PRELIMINARY CONCEPTUAL MODEL FOR AN ECOLOGICAL RISK ASSESSMENT FOR MICROPLASTICS ON STRIPED BASS IN THE POTOMAC RIVER ESTUARY

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TETRA TECH

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

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1. 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).

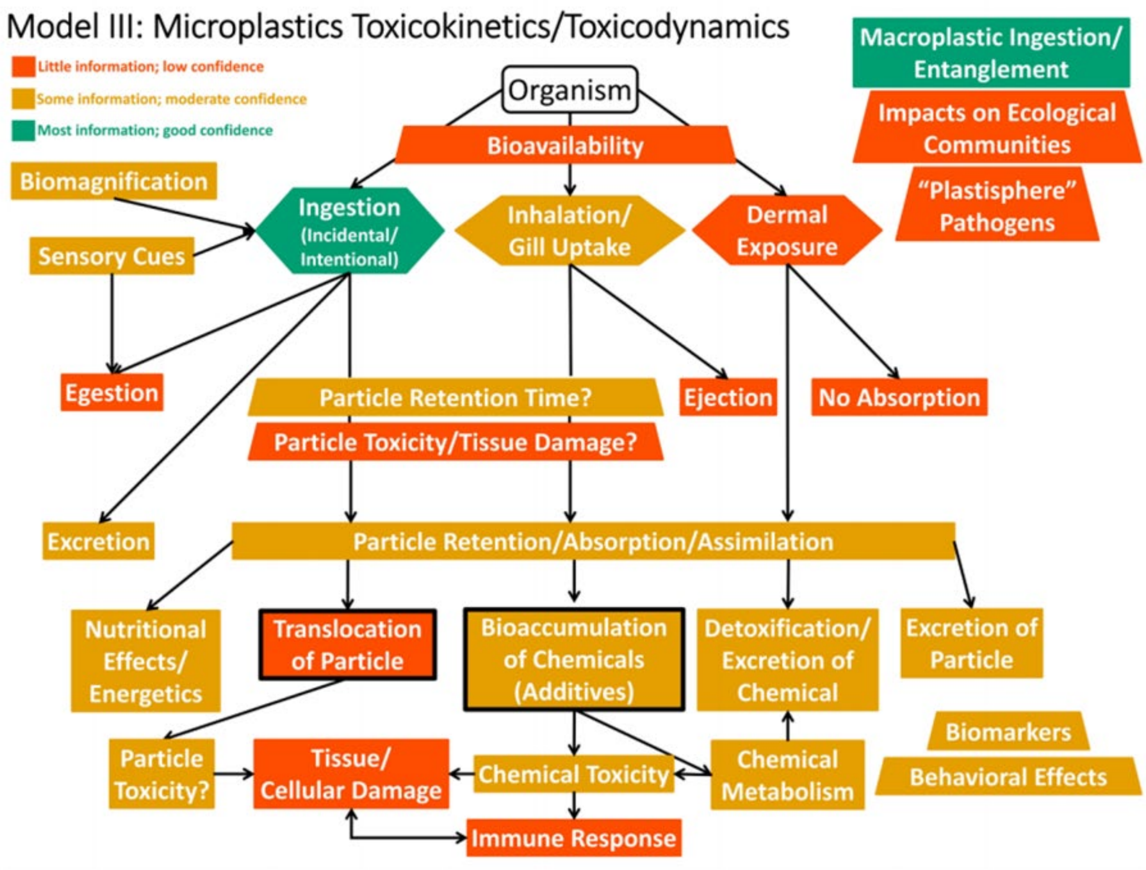


Figure 1-1. Conceptual model describing pathways and complexities regarding plastic exposure and potential outcomes (from EPA 2017)

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.

1.1. 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.

1.2. Temporal Coverage

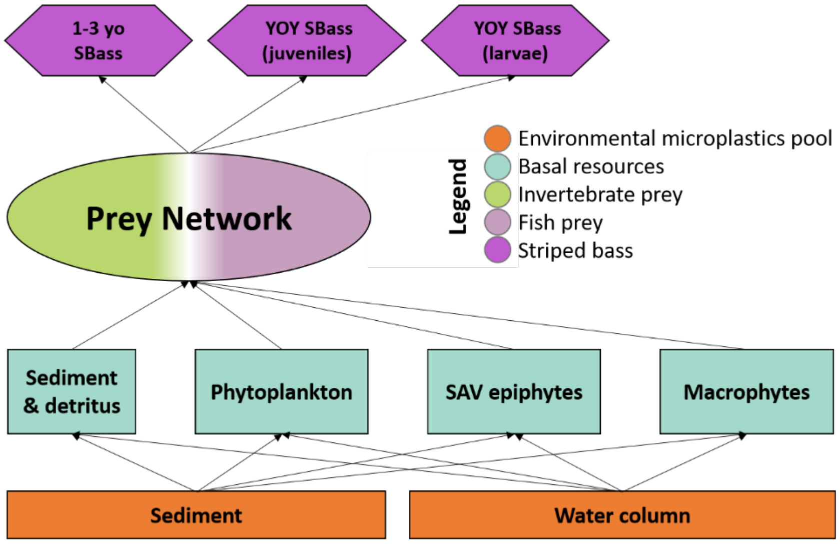
As noted, Chesapeake Bay fisheries, including the Potomac River estuary, has been well-studied for several decades, going back to at least the 1920's with rigorous population data available. In addition, ecological studies of economically important species (e.g. Striped Bass) span a long timeframe, with 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.

1.3. 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). 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.



Potential Assessment Endpoints

Individual Assessment Endpoints
- Growth rates
- Fecundity
- Predator susceptibility
- Direct mortality
- Physiological condition
- Behavior change

Population Assessment Endpoints
- Catch-per-unit-effort
- Size-at-age
- Age-structure
- Mortality
- Spawning stock biomass

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.*

1.4. 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. 2020) 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.

2. Methods

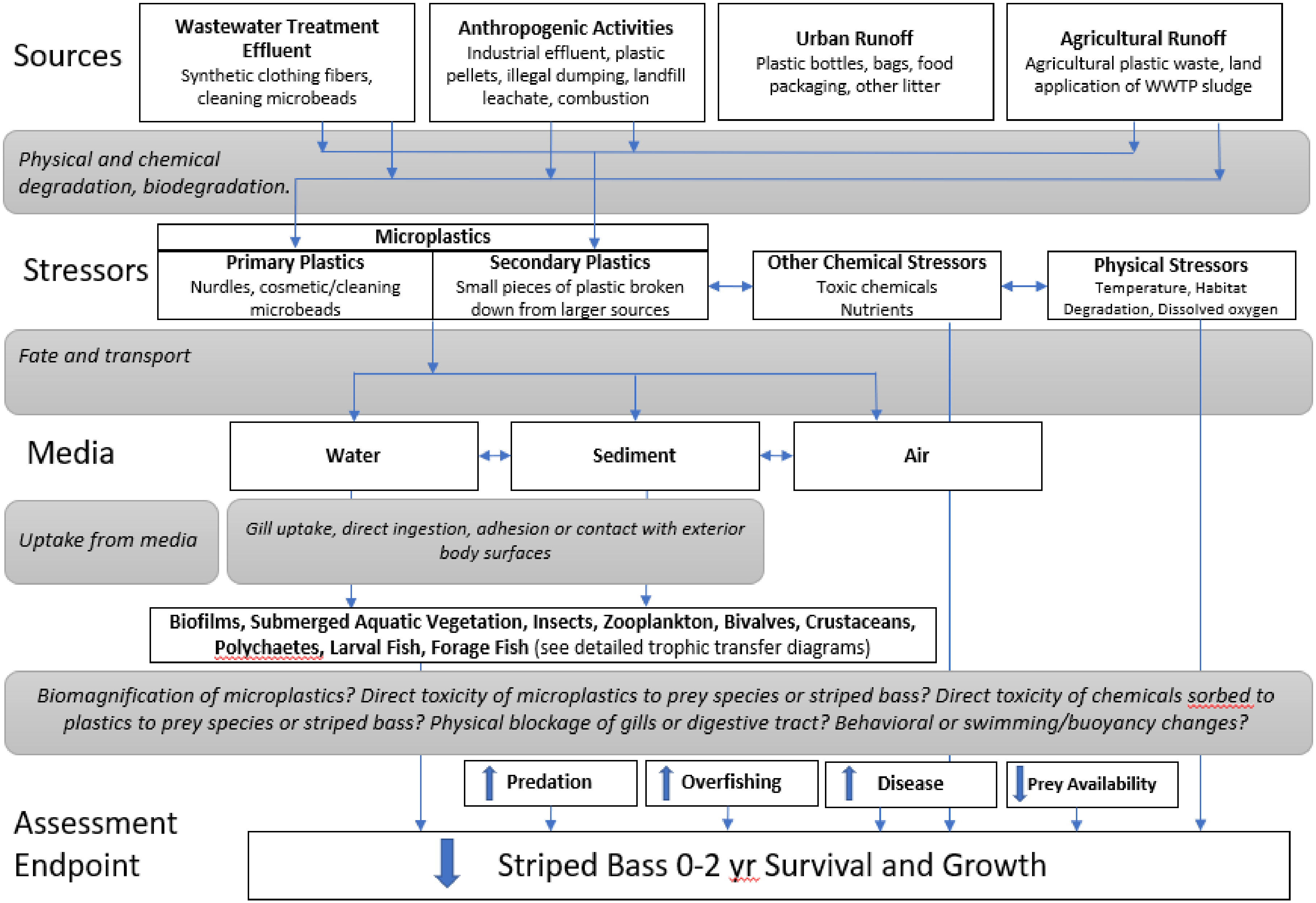
2.1. Literature review

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.

2.2. 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.

3. 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

3.1. 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.

3.2. 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.

3.3. 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.

3.4. 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.

4. Results

Trophic network supporting Striped Bass in the Potomac River

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

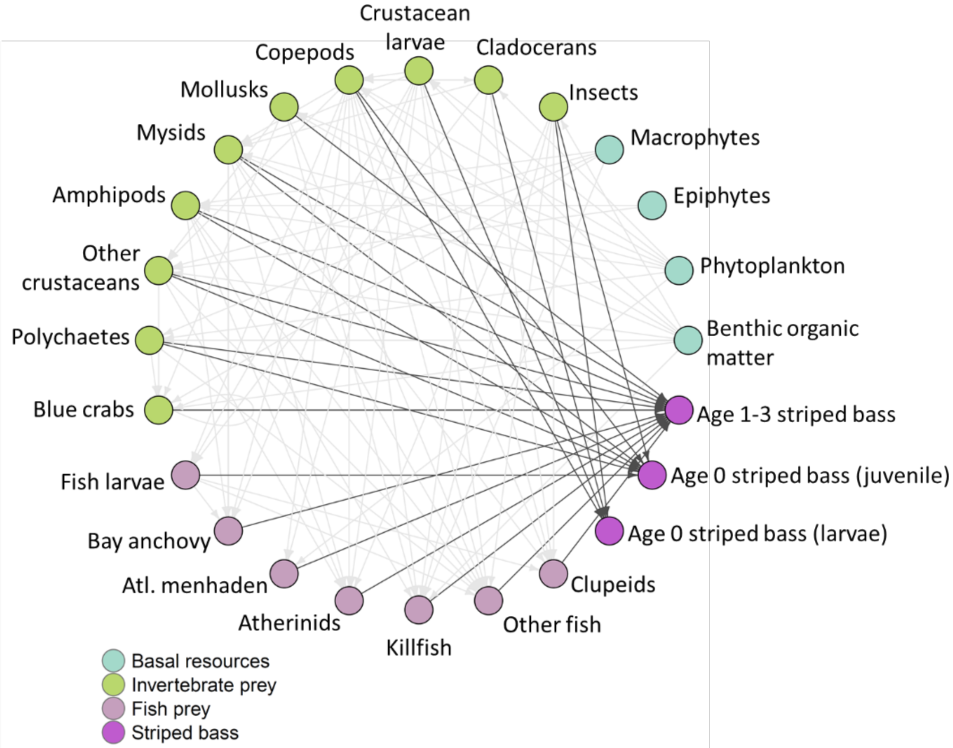


Figure 4-2. This diagram shows basal trophic resources that are also potential pools of microplastics in the environment and a generalized food web of prey items consumed by Striped Bass and organisms consumed by those prey items. Three age classes of Striped Bass (young of year [YOY] larvae, YOY juveniles,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

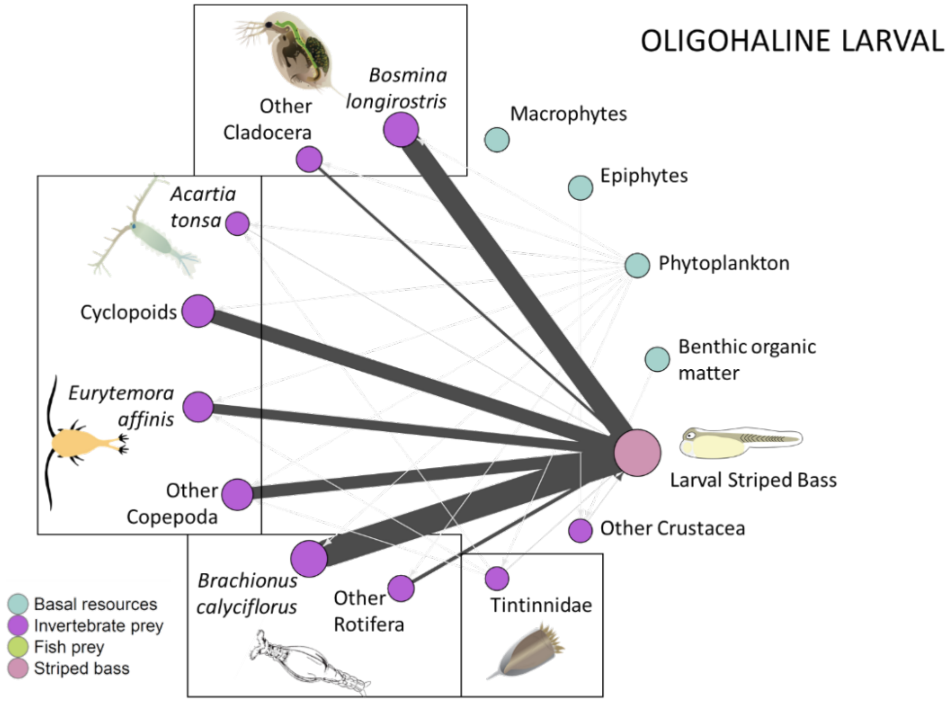


Figure 4-3. Larval Striped Bass food web from oligohaline portion of Potomac River estuary (adapted from Beaven and Mihursky 1980). Dark vectors connecting prey through direct consumption provides biomass-weighted percent contribution to diet for each prey category. Copepods are primarily adult-stages.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

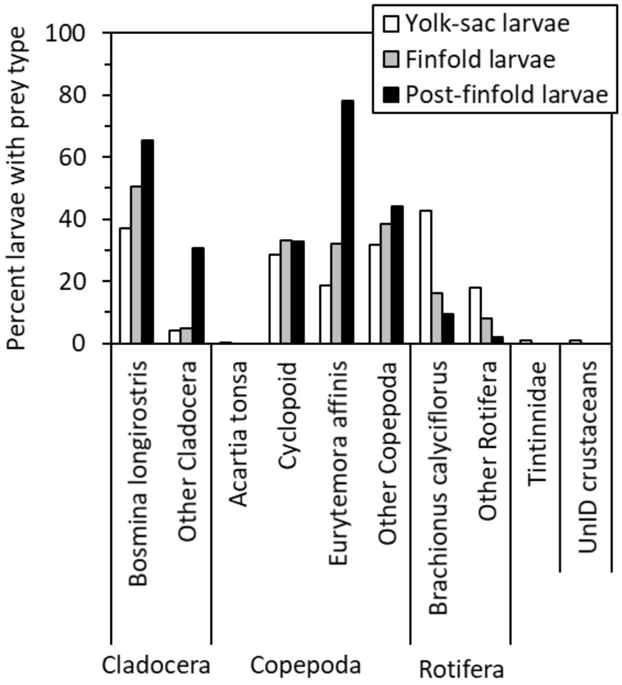


Figure 4-4. Larval Striped Bass diet composition as % frequency of occurrence of prey in fish with material in their stomachs from the oligohaline portion of Potomac River estuary (adapted from Beaven and Mihursky 1980).

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.

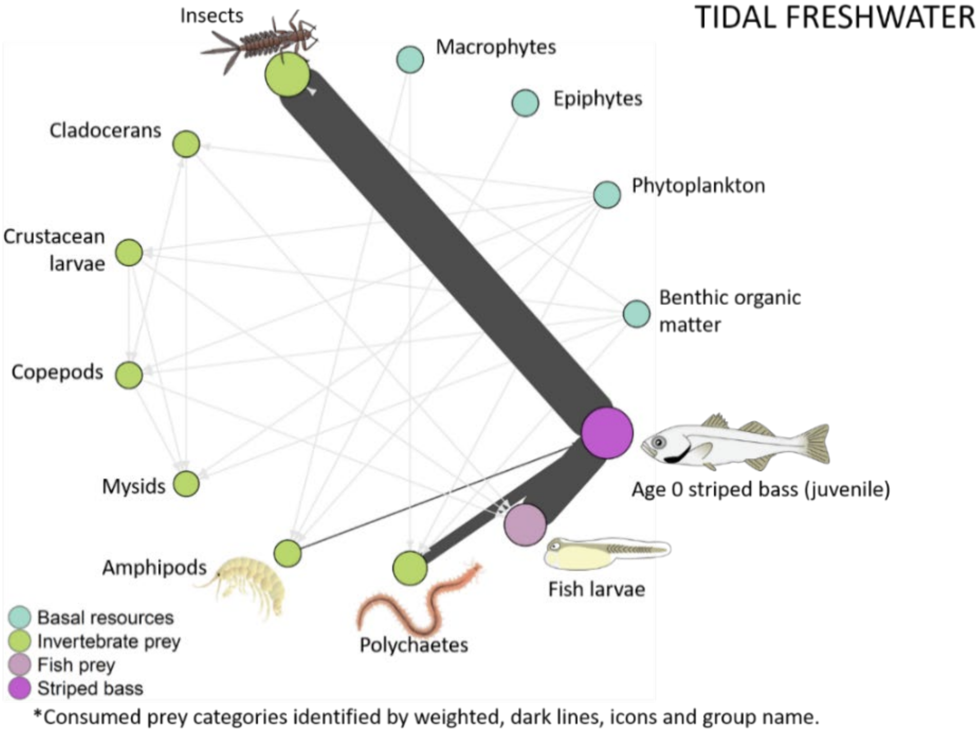


Figure 4-5. Juvenile Striped Bass food web from tidal fresh portion of Potomac River estuary (adapted from Boynton et al. (1981)). Dark vectors connecting prey through direct consumption provides biomass-weighted percent contribution to diet for each prey category. Insects are primarily Diptera larvae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

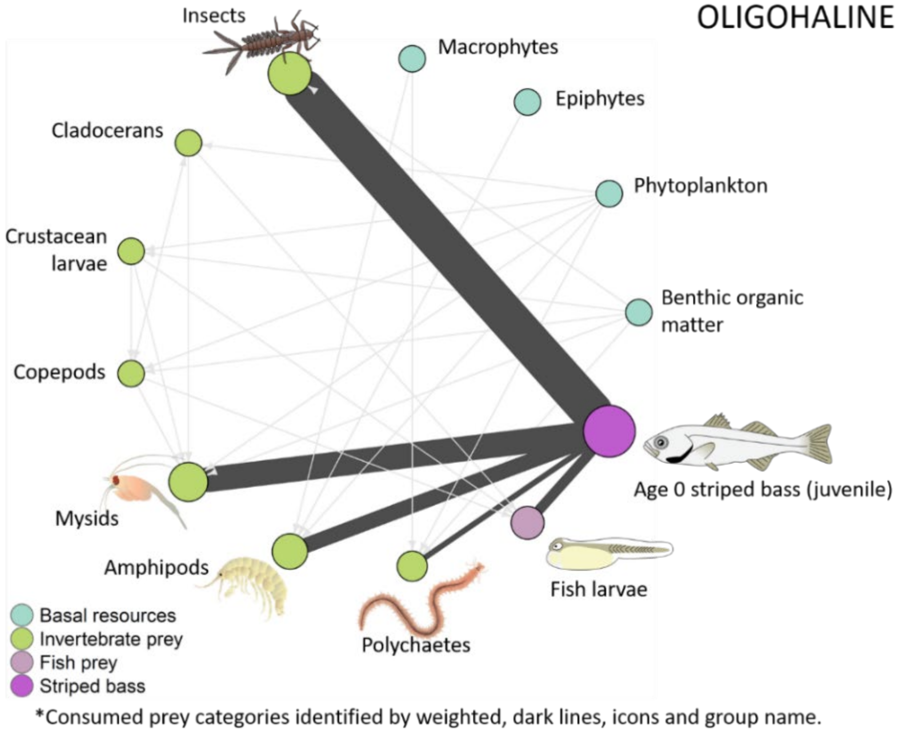


Figure 4-6. Juvenile Striped Bass food web from oligohaline portion of Potomac River estuary (adapted from Boynton et al. (1981)). Dark vectors connecting prey through direct consumption provides biomass-weighted percent contribution to diet for each prey category.

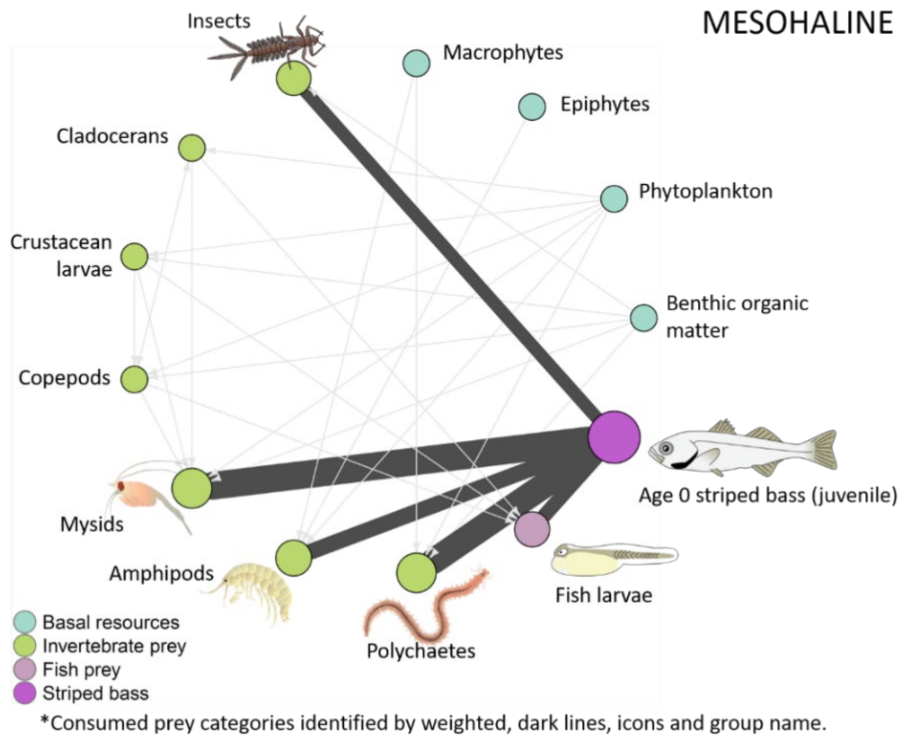


Figure 4-7. Juvenile Striped Bass food web from mesohaline portion of Potomac River estuary (adapted from Boynton et al. (1981)). Dark vectors connecting prey through direct consumption provides biomass-weighted percent contribution to diet for each prey category.

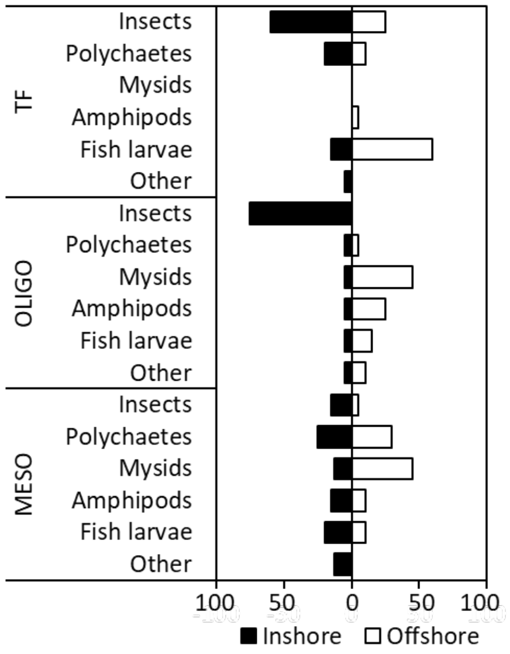


Figure 4-8. Juvenile Striped Bass % diet composition from fish collected at inshore and offshore locations in the tidal fresh (TF), oligohaline (OLIGO), and mesohaline (MESO) portion of Potomac River estuary (adapted from Boynton et al. (1981)).

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.

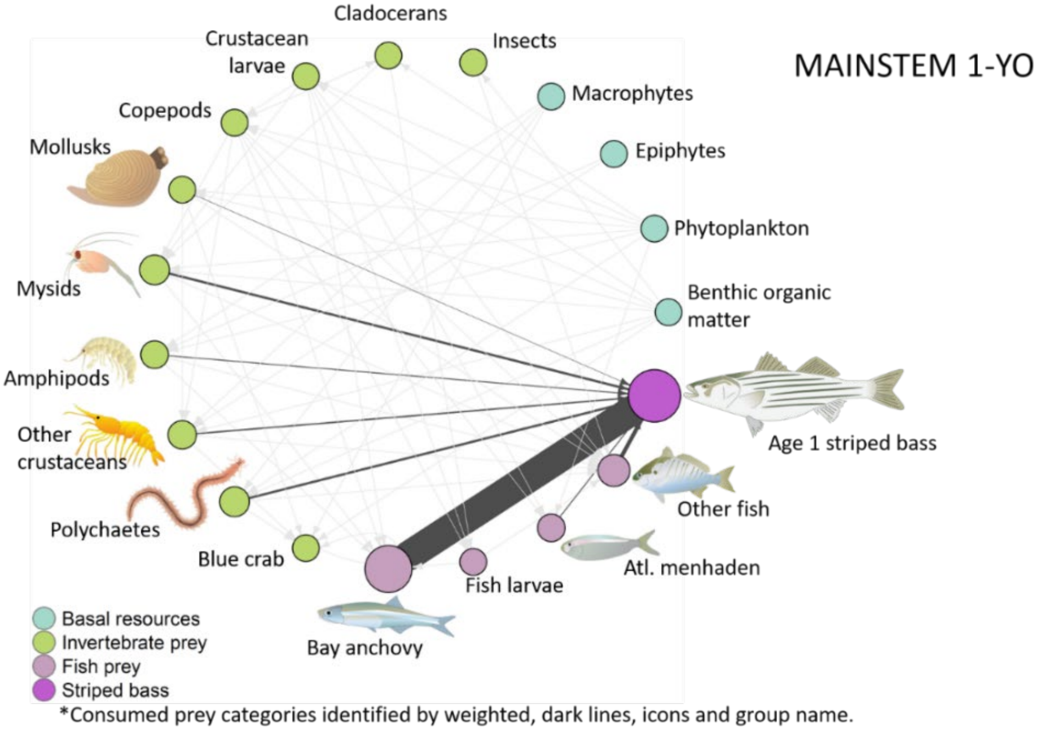


Figure 4-9. Food web with Striped Bass 1YO endpoint. Dark vectors connecting prey through direct consumption provides biomass-weighted percent contribution to diet for each prey category as found in Ihde et al. (2015).

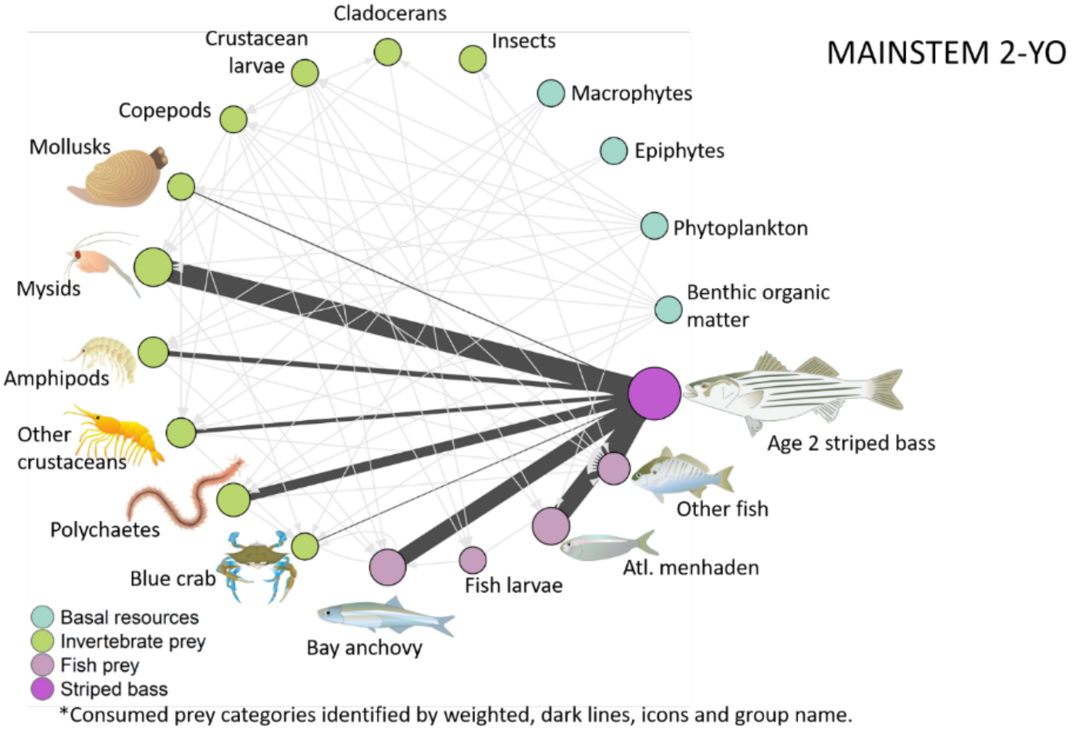


Figure 4-10. Food web with Striped Bass 2YO endpoint. Dark vectors connecting prey through direct consumption provides biomass-weighted percent contribution to diet for each prey category as found in Ihde et al. (2015). Note the change in dominant dietary components from 1YO.

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			🟨

4.1. 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.

4.1.1 Updated ERA model representing semi-quantitative pathways (updated October 2022)

A risk assessment conceptual model is a powerful tool that allows assessors the ability to recognize and highlight important trophic pathways to the biological endpoint of interest. In developing the initial conceptual model (2021) for microplastics accumulation in YOY striped bass in the Potomac River estuary, priority prey items enumerated in a few studies specific to the Potomac were identified, while making inferences from similar diet studies from the Chesapeake Bay. The next step, presented here, in model development is to focus on these taxa as the potential primary vectors of microplastics to YOY striped bass as these taxa make up the bulk of their diet. The model presented earlier in the document was critical to priority prey identification and the diet data within (Boynton et al. 1981, Ihde et al. 2015); however, several members of the Chesapeake Bay Program's Plastic Pollution Action Team (PPAT) have indicated the likelihood of a dietary regime shift due to plankton community changes in the Potomac since the early 1980's, the time of the Boynton study (1981). We therefore sought recent data on YOY striped bass to assess changes in diet and the magnitude of those changes since the 1980's. The

Smithsonian Environmental Research Center (SERC) recently completed (2022) a genetic analysis of striped bass stomach contents (M. Ogburn, pers. comm) finding that the top prey items did not differ markedly from the original Boynton study. This study used YOY Striped Bass sampled for various tributaries in the Bay via the juvenile index survey. Mysid shrimp and amphipods were primary prey, similar to the Boynton study; however, SERC found bay anchovy (*Anchoa mitchilli*) were also a dominant feature of YOY striped bass stomach contents. Bay anchovy were also represented by the Ihde et al. (2015) report that looked at the Chesapeake Bay mainstem.

As noted in the ERA model in an earlier section, there are few studies within the Chesapeake watershed that explicitly evaluated microplastic loading rates in prey taxa; therefore we looked beyond the region and included ecologically similar taxa (e.g., northern anchovy *Engraulis mordax*) as potential surrogates for Potomac River taxa. Here we provide inferential, semi-quantitative data and conceptual models, derived from peer-reviewed publications, that offer insight to potential microplastic loadings for YOY striped bass.

Mysid shrimp

Mysid shrimp (aka opossum shrimp) consist of an order (Mysida) of small crustaceans in the Class Malacostraca (Brusca and Brusca 1990). Mysids are found in virtually all aquatic environments (freshwater, marine, estuarine, riverine, deep sea) and have evolved multiple life history traits exploiting a variety of food sources (Brusca and Brusca 1990). Ecologically, mysid shrimp link multiple trophic pathways as omnivorous consumers, as prey for a wide range of vertebrate and invertebrate predators, and by transferring energy and materials across ecological boundaries through their vertical and horizontal migrations (Lasley-Rasher et al. 2015). As such, mysid shrimp often dominate the diets of several finfish species, including striped bass (Hostens and Mees 1999, Walter III et al. 2003). Recent diet studies for mysids have also indicated consumption of microplastics, though most have focused on laboratory studies (Wang et al. 2017, Lehtiniemi et al. 2018, Wang et al. 2020, Lee et al. 2021). The potential trophic transfer of these particles is examined here in relation to YOY striped bass.

Recent laboratory studies using the mysid *Neomysis japonica* demonstrate the impact of microplastics on larval shrimp swimming, in addition to a reduced ability to feed and reduced ability to evade predation (Wang et al. 2020). Similarly, the mysid *Americamysis bahia* (native to the Chesapeake Bay region) also uptake microplastics and show altered behavior (poor swimming) (Dickens 2021). There may be potential for striped bass to preferentially feed on impaired shrimp as suggested by this work.

There are few studies quantifying the number of particles found in environmentally-sampled mysids. Mesocosm studies (Setälä et al. 2016) mimicked habitat type and environmental conditions to gauge ingestion in littoral mysids exposed to three concentrations of microplastics.

The authors found that mysids ingested particles at the medium (μ=2) and high (μ=38) exposure concentrations. Microplastics have been shown to be transferred to mysids in the planktonic food web (Setälä et al. 2014), so there is little dispute about entrance into the lower trophic food web. However, as a primary food source to striped bass, changes in availability due to toxicity to mysids (Wang et al. 2017) may impact feeding and fitness of striped bass, either through reduction of the prey population or toxicity from ingestion.

Bay anchovy

Bay anchovy are the most abundant fish found in the Chesapeake Bay, and by extension, the Potomac River, by number and biomass (Jung and Houde 2003). As forage for piscivorous species, bay anchovy are a dominant component of the prey taxa. Recent genetic analyses from SERC have confirmed that this includes YOY striped bass. Bay anchovy feed primarily on meso-zooplankton, including copepods and mysid shrimp (Morton 1989, Houde and Zastrow 1991). However, it should be noted that prey items change as bay anchovies grow; Morton (1989, and references within) noted that bay anchovies 35-40mm length fed primarily on rotifers, copepods, and other organic matter and those 60-65 mm length fed primarily on mysid shrimp. This ontogenetic shift in feeding may be an important factor in terms of microplastic ingestion since both copepods and mysid shrimp are known consumers of microplastics (Setälä et al. 2014, Bai et al. 2021).

There is some literature on bay anchovy ingestion of microplastics, in addition to other anchovy (Engraulid) species that can be used to infer trophic pathways of microplastics via bay anchovy in the Potomac River. European anchovy (*Engraulis encrasicolus*), from South African water were found to carry 1.13 items per individual on average, with microplastic particles occurring in 57% of specimens (Bakir et al. 2020). *Anchoa* sp. from the Gulf coast of Texas (Corpus Christi Bay and Laguna Madre) were observed to ingest ~6 particles at length 25mm, but this decreased with growth to ~ 1 at 40mm (Hajovsky 2015). It seems that *Anchoa* sp. select against direct consumption of microplastic particles as they grow, leaving only the juvenile as a significant source for microplastics available through trophic transfer. This is similar to recent findings in the St. Lawrence River, where an ontogenic shift in feeding occurred after transition from larval to juvenile stage (Vanalderweireldt et al. 2019). Note that *Anchoa* sp. keep the same feeding mode throughout their life, namely direct feeding on planktonic organisms (Morton 1989). An increase in target size of prey items may likely occur during ontogeny but is comparatively small. However, this change may be large enough that smaller microplastic pieces are passed through the filter apparatus and therefore are not directly ingested with increasing body size. The implication here is that because YOY striped bass are feeding on the smaller bay anchovies, they are potentially ingesting more of the size-class most likely to carry a heavier body burden of microplastics. A 2020 study in the Mediterranean focusing on the European anchovy (*Engraulis encrasiclus)* found microplastics in 60% of fish sampled, with an average of 1-2 particles per fish, depending on location within the study are (Pennino et al. 2020). Another study of the European anchovy also found ingested microplastics embedded in the hepatic tissue, indicating microplastics are not restricted to digestive tracts only (Collard et al. 2017). In addition, an analysis of Japanese anchovy (*E. japonicus*) from Tokyo Bay showed a mean of 2.3 microplastic particles per fish (Tanaka and Takada 2016) .

A study in Charleston Harbor (a heavily urbanized estuary) evaluated microplastic loadings on bay anchovy specifically and determined mean loadings of 20.7 pieces of microplastic per gram of fish gut weight (Parker 2019). The authors found a significant relationship between fish weight and number of microplastic particles, with a mean 1.92 particles per individual (Parker 2019).

These relevant studies evaluating uptake by bay anchovy or their close relatives suggest that 1-2 microplastic particles are common levels of contamination found within the stomachs. This loading can be used to model microplastic loadings from bay anchovy to YOY striped bass. The finding of microplastic particles in hepatic tissue suggests that estimates of loading based on stomach contents alone may underestimate the potential exposure for a feeding striped bass.

Amphipods

Amphipods (order Amphipoda), like mysids, are another taxon of small organisms within the Class Malacostraca and are typically scavengers or detritovores (Brusca and Brusca 1990). They are found in almost every aquatic environment, including marine, freshwater, deep sea, and several genera are terrestrial (Brusca and Brusca 1990). Over 40 species of amphipods are found throughout the Chesapeake Bay estuary, inhabiting salt marshes, seagrass beds, deep channel, and mud flats (Lippson and Lippson 1997). These provide an enormous prey resource for small juvenile fish, including striped bass, and represent a conduit between detritus, benthic algae, and epibionts and the pelagic food web. Their feeding behavior also makes amphipods a likely vector for microplastics as their targeted food resource is of similar size to commonly-found microplastic particles (Botterell et al. 2022). Similar to mysid shrimp and bay anchovy, there are no current studies on microplastic uptake by amphipods in the Chesapeake Bay watershed, so we have used studies from other regions to infer uptake by amphipods in the Potomac River.

Microplastics have been found in all environments on Earth, including polar and deep sea systems, indicating the ubiquity of plastic pollution (Jones-Williams et al. 2020, Kane et al. 2020). In one recent study, deep sea lysianassoid amphipods averaged 1.0-3.2 particles per individual (Jamieson et al. 2019), suggesting a ready supply of particles at depth. *Gammarus duebeni* (a congener of several species found in the Potomac River and common inhabitant of estuarine systems in the northeastern Atlantic) were found to have an average of 1-30 particles per individual, while 48% had microplastics in the gastrointestinal tract (Mateos-Cárdenas et al. 2020). However these concentrations depended on microplastic density in laboratory experiments, and length of exposure time. Also, the number of particles increased between foregut and mid- to hindgut, but decreased in size due to fragmentation. This finding implies that amphipods are also a mechanism by which microplastic particles increase in number but decrease in size.

Fibers were found in the gut of *Gammarus fossarum* (a European freshwater Gammarid) after 0.5 hours of exposure and egestion was rapid and the digestive tract was empty 16 hours after exposure ended (Blarer and Burkhardt-Holm 2016). Microplastic ingestion varied by exposure concentrations but ranged from 0.93 (lowest concentration) to 7.18 (highest concentration). Plastic particles did not appear to pass through the gut lining. This provides one of the few studies on retention time of microplastics in an organism while it serves as a vector to predators. This study also showed that microplastics had deleterious health effects on the amphipod, which may lead to reduced availability as prey to fish.

Microplastics were found in 100% of *Gammarus setosus* sampled from the Svalbard archipelago with a mean abundance of 72.5 particles per individual (Iannilli et al. 2019). This study was the first to show ingestion of microplastics in this species within the environment and not in a laboratory setting. Furthermore, a recent study in the Arctic showed amphipods had ingested significantly more microplastics than copepods; ingested microplastics were all fragments and the majority were below 50 µm in size; comparison with water samples suggest selectivity of smaller-sized microplastics (Botterell et al. 2022).

Summary

While no studies on loadings of microplastics to priority prey taxa for YOY striped bass exist in the Potomac River or Chesapeake Bay, we can engage in some basic inferences based on research conducted globally with similar taxa, or in the case of bay anchovy, within the taxa but outside the region. The diagram in Figure 1 summarizes reported values of microplastic occurrences reported for these taxa (number of particles per individual) and how these common prey would serve as vectors for microplastics to reach striped bass. However, significant data gaps exist in understanding trophic transfer of microplastics, although recent research has demonstrated mysid shrimp as a strong source of microplastics and associated contaminants to fish (Hasegawa and Nakaoka 2021). Actual consumption by juvenile striped bass requires estimating feeding rates on all taxa, stomach size, and evacuation rates. Many of these

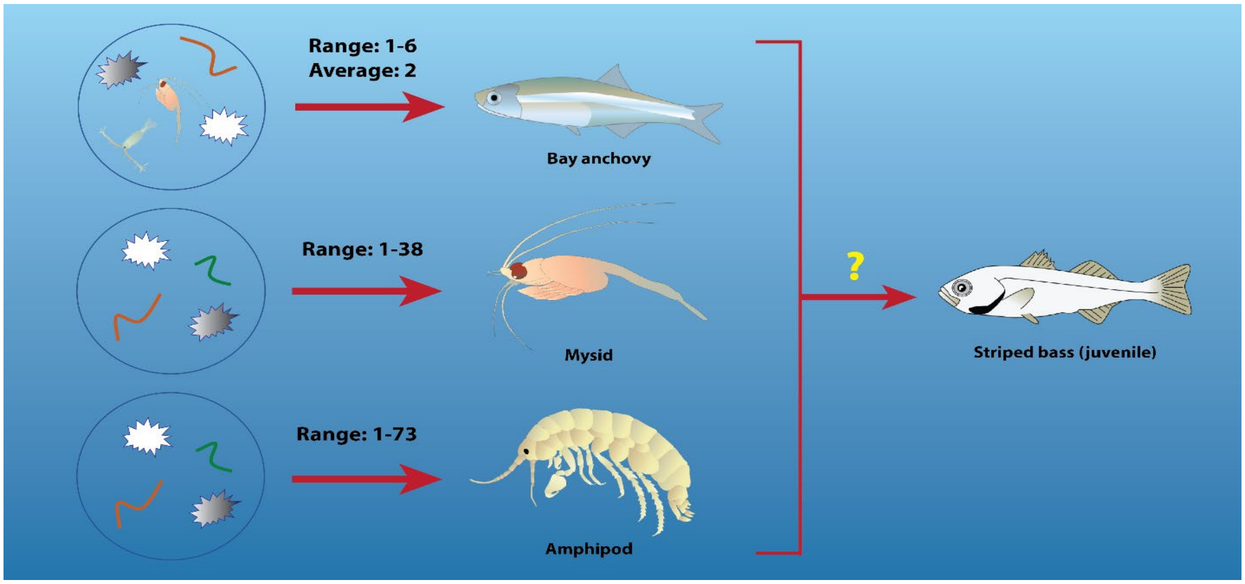


Figure 4-11. Estimated potential quantities of microplastic particles per individual for each of three common taxa (Bay Anchovy, Mysid, Amphipoda) reaching an individual feeding juvenile striped bass. Sources of microplastics for each taxa are displayed on the left, with most of it free-floating plastic particles, with the exception of mysid shrimp in bay anchovy diet (described in the text).

parameters can be estimated in laboratory studies to further quantify the risk. The next steps to further characterize risk to striped bass are to conduct combination field and laboratory studies within the Chesapeake watershed that will 1) elucidate the loadings of microplastics within the prey community, 2) measure uptake of microplastics in these taxa; 3) conduct behavioral studies of prey taxa after microplastic consumption, and 4) assess trophic transfer to YOY striped bass.

4.2. 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. 2014, 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	Freshwater regions

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
			2018, Jaikumar et al. 2019, Woods et al. 2020)	
Copepods	Y	Laboratory; Pacific Ocean	(Cole et al. 2015, Desforges et al. 2015)	
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	Santana et al found little

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**
			2020, Waddell et al. 2020)	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; 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 looked at

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**
			Rodrigues et al. 2019)	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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