Quality Criteria for the Analysis of Microplastic in Biota Samples: A Critical Review

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ABSTRACT: Data on ingestion of microplastics by marine biota are quintessential for monitoring and risk assessment of microplastics in the environment. Current studies, however, portray a wide spread in results on the occurrence of microplastic ingestion, highlighting a lack of comparability of results, which might be attributed to a lack of standardization of methods. We critically review and evaluate recent microplastic ingestion studies in aquatic biota, propose a quality assessment method for such studies, and apply the assessment method to the reviewed studies. The quality assessment method uses ten criteria: sampling method and strategy, sample size, sample processing and storage, laboratory preparation, clean air conditions, negative controls, positive controls, target component, sample (pre)treatment, and polymer identification. The results of this quality assessment show a dire need for stricter quality assurance in microplastic ingestion studies. On average, studies score 8.0 out of 20 points for “completeness of information” and 0 for “reliability”. Alongside the assessment method, a standardized protocol for detecting microplastic in biota samples incorporating these criteria is provided.

INTRODUCTION
The ubiquity of microplastic (plastic particles < 5 mm1), combined with associated effects, has raised concerns regarding marine species, ecosystems, and the impact it may have on human health. Microplastics have been detected in a wide variety of habitats in the ocean from shallow coasts to the deep sea.2–4 Increasing numbers of studies report the ingestion of microplastic by marine biota across multiple trophic levels, including animals often targeted by fisheries (Table 1).5–9 The ingestion of microplastics seemingly concerns a wider range of species than the ingestion of meso- and macroplastics; indeed, it is considered the most frequent interaction between plastic debris and marine organisms.10

Ingested microplastic particles are thought able to evoke a biological response through both physical and chemical mechanisms, although many of these effects have yet to be studied. Ingestion of microplastics is thought to cause physical damage in small organisms2 and has been speculated to provide a pathway for some associated chemicals to enter and spread in the food web all the way up to humans with microplastic particles as vectors.11–13 Additionally, ingestion by biota is considered a possible sink for microplastics.14 Therefore, measuring quantities of ingested plastic is of high priority to properly assess the risk of such hazards.

Physical impacts for small organisms like internal abrasions and blockages have been reported.2 Moreover, microplastic particles were shown to cause damage leading to cellular necrosis, inflammation, and lacerations of tissues in gastrointestinal tracts according to a review of plastic impact on biota.15 In bigger organisms, ingestion of larger objects (i.e., macroplastics) has been demonstrated too.

In addition to the impact of ingested microplastics proper, persistent organic pollutants (POPs) may concentrate on the particles. It is suggested this could pose a possible new route for POPs to enter the food chain;15,12 however, it has not been irrefutably shown that this actually happens.16–20 Contrary, evidence in Northern Fulmars (Fulmarus glacialis) suggests a transfer of POPs from the lipids in the animal to the plastic rather than the other way around.18

The concerns for the impacts of microplastic are reinforced by the hypothesis that microplastics may be able to spread through the food web by means of trophic transfer, a phenomenon that has been observed in a few instances.21,22
| criterion                  | Lusher et al. 48 | Tanaka and Takada 78 | Davidson and Dudas 59 | Rummel et al. 58 | Courtene-Jones et al. 49 | Devriese et al. 56 | Mathalon and Hill 8 | Wesch et al. 57 | Cannon et al. 43 | Desforges and Galbraith 50 | Li et al. 59 | Murphy et al. 81 | Van dermeersch et al. 27 | Davison and Asch 51 | Foekema et al. 60 | Karlsson et al. 53 | Nadal et al. 52 | Torre et al. 54 | Bellas et al. 57 | Jabon et al. 54 | Lusher et al. 5 | Van Cauwenberge et al. 60 | Braté et al. 83 | Anastasopoulou et al. 64 | Besseling et al. 51 | Peters et al. 70 | Vendel et al. 86 | Boerger et al. 82 | Liboiron et al. 55 | Neves et al. 5 | Wojcik-Fudaleswska et al. 87 | Romeo et al. 9 | Miranda and de Carvalho-Souza 88 |
|---------------------------|-----------------|--------------------|------------------------|-----------------|------------------------|-----------------|-----------------|-----------------|-----------------|------------------------|-----------------|--------------------|--------------------|----------------|-----------------|-----------------|-----------------|----------------|-----------------|----------------|-----------------|-----------------|----------------|-----------------|-----------------|----------------|-----------------|-----------------|----------------|-----------------|-----------------|----------------|
| study                     | 2016            | 2016               | 2016                   | 2016            | 2017                   | 2016            | 2014            | 2016            | 2016            | 2015                   | 2017            | 2017               | 2015               | 2011            | 2013            | 2017            | 2016            | 2016            | 2014            | 2015            | 2010            | 2015            | 2016            | 2014            | 2016            | 2016            | 2016            | 2016            | 2016            | 2016            | 2016            | 2016            | 2016            | 2016            |
| study year                | 2016            | 2016               | 2016                   | 2016            | 2017                   | 2016            | 2014            | 2016            | 2016            | 2015                   | 2017            | 2017               | 2015               | 2011            | 2013            | 2017            | 2016            | 2016            | 2014            | 2015            | 2010            | 2015            | 2016            | 2014            | 2016            | 2016            | 2016            | 2016            | 2016            | 2016            | 2016            | 2016            | 2016            | 2016            |
| sampling methods          | 2               | 2                  | 2                      | 2               | 0                      | 2               | 2               | 2               | 2               | 2                      | 2               | 2                  | 2                 | 2               | 0               | 1               | 2               | 1               | 0               | 1               | 2               | 1               | 0               | 1               | 2               | 1               | 2               | 1               | 0               | 1               | 0               |
| sample size               | 2               | 2                  | 2                      | 2               | 0                      | 2               | 2               | 2               | 2               | 2                      | 2               | 2                  | 2                 | 2               | 0               | 1               | 2               | 1               | 0               | 1               | 2               | 1               | 0               | 1               | 2               | 1               | 2               | 1               | 0               | 1               | 0               |
| sample processing and storage | 2           | 2                  | 2                      | 2               | 0                      | 2               | 2               | 2               | 2               | 2                      | 2               | 2                  | 2                 | 2               | 0               | 1               | 2               | 1               | 0               | 1               | 2               | 1               | 0               | 1               | 2               | 1               | 2               | 1               | 0               | 1               | 0               |
| laboratory preparation    | 1               | 2                  | 2                      | 2               | 0                      | 2               | 2               | 2               | 2               | 2                      | 2               | 2                  | 2                 | 2               | 0               | 1               | 2               | 1               | 0               | 1               | 2               | 1               | 0               | 1               | 2               | 1               | 2               | 1               | 0               | 1               | 0               |
| clean air conditions      | 1               | 2                  | 2                      | 2               | 0                      | 2               | 2               | 2               | 2               | 2                      | 2               | 2                  | 2                 | 2               | 0               | 1               | 2               | 1               | 0               | 1               | 2               | 1               | 0               | 1               | 2               | 1               | 2               | 1               | 0               | 1               | 0               |
| negative control          | 2               | 2                  | 2                      | 2               | 0                      | 2               | 2               | 2               | 2               | 2                      | 2               | 2                  | 2                 | 2               | 0               | 1               | 2               | 1               | 0               | 1               | 2               | 1               | 0               | 1               | 2               | 1               | 2               | 1               | 0               | 1               | 0               |
| positive control          | 2               | 2                  | 2                      | 2               | 0                      | 2               | 2               | 2               | 2               | 2                      | 2               | 2                  | 2                 | 2               | 0               | 1               | 2               | 1               | 0               | 1               | 2               | 1               | 0               | 1               | 2               | 1               | 2               | 1               | 0               | 1               | 0               |
| target component          | 1               | 2                  | 2                      | 2               | 0                      | 2               | 2               | 2               | 2               | 2                      | 2               | 2                  | 2                 | 2               | 0               | 1               | 2               | 1               | 0               | 1               | 2               | 1               | 0               | 1               | 2               | 1               | 2               | 1               | 0               | 1               | 0               |
| sample treatment          | 2               | 2                  | 2                      | 2               | 0                      | 2               | 2               | 2               | 2               | 2                      | 2               | 2                  | 2                 | 2               | 0               | 1               | 2               | 1               | 0               | 1               | 2               | 1               | 0               | 1               | 2               | 1               | 2               | 1               | 0               | 1               | 0               |
| polymer identification    | 2               | 2                  | 2                      | 2               | 0                      | 2               | 2               | 2               | 2               | 2                      | 2               | 2                  | 2                 | 2               | 0               | 1               | 2               | 1               | 0               | 1               | 2               | 1               | 0               | 1               | 2               | 1               | 2               | 1               | 0               | 1               | 0               |
| accumulated score         | 15              | 13                 | 12                     | 12              | 11                     | 11              | 11              | 11              | 11              | 11                     | 11              | 11                 | 11                | 10             | 10              | 10              | 10              | 10              | 10              | 10              | 10              | 10              | 10              | 10              | 10              | 10              | 10              | 10              | 10              | 10              | 10              | 10              | 10              | 10              |

Notes:
- *Scores of 0−2 were assigned to each publication in each of the 10 categories. The publications are sorted from high to low based on the "accumulated score". The overall reliability score was 0 for all studies and is not indicated.
- *Studies with involvement of 1 or more of the authors of the present paper.

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DOI: 10.1021/acs.est.8b01611

Environ. Sci. Technol. 2018, 52, 10230−10240
This is cause for concern especially in commercially valuable species as it possibly poses a threat to human food safety. To what extent this transfer occurs in the food web remains to be studied further.

Despite these worries concerning microplastic ingestion, the effects in the natural environment and implications for the food web remain poorly understood. Because of the absence of suitable standardized methods, data are too often incomparable, are not representative, and lack quality assurance. Hence, our knowledge on the fate and impacts of microplastics remains incomplete. The microplastic research field is young, and as research performed now lays down the foundations for later studies, there is a dire need for a standardized protocol for carrying out studies on the ingestion of microplastics by marine biota to mitigate this issue. Although first steps toward standardization of methodologies in environmental samples are being made, the comparability of current data is being impeded by the wide variety of methodologies, which has led to data of different quality. For dealing with the wide spread in quality of the data produced by studies, an example can be taken from the field of toxicology. In toxicology, it is common practice to assess the reliability of studies with consensus criteria, like the so-called Klimisch score, or the recently proposed Criteria for Reporting and Evaluating Ecotoxicity Data (CRED). These methods both offer scoring systems with different reliability categories, generating standardized documentation of validity evaluation. They were developed to guide risk assessors in performing unbiased, transparent, and detailed evaluations while guiding researchers in performing and reporting studies in a manner deemed appropriate. We argue that research and risk assessment with respect to the impacts of plastic debris are in urgent need for the development and use of such criteria.

The aim of the present study is to critically review the literature on ingestion of microplastic by marine biota. On the basis of this review, we develop a scoring method for ecological studies and the analytical methodologies employed to detect plastic debris in aquatic biota samples. The scoring method is subsequently applied retrospectively to the reviewed studies. This assessment does not result in an absolute judgment but is an indicator of the usefulness of these studies for risk assessment and monitoring purposes of microplastic ingestion in natural populations. We also provide average scores per evaluation criterion, illustrating which methodological aspects need improvements most. Finally, our synthesis provides the basis for a quality assurance protocol for the analysis of microplastic debris in biota samples.

### MATERIALS AND METHODS

An extensive literature review was undertaken by accessing the Web of Science, ScienceDirect, and Scopus databases for studies of microplastic ingestion in marine biota in natural populations, including studies from all years up until those published in June 2017. Queries included the following search terms: "microplastic AND ingestion AND marine", "microplastic AND uptake AND marine", "microplastic AND marine biota", and "microplastic AND biota AND monitor". Reference lists of the found articles, reviews, and reversed searches were consulted as well, resulting in a representative collection of 35 currently available studies. Laboratory exposure experiments were excluded from the collection. Furthermore, studies were only included if they provided data on the ingestion of microplastic. For these studies, the ingestion incidence was calculated as the fraction of sampled individuals containing microplastic. The 95% confidence intervals for these binominal proportions were assessed using the Wilson method. Subsequently, studies were scored according to method quality criteria discussed in the next section. All studies were assessed by two separate authors independently, after which differences in scoring were discussed and tuned until the assessment was done consistently across all studies. For maximizing transparency and traceability, the scoring explanations, scoring criteria, and scorings for all papers are provided as Supporting Information (Tables S1–S3, respectively). The eventual assessments do not express the value of studies. In hindsight, they only reflect the compliance of studies to reliability criteria as perceived by the authors of the present paper. Although we maximized our effort to be complete and thorough in this process, misinterpretations or misjudgements cannot be completely excluded.

The scoring method presented here was designed to assess current studies on reliability of their data on microplastic ingestion in marine field biota and is based on several aspects that define a reproducible and controlled study. The method evaluates the inherent adequacy of the employed methods for monitoring and risk assessment purposes relating to a standardized methodology and the description of the procedure and results. By scoring high in all categories, a study can be defined as "reliable", providing reproducibility, clarity, and plausibility of its findings.

### QUALITY ASSESSMENT SYSTEM

Previous scoring systems that have been proposed for assessing the reliability of ecotoxicology studies are the Klimisch and the more recent CRED scoring systems. The Klimisch criteria have received critiques for being unspecific and lacking essential criteria and guidance, leaving too much room for interpretation. The CRED evaluation method gives extensive guidance on how to use the set criteria and gives recommendations for reporting. Following the example set by the CRED method, the present evaluation method for microplastic ingestion studies provides several criteria that must be assessed, including guidance on how to assess each criterion. The quality assessment method is made up of ten criteria: (1) sampling method and strategy, (2) sample size, (3) sample processing and storage, (4) laboratory preparation, (5) clean air conditions, (6) negative controls, (7) positive controls, (8) target component, (9) sample (pre)treatment, and (10) polymer identification (Table 1). For each criterion, a score of 0, 1, or 2 can be assigned to the publication under review. Scores signify the following: 2 = reliable without restrictions, 1 = somewhat reliable but with restrictions, 0 = not reliable. If information is lacking on certain aspects in the publication, this is considered unreliable, leading to a lower score. After each criterion is scored, an overall reliability score is calculated by taking the product of all criteria scores, resulting in a maximum attainable overall theoretical reliability score of 1024 points, indicating a high reliability of a publication. This contrasts with both the CRED and Klimisch method: these methods assign a category of reliability to each criterion but do not quantify it with a score. In the evaluation method presented here, the quantification through scoring is deemed important because each criterion is considered crucial and equally important to the reliability of the results of a study. This means when a study scores 0 points on a criterion, too much uncertainty still surrounds the results of the study,
marking the results unreliable. This also means that when only one criterion is evaluated as “not reliable” (0 points) the overall reliability score of the study will be 0. Besides this overall reliability score, we provide an accumulated score calculated as the sum of the individual scores. This score has a maximum of 20 points and can be seen as a combination of the reliability and the completeness of information in a publication.

In the following ten paragraphs, argumentation is provided on each of the ten scoring categories, including explanation based on the currently reviewed studies and specification of scoring criteria. A supporting, more detailed overview of the scoring criteria is provided as Supporting Information (Tables S1 and S2).

**Sampling Methods and Strategy.** Several factors related to sampling method and strategy affect the results of microplastic detection in biota samples. For instance, because of differences in density and sinking as a result of biofouling, plastic is found at different depths of the water column. Microplastics are also known to accumulate in the sediment with deep sea bottoms likely to make up a sink for microplastics. Additionally, some species are known for diurnal vertical migration and are subjected to a wide variety of microplastic encountered, possibly affecting their ingestion rates. Nonecological factors such as mesh size will influence life stage of the caught individuals in the sample, whereas a small mesh size could lead to cod-end feeding. Sampling methods can greatly influence the outcome of a study; therefore, it is important that such characteristics of the sampling are recorded to create a reproducible study. Furthermore, by reporting such details, it could be easier to interpret the outcome and account for possible contamination in the results.

In this section, studies are scored on reportage, and therefore reproducibility, of the sampling, but also on choice of sampling method itself. Studies scoring high in this section reported extensively on their methods (e.g., type of gear, sampling location and depth) and controlled their own sampling or were fully aware of what had happened to the specimens during sampling. Articles with low scores either failed to report on (parts of) their sampling (Table 2), or used, for instance, store-bought individuals when making inferences for instance, store-bought individuals when making inferences on natural populations. The use of store- or market-bought individuals is not inherently wrong as long as the interest of the study lies on contamination of sea food and not on natural populations. Scores of 1 indicate that, for part of the sample, sampling was not performed correctly, whereas for another part of the sample it was: the aim of the study should be correctly matched to the sampling method. For example, Vandermeersch et al. (2015) partially used store-bought individuals while using self-sampled ones for a different part of the study. The microplastic uptake in mussels from different estuaries was compared with the uptake by commercial mussels. The commercial mussels were bought in stores, leading to uncertainty about the treatment of these mussels prior to the analysis: microplastic found in these mussels could have originated from contamination during handling in the production chain rather than from microplastic ingestion by the mussels themselves. Would the aim of this study have been

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**Table 2. Standardized Protocol for the Detection of Ingested Microplastic in (Marine) Biota**

| 1. Sampling methods | Sampling characteristics that should be recorded: |
|---------------------|--------------------------------------------------|
|                     |     - Gear                                      |
|                     |     - Mesh size and mesh size at cod-end (if applicable) |
|                     |     - Material                                  |
|                     |     - Location                                  |
|                     |     - Depth                                     |
|                     |     - Date and time of day                      |
|                     |     - Presence of plastic materials             |
| 2. Sample size      | A suitable sample size of 50 individuals per research unit (species, food web, ecoregion, feeding type, etc.) is required. The confidence interval of the ingestion incidences should be reported (Figure 1). |
| 3. Sample processing and storage | Between the moment of capture and the examination in the lab the biota samples should be stored on ice or frozen at ~20 °C. Smaller organisms can also be preserved in a glass container with ethanol or formaldehyde. Any sample handling, such as dissections, should be left for the lab. |
| 4. Laboratory preparation | All materials, equipment, and laboratory surfaces need to be thoroughly washed and rinsed; afterward, all materials should be kept under clean air conditions. |
| 5. Clean air conditions | The handling of samples should be performed in clean air facilities. Samples should not be taken out of the clean air facilities without being sealed off. If sampling processing and analysis cannot be conducted under clean air conditions, the implementation of negative controls (see criterion 6) will get even more important. |
| 6. Negative control | A replicate of 3 controls is advised that are included for each batch of samples and treated in parallel to the sample treatment. Additionally, if the samples have to be analyzed outside of the clean air facilities, clean Petri dishes should be placed next to the sample, and checked for any occurred air-borne contamination. |
| 7. Positive controls | A replicate of 3 is advised in which microplastics of known polymer identity and of targeted sizes are added to “clean” samples, which are then treated and analyzed the same way as the actual samples. The particle recoveries are calculated by tallying the numbers of retrieved particles to the amounts added. |
| 8. Target component | To ensure monitoring all ingested microplastic, the full gastrointestinal tract (esophagus to vent) of fish and the entire body of smaller species, e.g. bivalves, should be examined. |
| 9. Sample treatment | A digestion step must be included to dissolve organic matter in the sample when aiming in the detection of small microplastics (<300 μm). The digestion method described by Foekema et al. (2013) using a 10% KOH solution and enzymatic digestion methods (yet only for small organisms) are most suitable. In any case, heating or drying of the samples at high temperatures should be avoided. |
| 10. Polymer identification | Until now, most common methods in the field of microplastic research are FTIR or Raman spectroscopy, pyrolysis or TGA-GC-MS. The polymer identification is required for all, or at least a subsample of particles: When numbers of pre-sorted particles are <100, all particles should be analyzed. For particle numbers >100, >50% should be identified with a minimum of 100 particles. Particle counts with confidence intervals, detection limits for the count and for minimum particle size, polymer types and percentages (of different polymer types, of synthetic vs natural material), and particle sizes should be reported. |
to check microplastic content in store-bought individuals (i.e., checking on general contamination, not ingestion), this would not have been an issue. This study scored 1 in this section because part of the study can be considered reliable with sampling method correctly matched to the aim of the specific part of the study.

Sample Size. Both the International Council for the Exploration of the Sea ICES (2015) and the European Strategy Framework Directive’s Technical Subgroup on Marine Litter (MSFD-TSGML) (2013) recommend a sample size of at least 50 individuals. This sample size of 50 is arbitrarily chosen, since, due to the wide variety in microplastic ingestion reported by different studies, no clear indication of the true ingestion incidence of microplastic by biota can be estimated. When more clarity can be given in the future, this recommended sample size should be adjusted accordingly. If ingestion incidence appears to be low, higher sample sizes will be needed to give reliable results; if populations show high incidence of microplastic ingestion, lower sample sizes will suffice.

The scoring in this category is fairly straightforward using the recommended 50 individuals as a threshold until it is possible to perform a reliable power analysis to calculate a more appropriate sample size for ingestion studies. Too low a sample size may provide interesting data, but no conclusions can be drawn as the statistical power of such a study would be simply too low to infer any trends. A larger sample size is more appropriate sample size for ingestion studies. Too low a sample size over 50 specimens taken from a food web or ecoregion scored 2. A score of 0 was ascribed to studies using less than 50 specimens. Studies with >50 specimens in total and >25 specimens per research unit (e.g., a species, food web, or ecoregion) received a score of 1. For now, we also applied these criteria to a study that reported the presence of microplastic in a single-stranded whale, leading to a very wide confidence interval (Figure 1). However, for whales or for rare and protected species, the n = 50 criterion is difficult or even unethical to achieve in a sampling effort meant to assess trends in microplastic ingestion. For such big or protected organisms, retrospective data obtained from stranded animals and from bycatch through different reports need to be combined to reach a sample size with sufficient rigor.

Figure 1. Ingestion incidence and 95% confidence intervals recalculated from data provided in microplastic ingestion studies. Data are combined to obtain a “whole ocean” biota ingestion incidence value (○).

sample size over 50 specimens taken from a food web or ecoregion scored 2. A score of 0 was ascribed to studies using less than 50 specimens. Studies with >50 specimens in total and >25 specimens per research unit (e.g., a species, food web, or ecoregion) received a score of 1. For now, we also applied these criteria to a study that reported the presence of microplastic in a single-stranded whale, leading to a very wide confidence interval (Figure 1). However, for whales or for rare and protected species, the n = 50 criterion is difficult or even unethical to achieve in a sampling effort meant to assess trends in microplastic ingestion. For such big or protected organisms, retrospective data obtained from stranded animals and from bycatch through different reports need to be combined to reach a sample size with sufficient rigor. This would require harmonization of protocols to increase comparability of studies, guidance for which is beyond scope of the current review.

We further advise provision of the confidence interval in the reported count (e.g., refs 5 and 46); however, this was not yet included as criterion in the current scoring. On the basis of the total number of animals and the number of animals that ingested microplastics, we calculated the confidence intervals and provide an overview in Figure 1.

Sample Processing and Storage. After sampling, samples need to be stored until examination in the laboratory. Samples are often frozen, or whole specimens of smaller species are preserved in fixatives such as formalin, ethanol, or formaldehyde. ICES (2015) recommends storing biota samples on board using aluminum foil for freezing at −20 °C or preservation in ethanol in glass containers. In the present study, it was not considered necessary to wrap each individual in aluminum foil as long as specimens were quickly frozen after capture at −20 °C and stored in a closed container. If this is combined with a pre-examination rinse of the specimens (see “laboratory preparation”), it should suffice in mediating contamination of the exterior of the specimen. Under no circumstance should the specimen be opened on board. This is considered as a high and difficult to assess risk for contamination due to unregulated conditions on board. We further recommend avoiding the dissection of individuals outside clean air conditions at all times (see “clean air conditions”).

High scores were assigned to studies freezing their samples shortly after capture at −20 °C or storing them on ice, leaving any further handling until the laboratory. Alternative methods storing the samples in closed off containers with a fixative were also given the highest scores in case potential effects of these chemicals on different plastics were studied before application. Recently, the resistance of microplastics to formaldehyde/ethanol has been confirmed. ICES (2015) recommends storing biota samples on board using aluminum foil for freezing at −20 °C or preservation in ethanol in glass containers. In the present study, it was not considered necessary to wrap each individual in aluminum foil as long as specimens were quickly frozen after capture at −20 °C and stored in a closed container. If this is combined with a pre-examination rinse of the specimens (see “laboratory preparation”), it should suffice in mediating contamination of the exterior of the specimen. Under no circumstance should the specimen be opened on board. This is considered as a high and difficult to assess risk for contamination due to unregulated conditions on board. We further recommend avoiding the dissection of individuals outside clean air conditions at all times (see “clean air conditions”).

Laboratory Preparation. Contamination is a prevalent issue in microplastic research, creating uncertainty around the results of many studies. This risk and uncertainty have been dealt with in different ways. Different forms of prevention have been applied with varying degrees of success. ICES (2015) decided to exclude small fibers from analyses after finding a sharply decreased abundance when working under clean air conditions. ICES (2015) proposed in their preliminary protocol to exclude all fibers smaller than 5 mm in length from results. Although this may provide a way to reduce the issue of contamination in results, it is less than ideal; by excluding all small fibers from results, truly ingested fibers will be excluded from the results too. This could lead to an underestimation of ingestion rates and a potential knowledge gap in the ingestion of microplastic. Therefore, proper prevention is needed. In the laboratory, contaminations with synthetic polymers should be avoided as they may reduce the issue of contamination in results, it is less than

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Factors such as clothing should be considered. Often, contamination arises in the form of microfibers. Additional contamination originating from researchers’ clothing can easily be avoided by solely wearing 100% natural fiber clothing, such as cotton. Only wearing a 100% cotton lab coat may not suffice; if one was to wear a polyester shirt underneath, it would not be unimaginable that some fibers could end up in the samples. For the current scoring in this study, if all other precautions were met, a 100% cotton lab coat was considered sufficient.

In some studies, precautions were made by wiping surfaces and tools using alcohol. This method is probably not thorough enough to deal with contamination; merely wiping surfaces, be it with alcohol or water, could still leave particles. They could be missed, detach from the wipe during wiping, or the wipe itself could even prove to be a source of contamination (i.e., the material or dust already collected on the wipe before use). Rigorously washing and rinsing of the equipment are considered to be the only proper option here.

Additional to the preparation of surfaces and tools, the sample specimens themselves require some preparation. The exterior of the animal should be rinsed and checked for contamination. In the case of small specimens such as zooplankton, this is not an easy feat. In a study performed by Desforges et al. (2015), this issue was overcome by individually checking each specimen under a microscope and picking off any external contamination with a pair of tweezers.

In summary, a score of 2 was assigned when nonsynthetic clothing and a lab coat were used and equipment and organism exterior were rinsed. A score of 1 was assigned for solely wiping laboratory surfaces and equipment or not wearing a lab coat as long as negative control samples were run in parallel and examined for contamination. A score of 0 was assigned when no precautions were met.

Clean Air Conditions. Problems with airborne contamination are unavoidable unless work is performed under clean air conditions. To this end, sample handling should be done in a laminar flow cabinet or in a “clean room,” which is designed to minimize airborne contamination during sample handling and analysis. The use of such facilities is a necessity in microplastic research; any handling of samples outside clean air conditions creates a high risk of airborne contamination.

Other studies placed their samples in a fume hood to minimize the risk of contamination. However, because a fume hood draws air from the room into the hood (contrarily to a positive pressure laminar flow cabinet, which blows filtered air through the cabinet into the room), the risk of airborne contamination remains.

A few studies were seen that mitigated contamination by closing off samples as much as possible and handling them as fast as possible. These methods are not foolproof and should not be relied upon without further indication on results of negative samples treated in parallel to actual samples.

The proper use of clean air conditions was given a score of 2. A score of 0 was assigned to studies taking no regard for airborne contamination. Studies mitigating contamination by carefully keeping samples in a closed off situation as much possible scored 1 in this category, provided that negative controls were run in parallel and examined for contamination.

Negative Controls. Although increasing in recent studies, the use of controls in microplastic research is not standard practice. During sample handling, the chances of contamination by microplastic particles and fibers are high; thus, the use of controls, treated and analyzed in parallel to actual samples, is crucial.

For a study to score 2, proper blanks should be included for each batch of samples with at least three replicate blanks per batch. These controls should be performed without tissue, or with tissue that was confirmed to be devoid of microplastic, in parallel with samples containing the target component. By doing so, the controls are given the same full treatment as the studied specimens. Controls should be run regularly and with special attention to moments of high risk of contamination, such as moving specimens in and out of the laminar flow cabinet. Furthermore, the visual examination of samples forms a moment of high risk, which is why additionally placed and examined Petri dishes next to the sample might be advisable.

Scores of 1 indicate a blank analysis of some form, nevertheless deemed insufficient here. This includes, for instance, solely open Petri dishes or soaked paper that were placed next to the work surface and checked for contamination or the filtration of air. These do not account for contamination derived from used chemicals or equipment. Studies scored 0 when no form of negative control was included in the study.

Positive Controls. It is generally difficult to assess whether all microplastics present in a sample are effectively recovered from that sample. Small particles in particular may be overlooked or missed, and losses may occur during all steps of sample preparation, processing, and analysis. Therefore, it is considered crucial to include controls (triplicate) with added microplastic particles that are treated in parallel to the samples to determine the recovery rate (score of 2 points). Ideally, positive controls should also be included for the smallest targeted size class, and the limit in the detected size should be reported. We are aware of only three studies that included reliable positive controls. Davison and Asch, for instance, blindly added random numbers of spherical beads from two size classes into fish stomach contents, so that the researcher would not know this number, and were able to trace back all added particles to achieve 100% recovery. A score of 1 was assigned to studies with some form of a positive control (e.g., testing only a part of the protocol), and a score of 0 was assigned when no positive controls were included.

Target Component. Among the reviewed studies, different target components were described that are mainly (parts of) the digestive tracts for larger biota, like fish or whole specimens for smaller species, like bivalves. Choosing a suitable target component is an important part of the study setup. For accurate estimation of microplastic ingestion, it is important to examine the entire gastrointestinal tract (GIT) (esophagus to vent). By only examining the stomach, particles in the gut would be missed, leading to an underestimation of ingestion rate. When small animals such as bivalves and zooplankton are being studied, the entire specimen should be used.

Studies examining full specimens or entire GITs received the highest score. Examination of parts of the GIT were scored lower. In case a study examined a part of the GIT for a subsample yet full GITs for the rest of the sample, it was scored 1.

Sample (Pre)treatment. For extracting and characterizing microplastics in biological samples, a digestion step is a crucial component, namely, dissolving organic matter without
degrading plastic polymers. Detection of microplastic in a biological sample without getting rid of the organic matter makes for an unreliable method; the chance of missing particles is high, especially small particles that are not visually detectable. Therefore, it is advised to make use of a digestion pretreatment.42,61

Dehaut et al. (2016)62 performed a study testing six existing methods (including enzymatic, alkaline, and acidic digestion), comparing their effects on 15 different plastic polymers as well as their efficiency in biological samples. Their tests showed that, out of the six protocols, an adapted protocol of Foekema et al. (2013)6 was most successful. The original protocol involves the samples being left for digestion in 10% KOH solution and kept at room temperature for 3 weeks. The adapted protocol used 10% KOH solution with 24 h of incubation at 60 °C.63 This adaptation was made to shorten the incubation time. The heating of samples during digestion pretreatments to speed up the process is fairly common, and especially with acidic digestion methods, this is often part of the protocol. However, this practice may be ill advised because the heating of the samples could cause some microplastic particles to deform or clump together.63 Therefore, it is advised to apply the original protocol of Foekema et al. (2013).6 The adequacy of the 10% KOH protocol has recently been confirmed by Kühn et al. (2017)64 and Munno et al. (2018).63 However, for smaller organisms, like the soft tissue of mussels or plankton species, enzymatic methods have also been shown to provide high digestion rates with no damage to microplastic.65,66

On the basis of these findings, studies using a 10% KOH solution-based digestion, or an enzymatic digestion, received the highest score of 2. Studies not incorporating a digestion step received no points. Studies using other digestion methods were scored 1. A score of 1 was also assigned to studies that did not need a digestion step because the size of particles was large enough, which can be achieved by sieving the samples over 300 μm. This mesh size allows adequate particle sorting as is done frequently for, e.g., water samples.

**Polymer Identification**. Accurate identification of polymer types in environmental samples can be laborious. Hence, two aspects are relevant when assessing the polymer identities of a microplastic sample: (1) the quality of the method used for the identification (efficiency, sensitivity, accuracy, reproducibility) and (2) the quality of the selection of the subsample (representativeness).

**Polymer Identity**. Visual inspection (i.e., characterizing microplastic by eye under a dissection or stereomicroscope) was found to be a frequently used identification method.59,47,50,56,58,70 However, visual examination cannot be used to identify the (polymer) identity of a particle. Without formal evidence of polymer identity, a particle cannot be reported as being a microplastic particle. The quality of visual examination is influenced by the observer, properties of the plastic, targeted microplastic size, magnification of the microscope, and sample type.28 In a case study on microplastics in North Sea sediments, the usage of focal plane array (FPA) micro-Fourier transform infrared (micro-FTIR) spectroscopy revealed that only 1.4% of the particles visually sorted as microplastic were actually synthetic polymers.29 Fibers with a size over 500 μm were found to be of natural origin after an initial selection as microplastic.28,71 This uncertainty of visual identification further increases as particle size decreases, which illustrates the importance of verifying the chemical origin of potential microplastics.

To date, potential microplastics are identified mostly using spectroscopic29,69,72 or thermal degradation analyses.73–75 Particles sorted manually are mostly analyzed using attenuated total reflectance (ATR) Fourier transform infrared (FTIR) spectroscopy,9,58 but pyrolysis GC-MS is also applied.75 Both techniques result in a clear identification but are restricted to bigger particles due to the manual particle handling. When aiming for microscopic particle determination, the coupling of a microscope to FTIR or Raman spectroscopy reveals the chemical identity of particles and allows particle sizes to be estimated. Both techniques are limited by a certain minimum particle size.72,76,77 Alternatively, unsorted samples, i.e., where a polymer mixture might be present, can be analyzed using thermal degradation techniques.74,75 Because particles are not sorted manually, these techniques are not limited by a minimum particle size required; however, they do not provide information on microplastic size either. Furthermore, they do provide information on ingested polymer masses instead of presenting the numbers of ingested microplastic particles. One of these techniques should be applied and should always be favored over the so-called “hot point-test” applied by several studies.72,42,56 Plastic particles are “identified” when a particle shows a sticky dark mark when touched with a hot needle. However, this test does not allow polymer identification, is less suitable for thermoset and smaller plastics, and should therefore only be seen as a facilitation for visual sorting.

**Representative Subsample of Particles**. Many studies report polymer identities for a small subset of sorted particles only.7,53 This leaves considerable uncertainty with respect to the actual distribution of polymer types among samples. On the basis of practical experience using ATR-FTIR to determine polymer identities,6,16,46 we advise that when numbers of sorted particles are <100, all particles should be analyzed. For particle numbers >100, analysis becomes more laborious, but >50% should be identified for a representative subsample with a minimum of 100 particles being analyzed. The information given in the results section should contain the following: particle counts with confidence intervals, detection limits for the count and for minimum particle size, the polymer types determined, their percentages with regard to other polymer types and natural particles, and the microplastic size (classes).

If a study identified polymer identities and applied the latter criteria, 2 points were assigned. For insufficient numbers of identified particles that could result in an unrepresentative subsample, 1 point was assigned. Zero points were given if no polymer identification (i.e., purely visual sorting) was conducted.

**Protocol for Microplastic Ingestion Studies in Biota**

In this article, as a synthesis of our review and method assessment, we propose a standardized protocol for the detection of ingested microplastic in (marine) biota alongside the quality assessment method (Table 2). The protocol is adaptable for both vertebrates and invertebrates as long as the components of the quality assessment system are upheld. The protocol was developed taking the recommended protocol by ICES (2015)72 into account and accompanying with knowledge and evaluation of currently existing methodologies as outlined above. The protocol and quality assessment system are such
that, when following the protocol successfully, high reliability scores can be acquired. This protocol relies on the same literature analysis and argumentation as the assessment method and follows the categories step-by-step.

**GENERAL DISCUSSION**

Considerable uncertainty with respect to methodology was observed and quantified via the scoring system. Accumulated reliability scores ranged from 0 to 15 out of a maximum of 20 with an average of 8.0 (Table 1). As mentioned before, the results of such an assessment are not an absolute judgment, and the results should not be used as a ranking list of the value of studies. The scores are an indicator of the usefulness of these studies for risk assessment and monitoring purposes with respect to natural populations. The assessment evaluates common characteristics of a variety of studies. Not all decisions in a study can be captured in the scoring system; therefore, it is still important to critically look at a study and reflect upon its plausibility and comparability to other studies and not just upon its results.

Often studies could not be assigned a high score due to missing information on certain characteristics, such as details of the sampling or analytical procedures. Average scores (n = 35) per evaluation criterion were especially low (<1) for the criterion "positive controls" (0.17), "clean air conditions" (0.40), "sample treatment" (0.43), "laboratory preparation" (0.57), "polymer identification" (0.66), and "negative controls" (0.86) (Table 1). By leaving out such essential information, a study immediately becomes irreproducible and thus less reliable. One reason for initiating the present review was to systematically define this crucial information, such that future studies can avoid this by using standardized consensus methods.

On the basis of the assessment of reviewed papers (considered representative for currently available knowledge, Table S3), we conclude that all reviewed studies are not fully reliable. All studies scored 0 in at least one category, indicating an uncertainty around at least one of its aspects. Therefore, the overall reliability scores, calculated as the product of individual scores, were all 0 and thus were not included in Table 1. Each category of the assessment was defined by the consideration that if its set criteria were not up to par, the possibility of contamination could not be excluded. This is problematic, and for future studies the use of the proposed protocol is strongly recommended to obtain reliable and reproducible results. Following the proposed protocol, we conducted a study focusing on microplastic detection in North Sea fish while giving special attention to quality assurance and full reportage.

Our meta-analysis of microplastic ingestion data shows a wide variability among studies, which may be due to methodological, ecological, and/or spatial differences. Ingestion incidence ranges from 0 to 100% with confidence intervals that are narrower for higher sample sizes (Figure 1). On the basis of pooled data from all studies, an overall biota ingestion incidence of 16.6% (15.9–17.2 95% CI) was calculated. This "whole ocean" value can be interpreted as the percentage of the 13722 biota individuals sampled across all oceans in which microplastic was detected in the period of 2010–2017. The data underlying Figure 1 further reveal that, with sample sizes lower than 50, the confidence intervals can become as wide as 35–80% (Figure 1).

**PERSPECTIVE AND OUTLOOK**

We provided an evaluation method for the quality of studies reporting microplastic ingestion by biota. The applied quality criteria were defined based on a critical review of the literature available. Current studies are not of such a level of reliability that they could be used confidently for risk assessment or monitoring of microplastic by biota in the natural environment. Reliable ingestion rate studies are needed to define whether there is a risk posed by microplastic ingestion to the natural environment and to human food-safety. The proposed protocol can be used to perform these studies; the quality assessment system can be applied to control the quality of these data and enable an easier comparison of studies to move toward standardization and reliability. The quality assessment system may provide a tool and set an example that will help regulators and policy makers in their activities to mitigate contamination with plastic debris. Until now, the majority of studies focused on visually sortable microplastics. Our present scoring system is tuned to this research aim and used today’s best available information. However, we foresee that our recommendations may need adaptations when the focus is on much smaller microplastic, which is more difficult to detect. It is also conceivable that our proposed scoring system needs modification if the research field evolves, for instance, when new analytical technologies become available, just like the aforementioned CREED criteria for ecotoxicology studies. For now, all criteria were weighted equally as we considered all of them to be crucial for generating reliable results. Future research, however, may provide a rationale for using unequal weights, which thus would lead to another outcome of the scoring. Finally, we emphasize that a protocol and scoring system for microplastic analytical studies should be seen as a product of the scientific community rather than a product of a limited set of authors. In this sense, we see the present paper as a starting point in assessing quality assurance criteria for microplastic analytical studies rather than the final stage.

**ASSOCIATED CONTENT**

* Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b01611.

Explanation and definition of scores and scoring of individual papers (PDF)

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**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

S.M.M. and A.A.K. acknowledge funding from the Dutch Technology Foundation TTW (project number 13940) and additional support from KWR, IMARES, NVWA, RIKILT, the Dutch Ministry of Infrastructure and the Environment, The
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