source: Fossi et al., 2019 (PB MPAs Toolkit p. 31-34).

Each item was characterised according to the main list of litter categories (Galgani et al., 2013), which revised the original OSPAR and UNEP categories (Cheshire et al. 2009) and indicated the type (Artificial

Polymer Materials, Rubber, Cloth/Textile, Paper/Cardboard; Processed/Worked Wood and Metal) (Annex 1 for a detailed list of categories), size classes (B. 2.5-5 cm, C. 5-10 cm, D.10-20 cm, E. 20-30 cm, F. 30-50 cm, G. > 50 cm) and colours (W. White; T. Transparent; B. Black; C. Cyan/Blue; R. Red; G. Green; Y. Yellow; O. Other) of the floating objects. Finally, counts of scattered objects were converted to density values by dividing the total number of objects sighted by the effective area sampled in each transect:

$$Di = n \,/\, (Li \times W)$$

Where n is the number of items seen on the transect, L *is* the length of the transect, W (7 m) is the fixed width of the strip observed and expressed as items/km^2.

Sea surface floating microlitter

Floating microlitter samples were collected using a manta trawl (330 µm mesh size, 16 ×60 cm mouth opening) towed at 2-3 knots on the water surface for 30 min, held to the side of the boat to avoid the turbulence caused by the wake of the vessel (Fig. 12 A). At the end of sampling, the net was thoroughly rinsed from the outside to ensure that both plankton and microparticles were washed into the end of the net. Samples were filtered through a 300 µm metal sieve and stored in a 70% ethanol solution for synthetic particle analysis (Fig. 12 B). To avoid contamination throughout the sampling activities, all the materials used for sample collection, including the nets, were carefully cleaned and rinsed before each tow.

A **B**

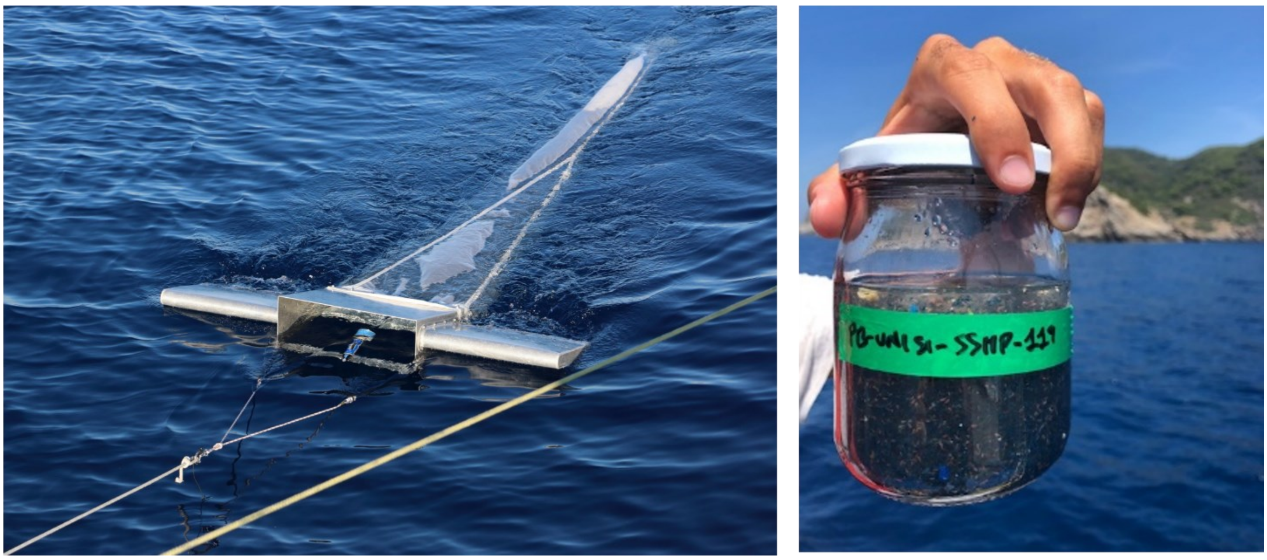


Fig. 12. Floating microlitter sampling activities carried out with a manta net (A); Manta net collected sample (B).

In the laboratory, the floating microlitter samples were filtered through a sieve (mesh size: 300 µm) and observed under an NBS stereo zoom microscope (Mod. NBS-STMDLX -T) equipped with an LED light and a micro metered eyepiece. The microparticles were manually isolated in a glass Petri dish and allowed to dry overnight at room temperature (Fig. 13). Each Petri dish was then photographed and analysed for particle size measurement (expressed in mm) using ImageJ software (Fiji Distribution). The isolated particles were characterised according to different size classes into small microparticles (SMPs) (0.3 - 1 mm), large microparticles (LMPs) (1 - 5 mm), mesoparticles (5 - 25 mm), and macroparticles (> 25 mm), shape (pellet, fragment, film, filament, microbead, and foam), and colour (black, blue, white/transparent, white/opaque, red, green, and others) and weighed using an OHAUS Explorer precision balance (± 0.1 mg).

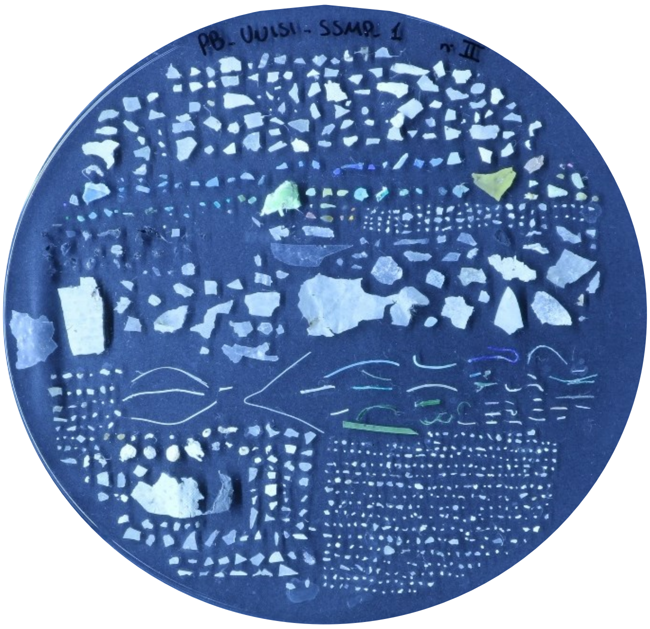


Fig. 13. Microparticles isolated from a surface sample.

Glassware was used in the laboratory procedures, and special care was taken to prevent airborne contamination by performing sample analysis in a clean airflow cabinet and using two glass Petri dishes placed on either side of the stereomicroscope as blank controls. Despite the use of contamination control procedures, fibres and paint were not included due to the risk of external contamination during sampling.

The data obtained, expressed as concentration items/km^2 and mg/km^2, were normalised, if necessary, by applying the correction factor proposed by Kukulka et al. (2012). This factor, widely accepted in the scientific literature (Baini et al., 2018; Faure et al., 2015; Fossi et al., 2017; Kooi et al., 2016) takes into account the unfavourable meteorological and maritime conditions (wave > 0.50 m and wind speed > 4 m/s) that may affect the accumulation of floating microparticles in surface waters due to the wind mixing effect, leading to an underestimation of their concentrations, and proposes an appropriate value to correct the final concentrations of the samples. Finally, the chemical composition of 10% of the isolated microplastics was evaluated, selected proportionally according to the relative abundance in the different size, shapes and colour classes for each sample. Using Fourier infrared spectroscopy (FTIR), each particle was scanned 16 times using an Agilent Cary 630 spectrophotometer. To identify the polymers, the spectrum obtained was processed using Agilent Micro Lab FTIR software and compared to a database of reference spectra. Only results that showed more than 80% overlap were accepted (Baini et al., 2018).

4.2.3 Quantification, and characterization of beach litter

Beach macrolitter

In accordance with the beach litter sampling protocols proposed by DeFishGear and the PB MPA projects, the presence and abundance of litter at the selected sites were assessed using performing two 100 m transects (only one transect if the total length of the beach was <100 m) (Tab. 6) separated by a 50 m stretch covering the entire area from the beach line to the back of the beach where natural vegetation or dunes begin.

Tab. 6. Beach litter sampling sites according to location area and classification (U: urban; Ru: rural; Re: remote). N. of transects carried out in different seasonal periods and years and the total number was reported. n.s. means not sampled.

			N. of transect sampled				
Sampling site	**Location area**	**Classification**	**Autumn**	**Winter**	**Spring**	**Summer**	**Total**
Vecchiano	Tuscan coast	Ru	2 (2019)	2 (2019)	2 (2019)	2 (2019)	8
San Vincenzo	Tuscan coast	Ru	2 (2019)	2 (2019)	2 (2019)	2 (2019)	8
Burano	Tuscan coast	Re	2 (2019)	2 (2019)	2 (2019)	2 (2019)	8
Stagnolu	Corse	Ru	1 (2020)	n.s.	1 (2019)	n.s.	2
Portu Novu	Corse	Ru	n.s.	1 (2021)	1 (2020)	n.s.	2
Cala di U Lioni	Lavezzi (Corse)	Re	1 (2019)	n.s.	1 (2021)	1 (2019)	3
Il Frate	Capraia	U	1 (2020)	1 (2020)	1 (2020)	1 (2021)	4
Capobianco	Elba	Ru	1 (2020)	1 (2020)	1 (2020)	1 (2021)	4
Lacona	Elba	Ru	2 (2020)	2 (2020)	2 (2020)	2 (2020)	8
Procchio	Elba	U	2 (2020)	2 (2020)	2 (2020)	2 (2020)	8
Cala Maestra	Montecristo	Re	1 (2020)	1 (2020)	1 (2020)	n.s.	3
Cala Giovanna	Pianosa	Re	1 (2020)	1 (2020)	1 (2020)	1 (2020)	4
Arenella	Giglio	Ru	1 (2020)	1 (2020)	1 (2020)	1 (2020)	4
Campese	Giglio	U	2 (2020)	2 (2020)	2 (2020)	2 (2020)	8

Any item larger than 2.5 cm was collected; heavy and/or larger items that could not be removed were categorized and the corresponding GPS coordinates recorded. Items found in the sampling unit were classified according to Master List categories (Galgani et al., 2013) (Annexe 2). In addition, each category of collected items was also classified using the corresponding Joint List codes proposed by Fleet et al. (2021) (Annexe 3). This current list uses a 2-to 4-letter code to indicate the level of detail that characterizes the collected object. The first level of detail in the list is one of nine material classes (Chemicals, Cloth/Textile, Food waste, Glass/ceramics, Metal, Artificial Polymer Materials, Paper/Cardboard, Rubber and Processed/Worked Wood), which are consistent with the categories set out in Commission Decision (EU) 2017/848. At the second level, the list uses 13 so-called "use categories" to which the wastes on the list are assigned (ag_agriculture related, aq_ aquaculture related, cl_ clothing, co_ building &construction-related, fc_ food consumption related, fi_ fisheries-related, hy_ personal hygiene and care-related, md_ medical-related, nn_ undefined use, re_ recreation-related, sm_ smoking-related, vk_ vehicle related, and hu_ hunting related). From the third level on, the characterization includes the physical properties of the object (e.g., shapes, volume, dimension). A detailed description of the categories can be found in Appendix 2. Objects made of mixed materials were assigned to the material classes according to their main component;

fragmented or broken but recognizable objects were assigned to the category corresponding to the larger part of the object. All objects belonging to the same category were counted and weighted; frequency was expressed in items/100m.

Beach microlitter

Beach microlitter have been monitored in the same 100 m transects conducted for macrolitter distribution evaluation and in the same four seasons (Tab. 6). However, due to the partial gravelly nature of the beaches and the mechanical cleaning procedures, some plots were not sampled as well as the whole sampling on the island of Capraia (Tab. 7). As for the samples collected in the southernmost part of the island of Corsica, the activities were carried out according to the aforementioned sampling protocol, but the data were not comparable with the others due to the different analytical techniques used to isolate and characterize the microparticles.

Tab. 7. Beach microliter sampling sites according to different seasonal periods. The total number of transects performed were reported. n.s. means not sampled.

		N. of plots sampled				
Sampling site	**Location area**	**Autumn**	**Winter**	**Spring**	**Summer**	**Total**
Vecchiano	Tuscan coast	9	9	9	9	72
San Vincenzo	Tuscan coast	9	9	9	9	72
Burano	Tuscan coast	9	9	9	9	72
Capobianco	Elba	n.s.	6	5	4	15
Lacona	Elba	5	18	5	8	36
Procchio	Elba	12	18	8	4	42
Cala Maestra	Montecristo	9	9	7	9	34
Cala Giovanna	Pianosa	9	9	9	9	36
Arenella	Giglio	3	9	4	2	15
Campese	Giglio	18	18	15	8	59

In each transect, three different zones to be sampled have been identified in correspondence of accumulation at low tide (AC 1), the zone of accumulation at high tide (AC 2), and an intermediate zone of no accumulation (OAC) (Fig. 14), to identify possible differences in the distribution of microlitter on the beaches.

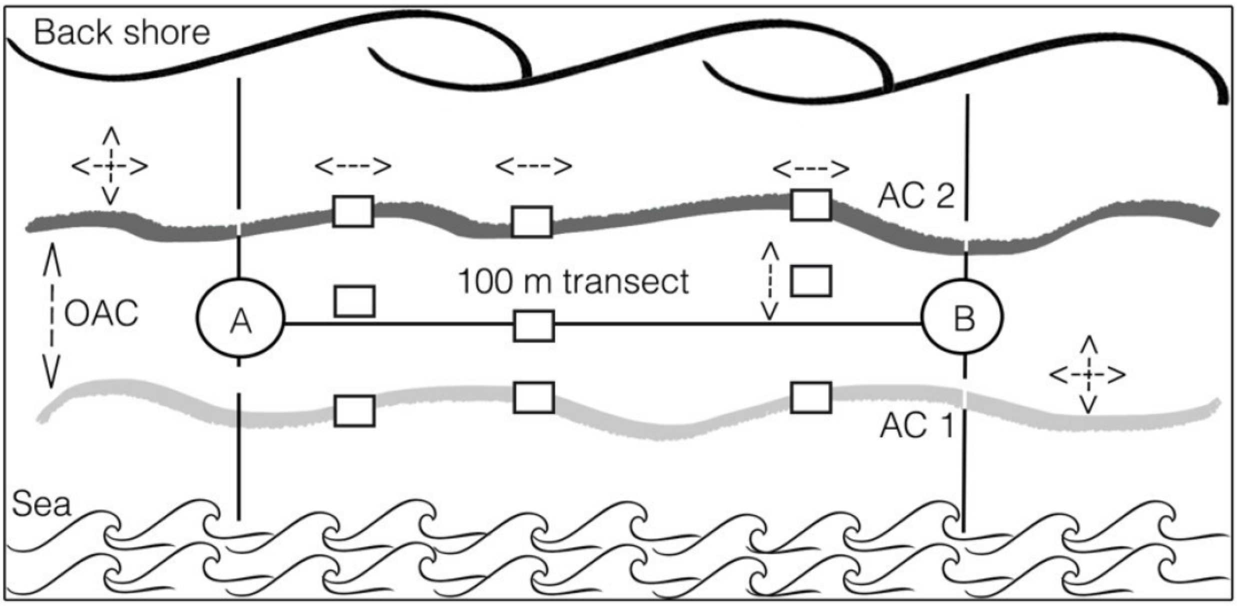


Fig. 14. Beach microlitter sampling sites according to the three different zones considered (AC 1, OAC and AC 2). Source: Frias et al., 2018.

Three plots of $1m^2$ were sampled in each zone, collecting the top 3-5 cm of sediment using a metal shovel. The sand was weighed (kg) and sieved through two metal sieves with mesh sizes of 5 mm and 1 mm, respectively. Large (> 25 mm) or non-plastic items were removed, and samples were stored in zip-lock bags. In the laboratory, samples were oven-dried (50°C overnight) and observed by the naked eye if they were large amounts of natural residues such as shells, leaves or twigs, or allowed to settle in NaCl hypersaline solution (1.2 g/cm^3) to allow flotation of synthetic particles. Meso and microlitter particles were carefully isolated in a glass Petri dish and analysed as described above for floating microlitter particles (see Section 3.1.4). All steps were performed using 100% cotton laboratory coats and precautions were taken to avoid cross-contamination of samples. Isolated particles were characterised according to different size classes into large microparticles (LMPs) (1 - 5 mm) and mesoparticles (5 - 25 mm), shape (pellet, fragment, film, fibre, filament, microbead, foam, and rubber), and colour (black, blue, white/transparent, white/opaque, red, green, and others) and weighed (mg) (± 0.1 mg) using an OHAUS Explorer precision balance. The chemical compositions were also evaluated as described above (see section 3.1.4). The data obtained were expressed in terms of concentrations as items/m^2 and items/kg dry sediment.

4.2.4 GIS (Geographical Information Systems) and Statistical analysis

Floating and beach marine litter concentration data were imported and processed using the Quantum GIS platform (version 3.10.1 A Coruña), and Rstudio (version 1.1.4.1106) to perform spatial and statistical analysis respectively.

Floating litter statistical analysis

Nonparametric analysis was used to examine the entire data set after checking for nonnormal distribution using the Shapiro-Wilk test. A Mann-Whitney-Wilcoxon test for pairwise comparisons was used to compare differences in floating litter, mean concentrations (items/km^2), and size classes, microlitter types, and macrolitter categories between the Pelagos Sanctuary and the Tuscan Archipelago National Park. In addition, the Kolmogorov-Smirnov test was used to compare the differences in the distribution of microlitter samples between the two selected areas. Statistical analysis was also performed considering the different islands of the Tuscan Archipelago National Park and the levels of protection. In particular, a Wilcoxon test was used to highlight the differences in the mean concentrations of floating litter (items/km^2) and size classes, type of microlitter and macrolitter categories between the samples carried out IN or OUT the National Park restricted zones and in protection zone 1 and zone 2. Any difference in concentrations of floating waste size classes between islands was assessed by performing a Kruskal-Wallis test for multiple comparisons. For classes that showed differences, a post hoc analysis was performed using Dunn's test. A significance level of 0.05 was used for all analyses.

Finally, the dataset related to the distribution of floating macro and microlitter throughout the studied area was examined considering the habitat types (bathyal, canyon, seamount, slope and continental shelf) and the main environmental (SST: sea surface temperature; SSH: sea surface height; MLD: depth of mixed layer and current velocity) and anthropogenic factors (vessel traffic, distance from ports, distance from the coast and distance from estuaries) that may influence their distribution. Data were taken from the Copernicus Marine Environmental Service, using daily products. Each floating macro and microlitter sample was associated with the corresponding daily value of the environmental variables considered. Vessel traffic and port data were downloaded from the European Marine Observation and Data Network (EMODnet). The vessel traffic data have a monthly resolution and include data from different vessel types (tankers, cargo vessels, fishing vessels, passenger vessels, sailing vessels and recreational vessels). Vessel densities are reported in hours/km^2/month; each floating waste concentration sample was linked to the corresponding monthly traffic data. Discharge location data were obtained in QGIS using river data downloaded from the ISPRA website (http://www.sinanet.isprambiente.it/it/sia-ispra/download-mais/reticolo-idrografico/view) for Italy and from the French government (https://www.data.gouv.fr/fr/datasets/cours-deau-metropole-2017-bd-carthage/) for France. In Italy, rivers were classified into two different groups (torrents and streams) according to their flow rate and classified as minor and major discharges, respectively. In France, rivers were divided into two classes according to their length: Rivers longer than 25 km belong to class 1 (major discharges), and those longer than 10 km belong to class 2 (minor discharges).

Descriptive statistics and normality tests (Shapiro-Wilk normality test and Anderson-Darling test) were performed for all datasets to determine whether parametric or non-parametric statistical analyses were appropriate. The Kruskal-Wallis test for multiple comparisons and post hoc test analysis was conducted to compare differences in the distribution of floating litter among different habitats. Spearman's rank correlation test was performed between each environmental variable and abundance of litter (items/km^2) and between the density of floating macro and MPs.

The analysis of environmental and anthropogenic factors affecting the distribution of floating litter was performed in two steps, following Kanhai et al. (2017). First, a Spearman's rank correlation test was performed between the factors considered and the scattering concentration. Then, generalised additive models (GAMs) were used to evaluate the influence of each variable on the distribution of floating litter. The response variable was always litter abundance (macrolitter or MPs), while the initial explanatory variables were pollution sources. The variables were considered separately so that a GAM was created for each variable for each type of floating litter (macrolitter or MPs). For the variables characterised by the presence of outliers, two models were created: one in which all values were included, the other in which the outliers were excluded. This procedure was chosen to exclude extreme situations that might not be representative of the general situation in the study area. To better understand the relationships represented by the GAM plots, a null line was used to define a positive effect of the predictors on litter accumulation, in a process called GAMvelope (Torres et al., 2008, Correia et al. 2015). The GAMvelope allowed highlighting areas favourable to litter accumulation in the Pelagos Conservation Area. A significance level ($p < 0.05$) was considered for all analyses.

Beach litter statistical analysis

Statistical analysis of macrolitter on beaches was carried out to assess differences in quantity (items/100m), composition and seasonal variation between the beach types considered (urban, rural and remote) and between the sites monitored (items/m^2), using a Kruskal-Wallis test for multiple comparisons. The same test was calculated for the mean concentrations of meso, and microlitter mean concentrations (items/m^2) between the different beaches and the three potential zones of particle accumulation (AC 1; OAC and AC 2). A significance level of 0.05 was used for all analyses performed with Rstudio (version 1.1.4.1106).

4.3 Results and discussions

The following sections present the main results of the sampling campaigns of floating litter and beach monitoring activities. The experimental designs performed *ad hoc* in the selected areas showed a valuable efficacy in the collection data providing reliable information on the abundance, distribution and composition of marine litter throughout the environmental compartments considered. Differences between the study areas (Pelagos Sanctuary and Tuscan Archipelago National Park) and among the different islands of the Tuscan Archipelago National Park are shown.

4.3.1 Floating macrolitter abundances and composition

A total of 273 transects were conducted to monitor the presence of floating macrolitter throughout the study area. A total of 2,169 items ranging from 0 to 3,974 items/km^2 were sighted, with an average concentration of 399.01 ± 485.84 items/km^2 (Fig. 15). This value ranged from one to two orders of magnitude higher than the threshold proposed by UNEP/MAP (2020) (5 objects/km^2) and the average concentration calculated considering the published data on the assessment of litter in the western part of the Mediterranean (29.7 ± 46.8 items/km^2). As far as we know, this value represents the highest amount of floating macrolitter recorded so far in the study area and could indicate a potential worsening of the macrolitter status in an important ecological area as the Pelagos Sanctuary (Arcangeli et al., 2018; Campanale et al., 2019; Di-Meglio and Campana 2017; Fossi et al., 2017; Suaria and Aliani 2014). As for the other Mediterranean basins, few studies reported similar litter concentrations in surface waters of MPAs in the Adriatic Sea (Palatinus et al., 2019) and near the islands of Malta and Gozo (Curmi and Axiak, 2021). Nevertheless, most published work has been conducted with oceanographic vessels sailing at > 6 knots and from an observing height of 6 to 25 m. Variability in observation conditions can affect the detection of small macrolitter (Class B. 2.5 - 5 cm), as previously acknowledged (Galgani et al. 2013; Zeri et al., 2018). Only recently studies have started to report the minimum size class detected (Arcangeli et al., 2017; Compa et al., 2019; Di-Méglio and Campana, 2017; Fossi et al., 2017; Vlachogianni et al., 2017, 2020; Zeri et al., 2018,), and relative information on the size characterization of sighted items (Zeri et al., 2018). Against this background, the application of harmonised monitoring protocols, as proposed and implemented in the PB MPAs project, will improve the accuracy and comparability of reported marine litter densities.

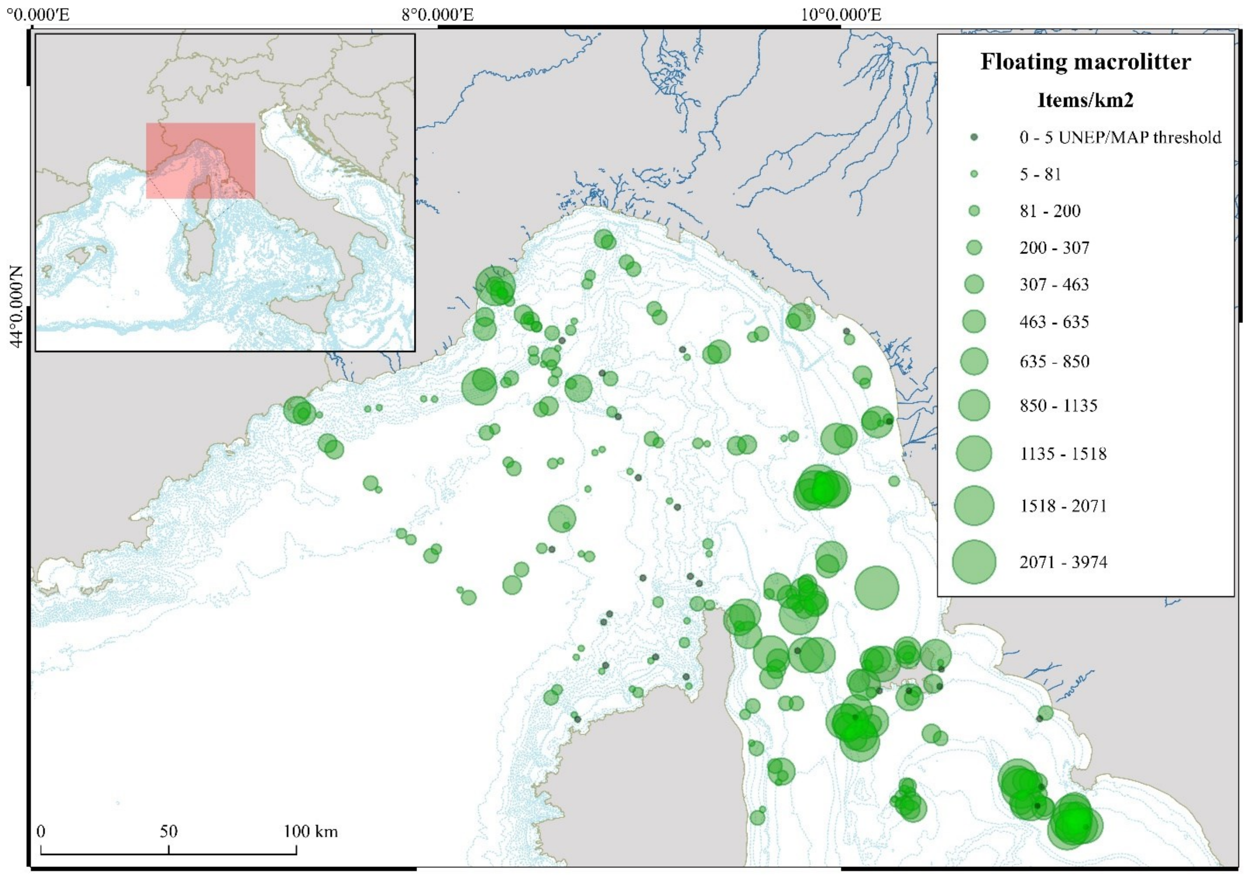


Fig. 15. Floating macrolitter spatial distribution in the whole study area considered. The concentrations of litter objects sighted were expressed in items/km^2, and the threshold proposed by UNEP/MAP (2020) was reported.

Litter and in particular artificial polymer materials items (99% of the total) were observed in 90% (245/273) of the transects conducted. These results are consistent with previous studies published throughout the Mediterranean Sea (Campanale et al., 2019; Compa et al., 2019; Fossi et al., 2017; Tata et al., 2020). 80% of the sighted objects had a size of less than 20 cm and a light-coloured characterization (> 80%), with size class B (2.5 - 5 cm; 58%) being the most common. The account of this dimensional range as the most frequently sighted is consistent with other studies conducted aboard small vessels at low speed, which allowed a homogeneous detection of all floating objects encountered in the sampled striped waters (Palatinus et al., 2019; Vlachogianni et al., 2017; Zeri et al., 2018).

Analysis of the most common objects revealed that more than 70% of all objects floating on the sea surface were represented by 10 categories of debris (Fig. 16). Objects of secondary origin belonging to the category "G67: Sheets and industrial packaging" and "G79: Plastic pieces 2.5 cm > < 50 cm" were most frequently sighted (Fig. 16). Their presence could be an indication of the degradation processes and fragmentation that affect the litter objects once dispersed in the marine environment, allowing the formation of small particles.

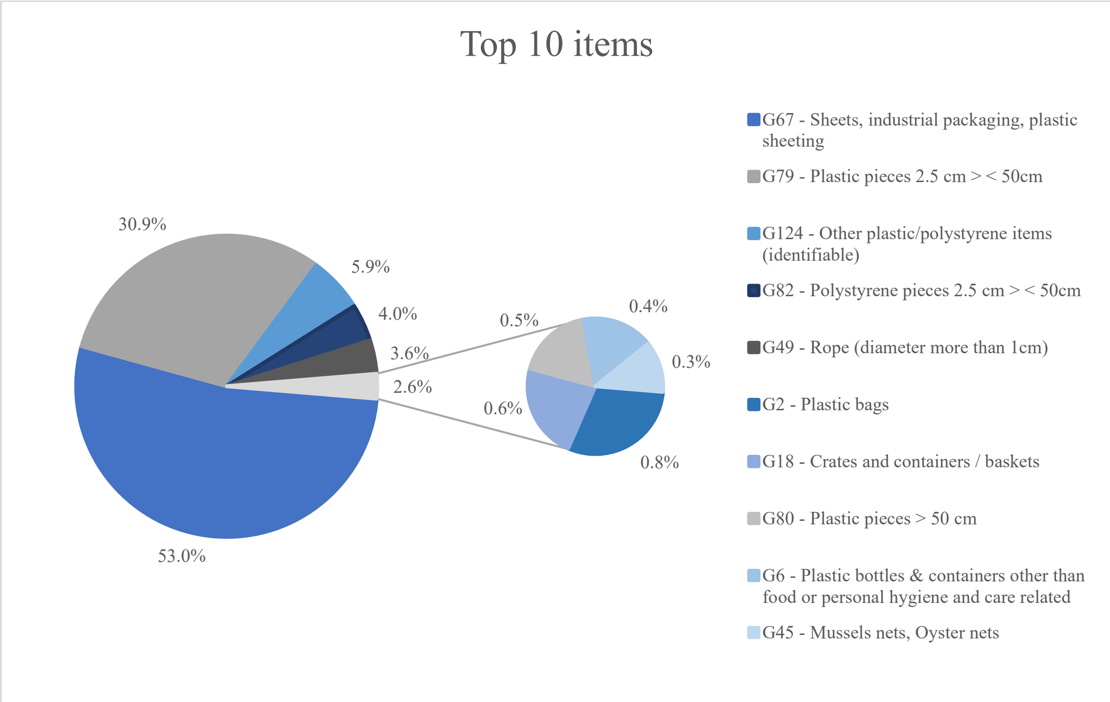


Fig. 16. Floating macrolitter top 10 items presence in the whole study area. G-code referring to the Master List of items characterization are displayed.

The Kruskal-Wallis test confirmed the difference in the distribution of samples between the two monitored areas (D = 0.3927, p-value = 1.968e-09). This difference was statistically significant (W = 5413.5, p-value = 9.707e-10) and confirmed a lower concentration of floating macrolitter in the Pelagos Sanctuary (280.36 ± 423.88 items/km^2) than in the surface waters of the Tuscan Archipelago National Park (617.76 ± 599.15 items/km^2) (Fig. 15). A difference in mean concentration was also statistically significant when considering different size classes (B. 2.5-5 cm, C. 5-10 cm, D. 10-20 cm, E. 20-30 cm, F. 30-50 cm, G. > 50 cm) between the two areas. Only for class E (20-30 cm), no difference in average concentration was observed, while the concentration for all other classes was higher in the Tuscan Archipelago National Park than in the Pelagos Sanctuary (Fig. 17). The highest abundances in surface waters off the islands of the Tuscany region, both in terms of the number of items and size classes, may be due to more recent inputs of pollution from land, as this area was particularly affected by tourist and recreational activities during the period of the sampling campaigns. Moreover, the stability of hydrodynamic features that characterise the Tuscan archipelago during the summer months could favour the floating of larger objects in coastal waters once they are dispersed in the marine environment, delaying their potential accumulation in pelagic areas.

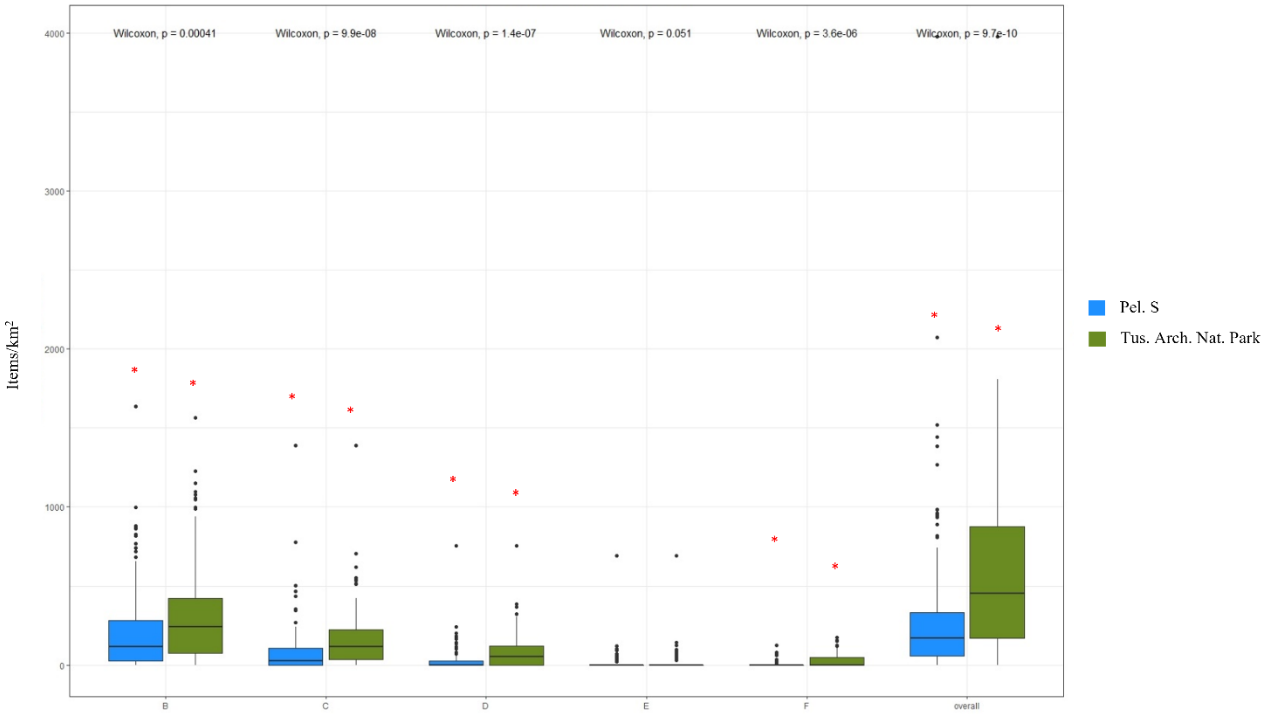


Fig. 17. Floating macrolitter different distribution among the two study areas considered (Pelagos Sanctuary: blue box plots; Tuscan Archipelago National Park: green boxplots) according to both size classes (B. 2.5-5 cm, C. 5-10 cm, D.10-20 cm, E. 20-30 cm, F. 30-50 cm, G. > 50 cm) and total avg. concentration. The boundaries of the boxes indicate the 25th and 75th percentiles, the whiskers above and below the boxes the 95th and 5th percentiles. Outliers are indicated by black dots. The horizontal line denotes the median value. * Indicates difference statistically significative ($p < 0.05$).

Considering the different types of litter, the categories with the highest average concentration in the Pelagos Sanctuary were "G67" and "G79", for which an average of more than 100 items / km^2 was recorded (Tab. 6). In the Tuscan Archipelago National Park as well for these categories highest concentrations were found, reaching more than 300 items/km^2 and 150 items/km^2, respectively. The categories "G58: Fish boxes", "G94: Tablecloth, "G145: other textiles" and "G149: Paper packaging" were only sighted in the Pelagos Sanctuary; while "G3: Buoys", "G74: foam packaging", "G135: Clothing", "G142: Rope, string, and nets" and "G160: Pallets" were only present in the Tuscan Archipelago.

Among the categories sampled in both study areas, "G6: Bottles", "G18: Crates and containers/baskets", "G45: Mussel nets/Oyster nets", "G48: Synthetic rope", "G67: Sheets, industrial packaging, plastic sheeting", "G79: Plastic pieces 2.5 cm > < 50 cm" and "G124: Other plastic/polystyrene items (identifiable)" were found to have a higher statistically significant concentration in the Tuscan Archipelago National Park than in the Pelagos Conservation Area (Tab. 8).

Tab. 8. Mean concentration of floating litter according to different categories, for the two considered study areas. Grey cells evidenced statistically significant higher values (Wilcoxon test; $p < 0.05$).

G-code and corresponding category	Concentration [Items / km^2] – mean (sd)		Wilcoxon test P (< 0.05)
	Pelagos Sanctuary	Tuscan Archipelago National Park	
G2-Bags	2.422 (13.771)	5.018 (19.075)	W = 9153, p-value = 0.1517
G6-Bottles	0.474 (4.582)	3.809 (13.923)	W = 8928.5, p-value = 0.006334
G18-Crates and containers / baskets	0.815 (6.476)	4.782 (15.669)	W = 8814.5, p-value = 0.004033
G38-Cover / packaging	0.336 (4.410)	0.345 (3.623)	W = 9484, p-value = 0.7541
G45-Mussel nets / Oyester nets	0.197 (2.585)	2.5 (2.585)	W = 9135.5, p-value = 0.02362
G48-Synthetic rope	10.572 (28.626)	20.036 (37.932)	W = 8516.5, p-value = 0.03621
G51-Fishing net	1.254 (9.728)	1.264 (9.370)	W = 9506.5, p-value = 0.9583
G58-Fish boxes - expanded polystyrene	0.191 (2.509)		n.a.
G63-Buoys		1.091 (11.442)	n.a.
G67-Sheets, indus. packaging, plastic sheeting	125.110 (289.537)	347.591 (435.738)	W = 5255.5, p-value = 1.628e-10
G74-Foam packaging/insulation/polyurethane		1.082 (11.346)	n.a.
G79-Plastic pieces 2.5cm ><50 cm	100.393 (167.452)	163.836 (193.097)	W = 7424.5, p-value = 0.001434
G80-Plastic pieces >50 cm	0.925 (6.465)	3.336 (23.555)	W = 9470.5, p-value = 0.8075
G82-Polystyrene pieces 2.5cm ><50 cm	17.012 (44.943)	19.400 (94.435)	W = 9920, p-value = 0.3895
G83-Polystyrene pieces >50 cm	1.127 (10.662)	0.618 (6.484)	W = 9539.5, p-value = 0.8402
G94-Table cloth	0.283 (3.725)		n.a.
G124-Other plastic/polystyrene items (identifiable)	16.156 (32.322)	36.918 (50.646)	W = 7607, p-value = 0.0006935
G125-Balloons and balloon sticks	1.491 (9.104)	1.645 (9.9)	W = 9529, p-value = 0.9441
G135-Clothing (clothes, shoes)		0.345 (3.623)	n.a.
G142-Rope, string and nets		0.618 (6.484)	n.a.
G145-Other textiles (incl. rags)	0.168 (2.205)		n.a.
G149-Paper packaging	0.769 (7.139)		n.a.
G158-Other paper items	0.150 (1.977)	0.936 (7.117)	W = 9396, p-value = 0.3195
G160-Pallets		0.355 (3.719)	n.a.
G168-Wood boards	0.214 (2.070)	1.555 (9.382)	W = 9362.5, p-value = 0.3209
G173-Other (specify)*	0.133 (0.1749)		n.a.
G197-Other (metal)	0.208 (2.737)	0.682 (5.088)	W = 9397, p-value = 0.3236

Considering the differences between the islands of the Tuscan Archipelago National Park, the highest concentration of macrolitter was found in the southern sector of the archipelago near the islands of Giglio and Giannutri (792.90 ± 610.13 items/km^2) and the northern sector between the islands of Gorgona and Capraia (726.42 ± 735.20 items/km^2) (Fig. 18). These patterns of accumulation may be influenced by the fresh inputs of litter originating directly from the coast due to the short distance of these islands and the proximity of the Tevere river identified as a plastic pollution source by De Lucia et al. (2018) in the southern sector. The influence of rivers on plastic distribution in this area was pointed out also by Galgani et al. (2019), evidencing how during the summer period, the northern part of the Tyrrhenian Sea was particularly affected by plastics riverine inputs originating from the Ombrone and Tevere rivers and spatially distributed by the superficial currents insisting on this area. Oceanographical features, and in particular the currents prevailing in the northern part of the Tuscan Archipelago National Park might be considered the main factors determining plastic distribution in this area. Other studies published in the literature have highlighted the temporary formation of a convergence area between the islands of Capraia, Corsica and Gorgona, which influences the distribution and concentration of litter (Fossi et al., 2017; Suaria et al., 2016).

The Kruskal-Wallis test confirmed the differences between islands in terms of floating litter concentration (Kruskal-Wallis chi-squared = 14.401, df = 6, p-value = 0.02547). The post - hoc Dunn test confirmed that Giannutri and Montecristo were statistically different from the other areas and were the islands with the highest (1040.35 ± 648.34 items/km^2) and lowest (264.93 ± 210.92 items/km^2) concentration values, respectively (p=0.001 - p adjusted=0.02) (Figs. 17 and 18 A). This difference could be explained both by the distance from the Tuscan coast and by the potential pollution sources that may affect these islands. Montecristo is the more pelagic island of the Tuscan archipelago and is located about 22 nm from the Tuscan coast. The waters of the island are fully protected up to a distance of 1 nautical mile and access is regulated and limited to 1000 visitors per year. For this reason, the presence and accumulation of litter could be limited, as confirmed by our data. The second-highest concentration was found in the waters facing the island of Pianosa (748.32 ± 522.32 items/km^2) (Figs. 17 and 18 A). The island is in the central sector of the Tuscan Archipelago and seems to be affected by intensive transport, accumulation and beaching ashore of litter items. So far, these data represent the first assessment of litter occurrence in this area, where surface currents seem to play a crucial role in marine debris accumulation.

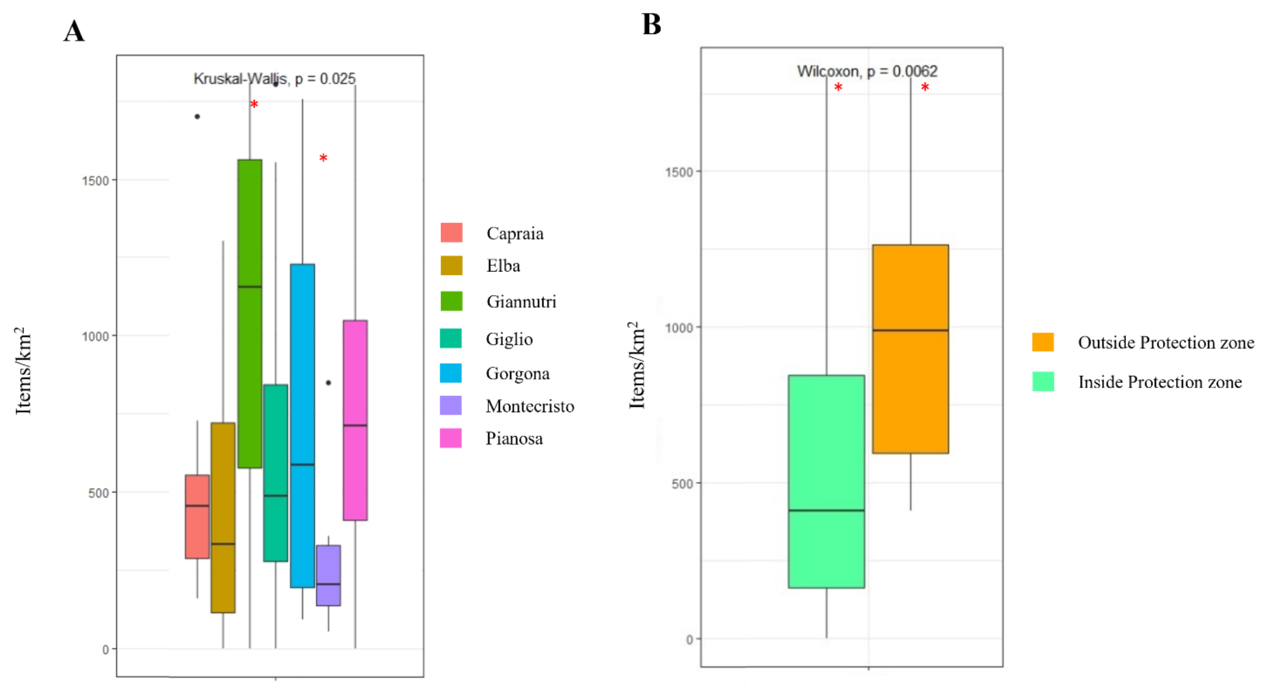


Fig. 18. Total mean concentrations of floating macrolitter distribution according to the Tuscan Archipelago National Park Islands (A) and presence of Protection Zone (B). The boundaries of the boxes indicate the 25th and 75th percentiles, the whiskers above and below the boxes the 95th and 5th percentiles. Outliers are indicated by black dots. The horizontal line denotes the median value. * Indicates difference statistically significative (p < 0.05).

As already highlighted for the whole study area, size class B was the most abundant in the different islands studied, especially in Giannutri Island with a concentration of 579.25 ± 402.43 items/km^2 (p = 0.001 - p adjusted= 0.02) (Tab. 8). Litter with larger dimensions (> 10 cm) was found statistically significant in the surface waters off the islands of Pianosa (classes D and E p = 0.002 p adjusted = 0.04) and again Giannutri (class D) (p adjusted = 0.023) (Tab. 9).

Tab. 9. Mean concentration of floating litter according to different size classes in the Tuscan Archipelago National Park. Grey cells evidenced statistically significant higher values (Kruskal-Wallis significance test for $p < 0.05$).

Dimension	Items / km² mean (sd)						
	Gorgona	Capraia	Elba	Montecristo	Pianosa	Giglio	Giannutri
B 2.5-5cm	463.25 (396.10)	268.15 (288.69)	193.71 (182.47)	121.83 (124.12)	356.07 (355.88)	384.13 (417.84)	579.25 (402.43)
C 5-10cm	136.75 (160.33)	177.77 (128.41)	134.10 (168.48)	95.83 (111.40)	187.57 (176.74)	153.75 (152.32)	249.25 (213.60)
D 10-20cm	79.00 (82.15)	64.92 (104.70)	81.67 (101.38)	18.67 (36.69)	122.29 (118.36)	36.19 (48.35)	110.83 (92.56)
E 20-50cm	17.33 (31.94)		11.67 (26.82)	5.08 (17.61)	30.93 (49.98)	6.50 (18.06)	39.00 (39.34)
F 50-100cm	27.75 (43.16)	14.38 (28.06)	11.19 (23.96)	17.33 (33.44)	38.86 (68.51)	30.31 (43.47)	38.00 (47.97)

Considering the samples carried out inside or outside the protected areas in the Tuscan Archipelago National Park, the concentration of floating litter showed statistically significant lower values inside the marine protected area than outside (p = 0.0062) (Fig. 18 B). Categories G67 and G79 were the most frequently found objects on the different islands. Their presence was assessed in higher concentrations in the samples conducted outside the protected areas in the Tuscan Archipelago National Park. However, statistical differences were only found for category G67 (679.08 ± 332.06 objects/km^2) (Wilcoxon test p-value = 0.0007492).

4.3.2 Floating microlitter abundances and composition

A total of 141 manta trawl samples were collected to assess the concentration of microlitter at the sea surface. A total of 56,084 particles were isolated, belonging respectively for 90% (n°. 50,985) and 10% (n°. 5,099) to microparticles and mesoparticles. No rubber particles were found, so the following results refer to MPs only. An average concentration of 259,490 ± 586,477 items/km^2 was found throughout the study area, ranging from 16,647 to 4,933,909 items/km^2 (Fig. 19). This value was in agreement with the mean value of floating MPs abundances in the western Mediterranean subregion, which was calculated considering the scientific studies available in the literature and set at 216,399 ± 284,360 items/km^2. Although no threshold values for MPs has yet been proposed, UNEP/MAP (2020) established a baseline of 340,000 items/km^2 for the Mediterranean Sea. Considering that the concentration shown in this study is lower than the mean of the whole basin suggests a potentially lower impact of these particles in the Pelagos Sanctuary. Despite that, as reported in a recent study by Caldweel et al. (2020) with a mean concentration of 233,927 ± 810,357 items/km^2, the occurrence and abundance of MPs in this area appear to be increasing compared to those reported during the 2018 sampling campaign carried out in 2018 (Caldwell et al., 2019) and by previous studies (Baini et al., 2018; Collignon et al., 2012, 2014; Fossi et al., 2017; Pedrotti et al., 2016; Tesán Onrubia et al., 2021). This threatening trend of particle accumulation may pose a threat to organisms living in this protected area throughout the marine trophic chain, as also highlighted by Fossi et al. (2017). Considering other MPAs in the Mediterranean Sea, the average concentration observed here (1.62 ± 3.67

items/m³), expressed as particles per m³ to allow a proper comparison with other studies (Tab. 2), is only lower than the values found by Fagiano et al. (2022) in Cabrera National Park (3.52 ± 8.81 items/m³), which is considered an area of high plastic waste density (Ruiz-orejon et al., 2018), confirming the potential trend of plastic waste accumulation compared to previous studies (Fossi et al., 2012, 2016; Panti et al., 2015).

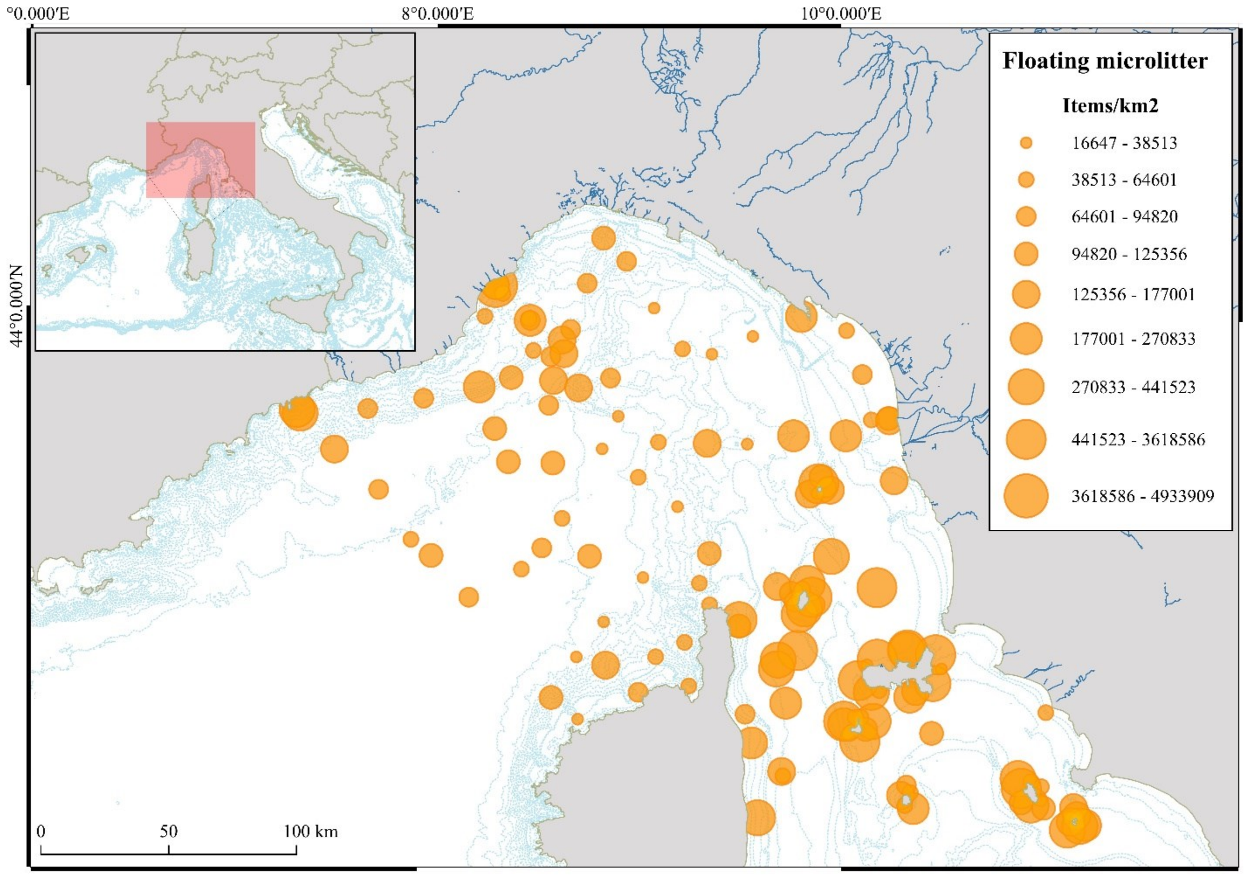


Fig. 19. Floating microlitter spatial distribution in the whole study area considered. The concentrations of litter objects sighted were expressed in items/km².

MPs characterization analysis revealed that large MPs were the most abundant size class (76%) (Fig. 20 A) and in particular the fraction ranging between 1 mm to 2.5 mm, accounting for 42% in total. Fragments (86%) and films (10%) are the most represented shapes with 96% of the isolated particles (Fig. 20 B). These results are consistent with other studies conducted in the Mediterranean Sea (Baini et al., 2018; Compa et al., 2020; Suaria et al., 2016) and elsewhere (Cózar et al. 2014; Eriksen et al., 2013; Lusher et al., 2014) and confirm that secondary microplastics are the most widespread in the marine environment and the most suitable for ingestion by marine organisms. Colours can also influence the uptake of plastic particles. Particularly brightly coloured items, which were represented in this study at a concentration of > 70%, could increase the likelihood of ingestion as they resemble prey (Marti et al., 2020; Wright et al., 2013).

Chemical composition analysis showed that polyolefin thermoplastics, represented by PE and PP (95% in total) (Fig. 20 C), were the most abundant. Their presence in the marine environment is widely recognised in all ocean basins (Baini et al., 2018; Enders et al., 2015; Gewert et al., 2017; Pedrotti et al., 2016; Suaria et al., 2016), reflecting the increasing production and use of these plastic polymers. They are mainly used in packaging and disposable products and their production in Europe represents ~50% of the total plastic demand (Plastics Europe, 2020). Moreover, as PE and PP positively buoyant polymers (0.90 - 0.99 g/cm^3; 0.85 - 0.92 g/cm^3) are sensitive to degradation in the marine environment and have a longer residence time at the sea surface, they tend to accumulate at the sea surface as confirmed by the plastic-type here found, mainly fragments and films, made of these materials.

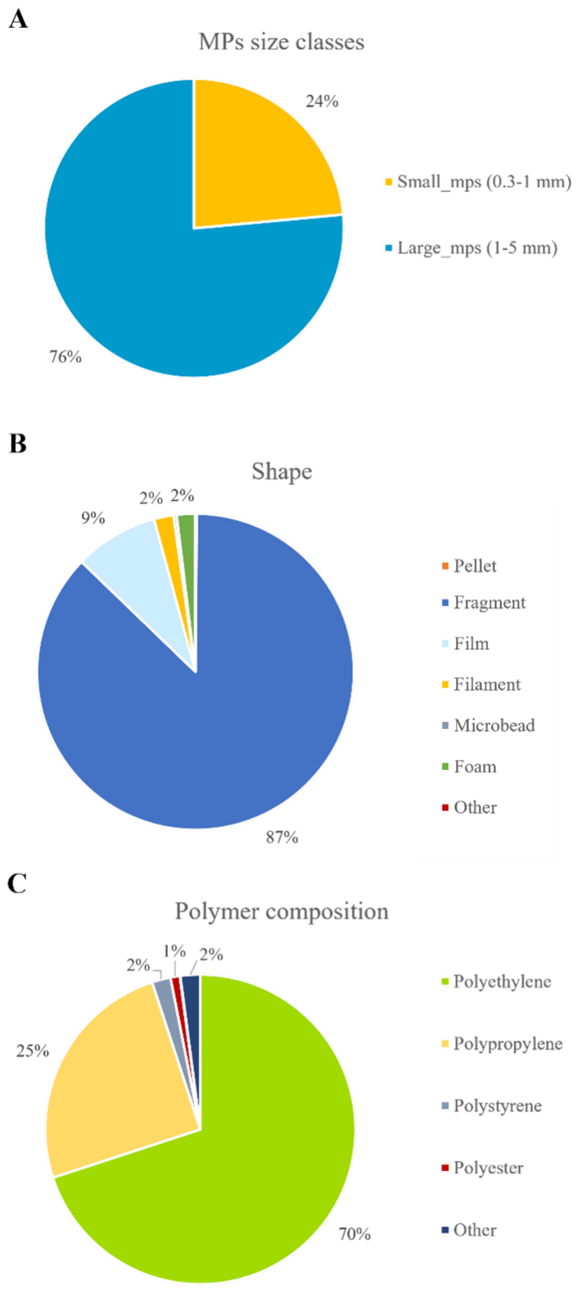


Fig. 20. Pie chart summarizing the percentages (in abundance) of MPs size classes (A), shape categories (B) and polymer composition (C) collected by manta trawl within the study area.

The two sampling campaigns conducted in the Pelagos Sanctuary (excluding the Tuscan Archipelago) and the Tuscan Archipelago National Park, respectively, highlighted some statistical differences in the abundance and characterization of MPs. The Kolmogorov-Smirnov test confirmed the difference in the distribution of samples between the two areas both in terms of weight density (mg/m^2) (D = 0.35402, p-value

= 0.0002725) and concentration (items/km^2) of particles (D = 0.25575, p-value = 0.01922), with a statistically significant difference observed for both parameters considered (weight density Wilcoxon test W = 1524, p-value = 1.884e-05; concentration Wilcoxon test W = 1524, p-value = 3.2 e-03). The average weight density and concentration values of MPs were lower in the Pelagos Sanctuary (0.068 ± 0.162 mg/m^2 and 226,075 ± 650,984 items/km^2) than in the surface waters of the Tuscan Archipelago National Park (0.152 ± 0.261 mg/m^2 and 355,281 ± 616,782 items/km^2) (Figs. 19 and 21 B). This result confirms what was observed for the distribution and concentration of floating macrolitter objects and strengthens the hypothesis that the presence of larger objects (categories G67 and G79) may influence the formation of MPs as a result of degradation and fragmentation processes. The same result was also underlined by the shape analysis of the isolated particles, which revealed a significant concentration of fragments (305,065 ± 522,863 items/km^2) (Wilcoxon test p-value = 9.2e-03) and film (37,479 ± 101,232 items/km^2) (Wilcoxon test p-value = 4.1e-05) (Fig. 21 A).

According to the classification of size classes, the difference in mean concentration between the two areas was statistically significant when considering only larger particles, which resulted in a higher concentration in the Tuscan Archipelago National Park (287,744 ± 497,983 items/km^2) than in the Pelagos Sanctuary (163,084 ± 466,917 items/km^2) (Fig. 21 B). This accumulation pattern was confirmed by the study of Pedrotti et al. (2016), analysing the size distributions of plastic particles at different distances from land and showing an increase in plastic abundance from large to small items moving from coastal to pelagic areas. Moreover, this result is consistent also with the general size distribution found by Cózar et al. (2014) for ocean surface waters. According to Pedrotti et al. (2016), the highest presence of large MPs in the nearshore areas could be due to the combination of efficient removals of small fragments from the surface due to their potential stratification along the water column, sinking due to the biofouling processes and the interactions with marine organisms such as invertebrates species. In addition, the gradual fragmentation processes due to physical and chemical degradation of plastic particles moving towards the pelagic areas may favourite the formation of smaller particles and their accumulation in offshore waters.

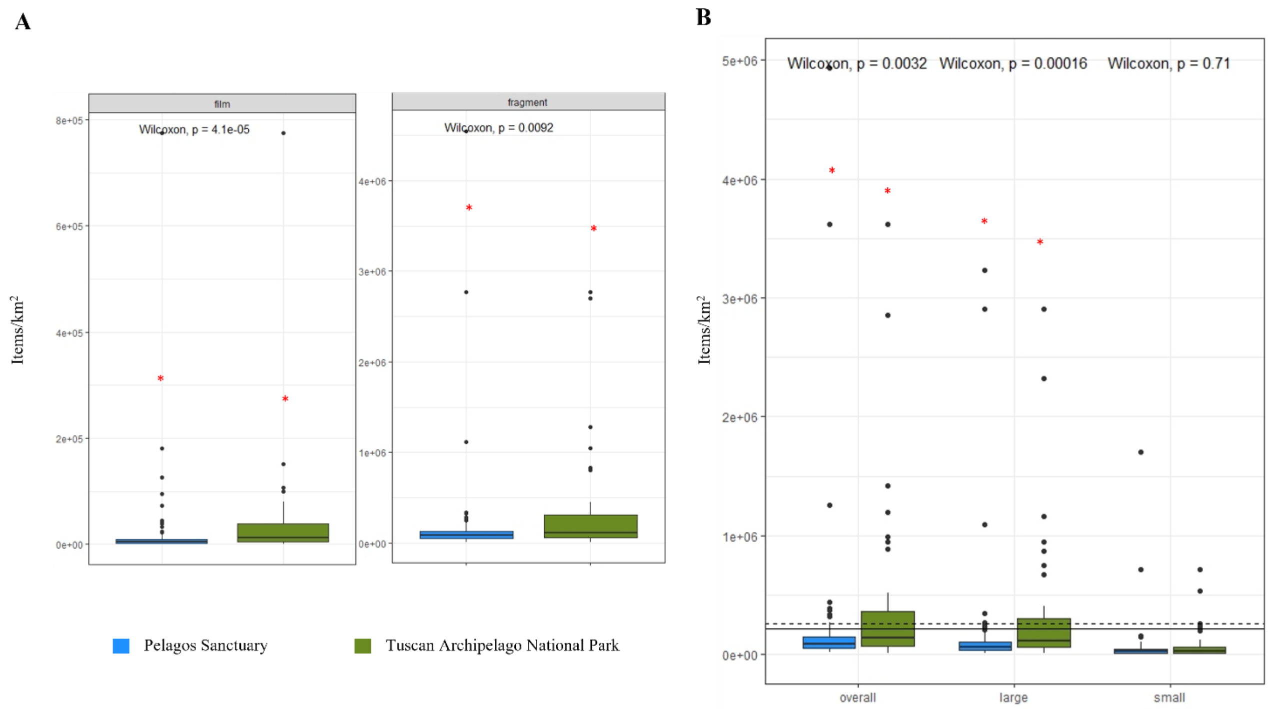


Fig. 21. Floating MPs different distribution among the two study areas considered (Pelagos sanctuary: blue boxplots; Tuscan Archipelago National Park: green boxplots) according to shape, size classes and total avg. concentration. The boundaries of the boxes indicate the 25th and 75th percentiles, the whiskers above and below the boxes the 95th and 5th percentiles. The horizontal line inside the boxplots denotes the median value. Outliers are indicated by black dots. The black line shows the reference value for mean MPs concentration in Northwestern Mediterranean Sea, while the dashed line represents the reference value for the standard deviation of MPs concentration in Northwestern Mediterranean Sea.* Indicates difference statistically significative ($p < 0.05$).

However, the Kruskal-Wallis test revealed no differences among the islands of the Tuscan Archipelago National Park (chi-squared = 10.483, df = 6, p-value = 0.1057 for density and chi-squared = 5.2042, df = 6, p-value = 0.5179 for concentration). The average weight density (mg/m^2) and item concentration (items/km^2) calculated for each island are shown in Tab. 10.

Tab. 10. Mean and SD weight density and concentration values for each considered Island in the Tuscan Archipelago National Park.

Island	Concentration (items/km^2)	Density (mg/m^2)
Gorgona	563,962 ± 1,123,234	0.317 ± 0.662
Capraia	211,650 ± 159,736	0.088 ± 0.052
Elba	469,624 ± 468,907	0.231 ± 0.219
Pianosa	290,966 ± 321,938	0.105± 0.103
Montecristo	102,966 ± 83,089	0.023 ± 0.019
Giglio	241,007 ± 292,466	0.101 ± 0.109
Giannutri	211,074 ± 106,240	0.129 ± 0.099

The highest concentrations for both number and weight density of particles were found in the northern part of the Tuscan Archipelago facing the island of Gorgona (Tab. 9). This area was previously described as the most affected by the presence of floating macroparticles, indicating the formation of a temporary accumulation zone previously described in the literature (Fossi et al., 2017; Suaria et al., 2016). High particle abundances were also detected around the islands of Elba and Pianosa in the central part of the archipelago, the second and third areas, respectively, where MPs seem to accumulate. While the first area is under strong anthropogenic pressure, especially during the summer months, leading to possible plastic pollution, the distribution and accumulation of particles on Pianosa again seem to be closely related to the surface currents that characterize the waters there. Differently from what was highlighted for macrolitter objects, the islands in the southern sector appear to be more vulnerable to recent inputs of plastic pollution from the coast. This is also confirmed by the greater extent of the sighted objects, which may be displaced to more pelagic areas of the Tyrrhenian Sea where fragmentation processes occur, as indicated by the litter dispersion model (Northern Tyrrhenian Gyre, described in Fossi et al., 2017). No differences in MPs distribution were found between the different levels of protection on the monitored islands.

4.3.3 Marine litter distribution: influence of marine habitats

The potential distribution of floating macrolitter and MPs was assessed considering the different marine habitats according to the topographic features within the Pelagos Sanctuary and the Tuscan Archipelago National Park. The monitored area was characterized by different habitat types in the bathyal, canyon, seamount, slope and continental shelf. The potential distribution of litter in these selected zones could provide preliminary information on the preferred distribution areas and marine species potentially affected by interaction and ingestion of litter. Concentrations for floating macrolitter and MPs are shown in Tab. 11.

Tab. 11. Floating macrolitter and MPs concentrations in the different habitats considered within the Pelagos Sanctuary.

Habitat	**Floating macrolitter (items/km^2)**	**MPs (items/km^2)**
Bathyal (200 m – 2000 m)	176 ± 158	88,508 ± 38,146
Canyon (1700 m - >2000 m)	238 ± 337	378,137 ± 1,107,092
Sea mount (400 m - > 2000 m)	205 ± 245	86,796 ± 59,482
Slope (200 m – 2000 m)	257 ± 340	161,176 ± 245,238
Continental shelf (0 m -200 m)	573 ± 572	310,489 ± 559,776

The Kruskal-Wallis test confirmed the difference between habitats only for floating macrolitter (Kruskal-Wallis chi-squared = 39.793, df = 4, p-value = 4.778e-08). Post-hoc analysis showed that the continental shelf and seamount areas were separated from all other habitats (Fig. 22 A). No difference was found for the distribution of MPs (Kruskal-Wallis chi-square = 8.9064, df = 4, p-value = 0.06348) in the Pelagos Sanctuary (Fig. 22 B).

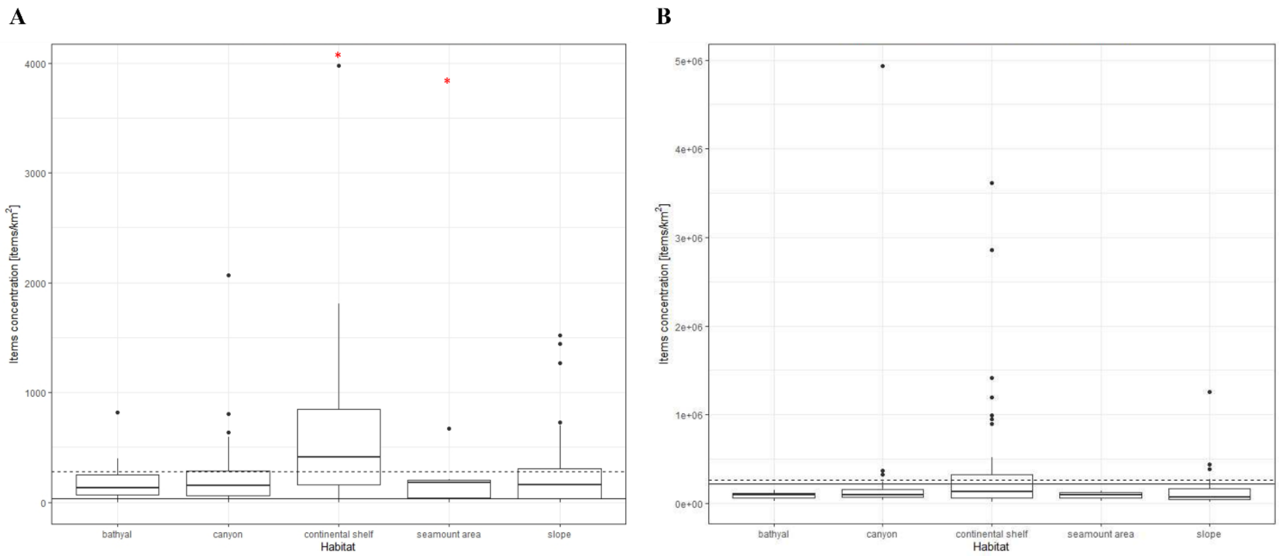


Fig. 22. Concentrations of floating macrolitter (A) and MPs (B) for different habitats within the Pelagos Sanctuary. The boundaries of the boxes indicate the 25th and 75th percentiles, the whiskers above and below the boxes the 95th and 5th percentiles. The horizontal line inside the boxplots denotes the median value. Black lines represent the reference value of mean floating macrolitter/MPs concentration in the Western Mediterranean sub-region and the dashed line represent mean concentration overall Pelagos Sanctuary from the present study.

The highest concentration of floating macrolitter was found in the correspondence of the continental shelf. This area, which is the natural extent of the mainland, from the coastline to a depth of 200 m, is the most sensitive habitat for the accumulation of floating debris that enters the marine environment via land-based sources. Previously described as an area characterized by low litter seafloor density (Galgani et al., 1996; Pham et al., 2104), could be considered a transition zone of buoyant litter towards pelagic habitats such as submarine canyons, where marine litter has been shown to sink and accumulate (Galgani et al., 2000; Gerigny et al., 2019).

4.3.4 Marine litter distribution: influence of environmental and anthropic factors

The distribution of the floating macrolitter and MP datasets throughout the monitored study area was examined considering the main environmental (SST: sea surface temperature; SSH: sea surface height; MLD: mixed layer depth and current velocity) and anthropogenic factors (vessel traffic, distance from ports, distance from the coast and distance from estuaries) that may influence their distribution. Correlation analyses show a statistically significant correlation between many of the variables considered (76%) and concentrations of floating macrolitter. In particular, SST, SSH, fishing vessel density and sailing vessel density showed a weak positive correlation ($0 < rho < 0.3$) with the amount of litter. Bathymetry (expressed as negative values) showed a stronger significant positive correlation ($0.3 < rho < 0.5$), while a weak negative correlation ($-0.3 > rho > 0$) was found between floating macrolitter concentration and mixed layer depth (MLD), current velocity, tanker density, cargo vessel density, distance from nearest major outfalls, and distance from the nearest minor outfall. The correlation of floating macrolitter abundance respectively with distance from the coast and distance from the nearest port was also negative and stronger ($-0.5 < rho < -0.3$). The descriptive statistical values of each environmental and anthropic variable considered and the corresponding correlation values and scatter plots with floating macro pollution concentration were summarised in Annexes 4 and 5. MPs concentration was significantly related to 47% of the variables studied. The statistically significant results show a weak positive correlation (0 < rho < 0.3) of microplastic density with sea surface temperature, sea surface height, bathymetry and sailing vessel density. Weak negative correlations (-0.3 < rho < 0) were found for currents, distance from the coast, distance from the nearest port and cargo ship density (Annexes 6 and 7).

Generalised additive models (GAM) were applied to further determine the influence of each variable on litter abundance (Annexes 8 and 9). In addition, to better highlight the relationships represented by GAM, a zero line was used to define a positive effect of the predictors on litter accumulation. This was done in a procedure called GAMvelope, described by Torres et al. (2008) (Figs. 23 A-C and 24 A-C), and allowed the identification of areas affected by the presence of litter in the Pelagos Sanctuary.

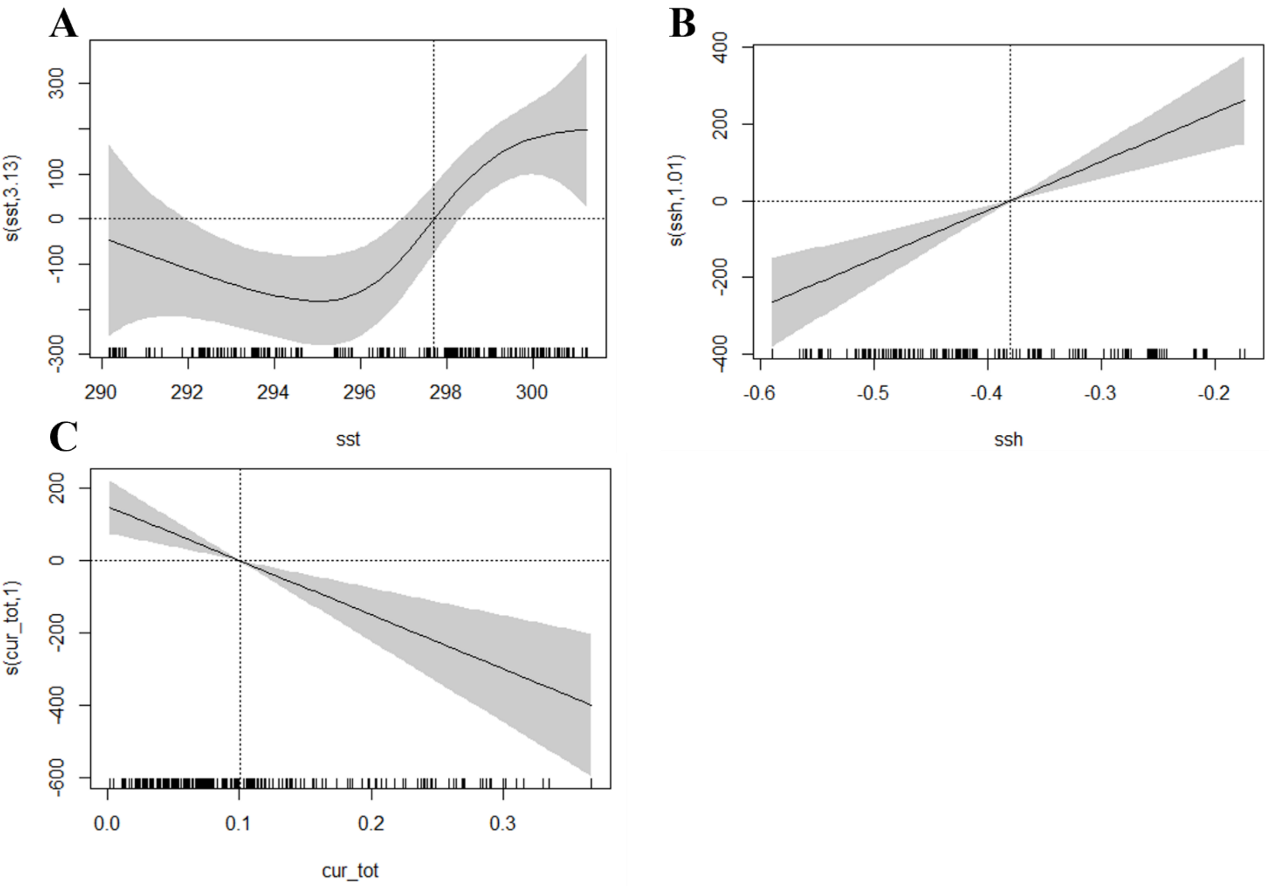


Fig. 23. GAMs plot of significative environmental variables (A: SST; B: SSH and C: current velocity) influencing the floating macrolitter accumulation. The degrees of freedom for non-linear fits are in parenthesis on the y-axis. Tick marks above the x-axis indicate the distribution of observations (with and without sightings). The shaded areas represent the 95% confidence intervals of the spline functions.

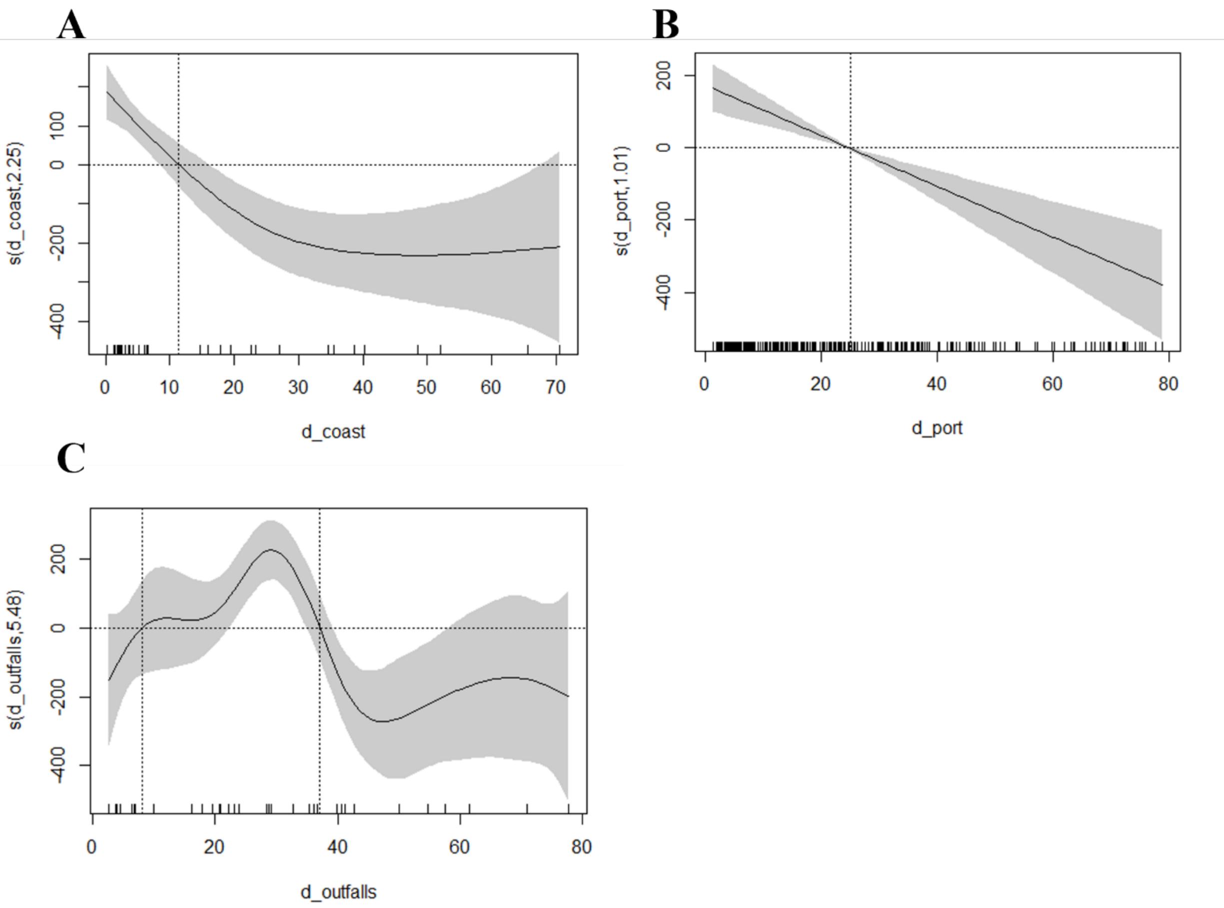


Fig. 24. GAMs plot of significative anthropic variables (A: distance to the coast; B: distance to the port; and C: distance to river outfall) influencing the floating macrolitter accumulation. The degrees of freedom for non-linear fits are in parenthesis on the y-axis. Tick marks above the x-axis indicate the distribution of observations (with and without sightings). The shaded areas represent the 95% confidence intervals of the spline functions.

Among the environmental variables, SST higher than 297.7 K (24.55°C), SSH higher than -0.38 m, and currents slower than 0.101 m/s have a positive effect on the accumulation of floating litter (Fig. 23 A-C). These results suggest that areas with warmer waters and weak oceanographic features such as lower wave height, slower currents, and no upwelling areas may favour macrolitter accumulation. The influence of certain physical and chemical parameters of oceanic waters on the distribution of litter and sampling activities was clearly outlined by van Sebille et al. (2020). The so-called "vertical mixing effect" of plastic particles, first described by Kukulka et al. (2012) and also emphasised by Enders et al. (2015) and Reisser et al. (2015), is closely related to wave height and direct wind force, which could facilitate the stratification of plastic particles along the water column according to their physical properties (Kooi et al., 2016). A significant increase in debris has also been observed during daily ocean warming, leading to an accumulation of particles at the warmer sea surface (Kukulka et al., 2016). Considering the anthropogenic factors, a positive relationship was found between the amount of floating macro debris and the distance from the coastline closer than 11 km, the distance from the nearest port closer than 25 km, and the distance from the river mouth between 8 and 37 km (Fig. 24 A-C). These results confirm the findings of the spatial analysis of litter (Figs. 15 and 19) and the distribution of floating plastics in the Mediterranean Sea modelled by Liubartesva et al. (2018), according to which more than 75% of the litter scattered in the sea is located in the

50 km of nearshore waters. These areas can potentially be affected by large amounts of litter originating from nearby land-based sources and coastal maritime activities associated with densely populated areas, as well as inputs from rivers (Jambeck et al., 2015; Lebreton et al., 2017). In the Pelagos Sanctuary, the commercial, tourist and maritime port of Livorno (one of the largest Italian ports with 30 million tonnes of cargo and 2 million people), the rivers Arno (240 km long and crossing several cities, agricultural areas and industrial zones) and Serchio, and the intensive aquaculture and fishing activities near La Spezia could be the main sources of waste and plastic pollution (Cincinelli et al., 2001; Cortecci et al., 2002; Giovacchini et al., 2018). Other minor litter inputs could be derived by the port of Genova, which is described to play an important role in litter distribution in the coastal areas of the northern part of the Pelagos Sanctuary, as well as the influence of the Magra river in the transport and accumulation of anthropogenic particles especially during the summer period (Galgani et al., 2019). Its contribution appears particularly evident in the Tuscan Archipelago National Park due to the mediated transport of plastic by currents towards the southern sector of the SPAMI monitored (Galgani et al., 2019). This area may also be characterized by litter originating from the Tevere and Ombrone rivers, despite their influences that seem heavy affect the Pelagos Sanctuary especially during the winter season (Galgani et al., 2019).

Sea surface temperature (SST), bathymetry and distance to the nearest port were shown to significantly influence MP distribution (Annexes 8 and 9). However, given the lower explained variance and the paucity of significant variables, no further analysis of MPs was conducted. Moreover, considering the existing predictive models for their distribution (Fossi et al., 2017, 2018; Liubartseva et al., 2018; Mansui et al., 2015; Politikos et al., 2020), the GAMvelope approach was not considered more effective and was applied only to floating macrolitter at the sea surface.

Nevertheless, a correlation between the spatial concentrations of floating macrolitter (n. 273 monitoring transects) and MPs (n. 141 manta trawl samples) collected in the whole study area was investigated to reveal a possible common distribution pattern. A significant strong direct correlation (p-value < 2.2e-16, rho = 0.6253157) was demonstrated by Spearman's rank test (Fig. 25), confirming the effectiveness of the experimental plan performed and highlighting the importance of the simultaneous floating litter sampling to better address the presence and distribution of plastic pollution in the marine protected areas, providing also preliminary information on the potential impacts on marine organisms.

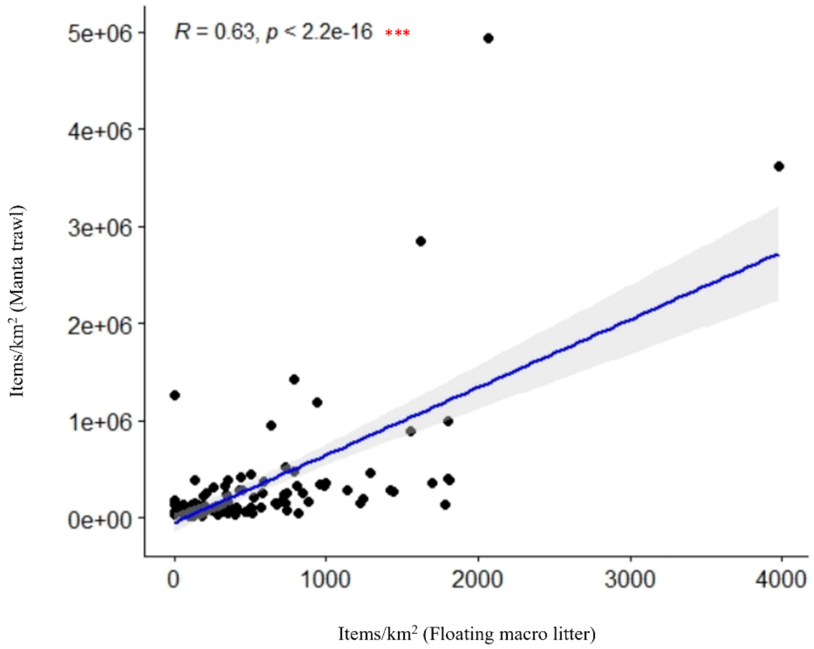


Fig. 25. Correlation scatterplots among floating macrolitter and MPs concentration. *** Statistical significance for p-values < 0.001

As a result, the overall risk maps produced for macrolitter floating at the sea surface can also provide a reliable indication for the accumulation of MPs. Average oceanographic conditions in terms of SST, SSH and current velocity were determined using monthly maps corresponding to the period of the sampling campaigns (June-September 2019). A 5 km grid was overlaid on the Pelagos Sanctuary area, assigning a value of 1 to each cell characterized by environmental (SST, SSH, and current velocity) and anthropogenic (distance to the coast, distance to the port, and distance to river outfall) variables that positively affect the litter dispersion distribution. A comprehensive hazard map was then generated, based on the distribution of floating macrolitter, with hazard indices ranging from 1 to 8 and indicating the areas at higher risk of exposure for the marine organisms (Fig. 26).

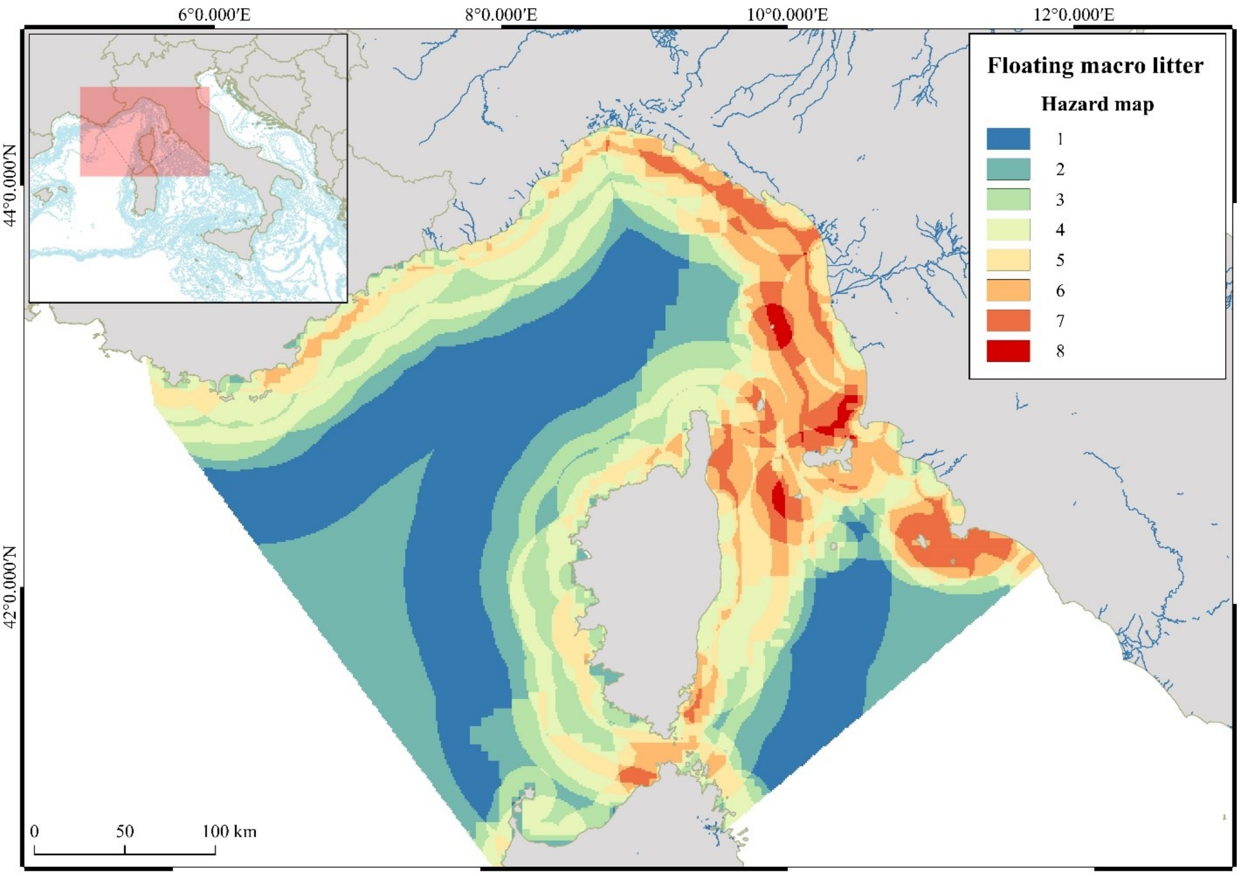


Fig. 26. Floating macrolitter spatial hazard map created considering the environmental and anthropic factors statistically influencing litter distribution. A hazard score, ranging from 1 to 8, was assigned highlighting areas with different impacts.

Overall, the study area was characterised by a high input of debris coming from the mainland (e.g., harbours and river inputs) and accumulating in coastal waters within about 10-15 nautical miles. The slope area off western Liguria, the continental shelf in the eastern part and the surrounding areas in the Tuscan Archipelago National Park and northeastern Corsica was shown to be particularly characterised by plastic debris accumulation. A moderate risk was present in the canyons of western Liguria and western Corsica, while the least accumulation of plastic was found in the offshore waters over the bathyal plane. The high concentration of litter evaluated on the coast may provide useful information on the ecological impact of plastics on biodiversity in the Pelagos Sanctuary and facilitate effective measures to prevent, reduce and dispose of marine litter in this MPA.

4.3.5 Beach macrolitter abundances and composition

The presence and distribution of macrolitter on the beach were evaluated in the Pelagos Sanctuary in three sites along the Tuscan coast and the southern part of the island of Corsica, and at 8 beaches on different islands of the Tuscan Archipelago National Park. A total of 31,769 items were recorded, removed and classified at the 14 sites surveyed. The average litter density was 521 ± 761 items/100m (median: 176 items/100m) and ranged from 20 items/100m to 3,166 items/100m.

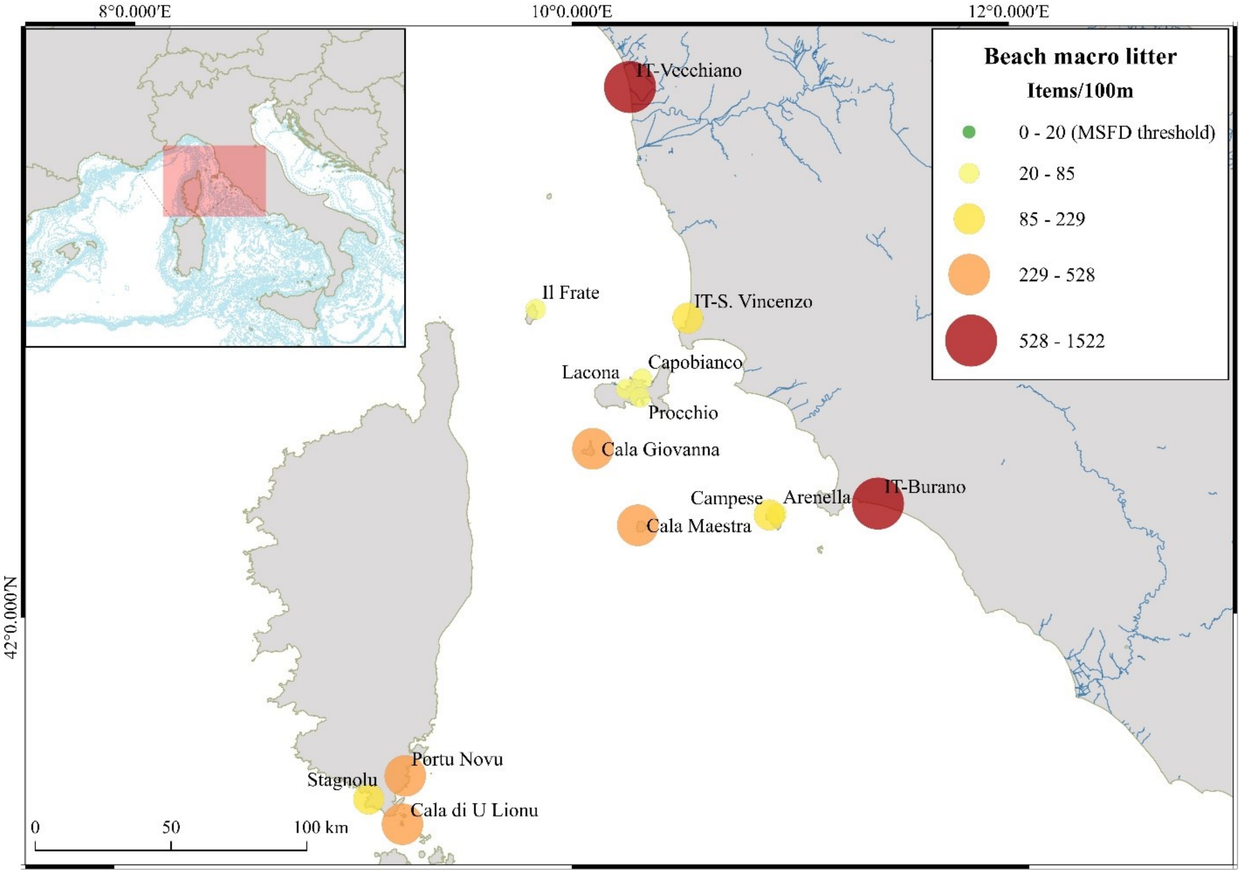


Fig. 27. Beach macrolitter concentrations (items/100m) in the sites monitored within the Pelagos Sanctuary and the Tuscan Archipelago National Park. The threshold proposed Task Group 10 of the MSFD (2021) was reported.

As shown in Fig. 27, the highest average litter density of 1033 ± 915 items/100m (0.53 ± 0.50 items/m^2) was recorded along the Tuscan coast, followed by the sites in the southern sector of the Corsican Islands (380 ± 205 items/100m or 0.34 ± 0.23 items/m^2). The lowest density of items found on a 100-metre stretch of coast was recorded in the northern sector of the Tuscan Archipelago on the island of Capraia (44 ± 205 items/100m). Discrepancies in the application of beach litter monitoring protocols and the marine litter densities in terms of units make the comparison of data difficult. However, the harmonised monitoring approach based on the indications of the MSFD protocol adopted in this study has allowed collecting reliable data on litter accumulation, providing baseline information of the pollution status of the selected sites comparable to the threshold value proposed by the Task Group 10 of the MSFD. The average litter concentration in the monitored area was in the same order of magnitude as those reported by Vlachogianni et al. (2020) with 714 items/100m in the whole basin, Fortibuoni et al. (2021) with 477 items/100m along the

Italian coasts, Camedda et al. (2021) with more than 39,000 items collected and Gioacchini et al. (2018) and Merlino et al. (2018) (1.06 items/m^2 and 0.72 ± 0.43 items/m^2) in the Pelagos Sanctuary. To date, there were very few data in the scientific literature evaluating the presence of beach litter in MPAs. Battisti et al. (2012, 2016) reported the presence of litter between 276 and 6700 items in the small protected area of Palude di Torre Flavia (Tyrrhenian Sea), although the concentration was not reported. Rizzo et al. (2021), focusing on the marine protected area of Torre Guaceto (Adriatic Sea), found a litter concentration of 47 items/m^2, mainly consisting of artificial polymers (> 70%).

All monitored sites exceed the threshold of EU MSFD TG 10 (20 items/100m) (Fig. 28) to protect beaches from ecological and socio-economic damage caused by litter. The average concentrations found in Burano (1522 ± 1032 items/100m) and Vecchiano (1398 ± 736 items/100m) exceed the above threshold by two orders of magnitude. Liubartseva et al. (2019) describe that these areas are critically affected by litter stranding and accumulation due to low to moderate plastic fluxes in coastal waters, varying from 2 kg (km day)$^{-1}$ to 3.3 kg (km day)$^{-1}$, increasing to 8 kg (km day)$^{-1}$ towards the Ligurian coast. Vecchiano beach (Migliarino-San Rossore-Massaciuccoli Regional Natural Park) has already been identified by Giovacchini et al. (2018) and Merlino et al. (2018) as the site most affected by litter along the Tuscan and Ligurian coasts, mainly due to potential pollution inputs from the Arno and Serchio rivers. On the other hand, no data were available on the distribution of macrolitter on the beaches of the WWF Oasis of Burano, although Cannas et al. (2017) found a high concentration of MPs in the sediments.

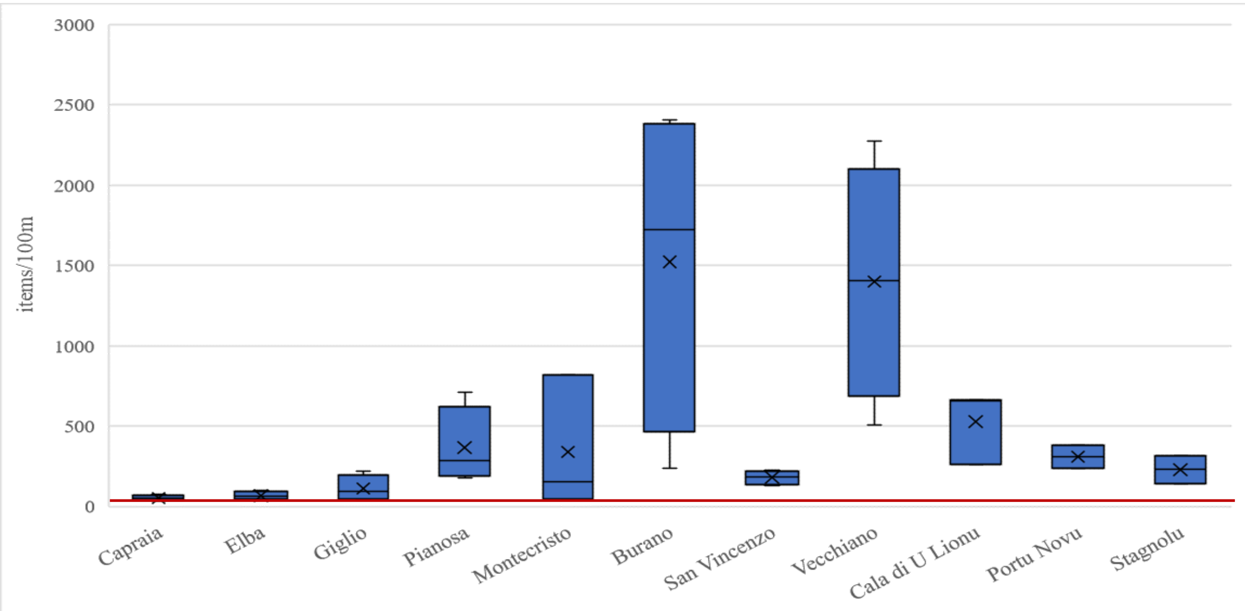


Fig. 28. Beach macrolitter concentrations (items/100m) in the sites monitored within the Pelagos Sanctuary and the Tuscan Archipelago National Park. The boundaries of the boxes indicate the 25th and 75th percentiles, the whiskers above and below the boxes the 95th and 5th percentiles. The horizontal line inside the boxplots denotes the median value and X the avg. value. The Red line indicates the threshold level defined by EU MSFD TG 10 (20 items/100m).

In the Bouches de Bonifacio Nature Reserve (the southern sector of the island of Corsica), the highest concentration of beach litter was found at Cala di U Lionu beach on the island of Lavezzi (528 ± 230 items/100m). This area is reported to have a low flux of plastics, 0.7 kg (km-day)$^{-1}$, mainly due to the intense maritime traffic that interests this area (Liubartseva et al., 2018). The distribution and accumulation of

wastes on this part of the Corsican coast seem to be strongly influenced by the two northward-flowing currents that rise along the opposite coastlines of Corsica. The interaction of hydrodynamic factors such as waves, wind and currents seem to regulate the distribution and accumulation of plastic also in the Tuscan Archipelago National Park. The highest densities of macrolitter on the beach were found on the islands of Pianosa and Montecristo, 365 ± 240 items/100m and 340 ± 419 items/100m, respectively. These sites, far from the coast and protected up to 1 nm, could be affected by the accumulation of litter objects dispersed in the facing waters, as suggested by Merlino et al. (2018), indicating a relatively low abundance of plastic objects on Pianosa Island (0.61 ± 0.29 items/m^2). Nevertheless, statistical analysis did not detect significant differences between the observed sites (Kruskal-Wallis chi-squared = 10, df = 10, p-value = 0.4405).
According to proximity to potential litter sources, potential mechanical cleaning, and tourism and recreational impacts, each monitored beach was categorized as remote, rural, and urban. Statistical analysis confirmed the differences between types using both the Kruskal-Wallis test (chi-square = 13.867, df = 2, p-value = 0.0009746) and pairwise comparison using the Wilcoxon test. This showed that remote beaches were the most affected by macrolitter (0.54 items/m^2 and 977 items/100m), followed by rural (0.25 items/m^2 and 484 items/100m) and urban (0.05 items/m^2 and 82 items/100m) (Fig. 29).

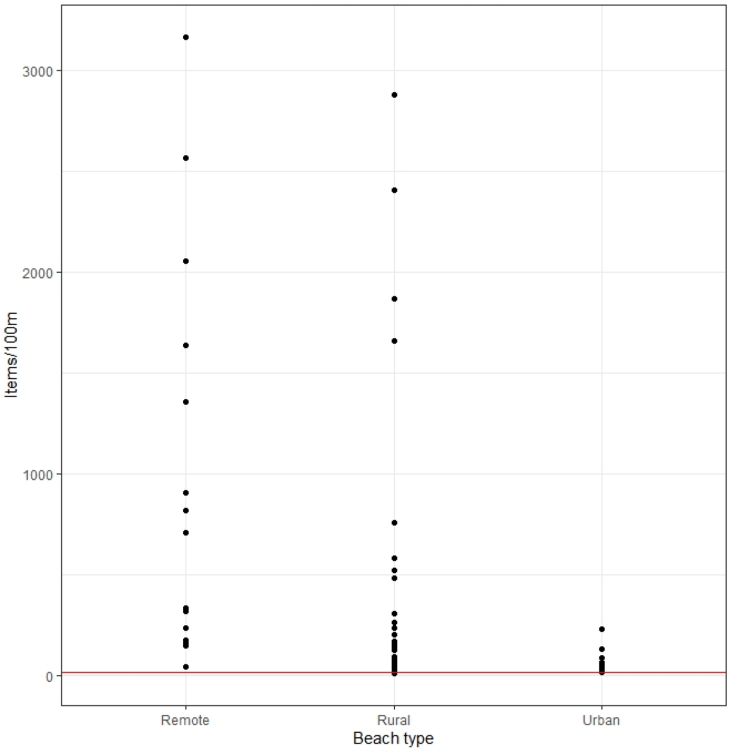


Fig. 29. Beach macrolitter concentration (items/100m) for each site is categorized according to the beach type. The Red-line represents the 20items/100m threshold defined by EU MSFD TG10.

This pattern of accumulation was also previously described by Giovacchini et al. (2018) and Vhlacogianni et al. (2017), who showed an increasing concentration of litter from urban (0.64 vs 0.11 items/m^2 respectively) to rural (0.87 vs 0.29 items/m^2 respectively) and remote (1.50 vs 0.55 items/m^2 respectively) beaches. The absence of regular cleaning activities (unlike urban beaches), and probably the large amounts of marine litter coming from the sea could deeply influence the accumulation of litter in these areas. This is the case of

Burano and Vecchiano beaches, where the highest litter density (0.82 ± 0.50 items/m^2 and 0.69 ± 0.35 items/m^2, respectively) is due to items generated by industries (e.g., textile factories, leather tanneries) located along the whole area crossed by the Fiora, Arno and Serchio rivers, or to other unexplained sources such as hazardous hospital or pharmaceutical wastes, whose management and disposal should be strictly regulated by national laws and regulations.
Artificial polymeric materials were the most abundant categories in all three beach types considered, i.e. remote (13,904), rural (12,520) and urban (728) areas, where values were 20 times higher than in other categories (Annexe 12). Glass and processed wood were the second and third most common categories in remote areas; paper, cardboard and glass in rural and urban areas (Annexe 12).
Within the study area, the overall seasonal distribution of litter shows a decreasing trend from winter to summer, although this is not confirmed by the Kruskal-Wallis test (Kruskal-Wallis chi-squared = 3.8026, df = 3, p-value = 0.2836). Looking at the different beach types between sites, different patterns can be seen, although these are not statistically significant (Fig. 30).

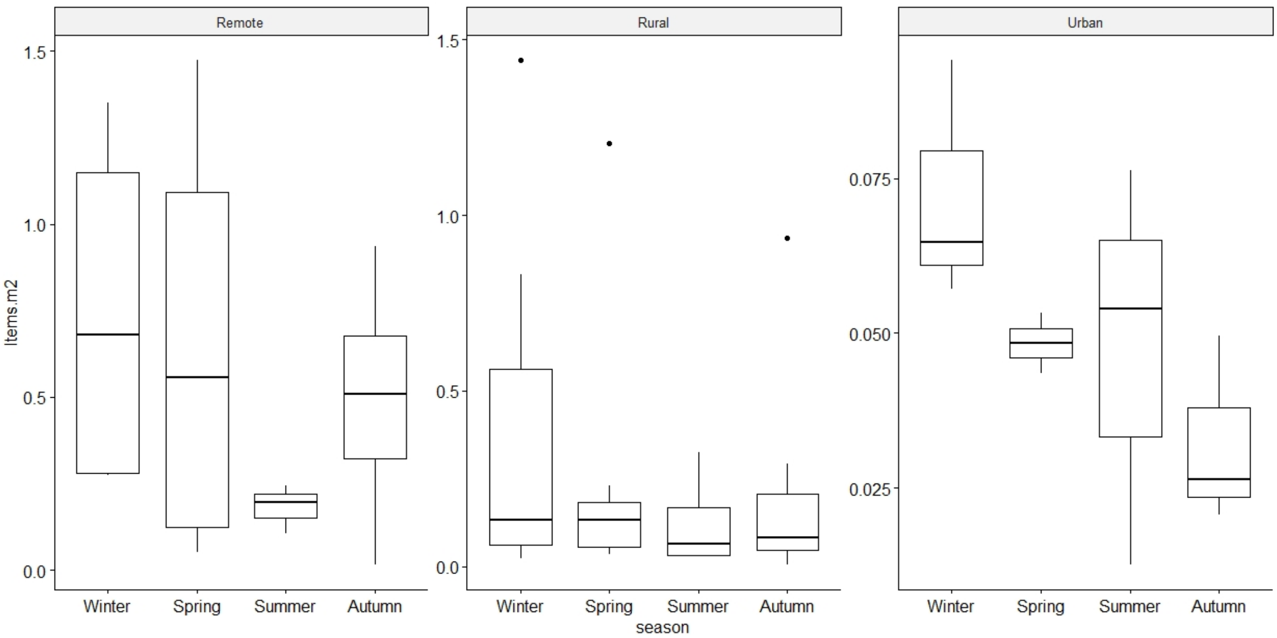
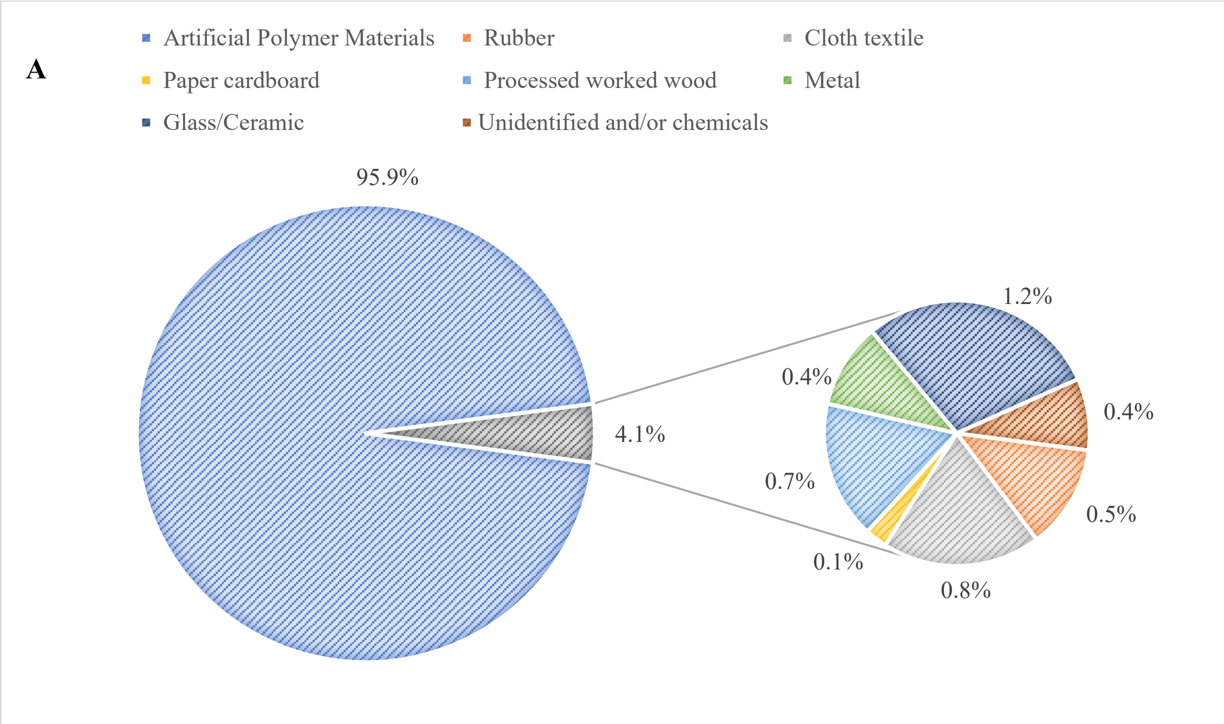


Fig. 30. Seasonal macrolitter concentration (items/m^2) for each site is categorized according to the beach type. The boundaries of the boxes indicate the 25th and 75th percentiles, the whiskers above and below the boxes the 95th and 5th percentiles. The horizontal line inside the boxplots denotes the median value.

Specifically, in urban beaches, an increase in presence of litter is evidenced during summer according to the increasing tourism flux rate, while it is evidenced as the season with less abundance of litter for remote and rural beaches. For these areas, the greater frequency and intensity of both sea storms and significant riverine inputs occurring during the winter and spring periods could explain the greater accumulation of litter found (Fig. 30).
The overall composition, expressed as a percentage of the total amount of litter collected, is shown in Fig. 31. The litter items collected in the study area (n°. 31,769 for a total weight > 340 kg) belong to 120 of the 159 categories of litter listed in the EU MSFD TG10 Master List. The vast majority of litter items (~96%) were made of artificial polymer materials and glass/ceramics (1.2%). The other categories accounted for

~3% in total (Fig. 31 A). Regarding the presence of artificial polymer materials, the 29,773 plastic items collected belong to 71 of the 86 typologies of artificial polymer materials presented in the reference classification (Galgani et al., 2013). At each of the beaches surveyed, this material was the most abundant category, accounting for more than 80% of the total (Fig. 31 B). These data highlight that plastics account for the majority of litter pollution in the study area, as is the case at many beaches worldwide (Asensio-Montesinos et al., 2019; Eriksen et al., 2013; Kumar et al., 2016; UNEP/MAP, 2015). The rubber (G125: Balloons and balloon sticks) and Cloth/textile (G137: Clothing/rags) categories were particularly prevalent at the San Vincenzo sampling site (2% and 5%, respectively) (Fig. 31 B), highlighting the potential impact of tourism and beachgoers. A relatively high concentration of glass and ceramic fragments was found at the monitored beaches in the Tuscan Archipelago (G208; 3% - 10%) (Fig. 31 B), except in the Capraia Islands, where Paper/cardboard (8%; G158: paper items) and Cloth/textile (6%; G137: Clothing/rags) (Fig. 31 B) were the most common categories found after artificial polymer materials.



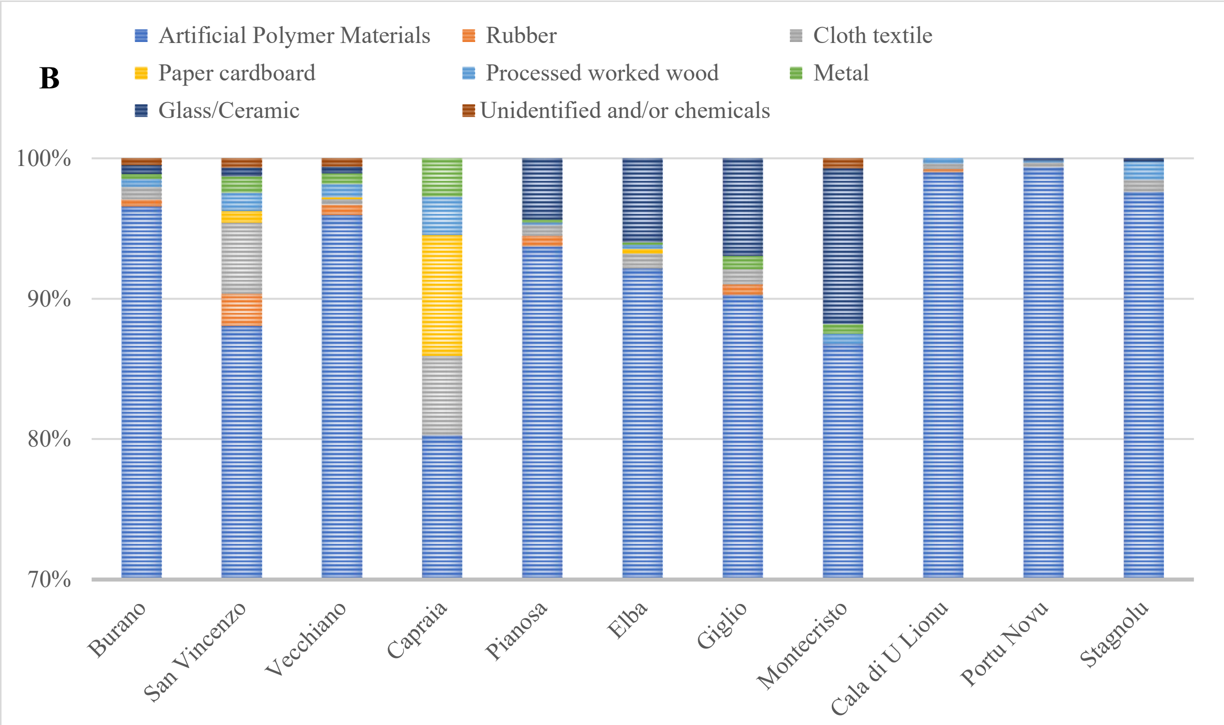


Fig. 31. Percentage (%) of total litter items per category type (artificial polymer material; rubber; cloth/textile; paper/cardboard; processed/worked wood; metal, glass/ceramics; unidentified and/or chemicals) in the whole study area (A) and each site monitored (B).

The top 10 items accounted for 89% of all items recorded (Tab. 12), similar to other studies where few item categories constitute the majority of the total amount of litter collected (Fortibuoni et al., 2021; Gjyli et al., 2020; Munari et al., 2016; Prevenios et al., 2017; Vhlacogianni et al., 2020).

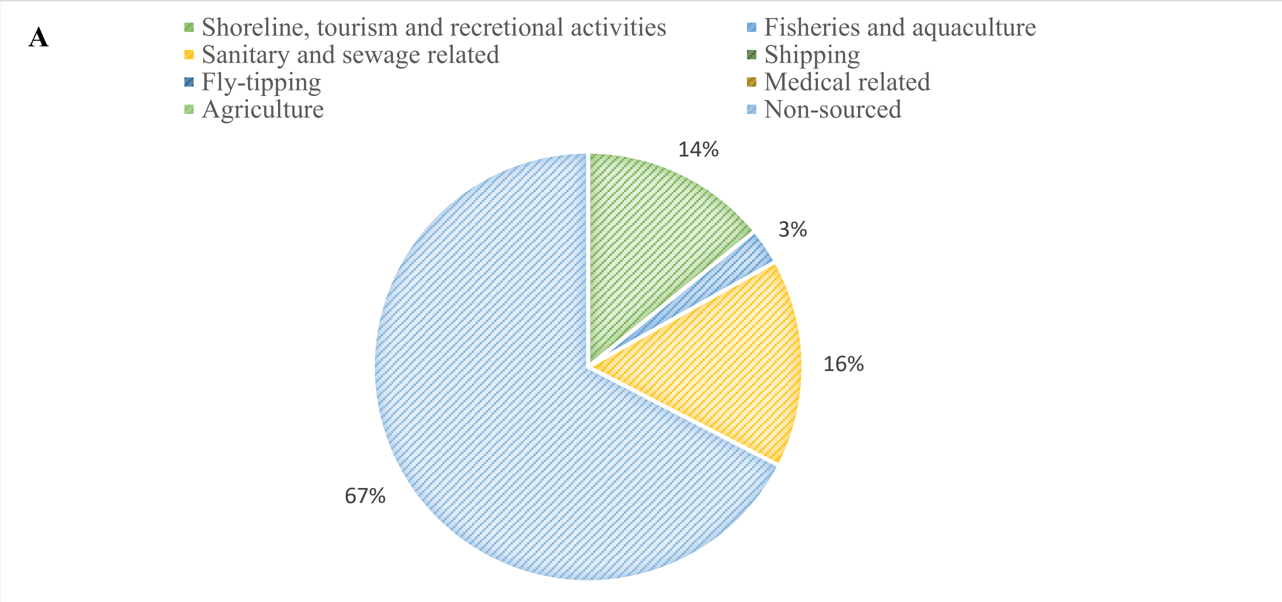
Tab. 12. Top 10 items found on the 14 surveyed beaches calculated on an aggregated basis of total litter counts in all beaches. G-code of the categories belonging to the Master List (Galgani et al., 2013) used for the litter objects classification and corresponding J-code (Joint list published by Fleet et al., 2021).

G-code (Master List)	J-code (Joint List)	Litter categories	%
G-79	J-79	Plastic pieces 2.5 cm > < 50cm	49
G-95	J-95	Cotton bud sticks	16
G-73	n.a.	Foam sponge	5
G-21	J-21	Plastic caps/lids from drinks	4
G-24	J-24	Plastic rings from bottle caps/lids	4
G-82	J-82	Polystyrene pieces 2.5 cm><50 cm	4
G-27	J-27	Cigarette butts and filters	3
G-70	J-70	Shotgun cartridges	2
G-22	J-22	Plastic caps/lids from chemicals	2
G-23	J-23	Plastic caps/lids unidentified	2

Plastic pieces measuring 2.5 cm > < 50 cm (G79) accounted for the highest proportion (49%) of litter collected in all surveys, followed by cotton bud sticks (G95) (16%). Commonly thrown into domestic sewage, they can end up in the marine and coastal environment due to the inefficiency of sewage treatment plants. This category represents the most collected in the beach of Burano accounting for more than 350 items (Annexe 10 B). Foams, including categories G73 and G82 (9% in total), were the third most collected item (Tab. 11), found mainly on Vecchiano beach with more than 450 items (Annexe 10 C). The low density of this material and its susceptibility to degradation once ashore could favour its transport from land, being blown by the wind and held by vegetation, especially on the dune. The remaining pieces of plastic beverage bottles (G21 and G24; 8%), cigarette butts and filters (G27; 3%), shotgun cartridges (G70; 2%) and plastic lids (G22 and G23; 4%), at the bottom of the list, could be related to the high tourism, recreational and hunting activities affecting the monitored areas, as well as the cutlery and trays (G34) and lighters (G26) found only at the San Vincenzo site (Annexe 10 D). Stagnolu beach, located in the western part of the Bouches de Bonifacio Nature Reserve, has the highest proportion of plastic fragments larger than 50 cm in the whole surveyed area (G80; 83 items). The beaches surveyed in the Tuscan Archipelago National Park, unlike the other sites, were particularly affected by shopping bags including pieces (G3; median 5 items/100m) and cigarette butts and filters (G27; median 4 items/100m) (Annexe 11 A). In particular, Pianosa Island appears to be affected by a high accumulation of pieces in the G3 category (16 items/100m). These data were confirmed by the highest concentration (523 ± 382 items/km^2) of category G67 (Sheets, industrial packaging, plastic sheeting) floating in the offshore waters of this area and probably washed ashore by waves and currents. Category G3 was found mainly on the 3 beaches on the Elba Islands (median 5 items/100m), reflecting the strong anthropogenic influence in this area, especially during the summer months. Cigarette filters pose a serious problem as cellulose acetate (the filter material) is photodegradable but has limited potential for biodegradation (Puls et al., 2011). In addition, cigarette butts/filters can harm the environment by serving as vectors for toxic chemicals such as trace metals, nicotine, and carcinogens when ingested by marine animals (Torkashvand et al., 2020).

Identifying the types and sources of marine litter is a critical step in devising effective management strategies aimed at preventing and reducing marine litter. Some litter has a unique function and can therefore be attributed to a specific source with a high degree of certainty. However, this is quite difficult for items that may come from a variety of sources or that are too small or damaged to be assigned. To reduce these gaps, in this study, the potential litter sources were investigated and identified by applying the MSFD Joint List proposed by Fleet et al. (2021). In total, 67% of the items collected could not be attributed to any particular source of pollution, 30% were attributable to land-based sources and 3% to marine sources. Contrary to other studies in the Mediterranean (Gjyli et al., 2020; Laglbauer et al., 2014; UNEP/MAP, 2015; Vlachogianni et al., 2017, 2020), waste from sanitary and sewage sources represent the main pollution input at the monitored sites in the Pelagos Sanctuary (Fig. 32 A). This finding was confirmed by the recent study performed by Fortibuoni et al. (2021) highlighting this source as the main affecting the Western Mediterranean sub-basin. Especially at Burano beach, improper disposal of sanitary items such as cotton swabs (G95), towels (G96) and tampons (G144), accounting for 28% in total (Fig. 32 B), have resulted in the principal source of litter pollution. Coastal sources, including inadequate waste management, tourism and recreational activities (14%

in total) (Fig. 32 A) resulted in the main inputs affecting the islands of the Tuscan Archipelago National Park, where they account on average for 25% (Fig. 32 B) of litter generated and dispersed in the marine environment. Inadequate waste management practices, including the irresponsible behaviour of tourists and residents, and activities related to coastal and marine tourism are undoubtedly the main sources of pollution, especially in summer. The relatively high concentrations of items related to food consumption (8%) and smoking (4%) confirm the hypothesis suggested above. However, this value is lower than the 38% and 58% reported by Vlachogianni et al. (2020) and Gjily et al. (2020), and the Mediterranean and global averages of 52% and 70%, respectively (Ocean Conservancy, 2018; UNEP/MAP MEDPOL, 2011).



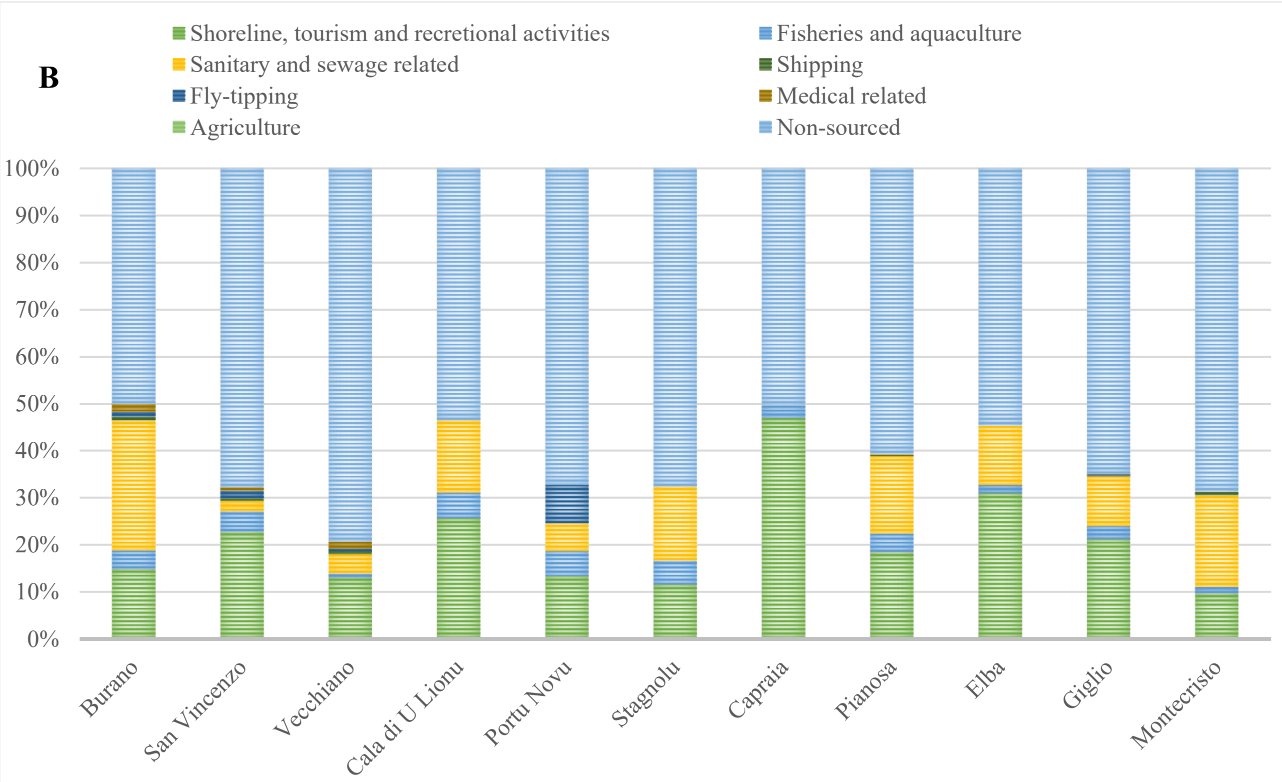
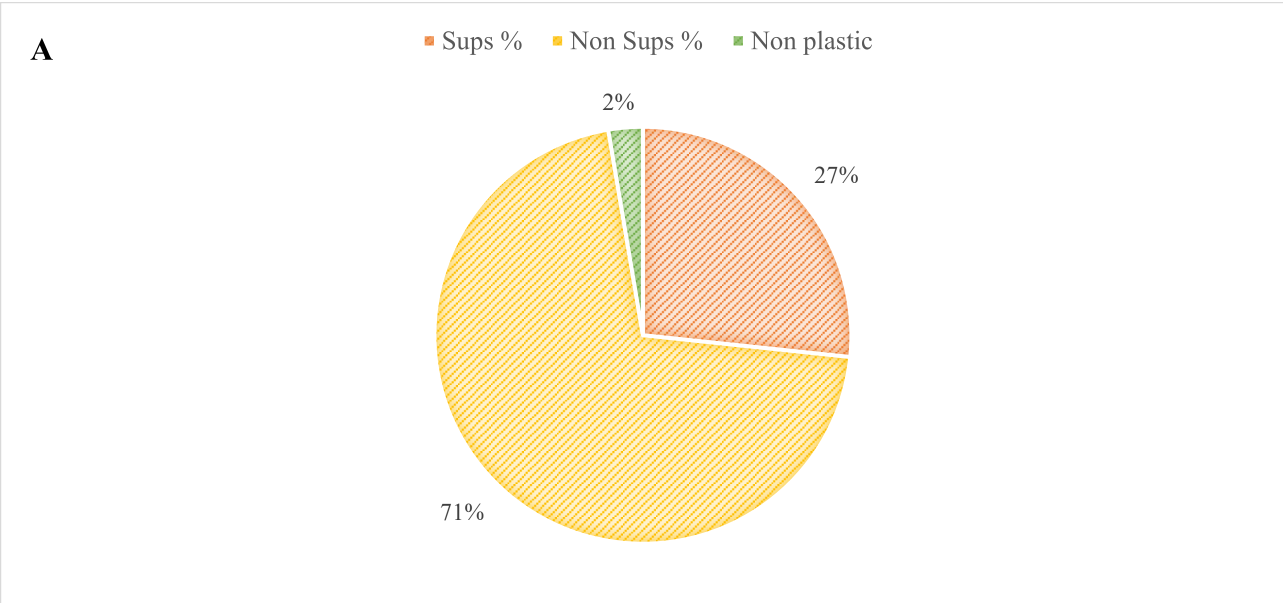


Fig. 32. Sources of marine litter in the whole study area (A) and each site monitored (B).

Finally, even if fly-tipping and inputs from fishing and aquaculture appear to have a lesser impact on monitored beaches. Items belonging to these sources, such as plastic construction material - G89 (8% on Portu Novu beach) and string and cord - G50 (average 5% on Corsican beaches), seem to affect mainly litter categories in the southern part of the island of Corsica, where shipping lanes were identified by Liubartseva et al. (2019) as the main sources of pollution. These data were by far lower than the levels reported by other studies for the Adriatic beaches (Munari et al., 2016; Prevenios et al., 2018; Vlachogianni et al., 2018).

In the study area, single-use plastics (SUPs) accounted for 27% of all items recorded (Fig. 33A), with values ranging from 7% to 44% at different beaches (Fig. 33 B). At an aggregate level, SUPs were most prevalent on the beaches of the Tuscan Archipelago National Park (30% of plastics on average), followed by the southern sector of the island of Corsica (26% of plastics on average) (Fig. 33 B). These data are consistent with the strong tourist flows that interest the monitored sites and that could strongly influence the dispersion of litter such as cotton bud sticks (G95) with 17%, plastic caps/ lids from drinks (G21) with 5% and cigarette butt and filters (G27) with 3%. Although the levels reported here are lower than those found by Addamo et al. (2017) on European beaches, where SUPs represent 50% of all marine litter collected and reported by Vlachogianni et al. (2020) on Mediterranean beaches at 38%, the adoption of EU strategies for plastics is needed to accelerate efforts towards a circular economy for these materials and lead to a drastic reduction in the use and impact of single-use plastics.



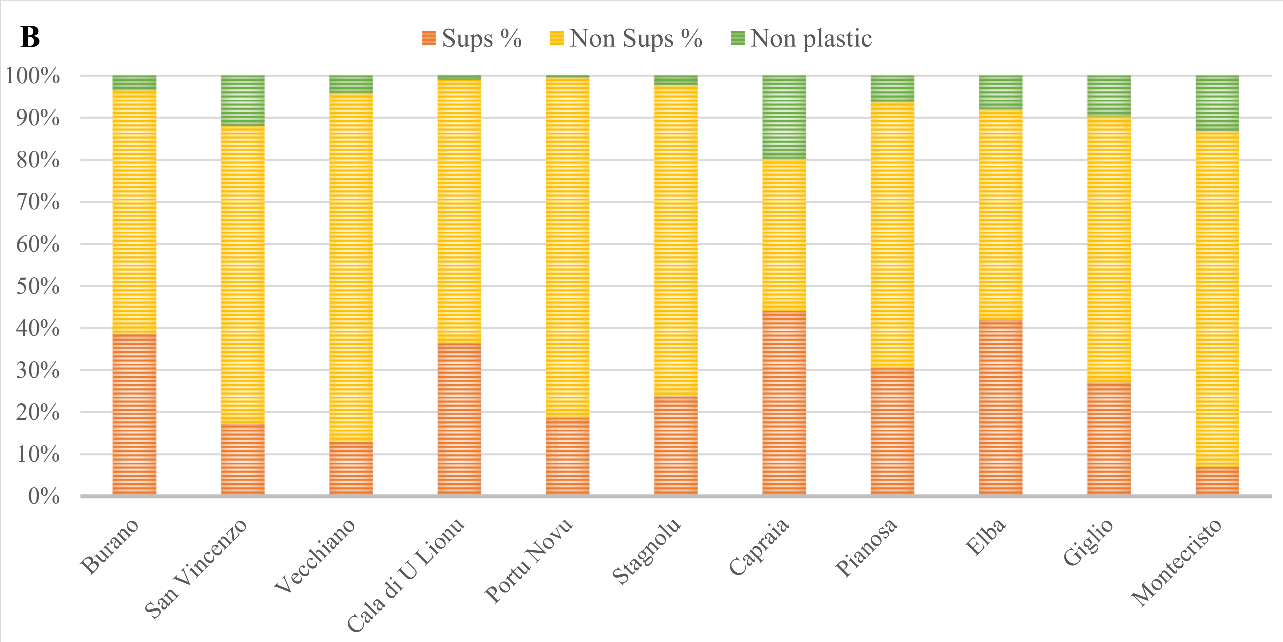


Fig. 33. Single-use plastics (SUPs) characterization in the whole study area (A) and each site monitored (B).

4.3.6 Beach microlitter abundances and composition

Visual separation of microlitter items from the sand was performed on samples collected at the three sites along the Tuscan coast (Vecchiano, San Vincenzo and Burano) and on four islands of the Tuscan Archipelago National Park (Elba, Pianosa, Montecristo and Giglio). A total of 19,159 plastic particles were isolated and initially characterized according to their size. Mesoplastics represented the majority of items found (56%; 10,812), while large MPs accounted for 8,347 particles (44%). Considering the two areas studied, the highest average concentrations of mesoplastics (30 ± 107 items/m^2) were found at sites along the Tuscan coast, while the Tuscan Archipelago showed the highest values for the abundance of MPs (22 ± 112 items/m^2) (Fig. 34).

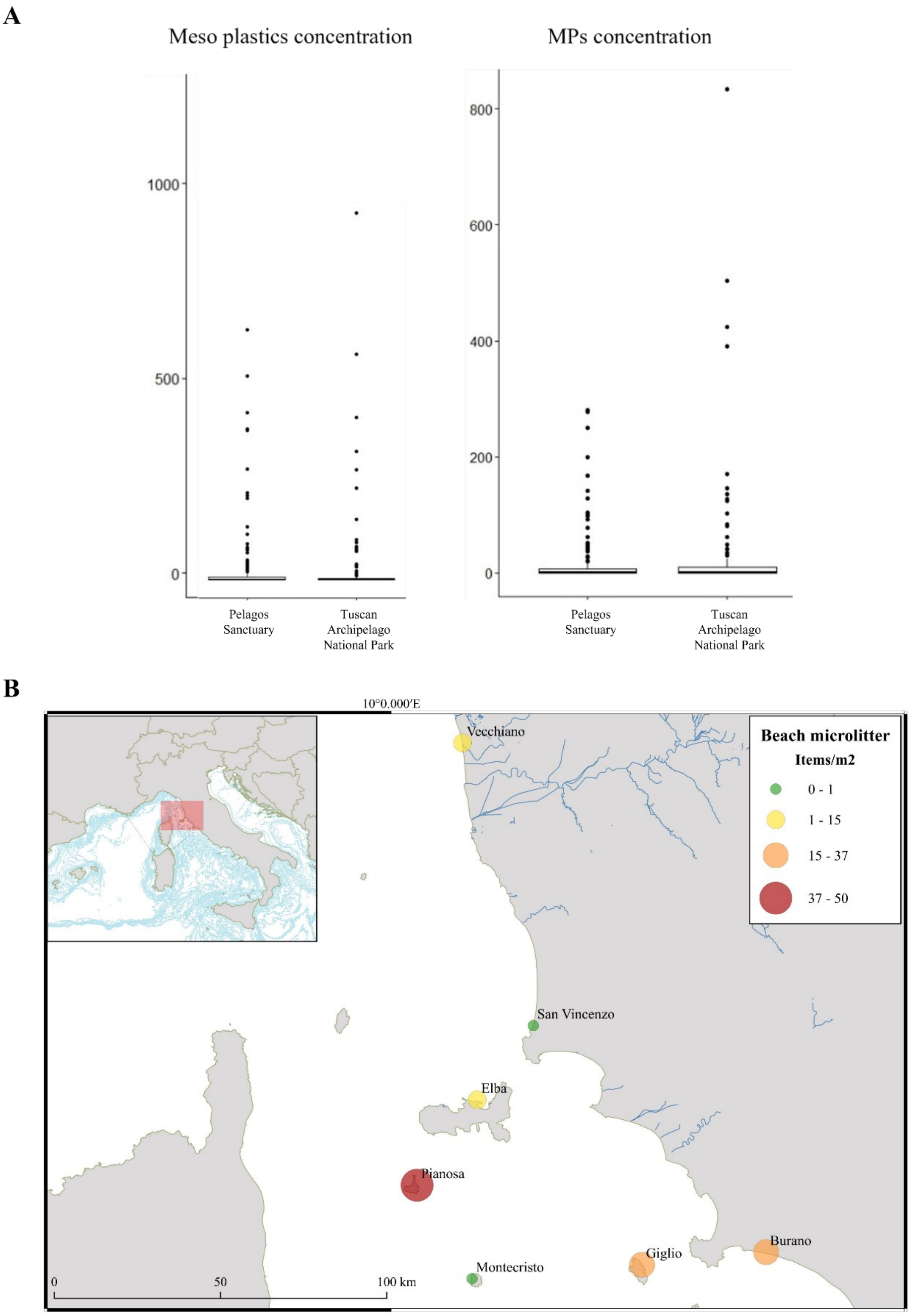


Fig. 34. Beach mesoplastic and MPs concentration (items/m^2) in the two areas monitored the Pelagos Sanctuary and the Tuscan Archipelago National Park (A). The boundaries of the boxes indicate the 25th and 75th percentiles, the whiskers above and below the boxes the 95th and 5th percentiles. The horizontal line inside the boxplots denotes the median value. MPs concentrations (items/m^2) in the beaches monitored in the Pelagos Sanctuary and Tuscan Archipelago National Park (B).

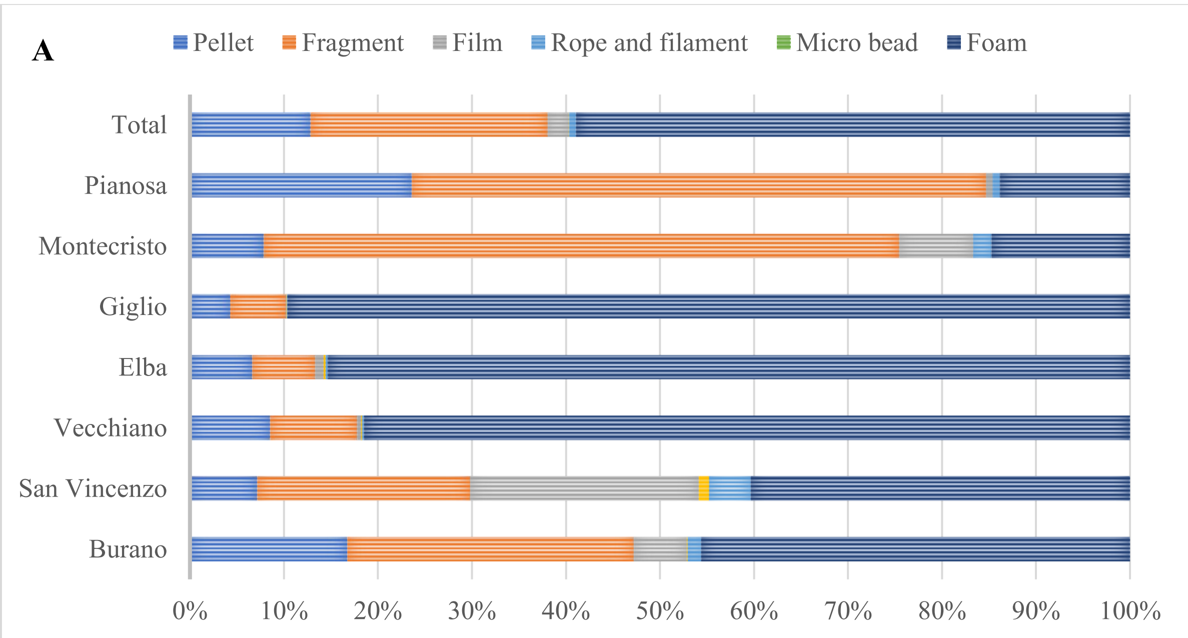
Comparing the results reported in the available literature presents some difficulties due to the use of different methods in data collection, classification and reporting of MPs, as well as different units of measurement (Alomar et al., 2016). Nevertheless, the concentrations reported in this study (Fig. 34 B and Tab. 12) are by far lower than those reported by Lots et al. (2017) on European beaches (291 ± 62 items/kg dry sediment) and in other MPAs in Spain (105.4 ± 9.2 items/kg dry sediment; Bayo et al, 2019), in the Pelagos Sanctuary (535.13 ± 389 items/m^2; Merlino et al., 2020) and the Tyrrhenian Sea (140 items/m^2; Cesarini et al., 2021) and comparable to the value reported by Turner and Holmes in 2011 for the island of Malta (14.2 items/m^2). Within the whole study area, the highest concentrations of MPs were isolated from the sediment of Pianosa (58 ± 95 items/m^2) and Giglio (37 ± 173 items/m^2) islands (Tab. 13). The presence of plastics on the Pianosa islands seems to be mainly related to the transport and distribution of particles in the offshore waters, as evidenced by the moderate accumulation of macrolitter objects. Tourism is strictly regulated and restricted throughout the year in this protected area and it may not be the main source of plastic pollution (Merlino et al., 2018).

Tab. 13. Beach MPs concentration (items/m^2 and items/kg dry sediment) within the whole study area and different sites monitored.

Sampling area	Sampling site	Items/m^2	Items/kg dry sediment.
Pelagos Sanctuary	Vecchiano	15 ± 35	1.6 ± 3.7
	San Vincenzo	1 ± 2	0.1 ± 0.2
	Burano	25 ± 59	2.6 ± 6.3
	Total	**14 ± 41**	1.5 ± 4.3
Tuscan Archipelago National Park	Elba	8 ± 58	0.8 ± 6.2
	Pianosa	50 ± 95	5.3 ± 10.1
	Montecristo	1 ± 3	0.1 ± 0.3
	Giglio	37 ± 173	3.9 ± 18.4
	Total	**22 ± 112**	2.4 ± 11.9
Total		**18 ± 86**	1.9 ± 9.1

Along the Tuscan coast, Burano was the site most affected by the presence of plastic particles (25 ± 59 items/m^2; 2.6 ± 6.3 items/kg dry sediment). Cannas et al. (2017) reported a high concentration of MPs in this area (466 ± 297 items/kg dry sediment), indicating the strong plastic pressure that could affect this important natural area. However, the different range of MPs sizes investigated (1 - 5 mm vs 63 µm - 5 mm) strongly influenced the final concentrations reported. Inputs from the Arno River, industrial activities and the urban environment can be considered as the main sources of plastic pollution in Vecchiano. MPs concentrations at this site were previously evaluated by Merlino et al. (2020) and Scopetani et al. (2021) (isolating particles up to 1.2 µm), which reported values ranging from thirty times (471 ± 333 items/m^2) to two orders of magnitude (207 ± 30 items/kg dry sediment) higher than those found in this study (15 ± 35 items/m^2 and 1.6 ± 3.7 items/kg dry sediment). Seasonal variation within the study area and between monitored sites was examined to highlight possible differences in beach accumulation. The data did not show a clear distribution pattern and seem to be related to the specific hydrodynamic characteristics and anthropogenic pressure affecting the sites studied (Annexe 13), as also highlighted by Merlino et al. (2020).

The characterization of the MPs revealed that the expanded material is the most abundant (59%) throughout the study area (Fig. 35 A). The distribution and accumulation of this type of material on the beaches could be strongly influenced by several factors. A large amount of larger foam objects (G73 and G82) at most of the studied sites, especially at Vecchiano, Burano and Giglio Island, could favour the formation of MPs due to the physical degradation processes that could affect these objects. Moreover, light polystyrene, the most abundant foamed polymer (52%; Fig. 35 B), may facilitate dispersion and transport by wind action (Merlino et al., 2020). The islands of Pianosa and Montecristo differ from the general characterization of MPs, showing a high percentage of fragments as the most abundant form (> 60%). As mentioned above, this accumulation in these areas could be due to local surface water circulation and stranding currents (Fig. 35 A). Fragments are resulted mainly composed of polyethylene and polypropylene (> 80%; Fig. 35 A), which make the particles susceptible to degradation processes. They are the main polymer used in the manufacture of disposable plastic items, which may influence their distribution in the marine environment. Pellets are quite common on beaches, where they are collected in higher concentrations than in the other environmental compartments. Their presence was found at all monitored sites and accounted for 13% of the total items characterised (Fig. 35 B). According to the data published in the literature, fibres are the predominant form found on the beaches of the Adriatic Sea and Tunisia (Abidli et al., 2018; Laglbauer et al., 2014;), reaching up to 99% of MP on different Mediterranean beaches (Lots et al., 2017). In this study, their presence was not detected being the large MPs the target particles to investigate and to the different identification and characterization method needed to correctly address their amount.



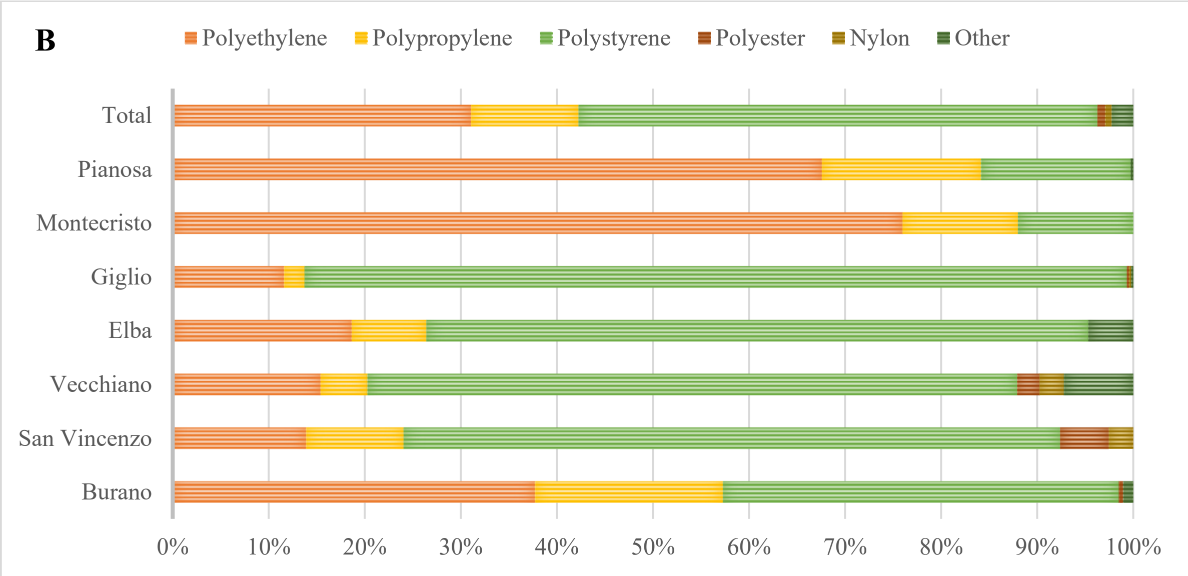


Fig. 35. Beach MPs characterization by shape (A) and polymer composition (B).

The distribution of MPs along the vertical profile of the monitored beaches was evaluated to highlight potential preferential accumulation zones. On the beaches of Pelagos Protected Area, a clear accumulation of plastic particles was detected in the line of high deposition AC 2 (Fig. 36 A), while on the beaches in Tuscan Archipelago National Park no differences were highlighted between the zones considered (Fig. 36 B). The same distribution pattern was demonstrated by Constant et al. (2019) and Merlino et al. (2020), who observed the presence of MPs on the beaches of the northwestern Mediterranean (Gulf of Lion and Pelagos Sanctuary). The authors state that the distribution of MPs could be influenced by meteorological (winter storm and wind) or hydrological events (sea storms and intense rainfall) occurring at a local scale in the studied area. This explanation could also be considered in the area investigated by this study, especially at the Vecchiano and Burano sites, where the highest concentrations of foamed MPs in the upper accumulation zone could be influenced by wind transport and vegetation entrapment since both are characterized by the presence of a natural dune. Considering the differences between sites, it is not possible to establish a general rule for accumulation in the different zones (Annexe 14).

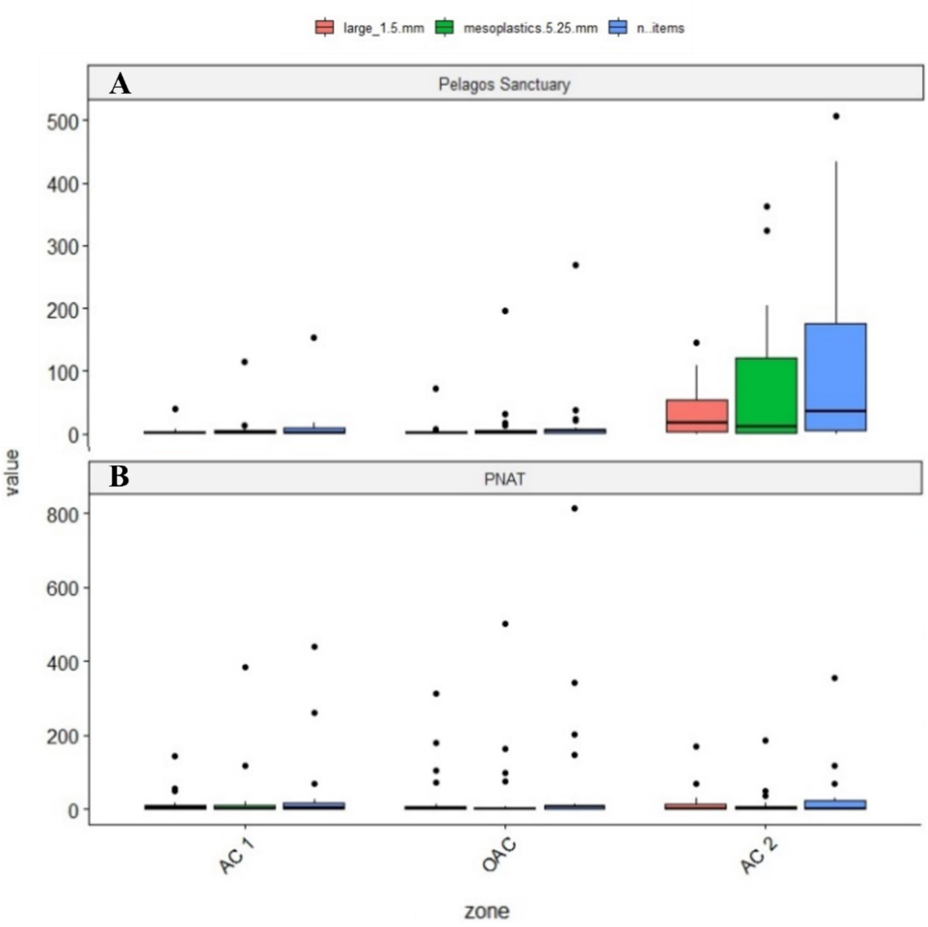


Fig. 36. Number of beach mesoplastics and MPs accumulation and distribution among the three different zones considered: AC 1 (low accumulation line), OAC (off accumulation zone) and AC 2 (high accumulation line) in the Pelagos Sanctuary (A) and Tuscan Archipelago National Park (B). The boundaries of the boxes indicate the 25th and 75th percentiles, the whiskers above and below the boxes the 95th and 5th percentiles. The horizontal line inside the boxplots denotes the median value.

In the Tuscan Archipelago National Park, a statistically significant difference (Wilcoxon test; W = 4111, p-value = 3.891e-05) was found between the beaches inside the protected zone established on the islands of Pianosa and Montecristo (74.5 ± 173.5 items/m^2) and outside this regulated area on the islands of Elba and Giglio (46.5 ± 232.7 items/m^2) (Fig. 37).

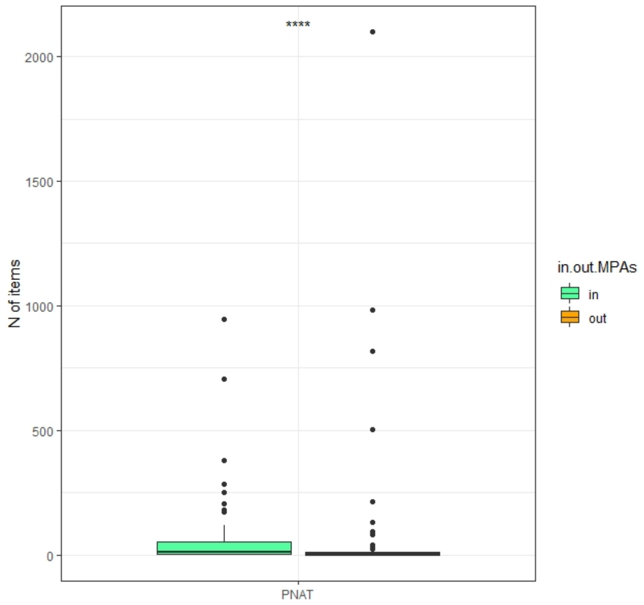


Fig. 37. MPs number per square meter found in the beaches located inside and outside protected islands in the Tuscan Archipelago National Park. The boundaries of the boxes indicate the 25th and 75th percentiles, the whiskers above and below the boxes the 95th and 5th percentiles. The horizontal line inside the boxplots denotes the median value.

The lack of regular beach cleaning activities compared to the monitored sites on the islands of Elba and Giglio, both tourist destinations characterised by an intense presence of beachgoers during the summer months and consistent cleaning of sediments, may have influenced the assessment of particle concentrations. However, it must be stressed that on the islands of Pianosa and Montecristo the transport of MP is mainly due to a contribution from the sea and that further studies are needed to better focus and limit the impact of litter and plastic on these islands.

4.3.7 Paraffin wax: a global assessment in the Pelagos Sanctuary

Introduction

Global production of industrial waxes currently stands at 4.79 million tons, with expected annual growth of 1.5-2%, driven primarily by increasing demand for single-use packaging applications (Grand View Research, Inc., 2017; Wei, 2012). Petroleum waxes (e.g., petrolatum, paraffin and microcrystalline products) are by far the most important in terms of production volume and economic impact, accounting for 85-90% of global wax consumption (Kline & Company, Inc., 2010). They are crude oil derivatives consisting mainly of a mixture of hydrocarbons with typical melting points between 35 and 95°C (Buchler and Graves, 1927; Mansoori et al., 2004). In particular, paraffin waxes are usually obtained as a by-product in the production of lubricating oils and consist mainly of saturated long-chain hydrocarbons (Cottom, 2000). These waxes are thermoplastic materials but are not usually considered plastics or polymers due to their relatively low molecular weight. Their great versatility and low reactivity make them suitable for a wide range of industrial applications (Kumar et al., 2005; Mansoori et al., 2004; Nasser, 1999; Wei, 2012). Every year, large quantities of fully refined or unrefined petroleum wax are transported around the world by tankers and cargo ships (Wei, 2012). After unloading, certain amounts of the product usually remain on the bottom of cargo tanks or crystallize on bulkheads and interior fittings and can then be treated in port reception facilities or discharged at sea under certain conditions. Operational practices are governed by the International Maritime Organization's (IMO) Annex II to the International Convention for the Prevention of Pollution from Ships (MARPOL 73/78), which contains provisions to control pollution from Noxious Liquid Substances (NLS) carried in bulk and sets out the standards and principles to be followed when discharging pollutants at sea. According to the latest version of Annex II (2007), petroleum waxes are classified as "high viscosity and solidifying substances" which fall into medium pollution category Y: "Noxious liquid substances which, if discharged into the sea during tank cleaning or ballast unloading, pose a risk to marine resources or human health or adversely affect amenities or other legitimate uses of the sea and therefore warrant a restriction on the quality and quantity of discharge into the marine environment". Therefore, cargo residues can be legally discharged into the sea provided the discharge is below the waterline, underway at a minimum speed of 7 knots and at least 12 nautical miles from the nearest land, and in water depths exceeding 25 meters. The only exception is Antarctica, where any discharge of NLS is prohibited. Bearing this in mind appears clearly how in basins, such as the Mediterranean Sea, heavy influenced by vessel traffics, the uncontrolled discharge of wax residues could represent a threatening issue for the marine organisms, especially in the protected areas such as the Pelagos Sanctuary. In this basin, 3,757,587 km of vessel traffic from 82,831 transits by 4205 distinct vessels (navigating under the flag of 90 different states) have been recorded by the study of Coomber et al. (2016). The authors show that the spatial and temporal distribution of marine traffic in this area varied according to the vessel type, identifying the passenger vessel as predominant, with 26,264 transits totalling 1,385,361 km, followed by cargo (21,753 transits totalling 1,427,681 km) and tanker (10,352 transits totalling 369,026 km) (Coomber et al., 2016).

To date, no reliable estimates of the number of petroleum waxes discharged at sea each year were assessed. The German Federal Maritime and Hydrographic Agency (BSH) reported that pieces of paraffin wax were found in 24 out of 33 trawls in the North Sea, as did Lorenz et al. (2020) in the southern sector of this basin. Data from the OSPAR beach litter database show that between 2001 and 2016, paraffin pieces were found in 371 out of 2,824 litter surveys on 151 different beaches, with a mean estimated frequency of 14.6 items per meter of beach line (maximum 738 items/m). The vast majority of these items were found in the North Sea region and originated from Denmark, Sweden, France, Germany, Belgium and the Netherlands. The presence of paraffin waxes was also detected on Lithuanian beaches, accounting for 63% of all isolated litter (Haseler et al., 2018), and in the Russian Baltic Sea by Esiukova (2017), as wax aggregates can concentrate microplastics. On average, 31.1 ± 18.8 MPs per sample or 11,479 ± 10,785 items per kg of wax were found.

In the Mediterranean Sea, the presence of wax in nearshore waters was first detected in 2013, when several fragments of white paraffin wax were found in a sample collected in the southern Adriatic Sea during a survey for floating microplastics (Suaria et al., 2016). Similar particles were also isolated from plankton samples by Zeri et al. (2018) and Vianello et al. (2018), who studied the distribution of microplastics in the northern Adriatic Sea. In the Ligurian Sea, and in particular, in the Pelagos Sanctuary, more than 350 kg of yellow wax were recovered in the surface waters off the northern side of Elba Island during the summer of 2017. Moreover, along a 200 km stretch of coastline in Tuscany, average densities of 15 kg/m^2 and 16,400 fragments/m^2, mainly with diameters between 5 and 30 mm, were found (Suaria et al., 2018). Nevertheless, their presence is rarely explicitly mentioned in the scientific literature and their actual occurrence in the marine environment is largely unknown.

The ingestion of wax particles has been reported in the stomach contents of northern fulmars (*Fulmarus glacialis*) (Avery-Gomm et al., 2017; van Franeker et al., 2011), in regurgitates from Black Legged Kittiwakes (*Rissa tridactyla*) and Great Cormorants (Phalacrocorax carbo) in Ireland (Acampora et al., 2017) and in a post-hatchling loggerhead turtle *(Caretta caretta*) on a South African beach (Ryan et al., 2016). The study of Nunes et al. (2020), in which the filter-feeding *Mytilus galloprovincialis* was exposed to paraffin microparticles, showed no ecotoxicological effects. Although ingestion was confirmed, toxicity was not demonstrated by measuring the activities of four enzymes involved in important cellular processes (e.g., antioxidant defence, glutathione reductase and peroxidase, and phase II metabolism).

Materials and methods

Briefly, the presence and distribution of paraffin waxes were assessed during the sampling campaigns described above. Floating wax debris was identified during the macrolitter monitored transects and isolated from the neustonic samples performed with the Manta Net. Particles were characterized by size, colour, and chemical composition according to analytical procedures described for macro and microlitter (see Section 4.2.2). In addition, stranded wax microparticles were isolated during sediment analysis and characterized as described in Section 4.2.3. Finally, chemical composition was assessed using the FTIR spectrometry technique as suggested by Baini et al. (2018).

Results and discussions

Wax residues are generally considered as chemical pollution rather than litter, as indicated by the recent Joint (J216 - J218) and Master List of litter items (G213) from EU MSFD TG 10 (Fleet et al., 2021; Galgani et al., 2013), as well as OSPAR (codes 109, 110 and 181) (Cheshire et al., 2009) and UNEP beach litter monitoring protocols (code OT01). During the sampling campaigns carried out in the Pelagos Sanctuary during the summer of 2019, different concentrations of yellow-coloured wax residues were found floating in the surface water and stranded on the Tuscan coast. Their presence was detected in 22% of the macrolitter monitoring transects with a total of 187 macro residues in the Ligurian Sea, corresponding to an average concentration of 29.8 ± 87.1 items/km^2. The spatial distribution analysis revealed a higher density of wax in the northwestern sector of this basin, close to the Ligurian coast, where an average of 44.1 ± 105.9 items/km^2 were sighted (Fig. 38 A). This accumulation pattern could be due to the potential discharge of wax residues by tankers and the subsequent dispersion by superficial currents in the surrounding waters. According to that, this area was particularly affected by the navigation routes of these vessels moving toward the ports of Genova and Savona (Fig. 38 D), where more than 1,000 tankers in total have registered to land from 2018 to 2020 (EMODnet Human activities portal; https://www.emodnet.eu/). The 80% of these residues had a size between 2.5 cm and 5 cm, no objects larger than 30 cm were found. Approximately 2,200 wax particles were isolated from the neustonic samples collected with the manta net. The majority (90%) had a size of less than 5 mm and 54% belonged to the size class of MPs between 1 mm and 5 mm. Analysis of the spatial distribution revealed a comparable average concentration of wax debris floating in the Tuscan Archipelago National Park (13,206 ± 50,824 items/km^2) and in the Pelagos Sanctuary (12,201 ± 20,170 items/km^2) (Fig. 38 B). However, it is possible to identify a particular area of accumulation in the northern part of the Tuscan Archipelago, in particular around the island of Gorgona, where the average concentration is five times higher (68,676 items/km^2 vs 12,586 items/km^2) than the average concentration measured in the whole study area. This area of the Ligurian Sea, already highlighted as a site of transient accumulation of MPs, was also identified by Suaria et al. (2018) as particularly affected by the presence of wax. Focusing on the tanker routes passing through this area (Fig. 38 D) appears particularly evident as the high number of these vessels moving towards the port of Livorno, exceeding the 600 units from 2018-2020 (EMODnet Human activities portal; https://www.emodnet.eu/), may represent a potential source of pollution of this material legally discharged in the offshore waters. Residues of these petroleum materials (n. 161) were also found stranded along the Tuscan coast and on three islands of the Tuscan archipelago (Elba, Pianosa and Giglio) (Fig. 38 C). Mainly particles with a size between 1 and 5 mm (68%) were found. The highest abundance was found on the monitored beach of Cala Giovanna on the island of Pianosa (110 items/m^2), already described in this study as affected by massive strandings of litter and MP particles.

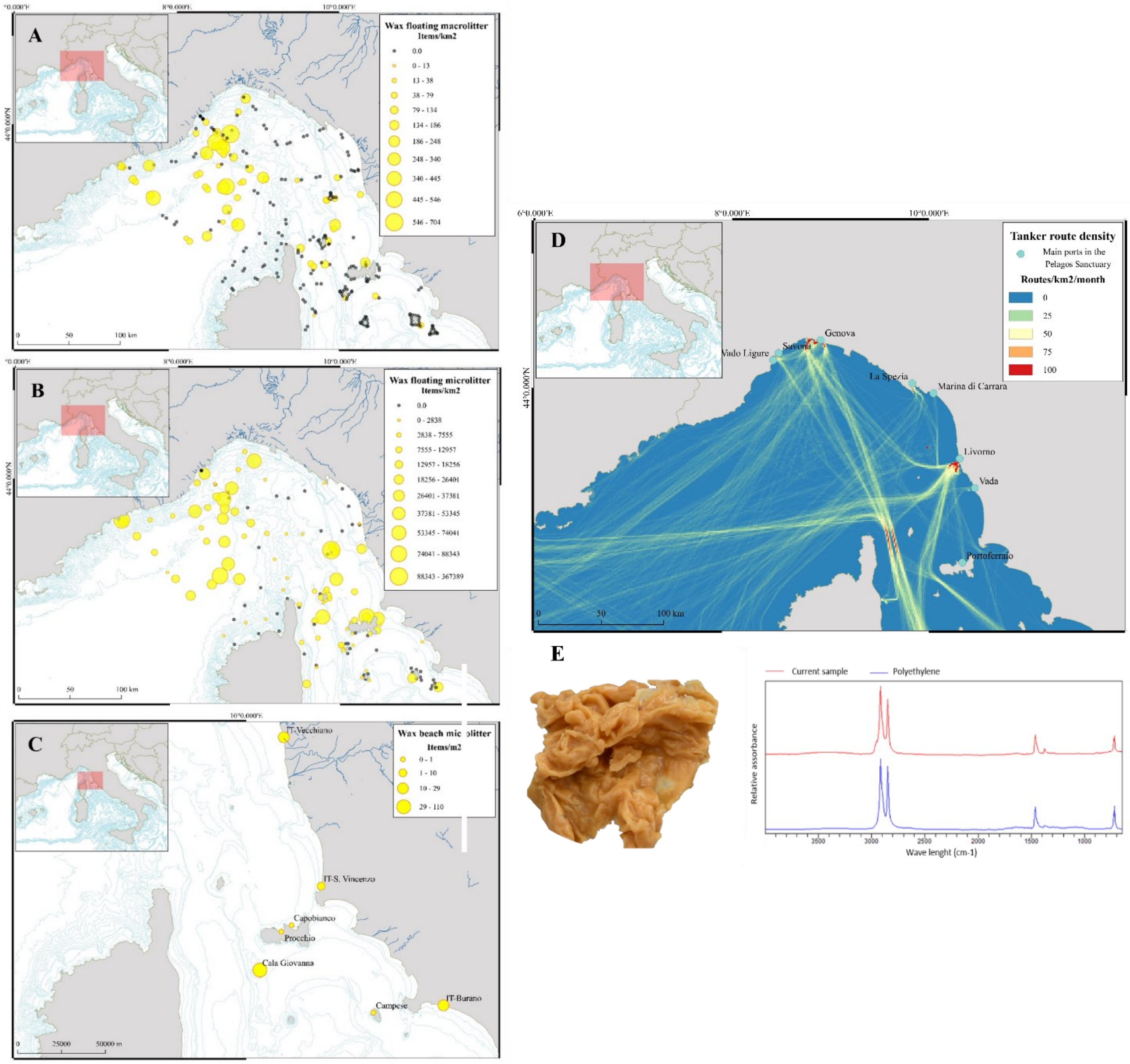


Fig. 38. Concentration of paraffin waxes macroparticles (A) and microparticles (B) detected on the sea surface and isolated from beach sediments (C) within the Pelagos Sanctuary. Tanker route density during summer 2019 in the Pelagos Sanctuary (D) (data source: EMODnet Human activities portal; https://www.emodnet.eu/). An example of a residue of paraffin wax collected during monitoring of floating macrolitter and the correspondent polymer analysis (E).

Chemical composition analysis revealed that the residue found was paraffinic polyethylene wax (Fig. 38 E), as previously suggested by Suaria et al. (2018) using the Fourier transform spectrometry technique. To date, this is the second detection of this material in the Pelagos Sanctuary and the first to provide detailed information on its spatial distribution and characterization. Although gas chromatography analysis of the same residues ruled out both acute toxicity and the presence of volatile organic compounds and inorganic contaminants (Suaria et al., 2018), the potential accumulation and prolonged residence time in the marine environment (5 months after the stranding event described by Suaria et al., 2018, many wax particles are still present) may pose a threatening risk for ingestion by organisms inhabiting this protected area.

4.4 Conclusions

Within the Plastic Busters MPAs Interreg-MED Project, a comprehensive analysis of the abundance, distribution and composition of floating marine litter in surface waters and stranded litter on several beaches of the SPAMI Pelagos Sanctuary and Tuscan Archipelago National Park was performed. Specifically:

- The high heterogeneity of marine litter evidence in the available literature strengthens the need to create and adopt shared monitoring protocols among the scientific community to collect reliable and consistent data. The application of a harmonized protocol for simultaneous macro and microlitter assessments in different marine compartments carried out in the frame of this thesis covers the existing gaps originating accurate and comparable data. These valuable data have been correlated with several driving factors such as the physical characteristics (study areas extent and habitat diversity), potential litter sources (ports, estuaries, urban and tourist coastal activities), and different levels of protection (SPAMI and Nationa Park and pelagic and coastal protected areas) of the marine environments investigated within the Pelagos Sanctuary and Tuscan Archipelago National Park, providing a more comprehensive representation of the distribution, abundances, and potential sources of plastic litter in the monitored areas. The simultaneous methodologies adopted allowed to highlight differences in the pattern of litter accumulation between pelagic and coastal waters and to give preliminary information on the areas most at risk.

- The average concentration of floating macrolitter (399.01± 485.84 items/km^2) identified in this study represents the highest amount of floating macrolitter recorded so far in the study area, largely exceeding the estimated average concentration in the western Mediterranean Sea (29.7 ± 46.8 items/km^2). Under the UNEP/MAP IMAP framework, a threshold value of 5 pieces/km^2 was set for the Common Indicator (CI) 23 in 2020, as the target threshold value to be achieved. This can be referred to as a “GES boundary”, enabling for quantitative and integrated analysis of the state of floating macrolitter pollution in the marine environment. Our survey reveals that GES for CI 23, is a long way from being achieved in the Pelagos Sanctuary and could indicate potential worsening litter pollution in the investigated area. Items of secondary origin belonging to the category "G - 67: Sheets and industrial packaging" and "G - 79: Plastic pieces 2.5 cm > < 50 cm" were most frequently sighted during the monitoring activities. Their presence could be indicative of the degradation processes and fragmentation that affect the litter objects after their dispersion in the marine environment, allowing the formation of smaller particles. The highest abundances were found in the surface waters off the islands of the Tuscan Archipelago National Park, both in terms of the number of objects and size classes, specifically near the islands of Giglio and Giannutri (792.90 ± 610.13 items/km^2) and the northern sector between the islands of Gorgona and Capraia (726.42 ± 735.20 items/km^2). This pattern of accumulation could be due to a recent input of pollution from land, as this area was particularly affected by tourism and recreational activities during the period of the sampling campaigns. Furthermore, riverine discharges from major rivers such as Arno, Serchio and

Tevere, as well as plastic accumulation driven by superficial currents insisting on these areas, are significant factors to consider to explain the pollution status of the Tuscan Archipelago National Park.

- The presence and abundance of MP in the coastal and pelagic waters of Palagos Sanctuary were investigated by 141 manta trawl samples, representing the larger dataset collected in the monitored area. Floating microparticles were detected in coastal and pelagic waters with an average concentration of 259,490 ± 586,477 items/km^2. As noted for macrolitter objects the MP concentration appears to be increasing compared to levels reported in previous studies, posing a potential risk to organisms living in this protected area throughout the marine trophic chain, as also highlighted by Fossi et al. (2017). Secondary MPs (fragments and films) ranging in size from 1 mm to 2.5 mm, mainly composed of PE and PP, were the most abundant classes throughout the study area. The highest MPs concentration was found in the Tuscan Archipelago National Park, confirming the observations on the distribution and concentration of floating macrolitter objects and strengthening the hypothesis that the presence of larger objects (categories G67 and G79) may influence the formation of MP as a result of degradation and fragmentation processes. The most affected islands were Gorgona (563,962 ± 1,123,234 items/km^2) and Pianosa (290,966 ± 321,938 items/km^2), where the accumulation of MPs seems to be closely related to the role of currents and other hydrodynamic features that exist in these areas.

- Statistical analysis revealed a strong correlation between the distribution of floating macrolitter objects and MPs, highlighting the significance and effectiveness of the simultaneous floating litter sampling design to better address the presence and distribution of plastic pollution in the marine protected areas. Considering the potential influence of environmental and anthropogenic factors on the distribution of plastics, a risk map was constructed based on the spatial concentration of macrolitter objects, to provide a reliable indication of the accumulation of MPs. Strong litter inputs were identified to originate from the mainland, with significant contribution of ports (e.g., Livorno, La Spezia and Genova) and estuaries (e.g., Magra, Serchio, Arno and Ombrone). Areas with warmer waters and weak oceanographic features (e.g., continental shelf) can facilitate plastic accumulation. Coastal waters -within 10-15 nautical miles- seem to represent litter retention zones, which in turn causes concerns about the underlying risk for marine biodiversity especially considering the key ecological role of the protected areas of the Pelagos Sanctuary and the Tuscan Archipelago. A major advantage of this integrated analysis is that the results can serve as an affordable basis for implementing effective marine litter prevention, reduction and disposal policies in this SPAMI.

- All monitored sites exceed the threshold defined by EU MSFD TG 10 for macrolitter pollution of beaches (20 items/100m), highlighting the need to protect the beaches of the Pelagos Sanctuary from the ecological and socio-economic impacts of litter. The overall composition shows that the vast majority of the litter (~96%) consists of artificial polymeric materials, mainly plastic pieces of 2.5

cm > < 50 cm (G79) and cotton bud sticks (G95). The Tuscan coast was the most affected by macrolitter, with an average litter density of 1,033 ± 915 items/100m (0.53 ± 0.50 items/m^2). In this area, litter appears to originate mainly from sanitary and sewage sources associated with the improper disposal of personal care products such as cotton buds (G95), towels (G96) and tampons (G144), accounting for 28% in total. In the Tuscan Archipelago National Park, inadequate waste management practices, together with coastal and marine tourism activities, are undoubtedly the main contributors to pollution, especially during the summer months. The relatively high concentrations of SUPs items related to food consumption (8%) and smoking (4%) required the adoption of EU plastic strategies to accelerate efforts towards a circular economy for these materials and lead to a drastic reduction in the use and impact of single-use plastic items.

- A total of 19,159 plastic particles were isolated from beach sediments, with an average concentration of 18 ± 86 items/m^2. Despite some difficulties in comparing the results reported in the literature, due to the use of different methods in data collection, classification and reporting of MP, as well as different units of measurement, the concentrations reported in this study are much lower than those found on European beaches and in other Mediterranean MPAs. Particle characterization revealed that expanded material was the most abundant (59%) throughout the study area. The distribution and accumulation of this type of material on the beaches could be strongly influenced by the presence of larger foamed objects (e.g., G73 and G82), which could favour the formation of MP through physical degradation processes. In addition, their lightweight may facilitate wind dispersal and transport and consequent accumulation in the upper parts of the beach (AC 2 zone). Remote beaches were the most affected by pollution from MPs, as also highlighted in the macrolitter pollutions. The lack of regular cleaning activities and protection from direct input (e.g., by beachgoers) could indicate an important contribution of litter coming from the sea.
- More than 2,500 yellow wax residues were found in the surface waters and on the beaches of the Pelagos Sanctuary. Chemical composition analysis revealed that these objects are petroleum, classified as paraffinic polyethylene wax. Preliminary information on the relationship between main tanker density routes and wax distribution in the Pelagos Sanctuary was provided, suggesting a potential discharge of these residues by vessels in the offshore waters facing the Savona, Genova and Livorno ports. Nevertheless, the lack of clear definition and identification through monitoring protocols and the paucity of data available in the literature highlight the need to gather information on their presence, distribution and potential sources in the Pelagos Sanctuary and their potential impact on the organisms inhabiting it.

The relevant information here achieved provided a scientific basis for addressing plastic pollution in MPAs and facilitating future management recommendations and use of the marine and coastal environments of these protected areas.

CHAPTER 5: PLASTIC INGESTION AND PRESENCE OF PHTHALATE ACID ESTERS (PAEs) IN BIOINDICATOR SPECIES OF THE PELAGOS SANCTUARY PROTECTED AREA

This chapter assesses the occurrence and impact of plastic debris and MPs on marine organisms inhabiting the Pelagos Sanctuary and the Tuscan Archipelago National Park, from invertebrate species to apex predators such as marine mammals. The potential release of plastic-associated compounds (PAEs: phthalate acid esters) was also assessed in a wide range of organisms and different biological tissues to provide information on the potential link between plastic ingestion and the release of toxic addictive compounds.

5.1 Introduction

5.1.1 Description of selected species as potential plastic impact bioindicators

A total of 14 species were collected (Fig. 39) to evaluate the potential plastic impact in terms of particle ingestion and PAE levels. The description of each species considered is summarized below according to their taxonomic classification.

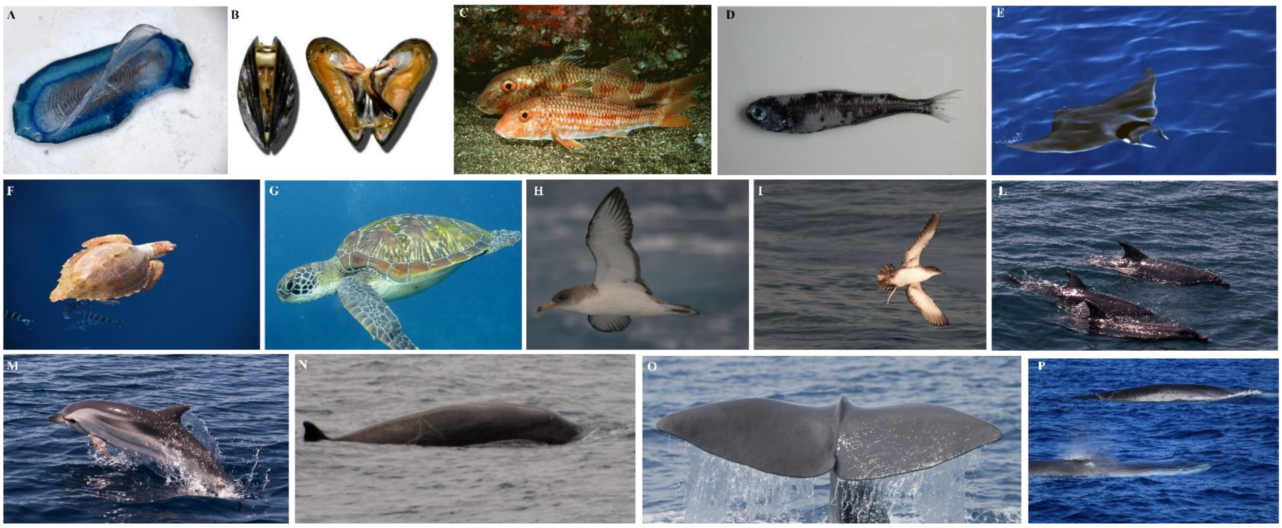


Fig. 39. Plastic ingestion impacts bioindicators species collected: *Velella velella* (A), *Mytilus galloprovincialis* (B), *Mullus surmuletus* (C), *Myctophum punctatum* (D), *Mobula mobular* (E), *Caretta caretta* (F), *Chelonia mydas* (G), *Calionectris diomedea* (H), *Puffinus yelkouan* (I), *Tursiops truncatus* (L), *Stenella coeruleoalba* (M), *Ziphius cavirostris* (N), *Physeter macrocephalus* (O) and *Balaenoptera physalus* (P).

Hydrozoans:

Velella velella (Linnaeus, 1758)

It is a cosmopolitan holoplanktonic marine hydrozoan that lives in open water. Floating colonies gather offshore in huge swarms, often consisting of millions of individuals (Purcell et al., 2012). *Velella velella* is an active predator of zooplankton, fish eggs and juveniles (Purcell et al., 2014), and are known to strongly influence the marine trophic chain. It represents the prey on some gastropods belonging to the genus *Janthina* and other marine species such as *Caretta caretta*, nudibranchs, and the sunfish *Mola mola* (Betti et al., 2019). As they disperse through the combined action of wind and currents and accumulate in regions with high concentrations of floating debris, potentially exposing them to MPs ingestion (Sabatés et al., 2010), it could be tested and considered as a potential mid-scale bioindicator species.

Mollusca:

Mytilus galloprovincialis (Lamark, 1819)

The usefulness of the Mediterranean mussel as a sentinel organism for monitoring MPs distribution in coastal areas has been demonstrated in several laboratory and field studies. This suspension feeder invertebrate is known to accumulate high levels of pollutants and microplastic (Avio et al., 2015; von Moos et al., 2012), providing a time-integrated indication of pollution. Due to its wide geographic distribution, abundance, basal position in the food web, accessibility, ability to conduct cage studies, and well-understood biology, the Mediterranean mussel can be selected as a bioindicator of microplastics in coastal areas. It is therefore an internationally recognised sentinel early warning species for marine pollution monitoring, used both in the U.S. Mussel Watch and the Mediterranean Marine Pollution Assessment and Control (MEDPOL).

Teleosts:

Mullus surmuletus (Linnaeus, 1758)

It is a heavily exploited demersal fish in the Mediterranean Sea, inhabiting sandy and muddy substrates, usually between 10 and 80 m, where it feeds mainly on benthic species such as shrimps and amphipods, polychaetes, molluscs, and benthic fishes (Alomar et al., 2017). It is one of the main target species of bottom trawling along the continental shelf and can also be caught by artisanal fisheries in shallower waters using gillnets and trammel nets (Biagi et al., 2002). The red striped mullet can be considered as a small-scale indicator of the presence and impact of MPs in the benthic environment of the Mediterranean Sea, where the ingestion of MP has been recorded in specimens from different areas (Alomar et al. 2017; Anastasopoulou et al. 2018; Güven et al. 2017).

Myctophum punctatum (Rafinesque,1810)

Based on their trophic level and habitat use, mesopelagic fishes can be used as indicators of the presence and impact of MPs in the Mediterranean pelagic environment at a medium scale. Lanternfishes, belonging to the family Myctophidae, perform extensive vertical migrations from the epipelagic zone at night to feed on zooplankton and micronekton (Battaglia et al., 2016; Van Noord, 2013). They play a key role in the pelagic trophic web and are an important link between primary consumers and tertiary consumers, such as commercially exploited fish, sharks and cetaceans (Catul et al., 2011). Therefore, they are thought to ingest plastic particles with zooplankton and possibly pass them on to higher trophic levels (Rochman et al., 2014; Romeo et al., 2016).

Elasmobranchs:

Mobula mobular (Bonnaterre, 1788)

The Giant devil ray is a pelagic species that resides in coastal and continental shelf waters. It spends the majority of its time in water less than 50 m, occasionally diving to higher depths (Notarbartolo di Sciara et al., 2015). The species exhibits large-scale movements, driven by seasonal patterns in prey availability, mainly small fishes, and zooplankton (Canese et al. 2011). To date, no evidence of plastic ingestion have been reported in the Mediterranean Sea and worldwide, although its habitat range may overlap with areas of heavy plastic pollution as highlighted by Germanov et al. (2019; 2018). Being a filter feeder organism, it could be particularly susceptible to direct microplastic ingestion during the feeding activities or indirectly through ingestion of already contaminated plankton.

Sea turtles:

Caretta caretta (Linnaeus, 1758)

Depending on its developmental stage and food availability, this species uses different ecological marine compartments. Because the loggerhead sea turtle is a carnivore to omnivore, it can ingest a large amount of waste mistaken for gelatinous prey or encrusted with food. In the Mediterranean, the frequency of ingestion varied from 35% in the Adriatic to almost 80% in the Spanish Mediterranean (Galgani, 2017). Due to its wide distribution and propensity to ingest marine litter, the loggerhead turtle was proposed as a target indicator species under the MSFD (indicator D10 C3), OSPAR (Claro, 2016) and UN Environment/MAP IMAP regulations (Indicator 24) (Fossi et al., 2018b) to assess the impact of litter in the Mediterranean Sea at a wide scale.

Chelonia mydas (Linnaeus, 1758)

The green sea turtle is a highly migratory species, undertaking complex movements and migrations through geographically diverse habitats. It has a worldwide distribution and is found in tropical and, to a lesser extent, subtropical waters including the Mediterranean Sea. It feeds mainly on seagrasses and is therefore found mainly in areas rich in underwater meadows. This species is classified as endangered by the IUCN (Seminoff, 2004), and is highly vulnerable to human impacts during their lives. Direct threats include fishing, collisions with boats, habitat destruction, and marine and coastal pollution. Only few studies report ingestion of litter in this species in the Mediterranean Sea (Duncan et al., 2019; Russo et al., 2003).

Seabirds:

Calonectris diomedea (Scopoli, 1769) and *Puffinus yelkouan* (Acerbi, 1827)

Globally, litter ingestion has been best studied in seabirds (Kuhn et al., 2020). In the Mediterranean, unfortunately, there is little information on the extent of plastic ingestion by seabirds (Codina-Garcia et al., 2013). However, species that largely inhabit the Mediterranean Sea, such as the Scopoli's shearwater and Yelkouan shearwater, could represent an interesting target species at a wide-scale considering also the large scale movements that they exhibit to feed in the foraging areas. As they are concentrated in breeding colonies in the central and eastern basin of the Mediterranean Sea from Sardinia through the central Mediterranean, the Adriatic, and the Aegean (Borg et al. 2010) and feed mainly on squid and small fishes (BirdLife International, 2018a,b), they are interesting candidates for indicators to monitor MPs and mesoplastics, although their availability for plastic ingestion analysis in the Mediterranean Sea remains quite a challenging task.

Cetacean odontocetes:

Tursiops truncatus (Montagu, 1823)

Bottlenose dolphins are found in a wide range of habitats, from coastal waters to pelagic waters, where they eat a variety of prey, primarily fish and squid, but also shrimp and other crustaceans (Wells et al., 2019). The subpopulation in the Mediterranean Sea is currently classified as Vulnerable (Bearzi et al. 2012). The main threats to this species are accidental capture in fishing gear, overfishing of prey, disturbance by shipping traffic and heavy contaminants pollution (Bearzi et al. 2012). Interactions with marine litter and ingestion of MPs have been reported in the Mediterranean (Alexiadou et al., 2019; Corazzola et al., 2021; Duras et al., 2021) and in a few cases described as a cause of mortality (Jerbi et al., 2021; Levy et al. 2009).

Stenella coeruleoalba (Meyen, 1833)

In the Mediterranean, this species is associated with highly productive oceanic waters beyond the continental shelf (Notarbartolo di Sciara et al., 1993, Panigada et al., 2008). It feeds in pelagic to benthopelagic zones, to depths of 200-700 m, on the continental slope and its diet consists of a variety of small organisms from the midwater and pelagic or benthopelagic zones, especially lanternfish, cod and squid (Archer 2018). Currently classified as Vulnerable on the IUCN Red List, the presence of MPs in their gastrointestinal tract has recently been highlighted (Baini et al. 2017; Corazzola et al., 2021; Duras et al., 2021; Novillo et al., 2020; Pribanic et al., 1999).

Ziphius cavirostris (Couvier, 1823)

Cuvier's beaked whales are deep-diving pelagic cetaceans that inhabit offshore waters of all oceans, feeding mainly on deep-sea squid and less frequently on fish and crustaceans (Baird et al., 2020). In the Mediterranean Sea, this species is mostly observed in waters deeper than 500 m, with a marked preference for depths of 1000 m to 2000 m (Tepsich et al. 2014). In this basin, it should be considered as a separate evolutionarily significant unit, distinct from other populations, and classified as Vulnerable according to IUCN criteria (Cañadas and Notarbartolo di Sciara, 2018; Carroll et al. 2016). The main threat to this species is noise pollution (Cañadas and Notarbartolo di Sciara 2018). Other potential threats include the occasional risk of bycatch in pelagic driftnets (Karaa et al., 2021), ingestion of plastic waste and MP (Cagnolaro et al. 1986; Corazzola et al., 2021; Duras et al., 2021).

Physeter macrocephalus (Linnaeus, 1758)

The species is mainly distributed along the continental slope, especially abundant in submarine canyons. It feeds mainly on mesopelagic squid, which it usually hunts at depths between 400 and 800 meters (Tepsich et al., 2014). The main threats to sperm whales in the Mediterranean Sea are entanglement and underwater noise from oil and gas drilling, but ingestion of plastic waste is another cause of concern for this species (Alexiadou et al., 2019; de Stephanis et al., 2013; Fossi et al., 2020; Jerbi et al., 2021; Mazzariol et al., 2011; Simmonds, 2012). It has been recently proposed as a potential bioindicator species for macroplastic ingestion in the Mediterranean Sea (Fossi et al., 2018b).

Cetacean mysticetes:

Balaenoptera physalus (Linnaeus, 1758)

Fin whales occur in the central and western Mediterranean Sea, mainly north and east of the Balearic Islands, and appear to be a mixture of a resident population and a population that migrates between the

Mediterranean Sea and the Atlantic Ocean (Notabartolo di Sciara et al. 2016). The range appears to be concentrated in the Ligurian Sea and the Gulf of Lyon in summer but expands to cover much of the western and central Mediterranean in winter. They feed primarily on planktonic euphausiids and are described as vulnerable to ingestion of MPs that accumulate in pelagic areas and absorption of chemical additives potentially released (Fossi et al., 2012, 2014, 2016). For this reason, they are proposed as wide-scale indicators of the presence and impact of microplastics throughout the pelagic environment of the Mediterranean Sea (Fossi et al., 2018b).

5.2 Materials and methods

5.2.1 Marine species sampling activities

Invertebrate and fish species

The hydrozoans *Velella velella* were collected during the June 2019 sampling campaign in the northwestern sector of the Pelagos Sanctuary, concurrently with MPs sampling conducted with a manta net (330 µm mesh). Specimens were individually isolated from the nesutonic samples, rinsed with pre-filtered water (0.45 µm) and stored in pools of 5-10 organisms each, resulting in a total of 62 pools (Tab. 14). In the same area, 21 specimens of *Myctophum punctatum* were collected at night with a plankton net in summer 2019 (Tab. 14). Invertebrates and fish were stored in liquid nitrogen for MPs and PAEs detection.

Tab. 14. Invertebrate and fish species collected during the sampling campaign carried out in the Pelagos Sanctuary. The common and scientific names, the number of organisms collected, and the analysis performed are shown.

Common name	Scientific name	N. of organisms collected	Analysis performed (n°. samples analyzed)
Mediterranean mussel	*Mytilus galloprovincialis*	54	MP ingestion (54) PAE detection (7 pools)
By-the-Wind sailor	*Velella velella*	62 pools	MP ingestion (53) PAE detection (6 pools)
Spotted lanternfish	*Myctophum punctatum*	21	MP ingestion (21)
Red striped mullet	*Mullus surmuletus*	47	MP ingestion (47) PAE detection (16)

During the sampling campaign conducted in July 2019 in the Tuscan Archipelago National Park, a total of 54 specimens of *Mytilus galloprovincialis* were sampled (Tab. 14). Mussels from wild populations were collected on the island of Capraia near an aquaculture fish farm (n. 24) and from a buoy in the islands of Montecristo (n. 27) and Giglio (n. 3) and immediately stored at -20°C before MPs detection. Mussels were dissected by cutting off the two adductor muscles to extract the soft tissue. The maximum shell length and width (cm) and total wet weight (g) and shell weight (g) of each mussel were measured. Forty-seven individuals of *Mullus surmuletus* were collected in collaboration with local artisanal fishermen using trammel nets from Capraia Island and Porto Ercole (the southern sector of the Tuscan Archipelago National Park) (Tab. 14). Live fish were immediately stored in dry ice before dissection. The biological parameters of both the fish species (total length of the specimen (cm), fork length (cm) and weight (g)), as well as the visible deformities and external condition, were recorded. The gastrointestinal tract (GIT) of each fish was removed from the oesophagus to the end of the intestine, and other tissues (muscle and liver) were saved for future analysis. To avoid contamination of the sample, the GIT of each specimen was rinsed with pre-filtered (0.45 µm) deionized water.

Stranded organisms

Several marine species stranded between 2016 and 2021 along the coasts of Tuscany were examined to assess the potential ingestion of plastic. Animals were stored at -20°C until processing, and dissections were carried out in a necropsy facility at the Laboratory of the *Accademia dei Fisiocritici Siena Onlus* in collaboration with the Environmental Protection Agency of Tuscany Region (ARPAT) and "*Osservatorio Toscano Cetacei e Tartarughe Marine* (OTC)" according to standard procedures (Ijsseldijk et al., 2019). In addition, the stomach and a sub-sample of the intestine of a fin whale (approximately 30% of the total length) stranded in Sorrento (Naples) on the 14th January 2021 were analysed for MPs detection. During dissection, different biological tissues were collected for the determination of PAEs contaminants. The list of specimens is reported in Tab. 15.

Tab. 15. Stranded species collected along the Tuscan coast in the Pelagos Sanctuary. The common and scientific names, IUCN conservation status, number of organisms collected, and analysis performed were shown.

Common name	Scientific name	IUCN status	N. of organisms collected	Analysis performed (n°. samples analyzed)
Giant devil ray	*Mobula mobular*	EN - Endangered	1	MP ingestion (1)
Loggerhead sea turtle	*Caretta caretta*	VU - Vulnerable	21	MP ingestion (21) PAE detection (6)
Green turtle	*Chelonia mydas*	EN - endangered	3	MP ingestion (3)
Scopoli shearwater	*Calonectris diomedea*	LC - Least Concern	1	MP ingestion (1)
Yelkouan Shearwater	*Puffinus yelkouan*	VU - Vulnerable	2	MP ingestion (1)
Striped dolphin	*Stenella coeruleoalba*	VU - Vulnerable	18	MP ingestion (18) PAE detection (14)
Bottlenose dolphin	*Tursiops truncatus*	VU - Vulnerable	9	MP ingestion (9) PAE detection (2)
Cuvier's Beaked Whale	*Ziphius cavirostris*	VU - Vulnerable	1	MP ingestion (1) PAE detection (1)
Fin whale	*Balaenoptera physalus*	VU - Vulnerable	1	MP ingestion (1) PAE detection (1)

Free-ranging organisms

Fin whale and sperm whale skin biopsies from free-ranging cetaceans have been collected in the Pelagos Sanctuary area (Tab. 16) using a non-destructive method by remote dart sampling, using a modified dart with an aluminium tip (8 mm diameter) launched with a Panzer V crossbow.

Tab. 16. Free-ranging species collected during the sampling campaigns carried out in the Pelagos Sanctuary. The common and scientific names, IUCN conservation status, number of skin biopsies collected and analysis performed were shown.

Common name	Scientific name	IUCN status	N. of skin biopsies collected	Analysis performed
Fin whale	*Balaenoptera physalus*	VU - Vulnerable	15	PAEs detection
Sperm whale	*Physeter macrocephalus*	VU - Vulnerable	4	PAEs detection

To avoid any possible infection, the tip is sterilised each time with alcohol before shooting. During the sampling process, attention was paid to taking the biopsy sample in the dorsal area close to the dorsal fin. Each biopsy (1-2 g of epidermal, dermal and blubber tissue) has been subdivided into different aliquots according to the different analyses: blubber tissue for contaminants analysis and epidermal, dermal part for ecotoxicological biomarkers, stable isotopes and sex determination. All the aliquots were immediately stored in liquid nitrogen and at -80°C until the analysis.

5.2.2 Plastic litter extraction and characterization: innovative methods

Plastic ingestion by *Velella velella*: setup and validation of multidisciplinary approach

To date, there is no validated digestion method for the extraction and isolation of MPs in *Velella velella*. Following the procedures as shown by Tsangaris et al. (2021), two different extraction methods using 15% H_2O_2 and 10% KOH were tested to select the optimal analytical procedures to extract ingested plastic particles from these organisms and to validate the species *Velella velella* as a potential bioindicator of MPs pollution in pelagic areas. Plastic fragments of the three most common polymers (PE, PP and PS) found floating at the sea surface in this study were obtained by cutting industrial pellets with a surgical blade. The largest dimension of each fragment was measured using ImageJ software. White/opaque fragments with a median size of 320 μm and a size range of 101-538 μm were divided into three size classes (100 - 200 μm, 200 - 300 μm, 300 - 500 μm). Organisms were measured individually (cm) and the total weight of each pool was recorded (g). Six pools of *Velella velella* (mean 7 ± 3 individuals/pool) were spiked with 5 plastic fragments of each polymer from each size class to reflect the variability of MPs found in field biota samples. Extraction of microplastics with H_2O_2 digestion was performed according to Tsangaris et al. (2021): 20 ml of 15% H_2O_2 per 1 g wet weight of tissue was added (1:20 w/v). The samples were heated on a hot plate at 50°C until all organic matter was removed (24 h heating). After digestion, the samples were diluted with 100 ml of purified water (Milli-Q) and filtered under a vacuum pump system onto a glass fibre filter (Whatman GF/C, pore size 1.6 μm). KOH digestion was performed according to Giani et al. (2019) (see Section 5.2.3). The criteria used to determine the most appropriate method were digestion efficiency (% DE) and membrane clogging (MC), recovery rates (% RE) and duration of total analytical procedures. The DE and MC were calculated following the studies of Karami et al. (2017) and Bianchi et al. (2020). For these parameters, the presence of undigested organic material on the filter membrane was considered as a potential factor that could limit the optical examination of MPs. Before and after filtration, the filter membranes were dried at 50 °C for 5 h and weighed on a balance with an accuracy of 0.1 mg. As control experiments, the same treatments were also performed on blank membranes (procedural blanks) to evaluate the possible weight gain of the membranes only due to the different solvents used. The digestion efficiency was calculated as follows:

$$\% \, DE = ((wi-(Wa- Wb)) /wi) *100$$

wi= weight of Velella organism pool; Wa= filter weight after digestion; Wb= filter weight before digestion.

Membrane clogging was also calculated by determining the total number of filter membranes required for optimal filtration of the total digested organic material.

$$\text{Membrane clogging (filter/g)} = N / (wi-(Wa- Wb)) /wi)$$

N= number of filters used; wi= weight of the pool of Velella organisms; Wa= weight of the filter after digestion; Wb= weight of the filter before digestion.

Finally, the recovery rates were evaluated as follows:

$$\text{Recovery rate } \% = (Na/ Nb) *100.$$

Na= number of spiked fragments; Nb= number of recovered fragments.

The recovered MPs were counted under a stereomicroscope and measured using ImageJ software. Finally, the chemical composition of the isolated MPs was evaluated using Agilent Cary 630 FTIR spectrometer to assess the recovery based on chemical composition. The identification of the polymers was accepted when the match with the reference spectra had a confidence level > of 70% (Baini et al., 2018).

Plastic ingestion by stranded organisms: setup and validation of dissection methods

As advocated by international agreements and European environmental policies, a systematic and standardized approach to investigate stranded marine organisms is needed with the aim of a shared data collection and interpretation, and common conservational strategies. In particular, the gastro-intestinal tract analysis is relevant for a variety of investigations such as pathological evaluation, diet analysis, marine litter detection, parasitological, microbiological and virological detection. The lack of a multidisciplinary and standardized approach to the analysis of GIT became even more relevant after the increase of the global concern about the ingestion of marine litter by marine megafauna (Fossi et al., 2018b; 2020; Panti et al., 2019). To cover this gap, an innovative protocol for a multidisciplinary sample collection based on a multi-sieves filtration system was implemented in collaboration with the Department of Comparative Biomedicine and Food Science of the University of Padua and the IZS (Experimental Zooprophylactic Institute) of Piemonte, Liguria and Valle d'Aosta. The multi-sieves tool consists of sequential sieves of different mesh sizes inserted on specific support created *ad hoc* (Fig. 40).

Fig. 40. The multi-sieves tool developed in collaboration with the Department of Comparative Biomedicine and Food Science of the University of Padua and the IZS (Experimental Zooprophylactic Institute) of Piemonte, Liguria and Valle d'Aosta. Source: Corazzola et al. (2021).

The meshes sequence selected was: 20 mm, 5 mm, 1 mm, 0.5 mm (optional, to be used when the volume of gastro-intestinal content is abundant, and a better separation of the material is necessary), 0.25 mm and 0.1 mm. A comprehensive description can be found in Corazzola et al. (2021).

Following this analytical protocol, stranded organisms examined in this thesis were analyzed at the *Accademia dei Fisiocritici in Siena Onlus*, by filtering GIT contents through three stacked stainless steel sieves (1, 0.25 and 0.1 mm). Briefly, the protocol involved the following steps:

- Transfer the GIT to a clean stainless steel necropsy table and thoroughly rinse the external part of the organ (i.e., stomachs or intestine) with filtered water (0.45 µm);
- Open the organ longitudinally, throughout the entire length, using scissors or scalpels;
- Collect gastric content sample in a tank, rinsing intensely the mucosa with current water to facilitate the complete exit of material;
- Transfer the organ contents from the tank/container into the multi-sieves system;
- Separate marine litter items visible to the naked eye (macroplastics) that are eventually retained by the sieves, wash them with filtered water and let them dry in a Petri dish;
- Collect all the material retained by each sieve and transfer it to jars for the MPs extraction.

5.2.3 Plastic litter extraction and characterization: harmonized methodology

Plastic litter extraction in all the species collected, from invertebrates to cetaceans, was performed according to Giani et al. (2019) and Tsangaris et al. (2021). Briefly, 5-10 ml of 10% KOH was added per 1 g of tissue fresh weight (1:5 w/v or 1:10 w/v for stranded organisms). Samples were heated on a thermostatic water bath (50°C) until all organic matter was removed (6 - 12 h heating). After digestion of the organic matter, the samples were filtered onto glass fibre filters (1.6 µm mesh) or a net (100 µm mesh, for stranded organisms) using a vacuum pump. To better separate the potential MPs ingested by stranded organisms from the digested organics left on the net, an additional step was performed in which the organics were resuspended with 100 ml of a hypersaline solution (NaCl: 1.2 g/cm^3). Samples were then re-filtered and visually observed under a stereomicroscope (Mod. NBS-STMDLX-T). Plastic particles larger than 100 µm were characterised according to different size classes into small microplastic (SMPs) (< 0.3 mm; 0.3 – 0.5 mm; 0.5 - 1 mm), large microplastic (LMPs) (1 – 2.5 mm and 2.5 - 5 mm) and mesoplastic (5 - 25 mm), shape (pellet, fragment, film, filament, microbead and foam) and colour (black, blue, white/transparent, white/opaque, red, green and others). For the items larger than 2.5 mm, classified as macroplastic, the MSFD protocol (Galgani et al., 2013) was adopted to categorize the plastic particles isolated following the category: sheetlike user plastics, threadlike user plastics, foamed user plastics, fragments and others. Finally, the chemical composition of the plastics isolated was evaluated using Agilent Cary 630 FTIR spectrometer according to Baini et al. (2018). Items with properties similar to those of plastic polymers (i.e., colour, regularity of shape, surface texture, ductility, and fracture strength) but too small to be chemically analysed by FTIR spectroscopy were isolated and tested using the hot needle technique (Bellas et al., 2016). The frequency of ingested plastic occurrence in each species was estimated following Pedà et al. (2020) as the proportion, on the total sample of the individuals which ingested plastics: (% O = N. individuals which ingested plastics/N. total samples x100).

5.2.4 Quality assurance and control

All glassware was rinsed with filtered water (0.45 µm) and cotton lab coats were used during all laboratory procedures. Tissue samples were covered with aluminium foil paper during digestion and filtration procedures took place in a laminar flow cabinet. Filters or nets were maintained in a Petri dish during observation under a stereomicroscope. Blank samples were taken during both GIT dissection and filtration to quantify airborne contamination.

5.2.5 PAEs detection

A new PAEs extraction method has been developed, tested and applied to evaluate the presence of phthalate acid esters in different biological tissues of the collected organisms (Tab. 17).

Tab. 17. Alive organisms and biological tissues of stranded species collected along the Tuscan coast in the Pelagos Sanctuary.

Biological tissue analysed	**Species analysed**
Whole organisms	*Mytilus galloprovincialis* and *Velella velella*

Muscle	*Mullus surmuletus*
Liver and fat	*Caretta caretta, Stenella coeruleoalba, Tursiops truncatus, Ziphius cavirostris*
Skin, muscle, heart, liver, kidney, lung, and faeces	*Balaenoptera physalus*
Skin biopsy	*Balaenoptera physalus* and *Physeter macrocephalus*

Sample processing

The levels of Dimethyl phthalate (DMP), Diethyl phthalate (DEP), Diallyl phthalate (DAP), Dipropyl phthalate (DPrP), Diisobutyl phthalate (DIBP), Dibutyl phthalate (DBP), Benzyl butyl phthalate (BBzP), Dicyclohexyl phthalate (DCHP), Bis(2-ethylhexyl) phthalate (DEPH), Di-n-octyl phthalate (DNOP) Diisononyl phthalate (DNIP) (Annexe 15) were evaluated in different biological tissues of several marine organisms. Samples were analysed adopting a new extraction method developed modifying the procedures proposed by Baini et al. (2017). Briefly, all analyzed biological tissues were freeze-dried for 48-96 hours, their fresh weight and dry weight were measured and water content (%) was calculated. Approximately 0.1 g of the freeze-dried tissue (0.03 g for skin biopsies and 0.15 g for *Velella velella*) of each sample was homogenized and spiked with 100 µl of 11 deuterated PAEs standards (mix-D4, 1 ppm) as an internal recovery standard before extraction in an ultrasonic bath (15 minutes) with 4 ml acetonitrile. The mixture was centrifuged (5 minutes at 4,500 rpm) and the upper phase containing the extracted PAEs was collected. This procedural step was repeated three times. The resulting extracts were purified using an Agilent extraction kit 5982-0029 (QuEChERS) and centrifuged at 5,000 rpm for 5 minutes. The extract was then evaporated under a gentle stream of nitrogen and resuspended in 300 µl of hexane.

Instrumental determination

Final extracts were analysed using an Agilent 8890A series gas chromatograph coupled to an Agilent 5977B Inert Plus low-resolution mass spectrometer with a simple quadrupole analyzer equipped with a versatile HP -5MS capillary column (30 m, 0.25 mm). The injection volume was 1 µl (splitless mode), and ultra-pure helium with a constant flow of 1 ml/min was used as the carrier gas. The oven temperature was set at 80°C and increased to 210°C at 20°C/min, and 240°C at 15°C/min. (holding time: 2 min.) and to 310°C at 15°C/min. (holding time: 3 min.). Quantitative analysis was performed by single ion monitoring (SIM).

Quantification

Quantification was based on 7-point calibration curves generated for each analyte, ranging from 10 to 1000 ppb. The correlation coefficient (R_2) and relative standard deviation (RSD) of the calibration curve were higher than 0.99. One spiked and procedural blanks were analysed in each batch. Internal recovery standards were added to all samples and the concentrations of PAEs were all corrected for recovery. The recoveries ranged from 76% to 123%. Limits of detection (LODs) for individual PAEs are listed in Tab. 18. Concentration values that were below the LOD were labelled as below the limit of detection (BDL) and a value of one-half of the BDL was used for statistical analysis.

Tab. 18. PAEs limit of detection according to the quantity (g) of freeze-dried sample extracted.

Biological tissue (g)	DMP (ng/g)	DEP (ng/g)	DAP (ng/g)	DPrP (ng/g)	IBP (ng/g)	DBP (ng/g)	BBzP (ng/g)	DChP (ng/g)	DEHP (ng/g)	DINP (ng/g)	DNOP (ng/g)
Skin biopsy (0.03 g)	1.14	1.14	2.86	1.14	1.14	1.14	11.43	11.43	2.86	57.14	11.43
V. velella (0.15 g)	0.02	0.02	0.05	0.02	0.02	0.02	0.20	0.20	0.05	1.00	0.20
Other tissue (0.1 g)	0.343	0.343	0.857	0.343	0.343	0.343	3.429	3.429	0.857	17.143	3.429

Quality assurance

Several laboratory and sampling procedures were used to ensure the integrity of the results concerning the ubiquitous nature of plasticizers. All glassware was previously cleaned with Alconox® and after a rinse with tap water followed by Milli-Q water, was washed with Contrad® 2000, rinsed several times with Milli-Q water, and dried overnight at 250°C. Prior to use, all glassware was rinsed with ultra-pure hexane (Fluka). Blank samples were also evaluated to check for possible laboratory contamination and interference; the blank value was subtracted from the analysed samples.

5.2.6 Statistical analysis

The normality distribution of both datasets referring to plastic ingestion and PAE concentrations in the analysed species was tested using the Shapiro Wilk test. According to this result parametric (Paired and Unpaired Student T-test) or non-parametric (Kruskal-Wallis Test, Wilcoxon Signed-Rank Exact Test for paired samples and Wilcoxon Rank Sum Test for unpaired samples), statistical tests have been applied. Any differences in the occurrence of plastic ingestion and PAE concentrations among species, different sampling sites, and biological tissue analysed as well as differences among the parameters considered for the setup and validation of the digestion model in *Velella velella* were computed. To validate *Velella velella* as potential bioindicators of plastic ingestion in surface waters of pelagic areas, a Spearman Rank Correlation Test was performed among the PAE levels, the average number of items ingested by *Velella velella* specimens and the MPs concentrations found in the corresponding manta trawl from which the organisms were isolated. Principal component analysis (PCA) was performed to assess potential influences of plastic ingestion and sampling site on PAEs concentration and composition, and to better identify the suitable biological tissues for the detection of plasticizers amond stranded organisms. A significance level of 0.05 was used for all analyses performed with Rstudio (version 1.1.4.1106).

5.3 Results and discussions

5.3.1 Plastic ingestion and PAE levels in local and small-scale bioindicators

Mytilus galloprovincialis: plastic ingestion and characterization

Among the 54 individuals of *Mytilus galloprovincialis* analyzed, plastic was found in 30 organisms, accounting for an overall frequency of ingestion of 56%. A total of 93 plastic particles were isolated, accounting for 96% of MPs size classes. Considering the different sampling sites, the ingestion rate was higher in Capraia (71%, n°. 17/24 organisms) than in Montecristo (48%, n°. 13/27 organisms). No plastic particles were found in the mussels sampled in the facing waters of the island of Giglio, however, the lower number of samples must be considered (n°. 3). The different plastic ingestion rates could be due to the different availability of plastic particles floating in the surface waters off the islands, as highlighted for the northern sector of the Tuscan Archipelago National Park in section 4.3.2 (Capraia: 211,650 ± 159,736 items/km^2; Montecristo: 102,966 ± 83,089 items/km^2). Moreover, the proximity of the aquaculture site in Capraia Island could have influenced the uptake of synthetic particles by filter-feeder organisms.

No statistical differences were highlighted between the mean number of plastic ingested by mussels in the two islands monitored, 2.2 ± 2.6 items/ind. in Capraia and 1.5 ± 3.1 items/all ind. in Montecristo islands (Fig. 41).

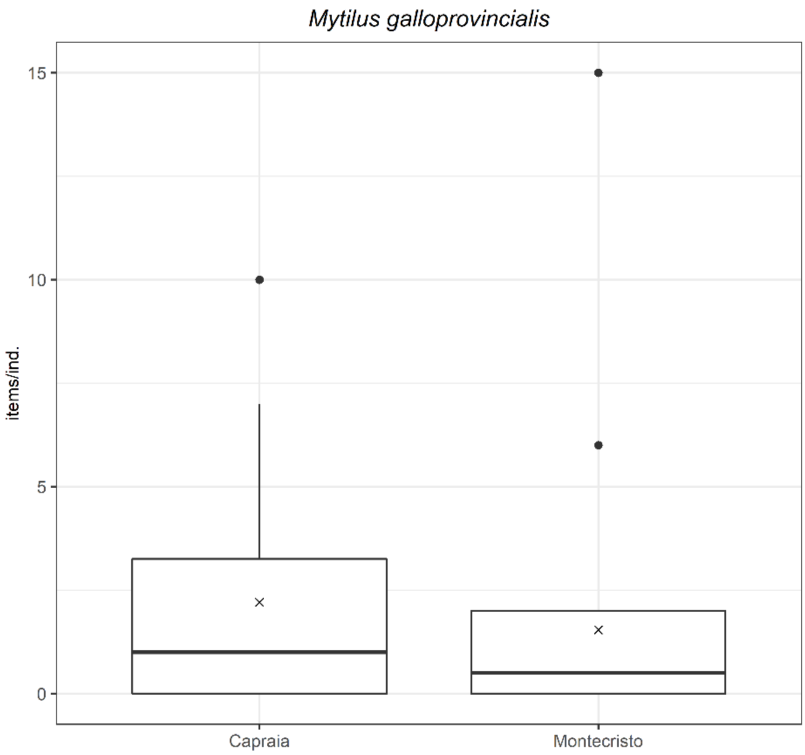
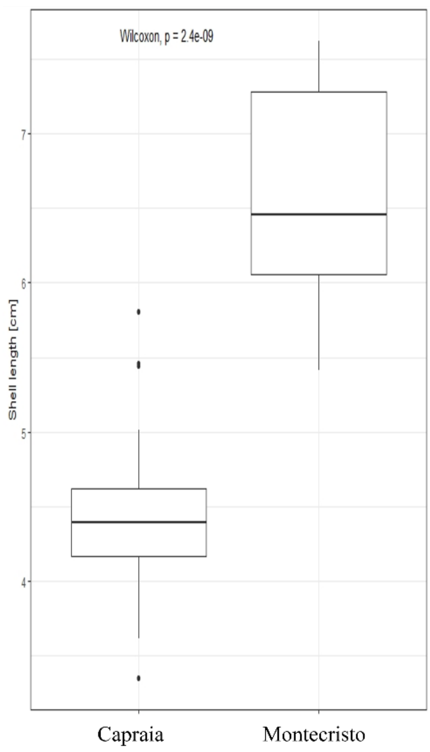


Fig. 41. MPs ingestion (items/ind.) in the mussels sampled in the Capraia and Montecristo islands. The boundaries of the boxes indicate the 25th and 75th percentiles, the whiskers above and below the boxes the 95th and 5th percentiles. The horizontal line inside the boxplots denotes the median value and X the avg. value.

The data here showed, appear to be higher than those obtained in other studies in the Mediterranean Sea. Digka and co-workers had found an occurrence of 45-47% and an average concentration of MPs per individual of 1.7-2.2 pieces analyzing organisms sampled in the Ionian Sea (Digka et al., 2018). Slightly further north, a study conducted by Vandermeersch in 2015 on mussels sampled at the mouth of the Po' (Adriatic Sea) had revealed a concentration of 0.14 - 0.18 items/g w.w. tissue (Vandermeersch et al., 2015). These differences could be due to both the different concentrations of microplastics in the surface waters of the sampled areas and the different availability for ingestion by this species. Other studies on ingestion by filter-feeding bivalves near the Gulf of La Spezia (*Crassostrea gigas* 0.11 items/g w.w. tissue, *Mytilus galloprovincialis* 0.05 items/g w.w. tissue and *Anomia ephippium* 0.12 items/g w.w. tissue) are reported by the study of Bonello et al. (2018).

A significant difference in the size of *Mytilus galloprovincialis* (Wilcoxon test, p = 2.4e-09) was found, with individuals from Capraia and Montecristo showing a mean shell length of 4.45 ± 0.56 cm and 6.61 ± 0.75 cm (Fig. 42 A). If the size seems not to affect the MPs ingestion in terms of number, 75% of mesoplastics was found in the larger organisms sampled in Montecristo Island, suggesting potential ingestion of plastic particles linked to the size of specimens (Fig. 42 B). Small MPs (< 1 mm) has been the size classes mostly found in both the areas considered, representing the 55% and the 64% of the total items isolated in Montecristo and Capraia, respectively (Fig. 42 B).

A



B

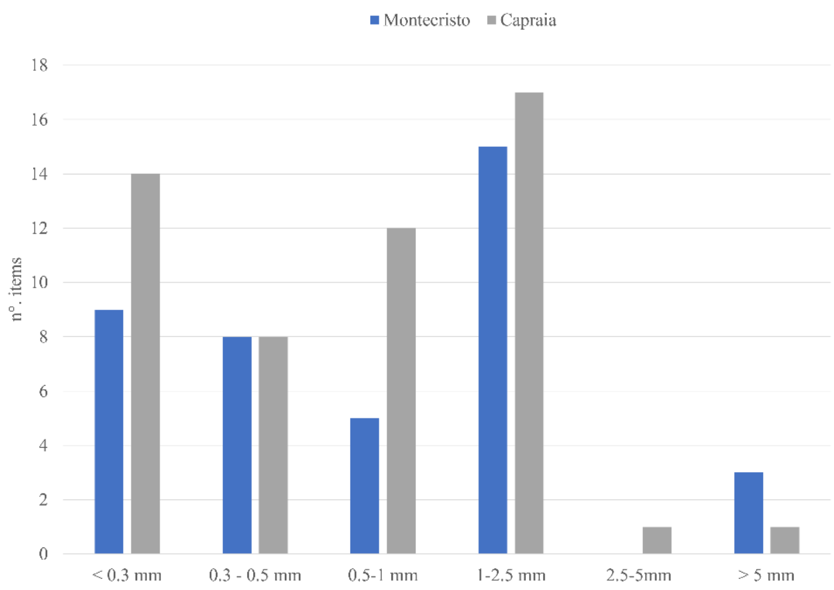


Fig. 42. Shell length (cm) of *Mytilus galloprovincialis* organisms sampled in the monitored areas and MPs ingestion (items/ind.) according to the size classes considered (B). The boundaries of the boxes indicate the 25th and 75th percentiles, the whiskers above and below the boxes the 95th and 5th percentiles. The horizontal line inside the boxplots denotes the median value. * Indicates the statistical significativity (p < 0.05).

These data are consistent with the results of the studies conducted by Digka et al. (2018) in the Adriatic Sea, where 60% of the microplastics found in mussels were between 0.1 - 0.5 mm in size, and Gedik & Eryasar, (2020) on the Turkish waters, showing the size classes lower than 0.5 mm as the most abundant. Processing data on the size classes of ingested particles is an important parameter to obtain information on their distribution and potential impact on marine organisms. In particular, more than 20% of the particles found in the analyzed organisms of both islands, had a size less than 300 µm (Fig. 42 B), the threshold for the mesh size of the usual plankton nets used for sampling microplastics at the surface. This confirms the importance of using bioindicators for the assessment of the presence and potential impact of plastic particles to obtain a comprehensive overview of this issue, especially on the possible fate of small plastic particles. The most common types of MPs (Fig. 43 A-C) were fibres, both in Capraia Island (72% of the total isolated particles) and in Montecristo Island (88%). Their presence, representing more than 70% of the total isolated particles (Figs. 42 A and 43 A), confirms that this type of microplastic is particularly prone to sink (Kooi et al., 2016) and accumulate in the water column, making it potentially available for ingestion by sessile organisms such as bivalves. Differently from the studies of Digka et al. (2018) and Gedik & Eryasar, (2020), where fragments are described as the most abundant plastic type, here this category represents 25% in Capraia and 10% in Montecristo of the total particles isolated.

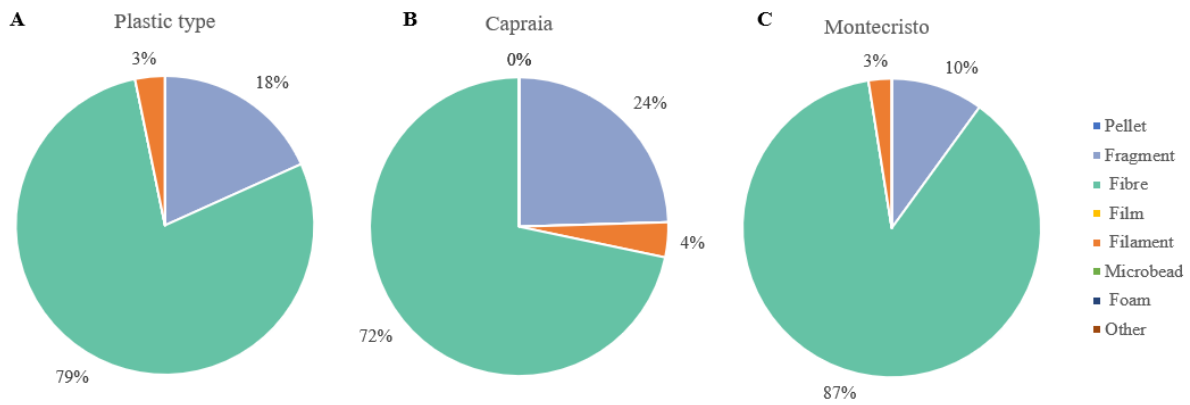


Fig. 43. MPs characterization according to the type categories in the *Mytilus galloprovincialis* organisms considered both the sites monitored (A), the Capraia (B) and Montecristo (C) islands sites.

Polymer analysis of the fibres was not performed and only particles larger than 200 µm were chemically characterised. Nevertheless, the synthetic origin of the isolated items was tested using the hot needle technique according to Bellas et al. (2016). Polypropylene was the most abundant polymer on both islands, accounting for 89% (Fig. 44 B).

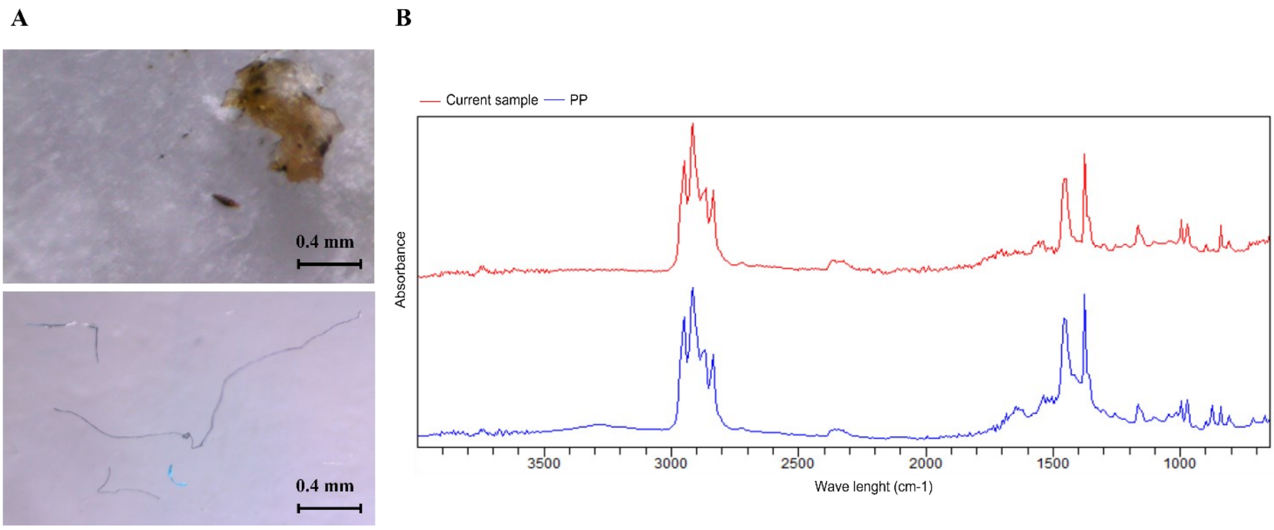


Fig. 44. MPs isolated from mussel organisms (A); Polypropylene spectrum obtained through FTIR analysis (B).

This polymer and polyethylene are the main materials of the fragmented MPs and were found in all marine environments of the oceans. Their presence in MPs ingested by bivalves has already been demonstrated by Gedik and Eryasar, (2020) and Digka et al. (2018). Polyvinyl chloride (PVC) was the second most abundant polymer (11%). This material, which is rarely found at the surface due to its high density, tends to sink and thus becomes a potential prey for sessile bivalve feeding organisms such as mussels (Suaria et al., 2016; Baini et al., 2018).

Mytilus galloprovincialis: PAE levels detection

Filter-feeder organisms tend to bioaccumulate anthropogenic particles and many persistent contaminants at higher concentrations than those found in the surrounding water. For this reason, phthalate acid ester levels were detected in different pools of Mediterranean mussels collected in Capraia (n°. 3 pools), Montecristo (n°. 3 pools) and Giglio islands (n°. 1 pool) of the Tuscan Archipelago National Park. Eight out of eleven compounds investigated were detected (Tab. 19) and represented mainly by Diisobutyl phthalate (DIBP) (42%), Dibutyl phthalate (DBP) (37%) and Diallyl phthalate (DAP) (12%).

Tab. 19. PAE concentrations (ng/g) for each compound considered in the Mediterranean mussel analysed according to the sampling site.

Sampling site	**ng/g w.w.**											
	DMP	**DEP**	**DAP**	**DPrP**	**DIBP**	**DBP**	**BBzP**	**DChP**	**DEHP**	**DINP**	**DNOP**	**∑ PAEs**
Capraia	<LOD	11.80	207.31	<LOD	701.11	512.25	5.30	15.80	63.91	<LOD	<LOD	**1519.65 ± 243.21**
Montecristo	0.60	5.40	45.79	<LOD	162.64	174.15	<LOD	27.50	13.86	<LOD	<LOD	**432.54 ± 90.77**
Giglio	0.42	10.30	40.87	<LOD	219.77	248.95	<LOD	31.05	42.84	<LOD	<LOD	**597.63 ± 65.46**

The highest concentration of ∑ PAEs was found in the organisms sampled in Capraia Island with a total mean of 1,519.65 ± 243.21 ng/g w.w (Fig. 45). By contrast, the lowest concentrations were found in the mussels of Montecristo Island with a total mean of 432.54 ± 65.46. ng/g w.w (Fig. 41). Despite that, the statistical analysis does not confirm the significant difference in the PAE concentrations among the three sites considered (Kruskal-Wallis Test chi-squared = 4.5714; p = 0.1017).

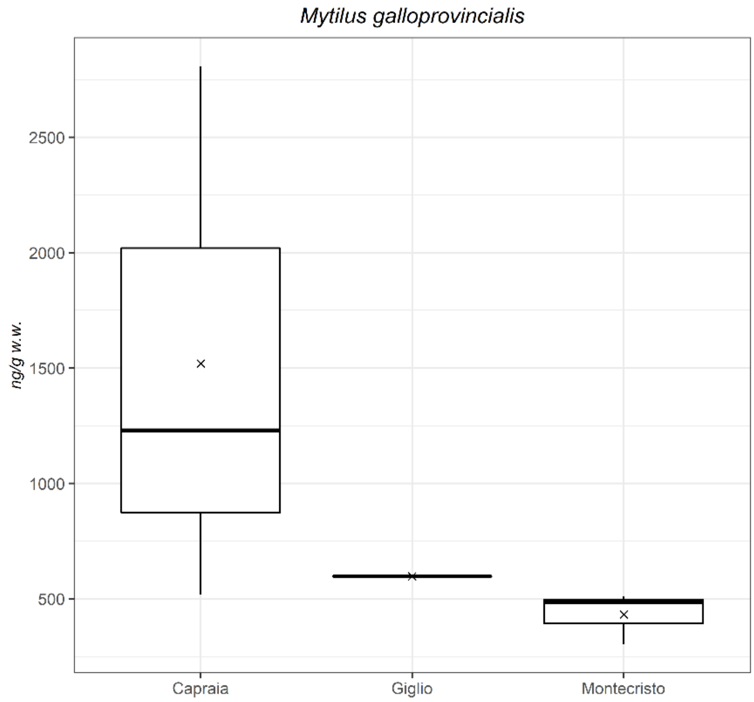


Fig. 45. PAEs levels in the Mediterranean mussel investigated according to the sampling site. The boundaries of the boxes indicate the 25th and 75th percentiles, the whiskers above and below the boxes the 95th and 5th percentiles. The horizontal line inside the boxplots denotes the median value and X the avg. value.

The obtained results may reflect the different plastic pressures that could insist on these areas, both in terms of the number of particles ingested by organisms and the concentrations of floating plastic in coastal waters. Mediterranean bivalves were reported to ingest an average of 2.2 ± 2.6 items/ind. in the Capraia Islands (occurrence of 71%), where a concentration of 211,650 ± 159,736 items/km^2 was measured. In contrast, organisms sampled at Montecristo showed a lower uptake rate of 48%, likely due to the lower number of floating MPs, which was reported to be 102,966 ± 83,089 items/km^2. Regarding the PAE levels detected in Giglio Island, an area particularly highlighted in this study for the presence of floating larger plastic objects, the small number of samples analysed (only one pool) may have influenced the results. Although this study can not state the direct transfer of PAEs from particles ingested by *Mitylus galloprovincialis* organisms, the results demonstrate that these lipophilic compounds are freely present in relatively high concentrations in marine waters and can be transferred to tissues as a consequence of the filtering activity of the biota (Gobas et al., 2003). The different anthropogenic influences affecting the considered islands can strongly affect the release in the surrounding waters and the accumulation of these pollutants by marine organisms. In Capraia Island, the proximity of the mussels sampling site to the harbour and the aquaculture fish farm could have influenced the high concentrations of PAEs found in the specimens analysed. Islands such as Giglio, which is subject to strong tourist pressure, especially in summer, could be affected by higher availability of plasticizers in the waters than the more protected island of Montecristo, where the possible sources of pollution are certainly not related to inland activities and can only originate from the sea. A large heterogeneity of PAE compounds was observed in the three pools sampled in the Capraia Islands as demonstrated by the confidence ellipse of the PCA analysis (Fig. 46), while no differences in the fingerprint of plasticizers were observed among the three sampling sites studied (Fig. 46).

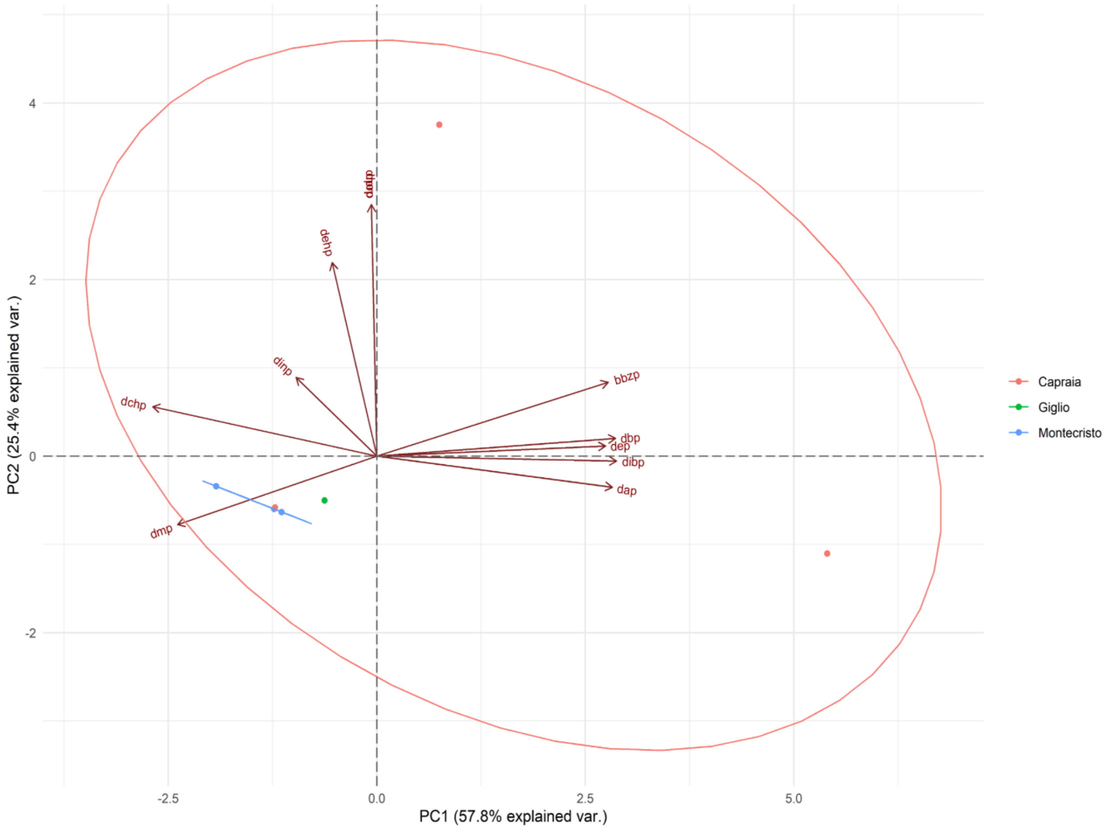


Fig. 46. Principal Component Analysis (PCA) biplot showing the multivariate variation among the 3 sampling sites in terms of PAEs compositions. Driving vectors indicate the direction and strength of each PAE compound considered are shown. The first two principal axes explained 83% of the variance.

DIBP was the most abundant compound throughout the study area, with levels ranging from 162.64 ng/g w.w. to 701.11 ng/g w.w. It is considered a specific plasticizer too volatile to be used in PVC and often combined with other phthalates and used in nitrocellulose, cellulose ether, and polyacrylate and polyacetate dispersions (ECPI, 2009). Relatively high concentrations of this plasticizer have been detected in the waters of the Bay of Marseille and the Rhone River (Paluselli et al., 2018a,b), confirming the hypothesis of intake by mussels during the filtering activity of the waters. In a recent article investigating the presence of several plasticizers in amphipods (*Talitrus saltator, Parhyale plumicornis, Parhyale aquilina, Speziorchestia stephenseni* and *Orchestia montagui*), this compound is reported as one of the most present in these organisms with an average concentration of 0,097 ± 0.076 mg/kg, highlighting the potential impact and bioaccumulation along the food web, being amphipod species the primary consumers in the marine environment (Lo Brutto et al., 2021). DBP, which is mainly used in the manufacture of plastics and paints, cosmetics, medical products, textiles, propellants, food packaging, dental materials and paper (OSHA, 2009), was the second most common plasticizer (174.15 - 512.25 ng/g w.w), followed by DAP (40.87 - 207.31 ng/g w.w). It is mainly used in industry as a monomer in the processing of thermoset plastics, polyester resins and paints (Bingham et al., 2011). Although Bis (2-ethylhexyl)phthalate (DEPH) was reported as one of the most abundant in various environmental matrices (Paluselli et al., 2018, Schimdt et al., 2021) and organisms, including Mediterranean mussels (Rioz-Fuster et al., 2022), its concentration does not exceed 5% of the total PAEs load in this study. Despite their ecological importance, molluscs have received relatively little attention in assessing the effects of plasticizers, including mitotic inhibition, induction of chromosomal aberrations, and effects on larval development (Hermabessiere et al., 2017; Ohelmans et al., 2009).

Mullus surmuletus: plastic ingestion and characterization

Plastic ingestion was investigated in *Mullus surmuletus* to evaluate the possible occurrence of MPs in a fish species inhabiting the coastal waters of the Pelagos Sanctuary. A total of 47 specimens of red striped mullet were collected in collaboration with the artisanal fisheries of the islands of Capraia (n. 25) and Porto Ercole (n. 22) in the southern sector of the Tuscan Archipelago National Park. At the aggregate level, 20% of the total number of fish analysed contained MPs in their gastrointestinal tract. On average, 1.1 items/ all ind. were identified and the majority of plastic particles found had a size between 1 and 2.5 mm, corresponding to the category of large MPs (44 %) (Fig. 47 A). No significant differences were found between the two areas surveyed.

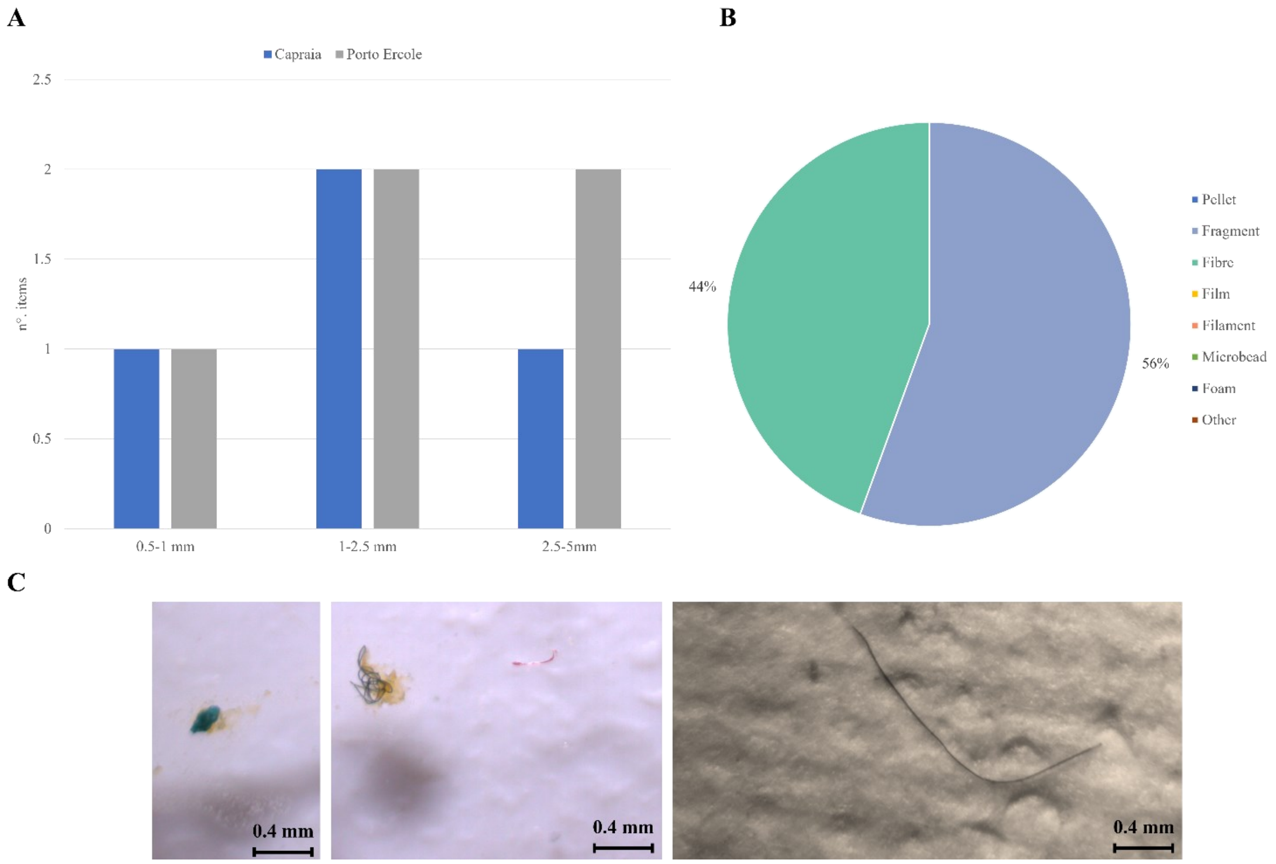


Fig. 47. Number of ingested MPs according to the size classes considered in the two monitored sites (A); total shape characterization of MPs isolated in *Mullus surmuletus* (B); fragments and fibres isolated analysing the GITs of the *Mullus surmuletus* (C).

The results found here are similar to the uptake values previously reported in the Mediterranean Sea. Alomar et al. (2017) reported that 27% of *Mullus surmuletus* sampled in the Balearic Islands had ingested a mean of 0.42 ± 0.04 items/ind.; Güven et al. (2017) showed that 35% of fish sampled in Turkish waters had ingested MPs with a mean of 1.22 ± 0.04 items/ind. In the Adriatic Sea, 70% of the red-striped mullet sampled had ingested MPs, with a mean of 2.7 ± 1.8 items/ind (Anastasopoulou et al., 2018). Similar concentrations were also found in other demersal species inhabiting the study area. Giani et al. (2019) reported an occurrence of MPs (20%) in *Mullus barbatus*, isolating a total of 28 synthetic particles, while in the study conducted by Bellas et al. (2016), the number of red mullets affected by plastic ingestion was 19%. Fragments (56%) and fibres (44%) were the only two shape classes of particles isolated (Fig. 47 B and C) and blue (38%), black (15%) and white/transparent (22%) the most common colours. Light coloured and blue fibres potentially resembling prey have also been identified in other planktivorous fishes (Boerger et al., 2010), suggesting visual confusion between prey and microplastics. In the case of *M. surmuletus*, they could indirectly ingest microplastics if they detect prey in the same way red mullet do with their barbels (Bellas et al., 2016). The synthetic origin of the isolated items was tested using the hot needle technique according to Bellas et al. (2016). Seafloor habitats, as well as coastal sediments, are considered the ultimate sink for plastics in the marine environment (Bellas et al., 2016) and ingested microplastics in marine species can indirectly provide information on microplastic pollution in these environments (Van Sebille et al., 2015). Mistri et al. (2020) previously reported the presence of plastic in several sediment samples collected in the Tuscan Archipelago National Park. Microplastics represented more than 80% of the isolated particles and were mainly composed of filaments and fragments, the same type categories found in the species analysed in this study. However, the low number of MPs isolated from the GITs of fish analysed and the lack of information on plastic particles presence on the feeding ground of this species in the sites monitored, despite the evidence highlighted by the study conducted by Mistri et al. (2020), did not allow to a reliable validation of this demersal species as a potential bioindicator of the benthic environment in the Pelagos Sanctuary.

Mullus surmuletus: PAE levels detection

PAEs detection in *Mullus surmuletu*s was evaluated in a total of 16 specimens sampled in two areas near the island of Capraia and the south of the Tuscan Archipelago National Park, near Porto Ercole (Grosseto). Muscle samples were properly selected according to the data of plastic ingestion performed on the GITs of fish organisms. A total of 8 individuals for each sampling site (n°. 4 that had ingested plastic and n°. 4 not affected by plastic ingestion) were analysed for a total of 16 specimens. Each PAE compound concentration was shown in Tab. 20 according to the sampling site and plastic ingestion.

Tab. 20. PAE concentrations (ng/g) for each compound considered in the *Mullus surmuletus* analysed according to the sampling site and the ingestion of plastic.

Sampling site	**MPs ingestion**	**ng/g w.w.**											
		DMP	**DEP**	**DAP**	**DPrP**	**DIBP**	**DBP**	**BBzP**	**DChP**	**DEHP**	**DINP**	**DNOP**	**∑PAEs**
Capraia	np	8.25	8.97	41.79	<LOD	51.48	45.11	6.24	<LOD	55.83	<LOD	<LOD	**221.80 ± 22.96**
	p	8.71	11.91	50.06	<LOD	47.85	47.20	8.84	<LOD	63.97	<LOD	<LOD	**242.03 ± 24.68**
Porto Ercole	np	<LOD	1.09	4.34	<LOD	16.35	22.85	3.42	<LOD	12.37	<LOD	<LOD	**63.29 ± 7.84**
	p	0.52	3.53	7.66	<LOD	24.07	37.42	2.21	<LOD	15.14	<LOD	<LOD	**93.40 ± 12.83**

All 11 compounds considered were detected with total mean concentrations of PAEs higher in the island of Capraia (231.92 ± 23.77 ng/g w.w.) than in Porto Ercole (78.35 ± 10.01 ng/g w.w.) (Fig. 48 A). Statistical analysis revealed a significant difference between the sampling site considered (Unpaired t-test; $t = 7.3262$, $p = 3.799\ e^{-06}$), while no statistical differences in PAE levels were detected considering the organisms according to the plastic ingestion (Capraia $p = 0.5383$; Porto Ercole $p = 0.3693$). This data was confirmed also by the PCA analysis that revealed a clear separation by sampling sites along the first dimension (Dim. 1), as expected (Fig. 45 B), while any clustering effect was observable due to the ingestion of plastic in the organisms analysed along the second dimension (Dim. 2) (Fig. 48 B).

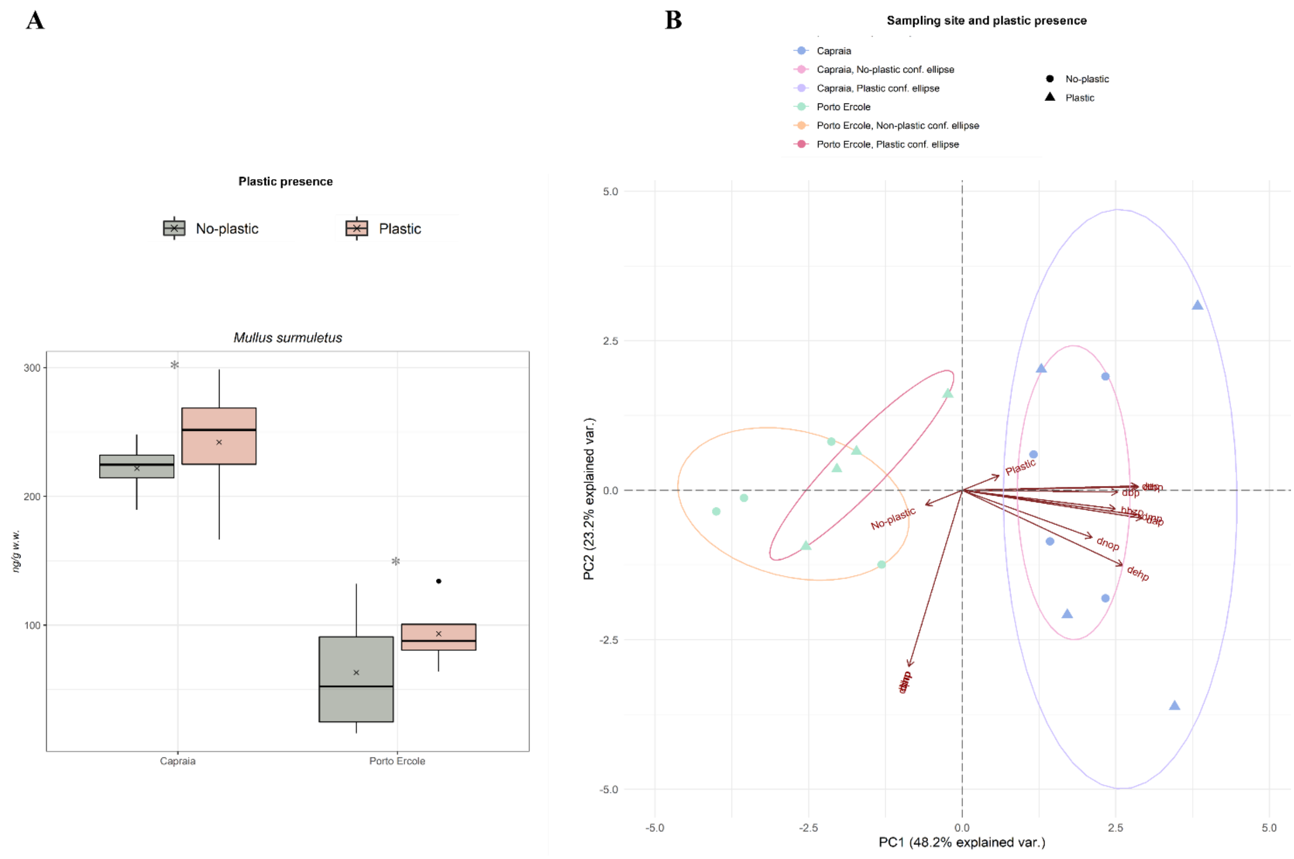


Fig. 48. Boxplots showing the different concentrations of PAEs in the *Mullus surmuletus* specimens analyzed according to the sampling site and the ingestion of plastic (A). The boundaries of the boxes indicate the 25th and 75th percentiles, the whiskers above and below the boxes the 95th and 5th percentiles. The horizontal line inside the boxplots denotes the median value and X the avg. value. Principal Component Analysis (PCA) biplot showing the multivariate variation among the two sampling sites and plastic ingestion in terms of PAEs concentrations (B). Driving vectors indicate the direction and strength of each PAE compounds considered. The first two principal axes explained 72% of the variance. * Indicates the statistical significativity ($p < 0.05$).

Since these substances are largely degraded in the marine environment, the concentrations in fish muscle reported in this study appear to be related to potentially high concentrations in the water column and via the food chain rather than a direct release from ingested particles. PAE congeners such as DIBP, DBP, DMP, DEP and DEHP are readily leached from plastics such as polyethylene bags and PVC cables once dispersed in the marine environment under certain light and bacterial community conditions (Paluselli et al., 2018). Moreover, a significant positive correlation was found between PAE concentration in different fish species and total PAE concentration, especially DBP, in surface water at the corresponding sampling sites, indicating a greater potential to harm aquatic organisms in areas with high PAE pollution (Sun et al., 2021).

DEHP (12.37 - 63.97 ng/g w.w.), DIBP (16.35 - 51.48 ng/g w.w.), DBP (22.85 - 47.20 ng/g w.w.), and DAP (4.34 ± 50.06 ng/g w.w.) were the most abundant plasticizers in fish organisms sampled in both areas (Tab. 20). These data are in agreement with those reported by other studies in Mediterranean fish species such as *Thunnus thynnus* (Guerranti et al., 2016), *Sparus aurata* (Gugliandolo et al., 2020) and *Lepidopus caudatus* (Salvaggio et al., 2019). These results are attributed to the feeding habits, aquatic life, bioavailability, physical and chemical properties of PAEs. In the case of *Mullus surmuletus*, which feeds benthically and has no migratory habits, it can better reflect the PAE content in the water and sediments of the habitat; therefore, it can be used as an indicator of PAE pollution in certain polluted areas. Interestingly, the ecological risk of PAEs in Tunisian seawater calculated by Gugliandolo et al. (2020) highlights DEHP, DiBP, DBP and DEP as the most abundant congeners found in both waters and fish muscles. This is also consistent with previous studies (Zhang et al., 2021) reporting a high risk of DiBP to sensitive fish from the Jiulong River and the East China Sea, respectively. Since 2006, the same phthalates have been listed in the European Commission's category 1B (Annex XIV) as substances of very high concern because they are toxic to reproduction and pose a high risk to fish species.

Myctophum punctatum: plastic ingestion and characterization

The plastic occurrence was investigated in *Myctophum punctatum* to evaluate the possible ingestion of MPs in a poorly investigated fish species inhabiting the pelagic waters of the Pelagos Sanctuary. A total of 13 MPs were isolated from the 21 *Myctophum punctatum* specimens analysed, accounting for a total occurrence of 29%. On average 2.16 items/ind, mainly composed of fragments (46%), fibres (39%) and film (15%) (Fig. 49 A-C) were found in the analysed organisms. The synthetic origin of the isolated items was tested using the hot needle technique according to Bellas et al. (2016). Predatory fish are reported to potentially ingest plastic pieces resembling prey such as salps or siphonophores (Choy and Drazen, 2013). In the case of lanternfishes, they may ingest brightly coloured and black plastic resembling zooplankton species such as copepods, which represent the primary source of energy for these fish (Battaglia et al., 2016; Romeo et al., 2016). According to that, the most common colours of isolated microplastic were white/transparent, blue and black, suggesting potential secondary ingestion throughout contaminated prey.

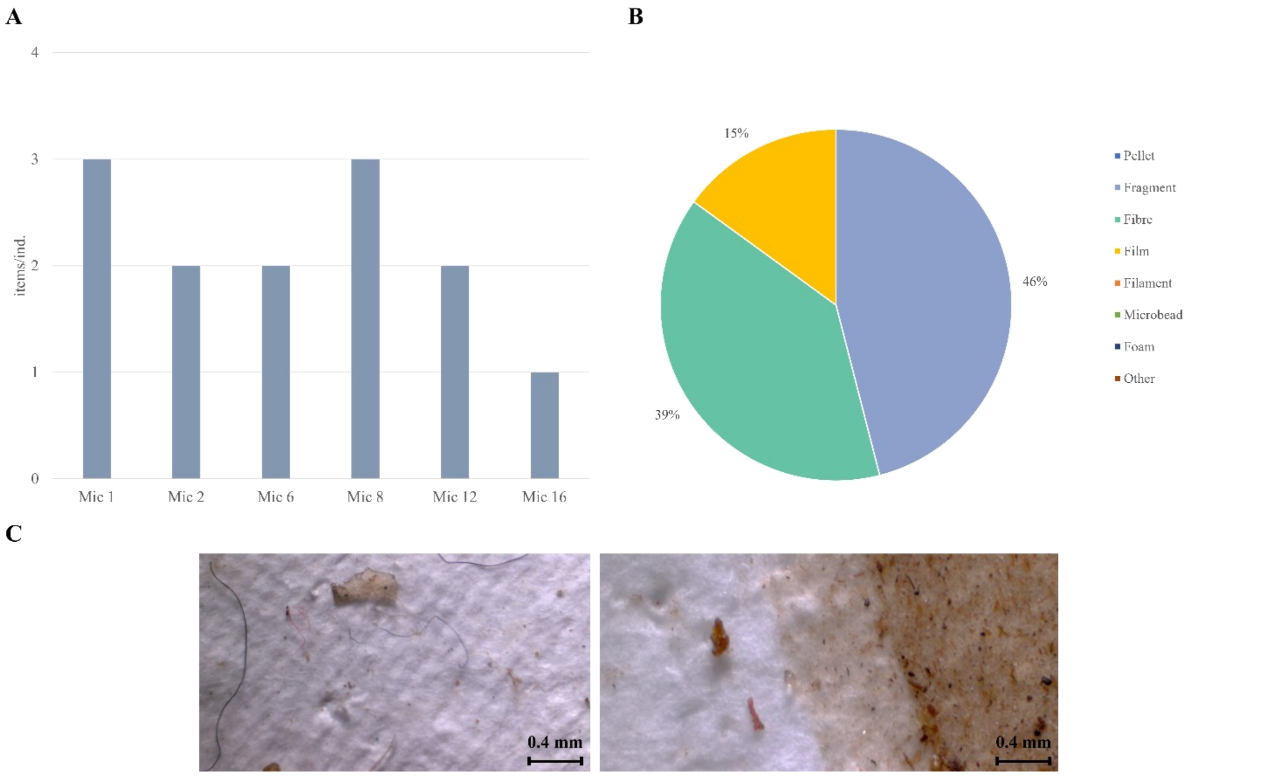


Fig. 49. Number of ingested MPs in each specimen of *Myctophum punctatum* affected by plastic ingestion (A); total shape characterization of MPs isolated (B); Fragments and fibres isolated analysing the GITs of the *Myctophum punctatum* (C).

The only study investigating plastic ingestion by lanternfishes in the Mediterranean Sea refers to a massive stranding of these species on the coast of Sicily (Romeo et al., 2016) between 2010 and 2011. A total of 226 organisms were collected and plastic particles were found in 5.8% of the GITs examined. Ingestion of plastic by mesopelagic fishes has been also previously reported in the Pacific (Boerger et al., 2010; Davison and Asch, 2011; Gassel et al., 2019), Atlantic (Lusher et al., 2015) and Arabian Seas (Jawad et al., 2021). Myctophids are the predominant vertically migratory taxa and account for the largest proportion of fishes in the euphotic zone at night. They have the potential to export significant amounts of organic and inorganic carbon as well as microplastics to the deep sea (Lusher et al., 2015). Moreover, these species are the main food source for several top pelagic predators and an important prey item for Mediterranean bluefin tuna and cetaceans representing a potential MPs bioaccumulation factor along the marine trophic chain (Romeo et al., 2016).

5.3.2 Plastic ingestion and PAE levels in medium and wide-scale bioindicators

Velella velella: plastic ingestion and characterization

Validation of the best digestion method for the extraction and isolation of MPs in *Velella velella* revealed a slightly greater efficiency of 10% KOH digestion protocol compared to 15% H_2O_2 for the extraction of MPs from this species. However, no statistical differences were found between the two treatments used and the parameters considered to evaluate the best digestion method (% DE Wilcoxon test w = 16; p = 0.5476; MC Wilcoxon test w = 15, p = 0.6905; recovery rate Wilcoxon test w = 1, p = 0.1573; recovery rate of size Kruskal Wallis test chi-squared: 1.5889, p = 0.2075; recovery rate polymer type Kruskal Wallis test chi-squared: 1.4689, p = 0.2456). The DE was higher than 98% for both tested methods (Fig. 50 A), confirming the complete digestion of the analyzed samples. Membrane clogging, referring to the total number of filter membranes required for optimal filtration of the total digested organic matter, was slightly lower for the 10% KOH digestion protocol than for the 15% H_2O_2 digestion protocol (mean number of filters 5 and 6, respectively) (Fig. 50 B). A clear difference was measured when evaluating the recovery rate of spiked MPs. On average, 91% of particles were recovered with the potassium hydroxide digestion protocols compared to 82% with the H_2O_2 protocol (Fig. 50 C). This relatively low recovery rate could be partly due to a dense foam that formed after the addition of hydrogen peroxide and hindered the filtration and further processing of the samples, as also confirmed by Avio et al. (2015) and Tsangaris et al. (2021).

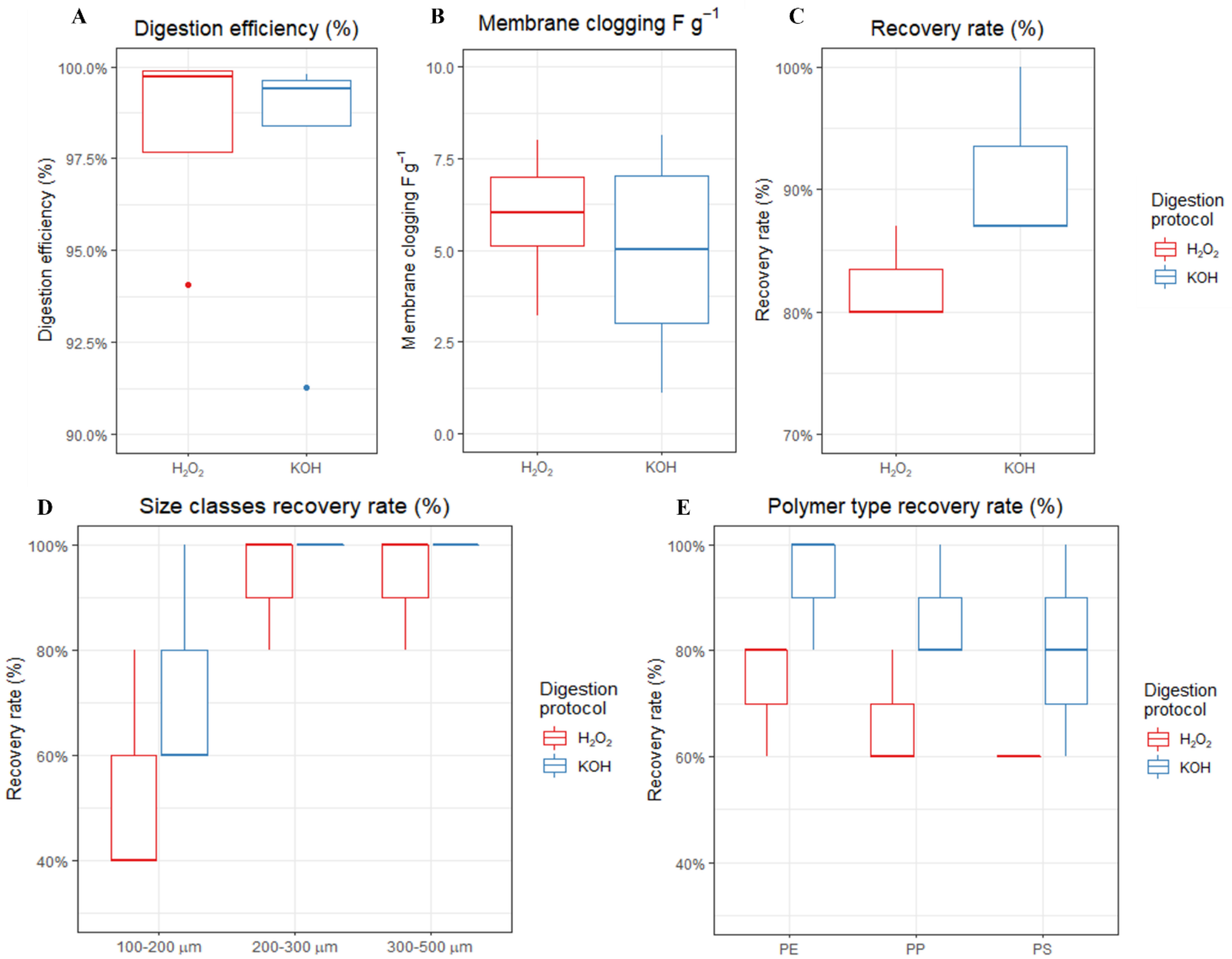


Fig. 50. Protocol selection. Digestion efficiency (%) (A), Membrane clogging (filter/g) (B); plastic particles recovery rate by number (%) (C), size class (%) (D) and polymer type (%) (E) data comparison between the two tested treatments on *Velella velella* pools . The boundaries of the boxes indicate the 25th and 75th percentiles, the whiskers above and below the boxes the 95th and 5th percentiles. The horizontal line inside the boxplots denotes the median value.

A quality assessment of the spiked microplastic was performed considering the recovery rate according to size class and polymer type. For both parameters considered, the digestion protocol based on 10% KOH proved to be the best option. It showed a percentage of 100% in the recovery of particles larger than 200 µm (Fig. 50 D) and more than 80% when considering the polymer type. Some particles have not been chemically analysed due to the FTIR limitation in the polymer detection of particles smaller than 200 µm (Fig. 50 E). A relatively low recovery (80% at 10% KOH) was observed for MPs between 100 µm and 200 µm. Finally, the time factor of the digestion procedures (6 h for 10% KOH and 12 h for 15% H_2O_2) was also considered in the selection of the best method. Considering the differences described above and the relatively low cost of the analytical procedure (Tsangaris et al., 2021), the 10% KOH digestion protocol was selected and used for the digestion of the 53 pools of *Velella velella* studied for the uptake of MPs.

A total of 237 plastic particles were isolated from the hydrozoan organisms analysed, accounting for a total occurrence of 81% (43/53 pools) and 0.71 ± 1.48 items/individual. These data represent the first report of plastic ingestion in this species in the Mediterranean Sea and worldwide. This organism is a free-floating marine hydrozoan, dispersed by currents and winds in the pelagic areas and characterized by annually blooms. In the Ligurian Sea, blooms show an annual periodicity, with a spring peak (Betti et al. 2017; Brian 1923; Issel 1928;). These high biomasses may have important consequences on the entire planktonic trophic web of this basin being an active predator of zooplankton, including fish eggs and juveniles (Purcell et al., 2015). As neustonic organisms, they passively accumulate in response to wind and current patterns and their distribution may coincide with that of floating MPs already driven by these parameters, suggesting potential ingestion of particles mistaken for prey. In cnidarians, the prey capture and feeding consist of a sequence of chemically mediated behaviours comprehending discharge of cnidocytes by compounds usually associated with cell membranes, retraction of tentacles triggered by endogenous compounds to move captured prey to the mouth and ingestion of the prey, promoted by a reversed ciliary beating on the mouth and pharynx (Macali et al., 2018). Due to its specific chemical and physical properties, plastic may be mistaken by nematocysts as food. Laboratory experiments conducted on hard corals showed nematocyst discharge and ingestion of different microplastics, suggesting that the presence of phagostimulants potentially related to toxic compounds found in the polymer materials are promoting chemoreception and subsequent ingestion of particles (Allen et al., 2017). This evidence has been highlighted in the Mediterranean Sea for the jellyfish species *Pelagia noctiluca* in which 4 plastic items have been found inside the gastrovascular cavity by Macali et al. (2018) and 55 debris in fibrous shape (53 MPs and 2 mesoplastics) ranging in size between 0.09 and 9.4 mm were found on stranded organisms along the Messina Straits coast (Albano et al., 2021). Plastic ingestion was already reported for other species such as *Aurelia aurita*, *Physalia physalis*, *Cassiopea xamachana* and *Crambionella orsini* in the Atlantic Ocean (Awuor et al., 2021; Illiff et al., 2020; Tarí Alcazo et al., 2019). MPs particles isolated belonging the 66% to large MPs and 30% to small MPs have been the size classes mostly found (Fig. 51 A). Among the large MPs, 44% of the particles found have a size ranging from 1 to 2.5 mm, reflecting the most abundant categories recovered floating in the sea surface of the Pelagos Sanctuary and confirming the suitability of this species to act as a potential bioindicator of plastic pollution in the pelagic realm of the monitored protected areas. Moreover, the presence of particles smaller than 1 mm, accounting for 33% in total (Fig. 51 A), provide important indications of the availability of MPs for the organisms inhabiting the surveyed area and the associated risk connected with smaller particles, usually found floating in lower concentrations as demonstrated by this study. Only 8 items larger than 5 mm were isolated. Fibres have been the most common type recovered (78%), followed by fragments (17%), and other filaments (4%). Particles of expanded material (0.8%) and plastic films (0.4%) accounted for very small percentages (Fig. 51 B). The higher presence of fibres ingestion was highlighted also by Tarì Alcazo et al. (2019) in organisms sampled near Gran Canaria and Illiff et al. (2020) in Florida.

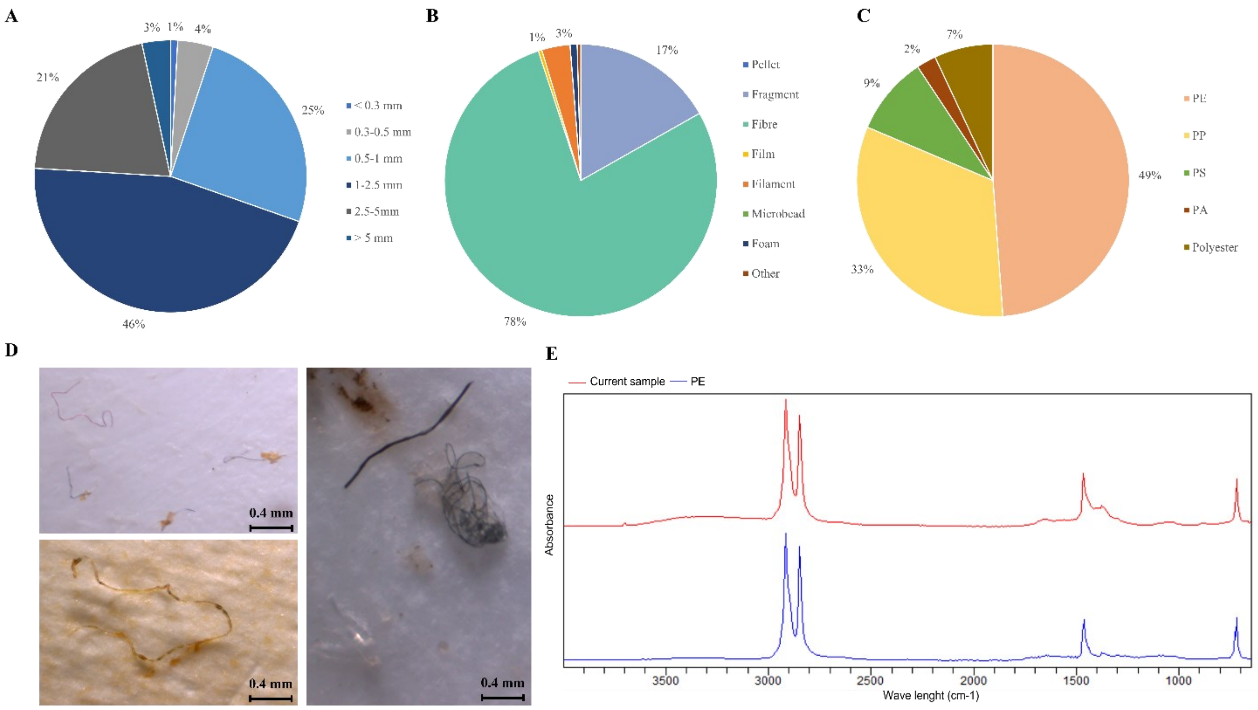


Fig. 51. Characterization by size classes (A), type (B) and polymer composition (C) of MPs isolated from *Velella velella* organisms. Fibres and fragments isolated from organisms analysed (D); PE spectrum obtained through the FTIR analysis (E).

Analysis of plastic polymers was performed on 43 isolated items (Fig. 51 D). Polyethylene (49%), polypropylene (33%) and polystyrene (9%) were the three most frequently found materials. Polyamide (7%) and polyester (2%) were also found (Fig. 51 C and E). Polyolefin plastics, found by this study as the most abundant polymers floating in the surface waters of the monitored areas, may behave like neuston organisms, possibly triggering a mechanical and chemoreceptor response from cnidarians and *Velella velella* in particular, which might consider these items as prey as also suggested by Macali et al. (2018).

Cnidarians such as *Pelagia noctiluca* and *Aurelia aurita* have recently been proposed as bioindicators of plastics in pelagic waters (Macali et al., 2020) and are widely considered model organisms in ecotoxicology (Echols et al., 2015, 2019; Faimali et al., 2014, 2017; Smith et al., 2016). To investigate the role of *Velella velella* as potential bioindicators of plastic ingestion in pelagic areas, the average number of items ingested was compared to the MPs concentration measured in each corresponding manta trawl. Samples isolated from the same manta trawl were considered replicates (Fig. 52 A).

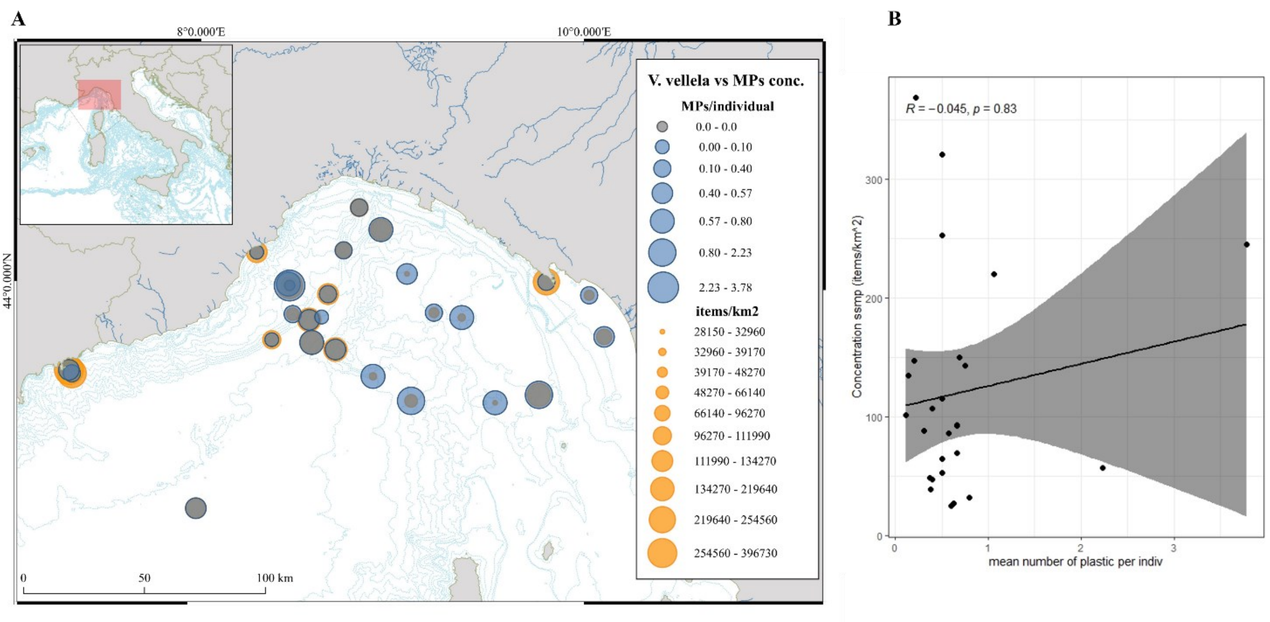


Fig. 52. Spatial analysis (A) and Spearman rank correlation test (B) between the mean number of items per *Velella velella* individual and MPs concentration in the corresponding manta trawl samples.

The Spearman rank correlation test (R = -0.045; p = 0.83) does not prove a relationship between the average number of items per individual and MPs concentration in the manta trawl samples (Fig. 52 B). Further analysis is needed to better investigate the role of this organism as a potential bioindicator of plastic ingestion, although this species has resulted heavily affected by particles ingestion. In addition, its wide spatial distribution in the Pelagos Sanctuary and the relevant role that this species has in the marine trophic chain, being an important food source for pelagic top predators, may contribute to consider *Velella velella* as an important indicator to better address the impact of MPs pollution in this protected area.

Velella velella: PAE levels detetction

PAE levels were detected for the first time in the *Velella velella* organisms sampled in the Pelagos Sanctuary (Tab. 21). All 11 compounds considered were detected in each pool analysed accounting for a total mean of 313.05 ± 65.89 ng/g w.w.

Tab. 21. PAE concentrations (ng/g w.w) for each compound considered in the *Velella velella* pools analysed. The corresponding value of floating MPs (items/km^2) found in the corresponding neustonic sample and the number of items/ind. ingested from organisms isolated from the same pools processed for PAEs detection were also shown.

Pool	ng/g w.w.											items/ind.	items/km^2
	DMP	DEP	DAP	DIBP	DBP	BBzP	DChP	DEHP	DINP	DNOP	∑PAEs		
V.v_1	0.58	5.86	1.97	139.10	157.98	2.06	3.14	61.02	<LOD	16.25	**387.62**	**3.78**	**245,280**
V.v_2	0.06	3.42	1.51	107.78	102.00	0.32	1.77	15.43	<LOD	1.36	**233.30**	**0.1**	**146,879**
V.v_3	0.09	4.96	1.49	101.11	108.11	1.00	1.63	19.24	<LOD	1.53	**238.79**	**0.5**	**143,163**
V.v_4	0.05	4.87	1.72	134.55	139.99	0.99	2.28	15.29	<LOD	2.41	**301.75**	**2.23**	**56,814**
V.v_5	<LOD	4.11	2.56	142.64	149.96	1.26	2.49	46.62	<LOD	5.11	**354.43**	**1.1**	**219,641**

V.v_6	0.21	8.62	2.27	134.98	131.62	0.57	0.01	80.88	<LOD	3.62	**362.39**	**0.36**	**47,249**
Tot. mean	0.13 ± 0.18	5.10 ± 1.76	1.89 ± 0.60	122.78 ±32.52	128.43 ±34.03	1.05 ± 0.67	1.90 ± 1.15	35.95 ±25.58	-	4.27 ± 4.75	**313.05 ± 65.89**	**1.35 ± 1.41**	**143,171 ± 81,174**

As highlighted for the other species, DBP (102.00 - 157.98 ng/g w.w), DIBP (101.11 - 142.64 ng/g w.w), and DEHP (15.29 - 80.88 ng/g w.w) resulted in the three most abundant compounds representing the 95% of the total PAEs load detected. These results are consistent with the plasticizers availability found in waters in the Western Mediterranean Sea, mainly represented by these compounds (Paluselli et al., 2018; 2019; Schimdt et al., 2021). The high presence of polyethylene fragmented particles revealed by this study both in the Pelagos Sanctuary surface waters and among the items ingested by *Velella velella* (49% of the total polymers found) could deeply affect the release both in waters and directly in the organisms once the plastic has been ingested of certain PAEs such as the DIBP and DBP described as the main compounds released from PE materials in the marine environment (Paluselli et al., 2018).

The spatial distribution of *Velella velella* pools investigated for the presence of phthalates was compared to the corresponding values in terms of avg. number of items ingested by these organisms and the MPs concentrations isolated from the corresponding manta trawl from which specimens have been collected to highlight potential correlations among PAEs levels, particles ingested and real on-field plastic abundances (Fig. 53 A-C).

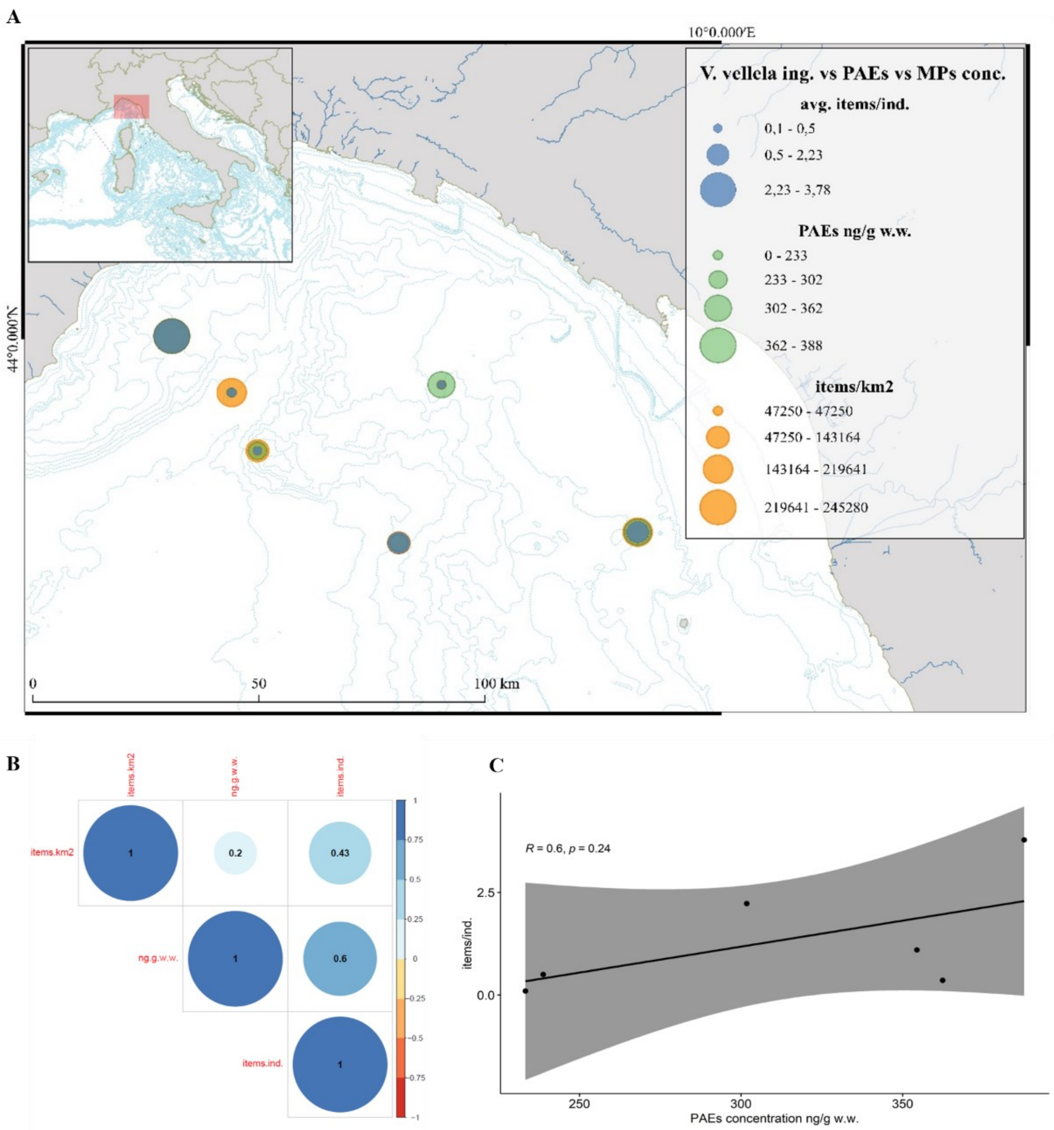


Fig. 53. Spatial distribution of PAEs concentration and mean number of items ingested in *Velella velella* organisms and the avg. concentrations of MPs isolated from the corresponding manta trawl samples (A). Potential correlation among the considered parameters (B). Correlation scatterplot between the number of items/ind. in *Velella velella* and PAEs concentration in the corresponding pools (C).

The results obtained show any correlations among the variables considered as confirmed by the Spearman correlation test applied, even if the number of particles ingested and the total PAE loads detected in the organisms seem to be slightly positively related ($r = 0.60$ $p = 0.24$) (Fig. 53 B and C). The low number of samples considered may have affected this result and further analysis and larger datasets are needed to better investigate the role of the *Velella velella* as bioindicators of plastic pollution in open waters and link the PAEs concentration to a direct release by ingested plastic particles.

Mobula mobular: plastic ingestion

Plastic ingestion was evaluated in a specimen of Giant devil ray (order: Myliobatiformes), stranded alive and subsequently dead in the southern part of the Tuscan coast, in October 2020. The organism was a female, weighing approximately 300 kg and having a wingspan of 3.4 m. A total of 23 plastic particles were isolated from the entire GIT. MPs were the most found items accounting for a total of 87%, mesoplastics were represented by the 13% of the items characterized, while no macroplastics were found (Fig. 54 A). The available information on plastic ingestion in species belonging to the order of myliobatiformes in the Mediterranean Sea are scarce and refer only to the data reported by Anastasopoulou et al. (2013), analysing two specimens of Pelagic stingray (*Pteroplatytrygon violacea*) and assessing the presence of synthetic particles in one of them. In the Atlantic Ocean, the species *Raja asterias* and *Leucoraja naeves* (order: Rajiformes) were reported to be affected by meso and microplastic items, estimating a frequency of occurrence of 43% and 1%, respectively (Lopez-Lopez et al., 2017; Neves et al., 2015).

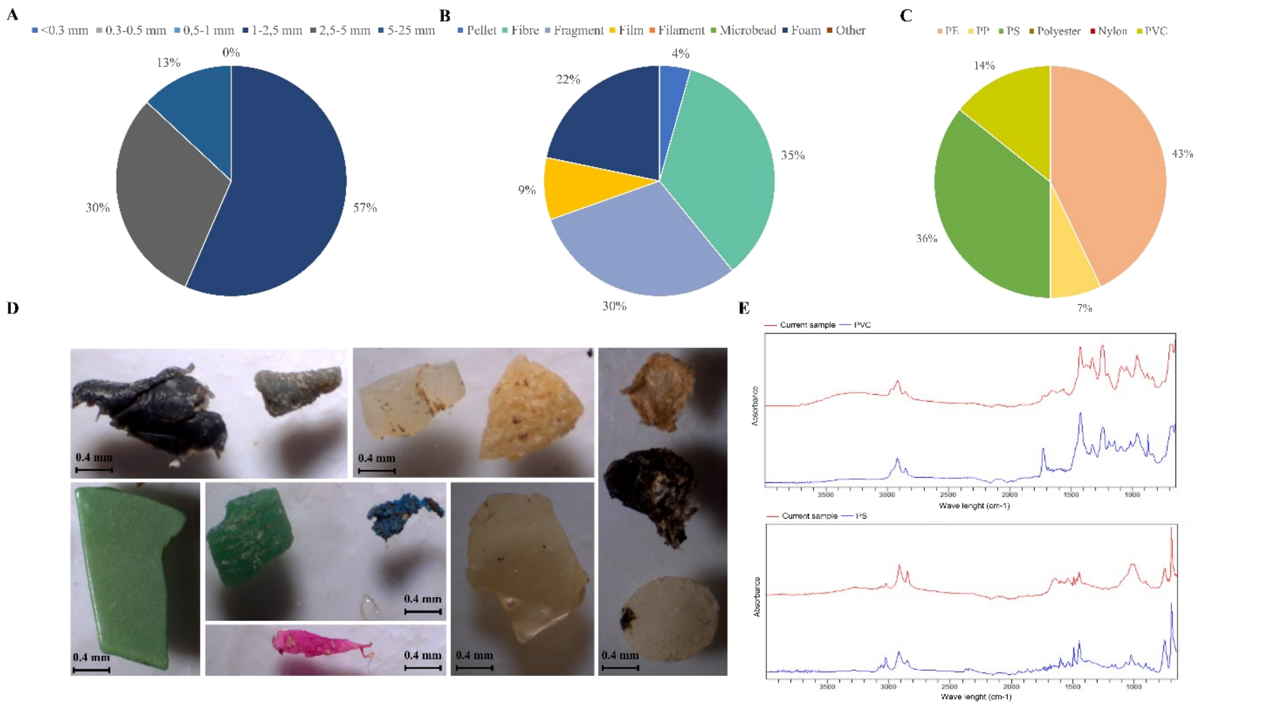


Fig. 54. Characterization by size classes (A), type (B) and polymer composition (C) of MPs isolated from the *Mobula mobular*. MPs isolated from the organism analysed (D); PVC and PS spectra obtained through the FTIR analysis (E).

In the *Mobula mobular*, the prevailing presence of plastic items ranging from 1 mm to 2.5 mm (57%) (Fig. 54 A) may suggest the attitude of this species to ingest floating MPs, being this size classes the most found floating in the waters of the Pelagos Sanctuary and in particular in the Tuscan Archipelago National Park, as demonstrated by this study. Fibres and fragments were the two most common plastic types found representing 35% and 30% of the total items isolated, respectively (Fig. 54 B-D). A relatively high abundance of foamed particles (22%, n. 5 items) was found, while only one primary microplastic, a pellet, was isolated (Fig. 54 B-D). The polymer analysis revealed the exclusive polyolefins composition, PE (43%) and PP (7%) (Fig. 54 C), of fragmented particles analysed according to the main materials used in the productions of hard plastics objects and the most common polymers, found floating in the study areas as reported by this study. The foamed composition of the five particles found was confirmed indicating the PS as the polymer materials (Fig. 54 C and E). Surprisingly, two plastic films made of PVC (Fig. 54 B-E) were identified by the FTIR analysis. This polymer is described to be negatively buoyant plastics, having a density higher than the seawaters (1.5 g cm^{-3}), and generally tends to sink along the water column (Suaria et al., 2016, Zeri et al., 2018). Its presence could be due to the potential ingestion during the stranding in the shallow waters. To the best of our knowledge, these data represent the first report of plastic ingestion by mobulids species in the Mediterranean Sea. The potential risk of plastic ingestion by filter-feeding organisms inhabiting the Pelagos Sanctuary was largely affirmed by Fossi et al. (2012, 2014 and 2016) for the fin whale and basking shark, and may represent a threatening issue also for this species due to the inefficiency of selectively exclude microplastics from zooplankton during its filter-feeding activities. According to that, the study conducted in the Indian ocean by Germanov et al. (2019) confirms the attitude of manta ray organisms to interact with floating synthetic particles estimating a theoretical hourly uptake of MPs ranging from 4.4 pieces h^{-1} to 62.7 pieces h^{-1}. Moreover, several plastic particles were isolated from egested material by *Mobula alfredi*, highlighting the effective capability of this species to ingest plastic particles, even greater than 2.5 mm, but also their ability to expel at least some of what is ingested through regurgitation or passing it in faecal matter (Germanov et al., 2019). The preliminary findings reported by this thesis on plastic ingestion by *Mobula mobular* represent the first data assessment on this charismatic species inhabiting the Pelagos Sanctuary. Data clearly show its capability to ingest plastic due to its filter-feeding behaviour and highlight the potential risks connected with the high MPs accumulation in their feeding ground, as previously reported by this study.

Caretta caretta and *Chelonia mydas*: plastic ingestion and characterization

Of the 21 loggerhead turtles examined, 14 (66%) had ingested plastic litter, while no evidence of the presence of anthropogenic items was found in 7 organisms. A total of 130 plastic particles were isolated, with an avg. abundance of 6.2 ± 12.3 items/ind (Fig. 55). Plastic litter was categorised for the majority as macroplastic (72%) and mesoplastic (9%). MPs were found in only 4 individuals (19%) accounting for 24 isolated items ranging in size from 1.1 mm to 5 mm (Fig. 56 A).

Fig. 55. Plastics isolated from the GITs of *Caretta caretta* and *Chelonia mydas.*

The loggerhead turtle has been widely considered as a target indicator species within the MSFD (D10 C3 indicator) to evaluate the impact of litter in the Mediterranean Sea as confirmed by the several studies evaluating the marine litter ingestion shown in Tab. 22. Moreover, this species was also selected as a candidate indicator by OSPAR in 2016 (Claro, 2016) and it has been identified as a key species to be used for the development of the UN Environment/MAP IMAP Candidate Indicator 24. In the western sector of the Mediterranean Sea (Spanish coast), the frequency of occurrence of litter on sea turtles reported by Domenech et al. (2019) was 78%. In the Tyrrhenian Sea, data collected from 2010 to 2011 by Campani et al. (2013) and from 2011 to 2014 by Matiddi et al. (2017) revealed values of ingestion of litter ranging from 71% to 85%. In the Sardinia sub-region, the occurrence varied between 14% (2008-2012) (Camedda et al., 2014) and 89% (2011-2014) (Matiddi et al., 2017).

Tab. 22. Current status of peer-reviewed papers published on marine litter ingestion in sea turtles in the Mediterranean Sea Sub Regions proposed by MSFD.

Species	Mediterranean Sea sub-region	N°. organisms analyzed	Occ. %	Avg. items ± sd per turtle	References
Carettac caretta	Western Mediterranean Sea	226	41 % (92)	10.17 ± 2.17	Camedda et al.,2022
	Western Mediterranean Sea	155	78% (121)	10.6 ± 16.3	Domenech et al., 2019
	Western Mediterranean Sea	150	85% (120)	16 ± 3	Matiddi et al., 2017
	Western Mediterranean Sea	121	14% (17)	19.58 ± 10.97	Camedda et al.,2014
	Western Mediterranean Sea	31	71% (22)	16.5 ± 29.1	Campani et al., 2013
	Western Mediterranean Sea	19	37% (7)	n.a.	Revelles et al.,2007
	Western Mediterranean Sea	54	76% (43)	2.51 ± 1.75	Tomas et al.,2002
	Ionian and Central Mediterranean Sea	36	72% (26)	7.94 ± 3.85	Digka et al., 2020
	Ionian and Central Mediterranean Sea	79	48% (35)	n.a.	Casale et al., 2008
	Ionian and Central Mediterranean Sea	44	16% (7)	n.a.	Russo et al., 2003
	Ionian and Central Mediterranean Sea	99	6% (6)	n.a.	Gramentz, 1988
	Adriatic sea	28	100%		Di Renzo et al., 2021
	Adriatic sea	45	98% (44)	6 ± 6.09	Biagi et al., 2021
	Adriatic sea	54	35% (19)	4.3 ± 6.6	Lazar and Graĉan., 2011
	Aegean Levantine Sea	22	n.a.	n.a.	Duncan et al., 2019a
	Aegean Levantine Sea	42	5% (2)	n.a.	Kaska et al.,2004
Chelonia mydas	Aegean Levantine Sea	34	n.a.	n.a.	Duncan et al., 2019a
	Aegean Levantine Sea	19	100%	61.8 ± 15.8	Duncan et al., 2019b

Despite the data available in the literature reporting a high uptake of plastic for this species, it shows a high tolerance to the ingestion of anthropogenic waste and the ability to excrete plastic particles (Biagi et al., 2021; Campani et al., 2013; Digka et al., 2020; Nelms et al., 2016). The presence of MPs in faecal samples of this species was recently highlighted by Biagi et al. (2021), where a mean value of 6 ± 6.09 items/ind. was isolated in samples from 44 specimens rescued in the Adriatic Sea. In the southern part of this basin, Di

Renzo et al. (2021) extracted more than 600 particles (0.45 µm to 1 mm) from 28 stranded specimens and Digka et al. (2020) reported that 16% of the total isolated items consisted of MPs.

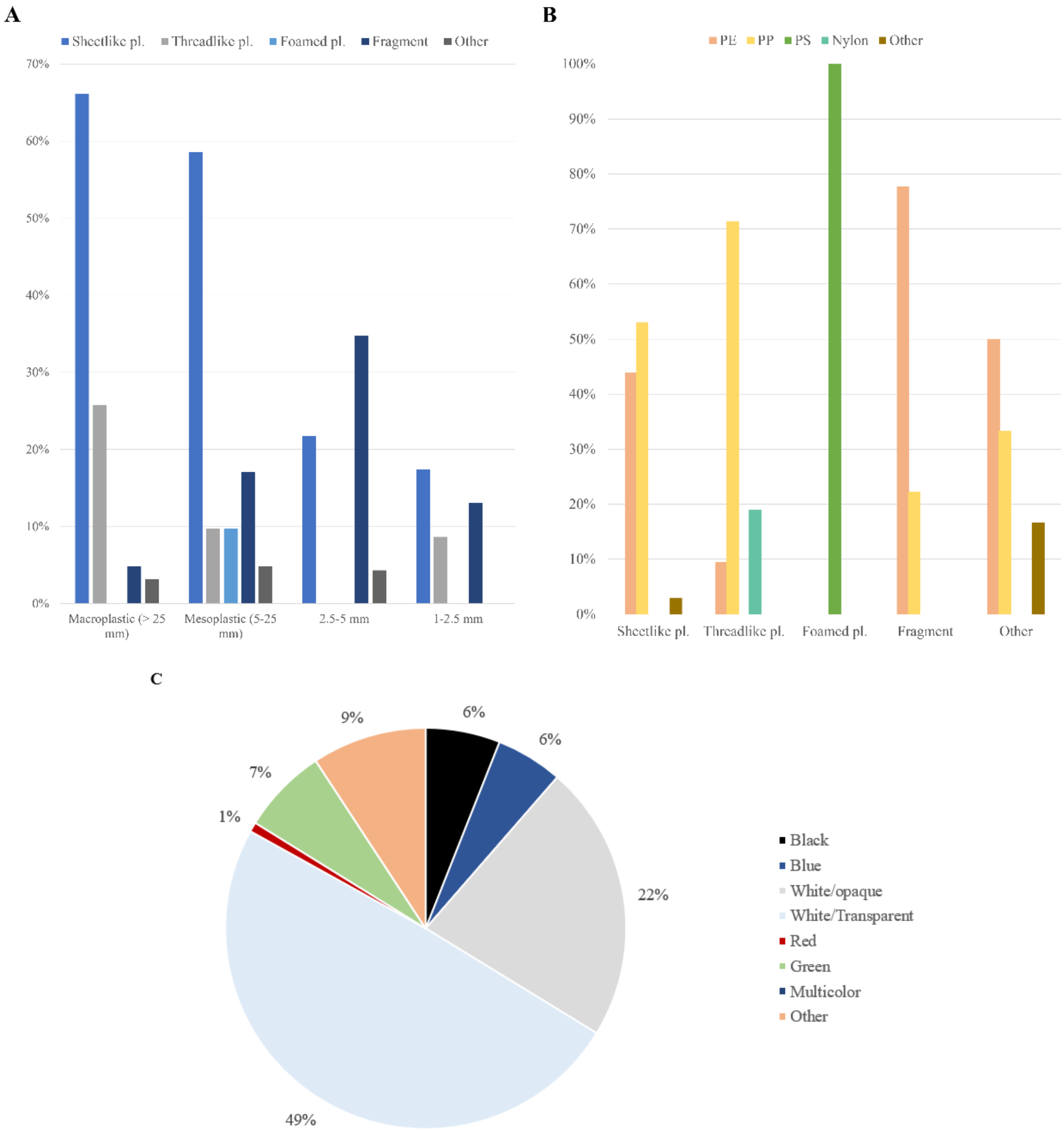


Fig. 56. Characterization of plastic litter isolated from the GITs of the *Caretta caretta* organisms. Plastic-type according to the different size classes considered (A), polymer composition according to plastic-type (B) and plastic colour (C).

Sheet-like plastic was the most abundant category for both macroplastics and mesoplastics (66% and 59%, respectively) (Fig. 56 A). These data confirm what has been observed in other studies on this species (Camedda et al., 2022; Digka et al., 2020; Campani et al., 2013). Plastic sheets or pieces are among the most frequently observed waste objects in the study areas, as confirmed by the macrolitter distribution data, and the predominance of this category in *C. caretta* could likely be explained by the actual high distribution of these items, apart from the fact that sea turtles could mistake them for jellyfish (Schuyler et al., 2012).

Higher concentrations of filaments (26%) found in the larger size classes (> 25 mm) could be mainly related to commercial and recreational fishing activities (Fig. 56 A). Fragments were the second most common category for mesoplastics (17%), while foamed materials made of polystyrene particles accounted for 3% of the total items found (Fig. 56 A).

For items ranging from 1 to 5 mm, the most common plastic types were fragments (48%), followed by films (39%) (Fig. 56 A). These data are consistent with those of Digka et al. (2020) and could be influenced by the high abundance and distribution of these plastic particles on the sea surface of the studied area.

Light-coloured plastics (mainly white and transparent) were the most common colour found (> 70%) (Fig. 56 C) in all size classes considered. These results confirm what has been previously described in other areas of the Mediterranean Sea, where the most frequently recorded colour of plastic was transparent and white (Camedda et al., 2017; Campani et al., 2013; Digka et al., 2020;). Although laboratory analyses have shown that turtles can discriminate between colours when actively selecting prey for ingestion (Schluyer et al., 2012; Swimmer et al., 2005), it is not yet clear whether the common ingestion of bright plastic items could be related to their intentional selection during feeding activities or their high availability in the environment.

Polymer analysis was closely related to the type of plastic isolated from the GITs of *Caretta caretta* organisms. Sheet-like plastics and fragments consist mainly of PP (54%) and PE (66%), respectively (Fig. 56 B). Nylon polymers were found to be one of the main materials of filament items and PS represent the only polymer in the isolated foam particles (Fig. 56 B). Among MPs, PE (61%) and PP (39%) were the only two plastic polymers found according to the most abundance categories (fragments and films).

A total of 3 green sea turtle organisms were analysed. Plastics were found in only one specimen (33%) and a total of 5 macroplastics were isolated. They were classified as white/transparent (80%) and black (20%) sheetlike plastic and consisted of PP (60%) and PE (40%). This species is very rare in the western part of the Mediterranean and the ingestion of transparent soft plastic could be due to the similarity with their natural food, which consists of seagrass or algae (Duncan et al., 2019b). Specimens stranded on the island of Cyprus were found to have an average of 61.8 ± 15.8 items/ind. and an MPs occurrence of 100% (Duncan et al., 2019a, b).

Caretta caretta and *Chelonia mydas*: PAE levels detection

PAE levels were detected in two species of sea turtles stranded on the Tuscan coast. Two different tissues (liver and fat) were analyzed in six specimens of *Caretta caretta* and one *Chelonia mydas*.

Phthalates were detected in all individuals analysed with concentrations ranging from 0.67 ng/g w.w. and 260.57 ng/g w.w. All tested compounds were detected, with five major congeners accounting for more than 80% of the total concentration: DIBP, DEHP, DBP, BBzP and DChP. Tab. 23 summarizes the results obtained.

Tab. 23. PAE concentrations (ng/g w.w.) for each compound considered in sea turtle species (*Caretta caretta* and *Chelonia mydas*) according to the different tissue analysed (fat and liver).

Species	Tissue	ng/g w.w.											
		DMP	**DEP**	**DAP**	**DPrP**	**DIBP**	**DBP**	**BBzP**	**DChP**	**DEHP**	**DINP**	**DNOP**	**∑PAEs**
C.caretta	fat	3.82	75.50	33.68	<LOD	243.03	192.42	33.71	18.17	132.99	6.17	13.13	**752.74 ± 84.14**
	liver	1.34	51.47	10.38	<LOD	117.42	77.23	40.39	3.48	41.30	38.79	0.67	**382.55 ± 37.50**
C.mydas	fat	0.79	31.73	62.36	<LOD	92.60	142.93	85.74	26.07	260.57	2.99	13.12	**718.95 ± 79.38**
	liver	<LOD	15.92	<LOD	4.23	63.07	31.33	71.95	41.93	70.86	2.14	2.63	**304.21 ± 29.64**

No statistical differences were highlighted between the PAEs load in the two species considered due to the low number of samples analysed. However, in accordance with the physical characteristics of this lipophilic family of contaminants, the highest concentrations in this study were detected in the adipose tissue of both species (0.12 - 243.03 ng/g w.w. and 0.06 - 260.57 ng/g w.w.) (Fig. 57).

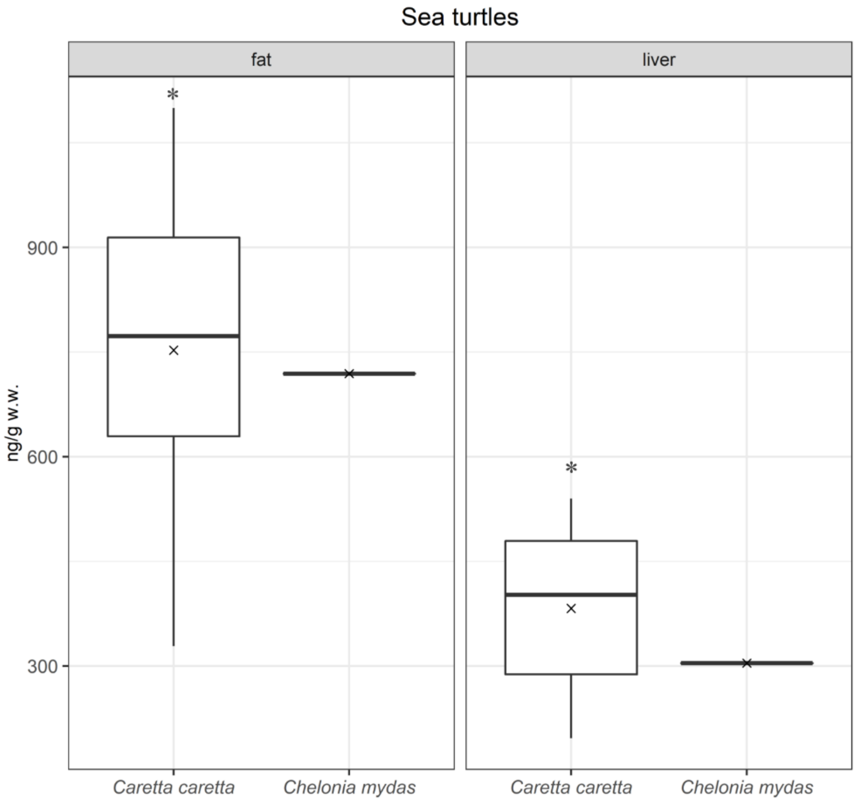


Fig. 57. PAE levels in fat and liver (ng/g w.w.) of stranded sea turtles. * Indicates the statistical significativity ($p < 0.05$). The boundaries of the boxes indicate the 25th and 75th percentiles, the whiskers above and below the boxes the 95th and 5th percentiles. The horizontal line inside the boxplots denotes the median value and X the avg. value.

In the loggerhead turtles, in particular, the paired t-test applied revealed significant differences between the phthalates content detected in the liver (382.55 ± 37.50 ng/g w.w.) and fat (752.74 ± 84.14 ng/g w.w.) ($t = 2.8717$; $df = 5$; $p = 0.03492$), suggesting its use as typical tissue to be monitored for the evaluation of these pollutants. Higher molecular weight phthalate esters such as DEHP, DChP and BBzP, showing high octanol-water partition coefficients (Annexe 16), are very hydrophobic substances described to strongly sorb to organic matter and lipidic tissues. Their presence was already highlighted in fat samples of *Caretta caretta* analysed by Savoca et al., (2018) where a major prevalence of the most lipophilic phthalates such as DEHP and DOTP were reported. Since sea turtles species are particularly affected by plastic ingestion, it is reasonable to suppose that phthalate here detected may come from plastic materials either by direct ingestion of plastics or by exposure to phthalates released in the environment during plastics degradation. Three out of five most abundant compounds are well described to be easily released by plastic in the marine environment especially from plastic bags (Paluselli et al., 2018). Sheet-like plastic represents the most frequent type of litter ingested by sea turtles suggesting a potential threat to marine organisms, not only due to the physical impact but also for the potential chemical release. To date, information on the presence, composition and tissue accumulation of PAEs in Mediterranean turtles is limited to the study by Savoca et al. (2018). The authors reported concentrations significantly higher than those found in this study (total concentration of 4,516 ng/g in fat), examining different tissues from stranded specimens of *Caretta caretta* (total concentration of 22,000 ng/g w.w. in fat) and *Dermochelys coriacea* (total concentration of 36,000 ng/g w.w. in gonads) along the Sicilian coast. Considering the fingerprint of PAEs in the two species monitored, the high molecular weight compounds (DEHP, DChP and BBzP) were detected mainly in *Chelonia mydas* (54% of the total concentration) while relatively low molecular weight plasticizers such as the DiBP and DBP were prevalent in *Caretta caretta* (56% of the total concentration). These differences could be influenced by the different feeding habits of the two species being the *Chelonia mydas* mainly herbivorous than the omnivorous *Caretta caretta*, feeding mainly on jellyfish and reported to be affected by relatively high loads of plasticizers (Savoca et al., 2018). According to the tissue analyzed, DEPH (132.99 ng/g w.w. in the *C. caretta* and 260.57 ng/g w.w. in the *C. mydas*), DIBP (243.03 ng/g w.w. in the *C. caretta* and 92.60 ng/g w.w. in *C. mydas*), and DBP (192.42 ng/g w.w. in *C. caretta* and 142.93 ng/g w.w. in *C. mydas*) were found to be more present in the adipose tissue of both species. These results are in line with those reported by Savoca et al. (2018) where DEHP and DBP are the two most detected congeners. On the contrary, BBzP was detected in a relatively higher concentration in the liver of *Caretta caretta* as confirmed once again by the findings of Savoca et al. (2018) analysing this tissue in the same species and *Dermochelis coriacea*. This congener is reported to be statistically correlated with the environmental distribution of MPs, suggesting a potential release in waters and directly in the tissues of the organism once particles have been ingested (Baini et al., 2017). Reported results showed how the monitoring of phthalates concentration in sea turtles could be chosen as a benchmark for exposure to plastics in the marine environment. The different phthalates distribution among the tissues opens the way to the interpretation of the impact that metabolic pathways of these substances may have on marine organisms, although further studies are needed to better understand the fate of PAEs in sea turtles, their relationship with marine plastic debris, and the potential toxicological effects that these contaminants can cause in these sentinel species.

Calonectris diomedea and *Puffinus yelkouan*: plastic ingestion and characterization

The presence of plastic particles was evaluated in two seabird species *Calonectris diomedea* (n°. 1) and *Puffinus yelkouan* (n°. 2) found stranded along the coast of Tuscany. Plastic particles were found in each specimen analysed, for a total of 328 items isolated, accounting for 97% of MPs and 3% of mesoplastics. Plastic ingestion by seabirds is well-documented in the North Sea where species as the Northern Fulmar has been known as a species that readily ingests marine plastic debris and it is considered an ecological indicator of plastic pollution (Kühn & van Franeker 2020; Van Franeker et al. 2011, 2021; Van Franeker & Law 2015). Despite that, in the Mediterranean Sea, these data represent the second report of plastic ingestion by Mediterranean seabirds, after the published research by Codina-Garcia et al., 2013. The plastic results obtained for each organism are summarized in Tab. 24.

Tab. 24. Age, sex and GITs weight (g) of seabirds species analyzed. The total number of plastic items and the corresponding weight (g) in each specimen are recorded.

Species	Age	Sex	GIT weight (g)	Total n°. plastics	Plastic weight (g)
Calonectris	chick	male	24.8	301	0.04

diomedea					
Puffinus yelkouan	adult	female	12.0	20	0.014
Puffinus yelkouan	adult	male	28.6	7	0.012

The Scopoli's shearwater was the species most affected by plastic ingestion, with a total of 301 pieces isolated from the gastrointestinal tract (Fig. 58 A). The only available data on plastic ingestion in this species relate to 49 individuals accidentally caught by longliners on the Catalan coast, where a frequency of plastic ingestion of 96% and an average number of 15.3 ± 24.4 items/individual were recorded (Codina-Garcia et al., 2013). Chicks of this species are fed at night by both parents for about 90 days before fledging (Magalhães et al., 2008). During the chick-rearing period, Scopoli's shearwater alternate short trips (ca. 1-4 days) over coastal waters to provide food to chicks with long excursions (ca. 10 days) over pelagic waters to replenish their reserves (Dell'Ariccia et al., 2010). Feeding usually on squid and small pelagic fishes, this species undergoes a high probability to directly ingest MPs floating at the sea surface especially on nearshore areas, during feeding activities and secondary ingestion preying fishes where plastic presence has been documented, as in the case of mackerel (Anastasopoulou et al. 2018; Deudero 1998; Deudero and Alomar 2015; Güven et al., 2017). Breeding adults may lose part of their plastic burden by feeding it to chicks which may explain the high number of plastic pieces found in the young specimens studied, as suggested by Van Franeker et al. (2011) for Dutch fulmar.

Fig. 58. MPs isolated from GITs of *Calionectris diomedea* (A) and *Puffinus yelkouan* (B and C).

A total of 20 and 7 pieces of plastic were isolated from the female and male organisms of Yelkouan shearwaters, respectively (Fig. 58 B and C). Plastic ingestion by this species was previously documented by Codina-Garcia et al. (2013) in 31 individuals found dead in the western Mediterranean Sea, who reported a

plastic ingestion rate of 71% and an average number of 7.0 ± 7.9 items/ individual. Characterised by conspicuous nearshore habits, partial migration, unsuspected diving abilities (often > 10 m), and a broad diet ranging from zooplankton to small pelagic fishes such as European anchovy and pilchard (Péron et al., 2013), this species may also be threatened by plastic ingestion as it feeds in the water column and at the water surface where plastic accumulates in large quantities (Baini et al., 2018; Suaria et al., 2016).
Relating our data to the threshold established by Van Franeker et al. (2021) for fulmars (0.1 g of plastic particles in the stomach), the weight of plastic isolated from the GITs of the species studied does not exceed the 40 mg found in Scopoli's shearwaters, which corresponds to 301 particles (Tab. 22). However, our results are below the above threshold, the lack of data on plastic ingestion in seabirds in the Mediterranean Sea and specimen collection makes it difficult to understand the real impact of plastic pollution on these species.
Plastic characterization revealed a high presence of SMPs in Scopoli's shearwaters (more than 60%), while particles larger than 1 mm up to 25 mm were found mainly in the Yelkouan shearwaters (80% on average) (Fig. 59 A). The same result was highlighted by Codina-garcia et al. (2013), where high frequencies of smaller MPs were isolated from the GITs of specimens of this species. This difference could be due to the different feeding behaviour and area of the shearwater species considered; moreover, seabirds are capable to decompose MPs through stomach acids and excrete plastic items approximately one month after the ingestion, depending on the plastic-type (Provencher, 2010; van Franeker et al., 2011), and a potential reduction in size could be a consequence of this process.

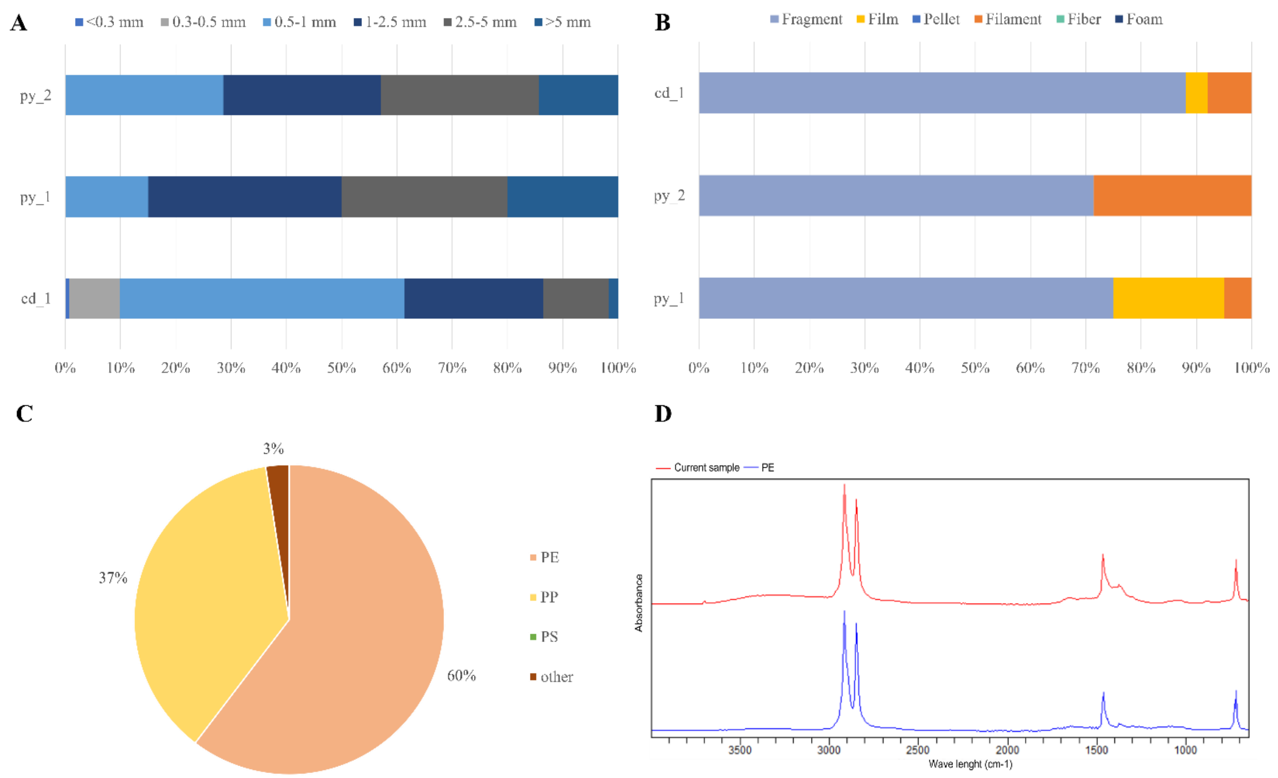


Fig. 59. Characterization by size classes (A), type (B) and polymer composition (C) of MPs isolated from sea birds organisms. Py: *Puffinus yelkouan* and Cd: *Calionectris diomedea*. FTIR spectra of PE, the most common polymer found (D).

Fragment (71 – 88%), filament (5 – 29%) and film (4 – 20%) have resulted in the categories of plastic most commonly ingested in the two species of seabirds (59 B). Plastic polymers analysis confirm their synthetic origin mainly made of polyolefins materials (PE and PP > 95%) (Fig. 59 C and D). These data reflect the higher abundance of these plastic categories on the sea surface and in the main preys of these Procellariiformes species (Baini et al., 2018; Collard et al. 2017; Compa et al. 2018; Fossi et al., 2017; Lefebvre 2019; Renzi et al. 2019) suggesting a potential plastic transfer along the marine trophic chain. Although previous data or long time monitoring studies within the Mediterranean Sea on plastic ingestion by seabirds are lacking, this result suggests an urgent need to assess the impacts and effects on their health, particularly in the case of threatened species such as the Scopoli's and Yelkouan shearwaters. In this regard, these species could be used as bioindicator species of the trends in plastic contamination at sea, despite the difficulties in their collection throughout the Mediterranean basin.

Cetaceans species: plastic ingestion and PAEs detection

Plastic ingestion was evaluated in four cetacean species (n°. 18 striped dolphins, n°. 9 bottlenose dolphins, n°. 1 Cuvier's beaked whale) stranded on the coast of Tuscany in the Pelagos Sanctuary, as well as in a fin whale found dead in Sorrento in winter 2021. A total of 449 plastic items were isolated from the GIT contents of all organisms, most of which were LMPs (63%) and SMPs (31%). Only 2 items were found that were larger than 25 mm and ranged in size from 37 to 39 mm. These were a piece of thread, which was probably a fishing line, and a fragment of sheetlike plastic. Among the delphinid species, the highest average number of MPs was isolated from GIT of *Ziphius cavirostris* (59 items/ind.), followed by *Tursiops truncatus* (29.4 ± 16.9 items/ind.) and *Stenella coeruleoalba* (5 ± 6 items/ind.) (Fig. 60).

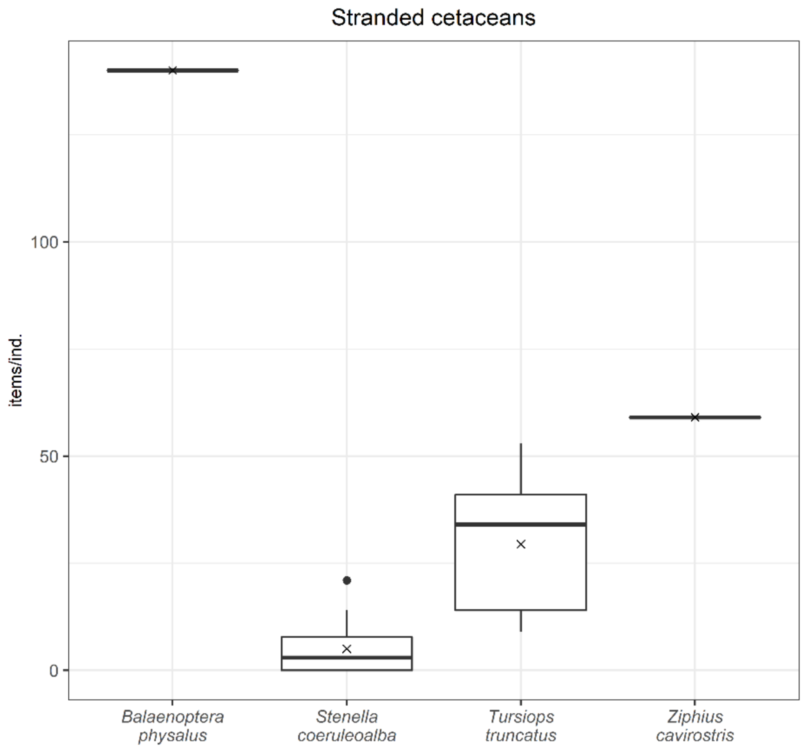


Fig. 60. Number of plastics isolated from the GITs of the cetacean species analysed. The boundaries of the boxes indicate the 25th and 75th percentiles, the whiskers above and below the boxes the 95th and 5th percentiles. The horizontal line inside the boxplots denotes the median value and X the avg. value.

LMPs ranging from 1 mm to 2.5 mm were the most abundant size classes in the species *Tursiops truncatus* (45%) and *Stenella coeruleoalba* (38%) (Fig. 62 A). Fibres and filaments were the two most common categories in the bottlenose dolphin (83% and 8%, respectively), while fragments (23%) were the second most common type of plastic in the striped dolphin (Figs. 61 and 62 B). In *Ziphius cavirostris*, the majority of MPs particles were less than 1 mm in size (44%) (Figs. 61 and 62 A) and consisted mainly of fragments (69%) and films (19%) (Figs. 61 and 62 B). Polymer analysis revealed that nylon and PET (7% and 5% of the total) (Fig. 62 C and D) were present only in the particles isolated from the bottlenose dolphin. These polymers mainly accounted for the isolated filament particles could be due to the interaction of this species with fishing activities, as well described in the Pelagos Sanctuary (Pennino et al., 2017). In striped dolphin and Cuvier's beaked whale, polyolefin polymers were the most frequently detected materials, mainly due to the higher presence of fragments and films. Fibre particles were not chemically analysed due to the insufficient spectral data gained by the FTIR instrument used.
Despite some recent scientific publications, information on the ingestion of MPs by dolphins in the Mediterranean Sea is scarce, not specifically focused on the characteristics of the items found (e.g. type, colour, chemical composition and possible sources) and limited to their frequency of occurrence. Corazzola et al. (2021), who analysed various organisms stranded on the Ligurian coast in the Pelagos Sanctuary, report a number of MPs ranging from 14 items in the striped dolphin to 59 items in the bottlenose dolphin (Tab. 18). Similarly, Novillo et al. (2020) report an average number of 14.9 ± 22.3 items/ind. isolated from the GITs of 43 stranded specimens on the Spanish coast (Tab. 25). The presence of macroplastics, especially sheetlike items, was reported in cuvier's beaked whale, striped dolphin and bottlenose dolphin in the studies by Duras et al. (2021) and Alexiadou et al. (2019). Odontocetes, which exhibit an active feeding linked to a highly developed echolocation system used for predation and orientation (Walker and Coe, 1990), could be mainly affected by secondary ingestion of plastic when they consume contaminated prey, as reported in the available literature (Bellas et al., 2016; Compa et al., 2018; Romeo et al., 2016).

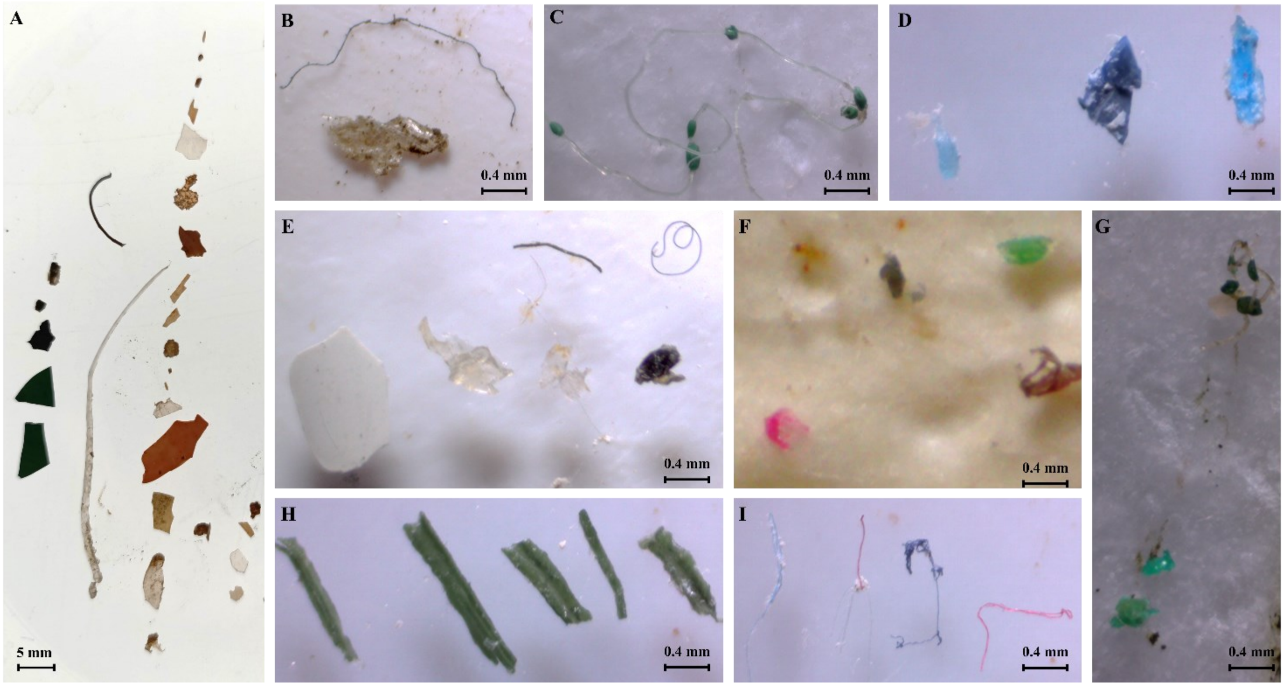


Fig. 61. MPs isolated from GITs of *Balaenoptera physalus* (A and B), *Tursiops truncatus* (C, D, E, H, I), *Stenella coeruleoalba* (F) and *Ziphius cavirostris* (G).

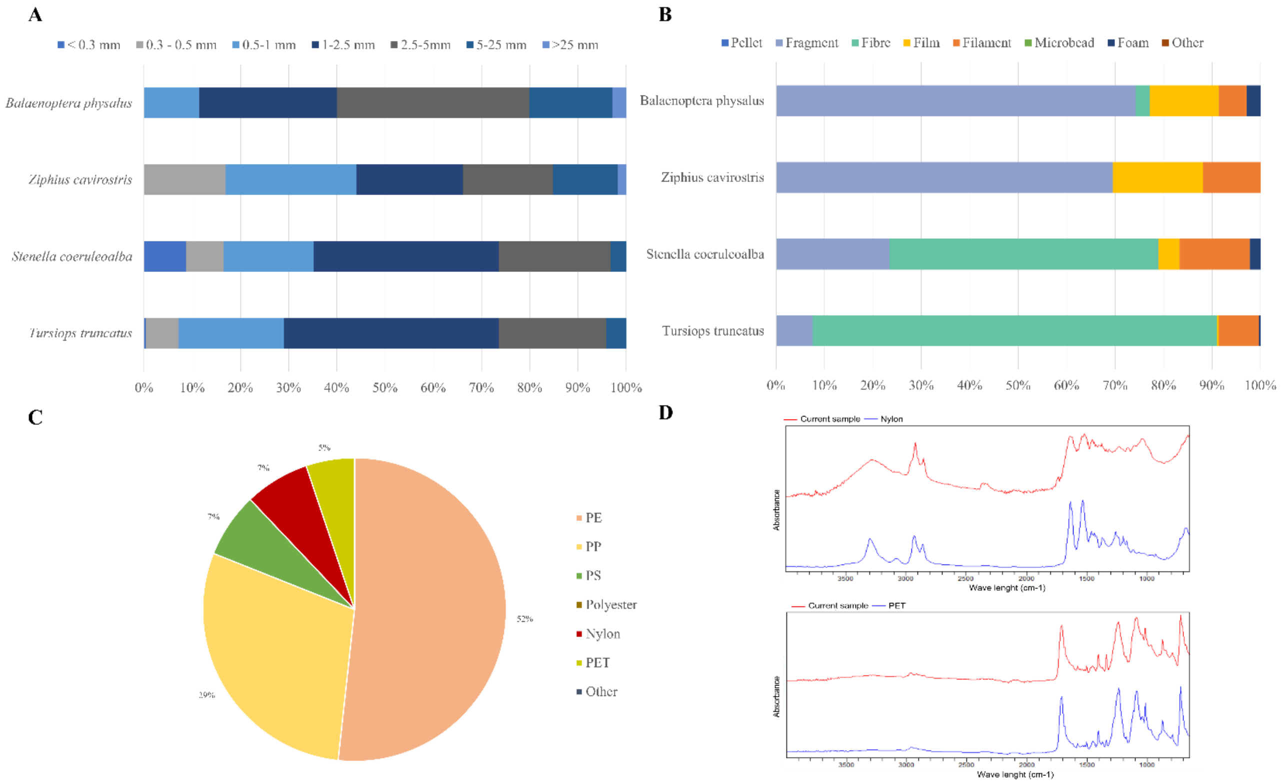


Fig. 62. Characterization of plastic litter isolated from the GITs of the cetacean species analysed according to the different size classes considered (A), plastic-type (B), polymer composition (C) and Nylon and PET FTIR spectrum (D).

In addition, differential feeding behaviour and spatial distribution could be other factors influencing their susceptibility to the impact of plastic litter. *Ziphius cavirostris*, for which deep-diving behaviour and suction mode of feeding pattern has been described, seems to be more subjected to plastic consumption (Poeta et al., 2017; Puig-Lozano et al., 2018) than the other two species considered. *Stenella coeruleoalba* and *Tursiops truncatus* prefer a raptorial feeding pattern (Werth, 2006), which could reduce the likelihood of plastic ingestion (Alexiadou et al., 2019). In addition, species inhabiting areas where litter accumulates, such as coastal areas and the seafloor, might be more prone to ingest plastic items, as confirmed by the highest number of particles found in the bottlenose dolphin and Cuvier's beaked whale, respectively, in this study and also highlighted by Puig-Lozano et al. (2018) and Alexiadou et al. (2019).

Tab. 25. Current status of peer-reviewed papers published on marine litter ingestion in cetaceans in the Mediterranean Sea Sub Region proposed by MSFD.

Species	Mediterranean Sea sub-region	Year of Study	*n*°. specimens	Occurrence %	Total n°. items	Avg. items/ind. ± sd	Plastic size	References
Physeter macrocephalus	Western Mediterranean Sea	2009-2016	13	77% (10)	n.a.	n.a.	> 25 mm	Panti et al., 2019
	Western Mediterranean Sea	2012	1	100%	59	n.a.	> 25 mm	de Stephanis *et al.*,2013
	Western Mediterranean Sea	1989	1	100%	n.a.	n.a.	> 25 mm	Viale *et al.,* 1992
	Aegean Levantine Sea	1993-2014	10	60% (6)	155	15.2 ± 42.2	> 25 mm	Alexiadou et al., 2019
	Aegean Levantine Sea	2006	8	50% (4)	n.a.	n.a.	> 25 mm	Notarbartolo-di-Sciara *et al.,* 2012
	Aegean Levantin Sea	2003	1	100%	1	n.a.	> 25 mm	Roberts 2003
	Adriatic Sea (Italy)	2014	3	100%	n.a.	n.a.	> 25 mm	Podestà *et al.*,2015
	Adriatic Sea (Italy)	2009	6	100%	n.a.	n.a.	> 25 mm	Mazzariol *et al*., 2011
	Adriatic Sea (Italy)	1981-1985	1	100%	n.a.	n.a.	> 25 mm	Cagnolaro *et al.*,1986
Globicephala melas	Western Mediterranean Sea	2019	1	100%	41	n.a.	< 5 mm	Corazzola et al., 2021
Ziphius cavirostris	Western Mediterranean Sea	2017	1	100%	59	n.a.	< 5 mm	Corazzola et al., 2021
	Adriatic Sea	1981-1985	1	100%	n.a.	n.a.	> 25 mm	Cagnolaro *et al.*,1986
	Aegean Levantine Sea	1990-2019	4	25% (1)	n.a.	n.a.	> 25 mm	Duras et al., 2021
	Aegean Levantine Sea	1993-2014	5	20% (1)	n.a.	n.a.	> 25 mm	Alexiadou et al., 2019
Grampus griseus	Ionian and Central Mediterranean Sea	1993-2014	5	20% (1)	n.a.	2.6 ± 5.8	> 25 mm	Alexiadou et al., 2019
	Aegean Levantine Sea	1993-1999	1	100%	1	n.a.	> 25 mm	Shoham-Frieder *et al.*,2002
Tursiops truncatus	Western Mediterranean Sea	2019	2	100%	59	n.a.	< 5 mm	Corazzola et al., 2021
	Ionian and Central Mediterranean Sea	1993-2014	4	0%	n.a.	n.a.	no items	Alexiadou et al., 2019
	Aegean Sea	1990-2019	253	1% (2)	n.a.	n.a.	> 25 mm	Duras et al., 2021
	Aegean Levantine Sea	2007	1	100%	n.a.	n.a.	> 25 mm	Levy *et al.*,2009
Stenella coeruleoalba	Western Mediterranean Sea	1988-2017	43	90.5% (40)	672	14.9 ± 22.3	< 5 mm	Novillo et al., 2020

	Western Mediterranean Sea	2019	1	100%	14	n.a.	< 5 mm	Corazzola et al., 2021
	Adriatic Sea	1998	1	100%	n.a.	n.a.	> 25 mm	Pribanic et al., 1999
	Ionian and Central Mediterranean Sea	1993-2014	4	0%	n.a.	n.a.	no items	Alexiadou et al., 2019
	Aegean Levantine Sea	1990-2019	33	3% (1)	n.a.	n.a.	> 25 mm	Duras et al., 2021
Delphinus delphis	Ionian and Central Mediterranean Sea	1993-2014	2	0%	n.a.	n.a.	no items	Alexiadou et al., 2019
Phocoena phocoena	Ionian and Central Mediterranean Sea	1993-2014	5	20% (1)	n.a.	0.2 ± 0.4	> 25 mm	Alexiadou et al., 2019
	Black Sea	2002-2003	43	11.63% (5)	n.a.	n.a.	> 25 mm	Tonay *et al.,*2007

In the analysed part of the intestinal tract of *Balaenoptera physalus*, about 30 m, a total of 35 plastic particles were isolated (Figs. 60 and 61). Cetaceans have an intestinal length that is approximately 5.5 times their body length (Slijper, 1979), considering that the estimated intestinal length of the studied fin whale accounts for approximately 110 m. Since 27% of this length was sampled, there was probably three to four times as much plastic in the entire organism than the total number of items found in the tract analysed. This would lead to an estimate of up to 140 small plastic particles. These were mainly LMPs (68%) between 1 mm and 5 mm, belonging in particular to the size classes ranging from 2.5 mm to 5 mm (Fig. 62 A). Fragments, films, and filaments were the most abundant plastic types (74%, 14%, and 6%, respectively) (Fig. 62 and B). Only one foamed particle (polystyrene) was isolated and a lower presence of fibres was observed. Polymer analysis confirmed PE and PP as the most common material (Fig. 62 C) even among the particles isolated from this species, accounting for 96%. These data represent the first assessment of direct ingestion of plastics by fin whales in the Mediterranean Sea, where they are considered sentinel species for exposure to MPs potentially assumed directly from the water column and via prey (Fossi et al., 2012, 2014, 2016, 2020). To date, few studies have addressed the ingestion of synthetic particles by mysticetes. Fossi et al. (2014) calculated the potential amount of microplastics ingested by fin whales in the Mediterranean Sea based on the concentration of microplastics in the waters in which they fed computing an ingestion rate of 3,653 microplastics/day. A different approach was described by Garcia-Garin et al. (2021), who examined the number of plastics ingested by krill organisms (*Meganyctiphanes norvegica*) from the stomach contents of 25 fin whales in western Iceland. The average MPs concentration in krill was 0.057 items/g of samples, suggesting daily ingestion of 38,646 to 77,292 particles by the whales. Similarly, Desforges et al. (2015) calculated the ingestion of microplastics by humpback whales (*Megaptera novaeangliae*) off the coast of British Columbia and based their estimates on the potentially ingested krill (*Euphasia pacifica*) collected from water samples. The authors indirectly estimated much higher ingestion of microplastics (over 300,000 items/day) than fin whales. Finally, Besseling et al. (2015) analysed the stomach contents of a stranded humpback whale in the Netherlands. They found a total of 16 microplastics in samples from a gastrointestinal tract that was only 5-10% of the total length, leading them to estimate a total of 160 microplastics in the entire tract.

While the presence of macroplastic ingestion in cetaceans is well understood both in the Mediterranean (Tab. 25) and globally (Baulch and Perry, 2014), microplastic ingestion in these species, particularly mysticetes, remains poorly studied due to difficulties in sampling and analysis and lack of standardisation methods (Zantis et al., 2021). Most of the ingested particles may be excreted in the faeces, and the effective rates of ingestion and excretion remain unknown. Despite that, the large number of synthetic particles in the feeding ground of marine mammals, secondary ingestion by contaminated prey and the potential release and accumulation of contaminants from ingested plastic poses a serious threat to these organisms. Promoting these types of studies and harmonising quantification systems to allow more accurate intra- and interspecific comparisons among cetacean species should be a priority for the future to better assess the impacts of plastic debris and the consequences for these sentinel species, strengthening their potential role as plastic bioindicators at a wide-scale (Fossi et al., 2020).

Stranded organisms: PAE levels detection

PAE levels were detected in four cetacean species stranded on the Tuscan coast. Two different tissues (liver and blubber) were analysed in one specimen of *Balaenoptera physalus*, 14 individuals of *Stenella coeruleoalba*, 2 individuals of *Tursiops truncatus* and one *Ziphius cavirostris*. Phthalates were detected in all species analysed at concentrations ranging from 0.34 ng/g w.w. and 463.10 ng/g w.w (Tab. 26). The highest concentrations were found in the bottlenose dolphin in both tissues examined: 1,032.24 ± 158.86 ng/g w.w. in blubber and 356.68 ± 45.51 ng/g w.w. in liver.

Tab. 26. PAE avg. concentrations (ng/g w.w.) for each compound considered in cetacean species (B.p.: *Balaenoptera physalus;* S.c.: *Stenella coeruleoalba;* T.t.: *Tursiops truncatus*; Z.c.: *Ziphius cavirostris*) according to the different tissue analysed (blubber and liver).

Species	Tissue	ng/g w.w.											
		DMP	**DEP**	**DAP**	**DPrP**	**DIBP**	**DBP**	**BBzP**	**DChP**	**DEHP**	**DINP**	**DNOP**	**∑PAEs**
B.p.	blubber	0.39	13.24	2.45	<LOD	63.30	39.79	10.74	18.07	75.16	2.49	0.50	**226.18 ± 26.87**
	liver	2.45	26.14	25.87	<LOD	70.91	46.00	<LOD	<LOD	38.83	33.67	0.55	**245.58 ± 23.79**
S.c.	blubber	3.12	22.19	12.72	0.80	88.68	154.28	9.16	22.81	77.49	5.86	2.25	**399.34 ± 49.49**
	liver	0.50	14.23	6.12	0.41	40.48	33.22	20.32	21.19	40.17	5.38	3.40	**185.42 ± 15.4**
T.t.	blubber	0.34	25.93	<LOD	2.48	40.19	347.77	463.10	27.33	101.37	22.38	1.08	**1032.24 ± 158.86**
	liver	<LOD	21.62	1.11	1.01	73.37	15.43	8.40	135.34	87.46	12.06	0.56	**356.68 ± 45.51**
Z.c	blubber	<LOD	6.17	8.64	1.10	106.55	77.28	7.96	1.37	146.79	6.87	1.37	**364.24 ± 52**
	liver	0.39	13.81	42.99	4.08	18.94	21.13	21.41	33.97	160.64	8.81	0.47	**326.64 ± 45.45**

Any statistical differences were found in the total PAEs load detected among species considering liver and fat respectively (Kruskal Wallis test, chi-squared = 6.1228, p = 0.1058 in the liver and chi-squared = 2.7043, p = 0.4393 in blubber). Nevertheless, higher concentrations of PAEs were detected in the blubber samples of all specimens, except for the fin whale, where a slightly higher concentration in liver tissue was found (Fig. 63 A). Low molecular weight phthalates (DIBP, DBP and DEP) have been detected mainly in the blubber samples, with high concentrations of BBzP in the *Tursiops truncatus* (463.10 ng/g w.w.) (Fig. 63 B). On the contrary in liver tissue, DEHP and DcHP showed higher abundances ranging from 21.19 ng/g w.w to 160.64 ng/g w.w (Fig. 63 B). Potential differences in the accumulation of phthalates between the tissues studied were also evaluated considering each species analysed (Kruskal Wallis test, chi-squared = 13.009, p = 0.00031). Post-hoc analysis revealed statistically significant differences only for the phthalate concentrations detected in the blubber and liver samples of *Stenella coeruleoalba* (Wilcoxon Signed Rank Exact Test, V = 103, p = 0.0003662) (Fig. 63 A). Although the PAE levels found in *Tursiops truncatus* may indicate significant differences in the accumulation of these chemicals in target tissues, the small number of samples analysed does not allow for reliable statistical analysis.

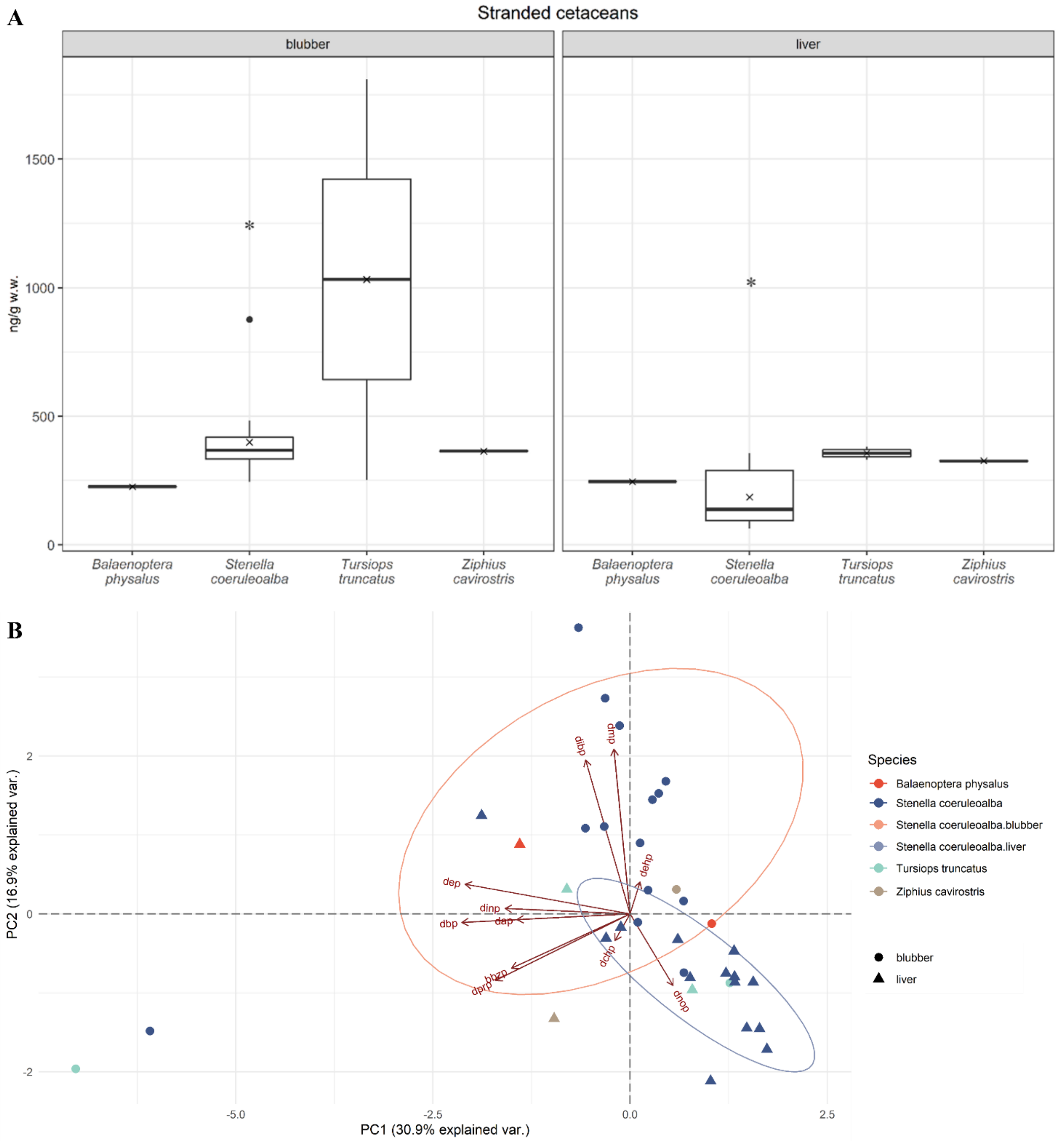


Fig. 63. PAE concentrations in the different tissues analysed among the species considered (A). The boundaries of the boxes indicate the 25th and 75th percentiles, the whiskers above and below the boxes the 95th and 5th percentiles. The horizontal line inside the boxplots denotes the median value and X the avg. value. * Indicates the statistical significativity ($p < 0.05$) between PAE concentrations in the liver and fat of *S. coeruleoalba*. Principal component analysis biplot showing the multivariate variation of PAEs concentration among the species and tissues analysed (B). Driving vectors indicate the direction and strength of each PAE compounds considered. The first two principal axes explained 48% of the variance.

Feeding habits, long-life span, spatial distribution and plastic ingestion can be considered as the most important factors influencing the intake of PAEs in cetacean species. Considering the proximity to inland sources, such as industrial and maritime activities, river runoff and the distribution of plastics, the PAE concentrations detected in *Tursiops truncatus* may reflect the different pressures to which this species, living in coastal waters, is exposed. The transport of plasticizers by sewage, rivers crossing industrial areas, and the direct release from plastic pollution that accumulates in the first few kilometres from the coast, as found in this study, may facilitate the accumulation of these lipophilic pollutants in bottlenose dolphins. In addition, both specimens studied were shown to ingest MPs (maximum 41 items), suggesting the possible leaching of plastic additives from these particles. The only data available in the literature on phthalate exposure in this species refer to the studies conducted by Montolo-Martinez et al. (2021) and Page-Karjian et al. (2020) on stranded organisms from the Atlantic Ocean, where concentrations of 0.19 - 1.35 μg/g d.w. (referring to DEHP only) and 0.02 - 46.3 μg/g d.w. (referring to DEP only) were found. Very high levels were detected in skin biopsy of living organisms of this species in the Mediterranean Sea, with values exceeding the 29,000 ng/g w.w. (Baini et al., 2017).

Pelagic Odontocetes species such as *Ziphius cavirostris* (364.24 ± 52 ng/g w.w. in blubber and 326.64 ± 45.45 ng/g w.w. in liver) and *Stenella coeruleoalba* (399.34 ± 49.49 ng/g w.w. in blubber and 185.42 ± 15.4 ng/g w.w. in liver), could be susceptible to PAEs accumulation through direct ingestion of plastic. In this study, the striped dolphins that had ingested synthetic particles showed higher concentrations of PAEs in adipose tissue, indicating the potential release of these compounds (Wilcox test, W = 67, p = 0.5169) (Fig. 64). Moreover, odontocete could be exposed to PAEs indirectly through the food chain. Indeed, high concentrations of plasticizers have been detected in the main preys of these species, such as squid (Savoca et al., 2018) and pelagic fish (Romeo et al., 2016; Savoca et al., 2018). To the best of our knowledge, PAE levels have been previously analysed in stranded striped dolphins only by Montolo-Martinez et al. (2021) who detected DEPH as the most abundant compound and DEP ranging from 13 to 225 ng/g. In the Mediterranean Sea PAEs load in free-ranging organisms of this species were reported by Baini et al. (2017). In this study plasticizers levels were represented mainly by the DEHP (80% of the total compounds considered), however, due to the units adopted to report the concentrations of PAEs analysed (ng/g dry weight), the results are not directly comparable.

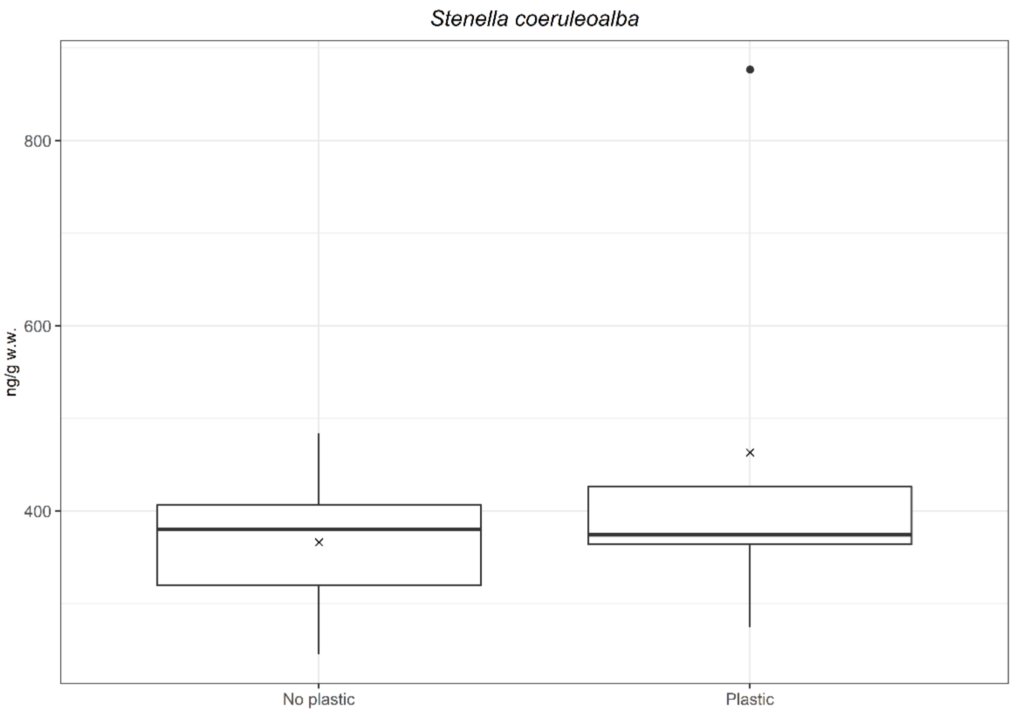
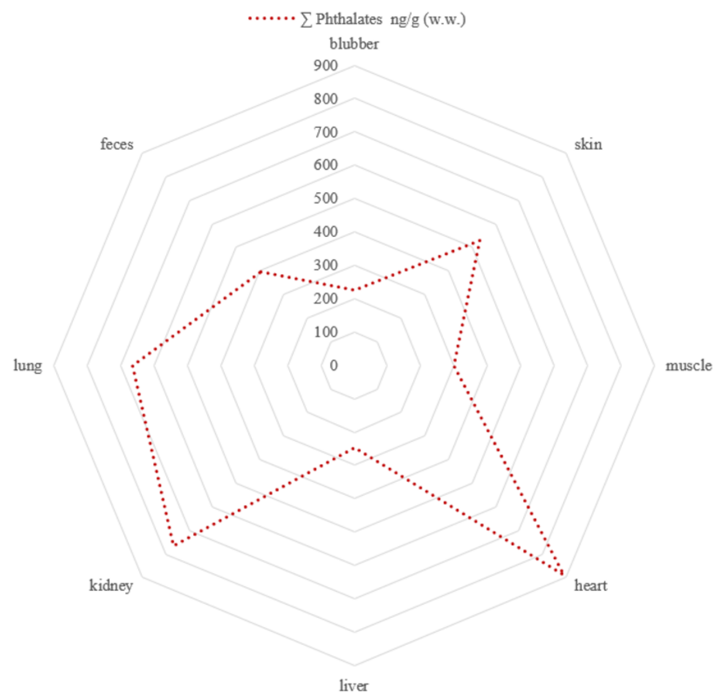


Fig. 64. PAE concentrations in the *Stenella coruleoalba* organisms (n° 5. had ingested plastic and n°.8 without plastic in the GITs) according to plastic ingestion. The boundaries of the boxes indicate the 25th and 75th percentiles, the whiskers above and below the boxes the 95th and 5th percentiles. The horizontal line inside the boxplots denotes the median value and X the avg. value.

Euphausiids represent the largest proportion of the diet of most baleen whales (Hewitt and Lipsky, 2018), which need to filter thousands of cubic meters of water every day to capture their food. During this activity, whales may ingest synthetic particles directly from the water (Fossi et al., 2012, 2014), or indirectly from their prey, if they are already contaminated with plastic particles (Besseling et al., 2015; Germanov et al., 2018). These may be considered the two preferential ways for the fin whale to intake the PAEs and accumulate these chemicals in tissues (226.18 ± 26.87 ng/g w.w. in blubber and 245.58 ± 23.79 ng/g w.w in liver). Phthalates presence in the surrounding waters of the feeding ground of this species, in the Pelagos Sanctuary, have been highlighted by Schimdt et al. (2021), reporting a mean value of 191 ± 123 ng/L mainly composed by DEPH and DBP. The same research report also high concentrations of these lipophilic contaminants in zooplankton species 7,230 ± 10,100 ng/g d.w. as well as the studies conducted by Baini et al. (2017) showed concentrations of 17 – 4,580 ng/g d.w., with DEHP levels of up to 2700 ng/g d.w., and Fossi et al. (2012) reporting concentration of DEPH and its primary metabolite MEHP ranging from 18.38 ± 44.39 ng/g to 61.93 ± 124.26 ng/g.

To better investigate the repartitions of phthalate esters in the whole organisms of the fin whale, six additional tissues were examined (lung, heart, kidney, faeces, skin and muscle). The highest concentrations were detected in the heart (889.87 ng/g w.w.), kidney (767.32 ng/g w.w) and lung (665.02 ng/g w.w.) (Fig. 65 A). The kidney, in particular, performs a variety of detoxification and compensatory functions and plays an essential role in the metabolism of exogenous toxic substances, including phthalates as demonstrated by the study of Hart et al. (2018) evaluating the excretion of these compounds in the urine of bottlenose dolphin. The biological tissues mentioned above differ from the PAEs composition, being the heart and kidney characterized mainly by high molecular weight such as the DEHP and DCHP (ranging from 277.30 ng/g w.w. to 332.94 ng/g w.w. and represent more than 65% of the total concentration) and the lung mainly prone to the accumulation of low molecular weight compounds as DIBP, DBP and DEP (ranging from 124.05 to 329.83 ng/g w.w. and accounting for the 92% of the total concentration) (Fig. 65 B). The same congeners resulted as the most abundant also in the blubber, liver and faeces. Relative high concentrations of DAP was detected in the faeces (194.22 ng/g w.w.), while DiNP was particularly present in the liver (33.67 ng/g w.w.) and kidney (19.86 ng/g w.w.). These two organs are considered the most sensitive to the toxicity of this compound even at low concentrations (Ambe et al., 2019). Muscle and skin do not show any defined fingerprints, being affected by the accumulation of both high and low molecular weight phthalates, although the total concentration of PAEs in the skin tissue show quite high values, especially for DEHP (266.81 ng/g w.w.).

A



B

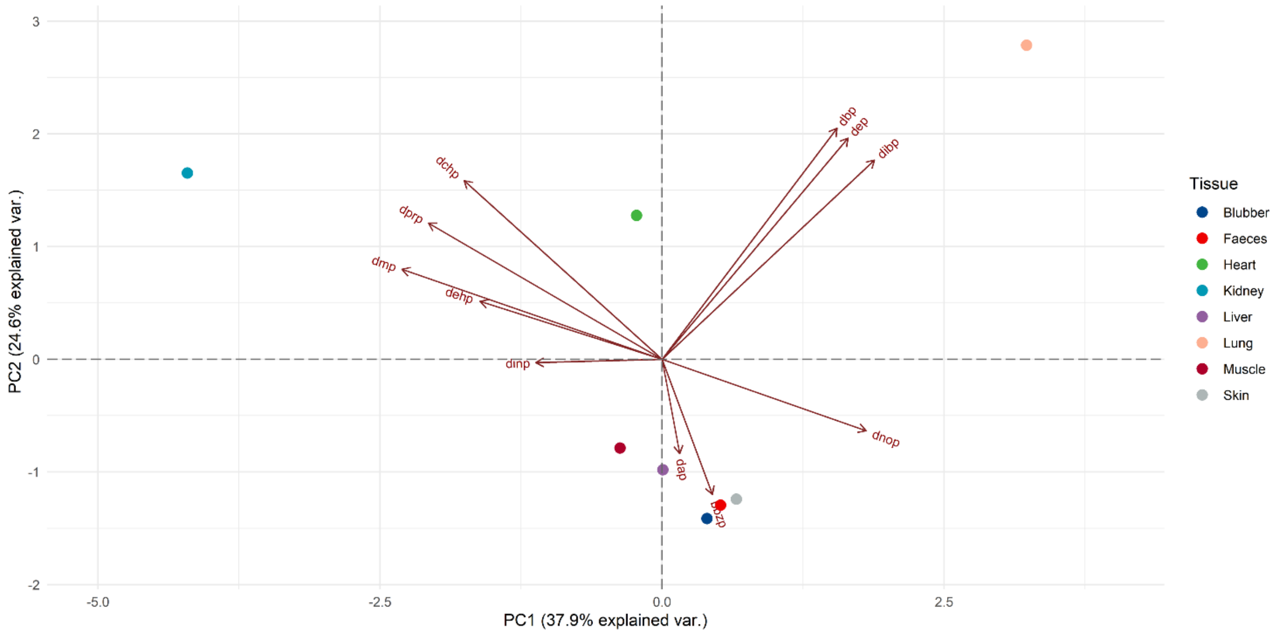


Fig. 65. PAEs levels in the different tissues of the *Balaenoptera physalus* (A). Principal component analysis biplot showing the multivariate variation among the tissue analysed and the PAEs concentrations (B). Driving vectors indicate the direction and strength of each PAE compounds considered. The first two principal axes explained 62.5% of the variance.

The present results provide reliable information on PAE concentrations in Mediterranean marine mammals, reducing the gap in the literature on this important topic. The differential distribution of phthalates in tissues allows an interpretation of the impact that the metabolic pathways of these substances may have on marine organisms, as well as the potential toxicological effects that these contaminants may cause in these sentinel species.

Free-ranging organisms: PAE levels detection

PAEs presence was detected also in skin biopsies of free-ranging organisms, the *Balaenoptera physalus* (n°. 15) and the *Physeter macrocephalus* (n°. 4) sampled in the Pelagos Sanctuary from 2018 to 2020. The levels of each compound detected are shown in Tab. 27.

Tab. 27. PAE concentrations (ng/g w.w.) for each compound considered in skin biopsies of cetaceans species (B.p.: *Balaenoptera physalus;* P.m.: *Physeter macrocephalus*).

Species	ng/g w.w.											
	DMP	DEP	DAP	DPB	DIBP	DBP	BBzP	DChP	DEHP	DINP	DNOP	∑PAEs
B.p.	13.04	68.08	17.68	<LOD	1159.22	293.62	14.82	35.08	351.46	<LOD	13.12	2764.97 ± 515.57
P.m.	<LOD	23.32	4.11	<LOD	622.80	139.87	<LOD	<LOD	160.88	<LOD	23.50	990.36 ± 85.85

The Wilcoxon rank-sum test (W = 57, p = 0.003612) revealed significantly higher concentrations in the skin biopsies of fin whales, where a total of 2,764.97 ± 515.57 ng/g w.w was detected (Fig. 66). This result could be due to the different feeding habits and exposure pathways of the two species studied. The fin whale is a mysticetes filter-feeding organism potentially exposed to ingestion and degradation of MPs and intake of PAEs from water and zooplankton (Fossi et al., 2012, 2014, 2016), while the sperm whale is an odontocete that feeds mainly on deep-sea squid (Taylor et al., 2019) and may be exposed to accumulation of these chemicals mainly through direct ingestion of plastic.

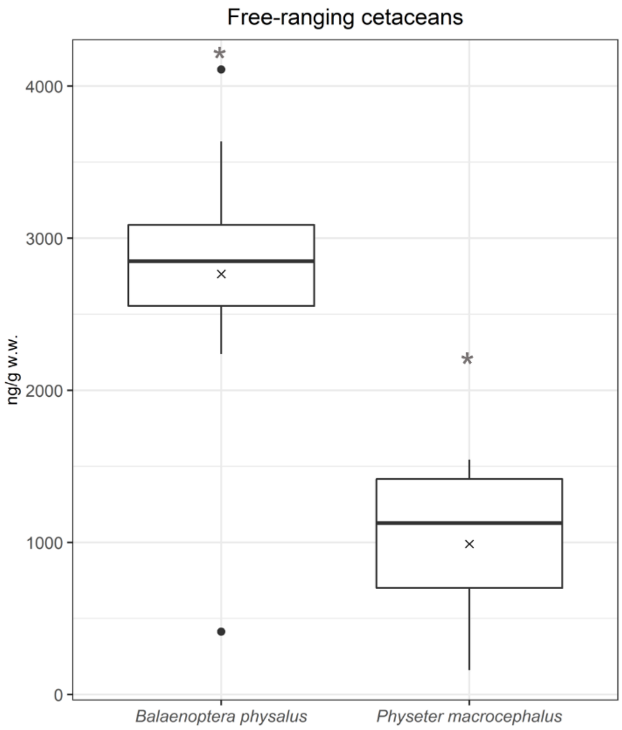


Fig. 66. PAE concentrations in skin biopsies of *Balaenoptera physalus* and *Physeter macrocephalus*. The boundaries of the boxes indicate the 25th and 75th percentiles, the whiskers above and below the boxes the 95th and 5th percentiles. The horizontal line inside the boxplots denotes the median value and X the avg. value. * Indicates the statistical significativity (p < 0.05).

The presence of PAE chemicals in Mediterranean free-ranging organisms was demonstrated for the first time by Fossi et al. (2012, 2014) analysing fin whale skin biopsies reporting a MEPH concentration ranging from

54.8 ng/g to 58 ng/g. In the same area, Baini et al. (2017) found heavy higher abundances of DEPH 7,051 ng/g d.w. rather than the metabolite compounds of phthalate esters accounting in total for 1,016 ng/g d.w. Although this study does not investigate the presence of phthalate acid monoester, the highest concentrations of non metabolised congeners may suggest a potential fresh input of phthalates in the feeding ground of the species analysed as a result of the direct plastic ingestion and both exposure to their presence in the surrounding waters and preys. Considering the fingerprints of these chemicals between the two species, no differences were highlighted. DIBP, DBP and DEHP have been the three most present compounds accounting for more than 90% of the total concentration detected. Comparing the results obtained for the free-ranging organism with those found in tissues of stranded species, it becomes clear how the concentrations differ by an order of magnitude. This opens the discussion regarding a possible degradation of these molecules once the organisms have died or their different binding to the tissue analyzed determining a lower recovery during the analytical phases of extraction. Despite that, the results showed as skin biopsy was a powerful tool to detect the PAEs concentration in free-ranging protected species, highlighting the warning of this emerging threat to baleen whales.

5.3.3 General remarks on plastic ingestion and PAE levels in the Pelagos Sanctuary

The results achieved by this study clearly showed plastic ingestion and the potentially related chemical impact are threatening issues that may affect marine organisms in the Pelagos Sanctuary. The highest concentration of plastic and in particular MPs found floating in the surface pelagic waters and near the coast may seriously represent an emerging risk for species inhabiting these areas as demonstrated by the presence of synthetic particles in all the taxa considered (Fig. 67). Feeding behaviour (e.g. filterers, visual predators and echolocators), habitat preferences and size distribution of prey items may deeply influence the availability of this synthetic material for marine organisms (Jâms et al., 2021).

Fig. 67. Average. number of plastic items/individuals found in all the species analysed.

Filter-feeding organisms such as *Mitylus galloprovincialis* (56% FO and 1.7 items/ind.) among invertebrates, *Mobula mobular* (23 items/ind.) among elasmobranchs, and *Balaenoptera physalus* (140 items/ind.) among cetaceans are particularly susceptible to plastic ingestion during active filtration of waters. Seabirds, which were found to have the highest concentration of plastic particles (157 items/ind.), could be mainly affected by secondary ingestion due to contaminated prey, as well as odontocetes species. Lanternfishes as *Myctophum punctatum*, where a plastic occurrence of 29% and a presence of 2.16 items/ind. were evaluated, could represent a possible source of plastic pollution for *Stenella coeruleoalba* (61% FO and 5 items/ind.), suggesting a possible transmission of MPs along the food chain. The mistake with their natural preys (i.e. jellyfish) was one of the main pathways of ingestion of sheet-like macroplastic (66% and 6.2 items/ind. for *C. caretta*) in sea turtles, while secondary ingestion could interest mainly the MPs uptake as confirmed by the high frequency of occurrence in *Velella velella* (80%, 1 items/ind.), which is described to be fed by these organisms (Frick et al., 2009).

Moreover, the physical properties of plastic (e.g. size, shape and colour) and its degree of degradation must be considered to evaluate the ingestibility of plastic litter. Comparing the distribution of size particles isolated from the manta trawl samples with those of MPs found in the GITs of the monitored species is possible highlighted as all the species analysed well reflect the size of plastic particles found in the marine environment strengthen their role as potential bioindicator of MPs pollution in the Pelagos Sanctuary (Fig. 68 A). This pattern of accumulation is particularly evident for the pelagic invertebrates species *Velella velella* (Fig. 68 A) and the coastal cetacean species *Tursiops truncatus* (Fig. 68 A), represent a first indication of the threat that MPs could cause on these species.

Moreover, the data reported by this thesis clearly shows how the use of well-known and new potential sentinel species of MPs pollution, may allow gathering important information on the distribution and availability for the marine organisms of the lower fractions of plastic particles (< 300 μm) usually not sampled by the common nets, stressing the urgency to better address the potential fate of these particles in the environment and the ecological risk that they may represent. The higher presence of macroplastics isolated from the GIT of the *Caretta caretta* confirms this species as a reliable indicator to assess the impact of macrolitter at a wide scale in the Mediterranean Sea as indicated by the MSFD (indicator D10 C3), OSPAR (Claro, 2016) and UN Environment/MAP IMAP regulations (Indicator 24) (Fossi et al., 2018) (Fig. 68 B).

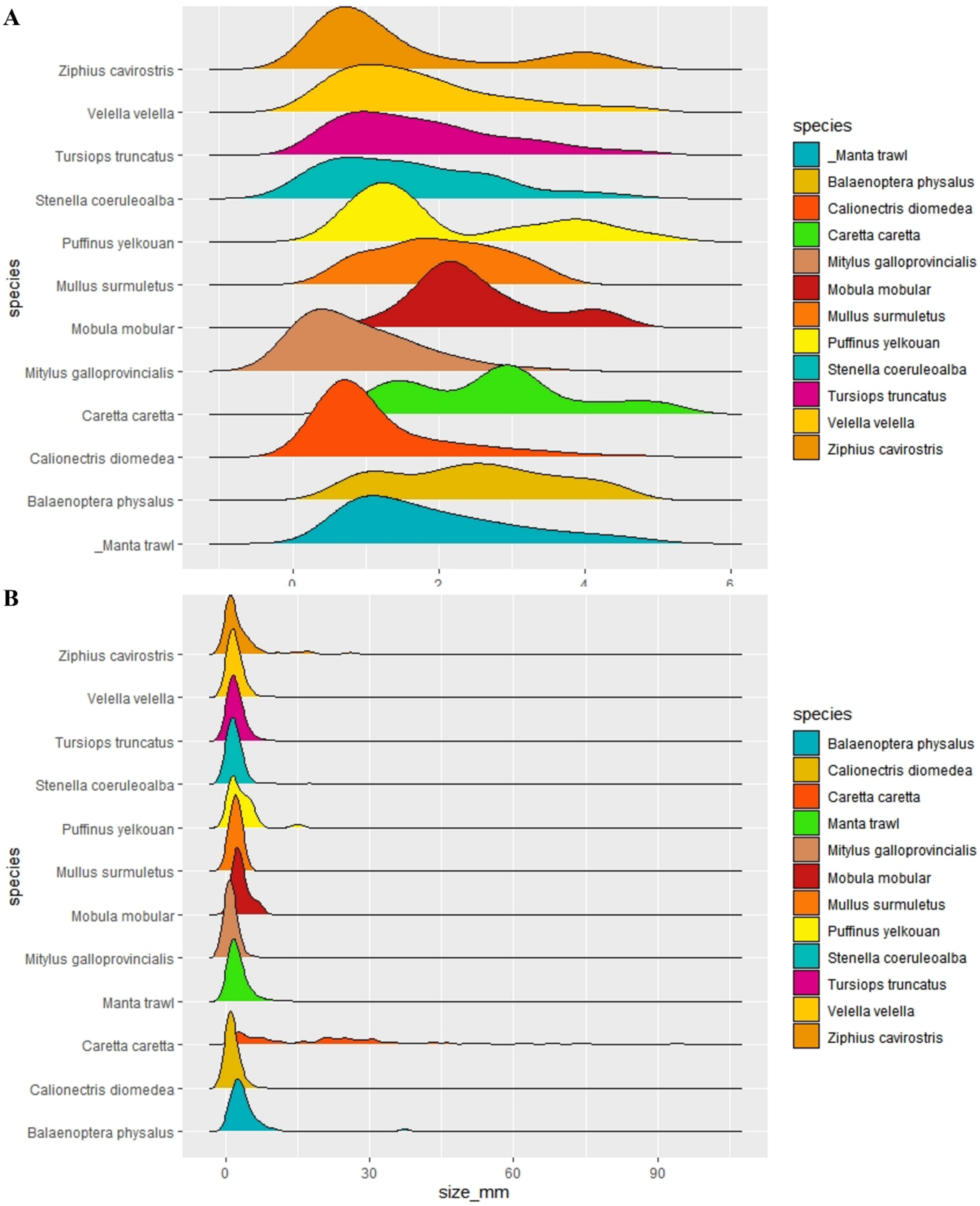


Fig. 68. Comparison among the plastic size classes distribution found in the manta trawl samples and each species analysed considering only MPs (A) and all size classes of plastic (B).

The PAE levels detected shed light on the chemical impact that plastic can have on marine species whether through direct release into organisms after ingestion or indirectly through its presence in surrounding waters. Filter-feeding organisms such as *Mitylus galloprovincialis* and *Balaenoptera physalus* showed the highest concentrations (922.03 ng/g w.w. and 2,764.97 ng/g w.w.) (Fig. 69), which is likely related to their filtration activity and their ability to accumulate pollutants in adipose tissue according to their different long life span. Proximity to potential pollution sources such as coastal anthropogenic activities, hotspot areas for plastic accumulation and inputs from rivers could be the main factors for the high PAE levels detected in the coastal species *Tursiops truncatus* (Fig. 69) compared to other stranded cetaceans, and the relatively high PAE levels detected in *Caretta caretta* could be due to the sensitivity of this species to frequently ingest plastic.

The results make clear the levels of exposure to PAEs in the marine organisms of the Pelagos Sanctuary and provide reliable information on the concentration and distribution of these substances in the different species and tissues, highlighting the risk that these threatening endocrine-disrupting chemicals can cause in the marine trophic chain (Porte et al., 2006).

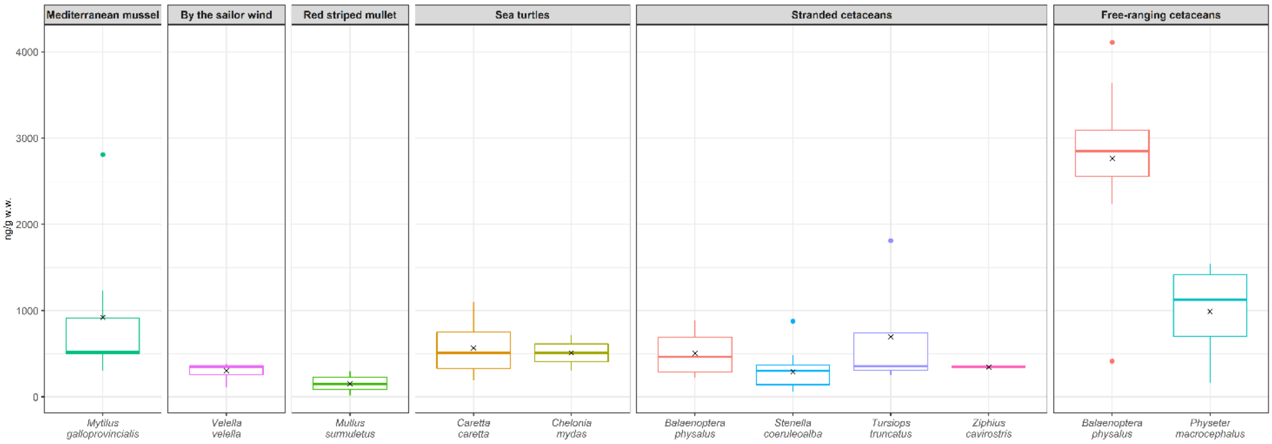


Fig.69. PAE concentrations found in each species analysed. The boundaries of the boxes indicate the 25th and 75th percentiles, the whiskers above and below the boxes the 95th and 5th percentiles. The horizontal line inside the boxplots denotes the median value and X the avg. value.

5.4 Conclusions

Within the frame of this thesis, a comprehensive analysis of the frequency of plastic ingestion and PAEs load in several organisms inhabiting the Pelagos Sanctuary protected area was assessed. A twofold monitoring approach was performed, addressing at the same time, plastics and MPs ingestion in multiple species and plasticizer concentrations stressing the direct potential relationship between the uptake of synthetic particles and the release of toxic substances. The 14 species considered from invertebrates to marine mammals were affected by plastic ingestion and phthalate esters presence, confirming their sensitivity and the suitability as bioindicators of plastic pollution according to their feeding behaviour, long life span and spatial distribution. Specifically:

- The *Mytilus galloprovincialis* revealed a total plastic occurrence of 56% in both islands considered, with the highest concentrations found in the specimens sampled in the facing waters of Capraia Island (2.2 ± 2.6 items/ind.). Any differences in the frequency of ingestion were highlighted according to the size of mussels, although the individuals larger in size sampled in the Montecristo Island seem to be more prone to ingest mesoparticles. The analysis of the size classes and shapes of the plastic ingested confirm the suitability to use bioindicators to reflect the effective pollution of the areas considered giving a comprehensive overview of the MPs availability for marine organisms, especially on the possible fate of small plastic particles (< 0.3 mm) and fibres that were not collected by the most common nets used to assess the plastic distribution on the sea surface. Accordingly, to the plastic ingestion results, the highest concentration of PAEs was found in the organisms sampled in Capraia Island with a total mean of 1,519.65 ± 243.21 ng/g w.w. This finding may reflect the different plastic pressures that could insist on the monitored areas, both in terms of the number of particles ingested by organisms and the concentrations of floating plastic in coastal waters, allowing to consider this species as a potential bioindicator at the local scale. The presence of plasticizers (mainly DIBP, DBP and DAP) in the Mediterranean mussel demonstrates that these lipophilic compounds are freely present in relatively high concentrations in marine waters and can be transferred to mussels tissues as a consequence of the filtering activity.
- Plastic ingestion analysis in the *Mullus surmuletus* revealed a quite lower number of MPs isolated, accounting for a total plastic occurrence of 20%. Any significant differences among the two sampling sites (Capraia and Porto Ercole) were shown both in terms of plastic ingestion and the number of particles isolated, with fragments and fibres as the two most abundant plastic-type. The significant difference between the sampling sites was revealed, instead, by the PAEs detection in the flesh of this species, showing the organisms sampled near the Capraia Islands as the most polluted (231.92 ± 23.77 ng/g w.w.). No statistical differences were detected considering the organisms according to the plastic ingestion, reporting the DEHP, DIBP, DBP and DAP as the most abundant plasticizers in this fish species. Results obtained for the *Mullus surmuletus*, about the plastic ingestion and PAE levels represent a first indication of the suitability of this species as a potential indicator of plastic and PAEs pollution in the benthic environment feeding exclusively on sediments

and having no migratory habits, although further studies on the real capability of this species to reflect the MPs pollution of their habitat are needed.

- The 29% of the lanternfishes analysed, belonging to the species *Myctophum punctatum,* were affected by plastic ingestion. Fragments and fibres were the two most abundant plastic-type found with a size dimension ranging for the majority of items from 1 to 2.5 mm. Light coloured, blue and black particles were the most present items, suggesting potential ingestion of MPs due to the resembling of prey such as copepods. Being this species the predominant vertically migratory taxa accounting for the largest proportion of fishes in the euphotic zone at night, the MPs found in their GITs could provide important information about the potential availability of synthetic particles for marine organisms in pelagic waters and along the water column. Moreover, representing the main food source for several top pelagic predators, this species could play a key role as MPs bioaccumulation factors along the marine trophic chain.

- A digestion method to isolate MPs from *Velella velella* organisms was set up and tested for the first time to achieve both an optimal digestion efficiency of the organic matter and recovery of spiked MPs in samples. The 10% KOH resulted in the best digestion methods (%DE > 98% and recovery rate > 91%) and it was adopted for the MPs extraction analysis. A total of 237 plastic particles were isolated from the hydrozoan organisms analysed, accounting for a total occurrence of 81% (43/53 pools) and 0.71 ± 1.48 items/ind. These data represent the first report of plastic ingestion in this species in the Mediterranean Sea and worldwide. As neustonic organisms, they passively accumulate in response to wind and current patterns and their distribution may coincide with areas heavy affected by floating MPs, suggesting potential ingestion of particles mistaken for prey such as zooplankton organisms and fish juveniles. MP particles isolated belonging for 66% to LMPs and 33% to SMPs, provide an important indication of the usefulness of this species to reflect the plastic pollution of the pelagic waters, giving additional information on the availability of smaller synthetic particles (< 1 mm) for marine organisms usually found floating in smaller concentration on the sea surface. Fibres have been the most common type recovered (78%), followed by fragments (17%), and filaments (4%) and the polyolefin polymer the plastic material most found (82%). Although the statistical analysis does not prove a strong relationship between the average number of ingested items per individual and MPs concentration in the corresponding manta trawl samples, due to its wide spatial distribution and the relevant role in the marine trophic chain, *Velella velella* may be considered as a potential indicator of MPs pollution in the neustonic waters of the Pelagos Sanctuary. A total of PAEs load equal to 313.05 ± 65.89 ng/g w.w. was detected in this hydrozoan species. DBP, DIBP and DEHP resulted in the three most abundant compounds representing 95% of the total PAEs detected. Their concentrations seem to be slightly positively related to the number of particles ingested by this species, suggesting their potential direct release from plastic.

- For the first time in the Mediterranean Sea, the ingestion of plastic particles by a mobulids species, the Giant devil ray, was reported. A total of 23 plastic particles were isolated from the entire GIT, represented for the majority by MPs (87%) and mesoplastics (13%). The prevailing abundance of plastic items ranging from 1 mm to 2.5 mm (57%), suggests the attitude of the *Mobula mobular* to ingest floating MPs, being this size classes the most found in the waters of the Tuscan Archipelago National Park, as demonstrated by this study. The higher presence of polyolefins fragments (50% in total), and foamed items made of PS (22%), commonly found floating on the sea surface, strengthen the hypothesis of MPs ingestion during the filter-feeding activities, highlighting the potential risk of this species to ingest plastic particles.
- The 66% of loggerhead turtles examined had ingested plastic litter, while plastic presence in the Green turtle was found in only one specimen (33%). A total of 135 plastic particles were isolated, with an avg. abundance of 6.2 ± 12.3 items/ind. for the *Caretta caretta* and 1.6 items/ind. for the *Chelonia mydas*. MPs were found in only 4 individuals (19%) of *Caretta caretta* accounting for 24 isolated items. Sheet-like plastic was the most abundant category for both macroplastics and mesoplastics (66% and 59%, respectively), while fragments (48%) and films (39%) were the most common plastic types among MPs. Light-coloured plastics were the most common colour found (> 70%) in all size classes considered and PE and PP were the principal polymers detected. Phthalates were detected in all individuals analysed with concentrations ranging from 0.79 ng/g w.w. and 260.57 ng/g w.w. in fat tissue, with five major congeners accounting for more than 80% of the total concentration: DIBP, DEHP, DBP, BBzP and DChP. Significant differences in the PAEs load were detected between liver and fat, suggesting the adipose tissue as the optimal tissue to be monitored for the evaluation of these pollutants. Reported results confirm the suitability of the PAEs detection as a benchmark for plastics exposure in sea turtles species and the different phthalates distribution among the tissues opens the way to the interpretation of the impact that metabolic pathways of these substances may have on marine organisms.
- Plastic ingestion was evaluated in two seabirds species accidentally found stranded along the Tuscan coast. The Scopoli's shearwater was the species most affected by plastic ingestion, with a total of 301 pieces, while in the Yelkouan shearwaters a total of 27 pieces were extracted from two organisms. The weight of plastic isolated from the GITs of the species studied does not exceed the 40 mg found in Scopoli's shearwaters and was lower than the threshold established by Van Franeker et al. (2021) for fulmars (0.1 g of plastic particles in the stomach). SMPs were found mainly in Scopoli's shearwaters (> 60%), while particles larger than 1 mm up to 25 mm were most abundant in the Yelkouan shearwaters (80%). Fragment (71 – 88%), filament (5 – 29%) and film (4 – 20%) have resulted in the categories of plastic most commonly ingested in the two species of seabirds and made of polyolefins materials (PE and PP > 95%). Despite the lack of data on plastic ingestion by the Mediterranean seabirds due to the limited availability of samples to be analysed and the difficulties

in their collection, this result suggests an urgent need to assess the impacts and effects of plastic on their health, and in particular for threatened species such as the Scopoli's and Yelkouan seawaters.

- Plastic ingestion and PAE concentrations were evaluated in different cetacean species and biological tissue. A total of 449 plastic items were isolated from the GIT contents of all cetacean organisms. LMPs ranging from 1 mm to 2.5 mm were the most abundant size classes in the species *Tursiops truncatus* (45%) and *Stenella coeruleoalba* (38%), while particles ranging from 2.5 mm to 5 mm were the most found in the *Balaenoptera physalus* (40%). MPs less than 1 mm in size, represent the majority (44%) among the particles isolated from the GIT of the *Ziphius cavirostris*. Only two macroplastics, probably a piece of a fishing line, and a fragment of sheetlike plastic were isolated among all the specimens analysed, from the GITs of the fin whale and Cuvier's beaked whale, respectively. A great heterogeneity of plastic types was found, represented mainly by fibres, fragments and films. Polymer analysis revealed the polyolefin polymers as the most frequently detected materials in all the species considered, confirming their widespread distribution in marine environments. Despite that, a relatively high presence of nylon and PET were detected among the particles isolated from the bottlenose dolphin probably due to the interaction of this species with fisheries activities. Feeding habits, long-life span, spatial distribution and plastic ingestion can be considered as the most important factors influencing the intake of PAEs in cetacean species. Phthalates were detected in all species analysed at concentrations ranging from 0.05 ng/g w.w. and 463.10 ng/g w.w. The highest concentrations were found in the bottlenose dolphin suggesting a potential accumulation due to the proximity of PAEs potential sources originating from the mainland. Low molecular weight phthalates (DIBP, DBP and DEP) have been detected mainly in the blubber samples, with high concentrations of BBzP in the *Tursiops truncatus* (463.10 ng/g w.w.). On the contrary in liver tissue, DEHP and DcHP showed higher abundances. In the fin whale the highest concentrations were detected in the heart (889.87 ng/g w.w.), kidney (767.32 ng/g w.w) and lung (665.02 ng/g w.w.) organs. These three tissues differ from the PAEs composition, being the heart and kidney characterized mainly by high molecular weight such as the DEHP and DCHP and the lung mainly prone to the accumulation of low molecular weight compounds as DIBP, DBP and DEP as well as the blubber, liver and faeces. Relative high abundances of DiNP was detected in the liver (33.67 ng/g w.w.) and kidney (19.86 ng/g w.w.) considered the most sensitive organs to the toxicity of this compound.
- PAEs presence was detected in skin biopsies of free-ranging organisms (*Balaenoptera physalus* and *Physeter macrocephalus*), revealing significantly higher concentrations in the fin whales (2,764.97 ± 515.57 ng/g w.w). The higher concentrations of phthalate compounds found in the surrounding waters of the feeding ground of the fin whale in the Pelagos Sanctuary and the filter-feeding behaviour of this species may explain the different load of toxic substances detected. DIBP, DBP and DEHP have been the three most present compounds accounting for more than 90% of the total concentration in both species. Heavy PAE concentrations found in the skin biopsy compared to

tissues of the stranded cetaceans, confirm this non-invasive sampling technique as a powerful tool to detect the concentration of the plasticizer in free-ranging protected species, highlighting the warning of this emerging threat to baleen whales. Moreover, the achieved results also stress the suitability of these species as indicators of the chemical impact of plastic additives in the pelagic environment considering the distinct feeding habits and exposure pathways characterizing the two monitored species.

CHAPTER 6: SPATIAL RISK ASSESSMENT OF MARINE LITTER AND MARINE MEGAFAUNA IN THE PELAGOS SANCTUARY AND TUSCAN ARCHIPELAGO NATIONAL PARK

This chapter provides innovative information on the species richness of the marine megafauna surveyed during the sampling campaigns carried out in the summer of 2019 in the two study areas. The experimental plan performed, allowed the simultaneous collection of information on the spatial distribution of species and floating marine litter to highlight the vulnerability of marine fauna to floating plastic in the context of a spatial risk assessment. While the identification of biodiversity hotspots and sensitive areas for coastal areas is an established process that usually leads to the designation of marine protected areas, the offshore waters are usually less studied. Marine megafauna, such as cetaceans and sea turtles, are often used as umbrella species because they not only play an ecological role but also have a charismatic impact on conservation efforts (Germanov et al., 2018). Identification of particularly sensitive areas and biodiversity hotspots in the pelagic realm can be effectively carried out by focusing on these valuable species.

6.1 Introduction

Ecological risk analysis has become an important tool for ecosystem-based management. By quantifying the likelihood of an adverse event or impact, risk assessment is very useful in determining mitigation measures to avoid or limit these impacts (Holsman et al., 2017). However, risk assessment is highly dependent on the specific methodology used to calculate it, especially the definition of the different parameters that characterize the risk. In the case of marine litter ingestion, the risk assessment should indicate where and when harm may occur. This involves not only the possible encounter of marine organisms with litter but also the assessment of the potential harmfulness of the litter, such as the type and shape (Fossi et al., 2018a). Previous studies on the global ocean and the Mediterranean Sea have addressed the assessment of the risk of marine litter pollution, using different methodologies and definitions of the factors involved in the risk assessment. The hazard definition is generally very similar for all of them, as the presence or absence of litter in the sea is the main starting point for any pollution study. Determining the amount of litter in the study area is a challenging task. Available observational data are spatially and temporally discontinuous and therefore insufficient to provide accurate information on the distribution of litter in the sea over larger regions and periods. To address this problem, most studies rely on indirect methods and numerical models. Wilcox et al. (2013) assessed the risk of entanglement in abandoned fishing nets in northern Australia by combining beach observations and bycatch records with numerical Lagrangian models to estimate the density of lost fishing gear in their study area. At the global scale, Wilcox et al. (2015) and Schuyler et al. (2016) analyzed the risk of plastic ingestion to seabirds and sea turtles, respectively. Both papers used plastic concentrations based on Lagrangian simulations and estimated the probability of ingestion using a binomial model that takes into account the biological characteristics of the different species and the litter distribution. Darmon et al. (2017) investigated the co-occurrence of sea turtles and plastic in French Mediterranean and Atlantic waters by analyzing aerial observations and assessing the probability of sea turtles encountering floating litter.

Compa et al. (2019) produced risk maps for the entire Mediterranean Sea for several species by using the global plastic distribution model of Lebreton et al. (2012). Generalized additive models (GAM) were used to determine the exposure and risk of each species, defined as the ingestion rate considering biological characteristics such as mobility, body size, class and habitat. Fossi et al. (2017) and Guerrini et al. (2019) investigated the impact of plastic pollution and MPs on the feeding grounds of fin whales in the Pelagos Sanctuary and used plastic distribution simulations to compute plastic concentration in their study region. The authors calculate risk as to the product of the average concentration of marine debris determined by their models and the presence/absence of fin whales in their study area. Finally, Soto-Navarro et al. (2021) created a global risk map for the Mediterranean Sea by using a high-resolution 3D marine litter dispersion model to estimate the concentration of particles according to their physical properties, combined with a larger dataset of species to estimate their exposure and vulnerability. Despite the valuable scientific relevance of the studies described, the approach adopted in most of them is still based on the numerical estimation of litter in the investigated areas and/or the predicted ingestion rate, which may not reflect the effective pressures to which marine species are exposed in the Mediterranean Sea. To produce a more accurate and reliable risk scenario, the collection of empirical data on the extent and typology of plastic litter, the spatial distribution of organisms and the potential impact on them are needed. To cover this gap, the experimental design adopted during the described sampling campaigns aims to collect simultaneous data on floating litter and biota distribution to perform a comprehensive risk assessment highlighting critical areas and providing information for the protection and mitigation measures to be taken forward.

6.2 Materials and methods

6.2.1 Marine species visual survey

Visual census of marine species presence was carried out simultaneously with litter monitoring activities (Fig. 70), by at least 3 trained Marine Mammal Observers (MMOs), covering 360° all around the R/V. MMOs shifted position every 30 minutes to avoid fatigue, alternating 90’ effort to 30’ resting. The searching effort was performed naked eye and with 7*50 binoculars. Monitored species included marine mammals, sea birds, and all marine fauna sighted at the sea surface.

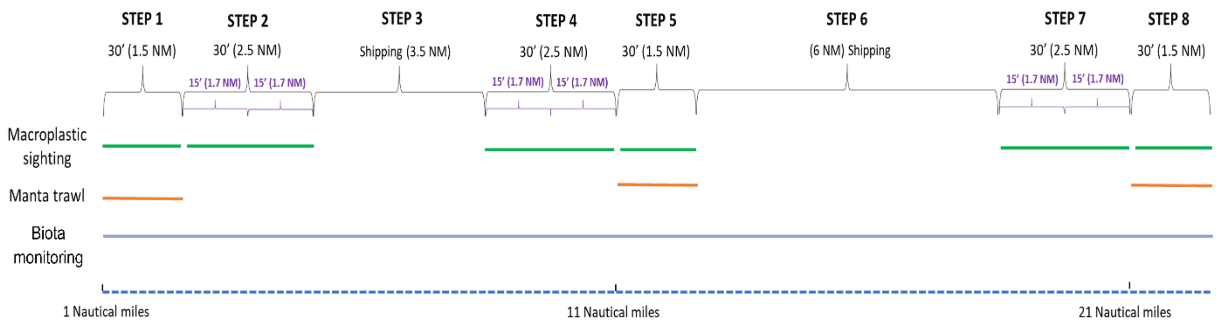


Fig. 70. Experimental design adopted in the study areas evaluating the presence and spatial distribution of both floating litter and biota (cetaceans and associated species).

6.2.2 Home range analysis for marine megafauna species

The analysis of the home ranges of the sighted species involved two steps. First, the relative densities of cetaceans and other marine organisms were calculated by determining the encounter rate (ER). This index of relative abundance has been used in several cetacean studies to make comparisons between different areas or to monitor changes in populations over time (Dwyer et al., 2016). Kernel density estimation (KDE) was then performed to reveal the spatial clustering of marine megafauna distribution and litter accumulation, identifying areas with a higher probability of occurrence. Sampling effort throughout the study area was weighted according to the ER of each species sighted and grouped as follows:

- Cetaceans: fin whale (bp), bottlenose dolphin (tt), striped dolphin (sc), and deep divers (deepd) including sperm whales, cuvier's beaked whale, and risso's dolphin;
- Associated species: sun fish (mm), giant devil ray (mb) and jellyfish (jellyf).
- Seabirds (seab)*:* Scopoli's shearwater (cd), Yelkouan shearwater (py), common tern, Audouin's gull (ia) and european shag.

The 1 km European Environment Agency (EEA) INSPIRE compliant reference grid was chosen for density analysis. Encounter rates were calculated for each cell i by dividing the total number of individuals of species j in cell *i* (*Nij*) by the kilometres (*Li*) surveyed in cell *i* and normalising to the highest value:

$$ER_{ij} = \frac{\frac{N_{ij}}{L_i}}{ER_{\max(j)}}$$

The total number of individuals of the same species sighted throughout the campaign was calculated for each cell of the grid. The KDE was calculated to determine the general and core area of distribution of megafauna species, using the centroids of the cells as a reference, with a radius of 20 km, and weighted considering ER. The radius was considered an appropriate range to account for the distribution of marine species and the distribution of plastic litter. Density estimation was performed separately for each species (sc, bp, tt, mm, mb, cd, py, ia) or species group (deepd, seab, jellyf). The 50% contour was then used to determine the core area (HR50) of species/species group distribution and the 90% contour to determine the general distribution (HR90). To assess the vulnerability to the marine litter for the two HRs considered, the density of floating macrolitter and MPs was related to the HR50 and HR90 of each species/species group.

6.2.3 Spatial risk assessment

The spatial risk analysis was carried out by combining the hazard map for macrolitter floating at the sea surface obtained by the GAMs analysis (Fig. 25) (Compa et al., 2019) and the sensitive maps of exposure obtained by the KDE for each species. Two different spatial scale risks were assessed considering the overall Pelagos Sanctuary and the surveyed area in the frame of this study. To obtain exposure maps for the entire Pelagos Sanctuary, species richness data were downloaded from the free Aquamaps database (https://www.aquamaps.org/) and used to calculate the spatial risk analysis. The 90% density contours of each species were overlaid on a 5 km grid to compute the risk analysis. Each cell was assigned a value of 1 if each time it overlaps within the H90 density area of a species. The final exposure map was calculated by assigning values starting from 1 to 8 (maximum number of species recorded for a single cell) to all cells each time they overlap within the H90 density areas of a species. All unsampled cells were assigned a value of 1 to exclude results with zero risk.

6.2.4 Statistical analysis

The Kolmogorov-Smirnov test was applied to evaluate possible differences between concentrations of floating macrolitter and MPs measured in the general (HR90) and core (HR50) distribution areas of each species, compared to the average concentration throughout the Pelagos Sanctuary. A pairwise post-hoc analysis using the Wilcox test was conducted to identify significant differences between concentrations measured in the HR50 or HR90 areas of each species, as highlighted by the Kolmogorov-Smirnov tests. The alternative hypothesis was that macrolitter and MPs concentrations measured in the HR50 or HR90 areas were higher than those measured in the Pelagos Sanctuary. Statistical analysis was performed with Rstudio (version 1.1.4.1106).

6.3 Results and discussions

6.3.1 Biodiversity richness and marine litter potential interactions

In the Pelagos Sanctuary and Tuscan Archipelago National Park, 908 sightings were recorded during the sampling campaigns, distributed among 17 different species: 6 species belong to the order Cetacea and the remaining 11 species belong to different taxa: birds, elasmobranchs, fish and cnidarians (Tab. 28).

Tab. 28. Species sighted during the sampling campaigns. The number of individuals per species, number of sightings and relative ER were shown.

Species	N°. individuals	N°. sightings	Cells with $ER_{ij} > 0$
Balaenoptera physalus (bp)	30	18	14
Deep divers (deepd) *Physeter macrocephalus, Ziphius cavirostris* and *Grampus griseus*	27	15	14
Setenella coeruleoalba (sc)	829	56	50
Tursiops truncatus (tt)	77	15	15
Seabirds (seab) *Scopoli's shearwater, Yelkouan shearwater, Audouin's gull, Sterna hirundo, and Gulosus aristotelis*	657	332	257
Scopoli's shearwater (cd)	471	258	204
Yelkouan shearwater (py)	169	65	57
Ichthyaetus audouinii (ia)	48	34	27
Mobula mobular (mb)	22	22	17
Mola mola (mm)	47	42	33
Jellyfish (jellyf) *Velella velella, Rhizostoma pulmo, Pelagia noctiluca and Cothyloriza tubercolata*	1360	51	43

Sensitivity maps obtained through the KDE analysis (Fig. 71) revealed a wide distribution of the species sighted in the Pelagos Sanctuary area and near the coast of the islands of the Tuscan Archipelago National Park. Focussing on the cetaceans distribution, it is evident the importance of the continental slope and submarine canyons as preferred habitat for the fin whale and deep divers organisms (Fig. 71 A and B). Their presence associated with these areas in the Pelagos Sanctuary is well known as they represent their feeding ground, especially during the summer season where the high productivity that characterized the Northwestern sector of the Mediterranean basin highly influences the distribution of the fin whale (Notarbartolo di Sciara et al. 2003; 2016). Deep divers species showed a preference for the deepest portions of the slope, the preferred habitat of mesopelagic cephalopods, their preferred prey (Pirotta et al. 2011), and deep offshore waters, likely in association with frontal systems (Gannier & Praca 2007). Especially, for the cuvier's beaked whale its distribution concern very restricted ranges indicating the strong preference of this species for specific habitats such as the Genova canyon (Cañadas et al. 2005). Differently from the pelagic species, the bottlenose dolphin distribution is limited to the continental shelf (Fig. 71 D), as demonstrated by the high presence along the Tuscan coast. Seabirds distribution are strictly connected with the proximity of the breeding colonies (Fig. 71 E, I, L and M) on the island of Cape Corse, the Parc National de Port-Cros (France) and in the islands of Capraia and Montecristo in the Tuscan Archipelago National Park (Leonzio et al., 1989; Péron et al., 2013; Thibault et al., 1996). Interesting is the overlapped of the distribution of jellyfish species and its natural predator sunfish, concentrated in the pelagic waters of the Pelagos Sanctuary.

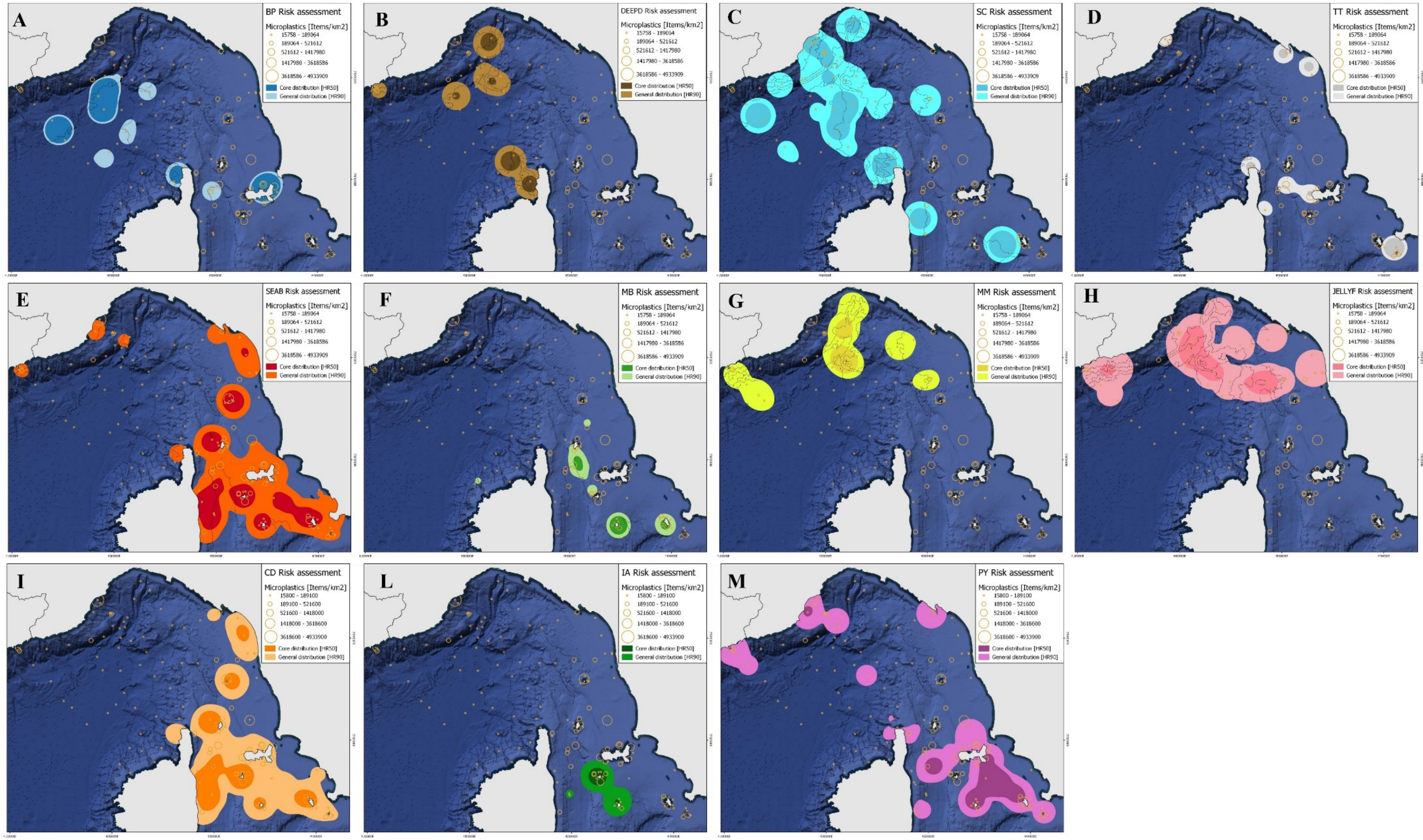


Fig. 71. Sensitivity maps for *Balaenoptera physalus* (BP) (A), Deep diver cetacean species (DEEPD) (B), *Stenella coeruleoalba* (SC), *Tursiops truncatus* (TT), seabirds (SEAB) (E), *Mobula mobula* (MB) (F), *Mola mola* (MM) (G), Jellyfish (JELLYF) (H), *Calionectris diomedea* (CD) (I), *Audouin's gull* (IA) (L) and *Puffinus yelkouan* (PY) (M). General and core distribution areas of the sighted species that overlapped with the density of the sea surface microlitter in the study area.

To investigate the potential interactions and impacts of floating litter on the observed species during summer 2019, the mean density of sea surface floating macrolitter and MPs in the general (H90) and core (H50) distribution areas of each species were compared to the overall mean density observed in the Pelagos Sanctuary (Annexe 17). Kolmogorov-Smirnov test and posthoc analysis through Wilcox test results are shown in Tabs 29 and 30.

Tab. 29. Summary of the p values resulted from the Kolmogorov-Smirnov between the distribution of sea surface floating macrolitter and MPs in the HR50 and HR90 of each species and the overall concentrations in the Pelagos Sanctuary. The number of * indicates the strength of the significant value: p < 0.001 '***', p < 0.01 '**' and p < 0.05 '*'.

Species	Sea surface floating litter		MPs	
	KS H90 vs Pel	**KS H50 vs Pel**	**KS H90 vs Pel**	**KS H50 vs Pel**
Fin whale (bp)	0.9527	0.7576	0.1869	0.3397
Deep divers (deepd)	**0.0004*** **	0.1437	0.3532	0.4264
Striped dolphin (sc)	**< 0.001*** **	**0.0035** **	**0.0224***	0.3624
Bottlenose dolphin (tt)	**0.0204** **	**0.0110***	0.1679	0.0842
Giant devil ray (mb)	**0.0292***	0.5286	0.3450	0.8161
Sunfish (mm)	**0.0122***	**0.0352***	0.4360	0.2115
Seabirds (seab)	**< 0.001*** **	**0.0176***	**0.0004*** **	0.9461
Scopoli's shearwater (cd)	**< 0.001*** **	**0.0209***	**0.0012** **	0.9681
Yelkouan shearwater (py)	**0.0142***	0.2900	**0.0032** **	0.5899
Audouin's gull (ia)	0.1248	0.0627	0.7478	0.8209
Jellyfish (jellyf)	**0.0003*** **	**0.0048** **	0.1007	0.1747

Tab. 30. Summary of the p values resulted from the Wilcoxon test between the distribution of sea surface floating macrolitter and MPs in the HR50 and HR90 of each species and the overall concentrations in the Pelagos Sanctuary. The number of * indicates the strength of the significant value: p < 0.001 '***', p < 0.01 '**' and p < 0.05 '*'.

Species	Sea surface floating litter		MPs	
	KS H90 vs Pel	**KS H50 vs Pel**	**KS H90 vs Pel**	**KS H50 vs Pel**
Deep divers (deepd)	0.9997	/	/	/
Striped dolphin (sc)	0.9998	0.9962	0.9581	/
Bottlenose dolphin (tt)	**0.0057** **	**0.0007*** **	/	**/**
Giant devil ray (mb)	**0.0074** **	/	/	/
Sunfish (mm)	0.9827	0.9416	/	/
Seabirds (seab)	**< 0.001*** **	**0.0006*** **	**0.0001*** **	/
Scopoli's shearwater (cd)	**< 0.001*** **	**0.0006*** **	**0.0002*** **	/
Yelkouan shearwater (py)	**0.0140***	/	**0.0008*** **	/
Jellyfish (jellyf)	0.9971	0.9979	/	/

The obtained results from the statistical tests revealed significative higher concentrations of floating sea surface macrolitter both in the H90 and H50 distribution areas of the bottlenose dolphin and seabird species Scopoli's shearwaters and Yelkouan shearwaters. These data confirm the potential higher risk of plastic interaction and ingestion in these species, characterized by a coastal spatial distribution where litter tend to accumulate as shown in chapter 3. Moreover, for the seabirds species, a statistical difference was also highlighted for the MPs concentrations confirming the attitude of these species to directly ingest plastic particles smaller than 5 mm during the feeding activities or indirectly through contaminated prey such as cephalopods and fishes, as demonstrated by the GITs content analysis performed in this study. A risk of plastic ingestion was also highlighted for the filter-feeder giant devil ray in the H90 distribution areas of this species, probably connected with the active filtration of waters, as confirmed by the ingestion data reported by this study for the first time in the Mediterranean Sea.

6.3.2 Spatial risk assessment in the Pelagos Sanctuary

To understand the levels of risk to marine organisms associated with floating litter hazard (Fig. 26), exposure maps were calculated to better represent species and plastic distribution data. Regarding the Pelagos Sanctuary scale (Fig. 72), species distribution data were obtained from online databases. The resulting risk map showed an elevated risk area in the coastal waters of eastern Liguria, coinciding with the Gulf of La Spezia (Fig. 72 C and F), especially for the bottlenose dolphin, being the only cetacean species considered with a preferential coastal distribution, and other species. A relative moderate risk insisted on the slope area in western Liguria, while the west and northeast of Corsica and the continental shelf in the eastern Pelagos Sanctuary showed the lower risk for marine mammals. Looking at the other marine organisms, due to the high number of species present, a higher risk was widely distributed across the Pelagos Sanctuary, encompassing the entire slope and continental shelf areas (Fig. 72 F).

Focusing on the surveyed area and aiming to draw a picture of the current risk that existed in the surveyed area during the summer of 2019, an exposure map was created based on the marine megafauna encountered (Fig. 73). The resulting risk map (Fig. 73 C) shows moderate to high risk in Genova Canyon, including its deepest part (2000 m depth), which was not highlighted in the global risk map. These results are consistent with the risk assessment of Fossi et al. (2017) and Guerrini et al. (2019), which demonstrate that the Liguro-Provencal basin was particularly vulnerable to plastic accumulation and may pose a serious threat to marine organisms, especially the fin whale. Indeed, this area is known to play a crucial role for this species, especially during the summer feeding season (Panigada et al., 2005; Druon et al., 2012). Similarly, the western canyon regions were highlighted as affected by a moderate risk according to the results of the whole Pelagos Sanctuary (Fig. 72 C and F). These areas are were reported to be particularly impacted by floating debris by Darmon et al. (2017), who assessed the risk associated with sea turtle distribution, and Angiolillo et al. (2021), evaluating the litter distribution including MPs in different canyons of the Ligurian Sea. These areas may act as a sink for litter and plastic pollution (Angiolillo et al., 2021), representing a serious threat for all the species inhabiting and feeding in these habitats such as the deep diver organisms (e.g., sperm whales and Cuvier's beaked whales). In the eastern part, the highest risk was confirmed on the continental shelf in the Gulf of La Spezia, in the Tuscan Archipelago National Park and the northeastern sector of the island of Corsica evidenced also by the recent studies of Soto-Navarro et al. (2021). The high concentration of floating litter measured in their surrounding waters may represent a serious threat affecting a wide range of organisms. Indeed, these areas represent the breeding site of many seabird species (e.g. the Yelkouan shearwater and the Audouin's gull), feeding grounds for the giant devil ray and the bottlenose dolphin, that could be seriously affected by the ingestion of plastic litter. Overall, the assessed risk analysis provides reliable information on the threat posed by plastic litter in the SPAMI Pelagos Sanctuary and the potential exposure that may affect a wide range of organisms and highlights the need to implement specific measures aimed at reducing the potential sources of plastic pollution and its impact on marine wildlife.

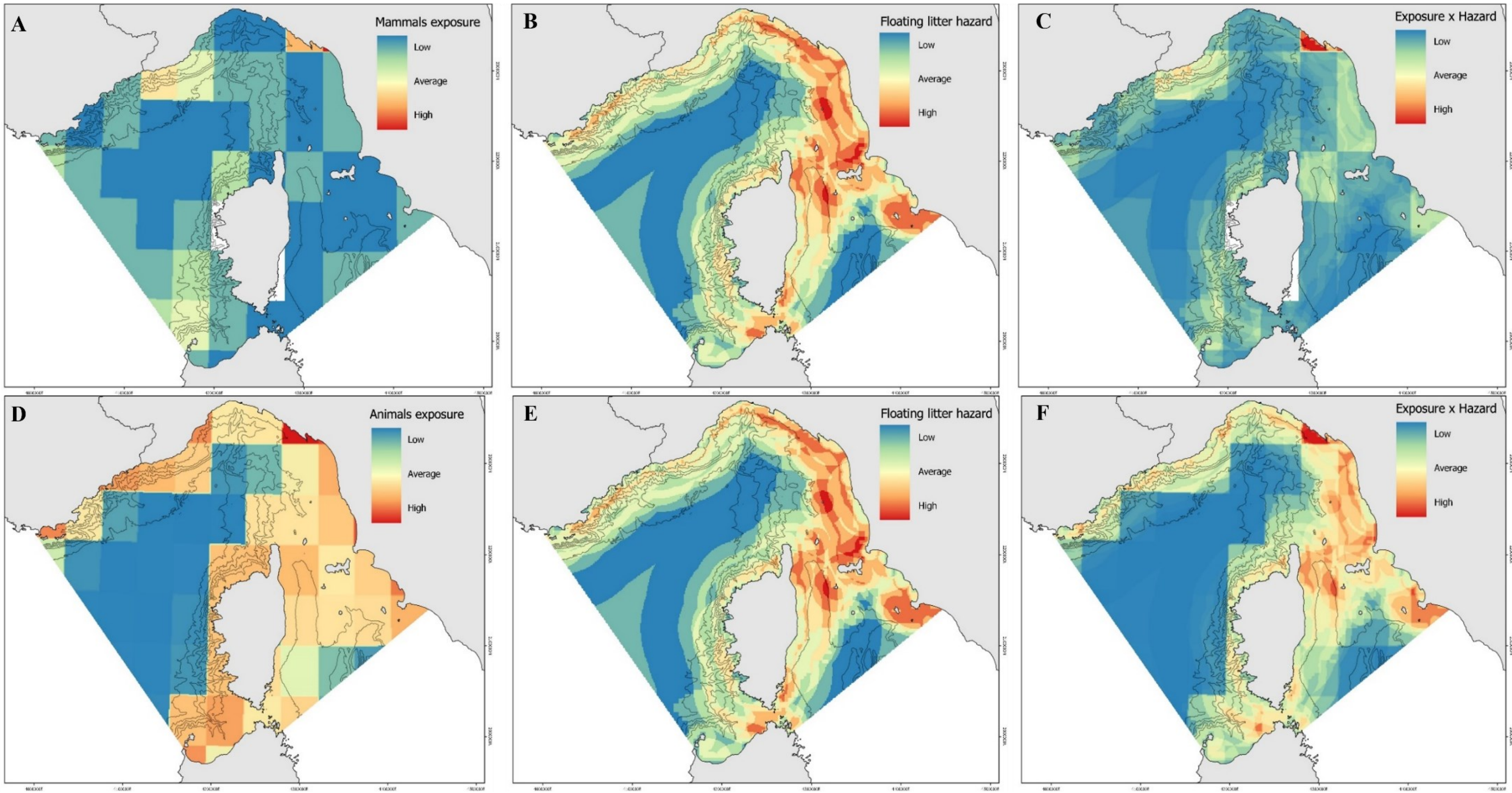


Fig. 72. Species richness of cetacean species (A) and other marine organisms (D) in the Pelagos Sanctuary. Hazard map referring to the sea surface floating macrolitter distribution evaluated during the sampling campaigns in summer 2019 (B and F). Spatial risk assessment for the Pelagos Sanctuary area combining the exposure and hazard maps (C and F).

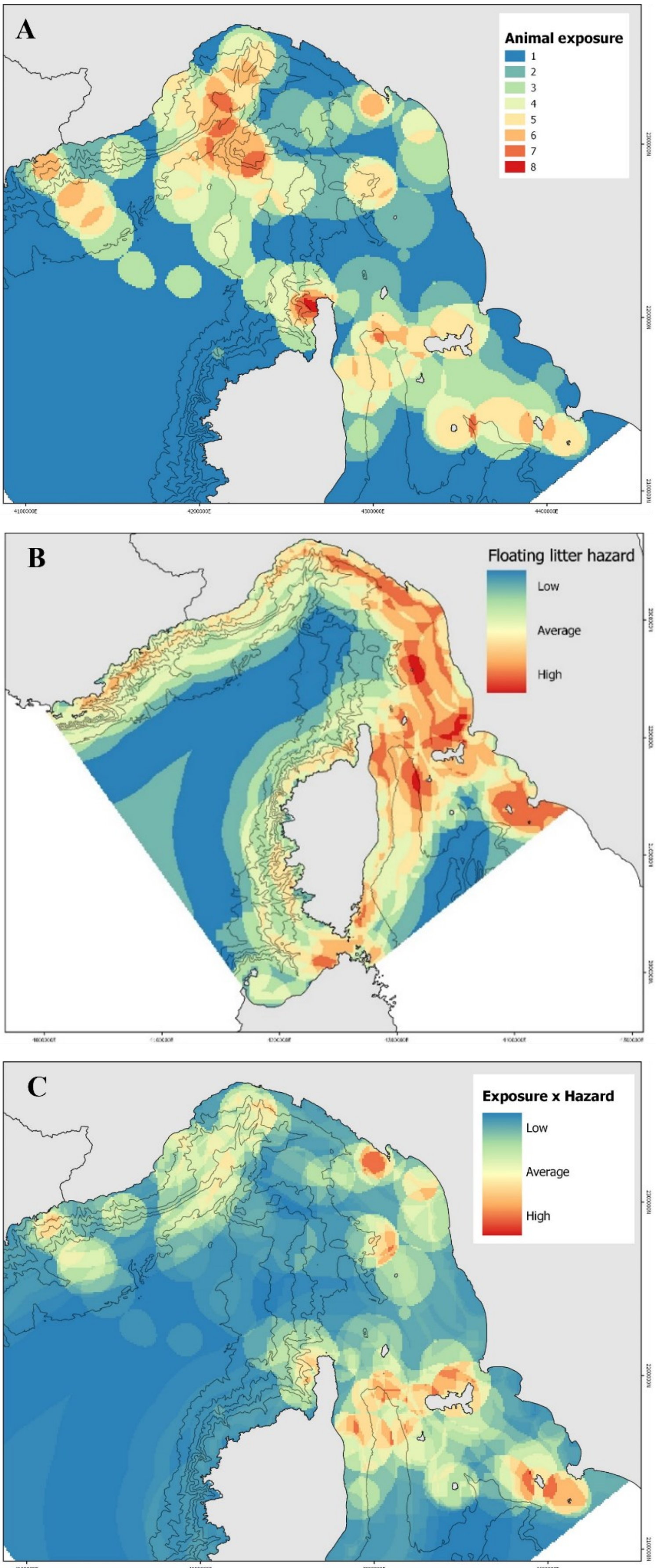


Fig. 73. Species richness according to the H90 distribution area (A) evaluated in the Pelagos Sanctuary during the sampling campaigns in summer 2019. Hazard map referring to the sea surface floating macrolitter distribution (B). Spatial risk assessment for the Pelagos Sanctuary area combining the exposure and hazard maps during the PB MPAs surveys (C).

6.4 Conclusions

The experimental design adopted to simultaneously collect data on floating marine litter and species distribution allowed, for the first time, an assessment of the risk of plastic exposure for several organisms inhabiting the Pelagos Sanctuary protected area. The assessment of species richness confirms the high ecological value of this SPAMI. Seventeen different species belonging to the marine megafauna were detected, providing useful information on their spatial distribution and their suitability to interact with floating plastic litter. The extensive sampling effort and overall dataset collected has been effective in predicting the risk of plastic pollution both at a general level in the Pelgaos Sanctuary and at a small scale focusing only on the areas effectively sampled. Both the risk analysis consistently indicate the Gulf of La Spezia and the Tuscan Archipelago National Park as the areas most affected by the accumulation of plastic litter and at higher risk of exposure. Interestingly, the Genova canyon area and seamount, widely known as an important habitat for various marine species and in particular cetaceans, was highlighted as a risk area for floating plastic litter. This finding, confirmed by the plastic ingestion data and PAE levels detected in several species collected in the SPAMI Pelagos Sanctuary, needs to be taken into account to strengthen protection efforts in this particular area, considering the other threats such as fishing activities, marine traffic and noise pollution that affect this part of the Pelagos Sanctuary. In addition, the species-specific analysis provided further evidence of the threat that plastic pollution can pose to a wide range of organisms, allowing the identification of critical areas and providing the basis for the development of effective protection and mitigation measures to be taken forward.

CHAPTER 7: FINAL CONCLUSIONS

Marine Protected Areas (MPAs) are recognised as an important strategy to minimise the impact of human activities on coastal and marine environments. Despite their special status, MPAs are not exempt from processes that can seriously threaten them, such as the pollution from anthropogenic litter. The present study, developed within the PB MPAs project, provided valuable data on the occurrence, abundance and composition of marine litter floating in surface waters and accumulating on the beaches of the SPAMI Pelagos Sanctuary and the Tuscan Archipelago National Park. In addition, the threats that marine litter poses to different marine species were highlighted by assessing the frequency of plastic ingestion in different bioindicator species and the chemical impacts related to the potential release of toxic substances, such as PAEs. The experimental designs, planned *ad hoc* for the selected study areas, harmonised and implemented the current methods for sampling marine litter in the different environments and defined a new simultaneous multilevel approach reflecting the strong pressure that marine litter, and in particular plastics, exert on organisms inhabiting the protected areas. Strong litter inputs were identified to originate from the mainland and accumulate in coastal waters within about 10-15 nautical miles. Harbours and riverine outfalls may contribute significantly to plastic pollution being identified as the main pathways for the input of litter into the marine environment, as are areas with warmer waters and weak oceanographic features (e.g. continental shelf) that could facilitate the accumulation of litter. The high concentrations of plastics floating on the sea surface and stranded on beaches (exceeding the threshold defined by EU MSFD TG 10) indicate a potentially threatening trend of particle accumulation that may pose a serious risk to organisms living in the Pelagos Sanctuary. As they are mainly objects of secondary origin, their presence indicates potential degradation and fragmentation processes that may favour the formation of small particles, as confirmed by the strong correlation between macrolitter and the spatial distribution of MPs. It has been shown that determining the typology and sources of marine litter is a crucial step towards the elaboration of effective management strategies, although it is still a rather difficult task. Campaigns to raise awareness among tourists, residents and other inland and marine users to change behaviour to reduce the consumption of single-use plastic are essential, especially in areas with heavy touristic fluxes as the Tuscan Archipelago National Park. The dual monitoring approach, simultaneously investigating plastic and MP ingestion in several species and concentrations of plasticizers highlighted the direct potential link between synthetic particle ingestion and toxic substance release. These data will constitute a strong basis for further analysis that will investigate the effects of plastic ingestion and the associated toxic compounds. All species studied, from invertebrates to marine mammals, were affected by the ingestion of plastics and phthalate esters, providing important indications on their sensitivity and attitude to interact with plastic pollution due to their feeding behaviour, long life span and spatial distribution. The reported results showed how the monitoring of phthalate concentration in sentinel species may represent a useful tool to gather information on the potential exposure to plastics in the marine environments, and the differential distribution of phthalates in tissues allows the interpretation of the effects that the metabolic pathways of these substances may have on marine organisms. Finally, the spatial risk assessment has revealed the Gulf of La Spezia and the National Park of the Tuscan Archipelago as the coastal areas most affected by the accumulation of plastic waste and at higher risk of exposure to organisms. In the pelagic realm, the Genova canyon and the seamount area, considered important habitats for various marine species and in particular for cetaceans, are resulted particularly affected by the presence and distribution of floating litter constituting the areas at higher risk of plastic exposure. The information obtained here provided a scientific basis for dealing with plastic pollution in MPAs and could facilitate future recommendations for the management and use of the marine and coastal environment of these protected areas.

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ANNEXES

Annexe 1.
Published peer-reviewed papers evaluating the plastic ingestion on Mediterranean marine organisms.

Taxa	Classes	Order	Species	References
Invertebrates	*Ascidiacea*	*Enterogona*	*Ascidia spp.*	Bonello et al. 2018
	Bivalvia	*Mytilida*	*Mytilus galloprovincialis*	Capo et al., 2021; Avio et al. 2017; Bonello et al. 2018; Digka et al. 2018; Vandermeersch et al. 2015
		Ostreida	*Crassostrea gigas*	Bonello et al. 2018
		Pectinida	*Anomia ephippium*	Bonello et al. 2018
	Hexanauplia	*Harpacticoida*	*n.a.*	Gusmão et al. 2016
	Holothuroidea	*Aspidochirotida*	*Holothuria forskali*	Alomar et al. 2016
			Holothuria tubulosa	Renzi et al. 2018
	Hoplonemertea	*Monostilifera*	*Ototyphlonemertes sp*	Gusmão et al. 2016
	Malacostraca	*Decapoda*	*Aristeus antennatus*	Carreras-Colom et al. 2018
			Carcinus aestuarii	Piarulli et al. 2019
			Nephrops norvegicus	Cristo and Cartes 1998
			Plesionika narval	Bordbar et al. 2018
	Nematoda	*n.a.*	*n.a.*	Gusmão et al. 2016
	Polychaeta	*incerta sedis*	*Claudrilus*	Gusmão et al. 2016
			Megadrilus schneideri	Gusmão et al. 2016
			Meiodrilus gracilis	Gusmão et al. 2016
			Nerilla mediterranea	Gusmão et al. 2016
			Protodrilus albicans	Gusmão et al. 2016
			Protodrilus oculifer	Gusmão et al. 2016
			Saccocirrus pussicus	Gusmão et al. 2016
		Phyllodocida	*Hesionura sp.*	Gusmão et al. 2016
			Syllidae	Gusmão et al. 2016
	Shypozoa	Semaeostomeae	*Pelagia noctiluca*	Albano et al., 2021; Macali et al., 2018
	Turbellaria	*Proseriata*	*Otoplanidae*	Gusmão et al. 2016
Marine mammals	*Mammalia*	*Cetartiodactyla*	*Balaenoptera physalus*	Baini et al. 2017
			Grampus griseus	Corazzola et al., 2021; Baini et al. 2017; Shoham-Frieder et al. 2002
			Physeter macrocephalus	Jerbi et al., 2021; Alexiadou et al., 2019; Cagnolaro et al. 1986; De Stephanis et al. 2013; Mazzariol et al. 2011; Notarbartolo-di-Sciara et al. 2012; Podestà et al. 2015; Roberts 2003; Viale et al. 1992;
			Stenella coeruleolba	Corazzola et al., 2021; Duras et al., 2021; Novillo et al., 2020; Baini et al. 2017; Pribanic et al., 1999
			Tursiops truncatus	Jerbi et al., 2021; Corazzola et al., 2021; Duras et al., 2021; Alexiadou et al., 2019; Baini et al. 2017; Levy et al. 2009
			Delphinus delphis	Alexiadou et al., 2019
			Grampus griseus	Alexiadou et al., 2019; Shoham-Frieder et al.,(2002)
			Globicephala melas	Corazzola et al., 2021
			Ziphius cavirostris	Corazzola et al., 2021; Duras et al., 2021; Cagnolaro et al. 1986

			Phocoena phocoena	Alexiadou et al., 2019; Tonay et al., 2007
Fish	*Chondrichthyes*	*Carcharhiniformes*	*Galeus melastomus*	Pedà et al., 2021; Alomar and Deudero 2017; Anastasopoulou et al. 2013; Capillo et al. 2020; Carrasson et al. 1992; Cartes et al. 2016; Deudero and Alomar 2015; Madurell 2003
			Prionace glauca	Bernardini et al. 2018
			Scyliorhinus canicula	Pedà et al. 2021; Alomar and Deudero 2017; Anastasopoulou et al. 2013; Capillo et al. 2020;
		Chimaeriformes	*Chimaera monstrosa*	Alomar and Deudero 2017
		Lamniformes	*Cetorhinus maximus*	Fossi et al. 2014
		Rajiformes	*Pteroplatytrygon violacea*	Anastasopoulou et al. 2013
			Raja clavata	Pedà et al., 2021; Anastasopoulou et al. 2013
			Raja miraletus	Capillo et al. 2020
			Raja oxyrinchus	Anastasopoulou et al. 2013
		Squaliformes	*Centrophorus granulosus*	Anastasopoulou et al. 2013
			Centroscymnus coelolepis	Cartes et al. 2016; Cliff et al. 2002
			Etmopterus spinax	Pedà et al., 2021; Alomar and Deudero 2017; Anastasopoulou et al. 2013; Carrasson et al. 1992; Cartes et al. 2016; Deudero and Alomar 2015; Madurell 2003
			Squalus acanthias	Anastasopoulou et al. 2013; Avio et al. 2015
	Teleosts	*Anguilliformes*	*Conger conger*	Anastasopoulou et al. 2013
			Nettastoma melanurum	Anastasopoulou et al. 2013; Cartes et al. 2016
		Aulopiformes	*Sudis hyalina*	Anastasopoulou et al. 2013
			Saurida undosquamis	Güven et al. 2017
		Clupeiformes	*Engraulis encrasicolus*	Collard et al. 2015; Collard et al. 2017; Compa et al. 2018; Lefebvre 2019; Renzi et al. 2019; Rios-Fuster 2019
			Sardina pilchardus	Anastasopoulou et al. 2018; Avio et al. 2015; Avio et al. 2020; Compa et al. 2018; Digka et al. 2018; Güven et al. 2017; Lefebvre 2019; Renzi et al. 2019; Rios-Fuster 2019
			Sardinella aurita	Avio et al. 2020
		Gadiformes	*Liza aurata*	Anastasopoulou et al. 2013
			Merluccius merluccius	Anastasopoulou et al. 2013; Avio et al. 2015; Avio et al. 2020; Giani et al. 2019; Mancuso et al. 2019
			Micromesistius poutassou	Anastasopoulou et al. 2013
			Mora moro	Anastasopoulou et al. 2013; Cartes et al. 2016
			Trachyrincus scabrus	Cartes et al. 2016
		Myctophiformes	*Diaphus metopoclampus*	Romeo et al. 2016
			Electrona risso	Romeo et al. 2016
			Hygophum benoiti	Romeo et al. 2016
			Myctophum punctatum	Romeo et al. 2016
		Ophidiiformes	*Cataetyx laticeps*	Cartes et al. 2016
		Osmeriformes	*Alepocephalus rostratus*	Cartes et al. 2016
		Perciformes	*Argyrosomus regius*	Güven et al. 2017

			Boops boops	Tsangaris et al., 2020; Garcia-Garin et al. 2019; Nadal et al. 2016; Rios-Fuster 2019; Sbrana et al. 2020
			Brama brama	Anastasopoulou et al. 2013
			Caranx crysos	Güven et al. 2017
			Coryphaena hippurus	Deudero 1998; Deudero and Alomar 2015; Massuti et al. 1998
			Dentex dentex	Güven et al. 2017
			Dentex gibbosus	Güven et al. 2017
			Epigonus telescopus	Anastasopoulou et al. 2013
			Lepidopus caudatus	Anastasopoulou et al. 2013; Bottari et al. 2019
			Lithognathus mormyrus	Avio et al. 2020; Güven et al. 2017
			Liza aurata	Anastasopoulou et al. 2018; Güven et al. 2017
			Mullus barbatus	Anastasopoulou et al. 2018; Avio et al. 2015; Avio et al. 2020; Bellas et al. 2016; Capillo et al. 2020; Digka et al. 2018; Giani et al. 2019; Güven et al. 2017
			Mullus surmuletus	Alomar et al. 2017; Anastasopoulou et al. 2018; Güven et al. 2017
			Naucrates ductor	Deudero 1998; Deudero and Alomar 2015
			Nemipterus randalli	Güven et al. 2017
			Pagellus acarne	Güven et al. 2017
			Pagellus bogaraveo	Anastasopoulou et al. 2013
			Pagellus erythrinus	Anastasopoulou et al. 2018; Avio et al. 2020; Digka et al. 2018; Güven et al. 2017
			Pagrus pagrus	Güven et al. 2017
			Pelates quadrilineatus	Güven et al. 2017
			Polyprion americanus	Anastasopoulou et al. 2013; Deudero 1998; Deudero and Alomar 2015
			Pomadasys incisus	Güven et al. 2017
			Saurida undosquamis	Anastasopoulou et al. 2013; Deudero 1998; Deudero and Alomar 2015
			Sciaena umbra	Güven et al. 2017
			Scomber japonicus	Anastasopoulou et al. 2018; Güven et al. 2017
			Scomber scombrus	Avio et al. 2020
			Seriola dumerili	Deudero 1998; Deudero and Alomar 2015
			Serranus cabrilla	Güven et al. 2017
			Siganus luridus	Güven et al. 2017
			Sparus aurata	Anastasopoulou et al. 2018; Güven et al. 2017
			Thunnus alalunga	Romeo et al. 2015
			Thunnus thynnus	De la Serna et al. 2012; Karakulak et al. 2009; Romeo et al. 2015
			Trachinotus ovatus	Battaglia et al. 2016
			Trachinus draco	Avio et al. 2020
			Trachurus mediterraneus	Chenet et al., 2021; Anastasopoulou et al. 2018; Deudero 1998; Deudero and Alomar 2015; Güven et al. 2017; Rios-Fuster 2019
			Trachurus picturatus	Anastasopoulou et al. 2018; Avio et al. 2020
			Umbrina cirrosa	Güven et al. 2017
			Upeneus moluccensis	Güven et al. 2017
		Pleuronectiformes	*Upeneus pori*	Güven et al. 2017

			Xiphias gladius	Anastasopoulou et al. 2013; Romeo et al. 2015
			Citharus linguatula	Anastasopoulou et al. 2018
			Solea solea	Anastasopoulou et al. 2018; Avio et al. 2020; Digka et al. 2018; Güven et al. 2017; Pellini et al. 2018
		Scorpaeniformes	*Chelidonichthys lucerna*	Anastasopoulou et al. 2018; Avio et al. 2015; Avio et al. 2020
			Helicolenus dactylopterus	Anastasopoulou et al. 2013; Madurell 2003; Deudero and Alomar 2015
			Scorpaena elongata	Anastasopoulou et al. 2013
			Trigla lucerna	Güven et al. 2017
			Trigla lyra	Capillo et al. 2020
		Tetraodontiformes	*Balistes capriscus or Balistes carolinensis*	Deudero 1998; Deudero and Alomar 2015
			Lagocephalus spadiceus	Güven et al. 2017
		Zeiformes	*Zeus faber*	Bottari et al. 2019
Reptiles	*Reptilia*	*Testudines*	*Caretta caretta*	Biagi et al., 2021; Di Renzo et al., 2021; Digka et al., 2020; Camedda et al. 2014; Campani et al. 2013; Casale et al. 2008; Casale et al. 2016; Domenech et al. 2019; Gramentz 1988; Kaska et al. 2004; Lazar and Graĉan 2011; Matiddi et al. 2017; Revelles et al. 2007; Russo et al. 2003; Tomas et al. 2002
			Chelonia mydas	Russo et al. 2003
			Dermochelys coriacea	Russo et al. 2003
Birds	*Aves*	*Charadriiformes*	Ichthyaetus audouini	Codina-Garcia et al., 2013
			Ichthyaetus melanocephalus	Codina-Garcia et al., 2013
			Larus michahellis	Codina-Garcia et al., 2013
			Rissa tridactyla	Codina-Garcia et al., 2013
			Catharacta skua	Codina-Garcia et al., 2013
		Procellariiformes	*Cory's shearwaters*	Codina-Garcia et al., 2013
		Procellariiformes	*Yelkouan shearwaters*	Codina-Garcia et al., 2013
		Procellariiformes	*Balearic shearwaters*	Codina-Garcia et al., 2013
		Suliformes	*Northern gannets*	Codina-Garcia et al., 2013

Annexe 2.

Floating marine macrolitter sampling sheet according to the Master List of litter categories (Galgani et al., 2013).

Toolkit for monitoring marine litter and its impacts on biodiversity in Mediterranean MPAs

TYPE OF MATERIAL																																												SIZE	COLOR	GPS time hh:mm:ss
ARTIFICIAL POLYMER MATERIALS																				RUBBER					CLOTH/TEXILE					PAPER/ CARDBOARD				PROCESSED/ WORKED WOOD					METAL					CLASSES		
G2	G6	G18	G38	G39	G45	G48	G51	G57	G58	G63	G67	G74	G79	G80	G82	G83	G94	G123	G124	G125	G126	G127	G128	G134	G135	G141	G142	G143	G145	G148	G149	G154	G158	G160	G162	G168	G169	G173	G175	G182	G191	G192	G197	B. 2.5-5 cm; C. 5-10 cm; D. 10-20 cm; E. 20-30 cm; F. 30-50 cm; G. 50-100; H. >100 cm	W. White; T. Transparent; B. Black; C. Cyan/Blue; R. Red; G. Green; Y. Yellow; O. Other	
Bags	Bottles	Crates and containers / baskets	Cover / packaging	Gloves	Mussel nets / Oyester nets	Synthetic rope	Fishing net	Fish boxes - plastic	Fish boxes - expanded polystyrene	Buoys	Sheets, indus. packaging, plastic sheeting	Foam packaging/insulation/polyurethane	Plastic pieces 2.5cm ><50 cm	Plastic pieces >50 cm	Polystyrene pieces 2.5cm ><50 cm	Polystyrene pieces >50 cm	Table cloth	Polyurethane granules <5mm	Other plastic/polystyrene items (identifiable)	Balloons and balloon sticks	Balls	Rubber boots	Tyres and belts	Other rubber pieces	Clothing (clothes, shoes)	Carpet & Furnishing	Rope, string and nets	Sails, canvas	Other textiles (incl. rags)	Cardboard (boxes & fragments)	Paper packaging	Newspapers & magazines	Other paper items	Pallets	Crates	Wood boards	Beams / Dunnage	Other (specify)	Cans (beverage)	Fishing related (weights, hooks, sinkers, lures	Wire, wire mesh, barbed wire	Barrels	Other (metal)			

Annexe 3.

Joint List of categories (Fleet et al., 2021) reporting also the correspondent G-code of Master List (Galgani et al., 2013).

Type-Code	J-Code	G-Code	Name	Definition
CHEMICALS				
ch_nn_drk_	J216		unidentified generally dark-coloured oil-like chemicals	Unidentified generally dark-coloured oil-like chemicals, i.e. no chemical analysis carried out.
ch_nn_lig_	J217		unidentified generally light-coloured paraffin-like chemicals	Unidentified generally light-coloured paraffin-like chemicals, i.e. no chemical analysis carried out.
ch_nn_uch_	J218		unidentified chemicals	Any unidentified chemicals, i.e. no chemical analysis carried out.
Cloth/Textiles				
ct_cl_clg_	J137	G137	clothing	Any type of clothes, garments and headwear made of natural or artificial polymer materials.
ct_cl_ftw_	J138	G138	shoes & sandals made of leather and/or textile	Various types of footwear, such as shoes and sandals made of leather and/or textile.
ct_nn_cpt_	J141	G141	cloth textile carpet & furnishing	Thick woven fabric used for covering the floor or other fabric used for furniture, fittings, and other decorative house accessories such as curtains.
ct_nn_sac_	J140	G140	hessian sacks/packaging	Sacks and other packaging items made of a strong, coarse fabric from hemp or jute (Hessian).
ct_nn_sal_	J143	G143	sails, canvas	A heavy durable cloth made of cotton, hemp, or jute, used for sails, tents, etc.
ct_nn_tex_	J145	G145	other textiles	Other textile items, including pieces of cloth, rags, etc. that are unidentifiable, as well as other identifiable cloth textile items, which do not fit in any other category of this list.
ct_re_bps_	J139	G139	cloth textile backpacks & textile bags	Textile receptacles with an opening at the top, shoulder straps or a handle, used for carrying things.
FOOD WASTE				
fw_	J215	G215	food waste	All types of anthropogenic non-packaged food and food remains.
GLASS/CERAMICS				
gc_co_btc_	J204	G204	glass ceramic construction materials (bricks, tiles, cement)	Any glass and ceramic material which is used for construction purposes such as brick, roof tiles, floor tiles, bricks, cement, etc.
gc_fc_tab_	J203	G203	glass and ceramic tableware (plates/cups/glasses)	Glass or ceramic dishes or dishware used for serving food and dining, plates, cups, glassware, serving dishes and other useful items for practical as well as decorative purposes.
gc_fi_trp_octo_	J207	G207	ceramic or glass octopus pots	Pots made of pottery, weighted with concrete, and typically having a volume of 4 litres. Octopus seeking refuge in the pots can be trapped.
gc_nn_b&c_bott_	J200	G200	glass bottles	Glass or ceramic containers with a narrow neck, used for storing drinks or other liquids. Includes pieces of glass that can be identified as coming from a bottle.

Type-Code	J-Code	G-Code	Name	Definition
gc_nn_b&c_jars_	J201	G201	glass jars	Wide-mouthed cylindrical containers made of glass or pottery, especially used for storing food. Includes pieces of glass that can be identified as coming from a jar.
gc_nn_gfr_	J208	G208	pieces of glass/ceramic (glass or ceramic fragments ≥ 2.5 cm)	Fragments of pottery or glass items that cannot be identified (≥ 2.5 cm).
gc_nn_lit_flbu_	J205	G205	glass fluorescent light tube	A low-pressure mercury-vapour gas-discharge lamp that uses fluorescence to produce visible light.
gc_nn_lit_libu_	J202	G202	glass light bulbs	A glass bulb inserted into a lamp or a socket in a ceiling, which provides light by passing an electric current through a filament or a pocket of inert gas. Includes all types, also halogen, LED, etc.
gc_nn_occ_ocet_	J219		other ceramic items	Other identifiable ceramic items, which do not fit in any other category of this list.
gc_nn_occ_ogli_	J210	G210	other glass items	Other identifiable glass items, which do not fit in any other category of this list.
METAL				
me_co_cab_	J194	G194	metal cables	A thick metal wire or a group of wires usually inside a rubber or plastic covering, which is used to carry electricity or electronic signals.
me_fc_b&c_cans_bevg_	J175	G175	metal drinks cans	Metal containers that are used for storing and selling, e.g. beer or soft drinks.
me_fc_b&c_cans_fcan_	J176	G176	metal food cans	Metal containers that are used for storing and selling food such as beans, soup, fish, corn, etc.
me_fc_tab_	J181	G181	metal tableware (e.g. plates, cups & cutlery)	Metal dishes or dishware used for serving food and dining, including cutlery, plates, cups, serving dishes and other useful items.
me_fi_trp_	J184	G184	metal lobster/crab pots	A portable trap that traps lobsters or crayfish. It can be constructed of wire or metal and netting. An opening permits the lobster or crab to enter a tunnel of netting.
me_fi_wsl_	J182	G182	metal fisheries related weights/sinkers, and lures	fisheries related items such as: weights/sinkers (a metal weight used in conjunction with a fishing lure or hook to increase its rate of sink, anchoring ability, and/or casting distance); lures (any bright artificial bait consisting of metal mounted with hooks and trimmed with feathers.
me_nn_app_	J180	G180	metal appliances (refrigerators, washers, etc.)	Metal (mostly electrical) devices or pieces of equipment designed to perform a specific task such as air conditioners, dishwashers, clothes dryers, freezers, refrigerators, kitchen stoves, water heaters, washing machines, trash compactors, microwave oven, etc.
me_nn_b&c_barl_	J187	G187	metal drums & barrels	Large cylindrical metal containers used for storing or shipping bulk cargo, i.e. oil, chemicals, etc.
me_nn_b&c_cans_aesp_	J174	G174	metal aerosol/spray cans	A type of dispensing system which creates an aerosol mist of liquid particles; used with a can or bottle that contains a payload and propellant under

Type-Code	J-Code	G-Code	Name	Definition
				pressure. Indicative examples of such items are: spray paints, cleaning spray foam, engine oil spray, etc.
me_nn_b&c_cans_ocan_	J188	G188	other metal cans	Other metal containers that are used for storing and selling products that are not food or drinks or paints
me_nn_b&c_cans_ptin_	J190	G190	metal paint tins	Metal containers that are used for paint
me_nn_b&c_lids_	J178	G178	metal bottle caps, lids & pull tabs from cans	Metallic caps and lids from bottles and containers, including the pull tabs from cans
me_nn_bat_	J195	G195	metal household batteries	Small-sized batteries that are typically used in small electronic devices such as flashlights, cameras, etc.
me_nn_foi_	J177	G177	metal foil wrappers, aluminium foil	Thin aluminium sheeting or leaves used, especially, to cover and wrap food.
me_nn_ome_larg_	J199	G199	other metal pieces > 50cm	Other identifiable metal items that are bigger than 50 cm in the longest dimension and do not fit in any other item category of this list.
me_nn_ome_smal_	J198	G198	other metal pieces 2.5cm ≥≤ 50cm	Other identifiable metal items that are smaller than 50 cm in the longest dimension and do not fit in any other item category of this list.
me_nn_srp_	J186	G186	metal industrial scrap	Metal resulting from the disuse of metal products such as parts of vehicles, building supplies, and surplus materials.
me_nn_wir_	J191	G191	wire, wire mesh, barbed wire	A metal mesh woven, knitted, welded, expanded, photo-chemically etched or electroformed steel or other (wire mesh); a metal wire with or without clusters of short, sharp spikes set at short intervals along it, used to make fences.
me_re_bbq_	J179	G179	metal disposable BBQs	A single-use barbecue grill made from lightweight aluminium material.
me_vk_prt_	J193	G193	metal vehicle parts / batteries	Any part of a car or other transport vehicle (i.e., boat) made predominantly of metal, including vehicle batteries. Excluding wheels.
ARTIFICIAL POLYMER MATERIALS /PLASTIC				
pl_ag_ghs_	J220		plastic sheeting from greenhouses	Plastic sheeting used to cover greenhouses generated during the construction, renovation, and demolition. This category is possibly only separable from other plastic sheeting by experienced workers.
pl_ag_irg_	J221		plastic irrigation pipes	Plastic irrigation pipes from agriculture generated during construction, renovation, and demolition.
pl_ag_oag_	J222		other plastic items from agriculture	Other plastic items from agriculture generated during construction, renovation, and demolition.
pl_ag_pot_	J90	G90	plastic flower pots	A plastic container in which plants are grown.
pl_ag_tra_	J223		trays for seedlings of foamed plastic	A foamed plastic tray in which seedlings are grown.
pl_aq_shf_oyst_	J46	G46	plastic oyster trays	A special tray made of square mesh used for growing oysters. Trays may be single, double or triple stacked, with or without feet, doors, v-braces and hooks.
pl_aq_shf_sack_	J45	G45	plastic mussels/oyster mesh bags, net sack, socks	A special bag or sack made of extruded net which is used for growing (underwater) oysters and other shellfish species. These bags can have different sizes and shapes, e.g. sack-like and tubular and the mesh net can have different sizes.

Type-Code	J-Code	G-Code	Name	Definition
pl_nn_pai_	J166	G166	plastic paint brushes	A brush used for painting, typically consisting of bristles fastened into a wooden or plastic handle. Can be made of a mixture of materials including metal.
pl_nn_pen_	J28	G28	plastic pens and pen lids	Any writing or drawing utensils, their parts and lids, made predominately from artificial polymer materials.
pl_nn_rps_rope_	J49	G49	plastic rope (diameter more than 1cm)	A stout cord of strands of plastic fibres twisted or braided together, with a diameter larger than 1 cm.
pl_nn_rps_strg_nodr_	J242		plastic string and cord (diameter less than 1cm) not from dolly ropes or unidentified	A material consisting of threads made of plastic twisted together to form a thin length, with a diameter smaller than 1 cm; excluding string and cord from dolly ropes.
pl_nn_stb_	J66	G66	plastic strapping bands	Plastic bands and straps used for fastening any type of package. Polypropylene and polyester strapping is the most commonly used plastic strapping on the market. Usually made of quite hard plastic.
pl_nn_tag_	J43	G43	plastic tags (fishing, shipping, farming and industry)	Plastic tags used to mark fish and shellfish such as lobsters and plastic cargo seals (pull-tight) both usually with a serial number. Also animal tags from farming.
pl_nn_tap_	J87	G87	plastic masking/duct/packing tape	Different sorts of plastic adhesive tape: used in painting, to cover areas on which paint is not wanted (masking tape); strong cloth-backed waterproof adhesive tape (duct tape); box-sealing tape, parcel tape or packing tape used for closing or sealing corrugated fibreboard boxes.
pl_nn_tel_	J88	G88	telephone	Mobile phone devices and any other type of telephones.
pl_nn_tfk_	J72	G72	plastic traffic cones	Plastic cone-shaped objects that are used to separate off or close sections of a road.
pl_re_div_	J86	G86	plastic fin trees (from fins for scuba diving)	The plastic supports placed inside diving flippers or fins to keep them in shape.
pl_re_fwo_	J243		plastic remains of fireworks	The plastic remains of fireworks such as caps of rockets, covers for fuses, exploding parts of battery fireworks.
pl_re_toy_	J32	G32	plastic toys and party poppers	Any plastic object that children play with, as well as objects commonly used at parties. Party poppers are small devices used as an amusement at parties, which explode when a string is pulled, ejecting thin paper streamers.
pl_sm_but_	J27	G27	tobacco products with filters (cigarette butts with filters)	A cigarette filter, also known as a filter tip, is a component of a cigarette, placed at the one tip of the cigarette in order to absorb vapours and accumulate particulate smoke components. The filter is commonly made from synthetic plastic cellulose.
pl_sm_lht_	J26	G26	plastic cigarette lighters	Small objects that produce a flame, commonly used for lighting cigarettes or cigars.
pl_sm_tob_	J25	G25	plastic tobacco pouches / plastic cigarette packet packaging	Plastic containers (pouches, boxes) used for cigarettes and tobacco.

Type-Code	J-Code	G-Code	Name	Definition
pl_nn_cbt_	J93	G93	plastic cable ties	A cable tie (also known as a wire tie, hose tie, steggel tie, zap strap or zip tie, and by the brand names Ty-Rap and Panduit strap) is a type of fastener, for holding items together, primarily electrical cables or wires.
pl_nn_cds_	J84	G84	plastic CDs & DVDs	Small plastic discs (and their keep cases) on which sound and data can be stored (CDs & DVDs).
pl_nn_cpa_shet_	J67	G67	plastic sheets, industrial packaging, sheeting	Large plastic packaging or sheeting used for the protection or covering/wrapping of large cargo objects. Plastic sheeting is used for a variety of industrial and commercial applications. It comes in many sizes, strengths, styles, and colours depending on the application.
pl_nn_fen_	J64	G64	plastic fenders	Plastic cushions (such as foam rubber) placed between a boat and a dock or between two boats to lessen shock and prevent chafing.
pl_nn_fib_	J68	G68	fibre glass items	Items made of fibreglass, a common type of fibre-reinforced plastic using glass fibre. Examples of fibreglass items include water pipes, pods, domes, traffic lights, pieces of boats etc.
pl_nn_flb_	J63	G63	plastic floats/buoys other source than fishing or not known	Plastic floats/buoys other source than fishing or not known. Floating devices that serve as navigation marks, marking reefs or other hazards, mooring locations. They can be anchored (stationary) or allowed to drift with ocean currents.
pl_nn_fom_nfpy_	J239		other foamed plastic items and fragments not made of foamed polystyrene	Items and fragments not made of foamed polystyrene (other than packaging or insulation related) made out of foamed sponge-like plastic, such as mattresses, bathing sponges, etc.
pl_nn_fom_pain_pack_	J257		foamed plastic packaging	Lightweight cellular foam (mainly foamed PU and PE materials) used as a packing material.
pl_nn_frg_fopy_larg_	J83	G83	fragments of foamed polystyrene > 50cm	Fragments of foamed polystyrene that are bigger than 50 cm in the longest dimension and originate from unidentifiable polystyrene items.
pl_nn_frg_fopy_smal_	J82	G82	fragments of foamed polystyrene 2.5 cm ≥≤ 50 cm	Fragments of foamed polystyrene that are bigger than 2.5 cm and smaller than 50 cm in the longest dimension and originate from unidentifiable polystyrene items.
pl_nn_frg_nofp_larg_	J80	G80	fragments of non-foamed plastic > 50cm	Fragments of plastic that are larger than 50 cm in the longest dimension and originate from unidentifiable plastic non-foamed polystyrene items.
pl_nn_frg_nofp_smal_	J79	G79	fragments of non-foamed plastic 2.5cm ≥≤ 50cm	Fragments of plastic that are bigger than 2.5 cm and smaller than 50 cm in the longest dimension and originate from unidentifiable plastic non-foamed polystyrene items.
pl_nn_idp_idfd_	J240		other identifiable foamed plastic items	Items that are made of foamed polystyrene, which are identifiable but do not fit in any other litter type category in this list.
pl_nn_idp_idnf_	J241		other identifiable non-foamed plastic items	Items that are made of non-foamed artificial polymers, which are identifiable but do not fit in any other litter type category in this list.

Type-Code	J-Code	G-Code	Name	Definition
pl_nn_b&c_eoil_smal_	J14	G14	plastic engine oil bottles & containers 2.5 cm ≥ ≤ 50 cm	Plastic bottles and containers smaller than 50 cm in any dimension, used for packaging motor oil, engine oil, or engine lubricant.
pl_nn_b&c_injn_	J17	G17	plastic injection gun containers/cartridges	A cartridge made of plastic for devices that are used to inject grease, silicone, or other fluids. Includes their nozzles.
pl_nn_b&c_jery_	J16	G16	plastic jerry cans	Large plastic flat-sided containers with a handle used for storing or transporting liquids, typically petrol or water.
pl_nn_b&c_lids_dtgt_	J22	G22	plastic caps/lids chemicals, detergents (non-food)	Plastic caps and lids from bottles and containers of cleaning products (i.e. detergents, toilet cleaners, glass cleaners, etc.) and chemicals.
pl_nn_b&c_lids_olid_	J23	G23	plastic caps/lids unidentified	Plastic caps and lids from unidentified bottles and containers.
pl_nn_b&c_lids_ring_	J24	G24	plastic rings from bottle caps/lids	Plastic structures around the circumference (usually) of the closure that is often found attached below a closure in bottles, jars, and tubs. The bottom part of a cap that breaks off when the cap is screwed off.
pl_nn_b&c_ob&c_	J13	G13	other plastic bottles & containers (drums)	Other plastic bottles and containers such as drums (cylindrical containers) generally used for the transportation and storage of liquids and powders.
pl_nn_bag_cabg_	J3	G3	plastic shopping/carrier/grocery bags	Shopping bags are medium-sized bags, typically around 10–20 litres in volume (though much larger versions exist, especially for non-grocery shopping), that are used by shoppers to carry home their purchases. Shopping bags can be made with a variety of plastics.
pl_nn_bag_dogb_	J101	G101	plastic dog/pet faeces bag	A plastic bag used for picking up and removing the faeces of a dog or other pet.
pl_nn_bag_ends_	J5	G5	the part that remains from tear-off plastic bags	Plastic packing bags are commonly found on the market in packs of 10, 20, 50, etc. This litter item refers to the part that remains after tearing-off the bags.
pl_nn_bag_hdsa_ohds_	J36	G36	other plastic heavy-duty sacks	Non-salt heavy duty plastic sacks for content such as animal feed, fertilizers, garden rubbish, etc.
pl_nn_bag_mesh_vege_	J238		plastic mesh bags for vegetable, fruit and other products	A special mesh bag made out of polypropylene, polyethylene or high-density polyethylene used for packaging and transporting agricultural products such as vegetables, fruit, bird feed, etc.
pl_nn_bag_smbg_	J4	G4	small plastic bags	Small plastic bags refer to small-sized bags such as freezer bags, zip-lock re-sealable food bags, poly bags, etc.
pl_nn_bio_	J91	G91	plastic biomass holder from sewage treatment plants and aquaculture	Plastic Filter Media or Biofiltration Media are small (1-4 cm diameter ca. 1 cm high) usually round plastic items that look a bit like a cake. https://www.bing.com/images/search?q=Plastic+Filter+Media+or+Biofiltration+Media&FORM=HDRSC2
pl_nn_box_	J18	G18	plastic crates, boxes, baskets	Plastic containers typically used to transport or store different types of items and products, other than fisheries and aquaculture related.
pl_nn_buc_	J65	G65	plastic buckets	A roughly cylindrical open container with a handle made of plastic and used to hold and carry liquids

Type-Code	J-Code	G-Code	Name	Definition
pl_hy_com_	J29	G29	plastic combs/hair brushes/sunglasses	Plastic items used for untangling or arranging the hair, as well as plastic glasses tinted to protect the eyes from sunlight or glare.
pl_hy_dap_	J98	G98	plastic diapers/nappies	Basic garments for infants consisting of absorbent synthetic polymer material drawn up between the legs and fastened about the waist, used to retain urine and faeces.
pl_hy_ohy_	J236		other plastic personal hygiene and care items	Other identifiable personal hygiene and care items that do not fit in any other category of this list. Can be made of other materials than artificial polymers.
pl_hy_stt_sant_	J96	G96	plastic sanitary towels/panty liners/backing strips	Sanitary towels/panty liners/backing strips.
pl_hy_stt_tamp_	J144	G144	plastic tampons and tampon applicators	A feminine hygiene product designed to absorb the menstrual flow or a plug of material used to stop a wound or block an opening in the body and absorb blood or secretions. The tampon applicator should be recorded within this category.
pl_hy_tfr_	J97	G97	plastic toilet fresheners	Toilet bowl fresheners, which are attached inside the toilet bowl to keep it smelling fresh.
pl_hy_wws_	J237		plastic wet wipes	A small disposable synthetic cloth treated with a cleansing agent, used especially for personal hygiene.
pl_md_msk_	J253		plastic single-use face-mask	Single-use facemask used to protect against, for example, dust, chemicals and pathogens (e.g., COVID-19 pandemic).
pl_md_omd_	J211	G211	other plastic medical items (swabs, bandaging, adhesive plasters etc.)	Items deemed necessary for the treatment of an illness or injury. These may include swabs, bandaging, adhesive plasters, etc. Can be made of other materials than artificial polymers.
pl_md_pha_	J100	G100	plastic medical/ pharmaceuticals containers/tubes/ packaging	A wide variety of artificial polymer packages used for the packaging of a wide variety of pharmaceutical solids, liquids, and gasses. Some of the common primary plastic packages are: blister packs, small bottles and containers, tubes, ampoules, etc.
pl_md_syg_	J99	G99	plastic syringes/needles	A plastic tube with a nozzle and piston or bulb for sucking in and ejecting liquid in a thin stream, used for cleaning wounds or body cavities, or fitted with a hollow needle for injecting or withdrawing fluids. Includes all parts of syringes (e.g. syringe plunger and the metal needle with plastic adapter) found separately.
pl_nn_b&c_clng_	J9	G9	plastic bottles and containers of cleaning products	Bottles and containers of cleaning products such as detergents, toilet cleaners, glass cleaners, etc.
pl_nn_b&c_eoil_larg_	J15	G15	plastic engine oil bottles & containers >50cm	Plastic bottles and containers bigger than 50 cm in any dimension, used for packaging motor oil, engine oil, or engine lubricant.

Type-Code	J-Code	G-Code	Name	Definition
pl_fi_net_smal_	J53	G53	plastic nets and pieces of net 2.5 cm ≥ ≤ 50 cm	Pieces of plastic open-meshed material made of twine, cord, or something similar, used typically for catching fish; smaller than 50 cm in the longest dimension.
pl_fi_net_strg_drop_	J232		plastic string and filaments exclusively from dolly ropes	Strings and filaments from blue, black or orange string that are used to protect bottom trawling nets against wear and tear. A dolly rope consists of around 30 strings; each string has around 25 threads.
pl_fi_net_strg_fish_	J233		other plastic string and filaments exclusively from fishery	Other string and filaments exclusively from fishery.
pl_fi_net_tang_mixd_	J234		plastic tangled nets and rope without dolly rope or mixed with dolly rope	Tangled pieces of plastic open-meshed material made of twine, cord, or something similar, used typically for catching fish. They may be found tangled with rope or dolly rope.
pl_fi_net_tang_tadr_	J235		plastic tangled dolly rope	Tangles of blue, black or orange rope that are used to protect bottom trawling nets against wear and tear. A dolly rope consists of around 30 strings; each string has around 25 threads. The dolly rope string as well as the separated threads, can occur in tangles in the marine environment. Tangles of dolly rope should consist entirely of dolly rope.
pl_fi_ofi_	J61	G61	other plastic fisheries related items not covered by other categories	Other fisheries related litter items that are not explicitly addressed by the fisheries related items included on this list, e.g. soft and hard plastic baits such as wobblers, spinners, etc.
pl_fi_trp_crab_	J42	G42	plastic crab/lobster traps (pots) and tops	Stationary plastic traps or pots used to catch crustaceans such as lobsters and crabs. Though the size and shape of the traps may vary, most feature a net covering and a cone-shaped entrance tunnel through which a crab or lobster is enticed with bait but cannot escape from.
pl_fi_trp_octo_	J44	G44	plastic octopus pots	Pots made of plastic or PVC tubing, weighted with concrete, and typically having a volume of 4 litres. Octopus seeking refuge in the pots can be trapped.
pl_hu_car_	J70	G70	plastic shotgun cartridges	A shotgun cartridge is a self-contained cartridge often loaded with multiple metallic "shot", which are small, generally spherical projectiles. The shells consist of a plastic tube mounted on a brass base holding a primer. Also plastic wads from shotgun cartridges can be found on their own.
pl_hy_b&c_bech_	J11	G11	plastic beach use related body care and cosmetic bottles and containers	Bottles and containers of body care and cosmetics products used at the beach such as sunscreen, suntan or after sun lotion, etc.
pl_hy_b&c_obch_	J12	G12	plastic non-beach use related body care and cosmetic bottles and containers	Bottles and containers of body care and cosmetics products such as shampoo, shower gel, toothpaste, perfume and others that are not explicitly used at the beach.
pl_hy_cbs_	J95	G95	plastic cotton bud sticks	A short plastic stick with a small amount of cotton on each end that is used for cleaning, especially the ears. The cotton is usually no-longer attached. The ends are rough when touched, where the cotton was attached. This feature can be used to separate from lolly sticks.

Type-Code	J-Code	G-Code	Name	Definition
pl_fc_tab_cups_fcup_	J226		cups and cup lids of foamed polystyrene	Single-use cups and their lids for coffee and other drinks; made of foamed polystyrene. They have a wide range of uses in restaurants, bakeries, or catering settings.
pl_fc_tab_cups_hpcp_	J227		cups and lids of hard plastic	Single-use cups and their lids for coffee and other drinks; made of non-foamed artificial polymer materials. They have a wide range of uses in restaurants, bakeries, or catering settings.
pl_fc_tab_cupt_cutl_	J228		plastic cutlery	Single-use knives, forks, and spoons.
pl_fc_tab_cupt_plat_	J229		plastic plates and trays	Single-use plates and trays made of artificial polymer material.
pl_fc_tab_stst_stir_	J230		plastic stirrers	Stirrers are used when serving hot drinks such as tea and coffee or other drinks such as cocktails.
pl_fc_tab_stst_strw_	J231		plastic straws	A drinking straw or drinking tube is a small pipe that allows its user to more conveniently consume a drink.
pl_fc_wrp_cwls_crsp_	J30	G30	plastic crisps packets/sweets wrappers	Plastic food packets and wrappers created and designed in various colours, materials, shapes, sizes and styles for crisp food products (i.e., potato chips, etc.) or sweets (i.e., chocolates, candy, ice-creams, etc.).
pl_fc_wrp_cwls_loly_	J31	G31	plastic lolly & ice-cream sticks	A plastic stick attached to the bottom of a popsicle/lolly/ice-cream or lollypop used as a handle to facilitate the eating process.
pl_fi_bag_hdsa_salt_	J85	G85	plastic commercial salt packaging	Heavy-duty sacks and other containers used for packaging and shipping salt.
pl_fi_box_fbox_	J58	G58	fish boxes - foamed polystyrene	Boxes made of foamed polystyrene, which are used for packaging fish or other seafood.
pl_fi_box_plbx_	J57	G57	fish boxes - hard plastic	Boxes made of plastic materials (other than expanded polystyrene), which are used for packaging fish or other seafood.
pl_fi_bte_	J92	G92	plastic bait containers/packaging	Plastic packaging (pouches, bags) and plastic containers suitable for storing, transporting, selling fishing baits.
pl_fi_fil_	J60	G60	plastic fishing light sticks / fishing glow sticks incl. packaging	An item that is used by anglers in order to make baits more attractive to fish. Fishing light sticks or glow sticks are typically tubes filled with fluorescent fluid. They can be found in a variety of sizes.
pl_fi_flb_	J62	G62	plastic floats for fishing nets	An item attached to the top of some types of fishing nets, like seine and trammel, that keeps them hanging vertically in the water. Floats come in different sizes and shapes.
pl_fi_lin_	J59	G59	plastic fishing line	A long nylon thread, usually attached to a baited hook, with a sinker or float, and used for catching fish. The fishing line may be found tangled or not and with or without hooks, sinkers and floats.
pl_fi_net_larg_	J54	G54	plastic nets and pieces of net > 50cm	Pieces of plastic open-meshed material made of twine, cord, or something similar, used typically for catching fish; bigger than 50 cm in the longest dimension.

Type-Code	J-Code	G-Code	Name	Definition
pl_aq_shf_tahi_	J47	G47	plastic sheeting from mussel culture (Tahitians)	Pieces of plastic sheeting about 50X40 cm which are cut at one end into fringes or stripes, so they look a little like a grass skirt from Hawaii. They are used to protect mussel cultures from animals that feed on mussels.
pl_cl_ftw_flip_	J102	G102	plastic flip-flops	A light sandal made of plastic, with a thong between the big and second toe.
pl_cl_ftw_shoe_	J136	G136	footwear made of plastic - not flip flops	Items of footwear made of plastic - not flip flops.
pl_cl_glv_hogl_	J40	G40	plastic gloves (household/dishwashing, gardening)	Gloves used to do household chores such as dishwashing, gardening, etc. They are typically made of different polymers, including latex, nitrile rubber, polyvinyl chloride. Less heavy-duty than industrial gloves.
pl_cl_glv_ingl_	J41	G41	plastic gloves (industrial/professional applications)	Gloves specifically dedicated to industrial applications, mechanical, engineering, agriculture, fisheries and aquaculture and construction. They are typically made of different polymers, including latex, nitrile rubber, polyvinyl chloride and neoprene.
pl_cl_glv_sugl_	J252		single-use plastic gloves	Single-use plastic gloves used, for example, in relation to the COVID-19 pandemic.
pl_cl_hdw_helm_	J69	G69	plastic hard hats/helmets	A hard or padded protective hat, various types of which are worn by construction workers, workers from offshore installations, soldiers, police officers, motorcyclists, sports players, and others.
pl_co_fom_pain_insu_	J256		foamed plastic insulation, including spray foam	Lightweight cellular foam (mainly foamed PU and PE materials) used especially for insulation (i.e. in walls, roofs, and foundations as thermal insulation and water barrier). Includes spray foam.
pl_co_oco_	J89	G89	plastic construction waste (not foamed insulation)	Plastic waste materials generated during the construction, renovation, and demolition of buildings or structures. These may include drainage pipes, waste pipes, plastic tubes for cables, etc. Not foamed insulation materials.
pl_fc_b&c_dbot_lage_	J8	G8	plastic drink bottles >0.5 l	Plastic bottles and containers with a volume larger than 0.5 litres, used to hold water, juice or other drinks for consumption.
pl_fc_b&c_dbot_smll_	J7	G7	plastic drink bottles ≤ 0.5 l	Plastic bottles and containers with a volume of 0.5 litres or less, used to hold water, juice or other drinks for consumption.
pl_fc_b&c_ffmd_	J224		plastic food containers made of foamed polystyrene	Foamed polystyrene containers used for carrying or storing food, such as fast food containers, lunchboxes, etc.
pl_fc_b&c_lids_drnk_	J21	G21	plastic caps/lids drinks	Plastic caps and lids from bottles and containers, used to hold water, juice or other drinks for consumption
pl_fc_b&c_pfoc_	J225		plastic food containers made of hard non-foamed plastic	Plastic containers used for carrying or storing food, such as fast-food containers, Tupperware, lunchboxes, etc. Made of non-foamed plastic.
pl_fc_sxp_	J1	G1	plastic 4/6-pack yokes & six-pack rings	Four or six-pack rings or yokes are a set of connected plastic rings that are used in multi-packs of drinks, particularly of drinks cans, to hold the cans together.

Type-Code	J-Code	G-Code	Name	Definition
PAPER/CARDBOARD				
pp_fc_b&c_tpak_milk_	J150	G150	paper cartons/Tetrapak milk	Containers made of carton with a plastic-lining used for milk.
pp_fc_b&c_tpak_otpk_	J151	G151	paper cartons/Tetrapak (non-milk)	Containers made of carton with a plastic-lining used for food products, other than milk.
pp_fc_tab_cups_	J244		paper cups	Cups for coffee and other drinks; made of cardboard. They have a wide range of uses in restaurants, bakeries, or catering settings.
pp_fc_tab_tray_	J245		paper food trays, food wrappers, drink containers	Single-use food trays, food wrappers and drink containers, made of paper.
pp_hy_cbs_	J246		paper cotton bud sticks	A short paper stick with a small amount of cotton on each end that is used for cleaning, especially the ears.
pp_nn_b&c_	J247		other paper containers	Other paper containers.
pp_nn_bag_	J147	G147	paper bags	A small bag made of paper, commonly used as shopping bags, packaging, etc.
pp_nn_box_	J148	G148	cardboard boxes	Boxes made of cardboard (a thick, stiff paper or material containing multiple layers of corrugated paper).
pp_nn_frg_	J156	G156	paper fragments	Fragments of paper items that cannot be identified.
pp_nn_new_	J154	G154	paper newspapers & magazines	Printed publications consisting of paper sheets and containing news, articles, advertisements.
pp_nn_opp_	J158	G158	other paper items	Other identifiable paper and cardboard items, which do not fit in any other category of this list.
pp_re_fwo_	J155	G155	paper tubes and other pieces of fireworks	Small paper/cardboard containers/tubes filled with explosive chemicals that produce bright coloured light patterns or loud noises when they explode (fireworks).
pp_sm_cig_	J152	G152	paper cigarette packets	A rectangular container made of paperboard, used as packaging for cigarettes. It may also include a plastic covering.
RUBBER				
ru_cl_ftw_rubo_	J127	G127	rubber boots	A tall boot that is made of rubber and that keeps the feet and lower legs dry.
ru_hy_con_	J133	G133	rubber condoms (incl. packaging)	A thin rubber sheath, used during sexual intercourse as a contraceptive or as a protection against infection. Within this category also the packaging should be recorded.
ru_nn_bnd_	J131	G131	rubber band (small, for kitchen/household/post use)	A thin, flexible loop that is made of rubber and used to hold things together.
ru_nn_its_rush_	J248		rubber sheet	Rubber sheeting made of rubber (or rubber-like artificial polymer). Rubber sheets are used for varied purposes, e.g.flooring, under shower pans, drainage systems, as lining for water containers and in construction.
ru_nn_oru_	J134	G134	other rubber pieces	Other identifiable rubber pieces, which do not fit in any other category of this list.
ru_nn_tyr_belt_	J249		rubber belts	Rubber belts are elongated rectangular rubber items.

Type-Code	J-Code	G-Code	Name	Definition
ru_re_bln_	J125	G125	rubber balloons	A small, coloured, rubber sack-like object which is inflated with air or gas and then sealed at the neck, used as a child's toy or for decoration. Within this category balloon ribbons, strings, plastic valves and balloon sticks that are or were attached to balloons are included.
ru_re_bls_	J126	G126	rubber balls	A spherical toy ball, usually fairly small, made of elastic material which allows it to bounce against hard surfaces.
ru_vk_its_intu_	J250		rubber inner-tubes	An inflatable usually ring-shaped rubber tube designed for use inside a pneumatic tire.
ru_vk_tyr_tyre_	J251		rubber tyres	Rubber tyres from all types of vehicles.
PROCESSED/WORKED WOOD				
wo_fc_b&c_cork_	J159	G159	wooden corks	A bottle stopper made of cork or a similar material. Note that plastic corks should be recorded under plastic caps and lids
wo_fc_ice_	J165	G165	wooden ice-cream sticks, chip forks, chopsticks, toothpicks	Various wooden sticks, including sticks from ice-creams, small wooden forks from fast food suppliers (chip forks), tapered sticks of wood held together in one hand and used as eating utensils in Asian cuisine (chopsticks), short pointed pieces of wood used for removing bits of food lodged between the teeth (toothpicks).
wo_fi_box_	J164	G164	wooden fish boxes	Boxes made of wood, which are used for storing or transferring fish or other seafood.
wo_fi_trp_	J163	G163	wooden crab/lobster pots	Stationary wooden traps used to catch crustaceans such as lobsters and crabs. Usually covered in a net.
wo_nn_box_	J162	G162	wooden crates, boxes, baskets for packaging	Wooden containers typically used to transport or store different types of items and products. Not fish boxes.
wo_nn_owo_larg_	J172	G172	other processed wooden items > 50cm	Other identifiable processed, worked or treated wooden items larger than 50 cm in the longest dimension, which do not fit in any other category of this list, e.g., planks, boards, beams.
wo_nn_owo_smal_	J171	G171	other processed wooden items 2.5 cm ≥ ≤ 50 cm	Other identifiable processed, worked or treated wooden items smaller than 50 cm in the longest dimension, which do not fit in any other category of this list, e.g. planks, boards, beams.
wo_nn_pal_	J160	G160	wooden pallets	A flat wooden structure on which heavy goods are put so that they can be moved using a fork-lift truck.
wo_re_fwo_	J167	G167	wooden fireworks & matches	A small thin piece of wood or cardboard tipped with flammable chemicals that catch fire with friction (match); any wooden remains of fireworks, e.g. sticks from rockets.

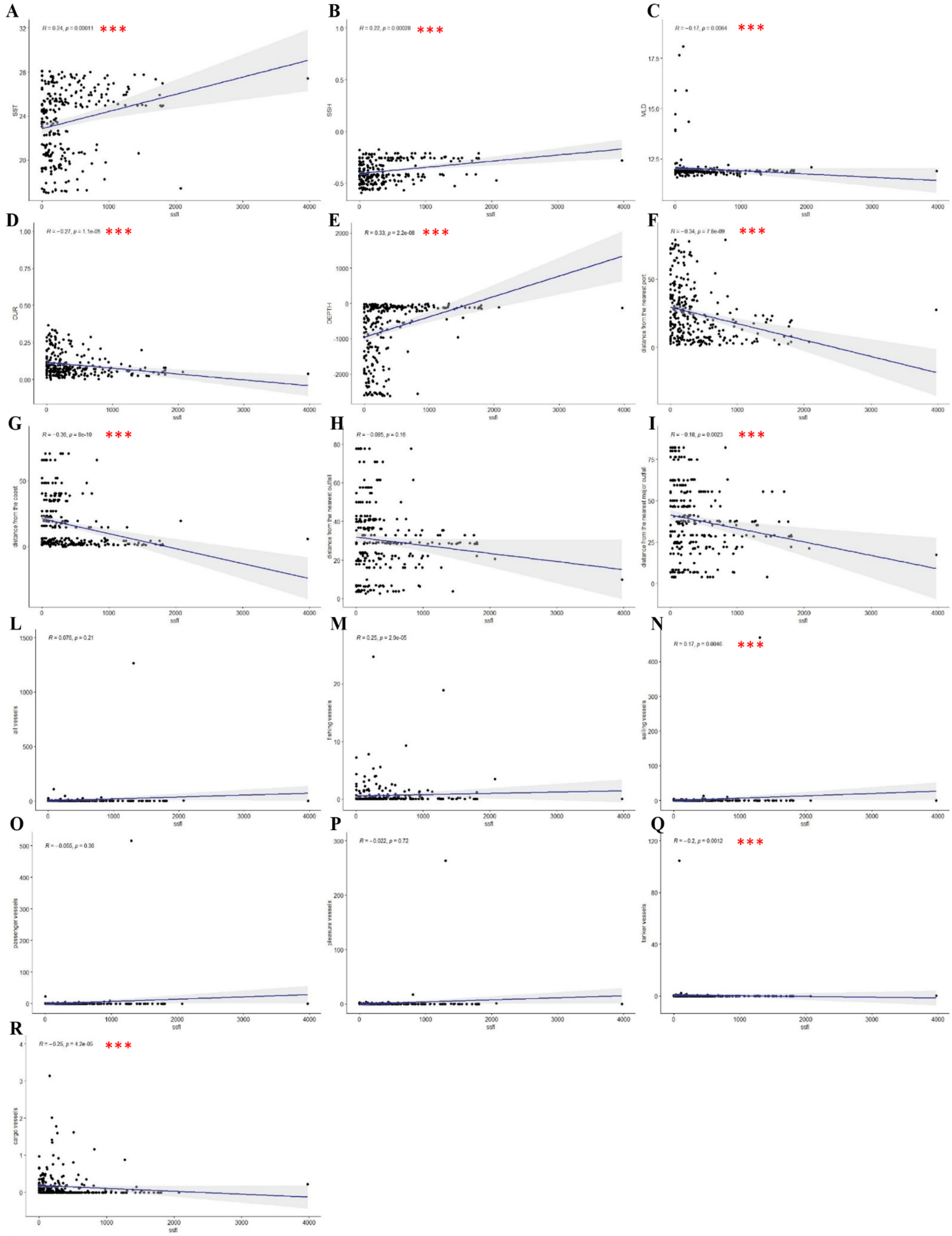
Annexe 4.

Summary descriptive statistics of environmental and anthropic variables and their correlation with floating macrolitter concentration. The strength of Spearman's rank correlation is reported using different shades of blue. Lighter blue corresponds to weak correlations (rho < 0.03), light blue to correlations (0.3< rho <0.5) and blue to strong correlations (rho > 0.5). The statistical significance is reported with different shades of grey. Light grey corresponds to p-values < 0.05, medium grey to p-values < 0.02, dark grey to p-values < 0.01.

Floating macrolitter																		
Descr. statistics	**SST (°C)**	**SSH (m)**	**MLD (m)**	**Curr. velocity (m/s)**	**Bath. (m)**	**Vess. all**	**Vess. fishing**	**Vess. sailing**	**Vess. pleasure**	**Vess. passenger**	**Vess. cargo**	**Vess. tanker**	**Dist. port (km)**	**Dist. coast (km)**	**Dist. outfalls (km)**	**Dist. Big outfalls (km)**	**Dist. Little outfalls (km)**	**Plastic density (items/ km^2)**
mean	23.47	-0.38	12.00	0.10	-723.21	7.59	0.62	2.34	1.45	2.29	0.15	0.46	24.72	16.70	30.20	38.15	46.15	399.01
sd	3.17	0.11	0.68	0.08	843.70	76.93	2.20	28.37	15.97	31.22	0.35	6.34	20.12	18.59	17.37	21.14	32.00	485.84
median	24.48	-0.41	11.89	0.07	-216.60	1.13	0.00	0.26	0.27	0.04	0.00	0.00	19.87	5.98	28.83	37.31	37.86	219.37
trimmed	23.64	-0.38	11.90	0.09	-588.92	1.73	0.17	0.36	0.32	0.12	0.07	0.02	21.90	13.52	28.93	37.14	41.98	305.39
mad	3.57	0.13	0.07	0.05	287.52	1.36	0.00	0.31	0.30	0.06	0.00	0.00	19.36	6.91	16.12	22.43	30.00	248.66
min	17.03	-0.59	11.58	0.00	-2617.4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.38	0.26	2.69	3.94	2.69	0.00
max	28.12	-0.17	18.10	0.37	-3.98	1267.23	24.70	468.95	263.90	515.59	3.14	104.75	78.84	70.59	77.76	82.05	132.49	3,974.3 9
range	11.09	0.42	6.52	0.37	2613.47	1267.23	24.70	468.95	263.90	515.59	3.14	104.75	77.46	70.33	75.07	78.11	129.80	3,974.3 9
skew	-0.41	0.08	6.64	1.31	-1.08	16.09	7.56	16.29	16.22	16.28	4.40	16.31	1.00	1.24	0.63	0.35	0.99	2.61
kurtois	-1.07	-1.23	47.50	0.87	-0.31	260.37	69.53	264.95	263.29	264.56	25.31	265.40	0.15	0.66	0.34	-0.62	0.40	11.09
se	0.19	0.01	0.04	0.00	51.06	4.66	0.13	1.72	0.97	1.89	0.02	0.38	1.22	1.13	1.05	1.28	1.94	29.04
Shapiro-Wilk normality test																		
p-value	1.11 e-06	8.88 e-09	<2.2e-16	1.39e-12	<2.2e-16	<2.2e-16	< 2.2e-16	<2.2e-16	<2.2e-16	< 2.2e-16	<2.2e-16	<2.2e-16	2.45e-10	<2.2e-16	4.01e-05	5.62e-04	7.92e-12	<2.2e-16
Anderson-Darling test																		
p-value	7.22e-12	4.28e-11	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	< 2.2e-16	<2.2e-16	< 2.2e-16	< 2.2e-16	< 2.2e-16	< 2.2e-16	< 2.2e-16	<2.2e-16	7.89e-05	1.46e-06	3.35e-16	<2.2e-16
*Spearman's rank correlation																		
rho	0.24	0.22	-0.17	-0.27	0.33	0.076	0.25	0.17	-0.022	-0.055	-0.24	-0.19	-0.34	-0.36	-0.085	-0.18	-0.12	
p value	0.0001	0.00028	0.0064	1.09e-05	2.22e-08	0.21	2.90e-05	0.004579	0.7224	0.3621	4.23e-05	0.001198	7.80E-09	8.023E-07	0.1605	0.002301	0.04737	

Annexe 5.

Correlation scatterplots among floating macrolitter concentration and environmental (SST (A), SSH (B), MLD (C), Current velocity (D), and Depth (E)) and anthropic (distance from ports (F), distance from the coast (G), distance from rivers outfalls (H and I) and marine traffic (L - R)) factors. The crescent number of * symbol indicates the statistical significance strength (* p-values < 0.05, ** p-values < 0.02, *** p-values < 0.01).



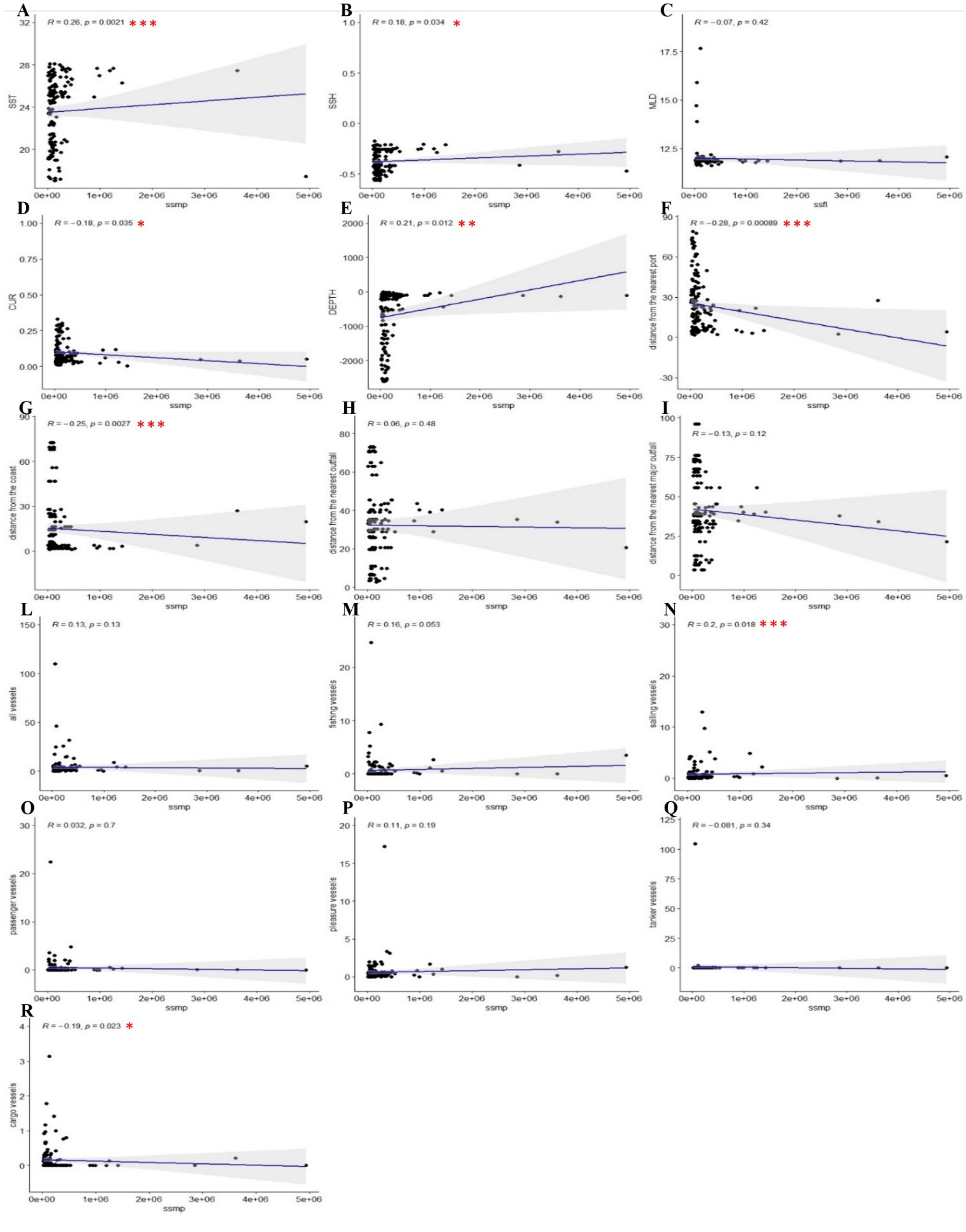
Annexe 6.

Summary descriptive statistics of environmental and anthropic variables and their correlation with MPs concentration. The strength of Spearman's rank correlation is reported using different shades of blue. Lighter blue corresponds to weak correlations (rho < 0.03), light blue to correlations (0.3 < rho < 0.5) and blue to strong correlations (rho > 0.5). The statistical significance is reported with different shades of grey. Light grey corresponds to p-values < 0.05, medium grey to p-values < 0.02, dark grey to p-values < 0.01.

MPs																		
Descr. statistics	**SST (°C)**	**SSH (m)**	**MLD (m)**	**Curr. velocity (m/s)**	**Bath. (m)**	**Vess. all**	**Vess. fishing**	**Vess. sailing**	**Vess. pleasure**	**Vess. passenger**	**Vess. cargo**	**Vess. tanker**	**Dist. port (km)**	**Dist. coast (km)**	**Dist. outfalls (km)**	**Dist. Big outfalls (km)**	**Dist. Little outfalls (km)**	**Plastic density (items/ km^2)**
mean	23.59	-0.38	12.00	0.10	-681.73	3.90	0.68	0.77	0.59	0.45	0.15	0.81	23.94	14.73	32.20	41.21	45.59	259,490
sd	3.21	0.11	0.67	0.08	835.86	10.74	2.41	1.62	1.52	1.99	0.38	8.82	19.94	19.21	19.61	21.80	31.84	586,477
median	24.57	-0.41	11.89	0.07	-173.60	1.33	0.00	0.30	0.32	0.04	0.00	0.00	19.00	3.84	30.38	39.16	37.86	105,195
trimmed	23.78	-0.38	11.90	0.08	-539.13	1.88	0.21	0.39	0.36	0.12	0.06	0.02	21.01	10.20	30.77	40.88	41.43	139,875
mad	3.55	0.14	0.06	0.05	208.67	1.40	0.00	0.40	0.38	0.06	0.00	0.00	19.26	3.38	14.71	16.90	29.28	85,294
min	17.05	-0.57	11.62	0.00	-2,607.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.92	1.19	2.69	3.44	2.69	15,757
max	28.09	-0.17	17.67	0.33	-3.98	109.75	24.70	12.95	17.23	22.47	3.14	104.75	78.84	72.57	73.08	96.13	132.35	4,933,909
range	11.04	0.39	6.04	0.33	2,603.6	109.75	24.70	12.95	17.23	22.47	3.14	104.75	76.92	71.38	70.39	92.69	129.66	4,918,152
skew	-0.43	0.04	6.49	1.32	-1.16	7.47	7.60	4.77	9.37	9.77	4.68	11.61	1.04	1.85	0.55	0.25	0.99	5.67
kurtois	-1.09	-1.27	44.98	0.72	-0.13	66.14	68.61	27.86	98.60	104.03	28.19	133.85	0.24	2.48	-0.33	-0.49	0.47	36.23
se	0.28	0.01	0.06	0.01	70.39	0.90	0.20	0.14	0.13	0.17	0.03	0.74	1.68	1.62	1.65	1.84	2.68	49,390
Shapiro-Wilk normality test																		
p-value	1.627e-03	1.209e-02	<2.2e-16	2.81e-08	3.99e-11	<2.2e-16	<2.2e-16	<2.2e-16	< 2.2e-16	< 2.2e-16	<2.2e-16	< 2.2e-16	2.44e-09	7.78E-13	9.622E-04	0.00014	1.21e-07	<2.2e-16
Anderson-Darling test																		
p-value	2.43e-05	1.57e-04	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	< 2.2e-16	<2.2e-16	< 2.2e-16	< 2.2e-16	<2.2e-16	<2.2e-16	1.40e-09	<2.2e-16	1.19e-07	6.40e-03	3.80e-08	< 2.2e-16
*Spearman's rank correlation																		
rho	0.26	0.18	-0.070	-0.18	0.21	0.13	0.16	0.20	0.11	0.032	-0.19	-0.081	-0.28	-0.25	0.060	-0.13	-0.013	
p value	0.0021	0.034	0.42	0.035	0.012	0.13	0.052	0.017	0.19	0.70	0.023	0.34	0.00085	0.0027	0.48	0.12	0.88	

Annexe 7.

Correlation scatterplots among floating macrolitter concentration and environmental (SST (A), SSH (B), MLD (C), Current velocity (D), and Depth (E)) and anthropic (distance from ports (F), distance from the coast (G), distance from rivers outfalls (H and I) and marine traffic (L - R)) factors. The crescent number of * symbol indicates the statistical significance strength (* p-values < 0.05, ** p-values < 0.02, *** p-values < 0.01).



Annexe 8.

Generalized additive models (GAMs) results for each environmental and anthropic factor potentially influencing the floating macrolitter distribution. The corresponding p-value of each variable and the deviance of data explained (%) were shown. Variables significantly influencing the floating macrolitter concentrations were highlighted in blue. The crescent number of * symbol indicates the statistical significance strength (* p-values < 0.05, ** p-values < 0.02, *** p-values < 0.01).

GAM for environmental variables	p-value	Deviance explained
Floating macrolitter conc. ~ s(sst)	6.45e-05 ***	10.2%
Floating macrolitter conc. ~ s(ssh)	6.14e-06 ***	7.55%
Floating macrolitter conc. ~ s(mld)	0.0597	1.34%
Floating macrolitter conc. ~ s(mld without outliers)	0.221	0.578%
Floating macrolitter conc. ~ s(cur)	6.64e-05 ***	5.9%
Floating macrolitter conc. ~ s(bath)	0.0597	1.34%
GAM for anthropic variables sources	**p-value**	**Deviance explained**
Floating macrolitter conc. ~ s(v_all conc.)	0.071	1.2%
Floating macrolitter conc. ~ s(v_all conc. without outliers)	0.682	0.0652%
Floating macrolitter conc. ~ s(v_fishing conc.)	0.415	0.246%
Floating macrolitter conc. ~ s(v_fishing conc. without outliers)	0.419	0.506%
Floating macrolitter conc. ~ s(v_sailing conc.)	0.0567	1.36%
Floating macrolitter conc. ~ s(v_sailing conc. without outliers)	0.256	0.482%
Floating macrolitter conc. ~ s(v_pleasure conc.)	0.0601	1.3%
Floating macrolitter conc. ~ s(v_pleasure conc. without outliers)	0.273	0.45%
Floating macrolitter conc. ~ s(v_passenger conc.)	0.0642	1.26%
Floating macrolitter conc. ~ s(v_passenger conc. without outliers)	0.383	0.292%
Floating macrolitter conc. ~ s(v_tanker conc.)	0.241	1.44%
Floating macrolitter conc. ~ s(v_tanker conc. without outliers)	0.108	2.06%
Floating macrolitter conc. ~ s(v_cargo conc.)	0.128	2.19%
Floating macrolitter conc. ~ s(v_cargo conc. without outliers)	0.0412 *	3.29%
Floating macrolitter conc. ~ s(d_coast)	1.19e-06 ***	11.8%
Floating macrolitter conc. ~s(d_port)	1.47e-06 ***	8.44%
Floating macrolitter conc. ~ s(d_outfall)	2.42e-05 ***	13.9%
Floating macrolitter conc. ~ s(d_big_outfall)	0.000132 ***	9.02%
Floating macrolitter conc. ~ s(d_little_outfall)	3.96e-05 ***	11%

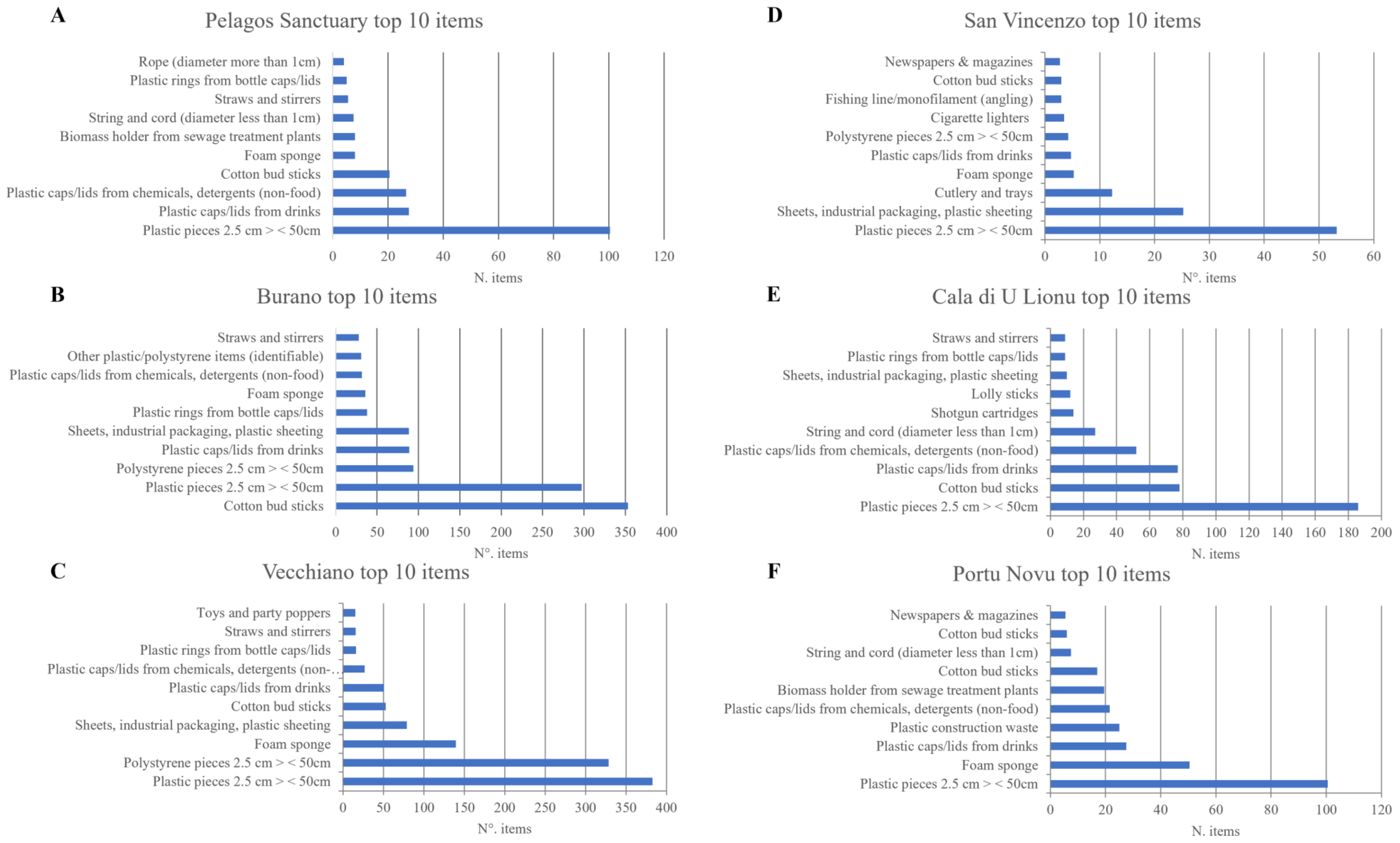
Annexe 9.

Generalized additive models (GAMs) results for each environmental and anthropic factor potentially influencing the MPs distribution. The corresponding p-value of each variable and the deviance of data explained (%) were shown. Variables significantly influencing the MPs concentrations were highlighted in blue. The crescent number of * symbol indicates the statistical significance strength (* p-values < 0.05, ** p-values < 0.02, *** p-values < 0.01).

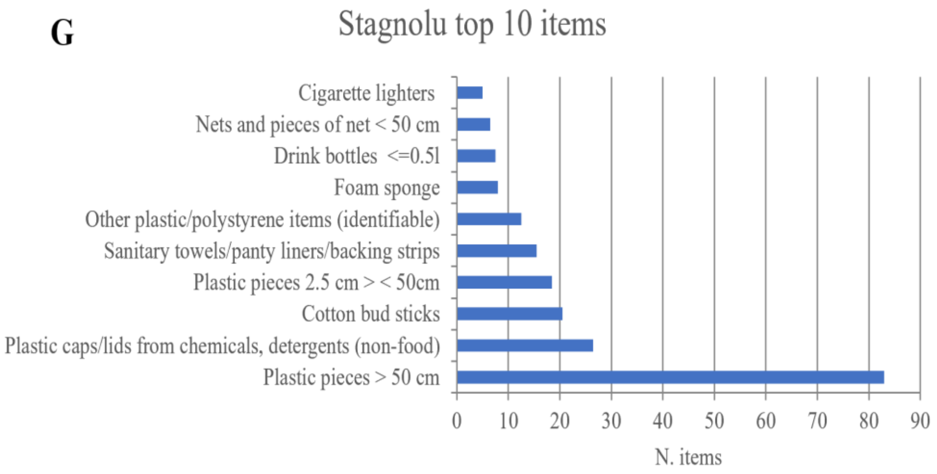
GAM for environmental variables	p-value	Deviance explained
MPs concentration ~ s(sst)	0.0278 *	7.97%
MPs concentration ~ s(ssh)	0.217	1.14%
MPs concentration ~ s(mld)	0.609	0.197%
MPs concentration ~ s(mld without outliers)	0.439	0.464%
MPs concentration ~ s(cur)	0.0647	2.53%
Floating litter density ~ s(bath)	0.0242 *	3.61%
GAM for pollution sources	**p-value**	**Deviance explained**
MPs concentration ~ s(v_all density)	0.844	0.0297%
MPs concentration ~ s(v_all density without outliers)	0.112	1.93%
MPs concentration ~ s(v_fishing density)	0.298	2.9%
MPs concentration ~ s(v_fishing density without outliers)	0.283	0.872%
MPs concentration ~ s(v_sailing density)	0.644	0.155%
MPs concentration ~ s(v_sailing density without outliers)	0.521	0.303%
MPs concentration ~ s(v_pleasure density)	0.593	0.209%
MPs concentration ~ s(v_pleasure density without outliers)	0.227	1.08%
MPs concentration ~ s(v_passenger density)	0.645	0.154%
MPs concentration ~ s(v_passenger density without outliers)	0.572	0.237%
MPs concentration ~ s(v_tanker density)	0.715	0.0981%
MPs concentration ~ s(v_tanker density without outliers)	0.509	0.322%
MPs concentration ~ s(v_cargo density)	0.478	0.364%
MPs concentration ~ s(v_cargo density without outliers)	0.469	0.388%
MPs concentration ~ s(d_coast)	0.455	0.402%
MPs concentration ~s(d_port)	0.0237 *	3.64%
MPs concentration ~ s(d_outfall)	0.236	3.06%
MPs concentration ~ s(d_big_outfall)	0.250	2.9%
MPs concentration ~ s(d_little_outfall)	0.402	0.507%

Annexe 10.

Top 10 items in each beach monitored within the Pelagos Sanctuary.



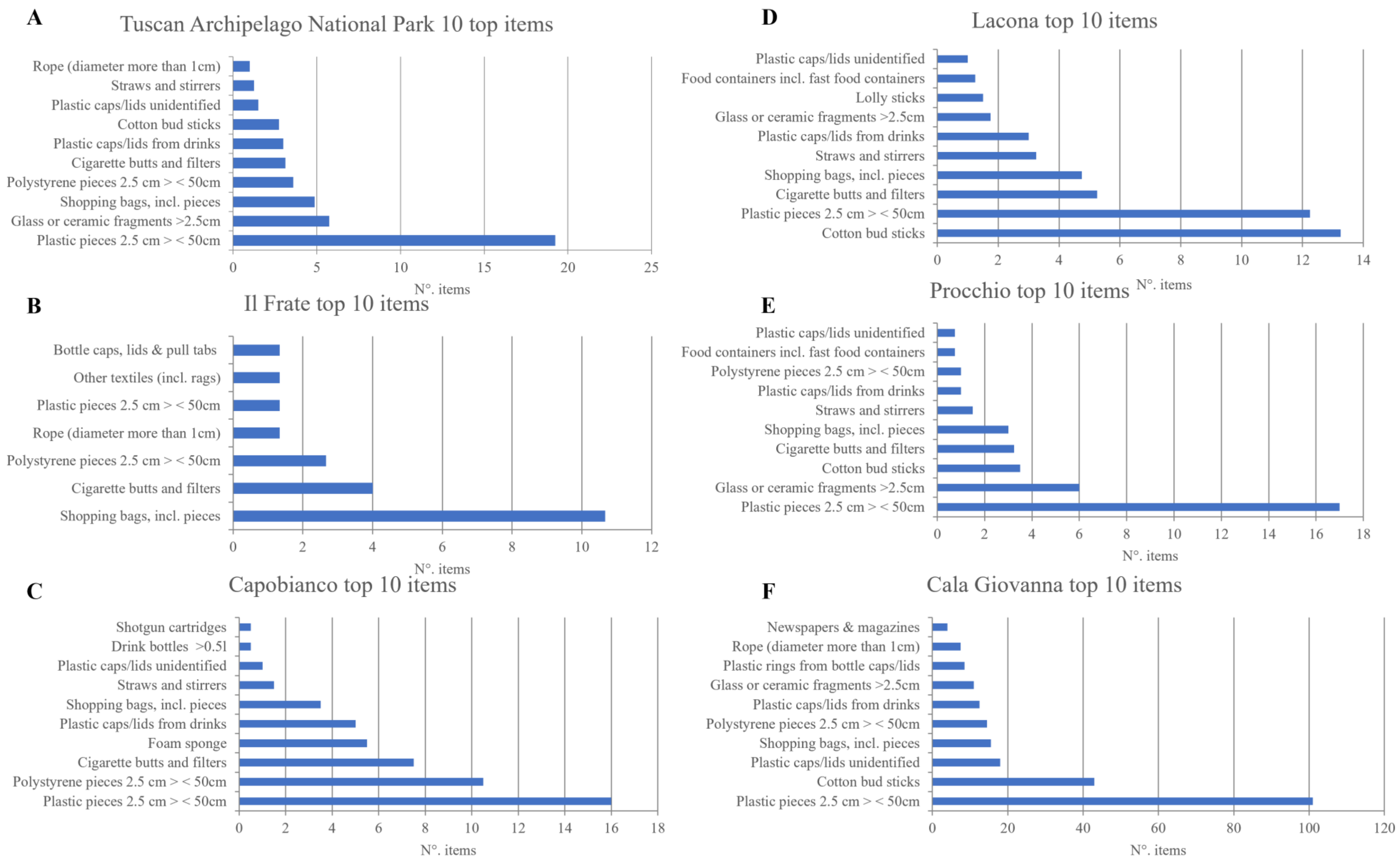
G



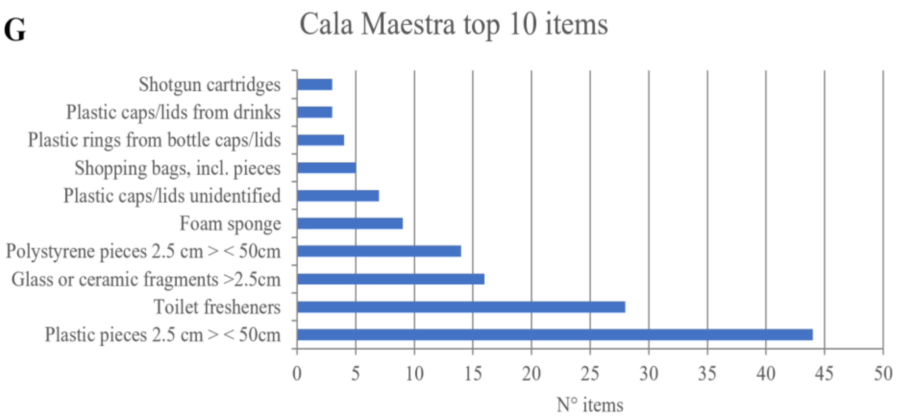
Stagnolu top 10 items
Cigarette lighters
Nets and pieces of net < 50 cm
Drink bottles <=0.5l
Foam sponge
Other plastic/polystyrene items (identifiable)
Sanitary towels/panty liners/backing strips
Plastic pieces 2.5 cm > < 50cm
Cotton bud sticks
Plastic caps/lids from chemicals, detergents (non-food)
Plastic pieces > 50 cm
0 10 20 30 40 50 60 70 80 90
N. items

Annexe 11.

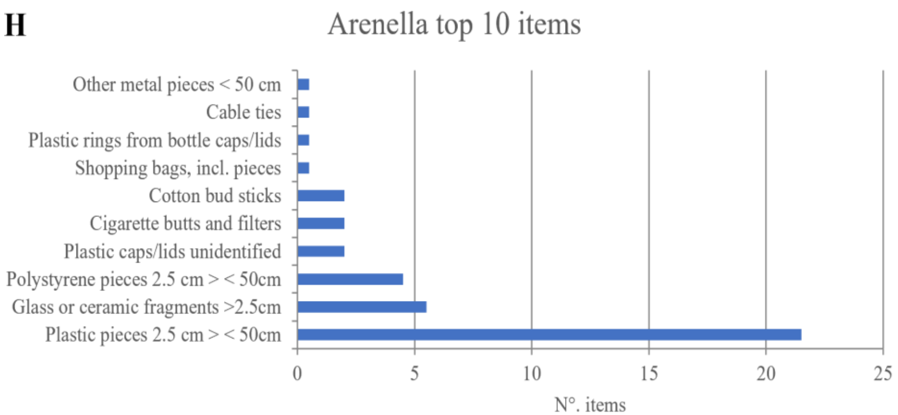
Top 10 items in the Tuscan Archipelago National Park and each beach monitored.



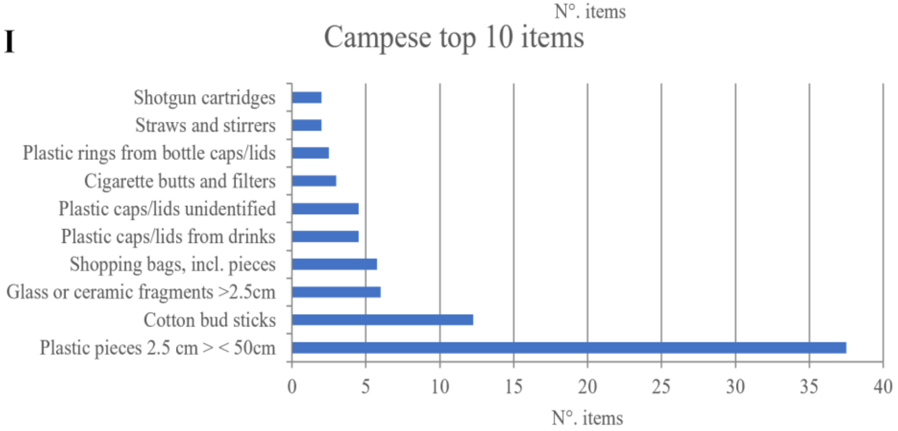
G


Cala Maestra top 10 items
Shotgun cartridges
Plastic caps/lids from drinks
Plastic rings from bottle caps/lids
Shopping bags, incl. pieces
Plastic caps/lids unidentified
Foam sponge
Polystyrene pieces 2.5 cm > < 50cm
Glass or ceramic fragments >2.5cm
Toilet fresheners
Plastic pieces 2.5 cm > < 50cm
0 5 10 15 20 25 30 35 40 45 50
N° items

H

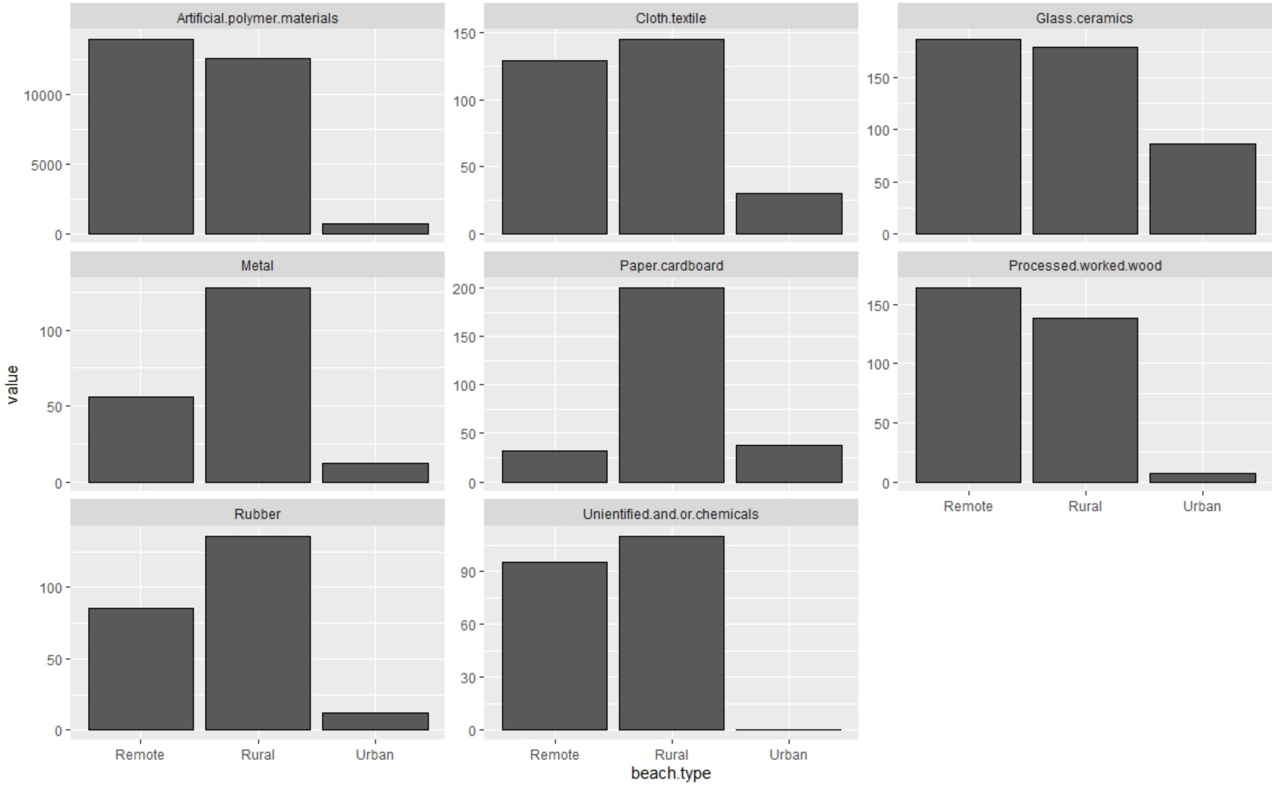

Arenella top 10 items
Other metal pieces < 50 cm
Cable ties
Plastic rings from bottle caps/lids
Shopping bags, incl. pieces
Cotton bud sticks
Cigarette butts and filters
Plastic caps/lids unidentified
Polystyrene pieces 2.5 cm > < 50cm
Glass or ceramic fragments >2.5cm
Plastic pieces 2.5 cm > < 50cm
0 5 10 15 20 25
N°. items

I


Campese top 10 items
Shotgun cartridges
Straws and stirrers
Plastic rings from bottle caps/lids
Cigarette butts and filters
Plastic caps/lids unidentified
Plastic caps/lids from drinks
Shopping bags, incl. pieces
Glass or ceramic fragments >2.5cm
Cotton bud sticks
Plastic pieces 2.5 cm > < 50cm
0 5 10 15 20 25 30 35 40
N°. items

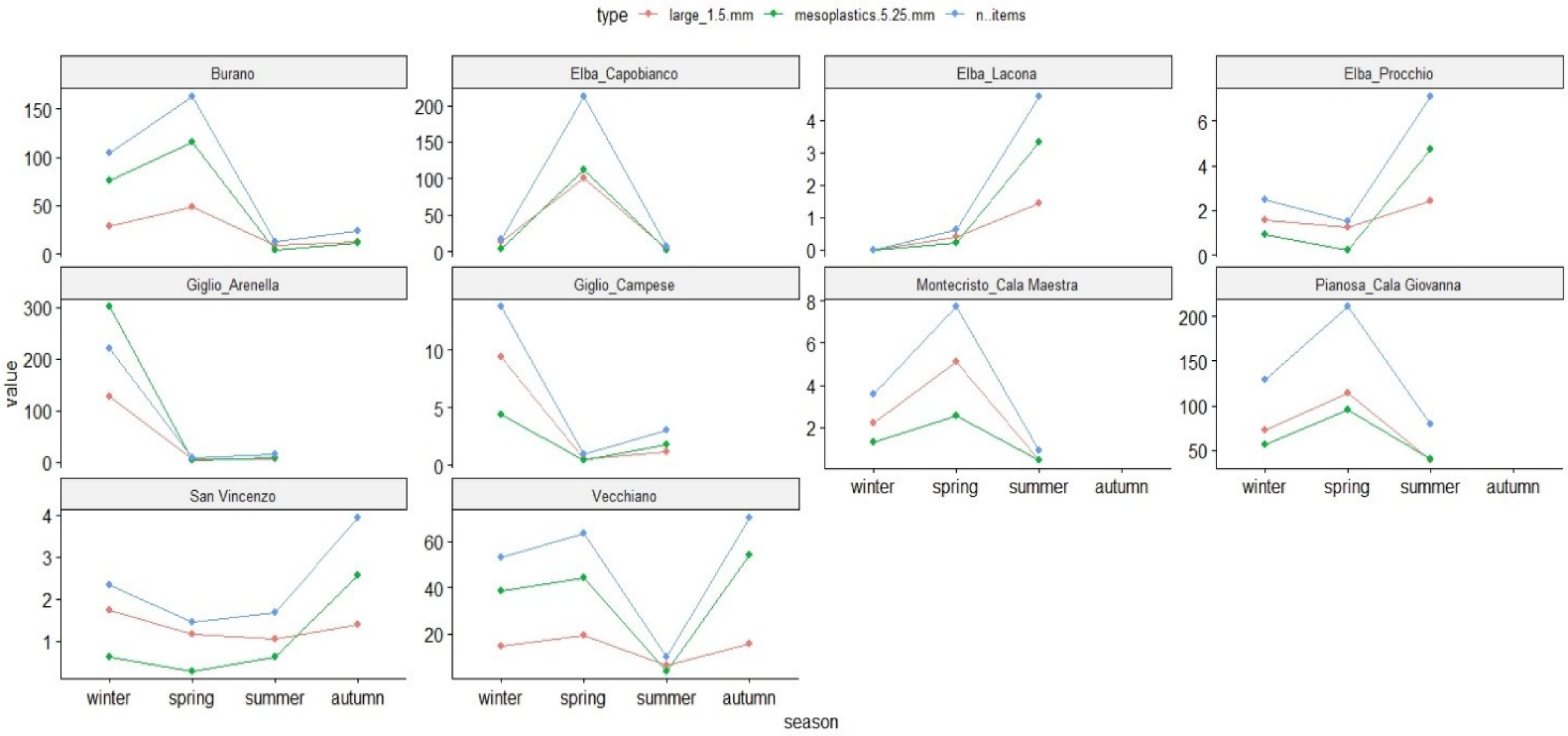
Annexe 12.

Number of items according to different materials collected for each beach type.



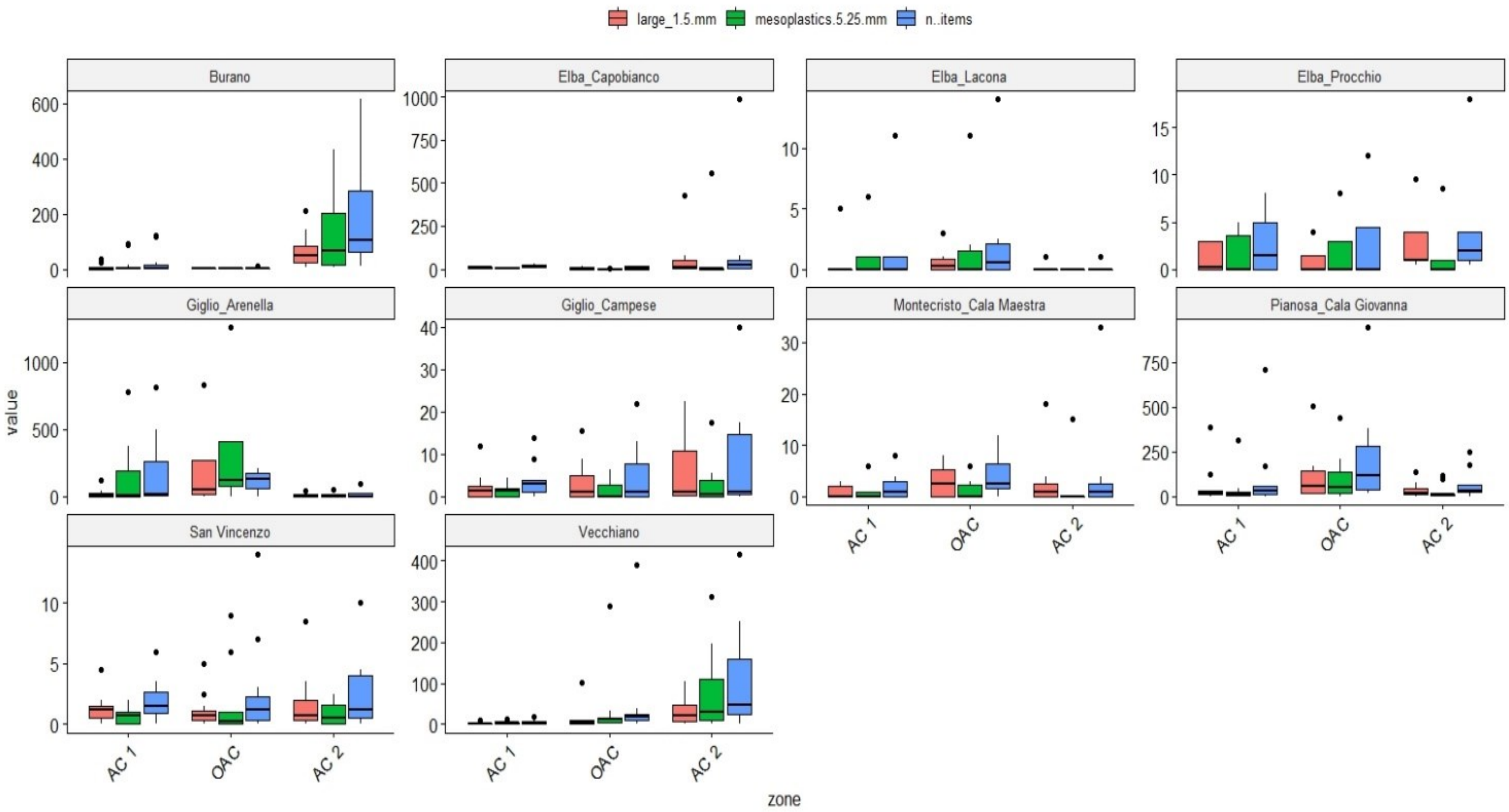
Annexe 13.

Seasonal differences in MPs distribution among the beaches monitored in the whole study area.



Annexe 14.

The number of items (total, mesoplastics and MPs) in the monitored beaches according to different accumulation zones considered.



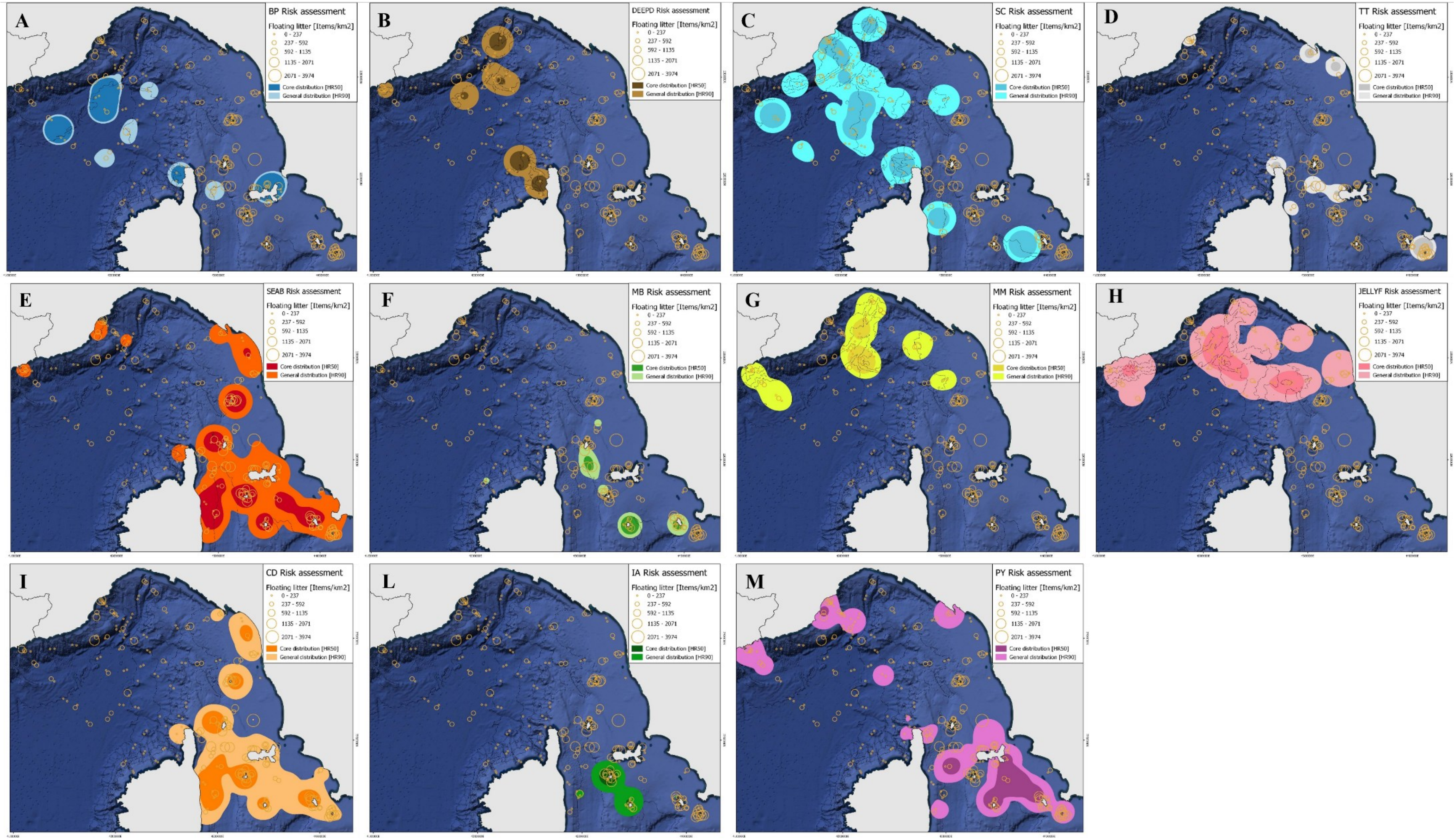
Annexe 15.

PAEs physiochemical properties: quantifier and qualifier ion (m/z), molecular weight (g/mol), number of carbon atom per chain, octanol−water partitioning (K_{OW}), octanol-air partition (K_{OA}) and air−water partitioning (K_{AW}).

PAEs compound	CAS number	Quantifier ion (m/z)	Qualifier ion (m/z)	Molecular weight (g/mol)	Carbon atom per chain	$logK_{OW}$	$logK_{OA}$	$logK_{AW}$
Dimethyl phthalate_DMP	131-11-3	163	194	194.2	1	1.61	7.01	-5.40
Diethyl phthalate_DEP	84-66-2	149	177	222.2	2	2.54	7.55	-5.01
Diallyl phthalate_DAP	131-17-9	149	189	246.3	3	3.11	7.87	-4.76
Dipropyl phthalate_DPrP	131-16-8	149	191	250.3	3	3.4	8.04	-4.64
Diisobutyl phthalate_DIBP	84-69-5	149	223	278.4	4	4.27	8.54	-4.27
Dibutyl phthalate_DBP	84-74-2	149	223	278.4	4	4.27	8.54	-4.27
Benzyl butyl phthalate_BBzP	85-68-7	149	206	312.4	4,6	4.7	8.78	-4.08
Dicyclohexyl phthalate_DCHP	84-61-7	149	167	330.4	6	6.2		
Bis(2-ethylhexyl) phthalate_DEHP	117-81-7	149	113	390.6	8	7.73	10.53	-2.8
Di-n-octyl phthalate_DNOP	117-84-0	149	279	390.6	8	7.73	10.53	-2.8
Diisononyl phthalate_DINP	28553-12-0	293	127	418.6	9	8.6	11.03	-2.43

Annexe 16.

Sensitivity maps for *Balaenoptera physalus* (BP) (A), Deep diver cetacean species (DEEPD) (B), *Stenella coeruleoalba* (SC), *Tursiops truncatus* (TT), seabirds (SEAB) (E), *Mobula mobula* (MB) (F), *Mola mola* (MM) (G), Jellyfish (JELLYF) (H), *Calionectris diomedea* (CD) (I), Audouin's gull (IA) (L) and Puffinus yelkouan (PY) (M). General and core distribution areas of the sighted species that overlapped with the density of the sea surface macrolitter in the study area.



Annexe 17.

Summary of the mean concentration (items/km^2) and standard deviation (sd) of sea surface floating litter and MPs measured in transects overlapping the home range (HR90) and core area (HR50) distribution. Values higher than the overall mean concentration found in the Pelagos Sanctuary were highlighted.

Species	Sea surface floating macrolitter Mean (sd) – Items/km^2			MPs Mean (sd) – Items/km^2		
	HR90	HR50	Overall Pelagos	HR90	HR50	Overall Pelagos
Fin whale (bp)	337 (332)	315 (333)	399 (486)	333,004 (427,767)	366,892 (465,194)	259,489 (586,476)
Deep divers (deepd)	168 (195)	204 (189)		119,374 (93,078)	87,964 (48,093)	
Striped dolphin (sc)	253 (332)	224 (333)		208,495 (700,302)	360,962 (1,142,531)	
Bottlenose dolphin (tt)	599 (593)	784 (625)		449,550 (1,000,097)	187,215 (104,266)	
Giant devil ray (mb)	553 (522)	489 (492)		251,675 (307,707)	191,945 (261,693)	
Sunfish (mm)	214 (185)	179 (160)		121,992 (89,946)	118,851 (30,434)	
Seabirds (seab)	523 (484)	510 (480)		282,667 (410,033)	250,158 (489,132)	
Scopoli's shearwater (cd)	559 (568)	524 (502)		329,329 (565,761)	259,069 (495,653)	
Yelkouan shearwater (py)	476 (571)	435 (432)		386,384 (814,068)	211,338 (232,855)	
Audouin's gull (ia)	535 (494)	616 (524)		218,835 (239,688)	255,563 (293,783)	
Jellyfish (jellyf)	228 (215)	157 (153)		115,188 (83,987)	100,619 (48,949)	