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Review

Detection of anthropogenic fibres in marine organisms: Knowledge gaps and methodological issues

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ABSTRACT

Most studies examining the occurrence of plastics and microplastics in marine organisms have identified anthropogenic fibres, of natural and synthetic origin, as the most commonly occurring category. Anthropogenic fibres may have been chemically treated with additives making them more persistent and a potential threat to marine organisms. However, fibres have often been excluded from analytical data for the difficulties related to the sampling and analytical procedures, including potential overestimation of the results due to airborne contamination. This review aimed to collect and analyse all studies focusing on the interaction between anthropogenic fibres and marine organisms worldwide, highlighting critical issues that need to be overcome for the analysis fibres on marine organisms. Furthermore, emphasis was placed on the species studied in the Mediterranean Sea, which is particularly affected by this type of pollution. Overall, this review shows that fibre pollution is an underestimated threat to marine organisms and that a specific, harmonised protocol for the analysis of different anthropogenic fibres needs to be developed.

1. Introduction

Marine litter pollution is one of the biggest threats to the marine environment and consists mainly of plastic. Starting from the study by Thompson (2004) the scientific community began to focus on the smaller plastics, microplastics, and fibres that could pose a threat to marine ecosystems. The first article reporting the ingestion of microplastics by marine organisms (planktivorous fish) was published in 2010 (Boerger et al., 2010). Since then, many other studies have been conducted on the interaction of marine organisms with microplastics. Due to their small size, microplastics can be easily ingested by marine organisms (Kühn and van Franeker, 2020) and can be excreted or accumulated, causing many different effects (Guzzetti et al., 2018). Plastics isolated from the environment and marine organisms are generally characterised by different dimensions, colours, shapes, and polymers (Probst et al., 2013).

Most studies examining the occurrence of plastics and microplastics in the marine environment and organisms have identified anthropogenic fibres as the most common category (Compa et al., 2018; Gago et al., 2016; Giani et al., 2019; Mathalon and Hill, 2014; Suaria et al., 2020).

The main sources of anthropogenic fibres in the marine environment are household laundry, textile and tyre industries, fragmentation of large plastic items, illegal dumping, and landfills; it is estimated that about 5 million tonnes of fibres enter the oceans each year (Carr, 2017; Mishra et al., 2019). The results of various studies have shown that each washing cycle of 1 kg of synthetic textiles releases 23,333–116,666 microfibres (Yang et al., 2019). Although the number of fibres in the environment will increase with the expected increase in the production of fibres, especially textile fibres, in the coming years (Mishra et al., 2019; Textile Exchange, 2021), there will be a lack of technologies to control fibre pollution (Kwak et al., 2022). The most produced category of textile fibres since 1990 is synthetic fibres, which are mainly composed of polyester and polyamide and accounted for 62 % of all fibres produced in 2020. The second most important fibre in terms of volume is cotton (a natural fibre) and man-made cellulosic fibres (MMCF), an increasingly important category composed mainly of viscose, with a global production volume of about 6.5 million tonnes (Textile Exchange, 2021).

Despite their natural origin, fibres may have been chemically treated with dyes, additives, and flame retardants. These treatments can alter the time of natural degradation and durability in the environment, making them more durable and posing a potential hazard to marine

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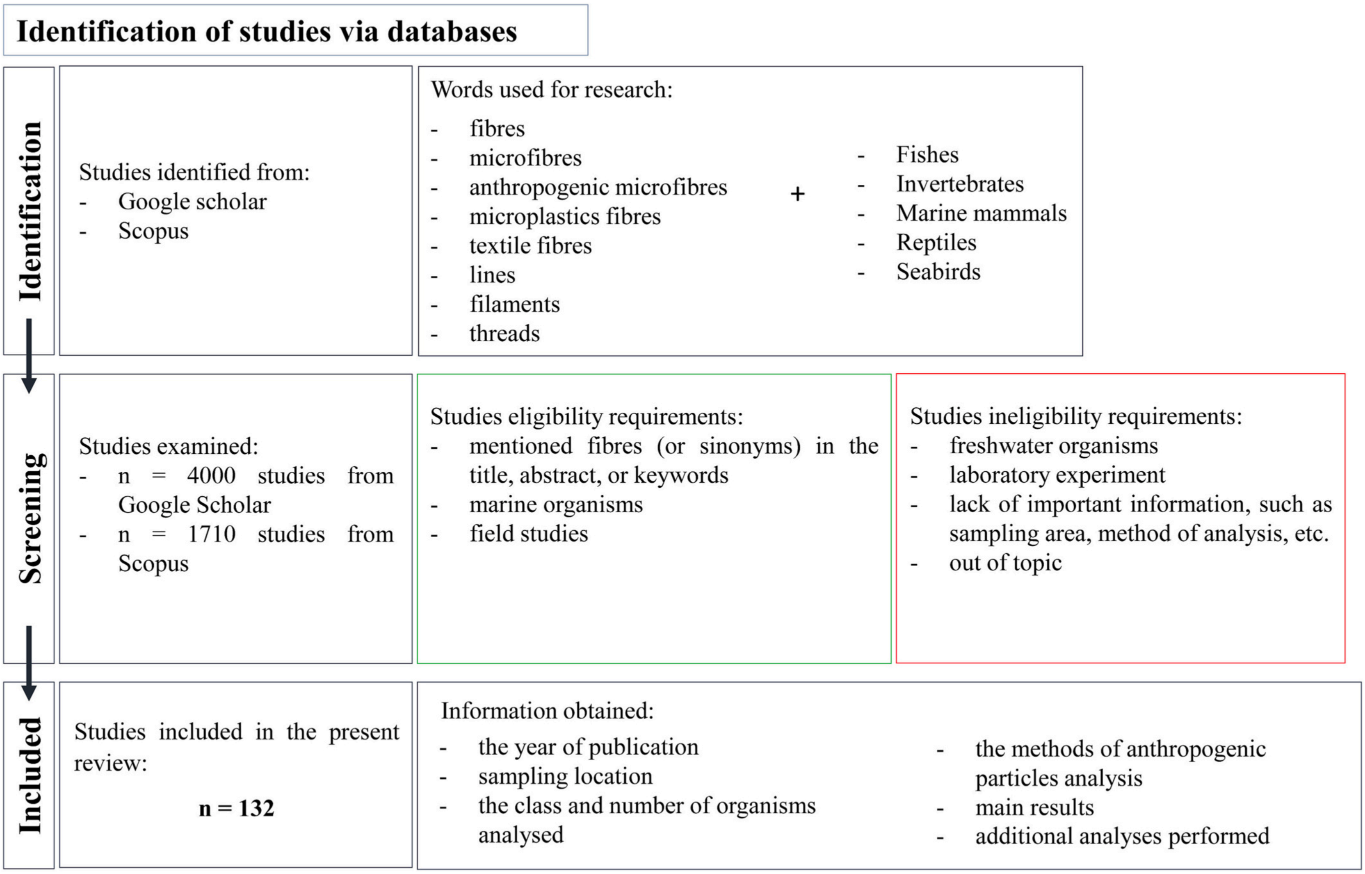

Fig. 1. The diagram graphically represents the main steps used to select the studies for this review.

organisms.

Due to limitations in on-field sampling, isolation, and chemical characterization from biological samples, and potential overestimation associated with airborne contamination during both on-field and laboratory activities, fibres were very often excluded by analytical data and/or poorly characterised, especially in the less recent articles (Avio et al., 2015; Waldschläger et al., 2020). Non characterization of fibres could lead to an incorrect estimate of the determination of the origin of this type of pollution, often associated exclusively with plastic ones (Athey and Erdle, 2022; Rebelein et al., 2021).

Recent studies have shown that not all anthropogenic fibres found in the marine environment and organisms are synthetic: fibres of natural origin have also been observed (Compa et al., 2018; Suaria et al., 2020). A study to characterise the polymeric nature of fibres sampled in surface waters around the world showed that fibre pollution is widespread worldwide and that the Mediterranean Sea is particularly affected by this type of pollution. It became clear that most of these fibres are not made of plastic but have a natural origin, such as cellulose (Suaria et al., 2020). Some researchers have begun to distinguish synthetic fibres from natural ones (Avio et al., 2020; Capillo et al., 2020; Compa et al., 2018; Savoca et al., 2019), and to develop specific methods to effectively isolate and characterise fibres from the environment and organisms (Corami et al., 2022; Tamminga et al., 2019), although a single, harmonised protocol that allows for comparable data has not yet been defined. In addition, there is much uncertainty about the terminology that should be used to define this form of particle. Fibres are also defined as microfibres and anthropogenic microfibres (which exclude all fibres longer than 5 mm) (Kershaw et al., 2019), microplastic fibres (which exclude all fibres of natural origin longer than 5 mm), textile fibres (which exclude all fibres that do not originate from the textile industry), lines, filaments, threads.

The various threats posed by anthropogenic fibres, both synthetic and natural to marine organisms represent one of the major gaps to be addressed by future research. The presence and impact of this type of debris on marine organisms have received very limited attention and require more in-depth investigation. A review of microplastic fibres by Rebelein et al. (2021) reported that adverse effects were observed in organisms, invertebrates, and fish exposed to synthetic fibres, especially in species at the lower end of the food chain. Laboratory experiments have shown that this type of pollution can negatively affect the fitness of zooplankton populations (Ziajahromi et al., 2017), decrease filtration rates and thus energy balance in bivalves (Woods et al., 2018), increase mortality in crabs (Horn et al., 2020), and cause gill damage, an increase in oxidative stress, and alter metabolism in fish (Hu et al., 2020). Further studies are needed to evaluate the differential effects of fibres and their chemical additives (such as dryers, plasticizers, and flame retardants) on biota (Kwak et al., 2022; Rebelein et al., 2021). As far as we know, there are no data on the effects of natural and semisynthetic anthropogenic fibres. Overall, there is very limited knowledge of the effects associated with fibre biological risk on natural populations' organisms (Kwak et al., 2022). In addition, many of these species are commonly consumed by humans and could ingest contaminants and fibres through food.

Due to the technical limitations in assessing the presence of microplastics and microfibres in the environment, marine species could play an important role in monitoring fibre pollution. The selection of bioindicator species is critical to represent the impact of fibres on the marine ecosystem.

In this review, all studies in which anthropogenic fibres were isolated from marine organisms were considered and analysed. The aim was to: assess the current status of studies on marine fibre pollution in different groups of marine organisms (invertebrates, fish, seabirds, reptiles, and marine mammals) worldwide, with a particular focus on the

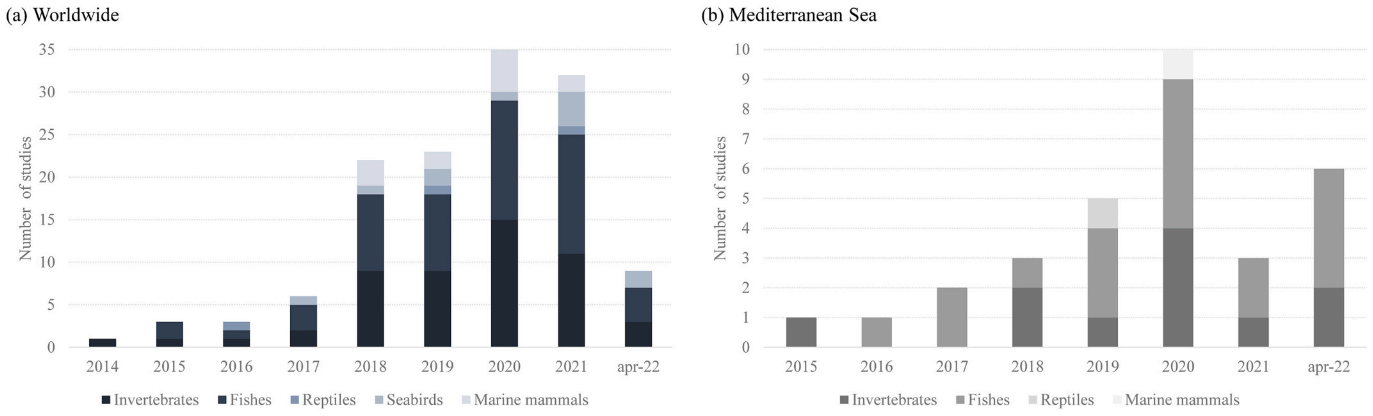

Fig. 2. Temporal distribution of the number of articles found worldwide (a) and in the Mediterranean Sea (b) in different colours according to the group of organisms analysed from January 2014 to April 2022.

Mediterranean Sea; show the distribution of studies and determine if there are some insufficiently studied areas; to define the most commonly used methods to isolate and chemically characterise this type of anthropogenic pollution and the main methodological criticisms; to evaluate if the collected studies have also assessed the presence of some effects or chemicals related to fibre pollution.

2. Materials and methods

Publications since 2010, the year of the first article on microplastics in marine biota, to April 2022 were considered. All articles that addressed interactions between marine organisms (invertebrates, fishes, seabirds, reptiles, and marine mammals) and marine debris, and mentioned fibres (or the most common synonym) in the title, abstract, or keywords were selected.

Systematic literature searches were conducted using general search engines and databases such as Google Scholar and Scopus, graphically represented in Fig. 1.

The studies reviewed and key data are listed in Table 1 of the Supplementary Material.

The spatial distribution of publications was graphically represented by employing the Quantum GIS platform (Version 3.10.1 A Coruña, 2019): the Food and Agriculture Organisation of the United Nations (FAO) subdivision of the world's oceans into Major Fishing Areas (MFA) and, for the Mediterranean Sea, Geographic Subareas (GSAs) were used.

All the major methods for isolating and analysing anthropogenic fibres in marine organisms (the taxa of marine organisms, the target tissue, the extraction method, the density separation solution, and the method and instruments for polymer composition analysis) are shown graphically using a Sankey diagram (SankeyMATIC, 2022, an open source software available at https://sankeymatic.com.). In this type of flowchart, the width of the arrows is proportional to the flow rate. An additional database was created for studies conducted in the Mediterranean Sea: data from each of the studied species were collected and compared in order to analyse the impact of anthropogenic fibres on marine biota.

3. Results

3.1. Temporal and spatial distribution

Many studies on anthropogenic particles and marine litter interaction with marine organisms pointed out the presence of fibres/filaments in the target organisms since 2010 (Lusher et al., 2013; Boerger et al., 2010) when the research on this topic started to increase and become an emerging scientific issue. However, they focused on plastic and microplastic particles and marginally considered the presence of fibres, not including this term or synonyms in the title, abstract or keywords. A total of 132 publications were selected in this bibliographic search and among the publications examined the first paper that accomplished the selection criteria used in the present review was published in 2014. This study showed the presence of fibres in a bivalve species, *Mytilus edulis*, without specifying the polymeric nature of the fibres found and classifying them as microplastic fibres (Mathalon and Hill, 2014). Since 2014, the number of publications has increased. All the papers analysed dealt with the interaction between marine litter and marine organisms and demonstrated the presence of fibres in the specimens studied. More than 30 articles were published in 2020 and in 2021, and 9 studies were already published in the first 4 months of 2022. The taxa of organisms studied have also increased, most publications analysed fish species ($n = 56$) and invertebrates ($n = 52$), followed by marine mammals ($n = 12$) and seabirds ($n = 11$), while only three articles focused on reptiles and, in particular, on sea turtle species. Fig. 2 graphically represents the number of publications for each group of organisms by year of publication.

Each investigated site by the studies considered was reassigned according to the FAO Major Fishing Areas (MFA), to highlight both the most and less monitored areas (Fig. 3a). The fibres were found in organisms from almost all FAO fishing areas, except for Antarctica and the southern Indian Ocean (MFA 58), and the Antarctic Pacific (MFA 88). The Mediterranean (MFA 37) is the most studied area followed by the Northeast Atlantic (MFA 27), and the Northwest to the Pacific (MFA 61).

The majority of articles selected for the literature search, 30 out of 132, concerned organisms sampled in the Mediterranean Sea (MFA 37). Therefore, we decided to elaborate in detail the data from the Mediterranean basin, in particular, the geographical distribution of the studies within the basin (Fig. 3b) and the impact on organisms (described below, in Section 3.2.5, Table 1). The first study conducted in this basin was published in 2015 (Fig. 2b). Anthropogenic fibres were isolated from 77 Mediterranean species: 49 fish species (including 5 cartilaginous fish species), 26 invertebrates, 2 reptiles, and only one marine mammal species. No studies evaluating anthropogenic fibres in seabirds were found.

According to the Mediterranean GSAs, the southern and central Tyrrhenian Sea (GSA10) is the area in this basin from which most of the organisms studied originated (Fig. 3b). Overall, anthropogenic fibres were found in organisms from the western Mediterranean Sea (GSA 1, 5, 6, 7, 8, 9, 10 and 11.2), the Adriatic Sea (GSA 17 and 18), the Ionia Sea (GSA 20) and from the Aegean-Levantine Sea (GSA 22, 24 and 25). No data were available for the central and southern parts of the Mediterranean.

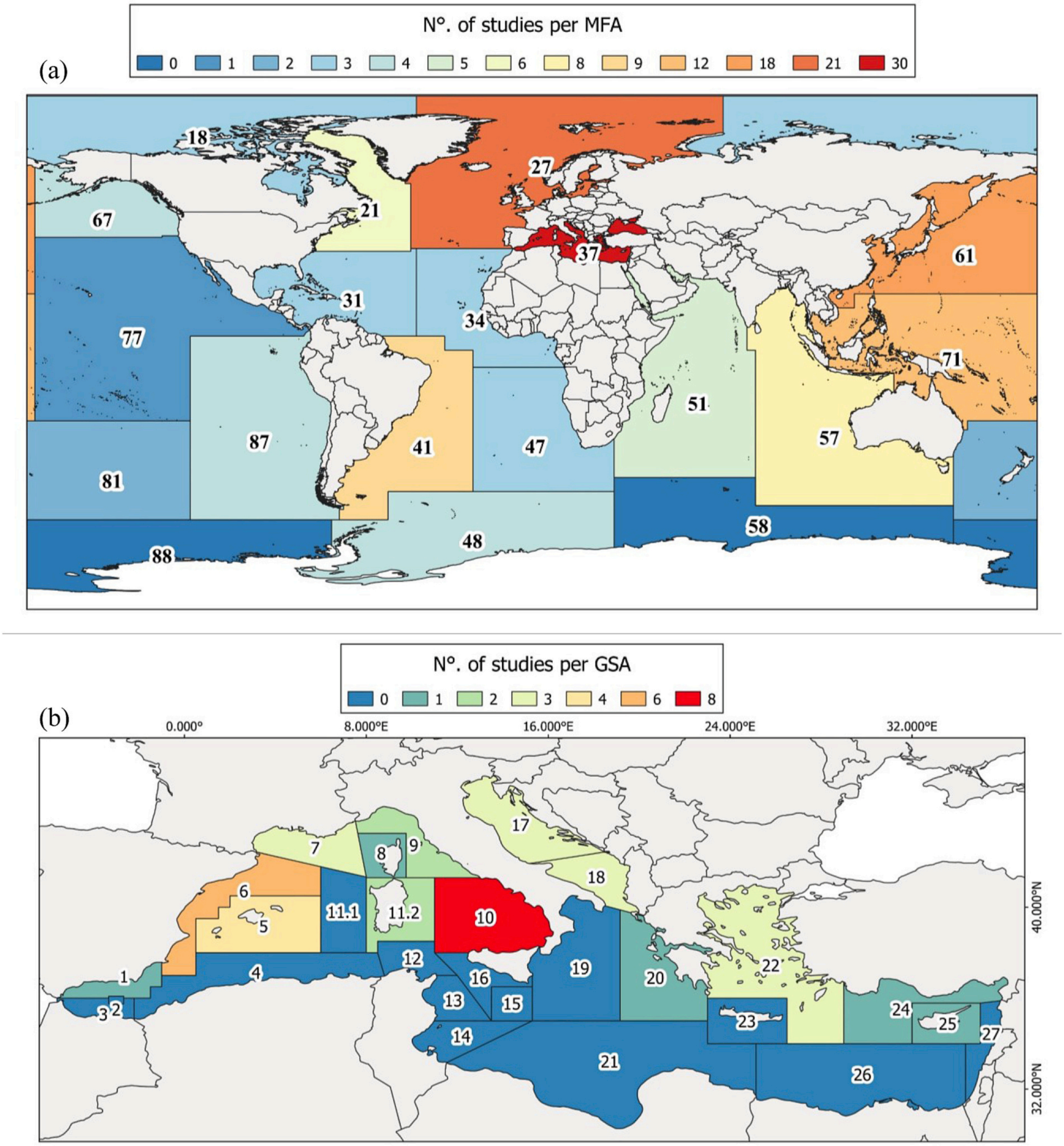

Fig. 3. Worldwide (a) and Mediterranean (b) distribution of studies examined; scale colours were assigned to each area (MFA or GSA) according to the number of studies from zero (blue) to the maximum number found (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Methodology and issues to isolate fibres from marine organisms

Many different methods have been developed and used to isolate marine litter from various tissues of marine organisms. Due to the large number of methods used to isolate fibres from marine organisms, a multi-fluxes diagram was created to graphically represent all the main steps and the different methods used (Fig. 4). The main nodes of analysis are the target organism's taxa, tissues, particle extraction procedures, and polymer composition analysis. However, as mentioned above, most of these methods have been developed to isolate plastic particles, and very often the effectiveness of isolating anthropogenic fibres, even of natural origin, is unknown.

3.2.1. Target tissues

Depending on the group of organisms under study, different types of tissues can be used to evaluate the route of uptake and the number of fibres in the samples, as well as the possible effects depending on their localization in the organism. Fig. 4 shows the different taxa (Node 1) and the main tissues (Node 2) examined in the studies analysed. Eighty-five out of 132 (63,4 %) papers analysed had the gastrointestinal tract (GIT) as the target tissue, obtained by dissection or necroscopy of the organisms. The GIT was the only tissue examined in the reptiles studied included in this review. In fish species, GIT was the target tissue in all the studies, in combination with gills (8 %) and other tissues such as the liver and muscle (1 %). Seventy per cent of the studies on invertebrate species used the whole organism or all soft tissues for analysis, 24 %

Table 1
Mean number of fibres per individual in each Mediterranean species and the study examined.

Taxa	Class	Species	Living domain	Reference	n° of organisms	N° fibres/n° organisms
Invertebrates	*Ascidiacea*	*Actinia* sp.	Benthic	(Avio et al., 2020)	29	5.6
	Bivalvia	*Mytilus galloprovincialis*	Benthic	(Avio et al., 2020)	48	11.5
				(Gedik and Eryaşar, 2020)	342	0.2
				(Santonicola et al., 2021)	15	7.7
		Ostrea eduli	Benthic	(Avio et al., 2020)	33	1.0
	Cephalopoda	*Octopus vulgaris*	Benthic	(Pedà et al., 2022)	6	3.9
	Echinoidea	*Paracentrotus lividus*	Benthic	(Avio et al., 2020)	18	3.2
	Malacostraca	*Nephrops norvegicus*	Benthic	(Carreras-Colom et al., 2018)	224	7.7
		Palaemon sp.	Benthic	(Avio et al., 2020)	21	4.6
		Squilla mantis	Benthic	(Avio et al., 2020)	10	0.0
		Aristeus antennatus	Benthopelagic	(Carreras-Colom et al., 2018)	148	0.1
	Polychaeta	*Sabella spallanzanii*	Benthic	(Avio et al., 2020)	23	0.0
	Scyphozoa	*Rhizostoma pulmo*	Pelagic	(Avio et al., 2020)	14	6.2
		Macrocrustaceans 9 sp.		(Remy et al., 2015)	235	0.4
Fishes	*Actinopteri*	*Boops boops*	Benthopelagic	(Nadal et al., 2016)	337	2.2
				(Savoca et al., 2019)	30	2.7
				(Tsangaris et al., 2020)	884	2.1
	Actinopteri	*Chelidonichthys lucerna*	Demersal	(Avio et al., 2020)	16	1.7
	Actinopteri	*Diplodus sargus*	Demersal	(Constant et al., 2022)	2	0.0
	Actinopteri	*Diplodus vulgaris*	Benthopelagic	(Avio et al., 2020)	14	4.6
	Actinopteri	*Engraulis encrasicolus*	Pelagic	(Lefebvre et al., 2019)	84	0.1
				(Santonicola et al., 2021)	15	9.1
	Actinopteri	*Lithognathus mormyrus*	Demersal	(Avio et al., 2020)	7	1.5
	Actinopteri	*Merluccius merluccius*	Benthopelagic	(Avio et al., 2020)	20	0.7
	Actinopteri	*Mugil cephalus*	Demersal	(Kılıç and Yücel, 2022)	20	27.9
	Actinopteri	*Mullus barbatus*	Demersal	(Avio et al., 2020)	28	3.4
				(Kılıç and Yücel, 2022)	43	6.7
				(Rodríguez-Romeu et al., 2020)	118	1.4
	Actinopteri	*Mullus surmuletus*	Demersal	(Kılıç and Yücel, 2022)	41	11.5
	Actinopteri	*Pagellus erythrinus*	Benthopelagic	(Avio et al., 2020)	6	2.0
				(Constant et al., 2022)	6	0.0
	Actinopteri	*Sardina pilchardus*	Pelagic	(Avio et al., 2020)	33	1.4
				(Lefebvre et al., 2019)	85	0.2
				(Savoca et al., 2020)	19	0.5
	Actinopteri	*Sardinella aurita*	Pelagic	(Avio et al., 2020)	9	2.2
	Actinopteri	*Saurida undosquamis*	Demersal	(Kılıç and Yücel, 2022)	39	5.8
	Actinopteri	*Scomber scombrus*	Pelagic	(Avio et al., 2020)	10	4.2
	Actinopteri	*Solea solea*	Demersal	(Avio et al., 2020)	20	0.4
	Actinopteri	*Sparus aurata*	Demersal	(Savoca et al., 2021)	80	0.3
	Actinopteri	*Spondyliosoma cantharus*	Benthopelagic	(Avio et al., 2020)	9	2.8
	Actinopteri	*Symphodus roissali*	Reef-associated	(Constant et al., 2022)	13	0.0
	Actinopteri	*Trachurus trachurus*	Pelagic	(Avio et al., 2020)	28	2.4
	Actinopteri	*Tracinus draco*	Demersal	(Avio et al., 2020)	6	3.4
	Actinopteri	*Trigla lyra*	Demersal	(Capillo et al., 2020)	16	0.2
	Actinopteri	*Uranoscopus scaber*	Benthic	(Constant et al., 2022)	1	0.0
	Elasmobranchii	*Galeus melastomus*	Demersal	(Alomar and Deudero, 2017)	125	0.1
Marine mammals	*Mammalia*	*Stenella coeruleoalba*	Pelagic	(Novillo et al., 2020)	43	11.5

analysed the GIT, and 6 % of papers used other tissues for analysis, such as the gastrovascular cavity and tentacles. Studies on seabirds and interactions with litter used non-invasive sampling methods in 50 % of the cases, faeces analysing (42 %) and regurgitated pellets (8 %); the remaining studies used GIT from stranded or hunted organisms. Research papers on marine mammals analysed GIT (85 % of the papers) and faecal samples (15 %), obtained after the necroscopy of stranded specimens.

3.2.2. *Extraction methods and density separation*

After the proper collection procedure, the target tissues are processed according to different methods in order to isolate the anthropogenic particles (Fig. 4). In 99 out of 132 (76 %) studies reviewed, chemical and enzymatic solutions were used to digest the organic material in the samples. For chemical digestion of organic material in biota samples, KOH was mainly used, ranging from 10 to 20 % (n = 50 papers) in concentration, followed by H_2O_2 ranging from 15 to 35 % (n = 22), NaOH was used in 6 articles (ranging from 1 M to 10 M), and 20 % HNO_3 solutions were used in only one study. Enzymatic digests were used in 7 of the 132 papers studied, including amylase, lipase, proteinase, naturase, and enzyme mixtures. Visual sorting with a microscope was used to examine the tissue and isolate the anthropogenic particles without digesting the organic matter. In addition, 14 publications used a combination of agents, using different chemicals or both chemicals and enzymes. All these digestion solutions could be incubated with organic tissue at various temperatures up to 60 °C. In 22 % of the articles, a density separation solution was used after digestion to better separate organic material and anthropogenic components. This was usually a saturated solution of NaCl (n = 25 articles); CaCl (n = 2), ZnCl (n = 1), or NaI (n = 1). Subsequently, all digested materials were vacuum filtered, using different types of filters such as glass-fibre, nitrocellulose, or silicon filter with different mesh sizes. The minimum mesh size used was 0.45 μm, despite the 15 % of the articles did not provide information on the mesh size of the filters.

3.2.3. *Analysis of polymer composition*

Most of the selected papers focused on the study of plastic particles, so anthropogenic fibres of natural origin were either not considered in the data processing or the chemical nature of isolated particles was not determined, leading to an underestimation of anthropogenic fibres of natural origin. Anthropogenic particles, including fibres, could be analysed to determine their polymeric composition (Fig. 4). Twelve of the 132 papers reviewed did not perform this type of analysis. Some papers classified anthropogenic fibres isolated from marine organism samples

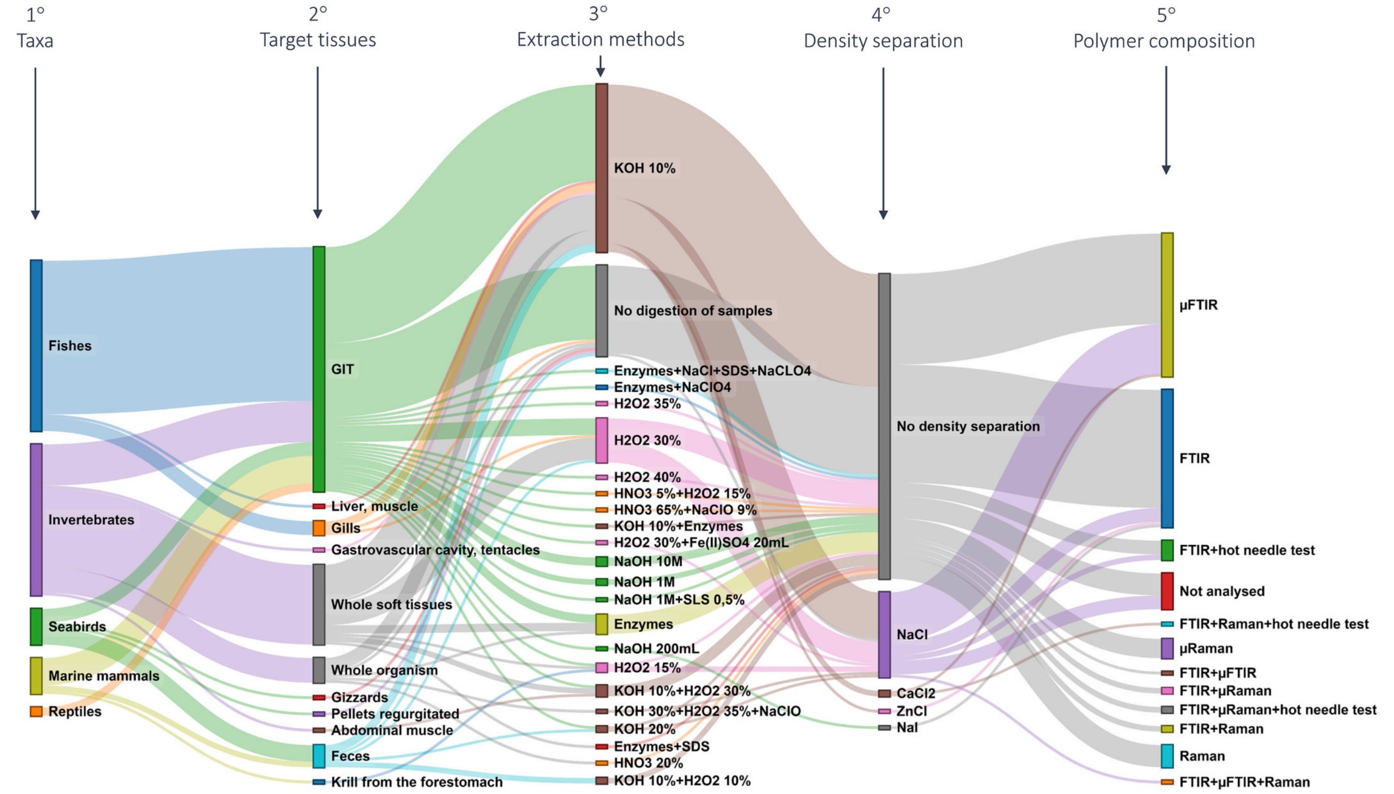

Fig. 4. Sankey diagram showing the main steps (nodes) and methods used for each organism class. The width of the arrows connecting one node to another is proportional to the number of studies in which a tissue or method was used: the first node shows the marine organism taxa, the second node shows the target tissue studied, the third node shows the extraction method, the fourth node shows the use of the density separation solution, and the fifth node shows the method and instrument used for polymer composition analysis. These data were also given in Table 1 of the supplementary material with the bibliographic reference.

as synthetic, using only visual identification (e.g., assuming that all coloured particles are plastic) or the "hot needle technique" (which can determine the synthetic nature of an object by observing its response to a hot metal tip) (Bellas et al., 2016; Hermsen et al., 2018; Santonicola et al., 2021). To perform the polymer composition analysis, potentially anthropogenic particles were isolated from the samples. This is one of the less harmonised steps, the results of which could be subjectively influenced by users. The polymer composition was determined using a spectroscopy method. The most commonly used instruments to perform spectroscopy analysis are Fourier transform infrared spectroscopy (FTIR) ($n = 50$) and μFTIR ($n = 45$), as well as μRaman ($n = 6$) and Raman ($n = 8$). In recent years, other methods combining two different instruments have also been used (e.g., FTIR and μFTIR or other techniques such as the hot needle test). Of the 117 publications that performed polymer composition analysis, 79 studies sampled subsets of particles in different ways: 35 randomly selected a percentage of total particles between 1 % and 95 % (1 article selected only 89 % of potential anthropogenic fibres), 29 articles selected anthropogenic particles or only fibres or only microfibres in different amounts without considering the total amount of particles isolated from the samples. Finally, the Hit Quality Index (HQI), reported by the investigated studies and showing the lower acceptable overlapping percentage of the particle spectra with those reported in the instrumental library was considered. Only 40 articles reported this value and in almost all cases it was above 70 %.

3.2.4. *Expression of the results*

Depending on the objectives of the study, the presentation of results may vary. The total amount of particles was generally described, but in

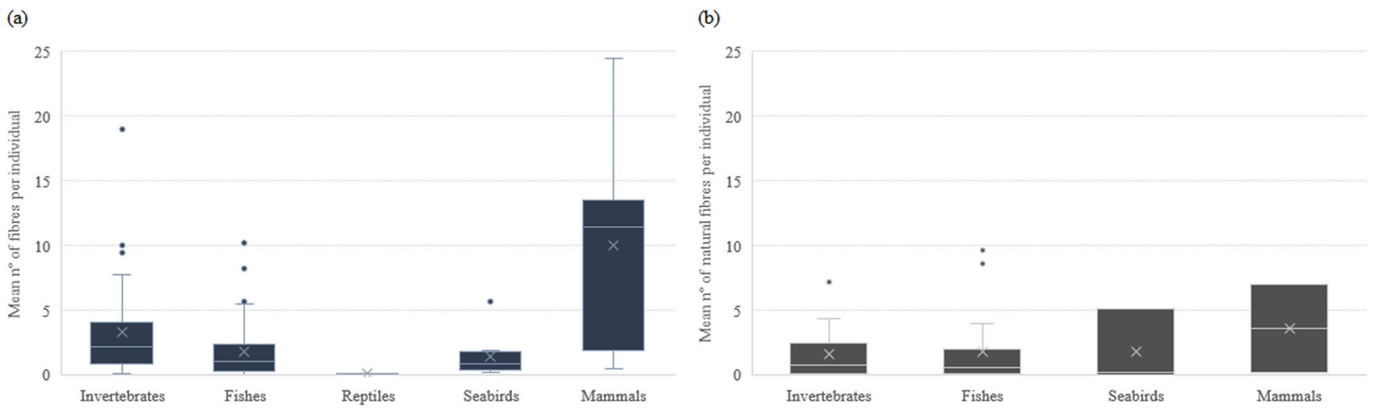

Fig. 5. Boxplot of the average number of total anthropogenic fibres (synthetic and natural) (a) and natural fibres (b) per individual in each group of marine species.

some cases (30 % of the total articles studied) these data were not provided or only partially described, e.g., only the total amount of microplastics or the total amount of fibres. Some of the publications also calculated the average particle abundance, usually reporting the number of particles per individual. However, in 42 % of the articles, these data were not calculated or differently expressed: number of fibres per individual or particles per g wet weight. In 83 % of the studies reviewed, the average number of fibres per sample could be calculated. However, not all studies reported or considered the number of natural fibres found. Only 40 % of the 132 studies examined reported the percentage of natural fibres, and the average number of natural fibres per sample could be determined in only 28 % of the publications. For the studies conducted in the Mediterranean Sea, an attempt was made to extrapolate the average number of fibres per individual for each of the species studied, in order to examine the extent to which the reported data on the impact of fibres (synthetic and natural) on the species studied are comparable and to try to highlight the species most affected (Table 1). It was not possible to extrapolate the average number of fibres (synthetic and natural) per individual for 37 % of the studies examined from the Mediterranean Sea. Among these studies, only in 5 articles it has been possible to determine the average number of fibres of natural origin per individual for each Mediterranean species studied.

3.2.5. *Impact of fibres on organisms*

To determine the impact of fibre pollution on marine organisms, data on the number of fibres found in the specimens analysed were considered for each publication studied. Fig. 5 shows the average number of total anthropogenic fibres (Fig. 5a) and natural fibres (Fig. 5b) for each group of marine organisms considered. Marine mammals appear to be the most contaminated group of organisms, with the highest average number of 10 fibres (synthetic and natural) per individual and 3.6 natural fibres. Seabirds are the second group of organisms according to the number of natural fibres per individual; no data on natural fibres are available for reptile species. The number of anthropogenic fibres per individual does not consider the potential effect of these particles on organisms depending on their size.

Table 1 shows the average number of anthropogenic fibres per individual of each Mediterranean species in each publication. As mentioned above, the presentation of results on fibre-organism interactions can be performed in different ways. *Mugil cephalus* appears to be the most impacted fish species according to the mean number of fibres per individual ($n = 27.9$), followed by *Mullus surmuletus* ($n = 11.5$). High values were also found in invertebrate species, only in 5 out of 12 cases, the average number of fibres per individual was lower than 3 and even reached 11.5 fibres per individual in specimens of *Mytilus galloprovincialis* (Avio et al., 2020). As for marine mammals, only one study reported an average of 11.5 fibres per specimen of *Stenella coeruleoalba* (Novillo et al., 2020). No data are available for reptile and seabird species. Only 11 out of 30 studies conducted in the Mediterranean Sea considered the presence of natural fibres in their data processing, but only in 5 articles (17 % of the studies examined from the Mediterranean Sea), it was possible to determine the average number of natural fibres per individual for each species analysed. In fish species, an average of 0.5 and 0.1 natural fibres per individual were found in specimens of *Mullus barbatus* (Rodríguez-Romeu et al., 2020) and *Sparus aurata* (Savoca et al., 2021), respectively. In invertebrates, an average of 0.01 to 0.03 natural fibres per individual was found in two decapod species, *Aristeus antennatus* and *Nephrops norvegicus* (Carreras-Colom et al., 2020; Carreras-Colom et al., 2018; Remy et al., 2015).

3.2.6. *Assessment of effects and contaminants associated with the presence of anthropogenic fibres*

Marine organisms are exposed to anthropogenic fibres, but the knowledge of the effects associated with the biological risk of fibres on organisms in natural populations is very limited (Kwak et al., 2022). The results obtained in this review confirm this gap; the assessment of the effects of exposure to anthropogenic fibres in marine organisms, coupled with the presence of microplastics, was performed in 8 out of the 132 articles analysed (Alomar et al., 2017; Alomar and Deudero, 2017; Chen et al., 2021; Cabansag et al., 2021; Compa et al., 2018; Hipfner et al., 2018; Nelms et al., 2019; Tsangaris et al., 2020). Only 5 studies, instead, focused exclusively on the interaction of anthropogenic fibres with marine organisms (Bordbar et al., 2018; Carreras-Colom et al., 2020; Iliff et al., 2020; Lefebvre et al., 2019; Rodríguez-Romeu et al., 2020). Most of these studies evaluated general fitness index values such as gonad somatic index (GSI), hepatosomatic index (HSI), Fulton factor (K), stomach fullness index (FULL). The results on the effects of fibres and microplastics on fitness index are in opposition to each other. Some associations have been found between the presence of microplastics and fibres and the weight or size of the organisms. For instance, more anthropogenic fibres and microplastics were found in smaller individuals of the same fish species (Compa et al., 2018; Hipfner et al., 2018), whereas in the jellyfish *Cassiopea xamachana*, specimens with a larger bell size presented a higher number of anthropogenic particles (Iliff et al., 2020). Furthermore, higher litter presence was associated with higher abundance stomach fullness index values in cartilaginous fish species, the fuller stomachs contained more microplastics or fibres (Alomar and Deudero, 2017). In contrast, other studies have found no association between body condition and microplastics or fibres ingestion (Bordbar et al., 2018; Cabansag et al., 2021; Carreras-Colom et al., 2020; Chen et al., 2021; Lefebvre et al., 2019; Rodríguez-Romeu et al., 2020; Tsangaris et al., 2020). Another study conducted by Alomar et al. (2017) linked the presence of fibres and microplastics to increased oxidative stress in *Mullus surmuletus* specimens. A study conducted on stranded marine mammals correlated the presence of infectious diseases with an average abundance of a slightly higher amount of anthropogenic particles than other specimens (Nelms et al., 2019). Concerning chemical additives associated with the interactions between fibres, microplastics and marine organisms, only one paper out of 132 studies selected attempted to link fibre and microplastic pollution to the presence of contaminants: no significant correlation was found with the presence of mercury in anchovy samples (Ningrum et al., 2019).

4. Discussion

The data collected during this literature analysis show that the interest of the scientific community in fibre contamination of marine organisms is steadily increasing, even though the number of publications decreased in 2021 in Mediterranean Sea, probably due to the curtailment of research activities by the Covid-19 pandemic. Regarding the worldwide geographical distribution of the studies reviewed, more than 50 % of them are from the Mediterranean Sea and the northern part of the Atlantic and Pacific Oceans, this reflects the distributions of studies concerning the interactions between microplastics and marine organisms (Ugwu et al., 2021). Some areas are poorly studied, such as the Indian Ocean, from which only 9 % of the studies examined are from, and no data are available for the southern part. Some of the countries bordering the Indian Ocean are among the largest producers of fibre in the world, through the textile industry, which is an important source of fibre in the marine environment (Mishra et al., 2019). Therefore, monitoring the presence and impact of anthropogenic fibre pollution on the environment and organisms in these areas is of great importance. There are also areas in the Mediterranean Sea where the presence of fibres in marine organisms has not been studied. No data are available for the southern areas of this basin, the waters bordering the African continent (GSA 3, 4, 12, 13, 14, 21, 26, 27). Monitoring the presence and impact of fibre pollution on organisms in less studied areas is a knowledge gap that needs to be filled by future research.

The number of species studied has increased over time, especially in invertebrates and fishes, although there are still few studies on reptiles, marine mammals, and seabirds. These groups include species that are not of commercial interest as well as protected species, and most studies deal with stranded organisms or bycatch. It is also likely that the paucity of studies on large animals such as cetaceans and sea turtles is due to the high volumes of gastrointestinal contents, which are generally difficult to handle and analyse (Corazzola et al., 2021). There is a need to develop a harmonised methodology to effectively isolate anthropogenic fibre from large quantities of samples. In seabirds, studies on the presence of marine litter and fibres have also been conducted using non-invasive methods by analysing samples of faeces and regurgitated pellets (Álvarez et al., 2018; Bessa et al., 2019; Caldwell et al., 2022; Fragão et al., 2021; Le Guen et al., 2020; Lourenço et al., 2017), also found high levels of anthropogenic fibres, both synthetic and natural. The use of non-invasive techniques is an advantage for seabird surveys, as a large number of samples can potentially be collected. However, in the Mediterranean, the area with the most studies, no publications are investigating the presence of fibres in seabirds. The importance of studying seabird species also stems from the fact that many of these species can be a vehicle for contamination between the marine and terrestrial environments (Bourdages et al., 2021) and are indicated as potential bioindicators of the presence of marine litter presence in the Mediterranean basin at the sea surface and in coastal waters (Fossi et al., 2018).

As mentioned above, the gastrointestinal tract is the tissue most commonly used to analyse the presence of plastic litter and fibres in marine organisms, as ingestion is considered the most likely interaction, especially for smaller particles (Kühn et al., 2015). The presence of smaller particles has also been detected in the gills, suggesting that these particles may also be ingested through the ventilation system (Abbasi et al., 2018). Concerning fish species, studies have been found since 2020 that examined the gills in addition to the GIT; in all of them, the percentage of fibres was more than 70 % of the total particles found, both in the GIT and in the gills (Capillo et al., 2020; Huang et al., 2020; Jaafar et al., 2021; Kılıç and Yücel, 2022; Koongolla et al., 2020). Simultaneous examination of different tissues of a specimen could provide a more complete overview of the effects of fibres on organisms (Jaafar et al., 2021). It should also be considered that in marine species, the abundance of microparticles in the GIT is influenced by several factors such as habitat, feeding strategy, and colour, while the presence of microplastics or fibres in the gills seems to depend on the abundance of this pollutant in the environment (Kılıç and Yücel, 2022). However, Capillo et al. (2020) hypothesised that the high fibre content of *Trigla lyra* may be related to its feeding behaviour: it ingests prey and sediment, which it then excretes through its gills. Furthermore, the effects of anthropogenic particles on the gills have not yet been well defined; it has been shown to have negative effects, including physical injury to the gill filaments and reduced respiratory efficiency (Barboza et al., 2020).

The most common methods for extracting fibres from marine organisms involve the use of chemical agents that promote digestion of the organic matter and facilitate isolation of the microparticles after filtration. KOH and H_2O_2 are the most used compounds at concentrations ranging from 10 to 20 % for KOH and from 15 to 40 % for H_2O_2. To catalyse the digestion reaction, the organic material is incubated with the digestion solution at temperatures above 50 °C in more than 60 % of the studies using KOH and H_2O_2. These methods were developed to isolate mainly synthetic polymers (Tsangaris et al., 2021). Other studies have shown that the use of high temperatures can alter the physicochemical properties of certain particles by changing their colour and polymer composition: for example, polyamide was found to begin denaturing at temperatures above 55 °C (Corami et al., 2020), while complete denaturation of wool occurs at temperatures above 40 °C (Treilles et al., 2020). Corami and coworkers developed a new method to simultaneously isolate synthetic and natural particles from fish and invertebrates and recommended the use of temperatures between 30 and 40 °C to avoid altering the chemical composition of anthropogenic particles (Corami et al., 2022; Corami et al., 2020). Also, Treilles et al. (2020), after testing different extraction methods to isolate scattering particles from organic material, recommended not exceeding 40 °C when digesting samples to avoid loose particles and underestimate the number of fibres detected. The use of density separation solutions could help to better separate the undigested part of the samples, such as sand residues, from the anthropogenic particles.

The final steps of the analysis, quantification and characterization of the particles found, are the less harmonised ones. In many of the studies reviewed, the size range on which the analysis is focused is not specified, especially the minimum detection limit given by the mesh size of the filters in the filtration, or even the detection limits of the instrument used for the polymer characterization.

Moreover, many studies select in different and, very often, arbitrary ways a partial aliquot of the sample on which polymer composition analyses are performed, without specifying some essential data that make it impossible, in some cases, to understand the total number of particles isolated or the number of particles on which the analysis was performed.

One of the main issues in the study of fibres pollution is how to distinguish anthropogenic fibres of natural origin from natural fibres (not anthropogenic), Zhao et al. (2016) identified some criteria for the isolation of man-made fibres from the samples using visual census under a stereomicroscope (e.g. no cellular or organic structures are visible, fibres must have the same thickness, fibres appear homogenous in colour and have the same diameter) that could be used for the development of a standard method and guideline for distinguishing anthropogenic fibres of natural origin from natural fibres. Using uniform criteria for fibre isolation could help develop guidelines to isolate anthropogenic fibres before polymer composition analysis and harmonise the final stages of analysis. Most of the studies analysed used the spectroscopy method to determine the chemical composition of particles isolated from biota samples. Sixty-three per cent of the publications did not report the HQI value, a very important value that can be used to define the minimum overlap limit that the spectra of the analysed material must have with those of the library for polymer identification. When the HQI value is specified, in most cases it is set to >70 % overlap. The presence of dyes, which are very common in fibres, may interfere with and/or reduce the HQI value. In addition, many natural materials may provide a less intense signal than synthetic ones (Athey and Erdle, 2022). Therefore, further studies are needed to define an HQI value that can effectively identify all types of fibres, both synthetic and natural.

Sometimes the spectroscopy method is combined with the hot needle test, especially with FTIR, which does not allow the analysis of smaller particles (especially fibres). The synthetic nature of a particle was determined by observing its response to a hot metal tip; polymeric compositions were not determined, so anthropogenic particles of natural origin were excluded from analysis or data processing (Battaglia et al., 2020; Jaafar et al., 2021; Kumar et al., 2018; Naji et al., 2018; Patterson et al., 2019; Pedà et al., 2022). In addition, the study of species sampled in the Mediterranean Sea has shown that there is no single, comparable unit of measurement to express the number of anthropogenic fibres isolated from organisms, make abundance estimates, and compare data from different study areas.

Almost all of the studies reviewed point to the problem of contamination during the analytical process, which could lead to incorrect estimates of the fibre load of marine organisms due to the high fibre content in the environment. Although several precautions are taken in some studies to control this type of contamination during laboratory activities, such as the use of white cotton suits or a clean filter to control airborne fibre concentrations, frequently no precautions were taken to control contamination during sampling, and field activities were often not taken. This is another issue that needs to be standardised to avoid altering estimates of fibre pollution in marine organisms and to obtain data that are comparable to each other. Finally, microparticles could also be a vehicle for plastic additives in organisms, further studies are essential to understand the effects of the presence of anthropogenic fibres on marine fauna and associated contaminants, in part because of their high propensity to interact with toxic pollutants (Syberg et al., 2015). The various threats posed by anthropogenic fibres to marine

DISTRIBUTION OF THE STUDIES:
! There are under-studied areas
! Still few studies on reptiles, marine mammals, and seabirds

TARGET TISSUES:
! Lack of methodology to isolate fibres from a large amount of tissue
! No precautions are taken to control contamination during sampling, and field activities

EXTRACTION METHODS:
! The incubation temperatures often do not allow the isolation of man-made fibres of natural origin
! Lack of standard criteria to isolate fibres before the polymer analysis to obtain comparable data
! To define criteria for the correct identification of anthropogenic fibres of natural origin and natural fibres (not anthropogenic)

POLYMER COMPOSITION:
! Analyses of polymer compositions are not always performed on particles with fibrous shapes
! There is no standard method for selecting fibres for polymer composition analysis
! It is necessary to identify an HQI value that can effectively identify all types of fibres

EXPRESSION OF THE RESULTS:
! Detection limits of the analysis are not always specified (size range, HQI, instruments limits)
! There is no single, comparable unit of measurement to express the number of anthropogenic fibres isolated from organisms

EFFECTS AND CONTAMINANTS ASSOCIATED:
! Almost no knowledge on the effects and contaminants related to the interaction between marine organisms and anthropogenic fibres

Fig. 6. Main knowledge gaps and methodological issues identified in this study.

organisms represent a major gap that needs to be addressed by future research (Kwak et al., 2022; Rebelein et al., 2021).

This review highlighted that there are many methodologies to isolate and characterise fibres from samples of marine organisms, but these methodologies are not harmonised and often their effectiveness has not been tested on all types of anthropogenic fibres, both natural and synthetic. Future research should aim to address the knowledge gaps and methodological issues identified in this study and summarised in Fig. 6. Recovery tests could be an important tool to identify a specific, robust and harmonised methodology to assess the impact of anthropogenic fibres in different tissues of marine organisms and to obtain comparable data.

5. Conclusions

The impact of anthropogenic fibres on marine organisms has gained worldwide attention in recent years. Most of the reviewed papers aimed to investigate the impact of plastic debris on marine organisms not specifically focusing on fibres. Although fibres are the most abundant category of microparticles found in the environment and organisms, they are often insufficiently considered or incompletely described, especially anthropogenic fibres of natural origin. For these reasons, the development of a specific methodology to fully describe these types of contaminants and their impacts on marine organisms is needed. This literature review has identified knowledge gaps and some critical points that should be considered in future research. Furthermore, the development of a harmonised methodology is necessary to obtain comparable data among different studies and to properly assess the impact of fibres on marine organisms. The harmonised methodology must include a protocol to prevent and control the contamination of samples during field and laboratory activities. All studies should also specify detection limits to obtain comparable data and verify the chemical composition of isolated fibres by spectroscopy. The adoption of a harmonised method and filling the gaps identified in this review would improve knowledge of fibre pollution and support the development of specific mitigation actions.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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