Discovering Preliminary Spatial and Demographic Relationships in Suspected Microplastic Consumption in Smallmouth Bass in the Monongahela River

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**Introduction**

         Microplastic research has exploded since the early 2000’s in part because of a growing social concern fueled by social media campagins (Petersen & Hubbart, 2020). References to microplastics jumping from just 2 in 2009 to 939 in 2019 (Peterson & Hubbart, 2020). Microplastics are any plastic particles under 5mm in length (Estahbanati & Fahrenfeld, 2016). There is no limit to the size of microplastics and new size categories are currently under debate to incorperate the wide range of sizes they encompass (Barboza et al., 2018; Li et al., 2018). Freshwater research of microplastics is just beginning to gain momentum, previously only making up 4% of the total research body on microplastics (Li et al., 2018). Currently of the 39 articles published about freshwater biota and microplastics, 25 are lab experiments assessing impacts on fish (Yao et al., 2020). Although lab experiments are necessary for comprehending the threat microplastics pose, an understanding of the reality of microplastics within our freshwater systems is clearly needed. Recently microplastics in freshwater systems were labeled an emerging threat due to their determimental physical impact and potential to act as a vector for other contaminents (Grigorakis & Drouillard, 2018).

         The microplastics themselves have the potential to irritate the stomach and intestines, damage tissue and cause feelings of false satiation further impacting the individuals energy intake (Bellasi et al.,2020). They can also accumulate and create blockages. Microplastics have also been shown to accumulate and transport polychlorinated biphenyls (PCBs), persistent organic pollutants (POPs), polybrominated diphenyl ethers (PBDEs), metals, bacteria and other pathogens into aquatic life(Bellasi et al.,2020). The impact of its vector status is debated and is dependent on the characteristics of the plastic in question and the environment. Characteristics influencing the vector efficiency include size, polymer composition, shape, and age. These characteristics are also intrisically tied to their hydrodynamics (Bellasi et al.,2020).

         Using the catch all term microplastics does not convey the complexity of existance under that umbrella. The complexity in microplastics characteristics changes their movement through the watershed affecting their bioavailability. Microplastics in riverine habitats in particular have been greatly ingnored. 80% of microplastic pollution in the ocean comes from terrestrial sources mainly through rivers (Wagner et al., 2014). Previously designated as highways for microplastic pollution in the ocean, the potential for storage of microplastics within the system was greatly ignored. Currently fisheries biologists and management agencies lack an understanding of abundance and exposure to microplastics and their potential to accumulate within an organism. This study aims to determine if there are preliminary spatial patterns that occur with microplastic consumption based on microplastics size in Smallmouth Bass, *Micropterus dolomieu*, in the upper Monongahela River, WV. It also aims to explore preliminary demographic data to understand if portions of the population are more susceptible to plastic accumulation based on their life history. By identifying vulnerable portions of the population as well as locations of increased bioavailablity through consumption this study should aid management in prioritizing further research and mitigation efforts.

         The study aims to answer four questions. Firstly it will determine if there differences between sites on the upper Monongahela River in total count of suspected microplastics in Smallmouth Bass. It is generally accepted that total microplastics increases as one moves from higher order streams to lower order streams and that it will increase past a dense urban center (Kowalski et al., 2016). It is predicted that the results will support this finding.

         Secondly this study will determined if there are differences between sites in the count of suspected microplastics when exaimed by size in Smallmouth Bass. It is suspected that differences may arise when smallmouth bass consumption of suspected microplastics are exaimened on a size basis. Size impacts the gravitational settling of microplastics and its hydro-cycling in the riverscape. Additionally locks and dams have been shown to alter accumulation and settling rates (McNeish et al., 2018). The Monongahelia River has a number of locks and dams that could alter the cycling of plastic within the environment, creating “stop lights” on the highway, retaining certain size classes within the locks making them more bioavailable for ingestion. Generally all size classes will not be equally represented in the various locations. We expect that larger plastics will be more unbiquitous with little difference between sites, while smaller plastics will have site distinct accumulation and more variablity due to differences in point and nonpoint source pollution.

         Thridly this study will examine the population demographics of pooled data and determine if there are significant relationships between the accumulation of total microplastics based on sex, length, weight, condition. It is hypothesizeed that total suspected plastics will be higher in females than males and in shorter individuals than longer ones. Smallmouth bass are zoobenthivores, meaning that benthic macroinvertebrates like crayfish make up a significant porition of their diet throughout their life. Benthic macroinvertebrates have been shown to accumulate microplastics (Zhang ET AL., 2021; Windsor et al., 2019). Additionally accumulation of microplastics in the round goby, *Neogobius melanostomus*, has been attributed to its zoobenthivory (McNeish et al., 2018). Since this sampling was conducted in May, prior to spawning, It is believed that females will have higher accumulations of microplastics because they are potentially consuming more prey in preparation for the high energetic cost of spawning. Length will be used as a proxy for ontogenetic shift and gape width. Shorter individuals are assumed to have smaller gapes that longer individuals would be more heavily reliant on macroinvertebrates. Shorter individuals will accumulate more total suspected microplastic than longer individuals because their diet compsition will have a higher percentage of macroinvertebrates.

         Finally this study will determine if there significant relationships between the accumulation of different microplastic sizes based on sex, length, and weight. It is hypothesized that there will be differences in size accumulation of suspected microplastics between the sexes. It is suspected that differences will be most prevaent in the smaller size ranges of plastics specifically the 212µm & 20µm due to either non descriminatory feeding to meet spawning energy requirements or a switch to prey that require less energy to capture. Smaller size ranges of plastic would bioaccumulate in the macroinvertebrates and crayfish because of the size of their feeding appendages and mouth. Plastics in the 106 µm sieve will be more ubiquitious distributed since plastics in this size range have been related to blockages in lab experiments and may be more likely to be retained in the intestines for longer. Similarly it is believed that there will be similar trends where smaller smallmouth bass will consume more of the smaller size range of plastic than larger smallmouth because of a higher reliance on macroinvertebrates.

**Study Area**

Samples were collected from Opepiska Pool, Morgantown Pool and the Point Marion Pool on the Monongahela River using boat electroshocking in May 2020 (Figure 1).

**Methods**

         Samples were taken back to West Virginia University where length, weight, and sex were recorded. Otliths were removed and cracked and burned to determine age. Viscera were then removed, marked and stored in whirlbags in a freezer at -4°C until analysis. Samples were defrosted in a warm water bath and processed for future diet analyses prior to digestion in a (w/v) 10% KOH solution in a petri dish in an oven at 40°C for 24 hours. Post digestion, samples were diluted and run through a sieve tower containing 500μm, 300μm, 212μm, 106 μm, and 20μm sieves to separate particles based on size. Each sieve was then washed into a Buchner funnel and collected onto a 47mm 11 μm filter paper and dried prior to visual inspection. Strict contamination protocol were followed including washing down surfaces with ethanol as well as UltraPure water andwearing nitrile gloves. A series of blanks were run as controls including a parallel air blank, transfer blank and blanks of UltraPure water. Microplastic counts will be refered to as suscpected microplastic counts since chemical confirmation is current not complete.

         All analyses for site location differences were run using poisson general lineralized models using the glm function in the package MASS in RStudio since total count of suspected microplastics as well as each size class was not normal according to the Shaprio-Wilkes test (alpha<.05) and the data was composed of counts (Table 1). Total counts of suspected microplastic were modeled as a Poisson random variable (yi ∼ Poisson(λi)). Null hypothesis testing was preformed to evaluate differences based on location with total plastics as the response variable and location as the predictor with an alpha value of .05. Since modeling output only reports differences between the reference site and the other two sites, contrasts were also preformed to determine if significant differences existed between the non-reference sites using null hypothesis testing and an alpha value of .05. All contrasts were preformed using the function glht in the package multcomp in R. Data was then further analyzed based on size with each sieve size modeled as a Poisson random variable. Individual poisson models were fit with the count of suspected microplastics in each sieve size as the response and location as a predictor. For example, a poisson model was fit to describe the count of suspected microplastics for the 500μm sieve predicted by location. Null hypothesis significance testing was conducted for each sieve size model with an alpha level of .05. Contrasts were preformed again to determine if significant differences existed using null hypothesis testing between the non-reference sites using an alpha value of .05.

         Prior to analyzing suspected microplastic consumption based on demographics, the demographics of the smallmouth bass from each lock were analyzed per pool. It was assumed that if no site specific differences occured for sex, weight or length then samples could be pooled without concern. Each variable, sex, weight, or length, was then modeled as a response based on the predictor, location. Sex was modeled as a Binomial random variable: yi∼Binomial(n,p) using the glm function in the package MASS in RStudio. Null hypothesis testing was preformed with an alpha value of .05. Weight and length were modeled as Gamma random variables: yi~Gamma(α, β) and modeled using the glm function in the package MASS in RStudio. Null hypothesis testing was preformed with an alpha value of .05. Contrasts were also preformed to determine if significant differences existed between the non-reference sites using null hypothesis testing. No significant differences were found when demographic variables (sex, weight, length or condition), were explained by pool location and so all samples were pooled for the demographic analysis (Table 2).

         All demographic analyses were also run using poisson general lineralized models using the glm function in the package MASS in RStudio. Sex, weight and length were each modeled as a predictor of total suspected microplastics with total count of suspected microplastic modeled as a Poisson random variable response (yi ∼ Poisson(λi)). An additional poisson model was run with total suspected microplastics as the response and an interaction between weight and length as the predictor. Null hypothesis significance testing was conducted on each model with an alpha level of .05. Afterwards, sex, weight and length were each modeled as a predictors and each seive size of plastic was modeled as a response. For example a poisson model was fit to describe the count of suspected microplastics for the 500μm sieve predicted by sex and then a separate poisson model was fit to describe the count of suspected microplastics for the 500μm sieve predicted by weight as well as by length. An additional poisson model was run with total suspected microplastics as the response and an interaction between weight and length as the predictor. Null hypothesis significance testing was conducted on each model with an alpha level of .05.

**Results**

Significant differences were found in the count of total suspected microplastics between Opekiska pool and the Morgantown pool

**Figures and Tables**

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