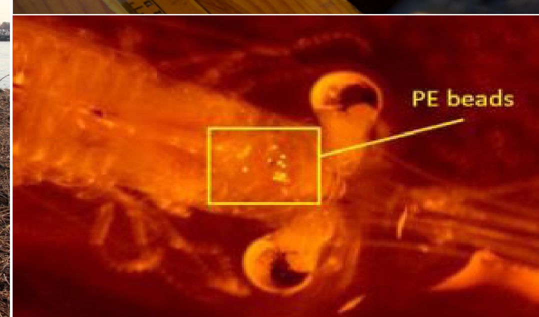
MICROPLASTIC MONITORING & SCIENCE STRATEGY FOR THE CHESAPEAKE BAY



TETRA TECH

Tetra Tech, Inc.
10711 Red Run Bvld.
Suite 105
Owings MIlls, MD 21117

Microplastic Monitoring & Science Strategy for the Chesapeake Bay

Tetra Tech, Inc.

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Prepared by:

Bob Murphy

Jennifer Flippin

Ryan Woodland (UMCES)

Paige Hobaugh

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Region 3 (Mid-Atlantic), Water Division, State Assistance & Partnerships Branch

Executive Summary

World production of plastic surpassed the 368 million tons mark in 2019, most of which is intended for packaging, for single use. Most plastic that escapes into the environment eventually breaks down into progressively smaller fragments that make their way into waterways. The occurrence of small plastic particles on beaches and in coastal waters was first reported in the 1970s although the term 'microplastics' was not used until relatively recently. Microplastics are currently defined as particles <5 mm (with the definition of nanoplastic still evolving, but generally <330 µm). It is likely that the amount of plastic waste in the ocean will continue to increase, driven primarily by the inexorable rise in plastics consumption and the continued inadequacy of re-use, recycling and waste management practices in many parts of the world. In 2019, the Chesapeake Bay Program (CBP) Scientific & Technical Advisory Committee (STAC) sponsored a workshop to evaluate the state of the knowledge within the Chesapeake watershed, resulting in several action items including developing a science strategy on microplastics.

The Plastic Pollution Action Team (PPAT) was formed at the directive of the CBP Management Board and was charged to develop a preliminary ecological risk assessment model (Appendix C), in addition to a size classification document (Appendix B), and this science strategy. Many of the topics from those first two documents are found in this document to formulate a strategy to address microplastics bay-wide.

The PPAT recommends the following priorities for the CBP to undertake:

1. Design and implement a microplastic monitoring program, integrated into the existing Chesapeake Bay watershed monitoring framework;
2. Support research to understand microplastic pathways in the Bay, including trophic pathways that may affect living resources such as Striped Bass, Blue Crabs, Oysters, and other species critical to the Bay ecosystem;
3. Ensure adequate infrastructure resources are available to process microplastic samples, including analytical equipment; and
4. Continue to support the PPAT in order to direct research, management, and policy development;

This strategy document provides an overview of management needs regarding implementing policies to reduce plastic pollution, which would result in reduction in microplastics. Answering these knowledge gaps will provide a defensible position for policy development. To do this, PPAT recommends implementing a monitoring program within the framework of the existing bay-wide monitoring programs. Additionally, data gaps important for basic research are noted as these can be filled by specific studies or within a monitoring program. This strategy is intended to be a starting point to develop research priorities, monitoring efforts, and policy development. It is expected to be updated in the future as more work is completed and new paradigms emerge.

I. Introduction

The global production and disposal of plastics has increased by orders of magnitude over the past 60 years (Rochman and Browne 2013, Li et al. 2016) and a large portion of plastic waste (11% or 19 to 23 Mt (Borrelle et al. 2020)) makes its way into waterways and coastal systems annually (Andrady 2011, Jambeck et al. 2015, Lebreton and Andrady 2019). Aside from the deleterious impacts on the aesthetics of the environment, there are concerns about the ecological harm posed by plastics. It is well-documented that larger plastic debris has significant and negative impacts on a variety of wildlife (Li et al. 2016), ranging from entanglement to increased mortality through ingestion (Davison and Asch 2011, Kühn and van Franeker 2020). An emerging concern, however, has shifted focus from large, visible plastic debris to the largely unseen microplastic contamination of the aquatic environment.

In 2019, the Chesapeake Bay Program's Scientific & Technical Advisory Committee (STAC) convened a two-day workshop focused on sharing the current state of the science regarding microplastics in the Bay and its watershed (Murphy et al. 2019). Several recommendations emerged from the workshop, including:

- formation of a Plastic Pollution Action Team (PPAT);
- development of a preliminary ecological risk assessment model (eventually targeting Striped Bass, age 0-2);
- standardizing terminology;
- developing a source reduction strategy; and
- utilizing the existing monitoring networks to monitor for plastic pollution.

This document summarizes the state of the science of microplastics, both in a global context and within the Chesapeake Bay region. It also builds off the completed preliminary risk assessment model to identify data gaps that will aid our understanding of the trophic pathways, sinks and sources of microplastics that potentially impact important Bay resources. The PPAT also identified management questions that helped identify additional data gaps that, once better understood, might lead to policy decisions. We also provide a framework to build microplastic monitoring into the existing monitoring efforts with regard to water quality, particle transport, and living resources. Lastly, using our current understanding of the magnitude of the issue, we also identify potential partnerships, technical, and financial resources. Our aim is to provide a path forward that ultimately informs management decisions.

II. State of the Science

Recent research has shown microplastics to be ubiquitous in habitats around the world (Castaneda et al. 2014a, Anderson et al. 2016, Jabeen et al. 2016), posing an emerging concern for aquatic life, and potentially, human health (Barboza et al. 2018). There has been a significant increase in the concentrations of microplastic particles in the surface waters of oceans within the last four decades and concern about the potential impact on the marine environment has increased during the past few years. Currently, plastic makes up about 80 to 85% of marine litter (Cole et al. 2011). Scientific investigations about the impact of microplastics on ecosystems have increased, along with public interest (GESAMP 2015). Plastics became the fastest growing segment of the municipal waste stream between 1950 and 2003, and its global production has increased significantly over the past decades (Auta et al. 2017). Despite filtration methods, wastewater effluent is estimated to release, on average, 4 million microparticles per facility per day (Sun et al. 2019). With 516 major wastewater treatment plants (WWTP) discharging effluent into the watershed, this is a significant concern for the Chesapeake Bay ecosystem. Additionally, the Chesapeake Bay watershed contains numerous urban and suburban areas that, via storm drains and non-point source surface run-off, are sources of plastic waste to the bay (Peters and Bratton 2016). These larger, visible plastic items degrade into smaller microplastics over time and are hypothesized to affect the bay in a variety of ways, both at the organismal and ecosystem level. While microplastics themselves could be directly harming bay species physically and chemically, recent research has also shown that organic toxic contaminants (e.g. polycyclic hydrocarbons [PAHs])—already known to pollute the bay—adsorb to microplastic particles. Once consumed by bay fauna, these compounds may have physiological and neurological effects, and may be magnified up the food chain (Batel et al. 2016, Windsor et al. 2019). De Frond et al., 2019 estimate that 190 tons of chemical additives are introduced to the ocean annually because of plastic materials.

Numerous attempts have been made to assess their potential effects not only to the environment, but specifically to biota and, ultimately, to humans. Due to their small size, these particles can be ingested by numerous marine species, leading to direct physical damage and potential toxicity effects (Wright et al. 2013). Microplastics may also leach plastic additives, including persistent organic pollutants (POPs) and potentially toxic elements that are adsorbed in higher concentrations than those found in the surrounding environment. These pollutants may transfer and accumulate in different tissues of organisms, possibly undergoing biomagnification along the food chain (GESAMP 2015). Hence, consumption of contaminated seafood poses a route for human exposure to microplastics, POPs, and potentially toxic elements (GESAMP 2015). POPs, including polychlorinated biphenyl (PCBs) and PAHs, have also been shown to accumulate on microplastics, thus enhancing their potential toxic effect in the environment (Hartmann et al. 2017). Such dangers have been demonstrated for numerous organisms, such as blue mussels, in which von Moos et al. (von Moos et al. 2012) verified that microplastics, namely, high-density polyethylene (PE), ranging from <1 to 80 mm were ingested and taken up into the cells and tissues of these organisms. Microplastic particles were drawn into the gills, transported into the stomach and into the digestive gland, where they accumulated in the lysosomal system after 3 hours of exposure (von Moos et al. 2012).

Furthermore, the authors demonstrated impacts to organismal health including histological changes and increased inflammation. Jovanovic (2017) reported potential negative effects of the ingestion of microplastics and nanoplastics by fish, including possible translocation of microplastics to the liver and intestinal blockage, yielding not only physical damage, but also histopathological alterations in the intestines and modification in lipid metabolism. It should be noted, however, that, despite demonstrating the potential fate and effects of microplastics on biota, these studies, as well as other numerous reports described in the scientific literature, focus on experiments on the use of polymeric particles at concentrations that far exceed those determined in the environment, thus not accurately simulating natural settings regarding composition, morphology, and concentration.

The state of the science of understanding microplastic distribution and potential harm is in its infancy in the Chesapeake Bay (Murphy et al. 2019). Recent studies have shown that microplastics are ubiquitous in the Chesapeake Bay (Bikker et al. 2020a) . A 2014 survey showed microplastics to be present in four tidal tributaries to the bay, with 59 of the 60 samples collected showing presence of particles (Yonkos et al. 2014). This study also found concentrations of microplastics to be highly correlated with population density and presence of suburban and urban development (Yonkos et al. 2014, Peters and Bratton 2016). Bikker et al. (2020a) conducted a bay-wide survey conducted a survey in 2020 and found microplastics in every sample collected (n=30). A 2017 study conducted by Tetra Tech, the Metropolitan Washington Council of Governments (MWCOG), and the DC Department of Energy & Environment (DOEE) found that microplastics accumulate on the plant leaves in submerged aquatic vegetation (SAV) beds in the tidal Potomac River. SAV is one of the bay's most important habitats and provides food and refuge for some of the region's most commercially and ecologically significant fisheries. Lastly, recent research has shown that potential human pathogens, such as *Vibrio* spp., have also been found to colonize microplastics in the Chesapeake Bay, providing evidence that particles could help disperse disease (Kirstein et al. 2016). Additionally, recent research by Seeley et al (2020) has demonstrated microplastic disruption in the nitrogen cycle within estuarine sediments. These effects may have substantial impacts on nitrogen dynamics throughout the estuary.

The recent ecological risk assessment conceptual model developed by Tetra Tech and the University of Maryland examined potential impacts of microplastics on age 0-2 Striped Bass (*Morone saxatillis*) in the Potomac River. Developed in consultation with the PPAT and the CBP Scientific and Technical Advisory Committee (STAC), this document evaluated a range of potential trophic pathways that microplastics may follow to reach Striped Bass, causing a potential ecological impact, as well as a potential risk to human health. This preliminary model demonstrated the paucity of data necessary to make informed decisions with regards to microplastics in the Bay and this document will build on that effort to describe data gaps and how the science may move forward.

III. Management Questions

This document has been developed to provide guidance in support of the Chesapeake Bay Program's PPAT. The PPAT recently completed a preliminary ecological risk assessment for microplastics and age 0-2 Striped Bass in the Potomac River, along with a companion document that recommended standardized terminology to be used for microplastics monitoring and research in the Chesapeake Bay. Furthermore, based on these two documents and interests from multiple agencies, the PPAT provided additional management questions and concerns that are addressed in this document.

1. *How can government and resource managers develop sound policies to reduce [micro]plastic pollution and assessing the economic impacts?*

To answer this question, data on the source and composition (i.e. polymer type) of plastic is required as limits on plastic availability or behavior change of a population are likely policy options. This document also recommends the establishment of a monitoring program to answer management questions in more detail, with particular emphases on hotspots and sources. Current modeling efforts of microplastic transport and fate bay-wide is also a valuable tool and it is recommended that this effort is expanded to include each of the tributaries and extend up into the watershed. It has been noted that stormwater control is one action that can reduce microplastic movement into waterways, provided the particles can be effectively removed from control structures. The economics of policy-making is somewhat beyond the scope of this document; however, we do recognize that socio-economic studies be conducted simultaneously to determine cost-benefit models for plastic reduction. Coupling these models with source-transport-fate models can likely help policy-makers determine the impact of current and future plastic reduction strategies. Similar approaches with nutrient reduction have proven successful, both economically and ecologically. Several PPAT members have recommended that a regional strategy will have greater impact over a broader area; this approach, using existing regional partnerships (such as CBP, Partnership for the Delaware Estuary, Mid-Atlantic Regional Council on the Oceans Marine Debris Workgroup, river commissions, etc.) will only strengthen the impacts of strong multi-jurisdictional policies. Initial regional policies and trash reduction programs, including best management practices, will provide a good starting point for full policy development.

2. *What health risks are posed by microplastics?*

The health effects of microplastics to humans is only recently being evaluated and better understood, although we are a long way from complete understanding of this highly variable impact (Campanale et al. 2020). Ecological risk (Bucci et al. 2020, de Ruijter et al. 2020) to wildlife is an ongoing area of research and we strongly recommend that research into trophic linkages, mortality rates, and other biological effects take place. As we have seen with Striped Bass, the unknowns are vast, but understanding the basics of transfer, or laboratory studies on mortality/reduced fitness will be especially valuable to fisheries managers. The link from Striped Bass (and other organisms harvested for consumption) to human health will be one component of this evolving question.

3. What are the sources, pathways, composition, and fate of microplastic loadings into the Chesapeake Bay?

This question addresses the crux of the issue in terms of our incomplete understanding the scale and nature of microplastic loadings within the watershed. This question drives the need to establish a monitoring program to answer these, as this data can be used to answer a host of other questions associated with understanding these processes. Current modeling of microplastic transport has provided excellent insight into general trends that we would expect in the mainstem of the Bay. However, model outputs are only as robust as the parameterized data that drive them. Understanding, through monitoring data, the sources, type, and abundance of microplastics will help refine models and increase spatial applicability to include the tributaries. Additional PPAT concerns centered mainly on the fate of microplastics and how physical properties (e.g. salinity, freshets, storms) impact microplastic distribution. Similar questions have been raised in terms of understanding impacts on benthic habitats (e.g. SAV beds, oyster reefs). Directed monitoring (addressed in a separate section) can potentially answer these questions, as can research on topics discussed under Data Gaps.

4. What management actions or policies may be effective in reducing microplastic pollution?

This question is shared by the PPAT and the group that drafted the science strategy for microplastics in San Francisco Bay. This is not surprising as it will take some form of intervention to reduce plastic loading throughout the watershed. Several ideas have been brought forth and many more are in development and should be evaluated. Foam food container bans, microbead bans, recycling efforts, and plastic bag bans have all been management strategies implemented to reduce plastic pollution. Additional ideas include engaging businesses that either produce or rely on plastic bottles to consider adopting alternate materials (e.g. glass) as these changes do not appear to create major economic hardship (Schuyler et al. 2018).

IV. Sampling and Quantification Methods

Quantification of microplastics is necessary to understand abundance in environmental matrices and develop a better correlation between exposure and effects including potential dose-response relationships in exposed organisms. Methods for sampling and analyzing microplastics across studies remain inconsistent. This is due to varying study goals, media, or plastic types/sizes considered; rapidly evolving technology; lack of standardized methodology; and reflective of the fact that the study of microplastics is relatively recent.

The 2019 *Microplastics in the Chesapeake Bay and its Watershed: State of the Knowledge, Data Gaps, and Relationship to Management Goals* workshop proceedings noted that inconsistencies such as the tendency to report mass/unit volume or particles/unit area in similar studies. Mixed unit estimates like these pose problems as number of particles m^{-3} cannot directly correspond to aquatic surface area since the volume of water in one area may be more or less than in another of the same area (Bikker et al. 2020a). Similarly, Burns and Boxall (2018) explain that because ecotoxicity tests are reported in measures of mass or number of particles per volume, measures reported in "items per square meter" are not as easily comparable. Consistency in estimation of particles per unit area or volume is critical since organisms respond differentially depending on the impact measured. For example, number of particles per unit area is appropriate when measuring impact on respiration since gill area is the determining factor for gas exchange. However, volumetric estimates are more appropriate for gastrointestinal studies since ingested "food" is more biologically relevant when measured by volume (although number of particles can also be relevant when assessing ingestion effects). Most microplastic analysis methods lack standardization and continue to update as new analytical technologies become available. Thus, the sampling, identification, and quantification of microplastics in different media remains inconsistent.

A companion document to the initial ecological risk assessment of microplastics in the Chesapeake Bay, *Uniform Size Classification and Concentration Unit Terminology for Broad Application in the Chesapeake Bay Watershed,* preliminarily provides recommendations and considerations for future research related to study design, analysis, and sampling. These considerations are as follows:

General Considerations

- Environmental plastics are complex because they encompass a diverse group of materials with different physical/chemical properties along with fate, transport, and bioavailability characteristics.
- A convention for grouping plastic debris based on size or other descriptors will improve communication, ability to compare the results of studies in a meaningful manner, and determine which plastics are potentially associated with risk.
- The lower size limit of microplastics reported by studies is often functionally constrained by limitations of sampling technology. In the case of Chesapeake Bay and tributary studies, researchers used manta trawl nets to capture and report microplastic fragments

with a lower limit of 333 µm (Bikker et al. 2020, Yonkos et al. 2014). However, this means that microplastics smaller than 333 µm are not explicitly quantified (though may have been depending on processing and analytical methods).

- The findings of some studies, especially field -based ecological studies where perhaps only visible plastics are currently quantified likely underestimates true exposure because smaller microplastics or nanoplastics could contribute to biological effects observed in a study but are not measured.
- Microplastic and nanoplastic designations will be the most useful terminology to describe plastics that are potentially biologically relevant via ingestion or inhalation/gill uptake. However, for the purposes of sampling, monitoring, and prediction of plastic loading to a particular system, it will be informative to classify larger plastics that are easily visible to the naked eye (e.g., foams, plastic bags, bottles, packaging materials, etc.), as these are the precursors to microplastics and nanoplastics, and are easily visible during shoreline or open water monitoring.

Potential Units of Measurement for Focus in Future Studies

Different characteristics of media or study objectives influence the decision of how environmental plastics are analyzed or reported. The following units were identified for consideration of inclusion in future research to ensure comparability:

- **Water:** Number of particles m^{-3}; Number of particles l^{-1} ; particles per unit volume concentrations are recommended for standardized monitoring strategies for the Chesapeake Bay watershed. This unit of measurement potentially accounts for particles throughout the water column, including those at the surface.
- **Sediment:** The number of particles in sediment should be measured volumetrically since organisms exist in a three-dimensional environment within the sediment. The exception to this would be to assess abundances of microplastics on the sediment surface as this region is exploited by a variety of deposit-feeding polychaetes, bivalves, crustaceans, and benthic fish.
- **Organism:** The mass of particles per individual is a general measurement that does not discriminate between organ or tissue as the site of accumulation and accounts for an organism's total exposure to microplastics. This measurement may serve as an informative tool for monitoring the prevalence of microplastic accumulation in organisms. This approach has advantages from a toxicology/risk standpoint.
- **SAV:** Measuring microplastics within SAV beds mostly depends on the research objectives. The most common measurement would assess the area covered on blades of plants. Goss et al. (2018) reported # particles blade $^{-1}$ which provides insight to loadings. However, area covered by microplastic particles is more biologically relevant because a) area of microplastics will block the surface of blades from sunlight, and b) larger particles can potentially be consumed by grazers, therefore area estimates can serve as a proxy for mass (as recommended above). One exception to this recommendation is in

the case of studies that are comparing SAV bed metrics (e.g. canopy capture of microplastics) to similar conditions elsewhere, which would entail measuring # particles unit volume^{-1}. Microplastics that settle to sediments in SAV beds would be captured under “Sediment” above.

- **Shoreline:** Quantifying plastics debris on shorelines will depend on the research, policy, or monitoring objective of interest. Options may include number or mass of particles per unit volume or area of shoreline substrate, or number of items per unit length of shoreline.

V. Data Gaps

The ecological effects of microplastics on living resources in Chesapeake Bay are relatively unknown. Much of this uncertainty arises from a lack of observational and experimental data on the types, sources, and fates of microplastics in the ecosystem. With few exceptions, a lack of studies on the size-distribution and associated contaminant characteristics of microplastics in Chesapeake Bay is another important knowledge gap that hinders our understanding of the toxicological effects of microplastics in Chesapeake Bay. In some instances, ecological data on individual species are either absent, incomplete, or dated, contributing to the uncertainty surrounding potential exposure to microplastics for these populations. For example, spatial, temporal, and ontogenetic patterns in diet, habitat use, and movements can all influence the relative exposure of individuals to microplastics in the environment. Highlighted below are several key data gaps facing the implementation of a robust microplastics ERA for the resident life-stages of Striped Bass in the Potomac River.

<u>Ecological niche</u>: There is an incomplete understanding of the diet of juvenile and resident sub-adult Striped Bass in the Potomac River. Previously published studies of young-of-the-year Striped Bass diet from the Potomac (e.g., Beaven and Mihursky 1980, Boynton et al. 1981) provide useful insight into ontogenetic and spatial patterns in diet. Specific to juvenile diet, results from the Boynton et al. (1981) study and those from other systems suggest this life-stage is highly opportunistic and suggests local prey availability will have a strong effect on local diet composition. It also suggests that any ecosystem-scale changes in the availability of prey in recent decades is likely to alter the realized diet of juvenile Striped Bass. Further, there is very little information available on diet of yearling and age-2 Striped Bass in the Potomac River although data from the adjacent mainstem of Chesapeake Bay suggests these age-classes are also quite opportunistic in their prey choice (Ihde et al. 2015). This generalist feeding behavior underscores the need for a contemporary and robust spatiotemporal assessment of resident Striped Bass diet composition along the Potomac River estuary in order to accurately quantify the trophic niche, and by extension the potential for trophic transfer of microplastics, of Striped Bass in this system.

<u>Microplastics in the Potomac River food web</u>: In order to link Striped Bass feeding to microplastics exposure, an understanding of in situ microplastics contamination in key trophic resources is needed. Previous studies have identified microplastics associated with basal trophic resources such as macrophytes and associated epiphytes, benthic organic matter, and suspended particulate organic matter (Table 1). While not intentionally consumed by Striped Bass, these basal resources represent sources of microplastics at the base of the Potomac River food web. Direct trophic transfer of microplastics from prey to fish consumers has been verified in numerous laboratory studies and recent evidence suggests that the trophic transfer of microplastics can be more important than passive uptake through non-trophic mechanisms in some situations (e.g., Hasegawa et al. 2021). Therefore, empirical measurements of microplastics loadings in dominant prey taxa are needed in order to track routes of microplastics exposure through trophic transfers to Potomac River Striped Bass.

A broad range of prey have been identified for Striped Bass in the Potomac River (Table 1). These prey all represent potential pathways for microplastics exposure for Striped Bass; however, those prey that contribute most to diet are considered the most critical data gaps regarding trophic exposure to microplastics. During exogenous feeding larval stages, spanning yolk-sac to post-finfold stages, *Bosmina longirostris* (Cladocera), *Eurytemora affinis* (Copepoda), and unidentified cyclopoid copepods were the dominant prey identified in a study by Mihursky and Beaven (1980). Young-of-the-year juveniles and resident subadults feed heavily on *Neomysis americana* (Mysida), polychaetes, and amphipods. Piscivory increases during this period, with Bay Anchovy (*Anchoa mitchilli*) and Atlantic Menhaden (*Brevoortia tyrannus*) contributing substantially to diets as well. These prey represent the most important knowledge gaps in our understanding of the potential for trophic transfer of microplastics to resident Striped Bass in the Potomac River.

Table 1. *Primary prey taxa, type of study verifying ingestion of microplastics by prey, and critical prey status (Juvenile/resident sub-adult Striped Bass: 1 = low priority, 2 = moderate priority, 3 = high priority; Lx = larval-stage Striped Bass with values matching the juvenile priority levels). Table modified from Tables 2-4 of the Preliminary Conceptual Model for an Ecological Risk Assessment for Microplastics on Striped Bass in the Potomac River Estuary).*

Major Taxa	**Type of study**	**Critical Prey status**	**References**
Habitat			
Macrophytes	Field		4 – 10
Epiphytes	Field		4,11
Benthic organic matter	Field		12,13
Phytoplankton	Laboratory		14,15
Invertebrate Prey			
Insects	Field	2	2
Crustacean larvae	Laboratory	1	1,16-18
Cladocerans	Laboratory	2	1,18-20
Bosmina longirostris		L3	1
Copepods	Laboratory; Field	2	1,21,22
Acartia tonsa		L1	1
Cyclopoid		L3	1
Eurytemora affinis		L3	1
Amphipods	Laboratory	3	2,3,23,24
Mysids	Laboratory	3	2,3,25-27

Table 1. *Primary prey taxa, type of study verifying ingestion of microplastics by prey, and critical prey status (Juvenile/resident sub-adult Striped Bass: 1 = low priority, 2 = moderate priority, 3 = high priority; Lx = larval-stage Striped Bass with values matching the juvenile priority levels). Table modified from Tables 2-4 of the Preliminary Conceptual Model for an Ecological Risk Assessment for Microplastics on Striped Bass in the Potomac River Estuary).*			
Major Taxa	**Type of study**	**Critical Prey status**	**References**
Polychaetes	Laboratory; Field	3	2,3,25,28,29
Blue crab	Field	1	3,30-32
Crustacea (other)	Field	1	3,33,34
Mollusks	Laboratory	1	3,35,36
Fish			
Bay anchovy	Field	2	3,37,38
Atlantic menhaden	Field	2	3,38
Fish larvae	Laboratory; Field	2	2,39

References: critical prey status designation: **1 – Beaven and Mihursky 1980, **2** – Boynton et al. 1981, **3** – Ihde et al. 2015; microplastics in prey: **4** – Goss et al. 2018, **5** – Reynolds and Ryan 2018, **6** – Murphy 2019, **7** – Townsend et al. 2019, **8** – Cozzolino et al. 2020, **9** – Huang et al. 2020, **10** – Jones et al. 2020, **11** – Seng et al. 2020, **12** – Castaneda et al. 2014, **13** – Murphy 2020, **14** – Long et al. 2015, **15** – Shiu et al. 2020, **16** – Jemec et al. 2016, **17** – Gambardella et al. 2017, **18** – Woods et al. 2020, **19** – Martins and Guilhermino 2018, **20** – Jaikumar et al. 2019, **21** – Cole et al. 2015, **22** – Desforges et al. 2015, **23**– Jeong et al. 2017, **24** – Mateos Cárdenas et al. 2019, **25** – Setälä et al. 2014, **26** – Lehtiniemi et al. 2018, **27** – Wang et al. 2020, **28** – Mathalon and Hill 2014, **29** – Knutsen et al. 2020, **30** – Santana et al. 2017, **31** – Cohen 2020, **32** – Waddell et al. 2020, **33** – Devriese et al. 2015, **34** – Waite et al. 2018, **35** – Avio et al. 2015, **36** – Gutow et al. 2016, **37** – Gray et al. 2018, **38** – Parker et al. 2020, **39** – Rodrigues et al. 2019*

Field studies that verify the presence of microplastics in the stomachs and guts of Striped Bass and their dominant prey should be considered the highest priority data gaps needed to be addressed through field studies. In order to be most effective, field collections should span the range of life-stages of resident Striped Bass and the habitats occupied by these life-stages throughout the year. Establishing spatiotemporal 'hotspots' of microplastics contamination of prey taxa would provide the information needed for resolving potential exposure of Striped Bass through trophic transfer.

Individual and population-level effects: Laboratory studies on the toxicological effects of microplastics on Striped Bass (and other Bay species) are needed to place the exposure of Striped Bass into a risk context. Studies of acute and chronic exposure of different types and concentrations of microplastics are necessary to determine individual-level effects on Striped Bass. Endpoints that span ecological (e.g., slower growth, smaller size at age) and more traditional morbidity and mortality estimates need to be established although care should be taken when extrapolating laboratory studies to field conditions. Population-level effects of microplastics exposure, such as changes in natural mortality rates, are difficult to test but represent key data gaps that are ultimately of greatest potential benefit to natural resource managers seeking to model the effect of microplastics on Potomac River Striped Bass population dynamics

Chesapeake Bay ecosystem: This preliminary ERA focused on Striped Bass in the Potomac River but there is an urgent need to replicate and build upon this process for other living resources. These include living resources with identified Outcomes under the framework of the Chesapeake Bay Agreement, such as Blue Crabs (*Callinectes sapidus*), Forage fish (also includes benthic invertebrate forage taxa), and Eastern Oyster (*Crassostrea virginica*), as well as resources that are ecologically or economically important but lack stated Outcomes. Potential species of interest under this latter group of taxa includes both native and invasive species. For example, native species such American Eel (*Anguilla rostrata*), river herring (*Alosa* spp.), American Black Duck (*Anas rubripes*), White Perch (*Morone americana*), and White Catfish (*Ameiurus catus*) occupy a range of ecological niches and some are harvested directly for human consumption. Focused ERAs on non-native species are also important, particularly for species such as Blue Catfish (*Ictalurus furcatus*) that are ecologically invasive and support growing commercial and recreational fisheries.

The lack of empirical data on microplastic contamination for Striped Bass prey was a critical data gap for this preliminary ERA. This data gap will be present for any ERA conducted in the Chesapeake Bay region and underscores the need for research on microplastic contamination and trophic transfer by lower trophic level taxa, in addition to research focused on species or groups of interest (e.g., Striped Bass). Overall, directed studies on the prevalence, intensity, and effects of microplastic contamination on focal species, their prey, and the environment are needed to support robust ERAs and continue developing our understanding of the risks of microplastics to humans in the Chesapeake Bay ecosystem.

VI. Monitoring Strategy

The 2019 STAC workshop (Murphy et al. 2019) participants identified microplastic monitoring throughout the Chesapeake Bay watershed as a primary need for several reasons. At present, the Bay community recognizes that baseline data showing the abundance and distribution of microplastic occurrence baywide is data poor (although, see (Yonkos et al. 2014, Bikker et al. 2020a) while only few studies have focused on tributaries or very localized assessments (Murphy 2020). For the management community, understanding baseline conditions of distribution and composition of microplastics will serve as a starting point for policy development and implementation. The monitoring strategy outlined here represents a starting point for this effort, although it is understood that monitoring programs may evolve as more data is collected, understanding of the conditions change, or resources to conduct such a program fluctuate. Members of the PPAT offered multiple ideas and endpoints that would be useful for both the scientific understanding and the data needs for managers.

Framework

The Chesapeake Bay Program, in partnership with the member states and Washington, DC, federal agencies, and several academic institutions, maintains a water-quality monitoring program that assesses current status of potential pollutants (e.g., nutrients), harmful algal blooms, sediment loads, dissolved oxygen, and numerous biological indicators. Because the nature of existing monitoring programs includes probable pathways or sinks for microplastics, working within the existing monitoring framework would be the most effective approach. In addition, as many PPAT members have indicated, the CBP is the logical home for driving multi-jurisdictional programs that address the shared resources of the watershed. Not only should a robust monitoring effort be addressed by CBP, it is also would serve (as it currently does) as a repository for microplastic monitoring data that would be available for analyses and potentially for policy development. Similar to previous bay agreements that set goals to be met, microplastic data collection can be a goal in itself; that is, having a spatially explicit map of microplastic distribution within 5 years of monitoring commencement.

Water quality

PPAT members frequently noted that monitoring the waters of the Chesapeake watershed is a top priority. This will address specific questions, including source identification (point source and non-point source), hotspots and 'hot moments' (temporal events associated with high concentrations), and polymer type within the water column. It was also noted that water monitoring include the non-tidal portions of the watershed as this will aid in identifying sources and potential impacts on living resources not addressed in the initial ecological risk assessment model. Given the numerous monitoring stations around the bay and tributaries, spatial resolution of suspended microplastics can be better understood following this network. This may also assist with refining existing models on microplastic movement within the estuary.

Benthos

The Chesapeake Bay Program Office currently supports a long-term benthic monitoring program of the tidal portion of the Chesapeake Bay and tributaries. Benthos monitoring is designed to give comprehensive spatial and temporal information on benthic conditions in the Bay and tributaries and includes taking a sample of sediment to be analyzed for organismal composition and sediment characteristics. This is an ideal sampling design whereby microplastic occurrence and quantity could be quantified within the existing program. This data would provide excellent spatial information and be critical for identifying hotspots and sinks.

Fish

The ERA conceptual model development for Striped Bass in the Potomac River identified major data gaps in the understanding of microplastic ingestion and trophic transfer in finfish (as noted elsewhere in this document). Most fish monitoring programs run by the states focus on population estimates, biodiversity, spatial distributions, stock assessments, and long-term changes in fish community structure. A subset of programs (e.g. VIMS' ChesMMAP) collects biological data on fish populations that include trophic information (stomach content analyses). Within the framework of existing programs, these collections might be exploited to garner microplastic ingestion data across the tidal portion of the Chesapeake Bay.

Non-Tidal

Sampling in the non-tidal portions of the watershed is critical to understanding the sources and loadings of microplastics reaching the estuary. Several ongoing programs throughout the states actively collect water samples in streams and rivers which could theoretically be used for microplastic analyses. While most current methods for sampling streams for microplastics include deployment of nets so the number of particles captured can be related to volume, recent methods developed by ASTM for pumping water through sieves in a traditional cross-section with vertical profiles across a stream (ASTM 2020) could more closely align water-quality data with microplastics data and better inform load estimations (particularly for more ubiquitous particles like fibers). Although not noted under fish above, several jurisdictions routinely sample non-tidal streams to assess fish and macroinvertebrate populations (e.g. local governments, Maryland Biological Stream Survey—MBSS). It could be feasible to include subsampling of fish and stream benthos for microplastic accumulation via these programs.

Zooplankton

Zooplankton population parameters were historically assessed through a CBP monitoring program that is no longer active. However, other ongoing programs (e.g. George Mason University's Gunston Cove program on the Potomac: Potomac Environmental Research and Education Center – Gunston Cove Reports (gmu.edu)) have shown downward trends in several zooplankton taxa that are preferred prey items for larval Striped Bass. While this data is something that is also noted in the Data Gaps section, restarting zooplankton monitoring programs throughout the Bay, and incorporating microplastics would be fairly easy to implement (provided adequate funding). Microplastics "inhabit" similar parts of the water column and are often mistaken as zooplankton prey by predators; current sampling methods for plankton can be used to include microplastics captured within the same samples and analyzed in conjunction with plankton identification.

VII. Current and Potential Future Management Actions

Many plastic particles are already found in the environment and range from intact macroplastics, primary microplastics that were intentionally created as small pieces, and secondary microplastics (and smaller) that have been degraded from macroplastics by exposure to sunlight, mechanical abrasion or actions, etc. The study of their effects is ongoing. Reduction of potential ecological exposure can be achieved by control measures that address sources and pathways to local waterbodies.

Addressing the source of plastics is one method to control their abundance in the environment, and these measures can occur through national or local legislative actions. For example:

- Microbead-Free Waters Act of 2015 was passed by congress in December 2015 and prohibits the manufacture, packaging, and distribution of over the counter products including cosmetics, medicines, and toothpaste that contain plastic microbeads (The Microbead-Free Waters Act: FAQs | FDA).
- The Anacostia River Clean Up and Protection Act of 2009 in Washington DC requires all businesses selling food or alcohol to charge a five cent fee if a disposable bag is provided with any purchase (Anacostia River Clean Up and Protection Act of 2009 | DOEE (dc.gov)). The goal of this act is to reduce plastic waste in the Anacostia and other local water ways, and the fee is distributed to the Anacostia River Clean Up and Protection Fund, which provides education along with trash capture and stream restoration projects.
- Other counties or municipalities in Maryland including Montgomery County, Howard County, Baltimore City, Takoma Park, Chestertown, and Westminster also have legislation that limits usage of plastic bags.
- In 2014, Washington DC enacted the Sustainable DC Omnibus Amendment Act which included requirements for various types of food service ware that were implemented in phases. In 2016, Washington, DC businesses and organizations that serve food or beverages from using disposable food service ware made of expanded polystyrene (also known as foam or by its trade name Styrofoam™) to serve consumers. An amendment to the Foam Ban, with new requirements for stores and retail establishments, went into effect on January 1, 2021. The new requirements ban the retail sale of foam food service ware; foam storage containers, such as coolers and ice chests; and foam loose-fill packaging material, commonly known as packing peanuts. The purpose of the act was to control the source of foam and plastic items that are wind-blown or carried by stormwater to streams and rivers. In addition, under this law, DC implemented a ban on plastic straws from being served in restuarants and other food-related businesses.
- On July 1, 2020, Prince George's County, Maryland, banned straws and stirrers that are not reusable or meet home compostable standards. On May 1 2021, Montgomery County single-use straw banned goes into effect. This ban applies to food service and retail businesses from providing or selling these items (Plastic Straw Ban | Prince

George's County, MD). In addition, Charles County (MD) and the City of Takoma Park also have single use plastic straw bans in place.

- The State of Maryland is considering a bill that would ban plastic bags statewide by 2022.
- Maryland House Bill 391: Solid Waste Management—Prohibition on Releasing Balloons into the Atmosphere passed the House on 4/8/2021 and was sent to the Governor for signature.
- Virginia State Code 29.1-556.1 bans the release of 50 or more balloons within a one hour period if they are made of “a nonbiodegradable or nonphotodegradable material or any material which requires more than five minutes' contact with air or water to degrade.” (§ 29.1-556.1. Release of certain balloons prohibited; civil penalty (virginia.gov).
- Executive Order No. 77- Virginia Leading by Example to Reduce Plastic Pollution and Solid Waste calls for the cessation of use of plastic bags, single use foam food containers, plastic straws and cutlery, and plastic bottles by the Virginia State Government. In addition, purchase and use of single use plastics and foam containers is being phased out with a goal of 25% reduction in 2022 and 100% by 2025.

Controlling the pathways that link trash to waterbodies is another method for reducing plastic in aquatic ecosystems. For example:

- Skimmer boats on the Anacostia river remove floating plastic trash.
- Trash wheels in Baltimore’s Inner Harbor
- Street Sweeping programs target high-trash urban areas.
- Clean teams program in Washington DC collects plastic, recyclables, and other trash from public spaces and gutters to prevent it from washing into storm drains or streams.
- Trash traps, including one on Nash Run—a tributary of the Anacostia River, remove trash runoff from urban areas before entering larger waterways, such as the Anacostia River.

Future efforts to reduce the source of environmental plastics could follow the examples provided above, with similar efforts expanding to other cities, municipalities, and states. Bans on single-use plastic in retail and food service sectors show promise for reducing the load of trash that is a parent material for some environmentally bound microplastic particles. Polymers like synthetic fibers from clothing materials are prevalent in wastewater or effluent, which serve as another source of microplastics; thus, improved fabric technologies and upgrades to wastewater treatment facilities may be necessary to further address this potential point-source. Because of the vast and variable nature of microplastics, additional sources may eventually be correlated with specific industries, processes, products, or activities. Additionally, very little is currently known regarding fate and transport of microplastics in the Potomac River and larger Chesapeake Bay although ongoing hydrodynamic modelling efforts appear well-suited to begin addressing this knowledge gap. Management decisions should be informed by the most up-to-date science in order to ensure that policies and laws target those materials that are most likely to result in environmental and ecological degradation.

VIII. Partnerships and Resources

As noted earlier, the scope of plastic pollution is only growing, with annual increases in plastic production and the ubiquity plastic pollution in all parts of the Bay. Microplastic persistence and related risks in the environment is a fact that managers need to understand and must contend with, in addition to all other anthropogenic stressors currently being addressed. And these efforts must be undertaken with constrained resources and available technology.

The Chesapeake Bay watershed is fortunate in that it is home to the seat of the federal government in Washington, DC. This allows lawmakers to easily experience firsthand the issues surrounding Bay pollution and restoration efforts. The 2019 STAC workshop brought together experts from the Bay states in addition to federal representatives from NOAA (Marine Debris Program), US EPA, and USGS. Each of these federal agencies is actively involved in conducting or funding research into basic science of understanding aspects of microplastics in the environment. These funding sources are crucial to leveraging state and municipal resources to study microplastic distribution; this is particularly important within the existing monitoring framework, to which microplastic monitoring may be added. The watershed is also home to a range of academic institutions, consulting companies, and non-government organizations that have the technical resources to address many of the issues outlined in this document. These groups also have relationships with private foundation funding sources that are keen to support research into emerging contaminants. Adequate funding resources, in addition to technical capabilities (e.g. specialized instrumentation) will allow the CBP and its partners to address microplastic pollution on the same scale it did in the 1970s and 80s with regard to eutrophication. We must understand the current (baseline) conditions, better understand linkages to living resources (e.g. as described for Striped Bass), understand source, fate, and transport through robust modeling (ongoing), and answer the critical questions management needs to effect sound, defensible policy.

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Appendix A: Plastic Pollution Action Team Participants

Plastic Pollution Action Team Participants	
Participant	**Affiliation**
Matt Robinson	DC DOEE (Team Chair)
Kelly Somers	EPA (Team Vice-chair)
Linsay Haram	SERC
Rob Hale	VIMS
Kirk Havens	VIMS
Meredith Seeley	VIMS
BK Song	VIMS
Julianna Greenberg	CRC
Denice Wardrop	CRC
Alex Lopez	Penn State
Phong Trieu	COG
Claire Buchanan	ICPRB
Christina Davis	ICPRB
Michael Gonsior	UMCES
Kristin Saunders	UMCES
Ryan Woodland	UMCES
Jonathan Cohen	University of Delaware
Donna Morrow	MD DNR
Mark Trice	MD DNR
Brooke Landry	MD DNR
Anna Kasko	MDE
Tish Robertson	VA DEP
Dann Sklarew	George Mason University
Jennifer Starr	CBP Local Government Advisory Committee/Alliance for the Chesapeake Bay
Marty Gary	PRFC
Christy Kehoe	NOAA
Amy Uhrin	NOAA
Carlie Herring	NOAA
Shawn Fisher	USGS
Rebecca Whiteash	PA DEP
Doug Austin	EPA
Bill Jenkins	EPA
Emily Trentacoste	EPA
Jennifer Flippin	Tetra Tech
Bob Murphy	Tetra Tech
Paige Hobaugh	Tetra Tech
Julie Lawson	CAC/DC Mayor's Office of the Clean City
Anthony Johnson	CRC
Justin Shapiro	CRC
Claire Svecik	DNREC

Appendix B: Uniform Size Classification and Concentration Unit Terminology for Broad Application in the Chesapeake Bay Watershed

Uniform Size Classification and Concentration Unit Terminology for Broad Application in the Chesapeake Bay Watershed

Tetra Tech

January 25, 2021

1. Introduction and Purpose

Plastic debris adversely affects aquatic and terrestrial organisms as a physical entanglement hazard, source of gastrointestinal distress, and potential for toxicity/ adverse physiological effects following uptake of smaller pieces through oral ingestion, inhalation/gills, or contact with external body surfaces (GESAMP 2015). Signs of toxicity potentially occur following uptake of chemical ingredients in plastic or via chemicals found in the environment like hydrophobic persistent, bioaccumulative, and toxic (PBT) compounds that tend to sorb to plastic debris (Batel et al. 2016). EPA conceptualized a summary of these pathways and complexities regarding plastic exposure and potential adverse outcomes (Figure 1) in their *Microplastics Expert Workshop Report* (USEPA 2017).

Plastic trash and its breakdown products are found in many terrestrial and aquatic habitats including fresh, estuarine, and marine waters. These plastics typically occur as the result of two broad sources-- primary and secondary plastics. Primary plastics are intentionally designed as small particles for use in industrial applications (e.g., "nurdles", small pellets used as raw material to produce plastic goods) or consumer product ingredients (e.g., abrasives in cosmetics, personal care products, and cleaners). Secondary plastics occur as fragments or fibers from the breakdown of larger debris like water bottles, synthetic fabrics, plastic bags, and single use food packaging.

The term "macrolitter" was first discussed in 2003 to describe plastic debris found in marine environments ranging in size from 63-500 µm (Gregory and Andrady 2003) and "microplastic" was introduced by Thompson et al. (2004) to describe the small pieces of plastic found in marine waters (Thompson et al. 2004). Subsequent efforts to consistently define "microplastics" have yet to result in a robust, specific definition, or method for consistently describing them. The use of the term "microplastics" causes some confusion because it can refer to the general classification of small plastic pieces found in the environment (as in Thompson et al. 2004); a size of plastic less than 5 mm (as in Arthur et al. 2009); or a specific size range, generally between 1 micron and 1-5 mm (Figure 2). In this document, the term "microplastic" is used to describe a specifically defined size class, while more general terms like "plastics" or "environmental plastics" are used to describe the general concept of small plastics in the environment.

Plastics constitute a complex and diverse group of substances that vary in size, shape, color, composition, source, age, along with other physical or chemical factors. These variables further increase in natural ecosystems as plastics weather and degrade, where they potentially release chemicals like phthalates, flame retardants, bisphenol A, serve as an absorptive surface for chemical contaminants like PCBs, and develop colonies of biofilm that are consumed by aquatic organisms (Velzeboer et al. 2014, Jang et al. 2017, Yu et al. 2018).

Environmental plastics research addresses a range of scenarios including many aquatic organisms, environmental compartments, plastic types, and the field is rapidly evolving. It is useful to consider meaningful ways to define, categorize, and measure plastics in the field and laboratory in order to interpret and compare study results. The purpose of this document is to describe and recommend a uniform size classification and concentration unit terminology for plastics and apply it to the parallel effort to develop an environmental risk assessment (ERA) framework and eventual monitoring plan for environmental plastics in the tidal Potomac River. It is understood that classification of plastics is very complex continuously evolving, and reconsideration of terminology will be necessary as the science advances. Creating bounds for size classifications is expected to be a useful tool for determining which size of plastics are most likely to cause an adverse physiological responses at different levels of biological organization. A systematic nomenclature is not meant to exclude or draw conclusions about smaller plastic particles that were not quantified in a particular study; it is only meant as a tool to classify and compare studies when appropriate data exist.

Many researchers acknowledge the need to classify beyond only particle size. While this document briefly acknowledges other classification factors, it is not intended to serve as an exhaustive resource for plastic classification based on multiple factors. The proposed terminology is recommended to standardize monitoring and research efforts to inform future iterations of this ERA or ERAs focused on other endpoints.

This document provides the next steps to addressing urgent needs recommended by the STAC following the April 2019 workshop, *Microplastics in the Chesapeake Bay and its Watershed: State of the Knowledge, Data Gaps, and Relationship to Management Goals*--

> *The Scientific, Technical Assessment and Reporting Team should incorporate development of ERAs of microplastics into the CBP strategic science and research framework, and the Plastic Pollution Action Team should oversee the development of the Ecological Risk Assessments (ERAs) focused on assessment of microplastic pollution on multiple living resource endpoints.*
>
> *STAC should undertake a technical review of terminology used in microplastic research, specifically size classification and concentration units, and recommend uniform terminology for the CBP partners to utilize in monitoring and studies focused on plastic pollution in the bay and watershed.*

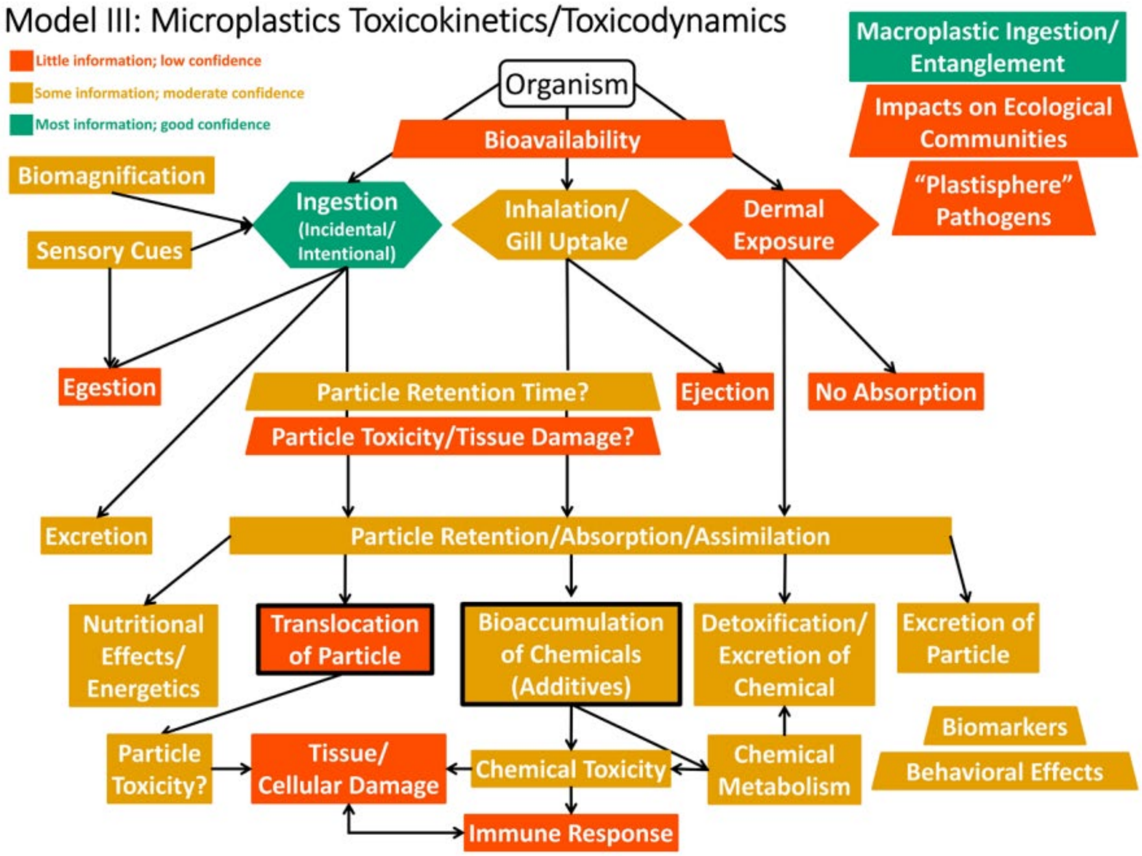

Figure 1. Conceptual model describing pathways and complexities regarding plastic exposure and potential outcomes (from USEPA 2017).

2. Classification of Plastics

Size, shape, density, composition, color, age, or a combination of several of these factors are frequent descriptors in the results of environmental plastics research. The purpose of this section is to describe current recommendations for size classifications of these plastics and briefly discuss other physical/chemical properties that may be important to consider in future ERA and monitoring efforts. Recent literature suggests that scrutiny of a single attribute is perhaps too simplistic of a view for drawing conclusions about the entire field of environmental plastics (Burton 2017, Hale 2018). However, size is expected to significantly influence the bioavailability of plastic fragments and dictate whether they are ingestible, inhalable/capable of interaction with gills, or able to cross cellular membranes. Thus, understanding implications of size and standardizing the terminology used to describe that parameter are important for moving forward with research that produces results that are comparable between studies. New studies are continually emerging, and evidence related to other individual attributes are building blocks that will improve future insight regarding ecological effects associated with the conglomeration of environmentally relevant mixtures of plastic particles.

2.1. Size

Grouping environmental plastics by size (at least in part) is one method to reduce complexity, understand exposure, and organize the universe of plastics (Arthur et al. 2009, Hartmann et al. 2019). Particle size is one factor that determines environmental fate and ecological relevance of plastic fragments and serves as a logical method for regulatory agencies to implement guidelines, as in the Microbead-Free Waters Act of 2015 which provided a plan for phasing out microbeads in cosmetics and personal care products.

The terms most frequently used to describe size of environmental plastics include megaplastics, macroplastics, mesoplastics, microplastics, and nanoplastics. These group names and corresponding sizes are not consistently applied across all studies, as demonstrated in the review by Hartmann et al. (2019) (Figure 2). The use of ambiguous and potentially conflicting definitions causes challenges in the interpretation and comparison among studies. For example:

- *Macroplastic* sizes have been defined as 1-15 cm (10-150 mm), >5 mm and anywhere from 2.5 to 100 cm (25-1,000 mm).
- *Mesoplastics* generally refer to a small range of sizes between 1-25 mm.
- *Microplastics* have been defined as 67-500 µm, 1-5000 µm, 20-5000 µm, or more broadly as <5,000 µm (the definition supported by NOAA).
- *Nanoplastics* have included sizes ranging from an upper limit of <20 µm to as small as 1 nm.

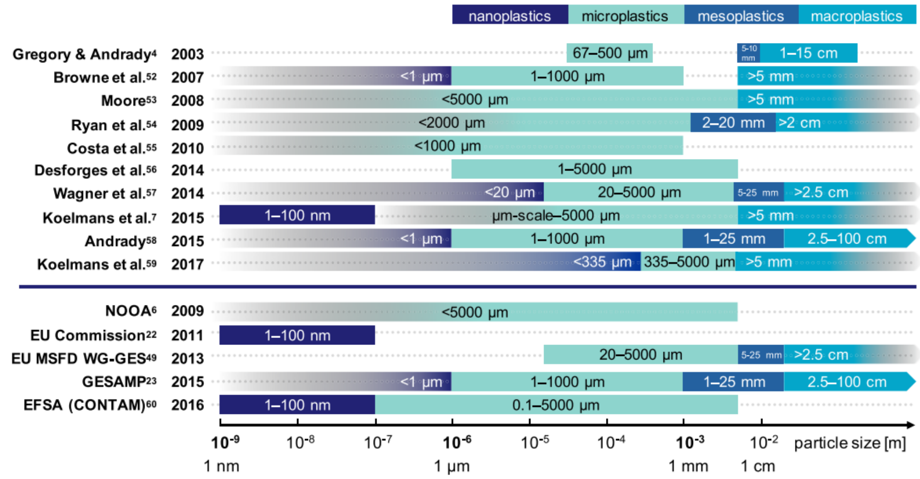

Figure 2. Examples of differences in the categorization of plastic debris according to size as applied (an/or defined) in scientific literature and in institutional reports (not exhaustive). From Hartmann et al. 2019.

Primary environmental plastics are often produced with discreet sizes to fulfil a specified purpose. Nurdles, the pelletized resin used by manufacturers, have been reported as 5 mm in diameter with a weight of 20 mg each (Hammer et al. 2012). Small plastics used in cosmetics are much smaller but are being phased out of production and use in many products including rinse-off cosmetics and toothpaste (Moore 2008, Duis and Coors 2016, Wardrop et al. 2016). However, upon entry into the environment, primary plastics break down to smaller fragments becoming secondary plastics. Even with the decreased production of certain primary plastics, those previously released are likely to persist in the environment (Besseling et al. 2017).

Size classification efforts of plastic fragments generally fall into three categories, influenced by the desire to capture 1) biological relevance of plastic pieces; 2) limitations of sampling or analytical detection capabilities; 3) a consistent naming framework.

The outcome of an international research workshop organized by NOAA defined microplastics as pieces of plastic less than 5 mm in length, with the rationale that 5 mm and smaller are those most likely to be ingested by animals and potentially cause adverse biological effects beyond physical blockage of the gastrointestinal tract (Arthur et al. 2009). It was agreed that setting a lower boundary for microplastic size was not appropriate but acknowledged that 333 µm was a practical lower boundary due to sampling equipment limitations. Mesh neuston nets with size of 333 µm are commonly employed in the collection of plankton and floating debris, thus plastics smaller than that mesh size are not necessarily captured during sampling (Hidalgo-Ruz et al. 2012). The use of sampling equipment with this lower limit is illustrated by two studies that quantified microplastics in the Chesapeake Bay and four estuarine river tributaries. In both cases, researchers used manta trawl nets to capture and report microplastic fragments as those ranging from 0.3-5 mm (Yonkos et al. 2014, Bikker et al. 2020a).

The Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) recommended a system of size classification that encompasses the range of mega to nanoplastics (Table 1) (GESAMP 2015, 2019). In three global assessment reports (GESAMP 2015, 2016, 2019), they recommended that all particles <5 mm should be included in an assessment of sources along with fate and effects of microplastics because using a different cutoff could exclude data from some pertinent published studies.

Table 2. Size classification as recommended by GESAMP.

Terminology	Size Classification
Megaplastics	>1 m
Macroplastics	25-1000 mm
Mesoplatics	5-25 mm
Microplastics	<5 mm
Nanoplastics	<1 µm

Frias and Nash (2019) reviewed current and previous methods for describing microplastics and proposed a definition that includes size and origin along with other chemical and physical properties. The authors define microplastics as "synthetic solid particle of polymeric matrix, with regular or irregular shape and with size ranging from 1 µm to 5 mm of either primary or secondary manufacturing origin, which are insoluble in water" (Frias and Nash 2019).

Hartmann et al. (2019) also reviewed current studies and provided recommendation for a framework of unified terminology for size but cautioned that categorizing plastic debris using one component, such as size, may result in oversimplification and suggest similarity between microplastics when it may not exist. The authors note that plastics within the same category may still differ widely because of their differences in hazardous properties or environmental behavior. However, their recommended size classification is shown in Table 2, and reflect consistent use of SI prefixes in the size designation of micro and nanoplastics.

Table 3. Size classification terminology recommended by Hartmann et al. (2019).

Terminology	Size Classification
Macroplastics	1 cm and larger
Mesoplatics	1 to 10 mm
Microplastics	1 to <1000 µm
Nanoplastics	1 to <1000 nm

The definition of nanoplastics is debated but has most often been described as <1000 nm (Browne 2007, Andrady 2011, Cole et al. 2011) or <100 nm (in at least one of its dimensions) as defined for non-polymer nanomaterials in the field of engineered nanoparticles (Koelmans et al. 2015).

One proposed system suggests that using a strict classification based on size is not a satisfactory approach since it does not capture the continuous nature of mixtures of environmental plastics or predict the fate of particles (Kooi and Koelmans 2019). Instead the study proposes a three-dimensional probability distribution that considers size, shape, and density along continuous scales. A discreet size classification system was not provided in this study and is beyond the current scope of this document which is intended to provide review of frequently used size classifications. However, the study warrants further investigation as a tool for use in future probabilistic risk assessments and should be further evaluated in the Science Strategy.

2.2. Other Methods of Classification

This section briefly outlines other considerations that are presumably meaningful elements necessary to illustrate the relationship between plastics and adverse ecological effects. The discussion is not intended to be exhaustive but rather serve as a point of consideration for inclusion of alternative classification methodologies that influence future ERA and monitoring activities.

2.2.1. Chemical composition

Chemical composition is an important identifying characteristic of plastics. Chemical and physical properties associated with different materials influence the fate, transport, exposure, and toxicity of particles. Common polymers described in the marine environment include the following (GESAMP 2016). Future ERA or monitoring efforts may focus on one or several types of parent materials if evidence suggests a potential for greater exposure or risk in particular organisms or ecosystems.

- ABS - Acrylonitrile butadiene styrene
- AC - Acrylic
- EP - Epoxy resin
- PA - Polyamide
- PCL - polycaprolactone
- PE (LD - low density, LLD - linear low density, HD - high density) - Polyethylene
- PET - Polyethylene terephthalate
- PGA - Polyglycolic acid
- PLA - Polylactide
- PP - Polypropylene
- PS - Polystyrene
- EPS (PSE) - Expanded polystyrene
- PU (PUR) - Polyurethane
- PVA - Polyvinyl alcohol
- PCV - Polyvinyl chloride
- PU (PUR) - Polyurethane
- SBR - Styrene-butadiene rubber

2.2.2. Shape or Structure

The identity of primary or secondary plastics and their susceptibility to environmental degradation influence the ultimate shape or structure of resultant plastics found in aquatic environments. Shape and structure may also indicate potential sources of debris including resin pellets, spheres/beads, fibers, etc. (Rochman et al. 2019). These attributes also influence the fate, transport, exposure, and potential toxicity of particles. Future ERA or monitoring efforts may focus on one or several types of shapes or structures if evidence suggests a potential for greater exposure or risk in organisms or ecosystems of interest. Those morphologies commonly described in literature include the following:

- Fibers, lines, filaments, threads
- Fragments
- Films
- Foam
- Beads, spheres, pellets
- Spheroid
- Cylindrical

2.2.3. Color

Plastic is produced in all colors, including clear and translucent variations, which means that a variety of colors are observed in environmental plastics. Color may be important in a biological context if certain colors are more likely to be mistaken for the food source of an organism (e.g., Xiong et al. 2019. In such case, color could be considered for closer study in a future ERA or monitoring effort.

3. Units of Concentration

Microplastics research report measurements in a variety of media including water, sediment, fish, invertebrates, phytoplankton, and plants along with different concentration units (Figure 3). Quantification of microplastics is necessary to understand abundance in environmental matrices and develop a better correlation between exposure and effects including potential dose-response relationships in exposed organisms.

Reported microplastic units tend to vary by study and media type, causing challenges for monitoring or identification of comparisons, correlations, or trends between studies with different objectives. The 2019 *Microplastics in the Chesapeake Bay and its Watershed: State of the Knowledge, Data Gaps, and Relationship to Management Goals* workshop proceedings noted that inconsistencies such as the tendency to report mass/unit volume or particles/unit area in similar studies. Mixed unit estimates like these pose problems as number of particles m^{-3} cannot directly correspond to aquatic surface area since the volume of water in one area may be more or less than in another of the same area (Bikker et al. 2020a). Similarly, Burns and Boxall (2018) explain that because ecotoxicity test are reported in measures of mass or number of particles per volume, measures reported in “items per square meter” are not as easily comparable. Consistency in estimation of particles per unit area or volume is critical since organisms respond differentially depending on the impact measured. For example, number of particles per unit area is appropriate when measuring impact on respiration since gill area is the important factor for gas exchange. However, volumetric estimates are more appropriate for gastrointestinal studies since ingested “food” is more biologically relevant when measured by volume (although number of particles can also be relevant when assessing ingestion effects). Most microplastic analysis methods lack standardization and continue to update as new analytical technologies become available. Thus, the sampling, identification, and quantification of microplastics in different media remains inconsistent.

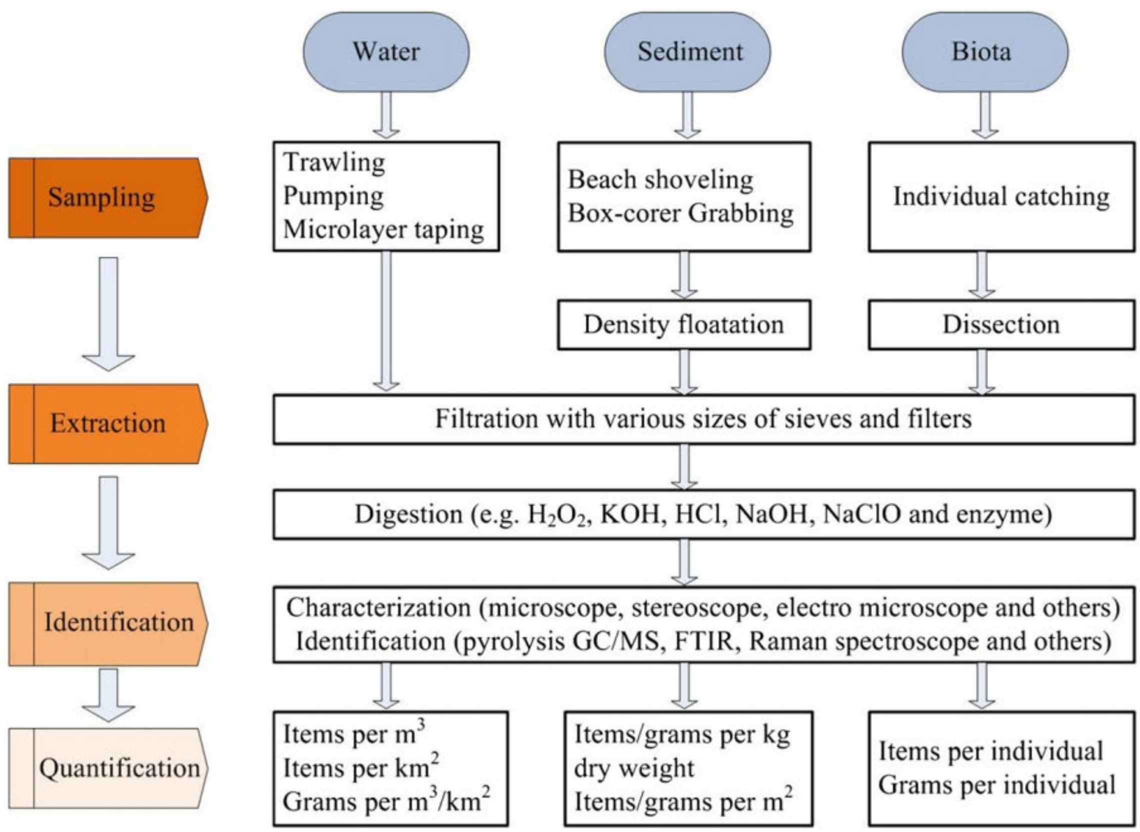

Figure 3. Analytical processes and example quantification processes in water, sediment, and biota (Mai et al. 2018).

3.1.1. Water Column

Surface water studies commonly measure microplastics at either a single depth profile or throughout a larger portion of the water column. Collection of plastic fragments for quantification is performed via surface water collection/filtration or by a trawl net in open water. For example, Bikker et al. (2020) characterized plastic fragments from 30 water samples collected throughout the Chesapeake Bay by using a surface manta trawl with 330 µm mesh net, and reported amount as particles/m^3 as a volume-based estimate and particles/km^2 as an area-based estimation of particles. Units used to describe the amount of microplastics in water include:

Number of particles per volume of water

- *Number of particles m^{-3}; Number of particles l^{-1}*
  Quantifies number of plastic particles in water by volume.
  This unit of measurement potentially accounts for particles throughout the water column.

Number of particles per area of water

- *Number of particles m^{-2}*
  Quantifies number of plastic particles on the surface area of water.
  Since water, is more than area (I.e. not two-dimensional), this metric is less informative for understanding the overall amount of microplastics and may exclude particles that are lower density and not at the surface of the water column.

3.1.2. Sediments

Plastic fragment quantification in sediment is typically evaluated based on surface area of a specified quadrat or a volume of bulk collected sediment. Units used to describe the amount of microplastics in sediment include:

Number of particles per volume of sediment

- *Number of particles l^{-1}*
  Quantifies number of plastic particles in sediment samples and based on a liquid volume of sediment.
- *Number of particles kg^{-1} dry weight*
  Quantifies number of plastic particles in sediment samples and based on dry weight of sediment.
- *Number of particles kg^{-1} wet weight*
  Quantifies number of plastic particles in sediment samples and based on wet weight of sediment.

Number of particles per area of sediment

- *Number of particles m^{-2} sediment surface*
  Quantifies number of plastic particles on the surface of a quadrate area of sediment.
- *Mass m^{-2} sediment surface*
  Quantifies mass of plastic particles on the surface of a quadrate area of sediment.

3.1.3. Organisms

Microplastic uptake ingestion or gill uptake in aquatic organisms can be used to monitor microplastic contamination. Commonly monitored biota include fish, sea turtles, sea birds, bivalves and other invertebrates, and plankton. Microplastics are most frequently evaluated in the digestive tract or gills. Units used to describe the amount of microplastics in animals or their tissues include:

Number of particles per individual

The number of particles per individual is a general measurement that does not discriminate between organ or tissue as site of accumulation. It can be a useful measurement for general exposure estimates and is currently comparable between studies. We include mass of microplastics but recognize that this is a difficult measurement to capture with existing technology. In the near term, number of particles is a preferred measurement since these pose significant toxicological issues.

- *Number of particles/individual*
  Quantifies abundance of plastic particles within a whole individual.

Mass of plastics per stomach or gastrointestinal tract

- *Mass of plastics in stomach*
  Quantifies abundance of plastic particles within stomach contents.
- *Mass of plastics in GI tract*
  Quantifies mass of plastic particles within the entire gastrointestinal tract.

Number of stomachs with particles

- *Number of organisms within a study in which plastics were found*
  Quantifies abundance of individual stomachs in which plastic particles were observed. A very useful metric that serves as an index to selectivity of fish (Hyslop 1980, Chesson 1983, Deudero and Morales-Nin 2001, Liao et al. 2001).

Number of particles per wet or dry tissue weight

The measurement of the number or mass of microplastic relative to body mass of an organism is intrinsically useful as it provides a standardized assessment per individual. Additionally, it allows for comparisons between studies.

- *Number of particles g^{-1} wet weight*
  Quantifies number of plastic particles in tissue samples and based on wet weight of tissue.
- *Number of particles g^{-1} dry weight*

Quantifies number of plastic particles in tissue samples and based on dry weight of tissue.

Total mass per unit of tissue

- *Mass of plastics/g wet weight*
  Quantifies mass of plastic particles in tissue samples and based on wet weight of tissue.
- *Mass of plastics/g dry weight*
  Quantifies mass of plastic particles in tissue samples and based on dry weight of tissue.

Number of particles in stomach or gastrointestinal tract

- *Number of particles in stomach*
  Quantifies the number of plastic particles in the stomach of an animal.
  This measurement provides insight to available plastics for ingestion and perhaps selectivity of plastic types by fish. However, it may not yield an ideal relative measure of impact given variability in size, whereby total microplastic mass may be more informative.
- *Number of particles in GI tract*
  Quantifies the number of plastic particles in the GI tract of an animal.
  This measurement shares many of the same issues as those previously described for number of particles in stomach or GI tract.

Number of particles on gill surfaces

- *Number of particles/gill surface*
  Quantifies the number of plastic particles on or in the gill surfaces of an animal.
  This methodology can potentially serve as a proxy for area of gill surface covered (and may be easier to measure than particle area).

Mass of particles on gill surfaces

- *Mass of plastics/ gill surface*
  Quantifies the mass of plastic particles on or in the gill surfaces of an animal.
  This is biologically informative measurement as gill surface area is critical for sufficient respiration (Avio et al. 2015).

3.1.4. Submerged Aquatic Vegetation

Number of Particles per Area of Blade/volume of plant canopy

- *Number of particles cm^{-2} of plant surface area*
  Quantifies the number of particles attached to plant surface.

Can be used to assess impacts directly to plant health or as pathway for organisms feeding on plant tissue or surface (Goss et al. 2018).

- *Number of particles l^{-1} of samples SAV canopy*
  If comparing the canopy filtration of particles, then a volumetric approach is more robust as one would be comparing # particles per volume of canopy sampled vs nearby similar volume of unvegetated water column (Murphy 2019).

3.1.5. Shoreline

Shoreline reaches, including beaches, are routinely surveyed for microplastic abundance. Beaches and shorelines are frequently treated in the same manner as sediments sampling since the nature of the environment is so similar. Therefore, the concentration units will be similar. However, if a consistent depth (i.e. volume) of substrate is sampled for each quadrat along a transect, density can also be recorded by unit area of shoreline, noting the volumetric concentrations

Number of particles or Mass of particles per unit volume of shoreline substrate

- *Number of particles kg^{-1} dry weight or l^{-1}*
  Quantifies number of plastic particles in beach samples and based on dry weight of sand/substrate.
- *Mass of particles as kg per dry weight or l volume of substrate*
  Quantifies mass of plastic particles in beach samples, based on dry weight or mass of sand/substrate.

Number of particles or Mass of particles per area of shoreline substrate

- *Number of particles m^{-2} or km^{-2} substrate surface (valid when depth of samples remains constant)*
  Quantifies number of plastic particles on the surface of a quadrat area of sediment.

Number of item/Mass of items per unit length of shoreline

- *Number of items per m or km of shoreline*
  Quantifies the number of plastic particles in a given measurement of shoreline
- *Mass of items kg per m or km of shoreline*
  The amount of plastic particles based on weight of items in a given measurement of shoreline.

4. Summary

- Environmental plastic classification remains complex and a unified classification/ descriptive system is still young, and it may be necessary to reconsider size and concentration units as research needs develop.

- It is not possible to exhaustively consider all chemical and physical properties in the current effort because plastics encompass a diverse group of materials with different physical/chemical properties along with fate, transport, and bioavailability characteristics.
- The upper cutoff for microplastics in most contemporary literature or recommendations is either 5 mm or 1 mm. The use of 5 mm has been acknowledged for its biological relevance in terms of potential uptake and is also the upper limit reported by two Chesapeake Bay studies. The designation of 1 mm (1000 µm) is convention driven and consistent with SI prefix "micro" but not necessarily consistent with results of current research. Constraining the definition to 1mm potentially leaves out biologically/ecologically relevant sizes that can be termed conceptually as "micro".
- Concentration units tend to vary by type of media investigated but are most generally reported (e.g., water, sediment, tissue, etc.) as mass/unit volume or particles/unit. The use of standardized terminology is necessary for the Chesapeake Bay Program and its partners to implement consistent monitoring and research in the bay and its watershed.

5. Recommendations

5.1. Size Classification for ERA and Future Research and Monitoring

- For the purposes of the current activities, we recommend defining a microplastic as <5 mm, as consistent with the recommendations of NOAA and the GESAMP observation that all particles <5 mm should be included in an assessment to ensure that data from pertinent published studies are not excluded. Two microplastic monitoring studies in the Chesapeake Bay and tributaries were consistent in reporting results with 5 mm as the upper cutoff (Bikker et al. 2020, Yonkos et al. 2014). Thus, using a 1 mm cutoff would mean that two highly relevant studies might be excluded or cause a point of uncertainty. While 1 mm is a more clear-cut representation of the SI prefix "micro," and arguably more appropriate in the sense of a naming convention, 1 mm and 5 mm represent the same order of magnitude. The inclusion of plastic particles up to 5 mm does not represent an order of magnitude change compared to 1 mm, is noted for biological relevance, and if included is unlikely to cause a significant change in the overall outcome of the ERA.
- The lower limit of microplastics research is often functionally constrained by limitations of sampling technology. In the case of Chesapeake Bay and tributary studies, researchers used manta trawl nets to capture and report microplastic fragments with a lower limit of 333 µm (Bikker et al. 2020, Yonkos et al. 2014). However, this field sampling limit should not prohibit the inclusion of laboratory data in the ERA that include microplastic particles smaller than 333 µm but greater than the defined size of nanoplastics.
- The lower limit of microplastic monitoring in the Chesapeake Bay would likely be limited to existing sampling equipment, i.e. 330µm. For the purposes of this assessment, the

definition of nanoplastics is recommended as 1 to <1000 nm, which is consistent with the SI naming convention and also inclusive of the alternative definition of <100 nm as defined for non-polymer nanomaterials in the field of engineered nanoparticles (Koelmans et al. 2015, Besseling et al. 2017). These compounds are not yet monitored in the Chesapeake Bay, tidal Potomac or in many species of interest, which leaves uncertainty about their ecological relevance.

- The findings of some studies, especially field -based ecological studies where perhaps only visible plastics are currently quantified, could be categorized as “Less than or equal to microplastic size” to acknowledge that nanoplastics or smaller may contribute to biological effects observed in a study, but were not measured. A systematic nomenclature is not meant to exclude or draw conclusions about smaller plastic particles that were not quantified; it is only meant as a tool to classify and compare studies when appropriate data exist.
- Microplastic and nanoplastic designations will be the most useful terminology to describe plastics that are potentially biologically relevant. However, for the purposes of monitoring and prediction of plastic loading to a particular system, it will be useful to classify larger plastics that are easily visible to the naked eye (e.g., bottles, packaging materials, etc.). This is generally outside of the scope of the current document. However, the designations presented by Hartmann et al. (2019) and GESAMP (2015, 2016, 2019) designating sizes associated with meso-, macro-, and mega-plastics warrant further discussion.
- The following classifications (Table 3) are recommended for the purpose of discussing a uniform size classification in the Chesapeake Bay watershed.

Table 3. Recommended Chesapeake Bay watershed size classification terminology.

Classification	Size	Rationale
Microplastic	5 mm - 1000 nm (1µm)	--NOAA and GESAMP precedence --Upper size limit is consistent with previous monitoring studies in Chesapeake Bay and tributaries --Use of 333 µm as a lower bound potentially excludes the inclusion of laboratory or monitoring studies that include data below that value -- The lower size limit is consistent with the SI naming convention.

Classification	Size	Rationale
Nanoplastic	1 nm - <1000 nm (1µm)	--The upper limit is consistent with the SI naming convention. --Limit is inclusive of particles <100 nm as defined for non-polymer nanomaterials in the field of engineered nanoparticles -- The lower size limit is consistent with the SI naming convention.

5.1. Units of Measurement for Future Research and Monitoring

- **Water:** Number of particles m^{-3} ; Number of particles l^{-1} which quantifies number of plastic particles in water by volume is recommended for standardized monitoring strategies in the Chesapeake Bay and watershed. This unit of measurement potentially accounts for particles throughout the water column, including those at the surface.
- **Sediment:** The number of particles in sediment should be measured volumetrically since organisms exist in a three-dimensional environment within the sediment. The exception to this would be to assess abundances of microplastics on the sediment surface as this region is exploited by a variety of errant polychaetes, crustaceans, and benthic fish.
- **Organism:** The mass of particles per individual is a general measurement that does not discriminate between organ or tissue as site of accumulation and accounts for an organism's total exposure to microplastics. This measurement may serve as an informative tool for monitoring the prevalence of microplastic accumulation in organisms. This approach has advantages from a toxicology/risk standpoint.
- **SAV:** Measuring microplastics within SAV beds mostly depends on the research objectives. The most common measurement would assess the area covered on blades of plants. Goss et al. (2018) reported # particles blade $^{-1}$ which provides insight to loadings. However, area covered by microplastic particles is more biologically relevant because a) area of microplastics will block the surface of blades from sunlight, and b) larger particles can potentially be consumed by grazers, therefore area estimates can serve as a proxy for mass (as recommended above). One exception to this recommendation is in the case of studies that are comparing SAV bed metrics (e.g. canopy capture of microplastics) to similar conditions elsewhere, which would entail measuring # particles unit volume^{-1}.
- **Shoreline:** Quantifying plastics debris on shorelines will depend on the research, policy, or monitoring objective of interest.

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Appendix C: Preliminary Conceptual Model for an Ecological Risk Assessment for Microplastics on Striped Bass in the Potomac River Estuary

Preliminary Conceptual Model for an Ecological Risk Assessment for Microplastics on Striped Bass in the Potomac River Estuary

Tetra Tech

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IX. Introduction

Plastic debris adversely affects aquatic and terrestrial organisms as a physical entanglement hazard, source of gastrointestinal effects, and potential for toxicity/ adverse biological effects following uptake of smaller pieces through oral ingestion, inhalation/gills, or contact with external body surfaces. EPA conceptualized a summary of these pathways and complexities regarding plastic exposure and potential adverse outcomes (Figure 1-1) in their *Microplastics Expert Workshop Report* (USEPA 2017).

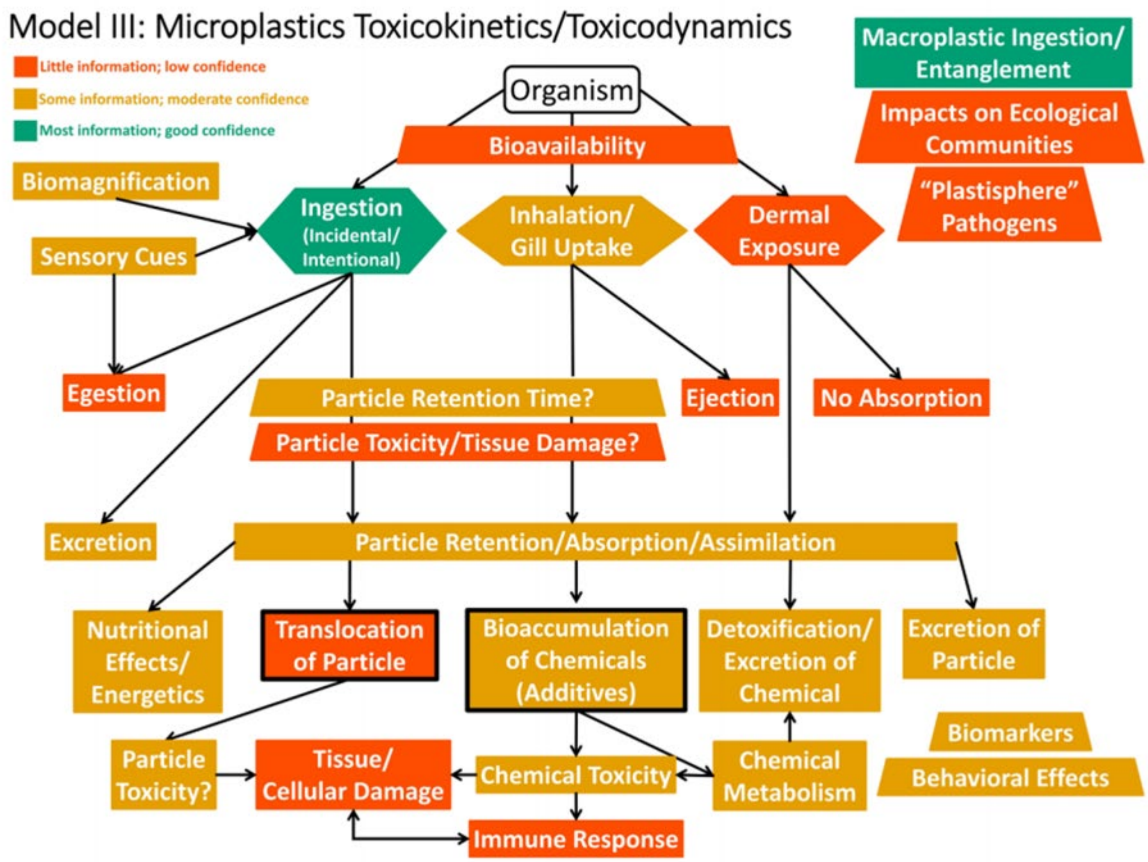


Figure 1-1. Conceptual model describing pathways and complexities regarding plastic exposure and potential outcomes (from EPA 2017)

Plastic trash and its breakdown products are found in many terrestrial and aquatic habitats including fresh, estuarine, and marine waters. These plastics typically occur as the result of two broad sources-- primary and secondary plastics. Primary plastics are intentionally designed as small particles for use in industrial applications (ex."nurdles", small plastic pellets used as raw material to produce plastic goods) or consumer product ingredients (ex. abrasives in cosmetics, personal care products, and cleaners). Secondary plastics occur as fragments or fibers from the breakdown of larger debris like water bottles, synthetic fabrics, plastic bags, and single use food packaging.

The ecological risk of these plastics, specifically those in the size range of microplastics (5 mm - 1000 nm [1µm]) and nanoplastics (1 nm - <1000 nm [1µm]) as defined in the companion document *Uniform Size Classification and Concentration Unit Terminology for Broad Application in the Chesapeake Bay Watershed*, is largely unknown. However, these are size ranges known or expected to be ingested or taken in through gills of aquatic organisms. The purpose of this project is to expand upon the needs identified in the *Microplastics Expert Workshop Report* (USEPA 2017) and develop a preliminary conceptual ecological risk assessment model to identify pathways, sources, effects, and unknowns related to environmental plastic debris, specifically microplastics and smaller, in the tidal portion of the Potomac River. The Potomac is a major tributary to the Chesapeake Bay, and this conceptual risk assessment will serve as a starting point for understanding the potential ecological effects of microplastics on the aquatic resources in the larger Bay. This initial effort is expected to inform a science strategy for microplastics in the Potomac River and provide insights regarding restoration efforts around the Chesapeake Bay and contributing watersheds, a need outlined recently by the Chesapeake Bay Program (Murphy et al. 2019).

A variety of individual organisms, species, populations, and/or life stages may be at risk due to microplastic exposures. Multiple species/life stages were considered for inclusion as an ideal biological endpoint for the ERA of microplastics in the Potomac River, with implications for the broader Chesapeake Bay. The first consideration was whether to include a semi-aquatic or aquatic species endpoint. While there are several good candidates for semi-aquatic species, more aquatic species are covered by the 2014 Chesapeake Bay Agreement. In addition, of the few studies carried out in the Chesapeake Bay watershed looking into microplastic occurrence, almost all have assessed the aquatic component. For these reasons, an aquatic species was chosen as the ERA endpoint.

Several fish and shellfish species were initially discussed as candidate endpoints:

1. Blue crabs (*Callinectes sapidus*) are an iconic species for the Bay and evoke strong interest by the general public. In addition, they are a very well-studied species in the Bay and elsewhere. Lastly, the 2014 Agreement established restoration outcomes for blue crabs and thus they should be considered.

2. American Shad (*Alosa sapidissima*). Shad are an abundant group that are regular components of the Potomac River estuary and the state fish of the District of Columbia. A major drawback of using American Shad (or other Alosines) is the nature of their life cycle--while Alosines are important in the ecosystem, they are transient. Adults enter the system from the ocean to spawn and then leave again. The young-of-year remain in the estuary, but eventually depart for life primarily in the ocean.

3. Forage fish (e.g. anchovies, silversides, etc) are an integral part of the coastal ecosystem, feeding on zooplankton while serving as primary food for Striped Bass (*Morone saxatilis*), Bluefish (*Pomatomus saltatrix*), and other piscivores. The 2014 Agreement identified the importance of forage fish and recommended further research to better understand their abundance. Research in other parts of the world have demonstrated ingestion of microplastics by forage species. By definition, forage species occur lower on the food chain and therefore might artificially represent a truncated pathway for microplastics.

4. American Eel (*Anguilla rostrata*) is a major component of estuarine and non-tidal ecosystems. However, the American Eel has a very complex life cycle that will make it very difficult to develop a strong risk assessment model. Adults reproduce in the Sargasso Sea, then larval stages are advected and migrate along the east coast of North America before entering estuaries and continuing into non-tidal waters where they develop into mature adults. American Eel then migrate out of the streams and rivers into the estuaries, followed by a long migration back to the Sargasso Sea to spawn and die. This species is not ideal for the current risk assessment because of their extensive movement between different habitats and geographical locations.

5. Eastern oysters (*Crassostrea virginica*) are an iconic species in the Chesapeake Bay and once supported large fishery, including one for the Potomac River. Pollution, overharvesting, and disease have reduced oyster populations to a remnant of their historical abundance. As filter feeders, they are likely to be more exposed to contaminant particles in the water column, such as microplastics. However, oysters are also selective feeders that will egest foreign or non-nutritive material in the form of pseudo-feces. In addition, while well-known and studied, the population resides in a restricted portion of the Potomac River (M. Gary, pers. comm) and lack the distribution within the system to provide a broader picture of the fate of microplastics. Lastly, oysters feed mostly on phytoplankton, thus an ERA model with oysters as the endpoint would miss the flow of microplastics through the larger food chain.

6. White Perch (*Morone americana*) are one of the most common estuarine finfish species in the Chesapeake Bay and Potomac River (Stanley and Danie 1983, Kraus and Secor 2004). They are a well-studied species and are known to the public due to their desirability for human consumption. White Perch remain in the estuary for their entire life cycle feeding on benthic organisms, invertebrates, small fish, and fish eggs. They are prey for larger piscivores such as Weakfish (*Cynoscion regalis*), Bluefish, and Striped Bass.

7. Striped Bass are one of the top-level piscivores found in the Chesapeake Bay and tributaries. The Chesapeake Bay is also a major center of reproduction along the western Atlantic. The species has been recognized as a major success story in terms of aggressive multi-jurisdictional management, when the population crashed in the late 1980's. The species recovered after a fishing moratorium was imposed for several years and is highly managed today. Striped Bass, as one of the highest trophic-level organisms in the Bay, provide a very good model endpoint as they will naturally include blue crabs and forage fish (both having

specific outcomes in the 2014 agreement). In addition, there is considerable literature on the trophic dynamics of striped bass (Fay et al. 1983, Hartman and Brandt 1995, Cooper et al. 1998, Secor 2000) along the east coast, including the Chesapeake Bay. While Striped Bass are also migratory, they tend to remain in the estuary the first several years of their life (Fay et al. 1983), thereby providing an organism that can reflect the potential impact of microplastics in a specific location. Lastly, recent research has demonstrated microplastics to be found in Striped Bass, although this work was not from the Chesapeake Bay (Baldwin et al. 2020).

After considering these species or species groups five criteria were considered to select the final ecological endpoint for the preliminary conceptual risk assessment model:

1) Upper trophic level species
    - Incorporation of all trophic ingestion, potentially
    - Includes lower trophic levels of interest
    - Likely to be targeted by humans

2) Represented in Chesapeake Bay Agreement Restoration Goals
    - forage fish
    - blue crabs
    - Striped Bass do not have a specific bay restoration goal, but consume species above

3) Data rich
    - Chesapeake Bay fisheries resources well- surveyed
    - Habitat associations well-known for many species
    - Adequate data to detect population fluctuations

4) Common, including recognition by the general public
    - Eels – fishery species of concern due to declining population bay-wide
    - Blue crab - iconic bay species
    - Oysters - known for water quality benefits as well as habitat and as a direct fishery
    - White Perch - ubiquitous
    - Striped Bass – prime example for aggressive fisheries management; highly sought-after game fish

5) Wide distribution
    - Eels
    - White Perch
    - Striped Bass
    - Blue crab
    - Forage fish

Striped Bass was selected as the receptor of interest for the initial assessment. It is likely that other species would also serve as excellent potential endpoints but may not fulfill the criteria described above. For example, oysters are an important component of the estuarine ecosystem, provide habitat and are consumed by some fish and humans. However, the population of oysters in the Potomac River is low and would likely not provide as much insight to microplastic movement through trophic pathways (Waite et al. 2018). Additionally, blue crabs and forage fish, both recognized under the 2014 Agreement, are lower trophic level species and would not provide a full picture of potential microplastic vectors. In general, these lower-level species would be included by using Striped Bass as the receptor of interest.

Striped Bass are an apex predator that feed on several important recreational and commercial fishery species in the Chesapeake Bay, which also have goals under the 2014 Chesapeake Bay watershed agreement. Striped Bass have witnessed a decline in abundance recently (M. Gary, Pers. Comm.) and the additional insight provided by the risk assessment will contribute to a better understanding of the suite of stressors facing the population as this species is under increased management scrutiny. Furthermore, The Potomac River and the upper Chesapeake Bay are the two most important nursery areas for Striped Bass. Lastly, by addressing Striped Bass, ecological risk to a myriad of species of interest and lower trophic levels (ex. blue crabs, forage fish, and oysters) can also be addressed by constructing food web models and identify potential trophic transfer.

Spatial Boundary

The risk assessment conceptual model is focused on the Potomac River estuary, including the tidal portions of any tributaries. Constraining the spatial extent of the assessment is necessary to reduce the amount of uncertainty and variability in the model development. This is particularly critical as data availability and gaps specific to the region are identified. Similarly, Striped Bass demonstrate variability in feeding across latitudes, thereby skewing the accuracy of an assessment for the Potomac using trophic pathways outside the Chesapeake region.

Temporal Coverage

As noted, Chesapeake Bay fisheries, including the Potomac River estuary, has been well-studied for several decades, going back to at least the 1920's with rigorous population data available. In addition, ecological studies of economically important species (e.g. Striped Bass) span a long timeframe, with relevant studies beginning in the 1960's and continuing to the present. In light of this, it was decided that all robust and comparative literature be reviewed for relevant trophic data. The studies by Beaven and Mihursky (1980) and Boynton et al (1981), while being older, are still relevant and remain the most comprehensive sources of data on the trophic ecology of Striped Bass in the Potomac River, particularly for the ages of interest. Recent analyses (Ihde et al. 2015) provides more recent analyses of age 1+ Striped Bass from the

Chesapeake Bay mainstem that sheds light on other diet trends. We expect the ecological risk assessment to be useful in future years as the understanding of microplastic sources and fates is better understood and quantified within the Potomac River estuary.

Assessment and Measurement Endpoints

Potential ecological assessment endpoints were identified based on the scope of the ERA. These assessment endpoints build off previously defined generic ecological assessment endpoints (USEPA 2003) which were developed to serve as broadly applicable endpoints for a range of ecological assessments. Specifically, Individual- and Population-level Generic Ecological Assessment Endpoints (GEAEs) (USEPA 2003)were modified to yield potential assessment endpoints identified for the Potomac River Striped Bass population (Figure 1-2). These proposed assessment endpoints reflect the potential individual and population-level effects of microplastics and ultimately reflect an overall assessment of the health of the Potomac River Striped Bass population. This includes recruiting early life stages, resident subadults, and returning adult Striped Bass that use the Potomac River as both spawning and foraging habitat. The strong fisheries management interests focused on Striped Bass support the specification of potential measurement endpoints that are common fisheries population assessment measurements (e.g., age-structure, catch-per-unit-effort, spawning stock biomass). Some of the potential assessment endpoints that are identified will be difficult to measure (e.g., behavior change, changing susceptibility to predation) but could provide useful contextual information for other assessment endpoints such as mortality rates.

Measurement endpoints represent specific measurements required to inform assessment endpoints. Measurement endpoints for some Individual-level assessment endpoints for Striped Bass include data necessary to estimate growth rates, fecundity, mortality and condition. For many of these assessment endpoints, multiple potential empirical measurements are suitable. For example, assessing juvenile growth rates could be accomplished by either collecting weekly-to-monthly cohort length data which would allow the application of modal length progression analysis, or the collection of otoliths from juveniles and the analysis of daily increment widths (assuming proper validation of the approach). Further, experimental options such as in situ caging experiments (to measure differences in body size before and after caged fish are held in the environment) or laboratory-based approaches such as RNA:DNA ratios could be used to help inform estimates of short term growth after appropriate experimental validation. Another example of a single individual assessment endpoint that can be evaluated using multiple measurement endpoints is physiological condition. Fish condition is often evaluated as the ratio of body weight to the cube of body length, a ratio that is sometimes scaled using a constant (i.e., Fulton's condition factor or *K*). Use of K or any related condition factor based on the relationship between fish weight and body length requires the collection of individual fish length and weight data, preferably at multiple times during the year and for each life-stage of interest. Alternative condition metrics include laboratory-based measurements of percent body (or tissue) lipid composition, energetic content (calorimetry), or stoichiometric ratios such as carbon:nitrogen tissue composition.

Measurements for population assessment endpoints such as population estimates rely on standardized sampling efforts conducted at sufficient spatial and temporal resolution to calculate robust measurements of number of fish caught per unit effort (e.g., square meter, cubic meter, deployment minutes). Age-structure endpoints require measurements that yield data on age distributions of target life stages (days for YOY, years for age-1+), while size-at-age assessment endpoints require that the age data be paired with individual length data. These data needs require a dedicated monitoring survey that collects each life stage at different times of the year, paired with laboratory approaches to determine age of captured individuals (e.g., otolith analysis). Standard fisheries methods are available that provide detailed descriptions of the data needs and associated survey designs for all the assessment endpoints identified here.

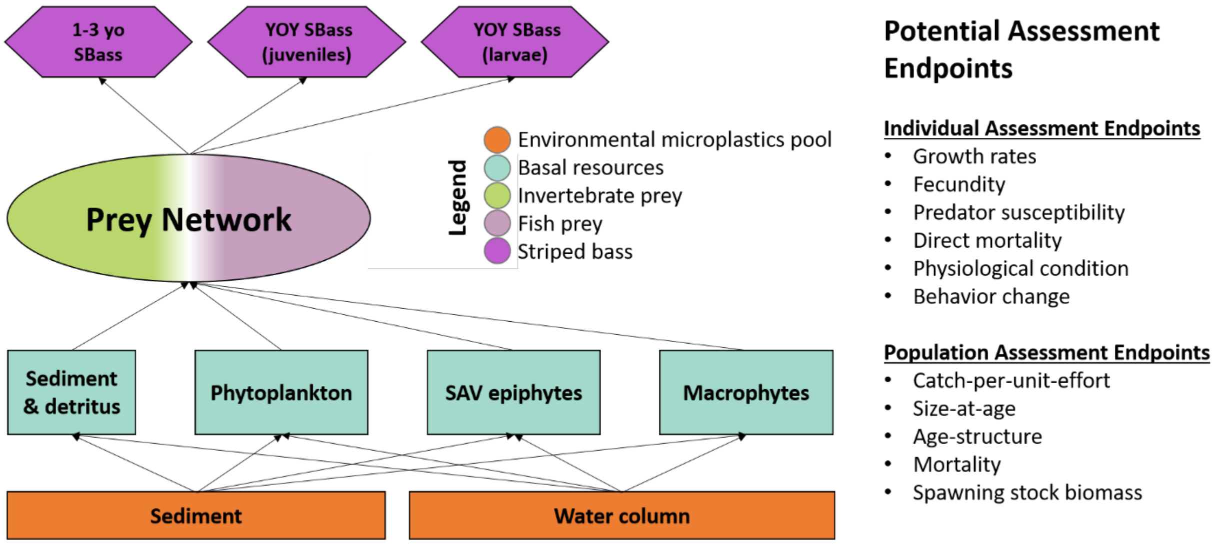


Figure 1-2. This diagram shows a simplified conceptual model of expected environmental pools of microplastics and generalized uptake through the food chain to Striped Bass. The large oval labeled "Prey Network" is further expanded in the next figure. Ecological assessment endpoints quantifiable at the individual (ex. growth, fecundity, etc.) and management-focused population level (ex. catch-per-unit-effort, size at age, etc.) are highlighted as potential endpoints to evaluate the effects of microplastics on the Potomac River population of Striped Bass. In many cases, it is expected that these represent data gaps without a known relationship to microplastic exposure and may not yet be quantifiable.

Stressors of Concern

Microplastics, both primary and secondary, and consisting of many polymers are the focus of this conceptual model. Microplastics encompass a very diverse group of materials with different physical/chemical properties along with fate, transport, and bioavailability characteristics. It is recognized that nanoplastics likely occur in ecological matrices, and they are acknowledged as a potential stressor, but are not addressed extensively in the current effort due to lack of data. It is also acknowledged that other co-occurring stressors in the Potomac are very important and result in ecological effects, including changes related to the assessment endpoint, growth and survival of striped bass. Such stressors include toxic chemicals, dissolved oxygen, parasites, temperature, and changing hydrological conditions.

Future efforts may focus on specific microplastics, but the initial conceptual model generally acknowledges the contributions of all microplastics. Inclusion of new studies will allow subsequent iterations to tailor the established framework to specific shapes, sizes, and polymers. For example, two initial studies show potential differences in the prevalence of particular microplastics in different portions of the Chesapeake Bay and its tributaries. A recent report of microplastic abundance in submerged aquatic vegetation in the Anacostia River showed that almost 75% of the identified particles were fibers, followed by a smaller percentage of fragments, and beads (Murphy 2020). Another recent study (Bikker et al. 2020b) identified morphology of plastic particles from water samples around the Chesapeake Bay and showed that the greatest abundance of particles were fragments, followed by film, and fibers.

It is noteworthy that the proportion of abundances in Bikker et al. (2020) were different than those found by Murphy (2020). This observation could be related to several factors. First, physical and chemical characteristics of plastics govern where they are found in the water column and how far they travel from their source. Currently, little is known about the quantitative transport of different types of plastic between the Potomac River and larger Chesapeake Bay, but differences could be due to distance from source and transport dynamics. Another potential explanation for the differing observation is that sampling methods used to capture plastics were not the same. Bikker et al. (2020) collected plastics from surface water using a manta trawl while Murphy (2020) collected grab sample cores from submerged aquatic vegetation beds. Due to the mesh size (i.e. typically > 300um) of netting used, surveys conducted with manta trawls could miss smaller particles like fibers.

While other types of microplastics might be more prevalent in the main portion of the Chesapeake Bay, preliminary evidence suggests that fibers could be more abundant in river systems. Additionally, field studies evaluating incidental microplastic consumption suggested that the majority of ingested microplastics were fibers (Baldwin et al. 2020, Desforges et al. 2015, Peters et al. 2017). These and future pieces of evidence will allow new iterations of the conceptual model to focus on risk associated with microplastics that may be associated with the greatest risk.

X. Methods

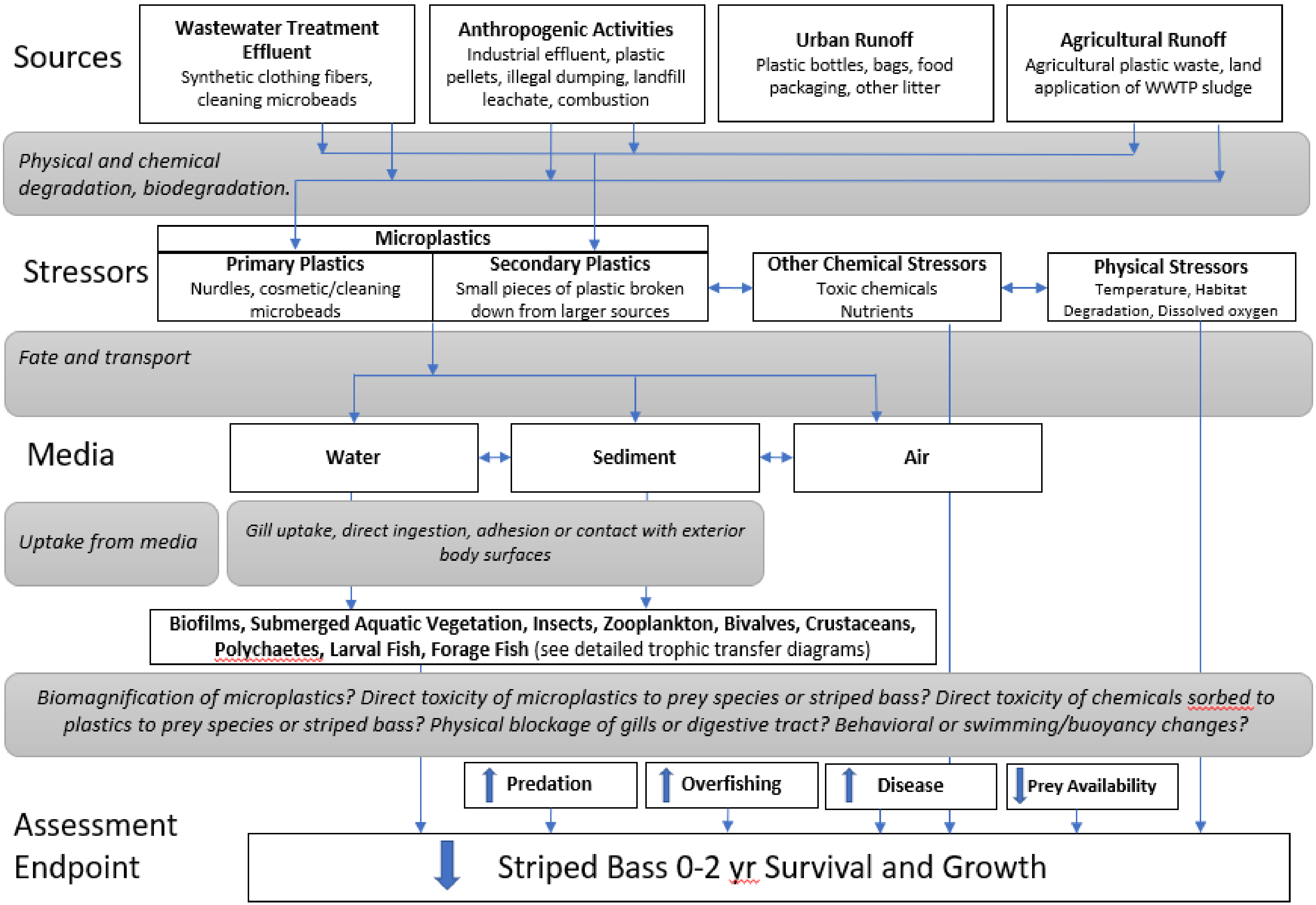
Literature review

A literature search was completed following the methodology approved under the quality assurance project plan (QAPP—see Appendix 1 for full discussion) developed using EPA guidance. The search of primary and gray literature was conducted to identify prey items consumed by Striped Bass, with an emphasis on data collected from the tidal Potomac River and Chesapeake Bay, and supplemented with information from east coast estuaries and other geographical locations, as appropriate. Prey items for the 0-2 year age class were emphasized in the draft diagrams, but information related to older age classes (and prey organisms) not resident in the Chesapeake Bay were retained for future reference. Relative contribution of prey items to Striped Bass diet was quantified where possible. Additional literature searches reviewed the current understanding of the sources for many microplastics in coastal regions that would subsequently affect the construction of the conceptual model.

R Script

Trophic networks were constructed using the *igraph* package in the *R* environment. The igraph package is a specialized network visualization package (Csárdi and Nepusz 2006). Trophic networks are graphical representations of static prey composition matrices; therefore, the network structure is not determined by statistical fitting. These network figures provide a reader-friendly representation of complex diet data and have been designed to emphasize important prey for each life-stage or habitat type.

XI. Conceptual model


Sources
Wastewater Treatment Effluent
Synthetic clothing fibers, cleaning microbeads
Anthropogenic Activities
Industrial effluent, plastic pellets, illegal dumping, landfill leachate, combustion
Urban Runoff
Plastic bottles, bags, food packaging, other litter
Agricultural Runoff
Agricultural plastic waste, land application of WWTP sludge
Physical and chemical degradation, biodegradation.
Stressors
Microplastics
Primary Plastics
Nurdles, cosmetic/cleaning microbeads
Secondary Plastics
Small pieces of plastic broken down from larger sources
Other Chemical Stressors
Toxic chemicals
Nutrients
Physical Stressors
Temperature, Habitat Degradation, Dissolved oxygen
Fate and transport
Media
Water
Sediment
Air
Uptake from media
Gill uptake, direct ingestion, adhesion or contact with exterior body surfaces
Biofilms, Submerged Aquatic Vegetation, Insects, Zooplankton, Bivalves, Crustaceans, Polychaetes, Larval Fish, Forage Fish (see detailed trophic transfer diagrams)
Biomagnification of microplastics? Direct toxicity of microplastics to prey species or striped bass? Direct toxicity of chemicals sorbed to plastics to prey species or striped bass? Physical blockage of gills or digestive tract? Behavioral or swimming/buoyancy changes?
Predation
Overfishing
Disease
Prey Availability
Assessment Endpoint
Striped Bass 0-2 yr Survival and Growth

Sources of Microplastics

Sources of plastics considered microplastic size or smaller include wastewater treatment plant effluents (e.g. synthetic clothing fibers, cleaning microbeads); anthropogenic activities (e.g. industrial effluent, plastic pellets, illegal dumping of garbage, landfill leachate, combustion); urban runoff (e.g. plastic bottles, bags, single use food packaging/utensils, and litter); agricultural runoff (e.g. agricultural plastics, land application of sludge from wastewater treatment plants). Primary plastics are most often associated with treated wastewater or industrial processes while secondary plastics are associated with sources of large plastics that break down into smaller pieces in the environment. Both primary and secondary plastics are subject to degradation, creating smaller fragments by physical, chemical, and biological factors. Such processes include ultraviolet radiation, abrasion due to movement of wind or water, and degradation by microbes.

Media

Primary pools of bioavailable microplastics were identified in different media including settled (sediment) and suspended particles (water column). Atmospheric deposition (air) is expected to contribute microplastics to aquatic systems, but striped bass and other aquatic organisms do not directly interface with air.

Stressors

Both primary and secondary microplastics are the stressor of concern. It is also acknowledged that other co-occurring stressors in the Potomac are very important and result in ecological effects, including changes related to growth and survival in striped bass. Such stressors include toxic chemicals, dissolved oxygen, disease, predation, prey availability, temperature, and changing hydrological conditions.

Development of trophic transfer pathways

Lower level trophic organisms and Striped Bass are exposed to microplastics via gills, direct ingestion, or surface/skin contact, potentially causing toxicity or behavioral changes. Understanding the trophic pathways contributing to Striped Bass diets are important to identify the most important dietary microplastic exposure routes. Thus additional, detailed trophic transfer models were developed to better understand potential microplastic transfer within the food web.

The compiled literature was examined for Potomac River relevant data on resident age-classes of Striped Bass, including food web interactions and potential assessment endpoints. Resident age-classes were defined as including: all young-of-the-year (YOY) stages (both larval and post-metamorphosis juvenile), and ages 1 through 3 fish. Though the 0-2 age class was the original focus, age-3 fish were included in the analysis because evidence suggests the majority of age-3 males remain resident in Chesapeake Bay (Secor and Piccoli 2007), indicating that primary exposure to microplastics for males of that age class is still limited to the geographic area of interest. Diet data for age 0-3 Striped Bass reported in several key studies were used to develop an unweighted, qualitative prey network (multigraph) linking dominant primary producers at the base of the food web, prey taxa, and Striped Bass: Markle and Grant (1970), Beaven and Mihursky (1980), Boynton et al. (1981), Walter III and Austin (2003, 458-710 mm size classes only), Muffelman (2006), Martino (2008), Shideler and Houde (2014), and Ihde et al. (2015). These regional studies were conducted in the Potomac River (Beaven and Mihursky 1980, Boynton et al. 1981), adjacent Virginia tributaries (Markle and Grant 1970, Muffelman 2006), and the Chesapeake Bay mainstem (Walter III and Austin 2003, Martino 2008, Shideler and Houde 2014). One study did note the direct consumption of microplastics by older Striped Bass, although it was in a reservoir system outside of the Chesapeake Bay region (Baldwin et al. 2020).

For each focal age-class, quantitative diet data were used to create positive (weighted) network diagrams. These positive networks have edges (lines between nodes) and nodes that vary in thickness as a function of the amount of each prey type consumed by the focal age-class, however, predator-prey linkages for non-focal age-classes or between prey nodes were not weighted. Prey importance for each positive network diagram was determined using % diet composition by biomass or, if biomass was not reported, volume or number. Dominant prey species were assigned individual categories (e.g., Bay Anchovy *Anchoa mitchilli*, Atlantic Menhaden *Brevoortia tyrannus*). Where prey groups were reported as lower taxonomic resolution aggregates, these aggregate prey taxa were maintained (e.g., polychaetes, insects) or were further aggregated to reflect diverse functional groups of taxonomically similar prey that contributed relatively little to diet individually but could be important together (e.g., other crustaceans, other fish, Table 3-1). Among sub-adult age-classes, data for age 3 males were not available at sufficient resolution to develop a positive network diagram; therefore, age-specific positive network diagrams were only developed for age 1 and 2 fish. Multigraph and positive networks linking prey groups to lower trophic position prey and, ultimately, to primary producers were based on compiled literature (Baird and Ulanowicz 1989) and professional knowledge of the project PIs.

Table 3-1. Aggregated Striped Bass prey table identifying specific taxa included in aggregate groups and associated references.

Aggregate group	Included taxa	Reference
Other fish	Teleostei *(Morone americana, Leiostomus xanthurus, Micropogonias undulatus, Urophycis regia, Notropis hudsonius, Lepomis gibbosus, Cynoscion regalis, Gobiosoma bosci)*	(Markle and Grant 1970, Walter III and Austin 2003, Ihde et al. 2015)
Insects (larvae and pupae)	Diptera (e.g., Muscidae, *Chironomus* sp., *Chaoborus* sp.), Hemiptera, Ephemeroptera	(Markle and Grant 1970, Boynton et al. 1981, Muffelman 2006)
Larval zooplankton	Cirripedia (barnacle larvae cirri), copepodites*, copepod nauplii*	(Markle and Grant 1970)
Other crustaceans	Mud crab, Palaemonidae (*Palaemonetes* sp.), sand shrimp (*Crangon septemspinosa*), mantis shrimp, isopods, xanthids*, Ovalipes ocellatus*	(Markle and Grant 1970, Walter III and Austin 2003, Muffelman 2006, Ihde et al. 2015, Lehtiniemi et al. 2018)

**based on literature from other estuaries ((Hjorth 1988, Limburg et al. 1997) - Hudson River)*

Very little data exist on microplastics loads in prey taxa in the Chesapeake Bay region, particularly for the Potomac River basin. Therefore, an initial evaluation of the relative importance of each prey category across Striped Bass age-classes was used to identify key data gaps for the species' forage base. A 'priority level' was assigned to each prey category based on the following criteria:

- High priority (> 5% diet composition across multiple life-stages)
- Moderate priority (> 10% diet comp within one life stage)
- Lower priority (< 10% diet comp within one life stage)

Due to differences in the way that the diet data were reported (i.e., % biomass, % number) and the subjective nature of the threshold values, these priority rankings are only intended to provide a summary of the available data. A life stage-specific breakdown of larval diet data is available in Beaven and Mihursky (1980), reported as % occurrence of each prey taxon in the stomachs of yolk-sac, finfold, and post-finfold larvae. Due to this method of reporting, these stage-specific data were not used to develop positive network diagrams but the data were used to create a prey priority ranking table for Striped Bass larvae in the Potomac River.

- High priority (> 5% frequency of occurrence across multiple larval-stages)
- Moderate priority (> 10% frequency of occurrence within one larval-stage)
- Lower priority (< 10% frequency of occurrence within one larval-stage)

These priority-level rankings are meant to provide guidance for future research priorities for trophic studies of Striped Bass microplastics exposure in the Potomac River.

XII. Results

Trophic network supporting Striped Bass in the Potomac River

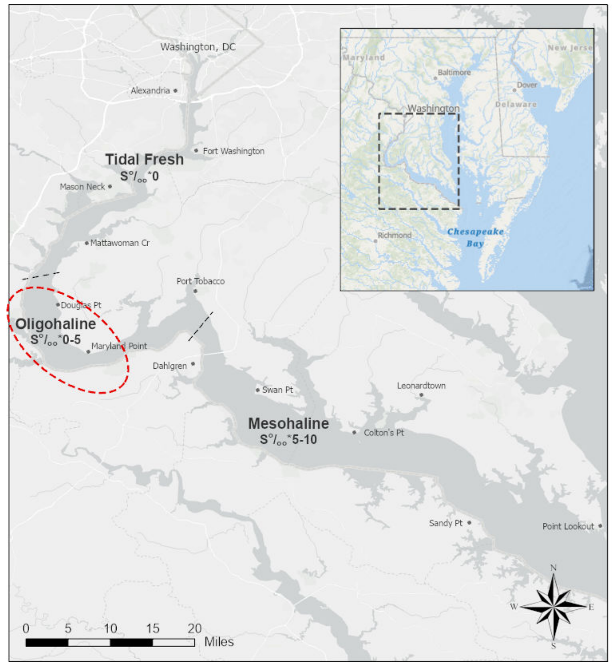


Figure 4-1. Potomac River showing the general extent of the salinity regimes used in this analysis, in addition to the estuarine turbidity maximum (red oval)

This series of diagrams is based on the dietary studies described above. The initial multigraph is a complete compilation of the available regional literature and highlights the number of potential trophic pathways through which microplastics could be ingested by different age-classes of Striped Bass (Figure 4-1). These diagrams do not explicitly include other potential microplastics exposure mechanisms such as directed consumption (i.e. mistaking microplastics for prey), passive uptake during feeding, uptake through the mouth during non-feeding activities, or exposure via other surfaces such as gills (Roch et al. 2020). Despite this, these diagrams can be used to infer the potential of such pathways. For example, microplastics in the surface sediments may be passively ingested by Striped Bass feeding on benthic polychaetes. The potential sediment-Striped Bass pathway is not specifically identified as an edge in either the multigraph or the positive network diagrams because there are not sufficient data on microplastics presence or concentration associated with the basal resources in the Potomac River or the amount of basal resource material typically ingested during Striped Bass feeding. The relative importance of trophic versus passive uptake of microplastics is unknown; however, several recent studies have documented the importance of trophic transfer as a major mechanism for microplastics exposure(Nelms et al. 2018, Hasegawa and Nakaoka 2021) Hasegawa and Nakaoka (2021) showed that trophic transfer of microplastics to fish via predation on mysid shrimp was 3–11 times greater than passive uptake from the water column. For Striped Bass in the Potomac River and the broader Chesapeake Bay, the relative importance of these potential exposure routes remain as critical data gaps but the structure of the trophic network(s) provided here can be used to identify where some of these passive pathways could exist.

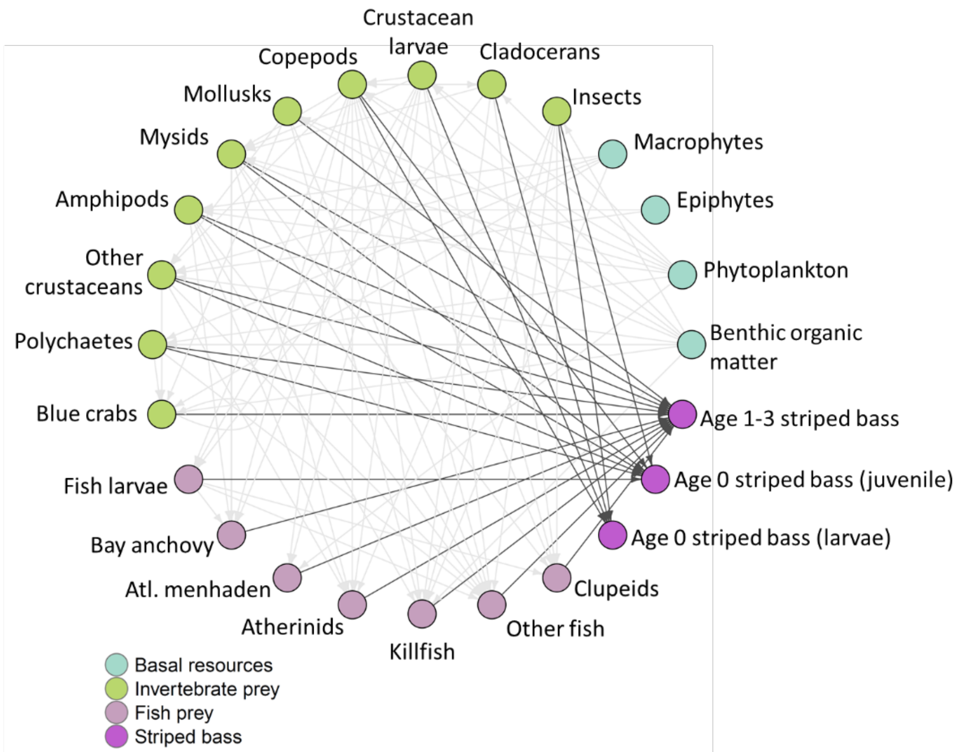


Figure 4-2. This diagram shows basal trophic resources that are also potential pools of microplastics in the environment and a generalized food web of prey items consumed by Striped Bass and organisms consumed by those prey items. Three age classes of Striped Bass (young of year [YOY] larvae, YOY juveniles, and 1-3-year-old) are shown with connections to their known prey items.

The reticulated structure of the Striped Bass food web indicates that microplastics exposure could follow a range of different trophic pathways. Many of the prey consumed directly by Striped Bass also contribute to diets indirectly (separated by at least one trophic transfer). For example, mysid shrimp are depredated by multiple age-classes of Striped Bass as well as by many of the forage fish that are directly consumed by Striped Bass such as bay anchovy, clupeids, and other small or juvenile fishes. If microplastics accumulate in prey, either in the gut or in body tissues following assimilation of very small fragments across the gut wall, indirect trophic transfers may represent an important 'source' of microplastics trophic exposure (Setälä et al. 2014). Finally, many of the prey taxa identified here rely on multiple basal resources (e.g., phytoplankton and benthic organic matter). By linking multiple basal resources, individual prey taxa will potentially be exposed to different pools of microplastics available in the environment.

Diet of larval Striped Bass in the Potomac River is dominated by small zoofauna (Beaven and Mihursky 1980). Dominant prey taxa include cladocerans such as *Bosmina longirostris*, copepods such as the calanoids *Eurytemora affinis* and *Acartia tonsa*, and rotifers such as *Brachionus calyciflorus* (Figure 4-2). Data from other salinity zones in the Potomac River for larval Striped Bass are not available but olighaline reaches in the vicinity of the estuarine turbidity maximum (ETM) are known to be important reaches for the concentration, growth and survival of this life stage. Therefore, diet composition of larval Striped Bass from this salinity zone is likely to be a good representation of diet for this life-stage. Due to differences in the reporting of diet composition by larval stage, there is not a stage-specific positive network diagram for yolk-sac, finfold, and post-finfold larvae, but the % frequency of occurrence of each prey type by larval stage is provided (Figure 4-3). Figure 4-3 shows that diet composition changes across these three different larval stages, with yolk-sac larvae feeding primarily on rotifers and cladocerans. Diet of finfold larvae demonstrates a reduced contribution of rotifers and increasing importance of cladocerans and copepods, while diet of post-finfold larvae of dominated by copepods and cladocerns. Among the copepods identified, *E. affinis* occurred much more frequently than *A. tonsa*. Many of these same zooplankton taxa were identified as dominant prey in the stomachs of larval Striped Bass in the oligohaline region of the Chesapeake Bay mainstem (Martino 2008, Shideler and Houde 2014).

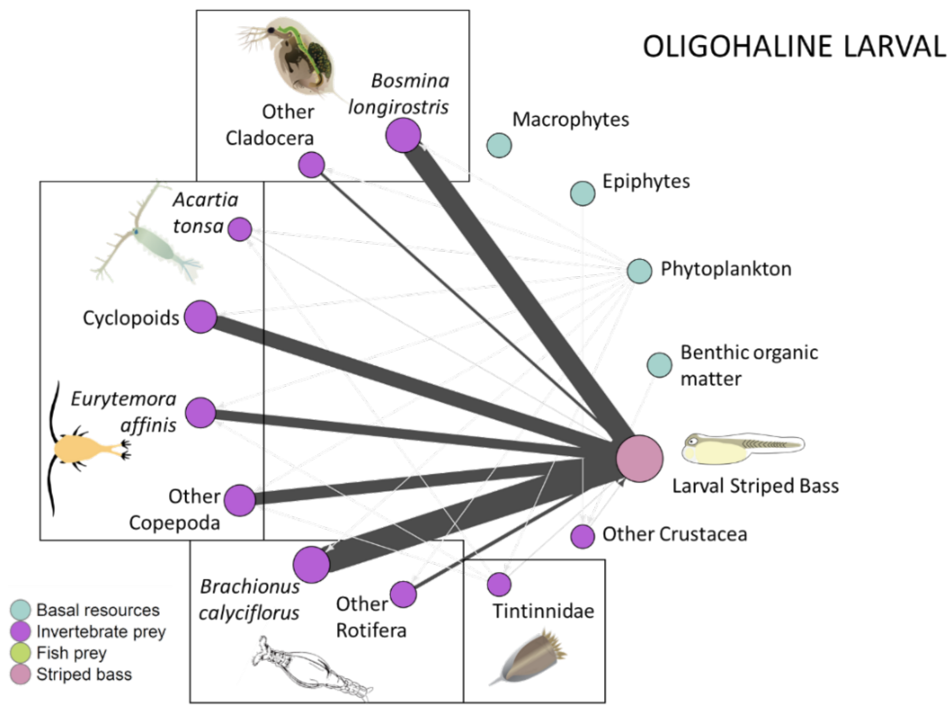


Figure 4-3. Larval Striped Bass food web from oligohaline portion of Potomac River estuary (adapted from Beaven and Mihursky 1980). Dark vectors connecting prey through direct consumption provides biomass-weighted percent contribution to diet for each prey category. Copepods are primarily adult-stages. Boxes around prey group taxonomically similar prey: Cladocera, Copepoda, Rotifera, Tintinnidae.

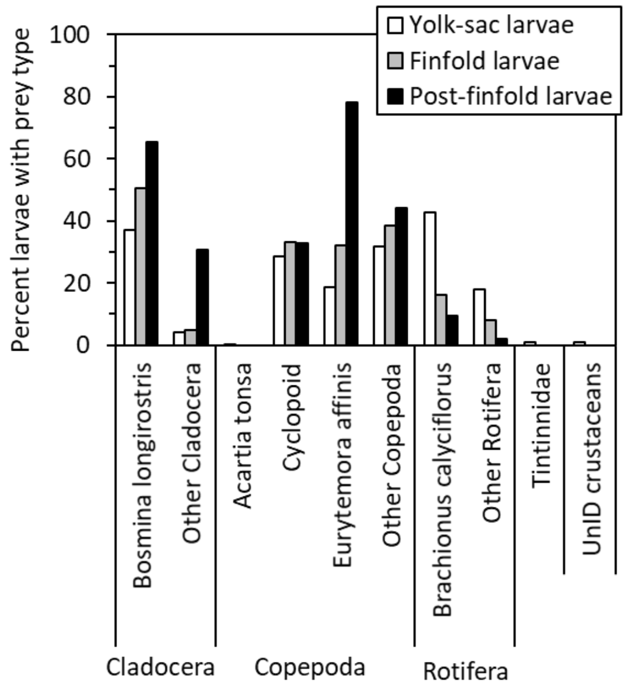


Figure 4-4. Larval Striped Bass diet composition as % frequency of occurrence of prey in fish with material in their stomachs from the oligohaline portion of Potomac River estuary (adapted from Beaven and Mihursky 1980).

Research conducted by Boynton et al. (1981) described quantitative dietary preferences of YOY Striped Bass (25-99 mm) foraging in three Potomac River salinity regimes—mesohaline, oligohaline, and tidal freshwater Figure 4-4, Figure 4-5, Figure 4-6). In these positive networks diagrams, dashed lines connecting the Striped Bass node to a prey item indicate the prevalence of that organism as a food item, with thicker lines and larger nodes indicating a greater contribution than thinner lines. These diagrams demonstrate that the diet of YOY Striped Bass varies in composition depending on salinity zone. For example, mysids and polychaetes make up most of the diet in mesohaline areas while fish larvae and insects are the most dominant dietary components in tidal freshwater areas. Diet composition in oligohaline areas were intermediate between the mesohaline and tidal freshwater with declining importance of insect larvae, increased importance of amphipods and polychaetes and the appearance of mysids. In addition to diet differences across salinity zones, Boynton et al. (1981) also found inshore-offshore differences in diet composition within each salinity zone (Figure 4-7). These differences are likely related to local prey availability, with insect larvae a larger component of the diet in juvenile Striped Bass collected in shallow inshore habitats and mysids a larger component in fish collected offshore (up to 5m depth).

The differing dietary preferences associated with salinity-based and inshore-offshore habitats are potentially important given ongoing research on the fate and transport of microplastics within the Potomac River. Boyton et al (1981) defined nearshore as the As data gaps close for fate and transport and uptake by prey items, an ecological risk assessment can be tailored to specific habitats that might be disproportionately affected by different varieties or concentrations of microplastics.

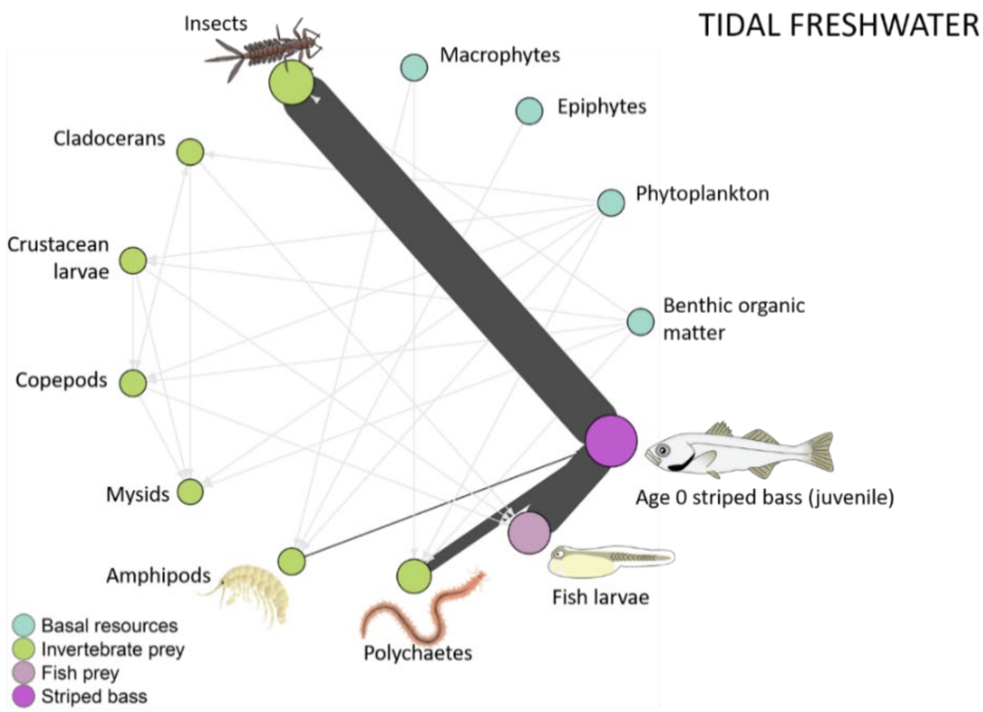


Figure 4-5. Juvenile Striped Bass food web from tidal fresh portion of Potomac River estuary (adapted from Boynton et al. (1981)). Dark vectors connecting prey through direct consumption provides biomass-weighted percent contribution to diet for each prey category. Insects are primarily Diptera larvae (e.g., Muscidae).

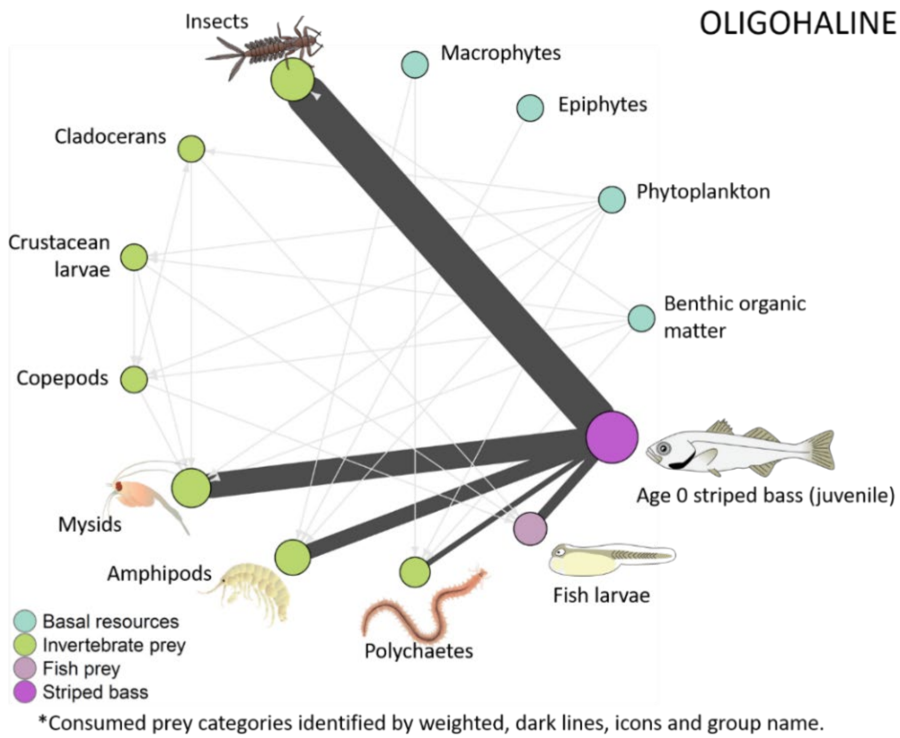


Figure 4-6. Juvenile Striped Bass food web from oligohaline portion of Potomac River estuary (adapted from Boynton et al. (1981)). Dark vectors connecting prey through direct consumption provides biomass-weighted percent contribution to diet for each prey category.

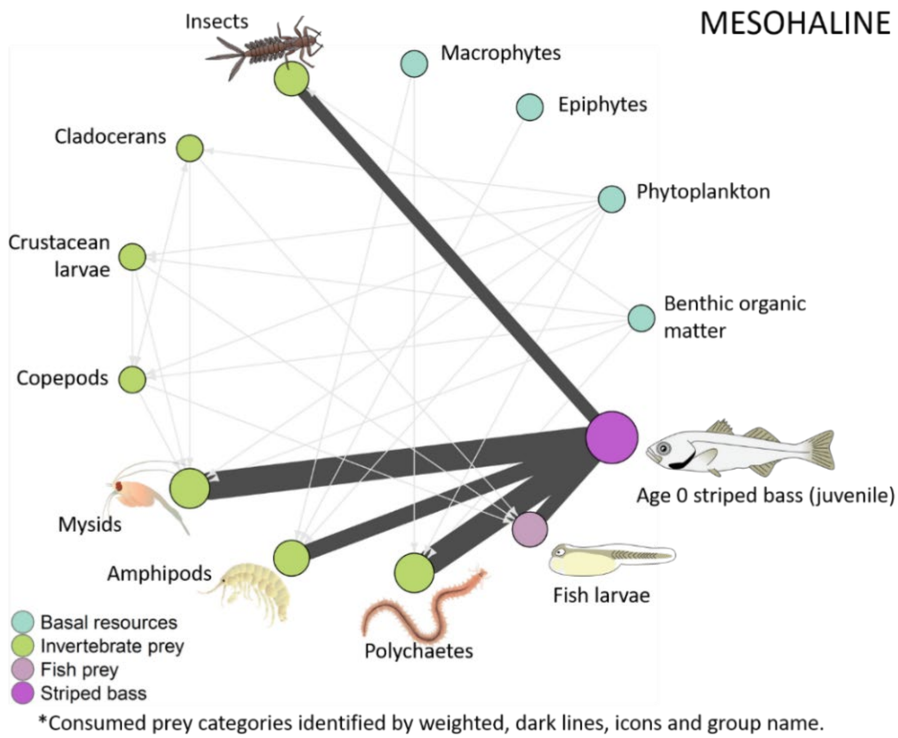


Figure 4-7. Juvenile Striped Bass food web from mesohaline portion of Potomac River estuary (adapted from Boynton et al. (1981)). Dark vectors connecting prey through direct consumption provides biomass-weighted percent contribution to diet for each prey category.

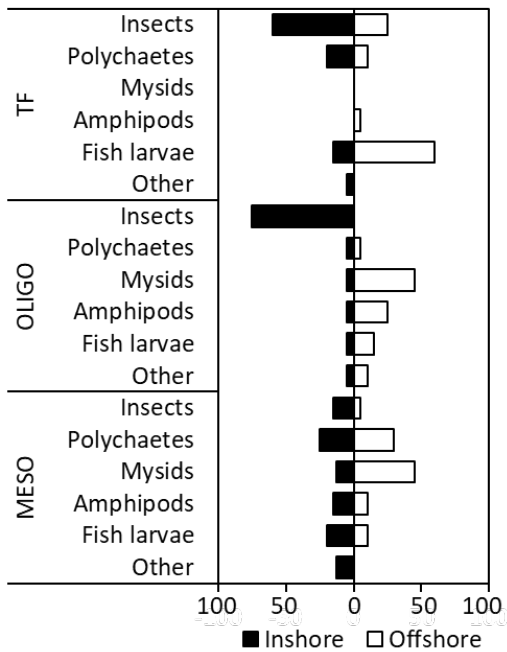
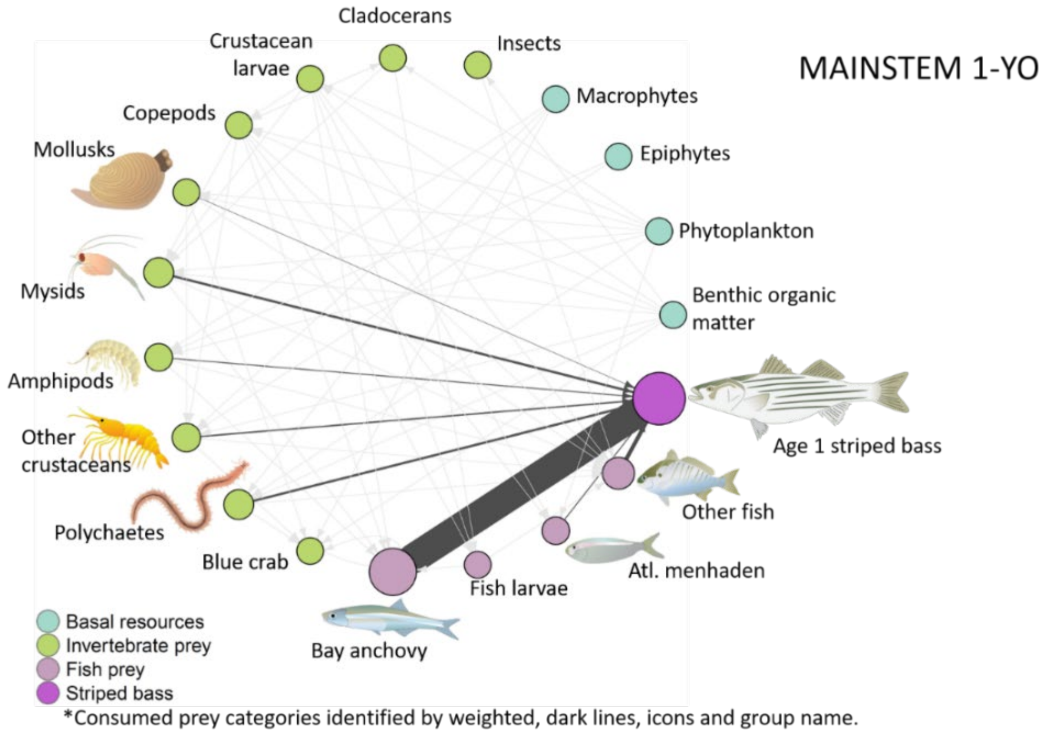


Figure 4-8. Juvenile Striped Bass % diet composition from fish collected at inshore and offshore locations in the tidal fresh (TF), oligohaline (OLIGO), and mesohaline (MESO) portion of Potomac River estuary (adapted from Boynton et al. (1981)).

As previously noted, the literature review evaluated a number of studies on Striped Bass diets and those that focused on Potomac River populations (Beaven and Mihursky 1980, Boynton et al. 1981) were used to develop the YOY positive network diagrams. However, few to no studies were found for older fish (1YO-2YO) from the Potomac River. Ihde et al. (2015) reviewed Striped Bass diets along the entire mainstem of the Chesapeake Bay, from tidal fresh to polyhaline regions, that can be utilized as a proxy for the Potomac River estuary. Those findings were used to develop positive network diagrams for ages 1 and 2 (Figure 4-8, .Figure 4-9). Identities of dominant prey taxa were very similar between the two age classes, with the inclusion of a relatively small amount of blue crab in the diet of age-2 Striped Bass being the major difference (Figure 4-10) (Ihde et al. 2015). Despite these close similarities in prey identity, there were substantial differences in the relative contribution of different prey to each age-class. Some key differences were an increased importance of benthic and invertebrate prey for the age-2 Striped Bass. Mysids, amphipods, and polychaetes all contributed more to the diet of age-2 Striped Bass. Atlantic Menhaden (*Brevoortia tyrannus*) also became more important to the diet of age-2 Striped Bass while Bay Anchovy (*Anchoa micthilli*) declined in importance relative to the age-1 diet.



.Figure 4-9. Food web with Striped Bass 1YO endpoint. Dark vectors connecting prey through direct consumption provides biomass-weighted percent contribution to diet for each prey category as found in Ihde et al. (2015).

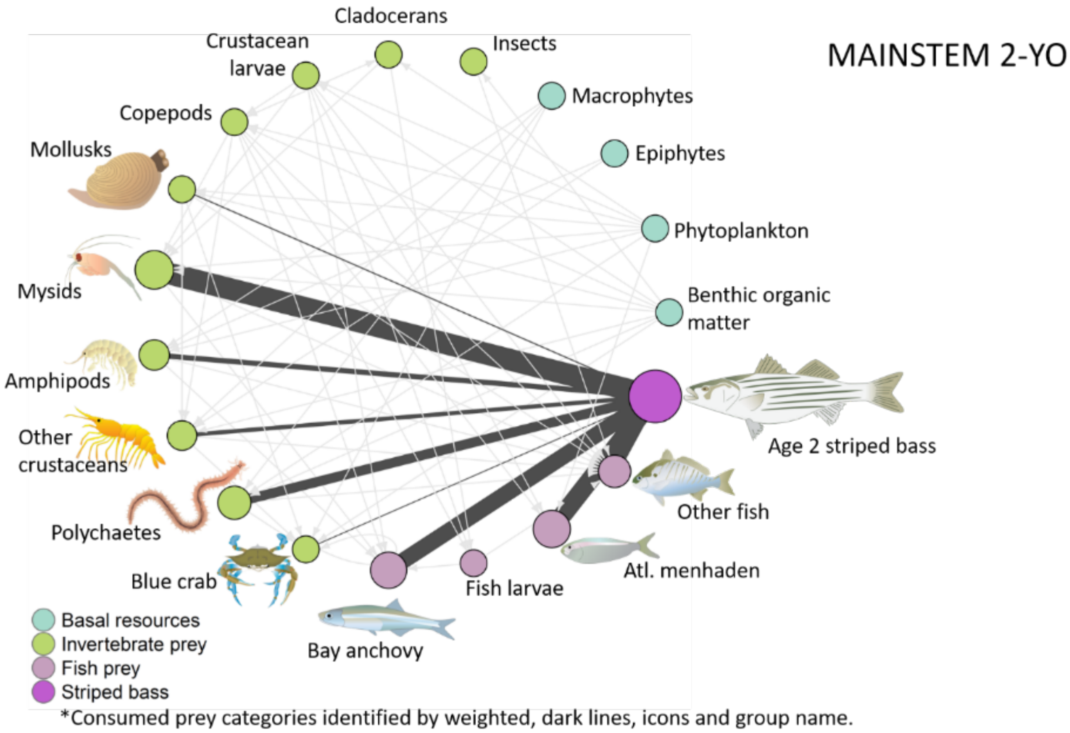


Figure 4-10. Food web with Striped Bass 2YO endpoint. Dark vectors connecting prey through direct consumption provides biomass-weighted percent contribution to diet for each prey category as found in Ihde et al. (2015). Note the change in dominant dietary components from 1YO.

An evaluation of diet composition data revealed several prey taxa that were important across multiple life-stages and age-classes of Striped Bass (Table 4-1Three prey taxa – mysids, amphipods, and polychaetes – were ranked as High priority taxa for future research on potential microplastics exposure of Potomac River Striped Bass through trophic transfer. These three prey taxa were important during the YOY juvenile and ages 1 and 2 subadult age-classes. Six prey taxa were identified as Moderate priority taxa because they were a dominant prey for at least one age-class of Striped Bass. These moderate priority prey included invertebrates (aquatic insect larvae, cladocerans, copepods) and fishes (fish larvae, Bay Anchovy, Atlantic Menhaden). Low priority prey contributed relatively little to diet and were only associated with one life stage (larval zooplankton, bivalves, other crustaceans, other fish).

Table 4-1. Percent diet composition by major prey category for each life-stage of Striped Bass (larval [inclusive of yolk-sac, finfold, post-finfold larvae], age-0 juvenile, age-1 and age-2 subadults [SA]). Salinity zone within the Potomac River is provided for age-0 life-stage diet data (Tidal fresh – TF, Oligohaline – OLIGO, Mesohaline – MESO) but not for age-1 or age-2 Striped Bass diet data originating from the mainstem of Chesapeake Bay (MAIN). Priority-level reflects proposed priority need for empirical measurement of microplastics loading in each prey category (High priority – red, Moderate priority – orange, Lower priority – yellow; classification levels described further in the text).

	Age-0				Age-1	Age-2	
	Larval	Juvenile			SA	SA	
Prey category	**OLIGO**	**TF**	**OLIGO**	**MESO**	**MAIN**	**MAIN**	**Priority-level**
Insects		47.5	40	12.5			
Cladocerans	26.2						
Larval zooplankton	1						
Adult copepods	40.3						
Bivalves					0.9	1.2	
Mysids		0	24.5	27	4.5	21	
Amphipods		1.5	15	15.5	1.9	5	
Other crustaceans					2.8	4	
Polychaetes		12	5.5	25	4.4	9.4	
Bay Anchovy					57.8	15.6	
Fish larvae		35.5	10	14			
Atl. Menhaden					1.9	17.9	
Other fish					7.6	8	

A separate evaluation of diet percent frequency of occurrence data for each larval stage of Striped Bass was conducted to provide higher resolution of dominant prey for this critical life-stage (Table 4-2). Four zooplankton prey taxa, including *B. longirostris*, *E. affinis*, Cyclopoid copepods, and other copepods, were ranked as High priority taxa for future research. These four prey taxa were important during the yolk-sac, finfold, and post-finfold larval stages. Other cladocerans and rotifers, including *B. calyciflorus*, were identified as Moderate priority taxa because they were a dominant prey for at least one larval stage of Striped Bass. Tintinnids, *A. tonsa*, and other (unidentified) crustaceans were classified as Low priority prey because they contributed relatively little to diet during one larval stage.

Table 4-2. Percent frequency of occurrence by major prey category in the diet of each larval-stage of Striped Bass (yolk-sac, finfold, post-finfold). Larval data are from the oligohaline zone within the Potomac River. Priority-level reflects proposed priority need for empirical measurement of microplastics *loading in each prey category (High priority – red, Moderate priority – orange, Lower priority – yellow; classification levels described further in the text).*

		Age 0 (OLIGOHALINE)			
Taxonomic group	**Prey taxon**	**Yolk-sac**	**Finfold**	**Post-finfold**	**Priority-level**
Cladocera	*Bosmina longirostris*	36.9	50.5	65.4	
	Other Cladocera	4	5	30.7	
Copepoda	*Acartia tonsa*	0.4			
	Cyclopoid	28.5	33.3	32.8	
	Eurytemora affinis	18.8	32.2	78.1	
	Other Copepoda	31.7	38.4	44.2	
Rotifera	*Brachionus calyciflorus*	42.6	16.2	9.6	
	Other Rotifera	17.8	8.1	1.9	
Ciliophora	Tintinnidae	0.8			
UnID crustaceans	UnID crustaceans	0.8			

Assessment Endpoint and Potential Effects of Microplastics

It is hypothesized that microplastics may contribute to decreased growth and survival of striped bass by several mechanisms. First, microplastics are known to cause physical blockage of the gut resulting in blockage or potentially reduced feeding due to a full gut. Microplastics could also cause behavioral changes in small organisms if the physical presence of microplastics changes buoyancy or swimming behavior, leading to increased susceptibility to predicators. Additionally, toxicity to striped bass could occur because of organic contaminants like PCBs, PCDEs, or other organic contaminants that strongly partition to plastics.

Analysis Plan

The conceptual model for microplastic risk assessment on Striped Bass demonstrates wide-ranging data gaps in our understanding of current microplastic abundance, distribution, and biological interactions. The model highlights potential pathways for microplastics to impact Striped Bass and which endpoints are potentially impacted. However, there is no data on the basic uptake of microplastics within the trophic ecology of Striped Bass in the Potomac River. Recent research, on the other hand, has evaluated the uptake of microplastics in species of the same genera seen in the Potomac River Striped Bass diets, although these studies were done elsewhere. These values begin to shed light on some aspects of how microplastics may enter the food chain, eventually reaching Striped Bass (Table 4-3).

Table 4-3. Studies on microplastic ingestion for taxa identified in the Potomac River estuary trophic pathways for Striped Bass, ages 0-2 YO.

Major Taxa	Confirmed MP presence or consumption? (Y/N)	Location	Citation	Notes
Habitat				
Macrophytes (includes SAV and wetlands)	Y	(SAV) Caribbean; UK, Korea; Washington, DC; (wetlands)South Africa; multiple	(Goss et al. 2018, Reynolds and Ryan 2018, Murphy 2019, Townsend et al. 2019, Cozzolino et al. 2020, Huang et al. 2020, Jones et al. 2020)	Macrophytes include a compilation of SAV and wetlands given similar roles for microplastic adherence
Epiphytes	Y	Caribbean;	(Goss et al. 2018, Seng et al. 2020)	Found in epiphytes on seagrass
Benthic organic matter	Y	St. Lawrence River; Washington DC;	(Castaneda et al. 2014b, Murphy 2020)	
Phytoplankton	Y	Laboratory;	(Long et al. 2015, Shiu et al. 2020)	Diatoms; aggregation of cells on MPs
Invertebrate Prey				
Insects	Y	Germany	(Ehlers et al. 2019)	Field collected caddisfly cases
Crustacean larvae	Y	Laboratory	(Jemec et al. 2016, Gambardella et al. 2017, Woods et al. 2020)	Lobsters; barnacle nauplii;
Cladocerans	Y	Laboratory	(Martins and Guilhermino 2018, Jaikumar et al. 2019, Woods et al. 2020)	Freshwater regions
Copepods	Y	Laboratory; Pacific Ocean	(Cole et al. 2015, Desforges et al. 2015)	

Table 4-3. Studies on microplastic ingestion for taxa identified in the Potomac River estuary trophic pathways for Striped Bass, ages 0-2 YO.

Major Taxa	Confirmed MP presence or consumption? (Y/N)	Location	Citation	Notes
Amphipods	Y	Laboratory	(Jeong et al. 2017, Mateos Cárdenas et al. 2019)	Jeong et al proposed an adverse outcome pathway for microplastic exposure that covers molecular and individual levels.
Mysids	Y	Laboratory	(Setälä et al. 2014, Lehtiniemi et al. 2018, Wang et al. 2020)	Hasegawa et al (2021) demonstrated trophic transfer of microplastics between mysids and fish predator
Polychaetes	Y	Newfoundland; laboratory; Norway	(Mathalon and Hill 2014, Setälä et al. 2014, Knutsen et al. 2020)	
Blue crab	Y	Murderkill and St. Jones Rivers, DE; Texas;	(Santana et al. 2017, Cohen 2020, Waddell et al. 2020)	Santana et al found little trophic cascade; Cohen's work in similar systems to tidal Potomac;
Crustacea (other)	Y	Florida; North Sea	(Devriese et al. 2015, Waite et al. 2018)	Waite et al found MPs in *Panopeus*, a known prey item for striped bass;

Table 4-3. Studies on microplastic ingestion for taxa identified in the Potomac River estuary trophic pathways for Striped Bass, ages 0-2 YO.

Major Taxa	Confirmed MP presence or consumption? (Y/N)	Location	Citation	Notes
				Devriese looked at *Crangon* shrimp, known prey ofr striped bass.
Molluscs	Y	Laboratory;	(Avio et al. 2015, Gutow et al. 2016)	Gutow looked at *Littorina*; Avio looked at mussels
Fish				
Bay anchovy	Y	South Carolina;	(Gray et al. 2018, Parker et al. 2020)	Other literature available for proxies to bay anchovy
Atlantic menhaden	Y	South Carolina	(Parker et al. 2020)	
Fish larvae	Y	Laboratory; Portugal	(Lonnstedt and Eklov 2016, Rodrigues et al. 2019)	Rodrigues looked at urbanized estuaries, multiple fish species;
Striped Bass				
Striped Bass	Y	Lake Meade	(Baldwin et al. 2020)	Freshwater impoundment

Applying the trophic pathways for Striped Bass risk assessment endpoints allows us to identify gaps in data, but more importantly, the ability to prioritize the particular taxa that overlap salinity regimes. For example, mysid shrimp are a priority taxon in Striped Bass diets throughout the Potomac River estuary, but we have no data on microplastic uptake by mysids within the Potomac. Recent research (Lehtiniemi et al. 2018, Hasegawa and Nakaoka 2021) has shown that mysids not only ingest microplastics, but that consumption by fish results in bioaccumulation since the microplastic particles are transferred to fish tissue. This implies the same mechanism can take place in the Potomac and therefore this pathway requires further investigation.

Prepared by:

Bob Murphy (Tetra Tech): Technical author
Jennifer Flippin (Tetra Tech): Technical author
Ryan J. Woodland, PhD (UMCES): Technical author
Jerry Diamond, PhD (Tetra Tech): Technical reviewer
Carol Gallardo (Tetra Tech): Technical editor
Paige Hobaugh (Tetra Tech): Graphic support
Alexis Walls (Tetra Tech): Graphic support

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