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Consumption and degradation of different consumer plastics by mealworms (*Tenebrio molitor*): Effects of plastic type, time, and mealworm origin

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ABSTRACT

The larvae of the Tenebrionidae-family beetles, i.e., mealworms, have been identified as potential consumers of polystyrene (PS). This may have major implications for reduction of PS waste in the environment. However, there is a lack of information on the ability of mealworms to consume other types of plastics beyond PS and quantify their degradation rates. The purpose of this work is to systematically determine the ability of mealworms to consume other sources of commonly used commercial plastics [e.g., polypropylene (PP), polyvinyl chloride (PVC), high density polyethylene (HDPE), and low-density polyethylene (LDPE)] over various periods of time (e.g., 10-, 20-, and 30-days). Additionally, this work addresses the effects of specific plastic type on changes in mealworm mass and viability. Mealworms from three different commercial sources were used. All five plastics were discovered to be consumable by mealworms to various degrees. Overall, PS was consumed most, with average consumption rates of 7.02 (± 0.66), 7.13 (± 2.37), and 8.70 (± 1.38) mg plastic consumed per 100 mealworms per day from three different sources. This was followed by PP, which had average consumption rates of 2.53 (± 0.03) , 4.55 (± 2.97) , and 3.21 (± 1.39) mg plastic consumed per 100 mealworms per day. Overall, 9.11 mg PS were consumed by 100 mealworms per day, and 0.85 mg PS were consumed by 1 g mealworm per day. The other three plastics (PVC, HDPE, and LDPE) were the least favorable for mealworm consumption. Novel findings from this work indicate that mealworms lost mass during the first 10 days of the experimental time period, yet they still consumed plastics. Additionally, larger-sized mealworms displayed a lower viability rate. Overall, this work contributes new understanding for the capabilities of mealworms to degrade several common and environmentally problematic (i.e., recalcitrant) plastics. Additionally, it provides critical information, such as Specific Consumption Rates (SCRs), for several plastics that have not been previously reported.

1. Introduction

Microplastic pollution has been one of the most major environmental issues besides air pollution, water pollution, and soil pollution (Amobonye et al., 2021; Wright et al., 2013; Yu et al., 2022). Wastes from plastics or polymer-based compounds are challenging to degrade, and they can last for hundreds of years, which causes environmental issues. Plastics have been shown to be detrimental to the environment on many levels, including to marine and terrestrial animals, plants, as well as to humans (Macali et al., 2018; McCormick et al., 2014; Nizzetto et al., 2016; Yang et al., 2021). Common plastic products require from 20 to 600 years to naturally decompose (Chamas et al., 2020; Ruiz-Orejón et al., 2016; Thompson et al., 2009). Specifically, in the south ocean of Italy, 78.6% mass of polluted plastic are polypropylene and

polyethylene, and the other 21.4% consists of other 12 plastic-based polymers (Pietrelli et al., 2017). Broadly, it was recorded that 4.8 to 12.7 million metric tons of plastic waste were disposed into the ocean in 2010 worldwide (Jambeck et al., 2015). Worldwide, plastic products such as foam boxes, food containers, straws, and plastic bags are the most common type of wastes and can be found in sewages in developing countries (Browning et al., 2021). With a consumptive human culture and inability to process thin-film plastics, this amount of waste is continually increasing.

Plastic wastes have threatened marine creatures and plastics have been found in the stomachs of dead whale and straw stuck in tortoise nostrils (GESAMP, 2015). Also, coral reefs are 85% more likely to acquire diseases due to microbial pathogens after being in contact with plastic waste (Lamb et al., 2018). Further, plastic nanoparticles from

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face soaps and make-up, which contain nano plastic beads are rinsed down to the sink and go toward the oceans (Rochman et al., 2016). Fish, shrimp, and other animals accidently swallow these particles, and they are later served for human consumption. Because of this condition, human exposure to plastics is chronic and means indirect consumption of plastics remains a human health problem (Campanale et al., 2020; Smith et al., 2018). Besides the effects on humans and marine creatures, plastic wastes buried under grounds and soils can be harmful to aboveground vegetation (Chae and An, 2020; de Souza Machado et al., 2019).

Polystyrene (PS) has been discovered to be consumed by mealworm larvae of darkling beetles of the Tenebrionidae family, the yellow mealworm beetle (Rumbos et al., 2020). The beetle larvae guts contain bacteria which help degrade plastics, specifically PS (Peng et al., 2019, 2020b; Tsochatzis et al., 2021; Yang et al., 2015a, 2015b). Many studies have been performed on PS. For example, Palmer et al. (2022) studied expanded polystyrene (EPS) in foam and acid-treated cup consumption by mealworms and beetles. The consumption ability between mealworms and beetles was well shown in this work, but that of other plastic types (PP, PVC, HDPE, and LDPE) were not reported in this study. Wang and Tang (2022) and Zhong et al. (2022) focused on PS where the transcriptome of the bacteria in mealworm guts was analyzed. Midgut imaging, muscle, and membrane were investigated in pre- and post-plastic digestion, was the focus of Wang and Tang (2022) and Zhong et al. (2022) analyzed the molarity and average size of functional groups to understand the depolymerization of plastic types after being consumed by mealworms. Sugumar et al. (2022) proved that mealworms are able to consume polystyrene foam and Styrofoam. Lastly, Yang et al., 2018 studied in depth the factors that affect mealworm ability to consume PS. While these studies provide timely and critical evidence for PS consumption, we provide additional information related to other plastic types.

We conducted a similar experiment to Yang et al. (2018) where mealworms were tested to consume PS foam. Additionally, we compared consumption of this plastic with four other types of plastics used in commercial and retail applications. Yang et al. (2018) found that viability rates of mealworms were significantly decreased when they were fed with only PS. This was compared to 1:1 mix of PS and bran versus only bran fed mealworms. Experiments with mealworms were carried out for 32-days, during which time the viability of mealworms and plastic consumption were measured. However, it remains unclear the specific time frame over which the viability rate of the mealworm begins to decrease. While the amount of consumed plastic was collected at 32-days, which was beneficial to illustrate total plastic consumption, additional detailed measurements for the rate of plastic degradation over this time period would help to provide a clearer understanding of plastic consumption. Furthermore, viability rates gradually decreased during that period, and not all of the original mealworms continuously consumed plastic. Mealworms used by Yang et al. (2018) from throughout the world indicate similar trends of PS plastic consumption by mealworms. These findings are promising, considering that PS is one of the six types of plastics that compose up to 80% of total plastic production globally (Leal Filho et al., 2019). Based on these findings, a need exists to determine if mealworms might consume other types of plastics in addition to PS.

While polypropylene has received some attention, for example Yang et al. (2021) studied consumption of polypropylene foam by mealworms, the novelty of our work stands out because foams are usually made of polystyrene while polypropylene is widely used to produce container sheet-like bags. Therefore, the study of propylene foam is necessary, and additionally, our work reveals novelty and insights of mealworm consumption ability of PP plastic bags, which is a highly produced type of plastic product. Brandon et al. (2018) is one of the very few works on polyethylene (PE). In this work, the chemical compositions of mealworm guts were analyzed after consuming PS and PE. While PE was used in this study, and the changes in molecular weight of polymer

chains were analyzed, consumption rates and mass of consumed plastic were not discussed. Our work not only includes the rates of consuming PE, viability rates and mealworm mass changes were also included after the effect of consuming a non-nutritious feeding source (i.e., PE). Additionally, our work provides and differentiates two types of polyethylene (low-density polyethylene and high-density polyethylene), thereby providing more detail for this type of plastic. Brandon et al. (2018) consider all commercially produced polyethylene as one type, but HDPE and LDPE are different beyond their density (Salih et al., 2013). The tensile strength, rigidness, elasticity, and melting points are factors that affect mealworm consumption ability (i.e., mandible and gut physical and biological digestion ability) and differentiate between HDPE and LDPE.

While Przemieniecki et al., 2022 tested consumption of PS and PE, PS was in the form of carboard, and PE was in the form of oxo-degradable and regranulated (i.e., recycled) bags. In our work, PS was in foam form and PE, LDPE, and HDPE were in commercial bag form, whose tensile strength and elasticity are different. Additionally, the study from Przemieniecki et al., 2022 was conducted for one time interval (45 days), whereas our study had three different time intervals (10-, 20, and 30-days), thereby allowing us to analyze for changes in consumption rates at shorter time intervals. Also, the focus of Przemieniecki et al., 2022 was to analyze the protein and fatty acid contents within mealworm and identify the bacteria strains in mealworm guts. Our work is distinguished by having essential observations from the comparison between plastic consumption rates and mealworm viability at different time intervals. Lastly, we were able to indicate the size of the mealworm affects their viability (i.e., the bigger the mealworm, the lower the viability) from studying mealworm from three different sources. In this study, the consumption ability of mealworms for five types of plastics was investigated. Four of the plastics were plastic bags used in commercial retail: high-density polyethylene (HDPE), low-density polyethylene (LDPE), Polyvinyl chloride (PVC), and polypropylene (PP). The last type of plastic was polystyrene (PS) in a foam-bead structure. The novel aspects of this study are the multiple types of plastics tested and the experimental conditions: three different time intervals and five different plastic types.

The objective of this work was to determine total consumption of plastics by mealworms over three-time intervals and measure the changes of the mealworm mass in relation to consumption. An additional novel aspect of this work is the time intervals used (e.g., 10-, 20-, and 30-days). Other works (e.g., Yang et al., 2021; Yang et al., 2018; and Peng et al., 2022, tested on only one time period: 35, 32, and 24 days, respectively, to study degradation; however, to identify how plastic consumption rates may change over time, we have included three different time points. Based on the shorter experiments, we are able to measure changes in mealworm mass due to plastic consumption. Findings support a better understanding of which types of plastics could be more biodegradable from mealworm consumption. This study also aims to better understand the effects on mealworm viability rate and weight change when nutritional resources are not available. Mealworms fed only PS in 32 days would result in the reduction of 28.3% of the experimental population numbers compared to only 9.2% for bran and only 2% for the bran and PS mixture (Yang et al., 2018). Plastics do not contain enough nutrition as they lack vitamins, minerals, and protein, which compromises growth and therefore, lead to decrease in mealworm viability. The central hypotheses for this work are that while mealworms can consume PS, there are other plastics which they can also potentially consume. Additionally, mealworms may lose mass while consuming plastic, which is in contrast to the results in Yang et al. (2018). This mass loss, potentially due to the lack of essential nutrition, such as vitamins, minerals, and fibers in plastic, may result in decreased mealworm viability.

2. Material and methods

2.1. Mealworms and plastic types

The study aims to measure changes in both mealworm mass and plastic consumption over three time periods under controlled laboratory conditions. Mealworms (*Tenebrio molitor*) from three different sources were obtained to ensure the experiment employed specimens that were raised in diverse conditions. This broadens the geographical scope in mealworm origin to measure their ability to digest plastic. Mealworms were purchased from three different providers: (1) Basset Cricket Ranch, Visalia, California, United States; (2) DBDPet, Reptile store in Mountainside, New Jersey, United States; (3) Uncle Jim's Worm Farm, Spring Grove, Pennsylvania, United States. These providers are called Source A, Source B, and Source C, respectively. All mealworms were shipped to Texas Tech University, Department of Plant and Soil Science, where the experiments were conducted.

Five types of plastics were identified as important polluters in the environment and relevant to this study: (1) HDPE, (2) LDPE, (3) PP, (4) PVC, and (5) PS. These five plastics were tested in this experiment. Of these five plastics, typically they are produced in the form of plastic bags; therefore, in this study, plastic bags of (1)–(4) were purchased and used as-is. PS bricks were purchased from the Juvale Store. PP bags (2 mil thick) were purchased from the Bonison Store. LDPE bags were purchased from the Infitite Pack Store. HDPE bags were purchased from the Cleanwrap Store. PVC bags were purchased from Jiakai. The five venders sell their products through the Amazon store online. These four types of plastic are widely used in supermarkets, grocery stores, and packaging companies. The last plastic (PS), however, is commonly used as a foam pellet or brick. Additionally, prior studies have used the foam version of PS (Yang et al., 2018). Therefore, PS Styrofoam was also used in this work.

2.2. Experimental setup

For the experimental setup (Fig. 1), each sample was carried out in



Fig. 1. An example of the preliminary experimental setup. For these experiments, 7,500 mealworms were placed into 75, 16-oz. polyethylene terephthalate (PET) storage jars (100 mealworms per jar) in the laboratory under normal room conditions at 23 °C. The jars were randomly placed on a laboratory benchtop under natural lighting conditions. PET is denser and thicker than the plastic sheets and polystyrene (PS) tested for consumption; therefore, the mandibles of mealworms could not penetrate the PET, nor could mealworms crawl out of the jars. Each jar contained one of the five types of plastic tested [PS, polypropylene (PP), polyvinyl chloride (PVC), high density polyethylene (HDPE), or low-density polyethylene (LDPE)].

triplicate. Each triplicate contained 100 mealworms from a particular source. The mass of the 100 mealworms was recorded. The mealworms were placed into jars that contained 2 g of plastic, and each of the five types of plastic were tested. No additional plastic was added to the jars after the experiment began. The jars were randomly arranged on the laboratory bench. Mealworms from Source A, Source B, and Source C were all used for the 30-day experiment. For the shorter interval experiments, which were carried out in order to examine the mass change of the mealworms and plastic consumption over time, only the Source A mealworms were used (i.e., for 10- and 20-day experiments). The 10-, 20-, and 30-day experimental time intervals were carried out separately due to the numerous sample size. The mealworms for the experiments were placed into jars on Feb 16th and Feb 26th (set of 30-day experiments), April 28th and April 30th (sets of 20-day experiments), and May 1st (10-day experiments), 2022. In total, there were 75 jars and 7,500 included for all of the experiments. The 7,500 mealworms were individually counted and weighted over the experimental period. This is the total for the comparison of both mealworm source and time interval experiments Thus, each time interval and experiment consisted of separate batches of mealworms.

To conduct the experiments, the 7,500 mealworms were placed into 75, 16 oz polyethylene terephthalate (PET) storage jars (100 mealworms per jar) and stored indoor under normal room conditions at 23 $^{\circ}$ C. The PET was more dense and thicker than the other plastics tested, thus the mandibles of the mealworms were not capable of biting the PET. Additionally, the mealworms were not able to crawl out of the jars; the jars were not sealed (i.e., open to the atmosphere). Ambient light and relative humidity were employed as mealworm jars were placed on laboratory bench spaces. The total mass of mealworms added each jar was obtained prior to commencing the experiment and after the experimental time interval had concluded. Thus, pre- and postexperimental mealworm masses were obtained. Changes in the mass of mealworms over the duration of the experiment were normalized to (i.e., divided by) the number of live mealworms. At the end of the experimental time interval, the plastic that was not consumed was also obtained and weighed. Thus, pre- and post-experimental masses of the plastics that were also added to the PET jars were obtained to quantify how much plastic the mealworms consumed during the experimental time periods. Fig. 2 visualizes several holes in the plastic sheets and PS foam caused by mealworms. In addition to the masses of the mealworms and plastics, the number of live mealworms were counted to calculate viability rate, as compared to the 100 individuals that were added at the start of the experimental time interval. All masses were recorded using an Ohaus (Parsippany, NJ) analytical balance. The jars were randomly placed on the laboratory bench at the beginning of the time period and remained in those positions for the entire assigned time interval. Jars were not placed near windows, and all jars were contained within 1 m², unobstructed from the ceiling. Thus, there was no environmental variability across the laboratory bench. Data analysis (i.e., averages, standard deviations, and figures) were carried out in Excel.

3. Results and discussion

3.1. Effects of plastic type on mealworm mass change and viability

Source A mealworms were fed for 10-, 20-, and 30-day time intervals with the five different types of plastic, and when considering these mealworms for three different time intervals (Fig. 3, Table 1), several important observations can be seen. Firstly, mealworms lost mass after 10 days of consuming plastic. Specifically, the mealworms lost 4.6 mg, 7.1 mg, 8.0 mg, 9.9 mg, and 8.3 mg for PS, PVC, PP, HDPE, and LDPE, respectively. Importantly, all mass loss for mealworms is reported on a per-mealworm basis. That is, the values of mass loss are calculate from the total mass loss divided by the number of viable mealworms at each specific time interval. The decrease in mealworm mass during the 10-day time interval is different than what occurred during the 20-day

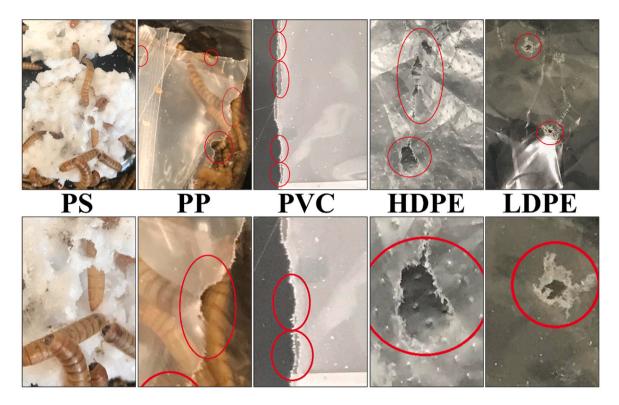


Fig. 2. Images of the different types of plastics used in the experiments. The red markings indicate where mealworms consumed portions of each plastic. Mealworms can be seen actively climbing onto the polystyrene (PS) foam and chewing through it, leaving visible holes. Images of polypropylene (PP) bags indicate where their edges have been actively chewed by the mealworms. Chewed edges of the polyvinyl chloride (PCV) bag by the mealworms and absence of holes at the center of the bag are notable. In the high-density polyethylene (HDPE) bags, several marks and holes caused by the mealworms can be observed. Several holes and scratches in the low-density polyethylene (LDPE) bags can be seen in the image. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

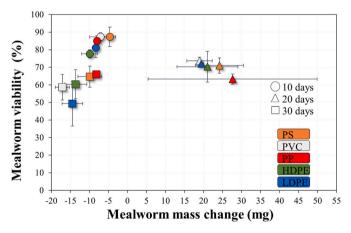


Fig. 3. The change in mealworm mass vs mealworm viability rate from Source A for 10, 20, and 30 days with five types of plastic. PS - polystyrene, PP – polypropylene, HDPE – high-density Polyethylene, LDPE – low-density polypropylene, and PVC – polyvinyl chloride.

period. During the 20-day time interval the mealworms gained mass (24.2 mg, 19.0 mg, 27.7 mg, 21.0 mg, and 19.5 mg for PS, PVC, PP, HDPE, and LDPE fed plastic respectively). Lastly, after the 30-day time interval, the mealworms drastically lost mass (9.9 mg, 17.1 mg, 8.2 mg, 13.6 mg, and 14.5 mg, respectively).

These changes in mealworm mass over the different time periods can be due to several factors. Before the mealworms were used in the experiment, the mealworm growers fed them with cardboard and molded pulp, which contain natural fibers from, such as, sugarcane, bamboo, white wheat, whose nutritional contents are higher than that of raw plastic polymers (Zhang et al., 2022). This difference in nutritional value could explain why the mealworms lost mass during the first 10-day time interval. It is likely that the mealworms lost mass because they were not accustomed to consuming the plastic. After the first 10-day time interval, it appears that the mealworms began to increase in mass during the 20-day time interval (Fig. 3, triangles). This increase in mass could be due to overcoming an initial hesitation to consume the nutritionally depleted plastic matrices and potentially differences in assimilation of mass associated with plastic constituents.

In addition, plastic with covalent pairs of carbon bonds, which all five plastics tested in this experiment contain, are very low in bioreactivity; specifically, bioreactivity refers to the ability of enzymes to degrade synthetic polymers (Chen et al., 2020; Stubbins et al., 2021). It may take time for the intestine microbiome in mealworm guts to become accustomed and digest these plastics, thus delaying the increase in their masses. Additionally, this might explain the increase in mealworm mass after 20-days. However, the lack of nutrition from plastic polymers does not provide enough nutritional value for their metabolism to function adequately (Arrese and Soulages, 2010), and eventually this causes the mealworm mass to decrease.

For the first 10-days' time interval (Fig. 3), the amount of mass lost also appeared to depend on the type of plastic. Specifically, from least mass lost to most mass lost, the effects of each plastic were: PS < PVC < PP < LDPE < HDPE. For 20-day time interval, mealworm mass gained was affected by each plastic in the order: PP > PS > HDPE > LDPE > PVC. Plastics that led mealworms to lose mass are ranked: PP < PS < HDPE < LDPE < PVC for 30-day testing time. This shows that PS and PP appear to be the most compatible types of plastics as feeding sources, among the five plastics tested. Consistently, those two plastics ranked high for mealworms such that they lose the least mass for 10-day

Table 1

Amount of consumed plastic (mg) by mealworm from Source A for three time intervals. (s.d. = standard deviation); PS - polystyrene, PP – polypropylene, HDPE – high-density polyethylene, LDPE – low-density polypropylene, and PVC – polyvinyl chloride; s.d. – standard deviation).

Plastic Type	Time interval	Plastic consumed (mg)	s.d.	Change in mealworm mass (mg)	s.d.	Mealworm viability (%)	s.d.
PS	10 days	108.1	4.3	-4.6	1.6	87.3	5.5
	20 days	139.1	58.3	24.2	6.3	71.0	4.4
	30 days	173.5	21.4	-9.9	3.1	64.7	6.0
PVC	10 days	5.1	1.4	-7.1	2.8	87.3	2.3
	20 days	6.5	1.8	20.2	3.3	73.7	2.1
	30 days	20.9	0.1	-17.1	1.9	58.7	7.2
PP	10 days	17.4	10.2	-8.0	0.7	85.0	3.0
	20 days	12.3	10.5	16.9	22.2	63.3	2.9
	30 days	63.0	1.2	-8.2	0.7	66.0	1.7
HDPE	10 days	8.3	2.6	-9.9	2.1	77.7	2.3
	20 days	7.4	2.2	21.0	8.0	70.3	8.7
	30 days	23.0	4.5	-13.6	2.9	60.3	8.3
LDPE	10 days	5.2	1.7	-8.3	0.9	81.0	5.6
	20 days	2.3	0.4	19.5	0.6	72.0	0.0
	30 days	13.9	0.5	-14.5	2.7	49.3	12.7

interval and 30-day intervals. Additionally, these plastics indicated the highest gained mass in the 20-day interval. Even though PP caused mealworms to lose 72.4% more mass than PS in the 10-day interval, PP had the least negative effect on mealworm mass for 30 days, just in front of PS. In the 20-day interval, PS was only behind PP in increasing mealworm mass (14.4% less mass gained).

Mealworms consuming PVC did not lose much mass during the first 10 days (Fig. 3), and individuals did not gain much mass during the 20day time interval. However, the PVC caused the most mass reduction during the 30-day time interval. This observation can be supported by (Peng et al., 2020a) who observed that PVC caused mealworms to lose mass because of release of organic compounds, such as carboxylic acids. While PVC contains some common functional groups (e.g., carboxyl and hydroxyl) that other plastics also contain, the chloride group in PVC, which takes up to more than half of the chemical mass, is the distinguishing property and might potentially cause this fluctuation in mealworm mass (Harper, 2002; Sugiura et al., 2001). Additionally, PVC was found to not contain high amount of nutrients or supply enough energy for mealworms; thus it is insufficient for their growth (Peng et al., 2020a). This explains the small gain in mass of mealworm during the 20-day interval. Mealworms appear to be less likely to consume polyethylene because both HDPE and LDPE caused the large reduction in mass (e.g., at 10- and 30-days) and less mass gained (e.g., at 20-days) than the other plastics. The digestive ability of the intestinal microbiome in mealworms may explain this observation because polyethylene has been shown to release components that significantly inhibit microbial growth (Zhu et al., 2020). Between two specific manufactured polyethylenes, HDPE and LDPE, mealworms were observed to lose 19.1% less mass from consuming LDPE than HDPE at the 10-day interval. However, mealworms gained (8.1%) more mass for 20-days and lost (5.9%) less mass for 30-days from consuming HDPE than LDPE. The mealworm digestive biosystem seems to be keener to digest HDPE than LDPE because HDPE has less branching, which makes it less bioresistant; plastics with more carbon-carbon bonds (i.e., more branching) tend to be more bioresistant (not reactive biologically) (Cnudde et al., 2018; Stubbins et al., 2021).

The viability rates were observed to decrease consistently throughout time: 77.7%–87.3% (9.6% range) for 10 days, 73.7%–63.3% (10.4% range) for 20 days, and 49.3%–66% (16.7% range) for 30 days. Because of the relatively small range in mealworm viability, the type of plastic did not appear to have as large an effect on the mealworms as time interval. This result partially agrees with another study which found no significant different in development stages (78.8%–73.9%; 4.9% range) of mealworms from feeding different food sources (Liu et al., 2020). A noticeable point is that the higher the viability rate, the

less the mealworm mass changed (either increase or decrease). The specific biological explanation for this observation remains unclear. Mealworm metabolism might be at its most stable state when the larvae mass does not significantly change during its lifetime.

3.2. Effects of plastic type on plastic consumption

The amount of plastic that mealworms consumed was 108.1, 5.1, 17.4, 8.3, and 5.2 mg for the 10-day interval; 139.1, 6.5, 12.3, 7.4, and 2.3 mg for 20-day interval; and 173.5, 20.9, 63.0, 23.0, and 13.9 mg for 30-day interval for PS, PVC, PP, HDPE, and LDPE, respectively (Fig. 3). Mealworm consumption of PS steadily increase as the set time interval increased ($108.1 \rightarrow 139.1 \rightarrow 173.5$ mg). Also, the amount of consumed PS greatly surpassed that of the other four plastics. This positive trend is in agreement with the analysis of the change in mealworm mass (Section 3.1), which also showed how favorable mealworms consume the PS plastic. However, the other four plastic types (PP, PVC, HDPE, and LDPE) do not show this same consistency, and the amount of plastic consumption for 10- and 20-day intervals were nearly equal (Fig. 3).

While PP was consumed at least 2.7 times less (on a mass basis) than PS, PP is the second highest favorable plastic feeding source for mealworms in this study (red square, 30-days, Fig. 4). This also matches with the compatibility of the mealworm plastic consumption that affected

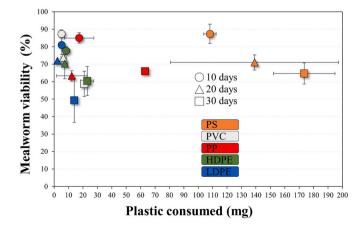


Fig. 4. Mass of plastic consumed vs mealworm viability rate from Source A for 10, 20, and 30 days with five types of plastic. PS - polystyrene, PP - polypropylene, HDPE - high-density Polyethylene, LDPE - low-density polypropylene, and PVC - polyvinyl chloride. Standard deviation values for the masses of plastics consumed are given in Tables 1 and 2

their mass change (discussed above in Fig. 3). The amount of plastic consumed is similar for PVC and HDPE, which are slightly higher than that of LDPE. Again, mealworms appear to favor digesting HDPE more than LDPE, which matches the observation of mealworm mass change versus mealworm viability shows above in Fig. 3. While PP appears to be the second most favorable plastic among the five experimented plastics, it (along with PVC, HDPE, and LDPE) appears to be an insalubrious food for mealworms because they consume nearly the same amount of each of those plastics over the 10- and 20-day intervals. Only at the 30-day interval does the consumption of PP increase substantially. This also illustrates both the limitations and endurance of mealworms when they are fed with unfavorable food.

An inverse relationship is observed with plastic consumption and viability rate (Fig. 4, Table 1): the lower the plastic consumption, the higher the viability rate. This might lead to the conclusion that higher consumption of plastic causes a decrease in mealworm viability. However, this conclusion would be shortsighted and not accurate because of additional variables (i.e., time), which ultimately impacts mealworm viability rates. Mealworm numbers may gradually decrease because stronger larvae can withstand the nutrient-deficient living conditions, while the weaker larvae cannot. The surviving larvae continued to consume plastic, and this results in an increase in the mass of consumed plastic over time. The relationship between plastic consumption and viability rate is, therefore, indirectly related to each other.

In addition to mealworm viability vs the amount of plastic consumed (Fig. 4), the amount of plastic consumed can also be plotted against the change in mealworm mass (Fig. 5, Table 1) to better clarify how those two variables are related. For the 10-day interval, because the time interval was shorter, the mealworm change in mass was not affected much. Therefore, their mass change affected by the five plastics is not so varied (e.g., 4.6-9.9 mg, 5.3 mg in range), where 4.6 mg is from PS consumption, which appeared to cause mealworms to lose the least mass. Despite the similarity in mass change, the amount of PS consumed is significantly higher (108.1 mg, orange circle) than the other four plastics. This shows that the effect on mealworm mass change is mostly independent from the plastic consumption. Although PS and PP was identified as the most gut-compatible plastics for mealworm among five experimented plastics (Section 3.1), mealworms did not show favor to consume PP as equal as PS in the 10-day interval (17.4 mg, red circle compared to 108.1 mg). Nonetheless, PP is still more favorable to mealworms than HDPE, LDPE and PVC as its consumption is more than two times higher (17.4 mg versus 8.3, 5.2, and 5.1 mg respectively).

The 20-day interval shows similar observations as the 10-day interval such that the range of mealworm mass change is low (5.2 mg) and PS is significantly consumed (139.1 mg) compared to other four plastics

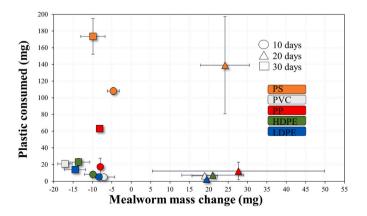


Fig. 5. The change in mealworm mass vs the mass of consumed plastic from Source A for 10, 20, and 30 days with five types of plastic. PS - polystyrene, PP – polypropylene, HDPE – high-density Polyethylene, LDPE – low-density polypropylene, and PVC – polyvinyl chloride. Standard deviation values for the masses of plastics consumed are given in Tables 1 and 2

(6.5, 12.3, 7.4, and 2.3 mg for PVC, PP, HDPE, and LDPE, respectively). The effect on mealworm mass change from consuming plastic can be clearly seen in the 30-day time interval where the range is 9.0 mg. Also, the favorability of PP is observed in 30-day time interval (Fig. 5, red square) where significant amounts of PP is consumed (63.0 mg) compared to PVC, HDPE, and LDPE (20.9, 23.0, and 13.9 mg, respectively).

3.3. Effects of three mealworm sources (CA, NJ, and CT) for 30-day time interval

Mealworms from three different commercial providers in different geographic locations (all in the US) were used in the experiment (Section 2.1) to observe whether local conditions and environment affected their plastic consumption ability. Mealworms from Source A show the highest viability rate (64.7-49.3%) among the three sources and across all types of plastics (Table 2). This was followed by mealworms from (Source B) (52.3-46.3%), and (Source C) mealworms displayed the lowest viability (39.3-23.0%) (Table 2). This observation was important because the mealworms from (Source C) actually displayed the largest average initial mass (93.1 mg per mealworm). Source B mealworms had an average of 87.9 mg in initial mass, whereas that of Source A mealworms were the lightest at 60.9 mg. This shows that the larger (or heavier) the mealworm appears to have a lower viability rate under the experimental conditions employed here. The experimental conditions were completely isolated from the natural environment, where there are other factors (e.g., predators such as lizards or larger insects, and extreme weather conditions) compared to the controlled laboratory conditions. Therefore, those factors are eliminated from affecting mealworm viability due to their size and anthropogenic activities (Gantchoff et al., 2020). Given these external (environmental) factors do not come into play with respect to mealworm viability, it is likely that solely the feeding material (i.e., type of plastic) caused differences in viability rates between the three sources of mealworms. Based on these findings, it appears that in this work, larger mealworms require broader food sources to remain viable and when the plastic materials were not a sufficient energy supply, the larger mealworms were the first to lose viability.

The change in mass of Source A mealworms over the 30-days (Table 2) depended on the type of plastic; the trend in mealworm mass loss was PVC > LDPE > HDPE > PS > PP. The loss in mass for Source B and Source C mealworms followed LDPE > PP > HDPE > PVC > PS and HDPE > PS > PP > LDPE > PVC, respectively. Based on the data in Table 2, mealworms from the three sources have diverse interests in consuming various plastic types, and a clear change in mealworm mass with respect to plastic type is not readily evident. The Source A mealworms appear to prefer PS and PP, which is in agreement with Figs. 3-5. However, the Source B mealworms appear to favor PS. The mealworms from Source C gained the most weight with PVC. These data indicate that geographic location and source of mealworms seems not to be a factor for favor of one type of plastic for another. An additional variable to consider may be the original feedstock for the mealworm provided by the producer. The effects of plastic compositions towards mealworm digestive system could also be the reason for this variability. However, this biological effect on mealworm mass remains unclear. While the change in mealworm mass varies by consuming different plastic types, the amount of consumed plastic showed clear consistencies (Table 2). Mealworms from all three experimented sources consumes PS as the highest consumed plastic: 173.5, 159.2, and 159.5 mg for Source A, B, and C, respectively. PP is the second highest consumed plastic, with 63.0, 99.3, 66.9 mg consumed for Source A, B, and C, respectively. While the viability of mealworms is similar to each other, independent of the type of plastic they consumed, the amount of consumed PCV, HDPE, and LDPE are significantly lower than PS and PP. PVC and HDPE are similar in mass consumption, and they were both consumed the most by Source A mealworms, followed by Source B, and C. This shows the consistent

Table 2
Amount of consumed plastic (mg) by mealworm from three sources for 30 days. (s.d. = standard deviation); PS - polystyrene, PP - polypropylene, HDPE - high-density polyethylene, LDPE - low-density polypropylene, and PVC - polyvinyl chloride).

Plastic Type	Mealworm source	Plastic consumed (mg)	s.d.	Change in mealworm mass (mg)	s.d.	Mealworm viability (%)	s.d.
PS	A	173.5	21.4	-9.9	3.1	64.7	6.0
	В	159.2	63.9	-16.0	2.8	49.0	3.6
	С	159.5	24.2	-17.7	6.5	23.0	6.2
PVC	A	20.9	0.1	-17.1	1.9	58.7	7.2
	В	14.5	2.5	-20.8	2.6	52.3	5.0
	С	9.9	1.2	2.6	45.4	24.0	11.5
PP	A	63.0	1.2	-8.2	0.7	66.0	1.7
	В	99.3	78.6	-22.3	2.1	46.3	5.1
	C	66.9	36.0	-16.6	3.5	38.3	3.1
HDPE	A	23.0	4.5	-13.6	2.9	60.3	8.3
	В	16.7	6.0	-21.0	2.5	52.3	3.8
	С	12.8	5.2	-20.2	5.4	39.3	4.5
LDPE	A	13.9	0.5	-14.5	2.7	49.3	12.7
	В	20.7	8.8	-24.1	3.7	49.0	1.0
	С	16.3	6.5	-10.2	5.8	34.3	10.4

consumption independent from the source of mealworms. This also agrees with the result discussed in Section 3.1 and 3.2 that consumption of HDPE and PVC are similar with time variances. One similar trend observed for PP and LDPE was that Source B mealworms consumed more of those plastics than the Sources A and C.

One noticeable trend between the changes in mealworm mass and the amount of plastic consumed can be seen in Table 2 where the mealworms can lose mass while at the same time consume plastic. This is evident particularly for PS and PP over all three time intervals of the experiment. PS and PP were preferred for consumption more than the other types of plastic. Mealworms from Source A lost between 8.2 and 17.1 mg, and from Source B, they lost 16.0–24.1 mg, depending on the type of plastic. The range of mass loss for Source A and B were similar (9.0 mg for Source A and 8.0 mg for Source B). As indicated in Table 2, it was evident that the larger mealworms had the lowest viability over time. Because mealworms from Source C were the largest ones (i.e., they had the highest initial mass), their large initial mass may be a potential cause/factor for this large range of mass-change dependent on type of plastic.

3.4. Statistical analyses and specific consumption rates

Statistical analyses were carried out to test for differences in the amounts of plastic consumed by mealworms and for changes in mealworm mass during the 10-, 20-, and 30-day experiments with Source A. Specifically, one-way ANOVA was coupled with a Post-hoc test (i.e., the Bonferroni corrected method) to test for significant differences in both plastic consumed and change in mealworm mass. The Bonferroni correction is needed to account for the multiple statistical tests performed between each type of plastic. For ANOVA, the alpha value was set to 0.05, and for the Bonferroni correction, the alpha level is divided by number of Post-hoc tests, which in our case is 10 (e.g., PS vs. PVC, PS vs. PP, PS vs. HDPE, etc.). Therefore, if the P-value is less than the Bonferroni corrected alpha level of 0.005, the result is significant.

Through these analyses, there was a significant difference between consumption of plastic and change in mealworm mass for several pairs of comparisons during the 30-day experiment only. The P values from the one-way ANOVA for plastic consumed for Source A at 10-, 20-, and 30-days were 1.0×10^{-9} , 9.6×10^{-9} , and 0.0003, respectively. This indicates that with an alpha value of 0.05, there were significant differences between the amounts of plastic consumed. However, to determine where those differences were, the Bonferroni correction, which utilizes an alpha value of 0.005, indicated that at 10-days, only PS had significantly more consumption than the other plastics. At 30-days, Posthoc analyses with the Bonferroni method determined that the

mealworms consumed more PS than the other plastics. Additionally at 30-days, mealworms consumed more PP than PVC, HDPE, and LDPE. The P values for the one-way ANOVA for change in mealworm mass for Source A at 10-, 20, and 30-days were 0.050, 0.947, 0.008, respectively. This indicates that only at 30-days was there a significant difference between mealworm mass caused by plastic type at each time point. Based on the Bonferroni correction, PVC caused more mass loss than PP.

The specific consumption rates (SCRs) are divided into two calculations. The first SCR is to observe how much plastic 100 mealworms consume in one day. In this calculation, the amount of plastic consumed is divided by the number of mealworms, then divided by the number of experimental days. This value is multiplied by 100 (mealworms). The number of mealworms is the average of the starting number of mealworms (100) and the number of viable mealworms at the end of the experimental time period. The second SCR calculates how much plastic was consumed by 1 g of mealworms in one day. This value is determined via the amount of consumed plastic divided by the mass of mealworms divided by the number of days in the experimental period. The mass of mealworms is the average mass of mealworms at the beginning and the end of the experimental time period. These two types of SCRs have been used by others to assess consumption rates (Yang et al., 2018). As the mealworm viability gradually decreased over time, and the number of mealworms was counted at the end of the experiment, it is important to use the average values from pre- and post-experimental time periods to not over- or under-calculate the SCR.

Overall, the amount of plastic consumed everyday per 100 mealworms (Table 3) does not significantly change throughout time intervals for all types of plastics. The SCRs are notably different over the experimental time periods. For example, for PS, the SCR is 51.7% higher in the 10-day interval compared to the 30-day interval. For PP, the highest SCR is 351.3% higher for the 30-day interval as compared to the 20-day interval. However, the SCRs over each time period are within each other ranges when considering their standard deviations (s.d.). A similar observation was seen for the amount of plastic consumed by 1 g of mealworms (Table 3). Although a 276% higher SCR was observed for the 30-day time interval versus the 10-day interval for PS, and a 488% higher SCR was observed for the 30-day interval than 20-day interval for PP, the SCR values overlapped each other with the consideration of the standard deviation. The SCRs of PS are the highest of all the plastics; on average, 9.11 mg of plastic were consumed by 100 mealworm per day, and 0.85 mg of plastic were consumed by 1 g of mealworm per day. This was followed by PP, which on average, 2.02 mg of plastic were consumed by 100 mealworms per day, and on average, 0.22 mg of plastic were consumed by 1 g of mealworm per day, using the three experimental time periods. These SCRs agree with the data and

Table 3Specific consumption rates of five types of plastics by Source A mealworms (SCR = specific consumption rates; s.d. = standard deviation; PS - polystyrene, PP - polypropylene, HDPE - high-density polyethylene, LDPE - low-density polypropylene, and PVC - polyvinyl chloride).

Plastic	Day	(mg plastic) x (100 mealworms) $^{-1}$ (d) $^{-1}$	s.d.	(mg plastic) x (g mealworm) $^{-1}$ (d) $^{-1}$	s.d.
PS	30	7.62	1.80	1.09	0.29
	20	8.17	2.91	0.78	0.20
	10	11.56	0.67	0.67	0.03
PP	30	3.43	2.07	0.47	0.24
	20	0.76	0.53	0.08	0.06
	10	1.89	0.93	0.11	0.05
HDPE	30	0.77	0.25	0.11	0.05
	20	0.44	0.12	0.05	0.01
	10	0.94	0.24	0.05	0.01
LDPE	30	0.79	0.28	0.10	0.03
	20	0.14	0.02	0.02	0.00
	10	0.57	0.14	0.03	0.01
PVC	30	0.68	0.16	0.09	0.04
	20	0.38	0.08	0.04	0.01
	10	0.54	0.13	0.03	0.01

observations above that PS is the most consumed plastic and PP is the second most consumed plastic.

Specific consumption rates from the variety of mealworm origins is also essential to consider. Table 4 lists SCR values of Source A, B, and C mealworms, which consumed five types of plastic for 30 days. There is a slight difference in SCR observed between sources for all types of plastic. For example, 7.02–8.7 mg of plastic were consumed per 100 mealworms per day for all three sources, and 0.9-1.41 mg of plastic were consumed per 1 g of mealworms for all sources. However, the SCR values are not distinguishable after standard deviation is taken into account (i.e., s.d. values overlap). This also indicates that the origin of mealworms does not affect their SCR. Both considerations of time interval and mealworm origin show that the SCRs of HDPE, LDPE, and PVC are significantly lower than SCRs of PS and PP. The values of 7.02-8.7 mg plastic/100 mealworms/day are in agreement with other published values for PS consumption by mealworms in a similar situation (i.e., without supplemental feed), which are 8.46 \pm 0.14 mg plastic/100 mealworms/day (Yang et al., 2018). In addition to the PS values, data presented here also provide SCRs for four other types of common plastic contaminants over three experimental time intervals, which have not been established to

Table 4
Specific consumption rates of five types of plastic for 30 days by mealworms where data are averages of Sources A, B, and C (SCR = specific consumption rates, s.d. = standard deviation; PS - polystyrene, PP - polypropylene, HDPE - high-density Polyethylene, LDPE - low-density polypropylene, and PVC - polyvinyl chloride).

Source	Plastic	(mg plastic) x (100 mealworms) $^{-1}$ (d) $^{-1}$	s.d.	(mg plastic) x (g mealworm) $^{-1}$ (d) $^{-1}$	s.d.
A	PS	7.02	0.66	1.41	0.12
	PP	2.53	0.03	0.49	0.03
	HDPE	0.96	0.17	0.16	0.03
	LDPE	0.62	0.04	0.11	0.00
	PVC	0.88	0.03	0.14	0.00
В	PS	7.13	2.37	0.90	0.27
	PP	4.55	2.97	0.55	0.36
	HDPE	0.74	0.23	0.09	0.03
	LDPE	0.93	0.33	0.11	0.04
	PVC	0.64	0.09	0.08	0.01
С	PS	8.70	1.38	0.98	0.14
	PP	3.21	1.39	0.37	0.16
	HDPE	0.61	0.19	0.07	0.02
	LDPE	0.82	0.29	0.09	0.03
	PVC	0.53	0.06	0.05	0.00

date.

4. Conclusions and future direction

This work illustrates the ability of mealworms to consume several types of plastics commonly used in commercial applications. This process would be beneficial to the environment by providing one option in reducing the growing volume of plastic waste. On average, from the three mealworm sources, one mealworm can consume 0.0762 mg (± 0.018) of PS foam per day. Excluding the possibilities of stage-development change and other environmental factors, in one year, a population of one million mealworms could thus consume 27.8 (± 6.56) , 12.5 (± 7.55) , 2.81 (± 0.90) , (± 1.02) , and 2.49 (± 0.58) kg of PS, PP, HDPE, LDPE, and PVC, respectively, per year.

The hypotheses statements were partially supported that mealworms would lose weight after being fed with plastic due to its lack of nutrition. While mealworms did lose weight during the total 30-day time interval, mealworms gained weight during the 20-day interval; this reveals several implications about plastic effects on mealworm. There may be adaptation of mealworms to the new and unfavorable food reflected by their fluctuation of mass change. Further investigation will be necessary to specify the chemical structures or groups that the microbiome in mealworm intestinal gut prefer to digest as well as any detoxification processes that might be influencing mass-gains.

The composition and chemical structure of the plastics also reveal several observations and implications about mealworm's digestion ability. There might be some similarities in the reaction of HDPE and PVC compositions toward mealworm metabolism. While PVC and HDPE are different in chemical structure, both of these plastics resulted in similar mealworm weight changes independently from their origins and experimental time intervals. The change in mass of mealworm is an important factor that impacts their viability; specifically, mealworm viability is high when their change in mass is small. Further study needs to be taken to examine the biological effects of mealworm metabolism that helps them remain viable when they do not change in weight.

Closer time interval testing would be beneficial to discover more about mealworm mass change from consuming plastics. The data showed that mealworms lost weight during the first 10-day interval. A period before 10 days is needed to observe if mealworms gain weight before that time. This would provide higher resolution data to further illustrate the rate of mass change for this species. A noticeable gain in mealworm weight was seen for the 20-day interval. An experiment for a time interval between 10 and 20 days is desired to understand what exact time period mealworms recover to their initial mass and begin to gain mass. This would provide essential information because the time it takes for mealworms to digest and transform plastic into energy affects their weight. Similarly, an experiment for a time interval between 20 and 30 days is needed to show how long after 20 days mealworms continue to gain weight and when they start to lose weight. This would illustrate when the mealworm body starts to reflect its need of more nutrition after obtaining the inadequate nutrition from plastic. A continuous experiment over 30-day with the same batch of mealworm in addition to adding nutrient-dense substrate along with the plastics will yield a better understanding of long-term mealworm viability and plastic consumption.

Finally, the structures of the plastics were different, with common PS plastics being consumed at greater rates. All other plastics were thin films or sheet-like in form. The mandibles of the mealworms are small and most of the mass lost from the film plastics were at edges (Fig. 2), while in PS plastics the mealworms consumed material throughout the substrate. This suggests that the physical form of the plastics could influence feeding, and therefore consumption rates, and changes to other plastic forms could induce feeding at higher rates.

CRediT authorship contribution statement

Thanh Quang Pham: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration. Scott Longing: Methodology, Resources, Writing – review & editing. Matthew G. Siebecker: Methodology, Software, Formal analysis, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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