Department
for Environment
Food & Rural Affairs

Evidence Reviews on Analysis, Prevalence & Impact of Microplastics in Freshwater and Estuarine Environments

Evidence Review 3

What is/are the impact(s) of microplastics on freshwater and estuarine biota?

December 2019

This publication is available at www.gov.uk/government/publications

Any enquiries regarding this publication should be sent to us at

Any enquiries regarding this publication should be sent to us at

defra.helpline@defra.gov.uk

www.gov.uk/defra

This research project was produced for Defra by Dr John Iwan Jones[1], Dr John Francis Murphy[1], Dr Amanda Arnold[1], Dr James Laurence Pretty[1], Prof Kate Spencer[2], Dr Adriaan Albert Markus[3] and Prof Dr Andre Dick Vethaak[3,4].

[1]School of Biological and Chemical Sciences, Queen Mary University of London, London E14NS

[2]School of Geography, Queen Mary University of London, London E14NS

[3]Deltares, Marine and Coastal Systems, Boussinesqweg 1, 2629 HV Delft, the Netherlands

[4]Vrije University Amsterdam, Department of Environment and Health, De Boelelaan 1085, 1081 HV Amsterdam, the Netherlands

Contents

Executive Summary

This Rapid Evidence Assessment used the systematic review procedure to assess the current evidence available on the impact of microplastics on freshwater and estuarine biota. It is important to understand what consequences microplastics may cause in the environment. Furthermore, we need to understand which types of microplastics cause impacts and at what concentrations.

A review was conducted of the primary literature, including grey literature, which reported evidence of the impact of microplastics on freshwater and estuarine biota. A particular focus were those publications which reported evidence on the extent to which microplastics influence the behaviour, feeding, growth, reproduction and survival of freshwater and estuarine biota, and any thresholds at which impacts occurred. Publications released prior to April 2019 were included in this review.

Evidence was acquired according to a predefined set of questions, compiled into a database containing full details of the source and its relevance to the project questions, and the evidence analysed, taking into account reporting biases in the literature, to produce a digestible summary of the evidence base available to answer the main project question and sub-questions, namely,

What is/are the impact(s) of microplastics on freshwater and estuarine biota?

> *a) To what extent do microplastics influence the feeding, growth, reproduction and survival of freshwater and estuarine biota? Do we know trigger levels or threshold values for microplastic impacts on biota?*
> *b) Are any differences between different taxonomic groups observed?*
> *c) Are results from laboratory studies relevant to microplastics at environmentally relevant field concentrations?*
> *d) Are any adverse impacts attributable to the particles or to adsorbed chemicals/microbes on the particles?*
> *e) Is there evidence to suggest impacts on populations of aquatic organisms?*

A set of pre-defined terms were used to search various databases and 2,172 potential evidence sources were identified. Further screening resulted in the identification of 105 unique sources that were used to provide evidence of the impact of microplastics on freshwater and estuarine biota.

The reliability of studies was scored using the CRED (Criteria for reporting and evaluating ecotoxicity data) method. Half of the studies achieved two thirds or less of the available points, indicating that the majority of studies of the impact of microplastics on freshwater and estuarine biota were unreliable in several aspects. Reliability scores indicated that published studies have become less reliable over time. Only studies which achieved reliability scores equal to or better than median reliability scores (corresponding to a total accumulated score of 20 or more out of a possible 30, and 3 zeros or less, out of a

possible 15) were used to derive relationships between the size of particles and ecotoxicological thresholds, to ensure that the relationships were based on valid data.

To what extent do microplastics influence the feeding, growth, reproduction and survival of freshwater and estuarine biota? Do we know trigger levels or threshold values for microplastic impacts on biota?

Threshold values reported from dose-response experiments were compiled and grouped according to ecotoxicological endpoints, namely effects on behaviour, feeding, growth, reproduction and survival. Other endpoints have been considered (metabolism and gene expression), but the link between the measured characteristic and a negative biological consequence for these was less strong. By far the largest number of tests were conducted where the test organisms were exposed to microplastics suspended in water, rather than introduced into the sediment or food. Hence, further analysis focussed on studies which considered waterborne exposure. Various units were used to report the dose of microplastics used in the ecotoxicological tests, making comparison among the different evidence sources more difficult. Furthermore, quantification of the exposure dose in terms of mass per unit volume (or mass), as is typical for most environmental contaminants, is not appropriate for microplastics. Microplastics occur as particles of various sizes and, therefore, vary in mass per particle. Thus, for a given mass the number of particles that organisms are exposed to will vary dependent on their size. From evidence review 1 (ER1) it was apparent also that the concentration of particles found in the environment was related to their size, indicating that environmental exposure concentrations are size dependent. Hence, to enable comparison amongst studies, thresholds expressed as mass of plastic were converted to count of particles.

Where thresholds were identified, the type of threshold values reported varied among the different evidence sources (e.g. Lowest Observed Adverse Effect Level, EC_{50}). As it is not possible to convert between threshold types, all threshold types were considered.

For the ecotoxicological endpoints behaviour, feeding, growth, reproduction and survival there was a clear relationship between the size of the particles used in the test and the threshold at which an effect was seen: the concentration required to cause an impact is related to the size of the particles of microplastic. The relationships between the size of particles used in tests and threshold effect concentrations amongst the five endpoints did not follow the pattern that might be expected assuming that the concentration necessary to illicit a response in an endpoint is positively related to the severity of the endpoint.

Are any differences between different taxonomic groups observed?

Taxonomic coverage of test organisms was limited. However, there were sufficient data to test the influence of taxonomic group used on size-specific thresholds for Crustacea, fish and algae. There was no significant effect of either the endpoint measured or the taxonomic group used, suggesting that there is no difference in sensitivity among different species. However, there was evidence to indicate that taxa are selective in their uptake of microplastics from the environment which may influence the susceptibility of biota to the impact of microplastics.

Are results from laboratory studies relevant to microplastics at environmentally relevant field concentrations?

A limited range of particle types and polymers have been used in ecotoxicological tests. Most tests used virgin, bead shaped particles and, as such, the types of microplastics used in ecotoxicological tests do not reflect the types of microplastics that are found in the environment well. However, there were sufficient data for specific polymers to test the influence of the polymer used on size-specific thresholds: no significant effect of polymer was found.

The range of sizes of plastic particles used in ecotoxicological studies included both micro- and nanoplastic particles. The median size used across all laboratory studies was 10 µm, whereas the median smallest particle size considered by monitoring studies of microplastics in estuaries and freshwaters were 200 and 100 µm respectively, largely constrained by the methods used. Therefore, the size range of particles used in ecotoxicological tests did not compare well with the size range of particles for which concentrations have been quantified in the environment. This mismatch adds uncertainty to our understanding of risk from microplastics. However, the mean concentrations at which effects were seen in ecotoxicological tests were more than 6 orders of magnitude higher than mean concentrations observed in the environment: most tests have been conducted at concentrations of microplastics that are not environmentally relevant.

Are any adverse impacts attributable to the particles or to adsorbed chemicals/microbes on the particles?

The majority of laboratory based ecotoxicological studies used primary (virgin) microplastic particles, such that any effects observed could only be attributable to the particles, and not to any adsorbed chemicals or microbes. However, there was evidence that considered the effect of chemicals adsorbed onto microplastics. The evidence sources reported both positive and negative effects, as well as no consistent dose response. However, due to variety of designs used in studies, it was not possible to draw substantial conclusions with regards the interaction between microplastics and chemicals. No studies considered effects of microbes attached to particles.

The limited data available indicate that the polymer of which virgin microplastic particles are comprised does not have an influence on their toxicity.

Is there evidence to suggest impacts on populations of aquatic organisms?

No studies provided evidence to suggest that microplastics had an impact on populations of aquatic organisms. Currently, there is insufficient evidence (particularly of damage in field populations associated with high concentrations of microplastics) to draw any conclusions regarding the impacts of microplastics on populations of freshwater or estuarine biota.

What is/are the impact(s) of microplastics on freshwater and estuarine biota?

In order to establish a threshold concentration where microplastics present a hazard to a limited number of taxa, quantile regression (based on the 10th percentile) was used to determine the size-specific concentration of microplastics that was lower than 90% of the thresholds identified for survival and, as a more conservative limit, across all the endpoints tested including sublethal effects. By comparing these thresholds with the data on mean concentrations of microplastics reported from field samples, it was apparent that the calculated size specific threshold concentration for lethal effects was considerably higher than 99% of reported environmental concentrations. Lethal effects of microplastics on freshwater and estuarine biota are highly unlikely. Over certain size ranges the calculated size specific threshold concentration for sublethal effects was exceeded by the highest 10% of concentrations reported from environmental samples, suggesting that there may be a possible risk of some sublethal effects in a small proportion of sites. However, there are a number of caveats on this result regarding sublethal effects, in particular the confidence in the size specific lethal threshold was lower in the size range where exceedance occurred.

1 Introduction

1.1 Background

Plastics are synthetic polymers which can be made into a vast range of inexpensive, light-weight and durable products that bring numerous societal benefits by providing important components for a multitude of applications in modern life. Since the 1950s, the plastics industry has grown exponentially to a global usage of 348 million tonnes annum^{-1} in 2017 (Plastics*Europe* 2018). A great variety of polymers and products are encompassed within the term "plastics", some of which will have a long service life, whereas others (around 40% of all the plastic produced) are used for packaging, which is predominantly single use.

It has been discovered that microscopic particles of plastic, microplastics, have been released into the environment (Thompson et al. 2004). Here we use the European Chemical Agency working definition of microplastic as "any polymer, or polymer-containing, solid or semi-solid particle having a maximum size of 5 mm or less in any dimension" (ECHA 2018). Additionally, the definition includes both those microplastics that have been intentionally created (i.e. primary microplastic), and those that are derived from degradation of larger plastic particles (i.e. secondary microplastic). It is estimated that 12 billion tonnes of microplastic will be discarded globally by 2050 (Geyer et al. 2017), with additional particles derived through degradation of larger material, resulting in impacts on biota predicted to cost in excess of $13 billion annum^{-1} (Nizzetto et al. 2016). Microplastics are now ubiquitous and microplastic particles have been reported from throughout the aquatic environment, from surface freshwaters (Hurley et al. 2018) to the deepest and most remote regions of the sea (Ivar do Sul and Costa 2014).

As microplastics are likely to originate from a variety of sources they comprise a variety of different polymer types, including polyethylene (PE), polypropylene (PP), acrylic, polyacrylamide (PAM), polyamide (PA), polyester (PES), polytetrafluoroethylene (PTFE), and polystyrene (PS) amongst others. Yet such microplastics are not naturally occurring. Hence, there is a need to understand how microplastics interact with the biota of freshwaters and estuaries, in order to identify any potential impacts on aquatic organisms and ecosystems. To fully comprehend the risk that microplastics present to freshwater and estuarine environments, it is important to understand what biological impacts manifest, as a consequence of which type of microplastics and at what concentrations. Furthermore, we need to understand if the impacts are a consequence of the microplastic particles themselves or chemicals/microbes associated with the microplastic particles. It is also necessary to establish if any impacts are apparent at the population level.

Within the above wider context, this third evidence review (ER3) is one of three reviews that aim to provide a robust review of the evidence base for informing policy development. This evidence is needed to inform decision making to effectively manage any potential risks stemming from microplastics.

1.2 Objectives

The overarching aim of this evidence review, commissioned by Defra, was to improve our understanding of the impact(s) of microplastics on freshwater and estuarine biota. The evidence available was assessed using the systematic review procedure.

The objectives were to:

1) undertake a Rapid Evidence Assessment for each of the primary research questions,

2) produce a database of evidence.

The objectives of the evidence review were delineated through the following Primary and Secondary questions.

Primary question:

What is/are the impact(s) of microplastics on freshwater and estuarine biota?

Secondary questions:

a) To what extent do microplastics influence the feeding, growth, reproduction and survival of freshwater and estuarine biota? Do we know trigger levels or threshold values for microplastic impacts on biota?

b) Are any differences between different taxonomic groups observed?

c) Are results from laboratory studies relevant to microplastics at environmentally relevant field concentrations?

d) Are any adverse impacts attributable to the particles or to adsorbed chemicals/microbes on the particles?

e) Is there evidence to suggest impacts on populations of aquatic organisms?

2 Methodology

2.1 Review methodology applied

This evidence review is a Rapid Evidence Assessment (REA) which aims "to provide an informed conclusion on the volume and characteristics of an evidence base together with a synthesis of what that evidence indicates following a critical appraisal of that evidence" (Collins et al., 2015). The review followed the methodology outlined in Collins et al. (2015). The primary and secondary questions considered (see Section 1), the Population, Intervention, Comparator and Outcome (PICO) elements (Table 2.1) and search terms to

be used were detailed in a protocol document, which was used to guide the review process. The REA work encompassed two components: a literature review and interviews with academic experts. Details of the approach used for the two REA components are provided in the Sections below.

Table 2.1 REA PICO elements.

PICO element	PICO element for this REA
Population	Microplastics
Intervention	Identification of the types and concentrations of microplastics that cause impacts on the biota found in freshwater and estuarine environments and the nature of those impacts
Comparator	Identification of a lack of impacts of microplastics on the biota found in freshwater and estuarine environments
Outcome	Robust evidence base on the types and concentrations of microplastics that cause impacts on the biota found in freshwater and estuarine environments and the nature of those impacts

2.2 Literature Review

The quality of the literature, including grey literature, which reported investigations into the impacts of microplastics on the biota found in freshwater and estuarine environments was systematically reviewed and assessed, noting in particular those that identified any thresholds in impacts.

2.2.1 Capturing the evidence base

The first step in the evidence reviews on analysis, prevalence & impact of microplastics in freshwater and estuarine environments was to assess the overall evidence base detailing research on microplastics in freshwaters and estuarine (transitional) waters. A wide search using population search terms (Table 2.2) was used at this stage to capture as much of the evidence as possible, with the results of these searches saved and interrogated further to answer each of the three more detailed key questions and their sub-questions from the three evidence reviews on microplastics in freshwaters and estuaries (the third of which is relevant here), thus reducing the effort required to establish the evidence base for each evidence review.

Publications released prior to April 2019 were included in this review. As microplastics have only been studied relatively recently (Thompson et al. 2004), no earliest date was used to define the date range of publications included. An exception on the date range was made to include two works of high relevance to the UK that were released after April 2019, namely Ball et al. 2019 (Sink to River - River to Tap. A review of potential risks from nanoparticles and microplastics. UK Water Industry Research Limited Report No. EQ01A231) and Santillo et al. 2019 (Plastic pollution in UK's rivers: a 'snapshot' survey of

macro- and micro-plastic contamination in surface waters of 13 river systems across England, Wales, Scotland and Northern Ireland. Greenpeace Research Laboratories Technical Report 04-2019).

Table 2.2 Population level search terms used with Boolean operators to identify the population of evidence available on microplastics in freshwaters and estuaries.

Population			
plastic*	freshwater*	wetland	potable
micro*	river*	marsh	reservoir
microplastic	stream*	swamp	aquifer
nanoplastic	brook	wastewater*	groundwater
*plastic	lake*	drinking water	sewage
	pool	aquatic	outfall
	pond	ecosystem*	estuar*
			transitional

The databases used for the searches, which encompassed both published and grey literature, included:

BioOne, COPAC, DART-Europe E-theses Portal, EBSCO Open dissertations, EThOS: Electronic Theses Online Service, European Commission Research Publications, European Sources Online, GoogleScholar, MedLine, JStor, SciFinder, Open Access Theses and Dissertations, OpenGrey, PubMed, PLoS, Scopus, SciFinder, Web of Science.

To capture grey literature, additional to that included in the list of databases to be searched (i.e. databases detailing unpublished theses and reports) undertook directed searches of holdings of relevant environmental regulators (e.g. Rijkswaterstaat (Dutch water authorities): http://www.rws.nl, Vlaamse Milieumaatschappij (Flemish Environmental Agency): http://www.vmm.be Bundesanstalt für Gewässerkunde (German Federal Institute of Hydrology): http://www.bafg.de RIVM (Dutch Environment Agency): http://www.rivm.nl)

The results of all searches were a) downloaded and saved in a searchable database for use in further searches and b) used to map the evidence record.

The overall evidence base on microplastics in freshwaters captured 3456 unique sources. The search engines Scopas, Scifinder and Web of Science produced the most hits. Some of the terms used produced a large number of hits, e.g. the combination micro AND plastic, but a brief inspection revealed that a large proportion of these sources were not relevant, so these terms were only used further in combination with other qualifying terms. Of the retained searches, *microplastic* produced the most hits (total across all engines 11,636).

To capture the evidence base to address the primary and secondary questions of this evidence review, the overall evidence base on microplastics in freshwaters and estuaries captured in the first phase was searched further using search terms specific to the questions of this evidence review (Table 2.3).

The results of all searches were saved for further use and used to map the evidence record. Those evidence sources that were identified by searches for evidence review 1 (ER1) or evidence review 2 (ER2) and scored as potentially relevant to ER3 during the screening process were transferred to an MS Excel spreadsheet formatted with columns corresponding to information fields relevant to the key question and sub-questions being addressed (See Appendix A ER3_Capture.xls) for consideration in this review. The information fields of the evidence capture form included information relevant to

1. The evidence
2. The biota studied,
3. The type of study and design
4. The microplastics considered and any other potential stressors
5. Toxicological endpoints considered,
6. Threshold values,
7. The location of the study

Those evidence sources that were not identified by searches as potentially relevant to ER1 or ER2 were transferred to the evidence capture form, but subject to screening before being included in the evidence review. The evidence base potentially relevant to ER3 identified through the searches was divided among the members of the ER3 review team in such a way that 10% of records were allocated twice (for quality assurance purposes). The reviewers screened the evidence and completed the evidence capture form.

The evidence capture form comprised two steps. The first initial screen of evidence sources not considered for ER1 or ER2 was used to:

a) Identify reviews, which were used for further identification of evidence sources, but not included in data capture *per se*, unless some novel data was presented.
b) Remove evidence sources specific to marine waters and not relevant to freshwaters or estuarine (transitional) waters.
c) Identify evidence sources that were likely to be relevant to ER1 (sampling and analytical methodology) and/or ER2 (sources and fate).
d) Identify evidence sources that were likely to be relevant to ER3.

Of the 2,172 evidence sources identified as potentially relevant, the initial screening identified 423 as likely to be relevant to the question of ER3 (Fig. 1).

Table 2.3 Search terms used to identify the evidence available on the impact of microplastics on the biota found in freshwaters and estuaries.

Population	Intervention	Comparator	Outcome
*invertebrate	contamina*	Lack of impact on biota	reproduct*
fish*	uptake		growth
bird*	sorption		feeding*
crustace*	toxic*		surviv*
population	ecotoxic*		death
threshold	consump*		population
bivalve	filter*		threshold
worm	detritiv*		trigger
diptera*	diet*		
biot*	foodweb		
plankton*	food web		
*plankton			
plankton			
microb*			
*fibre			
*fiber			
*bead			
fragment*			
pellet*			
flake*			
nurdle			
dust			

Those evidence sources that passed the initial screen were searched in detail to capture the evidence relevant to the question and sub-questions, and any relevant information recorded under the appropriate fields on the evidence capture form (Appendix A: ER3_Capture.xls). In particular, numerical information was captured where effects were quantified in the literature (e.g. proportions of microplastics from different sources). These evidence sources were supplemented with sources identified as relevant to the questions of this review through the searches undertaken in ER1 and ER2.

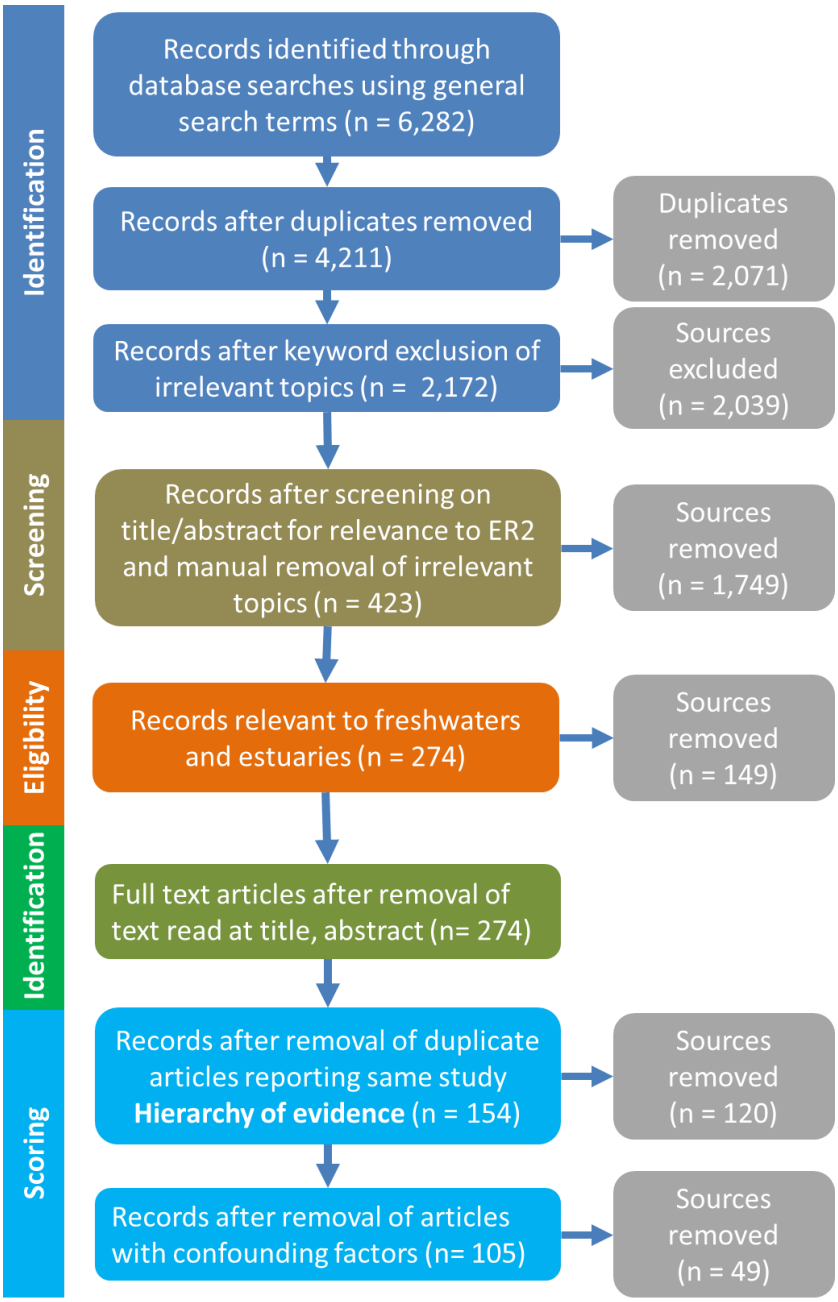

Fig 1. Map of evidence identified as relevant to ER3 during initial screening.

Of the sources likely to contain evidence relevant to freshwaters and estuaries, 105 unique sources were used to extract evidence (Fig. 1). Of these, 80 unique sources contained evidence relevant to biota found in freshwaters, and 27 unique evidence sources were used where the evidence was relevant to biota found in estuaries. Four sources contained evidence that was relevant to both habitats.

All the evidence was transferred from the evidence capture form into a searchable MS Access relational database, which was spatially referenced where appropriate (i.e. linked to a GIS data layer illustrating the field locations where evidence was obtained from). This database linked literature sources to the key questions and was used to produce extractable summaries of the evidence base underlying each of the key questions and sub

questions. After evidence capture, the total evidence base was compiled and quantified, and meta-analyses undertaken where appropriate (see Section 4).

In terms of volume of evidence, there has been an exponential increase in the number of publications relevant to the impact of microplastics on freshwater and estuarine biota in recent years (Fig 2). However, the growth in relevant evidence occurred at a slightly later date than for the evidence relevant to ER1 and ER2, appearing to accelerate from 2015 onwards. Furthermore, the increase in relevant publications has been driven by those relevant to freshwater biota, with a relatively small proportion of studies considering impacts on estuarine biota.

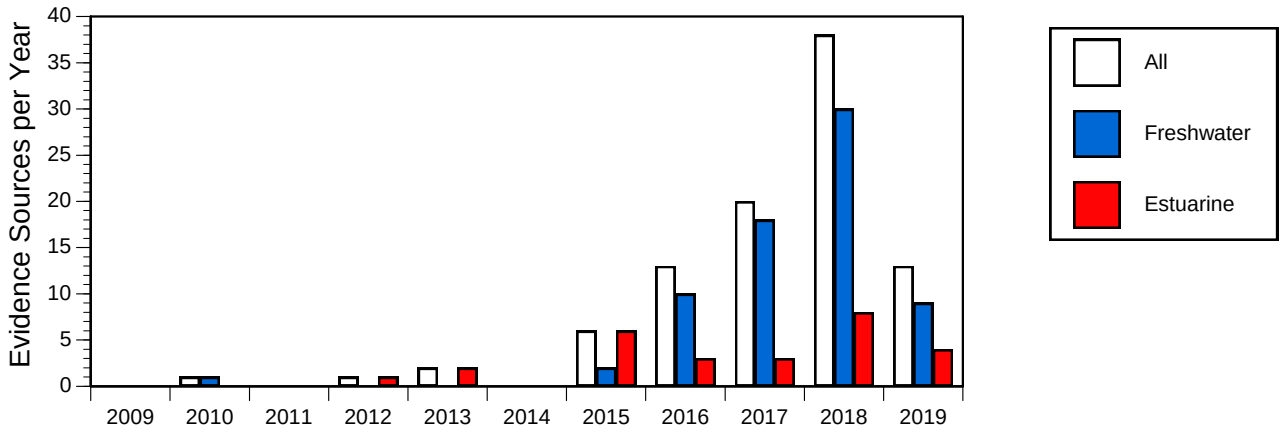

Fig 2. Number of evidence sources per year.

2.2.2 Reliability scores

Additional information on the reliability of the evidence provided by the source was captured using a separate spreadsheet, based on the CRED (Criteria for reporting and evaluating ecotoxicity data) method outlined by Moermond et al. (2016). The quality assessment was made up of fifteen criteria covering (a) General information, (b) Test compound used, (c) Test organism used and exposure condition, and (d) Statistical design and biological response (Table 2.4). For each criterion, a score of 0, 1, or 2 was assigned to the evidence source under review. Scores signified the following: 2 = reliable without restrictions, 1 = somewhat reliable but with restrictions, 0 = not reliable. If information was lacking on certain aspects in the evidence source, it was considered as unreliable, leading to a lower score. For each evidence source the Total Accumulated Score was calculated by adding scores for individual criteria (maximum 30 points). For the data provided by an evidence source to be considered sufficiently reliable, it should preferably have no 'zero' values for any of the individual scores. To assess the overall reliability of the evidence sources the number of zeros was calculated for each. Furthermore, the product of the scores in all relevant criteria was calculated, following the methods of Hermsen et al. (2018), to give a potential maximum Reliability Product Score of 32,768, but if any one criterion was evaluated as "not reliable" (0 points) the overall Reliability Product Score for the study was 0.

Table 2.4 Criteria used to assess reliability of evidence sources.

1. Validity criteria	Are valid controls used that do not compromise results through use of stressful or variable conditions? If applicable, are validity criteria fulfilled (e.g. control survival, growth)? Are control survival and/or other parameters within the range of what is normal for the species such that other confounding (stress) factors can be ruled out?
2. Adequate controls	Are controls adequate and sufficient to attribute any effects to the test substance (e.g. solvent control, negative and positive control)?
3. Identity of test substance	Is the test substance identified clearly with characteristics described? Are test results reported for the appropriate substance?
4. Source of test substance	Is the source of the test substance reported and is it trustworthy?
5. Identity of test organisms	Are the organisms well described (e.g. scientific name, weight, length, growth, age/life stage, strain/clone, gender if appropriate)?
6. Source of test organisms	Are the test organisms from a trustworthy source and acclimatized to test conditions? The place of origin should be described for field-collected organisms. Have the organisms not been pre-exposed to test compound or other unintended stressors?
7. Appropriate for test substance	Is the experimental system appropriate for the test substance, taking into account its physico-chemical characteristics? Avoidance of plastic in set-up etc. and minimising possibility of microplastic contamination.
8. Appropriate for test organism	Is the experimental system appropriate for the test organism (e.g., choice of medium or test water, feeding, water characteristics, temperature, light/dark conditions, pH, oxygen content)? Have conditions been stable during the test?
9. Gradient of exposure	Is the exposure gradient appropriately scaled from control to high exposure with ≥ 3 treatment levels + control? Is a correct spacing between exposure concentrations applied? A scaling factor of 3.2 (=√10) is often recommended. As a rule of thumb, a maximum scaling factor of 10 should be applied.
10. Exposure duration	Is the duration of exposure clearly stated?
11. Verification of exposure	Are analyses adequate to verify concentrations of the test substance over the duration of the study? It is important to know the actual exposure concentrations, and it should be clear if the reported concentrations are initial or final concentrations, whether they are mean or geometric mean concentrations, and which of these concentrations are used to calculate the effect concentrations. For microplastics, nominal concentrations without measurements can be acceptable, only if robust efforts to minimise contamination are in place.

12. Biomass loading	Is the density of test organisms in experimental units acceptable (no indication of stress in controls)? Is the biomass loading of the organisms in the test system within the appropriate range (e.g. < 1 g of fish/L)?
13. Adequate replication	Is a sufficient number of replicates used? Is a sufficient number of organisms per replicate used for all controls and test concentrations?
14. Appropriate statistical methods	Is a detailed description of statistics used given with confirmation that they are fit-for-purpose?
15. Raw data available	Are sufficient data available to check the calculation of endpoints (e.g. mortality, growth, reproduction, feeding rate, behaviour) and (if applicable) validity criteria (e.g., control data, concentration-response curves)? By "raw data" we mean the data needed to assess the statistics and variability in the controls, recalculate the reported endpoints, and calculate alternative endpoints.

2.3 Interviews

Interviews with academics working in the field of microplastics were conducted to get their expert opinion on the primary and secondary questions. Four academic experts were consulted:

Prof Amanda Callaghan, University of Reading, UK

Prof Isabelle Durance, University of Cardiff, UK

Dr Ika Paul-Pont, CNRS, Brest, France

Dr Katrin Wendt-Potthoff, Helmholtz Centre for Environmental Research GmbH –UFZ, Leipzig, Germany

Interviews (lasting 30-45 minutes) were held via phone with all the academics above. During the telephone interviews, the academics were requested to: provide their expert view on each of the primary and secondary questions; comment on key published literature relating to the questions; and provide information on ongoing or unpublished work relating to this evidence review, if applicable. The interviewee responses were recorded as notes during the interviews. The key messages/highlights derived from the interviews are outlined in Section 3.

3 Key messages from interviews with academic experts

Primary question:

What is/are the impact(s) of microplastics on freshwater and estuarine biota?

All four academic experts interviewed were in agreement that effects of microplastics on biota have been demonstrated through laboratory experiments, but that there is no robust evidence that such effects manifest in the environment. The academic experts interviewed expressed concerns that the experiments undertaken to date have used particle types, sizes and concentrations that do not reflect those reported by studies of microplastics in the environment. It was noted that some effects of microplastics may be temporary, for example *Daphnia* will depurate rapidly if left to recover. The influence of bias in publication was commented on by the experts interviewed, and in particular how this may influence perceptions of impacts.

Secondary questions:

a) To what extent do microplastics influence the feeding, growth, reproduction and survival of freshwater biota? Do we know trigger levels or threshold values for microplastic impacts on biota?

All the experts interviewed were of the opinion that there was insufficient evidence to draw any robust conclusions regarding thresholds. In the opinion of the experts, there has been a focus on short-term immediate effects, with less evidence on effects that take longer to manifest, such as reproduction. The experts noted that laboratory experiments have been undertaken at very high concentrations such that thresholds identified to date may be irrelevant to field conditions.

b) Are any differences between different taxonomic groups observed?

The four academic experts interviewed commented that laboratory tests have focussed on a few species, and there are gaps in the taxonomic coverage of the current knowledge. As such, it was the opinion of the academic experts that it is difficult to draw general conclusions. It was noted by the academic experts that feeding strategy affected exposure to microplastics and, therefore, likely to influence which taxa were affected.

c) Are results from laboratory studies relevant to microplastics at environmentally relevant field concentrations?

All four academic experts interviewed were of the opinion that the size and types (density, polymer, morphology) of microplastics used in experimental tests, together with the concentrations used, do not reflect those described by field studies. It was also noted that comparison with natural particles was lacking, so it is not possible to determine if any

effects are due to the plastics or the particles. The academic experts also noted that the presence of alternative food sources influences the uptake of microplastics: more realistic experiments are necessary. Furthermore, the need to determine risk, based on both exposure and hazard, was highlighted by the academic experts interviewed.

d) Are any adverse impacts attributable to the particles or to adsorbed chemicals/microbes on the particles?

All the interviewees said that there were potential concerns about adsorbed chemicals/additives. Such effects may be specific to the chemical in question. Most experiments have used virgin particles, so there is still insufficient evidence to determine how much of any impact may be due to adsorbed chemicals/additives. The experts felt that more work is needed to draw robust conclusions.

e) Is there evidence to suggest impacts on populations of aquatic organisms?

The four academic experts interviewed were in agreement that there was insufficient evidence to conclude that there are impacts on populations of aquatic organisms.

4 Literature Review

The outcomes of the literature review undertaken are outlined below, the structure being based on the primary and secondary questions. At the end of each question, a summary of the evidence is provided in a text box for clarity. This literature review is based on the 105 unique sources that were used to extract evidence (Fig. 1). The findings presented are summaries of the evidence available and, therefore, are influenced by the reliability of the primary literature, including grey literature, on which this report is based.

4.1 Reliability

Cumulative reliability scores ranged from 5 to 28 out of a possible 30 (Fig. 3), with a median total score of 20, indicating that half of the studies achieved two thirds or less of the available points. The number of reliability categories that scored zero ranged from 10 to 0 per study out of a possible 15 (Fig. 3). A zero score in any criterion indicated it was evaluated as “not reliable”: the median was 3 zeros per study, which indicates that half of the studies were based on methods that were unreliable in one fifth or more of the aspects considered. Using the product of the scores in all categories, which unambiguously identifies those studies that were reliable across all criteria, only 6 studies did not score 0. Overall, the majority of studies regarding the impacts of microplastics on the biota of freshwaters and estuaries were based on methods that were in some aspects not reliable. Reliability scores of studies appeared to decline over time, both in terms of cumulative scores and the number of zeros (Fig 4): whilst the highest scores achieved in each year remained more or less constant, the lowest scores declined with time, suggesting that less reliable studies were being published as the field developed.

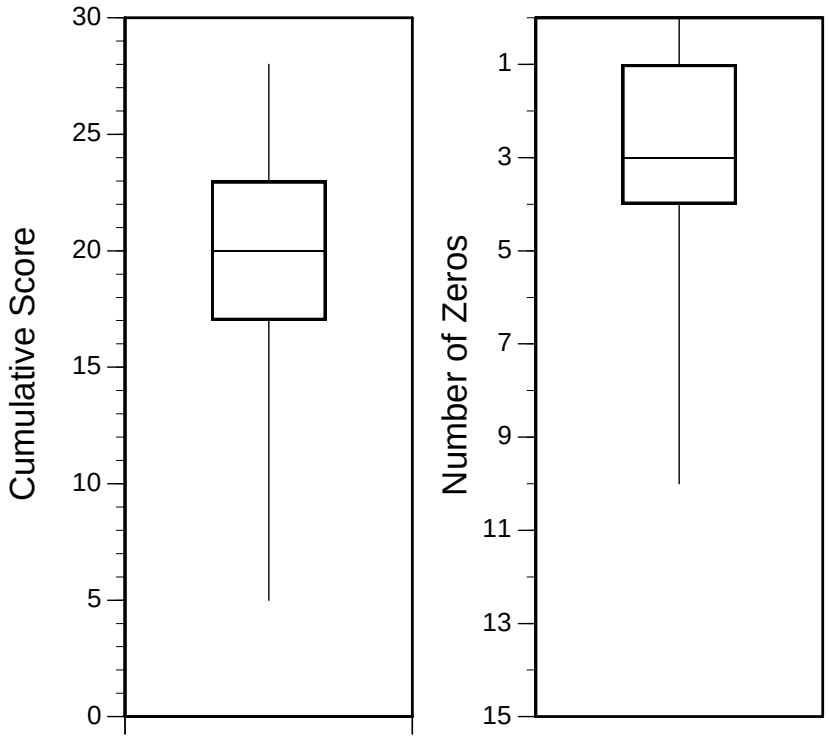

Fig 3. Box plots of reliability scores of studies investigating impacts of microplastics on estuarine and freshwater environments. Box indicates 25th and 75th percentiles, whiskers minimum and maximum, and line median size of particles (n = 103).

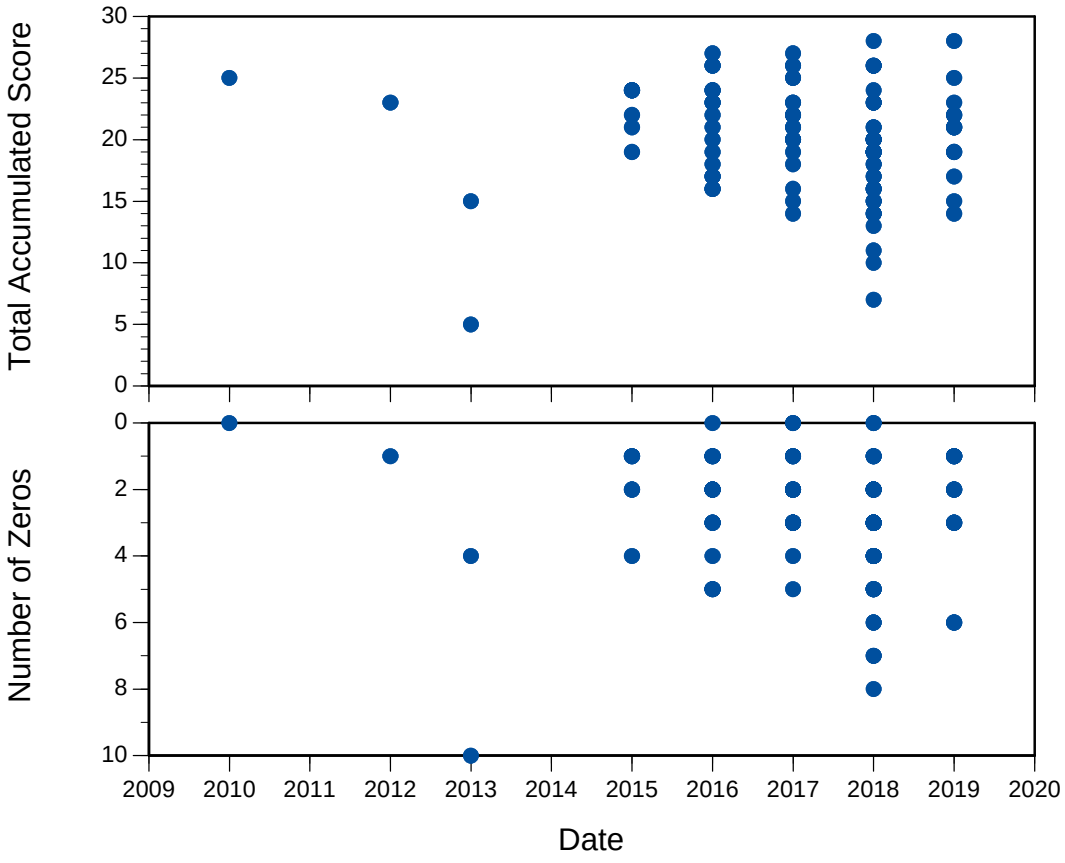

Fig 4. Change in reliability scores of studies investigating impacts of microplastics on estuarine and freshwater environments over time.

In many instances the statistical anlyses undertaken were not sufficiently robust to draw the conclusions that were derived. Of particular note were studies where a lack of a consistent dose response was ignored in preference for highlighting effects at specific individual dose levels. Similarly, studies often presented a number of different measured endpoints (e.g. expression of multiple genes) from the same experiment, yet did not correct for additive errors, increasing the likelihood of detecing a significant effect in one of the response variables.

It was also apparent that in many instances (particularly with molecular and biochemical biomarkers) the authors of studies did not establish any hypotheses *a priori*, in terms of the mechanistic connection with the endpoint measured and the expected directional change as a consequence of damage. Thus, interpretation of change in the endpoint measured could not be linked robustly to a negative effect of microplastics.

Another important issue that was rarely addressed, was that in order to attribute the effects observed in the tests undertaken to microplastics it is of fundamental importance that adequate controls are used. With few notable exceptions, natural inert particles were not used as a control (ideally in a dose response manner). Such a control is critical in order to determine if any effects were attributable to the microplastic *per se*, or simply an effect caused by particles of no nutritional value.

Furthermore, the tests undertaken to date used organisms that had been starved before use (as is standard prectice), and were typically presented with microplastics alone with no other food source. In the instances where natural food sources were made available (e.g. (Carlos et al. 2015, Aljaibachi and Callaghan 2018), there were indications that the biota selected those, rather than the microplastics. Hence, typical test conditions maximised the likelihood that microplastics were consumed: under field conditions, where alternative food sources may be available, such uptake and consequent effects may not be realised.

The majority of studies on the impact of microplastics on freshwater and estuarine biota were unreliable in several aspects.

Published studies have become less reliable over time.

4.2 Secondary question: To what extent do microplastics influence the feeding, growth, reproduction and survival of freshwater and estuarine biota? Do we know trigger levels or threshold values for microplastic impacts on biota?

To assess the extent of influence that microplastics have on freshwater and estuarine biota, the threshold values reported from dose-response experiments were compiled and grouped according to ecotoxicological endpoints, namely effects on behaviour, feeding, growth, reproduction and survival, as well as the additional endpoints of metabolism and other (largely gene expression), which in many cases were less strongly linked to negative biological consequences. The most frequently tested endpoints were those associated with metabolism (Fig 5), where it was common for a single evidence source to report tests assessed using multiple different metabolic endpoints. Similarly, evidence sources that used genetic endpoints each tended to report expression of multiple genes in response to exposure to microplastics.

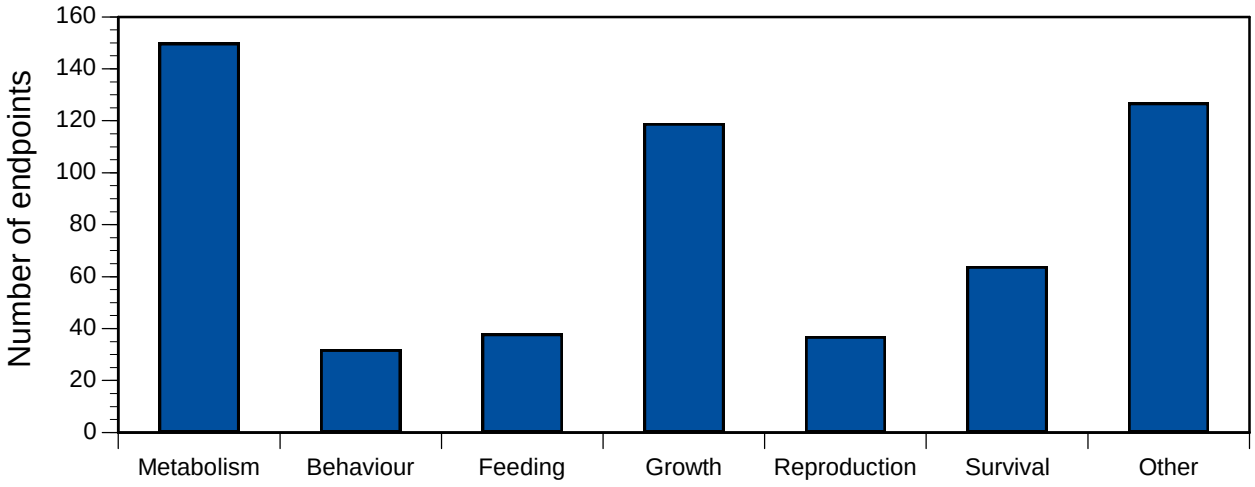

Fig 5. Number of endpoints tested.

By far the largest number of tests were conducted where the test organism were exposed to microplastics suspended in water, rather than introduced into the sediment or food (Fig 6). In one study, the microplastics were introduced directly into the tissues of the test organism by injection.

Across all endpoints the majority of experimental responses that were reported were characterised as “no consistent dose response relationship”. Only in tests using behavioural endpoints were negative responses (a downward trend in the characteristic measured) more frequent. Positive responses (an upward trend in the characteristic measured) were seen across all endpoints measured.

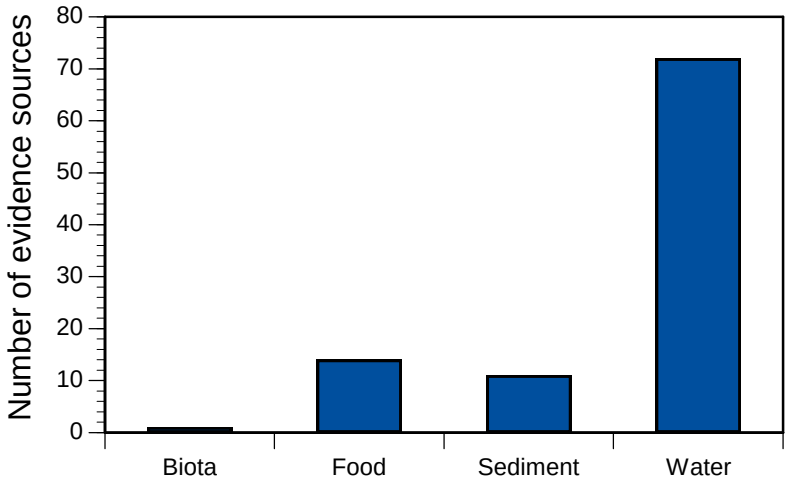

Fig 6. Matrix used to introduce microplastics to test organisms.

The evidence sources reported the dose of microplastics used in various units (Fig 7). As a consequence of most studies exposing test organisms to microplastics in water, the two most commonly used units were mass per unit volume and particles per unit volume. The variation in units used to quantify the dose of microplastics used in the ecotoxicological tests makes comparison among the different evidence sources more difficult. Furthermore, quantification of the exposure dose in terms of mass per unit volume (or mass), as is typical for most environmental contaminants, is not appropriate for microplastics. Microplastics occur as particles of various sizes and, therefore, vary in mass per particle. Thus, for a given mass, the number of particles that organisms are exposed to will vary dependent on their size. From ER1 it was apparent also that the concentration of particles found in the environment was related to their size, indicating that environmental exposure concentrations are size dependent. Hence, to enable comparison amongst studies, thresholds expressed as mass of plastic were converted to count of particles. This was possible for studies that had used beads and gave the polymer used and dimensions, by assuming that particles were spherical and calculating the mass of individual particles using the formula

$$\text{Mass} = \frac{4}{3} \pi r^3 \rho$$

where r is the mean radius of the particles used and ρ is the density of the polymer. In those cases where the density of the polymer used was not given, a standard density for that polymer was used. For other particle morphologies it was not possible to calculate the mass of individual particles as the dimensions were not given in sufficient detail.

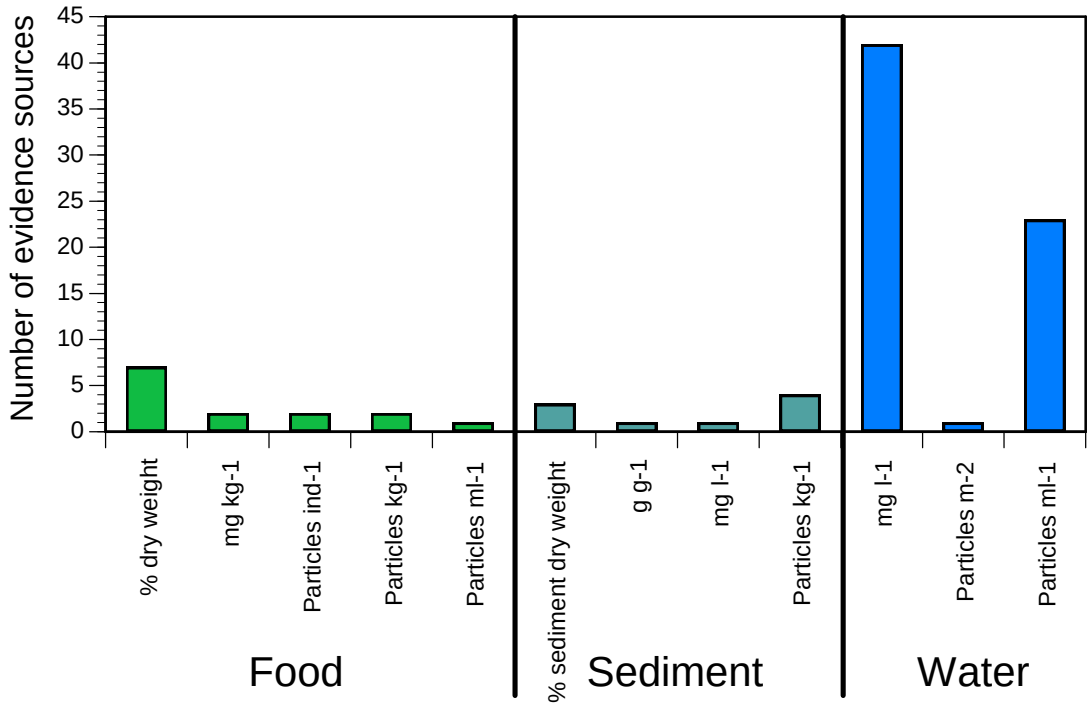

Fig 7. Units used to describe test exposure dose of microplastics.

Where thresholds were identified, the type of threshold values reported varied among the different evidence sources, with some reporting EC_{50} values (statistically derived dose at which 50% of the test organisms will be expected to respond), some reporting predicted no effect concentrations (PNEC: modelled concentration below which no adverse effects of exposure would be expected), and some reporting Lowest Observed Adverse Effect Level (LOAEL: lowest exposure level at which there are significant increases in adverse effects). As it is not possible to convert between threshold types, all threshold types were considered.

The initial objective of this review was to use the evidence compiled to undertake a species sensitivity distribution (SSD) analysis for each endpoint, as this is a standard model approach for establishing the variability in the sensitivity of multiple species to a single toxicant or stressor (Posthuma et al. 2002). The SSD approach can be used to establish a threshold concentration where the toxicant presents a hazard to a limited number of taxa (with the threshold typically set at a concentration that protects 90% or 95% of taxa). However, it soon became obvious that such an approach, although possible, was not appropriate for microplastics. As environmental exposure concentrations of microplastics are size dependent (following a Log-Log relationship: see ER1), a single threshold concentration is unlikely to be relevant to all particle sizes. Hence, using the evidence compiled from tests conducted on microplastics in water, the relationship between the mean size of particles used in the tests and reported threshold concentrations was established for the impact on feeding, behaviour, growth, reproduction and survival (irrespective of test organism and polymer used) using least squares regression on Log_{10}

transformed data (Fig 8)[1]. Such relationships were not derived for endpoints of metabolism or other (mainly gene expression) because, in most cases, the link between the measured characteristic and a negative biological consequence was not clearly defined. To ensure that the relationships derived were based on valid data, the reliability scores obtained by the studies used were assessed. All studies achieved reliability scores equal or better than the median (see section 4.1), a total accumulated score of 20 or more (out of a possible 30) and 3 zeros or less (out of a possible 15), with the exception of four studies (three scored 19 total accumulated score and one had 4 zeros). These four studies were not used to establish relationships between threshold concentrations and particle size.

There were insufficient data to establish the relationship between the mean size of particles used in the tests and reported threshold concentrations for microplastics in sediment.

For the toxicological endpoints behaviour, feeding, growth, reproduction and survival there was a clear relationship between the size of the particles used in the test and the threshold at which an effect was seen (Table 4.1), such that a higher concentration of smaller particles was required to cause an effect (Fig 8). Of the five endpoints, reproduction had the lowest number of threshold values on which to base the relationship with the size of particles (Fig 8d). However, the relationship between the size of particles and threshold concentrations was significant for all endpoints (Table 4.1).

The relationships between the size of particles used in tests and threshold effect concentrations amongst the five endpoints did not follow the pattern that might be expected assuming that the concentration necessary to illicit a response in an endpoint is positively related to the severity of the endpoint. Behaviour had the highest intercept and reproduction the lowest (Table 4.1).

Whilst the above analysis indicates that the size of microplastic particles influences the concentration at which effects manifest, the relationships were determined using least squares regression and, thus, reflect a position of central tendency. In order to establish a threshold concentration where microplastics present a hazard to a limited number of taxa, quantile regression[2] (based on the 10th percentile) was used to determine the size-specific concentration of microplastics that was lower than 90% of the thresholds identified for survival and, as a more conservative limit, across all the endpoints tested including sublethal effects (Fig 9). Using survival as an endpoint, this approach is an approximation to a species sensitivity distribution (SSD) whilst accounting for the effect of particle size.

---

[1] Least squares regression provides a line of best fit based on the conditional mean response by minimizing the sum of squared residuals (a residual being: the difference between an observed value, and the fitted mean value provided by the model). In this case the data used were Log_{10} of the actual values.

[2] Quantile regression provides a line of best fit through the conditional median or a specified percentile (in this case the 10 percentile, where 90% of observations are greater than this value) based on the distribution of the data, and uses a different mathematical approach to derive a solution based on minimizing the sum of absolute residuals.

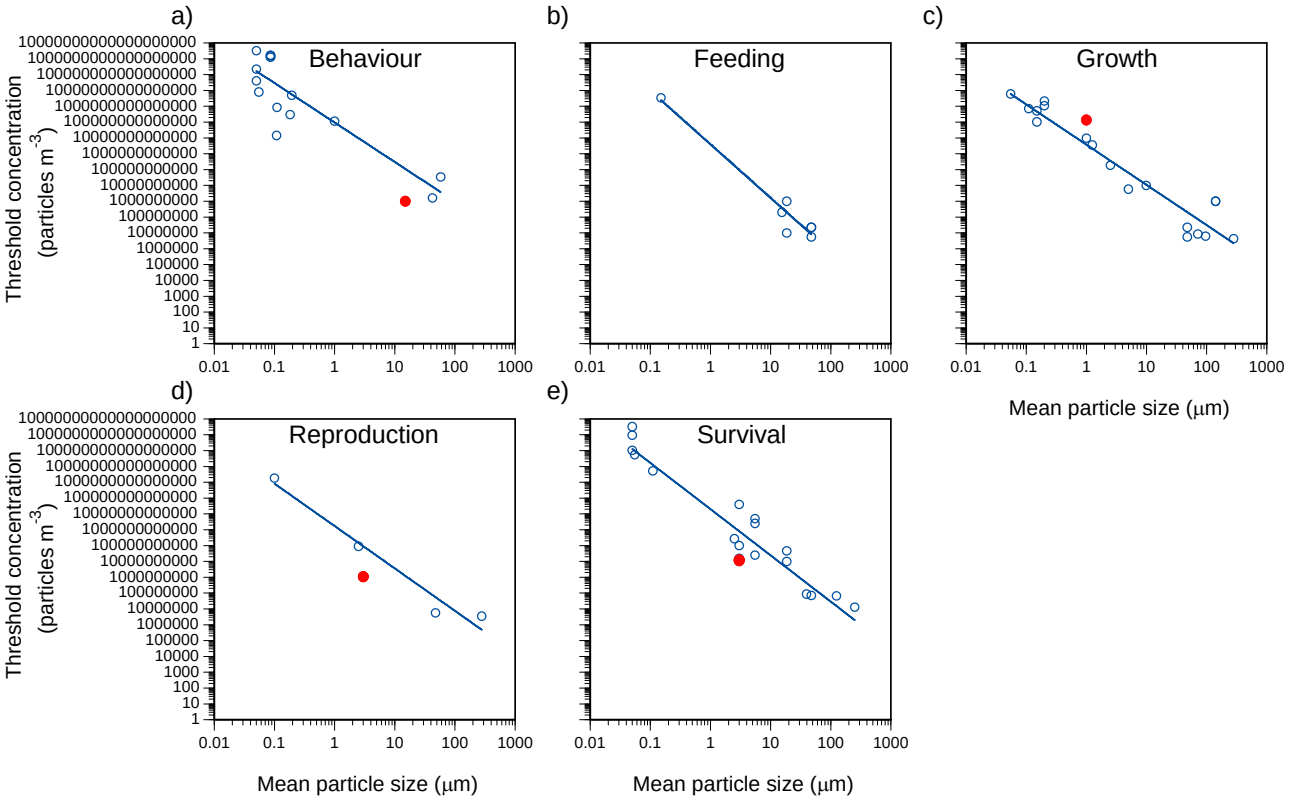

Fig 8. Relationship between the size of microplastic particles tested and reported toxicological thresholds investigating impacts on behaviour, feeding, growth, reproduction and survival of freshwater and estuarine biota. Filled symbols indicate studies which failed to achieve median reliability scores. Nanoparticles ≤0.1 µm.

Table 4.1 Parameter estimates (slope and intercept of line of best fit) and statistical results (F statistic, p value and R^2) from least squares regression for relationships between the particle size and threshold concentrations for different toxicological endpoints.

	Slope	Intercept	*F*	*p*	R^2
Behaviour	-2.493	13.98	39.45	≤ 0.0001	0.733
Feeding	-3.389	12.60	101.67	0.0002	0.944
Growth	-2.542	12.57	190.70	≤ 0.0001	0.918
Reproduction	-2.678	12.24	51.68	0.019	0.944
Survival	-2.640	13.68	126.05	≤ 0.0001	0.880

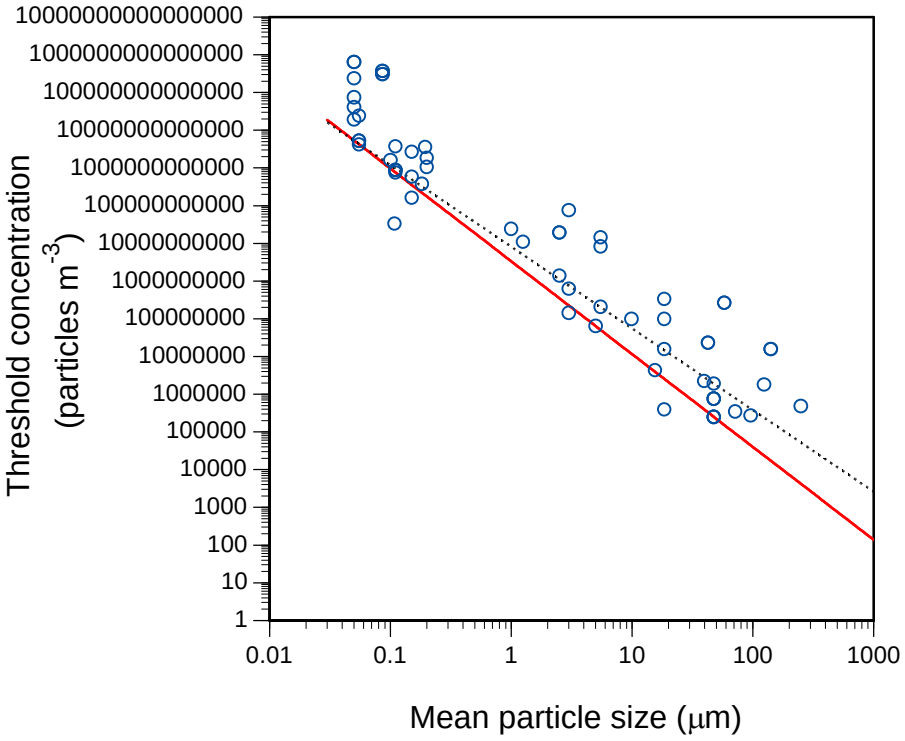

Fig 9. Size of microplastic particles tested and reported toxicological thresholds showing 10%ile relationship fitted by quantile regression to all endpoints (red line) and survival (dashed line). Nanoparticles ≤0.1 µm.

Under experimental conditions, at high concentrations microplastics can have a negative impact on the feeding, behaviour, growth, reproduction and survival of freshwater and estuarine biota. For all endpoints, there was sufficient evidence to conclude that the concentration required to cause such impacts is related to the size of the particles of microplastic.

4.3 Secondary question: Are any differences between different taxonomic groups observed?

Details of all test organisms used were compiled. Whilst a range of taxonomic groups were used including microbes, plants, invertebrates and vertebrates, the most frequently used test organisms were fish and Crustacea (Fig 10). The taxa used were not evenly distributed across the different toxicological endpoints, and there was an uneven distribution of taxonomic groups across the thresholds identified for effects of microplastics. Hence, there were only sufficient data to test the influence of taxonomic

group used on size-specific thresholds for Crustacea, fish and algae. Here, an analysis of covariance (ANCOVA)[3] was undertaken using general linear models to determine if either the taxonomic group of the test organism used (Crustacea, fish and algae), or the endpoint measured, had a significant influence on the relationship between the mean particle used in the test and the threshold concentration for an effect (Fig 11). There was no significant effect of either the endpoint measured or the taxonomic group used in the test (Table 4.2). Nevertheless, there was evidence to indicate that taxa are selective in their uptake of microplastics from the environment (e.g. Au 2017, Straub et al. 2017), with some organisms apparently only consuming microplastic particles within certain size ranges. Such size selectivity may influence the susceptibility of biota to the impact of microplastics if negative effects manifest through consumption of particles: biota would not be sensitive to impacts from particles of sizes that are avoided. The analysis undertaken here would not detect such a phenomenon, as data were only included if a threshold was detected.

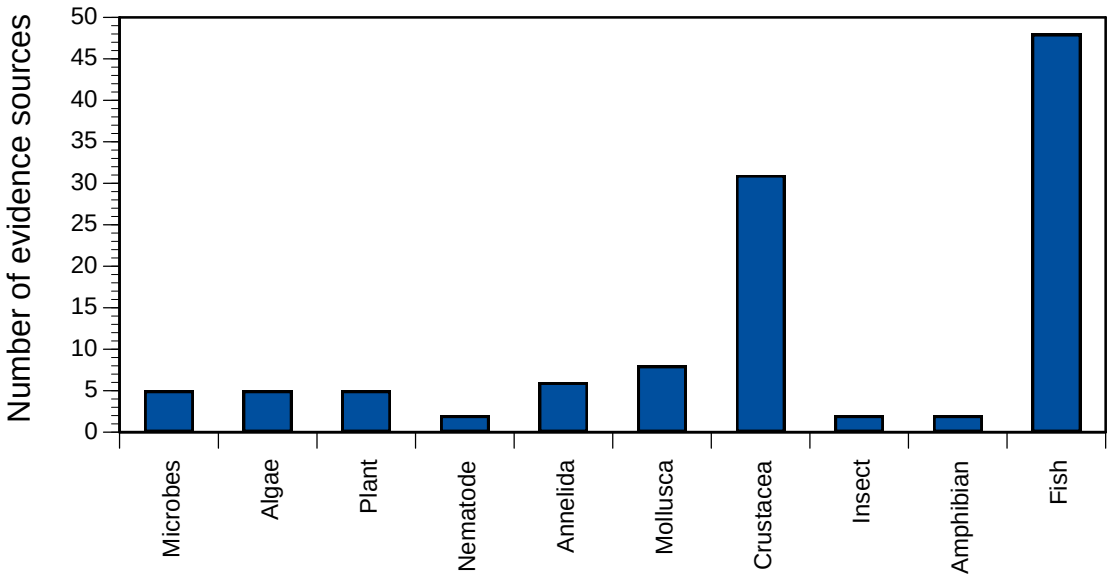

Fig 10. Test organism used to determine effects of microplastics on biota.

Table 4.2. Results of ANCOVA testing the influence of endpoint measured or taxonomic group of test organism (Crustacea, fish, algae) on the relationship between particle size and threshold effect concentration.

	F value	*p*
Particle Size	569.85	≤ 0.0001
Particle Size * Endpoint	0.30	0.8778
Particle Size * TaxaGp	1.71	0.2109

[3] ANCOVA uses a least squares approach to evaluate if the mean response differs across levels of a categorical independent variable, while statistically controlling for the effects of another continuous variable.

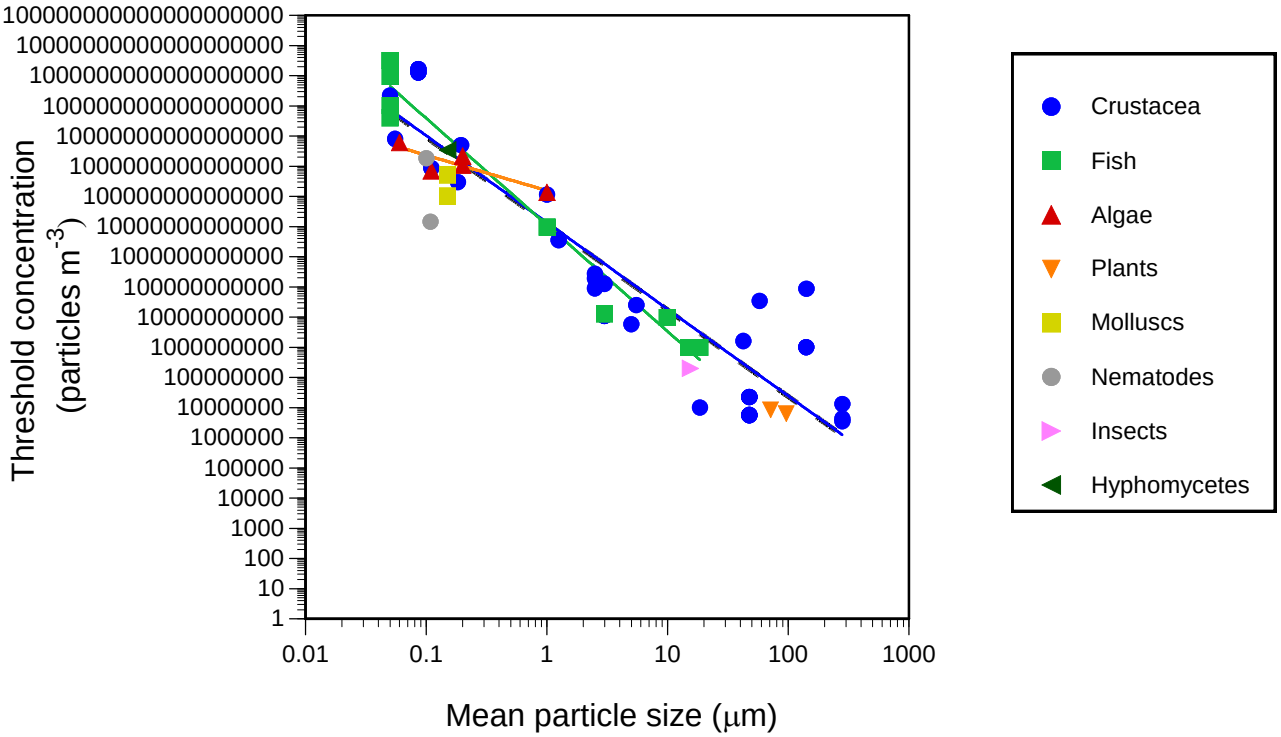

Fig 11. Effect of taxonomic group on relationships between particle size and effect threshold concentration. Relationships shown for Crustacea, fish and algae, and all taxa (dashed black line). Nanoparticles ≤0.1 µm.

There is evidence from ecotoxicological tests to indicate that taxonomic groups do not differ in their sensitivity to microplastics.

4.4 Secondary question: Are results from laboratory studies relevant to microplastics at environmentally relevant field concentrations?

Most studies used primary microplastics (Fig 12) of bead morphology (Fig 13) and comprised of a limited range of polymers, with the majority of studies using either polystyrene or polyethylene (Fig. 14). Even where secondary microplastics were used, they were typically derived from new (virgin) materials. From ER2 it was apparent that most microplastics found in environmental samples from freshwaters and estuaries were likely to be of secondary origin and, as such, the types of microplastics used in ecotoxicological tests do not reflect the types of microplastics that are found in the environment well.

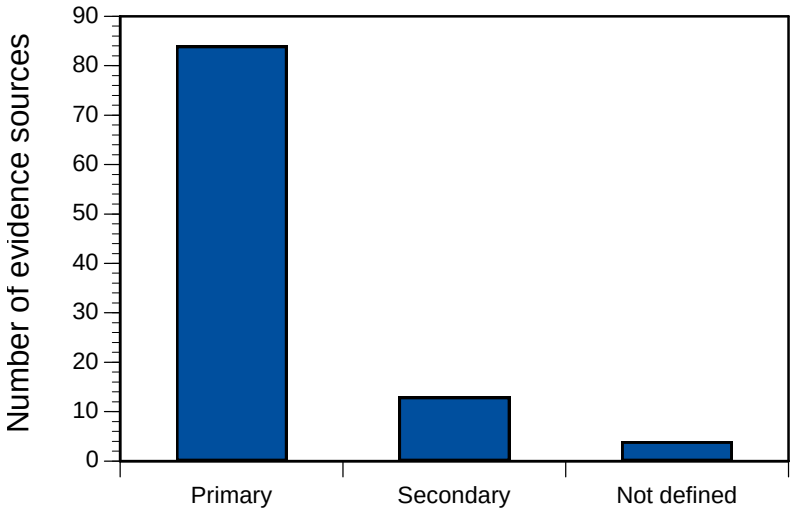

Fig 12. Source of microplastics used in ecotoxicological studies.

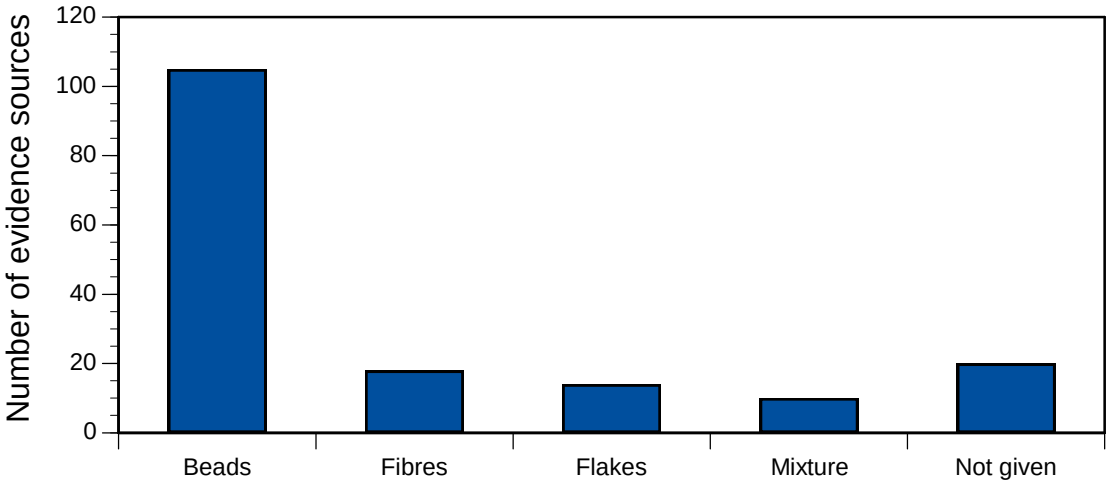

Fig 13. Morphology of microplastics used in ecotoxicological studies.

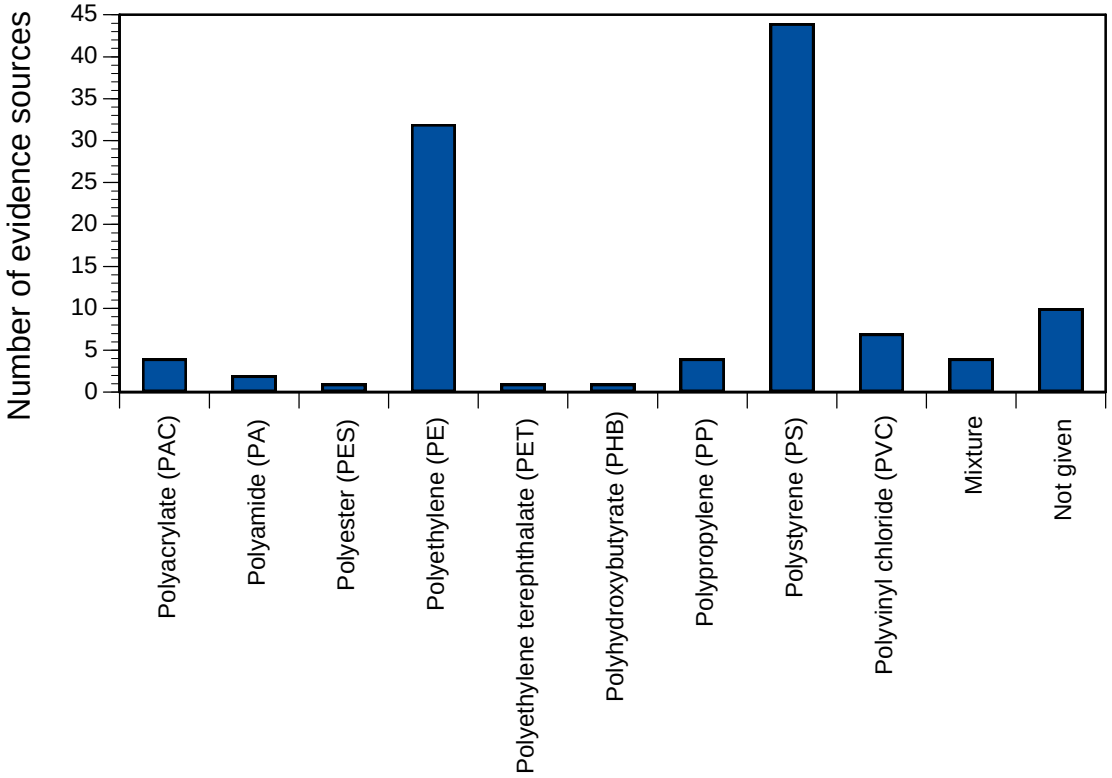

Fig 14. Polymers used in ecotoxicological studies.

The range of sizes of plastic particles used in the ecotoxicological studies included both micro- and nanoplastic particles (Fig 15a). The median size used across all laboratory studies was 10 µm, with 75% of studies using particles that were smaller than 82.5 µm, and 25% of studies used particles that were < 2 µm. These size ranges do not compare well with the size ranges of particles which have been quantified in the environment, where the methods used to date have been limited to describing concentrations of larger particles. Using data from ER1, the median smallest particle size quantified by studies of microplastics in estuaries and freshwaters were 200 and 100 µm respectively (Fig 15b), an order of magnitude larger than that used in toxicity tests. Only 25% of studies of microplastics in estuaries and freshwaters considered particles that were less than 50 µm. It was apparent that the majority of laboratory based ecotoxicological studies have been undertaken using plastic particles of sizes that do not reflect the sizes of the microplastic particles for which concentrations have been described from environmental samples collected in estuaries and freshwaters. This is most likely due to size constraints on the methodologies used to characterise microplastics from environmental samples (ER1). This mismatch adds uncertainty to our understanding of risk from microplastics. The majority of evidence describing toxicological thresholds is for smaller particles but we do not know the concentrations at which such particles are present in the environment and, correspondingly, we don't know the toxicological thresholds for the larger particles for which we do know the concentrations present in the environment.

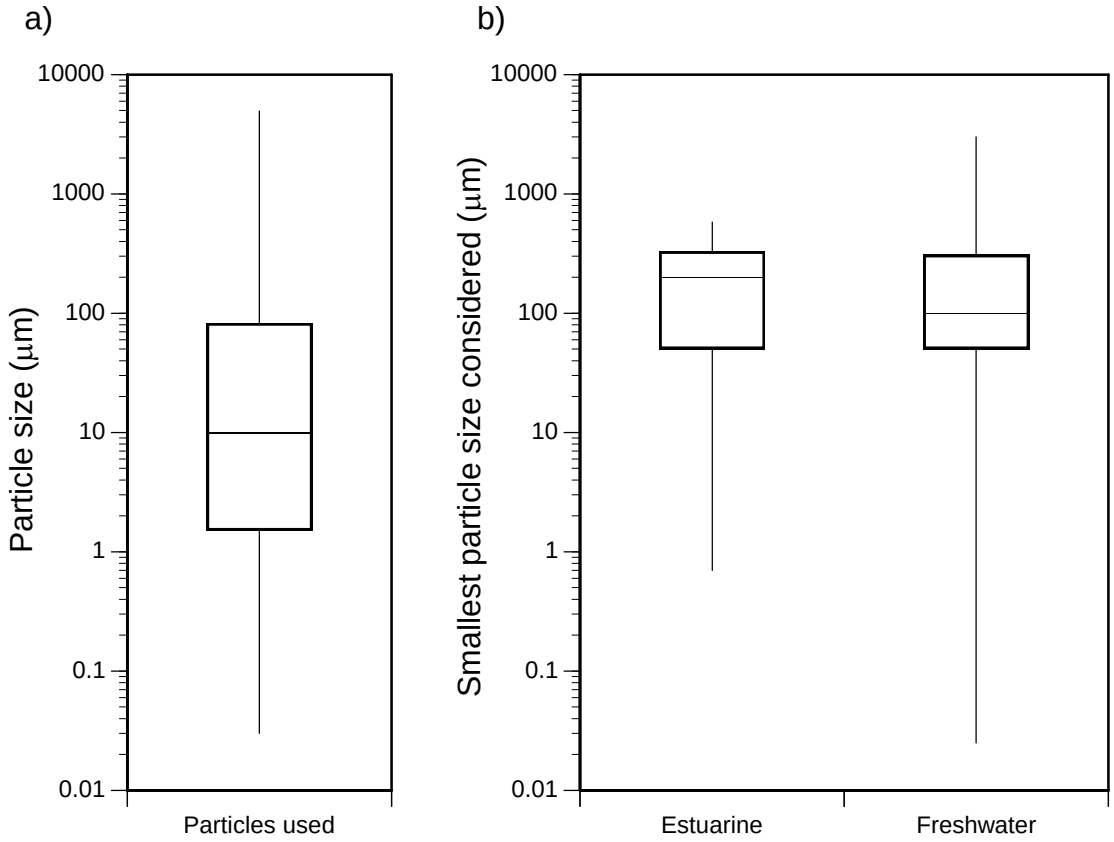

Fig 15. Box plots of size ranges of a) particles used in ecotoxicological studies (n = 125), and b) smallest particles considered in studies of microplastics in estuarine and freshwater environments (from ER1, n = 185). Nanoparticles ≤0.1 µm. Box indicates 25th and 75th percentiles, whiskers minimum and maximum, and line median size of particles.

To assess if ecotoxicological studies have been undertaken at concentrations that are relevant to microplastics at environmentally relevant field concentrations, the size of the particles used has to be taken into consideration. The data describing the relationship between identified thresholds in toxicological endpoints and the size of particles used in experimental tests was compared with the data compiled in ER1 describing the size of the smallest particles considered and the concentration of microplastics observed in freshwaters and estuaries.

It was apparent that the experimental tests that have been undertaken have used concentrations of microplastics that were far greater than the concentrations that have been reported from samples collected from freshwater and estuarine environments (Fig 16). Taking into account the size of particles, the mean concentration at which the effects of microplastics were observed in experimental tests was approximately 6 to 8 orders of magnitude greater than the mean concentrations reported from field samples collected from freshwater and estuarine environments (calculated from difference in relationships: Table 4.3).

Table 4.3 Slope and intercept of relationships derived by least squares regression between size of particles and either threshold effect concentrations for all endpoints or environmental concentrations. [Lines shown in Fig. 16]

	Slope	Intercept
Threshold effect concentrations from ecotoxicological tests	-2.85	13.195
Environmental concentrations	-1.68	5.258

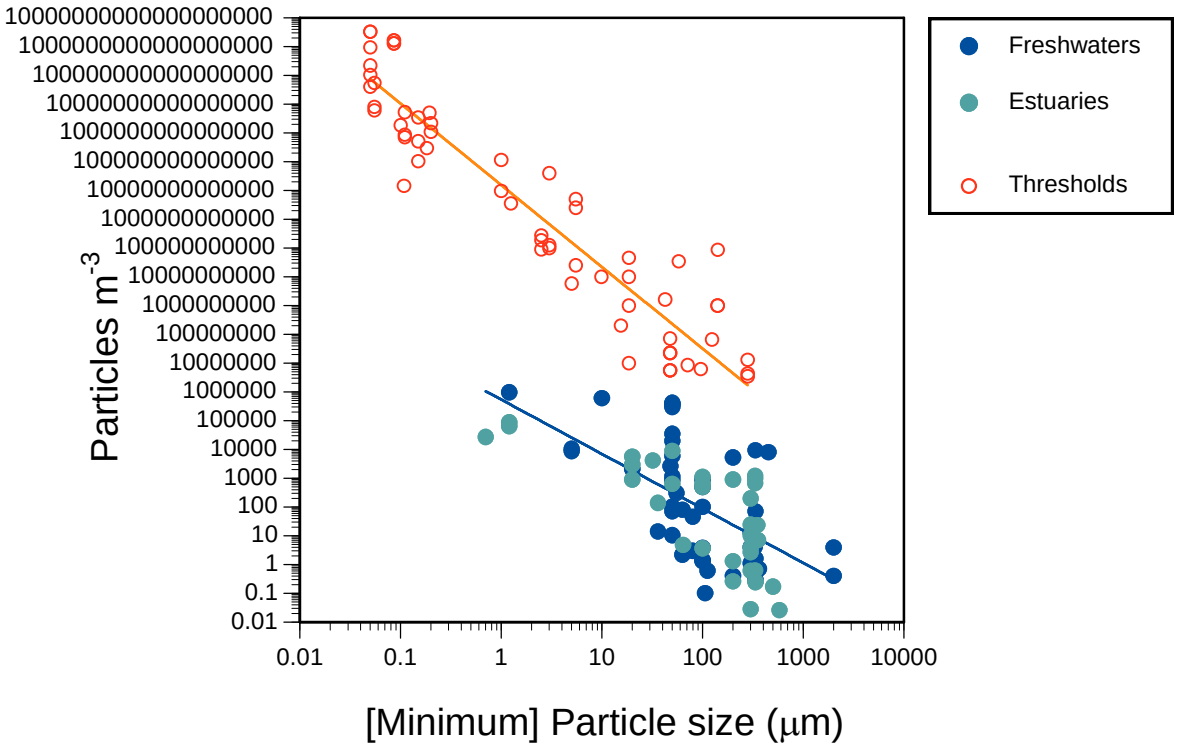

Fig 16. Relationship between the size of particles used in ecotoxicological experiments and threshold effect concentrations, together with the minimum particle size considered and mean concentrations of microplastics from samples of freshwater and estuarine environments. Nanoparticles ≤0.1 µm. Lines fitted by least squares regression through all endpoints and all environmental samples.

The majority of laboratory based toxicological studies have been undertaken using plastic particles that do not reflect the size and type of the microplastic particles that have been described from environmental samples collected in estuaries and freshwaters. This mismatch adds uncertainty to our understanding of risk from microplastics.

Laboratory based toxicological studies have been undertaken using concentrations of microplastics that are many orders of magnitude greater than the concentrations that have been reported from samples collected from freshwater and estuarine environments.

4.4 Secondary question: Are any adverse impacts attributable to the particles or to adsorbed chemicals/microbes on the particles?

The majority of laboratory based ecotoxicological studies used primary (virgin) microplastic particles (Fig 12), such that any effects observed (as detailed in section 4.2) could only be attributable to the particles, and not to any adsorbed chemicals or microbes. Nevertheless, a substantial number of studies considered the interaction between chemicals and microplastics (Fig 17). No studies considered the impact of microbes attached onto microplastic particles.

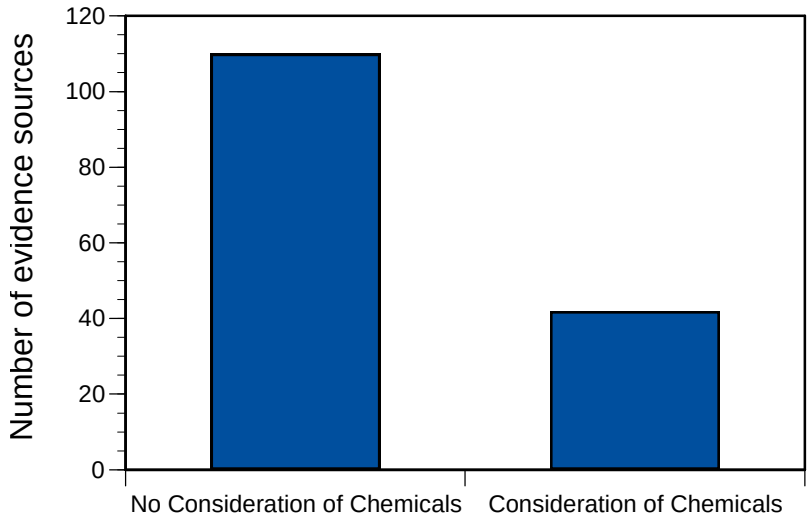

Fig 17. Volume of evidence that considered the effect of chemicals.

The consideration of chemicals in experimental studies followed four designs, dependent on the questions being addressed:

Chemical adsorbed onto (or combined within) microplastics before test exposure
Chemical added to water during test exposure to microplastics
Organism exposed to chemical before test exposure to microplastics
Leachate derived from microplastics used in test exposure

The chemicals considered included polyaromatic hydrocarbons, polychlorinated biphenyls, metals, insecticides, personal care products and medicines, as well as uncharacterised mixtures. Studies rarely used both microplastics and chemicals in an experimental design that enabled a dose response effect of both independently and in combination to be established. Furthermore, the environmental relevance of the concentrations of both the microplastics (see section 4.4) and chemicals used (including leachate from plastics) were rarely demonstrated. The effect of microplastics and chemicals in combination was not straightforward. The evidence sources reported both positive and negative effects, as well as no consistent dose response. Positive effects included a reduction in the negative effect of the chemical in the presence of microplastics (e.g. reduced toxicity of phenol: Sinche 2010). Negative effects included microplastics acting as a vector for the chemical (e.g. uptake of nicotine from cigarette filter fibres: Wright et al. 2015). However, due to variety of designs used in studies, it was not possible to draw substantial conclusions with regards the interaction between microplastics and chemicals.

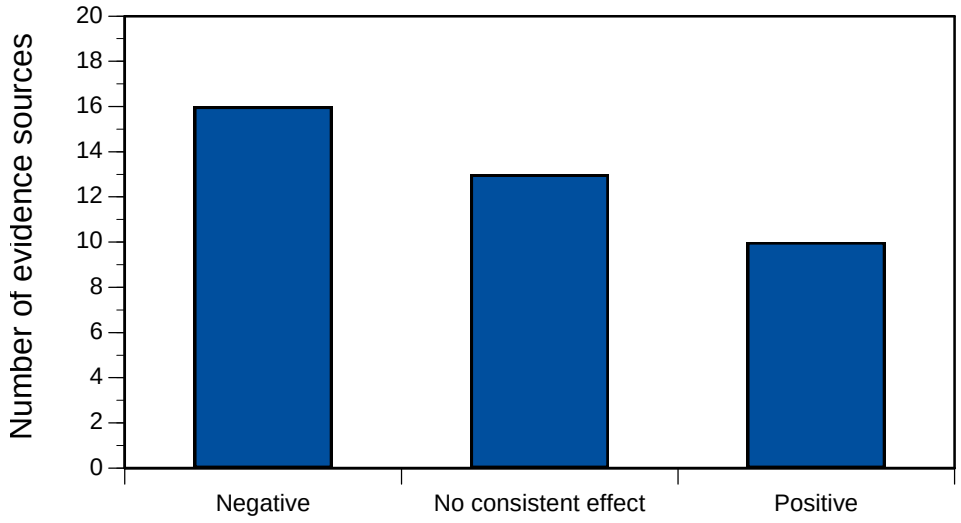

Fig 18. Effect of chemicals on responses recorded.

As detailed in section 4.4, studies used a limited range of polymers, with the majority of studies using primary microplastics of either polystyrene or polyethylene (Fig. 12). Furthermore, the polymers used were not evenly distributed across the different toxicological endpoints. Nevertheless, as studies used particles of polyethylene (PE), polystyrene (PS) and polyamide (PA) of various sizes and without any chemicals added,

there were sufficient data to test the influence of the polymer used on size-specific thresholds. Analysis of covariance (ANCOVA)[4] using general linear models was used to determine if either the polymer used in the test, or the endpoint measured, had a significant influence on the relationship between particle size and the threshold concentration for an effect (Fig 19). There was no significant effect of either the polymer used or the endpoint measured on the relationship between the size of particles and threshold concentrations (Table 4.4), suggesting that there is no difference among polymers in terms of their toxicity. The inclusion of data from further ecotoxicological tests using particles of various sizes comprised of a wider range of polymers would improve the confidence in this conclusion.

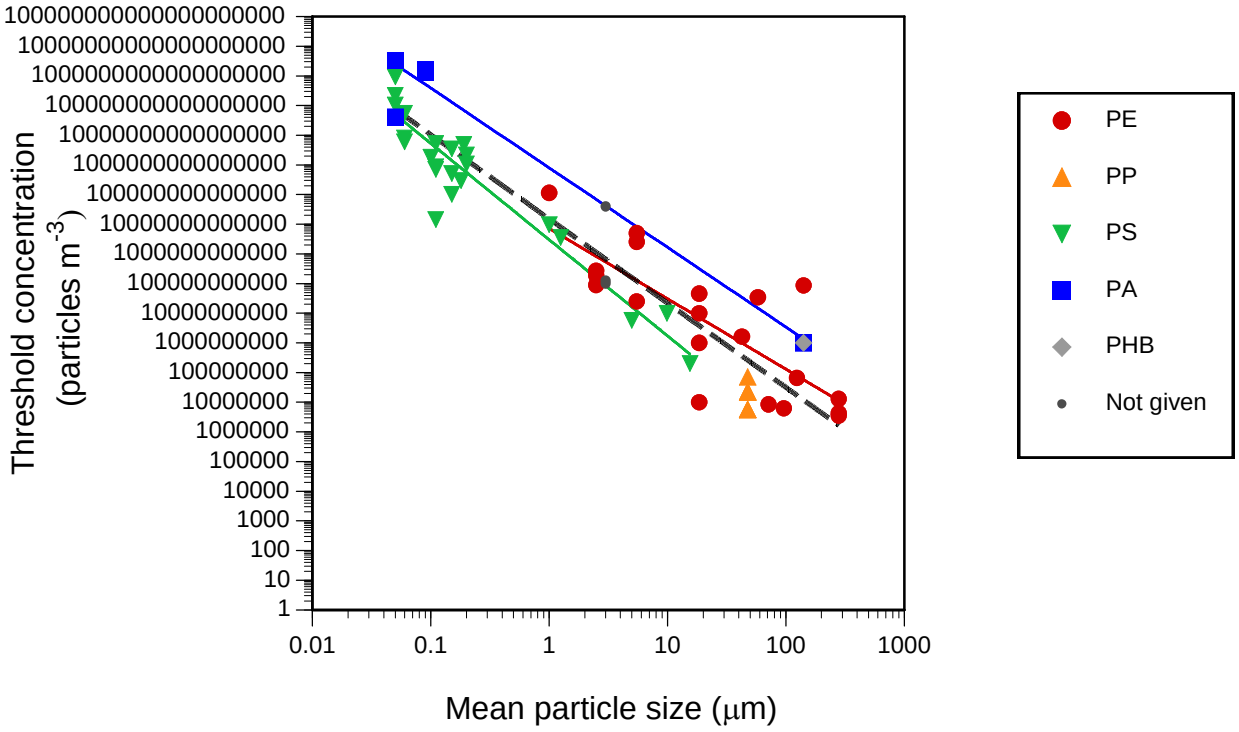

Fig 19. Effect of the polymer on the relationship between particle size and effect threshold concentration. Relationships shown for polyethylene (PE), polystyrene (PS), polyamide (PA), and all polymers (dashed black line): the range of sizes used for polypropylene (PP), polyhydroxybutyrate (PHB) and an unnamed proprietary polymer were insufficient to establish independent relationships. Nanoparticles ≤0.1 µm.

[4] ANCOVA uses a least squares approach to evaluate if the mean response differs across levels of a categorical independent variable, while statistically controlling for the effects of another continuous variable. Here, does the mean threshold concentration vary among the polymers used whilst taking into account the size of the particles.

Table 4.4. Results of ANCOVA testing the influence of polymer used or endpoint measured on the relationship between particle size and threshold effect concentration.

	F value	*p*
Particle Size	807.84	≤ 0.0001
Particle Size* Endpoint	0.80	0.5360
Particle Size* Polymer	0.91	0.4106
Particle Size*Endpoint*Polymer	0.69	0.5085

The majority of laboratory based toxicological studies used primary (virgin) microplastic particles, such that any effects observed could only be attributable to the particles, and not to any adsorbed chemicals or microbes. No studies considered effects of microbes attached to particles. A number of studies considered the effect of chemicals adsorbed onto microplastic particles and reported positive, negative and no response. However, due to variety of designs used in studies, it was not possible to draw substantial conclusions regarding underlying mechanisms.

There is evidence from ecotoxicological tests to indicate that microplastics comprised of different polymers do not differ in their toxicity.

4.6 Secondary question: Is there evidence to suggest impacts on populations of aquatic organisms?

No studies provided evidence to suggest that microplastics had an impact on populations of aquatic organisms. Most studies were solely based on laboratory experiments. Where field evidence was available, it either did not demonstrate that microplastics had a negative effect (e.g. Kazour et al. 2018) or did not discount other potentially confounding effects on populations sufficiently to ascribe any effect solely to microplastics (e.g. Hurley et al. 2017). To assess the potential impact of environmental pollutants in the receiving environment, three lines of empirical evidence (exposure, toxicity and damage) form the basis of any assessment (Long and Chapman 1985, Chapman 2007). Currently, there is insufficient evidence (particularly of damage in field populations associated with high concentrations of microplastics) to draw any conclusions regarding the impacts of microplastics on populations of freshwater or estuarine biota.

Currently, there is insufficient evidence (particularly of damage in field populations associated with high concentrations of microplastics) to draw any conclusions regarding the impacts of microplastics on populations of freshwater or estuarine biota.

4.7 Primary question: What is/are the impact(s) of microplastics on freshwater and estuarine biota?

As detailed in section 4.2, it was apparent that experimental studies indicate that high concentrations of microplastics can affect feeding, behaviour, growth and survival of freshwater and estuarine biota in a size specific manner. It is also likely that reproduction follows a similar pattern, but there is insufficient evidence at this time to draw this conclusion. With respect to sublethal effects, it is not clear whether such effects are lasting: whilst there was clear evidence of uptake of microplastics into the guts of biota (e.g. Sinche 2010, Hu et al. 2016, Imhof et al. 2017, Magni et al. 2018), it was also apparent that they rapidly void them from their guts (e.g. Wegner et al. 2012, Booth et al. 2016, Frydkjær et al. 2017, Bruck and Ford 2018, Revel et al. 2018) such that acute effects on behaviour and feeding may be reversible if exposure is reduced. The extent to which the sublethal effects measured manifest as damage at the population level is yet to be established.

Nevertheless, using threshold effect concentrations for the toxicological endpoints of feeding, behaviour, growth, reproduction and survival it was possible to establish a size-specific threshold for the effect of microplastics in water on freshwater and estuarine biota (see section 4.2). These size-specific thresholds corresponded to concentrations that were lower than 90% of threshold effect concentrations recorded for all endpoints including sublethal effects, and the equivalent for lethal effects (survival) of freshwater and estuarine biota.

By comparing these thresholds with the data on mean concentrations of microplastics per study reported from field samples (compiled in ER1), it was apparent that the threshold for lethal effects was at a higher concentration than any reported concentration of microplastics from freshwater and estuarine environments (Fig 20a). However, there were some studies of microplastics in freshwater and estuarine environments which reported environmental concentrations that were greater than the calculated 10%ile threshold of all endpoints including sublethal effects. By using quantile regression to determine the distribution of mean concentrations reported from field samples collected from freshwater and estuarine environments, it was possible to provide more confidence in the estimated proportion of sites that exceed the calculated 10%ile thresholds (Fig 20b). The calculated threshold concentrations for lethal effects were considerably higher than 99% of reported environmental concentrations, suggesting that lethal effects are highly unlikely. Over certain size ranges the calculated threshold concentration including sublethal effects was

exceeded by the highest 10 percentile of reported environmental concentrations (i.e. the calculated sublethal threshold was between the 99%ile and 90%ile), suggesting that there is a risk that sublethal effects may occur in a small proportion of sites.

It should be noted, however, that the results of quantile regression are heavily influenced by the density of data along the y axis. Individual points have more influence on the results where data are sparse, particularly towards the extremes of the range. This was true of the upper end of the size range of particles used to calculate the 10%ile threshold of all endpoints including sublethal effects, and of the upper and lower ends of the range of reported concentrations of microplastics from freshwater and estuarine environments. This is important as it was towards the upper end of the range of particle sizes used in ecotoxicological tests where the 10%ile threshold of all endpoints including sublethal effects was exceeded. The inclusion of further ecotoxicological tests using larger particles would improve the confidence in the threshold across this critical size range.

The calculated size specific threshold concentration for lethal effects was considerably higher than 99% of reported environmental concentrations, suggesting that lethal effects of microplastics on freshwater and estuarine biota are highly unlikely. Over certain size ranges the calculated size specific threshold concentration for sublethal effects was exceeded by the highest 10% of concentrations reported from environmental samples, suggesting that there may be a possible risk of some sublethal effects in a small proportion of sites.

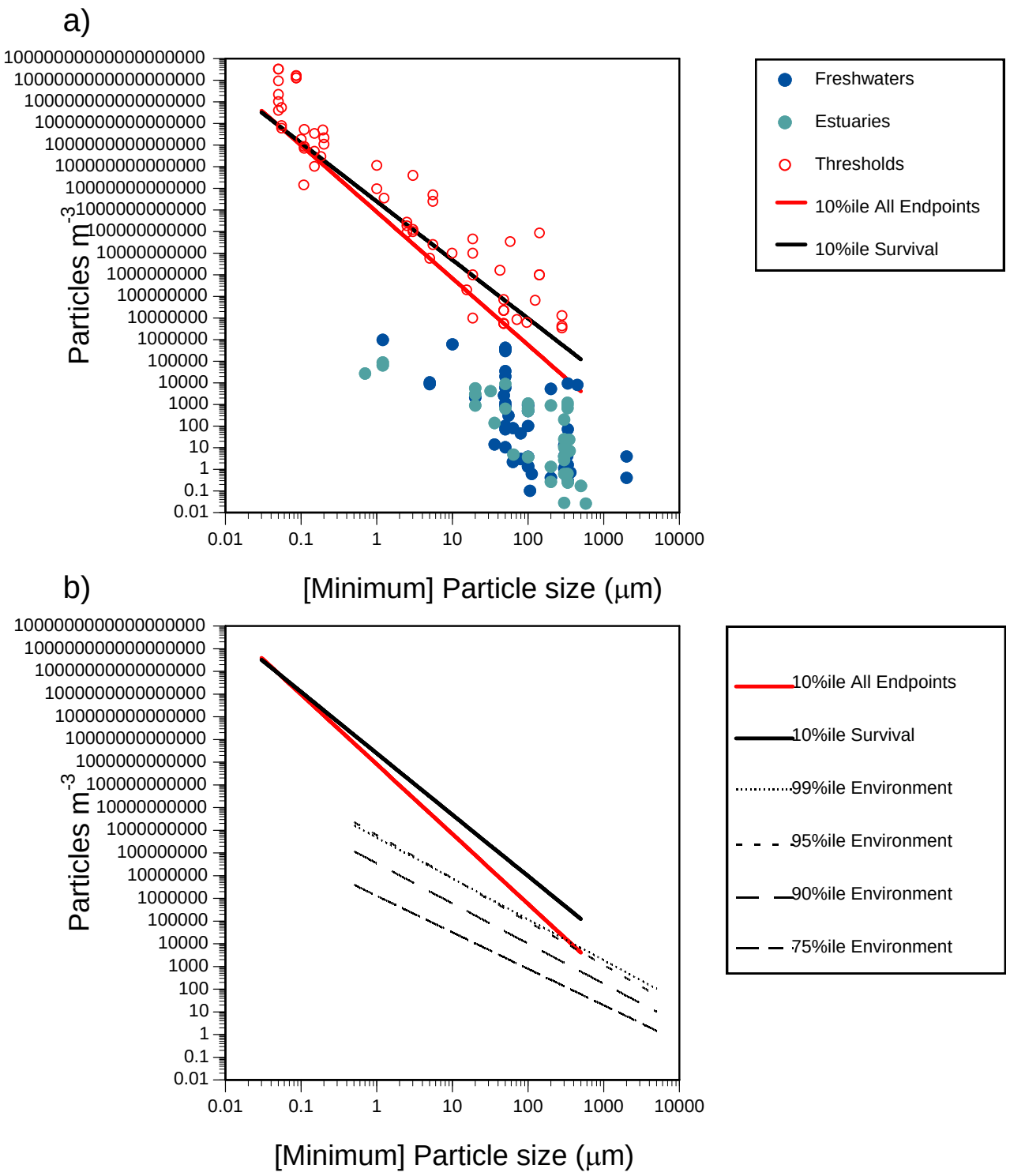

Fig 20. Concentrations of microplastics from samples of freshwater and estuarine environments (mean per study considered in ER1) together with size specific 10 %ile thresholds concentrations for all (including sublethal) and lethal endpoints, showing a) all data, and b) percentile distributions of reported concentrations of microplastics from freshwater and estuarine environments. Nanoparticles ≤0.1 µm.

5. Limitations

Key limitations of this review are outlined below; these stem primarily from the fact that this is a relatively new and developing scientific field.

The size range of microplastic particles used in laboratory studies do not compare well with the size ranges of particles which have been quantified in the environment, where the methods used to date have focussed on describing concentrations of larger particles. Similarly, a limited range of particle types (polymers, morphologies, origin, age) have been used in laboratory studies compared with those found in the environment. This mismatch adds uncertainty regarding how applicable the findings from laboratory studies are to the conditions found in the environment.

A limited range of taxa have been used in laboratory studies, which adds uncertainty regarding the generality of any conclusions for the range of species found in the environment.

There were inconsistencies in the way methods and results were reported in different studies. Whilst efforts were made to extract information in a consistent way, this inconsistency in reporting among primary sources has constrained the comparisons that could be made, and will have added uncertainty when comparing among studies.

The design of the studies considered, and the preconceptions underlying these designs, are likely to have influenced the results obtained by those studies. This is particularly true of the exposure conditions used in tests, which may have enhanced effects through the use of starved organisms without access to alternative food sources.

The findings presented are infuenced by the reliability of the primary literature, including grey literature, on which this report is based. An assessment of the reliability of the studies included in this review was undertaken (see section 4.1), which indicated a decline in reliability over time. To limit the effect of unreliable studies, this assessment of reliability was used to exclude studies from critical analyses in the review. However, the field would benefit substantially from improvements in the reliability of the primary literature.

6. Conclusions

The aim of this evidence review was to address the question "What is/are the impact(s) of microplastics on freshwater and estuarine biota?" using the evidence available from studies relevant to the biota of freshwaters and estuaries. It was clear from this evidence that the concentration of microplastics required to cause detrimental effects was dependent upon the size of the particles. As such, a single threshold concentration is not relevant to microplastics, and standard approaches to define hazard limits are not applicable. Rather a size specific threshold should be used to describe the hazard, best described by a relationship between particle size and the threshold concentration.

Although limited data were available, the evidence from the literature review indicated that there was no difference among taxonomic groups in their susceptibility to the effects of microplastics. This finding contrasts with the opinion of the academic experts interviewed, who expected differences among taxa based on their feeding strategy, but may be due to the limited range of taxa used in studies, as acknowledged by the experts interviewed.

Whilst the experts expressed concerns about the effect of chemicals adsorbed onto microplastic particles, the evidence from the primary literature was not conclusive: studies indicated positive, negative and no interaction between chemicals and microplastics. However, due to the variety of designs used in studies, it was not possible to draw substantial conclusions regarding underlying mechanisms.

Both the systematic review and academic experts interviewed indicated that the ecotoxicological studies undertaken to date have used microplastic particles of types, sizes and concentrations that do not reflect those described by studies of microplastics in environmental samples from freshwaters and estuaries. However, using data accumulated through the systematic review it was possible to identify size specific thresholds for both lethal and sublethal effects on biota. Quantile regression was used to define the size specific concentrations corresponding to an effect in 10 percent of ecotoxicological tests, i.e. concentrations at this limit are lower than those that caused an effect in 90% of tests reported.

By comparing these thresholds with reported concentrations of microplastics from freshwater and estuarine environments it was evident that the risk of lethal effects of microplastics on biota is very low: the size specific lethal threshold was considerably higher than the 99%ile of reported mean concentrations. However, the evidence suggested that for some particle sizes, the reported concentrations of microplastics in freshwater and estuarine environments might be in exceedance of the size specific threshold for sublethal effects on biota. It was estimated (using quantile regression) that this may be relevant to the highest 10 percentile of reported environmental concentrations globally. As such, by inference, there is a possible risk that concentrations may be sufficiently high to potentially cause sublethal effects in more than 10% of taxa at a small proportion of sites.

However, there are a number of caveats on this result, particularly regarding sublethal effects.

i) The range of particle sizes used in laboratory ecotoxicological tests did not correspond well with those reported for environmental samples. This mismatch adds uncertainty to our understanding of risk from microplastics. The majority of evidence describing toxicological thresholds is for smaller particles but we do not know the concentrations at which such particles are present in the environment and, correspondingly, we don't know the toxicological thresholds for the larger particles for which we do know the concentrations present in the environment. In particular, the confidence in the size specific lethal threshold was lower in the size range where exceedance occurred.
ii) The estimates of environmental concentrations used were based on sampling and analytical methods that were rarely scientifically robust and appropriate (see ER1).
iii) It is not clear if the sublethal effects measured in ecotoxicological tests are permanent, nor the extent to which the sublethal effects measured manifest as damage at the population level.
iv) Tests were typically conducted under conditions that would enhance the uptake of microplastics relative to field conditions and, hence, may represent a "worst case scenario".

As no studies provided evidence to suggest that microplastics had an impact on populations of aquatic organisms in freshwaters and estuaries, it is not possible to verify the conclusions regarding sublethal effects.

This report also concludes that the evidence regarding the impact of microplastics on freshwater and estuarine biota is generally of low reliability. Of concern is the fact that the quality of published works appears to be declining.

7. Recommendations

It is strongly recommended that the authors and publishers of ecotoxicological studies of microplastics follow robust criteria for reporting and evaluating ecotoxicity data such as the CRED method outlined by Moermond et al. (2016). We also recommend that authors of reports present methods and results in a more consistent manner, for example the units used to express dose concentrations and the type of threshold values reported.

A single threshold concentration is not relevant to microplastics, and standard approaches to define hazard limits are not applicable: it is recommended that any thresholds used by regulators to describe the hazard presented by microplastics when setting safe limits should take the size of particles into account.

It is also recommended to the research community that evidence is gained from further robust tests of the effects of microplastic particles of a size, type, polymer and age that is relevant to those described by studies of microplastics in environmental samples from

freshwaters and estuaries. This is particularly true of larger microplastic particles, as very few tests have been conducted with particles greater than 100 µm.

It is also recommended to the research community that natural inert particles are used as a control in a dose response manner in order to determine if any effects are attributable to the microplastic *per se*, or simply an effect caused by particles of no nutritional value.

8. References

Aljaibachi, R. and A. Callaghan. 2018. Impact of polystyrene microplastics on *Daphnia magna* mortality and reproduction in relation to food availability. PeerJ **2018**.

Au, S. 2017. Toxicity of Microplastics to Aquatic Organisms. text. Clemson University.

Ball, H., R. Cross, E. Grove, A. Horton, A. Johnson, M. Jürgens, D. Read, and C. Svendsen. 2019. Sink to River - River to Tap. A review of potential risks from nanoparticles and microplastics. EQ01A231, UK Water Industry Research Limited, London.

Booth, A. M., B. H. Hansen, M. Frenzel, H. Johnsen, and D. Altin. 2016. Uptake and toxicity of methylmethacrylate-based nanoplastic particles in aquatic organisms. Environmental Toxicology and Chemistry **35**:1641-1649.

Bruck, S. and A. T. Ford. 2018. Chronic ingestion of polystyrene microparticles in low doses has no effect on food consumption and growth to the intertidal amphipod Echinogammarus marinus? Environmental Pollution **233**:1125-1130.

Carlos, d. S. L., L. G. Luis, and L. Guilhermino. 2015. Effects of microplastics on juveniles of the common goby (Pomatoschistus microps): confusion with prey, reduction of the predatory performance and efficiency, and possible influence of developmental conditions. Environ Pollut **196**:359-362.

Chapman, P. M. 2007. Do not disregard the benthos in sediment quality assessments! Marine Pollution Bulletin **54**:633-635.

Collins, A.M., Coughlin, D., Miller, J., Kirk, S. 2015. The Production of Quick Scoping Reviews and Rapid Evidence Assessments: A How to Guide. JWEG.

ECHA (European Chemical Agency) 2018. Note on substance identification and the potential scope of a restriction on uses of 'microplastics' Version 1.1 – 16/10/2018.

Frydkjær, C. K., N. Iversen, and P. Roslev. 2017. Ingestion and Egestion of Microplastics by the Cladoceran Daphnia magna: Effects of Regular and Irregular Shaped Plastic and Sorbed Phenanthrene. Bulletin of Environmental Contamination and Toxicology **99**:655-661.

Geyer, R., J. R. Jambeck, and K. L. Law. 2017. Production, use, and fate of all plastics ever made. Science Advances **3**.

Hermsen, E., S. M. Mintenig, E. Besseling, and A. A. Koelmans. 2018. Quality Criteria for the Analysis of Microplastic in Biota Samples: A Critical Review. Environmental Science & Technology **52**:10230-10240.

Hu, L., L. Su, Y. Xue, J. Mu, J. Zhu, J. Xu, and H. Shi. 2016. Uptake, accumulation and elimination of polystyrene microspheres in tadpoles of *Xenopus tropicalis*. Chemosphere **164**:611-617.

Hurley, R., J. Woodward, and J. J. Rothwell. 2018. Microplastic contamination of river beds significantly reduced by catchment-wide flooding. Nature Geoscience **11**:251-257.

Hurley, R. R., J. C. Woodward, and J. J. Rothwell. 2017. Ingestion of Microplastics by Freshwater Tubifex Worms. Environmental Science & Technology **51**:12844-12851.

Imhof, H. K., J. Rusek, M. Thiel, J. Wolinska, and C. Laforsch. 2017. Do microplastic particles affect Daphnia magna at the morphological, life history and molecular level? Plos One **12**:e0187590.

Ivar do Sul, J. A. and M. F. Costa. 2014. The present and future of microplastic pollution in the marine environment. Environmental Pollution **185**:352-364.

Kazour, M., S. Jemaa, M. El Rakwe, G. Duflos, L. Hermabassiere, A. Dehaut, F. Le Bihanic, J. Cachot, V. Cornille, K. Rabhi, G. Khalaf, and R. Amara. 2018. Juvenile fish caging as a tool for assessing microplastics contamination in estuarine fish nursery grounds. Environmental Science and Pollution Research **27**: 3548–3559.

Long, E. R. and P. M. Chapman. 1985. A sediment quality triad - measures of sediment contamination, toxicity and infaunal community composition in Puget Sound. Marine Pollution Bulletin **16**:405-415.

Magni, S., A. Binelli, F. Gagne, C. Andre, J. Auclair, H. Hanana, T. C. Della, C. C. Parenti, and F. Bonasoro. 2018. Evaluation of uptake and chronic toxicity of virgin polystyrene microbeads in freshwater zebra mussel *Dreissena polymorpha* (Mollusca: Bivalvia). Sci Total Environ **631-632**:778-788.

Moermond, C. T. A., R. Kase, M. Korkaric, and M. Agerstrandk. 2016. CRED: criteria for reporting and evaluating ecotoxicity data. Environmental Toxicology and Chemistry **35**:1297-1309.

Nizzetto, L., G. Bussi, M. N. Futter, D. Butterfield, and P. G. Whitehead. 2016. A theoretical assessment of microplastic transport in river catchments and their retention by soils and river sediments. Environmental Science-Processes & Impacts **18**:1050-1059.

Plastics*Europe* Association of Plastic Manufacturers. 2018. Plastics – the Facts 2018. An analysis of European plastics production, demand and waste data. PlasticsEurope AISBL, Belgium.

Posthuma L., Suter G.W. II and Traas T.P. (2002). Species Sensitivity Distributions in Ecotoxicology. Lewis Publishers, Boca Raton, FL. 587 pp.

Revel, M., N. Yakovenko, T. Caley, C. Guillet, A. Châtel, and C. Mouneyrac. 2018. Accumulation and immunotoxicity of microplastics in the estuarine worm *Hediste diversicolor* in environmentally relevant conditions of exposure. Environmental Science and Pollution Research.

Santillo, D., K. Brigden, V. Pasteur, F. Nicholls, P. Morozzo, and P. Johnston. 2019. Plastic pollution in UK's rivers: a 'snapshot' survey of macro- and micro-plastic contamination in surface waters of 13 river systems across England, Wales, Scotland and Northern Ireland. Greenpeace Research Laboratories Technical Report 04-2019, Greenpeace http://www.greenpeace.to/greenpeace/wp-content/uploads/2019/06/GRL-TR-04-2019-plastics-in-UK-rivers.pdf.

Sinche, F. 2010. Impact of Microsparticle Concentration Levels upon Toxicity of Phenol to Artemia. MSc Thesis, Clemson University, South Carolina, USA.

Straub, S., P. E. Hirsch, and P. Burkhardt-Holm. 2017. Biodegradable and petroleum-based microplastics do not differ in their ingestion and excretion but in their biological effects in a freshwater invertebrate *Gammarus fossarum*. International Journal of Environmental Research and Public Health **14**.

Thompson, R. C., Y. Olsen, R. P. Mitchell, A. Davis, S. J. Rowland, A. W. G. John, D. McGonigle, and A. E. Russell. 2004. Lost at sea: Where is all the plastic? Science **304**:838-838.

Wegner, A., E. Besseling, E. M. Foekema, P. Kamermans, and A. A. Koelmans. 2012. Effects of nanopolystyrene on the feeding behavior of the blue mussel (*Mytilus edulis* L.). Environmental Toxicology and Chemistry **31**:2490-2497.

Wright, S. L., D. Rowe, M. J. Reid, K. V. Thomas, and T. S. Galloway. 2015. Bioaccumulation and biological effects of cigarette litter in marine worms. Scientific Reports **5**: 14119.

Appendix A ER3_Capture.xls

See Excel spreadsheet ER3_Capture.xls. Column headers reproduced here for convenience

Evidence							
Ref No	Reference	Year	Title	Journal	Vol	Pages	URL
free	free	free	free	free	free	free	free

Taxonomic Group	Species detail	Size of organism		Functional group	Study Type	Waterbody Type
			units			
menu	free	free	menu	menu	menu	menu

Matrix exposed to	Plastic			Size of particles considered (in µm)		
	Macro-	Micro-	Nano-	mean	smallest	largest
menu	Y/N	Y/N	Y/N	free	free	free

Morphology of particles considered		Polymers considered		Sources/products considered	Character	
	detail		detail		Primary/secondary	
menu	free	menu	free	menu	menu	

Adsorbed chemicals		Adsorbed microbes		Other multiple-stressors		Toxicological endpoint	
	Which Chemicals?		Which Microbes		Which other Stressors		detail
Y/N	free	Y/N	free	Y/N	free	menu	free

Concentration used							Concentration consumed			
Lowest	Highest	Number of treatment levels	Threshold	Relationship	Details	Units	Lowest	Highest	Threshold	Units
free	free	free	free	menu	free		free	free	free	menu

Effect attributed to adsorbed			Effect of multiple stressors		Population level effects?	
chemicals	microbes	detail		detail		detail
Y/N	Y/N	free	Y/N	free	Y/N	free

Continent	UK Location			
	UK	Lat	Long	Comments
menu	Y/N	free	free	free

menu = choice of options from pull down menu

Y/N = choice of Yes or No from pull down menu

free = any information can be entered into the field

Appendix B Evidence Sources Used

Reference	Year	Title	Journal	Vol	Pages
Aljaibachi, R and Callaghan A	2018	Impact of polystyrene microplastics on *Daphnia magna* mortality and reproduction in relation to food availability	PeerJ	6	e4601
Al-Jaibachi, R, Cuthbert RN and Callaghan A	2019	Examining effects of ontogenic microplastic transference on *Culex* mosquito mortality and adult weight	Sci Total Environ	651	871-876
Asmonaite, G, Larsson K, Undeland I, Sturve J and Almroth BC	2018	Size Matters: Ingestion of Relatively Large Microplastics Contaminated with Environmental Pollutants Posed Little Risk for Fish Health and Fillet Quality	Environmental Science & Technology	52	14381-14391
Asmonaite, G, Sundh H, Asker N and Carney Almroth B	2018	Rainbow Trout Maintain Intestinal Transport and Barrier Functions Following Exposure to Polystyrene Microplastics	Environ Sci Technol	52	14392-14401
Au, S	2017	Toxicity of Microplastics to Aquatic Organisms	Clemson University	PhD	
Barboza, LGA, Vieira LR, Branco V, Carvalho C and Guilhermino L	2018	Microplastics increase mercury bioconcentration in gills and bioaccumulation in the liver, and cause oxidative stress and damage in *Dicentrarchus labrax* juveniles	Scientific Reports	8	15655
Barboza, LGA, Vieira LR, Branco V, Figueiredo N, Carvalho F, Carvalho C and Guilhermino L	2018	Microplastics cause neurotoxicity, oxidative damage and energy-related changes and interact with the bioaccumulation of mercury in the European seabass, *Dicentrarchus labrax* (Linnaeus, 1758)	Aquatic Toxicology	195	49-57
Batel, A, Borchert F, Reinwald H, Erdinger L and Braunbeck T	2018	Microplastic accumulation patterns and transfer of benzo a pyrene to adult zebrafish (*Danio rerio*) gills and zebrafish embryos	Environmental Pollution	235	918-930

Reference	Year	Title	Journal	Vol	Pages
Batel, A, Linti F, Scherer M, Erdinger L and Braunbeck T	2016	Transfer of benzo a pyrene from microplastics to *Artemia* nauplii and further to zebrafish via a trophic food web experiment: CYP1A induction and visual tracking of persistent organic pollutants	Environmental Toxicology and Chemistry	35	1656-1666
Beckingham, B and Ghosh U	2017	Differential bioavailability of polychlorinated biphenyls associated with environmental particles: Microplastic in comparison to wood, coal and biochar	Environmental Pollution	220	150-158
Blarer, P and Burkhardt-Holm P	2016	Microplastics affect assimilation efficiency in the freshwater amphipod *Gammarus fossarum*	Environ Sci Pollut Res	23	23522-23532
Booth, AM, Hansen BH, Frenzel M, Johnsen H and Altin D	2015	Uptake and toxicity of methylmethacrylate-based nanoplastic particles in aquatic organisms	Environmental Toxicology and Chemistry	35	1641–1649
Browne, MA, Niven SJ, Galloway TS, Rowland SJ and Thompson RC	2013	Microplastic Moves Pollutants and Additives to Worms, Reducing Functions Linked to Health and Biodiversity	Current Biology	23	2388-2392
Bruck, S and Ford AT	2018	Chronic ingestion of polystyrene microparticles in low doses has no effect on food consumption and growth to the intertidal amphipod *Echinogammarus marinus*?	Environmental Pollution	233	1125-1130
Canniff, PM and Hoang TC	2018	Microplastic ingestion by *Daphnia magna* and its enhancement on algal growth	Sci Total Environ	633	500-507
Carlos, dSL, Luis LG and Guilhermino L	2015	Effects of microplastics on juveniles of the common goby (*Pomatoschistus microps*): confusion with prey, reduction of the predatory performance and efficiency, and possible influence of developmental conditions	Environ Pollut	196	359-362

Reference	Year	Title	Journal	Vol	Pages
Casado, MP, Macken, A and Byrne HJ	2013	Ecotoxicological assessment of silica and polystyrene nanoparticles assessed by a multitrophic test battery	Environment International	51	97–105
Chen, Q, Gundlach M, Yang S, Jiang J, Velki M, Yin D and Hollert H	2017	Quantitative investigation of the mechanisms of microplastics and nanoplastics toward zebrafish larvae locomotor activity	Sci Total Environ	584-585	1022-1031
Dantas, DV, Ribeiro CIR, Frischknecht CCA, Machado R and Farias EGG	2019	Ingestion of plastic fragments by the Guri sea catfish *Genidens genidens* (Cuvier, 1829) in a subtropical coastal estuarine system	Environmental Science and Pollution Research	26	8344–8351
De Felice, B, Bacchetta R, Santo N, Tremolada P and Parolini M	2018	Polystyrene microplastics did not affect body growth and swimming activity in *Xenopus laevis* tadpoles	Environ Sci Pollut Res	25	34644-34651
Détrée, C and Gallardo-Escárate C	2017	Polyethylene microbeads induce transcriptional responses with tissue-dependent patterns in the mussel *Mytilus galloprovincialis*	Journal of Molluscan Studies	83	220-225
Ding, J, Zhang S, Razanajatovo RM, Zou H and Zhu W	2018	Accumulation, tissue distribution, and biochemical effects of polystyrene microplastics in the freshwater fish red tilapia (*Oreochromis niloticus*)	Environ Pollut (Oxford, U K)	238	1-9
Espinosa, C, Cuesta A and Esteban MÁ	2017	Effects of dietary polyvinylchloride microparticles on general health, immune status and expression of several genes related to stress in gilthead seabream (*Sparus aurata* L.)	Fish and Shellfish Immunology	68	251-259
Ferreira, P, Fonte E, Soares ME, Carvalho F and Guilhermino L	2016	Effects of multi-stressors on juveniles of the marine fish *Pomatoschistus microps*: Gold nanoparticles, microplastics and temperature	Aquatic Toxicology	170	89-103

Reference	Year	Title	Journal	Vol	Pages
Fonte, E, Ferreira P and Guilhermino L	2016	Temperature rise and microplastics interact with the toxicity of the antibiotic cefalexin to juveniles of the common goby (*Pomatoschistus microps*): Post-exposure predatory behaviour, acetylcholinesterase activity and lipid peroxidation	Aquatic Toxicology	180	173-185
Frydkjaer, CK, Iversen N and Roslev P	2017	Ingestion and Egestion of Microplastics by the Cladoceran *Daphnia magna*: Effects of Regular and Irregular Shaped Plastic and Sorbed Phenanthrene	Bulletin of Environmental Contamination and Toxicology	99	655-661
Gerdes, Z, Ogonowski M, Nybom I, Ek C, Adolfsson-Erici M, Barth A and Gorokhova E	2019	Microplastic-mediated transport of PCBs? A depuration study with *Daphnia magna*	PLoS ONE	14	e0205378
Gonçalves, APC	2017	Effects of polymeric nanoparticles on fish : a multiparametric approach	Universidade de Aveiro	MSc	
Grigorakis, S, Mason SA and Drouillard KG	2017	Determination of the gut retention of plastic microbeads and microfibers in goldfish (*Carassius auratus*)	Chemosphere	169	233-238
Guilhermino, L, Vieira LR, Ribeiro D, Tavares AS, Cardoso V, Alves A and Almeida JM	2018	Uptake and effects of the antimicrobial florfenicol, microplastics and their mixtures on freshwater exotic invasive bivalve *Corbicula fluminea*	Sci Total Environ	622-623	1131-1142
Guven, O, Bach L, Munk P, Dinh KV, Mariani P and Nielsen TG	2018	Microplastic does not magnify the acute effect of PAH pyrene on predatory performance of a tropical fish (*Lates calcarifer*)	Aquatic Toxicology	198	287-293

Reference	Year	Title	Journal	Vol	Pages
Horton, AA, Vijver MG, Lahive E, Spurgeon DJ, Svendsen C, Heutink R, van Bodegom PM and Baas J	2018	Acute toxicity of organic pesticides to *Daphnia magna* is unchanged by co-exposure to polystyrene microplastics	Ecotoxicology and Environmental Safety	166	26-34
Hu, L, Su L, Xue Y, Mu J, Zhu J, Xu J and Shi H	2016	Uptake, accumulation and elimination of polystyrene microspheres in tadpoles of *Xenopus tropicalis*	Chemosphere	164	611-617
Hurley, RR, Woodward JC and Rothwell JJ	2017	Ingestion of Microplastics by Freshwater *Tubifex* Worms	Environ Sci Technol	51	12844-12851
Imhof HK, Rusek J, Thiel M, Wolinska J and Laforsch C	2017	Do microplastic particles affect *Daphnia magna* at the morphological, life history and molecular level?	PLoS One	12	e0187590
Imhof, HK and Laforsch C	2016	Hazardous or not – Are adult and juvenile individuals of *Potamopyrgus antipodarum* affected by non-buoyant microplastic particles?	Environmental Pollution	218	383-391
Jabeen, K, Li B, Chen Q, Su L, Wu C, Hollert H and Shi H	2018	Effects of virgin microplastics on goldfish (*Carassius auratus*)	Chemosphere	213	323-332
Jaikumar, G, Baas J, Brun NR, Vijver MG and Bosker T	2018	Acute sensitivity of three Cladoceran species to different types of microplastics in combination with thermal stress	Environmental Pollution	239	733-740
Jemec, A, Horvat P, Kunej U, Bele M and Krzan A	2016	Uptake and effects of microplastic textile fibers on freshwater crustacean *Daphnia magna*	Environ Pollut	219	201-209
Jin, Y, Xia J, Pan Z, Yang J, Wang W and Fu Z	2018	Polystyrene microplastics induce microbiota dysbiosis and inflammation in the gut of adult zebrafish	Environmental Pollution	235	322-329

Reference	Year	Title	Journal	Vol	Pages
Kalcikova, G, Gotvajn AZ, Kladnik A and Jemec A	2017	Impact of polyethylene microbeads on the floating freshwater plant duckweed *Lemna minor*	Environmental Pollution	230	1108-1115
Karami, A, Groman DB, Wilson SP, Ismail P and Neela VK	2017	Biomarker responses in zebrafish (*Danio rerio*) larvae exposed to pristine low-density polyethylene fragments	Environmental Pollution	223	466-475
Karami, A, Romano N, Galloway T and Hamzah H	2016	Virgin microplastics cause toxicity and modulate the impacts of phenanthrene on biomarker responses in African catfish (*Clarias gariepinus*)	Environmental Research	151	58-70
Katzenberger, TD	2015	Assessing the biological effects of exposure to microplastics in the three-spined stickleback (*Gasterosteus aculeatus*) (Linnaeus 1758)	University of York	PhD	
Kazour, M, Jemaa S, El Rakwe M, Duflos G, Hermabassiere L, Dehaut A, Le Bihanic F, Cachot J, Cornille V, Rabhi K, Khalaf G and Amara R	2018	Juvenile fish caging as a tool for assessing microplastics contamination in estuarine fish nursery grounds	Environmental Science and Pollution Research	27	3548–3559
Kim, D, Chae Y and An YJ	2017	Mixture Toxicity of Nickel and Microplastics with Different Functional Groups on *Daphnia magna*	Environ Sci Technol	51	12852-12858
Kleinteich, J, Seidensticker S, Marggrander N and Zarfl C	2018	Microplastics Reduce Short-Term Effects of Environmental Contaminants. Part II: Polyethylene Particles Decrease the Effect of Polycyclic Aromatic Hydrocarbons on Microorganisms	Int J Environ Res Public Health	15	

Reference	Year	Title	Journal	Vol	Pages
Kokalj, AJ, Kunej U and Skalar T	2018	Screening study of four environmentally relevant microplastic pollutants: Uptake and effects on *Daphnia magna* and *Artemia franciscana*	Chemosphere	208	522-529
Lei, L, Wu S, Lu S, Liu M, Song Y, Fu Z, Shi H, Raley-Susman KM and He D	2018	Microplastic particles cause intestinal damage and other adverse effects in zebrafish *Danio rerio* and nematode *Caenorhabditis elegans*	Sci Total Environ	619-620	1-8
LeMoine, CMR, Kelleher BM, Lagarde R, Northam C, Elebute OO and Cassone BJ	2018	Transcriptional effects of polyethylene microplastics ingestion in developing zebrafish (*Danio rerio*)	Environ Pollut	243	591-600
Liu, Y, Wang Z, Wang S, Fang H, Ye N and Wang D	2019	Ecotoxicological effects on *Scenedesmus obliquus* and *Danio rerio* co-exposed to polystyrene nano-plastic particles and natural acidic organic polymer	Environmental Toxicology and Pharmacology	67	21-28
Liu, Z, Cai M, Yu P, Chen M, Wu D, Zhang M and Zhao Y	2018	Age-dependent survival, stress defense, and AMPK in *Daphnia pulex* after short-term exposure to a polystyrene nanoplastic	Aquatic Toxicology	204	1-8
Lu, C, Kania PW and Buchmann K	2018	Particle effects on fish gills: An immunogenetic approach for rainbow trout and zebrafish	Aquaculture	484	98-104
Lu, K, Qiao R, An H and Zhang Y	2018	Influence of microplastics on the accumulation and chronic toxic effects of cadmium in zebrafish (*Danio rerio*)	Chemosphere	202	514-520
Lu, Y, Zhang Y, Deng Y, Jiang W, Zhao Y, Geng J, Ding L and Ren H	2016	Uptake and Accumulation of Polystyrene Microplastics in Zebrafish (*Danio rerio*) and Toxic Effects in Liver	Environmental Science & Technology	50	4054-4060

Reference	Year	Title	Journal	Vol	Pages
Luan, L, Wang X, Zheng H, Liu L, Luo X and Li F	2019	Differential toxicity of functionalized polystyrene microplastics to clams (*Meretrix meretrix*) at three key development stages of life history	Marine Pollution Bulletin	139	346-354
Luis, LG, Ferreira P, Fonte E, Oliveira M and Guilhermino L	2015	Does the presence of microplastics influence the acute toxicity of chromium(VI) to early juveniles of the common goby (*Pomatoschistus microps*)? A study with juveniles from two wild estuarine populations	Aquat Toxicol	164	163-174
Ma, Y, Huang A, Cao S, Sun F, Wang L, Guo H and Ji R	2016	Effects of nanoplastics and microplastics on toxicity, bioaccumulation, and environmental fate of phenanthrene in fresh water	Environ Pollut	219	166-173
Magni, S, Binelli A, Gagne F, Andre C, Auclair J, Hanana H, Della TC, Parenti CC and Bonasoro F	2018	Evaluation of uptake and chronic toxicity of virgin polystyrene microbeads in freshwater zebra mussel *Dreissena polymorpha* (Mollusca: Bivalvia)	Sci Total Environ	631-632	778-788
Mao, Y, Ai H, Chen Y, Zhang Z, Zeng P, Kang L, Li W, Gu W, He Q and Li H	2018	Phytoplankton response to polystyrene microplastics: Perspective from an entire growth period	Chemosphere	208	59-68
Murphy, F and Quinn B	2018	The effects of microplastic on freshwater *Hydra attenuata* feeding, morphology & reproduction	Environ Pollut	234	487-494
Nasser, F and Lynch I	2016	Secreted protein eco-corona mediates uptake and impacts of polystyrene nanoparticles on *Daphnia magna*	Journal of Proteomics	137	45-51
Nematdoost Haghi, B and Banaee M	2017	Effects of micro-plastic particles on paraquat toxicity to common carp (*Cyprinus carpio*): biochemical changes	International Journal of Environmental Science and Technology	14	521-530

Reference	Year	Title	Journal	Vol	Pages
O'Donovan, S, Mestre NC, Abel S, Fonseca TG, Carteny CC, Cormier B, Keiter SH and Bebianno MJ	2018	Ecotoxicological effects of chemical contaminants adsorbed to microplastics in the Clam *Scrobicularia plana*	Frontiers in Marine Science	5	143
Ogonowski, M, Schur C, Jarsen A and Gorokhova E	2016	The Effects of Natural and Anthropogenic Microparticles on Individual Fitness in *Daphnia magna*	Plos One	11	e0155063
Oliveira, M, Ribeiro A, Hylland K and Guilhermino L	2013	Single and combined effects of microplastics and pyrene on juveniles (0+group) of the common goby *Pomatoschistus microps* (Teleostei, Gobiidae)	Ecological Indicators	34	641-647
Pacheco, A, Martins A and Guilhermino L	2018	Toxicological interactions induced by chronic exposure to gold nanoparticles and microplastics mixtures in *Daphnia magna*	Science of the Total Environment	628-629	474-483
Paul-Pont, I, Lacroix C, González Fernández C, Hégaret H, Lambert C, Le Goïc N, Frère L, Cassone AL, Sussarellu R, Fabioux C, Guyomarch J, Albentosa M, Huvet A and Soudant P	2016	Exposure of marine mussels *Mytilus* spp. to polystyrene microplastics: Toxicity and influence on fluoranthene bioaccumulation	Environmental Pollution	216	724-737
Pikuda, O, Xu EG, Berk D and Tufenkji N	2019	Toxicity assessments of micro- and nanoplastics can be confounded by preservatives in commercial formulations	Environ Sci Technol Lett	6	21-25

Reference	Year	Title	Journal	Vol	Pages
Qu, H, Ma R, Wang B, Yang J, Duan L and Yu G	2018	Enantiospecific toxicity, distribution and bioaccumulation of chiral antidepressant venlafaxine and its metabolite in loach (*Misgurnus anguillicaudatus*) co-exposed to microplastic and the drugs	J Hazard Mater	370	203-211
Qu, M, Xu KN, Li YH, Wong G and Wang DY	2018	Using acs-22 mutant *Caenorhabditis elegans* to detect the toxicity of nanopolystyrene particles	Science of the Total Environment	643	119-126
Redondo-Hasselerharm, PE, Falahudin D, Peeters E and Koelmans AA	2018	Microplastic Effect Thresholds for Freshwater Benthic Macroinvertebrates	Environ Sci Technol	52	2278-2286
Rehse, S, Kloas W and Zarfl C	2016	Short-term exposure with high concentrations of pristine microplastic particles leads to immobilisation of *Daphnia magna*	Chemosphere	153	91-99
Rehse, S, Kloas W and Zarfl C	2018	Microplastics reduce short-term effects of environmental contaminants. Part I: Effects of Bisphenol A on freshwater zooplankton are lower in presence of polyamide particles	Int J Environ Res Public Health	15	280
Revel, M, Yakovenko N, Caley T, Guillet C, Chatel A and Mouneyrac C	2018	Accumulation and immunotoxicity of microplastics in the estuarine worm *Hediste diversicolor* in environmentally relevant conditions of exposure	Environmental Science and Pollution Research	27	3574–3583
Romano, N, Ashikin M, Teh JC, Syukri F and Karami A	2018	Effects of pristine polyvinyl chloride fragments on whole body histology and protease activity in silver barb *Barbodes gonionotus* fry	Environmental Pollution	237	1106-1111
Schiavo, S, Oliviero M, Romano V, Dumontet S and Manzo S	2018	Ecotoxicological assessment of virgin plastic pellet leachates in freshwater matrices	Journal of Environmental Accounting and Management	6	345-353

Reference	Year	Title	Journal	Vol	Pages
Seena, S, Graça D, Bartels A and Cornut J	2019	Does nanosized plastic affect aquatic fungal litter decomposition?	Fungal Ecology	39	388-392
Sinche, F	2010	Impact of Microsparticle Concentration Levels upon Toxicity of Phenol to Artemia	Clemson University	PhD	
Sleight, VA, Bakir A, Thompson RC and Henry TB	2017	Assessment of microplastic-sorbed contaminant bioavailability through analysis of biomarker gene expression in larval zebrafish	Marine Pollution Bulletin	116	291-297
Straub, S, Hirsch PE and Burkhardt-Holm P	2017	Biodegradable and petroleum-based microplastics do not differ in their ingestion and excretion but in their biological effects in a freshwater invertebrate *Gammarus fossarum*	Int J Environ Res Public Health	14	774
Tang, J, Wang X, Yin J, Han Y, Yang J, Lu X, Xie T, Akbar S, Lyu K and Yang Z	2019	Molecular characterization of thioredoxin reductase in waterflea *Daphnia magna* and its expression regulation by polystyrene microplastics	Aquat Toxicol	208	90-97
Thaysen, C, Stevack K, Ruffolo R, Poirier D, De Frond H, DeVera J, Sheng G and Rochman CM	2018	Leachate from expanded polystyrene cups is toxic to aquatic invertebrates (*Ceriodaphnia dubia*)	Frontiers in Marine Science	5	71
Thiagarajan, V, Iswarya V, P AJ, Seenivasan R, Chandrasekaran N and Mukherjee A	2019	Influence of differently functionalized polystyrene microplastics on the toxic effects of P25 TiO_2 NPs towards marine algae *Chlorella* sp	Aquatic Toxicology	207	208-216
Tosetto, L, Brown C and Williamson JE	2016	Microplastics on beaches: ingestion and behavioural consequences for beachhoppers	Marine Biology	163	199

Reference	Year	Title	Journal	Vol	Pages
Van Cauwenberghe, L, Claessens M, Vandegehuchte MB and Janssen CR	2015	Microplastics are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) living in natural habitats	Environmental Pollution	199	10-17
van Weert, S, Redondo-Hasselerharm PE, Diepens NJ and Koelmans AA	2019	Effects of nanoplastics and microplastics on the growth of sediment-rooted macrophytes	Sci Total Environ	654	1040-1047
Veneman, WJ, Spaink HP, Brun NR, Bosker T and Vijver MG	2017	Pathway analysis of systemic transcriptome responses to injected polystyrene particles in zebrafish larvae	Aquatic Toxicology	190	112-120
Wan, Z, Wang C, Zhou J, Shen M, Wang X, Fu Z and Jin Y	2019	Effects of polystyrene microplastics on the composition of the microbiome and metabolism in larval zebrafish	Chemosphere	217	646-658
Wardrop, P, Shimeta J, Nugegoda D, Morrison PD, Miranda A, Tang M and Clarke BO	2016	Chemical Pollutants Sorbed to Ingested Microbeads from Personal Care Products Accumulate in Fish	Environmental Science & Technology	50	4037-4044
Watts, TJ	2017	Effects of Multiple Stressors on the Freshwater Amphipod, *Gammarus pulex*: Microplastics and Temperature	QMUL	MSc	
Weber, A, Scherer C, Brennholt N, Reifferscheid G and Wagner M	2018	PET microplastics do not negatively affect the survival, development, metabolism and feeding activity of the freshwater invertebrate *Gammarus pulex*	Environ Pollut	234	181-189
Wegner, A, Besseling E, Foekema EM, Kamermans P and Koelmans AA	2012	Effects of nanopolystyrene on the feeding behavior of the blue mussel (*Mytilus edulis* L.)	Environ Toxicol Chem	31	2490-2497

Reference	Year	Title	Journal	Vol	Pages
Wei, W, Huang QS, Sun J, Wang JY, Wu SL and Ni BJ	2019	Polyvinyl Chloride Microplastics Affect Methane Production from the Anaerobic Digestion of Waste Activated Sludge through Leaching Toxic Bisphenol-A	Environmental Science and Technology	53	2509-2517
Wen, B, Jin S-R, Chen Z-Z, Gao J-Z, Liu Y-N, Liu J-H and Feng X-S	2018	Single and combined effects of microplastics and cadmium on the cadmium accumulation, antioxidant defence and innate immunity of the discus fish (*Symphysodon aequifasciatus*)	Environmental Pollution	243	462-471
Wen, B, Zhang N, Jin SR, Chen ZZ, Gao JZ, Liu Y, Liu HP and Xu Z	2018	Microplastics have a more profound impact than elevated temperatures on the predatory performance, digestion and energy metabolism of an Amazonian cichlid	Aquat Toxicol	195	67-76
Wright, SL, Rowe D, Reid MJ, Thomas KV and Galloway TS	2015	Bioaccumulation and biological effects of cigarette litter in marine worms	Scientific Reports	5	14119
Yin, L, Chen B, Xia B, Shi X and Qu K	2018	Polystyrene microplastics alter the behavior, energy reserve and nutritional composition of marine jacopever (*Sebastes schlegelii*)	Journal of Hazardous Materials	360	97-105
Yu, P, Liu Z, Wu D, Chen M, Lv W and Zhao Y	2018	Accumulation of polystyrene microplastics in juvenile *Eriocheir sinensis* and oxidative stress effects in the liver	Aquatic Toxicology	200	28-36
Zhang, Q, Qu Q, Lu T, Ke M, Zhu Y, Zhang M, Zhang Z, Du B, Pan X, Sun L and Qian H	2018	The combined toxicity effect of nanoplastics and glyphosate on *Microcystis aeruginosa* growth	Environmental Pollution	243	1106-1112
Zhang, S, Ding J, Razanajatovo RM, Jiang H, Zou H and Zhu W	2019	Interactive effects of polystyrene microplastics and roxithromycin on bioaccumulation and biochemical status in the freshwater fish red tilapia (*Oreochromis niloticus*)	Sci Total Environ	648	1431-1439

Reference	Year	Title	Journal	Vol	Pages
Zhao, L, Qu M, Wong G and Wang D	2017	Transgenerational toxicity of nanopolystyrene particles in the range of μg L^{-1} in the nematode *Caenorhabditis elegans*	Environ Sci: Nano	4	2356-2366
Zhao, T, Tan L, Huang W and Wang J	2019	The interactions between micro polyvinyl chloride (mPVC) and marine dinoflagellate *Karenia mikimotoi*: The inhibition of growth, chlorophyll and photosynthetic efficiency	Environmental Pollution	247	883-889
Ziajahromi, S, Kumar A, Neale PA and Leusch FDL	2017	Impact of microplastic beads and fibers on waterflea (*Ceriodaphnia dubia*) survival, growth, and reproduction: Implications of single and mixture exposures	Environ Sci Technol	51	13397-13406
Ziajahromi, S, Kumar A, Neale PA and Leusch FDL	2018	Environmentally relevant concentrations of polyethylene microplastics negatively impact the survival, growth and emergence of sediment-dwelling invertebrates	Environ Pollut	236	425-431