REVIEWS IN FISHERIES SCIENCE & AQUACULTURE
https://doi.org/10.1080/23308249.2023.2216301

A Critical Assessment of Microplastics in Molluscan Shellfish with Recommendations for Experimental Protocols, Animal Husbandry, Publication, and Future Research

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ABSTRACT

Microplastics (MP) are a contaminant of emerging concern and, as such, there has been a rush to action and publication. Over the past two decades, this haste has resulted in a chaotic and cluttered literature rife with inappropriate methodologies, poor experimental protocols, misinterpreted results, overstated significance, and subsequent damaging media stories. This review provides a critical assessment of the current scientific literature on interactions between particle-feeding molluscs and MP and their purported impacts (>750 publications), and recommendations for future efforts. Experimental studies were critically assessed and assigned scores ranging from 0 to 2 as indicators of their veracity. The mean ratio for the 84 papers included in this analysis was 0.9, indicating that most publications contained too many flaws. It is not surprising that MP have been noted in shellfish guts globally. What is surprising is the extremely low level of particles routinely recorded (see Table 1 and references therein). The presence of MP in molluscs has been shown repeatedly, with little regard for quality assurance and control measures. The inconsistencies across studies and lack of proper sampling design have inundated the literature with incomparable studies and inappropriate claims. Common mistakes in field studies from collection through digestion and MP characterization are discussed and identified in 128 studies. Suggestions are made to improve field studies at every stage. The data to date clearly demonstrate extremely low numbers (<10 per individual) of MP in filter-feeding bivalve molluscs globally. There are no data demonstrating presence of MP in these molluscs is a serious risk to human health, and few data to demonstrate negative impacts on the shellfish at environmentally relevant concentrations. Many of the studies on suspension-feeding bivalve molluscs and other invertebrates are weak or fatally flawed. There is a recurring presence in the published literature of misunderstanding of the feeding processes, capabilities for particle selection and rejection, and species-specific differences that all lead to misinformation, misinterpretation, and incorrect assumptions regarding potential impacts. There are major shortcomings to many laboratory studies that examined uptake and accumulation of MP by bivalves and their subsequent effects. The shortcomings have led to a seriously flawed literature on purported interactions and impacts of MP on these animals. If potential investigators do not possess the necessary knowledge and skills to carry out the study, they should engage a collaborator that has the requisite expertise. Bivalves and other particle-feeding molluscs are complex living organisms with extraordinary capabilities for the control of selective capture, ingestion, and egestion of particulate material. They should be recognized and treated as such in any attempt to describe impacts of stressors, including different particle types, on their feeding and ability to accumulate materials. Any future experimental studies need to be focused carefully, based upon clear questions, use standardized analytical procedures, demonstrate a knowledge of the animals being studied, and an understanding of the literature extant. The hype needs to be curtailed and scientists should not imply impacts or potential impacts when there are no data to support the suppositions at environmentally relevant concentrations of MP. The case is further strengthened to stop advocating for the use of bivalve molluscs as reliable indicators of MP in the environment. Recommendations are offered for future efforts including harmonization of methodologies. Finally, a plea is made for editors of scientific journals to make a stronger effort to engage qualified peer-reviewers and stop the flow of poorly done studies and superficial reviews that do nothing more than confuse the literature and reinforce inadequate studies and prior reviews. This review is presented from the viewpoint and consideration of experts in shellfish physiology, and represents the opinions of, and assessments made by, the authors.

KEYWORDS

Microplastics; molluscs; peer review; bioindicators; methodology

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The peer review process for this manuscript was handled by Editorial Board member, Dr. Jay Parsons.

1. Introduction

As a contaminant of emerging concern, microplastics (MP) have attracted considerable attention and there has been a rush to action and publication that has resulted in a chaotic and cluttered literature rife with incorrect information, inappropriate methodologies, poor experimental protocols, misinterpreted results, and overstated significance. The vast majority of the reported field values are extremely low. Additionally, many of the studies used different collection methods, detection methods, and reported results with variable units of measure (e.g., MP·individual^{-1}, items·g wet weight^{-1}) making comparisons difficult, if not impossible (see Table 1 and references therein).

Plastic is a mainstay in current civilization globally and, as with many other products, its presence is both a great asset and an environmental concern. The term "plastic" is far encompassing, describes a range of materials, and descriptions are provided elsewhere (Andrady and Neal 2009; Thompson et al. 2009). Borrelle et al. (2020) recently estimated that 19–23 million Mt (11%) of total plastic waste generated globally in 2016 alone entered aquatic ecosystems. Production of plastic, specifically resins and fibers, has increased exponentially since the 1950s (ca. 2 Mt), reaching 380 Mt as of 2015 (Geyer et al. 2017). These production levels are expected to reach 26,000 Mt of resins, 6000 Mt of polypropylene and acrylic fibers, and 2000 Mt of additives by 2025 (Geyer et al. 2017). The increased production and use of plastics over the past several decades has led to increasing loads of mismanaged plastic waste. A portion of this waste enters the marine environment, with cumulative amounts in marine systems estimated at between 5 and 13 million metric tons (Jambeck et al. 2015). Plastic debris such as bags, bottles, caps, and fishing line are not only eyesores, but can have ecological impacts, directly affecting marine fauna, for example, seabirds, fishes, sea turtles, which become tangled or mistake plastic materials for food (Secretariat Convention Biological Diversity [SCBD] 2012). Additionally, within the environment plastic debris degrades and fragments into MP (Andrady 2003).

Microplastics have been defined traditionally as particles less than 5 mm (Arthur et al. 2009). More recently, Hartmann et al. (2019) redefined the size as less than 1 mm, and in this review, the range of size for MP is set between 1 µm and 1 mm, with those particles less than 1 µm being defined as nanoparticles. Microplastics are either manufactured directly for use in personal-care products (e.g., microspheres), or produced indirectly as larger plastic debris and synthetic garments degrade and fragment (e.g., microfibers, films, irregular particles; see Lambert et al. 2014; Lusher et al. 2017b). Within environmental samples, a mixture of MP can be found with various shapes (e.g., spherical, angular, fibers) and compositions (e.g., nylon, low- and high-density polyethylene, polystyrene, polypropylene; see Hidalgo-Ruz et al. 2012; Andrady 2017; Gago et al. 2018; Mishra et al. 2019 for reviews). Plastic particles are a widespread contaminant in marine ecosystems, being well-documented in biota, sediment, the water column, and marine aggregates (Ryan et al. 2009; Browne et al. 2011; Cole et al. 2013; Farrell and Nelson 2013; Van Cauwenberghe et al. 2013; Desforges et al. 2014; Lusher et al. 2017a, 2017b; Zhao et al. 2017, 2018; Phuong et al. 2018a; Renzi et al. 2018). Some reports estimate that MP make up approximately 92% of plastic debris floating at the sea surface, with losses from the surface to the benthos and shoreline mediated by physical and biological processes (Cole et al. 2011; Eriksen et al. 2014; Law and Thompson 2014; Zhao et al. 2018; Kane et al. 2020).

Microplastic pollution has received considerable attention in multiple environments (see reviews by Rands et al. 2010; Sutherland et al. 2010; Cole et al. 2011; Eerkes-Medrano et al. 2015; Horton et al. 2017; Zhang 2017) with particular focus on the marine environment. Given the ubiquitous distribution of MP and microfibers in aquatic environments, there has been a surge in the number of papers published on the incidence of MP in surface waters, the water column, sediments, estuaries, beaches, and in various species of aquatic organisms, as well as surveys of shellfish in markets (see GESAMP 2015a, 2015b; Lusher 2015 for reviews; Tables 1 and 2 this review), and on the potential impacts of these particles on aquatic organisms.

Interactions between MP and numerous marine taxa have been investigated, with many studies focusing on particle-feeding invertebrates. Within that realm, the role of marine molluscs, especially bivalves, has been the focus of many surveys and experimental efforts to establish the presence of MP and their potential impacts (see Tables 1 and 2, and References). This is in part because they are the most abundant suspension feeding guild in the marine environment, and often dominate the macrobenthos, performing important ecosystem services (Dame 1993; Prins et al. 1998; Newell 2004; Smaal et al. 2019), and are sensitive to changes in both abiotic and biotic parameters of the environment (Davids 1964; Newell 1979; Doering and Oviatt 1986;

Jørgensen 1990; Shumway and Parsons 2016; Bayne 2017). They also garner special attention because they are most often consumed whole, that is, with the digestive system intact, and are a potential source of transfer of accumulated materials to other organisms including humans. It is also because there has been an assumption since the early days of reporting that bivalve molluscs would be the most obvious indicator of MP in the environment.

The early studies of MP and suspension feeding molluscs were based upon an assumption that these animals would be the most obvious indicator of MP in the environment. Additionally, many bivalve species are commercially important, with the bivalve shellfishery estimated to be worth over US$350M (NOAA/NMFS 2020; Naylor et al. 2021). For 2019, UN-FAO estimated global seafood consumption at 20.5 kg per capita of which 12% were molluscs (excluding cephalopods). Globally, they estimated global seafood consumption was 157.7 million tonnes, live weight equivalent (FAO 2022). Finally, as primary consumers at the base of the food chain, there is a concern that bivalves may transfer MP to organisms at higher trophic levels including humans; however, evidence for this transfer is limited, if not simply assumed, and controversial (e.g., Talsness et al. 2009; van Cauwenberghe and Janssen 2014; Bouwmeester et al. 2015; Galloway 2015; Sharma and Chatterjee 2017; Wright and Kelly 2017; Barboza et al. 2018; Carbery et al. 2018; Murphy 2018; Smith et al. 2018; Fang et al. 2019; Robledo et al. 2019; Campanale et al. 2020; Danopoulous et al. 2020a; De-La-Torre 2020; Daniel et al. 2021; Vethaak and Legler 2021). For these reasons, numerous studies have attempted to quantify the presence of MP in bivalves under natural conditions and in markets, and to assess the potential impacts of plastic particles on the bivalves under experimental conditions. While MP have been identified in numerous species globally (see Table 1), the levels of contamination are extremely low. Gouin (2020) reported that the number of MP particles per individual across all studies and species (including fish and shellfish) was estimated to be 4, with studies typically reporting means ranging from 0 to 10 particles per individual. A closer examination of the supplemental material provided with that review indicates that very few suspension feeding shellfish were included in the assessment.

Further, there are questions and major shortcomings with regard to the rigor and methodologies used for detection of MP, and also concerning laboratory experiments and animal husbandry (see Table 3 and discussion below). Microplastics are difficult to identify and quantify, and the current literature on the presence and impacts of MP on marine organisms, especially that concerning molluscs, is seriously flawed.

Bivalve molluscs are complex living organisms with extraordinary capabilities for the control of uptake, capture, consumption, and sorting of particulate material (Beninger and St-Jean 1997a, 1997b; Ward and Shumway 2004; Cranford et al. 2011; Rosa et al. 2018). They should be recognized and treated as such in any attempt to describe impacts of stressors, including different particle types, on their feeding and ability to accumulate materials. There is a recurring presence in the published literature of misinformation and misunderstanding of the feeding processes of bivalves, capabilities for particle selection and rejection, and species-specific differences, all of which is discussed in detail below.

Numerous publications have appeared that claim to be reviews, but most are not comprehensive, lack any critical assessment or insightful discussion, and many are written by individuals with no expertise on the topics presented. The authors frequently indicate that they are providing meta-reviews and that they have followed some strict protocols, have searched various databases using specific key words, and then do little more than provide a listing of what they found. No matter how strict the protocol, without a knowledgeable and critical assessment of the information being reviewed, the efforts serve little purpose. These publications do a great disservice to future readers who rely upon the published information and accept it as accurate (Underwood et al. 2017). Publication of these superficial and non-critical efforts that perpetuate poor studies also gives credence to poor science.

Reviews can provide clarity, synthesis, and insight if prepared by experts and presented in a clear manner. The preparation of reviews can be daunting, especially when the literature extant is copious and of highly variable quality, with some of it fatally flawed (Glenn Richey and Davis-Sramek 2021). The European Food Safety Authority (EFSA 2010) differentiated between narrative reviews and systematic reviews, and presented guidance on the preparation of systematic reviews for use by food and feed safety assessments to support decision-making. Their guidance was considered in the present review where the quality of evidence in terms of methodological soundness and strength of evidence provided in published studies was assessed, and results from studies of higher quality noted. This level of analysis does not typically occur

narrative reviews (= literature surveys; see Sargeant et al. 2005). This review provides a comprehensive assessment of the extant literature that reports on the presence of MP in suspension-feeding molluscs, particularly bivalve molluscs, and purported impacts of MP particles on these animals in laboratory and mesocosm experiments. It provides a critical assessment of methodologies, interpretations, and publication practices. Over 750 peer-reviewed papers were assessed and analyzed, and the synthesis presented is as comprehensive and objective as possible, following the guidelines of EFSA (2010). This review presents original information from published documents (both peer-reviewed and reports) and assesses both the quality of the data and the synthesis and interpretation as presented by the original authors (see EFSA 2010). The data were synthesized to clarify links between the original research and the authors' conclusions. There are comprehensive tables that summarize available published data and a detailed bibliography. Full transparency is provided in that all published papers identified by the authors have been included in the analyses, that is, not presorted according to any arbitrary standards or protocols.

Previous field collection studies were examined, and suggestions provided on methodology and reporting. The benefits and consequences of the continually developing methods used for MP collection and detection are discussed. Further, basic information that should be considered by researchers who undertake studies on MP and particle-feeding molluscs, especially with regard to animal husbandry, physiology, and experimental protocols is provided. This review primarily focuses on bivalves but incorporates the few studies of other particle-feeding molluscs (e.g., Calyptraeidae), notes shortcomings in methodologies, and provides a critical assessment of experimental studies on the association, ingestion, uptake, and purported impacts of MP on the biology of these animals. One goal of the review is to point out where serious errors have occurred and led to erroneous conclusions which continue to be propagated through subsequent literature.

As pointed out by Donnelly et al. (2018), synthesis which is not rigorous is bad science, and it is also bad for policy, as policy informed by flawed science can lead to avoidable mistakes. They provide four principles for evidence syntheses noting that they should be inclusive, rigorous, transparent, and accessible. This review is an effort to provide this synthesis for MP and molluscs.

2. Molluscs as bioindicators

Choice of indicator species for any environmental perturbance or pollutant is based upon cumulative effects of changes on some organism or population of organisms in which measurable changes in specific and measurable criteria are known, for example, abundance, reproductive success, growth rate, or some physiological parameter(s) (see Cairns and Pratt 1993; Bartell 2006; Burger 2006).

More recently, Siddig et al. (2016 and references therein) reviewed the selection criteria for, and use of, indicator species to monitor ecological change by assessing a long-term dataset (2000 papers published in *Ecological Indicators*) and concluded that in 99% of the publications they reviewed, statistical methods were used to assess the performance of selected indicators, and reported that indicator species have been chosen based primarily on four criteria: past research, ecological importance, abundant species, or a combination of these factors. They also provided recommendations regarding selection and evaluation of ecological indicators, including a 5-step process: selection of clear monitoring goals; identification of the ecological setting; selection of the candidate indicator species based on clear criteria; selection of ecological covariates and predictors to which the indicator species is particularly responsive; and simultaneous sampling of species abundance and ecosystem covariates to identify the indicator species analysis to obtain the indicator value for each species.

Goodsell et al. (2009) carefully reviewed the use of specific taxa as reliable indicators of environmental conditions or impacts and provided a clear and thoughtful summary and assessment of the evidence necessary for a species to be a reliable indicator of environmental conditions or impacts. They argued that variables measured as indicators need to be strongly and consistently correlated (through space and time) with levels of the environmental variables; that appropriate experiments must be done to establish that an observed correlation is causal; and that it must be established that the taxa respond directly to changes in the environmental variables they are supposed to indicate, that is, it must be clearly established through careful experimentation that an observed correlation is causal before the taxa can be deemed a useful indicator. They further presented a sensible approach to choose indicator species including assessment of reliability, and verification of the veracity of the species.

Bivalve molluscs are one of the most abundant groups of suspension feeding organisms globally, easily

accessible, and readily filter a wide size range of microscopic particles (see Ward and Shumway 2004; Cranford et al. 2011; Rosa et al. 2018) and references therein for a complete review). Bivalves have previously been commonly used as effective indicators or sentinels of marine pollution (e.g., Goldberg 1975, 1980, 1986; Kimbrough et al. 2008; Andral et al. 2011; Farrington et al. 2016; Beyer et al. 2017), and have been used as bioindicators in several biomonitoring programs globally including the United States. Mussel Watch, Assessment and Control of Pollution in the Mediterranean region (MEDPOL), and the North East Atlantic Oslo and Paris Commission (OSPAR). Based upon these prior uses, it has been automatically assumed by the community-at-large that these same filter-feeders will be suitable bioindicators for the presence of microplastic (MP) in the water column or resuspended sediments, that is, material made available to the filter-feeders (see Tables 1 and 2 and refs therein). For example, Vandermeersch et al. (2015) and others have encouraged use of mussels as bioindicators (Tanabe et al. 2000; Thain et al. 2008; OSPAR Commission 2013). Beyer et al. (2017) included mussels as good candidates for assessment of MP exposure in the environment, but also provided a number of factors that should be taken into account if doing so, for example, bioavailability, shape, induced histological alterations, use of appropriate controls, and whether MP particles could act as vectors for transport of other environmental contaminants (see also Bowley et al. 2021). Based on prior successful use as monitors for other contaminants, Catarino et al. (2018) also advocated for the use of mussels as reliable indicators of MP in the marine environment. This advocacy is mistaken. Use of filter-feeders as bioindicators is only reliable if the animals integrate the pollutants into their bodies, for example, dissolved chemical pollutants. In the case of MP, the particles are solid, transient, selected for or against on a species-specific basis by the shellfish, and excreted rapidly, that is, they cannot be used as reliable indicators (see Ward et al. 2019b for detailed assessment).

The rationale for using filter-feeders as bioindicators is that they will serve as environmental integrators over time. This assumption is based upon prior uses with dissolved pollutants that accumulate in tissues and has only recently been tested experimentally. While mussels are good integrators of many pollutants, the majority are compounds that are integrated into the body tissues of the mussels over time. From the beginnings of the MP barrage, there was no good reason to assume that these same filter-feeders would be good bioindicators of refractory particles such as MP which are known to pass through these animals freely and rapidly. Indeed, MP have been used as markers in suspension feeding studies for decades (see Tamburri and Zimmer-Faust 1996; Brillant and MacDonald 2000; Ward and Shumway 2004; Ward et al. 2019a and references therein). The study by Ward et al. (2019b) specifically designed to elucidate the ability of mussels (*Mytilus edulis*) and oysters (*Crassostrea virginica*) to filter and consume MP (spheres and fibers), clearly demonstrated that mussels are not good indicators of MP, based upon their ability to selectively ingest or reject plastic particles coupled with rapid gut evacuation (see also Subsection 4.1). Similarly, Weinstein et al. (2022) examined uptake and depuration of MP (fragments, fibers) by *C. virginica* and found that the rates of these processes were size- and shape-dependent. They concluded that oysters would not be a useful bioindicator of MP pollution in the environment.

Based upon early assumptions regarding their suitability as bioindicators, numerous studies have reported on the use of filter feeders as biomonitors for MP. Su et al. (2018) reported very low levels of plastic particles (0.3–4.9 items·g^{-1} or 0.4–5 items·individual^{-1}) in Asian clams (*Corbicula fluminea*) in China with microfibers being the dominant type and stated "we demonstrated the Asian clam as an (sic) bioindicator of MP pollution in freshwater systems, particularly for sediments." Most recently, Cho et al. (2021) began their abstract with "Bivalves are useful bioindicators of microplastic contamination in the marine environment for several reasons" and concluded that "bivalves can reflect the microplastic pollution characteristics of the surrounding waters where they live." They cite the paper by Ward et al. (2019b) to note the possibility of particle rejection, but clearly missed the significance of the results contradicting their conclusions regarding the use of bivalves as bioindicators.

Digka et al. (2018a, 2018b) recommended the use of mussels as bioindicators for MP and as a means to establish a baseline for future assessment. Li et al. (2019a) subsequently stated unequivocally that "Laboratory studies collectively demonstrate that mussels may be good model organisms in revealing microplastic uptake, accumulation and toxicity" and they proposed the use of mussels as target species to monitor MP. Not only is their own study flawed, some of the studies they include in reaching their conclusions are also flawed, that is, this over-arching generalization based upon no strong data is unwarranted. Ding et al. (2021) reported MP abundance ranging between 0.5 and 3.3 items·individual^{-1} in four species of

bivalves, showed high variability of results regionally and between animals, and, coupled with a very superficial literature review, still recommended that clams and mussels can serve as bioindicators for MP in the sediment and water, respectively. Most recently, Joshy et al. (2022) ended their abstract claiming that "the usefulness of sedentary bivalves in assessing the aquatic pollution (MP) has been validated through this study" with no mention of published work that shows otherwise. Their study also indicates MP "in the gill" which could not be determined using the described methods and was likely particles on and between the gill filaments. Their discussion, like many others that rely upon the veracity of published literature, cites numerous flawed studies regarding impacts of MP on bivalves.

Wesch et al. (2016) summarized current practices employed in the monitoring of MP ingestion by marine biota. They provided a comprehensive overview and critique of ingestion studies and associated methodologies, noted the necessity for standardized procedures, and provided a focused list of recommendations including standardization of the monitoring process, implementation of standard operating procedures for MP ingestion studies, a conceptual framework for selecting a group of suitable indicator species, optimization of analytical techniques, and development of clean air techniques in the laboratory to prevent background and airborne contamination. Gago et al. (2016) describes how there are few recognized approaches for monitoring MP and calls for the harmonization and coherence of methodologies to reliably monitor the abundance of MP. Bonanno and Orlando-Bonaca (2018) recently provided a refreshing recommendation that biomonitoring should rely upon the combination of several bioindicator species with different characteristics that complement each other. The recommendations put forth in these papers should be carefully considered when attempting to find a suitable bioindicator for MP pollution.

3. Methods used to collect and process field samples

3.1. Field collection and preservation considerations

Bivalve molluscs are easily collected by dredge, SCUBA, or by hand along the shore. The efficiency of these collection methods will not be discussed in this section. Instead, this section focuses on the external factors that contribute to microplastic contamination and measures that can be taken in the field, during transportation, and in the laboratory to reduce contamination. Almost all of the studies reviewed (Table 1) examined the entire soft body of the animal and report the number of microplastics (MP) per individual or per gram weight. Overall, the number of MP identified in bivalves is low and highly variable.

3.1.1. Issues and problems

The collection of molluscs from the field has become common practice in microplastics (MP) research, yet there are still discrepancies in how best to collect specimens and dissect and preserve the animals in the laboratory. Many studies have collected molluscs from the wild and from aquaculture facilities off the coast of urban areas in an effort to attribute MP loads to urbanization and relevant industries (e.g., Li et al. 2016, 2018a; Chen et al. 2018; Kolandhasamy et al. 2018; Phuong et al. 2018b; Qu et al. 2018; Renzi et al. 2018; Su et al. 2018; Waite et al. 2018; Zhao et al. 2018; see Table 1), while others sampled molluscs from supermarkets or local fish markets to compare the number of MP found in natural and store-bought shellfish (e.g., De Witte et al. 2014; Mathalon and Hill 2014; Van Cauwenberghe and Janssen 2014; Rochman et al. 2015; Vandermeersch et al. 2015; Ding et al. 2018; Li et al. 2018b). There are different considerations to be made for molluscs collected from the natural environment compared to those that are store bought, but in both cases the potential for background contamination is high.

Potential sources of contamination in the field include, but are not limited to, synthetic clothing worn by the researchers (see Scopetani et al. 2020 for discussion), plastic containers used for sampling or transport, synthetic rope (example of potential contamination: rope used in fishing for jumbo squid; Gong et al. 2021), gloves, and other materials. Some studies used plastic bags to transport their samples (e.g., Mathalon and Hill 2014; Li et al. 2016; Phuong et al. 2018a; Waite et al. 2018; Scott et al. 2019; Bagheri et al. 2020; Ding et al. 2021). Although most researchers have adopted the quality assurance measure of wearing cotton laboratory coats or full body

Table 1. Field studies of microplastics in molluscs and their methods are presented in this table.

Species	Microplastic concentration measured in tissue	Particle size	Isolation method	Detection method	Location	Criticisms	Reference
Aequiyoldia eightsii (Antarctic yoldia)	Mean: 2.25 MP·ind^{-1} (estimated from figure 5 in study)	33–5000 µm Highest relative abundance: 50–100 µm	Nile Red digestions: Stored in EtOH; dried at 80 °C for 1 hr; 1% NaOH digestion at 40 °C for 24 hr; NaCL density separation; filtered onto glass fiber filters (GF/F, 0.7-µm pore size) For FTIR analysis: 30% H_2O_2 digestion for 72 hr at room temperature; filtered onto ANODISCS (0.2-µm pore size, 47-mm diameter)	Visual-stereomicroscope; Nile Red staining; FTIR on most contaminated species	Terra Nova Bay (Ross Sea), Antarctica	Pooled individuals Missing from QA/QC: 3, 6, 11	Sfriso et al. (2020)
Alectryonella plicatula (fingerprint oyster)	Mean: 10 ± 5 MP·ind^{-1} (estimated from figure 3 in study)	5 µm–5 mm; majority > 250 µm	30% H_2O_2 digestion at 65 °C for 24 hr; then, at room temperature for 24–48 hr (if needed); NaCl density separation; filtered onto cellulose nitrate membrane filter (5-µm pore size, 47-mm diameter)	Visual-stereoscope; FTIR on subset of particles	China	1–5 individuals were pooled as one replicate; density of NaCl solution not defined; tools not listed as glass (e.g., petri dish) Missing from QA/QC: 3, 6, 9, 10, 11, 12	Li et al. (2015)
Amiantis purpuratus	Fibers (mean): 4.2 ± 3.2 MP·ind^{-1} 3.3 ± 2.8 MP· g ww^{-1} Fragments (mean): 1.1 ± 1.0 MP·ind^{-1} 6.8 ± 7 MP·g ww^{-1}	10–5000 µm	30% H_2O_2 digestion; filtered onto filter paper (25-µm pore size) and again onto a nitrocellulose filter (0.45-µm pore size)	Visual-dissection microscope; hot needle test; SEM-EDX; FTIR on subset of particles	Persian Gulf	3–11 individuals per replicate Missing from QA/QC: 3, 4, 6, 10, 11, 13	Naji et al. (2018)
Amiantis umbonella (venerid clam)	Fibers (mean): 4.2 ± 3.4 MP·ind^{-1} 11.8 ± 2.5 MP·g ww^{-1} Fragments (mean): 1.7 ± 1.2 MP·ind^{-1} 6.0 ± 2.2 MP·g ww^{-1}	10–5000 µm	30% H_2O_2 digestion; filtered onto filter paper (25-µm pore size) and again onto a nitrocellulose filter (0.45-µm pore size)	Visual-dissection microscope; hot needle test; SEM-EDX; FTIR on subset of particles	Persian Gulf	3–11 individuals per replicate Missing from QA/QC: 3, 4, 6, 10, 11, 13	Naji et al. (2018)
Amarilladesma mactroides (yellow clam)	Mean: 0.33 ± 0.1 MP·g ww^{-1}	0.013–9 mm Fibers most abundant: <0.5 mm and 0.5–1 mm	10% KOH digestion at 50°C for 48 hr; filtered onto a glass fiber filter (0.7-µm pore size)	SEM-EDX on a subsample of fibers; FTIR on a random subsample of recovered particles >500 µm	Buenos Aires Province, Argentina	10 animals pooled per replicate Missing from QA/QC: 3, 6, 7, 9, 10, 11, 12, 13	Truchet et al. (2021)
Anadara antiquata (cockle)	MP in 48% of cockles sampled, total of 138 microplastics from 160 cockles Highest mean site concentration: 2.1 ± 1.8 particles·ind^{-1} Range: 0–5 particles·ind^{-1}	>0.5 mm	10 M NaOH digestion at 60 °C for 24 hr; neutralized with 10 M H_2SO_4; filtered onto filter paper (90-mm diameter, 11-µm pore size)	Visual-light microscope; FTIR on subset of particles	Tanzania, Africa	Missing from QA/QC: 2, 6, 10, 11	Mayoma et al. (2020)

(*Continued*)

Table 1. Continued.

Species	Microplastic concentration measured in tissue	Particle size	Isolation method	Detection method	Location	Criticisms	Reference
Anodonta anatine (duck mussel)	Värpinge (downstream of wastewater) Mean number of particles "across objects": 12.2 ± 9.1 Mean number of fibers "across objects": 63.3 ± 29.6 *SD* Genarp (upstream, rural) Mean number of particles "across objects": 3.3 ± 3.5 Mean number of fibers "across objects": 38.0 ± 34.2 *SD*	<5 mm	65% HNO_3 for 24–72 hr diluted to 32.5% with water after 48 hr; filtered onto glass fiber filters (GF/C, 1.2-µm pore size)	Visual-light microscopy; hot needle test	Höje å River, Sweden	Pooled two individuals per replicate Missing from QA/QC: 2, 3, 4, 6, 9, 10, 11, 12, 13	Berglund et al. (2019)
Anomalodiscus squamosus	Mean: 3.48 ± 0.89 MP·g^{-1} ww	93.3% were fibers Mean length: 1004.2 ± 464.8 µm Mean diameter: 21.8 ± 7.3 µm	10% KOH digestion at 40 °C for 48 hr; sieved through a 38-µm sieve; filtered onto a cellulose ester filter (0.45-µm pore size)	Visual-stereomicroscope; FTIR on a subset of particles	Hong Kong, China	Low percent recovery for gastropods (50%) because of shell debris on filter not removed for digestion; text states 5.37 ± 1.24 MP·g^{-1} ww; does not match table data; species listed in figures 4 and 5 differ; mislabeled gastropods as crabs Missing from QA/QC: 3, 4, 6, 11, 12	Xu et al. (2020)
Anomia ephippium (saddle oyster)	Mean: 0.12 MP·g dw^{-1}	<5 mm Mean fragment size: 60.01 ± 38 µm Mean filament size: 530 ± 470 µm	30% H_2O_2 digestion at 60 °C; NaCl density separation; filtered onto 5-µm pore size cellulose nitrate membrane filter	Visual-epifluorescence reverse microscope	Gulf of La Spezia, Italy	Missing from QA/QC: 3, 4, 6, 10, 11, 13	Bonello et al. (2018)
Arca noae (Noah's ark shell)	Mean: 1.65 ± 0.28 MP·ind^{-1} 0.58 ± 0.4 items·g ww^{-1}	300–1000 µm	10% KOH digestion at 60 °C for 48 hr; NaCl density separation for 15 min; filtered onto glass microfiber filter paper (pore size not stated)	Visual-stereomicroscope; FTIR	Southeastern Mediterranean coasts, Alexandria, Egypt	Suggest use as bioindicator No mention of QA/QC measures	Said et al. (2022)
Argopecten purpuratus (Peruvian scallop)	Mean: 2.25 ± 0.54 MP·ind^{-1}	<5 mm, fibers most abundant	10% KOH at 60 °C overnight; filtered onto glass fiber filter paper (20-µm pore size)	Visual-microscope; FTIR on a total of 10 randomly selected particles	Lima, Peru	Pooled two muscles and two gonads per sample Missing from QA/QC: 3, 4, 6, 10, 11, 12	De-la-Torre et al. (2019)
Asthenometis asthenodon (tellin clam)	2.1 ± 2.2 MP·ind^{-1} 0.5 ± 0.5 MP·g ww^{-1} Mean across 6 species: 4.8 ± 8.0 MP·ind^{-1} 2.1 ± 3.3 MP·g ww^{-1}	0.1–5 mm	10% KOH digestion for 48 hr; filtered onto a glass fiber filter (GF/C, 1.2-µm pore size)	Visual-stereomicroscope; FTIR on a random subsample of 50 particles	Golgo de Nicoya, Costa Rica	Missing from QA/QC: 1, 5, 6, 8, 9, 10,	Rojas-Jimenez et al. (2022)

Aulacomya atra (ribbed mussel)	Mean: 2.8 MP·g ww^{-1} 2.9 MP·ind^{-1}	50–1000 µm, fibers most common	10% KOH digestion at 60 °C for 24 hr; filtered through nylon mesh (20-µm pore size)	Visual-stereomicroscope; hot needle test	Cape Town, South Africa	Pooled three individuals per replicate Missing from QA/QC: 2, 4, 5, 6, 10, 11, 12, 13	Sparks (2020)
	Mean: 0.3 items·g ww^{-1}	0.5–4.97 mm	30% H_2O_2 digestion at 60 °C for 24–48 hr; filtered through 30-µm mesh	Visual-optical microscope; UV light to examine transparency of potential microplastics; SEM-EDS	Patagonia, Argentina	Pooled 5–10 mussels per replicate Missing from QA/QC: 1, 2, 5, 6, 10, 11, 13	Ríos et al. (2020)
Barbatia sp. (bearded ark clam)	Mean: 1.0 ± 0.3 MP·g^{-1} ww (estimated from figure 4 in study)	93.3% were fibers Mean length: 1004.2 ± 464.8 µm Mean diameter: 21.8 ± 7.3 µm	10% KOH digestion at 40 °C for 48 hr; sieved through a 38-µm sieve; filtered onto a cellulose ester filter (0.45-µm pore size)	Visual-stereomicroscope; FTIR on a subset of particles	Hong Kong, China	Low percent recovery for gastropods (50%) because of shell debris on filter not removed for digestion; some discussed data does not match table data; species listed in figures 4 and 5 differ; mislabeled gastropods as crabs Missing from QA/QC: 3, 4, 6, 11, 12	Xu et al. (2020)
Batillaria multiformis	Mean: 5.37 ± 1.24 MP·g^{-1} ww	93.3% were fibers Mean length: 1004.2 ± 464.8 µm Mean diameter: 21.8 ± 7.3 µm	10% KOH digestion at 40 °C for 48 hr; sieved through a 38-µm sieve; filtered onto a cellulose ester filter (0.45-µm pore size)	Visual-stereomicroscope; FTIR on a subset of particles	Hong Kong, China	Low percent recovery for gastropods (50%) because of shell debris on filter not removed for digestion; some discussed data does not match table data; species listed in figures 4 and 5 differ; mislabeled gastropods as crabs Missing from QA/QC: 3, 4, 6, 11, 12	Xu et al. (2020)
Batillaria zonalis	Mean: 2.5 ± 0.5 MP·g^{-1} ww (estimated from figure 4 in study)	93.3% were fibers Mean length: 1004.2 ± 464.8 µm Mean diameter: 21.8 ± 7.3 µm	10% KOH digestion at 40 °C for 48 hr; sieved through a 38-µm sieve; filtered onto a cellulose ester filter (0.45-µm pore size)	Visual-stereomicroscope; FTIR on a subset of particles	Hong Kong, China	Low percent recovery for gastropods (50%) because of shell debris on filter not removed for digestion; some discussed data does not match table data; species listed in figures 4 and 5 differ; mislabeled gastropods as crabs Missing from QA/QC: 3, 4, 6, 11, 12	Xu et al. (2020)
Bolinus (*Murex*) *brandaris* (purple dye murex or spiny dye-murex)	Mean: 1100 items·ind^{-1} (estimated from figure 2 in study)	0.05–5 mm	10% KOH digestion at 65 °C for 24 hr and then at room temperature for another 24 hr; NaCl density separation; filtered onto a 1-µm filter	Visual-stereomicroscope; FTIR on 30 particles	Tunisia	Each individual treated as a replicate Missing from QA/QC 3, 6, 9, 10, 11, 12	Abidli et al. (2019)
Brachidontes rodriguezii (scorched mussel)	Mean: 0.17 ± 0.07 MP·g ww^{-1}	0.013–9 mm; fibers most abundant: <0.5 mm and 0.5–1 mm	10% KOH digestion at 50°C for 48 hr; filtered onto a glass fiber filter (0.7-µm pore size)	SEM-EDX on a subsample of fibers; FTIR on a random subsample of recovered particles >500 µm	Buenos Aires province, Argentina	Pooled 30 animals per replicate Missing from QA/QC: 3, 4, 6, 7, 9, 10, 11, 12, 13	Truchet et al. (2021)
Cerastoderma edule (common cockle)	4.3 ± 4.32 microfibers·ind^{-1} 374 ± 563.6 microfibers in relation to biomass (ash free dry weight)	>5 mm Mean length: 2.37 ± 2.27 mm Mean section: 20 ± 8 µm	Protease enzyme digestion (neutrase) at 40 °C for 3–6 hr	Visual-stereomicroscope; FTIR on a subset of particles	Tejo Estuary, Portugal Banc d' Arguin Mauritania Bijagos Archipelago, Guinea-Bissau	Missing from QA/QC: 1, 2, 4, 5, 6, 9, 10, 12	Lourenço et al. (2017)

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Table 1. Continued.

Species	Microplastic concentration measured in tissue	Particle size	Isolation method	Detection method	Location	Criticisms	Reference
Cerastoderma edule (common cockle)	Mean: 2.46 ± 1.16 MP·ind^{-1} 0.74 ± 0.35 MP·g ww^{-1}	≥15 µm	10% KOH at 60 °C for 24 hr with agitation; filtered onto a glass fiber filter (GF/A, 1.6-µm pore size)	Visual-stereomicroscope; µ-Raman	Channel coastline, France	Missing from QA/QC: 10, 12	Hermabessiere et al. (2019)
Cerastoderma lamarcki (common cockle)	Highest MP levels: 19.8 MP·g dw^{-1} Mean: 11.3 MP·g ww^{-1} (calculated from Table 2 in study)	Most common size ranges: 1–2 mm (42.2%) 2–5 mm (26%); fibers most common	10% KOH digestion at 40 °C for 48–72 hr; filtered onto Whatman PLC filter paper; NaI density separation and filtered again	Visual-stereomicroscope; hot needle test; FTIR	Gorgan Bay, Caspian Sea	Confirmed limited contamination with "aquracy test" (sic) so not sure of exact methods; confusing methodology; authors report $n = 22$ but Table 2 shows MP per individual; digestion method not clear, appears to have used fish protocol for molluscan tissue Missing from QA/QC: 1, 3, 5, 6, 10, 11, 12	Bagheri et al. (2020)
Cerastoderma spp.	Mean: 11.9 ± 5.5 MP·g ww^{-1}	0.037–4.7 mm; fibers most prevalent	1.8 M KOH digestion at 60 °C for 24 hr; filtered onto glass fiber filters (GF/C, 1.2-µm pore size)	Visual-stereomicroscope; µ-Raman on subsample for each species; FTIR if needed	Ria Formosa Lagoon, Portugal	Each sample contained anywhere from 1–4 individuals Missing from QA/QC: 3, 4, 6, 10, 11	Cozzolino et al. (2021)
Cerithidea obtuse (obtuse horn shell)	Mean: 167 ± 16.01 MP·ind^{-1} Films most abundant: 71.1 ± 14.57 particles·ind^{-1}	Not defined, <5 mm	HNO_3 digestion for 12 hr; diluted 10x	Visual-microscope	Pangkal Babu Mangrove Forest, Indonesia	No mention of QA/QC measures	Fitri and Patria (2019)
Chlamys farreri (Farrer's scallop)	Range of means from five markets: 5.2–19.4 items·ind^{-1} 3.2–7.1 items·g ww^{-1}	25 µm–5 mm	10% KOH digestion at 60°C for 24 hr; filtered onto GF/F filters (0.7-µm pore size)	Visual-stereomicroscope; FTIR	Qingdao, China	Missing from QA/QC: 1, 3, 4, 5, 6, 11, 12	Ding et al. (2018)
	Range of MP: 8–13 particles·ind^{-1}	5 µm–1 mm	30% H_2O_2 digestion at 60°C for 24 hr; ZnCl density separation; digested individual tissues (muscle, kidney, hepatopancreas, intestine, gonad, anus, hemolymph, mantle, and gill); filtered onto a mixed membrane (5-µm pore size)	Visual-stereomicroscope; hot needle test	Shandong Peninsula, China	"Each tissue of the scallop was divided into 4 parallel samples." Missing from QA/QC: 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12,13	Sui et al. (2020)
	Range of mean MP across season and location: 0.5–2.9 items·ind^{-1} 0.4–3.4 items·g ww^{-1}	7 µm–5 mm	10% KOH digestion at 60°C for 24 hr; filtered onto GF/F filters (0.7-µm pore size)	Visual-stereomicroscope; FTIR	Qingdao, China	Missing from QA/QC: 1, 5, 6, 10, 11	Ding et al. (2021)
Chicoreus capucinus (mangrove murex)	Reported range of MP abundance for all three species: 0.50–1.75 particles·g ww^{-1} 0.25–0.88 particles·ind^{-1}	30–1850 µm; majority (57%) 30–1000 µm	69% HNO_3 and 30% H_2O_2 (4:1 v/v) digestion heated to 50 °C for 30 min; NaCl density separation; filtered onto 0.45-µm pore size (47-mm diameter) cellulose nitrate membrane filters	Visual-stereomicroscope; FTIR on a subset of particles	Klang River Estuary, Malaysia	Pooled the gastropods at each site Missing from QA/QC: 3, 6, 9, 11, 12	Zaki et al. (2021)

Chiton granosus (skunk sea cradle)	Mean: 6.92 ± 2.13 particles·g ww^{-1}	<5 mm; fibers most common, followed by fragments, spheres, and films	10% KOH digestion at 60 °C overnight; filtered onto glass fiber filter paper (20-µm pore size)	Visual-microscope	Lima, Peru	Missing from QA/QC: 6, 10, 11, 13 *(based on referenced Dioses-Salinas et al. 2020)*	De-la-Torre et al. (2020)
Choromytilus meridionalis (black mussel)	Mean: 1.8 MP·g ww^{-1} 5.6 MP·ind^{-1}	50–1000 µm; fibers most common	10% KOH digestion at 60 °C for 24 hr; filtered through nylon mesh (20-µm pore size)	Visual-stereomicroscope; hot needle test	Cape Town, South Africa	Pooled three individuals per replicate Missing from QA/QC: 2, 4, 5, 6, 10, 11, 12, 13	Sparks (2020)
	Mean MP concentration for all samples: 3.83 ± 0.2 MP·ind^{-1} 0.04 ± 0.003 MP·g ww^{-1}	>500 µm	10% KOH digestion at 60 °C for 48 hr; filtered through nylon mesh (20-µm pore size)	Visual-stereomicroscope; FTIR	Cape Town, South Africa	Combined species Missing from QA/QC: 6, 11	Sparks et al. (2021)
Cicre scripta (circular tapestry shell)	Mean: 0.25 MP·g^{-1} ww (estimated from figure 4 in study)	93.3% were fibers Mean length: 1004.2 ± 464.8 µm Mean diameter: 21.8 ± 7.3 µm	10% KOH digestion at 40 °C for 48 hr; sieved through a 38-µm sieve; filtered onto a cellulose ester filter (0.45-µm pore size)	Visual-stereomicroscope; FTIR on a subset of particles	Hong Kong, China	Low percent recovery for gastropods (50%) because of shell debris on filter not removed for digestion; some discussed data does not match table data; species listed in figures 4 and 5 differ; mislabeled gastropods as crabs Missing from QA/QC: 3, 4, 6, 11, 12	Xu et al. (2020)
Corbicula fluminea (Asian clam)	Range: 0.2–12.5 items·g ww^{-1}	<5 mm; dominant size class: 100–1000 µm (fibers)	30% H_2O_2 digestion at 65 °C for 72 hr; filtered onto polycarbonate filters (100-µm pore size)	Visual-stereomicroscope; FTIR or SEM-EDS on subset of particles	Taihu Lake, China	Pooled 2–20 individuals per replicate depending on the season Missing from QA/QC: 3, 6, 10, 11, 12, 13	Su et al. (2016)
	Range: 0.4–5.0 MP·ind^{-1} 0.3–4.9 items·g ww^{-1}	0.021–4.02 mm; majority: 0.25–1.0 mm	30% H_2O_2 digestion at 65 °C for maximum of 72 hr; filtered onto a 20-µm nylon filter	Visual-microscope; FTIR on subset of particles	Taihu Lake, China	Fibers most dominant; also found in blanks and not removed from analysis; filtered water and sediment through nylon net filters; digestion solutions not filtered; claimed freshwater bioindicator for MP, 2–4 clams per replicate digestion, three replicates per site Missing from QA/QC: 3, 6, 9, 10, 11, 12	Su et al. (2018)
	1404 MP total from 9 samples Range: 0–24 particles·ind^{-1} Mean: 6.40 particles·ind^{-1}	<5 mm	10% KOH digestion at 60 °C for 12–24 hr; filtered onto polycarbonate membrane filters (10-µm pore size)	Visual-stereomicroscope; FTIR on subset of particles	River Thames, England	Wet wipes pollution Missing from QA/QC: 1, 2, 5, 6, 10, 11	McCoy et al. (2020)
	Mean: 0.3 ± 0.2 MP·ind^{-1} (resident clams)	<5 mm fibers most dominant	20% KOH digestion at room temperature for 14 d; sieved through a stainless-steel sieve (125-µm pore size) and then filtered onto a polycarbonate filter (10-µm pore size)	Visual-dissecting microscope; Raman on 25% of all particles, first 10 particles of each microplastic category (by color and shape)	San Francisco Bay, California, USA	2–3 composite samples per site; each composite sample was composed of 4 -10 individual bivalves; let animals depurate before digestion (not representative) Missing from QA/QC: 3, 4, 5, 6, 10, 11	Klasios et al. (2021)
Corbicula japonica (basket clam)	Microplastics found in 10% of bivalves sampled; one microplastic piece in each of three specimens	300 µm–5 mm; mean size: 760 ± 420 µm	30% H_2O_2 digestion until completely dissolved; sieved through a 300-µm mesh sieve	Visual-stereomicroscope; FTIR	Osaka Bay, Japan	No mention of QA/QC measures	Nakao et al. (2020)

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Table 1. Continued.

Species	Microplastic concentration measured in tissue	Particle size	Isolation method	Detection method	Location	Criticisms	Reference
Crassostrea angulata (Portuguese oyster)	Mean: 0.62 items·g ww^{-1} 2.93 items·ind^{-1}	20.34–4807.22 µm majority: <500 µm	10% KOH and 30% H_2O_2 digestion at 60 °C for 48 hr; filtered onto a glass microfiber filter (GF/B; 1-µm pore size)	Visual-stereomicroscope; FTIR	China	Pooled samples; did not distinguish between species Missing from QA/QC: 3, 4, 6, 10, 11, 12	Teng et al. (2019)
	Mean: 38.4 ± 5.9 items·5 ind^{-1} (calculated from Table 1)	Over half were <100 µm	30% H_2O_2 digestion at 65 °C for 24 hr or longer; NaCl density separation overnight; filtered onto a mixed cellulose ester membrane filter (5-µm pore size)	Visual-microscope; µ-Raman on subset of particles	Taiwan	Five animals per replicate Missing from QA/QC: 3, 6, 10, 11, 12	Liao et al. (2021)
Crassostrea gasar (mangrove oyster)	Mean: 9.6 items·150 mg·hepatopancreas^{-1}	0.03–5.0 mm	10% KOH digestion at 40 °C for 48 hr; NaCl density separation for 15 min; filtered onto cellulose nitrate membranes (8-µm pore size)	Visual-stereomicroscope; SEM-EDS	Paranaguá estuarine system, Brazil	Pooled hepatopancreas dissected from multiple oysters Missing from QA/QC: 2, 3, 4, 6, 8, 10, 11, 13	Vieira et al. (2021)
Crassostrea gigas (Pacific oyster)	2 particles·ind^{-1} Mean: 0.47 ± 0.16 particles·g ww^{-1} (without depuration) 0.35 ± 0.05 particles·g ww^{-1} (3-day depuration)	11–20 µm	69% HNO_3 digestion overnight and then 2 hr of boiling in 80 °C filtered deionized water; filtered onto cellulose nitrate membrane filter (5-µm pore size)	Visual-microscope; µ-Raman on a subset of particles	Brittany, France	Acid digestion is known to be destructive to plastic polymers; also boiled digestate, further destroying plastics Missing from QA/QC: 3, 5, 6, 8, 10, 11	Van Cauwenberghe and Janssen (2014)
	Mean: 0.6 ± 0.9 fibers·ind^{-1}	5.5 mm long 0.02–0.05 mm wide	10% KOH digestion at 60 °C overnight; collected down to 0.5 mm	Visual-dissecting microscope	California, USA	Debris over 0.5 mm because of no spectroscopy access, only claiming anthropogenic - not specified as plastic Missing from QA/QC: 3, 8, 10, 11, 12, 13	Rochman et al. (2015)
	Total MP at two sites: 87 and 30 particles·g dw^{-1}	Majority: <300 µm	45-minute HNO_3 and microwave destruction protocol followed by neutralization with NaOH and 30% H_2O_2; filtered onto glass fiber filters (0.7-µm pore size)	Visual-light microscope; FTIR on subset of particles	Dutch coast	Acid digestion is known to be destructive to plastic polymers Missing from QA/QC: 3, 4, 6, 9, 10, 11, 12	Leslie et al. (2017)
	Mean: 0.11 MP·g dw^{-1}	Mean fragment size: 60.01 ± 38 µm Mean filament size: 530 ± 470 µm	30% H_2O_2 digestion at 60 °C; NaCl density separation; filtered onto cellulose nitrate membrane filter (5-µm pore size)	Visual-epifluorescence reversed microscopy	Gulf of La Spezia, Italy	Missing from QA/QC: 3, 4, 6, 10, 11, 13	Bonello et al. (2018)
	Mean: 77.12 ± 126.0 particles·g ww^{-1} (wild) 212.80 ± 153.80 particles·g ww^{-1} (farmed)	10 µm–5 mm	68–70% HNO_3 digestion at room temperature overnight; boiled for 2 hr (100 °C); filtered onto cellulose paper (2.5-µm pore size)	Visual-microscope; FTIR on subset of particles	Vancouver Island	2–5 individuals per replicate Missing from QA/QC: 3, 5, 6, 10, 11	Murphy (2018)

Crassostrea gigas (Pacific oyster)	Mean: 0.18 ± 0.16 MP·g ww^{-1} 2.1 ± 1.71 MP·$oyster^{-1}$	50–100 µm	10% KOH at 60 °C for 24 hr; separatory funnel for density separation and a KI addition; filtered onto a cellulose nitrate filter (12-µm pore size, 25 mm diameter)	FTIR	Pen-Be and Aiguillon Bay, France	Stored samples in plastic bags (ran blanks and FTIR for the bags) Missing from QA/QC: 3, 4, 5, 6, 11, 12	Phuong et al. (2018b)
	Mean: 1482.82 ± 19.20 items·kg ww^{-1}	0.05–5 mm in length	10% KOH digestion at 65 °C for 24 hr; then at room temperature for another 24 hr; NaCl density separation; filtered onto a 1-µm filter	Visual-stereomicroscope; FTIR on 30 particles	Tunisia	Each individual treated as a replicate Missing from QA/QC: 3, 6, 9, 10, 11, 12	Abidli et al. (2019)
	Mean: 0.15 ± 0.20 n·g ww^{-1} 0.97 ± 0.74 n·ind^{-1}	Majority: <300 µm	10% KOH digestion at 60 °C overnight; sieved through 20-µm sieve; lithium meta-tungstate density separation overnight; filtered onto polycarbonate membrane filters (20- µm pore size)	Visual-microscope; FTIR	South Korea	Pooled five individuals per sample Missing from QA/QC: 6, 11	Cho et al. (2019)
	310 potential microplastics found in all oysters Before outplant (mean): 0.02 ± 0.03 MP·g dw^{-1} After outplant (mean): 0.22 ± 0.28 MP·ind^{-1} 0.04 ± 0.06 MP·g dw^{-1}	10–5000 µm particles (largest dimension measured); primarily 100–499 µm; fibers most dominant	Dried tissue digested in 10% KOH at 60 °C for 24 hr; filtered onto polycarbonate membrane filters (8-µm pore size)	Visual-compound microscope; FTIR on subset of particles	Vancouver Island, Canada	Missing from QA/QC: 3, 5, 6, 10, 11	Covernton et al. (2019)
	Fibers: 4.2 MP·ind^{-1} Fragments: 2 MP·ind^{-1} Beads: 0.5 MP·ind^{-1} HNO_3 digestion: 7 MP·ind^{-1} H_2O_2 digestion: 2 MP·ind^{-1}	Water sample size range: 0.17–5 mm Mean size: Fibers: 2.3 ± 0.87 mm Fragments: 1.6 ± 0.97 mm Pellets: 0.3 ± 0.2 mm Beads: 0.24 ± 0.1 mm	22.5 M HNO_3 digestion at room temperature for 12 hr; boiled at 100 °C for 30 min; diluted 1:10 with warm deionized water and filtered; 30% H_2O_2 digestion at 55 °C for 7 d; filtered sample	Visual-stereomicroscope and optical microscope	Bahía Blanca Estuary, Argentina	Methods are not descriptive enough to replicate; did two digestions on each oyster gut – appears tissue from individual oysters were split for two independent analyses; did not specify filters for molluscan tissue Missing from QA/QC: 2, 4, 6, 9, 10, 12, 13	Fernández Severini et al. (2019)
	Mean: 0.62 items·g ww^{-1} 2.93 items·ind^{-1}	20.34–4807.22 µm majority <500 µm	10% KOH and 30% H_2O_2 digestion at 60 °C for 48 hr; filtered onto a glass microfiber filter (GF/B; 1-µm pore size)	Visual-stereomicroscope; FTIR	China	Pooled samples; did not distinguish between species Missing from QA/QC: 3, 4, 6, 10, 11, 12	Teng et al. (2019)

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Table 1. Continued.

Species	Microplastic concentration measured in tissue	Particle size	Isolation method	Detection method	Location	Criticisms	Reference
Crassostrea gigas (Pacific oyster)	Whole oyster (mean) 10.95 ± 0.77 MP·ind^{-1} 0.35 ± 0.04 MP·g ww^{-1}	0.063–8.72 mm	Separated gut and non-gut tissue; 10% KOH digestion for 24 hr; 63-µm sieved and retained on sieve was dried in a Petri dish at 40 °C for 24 hr; second 10% KOH digestion and NaCL density separation	Visual-stereomicroscope; FTIR on subset (10%) of suspected microfibers	Oregon, USA	Contamination quantified, but not subtracted Missing from QA/AC: 4, 6, 10, 11	Baechler et al. (2020a)
	Mean: Qingdao: 4.1 ± 2.6 items·ind^{-1} Xiamen: 6.0 ± 10.5 items·ind^{-1} Mean MP among all six species: Qingdao: 0.8–4.4 items·g ww^{-1} 1.2–4.1 items·ind^{-1} Xiamen: 2.1–4.0 items·g ww^{-1} 1.3–6.0 items·ind^{-1}	Most abundant size range: 100–200 µm Qingdao: 10–4377 µm Xiamen: 58–5000 µm	10% KOH digestion at 60 °C for more than 24 hr; filtered the sample	Visual-stereomicroscope; FTIR	Qingdao and Xiamen, China	Did not report what filter pore size was used; measured MP in six species (figure 2), but only discussed highest MP abundance in two species Missing from QA/QC: 3, 6, 10, 11, 12	Ding et al. (2020)
	Mean: 1.13 ± 0.84 particles·g ww^{-1}	<1000 µm; most common size: 100–200 µm	Enzymatic digestion (lipase and protease) at 60 °C overnight; sieved through a 20-µm sieve; density separation overnight with NaCl; filtered onto filter paper (20-µm pore size; polycarbonate)	FTIR on all particles ≤50 µm	South Korea	Missing from QA/QC: 3, 6, 11, 12	Jang et al. (2020)
	Mean: 1.75 particles·oyster^{-1}	Mean microfiber length: 621.93 µm; fibers most dominant	30% H_2O_2 digestion at 65 °C for 24–48 hr; density separation with 25% saline solution; 2 hr later filtered onto nitrate cellulose membrane (5-µm pore size)	Visual-dissecting microscope; FTIR and Raman	Puget Sound, Washington, USA	Missing from QA/QC: 3, 4, 6, 10, 11, 12	Martinelli et al. (2020)
	Total concentration in oysters: 0.1 mg·g ww^{-1}	N/A-- Can't quantify size with Py-GC/MS	10% KOH digestion at 60 °C for 24 hr; filtered onto glass fiber filters (GF/D, 2.7-µm pore size)	Py-GC/MS	Australia	Missing from QA/QC: 3, 12	Ribeiro et al. (2020)

Crassostrea gigas (Pacific oyster)	4.53 items·g ww^{-1} 24.49 items·g dw^{-1} Freeze dried tissues: 35.6 items·g dw^{-1}, 7.12 items·g ww^{-1} Digestive gland: 3.31 items·g ww^{-1} Muscles: 4.05 items·g ww^{-1} Gill and Mantle: 9.5 items·g ww^{-1} (estimated from figure 1 in study, 56.06% of MP)	Fragments <0.2 mm; fiber size not mentioned	30% H_2O_2 digestion; filtered onto glass fiber filters (GF/B, 1-µm pore size, 47-mm diameter); after 24 hr at room temperature the digestate was diluted with a saturated NaCl solution and filtered over GF/B filters; after another 12 hr, the solution was filtered onto a cellulose ester membrane (5-µm pore size, 47-mm diameter)	Visual-stereomicroscope; FTIR on subset of particles; SEM-EDX for surface characteristics of 9 representative microplastics	Yantai, China	Isolated "3 parts": digestive gland, gills and mantle, and muscles No mention of QA/QC measures	Zhu et al. (2020)
	Mean across seasons and location: 1.2–3.3 items·ind^{-1} 0.3–3.0 items·g ww^{-1}	7 µm–5 mm	10% KOH digestion at 60 °C for up to 24 hr; filtered onto glass fiber membrane filters (GF/F, 0.7-µm pore size)	Visual-stereomicroscope; FTIR	Qingdao, China	Recommend mussels and clams as bioindicators; isolated the digestive system Missing from QA/QC: 1, 5, 6, 10, 11	Ding et al. (2021)
Crassostrea gigas/Mytilus edulis (ran as "composite" samples - pooled)	Mean: 0.33 ± 0.23 MP·g ww^{-1} 1.21 ± 0.68 MP·ind^{-1}	Longest axis size range: 46–13,298 µm; most common size: 100–200 µm	10% KOH digestion at 60 °C; sieved through 20-µm pore size sieve; lithium meta-tungstate density separation for 24 hr; filtered onto polycarbonate filter (20-µm pore size)	Manually counted and analyzed particles with FTIR	South Korea	Claim bivalves are good bioindicators; pooled five individuals per sample; pooled oysters and mussels together Missing from QA/QC: 11, 12	Cho et al. (2021)
Crassostrea hongkongensis (Hong Kong oyster)	Mean: 0.62 items·g ww^{-1} 2.93 items·ind^{-1}	20.34–4807.22 µm majority <500 µm	10% KOH and 30% H_2O_2 digestion at 60 °C for 48 hr; filtered onto a glass microfiber filter (GF/B; 1-µm pore size)	Visual-stereomicroscope; FTIR	China	Pooled samples; did not distinguish between species Missing from QA/QC: 3, 4, 6, 10, 11, 12	Teng et al. (2019)
	Mean: 4.7 ± 0.3 particles·ind^{-1} 0.8 ± 0.2 particles·g ww^{-1}	Most abundant <0.25 mm	10% KOH digestion at 40°C for 48–96 hr; filtered onto a filter membrane (5-µm pore size)	Visual-stereomicroscope; FTIR	Maowei Sea, China	Missing from QA/QC: 3, 6, 9, 10, 11, 12	Zhu et al. (2019)
(Pers. comm. Authors did not specify in publication)	Year-long study at different sites Digestive gland (mean): 2.84 ± 0.44 particles·g ww^{-1} Gills (mean): 7.05 ± 1.21 particles·g^{-1} ww Other tissues (mean): 0.59 ± 0.08 particles·g ww^{-1}	>5 µm	10% KOH digestion at 40 °C with constant agitation for 48–72 hr; filtered onto a nylon membrane (5-µm pore size, 47-mm diameter)	Visual-stereomicroscope; analyzed a subset of fibers and analyzed all other particles using FTIR	Maowei Sea, China	Pooled oyster samples; did not mention the species examined Missing from QA/QC: 6, 10, 11, 12	Zhu et al. (2021)
Crassostrea sikamea (Kumomoto oyster)	Mean: 0.62 items·g ww^{-1} 2.93 items·ind^{-1}	20.34–4807.22 µm majority <500 µm	10% KOH and 30% H_2O_2 digestion at 60 °C for 48 hr; filtered onto a glass microfiber filter (GF/B; 1-µm pore size)	Visual-stereomicroscope; FTIR	China	Pooled samples; did not distinguish between species Missing from QA/QC: 3, 4, 6, 10, 11, 12	Teng et al. (2019)
Crassostrea virginica (eastern oyster)	Mean: 16.5 MP·ind^{-1} 3.84 ± 3.39 MP·g ww^{-1}	Claims can identify down to 0.001 mm	30% H_2O_2 digestion at 65 °C for 24 hr and then at 25 °C for 24–48 hr; filtered onto a nitrocellulose membrane filter (0.45-µm pore size)	Visual-dissecting microscope; hot needle test	Florida, USA	Fibers found in their samples are the same color as their recovery test Missing from QA/QC: 1, 2, 3, 5, 6, 9, 11, 12, 13	Waite et al. (2018)

(*Continued*)

Table 1. Continued.

Species	Microplastic concentration measured in tissue	Particle size	Isolation method	Detection method	Location	Criticisms	Reference
Crassostrea virginica (eastern oyster)	Mean: 0.72 MP·ind^{-1} 0.18 MP·g ww^{-1}	Mean fiber length: 1.64 ± 1.28 mm Mean fragment length: 0.405 ± 0.521 mm	10% KOH digestion at 40 °C for 72 hr; sieved through a 35-µm sieve; 4.4 M NaI density separation in centrifuge for 5 min; filtered onto a Whatman No. 540 filter paper	Visual-dissecting microscope; hot needle test	Georgia, USA	Pooled oysters to provide 14 g samples at each tide level; built a "ventilation chamber" for work area made of plastic and did not account for the plastic used in their QA/QC Missing from QA/QC: 1, 3, 4, 6, 10, 11, 12, 13	Keisling et al. (2020)
Cyclina sinensis (Chinese venus)	Mean: 4.82 ± 2.17 MP·ind^{-1} (estimated from figure 3 in study)	5 µm–5 mm; majority >250 µm	30% H_2O_2 digestion at 65 °C for 24 hr and then at room temperature for 24–48 hr (if needed); NaCl density separation; filtered onto cellulose nitrate membrane filter (5-µm pore size, 47-mm diameter)	Visual-stereoscope; FTIR on subset of particles	China	1–5 individuals pooled as one replicate; density of NaCl solution not defined; tools not listed as glass (e.g., petri dish) Missing from QA/QC: 3, 6, 9, 10, 11, 12	Li et al. (2015)
Cypraea tigris (tiger cowrie)	Mean: 0.3 MP·ind^{-1}	<5 mm	10% KOH digestion at 60°C overnight; poured into a petri dish	Visual-stereomicroscope	Makassar Strait, Indonesia	No mention of QA/QC measures	Tahir et al. (2019)
Cyamiocardium denticulatum	Mean: 0.75 MP·ind^{-1} (estimated from figure 5 in study)	33–5000 µm; highest relative abundance: 50–100 µm	Nile Red digestions: Stored in ethanol Dried at 80 °C for 1 hr; 1% NaOH digestion at 40 °C for 24 hr; NaCL density separation; filtered onto glass fiber filters (GF/F, 0.7-µm pore size) For FTIR analysis: 30% H_2O_2 digestion at room temperature for 72 hr; filtered onto ANODISCS (0.2-µm pore size, 47-mm diameter)	Visual-stereomicroscope; Nile Red staining; FTIR on most contaminated species	Terra Nova Bay (Ross Sea), Antarctica	Pooled individuals Missing from QA/QC: 3, 6, 11	Sfriso et al. (2020)
Donax cuneatus (Cuneate wedge shell)	Range MP abundance in body of clam: 0.29–2.7 items·ind^{-1} 0.6–1.3 MP·g ww^{-1}	Fibers majority: 100–250 µm	Visual removal of MP on exterior of soft tissues prior to digestion; 10% KOH digestion at 40°C for 72 hr; filtered onto filter paper; NaI density separation at room temperature for 24 hr; filtered onto cellulose nitrate filter paper (0.8-µm pore size)	Visual-stereomicroscope; hot needle test; FTIR on MP >0.5 mm; SEM-EDAX for MP surface morphology	Gulf of Mannar, India	Missing from QA/QC: 3, 6, 11, 13	Sathish et al. (2020)

Donax spp. (wedge shell)	Mean: 2.89 ± 0.54 MP·g^{-1} ww	93.3% were fibers Mean length: 1004.2 ± 464.8 µm Mean diameter: 21.8 ± 7.3 µm	10% KOH digestion at 40 °C for 48 hr; sieved through a 38-µm sieve; filtered onto a cellulose ester filter (0.45-µm pore size)	Visual-stereomicroscope; FTIR on a subset of particles	Hong Kong, China	Low percent recovery for gastropods (50%) because of shell debris on filter not removed for digestion; some discussed data does not match table data; species listed in figures 4 and 5 differ; mislabeled gastropods as crabs Missing from QA/QC: 3, 4, 6, 11, 12	Xu et al. (2020)
Dosidicus gigas (Jumbo squid)	Gill (mean): 5.42 ± 5.96 MP·ind^{-1} 0.20 ± 0.24 MP·g dw^{-1} Intestine (mean): 3.96 ± 3.62 MP·ind^{-1} 0.74 ± 0.77 MP·g dw^{-1} Stomach (mean): 7.42 ± 4.88 MP·ind^{-1} 0.30 ± 0.24 MP·g dw^{-1}	80.75–4632.27 µm	10% KOH digestion at 60 °C for 24 hr; filtered onto a glass fiber filter (2.7-µm pore size, 47-mm diameter)	Visual-stereomicroscope; FTIR on a subset of particles	Northern Humbolt Current, Western South America	Say they applied strict contamination prevention measures, but not listed Missing from QA/QC: 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12	Gong et al. (2021)
Dosinia isocardia	1.5 ± 1.96 microfibers·ind^{-1} 286.3 ± 280.0 microfibers in relation to biomass (ash free dry weight)	>5 mm Mean microfiber length: 2.37 ± 2.27 mm Mean microfiber section: 20 ± 8 µm	Protease enzyme digestion (neutrase) at 40 °C for 3–6 hr	Visual-stereomicroscope; FTIR on a subset of particles	Tejo Estuary Portugal Banc d' Arguin Mauritania, Bijagos archipelago, Guinea-Bissau	Missing from QA/QC: 1, 2, 4, 5, 6, 9, 10, 12	Lourenço et al. (2017)
Dreissena bugensis (quagga mussel)	No microbeads found in any animals	N/A	N/A	Visual-dissecting scope of dissected soft tissues	St. Lawrence River, New York, USA	Animals were 10.59–22.9 mm so authors believe the mussels were too small for beads >35 µm; transport in plastic bags and left animals in buckets of water for 48 hr before dissections No mention of QA/QC measures	Schessl et al. (2019)
Dreissena polymorpha (zebra mussel)	No microbeads found in any animals	N/A	N/A	Visual-dissecting scope of dissected soft tissues	St. Lawrence River, New York, USA	Animals were 10.59–22.9 mm so authors believe the mussels were too small for beads >35 µm; transported in plastic bags and left animals in buckets of water for 48 hr before dissections No mention of QA/QC measures	Schessl et al. (2019)
Eatoniella sp.	Mean: 1.1 MP·ind^{-1} (estimated from figure 5 in study)	33–5000 µm; highest relative abundance: 50–100 µm	Nile Red digestions: Stored in EtOH; dried at 80°C for 1 hr; 1% NaOH digestion at 40°C for 24 hr; NaCL density separation; filtered onto glass fiber filters (GF/F, 0.7-µm pore size) For FTIR analysis: 30% H_2O_2 digestion at room temperature for 72 hr; filtered onto ANODISCS (0.2-µm pore size, 47-mm diameter)	Visual-stereomicroscope; Nile Red staining; FTIR on most contaminated species	Terra Nova Bay (Ross Sea), Antarctica	Pooled individuals Missing from QA/QC: 3, 6, 11	Sfriso et al. (2020)

(*Continued*)

Table 1. Continued.

Species	Microplastic concentration measured in tissue	Particle size	Isolation method	Detection method	Location	Criticisms	Reference
Ellobium chinense	Mean total content: 95.6 ± 5.0 items·kg ww^{-1} with and without shells Range: 38 ± 2 to 320 ± 3 items·kg ww^{-1}	Dominant size range: 500–1000 µm	10% KOH digestion; filtered onto glass fiber filters (1.2-µm pore size)	Visual-stereomicroscope; µ-Raman	Beibu Gulf, China	Muffled filters used but did not mention equipment; digested shell Missing from QA/QC: 3, 4, 9, 11, 12	Li et al. (2020)
Ennucula tenuis (smooth nutclam)	Range: 1–7 MP·organism^{-1}	41 µm–9 mm plastics found across different species sampled in this study	10% KOH digestion at 50 °C overnight; NaCl density separation with vacuum filtration onto a cellulose membrane filter (8-µm pore size)	Visual-stereomicroscope; FTIR	Oslofjord, Norway	Missing from QA/QC: 3, 6, 11, 12	Bour et al. (2018a)
Gafrarium spp.	Mean: 0.26 ± 0.08 MP·g^{-1} ww	93.3% were fibers Mean length: 1004.2 ± 464.8 µm Mean diameter: 21.8 ± 7.3 µm	10% KOH digestion at 40 °C for 48 hr; sieved through a 38-µm sieve; filtered onto a cellulose ester filter (0.45-µm pore size)	Visual-stereomicroscope; FTIR on a subset of particles	Hong Kong, China	Low percent recovery for gastropods (50%) because of shell debris on filter not removed for digestion; some discussed data does not match table data; species listed in figures 4 and 5 differ; mislabeled gastropods as crabs Missing from QA/QC: 3, 4, 6, 11, 12	Xu et al. (2020)
Hexaplex (*Murex*) (banded dye-murex)	Mean: 703.95 ± 109.80 items·kg ww^{-1}	0.05–5 mm in length	10% KOH digestion at 65 °C for 24 hr; then at room temperature for another 24 hr; NaCl density separation; filtered onto a 1-µm filter	Visual-stereomicroscope; FTIR on 30 particles	Tunisia	Each individual treated as a replicate Missing from QA/QC 3, 6, 9, 10, 11, 12	Abidli et al. (2019)
Hiatella arctica (wrinkled rock-borer, arctic hiatella, or arctic saxicave)	Mean at 27 m depth: 8.1 ± 1.8 MP·ind^{-1} Mean at 40 m depth: 112.8 ± 33.4 MP·ind^{-1}	10–5000 µm	10% SDS solution at 50 °C for 6 d; enzyme digestion at ≤40 °C (protease, lipase, cellulase, chitinase) takes ~8 d; 30% H_2O_2 digestion at 37°C for 24 hr; after chitinase digestion, 30% H_2O_2 digestion with Fe (II) catalyst below 60°C until end of reaction; filtered onto stainless steel filter (10-µm pore size) and then wet sieved with stainless steel sieve (500-µm pore size; <500 and >500 separated); ZnCl density separation for <500 µm particles; Filtered onto anodisc filter (0.2 µm-pore size)	Visual-stereomicroscope; FTIR	Northern Svalbard, Arctic	Missing from QA/QC: 3, 10, 11	Teichert et al. (2021)

Laevistrombus turturella (Gonggong snail)	Madong Village (mean): 492 ± 107.68 MP·ind ww^{-1} Pengudang Village (mean): 476 ± 171.34 MP·ind ww^{-1} Busung Village (mean): 360 ± 118.43 MP·ind ww^{-1} Kawal Village (mean): 628 ± 191.93 MP·ind ww^{-1}	1 µm–5 mm	65% HNO_3 digestion for 24 hr; diluted	Visual-light microscope analysis of diluted samples	Bintan Island, Indonesia	Confusing methods description No mention of QA/QC measures	Al Hamra and Patria (2019)
Lasmigona costata (fluted-shell mussels)	Mean range: 0–0.16 MP·g ww^{-1} 0–7 MP·ind^{-1}	21–298 µm Mean size: 114 ± 63 µm	Protease enzyme digestion at 37 °C for 16 hr; filtered onto Nitex® screens (250, 100, 50 µm pore sizes)	Visual-stereomicroscope with fluorescence; picked particles onto silicon wafer for analysis; Raman	Grand River watershed, Ontario, Canada	Missing from QA/QC: 3, 4, 6, 10, 11	Wardlaw and Prosser (2020)
Leukoma asperrima (Delesserti venus clam)	12.2 ± 11.5 MP·ind^{-1} 4.9 ± 5.3 MP·g ww^{-1} Mean across six species: 4.8 ± 8.0 MP·ind^{-1} 2.1 ± 3.3 MP·g ww^{-1}	0.1–5 mm	10% KOH digestion for 48 hr; filtered onto a glass fiber filter (GF/C, 1.2-µm pore size)	Visual-stereomicroscope; FTIR on a random subsample of 50 particles	Golgo de Nicoya, Costa Rica	Missing from QA/QC: 1, 5, 6, 8, 9, 10,	Rojas-Jimenez et al. (2022)
Leukoma ecuadoriana (venerid clam)	18 ± 10.5 MP·ind^{-1} 5.5 ± 3.2 MP·g ww^{-1} Mean across six species: 4.8 ± 8.0 MP·ind^{-1} 2.1 ± 3.3 MP·g ww^{-1}	0.1–5 mm	10% KOH digestion for 48 hr; filtered onto a glass fiber filter (GF/C, 1.2-µm pore size)	Visual-stereomicroscope; FTIR on a random subsample of 50 particles	Golgo de Nicoya, Costa Rica	Missing from QA/QC: 1, 5, 6, 8, 9, 10,	Rojas-Jimenez et al. (2022)
Limnoperna fortune (golden mussel)	Mean: 2.08 ± 1.33 MP·g ww^{-1} 0.43 ± 0.35 MP·ind^{-1}	>500 µm; most frequent size ≤1000 µm	30% H_2O_2 digestion until fully dissolved at 65 °C; filtered onto cellulose nitrate filter (0.45-µm pore size)	Visual-light microscope	Río de la Plata estuary, Argentina	Pooled 10 mussels per replicate Missing from QA/QC: 1, 3, 4, 5, 9, 10, 11, 13	Pazos et al. (2020)
Littorina littorea (common periwinkle)	Mean: 2.14 MP·g ww^{-1}	Fiber lengths: 60–15,585 µm Investigated particles from 1–5000 µm	10% KOH digestion at room temperature for 24 hr; filtered onto a glass fiber filter (GF/C)	Visual-stereo microscope; FTIR on subset of particles	Galway Bay, Ireland	Missing from QA/QC: 2, 4, 6, 8, 9, 10, 12	Doyle et al. (2019)
Littoraria scabra (mangrove periwinkle)	Mean: 86.88 MP·g body weight 75.5 MP·ind^{-1}	1–5 mm	65% HNO_3 digestion for 24 hr; NaCl density separation	Visual-microscope	Pramuka Island, Jakarta Bay, Indonesia	Unclear methodology; appears to be wet weight, but not specified No QA/QC mentioned except the use of aluminum foil to cover glass beakers during digestion	Patria et al. (2020)
Littoraria sp. (periwinkle)	Angsila (mean): 0.23 ± 0.02 particles·g ww^{-1} Bangsean (mean): no particles found Samaesarn (mean): 0.17 ± 0.08 particles·g ww^{-1}	<5 mm	69% HNO_3 digestion at room temperature overnight; boiled for 2 hr (100 °C); diluted with 80 °C deionized water; vacuum filtered onto cellulose nitrate membrane (5-µm pore size)	Visual-stereomicroscope; Raman	Thailand	Pooled individuals before analysis Missing from QA/QC: 3, 4, 6, 9, 10, 11, 12	Thushari et al. (2017)

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Table 1. Continued.

Species	Microplastic concentration measured in tissue	Particle size	Isolation method	Detection method	Location	Criticisms	Reference
Lunella coronate (crowned turban shell or coronate moon turban)	Mean: 3.0 ± 0.4 MP·g^{-1} ww (estimated from figure 4 in study)	93.3% were fibers Mean length: 1004.2 ± 464.8 µm Mean diameter: 21.8 ± 7.3 µm	10% KOH digestion at 40 °C for 48 hr; sieved through a 38-µm sieve; filtered onto a cellulose ester filter (0.45-µm pore size)	Visual-stereomicroscope; FTIR on a subset of particles	Hong Kong, China	Low percent recovery for gastropods (50%) because of shell debris on filter not removed for digestion; some discussed data does not match table data; species listed in figures 4 and 5 differ; mislabeled gastropods as crabs Missing from QA/QC: 3, 4, 6, 11, 12	Xu et al. (2020)
Magallana bilineata (Indian edible oyster)	Mean: 6.9 ± 3.84 items·ind^{-1} 0.81 ± 0.45 items·g ww^{-1}	<5 mm; most common size: 0.25–0.5 mm	10% KOH digestion at 50 °C for 72 hr; NaI density separation for 24 hr; filtered onto nitrate cellulose filter papers (0.8-µm pore size)	Visual-stereomicroscope; hot needle test; FTIR on 60 particles; subset analyzed with SEM-EDAX for surface morphology	Gulf of Mannar, India	Pooled five individuals per replicate Missing from QA/QC: 3, 4, 6, 10, 11, 12, 13	Patterson et al. (2019)
	Mean: 15 MP·ind (estimated from figure 2 in study, not mentioned in text) Overall mean of three species: 15.9 items·g tissue	Majority <2 mm; largest were 4 mm	Incubation at 50 °C for 15 min; proteinase-K enzyme digestion 50 °C for 2 hr; deproteinized at 60 °C for 20 min; filtered onto glass fiber filters (1.2-µm pore size)	Visual-stereomicroscope; µ-Raman	Southwest coast of India	Claim microplastics "in" the gills; axis labels of figure 2 do not line up; not clear from methods what was sampled Missing from QA/QC: 3, 4, 10, 11, 12	Joshy et al. (2022)
Magallana (*Crassostrea*) *gigas* (Pacific oyster)	Mean: 0.07 ± 0.03 MP·g ww^{-1}	>75% of fibers; >250 µm length; all fragments <500 µm	Mixture of 30% KOH and 30% H_2O_2 digestion at 40 °C for at least 72 hr; filtered onto 4.7-cm filter (GF/D)	Visual-stereomicroscope; FTIR on subset of particles; hot needle test	Baja California, Mexico	Included gonad into dissected "digestive system," but gonad enmeshed in mantle, not a separate organ Missing from QA/QC: 10, 11	Lozano-Hérnandez et al. (2021)
Marcia spp.	Mean: 0.25 ± 0.05 MP·g^{-1} ww (estimated from figure 4 in study)	93.3% were fibers Mean length: 1004.2 ± 464.8 µm Mean diameter: 21.8 ± 7.3 µm	10% KOH digestion at 40 °C for 48 hr; sieved through a 38-µm sieve; filtered onto a cellulose ester filter (0.45-µm pore size)	Visual-stereomicroscope; FTIR on a subset of particles	Hong Kong, China	Low percent recovery for gastropods (50%) because of shell debris on filter not removed for digestion; some discussed data does not match table data; species listed in figures 4 and 5 differ; mislabeled gastropods as crabs Missing from QA/QC: 3, 4, 6, 11, 12	Xu et al. (2020)
Meretrix lusoria (Asian hard clam)	Mean: 9 MP·ind^{-1} (estimated from figure 3 in study)	5 µm–5 mm; majority >250 µm	30% H_2O_2 digestion at 65 °C for 24 hr; then, at room temperature for 24–48 hr (if needed); NaCl density separation; filtered onto cellulose nitrate membrane filter (5-µm pore size, 47-mm diameter)	Visual-stereoscope; FTIR on subset of particles	China	1–5 individuals were pooled as one replicate; density of NaCl solution not defined; tools not listed as glass (e.g., petri dish) Missing from QA/QC: 3, 6, 9, 10, 11, 12	Li et al. (2015)
Meretrix lyrata (lyrate Asiatic hard clam)	Mean: 0.35 ± 0.08 particles·g ww^{-1} (before three-day depuration) 0.23 ± 0.07 particles·g ww^{-1} (after three-day depuration)	5–1000 µm; most common 5–20 µm	Digested - does not say how	Not specified for clams, but identified potential MP in sediment under the microscope	Carey Island, Selangor, and west coast Peninsular, Malaysia	Methods are unclear No mention of QA/QC measures	Hamid et al. (2020)

Meretrix lyrata (lyrate Asiatic hard clam)	Small clams (mean): 0.67 ± 0.15 items·ind^{-1} 0.28 ± 0.06 items·g ww^{-1} Large clams (mean): 0.23 ± 0.09 items·ind^{-1} 0.03 ± 0.01 items·g ww^{-1}	<5 mm	30% H_2O_2 digestion at 60 °C for 48 hr; then, at room temperature for 48 hr; NaCl density separation left for 24 hr; filtered onto cellulose nitrate membrane filter (5-µm pore size, 47-mm diameter)	Visual-stereomicroscope; hot needle test; FTIR on subset of particles	Tapi-Phumduang River and Bandon Bay, Thailand	Report a MP recovery rate of 100% but do not describe in methods Missing from QA/QC: 6, 10, 11, 12	Chinfak et al. (2021)
Meretrix meretrix (great clam)	Mean: 0.4 MP·g ww^{-1} (estimated from figure 1b in study)	0.1–16.4 mm Bivalves contained more 0.1–1 mm than fish examined	10% KOH digestion at 60 °C for 6 hr; NaCl density separation overnight; filtered onto a cellulose nitrate filter (GF/A, 1.6-µm pore size)	Visual-stereomicroscope; Raman	Fujian Province, China	Discussed only pooled sample data Missing from QA/QC: 3, 6, 11, 12	Fang et al. (2019)
	Panithittu Estuary (mean): 1.84 ± 0.61 MP·g ww^{-1} 1.75 ± 0.35 MP·ind^{-1} Chunnambar Estuary (mean): 1.76 ± 0.48 MP·g ww^{-1} 4.80 ± 1.39 MP·ind^{-1}	61.02% of particles <100 µm	10% KOH digestion for 72 hr at 40 °C, added more KOH and digested an additional 24 hr if tissue remained; filtered onto Whatman filter papers (11-µm pore size)	Nile Red staining; visual-fluorescent microscope; Raman on subset of particles	Pondicherry, India	Pooled 10 individuals per replicate Missing from QA/QC: 1, 3, 4, 5, 6, 10, 11, 12	Dowarah et al. (2020)
Modiolus modiolus (northern horsemussel)	Mean: 0.086 ± 0.031 particles·g ww^{-1} 3.5 ± 1.29 particles·ind^{-1}	From validation: 0.2–2 mm fibers with 0.01–0.05 mm widths	Corolase 7089 enzyme digestion at 60 °C overnight; filtered onto cellulose membrane filter (0.8-µm pore size)	Visual-dissecting microscope; FTIR on subset of particles; Nile Red dye with fluorescent microscope	Scotland	Missing from QA/QC: 3, 4, 6, 10, 11, 12	Catarino et al. (2018)
Monodonta labio	Mean: 3.5 ± 0.6 MP·g^{-1} ww (estimated from figure 4 in study)	93.3% were fibers Mean length: 1004.2 ± 464.8 µm Mean diameter: 21.8 ± 7.3 µm	10% KOH digestion at 40 °C for 48 hr; sieved through a 38-µm sieve; filtered onto a cellulose ester filter (0.45-µm pore size)	Visual-stereomicroscope; FTIR on a subset of particles	Hong Kong, China	Low percent recovery for gastropods (50%) because of shell debris on filter not removed for digestion; some discussed data does not match table data; species listed in figures 4 and 5 differ; mislabeled gastropods as crabs Missing from QA/QC: 3, 4, 6, 11, 12	Xu et al. (2020)
Mussels *Authors do not specify species	Quantified mass in mussels: 3.59 mg PVC	<5 mm	1 M NaOH digestion at 50 °C for 15 min; 16 M HNO_3 digestion at 50 °C for 15 min then heated to 80 °C for 15 min; diluted and filtered onto cellulose nitrate filter (0.45-µm pore size); NaI density separation, diluted with 80 °C ultrapure water and filtered onto quartz filters (no pore size listed)	FTIR	China	Methods development study with insufficient method descriptions Missing from QA/QC: 2, 3, 4, 11, 12	Yu et al. (2019a)
Mytella strigata (charru mussel)	1.3 ± 1.2 MP·ind^{-1} 2.8 ± 3.9 MP·g ww^{-1} Mean across six species: 4.8 ± 8.0 MP·ind^{-1} 2.1 ± 3.3 MP·g ww^{-1}	0.1–5 mm	10% KOH digestion for 48 hr; filtered onto a glass fiber filter (GF/C, 1.2-µm pore size)	Visual-stereomicroscope; FTIR on a random subsample of 50 particles	Golgo de Nicoya, Costa Rica	Missing from QA/QC: 1, 5, 6, 8, 9, 10,	Rojas-Jimenez et al. (2022)

(*Continued*)

Table 1. Continued.

Species	Microplastic concentration measured in tissue	Particle size	Isolation method	Detection method	Location	Criticisms	Reference
Mytella guyanensis (mangrove ussel)	1.5 ± 1.6 MP·ind^{-1} 0.7 ± 0.7 MP·g ww^{-1} Mean across six species: 4.8 ± 8.0 MP·ind^{-1} 2.1 ± 3.3 MP·g ww^{-1}	0.1–5 mm	10% KOH digestion for 48 hr; filtered onto a glass fiber filter (GF/C, 1.2-µm pore size)	Visual-stereomicroscope; FTIR on a random subsample of 50 particles	Golgo de Nicoya, Costa Rica	Missing from QA/QC: 1, 5, 6, 8, 9, 10,	Rojas-Jimenez et al. (2022)
Mytilus californianus (California mussel)	Mean: 1.5 ± 0.8 MP·ind^{-1} (transplanted mussels)	<5 mm, fibers most dominant	20% KOH digestion at room temperature for 14 d; sieved through a stainless-steel sieve (125-µm pore size); filtered onto a polycarbonate filter (10-µm pore size)	Visual-dissecting microscope; Raman on 25% of all particles, first 10 particles of each microplastic category (by color and shape)	San Francisco Bay, California, USA	2–3 composite samples per site; each composite sample was composed of 4 -10 individual bivalves; let animals depurate before digestion (not representative) Missing from QA/QC: 3, 4, 5, 6, 10, 11	Klasios et al. (2021)
Mytilus chilensis (Chilean mussel)	Mean: 8.6 ± 3.53 items·ind^{-1}	Mean fiber length: 742.3 ± 702.1 µm	30% H_2O_2 digestion at 45°C for 48 hr; 1 hr at room temperature; at 55°C for 15 min; filtered onto filter paper (22-µm pore size)	Visual-stereomicroscope; FTIR and or Raman on a subset of suspected particles	Ushuaia Bay, Argentina	Transported to the laboratory in seawater, can still feed or egest so not a true snapshot Missing from QA/QC: 3, 4, 6, 10, 11, 12	Pérez et al. (2020)
	Processed mussels (mean): 0.82 ± 0.96 MP·5 ind^{-1}	150–500 µm most detected	30% H_2O_2 digestion for 36–48 hr; NaCl density separation; filtered onto cellulose nitrate filters (5-µm pore size)	Visual-stereomicroscope; FTIR	Sicily, Italy	Five mussels per replicate Missing from QA/QC: 6, 9, 11, 12	Nalbone et al. (2021)
Mytilus edulis (blue mussel)	Mean: 170 fibers·5 ind^{-1} (wild) 375 fibers·5 ind^{-1} (farmed)		30% H_2O_2 at 55–65 °C until H_2O_2 evaporated; NaCl density separation; filtered onto gridded nitrocellulose membrane filters (0.8-µm pore size)	Visual-dissecting microscope (2x magnification)	Newfoundland & Labrador	Incomplete digestions; high fiber contamination from air (~100 fibers per blank) Missing from QA/QC: 1, 3, 4, 5, 6, 9, 10, 11, 13	Mathalon and Hill (2014)
	Mean: 0.36 ± 0.07 particles·g ww^{-1} (without depuration) 0.24 ± 0.07 particles·g ww^{-1} (3-day depuration)	5–10 µm	69% HNO_3 digestion overnight and then 2 hr of boiling in 80 °C filtered deionized water; filtered onto cellulose nitrate membrane filter (5-µm pore size)	Visual-microscope; µ-Raman on a subset of particles	Germany	Nitric acid digestion known to be destructive to plastic polymers; also boiled digestate, further destroying plastics Missing from QA/QC: 3, 5, 6, 8, 10, 11	Van Cauwenberghe and Janssen (2014)
	Mean: 0.2 particles·g ww^{-1} tissue 0.1 particles·g^{-1}	20–90 µm (tissue) 15–500 µm (feces)	69% NHO_3, boiled for 2hr; dilution with warm (~80°C) filtered deionized water; filtered over a cellulose nitrate membrane filter (5-µm pore size)	Visual-microscope; µ-Raman on a subset of particles	French-Belgian-Dutch coastline	Allowed 24-hr depuration in seawater before dissection- at least collected feces; reported MP in feces as "tissue" Missing from QA/QC: 3, 4, 6, 7, 8, 9, 10, 11, 12	Van Cauwenberghe et al. (2015) Environ Poll
	Range: 1.5–7.6 items·ind^{-1} tissue 0.9–4.6 items·g ww^{-1} Mean: 4 items·ind^{-1} 2.2 items·g ww^{-1}	<250 µm	30% H_2O_2 at 65 °C digestion in oscillation incubator for 24–48 hr; NaCl density separation; filtered onto cellulose nitrate membrane filter (5-µm pore size, 47-mm diameter)	Visual-stereomicroscope; FTIR on subset of particles; SEM with EDS confirmation of elemental composition	China	2–5 mussels per replicate Missing from QA/QC: 1, 5, 6, 9, 11, 12, 13	Li et al. (2016)

Mytilus edulis (blue mussel)	Mean: 1.05 ± 0.66 to 4.44 ± 3.03 particles·g ww^{-1}	0.2–10.67 mm	Trypsin digestion at 38–42 °C for 30 min; filtered through 52-µm mesh gauze; visually selected particles from gauze onto 30-mm filter paper	Visual-dissecting microscope; FTIR	Clyde Estuary, Scotland	Stored mussels in freezer bags (wrapped in aluminum foil in bags) Missing from QA/QC: 2, 4, 6, 12	Courtene-Jones et al. (2017)
	Mean: 37,000 MP·kg dw^{-1}	Length: 30–2000 µm	Incubated dried/ground tissue at 60 °C in a homogenization buffer (400 mM Tris-HCl, 0.5% SDS) for 60 min; proteinase K digestion for at 50 °C for >2 hr and then at 60 °C for 20 min; 30% H_2O_2 digestion at room temperature overnight; filtered onto GF/C filters (1.2-µm pore size)	Visual-light microscope; reference microplastic pictures; hot needle test	Dutch North Sea	Did not use FTIR/Raman for mussel samples Missing from QA/QC: 3, 4, 6, 9, 11, 12, 13	Karlsson et al. (2017)
	Total MP at two sites: 105 and 19 particles·g dw^{-1}	Majority <300 µm	HNO_3 digestion for 45 min and microwave destruction protocol followed by neutralization with NaOH and 30% H_2O_2 digestion; filtered onto glass fiber filters (0.7-µm pore size)	Visual-light microscope; FTIR on a subset of particles	Dutch coast	Acid digestion is known to be destructive to plastic polymers Missing from QA/QC: 3, 4, 6, 9, 10, 11, 12	Leslie et al. (2017)
	9.2 items·g ww^{-1} (intestine)	0.05–5 mm	30% H_2O_2 digestion at 65 °C for 48 hr; NaCl density separation; filtered onto cellulose nitrate membrane filter (5-µm pore size)	Visual-stereomicroscope; FTIR	East China Sea	Six replicates of 30 mussels; fibers noted in adductor and foot most likely from lab exposure/ contamination Missing from QA/QC: 3, 6, 9, 10, 11, 12	Kolandhasamy et al. (2018)
	Mean: 0.7–2.9 items·g ww^{-1} 1.1–6.4 items·ind^{-1} (wild) 0.9 items·g ww^{-1} (supermarket live) 1.4 items·g ww^{-1} (supermarket processed)	5–250 µm; majority 8 µm–4.7 mm	30% H_2O_2 digestion at 65 °C in oscillation incubator for 24–48 hr; NaCl density separation; filtered onto cellulose nitrate membrane filter (5-µm pore size); particles on filter washed into glass beaker with 30% H_2O_2 for extra digestion before the density separation	Visual-dissecting microscope; undeterminable particles through FTIR; SEM-XRD	U.K. coastline	1–5 mussels per replicate Missing from QA/QC: 3, 6, 9, 11, 12, 13	Li et al. (2018b)
	Mean: 138 ± 202.30 particles·g ww^{-1} (wild) 259.4 ± 114.10 particles·g ww^{-1} (farmed)	10 µm–5 mm	68–70% NHO_3 digestion overnight at room temperature, boiled for 2 hr (100 °C); filtered onto cellulose paper (2.5-µm pore size)	Visual-microscope; FTIR on subset of particles	Vancouver Island	2–5 individuals per replicate Missing from QA/QC: 3, 5, 6, 10, 11	Murphy (2018)

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Table 1. Continued.

Species	Microplastic concentration measured in tissue	Particle size	Isolation method	Detection method	Location	Criticisms	Reference
Mytilus edulis (blue mussel)	Mean: 0.23 ± 0.2 MP·g ww^{-1} 0.60 ± 0.56 MP·$mussel^{-1}$	50–400 µm	10% KOH digestion at 60°C for 24 hr; separatory funnel for density separation and a KI addition; filtered onto cellulose nitrate filter (12-µm pore size, 25-mm diameter)	FTIR	Pen-Be and Aiguillon Bay, France	Stored samples in plastic bags (ran blanks and FTIR for the bags) Missing from QA/QC: 3, 4, 5, 6, 11, 12	Phuong et al. (2018b)
	Range: 1.52–5.36 MP·g ww^{-1} 0.77–8.22 MP·ind^{-1}		30% H_2O_2 digestion at 65°C for 72 hr; NaCl density separation; filtered onto cellulose nitrate membrane filter (5-µm pore size)	Visual-stereomicroscope; LUMOS microscopy (ATR mode) on subset of particles	China	2–5 mussels pooled as a replicate Missing from QA/QC: 3, 6, 9, 10, 11, 12	Qu et al. (2018)
	Digestive gland/gut mean (spring): 0.4 ± 0.7 particles·g ww^{-1} 0.4 ± 0.7 particles·ind^{-1} Digestive gland/gut mean (fall): 0.8 ± 1.4 particles·g ww^{-1} 0.3 ± 0.6 particles·ind^{-1} Overall mean: 0.6 ± 1.2 particles·g ww^{-1} 0.4 ± 0.6 particles·ind^{-1}	47.9–1030.1 µm	30% H_2O_2 digestion at ~75°C for 24 hr; then, room temperature for 36–48 hr; added 15% H_2O_2 as needed; NaI density separation (1.6 g·cm^3); filtered onto polycarbonate filters (0.8-µm pore size) and particles transferred to calcium fluoride window	Visual-stereomicroscope; Raman and FTIR	Connecticut, USA	Missing from QA/QC: 10	Zhao et al. (2018)
	Mean: 0.15 ± 0.20 particles·g ww^{-1} 0.97 ± 0.74 particles·ind^{-1}	Majority <300 µm	10% KOH digestion at 60°C overnight; sieved through 20-µm sieve; lithium meta-tungstate density separation overnight; filtered onto polycarbonate membrane filters (20- µm pore size)	Visual-microscope; FTIR	South Korea	Missing from QA/QC: 6, 11	Cho et al. (2019)
	Mean: 0.1 MP·g ww^{-1} (estimated from figure 1b in study)	0.1–16.4 mm Bivalves contained more 0.1–1 mm than did fish examined	10% KOH digestion at 60°C for 6 hr; NaCl density separation overnight; filtered onto cellulose nitrate filter (GF/A, 1.6-µm pore size)	Visual-stereomicroscope; Raman	Fujian Province, China	Discussed only pooled sample data Missing from QA/QC: 3, 6, 11, 12	Fang et al. (2019)
	Mean: 0.76 ± 0.40 MP·ind^{-1} 0.15 ± 0.06 MP·g ww^{-1}	≥15 µm	10% KOH digestion at 60°C for 24 hr with agitation; filtered onto a glass fiber filter (GF/A, 1.6-µm pore size)	Visual-stereomicroscope; µ-Raman	Channel coastline, France	Missing from QA/QC: 6, 10, 12	Hermabessiere et al. (2019)
	Range: 1.43–7.64 items·$mussel^{-1}$	50 µm–8.7 mm	10% KOH digestion at 70°C for up to 48 hr; filtered through nylon mesh (50-µm pore size)	Visual-dissecting microscope at 30x magnification; FTIR on subset of particles	England (southwest coast)	Missing from QA/QC: 1, 4, 5, 6, 9, 11	Scott et al. (2019)

Mytilus edulis (blue mussel)	Mean: 1.43 ± 1.45 particles·g ww^{-1}	<1000 µm; most common size: 100–200 µm	Enzymatic digestion with lipase and protease at 60 °C overnight; sieved through a 20-µm sieve; NaCl density separation overnight; filtered onto filter paper (20-µm pore size; polycarbonate)	FTIR on all particles ≤50 µm	South Korea	Missing from QA/QC: 3, 6, 11, 12	Jang et al. (2020)
	Fresh mussels (mean): 0.83 ± 0.96 MP·5 ind^{-1} Processed mussels (mean): 1.46 ± 1.02 MP·5 ind^{-1}	150–500 µm most detected	30% H_2O_2 digestion for 36–48 hr; NaCl density separation; filtered onto cellulose nitrate filters (5-µm pore size)	Visual-stereomicroscope; FTIR	Sicily, Italy	Five mussels per replicate Missing from QA/QC: 6, 9, 11, 12	Nalbone et al. (2021)
Mytilus galloprovincialis (Mediterranean mussel)	Mean: 4 ± 2 MP·ind^{-1} (estimated from figure 3 in study)	5 µm–5 mm; majority >250 µm	30% H_2O_2 at 65 °C digestion for 24 hr; then at room temperature for 24–48 hr (if needed); NaCl density separation; filtered onto cellulose nitrate membrane filter (5-µm pore size, 47-mm diameter)	Visual-stereomicroscope; FTIR on subset of particles	China	1–5 individuals were pooled as one replicate; density of NaCl solution not defined; tools not listed as glass (e.g., petri dish) Missing from QA/QC: 3, 6, 9, 10, 11, 12	Li et al. (2015)
	Acid Mix Method (mean): 0.18 ± 0.15 particles·g ww^{-1} (wild) Nitric Acid Method (mean): 0.12 ± 0.04 particles·g ww^{-1} (wild)	<5 mm	Mixed Acid Method: 65% HNO_3: 68% $HCLO_4$ (4:1, v:v) digestion overnight at room temperature, boiled 10 min; diluted boiled until tissue gone; filtered cool over qualitative filter (VWR, Grade 310, 10–20 µm pore size) Nitric Acid Method: 69% HNO_3 digestion overnight; then 2 hr of boiling; diluted with 80 °C filtered deionized water; filtered warm onto cellulose nitrate membrane filter (5-µm pore size)	Visual-microscope (200X mag); hot needle test	Italy, Portugal, Spain (wild)	Claim particles were too small for Raman but defined the particles as plastic Missing from QA/QC: 6, 10, 11, 13	Vandermeersch et al. (2015)
	Transplanted mussels (mean): 1–2 items·ind^{-1}	<5 mm	NaCl density separation of ground tissue; filtration and 15% H_2O_2 digestion of remaining organics in a petri dish	Visual sorting; FTIR	Giglio Island, Italy	Missing from QA/QC: 1, 2, 3, 5, 6, 9, 11	Avio et al. (2017)
	Mean: 0.05 MP·g dw^{-1}	Mean fragment: 60.01 ± 38 µm Mean filament: 530 ± 470 µm	30% H_2O_2 digestion at 60 °C digestion for 1 hr; then at room temperature until fully digested; NaCl density separation; filtered onto cellulose nitrate membrane filter (5-µm pore size)	Visual-epifluorescence reversed microscopy	Gulf of La Spezia, Italy	Missing from QA/QC: 3, 4, 6, 10, 11, 13	Bonello et al. (2018)

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Table 1. Continued.

Species	Microplastic concentration measured in tissue	Particle size	Isolation method	Detection method	Location	Criticisms	Reference
Mytilus galloprovincialis (Mediterranean mussel)	Range: 1.7–2 items·ind^{-1} Mean: 1.9 ± 0.2 items·ind^{-1}	0.1–0.5 mm most abundant	Dissected digestive gland and gills; 30% H_2O_2 digestion at 55–65 °C until evaporation; added 1–2 mL more of H_2O_2 with 100 mL Milli Q; filtered onto glass fiber filter (1.2-µm pore size)	Visual-stereomicroscope; FTIR	North Ionian Sea	Missing from QA/QC: 3, 4, 6, 9, 11, 12	Digka et al. (2018b)
	Range of mean MP from five markets: 1.9–9.6 items·ind^{-1} 2.0–12.8 items·g ww^{-1} Wild mussels (mean): 0.53 items·ind^{-1} 2.0 items·g ww^{-1}	25 µm–5 mm; fibers most dominant	10% KOH digestion at 60°C for 24 hr; filtered onto GF/F filters (0.7-µm pore size)	Visual-stereomicroscope; FTIR	Qingdao, China	Missing from QA/QC: 1, 3, 4, 5, 6, 11, 12	Ding et al. (2018)
	Median uptake: 6.2–7.2 MP·g ww^{-1}	1150–2290 µm (avg size)	H_2O_2 (30% per gram of tissue) digestion at 50 °C for 48 hr or longer (if needed); filtered onto 0.45-µm size pore paper	Visual-stereomicroscope; "four-eye approach"	Italy	Missing from QA/QC: 3, 4, 6, 7, 8, 10, 11, 12, 13	Renzi et al. (2018)
	Mean: 800 items·kg ww^{-1} (estimated from figure 2 in study)	0.05–5 mm in length	10% KOH digestion at 65 °C for 24 hr; then at room temperature for another 24 hr; NaCl density separation; filtered onto a 1-µm filter	Visual-stereomicroscope; FTIR on 30 particles	Tunisia	Each individual treated as a replicate Missing from QA/QC: 3, 6, 9, 10, 11, 12	Abidli et al. (2019)
	Mean: 0.69 MP·mussel^{-1} 0.23 MP·g ww^{-1}	<0.5 mm most common size	30% H_2O_2 digestion at 65 °C for 3 d; filtered onto GF/C filters (1.2-µm pore size)	Visual-stereomicroscope; FTIR	Turkey	Pooled three individuals per replicate Missing from QA/QC: 6, 10, 11	Gedik and Eryasar (2020)
	Coastal organisms (mean): 1.06–1.33 fragments·g ww^{-1} 0.62–0.63 fibers·g ww^{-1} Offshore organisms (mean): 0.65–0.66 fragments·g ww^{-1} 0.24–0.35 fibers·g ww^{-1}	Majority: 20–40 µm	Protease enzyme digestion at 50°C for 48 hr; followed by a 20% KOH digestion at 50°C for 36 hr; filtered through aluminum oxide filter (0.2-µm pore size)	Visual-stereomicroscope; FTIR	Adriatic Sea, Italy	Pooled 10 mussels for analysis Missing from QA/QC: 11, 12	Gomiero et al. (2019)

Mytilus galloprovincialis (Mediterranean mussel)	Mean: 0.6 ± 0.1 MP·mussel^{-1}	Mean: 1.7 ± 0.1 mm Mean max size fiber-type MP: 1.6 ± 0.1 mm Mean max size fragment-type MP: 1.8 ± 0.1 mm	30% KOH:NaCLO digestion at room temperature for 10 d; KI density separation, settled for 1 d; filtered through membrane filter (0.45-µm pore size)	Visual-microscope; µ-Raman on subset of particles	Turkey	Collected (ready-to-eat) stuffed mussels from markets; pooled 7–10 mussels per sample Missing from QA/QC: 9, 10, 11	Gündoğdu et al. (2020)
	Mean: 2.8 MP·g ww^{-1} 3.4 MP·ind^{-1}	50–1000 µm; fibers most common	10% KOH digestion at 60 °C for 24 hr; filtered through nylon mesh (20-µm pore size)	Visual-stereomicroscope; hot needle test	Cape Town, South Africa	Three individuals per replicate Missing from QA/QC: 2, 4, 5, 6, 10, 11, 12, 13	Sparks (2020)
	Mean: 7.7 ± 3.8 items·mussel^{-1}	300 µm–5 mm	10% KOH digestion at 60ºC for at least 48 hr; shaken every 8 hr for 40 s; filtered through 300 µm stainless steel mesh and washed into a Petri dish with MilliQ water; NaI density separation overnight; filtered onto glass fiber filter (0.8-µm pore size, 55-mm diameter)	Visual-stereomicroscope; FTIR	Bizerte Lagoon, Tunisia	Missing from QA/QC: 10, 11	Wakkaf et al. (2020)
	Mean across seasons and location: 0.8–2.1 items·ind^{-1} 1.6–2.6 items·g ww^{-1}	7 µm–5 mm	10% KOH digestion at 60 °C for up to 24 hr; filtered onto glass fiber membrane filters (GF/F, 0.7-µm pore size)	Visual-stereomicroscope; FTIR	Qingdao, China	Recommend mussels and clams as bioindicators; isolated the digestive system Missing from QA/QC: 1, 5, 6, 10, 11	Ding et al. (2021)
	41 ± 13 items·g dw^{-1}	<5 mm Maximum mean percentage of MP as a function of mussel size at 80–150 µm size range	2.5% KOH and 5% H_2O_2 and 2.7% methanol digestion in microwave (60 °C) for 3 hr; cooled at room temperature and neutralized with 10% citric acid; filtered through glass fiber filters (1.2-µm pore size)	Visual-microscope; Raman	Italy	Mussels purchased at the market; tested a microplastic digestion method Missing from QA/QC: 3, 6, 11, 12	Fraissinet et al. (2021)
	Fresh mussels (mean): 3.26 ± 2.76 MP·5 ind^{-1} Processed mussels (mean): 0.65 ± 0.98 MP·5 ind^{-1}	150–500 µm most detected	30% H_2O_2 digestion for 36–48 hr; NaCl density separation; filtered onto cellulose nitrate filters (5-µm pore size)	Visual-stereomicroscope; FTIR	Sicily, Italy	Five mussels per replicate Missing from QA/QC: 6, 9, 11, 12	Nalbone et al. (2021)
Mytilus meridionalis (black mussel)	Mean MP concentration for all samples: 3.83 ± 0.2 MP·ind^{-1} 0.04 ± 0.003 MP·g ww^{-1}	>500 µm	10% KOH digestion at 60 °C for 48 hr; filtered onto nylon mesh (20-µm pore size)	Visual-stereomicroscope; FTIR	Cape Town, South Africa	Authors combined species Missing from QA/QC: 6, 11	Sparks et al. (2021)
Mytilus trossulus (Pacific blue mussel)	Mean: 0.4 ± 0.19 MP·g ww^{-1} 0.1 ± 0.2 MP·ind^{-1}	<5 mm	Tested multiple digestion methods (Table 1 in their paper); SDS and detergent enzyme digestion at 37.5 °C for 48 hr with occasional agitation	Visual-light microscope; FTIR	Baltic Sea	Caging experiment in field near wastewater drainage pipe Missing from QA/QC: 3, 6, 10	Railo et al. (2018)

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Table 1. Continued.

Species	Microplastic concentration measured in tissue	Particle size	Isolation method	Detection method	Location	Criticisms	Reference
Mytilus spp. *Authors pooled with *M. galloprovincialis*	Acid Mix method (mean): 0.13 ± 0.14 particles·g ww^{-1}		Mixed Acid Method: 65% HNO_3: 68% $HCLO_4$ (4:1, v:v) overnight at room temperature; boiled 10 min; diluted and boiled until tissue gone; filtered cool over qualitative filter (VWR, Grade 310, 10–20 µm pore size) Nitric Acid Method: 69% HNO_3 digestion overnight; then 2 hr of boiling; diluted with 80 °C filtered deionized water; filtered warm onto cellulose nitrate membrane filter (5-µm pore size)	Visual-microscope (200X mag); hot needle test	Spain, Italy, France, Denmark, The Netherlands (farmed)	Authors combined farmed species; claim particles were too small for Raman but defined the particles as plastic Missing from QA/QC: 6, 10, 11, 13	Vandermeersch et al. (2015)
	Ria of Vigo (mean): 2.19 ± 1.57 MP·ind^{-1} 1.59 ± 1.28 MP·g ww^{-1} (KOH) Cantabrian Sea (mean): 2.81 ± 2.80 MP·ind^{-1} 2.55 ± 2.8 MP·g ww^{-1} (KOH) HNO_3 digestion (mean): 1.22 ± 1.42 MP·g ww^{-1} KOH digestion (mean): 2.07 ± 2.21 MP·g ww^{-1}	Observed: 500–5000 µm; most common: 200–500 µm;	65% HNO_3 digestion at room temperature for 24 hr; 10% KOH digestion at room temperature for 21 d; filtered onto GF/C filters (0.7-µm pore size)		Spain (north coast)	Authors combined species; note digestions resulted in different microplastic concentrations; claim use as bioindicator Missing from QA/QC: 4, 6, 9, 10, 11, 13	Reguera et al. (2019)
Mytilus spp. *Authors guess hybrid of *M. galloprovincialis* & *M. trossulus*	Mean: 0.9 ± 0.6 MP·ind^{-1} (resident mussels)	<5 mm; fibers most dominant	20% KOH digestion at room temperature for 14 d; sieved through a stainless-steel sieve (125-µm pore size) and then filtered onto a polycarbonate filter (10-µm pore size)	Visual-dissecting microscope; Raman on 25% of all particles, first 10 particles of each microplastic category (by color and shape)	San Francisco Bay, California, USA	2–3 composite samples per site Each composite sample was composed of 4 -10 individual bivalves Let animals depurate before digestion (not representative) Missing from QA/QC: 3, 4, 5, 6, 10, 11	Klasios et al. (2021)
Mytilus spp.	Range: 2.6–5.1 fibers·10 g ww^{-1}	200–1500 µm	Acid destruction with HNO_3:$HClO_4$ (4:1) overnight at room temperature and then boiled twice; filtered onto qualitative filter (VWR, Grade 310, 10–20 µm pore size)	Visual-stereomicroscope; hot needle test	Belgium	No polymer ID; depurated mussels for 24 hr before freezing Missing from QA/QC: 6, 10, 11, 13	De Witte et al. (2014)
	Mean: 1.5 ± 2.3 MP·ind^{-1} 0.97 ± 2.61 MP·g ww^{-1} Range: 0–6.9 MP·ind^{-1} 0–7.9 MP·g ww^{-1}	70–3870 µm	10% KOH digestion at 60 °C for 24 hr; filtered onto GF/D filter (2.7-µm pore size)	Visual-stereomicroscope; FTIR	Norway	Missing from QA/QC: 6, 11, 12	Bråte et al. (2018b)

	Mean: 3.0 ± 0.9 particles·g ww^{-1} 3.2 ± 0.52 particles·ind^{-1}		Corolase 7089 enzyme digestion at 60 °C overnight; filtered onto cellulose membrane filter (0.8-µm pore size)	Visual-dissecting microscope; FTIR on subset of particles; Nile Red dye with fluorescent microscope	Scotland	Missing from QA/QC: 3, 4, 6, 10, 11, 12	Catarino et al. (2018)
Mytilus spp. (*M. californianus* & *M. edulis*) *Authors pooled data from both species	Mean for all samples: 4.47 fibers·$sample^{-1}$ 1.02 fibers·g ww^{-1} Mean at harbor sites: 6.04 fibers·$sample^{-1}$ 1.38 fibers·g ww^{-1} Mean at beach sites: 3.62 fibers·$sample^{-1}$ 0.55 fibers·g ww^{-1} Supermarket mussels: 3.21 fibers·$sample^{-1}$ 0.56 fibers·g ww^{-1}	<5 mm	30% H_2O_2 digestion at 65 °C for 24 hr; NaCl density separation for 24 hr; filtered onto a cellulose nitrate filer (5-µm pore size)	Visual-stereomicroscope	Southern California Harbors, California, USA	Only examined microfibers; depurated before dissection; two mussels per replicate Missing from QA/QC: 2, 3, 4, 6, 10, 11, 13	Mankin and Huvard (2020)
Mytilaster lineatus	9.30 MP·g ww^{-1}	Mostly fibers, most common size ranges observed: 1–2 mm (42.2%) and 2–5 mm (26%)	10% KOH digestion at 40 °C for 48–72 hr; Whatman PLC filter paper used; NaI density separation and filtered again	Visual-stereomicroscope; hot needle test; FTIR	Gorgan Bay, Caspian Sea	QA/QC based on Wang et al. (2019); refers to both species indiscriminately as "clams" and "mussels"; confirmed limited contamination with "aquracy test" (sic) so not sure of exact methods Missing from QA/QC: 1, 3, 5, 6, 10, 11, 12	Bagheri et al. (2020)
Neotrapezium liratum (quadrate trapezium)	Mean: 1.25 ± 0.1 MP·g^{-1} ww (estimated from figure 4 in study)	93.3% were fibers Mean length: 1004.2 ± 464.8 µm Mean diameter: 21.8 ± 7.3 µm	10% KOH digestion at 40 °C for 48 hr; sieved through a 38-µm sieve; filtered onto a cellulose ester filter (0.45-µm pore size)	Visual-stereomicroscope; FTIR on a subset of particles	Hong Kong, China	Low percent recovery for gastropods (50%) because of shell debris on filter not removed for digestion; some discussed data does not match table data; species listed in figures 4 and 5 differ; mislabeled gastropods as crabs Missing from QA/QC: 3, 4, 6, 11, 12	Xu et al. (2020)
Nerita articulata (lined nerite)	Reported range of MP abundance for all three tested species: 0.50–1.75 particles·g ww^{-1} 0.25–0.88 particles·ind^{-1}	30–1850 µm; majority (57%) 30–1000 µm	69% HNO_3 and 30% H_2O_2 (4:1 v/v) heated to 50 °C for 30 min; NaCl density separation; supernatant filtered through cellulose nitrate membrane filters (0.45-µm pore size, 47-mm diameter)	Visual-stereomicroscope; FTIR on subset of particles	Klang River Estuary, Malaysia	Pooled gastropods at each site Missing from QA/QC: 3, 6, 9, 11, 12	Zaki et al. (2021)
Nerita chamaeleon (cameleon nerite)	Mean: 1.50 ± 0.20 MP·g^{-1} ww	93.3% were fibers Mean length: 1004.2 ± 464.8 µm Mean diameter: 21.8 ± 7.3 µm	10% KOH digestion at 40 °C for 48 hr; sieved through a 38-µm sieve; filtered onto a cellulose ester filter (0.45-µm pore size)	Visual-stereomicroscope; FTIR on a subset of particles	Hong Kong, China	Low percent recovery for gastropods (50%) because of shell debris on filter not removed for digestion; some discussed data does not match table data; species listed in figures 4 and 5 differ; mislabeled gastropods as crabs Missing from QA/QC: 3, 4, 6, 11, 12	Xu et al. (2020)

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Table 1. Continued.

Species	Microplastic concentration measured in tissue	Particle size	Isolation method	Detection method	Location	Criticisms	Reference
Nerita polita (polished nerite)	Reported range of MP abundance for all three species: 0.50–1.75 particles·g ww^{-1} 0.25–0.88 particles·ind^{-1}	30–1850 µm; majority (57%) 30–1000 µm	69% HNO_3 and 30% H_2O_2 (4:1 v/v) heated to 50 °C for 30 min; NaCl density separation; supernatant filtered through cellulose nitrate membrane filters (0.45-µm pore size, 47-mm diameter)	Visual-stereomicroscope; FTIR on subset of particles	Klang River Estuary, Malaysia	Pooled the gastropods at each site Missing from QA/QC: 3, 6, 9, 11, 12	Zaki et al. (2021)
Nototodarus gouldi (Gould's squid)	Σ 0.04 mg·g ww^{-1}	N/A-- Can't quantify size with Py-GC/MS	10% KOH digestion at 60 °C for 24 hr; filtered onto glass fiber filters (GF/D, 2.7-µm pore size)	Py-GC/MS	Australia	Missing from QA/QC: 3, 12	Ribeiro et al. (2020)
Nudibranch	No particles detected	<5 mm	10% KOH digestion at 60°C overnight; poured into a petri dish	Visual-stereomicroscope	Makassar Strait, Indonesia	No mention of QA/QC measures	Tahir et al. (2019)
Nuttallia obscurata (varnish clam)	Mean: 0–2.92 MP·$clam^{-1}$	50–600 µm in diameter (fibrous, pellet and fragment); did not examine microfibers (high airborne contamination)	10% KOH digestion at 60 °C for 48 hr; dyed with Rit Dye More to reveal synthetic polymers; cellulose filter paper (11-µm pore size); rinsed with 30% H_2O_2	Visual-microscope; FTIR	Burrard Inlet and Baynes Sound, British Colombia	Suggest species as bioindicators for microfragments and microspheres (but then cite Ward et al., (2019a) that states they are not good bioindicators); dye can interfere with FTIR, excluded textile microfibers >0.5 mm from analysis; stated limitations for QA/QC Missing from QA/QC: 3, 4, 5, 6, 11	Bendell et al. (2020)
Ostrea denselamellosa (lamellated oyster)	0.31 ± 0.10 items·g ww^{-1} 1.67 ± 0.44 items·ind^{-1}	74–2000 µm found; razor clams had more particles <500 µm	10% KOH and 30% H_2O_2 digestion at 60 °C for at least 24 hr; filtered onto a glass fiber filter (GF/F, 0.7-µm pore size)	Visual-stereomicroscope; FTIR	Xiangshan Bay, China	Missing from QA/QC: 1, 3, 5, 6, 11, 12	Wu et al. (2020)
Patinopecten yessoensis (Yesso scallop)	Mean: 57.2 MP·ind^{-1}	5 µm–5 mm; majority >250 um	30% H_2O_2 digestion at 65 °C for 24 hr; then, at room temperature for 24–48 hr (if needed); NaCl density separation; filtered onto cellulose nitrate membrane filter (5-µm pore size, 47-mm diameter)	Visual-stereoscope; FTIR on subset of particles	China	1–5 individuals pooled as one replicate; density of NaCl solution not defined; materials not listed as glass (e.g., petri dish) Missing from QA/QC: 3, 6, 9, 10, 11, 12	Li et al. (2015)
	Mean: 0.15 ± 0.20 particles·g ww^{-1} 0.97 ± 0.74 particles·ind^{-1}	Majority <300 µm	10% KOH digestion at 60 °C overnight; sieved through 20-µm sieve; lithium meta-tungstate density separation overnight; filtered onto polycarbonate membrane filters (20- µm pore size)	Visual-microscope; FTIR	South Korea	Missing from QA/QC: 6, 11	Cho et al. (2019)

Pecten maximus (King scallop)	Mean: 6.6 MP·ind^{-1} 0.2 MP·g ww^{-1}	1–5000 µm	30% H_2O_2 digestion at 65 °C for 24 hr; then, room temperature for 24–48 hr; filtered onto a cellulose membrane filter (5-µm pore size)	Visual-stereomicroscope; FTIR on subset of particles	Chile	Missing from QA/QC: 1, 3, 5, 6, 9, 10, 11, 13	Akoueson et al. (2020)
Perna canaliculus (green lipped mussel)	Mean: 0.2 ± 0.2 MP·mussel^{-1} Range of MP abundances: 0–1.5 MP·mussel^{-1} 0–0.48 MP·g ww^{-1}	50–700 µm diameter; median diameter: 100 µm	HNO_3 digestion at room temperature; 2 hr boiling; diluted with ultra-pure water at 80 °C; filtered onto a glass fiber filter (GF/C, 1.2-µm pore size, 47-mm diameter)	Visual-fluorescent microscope but without fluorescence; FTIR	New Zealand	Pooled mussels Missing from QA/QC: 3, 4, 6, 7, 9, 10, 11, 12	Webb et al. (2019)
Perna perna (brown mussel)	75% of mussels had MP- did not quantify the MP	<5 mm	HNO_3 digestion at room temperature and then 15 minutes of boiling; filtered onto glass fiber filters (0.7-µm pore size)	Visual-polarized light microscopy to determine the difference between tissue and synthetic materials	Santos Bay, Brazil	Only assessed percent of contaminated mussels; no polymer identification; excluded fibers from the study Missing from QA/QC: 2, 3, 4, 6, 10, 11, 13	Santana et al. (2016)
	Farmed: 4.12 MP·g ww^{-1} 25.9 MP·ind^{-1} (before depuration) 2.83 MP·g^{-1} 18.4 MP·ind^{-1} (after depuration) Wild: 6.67 MP·g ww^{-1} 31.2 MP·ind^{-1} (before depuration) 3.12 MP·g ww^{-1} 16.6 MP·ind^{-1} (after depuration)	<5 mm	30% H_2O_2 digestion at room temperature for 7 d; hypersaline NaCl density separation; filtered onto mixed cellulose esters filter (0.45-µm pore size)	Visual-stereomicroscope; FTIR	Guanabara Bay, Brazil	Missing from QA/QC: 3, 4, 6, 10, 11, 12	Birnstiel et al. (2019)
Perna spp. (*P. perna* & *P. viridi*)	Mean: 0.1 ± 0.03 to 2.05 ± 0.33 items·g ww^{-1} 0.87 ± 0.55 to 10.02 ± 4.15 items·30 ind^{-1}	Fibers from 500–1000 µm were dominant; fibers >500 µm were analyzed with FTIR	10% KOH and 30% H_2O_2 digestion at 40ºC for 72 hr with continual 30% H_2O_2 additions; filtered onto a cellulose nitrate filter paper (0.8-µm pore size)	Visual-stereomicroscope; hot needle test; FTIR; SEM-EDAX	Southern India	30 individuals per sample; authors pooled data across mussel size classes and collection sites Missing from QA/QC: 3, 6, 11, 12, 13	Patterson et al. (2021)
Perna viridis (Asian green mussel)	Range: 1.52–5.36 MP·g ww^{-1} 0.77–8.22 MP·ind^{-1}		30% H_2O_2 digestion at 65 °C for 72 hr; NaCl density separation; filtered onto size cellulose nitrate membrane filter (5-µm pore size)	Visual-stereomicroscope; LUMOS microscopy (ATR mode) on subset of particles	China	2–5 mussels pooled as a replicate Missing from QA/QC: 3, 6, 9, 10, 11, 12	Qu et al. (2018)
	<1 mm	10–500 µm	70% HNO_3 digestion for 40 hr; boiled for 15–20 min; added hot water at 80 °C to dilute sample; filtered onto filter paper (11-µm pore size)	Visual-stereomicroscope	Bacoor, Philippines	Bought from Sineguelasan Seafood Terminal; three mussels per replicate No mention of QA/QC measures	Argamino and Janairo (2016)

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Table 1. Continued.

Species	Microplastic concentration measured in tissue	Particle size	Isolation method	Detection method	Location	Criticisms	Reference
Perna viridis (Asian green mussel)	Mean: 5 particles·0.25 g ww^{-1}	51.31–87.43 µm	30% H_2O_2 digestion; density separation with supersaturated solution; filtered onto filter paper	Visual-light microscope; SEM with EDX	Java Sea, Indonesia	Missing from QA/QC: 2, 3, 4, 6, 7, 8, 9, 11, 12, 13	Khoironi et al. (2018)
	Mean: 0.29 MP·g ww^{-1} (estimated from figure 1b in study)	0.1–16.4 mm; bivalves contained more 0.1–1 mm than fish examined	10% KOH digestion at 60 °C for 6 hr; NaCl density separation overnight; filtered through a GF/A cellulose nitrate filter (1.6-µm pore size)	Visual-stereomicroscope; Raman	Fujian Province, China	Discussed only pooled sample data Missing from QA/QC: 3, 6, 11, 12	Fang et al. (2019)
	Mean: 2.60 ± 1.14 MP·ind^{-1} 0.29 ± 0.14 MP·g ww^{-1}	15–400 µm	10% KOH digestion; 50% KI density separation; filtered onto cellulose nitrate filters (12-µm pore size)	Directly identified particles from filters using FTIR	Vietnam	Missing from QA/QC: 3, 4, 6, 10, 11	Phuong et al. (2019)
	Particles and colorants: Direct observation of tissue: 1.8 ± 1.7·0.5 g ww^{-1} Acid digestion: 3.2 ± 3.2·10 g ww^{-1} Fibers: Direct observation of tissue: 1.4 ± 0.9·0.5 g ww^{-1} Acid digestion: 0.9 ± 0.3·10 g ww^{-1}	Particles and colorants: 30–103 µm Fibers: 5–25 µm	69% NHO_3 digestion overnight; then 2 hr of boiling in 80 °C filtered deionized water; filtered onto cellulose nitrate membrane filter (5-µm pore size)	Visual inspection of tissue prior to digestion; Raman on filtered/processed samples	India	Missing from QA/QC: 2, 3, 6, 10, 11, 12	Naidu (2019)
	Mean: 0.18 ± 0.04 MP·g ww^{-1} 0.50 ± 0.11 MP·ind^{-1}	77.42% of particles <100 µm	10% KOH digestion at 40 °C for 72 hr; added more KOH and digested an additional 24 hr if tissue remained; filtered through Whatman filter papers (11-µm pore size)	Nile Red staining; visual-fluorescent microscope; Raman on subset of particles	Pondicherry, India	Pooled 10 individuals per replicate Missing from QA/QC: 1, 3, 4, 5, 6, 10, 11, 12	Dowarah et al. (2020)
	Digestive gland: 5.6 items·g Gill: 8.5 items·g Overall mean of three species: 15.9 items·g tissue	Most particles were <2 mm; largest were 4 mm	Incubation at 50 °C for 15 min; proteinase-K enzyme digestion at 50 °C for 2 hr; deproteinized at 60 °C for 20 min; filtered onto glass fiber filters (1.2-µm pore size)	Visual-stereomicroscope; µ-Raman	Southwest coast of India	Claim microplastics "in" the gills; axis labels of figure 2 do not line up; not clear from methods what was sampled Missing from QA/QC: 3, 4, 10, 11, 12	Joshy et al. (2022)
	Small mussels (mean): 5.70 ± 0.49 items·ind^{-1} 0.86 ± 0.08 items·g ww^{-1} Large mussels (mean): 1.77 ± 0.30 items·ind^{-1} 0.14 ± 0.02 items·g ww^{-1}	<5 mm	30% H_2O_2 at 60 °C digestion for 48 hr; then at room temperature for 48 hr; NaCl density separation left for 24 hr; filtered onto cellulose nitrate membrane filter (5-µm pore size, 47-mm diameter)	Visual-stereomicroscope; hot needle test; FTIR on subset of particles	Tapi-Phumduang River and Bandon Bay, Thailand	Report a MP recovery rate of 100%, but do not describe in methods Missing from QA/QC: 6, 10, 11, 12	Chinfak et al. (2021)
Pinna sp.	Mean: 0.5 MP·ind^{-1}	<5 mm	10% KOH digestion at 60°C overnight; poured into a petri dish	Visual-stereomicroscope	Makassar Strait, Indonesia	No mention of QA/QC measures	Tahir et al. (2019)

Pinctada radiata (Atlantic pearl oyster)	Fibers (mean): 2.6 ± 0.6 MP·ind^{-1} 0.1 ± 0 MP·g ww^{-1} Fragments (mean): 0.8 ± 0.4 MP·ind^{-1} 0 ± 0.1 MP·g ww^{-1}	10–5000 µm	30% H_2O_2 digestion; filtration through filter paper (25-µm pore size) and secondary filtration through a nitrocellulose filter (0.45-µm pore size)	Visual-dissection microscope; hot needle test; SEM-EDX; FTIR on subset of particles	Persian Gulf	3–11 individuals per replicate Missing from QA/QC: 3, 4, 6, 10, 11, 13	Naji et al. (2018)
Pinctada sp. (pearl oyster)	Mean: 0.3 MP·ind^{-1}	<5 mm	10% KOH digestion at 60°C overnight; poured into a petri dish	Visual-stereomicroscope	Makassar Strait, Indonesia	No mention of QA/QC measures	Tahir et al. (2019)
Pirenella alata (horn snail)	Mean: 2.5 ± 0.3 MP·g^{-1} ww (estimated from figure 4 in study)	93.3% were fibers Mean length: 1004.2 ± 464.8 µm Mean diameter: 21.8 ± 7.3 µm	10% KOH digestion for 48 hr at 40 °C; sieved through a 38-µm sieve; filtered onto a cellulose ester filter (0.45-µm pore size)	Visual-stereomicroscope; FTIR on a subset of particles	Hong Kong, China	Low percent recovery for gastropods (50%) because of shell debris on filter not removed for digestion; some discussed data does not match table data; species listed in figures 4 and 5 differ; mislabeled gastropods as crabs Missing from QA/QC: 3, 4, 6, 11, 12	Xu et al. (2020)
Pirenella cingulata (horn snail)	Mean: 2.5 ± 0.3 MP·g^{-1} ww (estimated from figure 4 in study)	93.3% were fibers Mean length: 1004.2 ± 464.8 µm Mean diameter: 21.8 ± 7.3 µm	10% KOH digestion for 48 hr at 40 °C; sieved through a 38-µm sieve; filtered onto a cellulose ester filter (0.45-µm pore size)	Visual-stereomicroscope; FTIR on a subset of particles	Hong Kong, China	Low percent recovery for gastropods (50%) because of shell debris on filter not removed for digestion; some discussed data does not match table data; species listed in figures 4 and 5 differ; mislabeled gastropods as crabs Missing from QA/QC: 3, 4, 6, 11, 12	Xu et al. (2020)
Polititapes spp. (carpet shell)	Mean: 10.4 ± 10.4 MP·g ww^{-1}	0.037–4.7 mm; fibers most prevalent	1.8 M KOH digestion at 60 °C for 24 hr; filtered through glass fiber filters (GF/C, 1.2-µm pore size)	Visual-stereomicroscope; µ-Raman on subsample for each species FTIR if needed	Ria Formosa Lagoon, Portugal	Each sample contained anywhere from 1–4 individuals Missing from QA/QC: 3, 4, 6, 10, 11	Cozzolino et al. (2021)
Reisha clavigera (Asian rock shell)	Mean: 3.5 ± 0.5 MP·g^{-1} ww (estimated from figure 4 in study)	93.3% were fibers Mean length: 1004.2 ± 464.8 µm Mean diameter: 21.8 ± 7.3 µm	10% KOH digestion at 40 °C for 48 hr; sieved through a 38-µm sieve; filtered onto a cellulose ester filter (0.45-µm pore size)	Visual-stereomicroscope; FTIR on a subset of particles	Hong Kong, China	Low percent recovery for gastropods (50%) because of shell debris on filter not removed for digestion; some discussed data does not match table data; species listed in figures 4 and 5 differ; mislabeled gastropods as crabs Missing from QA/QC: 3, 4, 6, 11, 12	Xu et al. (2020)
Ruditapes decussatus (grooved carpet shell)	Mean: 1400 items·kg ww^{-1} (estimated from figure 2 in study)	0.05–5 mm in length	10% KOH digestion at 65 °C for 24 hr, then, at room temperature for another 24 hr; NaCl density separation; filtered onto a 1-µm filter	Visual-stereomicroscope; analyzed 30 particles with FTIR	Tunisia	Each individual treated as a replicate Missing from QA/QC: 3, 6, 9, 10, 11, 12	Abidli et al. (2019)
	Mean: 18.4 ± 21.9 MP·g ww^{-1}	0.037–4.7 mm; fibers most prevalent	1.8 M KOH digestion at 60 °C for 24 hr; filtered onto glass fiber filters (GF/C, 1.2-µm pore size)	Visual-stereomicroscope; µ-Raman on subsample for each species FTIR if needed	Ria Formosa lagoon, Portugal	Each sample contained anywhere from 1–4 individuals Missing from QA/QC: 3, 4, 6, 10, 11	Cozzolino et al. (2021)

(*Continued*)

Table 1. Continued.

Species	Microplastic concentration measured in tissue	Particle size	Isolation method	Detection method	Location	Criticisms	Reference
Ruditapes philippinarum (Manila clam)	Mean: 5 ± 3 MP·ind^{-1} (estimated from figure 3 in study)	5 µm–5 mm, majority >250 µm	30% H_2O_2 digestion at 65 °C for 24 hr; then, at room temperature for 24–48 hr (if needed); NaCl density separation; filtered onto cellulose nitrate membrane filter (5-µm pore size, 47-mm diameter)	Visual-stereomicroscope; FTIR on subset of particles	China	1–5 individuals pooled as one replicate; density of NaCl solution not defined; materials not listed as glass (e.g., petri dish) Missing from QA/QC: 3, 6, 9, 10, 11, 12	Li et al. (2015)
	Range: 0.07–5.5 MP·g ww^{-1}		HNO_3 (69.7%) digestion at 90 °C for 4 hr; filtered onto glass fiber filters (1.2-µm pore size)	Visual-dissecting microscope and compound microscope	British Columbia	Recovery tested with spiked samples (PE and PS)-not reported Missing from QA/QC: 3, 4, 6, 11, 13	Davidson and Dudas (2016)
	Mean: 39.20 ± 27.08 particles·g ww^{-1} (wild) 89.42 ± 50.88 particles·g ww^{-1} (farmed)	10 µm–5 mm	68–70% NHO_3 digestion overnight at room temperature; boiled for 2 hr (100 °C); filtered onto cellulose paper (2.5-µm pore size)	Visual-microscope; FTIR on subset of particles	Vancouver Island	2–5 individuals per replicate Missing from QA/QC: 3, 5, 6, 10, 11	Murphy (2018)
	Mean: 0.43 ± 0.32 MP·ww^{-1} 2.19 ± 1.20 MP·ind^{-1}	Longest axis size range: 46–13,298 µm; most common size: 100–200 µm	10% KOH digestion at 60 °C; sieved through 20-µm pore size sieve; lithium meta-tungstate density separation for 24 hr; filtered onto polycarbonate filter (20-µm pore size)	Manually counted and analyzed particles with FTIR	South Korea	Claim use as bioindicators; pooled 5 individuals/sample and pooled separate species (oysters and mussels) Missing from QA/QC: 11, 12	Cho et al. (2021)
	Mean across seasons and location: 1.2–3.2 items·ind^{-1} 4.5–20.1 items·g ww^{-1}	7 µm–5 mm	10% KOH digestion at 60 °C for up to 24 hr; filtered onto glass fiber membrane filters (GF/F, 0.7-µm pore size)	Visual-stereomicroscope; FTIR	Qingdao, China	Recommend mussels and clams as bioindicators; isolated the digestive system Missing from QA/QC: 1, 5, 6, 10, 11	Ding et al. (2021)
	Mean based on FTIR imaging: Small clams 2.70 ± 1.66 MP·g^{-1} 15.64 ± 9.25 MP·ind^{-1} Large clams 3.65 ± 1.59 MP·g^{-1} 41.63 ± 16.90 MP·ind^{-1}	20–100 µm, most dominant sizes	10% KOH digestion for 24 hr at 60 °C; filtered onto a glass fiber filter (GF/A, 162-µm pore size); $ZnCl_2$ density separation; stainless steel mesh filters; zinc chloride for Key	Visual-fluorescence microscope; Nile Red; FTIR imaged whole filters	Incheon Market, South Korea	Used some spiked sample as the negative control during processing; composite samples analyzed with FTIR ($n = 4$–13 per sample) Missing from QA/QC: 3, 6, 11	de Guzman et al. (2022)
Ruditapes variegatus (variegated carpet shell)	Mean: 2.75 ± 1.5 MP·g^{-1} ww (estimated from figure 4 in study)	93.3% were fibers Mean length: 1004.2 ± 464.8 µm Mean diameter: 21.8 ± 7.3 µm	10% KOH digestion at 40 °C for 48 hr; sieved through a 38-µm sieve; filtered onto a cellulose ester filter (0.45-µm pore size)	Visual-stereomicroscope; FTIR on a subset of particles	Hong Kong, China	Low percent recovery for gastropods (50%) because of shell debris on filter not removed for digestion; some discussed data does not match table data; species listed in figures 4 and 5 differ; mislabeled gastropods as crabs Missing from QA/QC: 3, 4, 6, 11, 12	Xu et al. (2020)

Saccostrea cucullata (hooded oyster)	Range: 1.5–7.2 items·g ww^{-1} 1.4–7.0 items·ind^{-1}	20–5000 µm; majority <100 µm	10% KOH digestion at 65 °C for 24 hr; then, at room temperature for another 24 hr; NaCl density separation; filtered onto a 20-µm filter	Visual-stereomicroscope; FTIR on subset of particles	Pearl River, China	Recommend use as biomonitor; five individuals per replicate (six replicates per site) Missing from QA/QC: 3, 6, 10, 11, 12	Li et al. (2018a)
	Range: 0.43–7.20 particles·g ww^{-1}	<5 mm; more than 50% between 10 and 49 µm	10% KOH digestion at 40 °C for 48 hr; filtered onto filter paper (11-µm pore size)	Visual-compound microscope; FTIR	Brunei, South China Sea	Pseudo-replication (75 g of tissue from each site divided into three samples) Missing from QA/QC: 3, 6, 9, 10, 11, 12	Lee et al. (2022)
	Mean: 1.84 MP·g ww^{-1} Range: 0.14–7.90 MP·g ww^{-1} 1–14 particles in each individual across all sites	Majority: 101–500 µm	10% KOH and 30% H_2O_2 digestion at 65 °C for 8 hr with 30% H_2O_2 additions every 4 hr; filtered onto a glass fiber filer membrane (GF/D, 2.7-µm pore size)	Visual-optical microscopy; FTIR	Zhuhai, China	Missing from QA/QC: 3, 6, 10, 11, 12	Wang D, et al. (2021)
Saccostrea forskalii (rock oyster)	Angsila (mean): 0.57 ± 0.22 particles·g ww^{-1} Bangsean (mean): 0.37 ± 0.03 particles·g ww^{-1} Samaesarn (mean): 0.43 ± 0.04 particles·g ww^{-1}	<5 mm	69% HNO_3 digestion at room temperature overnight; boiled for 2 hr (100 °C); diluted with 80 °C deionized water; vacuum filtered onto cellulose nitrate membrane (5-µm pore size)	Visual-stereomicroscope; Raman	Thailand	Pooled individuals before analysis Missing from QA/QC: 3, 4, 6, 9, 10, 11, 12	Thushari et al. (2017)
Saccostrea glomerata (Sydney rock oyster)	Range: 0.15–0.83 MP·g ww^{-1}	>5 mm; most common 0.1–0.5 mm; fibers most dominant	10% KOH digestion at 60–65 °C for 24 hr; then another 24 hr at room temperature; NaI density separation; filtered through glass fiber filters (GF/B, 1.0-µm pore size)	Visual-stereomicroscope; Nile Red fluorescence; FTIR on subset of particles	New South Wales, Australia	Pooled five individuals per replicate Missing from QA/QC: 3, 4, 6, 11, 12	Jahan et al. (2019)
Saccostrea spp. (rock oyster)	Mean: 75.9 ± 28.5 items·5 ind^{-1} (calculated from Table 1 in study)	Over 50% were <100 µm	30% H_2O_2 digestion at 65 °C for 24 hr or longer; NaCl density separation overnight; filtered onto a mixed cellulose ester membrane filter (5-µm pore size)	Visual-microscope; µ-Raman on subset of particles	Taiwan	Five animals per replicate Missing from QA/QC: 3, 6, 10, 11, 12	Liao et al. (2021)
Scapharca cornea (Corneous ark)	Mean MP: Station 1: 557.98 particles·g dw^{-1} Station 2: 261.22 particles·g dw^{-1} Station 3: 86.27 particles·g dw^{-1}	Mostly filaments 0.12–9.5 mm in length	10 M NaOH digestion at 60 °C for 24 hr; filtered twice with 500-µm pore size filter; density separation in saltwater and fresh distilled water; filtered onto cellulose nitrate membrane filter (0.45-µm pore size)	Visual-dissecting microscope; FTIR	Setiu Wetland, Terengganu, Malaysia	No mention of QA/QC measures	Ibrahim et al. (2016)

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Table 1. Continued.

Species	Microplastic concentration measured in tissue	Particle size	Isolation method	Detection method	Location	Criticisms	Reference
Scapharca subcrenata (half-crenated ark or bloom clam)	Mean: 45 ± 15 MP·ind^{-1} (estimated from figure 3 in study) 10.5 particles·g ww^{-1}	5 µm–5 mm; majority >250 µm	30% H_2O_2 digestion at 65 °C for 24 hr; then at room temperature for 24–48 hr (if needed); NaCl density separation; filtered onto cellulose nitrate membrane filter (5-µm pore size, 47-mm diameter)	Visual-stereoscope; FTIR on subset of particles	China	1–5 individuals pooled as one replicate; density of NaCl solution not defined; tools not listed as glass (e.g., petri dish) Missing from QA/QC: 3, 6, 9, 10, 11, 12	Li et al. (2015)
	Mean: Qingdao: 4.4 ± 1.9 items·g^{-1} ww Xiamen: 4.0 ± 3.4 items·g^{-1} ww Mean MP among all six species: Qingdao: 0.8–4.4 items·g ww^{-1} 1.2–4.1 items·ind^{-1} Xiamen: 2.1–4.0 items·g ww^{-1} 1.3–6.0 items·ind^{-1}	Majority: 100–200 µm Qingdao: 10–4377 µm Xiamen: 58–5000 µm	10% KOH digestion at 60 °C for over 24 hr; filtered the sample	Visual-stereomicroscope; FTIR	Qingdao and Xiamen, China	Did not say what filter they used; measured MP in six species (figure 2), but only discussed highest MP abundance in two species Missing from QA/QC: 3, 6, 10, 11, 12	Ding et al. (2020)
Scrobicularia plana (peppery furrow shell)	3.3 ± 2.06 microfibers·ind^{-1} 197 ± 323.7 microfibers in relation to biomass (ash free dry weight)	>5 mm Mean length: 2.37 ± 2.27 mm Mean section: 20 ± 8 µm	Protease enzyme digestion (neutrase) at 40 °C for 3–6 hr	Visual-stereomicroscope; FTIR on a subset of particles	Tejo estuary Portugal Banc d' Arguin Mauritania, Bijagos archipelago, Guinea-Bissau	Missing from QA/QC: 1, 2, 4, 5, 6, 9, 10, 12	Lourenço et al. (2017)
Semimytilus algosus (dwarf mussel)	Mean: 1.65 ± 0.22 particles·g ww^{-1}	<5 mm; fibers most common, followed by fragments, spheres, and films	10% KOH digestion at 60 °C overnight; filtered onto glass fiber filter paper (20-µm pore size)	Visual-microscope	Lima, Peru	Pooled three individuals per sample Missing from QA/QC: 6, 10, 11, 13 *(based on referenced Dioses-Salinas et al. 2020)*	De-la-Torre et al. (2020)
Senilia senilis (West African bloody cockle)	1.0 ± 2.55 microfibers·ind^{-1} 163 ± 452.8 microfibers in relation to biomass (ash free dry weight)	>5 mm Mean length: 2.37 ± 2.27 mm Mean section: 20 ± 8 µm	Protease enzyme digestion (neutrase) at 40 °C for 3–6 hr	Visual-stereomicroscope; FTIR on a subset of particles	Tejo Estuary Portugal Banc d' Arguin Mauritania, Bijagos archipelago, Guinea-Bissau	Missing from QA/QC: 1, 2, 4, 5, 6, 9, 10, 12	Lourenço et al. (2017)
Sepia officinalis (common European cuttlefish)	Mean: 700 items·kg ww^{-1} (estimated from figure 2 in study)	0.05–5 mm in length	10% KOH digestion at 65 °C for 24 hr; then at room temperature for another 24 hr; NaCl density separation; filtered onto a 1-µm filter	Visual-stereomicroscope; FTIR on 30 particles	Tunisia	Each digestive tract was treated as a replicate Missing from QA/QC 3, 6, 9, 10, 11, 12	Abidli et al. (2019)

Sepia officinalis (common European cuttlefish)	Median concentration: 28.5 particles·ind^{-1} (cultured) 39 particles·ind^{-1} (wild-caught)	<5 mm; fibers most common	Homogenized at 50 °C for 30 min; protease K enzyme digestion at 37 °C for 24 hr; 5 M $NaClO_4$ added at 60 °C for 30 min at room temperature, overnight; filtered through Whatman grade 4 filters (25-µm pore size)	Visual-light microscope; FTIR on subset of particles	Portugal	Missing from QA/QC: 3, 6, 9, 10, 11	Oliveira et al. (2020)
Siliqua patula (Pacific razor clam)	Whole clam (mean): 8.84 ± 0.45 MP·ind^{-1} 0.16 ± 0.02 MP·g ww^{-1}	0.063–8.72 mm	10% KOH digestion for 24 hr; sieved through 63-µm sieved and retained on sieve was dried in a Petri dish at 40 °C for 24 hr; second 10% KOH digestion and NaCL density separation	Visual-stereomicroscope; FTIR on subset (10%) of suspected microfibers	Oregon, USA	Contamination quantified, but not subtracted; separated gut and non-gut tissue Missing from QA/AC: 4, 6, 10, 11	Baechler et al. (2020a)
	Whole clam (mean): 6.75 ± 0.60 MP·sample^{-1} Gut tissue (mean): 7.88 ± 0.71 MP·sample^{-1} Non-gut tissue (mean): 4.96 ± 0.56 MP·sample^{-1} "Cleaned" samples (mean; prepared for human consumption): 3.44 ± 0.25 MP·sample^{-1}	<5 mm; microfiber lengths: 0.11–7.84 mm	10% KOH digestion two times and then a NaCL density separation	Visual-stereomicroscope; FTIR on subset (10%) of suspected particles in whole and cleaned clams	Olympic Peninsula, Washington, USA	GOOD: do say the MP exposure is low from harvested razor clams; methods based on Baechler et al. (2020a) Missing from QA/QC: 4, 6, 10, 11, 12	Baechler et al. (2020b)
Sinonovacula constricta (Chinese razor clam)	Mean: 15 ± 5 MP·ind^{-1} (estimated from figure 3 in study)	5 µm–5 mm; majority >250 µm	30% H_2O_2 digestion at 65 °C for 24 hr; then, at room temperature for 24–48 hr (if needed); NaCl density separation; filtered onto cellulose nitrate membrane filter (5-µm pore size, 47-mm diameter)	Visual-stereomicroscope; FTIR on subset of particles	China	1–5 individuals pooled as one replicate; density of NaCl solution not defined; tools not listed as glass (e.g., petri dish) Missing from QA/QC: 3, 6, 9, 10, 11, 12	Li et al. (2015)
	0.21 ± 0.05 items·g ww^{-1} 1.8 ± 0.34 items·ind^{-1}	74–2000 µm; razor clams had more particles <500 µm	10% KOH and 30% H_2O_2 digestion at 60 °C for at least 24 hr; filtered onto a glass fiber filter (GF/F, 0.7-µm pore size)	Visual-stereomicroscope; FTIR	Xiangshan Bay, China	Missing from QA/QC: 1, 3, 5, 6, 11, 12	Wu et al. (2020)
Spondylus spinosus (spiny oyster)	Mean: 7.2 ± 1.4 items·ind^{-1} 0.45 ± 0.3 items·g ww^{-1}	>5 mm; majority 0–200 µm	10% KOH digestion at 60 °C for 24 hr; filtered onto glass fiber filters (GF/A)	Visual-stereomicroscope; µ-Raman	Levantine Basin, Lebanon	Missing from QA/QC: 3, 10, 11	Kazour et al. (2019)
Tagelus affinis (neighbor tagelus)	2.0 ± 1.9 MP·ind^{-1} 0.5 ± 0.4 MP·g ww^{-1} Mean across six species: 4.8 ± 8.0 MP·ind^{-1} 2.1 ± 3.3 MP·g ww^{-1}	0.1–5 mm	10% KOH digestion for 48 hr; filtered onto a glass fiber filter (GF/C, 1.2-µm pore size)	Visual-stereomicroscope; FTIR on a random subsample of 50 particles	Golgo de Nicoya, Costa Rica	Missing from QA/QC: 1, 5, 6, 8, 9, 10,	Rojas-Jimenez et al. (2022)

(*Continued*)

Table 1. Continued.

Species	Microplastic concentration measured in tissue	Particle size	Isolation method	Detection method	Location	Criticisms	Reference
Tapes philippinarum (Manila clam)	Mean: 0.15 ± 0.20 particles·g ww^{-1} 0.97 ± 0.74 particles·ind^{-1}	Majority <300 µm	10% KOH digestion at 60 °C overnight; sieved through 20-µm sieve; lithium meta-tungstate density separation overnight; filtered onto polycarbonate membrane filters (20- µm pore size)	Visual-microscope; FTIR	South Korea	Estimated yearly intake of MP from shellfish consumption to be 212 *n*/ person per year; purchased from fish markets; five individuals per sample Missing from QA/QC: 6, 11	Cho et al. (2019)
Tegillarca granosa (blood cockle)	Mean: 5 ± 2 MP·ind^{-1} (estimated from figure 3 in study)	5 µm–5 mm, majority >250 µm	30% H_2O_2 digestion at 65 °C for 24 hr; then, at room temperature for 24–48 hr (if needed); NaCl density separation; filtered onto cellulose nitrate membrane filter (5-µm pore size, 47-mm diameter)	Visual-stereomicroscope; FTIR on subset of particles	China	1–5 individuals pooled as one replicate; density of NaCl solution not defined; tools not listed as glass (e.g., petri dish) Missing from QA/QC: 3, 6, 9, 10, 11, 12	Li et al. (2015)
	Mean: 0.12 MP·$g\ dw^{-1}$	Mean fragment: 60.01 ± 38 µm Mean filament: 530 ± 470 µm	30% H_2O_2 digestion at 60 °C for 1 hr; then at room temperature until fully digested; NaCl density separation; filtered onto cellulose nitrate membrane filter (5-µm pore size)	Visual-epifluorescence reversed microscopy	Gulf of La Spezia, Italy	Missing from QA/QC: 3, 4, 6, 10, 11, 13	Bonello et al. (2018)
Tegula atra (smooth black tegula)	Mean: 0.88 ± 0.20 particles·g ww^{-1}	<5 mm; fibers most common, followed by fragments, spheres, and films	10% KOH digestion at 60 °C overnight; filtered onto glass fiber filter paper (20-µm pore size)	Visual-microscope	Lima, Peru	Missing from QA/QC: 6, 10, 11, 13 *(based on referenced Dioses-Salinas et al. 2020)*	De-la-Torre et al. (2020)
Tegula funebralis (black turban snail or black tegula)	Mean: 9.91 ± 6.31 MP·$g\ ww^{-1}$	<5 mm	10% KOH digestion for 4 weeks	Visual-dissecting microscope	Sonoma County, California, USA	Boiled snails to euthanize-melt plastic Missing from QA/QC: 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13	Saley et al. (2019)
Terebralia sulcate (sulcate swamp cerith)	Mean: 1.75 ± 0.5 MP·g^{-1} ww (estimated from figure 4 in study)	93.3% were fibers Mean length: 1004.2 ± 464.8 µm Mean diameter: 21.8 ± 7.3 µm	10% KOH digestion at 40 °C for 48 hr; sieved through a 38-µm sieve; filtered onto a cellulose ester filter (0.45-µm pore size)	Visual-stereomicroscope; FTIR on a subset of particles	Hong Kong, China	Low percent recovery for gastropods (50%) because of shell debris on filter not removed for digestion; some discussed data does not match table data; species listed in figures 4 and 5 differ; mislabeled gastropods as crabs Missing from QA/QC: 3, 4, 6, 11, 12	Xu et al. (2020)

Thyasira debilis (northern hatchet-shell)	Mean: 2.9 MP·ind^{-1} (estimated from figure 5 in study)	33–5000 µm; highest relative abundance: 50–100 µm	Nile Red digestions: Stored in EtOH; dried at 80 °C for 1 hr; 1% NaOH digestion at 40 °C for 24 hr; NaCL density separation; filtered onto glass fiber filters (GF/F, 0.7-µm pore size) For FTIR analysis: 30% H_2O_2 digestion at room temperature for 72 hr; filtered onto ANODISCS (0.2-µm pore size, 47-mm diameter)	Visual-stereomicroscope; Nile Red staining; FTIR on most contaminated species	Terra Nova Bay (Ross Sea), Antarctica	Pooled individuals Missing from QA/QC: 3, 6, 11	Sfriso et al. (2020)
Topshells (*Phorcus lineatus* and *Steromphala umbilicaris*) *Authors pooled species	Range: 0.56–148.28 particles·g ww^{-1} 1–71 items·ind^{-1}	>50 µm	30% H_2O_2 digestion at 60 °C for 3–4 d; diluted in 1 L distilled water and filtered onto hydrophilic polyether sulfone membranes (0.45-µm pore size)	Visual-microscope; FTIR on subset of particles	North coast of Spain	Missing from QA/QC: 3, 6, 10, 11, 12	Janssens and Garcia-Vazquez (2021)
Unionidae (freshwater mussels) *Authors did not specify species	Mean: 3.5 ± 2.2 items·ind^{-1} Range: 1–7 items·ind^{-1}	≤150 µm- used to validate methodology, did not report much on particles found in field samples	69% HNO_3 and 30% H_2O_2 (4:1 v/v) digestion heated to 50 °C and stirred for 30 min; NaCl density separation; filtered onto cellulose nitrate membrane filters (5-µm pore size, 47-mm diameter)	Visual-microscope; FTIR	Pearl River Delta, China	Method development paper, tested method on some sample mussels presented here Missing from QA/QC: 3, 4, 6, 11, 12	Yu et al. (2019b)
Uroteuthis (*Photololigo*) *duvaucelii* (Indian squid)	Mean: 0.18 ± 0.48 MP·ind^{-1} 0.008 ± 0.02 MP·g ww edible tissue^{-1}	100–300 µm	10% KOH digestion at 60 °C for 24 hr; filtered onto filter paper (11-µm pore size)	Visual-stereomicroscope; FTIR	Kerala, India	Missing from QA/QC: 6, 10, 12	Daniel et al. (2021)
Venerupis philippinarum (Manila clam)	212 potential MP found in all clams Before outplant (mean): 0.16 ± 0.18 MP·g dw^{-1} After outplant (mean): 0.16 ± 0.22 MP·ind^{-1} 0.22 ± 0.31 MP·g dw^{-1}	10–5000 µm particles (largest dimension measured); majority 100–499 µm; fibers most dominant	Dried tissue digested in 10% KOH at 60 °C for 24 hr; filtered onto polycarbonate membrane filters (8-µm pore size)	Visual-compound microscope; FTIR on subset of particles	Vancouver Island, Canada	Missing from QA/QC: 3, 5, 6, 10, 11	Covernton et al. (2019)
	Mean: 0–2.92 MP·clam^{-1}	50–600 µm in diameter (fibrous, pellet and fragment) Did not examine microfibers (high airborne contamination)	10% KOH digestion at 60 °C for 48 hr; dyed with Rit Dye More to reveal synthetic polymers; filtered onto cellulose filter paper (11-µm pore size); rinsed with 30% H_2O_2	Visual-microscope; FTIR	Burrard Inlet and Baynes Sound, British Colombia	Suggest species as bioindicators for microfragments and microspheres (but then cite Ward et al. (2019a) that states they are not good bioindicators); dye can interfere with FTIR, excluded textile microfibers >0.5 mm from analysis; stated limitations for QA/QC Missing from QA/QC: 3, 4, 5, 6, 11	Bendell et al. (2020)

(*Continued*)

Table 1. Continued.

Species	Microplastic concentration measured in tissue	Particle size	Isolation method	Detection method	Location	Criticisms	Reference
Villorita cyprinoides (black clam)	Digestive gland: 22.8 items·g Gill: 29.6 items·g Overall mean of three species: 15.9 items·g tissue	Majority <2 mm; largest were 4 mm	Incubation at 50 °C for 15 min; proteinase-K enzyme digestion 50 °C for 2 hr; deproteinized at 60 °C for 20 min; filtered onto glass fiber filters (1.2-µm pore size)	Visual-stereomicroscope; µ-Raman	Southwest coast of India	Claim microplastics "in" the gills; axis labels of figure 2 do not line up; not clear from methods what was sampled Missing from QA/QC: 3, 4, 10, 11, 12	Joshy et al. (2022)
Villorita sp. (black clam)	Mean: 23 ± 20 particles·ind^{-1} Mean in "digestive" tissues (gills, intestine, stomach): 12 ± 15 particles·ind^{-1} Mean in "non-digestive" tissues (mantle, gonad, adductor, visceral tissue): 11 ± 10 particles·ind^{-1}	In "digestive" tissues: 0.012–2.85 mm In "non-digestive" tissues: 0.013–4.35 mm	10% KOH digestion at 60 °C for 24 hr; filtered onto a 20-µm mesh and then onto a filter	Visual-stereomicroscope; Raman on a subset of particles (~5%)	Vembanad Lake, India	Mention two types of filters; methods unclear Missing from QA/QC: 6, 10, 12	Nikki et al. (2021)
Yoldiella antarctica	Mean: 1.5 MP·ind^{-1} (estimated from figure 5 in study)	33–5000 µm Highest relative abundance: 50–100 µm	Nile Red digestions: Stored in EtOH; dried at 80 °C for 1 hr; 1% NaOH digestion at 40 °C for 24 hr; NaCL density separation; filtered onto glass fiber filters (GF/F, 0.7-µm pore size) For FTIR analysis: 30% H_2O_2 digestion at room temperature for 72 hr; filtered onto ANODISCS (0.2-µm pore size, 47-mm diameter)	Visual-stereomicroscope; Nile Red staining; FTIR on most contaminated species	Terra Nova Bay (Ross Sea), Antarctica	Pooled individuals Missing from QA/QC: 3, 6, 11	Sfriso et al. (2020)
Zygochlamys patagonia (Patagonian scallop)	Mean: 2.1 MP·ind^{-1} 0.3 MP·g ww^{-1}	1–5000 µm	30% H_2O_2 digestion at 65 °C for 24 hr; at room temperature for 24–48 hr; filtered onto cellulose membrane filter (5-µm pore size)	Visual-stereomicroscope; FTIR on subset of particles	Chile	Missing from QA/QC: 1, 3, 5, 6, 9, 10, 11, 13	Akoueson et al. (2020)

Taxonomic names are as in original publications, and table is organized by species. All studies were compared to a list of quality assurance and quality control measures (QA/QC; see Table 2). Each precaution was assigned a number, and if the study did not mention that QA/QC measure, the appropriate number was added to the criticism column. The numbers assigned to each measure do not signify priority over the other measures. See text in Subsection 3.5.1 under Summary Recommendations for more details on this numbering system. Detection method abbreviations: FTIR (Fourier transform infrared spectroscopy); SEM-EDX; SEM-EDS; SEM-EDAX; (scanning electron microscopy with energy dispersive X-ray spectroscopy); SEM-XRD (scanning electron microscopy with energy dispersive X-ray diffractometry); Py-GCMS (pyrolysis gas chromatography mass spectrometry).

suits during sampling, others do not mention whether non-synthetic fabrics were worn during the study (e.g., Mathalon and Hill 2014; Argamino and Janairo 2016; Waite et al. 2018; Reguera et al. 2019; Saley et al. 2019; Zhu et al. 2019; Truchet et al. 2021; Rojas-Jimenez et al. 2022). Given that fibers are the predominant type of MP found in many studies of the marine environment (e.g., Mathalon and Hill 2014; Gewert et al. 2017; Lourenço et al. 2017; Barrows et al. 2018) this issue is most concerning. It is likely that a large subset of fibers reported in many studies on shellfish could be from contamination (see for discussion: Scopetani et al. 2020; Gwinnett and Miller 2021). Microplastic contamination cannot be fully avoided, but the introduction of plastic particles during sample collection, specimen dissection, sample preparation, and analyses can be minimized or controlled with appropriate methods.

No matter the collection method, all studies in Table 1 reported that glassware was rinsed with a form of filtered water (e.g., deionized, ultra-pure) before use. This is the minimum required for tools used for collection. Simply covering cleaned glassware and tools with aluminum foil can prevent contamination and is considered a common practice in MP research; almost all the studies in Table 1 accounted for this precaution (exceptions: Van Cauwenberghe and Janssen 2014, Argamino and Janairo 2016, Renzi et al. 2018, Saley et al. 2019, Sui et al. 2020, Vieira et al. 2021).

In most studies, molluscan samples were transported on ice and frozen upon returning to the laboratory (e.g., Davidson and Dudas 2016; Ding et al. 2018; Qu et al. 2018; Su et al. 2018; Abidli et al. 2019; Doyle et al. 2019; Hermabessiere et al. 2019; Scott et al. 2019; Thiele et al. 2019; Teng et al. 2019; Webb et al. 2019; Baechler et al. 2020b; Bendell et al. 2020; Sathish et al. 2020; Zaki et al. 2021). If present, the shells of the animals were rinsed prior to dissection and freezing. Few studies digest the shell along with tissues as shells are difficult to digest and typically will not contain MP (but see Han et al. 2022). Railo et al. (2018) compared MP concentrations in shelled and unshelled mussels and found higher microfiber concentrations in unshelled mussels, suggesting that shell removal introduces contamination. Therefore, it is important to carefully rinse tissues prior to digestion to minimize contamination.

A few studies suggested the use of ethanol and formaldehyde for preserving the animals until analysis (ICES Special Request Advice 2015; Courtene-Jones et al. 2017; Karlsson et al. 2017; Lourenço et al. 2017; Bonello et al. 2018; Hermsen et al. 2018; Khan and Prezant 2018; Patria et al. 2020; Revel et al. 2020; Sfriso et al. 2020). Courtene-Jones et al. (2017) suggested that MP can withstand formaldehyde and ethanol preservation although loss of color has been observed (personal observations) in fibers after prolonged ethanol exposure. Additionally, the use of ethanol for sample preservation may interfere with chemical digestion of the samples (see for discussion: Lusher et al. 2020b), so it may be best to avoid the use of preservatives if freezing is sufficient to address the goals of the study.

3.1.2. Recommendations

The use of plastic should be avoided when collecting samples from the natural environment. Plastic bags can be replaced with aluminum foil, glass containers, or cotton, paper, or burlap bags. Aluminum and glass will contribute fewer particles that could be mistaken for microplastics than paper or burlap, but any non-plastic material would be preferable because particles could be identified as non-synthetic with spectroscopy.

In cases where plastic is unavoidable or mistakenly used, tracking can be used to account for contamination (e.g., Covernton et al. 2019). Sparks (2020) used plastic bags to transport collected animals but examined plastic particles from the bag under a microscope and removed similar particles from the analyses. Klasios et al. (2021) used polypropylene containers during the digestion step but accounted for any potential contamination in their results. Any source of potential contamination should be sampled and accounted for during processing, including gear used in sampling (see also Hermsen et al. 2018). Optimally, small pieces of each potential contaminant source should be collected, marked appropriately, examined under the microscope, and identified using a chemical identification method. For example, plastic is often used to package store-bought samples, thus the plastic wrapping should be characterized for comparison with MP found in the samples, even if the exterior of the animal is rinsed prior to dissection (Phuong et al. 2018a). If the same polymer is found in the sample and resembles the color and shape of fragments from the packaging, it can be excluded from the MP count to reduce overestimation of plastic particles. The researchers also need to consider the question addressed by their work. If the goal is to determine MP concentrations in mussels from their natural environment, then collecting seafood from the supermarket is not an appropriate

sampling strategy (e.g., Vandermeersch et al. 2015; Ding et al. 2018; Hermsen et al. 2018).

A recommended practice is to use pre-cleaned glassware and metal tools during sampling and processing. Rinsing equipment at least 3 times with a filtered water such as Milli-Q water can remove contamination, but there are other methods that can be used to efficiently remove microplastic contamination. Heating all glass and metal equipment at 450 °C will burn off MP and should be considered for most glassware and metal equipment (Rochman et al. 2015; Zhao et al. 2017, 2018). In cases where large glassware will not fit within a muffle furnace (e.g., glass carboys) or high temperature would damage metal equipment, acid-washing (e.g., Catarino et al. 2018; Joshy et al. 2022) or rinsing with acetone or ethanol (e.g., De Witte et al. 2014; Vandermeersch et al. 2015; Kazour et al. 2019; Nikki et al. 2021; Zaki et al. 2021) are reasonable alternatives. As noted above, covering cleaned equipment with aluminum foil will help prevent contamination by plastic particles and is a common practice in MP research.

To obtain a valid snapshot of any potential MP in field samples, it is best to transport animals on ice and perform dissections in the laboratory in a clean-air environment as soon as possible. Otherwise, samples should be frozen until processed to avoid issues associated with the animals gaping, which could allow plastic particles to enter between the valves or leave the mantle cavity in leaking pallial fluid (e.g., Van Cauwenberghe and Janssen 2014; ICES Special Request Advice 2015; Hermsen et al. 2018; Hamid et al. 2020; Pérez et al. 2020). Prior to isolation of tissues and chemical digestion, the exterior of the shells should be thoroughly rinsed with filtered water (e.g., Patterson et al. 2019; Wang D, et al. 2021). Whether or not to rinse the isolated tissues will depend on the question being asked. If the goal is to determine the number of plastic particles with which the bivalve has come in contact, including those captured but not ingested (e.g., in pseudofeces), then rinsing should not occur. If the goal is to determine if the MP were ingested or taken up by some other means (e.g., phagocytosis), then the tissues should be thoroughly rinsed. Thorough rinsing of the gills and mantle is important as these tissues contain numerous ciliated tracts that transport and process particles (Owen 1978; Beninger et al. 1992; Ward 1996), some of which are destined for rejection. Rinsate can be collected and analyzed separately to distinguish those MP that are on the tissues from those that are within tissues or lumina. Other methods to distinguish between plastic particles in vs on tissues are possible, such as allowing the animals to depurate for a short period of time in filtered seawater (~2 hr; Zhao et al. 2018). During depuration, pseudofeces and feces should be collected with the aid of a microscope to quantify plastic particles in these compartments (i.e., pseudofeces = particles on the tissues; feces = particles in the gut). Depuration periods of more than a couple of hours should be avoided because of increased chance of contamination with laboratory-generated MP (e.g., Van Cauwenberghe et al. 2015; Hamid et al. 2020; Klasios et al. 2021). Hermsen et al. (2018) provided comprehensive guidelines for appropriate procedures that should be used during sample collection to monitor for MP contamination. "Wipe tests" can be performed by wiping a wet filter (e.g., 47 mm diameter polycarbonate filters) on surfaces that could introduce contamination during sampling (e.g., deck or railing of boat; Zhao et al. 2017). This would be an additional monitoring effort. If any microplastics picked up by the filter matched the color, type, and shape of polymer found in a sample processed in tandem, the particle would be removed from analysis in the same way that blanks are monitored.

3.2. QA/QC measures in the laboratory

Once animals have been collected, dissected, and properly stored, the laboratory needs to be prepared for microplastic (MP) extractions. A thoroughly cleaned laboratory will reduce the chances of MP contamination. No matter the scientific question being addressed, there are simple QA/QC measures that can be taken to combat MP contamination in the laboratory. Below, common mistakes and simple strategies to prevent and account for MP contamination in the laboratory are discussed.

3.2.1. Issues and problems

There are many sources of microplastic (MP) contamination in the laboratory, particularly microfibers in the form of dust. The wearing of any synthetic clothing during the research process will likely lead to microfiber contamination. Wearing a cotton laboratory coat will help minimize this contamination and has become the minimum standard for MP research (Hermsen et al. 2018; Gwinnett and Miller 2021). Nonetheless, there are studies that do not mention whether cotton laboratory coats were worn (e.g., Karlsson et al. 2017; Leslie et al. 2017; Digka et al. 2018b; Doyle et al. 2019; Saley et al. 2019; Webb et al. 2019; Gong et al. 2021; Liao et al. 2021). A "clean" laboratory space can refer to various levels of

cleanliness from merely wiping down surfaces to having the area professionally cleaned. Wiping surfaces can leave particles behind and is not an adequate quality assurance measure, particularly if the cloth used is made of plastic fibers (Hermsen et al. 2018). Many recent studies did not perform their analysis in clean-air conditions or to include procedural blanks to account for contamination during sample processing (Argamino and Janairo 2016; Lourenço et al. 2017; Khoironi et al. 2018; Berglund et al. 2019; Fernández Severini et al. 2019; Yu et al. 2019a; McCoy et al. 2020; Sparks 2020; Gong et al. 2021; Sparks et al. 2021). Fume hoods are not considered a clean air environment because air is drawn into the hood from the room, increasing the risk of dust contamination. Therefore, the need for a blank would be even greater for studies where isolations were carried out under these conditions (examples of studies that used a fume hood: Webb et al. 2019; Yu et al. 2019b; Keisling et al. 2020; Martinelli et al. 2020; Nikki et al. 2021; Vieira et al. 2021; Zaki et al. 2021). Many studies do not mention whether the samples or tools were covered during processing (e.g., Van Cauwenberghe and Janssen 2014; Argamino and Janairo 2016; Renzi et al. 2018; Saley et al. 2019; Tahir et al. 2019; Patria et al. 2020; Sui et al. 2020; Vieira et al. 2021; Said et al. 2022), or indicate whether processing and analyses were performed under clean-air conditions (e.g., Su et al. 2016; Lourenço et al. 2017; Berglund et al. 2019; Fang et al. 2019; Jahan et al. 2019; Zhu et al. 2019; Pérez et al. 2020; Wu et al. 2020; Xu et al. 2020; Daniel et al. 2021; Fraissinet et al. 2021; Janssens and Garcia-Vazquez 2021; Liao et al. 2021; Patterson et al. 2021; Truchet et al. 2021; Joshy et al. 2022; Lee et al. 2022). Several studies also do not indicate whether an open petri dish or filter was placed on the bench top to account for airborne contamination of plastic particles (e.g., Digka et al. 2018b; Kazour et al. 2019; Phuong et al. 2019; Dowarah et al. 2020; Oliveira et al. 2020; Wardlaw and Prosser 2020; Teichert et al. 2021; Joshy et al. 2022). While this may be a secondary precaution, it can help the researcher keep track of dust fallout.

Microplastic contamination can be introduced through reagents used in the study, and many studies do not filter reagents prior to use (e.g., Ding et al. 2018; Renzi et al. 2018; De-la-Torre et al. 2019; Reguera et al. 2019; Teng et al. 2019; Xu et al. 2020; Cozzolino et al. 2021; Vieira et al. 2021). Filters themselves can also be a source of contamination and most studies do not account for this possibility (e.g., Bråte et al. 2018b; Gedik and Eryasar 2020; Lozano-Hérnandez et al. 2021). Such sources of plastic particles introduced during laboratory preparation may falsely inflate the number of MP, particularly microfibers, found in biotic samples. For this reason, some researchers do not include microfibers in their analysis (e.g., Foekema et al. 2013; Avio et al. 2015b; ICES Special Request Advice 2015; Bendell et al. 2020). In addition to procedural blanks, positive controls are necessary to determine how many MP can be recovered from the samples (Hurley et al. 2018; Provencher et al. 2020). By adding a known number of plastic particles of a given size range to control samples, the losses throughout preparation, processing, and analysis can be determined (Hermsen et al. 2018; Plee and Pomory 2020). Unfortunately, only 23 of the reviewed studies included positive controls (Davidson and Dudas 2016; Li et al. 2016, 2018b, 2020; Phuong et al. 2018a; Waite et al. 2018; Fang et al. 2019; Gomiero et al. 2019; Jahan et al. 2019; Yu et al. 2019b; Bendell et al. 2020; Jang et al. 2020; Ribeiro et al. 2020; Sathish et al. 2020; Sfriso et al. 2020; Wu et al. 2020; Xu et al. 2020; Cho et al. 2021; Fraissinet et al. 2021; Nalbone et al. 2021; Patterson et al. 2021; Sparks et al. 2021; Zaki et al. 2021). All other studies (Table 1) failed to include positive controls or even reference the percent recovery determined by previous studies using the same method. Failure to use appropriate controls introduces doubt that the reported MP concentrations in the sampled molluscs are accurate or comparable across a broad range of publications. Whereas many studies have adopted basic QA/QC measures such as rinsing glassware and running procedural blanks along with field samples, others do not mention QA/QC precautions but report MP in molluscan tissue (e.g., Ibrahim et al. 2016; Rosas-Luis 2016; Al Harma and Patria 2019; Fitri and Patria 2019; Schessl et al. 2019; Tahir et al. 2019; Akoueson et al. 2020; Hamid et al. 2020; Nakao et al. 2020; Patria et al. 2020; Zhu et al. 2020).

3.2.2. Recommendations

Simple QA/QC measures include covering samples, glassware and dissection equipment with aluminum foil when not in use (e.g., Li et al. 2018b; Zhao et al. 2018), and wiping down benches with ethanol and cotton cloths. Even with these measures, particles can detach from cleaned surfaces, equipment, or the cloth itself as mentioned previously (Hermsen et al. 2018). Cotton laboratory coats help to reduce microfiber contamination from synthetic clothing and have become the standard for MP research. As the field develops, suggestions have been made for a full-coverage solution such as brightly colored, cotton jumpsuits (Hermsen et al. 2018; Gwinnett

and Miller 2021). Laminar flow cabinets, biosafety cabinets, and clean air rooms provide a good environment for handling and preparation by preventing MP-laden dust from settling on the samples (Hermsen et al. 2018; GESAMP 2019). If the work is not performed under clean air condition, it becomes even more important to monitor for dust contamination by placing wet filters or open petri dishes on bench tops to collect plastic particles that settle out of the air or are shed from clothing. Although all studies reviewed rinsed their glassware with some form of filtered water (e.g., deionized water), the number of rinse steps is not mentioned often. Rinsing glassware three times with a filtered form of water should remove most plastics. Burning off plastics using a muffle furnace is an efficient way to clean glassware and tools. Alternatives include employing a rigorous cleaning system such as acid washing (e.g., Yu et al. 2019a; Joshy et al. 2022) or multiple rinse steps with water and ethanol (e.g., Hermabessiere et al. 2019; Kazour et al. 2019; Pazos et al. 2020; Wakkaf et al. 2020; Teichert et al. 2021) as described above (Sampling and Preservation suggestions; De Witte et al. 2014; Rochman et al. 2015; Vandermeersch et al. 2015; Zhao et al. 2017, 2018). Prior to analyses, prefiltering liquid reagents through muffled glass fiber filters into clean glassware is another simple step to limit procedural contamination and can be monitored in the form of procedural blanks (as in De Witte et al. 2014; Van Cauwenberghe and Janssen 2014; Vandermeersch et al. 2015; Li et al. 2015, 2016, 2018a, 2018b; Kolandhasamy et al. 2018; Qu et al. 2018; Su et al. 2018; Waite et al. 2018). A submicron filter or one below the size range of the particles being examined is preferable. Clean equipment and filtered reagents should be covered with aluminum foil and placed in the clean air environment before use.

Positive controls and procedural blanks are also an important part of QA/QC. Procedural blanks are important for identifying contamination that occurred during sample preparation, so that the particles can be removed from the analysis. Procedural blanks are considered a standard quality control practice (Brander et al. 2020; Cowger et al. 2020a). This step was reported to have been done in most studies (see Table 1). Blanks should be run in tandem with real samples. An extra precaution would be to place wet filters in open glass petri dishes on bench tops near microscopes and other equipment when processing samples to monitor MP in settling dust (examples of potential dust contamination: Davidson and Dudas 2016; Wesch et al. 2016; Renzi et al. 2018), or periodically wiping surfaces with a clean filter. Positive controls need to be included to understand how many particles can be recovered using the described methods (Mintenig et al. 2018; Rochman et al. 2019). Ideally, new positive controls should be used for every study, even if the researchers have used the method previously. The percent recovery of the method should be determined to assess what MP (different sizes, shapes, and polymer types) and how many will be recovered successfully. The particles used should reflect the objectives of the study. For example, if the purpose of the study is to identify particles 40–150 μm in size then particles in that size range should be used in the positive controls. The percent recovery is influenced by several factors including polymer density if a density separation is used, and particle size and filter pore size if filtration is used for extraction and isolation (see below). At the very least, a percent recovery should be reported from a previous study that used the specific method, ideally for several different MP sizes, shapes, and polymer types. Percent recovery must be reported for each method so that the efficiency with which different types of MP can be recovered is known.

3.3. Microplastic extraction and isolation

During the extraction stage, chemical digestion is used to break down the tissue samples and isolate the microplastics (MP). The more efficient the digestion, the easier MP identification will be as organics can interfere with spectroscopic methods (Primpke et al. 2020a). The literature has been flooded with different MP extraction methodologies over the last decade. Different digestion methods reported in the literature include acidic (e.g., nitric acid, hydrochloric acid, perchloric acid; Cole et al. 2014; Enders et al. 2017; Karami et al. 2017), enzymatic (e.g., proteinase K, trypsin; Cole et al. 2014), oxidative (e.g., hydrogen peroxide; Mathalon and Hill 2014; Zhao et al. 2018), and alkaline (e.g., sodium hydroxide, potassium hydroxide; Foekema et al. 2013; Kühn et al. 2017). All of these methods have advantages and disadvantages depending upon factors such as the molarity, concentration, and temperature of the chemical-digestion procedure.

Tissue digestions are often coupled with a density separation to isolate lower-density plastic particles from denser inorganics such as sand. If the digestion is successful and there is little inorganic material, a density separation step may not be necessary. The following section discusses the advantages and limitations associated with different extraction and isolation methods for MP.

3.3.1. Issues and problems

Animal tissue and other organic components can interfere with spectroscopic analysis and identification of plastic polymers making the removal of these materials prior to identification an important step (Cabernard et al. 2018; Lusher et al. 2020a, 2020b; Primpke et al. 2020a). Many studies digested several animals together instead of processing individual animals as replicates (e.g., Li et al. 2015; Su et al. 2016, 2018; Naji et al. 2018; Berglund et al. 2019; Cho et al. 2019; De-la-Torre et al. 2019, 2020; Teng et al. 2019; Keisling et al. 2020; Pazos et al. 2020; Ríos et al. 2020; Sparks 2020; Vieira et al. 2021). This practice not only masks variation in plastic-particle load between samples but, depending on how the data are treated, can result in pseudoreplication. There are numerous options for chemical digestion of organics, however, many can cause damage to different polymers and synthetic coloring. For example, although efficient at digesting organics (e.g., 94–98%; Lusher et al. 2020b), compounds such as nitric acid (HNO_3) have been shown to dissolve or melt polystyrene and polyamide particles, common polymers found in environmental samples (Claessens et al. 2013; Dehaut et al. 2016; Lusher et al. 2020b). In particular, acidic digestion in combination with boiling is too aggressive for many types of microplastics (MP). In recent years, several studies have used acidic digestion methods in combination with boiling despite evidence that these methods destroy most of the polymers in their samples (Claessens et al. 2013; De Witte et al. 2014; Van Cauwenberghe and Janssen 2014; Van Cauwenberghe et al. 2015; Vandermeersch et al. 2015; Argamino and Janairo 2016; Santana et al. 2016; Thushari et al. 2017; Murphy 2018; Fernández Severini et al. 2019; Naidu 2019; Webb et al. 2019). Even boiling tissue without acids, such as for euthanasia, can damage plastic particles (as in Saley et al. 2019). Oxidative methods also can destroy some polymers and affect synthetic colors, making some MP transparent (Nuelle et al. 2014). Nuelle et al. (2014) found that after seven days of exposure to 35% hydrogen peroxide (H_2O_2), particles of polyacrylate, polycarbonate and polypropylene were more transparent, polyethylene terephthalate (PET) became discolored, and linear low-density polyethylene fragmented. Color is often used as a categorical variable for sorting MP from environmental samples (e.g., Klasios et al. 2021), but as evidence shows, color can be affected during processing. Alkaline digestions can alter and destroy condensation polymers such as polyester or polyamides (as shown by Dehaut et al. 2016; Hurley et al. 2018). Temperatures over 60 °C can melt polyethylene-based microspheres and should be avoided (see also Munno et al. 2018; Lusher et al. 2020b). Claessens et al. (2013) performed a series of acidic, basic, and oxidative digestions in various combinations on the tissues of blue mussels, heating them all to 60 °C, and then, to 100 °C. Within the samples, polystyrene spheres melted, and nylon fibers were destroyed. Despite this outcome, the study suggested using HNO_3 acid for 2 hr and then boiling for 1 hr to digest organics and isolate MP from environmental samples. Many studies have used digestion temperatures over 60 °C after it been shown multiple times that these temperatures are damaging to multiple polymer types (e.g., Su et al. 2018; Waite et al. 2018; Abidli et al. 2019; Martinelli et al. 2020; Pazos et al. 2020; Chinfak et al. 2021). The use of destructive digestion methods can result in underestimating the number of plastic particles in samples and biasing counts in favor of polymers that are able to withstand aggressive chemical digestion. Additionally, particle fragmentation caused by some digestion methods can also lead to inaccurate counts of MP in environmental samples. Such issues are outlined below.

As summarized above, the concentration of reagents can influence digestion efficiency and the destruction of polymers. Higher concentrations and temperatures result in more efficient digestions (Lusher et al. 2020b), but at a cost. Hydrochloric acid (HCl) destroys nylon and polyester at concentrations above 37% but fails to digest tissue efficiently at lower concentrations (5–20%) making it a poor option (Nuelle et al. 2014; Karami et al. 2017). Enders et al. (2017) tested multiple digestion methods on fish stomachs and showed that the acid mixture recommended by ICES Special Request Advice (International Council for the Exploration of the Sea 2015) of 69% nitric acid:70% perchloric acid (69% HNO_3:70% $HClO_4$) altered or destroyed polyamide, polyurethane, rubber elastomers, polyvinyl chloride, and polymethyl methacrylate even before heat was applied. Therefore, studies that have used concentrated acids during digestion likely destroyed certain polymers in their samples and do not provide a complete picture of MP in the environment (e.g., Van Cauwenberghe and Janssen 2014; Van Cauwenberghe et al. 2015; Thushari et al. 2017; Murphy 2018; Fernández Severini et al. 2019; Zaki et al. 2021). At higher concentrations, H_2O_2 foams excessively, lowering the digestion efficiency and leading to potential particle loss (Claessens et al. 2013; Karami et al. 2017). At 30%, H_2O_2 degrades nylon and oxidizes polyester particles at 50 °C (Karami et al. 2017). As H_2O_2 becomes unstable overtime, oxidation causes temperatures to rise. The addition of an iron

catalyst has been suggested as it combats the temperature rise to reduce the risk of polymer damage (e.g., Fenton's reagent; see Lusher et al. 2020b for more information). Even with an iron catalyst, wet peroxide oxidation techniques can be arduous and must be carefully monitored to regulate the temperature properly (Lusher et al. 2020b). Many studies have used high concentrations of H_2O_2 (30% or higher) and heat for tissue digestion in shellfish (Li et al. 2015; Bonello et al. 2018; Naji et al. 2018; Su et al. 2018; Zhao et al. 2018; Teng et al. 2019; Martinelli et al. 2020; Pazos et al. 2020; Ríos et al. 2020; Chinfak et al. 2021). For alkaline digestion, a 10% potassium hydroxide (KOH) solution has been used in several studies (Foekema et al. 2013; Dehaut et al. 2016; Kühn et al. 2017); however, 10% KOH has been shown to discolor polyamides at 50 °C and 60 °C (Karami et al. 2017). Despite discoloration, lower concentrations of KOH (≤10%) do not appear to affect polymer identification by means of micro-Raman spectroscopy (μRaman) or micro-Fourier transform infrared spectroscopy (μFTIR;Dehaut et al. 2016; Munno et al. 2018). A saturated KOH solution lowers Raman spectral quality and causes spectral deviations compared to undigested polymers, but the particles can still be identified (Karami et al. 2017; Munno et al. 2018). For example, at high concentrations (224 g·L^{-1}) KOH can alter the FTIR spectrum of polystyrene foam (Munno et al. 2018). Sodium hydroxide (NaOH) is another common alkaline solution used for the digestion of organic matter. Ten-molar NaOH can degrade polycarbonate and PET (Dehaut et al. 2016; Hurley et al. 2018), two polymers commonly found in the marine environment. Ibrahim et al. (2016) and Mayoma et al. (2020) isolated MP from bivalve tissues using 10 M NaOH, citing its high digestion efficiency and identified both polyethylene and polyamides. Although the choice of any digestion method comes with tradeoffs, it is unclear if either of these studies considered the potential loss of polycarbonate or PET. Ibrahim et al. (2016) mentioned no precautions to prevent contamination, and neither study mentioned testing their NaOH digestion method on any polymer type. The lack of methodological information in many of the studies reviewed makes it impossible to determine what results are reliable and whether the researchers considered best practices.

Inorganic material can also interfere with spectroscopic analysis, and density separation is often used after chemical digestion to isolate MP from inorganic particles (e.g., silt, sand). Tissues from deposit feeding animals, for example, may have higher concentrations of inorganic material that need to be removed with one or more density separation steps. All of the studreviewed that used density separation, employed two saline-based steps to separate MP from the inorganic particles, with the exception of Ibrahim et al. (2016) who used one and a water-separation as their second step. Various time frames were used without explanation, and many authors failed to mention the density of the solution used for separation (see Table 1). Of these studies, 14 did not report the density of the solution used in their method (Li et al. 2015, 2018a, 2021; Vandermeersch et al. 2015; Bonello et al. 2018; Phuong et al. 2018a; Birnstiel et al. 2019; Tahir et al. 2019; Webb et al. 2019; Yu et al. 2019b; Martinelli et al. 2020; Zhu et al. 2020; Vieira et al. 2021; Zhu et al. 2021). A saturated NaCl solution (density = 1.2·g cm^{-3}), which can isolate polymers such as polystyrene, polyamides and acrylics, was used in many studies (Mathalon and Hill 2014; Li et al. 2015, 2016, 2018a, 2018b; Ibrahim et al. 2016; Avio et al. 2017; Bonello et al. 2018; Bour et al. 2018a; Kolandhasamy et al. 2018; Qu et al. 2018; Abidli et al. 2019; Birnstiel et al. 2019; Fang et al. 2019; Yu et al. 2019b; Baechler et al. 2020a, 2020c; Jang et al. 2020; Martinelli et al. 2020; Patria et al. 2020; Zhu et al. 2020; Chinfak et al. 2021; Nalbone et al. 2021; Vieira et al. 2021; Zaki et al. 2021). Ibrahim et al. (2016) followed the NaCl separation with a freshwater separation to isolate polymers with lower densities like polypropylene, ethyl vinyl acetate, and polyethylene (<0.98 g cm^{-3}; Hurley et al. 2018). Plastics typically found in the marine environment range in densities from 0.8 to 1.4 g cm^{-3} and are differentially separated by NaCl (Hidalgo-Ruz et al. 2012). Selecting a solution with too low a density, therefore, can introduce bias as denser polymers would not be included in the MP counts. A potassium iodide solution (KI, density = 1.6·g cm^{-3}) was used by three studies and would separate polyoxymethylene along with less dense polymers (Phuong et al. 2018a, 2019; Gündoğdu et al. 2020; Uddin et al. 2020). Lithium meta-tungstate (LMT, density = 1.6·g cm^{-3}) was used in one study and would isolate the same range of densities as KI (Cho et al. 2019). A few other studies used a denser sodium iodide solution (NaI, density = 1.8·g cm^{-3}; Zhao et al. 2018; Gomiero et al. 2019; Jahan et al. 2019; Patterson et al. 2019; Bagheri et al. 2020; Keisling et al. 2020; Sathish et al. 2020; Wakkaf et al. 2020) which is expensive, and a health hazard, but can isolate denser polymers such as polyester (Claessens et al. 2013; Kedzierski et al. 2017). Even with this method, some very dense polymers such as Teflon can be lost (Lusher et al. 2020b). Density separations add time and effort to method protocols and

are better suited for sediment extractions where inorganic particles can interfere with MP sorting under the microscope. Additionally, some salts can be costly (Quinn et al. 2017). Fouling and biofilms alter particle densities and interfere with density separations, adding to the long list of limitations associated with MP isolation.

The final step in MP extraction and isolation is vacuum filtration onto a filter for visual identification and chemical validation. Filters can clog during this process, and the degree of blockage is dependent on the efficacy of chemical digestion, the volume and temperature of digestate filtered, and pore size of the filter (Claessens et al. 2013; Dehaut et al. 2016). Filter pore size will also affect the size of plastic particles isolated. Studies presented in Table 1 used a variety of filters with pore sizes ranging from 0.2 µm to 500 µm without explanation (e.g., Ibrahim et al. 2016; Pazos et al. 2020) making it difficult to compare the number of smaller particles found in samples across studies.

3.3.2. Recommendations

There have been numerous methodologies published for the extraction of microplastics (MP) from tissues (e.g., Cole et al. 2014; Dehaut et al. 2016; Karami et al. 2017; Munno et al. 2018), and even more papers reviewing, testing, and discussing the validity of the methods (Claessens et al. 2013; Dehaut et al. 2016; Lusher et al. 2017c, 2020a, 2020b; Hermsen et al. 2018; Dellisanti et al. 2023). As the field has developed, multiple attempts have been made to standardize extraction methods, with few suggesting the use of destructive chemical methods (e.g., Claessens et al. 2013; ICES Special Request Advice 2015; Vandermeersch et al. 2015). Several factors make it difficult to establish standard methods across animal species. For example, digestion efficiency can vary across species using the same method. Similar sized tissues of the mussel (*Mytilus edulis*) and oyster (*Crassostrea virginica*) are differentially digested using the same method (15% H_2O_2 and 10% KOH; personal observation). Altered digestion procedures are needed for tissues of salmon compared to herring because of differences in fat and oil content (Thiele et al. 2019). Regardless of the extraction method chosen, individual animals should be treated as replicates and digested and processed separately in order to capture the true variation in MP concentration among samples. This may be a tradeoff between the resources needed to process a larger number of samples and the study objective. All procedures should be validated with positive controls prior to conducting the study and researchers should follow the same reporting guidelines and QA/QC measures (Table 2; Hermsen et al. 2018; Rochman et al. 2019; Cowger et al. 2020a). The chosen method should address the research question at hand and minimize the number of tradeoffs necessary to achieve a reasonable answer.

The choice of a digestion method will depend on the complexity of the matrix. Simple steps can be taken to increase the speed and efficiency of the digestion such as processing animals individually to reduce tissue mass, finely chopping tissues before digestion, using a larger volume of reagent, or consistently mixing or vortexing the digestate. There is a growing body of literature regarding which chemical digestion methods are destructive and should not be used, such as acids and high temperatures. Yu et al. (2019b) successfully recovered and identified plastic particles in spiked biotic samples using a combination of nitric acid and hydrogen peroxide at 50 °C. They suggested that without boiling, nitric acid may be used to effectively recover MP from field samples, but numerous other studies have reported major losses and deformed plastic particles when using acid digestion methods (see above section). Although acids have a high digestion efficiency, the consequences of particle loss are too high. It is best to avoid the use of acid for chemical digestion of organic matter, as recommended by numerous papers (e.g., Nuelle et al. 2014; Enders et al. 2017; Karami et al. 2017; Munno et al. 2018). With the addition of Fenton's reagent at low temperatures (≤50 °C), H_2O_2 has been successfully used for complex matrices (Lusher et al. 2020b). Samples must be monitored when using H_2O_2 to avoid unplanned temperature increases as a result of chemical reactions. Use of low concentration (<35%) avoids excessive polymer damage and foaming and does not affect Raman spectra (Zhao et al. 2017). Ten percent KOH has been shown to be effective and cause minimal damage to plastic polymers, particularly at lower temperatures of <50 °C (Rochman et al. 2015; Dehaut et al. 2016; Karami et al. 2017; Bråte et al. 2018b; Hermabessiere et al. 2019; Lusher et al. 2020b). Although, Dehaut et al. (2016) found 10% KOH to be efficient at digesting mussel tissue with little to no impact on subsequent Raman spectra when used at 60 °C. Gündoğdu et al. (2020) used a previously tested solution of 30% KOH:NaClO with good digestion efficacy and no effects on Raman (Enders et al. 2017). Procedures using enzymes are appealing compared to acid, alkaline, and oxidative methods because the digestion can be run at a neutral pH and moderate temperatures, leaving polymers unaffected (Cole et al.

2014; Qiu et al. 2016; Courtene-Jones et al. 2017; von Friesen et al. 2019). For example, enzymatic digestions with proteinase K (Cole et al. 2014) and trypsin (Courtene-Jones et al. 2017) can be used to digest biological material with efficient recovery and without the destruction of plastic polymers. Enzymatic digestions are expensive and time consuming, and therefore, are not as common in the literature. Such drawbacks are not unique to enzymatic techniques. There are disadvantages to almost all current chemical digestion methods (Dehaut et al. 2016; Lusher et al. 2017a), and there is a need for further testing and validation of digestion methods. It is clear, however, that the use of reagents and methods that are known to damage polymers, such as boiling plastics, should be avoided.

Density separation methods may also need to be used to remove inorganic material, although adding more steps to any method makes it more complex and can lead to higher contamination and lower recovery of MP (Hidalgo-Ruz et al. 2012; GESAMP 2019). A saturated sodium chloride (NaCl) solution is the most common, low-cost option used to isolate particles less dense than 1.2 g·cm^{-3}. Denser salts such as sodium iodide and zinc chloride are used to recover denser MP (e.g., polyvinyl chloride; Quinn et al. 2017; Sui et al. 2020). The reagent chosen for a density separation should be picked based on the question asked, compatibility with other reagents used, and only if it improves the isolation method.

Collection of MP onto a filter typically follows digestion and density separation steps, and the choice of filter is another important consideration. There are several different types of filters with various pore sizes; 5 µm being fairly common, although some studies use larger (e.g., 20 µm) or smaller (e.g., 0.45 µm) pore sizes. Comparing results across different studies would be more reliable if the selected filter type and pore size were standardized (Metz et al. 2020). In concert with this, researchers must consider the identification method used because methods have different spatial resolutions and are influenced by the filter composition (discussed below). In theory, µFTIR can identify particles >10 µm, but µRaman has a spatial resolution below 1 µm (Lenz et al. 2015). In reality, the lower size limits are set to 20–50 µm for µFTIR or 10 µm with µRaman to make more accurate identifications (State Water Resources Control Board 2021a, 2021b). Filters can introduce contamination just as easily as any other equipment. Therefore, filters must be checked for contamination under a microscope before use and cleaned if necessary (Zhao et al. 2017). Filters can be sonicated with methanol or ethanol (70%) to remove contamination or combusted if they are made of glass fibers to prevent contamination.

A final recommendation for the extraction of MP from tissues and other samples is that all methods should be tested and validated before use by checking digestion efficiency and percent recovery of relevant polymer types and shapes using positive controls. Recovered control particles should then be examined for damage and tested for polymer degradation using methods such as µFTIR or µRaman spectroscopy (e.g., Dehaut et al. 2016; Munno et al. 2018; Yu et al. 2019b). Such steps are important to report in support of extraction methods, particularly because MP abundance is often under 10 particles per sample (Hermsen et al. 2018; Lusher et al. 2020b). Methods can vary depending on the target species and user, which contributes to the disparity of results reported in the literature. Whereas it is important to alter methods so that they work for the target species, it is also essential to meet scientific standards by testing and reporting recoveries, limitations, QA/QC measures taken and reasonable explanations for the method chosen. No method is perfect and there will be tradeoffs. While a complete digestion of organic tissue allows for a more efficient polymer identification, a method that results in an incomplete digestion will not destroy more fragile polymers.

3.4. Microplastic identification

Once the suspected microplastics (MP) have been isolated from the samples, proper identification is the next challenge. While particles may need to be isolated individually, MP should be identified using some form of chemical characterization. Spectroscopy provides a distinctive fingerprint that can be compared to known polymer spectra for a more accurate characterization and is the predominant method used in environmental MP research. There are tradeoffs when using any form of spectroscopy, as there are for every step of MP research, but all spectroscopic methods surpass visual identification alone.

3.4.1. Issues and problems

Using visual identification as the sole criterion to identify microplastics (MP) is a common method described in the literature (e.g., Argamino and Janairo 2016; Davidson and Dudas 2016; Rosas-Luis 2016; Santana et al. 2016; Bonello et al. 2018; Renzi et al.

2018; Al Hamra and Patria 2019; Fernández Severini et al. 2019; Fitri and Patria 2019; Saley et al. 2019; Schessl et al. 2019; Tahir et al. 2019; De-la-Torre et al. 2020; Hamid et al. 2020; Mankin and Huvard 2020; Patria et al. 2020; Pazos et al. 2020). Visual identification of MP is subjective, inaccurate for particles smaller than 500 µm, and results in high rates of misidentification (ca. 20–99% error rate; Hidalgo-Ruz et al. 2012; Lenz et al. 2015; Löder and Gerdts 2015; Song et al. 2015; Qiu et al. 2016; Comnea-Stancu et al. 2017; Primpke et al. 2017; Shim et al. 2017; Kroon et al. 2018; Cowger et al. 2020b; Lusher et al. 2020a). Visual sorting and counting of MP introduces human error, including bias for easily identified plastic particles (e.g., brightly colored fibers and fragments; Qiu et al. 2016) and incorrect identification of natural particles (e.g., fragments of algal filaments and shells) as synthetic. For example, particles are incorrectly labeled as MP 32% to 52% more often when using visual identification over chemical characterization techniques (Pyrolysis gas chromatography coupled with mass spectrometry (GC/MS) and µRaman; Dekiff et al. 2014; Lenz et al. 2015). It may be necessary to sort particles visually before verifying with spectroscopy if the available instrument does not have imaging or scanning technologies, or if filtered samples exhibit high levels of background noise (e.g., incompatible filter choice, high number of inorganic particles, incomplete digestion of organics). Although pre-sorting particles for these reasons is valid, researchers should be aware that the number of plastic particles ultimately identified may be underestimated. Visual sorting also can increase the amount of dust contamination in the sample because of longer air exposure times. Another common practice that can lead to errors in estimating MP abundance is visually analyzing only a small portion of each sample (<50% of the sample; e.g., Scott et al. 2019; Akoueson et al. 2020; Baechler et al. 2020a, 2020c; Dowarah et al. 2020; Gündoğdu et al. 2020; McCoy et al. 2020; Oliveira et al. 2020; Pérez et al. 2020; Xu et al. 2020; Janssens and Garcia-Vazquez 2021; Klasios et al. 2021; Nalbone et al. 2021; Nikki et al. 2021; Zaki et al. 2021; Lee et al. 2022). Although many studies are limited by processing time and analytical cost, estimating concentrations from subsamples can result in inaccurate concentration as MP abundance can be variable between samples and subsamples. Due to the subjective nature of particle picking, the reported MP concentrations could be slightly underestimated (likely <10% underestimation; Qiu et al. 2016; Lusher et al. 2020a). This is a tradeoff that is often made due to limited resources for analysis and automated identification processes. Finally, there are no standard reference libraries for the visual identification of environmental MP and many laboratory groups are self-trained, causing large discrepancies across studies (Cowger et al. 2020b).

Investigators limited by the cost of spectroscopic analyses often turn to physical characterization methods. The hot-needle method is used in addition to visual identification as an inexpensive alternative to spectroscopy (De Witte et al. 2014; Vandermeersch et al. 2015; Karlsson et al. 2017; Waite et al. 2018; Berglund et al. 2019; Keisling et al. 2020; Sparks 2020; Sui et al. 2020). The particles are identified as plastic based upon melting point. This method is slow, will only work if the needle remains hot, can only be used on larger particles that can be visually sorted (≥200 µm, or >500 µm for a more conservative estimate), cannot differentiate between specific polymers, and can misidentify natural materials that have similar melting points to plastic (e.g., natural rubbers; Lusher et al. 2017a, 2017b). Nile red is another method used to stain and identify plastic particles in samples (Shim et al. 2016; Maes et al. 2017). This method should be used with caution, however, as it cannot differentiate between specific polymers and can also stain organic particles like wood and chitin leading to an overestimation of MP (Lusher et al. 2017a; Araujo et al. 2018; Nel et al. 2021; de Guzman et al. 2022). Nile red also is sensitive to lipids making it a poor choice for a complex sample such as molluscan tissue. Use of both Nile red and the hot-needle method introduce doubt with the accuracy of MP counts. Density separations can be used to isolate plastics based on density, providing a low-cost option for separating out polymer groups (Barnett et al. 2021), but none of these methods should be used as the sole means to identify MP in future research.

As has been stated in many different studies and reviews, spectroscopy is the preferred method for polymer characterization, as it has been shown to be very accurate (>95% accurate identification of known plastic polymers; Langknecht et al. 2023). Whereas it can be costly and requires training, spectroscopy makes it possible to determine the polymer composition of the particle, thereby providing more accurate MP counts. There are limitations to each chemical characterization method such as spectral interference as a result of degraded polymers, biofilms, and adsorbed contaminants (see Cowger et al. 2020b; Lusher et al. 2020a; Primpke et al. 2020a). Additionally, library reference spectra are typically based on virgin polymers making it difficult to find a distinctive

library match for degraded environmental plastics (Araujo et al. 2018; Chabuka and Kalivas 2020). Library matching is based on a correlation which can vary for the same polymer with different molecular and conformational characteristics (Mecozzi et al. 2016). Polyethylene, for example, can have an amorphous and crystalline structure, both of which produce different spectra (Mecozzi et al. 2016). Identification is further complicated by weathering, additives, other chemical contaminants, and any inorganic or organic debris attached to the sample extracted from a living organism (Fotopoulou and Karapanagioti 2012; Mecozzi et al. 2016; Chabuka and Kalivas 2020). The composition of the filter on which the MP are collected can also introduce interference. For example, glass fiber filters mask transparent and white fibers from visual identification and are incompatible when analyzing particles in reflection mode with μFTIR (Lusher et al. 2020a). These issues complicate polymer identification. Currently, there is no standard for reporting a minimum acceptable percent match with library spectra, or "hit-quality index" (Renner et al. 2019; De Frond et al. 2021). If the reviewed studies did report an acceptable minimum match, it fell between 60% (e.g., Phuong et al. 2019; Zhu et al. 2019; Nakao et al. 2020; Zhu et al. 2020) and 80% (e.g., Patterson et al. 2019, 2021; Bendell et al. 2020; Sathish et al. 2020), often with no explanation as to why this percentage was chosen. Expertise of the researcher can affect the validity of a match. For example, a spectrum might have a low match value, but if the peaks are indicative of a certain polymer, the user may identify it as such (see Renner et al. 2019). Spectra can be manipulated (e.g., baseline correction, smoothing) to improve the hit-quality index. Over modification can, however, lead to loss of important peaks, and very few studies disclose how their spectra were treated prior to library matching (see Renner et al. 2019).

Gas chromatography combined with mass spectroscopy (GC/MS) provides polymer identification and mass quantification. There are multiple forms of GC/MS available depending on the research questions. Pyrolysis-GC/MS is a thermochemical technique that can identify both the polymer and organic additives (Hermabessiere et al. 2018). This technique was originally used to analyze one particle at a time with mass limitations (mass limit of 0.5 mg; Nuelle et al. 2014). Each particle was manually inserted into the pyrolysis tube; a time-consuming process (Fries et al. 2013; Löder and Gerdts 2015; Dümichen et al. 2017; Mai et al. 2018). Pyrolysis-GC/MS has now been developed for whole sample mass quantification and is used to study low molecular weight components in a sample. Thermal-desorption-GC/MS can be used to study higher molecular weight components (Dümichen et al. 2015; Mai et al. 2018). With both techniques, the analyzed material is destroyed, and the organic matrix of samples can interfere with peaks and influence calibration (Löder and Gerdts 2015; Fischer and Scholz-Böttcher 2017; La Nasa et al. 2020). Additionally, because polymer composition can be altered if plastic particles are heated prior to analysis, researchers need to select appropriate chemical digestion and MP isolation procedures carefully (La Nasa et al. 2020). GC/MS does not determine particle size, shape or provide particle counts, making it difficult to compare results using this method with those using other techniques (Dümichen et al. 2015; Lusher et al. 2020a). If, however, mass quantification of polymers is the research goal, then GC/MS methods would be the best way to achieve accurate results.

Scanning electron microscopy and energy dispersive spectroscopy (SEM/EDS), often combined with X-ray diffraction, can be used in MP research for elemental identification purposes. The technique can differentiate carbon-based particles from minerals, but cannot be used to determine if the particle is in fact plastic (Li et al. 2016; Wang et al. 2017; Abbasi et al. 2018; Naji et al. 2018; Vieira et al. 2021). SEM/EDS has been used to prescreen particles before analysis and definitive polymer identification by means of μRaman or μFTIR, as both spectroscopic methods are time consuming (Wang et al. 2017; Vieira et al. 2021). SEM imaging is beneficial for investigating the physical characteristics of particles as well, and can be used to prescreen for MP in a mineral-dense sample.

Micro-Raman spectroscopy (μRaman) is a common method for MP identification but does have limitations. μRaman is sensitive to fluorescence from organic matter, making it difficult to collect distinctive spectra in environmental samples that have not been completely digested (Cabernard et al. 2018). The background fluorescence can create noise that masks parts of the MP spectrum, restricting particle identification (Käppler et al. 2016). μRaman spectroscopy can be used to identify particles as small as 10 μm, providing a better size resolution than μFTIR, but longer processing times are required (e.g., 38 hr for μRaman imaging compared to 20 min for μFTIR imaging; Käppler et al. 2016; Elert et al. 2017). Pigments or fillers associated with plastics can also produce a Raman scattering that is stronger than the polymer matrix of particle being analyzed, thus, masking its composition (Lenz et al. 2015; Munno et al. 2018; Lusher et al. 2020a).

Micro-Fourier transform infrared spectroscopy (μFTIR) is one of the most used spectroscopy methods for MP analysis. Particles as small as 20–50 μm can be identified using different modes of μFTIR, which is larger than the size that can be analyzed using μRaman. Therefore, μFTIR would not be the preferred method if investigating the smallest plastic particles that are often neglected in MP research (Harrison et al. 2012; Käppler et al. 2016; Primpke et al. 2017; Cabernard et al. 2018). Käppler et al. (2016) also found a significant underestimation in quantifying plastic particles using μFTIR transmission imaging when compared to μRaman, particularly for plastics <20 μm. Although even with μRaman, particles under 10 μm are not analyzed. In addition, the thickness of particles is a concern when using μFTIR in transmission mode because the infrared light must pass through the sample (Käppler et al. 2016). Thickness is less of an issue when using μRaman imaging as only the surface of the particle is analyzed (Cabernard et al. 2018). Different modes of μFTIR are less affected by particle thickness such as attenuated total reflectance (ATR) but this mode requires physical contact with the particle which can adhere to the crystal and disrupt analysis if using imaging or scanning technology (Käppler et al. 2016).

3.4.2. Recommendations

Visual identification should not be used as the sole means of microplastic (MP) identification and quantification. Polymer composition can only be determined using chemical characterization methods. Previous studies that used visual identification, alone or in combination with Nile Red staining or the hot-needle method, should be interpreted with caution (e.g., MP concentrations are likely inflated; e.g., Al Hamra and Patria 2019; Fitri and Patria 2019), and limitations of the study should be fully recognized (Ivar do Sul 2021). For example, Rochman et al. (2015) acknowledges the limitations of their study and do not designate any particles as actual plastics because they did not have the instruments necessary to confirm identifications.

Visual sorting may be necessary prior to chemical characterization depending on the sample type (e.g., higher number of inorganic particles), digestion efficiency and filter composition. Studies should acknowledge the limitations of visual sorting and identification and clearly state this in the methods. During visual sorting, appropriate QA/QC measures should be followed (as described above) and if possible, a visual guide should be referenced to aid in selecting potential plastic particles. These guides describe relevant MP characteristics such as shape, size, and other physical properties (e.g., flexibility, hydrophobicity, reflective properties; Rochman et al. 2019). Lusher et al. (2020a) emphasizes the need to harmonize visual identification and provides more concrete physical descriptors to aid in the selection of potential MP. This work and others should be consulted to help with more consistent visual sorting methods as the field works toward standard methods (Laura Markley and Ellenora Huth, personal communication; also see Kroon et al. 2018; Rochman et al. 2019; Karlsson et al. 2020). In addition to identifying suspected MP, analyzing a range of particle types suspected as non-synthetic for confirmation can lower the chances of underestimating the number of MP in a sample (Qiu et al. 2016; Hermsen et al. 2018; Lusher et al. 2020a). Color should not be used as an important categorical variable because color can be affected by the chemicals used for sample storage and digestion, temperature, and other factors. The color of plastic particles in blank samples, however, can aid in identifying a source of contamination.

Chemical characterization must be used to positively identify synthetic polymers. The various techniques described above are recommended and can be used to determine the types and concentrations of MP in environmental samples. Suspected particles cannot be considered MP without this type of confirmation. In particular, spectroscopic methods such as μFTIR and μRaman are common and reliable methods for polymer identification (Lusher et al. 2017b). Both are non-destructive molecular vibrational techniques that function complementary with one another, meaning the molecular bonds that produce strong FTIR intensities will result in weak Raman intensities (Koenig 1992). Prior to chemical characterization, researchers should establish standard protocols for selected methods which should meet basic MP-analysis requirements and be validated with positive control recoveries from each individual researcher (Isobe et al. 2019). The specific technique used to characterize the MP will influence other sample processing methods. For example, enzyme digestion methods are preferable for Pyrolysis-GC/MS, but are also expensive, can have low digestion efficiency, and have a complex methodology (Catarino et al. 2017; La Nasa et al. 2020; Lusher et al. 2020b). With regards to spectroscopic analyses, choice of filter on which the suspected plastic particles will be collected is an important consideration. Gold and silver filters are recommended when using reflection mode for μFTIR because they will not cause interference, but can cause spectral artifacts

with Raman spectroscopy. Filters transparent to IR, such as silicon, are needed for transmission μFTIR and do not create noise when used with μRaman (Thermoscientific, Käppler et al. 2015, 2016). Additionally, silicon filters are more affordable than gold and silver, but are brittle and need to be handled with care. The pore size of selected filters will delineate the minimum particle size for analyses and should be chosen based on digestion method and goals of the study. Various products are available to simplify processing and preventing sample loss such as the use of EasyLift ® tape to securely transfer particles from filters for analysis by μRaman spectroscopy and other methods (Gwinnett et al. 2021). Skintac® is another useful product for securing particles onto a slide prior to analysis by μRaman (Thaysen et al. 2020). Both of these products, however, can interfere with μFTIR analyses (authors' experiences). Regardless of the technique selected, testing the chemical characterization with spiked samples is recommended to identify potential problems such as loss of particles because of improper digestion and extraction techniques (Cabernard et al. 2018; Cui et al. 2022).

Researchers should develop a reference library of weathered plastic polymers to reduce the misidentification of polymers in environmental samples (Araujo et al. 2018; Primpke et al. 2018). There are kits and open-source libraries available that provide a variety of environmentally relevant (weathered) polymers to build a reference library for the chosen methods. Spectra obtained from analyzing these polymers can be compared to spectra from other laboratory libraries (e.g., Polymer Kit 1.0, openspecy.org, FLOPP/FLOPP-e, SLOPP/SLOPP-e; Hawaii Pacific University: Center for Marine Debris Research, Cowger et al. 2021; De Frond et al. 2021; Miller et al. 2022). Primpke et al. (2020b) created a software tool called siMPle which makes it possible to identify MP from spectra collected by different μFTIR instruments. Different modes of μFTIR will require different libraries to accurately identify polymers, and the chosen mode will depend on the particle characteristics. Attenuated total reflectance (ATR) μFTIR is useful for analyzing irregular particles and is commonly used in MP research, while transmission mode μFTIR is suitable for thinner particles like films or flattened fibers (Löder and Gerdts 2015; Andrade et al. 2020). Other strategies also should be considered such as using algorithms to sort collected spectra into polymer classes based on spectral measurements across a range of environmental MP (Chabuka and Kalivas 2020). As more environmentally relevant polymer libraries become available, a standard minimum percent match should be established for acceptable polymer identification. Renner et al. (2019) discusses how to preprocess spectra prior to library matching using treatments such as baseline corrections and smoothing. These methods help with signal-to-noise ratio and provide a higher library match.

As technologies improve, automated analyses are becoming more available and provide several advantages. For example, twice as many MP (≤500 μm) can be identified using automated μRaman analysis compared to μFTIR imaging analysis, but requires quadruple the analysis time (Cabernard et al. 2018). Automatic-scanning software maps the entire sample filter reducing the subjectivity introduced by visually sorting and selecting individual particles for analysis (Käppler et al. 2016; Primpke et al. 2017, 2018; Araujo et al. 2018; Brandt et al. 2020; Song et al. 2021). An additional QA/QC measure that should be considered when using scanning technology is to photograph the scanned filters to double check and account for any inconsistencies in the software's output (Lusher et al. 2020a). Analyses using μFTIR can be performed in a timelier manner but may underestimate the number of small MP in the samples, especially those <20 μm (Käppler et al. 2016; Cabernard et al. 2018). When faced with limited resources for analytical costs, it is sometimes necessary to analyze only a portion of each sample. When doing so, identifying a minimum of 50% of the sample or at least 100 suspected MP particles using one of the chemical characterization methods described above has been recommended (see Hidalgo-Ruz et al. 2012; Hermsen et al. 2018; Koelmans et al. 2019). While automated processes are available, visual sorting and analysis of individual particles with μFTIR or μRaman is much more common. At least every type of suspected MP should be identified in the sample and confirmations should be made for particles believed not to be MP to check for user error rates. Only particles that are confirmed as MP should be included in particle counts.

Use of multiple techniques for MP identification is another option. Combining μRaman and μFTIR analyses, for example, would provide more comprehensive identification and quantification as each method is better suited for different challenges (e.g, fluorescence, different polymers, additives, absorbance; Käppler et al. 2015, 2016; Cabernard et al. 2018; Yu et al. 2019a). Pretreatment methods are being developed for pyrolysis-GC/MS sample preparation making it a more appealing method (Fischer and Scholz-Böttcher 2017; Hermabessiere and Rochman 2021; La Nasa et al. 2021). Despite sample loss, pyrolysis-GC/MS is rising in popularity as it can

identify the entire chemical composition of polymers from the surface to core and may be the best method for identification of microplastics <10 µm (Dehaut et al. 2020). Again, this is a tradeoff between knowing how many particles are present and the particle shapes and sizes for a more accurate estimation of polymer composition by mass quantification. Combining the mass-based quantitative data provided by GC/MS with particle morphology and count data gathered by µFTIR or µRaman is another powerful option (Fischer and Scholz-Böttcher 2017; Mintenig et al. 2018). Such approaches, however, are often not realistic for many studies because of the cost of analyses and instrument availability. As individual techniques, both µFTIR and µRaman are excellent for identification and quantification of plastic particles and have become standard methods for MP research.

There are many options for chemical characterization of MP, and each has benefits and limitations. New methods are being developed and implemented such as the laser direct infrared (LDIR) technique which is supposed to be faster than FTIR or Raman and could be easier to automate (Ourgaud et al. 2022). Researchers need to decide the technique that will best address the research question at hand considering analytical costs and time necessary to process the number of samples collected. These constraints and limitations need to be acknowledged and considered when drawing conclusions from a study. With regards to spectroscopic techniques, a number of methods have been developed and reviewed by experts in the field and should be considered carefully before selecting a final methodology (see Lenz et al. 2015 [µRaman]; Löder and Gerdts 2015 [critical perspective of all methods]; Löder et al. 2015 [µFTIR]; Käppler et al. 2016 [µFTIR and µRaman]; Dümichen et al. 2017 [GC/MS]; Primpke et al. 2017 [µFTIR]; Käppler et al. 2018 [GC/MS and µFTIR]; Mintenig et al. 2018 [µFTIR and Pyrolysis-GC/MS]; Renner et al. 2019 [review of preprocessing measures]; Andrade et al. 2020 [IR reporting guidelines and descriptions]; Dehaut et al. 2020 [Pyrolysis-GC/MS]; Cowger et al. 2020b [overview of all]; La Nasa et al. 2020 [GC/MS]). There is now enough information available to avoid methods that are known to be flawed.

3.5. Summary recommendations

Many of the issues and problems discussed in this section are the consequence of a developing research field. Few researchers have prior experience working with microplastics (MP) or the analytical tools used to extract and identify polymers (Lusher et al. 2020a). These problems can be addressed through collaborations and a thorough review of the literature. There are a number of publications that focus on the standardization of methods from sample collection to MP identification, and they recommend best practices to improve digestion efficiency, detection limit, and comparability (see Hidalgo-Ruz et al. 2012; Woodall et al. 2015; Dehaut et al. 2016, 2019; Rochman et al. 2017; Hermsen et al. 2018; Mai et al. 2018; Isobe et al. 2019; Koelmans et al. 2019; Renner et al. 2019; Weis 2019; Zhang et al. 2019; Brander et al. 2020; Lusher et al. 2020b; Primpke et al. 2020a; Provencher et al. 2020; Prata et al. 2021). Reporting guidelines have been suggested to make publications more coherent across the broad range of methods and research goals (Weis 2019; Cowger et al. 2020a; Provencher et al. 2020) as MP are derived from many materials and degraded into many forms (e.g., different shapes, sizes, colors, densities, chemical properties; Rochman et al. 2019). The aforementioned papers can be reviewed prior to study design to make sure methods can be reproduced and are interpreted correctly.

3.5.1. Overview of QA/QC measures

Methods from all of the studies critically reviewed above (Section 3) and listed in Table 1 were assessed with respect to quality assurance and control (QA/QC) measures listed in Table 2. The list not only outlines the criteria by which studies were evaluated, but is designed to improve the quality of microplastics (MP) research in the future and provide guidance from experiences of the authors and relevant research in the field. The first three items on the QA/QC list are focused on monitoring for MP contamination whereas the remaining 10 items provide measures to reduce sample contamination throughout the collection of samples and identification of MP (Table 2). The studies in Table 1 used a variety of methods, many of which were potentially destructive of MP and not reproducible. The QA/QC criteria listed in Table 2 and further explained below should be considered when evaluating the conclusions of and referencing the studies summarized in Table 1.

Criterion #1 (Table 2) was indicated next to studies that did not mention accounting for plastic contamination from equipment used in the field or laboratory. This criterion is linked with #5 as described below. The QA/QC list does not address the number of procedural blank replicates used in the study (#2). If the researchers claimed to use a procedural blank (or negative control), the criterion was considered to be met and was not noted next to that study. At least

Table 2. The quality assurance and control (QA/QC) criteria used to assess the quality of research papers in Table 1.

QA/QC measures
Monitor contamination
1. Account and monitor for plastic used throughout collection, extraction, and identification
2. Procedural blank samples processed alongside collected samples (negative controls)
3. Monitor airborne contamination with a wet filter and/or an open petri dish in the field and laboratory
Prevent contamination
4. Prefilter liquid reagents into clean glassware
5. Use glass and metal instead of plastic whenever possible
6. Muffle glassware, glass filters, and metal (450 °C for minimum 5 hr) prior to use or rigorously clean glassware and equipment (acid wash protocol, multiple rinses with filtered water and ethanol)
7. Rinse glassware prior to use and in-between samples
8. Cover all containers (e.g., aluminum foil) when not in use
9. Wear cotton laboratory coats or non-synthetic clothing when interacting with samples
10. Use positive controls and test and report method recovery
11. Check filters for contamination under microscope and clean prior to use (not necessary if can be muffled)
12. Perform extractions in clean air environment (laminar flow hood, air-controlled space)
13. Identify polymers with chemical characterization method (e.g., FTIR, Raman, Pyrolysis-GC/MS)

Each QA/QC measure was assigned a number. If the study did not mention a particular QA/QC measure, the appropriate number was added to the criticism column in Table 1. The numbers assigned to each measure do not signify priority over the other measures. One hundred thirty studies were reviewed against this QA/QC list.

three procedural blanks should be used and treated in parallel with the samples (Hermsen et al. 2018). Wipe tests have been recommended in one study (Zhao et al. 2017), whereas placing an open petri dish or wet filter out during sample collection and processing to monitor contamination was more common. Therefore, criterion #3 was only marked if the study did not monitor airborne contamination. Liquid reagents can harbor potential contamination and should be filtered through a submicron filter prior to use. For this reason, criterion #4 was noted if the study did not mention prefiltering liquid reagents, although studies that did indicate a prefiltering step did not always report the pore size of the filter. If studies mentioned the use of plastic, such as plastic bags for sampling, the study was marked for violating criterion #5 and checked for #1. Otherwise, studies were assumed to avoid the use of plastic materials and supplies. Glassware should be muffled to remove plastic materials (#6), however, it is possible to clean glassware thoroughly with filtered water and prefiltered ethanol or an acid washing protocol. Criterion #6 was noted in the table if the studies did not muffle glassware or apply an alternative cleaning protocol. Microplastics can stick to different containers as samples are processed, making it necessary to rinse containers, funnels, filtration stands and filters multiple times with filtered water and/or ethanol. Not one study mentioned the use of multiple rinse steps in the prevention of MP sample loss, so any mention of rinsed glassware was considered adequate to meet criterion #7. Researchers of future studies that cannot meet criterion #6 should rinse glassware and filters at least 3x with high-purity, filtered water (e.g., Milli-Q). The cleaning of laboratory spaces was not included in the QA/QC list (Table 2) because depending on the method (e.g., cotton fiber cloth used for cleaning) it is not clear if the cleaning procedures could contribute to airborne contamination. All containers should be covered when not in use and this is often done with aluminum foil. If the study did not mention covering containers, criterion # 8 was noted. The wearing of cotton laboratory coats is considered the minimum coverage necessary to reduce contamination from synthetic clothing (see Gwinnett and Miller 2021). The use of full body suits composed of cotton would be desirable, however, failure to meet criterion #9 was indicated only if there was no mention of non-synthetic clothing coverage. If the study did not mention the use of positive controls or report method recoveries it was marked with criterion #10. Any non-muffled filters should be checked for contamination prior to use and cleaned if necessary to meet criterion #11. To minimize contamination, it is best to prepare and process samples in laminar flow hood or biosafety cabinet. Failure to meet criterion #12 was noted if the study did not mention a "clean air environment" or that they monitored air flow. Importantly, polymers should be positively identified using a chemical characterization method, and studies that failed to do so were indicated as such (#13). For studies that used chemical characterization, the assumption was that the MP were identified properly. The QA/QC list (Table 2) does not account for the percent of properly identified MP using spectroscopy. Studies that identified a subset of MP from their samples were not indicated, but as discussed above, if the sample contains over 100 particles at least 50% of the sample should be analyzed (Hermsen et al. 2018) or all suspected particles confirmed as MP along with validation of potential error sources. For example, a subset of suspected minerals

should be confirmed as minerals and not a misidentified MP using a chemical characterization method.

3.6. Conclusions

Many of the studies reviewed above (Section 3) did not meet basic reporting standards or provided insufficient descriptions of methods and techniques, and/or referenced publications for their QA/QC measures (e.g., Andrade et al. 2020; Bagheri et al. 2020; Ding et al. 2021; Zaki et al. 2021). The inconsistencies in QA/QC measures, and in many cases lack thereof, lead to questions concerning the validity, significance, and interpretation of the results. Additionally, many studies only reported the presence and body burden of microplastics (MP) found in certain molluscs in the natural environment (e.g., Davidson and Dudas 2016; Ibrahim et al. 2016; Abbasi et al. 2018; Li et al. 2018a; Abidli et al. 2019; Borja and Elliott 2019; Fernández Severini et al. 2019; Fitri and Patria 2019; Chinfak et al. 2021; Gong et al. 2021; Janssens and Garcia-Vazquez 2021; Joshy et al. 2022). Although such studies were part of the maturation of the field, simply reporting the relatively low concentration of plastic particles in yet another species of mollusc is of limited value. Future studies should address the implications of MP in the marine environment and potential consequences for the resident communities. Finally, this section of the review should not be used as a sole reference for MP research in molluscs, but should provide a solid starting point. The field of science concerned with MP in the environment is growing and developing, and the literature should be thoroughly reviewed before selecting methods and defining QA/QC procedures.

4. Laboratory studies on particle feeding molluscs

4.1. Background

There are several issues that have influenced and impacted the current state of the science and published literature concerning microplastics (MP) and particle-feeding molluscs. First, few investigators consult the older literature (pre-2000) and are unaware of the rich body of literature that exists on particle capture, ingestion, and egestion of natural and synthetic particles by bivalve molluscs. For example, plastic particles have been used in laboratory feeding studies with molluscs for decades (for reviews see Ward and Shumway 2004; Rosa et al. 2018; Ward et al. 2019b, and references therein). Polystyrene microspheres and other plastic particles have been employed as food-surrogates to study a range of feeding processes including particle capture, selection, and gut residence time. One of the first researchers to use synthetic latex microspheres (polystyrene) to study feeding behavior of the suspension-feeding molluscs (*Crepidula fornicata*) was Williams (1978). Since that time, polystyrene microspheres and other MP particles have been used to investigate feeding processes of holoplanktonic, meroplantkonic, and benthic animals (e.g., Gerritsen and Porter 1982; Holland et al. 1986; Ward and Targett 1989; Solow and Gallager 1990; Hart 1991; Mayer 1994; Ward 1996; Milke and Ward 2003; Baer et al. 2008; Rosa et al. 2013, 2017; Shumway et al. 2014). Lack of attention to these instructive papers has led to poorly designed studies, weak and erroneous conclusions, and results that duplicate previous research. For example, it has been known for decades that suspension feeders will capture and ingest certain types of plastic particles, and that capture efficiency is dependent upon particle size (e.g., Ward and Targett 1989; Zankai 1994; Lei et al. 1996; Tamburri and Zimmer-Faust 1996; Cranford et al. 1998; Baker et al. 2000; Kach and Ward 2008). Yet, some recent works have "rediscovered" these facts (e.g., Browne et al. 2008; Van Cauwenberghe et al. 2013, 2015; Setälä et al. 2016; Khan and Prezant 2018; Fernández and Albentosa 2019a, 2019b; Gonçalves et al. 2019; Kinjo et al. 2019; Scanes et al. 2019; Moreschi et al. 2020; Song et al. 2020; Wang et al. 2021a; Jiang et al. 2022), implicitly suggesting that they are the first to record such events. A firm understanding of the accumulated knowledge regarding ingestion of MP, and basic biology and physiology of bivalves and other particle feeders, is imperative for the integrity of future studies. Below, a short primer and salient references are provided so that researchers of future studies may benefit from and build upon their predecessors.

4.1.1. Suspension-feeding processes

Bivalves are well-adapted to a diet of relatively dilute, heterogenous particles, and cells (Morton 1960; Jørgensen 1976; Winter 1978; Bayne et al. 1989; Cranford et al. 2011). These animals employ upstream mechanisms to encounter, retain, and transport particles on the gill (= ctenidium). The lateral cilia of the gill filaments create a current of water that flows into the inhalant aperture or siphon, through the interfilamentary spaces of the gills, into the suprabranchial cavity, and out the exhalant aperture or siphon. Particles suspended in this flow are captured

as they encounter and are retained by the frontal surfaces of the gill filaments. Currents produced by the action of the laterofrontal cilia or cirri of the filaments facilitate this process by redirecting particles from the through current, laterally onto the filaments (Riisgård et al. 1996; Ward et al. 1998b; Riisgård and Larsen 2000a, 2000b). Capture efficiency increases asymptotically with increasing particle size above ca. 1 µm to a maximum efficiency of close to 100%. In general, particles < ca. 1 µm are inefficiently captured by most bivalve species unless they are incorporated into aggregates or are highly agglomerated (Kach and Ward 2008; Ward and Kach 2009; Doyle et al. 2015). Capture efficiency for particles between ca. 1 µm and 6 µm is species-specific, and dependent upon gill architecture and structure of the laterofrontal cilia or cirri (e.g., Vahl 1972; Møhlenberg and Riisgård 1978; Palmer and Williams 1980; Riisgård 1988; Silverman et al. 1995; Lei et al. 1996). Surface characteristics of particles <6 µm also can affect capture efficiency (Hernroth et al. 2000; Yahel et al. 2009; Rosa et al. 2017). Particles between ca. 6 and 500 µm are captured efficiently by most species, as are particles >500 to 1000 µm, but many of these are rejected in pseudofeces and thus ingested in low proportions.

Particles captured by the gills are transported to the ventral or dorsal margins by frontal ciliary tracts, and then directed by marginal tracts toward the mouth in either a cohesive mucous string (ventral), or less cohesive mucus–water slurry (dorsal; Ward 1996; Ward et al. 1993). In a few groups of bivalves (e.g., Arcidae, Anomiadae) the ventral tracts direct particles posteriorly for rejection. The feeding processes of bivalves are accomplished by means of both mucociliary and hydrodynamic mechanisms. The use of mucus of varying qualities and viscosities at different anatomical locations gives the bivalve maximum flexibility in rejecting, ingesting, and processing particulate matter (Beninger and St-Jean 1997b; Beninger et al. 1997).

Not all captured particles are ingested. In fact, few species of bivalves simply encounter and engulf particulate matter. Rather, there is a large body of evidence demonstrating that bivalves rapidly sort particles based on physical and chemical factors, with material of higher quality being ingested and digested preferentially over that of lower quality (for reviews see Ward and Shumway 2004; Rosa et al. 2018; Ward et al. 2019b). This capability for selection is probably unsurpassed by any other particle-feeding group. Particle size, shape, and surface properties (both specific and non-specific) interact in complex ways to affect preferential rejection and ingestion of material. It should be emphasized, however, that selection is never 100% efficient and inevitably some undesirable material will be ingested. Particles destined for rejection are bound in cohesive mucus, transported *via* well-developed mucociliary processes to specific sites on the mantle, and expelled as pseudofeces (Galtsoff 1964; Ward et al.1994b; Beninger and St-Jean 1997a; Beninger et al. 1997; Garrido et al. 2012). The loci of selection are species specific, and depend upon the architecture and ciliary tracts of the gill. Some species of bivalves (e.g., oysters, scallops) can select particles on both gills and labial palps (paired structures surrounding the mouth), whereas other species (e.g., mussels) only select on the palps (Ward et al. 1997, 1998a, 1998b; Beninger et al. 2004, 2008). Importantly, at high concentrations of particles, selection based upon quality diminishes in many species and indiscriminate rejection occurs (e.g., Urban and Kirchman 1992; Newell and Shumway 1993; Hawkins et al. 1998; Beninger et al. 2008).

The feeding process summarized above is by far the major avenue by which suspension-feeding bivalves (and some deposit-feeding bivalves) ingest or take up particulate matter. In adult bivalves, few if any particles destined for accumulation or ingestion are collected by the foot, despite what has been stated in the literature (Kolandhasamy et al. 2018) and amplified in subsequent reviews (Zhang et al. 2020). Indeed, ciliary tracts associated with keeping the pallial cavity cleared of pseudofeces and errant particles are numerously distributed on the mantle, epidermis of the visceral mass, and foot (if one exists). These tracts, however, lead particulate matter to rejection areas. The discovery of microplastics (MP) on the mantle, foot, visceral mass, or even gills does not mean that the animal is "taking up" or "accumulating" these particles (see below) *via* these organs.

4.1.2. Digestive processes

Bivalves possess a complex gut (e.g., Graham 1949; Yonge 1949; Owen 1956; Reid 1965). Within the stomach are abundant ciliary tracts aligned on ridged sorting areas and pouches, a chitinized gastric shield, and openings to the digestive diverticula (digestive gland). A crystalline style protrudes into the stomach from the ciliated style sac that lies adjacent to the midgut. The mechanical action between the rotating crystalline style and teeth of the gastric shield, along with the chemical action of liberated enzymes, breaks apart large particles and particle aggregates and begins the process of extracellular digestion. Particles within the rotating slurry are subjected to ridged sorting areas

of the stomach. Lighter particles and particle fragments enter the digestive diverticula (digestive gland) where more complete intracellular digestion occurs. Larger and more dense material passes into the intestinal groove and is transported to the midgut where it mixes with other undigested material and is incorporated into fecal pellets. As a result of the complex anatomy of the gut, bivalves void feces from two compartments (van Weel 1961). Intestinal feces are composed of undigested material subjected to extracellular digestion in the stomach, and are voided on short timescales (e.g., 0.2–2 hr). Glandular feces are composed of undigested material subjected to intracellular digestion by phagocytic cells that line the sacs of the digestive diverticula, and are voided on longer timescales (e.g., 2–9 hr or longer; Menzel 1955; Owen 1974; Foster-Smith 1975; Gagnon and Fisher 1997). The production of two types of feces, voided over different time intervals makes determining gut residence time more challenging (Bricelj et al. 1984; Hawkins et al. 1990; Decho and Luoma 1991; Gagnon and Fisher 1997).

Numerous studies have demonstrated that bivalves can discriminate among particles in the gut. Selection can occur between different species of microalgae (Cucci et al. 1985; Shumway et al. 1985; Cognie et al. 2001), living and heat-killed microalgae (Brillant and MacDonald 2003; Beninger et al. 2008), microalgae and inorganic material (Menzel 1955; Foster-Smith 1975), and sediment with and without organic coating (Gagnon and Fisher 1997). Discrimination of microplastics (MP) in the gut has also been demonstrated, with some plastic particles having much shorter gut residence times than others (Cranford et al. 1998; Brillant and MacDonald 2000, 2002; Ward and Kach 2009; Ward et al. 2019a). The selection mechanism seems to be based upon size, density, and chemical properties of the particles and cells.

The residence time of refractory particles, including microplastics, in the gut of bivalves has been studied previously. Results indicate that >85% of >2-μm polystyrene, HDPE, silica, and aluminum spheres are egested within 48 hr post exposure (Ward and Targett 1989; Cranford et al. 1998; Brillant and MacDonald 2002; Ward and Kach 2009; Fernández and Albentosa 2019a, 2019b). The remaining particles are egested within 96 hr (op cit), with only residual amounts remaining after that. Whether small particles within the 2- to 100-μm range are retained longer than larger particles is unclear. For the scallop, *Placopecten magellanicus*, one study found that gut retention time (GRT) of larger (20 μm) polystyrene spheres was significantly longer than that of smaller spheres (5 μm, Brillant and MacDonald 2000). Another study, however, found that scallops egested 6-μm polystyrene spheres at a slower rate than 10-μm spheres (Cranford et al. 1998).

Translocation of nanoplastic and microplastic to other tissues has received recent attention. Unsurprisingly, several studies have reported that plastic particles to which bivalves are exposed can be found in the mucus on the outer side of the gill epithelium (Paul-Pont et al. 2016; Guilhermino et al. 2018), and in the digestive tract, including the digestive gland tubules and within the epithelial cells lining the tubules (von Moos et al. 2012; Avio et al. 2015a; Guilhermino et al. 2018; Khan and Prezant 2018; Pittura et al. 2018). Microplastics have also been reported in the hemolymph, hemolymphatic sinuses, and hemocytes after experimental exposure (Browne et al. 2008; Avio et al. 2015a; Ribeiro et al. 2017; Guilhermino et al. 2018; Pittura et al. 2018). A few studies also reported that after exposure plastic particles (NP, MP) can be found in the gills and other tissues (von Moos et al. 2012; Avio et al. 2015a; Al-Sid-Cheikh et al. 2018; Pittura et al. 2018; Fernández and Albentosa 2019b). Retention of plastic particles in the hemolymph and other tissues seems to be longer than GRT. Residual amounts can be found in the hemolymph of the mussel, *M. edulis*, 48 days post exposure (<1%, <10 μm; Browne et al. 2008), the gills of the mussel, *M. galloprovincialis*, 7 days post exposure (<3%, <3 μm; Fernández and Albentosa 2019b), and in body tissues of the scallop, *Pecten maximus*, up to 48 days post exposure (<5%, 248 nm; Al-Sid-Cheikh et al. 2018). Of note, however, some studies have found no evidence that MP were phagocytized by cells of the digestive gland or translocated to other tissues (Brillant and MacDonald 2000; Paul-Pont et al. 2016; Gonçalves et al. 2019; Revel et al. 2020). Future studies on retention and accumulation of plastic particles in bivalves must explore realistic concentrations, make comparisons with natural refractory particles, and study a wider range of species to provide a better understanding of uptake and depuration kinetics.

4.2. Overview of studies on physiological and cellular processes

Many laboratory studies have examined the ingestion, uptake, and elimination of microplastics (MP) by bivalves, and have investigated potential impacts of plastic particles on various biological processes. Unfortunately, the current literature has been inundated by inappropriate husbandry, experimental

designs, and methodologies. Authors of many published studies demonstrate little, if any, knowledge of molluscan biology or physiology. Indeed, many do not feed the animals during extended experimental protocols, or recognize when the animals are stressed, feeding unnaturally (or not at all), do not know the anatomy and function of the animals, or recognize the difference between feces (glandular vs. intestinal), pseudofeces, and general debris. Additionally, a wide range of putative effects of MP on bivalves have been reported in the literature, even for the same plastic polymer. This disparity is in part a result of the different physiological condition of the experimental bivalves (from starved, to stressed, to good health), the wide range of MP concentrations tested (from environmentally relevant to ten orders of magnitude higher), and the quality of the methods employed for measuring end points (from poor to excellent). Consequently, many published results are questionable. In this section, examples are provided of studies that fall short with regard to several important experimental methods and procedures. The section focuses on MP but provides several references to studies that examined nanoplastics for comparison. A summary of the studies discussed below, along with a relative rating (0–2) can be found in Table 3. Of note, is that the mean rating for the 84 papers reviewed for this table was 0.9, indicating that most publications contained some to many flaws.

4.3. Poor husbandry before, during, and after exposure experiments

Bivalves are omnivorous, but their diet consists mainly of phytoplankton. Field studies that have determined the actual food items of bivalves, including those for oysters (Lotsy 1893; Moore 1931; Galtsoff 1964), mussels (Field 1911, 1924; Newell et al. 1989), surfclams (Shumway et al. 1994), and sea scallops (Shumway et al. 1987), all clearly demonstrate that these animals ingest phytoplankton, detritus, and resuspended benthic materials to greater or lesser degrees. Additionally, there is a rich body of literature regarding the most nutritious food for maintaining and growing suspension-feeding bivalves under laboratory conditions (e.g., Ukeles 1969; Epifanio and Mootz 1976; Urban and Langdon 1984; Wikfors et al. 1996; Castagna 2001). In short, these diets consist of a mixture of phytoplankton species of a size and nutritional value that are appropriate for the life stage of the animal. Unfortunately, several studies examining the effects of microplastics (MP) on bivalves have neglected the above information, and report feeding bivalves a range of inappropriate diets including those consisting of zooplankton (Setälä et al. 2016; Pittura et al. 2018) or food stuff that includes phytoplankton diluted with one or more of the following: flour, herbs, yeast, egg derivatives, Ca-caseinate, bivalve meal or shrimp by-products (e.g., Liquifry Marine®, Interpet House, used in study by von Moos et al. 2012; Pro-coral Phyton®, Tropic Marin, used in study by Khalid et al. 2021; Coraliquid, Sera Marin used in the study by Capolupo et al. 2021). Use of inappropriate food means that over the course of acclimation and experimentation the bivalves in these studies were under starvation conditions (e.g., up to 43 days, Pittura et al. 2018; 84 days, von Moos et al. 2012). The consequences of using inappropriate diets are highlighted in the study of Khalid et al. (2021). Mussels, *Mytilus edulis*, were purchased from the grocery store and fed an unsuitable food (Pro-coral Phyton) during acclimation (1 week) and experimentation. Mussels were then exposed to high concentrations of Poly(L-lactide) particles for 8 days. During the course of the experiment, over half of the mussels died in each treatment, including the control. Undeterred, the researchers measured a range of cellular endpoints on these stressed animals, reporting downregulation of glycerophospholipids in mussels exposed to the MP. Given the poor husbandry in this study, and the fact that no measurements were made on mussels initially collected (baseline controls) from the supermarket or on mussels freshly collected from the environment, results of this research provide no useful information on the possible effects of MP on mussels in nature.

In addition to food quality, researchers must understand how food quantity affects bivalve feeding and condition (e.g., Bayne et al. 1993; Iglesias et al. 1996; Cranford et al. 1998; Hawkins et al. 1999). Bivalves, as well as many other suspension feeders, are adapted to a continuous supply of phytoplankton and other nutritious particles that are available at rather low concentrations (e.g., typical near-shore concentrations of microplankton [2–200 µm] are $<1 \times 10^4$ cells·mL^{-1}; Beers et al. 1980; Gin et al. 2000; Dennett et al. 2001; Buchanan et al. 2005; Leblanc et al. 2018). A common mistake of inexperienced researchers working with bivalves is to provide the animals a concentration of phytoplankton once a day or less frequently that is either too low or too high. A low concentration of phytoplankton food avoids excess pseudofeces production, ensuring that most of the diet is ingested; however, in low-volume static systems (<25 L), which most studies employ, the clearance capacity of bivalves (2–11 L·hr^{-1}·g dry mass^{-1}) will rapidly deplete

Table 3. Laboratory studies of interactions between marine molluscs and microplastics with some references to nanoplastics.

Species	Experiment	Particle types, sizes, and concentrations tested	Reported results	Notes	Reference and rating
Abra nitida (semele clam)	Effects of MP on behavior and physiological processes	PE particles: 4–6 µm, 20–25 µm, 125–500 µm 1, 10, and 25 mg·kg^{-1} sediment For 1 mg·kg^{-1} sediment equivalent to 9.3×10^6, 2.4×10^6, and 94 MP·kg^{-1} for small, medium, and large particles, respectively	Protein content decreased in clams exposed to largest MP at all concentrations; no effect on total energy reserves, survival, CI or burrowing behavior	Deposit-feeding clams maintained in natural sediment during 1-month acclimation and 4-wk exposure	Bour et al. (2018b) **Rating = 2**
Anodontites trapesialis (freshwater mussel)	Uptake and elimination of MP	PE (ultra-high molecular weight) microspheres: 55–100 µm diameter 75 mg·L^{-1} Equivalent to 3.3×10^5 MP·L^{-1}	Mussels ingested MP; MP eliminated through pseudofeces and feces	Insufficient information regarding diet quantity (i.e., number cell·mL^{-1}) during 7-d acclimation and 8-d exposure; no mention of volume of water or number of mussels held during acclimation; no separation of pseudofeces from feces, pathway of elimination cannot be determined; confused particles filtered with particles ingested; poor histological sections; confusing and inaccurate description of suspension feeding	Moreschi et al. (2020) **Rating = 0.5**
Argopecten irradians (bay scallop)	Ingestion of MP and effects on cellular processes	PS microspheres: 1 µm diameter 10, 100, and 1000 MP·mL^{-1} Equivalent to 1×10^4, 1×10^5, and 1×10^6 MP·L^{-1}, respectively	Scallops ingested MP, number ingested depended on exposure concentration; oxidative stress increased with MP concentration and duration	Poorly designed study resulting from failed understanding of molluscan feeding literature; scallops starved during 24-hr acclimation and likely under stress; no indication of diet quantity or quality delivered during 7-d exposure, scallops likely under starvation stress; experimental design includes pseudoreplication	Song et al. (2020) **Rating = 0**
Atactodea striata (striate beach clam)	Effects of MP on physiological processes	PS particles: 63–250 µm 10 or 1000 MP·L^{-1}	Clams ingested MP; MP at high concentrations reduced CR; no effects on RR or AE	Clams starved during 2-d acclimation and likely under stress; inadequate diet quantity delivered during 2-wk exposure, clams likely under starvation stress; CR unreliable, 3 clams (ca. 3.8 cm) placed in 225 mL seawater (crowded conditions), one sample taken after only 10 minutes; high diet quantity (6.5×10^5 cells·mL^{-1}) delivered during clearance studies likely depressed feeding and stimulated excess pseudofeces production; AE unreliable, collected feces over only a 2.5-hr period; digesting tissues in boiling concentrated nitric acid (22.5 M) can cause polystyrene to dissolve	Xu et al. (2017) **Rating = 0 #**
Choromytilus chorus (giant mussel)	Effects of MP on tissues and physiological processes	Fluorescent PS microspheres: 8.1–12 µm diameter 100 or 1000 MP·L^{-1}	MP had no effects on physiological processes of scallops (CR, IR, AE, AmEx, SFG); some histopathological differences at 1000 MP·L^{-1}	Mussels starved for 2 d after collection and then delivered adequate diet during 6-d acclimation; adequate diet delivered during 40-d exposure; mussels starved for 24 hr after exposure and before physiological measurements; contrary to authors' statements, MP concentrations were not close to environmental levels	Opitz et al. (2021) **Rating = 1**

(*Continued*)

Table 3. Continued.

Species	Experiment	Particle types, sizes, and concentrations tested	Reported results	Notes	Reference and rating
Corbicula fluminea (Asian clam)	Effects of MP and florfenicol on physiological and cellular processes	Fluorescent microspheres (polymer type not given): 1–5 µm diameter 0.2 and 0.7 mg·L^{-1} Equivalent to 3.7×10^{7} and 1.3×10^{8} MP·L^{-1}, respectively	Clams ingested MP; MP found in the gut, digestive gland, connective tissue, hemolymphatic sinuses and on gill surface; MP at 0.2 mg·L^{-1} inhibited cholinesterase activity; mixtures of MP and florfenicol caused feeding inhibition and other effects on cells	Inadequate diet quantity delivered during 14-d acclimation, clams likely under starvation stress; clams starved during 96-hr exposure and likely under stress; polymer type used in study not given	Guilhermino et al. (2018) **Rating = 0.5**
	Ingestion of MP (fibers) of different length and polymer type	AC, PA, PEA, PET, PVA and RA microfibers: 5–100, 100–250, 250–500, 500–1000, and 1000–5000 µm length (width 12–26 µm) 100 and 1000 MP·L^{-1}	Clams ingested MP; abundance of PET fibers in gut higher than other polymer types; abundance of 100–250 µm PET fibers in gut higher than other sizes at 1000 MP·L^{-1}	Adequate diet delivered during 7-d acclimation; no indication of diet quantity delivered during 2-d exposure; feces not collected so actual number of microfibers ingested not determined	Li et al. (2019b) **Rating = 1**
Crassostrea gigas (Pacific oyster)	Ingestion of NP and MP and effects on larval feeding and growth	Aminated (-NH_2) and carboxylated (-COOH) fluorescent and unlabeled PS microspheres: 70 nm-20 µm diameter 1, 10, 100, and 1000 MP·mL^{-1} (depending on experiment) Equivalent to 1.0×10^{3}, 10^{4}, 10^{5}, and 10^{6} MP·L^{-1}, respectively	Oyster larvae ingested NP and MP; MP size/surface properties and larval age determined number of MP consumed; no effects on feeding or growth	Adequate diet delivered during 1- to 8-d exposures	Cole and Galloway (2015) **Rating = 2**
	Effects of MP on reproduction and larval offspring	Fluorescent PS microspheres: 2 and 6 µm diameter 0.023 mg·L^{-1} (continuously supplied) 0.01 mg·L^{-1} (surrounding water) Equivalent to 1.8×10^{6} and 2.1×10^{4} MP·L^{-1} for 2- and 6-µm spheres, respectively	Oysters ingested more 6-µm than 2-µm spheres; MP increased ingestion of microalgae and AE; oocyte number, diameter, and sperm velocity decreased; offspring (D-larva) yield and development decreased; expression of some genes altered	Adequate diet delivered during 2-month acclimation and 2-month exposure; well-established methods used for feeding and physiological measurements	Sussarellu et al. (2016) **Rating = 2**
	Effects of MP on larval behavior and development	Fluorescent microspheres (polymer type not given): 1–5 µm diameter 0.1, 1, and 10 mg·L^{-1} Equivalent to 7.2×10^{6}, 6.9×10^{7}, and 5.4×10^{8} MP·L^{-1}, respectively	MP at 1 and 10 mg·L^{-1} increased malformations and developmental arrest of oyster larvae; MP at 0.1 and 1 mg·L^{-1} decreased swimming speed	Adequate diet delivered during 1- to 24-hr exposures; high concentrations of MP used, especially for larvae; inappropriate terminology used for larval anatomy; polymer type used in study not given	Bringer et al. (2020a) **Rating = 1**

Crassostrea gigas (Pacific oyster)	Effects of MP on larval behavior and development	HDPE powder: 4–6 µm, 11–13 µm, and 20–25 µm 0.1, 1, and 10 mg L^{-1} Equivalent to 3.0×10^5, 2.6×10^6, 2.4×10^7, 3.0×10^5, 9.0×10^5, 1.6×10^7, 2.0×10^5, 1.2×10^6, and 8.2×10^6 MP·L^{-1}, respectively for each of the three sizes and three concentrations used Cu solution as positive control	Smaller MP (4–13 µm) caused increased malformations and developmental arrest of oyster larvae compared to larger MP (20–25 µm); smaller MP (4–13 µm) caused dose-dependent decrease in swimming speed; larger MP (20–25 µm) had no effect on swimming speed	No indication of diet quantity or quality delivered during 24-hr exposures, larvae likely under starvation stress; inappropriate terminology used for larval anatomy	Bringer et al. (2020b) **Rating = 1**
	Effects of MP on physiological and cellular processes	PS microspheres: 6 µm diameter 1×10^4, 1×10^5, and 1×10^6 MP·L^{-1}	MP found in intestines and digestive tubules of oysters; MP at highest concentration decreased CI and LS, and increased mortality; no effects on cellular inflammation or growth	Adequate diet delivered during 1-d acclimation and 80-d exposure; no indication of how biodeposits were collected; contrary to authors' statements, MP concentrations were not environmentally relevant	Maes et al. (2020) **Rating = 1.5**
	Uptake of MP and effects on tissues and physiological and cellular processes	PE and PP particles: <400 µm 0.008, 10, and 100 µg·L^{-1} (50:50 PE:PP) Equivalent to 9.0×10^0, 1.1×10^4, and 1.1×10^5 MP·L^{-1}, respectively	MP found in biodeposits of oysters at all concentrations; MP not found in the gills, digestive gland, or other tissues; no effects on feeding, CI, tissues, or cellular biomarkers (e.g., AcP, CAT, GST, ROS, SOD, DNA damage)	Inadequate diet quantity delivered during 15-d acclimation, 10-d exposure and 10-d depuration, oysters likely under starvation stress; robust methods and procedures.	Revel et al. (2020) **Rating = 1.5** #
	Effects of MP on larval settlement and juvenile (spat) growth	Field-collected PP, PVC, and HDPE particles: 138 µm ($\bar{x}$) 0.1 and 10 mg L^{-1} (theoretical) Equivalent to $8.8–9.7\times10^5$ and $7.8–8.9\times10^6$ MP·L^{-1}, respectively depending on polymer type	MP reduced settlement success of oyster larvae exposed to 10 but not 0.1 mg·L^{-1}; no effect on metamorphosis; MP decreased spat growth after 28 d; increased growth of spat after 89 d	No indication of diet quantity delivered to larvae during 7-d exposure; contrary to authors' statements, MP concentrations were not "realistic"	Bringer et al. (2021) **Rating = 1**
	Uptake of MP and effects on tissues and cellular processes.	PE and PET powder: 37 µm (PE) and ~ 31 µm (PET) 10 and 1000 µg·L^{-1} Equivalent to $4–5\times10^2$ and $4–5\times10^4$ MP·L^{-1}, respectively depending on polymer type	MP found in oyster gills and digestive glands; MP inhibited lipid metabolism and altered metabolic profiles (energy metabolism, inflammatory response); MP caused tissue damage to gills and digestive gland; toxicity, assessed by biomarker responses, increased with increasing MP concentration; PET more toxic than PE	Little information provided regarding diet quality delivered during 2-wk acclimation and 21-d exposure; high diet quantity (3×10^8 cells·mL^{-1}) delivered during acclimation and exposure likely depressed feeding and stimulated excess pseudofeces production; accurate enumeration of MP in gill and digestive gland samples using stereomicroscope suspect; integrated biomarker response analysis used	Teng et al. (2021) **Rating = 0.5**

(*Continued*)

Table 3. Continued.

Species	Experiment	Particle types, sizes, and concentrations tested	Reported results	Notes	Reference and rating
Crassostrea gigas (Pacific oyster)	Effects of MP (mixtures) on physiological processes, cellular biochemistry, and behavior and development of larval offspring	HDPE (28%), PP (40%) and PVC (32%) particle mixture: 139 µm (± 2.3 µm) 0.1 and 10 mg·L^{-1} Equivalent to 7.6×10^{5} and 3.3×10^{6} MP·L^{-1}, respectively	Oysters ingested MP; MP increased mortality, highest mortality (23%) in 10 mg·L^{-1} treatment; effects on GST, MDA, and LAC activity depended on time and concentration; no effects on CI or SOD activity; larvae from exposed adults had decreased swimming speed and growth; larvae from adults exposed to 10 mg·L^{-1} exhibited more abnormalities	No indication of diet quantity or quality delivered to adults during 7-d acclimation; no indication of diet quantity delivered to adults during 56-d exposure; no indication of diet quantity or quality delivered to larvae; adults and larvae likely under starvation stress	Bringer et al. (2022) **Rating = 0.5**
Crassostrea virginica (eastern oyster)	Uptake and effects of NP and MP on lysosomal stability (LS)	Fluorescent PS nanospheres and microspheres: 50 nm and 3 µm diameter 10, 50, and 100 µg·L^{-1} Equivalent to 1.5 x 10^{11}, 7.3×10^{11}, 1.5×10^{12} NP·L^{-1}, and 6.7×10^{5}, 3.4×10^{6}, 6.7×10^{6} MP·L^{-1}, respectively.	NP accumulated in digestive-gland (hepato-pancreas) cells of oysters; no accumulation of MP in digestive gland cells; no effect on LS	Inadequate diet quantity delivered during 1- to 2-wk acclimation, oysters likely under starvation stress; no indication of diet quantity or quality delivered during 2-d exposure; examined both *in vitro* & *in vivo* exposures	Gaspar et al. (2018) **Rating = 1**
	Selective ingestion and egestion of MP	PS microspheres and PA (nylon) microfibers: 19–1000 µm diameter (spheres), 75–1075 µm length (fibers, 30-µm width) MP offered directly; 7 to 440 particles per dose depending on size	Proportion of spheres rejected and egested <3 hr by oysters increased with sphere size; proportion of fibers rejected and egested <3 hr showed no trend with fiber length	Adequate diet delivered during acclimation, 2-hr exposure and 2-d depuration; pseudofeces and feces collected and identified under stereomicroscope; MP aged in seawater for 3 d	Ward et al. (2019b) **Rating = 2**
	Ingestion and egestion of MP (dock-side experiment)	MP in natural seawater: size and concentration of MP in delivered seawater not determined	Oysters ingested and egested MP; after exposure <~2 MP (mostly fibers) found in samples of pseudofeces, feces, and tissues; PET was most abundant polymer found in biodeposits and tissues	Oysters held in natural seawater during ca. 1-wk acclimation, and in flowing natural seawater during 2-hr exposure; pseudofeces and feces collected without aid of microscope, cross-contamination and underestimation likely; size, concentration and polymer type of MP in seawater not determined; only 10% of suspected MP in samples identified with FTIR; errors in reporting size range of MP in samples (e.g., 0.5–6 mm or 0.05–6 mm?)	Craig et al. (2022) **Rating = 0.5**
	Uptake and depuration of MP	PET microfibers, PA (nylon) particles and tire crumb particles: 301 µm length (width = 19.8 µm), 321 µm and 226 µm, respectively. 5×10^{3} MP·L^{-1}	Oysters accumulated longer PET fibers and smaller PA and tire particles; mean accumulation of each MP type was <4 MP·g^{-1}; after depuration, 64–81% of all MP eliminated; accumulation and depuration of MP was size and shape dependent	Insufficient information provided regarding diet quantity (i.e., number cell·mL^{-1}) during 72-hr acclimation, 96-hr exposure and 96-hr depuration; number of MP accumulated in oysters included particles captured but not necessarily ingested	Weinstein et al. (2022) **Rating = 1.5**

Crassostrea rivularis (jinjiang oyster)	Combined effects of MP and seawater acidification on embryonic development and larval behavior and physiology	Fluorescent microspheres (polymer type not given): 10 µm diameter 10 and 1000 $MP \cdot L^{-1}$ pH: 7.3 and 8.1	MP had no effect on hatching rate of oyster eggs (either concentration or pH); MP at 1000 $MP \cdot L^{-1}$ and pH 8.1 affected larval deformation, shell length, and swimming speed; no effects on these endpoints at 10 $MP \cdot L^{-1}$; no effects on total antioxidant capacity or MDA activity (either concentration or pH); MP has some effect on ALP activity	Husbandry of larvae during 9-d exposure poorly defined (diet, water changes, density in tanks); details of MP exposure poorly described; polymer type used in study not given	Sui et al. (2022b) **Rating = 0.5 #**
Crepidula onyx (onyx slippersnail)	Effects of MP on growth and early development	PS microspheres: 2–5 µm diameter 1.0×10^1, 6.0×10^4, and 1.4×10^5 $MP \cdot mL^{-1}$ Equivalent to 1.0×10^4, 6.0×10^7, and 1.4×10^8 $MP \cdot L^{-1}$, respectively.	MP at the two highest concentrations decreased growth of snail larvae, affected settlement, and decreased juvenile growth; MP at 1×10^4 $MP \cdot L^{-1}$ had no effects on larvae or juveniles	Adequate diet delivered during 1-d acclimation and 65-d exposure; diet concentration increased over time to account for larval growth; high concentrations of MP used, especially for larvae; contrary to authors' statements, MP concentrations were not environmentally relevant	Lo and Chan (2018) **Rating = 1.5**
Dreissena bugensis (quagga mussel)	Uptake and effects of MP on physiological processes	Fluorescent HDPE microspheres: 10–45 µm diameter 0.1 $g \cdot L^{-1}$, 0.4 $g \cdot L^{-1}$, and 0.8 $g \cdot L^{-1}$ Equivalent to 9.2×10^6, 3.6×10^7, and 7.3×10^7 $MP \cdot L^{-1}$, respectively	MP found on gills and in intestine of mussels; complete elimination of MP did not occur in 24 hr; some effects on FR, but inconsistent across the 3 d of exposure; no effects on mortality, reproduction, or RR	Insufficient information provided regarding diet quantity (i.e., diet volume) delivered during several day acclimation; mussels starved for 2 d prior to physiological experiments and likely under stress; mussels starved during 24-hr exposures and during depuration and likely under stress; no indication of diet quantity used during physiological measurements; poor methodology for physiological measurements	Pedersen et al. (2020) **Rating = 0**
Dreissena polymorpha (zebra mussel)	Uptake and effects of MP on cellular processes	PS microspheres: 1 and 10 µm diameter 1×10^6 $MP \cdot L^{-1}$ and 4×10^6 $MP \cdot L^{-1}$ (50:50 mix of both sizes)	MP found in gut, tissues and hemolymph of mussels; MP at 1×10^6 $MP \cdot L^{-1}$ affected CAT and glutathione peroxidase activities; both MP concentrations increased levels of dopamine; no effects on oxidative stress or genotoxicity	No indication of diet quantity delivered during 2-wk acclimation and 6-d exposure; mussels fed only 1x every 2–3 d and likely under starvation stress; well-established methods used to evaluate biomarker responses	Magni et al. (2018) **Rating = 1**

(*Continued*)

Table 3. Continued.

Species	Experiment	Particle types, sizes, and concentrations tested	Reported results	Notes	Reference and rating
Dreissena polymorpha (zebra mussel)	Ingestion and depuration of MP, and effects on physiological and cellular processes	Fluorescent PS microspheres, PS particles, and diatomite (natural) particles: 5, 10, 45, and 90 µm diameter (spheres); ≤63 µm (particles) 0.3 and 3 MP·mL^{-1} (spheres); 6.4, 160, 4000, or 100,000 MP·mL^{-1} (PS particles); 100,000 particles·mL^{-1} (diatomite) Equivalent to 3×10^{2} and 3×10^{3} MP·L^{-1} (spheres); 6.4×10^{3}, 1.6×10^{5}, 4.0×10^{6}, and 1.0×10^{8} MP·L^{-1} (PS particles); 1.0×10^{8} particles·L^{-1} (diatomite), respectively	Mussels ingested MP, number depended on particle size and exposure time; ingestion of MP increased with concentration and decreased with increasing microalgal diet; <10 % of MP remained in gut 24 hr post exposure; highest concentration of MP increased clearance of microalgae; no effects of MP or diatomite (up to 42-d exposure) on energy reserves or oxidative stress	No indication of diet quantity delivered during 2-wk and 4-wk acclimation; adequate diet delivered during exposures that examined ingestion (up to 168 hr), depuration (up to 120 hr) and toxicity (up to 42 d); CR unreliable, no controls for microalgal settling or growth (i.e., beakers without mussels); high concentration of diet delivered during CR experiments (1.2×10^{7} cells·mL^{-1}) likely depressed feeding and stimulated excess pseudofeces production; robust methods, procedures and QA used for other measurements	Weber et al. (2021) **Rating = 1***
Ennucula tenuis (smooth nutclam)	Effects of MP on behavior and physiological processes	PE particles: 4–6 µm, 20–25 µm, and 125–500 µm 1, 10, and 25 mg·kg^{-1} sediment For 1 mg·kg^{-1} sediment, equivalent to 9.3×10^{6}, 2.4×10^{6}, and 9.4×10^{1} MP·kg^{-1} for the three particle sizes, respectively	Total energy reserves of clams decreased with increasing particle concentration when exposed to largest particles; overall, no effects on protein, carbohydrate, or lipid contents; no effects on survival, CI, or burrowing behavior	Deposit-feeding clams maintained in natural sediment during 1-month acclimation and 4-wk exposure	Bour et al. (2018b) **Rating = 2**
Geukensia demissa (Atlantic ribbed mussel)	Ingestion and rejection of MP	PS and PE microspheres: 5 µm and 250–300 µm diameter, respectively 0.167 g·L^{-1} and 3.3 g·L^{-1}, respectively Equivalent to 2.4×10^{9} and 3.3×10^{5} MP·L^{-1}, respectively	Mussels ingested both sizes of MP; both sizes of MP found in tissues, pseudofeces, and feces; large MP eliminated after 24-hr depuration; a few small MP remained in gut.	Adequate diet delivered during 1-wk acclimation and 2-hr exposure; mussels starved during 24-hr depuration; high concentrations of diet and MP in containers during 2-hr exposure (10^{6} particles·mL^{-1}) likely depressed feeding and stimulated excess pseudofeces production; performed histology to verify MP in various tissues and hemolymph	Khan and Prezant (2018) **Rating = 1**
Macoma balthica (Baltic clam)	Ingestion of MP	Fluorescent PS microspheres: 10 µm diameter 5, 50, and 250 MP·mL^{-1} Equivalent to 5×10^{3}, 5×10^{4}, and 2.5×10^{5} MP·L^{-1}, respectively	Clams ingested MP; MP found in digestive tract and in or on the gills; number of MP ingested increased with exposure concentration	Deposit-feeding clams maintained in natural sediment during acclimation and exposure; 6 clams held in an aquarium with 20 polychaetes, 6 amphipods, 6 foolish mussels, 2 gammarids and 4 mysid shrimp; authors confuse MP found on the gill filaments with "in the gills"	Setälä et al. (2016) **Rating = 1.5**

Meretrix meretrix (Asiatic hard clam)	Effects of NP on hatching, development, metamorphosis, and malformation of eggs and larvae	Aminated (-NH_2) and carboxylated (-COOH) PS nanospheres: 100 nm and 200 nm diameter, respectively. 0.02, 0.2, 0.5, 1, and 2 mg·L^{-1} Equivalent to 3.6×10^{10} to 10^{12} NP·L^{-1}, and 4.5×10^{9} to 10^{11} NP·L^{-1}, respectively for the two sphere diameters	Both types of NP affected hatching of clam eggs and development, malformations, and shell size of larvae depending on larval stage and dose; PS-NH_2 produced greater negative effects on developing larvae than PS-COOH; NP at 0.02 mg·L^{-1} produced fewer effects on developmental endpoints	Adequate diet delivered to larvae during 1- to 7-d exposure; contrary to authors' statements, extremely high NP concentrations were likely not environmentally relevant	Luan et al. (2019) **Rating = 1.5**
Mytilus coruscus (thick shell mussel)	Effects of MP and starvation on CI, and byssus production and adhesion	Fluorescent PS microspheres: 2 µm diameter 2.5 µg·L Equivalent to 5.6×10^{5} MP·L^{-1}	MP negatively affected CI and byssus adhesion in well-fed mussels; MP had no effects on length or number of byssal threads produced in well-fed mussels, and mixed effects on transcript levels of genes encoding for byssus-formation proteins; starvation exacerbates the adverse effects	Adequate diet delivered during 2-wk acclimation and 3-wk exposure; contrary to authors' statements, MP concentrations were not environmentally relevant	Shang et al. (2021) **Rating = 1.5**
	Ingestion of NP and MP	Fluorescent PS nanospheres and microspheres: 0.07, 0.5, 5, 10, and 100 µm diameter 0.2 mg·L^{-1} Equivalent to 2.6×10^{15}, 7.3×10^{12}, 7.3×10^{9}, 3.6×10^{8}, and 9.1×10^{5} NP/MP·L^{-1} for the five sphere diameters, respectively	Mussels ingested NP and MP, number depended on sphere diameter and exposure time; majority of NP and MP found in digestive tract compared to mantle or gill	Adequate diet delivered during 1-wk acclimation, 3.5-d exposure and 3.5-d depuration; calculated intake ratio unreliable, no controls for particle agglomeration and settling (i.e., aquaria without mussels); calculated uptake unreliable, unclear whether controls for natural fluorescence of tissues (i.e., mussels without NP/MP) were used; failed to consider repeated re-filtration of water by mussels, design did not allow for direct comparison of capture efficiency of NP and MP; contrary to authors' statements, NP/MP concentrations were not environmentally relevant	Wang et al. (2021a) **Rating = 0**

(*Continued*)

Table 3. Continued.

Species	Experiment	Particle types, sizes, and concentrations tested	Reported results	Notes	Reference and rating
Mytilus coruscus (thick shell mussel)	Effects of NP and MP, delivered with and without food, on energy budget, digestive enzymes, and oxidative stress	Fluorescent PS nanospheres and microspheres: 70 nm and 10 µm diameter 0.2 mg·L^{-1} and 0.02 mg·L^{-1} Equivalent to 2.6×10^{10} NP·L^{-1} and 3.6×10^{3} MP·L^{-1}, for the two sphere diameters, respectively, at the highest concentration	In starved mussels, NP and MP reduced SFG and increased production of some antioxidants; presence of food reduced some negative effects of NP and MP	Adequate diet delivered during 2-wk acclimation and 2-wk exposure; calculated intake/clearance ratio unreliable, no controls for particle agglomeration and settling (i.e., chambers without mussels); no feces collected over 3-d ingestion experiment, results show only residual amounts of NP and MP in gut after exposure period; AE unreliable, feces with incorporated NP/MP ashed, loss of NP/MP mass included in "organic" fraction; RR unreliable, used YSI probe and gave no indication that water in containers was kept at constant temperature or stirred; contrary to authors' statements, MP concentrations were not environmentally relevant	Wang et al. (2021b) **Rating = 0**
	Combined effects of MP and seawater acidification on physiological processes and energy budget	Fluorescent PS microspheres: 10 µm diameter 10 and 1000 MP·L^{-1} pH: 7.3 and 8.1	MP at 1000 MP·L^{-1} and pH 8.1 decreased CR and SFG after 21 d; increased AE (days 7–21), RR (days 3,7) and AmEx (day 7); MP at 10 MP·L^{-1} and pH 8.1 had no effects on mussel CR, AE, AmEx, or SFG; RR decreased after 21 d; few consistent effects found at pH 7.3 with either MP concentration	Adequate diet delivered during 3-wk acclimation and 3-wk exposure; no indication of how diet and MP were delivered in the flow-through exposure system (260 L·hr^{-1}); mussels starved for 6 hr prior to physiological measurements; CR likely underestimated, sampling interval too long (1 hr), no indication of volume of water or number of animals in tanks; AE unreliable, feces with incorporated MP ashed, loss of MP mass included in "organic" fraction; RR unreliable, used YSI probe and gave no indication that water in containers was kept at constant temperature or stirred	Sui et al. (2022a) **Rating = 0 #**
Mytilus edulis (blue mussel)	Uptake of MP and effects on physiological and cellular processes	Fluorescent and unlabeled PS microspheres: 2 and 4–16 µm diameter (uptake), and 3 and 9.6 µm diameter (trans-location) 0.51 g·L^{-1} (uptake) and 43 MP·mL^{-1} (translocation) Equivalent to 1.2×10^{11} and 9.3×10^{8} MP·L^{-1} for the two sphere diameters, respectively (uptake); and 4.3×10^{4} MP·L^{-1} for both sphere diameters (translocation)	Mussels ingested all sizes of MP; low numbers of both 3 and 9.6 µm spheres found in hemolymph and hemocytes after 3-hr exposure, 3 µm spheres found in greater abundance; low % of both 3 and 9.6 µm spheres remained in hemolymph after 48 d of depuration; MP had no effects on CR, oxidative status of hemolymph, or viability and phagocytic activity of hemocytes	No indication of diet quantity delivered during 48-hr acclimation and 48-d depuration; mussels starved during 3- and 12-hr exposures; feces collected without aid of microscope and likely contaminated with pseudofeces; description of mussel anatomy contains several errors in terminology (e.g., inhalant siphon, abductor muscle); performed histology to verify MP in various tissues and hemolymph	Browne et al. (2008) **Rating = 0.5**

Mytilus edulis (blue mussel)	Uptake of MP and effects on physiological processes, tissues, and cellular biochemistry	HDPE particles: >0–80 µm 2.5 g·L^{-1} Equivalent to 7.8×10^{7} MP·L^{-1}	Mussels ingested MP; MP found on and in the gills, stomach, intestine, and digestive gland; smallest particles found in the blood lacunae; MP caused histological changes and inflammatory responses and affected LS; effects increased with exposure times; no effects on lipofuscin accumulation, lipid metabolism, or CI	Inappropriate diet quantity and quality (Liquifry) delivered during 12-wk acclimation and 96-hr exposure, mussels under starvation stress; performed histology and used polarized-light microscopy to verify MP in various tissues	von Moos et al. (2012) **Rating = 0.5**
	Effects of different NP and microalgal concentrations on feeding behavior	PS nanospheres: 30 nm 0.1, 0.2, and 0.3 g·L^{-1} Equivalent to 6.4×10^{15}, 1.3×10^{16}, and 1.9×10^{16} NP·L^{-1}, respectively	Mussels captured and ingested agglomerated NP; high concentrations of NP stimulated excess pseudofeces production	Mussels starved during 15-hr acclimation; high concentrations of diet (ca. 6.0×10^{4} and 1.2×10^{5} cells·mL^{-1}) delivered during 8-hr exposure likely affected feeding; valve opening is a poor indicator of filtering activity	Wegner et al. (2012) **Rating = 0.5**
	Ingestion of MP and effects on cellular energy allocation (CEA)	PS microspheres: 10, 30, and 90 µm diameter 110 MP·mL^{-1}, total (50, 50, and 10 MP·mL^{-1} for 10, 30, and 90 µm spheres, respectively) Equivalent to 1.1×10^{5} MP·L^{-1}, total	Mussels ingested MP, number ingested depended on diameter; low number of MP found in tissues and hemolymph (<0.3%); MP increased RR; no effects on energy reserves (protein, lipid, carbohydrate) or CEA	No indication of diet quantity or quality delivered during 2-wk exposure; mussels starved during 24-hr depuration; calculated ingestion ratios unreliable, no controls for particle settling (i.e., beakers without mussels); detailed methods for hemolymph removal and CEA determination lacking; digesting tissues in boiling concentrated nitric acid (69%) can cause polystyrene to dissolve	Van Cauwenberghe et al. (2013) **Rating = 0**
	Occurrence of MP in field-collected mussels and effects on cellular energy allocation (CEA; lab exposure)	PS microspheres: 10, 30 and 90 µm diameter 110 MP·mL^{-1}, total (50, 50, and 10 MP·mL^{-1} for 10, 30, and 90 µm spheres, respectively) Equivalent to 1.1×10^{5} MP·L^{-1}, total	Field-collected mussels contained low levels of MP (0.2 MP·g^{-1}); MP increased energy consumption in digestive gland cells compared to controls (lab exposure); no effects on energy reserves or CEA (lab exposure)	No indication of diet quantity delivered during 2-wk acclimation and 2-wk exposure; mussels starved during 24-hr depuration; depuration period (24 hr) not sufficient to allow all MP to be eliminated from gut; digesting tissues in boiling concentrated nitric acid (69%) can cause polystyrene to dissolve	Van Cauwenberghe et al. (2015) **Rating = 0.5**
	Effects of MP on FR and measures of ecosystem function	HDPE and PLA particles: 103 µm (range = 0.5 – 316 µm) and 66 µm (range = 0.6 – 363 µm), respectively 2.5 and 25 µg·L^{-1} Equivalent to 9.0×10^{1} and 8.0×10^{2} MP·L^{-1} (HDPE) and 1.5×10^{2} and 1.2×10^{3} MP·L^{-1} (PLA), respectively	Both types of MP delivered at 25 µg·L^{-1} decreased mussel FR; no effects at 2.5 µg·L^{-1}; no effects of either type of MP at either concentration on infaunal invertebrate assemblages	Adequate diet delivered during 50-d exposure in outdoor mesocosms; erroneously reported that environmental MP concentrations as high as 7.8×10^{3} MP·L^{-1} have been found; actual reported field values (<2 mm) were <1 MP·L^{-1} (Kang et al. 2015a)	Green et al. (2017) **Rating = 1.5**

(*Continued*)

Table 3. Continued.

Species	Experiment	Particle types, sizes, and concentrations tested	Reported results	Notes	Reference and rating
Mytilus edulis (blue mussel)	Occurrence of MP in field-collected mussels, and uptake of MP in and on specific organs (lab exposure)	PET microfibers: >100 µm length (width not provided) 2.0×10^3 MP·L^{-1}	Field-collected mussels contained low levels of MP; Fibers found in or on various organs within the pallial cavity (lab exposure and depuration)	No indication of diet quantity or quality delivered during 5-d acclimation, 48-hr exposure or 48-hr depuration, mussels likely under starvation stress; starvation likely affected filtration and elimination of fibers; actual isolation of stomach from intestine, and gonad from mantle suspect; no histology performed to differentiate MP on versus in various organs; crude preparation of fibers, no indication of actual size range; lack of knowledge of particle-feeding processes lead to erroneous conclusions	Kolandhasamy et al. (2018) **Rating = 0**
	Occurrence of MP in mussels collected in the field and exposed in the laboratory	PS microfibers, PVC particles and PS microspheres: 100–3500 µm length (fibers, width not provided), 20–500 µm size (particles), 10 µm diameter (spheres) 100 and 1000 MP·L^{-1} (ratio of 1:1:8 spheres, particles, and fibers, respectively)	Field-collected mussels contained low levels of MP (ca. 3 MP·g^{-1}); all types of MP tested were found in or on sampled tissues (lab exposure); smaller fibers and spheres taken up in preference to larger particles; positive correlation between MP in the water and mussels	No indication of diet quantity or quality delivered during 5-d acclimation or 5-d exposure; ingestion of MP not verified by collecting feces; identified MP could have been on the gills or mantle (post capture)	Qu et al. (2018) **Rating = 1**
	Ingestion and elimination of MP (fibers)	PET microfibers 459 µm length (20 µm width) 3–30 MP·mL^{-1} Equivalent to 3×10^3 to 3×10^4 MP·L^{-1}	Mussels ingested MP; majority of fibers rejected in pseudofeces (71%); fibers reduced FR	Inadequate diet quantity delivered during 7- to 10-d acclimation and 72-hr exposure, mussels likely under starvation stress; pseudofeces cannot be "counted" as it is amorphous and of different size and shape; pseudofeces and feces collected without aid of microscope, cross-contamination and underestimation likely; uptake measurements unreliable, mussel (ca. 30 mm) would clear 1 L of water in ca. 2 hr leaving very few MP between 3 and 72 hr; reported FR (50 mL·min^{-1}) much higher than literature values for ca. 30 mm shell-length mussel (Cranford et al. 2011); confusing explanation of experimental methods and use of non-standard terminology	Woods et al. (2018) **Rating = 0**
	Effects of MP on byssus production and adhesion, and hemolymph proteome	HDPE and PLA particles: 103 µm (range = 0.5 – 316 µm; HDPE) and 66 µm (range = 0.6 – 363 µm; PLA) 25 µg·L^{-1} Equivalent to 8.4×10^2 and 1.3×10^3 MP·L^{-1} for the two polymer types, respectively	HDPE reduced the number and attachment strength of mussel byssal threads; both types of MP altered the hemolymph proteome, including putative immune, metabolic, and detoxification proteins; overall HDPE caused more changes to protein abundances than PLA	Adequate diet delivered during 52-d exposure in outdoor mesocosms	Green et al. (2019) **Rating = 2**

Mytilus edulis (blue mussel)	Ingestion and egestion of NP and MP and effects on larval growth and development	PS nanospheres and microspheres: 100 nm and 2 µm diameter Exp. 1 & 2: 10^{11} NP·mL^{-1} and 10^{8} MP·mL^{-1}; Exp. 3: 7.7×10^{5} to 5.1×10^{8} NP·mL^{-1}, and 9.6×10^{1} to 6.4×10^{4} MP·mL^{-1} Equivalent to [Exp. 1 & 2]: 10^{14} NP·L^{-1} and 10^{11} MP·L^{-1}; [Exp. 3]: 7.7×10^{8} to 5.1×10^{11} NP·L^{-1}, and 9.6×10^{4} to 6.4×10^{7} MP·L^{-1}	Mussel larvae ingested more MP than NP; egestion was independent of sphere size; NP and MP increased number of abnormal larvae, more pronounced malformations caused by NP; no effects on larval growth or feeding rates	Adequate diet delivered to larvae during 4-hr, 48-hr, and 2-wk exposures; high concentrations of NP and MP used, especially for larvae	Rist et al. (2019) **Rating = 2**
	Selective ingestion and egestion of MP	PS microspheres and PA (nylon) microfibers: 19–1000 µm diameter, 75–1075 µm length (30 µm width), respectively. MP offered directly: 7–440 particles per dose depending on size	Proportion of microspheres rejected and egested <3 hr by mussels increased with sphere size; proportion of fibers rejected and egested <3 hr showed no trend with fiber length	Adequate diet delivered during acclimation, 2-hr exposure and 2-d depuration; pseudofeces and feces collected and identified under stereomicroscope; MP aged in seawater for 3 d	Ward et al. (2019b) **Rating = 2**
	Effects of MP on CI and gut microbiota	HDPE particles (virgin and 1-month seawater aged): 4–6 µm and 20–25 µm 0.2 and 20 mg·L^{-1} Equivalent to 1.2×10^{6} and 1.2×10^{8} MP·L^{-1}, respectively	Virgin and aged MP altered gut microbiota of mussels after 1, 3, and 6 wks; greatest effects found at highest concentration and with aged MP; no effect on CI	Inadequate diet quantity delivered during 7-d acclimation, 6-wk exposure and 8-d depuration; mussels fed only 3x per wk and likely under starvation stress; contrary to authors' statements, MP concentrations were not environmentally relevant	Li et al. (2020b) **Rating = 1**
	Effects of MP on various cellular and biochemical processes	PLA particles: 0.8–10 µm 10 and 100 µg·L^{-1} Equivalent to 1.9×10^{6} and 1.9×10^{7} MP·L^{-1}, respectively	Neither concentration of MP affected mussel CI or biomarkers associated with oxidative stress, neurotoxicity, or immunotoxicity; MP caused a down-regulation of features associated with glycerophospholipids.	Inappropriate diet quality (Pro-coral phyton) delivered during 1-wk acclimation and 8-d exposure, mussels under starvation stress; high mortality in all groups (including control) after 8 d (ca. 18–33%) likely caused by inappropriate diet; confusing description of mortality and number of replicates used	Khalid et al. (2021) **Rating = 0**

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Table 3. Continued.

Species	Experiment	Particle types, sizes, and concentrations tested	Reported results	Notes	Reference and rating
Mytilus galloprovincialis (Mediterranean mussel)	Uptake of MP, transfer of adsorbed pyrene to tissues, and effects on cellular processes	PE and PS microparticles: <100 µm (no size distribution provided) 1.5 g·L^{-1} (exposure exp.) Equivalent to 2×10^{7} MP·L^{-1} (assuming a mode of 50 µm)	Mussels ingested MP; both PE and PS found in hemolymph, gills, and digestive tissues; both PE and PS transferred pyrene to mussels; both virgin and contaminated MP affected some of the measured biomarkers (e.g., immunological responses, neurotoxic effects, onset of genotoxicity, gene expression profile); weight-of-evidence index indicated overall risk classification of: PE-virgin (slight), PS-virgin (moderate), PE-pyrene (moderate), PS-pyrene (severe)	No indication of diet quantity or quality delivered during 10-d acclimation and 7-d exposure, mussels likely under starvation stress; crowded experimental conditions (10, 5-cm mussels in 6-L water), no mention of aeration or means to keep particles in suspension during exposure; methodology regarding collection of hemolymph and isolation of tissues not defined; performed histology to verify MP in various tissues and hemolymph	Avio et al. (2015a) **Rating = 0.5**
	Effects of NP on immune function of hemocytes *in vitro*	Aminated (-NH_2) PS nanospheres: 50 nm diameter 1, 5, and 50 µg·mL^{-1} Equivalent to 1.5×10^{13}, 7.3×10^{13}, and 7.3×10^{14} NP·L^{-1}, respectively	NP caused a dose-dependent decrease in phagocytic activity of hemocytes, and an increase in lysozyme activity, ROS, and NO production; NP induced apoptotic processes at highest concentration	No indication of diet quantity or quality delivered during 1- to 3-d acclimation, mussels likely under starvation stress; hemocytes exposed to NP for periods of 30 min to 4 hr	Canesi et al. (2015) **Rating = 1.5**
	Effects of MP on gene expression	HDPE microparticles: 1–50 µm 1.5×10^{7} MP·L^{-1}	MP caused up-regulation of mussel genes associated with carbon metabolism, oxidative stress, immune response, and apoptosis (mantle, digestive gland); genes associated with carbon metabolism were down-regulated (hemolymph, gills)	Adequate diet delivered during 1-wk acclimation and 24-hr exposure; mussels starved for 24 hr prior to experiments	Détrée and Gallardo-Escárate (2017) **Rating = 1.5**
	Ingestion of MP and effects on tissues	PE microparticles (virgin and weathered): 50–570 µm 0.01 mg·mL^{-1} Equivalent to 1.2×10^{3} and 1.9×10^{3} MP·L^{-1} for virgin and weathered PE, respectively	Mussels ingested same number of virgin and weathered MP; virgin and weathered MP caused structural changes to gills and digestive gland; necrosis in other tissues (e.g., mantle); no effect on CI	No indication of diet quantity or quality delivered during 20-d acclimation, mussels likely under starvation stress; inadequate diet quantity delivered during 21-d exposure, mussels fed only 3x per wk and likely under starvation stress; isolated PE particles from toothpaste; unclear if hemolymph was actually collected, authors indicate that needle was "….*inserted into the sinus* near *to the posterior adductor muscle*…."; inaccurate description of particle capture	Bråte et al. (2018a) **Rating = 0**

Mytilus galloprovincialis (Mediterranean mussel)	Ingestion of MP and effects on larval feeding, development, and cellular processes	Fluorescent PS microspheres: 3 µm diameter 50–10,000 MP·mL^{-1} Equivalent to 5×10^{4} to 1×10^{7} MP·L^{-1}, respectively	Ingestion of MP by mussel larvae increased with increasing plastic concentration; transcriptional alterations found at 50 and 500 MP·mL^{-1} including up-regulation of genes involved in shell biogenesis and immune-modulation; lysosomal enzymes also inhibited; no effects on feeding at 2000 MP·mL^{-1} or development at 50–10,000 MP·mL^{-1}	No indication of diet quantity or quality delivered to adults during 3-d acclimation; adequate diet delivered to larvae during holding period; no indication of diet quantity or quality delivered to larvae during 9-d uptake and 8-d retention experiments, larvae likely under starvation stress	Capolupo et al. (2018) **Rating = 0.5**
	Uptake of MP, transfer of adsorbed benzo(a)-pyrene (BaP) to tissues and effects on cellular processes	LDPE microparticles: 20–25 µm 10 mg·L^{-1} Equivalent to 2.3×10^{7} MP·L^{-1}	Mussels ingested MP; found in hemolymph, gills, and digestive tissues; MP transferred BaP to gills and digestive gland; both virgin and contaminated MP affected immunological biomarkers depending on exposure time; limited effects were found for oxidative status, neurotoxicity, and genotoxicity; weight-of-evidence index indicated risk classification of: virgin LDPE (slight) and contaminated LDPE (major)	No indication of diet quantity or quality delivered during 15-d acclimation, mussels likely under starvation stress; inappropriate diet quantity and quality (zooplankton, 50–300 µm) delivered during 4-wk exposure, mussels under starvation stress; crowded experimental conditions (60, 6-cm mussels in 20 L water); performed histology to verify MP in various tissues	Pittura et al. (2018) **Rating = 0.5**
	Uptake and egestion of MP	HDPE particles: 2–22 µm ($\bar{x}$ = 4–6 µm) 2 and 4 mm^{3}·L^{-1} Equivalent to 3.8×10^{4} and 7.6×10^{4} MP·L^{-1}, respectively.	Mussels ingested and egested MP in a manner similar to microalgae; elimination depended on size, MP <6 µm remained in the gut longer	Adequate diet delivered during 8-wk acclimation; mussels starved during 3-d acclimation, 4-hr exposure and 6-d depuration, and likely under stress; calculated clearance "rates" unreliable, no controls for particle settling or other losses (i.e., beakers without mussels); elimination results unreliable given starvation of mussels during depuration; lack of knowledge of particle-feeding rates and processes lead to erroneous conclusions (e.g., "clearance rate" of MP increased with increasing concentration); all data based on counts from electronic particle counter, counted particles not definitively ID as MP	Fernández and Albentosa (2019a) **Rating = 0**

(*Continued*)

Table 3. Continued.

Species	Experiment	Particle types, sizes, and concentrations tested	Reported results	Notes	Reference and rating
Mytilus galloprovincialis (Mediterranean mussel)	Uptake and egestion of MP	HDPE particles: 2–22 µm ($\bar{x}$ = 4–6 µm) 3 mg·L^{-1} Equivalent to 5.4 × 10^{7} MP·L^{-1}	Mussels ingested and egested MP; elimination depended on size, MP <4 µm remained in the gut longer; small amount (2.2%) of MP translocated to gills; MP <3 µm remained in gills after 7 d depuration	Adequate diet delivered during 8-wk acclimation; mussels starved during 4-hr exposure and 7-d depuration and likely under stress; calculated % particles cleared unreliable, no controls for particle settling or other losses (i.e., beakers without mussels); elimination results unreliable given starvation of mussels during depuration; all data based on counts from electronic particle counter, counted particles not definitively ID as MP	Fernández and Albentosa (2019b) **Rating = 0.5**
	Uptake of MP and effects on the multixenobiotic resistance (MXR) system in larvae and adults	Fluorescent PS microspheres: 3 and 45 µm diameter 50 and 500 MP·mL^{-1} (larvae); 1 and 10 MP·mL^{-1} (adults), respectively. Equivalent to 5 × 10^{4} and 5 × 10^{5} MP·L^{-1} (larvae); 1 × 10^{3} and 1 × 10^{4} MP·L^{-1} (adults), respectively	MP found in hemolymph of adult mussels; particle size and concentration affected accumulation; smallest MP (3-µm) reduced MXR activity and down-regulated MXR-related transporters in embryos (both concentrations); effects of MP on MXR activity in adults depended on particle size, concentration, and tissue (hemocytes, gills)	Little information provided regarding diet quantity delivered to adults during acclimation (unknown duration); no indication of diet quantity or quality delivered to larvae during 48-hr embryotoxicity assay, larvae likely under starvation stress; no indication of diet quantity or quality delivered to adults during 4-d exposure, mussels likely under starvation stress	Franzellitti et al. (2019) **Rating = 1**
	Ingestion of MP and effects on tissues and cellular processes	Fluorescent and unlabeled PS microspheres: 2, 5, 6, and 10 µm diameter 10 and 1000 MP·mL^{-1} Equivalent to 1 × 10^{4} and 1 × 10^{5} MP·L^{-1}, respectively	Mussels ingested and egested MP; MP found in various gut tissues depending on exposure time; MP not found in gills or gonads regardless of exposure time, or microsphere size or concentration; no histopathological damage or increased oxidative stress	No indication of diet quantity or quality delivered during acclimation (unknown duration) and short-term (up to 48 hr) assays, mussels likely under starvation stress; no indication of diet quantity delivered during 21-d exposure and 7-d depuration; performed histology to verify MP in various tissues	Gonçalves et al. (2019) **Rating = 1**
	Ingestion and elimination of MP	Fluorescent PS microspheres: 1, 10, and 90 µm 250 MP·mL^{-1} Equivalent to 2.5 × 10^{5} MP·L^{-1}	Mussels ingested and egested MP; gut retention time and egestion rate varied with size of microspheres	Inadequate diet quantity delivered during acclimation and 40-d depuration, mussels likely under starvation stress; mussels starved during 3-hr exposure; experimental set up for feces collection seriously flawed, inhalant aperture sealed within a 25-mL tube for up to 40 d isolating mussel from diet and clean water in holding container; reported results unreliable	Kinjo et al. (2019) **Rating = 0**

Mytilus galloprovincialis (Mediterranean mussel)	Incorporation of MP into biodeposits and effects on sinking velocity and uptake by a polychaete	Fluorescent PA and unlabeled PP particles: 41 and 128 µm 600 and 300 MP·L^{-1}, respectively.	Mussels ingested and egested MP; incorporation of MP into fecal pellets increased MP sinking velocity but decreased fecal-pellet sinking velocity; incorporation of MP into fecal pellets doubled uptake by polychaete	Mussels starved during 24-hr acclimation and 48-hr exposure and likely under stress; MP aged in seawater for 48 hr	Piarulli and Airoldi (2020) **Rating = 1**
	Ingestion of MP and red clay, and effects on byssus production and physiological processes	PVC and red clay particles: 0.1–100 µm 1.5, 15 and 150 mg·L^{-1} Equivalent to 1.2×10^{6}, 1.2×10^{7}, and 1.2×10^{8} MP·L^{-1}, respectively	PVC and clay had no effects on survival, RR, or CI of mussels at any concentration; no effect of particle type on byssus production; for both particle types more byssal threads produced at highest concentration; no differences in response variables between mussels exposed to PVC and clay	No indication of diet quantity or quality delivered during 11- to 14-d acclimation, mussels likely under starvation stress; inadequate diet quantity delivered during 5-wk exposure, mussels likely under starvation stress	Yap et al. (2020) **Rating = 1***
	Effects of MP (fibers) on feeding, tissues, and DNA	Microfibers of undetermined composition (from laundromat lint): 20–750 µm length (<30 µm width) 56, 100, 180 mg·L^{-1} Equivalent number not possible to calculate	Fibers caused DNA damage and increased percentage of mussels with abnormal gills and digestive glands; no effect on CR	Diet quantity delivered during 2-wk acclimation unclear; mussels fed only 3x per wk and likely under starvation stress; no indication of diet quantity or quality delivered during 7-d exposure, mussels likely under starvation stress; study difficult to replicate	Alnajar et al. (2021) **Rating = 0.5**
	Ingestion of MP and effects on hemocytes and gill tissue	Fluorescent PET microfibers: 50 and 100 µm length (13 µm width) 0.5 µg·L^{-1} and 100 mg·L^{-1} Equivalent to 2.2×10^{1} and 4.5×10^{6} MP·L^{-1} (50 µm), and 1.1×10^{1} and 2.2×10^{6} MP·L^{-1} (100 µm), respectively	Mussels ingested MP; high concentration of both sized fibers increased cell necrosis, DNA damage, ROS, NO, and AChE activity; variable effects at low concentration	Adequate diet delivered during 14-d acclimation; mussels starved during 4-d exposure and likely under stress; crowded experimental conditions (13, 3.7-cm mussels in 3 L water); confusing terminology used to describe mussel digestive system; performed histology to verify microfibers in digestive system	Choi et al. (2021) **Rating = 0.5 #**
	Uptake of plastic additive (hexabromocyclo-dodecane; HBCD) through MP ingestion or indirect leaching	Expanded PS particles: 20–770 µm and 4–5 mm 0.05 and 0.1 mg·mL^{-1}, respectively Equivalent to 6.8×10^{4} and 8.9×10^{1} MP·L^{-1}, respectively	HBCD levels increased in mussels exposed to both mm- and µm-sized PS; uptake through the aqueous phase (i.e., mm sized PS) more significant pathway for HBCD accumulation than particle ingestion; HBCD concentrations higher than control group	Adequate diet delivered during 5-d acclimation; mussels starved during 4- to 10-d exposure and 72-hr depuration and likely under stress	Jang et al. (2021) **Rating = 1**

(*Continued*)

Table 3. Continued.

Species	Experiment	Particle types, sizes, and concentrations tested	Reported results	Notes	Reference and rating
Mytilus galloprovincialis (Mediterranean mussel)	Effects of MP on digestive enzymes	PE and PS microspheres: 20 and 75 µm diameter 1×10^{4} and 5×10^{4} MP·L^{-1}	Low concentration of PS, but not PE, reduced amylase and xylanase activities in mussels, and increased cellulase activity; high concentrations of PE (75 µm) increased protease activity; no effects of MP type, size, or concentration on activities of laminarinase, lipases, or lipolytic esterases	Adequate diet delivered during 8-d acclimation and 7-d exposure; low volume of water (200 mL) used in experiments for 6 cm mussels; compared enzyme activity between initial field-collected and lab-acclimated mussels	Trestrail et al. (2021) **Rating = 2**
Mytilus trossulus (foolish mussel)	Ingestion of MP	Fluorescent PS microspheres: 10-µm diameter 5, 50, and 250 MP·mL^{-1} Equivalent to 5×10^{3}, 5×10^{4}, and 2.5×10^{5} MP·L^{-1}, respectively	MP found on the gills and in the digestive tract of mussels; number of microspheres ingested increased with exposure concentration	Six mussels held in an aquarium with 20 polychaetes, 6 amphipods, 6 Baltic clams, 2 gammarids and 4 mysid shrimp; mussels starved during 24-hr exposure; authors confuse MP found on the gill filaments with "in the gills"	Setälä et al. (2016) **Rating = 1.5**
	Ingestion of MP and natural silt, and effects on CR	Fluorescent PE microspheres and natural silt: 32–38 µm diameter and 30–37 µm size, respectively 1–2500 MP·mL^{-1} and 1–11,250 particles·mL^{-1} (silt) Equivalent to 1×10^{3} to 2.5×10^{6} MP·L^{-1} and 1×10^{3} to 1.1×10^{7} particles·L^{-1}, respectively	PE reduced CR of mussels at high concentrations (>1250 MP·mL^{-1}); silt at high concentration (>1250 particles·mL^{-1}) had no effect on CR; no difference between PE and silt at lower concentrations	Mussels held in flowing seawater during 48-hr acclimation; adequate diet delivered during 1-hr exposure; mussels starved for 12-hr prior to exposure; both MP and silt delivered with microalgae	Harris and Carrington (2020) **Rating = 2***
Mytilus spp.	Uptake of MP, transfer of adsorbed fluoranthene (FLU) to tissues and effects on cellular processes.	Fluorescent PS microspheres: 2 µm and 6 µm diameter 1800 and 200 MP·mL^{-1}, respectively Equivalent to 1.8×10^{6} and 2×10^{5} MP·L^{-1}, respectively	MP found in digestive tract of mussels; MP not found in gills, mantle, or gonad; some MP remained in intestine after depuration; a few found on gills; MP increased hemocyte mortality, ROS production, antioxidant, and glutathione-related enzymes, and caused tissue abnormalities; MP had minor role in transferring FLU to mussel tissues compared to water and foodborne exposure; interactive effects of MP and FLU observed; some effects changed after depuration	Adequate diet delivered during 6-wk acclimation, 7-d exposure and 7-d depuration; robust methods and procedures; performed histology to verify MP in various tissues	Paul-Pont et al. (2016) **Rating = 2**

Mytilus spp.	Uptake of MP and effects on tissues and physiological and cellular processes	PE and PP particles: <400 µm 0.008, 10, and 100 µg·L^{-1} (50:50 PE:PP) Equivalent to 9.0×10^{0}, 1.1×10^{4}, and 1.1×10^{5} MP·L^{-1}, respectively	MP found in mussel biodeposits at all concentrations; MP found in digestive gland only at highest concentration; MP not found in gills or other tissues; MP at low and medium concentrations increased SOD and CAT in digestive gland, no effects at highest concentration; MP at highest concentration increased SOD and CAT in gills, no effects at low and medium concentrations; no effects on feeding, tissues, CI, or DNA	Error in calculated number of microalgal cells delivered to each mussel each day; inadequate diet quantity delivered during 8-d acclimation, 10-d exposure and 10-d depuration, mussels likely under starvation stress; robust methods and procedures	Revel et al. (2019) **Rating = 1.5** #
	Comparative toxicity of different sized and shaped MP	Fluorescent PS nanospheres (sulfated), unlabeled PS microspheres and PA (nylon) microfibers: 50 nm diameter (spheres), 20 µm diameter (spheres) and 30 µm length (fibers, 10 µm width), respectively. 7.3×10^{9}, 1.1×10^{2}, and 1.9×10^{2} NP/MP·mL^{-1}, respectively Equivalent to 7.3×10^{12}, 1.1×10^{5} and 1.9×10^{5} NP/MP·L^{-1}, respectively	MP (spheres, fibers) found in digestive gland of mussels; NP stimulated an immune response; all plastic types increased SOD activity after 24-hr exposure, SOD levels returned to control levels after 7 d of exposure; no evidence of genotoxicity or effects on LS after exposure to any form of plastic particle	Mussels starved for 24 hr prior to acclimation; diet quantity delivered during 1- to 2-wk acclimation unclear; no indication of tank volume or biomass; high concentration of diet delivered during 7-d exposure (4×10^{5} cells·mL^{-1}) likely depressed feeding and stimulated excess pseudofeces production	Cole et al. (2020) **Rating = 1.5**
	Effects of MP on physiological processes, byssus production, and cells of digestive system and gills	PS microspheres and PVC particles: 40 µm diameter and 11–60 µm size, respectively 1.5×10^{1}, 1.5×10^{3}, 1.5×10^{4} MP·ind^{-1}·wk^{-1} (PS); and 1.5×10^{1}, 1.5×10^{3}, 1.5×10^{4}, 1.5×10^{5}, 1.5×10^{6} MP·ind^{-1}·wk^{-1} (PVC) Equivalent to 1×10^{1}, 1×10^{3}, 1×10^{4} MP·L^{-1}·wk^{-1} (PS), and 1×10^{1}, 1×10^{3}, 1×10^{4}, 1×10^{5}, 1×10^{6} MP·L^{-1}·wk^{-1} (PVC), respectively	PS at 1500 MP·ind^{-1}·wk^{-1} affected byssal-thread production of mussels after 36 wks; PS at 15,000 MP·ind^{-1}·wk^{-1} affected CR after 36 wks and SOD levels in gills and digestive system after 42 wks; PS at 15 MP·ind^{-1}·wk^{-1} also affected SOD levels in gills after 42 wks; no effects of PS at any concentration on growth rates, CI, or MDA levels; no effects of PVC at any concentration on any biomarker; overall, small effect sizes suggest that PS and PVC pose a minor threat to mussel population	Adequate diet delivered during 12-wk acclimation and 42-wk exposure; diet concentration increased over time to account for growth; conducted pilot study to confirm that mussels ingested MP; design includes environmentally relevant concentration of MP (10 MP·L^{-1}) and chronic exposure (42 wks)	Hamm and Lenz (2021) **Rating = 2** #

(*Continued*)

Table 3. Continued.

Species	Experiment	Particle types, sizes, and concentrations tested	Reported results	Notes	Reference and rating
Ostrea edulis (European flat oyster)	Effects of MP on growth, FR, RR and associated benthic communities	HDPE and PLA particles: 103 µm (range = 0.5 – 316 µm) and 66 µm (range = 0.6 – 363 µm), respectively 0.8 and 80 µg·L^{-1} Equivalent to 2.1 × 10^{1} and 2.1 × 10^{3} MP·L^{-1} (HDPE), and 3.1 × 10^{1} and 3.2 × 10^{3} MP·L^{-1} (PLA), respectively	HDPE and PLA had no effects on oyster growth, FR, or RR at either concentration; both types of MP delivered at high concentration affected structure of benthic assemblages, including species richness and total abundance of organisms	Adequate diet delivered during 60-d exposure in outdoor mesocosms; erroneously reported that environmental MP concentrations as high as 7.8 × 10^{3} MP·L^{-1} have been found, actual maximum reported values (<2 mm) were 7 MP·L^{-1} ($\bar{x}$ <1 MP·L^{-1}; Kang et al. 2015b)	Green (2016) **Rating = 1**
	Effects of MP on FR and measures of ecosystem function	HDPE and PLA particles 103 µm (range = 0.5 – 316 µm) and 66 µm (range = 0.6 – 363 µm), respectively 2.5 and 25 µg·L^{-1} Equivalent to 9.0 × 10^{1} and 8.0 × 10^{2} MP·L^{-1} (HDPE) and 1.5 × 10^{2} and 1.2 × 10^{3} MP·L^{-1} (PLA), respectively	Both types of MP delivered at both concentrations increased oyster FR; MP decreased the number of some infaunal invertebrates	Adequate diet delivered during 50-d exposure in outdoor mesocosms; FR of control oysters low; erroneously reported that environmental MP concentrations as high as 7.8 × 10^{3} MP·L^{-1} have been found; actual reported field values (<2 mm) were <1 MP·L^{-1} (Kang et al. 2015a)	Green et al. (2017) **Rating = 1.5**
	Uptake of virgin and *E. coli*-coated MP and effects on physiological processes	PMMA microspheres: 45 µm diameter 0.33 mg MP·L^{-1} Equivalent to 2.5 × 10^{2} MP·L^{-1}	Oysters ingested higher number of *E. coli*-coated MP compared to virgin MP; *E. coli*-coated MP increased RR after 10 d, virgin MP had no effect; no effects of either coated or virgin MP on CR; no accumulation of either coated or virgin MP	Oysters starved during 48-hr acclimation and likely under stress; adequate diet delivered during 10-d exposure; CR likely underestimated, sampling interval too long (1 hr) for 1 L of water	Fabra et al. (2021) **Rating = 1.5**
Pecten maximus (great scallop)	Uptake and elimination of NP	PS nanospheres (radiolabeled): 24 nm and 248 nm diameter 15 µg·L^{-1} Equivalent to 2 × 10^{12} and 1.8 × 10^{9} NP·L^{-1}, respectively	Uptake of 24-nm spheres by scallops greater than 248-nm spheres; overall, 24-nm spheres were distributed throughout whole body, whereas 248-nm accumulated in intestine; majority of both NP sizes were depurated within 3 d	Adequate diet delivered during 2-wk acclimation, 6-hr exposure and 48-d depuration; scallops starved 2-d before exposure and likely under stress; radiolabeled NP allowed for precise determination of tissue distributions and biokinetics	Al-Sid-Cheikh et al. (2018) **Rating = 2**
Perna perna (brown mussel)	Effects of virgin and aged plastic pellets on embryo development	Plastic pellets collected from beaches and virgin PP pellets: size of pellets not provided 0.5, 1, and 2 mL of pellets per 8 to 10 ml of water (note: number·mL^{-1} not possible to determine)	Mussel embryos affected by leachates from both virgin and beach pellets; toxicity of the leachate from beach pellets higher than that of virgin pellets	No indication of diet quantity or quality delivered during 48-hr exposure, larvae likely under starvation stress; no size information provided for collected pellets or for PP pellets; little information provided on specific polymer composition of collected pellets; contaminants leached from collected pellets not identified; study difficult to replicate.	Gandara e Silva et al. (2016) **Rating = 0**

(*Continued*)

Perna perna (brown mussel)	Uptake of NP/MP and bio-transference to predators	PVC nano/microparticles: 0.1-1.0 µm 0.5 g·L^{-1} Equivalent to 4.4 × 10^{10} NP/MP·L^{-1}	Mussels ingested NP/MP; after 12-d depuration, no NP/MP present in soft tissues; NP/MP only found in hemolymph; NP/MP transferred to predators (crab, fish) but did not accumulate in their tissues	Mussels held in flowing seawater during 3-d acclimation and 12-d depuration; description of mussel anatomy contains error in terminology (i.e., abductor muscle)	Santana et al. (2017) **Rating = 1**
	Effects of NP/MP on physiological processes and cells of gills and hemolymph	PVC nano/microparticles: 0.1-1.0 µm 0.125 g·L^{-1} Equivalent to 1.1 × 10^{10} NP/MP·L^{-1}	No effects of NP/MP on mussel CR, AE, CI, or growth; no effects of NP/MP on LS, LPO, or DNA of cells	Mussels held in natural seawater during 5-d acclimation; adequate diet delivered during 90-d exposure, but mussels fed every other day and likely under starvation stress; mussels starved for 24 hr prior to CR study; CR unreliable, concentration of microalgae delivered very low (140 cells·mL^{-1}), reported rates (1.6–1.8 L·hr^{-1}) higher than literature values for ca. 25 mm shell-length mussel (Resgalla et al. 2006); AE unreliable, feces with incorporated NP/MP ashed, loss of NP/MP mass included in "organic" fraction	Santana et al. (2018) **Rating = 0.5**
Perna viridis (green mussel)	Effects of fluoranthene-contaminated and virgin MP on physiological processes and survival [they soaked the PCV in fluoranthene-contaminated seawater for 24 d]	PVC particles: 1–50 µm 21.6, 216 and 2160 mg·L^{-1} Equivalent to 1.2 × 10^{7}, 1.2 × 10^{8} and 1.2 × 10^{9} MP·L^{-1}, respectively	After 40- to 44-d exposure, fluoranthene-contaminated MP caused concentration-dependent decreases in CR, RR, and byssus production; highest concentration of virgin MP produced same effects; over 91 d of exposure, contaminated MP caused concentration-dependent decrease in survival; highest concentration of virgin MP produced same effect	Inadequate diet quantity delivered during 2-wk acclimation and 91-d exposure, mussels likely under starvation stress; MP added 1x per wk with two simulated resuspension events per d; mussels starved for 2 hr prior to clearance studies; CR unreliable, no controls for microalgal settling or growth (i.e., beakers without mussels) and no indication of formula used for calculation; high mortality in all groups (including control) after 40 d (ca. 50–100%) likely caused by inadequate diet; conducted pilot study to confirm that mussels ingested MP	Rist et al. (2016) **Rating = 0.5**
	Uptake of MP and effects on mortality	PBS, PP, PP particles: <30 µm, 30–300 µm and 300–1000 µm 66, 333, 666 and 1333 MP·L^{-1}, respectively	Mussels ingested MP, number ingested depended on exposure concentration and size; only 10% of MP accumulated in tissues, very few found in or on gills; after 96-hr exposure, all sizes, types, and concentrations of MP caused high mortality (70–100%) except for <30 µm PS at 66 MP·L^{-1} (ca. 60% mortality)	High diet quantity (2.3 × 10^{6} cells·mL^{-1}) delivered during 1-wk acclimation likely depressed feeding and stimulated excess pseudofeces production; no mention of volume of water or number of mussels held during acclimation; no indication of diet quantity delivered during 96-hr exposure, figure 2 suggests number of cells delivered was inadequate (e.g., small fraction of 1333 items·L^{-1}); confusing descriptions of experimental methods; pseudofeces and feces collected without aid of microscope, cross-contamination and underestimation likely; high mortality (ca. 70–100%) after 96-hr exposure regardless of MP size, type or concentration suggest presence of confounding factors; results unreliable	Phothakwanpracha et al. (2021) **Rating = 0**

(*Continued*)

Table 3. Continued.

Species	Experiment	Particle types, sizes, and concentrations tested	Reported results	Notes	Reference and rating
Perna viridis (green mussel)	Effect of non-biodegradable and biodegradable MP on physiological processes	PLA and PET particles: 200 µm 100 and 1000 µg·L^{-1} Equivalent to 1.7–2.0 × 10^{1} and 1.36–1.4 × 10^{2} MP·L^{-1}, respectively for the two polymer types	No effects of MP type or concentration on mussel mortality, CI, CR, or RR	Mussel held in flowing natural seawater during 10-d acclimation; adequate diet delivered during 4-wk exposure; high concentration of diet in tanks (2 × 10^{5} cells mL^{-1}) likely stimulated excess pseudofeces production; robust methods and procedures	Joyce and Falkenberg (2022) **Rating = 1.5** #
Pinctada fucata (Akoya pearl oyster)	Occurrence of MP in shells and pearls, and effects on biominerali-zation	PE particles: 20 µm 0.2, 0.5, and 1.0 g·L^{-1} Equivalent to 5 × 10^{7}, 1.2 × 10^{8}, and 2.5 × 10^{8} MP·L^{-1}, respectively	After 96-hr exposure, MP increased expression of oyster genes related to biomineralization; after 7-d exposure, MP were found on and in the mantle, and on the internal surface of shells; *In vitro*, high concentrations of MP interfered with $CaCO_3$ crystallization	No indication of diet quantity delivered during 2-wk acclimation, oysters fed only 2x per wk and likely under starvation stress; no indication of diet quantity or quality delivered during 96-hr and 7-d exposure, oysters likely under starvation stress; high concentrations of MP used likely affected feeding processes; performed histology to verify MP in various tissues	Han et al. (2022) **Rating = 0.5**
Pinctada magaritifera (pearl oyster)	Effects of MP on energy balance and gametogenesis	PS microspheres: 6 and 10 µm diameter 0.25, 2.5 and 25 µg·L^{-1} Equivalent to 3.2 × 10^{2}, 3.2 × 10^{3} and 3.2 × 10^{4} MP·L^{-1}, respectively	MP decreased assimilation efficiency and SFG of oysters in a dose-dependent manner; abnormalities of gonadal tissue also found; no effects on IR, RR, shell growth, or gonadal development	Oysters held in natural seawater during 2-wk acclimation; adequate diet delivered during 2-month exposure; robust methods and procedures; feces with incorporated MP ashed for AE, loss of MP mass included in "organic" fraction but correction factor applied based on digestion of organics with KOH	Gardon et al. (2018) **Rating = 2**
Ruditapes philippinarum (Manila clam)	Ingestion of MP, transfer of adsorbed mercury to tissues, and effects on feeding rate, tissues, and cells	Fluorescent PE microspheres: 10–45 µm diameter 25 mg·L^{-1} Equivalent to 1.4 × 10^{5} MP·L^{-1}	Clams ingested MP; few MP found in or on gill and mantle tissues; contaminated MP transferred little mercury to tissues; virgin and contaminated MP, as well as mercury, decreased FR, caused alteration of gill and digestive gland tissues, and reduced hemocyte viability; no effects on biomarkers of oxidative stress	Adequate diet delivered during 12-d acclimation and 7-d exposure; FR unreliable, sampling interval too long (1 hr) for 15 clams in 12 L water, no controls for microalgal settling or growth (i.e., beakers without clams); experimental design includes pseudo-replication; erroneously reported that environmental MP concentrations between 0.5 and 10 MP·mL^{-1} have been found; actual reported field values were <0.5–10 MP·L^{-1} (Enders et al. 2015; Nel and Froneman 2015; Gorokhova 2015)	Sikdokur et al. (2020) **Rating = 0.5**
	Effects of MP and red clay particles on physiological processes and byssus production and adhesion	PMMA, PVC, Celite and red clay particles: 120 µm (PMMA), 12 µm (PVC), 87 µm (Celite) and 15 µm (clay; range = 0.1–400 µm) 1.5, 15 and 150 mg·L^{-1} Equivalent to [PMMA & Celite]: 5.2–6.2 × 10^{6}, 10^{7}, 10^{8} MP·L^{-1}, respectively; [clay and PVC]: 2.7–3.3 × 10^{7}, 10^{8}, 10^{9} MP·L^{-1}, respectively	PVC at 150 mg·L^{-1} reduced CI of mussels whereas clay did not; MP and natural particles had no effects on RR, CR, mortality, or byssus production and strength at any concentration; no differences found between responses to MP and natural particles at any concentration	Mussels held in flow-through conditions and delivered adequate diet during 12- to 23-d acclimation; adequate diet delivered during 68-d exposure; calculated clearance "rates" unreliable, no controls for particle settling or other losses (i.e., beakers without mussels)	Barkhau et al. (2022) **Rating = 1.5***

Ruditapes philippinarum (Manila clam)	Effects of MP on physiological processes, growth, and reproduction	Fluorescent PS 5 and 10 µm diameter 25 µg·L^{-1} Equivalent to 3.6×10^{5} and 4.5×10^{4} MP·L^{-1}, respectively.	Clams ingested MP; more 5-µm spheres found in digestive gland and intestine compared to 10-µm spheres; few MP found in or on the gills; both sizes of MP decreased IR and AE, and increased RR and AmEx, stronger effects at 30 d and with 10-µm spheres; calculated SFG and dynamic energy budget demonstrated less energy available for growth and reproduction; MP affected transcriptomic profiles of genes involved in metabolism and insulin-related signaling	No indication of diet quantity delivered during 7-d acclimation or 30-d exposure, clams likely under starvation stress; high concentration of diet delivered during feeding assays (2×10^{5} cells·mL^{-1}) likely stimulated excess pseudofeces production; IR unreliable, uningested microalgae lost through production of pseudofeces not considered, no controls for microalgal settling (i.e., beakers without clams); AE unreliable, feces with incorporated MP ashed, loss of MP mass included in "organic" fraction; erroneously reported that environmental MP concentrations between 3 and 34 µg·mL^{-1} have been found, actual reported values were 0.003–0.034 µg·mL^{-1} (Lattin et al. 2004; Moore et al. 2001); contrary to authors' statements, MP concentrations were not environmentally relevant	Jiang et al. (2022) **Rating = 0**
Saccostrea glomerata (Sydney rock oyster)	Uptake of MP to tissues	Fluorescent carboxylated (-COOH) PS microspheres: 0.5 and 2 µm 200 µL·L^{-1} Equivalent to 7.3×10^{10} and 1.1×10^{9} MP·L^{-1}, respectively	Oysters ingested MP; both sizes of MP found in hemolymph	No indication of diet quantity delivered during 24-hr acclimation or 5-d exposure, oysters likely under starvation stress; poor husbandry, oysters held in 1 L of water which was not changed during 5-d exposure	Scanes et al. (2019) **Rating = 0.5**
Scrobicularia plana (peppery furrow shell)	Uptake and elimination of MP and effects on cellular processes	PS microspheres: 20 µm diameter 1 mg·L^{-1} (4 MP·mL^{-1}; Note: authors' calculation is in error; should be ca. 230 MP·mL^{-1}) Equivalent to 2.3×10^{5} MP·L^{-1}	MP found in the hemolymph, digestive gland and in or on the gills of bivalve; MP had inconsistent effects on antioxidant enzymes and LPO levels; increased SOD levels in gills most consistent; MP decreased AChE activity; overall, no genotoxic effects during exposure; genotoxic effects found after depuration; no effects on CI	Authors fail to recognize that *S. plana* feeds mainly as a deposit feeder, not as a suspension feeder; bivalves starved during 7-d acclimation, 14-d exposure and 7-d depuration and likely under stress; poor husbandry, no sediment provided in which deposit feeders could burrow, crowded experimental conditions with 60 (4 cm) animals in 20 L water; no indication of how much MP ingested, especially considering that MP was suspended and bivalve is a deposit feeder; used FTIR to ID MP in tissue samples; calculated MP·mL^{-1} in error, should be 230 MP·mL^{-1} (PS, 20 µm, 1 mg·L^{-1})	Ribeiro et al. (2017) **Rating = 0**

(*Continued*)

Table 3. Continued.

Species	Experiment	Particle types, sizes, and concentrations tested	Reported results	Notes	Reference and rating
Scrobicularia plana (peppery furrow shell)	Ingestion of MP, transfer of adsorbed benzo(a)-pyrene (BaP) to tissues and effects on cellular processes	LDPE particles: 4–6 and 20–25 µm 1 mg·L^{-1} Equivalent to 1.7×10^{7} and 1.8×10^{5} MP·L^{-1}, respectively	MP found in whole body of bivalve; both MP sizes transferred BaP to tissues, larger size transferred more; virgin and contaminated MP of both sizes affected some biomarkers (AChE, CAT, GST, LPO, SOD) in gills and digestive gland after 7 and/or 14 d; no effects on CI; overall, effects depended on size of MP	Deposit-feeding bivalves maintained in natural sediment during 5-d acclimation and 2-wk exposure; incorrectly state that bivalve is a suspension feeder; MP delivered in suspension every-other day, not in sediment; figure 1 poorly labeled and confusing; MP found in whole body could have included those on gills and mantle, not in gut	Rodrigues et al. (2022) **Rating = 1**

Taxonomic names are as in original publications, and table is organized by species. Plastic particle concentrations as in original publications and standardized to MP·L^{-1}. Unless otherwise indicated, no natural particle control was used in the study. Ratings: 0 = poor study with many serious flaws; 1 = study with some flaws; 2 = very good study with no major flaws; asterisks (*) next to rating indicates use of natural particle control; hashtag (#) next to rating indicates use of environmentally relevant MP concentration(s).

Key: Plastic particles - MP: microplastic; NP: nanoplastic; AC: acrylic; HDPE: high-density polyethylene; LDPE: low-density polyethylene; PA: polyamide (PA = nylon); PBS: polybutylene succinate; PE: polyethylene; PA: polyester-amide; PET: polyester; PLA: polylactic acid; PMMA: polymethyl methacrylate (acrylic); PP: polypropylene; PS: polystyrene; PVA: polyvinyl alcohol; PVC: polyvinyl chloride; RA: rayon; Biomarkers - AChE: achace; AcP: acid phosphatase; ALP: alkaline phosphatase; CAT: catalase; GST: glutathione S-transferase; LAC: laccase; LPO: lipid peroxidation; LS: lysosomal stability; NO: nitric oxide; ROS: reactive oxygen species; SOD: superoxide dismutase; MDA: malondialdehyde; Physiological processes - AE: absorption efficiency; AmEx: ammonia excretion; CR: clearance rate; CI: condition index; FR: filtration rate; IR: ingestion rate; RR: respiration rate; SFG: scope for growth.

Notes: (1) When not provided in the publication, concentrations in mass per unit volume were converted to an estimated number per unit volume by assuming a spherical particle and using the equation given in Polysciences Data Sheet #238 (Polysciences Inc. 2009). (2) Control concentrations (e.g., 0 mg·L^{-1}) not listed in third column. (3) Only significant effects ($p < 0.05$) and no effects listed in fourth column.

delivered food in a matter of hours (Bricelj and Malouf 1984; Riisgård 1988; Newell 2004; Pan and Wen-Xiong 2004; Cranford et al. 2011). Over the remaining time of acclimation or experimentation, the animals will be under starvation conditions as in the studies by Xu et al. (2017), Bråte et al. (2018a), Gaspar et al. (2018), Santana et al. (2018), Kinjo et al. (2019), Revel et al. (2019, 2020), Sikdokur et al. (2020), and Alnajar et al. (2021; see Table 3).

Delivering food at too high a concentration can result in excess pseudofeces production, decreased feeding rates, or both responses depending upon species (see Subsection 4.3.1). The study of Phothakwanpracha et al. (2021) is a good example of how high food concentration can affect experimental outcomes. In this work, researchers examined the effects of particle size and concentration on uptake of polystyrene (PS), polypropylene (PP), and polybutylene succinate (PBS) particles by the green mussel, *Perna viridis*. During the four-day exposure period, animals were held in recirculating tanks (10 mussels·tank^{-1}, ca. 5.7 cm shell length) with 11 L of water and fed the microalga *Isochrysis galbana* at a concentration of ca. 2.3×10^6 cell·mL^{-1}. Such a high food concentration would have resulted in excess pseudofeces production and depressed feeding rates. It is not surprising that even those mussels exposed to the smallest (<30 µm) and lowest concentration of MP (66 particles·L^{-1}) rejected ca. 90% of the plastic particles and exhibited >50% mortality. Mussels exposed to larger (30–300 µm, 300–1000 µm) and slightly higher concentrations (333–1333 particles·L^{-1}) of the MP exhibited even higher mortality of up to ca. 90%. High levels of mortality in response to PS and PP particles, even at much higher concentrations, have not been reported previously (e.g., Van Cauwenberghe et al. 2015; Revel et al. 2019, 2020; Jiang et al. 2022), and suggest that poor husbandry or other confounding factors of the Phothakwanpracha et al. (2021) study were responsible for the results.

One common practice in MP studies is the use of multiple animals in static or flow-through conditions. Failure to consider biomass in the diet calculations, however, can lead to inadequate food supply and starvation. For example, during the experiments of Revel et al. (2020), 10 oysters (*Crassostrea gigas*; ca. 10 cm shell height) were held in 10 L of water and fed once per day with the phytoplankton *Tetraselmis suecica* at a stock concentration of 10^8 cells·mL^{-1}. A dose of 40 µL·L^{-1} was added to each tank resulting in a total concentration of 4.0×10^7 cells per tank, or 4×10^6 cells per animal per day. For oysters of 10 cm size, this diet is not adequate to maintain good condition over a 10-day period. Similarly, Rist et al. (2016) acclimated groups of 30 mussels (*Perna viridis*; 3.5–4.0 cm shell length) in 20 L of seawater, feeding them 1×10^6 cells of *Isochrysis galbana* twice per day. This concentration resulted in a diet of ca. 6.7×10^4 cells per animal per day for a period of two weeks, an insufficient food supply to maintain good condition. During the 91 days that mussels were exposed to MP, food supply was only slightly better. Within individual containers, each mussel was delivered 4×10^6 cells per day. It is no wonder that over the course of this experiment, mussels in all experimental groups exhibited 100% mortality, and those in the control group exhibited ca. 70% mortality. Notwithstanding these mortality events, physiological processes were measured and compared. Xu et al. (2017) held clams (*Atactodea striata*, 3.5–4.0 cm shell length) in tanks with 3 L of seawater. They were fed daily with *Dunaliella tertiolecta* at a stock concentration of 1.5×10^6 cells·mL^{-1}. Fifty mL of stock were added to each tank containing 10 clams, resulting in a diet of 7.5×10^6 cells per animal per day. This food supply is inadequate to maintain clams over a 2-week experimental period, especially considering that the clams were not fed during weekends. In a final example, Woods et al. (2018) held 25 mussels (*M. edulis*, 2.8 cm shell length) in 4-L jars and fed them daily with *Rhodomonas salina* at 8.0×10^3 cells·mL^{-1}. This diet translates to ca. 1.3×10^6 cells per animal per day which is barely adequate for maintenance. Consequently, when evaluating the results from studies that fail to deliver an adequate food supply, the confounding effects of food deprivation must be considered.

In the most extreme examples of poor husbandry, some studies also report that the bivalves were starved for a day or more before experimentation or during exposure to plastic particles (Détrée and Gallardo-Escárate 2017; Ribeiro et al. 2017; Xu et al. 2017; Al-Sid-Cheikh et al. 2018; Guilhermino et al. 2018; Auguste et al. 2020; Cole et al. 2020; Pedersen et al. 2020; Piarulli and Airoldi 2020; Song et al. 2020; Fabra et al. 2021; Jang et al. 2021). In a study of the effects of polyethylene terephthalate microfibers on the mussel, *Mytilus galloprovincialis*, Choi et al. (2021) starved their experimental animals for the entire exposure period (4 days). They cite ASTM E2455-06 (2013) guidelines as justification for starving the 3- to 4-cm mussels during exposure. The ASTM guidelines cited, however, are for water-only toxicity tests with freshwater mussels, specifically glochidia and post-metamorphic juveniles. Glochidia are parasitic on the gills of freshwater fishes, and the microscopic

juveniles feed on deposited material – including bacteria, algae and detritus – using ciliary currents on the foot and mantle (Reid et al. 1992; Yeager et al. 1994). Additionally, the endpoint for these types of studies is survival and in some cases metamorphosis and growth, not biomarkers of stress. Therefore, the ASTM guidelines do not apply to the Choi et al. (2021) study, and the starvation conditions employed taint the results of their research. The practice of starving bivalves has no benefit to the animals or the experimental procedures and should be dropped from future designs.

Finally, some studies provide little or no information regarding the diet quantity and quality that was delivered to animals during the laboratory holding period, acclimation, or experimentation, creating doubt about adequate husbandry. Uncertainties arise when researchers fail to report whether experimental animals were fed, indicate a food regime but neglect to indicate the volume of the holding or experimental tanks, fail to report the biomass held in each tank, or indicate the diet used for acclimation but do not indicate the diet used during exposure (Browne et al. 2008; Van Cauwenberghe et al. 2013, 2015; Avio et al. 2015a; Canesi et al. 2015; Gandara e Silva et al. 2016; Setälä et al. 2016; Détrée and Gallardo-Escárate 2017; Bråte et al. 2018a; Capolupo et al. 2018, 2021; Gaspar et al. 2018; Kolandhasamy et al. 2018; Magni et al. 2018; Qu et al. 2018; Gonçalves et al. 2019; Franzellitti et al. 2019; Kinjo et al. 2019; Li et al. 2019b; Scanes et al. 2019; Cole et al. 2020; Moreschi et al. 2020; Pedersen et al. 2020; Piarulli and Airoldi 2020; Song et al. 2020; Yap et al. 2020; Alnajar et al. 2021; Bringer et al. 2021; Teng et al. 2021; Han et al. 2022; Jiang et al. 2022; Sui et al. 2022b; Weinstein et al. 2022). Given the critical importance of food supply in the general condition of any animal, and its potential impact on the outcome of toxicological studies, results of the aforementioned studies should be interpreted with caution.

The issues regarding husbandry and diets demonstrate two points. The first is the inexperience of many researchers with techniques for holding and caring for bivalve molluscs. Bivalves, like any animal, require proper care and attention to be used in studies which attempt to determine how they interact with MP in nature. Second, given the poor conditions under which experimental bivalves of many studies have been held, it is likely that the reported effects were in part a result of physiological stress (e.g., von Moos et al. 2012; Rist et al. 2016; Setälä et al. 2016; Détrée and Gallardo-Escárate 2017; Ribeiro et al. 2017; Xu et al. 2017; Guilhermino et al. 2018; Pittura et al. 2018; Auguste et al. 2020; Alnajar et al. 2021; Khalid et al. 2021; Han et al. 2022; Jiang et al. 2022). In fact, Shang et al. (2021) demonstrated that starvation exacerbates the adverse effects of polystyrene microspheres (2 µm; 5.6×10^5 MP·L^{-1}) on the mussel, *Mytilus coruscus*. Some researchers try to dismiss the poor conditions under which their experimental bivalves were held. Ribeiro et al. (2017), for example, starved their bivalves during acclimation, exposure and depuration. They noted that there was no significant change in the condition index (CI) of clams (*Scrobicularia plana*) over the 21-day exposure and depuration periods, but mean CI did decline by ~13% in the unexposed animals during this time suggesting stressful conditions. Pittura et al. (2018) and Jiang et al. (2022) delivered inappropriate diets to their experimental bivalves and reported little or no mortality during acclimation and experimentation. Although lack of mortality is a good benchmark, it does not mean the condition of the animals was satisfactory. Therefore, without controls for the combined effects of MP and food deprivation, many of the reported effects could have been caused entirely or in part by stress associated with starvation or near-starvation conditions.

4.3.1. Recommendations

Researchers attempting to conduct experiments on the interactions between microplastics (MP) and bivalves, including toxicological effects, should thoroughly review the literature regarding the maintenance of these animals (e.g., Kennedy et al. 1996; Kraeuter and Castagna 2001; Shumway and Parsons 2016; Bayne 2017). Ideally, bivalves should be maintained under flow-through conditions utilizing a water flow rate that exchanges all water several times per day. Infaunal animals should be provided with a suitable substratum into which they can burrow. In the laboratory, bivalves can be maintained under static conditions in well aerated tanks that provide at least 1 L of water per animal and is exchanged at least every other day, depending upon size and biomass of the animals. In all cases, bivalves should be fed a diet consisting of cultured or commercially-available stabilized phytoplankton that includes species known to be an excellent source of nutrition (e.g., diatoms, flagellates; Ukeles 1969; Wikfors et al. 1996). Diet levels for holding animals and experimentation should be based upon the mass of the bivalves. A phytoplankton dry mass of between 0.1% and 0.2% of the animal live mass, which is equivalent to approximately 3% of the dry tissue mass, should be provided daily (e.g., Helm and Bourne 2004). For bivalves in the size range of 3 to 8 cm (shell length or height), a rough estimate

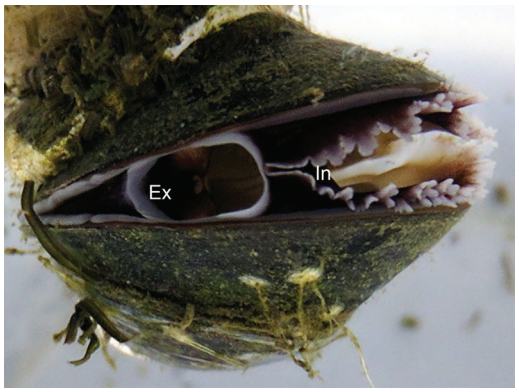

Figure 1. A well-fed, actively feeding blue mussel. Note the wide inhalant aperture (In, right) and well-formed exhalent siphon (Ex, left).

is approximately 10^7 to 10^8 cells per animal per day. Of course, these concentrations will depend upon the species of phytoplankton delivered (biovolume, quality), and the species and size of the bivalve being held. For longer studies (weeks, months), increased diet levels should be considered. Diets should be delivered either *via* a peristaltic pump or in batches over the course of the day so that the concentration within the experimental containers does not exceed 5×10^4 cells·mL^{-1}. This practice will prevent excess pseudofeces production and ensure that most of the delivered food is ingested. Examples of studies that have delivered an adequate food supply during acclimation and experimentation include those by Cole and Galloway (2015); Paul-Pont et al. (2016); Sussarellu et al. (2016); Green et al. (2017, 2019); Ward et al. (2019b); Maes et al. (2020); Fabra et al. (2021); Hamm and Lenz (2021); Trestrail et al. (2021). Finally, researchers need to monitor the experimental animals on a daily, if not more frequent, basis noting those that are consistently closed, have spawned, or are producing excessive pseudofeces. By doing so, researchers can identify those experimental bivalves that are in good vs. poor condition (Figure 1).

4.4. Issues and problems regarding anatomical structures and their terminology

Some of the literature pertaining to bivalves and microplastics (MP) demonstrates deficient knowledge of the anatomy and physiology of these animals. A fundamental understanding of the biology of the study organism is needed before experimentation to avoid errors. In the least offensive examples, a disregard for the wealth of knowledge of bivalve biology, assembled by researchers dating back well over 100 years, has led to incorrect terminology, and thus uncertainty, with experimental designs. Some studies make note of careful dissections and removal of the digestive gland, stomach, or gut with no obvious understanding of the complex anatomy and difficulty in dissecting the animals to this level of precision (e.g., Zhu et al. 2020). Errors in morphological terminology also exist. Various species of mussels have been used in a large number of studies with MP because of their availability, abundance, and amenability to laboratory experimentation. True mussels (Mytilidae) do not possess an inhalant siphon; they possess an inhalant aperture and exhalant siphon (Yonge 1957; Figure 1). Yet, several publications erroneously assert that an inhalant siphon is present. In a study on the putative translocation of MP to the circulatory system of *M. edulis*, Browne et al. (2008) state that, "Studies examining feeding in *M. edulis* have shown that microspheres of polystyrene are drawn through the inhalant siphon and filtered via the gill." Cole et al. (2011) and Wright et al. (2013) also mention an inhalant siphon of *M. edulis*, perpetuating this error. Ribeiro et al. (2019) also state that for bivalves in general, plastic particles "….can also be ingested through the inhalant siphon….," which is not accurate, as many bivalve species, in addition to mussels, do not have an inhalant siphon. Ribeiro et al. (2020) perpetuates this misconception by indicating that oysters also have an inhalant siphon, which they do not possess. Santana et al. (2017) refers to mussels (*Perna perna*) as having abductor muscles. In fact, no bivalve has abductor muscles (i.e., moving the shells away from the midline), they possess only adductor muscles (i.e., moving the shells toward the midline). Abduction occurs by means of the hinge ligament which antagonizes the adductor muscles (Morton 1979). Using histological techniques, Choi et al. (2021) visualized microfibers in the gut of mussels (*M. galloprovincialis*) suggesting that short fibers accumulated in the stomach, gut, and "secondary duct" (referred to as lower intestinal organs), whereas long fibers were only observed in the stomach and gut (referred to as upper intestinal organs). Not only are the terms lower and upper intestinal organs ambiguous with regard to bivalves, but it is also unclear to which organ "secondary duct" refers. Presumably, the authors are referring to the digestive gland or the ducts leading to that organ. In a final example, Bringer et al. (2020a) exposed oyster larvae to high concentrations of MP, stating that for the D-larval stage, "….MP agglomerates were stuck to the D-larvae coat and locomotor eyelashes." Bringer et al. (2020b) also refers to "locomotor eyelashes" of D-stage larvae. It is unclear to which structures the authors are referring, but perhaps they mean the larval shells and cilia of the velum. The

infiltration and perpetuation of such erroneous terminology in the MP literature is an unfortunate situation that will lead to ambiguity and confuse future students of molluscan biology.

Disregard for the anatomy of bivalves can also lead to misleading statements and flawed studies. Working with the freshwater clam, *Corbicula fluminea* (mean size = 2.2 cm), Li et al. (2019b) stated that the diameter of the siphons was 620 µm without providing any supporting evidence of how they arrived at this diameter. The stated diameter is far smaller than what has been recorded previously by malacologists for *Corbicula* spp. of that size (inhalant siphon ca. 2–4 mm diameter; Fox 2001; Korniushin 2004). This error leads Li et al. (2019b) to conclude that, "It was hard for the clams to take up fibers >1000 µm due to the limitation of their feeding apparatus," a dubious assertion at best. In another study, Kinjo et al. (2019) attempted to study the size-dependent elimination of MP in the Mediterranean mussel, *Mytilus galloprovincialis*. During a depuration period of up to 240 hr (short-term) and 40 days (long-term) mussels were situated with the posterior portion of their body sealed within a 25-mL tube in order to collect feces. Tubes were then placed in larger 1.2 L containers. Such an orientation, however, would also seal the inhalant aperture of the animal into the tube, effectively isolating it from food and oxygen available in the larger container. The present authors suggest that a more thorough peer review would have identified these issues.

4.4.1. Recommendations

Researchers of future studies should thoroughly familiarize themselves with the basic anatomy and established terminology of their experimental animals. Assumptions regarding the structure and function of pallial-cavity and internal organs should be verified with literature published by experts in molluscan biology. Good starting points are the many textbooks and technical treatises that have been published over the past 50 years (e.g., Morton 1967, 1976; Kennedy et al. 1996; Kraeuter and Castagna 2001; Shumway and Parsons 2016; Bayne 2017). For less common species, a thorough literature search will yield relevant information.

4.5. Issues and problems regarding physiological and toxicological terms

In many studies, standard terminology regarding physiological rates is not used, or used incorrectly. These rates include (each per unit time), (1) pumping (volume water pumped), (2) clearance (volume of water cleared of particles), (3) filtration (mass or number of particles cleared), (4) ingestion (mass or number of particles cleared minus mass or number of particles rejected in pseudofeces), and (5) respiration (mL or mg oxygen consumed; Bayne et al. 1988, 1993; Ward and Shumway 2004; Cranford et al. 2011; Rosa et al. 2018). Unfortunately, some papers incorrectly use these terms, leading to ambiguity and confusion, and stems from the lack of knowledge of feeding and digestive processes of bivalves (Green 2016; Gardon et al. 2018; Kolandhasamy et al. 2018; Woods et al. 2018; Li et al. 2019b; Pedersen et al. 2020; Sikdokur et al. 2020; Zhu et al. 2020; Wang et al. 2021a). Additionally, several publications misuse standard toxicological terms, including: (1) accumulation/bioaccumulation, which is the "progressive increase in the amount of a substance in an organism or part of an organism that occurs because the rate of intake exceeds the organism's ability to remove the substance from the body"; (2) assimilation, which is the "uptake and incorporation of substances by a living organism"; and (3) uptake, which is the "entry of a substance into the body, an organ, a tissue, a cell, or the body fluids by passage through a membrane or by other means" (Duffus et al. 2007). Improper use of terminology compounds ambiguity making it difficult to decipher or compare methodologies and results.

4.5.1. Recommendations

As with proper understanding of anatomical structures and their terminology, researchers of future studies should thoroughly familiarize themselves with current physiological and toxicological terms. The literature cited above is a good starting point for learning about the functional significance of these terms (see also Subsection 4.1).

4.6. Issues and problems regarding feeding behavior and physiology

Deposit- and suspension-feeding bivalves are well adapted to collect/capture, sort and ingest a range of living, non-living, organic, and inorganic particular matter. Some of the anatomical structures devoted to particle feeding are housed within the pallial cavity (mantle cavity; e.g., gills, labial palps, lips, proboscides), whereas others are found within the visceral mass (e.g., stomach, digestive gland, intestine). The integrated processes that these organs perform are responsible for the rates measured in physiological studies. Captured particles are processed by a manifold of ciliary tracts on the gills, labial palps, and lips. The outcome will either be ingestion (entering the mouth,

i.e., uptake) or rejection of the particles. Rejected particles are carried to the mantle, directed by ciliary tracts to specific locations (e.g., principal discharge area, inhalant aperture or siphon; see below), and expelled back to the environment (Beninger et al. 1999). Similarly, particles that adhere to the foot (if present) during movement are carried by cilia to rejection tracts on the mantle or taken directly back to the environment. The discovery of microplastics (MP) *on* organs within the pallial cavity does not mean that they have been "taken up" in the strict sense. Rather, they represent particles undergoing the normal sorting process that mediates the flux of particulate matter in and out of the pallial cavity. When collected by a researcher, this flux is halted and some particles, including MP, are trapped within the pallial cavity. The time course over which material destined for ingestion is processed within the pallial cavity ranges from ca. 1 to 25 min, depending upon shellfish species and diet quantity and quality (Milke and Ward 2003; Ward et al. 2003). Reprocessing of some particulate matter can occur in some species of bivalves, which could extend the residence time of material within the pallial cavity (Ward et al. 1994a, 1994b; Levinton et al. 1996). Additionally, the way in which pseudofeces are accumulated and stored temporarily (e.g., oysters) or continuously released (e.g., mussels) will affect the residence time within the pallial cavity of particles destined for rejection. Rinsing the pallial organs after dissection and prior to analysis does not guarantee removal of all particles from the epithelial surfaces, especially from the gills, labial palps, and mantle. These surfaces are abundantly supplied with mucocytes and covered in high-viscous mucus (Beninger and St-Jean 1997b; Beninger et al. 1993). Particles can be trapped on or within this fine layer of mucus, making removal by rinsing difficult, if not impossible. Some particles will also be trapped within the dorsal and ventral ciliated tracts and grooves (if present) of the gill and be resistant to removal by irrigation.

The above points have been unappreciated, if not misunderstood, by many researchers leading to questionable conclusions regarding the "uptake" and "accumulation" of MP by bivalves (Setälä et al. 2016; Ribeiro et al. 2017; Kolandhasamy et al. 2018; Woods et al. 2018; Li et al. 2019b; Zhu et al. 2020; Sendra et al. 2021; Teng et al. 2021; Jiang et al. 2022). Some true uptake of colloidal and particulate matter is possible across the epithelial surfaces of the pallial organs as a result of the action of wandering amebocytes, and perhaps endocytosis by epithelial cells; but, such uptake of material is typically restricted to particles and bacteria ≤ a few micrometers in size (Nakahara and Bevelander 1967; George et al. 1976; McLean 1980; Grenon and Walker 1982; Le Pennec et al. 1988; Pan and Wen-Xiong 2004; von Moos et al. 2012; Gaspar et al. 2018). To determine true uptake of MP across the epithelial surfaces of the pallial organs definitively, histological techniques should be applied to verify that particles are within the tissues proper (intra- or intercellular). Good examples of studies that have applied such techniques include those of Browne et al. (2008), von Moos et al. (2012), Avio et al. (2015a), Paul-Pont et al. (2016), Gaspar et al. (2018), Guilhermino et al. (2018), and Magni et al. (2018). Of course, uptake *via* ingestion also occurs, which can be verified by isolating and processing gut tissues, followed by MP verification by means of microscopy (e.g., white light, fluorescent, μFTIR, μRaman) or other methods with appropriate controls (e.g., Fernández and Albentosa 2019b). Histology and other techniques (e.g., autoradiography; Al-Sid-Cheikh et al. 2018) can also be applied to verify uptake and translocation of plastic particles.

Poor understanding of particle-feeding processes has led to several studies with questionable results and incorrect assertions. One example is the study by Ribeiro et al. (2017), in which the researchers attempted to examine the uptake of MP by the peppery furrow shell (clam), *Scrobicularia plana*, and subsequent effects on a suite of biomarkers. The researchers carried out suspension-feeding experiments on the clam which is a well-known deposit-feeding species (Hughes 1969, 1970). Although *S. plana* can remove some particles from suspension (Worrall et al. 1983), the clam derives its nutrition from sediment collected *via* its mobile inhalant siphon. Any attempt to determine the uptake of MP by *S. plana* would need to include sediment with incorporated plastic particles, but none was provided. Such disregard for the actual feeding processes of this species calls into question the results of this study. Ribeiro et al. (2019) comment upon feeding mechanisms, yet demonstrate a serious lack of understanding of the processes. In reference to bivalves in general, the authors note that plastic particles, "….can also be ingested through the inhalant siphon, transported to the mouth and once in the haemolymph, transferred to the digestive tract for intracellular digestion." (Ribeiro et al. 2019). Ingestion occurs when particles enter the mouth, and particulate matter most certainly does not first travel to the hemolymph and then to the digestive tract. Ribeiro et al. (2020) also make several inaccurate statements regarding feeding in the Pacific oyster (*Crassostrea. gigas*), indicating that, "Oysters are deposit feeders, which means that they

filter particulate matter (including microplastics) from the water and sediments. The particles are first taken up through the inhalant siphon from the surrounding water and trapped in the gills, which is the first contact organ. In the gills, the particulate matter transfers to the hemolymph that goes to the heart and from there it is distributed to the rest of the body because of oyster's open circulatory system." There are several errors in these statements. First, oysters are not deposit feeders, they are suspension feeders (Galtsoff 1964; Kennedy et al. 1996; Bayne 2017). Second, they do not possess an inhalant siphon. Third, particulate matter is taken up mainly through the mouth (see above) and enters the digestive system where extracellular and intracellular digestion occurs. Nutrients from this material then enter the hemolymph for distribution to the body. As a final example of erroneous statements, Bråte et al. (2018a) writes that "When mussels are feeding they transport particles through the inter-filamentary canals of the gills to the mouth and down the esophagus." In fact, particles that are transported "through" the interfilamentary canals are not captured and would pass into the suprabranchial chamber and out the exhalant siphon or aperture. Only those particles that are captured on the gill filaments would be passed to the labial palps and mouth for ingestion.

Zhu et al. (2020), studying the uptake of different types of MP by oysters (*C. gigas*), make similar mistakes. Oysters were collected in the field and soft tissues isolated by dissection without rinsing or allowing the animals to purge the pallial cavity of pseudofeces (cf., Zhao et al. 2018). Tissues were chemically digested and MP identified by means of μFTIR. The authors then go on to describe the different types of MP found in various tissues without recognizing that the plastic particles could have been on the outer epithelial surfaces and being processed as pseudofeces. Although one focus of this study was to determine the total amount of MP found within the edible portions of the oyster that could be transferred to humans, the authors conclude that, "The recorded differences suggest that microplastics in the form of fibers and fragments utilize different pathways to enter the oysters." It is unclear to which different pathways they are referring, but given the problems associated with their techniques, the published data provide little insight into the ingestion, egestion, and tissue distribution of plastic particles in oysters.

The confused and inaccurate statements cited above will further damage the MP literature moving forward. Perpetuation of such errors also is common in published reviews by authors who have little knowledge of feeding processes in bivalves and accept the material in published papers without question or critical assessment. As an example, in a review of the internalization and effects of plastic particles on bivalves, Sendra et al. (2021) attempt to describe suspension-feeding processes. Among the inaccurate descriptions, they indicated that on the gill, MP are transported by endocytosis, noting that this is the main pathway for dust and small particles. They then separate that route (endocytosis) from a second uptake route involving inhalation *via* the siphon and transport to the mouth. By their assertions, it is clear that the authors fail to understand the series of events that lead to particle ingestion, which are: (1) movement through the inhalant aperture or siphon, (2) capture by the gills, (3) transport to the labial palps, and (4) internalization *via* the mouth. In several sections, they also confuse particle retention on the gills with gut retention time, suggesting that retention in the gut is somehow connected to the laterofrontal cilia/cirri of the gill. As with the incorrect use of terminology, the inaccurate descriptions of bivalve feeding damages the collective literature on the subject and will lead to confusion in the future.

Particle-selection by bivalves is another process often under-appreciated or unknown by researchers. Inaccurate statements regarding particle selection by Ribeiro et al. (2019) demonstrate this point. In this paper the authors noted "The ability for marine invertebrates, such as bivalves to distinguish between organic and inorganic particles, but not microplastics, poses the question of by (sic) which mechanism they do so. It has been suggested that the shape and charge of particles may play a role in the ingestion and consequently translocation in the organism (citing Browne et al. 2008), but this hypothesis hasn't been tested thus far." This assertion is simply incorrect. It disregards the decades of research on particle selection capabilities of bivalves (e.g., Shumway et al. 1985, 1997; Ward and Targett 1989; Iglesias et al. 1992; MacDonald and Ward 1994; Defossez and Hawkins 1997; Hernroth et al. 2000; Levinton et al. 2002; Cognie et al. 2003; Beninger et al. 2008; Rosa et al. 2017), some of which specifically address particle shape and charge of MP (Solow and Gallager 1990; Pales-Espinosa et al. 2010a, 2010b; Rosa et al. 2013; Ward et al. 2019b). The influence of other physicochemical surface properties on the selection of phytoplankton cells and MP has received much attention. In brief, both non-specific (i.e., charge, wettability, hydrophobicity) and specific (carbohydrates) surface characteristics interact with mucus covering the feeding structures to affect preferential rejection or ingestion of particles (for reviews

see Ward and Shumway 2004; Rosa et al. 2018; Ward et al. 2019a). This body of knowledge not only reveals the impressive capability of bivalves for sorting particles pre- and post-ingestively, but it also demonstrates why virgin MP should not be used in feeding studies. Plastic particles that are not aged in seawater, for example, will not have a biofilm and will have surface properties that could diverge from those of MP in the environment.

Given the selective feeding capabilities of bivalves, the concentration to which the animal is exposed in a vessel does not necessarily equate to the internal exposure. Pseudofeces are produced under almost all conditions, even at a relatively low concentration of particles (<8000 particles·mL^{-1}; Ward et al. 2019a), and the rejected material is often too small to be seen by the unaided eye. Given the amorphous nature and the range of size and shapes of pseudofecal material, it cannot be "counted," nor can its production be assessed by casual observations as some studies have suggested (Wegner et al. 2012; Xu et al. 2017; Woods et al. 2018; Phothakwanpracha et al. 2021). These points have not been appreciated by several researchers whose studies have likely underestimated the number of plastic particles rejected, because not all of the pseudofeces was collected, and overestimated the number of plastic particles that were ingested because the feces were contaminated with pseudofeces (Browne et al. 2008; Xu et al. 2017; Woods et al. 2018; Kinjo et al. 2019; Craig et al. 2022).

The anatomical location of pseudofeces release and the way in which it is released also need to be considered. For example, the pseudofeces-discharge site for oysters (aka, principal-discharge area) is near the most anterior portion of the inhalant aperture, adjacent to the intersection of the labial palps and the gills (Galtsoff 1964; Ward et al. 1994b). In these animals, MP destined for rejection is accumulated in mucous boluses of various sizes and stored temporarily at this site before being expelled by means of rapid valve adductions. For many other, but not all, bivalve species, the pseudofeces-discharge site is located at the inhalant aperture or siphon (e.g., Ward et al. 2019a). For mussels, MP destined for rejection is released more continuously as individual particles, small particle masses, or in small boluses of mucus. Therefore, once placed in MP-free water, rejection of plastic particles by mussels will diminish over several minutes, whereas rejection of particles by oysters may occur after a longer period of time, depending upon how rapidly the oyster opens after transfer.

4.6.1. Recommendations

Researchers studying the uptake of microplastics (MP) by bivalves should have knowledge of the efficiency at which the plastic particles being tested are retained and ingested by the species of bivalve being investigated. Different species of molluscs process particles differently based upon different physical and chemical characteristics (see Shumway et al. 1985; Beninger and Decottignies 2004; Ward and Shumway 2004; Beninger et al. 2008; Rosa et al. 2018 and references therein), and researchers need to consider the physicochemical factors that mediate feeding processes. Identification and collection of pseudofeces should be carried out under a stereomicroscope. This procedure will ensure that the majority of pseudofeces is collected and reliably separated from feces and other debris (see Arakawa 1963, 1965, 1970; Kraeuter and Haven 1970). Additionally, given the selective capabilities of bivalves, aged plastic particles should be used in MP exposure experiments to be more representative of the natural environment where weathered plastic particles can exhibit different surface chemistry compared to those newly purchased. The selective capabilities of bivalves also argue against relying on these animals as bioindicators of MP in the environment (Ward et al. 2019b; Li et al. 2020). That is, quantifying the number and types of plastic particles in the pallial cavity and gut of bivalves is not necessarily a good proxy for the number and type suspended in the aqueous environment. Researchers designing and completing future studies should be well versed in the extensive literature pertaining to this topic.

4.7. Issues and problems regarding digestive processes

Another issue is the lack of understanding of the way in which ingested material is egested and the amount of time over which this process occurs. The production of two types of feces, voided over different time intervals, has been neglected by most studies examining egestion of microplastics (MP) by bivalves (see Subsection 4.1.2). Intestinal feces containing MP can be egested within 20 min of exposure and can continue for several hours (Menzel 1955; Owen 1974; Foster-Smith 1975; Gagnon and Fisher 1997). Therefore, researchers who neglect to collect feces during the exposure period (e.g., Kinjo et al. 2019; Wang et al. 2021b), risk underestimating the number of plastic particles ingested and egested. For example, Li et al. (2019b) examined the ingestion of microfibers of various polymer types and lengths (12–26 μm in diameter) by the clam, *Corbicula fluminea*. They

exposed clams to fibers over a two-day period, then sacrificed the animals to determine the number contained within their soft tissues, claiming that this represented the quantity and type taken up by the clams. There are, however, several errors associated with this study. First, because no feces were collected during exposure, there is no way of knowing the actual types of fibers that were ingested and egested over the experimental period. Additionally, because all soft tissues were digested for analysis, there is no way of determining if the observed fibers were in fact in the gut or if they were simply residing in the pallial cavity (see Subsection 4.6). Although the authors state that the tissues "….were rinsed gently with ultrapure Milli-Q water to remove their invervalvular water," this process in no way guarantees that all microfibers were removed from the organs of the pallial cavity.

4.7.1. Recommendations

Gut passage time of material that enters the digestive gland has been studied previously (Bricelj et al. 1984; Decho and Luoma 1991; Gagnon and Fisher 1997), and researchers examining this issue with respect to microplastics (MP) should thoroughly understand the complexities of this process. The selection of different types of MP in the gut of bivalves and its effect on determining gut residence times needs to be understood and relevant publications consulted (e.g., Cranford et al. 1998; Brillant and MacDonald 2000, 2002; Ward and Kach 2009; Ward et al. 2019a). For studies that are designed to evaluate the total ingestion and egestion of plastic particles, feces must be collected soon after the start of the exposure period. Identification and collection of feces should be carried out under a stereomicroscope so as to separate this biodeposit reliably from pseudofeces and other debris.

4.8. Issues and problems with experimental design and calculating physiological processes

Examples of the use of inappropriate methodologies to measure physiological processes and the use of incorrect equations to calculate rates can be found in several published studies. Additionally, some authors provide little or no information regarding the equation(s) used to calculate feeding, creating doubt about the accuracy of the estimated rates (Rist et al. 2016).

As an example of inappropriate methodologies, Wang et al. (2021a) attempted to examine the effect of particle size on the removal of nanoparticles and microparticles from suspension. The mussels (*Mytilus coruscus*) were provided with particles of five different diameters (0.07 µm, 0.5 µm, 5 µm, 10 µm, 100 µm) in static tanks, each containing 40 L of seawater and 30 mussels (ca. 7.7 cm shell length). Delivered concentration of particles varied over several orders of magnitude and ranged from ca. 2.6×10^{12} to 9.1×10^{2} particles·mL^{-1} for the 0.07-µm and 100-µm particles, respectively. Water samples were taken at the start and then 3 and 12 hr after mussels had begun to feed. The authors concluded that the smaller plastic particles were ingested more readily by mussels and that particle ingestion was negatively dependent on size, a result that is counter to decades of published studies. There are many problems associated with this study including lack of controls for particle agglomeration, settling, and other losses, but the most troubling is the design itself, which does not allow for a direct comparison of capture efficiency between particle sizes. In the closed-system tanks (static) 30 mussels would have filtered all 40 L of seawater in about 40 min (7.7 cm mussel clears ca. 2 L·hr^{-1}; Cranford et al. 2011). Large particles that are retained with high efficiency would have been removed first, with smaller particles being removed over time with each subsequent refiltration of the water. This result can be seen in figure 1 of Wang et al. (2021a). After 3 hr, there is a difference in percent removal between size class, but at 12 hr, the % removal of the smaller size classes has "caught up" with the larger sizes. This very point was discussed in the mathematical analysis of capture efficiency and clearance rate by Williams (1982) and reinforces the need for researchers to read and understand older literature. The anomalous result for the 100-µm diameter particles was likely caused by the rejection and subsequent resuspension of these larger particles (see Ward et al. 2019b), or an error in trying to measure so few remaining particles. Given these issues, the conclusions of Wang et al. (2021a) should be interpreted with caution, if not discounted entirely.

Similarly, a lack of knowledge of particle-feeding rates led Fernández and Albentosa (2019a) to erroneously conclude that "clearance rate of MPs increased with increasing concentration (p. 328)." First, the authors did not measure clearance rate, only a decrease in particle concentration (~5 µm MP, similar-sized microalga) over time. As expected, there was a greater drop in particle concentration over time in beakers that were delivered a higher concentration. The actual clearance rate (e.g., mL·min^{-1}; Coughlan 1969), however, was exactly the same. Consequently, there was no physiological change by the mussels and thus no indication of "faster clearing of both types of particles

at increasing concentration (p. 324)." Of course, the filtration rate (e.g., number particles·min^{-1}) was different, but such change would have been achieved if the suspensions were simply passed through a mechanical filter.

In at least five publications, problems were found with the determination of absorption efficiency (AE). Gardon et al. (2018), Santana et al. (2018), Wang et al. (2021b), Jiang et al. (2022), and Sui et al. (2022a) examined the effects of plastic particles on absorption efficiency using the method of Conover (1966), which measures the mass of organic matter lost from food and feces after ignition in a muffle furnace. This method, however, is not appropriate when using particles that are not bioavailable (i.e., not digested or absorbed), but are organic and will burn off during the ashing process. The result will be an overestimate of the organic content in the feces and an underestimate of absorption efficiency. Gardon et al. (2018) recognized this problem and applied a correction factor to the organic content of feces obtained by ashing. Given the difficulty in measuring absorption efficiency even under the best of circumstances, however, applying a correction factor renders the results less reliable. Neither Santana et al. (2018), Wang et al. (2021b), Jiang et al. (2022), or Sui et al. (2022a) make mention of the problem, and as such the reliability of their results is in doubt. Wang et al. (2021b) did wait approximately 14 hr after the end of a two-week exposure period before collecting feces. Nonetheless, it is likely that plastic particles remained in the gut and were egested in feces after the 14-hr depuration period, thus undermining accurate AE calculations. Because of the errors introduced by the loss of plastic mass from feces after ashing, the Conover (1966) method is not recommended for absorption efficiency measurements in experiments with plastic particles.

Another error associated with some studies stems from the use of multiple animals in the same vessel. Although individual bivalves have individual physiologies, in some cases this design is appropriate as long as good husbandry is followed (see above); however, in several experiments, the researchers treated individual animals within a shared container as replicates (e.g., Détrée and Gallardo-Escárate 2017; Bråte et al. 2018a; Woods et al. 2018; Sikdokur et al. 2020; Song et al. 2020; Khalid et al. 2021; Han et al. 2022; Jiang et al. 2022). Such a design in which the animals are not strictly independent of each other is a form of pseudo replication that can invalidate statistical comparisons and result in spurious effects rather than true treatment effects (see Hurlbert 1984; Tincani et al. 2017). In other cases, the number of actual replicates is difficult to decipher from the methodology described (e.g., Xu et al. 2017).

4.8.1. Recommendations

Well-established equations should be used when calculating physiological rates (for review see Bayne et al. 1976; Bayne and Newell 1983). For feeding rates (clearance, filtration, ingestion), appropriate controls for settling and the division of phytoplankton need to be included in the experimental design. Settling and division rates need to be accounted for in the final estimates of feeding. In static systems, the equation of Coughlan (1969) should be used to determine clearance rate. Importantly, researchers need to understand the underlining concepts regarding the calculation of clearance rates (see Rosa et al. 2018 for review). Briefly, the relationship between the decrease in particle concentration and time is not linear over its entire range. Rather, the relationship is an exponential decay function with the concentration approaching a value of zero (Williams 1982). To measure clearance rate, only the linear portion of that relationship (curve) should be used, which gives a value that is comparable to what one would obtain in a volume of water with a constant number of particles (e.g., natural environment). This methodology is particularly important for designs that use small volumes of water (e.g., <2 L), or larger volumes but more than one bivalve per container. Considering the clearance rates of many bivalves (2–11 L·hr^{-1}·g dry mass^{-1}), the depletion of particles that are retained with ca. 100% efficiency can occur over a period of minutes depending on experimental conditions and size of the animal under study. Therefore, water samples for clearance rate calculations need to be taken within this timeframe to capture the linear portion of the relationship between decrease in particle concentration and time. Sampling at the start and then 30 or more minutes after feeding has begun (e.g., Green 2016; Rist et al. 2016; Green et al. 2017; Pedersen et al. 2020; Sikdokur et al. 2020; Fabra et al. 2021; Wang et al. 2021a, 2021b), can result in significant underestimation of true clearance and filtration rates. In addition, sampling on a timescale that exceeds the turnover time of water in the chamber can result in calculation of erroneously high capture efficiencies for inefficiently retained particles (e.g., <2 μm). As mentioned above, this outcome is a result of water and particles passing through the gills multiple times, inflating capture efficiency with each pass. For experimental designs using a flow-through

method, the equation of Hildreth and Crisp (1976) or Riisgård (1977) should be used. Importantly, researchers should fully investigate the many papers that describe specific conditions for such a design to avoid artifacts, including flow rate, chamber geometry, and sampling protocols (Filgueira et al. 2006; Larsen and Riisgård 2011).

For absorption efficiency, a wet oxidation method should be used to determine the organic content of food and feces. Methods such as those of Newell (1982) and Dehaut et al. (2016) should be applied after determining that plastic particles are not affected by the oxidizing agent. Using multiple animals per container and dividing by the number of individuals to obtain a rate per animal should be avoided. First, individual bivalves have individual behaviors and physiologies. Second, given the strong influence of suspension feeding on particles in the water column, interactive effects between one bivalve and another are possible. Finally, treating individual animals in the same aquarium or chamber as replicates can present statistical problems because of pseudo replication. More information about this issue can be found in the papers of Hurlbert (1984) and Tincani et al. (2017).

4.9. Issues and problems regarding use of high concentrations of plastic particles and lack of appropriate particle controls

One of the most pervasive issues with studies on the uptake and subsequent effects of microplastics (MP) on bivalves is the unrealistic level of suspended MP to which the animals were subjected. In most publications, the experimental exposures have ranged from two to eight orders of magnitude higher than environmental levels (Table 3). There are several problems with studies that use concentrations that far exceed natural loads. First, many studies lack controls to test the effects of natural refractory particles on the measured endpoints. Second, it is unlikely that environmental loads of MP will reach such high levels so the practical significance of experimental results and conclusions is questionable. Third, the particle concentrations are often well in excess of the level that stimulates production of copious pseudofeces or triggers a decrease in feeding activity, both of which would reduce ingestion rate and affect digestive processes.

With regard to the first issue, measured concentrations of suspended MP verified with spectroscopy (e.g., μRaman, μFTIR) in a variety of coastal and open-ocean waters, range from < 1 to approximately 15 particles·L^{-1} (Doyle et al. 2011 [>500 μm]; Lusher et al. 2014 [250–1000 μm]; Song et al. 2014 [<50–1000 μm]; Enders et al. 2015 [10–1000 μm]; Kang et al. 2015a, 2015b [<2000 μm]; Phuong et al. 2016 [their Table 1]; Miller et al. 2017 [100–4000 μm]; Zhao et al. 2017 [100–500 μm]; Cai et al. 2018 [2–300 μm]; Qu et al. 2018 [20–5000 μm]; Zhao et al. 2018 [73–5100 μm]; Wakkaf et al. 2020 [>300 μm]). Higher concentrations have been reported for a few near-shore locations, including: (1) coastal waters of Sweden adjacent to a PE production plant where ~ 100 particles·L^{-1} were found (500–2000 μm; visual identification only; Noren 2007), and 2) highly polluted coastal regions of Tuticorin (India) where 10 to 30 particles·L^{-1} were found ($\bar{x}$ ~ 19 particles·L^{-1}; 100–5000 μm; Sathish et al. 2020; note: only suspected MP > 500 μm were verified with FTIR). These reported concentrations, however, are outliers and are not the norm. Several studies erroneously reported that MP concentrations in the environment can be as high as approximately 1×10^4 MP·L^{-1} (Green 2016; Green et al. 2017; Sikdokur et al. 2020) and 3 to 34 μg·mL^{-1} (Jiang et al. 2022), citing many of the papers referenced above. The errors seem to stem from a misunderstanding of the unit volume reported in the literature, with the authors confusing m^3 (1000 L) with one L, confounding L with mL, and incorrectly converting g·L^{-1} to μg·mL^{-1}. Unfortunately, such inaccuracies have entered the literature and are being propagated by some authors (e.g., Jiang et al. 2022). Abundance of suspended MP in marine environments is expected to increase 50-fold by 2100 (Everaert et al. 2018), and arguments have been made for exposing bivalves and other animals to high concentrations of MP as a way of examining future environmental scenarios; however, predicted mean concentrations under a worst-case scenario will still be <1 particle·L^{-1} (Everaert et al. 2018), far lower than those used in most studies. Given this reality, claims of environmental relevancy by researchers who exposed bivalves to concentrations of MP orders of magnitude higher than current and expected loads are disingenuous at best (e.g., Lo and Chan 2018; Gonçalves et al. 2019; Li et al. 2019b, 2020; Luan et al. 2019; Maes et al. 2020; Khalid et al. 2021; Shang et al. 2021; Teng et al. 2021; Wang et al. 2021a, 2021b; Jiang et al. 2022).

In reference to the second issue, it is well known that changes in suspended particulate matter affect feeding and rejection rates of suspension-feeding bivalves (e.g., Widdows et al. 1979; Kiørboe et al. 1980; Bricelj and Malouf 1984; Bayne et al. 1987; Ward and MacDonald 1996). The way in which bivalves respond to changes in particle concentration depends upon species and environmental conditions, but typically involves regulating ingestion by reducing

clearance rates, increasing production of pseudofeces, or both of these responses (Møhlenberg and Kiørboe 1981; Bricelj and Malouf 1984; Iglesias et al. 1992; Navarro et al. 1992; de Villiers and Hodgson 1993; Ward and MacDonald 1996; Bacon et al. 1998). In turn, pre-ingestive responses can influence post-ingestive processes such as gut retention time and absorption efficiency (Bayne and Hawkins 1992; Navarro and Iglesias 1993). Importantly, feeding rates of some bivalve species are negatively affected by inorganic particles with similar size (0.1–30 μm), density (wet, ca. 1.2–2.4 $g \cdot mL^{-1}$), and concentration (e.g., 1–2500 $mg \cdot L^{-1}$; Loosanoff 1962; Bricelj and Malouf 1984; Robinson et al. 1984; Cranford and Gordon 1992; Shumway et al. 2003; Benitez-Polo and Velasco 2020; Goldsmith et al. 2021) to those of MP used in studies that have reported negative impacts (e.g., von Moos et al. 2012; Wegner et al. 2012; Rist et al. 2016; Pittura et al. 2018; Fernández and Albentosa 2019b; Luan et al. 2019; Sikdokur et al. 2020; Alnajar et al. 2021; Teng et al. 2021). Similarly, high numeric concentrations of phytoplankton cells, ranging in size from approximately 2 to 60 μm, can negatively affect feeding and digestive processes of bivalves (e.g., Loosanoff and Engle 1947; Davids 1964; Winter 1977, 1978; Khalil 1996). The concentration of cells that elicit effects range from 10^4 to 10^8 cells$\cdot L^{-1}$, depending on species, and are comparable to, if not below, the concentrations of MP that recent studies have used in exposure experiments (e.g., Paul-Pont et al. 2016; Rist et al. 2016; Détrée and Gallardo-Escárate 2017; Guilhermino et al. 2018; Lo and Chan 2018; Magni et al. 2018; Pittura et al. 2018; Cole et al. 2020; Li et al. 2020; Maes et al. 2020; Pedersen et al. 2020; Han et al. 2022). Chemical compounds from natural particulate matter can also significantly reduce clearance rates in several species of bivalves (Levinton et al. 2002).

The impact of natural particulate matter (cells, refractory particles) on bivalve feeding and digestive processes brings up a crucial point regarding the third issue noted above. Almost all studies reporting negative impacts of MP fail to employ a natural particle control to differentiate particle effects from those potentially caused by properties of the MP (e.g., specific polymers, leachates). Therefore, claims that plastic particles *per se* affect the experimental animal cannot be substantiated because any similar sized and shaped particle, at the concentration tested could have produced the same effect. Notable exceptions include the studies of Harris and Carrington (2020), Yap et al. (2020), Barkhau et al. (2022), and Collins (2022) who used appropriate control particles to compare with the MP under investigation. Yap et al. (2020) exposed mussels, *Mytilus galloprovincialis*, to polyvinyl chloride particles (~12 μm) and Moroccan red clay (~14 μm) at 1.5, 15, and 150 $mg \cdot L^{-1}$ for 5 wk. Overall, they found no significant effect of either particle type on survival, byssus production, respiration rate, or condition index. Collins (2022) exposed mussels (*Mytilus edulis*) to nylon microfibers (length = 500 μm, diameter = 30 μm), or particles prepared from ground *Spartina* spp. leaves and stems at a concentration of 50 or 100 particles$\cdot L^{-1} \cdot hr^{-1} \cdot mussel^{-1} \cdot day^{-1}$. The *Spartina* spp. particles were of comparable size and aspect ratio to nylon fibers and were used to control for the presence of indigestible particles. After 21 days of exposure, neither particle type affected the microbial community of the gut (analysis of 16S rRNA gene sequencing) or stomach or digestive gland tissues (histological analysis) compared to the control group that received no test particle. Harris and Carrington (2020) exposed mussels, *Mytilus trossulus*, to polyethylene spheres (32–38 μm) at concentrations ranging from 1.0×10^3 to 2.5×10^6 particles$\cdot L^{-1}$ and natural silt particles (30–37 μm) at concentrations ranging from 1.0×10^3 to 1.1×10^7 particles$\cdot L^{-1}$ for 1 hr. Under this brief exposure scenario, neither MP or silt affected mussel clearance rates below a concentration of 1.2×10^6 particles$\cdot L^{-1}$. At higher concentrations, clearance rates were reduced significantly by MP compared to silt and microalgae (*Dunaliella* spp). Barkhau et al. (2022) included red clay (~15 μm) and Celite (diatomaceous earth, ~87 μm) in their study with the dwarf mussel, *Semimytilus algosus*, as natural particle controls for polyvinyl chloride (PVC, ~12 μm) and polymethyl methacrylate (PMMA, ~120 μm) particles, respectively. Mussels were exposed to high concentrations of MP ranging from 1.5 to 150 $mg \cdot L^{-1}$, equivalent to 6.2×10^6 to 3.3×10^9 particles$\cdot L^{-1}$, and similar concentrations of natural particles. After 63 to 68 days of exposure, there were no significant effects of any particle type on respiration rates, clearance rates, byssus strength, or mortality. For condition index (CI), only mussels exposed to PVC at the highest concentration (150 $mg \cdot L^{-1}$) showed a significant decrease in CI compared to control mussels. The reduction in CI was not observed in mussels exposed to the same concentration of red clay. The results of Harris and Carrington (2020) and Barkhau et al. (2022) are the first to demonstrate differential effects of plastic particles and natural particles on mussels, albeit at very high concentrations. In summary, data and conclusions from studies that used high concentrations of plastic particles have led to

inappropriate speculation regarding the impacts of MP on marine animals in natural environments. Additionally, given the body of literature cited above, studies that reported negative effects of MP on bivalves and failed to include a natural particle control should be considered inconclusive.

As concisely summarized by Lenz et al. (2016), there is a critical need for laboratory studies that use concentrations of MP that are environmentally relevant. To emphasize this point, several studies using lower MP concentration (10^1–10^5 particles·L^{-1}), albeit many still not at environmentally relevant concentrations, have reported minimal or no apparent effects on behavior, physiology or cell biology of suspension-feeding molluscs (Van Cauwenberghe et al. 2015; Green 2016; Green et al. 2017; Lo and Chan 2018; Gonçalves et al. 2019; Harris and Carrington 2020; Revel et al. 2020; Bringer et al. 2021; Fabra et al. 2021; Hamm and Lenz 2021; Opitz et al. 2021; Collins 2022; Joyce and Falkenberg 2022; Sui et al. 2022b [results at pH 8.1]). Green et al. (2017), however, did report a significant decrease in filtration rate of the mussel, *M. edulis*, exposed for 50 days to polylactic acid (PLA, ca. 65 µm) and high-density polyethylene (HDPE, ca. 103 µm) at 25 µg·L^{-1} (ca. 10^3 particles·L^{-1}), but no effect at 2.5 µg·L^{-1} (ca. 10^2 particles·L^{-1}). Interestingly, under the same conditions, the filtration rates of the oyster, *O. edulis*, significantly increased. Fabra et al. (2021) exposed *O. edulis* to polymethyl methacrylate (PMMA) microspheres (ca. 45 µm), either virgin or coated with *E. coli*, for 10 days at ca. 250 particles·L^{-1}. PMMA particles coated with *E. coli* caused a significant increase in respiration rate of oysters, however, virgin particles produced no significant physiological responses. In a study on early ontogeny of the Pacific oyster, *Crassostrea gigas,* Bringer et al. (2021) exposed pediveliger larvae for 7 days to a cocktail of MP created from plastic beach debris which included polypropylene (PP, 40%), polyvinyl chloride (PVC, 32%), and high-density polyethylene (HDPE, 28%). Even at a concentration of approximately 9.2×10^5 particles·L^{-1} (ca. 139 µm), there was no effect on settlement success, and only temporary reduction in growth during the first 21 days post-exposure. After 11 months, larvae exposed to the MP (for 7 days) exhibited a total growth greater than the control group. Collins (2022) exposed mussels (*M. edulis*) to nylon microfibers (length = 500 µm, diameter = 30 µm) for 21 days at concentrations of 50 or 100 particles·L^{-1}·hr^{-1}·$mussel^{-1}$·day^{-1}. Results indicated that the nylon MP had no effect on the microbial community of the gut nor the stomach or digestive gland tissues compared to the control group that received no test particle. Finally, in a well-executed study in which good animal husbandry was applied, Hamm and Lenz (2021) exposed juvenile *M. edulis* to polystyrene spheres (PS, 40 µm) and polyvinyl chloride particles (PVC, ca. 11–60 µm) at concentrations ranging from 15 (fifteen) to 1.5×10^6 particles·$individual^{-1}$·$week^{-1}$ (p/i/w). The lower concentrations were at (15 p/i/w) or slightly above (1500 p/i/w) environmentally relevant concentrations. Over a 36-week exposure period, the researchers found no significant effects of the lower concentrations of MP (15, 1500 p/i/w) on growth rates, condition index, or clearance rates. A significant decrease in superoxide dismutase (SOD) was found, however, in the gills of mussels exposed to PS at 15 and 15,000 p/i/w after 42 wk. The PVC at these concentrations had no effect on gill SOD. Authors concluded that, "The small effect sizes we observed for the response variables assessed suggest that these specific microplastics pose only a minor threat to blue mussel populations." The study of Hamm and Lenz (2021) and others cited above strongly suggest that there are minimal, if any, effects of environmentally-relevant concentrations of many MP types on bivalve physiology and health.

4.9.1. Recommendations

Previous calls for more standardized methodologies, experimental protocols, and realistic exposures at environmentally relevant conditions have been made (e.g., GESAMP 2015a, 2015b; Van Cauwenberghe et al. 2015; Koelmans et al. 2016; Lenz et al. 2016; Lohmann 2017; Underwood et al. 2017; Li et al. 2019a; Rochman et al. 2019; Provencher et al. 2020; Baroja et al. 2021). These recommendations are amplified here, and they should be heeded by researchers in the future. In particular, the concentrations of microplastics (MP) to which animals are exposed should be expressed as number per unit volume (e.g., particles·L^{-1}) along with appropriate dimensions. Presenting concentrations only as mass per volume (e.g., µg·L^{-1}) is not biologically relevant for several reasons. First, suspension feeders interact with individual particles, capturing, selecting, and ingesting them based on physicochemical properties. Delivering 10 plastic spheres with a diameter of 200 µm to a bivalve is very different from delivering 10,000 spheres with a diameter of 20 µm but of equal mass. Second, the density of most MP is close to that of water (e.g., freshwater ca. 1 g·mL^{-1}; seawater ca. 1.03 g·mL^{-1}). Therefore, gravitational forces are negligible and have little bearing on particle feeding. Surface area and volume of the delivered MP also may be of interest. Surface area affects adsorption

of dissolved chemicals and influences interactions with tissues and cells. The three-dimensional aspect of the plastic particles (volume) determines the room that is occupied within the gut and can affect digestive processes. Therefore, number of MP delivered per unit volume and appropriate dimensions should always be provided so that inter-study comparisons can be made. Finally, and importantly, natural refractory particles with similar size and shape to those of the MP should be used as a control in all future studies examining effects of plastic particles on bivalves and other suspension feeders (see also Doyle et al. 2022; Waldschläger et al. 2022). Such action will allow researchers to disentangle effects caused by high concentrations of any refractory particle from those caused by properties of the MP.

5. Trophic transfer

Gouin (2020) reviewed ingestion and trophic transfer of plastic particles and concluded that, while there is strong evidence for biological ingestion of microplastics (MP), they do not bioaccumulate and do not appear to be subject to biomagnification. He noted that >99% of observations from field-based studies included in his review reported MP located within the gastrointestinal tract. A survey of supplemental material provided with that review indicates that few bivalve molluscs were included in the assessment; however, the studies published regarding bivalves and possible food web transfer presented in the present review support his conclusion regarding lack of biomagnification *via* bivalve molluscs. In a few studies, authors attempted to demonstrate food web connections and transfer of MP between bivalves and higher trophic levels, for example, *via* crabs (Farrell and Nelson 2013; Setälä et al. 2014; Watts et al. 2014).

Farrell and Nelson (2013) exposed mussels (*Mytilus edulis*) to 0.5-µm fluorescent microspheres (note: mussels cannot filter particles below approximately 2 µm efficiently; see Subsection 4.1.1) at high concentrations, and then fed the mussel meat to green crabs (*Carcinus maenas*). Particles were transferred to the crabs and were found predominantly in the hemolymph, but also noted in the stomach, hepatopancreas, ovary, and gills at very low levels (0.04% of the level fed to the mussels). The particles were no longer present in the crabs after 21 days. Santana et al. 2017 presented a study on trophic transference of MP in food webs using mussels (*Perna perna*), crabs (*Callinectes ornatus*), and the puffer fish (*Spheoeroides greeleyi*). Mussels were exposed to a high concentration of MP (4.4×10^7 particles·mL^{-1}) and their contaminated tissues fed to the predators. Both crabs and puffer fish egested MP in their feces when feeding on mussel tissue, but did not retain MP after a depuration period. Authors suggested that trophic cascading of MP was unlikely given their results. Troost et al. (2018) developed a model that predicted no effect of MP on total primary or secondary production of the North Sea. The authors noted that strong assumptions were required in developing the model due to the scarcity of field data on MP and identified knowledge gaps in need of resolution. In a recent review, Carbery et al. 2018 noted that no studies have tracked the fate of MP and environmentally relevant chemical mixtures through a complex marine food web. The dearth of studies is probably for very good reason – it would be extremely difficult to carry out with any accuracy and, given the extremely low levels noted in primary consumers such as bivalve and the fact that they retain most plastic particles for a short length of time (hours; see Ward et al. 2019b), unlikely to yield useful information.

The potential for trophic transfer of MP through the food web has received relatively little attention, but some authors have invoked this possibility, again, in justification for the importance of studying suspension feeding animals at the base of the food chain. There are numerous diagrams of theoretical pathways for trophic transfer of MP; however, there is no reliable evidence for transfer and bioconcentration of MP through the food chain as a result of consuming molluscs, either by invertebrates, lower vertebrates, or humans. Moreover, given the extremely low levels of MP in bivalves under field conditions, it is considered highly unlikely that transfer and amplification of levels will be prevalent.

6. Aquaculture

Aquaculture of bivalve molluscs is a global activity, and the general sentiment is regularly put forth that "there is concern regarding the influence of fisheries and aquaculture on microplastic (MP) pollution and seafood." While this sounds to be a reasonable concern, it is most often iterated as the justification for a scientific study, a popular press article, or a press conference. Generally speaking, there are no data to support a claim that shellfish aquaculture increases the presence of MP in the cultured animals (see below). Yet, some scientists, and those in search of evidence to denounce the practice of aquaculture, still make statements that imply shellfish aquaculture is a scourge and pollutes the animals and the environment. While aquaculture facilities and their associated gear

(mostly polyethylene or a mix of polyethylene and polypropylene) have been suggested as potential sources of MP contamination (Mathalon and Hill 2014; Castro et al. 2016), there is very little known about the degradation rates of materials used in the shellfish aquaculture industry, and no substantial or credible evidence to suggest that shellfish aquaculture serves as a major source of plastic particles.

A summary of available information on MP in fisheries and aquaculture was commissioned by FAO as a Technical Paper (Lusher et al. 2017b). This document provided a wealth of information, but little in the way of a critical analysis of much of that information, other than to point out gaps in the existing literature. A table was presented showing the occurrence of MP in species of bivalves destined for human consumption showing a range of particle totals from 0.2–34 per g soft tissue. A brief summary of the literature on uptake and exposure experiments was presented, but with no assessment of the veracity (or lack thereof) of the studies noted.

Several studies have purported to study depuration of MP from shellfish including mussels, oysters, and clams (see, e.g., De Witte et al. 2014; Van Cauwenberghe and Janssen 2014; Van Cauwenberghe et al. 2015; Davidson and Dudas 2016; Birnstiel et al. 2019; Covernton et al. 2022), with an eye toward some application to aquaculture or other industry need. While these studies all reported a reduction in the numbers of particles relative to the starting points, the initial concentrations were all extremely low (most less than 10 particles·individual^{-1}), some reported as low as <1 particle·individual^{-1}. Some studies actually discuss the "significance" of depuration of particles from initial levels of 1–5 particles to levels of 1–3 particles. Not only is the accuracy of retrieving this low number of particles questionable, but also the thought that such a reduction is in any way meaningful is quite unrealistic. The significance of such low levels and any associated reduction is questionable at best and has little to no value in any practical application.

Farmed mussels and oysters have received the most attention with widely variable results, and all studies report very low quantities of MP in both groups. In one of the first studies, Van Cauwenberghe and Janssen (2014) reported MP in mussels (*Mytilus edulis*; $\bar{x} = 0.36 \pm 0.07$ [*SD*] particles·g ww^{-1}) and Pacific oysters (*Crassostrea gigas*; $\bar{x} = 0.47 \pm 0.16$ [*SD*] particles·g ww^{-1}) at the time of human consumption and suggested that a European shellfish consumer could consume 11,000 MP·year^{-1}. De Witte et al. (2014) reported on differences in MP contained in "consumption mussels" and wild-type mussels collected at department stores and from the field, respectively. While they noted a higher prevalence of fibers related to local fisheries activities in the field animals, no information was provided on the original source of the commercially purchased mussels. Mathalon and Hill (2014) reported purported plastic particles from farmed mussels (*Mytilus edulis*) and wild mussels. Their numbers were expressed per five mussels, and even after calculating per individual, their reported concentrations are higher than any other publications. They did not confirm the identity of the particles using either μRaman or μFTIR, and relied upon visual assessment which is notoriously unreliable and thus their numbers are probably overestimated. Li et al. (2016, 2018b) reported more MP particles in wild mussels (1–6 MP·individual^{-1}) than in cultured mussels (0.9 items·g^{-1}), and suggested it was representative of the locations sampled. Farmed mussels were in a location less affected by human activity and without plastic ropes, although a reasonable explanation, assigning significance to a comparison of such low numbers seems moot. Ding et al. (2018) also collected samples from markets and analyzed wild vs. farmed animals and reported that farmed mussels contained more MP (3.17 MP·g^{-1}) than wild mussels (2 MP·g^{-1}), and Li et al. (2018b) reported higher debris in wild mussels (1.6 items·g^{-1}) than farmed (1.1 items·g^{-1}). Both of these studies are further examples of minor differences being presented as meaningful.

Vandermeersch et al. (2015) assessed MP concentrations from three European estuaries identified as "hotspots" or worst-case scenarios for accumulation of MP, as well as commercial mussels from five European countries and reported values of <1 plastic particle·g ww^{-1} tissue. Renzi et al. (2018) noted no differences between MP contents in cultured versus natural mussels (*Mytilus galloprovincialis*). Li et al. (2015) collected molluscs (mussels, clams, scallops, oysters) only from markets and stated that MP content was significantly higher in farmed samples compared to wild samples but did not report data for the MP contents of the two groups. The data presented by Phuong et al. (2018b) are in percentages and difficult to interpret, but they claim higher MP detection rates in farmed oysters (93%) and mussels (90%) than in wild animals (80% and 90%), respectively. Digka et al. (2018b) also reported results as percentages and did not detect a difference in MP levels between wild (47.5%) and farmed (45%) mussels. There was no significant difference in uptake between wild and cultured mussels (*Perna perna*) in Brazil (Birnstiel et al. 2019).

Davidson and Dudas (2016) compared the levels of MP in Manila clams (*Venerupis philippinarum*) and found no difference between cultured and wild caught animals. It should also be noted that the levels recorded were very low (range 0.7–5.47 particles·g soft tissue^{-1}; approximately 6–15 particles·clam^{-1}). They used neither μRaman nor μFTIR to identify the MP positively. In their materials and methods, they indicated that "values were expressed as the mean number of MP particles per 1 g of clam tissue to standardize for clam size (particles·g^{-1})." Yet in their Table 1 they show data for number of putative "MP" per animal. They did conclude that shellfish aquaculture operations did not appear to be increasing MP concentrations in farmed clams. In a subsequent study, Covernton et al. (2019) reported on bivalve molluscs (Manila clams, *V. philippinarum,* and Pacific oysters, *Crassostrea gigas*) grown on commercial shellfish beaches in British Columbia, Canada. Reported numbers of particles were very low (<1·individual^{-1}; 0.05 vs. 0.03·g dry tissue weight^{-1}), and they concluded that the levels of MP in shellfish from aquaculture and non-aquaculture sites did not differ for either species. They suggested that observed MP concentrations may be related to factors other than the aquaculture facility and gear deployed. Bendell et al. (2020) published on the use of infaunal bivalves as biomonitors of plastic particle pollution and their results directly contradicted Covernton et al. (2019). They suggested that poor survival of animals in the Covernton study was part of the explanation for the differences in results, but also pointed to the use of oysters as monitors while citing the works of Ward et al. (2019a, 2019b) who demonstrated that selective feeding precludes the use of oysters as bioindicators of MP. Bendell et al. (2020) went on to carry out a study with two species of clams (Manila clam *Venerupis* = *Ruditapes philippinarum* and varnish clam, *Nutallia obscurata*) under the incorrect premise that "They are non-discriminatory feeders that don't egest excess food through pseudofeces." Unfortunately, this is patently untrue as these and all other bivalves produce pseudofeces under certain conditions. Bendell et al. (2020) cite Gillespie et al. (1999) who say nothing about pseudofeces production or lack thereof. In fact, pseudofeces production by *Ruditapes* has been reported previously (see Defossez and Hawkins 1997). They also stated that the diversity in size and shape of particles found in the clams indicated that they are non-discriminatory in their feeding. This statement is also not necessarily true, that is, there is no evidence to show what particles were or may have been rejected during the feeding process. Based upon the erroneous assumption that no pseudofeces are produced by these clams, Bendell et al. (2020) incorrectly concluded that both clams might be suitable biomonitors for tracking MP in the field.

Chen et al. (2018) reported on the contributions of aquaculture operations to the MP found in surrounding waters. They reported not only low numbers of particles in seawater ($\bar{x} = 8.9 \pm 4.7$ [*SD*] per L) and sediment ($\bar{x} = 1739 \pm 2153$ [*SD*] per kg, but noted a mean particle size of 1.54 ± 1.53 (*SD*) mm and 1.33 ± 1.69 (*SD*) mm in seawater and sediment, respectively. While the study showed that the aquaculture gear poses a source of MP to the marine environment, it also clearly demonstrated that the particles produced are too large to be consumed by many suspension feeding bivalves (Ward et al. 2019a). Yet, they still end their discussion noting that the "high level of microplastic in the mariculture farms … pose a potential risk to cultured seafood and consumer health." In a later study, Chen et al. (2022) published on MP pollution in Zhanjiang Bay, an area supporting intensive oyster culture. They reported 0 to 2.65 MP·m^{-3} ($\bar{x} = 0.37 \pm 0.57$ [*SD*] MP·m^{-3}) with higher levels in May (0.50 MP·m^{-3}) and lowest in January (0.28 MP·m^{-3}). On a per L basis, the mean concentration works out to be 3.7×10^{-4} MP·L^{-1}. Using these miniscule levels and insignificant differences, they proceeded to discuss the data at great length including trends in distribution and hydrodynamic processes and possible contributions from the aquaculture industry. What this study actually demonstrated was the general lack of contribution of MP pollution from the aquaculture activities to the local environment.

Cho et al. (2019) suggested, based on no data, that the intake of MP through consumption of seafood could be reduced by depuration or cooking. They also suggested that depuration might have taken place during transportation or storage, but with no explanation as to how they believe this could possibly have happened unless the animals were shipped in water and actively pumping.

Birnstiel et al. (2019) discussed the purported importance of depuration in the process of removing MP from mussels (*Perna perna*). They noted that nylon fibers were the most abundant particles in mussels and that blue fibers were more readily depurated, insinuating that color was in some way a determining factor. They used FTIR to identify MP polymer types and reported concentrations of approximately 30 ± 18 (*SD*) MP·mussel^{-1}. They also reported no difference between levels of MP in farmed vs. wild mussels. Based upon a depuration

period of 96 hr, when approximately half of the already low concentration of particles were removed, they concluded that the mussels in their study (*Perna perna*) had high quantities of MP, "that mussels from Guanabara Bay may not be adequate for human consumption," and that their results "highlight the importance of depuration in reducing microplastic pollution in seafood." Wu et al. (2020) studied a long-standing aquaculture facility in China and, in addition to fish and shrimp, they reported mean values for two bivalve species, *Ostrea denselamellosa* and *Sinonovacula constricta*, of 1.67 ± 0.44 (*SE*) and 1.8 ± 0.34 (*SE*) MP·animal^{-1}, respectively. They begin their abstract with "…the aquaculture industry may suffer from microplastic pollution, especially when plastic products are widely used for aquaculture" to justify their study, but end with the more positive statement that "microplastics may not increase the health risk of consuming seafood and their impacts on commercial species may be less deleterious than previously thought." This effort is based upon a long-term facility with animals that inhabited the facility for almost a year, and provides strong evidence for lack of accumulation of MP in the shellfish and subsequent impacts.

Zhu et al. (2021) reported on long-term monitoring (one year) trends of MP concentrations in seawater and farmed oysters (species not specified) in the Maowei Sea, an aquaculture region in China. They reported no significant correlation of MP abundances between the water and oysters, a result consistent with the knowledge that suspension-feeding bivalves are not robust indicators of MP in the surrounding waters (Ward et al. 2019a, 2019b). Their conclusion is, however, based upon erroneous data collection and understanding of the biology of the oysters. They pooled three oysters as one sample and "divided by 3" to provide individual loads, used wet weights as their standard, and reported concentrations of MP in separate tissues. It is highly unlikely that there were MP *in* tissues other than the digestive gland, but rather *on* those tissues and merely an artifact of methodology and lack of knowledge of oyster feeding physiology. Indeed, they reported more MP in the gills ($\bar{x}$ = 7.05 ± 1.21 [*SE*] particles·g^{-1}), followed by digestive glands ($\bar{x}$ = 2.84 ± 0.44 [*SE*] particles·g^{-1}), and other tissues ($\bar{x}$ = 0.59 ± 0.08 [*SE*] particles·g^{-1}). All of these values are to be expected as the animals were filtering the particles and concentrating them *on* the gills and moving some of them (see Subsection 4.1.1) to the mouth and then digestive gland (thus lower concentration). Microplastics found *in* the other tissues were likely those *on* the epithelial surface being transported by ciliary rejection tracts. Moreover, as with all other studies counting MP in field samples of shellfish, the total levels of particles recorded were extremely low, as were the levels in the surrounding seawater (1.47–7.61 particles·L^{-1}).

All of these studies on the levels of MP in shellfish from aquaculture systems have one result in common – extremely low, if not miniscule, concentrations of MP. Some authors try to make claims of ecological or environmental significance based on the difference between minute numbers of particles and carry out varied statistical analyses in an effort to give their results more meaning. In many studies, sweeping statements are made regarding potential detrimental impacts of aquaculture gear on the environment and the levels of MP in the surrounding waters and cultured shellfish based upon these very low and most likely inconsequential numbers. In fact, most studies comparing concentration, types, and shapes of plastics between wild and farmed bivalves did not identify significant differences.

False claims or negative publicity can have far-reaching and hugely detrimental impacts on the aquaculture industry. There are no convincing data to support any negative claims being made with regard to the contributions that the aquaculture industry may be making to the levels of MP in the environment. Further, there are no data to support claims that there are negative impacts on the shellfish crops or the environment, or that the extremely low levels of MP accumulated in the shellfish pose any threat to human health. Most unfortunately, it is difficult, if not impossible, for different user groups including scientists, managers, outreach specialists, and policy makers to make sense of these published materials or know what is reliable and what is not. Too many papers include opinions and arm-waving, conclusions are extrapolated well beyond what the data justify, and others are non-definitive, relying upon descriptors such as "potential," "possible," "maybe," or "could," which enhances the possibility of future funding, but confuses readers and leads to inappropriate or misleading conceptions of actual impacts. Negative perception is fueled by these inflammatory comments, publicity, and stories based upon speculation that are not supported by data. This in turn can have devastating and long-lasting impacts on the shellfish industry and public understanding of the real concerns regarding MP in the marine environment. The aquaculture industry has acknowledged the challenge, recognized the benefits offered by plastic tools and gear, recommended sensible actions, and recommended focused research (NAA 2021). These unwarranted and

unsupported claims need to stop until and unless there are solid data to support them (see also Shumway et al. 2018).

7. Potential transfer of toxic materials

This area of inquiry is an emerging field of study and available data are still scant. Adsorbed environmental pollutants on the surface of the microparticles is not covered in this review. There have been numerous reports regarding the adhesion and adsorption of pollutants to microplastic (MP) or their potential for the same, and their transfer through the food web, and transport of chemicals, for example, hydrophobic organic chemicals and others (Teuten et al. 2009; Cole et al. 2011; Koelmans et al. 2015; Andrady 2017; Lohmann 2017). Good reviews are provided by Engler (2012), Koelmans et al. (2015), Lusher (2015), and Carbery et al. (2018). There are also reports demonstrating that ingestion of MP is not likely to increase exposure to these chemicals and thus risks in the marine environment. Koelmans et al. (2016) provided a strong critical review on the topic of hydrophobic organic chemicals (HOC) and the perceived hazard and risk of plastic in the marine environment. They presented a model based upon empirical studies and concluded that ingestion of plastic particles is not likely to increase the exposure to, and thus risks of, HOC in the marine environment. In a subsequent paper (Koelmans et al. 2017), it was noted that the actual environmental risks of different plastics and their associated chemicals remain largely unknown. The literature associated with MP and potential contamination is not covered in this review; however, Pittura et al. (2018) reported on the role of MP as vehicles for the transfer of environmental PAH to mussels and included data on both virgin and pre-contaminated MP (LDPE, 20–25 μm). They fed the animals on zooplankton throughout the experiments – a highly unsuitable food for mussels. They noted that both virgin and contaminated MP affected immunological biomarkers depending on exposure time. Limited effects were found for oxidative status, neurotoxicity, and genotoxicity. The lack of food and poor experimental conditions, however, make these results questionable or tenable at best.

Some authors couch their arguments by noting the potential for contaminants to adhere to the MP (see, e.g., Mato et al. 2001; Ríos et al. 2007; Barnes et al. 2009; Arienzo et al. 2021). Studies have demonstrated that the argument that MP can serve as significant concentrating vectors for "chemicals and other pollutants" has been debunked. Several recent reviews have summarized the potential for nanoplastics and microplastics to act as vectors of pollutants, and all have concluded that they will only have a negligible contribution to chemical exposures compared to other sources, especially the diet (Koelmans et al. 2016; Besseling et al. 2019; Utne Skåre et al. 2019). Yet, the argument is still put forth to justify more superfluous experiments.

As proposed by Connors et al. (2017) in discussing ecotoxicological studies, more attention needs to be paid to detail of reporting information regarding particle concentrations, descriptions of test particles, delivery techniques, use of environmentally relevant concentrations, and inclusion of appropriate controls for all studies on interactions between MP and animals, that is, a need to evaluate the quality of experimental studies. They provide guidance to improve the reliability and relevance of ecotoxicological studies, and also discuss areas of research where improvement is needed, for example, comparing and constrasting MP vs. natural particles as delivery vehicles should be considered.

8. Human health

Human uptake of microplastic (MP) from seafood has received more attention from the media and public outreach efforts than from the scientific community. Numerous authors have tried to demonstrate trophic transfer of MP and links to human health associated with consumption of seafood. There are currently no strong data to demonstrate significant uptake or impacts of MP by humans through the consumption of shellfish, and, to date, there are no credible data from field or laboratory observations to indicate detrimental impacts of MP in shellfish on human health. Cox et al. (2019) provided a summary of human uptake of MP from a variety of sources including inhalation and water and food consumption and estimated that 74,000 to 121,000 particles are taken up with a potential increase of 90,000 particles·year^{-1} for those who consume only bottled water. They also noted that these values are probably underestimates.

Lusher et al. (2017b) stated in the executive summary of an FAO report that "trophic transfer of MP will not lead to accumulation in seafood, and associated PBT and additives have a negligible effect on the total human dietary intake of these compounds." The report by the EFSA (2016) concluded that there are insufficient data available to characterize potential toxicity in humans, and that data demonstrating occurrence in food is not sufficient to estimate exposure, and any risk from exposure to MP could not be characterized. The SAPEA (2019) report supported

this conclusion and further noted that an environmental risk of MP was low on a global scale, but that risk may exist in a few very polluted locations. Thus, three comprehensive assessments (EFSA 2016; Lusher et al. 2017b; SAPEA 2019) all concluded that there is a general lack of exposure and hazard data and the risk of both nano- and MP to human health cannot be evaluated.

Subsequent analyses by Danopoulos et al. (2020a; one of a series of reviews: Danopoulos et al. 2020b, 2020c, 2022) estimated human consumption of MP through molluscs from literature values as 0 to 27,825 MP per year and noted that it varies greatly between geographic regions.

Yet, despite a lack of evidence for concentrated accumulation and magnification of MP in food webs, or any data to support such hypotheses, there are still regular and on-going statements published that present and/or discuss hypotheses as facts. Many authors add the general statement that "microplastics in shellfish pose a potential threat to human health" as a matter of course in the hopes of giving their effort more significance. As examples, Thushari et al. (2017) state that "contaminated bivalves pose potential health risks for seafood consumers" and, based on a predicted risk of consuming 5.8 MP·portion^{-1} of mussels, Gündoğdu et al. (2020) assert that "MP pollution is a serious problem in seafood." More general publications and outreach documents regularly include statements to garner attention to the purported significance of the MP-shellfish interactions by making tenuous and blatantly inflamed links to potential human health risks. In an example from a recent posting on Science Daily (December 23, 2020) the headline read "Highest levels of microplastics found in molluscs, new study says." First, this headline was not news, it has been known for over a decade that MP are commonly found in molluscs in the marine environment, and further, it gives the impression to the uninformed that there is some inherent threat in their presence. The author of the study, E. Danopoulos (cited elsewhere), is quoted as saying "No-one yet fully understands the full impact of microplastics on the human body, but early evidence from other studies suggest they do cause harm." The results of the study and possible implications could easily have been presented without the need to alarm the public. Such activities serve only to confuse readers and can be a great detriment to the shellfish and aquaculture industries as public perception is unnecessarily and unjustly negatively influenced.

As noted in Koelmans et al. (2017), the discussion should be had regarding impacts of MP on human health and the environment. To this should be added that the discussion be based on solid evidence, not hyperbole. There should be great concern regarding the influence of the current hype regarding impacts of MP on shellfish and hypothesized potential impacts on human health (see Galloway 2015; Clark et al. 2016 for general reviews; and Cole et al. 2011; Wright and Kelly 2017 for reviews on potential human health issues). The statements of Van Cauwenberghe and Janssen (2014); Rochman et al. (2015) are reiterated in even more general terms stating that "either through direct ingestion, or *via* trophic transfer, microplastics are also ending up in commercial seafood, including European mussels and oysters and fish and shellfish sourced at markets in the United States and Indonesia." While there may have been some reliably sampled and reported presence of minute numbers of MP considered when making these statements, the vague generalities do not provide the whole story such as uncertainties and cause and effect data. Perpetuation of inaccurate vagaries has resulted in undue and unjustified negative publicity on the shellfish aquaculture industry (e.g., Wiedenhoft 2018), as anti-aquaculture activists are quick to seize upon any scientific "evidence" no matter the veracity, that shellfish aquaculture results in environmental damage or human health concerns, but are rarely in a position to assess or analyze the quality and validity of the available data. Unfortunately, these questionable data and interpretations fan the fires of unwarranted negative perception and panic (see Shumway et al. 2018). Wright and Kelly (2017) provided a summary of cross-disciplinary scientific literature to assess knowledge of any human health issues related to MP. Their thoughtful assessment notes that a robust evidence base of exposure levels is currently lacking and that assessing current exposure levels and burdens is key.

Numerous authors have published comments with regard to MP and human health with no supporting data to indicate a threat other than to note that MP can potentially be transferred to humans *via* consumption of shellfish. Some papers report that eating shellfish present a potential risk with regard to consumption of MP, but give no regard to the extremely low actual levels of MP reported and the unlikely event that these "potential risks" are in any way real.

Frias et al. (2014), Carbery et al. (2018), and Smith et al. (2018) all suggested that MP and contaminants could have significant consequences with regard to seafood consumption. Levels of approximately 0.4 MP·g^{-1} (see Van Cauwenberghe and Janssen 2014) or 2 to 10 MP·g^{-1} (Li et al. 2015; Do et al. 2022) of shellfish tissue bordered on non-detectable and should

not be given undue significance with regard to human health (see summary of levels in Table 1). Van Cauwenberghe and Janssen (2014) clearly noted that estimations of the potential risks for human health posed by MP in food is not yet possible (see also Smith et al. 2018 for a list of suggested research needs for MP and their effects on human health).

Santillo et al. (2017) noted in a commentary that the potential for humans to consume MP in seafood and state that implications for health need to be considered, citing several questionable studies in their discussion of the topic. Teng et al. (2019) reported on MP in cultured oysters from several areas in China, and begin their abstract with the statement that "the presence of microplastics in seafood may pose a threat to food safety, and there is an urgent need to evaluate the potential risks of microplastics to human health." They further note (incorrectly) that field studies have demonstrated a high abundance of MP ingestion in bivalves, go on to list studies that reported very low levels of MP, and then state that "Our results suggest that microplastics might pose potential risks to human health when humans consume these contaminated oysters." In fact, all their results do demonstrate is that there were low levels of MP in oysters and nothing regarding human health. In a laboratory study, Scanes et al. (2019) reported on MP detected in hemolymph in the rock oyster *Saccostrea glomerata;* however, they exposed the oysters to very high levels of plastic particles (0.5 and 2.0 μm; at 10^9–10^{10} MP·L^{-1}) so contamination of the hemolymph by other fluids was likely. They state at the end of their abstract that their study "highlights the need to monitor microplastics in the marine environment and aquaculture to safeguard the seafood industry."

Li et al. (2018b) stated, with no caveats, that mussels are a vector for transfer of MP into the human food chain. They noted levels of total debris items from 1.1 to 6.4 items·individual^{-1} in wild mussels. They also reported levels in pre-cooked mussels of 1.4 items·g^{-1} and 0.9 items·g^{-1} in live mussels and claim that these miniscule differences are significantly different. Cho et al. (2019) suggest that MP pose a threat to human health through seafood consumption and go on to recommend depuration efforts. They estimated dietary intake of MP by the Korean population *via* shellfish as 212 particles per person per year.

Akoueson et al. (2020) provided data on MP in edible versus non-edible tissues from seafood, and yet analyzed scallops as whole tissue samples, noting that all tissues are edible. In fact, only the adductor muscle or the adductor muscle plus gonad are consumed in most geographic regions, and MP are known to occur primarily in the digestive glands. They included approximately 31 papers and summarize the literature on MP in fish and shellfish in one paragraph! They note that their results "validate microplastics as an emerging risk in the food chain and establish seafood as a vector for the exposure and uptake of MP through the ingestion route for humans," as if this was the first study to note MP in shellfish or uptake by consumers. These are only representative examples of aggrandizing statements invoking the potential human health threats to justify studies and their publication.

Hantoro et al. (2019) presented a review and noted clearly that data regarding levels of MP in coastal seafood and their toxicological effects are limited, and that dietary risk is still poorly explored. They provide a more extensive review of MP in fish and shellfish than most, but the coverage of shellfish is superficial at best and they include incorrect statements and assumptions based on prior publications, for example, "Filter feeders, such as bivalves, oysters, and clams, display a non-selective feeding behavior and are, therefore, more likely to ingest microplastics." They summarized a study on depuration of MP from mussels (Van Cauwenberghe and Janssen 2014; see Section 6) noting that after three days of depuration, the levels of MP in blue mussel was reduced from a mean of 0.36 ± 0.07 (*SD*) particles·g ww^{-1} to 0.24 ± 0.07 (*SD*) particles·g ww^{-1}, and in oysters (*C. gigas*) from 0.47 ± 0.16 (*SD*) particles·g ww^{-1} to 0.35 ± 0.05 (*SD*) particles·g ww^{-1} and concluded that "depuration reduces the number of microplastics in shellfish, most particles seem to remain in the animals," completely ignoring the fact that these levels are trivial at best. They further cite prior studies to demonstrate impacts of MP on shellfish, some of which are unreliable. They conclude that current data availability is insufficient to perform an adequate risk assessment for MP affecting seafood species and human health, but then recommend that food safety managers consider a provisional action level for MP in seafood.

In a more nuanced effort to assess potential impacts of MP on human health, Baechler et al. (2020b) investigated potential exposure of recreational harvesters and consumers of razor clams in a sparsely populated area of the Pacific Northwest (USA). They reported low levels of primarily microfibers at all sites sampled and stated that mean "suspected MP burden" differed by tissue type analyzed, but the concentrations found in all tissues were very small. The authors also carried out a survey to assess consumption habits. They estimated that an annual suspected exposure was 60 to

3070 MP for cleaned (digestive gland removed) and 120 to 6020 MP for whole clams. Based on their data, they posited that recreational razor clam harvester-consumers are exposed to low levels of MP, and that this particular exposure poses a minimal vector for MP. They offer their data as a reference to inform future recommendations and development of human health standards. Baechler et al. (2020c) later noted that the human health risks from consumption of commercial species containing MP are unknown and, as with many other papers, point out the need to understand potential human health risks posed by species contaminated with MP. Most recently, Zhang et al. (2022) presented a study on the association of zoonotic protozoan parasites with MP in seawater and discussed potential impacts for human health. Their study demonstrated a novel potential pathway by which anthropogenic pollutants may be serving as a source of transmission in the marine environment, but this transmission has not been seen. Zhang et al. make no mention of known comparable data for biotic and abiotic microparticles or even "natural particles" as platforms for zoonotics, that is, MP are but one more type of particle upon which zoonotics can attach and be transported or serve as a vector and readily available to consumer organisms.

Many authors invoke the potential for impacts on human health to justify their study; however, none of those studies addressed human health. Levels ranging from 1 to 30 particles·day^{-1} (1800 – 11,000 MP·year^{-1}) have been suggested for regular consumers of shellfish (see Van Cauwenberghe and Janssen 2014; Vandermeersch et al. 2015; Cho et al. 2019; see also Lusher et al. 2017b for a theoretical assessment of potential consumption and Dawson et al. 2021 for a review of Australian seafood consumption patterns and potential MP consumption). Lusher et al. (2017b) speculated that a worst-case estimate of human exposure to MP after eating a portion of mussels (225 g) would lead to ingestion of 900 MP particles representing approximately 7 µg of plastic and, while this is a very low number, it should still be noted that it is based on several assumptions and not solid data. Barboza et al. (2018) provided yet another review of the presence of MP in marine organisms, but again, this review does not assess the information critically. For many of the accounts reported in their summary table, they list the size range of particles at 5 to 5000 µm, apparently because specific size ranges were not provided in the original papers and they simply inserted the defined range for MP. They list the general term "soft tissue" as the site of MP accumulation and there is no discussion of the veracity of the data, only another list of the same papers cited in prior reviews. As with all prior papers and reviews, they note that MP ingestion has been observed in a wide range of animals, including bivalve molluscs, but no mention is made of the significance of the minute numbers of particles reported. Their review focused on the perceived potential impacts of ingestion of MP by humans through the consumption of marine species contaminated with these particles, again, raising the overall sense that this is an international crisis that may, in fact, be inconsequential. This is not to say that the issue should not be addressed, but its potential impacts should not be overstated or over worried until there are data to support these statements and concerns.

Even the few studies (van Cauwenberghe and Janssen 2014; Devriese et al. 2015; Vandermeersch et al. 2015; De Witte et al. 2014) that have reported theoretical numbers for the consumption of MP through seafood provide very low estimates, for example, 175 to 11,000 particles·year^{-1}. People consume more than this through their regular diets routinely (see Barboza et al. 2018 and references therein; Catarino et al. 2018). Continued use of the word "contaminated" to refer to the presence of 1 (or even a fraction thereof) MP particle in an entire oyster or mussel reinforces the false sense that this is an issue of grand proportions, and it is premature to suggest continuous monitoring programs, risk analysis frameworks, and regulatory frameworks to increase human food safety, based upon such data.

A review of nanoplastic and microplastic contamination in the food chain (Toussaint et al. 2019) was designed to explore the presence of plastic particles in animals and food products that are part of the human food chain. Bivalve molluscs were included in their assessment along with many other food items. One of the main findings of the review was that the data needed to assess exposure of humans to plastic particles through their diet cannot be produced until methods are standardized and clear definitions available. They claim (their Table 1) that only 22 papers on MP contamination in bivalves have been published since 2010, clearly a substantial underestimate (see Table 1 this review). The introduction presents inaccurate summaries of information on bivalve molluscs based upon citation of information known to be flawed. They note that *Mytilus edulis* can ingest MP particles of sizes ranging from 2 µm – to 10 µm. While this is true, it is but a mere fraction of the size range the mussels can accommodate. Flawed studies and conclusions from prior publications are included. As seen in other instances, this is not an unusual

situation and further reinforces the issues associated with publication of flawed and erroneous studies. It also highlights the subsequent inability of non-experts to distinguish between valid and non-valid data and conclusions in their efforts to make valid generalizations and recommendations.

Wright and Kelly (2017) provided a cross-disciplinary review to evaluate the potential human health impacts of MP and provided recommendations for future research. Their discussion pointed out that, while there is potential for MP to impact human health, assessing current exposure levels and burdens is key. Given the low levels of MP consumed by bivalve molluscs, the likelihood of this being a threat to human health seems minimal. Microplastics are found in other food items, drinking water (see reviews by Koelmans et al. 2019; Danopoulous et al. 2020c), and airborne MP are regularly inhaled, that is, the extremely low levels present in suspension feeding shellfish are of little concern with regard to seafood safety.

The World Health Organization (2022) and the Food and Agriculture Organization of the United Nations (FAO; Garrido Gammaro and Costanzo 2022) each published comprehensive reviews to assess the evidence for risks to human health concerning MP in food commodities including seafood. Both reviews presented in-depth and insightful analyses of published literature, and neither review presented alarmist statements regarding public health issues. While the WHO (2022) review focused primarily on nanoparticles, the Garrido Gammaro and Costanzo (2022) review included contributions from several leading experts and addressed the presence of MP in food. They concluded that hazards and exposure levels are generally low, that there are significant challenges including data paucity, knowledge gaps regarding toxicity of the particles, and lack of standardized analytical methods. They recommended development, fine-tuning, and harmonization of analytical techniques for MP in food, ongoing studies on the occurrence and toxicity of these substances in food value chains, and investigation of the acute and chronic exposures to MP in various foods.

Common sense dictates that, given the ubiquitous presence of these particles in coastal waters, not only is identification of one or even ten microparticles in a mussel, oyster, or other shellfish species an inconsequential concentration, but that the idea that these small levels of plastic particles identified could possibly harbor enough of any toxin at a level that could impact human health seems preposterous. If and until there are solid scientific data to the contrary, authors should cease using the guise of "possible and potential threats and impacts to human health" to justify their studies. Whereas it is entirely possible that future research could demonstrate impacts of MP on human health, there are currently no data to justify that thinking. Further, given the extremely low levels recorded in molluscan shellfish, it is highly unlikely that molluscs will serve as a significant vector for any such threat.

9. The status of peer reviewed literature and reviews

The VKM report (Utne Skåre et al. 2019) examined the state of knowledge regarding MP (<1 mm) in relation to environmental and human health. They reported that 60% of the scientific MP papers in the peer-reviewed literature were not of sufficient quality to include in the data analysis review. Their assessment revealed that the two most highly cited journals, *Marine Pollution Bulletin* and *Environmental Pollution*, were responsible for a large proportion of the poor-quality publications (both journals had >30% of the publications classified as poor quality). Of the 33 journals examined by Utne Skåre et al. (2019), 12 failed to publish any articles that received acceptable scores. None of the top three journals with the most publications at the time (*Marine Pollution Bulletin*, *Environmental Pollution*, and *Science of the Total Environment*) had any publications that ranked excellent quality condition (Utne Skåre et al. 2019). This assessment is strongly reinforced in the present review (see Tables 1 and 3; Literature Cited). Other journals could certainly be included based upon analyses presented in this review, for example, *Chemosphere* and others as delineated in Tables 1 and 3. A review by Zhou et al. (2022) recently provided yet another bibliometric analysis, noting the same three journals as publishing the most papers concerning MP research, but as with all other bibliometric reviews, there is no discussion or concern regarding the veracity of the science in the papers published. Published is not a synonym for quality and all readers need to be acutely aware of this fact.

Borja and Elliott (2019) posed the question "when will we have enough papers telling us that marine plastics, ocean litter and microplastics occur in the seas?" and provided a very well-conceived discussion of "shot-gun science," that is, the trends in publication of papers on marine stressors, for example, radioactivity, oil pollution, eutrophication, metals, and organic pollutants as they gained popularity – and now microplastics (MP) and ocean litter. They noted that studies

are needed to demonstrate the effects as well as the cause of pollutants. Their assessment is reinforced by the current review. There is a need for an objective, unbiased analytical analysis approach to the issue of MP and marine molluscs and seafood. There is no need for a further plethora of publications indicating that MP have been found in another species in another geographic location. If a region is identified where no MP are identified, that would be newsworthy! A database could be established where individuals enter their information in a specified format with standardized parameters on global distribution of MP and the species in which they were identified. At least one such platform exists at *Marine Pollution Bulletin* in the Baseline section (see Richardson 2012, 2022) and has been successful in making long-term data available for general use, the need for, and value of, which are discussed by Borja and Elliott (2021).

Koelmans et al. (2017) provided a thoughtful assessment of the actual risks of plastic debris vs. opinions, beliefs, and perceptions, and noted the already present hype associated with unintended overreaction and exaggeration (see also Connors et al. 2017). More importantly, they provided a clear path forward with a rigorous framework to approach risk assessment with respect to plastic debris of all sizes in all habitats. Rochman et al. (2019) presented a much needed and thoughtful commentary on the flaws currently associated with the field of MP, the consideration that all MP are the same, and the associated literature. They focused their discussion on the consideration of MP as a suite or class of contaminants and methods for sampling and analysis.

Solid risk assessments are still relatively new in this field. Everaert et al. (2018) carried out an environmental risk assessment for MP by estimating the order of magnitude of the past, present, and future concentrations. They included several studies on molluscs, many of which have serious shortcomings (see Table 3 in this review), as well as included the rotifer *Brachionus koreanus* among the molluscs in their table of data used for their calculations. They noted that a great number of studies concluded that MP are a threat or risk to these systems and refreshingly pointed out that these conclusions are mostly based upon conjecture and inadequate datasets. They further suggested that, on average, no direct effects of free-floating MP in the marine environment are expected up to the year 2100 and encourage research using realistic environmental MP concentrations to verify their findings.

Gouin et al. (2019) provided a comprehensive assessment of needs for an environmental risk assessment framework for MP based upon a symposium cosponsored by the International Council of Chemical Associations involving 39 scientists representing 8 countries. The multistakeholder group reached consensus with respect to interpretation of the current state of the science related to effects and exposure to MP particles which implies that it is unlikely that current levels of MP in the environment represent a risk. They presented a detailed framework for determining chemical environmental risk assessment. While they specifically addressed chemical risk assessment, their approach would be well considered by the biological community. They added that as increasing amounts of plastic reach the environment, risk calculation ratios can also be expected to increase. A subsequent paper by Adam et al. (2021) presented the first environmental risk assessment for marine waters based on measured concentrations. Interestingly and significantly, they concluded that risks toward marine organisms are unlikely given the available data. As with the assessment by Gouin et al. (op cit), Adam et al. (2021) note their conclusions could change as more data become available or as concentrations of MP in marine waters increase.

There have been numerous reviews that attempt to make sense of the cluttered literature, and the same publications, flawed or not, are cited repeatedly. The availability of computer searches and analyses has spawned an entire arena of new publications showing various metrics, many of which are of questionable need or use. As just one example, Pauna et al. (2019) provided a bibliometric network analysis that identified the top journals and authors in terms of publications on MP and concluded with the obvious, that an increasing trend of publications focusing on MP is expected in the future and that collaborative and interdisciplinary studies should be encouraged. The latest bibliometric analysis (Sorensen and Jovanović 2021) justifies their effort claiming it to be the first to analyze MP, microfibers, microspheres, and nanoplastic research trends in the environment. This is yet another desk exercise with no critical analysis of the literature, simply accepting the fact that the papers were published. They did note that the most papers were published in *Marine Pollution Bulletin, Environmental Pollution,* and *Science of the Total Environment*. It is thus not surprising that the majority of highly criticized publications noted in the present review were published in these journals (and others), including *Chemosphere*, and reinforces the onus on editors and reviewers of these journals to be judicious in their review processes (see discussion below).

There are reviews, meta-reviews, overviews, mini-reviews, surveys, preliminary analyses, and assessments, and, most recently, a "review of reviews." Many are written by individuals with little or no expertise on the topics covered. The authors frequently indicate that they are providing meta-reviews and that they have followed some strict protocols, have searched various databases using specific key words, and then do little more than provide a listing of what they found. No matter how strict the protocol, without a knowledgeable and critical assessment of what is being reviewed, the efforts serve little purpose. These efforts do a great disservice to future readers in that they rely upon the published information and accept it as accurate. Publication of these superficial and non-critical efforts also serves to give credence to poor science. Few are critical, nor do they assess the veracity of the papers cited or provide any insightful discussion. Many are grossly insufficient and do little more than reinforce errors and misconceptions. While review papers continue to be published, most do little more than summarize a (small) portion of the literature, and most do not even do that comprehensively. There is currently very little critical assessment of prior studies, and few reviews provide a critical analysis of the literature extant, the data presented, or provide any recommendations for the future. Many of the published studies include what appear to the non-expert to be detailed, careful, and informed methods, materials, husbandry, and experimental protocols; however, careful reading by experts reveal numerous issues with regard to animal husbandry, experimental protocols, instrumentation, or even basic understanding of the literature they are citing. Unfortunately, the papers were probably reviewed by equally inexperienced individuals and the dubious results in primary studies perpetuated in subsequent studies and reviews.

Most published review efforts, in fact, accept the published data at face value and thus serve to perpetuate poor science and invalid conclusions. Indeed, in one explicit example, Gall and Thompson (2015) in their review of the impacts of debris on marine life specifically stated that their literature review process took the publications at face value and "did not include assessment of the reliability of each report." Foley et al. (2018) included a mere six studies (see their Table 1) on bivalve molluscs in their purported meta-analysis of impacts on fish and aquatic invertebrates, several of which include critical flaws (see Table 3 this review). In their first "highlight" on the front page of the paper, they state that "microplastic may pose directly deleterious threat to aquatic organisms worldwide." They did not take into account the potential effect(s) of the concentration of particles to which organisms were exposed. Of the six studies included in their analysis, five demonstrated consumption of MP and several included questionable data (see Table 3 this review). They generalized that, for the parameters they examined, echinoderms and molluscs are not affected by exposure to MP. While the final conclusion is probably valid, the means to reach it were flawed.

Provencher et al. (2019) reviewed the literature concerning uptake and trophic transfer of plastic debris. Their effort included macroplastic and microplastic debris and concluded that most studies examined contamination, but few discussed fate or trophic transfer of the plastics. Their assessment included very little discussion of suspension feeding invertebrates *per se*, focusing more on fish and macrofauna (birds, turtles, marine mammals). They identified excretion, bioaccumulation, biomagnification, and trophic transfer as areas of research to be addressed in future efforts to assess the fate of plastic contamination through food webs.

A review by Paul-Pont et al. (2018) states that "substantial effects have been reported on feeding activity, reserve depletion, impairment of oxidative balance and the immune system, and inflammatory responses, with impacts on animal fitness, notably reproduction." This statement gives the reader the impression that impacts of MP on marine organisms are rampant and wide ranging when, in fact, their references for this statement refer to a worm, two prior non-critical reviews, and two references to their own work, wherein most of the impacts mentioned above were seen in oysters and mussels, not a wide range of species. Ribeiro et al. (2019) include a statement in their abstract that there is no conclusive evidence for the mode of accumulation of MP in either mammals or humans, which implies that there is demonstrable accumulation in humans and mammals – an area that is still very open to debate. In fact, demonstrated negative impacts of MP on marine organisms are few and limited, and, in many studies, the science needs critical evaluation before accepting the reported results and reporting them repeatedly as dogma.

One recent example of this perpetuation of weak studies as fact and superficial coverage of the literature is the overview by Carbery et al. (2018). These authors summarized a number of prior reviews and included a table purportedly to show the MP concentration, polymer type, size, and shape ingested by marine organisms in the natural environment; however, the table summarized the results of only 10 published

studies, a tiny fraction of the available published information. They present a long list of recommendations, some of which reiterate previous recommendations from prior studies, and others that appear to be more specific and aimed at their future research interests. These recommendations appear as a shopping list to justify any future research efforts regardless of their significance or potential usefulness. They note "particularly for the southern hemisphere," but without any discussion as to why, and their suggestion for studies on impacts of cooking on accessibility of possible contaminants lacks any foundation whatsoever. Anbumani and Kakkar (2018) reviewed the ecotoxicological effects of MP on biota and included only a superficial number of papers on molluscs, many of which are flawed, again, reinforcing erroneous results.

Ivar do Sul and Costa (2014) provide a particularly flawed discussion of the uptake and consumption of MP by suspension feeding mussels, in that they simply reiterated results from prior studies that contained significant experimental flaws and conclusions, and again flamed the fires of implications of consuming shellfish to human health.

A most recent example of highly experienced authors in the field of plastics and the marine environment relying upon flawed published literature is provided in a review by Steer and Thompson (2020). They included a long list of invertebrate species that ingest MP and noted studies that purported to show impacts of the MP – many of which contain errors, poor experimental protocols, and misinterpretations. The authors justifiably relied upon published studies that should have been more carefully vetted by reviewers and editors prior to publication. A similar situation is presented in the review by Bucci et al. (2020) in which the authors provided a broad review of published papers, but gave no consideration to the quality of the data in the papers cited. It is this continued, repeated, and non-critical citation of flawed studies that confuses inexperienced readers and perpetuates poor science.

In yet another review, Ribeiro et al. (2019) purportedly summarize both nanoplastics and microplastics: "Here we summarize available literature on the accumulation of microplastic and their associated contaminants in a variety of organisms including humans" – all in five pages of text and three pages of references. Any good editor or reviewer should have questioned the veracity of being able to summarize available literature from such a vast database in such a short communication. In addition to being a superficial coverage at best, there are inaccurately attributed references, and a schematic diagram showing, incorrectly, a mussel with an inhalant siphon (see also Subsections 4.4 and 4.6).

The recent review of direct and indirect effects of MP on bivalves, with a focus on edible species (put forth as a mini review) by Zhang et al. (2020) is a strong example of authors having no appreciation of the veracity of prior works, citing them blindly, and peer reviewers and editors not providing the oversight necessary to stop publication. None of the authors is cited in their reference list giving the reader pause as to their expertise in the field. As just a few examples of issues associated with this publication: the authors provide an abstract with what appears to be a strong statement regarding direct and indirect impacts of MP on bivalve molluscs; however, a careful read of the studies they cite to form these conclusions shows that they were chosen selectively, not comprehensively, and include several papers with major flaws. If the authors had any knowledge of the field, they might have recognized at least some of these flaws, and one might have hoped that seasoned and informed reviewers would have noted at least some of them as well. The authors stated that MP fibers are harder to remove from the digestive tract than granular particles – not necessarily true, based upon one flawed study by Li et al. (2015; see Section 6). They then cite an example of a species of *Daphnia,* most certainly not a mollusc. They continue by citing one of the most egregiously incorrect studies published to date (Kolandhasamy et al. 2018) which claims MP fibers were accumulated *in* the foot and mantle of blue mussels. This purported result is egregious and most likely a result of experimental and interpretational errors (i.e., particles on ciliary rejection tracts of the foot and mantle that were not properly washed from the epithelia; see Subsections 4.1, 4.5, and 4.6). The conclusions of this study also demonstrate the authors' lack of understanding of the way in which captured particles are transported by the mantle-cavity organs and ingested particles are conveyed through the digestive system by these animals. Yet, the paper continues to be cited. Further, the information Zhang et al. (2020) used to demonstrate the "global decline" in edible bivalve molluscs (by the title, the basis for their discussion) is based upon freshwater mussels (not eaten in most places), a mention of pearl oysters, and no careful analysis of actual bivalve production data from marine environments – the prime areas of bivalve production globally. Indeed, their entire discussion (justification) for declining bivalve populations globally is presented in two superficial paragraphs with no quantitative data, and it is, indeed, an incorrect assessment. Their superficial and uninformed discussion of purported

impacts of MP fibers on the physiology of molluscs does nothing more than further compound errors already published. In addition, their broad statements regarding potential human health concerns are unwarranted. The first line of their conclusions is simply wrong and, as they state it, gives the impression that there is ample solid evidence to support the statement.

Wong et al. (2020) presented a bibliometric analysis of nanoplastics and microplastics in global food webs and included a statement in their introduction that MP pollution in the aquatic environment is linked to the global scale decline in the bivalve populations of clams and oysters in the recent decade and cite Zhang et al. (2020; see above). This study is a desk exercise that identified the journals that publish the most papers in the field, but says nothing about the quality of the publications, and provides only superficial bibliographic data on citations. This is a classic example of how erroneous statements are reinforced by continued citation. One full paragraph in their introduction lists what the author calls physical impacts, then lists a series of physiological impacts, most erroneous, and all without attribution. Their conclusion that the field of MP is interdisciplinary and global are hardly unexpected or even a conclusion.

One group of authors published a meta review of reviews – in 10 pages including a page of references (Aretoulaki et al. 2020). This was another desk analysis prepared by a group of individuals with no apparent demonstrated expertise in marine plastic pollution that discusses the "how-to" of preparing a review. One of their assumptions was that, because the journals they used in their analyses "have a rationally high impact factor and thus, it is assumed that the selected studies have undergone meticulous and strict quality control by editors and peer-reviewers." One could write volumes on the value of impact factors, but it is sufficient to point out here that quality is in no way considered in the calculation of an IF, it is based upon citations. The information provided in the present review clearly refutes that assumption. Indeed, this "review of reviews" by Aretoulaki et al. (2020) included anything published and provided no critical assessment. As described in the present effort, several of the journals cited by these authors have published some of the most egregiously flawed studies and reviews. While very limited in scope (the assessment is based upon only one database), the effort by Aretoulaki et al. (2020) does provide a summary of review types published in recent years, pointing out that qualitative reviews are the most common, that is, non-critical. The authors indicate that they realize their study is limited, but unashamedly offer what they claim to be a synthesis of perspectives on MP pollution, an assessment of the research progress, and highlights for future research directions.

Sendra et al. (2021) reviewed uptake, translocation, and depuration of both nanoplastics and microplastics in bivalves. They presented a confused description of the pathways for uptake by, and distribution within, the bivalves that combines both nanoplastics and microplastics – particles that can be handled quite differently by the animals. There are numerous facts taken from various papers, but not presented as a cohesive discussion or correct description of the functionality in bivalves. They cite an equally confused and erroneous study (Ribeiro et al. 2017; see Subsections 4.3 and 4.6, Table 3) for their description of particle capture and transport. This is but one more example of flawed literature being cited repeatedly and becoming entrained in the literature. The discussions regarding accumulation of particles and interpretation of prior literature are equally confused. Published literature is cited throughout with no critical assessment or apparent understanding of its veracity, and incorrect summaries and interpretations made. They state in their summary (p. 11) that "plastic accumulation in oysters, mussels, scallops, and clams has been revealed, indicating the high levels of plastic ingestion by bivalves to which humans are directly and indirectly exposed *via* trophic webs and biomagnification." Not only is it incorrect to state that these shellfish contain high levels of particles, but the implication regarding human consumption is also not supported by any data to date. Their review is rife with errors, both typographical and substantive, for example, they cite "Ecol Ser et al. 1983" (this is a typographical error that lists part of the journal title as the author which should be Newell and Jordan (1983) and misspell Newell)), and write "MPs can be inhaled…" by bivalves. These are just a few examples of multiple issues that should have been addressed by competent reviewers and editors.

Yet another example of a questionable published review purportedly addressed potential impacts of MP in aquaculture (Zhou et al. 2021). It is a stellar example of a poorly written and unedited, superficially referenced product from authors with no demonstrated expertise in aquaculture. It is nothing more than a desk survey, provides an incomplete summary of published literature, and a weak, superficial, "conclusion and perspective" with no substantial contributions to understanding potential impacts of MP on aquaculture. Further, they include the commonly seen unsubstantiated statement that "undoubtedly, the plastics are hazardous to human

health due to their physical and chemical toxicity," and that "the safety of aquaculture products is closely related to human health because (sic) the residues of microplastics in fish leading to various potential hazards."

Most recently, Gabisa and Gheewala (2022) and Sangkham et al. (2022) published non-critical literature reviews of MP in ASEAN countries and exposure routes, toxic studies, and potential effects on human health, respectively. Gabisa and Gheewala (op. cit.) ended their introduction with a statement that the presence of MP in marine microorganisms is becoming a concern for human health. They went on to discuss MP in sediments, water systems, and macroorganisms. Their coverage of impacts of MP on humans is limited to two paragraphs and mix their discussion of MP with nanoplastics. Sangkham et al. (2022) also combine MP and nanoplastics in their discussion, but do provide a succinct list of unanswered questions regarding MP research and protocols. Bom and Sá (2022) published a baseline paper reporting MP concentrations in four bivalves from Brazilian markets. Mussels (*M. chilensis* from Chile and *P. perna* cultured in Brazil), oysters (*C. gigas*, cultured in Brazil) and scallops (identified as *Placopecten magellanicus* from Peru – likely misidentified as *Placopecten* are not native to Peru and not sold or exported whole). They reported a mean number of MP in all samples of 10.69 ± 0.43 (*SE*) MP·individual^{-1} and 1.64 ± 0.19 (*SE*) MP·g ww^{-1} and, based upon these very low levels of MP, recommended mandatory use of depuration techniques to protect human health.

The scientific literature concerning MP and molluscs is already severely polluted and, unfortunately, publication means that these citations will be available forever. A further problem associated with the literature extant is the promulgation of so-called reviews by individuals with no expertise in the field who decide to jump on the bandwagon, search the internet for publications, and prepare an incomplete, non-critical summary of literature. These efforts do nothing more than clutter the literature and reinforce citations for weak or incorrect studies and should be thwarted by diligent editors. There is no quick fix for this situation, but going forward, editors should engage qualified reviewers, make a concerted effort to accept only studies that have been carried out using valid and current methodologies, have engaged proper animal husbandry, included and critically assessed prior literature, and in general provide information that moves the field forward in a positive manner. Future reviews that do nothing more than summarize published papers are unnecessary. Peer-reviewers and editors should also stem the continued citation of publications known to be erroneous. In short, this means that editors need to know the field and if they do not, they should engage colleagues who do.

10. Conclusions

The published literature reviewed here confirms the current levels of microplastic (MP; >~ 10 µm) in molluscs, especially marine bivalves, under field conditions is extremely low globally, and this overarching fact seems to have eluded the community at large as they continue to search for impacts, effects, and adverse links to aquaculture and human health. Suspension feeding shellfish are not serving as reservoirs of MP in the natural environment. Further, the experimental exposures at environmentally relevant concentrations provide no strong evidence overall, that molluscs are impacted in any uniform or meaningful way by these low levels of microparticles. The distribution of MP (>~ 10 µm) is ubiquitous and they will be found everywhere they are sought and in very low concentrations. It is demonstrated here that these extremely low levels are highly unlikely to impose any measurable impacts on the shellfish in their natural habitats, nor are they currently a credible or demonstrated threat to human health. Coupled with the knowledge that these molluscs will not serve as reliable bioindicators for the presence of MP in the local environment, there is little need for further descriptive efforts. The continued stream of studies and reports on the presence of MP in bivalve molluscs is unnecessary without specific justification.

False claims or negative publicity can have far-reaching and hugely detrimental impacts on the aquaculture industry. There are no strong data to support any negative claims being made with regard to the contributions that the aquaculture industry may be making to the levels of MP in the environment. Further, there are no data to support claims that there are negative impacts on the shellfish crops or the environment, and these unwarranted and unsupported claims need to stop until and unless there are solid data to support them.

General commentaries are routinely presented noting that "laboratory studies have been performed and that MP can be taken up into cells by endocytosis, retained and translocated to different tissues, cause histological alterations, inflammatory reactions, and ecotoxicological response at cellular, biochemical, and molecular levels as well as alter physiological functions such as reproduction, respiration nutrition, and

growth," all in an effort to justify the study at hand, but with no consideration given to the veracity of the studies being cited. Any future experimental studies need to be focused carefully, based upon clear questions, use standardized analytical procedures and environmental levels of MP, and demonstrate a knowledge of the animals being studied and the literature extant. The "hype" needs to be curtailed and scientists should not imply impacts or potential impacts of environmentally relevant MP concentrations when there are little data to support the suppositions.

Seafood safety and public health are important considerations, but exaggerated claims with regard to the potential human health impacts of consumption of MP need to be stopped. There are currently no reliable data to indicate that MP associated with shellfish have any impact on human health, and it is highly unlikely that the extremely low levels of MP reported in bivalve molluscs globally presents any significant risk to either the shellfish or to human health. In reality, the number of MP found in shellfish is far outweighed by the MP inhaled and consumed by humans in everyday life. It is recommended that future societal efforts be focused on preventing thoughless discard of plastic, recovering plastic debris, creating easily recycled plastic products, and on education to reduce MP entering the environment.

Not all studies carried out provide useful or meaningful data and many are of questionable quality. As such, they do not warrant publication in peer-reviewed journals. Scientific studies that make rash generalizations or overextend their importance based on weak data, or use statistical comparisons of fractions of particles·individual^{-1} in an attempt to obscure "effect size" and add environmental meaning to their results and conclusions, is questionable, scientifically unsound and, in some cases, outright irresponsible. Such studies should be curtailed in favor of more meaningful assessments and presentations. Managers and policy makers are relying on the veracity of peer-reviewed scientific literature in their decision making, yet the quality and conclusions of that published research are, in many cases, questionable if not outright incorrect. Provencher et al. (2020) rightly and strongly argued that journal editors and reviewers must be proactive in ensuring the quality and veracity of contributions, including careful consideration of the quality of manuscripts and their place in the field extant. That sentiment is reinforced in the present review. They provide a comprehensive listing of critical aspects to address when reviewing and publishing MP research to be considered by authors, reviewers, and editors. Utilization of this list is strongly recommended as is their advice to engage senior reviewers in this critical field, not junior, less experienced reviewers. The science, both past and current, dealing with impacts and interactions of MP and molluscs is, in many instances, seriously flawed and the only way to stem this flood of erroneous, flawed, and confusing information is to stop publishing it.

11. Summary

- A critical review of the literature extant on the interactions between molluscs and microplastics (MP) revealed a vast, rapidly growing, and contradictory literature, much of which is based upon poor experimental procedures and animal husbandry, lack of knowledge of the literature extant, and often coupled with a quest for rapid publication of papers to enhance resumes and garner funding.
- The literature is rapidly being populated with weak and often incorrect and unreliable information. This makes it impossible for inexperienced investigators or readers to recognize or identify the valid vs. the invalid. Investigators should take notice of prior literature with regard to molluscan biology and physiology, utilize appropriate techniques and technologies, and practice good animal husbandry when planning and executing experiments. If the investigators have no prior experience in these fields, they should engage experts as collaborators.
- It is well-established that bivalve molluscs can and do consume MP, and nothing remarkable has been published regarding the uptake of MP by suspension-feeding and deposit-feeding shellfish, that is, it is not surprising that numerous investigators have noted MP in shellfish guts globally. What is remarkable are the extremely low levels of MP particles reported (>~ 10 per individual; See Table 1 and references therein). It is clear that the levels of accumulation are very low, often bordering on undetectable (and hence unreliable). It is this consistent reporting of low levels that is of significance, not the fact that they are present. Given the ubiquitous distribution of MP in the marine and freshwater environments, coupled with the knowledge that these molluscs will not serve as reliable bioindicators for the presence of MP in the local environment, the same results will be seen in most areas studied. The

current wave of papers demonstrating their presence serves to oversell the significance of the findings. No further studies are needed to demonstrate that MP occur on beaches, in sediments, in shellfish, fish or in a new geographic region, unless there is a clear driving force for doing so. Simply adding one more species or site to the collection is not sufficient cause.

- Bivalve molluscs are poor bioindicators of MP in the natural environment because of their ability to ingest and reject particles selectively. There is a limited size-range of microparticles and fibers that can be ingested (which most papers ignore or misinterpret), demonstrated rejection of particles, and very limited gut residence time. It is difficult and expensive to carry out experiments and identify particles reliably, and they have limited utility, as results will vary between species and habitat (benthic animals vs. those in the water column). Use of bivalve molluscs as reliable indicators or biomarkers of MP pollution is strongly discouraged.
- There is no evidence that the MP are a credible or demonstrated threat to human health. Authors should stop including vague statements and references to possible and potential impacts of MP on human health as means to aggrandize their studies.
- There are negligible data to support any negative claims being made with regard to the contributions that the aquaculture industry may be making to the levels of MP in the environment. Further, there are no data to support claims that there are negative impacts on the shellfish crops or the environment, and these unwarranted and unsupported claims need to stop until and unless there are solid data to support them. Exaggerated and unwarranted claims and negative publicity can have far-reaching and hugely detrimental impacts on the aquaculture industry.
- Basic information is provided on the biology, physiology, ecology, and aquaculture of bivalve molluscs, with recommendations for the execution of suspension feeding experiments.
- There is no evidence that the extremely low levels of MP reported in molluscs are likely to impose any measurable impacts on the shellfish in their natural habitats. There is little evidence to support the contention that environmentally relevant concentrations of plastic particles pose a serious threat to the cellular biology or physiology of bivalves. This conclusion is based upon the various problems in experimental design and methodologies, the exaggerated concentrations often used, and the lack of appropriate natural particulate controls. As such, a greater emphasis should be placed on studies using environmentally relevant levels of MP and exposure times of months to a year to investigate whether chronic exposures have impact on the biology and physiology of shellfish.
- The laboratory data extant on interactions between MP and bivalve molluscs is weak and, in some cases, outright unreliable. While bivalve molluscs are one of the most important members of the benthic community, they should not be unduly exploited to provide tenuous data and arguments regarding the impact of MP on the environment or be used to provide undue support for negative impacts.
- There needs to be a concerted effort to standardize methodologies, techniques, and exposure concentrations in the laboratory. Development of a risk assessment plan as proposed by Koelmans et al. (2017) to provide policy makers, funding agencies, scientists, and stakeholders with accurate assessments upon which to make informed decisions is strongly encouraged. It is obvious that MP concentrations (>~ 10 per individual) in molluscs are typically below 20 MP per individual when proper QA/QC measures and analytical methods are employed. These methods, along with proper experimental design, are essential to determine the concentration of MP in molluscs collected from the field. A recommendation is made to establish an international database to collect data on distribution of MP globally, and cease the publication of qualitative studies that do nothing more than identify another geographic location and another species found to contain MP.
- Individuals undertaking studies with live animals should be versed in the husbandry, behavior, and physiology of those organisms, as well as the literature extant and accepted technologies and techniques associated with the field.
- Editors need to be more careful and selective in what is accepted for publication and pay particular attention to (1) the methods employed; (2) the validity of the conclusions, (3) reference to prior publications, and (4) the significance of the results presented. They

should be diligent in engaging experts as peer-reviewers to limit the number of weak or unacceptable papers masquerading as reliable studies in the peer-reviewed literature, and thus, not only giving them undue credibility, but also confusing those who are looking to the peer-reviewed literature for reliable information. There used to be value in the term "peer-reviewed" and a sense of validity. Sadly, that concept is no longer a given. The recent paper by Provencher et al (2020) pleading for an effort to "raise the publications bar for microplastics research" is highly recommended reading for all editors and scientists involved in this field.

12. Epilogue

This review is not the first calling for a more rational approach to the research arena (Connors et al. 2017; Paul-Pont et al. 2018) and calling attention to the importance of providing solid data and not proliferating the terms "potential" and "possible" (see Koelmans et al. 2017) which perpetuate the myth that there are significant and profound impacts of microplastic (MP) on all things considered. Weis (2019) presented a refreshing overview of thoughts on improving MP research based on a lecture presented at a conference (Impacts of Microplastics in the Urban Environment). The paper is as much editorial as review and from a non-expert on MP *per se*, but provides insightful thoughts on the status of current MP research and presents a sensible approach to future efforts. She included some papers known to have questionable methods and results, yet still identified key questions, noted the high number of inconsequential studies published, and the need for more focus on important aspects moving forward. The article reinforced the benefit of having senior, experienced researchers providing insight and guidance.

Paul-Pont et al. (2018) and Weis and Palmquist (2021) reviewed constraints and priorities for conducting experimental exposures of marine organisms to MP and offered several recommendations for standardizing methodologies and protocols as the field moves forward. This included consideration of the complexity of MP particles *per se*, diversity of particles encountered by organisms, availability, and distribution of particles in experimental efforts, use of realistic concentrations of particles, and strict experimental procedures to verify the existence of genuine translocation. Everaert et al. (2018) noted that many of the studies that conclude that MP are a threat or risk to marine systems are based on conjecture and inadequate datasets. All of these are valid concerns and sensible recommendations. To these should be added identification of questions or issues that require investigation, choice of appropriate test organisms, procurement of sufficient expertise to carry out the experiments correctly and successfully, and an improved peer-review and editorial process.

Reduction of plastic in the environment will require major changes in human behavior globally and a stronger awareness and better understanding by the general public of the macroplastic litter issue and the resulting MP. Henderson and Green (2020) provide an interesting assessment of perceptions, knowledge, and understanding of MP through focus group study and the role of media in framing perceptions and socio-cultural dimensions in reducing single-use plastics. Their effort addresses how audiences engage with the issue of plastic pollution, the tools used by various groups to understand new information about MP, and the cultural aspect of mitigation solutions. They highlight the need for greater scientific literacy and the potential role of media in shaping that knowledge, stressing the need for scientific accuracy and compelling stories. Until public understanding is increased and there is a broad acceptance of the need to change habits and use of plastics, the problems associated with MP will continue to increase. As Mendenhall (2018) noted, it may be true that enough is known about marine plastic debris to know that something needs to be done, but there is not enough to generate an urgency to formulate and implement effective solutions. A deeper base of scientific evidence is needed to generate binding international conventions.

Joyce and Falkenberg (2022) recently provided a thoughtful and significant summary regarding future research endeavors regarding MP: "Although more research into the impacts of microplastics is required, the disproportionate advocacy of plastic pollution in comparison to other, potentially greater threats to organisms, ecosystems, and biodiversity (Barrios-O'Neill 2021) should be avoided owing to the small effects caused by microplastics at environmentally relevant concentrations." They ended with, "Further, we suggest that, while a high-profile threat to ecosystems, investigating the effects of microplastics on ecosystems should be conducted alongside, and not draw focus away from, other major threats such as climate change."

It is recommended, as also noted by Abalansa et al. (2020), that researchers consider using application of the Driver-Pressure-State-Impact-Response (DPSIR) framework as one means of standardizing collection

of information for effective, adaptive management of marine plastic pollution. The most important task facing everyone is reducing the amount of plastic (macro, micro, and nano) that enters the environment, not continuing a litany of inconsequential studies noting their presence and potential impacts or fueling media hype with unsubstantiated stories regarding possible or potential impacts on human health. As noted by Barnes (2019), this will require investment in scientific and technological research. To this should be added honest self-assessment by authors regarding their qualifications and available facilities before undertaking studies or reviews involving molluscs and MP. Finally, there needs to be a more critical and concerted effort by editors and reviewers to vet the science and stop the flow of erroneous, superfluous, and damaging efforts.

Acknowledgments

Dr. Jay Parsons of the Editorial Board handled the review process and the authors thank him and four anonymous reviewers for their input. Drs. Stephan Bullard, Joth Davis, Paul Dayton, Simone Duerr, Kay Ho, John Icely, Bill Lucey, Alice Newton, Robert Rheault, Brian Rothschild, and Paul Zajicek provided insightful comments on earlier versions of the manuscript.

Funding

This work was partially supported by grants from the National Oceanic and Atmospheric Administration, Marine Debris Program NA17NOS9990121 (J.E.W. and S.E.S.); Long Island Sound Study Award # 1157712/2/86473; Connecticut Sea Grant (R/A-48).

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