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Marine mussel-based biomarkers as risk indicators to assess oceanic region-specific microplastics impact potential

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ABSTRACT

Microplastics (MPs) pollution in the ocean is an area of growing concern. Marine fauna subject to MPs exposure from various environmental sources are most likely to have detrimental effects on their immune system. However, studies of potential risks of MPs on marine ecosystems in light of environmental concentrations are largely limited. To this end, we presented an approach for assessing potential impact of MPs on marine ecosystems based on marine mussel *Mytilus*-based probabilistic risk assessment framework. The immunotoxic-based biomarkers of *Mytilus* were used to assess the impact of MPs on marine environment appraised with datasets by compiling oceanic region-specific and comprehensive MPs – environment studies along with published toxicity experiments. The immunological effects of MPs on lysosomal destabilization and phagocytosis in hemocytes of *Mytilus* were reconstructed as the concentration–response functions. We assessed the risk for marine environment exceeding a threshold of *Mytilus*-based immunological toxicity based on the benchmark concentration (BMC) approach corresponding to a 10% inhibition effect (BMC_{10}). We estimated BMC_{10} values for inhibitions of lysosomal membrane stability and phagocytosis to be 1.4 and 0.4 mg L^{-1}, respectively. Here we showed that the overall MPs-associated impact potential was low among South Pacific Ocean, Mediterranean Sea, and South Atlantic Ocean. However, we found that MPs from North Pacific Ocean were very likely (> 90% probability) to pose a potential threat to marine mussels. Our findings have important implications for understanding the linked relationships between environmental MPs and likelihood of exposure risk for marine organisms in different oceanic regions around the world. We suggest that *Mytilus*-based risk indicator for estimating risk metrics of essential marine ecosystems posed by environmentally relevant MPs concentrations can help inform practices for the sustainable management and for mitigating the environmental MPs-induced negative impact on marine organisms.

1. Introduction

Worldwide plastic production has dramatically increased in decades and reached 359 million tons in 2018 (PlasticsEurope, 2019). Tremendous mass of mismanaged plastics has been generated with an annual estimate of 1.15–12.7 million metric tons entering the ocean, resulting in an accumulation of plastic wastes in marine (Jambeck et al., 2015; Lebreton et al., 2017). Plastic debris that exist in environments have been degraded into tiny plastic particles referring to as microplastics (MPs). Based on the National Oceanic and Atmospheric Administration (NOAA) marine debris program, MPs are defined as plastic particles with size < 5 mm in diameter (D_p < 5 mm) (Arthur et al., 2009). In the past decade, MPs have been found in vast quantities and become an emerging issue of international concern.

van Sebille et al. (2015) estimated that 15–51 trillion MP particles were afloat at the global ocean in 2014. It is known that prevailing winds and surface current systems can transport buoyant MPs to a long distance (Lusher et al., 2017a; Wichmann et al., 2019). Places where plastic pollutants drawn into a massive vortex turn into six plastic-filled subtropical gyres of the North Pacific and South Pacific Oceans, the North Atlantic and South Atlantic Oceans, the Indian Ocean, as well as the Mediterranean Sea gyres (van Sebille et al., 2015). While the overall accumulation patterns are consistent, MP concentrations show large differences at regional scales due partly to complexity of the ocean dynamics (Law et al., 2014).

The laboratory-based studies indicated that MPs burden was highly likely to pose significant effects on the development, fecundity, and mortality among invertebrates, shellfish, fish, and other organisms (Balbi et al., 2017; Beiras et al., 2019; Naidoo and Glassom, 2019; Sussarellu et al., 2016). However, little has been explored for potential

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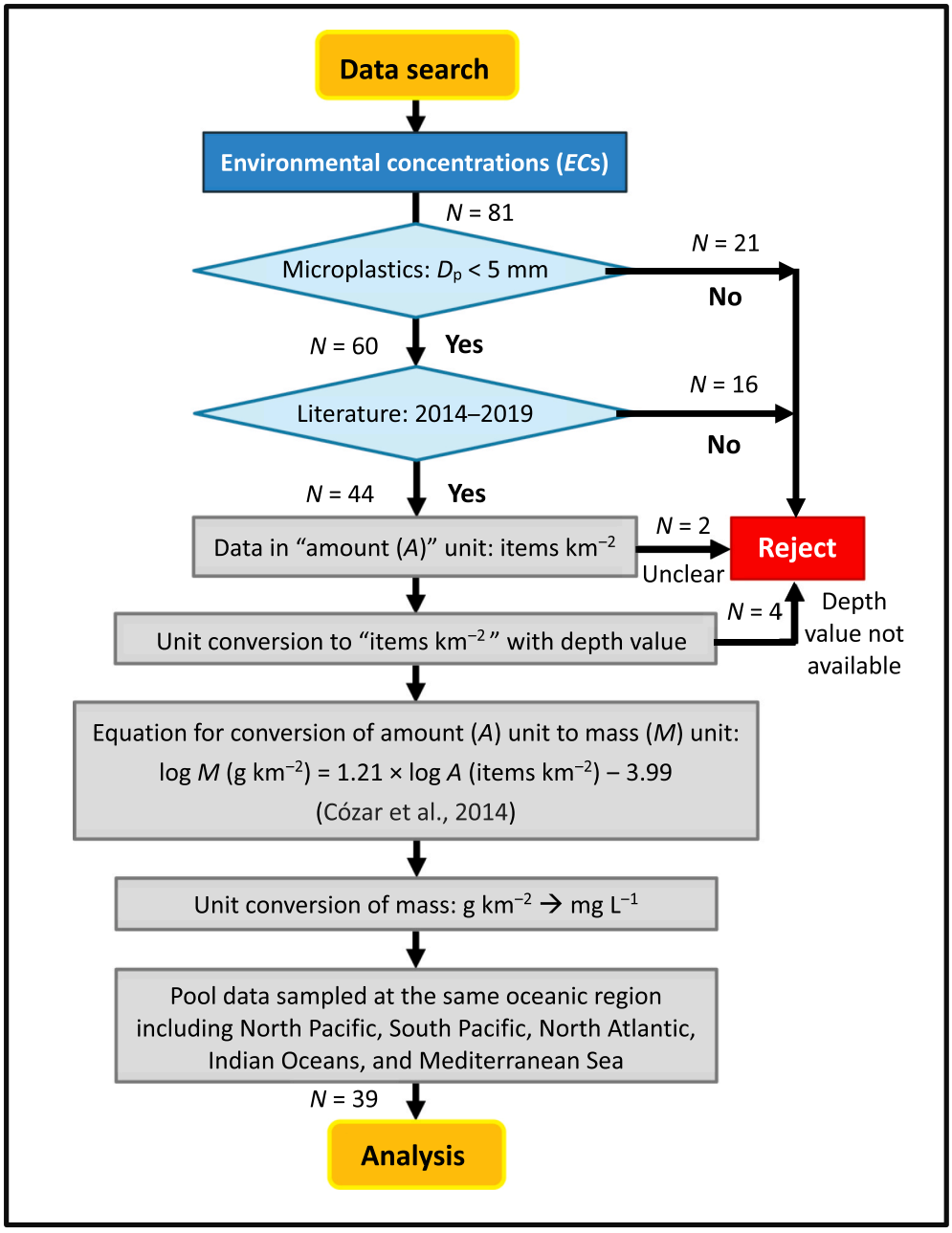

Fig. 1. Schematic showing the algorithm for data selection and treatment of oceanic region-specific microplastics (MPs).

toxicity of MPs based on environmentally relevant concentrations as to address the real exposure risks. To provide a more reliable prediction of risk assessment, employing environmentally realistic data of MPs concentrations is potentially practical to estimate the extent of adverse effects and health risks in marine biota.

By virtue of its widespread geographical distribution, abundance, and fundamental role in the functioning of the marine ecosystem, the mussel is the most commonly preferred biological indicator in field-monitoring and model organism among the experimental studies of MPs (Browne et al., 2008; Green et al., 2019; Li et al., 2019; Salazar and Salazar, 1996). In this study, we employed mussel *Mytilus galloprovincialis* as a model organism. This species is dominant intertidal organism in temperate regions, considered highly invasive all around the globe (CABI, 2020). Field investigations on MPs in mussels evidenced the susceptibility to MPs uptake from surrounding waters (Digka et al., 2018; Li et al., 2015; Sparks, 2020; Wakkaf et al., 2020). Therefore, *M. galloprovincialis* is virtually ideal for being a bioindicator of coastal MPs pollution.

A positive correlation was found between MPs in field-collected mussels and ambient waters (Li et al., 2019; Wakkaf et al., 2020). Their blood cells, hemocytes, represent the backbone of the mussel immune system and are highly responsive to the recognition of microbe-associated molecular patterns (Pipe and Coles, 1995). In recent years, the *Mytilus* cellular immunotoxicity in response to MPs exposure has been broadly reported (Canesi et al., 2015, 2016; Pittura et al., 2018). Thus, there is a strong need for a practical framework to provide an effective tool to rapidly assess the potential impacts of MPs pollution on marine environment. Although excellent progress has been made in specific regions, at the global scale this is not yet feasible.

We therefore derived ecological indicators based on marine mussel biomarkers covering relevant drivers of MPs exposure-associated cellular immunotoxicity. We combined data on the oceanic region-specific and comprehensive MPs – environment studies along with the published toxicity experiments to understand the potential impact posed by

environmental MPs based on marine mussel biomarker-based risk indicator. The purpose of this study was fourfold: (1) to derive the concentration–response functions to obtain *Mytilus*-based biomarkers as risk indicators, (2) to estimate thresholds of MPs appraised with the *Mytilus*-based immunological toxicity data, (3) to estimate exceedance risks and risk quotients of MPs among different oceanic regions worldwide, and (4) to implicate the potential impact of environmental MPs on marine ecosystems.

2. Materials and methods

2.1. Study data

The toxicity experimental data of mussels adopted from Canesi et al. (2015) were used to evaluate the immunological effects in *M. galloprovincialis* induced by polystyrene (PS)-MPs exposures. The PS is a common polymer type MPs debris in the marine environment and mussels (Enders et al., 2015; Kazour and Amara, 2020; Rudduck et al., 2017). In experimental studies, PS is commonly used as a representative MP since it is readily available with uniform size and specific surface properties (Sun et al., 2020; Ward et al., 2019). Despite the use of nano-size primary PS, agglomeration of PS particles with a Z-average of 200.3 nm in solution was indicated a real exposure condition (Canesi et al., 2015). Strictly speaking, aggregated PS were referred to as PS-MPs based on a general definition of nanomaterials in size range of 1–100 nm (European Commission, 2011).

The particle characteristics of 50 nm tested PS-MPs at 50 mg L^{-1} in artificial sea water were adopted to rederive the particle size distribution of PS-MPs. The frequency distribution values in percentage of size were calculated based on the intensity values at specific Z-average extracted from the particle size measurements (Table S1). A four-parameter log-normal (LN) function model was used to fit the experimental data (Table S1) to estimate particle size distribution with a geometric mean (gm) and a geometric standard deviation (gsd).

Given the lack of antigen–antibody-mediated immune responses, the hemocytes are regarded as the main immune cells in marine mussels (Shi et al., 2020). The common endpoints of the immune functional assays for mussels were lysosomal membrane stability (LMS) and phagocytosis. In brief, hemocytes were extracted from mussels and incubated at three PS-MPs concentrations of 1, 5, and 50 mg L^{-1} in artificial sea water (ASW) at 16 °C. Meanwhile, aliquots of hemolymph were incubated with the same PS-MPs concentrations in filtered hemolymph serum for LMS evaluation to mimic *in vivo* condition. The incubation times were 30 and 60 min for LMS and phagocytosis assays, respectively. The short-term *in vitro* effects of PS-MPs were normalized to control group and denoted as inhibition percentage (Table S2).

2.2. Oceanic region-specific microplastics estimation

To reflect the real abundances of MPs that organisms were most likely to expose in marine ecosystems, environmental concentrations of MPs could be obtained from abundantly related literature based on the proposed data search algorithm (Fig. 1). Generally, mussels most live in rocky coasts or intertidal zones in the shallow-water marine domain (Morton, 2019). As suspension-feeders, they are likely to filter pelagic particles and to deposit wastes in surface waters down to 10 m depth (Loo and Rosenberg, 1989; National Research Council, 2010).

Therefore, coastal environments were preferred as potential exposure sites of MPs for mussels rather than those sampled sites far from the shore. MPs abundances reported for surface oceanic waters across the world could be extracted from numbers of published literature and reports in the period 1974–2019. All data were collected by Google Scholar search with keywords of MP, marine, ocean, and surface water.

In response to the keyword queries, there were 81 papers that conducted ocean plastics debris sampling. As taking a holistic view of global MPs contamination without being biased toward specialization, an algorithm was designed subjecting to the following constraints (Fig. 1). The first criterion was that the size range of sampled plastics must meet the definition of MPs ($D_p < 5$ mm), because some studies included MPs counts during sampling. Secondly, literature had to be published in recent years, dated from 2014 to 2019 for the marine system.

Moreover, these extracted data were in various formats and units. Considering the use of selected data in the following risk assessment, environmental concentration expressed as the mean number of items per cubic meter or liter need to be converted into a metric unit of area by multiplying sampled depth. We assumed half of high of MPs-collected trawl net as sampled depth which was not provided in articles. This approach has been commonly used in MPs sampling studies (Eriksen et al., 2018; Gewert et al. 2017). If neither the sampled depth nor the dimension of sampling net could be found in studies, they would be rejected due to the difficulty in unit conversion. Data with unclear information such as a single value of concentrations without uncertainty would also be excluded. For those studies that were unable to meet the constraints would be rejected.

After the screening of 81 papers based on the above criteria, the remaining 39 studies with 41 values quantified as mean and standard deviation in an “amount” unit data need to be converted into a “mass” unit by using a linear regression model adopted from Cózar et al. (2014) as,

$$\log M = 1.21 \times \log A - 3.99, \quad (1)$$

where M is the mass concentration (g km^{-2}) and A is the amount concentration (items km^{-2}) given a depth value (Fig. 1). Finally, a volumetric measurement in “mg L^{-1}” was obtained by using sampled depth to express the unit of environmental concentration. The last process was to pool those concentration data sampling at the same ocean, classified into the North Pacific, South Pacific, North Atlantic, South Atlantic, Indian Oceans, and Mediterranean Sea. The Monte Carlo (MC) simulation was employed to simulate six predicted environmental concentrations (*PEC*s) that would be furtherly incorporated into the risk characterization scheme.

2.3. Effect analysis

To mathematically describe the non-linear concentration–response relationships of inhibitions of LMS and phagocytosis, a pharmacodyamically-based three-parameter Hill model was used,

$$I(C) = (I_{max} \times C^n)/(IC50^n + C^n) \quad (2)$$

where $I(C)$ is the inhibition of LMS or phagocytosis (%) posed by a given exposure concentration (C, mg L^{-1}) of PS-MPs, I_{max} is the maximum inhibition in the presence of PS-MPs (%), $IC50$ is the estimated exposure concentration at which the inhibition is half-maximal response (mg L^{-1}), and n is the Hill coefficient that characterizes the curvature of concentration–response, showing the potency of PS-MPs to the endpoint.

Moreover, to set an acceptable level of PS-MPs for the hemocyte immune function in mussel, the benchmark methodology has been used as an alternative to the no-observed-adverse-effect-level (NOAEL) in toxicological studies (Crump, 1995). The benchmark concentration (BMC) approach involves specifying the benchmark change in effect level. Based on the guidance from U.S. EPA, a level of 10% excess response has been commonly used as the benchmark change (EPA, 2012). Therefore, PS-MPs concentration corresponding to a 10% inhibition effect (BMC_{10}) could be estimated based on fitted parameters in the Hill model.

2.4. Risk characterization

A probabilistic risk model and a risk quotient (*RQ*) model were used

to quantitatively generate the numerical risk estimates. The variability and uncertainty in factors that influence risk were involved in risk estimates that were in terms of the probability and magnitude of immune responses based on a qualitative understanding of *in vitro* cytotoxicity testing. Given the environmental concentration data of MPs in sea water, the immunological effects as biomarkers in hemocytes incubated in ASW would be used for risk assessment. Based on the Bayesian inference, *PEC*s of oceanic region-specific MPs and the Hill-based concentration–response analysis were integrated into exceedance risk (ER) estimations. Therefore, risks of MPs-induced immunotoxicity in mussel ($\Phi(R)$) could be obtained by jointing prior probabilities of *PEC*s and conditional probabilities of $P(I|PEC)$, resulting in a posterior probability as,

$$\Phi(R) = P(PEC) \times P(I\,|PEC) \quad (3)$$

The ER can then be estimated as $1 - \Phi(R)$, describing an exceeding probability of a particular degree of effect at a certain exposure concentration.

Furthermore, we used *RQ*, the most commonly used risk index, to capture impact potential of oceanic regional specific MPs on marine environment. The mussel biomarker-based *RQ* can be written as

$$RQ = PEC/\mathrm{BMC}_{10} \quad (4)$$

where *PEC* and BMC_{10} were treated probabilistically. $RQ > 1$ indicates MPs posing a potential threat to the immune system of mussel, whereas $RQ < 1$ reveals no significant risk.

Fig. 2 summarizes the overall study framework for quantifying the oceanic region-specific potential impacts of MPs on marine environment based on marine *Mytilus* biomarkers-based risk indicators.

2.5. Uncertainty analysis

This study employed the TableCurve 2D (Version 5.01, AISN Software Inc., Mapleton, OR, USA) to simulate size-distribution of PS-MPs and the concentration–response model fittings to the published data adopted from Canesi et al. (2015). The Oracle® Crystal Ball software (Version 11.1, Oracle Corporation, Redwood Shores, CA, USA) was employed to implement the MC simulation performed with 10,000 iterations to sufficiently ensure the uncertainties of simulation results. Percentiles of 2.5th and 97.5th were generated as the 95% confidence intervals (CIs) for all model fittings.

3. Results

3.1. Size distribution

The size distribution of PS-MPs particle measured in Canesi et al. (2015) was well fitted by a LN function ($r^2 = 0.9$, p-value < 0.001) (Fig. 3; Table S1). The gm diameter of PS particles applied in toxicity experiments for mussels was 216.18 nm with a gsd of 2.19, denoted as LN(216.18 nm, 2.19). Based on the size definition of nanomaterials, for 50% or more of the particles in the number size distribution with at least one dimension < 100 nm (European Commission, 2011), results indicated that aggregation of PS-MPs in ASW resulted in micro-scale PS particles rather than primary particle diameter of 50 nm.

3.2. Concentration-effect relationship

The relationships between PS-MP concentrations and inhibitions of LMS and phagocytosis in mussels fitted with a three-parameter Hill model showed a good fitness ($r^2 > 0.9$; p-value < 0.001) (Fig. 4A–C). The effect assessment of PS-MPs incubation obtained in ASW showed that I_{max} estimates of LMS and phagocytosis were 48.0 ± 10.8% (mean ± SE) and 50.6 ± 3.6%, respectively (Fig. 4A,B; Table S3). The fitted *IC*50s of inhibited LMS and phagocytosis were 7.9 ± 5.3 and 1.9 ± 0.4 mg L^{-1}, respectively (Fig. 4A,B; Table S3). Moreover, BMC_{10}s of LMS and phagocytosis inhibitions were 1.4 ± 1.8 and 0.4 ± 0.2 mg L^{-1}, respectively (Table S3). In comparison, inhibition of phagocytosis was more sensitive to PS-MPs exposure than that of LMS, suggesting that PS-MPs with a less concentration were likely to cause the significant reaction.

On the other hand, mean estimates of I_{max} and *IC*50 of LMS inhibition induced by PS-MPs suspension in serum were 67.3% and 1.4 mg L^{-1}, respectively (Fig. 4C; Table S3). In addition, BMC_{10} of LMS in hemocytes with the presence of hemolymph serum was 0.15 ± 0.04 mg L^{-1}, appearing as the lowest BMC_{10} among three assays. All n values were > 1, implicating cooperative inhibitions of both biomarkers to the internalization process of PS-MPs in hemocytes of mussels (Fig. 4A–C; Table S3). Due to limited information on toxicokinetics of PS-MPs in mussels, a simple statistical relationship between PS-MPs concentrations in ASW and serum ($C_{serum} = 0.647ln(C_{ASW}) - 0.313$, $r^2 = 0.88$) may improve our ability to predict consequences of impacts of environmental MPs on mussels (Fig. 4D). A mechanistic relationship of how PS-MPs affect mussels representing as LMS inhibition can be further constructed (Fig. 4E).

3.3. Oceanic region-specific exposure risk estimates

Based on the systematic review and algorithm analysis, there were 41 sites of oceanic samples sorted by six open ocean surface regions (Fig. S1; Table S4). We showed that the North Pacific Ocean covered the most MPs data that were extracted from 12 papers, followed by the Mediterranean Sea (9 papers) and the North Atlantic Ocean (7 papers) (Fig. S1; Table S4). However, oceanic regions in the southern hemisphere were less investigated in that only 4 to 5 studies had provided valuable measurements (Fig. S1; Table S4). We found that three environmental concentrations of MPs in the North Pacific Ocean were higher than both BMCs, potentially contributing to a substantial fraction of ecological impacts (Fig. S1).

Moreover, *PEC*s of MPs in six oceanic regions were predicted via the 10,000 iterative MC simulations with a LN distribution. The values of *PEC*s among oceans were in the following order of the North Pacific, Indian, North Atlantic, South Atlantic Oceans, Mediterranean Sea, and South Pacific Ocean (Fig. 5A). We found that the maximum gm estimate of *PEC* was ~ 70 mg L^{-1} in the North Pacific Ocean, much greater than those in the others, whereas the South Pacific Ocean had the minimum gm estimate of *PEC* of 2.51×10^{-4} mg L^{-1} (Fig. 5Aiii, iv).

Results showed that the patterns of ER curves and their orders in both effects were the same, depending on the slopes and skewness of *PEC* distributions of MPs in different oceanic regions (Fig. 5B). The probabilities were above 50% (ER = 0.5) in that LMS and phagocytosis inhibitions ranged from 45.71 to 2.85×10^{-5} and 50.32–9.52×10^{-5}%, respectively, that the highest and lowest inhibitions were appeared at the North Pacific and South Pacific Oceans, respectively (Table S5).

We further showed that only the North Pacific Ocean had *RQ* exceeding 1 even at the 2.5th percentile, indicating the significant risk of mussel immunity posed by MPs (Fig. 6). Whereas the South Pacific, South Atlantic Oceans, and Mediterranean Sea had *RQ*s far < 1, implicating that MPs were less likely to pose potential risk on mussels. For LMS-based *RQ*, median *RQ*s were in the order of 82.36 for the North Pacific Ocean, 0.81 for the Indian Ocean, 0.30 for the North Atlantic Ocean, 6.55×10^{-3} for the South Atlantic Ocean, 2.28×10^{-3} for the Mediterranean Sea, and 2.88×10^{-4} for the South Pacific Ocean (Fig. 6A; Table S6). A similar fashion of *RQ* rank was also found based on BMC_{10} of phagocytosis inhibition among global oceans with median values of 187.1, 1.87, 0.67, 1.52×10^{-2}, 5.25×10^{-3}, and 6.53×10^{-4}, respectively (Fig. 6B; Table S6).

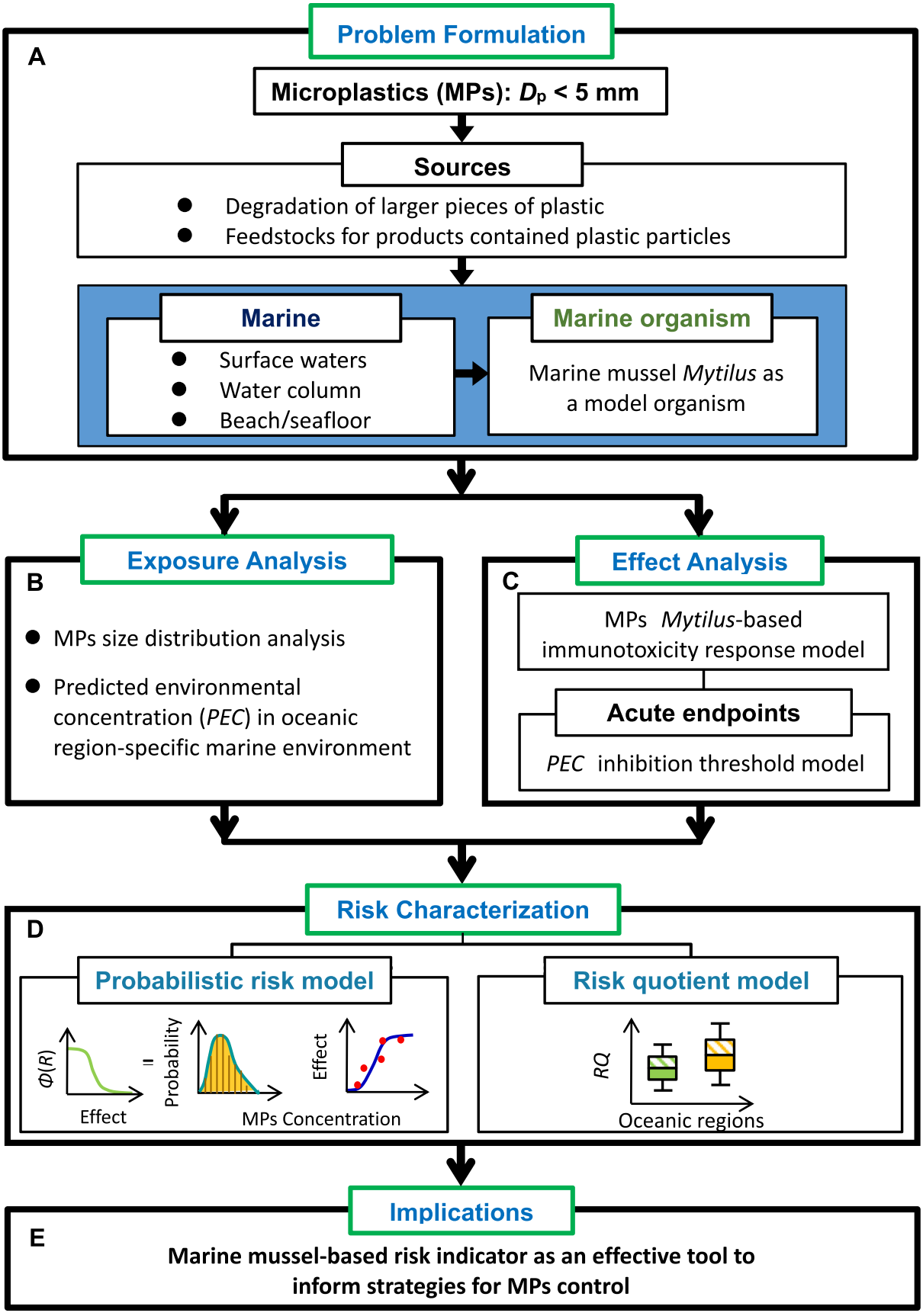

Fig. 2. Schematic showing framework for the oceanic region-specific potential impacts of MPs on marine environment based on *Mytilus* biomarker-based risk indicator.

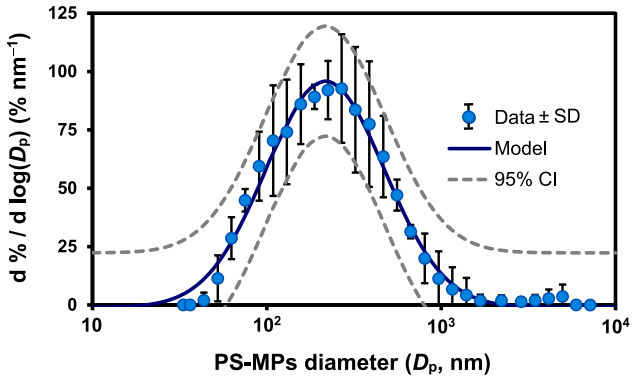

Fig. 3. Reanalyzed fraction of 50 mg L^{-1} polystyrene microplastics (PS-MPs) size distribution with particle diameter (D_p) with a lognormal (LN) distribution.

4. Discussion

4.1. Immunotoxicity in mussels posed by microplastics

In comparison to conventional animal-based approach, hemocyte exposure assays become powerful tools as surrogates for acute toxicity testing, detecting inherent toxicological properties (Figueras et al., 2019). Immunotoxicity is primarily governed by lysosomal destabilization and frustrated phagocytosis, decreasing the ability of animals to defend themselves against exogenous substances (Jovanović and Palić, 2012). Lysosomes, known to enclosure and detoxification of xenobiotics to prevent toxic injury, have been deemed as sensitive organelles toward micro-/nanoplastics (Avio et al., 2015; Pittura et al., 2018). Previous studies evidenced that MPs caused a significant reduction of LMS in mussels within hours (Avio et al., 2015; von Moos et al., 2012). Due to high exposure concentrations of PS-MPs adopted in this *in vitro* assessment of the mussel system, phagocytosis significantly declined in an hour. This is in consistence with the observations of a dose-dependent decrease in phagocytic activity under the same exposure condition (Canesi et al., 2016).

In natural conditions, MPs are bioavailable to mussels via their filter activity and adherence (Kolandhasamy et al., 2018). Uptake MPs are found to be translocated from the gut to the open circulatory system where hemolymph and hemocytes mediate the physiological responses to environmental stress (Browne et al., 2008; Fernández and Albentosa, 2019). An *in vivo* experiment evidenced the uptake, accumulation, and translocation of 3 and 9.6 μm PS-MPs in hemolymph and hemocytes (Browne et al., 2008). Due to the sensitive immune system against non-self-particles and the internalization of MPs by hemocytes, a molecular-level information of mechanism of immune dysfunction induced by MPs is essential for the interpretation of toxicity assessment (Figueras et al., 2019).

In vitro tests with hemocytes are the cornerstone of ecotoxicological studies, providing an in depth understanding of the mode of action to the response and the adaptation of mussels to environmental pollution (Figueras et al., 2019). Evidence showed that PS-MPs in size of 30 μm significantly stimulated total extracellular reactive oxygen species (ROS) production which may damage the membrane and cytoskeleton of hemocytes (Sun et al., 2020). Lysosomal membranes are highly susceptible to the over-production of ROS generated throughout metabolic process (Regoli et al., 2004). In addition, significant increase of the acid phosphatase activity in *Mytilus* exposed to 0.008 and 10 μg L^{-1} indicated the lysosomes overload, resulting in the release of hydrolases in extracellular environment (Revel et al., 2019). Hence, the lower LMS of hemocytes could be reasonably related to an overburden of lysosomes with MPs and the oxidative damage (Jovanović and Palić, 2012).

Moreover, the over-expression of several proteins involved in endosomes maturation, endocytic trafficking, and lysosomal degradation indicated MPs internalization via endolysosomal system (Avio et al., 2015; von Moos et al., 2012). The up-regulation of genes and the inhibition of those coding for lysosomal enzymes showed a coordinated increase modulated by MPs in *M. galloprovincialis* (Avio et al., 2015; Capolupo et al., 2018). Their findings of high variability in immune-related genes could explain the adaptation of mussel immune system to MPs-contaminated environment. Understanding the mode of action of MPs on hemocytes, the *in vitro*-to-*in vivo* correlation in the effect assessment could be enhanced by using toxicokinetic modeling or the application of quantitative structure activity relationships (Bale et al., 2014).

The present work was built on the valuable data from Canesi et al. (2015), providing the mathematical insight on the dynamics of cellular damage in marine mussels exposed to PS-MPs. Our findings implicated that phagocytosis was highly susceptible to PS-MPs than LMS with lower *EC*50 of approximately 4-fold. Smaller MPs are easily phagocytosed by phagocytosis for accommodating more particles (Browne et al., 2008). On the other hand, several notable immunological effects in mussels have been found in the recent MPs studies. Induction of apoptotic processes were responsible for cell-mediated immunity against the PS-MPs exposure (Canesi et al., 2015). Exposure to PE-MPs led to increased abundance of immune-responsive proteins in the haemolymph of mussels (Green et al., 2019). Significant immunological alterations in terms of strong inflammatory responses, formation of granulocytomas, and granulocytes/hyalinocytes ratio were also observed in *Mytilus* (Pittura et al., 2018; von Moos et al., 2012).

4.2. Toxicity thresholds for marine mussels

Generally, the ecotoxicological studies performed toxicity thresholds for test organisms with endpoint values in *EC*10 or *EC*20 directly based on the model fit (Azimonti et al., 2015; Beiras et al., 2019). The no observed effect concentrations (NOECs) of MPs also could be used as effect thresholds (Everaert et al., 2018). However, it is rough to select the critical thresholds from the experiments constrained by limited and discrete doses to responses. In this study, the benchmark modeling in the dose–response assessment was first provided for estimating the effect threshold of MPs concentration for mussels. This scientific and pragmatic approach has already been used to determine a biological threshold in many toxicological studies (Hsieh et al., 2017; Wang et al., 2019a).

A rapid boost of laboratory researches has documented the effects exerted by MPs in mussels. Based on the different experiment scenarios and the biomarker responses, selected critical concentrations of MPs varied among all present systems. Nonetheless, few studies analyzed their toxicity data for further evaluating the dose–response relationships along with deriving threshold values. To date, only one study calculated a 48-h *EC*50 of 0.142 mg L^{-1} on embryo development of *M. galloprovincialis* in response to 0.2 μm PS-MPs, similar to the size of used particles in this work (Balbi et al., 2017).

As for immunological effects in mussels, phagocytosis with a three-fold lower BMC_{10} was more easily inhibited by PS-MPs in medium of ASW than LMS. Notably, changing the exposure medium from ASW to hemolymph serum made BMC_{10} for LMS attenuate approximately a ten-fold decrease. Results of hemocytes incubated in hemolymph serum implicated that PS-MPs would cause more harm to mussels in case of higher bioavailability, although, large commitments of resources will be required to organize, standardize, and validate (Kroll et al., 2009).

4.3. Risk assessment of microplastics in marine ecosystems

As a sessile filter-feeder, mussel effectively reflects ambient environmental pollution and plays a crucial role in early warning for monitoring contaminants (Li et al., 2019). *M. galloprovincialis* has been recorded in Europe, South America, South Africa, China, Japan, California, and Australia whether wild caught or farm raised (Beyer et al., 2017). Our study had scanned all the available scientific literature and

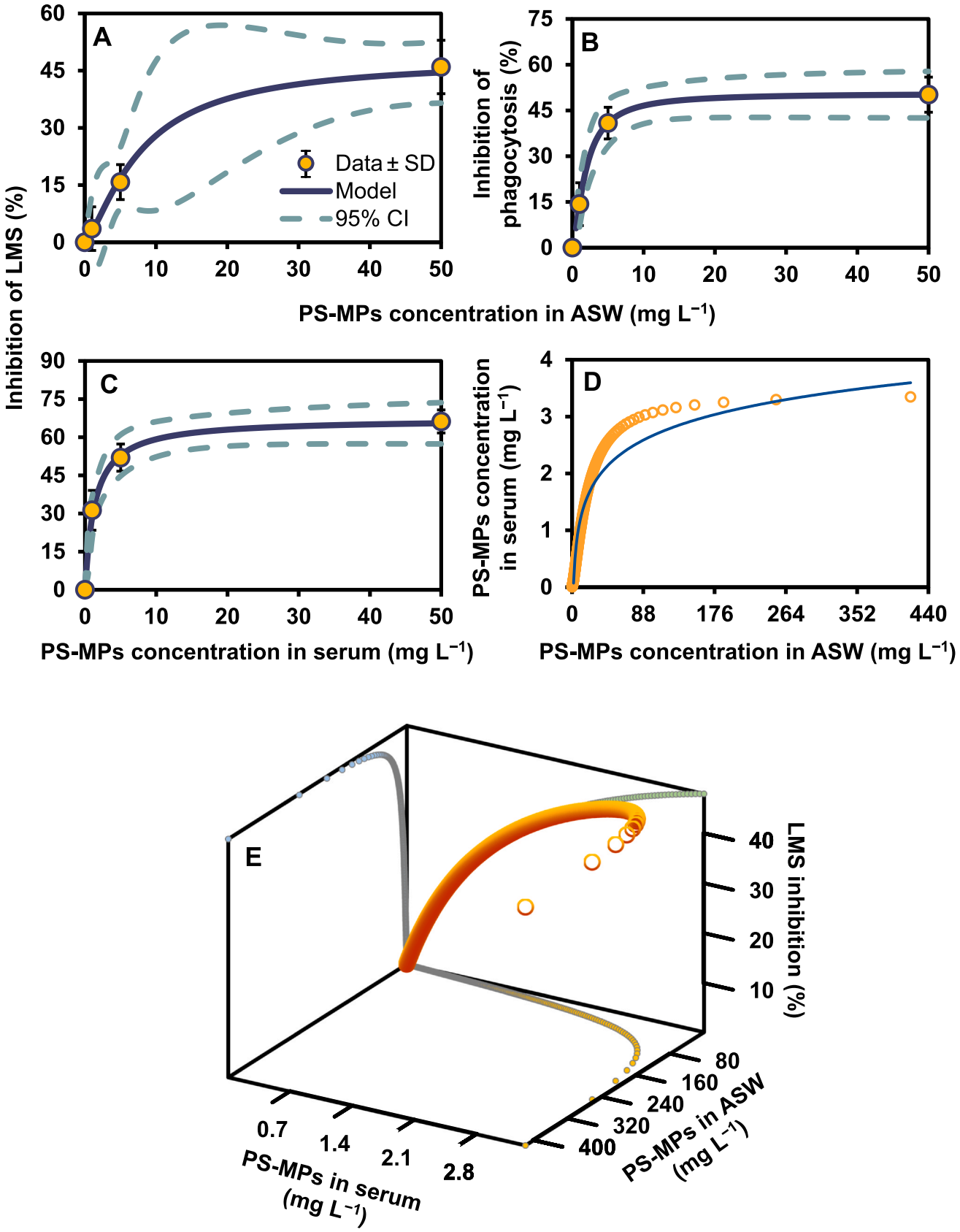

Fig. 4. Constructed the concentration–response profiles for relationships between PS-MP concentrations and inhibitions of (A) lysosomal membrane stability (LMS) and (B) phagocytosis in hemocytes that were incubated in artificial sea water (ASW). (C) The concentration–response relationship between PS-MP concentrations and inhibition of LMS in hemocytes incubated in serum of mussels. (D) Relationship between PS-MPs concentrations in ASW and serum. (E) A 3D scatter plot showing the inhibited LMS response to PS-MPs exposure in artificial sea water (ASW) and serum.

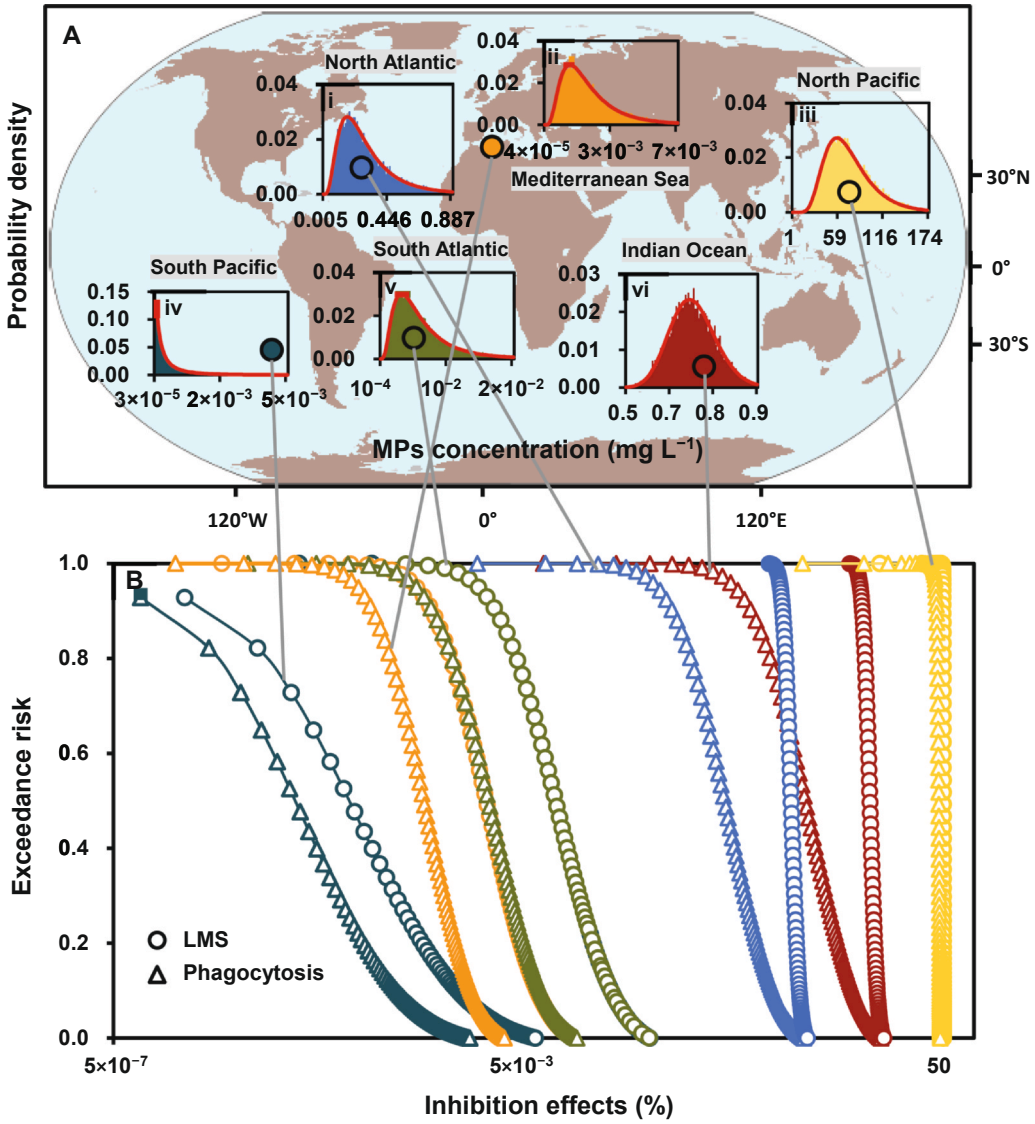

Fig. 5. (A) The lognormal (LN) distributions of MPs concentrations among (i) North Atlantic Ocean: LN(0.25 mg L^{-1}, 1.90), (ii) Mediterranean Sea: LN(1.97 × 10^{-3} mg L^{-1}, 1.88), (iii) North Pacific Ocean: LN(69.8 mg L^{-1}, 1.51), (iv) South Pacific Ocean: LN(2.51 × 10^{-4} mg L^{-1}, 3.93), (v) South Atlantic Ocean: LN(5.65 × 10^{-3} mg L^{-1}, 1.97), and (vi) Indian Ocean: LN(0.73 mg L^{-1}, 1.11). (B) Exceedance risks (ERs) of LMS and phagocytosis inhibitions.

extracted the eligible data for risk assessment at a global scale. We found that MPs from North Pacific Ocean were very likely (> 90% probability) to pose a potential threat to marine mussels. Meanwhile, the North Pacific Ocean has the maximal data whether prior or post algorithm-based selection. Interestingly, most of them were sampled near East Asia including Japan, Korea, and China (Isobe, 2016; Song et al., 2015; Wang et al., 2019b).

There are some literature indicating severe MPs contamination occurred in the North Pacific Ocean. Boucher and Friot (2017) reported that the total losses of MPs from China and East Asia were 462 kilotons that account for over 30% global emission. These densely populated land-based sources are mainly around the North Pacific Ocean known as the Great Pacific Garbage Patch. Among the major delivery channels, the Taiwan Strait is the most important pathway of the export of near-surface floating MPs from the East China Sea (Zhang et al., 2020). MPs account for 8% of the total mass, whereas 94% of the estimated 1.8 trillion pieces are floating in this area, potentially contributing a large fraction of MPs as a threat to biota (De Wolff, 2014; Lebreton et al., 2018). Another study also estimated a weight of 21,290 metric tons of floating MPs in the North Pacific Ocean (Law et al., 2014).

Contrastingly, MPs in the South Pacific and South Atlantic Oceans showed no significant risks to the immune system of mussels based on the present risk estimates. It is likely due to relatively lower MPs release in South America and Africa (Boucher and Friot, 2017). Eriksen et al. (2014) also suggested the same order of MPs weight in the world surface oceans, with the highest and lowest occurred in the North Pacific and South Pacific Oceans, respectively. However, limited information of MPs concentrations in the Indian and South Atlantic Oceans is a potential reason for certain reservations on health risks for marine organisms.

The model organism used in this study has commonly named Mediterranean mussel due to its native habitat. The Mediterranean Sea has the second most environmental concentration data with values most in the range of $10^{-3}-10^{-4}$ mg L^{-1} based on our *PEC* dataset. Therefore, no significant risk of MPs was presented. However, coastal species and those with larger home ranges were more at risk posed by plastic pollution than open-sea species and local ones in the Mediterranean Sea (Compa et al., 2019).

As we scrutinized the 41 data from each study, only five mass concentrations from Geoje and Jinhae Bays of Korea, Coastal British Columbia, North Atlantic Ocean, and the south-eastern coastline of South Africa were higher than threshold values of MPs for mussel

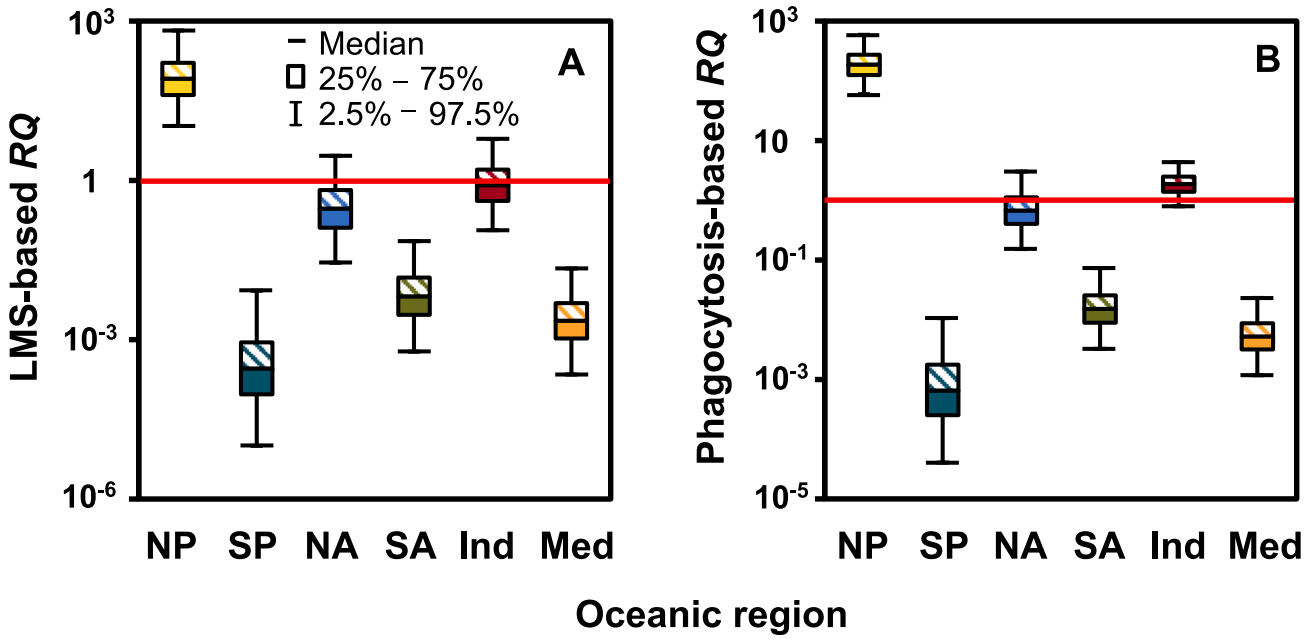

Fig. 6. Box and whisker plots of risk quotient (*RQ*) distributions in the criteria of (A) LMS and (B) phagocytosis-based threshold for inhibition in hemocytes of mussels exposed to *PECs* of MPs at different oceanic regions (NP: North Pacific Ocean; SP: South Pacific Ocean; NA: North Atlantic Ocean; SA: South Atlantic Ocean; Ind: Indian Ocean; and Med: Mediterranean Sea). The red line indicates $RQ = 1$.

immunotoxicology (Desforges et al., 2014; Nel and Froneman, 2015; Poulain et al., 2019; Song et al., 2014, 2015). Sources and types of MPs sampled in these areas have much in common. Desforges et al. (2014) and Song et al. (2014, 2015) demonstrated that marine-based sources including fisheries and aquaculture were dominant due partly to high abundance of paint resin particles. Synthetic fibers originated from sewage effluent were also identified with a preponderant percentage of MPs (Desforges et al., 2014; Nel and Froneman, 2015). Otherwise, MPs types collected in the South and Northwestern Pacific Ocean were dominated by fragments (Eriksen et al., 2018; Pan et al., 2019; Rudduck et al., 2017).

Whereas MPs concentrations in other sample locations were far less than the present thresholds, governments and relevant units should not trifle with MPs pollution and impacts on regional biota under no circumstances. A bioassessment showed that 75% of sampled mussels had ingested MPs from estuarine waters in Southern Brazil in which no significant health risk of MPs on mussels presented based on our assessment (Santana et al., 2016). Nevertheless, based on our effect analysis, acute toxicity was induced less than one hour. Once a massive pulse of MPs flowed into ocean, it is highly likely to pose a serious risk to mussel immunity.

4.4. Limitations and implications

The main reason of broadening the scope of risk analysis from PS-MPs to MPs was that few field studies have categorized MPs types from sampled locations, let alone PS-MPs observations. On the other hand, a previous report demonstrated that adverse effects of MPs on marine organisms potentially arise as the physical impacts (GESAMP, 2015). Similar immunological effects were found to different materials of MPs, indicating that responses might be triggered by physical abrasion of the tissue (Avio et al., 2015; Green et al., 2019; von Moos et al., 2012). Our study framework shed light on the potential impact and the estimated risks which could be improved in case more robust *in vivo* testing and environmental concentrations of MPs are available. We therefore strongly suggest that future researches of MPs impact on marine organisms should be required for adopting documented environmental concentrations for risk assessment to yield more realistic estimates.

In this study, the used PS-MPs in adopted animal experiments from Canesi et al. (2015) were all spherical MPs. Many laboratory studies regarding MPs exposure rely solely on pre-produced plastics that are easily purchased; however, these are not representative of the diverse forms currently present in the environment (Lusher et al., 2017b). MPs identified in both seawater and wild *M. galloprovincialis* showed that fragments and fibers were the predominant type (Digka et al., 2018; Sparks, 2020; Wakkaf et al., 2020). The exposure to irregular MPs with sharp surfaces could potentially contribute to exacerbate the adverse effects (von Moos et al., 2012). Therefore, further studies are required to introduce varied shapes of MPs in the toxicological experiments.

Based on our reanalyzed size distribution, the occurrence of agglomeration leading to micro-scale PS particles was confirmed as a real exposure condition. MPs tend to agglomerate due to the physiochemical properties, salinity, and particle size, as well as the collision probability between MPs and natural organic substances (Choi et al, 2020; Summers et al., 2018). Since the degradation of plastic particles during short-term bioassays is highly unlikely, the agglomeration process influences the physical properties of MPs which largely determine the mobility, bioavailability, and toxicity of particles during exposure (Karami, 2017; Ward and Kach, 2009). A study in mussels demonstrated that MP aggregates could enhance uptake and internal exposure (Ward and Kach, 2009).

In light of the exposure risk assessment, it is essential to acquire size and number of MPs per unit volume of water taken in by organisms (Syberg et al., 2015). Particle size of MPs found in wild *M. galloprovincialis* varied from 5 to 5000 μm which were corresponding to the size of environmental MPs in our *PEC* dataset (Li et al., 2019). However, among all selected *PECs* in aquatic systems, lower size limits of sampled MPs fixed by mesh size cutoff of the plankton nets were larger than those of modeled MPs in size of 0.2 μm in the present study. We found that the smallest MPs among 39 studies was 7 μm in the Northest Atlantic Ocean (Enders et al., 2015). On the other hand, the most dominant size ranges of MPs were found predominantly in the interval of smallest particle size. In addition, MPs abundance showed a power law negative relationship with net mesh size regardless of sampling region and time (Lindeque et al., 2020; Shim et al., 2018). Currently, it is difficult to reflect the real abundance of MPs in environments due

partly to their small size and lack of standard methods for sample collection, characterization, and quantification.

Furthermore, the abundance and distribution of MPs in marine environment are believed to be driven by peculiar morphodynamic variability and oceanographic features, such as prevailing surface currents and winds (Isobe et al., 2017; Rudduck et al., 2017). A complex seasonal pattern of MPs concentration was exhibited along coastlines of the Indian Ocean and the Red Sea (Martin et al., 2019; Veerasingam et al., 2016). Hence, implementation of recording surveillance data is of great importance in improving risk assessment of MPs. Given that the spatiotemporal variation and other environmental stressors are still difficult to be dealt with, more field data are needed to establish a robust database of worldwide environmental concentrations of MPs to comprehensively assess the health risks that marine organisms confront with.

A broad evidence has demonstrated bioaccumulations of MPs in marine species and trophic transfer effects in food chains of aquatic ecosystem (Browne et al., 2008; Cole et al., 2011; Farrell and Nelson, 2013; Rochman et al., 2014). It was reported that 0.05–7.2 and 2.1–10.5 items of MPs per gram soft tissue of wild caught and fishery market bought mussels, respectively (Li et al., 2015, 2019). It is expected that annual dietary exposure for European shellfish consumers is likely to reach 11,000 MPs per year (Van Cauwenberghe and Janssen, 2014). Miranda and de Carvalho-Souza (2016) indicated that the potency of MPs transferring through food chain was more likely to pose ecological and health-related risks. In finding MPs in mussels and other seafood, the consumption risks of MPs to human health need further explored (Hossain et al., 2020; Yang et al., 2019).

5. Conclusions

In this study we presented an approach for assessing potential impact of MPs on oceanic-specific marine ecosystems based on a marine mussel *Mytilus*-based probabilistic risk assessment framework. The extremely high exposure risk of MPs in the North Pacific Ocean where scientists showed a great concern evidenced the heavy MPs pollution having the potential to induce the significant toxicity effects on marine organisms. Our findings have important implications for understanding the linked relationships between environmental concentrations and likelihood of exposure risk of marine organisms in different oceanic regions around the world. We suggest that *Mytilus*-based indicator for estimating risk metrics of essential marine ecosystems posed by environmentally relevant MPs concentrations can help inform practices for the sustainable management and for mitigating the environmental MPs-induced negative impact on marine organisms. This work recommends that there is an urgent need to take immediate actions to minimize the detrimental consequences of marine MPs pollution, such as source control and removal management.

Declaration of Competing Interest

None.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2020.106915.

References

Arthur, C., Baker, J., Bamford, H., (Eds.), 2009. Proceedings of the international research workshop on the occurrence, effects, and fate of microplastic marine debris. Sep 9–11, 2008. NOAA Technical Memorandum NOS-OR&R-30.

Avio, C.G., Gorbi, S., Milan, M., Benedetti, M., Fattorini, D., d'Errico, G., Pauletto, M., Bargellon, L., Regoli, F., 2015. Pollutants bioavailability and toxicological risk from microplastics to marine mussels. Environ. Pollut. 198, 211–222. https://doi.org/10.1016/j.envpol.2014.12.021.

Azimonti, G., Galimberti, F., Marchetto, F., Menaballi, L., Ullucci, S., Pellicioli, F., Allessandra, C., Lidia, C., Allesio, I., Angelo, M., de Boer, W., van der Voet, H., 2015. Comparison of NOEC values to EC10/EC20 values, including confidence intervals, in aquatic and terrestrial ecotoxicological risk assessment. EFSA Support. Publ. 12 (12), 906E. https://doi.org/10.2903/sp.efsa.2015.EN-906.

Bale, A.S., Kenyon, E., Flynn, T.J., Lipscomb, J.C., Nedrick, D.L., Hartung, T., Patton, G.W., 2014. Correlating *in vitro* data to *in vivo* findings for risk assessment. ALTEX-Altern. Anim. Ex. 31 (1), 79–90. https://doi.org/10.14573/altex.1310011.

Balbi, T., Camisassi, G., Montagna, M., Fabbri, R., Franzellitti, S., Carbone, C., Dawson, K., Canesi, L., 2017. Impact of cationic polystyrene nanoparticles (PS-NH_2) on early embryo development of *Mytilus galloprovincialis*: Effects on shell formation. Chemosphere 186, 1–9. https://doi.org/10.1016/j.chemosphere.2017.07.120.

Beiras, R., Tato, T., López-Ibáñez, S., 2019. A two-tier standard method to test the toxicity of microplastics in marine water using *Paracentrotus lividus* and *Acartia clausi* larvae. Environ. Toxicol. Chem. 38 (3), 630–637. https://doi.org/10.1002/etc.4326.

Beyer, J., Green, N.W., Brooks, S., Allan, I.J., Ruus, A., Gomes, T., Schøyen, M., 2017. Blue mussels (*Mytilus edulis* spp.) as sentinel organisms in coastal pollution monitoring: a review. Mar. Environ. Res. 130, 338–365. https://doi.org/10.1016/j.marenvres.2017.07.024.

Boucher, J., Friot, D., 2017. Primary microplastics in the oceans: A Global Evaluation of Sources. International Union for Conservation of Nature and Natural Resources (IUCN), Gland, Switzerland, pp. 43p.

Browne, M.A., Dissanayake, A., Galloway, T.S., Lowe, D.M., Thompson, R.C., 2008. Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). Environ. Sci. Technol. 42 (13), 5026–5031. https://doi.org/10.1021/es800249a.

CABI, 2020. *Mytilus galloprovincialis* [original text by Vicki Bonham]. Invasive Species Compendium. CAB International, Wallingford, UK.

Canesi, L., Ciacci, C., Bergami, E., Monopoli, M.P., Dawson, K.A., Papa, S., Canonico, B., Corsi, I., 2015. Evidence for immunomodulation and apoptotic processes induced by cationic polystyrene nanoparticles in the hemocytes of the marine bivalve *Mytilus*. Mar. Environ. Res. 111, 34–40. https://doi.org/10.1016/j.marenvres.2015.06.008.

Canesi, L., Ciacci, C., Fabbri, R., Balbi, T., Salis, A., Damonte, G., Cortese, K., Caratto, V., Monopoli, M.P., Dawson, K., Bergami, E., Corsi, I., 2016. Interactions of cationic polystyrene nanoparticles with marine bivalve hemocytes in a physiological environment: role of soluble hemolymph proteins. Environ. Res. 150, 73–81. https://doi.org/10.1016/j.envres.2016.05.045.

Capolupo, M., Franzellitti, S., Valbonesi, P., Lanzas, C.S., Fabbri, E., 2018. Uptake and transcriptional effects of polystyrene microplastics in larval stages of the Mediterranean mussel *Mytilus galloprovincialis*. Environ. Pollut. 241, 1038–1047. https://doi.org/10.1016/j.envpol.2018.06.035.

Choi, J.S., Hong, S.H., Park, J.W., 2020. Evaluation of microplastic toxicity in accordance with different sizes and exposure times in the marine copepod *Tigriopus japonicus*. Mar. Environ. Res. 153, 104838. https://doi.org/10.1016/j.marenvres.2019.104838.

Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. Mar. Pollut. Bull. 62 (12), 2588–2597. https://doi.org/10.1016/j.marpolbul.2011.09.025.

Compa, M., Alomar, C., Wilcox, C., van Sebille, E., Lebreton, L., Hardesty, B.D., Deudero, S., 2019. Risk assessment of plastic pollution on marine diversity in the Mediterranean Sea. Sci. Total Environ. 678, 188–196. https://doi.org/10.1016/j.scitotenv.2019.04.355.

Cózar, A., Echevarría, F., González-Gordillo, J.I., Irigoien, X., Úbeda, B., Hernández-León, S., Palmae, Á.T., Navarrof, S., García-de-Lomasa, J., Ruizg, A., Fernández-de-Puelles, M.L., Duarte, C.M., 2014. Plastic debris in the open ocean. Proc. Natl. Acad. Sci. 111 (28), 10239–10244. https://doi.org/10.1073/pnas.1314705111.

Crump, K.S., 1995. Calculation of benchmark doses from continuous data. Risk Anal. 15 (1), 79–89. https://doi.org/10.1111/j.1539-6924.1995.tb00095.x.

De Wolff, K., 2014. Gyre Plastic: Science, Circulation and the Matter of the Great Pacific Garbage Patch. University of California, San Diego. ProQuest ID: DeWolff_ucsd_0033D_14538. Merritt ID: ark:/20775/bb0479603s. Retrieved from https://escholarship.org/uc/item/21w9h64q.

Desforges, J.P.W., Galbraith, M., Dangerfield, N., Ross, P.S., 2014. Widespread distribution of microplastics in subsurface seawater in the NE Pacific Ocean. Mar. Pollut. Bull. 79 (1–2), 94–99. https://doi.org/10.1016/j.marpolbul.2013.12.035.

Digka, N., Tsangaris, C., Torre, M., Anastasopoulou, A., Zeri, C., 2018. Microplastics in mussels and fish from the Northern Ionian Sea. Mar. Pollut. Bull. 135, 30–40. https://doi.org/10.1016/j.marpolbul.2018.06.063.

Enders, K., Lenz, R., Stedmon, C.A., Nielsen, T.G., 2015. Abundance, size and polymer composition of marine microplastics ≥ 10 μm in the Atlantic Ocean and their modelled vertical distribution. Mar. Pollut. Bull. 100 (1), 70–81. https://doi.org/10.1016/j.marpolbul.2015.09.027.

EPA, 2012. Benchmark dose technical guidance. US Environmental Protection Agency. https://www.epa.gov/risk/benchmark-dose-technical-guidance.

Eriksen, M., Lebreton, L.C., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan, P.G., Reisser, J., 2014. Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PloS One 9 (12),

e111913. https://doi.org/10.1371/journal.pone.0111913.
Eriksen, M., Liboiron, M., Kiessling, T., Charron, L., Alling, A., Lebreton, L., Richards, H., Roth, B., Ory, N.C., Hidalgo-Ruz, V., Box, C., Cummins, A., Thiel, M., Meerhoff, E., 2018. Microplastic sampling with the AVANI trawl compared to two neuston trawls in the Bay of Bengal and South Pacific. Environ. Pollut. 232, 430–439. https://doi.org/10.1016/j.envpol.2017.09.058.
European Commission, 2011. Recommendation on the definition of a nanomaterial. Official Journal of the European Union. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011H0696&from=EN (accessed 13 May 2020).
Everaert, G., Van Cauwenberghe, L., De Rijcke, M., Koelmans, A.A., Mees, J., Vandegehuchte, M., Janssen, C.R., 2018. Risk assessment of microplastics in the ocean: Modelling approach and first conclusions. Environ. Pollut. 242, 1930–1938. https://doi.org/10.1016/j.envpol.2018.07.069.
Farrell, P., Nelson, K., 2013. Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). Environ. Pollut. 177, 1–3. https://doi.org/10.1016/j.envpol.2013.01.046.
Fernández, B., Albentosa, M., 2019. Insights into the uptake, elimination and accumulation of microplastics in mussel. Environ. Pollut. 249, 321–329. https://doi.org/10.1016/j.envpol.2019.03.037.
Figueras, A., Moreira, R., Sendra, M., Novoa, B., 2019. Genomics and immunity of the Mediterranean mussel *Mytilus galloprovincialis* in a changing environment. Fish Shellfish Immun. 90, 440–445. https://doi.org/10.1016/j.fsi.2019.04.064.
Gewert, B., Ogonowski, M., Barth, A., MacLeod, M., 2017. Abundance and composition of near surface microplastics and plastic debris in the Stockholm Archipelago. Baltic Sea. Mar. Pollut. Bull. 120 (1–2), 292–302. https://doi.org/10.1016/j.marpolbul.2017.04.062.
GESAMP (Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection)., 2015. Sources, fate and effects of microplastics in the marine environment: a global assessment. In: Kershaw, P.J. (Ed.). (IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection). Rep. Stud. GESAMP No. 90, 96 p.
Green, D.S., Colgan, T.J., Thompson, R.C., Carolan, J.C., 2019. Exposure to microplastics reduces attachment strength and alters the haemolymph proteome of blue mussels (*Mytilus edulis*). Environ. Pollut. 246, 423–434. https://doi.org/10.1016/j.envpol.2018.12.017.
Hsieh, N.H., Chung, S.H., Chen, S.C., Chen, W.Y., Cheng, Y.H., Lin, Y.J., You, S.H., Liao, C.M., 2017. Anemia risk in relation to lead exposure in lead-related manufacturing. BMC Public Health 17 (1), 389. https://doi.org/10.1186/s12889-017-4315-7.
Hossain, M.S., Rahman, M.S., Uddin, M.N., Sharifuzzaman, S.M., Chowdhury, S.R., Sarker, S., Chowdhury, M.S.N., 2020. Microplastic contamination in Penaeid shrimp from the Northern Bay of Bengal. Chemosphere 238, 124688. https://doi.org/10.1016/j.chemosphere.2019.124688.
Isobe, A., 2016. Percentage of microbeads in pelagic microplastics within Japanese coastal waters. Mar. Pollut. Bull. 110 (1), 432–437. https://doi.org/10.1016/j.marpolbul.2016.06.030.
Isobe, A., Uchiyama-Matsumoto, K., Uchida, K., Tokai, T., 2017. Microplastics in the Southern Ocean. Mar. Pollut. Bull. 114 (1), 623–626. https://doi.org/10.1016/j.marpolbul.2016.09.037.
Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science 347 (6223), 768–771. https://doi.org/10.1126/science.1260352.
Jovanović, B., Palić, D., 2012. Immunotoxicology of non-functionalized engineered nanoparticles in aquatic organisms with special emphasis on fish—Review of current knowledge, gap identification, and call for further research. Aquat. Toxicol. 118, 141–151.
Karami, A., 2017. Gaps in aquatic toxicological studies of microplastics. Chemosphere 184, 841–848. https://doi.org/10.1016/j.chemosphere.2017.06.048.
Kazour, M., Amara, R., 2020. Is blue mussel caging an efficient method for monitoring environmental microplastics pollution? Sci. Total Environ. 710, 135649. https://doi.org/10.1016/j.scitotenv.2019.135649.
Kolandhasamy, P., Su, L., Li, J., Qu, X., Jabeen, K., Shi, H., 2018. Adherence of microplastics to soft tissue of mussels: a novel way to uptake microplastics beyond ingestion. Sci. Total Environ. 610, 635–640. https://doi.org/10.1016/j.scitotenv.2017.08.053.
Kroll, A., Pillukat, M.H., Hahn, D., Schnekenburger, J., 2009. Current *in vitro* methods in nanoparticle risk assessment: limitations and challenges. Eur. J. Pharm. Biopharm. 72 (2), 370–377. https://doi.org/10.1016/j.ejpb.2008.08.009.
Law, K.L., Morét-Ferguson, S.E., Goodwin, D.S., Zettler, E.R., DeForce, E., Kukulka, T., Proskurowski, G., 2014. Distribution of surface plastic debris in the eastern Pacific Ocean from an 11-year data set. Environ. Sci. Technol. 48 (9), 4732–4738. https://doi.org/10.1021/es4053076.
Lebreton, L.C., Van Der Zwet, J., Damsteeg, J.W., Slat, B., Andrady, A., Reisser, J., 2017. River plastic emissions to the world's oceans. Nat. Commun. 8, 15611. https://doi.org/10.1038/ncomms15611.
Lebreton, L., Slat, B., Ferrari, F., Sainte-Rose, B., Aitken, J., Marthouse, R., Hajbane, S., Cunsolo, S., Schwarz, A., Levivier, A., Debeljak, P., Maral, H., Schoeneich-Argent, R., Brambini, R., Reisser, J., Noble, K., 2018. Evidence that the Great Pacific Garbage Patch is rapidly accumulating plastic. Sci. Rep. 8 (1), 4666. https://doi.org/10.1038/s41598-018-22939-w.
Li, J., Yang, D., Li, L., Jabeen, K., Shi, H., 2015. Microplastics in commercial bivalves from China. Environ. Pollut. 207, 190–195. https://doi.org/10.1016/j.envpol.2015.09.018.
Li, J., Lusher, A.L., Rotchell, J.M., Deudero, S., Turra, A., Bråte, I.L.N., Sun, C., Hossain, M.S., Li, Q., Kolandhasamy, P., Shi, H., 2019. Using mussel as a global bioindicator of coastal microplastic pollution. Environ. Pollut. 244, 522–533. https://doi.org/10.1016/j.envpol.2018.10.032.
Lindeque, P.K., Cole, M., Coppock, R.L., Lewis, C.N., Miller, R.Z., Watts, A.J.R., Wilson-McNeal, A., Wright, S.L., Galloway, T.S., 2020. Are we underestimating microplastic abundance in the marine environment? A comparison of microplastic capture with nets of different mesh-size. Environ. Pollut. 265, 114721. https://doi.org/10.1016/j.envpol.2020.114721.
Loo, L.O., Rosenberg, R., 1989. Bivalve suspension-feeding dynamics and benthic-pelagic coupling in an eutrophicated marine bay. J. Exp. Mar. Biol. Ecol. 130 (3), 253–276. https://doi.org/10.1016/0022-0981(89)90167-6.
Lusher, A.L., Hollman, P.C.H., Mendoza-Hill, J.J., 2017a. Microplastics in fisheries and aquaculture: status of knowledge on their occurrence and implications for aquatic organisms and food safety. FAO Fisheries and Aquaculture Technical Paper. No. 615. Rome, Italy.
Lusher, A.L., Welden, N.A., Sobral, P., Cole, M., 2017. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. Anal. Methods 9 (9), 1346–1360. https://doi.org/10.1039/C6AY02415G.
Martin, C., Agustí, S., Duarte, C.M., 2019. Seasonality of marine plastic abundance in central Red Sea pelagic waters. Sci. Total Environ. 688, 536–541. https://doi.org/10.1016/j.scitotenv.2019.06.240.
Miranda, D.D.A., de Carvalho-Souza, G.F., 2016. Are we eating plastic-ingesting fish? Mar. Pollut. Bullet. 103 (1–2), 109–114. https://doi.org/10.1016/j.marpolbul.2015.12.035.
Morton, B., 2019. Bivalve. Encyclopædia Britannica, inc. https://www.britannica.com/animal/bivalve (accessed 26 December 2019).
Naidoo, T., Glassom, D., 2019. Decreased growth and survival in small juvenile fish, after chronic exposure to environmentally relevant concentrations of microplastic. Mar. Pollut. Bullet. 145, 254–259. https://doi.org/10.1016/j.marpolbul.2019.02.037.
National Research Council, 2010. Ecosystem Concepts for Sustainable Bivalve Mariculture. The National Academies Press, Washington, DC https://doi.org/10.17226/12802.
Nel, H.A., Froneman, P.W., 2015. A quantitative analysis of microplastic pollution along the south-eastern coastline of South Africa. Mar. Pollut. Bull. 101 (1), 274–279. https://doi.org/10.1016/j.marpolbul.2015.09.043.
Pan, Z., Sun, X., Guo, H., Cai, S., Chen, H., Wang, S., Zhang, Y., Lin, H., Huang, J., 2019. Prevalence of microplastic pollution in the Northwestern Pacific Ocean. Chemosphere 225, 735–744. https://doi.org/10.1016/j.chemosphere.2019.03.076.
Pipe, R.K., Coles, J.A., 1995. Environmental contaminants influencing immunefunction in marine bivalve molluscs. Fish Shellfish Immunol. 5 (8), 581–595. https://doi.org/10.1016/S1050-4648(95)80043-3.
Pittura, L., Avio, C.G., Giuliani, M.E., d'Errico, G., Keiter, S.H., Cormier, B., Gorbi, S., Regoli, F., 2018. Microplastics as vehicles of environmental PAHs to marine organisms: combined chemical and physical hazards to the Mediterranean mussels. Mytilus galloprovincialis. Front. Mar. Sci. 5, 103. https://doi.org/10.3389/fmars.2018.00103.
PlasticsEurope, Plastics – the Facts 2019 http://www.plasticseurope.org 2019 accessed 6 December 2019.
Poulain, M., Mercier, M.J., Brach, L., Martignac, M., Routaboul, C., Perez, E., Desjean, M.C., ter Halle, A., 2019. Small microplastics as a main contributor to plastic mass balance in the North Atlantic subtropical gyre. Environ. Sci. Technol. 53 (3), 1157–1164. https://doi.org/10.1021/acs.est.8b05458.
Regoli, F., Frenzilli, G., Bocchetti, R., Annarumma, F., Scarcelli, V., Fattorini, D., Nigro, M., 2004. Time-course variations of oxyradical metabolism, DNA integrity and lysosomal stability in mussels, *Mytilus galloprovincialis*, during a field translocation experiment. Aquat. Toxicol. 68 (2), 167–178. https://doi.org/10.1016/j.aquatox.2004.03.011.
Revel, M., Lagarde, F., Perrein-Ettajani, H., Bruneau, M., Akcha, F., Sussarellu, R., Rouxel, J., Costil, K., Decottignies, P., Bruno, C., Châtel, A., Mouneyrac, C., 2019. Tissue-specific biomarker responses in the blue mussel *Mytilus* spp. exposed to a mixture of microplastics at environmentally relevant concentrations. Front. Environ. Sci. 7, 33. https://doi.org/10.3389/fenvs.2019.00033.
Rochman, C.M., Kurobe, T., Flores, I., The, S.J., 2014. Early warning signs of endocrine disruption in adult fish from the ingestion of polyethylene with and without sorbed chemical pollutants from the marine environment. Sci. Total Environ. 493, 656–661. https://doi.org/10.1016/j.scitotenv.2014.06.051.
Rudduck, O.A., Lavers, J.L., Fischer, A.M., Stuckenbrock, S., Sharp, P.B., Banati, R.B., 2017. Inter-annual variation in the density of anthropogenic debris in the Tasman Sea. Mar. Pollut. Bull. 124 (1), 51–55. https://doi.org/10.1016/j.marpolbul.2017.07.010.
Salazar, M.H., Salazar, S.M., 1996. Mussels as bioindicators: effects of TBT on survival, bioaccumulation, and growth under natural conditions. In: Champ, M.A., Seligman, P.F. (Eds.), Organotin. Springer, Dordrecht, pp. 305–330.
Santana, M.F.M., Ascer, L.G., Custódio, M.R., Moreira, F.T., Turra, A., 2016. Microplastic contamination in natural mussel beds from a Brazilian urbanized coastal region: rapid evaluation through bioassessment. Mar. Pollut. Bull. 106 (1–2), 183–189. https://doi.org/10.1016/j.marpolbul.2016.02.074.
Shi, W., Han, Y., Sun, S., Tang, Y., Zhou, W., Du, X., Liu, G., 2020. Immunotoxicities of microplastics and sertraline, alone and in combination, to a bivalve species: size-dependent interaction and potential toxication mechanism. J. Hazard. Mater. 396, 122603. https://doi.org/10.1016/j.jhazmat.2020.122603.
Shim, W.J., Hong, S.H., Eo, S., 2018. Marine microplastics: abundance, distribution, and composition. In: Zeng, E.Y. (Ed.), Microplastic Contamination in Aquatic Environments. Elsevier, pp. 1–26.
Song, Y.K., Hong, S.H., Jang, M., Kang, J.H., Kwon, O.Y., Han, G.M., Shim, W.J., 2014. Large accumulation of micro-sized synthetic polymer particles in the sea surface microlayer. Environ. Sci. Technol. 48 (16), 9014–9021. https://doi.org/10.1021/es501757s.
Song, Y.K., Hong, S.H., Jang, M., Han, G.M., Shim, W.J., 2015. Occurrence and

distribution of microplastics in the sea surface microlayer in Jinhae Bay. South Korea. Archives Environ. Contam. Toxicol. 69 (3), 279–287. https://doi.org/10.1007/s00244-015-0209-9.

Sparks, C., 2020. Microplastics in mussels along the coast of Cape Town, South Africa. Bull. Environ. Contam. Toxicol. 104, 423–431. https://doi.org/10.1007/s00128-020-02809-w.

Summers, S., Henry, T., Gutierrez, T., 2018. Agglomeration of nano-and microplastic particles in seawater by autochthonous and de novo-produced sources of exopolymeric substances. Mar. Pollut. Bull. 130, 258–267. https://doi.org/10.1016/j.marpolbul.2018.03.039.

Sun, S., Shi, W., Tang, Y., Han, Y., Du, Z., Zhou, W., Hu, Y., Zhou, C., Liu, G., 2020. Immunotoxicity of petroleum hydrocarbons and microplastics alone or in combination to a bivalve species: Synergic impacts and potential toxication mechanisms. Sci. Total Environ. 728, 138852. https://doi.org/10.1016/j.scitotenv.2020.138852.

Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., Goïc, N.L., Quillien, V., Mingant, C., Epelboin, Y., Corporeau, C., Guyomarch, J., Robbens, J., Paul-Pont, I., Soudant, P., Huvet, A., 2016. Oyster reproduction is affected by exposure to polystyrene microplastics. Proc. Natl. Acad. Sci. 113 (9), 2430–2435. https://doi.org/10.1073/pnas.1519019113.

Syberg, K., Khan, F.R., Selck, H., Palmqvist, A., Banta, G.T., Daley, J., Sano, L., Duhaime, M.B., 2015. Microplastics: addressing ecological risk through lessons learned. Environ. Toxicol. Chem. 34 (5), 945–953. https://doi.org/10.1002/etc.2914.

Van Cauwenberghe, L., Janssen, C.R., 2014. Microplastics in bivalves cultured for human consumption. Environ. Pollut. 193, 65–70. https://doi.org/10.1016/j.envpol.2014.06.010.

van Sebille, E., Wilcox, C., Lebreton, L., Maximenko, N., Hardesty, B.D., van Franeker, J.A., Eriksen, M., Siegel, D., Galgani, F., Law, K.L., 2015. A global inventory of small floating plastic debris. Environ. Res. Lett. 10 (12), 124006. https://doi.org/10.1088/1748-9326/10/12/124006.

Veerasingam, S., Saha, M., Suneel, V., Vethamony, P., Rodrigues, A.C., Bhattacharyya, S., Naik, B.G., 2016. Characteristics, seasonal distribution and surface degradation features of microplastic pellets along the Goa coast, India. Chemosphere 159, 496–505. https://doi.org/10.1016/j.chemosphere.2016.06.056.

von Moos, N., Burkhardt-Holm, P., Köhler, A., 2012. Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. Environ. Sci. Technol. 46 (20), 11327–11335. https://doi.org/10.1021/es302332w.

Wakkaf, T., El Zrelli, R., Kedzierski, M., Balti, R., Shaiek, M., Mansour, L., Tlig-Zouaria, S., Bruzaud, S., Rabaoui, L., 2020. Microplastics in edible mussels from a southern Mediterranean lagoon: Preliminary results on seawater-mussel transfer and implications for environmental protection and seafood safety. Mar. Pollut. Bull. 158, 111355. https://doi.org/10.1016/j.marpolbul.2020.111355.

Wang, X., You, S.H., Lien, K.W., Ling, M.P., 2019a. Using disease-burden method to evaluate the strategies for reduction of aflatoxin exposure in peanuts. Toxicol. Lett. 314, 75–81. https://doi.org/10.1016/j.toxlet.2019.07.006.

Wang, T., Zou, X., Li, B., Yao, Y., Zang, Z., Li, Y., Yu, W., Wang, W., 2019b. Preliminary study of the source apportionment and diversity of microplastics: taking floating microplastics in the South China Sea as an example. Environ. Pollut. 245, 965–974. https://doi.org/10.1016/j.envpol.2018.10.110.

Ward, J.E., Kach, D.J., 2009. Marine aggregates facilitate ingestion of nanoparticles by suspension-feeding bivalves. Mar. Environ. Res. 68, 137–142. https://doi.org/10.1016/j.marenvres.2009.05.002.

Ward, J.E., Rosa, M., Shumway, S.E., 2019. Capture, ingestion, and egestion of microplastics by suspension-feeding bivalves: a 40-year history. Anthropo. Coasts 2 (1), 39–49. https://doi.org/10.1139/anc-2018-0027.

Wichmann, D., Delandmeter, P., van Sebille, E., 2019. Influence of near-surface currents on the global dispersal of marine microplastic. J. Geophys. Res. Oceans 124 (8), 6086–6096. https://doi.org/10.1029/2019JC015328.

Yang, Y.F., Chen, C.Y., Lu, T.H., Liao, C.M., 2019. Toxicity-based toxicokinetic/toxicodynamic assessment for bioaccumulation of polystyrene microplastics in mice. J. Hazard. Mater. 366, 703–713. https://doi.org/10.1016/j.jhazmat.2018.12.048.

Zhang, Z., Wu, H., Peng, G., Xu, P., Li, D., 2020. Coastal ocean dynamics reduce the export of microplastics to the open ocean. Sci. Total Environ. 713, 136634. https://doi.org/10.1016/j.scitotenv.2020.136634.