Where are we? Towards an understanding of the selective accumulation of microplastics in mussels

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Running title: microplastic's selective accumulation in mussels

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Abstract

Mussels are suggested as bioindicators of marine microplastic pollution. However, they are selective in regards to accumulation of microplastics. To make studies more targeted and comparable, ultimately helping to determine the suitability of the mussel as a bioindicator species for microplastic exposure, we review the published literature that has directly or indirectly demonstrated particle selection in mussels. The reported difference between microplastic levels in mussel tissues and environmental matrices provides evidence for their selective uptake characteristics. Both the organ-specific fate characteristics of microplastics, and the different movement pattern of microplastics in the same organ, show that selective translocation processes take place. The selective elimination is reflected in multiple aspects which include (1) the different characteristics of microplastics in excretion and mussel body; (2) the different retention time of various microplastics in mussels; and (3) the tissue-specific change in the numbers of microplastics during the depuration process. This selectivity is affected by the characteristics of the microplastics, the environmental, or laboratory exposure concentrations, feeding status, and other factors. There are still many research gaps and contradictory viewpoints in this field due to this complexity. The current methodology needs improvement and a breakthrough in standardization.

Keywords: microplastic; mussel; fate; selectivity; translocation

Capsule: Mussel has particle selection for microplastics

1. Introduction

Environmental microplastic (<5 mm) pollution has received a growing amount of scientific, public, and regulatory interest due to its potential ecological (Seeley et al., 2020; Yang et al., 2020; Huang et al., 2021) and human health risks (Bouwmeester et al., 2015; Carbery et al., 2018; Zhang et al., 2020). Microplastics are ingested by many organisms from different trophic levels with various feeding strategies (Setälä et al., 2016; Ter Halle et al., 2017). Laboratory studies have highlighted the adverse impacts of microplastics in diverse aquatic organisms, such as zooplankton, bryozoans, bivalves, crustaceans, turtles, fish, seabirds, and large mammals (Wright et al., 2013; Pedersen et al., 2020). In addition, the release of plastic additives and other toxic substances (such as phenanthrene and metals) from microplastics to biota poses a threat for marine biodiversity and human health (Browne et al., 2013; Luís et al., 2015; Gandara E Silva et al., 2016; Frère et al., 2017).

Mussels are commonly used to study transport characteristics and consequent biological effects of microplastics. Field investigations show that microplastics are detected in mussels from more than twenty countries (Mathalon and Hill, 2014; Van Cauwenberghe and Janssen, 2014; De Witte et al., 2014; Van Cauwenberghe et al., 2015; Vandermeersch et al., 2015; Li et al., 2016; Catarino et al., 2017; Karlsson et al., 2017; Leslie et al., 2017; Murphy, 2018; Bonello et al., 2018; Zhao et al., 2019; Berglund et al., 2019; Cho et al., 2019; Li et al., 2019a; Naidu, 2019; Webb et al., 2019; Gedik and Eryaşar, 2020; Pazos et al., 2020; Sparks, 2020). Laboratory studies have also reported adverse effects of microplastics on mussels including within the antioxidant system, the immune system, physiological responses, histological changes, energy alteration, genotoxicity and transcriptional responses, and neurotoxic effects (reviewed in Li et al., 2019a).

It has been recognized that there is a selectivity during the uptake, translocation and elimination processes of microplastics even though mussels are filter feeders (Woods et al., 2018; Gonçalves et al., 2019; Rist et al., 2019a, 2019b; Fernández and Albentosa, 2019a; Ward et al., 2019a; Ward et al., 2019b). However, our understanding of how mussels sort and translocate microplastics with different characteristics is still in the early stages. The selectivity results in different translocation routes and retention times of various microplastics, which affects the related adverse effects. In addition, the research on selectivity process will update knowledge about the extent to which microplastics in mussels reflect environmental loadings of microplastics. This is crucial to determine the applicable conditions of mussel as a bioindicator species for microplastic.

Herein the uptake, translocation and elimination process of microplastics in mussels is reviewed. The aims addressed are (i) what is the current knowledge regarding mussel's selectivity characteristics to microplastics; (ii) what are the key factors affecting this process; and (iii) how to improve current methodology, with targeted experimental designs.

For this review, publications from Web of Science, Science Direct, and Google Scholar were collected using the keywords 'mussel', 'bivalves', 'microplastics' and 'nanoparticles. In total, 116 studies were identified and further screened according to the contents. The literature selected met one of the following criteria: (i) it is a field investigation in which results compare the characteristics of microplastics between mussels and the environment, or between different mussel tissues, or between mussels and their excreta, (ii) it is a field investigation in which microplastics in mussels with different body size, weight, or life stage have been compared, (iii) it is a laboratory study which uses different microplastics for mussel exposures, and also compares

uptake curves, or translocation sites, or retention time for those various microplastics, (iv) it is a laboratory study in which tissue-specific characteristics of microplastics during exposure or clearance period have been reported, or (v) it is a laboratory study in which other variables are investigated in parallel (e.g., exposure concentration, feeding condition, sampling time), reporting that these variables may affect microplastics' uptake, translocation or elimination by mussels. After this final sift, 35 publications remained, and are considered herein.

2. The selective characteristics of mussels to microplastics

Although some studies have stressed that mussel has a selectivity to microplastics, only partial evidence has been demonstrated in the past. In this section, the uptake, translocation and elimination pathways of microplastics in mussels and the selective characteristics during different stages are summarized and highlighted. Microplastics are selected due to various factors, which will be discussed in section 3.

2.1 Microplastics from environment to mussels—uptake

Microplastic accumulation in mussels is impacted by ingestion, adherence and fusion (Kolandhasamy et al., 2018; Li et al., 2019b). Among them, ingestion is the only way for organisms to take in contaminants such as microplastics by active. Microplastics enter via the inhalent siphons of mussels along with surrounding seawater, and then are captured by the gills. On the gill surface, microplastics are assimilated over the gill epithelium or transported into the mouth and digestive system mediated by microvilli activity and endocytosis (Von Moos et al., 2012). According to this uptake route, siphons, gill, intestine and stomach are the main organs involved in ingestion process, while the other organs (including gonad, mantle, adductor, visceral and foot) are responsible for the adherence of microplastics (Kolandhasamy et al., 2018). Surprisingly, adherence plays the same role as ingestion in accumulation of

microplastics in mussels with similar uptake proportions through two pathways (Kolandhasamy et al., 2018). Fusion of microplastic into the byssus, as a novel mode of uptake, has also been demonstrated in a recent laboratory simulation study (Li et al., 2019b). Although adherence and fusion are regarded as two different pathways of microplastic uptake in mussels, the related mechanisms are not clear. More studies are needed to explore if microplastics sticking to the membrane of mussel tissues could enter cells or deep tissues, and if microplastics fusing to newly formed byssus can be transfered to the foot or other organs.

The differences between microplastics in mussel tissues and that observed in surrounding environmental matrices provide evidence for their selective characteristics (table 1). For example, mussels collected from China, the United Kingdom and Greece contained more of the smaller sized microplastics in their tissues when compared with the surrounding seawater (Digka et al., 2018; Li et al., 2018; Qu et al., 2018). In addition, many laboratory simulation experiments have demonstrated the differential uptake of microplastics in mussels (table 1). For instance, only microplastics of the smallest size (10 μm) were detected in mussels that were exposed to seawater containing three different-sized (10 μm, 30 μm, 90 μm) microplastics (Van Cauwenbergheet al., 2015). Sometimes, it is hard to determine which factor leads to observed differential uptake when microplastics of different materials and size are used together. It is not necessarily a size effect. For example, polystyrene spherules with 5 μm in diameter are more easily ingested by ribbed mussel (*Geukensia demissa*) compared with 250–300 μm polyethylene microspheres when they were exposed together (Khan and Prezant, 2018). Since different and limited size of microplastics are used in each study, it is hard to compare current studies and to determine which size range of all the microplastics is more likely to be

ingested or absorbed by mussels. In addition, it is necessary to test if the selectivity exists among different sized nanoplastics and the related mechanisms.

This difference also exists between 6 μm and 10 μm particles, with different uptake curves observed in mussels (Gonçalves et al., 2019). Apart from size, mussels also demonstrate selectivity of other characteristics such as shape and weathering degree (Bråte et al., 2018; Qu et al., 2018). Laboratory studies have shown, for instance, that *Mytilus galloprovincialis* ingested significantly more weathered polyethylene particles than virgin particles (Bråte et al., 2018). From the foregoing account it can be concluded that mussels have a selectivity to a certain property of microplastics (e.g. size, shape or weathering degree). However, microplastics are synthesis of many properties. When they are put together, how the selectivity works? It is difficult to deterimine to which property the selectivity is preferential. More attention to the comprehensive selectivity is suggeted.

While current studies provide evidence for different uptake characteristics of microplastics by mussels, their relative contributions to selecting particles have not been determined. Most studies focus on microplastic fate in the whole body or specific organs, such as the gills and digestive gland, yet a more systematic approach according to uptake mode and rate is needed to determine how selective uptake occurs. Up to now, some problems are far from being fully understood. For example, if microplastics' adhesion to mussels is a selection process due to some specific factors (e.g., surface charge, shape, size) or it happens at random. If it's the former, what kind of microplastics are more likely to be attached.

Table 1

2.2 Microplastics in different mussel tissues—translocation

Microplastics have been observed transfering to different organs and tissues after

uptake (Fig. 1). Gills, palps and stomach are important organs involved in selecting and transfering microplastics (Ward et al., 2019a). On the gills and palps of mussels, microplastics may be rejected as pseudofeces, or directly assimilated by the gill epithelium, or transported into the mouth and digestive system (Ward et al., 2019a). Microplastics in the stomach can also be selectively transferred to the digestive gland, or to the intestinal groove and mid-gut (Ward et al., 2019a). Microplastics entering the digestive gland, where complete intracellular digestion occurs, may be then transferred to lysosomes and the circulatory system (Browne et al., 2008). However, no studies have been conducted on the transport route of microplastics in haemolymph. Also, on occasion, phagocytosed particles in the digestive gland prove to be indigestible and are excreted via the pericardial gland and excretory organs (Merzel et al., 2019; Cole et al., 2020). According to Ward and Shumway (2004), microplastics directed to the intestine have a shorter gut retention time than those directed to the digestive gland.

Figure 1

Both the organ-specific characteristics of microplastics and different movement pattern of microplastics in the same organ show a selective translocation process. When the evidence of selective translocation of microplastics in mussels are collected from current studies, we can find that most of the studies focus on the selectivity to size of microplastics during translocation. For example, relatively large sized microplastics (of 10 μm) can be transferred to mussel's digestive tract after 5 min of exposure, while the smaller particles (of 2, 5, 6 μm in size) take 15 min following exposure (Gonçalves et al., 2019). Different size exposures (ranging from 2-22 μm) result in a higher proportion of smaller-sized microplastics in digestive gland, with no microplastics >8 μm detected (Fernández and Albentosa, 2019a). In addition, Browne

et al. (2008) observed that smaller (3.0 μm) microspheres could translocate from the gut cavity to the hemolymph more quickly than larger ones (9.6 μm). Different accumulation characteristics of microplastics in gills, rectum and siphons of mussels have also been demonstrated (Merzel et al., 2019). In fact, mussels maybe have a selectivity to other features of microplastics. Kolandhasamy et al. (2018) show that fibers, fragments, sheets, and spheres account for various proportions among different field mussel tissues, which implys mussels' selectivity to the shape of microplastics during translocation period. However, current laboratory studies still lack attention on other features except size. Furthermore, limited tissue types are analyzed in many studies, leading to data gaps.

2.3 Microplastics in pseudofeces and feces—elimination

Accumulation of microplastics in mussels is a dynamic process with both uptake and elimination occuring at the same time. It is a relatively complex process, where selective translocation exists throughout. However, understanding the mechanism is still in the early stages. Some interesting phenomenons require further testing and interpretation. For example, microplastics in the digestive gland of *M. galloprovincialis* decreased with time during depuration period, while that in gills showed an opposite trend, which implied a transfer of microplastics from other tissues to gills (Fernández and Albentosa, 2019b). In addition, accumulation of polystyrene (PS) beads in the siphons of quagga mussels (*Dreissena rostriformis bugensis*) was observed during both exposure and clearance experiments (Merzel et al., 2019). Microplastics rejected as pseudofeces and excreted as feces will respectively pass through the inhalant siphon and exhalant siphon, where particles are trapped. However, it is still not known how microplastics become trapped there.

Microplastics can be eliminated through pseudofeces or feces (Fernández and

Albentosa, 2019a). If microplastics captured by the gill are discriminated and rejected by mussels, they will be transported to specific sites on the mantle and expelled as pseudofeces (Ward et al., 2019a). Microplastics transported to mid-gut will be mixed with other undigested material and incorporated into fecal material (Ward et al., 2019a). Generally, different elimination paths lead to different retention times in the body (Kinjoet al., 2019). Several studies have used depuration experiments to explore the elimination role of microplastics in mussel (Table 2). Evidence suggests that microplastics can be efficiently ejected from mussels in a short time (Woods et al., 2018; Birnstiel et al., 2019; Fernández and Albentosa, 2019a; Rist et al., 2019a). However, the evidence also suggests that some proportions of the particles are retained in mussel tissues regardless of a long-duration depuration (Browne et al., 2008; Paul-Pont et al., 2016; Kinjo et al., 2019; Merzel et al., 2019). For example, microplastics (3, 9.6 μm microspheres) were still retained in the haemolymph of mussels (*M. edulis*) 48 days after exposure (Browne et al., 2008).

The selective elimination observed is reflected in multiple aspects (Table 2). Firstly, the most intuitive embodiment is the difference of microplastics in excretion and mussel body. The characteristics of microplastics in excretion differ from those in mussels. This is because mussel selectively eliminates part of microplastics in its body. For example, Zhao et al. (2019) reports that the size of microplastics in pseudofeces, feces, digestive glands and gut differ considerably. Larger microplastics were detected in feces of field *M. edulis* (15-500 μm) compared with those in the soft tissue (20-90 μm, Van Cauwenberghe et al., 2015). Secondly, the different retention times of various microplastics reflect particle selection. For example, Van Cauwenberghe et al. (2015) found that small-sized particles were more easily retained within mussels after 24 h gut clearance. This is supported by Fernández and

Albentosa (2019b), whereby the percentage of microspheres rejected in pseudofeces by mussels increased with size. The same trend has not, however, been observed for fibers (Ward et al., 2019b). In addition, spheres and fibers egested in feces also showed different change trend with varying size (Ward et al., 2019b). In another study, blue fibers were apparently more effectively eliminated from *Perna perna* than other types after depuration (Birnstiel et al., 2019). Finally, the tissue-specific change in number of microplastics during the depuration process shows particle selection as well. Microplastics in different tissues are likely to be eliminated with different efficency. If the mussels' digestive tract is subdivided into three main regions, only microplastics in the conjoined style sac and midgut (R2) can be eliminated compeletely after 7 days' depuration compared with separated midgut (R3) and rectum (R4, Gonçalves et al., 2019). In addition, reduced microplastics in digestive gland, gill and other soft tissue during depuration differ significantly (Woods et al., 2018; Wang et al., 2021).

Most of laboratory studies on selective elimination of microplastics just used spheres or particles, only 2/12 used fibers or fragments as well (Table 2). However, there are difference in elimination rule between spheres and fibers (Dimitrijevic, 2018; Ward et al., 2019b). More research on other shapes (fragments, fibers, films) may provide new findings in the future. Although the size of exposed microplastics from all studies ranges widely from 70 nm to 5 mm, there is always a limited size range used in each study (Table 2). This represents an obstacle to comprehensively reveal how mussels select microplastics to egest from all kinds of microplastics in the real environment or compare results among different studies.

Table 2

3. Factors affecting selective accumulation of microplastics in mussels

Mussels sort particles according to physical, chemical, and nutritional properties (Zhao et al., 2019; Ward et al., 2019a). It is well established that microplastic is a relatively diverse pollutant and comprises a complex mixture of particles, which could be categorised by size, shape, colour and so on (Scott et al., 2019). Hence, accumulation of microplastics in mussels demonstrates variety of characteristics. There are twenty-eight studies having shown, or implied that, the selective uptake, translocation or elimination of microplastics in mussels is affected by specific factors (Fig. S1, Table 3). Among which, the influence of size has been the most implicated factor in 19 papers, followed by the exposure concentration of microplastics with 6 corresponding papers. In addition, the shape, color, aggregation and weathering of microplastics and feeding condition of mussels are included in a few studies. Most focus on the influence of one factor (n=21), while fewer (n=7) use a multifactoral approach (Fig. S1).

Table 3

3.1 Size of microplastics

As mentioned above, the size of microplastics is considered to be one of the most important factors in affecting their uptake, translocation and elimination by mussels. This is attributable to the fact that ingestion of particulate matter is limited by the body structure and size. For example, mussel's capture for particles is constrained by size and complexity of the laterofrontal cirri (Ward and Shumway, 2004). Hence, mussels have different capture efficiency for microplastics of varying size. The capture efficiency shows an increasing trend with increasing particle size above 1 μm to a maximum efficiency (near 100%) at the size of 2.5-3.5 μm (Ward et al., 2019a). This optimal size range for efficient capture could have implications for nanoplastics uptake. Theoretically, mussels may not capture nanoplastics efficiently, yet laboratory

studies do report the incidence of nanoplastics in the digestive system and/or hemolymph (Santana et al., 2017; Merzel et al., 2019). One possible explanation is that nanoplastics could be passively ingested along with water flow in spite of the low capture efficiency. One study has also shown different movement patterns of the microplastics through the gills of mussels depending on the size of the PS beads between 1, 2 μm and 200 nm (Merzel et al., 2019). Both microbeads of 1 μm and 2 μm occur in the parallel ciliated grooves of the gills, while beads of 200 nm do not have the same effect (Merzel et al., 2019).

Anatomical constraints also exists in the gill, labial palps, and mouth of mussels, which could reduce the ingestion of particles larger than 100 μm (Ward et al., 2019a). Consequently, many studies report a relatively high proportion of smaller-sized particles ingested by mussels (Browne et al., 2008; Van Cauwenberghe et al., 2015; Khan and Prezant, 2018; Li et al., 2018; Qu et al., 2018; Graham et al., 2019; Scott et al., 2019). Although mussels have a high capture efficiency for the microplastics between 500 and 1000 μm, they tend to reject these particles as pseudofeces. Ward et al. (2019a) propose an upper size limit of particles ingested by mussels in the range of 600–900 μm. However, ingestion of fibers larger than 1000 μm have been demonstrated in another study (Ward et al., 2019b).

In addition, microplastics are sorted in the stomach and the light and small particles will translocate to the digestive gland and then undergo intracellular digestion (Ward et al., 2019a). Since cells are a limited size, there may be a upper limit to the size of particles that could be translocated to digestive diverticula (digestive gland) or to lysosomes and the circulatory system. Recent laboratory studies report that microplastics as large as 45 μm may enter the hemolymph (Franzellitti et al., 2019). Since only microplastics of a specific size range were used

in laboratory exposure, it is unknown if larger microplastics could enter the hemolymph as well. In addition, no information is available about the size range of microplastics in the hemolymph of field mussels due to the methodological limitations. It remains to be elucidated if laboratory and field exposure regimes result in different upper limits of microplastics size in determining uptake. We should pay more attention to the small particles (of the size range less than 1000 μm) in monitoring microplastics from field mussels.

In general, large and heavy microplastics have been observed to directly translocate to the gut and egested as feces without intracellular digestion (Ward et al., 2019a; Fernández and Albentosa, 2019b). Hence, large particles are reported to be eliminated more quickly than small ones in many studies (Wesch et al., 2016; Ward et al., 2019b; Fernández and Albentosa, 2019a, 2019b). In field investigations, mussels should be frozen quickly and avoid depuration prior to digestion, which could prevent preferential elimination of large-sized microplastics. However, Kinjo et al. (2019) found that small PS microspheres (1, 10 μm) could be excreted from mussel digestive tracts more quickly compared with those of 90 μm. The opposite results maybe resulted from other underlying factors except size of microplastic. Furthermore, these conclusions were drawn on the premise that all the microplastics are granulated or spherical (table 2), which does not apply to fibers. The egestion of fibers appears to be unaffected by size difference (Ward et al., 2019b).

To summarise, the size of microplastic may well determine its translocation route and residence time in the mussel. It may therefore be possible to predict the occurrence of selected particles (spheres rather than fibers) in specific organs according to their size range, within the laboratory environment. Extrapolating any such predictions to the environment must consider a more complex dynamic process

including continuous uptake, translocation and elimination. Microplastics with a high uptake proportion does not necessarily to accumulate in the body and vice versa. For example, high density polyethylene (HDPE) particles of 2-4 μm have the lowest uptake proportion among particles of 2-15 μm by mussels after exposure, yet account for the largest proportion in the digestive glands after a 6-day depuration (Fernández and Albentosa, 2019a). Building on size range, other factors, especially shape, must also be considered.

3.2 Shape of microplastics

The shape of microplastics is another important factor that influences particle selection. Diverse classification methods for reporting shape have been adopted and these include: fiber (filament), fragment, sphere (pellet, bead) and film (flake, sheet) (De Witte et al., 2014; Courtene-Jones et al., 2017; Lusher et al., 2017; Bråte et al., 2018; Khoironi et al., 2018; Kolandhasamy et al., 2018; Railo et al., 2018). Fiber refers to microplastic with a slender and elongated appearance; sphere is defined as round microplastic that looks like a ball in shape; film is a very thin layer or piece of large plastic debris; and a fragment is an isolated part of large plastic debris that could not be classified into other types (Li et al., 2016). Among them, fiber and fragment are the most dominant types detected in field mussels, while sphere is used most frequently in laboratory exposure experiments (Burns and Boxall, 2018; Li et al., 2019a).

To date, the size of microplastics has usually been defined by their longest dimension, but this fails to completely reflect their three-dimensional structure, especially for fibers. When particles are transported within mussels, they are likely to be rotated sporadically at different angles, potentially changing their original route and retention time. For instance, is has been speculated that fibers tend to become

trapped resulting in a longer retention time compared with other types, and that is why fibers represent the dominant type in field mussels (Birnstiel et al., 2019; Scott et al., 2019). Evidence has also shown that the shape of particles, especially nanoparticles, has an effect on their bioaccumulation kinetics (Albanese et al., 2012; Ma et al., 2014; Li et al., 2015; Gilbertson et al., 2016). For example, mesoporous silica nanoparticles show decreased liver distribution and urinal excretion with increasing aspect ratios from 1 to 5 (Li et al., 2015).

The majority of published studies have used microplastics of a single shape (which are usually spheres) to conduct exposure and deputation studies, which makes comparison among different shapes problematical. In a recent study, differential rejection and egestion patterns of fibers and spheres in *M. edulis* was demonstrated (Ward et al., 2019b). The proportion of microspheres rejected in pseudofeces was reported as increasing from approximately 10% for the smallest spheres (of 19 μm diameter), to 98% for the largest spheres (1000 μm). For rejected microfibers, these were observed to occur at a comparatively low value regardless of size. In feces, the proportion of microspheres egested was reported as increasing with sphere size from 10% to 50% (Ward et al., 2019b). A similar egestion proportion (50%) for fibers of 75 μm and 587 μm size range was also observed (Ward et al., 2019b). In contrast, a significantly lower egestion value (of 15%) was reported for fibers of 1075 μm in size (Ward et al., 2019b). Endoscopic examinations have shown that both spheres and fibers, captured by mussels, are transported to the ventral grooves of the gill (Ward et al., 2019b). At this point, there is subsequently a difference in that large spheres (e.g., 510 μm diameter) rotate on the frontal surface during ciliary transport, whereas large fibers (587 and 1075 μm length) orientate parallel to the anteriorposterior axis before entering the ventral grooves (Ward et al., 2019b). This phenomenon may explain why

more of the large fibers are observed to be ingested, compared with spheres of a similar size. These findings highlight how the shape of microplastics affects their uptake, translocation and elimination in mussels and merits future study.

3.3 Physicochemical surface properties of microplastics

Physicochemical surface properties of microplastics also have an effect on particle selection under selected conditions and stages. For example, *M. edulis* have a higher capture efficency for 2-3 μm diameter PS microspheres that are not bound with neoglycoprotein (NGP) compared with similar sized, more hydrophobic, microspheres with bound NGP containing D-mannose. In contrast, this rule did not apply to larger microspheres of 4-10 μm, which show similar capture characteristics regardless of coating (Rosa et al., 2017).

In addition to particle capture efficency, surface properties of microplastics also have an influence on their preferential rejection or ingestion by mussels (Ward et al., 2019a). Rosa et al. (2013) reported that *M. edulis* tend to ingest 10 μm microplastics with surfaces of a higher negative charge, or more hydrophobicity, preferentially. Pre-weathered microplastics are ingested by mussels significantly more than virgin microplastics (Bråte et al., 2018), which could be partially explained by the formation of a biofilm under the weathering process. Biofilm formation may change the density and hydrophilicity characteristics of microplastics and generate carbohydrate-enriched coatings (Ward et al., 2019a). Furthermore, some microalgae metabolites may be adsorbed to microspheres (10 μm) and affect selective ingestion of particles by mussels (Ward and Targett, 1989). Different surface properties may lead to different gut retention times and translocation routes (Ward et al., 2019a). However, the information on how surface properties of microplastics affect their postingestive process is still poorly defined. Related to this end, fluorescent particles are widely

used in laboratory exposure regimes, whereby a higher clearance efficiency of fluorescent particles (3-15 μm) in seawater by mussels has been observed compared with that of non-fluorescent particles (Ward and Shumway, 2004). It is possible that the fluorescent material changes the surface properties of microplastics, which, in turn, make the fluorescent particles easier to attach to the surface or internal organs of mussels. In addition, color dyes on the surface of microplastics may be released and enter mussels. The size of fluorescent particles detected in mussels should be analyzed carefully to make sure they are microplastics instead of color dyes. Color dyes wrapped in microplastics could avoid this problem. Hence, the effects of fluorescence should also be taken into account during experimental design and results analysis in future studies.

3.4 Environmental or exposure concentration

A number of field studies attempt to explore the relationship of microplastics concentration in the environment and in mussels. Qu et al. (2018) report a positive and quantitative correlation of microplastics in mussels and their surrounding waters. A similar positive correlation between mussels and sediments has also been shown in another study (Scott et al., 2019). However, many studies do not find a relationship between mussels and environment media because the number of sampling sites is insufficient or the levels of microplastics across all the sampling sites has no significant difference (Li et al., 2018). Current field investigations focus on how environmental concentration of microplastics affects the abundance in the whole mussel body or in selected mussel organs, but little is known about how environmental concentration affects the whole accumulation process, especially the translocation of microplastics in the real environment, which is another possible area for future study.

Some interesting findings about the effect of exposure concentration on uptake, translocation and elimination of microplastics have been recently reported in laboratory exposures. For example, a linear increase of microplastic uptake in mussel larva with increasing exposure concentrations was demonstrated (Capolupo et al., 2018). This is in line with many studies that showed an increased uptake rate of microplastics by adult mussels with increasing exposure concentrations (Woods et al., 2018; Fernández and Albentosa, 2019a; Pedersen et al., 2020).

Other studies have shown that microplastics exposure has no significant influence on the clearance rate (filtration rate) of mussels, defined as the volume of water cleared of suspended particles per unit time (Revel et al., 2019; Rist et al., 2019b). If so, the increase of microplastics uptake rate is proportional to the increase of their exposure concentration under constant clearance rate. The same uptake and elimination proportion of microplastic by mussels and the same change trend with time have been observed under different concentrations (Fernández and Albentosa, 2019a, Rist et al., 2019b). However, the opposite result showed that the clearance rate of mussels was inhibited under high concentrations of microplastic (Harris and Carrington, 2019; Gu et al., 2020). In addition, Woods et al. (2018) showed that the uptake rate of microfibers by mussels initially increased and then remained stable with increasing exposure concentration, which implied a saturation of the uptake rate. The information regarding whether saturation really exists for all microplastics, and if it is involved with inhibited clearance rate, is still unavailable and requires further testing.

Meanwhile in further studies, a higher uptake rate of microplastics by mussels, which was caused by higher exposure concentrations, leading to a higher elimination rate has been reported (Revel et al., 2019; Fernández and Albentosa, 2019a; Pedersen

et al., 2020). In addition, Dimitrijevic (2018) observed differentiated rejection and egestion of microplastics by mussels under low, medium and high exposure concentrations, applied to both fibers and spheres.

Another interesting finding is that the proportions of microplastics in mussel's gills, digestive gland and all other soft tissue differ under different exposure concentrations (Woods et al., 2018), though the underlying mechanism is unknown. In addition, microplastic could not be detected in mussel digestive gland until the highest exposure concentration (of 100 μg/L) was used in one study (Revel et al., 2019). Hence, exposure concentration is an important factor during experimental design when studying the translocation characteristics of microplastics within the organisms.

3.5 Feeding status

The availability of food in a laboratory exposure experiment can cause confounding issues by affecting the test organism's physiological status. The current studies usually provide microalgae cells to mussels as food. Studies have shown that microplastic interacts with microalgae, promoting the formation of hetero-aggregations constituted of microplastics, microalgae and exopolysaccharides (Lagarde et al., 2016; Long et al., 2017). This process can change the density, size and bioavailability of microplastics (Lagarde et al., 2016). Further aggregation of nanoplastics may also enhance uptake by mussels (Ward and Kach, 2009; Wegner et al., 2012).

Both microalgae and microplastics are particulate matter, whereby studies have found that mussels clear both in the water at the same rate (Fernández and Albentosa, 2019a, 2019b). It was suggested that the mussels did not distinguish them efficiently and regarded microplastics as food. The occurrence of either one may affect the uptake of the other. Significantly less microalgae cells (*Rhodomonas salina*) were

filtered by mussels when microplastics were present (Woods et al., 2018). Similarly, Rist et al. (2019a) tested the uptake of microplastics by mussel larvae under different ratios of algae to plastic particles including 0:1, 1:3 and 1:1. The result showed that microplastics co-exposed with algae significantly reduced uptake of microplastics, and the degree of each did not change with increasing concentration (Woods et al., 2018; Rist et al., 2019a).

In addition to uptake, feeding status during exposure period also has an effect on egestion of microplastics. Since sufficient food intake promotes gastrointestinal motility and fecal formation, the presence of food decreased the uptake and increased the elimination of microspheres in the tadpoles (Hu et al., 2016), which does not seem to be the case in mussels. A recent study has reported that food presence prolonged the gut retention time of microplastics in mussels, while it had no effect on ingestion of microplastics (Chae and An, 2020). This result is contrary to previous results and more research is therefore needed. As discussed above, microplastics and microalgae have a similar uptake rate by mussels (Fernández and Albentosa, 2019a, 2019b). However, it is not clear if there are different translocation routes or proportions between the two, relating to differential nutritional value, and/or whether microalgae could then affect translocation of microplastics in mussels. In order to improve such exposure experiments, especially for environmental relevance, mussels caged in the field would be more appropriate.

3.6 Other factors

There are additional potential factors affecting selective accumulation of microplastics in mussels, such as time set (e.g., exposure time, depuration time, sampling time), color of the microplastics, species, the size and life stage of the mussels, and natural variation factors. In laboratory simulations, all results may

change with time. For example, microplastics were not detected in the digestive gland of mussels following a 20 minute exposure, yet they were detected when the exposure time was extended to 21 days (Gonçalves et al., 2019). Hence, the time set involved in exposure, sampling and depuration should be considered in order to achieve the experimental objective.

Many field studies have reported different colors of microplastics in mussels, while few studies allow conclusions on whether mussels select microplastics according to their colors. While selected microplastics of specific colors (e.g. blue, orange) have been reported as most common in mussels (De Witte et al., 2014; Birnstiel et al., 2019; Kazour and Amara, 2020), there is no evidence that mussels select them actively. If it is an active selection process, there are probably other confounding factors with those particles that make them more susceptible of being ingested. More likely, it's because these colors of microplastics represent a higher proportion in the environment. Birnstiel et al. (2019) documented that blue fibers in field mussels could be eliminated more effectively after depuration, which seems to imply a preferential elimination of specific color microplastics. More studies are needed to verify this conclusion.

There are different species in the genus *Mytilus* including *M. edulis, M. trossulus, M. galloprovincialis, M. californianus, M. platensis* and *M. coruscus* (Gaitan-Espitia et al., 2016), which have different genomic composition and gene expression profiles, subsequently leading to differences in how they deal with environmental stress (De Witte et al., 2014). When exposed to microplastics, *M. galloprovinciallis* is able to reduce its clearance rate, which affects the accumulation of microplastics (Alnajar et al., 2021). However, Browne (2008) reported that the ingestion of microplastics did not cause significant changes in *M. edulis*' filter feeding activity. The different

response in feeding behavior will lead to difference in microplastics accumulation among such species. More studies are needed to test and compare how other species respond to microplastic exposure.

Whether the size of mussels affects the accumulation of microplastics is currently under debate. Some researchers conclude that there is no relationship between the abundance of microplastics and a mussel's size or weight (Scott et al., 2019; Webb et al., 2019), while others report that the number of fibers in the mussel is positively correlated with size (Berglund et al., 2019; Ding et al., 2020). Dowarah et al. (2020) show a positive correlation between the microplastics per mussel and the weight of the whole body. All of these studies focus on the number of microplastics. However, particle selection by mussels is reflected in both quality and quantity, adding another variable in determining translocation into different organs and the characteristics (e.g. size, shape).

4. Perspectives and conclusion

4.1 Methodological improvement

Microplastics studies have tended to adopt broadly consistent experimental schemes whereby the uptake, translocation or egestion characteristics in mussels have been explored. During the process of exposure and/or depuration, microplastics are either measured in selected tissues, the whole body, the surrounding seawater, or in excreta, at various, but usually limited numbers of time points. Issues arise in that the characteristics of the microplastics, their concentration, the time/duration of exposure, the depuration time, and the sampling time point and type are often very diverse, which can lead to contradictory conclusions and debate (see section 4.2).

As mentioned above, accumulation of microplastics in mussels is a dynamic

process and, added to this, experimental results may also vary with differing sampling time points. Shortening sampling interval and increasing sampling frequency could contribute to our understanding of how the dynamic process occurs. However, only a limited number of sampling time points are incorporated into the majority of studies conducted. In addition, comprehensive monitoring of all the mussel tissues, the seawater and excreta would ideally be carried out at each sampling time in order to track all the translocation routes of microplastics. Ideally, pseudofeces and feces would also be analyzed separately as they represent different transport routes.

Most of the studies pay much attention to gills, digestive gland and hemolymph, while microplastics could accumulate in other tissues or areas and these require more attention. For instance, a recent study has shown that microplastics can be trapped in mussel siphons, both in the exposure and depuration periods (Merzel et al., 2019). Specifically, fluorescence from the microplastics was detected in the mussel siphons after a 44 day depuration period, yet the feces were no longer fluorescent. Another study found that 15.4% microplastics were retained inside the shell cavity of Pacific oysters (add latin name here), while no microplastic was detected in the soft tissue after 3 days' depuration (Graham et al., 2019). Interestingly, microplastics in mussel larvae increased with prolonged depuration time in a study (Rist et al., 2019a). Measuring microplastics in the surrounding seawater and fecal matter will also help to verify the interpretation that any discharged microplastics are available and have been taken up again during depuration.

The exposure concentration, exposure time, and depuration time are important factors that affect the experimental results. Before deciding a time period, a pre-test to ensure whether the time is long enough, and the concentration is suitable for microplastics to be translocated into specific organs or egested, in order to meet any

study objectives. Gonçalves et al. (2019), for instance, were not able to detect microplastics in the digestive gland of mussels until the exposure time was extended. Similarly, Revel et al. (2019) found that microplastics only occurred in the digestive gland of mussels exposed to the highest concentration. Many studies highlight the quick elimination of microplastics in mussels, while Rist et al. (2019b) showed inefficient egestion of microplastics from mussels within two hours' depuration and suggested a longer depuration time for future study.

Finally, there are instances where the results of different studies have led to contradictory conclusions which need to be considered, and the underlying reasons that have led to this situation, better understood. Since particle selection of mussels exists, differing and opposing accumulation characteristics may occur for different microplastics. Hence, the result is determined according to the features of the microplastics used in the study. In addition, different microplastic analysis methods are widely used, which may contribute to different results. Single histological section observation, for instance, is not sufficient to judge the absence of microplastics in specific tissues. Alongside histological analysis, extraction and quantification of microplastics in mussel tissues are necessary steps to study particle selection of mussels in future studies. A breakthrough in the detection method of microplastics with smaller particle size (1-20 μm) will also contribute to collecting field evidence of particle translocation, especially in the hemolymph. Promisingly, Stamataki et al. (2020) have used a dynamic energy budget model to study the relationship between microplastics accumulation in mussels and characteristics of mussel's surrounding environment, which shows high seasonal fluctuations. This brings a new research approach and more comprehensive models can be envisaged in future studies.

4.2 Conflicting research viewpoints

Contradictory conclusions from the accumulated literature also require highlighting. For example, some report that mussels tend to ingest smaller sized microplastics from the environment (Digka et al., 2018; Li et al., 2018), while others demonstrate that microplastics in seawater, sediments and mussels had similar size (Kazour and Amara, 2020). Some research concludes that larger microplastics are more easily egested by mussels (Van Cauwenberghe et al., 2015; Fernández and Albentosa, 2019b), while others report the opposite (Kinjo et al., 2019). Woods et al. (2018) have demonstrated that microplastics in the gills of mussels were more easily eliminated than in the digestive gland. However, Fernández and Albentosa (2019b) report the opposite viewpoint. In addition, if the abundance of microplastics in mussels has a relationship with the size or weight of mussels is still for debate (Berglund et al., 2019; Scott et al., 2019; Webb et al., 2019). These are only three specific examples of contradictory findings and conclusions, others exist.

Currently, it is difficult to determine conclusions due to the limited number of studies and the differences in experimental conditions. In designing future experimental approaches, a more targeted and comparable experimental design approach would help to solve some of the contradictory conclusions. We suggest the use of a reference material approach, where the uptake/elimination properties have been well characterized in a model species and that is then used in parallel by investigators to calibrate their experiments, allowing confidence in the results and comparisons.

Another controversial area of debate is whether the mussel could serve as bioindicator species of microplastics exposure and impacts. On the one hand, some researchers propose the use of mussels as target species to monitor microplastics due to the positive and quantitative correlation of microplastics in field mussels and in

their surrounding waters (Lusher et al., 2017; Qu et al., 2018; Li et al., 2019a). In addition, similar morphotype and polymeric composition of microplastics have been found in mussels and their surrounding environmental media (Li et al., 2018; Leslie et al., 2017; Qu et al., 2018; Digka et al., 2018; Railo et al., 2018; Cho et al., 2021). Yet others hold the opposite view because mussels show particle selection (Zhao et al., 2019; Dimitrijevic, 2018).

Theoretically, any organism can only ingest a limited size range of microplastics dependent on their physical structure. A bias in microplastics internalization rates and particle selection will presumably exist in all animals, not only mussels. For example, Xiong et al. (2019) have shown that ingestion and egestion of polyethylene by goldfish are also both affected by morphological features of microplastics. The criteria for a bioindicator species of traditional dissolved pollutants does not apply to microplastics completely. A new criterion adapting for particle pollutants including microplastics is needed.

Despite these caveats for proposing mussels as bioindicator species, field caging studies provide a feasible protocol to understand mussels' selective accumulation to microplastics and their indicator function in the natural habitats. Perhaps an important key consideration is how long the mussels should stay deployed to reflect microplastic concentrations in their surrounding environment. Kazour and Amara (2020) kept depurated mussels caged in five different marine coastal areas for six weeks, reporting that the amount and properties of microplastics in transplanted mussels were similar to those in native mussels at the same site. This suggests that six weeks is a suitable deployment time for mussels to reach a steady-state in microplastics bioaccumulation in a certain environment. In addition, a good overlap in polymer type proportions between transplanted mussels and sediments was observed. On the other hand, the abundance and shapes of microplastics detected in the caged mussels did not show a quantitative relationship, or similarity, with those detected in the surrounding seawater or sediments, which reflects a selective accumulation of microplastics in mussels.

Mussel's particle selection will make it difficult to reflect all the microplastics

types in the environment. A thorough understanding of mussels' selectivity to microplastics will help determine the applicable conditions for the use of the mussels as bioindicator species for microplastics. In future studies, classification scheme of microplastics according to mussels' selectivity could be established and adopted. For example, selected microplastics with specific properties are more easily retained in mussels, which could be classified as one category. Once established, it would be possible to further explore the quantitative and qualitative relationship of microplastics belonging to a defined category in mussels and their environment in a more robustly comparative manner.

4.3 Conclusion

Mussel is one of the most commonly used biomonitoring organisms to study transport characteristics and effects of microplastics in organisms. The accumulation of microplastics in mussels is a relatively complex dynamic process with both uptake and elimination at the same time. Many field investigations and laboratory exposure studies have directly or indirectly demonstrated that there is a selectivity during the uptake, translocation and elimination process of microplastics in mussels. At the same time, there are many factors affecting mussel's particle selection in different ways. Although there is a good research foundation for this problem, there are still many research gaps and contradictory viewpoints in this field. In the next few years, more research should focus on the mechanism of how the particle selection occurs in mussel tissues and how various factors affect this selectivity. In addition, the current methodology needs improvement and a more concerted effort to ensure that studies are more targeted and comparable.

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Figure and Table Legends

Figure 1. Translocation of microplastics in mussels. There are various selectivity mechanisms in each step.

Table 1. Studies on selective uptake of microplastics in mussels.

Table 2. Laboratory studies on selective elimination of microplastics in mussels.

Table 3. Studies showing factors that affect selective accumulation of microplastics in mussels.

Supplemental Figure and Table Legends

Fig. S1 Published studies on factors affecting accumulation of microplastics in mussels. A. the number of papers for each factor. B. the multifactorial papers and their study factors.

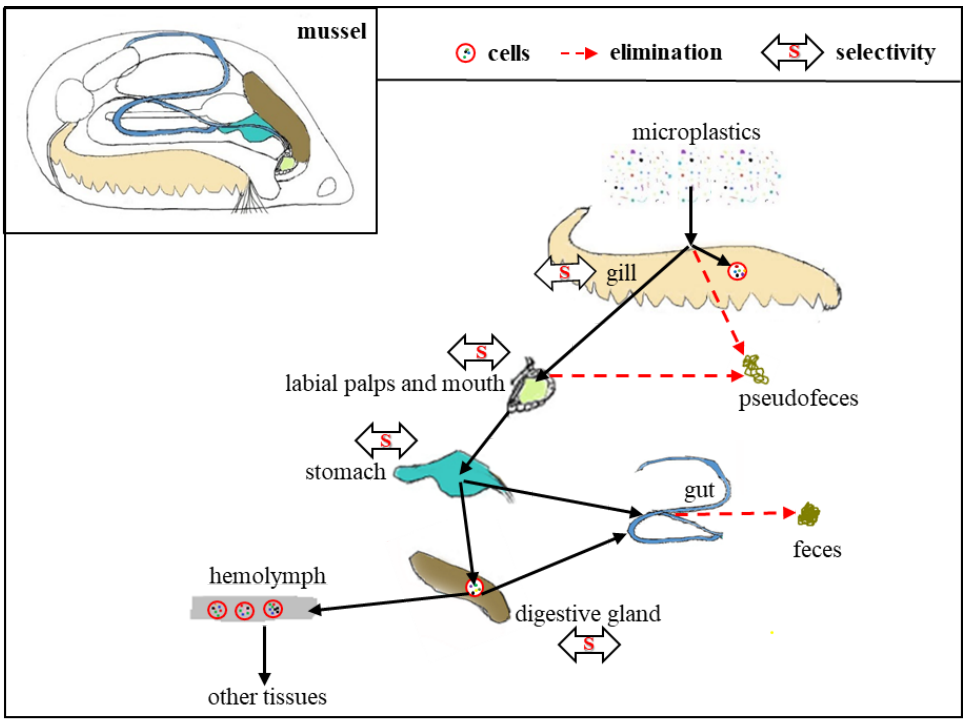

Figure 1. Translocation of microplastics in mussels. There are various selectivity mechanisms in each step.

Table 1 Studies on selective uptake of microplastics in mussels

Experiment type &species	Environmental /exposure media	Selective characteristics	Reference
Field investigation			
Mytilus edulis	seawater	larger percentage of small sized particles (5-250 μm) ingested by mussels (44-83%) compared to media (30-40%)	Li et al., 2018
M. edulis *Perna viridis*	seawater	larger percentage of small sized particles ingested by mussels compared to media (no accurate data available)	Qu et al., 2018
M. galloprovincialis	seawater sediment	larger percentage of small sized particles (<1 mm) ingested by mussels (>90%) compared to media (<70%)	Digka et al., 2018
M. edulis	seawater sediment	smaller sized fragments ingested by mussels (<500 μm) compared to media (about 1000-4000 μm)	Scott et al., 2019
M. edulis	seawater sediment	different color and shape characteristics of microplastics ingested by mussels compared to media	Kazour and Amara, 2020
P. viridis	seawater sediment	only fiber ingested by mussels in the media with both fibers and fragments	Chinfak et al., 2021
Laboratory simulation			
M. edulis	10, 30, 90 μm[a]	only particles of 10 μm in mussels	Van Cauwenberghe et al., 2015
Geukensia demissa	5, 250–300 μm	more particles of 5 μm in mussels	Khan and Prezant, 2018
M. edulis	beads, fragments, fibers	more fibers and beads in mussels	Qu et al., 2018
M. galloprovincialis	weathered particles, virgin particles	more weathered particles in mussels	Bråte et al., 2018
M. edulis	100 nm, 2 μm	more particles of 2 μm in mussels	Rist et al., 2019a
M. edulis	spheres (19, 113, 287, 510, 1000 μm) fibers (75, 587, 1075 μm)	varying uptake percentage with different sizes or shapes	Ward et al., 2019b
M. galloprovincialis	6, 10 μm	different uptake curves for two sizes	Gonçalves et al., 2019
M. coruscus	0.07, 0.5, 5, 10, 100 μm	varying uptake percentage with different sizes	Wang et al., 2021

[a]The sizes or types of microplastics in exposure media.

Table 2 Laboratory studies on selective elimination of microplastics in mussels

Test microplastics			Exposure time	Depuration time	Selective elimination	Reference
Polymer	Shape	Size				
Mytilus edulis						
PS	spheres	10, 30, 90 µm	14 d	24 h	longer RT[a] for smaller particles	Van Cauwenbergheet al., 2015
PS, nylon	spheres, fibers	19-1075 µm	3 h	48 h	varying elimination rate with size for both spheres and fibers	Ward et al., 2019b
PE, PET, ABS	fibers, spheres, fragments	129 µm-5mm, 45-106 µm,	5 h	24 h	differentiated rejection and egestion for fibers and spheres	Dimitrijevic, 2018
PS	particles, beads	100 nm, 10 µm	45 min	72 h	longer RT for nanoparticles	Ward and Kach, 2009
PET	fibers	459 µm	2 h, 9 h 3 h	1 h 9 h	different elimination rate for digestive gland, gill and other soft tissue	Woods et al., 2018
M. galloprovincialis						
HDPE	particles	≤22 µm	4 h	7 d	longer RT for smaller particles	Fernández and Albentosa, 2019b
PS	spheres	2, 5, 6, 10 µm	20, 90 min, 48 h, 21 d	7 d	different RT in different locations of digestive tract	Gonçalves et al., 2019
PS	spheres	1, 10, 90 µm	3 h	40 d	longer RT for larger particles	Kinjo et al., 2019
HDPE	particles	≤20 µm	4 h	6 d	longer RT for smaller particles	Fernández and Albentosa, 2019a
M. coruscus						
no	spheres	0.07, 0.5, 5, 10, 100 µm	87 h	87 h	faster elimination rate for 0.07 µm in digestive tract	Wang et al., 2021
Perna perna						
PVC	spheres	0.1-1 µm	3 h	12 d	longer RT for spheres in hemolymph	Santana et al., 2017
Dreissena rostrformis bugensis						
PS-COOH	beads	200, 1000, 2000 nm	24 h	20-44 d	different gut clearance time for different size	Merzel et al., 2019

Abbreviations: PS, polystyrene; PE, polyethylene; PET, polyethylene terephthalate; ABS, acrylonitrile butadiene styrene; HDPE, high-density polyethylene; PVC, polyvinyl chloride. [a] means retention time in mussels.

Table 3 Studies showing factors that affect selective accumulation of microplastics in bivalves

Species	Factor	Reference
Mytilus spp.	concentration	Revel et al., 2019
M. galloprovincialis	weathering	Bråte et al., 2018
M. galloprovincialis	concentration	Capolupo et al., 2018
M. galloprovincialis	size	Gonçalves et al., 2019
M. galloprovincialis	size	Kinjo et al., 2019
M. galloprovincialis	size	González-Soto et al., 2019
M. galloprovincialis	size, concentration	Fernández and Albentosa, 2019a
M. galloprovincialis	size	Fernández and Albentosa, 2019b

M.coruscus	size	Wang et al., 2021
M. edulis	size	Browne et al., 2008
M. edulis	aggregation, size	Ward and Kach, 2009
M. edulis	feeding	Wegner et al., 2012
M. edulis	color	De Witte et al., 2014
M. edulis	size	Van Cauwenberghe et al., 2015
M. edulis	aggregation	Porter et al., 2018
M. edulis	size	Li et al., 2018
M. edulis	size, concentration, shape	Dimitrijevic, 2018
M. edulis	concentration	Woods et al., 2018
M. edulis	size	Zhao et al., 2018
M. edulis	size, feeding	Rist et al., 2019
M. edulis	size	Scott et al., 2019
M. edulis	size, shape	Ward et al., 2019b
M. edulis *Perna viridis*	size, shape	Qu et al., 2018
P. viridis	size, color	Naidu, 2019
P. perna	color	Birnstiel et al., 2019
Dreissena rostrformis bugensis	size	Merzel et al., 2019
D. bugensis	concentration	Pedersen et al., 2020
Geukensia demissa.	size	Khan and Prezant, 2018

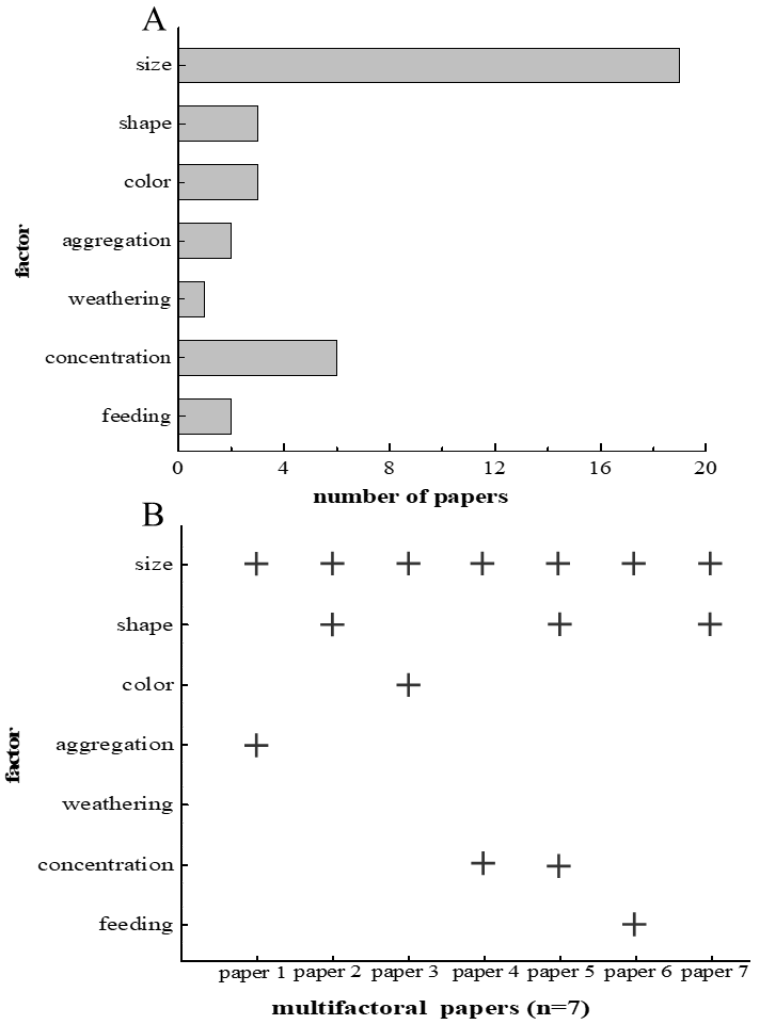

Fig.S1 Published studies on factors affecting accumulation of microplastics in mussels. A. the number of papers for each factor. B. the multifactorial papers and their study factors.