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Microplastic Contamination of Seafood Intended for Human Consumption: A Systematic Review and Meta-Analysis

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Background: Microplastics (MPs) have contaminated all compartments of the marine environment including biota such as seafood; ingestion from such sources is one of the two major uptake routes identified for human exposure.

Objectives: The objectives were to conduct a systematic review and meta-analysis of the levels of MP contamination in seafood and to subsequently estimate the annual human uptake.

Methods: MEDLINE, EMBASE, and Web of Science were searched from launch (1947, 1974, and 1900, respectively) up to October 2020 for all studies reporting MP content in seafood species. Mean, standard deviations, and ranges of MPs found were collated. Studies were appraised systematically using a bespoke risk of bias (RoB) assessment tool.

Results: Fifty studies were included in the systematic review and 19 in the meta-analysis. Evidence was available on four phyla: mollusks, crustaceans, fish, and echinodermata. The majority of studies identified MP contamination in seafood and reported MP content <1 MP/g, with 26% of studies rated as having a high RoB, mainly due to analysis or reporting weaknesses. Mollusks collected off the coasts of Asia were the most heavily contaminated, coinciding with reported trends of MP contamination in the sea. According to the statistical summary, MP content was 0–10.5 MPs/g in mollusks, 0.1–8.6 MPs/g in crustaceans, 0–2.9 MPs/g in fish, and 1 MP/g in echinodermata. Maximum annual human MP uptake was estimated to be close to 55,000 MP particles. Statistical, sample, and methodological heterogeneity was high.

Discussion: This is the first systematic review, to our knowledge, to assess and quantify MP contamination of seafood and human uptake from its consumption, suggesting that action must be considered in order to reduce human exposure via such consumption. Further high-quality research using standardized methods is needed to cement the scientific evidence on MP contamination and human exposures. https://doi.org/10.1289/EHP7171

Introduction

Microplastics (MPs) are broadly defined as synthetic polymeric particles <5 mm in diameter (Frias and Nash 2019; GESAMP 2015, 2016), often also including nanoplastics, which are <100 nm in diameter (Lusher et al. 2017a). They can be classified into two categories according to their origin: primary (intermediate feedstock, pellets/resin, by-products), and secondary (formed through fragmentation and degradation) (Carbery et al. 2018; Karlsson et al. 2018). MPs are diverse, originating from the wide variety of plastics produced for household products, construction material, and industrial applications. Human exposure is suggested to be principally via ingestion and inhalation (Abbasi et al. 2019; Wright and Kelly 2017). MPs are ubiquitous in the environment, with marine environments especially affected owing to the amount of plastic waste they receive (Burns and Boxall 2018; Gourmelon 2015; J Li et al. 2016). The degradation of plastic waste in the sea is the major source of MP contamination (Eriksen et al. 2014). The generation of plastic waste and mismanagement of its disposal is expected to triple by 2060, reaching 155–265 million metric tons per year (Lebreton and Andrady 2019). MPs are extremely persistent particles; over time they have contaminated all compartments of marine ecosystems, including the food web and biota across different trophic levels, such as bivalves (SY Zhao et al. 2018), crustaceans (F Zhang et al. 2019), fish, and mammals (Lusher et al. 2015; Nelms et al. 2018). MPs have been found in various parts of organisms such as the gastrointestinal (GI) tract (Sun et al. 2019), liver (Collard et al. 2017a), gills (Feng et al. 2019), and flesh (Akoueson et al. 2020; Karami et al. 2017b). Commercial seafood species are either consumed whole, such as bivalves, some crustaceans, and some small fish, or just parts of them, such as larger fish and mammals. Therefore, understanding the MP contamination of specific body parts, and their consumption by humans, is key.

Food safety is managed in terms of hazards and risk analysis, where hazards are classified into three categories according to their potential to cause a health effect: biological, chemical, and physical (EC 2002). The MP health effects that are currently under consideration include all three categories (Smith et al. 2018; Wright and Kelly 2017). MPs contain various chemicals with differing concentration (Hartmann et al. 2019), and their effects can come from the plastics' primary components (polymers), the additives that are used to enhance their attributes (plasticizers), the chemical contaminants absorbed while in the environment [e.g., polycyclic aromatic hydrocarbons (Hartmann et al. 2017; Ziccardi et al. 2016) and polychlorinated biphenyls (Engler 2012), or the microorganisms colonizing their surfaces (Viršek et al. 2017)]. MPs can thus be considered either the primary hazard or a pathway for a hazard, both linked to human health. The contamination of food intended for human consumption, with this emerging risk and the possible effects on health, has raised concern in the scientific community (Barboza et al. 2018; Diepens and Koelmans 2018; Santillo et al. 2017; Waring et al. 2018) as well as among stakeholders (GESAMP 2015, 2016) and policy makers globally (EFSA Panel on Contaminants in the Food Chain 2016). There is a growing body of evidence regarding effects in aquatic animals, but health effects on humans are still unclear (Karbalaei et al. 2018; Sharma and Chatterjee 2017; Smith et al. 2018). There is a clear need to address this emerging risk and promptly implement mitigation strategies for the protection of the environment and human health.

This systematic review focuses on seafood intended for human consumption. The aim is to map the existing evidence, appraise study quality using a standardized approach, identify knowledge

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gaps, and ultimately collate the data in order to quantify human exposures. Predicted human exposures calculated using modeling could consequently be used in a risk assessment framework to characterize the risk coming from MPs through the ingestion uptake route.

Methods

This review is based on a protocol published in PROSPERO (Danopoulos et al. 2019). The protocol was created in order to standardize the methods and protect against the inclusion of bias, according to the guidelines set by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses protocols (PRISMA-P) (Moher et al. 2015; Shamseer et al. 2015). In brief, only primary, peer-reviewed studies with descriptive and analytic observational study designs were eligible for inclusion. There were no publication date limits. Only studies that sampled commercially relevant seafood species were included, regardless of the species of the organism (e.g., fish, mollusks, crustaceans) or the part of the body that MPs were reported to be found in, for example, the gills, GI tract, liver, and flesh. If a study focused on the GI tract of a type of seafood, it was included only if the species of the seafood was small and it was reasonable to assume that it is usually eaten whole with the GI tract intact (e.g., anchovies, shrimps). Studies reporting on samples that were not collected as food, but are regularly consumed as such (e.g., mussels), were included. Studies must have used one of the four currently validated procedures for the identification of the chemical composition of particles: namely, Fourier-transform infrared spectroscopy (FT-IR), Raman spectroscopy (RM), pyrolysis gas chromatography/mass spectrometry, and scanning electron microscopy (SEM) plus energy-dispersive X-ray spectroscopy. All included studies must have reported the use of procedural control samples to avoid post-sampling contamination.

The following online databases/sources were searched from launch date: MEDLINE (OVID interface, 1946 onward), EMBASE (OVID interface, 1974 onward), and Web of Science core collection (Web of Science, 1900 onward). The initial search was executed on 10 July 2019. The searches were repeated on 5 October 2020 to include the most recently published papers. Search terms included: microplastic, nanoplastic, plastic/, micro*, fiber*, food contamination, and seafood. The full search strategy can be found in Tables S1 and S2. Study screening was completed by two independent reviewers (E.D. and L.J. for the original searches; E.D. and M.T. for the rerun of the searches) at two levels, initially reviewing titles and abstracts. Screening results were compared and disagreements discussed. Inter-rater agreement at the first level was 90%, Cohen's κ: 0.34, for the original searches, and 97%, Cohen's κ: 0.65, for the rerun. This was followed by a full paper review for potentially eligible papers. A third-party arbitrator (J.M.R.) resolved the discrepancies between the two reviewers (for both searches). Inter-rater agreement at this level was 100%, Cohen's κ: 1 for both searches. Corresponding authors were contacted when more information was required with a maximum of three emails sent. Data was extracted as sample characteristics, sampling and analysis methods, MP content in any quantified unit, composition analysis results, and procedural samples results.

Synthesis of Results

The primary outcome was reported as MP content in terms of particles per unit mass or individual organism expressed as the mean value [and standard deviation (SD) or standard error] or the range. Effort was made to convert all the data into the same unit of measurement of particles/g (wet weight) when it was appropriate, and the necessary raw data was available. The MP contents for species of the same family in the same study were pooled using the formulae for combining groups proposed by Higgins and Green (2011, Table 7.7a) (Table S3). When needed, the conversion of the five-number summary (sample minimum and maximum, median, lower and upper quartile) to the quantities needed for this review, was made using the methods and calculator developed by Shi et al. (2020). The calculator draws on the methods developed by Luo et al. (2018) for the estimation of the mean of the sample and the methods by Wan et al. (2014) for the estimation of the SD.

The results of the studies were weighted using the inverse of the variance method (Chen and Peace 2013). In order to collate and quantify the data, random-effects meta-analysis models were used (Higgins et al. 2019). Random-effects models were preferred over fixed-effects models because it was assumed that the samples did not share one common true effect size that was influenced equally by the same factors but, rather, a distribution of true effect sizes (Chen and Peace 2013; Harrer et al. 2019b; Veroniki et al. 2016). The DerSimonian-Laird t^2 estimator was used for all the random-effects models (DerSimonian and Laird 1986, 2015) because this accounts for variations both within and between studies. The Higgins I^2 test and the chi-squared Cochran's Q statistic were used to assess statistical heterogeneity (Higgins and Thompson 2002; Higgins et al. 2003). The I^2 test is the percentage of variability in the effect size that is not produced by sampling error. The Cochran's Q statistic refers to the null hypothesis of homogeneity and is expressed in the chi-square and p-values (Higgins et al. 2003).

The source of between-study statistical heterogeneity was investigated by examining statistical outliers and an influence analysis of studies. Statistical outliers were defined as studies where the 95% confidence interval (CI) of their effect size estimate, as calculated by the random-effects model, did not overlap with the 95% CI of the pooled effect size estimate (Harrer et al. 2019b). Statistical outliers of extremely large effects were specifically targeted to account for and avoid overestimations (where the lower bound of the 95% CI of the study was higher than that of the upper bound of the 95% CI of the pooled effect). To test the influence of individual studies, the models were rerun without these outliers, and the two pooled effect size estimates compared. To further test the influence of every study, the models were rerun excluding one study each time to assess each study's influence on the pooled effect size (Harrer et al. 2019b). Influence diagnostics included the I^2 and Q values (Baujat et al. 2002) and the contribution to the pooled effect size (Viechtbauer and Cheung 2010). The results of the influence analysis were examined numerically and graphically.

Methodological and sample heterogeneity were explored using subgroup analysis employing a fixed-effects (plural) model (mixed-effects model) (Harrer et al. 2019b). R (version 3.6.0; R Development Core Team) was used for all calculations and models executing all analysis via RStudio (version 1.2.1335; RStudio), using the additional packages meta (version 4.9-7; Schwarzer 2019), metaphor (version 2.1-0; Viechtbauer 2010), dmetar (Harrer et al. 2019a), robvis (McGuinness and Kothe 2019), and ggplot2 (Wickham et al. 2016). The code is provided in the Supplemental Material, "Code for R used in the meta-analysis." Each data set was assessed separately in order to determine its suitability for meta-analysis in terms of heterogeneity. The results of the meta-analysis are presented as the MP content (in MPs per gram) with a 95% CI and p-value. Maps were created in ArcGIS Desktop (version 10.8; Esri).

Risk of Bias/Quality Assessment

A bespoke risk of bias (RoB) assessment tool was created, rating the studies across four domains: study design, sampling, analysis, and reporting with a final overall assessment (Table S4). The tool comprises a checklist with questions covering all aspects of experimental protocol development, execution, and reporting. The rating of the studies was as follows: high, low, or unclear RoB, supported by a justification for each of the entries.

The construction of the RoB tool was based on up-to-date scientifically robust methodology by the Cochrane organization, which is the leading scientific body in the field of systematic reviews (Higgins et al. 2011, 2019). According to the guidance, the use of scales and scores (numerical) for the assessment was avoided. Instead, for each of the entries, a question was formulated in order to prompt a response that was used as the support for the judgment (Table S4). For each item in the tool, there were two entries: the answer, with additional notes when needed, and the rating. In the answer entry, a copy of the text from the study on which the decision was made is provided, allowing transparency on how the decision was made. The rating of the studies for each entry, domain, and overall study was as follows: high, low, or unclear RoB. RoB assessment was done both on the study and on the specific outcome level. This allowed for the direct comparison of the RoB rating of a specific domain of the study against a specific outcome. For example, when reviewing the sampling methodology, the sampling domain RoB rating is more relevant than that of the overall RoB rating. For the majority of the items in the tool, the rating of high or low was based on a yes/no answer or a numerical value. The rating unclear was assigned when the study did not report sufficient information to make a judgment or when the associated risk was unknown. In order to achieve maximum transparency, all items are discussed in the section "RoB tool additional explanation" in the Supplemental Material.

Weighting of Domains and Questions

A rating was given to each of the 21 items of the RoB tool; subsequently, a rating was given to each of the four domains on the basis of the rating of the individual items in it; and, finally, the overall rating was given according to the domains' rating. In order to decide the weighting of the individual entries in the checklist, three experts in the field were contacted and asked to provide their top three entries/questions of the table as the most important factors to judge the studies' RoB. All three experts concentrated on four questions: 4, 8, 13, and 15 (see "RoB tool additional explanation" in the Supplemental Material). The questions focused on two topics. First, the prevention of sample contamination and its validation by the use of procedural blank samples. Second, the use of a validated method for identifying the composition of the particles and how a spectra library would be employed to do so. This expert opinion on the importance of individual entries of the RoB tool was taken into consideration for the rating of the domain as well as the overall rating of the studies.

Publication bias was explored using the Egger's test (Egger et al. 1997) visualized in funnel plots and the precision of the effect estimate (Liberati et al. 2009). Overall assessment of the certainty of the evidence was based on the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) framework (Higgins et al. 2019) and the Environmental-GRADE (Bilotta et al. 2014) across five domains, categorized into four certainty ratings: high, moderate, low, and very low.

Results

Study Selection

The initial searches led to 2,467 publications, following the removal of duplicates. In the first level screening, 2,307 citations were excluded on the basis of their title and abstract. For the second level screening, the full text of the remaining 160 studies were evaluated, and a total of 34 studies that analyzed seafood samples met the eligibility criteria set for this review (see PRISMA flow diagram, Figure S1). The update of the searches identified 16 more studies eligible for the review, bringing the total number of included studies to 50 (Figure S1).

Study Characteristics

All the studies included are environmental field studies employing descriptive and analytic observational study designs, sampling and analyzing four phyla: mollusks, crustaceans, fish, and ehcinodermata (Table 1). Eight studies analyzed organisms coming from more than one phylum. Twenty-three studies sampled only mollusks, 15 only fish, 3 only crustaceans, and 1 only echinodermata. Five studies sampled both mollusks and crustaceans, 2 mollusks and fish, and 1 mollusks, crustaceans, and fish. The study characteristics are presented in Table 1. Twenty-eight studies used samples from Asia, 13 from Europe, 4 from the Americas, 2 from Africa, 1 from Australia/Oceania, and 2 from more than one continent (and their coasts). The overall sample size for fresh fish was $n = 1{,}269$ ($n = 665$ anchovies, $n = 274$ sardines, $n = 240$ painted comber, $n = 20$ sand lance, $n = 19$ bogue, $n = 19$ seabass, $n = 12$ haddock, $n = 10$ plaice, $n = 10$ mackerel); for dried fish, $n = 120$ ($n = 30$ mackerel, $n = 30$ croaker, $n = 30$ mullet, $n = 30$ anchovies); and for canned fish, $n = 842$ ($n = 608$ sprat, $n = 184$ sardines, $n = 45$ tuna, $n = 5$ mackerel). For the rest of the seafood, the overall sample size was $n = 4{,}543$ [mollusks $n = 3{,}882$ ($n = 1{,}728$ mussels, $n = 1{,}015$ oysters, $n = 702$ clams, $n = 171$ sea snails, $n = 166$ scallops, $n = 100$ cockles), crustaceans $n = 451$ ($n = 262$ shrimps, $n = 139$ crabs, and $n = 50$ barnacles), and echinodermata $n = 210$]. Two studies did not provide the exact sample size: Qu et al. (2018) reported $n \sim 760$ mussels and Wu et al. (2020) reported 10–20 samples for each species, and Teng et al. (2020) did not report sample sizes at all. Species for all samples are presented in Table 1. An additional phylogenetic tree is provided for the molluskan species in Figure S2 to facilitate reference to nomenclature. Sample size fluctuated between the studies. Although we are not aware of a gold standard as yet for the number of samples for such environmental studies, many studies used $n \geq 5$ per species, whereas others used $n \geq 30$. Only three studies in the review used <5 organisms per species (Abidli et al. 2019; Collard et al. 2017a; F Zhang et al. 2019).

FT-IR was used by 72% ($n = 36$) of the studies as the preferred method for identifying the chemical composition of the particles, followed by RM, which was used by 20% ($n = 10$) (Table 1). One study used both methods, and the other 3 combined the use of FT-IR and SEM. Twenty-three different particle-extraction processes were used (Table 1; Table S5). The most common method was that developed by Li et al. (2015), used by 11 studies. The method uses a 30% hydrogen peroxide (H_2O_2) treatment for the digestion of the samples, followed by a density-separation step using a sodium chloride (saline) solution and filtration.

RoB Within Studies

The summary of the results of the RoB assessment is illustrated in Figure 1 and in Table S6. The individual rating for each study across all domains is presented in Table S7; 13 studies (26%) were rated as having a high RoB, 26 (52%) a low RoB, and the remaining 11 (22%) an unclear RoB. The domain most often rated as of high RoB was reporting (20 studies; 47%), and the domain that was most rated as unclear RoB was analysis (20 studies; 47%). The most common issues were failure to report the results of the procedural blank samples (e.g., Hossain et al. 2020; HX Li et al. 2018; Thushari et al. 2017; J Wang et al. 2019; Wu

Table 1. Study characteristics for seafood studies.

References	Geographic location	Sample phylum/class	Sample species (common name)	Sampling location	Habitat	*N*	*n*	MPs extraction procedure references	MPs identification method	Outcome
Abidli et al. 2019	Tunisia			Environment	Wild			HX Li et al. 2018	FT-IR	Mean MPs content per mass with SD
		Bivalve mollusks				42				
			Mytilus galloprovincialis (mussel)				15			
			Ruditapes decussatus (clam)				24			
			Crassostrea gigas (oyster)				3			
		Gastropod mollusks				18				
			Hexaplex trunculus (sea snail)				9			
			Bolinus brandaris (sea snail)				9			
Akhbarizadeh et al. 2020	Iran	Fish		Market (canned)	NA	50		Karami et al. 2017b	RM	Mean MPs content per mass with SD
			Thunnus tonggol (longtail tuna)				25			
			Thunnus albacares (yellowfin tuna)				20			
			Scombermorus commerson (mackerel)				5			
Akoueson et al. 2020				Market	NA			J Li et al. 2018	FT-IR	Mean MPs content per mass and individual with SD
		Fish				42				
	Scotland		*Melanogrammus aeglefinus* (haddock)				12			
	Greece		*Dicentrarchus labrax* (seabass)				10			
	Iceland		*Pleuronectes platessa* (plaice)				10			
	Scotland		*Scromber scombrus* (mackerel)				10			
		Bivalve mollusks				20				
	Chile		*Zygochlamys patagonica* (scallop)				10			
	Scotland		*Pecten maximus* (scallop)				10			
Baechler et al. 2020	USA	Bivalve mollusks		Environment		283		Developed their own	FT-IR	Mean MPs content per mass and individual with SD
			C. gigas (oyster)		Farmed		141			
			Siliqua patula (razor clam)		Wild		142			
Birnstiel et al. 2019	Brazil	Bivalve mollusks	*Perna perna* (mussel)	Environment		20		Van Cauwenberghe et al. 2015	FT-IR	MPs content range per mass with SD
					Farmed		10			
					Wild		10			
Bour et al. 2018	Norway			Environment	Wild			Avio et al. 2015; Dehaut et al. 2016	FT-IR	Frequency of MPs occurrence
		Crustacean	*Crangon allmanni* (shrimp)				20			
		Bivalve mollusks	*Ennucula tenuis* (mussel)				12			
Bråte et al. 2018	Norway	Bivalve mollusks		Environment	Wild	332		Dehaut et al. 2016	FT-IR	Mean MPs content per mass and individual with SD
			M. edulis (mussel)				NA			
			M. trossulus (mussel)				NA			
			M. galloprovincialis (mussel)				NA			

Table 1. (*Continued.*)

References	Geographic location	Sample phylum/class	Sample species (common name)	Sampling location	Habitat	*N*	*n*	MPs extraction procedure references	MPs identification method	Outcome
Cho et al. 2019	South Korea	Bivalve mollusks		Market	Farmed	240		Karami et al. 2017a	FT-IR	Mean MPs content per mass and individual with SD
			C. gigas (oyster)				60			
			M. edulis (mussel)				60			
			Tapes philippinarum (clam)				60			
			Patinopecten yessoensis (scallop)				60			
Collard et al. 2017a	Mediterranean Sea, English Channel	Fish		Environment	Wild	15		Collard et al. 2015	RM	Frequency of MPs occurrence
			Engraulis encrasicolus (anchovy)				13			
			Sardina pilchardus (sardine)				2			
Collard et al. 2017b	English Channel, Mediterranean Sea, and Northeastern Atlantic	Fish		Environment	Wild	40		Collard et al. 2015	RM	Mean MPs content per individual
			E. encrasicolus (anchovy)				20			
			S. pilchardus (sardine)				20			
Digka et al. 2018	Northern Ionian Sea			Environment				Mathalon and Hill 2014	FT-IR	Mean MPs content per individual with SD
		Bivalve mollusks	*M. galloprovincialis* (mussel)		Wild/farmed		80			
		Fish	*S. pilchardus* (sardine)		Wild		36			
Ding et al. 2018	China	Bivalve mollusks				115		Developed their own	FT-IR	Mean MPs content per mass and individual
			Chlamys farreri (scallop)	Market	Farmed		50			
			M. galloprovincialis (mussel)							
				Market	Farmed		50			
				Environment	Wild		15			
Ding et al. 2019	China	Bivalve mollusks		Market	NA	40		Developed their own	FT-IR and SEM	Mean MPs content per mass and individual with SD
			M. galloprovincialis (mussel)				20			
			Ruditapes philippinarum (clam)				10			
			Mactra veneriformis (clam)				10			
Ding et al. 2020	China			Market	NA	80		Ding et al. 2018, 2019	FT-IR	Mean MPs content per mass and individual with SD
		Bivalve mollusks				120				
			M. galloprovincialis (mussel)				10			
			Perna viridis (mussel)				10			
			R. philippinarum (clam)				20			
			C. gigas (oyster)				20			
			Sinonovacula constricta (clam)				20			
			Scapharca subcrenata (clam)				20			
			Meretrix lusoria (clam)				20			
		Gastropod mollusks				20				
			Busycon canaliculatu (sea snail)				20			

Table 1. (*Continued.*)

References	Geographic location	Sample phylum/class	Sample species (common name)	Sampling location	Habitat	N	n	MPs extraction procedure references	MPs identification method	Outcome
Fang et al. 2018	Bering Sea and Chukchi Sea			Environment	Wild			Digestion: Dehaut et al. 2016; Phuong et al. 2018a Floatation/filtration: Li et al. 2015	FT-IR	Mean MPs content per mass with SD
		Bivalve mollusks				57				
			Astarte crenata (clam)				28			
			Macoma tokyoensis (clam)				29			
		Gastropod mollusks				43				
			Retifusus daphnelloides (sea snail)				24			
			Latisipho hypolispus (sea snail)				19			
		Crustaceans				80				
			Pandalus borealis (Arctic shrimp)				21			
			Chionoecetes opilio (snow crab)				59			
Feng et al. 2019	China	Fish	*Thryssa kammalensis* (rednose anchovy)	Environment	Wild	19		Dehaut et al. 2019; Foekema et al. 2013; Hermsen et al. 2018; Karami et al. 2017a	FT-IR	Mean MPs content per mass and individual with SD
Feng et al. 2020	China	Echinodermata		Environment	Wild	210		Foekema et al. 2013; Karami et al. 2017a	FT-IR	Mean MPs content per mass and individual
			Strongylocentrotus intermedius (sea urchin)				NA			
			Temnopleurus hardwickii (sea urchin)				NA			
			Temnopleurus reevesii (sea urchin)				NA			
			Hemicentrotus pulcherrimus (sea urchin)				NA			
Hermabessiere et al. 2019	France	Bivalve mollusks		Environment	Wild	200		Dehaut et al. 2016	RM (no fibers)	Mean MPs content per mass with SD
			M. edulis (mussel)				100			
			C. edule (cockle)				100			
Hossain et al. 2020	Bangladesh	Crustacean		Environment	Wild	30		Li et al. 2015; Su et al. 2016	FT-IR	Mean MPs content per mass with SD
			Metapenaeus monocerous (brown shrimp)				20			
			Penaeus monodon (tiger shrimp)				10			
Karami et al. 2017b	Malaysia	Fish		Market (packed dried)	NA	120		Karami et al. 2017a	RM	Frequency of MPs occurrence
			Chelon subviridis (greenback mullet)				30			
			Johnius belangerii (Belanger's croaker)				30			
			Rastrelliger kanagurta (*Indian mackerel)*				30			
			Stolephorus waitei (spotty-face anchovy)				30			

Table 1. (*Continued.*)

References	Geographic location	Sample phylum/class	Sample species (common name)	Sampling location	Habitat	N	n	MPs extraction procedure references	MPs identification method	Outcome
Karami et al. 2018	Product of Canada, Germany, Iran, Japan, Latvia, Malaysia, Morocco, Poland, Portugal, Russia, Scotland, Thailand, and Vietnam	Fish		Market (canned)	NA	792[a]		Karami et al. 2017a	RM	Frequency of MPs occurrence
			Canned sardines (species unknown)				184[a]			
			Canned sprats (species unknown)				608[a]			
Leslie et al. 2017	Netherlands			Environment	Wild			Van der Horst 2011, 2013	FT-IR	Mean MPs content per mass
		Bivalve mollusks				26				
			M. edulis (mussel)				20			
			C. gigas (oyster)				6			
		Gastropod mollusks	*Littorina littorea* (sea snail)			10				
		Crustacean	*Carcinus maenas* (crab)			10				
HX Li et al. 2018	China	Bivalve mollusks	*Saccostrea cucullata* (oyster)	Environment	Wild	330		Li et al. 2015	FT-IR	MPs content range per mass and individual
J Li et al. 2018	UK	Bivalve mollusks	*M. edulis* (mussel)			246		J Li et al. 2016	FT-IR	Mean MPs content per mass with SD
				Environment	Wild		162			
				Market						
					Farmed		54			
					Wild		30			
J Li et al. 2016	China	Bivalve mollusks	*M. edulis* (mussel)	Environment		390		Li et al. 2015	FT-IR	Mean MPs content per mass and individual
					Wild		222			
					Farmed		168			
Li et al. 2015	China	Bivalve mollusks		Market	Wild/farmed	144		Developed their own	FT-IR	Mean MPs content per mass with SD
			Scapharca subcrenata (clam)		NA		6			
			Tegillarca granosa (clam)		NA		18			
			Alectryonella plicatula (clam)		NA		18			
			R. philippinarum (clam)		NA		24			
			Sinonovacula constricta (clam)		NA		6			
			M. lusoria (clam)		NA		18			
			Cyclina sinensis (clam)		NA		30			
			M. galloprovincialis (mussel)		NA		18			
			P. yessoensis (scallop)		NA		6			
Lopes et al. 2020	Portugal	Fish		Environment	Wild	226		Dehaut et al. 2016	FT-IR	Mean MPs content per individual with SD
			S. pilchardus (sardine)				76			
			E. encrasicolus (anchovy)				131			
			Boops boops (bogue)				19			
McGoran et al. 2018	Thames Estuary, UK	Crustacean	*C. crangon* (brown shrimp)	Environment	Wild	116		Their own method without digestion	FT-IR	Mean MPs content per individual and frequency of occurrence

Table 1. (*Continued.*)

References	Geographic location	Sample phylum/class	Sample species (common name)	Sampling location	Habitat	*N*	*n*	MPs extraction procedure references	MPs identification method	Outcome
Naji et al. 2018	Persian Gulf			Environment	Wild			Li et al. 2015	FT-IR, SEM	Mean MPs content per mass
		Gastropod mollusk	*Amiantis umbonella* (sea snail)			30				
		Bivalve mollusks				63				
			Amiantis purpuratus (scallop)				30			
			Pinctada radiate (oyster)				33			
Nam et al. 2019	Vietnam	Bivalve mollusk	*P. viridis* (mussel)	Environment	Wild	5		Phuong et al. 2018b	FT-IR	Mean MPs content per mass and individual with SD
Phuong et al. 2018a	French Atlantic coasts	Bivalve mollusks		Environment	Wild/farmed	180		Phuong et al. 2018b	FT-IR	Mean MPs content per mass and individual with SD
			M. edulis (mussel)		NA		120			
			C. gigas (oyster)		NA		60			
Pozo et al. 2019	Chile	Fish	*Strangomera bentincki* (sardine)	NA	NA	10		Lindeque and Smerdon 2003	FT-IR	Frequency of MPs occurrence
Qu et al. 2018	China	Bivalve mollusks		Environment	Wild	~760		Li et al. 2015	FT-IR	MPs content range per mass and individual
			M. edulis (mussel)				~430			
			P. viridis (mussel)				~330			
Renzi et al. 2019	Adriatic Sea	Fish		Environment	Wild	160		Nuelle et al. 2014; Avio et al. 2015	FT-IR	Mean MPs content per individual
			S. pilchardus (sardine)				80			
			E. encrasicolus (anchovy)				80			
Su et al. 2018	Middle-lower Yangtze River Basin, China	Bivalve mollusk	*Corbicula fluminea* (Asian clam)	Environment	Wild	208		Li et al. 2015; Su et al. 2016	FT-IR	Mean MPs content per mass and individual with SD
Su et al. 2019	China	Fish	*Lateolabrax maculatus* (seabass)	Environment	Wild	9		Jabeen et al. 2017	FT-IR	Mean MPs content per mass and individual with SD
Sun et al. 2019	Yellow Sea, China	Fish		Environment	Wild	380		Desforges et al. 2015	FT-IR	Mean MPs content per individual
			Setipinna taty (anchovy)				20			
			Anchoviella commersonii (anchovy)				30			
			Engraulis japonicus (anchovy)				280			
			Ammodytes personatus (sand lance)				50			
Tanaka and Takada 2016	Tokyo Bay, Japan	Fish	*E. japonicus* (Japanese anchovy)	Environment	Wild	64		Foekema et al. 2013; Rochman et al. 2015	FT-IR	Mean MPs content per individual with SD
Teng et al. 2019	China	Bivalve mollusks		Environment	Farmed	306		Munno et al. 2018	FT-IR	Mean MPs content per mass and individual
			C. gigas (oyster)				NA			
			C. angulate (oyster)				NA			
			C. hongkongensis (oyster)				NA			
			C. sikamea (oyster)				NA			

Table 1. (*Continued.*)

References	Geographic location	Sample phylum/class	Sample species (common name)	Sampling location	Habitat	*N*	*n*	MPs extraction procedure references	MPs identification method	Outcome
Teng et al. 2020	China	Fish	*Sardinella zunasi* (Japanese scaled sardine)	Environment	Wild	NA		Munno et al. 2018	FT-IR	Mean MPs content per mass and individual with SD
Thushari et al. 2017	Gulf of Thailand			Environment	Wild			Claessens et al. 2013	RM	Mean MPs content per mass with SD
		Bivalve mollusk	*Saccostrea forskalii* (oyster)				15			
		Gastropod mollusk	*Littoraria* sp. (periwinkle, sea snail)				50			
		Crustacean	*Balanus amphitrite* (barnacle)				50			
Van Cauwenberghe and Janssen 2014	Germany	Bivalve mollusks			Farmed	93		Claessens et al. 2013	RM	Mean MPs content per mass with SD
	Germany		*M. edulis* (mussel)	Environment			72			
	France		*C. gigas* (oyster)	Market			21			
J Wang et al. 2019	South Yellow Sea, Korea and China			Environment	Wild			Claessens et al. 2013	FT-IR, SEM	Mean MPs content per mass with SD
		Bivalve mollusk	*Acila mirabilis* (clam)				20			
		Crustacean	*C. affinis* (sand shrimp)				10			
Q Wang et al. 2020	China	Fish		Environment	Wild	58		Munno et al. 2018	FT-IR	Mean MPs content per mass and individual with SD
			Konosirus punctatus (spotted sardine)				44			
			Thryssa mystax (Gangetic anchovy)				8			
			Sardinella zunasi (Japanese scaled sardine)				6			
Webb et al. 2019	New Zealand	Bivalve mollusk	*Perna canaliculus* (mussel)	Environment	Wild	96		Claessens et al. 2013	FT-IR	Mean MPs content per individual with SD and range of MPs per mass
Wu et al. 2020	China			Environment	Farmed			Li et al. 2015	FT-IR	Mean MPs content per mass and individual with SD
		Fish				NA				
			Larimichthys crocea (large yellow croaker)				10–20[b]			
			Konosirus punctatus (dotted gizzard shad)				10–20[b]			
		Bivalve mollusks				NA				
			Ostrea denselamellosa (oyster)				10–20[b]			
			Sinonovacula constricta (razor clam)				10–20[b]			
		Crustacean	*Parapenaeopsis hardwickii* (shrimp)				10–20[b]			

Table 1. (*Continued.*)

References	Geographic location	Sample phylum/class	Sample species (common name)	Sampling location	Habitat	N	n	MPs extraction procedure references	MPs identification method	Outcome
F Zhang et al. 2019	China	Crustaceans		Environment	Wild	136		Masura et al. 2015 (for crustaceans)	FT-IR	Frequency of occurrence per individual
			Oratosquilla oratoria (shrimp)				64			
			O. kempi (shrimp)				1			
			Portunus trituberculatus (crab)				30			
			Carcinoplax vestita (crab)				18			
			Charybdis bimaculata (crab)				15			
			Charybdis variegate (crab)				4			
			Portunus gracilimanus (crab)				3			
			Charybdis japonica (crab)				1			
SY Zhao et al. 2018	Avery Point Dock, USA	Bivalve mollusks	*M. edulis* (mussel)	Environment	Wild	37		Zhao et al. 2017	RM April samples FT-IR September samples	Mean MPs content per mass and individual with SD
Zhu et al. 2019	China, Maowei Sea	Bivalve mollusk	*C. hongkongensis* (oyster)	Environment	Wild	20		Foekema et al. 2013; Karami et al. 2017a	FT-IR	Mean MPs content per mass and individual with SD
Zitouni et al. 2020	Tunisia	Fish	*Serranus scriba* (painted comber)	Environment	Wild	240		Dehaut et al. 2016; Phuong et al. 2018b	RM	MPs content per mass with SD

Note: Outcome is the description of the MP content as reported by each study. FT-IR, Fourier-transform infrared spectroscopy; MP, microplastic; N, overall sample size expressed in number of organisms per phylum or class; n, sample size expressed in number of organisms per species, sampling location, or habitat accordingly; NA, not available; RM, Raman spectroscopy; SD, standard deviation; SEM, scanning electron microscopy.

[a]20 brands of canned fish were employed, 11 for sardines and 9 for sprats. Samples n was calculated based on the number of fish in one can per brand.

[b]10–20 per species (exact n was not reported).

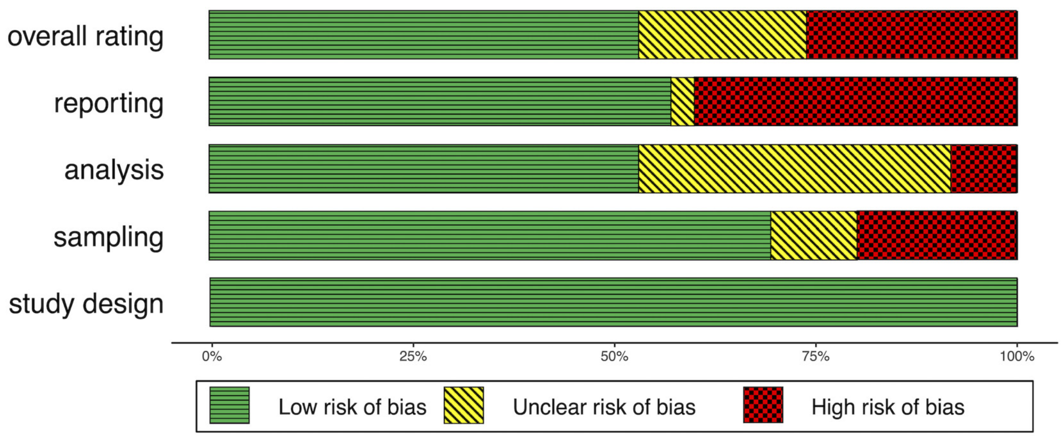

Figure 1. Risk of bias (RoB) assessment seafood studies. The three ratings are illustrated by percentage. The numerical data for the figure is provided in Table S6. Individual rating per study and per domain is provided in Table S7. Rating was executed according to the RoB tool (see Table S4 and the "RoB tool additional explanation" section in the Supplemental Material).

et al. 2020) and the specifications of the chemical composition analysis (e.g., Collard et al. 2017a; Renzi et al. 2019). The domain of study design was rated as of low RoB across all studies. Lack of space often precludes careful description of the sampling design development, and this was not reported in any of the studies, but the description of sampling activities was adequate to infer it. Further details of the RoB assessment are discussed in the narrative analysis and the results were used to inform both qualitative and quantitative analyses.

Results on MP Contamination Levels within Seafood

The MP content results are presented in three tables, one for each phylum to facilitate comparison (Tables 2–4). The results for the echinodermata phylum (Feng et al. 2020) are presented in Table 2 along with the molluskan phylum. Studies appear in more than one table if their samples included more than one phylum of organisms. The MP content is expressed as the number of MP particles per gram of sample or per individual organism. Studies provided either the mean content (with or without the SD) or the range of content or both. Lopes et al. (2020) reported only the median and the interquartile range; the methods and calculator developed by Shi et al. (2020) were used to estimate the mean and SD. A minority of the studies reported only the frequency of samples being positive for MP contamination and were excluded from the statistical summary.

In terms of procedural blank samples results, 18% of the studies ($n=9$ of 50) did not report their results, whereas a surprising 36% ($n=18$) reported that no MPs were found (Table S8). The 46% of the studies ($n=23$) that did report the discovery of specific MPs content in their blank samples used their results in different ways. Thirty-five percent of the studies (34.7%; $n=8$ of 23) corrected their final findings against the results of the procedural blank samples, whereas an additional 8.6% ($n=2$) subtracted the absolute number of discovered MPs from their results. Twenty-six percent ($n=6$) considered the results to be negligible without offering justification to that effect, whereas 4.3% ($n=1$) did not make use of the results and did not provide an explanation. On the other hand, 13% of the studies ($n=3$) tested the significance of their results statistically, and 8.6% ($n=2$) used the results to set detection limits. The remaining 4.3% ($n=1$) did not report if or how the results were used.

Molluskan Studies

Statistical summary of effects and narrative analysis. Thirty-one studies analyzed mollusks (Table 2), but only data from 27 studies (87%) were combined in a statistical summary. Four studies were excluded: Leslie et al. (2017) used a different approach for the analysis and reported their results as MP per gram of dry weight, two studies reported results per individual organism (Birnstiel et al. 2019; Digka et al. 2018), and one reported frequency of MP occurrence (Bour et al. 2018). The range of MP content for mollusks was 0–10.5 MPs/g of organism (wet weight). The means and ranges reported by the included studies were skewed toward the lower MP content. Sixteen studies reported values <1 MP/g, and the remaining 11 reported values >1 MP/g (Figure 2).

Seven studies were rated as having a high RoB because they did not report the results for the analysis of their procedural blanks (Hermabessiere et al. 2019; HX Li et al. 2018; Thushari et al. 2017; J Wang et al. 2019; Webb et al. 2019; Wu et al. 2020; SY Zhao et al. 2018) (Table S7), an analysis step that was rated as one of the most important questions in the RoB assessment tool. Five of these studies reported MP content >1 MP/g and the rest, <1 MP/g. The study by Baechler et al. (2020) was also found to have high RoB in the domains of analysis and reporting because the majority of the analysis details were not reported. The study reporting the highest MP mean content [6.9 MPs/g; J Wang et al. (2019)] and the study reporting the highest MP range of content [2–7.1 MPs/g; HX Li et al. (2018)] were both rated as having a high RoB in two domains: sampling and reporting. J Wang et al. (2019) was additionally rated as having an unclear RoB in the analysis domain. Omitting these two studies from the statistical summary decreased the MP content to 0–7.2 MPs/g wet wt.

In terms of geographical spread, 59.2% ($n=19$ of 27) of the studies sampled organisms off the coasts of Asia (52.6% of which were from China; $n=10$ of 19), 18.5% ($n=5$) off the coasts of Europe, 11.1% ($n=3$) from the Americas, 3.7% ($n=1$) from Africa, 3.7% ($n=1$) from Australia/Oceania, and 3.7% ($n=1$) from between the Americas and Asia (Table 2). Eighty-two percent of the studies ($n=9$ of 11) that reported MP content >1 MP/g came from the coasts of Asia. In contrast, only 20% of the studies ($n=1$ of 5) from Europe reported MP content >1 MP/g.

At least 15 different particle-extraction procedures were reported. The procedures can be divided into three broad categories depending on the chemical compound used to digest the samples: H_2O_2, potassium hydroxide (KOH), and nitric acid (HNO_3) (Table S5). There are further differences between these three categories, such as time period and temperatures for digestion, the use of a density-separation step and its specifications (physical/

Table 2. Molluskan seafood microplastic content results.

References	Geographic location	Sample species	Sample additional details[a]	*N*	Mean MPs/g	± SD	Range MPs/g	Composition
Abidli et al. 2019	Tunisia				1.03	0.36	0.70 ± 0.10 to 1.15 ± 0.02^{b}	Fibers: PP 100%; fragments: PP 60%, PE 40%; films: PP 50%, PE 50%
		Mytilus galloprovincialis		15	NA			
		Ruditapes decussatus		24	NA			
		Crassostrea gigas		3	1.48	0.02		
		Hexaplex trunculus		9	0.70	0.11		
		Bolinus brandaris		9	NA	NA		
Akoueson et al. 2020								PET, PE
	Scotland	*Zygochlamys patagonica*		10	0.29	0.10	0.16–0.47	
	Chile	*Pecten maximus*		10	0.17^{c}	0.9	0.06–0.35	
Baechler et al. 2020	USA							PET, acrylic, aramid
		C. gigas	Farmed	141	0.35	0.04	0.1 ± 0.02 to 0.85 ± 0.41	
		Siliqua patula	Wild	142	0.16	0.02	0.09 ± 0.01 to 0.62 ± 0.33	
Birnstiel et al. 2019	Brazil						16.6 ± 6.6 to 31.2 ± 17.8^{d}	Fibers: PA; fragments: PMMA
		Perna perna	Farmed	10				
		P. perna	Wild	10				
Bour et al. 2018	Norway	*Ennucula tenuis*		12			$41.1\%^{e}$	PE 54%, PP 16.8%
Bråte et al. 2018	Norway	*Mytilus* spp.		332	0.97	2.61	0–7.9	CP 63.9%, parking lot tar and EVA foam 18.7%, PET 9.9%, acrylic 2.9%, PP 1.2%, PE 1%, PA <1%
Cho et al. 2019	South Korea							PE, PP, PS, and polyester, accounting for >80% of MPs
		C. gigas		60	0.07	0.06	0–0.19	
		M. edulis		60	0.12	0.11	0–0.35	
		Tapes philippinarum		60	0.34	0.31	0.03–1.08	
		Patinopecten yessoensis		60	0.08	0.08	0.01–0.17	
		All species		240	0.15	0.20		
Digka et al. 2018	Northern Ionian Sea	*M. galloprovincialis*		80	1.9^{f}	0.2		75% PE, 12.5% PP, 12.5% PTFE
Ding et al. 2018	China							CP, PP, PTFE
		Chlamys farreri	Farmed	50			3.2–7.1	
		M. galloprovincialis					2.0–12.8	
			Farmed	50	3.17			
			Wild	15	2			
Ding et al. 2019	China						0.16–0.74	RY 48.92%, PET 33.87%, CPE 9.68%, PTFE 4.84%, PS 2.15%, PE+PP 0.54%
		M. galloprovincialis						
			Qingdao	10	0.16	0.13		
			Dongying	10	0.42	0.26		
		Ruditapes philippinarum	Qingdao	10	0.74	0.54		
		Mactra veneriformis	Dongying	10	0.31	0.27		
Ding et al. 2020	China		NA	140			0.8–4.4	Qingdao: RY 41.5%, PET, 16.4%, CPE, 11.8%, PVC, 10.3% Xiamen: RY 44.4%, PVDF 24.2%, CPE 14.0%, PVC 6.8%, PET 5.1%
		M. galloprovincialis		10				
		Perna viridis		10				
		R. philippinarum		20				

Table 2. (*Continued.*)

References	Geographic location	Sample species	Sample additional details[a]	N	Mean MPs/g	±SD	Range MPs/g	Composition
		C. gigas		20				
		Sinonovacula constricta		20				
		Scapharca subcrenata		20				
		Meretrix Lusoria		20				
		Busycon canaliculatu		20				
Fang et al. 2018	Bering Sea and Chukchi Sea							PA 46%, PE 23%, PET 18%, CP 13%
		Astarte crenata		28	0.08	0.07	0–0.12	
		Macoma tokyoensis		29	0.03	0.05	0–0.08	
		Retifusus daphnelloides		24	0.12	0.07	0.05–0.13	
		Latisipho hypolispus		19	0.02	0.002	0.02–0.03	
Feng et al. 2020[g]	China		Wild	210	1			CP 36.65%, PET/polyester 16.29% PE 14.03%, PP 13.12%, PP-PE 7.69%, PA 4.07%, RY 3.17%, PAN 2.71%, PU 1.36%, PVA-PE 0.90%
		Strongylocentrotus intermedius		NA				
		Temnopleurus hardwickii		NA				
		Temnopleurus reevesii		NA				
		Hemicentrotus pulcherrimus		NA				
Hermabessiere et al. 2019[h]	France							PE 36.8%, ABS 32.5% and SBR 26.3%, PP, PS, PET >5%
		M. edulis					0.15–0.25	
			Le Portel	50	0.25	0.16		
			Baie des Veys	50	0.15	0.06		
		C. edule					0.19–0.74	
			Baie d'Authie	50	0.74	0.35		
			Baie des Veys	50	0.19	0.08		
Leslie et al. 2017[i]	Netherlands							Not specified
		C. gigas						
			Eastern Scheldt	3	87			
			Rhine Estuary	3	30			
		M. edulis						
			Eastern Scheldt	10	105			
			Ter Heijde, North Sea	10	19			
		Littorina littorea	Eastern Scheldt	10	20			
HX Li et al. 2018	China	*Saccostrea cucullata*		330			1.5–7.2	PET 34%, PP 19%, PE, 14%, PS, 8%, CP, 8%, PVC 6%, PA, 4%, EPS 3%
J Li et al. 2018	UK						0.72-2.89	
		M. edulis		162				Polyester 43%, RY 26%, CL 14%
			Edinburgh	12	1.23	0.25		
			Filey	18	2.55	0.44		
			Hastings-A	30	1.59	0.51		
			Hastings-B	18	2.37	0.90		
			Brighton	18	0.95	0.18		
			Plymouth	24	0.72	0.16		
			Cardiff	30	2.89	0.62		
			Wallasey	12	1.65	0.23		
		M. edulis						PP 17%, polyester 17%, RY 17%, acrylic 13%, CL 9%, PE 4%, PGR 4%

Table 2. (*Continued.*)

References	Geographic location	Sample species	Sample additional details[a]	*N*	Mean MPs/g	± SD	Range MPs/g	Composition
			Supermarket live (farmed)	36	0.91	0.19		
			Supermarket processed (farmed/wild)	48	1.37	0.24		
J Li et al. 2016	China						0.9–4.6	CP 41.1%, PET 16.3%, PTA 10.9%, POM 7%, PE 3.1%, PNMA 2.3%
		M. edulis	Wild	222	2.7			
		M. edulis	Farmed	168	1.6			
Li et al. 2015	China						2.1–10.5	PE, PET, PA (no %)
		Scapharca subcrenata		6	10.45	4.4		
		Tegillarca granosa		18	4.13	1.72		
		M. galloprovincialis		18	2.39	1.32		
		P. yessoensis		6	2.34	0.78		
		Alectryonella plicatula		18	5.77	1.28		
		Sinonovacula constricta		6	2.08	1.18		
		R. philippinarum		24	2.52	1.07		
		M. lusoria		18	4.19	1.19		
		Cyclina sinensis		30	3.98	1.38		
Naji et al. 2018	Persian Gulf							PE, PET, nylon (no %)
		Amiantis umbonella		30	~2			
		A. purpuratus		30				
		Pinctada radiata		33				
Nam et al. 2019	Vietnam	*P. viridis*	Wild	5	0.29	0.14		PP 31%, Polyester 23%, PE 15%, PVA 8%, PA 8%, Rubber 8%, PS 7%
Phuong et al. 2018a	French Atlantic coasts							
		M. edulis		120	0.23	0.20		PP 47%, PE 38%
		C. gigas		60	0.18	0.16		PE ~50%, PP ~25%
Qu et al. 2018	China						1.52–5.36	PET 74%, RY, PE, PVC and PP
		M. edulis		~430				
		P. viridis		~330				
Su et al. 2018	China	*Corbicula fluminea*		208			0.3–4.9	Polyester 33%, PP 19%, PE 9%
			S1 Lake	NA	0.72	0.19		
			S2 Lake	NA	0.55	0.20		
			S3 River	NA	4.88	2.31		
			S4 River	NA	1.43	0.47		
			S5 River	NA	2.21	0.77		
			S6 River	NA	0.57	0.80		
			S7 River	NA	0.86	0.48		
			S8 Lake	NA	0.44	0.24		
			S9 Lake	NA	0.29	0.26		
			S10 Lake	NA	0.42	0.15		
			S11 Lake	NA	0.42	0.07		
			S12 Estuary	NA	1.11	1.10		
			S13 Estuary	NA	2.71	0.20		
			S14 Estuary	NA	0.99	0.57		
			S15 Lake	NA	0.55	0.02		
			S16 Lake	NA	0.78	0.13		
			S17 Lake	NA	1.72	1.15		
			S18 River	NA	1.22	0.53		

Table 2. (*Continued.*)

References	Geographic location	Sample species	Sample additional details[a]	*N*	Mean MPs/g	±SD	Range MPs/g	Composition
			S19 Lake	NA	3.70	2.33		
			S20 Lake	NA	2.19	1.32		
			S21 Lake	NA	0.68	0.32		
Teng et al. 2019	China	*C.* spp.		306	0.62	0.88	0.11–2.35	CP 41.34%, PE 22.97%
Thushari et al. 2017	Gulf of Thailand						0–0.57	PA, PET, PS (no %)
		Saccostrea forskalii		15				
			Angsila	NA	0.57	0.22		
			Bangsaen	NA	0.37	0.03		
			Samaesarn	NA	0.43	0.04		
		Littoraria sp.		50				
			Angsila	NA	0.23	0.02		
			Bangsaen	NA	0	—		
			Samaesarn	NA	0.17	0.08		
Van Cauwenberghe and Janssen 2014								Not specified
	Germany	*M. edulis*						
			No depuration	36	0.36	0.07		
			After depuration	36	0.24	0.07		
	France	*C. gigas*						
			No depuration	11	0.47	0.16		
			After depuration	10	0.35	0.05		
J Wang et al. 2019	South Yellow Sea	*Acila mirabilis*		20	6.9	2.1		Not specified
Webb et al. 2019	New Zealand	*Perna canaliculus*	Wild	96	0.03	0.04	0–0.48	PE, PA, acrylic, RY, nylon, PVA
Wu et al. 2020	China		Farmed					CL, PET, PP, PE, PA, acrylonitrile
		Ostrea denselamellosa (oyster)		10–20	0.31	0.10		
		Sinonovacula constricta (razor clam)		10–20	0.21	0.05		
SY Zhao et al. 2018	USA	*M. edulis*		37	0.6	1.2	0–5.1	PP 44.7%, polyester 21.2%, CL 11.8%, nylon 3.5%, PE 2.3%, PS 2.3 %, etc.
Zhu et al. 2019	China	*C. hongkongensis*		20	0.8	0.2	0.7–1.1	RY 50%, polyester 39%

Note: Studies reported either the mean MP content (with or without the SD) or the range of MP content or both. MP content is expressed as number of MP particles per gram of tissue (wet weight) unless otherwise stated. —, no data; CL, cellulose; CP, cellophane; CPE, chlorinated polyethylene; EPS, expanded polystyrene; EVA, Ethylene-vinyl acetate; MPs, microplastics; *N*, sample size expressed in number of organisms; NA, not available; PA, polyamide (nylon); PAN, polyacrylo-nitrile; PE, polyethylene; PET, polyethylene terephthalate; PGR, propylene glycol ricinoleate; PMMA, polymethyl methacrylate; PNMA, poly(*N*-methyl acrylamide); POM, polymerized oxidized material; PP, polypropylene; PS, polystyrene; PTA, Polyester terephthalic acid; PTFE, polytetrafluoroethylene; PU, polyurethane; PVA, polyvinyl alcohol; PVA-PE, poly-vinylacetate- ethylene; PVC, polyvinyl chloride; RY, rayon; SD, standard deviation.

[a]Additional details include further sample characteristics appropriate for each study regarding sampling further geographic location, sampling origin (environment, market), habitat (wild, farmed) and sample further processing information (depuration).

[b]Calculated from MPs/kg.

[c]Not significantly different for the procedural blank results.

[d]Range MPs/individual organism.

[e]Frequency of MPs/individual occurrence on the sample.

[f]MPs/individual organism.

[g]Echinodermata phylum.

[h]Expressed as mean ± 2 standard errors (95% confidence interval).

[i]Total number of particles per gram of dry tissue.

Table 3. Crustacean seafood microplastic content results.

References	Geographic location	Sample	*N*	Mean MPs/g	±SD	Freq.	Composition
Bour et al. 2018	Norway	*Crangon allmanni*	20			65%	PE 54%, PP 16.8%
Fang et al. 2018	Bering Sea and Chukchi Sea						PA 46%, PE 23%, PET 18%, CP 13%
		Chionoecetes opilio	59	0.14	0.08		
		Pandalus borealis	21	0.24	0.19		
Hossain et al. 2020	Bangladesh						PA, RY
		Metapenaeus monocerous	20	3.87	1.05		
		Penaeus monodon	10	3.40	1.23		
Leslie et al. 2017	Netherlands	*Carcinus maenas*	9	0			Not specified
McGoran et al. 2018	UK	*C. crangon*	116	1[a]	0	6%	Polyester 33%, nylon 20%, PP 15%
Thushari et al. 2017	Gulf of Thailand		50				PA, PET, PS (no %)
		Balanus amphitrite[b]	NA	0.57	0.22		
		B. amphitrite[c]	NA	0.37	0.03		
		B. amphitrite[d]	NA	0.43	0.04		
J Wang et al. 2019	South Yellow Sea, Korea and China	*Crangon affinis*	10	8.6	2.6		Not specified
Wu et al. 2020	China	*Parapenaeopsis hardwickii*	10–20	0.25	0.08		CE, PE
F Zhang et al. 2019	China					25%	PET 65%, PP 10%
		Oratosquilla oratoria	64				
		O. kempi	1				
		Portunus trituberculatus	30				
		Carcinoplax vestita	18				
		Charybdis bimaculata	15				
		C. variegate	4				
		P. gracilimanus	3				
		Charybdis japonica	1				

Note: Studies reported MP content results either as the mean MP content (with or without the SD) or the frequency of samples positive for MP presence. MP content is expressed as number of MP particles per gram of tissue (wet weight) unless otherwise stated. Freq., frequency of samples positive for MP presence; CP, cellophane; MPs, microplastics; *N*, sample size expressed in number of organisms; PA, polyamide (nylon); PE, polyethylene; PET, polyethylene terephthalate; PP, polypropylene; RY, rayon; SD, standard deviation.
[a]MPs/individual organism.
[b]Sampling site: Angsila.
[c]Sampling site: Bangsaen.
[d]Sampling site: Samaesarn.

chemical), the use of further chemicals, and the pore size of the filters. Many studies poorly reported the procedure used, in some cases, missing crucial details of the analysis protocol. In terms of MP content, of the 12 studies that used H_2O_2 for digestion (exclusively or not), 67% ($n=8$ of 12) reported MP content >1 MP/g. In most cases, the use of H_2O_2 was accompanied by a subsequent density-separation step (88% of the studies; $n=7$ of 8), suggesting that process was more effective in extracting MPs from biota than the methods using KOH and HNO_3 for digestion.

Samples examined by the studies came either directly from the environment or from markets, which opens up two associated issues: post-collection MP contamination, and the effects following any depuration period. It has been argued that depuration might be effective in extracting MPs from bivalves, with two studies testing this hypothesis (Birnstiel et al. 2019; Van Cauwenberghe and Janssen 2014). Birnstiel et al. (2019) concluded that depuration (over a 4-d period) significantly reduced MP content in their samples (*Perna perna*). Similarly, Van Cauwenberghe and Janssen (2014) found that a 3-d depuration was effective in removing a large proportion of MP contamination (in *Mytilus edulis* and *Crassostrea gigas*). Although the results of these two studies are promising in terms of the reduction of MP contamination, more research is needed to address a number of issues mainly around the methodology of the depuration procedure. For example, the time of depuration required may vary between different species, and the use of seawater that has already been filtered specifically to target MPs, is also key. The effect of depuration cannot be assessed in this review because in most cases, when bivalves have been acquired from markets, it is not known whether they have undergone a depuration process or not. Therefore, it is not clear whether MP contamination after the collection of seafood has a significant effect, or if it is mitigated by depuration. Five studies collected samples only from markets (Cho et al. 2019; Ding et al. 2019, 2020; Li et al. 2015; Akoueson et al. 2020), 3 from both the environment and markets (Ding et al. 2018; J Li et al. 2018; Van Cauwenberghe and Janssen 2014), and the other 23 from the environment (Table 1). The samples collected directly from the environment had a broader range of MP content (0.03–6.9 MPs/g) than the samples collected from a market (0.15–3.93 MPs/g) (Table 2).

The importance of the source (farmed or wild) has been highlighted in previous research (Mathalon and Hill 2014). From the studies that collected mollusks only from markets, only one reported sampling both farmed and wild organisms. Li et al. (2015) stated that MP content was significantly higher in farmed samples but did not report separate data for the MPs contents of the two groups. Ding et al. (2018) collected samples from markets and the environment but did not compare the two groups. Instead, they tested wild vs. farmed organisms and reported farmed mussels contained more MPs (3.17 MPs/g) than wild (2 MPs/g). In contrast, J Li et al. (2018) reported higher anthropogenic debris content in wild mussels per gram (1.6 items/g) than farmed (1.1 items/g), but more in farmed mussels per individual organism. The study by Van Cauwenberghe and Janssen (2014) sampled only farmed organisms. The results of the 23 studies that collected only environmental samples were contradictory. Four studies sampled both wild and farmed organisms of the same species. J Li et al. (2016) found more MPs in wild mussels (2.7 MPs/g) than in farmed ones (1.6 MPs/g). Phuong et al. (2018a) reported higher detection rates for MPs in farmed samples (oysters 93%, mussels 90%) compared with the wild ones (oysters 80%, mussels 65%). Digka et al. (2018) did not detect a difference between the ingestion of MPs in wild (47.5%) and farmed (45%) mussels. Birnstiel et al. (2019) also found the wild mussels to be more contaminated than farmed, but this difference was not significant (analysis of variance $F1,36=0.006$, $p=0.94$). Of the rest of the environmental studies, 1 analysed wild and farmed organisms of different species (Baechler et al. 2020), 2

Table 4. Fish microplastic content results.

References	Geographic location	Sample	N	MPs/g	± SD	MPs/ individual	± SD	Frequency	Composition
Akhbarizadeh et al. 2020	Iran			1.28	0.04				PET 36.6%, PS 17.6%, PP 13.5%, PS-PP 10.2%, PS-PET 7.9%, nylon 7.1%, PVC 3.9%, LDPE 3.2%
		Thunnus tonggol	25[a]	0.15	0.05				
		T. albacares	20[a]	0.10	0.04				
		Scombermorus commerson	5[a]	0.15	0.03				
Akoueson et al. 2020	Scotland	*Melanogrammus aeglefinus*	12	1.07[b]	0.12				CL 62%, PET 19%, CP 15%, polyolefin 4%
	Greece	*Dicentrarchus labrax*	10	1.04[b]	0.07				CL 43%, CP 14%, PET 11%
	Iceland	*Pleuronectes platessa*	10	1.31[b]	0.11				PE 41%, PET 14%, CL 14%, CP 9%
	Scotland	*Scromber scombrus*	10	0.58[b]	0.10				PET 25%, CL 25%, CP 25%, PP 9%, PA 8%, PAN 8%
Collard et al. 2017a								9 MPs found in 8 of the 10 livers	PE
	Mediterranean Sea	*Engraulis encrasicolus*	13						
	English Channel	*Sardina pilchardus*	2						
Collard et al. 2017b									PE 37%, PP 26%, PET 16%, PAN 7%, PS 5%, PA 5%, PEG 2%, PBMA 2%
	Mediterranean Sea, Bay of Biscay	*E. encrasicolus*	20			0.85			
	English Channel, Bay of Biscay	*S. pilchardus*	20			0.53			
Digka et al. 2018	Northern Ionian Sea	*S. pilchardus*	36			1.8	0.2		PE 55.5%, PP 27.7%, PET 5.5%, PS 5.5%, PTFE 5.5%
Feng et al. 2019	China	*Thryssa kammalensis*	19	11.19	1.28	22.21	1.70		CP 33.5%, PP 15.0%, PE 13%, nylon 8.0%, PET 4.5%
Karami et al. 2017b	Malaysia							29 MPs in flesh; 7 MPs in organs	PP, 47.2%, PE 41.6%, PS 5.56%, PET 2.77%, NY6 2.77%
		Chelon subviridis (packed dried)	30						
		Johnius belangerii	30						
		Rastrelliger kanagurta	30						
		Stolephorus waitei	30						
Karami et al. 2018	Product of Canada, Germany, Iran, Japan, Latvia, Malaysia, Morocco, Poland, Portugal, Russia, Scotland, Thailand, and Vietnam	sardines and sprats (canned, unknown species)	20[c]					MPs found in 35% of sample	PP 33.3%, PET 33.3%, PE 16.6%, PVC 16.6%
Lopes et al. 2020	Portugal								PP 21%, PE 16%, CL 16%, RY 13%, styrene/acrylic copolymer 11%, polyacrylate 8%, NY6 4%, PET 4%, polymeric epoxy plasticizer 4%
		S. pilchardus	76			0.23	0.04		
		E. encrasicolus	131			0.5	0.6		
		Boops boops	19			0.34	0.6		
Pozo et al. 2019	Chile	*Strangomera bentincki*	10					MPs found in 30% of sample	PET 75%, PE 25%

Table 4. (*Continued.*)

References	Geographic location	Sample	*N*	MPs/g	± SD	MPs/ individual	± SD	Frequency	Composition
Renzi et al. 2019	Adriatic Sea								
		S. pilchardus	80			4.63			PP 50%, PVC 30%, PTFE 10%, PA 10%
		E. encrasicolus	80			1.25			PVC 93%, PET 7%
Su et al. 2019	China	*Lateolabrax maculatus*	9	0	0				
Sun et al. 2019	China								Organic oxidation polymers 40%, PE 22%, PA 11%
		Setipinna taty	20			0.35			
		Ammodytes personatus	50			0.54			
		Anchoviella commersonii	30			0.40			
		Engraulis japonicus	280			0.39			
Tanaka and Takada 2016	Tokyo Bay, Japan	*E. japonicus*	64			2.3	2.5		PE 52.0%, PP 43.3%, PS 2.0%, E/P 2.0%, E/P/D 0.7%
Teng et al. 2020	China	*Sardinella zunasi*	NA	0.77	1.42	2.84	1.93	MPs found in 78.8% of sample	CP 61.0%, PET 29.0%, PP 6.0%, PA 2.4%, PAN 1.6%
Q Wang et al. 2020	China								CP 77.5%, PET 16.9%, PP 2.5%, PAN 0.9%, PE 0.5%, PVAc 0.5%, PA 0.4%, PS 0.4%, PB 0.2%, PC 0.2%
		Konosirus punctatus	44	0.12	0.14	3.71	3.39		
		Thryssa mystax	8	0.09	0.05	1.65	1.39		
		Sardinella zunasi	6	0.02	0.02	0.74	0.76		
Wu et al. 2020	China								
		Larimichthys crocea	10–20	0	0				
		Konosirus punctatus	10–20	0	0				
Zitouni et al. 2020	Tunisia	*Serranus scriba*	240	2.90	1.54				PEVA, HD-PE, LD-PE, PA or nylons, PEMA

Note: Studies reported MP content results either as the mean MP content (with or without the SD) or the frequency of samples positive for MP presence. MP content is expressed as number of MP particles per individual organism. CL, cellulose; CP, cellophane; E/P, ethylene/propylene copolymer; E/P/D, ethylene/propylene/diene terpolymer; HD, high-density; LD, low-density LDPE, low density polyethylene; MPs, microplastics; *N*, sample size expressed in number of organisms; NY6, nylon-6; PA, polyamide (nylon); PAN, polyacrylonitrile; PB, polybutene; PBMA, poly (butyl methacrylate); PC, polycarbonate; PE, polyethylene; PEMA, polyethylene-co-methyl acrylate PEG, polyethylene glycol; PET, polyethylene terephthalate; PEVA, polyethylene-vinyl-acetate; PP, polypropylene; PS, polystyrene; PTFE, polytetrafluoroethylene; PVAc, polyvinyl acetate; PVC, polyvinyl chloride; SD, standard deviation.

[a]Cans of fish.

[b]Not significantly different for the procedural blank results.

[c]Brands (4 cans per brand, 2–30 fish per can).

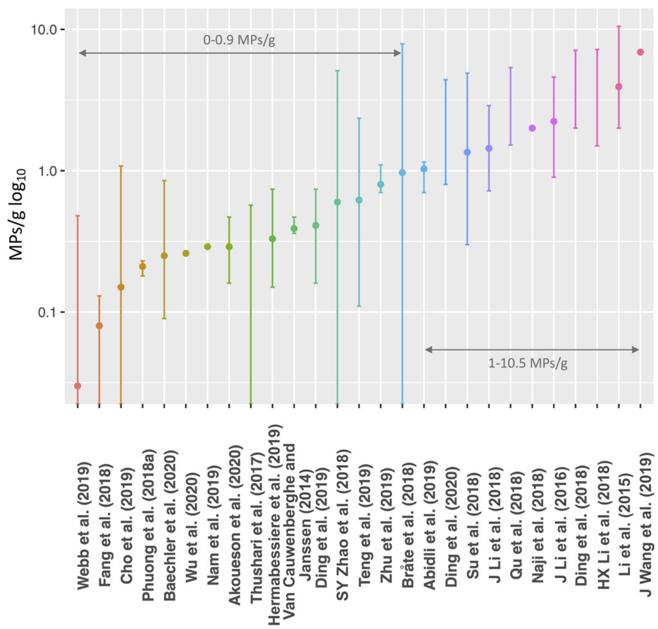

Figure 2. The overall microplastics per gram (MPs/g) content for mollusks illustrated in a $\log_{10}$ scale. Points represent mean MPs/g values for the studies, where reported. Whiskers represent the reported ranges of MPs/g.

analyzed farmed organisms (Teng et al. 2019; Wu et al. 2020), and the remaining 16 analyzed wild organisms. No pattern between wild and farmed organisms emerged in a review of the data.

In terms of validating the chemical composition as actual MPs, 10 studies (32%) did not report how many of the extracted particles were analyzed for polymeric composition. The remaining 21 studies (68%) reported percentages ranging from 0.9% to 100%. Eight studies (26%) analyzed 100% of the particles (Cho et al. 2019; Ding et al. 2018, 2019, 2020; Nam et al. 2019; Phuong et al. 2018a; Webb et al. 2019; Wu et al. 2020), one 80% (Hermabessiere et al. 2019), and the rest 0.9–36% (Table S9). Following on from this, it is noteworthy that 16 (52%) of the studies, once these particles have been isolated, did not state the percentage of similarity compared with the spectral library that was used as the level of acceptance.

To investigate the relationship between all these variables, a series of statistical tests were executed. Only seven studies reported all the variables needed for the analysis (Hermabessiere et al. 2019; J Li et al. 2016, 2018; Phuong et al. 2018a; Su et al. 2018; Teng et al. 2019; Zhu et al. 2019) (Table S9). Data was examined to detect whether they were normally distributed by fitting a series of Shapiro-Wilk's tests (Ennos and Johnson 2018). Pearson correlation analysis was used for the normally distributed data and Spearman correlation analysis for the data not normally distributed (Ennos and Johnson 2018). There was a significant negative correlation between the MPs-per-gram content, the percentage of the particles that were analyzed [$p = 0.024$, correlation coefficient $R = -0.86$ (Figure S3A)] and the number of particles analyzed [$p = 0.0004$, $R = -1$ (Figure S3B)]. There was also a significant positive correlation between the MP content and the similarity index of the spectral library ($p = 0.054$, $R = 0.75$) (Figure 3).

No significant correlation (Spearman correlation analysis) was found between the percentage of the verified MPs and the percentage of the particles that were analyzed ($p = 0.1667$), the number of particles analyzed ($p = 0.2357$), nor the similarity index of the spectral library ($p = 0.356$).

Ten percent of the studies ($n = 3$ of 31) did not report any results on the polymeric composition of the particles (Leslie et al.

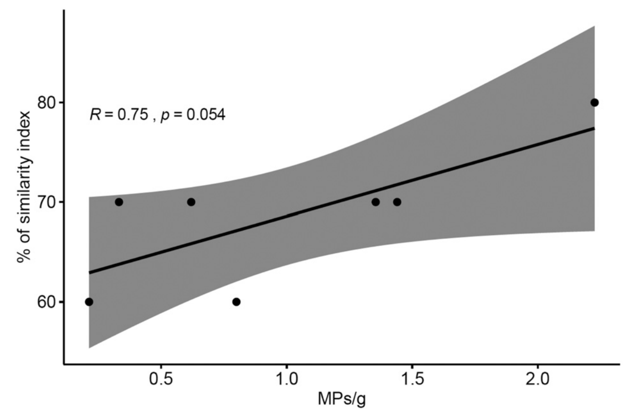

Figure 3. Pearson correlation analysis between the amount of microplastics per gram (MPs/g) in mussels and the percentage of similarity compared with the spectral library that has been used as the level of acceptance. R is the Pearson correlation coefficient with the corresponding p-value. The gray-shaded area represents the 95% confidence belt.

2017; Van Cauwenberghe and Janssen 2014; J Wang et al. 2019) (Table 2). A key difference between the rest of the studies is that 53.6% ($n = 15$ of 28) reported finding either cellulose, cellophane (CP), or rayon in their samples and reported them as part of the plastic material, whereas the other half did not. It is unclear whether this is because they were not considered plastic or because they were not found. Looking at the percentages of composition attributed to these materials, it became clear that their inclusion as MPs had a substantial effect on the MP content results. Across the studies that did not report cellulose-related material, polyethylene (PE) was the most abundantly discovered polymer, followed closely by polypropylene (PP). In the rest of the molluskan studies, CP was the most abundant material followed by polyethylene terephthalate (PET), rayon, and polyester.

Meta-analysis of MP content results. Two molluskan classes were included—bivalves and gastropods—constituting six molluskan families: clams, cockles, mussels, oysters, scallops, and sea snails (Table 1). The data for all the species of the same family per study were combined, resulting in 32 different sample data sets from 19 studies (Table S10). Sample heterogeneity between the classes and families was assessed in subgroup analyses using mixed-effects models that showed no significant difference between the overall effect between the two classes ($Q = 0.82$, $p = 0.37$) but a significant difference between the six families ($Q = 33.73$, $p < 0.01$) (Figure 4). Subgroup analysis was also used to identify whether further sample characteristics and methods variability might have affected heterogeneity. A significant difference was also identified between samples that were collected directly from the environment ($n = 23$) and those collected indirectly, that is, from a market ($n = 9$) ($Q = 29.33$, $p < 0.01$) (Table S11), coinciding with the findings of the narrative analysis concerning this sample characteristic. Significant differences were identified between the 16 different geographical origins of the samples, $Q = 698.52$, $p < 0.01$, and the three different RoB ratings $Q = 15.42$, $p < 0.01$ (Table S11). In light of these results, analyses using random-effects models were fitted separately for each of the six families of mollusks. In doing so, the heterogeneity between the different families of mollusks could be addressed. Further characteristics were explored within each family analysis separately. The effects that the habitat and feeding parameters had in terms of farmed vs. wild organisms could not be modeled owing to the lack of information because one study (Ding et al. 2019) did not report this characteristic and two studies (Phuong et al. 2018a; Li et al. 2015) collected both farmed and wild organisms and did not provide differentiated results (Table S10).

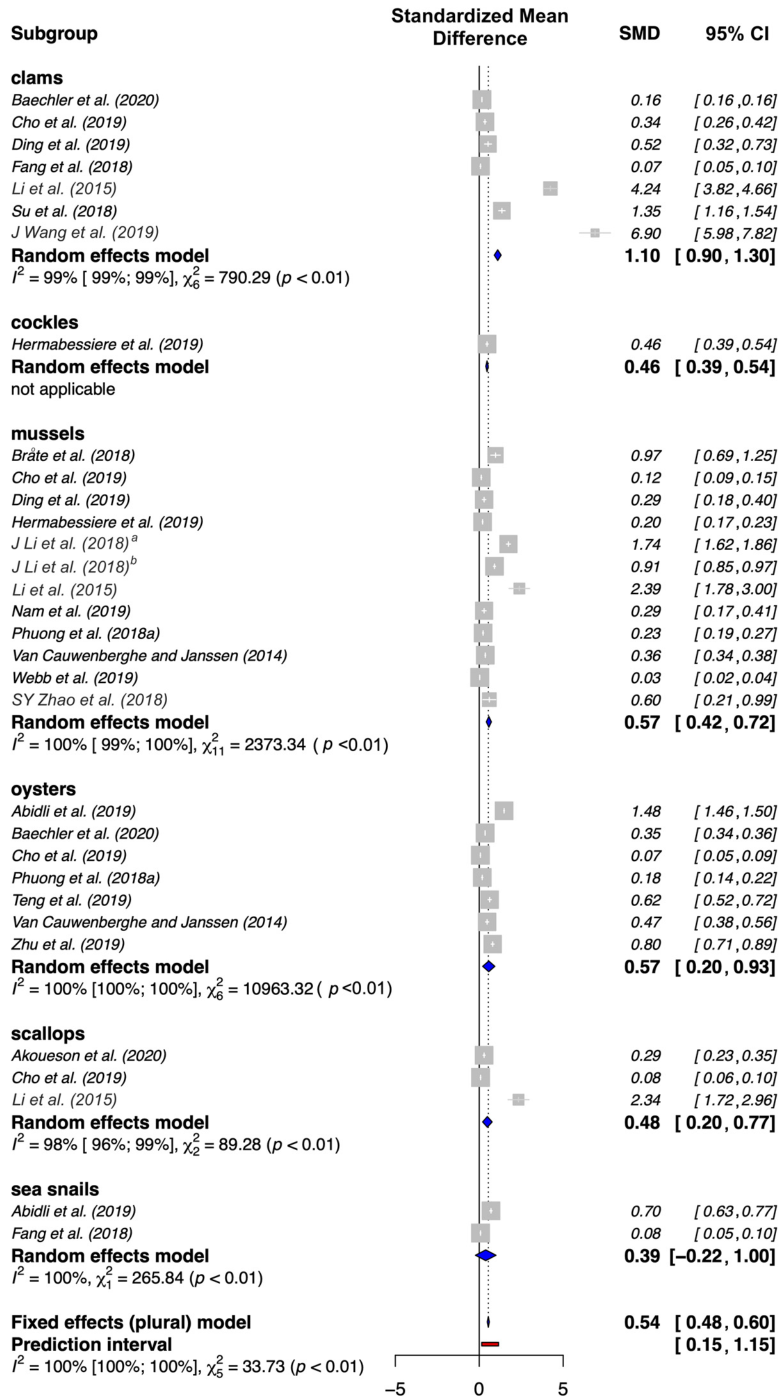

Figure 4. Forest plot for subgroup analysis between six molluskan families using a mixed-effects model (random-effects model for studies within each category and fixed-effect model between family categories). Studies were weighted using the inverse of the variance method (Chen and Peace 2013). The *x*-axis represents the standardized mean difference expressed in microplastics per gram (MPs/g). The vertical line is the line of null effect where MP content is 0. The gray boxes represent the pooled effect estimate and the lines the 95% confidence interval (CI). The size of the boxes is proportional to the study weight. The diamonds are the combined point estimates and CI for each of the subgroups. The dotted line is the overall pooled effect for all subgroups with a corresponding diamond. The red box is the 95% prediction interval. The a (superscript) samples collected form the environment; b (superscript) samples collected form the market (J Li et al. 2018).

Clams. Seven studies that analyzed clams were included in the meta-analysis (Figure 4). The model revealed high statistical heterogeneity of the pooled effect: $I^2 = 99.2\%$ and chi-square $= 790.29$, $p < 0.01$. Two statistical outlier studies of extremely large effects were detected: J Li et al. (2015) and J Wang et al. (2019); the overlap between the 95% CIs between the individual studies and the pooled results of the model are presented in the forest plot in Figure 4. An influence analysis revealed that they were also the most influential studies in terms of heterogeneity (I^2) and overall effect (Figure S4A,B) (Viechtbauer and Cheung 2010). Two studies were rated as of high RoB (Baechler et al. 2020; J Wang et al. 2019). Fitting the model without these studies increased the MP content from 1.1 MPs/g to 1.25 MPs/g [(95% CI: 0.70, 1.79), $p < 0.01$] but did not affect heterogeneity (Figure S5). Therefore, the results of the statistical outlier test, the influence analysis and the RoB rating justified the exclusion of the Baechler et al. (2020) and the J Wang et al.

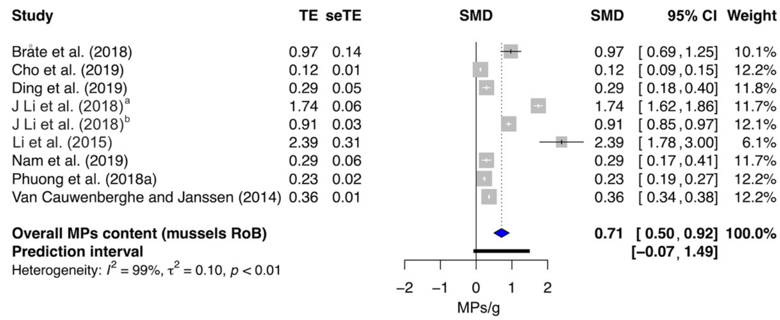

Figure 5. Forest plot for random-effects model results for mussels without the two high risk of bias (RoB) studies (Hermabessiere et al. 2019; SY Zhao et al. 2018). The x-axis represents the standardized mean difference (SMD) expressed in microplastics per gram (MPs/g). TE is the MP content reported by each study, and seTE is the calculated standard error. The vertical line is the line of null effect where MP content is 0. The gray boxes represent the pooled effect estimate and the whiskers, the 95% confidence interval (CI). The size of the boxes is proportional to the study weight. The diamond is the combined point estimate and 95% CI, and the dotted line is the overall pooled effect. The black box represents the 95% prediction interval. The a (superscript) samples collected form the environment; b (superscript) samples collected form the market (J Li et al. 2018).

(2019) data from the meta-analysis. A subgroup analysis using a random-effects model also revealed that there was a significant difference between the five countries/regions included in the meta-analysis ($Q = 274.41$, $p < 0.01$), the use of FT-IR ($n = 6$) and RM ($n = 1$) ($Q = 58.16$, $p < 0.01$), and the source of the samples [environment, $n = 5$; market, $n = 2$ ($Q = 44.96$, $p < 0.01$)] (Table S11).

Mussels. Eleven studies reporting mussel MP content were included in the meta-analysis. The analysis did not include the results of the processed mussel samples coming from supermarkets in the study by J Li et al. (2018) study nor the samples after depuration in the Van Cauwenberghe and Janssen (2014) study in order to improve the homogeneity of the data. The mean content was 0.57 MPs/g [95% CI: (0.42, 0.72), $p < 0.01$] with a high heterogeneity: $I^2 = 99.5\%$, chi-square $= 2{,}373.34$, $p < 0.01$ (Figure 4). The two studies by J Li et al. (2015, 2018) were determined to be statistical outliers of extremely large effect (Figure 4). An influence analysis also identified the same studies as the most influential studies in terms of contribution to the effect size (Figure S6A), whereas the study by Webb et al. (2019) was found to be the major contributor to the heterogeneity I^2 (Figure S6B) and a major influence on the pooled result (Figure S7). The geographical origin of the samples was also found to be associated with significant differences in the MP content ($Q = 949.96$, $p < 0.01$), but no significant differences of the source of the samples was found [environment, $n = 9$; market, $n = 3$ ($Q = 0.38$, $p = 0.54$)] (Table S11). The influence in choice of FT-IR ($n = 8$), RM ($n = 3$), or both ($n = 1$) also revealed a significant difference ($Q = 12.21$, $p < 0.01$; Table S11), where the use of FT-IR was associated with higher MP content. The RoB rating analysis showed that there was a significant difference between the three ratings ($Q = 13.11$, $p < 0.01$). In light of these results, in order to improve the quality of the data, we fitted a model omitting the results of the three studies rated as of high RoB (Hermabessiere et al. 2019; Webb et al. 2019; SY Zhao et al. 2018). The results of the model are shown in Figure 5, where MP content was 0.71 MPs/g [(95% CI: 0.50, 0.92), $p < 0.01$], and heterogeneity was high ($I^2 = 99.3\%$, chi-square $= 1{,}170.31$, $p < 0.01$). Although J Li et al. (2015, 2018) were identified as statistical outliers and the major influencers of the effect size, they were not omitted from the analysis because they were rated as having low RoB. Therefore, it was assumed that the difference in their results was due to variability in the measurements rather than methodological or experimental factors.

Oysters. Seven studies were included in the oysters' meta-analysis (Figure 4). The mean content was 0.57 MPs/g [95% CI: (0.20, 0.93), $p < 0.01$]. Heterogeneity was high ($I^2 = 99.9\%$, chi-square $= 10{,}963.32$, $p < 0.01$). One study (Abidli et al. 2019) was detected as a statistical outlier of extremely large effects (Figure 4) and which was also rated as having an unclear RoB. An influence analysis identified the same study to be the primary influencer in terms of I^2 heterogeneity and effect size results (Figure S8A,B). Excluding this study from the model resulted in a reduced mean content of 0.41 MPs/g (95% CI: 0.25, 0.57) with high heterogeneity ($I^2 = 99.6\%$, chi-square $= 1{,}308.55$, $p < 0.01$). One study was rated as having a high RoB (Baechler et al. 2020). Excluding this study from the model resulted in a higher content of 0.60 MPs/g with a broader CI [(95% CI: −0.06, 1.26), $p = 0.07$] and high heterogeneity ($I^2 = 99.9\%$, chi-square $= 10{,}570$, $p < 0.01$). Excluding both studies from the model in a further sensitivity analysis, justified by the previous findings, resulted in a mean content of 0.42 MPs [(95% CI: 0.19, 0.65), $p < 0.01$] and high heterogeneity ($I^2 = 99.1\%$, chi-square $= 432.73$, $p < 0.01$) (Figure 6). Subgroup analysis showed that there was a significant difference between the six different countries/regions of origin of the samples ($Q = 10{,}866.76$, $p < 0.01$). No significant difference was found between the use of

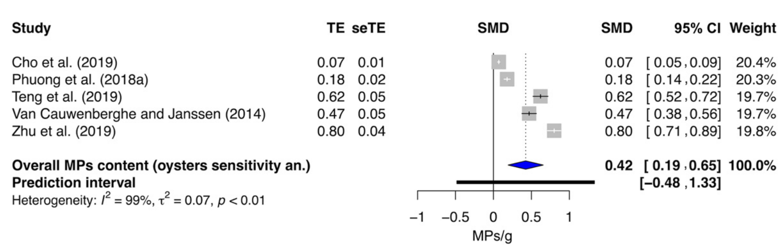

Figure 6. Forest plot for random-effects model for oysters, sensitivity analysis results without the high-risk of bias study (Baechler et al. 2020), and the statistical outlier of extremely large effects (Abidli et al. 2019). The x-axis represents the standardized mean difference (SMD) expressed in microplastics per gram (MPs/g). TE is the MP content reported by each study, and seTE is the calculated standard error. The vertical line is the line of null effect where MP content is 0. The gray boxes represent the pooled effect estimate and the whiskers the 95% confidence interval (CI). The size of the boxes is proportional to the study weight. The diamond is the combined point estimate and 95% CI, and the dotted line is the overall pooled effect. The black box represents the 95% prediction interval. Note: an., analysis.

FT-IR ($n = 5$) and RM ($n = 2$) ($Q = 1.33$ $p = 0.25$) nor between the origin of the sample [environment, $n = 5$; market, $n = 2$ ($Q = 1.78$, $p = 0.18$)] (Table S11). The results of the subgroup analysis were interpreted with caution owing to the low number of the studies, in a similar manner to the clams' family analysis.

Scallops/sea snails. Three studies were included in the scallops' meta-analysis and the mean content was 0.48 MPs/g [95% CI: (0.20, 0.77), $p < 0.01$] with high heterogeneity ($I^2 = 97.8\%$, chi-square = 89.28, $p < 0.01$) (Figure 4). All studies were rated as of low RoB. The study by J Li et al. (2015) was identified as a statistical outlier of extremely large effects (Figure 4). Further influence and subgroup analysis were not appropriate owing to the limited number of studies.

The results of the two studies on sea snails were not found to be appropriate for meta-analysis (Figure 4). The CIs for this family included negative values (95% CI: −0.22, 0.99) and the statistical heterogeneity was extremely high ($I^2 = 99.6\%$). Therefore, the studies were only included in the statistical summary and the narrative analysis.

After the completion of the separate analysis for each family of mollusks, a random-effects model was fitted, again including studies for all families but excluding the five high-RoB studies (Baechler et al. 2020; Hermabessiere et al. 2019; J Wang et al. 2019; Webb et al. 2019; SY Zhao et al. 2018) (Figure S9). The mean content was 0.78 MPs/g [(95% CI: 0.58, 0.97), $p < 0.01$] and heterogeneity was still high ($I^2 = 99.8\%$, chi-square = 14,491.45, $p < 0.01$). The results of this model represent the best estimation for MP content of all molluskan families.

Publication bias. The RoB across studies was examined using funnel plots (Borenstein et al. 2009), plotted separately for the different families of mollusks (Figure S10A–D). The results of the Egger's test of the intercept show that the asymmetry was not substantial for clams ($p = 0.07$), oysters ($p = 0.58$), and scallops ($p = 0.09$) but was substantial for mussels ($p < 0.01$) (Egger et al. 1997). The power of the Egger's test was lower for the clams, oysters, and scallops because the number of the included studies was <10. The robustness of the eligibility criteria of the review might have excluded studies that would possibly have improved the symmetry of the funnel plots. Regarding the crustacean and the fish studies, their results were not expressed in a way that they could be statistically appraised. Publication bias is addressed in the statistical summary/narrative analysis.

Crustaceans studies. Nine studies sampled crustaceans (Table 3), with three reporting the frequency of MP detection. McGoran et al. (2018) reported that only 6% of their samples tested positive for MP contamination, F Zhang et al. (2019) reported the level to be 25%, and the study by Bour et al. (2018) elevated the level to 65%. All three studies were rated as having an unclear RoB in the domains of sampling, analysis, and reporting (Table S7). Regarding the remaining six studies, the study by Leslie et al. (2017) could not be used for comparison owing to methodological issues in the particle-extraction protocol (as mentioned above). Therefore, the statistical summary included the other five studies (Fang et al. 2018; Hossain et al. 2020; Thushari et al. 2017; J Wang et al. 2019; Wu et al. 2020). The range of MP content was from 0.14 ± 0.08 to 8.6 ± 2.6 MPs/g (Figure 7). Four of these studies were rated as having a high RoB (Table S7) and could account for the major difference in these results. Three of these studies have already been appraised in the molluskan analysis previously (Thushari et al. 2017; J Wang et al. 2019; Wu et al. 2020). The study by Hossain et al. (2020) was found to have a high RoB in the domain of sampling and an unclear RoB in the domains of analysis and reporting (Table S7) because they did not report vital information of their analysis, such as the results of the procedural blank samples. Regarding the particle-

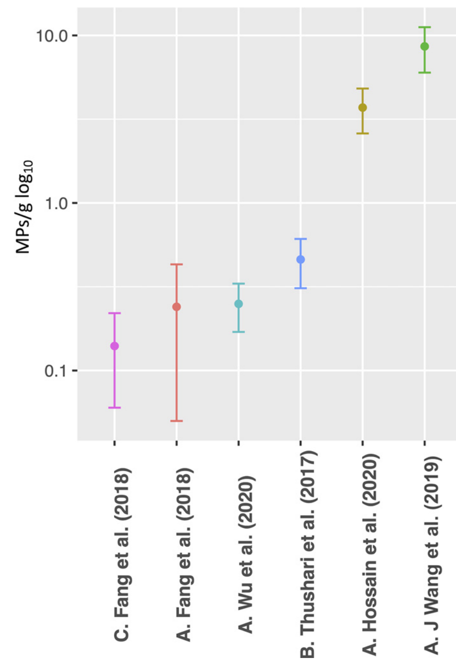

Figure 7. The overall microplastics per gram (MPs/g) content for crustacean families of shrimps, barnacles, and crabs; illustrated in a $\log_{10}$ scale. Points represent mean MPs/g values and whiskers represent the corresponding standard deviations (SDs). The results of Hossain et al. (2020) and Thushari et al. (2017) have been pooled per family and species, respectively. A, shrimps; B, barnacles; C, crabs.

extraction process, McGoran et al. (2018) did not use any type of digestion but, rather, dissected samples in 1-cm sections and examined them under a dissection microscope. This approach may have significantly affected the findings in that visual inspection in 1-cm dissections may not be adequate to discover and identify particles that can be <1-cm long. Three chemicals were used for digestion of the samples: H_2O_2 (37.5% of the studies; $n = 3$ of 8), KOH (25%; $n = 2$), and HNO_3 (25%; $n = 2$), and a combination of KOH and H_2O_2 (12.5%; $n = 1$). Fifty percent of the studies ($n = 4$) followed the digestion with a density-separation process (Table S5). Five studies (56%) sampled from the broader area off the coasts of Asia (Hossain et al. 2020; Thushari et al. 2017; J Wang et al. 2019; Wu et al. 2020; F Zhang et al. 2019), one between Asia and America (Fang et al. 2018), and the rest from Europe (33%) (Bour et al. 2018; Leslie et al. 2017; McGoran et al. 2018) (Table 3). All studies included in the statistical summary came from Asia and the Americas. All studies used samples collected directly from their habitat and all samples were wild apart from one (Wu et al. 2020), and 89% ($n = 8$ of 9) used FT-IR for spectral analysis. In terms of polymeric composition, the most abundant were PE and polyamide (nylon) (PA) followed by PP and PET (Table 1). Fifty-six percent of the studies ($n = 5$) (Fang et al. 2018; Leslie et al. 2017; McGoran et al. 2018; Thushari et al. 2017; J Wang et al. 2019) did not report the similarity index of the spectral library, and only 44% ($n = 4$) (Fang et al. 2018; Leslie et al. 2017; McGoran et al. 2018; Wu et al. 2020) reported the proportion of extracted particles analyzed for composition. Therefore, executing correlation analysis was not possible owing to the lack of data.

The statistical summary was based on five studies, four of which were rated as having a high RoB; therefore, the confidence in those results was deemed to be low. Sample heterogeneity could not be assessed in depth owing to the small number of studies. However, variability was identified throughout the research protocols as in the molluskan studies.

The available data on crustaceans were not found to be appropriate for meta-analysis. There were only three studies (Fang et al. 2018; Hossain et al. 2020; J Wang et al. 2019) that provided the necessary data (Table 3). These analyzed two different families (shrimps and crabs), comprising five different species: shrimps: *Crangon affinis, Metapenaeus monocerous, Pandalus borealis, Penaeus monodon*; crabs: *Chionoecetes opilio*, making it unreasonable to collate data with such sample heterogeneity.

Fish studies. Eighteen studies analyzed fish, with 4 reporting the discovery of MPs in the samples or the rate of discovery (Collard et al. 2017a; Karami et al. 2017b, 2018; Pozo et al. 2019) (Table 4). Two studies (Akhbarizadeh et al. 2020; Karami et al. 2018) used canned samples (whole fish), and 1 (Karami et al. 2017b) used dried fish (flesh and organs) (Table 1). Akhbarizadeh et al. (2020) reported 1.28 ± 0.04 MPs/g in canned tuna. Karami et al. (2017b, 2018) did not report MP content (Table 4). These samples had undergone substantial processing; therefore, it would not be reasonable to pool data including them because the fish might have been exposed to airborne MP contamination in some part of processing. From the remaining 13 studies, 7 reported MP content per mass, with a range of 0–11.9 MPs/g (Figure S11), 6 reported MP content only per individual organism, and 3 reported MP content expressed both per mass and per individual organism, with a range of 0.23–22.21 MPs/individual (Figure S12). Only 3 of the studies reported the weight of the samples used (Digka et al. 2018; Renzi et al. 2019; Sun et al. 2019), allowing a conversion from MP content per individual to MP content per mass (Table S12).

All the studies apart from one (Akoueson et al. 2020) collected organisms directly from the environment, and one study did not report the origin of their samples (Pozo et al. 2019). Sixty-one percent of the samples ($n = 11$ of 18 studies) were wild organisms (Table 1). Regarding the particle-extraction process, 39% used KOH ($n = 7$ of 18), 22% used H_2O_2 ($n = 4$), 17% ($n = 3$) a combination of KOH and H_2O_2, 11% ($n = 2$) used a combination of sodium hypochlorite and methanol, 5% ($n = 1$) used HNO_3, and 5% ($n = 1$) used the enzyme proteinase-K (Table S5). Forty-four percent ($n = 8$ of 18) combined the digestion with a density-separation process. Sixty-seven percent ($n = 12$) used FT-IR, and 33% ($n = 6$) used RM. Fifty percent of the samples came from Asia ($n = 9$), 33% from Europe ($n = 6$), 5.6% ($n = 1$) from Africa, 5.6% ($n = 1$) from South America, and 5.6% ($n = 1$) from multiple continents (Table 4).

There were seven studies that sampled anchovies (six species; Table 1) reporting a range of 0.35–22.21 MPs/individual. The highest MP content (22.21 ± 1.7 MPs/individual) was reported by Feng et al. (2019). It was the only study that used the gut, gills, and skin of the samples for analysis, reporting a significant difference of MPs in the different tissues of gut and gill ($F = 39.911$, degrees of freedom $= 2$, $p = 0.001$). They did not report the MP content per tissue per species; therefore, the direct comparison with the rest of the studies would be inappropriate. Feng et al. (2019) attributed the higher MP content to the highly polluted sampling area of Haizhou Bay, the habitat, and to the feeding habits of the species (*Thryssa kammalensis*). Excluding this study brings the range to 0.35–2.3 MPs/individual. The study reporting the second highest MP content was Tanaka and Takada (2016), which was rated as having an unclear RoB owing to missing information regarding sampling and analysis (Table S7). The higher amount of MP content could also be attributed to the fact the samples came from Tokyo Bay, which is situated off the highly urbanized and industrialized Tokyo metropolitan area.

Six studies sampled sardines (three species; Table 1), reporting a range of 0.23–4.63 MPs/individual. The relatively high value of 4.63 MPs/individual was reported by the study by Renzi et al. (2019), which was rated as having a high RoB. Information was not reported regarding sampling and analysis, the most important being the use of replicate samples, and any details around the composition identification process. Excluding this high-RoB study brings the range to 0.23–3.71 MPs/individual. Of the four studies that reported only MPs/individual, only two reported on the size of them (i.e., weight). The study by Renzi et al. (2019) used considerably larger samples ($20.22\ g \pm 4.2$) than Digka et al. (2018) ($9.63\ g \pm 1.46$), which would account for the higher MP content per individual. All the studies that sampled anchovies and sardines used the stomach or whole GI tract of the organism for the analysis.

Four studies sampled the flesh of larger fish. Two studies reported the absence of MP contamination in seabass (*Lateolabrax maculatus*) (Su et al. 2019), in yellow croaker (*Larimichthys crocea*) and dotted gizzard shad (*Konosirus punctatus*) (Wu et al. 2020), whereas Akoueson et al. (2020) did not discover MP content significantly different from the procedural blank samples results. Only the study by Zitouni et al. (2020) reported a content of 2.9 ± 1.54 MPs/g in painted comber (*Serranus scriba*). This study was rated as having an unclear RoB in two domains of sampling and analysis and a high RoB in the domain of reporting (Table S7), resulting in an overall high RoB. The main factor was the unclear reporting of the procedural samples results. Therefore, the results of the study were excluded from the statistical summary. Wu et al. (2020) was also rated as of high RoB owing to the lack of reporting of the procedural blank samples results.

Regarding the MPs polymer composition, the most prevalent polymers for fish were PE and PP, followed by PET and CP (Table 4). Forty-four percent of the studies ($n = 8$ of 18) did not report on the accepted similarity index to the spectra library, whereas 39% ($n = 7$) did not report how many suspected MP particles they analyzed (Table S13).

Comparison between species, different body parts used for analysis and the geographical origin of the samples was hindered because not all studies reported the MP content per mass but only MPs per individual organism. MP content was associated with the part of the organism used for analysis and the RoB rating. Methodological heterogeneity identified in sampling and analysis was similar to the molluskan and crustacean studies. Five studies (Akoueson et al. 2020; Feng et al. 2020; Su et al. 2019; Q Wang et al. 2020; Zitouni et al. 2020) provided the necessary data for meta-analysis of MP content per mass and five (Digka et al. 2018; Feng et al. 2020; Lopes et al. 2020; Tanaka and Takada 2016; Q Wang et al. 2020) per individual organism, but all of them sampled different families/species of fish (Table 1), which prevented comparison; therefore, meta-analysis was not attempted. One study (Feng et al. 2020) sampled the phylum echinodermata and reported a content of 0.82 MPs/individual or 1 MP/g in the edible part (gonad) of sea urchins (4 species; Table 2).

Summary of Evidence

The summary of evidence table (Table 5) presents the results of the systematic review, integrating the meta-analysis results as well as the results of the statistical summary and the narrative analysis. The description of the certainty of the evidence as well as the justification for downgrading and upgrading evidence can be found in the certainty framework assessment in Table S14. In brief, RoB rating downgraded the certainty of the evidence only in the case of the crustacean studies because 80% of the studies included ($n = 4$ of 5) were rated as having a high RoB. Heterogeneity was high across all the families of organisms and downgraded all the evidence by one grade. Conversely, data were not downgraded regarding the three domains of indirectness, imprecision, and publication bias because the evidence was not found to be affected by these factors.

Table 5. Summary of effects.

Seafood category	Number of studies	Outcomes	95% CI	Certainty of the evidence[a]
Average MPs/g content[b]				
Mollusks				Low[c]
Clams	5	1.25	±0.55	
Mussels	9	0.71	±0.21	
Oysters	5	0.42	±0.23	
Scallops	3	0.48	±0.29	
Overall	14	0.78	±0.2	
Range of MPs/g content[d]				
Mollusks	21	0–10.5		Moderate
Crustaceans	2	0.1–8.6		Low
Range of MPs/individual content[d]				
Fish				Moderate
Anchovies	6	0.35–2.3		
Sardines	6	0.23–4.63		
Lance	1	0.54		
Bogue	1	0.34 ± 0.6 SD		
Overall fish	9	0.23–4.63		
Echinodermata				Moderate
Sea urchins	1	0.82		
Range of MPs/g content				
Fish				Moderate
Anchovies	3	0.01–0.09		
Sardines	4	0.02–0.77		
Lance	1	0.08		
Comber	1	2.9 ± 1.54 SD		
Croaker	1	0		
Seabass	1	0		
Overall fish	10	0–2.9		
Echinodermata				Moderate
Sea urchins	1	1		

Note: Data represent MP content in global seafood samples (mollusks, crustaceans, fish), meta-analysis results, and statistical analysis results. Certainty of the evidence was rated according to Higgins et al. (2019). MP, microplastic; SD, standard deviation.
[a]All studies were upgraded owing to the absence of confounders according to the results of the assessment of the certainty of evidence. Details for the assessment are provided in Table S11.
[b]Meta-analysis results.
[c]Owing to high heterogeneity (see assessment of the certainty of evidence in Table S11).
[d]Statistical summary results.

Table 6. Yearly microplastic uptake from the consumption of seafood.

Yearly uptake	MPs	95% CI
Mean yearly uptake[a]		
Mollusks		
Clams	3,312	±1,431
Mussels	1,881	±557
Oysters	1,113	±610
Scallops	1,272	±769
Overall	2,067	±503
Range of yearly uptake[b]		
Invertebrates		
Mollusks	0–27,825	
Crustaceans	206–17,716	
Fish		
Anchovies	31–279	
Sardines	62–2,387	
Lances	230	
Combers	8,323	
Overall fish	31–8,323	

Note: The consumption has been calculated for each family and then pooled for each of the three phyla; mollusks, crustaceans, and fish corresponding to the yearly global seafood consumption data (FAO 2020a). CI, confidence interval; MPs, microplastics.
[a]Based on the meta-analysis results.
[b]Based on the statistical summary results.

Regarding the three upgrading domains, large effects and dose response did not apply in these studies, whereas all studies were upgraded by one grade owing to the lack of confounders.

Human Exposure to MPs through Seafood

According to the Food and Agriculture Organization of the United Nations (FAO 2020a), global human consumption for fish and seafood in 2017 was 20.38 kg/capita per year; breaking down as fish at 15.21 kg/capita per year, mollusks at 2.65 kg/capita per year, crustaceans at 2.06 kg/capita per year, and cephalopods at 0.47 kg/capita per year (live-weight equivalent). The data by the FAO cover 173 countries around the world (FAO 2020a) and indicate significant variability in fish and seafood consumption by country, ranging from 0.25 kg/capita per year in Afghanistan to 90.71 kg/capita per year in Iceland.

Combining the data for global human consumption of seafood with the outcomes of the statistical summary in this review results in an extrapolation of yearly MP uptake of 0–27,825 MPs from mollusks, 206–17,716 MPs from crustaceans, and 31–8,323 MPs from fish (Table 6). The total maximum yearly MP uptake from all seafood categories, based on FAO (2020a) data could be as high as 53,864 MPs. Seafood consumption between countries varies greatly and is predominantly connected to geography and culture. For example, it is estimated that people in Angola consume 0.01 kg of mollusks per year, whereas in Hong Kong this rises to 15.32 kg per year (FAO 2020a). The variations of projected maximum yearly MP uptake from global consumption of mollusks is illustrated in Figure 8, for crustaceans in Figure S13, and for fish in Figure S14. The numerical data for the maps can be found in Table S15.

Discussion

Although this is not the first review on this topic, it represents the first systematic review concerning MP contamination of seafood intended for human consumption. Two recent reviews (Hantoro et al. 2019; Toussaint et al. 2019) presented evidence of human exposure to MP through the consumption of seafood but did not critically collate evidence in order to quantify MP uptake. A recent review by Cox et al. (2019) reported MP content of 1.48 MPs/g for seafood, which is consistent with the higher end of the results reported here. The review by Cox et al. (2019) included studies that were rejected by the screening process for this review. For instance, the studies by De Witte et al. (2014) and Davidson and Dudas (2016) were rejected because a particle composition identification process was not included. Using only visual observation for the identification of MP particles can lead to overestimations (Rocha-Santos and Duarte 2015; Strungaru et al. 2019; S Zhang et al. 2019). The inclusion of such studies in these reviews could explain this overestimation.

Fifty studies were systematically reviewed, and the overall quality of the evidence was assessed as low to moderate (Table 5). RoB rating was correlated with fluctuations in the MP content results across all phyla. This suggests that the bespoke quality assessment tool was successful in detecting the most important parts of the studies' protocol and execution, from formulating the rationale to reporting of results. According to the meta-analysis, the MP content in mollusks was 0.78 MPs/g (95% CI: 0.58, 0.97) (Figure S9). Meta-analysis was executed primarily separately for the different molluskan families to address sample and statistical heterogeneity. The range of MP content was found to be 0–2.9 MPs/g in fish, 0.1–8.6 MPs/g in crustaceans, and 0–10.5 MPs/g in mollusks (Table 5), extrapolating to yearly consumptions of 31–8,323, 206–17,716, and 0–27,825 MPs, respectively (Table 6).

Seafood consumption between countries varies greatly. Countries that are the highest producers of seafood are not

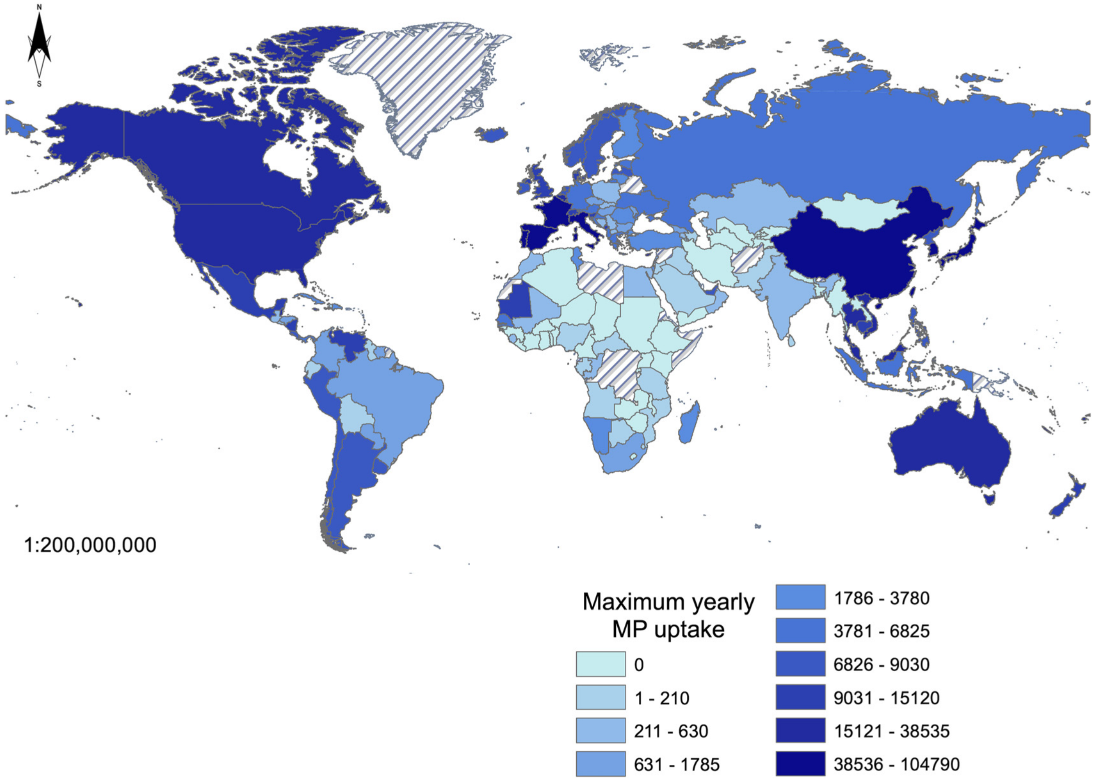

Figure 8. Predicted global yearly maximum microplastic (MP) particles uptake through mollusk consumption. The data have been calculated using the FAO (2020a) consumption data for the different mollusks' families per country and the maximum MPs/g content of mollusks derived from the statistical summary results herein. The numerical data is shown in Table S15. MP data were classified in 10 categories using quantile classification for illustration purposes. The hatched areas illustrate countries for which data on mollusk consumption were not available.

necessarily the ones that consume it. According to FAO (2020b), Spain is the leading producer of mussels for human consumption, reaching 250,000 metric tons per year, but it is not the highest consumer (42.38 kg/capita per year) (FAO 2020a). China is also a leader in mussel production (600,000 metric tons), but a large proportion is used as fish food. Other major producers are Chile, Thailand, and New Zealand (Guillen et al. 2019). Corrections for the calculation should include information on where the seafood is produced/caught and where it is consumed. Unfortunately, information at this level of granularity is not readily available. A recent study by Guillen et al. (2019) attempted to calculate the global seafood production and consumption footprint using FAO consumption data and modeling, reporting China to be the major global producer and consumer and also as being self-sufficient for the most part (Guillen et al. 2019).

Other media have also been identified as vectors of MPs via the ingestion route with varying MP concentrations, such as sugar (0.44 MPs/g) (Cox et al. 2019), whereas in our previous work, salt was found to have an MP content of 0–1,674 MPs/kg (Danopoulos et al. 2020a), tap water 0–628 MPs/L, and bottled water 0–4,889 MPs/L (Danopoulos et al. 2020b). Further systematic reviews are needed to robustly assess MP contamination and human exposures from all food categories.

In addition to food ingestion, atmospheric MP contamination presents an additional pathway for MP human exposures (Chen et al. 2020), related to direct exposures via inhalation (Wright et al. 2020) and indirect exposures via nondietary ingestion routes of hand-to-mouth behavior (Gasperi et al. 2018), inadvertent ingestion (Abbasi et al. 2019), and occupational exposures (Gallagher et al. 2015). Recent studies have started to quantify indoor and outdoor air MP levels, for example, Dris et al. (2017) reported concentrations of 1.0–60 MPs/m^3 (indoor) and 0.3–1.5 MPs/m^3 (outdoor) in air, whereas Liu et al. (2019) measured levels of 0–4.18 MPs/m^3 in outdoor air. A recent review has attempted to extrapolate to human exposures, reporting annual inhalation of 1.9×10^3–1.0×10^5 MPs (indoors) and 0–3.0×10^7 MPs (outdoors) (Zhang et al. 2020). These additional pathways must be included in an aggregate human exposure scenario to account for multiple pathways, routes, and media (U.S. EPA 2019; IPCS 2009). A direct comparison between the magnitude of exposure via different pathways is not advisable at this point given that the end point of the exposures might be different and the internal doses of MPs are likely to vary and depend on the physicochemical MP characteristics (e.g., size, hydrophilicity) (Galloway 2015) and the responses of the barrier organ, that is, the GI tract (Keshav and Bailey 2013; Vancamelbeke and Vermeire 2017) and the lower regions of the respiratory tract (Timbrell 2009). The presence of MPs has been confirmed in both human lung tissue (Pauly et al. 1998) and the GI tract (Schwabl et al. 2019). There is evidence that occupational exposure to high levels of airborne MPs can impact upon human health (Donaldson and Tran 2004; Gallagher et al. 2015; Pauly et al. 1998), but further research is needed to understand whether dietary MPs exposures can have a detrimental effect on the human GI system.

In terms of the most prevalent polymeric compositions in mollusks, discounting the studies that did not report cellulose-related material, PE was the most abundantly detected polymer, followed closely by PP. In the rest of the studies, CP was the most abundant material followed by PET, rayon, and polyester; their reported MP levels might have been inflated by the inclusion of these materials. In crustaceans, the more prevalent polymers were PE and PA, and in fish PE and PP. Consensus is needed in the definition of MPs because some studies included nonsynthetic or semisynthetic polymers in their results. Across the families of organisms, PE and PP were the most dominant, corresponding to the global plastic production trends (Plastics Europe 2019). According to the European Plastics Industry Association, for the past 14 y, the plastics with the highest demand and distribution by resin have been PE (combined low- and high-density PE) followed by PP, polyvinyl chloride, polyurethane, PET, and expanded polystyrene/polystyrene (Plastics Europe 2008, 2017, 2018, 2019).

Narrative analysis showed that molluskan MP contamination was skewed toward content of <1 MP/g and that there seemed to be a correlation of higher MP values in samples from Asia. A geographical variation in MP content was observed whereby a majority of studies (82%; $n = 9$ of 11) reporting an MP content of >1 MP/g were from the coasts of Asia, in contrast to only one study from Europe. It is important to note that this correlation might be artificial owing to more research being conducted in Asia. However, a recent report by the Ocean Conservancy and McKinsey Center for Business and Environment (2015) argued that more than 50% of the plastic pollution of the oceans, originating from land, comes from five Asian countries (Jambeck et al. 2015). The pattern of MPs contamination of the oceans (surface/column water and sediments) has been the subject of intensive recent research, but their results are contradictory (Li et al. 2019; Olivatto et al. 2019; Pan et al. 2019; Yu et al. 2018; C Zhang et al. 2019; J Zhao et al. 2018). The systematic review on MP environmental occurrence by Burns and Boxall (2018) point to higher contamination close to urban and industrial coastal areas and rivers for surface waters. In contrast, other research and reviews report higher MP and plastic concentrations in the convergence zones of the subtropical gyres and higher concentrations in the open ocean than in coastal areas (Avio et al. 2017; Barrows et al. 2018; Cózar et al. 2014; Eriksen et al. 2014). Therefore, it is not yet possible to draw conclusions on geographical patterns of MP contamination, and further research is needed.

The contamination of organisms is likely to be affected by the level of contamination of their environment, followed by their feeding habits and physiology. The differences in the amount of MPs between mollusks and the other two phyla can be attributed to the fact that they are filter or bottom feeders. Their physiology renders them a natural filtering system of the oceans, making them vulnerable to MP contamination. In fish, apparent organs for MPs aggregation include the GI tract and gills, which indeed were the focus of many of the studies (Digka et al. 2018; McGoran et al. 2018; Sun et al. 2019; Tanaka and Takada 2016). On the other hand, MPs were not discovered in the studies that analyzed the flesh of larger fish.

Sampling directly or indirectly from the environment and whether the organisms were wild or farmed were recognized as important factors for their contamination. Regarding wild vs. farmed organisms, analysis was inconclusive. A controlled environment might seem more protected against the contamination of farmed organisms, but if the farm is situated in an MP-contaminated area, the water quality will have an impact. In addition, Karbalaei et al. (2020) identified MP contamination in three brands of commercial fishmeal; the use of such fishmeal could have cumulative effects in farmed seafood (Karbalaei et al. 2020). A significant difference was found between the molluskan families collected directly from the environment and those collected indirectly (i.e., from markets), with the first found to be more heavily contaminated with MPs. The depuration procedure that some mollusks are subjected to before being commercially available was proposed as one possible mitigating factor.

A wide range of methodological heterogeneity was detected across the studies regarding sampling and analysis. The size of the sampling regime has a direct effect on the power of the study in terms of both internal and external validity, that is, whether the results can be used to extrapolate to a general population (Higgins et al. 2019). Sampling size is inherently connected to the overall sampling design of the study and is a function of the project's objective, sampling approach, cost, environmental variability, and tolerable error (U.S. EPA 2000, 2002; Zhang 2007). The European Commission, through the Institute for Environment and Sustainability (EC 2013), produced guidelines that raised the minimum amount of sampled specimens to 50 per species and age group, a level that was not reached by many of the studies in this review. It should be noted that this recommendation applies to monitoring the ingestion of litter by fish over time or between different locations. These guidelines speak to the need for more robust sampling. Furthermore, the majority of the studies did not use a robust sampling design, such as a simple random, stratified, or systematic design but, rather, used a judgmental sampling design. However, a judgmental sampling design should be avoided in environmental studies because it can affect the quality of the study and introduce bias (Zhang 2007).

Results were associated with the different particle-extraction procedures and the specifications of the composition identification methods, highlighting the varying effectiveness of research protocols. It has been argued in recent reviews (Miller et al. 2017; Lusher et al. 2017b; Silva et al. 2018) and method papers (Claessens et al. 2013; Collard et al. 2015; Dehaut et al. 2016) that the use of different chemical and physical treatments for the extraction of particles can influence the effectiveness of the procedure or even further degrade and damage the particles. Although the performance of these procedures is not the focus of this review, it highlights the methodological heterogeneity in the field and the need for consensus. These variations in methods are likely to affect results, under- or overrepresenting MP content. Major differences were found in the processes that were implemented to extract possible MP particles from the tissue of the organisms, specifically in the use of different chemicals for the digestion of the samples and the use of a density-separation process.

Further important variations were identified in the composition identification process in terms of the quantity of analyzed particles and the specification of the analysis protocol. Following on from the extraction step, there was a lack of consensus on the percentage of particles isolated that need to be analyzed for composition in order to extrapolate safely to the whole sample. In most cases, this would be a function of available time and resources given that composition analysis is time consuming, labor intensive, and expensive. Nevertheless, it can be assumed that the larger the number/proportion of the analyzed particles, the higher our confidence in the results. The number/proportion of particles undergoing composition analysis should also be considered in relation to the percentage of particles confirmed as MPs, as well as the accepted percentage of similarity compared with the spectral library. Correlation analysis found that as the absolute number of particles and the proportion of particles analyzed increased, the MPs/g content was reduced. This leads to the logical assumption that as the numbers of particles tested increase, the better the quality of the research protocol, and the less they are detected in samples. A further finding was that the use of higher spectral similarity indexes was found to be more robust. As the similarity index rose from 60% to 70% and 80%, the MPs-per-gram content also rose. This suggests that as inclusion criteria become more stringent, higher MPs content is identified. One would expect that the lower the similarity index, the more particles would be confirmed as MPs, and thus the greater the MPs-per-gram content would be observed. This is the opposite of what these results showed. In order to explore this further, correlation analysis was carried out between the percentage of the verified MPs and the rest of the variables (the percentage of particles that were analyzed, the number of particles analyzed, the similarity index of the spectral library), but no significant correlation was found. It should be noted that these results were based on the results of only seven studies, but this analysis can be repeated in the future when more data are available to produce more robust results.

RoB assessment revealed a few focal areas as the source of studies' weaknesses. The most frequently recognized issue was the use of procedural blank samples and the reporting, or not, of their results. In some cases (8.6%; $n = 2$ of 23; Table S8), studies that did report the results, did not further clarify how the results were used, whereas in many studies (26%, $n = 6$ of 23), the authors reported that the amount of MPs discovered in procedural samples was inconsequential without offering any more evidence to their conclusion (e.g., statistical tests). The specifics around their use also varied greatly in terms such as the number of samples used and whether they tested the reagents used in the experiments.

Recent reviews by Hermsen et al. (2018) and Koelmans et al. (2019) proposed quality assessment systems for MPs research regarding biota samples and water samples, respectively, similar to the RoB tool used in the present meta-analysis. Both reviews identified high levels of variability in methods and recognized the need for harmonization and transparency in methodology and reporting. There is an evident need for harmonization and/or standardization in all aspects of the research protocols in order to increase confidence in the results (Hartmann et al. 2019). There is a subtle but significant difference between the two terms. Although they both refer to reducing the variations in the methodology, harmonization is less stringent and allows some variation, whereas standardization implies complete absence of variations. Standardization cannot be achieved throughout all aspects of scientific experimental protocols, but best practices for analytical procedures and quality assurance and control tools can be set as the minimum standard for designing, executing, and reporting experiments (Johnson et al. 2020). The lack of such harmonized methods hinders the acquisition of reliable and reproducible data. This need is also highlighted by current interlaboratory efforts to achieve these goals by the Joint Research Center (JRC 2019) of the European Commission, the German Federal Institute for Materials Research and Testing, and the Vrije Universiteit Microplastics Interlaboratory Study and Workshops (https://science.vu.nl/en/research/environment-and-health/projects/microplastics-ws-and-ils/index.aspx). Our findings coincide with recent reviews by Hermsen et al. (2018) and Koelmans et al. (2019), who in proposing quality assessment systems for MPs research also identified a high level of variability in methods and the need for harmonization and transparency in reporting.

Statistical heterogeneity, which is the quantified variability of data, is the product of clinical and/or methodological variability among the studies of the meta-analysis (Higgins et al. 2019; Rücker et al. 2008). Clinical heterogeneity refers to the variability of the sample characteristics, and methodological heterogeneity refers to the variability of methods. Measuring the statistical heterogeneity in meta-analysis can be used to evaluate whether all the studies are measuring the same thing. In the present review, the effect measure of interest (MP content) was a tangible physical measure, and it is possible to be confident that the studies are indeed measuring the same thing. Specifically, in order to strengthen this confidence, the use of a chemical composition identification method was set as an inclusion criterion. Furthermore, heterogeneity can inform whether it is appropriate to combine data from different studies (Borenstein et al. 2009). The wide scope of the present review predetermined that the diversity of the included studies would be high. Diversity existed regarding both sample characteristics (e.g., more than 40 species of mollusks; Figure S2) and the studies' methods (e.g., 23 different particle-extraction processes; Table S5). Nevertheless, the studies were judged to be homogeneous enough to produce a meaningful summary. This decision was based on the similarity of the physiological characteristics of the sample population as well as the intended use of the organisms as seafood. Heterogeneity was recognized before the execution of the meta-analysis and was partially addressed by using random-effects models instead of fixed-effect models. Throughout the meta-analysis applied to the molluskan families, statistical heterogeneity given that measured by the I^2 value was found to be high. The confidence in the I^2 values was limited owing to the small number of studies. All attempts to decrease heterogeneity by excluding highly influential studies and statistical outliers were unsuccessful. Subgroup analysis showed that significant differences existed between the geographical origins of the samples across all the different molluskan families. Therefore, there is a high probability that the residual heterogeneity was caused by diversity in the geographical origin of the samples.

Human health effects related to MP exposures, and indeed the levels of MPs in human subjects, are only recently being investigated, but there is a growing body of literature to support evidence of uptake (Abbasi et al. 2019; Gallagher et al. 2015; Schwabl et al. 2019) and detrimental impacts (Dong et al. 2020; Gallo et al. 2018; Stock et al. 2019). Recently reported potential human effects include GI and liver toxicity (Chang et al. 2020; W Wang et al. 2019) as well as neurotoxicity (Prüst et al. 2020). The key identified exposure route is ingestion (along with inhalation) (Chang et al. 2020; Hale et al. 2020), with seafood being a major medium of exposure (van Raamsdonk et al. 2020; YL Wang et al. 2020). Key toxic mechanisms include cytotoxicity via oxidative stress (Chang et al. 2020), gene expression alteration and genotoxicity (YL Wang et al. 2020) changes to the gut microbiota (van Raamsdonk et al. 2020), metabolism disorders, and inflammatory reactions (Chang et al. 2020). Evidence comes from animal studies and human cell lines. Although the findings are in some cases contradicting (van Raamsdonk et al. 2020) and further research is undoubtedly needed, there is also no evidence that MP human exposure is safe (Leslie and Depledge 2020). Seafood is an important source of protein for populations around the world, and it may be time to implement the precautionary principle (Kriebel et al. 2001), based on the existing scientific evidence, and take steps in policy, industry, and society to minimize human exposures to foodborne MPs where possible.

Strengths and Limitations

This systematic review collates evidence from multiple studies and estimates human MP exposures via seafood consumption. The review used robust methodology and a bespoke RoB assessment tool to appraise the quality of the studies. Although heterogeneity was acknowledged throughout the review, the strategies used to remediate it had limited success. Extrapolating to human MP uptake through seafood was based only on the species for which evidence was available, thus affecting the external validity of the results.

Conclusions

Fundamentally, the vast majority of studies included in the present review found MPs in the seafood samples. The data support the hypothesis that seafood is a major verified vector for human exposure to MPs. The levels of MP contamination varied in different phyla of organisms from fish (0–2.9 MPs/g), to echinodermata (1 MPs/g), to crustaceans (0.1–8.6 MPs/g) and mollusks (0–10.5 MPs/g).

A key finding of this work is the need for harmonization and standardization of methods and procedures throughout the research process, starting from sampling design on through to reporting. The bespoke RoB assessment tool used in the present review and the narrative analysis along with the GRADE certainty framework identified the following areas that would benefit from improvement, clarification, and further research:

- In order to reduce RoB, there is a need for overall methodological improvement in study design (sampling and analysis) and execution.
- Sampling design must be linked to the aim of the study and a rationale should be provided, particularly for sample size and location.
- High standards of laboratory practices should be followed to avoid post-sampling contamination.
- The use and detailed reporting of procedural blank samples must be instituted to account for post-sampling MP contamination.
- There is a need for harmonization of the procedure that is used to extract particles from the tissues of organisms because varying effectiveness can significantly affect results and hamper comparisons across studies.
- The use of a verified technique for the identification of the composition of the particles is imperative to avoid under- or overrepresentation. In particular, a consensus is needed in the definition of MPs because some studies include nonsynthetic and/or nonsynthetic polymers in their results.
- Consensus is needed for the protocol of the composition identification process in the proportion of particles analyzed, which spectra library is used, and what minimum accepted similarity index to the spectra library is allowed.
- Consensus is needed on the definition of MPs in terms of size, which is perhaps also related to body compartment exposure/uptake characteristics.
- Reporting should include details of the organisms' characteristics, such as weight, to facilitate conversion to other units and comparison between studies.
- Further research is needed on the effectiveness of depuration on the mitigation of MP contamination of mollusks.

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