

Glow and Grow: The Effect Of Microplastics on Development and Lifespan in *C. elegans*

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Abstract:

Microplastics are the most prevalent type of artificial debris in most waterways. There is little knowledge about the effect microplastics have on organisms. This project evaluated the effect of microplastics on the development and lifespan of *C. elegans*. This model organism was selected because *C. elegans* are non-parasitic, non-hazardous, transparent, and share a large number of genes with humans. In this investigation, wild-type *C. elegans* were exposed to fluorescent plastic microbeads ranging in size from 1-5 μ m to determine if the worms would ingest them and what affects the beads would have on reproduction and development. We hypothesized that: 1) *C. elegans* will ingest the microplastics and 2) *C. elegans* exposed to the microplastics will develop twice as fast through their larval stages. L3/L4 worms were added to plates with or without microplastics and allowed to reproduce. The eggs and larvae were then counted three times a day and their stages of development were recorded until those worms became reproductive adults. Fluorescent microscopy showed the presence of microplastics in the digestive tract of the worms, confirming the ingestion of microplastics and supporting the first hypothesis. The data did not indicate an effect of microplastics ingestion on the development of *C. elegans*, and therefore the second hypothesis was not supported. This experiment will be repeated to reduce variation within control and experimental groups.

Introduction:

Microplastics are the most prevalent type of marine debris in our oceans, rivers, lakes and many other sources of water; including the ones we drink. Microplastics end up in almost every part of everyday life from cooking to washing your face. Because microplastics are small, measuring less than five millimeters in length, they can easily flow through filtration systems making it easier for them to be widespread in their effects (What Are Microplastics).

Caenorhabditis elegans or *C. elegans* are a model organism that was used in this project. They are transparent and measure one millimeter long when in adulthood. They are non-parasitic, non-pathogenic, non-hazardous, and free living. Most notably they are hermaphrodites, meaning they do not need another individual to reproduce as their body contains both male and female reproductive organs (Edgley). One benefit to using *C. elegans* for this particular experiment is that they are also consistent eaters, meaning they move around the plate eating anything that comes in their way using the pulse of their pharynx(Avery). This means if something is on their plate it will most likely be eaten if it is small enough, for example bacteria or bacteria-sized plastics. Another benefit to *C. elegans* is that they have many genes in common with humans and are similar on a cellular level. This could mean the results uncovered in *C. elegans* are applicable to humans. One last benefit is that the developmental stages of the *C. elegans* are very clear. The developmental stages are split into four larval stages and adulthood and each of the categories have features that make them identifiable from each other. L1s are tiny, move slowly, and are almost transparent, with very few organs that are noticeable. L2s have

noticeable internal organs, but are still small and move slowly. L3s have all visible internal organs besides eggs and are almost fully grown in size. L4s have all noticeable internal organs as well as eggs and move at a faster rate than previous stages. Adults are barely visible by the naked eye, reaching one millimeter in length and have fully developed eggs to lay (Figure 1). The *C. elegans* spend a specific amount of time in each stage that makes the life cycle predictable. In this project, I studied the effects of microplastics on the development and lifespan of *C. elegans*. Microplastics are a relatively new phenomenon that could have profound effects on people's health and the way they develop. Although *C. elegans* and humans have similar pathways they are not the same and this is only to ideate the effects.

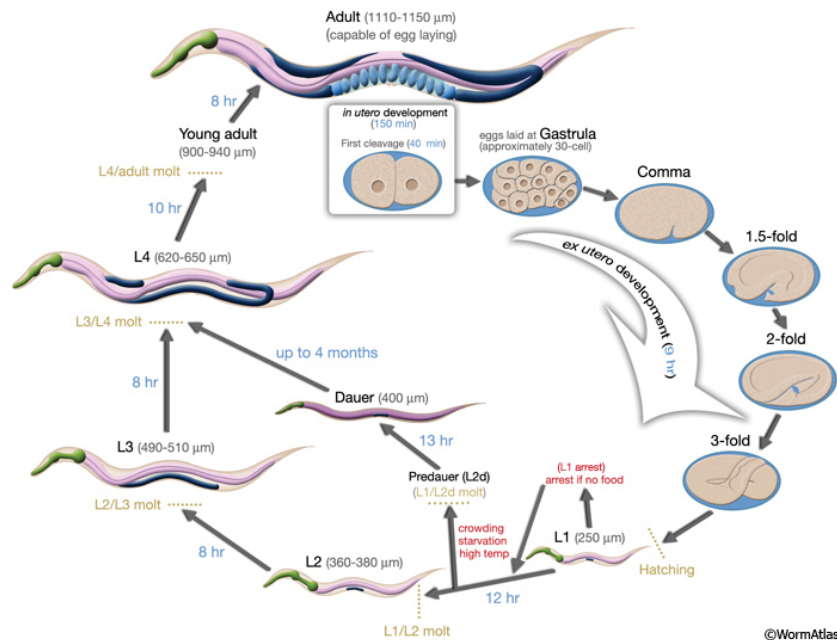


Figure 1. Illustration of the *C. elegans* life cycle. This figure shows each development stage as well as the timing. Figure from WormAtlas (Herndon)

When talking with Dr. Peter Faletra about this topic something interesting came up; how BPA in plastic can have an effect on children's development, making them develop faster. Embryos can develop much faster when exposed to BPA or BPS compared to non-exposed embryos (Schmidt, 2016). This information contributed to two hypotheses. My hypotheses were that 1) the worms will eat the microplastics and 2) when the microplastics are introduced, the larval development of *C. elegans* will be twice as fast. Here, we tested these hypotheses by feeding the worms fluorescent microbeads and observing the development of progeny.

Methods:

In the experiment, both the experimental and control groups had three plates, all filled with *C. elegans* nematode growth medium (NGM). Under a sterile environment the three experimental plates had 100 microliters of microplastic solution applied to them. A microplastic solution was made using fluorescent microbeads (Cospheric) at a concentration of 3.57 μg/ml in autoclaved tap water. These beads range in size from 1 μm to 5 μm, meaning they are largely small enough for the *C. elegans* to eat. Then all six of the plates had 150 microliters of OP50

E.coli bacteria, the standard *C. elegans* food source, applied to them. Five L3/L4 worms were put onto each plate and allowed to reproduce. When all of the plates had eggs on them, the worms were removed. Numbers of eggs, L1s, L2s, L3s, L4s, and adults were then counted three times a day at: 8:45am, 1:00pm, and 7:00pm. The numbers for each category were recorded in a spreadsheet. Once there were adults on the plates, the plate was retired from experimentation because these adults could now start laying their own eggs, resulting in a second generation.

After the experiment had been completed and the developed worms had been on the microplastic plates for all of their life they were moved to plates without microplastics for 30 minutes to allow microplastics stuck to the outside of their bodies to be shed off. Then, the worms were photographed using a Stereo Fluorescence Microscope Adaptor (Nightsea), a Nikon Stereozoom microscope, and a Canon Rebel T5i camera. Since the plastic microbeads are fluorescent, this setup allowed for their visualization inside the worms if they were ingested.

Results and Discussion:



Figure 2. *C. elegans* containing microplastics. This microscope image was taken with both white light and the fluorescent light setup for green fluorescence.

By imaging the *C. elegans* with the fluorescent light setup for green fluorescence and dim transmitted white light, we can see the worm itself and the fluorescent microbeads within it (Figure 2). The microplastics travel in a line down the body, which starts just after the head and pharynx, suggesting the microplastics are present throughout the digestive tract.

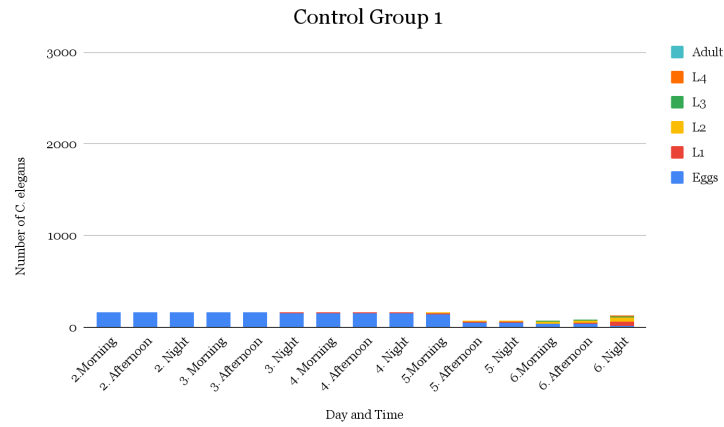


Figure 3A

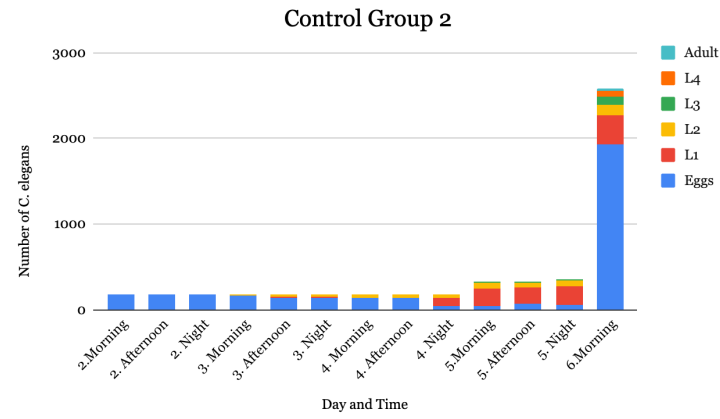


Figure 3B

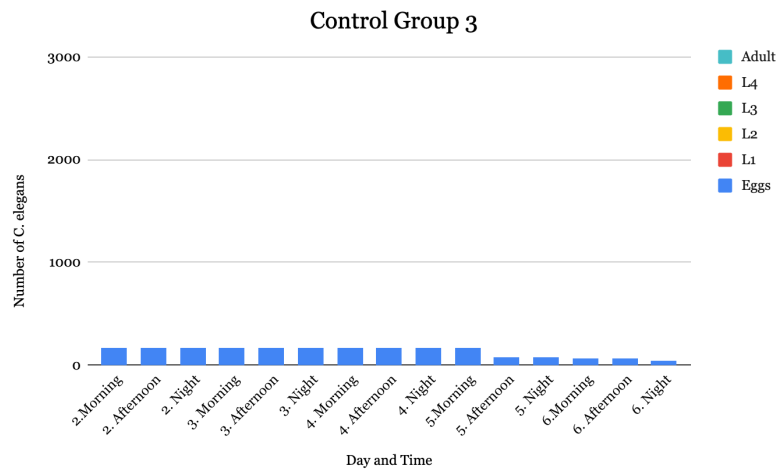


Figure 3C

Figure 2. Control group development over the course of the experiment. Each group is presented as its own graph: Control Group 1 (3A), Control Group 2 (3B), and Control Group 3 (3C). Stacked bars show the number of eggs and each stage of larval development over the experimental time points. All of the control data is on a scale of 3000.

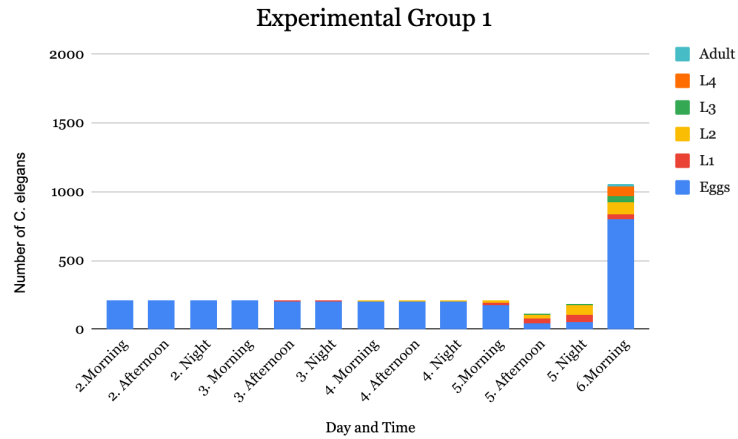


Figure 4A

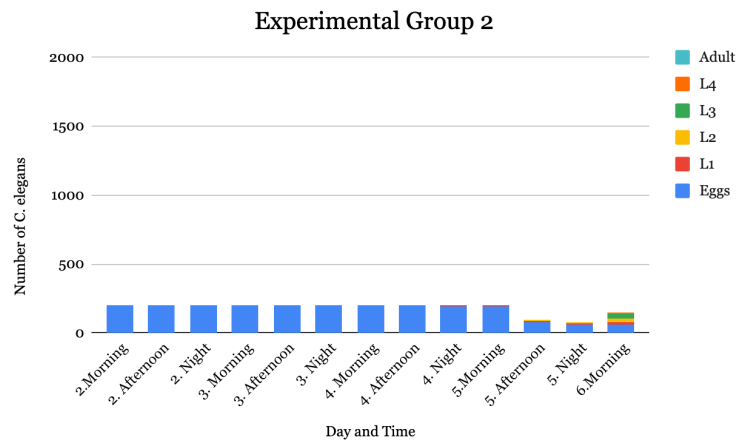


Figure 4B

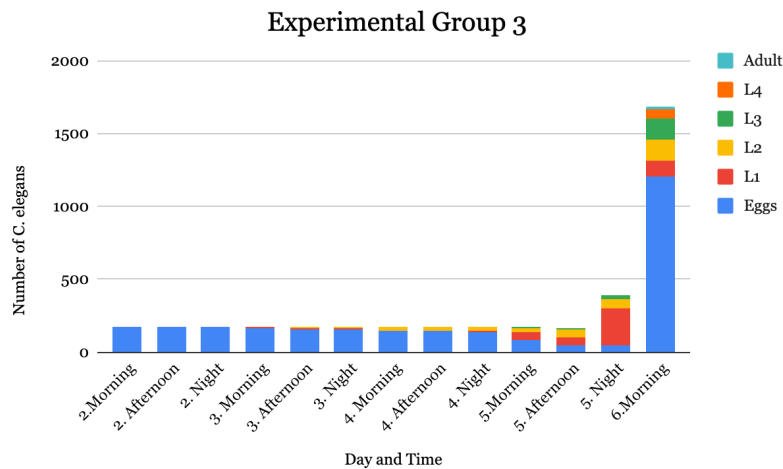


Figure 4C

Figure 3. Experimental group development over the course of the experiment. Each group is presented as its own graph: Experimental Group 1 (4A), Experimental Group 2 (4B), and Experimental Group 3 (4C). Stacked bars show the number of eggs and each stage of larval development over the experimental time points. All of the experimental data is on a scale of 2000.

The graphs above show each of the plates from the experiment and the amount in each of the categories over time. Control group one started showing worm development on the Day 3 night count and the experiment was stopped on the Day 6 night count (Figure 3A). I stopped the experiment when the first generation adults could reproduce because their eggs and young worms were second generation and I did not want them mixing with the first generation. Control group two started to show development beyond eggs on the Day 3 morning count and ended on Day 6 evening count (Figure 3B). The reason the last bar on this graph is so big is because the adult worms from generation one reproduced overnight before the final count of the plate. Control group 3 had eggs from the beginning similar to the other plates but none of the eggs hatched and grew into worms (Figure 3C). I retired this plate because the eggs were not hatching on Day 6 night. Experimental group one had eggs start hatching on Day 3 during the afternoon count. (Figure 4A) The plate was retired on Day 6 in the morning count. Experimental group two started showing egg hatching on Day 4 in the morning and was retired on Day 6 in the morning (Figure 4B). The final experimental group started hatching on Day 3 in the morning and was retired on Day 6 in the morning (Figure 4C).

When looking at the data, all of the plates were retired on day 6 at some point, all first showed worm development on day 3 or 4, and all of the data, except the unusual control plate (Figure 3C), had their highest numbers on the last day, due to the reproduction of the generation one adults. Control Group 3 was unusual because no eggs on that plate hatched. Few conclusions can be drawn from these data for the developmental speed. The unusual control plate prevented comparisons on start and finish time and the use of statistics for my conclusion benchmarks because there were not three on both sides to compare. From what this preliminary data shows, the microplastics do not have a dramatic effect on the developmental speed but more data collection needs to be done before those conclusions can be verified and supported with statistics.

Visualization of the worms clearly showed the microplastics inside of them (Figure 2). Due to the position of the microplastics we can assume that the microplastics are consumed because they appear to be in the digestive tract. Another reason the assumption of the microplastics being in the digestive tract can be made is because the *C. elegans* were moved to a plate with no microplastics for 30 minutes before imaging to allow microplastics stuck to the worms' cuticles to be shed. My original hypotheses were that 1) *C. elegans* will eat the microplastics and 2) when the microplastics are introduced, the development of *C. elegans* will be twice as fast. From my data, I can conclude that my first hypothesis is supported. The second hypothesis requires more experimentation. Lifespan studies also require more research. The experiment is currently in the process of being repeated.

Sources:

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