The Behavioral Effects of Microplastic Exposure in *Drosophila melanogaster*

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Author Note

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Abstract

Activity and manufacturing have caused an increase in micro plastic accumulation in the environment, specifically in rivers, streams, and oceans. As a result, microplastic exposure, including consumption, is inevitable. The substances in plastic have been found to cause adverse physical effects, some of which are altering the nervous system or causing cancer. This project aims to connect polyethylene, the most commonly used plastic worldwide, with a change in behavior. *Drosophila melanogaster* were exposed to polyethylene wax particles during their egg, larvae, pupae, and adult stages to quantify a change in behavior. After reaching maturity, three assays will be conducted to look at three behaviors: locomotion, feeding, and social interaction. *Drosophila* exposed to microplastics traveled less distance, consumed less food, and had abnormal social interactions. These findings demonstrate that there are behavioral concerns about plastic exposure. The methods used in this research can be manipulated by using different concentrations, mixtures, exposure periods, and types of plastics to see if those results remain consistent with the findings in this study. Future research could also investigate possible treatments to reverse the behavioral changes that occur as a result of microplastic exposure.

Keywords: polyethylene wax particles, fruit flies, locomotion, feeding, social space

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I would like to thank Dr. Kevin Crowthers for his continuous advisory throughout my research process. I would also like to thank Saint-Gobain for their donation of polyethylene wax articles.

The Behavioral Effects of Microplastic Exposure in *Drosophila melanogaster*

The use and production of plastics have increased greatly so that by 2019, humans were producing 460 million tons annually (Ritchie & Roser, 2018). Although some are recycled, many are mishandled are after use. Many of these plastics can arrive in the ocean. This happens in three main ways: plastic that is mishandled during movement to landfills, littered plastic that is transported by runoff and wind, and plastics such as microbeads and microfibers that go down the drain when consumer cosmetics are used, and laundry is washed. (*How Does Plastic End up in the Ocean?*, n.d.). In addition, larger pieces of littered plastic can be broken down into microplastics. These plastics can become small enough for an organism to eat as they mistake the plastic for food. Once an organism consumes microplastics, it can travel up the food chain up to human consumption. It has been estimated that there are 15 to 51 trillion microplastic particles floating on surface water worldwide (Lim, 2021). Exposure to microplastics has increased to the point where humans are consuming 39,000 to 52,000 particles annually (Cox et al., 2019).

But microplastics are not the only way that substances in plastic are consumed; when plastics are exposed to heat—in a microwave for example—the chemicals in microplastics can break down and enter the food humans consume. On top of that, the chemicals can be broken down even further over long-term use as, many takeout containers are not built to be washed and reused (Zanolli, 2020)

The Effects of Microplastic Exposure on Humans

The increased exposure to microplastics is alarming as research has shown that many of the common chemicals that make up plastics have adverse side effects on humans. This list of chemicals includes, but is not limited to, styrene, formaldehyde, and bisphenol A (Husain et al.,

2015). These substances have been labeled carcinogenic to humans and have been linked to disrupting the endocrine system, reproductive system, and nervous system (Husain et al., 2015).

Previous Research and Knowledge Gap

Most of the prior research into microplastic exposure has either investigated the physical effects in humans or the behavioral effects in model organisms. The research involving model organisms has found that even among similar species, exposure to microplastics can have varying results. For example, some studies have found that feeding behavior in amphipods, copepods, and coral species decreases after microplastic exposure (Cunningham et al., 2021). In contrast, no effect on feeding behavior was found in shore crabs (Cunningham et al., 2021). Copepods and shore crabs are crustaceans, yet the two organisms yielded different results. Additionally, the effects found could likely be even more severe if the organisms were exposed to a higher concentration of microplastics (Personal Communication, E. Cunningham, 2022).

This project focuses on the behavioral effects of microplastic exposure rather than physical effects as physical effects in humans have been widely studied (Husain et al., 2015). In addition, behavioral changes in humans could have significant implications as they could lead to susceptibility to various mental health illnesses. *Drosophila melanogaster* were used as a model organism to link a change in behavior to microplastic exposure,.

***Drosophila* melanogaster as a Model Organism**

Drosophila melanogaster, commonly known as the fruit fly, has been used as a model in biomedical sciences for over a hundred years. *Drosophila* has found success as a model organism due to its low cost, short lifespan, and easy maintenance (Tolwinski, 2017). *Drosophila* makes a good model for humans because the organism contains homologous genes that have a direct relation to human diseases. Out of all genes that affect human disease, more than 60% are

related to a *Drosophila* ancestor-specific gene (Mackay & Anholt, 2006). In the past, *Drosophila* has been widely used to study brain disorders such as Parkinson's and Alzheimer's disease because *Drosophila* exhibits complex traits similar to humans. These traits include a circadian rhythm, drug responses, locomotion, aggressive behavior, and longevity (Mackay & Anholt, 2006). *Drosophilae* are also easy to use as a model organism as animal welfare ethical review boards do not have to approve research involving the organism (Baenas & Wagner, 2019).

Polyethylene Wax Particles

Polyethylene was used as the microplastic for this project as polyethylene is the most widely used plastic in the world (*Polyethylene | Properties, Structures, Uses, & Facts | Britannica*, n.d.). Additionally, most plastic food containers, both takeout and reusable containers, are made out of either low-density polyethylene or polypropylene (Zanolli, 2020).

Polyethylene wax (PE wax) particles were used in this project. The PE wax particles utilized were 8-10 microns long and thus were small enough for the *Drosophila*—on average 3 mm long and 2 mm wide—to possibly consume (Miller, 2000). Even if the *Drosophila* did not end up consuming the particles, the organisms were exposed to the microplastic chemicals—such as ethyne—for the majority of their lifespan.

PE wax is a thermoplastic polymer consisting of long ethylene monomer chains. PE wax can be made with high-density polyethylene and low-density polyethylene (*POLYETHYLENE WAX*, n.d.) The material has applications such as a plastic additive, lubricant, resin additive, and more. The singular component of PE wax, ethylene ($H_2C=CH_2$), is the simplest of the alkenes—organic compounds that contain carbon-carbon double bonds. Sources of ethylene include natural gas and petroleum, as well as a naturally occurring hormone in plants. Polyethylene

plastic is the result of the polymerization of ethylene monomers (*Ethylene | Structure, Sources, Production, Uses, & Facts | Britannica*, n.d.).

Researchable Question

How does direct exposure to polyethylene wax microplastics affect the behavior of *Drosophila melanogaster*?

Objective

To address the researchable question, three assays—feeding, locomotion, and social behavior—were conducted with *Drosophila* exposed to PE wax particles. Microplastic exposure was conducted with three different exposures and in four different developmental stages: egg, larva, pupa, and adult. After the assays were completed, a t-test was conducted which validated the significance of the data. Additionally, a linear regression test quantified the correlation between polyethylene concentration and the assay results.

The three assays were chosen for this project because they gather data on three vital behaviors for human well-being and survival: locomotion, feeding, and social interactions (Glover, 2017; Petrovich, 2018; Young, 2008).

Hypothesis

If *Drosophila melanogaster* organisms are exposed to polyethylene wax microplastics, then the organisms will move around less, the organism will eat less, and the social distance between the organisms will be smaller because the exposure to microplastic particles will affect the organism's behavior.

Most prior research involving microplastic exposure has shown that cognitive function changes after exposure (Cunningham et al., 2021). Cognitive function has been modeled using the amount of food the model organism consumes and the locomotion of the organism.

Abnormal social behavior is expected as the brain is not processing information in the same way it was previously.

Section II: Methodology

Role of Student vs. Mentor

I spent four months culturing and conducting assays with *Drosophila melanogaster* to collect data that supported my hypothesis. This data needed to contain data concerning the behavior of organisms that were exposed to microplastics and organisms that were not in order for a comparison to be made. At first, my mentor taught me how to culture the *Drosophila*; afterwards, all culturing and handling of the *Drosophilae* was done by myself.

Equipment and Materials

In this project, wild-type Oregon R strain *Drosophila melanogaster* cultures with red eyes were obtained from Carolina Biological. Aside from the microplastics, which were accquired from Saint-Gobain, all other materials are easily accessible. Modifications to published assays were created based on time constraints and limitations of the lab I was working in.

Culturing the Drosophila

Drosophila cultures were maintained under a 12h/12h light and dark cycle, and the offspring were allowed to mature for 3 weeks. The control group matured with exposure to only the feed and distilled water, while the experimental groups were exposed to either a 50 µg/mL, 100 µg/mL, or 200 µg/mL PE wax microplastic in their feed. A stock solution—1000ug/mL PE wax in distilled water—was diluted with more distilled water to the aforementioned solutions; then the solutions were mixed with equal amounts of *Drosophila* feed. Due to material and time constraints, the same *Drosophilae* were used for all three assays.

Locomotion Assay

The assay used to quantify motion was based on Madabattula et al. (2015). 20 *Drosophilae* were placed into a graduated cylinder with a line marked 17.5 cm from the bottom of the tube. The *Drosophilae* were tapped to the bottom and after the last tap occurs, a 2-minute trial was conducted and recorded. During the trial, *Drosophilae* are expected to climb up the walls of the graduated cylinder. After all trials videos were analyzed to record the number of *Drosophilae* that climb above the 17.5 cm line every 10 seconds. After each trial, the *Drosophilae* will be, replaced; 5 such trials will be conducted. This assay makes use of the negative geotaxis—the natural behavior of *Drosophilae* to move in the direction opposite of gravity (Madabattula et al., 2015). This assay was chosen over similar locomotion assays because the increased trial period and threshold line create a more sensitive assessment of a *Drosophila*'s climbing ability. This assay makes use of a climbing height of 17.5 because the increase in assay difficulty aids in identifying minor changes that may occur (Madabattula et al., 2015).

Feeding Assay

The next assay I conducted was a feeding assay that made use of the CApillary FEeder assay (CAFE assay) created by Diegelmann et al. (2017). For the assay, four capillary tubes filled with a sucrose stock solution were placed into the fly vials containing the 8 *Drosophilae* each; then, after a 24-hour feeding period, the amount of the sugar solution remaining in the capillary tube was recorded; the lower the ending height of the solution, the more food the *Drosophilae* consumed. The sucrose stock solution was created at 3 M (10%, w/v), then the solution was diluted down to a 1 M sucrose stock solution for the *Drosophilae* to feed from.The sucrose solution will be dyed red and blue to counterattack the potential bias of the organisms.

Additionally, vials containing just capillary tubes without *Drosophilae* were made; this vials accounted for any evaporation that occured during the 24 hour feeding period.

Social Interaction Assay

The final assay utilized for this project is an assay based on the social space assay created by Simon et al. (2012). Social space is the distance between two individuals of the same species. This distance is determined by the ideal balance of attraction and repulsion (Kaur et al., 2015). For this model organism, Canton-s flies, regardless of gender, lie within two body lengths from each other (Simon et al., 2012). The preparation for the assay started off by allowing the *Drosophilae* to mature with interactions with both genders. The day prior to experimentation, the *Drosophilae* will be separated by gender for the assay—separating the *Drosophilae* by gender removes mating behaviors as a factor for the choices the *Drosophila* face. The following day, 40 *Drosophilae* of the same gender will be placed into a petri dish measuring 9 cm in diameter and 1.4 cm in depth. First, *Drosophilae* will be given 15 minutes to acclimate to the testing chamber; and after 15 minutes, a photo will be taken and digitally analyzed using the open-source software ImageJ to measure the distance between a *Drosophila* and its nearest neighbor.

Justification for Assays Used

The assays for the control group will be conducted first to gather preliminary data and practice handling the model organism. These three assays were chosen because they gather data on behaviors commonly seen in various behavioral disorders. If a connection can be made between the behaviors displayed in the assays, then exposure to microplastics could be linked to a behavioral disorder.

Statistical Tests

Z-test

After experimentation, I realized the best way to analyze the results of the locomotion assay was to conduct a z-test as the average number of flies would better represent the locomotion data.

Student's t-test

After all of the data was collected, a student's t-test was conducted between the control and the various experimental groups. As the student's t-test compares values from two groups, the data from the *Drosophilae* exposed to the PE wax microplastics will be compared to the control, the *Drosophilae* not exposed to the PE wax microplastics.

Linear Regression Analysis

Alongside the student's t-test, a Linear Regression Analysis was conducted to quantify the correlation between an increasing polyethylene concentration and the results of this study.

Section III: Results

This experiment involved three different assay to investigate how behavior can be impacted by micro plastic exposure. The raw data for all of the assays can be found in Appendix B.

Locomotion Assay Results

Locomotive behavior was quantified by recording how many flies climbed up above the threshold every 0 second interval for a total of 120 seconds (See Appendix B for raw data). From a first glance, Figure 1 shows that more flies reached the threshold line than the other

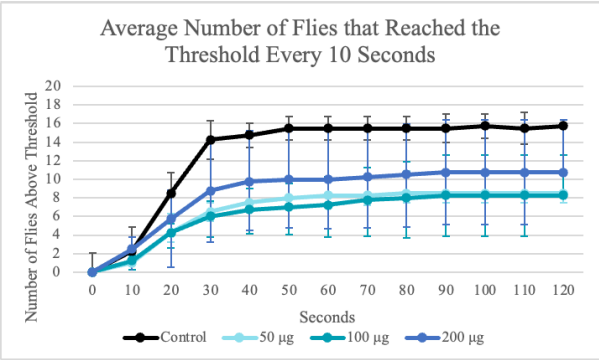

Figure 1: ***Average Number of Flies that Reached the Threshold Every 10 Seconds.*** This graph depicts the average number of flies that reached the 17.5 threshold line every 10 seconds (+/- standard deviation)

experimental groups. Specifically, at the 20 second mark, the control starts to separate from the other groups; then by the 30 second mark, the control has a visibly greater average number of *Drosophilae* above the threshold line. Table 1 depicts the *p*-values that were calculated utilizing the data from the 120 second mark, and it is seen that the results from the 50 µg/mL and 100 µg/mL resulted in statistically significant data, but the 200 µg/mL did not. The error bars shown in Figure 1 weaken the correlation as the 200 µg/mL error bars stretch up to the control data. The 200 µg/mL have the largest error bars due to the wide range of data (see Appendix B), while the control data has the smallest error bars. The control vs 50 µg/mL resulted in a *p*-value less than 0.01 while the control vs 100 µg/mL resulted in a *p*-value less than 0.05. As shown in Figure 2, a linear regression analysis was also conducted with the data from the 120 second mark to quantify the relationship between an increased concentration and a more significant change. The linear regression model suggests that there is a negative correlation between the number of flies that pass the threshold line at the 120 second mark and the concentration of exposure. However, the R^2 value for the model ended up being very low at 0.0838. This means that only 8.38% of the dependent variable—number of flies—is explained by the independent variable—the concentration of microplastics the *Drosophilae* were exposed to.

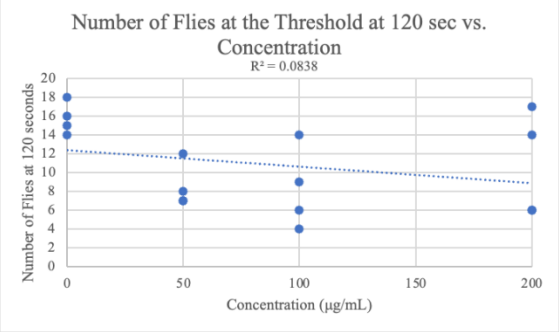

Figure 2: ***Number of Flies at the Threshold at 120 sec vs. Concentration***. This graph depicts data points from each trial at the 120 second mark. A trendline was created and a R^2 value was calculated.

Table 1: ***Z-test results for locomotion assay***. The table below compares the average of the number of flies above the threshold line. This data utilizes the number of flies from the 120 second mark.

	Control vs 50 µg	Control vs 100 µg	Control vs 200 µg
p-value	0.001161	0.042317	0.186815

Feeding Assay

Feeding behavior was quantified by placing *Drosophilae* into centrifuge tubes where they only had access to capillary tubes filled with a sucrose solution. The *p*-values were calculated with a student's t test by compaing the final heights' of the capillary tubes. All capillary tubes were filled to the 5 µL line, this corresponded to a height of 2.7 cm. Since they were all filled to the same height at the beginning of the assay, I only analyzed the final heights of the capillary tubes. In Table 2, the 50 µg/mL has a *p*-value less than 0.05, but when looking at just the blue solution tubes, all three conentrations resulted in *p*-values less than 0.001. In addtion none of the *p*-values for the red solution are stastically significant. I saw qualitatively (see Figure 2) that the capillary tubes filled with the red solution were much higher than the capillary tubes filled with the blue solution, so I decided to run additional t-tests to look at just the data from a certain solution. This allowed me to conclude whether or not a factor aside from evaporation decreased the height of the liquid in the capillary tubes. Table 3 highlights these *p*-values. When looking at all capillary tubes, it can be seen that only the control produced data that was statistically significant from the evaporation, but the concentration groups were not. When looking at just the blue solution, all experimental groups resulted in statistically significant results; and again, the

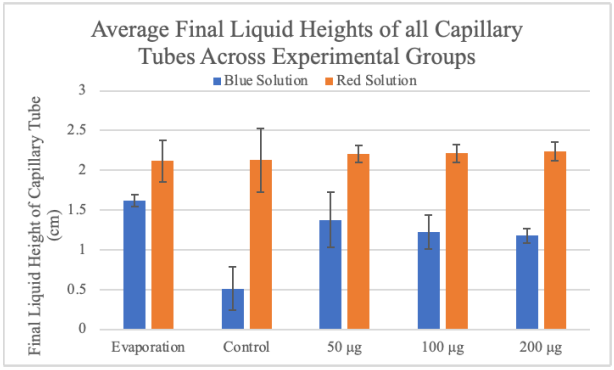

Figure 3: ***Average Final Liquid Heights of all Capillary Tubes Across Experimental Groups.*** This data compared the final heights of the control the concentrations.

Table 2: ***T-test results for feeding assay***. The table below compares the final liquid heights of the control to the experimental groups.

	Control vs 50 µg	Control vs 100 µg	Control vs 200 µg
p-value All Tubes	0.03342659	0.06151085	0.06958714
p-value Just Blue	0.00002353	0.00001033	0.00000202
p-value Just Red	0.12307883	0.09397269	0.05177601

Table 3: ***T-test results for feeding assay***. The table below compares the final liquid heights of the evaporation to all experimental groups.

	Evaporation vs. Control	Evaporation vs. 50 µg	Evaporation vs. 100 µg	Evaporation vs. 200 µg
p-value All Tubes	0.02251954	0.31611670	0.20222857	0.19089361
p-value Just Blue	0.00000063	0.06055869	0.00052745	0.00000022
p-value Just Red	0.46999097	0.42314631	0.31073727	0.35062686

data from the red solution is not statistically significant. Additionally, after the 24-hour feeding period, I noticed that the *Drosophilae* exposed to microplastics had experienced high mortality rates (shown in Figure 4), so I decided to run a t-test on the number of flies alive as all centrifuge tubes started out with eight *Drosophilae*. As seen in Table 4, the 50 µg/mL has a *p*-value less than 0.01 while the 100 µg/mL and 200 µg/mL concentrations have a *p*-value less than 0.001. Even with the error bars shown in Figure 4, the control on average had many more *Drosophila* survive through the entirety of the assay.

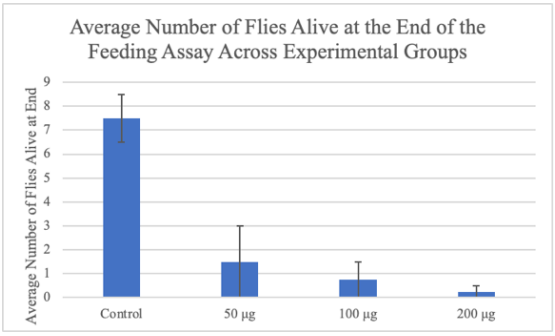

Figure 4: ***Average Number of Flies Alive at the End of the Feeding Assay Across Experimental Groups***. This data graphs the average number of flies alive.

Table 4: ***T-test results for feeding assay***. The table below compares the number of flies alive after the 24-hour period in the control to the experimental groups.

	Control vs 50 µg	Control vs 100 µg	Control vs 200 µg
p-value	0.0014395	0.0000196	0.0000129

Social Space Assay

The social space assay quantified social behavior by looking at the distance between organisms. This assay was limited as I ran into unexpected mortality so I had to limit the assay just to the control, 100 µg/mL, and 200 µg/mL concentrations. In Figure 5, the data from each experimental group is close in number and each group has a large error bar. A t-test was run to com pare the control to the concentration groups. As seen in Table 5, only the 200 µg/mL resulted in a *p*-value less than 0.05

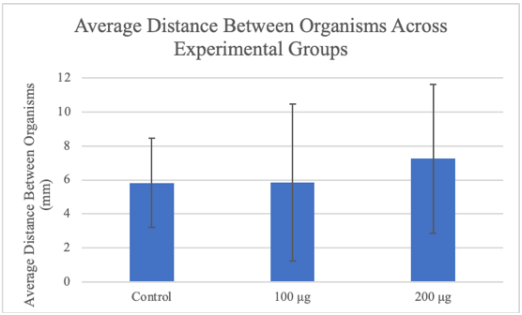

Figure 5: ***Average Distance Between Organisms Across Experimental Groups***. This data contains the average distance between a fly and its nearest neighbor.

Table 5: ***T-test results for social space assay***. The table below compares the number distance between flies from the control to the experimental groups.

	Control vs 100 µg	Control vs 200 µg
p-value	0.580716	0.040195

Section IV: Discussion

The experiments conducted in this assay were chosen and designed to collect data to quantify behavior. Locomotion is a behavior organisms need to move around. Feeding behaviors makes sure an organism has enough energy to sustain itself. Finally, a proper social space allows for effective communication. The objective for this research was to see whether behavior was changed as a result of PE wax microplastic consumption or exposure. The objectives were partially accomplished as the data was found to be statistically significant, but all assays were limited by the number of trials conducted.

In the locomotion assay, two groups saw statistically significant decreased locomotion at ($P<0.05$, student's t test)-50 µg/mL and 100 µg/mL concentrations. However, the 200 µg/mL did not pass the 0.05 significance level. This refutes my hypothesis as the 200 µg/mL was the largest concentration I created for exposure. Theoretically, the more microplastics the *Drosophilae* are exposed to, the more the behavior would change. The statistically insignificant data from the 200 µg/mL most likely occurs from the fourth trial I conducted with this concentration (see Appendix B). 17 *Drosophilae* at the end of the 120 second trial is a number I would expect from the control group. Ultimately, due to the minimal amount of data I had collected, I chose the keep the piece datum in my statistical tests. If more data had been collected, it would be possible to determine whether or not that piece of datum is an outlier; if it is, then it can be removed from any further data calculations. Another piece of data that also does not align with the hypothesis concerning an increasing concentration and change in behavior is that the 50 µg/mL experimental group had the most significant data compared to the control.

The Linear Regression Analysis was run to determine if the behavior would change if the *Drosophilae* were exposed more microplastics. For the Linear Regression Analysis, I decided

to utilize the same data points as the z-test, and due to the limited data points, the R^2 value for the trendline was 0.0838. The R^2 value meant that only 8.38% of the data for the dependent variable—number of flies above the threshold—could be explained by the independent variable—the concentration of microplastics the organisms were exposed to. Thus, I cannot definitively state that an increased concentration of microplastics will result in an increased change in behavior.

In the feeding assay, it can be seen from the evaporation data that the *Drosophilae* did consume food. Surprisingly, the *Drosophilae* consumed more blue solution than the red solution. As seen in Figure 3, the blue solution on average was much lower than the red solution. This is likely due to the two solutions being different. Looking at just the evaporation capillary tubes alone, it can be seen that the red solution was much higher on average. In the evaporation centrifuge tubes, there were only capillary tubes. Since there were only capillary tubes, the only way for the solution height to decrease is evaporation. If the solutions were similar, then the evaporation heights should be similar. The difference in solutions could be due to two factors. The first factor is that the dye I used. Although the dye was from the same brand and package, they may differ enough in composition enough to affect the final resulting solution. The other explanation is that when I created the red and blue solutions, the dilution of greater concentrated sucrose solution was not the same for both solutions and thus resulted in different rates of evaporation and consumption.

Limiting and Confounding Variables

The greatest limitation within my project is that with the resources available to me, I have no way of confirming if the *Drosophila* consumed the PE wax particles. As I stated in the Introduction, the PE wax particles were small enough for the *Drosophila* to potentially consume

but when conducting my research, I had no way of confirming microplastic consumption though. This means that it is possible that the results stemmed from PE wax exposure. A confounding variable that the dispersion of PE wax particles was most likely not uniform in the original solution that was created. When the PE wax particles were mixed in with the distilled water to create the original solution, I noticed that they were hydrophobic. This meant that it was difficult to ensure that the mixture was homologous. One failure that limited my ability to collect research is that out of the four 100 µg/mL *Drosophila* vials I cultured, two of them failed to produce offspring. Even though I had enough of the other concentrations and the control, the low 100 µg/mL population limited the number of trials I was able to conduct for each assay since the data had to be equalized. Another failure that occurred during experimentation is that I did not prepare the assay materials ahead of time—especially while the *Drosophilae* were culturing. As a result, I was limited on time to collect the data and the *Drosophilae* had unplanned exposure periods to the PE wax particles as I prepared the assay materials.

Connections to Prior Research

The conclusions drawn here today align with prior research from Kaur et al. in 2015 and Cunningham et al. in 2021 because each study found data that supported the conclusion that microplastic exposure results in a change in behavior. Cunningham et al. (2021) differed from my research as they focused on hermit crabs and a behavior that is unique to their physiology. On the other hand, the research presented here looks at multiple behaviors. Additionally, rather this research prioritized behaviors that are commonly seen in many organisms—locomotion, feeding, and social space—rather than the model organism. Kaur et al. in 2015 more closely resembles the assays in this study due to both studies utilizing *Drosophila* as their model organism. However, this study utilizes simpler methods and materials that are easier to replicate.

For example, Kaur et al. in 2015 use dimethyl sulfoxide to create a feed mixture for the *Drosophila* that contains bisphenol A. Dimethyl sulfoxide is not only harmful to humans, but is also another variable that impacts the resulting *Drosophilae* behavior.

Implications and Applications

When I take all the assays and statical tests altogether, I can conclude that after exposure to polyethylene wax particles, *Drosophila melanogaster* organisms experienced a change in behavior. I cannot confidently quantify how significant that change is, nor can I state that an increased concentration of microplastics will lead to a more significant change, as the amount of data I collected limits the validity and strength of the statistical tests I ran. The error bars for each graph that I created display that my results had a chance for high error due to the limited number of data points. In the future this means that my results could be strengthened or weakened.

Future Research

In the future, my research can be extended into different model organisms, different types of plastics, plastic concentrations, and different behaviors. In this project, new conclusions can be drawn by adjusting almost every single variable to collect new data. If I were to extend this project, I would first like to conduct the same assays again, but this time adjusting for the limitations and failures that occurred throughout experimentation. I would prioritize conducting the same assays again with a more rigorous procedure because I believe the results would be more conclusive. After conducting the same assays, I would look at different behaviors, such as mating or aggressive behaviors. Additionally, I would look at the same behaviors researched in this project with a different assay; for example, I would conduct a horizontal locomotion assay. Looking at the same behavior with a different lens would give me the ability to narrow down my conclusions; if I conduct a horizontal locomotion assay, then I can draw conclusions based on

just the locomotion of the organism and see how it compares to the negative geotaxis of the organism. Additionally, feeding assay would be conducted again, but this time I would include a filter paper or sponge in order for the *Drosophilae* to have access to water.

Section V: Conclusion

This research was conducted to look into possible changes in behavior. Changes in behavior were quantified by collecting data from a locomotion assay, feeding assay, and a social space assay. These assays collected data from a control, and *Drosophilae* that had been exposed to concentrations of 50 µg/mL, 100 µg/mL, or 200 µg/mL throughout their egg, larva, pupa, and adult stages. The results did partially prove my hypothesis as the data for 50 µg/mL and 100 µg/mL locomotion data were statistically significant when compared to the control data. On the other hand, the lack of supporting data from the 200 µg/mL experimental group from the same assay make it hard to draw conclusions. In addition, other results—such as the mortality during the feeding assay—were unexpected. The various results from all of the assays limits the strength in the conclusions that can be made. What can be certain is that the research presented here is justification that more data concerning behavior and microplastics. This research has huge implications as all organisms are exposed to plastic particles in the present day.

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Section VII: Appendices

Appendix A: Limitations and Assumptions

Limitations:

1. The number of *Drosophila melanogaster* available was limited; in turn, this limited the strength of the statistical tests that were ran.
2. Laboratory time was limited; 2:45-4:30 two days a week and 4 hours over the weekend.

3. Laboratory equipment was limited.

Assumptions:

1. The trends observed are predictive of the future.
2. After reaching the adult stage, *Drosophilae* would exhibit similar behavior no matter if they reached the adult stage 3 days ago or 3 weeks.
3. Even though the original microplastic mixture was not homogeneous, it was not enough to affect the concentration of microplastic in each vial greatly.

Appendix B: Raw Data

Table 6: ***Raw control data for the Locomotive Assay*****.** The table below contains the data for how many flies reached the threshold line during each 10 second period for each of the control trials. The rightmost columns contain the average for the time interval and the standard deviation for the time intervals.

	Control					
Seconds	Trial 1 Num Flies	Trial 2 Num Flies	Trial 3 Num Flies	Trial 4 Num Flies	Average	Stan Dev
10	4	0	1	4	2.25	2.06155
20	11	10	5	8	8.50	2.64575
30	15	15	11	16	14.25	2.21736
40	15	15	12	17	14.75	2.06155
50	16	15	14	17	15.50	1.29099
60	16	15	14	17	15.50	1.29099
70	16	15	14	17	15.50	1.29099
80	16	15	14	17	15.50	1.29099
90	16	15	14	17	15.50	1.29099
100	17	15	14	17	15.75	1.50000
110	16	15	14	17	15.50	1.29099
120	16	15	14	18	15.75	1.70783

Table 7: ***Raw 50 µg data for the Locomotive Assay*****.** The table below contains the data for how many flies reached the threshold line during each 10 second period for each of the 50 µg trials. The rightmost columns contain the average for the time interval and the standard deviation for the time intervals.

	50 µg					
Seconds	Trial 1 Num Flies	Trial 2 Num Flies	Trial 3 Num Flies	Trial 4 Num Flies	Average	Stan Dev
10	1	2	1	0	1	0.81650
20	2	7	4	4	4.25	2.06155
30	6	8	6	6	6.5	1.00000
40	6	11	6	7	7.5	2.38048
50	7	11	7	7	8	2.00000
60	7	11	8	7	8.25	1.89297
70	7	11	8	7	8.25	1.89297
80	7	12	8	7	8.5	2.38048
90	7	12	8	7	8.5	2.38048

100	7	12	8	7	8.5	2.38048
110	7	12	8	7	8.5	2.38048
120	7	12	8	7	8.5	2.38048

Table 8: ***Raw 100 µg data for the Locomotive Assay.*** The table below contains the data for how many flies reached the threshold line during each 10 second period for each of the 100 µg trials. The rightmost columns contain the average for the time interval and the standard deviation for the time intervals.

	100 µg					
Seconds	Trial 1 Num Flies	Trial 2 Num Flies	Trial 3 Num Flies	Trial 4 Num Flies	Average	Stan Dev
10	1	1	2	1	1.25	0.50000
20	4	5	5	3	4.25	0.95743
30	6	8	6	4	6	1.63299
40	9	8	6	4	6.75	2.21736
50	10	8	6	4	7	2.58199
60	11	8	6	4	7.25	2.98608
70	12	9	6	4	7.75	3.50000
80	13	9	6	4	8	3.91578
90	14	9	6	4	8.25	4.34933
100	14	9	6	4	8.25	4.34933
110	14	9	6	4	8.25	4.34933
120	14	9	6	4	8.25	4.34933

Table 9: ***Raw 200 µg data for the Locomotive Assay.*** The table below contains the data for how many flies reached the threshold line during each 10 second period for each of the 200 µg trials. The rightmost columns contain the average for the time interval and the standard deviation for the time intervals.

	200 µg					
Seconds	Trial 1 Num Flies	Trial 2 Num Flies	Trial 3 Num Flies	Trial 4 Num Flies	Average	Stan Dev
10	1	2	3	4	2.5	1.29099
20	3	4	6	10	5.75	3.09570
30	5	5	9	16	8.75	5.18813
40	6	5	11	17	9.75	5.50000
50	6	6	11	17	10	5.22813
60	6	6	11	17	10	5.22813
70	6	6	12	17	10.25	5.31507
80	6	6	13	17	10.5	5.44671

90	6	6	14	17	10.75	5.61991
100	6	6	14	17	10.75	5.61991
110	6	6	14	17	10.75	5.61991
120	6	6	14	17	10.75	5.61991

Table 10: ***Raw evaporation data for the Feeding Assay.*** The table below contains the data for how much of the sucrose mixture evaporated when left alone. The data for the red solution and the blue solution were separated for data analysis due to the visible difference after the assay had concluded.

	Amount Evaporated					
	Blue	Blue 2	Red	Red 2	Blue Average	Red Average
Tube 1	1.1	1.1	0.5	0.4	1.0833	0.5833
Tube 2	1	1.1	0.6	1.1		
Tube 3	1	1.2	0.5	0.4		
Stan Dev		0.07528		0.26394		

Table 11: ***Raw control data for the Feeding Assay.*** The table below contains the data for the final heights of the solutions in the capillary tubes for the control group.

	Ending Height Control (cm)					
	Blue	Blue 2	Red	Red 2	Blue Average	Red Average
Tube 1	0.6	0.6	2.1	2.3	0.5125	2.125
Tube 2	0.4	0.5	2.3	1.9		
Tube 3	0.8	0.3	2	2.1		
Tube 4	0.8	0.1	2.1	2.2		
Stan Dev		0.24165		0.13887		

Table 12: ***Raw 50 µg data for the Feeding Assay.*** The table below contains the data for the final heights of the solutions in the capillary tubes for the 50 µg group.

	Ending Height 50 µg (cm)					
	Blue	Blue 2	Red	Red 2	Blue Average	Red Average
Tube 1	1.5	1.4	2.1	2.2	1.375	2.2
Tube 2	1.3	1.4	2.1	2.1		
Tube 3	1.5	1.8	2.3	2.4		
Tube 4	0.6	1.5	2.2	2.2		
Stan Dev		0.34538		0.10690		

Table 13: ***Raw 100 µg data for the Feeding Assay.*** The table below contains the data for the final heights of the solutions in the capillary tubes for the 100 µg group.

	Ending Height 100 µg					
	Blue	Blue 2	Red	Red 2	Blue Average	Red Average
Tube 1	1.4	1.1	2.1	2.1	1.225	2.2125
Tube 2	1.3	1.2	2.2	2.2		
Tube 3	0.8	1.2	2.3	2.1		
Tube 4	1.5	1.3	2.3	2.4		
Stan Dev		0.21213		0.11260		

Table 14: ***Raw 200 µg data for the Feeding Assay.*** The table below contains the data for the final heights of the solutions in the capillary tubes for the 50 µg group.

	Ending Height 200 µg (cm)					
	Blue	Blue 2	Red	Red 2	Blue Average	Red Average
Tube 1	1.2	1.2	2.1	2.4	1.175	2.2375
Tube 2	1	1.2	2.2	2.2		
Tube 3	1.1	1.2	2.2	2.4		
Tube 4	1.2	1.3	2.3	2.1		
Stan Dev		0.08864		0.11877		

Table 15: ***Number of Drosophilae alive at the end of the Feeding Assay.*** The table below contains the data for all experimental groups on how many Drosophilae were alive at the end of the assay.

	Control	50 µg	100 µg	200 µg
Tube 1	8	2	1	1
Tube 2	8	0	1	0
Tube 3	8	0	1	0
Tube 4	6	4	0	0
Average	7.5	1.5	0.75	0.25

Table 16: ***Raw data for the Social Space Assay***. The table contains the data for all tested experimental groups on the distance from each fly to its nearest neighbor.

Fly Number	Control	100 µg	200 µg

1	4.7	3.9	18.4
2	4.7	3.9	14.6
3	2.2	5.7	10.0
4	2.2	12.2	4.4
5	8.5	12.9	4.4
6	4.0	11.7	8.2
7	2.0	23.9	3.6
8	2.0	7.1	3.1
9	6.1	7.1	3.1
10	7.8	10.6	11.4
11	11.4	4.2	12.5
12	3.7	4.2	12.5
13	4.2	10.5	8.2
14	3.2	5.2	8.2
15	5.8	2.3	9.6
16	4.1	1.7	4.0
17	5.3	2.2	4.0
18	4.6	3.8	2.6
19	4.0	2.3	4.3
20	4.0	6.2	4.5
21	9.9	1.6	4.2
22	4.2	1.6	4.2
23	4.2	1.6	8.5
24	10.0	3.0	5.3
25	10.7	3.5	5.4
26	4.7	7.5	4.6
27	4.7	8.7	8.1
28	7.8	3.5	5.4
29	8.3	3.5	5.6
30	6.9	7.7	2.7
31	2.8	3.1	2.7
32	2.8	3.1	17.1
33	3.0	3.1	14.0

Average	5.671	5.852	7.255
Stan Dev	2.6377	4.63264	4.38314