Section XX: Updated ERA model representing semi-quantitative pathways

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 for microplastics accumulation in YOY striped bass in the Potomac River estuary, we were able to identify prey items enumerated in a few studies specific to the Potomac, 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. We relied heavily on the current model presented earlier in the document 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 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. 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 Chesapeake Bay mainstem.

As noted in the ERA model in an earlier section, there are few studies within the Chesapeake region 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.

<u>*Bay anchovy*</u>

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

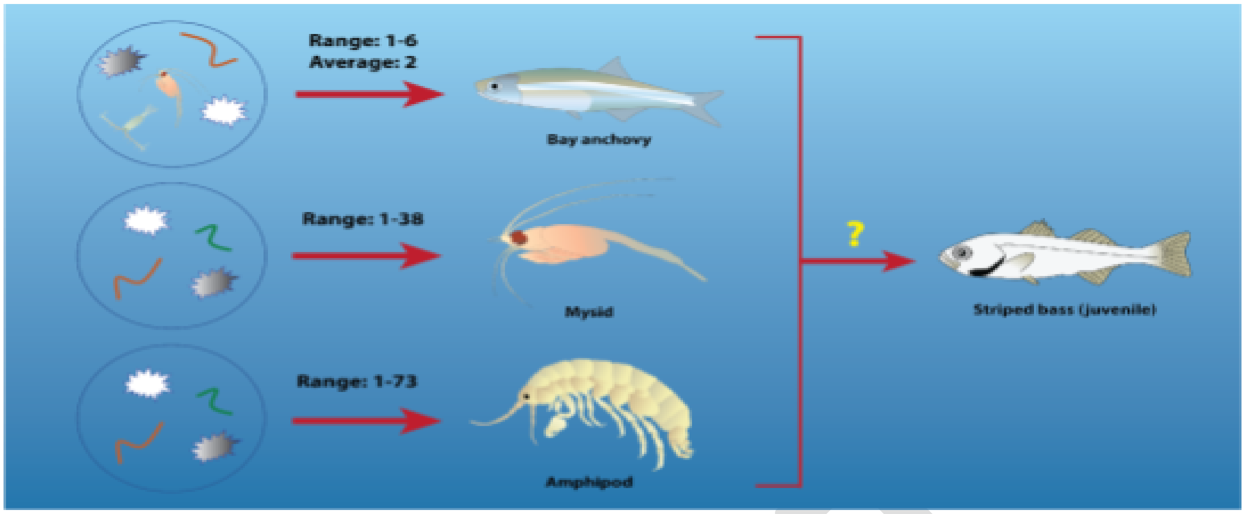

*Figure SEQ Figure * ARABIC 1. Estimated potential quantities of microplastic particles per individual for each of three common taxa (Bay Anchovy, Mysid, Amphipoda) reaching an individual feeding juvenile striped bass. Sources of microplastics for each taxa are displayed on the left, with most of it free-floating plastic particles, with the exception of mysid shrimp in bay anchovy diet (described in the text).*

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 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 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.

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