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.

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.