Ingestion of Tire Wear Microplastics Causes Potential Neurological Harm in Planarians, Manifested as Slower Phototaxis

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Abstract

Microplastic pollution has increased over the past decade and is causing detrimental effects on living organisms. While research on microplastics and their impacts on ecosystems is expanding, specific investigations of tire wear particles (TWP) are limited. This experiment focuses on tire wear particles' effect on planaria's phototaxis response. In an 8-week experiment, planarians were fed TWP-dosed food and then observed for a possible delay in phototactic behavior. Current research suggests that TWP will increase the time for planaria to move away from light due to possible neurological damage. To conduct this research, the liver paste was mixed with varying concentrations of tire wear as food for eight weeks. Each week, the duration of a repeatable phototactic response was measured in the control and test planaria. Results show that the 1.5% concentration of (TWP) in the liver paste negatively impacts the planaria phototaxis, reflected by increased time to evade a light source. These results can be applied directly to the evolutionary selection of wild planaria and indirectly to human health.

1.0 Introduction

Microplastic pollution has become a severe environmental issue with the continued use of plastics. Current estimates suggest that over 170 trillion plastic particles float in the ocean (Eriksen et al., 2023). Of this total, 5-10% come from tire wear particles (TWP), a type of plastic comprising a mixture of synthetic rubber, filters, and softeners (Kole et al., 2017; Robbins, 2023). While TWPs are among the most significant and common contributors to microplastic pollution, research on their effects is limited (Luo et al., 2021).

1.1 Background

1.1.1 Microplastic Composition and Impacts on Aquatic Organisms

Microplastics comprise plastic particles less than 5mm (Rogers, 2023). They are solid, water-insoluble, with a low degradation rate, and contain chemical additives harmful to the environment (Järlskog et al., 2022). Plastic has often persisted in the marine environment for hundreds of years (Bajt, 2021). Microplastics can also easily be washed into freshwater or ocean water, impacting aquatic ecosystems and causing neurological damage to aquatic species (Rogers, 2023). Previous studies suggest marine organisms' intake of microplastics could eventually change their eating behavior, inhibit growth, and cause genetic damage (Li et al., 2021; Gao et al., 2022).

1.1.2 Tire Wear Particles Composition and Concentration

Tire wear particles (TWP) are materials that arise from rubber and differ in the type of tire, size, and weathering condition. They range from 4μm to 265μm depending on factors like driving speed and tire composition (Kovochich et al., 2021). TWP concentration in the environment varies locally. For example, the concentration of environmental runoff on rural highways in Sweden has up to 38% tire wear particles (Järlskog et al., 2022). At Kraków, Poland, the tire wear particles in the sediments approach up to 50% (Worek et al., 2022). As these microplastics degrade over time, they become nanoparticles that directly interact with biological cells, such as tissue, blood, etc. For example, a recent study showed that 90% of the plastics found in bottled water were nano-sized (*Plastic Particles in Bottled Water*, 2024).

1.1.3 Planaria Anatomy and Physiology

Planaria are a type of flatworm found in saltwater and freshwater environments. With a central nervous system (CNS) similar to humans, the planaria nervous system affects behaviors such as negative phototaxis response (light avoidance). The ocelli (eyespots) absorb the light

signals from the planaria's pigment cells, which are received at the neural ganglia, and send a message to their motor system, creating a physiological response (Paskin et al., 2014). Unlike humans, their food is taken in through the pharynx, is digested at the gastrovascular cavity, and waste is ejected back at the pharynx. Nutrients and minerals are ingested through the tissue by diffusion(Reho et al., 2022). In addition, planaria typically glide in a straight line and sometimes use body contraction, similar to earthworms(Werner et al., 2014; *Planaria | the Wildlife Trusts*, n.d.). However, when planaria develops diseases, it may experience morphological changes, including spiraling, head twisting, shape change, and orientation problems(Grebe&Schaeffer, 1991; Wu et al., 2012).

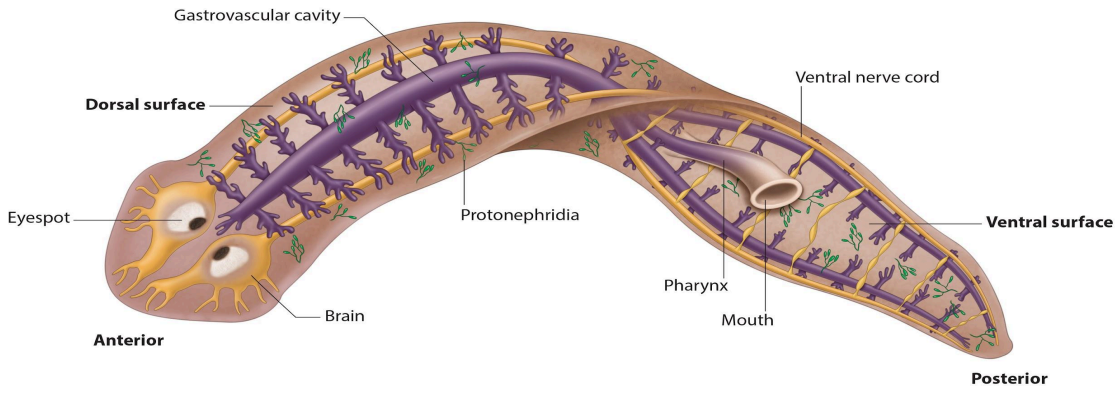


Figure 1: Anatomy of Planaria (*Anatomy | Planarian Educational Resource*, 2013)

1.1.5 Learning Ability of Planarians

Spatial localization refers to learning and remembering locations and is one of the essential survival and adaptation characteristics in living organisms. Qian et al. showed that planaria exhibit spatial localization by finding the entrance of the dark chamber in a shorter time after six days of one-hour-per-day training (Qian et al., 2023).

1.2 Literature Review

Planaria display different behavioral responses based on exposure to different wavelengths of light. A shorter wavelength will provide a more intense photophobic response, while longer wavelengths will elicit no response from planaria. Research by Paskin et al. in 2014 shows that planaria's photophobia has these two separate responses and demonstrates the negative phototaxis of planaria under different spectra of light, showing how reactions vary with color. It provides a general idea of planaria's phototaxis behavior under each color of light and the method to use when collecting data, which helps set our experimental protocols.

Shomrat and Levin studied planarians' long-term memory in 2013. They were investigating the effect of brain regeneration on memory and learning. For their study, planarians were trained continuously for 10-11 days in a specific paradigm. The result shows that the familiarized group takes a shorter time to reach the requirement. This research demonstrates planaria's learning ability and memory-storing behavior so that they can be trained and adapted for particular tasks. It also suggests that their learning ability could be a factor in this experiment.

1.3 Driving Question and Project Statement

Understanding how tire wear particles (TWP) affect organisms is crucial, as TWP significantly impacts the environment, wildlife, and human health. However, current research on TWP impacts primarily concerns fish and macroinvertebrates (Cunningham et al., 2022). To fill this gap, this project explores the effect of TWP on the phototaxis behavior of planaria. Planaria were chosen because they live in TWP-laden sediments and have a nervous system similar to humans.

Specifically, this experiment examines planaria's phototaxis behavior after ingestion of different concentrations of tire wear particles. We hypothesize that TWP will increase the time for planaria to move away from light due to damage in the nervous system.

2.0 Methods

The method used in this experiment involves collecting tire wear particles, caring for planaria in a controlled environment, setting up an experimental group, mixing the tire wear with the liver paste, and completing phototaxis observations.

2.1 Tire Wear Particles

(TWP)Tire wear particles were collected by abrading the rubber of an automobile tire. Jianing Xu, a classmate, counted the tire wear samples and categorized them into different sizes using a compound microscope. The tire wear particle distribution diameter, shown in Figure 2, reflects a visual count and classification of sizes under 40x magnification (Andrews et al., 2024). Different concentrations of TWP were mixed with blended liver paste for feeding. The detailed tire wear-liver paste preparation protocol can be found in the Appendix. TWP concentrations reported in sediment measurements range from less than 1% to 50% (see Figure 3). A concentration >2% was not pursued since the liver paste will be full of TWP, which might decrease the amount of liver paste the planaria consume during the feeding process.

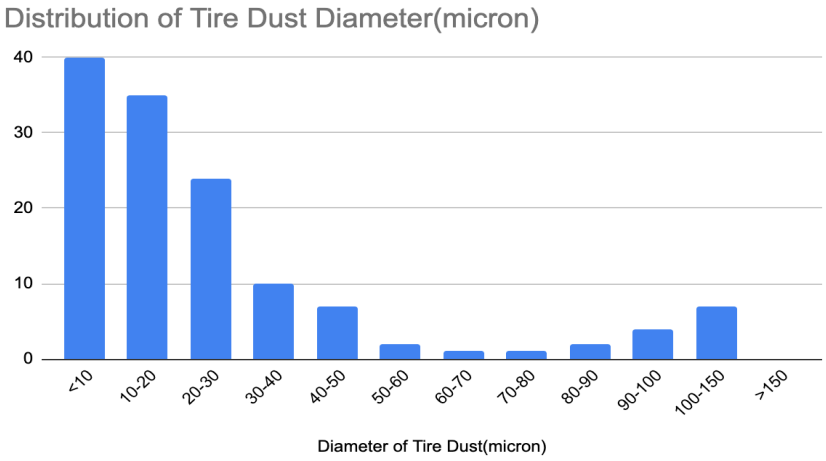


Figure 2: Size Distribution of Tire Wear Particles

Author	Tire Wear Concentration in the Sediments (g TWP /Kg liver paste)	Mass percentage concentration
Ni et al., 2008	0.4 - 2.2	0.04%- 0.22%
Redondo-Hasselerharm et al., 2018	0.3 - 155	0.03%- 15.5%
Worek et al., 2022	480	48%

Figure 3:Tire Wear Environmental Concentration Data

2.2 Habitat for Planaria Care

Dugesia tigrina purchased from Carolina Biological were placed in Petri dishes with Arrowhead spring water. Experimental groups of 5-10 planarians per dish were housed in a dark, insulated box (kept at 19-20°C), fed once a week, and changed their water twice a week. The detailed Planaria care protocol can be found in the Appendix.

2.3 Experimental Groups

The experimental group consisted of four planaria groups, each fed a different concentration of tire wear particles mixed into the liver paste. The experimenting concentrations were 1.5 g/kg(0.15%), 7.5g/kg(0.75%), 15 g/kg(1.5%), and one control group was fed with regular liver paste. The environmental concentration data we found during our research, explicitly stated in 2.1.0, helped us determine the concentration groups. For the experiment, 55 planaria of similar sizes are picked. In the first part of the experiment, five planaria are placed in each labeled petri dish (control, 0.15% TWP, and 1.5% TWP). In the second part of the experiment, ten planaria are placed in each labeled petri dish (control, 0.15% TWP, 0.75% TWP, and 1.5% TWP). During the phototaxis observation phase, planaria are fed every Friday and tested for light sensitivity (phototaxis) every Tuesday after the feeding.

2.4 Phototaxis Method

Phototaxis is measured using a diagram with four circles that have a diameter of 2, 4, 6, and 8 inches from the center point. The paper was taped below the rectangular petri dish, matching the flashlight to the center dot. The planaria were transferred to the center dot, moving the Petri dish if not initially centered. The time planaria traveled out of the light was recorded by simultaneously turning the flashlight and timer, measuring the time the head of planaria reached each consecutive ring. Maximum uncertainty in time measurement is expected to be ±1.5 seconds (including stopwatch reaction time and parallax). If the planaria does not reach line 2 within 90 seconds, time is automatically stopped, and the worm is removed from the data set for the week. Data was collected over eight weeks.

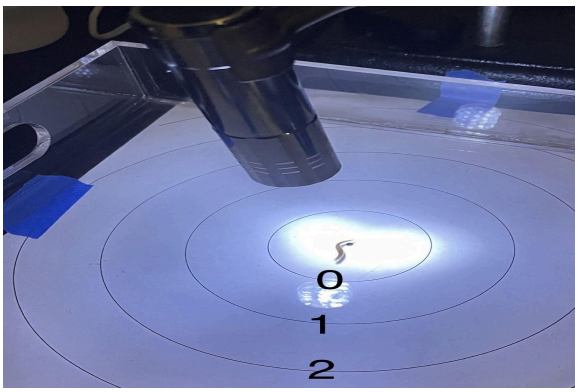


Figure 4: Phototaxis Observation

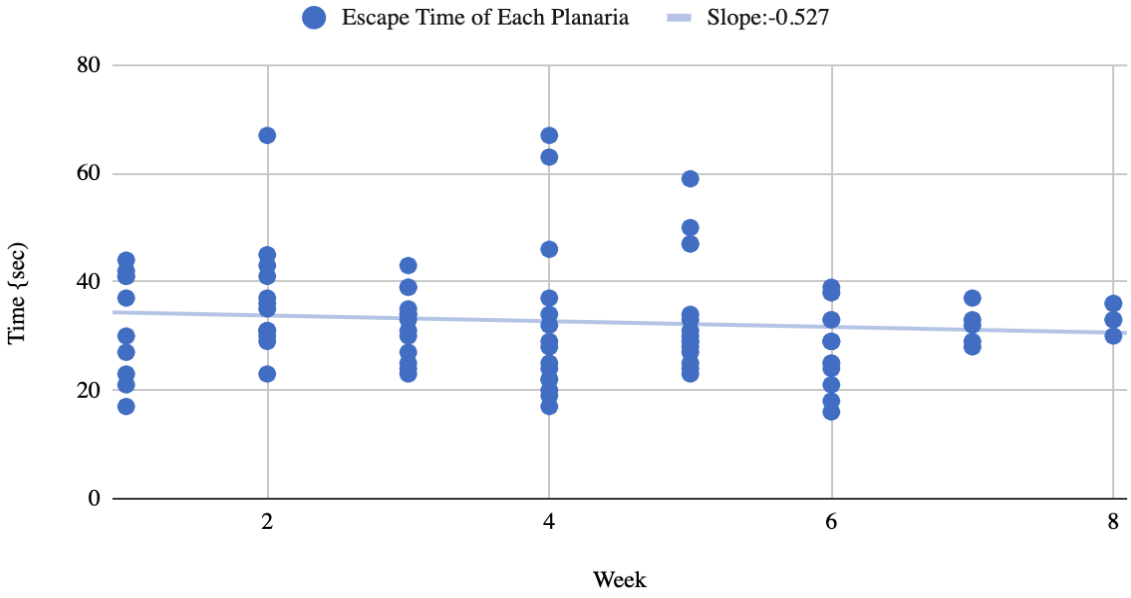
2.5 Morphological Data Collection

Starting in week 5, the abnormal behavior of planaria was observed and documented during the phototaxis testing. Determination of morphological issues was accomplished using the method found in other articles (Grebe&Schaeffer, 1991; Wu et al., 2012).

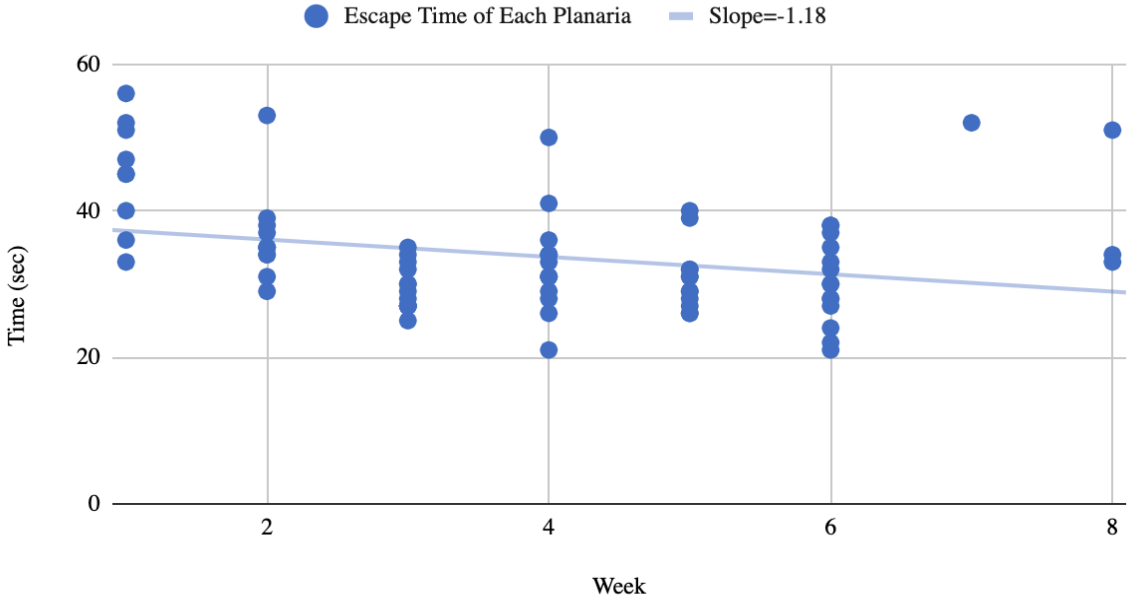
3.0 Results

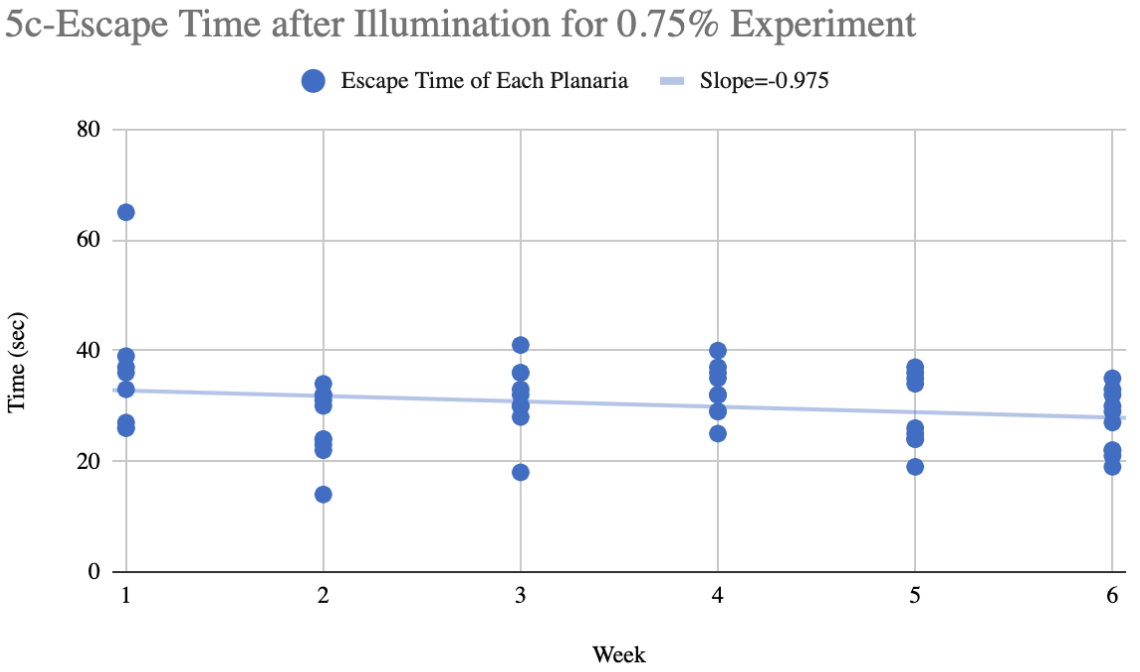
The escape time of planaria in each concentration group is shown in Figure 5. The first observations of phototaxis (week 1 in Figure 5) began after two weeks of TWP feeding.

5a- Escape Time after Illumination for Control Group



5b-Escape Time after Illumination for 0.15% Experiment





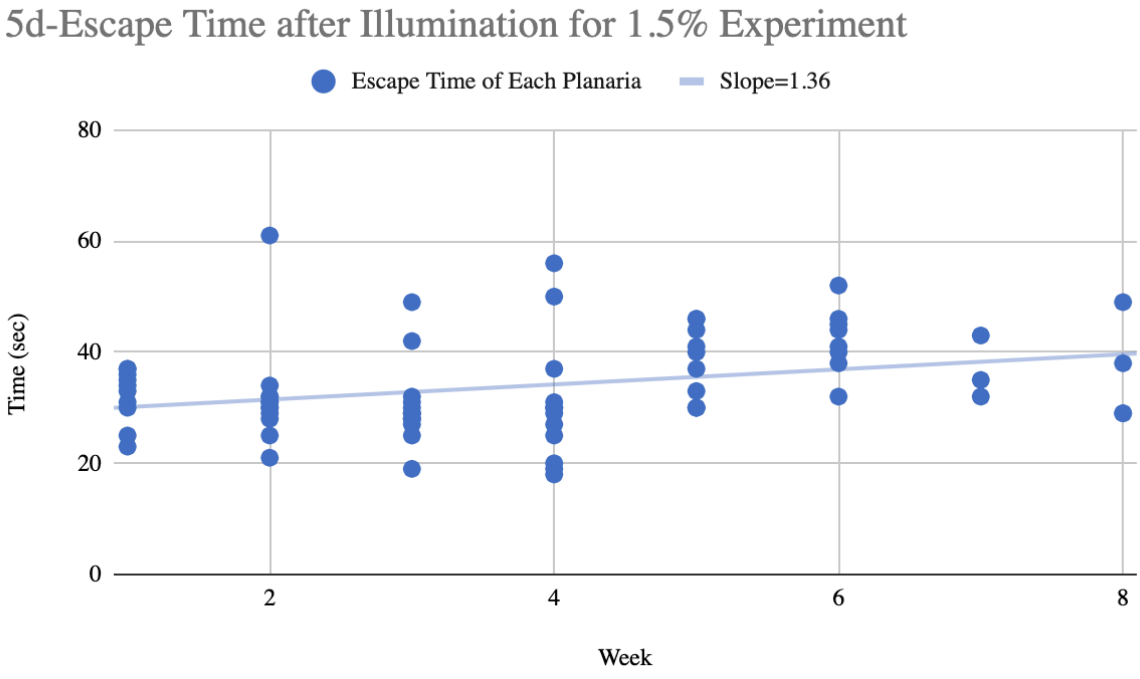


Figure 5a - 5d: Planaria escape travel time out of the light throughout the 8-week experiment for the control group (5a) and three experimental groups (5b, 5c, 5d).

The slopes in Figure 5 indicate the time change for planaria escaping out of the light for each concentration group. Positive slopes show that there has been an increase in time for planarians to move out of the light, while negative slopes show a decrease in time. Except for the 1.5% concentration groups, the other groups have a decrease in time, reflected by a negative slope. The number of data points varies weekly since some worms are lost to death, and others do not complete a trial within 90 seconds.

4.0 Discussion

4.1 Phototaxis Response Change

We expected that the higher the concentration of tire wear particles that planarians were exposed to, the more time it would take them to move out of the light. This expectation is consistent with the idea that TWP could damage musculature, photoreceptors, or cause neurological damage that decreases learning ability or inhibits the signal sent to the motor system. With this interpretation, the control group should show no change, and increasing TWP concentration should show increasing levels of slowing. Figure 6 shows each group's speed change (the slopes from data charts in Figure 5) throughout the experiment. Only the highest concentration of TWP exposure (1.5%) resulted in a slowing of the phototaxis response. The other experimental groups (including the control) got faster at avoiding light over the course of the experiment. This fact cannot be explained by the earlier interpretation, which leads to the idea of learning.

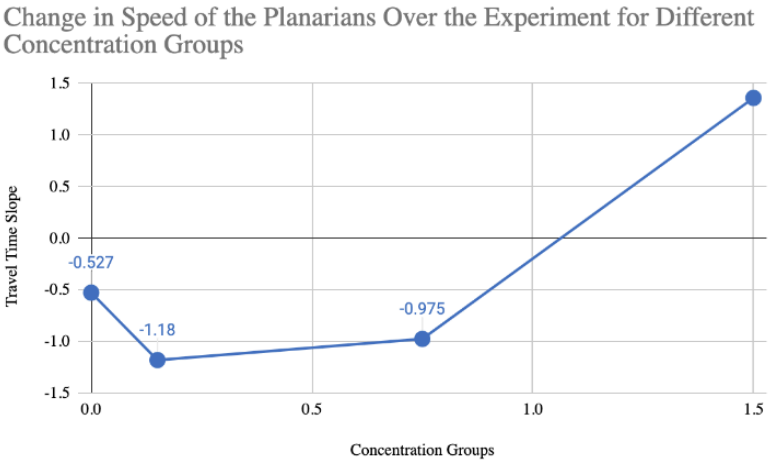


Figure 6: The Speed Change of the Planaria's Escape Time Out of the Light for Each Concentration Group

4.2 Morphological Data

According to Figure 7, planarians with higher concentrations experienced more behavioral issues than the control and experimental groups with lower concentrations. While moving out of the light, the planarians with 1.5% tire wear particles tend to move horizontally around the light circle rather than vertically out of the light circle. They also have a higher observed frequency of head twisting and spiraling issues. These types of motion are known to be the result of neurological damage (Grebe&Schaeffer, 1991; Wu et al., 2012).

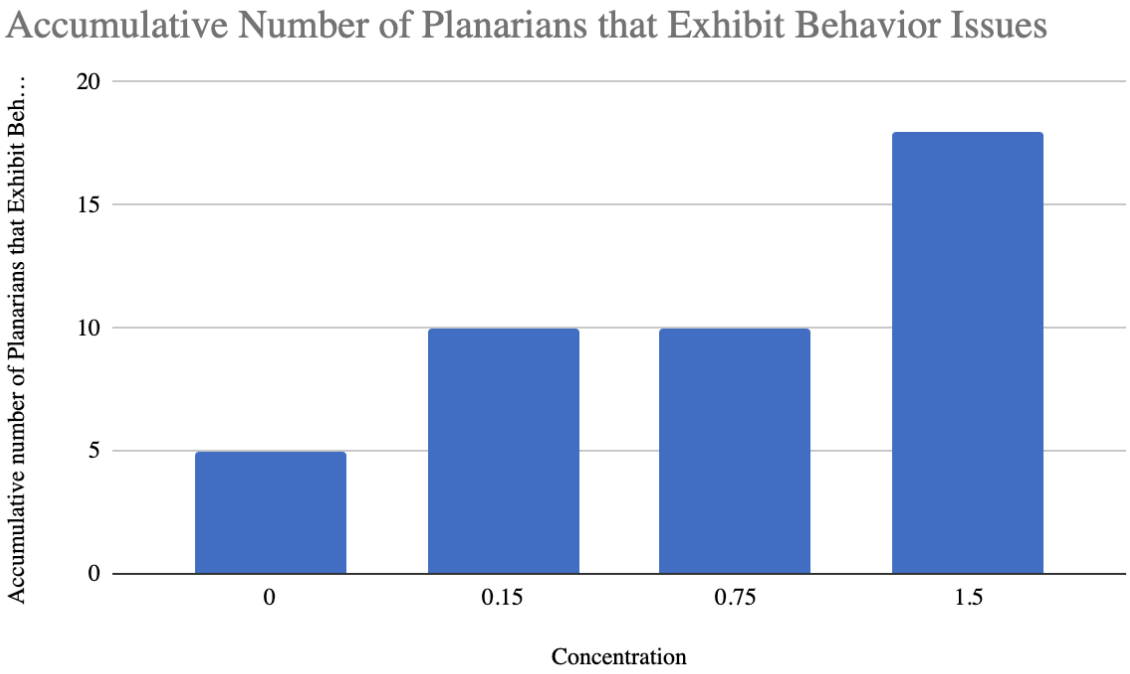


Figure 7: Number of Planaria that Experience Behavior Issues in Each Concentration Group

4.3 Neurological Harm

Even though several possible mechanisms might affect the planaria's phototaxis behavior, The two sets of data point to disruption in th learning ability of planaria. The morphological data

suggests a neurological effect instead of damage on musculature or photosensitivity damage. The time decrease of control and low concentration implies that planaria in these groups become familiar with the examination method throughout the experiment. Therefore, the most probable explanation is a negative neurological impact on planaria's learning ability. The phototaxis and morphology data would be stronger with a larger sample size for the experimental groups. Future studies are recommended to determine the exact cause of the observed changes since this experiment could not distinguish between the three interpretations we have suggested.

4.4 Significance

This experiment has two major implications. The wild planaria might be more vulnerable to predation if they suffer from phototaxis degradation. This may be an additional evolutionary selection pressure if TWPs are present in their environment. The suggestive result of damaging planaria's nervous system, potentially the learning ability, reflects the natural selection of planaria in the wild. The result can also imply the possible harm of tire wear particles on humans' nervous systems due to the similarity of the nervous systems between humans and planarians.

5.0 Conclusion

Compared to microplastics, research on Tire Wear Particles (TWP) possible harm to living organisms is limited. The main objective of this experiment is to indicate the possible harm of TWP on planaria's phototaxis behavior. In an eight-week experiment, planaria were continuously fed at different concentrations of TWP liver paste, and their phototaxis response was tested. The result shows that the planaria in the highest TWP concentration group showed a time increase when escaping from the light, while the other experimental groups (including the control) decreased in time. This difference is likely due to neurological harm to planaria's learning ability when exposed to a high concentration of TWP. Damage to a planaria's learning

ability could impact its survival in the environment, and due to similar nervous systems between planaria and humans, the result can be linked to the possible impact of TWP on the nervous system of humans. Further research could explore the effect of tire wear particles on planarians' morphological issues and motor systems, the reasons behind the cannibalism and abnormal shapes of planarians during the experiment, and the mechanism of how the tire wear particles impact the learning ability damage of planarians. This study underscores the importance of the adverse effect of tire wear particles on planarians' learning ability and phototaxis behavior. It provides an ongoing discourse for future research on the harm of tire wear particles.

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7.0 Appendices

Attach any artifacts your reader may need to examine - data, charts, code, etc.

7.1 Detail Planaria Protocol: Planaria Protocol

7.2 Tire Wear Liver Paste Preparation: Food Preparation Protocol