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Acute sublethal and lethal effects of tire wear particle leachate on larval fathead minnows (*Pimephales promelas*)

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ABSTRACT

Tire wear particles are a specific class of microplastics that are shed from tires when they are abraded by pavement. These particles contain a multitude of compounds, such as synthetic chemicals, heavy metals, and antioxidants. Many of these chemicals can be leached into water under certain conditions and enter waterways through stormwater runoff. To assess how tire wear particle leachate affects aquatic life, acute (24 hr) toxicity tests using leachate produced over 7-day and 14-day periods were performed on larval (7 day old - 30 day old) fathead minnows (*Pimephales promelas)*. To assess sublethal effects, EthoVision behavior tracking software was used, as well as feeding assays and manual ethograms. To assess lethal effects, the mortality rate of the different treatment groups (control, 7-day, 14-day) was assessed. A dilution study was conducted to identify the mortality rate of possibly more environmentally relevant leachate concentrations. Behavioral changes were observed in both the 7- and 14-day leachate treatments, with an overall decrease in mobility. Any time spent mobile was characterized by erratic behaviors that are indicative of neurological dysfunction. Leachate lethality increased with concentration, with the 75–100 % 14-day leachate solutions exhibiting the highest mortality. The LC_{50} of the 14-day leachate was determined to be 76 %. This study highlights both behavioral and lethal effects of tire wear particle leachate on early life stages of freshwater fish.

1. Introduction

Microplastics are classified as small plastic debris particles less than 5 mm in length that often result from the breakdown of larger plastics. They are difficult to remove from the environment and often find their way into the digestive tracts of numerous species. Tire wear particles (TWPs) are one of the largest sources of microplastics in the environment. They enter waterways via stormwater runoff or become airborne as particulate matter (US EPA O, 2017). TWPs have been listed as a contaminant of emerging concern by the US EPA O (2017), and are produced by the friction between a car tire and a road surface when driving and are composed of both tire tread and road pavement material. TWPs are generally characterized by a jagged elongated shape and black color.

Tires are comprised of many different natural and synthetic materials, but the components that cause the most harm to ecosystems include synthetic rubber polymers, heavy metals, and various other synthetic additives (Goßmann et al., 2021). When TWPs enter aquatic environments through stormwater runoff, water percolates through them and releases their chemical components into the waterways as leachate. The leaching of compounds into waterways can often be exacerbated by heat, UV exposure, pH changes, and degradation of the particles (Kolomijeca et al., 2020). Once this leachate has entered aquatic ecosystems, it has the potential to cause severe damage to organisms. As leachate is a mixture of many different chemical compounds, it is difficult to pinpoint what exactly is causing the most harm to organisms, especially since it is possible that the way the compounds interact with each other can increase toxicity (Chibwe et al., 2022). Once in the environment, leachate also undergoes significant modifications to its physical and chemical properties due to chemical reactions and biological activities (Thodhal Yoganandham et al., 2024). One example of the complexity of the toxicity of tire wear to aquatic species is the recent identification of 6PPD-quinone as a contaminant of concern specifically for juvenile coho salmon, which were observed to have increased mortality in the presence of the compound. 6PPD-quinone is a stabilizing additive in tires that prevents them from breaking down quickly (Foldvik et al., 2022). What makes this case complex is the unusual pattern of toxicity in species closely related to coho salmon,

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Table 1
Ethogram used for assessment of changes in fathead minnow larvae behavior when exposed to tire wear particle leachate.

Behavioral States	– Swimming – Immobile – Swimming in tight circles at high speeds
Behavioral Events	– Twitching/Spasms – Spontaneous acceleration from immobility – Spontaneous changes in swimming direction

Table 2
Post hoc Fisher's exact analytical test results for all diluted 14-day leachate mortality rate comparisons assessed.

Analysis Comparing	*p-value*
Control vs. 25 % 14-day leachate	0.237
Control vs. 50 % 14-day leachate	0.024
25 % vs. 50 % 14-day leachate	0.472
25 % vs. 75 % 14-day leachate	< 0.01
50 % vs. 75 % 14-day leachate	< 0.01
75 % vs. 100 % 14-day leachate	1

Table 3
Welch's ANOVA and Games Howell analytical test results for all behavioral endpoints assessed using EthoVision 11.5 XT.

Behavioral Parameter	Analysis Comparing	*p-value*
Average Distance Moved (cm)	All treatment groups	0.090
Time Spent in a Highly Mobile State (s)	All treatment groups	0.096
Average Velocity (cm/s)	All treatment groups	< 0.01
	Control vs. 7-day leachate	0.01
	Control vs. 14-day leachate	0.02
	7-day vs. 14-day leachate	0.89
Time Spent in a Mobile State (s)	All treatment groups	< 0.01
	Control vs. 7-day leachate	< 0.01
	Control vs. 14-day leachate	< 0.01
	7-day vs. 14-day leachate	0.68
Time Spent in an Immobile State (s)	All treatment groups	< 0.01
	Control vs. 7-day leachate	< 0.01
	Control vs. 14-day leachate	< 0.01
	7-day vs. 14-day leachate	0.09
Average Maximum Acceleration Rate (cm/s^2)	All treatment groups	0.01
	Control vs. 7-day leachate	0.01
	Control vs. 14-day leachate	0.5
	7-day vs. 14-day leachate	0.7

with some species being extremely sensitive to 6PPD-quinone and others showing no effects (Foldvik et al., 2022). Other studies have observed effects of TWP leachate on freshwater fish, specifically characterizing the acute response of coho and chem salmon through mortality and blood physiology tests (McIntyre et al., 2021).

This study focuses on the toxicological impacts of TWP leachate on fathead minnow larvae *(Pimephales promelas)*. Fathead minnows are relatively widely distributed, with populations occurring from central Canada to northern Mexico, and across most of the middle to eastern United States. Fathead minnows were chosen because they are native this region. This species is also commonly used in assessing the acute toxicity of chemicals and is ideal for use in a lab environment due to its tolerance of a wide range of water conditions (Ankley and Villeneuve, 2006). The use of fathead minnows in the larval stage is based on previous research which concluded that the nervous system of larval fathead minnows is particularly sensitive and is therefore more vulnerable to environmental contaminants such as TWP leachate (Rice and Barone, 2000). The fact that larval fathead minnows are more sensitive to neurotoxic effects than adults is useful because behavior relies on cognitive function. A more detectable shift in behavior in larvae than adults is expected following contaminant exposure (Krzykwa et al., 2018). Another benefit to using larval fish is that due to their smaller size, more individuals can be included in the study over a shorter period.

In this study we aimed to assess the impacts of leachate that has been leached for 7 days versus 14 days and different concentrations of TWP leachate on behaviors, including feeding and swimming activity, as well as mortality rate. Feeding activity and swimming assays after exposure to a toxic substance evaluated the possible alteration of neurological function (Hoang et al., 2022). EthoVision, a tracking software used for analyzing animal behavior, was used to track the percent activity after exposure. To assess feeding behavior after exposure previously described methods were used for fathead minnow larval feeding assays (Krzykwa and Sellin Jeffries, 2020). Previous studies have identified changes in freshwater organisms' physiology when exposed to TWP leachate, while this study aims to address the knowledge gap in the understanding of the behavioral changes seen in organisms. We hypothesize that individuals that have been exposed to leachate will experience erratic behaviors and higher mortality rates than those in the control group. Leachate that has leached for 14 days will cause the highest mortality rates as well as the most erratic behaviors.

2. Methods

2.1. Leachate production

TWP leachate was produced from cryo-milled tire tread (a standardized TWP surrogate produced by the U.S. Tire Manufacturers Association for researchers to use in lab experiments) using EPA Method 1315, *Mass Transfer Rates of Constituents in Monolithic or Compacted Granular Materials Using a Semi-Dynamic Tank Leaching Procedure* (US EPA O, 2019). Leachate production was executed by our partners at the University of Buffalo Department of Civil, Structural, and Environmental Engineering. 7-day leachate was produced by allowing tire wear particles to be in contact with water for 7-days, and 14-day leachate was produced by allowing tire wear particles to be in contact with water for 14-days. Leachate was stored in glass containers in a dark refrigerator to prevent the further degradation of any compounds present in the leachate solutions.

2.2. Larvae rearing and tank conditions

Fathead minnow larvae were reared by Aquatic BioSystems Inc. in Fort Collins, Colorado. Rearing conditions included a water temperature range of between 22°C - 25°C, a total water hardness (as $CaCO_3$) of approximately 117 mg/l, a total alkalinity (as $CaCO_3$) of approximately 95 mg/l, and a pH of 7.75. Feeding began one day after hatching; they were fed brine shrimp (*Artemia sp.*). Upon arrival in the laboratory from Aquatic BioSystems Inc., larvae were acclimated to their hold tank water conditions by drip acclimation for 45 min.

All larvae were kept in the same tank in a temperature-controlled room (24°C), with a 12:12 light cycle, an average pH of 7.5, and an air pump ensuring adequate dissolved oxygen. Feedings occurred twice a day on weekdays, and once a day on the weekend. They were fed < 24-hour-old brine shrimp (*Artemia sp.)*, that were cultured (500 mL of dechlorinated water and 5 g of API aquarium salt) with a small 1–5-gallon aquarium heater to keep the water temperature at about 30°C and an air stone to aerate the brine shrimp cysts to ensure a high yield. All methods involving *Pimephales promelas* were conducted in accordance with IACUC guidelines for the care and use of laboratory animals.

2.3. Acute toxicity tests

Acute toxicity tests were conducted over a 24-hour period, with three

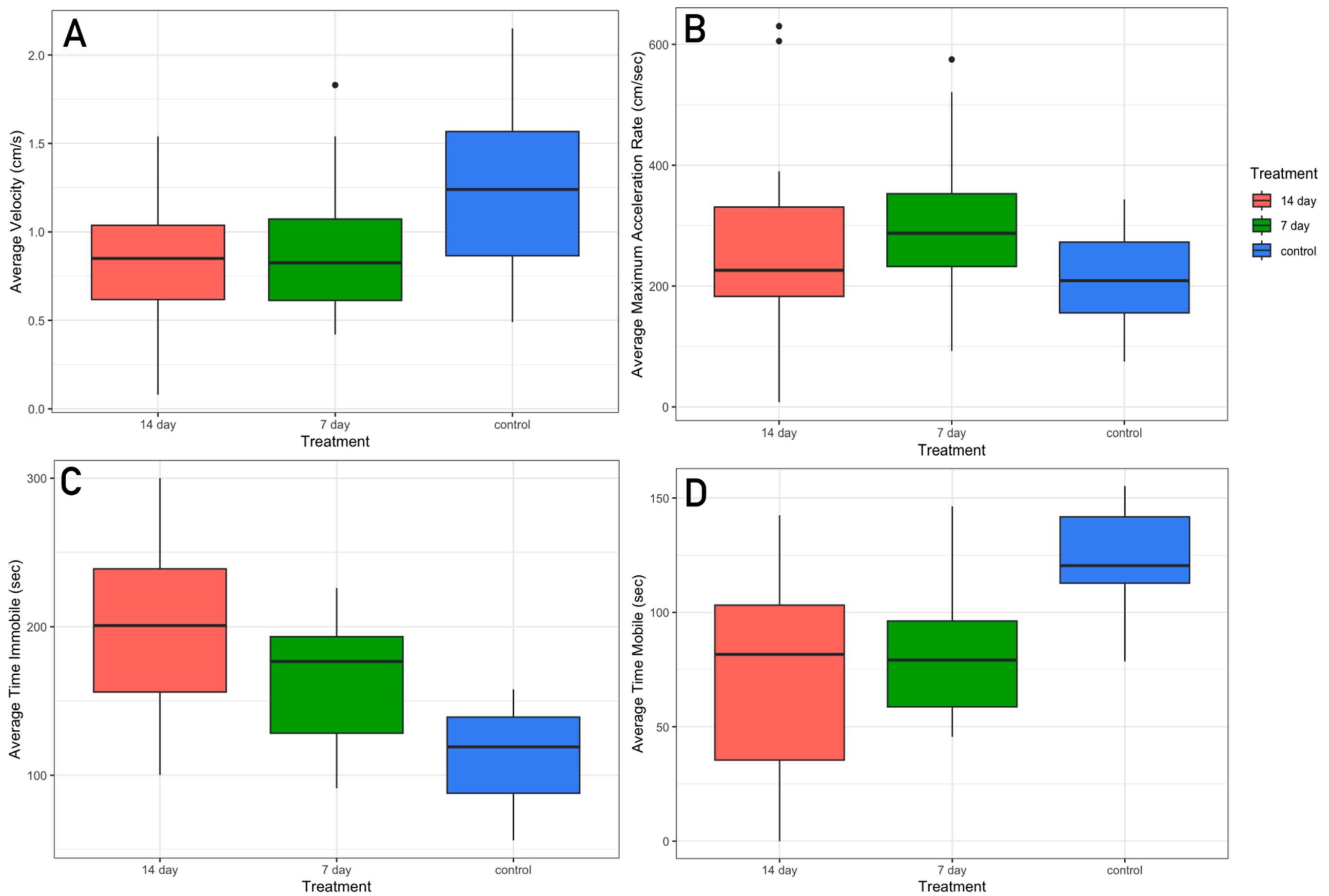

Fig. 1. Significant data collected using Noldus EthoVision XT 11.5. Control N = 24, 7-day leachate group N = 24, 14-day leachate group N = 14. Dots on graphs represent outliers.

treatment groups: control (tank water), 100 % of 7-day leachate, and 100 % of 14-day leachate. Five to six individuals were placed in 300 mL of each treatment (control n = 24, 7-day n = 35, 14-day n = 39). All other environmental conditions were the same between treatments: 24° C, 12-hour light cycle, and aeration. The behavior of the surviving exposed individuals (which did not die during the 100 % concentrated leachate solution acute toxicity tests), was then assessed using behavioral assays.

Acute toxicity tests were also conducted under the same conditions but using different concentrations of the leachates to assess the mortality rate at different concentrations of leachate and create a more ecologically relevant scenario, as leachate will never be at a concentration of 100 % in the environment. In this experiment, mortality was assessed at five different concentrations of 14-day leachates: 100 %, 75 %, 50 %, 25 %, and 0 %. Each beaker held 10 individuals per trial for a total of three trials and 30 individuals per treatment. The dilution tests were used only to assess mortality, and individuals in the dilution tests were not subjected to further behavioral and feeding tests.

2.4. EthoVision behavioral assays

Following the acute toxicity tests, behavioral assays were performed on the remaining individuals who did not die during the acute toxicity tests, control n = 22, 7-day n = 24, and 14-day n = 14. Noldus EthoVision XT 11.5 was used to assess any changes in swimming behaviors. Directly after the 24-hour acute toxicity test, behavior assays were performed on the exposed subjects one at a time. One individual was placed in the trial arena (a ramekin dish filled with 5 cm of tank water) and given 5 min to acclimate before the 5-minute behavior tracking began. Six different parameters were measured: average distance moved (cm); average velocity (cm/s); time spent in a highly mobile state (s); time spent in a mobile state (s); time spent in an immobile state (s); and maximum acceleration (cm/s^2). EthoVision also produced heatmaps showing the frequency of time the subject spent in different areas of the arena and the path the subject took.

2.5. Feeding assays

Directly after the EthoVision behavioral assessment, larvae were placed into their own individual well on a 6-well plate that contained tank water with 10 prey items (brine shrimp). The larvae were then given 15 min to eat the prey items. After this 15-minute period, the number of remaining prey items was recorded.

2.6. Manual ethogram analysis

To assess fish behavioral events not captured by EthoVision, a manual ethogram was created through observations of both control and exposed fish (Table 1). EthoVision video recordings were also used to assess smaller changes in fish behavior. All the behavioral data recorded for each fish was taken from the same 5-minute video recorded directly after a 24-hour exposure period to their respective treatment. The EthoVision videos were then analyzed by manually recording the changes in behavioral states, and at what time they were observed, as well as the number of times each behavioral event was observed. Duration of behavioral states were calculated using the embedded video time stamps.

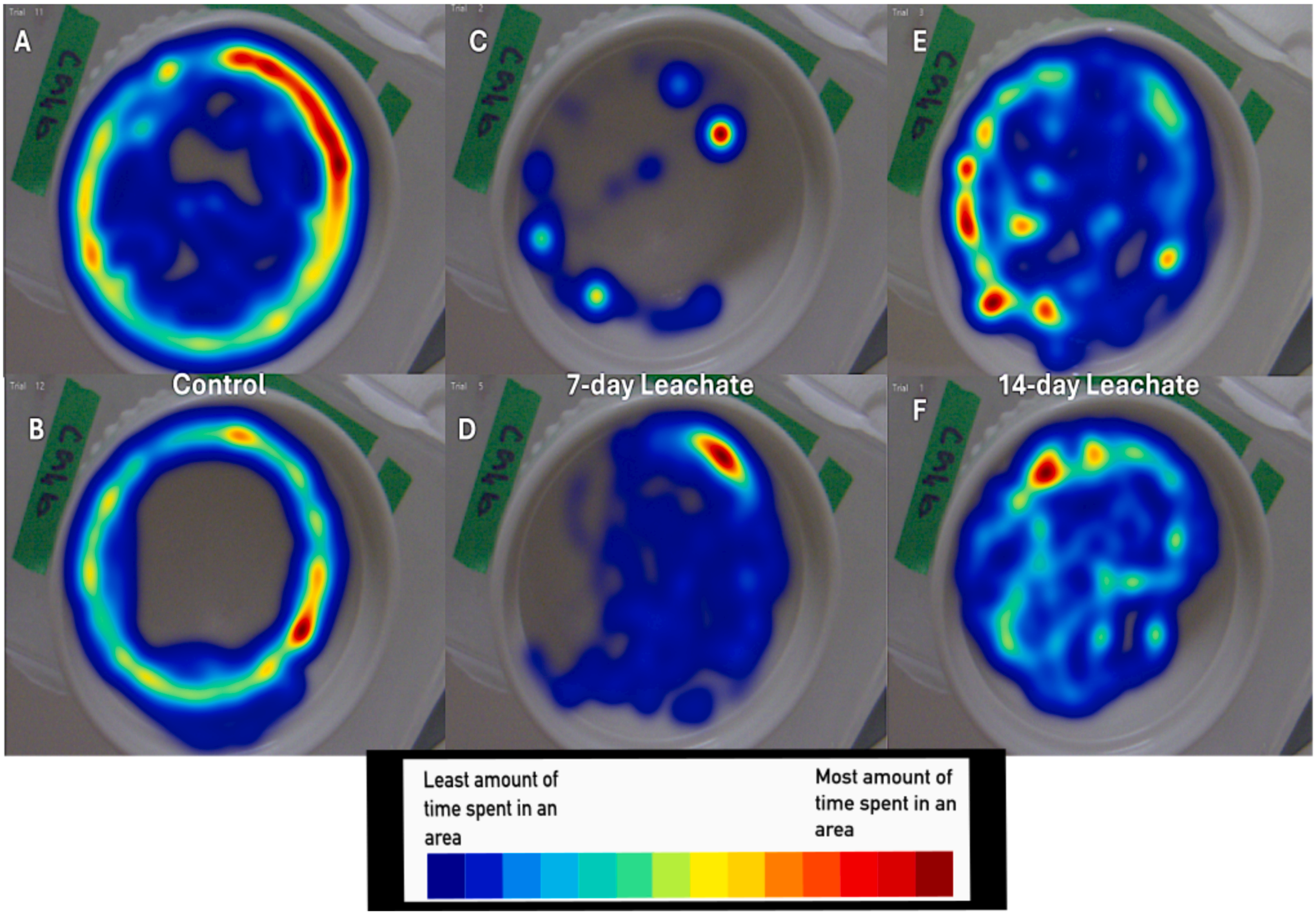

Fig. 2. Heatmap images generated by EthoVision from behavioral assays. Images A and B are heatmaps from control group individuals, C and D are from 7-day leachate group individuals, and E and F are from 14-day leachate group individuals.

2.7. Statistical analysis

All behavioral and mortality data were analyzed using RStudio for analysis. For all EthoVision and manual ethograms, Welch's ANOVA was used to assess the significance of the differences between exposure groups for each behavior measured. If significant differences were observed in the Welch's ANOVA test, a post hoc Games Howell test was performed to assess which groups were significantly different in the measured behavior. Feeding assay data was analyzed using a general linear model. Mortality and dilution study data were analyzed using Fisher's exact tests with post hoc Fisher's exact test analyses comparing two specific treatments. To determine the LC_{50} of the dilution study data, the data was graphed and the slope of the trendline was found. Using the slope equation, the LC_{50} of the 14-day tire wear particle leachate was determined.

3. Results

3.1. Acute toxicity test: mortality

There was a significant difference in the mortality rate between treatment groups ($p = <0.01$) (control n = 24, 7-day n = 35, 14-day n = 39). A post hoc Fisher's exact test revealed differences between the control and the 7-day leachate ($p = 0.016$) as well as between the control and the 14-day leachate ($p = <0.01$) and between the 7-day leachate and the 14-day leachate ($p = <0.01$). Overall, the 14-day leachate treatment group had the highest mortality rate.

3.2. Acute toxicity test: dilution study

There was an overall significant difference in mortality among the treatment groups (Table 2). The post hoc Fisher's exact test comparing the control to the 25 % 14-day leachate revealed no significant difference between them, but there were significant differences between the control and all other concentrations. There was no significant difference between the 25 % 14-day leachate and the 50 % 14-day leachate, but that there was a significant difference between the 25 % 14-day leachate and the 75 % leachate, as well as between the 25 % 14-day leachate and the 100 % leachate. There was also a significant difference between the 50 % 14-day leachate and the 75 % 14-day leachate, as well as between the 50 % 14-day leachate and the 100 % 14-day leachate. There was no significant difference between the 75 % 14-day leachate and the 100 % 14-day leachate. Based on the dilution study, the LC_{50} of this data was approximately 76 %.

3.3. EthoVision behavioral analysis

Of the six behavioral parameters assessed, there were two in which no significant differences were observed among treatment groups, and four in which significant differences were observed among treatment groups (Table 3). There was no significant difference in the average distance moved (cm) among treatment groups, nor in the amount of time spent in a highly mobile state (s) among groups.

There was a significant difference in the average velocity (cm/s) ($p < 0.01$), time spent in a mobile state (s) ($p < 0.01$), time spent in an immobile state (s) ($p < 0.01$), and the average maximum acceleration

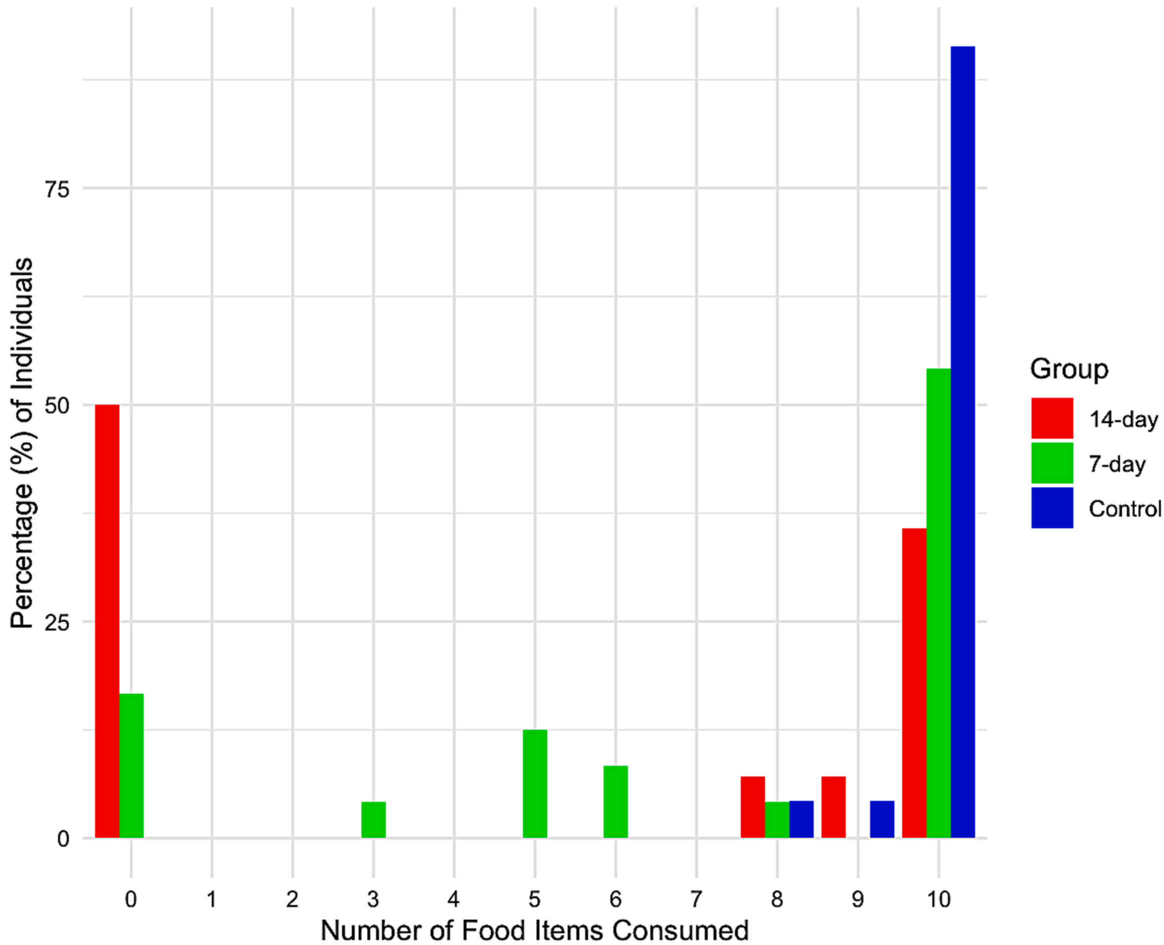

Fig. 3. Distribution of feeding assay data for treatment groups. Control group N = 24, 7-day leachate group N = 24, 14-day leachate group N = 14. There were no clear data trends and no significant differences among treatment groups.

rate (cm/s^2) ($p = 0.01$) among treatment groups (Table 2). A post hoc test revealed that the average velocity (cm/s), amount of time spent in a mobile state (s), and the amount of time spent in an immobile state (s) showed significant differences between the control and 7-day leachate, and the control and 14-day leachate (Fig. 1). The average maximum acceleration (cm/s^2) showed only a significant difference between the control and the 7-day leachate.

EthoVision also provided heatmap images which provide a visual representation of the differences in the amount and type of movement in different treatment groups (Fig. 2). The heatmap images demonstrate clear differences in the swimming behaviors of leachate-exposed individuals relative to the control. Control individuals exhibited more predictable behavior, staying close to the outside of the arena while leachate individuals exhibited more unpredictable and random movements.

3.4. Feeding assays

To assess differences in feeding behaviors between individuals in different treatment groups, a general linear regression analysis was performed. This test revealed that there were no significant differences between treatment group eating behaviors in the feeding assays. There were also no significant differences between the control and treatment groups. The number of prey items eaten per individual did not differ among treatment groups (Fig. 3).

3.5. Manual ethogram analysis

Of the six behavioral parameters (three behavioral states and three behavioral events) assessed manually, only one parameter was found to have no significant differences between treatment groups, amount of time (s) spent swimming in tight circles (Table 4). Though Welch's ANOVA came up with an N/A p-value (due to an average time spent swimming in tight circles being zero seconds for the control group), a post hoc test revealed that there was no significant difference between the control and the 7-day leachate, the control and the 14-day leachate, or the 7-day leachate and the 14-day leachate (Fig. 4).

There was a significant difference in the amount of time spent swimming (s), the amount of time spent immobile (s), the number of spontaneous accelerations from immobility events, the number of twitching/spasm events, and spontaneous changes in direction events. The amount of time spent swimming (s), amount of time spent immobile (s), number of spontaneous accelerations for immobility events, number of twitching/spasm events, and number of spontaneous change in direction events differed significantly between the control and the 7-day leachate as well as between the control and the 14-day leachate.

4. Discussion

4.1. Mortality

The mortality data indicates that the longer that tire wear particles are able to leach any compounds into water, the more lethal the leachate will be. There was a 0 % mortality rate for the control treatment, a 22 %

Table 4
Welch's ANOVA and Games Howell analytical test results for all behavioral endpoints assessed using manual ethogram observation techniques.

Behavioral Parameter	Analysis Comparing	*p*-value
Time (s) spent swimming in tight circles	All treatment groups	N/A
Time (s) spent swimming	All treatment groups	< 0.01
	Control vs. 7-day leachate	< 0.01
	Control vs. 14-day leachate	< 0.01
	7-day vs. 14-day leachate	0.64
Time (s) spent immobile	All treatment groups	< 0.01
	Control vs. 7-day leachate	< 0.01
	Control vs. 14-day leachate	< 0.01
	7-day vs. 14-day leachate	0.94
Number of spontaneous accelerations from immobility events	All treatment groups	< 0.01
	Control vs. 7-day leachate	< 0.01
	Control vs. 14-day leachate	< 0.01
	7-day vs. 14-day leachate	0.71
Number of twitching/spasm events	All treatment groups	< 0.01
	Control vs. 7-day leachate	< 0.01
	Control vs. 14-day leachate	< 0.01
	7-day vs. 14-day leachate	0.82
Number of spontaneous change in direction events	All treatment groups	< 0.01
	Control vs. 7-day leachate	< 0.01
	Control vs. 14-day leachate	< 0.01
	7-day vs. 14-day leachate	1

rate for the 7-day leachate treatment, and a 69 % rate for the 14-day leachate treatment. As tire wear particles are exposed to extreme environmental conditions, such as abnormal pH, high temperature, and high UV rays, when in water, they have a high potential for leaching any synthetic compounds, heavy metals, antioxidants, etc. With climate change, more locations may experience longer and more extreme hot weather events which can potentially lead to tire wear particles in the environment being exposed to more extreme environmental conditions for longer periods during summer months that allow them to potentially leach more of their ingredients into waterways. This is cause for concern considering the leachate that was given two weeks to develop was approximately 47 % more lethal than the leachate that was given one week to develop. However, it is important to bear in mind that 100 % leachate is unlikely to occur in the surface waters.

While the concentration of tire wear particle leachate that could be expected to be found in the environment is unknown due to the complex nature of the leachate, the dilution study makes this research more ecologically relevant. Of the five different diluted 14-day leachate groups used in the dilution study, the largest significant increase in mortality rate was from the 50 % dilution to the 75 % dilution, a 35 % increase in mortality. Leachate concentrations of 50 % or higher showed a significant increase in mortality rate compared to the control and 25 % leachate.

It is important to make the distinction between the length of time taken to produce the leachate and how concentrated the leachate is (Chibwe et al., 2022). The amount of time spent producing a leachate solution can influence how many different compounds are in the leachate solution as well their concentrations. The concentration of leachate solution is only determined by how diluted a leachate solution is. This difference is what distinguishes the 7-day and 14-day leachate study (the length of time taken to produce the leachate) from the diluted 14-day leachate study (how concentrated the leachate is) and shows that these two tests were identifying different scenarios.

Moving forward, it would be ideal to identify an environmentally relevant concentration of tire wear particle leachate, as well as pinpoint exact compounds within the leachate that are responsible for any adverse effects to aquatic life. However, as stated above, determining these compounds is difficult due to the number of compounds in tires as well as the fact that tire composition is highly variable based on the company that makes it, the type of tire (truck, car, bike, etc.), and the components within the tire (including treads, belts, inner liners, etc.) (Mayer et al., 2024). This variability is what has made characterizing an ecologically relevant concentration of tire wear particle leachate so difficult and would require the identification of endpoint concentrations for each tire type and brand.

4.2. Sublethal effects

Behavior tracking indicated a difference in how individuals exposed to leachate behaved compared to control individuals. Four of the six behaviors measured had significantly different values among the treatment groups and the control group, but not between the 7-day and 14-day leachate treatment groups; individuals exposed to either leachate treatment on average spent significantly less time in a mobile state and more time in an immobile state than those in the control group. Decreased mobility can negatively impact critical activities such as predator avoidance and prey capture, which can lead to a decreased survival rate (Fulton and Key, 2001). Also, individuals in both leachate treatment groups had a lower average velocity (cm/s), than those in the control group. This also indicates a decreased mobility in individuals exposed to leachate. Lastly, the average maximum acceleration rate (cm/s^2) was only significantly different between the control and 7-day leachate treatment, with individuals in the 7-day treatment showing

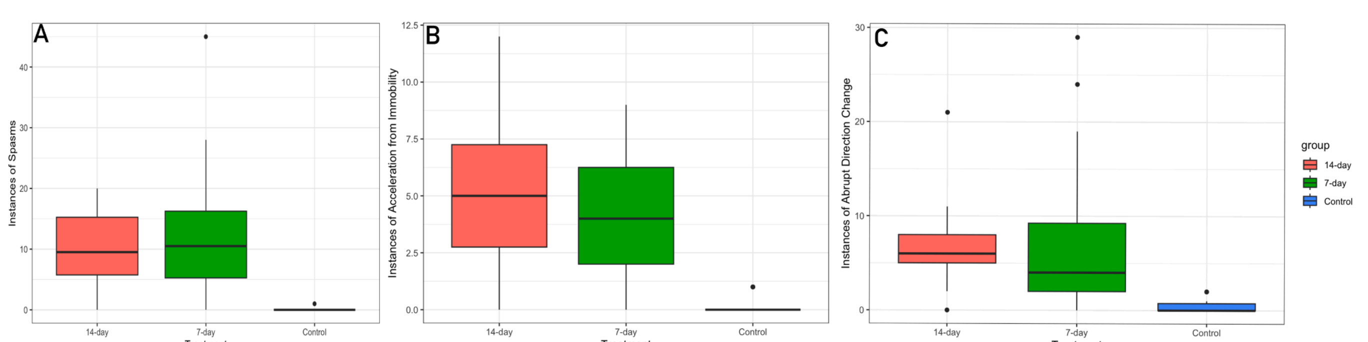

Fig. 4. Difference between treatment groups for the 3 unique significant data sets collected using the manual ethogram laid out in Table 4. Control N = 24, 7-day leachate group N = 24, 14-day leachate group N = 14. Dots on graphs represent outliers.

faster maximum acceleration rates than the control and the 14-day leachate. This behavioral change was also observed using the manual ethogram, and is likely indicative of metabolic dysfunction, which is commonly caused by chemicals that disrupt the coupling between electron transport and ATP synthesis in the mitochondria and effectively inhibit ATP production and release produced energy as heat in the place of ATP (Drummond and Russom, 1990).

Smaller changes in behaviors recorded in the ethogram data confirmed a difference in behaviors between exposed and control individuals. Five of the six behavioral endpoints showed significant differences among the control and treatment groups, but once again there were no significant changes between the 7-day and 14-day leachate treatment groups.

When looking closer at these behavioral endpoints, individuals that were exposed to either leachate treatment spent less time in a swimming state and more time in an immobile state than those in the control group. This reinforces the conclusion from the EthoVision data that individuals exposed to either leachate group exhibited decreased mobility, which can have negative impacts on survival. While overall movement was greatly inhibited in individuals exposed to leachate, the smaller movements observed manually were highly unpredictable and erratic. A prime example of this unpredictability is the significant increase in the occurrence of spasms in both the 7-day and 14-day leachate groups compared to the control group, but no significant difference between the 7-day and 14-day leachate groups. An increase in spasms is a commonly observed behavior often indicative of neurological dysfunction, that is commonly linked to acetylcholinesterase (AChE) inhibition (Drummond and Russom, 1990). There was also a significant increase in occurrences of acceleration from immobility among the 7-day and 14-day leachate groups compared to the control groups, but no significant difference between the treatment groups. An increase in sudden acceleration would be categorized as accelerated locomotor activity and can also be indicative of metabolic dysfunction, which as stated above, inhibits the production of ATP.

The last behavioral endpoint assessed was instances of abrupt directional change, in which individuals were observed taking sharp turns with little to no predictability. There was a significant increase in occurrences of abrupt directional change in individuals exposed to the 7-day and 14-day leachate groups when compared to the control group, though there was no significant difference between the treatment groups. This shows that individuals exposed to either leachate treatment can exhibit accelerated locomotor activity which, as with increases in sudden acceleration, can be indicative of metabolic dysfunction.

Both behavioral assays provided significant data for five different endpoints, suggesting that individuals exposed to either leachate treatment group exhibited decreased mobility overall. Decreased mobility can cause decreased prey capture and predator avoidance, which can lead to a decreased rate of survival. Though behaviors were decreased overall, the few behaviors that were observed were erratic and consistent with what has been described as "accelerated locomotor activity", a major sign of metabolic dysfunction among 30-day old fathead minnow larvae (Drummond and Russom, 1990). Drummond and Russom linked accelerated locomotor activity to metabolic dysfunction and, ultimately, inhibition of ATP production which can lead to cellular dysfunction and cell death. Another type of behavior observed has been linked to neurological dysfunction and an inhibition of AChE, which can cause continuous nerve firing and is typically only observed at near lethal contaminant concentrations (Olivares-Rubio and Espinosa-Aguirre, 2021). More research is needed into the exact mechanisms affected by tire wear leachate to confirm that these behaviors are truly linked to the inhibition of these vital functions.

5. Conclusion

Investigating the sublethal and lethal effects of tire wear particle leachate in fathead minnow larvae gives us a well-rounded understanding of the environmental implications of tire wear particles. In terms of sublethal behavioral effects, subjects exposed to either the 7- or 14-day leachate showed increased immobility, which can decrease prey capture and predator avoidance. When larvae were moving, the movements were more sporadic and unpredictable. These observed changes in behaviors can be signs of metabolic and neurological damage. In terms of lethal effects, the longer tire wear particle leached into water, the more lethal it was, and the more concentrated the leachate was the more lethal it was. Moving forward, there is need for an in-depth analysis of the chemical composition of TWP leachate that outlines the toxicant effects of the mixture. Further research is also needed to determine what environmentally relevant concentrations of tire wear particle leachate are, however, this research gives us a good starting point for quantifying the effects of the leachate on aquatic life.

CRediT authorship contribution statement

Susan Allen: Writing – review & editing, Supervision, Funding acquisition. **Grace Mattson:** Writing – original draft, Methodology, Investigation, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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