The Effect of Microplastic Type on the Heart Rate of Filial Generation *D. magna*

Grant Proposal

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Abstract

Microplastics are a persistent issue in the environment as they have had hundreds of years to form as much as they do right now. Due to this long period of development, marine organisms have consumed microplastics, which along with the inhalation of microplastics and consumption of bottled water has led to microplastics being found in humans. Microplastics have been shown to have negative effects on the cardiovascular and reproductive systems separately, however, not much has been done to look at how transferred microplastics can affect organ systems in offspring or how different microplastics types can affect these results, which is what this project aims to analyze. It was hypothesized that the smallest size of microplastic and polystyrene microplastics would decrease the heart rate the most. So far, polyethylene and polypropylene suspensions of 1µL/mL and 0.2µL/mL were administered to *D. magna* for an acute period of 24 hours and a chronic period of 48 hours with their heart and mortality rates being recorded each 24 hours. Both polymers of concentration 1µL/mL and polypropylene of 0.2µL/mL were found to significantly decrease the heart rate in acute exposure, as well as all suspensions having significantly decreased heart rates compared to day 0 in chronic exposure with polyethylene 1µL/mL having a 20% survival rate. These results provide evidence that microplastics can affect the heart rate and life expectancy of an organism, so it would be worthwhile to continue to investigate how these effects translate to polystyrene and different microplastic sizes in filial generation organisms.

Keywords: microplastics, polymer, *D. magna*, heart rate, offspring, filial generation

The Effect of Microplastic Type on the Heart Rate of Filial Generation *D. magna*

Microplastics:

The ocean contains 5 trillion plastic pieces, which is equivalent to 250,000 tons of plastic (Yu et al., 2024). The high levels of microplastics are due to the fact that 400 million tons of plastic is produced annually, but only 9% is recycled, meaning that the rest ends up in landfills and oceans (Yu et al., 2024). Microplastics are plastics that are smaller than 5 millimeters (Rosen, 2024), and can be spread through the air, water, and through food chains via consumption of organisms exposed to microplastics (Roslan, 2024). There are two different types of microplastics, being primary and secondary. Primary microplastics are plastics that are purposefully manufactured to be small for industrial and commercial use. Secondary microplastics are more common and are created through the degradation of bigger plastics. They cannot be broken down easily due to how they are made up of long chains of synthetic polymers, which are resistant to biodegradation. The decrease in particle size is an issue, as when less than 20 micrometers, microplastics can penetrate cell membranes, causing issues with metabolism, reproduction, and behavior (Hale et al, 2020). Microplastics can also vary in shape, as they can be found in films, fragments, spheres, and fibers. The shapes can lead to variations in the locations of where the microplastics end up in the human body. For example, microfibers are more likely to remain in the intestines, while irregular shaped microplastics are more likely to cause issues than sphere shaped microplastics (Chen et al., 2023). This means that overall, the toxicity of secondary microplastics are more severe than primary microplastics, as the nature of degradation yields irregular shapes. The most common microplastics are composed of polyethylene, polypropylene, polyethylene terephthalate, polystyrene, polyvinyl chloride, or polyamide (Chen et al., 2023).

Effects on Cardiovascular System:

Overall, microplastics have been found in 8 of the 12 organ systems in the human body, one of which being the cardiovascular system, as microplastics have been found in blood vessels, thrombi, veins, and the heart. The microplastics in the blood were found to increase the frequency of cell division errors, which were linked to infertility, diabetes, obesity, cardiovascular disease, cancer, and neurological diseases (Roslan et al., 2024). When looking at patients with carotid artery disease, Marfella et al. in 2024 noted that out of the 304 people enrolled in the study, 181 were detected to have microplastics in the plaque of their arteries. The patients who had microplastics in their plaque were also more likely to reach the end-point of the experiment than without microplastics in their plaque, the end point being myocardial infarction, stroke, or death. The microplastics in the plaque were found to be jagged and less than 1 micrometer.

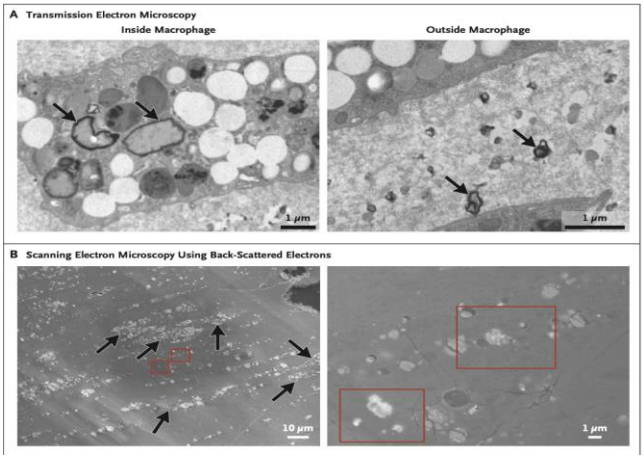

Figure 1: Close up images of plaque taken using two different methods. The image on the left of A depicts inside the tissue immune cell while the image on the right depicts outside the immune cell. The jagged shapes indicated by the arrows are microplastics. B depicts the overall images of the plaque.

In a study done by Wang et al. in 2022 on the toxicity of polyethylene microplastics on *D. magna*, it was found that when exposed to concentrations of polyethylene microplastics that were 20 micrometers in length, the heart rate of the *D. magna* decreased significantly, with the 30 micrometer concentrations decreasing the heart rate even further. In another study done by Hwang et al. in 2020 on the toxicity of polystyrene microplastic particles to human cells, particles smaller than 10 micrometers were found to penetrate through blood vessels, and

particles smaller than 5 micrometers changed cell membrane surface charges and aggregation of red blood cells, leading them to break down. However, when Chen et al. looked the effects of different polymers of microplastics on the embryo and larvae of zebrafish in their 2023 study, they found that the polyethylene and polystyrene microplastics increased the heart rate of zebrafish larvae at high enough concentrations, which contrasts the decrease in heart rate experienced by the *D. magna*. The heart rate can be a sign of a more underlying issue, such as tachycardia, bradycardia, and other heart rate arrythmias, which can correlate microplastic exposure to heart problems.

Effects on Reproduction:

Microplastics have also been found in and related to the reproductive system, as microplastics were detected in the testicles, breastmilk, meconium, infant feces, semen, and placenta. The continuous contact of microplastics could lead to the deterioration of sperm quality and might contribute to male fertility (Roslan et al., 2024). Also, due to the microplastics found in the placenta, microplastics overall could be transferred from parent to child. In a study conducted by Yu et al. in 2024 on how microplastic exposure to a parent mammal affected their offspring, they found that when the parent mice were exposed to the polystyrene microplastics, the children had microplastics found in their brain, liver, lungs, heart, and kidneys. They would display behaviors similar to anxiety, depression, and autism spectrum disorder behaviors. There were also connections between prenatal exposure to microplastics and gut damage as well as decreased reproductivity.

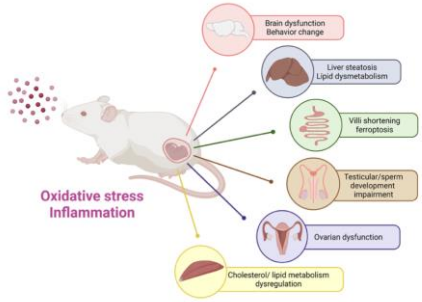

Figure 1. The impact of prenatal microplastic exposure on various organs of the offspring, including the brain, liver, intestine, reproductive system, and skeletal muscle. This diagram emphasizes the potential risks linked to maternal exposure to nanoplastics and microplastics, highlighting the necessity of further research to comprehensively clarify long-term effects on the health of offspring.

Figure 2: The visual results of prenatal exposure of microplastics on different organs in rats (Yu et al. 2024).

Why D. magna were chosen for this experiment:

Daphnia magna, or *D. magna,* are microcrustaceans that are common primary consumers in freshwater ecosystems (Nunes et al., 2022). They are good for tests involving toxicity in freshwater environments, due to their sensitivity to changes in the environment. They are also easy to handle in laboratory settings and have an important role in food webs (Nunes et al., 2022). Along with this, they are also a model organism for human heart rate, due to their heart being like that of a human (Kurien et al., 2018). They will therefore be used as the model organism due to their see-through body and relative ease to calculate heart rate. They also can reproduce relatively quickly, allowing for efficiency of testing.

Figure 3. Image of a *D. magna*. Their heart is above the eggs.

Section II: Specific Aims

This proposal's objective is to analyze how different types of microplastics affect the heart rate of the children of the affected generation of *D. magna*. The hypothesis is that the different

polymers and sizes will be present in the children of the affected generation, with the smaller the size the more present in the children. It is also hypothesized that the *D. magna* heart rates will increase and it will show behavioral changes when exposed to the different types of microplastics. The rationales is that with the smaller microplastics as well as polystyrene particles, the *D. magna* will become more stressed and its heart rate will increase, along with displaying more frantic behaviors. The work proposed here will help detail how microplastics could have cross-generational effects in different organisms, which could be tied to human effects.

Hypothesis 1: If *D. magna* are administered the smallest size of microplastic, then their heart rate will decrease the most.

Hypothesis 2: If *D. magna* are administered polystyrene microplastics, then their heart rate will decrease the most.

Section III: Project Goals and Methodology

Relevance/Significance

Microplastics have become a big issue as of late, with a lot of research being done in the field. With emerging worries and concerns with microplastics being found in human tissue and organs, as well as evidence pointing to cross-generational transfer (Yu et al., 2024), it is important to understand how different effects of microplastics on organs can interact, such as with cross-generational transfer and the heart. With a better understanding of how microplastic effects cross-generationally on organs work, it will be easier to take future steps into solving the issue.

Innovation

A lot of research has been done on how microplastics affect each organ system separately, such as the cardiovascular system and reproductive system, however, not much has been done to combine effects

from different organ systems to see if the effects are connected in any way. This project aims to connect the reproductive and cardiovascular systems through investigating how microplastics could transfer through generations and affect the heart of organisms.

Methodology

To achieve the goal of the project, *D. magna* were chosen as a model organism. Each *D. magna* will be selected and will be exposed to the fluorescent microplastic solutions of different sizes or different polymers for 4 days for their eggs to develop. Then, each *D. magna* will be washed off and transferred to clean water where every offspring within a 24-hour window will be collected. Finally, the offspring will have their heart rates counted and recorded, as well as having their bodies examined under a UV light to see if any of the microplastics transferred.

Hypothesis 1:

The objective is to see how different sizes of microplastics affect the heart of filial generation *D. magna*, specifically if the smallest size of microplastic decreases the heart rate the most. The approach is to expose *D. magna* to different microplastic size solutions and wait for them to reproduce before collecting their offspring and recording the heart rates as well as possible microplastic transference.

Justification and Feasibility. This section aims to see how different sizes of microplastics affect a parent *D. magna* heart rate before looking at how it would affect their offspring. In a study done by Wang et al. in 2022, they looked at how different sizes of polyethylene microplastics affected the heart rate of *D. magna*. This graph displays the average heart rate pretreatment and post 24-hour treatment and found that though both microplastic

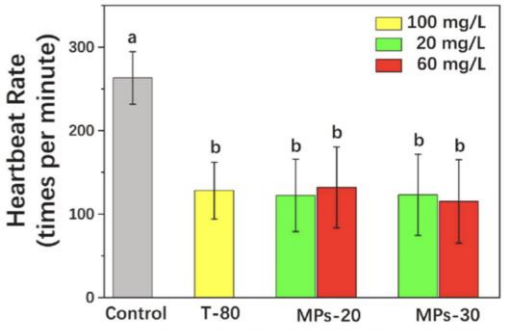

Figure 4. A bar graph from Wang et al. in 2022 that looks at how different sizes of MPs at different concentrations affect the heart rate.

sizes had a similar heart rate at 20mg/mL, the 30µm polyethylene microplastic (MP-30) decreased the heart rate slightly more than the 20µm microplastic did. However, Jahedi et al. in 2025 found that in a lot of zebrafish models, the smaller microplastic sizes induced bradycardia, so a microplastic size of <10µm would have a better chance of decreasing the heart rate when moving farther away from the threshold of the max size *D. magna* can consume.

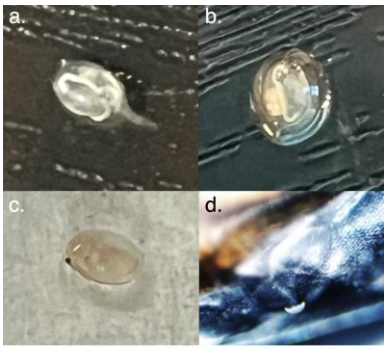

Figure 5. Images a-c depict qualitative data, where image c is a control *D. magna* whereas images a and b are *D. magna* post exposure to a microplastic suspension. The gut in image c is darker than the guts in images a and b, which are white. Image d is a *D. magna* heart under the microscope.

Summary of Preliminary Data. The maximum size of particle that a *D. magna* can intake is 50-70µm (Bethesda, 2005). Images a and b in the figure are *D. magna* that have been in microplastic suspensions for 24 hours. Unlike the non-exposed *D. magna* in image c who has a reddish gut, the *D. magnas* in images a and b have guts that are more of a whiteish color, suggesting that the microplastics administered to them were under 50-70µm.

Expected Outcomes. The overall outcome of this hypothesis is to show that smaller sizes of microplastics affect the heart rate much more than larger sizes. This knowledge can then be used for instigating research into strategies to mitigate the transference of microplastics into the body.

Potential Pitfalls and Alternative Strategies. Some potential issues are that the *D. magna* could all die due to factors such as too concentrated of a solution, too little food, or just natural causes. As death is a factor that cannot be avoided, preliminary data will be collected for the mortality of *D. magna* in the concentrations to troubleshoot how concentrated they should be. Another issue is that, though the microplastics administered were under 50-70µm, their sizes are still unknown and therefore nothing can be said about size effects. However, to combat this obscurity issue, microplastics of known sizes and polymers can be purchased for experimentation to show a more concrete correlation.

Hypothesis 2:

The objective is to see how different polymers of microplastics affect the heart of filial generation *D. magna*, specifically if polystyrene microplastics decrease the heart rate the most. The approach is to expose *D. magna* to different microplastic polymers and wait for them to reproduce before collecting their offspring and recording the heart rates as well as possible microplastic transference.

Justification and Feasibility. This section aims to analyze how different microplastic polymers could affect a parent *D. magna* before looking at how it would affect their offspring. In a study done by Chen et al. in 2023, they looked at how different microplastic polymers affect the larvae of zebrafish. The graph displays various polymers of microplastics, including polyethylene, polypropylene, and polystyrene, as well as different concentrations with images of each embryo chorion.

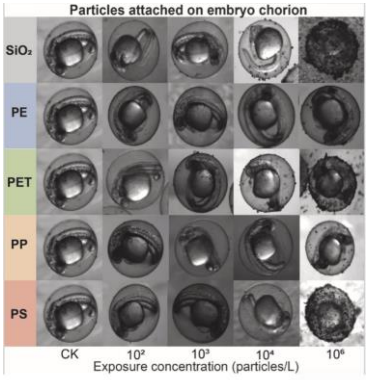

Figure 6. A figure from Chen et al. in 2023 that looks at different polymers and different concentrations of polymers on how much they stuck to the embryo chorion of zebrafish.

Out of polyethylene, polypropylene, and polystyrene, it was found that polystyrene stuck to the embryo the most at a 10^6 particles/L concentration. This could reflect in eggs in *D. magna*, where the polystyrene could be more likely to stick to the eggs and cause the embryos to be exposed to a higher concentration of microplastics, leading to a decrease in heart rate.

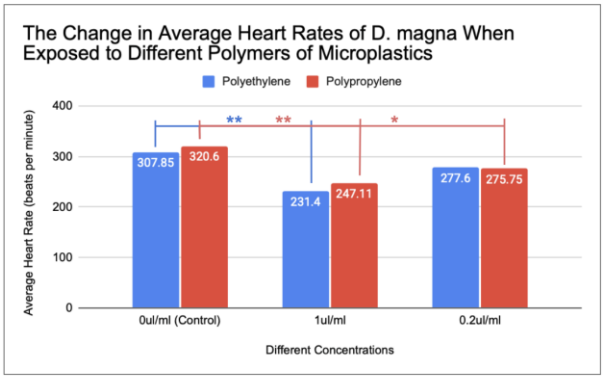

Figure 7. A bar graph that compares the average heart rates between the different concentrations of polyethylene and polypropylene microplastics in the acute test. Compared to the control, the polyethylene and polypropylene 1ul/ml concentrations were significant by a p value of <0.01 (p=0.002 and p=0.009), while the polypropylene concentration of 0.2ul/ml was significant by a p value of <0.05 (p=0.03).

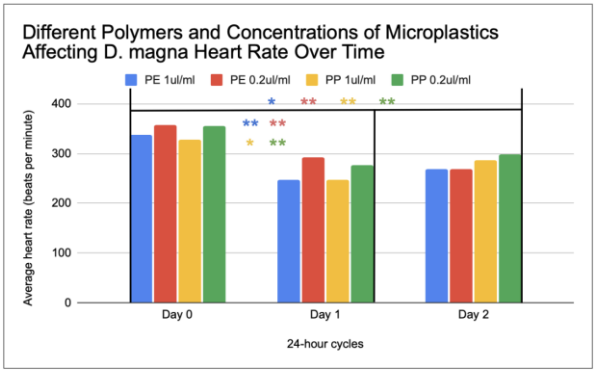

Figure 8. A line graph showing how the average heart rate of a *D. magna* changed over time in 24-hour periods when exposed to different concentrations and polymers of microplastics. All polymer and concentration combinations had a p value <0.01 from Day 0 to Day 1 and had a p value of <0.01 from Day 0 to Day 2 except for polyethylene at 1ul/ml, which was <0.05.

Preliminary Data. The data represents the average *D. magna* heart rates after being exposed to different concentrations of different concentrations for 24 hours and 48 hours. The data shows a decrease in heart rate after being exposed to microplastics, with polyethylene having a lower heart rate than polypropylene and retaining that lower heart rate for longer. This does give evidence that microplastics can decrease the heart rate, and that different polymers could change the severity and duration of the heart rate decreasing.

Expected Outcomes. It was expected for the heart rate to in general decrease, with polyethylene having a more pronounced effect, which was supported by the preliminary data collected.

Potential Pitfalls and Alternative Strategies. Some potential pitfalls are the avaliability of polystyrene microplastics, as well as keeping the size consistent amongst the polymers. Another pitfall could be the measurements of each microplastic to make them into solutions, as the amount needed to make solutions is a small amount. Some alternative strategies are to address consistent size and availability is to prioritize consistent size in acquiring microplastics regardless of color or fluorescence, as well as using a more powerful scale to be able to weigh the microplastics out better.

Section III: Resources/Equipment

Materials:

- *D. magna* (over 200)
- *D. magna* food (yeast and water mixture)
- Different polymers of fluorescent microplastics with consistent sizes
- Different sizes of fluorescent microplastics with consistent polymers
- Microscope with a record feature
- Well plate for isolation of *D. magna* and collection of second generation.
- Milli Q water mixed with salty shrimp RH/KH+ to add back minerals in the Milli Q water

Procedure:

1. Take *D. magna* and analyze them under a microscope to see if they have eggs. Take all *D. magna* with eggs in the egg sac.
2. Put one *D. magna* in each well and put the polymer solution in each well.
    - The polymer solution will be made by taking an equivalent amount of each polymer and mixing each in the Milli Q water RH/KH+ solution.
3. After exposing period, take the *D. magna* out and put each individual one into a new well of clean spring water.
4. Once they reproduce, take the parent *D. magna* out of the well and wait a few days for the offspring *D. magna* to be big enough to analyze the heart rate.
5. Take each offspring *D. magna* and record the heart rate under a microscope.
6. Take the offspring *D. magna* analyze under a UV light. This is to see if any of the microplastics transferred from parent to offspring.
7. Repeat steps 1-6 for each polymer solution and the control group in part 1 of the experiment. Part 2 of the experiment is relatively the same except instead of different polymer solutions, it is different microplastic size solutions.
8. Repeat steps 1-7 for each trial.

Section V: Ethical Considerations

The microplastics that will be used will be small enough to get absorbed through human skin, as well as microplastics overall being harmful for the environment. To address the health concerns, a lab coat, goggles, and nitrile gloves will be worn. To address the environmental risks, all equipment and tests that interacted with microplastics will be thoroughly washed and contained. All microplastics contained will be identified and isolated according to the WPI guidelines for hazardous materials and will be disposed of by WPI.

Section VI: Timeline

- Early November: Research, purchase materials, find a lab that fits the equipment requirements that I need.
- Mid-Late November: Research, preliminary data, trouble shooting.
- Early-Mid December: Finish Research, preliminary data, trouble shooting, preparing for December fair.
    - By Dec 5: Preliminary testing done on *D. magna* to analyze the effects of caffeine and ethanol on heart rate to provide baselines for increases and decreases in heart rate respectively.
    - By Dec 12: Preliminary testing done on *D. magna* to analyze the effects of caffeine and ethanol on the filial generation to troubleshoot the actual project procedure, as well as testing done on one microplastic type to troubleshoot the experiment with the actual independent variable.
- Early-Mid January: Start actual data collection.
    - By January 23 testing on different sizes and polymers of microplastics should be done.
- Late January: data analysis, prepare board and presentation.

- By January 30 data analysis and board should be made.
- Presentation will be practiced until February fair.

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