



Review

Microplastics in marine biota: A review

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ABSTRACT

Plastics are the most important component in marine debris. In turn, within plastics, microplastics (<5 mm) are those that most affect marine biota. Thus, this review has as its main objective to show the current state of studies of microplastics, as well as to determine the groups of vertebrates most affected by microplastics, and the type and predominant color of microplastics. For this research, we review a total of 132 articles, from 2010 to May of 2020. Our results show that the group more affected are turtles with 88% of the specimens contaminated by microplastics and median of 121.73 particles/individue. The predominant type is fibers (67.3%), polymer is polyethylene (27.3%), size is less than 2 mm (73.6%), and color is blue (32.9%).

1. Introduction

Marine litter has become a dilemma for the whole of society, affecting all sectors: economic, social, environmental, and even cultural, becoming a multigenerational problem (Hardesty et al., 2015). Within marine litter, plastics, a family of organic polymers, has become one of the main waste products, mainly due to the high demand for its use, which has caused an exponential growth, overcoming the rest of artificial materials (Geyer et al., 2017). This demand in the plastic industry has caused it to increase from 5 million tons in 1960 to 359 million in 2018 (Europe & EPRO, 2019). In addition, it is estimated that 275 million tons were generated in 2010, of which 12.7 million tons ended up in the marine environment (Jambeck et al., 2015). World plastics production in 2018 was distributed in: 51% Asia, 20% Europe, 18% North America, 7% Africa, 4% South America (Europe & EPRO, 2019). Thus, it is estimated that in 2014 there were 5.25 trillion plastic particles in the oceans, and North Pacific contained 37.9% of these particles, due to the dynamics of the thermohaline current (Eriksen et al., 2014). Plastic waste produces a massive environmental impact, due to its abundance and persistence in the environment, especially in the marine environment, becoming one of the most serious threats for the oceans and biodiversity (Carbery et al., 2018; Gall and Thompson, 2015). So, plastic pollution is one of the main environmental problems in most of the terrestrial and marine environments, causing damage of communities at both the macro and micro levels, with no known ecosystem which does not fall under the scope of this type of contamination (Taylor et al., 2016).

The origin of plastics that end up in the marine environment is mainly terrestrial, through wind, rivers, effluents, and wastewater, although recreational activities and fishing are also sources of plastics in the marine environment (Anbumani and Kakkar, 2018; Ryan et al., 2009). Thus, most of the plastics found in the oceans are fishing nets and gear, plastic bags, plastic bottles and plastics cooking utensils (Hardesty et al., 2015), all of these materials have been made from fossil fuels, and none of them are biodegradable (Geyer et al., 2017).

Microplastics can be divided according to their origin into three different groups: primary microplastics, which are those that are specifically created with a size lower than 5 mm, due to their abrasive qualities (Microbeads); secondary microplastics are those that originate from the disintegration or fragmentation of macro and mesoplastics due to the action of physical agents and UV rays (Fibers, fragments, films, foams); and tertiary microplastics, which are those used for the pre-production of plastics and reach the environment in the same state in which they were produced (Pellets) (Carbery et al., 2018; Anderson et al., 2016).

Finally, the worldwide production of microplastics according to their polymer composition is as follows: 36% polyethylene (PE), 21% polypropylene (PP), 12% polyvinyl chloride (PVC), <10% polyethylene terephthalate (PET), <10% polyurethane (PUR) and <10% polystyrene (PS) (Geyer et al., 2017).

Currently, all the oceans and seas in the world are contaminated by microplastics (Kühn and van Franeker, 2020; Rochman et al., 2015), accumulating in pelagic zones and sedimentary environments (Thompson et al., 2004). The main concern of plastics is their impact on biota,

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and it began in the 1960s, when plastic fragments were found in the gastrointestinal system of birds in the marine environment (Ryan et al., 2009). Moreover, microplastics have been described in the 17% of the species of International Union for Conservation of Nature (IUCN) Red List (Hardesty et al., 2015), so that it may contribute to the species extinction (Gall and Thompson, 2015).

In addition, the bioavailability of microplastics can increase due to flocculation with marine particles, creating aggregates that enter in food chain. In turn, fecal remains with microplastics can be ingested by detritivorous species (Wright et al., 2013). On the other hand, the ingestion of microplastics by marine zooplankton has been demonstrated (Desforges et al., 2015), as well as the transfer of microplastic particles from mesozooplankton to macrozooplankton, so exist a real risk of microplastics getting on marine food webs (Setälä et al., 2014). Likewise, microplastic transfer has been found in marine invertebrates, such as the species of *Mytilus edulis* (mussel) and *Carcinus maenas* (crab) (Farrell and Nelson, 2013), proving that there are higher trophic levels that ingest microplastics through their prey (Wright et al., 2013).

Likewise, the factors that have been defined as the main responsible for the ingestion or assimilation of microplastics by marine organisms are the following: size (the smaller are more bioavailable), the density (greater the quantity of microplastics lead to greater the possibility of ingestion and/or adsorption), abundance (greater variety of microplastics involves a greater possibility of organisms being attracted to this material), and color (it has been shown that there are certain colors that tend to attract certain groups of organisms), all these factors cause an increase in the bioavailability of microplastics in organisms with respect to other anthropogenic waste (Alomar et al., 2017; Ory et al., 2017; Wright et al., 2013). On the other hand, microplastics in their weathering process in the marine environment release volatile organic compounds, such as dimethyl sulfide (DMS), a compound present in algae, so that an olfactory mark is generated, causing that some organisms of zooplankton, such as copepods consume microplastics mistaking them for their prey (Procter et al., 2019). Furthermore, this behavior has also been demonstrated in seabirds, showing that the chemical aromatic signal released by the microplastics produces greater ingestion in marine fauna (Savoca et al., 2016).

Also, one has to take into account the difficulty in providing a standardized method of sampling about ingestion of microplastics by marine biota. However, it is possible to establish guidelines about the area, time, number and size of organisms indicating contamination by microplastics (Wesch et al., 2016a, b). In this sense, a quality assessment protocol has been described using several criteria: sampling method and strategy, sample size, sample storage and processing, laboratory preparation, controls, and polymer treatment and identification, providing a standardized protocol for the detection of microplastics in marine biota (Hermsen et al., 2018).

Furthermore, is important highlight that, given the characteristics of microplastics, a set of techniques for their detection in marine biota have been used since their discovery, among which those are: visual identification (human eye or microscopy), density separation and C:H:N análisis (separate by density), Pyrolysis-GC/MS (compare with pyrograms), Raman spectroscopy (monochromatic laser and compare the polymer spectra) and Fourier Transform Infrared (FTIR) spectrometer (infrared radiation producing molecular vibrations) (Rezania et al., 2018).

Finally, the impact of microplastics on marine fauna is mainly due to two issues: on the one hand, after ingestion of microplastics, these can accumulate in the animal's organs, generating mechanical obstruction and preventing them from feeding or breathing, which is a physical impact on the biology of the individual (Anbumani and Kakkar, 2018). Moreover, there is chemical impact, since it has been shown that microplastics can contain persistent organic pollutants (POPs), such as polychlorinated biphenyl (PCB) (Hermsen et al., 2018). In turn, it has been shown that microplastics from beaches around the world contained organochlorine compounds such as: dichlorodiphenyltrichloroethane

(DDT), its derivatives dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD), hexachlorocyclohexane (HCH), and polychlorinated biphenyls (PCB), all classified as persistent organic pollutants (POPs) (Ogata et al., 2009).

In addition, the potential of microplastics to transport hydrophobic contaminants such as phenanthrene in sediments has been demonstrated, so this can affect organisms living in these habitats (Teuten et al., 2007). Some specific plastic additives such as phthalates and bisphenol A (BPA), affect reproduction in various organisms, including crustaceans and fish, and produce genetic malformations, altering hormonal systems (Oehlmann et al., 2009). Likewise, an increase in epithelial cysts in plastic-feeding birds has also been reported (Roman et al., 2019). Different additives and microplastic by-products have been found in seabirds, among which UV stabilizers such as UV-328, UV-236, and UV-237 containing benzotriazole groups and BP-12 containing a benzophenone group, which alter the endocrine system, and flame retardants such as hexabromocyclododecane (HBCDD) and Decabromodiphenyl ether (deca-BDE), which are included in the list of persistent organic pollutants (POPs) (Tanaka et al., 2019). It is also important to highlight the occurrence of organic UV filters found in microplastics, such as benzophenone 3 (BP-3), 4-methylbenzylidene camphor (4-MBC), octocrylene (OC), octyl-methoxycinnamate (OMC) and ethylhexyl dimethyl p-aminobenzoic acid (OD-PABA) (Cadena-Aizaga et al., 2020). Likewise, up to 81 chemical compounds have been found in microplastics in the Canary Islands, in the North Atlantic, among which organochlorine compounds such as polychlorinated biphenyls (PCB), dichlorodiphenyltrichloroethane (DDT) and derivatives, organochlorine pesticides (OCP), polycyclic aromatic hydrocarbons (PAH) and bromodiphenyl esters (BDE) stand out (Camacho et al., 2019).

Therefore, the present study aims to carry out a review of the existing studies in microplastic (MP) pollution in the marine biota studied in its natural environment, focusing on marine vertebrates (sea birds, fish, marine mammals and turtles) and visualize temporal trend in the number of studies on microplastics. The second aim is to establish, the main types, polymers and colors of microplastics in marine vertebrates in order to support for decision making in management and future research. And finally, to determine the main methods for measuring microplastics in marine biota, in order to harmonize methodologies.

2. Methodology

To carry out this bibliographic review, a list of references obtained from the Web of Science Database (WOS) was used. The key search word was "microplastics", obtaining a total of 3623 references on May 17, 2020. The list obtained was then filtered by the fields "Plant Science", "Zoology", "Oceanography", giving a total of 1345 references.

Once this first selection was made, all those references that did not study species in the marine environment were first disregarded, that is, studies carried out in rivers, lakes or reservoirs were disregarded. At the same time, research carried out in the laboratory was also disregarded, since it has been demonstrated that significant differences exist between field studies and experiments on exposure to microplastics in the laboratory (Rezania et al., 2018). Finally, review articles and those which weren't complete were disregarded, making a final list of 213 articles. These 213 references were used to show in the results the distribution by fields, spatial and temporal of the research of microplastics in marine biota. Then, once we had these perfectly defined references, we limited ourselves to studying only vertebrates 132 articles (marine mammals, seabirds, turtles and fish) to study the use of advanced instrumentation, organ of analysis, size, type, polymers and color of particles.

The following information was obtained from each article: location, sample size (n), group (sea birds, fish, marine mammals and turtles), species, organ of analysis (feces, stomach, gastrointestinal tract, others), % of individuals with microplastics, number of microplastic items per individual, number of total microplastic particles, size of particles (mm),

predominant type of microplastic (fibers, fragments, pellets, films, and foams), predominant type of polymer, predominant color of microplastic, type of visual instrumentation (dissecting microscope, stereo microscope, rulers), use of QA/QC procedures, and use of advanced instrumentation (Raman spectroscopy/FT-IR spectroscopy), creating the data table shown in the results.

Once obtained this table, created in the Microsoft Excel spreadsheet program version Microsoft 365, the statistical program R studio was used in its version R version 3.6.1 (2019-07-05) with the set of packages tidyverse 1.3.0 (Wickham et al., 2019) and ggplot2 (Wickham, 2016) to make the graphs that are shown in the results.

3. Results

3.1. Field, spatial and temporal distribution

This study showed that of the 1345 references found (143 references for Oceanography, 219 for Plant Science, and 983 for Zoology), only 213 references were in biota in the natural environment, divided into the following: 20 marine mammals, 15 seabirds, 9 turtles, 97 fish, 69 invertebrates, and 3 plants (Fig. 1).

The data obtained from the 213 articles cover all oceans and continents, so that the most studied areas are the Atlantic Ocean (77), the Pacific Ocean (69) and the Mediterranean Sea (35), while the least studied are the Indian Ocean (17), the Arctic Ocean (8), the Baltic Sea (4) and the Antarctic Ocean (3) (Fig. 2).

In turn, if we study scientific production for years, we can see how the discipline of microplastics in marine fauna is very recent. The first article that studies microplastic waste specifically in vertebrates was published in 2010 (Boerger et al., 2010), and the first article about invertebrates in 2014 (Mathalon & Hill, 2014). Since 2010, the number of articles has increased rapidly, going from a single article in that year to 60 articles in 2019 and 42 articles in the first five month of 2020 (Fig. 3).

Thus, of the 132 studies reviewed in this work, 129 reported microplastics in organs of marine fauna, and only 3 articles (2 articles of marine mammals and 1 article of fish) reported that no microplastics had been found in any individual, that is, 97.73% of the articles found microplastic contamination in the organisms studied. Overall, the articles reviewed have studied a total of 25,907 individuals, finding microplastic particles in 7375 individuals, therefore, 28.47% of all individuals studied were contaminated with microplastic particles.

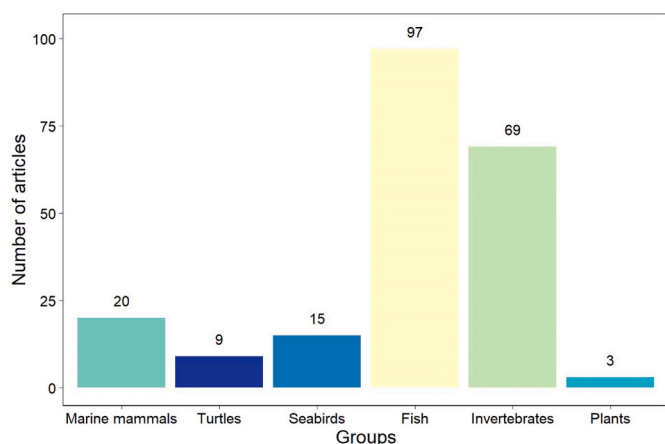


Fig. 1. Number of articles found on the Web of Science bibliographic information website in May 2020, classified with distribution by groups based on the following colors: bluish green: marine mammals (20), dark blue: turtles (9), blue marine: seabirds (15), yellow: fish (97), light green: invertebrates (69), turquoise: plants (3).

3.2. Instrumentation and QA/QC procedure

Of the 132 articles reviewed in this study, most, 83 articles, perform the analysis of microplastics on the gastrointestinal tract, 34 articles only in the stomach, and 9 in the feces, while only 5 in other organs such as gills, muscles and livers. However, this changes if we compare it by groups, in which case, in fish the study of the complete gastrointestinal system is 73%, the stomach 24% and other organs 3%. In turtles, 60% of the studies analyzed the entire gastrointestinal system and 40% the stomach. In seabirds, only 20% study the entire gastrointestinal system, 33% the stomach, 20% feces, and 20% other organs. Finally, in marine mammals, only 33% study the entire gastrointestinal system, 33% the stomach, and 33% feces. It is verified that most of the articles are focused on the gastrointestinal tract, which provides more information about the microplastic contamination of the individual than studying only the stomachs, but with great differences between groups (Fig. 4).

In turn, of the 132 articles, most of them used advanced techniques for the detection of microplastics, such as the Fourier Transform Infrared (FTIR) spectrometer, reaching 60.4% of the articles reviewed, and to a lesser extent Raman spectroscopy. It is important to highlight that an important percentage of the articles, 32.8%, did not use any advanced analysis technique, so the identification was based on the use of visual techniques such as the microscope. Moreover, the use of advanced methodologies differs by groups, in fish, 63% use FTIR, 7% Raman spectroscopy, and 30% none of these. In turtles 70% use FTIR, 20% Raman spectroscopy, and 10% none. In contrast, in seabirds only 47% use FTIR, and 53% do not use any. In marine mammals, 53% use FTIR, 7% use Raman spectroscopy, and 40% none (Fig. 5).

In addition, it was decided to take into account in this review the adoption of precautions to avoid secondary contamination of the samples and the validity of the QA/QC processes. Thus, in this review, a filtering of those articles that used different methodologies to avoid secondary contamination was carried out (Annex I). This is necessary, since it has been shown that contamination by plastic microfibers can exist if proper precautions are not taken (Foekema et al., 2013).

The results show that 25.4% of the studies do not take into account specific precautions to avoid contamination of the samples, 9.2% take into account precautions against cross contamination such as the use of nitrile gloves and a cotton coat, cleaning of the instrumentation, and filtering of the reagents used, but do not take into account possible airborne contamination. On the other hand, 44.6% of the studies take into account the precautions for cross contamination outlined above, and also use laboratory blanks to evaluate possible airborne contamination. Finally, 20.8% of the studies take into account both precautions against cross contamination, as well as precautions to evaluate possible airborne contamination through targets, in addition, they use laminar flow to minimize it.

The group distribution differs significantly. In seabirds, 60% of the studies do not take into account specific precautions to avoid contamination of samples, 6.7% take into account precautions for cross contamination but not for air pollution, and 33.3% take into account precautions for evaluating airborne and cross-contamination using blanks. In turtles the figures are similar, 62.5% of the studies do not take into account specific precautions to avoid contamination of the samples, 12.5% take into account precautions to avoid cross and air contamination, and 25% also use a hood laminar flow to avoid air pollution. In marine mammals, 26.7% do not take into account measures for contamination, 6.7% have measures for cross contamination but not air pollution, 46.7% take into account precautions to evaluate cross and air contamination through targets, and the 20% also use a laminar flow hood. Finally, fish is the group where precautions have been maintained the most, only 16.3% did not take into account specific precautions, 10.9% used measures against cross contamination but not air, 48.9% take into account cross and air contamination, and 23.9% take into account measures to avoid cross and air contamination, and use a laminar flow hood to minimize it.

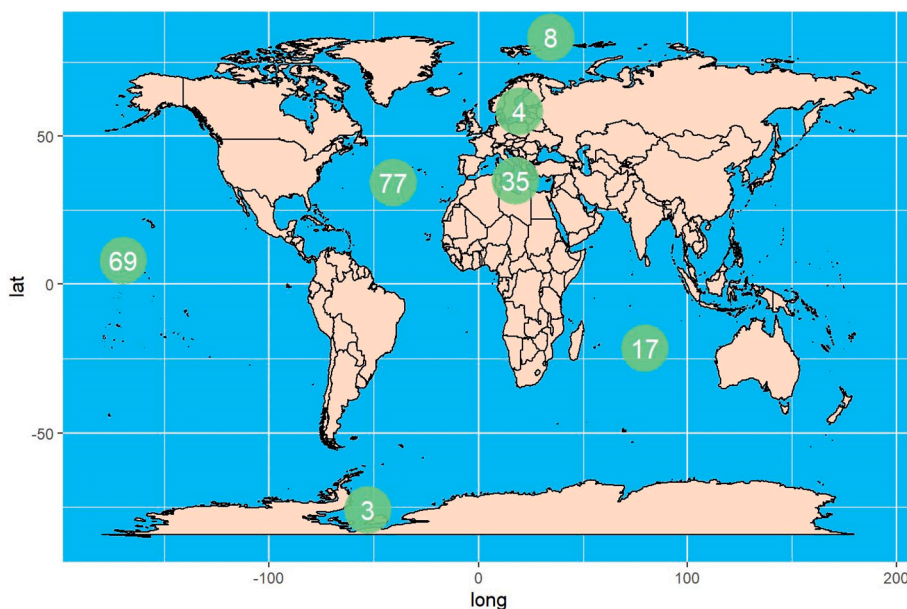


Fig. 2. Distribution of the number of articles found in the Web of Science bibliographic information website in May 2020, taking into account the 213 articles, including invertebrates, according to the oceans and seas studied. The following seas and oceans are represented: Atlantic Ocean (77), Pacific Ocean (69), Mediterranean Sea (35), Indic Ocean (17), Arctic Ocean (8), Baltic Sea (4) and Antarctic Ocean (3), based on latitude (lat) and longitude (long).

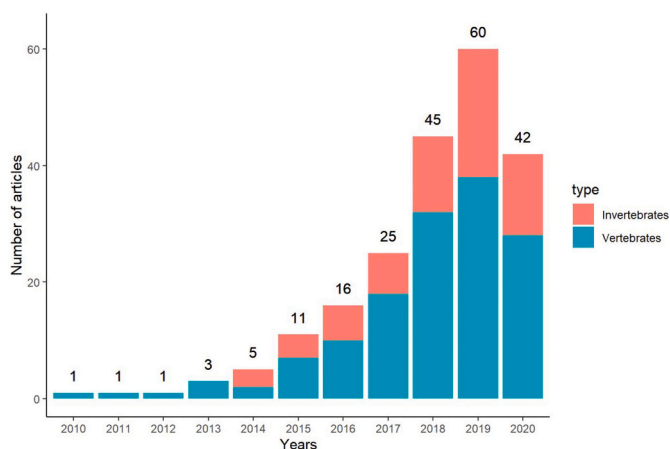


Fig. 3. Distribution of the number of articles found in the Web of Science bibliographic information website in May 2020, taking into account the 213 articles, according to the years when the articles were published. In blue the articles that analyzed vertebrates and in red the articles that analyzed invertebrates, with the number of articles in the top of the graph.

3.3. Concentration and sample size of microplastics

In general, all the articles carry out their respective studies with a fairly high number of specimens. The studies carried out on fish are noteworthy, since with an average of 233 specimens, there are outpoint articles that study up to 1429 specimens. The rest of the groups present very similar averages and medians: 121 in fish, 44 in marine mammals, 62 in sea birds, and 47 in turtles. It is also important to note that in all cases, except for the seabirds, there are studies that fall below 5 individuals (Fig. 6).

On the other hand, we show the average % of individuals with microplastics by groups, in which we can determine, that the group most affected by these particles are the turtles, 88% of the specimens studied were contaminated by microplastics. The rest of the groups present very similar values: 42% of the fishes affected, 59% of the marine mammals affected, and 50% of the sea birds affected (Fig. 7). Something

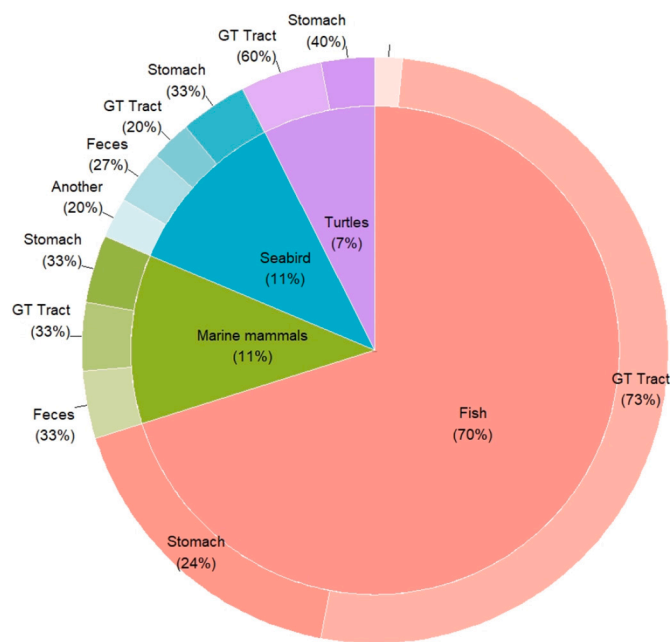


Fig. 4. Percentage of articles studied according to the organ of analysis (GT tract: gastrointestinal system, stomach, feces, another: gills, gizzard, skin, muscle) taking into account the 132 articles reviewed for vertebrate group (in red the fish, in green the marine mammals, in blue the seabirds and in purple the turtles).

fundamental to take into account is that all the groups consist of articles where all the studied individuals were contaminated by microplastics.

In turn, it is interesting to determine the average number of microplastic particles found in the individuals of each group. Since they show us the great potential that many organisms have to accumulate microplastics, the most controversial case is that of the turtles, whose average number of particles differs by two orders of magnitude from the rest of the groups. Thus, the average of microplastic particles found in turtles is 121.7 particles per individual, while in fishes it is 2.6 particles, in marine

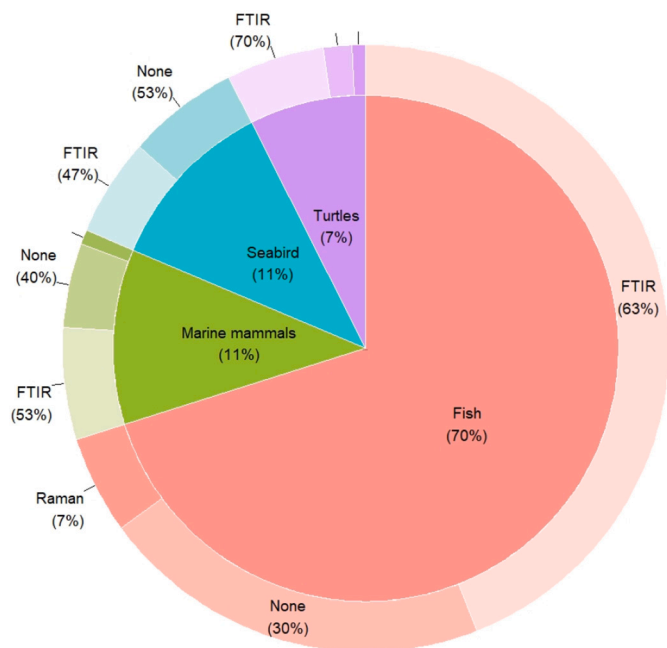


Fig. 5. Percentage of articles studied according to the use of advanced technology instrumentation (FTIR: Fourier Transform Infrared (FTIR) spectrometer, Raman: Raman spectroscopy, None: none of the technologies described above) taking into account the 132 articles reviewed for vertebrate group (in red the fish, in green the marine mammals, in blue the seabirds and in purple the turtles).

Group	mean	minimum	maximum	median
Fish	233.09	5.00	1,429.00	121.00
Marine mammals	100.73	1.00	654.00	44.00
Seabird	143.27	9.00	850.00	62.00
Turtles	57.00	2.00	102.00	46.50

Fig. 6. Sample size (number of individuals) in the 132 articles studied, as a function of the mean, the minimum size, the maximum size and the median, depending on the groups of vertebrates (fish, marine mammals, seabirds, and turtles).

Group	mean	minimum	maximum	median
Fish	41.99	0.00	100.00	33.50
Marine mammals	59.49	0.00	100.00	67.80
Seabird	50.38	0.50	100.00	47.70
Turtles	88.17	58.30	100.00	100.00

Fig. 7. Percentage of individuals affected by microplastics contamination in relationship with the total of individuals studied in the 132 articles, as a function of the mean, the minimum size, the maximum size and the median, depending on the groups of vertebrates (fish, marine mammals, seabirds, and turtles).

mammals 9.7 particles and in sea birds 7.0 particles. Likewise, the minimum of particles found in turtles, 22.7 particles, is similar to the maximum of particles found in fishes 34.0 particles, in marine mammals 27.9 particles, and in sea birds 22.0 particles, being the maximum for turtles 220.7 particles (Fig. 8). Therefore, the group most affected by micro-plastic pollution is turtles.

Group	mean	minimum	maximum	median
Fish	2.61	0.03	34.00	1.64
Marine mammals	9.71	0.00	27.90	7.70
Seabird	7.04	1.19	22.00	1.78
Turtles	121.73	22.70	220.76	121.73

Fig. 8. Number of microplastic particles items (<5 mm) per individual in the 132 articles studied, as a function of the mean, the minimum size, the maximum size and the median, depending on the groups of vertebrates (fish, marine mammals, seabirds, and turtles).

3.4. Shape, polymer, size and colors of microplastics

As for the type of microplastic, it is interesting to determine the shape of the microplastic, since it is one of the best indicators about its origin. In case the ingestion of pellets prevails, it provides us with information that the area is affected by industrial processes, while if fibers prevail, the source can be residual water with remains of clothes, and when fragments and others prevail, we can estimate that it is a "fast" process of breakage of macro and mesoplastics. So, biomonitoring is a suitable method to determine the sources and speed of microplastics in marine ecosystems (Álvarez et al., 2018; Gouin, 2020; Herrera et al., 2019; Kazour et al., 2019; Rezanian et al., 2018).

At an average level of all the organisms studied in the articles reviewed, the predominant type of microplastic in each article has been obtained, the fibers predominant microplastics, which are found as predominant in 67.3% of the articles reviewed. The next important group of fragments, representing 25.7% of the articles reviewed. Pellets and films, represent only 3.5% each.

However, if we analyze the predominant type of microplastic according to the different groups, we obtain different trends from those generally expected. In this case, we can observe how the fish group shows a similar trend to the general average (71.1% fibers, 21.7% fragments, 3.6% pellets, 3.6% films), which makes sense given that it is the majority group of articles that have been studied.

In marine mammals, none of the articles show as predominant microplastic pellets or films, being the predominant microplastic fibers in 72.7% of the articles (Fig. 9). The group of marine birds is interesting, since it is the only group where the fibers do not represent the majority type of microplastics, since they are found in the same percentage as the fragments (45.5% each), and in addition pellets appear as the predominant microplastic in 9.1% of the articles. This shows us that birds are much more affected by microplastics in granular or fragment form, than by microplastics in fiber form. Finally, the turtle group also has fibers as the predominant microplastic, but to a lesser extent than fish or mammals, as it only represents 50% of the articles. Fragments representing 37.5% and films 12.5% become important in this group.

On the other hand, knowing the predominant polymer from which the microplastic particles are formed gives us an idea of the period of time that this microplastic will take to degrade, since each polymer has a certain period of degradation, as well as the possible organic compounds that can be released by the microplastic due exclusively to the polymer. The data show that the predominant polymer found in the studied vertebrates is polyethylene (PE) in 27.3% of the articles, followed by polypropylene (PP) in 14.3% of the articles, rayon in 11.7% of the articles and polyester in 10.4% of the articles (Fig. 10).

The size of microplastics is also an important factor to take into account. Our results show the following distributions: 25% of the articles describe that the main size of microplastics is between 0.3 and 0.5 mm, 26.4% between 0.6 and 1.0 mm, 6.9% between 1.1 and 1.5 mm, 15.3% between 1.6 and 2.0 mm, 11.1% between 2.1 and 2.5 mm, 4.2% between 2.6 and 3.0 mm, 4.2% between 3.1 and 3.5 mm, 2.8% between 3.6 and 4.0 mm (Fig. 11). None of the articles studied comment that the

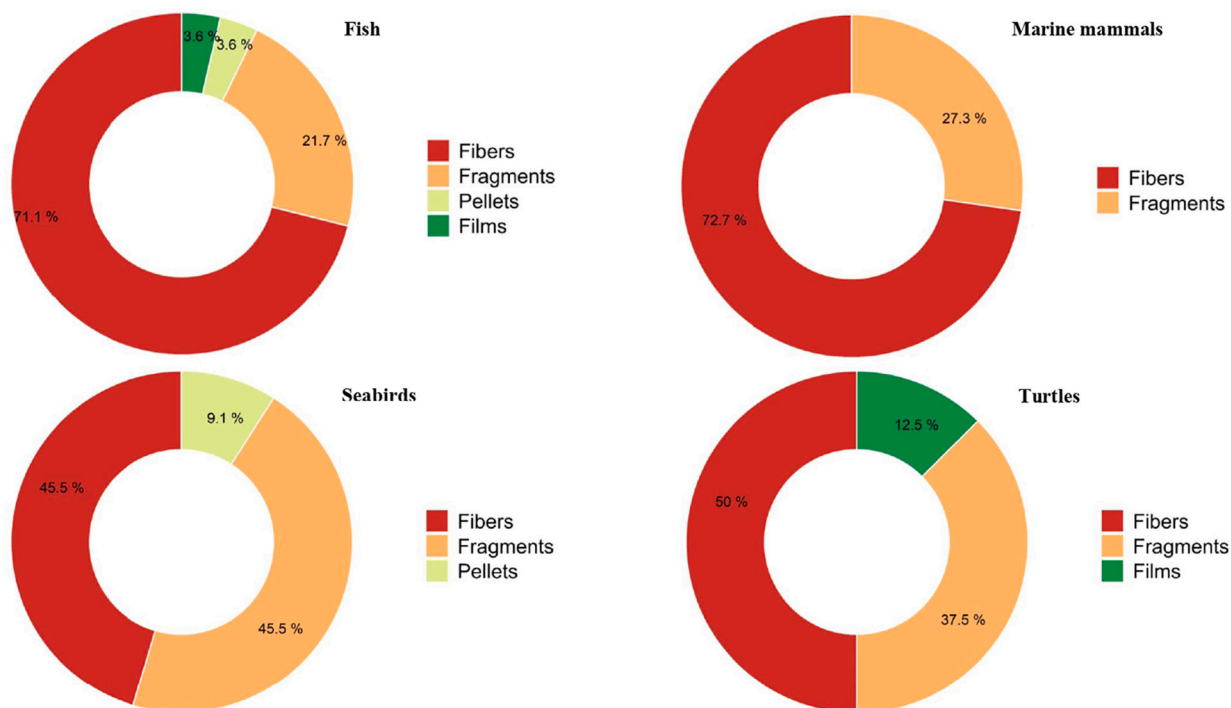


Fig. 9. Percentage of predominant shape of microplastic divided into the main categories: fibers in red, fragments in orange, films in dark green and pellets in light green, in the 132 articles analyzed, depending on the groups of vertebrates studied (fish, marine mammals, seabirds, and turtles).

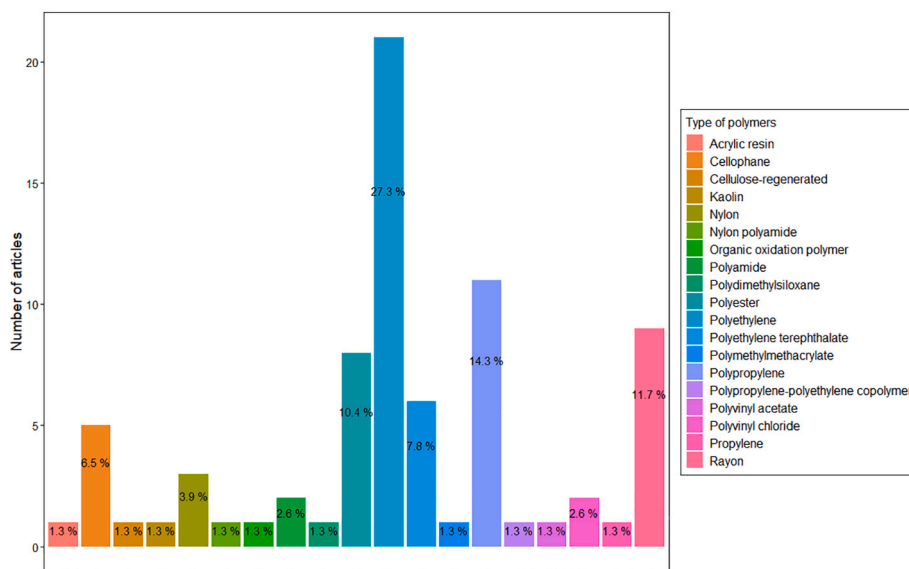


Fig. 10. Predominant polymers of microplastics described in the 132 articles studied, both synthetic polymers and semi-synthetic polymers (rayon) have been taken into account, all with the percentage of articles that describe each polymer as the main polymer.

main particle size is in the 4.0–5.0 mm range. However, 4.2% of the articles describe plastic particles between 5.0 and 8.0 mm as main particles. This shows that 73.6% of the articles describe the main microplastics smaller than 2.0 mm.

Finally, the predominant colour of the microplastics in the fauna studied in each of the articles was reviewed, obtaining the following results: 32.94% blue, 24.71% white, 18.82% black, 16.47% transparent, 3.53% green, 2.35% red, and 1.18% brown. Thus, it can be seen that the majority colour is blue, in 32.94% of the articles, followed by white, in 24.71% of the articles.

However, studying the groups separately we find very different

trends from the previous one. In fish, the distribution by colors are the following: blue (28.12%), black (23.44%), transparent (21.88%), white (18.75%), green (4.69%) and red (3.12%). In marine mammals, consist of blue (50%) and black (12.5%), and transparent (37.5%). Birds are the only group where transparent colour are the majority (55.56%), followed by blue (33.33%) and brown (11.11%). Finally, in the group of turtles blue colour represents 75% of the items and transparent colour represents the remaining 25% of the items (Fig. 12).

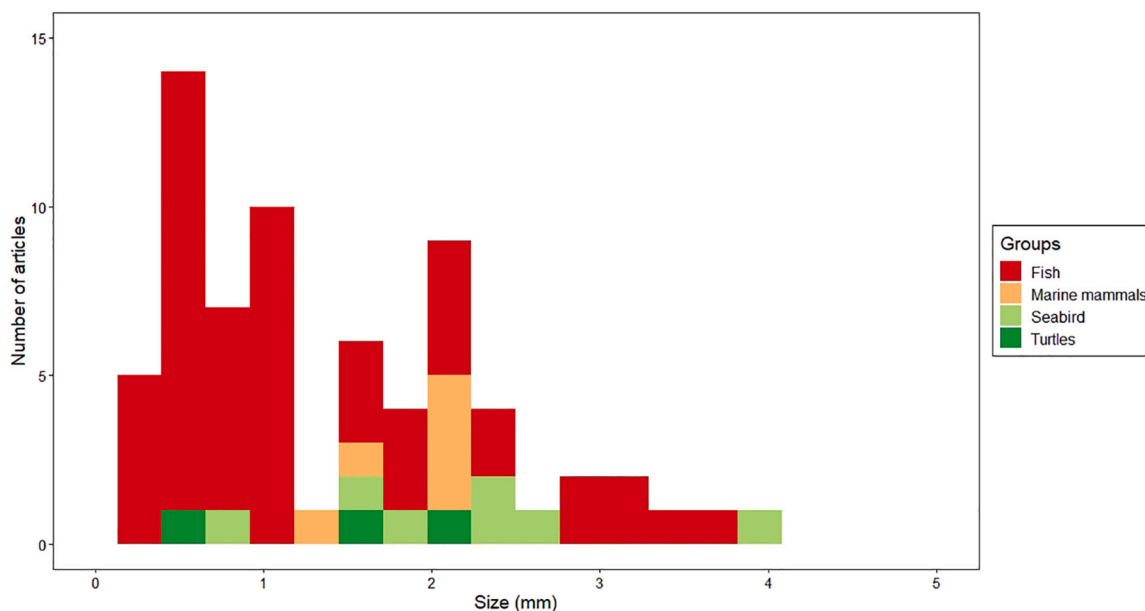


Fig. 11. Predominant size of microplastic described as main in the 132 articles studied, the minimum size of microplastics (0.3 mm) up to the maximum size of microplastic (5.0 mm) have been taken into account, all based on the groups of vertebrates studied (fish in red, marine mammals in orange, seabirds in light green, turtles in dark green).

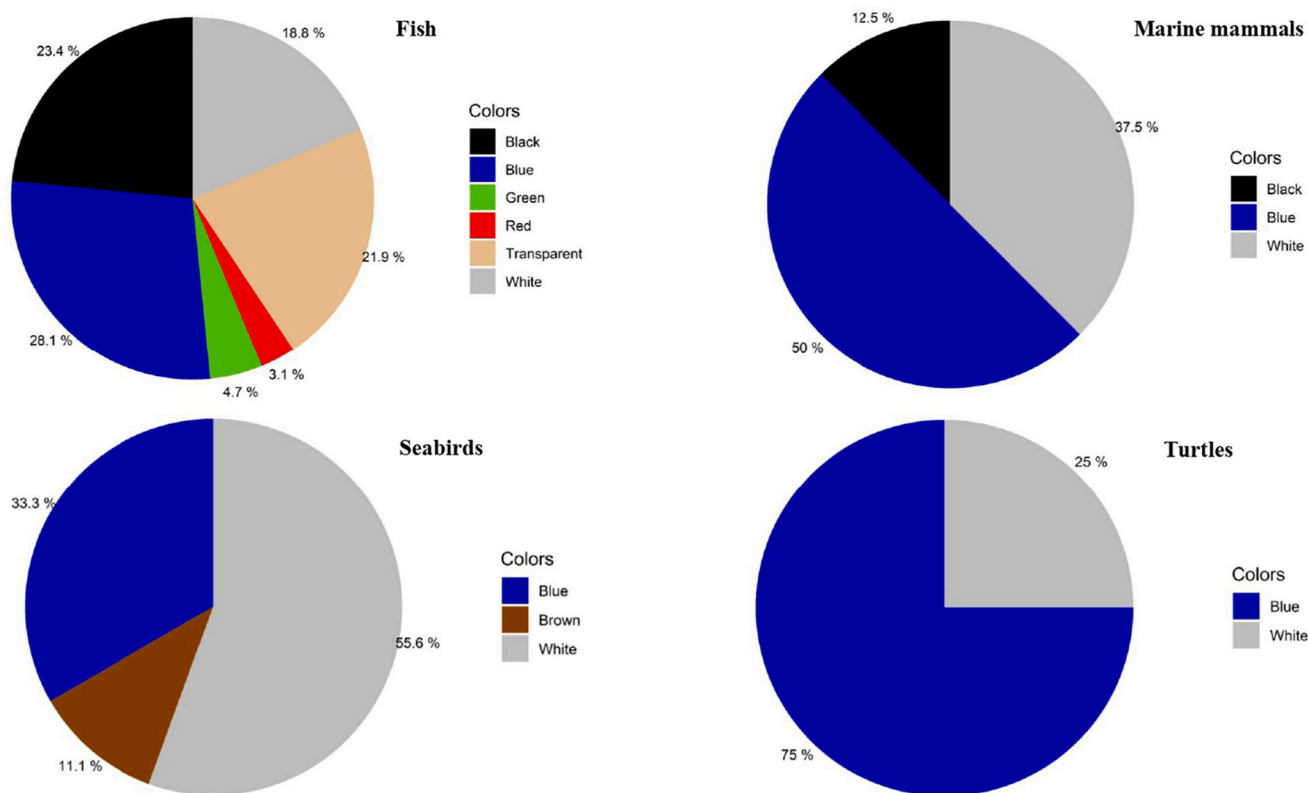


Fig. 12. Predominant colour of microplastic described as main in the 132 articles analyzed, each color represented corresponds to its usual color, except for transparent and white, which are light brown and grey respectively, all based on the groups of vertebrates studied.

4. Discussion

The present review makes an extensive revision of all the information contained of microplastics in marine biota. In this sense, our data show that 132 scientific articles make studies of microplastics in marine vertebrates (turtles, mammals, birds and fish), this number coincides

with other reviews of microplastics in vertebrates, which studying only cetaceans, fish and turtles reach 112 articles with a review methodology similar to ours (López-Martínez et al., 2020). On the other hand, our data show that, in the last decade, studies of microplastics in marine biota have increased exponentially, going from the first study specifically of microplastics in 2010 to 60 studies in 2019. This behavior

shows a growth rate in the number of articles that increases rapidly and significantly, so confirms the assumptions that the environmental threat of plastics is significant in marine animals and is acquiring great attention in recent years (Santos et al., 2016), other reviews show the same behavior in the study of microplastics in the last decade (Gouin, 2020).

As for the geographical distribution, it can be seen that most of the articles made (112/213) have been made in the Atlantic and Mediterranean, and in the Pacific (69/213). These data agree with reports from the Secretariat of the Convention on Biological Diversity, which reports that the largest number of articles describing plastic contamination in the world are from North America (117), Europe (52) and Australia (56) (Dias and Lovejoy, 2012). This behavior can be associated with the greater amount of resources for research by countries in Europe, Asia, and the Americas, and the greater pollution by plastics that occurs in these countries (89% of the world's plastics production comes from Asia, North America, and Europe) (Europe & EPRO, 2019).

Our results also show a clear tendency to study the complete gastrointestinal system of the species, with most studies (83/132) being reasonable considering the difficulty in evaluating all microplastics in incomplete samples, so that the gastrointestinal systems show to be the most effective organs to evaluate microplastics in biota (Hermesen et al., 2018). In turn, 67.2% of the articles used FTIR and Raman, which has two major benefits: the first is that visual examination by microscopy does not allow the identification of different polymers, a problem that is solved by the use of these advanced techniques, and also allows a distinction between natural and synthetic polymers (Gouin, 2020). On the other hand, visual identification techniques without subsequent verification by FTIR or Raman, are more likely to miss particles that are mixed in the digestive tracts with other materials, and whose extraction is more complicated (Wesch et al., 2016a, b). Moreover, the results show that it is necessary to carry out harmonized sampling protocols and quality controls in the analysis of microplastics in samples of marine biota, since there are currently different approaches to avoid contamination, but not all studies pay attention to air contamination by microfibers in biota samples (Foekema et al., 2013; Brander et al., 2020).

As for the number of individuals per group (median is 121 in fish, 44 in marine mammals, 62 in sea birds, and 47 in turtles). It is important to take into account the size of the sample when carrying out studies of contamination by microplastics, since an excessively small size can give us an idea of individual contamination but not group or species contamination. However, it is essential to take into account the ethical bases of the study of microplastics in living organisms, especially in the case of turtles and marine mammals, with the great difficulty in obtaining samples, because they are obtained from strandings or accidental fishing of these species. The information of this review can serve as a guide for future studies, since using the medians of the studies carried out as a reference, can give us an idea of the sample size that may be adequate for the study of these groups. This will avoid underestimating due to lack of data or investing unnecessary effort.

Research directed at marine mammals, turtles, and birds does not use hunting or fishing techniques, therefore they are based on studies of feces or acquire individuals that have died prior to the study. In fish, however, many studies carry out fishing and instead others acquire them in markets and fishmongers where the specimen had already been fished with a different objective than the study of microplastics, which makes it possible to have a greater number of specimens for research. In our opinion, and taking into account the logic of species conservation, in no case should you hunt or fish for individuals, whatever the group they belong to, since this could be a contradiction, carrying out scientific work to "protect" certain species through knowledge of the effect of microplastics on them, but on the other hand minimizing their populations and being able to provoke changes in the ecosystems from which they come depending on the number of individuals used. Therefore, for future studies, we recommend that the analysis of microplastics

be carried out on species that have been captured or have died previously, and that therefore the study of microplastics is not one of the reasons for the death of individuals.

Our results also show that turtles are the group most affected by microplastics, as it is the group with the highest percentage of individuals affected by microplastics (88% turtles, 59.5% marine mammals, 50.4% sea birds, 42% fish). However, the high prevalence of microplastics in turtles is shown not only in the proportion of contaminated individuals, but also in the mean number of microplastic particles found in individuals (121.7 items in turtles, 2.6 items in fishes, 9.7 items in marine mammals, 7.0 items in sea birds). We associate this great difference with the rest of the groups mainly to two processes: first, the large spatial distribution of the turtles and their migratory movements, which allows them to be found in areas highly contaminated by microplastics at certain times of their lives, so that microplastic particles may be present due to environmental exposure (Pham et al., 2017), and on the other hand the diet, turtles can feed on pelagic organisms when they are young, so that the shape of the plastic bags can be confused with organisms such as jellyfish, and they can feed on benthic organisms when they are adults, so that they can acquire the microplastics when they are swallowed, since an important part of the microplastics remains in the sediments and sedimentary organisms (Duncan et al., 2019). For the remaining groups, the percentages are relatively similar between them, in all cases exceeding 40% of affected individuals. This provides us with quite clear information on the enormous impact that microplastics have on marine vertebrate biota.

Although the proportion of individuals is similar, the mean number of microplastic particles in birds, mammals and fish differ, being mammals (9.7) and seabirds (7.0) larger than fish (2.6), in mammals this increase of microplastic particles compared to fish is associated with a trophic transfer (Moore et al., 2020), since they consume the entire prey, and can contain this microplastic, obtaining them through the diet (Hernandez-Milian et al., 2019). In birds it is associated with trophic transfer through the consumption of prey with microplastics, but also with the direct ingestion of the microplastics, which can be confused by their shape and colour with plankton organisms (Amélineau et al., 2016). It has also been demonstrated that the microplastics can come from pieces of macro and mesoplastics that are broken down into pieces in the gastrointestinal system of birds (Provencher et al., 2018). These arguments could explain the difference between the fish group and the marine mammal and seabird groups, but there is no doubt that more research is needed on the sources and mechanisms of microplastics in marine fauna. In turn, it is necessary to comment that the number of particles in fish fits with other studies, which describe from 1 to 20 particles depending on the fish species (Rezania et al., 2018).

The predominant type of microplastics found in all groups are fibers (71.1% in fish, 72.7% in marine mammals, 45.5% in seabirds, 50.0% in turtles), we associate this with the fact that most microplastic particles identified in the marine environment are fibers (Wright et al., 2013), and also match to with other studies that state that the predominant microplastic in fish was fibers (Rezania et al., 2018; Rochman et al., 2015). This can be explained by the fact that most plastics come from land-based sources, through sewage and solid waste treatment plants, which could explain the prevalence of fiber-type microplastics from laundry. Moreover, the loading of microplastics from fishing nets also contributes to increase the proportion of fibers compared to other types of microplastics (Anbumani and Kakkar, 2018). Birds and turtles show different behaviors from the rest, they present a lower proportion of fibers compared to the proportion of fragments, this can be associated with two different processes: first, part of the studies observe species of coastal birds, and correlations have been shown between the type of microplastic predominant on the coast and that found in the stomach of birds (Kain et al., 2016). So that this decompensation against other groups may be due to the greater amount of fragments against fibers in coastal habitats, secondly, birds select plastic particles that resemble zooplankton prey (Floren and Shugart, 2017), so depending on the

similarity of the prey of each species there will be a prevalence towards one type of microplastic or another. This case is also notable in turtles, which have a greater attraction to components similar to gelatinous macro-zooplankton (Vélez-Rubio et al., 2018). Prevalence of pellets in birds with respect to the rest of the groups is due to the fact that the areas where the samples were analyzed are closer to plastic industries that use this type of microplastics in their production processes (Adika et al., 2020), and that they can release them in nearby areas, affecting the local marine fauna.

The predominant polymers in marine biota are directly related to their production and therefore to the quantities that reach the environment. The polyethylene match to being the largest polymer in world production (36%) and that found in marine vertebrate organisms (27.3%). The same happens with the polypropylene, which is the second polymer in world production (21%) and that found in marine vertebrate organisms (14.3%), and the same with polyester that is the third in world production (<10%) and fourth in marine vertebrate organisms (10.4%) (Geyer et al., 2017).

An important aspect in plastic polymers is the type of polymer. In this review, all the main polymers present in the articles have been considered, but it is necessary to take into account the nature of the polymer, since there are polymers such as Rayon that are considered semi-synthetic fibers, due to their origin from natural sources. Rayon is a biodegradable polymer, but it is associated with contamination by the textile industry and wastewater (Ding et al., 2019; Le Guen et al., 2020; Lusher et al., 2013), so it can be a useful indicator to monitor pollution by anthropogenic particles, as well as certain associated organic compounds, such as polycyclic aromatic hydrocarbons (PAH) (Naidoo et al., 2020; Martinelli et al., 2020).

Most of the microplastic particles are in the range between 1.1 and 4.75 mm (Eriksen et al., 2014). On the other hand, it is also reported that most of the microplastics in surface waters and in sediments have sizes less than 2 mm (Sagawa et al., 2018), as well as that the size smaller than 2 mm can be easily ingested by the marine biota, which can be confused with its usual prey (Gago et al., 2020; Naidoo et al., 2020; Ory et al., 2017). Thus, our results are consistent with those described above, since they show that 73.6% of the studies comment that the predominant size range is less than 2.0 mm.

As for the predominant color, we found that in most of the groups studied the blue colour stand out (28.12% fish, 50% marine mammals, 75% turtles), this fits with other studies that highlight the predominant color in marine fauna such as black and blue (Rezania et al., 2018), the main explanation for this is that marine fauna confuse their common prey with microplastic particles due to the color (Kain et al., 2016; Rios-Fuster et al., 2019). This has been demonstrated in plankton fish, which showed a preference for blue colored fragments, because their prey in the natural environment are blue copepods (Ory et al., 2017). However, birds are the only group with a preference for transparent colour (55.56%) with respect to the rest of the groups. These data are consistent with other studies that show a preference for transparent colour in birds compared to blue colour (Amélineau et al., 2016; Floren and Shugart, 2017; Kain et al., 2016). This can be explained by the probability that visual searchers locate the colors, thus animals that observe plastics from below ingest dark colored fragments, like blue colour, while animals that observe plastics from above ingest light colored plastics, like transparent colour (Santos et al., 2016), this fits our data perfectly, and provides a reasonable explanation for the difference in color preference by groups. More attention needs the sources of microplastics in the ocean, the origin of these microplastics, and the effects microplastics have on the health of marine organisms, and to assess and promote changes at the political and social levels that encourage real plastic reduction strategies (Gall and Thompson, 2015; Hardesty et al., 2015; Ryan et al., 2009).

5. Conclusions

The conclusions of this study are the following:

1. Research on microplastics has increased rapidly, but there is an urgent need to carry out studies of microplastic contamination in little studied areas such as Antarctica, the Arctic and the Indian Ocean.
2. It is required to create a common methodology for the study of microplastics in marine biota, making quality studies that do not underestimate the impact of contamination due to the methodology. In this sense, we suggest that all studies of microplastics have been constructed using advanced technologies: Fourier Transformed Infrared Spectroscopic, Raman Spectroscopy, or any other not foreseen in this study that identifies microplastics with great effectiveness.
3. It is necessary that future studies take into account the possible contamination by fibers associated with air contamination. Therefore, we believe the use of blanks and laminar Flow is required.
4. Microplastics have a great impact on marine biota, especially on vertebrate fauna such as turtles, that are affected in 88% of the individuals studied, as well as on species included in the IUCN Red List.
5. The prevalence of fiber type microplastics (67.3%), gives us a clear idea of the failures in the countries waster water treatments (WWT), which is why further research is needed on the sources of microplastics.
6. The predominant size is <2 mm (73.6%) and predominant colour is blue (32.9%), associated with the ease of consumption, as well as the resemblance to common prey.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2021.112540>.

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