Investigating the Impact of Tire-Wear Particles on the Regeneration of *Dugesia tigrina*

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Abstract Microplastics (MPs) pose a significant global health and environmental threat. Limited research has examined the impact of Tire Wear Particles (TWPs), a type of microplastics, on freshwater ecosystems. *Dugesia tigrina,* a common freshwater planaria species known for its remarkable regenerative abilities driven by neoblast stem cells, serves as the subject of this study which investigates the effects of TWP concentration and accumulation on the regeneration rate of planaria using a Head:Body color ratio. When exposed to TWPs over 2 weeks in different concentrations, *Dugesia tigrina* demonstrates an accelerated regeneration in higher concentration. Surprisingly, *Dugesia tigrina* that are exposed to TWPs for a longer period of 6 weeks shows signs of tolerance to TWP.

1.1 Introduction Due to plastics' flexibility, affordability, and durability, this synthetic material permeates most industries including healthcare, electronics, automotive, packaging, and even textiles. Their long lifespan with inadequate recycling allows plastics to degrade into microplastics, which are smaller than 5 millimeters, and nanoplastics (smaller than .001 millimeters) to make their way to land, air, freshwater, and oceans (US Department of Commerce, 2016). Tire Wear Particles (TWPs) have remained relatively understudied in comparison to other sources of microplastic pollution like Polyethylene (PE). TWPs are generated from friction between tires and the road, which includes both rubber from the tires and road wear particles (Kovochich et al, 2021). These microplastics are washed into bodies of water from the road and end up in

fish and other organisms, working up the food chain and affecting the freshwater ecosystem.

In 2017, tire wear and tear was reported to contribute to 5-10% of the global amount of plastics in the oceans and 3-7% of airborne particulate matter (PM2.5), indicating its prominent role in the global health burden of air and water pollution (Kole et al., 2017). Marfella et al. found direct evidence in 2024 that carotid artery plaque patients with microplastics and nanoplastics detected had a higher composite risk of myocardial infarction (a heart strain), stroke, or death from any cause at 34 months of follow-up than those in whom plastics were not detected. Despite being a significant source of microplastics, the impact of TWPs on organisms, especially in freshwater bodies, is not sufficiently addressed by research and evidence.

Dugesia tigrina is a freshwater planaria species renowned for their remarkable regenerative abilities capable of regenerating their body parts and each fractured piece becomes a new planaria after around two weeks. *Dugesia tigrina* are also very important decomposers and indicator species for water quality as they are sensitive to toxins (Goonetilleke et al., 2017). These qualities make them an intriguing subject for investigating the potential impacts of TWPs on biological processes.

1.2.1 Anatomy of Planaria Planarians are a group of living flatworms of the class Turbellaria in phylum Platyhelminthes. Planarian refers to any member of the family Planariidae and allied families, even though Planaria is the name of a single genus. Planarians are a common sight in freshwater, where they can occasionally be found in large groups. Planarians are bilaterally symmetric metazoans (digestive cavities with specialized cells) that have essentially infinite capacity for regeneration and have served

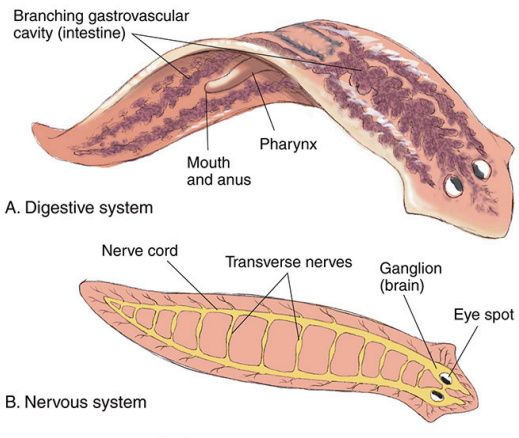

Figure 1: The anatomy of a planarian (Järlskog, et al.)

as a standard example of regeneration for more than a century. They contain two ventral nerve cords, many sensory neurons, and the brain or cephalic ganglia (Accorsi et al, 2017) (Figure 1).

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1.2.2 Tire-wear Particles Microplastics are minuscule pieces of plastic less than 5 millimeters in diameter (US Department of Commerce, 2016)**.** Tire Wear Particles totaling six million metric tons are emitted annually worldwide. Even while the majority of emissions remain localized near the sources, it has been demonstrated that even the tiniest particles may travel great distances via the atmosphere and end up in inland water bodies (Selonen et al., 2021).

1.3 Literature Review

1.3.1 Nanoplastic Effect on Planaria Cesarini et al. observed in 2023 that nano plastics (NPs), particles smaller than 1000 nanometers, cause a decrease in regeneration (as shown by decreased blastema length in Figure 2) and inhibit feeding at low and high concentrations. Expected to cause more harm to organisms than microplastics as they have a higher potential to cross biological membranes, this study shows planaria's feeding behaviors and regeneration rate can be indicators of toxicity in water. While this study did not observe microplastics or TWPs, it can be suggested from this study that we will observe similar but fewer effects of microplastics on regeneration rate.

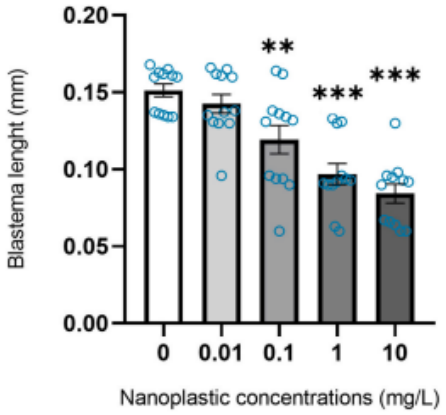

Figure 2: Blastema Regeneration in relationship to higher doses of nanoplastic concentrations. Blastma length declines in higher concentration levels

1.3.2 Microplastics in Prey May Delay Regeneration Rate in Planaria

Microplastics were fed to prey, and upon consumption by planaria - the planaria showed delayed regeneration. Using polyurethane microplastics (PU-MPs) measuring 7-9 μm in size and in the concentration of 375 mg per kilogram, the regeneration of auricles, which is the pair of sensory protrusions on the sides of the head, is noticeably delayed by the contaminated prey with MPs (Gambino et al, 2020). This study shows the effect of MPs on auricles and also that planaria still has remarkable regeneration abilities even at high concentrations of 375 mg/Kg of PU-MP (Figure 2). Gambino et al. (2020) also showed that upon consumption by planaria, the larger microplastics of spheres measuring 10 to

27 μm and long fibers were quickly expelled, likely because they are too large to be phagocytized or be engulfed into cells. Even though short fibers sized at 14 μm persisted for up to 4 days after ingestion without being detected in the intestinal lining, and smaller spheres less than 6 μm in diameter remained in the gut for an extended period as enterocytes absorbed them, accumulating in the intestinal lining. Observing a significant decrease in growth rate, the research provides some insights into the mechanism of microplastics' effect on planaria: it rules out reduced food intake as the cause because all meals

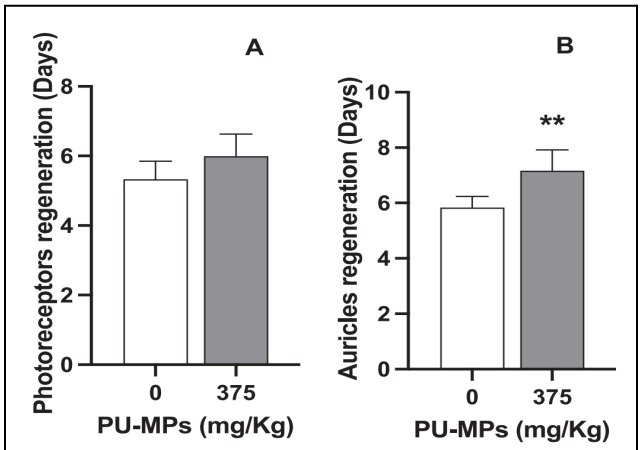

Fig. 4. *Girardia tigrina* regeneration after exposure to (un)contaminated prey (*Chironomus riparius* 4th instar larvae previously exposed to 0 or 375 mg PU-MP/kg sediment). (A) Time (days) for photoreceptors regeneration; (B) Time (days) for auricle regeneration ($n = 6$). All values are presented as mean ± standard deviation (n = 6). ** denotes significant differences from the control ($p < 0.01$).

Figure 3: The regeneration rate utilizing PU-MPs of planaria photoreceptors and auricles. (Gambino et al, 2020)

were constant but entirely ingested by all groups, and suggests microplastics negatively interfere with food absorption and with energetic metabolism in general. Gambino et al. (2020) recorded the days of absence or presence of photoreceptors, identifiable as dots on the head/eyes, and the presence of auricles, the sensory organs located on the sides of the head. Gambino's results revealed intriguing findings: while the regeneration of photoreceptors remained largely unaffected, the regeneration of auricles in planarians that consumed contaminated prey was significantly delayed, extending beyond 24 hours compared to the control group. This

delay was particularly notable in planarians fed prey contaminated with 375 mg of PU-MPs. The study utilized six planarians to assess the regeneration process.

1.4 Project Statement Understanding tire-wear particles' impact on the environment, especially freshwater organisms, is crucial. With a neurological system similar to humans and as a freshwater, bottom-dwelling primary consumer prone to ingest microplastics in sediments, *Dugesia tigrina* will allow investigation of TWPs' impacts on freshwater organisms and the ecosystem. While ingestion of polystyrene microplastics has been shown to slow the regeneration of planaria in some articles, the impacts of TWPs on regeneration have not been sufficiently studied. Our project investigates the effect of accumulated ingestion of TWP on the regeneration rate of *Dugesia tigrina* at environmentally relevant concentrations. After cutting the heads of planaria, we observed and compared the elapsed days for photoreceptor formation and trends in the pigment formation of the regenerated head.

2.0 Method

2.1 Care and Feeding *Dugesia tigrina* we bought from Carolina Biological Supply. The planarians were housed in labeled Petri dishes with Arrowhead spring water and kept in an insulated dark box. Additionally, the planaria were fed a high-protein diet of beef liver. Cleaning was completed after each feeding session and three days after each feeding.

2.2 Tire Wear Particles (TWP)

2.2.1 Tire Wear Particles Preparation TWP were obtained by using a wire brush on a rotary tool to abrade the rubber on a car tire. The resulting particles were collected and then mixed with liver paste to create mass percentage concentrations of 0.15%, 0.75%, and 1.5% TWP. Another liver paste has no TWP to be used with the control group.

2.2.2 Distribution of Tire Wear Particles Obtained

Microplastics are smaller than 5 millimeters or 5000 microns, and nanoplastics are smaller than 0.001 millimeters or 1 microns. As shown in Figure 4, about a third of TWPs fed to our planaria are smaller than 10 microns, which includes both TWP microplastics and nanoplastics.

2.3 Experimental Method Two experiments were performed to test the effect of TWP accumulation in planaria. For the first group, planaria were fed TWP concentrations for two weeks and the second group was fed for six weeks, after which they were cut and regeneration observations began. Group 1 (2 weeks) concentrations of 0%, 0.15%, 0.75%, and 1.5% of TWP. Group 2 (6 weeks): concentrations of 0%, 0.15%, and 1.5% of TWPs.

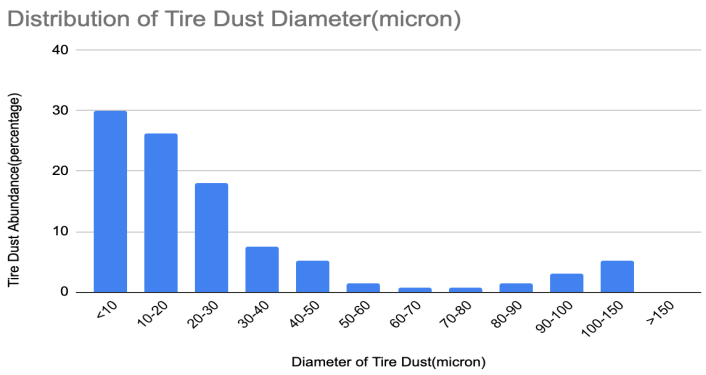

Figure 4: Distribution of Tire Dust Diameter Used in This Study. 1) 1 Millimeter = 1000 Microns. (Andrews et al., 2024)

2.4 Cutting Method The preparation for planaria dissection started by filling small Petri dishes with spring water and labeling them accordingly. Then the planaria were transferred from their housing using a plastic transfer pipette or wash bottle filled with spring water onto a larger Petri dish specifically for cutting. Using a sterilized knife, the planaria were cut just behind the auricles, ensuring not to make contact with the planaria using the pipette. Following the dissection, the Petri dish was cleaned with fresh spring water to eliminate any potential debris, allowing the dissected planaria to be rinsed

into their respective Petri dishes. Finally, each headless body was stored separately in its own small Petri dish. To avoid fragmentation, the scalpel was rinsed with spring water before each new cut, and a separate transfer pipette was used for each worm.

2.5 Measurement Method

2.5.1 Measurement of Eye Regeneration An optical microscope was used to take pictures that document each planarian's regeneration rate using a USB camera. The regeneration of black spots in the eye indicates full regeneration. This data is recorded in days and will have time uncertainty based on the last image taken.

2.5.2 Measurement of Head Regeneration through Color Intensity Collection The regeneration of planaria is dependent on the stem cells called blastema, which are transparent cell aggregates on the cut surface. Measurements of the darkness of the blastema in comparison to the darkness of the body of each planaria were collected and used as ratios to gauge the completeness of regeneration. This allowed the elimination of uncertainty introduced by variable lighting.

Figure 5: Example of ImageJ Data Collection. a) selection calculated for mean head color. b) selection calculated for mean body color

Using ImageJ, a public domain Java image processing program inspired by NIH Image for the Macintosh, polygon selections outline the area between the tip of the planaria's head and right before its eye spots (see Figure 5). The mean value of the color histogram was recorded for the head and body region, and a head-over-body ratio was calculated.

3.0 Results

3.1 Uncut Planaria's Mean Color Ratio Data Using the 6-week fed planaria from each experimental concentration group, the mean color ratio of each planaria was calculated and plotted. The slope of the trendline is 0.00637, which is almost equal to zero.

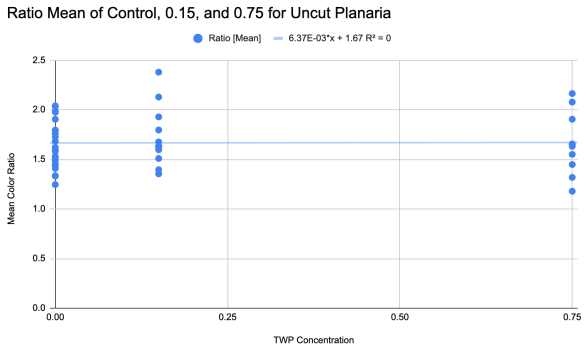

Figure 6: Mean Color Ratio Scatterplot of Uncut groups of 6 Weeks Control, 0.15, and 0.75 with Trendline

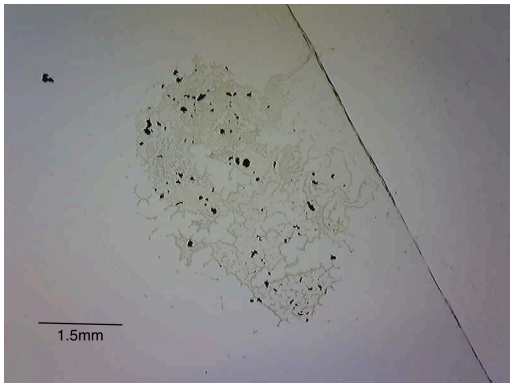

Figure 7: Mucus Emitted One Day After Feeding For 6 Weeks 0.15 group (Magnification 1)

3.2 Mucus with TWPs is Emitted After Feeding

One day after feeding, all groups (including the control) produced ejection mucus with TWPs in their dish. As seen in Figure 7, most TWP emitted is larger than 50 microns.

3.3 Eye Regeneration

While the regeneration of the eyes for both groups was equal, all eyespots were regenerated by 6 days after the initial cut.

3.4 Head: Body Color Ratio Results for 6-week and 2-week groups

As the stem cells or neoblasts mature, they develop pigment and become darker. Table 2 shows the change in Head:Body color ratio for each experimental group. A positive change in color ratio indicates that the head is getting darker compared to the body over time, and a negative change in color ratio indicates the head is getting lighter over time.

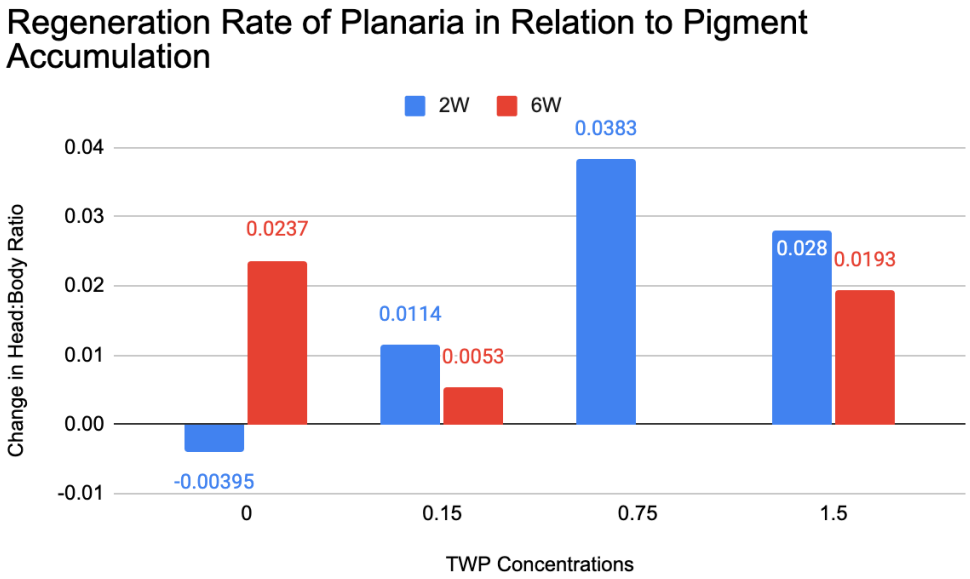

Figure 8: Change in Mean Color Ratio of 6-week and 2-week Experimental Groups

4.0 Discussion

Before cutting worms, the Body:Head color ratio was found to not be affected by TWP ingestion, as shown in Figure 6. This is important so any color change effects that are observed can be identified as part of the regeneration process. Gambino et al. (2020) showed in their study that larger microplastics from 10 to 27 µm are quickly expelled but small spheres less than 6µm stay in the gut. Our results show that ejected TWP are greater than 50µm, indicating that the smaller particles remain in the planaria and are potentially responsible for observed changes. The transparency of the blastema is used to analyze the progress of regeneration in planaria. The more positive the change in color ratio (cells become darker) from Figure 8, the fewer neoblasts are present and the more mature the regeneration progress is. This study expected the control group to have the most positive regeneration rate and most mature regeneration because we hypothesized

that higher dosage and accumulation of TWPs would delay the regeneration rate. Three interpretations of the data are:

1) For the 2-week feeding group, the higher TWP concentration experimental groups show an accelerated regeneration rate, as seen in the higher bars on Figure 8. This indicates a more robust regeneration response in these groups, potentially suggesting the worms are confronting an increased biological challenge that could lead to accelerated neoblast formation and maturation.
2) For the 6-week feeding group, all groups had positive rates of color change, with the control and 1.5% TWP concentration groups' color change rates similar (both groups experienced a darker head). It is possible that the planaria metabolism becomes more tolerant with the TWPs over 6 weeks, and thus, it has a smaller effect on the regeneration of stem cells.
3) Directly comparing the 2-week and 6-week feeding groups, the 2W experimental groups all show larger color changes than their 6W counterparts. If color change is in fact connected to the regeneration rate, then the accumulation of TWPs does delay the regeneration rate.

Open questions that this discussion raises include: What is the mechanism for the relationship between pigmentation and blastema formation, how does blastema tolerance to TWP affect regeneration rate and what causes the differences in data between the two control groups? In addition, repeating this study with higher numbers of worms would help eliminate uncertainties due to the small sample size.

5.0 Conclusion

Microplastics (MPs) pose an imperative environmental and global threat. Limited research has examined the impact of Tire Wear Particles (TWPs), a type of microplastics, on freshwater ecosystems. Using a head-to-body color ratio, this study examined the effects of TWP concentration and accumulation on the regeneration rate of planaria, well-known for its extraordinary regenerative abilities driven by neoblast stem cells. It was established that planaria dosed with TWPs did show changes in blastema pigmentation during regeneration. However, whether these changes were the result of bioaccumulation or the dosage concentration needs further study.

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