

Developmental Effects of Microplastic Ingestion on the Tropical House Cricket

Gryllobates sigillatus

By

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Abstract

Microplastic (MP) is a growing concern as an environmental contaminant as it is now considered ubiquitous in our ecosystems. Microplastics have been confirmed to be present in terrestrial environments, yet the majority of studies have focused on the adverse effects of MPs on aquatic biota. I tested the effect of prolonged dietary microplastic exposure on the growth of the tropical house cricket *Gryllobates sigillatus*. Freshly hatched crickets were fed a standard diet containing different concentrations of fluorescent polyethylene MP beads (75-105 μ m) or untreated polyethylene terephthalate microfibers mixed into their diet until adulthood. Weight and body length were measured weekly and MP ingestion was confirmed through fluorescence microscopy and visual inspection of frass. Surprisingly, I found no effect of polyethylene MP ingestion on growth rate or final body size of *G. sigillatus*, yet females experienced a reduction in size and weight at high concentrations of polyethylene terephthalate microfibers in their diet. These results suggest that high concentrations of polyethylene MP beads can be passed through the cricket's gut without a substantial negative effect on their growth and development time, but high concentrations of polyethylene terephthalate microfibers cannot. Although we report the effects of MP ingestion on the growth of *G. sigillatus*, it remains uncertain what threats microplastics pose to other insect life history traits such as fecundity.

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Introduction

Plastic pollution has become a major global environmental issue (Kumar et al., 2021). In 2017, it was estimated that seven billion metric tons of plastic waste had been produced to date with only 9% recycled and 12% incinerated. The remaining 79% was discarded in landfills or the environment (Geyer et al., 2017). A combination of plastic's poor natural degradation and limited waste recovery has attracted the public's attention to its risk to the environment and human health (Campanale et al., 2020; Chae & An, 2018; Ivar Do Sul & Costa, 2014). One major plastic waste that has led to the ubiquity of plastic pollution in our ecosystems is microplastic. Microplastics (MP) are plastic particles that range in size from 1 μ to 5 mm (Frias & Nash, 2019). They are produced as either primary (manufactured micro-sized plastic for commercial or domestic use) or secondary (resulting from breakdown of larger plastics from photolytic/mechanical fragmentation or biological degradation) plastics (Helmberger et al., 2020). Primary microplastics can originate from sources such as pellet spillage from air blasting machines or microbeads in facial cleansers (Auta et al., 2017), whereas secondary microplastics can have numerous sources, such as agricultural plastic films, synthetic rubber debris from tires, electric clothes dryers, or solid landfill/litter (Xu et al., 2020). Some researchers have gone so far as to define the current period as the "Plasticene" due to the ubiquitous prevalence of plastic fragments and their deposition in stratified rock (Haram et al., 2020).

Since we now realize the severity of plastic's integration into our ecosystems, there has been a rapid advancement in their study as environmental contaminants (Wang et al., 2021). Microplastic particles have been detected in wide range of environments such as marine water, freshwater (Rodrigues et al., 2018), agroecosystems (Ng et al., 2018), terrestrial systems (Rillig, 2012), food and drinking water (Almaiman et al., 2021; Smith et al., 2018) in a wide range of

shapes, sizes, polymers, and concentrations (Campanale et al., 2020). The majority of microplastics found in agricultural and natural soils are polyethylene (PE), polystyrene (PS), polypropylene (PP), polyvinyl chloride (PVC), and polyethylene terephthalate (PET) (Xu et al., 2020).

Adverse effects of microplastics on organisms can be categorized as either physical effects (caused by particle shape, size, or concentration) or chemical effects (caused by chemical additives originating from the plastic or absorbed from the surrounding environment). Chemical additives, such as BPA (bisphenol A), are added to plastic products during manufacture to give plastic qualities such as color and transparency, or to improve its resistance to abiotic and biotic degradation by, for example, ozone, temperature, or bacteria (Hahladakis et al., 2018). For example, PVC commonly has phthalates added to create the desired properties for the plastic (e.g., flexibility, transparency). Phthalate molecules are not chemically bound to PVC, but physically interact through van der Waals forces, so they can easily diffuse into their surrounding environment through the air, water, or soil (Henkel et al., 2019; Zhang & Chen, 2014). Since microplastics have hydrophobic properties, their surface can also adsorb and concentrate hydrophobic organic pollutants from the environment, such as organochlorine pesticides or polycyclic aromatic hydrocarbons (Zhang & Chen, 2014). Further, MPs can adsorb heavy metals such as Cd, Zn, Ni, or Pb from their immediate environment (Brennecke et al., 2016). One particularly concerning and recently discovered point of contamination is the capacity for pathogenic bacteria to aggregate and form biofilms on microplastics (Gong et al., 2019). Microplastics have been shown to generate microbial biofilms that differ significantly from those found on natural particles such as wood pellets (Oberbeckmann et al., 2018) or cellulose and glass beads (Ogonowski et al., 2018). Such biofilms have the potential to serve as threats to

animals and humans. For example, *Vibrio parahaemolyticus*, a sea water bacterium that causes gastrointestinal illness in humans, has been identified in biofilms on microplastics made of PE, PP, and PS (Kirstein et al., 2016). Microplastic biofilms were also found to have higher antibiotic and metal resistance gene abundance than the surrounding water, indicating that MPs can provide a substrate for antibiotic and toxic metal-resistant microorganisms (Yang et al., 2019). Taken together, these findings all suggest that MPs can serve as a vector to disperse toxins and pathogens to higher trophic levels, which can not only affect fauna, but may also threaten human health (Kirstein et al., 2016; McCormick et al., 2016; Zettler et al., 2013). The existence of these contaminants within or on MPs can potentially lead to complex interactions of indirect toxicity. Because of this complexity of plastic chemistry, physical form, and propensity for binding other contaminants there is an urgent need to carefully dissect whether and how microplastics pose a threat to biota.

The majority of studies to date have focused on MP effects on marine and freshwater animals (Cole & Galloway, 2015; Gaspar et al., 2018; Li et al., 2020; Lindeque et al., 2020; Mathalon & Hill, 2014; Sussarellu et al., 2016). Several have shown that MPs can be ingested by a variety of marine animals (e.g., invertebrates, sea birds, fish) with a wide range of indirect and indirect toxicities (Anbumani & Kakkar, 2018; Huang et al., 2021; Lusher et al., 2017; McCauley & Bjorndal, 1999; Phillips & Bonner, 2015; Provencher et al., 2010; Rochman et al., 2014; Rummel et al., 2016; Ryan et al., 1988). For example, high-density polyethylene microplastic particles (< 80 µm) have been found in the gills and digestive system of the blue mussel (*Mytilus edulis* L.) when MPs were introduced in their environment. This exposure produced damage to the lysosomal membrane of the mussel digestive system (von Moos et al., 2012). Sussarellu et al. (2016) exposed Pacific oysters (*Crassostrea gigas*) to polystyrene

microbeads for two months during their reproductive cycle and observed significant decreases in oocyte number and diameter as well as sperm velocity. Oyster larval yield and growth was also reduced by ~41% and ~18%, respectively. Haave et al. (2021) investigated concentrations of MP in organs and tissues of coastal birds, mammals, and fish and found 8 of the 13 animals tested had MP in one or several tissues.

Although microplastics have been studied extensively in marine environments, we now acknowledge that they are well dispersed in terrestrial environments as well. This has furthered the need to fill the gap of knowledge in MP's effects on terrestrial ecosystems (Wright et al., 2020). Microplastics are deposited in the soil from water and wind originating from different sources including consumer products, the deterioration of plastic in landfills, wastewater sludge deposited on agricultural soils (Corradini et al., 2019), or car tire wear (Jan Kole et al., 2017). A review by Koutnik et al. (2021) analyzed the concentrations of microplastics in 117 studies and showed that the abundance of MPs in soil varies by up to 8 orders of magnitude between locations tested with concentrations of ~13,000 MP items/kg dry soil found in agricultural and horticultural sites. These results were consistent with the MP concentration variation found in the surrounding surface water. PE, PP, and PET were found in nearly 80% of studies and in highest concentrations closer to urban areas. Soil and sediment samples from areas closer to industrial emission sites were found to have higher concentrations of MPs with as many as 690,000 MP items/kg found in soil near industrial areas in China (Büks & Kaupenjohann, 2020).

We currently lack a full understanding on the harm of microplastic ingestion in terrestrial animals. What has been done has demonstrated varied adverse effects of MPs on digestion, growth, reproduction, and behaviour. For example, a mouse model fed fluorescent polystyrene microparticle for 28 days showed an accumulation of plastics in the liver, gut, and kidneys (Deng

et al., 2017). When the terrestrial snail *Achatina fulica* were fed polyethylene terephthalate microplastic fibres, they drove the snails to reduce their food intake and caused significant damage in their gastrointestinal walls (Song et al., 2019). Another recent study showed that giant snails (*Achatina reticulata*) could ingest and egest irregularly shaped and sized PET MPs at 1 and 10% w/w in feed without inducing any change in mortality and actually showed an increase in growth of the snails (de Felice et al., 2021). Springtails (*Folsomia candida*) exposed to polyethylene microplastics in the soil showed avoidance behaviour towards soils with high concentrations of polyethylene MP (0.5% and 1% w/w in dry soil), reproduction was reduced by 70% and gut microbial communities were significantly altered when exposed to microplastics in soil (Ju et al., 2019). A study by Kwak and An (2021) showed microplastic ingestion caused damage to male earthworm (*Eisenia andrei*) reproductive organs, inhibiting spermatogenesis, but caused negligible damage to female reproductive tissues. While the earthworm *Lubricus terrestris* experienced a reduced growth rate and weight loss when high MP concentrations of 28, 45, and 60% w/w were mixed into the plant litter deposited on the soil surface. These earthworms were also able to transform the plastic and egest them deeper into the uncontaminated soil (Huerta Lwanga et al., 2016), showing that terrestrial organisms can transform plastics in the environment in addition to being negatively impacted by them. The studies presented specifically observed interactions between terrestrial animals and untreated MP particles, demonstrating that a full understanding of the toxicological effects of untreated MPs alone is necessary before we can understand the additive effects from toxins bound to MPs.

Although the studies described above mainly focused on mammalian models and soil dwelling organisms, there is a lack in knowledge on how insects are influenced by MPs. Insects represent the largest class of animals with an estimated 5.5 million species and approximately 1

million already described (Stork, 2018). As the most successful group of animals, they are found in nearly every terrestrial niche (Gaston & Lawton, 1988) and play an integral role in the balance of the world's terrestrial ecosystems through plant pollination, organic decomposition, and as a source of food for other taxa (Basset & Lamarre, 2019). Studies conducted in Europe and North America have suggested that potentially 40% of insect species in temperate countries may become extinct over the next few decades with one of the major drivers being pollution (Sánchez-Bayo & Wyckhuys, 2019). Therefore, it is important to understand whether MP pollution in terrestrial environments can contribute to the decline of insects, and how it may do so. Because they lie on a lower trophic level, insects that ingest MPs may also pose a risk for bioaccumulation to animals in higher trophic levels (Miller et al., 2020). As microplastics are deposited directly on the surface of soils, insects with life history traits that put them in close contact with the soil can be directly exposed to those plastics.

MP deposition by humans into soil is not the only way plastics can reach terrestrial systems; recent studies have also highlighted how insects may impact MP flow through aquatic and terrestrial systems. Freshwater environments have insects which spend their juvenile stages in water, but their adult stages in the terrestrial environment. These include mosquitoes, mayflies, dragonflies, and midges (Dijkstra et al., 2014). *Culex pipiens* have been found to oviposit on water that is contaminated by PS MP (Cuthbert et al., 2019), and MPs have been found to transfer between life stages on these aquatic larval stages to their terrestrial flying adult life stages (Al-Jaibachi et al., 2018, 2019). To my knowledge, most studies on microplastic's effects on terrestrial animals have focused on soil dwelling invertebrates or freshwater larval stage invertebrates, leaving a gap in the current body of knowledge of microplastics effect on different terrestrial organisms. While information on MP's effects on insects is scarce, what we

know from other animals suggests that growth and development are among the most common processes affected by MP ingestion (Horton et al., 2017).

The growth phases of insects are similar to that of other animals with stages of embryonic development, sexual maturation, and reproductive adulthood. Adult body size in insects is dependent on the growth that occurs during the juvenile stage (Texada et al., 2020). Insects of the same species produce individuals of different sizes depending on the environmental conditions, such as malnutrition or disease (Tennessen & Thummel, 2011). Plasticity in body size is thought to be an important mechanism by which fitness can be improved in response to short-term environmental variation. In general, insects that with poor diets grow to be smaller adults (Nijhout & Callier, 2015). The diet of insects varies widely, but a balance of proteins extracted from their respective food source is necessary to fuel the processes that promote growth (Mirth & Riddiford, 2007). In hemimetabolous insects, such as crickets, most body parts grow at the same time as the body and in the same proportion by each larval moult (Thompson, 2019). Growth in insects is tightly regulated by the physiological effects of two hormones, juvenile hormone (JH) and ecdysone. Moulting of insects' exoskeleton is caused by the periodic secretion of ecdysone to induce moulting, whereas JH inhibits insect metamorphosis until larvae have reached an appropriate stage and size. Once insects have reached their final instar, JH secretion lowers and larvae transform into adults either via a pupal stage (holometabolous) or directly (hemimetabolous) (Smykal et al., 2014). Hemimetabolous insects grow in stages called instars where each moult leads to an exponential size increase by about the same factor, making the last larval instar the largest accumulation of mass (Sturm, 2016).

Since quality of diet affects the growth of insects, the presence of MP in the diet may influence the growth rate and final size of insects. Microplastics should not contribute any

nutritional value to the insects, so its presence in food is likely to dilute the energy received from their diet. As well, plastics may cause blockages in the gut of insects, leading to a decrease in the capacity for nutrient intake. If insects can receive adequate nutrition while MP is in their diet, they may also experience a toxicological effect on their growth or metabolic pathways (e.g., through endocrine disruption) due to leaching of the plastic.

I hypothesized that as in earthworms, microplastic consumption is detrimental to insect growth and development. To test this hypothesis, I used the tropical house cricket (*Gryllobates sigillatus*) as a model. *G. sigillatus* are generalist omnivores that spend their entire life in close contact with the soil where they may be exposed to microplastics. *G. sigillatus* are thought to have originated from Southern Asia and now have a worldwide distribution in warmer climates (Smith & Thomas, 1988). Crickets have been used as a model in previous studies to analyze allometric growth in insects (Bertram et al., 2021). The effects of microplastic ingestion on growth may affect certain organs, such that changes in organ growth are more pronounced than changes seen in total body size (Whitman, 2008). As a larger insect with allometric growth, *G. sigillatus* are a prime candidate for studying changes in growth as a consequence of microplastic ingestion.

Crickets were exposed to either fluorescent PE microplastic beads (2.5, 5, or 10% w/w) or PET microfibers (0.25, 0.5 or 1% w/w) mixed into feed. PE and PET are two of the largest contributors to MP waste (Hamid et al., 2018). Ingestion of the MPs was confirmed by visual inspection of frass (Figure 3) while the growth rate was measured through mass and body length measurements. Because parts of the body can grow independent of one another, changes in growth can occur at different organs in the body. If the microplastics are being ingested by the

crickets, I expected that increasing amounts of MP will have negative effects on the number of crickets which survive, or change the rate of gain in body mass or size over developmental time.

Methods

Microplastics

Two types of plastics were tested in this experiment. Fluorescent polyethylene microspheres (Cospheric; 90-106 μ m, 1.10-1.14g/cc mean density, peak emission 445 nm when excited at 407 nm) were used in this experiment as polyethylene is one of the most used polymers in plastic material production (Horton et al., 2017) and the fluorescence allowed me to track plastic ingestion during this experiment and parallel studies. The second type of plastic used was untreated spun polyester (item #777, spun polyester type 54, Testfabrics, Inc., USA). PE and PET are both used commonly in aquatic experiments, allowing direct comparisons to previous studies. The polyester fabric was cut into small pieces with fabric scissors and blended using a Magic Bullet to simulate microfibers produced by domestic dryers and fabric industries (Kapp & Miller, 2020). The fabric was blended in three 5-6 second intervals and stored in an air-tight container until use.

Cricket rearing

Grylloides sigillatus eggs were supplied by Entomo Farms (Norwood, Ontario, Canada) and placed directly into an incubator maintained at \sim 31.5-33 $^{\circ}$ C and \sim 30-40% RH on a 14:10 L:D cycle. Eggs were moistened with water and gently stirred every other day to prevent desiccation and mold growth until emergence. Once emerged, individual crickets were housed in 3 $\frac{1}{4}$ oz plastic solo cups with matching plastic lids and provided a piece of egg carton for shelter along

with food and water *ad libitum* for the duration of the experiment. Because each cricket was reared under the same conditions, any effect of plastic contamination from their housing would be the same among treatment groups. Regardless, plastic solo cups used for cricket housing were washed and dried between feeding to prevent further plastic contamination. Water for crickets was provided in 0.65 mL microcentrifuge tubes with moistened dental cotton covering the opening and the base diet consisted of a proprietary mixture of corn, soybean, herring, and hog meal (Earth's Harvest Organic Cricket Grower).

Microplastic Bead Feeding

Within 24 h of emergence, 96 newly emerged cricket nymphs were selected at random and placed into individual housing. Crickets were divided into four treatment groups with 24 crickets in each group. Each group received either 0 (control), 2.5, 5, or 10% w/w fluorescent blue microplastic beads mixed into the dry feed (Figure 1). Food and water was replaced every 3-4 days for each cricket for the duration of the experiment.

Microplastic Fiber Feeding

A separate experiment was conducted with PET microfibers. Within 24 h of emergence, 96 newly emerged cricket nymphs were selected at random and placed into individual housing. Crickets were divided into four treatment groups with 24 crickets in each group. Each group received either 0 (control), 0.25, 0.5, or 1% w/w PET microfibers mixed into the wet feed. PET fibers were dispersed in water before mixed into feed to ensure an even distribution of fibers. A lowered concentration of microfibers was used to adjust for the difference in density of plastics. Food and water was replaced every 3-4 days for each cricket for the duration of the experiment. Sex of the crickets was determined at the 5-6 week point of the experiment when the ovipositor

was present in the female crickets. Male crickets lack an ovipositor and have pronounced wings on their abdomen that are used to produce their characteristic mating calls.

Cricket Body Measurement

Body mass and size of crickets was measured weekly starting from 24 h of initial cricket nymph hatch. Body mass was measured by placing live crickets into a pre-weighed 2 mL microcentrifuge tube and weighing them with a Sartorius ME-5 microbalance scale (Precision Weighing Balances, MA, USA). To take body size measurements, live crickets were placed into clear, flat 2.5" x 3.5" plastic bags and photographed using a pre-calibrated Stemi 508 trinocular dissecting microscope equipped with a camera (Zeiss, Jena, Germany). A scale bar was photographed with the cricket to accurately perform digital measurements. Digital measurements were then taken from the photos using the image analysis software ImageJ v.148 (National Institutes of Health, Bethesda, MD, U.S.A). Head width (maximal distance between the outer edges of the eyes), pronotum width (maximal distance across the coronal width of the pronotum) and length (maximal distance down the sagittal length of the pronotum), and abdomen length (maximal distance down the sagittal length of the abdomen) (Figure 2) were measured for each individual over the duration of 8 weeks. Crickets were euthanized by freezing after the final measurements.

Data Analysis

Data analysis was conducted in R version 4.0.2 using R Studio version 1.3.1073 (R Core Team, 2014) Data distributions and variance were assessed using Shapiro-Wilk tests and Q-Q plots. Body size and mass measurements were not normally-distributed within all groups (with and without logarithmic transformation). To increase confidence in my findings and test for interactive effects of developmental time and MP concentration on body size and mass, the

effects of MP fiber and bead concentration in feed on the growth of the crickets were analyzed using a linear mixed effects model with the lme() function in R (Bates et al. 2014). Then I analyzed the final week of measurements using a Kruskal-Wallis test with the kruskal.test() function in R. Cricket measurements were separated by sex to account for any differences in size caused by sexual dimorphism in *G. sigillatus* (Archer et al., 2012). The week of each measurement was treated as a fixed effect while each individual crickets measured was treated as a random effect to account for variability in growth per individual. Cricket mortality was not found to differ between treatment groups.

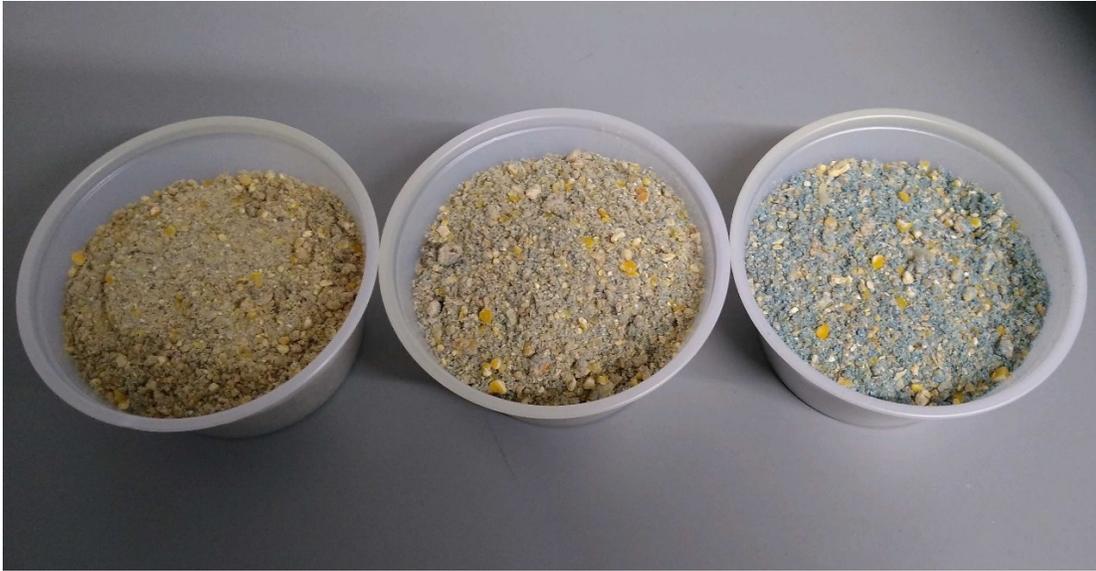


Figure 1: Cricket diet mixed with blue, fluorescent microplastic beads. Concentrations shown from left to right are 2.5, 5 and 10% w/w plastic mixed with base diet.



Figure 2: Example of measurements taken from crickets. Black = abdomen length, white = thorax width, green = thorax length, blue = head width.

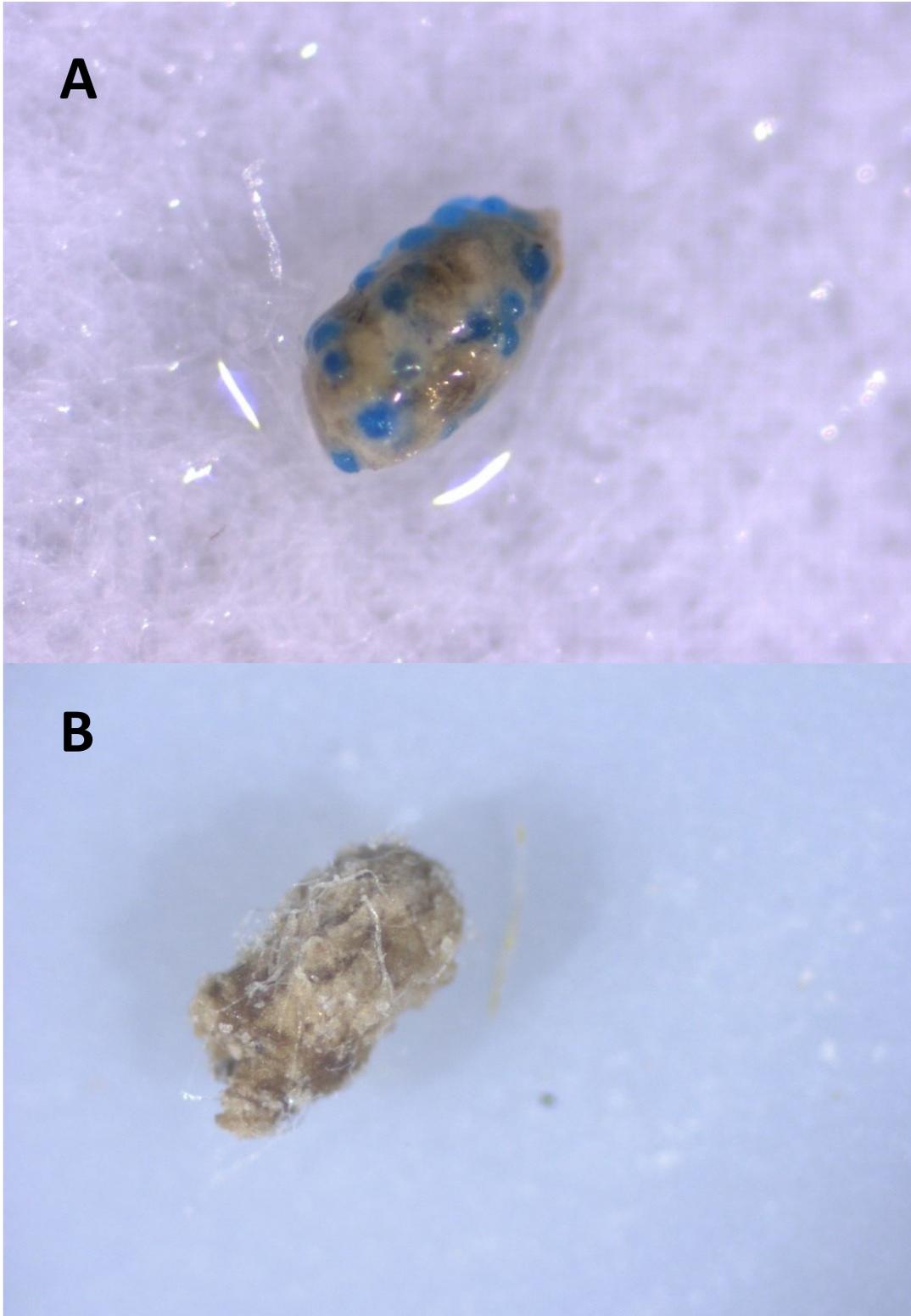


Figure 3: Frass from crickets feeding on 10% w/w fluorescent polyethylene microbeads in feed (A) and frass from crickets feeding on 1% w/w polyethylene terephthalate in feed (B).

Results

Microbead Ingestion

40 Male and 39 female crickets survived for the duration of the experiment while consuming fluorescent PE microplastic beads in their diet. The majority of crickets that did not survive died within the first week of growth. Subsequent loss of crickets during the study were caused by crickets escaping or damage caused during handling (one animal fed the 5% w/w diet and one control). The number of crickets that died during the experiment were not statistically significant from the control (Table 1) Contrary to my predictions, the growth of both males and females did not significantly change in any of the parameters tested for LME (Table 2 & 3). Likewise, the growth of males and females was not significantly different at any concentration of PE MP beads in feed when tested with a Kruskal-Wallis test. Regardless of sex, there was no obvious change in variation in body mass with increasing concentration of PE microplastic beads in their diet (Figure 8).

Microfiber Ingestion

47 male and 37 female crickets lived for the duration of the experiment while consuming PET microfibers in their diet. The majority of crickets that did not survive died within the first or second week of growth. Unlike the microbead mortality, crickets fed 0.5 and 1% microfiber diets tended to die more frequently than those fed the other diets, but too few animals died during the experiment to reliably test this result using a statistical analysis (Table 1). Subsequent loss of crickets during the study were caused by crickets escaping or damage caused during handling (three animals fed 1% w/w diet, one animal fed 0.5% w/w diet). There were no significant interactive effects of age and plastic dose on any measurement with the exception of 0.5% w/w

PET on body mass. Males fed 0.5% w/w PET showed a significant decrease in mass (LME; $t(321) = -2.4, p = 0.0301$) (Table 4). Female crickets showed no difference in growth for all concentrations of microplastics with the exception of the 1% microfiber concentration (Table 5). Females fed 1% PET were significantly smaller and less massive than control crickets. Female abdomen length showed the largest difference in mean size compared to the control (LME; $t(245) = -4.344, p < 0.0001$). Similarly, thorax width was significantly reduced in female crickets by a 1% w/w PET microfiber dose in the diet (LME; $t(245) = -3.4, p = 0.0009$). 1% w/w PET microfibers in diet also significantly reduced the thorax length (LME; $t(245) = -0.05, p = 0.0041$) and head width was significantly smaller in the same group of crickets (LME; $t(245) = -0.04, p = 0.0172$). Overall, higher microplastic doses significantly reduced body mass in female crickets (LME; $t(245) = -12.3, p = 0.0002$).

Following the results of the linear mixed effects model, a Kruskal-Wallis test was performed to validate the significance seen from the non-normal results. The results of the Kruskal-Wallis test showed that final week measurements for female crickets fed 1% w/w PET microfibers were significantly different from the control. High concentrations of PET microfibers in their diet caused a significant decrease in abdomen length (KW: $\chi^2 = 10.4, p = 0.0152$). Thorax length was significantly reduced by the 1% w/w PET microfibers in their diet (KW: $\chi^2 = 11.9, p = 0.0078$). As well, consumption of high concentrations of PET microfibers significantly reduced thorax width (KW: $\chi^2 = 10.4, p = 0.0155$) and head width was significantly smaller when feeding on 1% w/w PET microfibers (KW: $\chi^2 = 12.0, p = 0.0075$). Lastly, female crickets weighed significantly less than the control when fed 1% w/w PET microfibers (KW: $\chi^2 = 7.7, p = 0.0522$). These results help confirm the validity of the results shown from the LME by verifying that measurements of abdomen length, thorax length, thorax width, and mass of female

crickets fed 1% w/w PET microfibers differ significantly from the control at the end of their growth. Regardless of sex, there was no obvious change in variation in body mass with increasing concentration of PET microfibers in their diet (Figure 9), with the exception of the highest concentration of PET microfibers fed to the female crickets. The female crickets fed 1% w/w PET microfibers showed a higher variance than each other PET MP concentration (Figure 9b).

Table 1: Mortality of *Gryllodes sigillatus* during MP feeding experiments. Crickets that died or were lost during measurement were excluded from the experiment.

Microbead Concentration (% w/w)	# Of Crickets at Beginning of Experiment	# Of Crickets that Died During the Experiment	Ratio of Cricket Death	Average Age at Death (week)
0	24	5	0.21	2
2.5	24	5	0.21	2
5	24	6	0.25	2
10	24	4	0.17	2
Microfiber Concentration (% w/w)				
0	24	1	0.04	2
0.25	24	0	0.00	2
0.5	24	5	0.21	2
1	24	4	0.17	2

Table 2: Results of linear mixed effects model for microbead concentration vs. growth of the abdomen, thorax, head, and weight of male *G. sigillatus* with cricket identity as random effects and presented with interactions with the cricket age (week). Results considered statistically significant from the control ($p < 0.05$) are presented in bold.

Coefficient	Weight		Head Width		Thorax Width		Thorax Length		Abdomen Length							
	Estimates	Conf. Int. (95%)	P-Value	Estimates	Conf. Int. (95%)	P-Value	Estimates	Conf. Int. (95%)	P-Value	Estimates	Conf. Int. (95%)	P-Value				
(Intercept)	-43.22	-58.36 – -28.07	<0.001	0.20	0.02 – 0.38	0.032	-0.07	-0.30 – 0.16	0.530	-0.07	-0.22 – 0.08	0.343	-0.70	-1.52 – 0.12	0.093	
Concentration [2.5%]	0.87	-21.75 – 23.49	0.938	-0.05	-0.32 – 0.22	0.713	-0.04	-0.38 – 0.31	0.827	-0.04	-0.26 – 0.18	0.720	-0.19	-1.41 – 1.03	0.751	
Concentration [5%]	-8.54	-32.02 – 14.93	0.465	-0.12	-0.40 – 0.16	0.382	-0.12	-0.48 – 0.23	0.486	-0.13	-0.35 – 0.10	0.268	-0.48	-1.74 – 0.79	0.449	
Concentration [10%]	0.04	-20.70 – 20.77	0.997	0.02	-0.23 – 0.26	0.902	0.05	-0.27 – 0.36	0.767	0.01	-0.19 – 0.20	0.951	0.11	-1.01 – 1.23	0.844	
week	23.83	21.51 – 26.15	<0.001	0.49	0.46 – 0.53	<0.001	0.60	0.56 – 0.64	<0.001	0.34	0.32 – 0.37	<0.001	2.04	1.90 – 2.18	<0.001	
Concentration [2.5%] * week	-1.45	-4.77 – 1.87	0.392	-0.00	-0.06 – 0.05	0.872	-0.01	-0.07 – 0.05	0.661	-0.00	-0.04 – 0.04	0.961	-0.04	-0.25 – 0.16	0.668	
Concentration [5%] * week	2.70	-0.80 – 6.20	0.130	0.03	-0.02 – 0.08	0.286	0.03	-0.04 – 0.09	0.442	0.03	-0.01 – 0.07	0.141	0.10	-0.11 – 0.32	0.343	
Concentration [10%] * week	-0.86	-3.94 – 2.21	0.582	-0.01	-0.05 – 0.04	0.746	-0.02	-0.08 – 0.03	0.409	-0.00	-0.04 – 0.03	0.833	-0.05	-0.24 – 0.14	0.631	
Random Effects																
σ^2	550.14		0.13			0.19				0.08				2.09		
τ_{00}	249.36	cricket	0.00	cricket		0.02	cricket			0.01	cricket			0.42	cricket	
ICC			0.03			0.10				0.06				0.17		
N	40	cricket	40	cricket	40	cricket	40	cricket	40	cricket	40	cricket	40	cricket	40	cricket
Observations	306		306		306		306		306		306		306		306	
Marginal R^2 / Conditional R^2	0.845 / NA		0.906 / 0.909		0.900 / 0.910		0.885 / 0.892		0.898 / 0.915							

Table 3: Results of linear mixed effects model for microbead concentration vs. growth of the abdomen, thorax, head, and weight of female *G. sigillatus* with cricket identity as random effects and presented with interactions with the cricket age (week). Results considered statistically significant from the control ($p < 0.05$) are presented in bold.

Coefficient	Weight			Head Width			Thorax Width			Thorax Length			Abdomen Length		
	Estimates	Conf. Int (95%)	P-Value	Estimates	Conf. Int (95%)	P-Value	Estimates	Conf. Int (95%)	P-Value	Estimates	Conf. Int (95%)	P-Value	Estimates	Conf. Int (95%)	P-Value
(Intercept)	-69.62	-98.72 – -40.52	<0.001	-0.01	-0.27 – 0.25	0.947	-0.30	-0.64 – 0.03	0.074	-0.28	-0.48 – -0.08	0.006	-0.81	-1.89 – 0.27	0.142
Concentration [2.5%]	-3.50	-45.69 – 38.68	0.867	-0.05	-0.43 – 0.32	0.772	-0.10	-0.58 – 0.38	0.673	-0.05	-0.34 – 0.24	0.752	-0.34	-1.91 – 1.22	0.659
Concentration [5%]	5.57	-35.75 – 46.90	0.786	-0.04	-0.41 – 0.33	0.840	-0.05	-0.52 – 0.43	0.845	-0.03	-0.31 – 0.26	0.853	0.09	-1.45 – 1.62	0.911
Concentration [10%]	-4.49	-49.88 – 40.90	0.842	0.03	-0.38 – 0.44	0.872	0.01	-0.51 – 0.53	0.964	-0.01	-0.33 – 0.30	0.930	-0.34	-2.02 – 1.35	0.685
week	34.92	30.40 – 39.45	<0.001	0.56	0.52 – 0.61	<0.001	0.69	0.63 – 0.75	<0.001	0.42	0.38 – 0.46	<0.001	2.12	1.94 – 2.30	<0.001
Concentration [2.5%] * week	2.11	-4.09 – 8.31	0.503	0.03	-0.04 – 0.10	0.375	0.05	-0.03 – 0.13	0.225	0.02	-0.03 – 0.07	0.338	0.09	-0.16 – 0.33	0.495
Concentration [5%] * week	-1.89	-8.03 – 4.25	0.545	0.01	-0.06 – 0.07	0.869	0.01	-0.07 – 0.09	0.844	0.01	-0.04 – 0.05	0.834	-0.15	-0.39 – 0.10	0.236
Concentration [10%] * week	3.57	-3.17 – 10.31	0.298	0.02	-0.06 – 0.09	0.634	0.03	-0.06 – 0.12	0.516	0.03	-0.03 – 0.08	0.347	0.16	-0.11 – 0.43	0.246
Random Effects															
σ^2	1938.81		0.23				0.35			0.13			3.10		
τ_{00}	944.30	cricket	0.03	cricket			0.06	cricket		0.02	cricket		1.02	cricket	
ICC			0.12				0.16			0.16			0.25		
N	39	cricket	39	cricket			39	cricket		39	cricket		39	cricket	
Observations	289		289			289			289			289			
Marginal R ² / Conditional R ²	0.780 / NA		0.874 / 0.889			0.868 / 0.888			0.872 / 0.892			0.856 / 0.892			

Table 4: Results of linear mixed effects model for microfiber concentration vs. growth of the abdomen, thorax, head, and weight of male *G. sigillatus* with cricket identity as random effects and presented with interactions with the cricket age (week). Results considered statistically significant from the control ($p < 0.05$) are presented in bold.

Coefficient	Weight		Head Width		Thorax Width		Thorax Length		Abdomen Length						
	Estimates	Conf. Int (95%)	P-Value	Estimates	Conf. Int (95%)	P-Value	Estimates	Conf. Int (95%)	P-Value	Estimates	Conf. Int (95%)	P-Value			
(Intercept)	-60.62	-75.70 – -45.53	<0.001	0.38	0.27 – 0.49	<0.001	0.22	0.08 – 0.36	0.002	0.09	-0.01 – 0.20	0.090	0.26	-0.20 – 0.71	0.264
Concentration [0.25%]	-5.18	-25.29 – 14.94	0.606	-0.11	-0.26 – 0.04	0.138	-0.13	-0.31 – 0.06	0.170	-0.09	-0.23 – 0.05	0.180	-0.20	-0.81 – 0.40	0.506
Concentration [0.5%]	6.45	-15.96 – 28.85	0.565	-0.06	-0.22 – 0.11	0.496	-0.05	-0.25 – 0.16	0.634	-0.02	-0.18 – 0.13	0.768	-0.08	-0.75 – 0.60	0.817
Concentration [1%]	1.59	-20.95 – 24.13	0.888	-0.05	-0.22 – 0.11	0.531	-0.03	-0.24 – 0.17	0.749	-0.05	-0.20 – 0.11	0.545	0.01	-0.67 – 0.68	0.988
week	36.37	33.77 – 38.96	<0.001	0.33	0.31 – 0.35	<0.001	0.43	0.40 – 0.45	<0.001	0.26	0.24 – 0.28	<0.001	1.50	1.41 – 1.59	<0.001
Concentration [0.25%] * week	0.77	-2.61 – 4.14	0.655	0.01	-0.02 – 0.04	0.427	0.02	-0.02 – 0.05	0.362	0.02	-0.01 – 0.04	0.252	-0.00	-0.12 – 0.11	0.965
Concentration [0.5%] * week	-4.16	-7.92 – -0.40	0.030	-0.00	-0.03 – 0.03	0.944	-0.01	-0.05 – 0.03	0.666	-0.01	-0.04 – 0.02	0.633	-0.07	-0.20 – 0.05	0.259
Concentration [1%] * week	-2.37	-6.22 – 1.48	0.227	0.01	-0.02 – 0.04	0.545	-0.00	-0.04 – 0.04	0.999	0.01	-0.02 – 0.04	0.562	-0.07	-0.20 – 0.06	0.299
Random Effects															
σ^2	803.27		0.06			0.08				0.05				0.91	
τ_{00}	158.88	cricket	0.00	cricket		0.00	cricket			0.00	cricket			0.03	cricket
ICC			0.01			0.04				0.00				0.03	
N	47	cricket	47	cricket		47	cricket			47	cricket			47	cricket
Observations	372		372			372				372				372	
Marginal R^2 / Conditional R^2	0.891 / NA		0.915 / 0.916			0.918 / 0.922				0.875 / 0.875				0.923 / 0.926	

Table 5: Results of linear mixed effects model for microfiber concentration vs. growth of the abdomen, thorax, head, and weight of female *G. sigillatus* with cricket identity as random effects and presented with interactions with the cricket age (week). Results considered statistically significant from the control ($p < 0.05$) are presented in bold.

Coefficient	Weight		Head Width		Thorax Width		Thorax Length		Abdomen Length						
	Estimates	Conf. Int (95%)	P-Value	Estimates	Conf. Int (95%)	P-Value	Estimates	Conf. Int (95%)	P-Value	Estimates	Conf. Int (95%)	P-Value			
(Intercept)	-103.73	-125.92 – -81.54	<0.001	0.12	0.00 – 0.23	0.047	-0.11	-0.25 – 0.04	0.160	-0.17	-0.27 – -0.07	0.001	-0.16	-0.62 – 0.30	0.505
Concentration [0.25%]	-6.74	-42.98 – 29.50	0.708	0.06	-0.13 – 0.25	0.530	0.08	-0.16 – 0.32	0.526	0.02	-0.14 – 0.19	0.790	-0.28	-1.03 – 0.47	0.455
Concentration [0.5%]	3.52	-32.85 – 39.89	0.845	0.10	-0.09 – 0.29	0.276	0.14	-0.10 – 0.39	0.230	0.07	-0.09 – 0.24	0.382	0.02	-0.73 – 0.78	0.949
Concentration [1%]	29.28	-6.11 – 64.68	0.102	0.16	-0.02 – 0.34	0.082	0.25	0.02 – 0.49	0.034	0.15	-0.01 – 0.31	0.060	0.55	-0.18 – 1.29	0.134
week	51.38	47.44 – 55.31	<0.001	0.40	0.37 – 0.42	<0.001	0.51	0.48 – 0.53	<0.001	0.33	0.31 – 0.34	<0.001	1.56	1.47 – 1.64	<0.001
Concentration [0.25%] * week	3.92	-2.29 – 10.14	0.215	0.00	-0.03 – 0.04	0.834	0.00	-0.04 – 0.05	0.913	0.01	-0.02 – 0.04	0.612	0.10	-0.03 – 0.24	0.114
Concentration [0.5%] * week	1.42	-4.88 – 7.71	0.658	0.00	-0.03 – 0.04	0.869	-0.00	-0.05 – 0.04	0.864	0.01	-0.02 – 0.04	0.700	0.04	-0.09 – 0.17	0.537
Concentration [1%] * week	-12.29	-18.59 – -5.98	<0.001	-0.04	-0.08 – -0.01	0.017	-0.07	-0.12 – -0.03	0.001	-0.04	-0.08 – -0.01	0.004	-0.29	-0.42 – -0.16	<0.001
Random Effects															
σ^2	2008.71			0.06			0.10			0.05			0.88		
τ_{00}	303.58	cricket		0.00	cricket		0.01	cricket		0.00	cricket		0.12	cricket	
ICC				0.05			0.08			0.05			0.12		
N	37	cricket		37	cricket		37	cricket		37	cricket		37	cricket	
Observations	286			286			286			286			286		
Marginal R^2 / Conditional R^2	0.871 / NA			0.924 / 0.928			0.924 / 0.930			0.916 / 0.920			0.926 / 0.935		

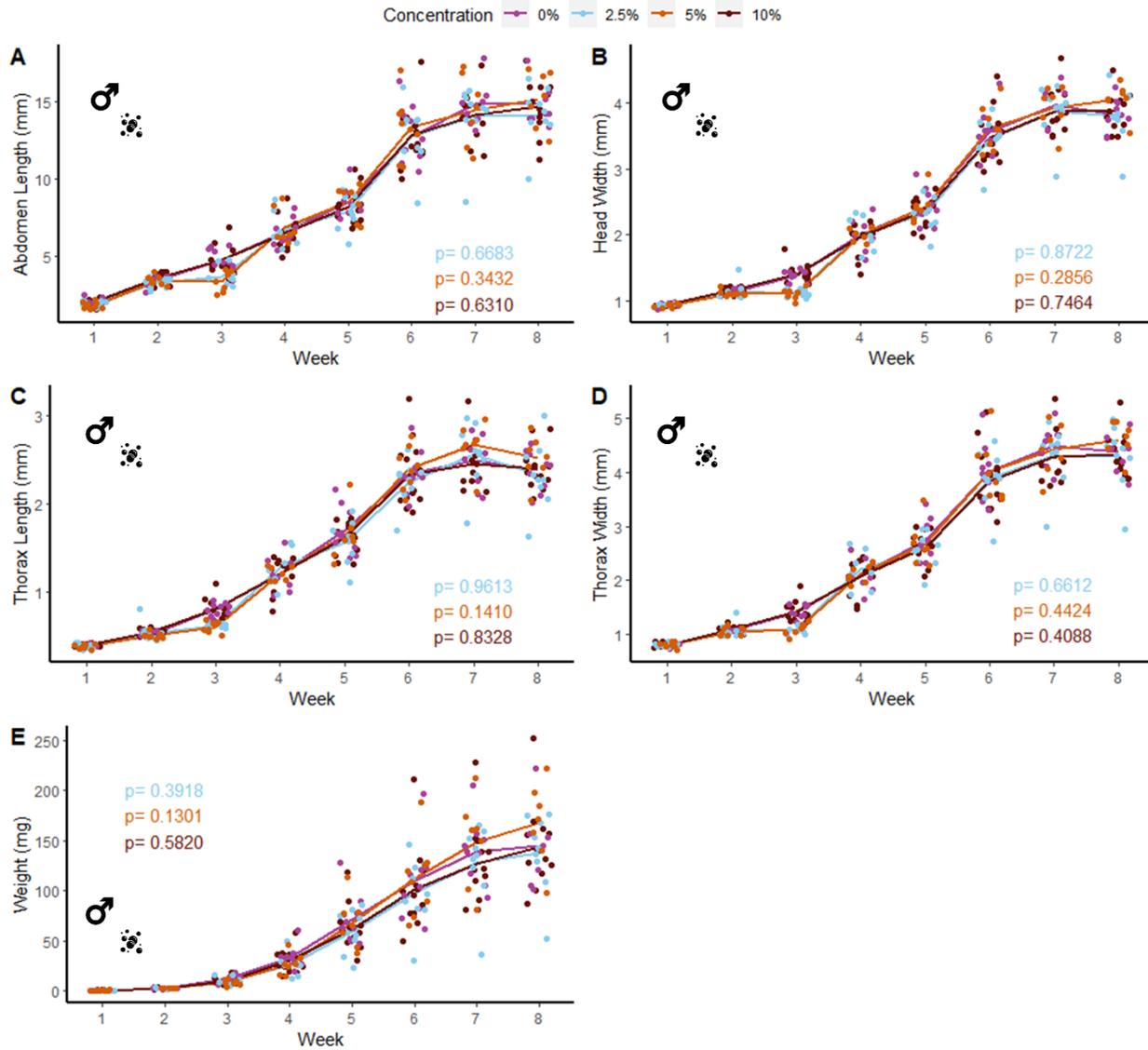


Figure 4: Observations of body size and mass in male *Grylloides sigillatus* fed a diet containing PE microbeads. Initial measurements of crickets were taken at week 1. P-values are presented as an interaction of the concentration of PE microbeads with the week of growth. Each letter indicates a different type of measurement with A = abdomen length, B = head width, C = thorax length, D = thorax width, E = weight.

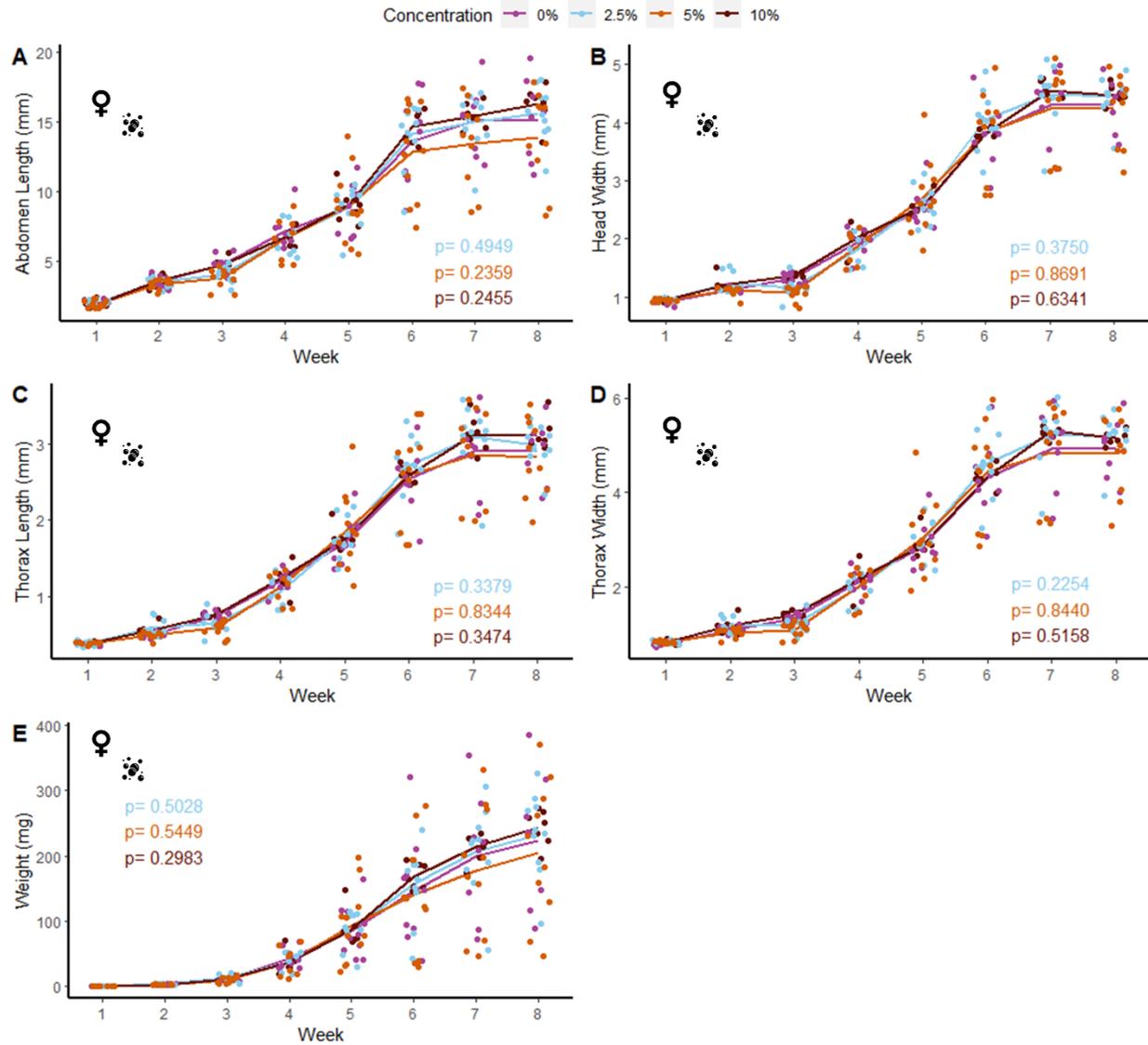


Figure 5: Observations of body size and mass in female *Grylodes sigillatus* fed a diet containing PE microbeads. Initial measurements of crickets were taken at week 1. P-values are presented as an interaction of the concentration of PE beads with the week of growth. Each letter indicates a different type of measurement with A = abdomen length, B = head width, C = thorax length, D = thorax width, E = weight.

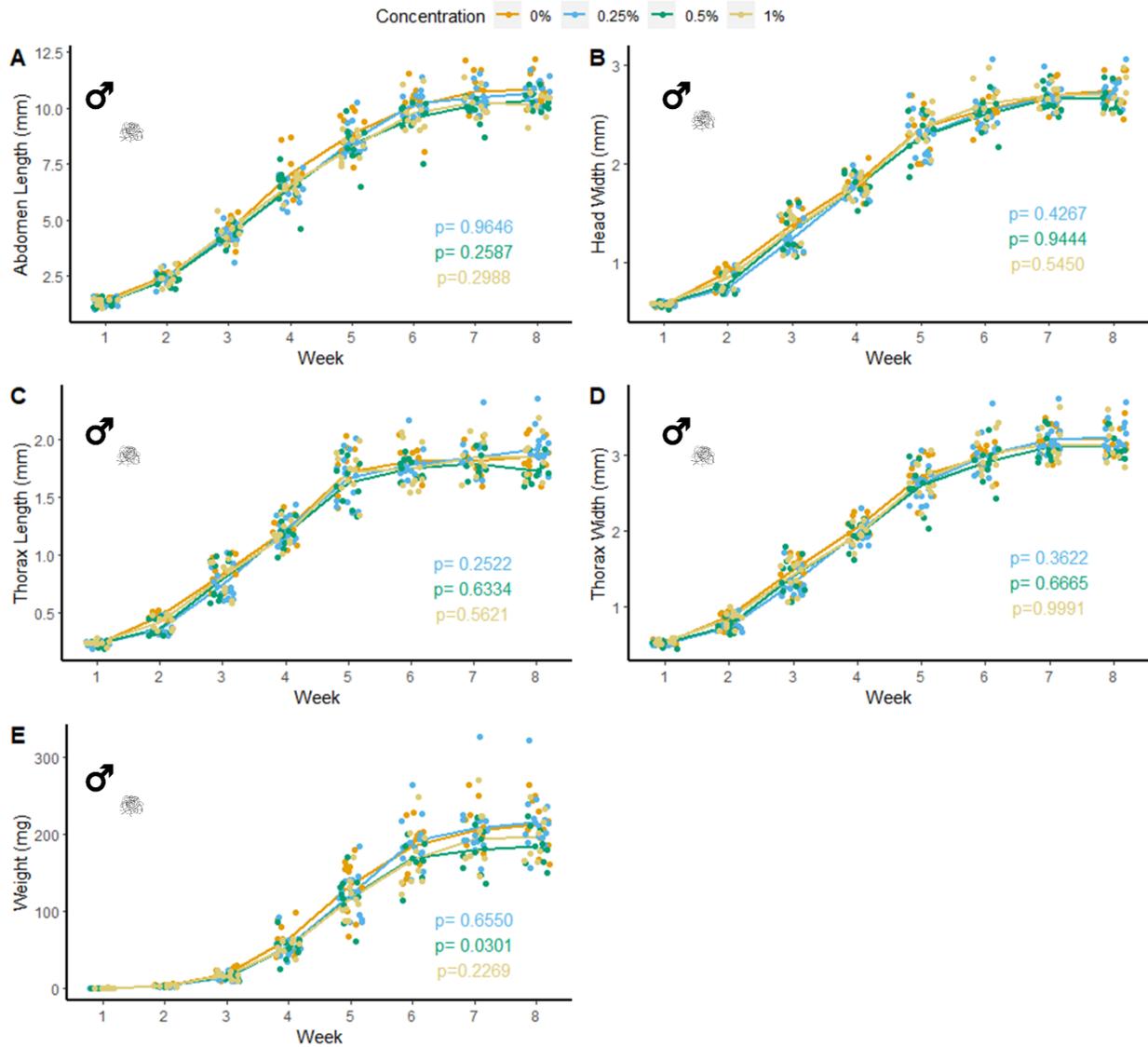


Figure 6: Observations of body size and mass in male *Gryllodes sigillatus* fed a diet containing PET microfibers. Initial measurements of crickets were taken at week 1. P-values are presented as an interaction of the concentration of PET microfiber with the week of growth. Each letter indicates a different type of measurement with A = abdomen length, B = head width, C = thorax length, D = thorax width, E = weight.

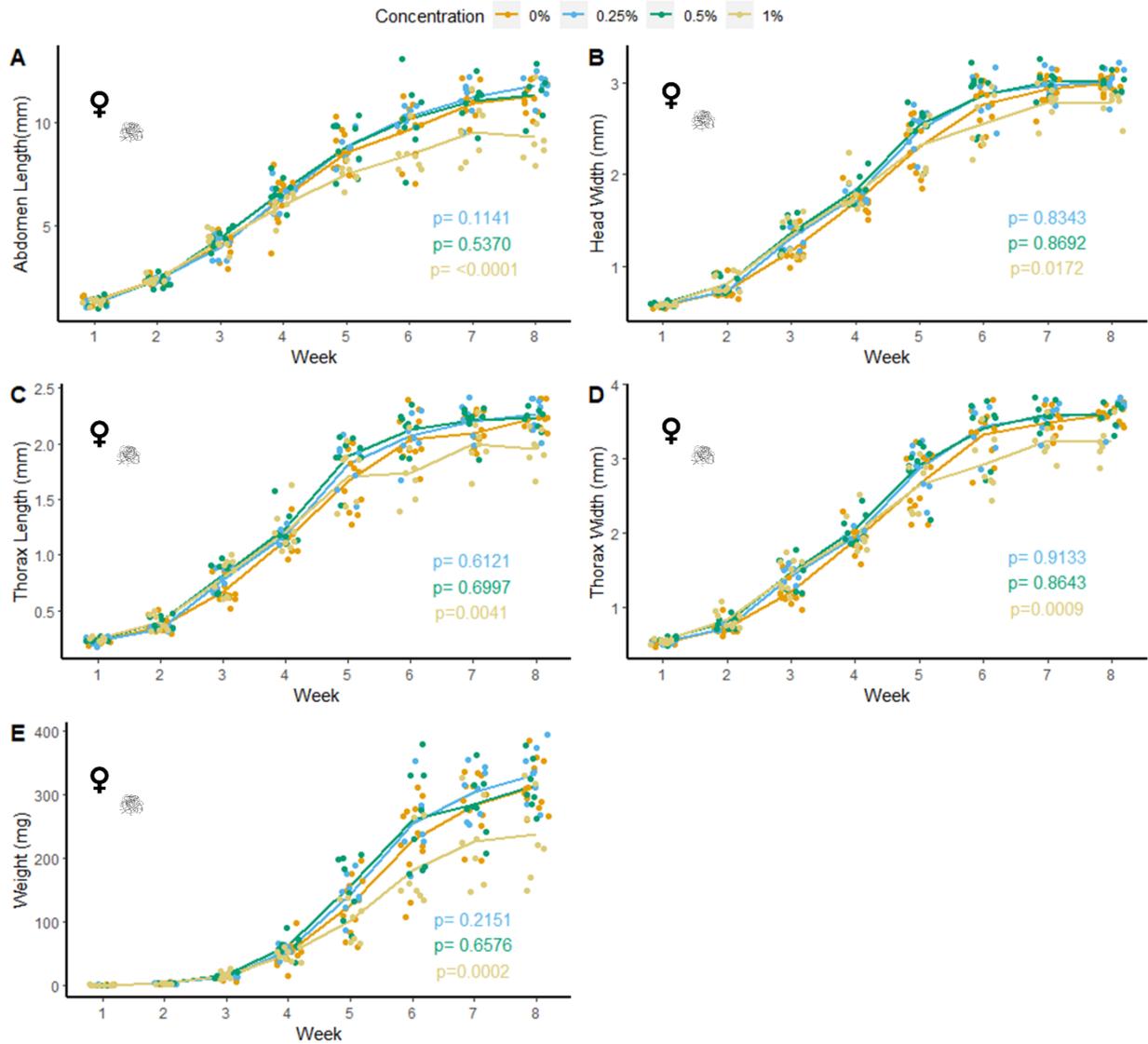


Figure 7: Observations of body size and mass in female *Grylloides sigillatus* fed a diet containing PET microfibers. Initial measurements of crickets were taken 1 week following emergence. P-values are presented as an interaction of the concentration of PET microfiber with the week of growth. Each letter indicates a different type of measurement with A = abdomen length, B = head width, C = thorax length, D = thorax width, E = weight.

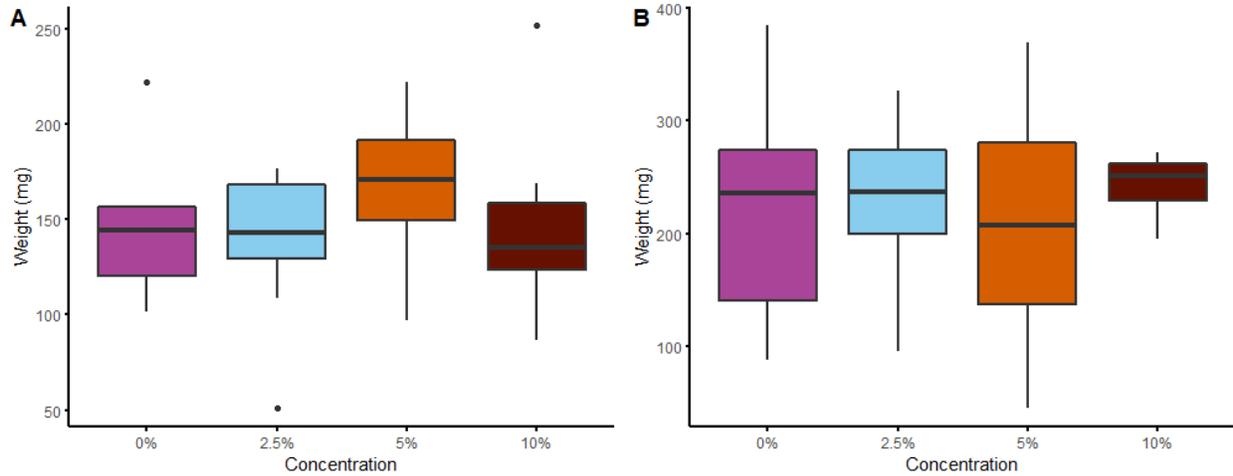


Figure 8: Final mass measurements of male (A) and female (B) *Gryllobates sigillatus* fed PE microbeads. Middle box lines represent the median and the 75th and 25th percentiles correspond to the upper and lower box boundaries, respectively. Whiskers extending past the box represent 1.5 * inter-quartile range. Individual outliers are represented as dots.

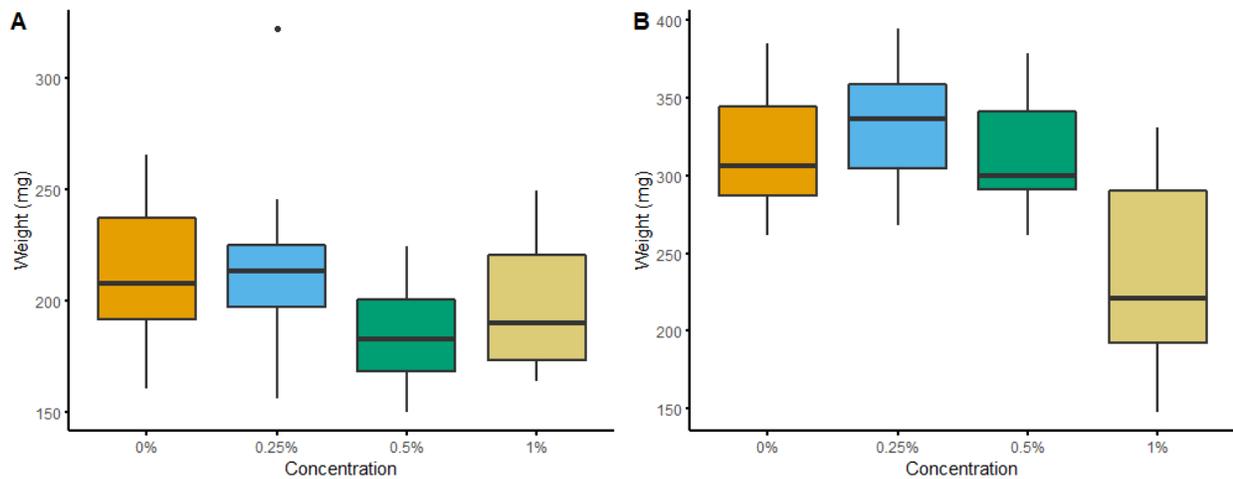


Figure 9: Final mass measurements of male (A) and female (B) *Gryllobates sigillatus* fed PET microfibers. Middle box lines represent the median and the 75th and 25th percentiles correspond to the upper and lower box boundaries, respectively. Whiskers extending past the box represent 1.5 * inter-quartile range. Individual outliers are represented as dots.

Discussion

Microplastic contamination now permeates nearly every environment in the world and has become a major environmental concern (Kumar et al., 2021). The findings presented here to my knowledge are the first to investigate the effects of microplastic ingestion on the growth and development of a hemimetabolous insect. The majority of previous studies that analyzed the toxicological effects of MP on insects focused on the freshwater larval stage of holometabolous insects. As with previous MP studies, the crickets were found to ingest and pass the plastics presented in their food. I found that when both male and female *G. sigillatus* ate high concentrations of PE MP, there was no change in body size, weight, or mortality. When female crickets were fed high concentrations (1% w/w) of PET microfibers, they experienced a significant reduction in total body size and weight throughout development, while males only experienced a reduction in weight, but not any measure of body size.

While a PET fiber diet caused a reduction in size for the females and the weight of males, to my surprise, I found that there was no significant change in the size or weight of either sex when they were fed diets of PE microbeads at high concentrations (up to 10% w/w). This raises questions regarding the mechanisms through which plastics reduce the growth of crickets and the toxicological effects caused by PET or PE. Because the PE microbeads did not cause any change in the size of the crickets, maybe the decrease in body size is caused by the configuration of the plastic (fiber vs. bead). Goldfish (*Carassius auratus*) chewed and often rejected MP fragments and pellets, but passively ingested MP fibers. The microfiber ingestion caused significant damage to the gastrointestinal tract and the liver of the goldfish (Jabeen et al., 2018). Because *G. sigillatus* readily ingested the beads, they may be causing damage to the gut walls allowing passage through the gut barrier without changing the growth trajectory of the crickets. As

another avenue to follow with this study, there should be an investigation into whether MPs are being transferred beyond the gut wall and into different organs of the crickets and whether such persists after acute or chronic MP feeding. Such an effect can occur without any obvious effect on life history traits. For example, PE MPs did not cause significant effects on the growth, survival, or reproduction of the worm, *Eisenia andrei*, but did show evidence of physical damage to the gut (Rodriguez-Seijo et al., 2017).

In the grasshopper, *Melanopus differentialis*, a lower quality diet led to a larger gut size (Yang & Joern, 1994). Changes in gut size may allow for insects to ingest more food at one point in development or increase the efficiency of digestion from a longer retention of food in the gut. As such, the *G. sigillatus* may be adjusting the size of their gut to allow for more food to be retained due to the lack of nutrition in their diet from the plastic. Since plastics have become a substantial environmental concern, there has also been parallel study into the biodegradation of plastic through fungi, bacteria, and insects (Khyade, 2018). A diet of only high-density PE for lesser waxworms (*Achroia grisella*) was not able to provide enough nutrients needed for growth and lowered the survival rate of the waxworms, even though degradation of the plastic was occurring (Kundungal et al., 2019). The lesser waxworm is a pest of honeycomb wax whose ability to digest the PE into polyethylene glycol is thought to stem from their ability to digest beeswax. As the lesser waxworm is unable to retain enough nutrition from plastic, it is very unlikely that the crickets would be receiving any type of nutritional value from ingested PE plastic.

G. sigillatus ate PE microbeads indiscriminately when presented in their food. Perhaps the size of the plastic in the diet was too small for the crickets to filter through. Microplastics in the environment are found in a wide range of sizes and *G. sigillatus* may avoid MP in their diet if

presented in these different sizes. Likewise, if provided an alternative food source simultaneously without the presence of MPs, *G. sigillatus* may also show a preference towards a diet free of plastic. The freshwater shrimp, *Gammarus pulex*, avoided a diet with acrylic microfibers when presented with an alternative diet without microfibers at the same time (Yardy & Callaghan, 2020). This provides an avenue to test feeding preferences in *G. sigillatus* when presented with differing sizes of plastic and diets with or without plastic. *G. sigillatus* may avoid food contaminated with MP if given the choice.

Experiments were performed separately with crickets grown on diets containing PE microbeads or PET microfibers. Crickets (both males and females) reared for the PET feeding experiment grew larger compared to crickets reared for the PE feeding experiment (Figures 8 & 9). This larger growth seen during the PET experimental group may be attributed to the water availability for the crickets. The PET diet had water mixed into the feed to aid in mixing the fibers into the base diet. McCluney and Date (2008) showed shorter periods of water availability reduced the final dry weight and body length for the cricket *Acheta domesticus*, showing that hydration plays an integral role in the growth of *A. domesticus*. While crickets in my experiment were provided water *ad libitum* in the form of water saturated in dental cotton, the additional water mixed with the feed in the PET fed population may have forced the crickets to intake higher amounts of water compared to the PE fed population. This increase in water consumption may have contributed to the larger size seen in the PET fed population.

I observed no differences in the size of crickets fed PET MP, but did detect a significant change in body weight in those fed the 0.5% plastic diet. This effect might reflect a change in body composition of male crickets fed MPs. For example, the males may have suffered a reduction in fat body in order to maintain their body size. The insect fat body is important to

maintain metabolic homeostasis. It functions as storage for excess nutrients and synthesizes lipids, glycogen, and most of the hemolymph proteins and circulating metabolites (Arrese & Soulages, 2010). These macromolecules can be broken down for energy during periods of high energy demand (e.g., flying) or starvation (Beenakkers et al., 1985). When insects experience periods of starvation, they undergo an increase in hemolymph lipid concentration due to an elevation in lipids or diglycerides from the stored fat body (Beenakkers et al., 1985). If *G. sigillatus* are experiencing a decreased nutrient intake due to high concentrations of PET providing no nutritional substance in their diet, then the concentration of lipids in the hemolymph could be measured to verify if reduced nutrient intake is driving the reduction in cricket weight. Alternatively, levels of lipids, or the dry mass of the fat body of crickets fed different concentrations of MP could be compared. The microfibers may be causing a blockage in the gut of the crickets, giving a false satiation, that leads them to consume less food than they require to sustain a typical growth rate. Food was provided *ad libitum* for the crickets; therefore, they may have compensated the lack of nutrition through increased feeding. Amphipods (*Gammarus mucronatus*, *Elasmopus levis*, and *Ampithoe longimana*) presented low-quality diets (lower protein, nitrogen, and total organic carbon) exhibited increased compensatory feeding, but still experienced a decline in growth and fecundity (Cruz-Rivera & Hay, 2000). *G. sigillatus* may compensate for the diluted nutrients in their food by increasing their food intake to maintain key biological functions. To determine if *G. sigillatus* adjust their diet by increasing their food intake, one could measure the quantity of food consumed when it is mixed with plastic.

Although females fed 1% microfibers experienced a decrease in each of the parameters measured, abdomen length showed the most significant reduction in size on average in the female crickets (Figure 7a). This can be indicative of the microfibers causing damage to the

reproductive system in the female *G. sigillatus* by depriving them of the energetic costs for the production of oocytes (Ziegler & van Antwerpen, 2006). In another cricket species, *Gryllus bimaculatus*, fat body mass peaked after adult eclosion then depleted over 48 h following a 16-fold increase in ovary weight (Lorenz & Anand, 2004). The reduced weight seen in female *G. sigillatus* fed 1% w/w microfibers may be an indicator of an insufficient production of fat body during development, which can later decrease the production of oocytes and thereby impact reproductive fitness (Ziegler & van Antwerpen, 2006). Abdomen size has also been directly correlated to reproductive output (Wickman & Karlsson, 1989), suggesting that smaller abdomen size in females can indicate a reduction in number of offspring. Recently, a PhD student in the MacMillan lab observed that abdomen length in *G. sigillatus* females increased when they were fed honeybee royal jelly, and the same animals had 60% more eggs in their ovaries (Muzzatti et al., unpublished observations). Future studies can investigate how production of eggs or viability of offspring are impacted by the same levels of MP ingestion used here. By measuring the number of eggs present in adult female crickets fed high concentrations of PET MP fibers, one could see if the reduction in abdomen size translates to a reduction in reproductive output. In support of this hypothesis, reproduction was inhibited in springtails (*Folsomia candida*). This was measured by the number of juveniles produced in the presence of increasing concentrations of PE MPs in the soil (Ju et al., 2019). Although PE MP ingestion did not lead to any significant decrease in the cricket's abdomen length, there is still potential for further testing to understand whether ingestion of PE beads can similarly affect the reproductive output of crickets. In nematodes (*Caenorhabditis elegans*), continuous exposure to PET microfibers caused a decrease in reproduction rates not only in the maternal generation, but also F2 and F3 generations (Liu et al., 2021) *G. sigillatus* undergo multiple matings and lay multiple batches of eggs throughout

adulthood (Sakaluk et al., 2002). Since *G. sigillatus* readily consume MPs, they can provide an ideal study system for the reproductive or multi-generational effects that MP ingestion may have on insect fitness.

My experiment specifically isolated the effects of untreated PE and PET on the growth of the cricket *G. sigillatus*. The effects of both the microbead and microfiber feeding observed here may not be completely indicative of how MPs ingested outside of a laboratory setting can affect *G. sigillatus*. The toxicity of plastics can be significantly altered through contact with other organic pollutants that can bind both MP particles and fibers (Vázquez & Rahman, 2021). For example, the most common type of litter on Earth, cigarette butts, potentially release about 0.3 million cellulose acetate microfibers a year with toxic leachates absorbed from the cigarette smoke (Belzagui et al., 2021). Because of MPs readiness to leach different contaminants from its environment in addition to the number of additives used in the production of plastic, it is unlikely that these animals will experience exposure to virgin, uncontaminated MPs in the wild (Hahladakis et al., 2018), and yet clearly these plastics alone can have significant effects on growth. Zhang et al. (2020) investigated the damage caused to fruit flies (*Drosophila melanogaster*) by not only MPs, but a combination of MPs and cadmium (Cd), a widely used heavy metal pigment that readily leaches into the environment. The combination of Cd with MP actually intensified the toxicity of the heavy metal, suggesting an increase in the bioavailability for *D. melanogaster* from the plastic. Similar results were seen when MPs were combined with Cd in the soil for the worm *Eisenia foetida* (Zhou et al., 2020). Thus, while MPs alone caused a decrease in growth rate and mortality, the combination of MPs and Cd enhanced the negative impacts of the MP. Since Cd is only one of the toxins that can be readily leached from MPs, characterizing and deeply understanding the toxicity of MP remains a huge challenge.

Regardless, virgin MP can have their own toxicological effects on biota that we must first understand before we can comprehend the combined toxicity it creates in the environment.

Conclusion

A challenge remains in fully assessing the impact of microplastics on terrestrial biota due to the complexity of interactions MPs can have with toxins in its environment. Microplastics have the capability to increase the bioavailability of toxins in their environment, but to understand the extent of their toxicity, we must first understand how unadulterated plastic can harm biota. In this thesis, I aimed to elucidate the potential effects microplastic feeding had on the growth and development of the cricket species *Gryllodes sigillatus* to identify whether toxicological effects of virgin MPs manifest at the organismal level. My results revealed that high concentrations of untreated PET microfibers can significantly alter the growth of female *G. sigillatus*, and the weight, but not size, of males. The mechanisms which cause this change in growth remain to be determined. To my surprise, PE microbead ingestion caused no change in the growth of both male and female crickets, which suggests that they may compensate for reduced nutrient content in the diet by ingesting more food, while receiving no nutritional value from the PE. Questions remain about how plastics can be transformed in the gut of terrestrial insects and whether there are other effects of MP feeding on insect fitness that cannot be observed through body size or weight measurements, such as effects on reproduction or behaviour.

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