Microplastics in aquatic organisms: Improving understanding and identifying research directions for the next decade

The study of environmental microplastics has increased over the past decade, with hundreds of new studies and resultant papers on the presence, fate, and sources of microplastics in marine and freshwater systems (Fig. 1). Despite the explosion of interest in the topic and in comparison to the research on the presence of microplastics in marine or fresh waters, there have been notably fewer studies on the extent to which these debris items are ingested by aquatic organisms and far fewer on the potential consequences, or response to their presence in organismal guts, tissues, and food webs. Even less research has focused on the smallest plastic debris items, nanoplastics (<1 μm). In this special issue on *Microplastics in marine and freshwater organisms: Presence and potential effects*, we highlight and address some of the many remaining questions. Articles in the issue examine the following: occurrence in freshwater fish (Hurt et al., Simmerman et al.), effects in freshwater plants (Dovidat et al.), reproductive effects, trophic transfer (Athey et al., Horn et al.), sensitivity in early life stages (Athey et al.), spatiotemporal variability in microplastics (Baechler et al. A, B), connections between feeding strategies, and microplastic ingestion (Caldwell et al., Not et al., Harris and Carrington) among others. As an introduction to this special issue, here we briefly discuss research gaps, challenges, and solutions to this important topic.

**Major research gaps**

Hundreds of marine and freshwater species encounter microplastics in their environment (Carbery et al. 2018). To date, a few hundred studies have documented microplastic assimilation, ingestion, or retention in the gut or tissue of a wide range of organisms in both laboratory and field settings. These studies include taxa across trophic levels, including algae, plants, invertebrates, fish, mammals, and birds, but many focus on invertebrates or a limited number of fish species (e.g., Baechler et al. A, B). The relatively fewer studies of microplastic exposure effects on biological responses (e.g., growth, swimming behavior, and molecular endpoints) focus on a few select invertebrates (e.g., daphnids) or fishes (e.g., zebrafish). Consequently, there are large gaps in understanding microplastic assimilation in organisms and their response to exposure, particularly in algae and plants, as well as limited knowledge on microbial colonization (e.g., Dudek et al.). In addition, less research related to freshwater vs. marine organisms exists for any of these topics (de Sá et al. 2018). The paper by Dovidat et al. and a review by Rogers et al. begin to address some of the species-based data gaps and highlight future research needs in freshwater plants and for microbe–plastic interactions. Finally, while still limited, studies are now being expanded to include important nonmodel species as some of the articles in this special issue demonstrate (e.g., Brandon et al., Dovidat et al., Waddell et al., Horn et al.).

Another notable research gap in the study of microplastics in organisms is understanding the implications of microplastic interactions spanning trophic levels, and particularly those that also consider additives or sorbed chemicals of microplastics. For example, studies on the trophic transfer of microplastic confirms that those items ingested by zooplankton, copepods, and mussels are transferred to predators, including shrimp, crab, and fish (Au et al. 2017). However, fewer studies evaluate the transfer across complex food webs, particularly in the field, and are often done by inferring trophic transfer from prevalence in the feces of top predators and their most common prey. Additionally, environmental plastics are known to contain additives and sorbed pollutants yet only a few studies have quantified or modeled the transfer of chemicals from microplastics across multiple species in a food web, limiting the overall understanding of toxicity and community- and ecosystem-level risk. Thus, the potential impact of microplastics on predator–prey dynamics and entire food webs remains largely unknown. Articles in this special issue that focused on trophic transfer provide important foundations on which to develop this understanding (e.g., Athey et al., Simmerman et al.) but more research is clearly needed.

Finally, a critical knowledge gap exists in background data on the prevalence of microplastic concentrations and effects...
in organisms across the globe. For example, microplastics are comparatively well studied in Europe and Australia, as well as in parts of Asia. However, there are major deficits in North and South America, Africa, and virtually no published studies in the Middle East. This special issue begins to address some of these deficits in both marine and freshwater organisms (e.g., Baechler et al. A, B; Horn et al., Hurt et al.)

**Research challenges**

To fill the above knowledge gaps and improve understanding, research is needed to establish baselines (e.g., Baechler et al. A and B); document trophic and geographic variability (e.g., Hurt et al., others); and test for individual-, community-, and ecosystem-level effects of microplastics (e.g., Athey et al., Dovidat et al., Horn et al., and Rogers et al.). Unfortunately, there are seven major challenges in conducting this important research. The first is **funding**. Although it is fairly inexpensive to collect and digest samples, it is far more expensive to perform necessary microplastic validation and polymer characterization. The second is **training and personnel**. We currently lack enough trained personnel to sample and analyze the diverse array of taxonomic groups and trophic levels across a broad geographic range of fresh and marine waters. In other words, there are not enough individuals trained in good QA/QC methods, nor enough funding for clean work spaces, to collect and process the necessary samples. A third challenge is **contamination**. It is now known that microscopic debris, particularly fibers, are ubiquitous across indoor and outdoor environments. Thus, studies lacking the means to sufficiently prevent and account for background contamination may be overreporting the amount of plastic debris in samples. Likewise, research conducted without access to proper microscopes or analytical confirmation may unintentionally misrepresent the source or type of microplastic pollution, or mischaracterize nonmicroplastic items (Shim et al. 2017).

The fourth major challenge is the fact that the size range of debris researchers are expected to detect and quantify keeps getting **smaller** and the importance of designing sampling regimes to account for a wider distribution of microscopic microplastics (< 1 mm) is increasing each year (Nguyen et al. 2019). This trend increases not only the cost but also the training and validation necessary to adequately study microplastics in these size ranges. For example, much of the current data available from field sampling exist for size fractions ≥ 300 μm with less information on smaller microplastics and even less on the nanoplastics (Fig. 1) the majority of polymers eventually degrade into. Along with problems with sampling regimes, this challenge is exacerbated by the analytical capacity of currently available or affordable technology. Namely, those technologies available for polymer characterization (e.g., FTIR or Raman spectroscopy) are unable to adequately characterize very small particles or fibers (< 20 μm for FTIR, < 1 μm for Raman) or the time needed for characterization is highly limiting for environmental samples. Therefore, researchers are currently faced with a trade-off between polymer confirmation for limited size classes or detection of smaller sizes using dyes (e.g., Nile Red) to help process samples in a timely manner. The challenges and costs associated with detecting smaller sized plastics or various morphologies create our fifth

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**Fig. 1.** Publications on micro- and nanoplastics (5 mm to 1 μm and < 1 μm, respectively) have increased exponentially over the past decade, but significant gaps still remain, particularly for the measurement of responses to microplastics. Searches were conducted in Web of Science on “Topic” to estimate the number of papers across the sub-categories of (1) Response [Nanoplastic* AND Response*, or Microplastic* AND Response*], (2) Ingestion [Nanoplastic* AND Organism* AND Ingest*, or Microplastic* AND Organism* AND Ingest*], and (3) Water [Microplastic* AND Water*, or Nanoplastic* AND Water*]. These are rough estimates and overlap in publications may exist between one or more categories.
challenge: realistic count and size-based exposure scenarios. Due to limited data on a range of size classes, experiments are unable to adequately examine organismal exposures using relevant concentrations in relevant size classes, limiting characterization of effects, trophic transfer, and overall risk assessment.

The sixth challenge is that microscopic debris can be comprised of materials that are plastic, blends of synthetic and natural materials, or natural (cotton, linen, chitin), which complicates methodologies, analyses, and inferences. For example, whether the composition of this debris makes an appreciable difference to exposed organisms is not yet fully understood. In fact, over the past few years the emphasis has shifted from a primary concern for plastic-associated chemicals to a call for describing plastics as a class of pollutants rather than a single pollutant type (Rochman et al. 2019), and thus gaining a better understanding of how size, shape, polymer, and even material types outside of polymers (synthetic, natural, blend of both) influence encounter rate, internalization, and subsequent effects, if any. Understanding the true complexity of these micro- and even nanosized items is a challenge that will continue to confront researchers as we enter a second decade of intensive microplastics study, as well as relating findings to concerns over the now-documented human exposure.

All of the above challenges make studying microplastics in aquatic ecosystems extremely difficult. However, because the study of microplastics is still relatively nascent, one area that may most severely limit forward progress is the lack of standardized methodologies because this greatly limits researchers’ ability to compare across studies. Although communication across research groups continues to increase, efforts to strengthen these connections are critical to improving the quality and comparability of research findings, eventually allowing for the meta-analyses that are necessary to accurately assess the hazard and risk of microplastics across freshwater and marine ecosystems (e.g., Bucci et al. 2019). Nevertheless, we are optimistic about these challenges, as we describe next.

Solutions

Many of the above challenges are being addressed through novel approaches and partnerships. For example, one tactic for addressing standardization and methodology challenges is the newly created US National Science Foundation funded Pacific Northwest Consortium on Plastic Pollution, whose goal is to conduct fundamental research on the fate and effects of micro- and nanoplastics in aquatic environments to inform risk management decisions that will be protective of the environment, essential fisheries, and aquaculture commodities. Another promising solution is an upcoming special issue on standard methodologies for sampling, digestion, and analysis in the Journal of Applied Spectroscopy. In addition, global interest groups in the Society of Environmental Toxicology and Chemistry focused on the study of nanoparticles and microplastics are linking investigators and labs across continents. Further, to address the personnel shortage for baseline and monitoring work and to increase the availability of consistent and reliable baseline data and cover a wider range of environments, there are efforts in process to develop clear protocols and QA/QC techniques for citizen science research.

Additionally, there are nascent efforts to assess and implement strategies to reduce the time and overall cost of conducting microplastic research. First, automation across the microplastic workflow in a small number of labs is beginning to take hold. This is primarily being developed for conducting accurate counts using fluorescent dyes coupled with imaging software (e.g., ImageJ) or using automated mapping technology for polymer characterization (e.g., FTIR). Second, open access data (such as the data repositories for articles in this special issue) and polymer libraries are being developed and shared to save costs associated with polymer identification and to again allow much needed transparency for meta-analysis. While these tools are still being developed and validated for different size classes, they hold great promise for reducing the time to process and eventually with increased use, should reduce the cost of automated technology.

Furthermore, there is a great need to establish a consistent framework for risk assessment of microplastic and nanoplastic exposures by organisms. Frameworks such as those established for the determination of Adverse Outcome Pathways (AOPs) or AOP networks (Ankley et al. 2010, Knappen et al. 2018) have been in existence for up to a decade or more for soluble pollutants such as pesticides, industrial chemicals, and pharmaceuticals that are present in effluent and stormwater runoff. Micro- and nanoplastics researchers can use these existing constructs to inform experimental design for sampling efforts and exposure regimes. Mechanistic information on endpoints relevant to fitness (e.g., development, swimming behavior, reproduction) is necessary to predict potential effects at organism and population levels. The importance of collecting comparable data on responses across environmentally relevant ranges of plastic concentrations has become evident as toxicologists and ecologists grapple with the need to determine what amount of micro- or nanoplastic pollution may pose a potential threat to aquatic organisms.

This special issue takes a step toward addressing some of the knowledge gaps and research challenges. We hope that the findings from the studies in this special issue generate more new questions than the number of answers they provide. We hope that this special issue will help steer microplastics research in the next decade and we look forward to the future research directions that these articles catalyze.

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