The effects of polyethylene microplastics on dragonfly nymphs

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Abstract

Plastic waste is entering our waterways at an unprecedented rate. This plastic comes from poor waste disposal and storm sewage drain off. Once in our waterways, the plastic breaks down into smaller pieces, which puts organisms at risk to microplastic (MP) exposure. Overtime, MP can accumulate within the organism. Previous studies have analyzed how growth, survivability, and reproductive fitness are impacted by microplastics, however few studies have considered how MPimpact respiration of aquatic organisms. I studied the effects of chronic polyethylene microplastic (PE) exposure on dragonfly nymph respiration. Dragonfly nymph's are ideal for this study because the ventilation of abdomen during respiration provides an avenue for MP to accumulate within the organism's abdomen and accrue on the internal gills. I predicted that the accumulation of MP in the abdominal chamber would decrease the surface area available for gas exchange and thereby increase the dragonfly nymph's ventilation rates and oxygen consumption. Twenty-four individuals were evenly split between two experimental groups. A control group (no PE exposure) and a microplastic exposure group. I then measured their ventilation and oxygen consumption rates on day 7 and day 14. Ventilation rates were measured by video recording the animal and counting the number of abdominal pumps within three minutes. Average oxygen consumption was measured with a fiber optic oxygen sensor. Additionally, I measured MP accumulation within the abdominal cavity by dissecting the abdomen and measuring the average proportional area taken up by the spheres. Neither exposure time nor MP exposure had a statistically significant effect on dragonfly nymph ventilation and oxygen consumption rates. However, MP accumulation was observed within the dragonfly nymphs, which could lead to biomagnification of PEs throughout freshwater ecosystems.

Introduction

Poor waste disposal practices and storm sewage drainage has allowed plastics to enter streams, rivers, and oceans (Wright et al., 2013). These plastics consist primarily of littered plastics (e.g., single-use plastics, food packages, etc) and personal care products (e.g. cosmetics, face soap, etc) (Baskaran & Sathiavelu, 2022; Fendall & Sewell, 2009). Once these plastics enter the waterways, they begin to decompose into smaller particles called microplastics (MP) (diameter < 5 mm, Baldwin et al., 2016). It is anticipated that MP will become more abundant as the human population continues to increase, therefore it will be more likely for an organism to encounter MP in aquatic environments (Wright et al., 2013; Ribeiro-Brasil et al., 2021).

When an organism encounters MP, the MP are often haphazardly ingested or used by the organism, often to its own detriment (Wright et al., 2013). In oysters (*Crassostrea gigas*) the ingestion of MP reduces reproductive fitness by decreasing oocyte size and production and sperm velocity (Sussarellu et al., 2016). In marine worms (*Arenicola marina*), the ingestion of MP reduces energy reserves by increasing inflammation (Wright et al., 2013). Rather than ingestion, some organisms may inadvertently use MP rather than natural material. For example caddisfly larvae create cases from substrate material for protection from predators (Ehlers et al., 2020). When caddisfly larvae (*Lepidostoma basale*) were exposed to polyvinyl chloride and polyethylene terephthalate MP, they incorporated the plastics into their cases, which reduced case stability by lowering case density and thereby lowered rates of larvae survival (Ehlers et al., 2020).

Not all MP impacts aquatic organisms equally. The size and concentration of the MP can determine the severity of their impact. Ziajahromi et al. (2018) presented chironomid larvae (*Chironomus tepperi*) with MP ranging in size classes from 1-126 μm. The smaller sized particles (1-54 μm) decreased larvae survival and body size, while the largest particles (100-126 μm) had little impact. The smaller polyethylene (PE) microplastics were ingested, which likely led to a decrease in quality food intake, while the larger particles were not ingested (Ziajahromi et al., 2018). Higher PE concentrations also correlate with higher mortality rates. Freshwater amphipods (*Hyalella azteca*) were exposed to MP concentrations ranging between 0-100,000 microplastics/mL. After 10 days of acute exposure the organisms exposed to high and intermediate concentrations (>1000 microplastics/mL) had significantly higher mortality rates compared to the organisms exposed to the lower concentrations (<100 microplastics/mL) (Au et al., 2015). After 28 days of chronic exposure, Au et al. (2015) found that 5000 and 10,000 microplastics/mL affected amphipods body size and reproduction. These results suggest that increased consumption of PE concentrations may lead to decreased ingestion of natural food leading to reduced growth rates (Au et al., 2015).

Most studies focus on how the ingestion or use of MP directly impacts growth, survivability, and reproductive fitness (Ribeiro-Brasil et al., 2021). Few studies, however, have considered how the accumulation of MP impacts respiration of aquatic organisms. Watts et al. (2016) found that MP accumulates on the gills of shore crabs (*Carcinus maenas*) resulting in significantly lower oxygen consumption after one-hour exposure. Paul-Pont et al. (2016) observed that marine mussels (*Mytilus edulis* and *Mytilus galloprovincialis*) exposed to polystyrene MP also accumulated MP on their gills. They did not, however, measure potential changes to oxygen consumption rates.

Here, I tested the effects of chronic PE on dragonfly nymph respiration. Dragonfly nymphs are ideal for exploring the impact of MP on respiration because of how they breathe. Dragonfly nymphs respire by ventilating their abdomen (Hughes & Mills, 1966). Specifically, as they contract and relax their abdomen, oxygenated water enters in through their anal opening and flows over their gills (Hughes & Mills, 1966). Their abdomen then pumps the water out. It is possible, therefore, for foreign objects within the water (e.g., microplastics) to enter the abdominal cavity via this method of gas exchange. In fact, Chagas et al. (2021) observed PE microplastics (diameter: 35.46 μm ± 18.17 μm) accumulated in the dragonfly nymph (*Aphylla williamsoni*) abdominal cavities likely through respiratory structures and ingestion (Chagas et al., 2021). This accumulation of MP in the abdominal chamber could decrease the surface area available for gas exchange and thereby increase the dragonfly nymph's ventilation rates. For example, Ubhi and Matthews (2017) found that dragonfly nymphs subjected to hypoxic water increased their ventilation rates to compensate for the lower oxygen levels.

Methods

Animal Culture

Forty-eight dragonfly nymphs (*Anax* sp.) were obtained from Carolina Biological Supply Inc. (Burlington, NC, USA) and kept in individual 266 mL plastic containers. These containers were subsequently distributed between larger 7.6 L plastic containers for storage purposes. These containers were kept in a climate controlled room at 21± 1°C on a 12:12 light:day cycle (Thorp, 2010). The dragonfly nymphs were fed mealworms twice per week, but not on the day of testing. Mealworms were removed from the cups approximately 24 hours prior to respiration rate or ventilation rate testing.

Polyethylene microplastic concentration

To explore the effects of MP on dragonfly nymphs, I chose a concentration approximately equal to the concentration used by Au et al. (2015), where they observed increased mortality. The control concentration (C) contained 0 microplastics/mL and the variable concentration contained 15,000 microplastics/mL. Au et al. (2015) tested the effects of microplastics on a benthic aquatic invertebrate (*H. azteca*), which would be subjected to sunken MP accumulating on the substrate. Here, I increased the concentration of MP to raise the number of suspended MP in the water column. Dragonflies are dynamic predators and do not feed on the substrate, therefore increasing the number of suspended MP may increase their contact with these MP.

The concentration was prepared by adding 3.6 g of dry, blue PE microplastics (27-45 μm, 1.00g/cc; Cospheric, CA, USA) to 20mL of moderately hard water (Ziajahromi et al., 2018). To prevent

aggregation of the MP in water, 2.4 mL Tween 20 was added to the solution (Au et al., 2015). The solution was mixed using a vortex mixer (BioCot, Stuart) for 2 min (Ziajahromi et al., 2018). Concentration was measured using a hemocytometer (Improved Neubauer; C. A. Hausser & Son, Philadelphia, PA, USA). Twenty-four individuals were kept in the microplastic concentrations.

Figure 1: Viewing chamber for recording ventilation rates of dragonfly nymphs. Left: Camera set-up. Right: Viewing chamber orientation under the camera.

Experimental design

To measure ventilation rates, individual nymphs were transferred to a petri dish filled with temperature-adjusted deionized water (Figure 2). The transparent lid permitted viewing from above and a white platform enhanced contrast against the nymph's body. The viewing chamber was lit with a red LED to create a dark silhouette of the nymph. They were given 10 minutes to acclimate to the new environment before ventilation rates were recorded (Ubhi & Matthews, 2017). After the acclimation period, the nymph was recorded for 15 minutes to track the abdominal pumping activity (Ubhi & Matthews, 2017). The nymph was filmed by an EOS 4000 D camera in video mode with a EF-S 18-55 mm lens (Canon, Tokyo, Japan). Once the recording was complete, the dragonfly nymph was returned to their original container. Recordings were reviewed and the number of abdominal pumps over a 3 minute period were counted. Ventilation rates were then calculated based on beats per minute divided by the nymph's mass (bpm/g).

To measure oxygen consumption, individual nymphs were transferred to a sealed 20mL vial filled with deionized water that was positioned within a 5 L water bath to help stabilize temperature. Oxygen consumption was measured via a 2-channel Firesting optode system (Pyroscience sensory

technology) using temperature-compensation and intermittent-flow respirometry (Svendsen et al., 2016). The nymphs were given 5 minutes to acclimate to the new environment and their oxygen concentration was measured over the following 20 minutes. After the acclimation period (and again at the 15 minute mark), the vial was briefly opened and the water inside was refreshed with fresh water using a 10 mL syringe. The optode connected to the second channel of the Firesting was used to measure oxygen changes in a sealed, and empty, 20 mL vial. This vial was also refreshed with water at the same intervals. Once the recording was complete, the dragonfly nymph was patted-dried, weighed, and returned to their original container. Recordings were reviewed and the most stable 2-minute period from the experimental chamber was analyzed. The resulting oxygen consumption was then calculated by subtracting the oxygen change in the control chamber from that of the experimental chamber and was reported in microliters per gram per hour (μL/g/hr).

Ventilation rates and oxygen consumption rates were measured on eight individuals kept in each concentration at 7 days and 14 days of exposure. The 14 day time interval was selected based on López-Rojo et al. (2020) experimental design that found significant negative effects on stream invertebrates exposed to MP for 14 days.

Visual and morphological analysis

To estimate the accumulation of MP within the dragonfly nymph abdomen, individuals were dissected after all measurements were recorded. Each dragonfly nymph was killed at 2:30 PM EST to avoid diurnal variations (Throp, 2010). The specimens were preserved in 70% EtOH and stored in separate containers. The abdomen was dissected and viewed under a stereomicroscope to identify the presence of any blue MP spheres. Individual spheres were unable to be counted due to their affinity for adherence to one another within the organism. This also made it difficult to measure the 3-dimensional impact of the spheres via volume measurements. To approximate the accumulation of MP within the dragonfly nymph's abdominal cavity, therefore, the proportional area taken up by spheres was measured by taking the area of the spheres and dividing by the area of the abdominal cavity.

Statistical analysis

To analyze potential differences in exposure duration and concentration, a 2-way ANOVA was conducted in R (R Core Team, 2016) for both ventilation rates (bpm/g) and oxygen consumption (μL/g/hr). The 2-way ANOVA assumptions were confirmed by testing for normality using a Shapiro-Wilk test and testing for variance homogeneity using a Bartlett test. Data was reported as mean ± standard error (SEM).

Results

Ventilation rates

The presences of PE microplastics and time of exposure had no significant impact on dragonfly nymph ventilation rates (Fig 3, Table 1. $F_{1,9} = 4.250$, $p = 0.066$).

Oxygen consumption

The presences of PE microplastics and time of exposure had no significant impact on dragonfly nymph oxygen consumption (Fig 4, Table 1, $F_{1,9} = 3.867$, $p = 0.081$).

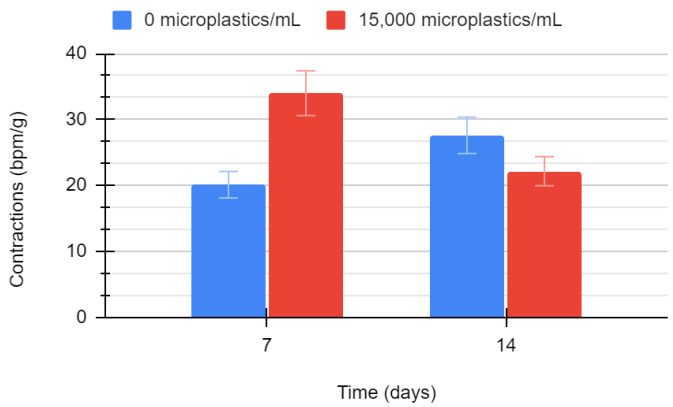

Figure 3: Ventilation Rate (bpm/g) (± SEM) of dragonfly nymphs exposed to different concentrations of MP over different time intervals. n=4 for all groups.

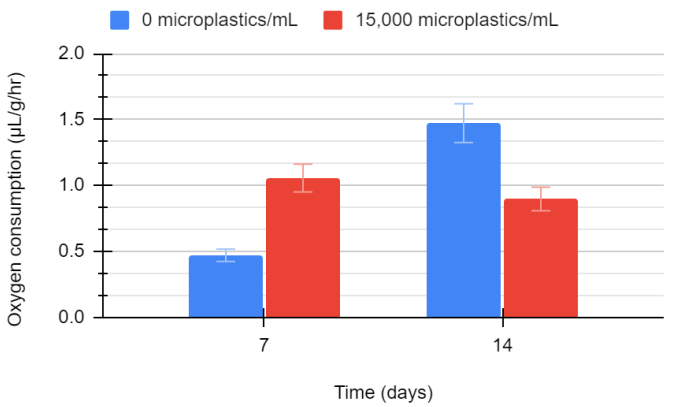

Figure 4: Average oxygen consumption (µL/g/hr) (± SEM) in dragonfly nymphs exposed to different concentrations of MP over different time intervals. n =3 for the 0 microplastics/mL groups, n=4 for the 15,000 microplastic/mL groups.

Microplastic accumulation

PE microplastics were observed adhered to the external and internal surfaces of the dragonfly nymphs. MP accumulated in the abdominal and thoracic cavities, which corresponded with Chagas et al. (2021) observations (Figure 5). The average proportional area taken up by spheres was 1.35% after 14 day exposure. Because no MP were observed or expected in the control animals, I did not subject density measurements to statistical analysis.

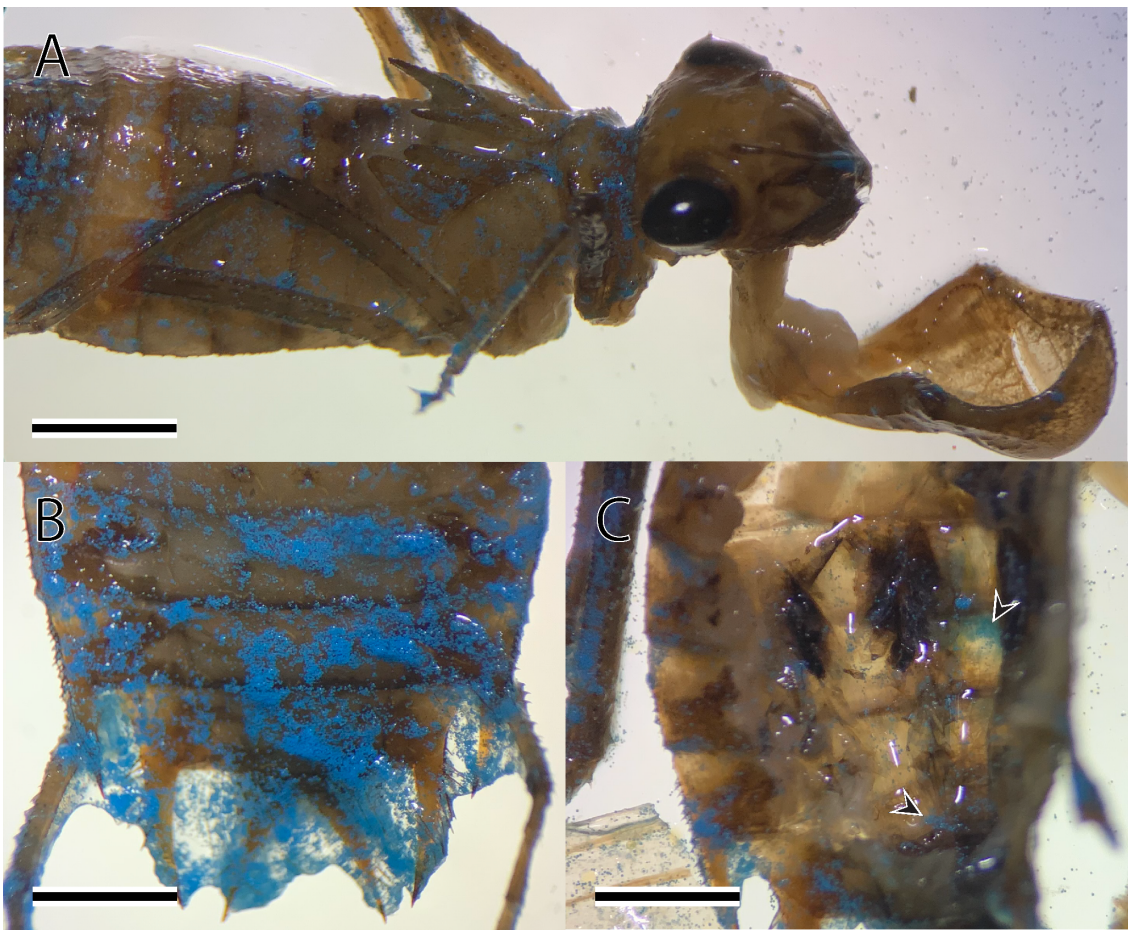

Figure 5: Whole organism images showing adherence of blue MP after 14 d exposure. A) head (with extended labium) and thorax. B) Posterior portion of abdomen (external). C) Posterior portion of abdomen with cuticle removed to show abdominal cavity. Arrows indicate example patches of internal MP. Scale bar = 3mm for all panels**.**

Table 1. 2-way ANOVA results for factors affecting ventilation rates and average oxygen consumption in dragonfly nymphs.

	Variable (d.f)	*F*	*P*
Average Ventilation Rate (bpm/g)	Concentration (1)	0.376	0.554
	Time (1)	0.214	0.654
	Concentration x Time (1)	4.250	0.066
Average Oxygen Consumption (µL/g/hr)	Concentration (1)	0.003	0.956
	Time (1)	1.692	0.226
	Concentration x Time (1)	3.867	0.081

Discussion

My hypothesis that MP accumulation in the abdomen would lead to less available surface area for gas exchange resulting in increased ventilation and oxygen consumption rates was not supported. The concentration of MP and time of exposure had no effect on dragonfly nymph average ventilation rate or average oxygen consumption (Table 1).

These results contradict those of other studies that found negative effects of MP on the respiration rates of other aquatic invertebrates. In shore crabs, Watts et al. (2016) found that even 1 hour of MP exposure (concentration: 10000 microspheres/mL) was enough to reduce respiration rates. This difference in results may be attributed to the different lifestyles of shore crabs and dragonfly nymphs. Shore crabs are active-search predators (Hedvall et al., 1998), whereas dragonfly nymphs are sit-and-wait or ambush predators (Ross & Winterhalder, 2015). These organisms have adapted feeding strategies that best support their biological and ecological lifestyles (e.g. predator locomotion, prey lifestyle, habitat) (Ross & Rogers, 2011). As an ambush predator, dragonfly nymphs are able to conserve energy and reduce metabolic costs (Ross & Rogers, 2011). Thus, the sit-and-wait feeding strategy supports the organism's biological processes and additional stress may be required to observe negative effects on dragonfly nymphs.

Here, I exposed dragonfly nymphs to 15000 microplastics/mL for 14 days. Previous studies have shown that even 10,000 microplastics/mL are often adequate to elicit negative effects. Au et al. (2015) observed that freshwater amphipods exposed to concentrations >10,000 microplastics/mL experienced higher mortality rates compared to the amphipods exposed to lower concentrations (>100 microplastics/mL) (Au et al., 2015). When lower MP concentrations are present, longer exposure may be necessary to observe the effects they have on dragonfly nymphs. Au et al. (2015) exposed freshwater amphipods to 10,000 microplastics/mL for 42 days compared to the 14 days of this present study. A significant difference was not detected until day 28 and a threshold effect was observed for the remaining days (Au et al., 2015). In North America, dragonflies can survive for 1-6 years as nymphs and undergo multiple stages in development (Thorp & Rogers, 2011). Therefore, 14 day exposure may not have been a long enough period to see significant changes due to MP exposure. Longer exposure may allow for the impacts of MP to accrue over time and also allow for observations throughout developmental stages.

Dragonfly nymphs typically develop in ponds, where water quality can vary substantially (Catling, 2005). Catling (2005) observed that odonate species diversity and the number of individuals decreased with increasing biological oxygen demand across different ponds. Dragonfly larvae seem to

be particularly sensitive to oxygen levels compared to other aquatic invertebrates (Chapman et al., 2004). Furthermore, the severity of the conditions determines the amount of stress the organisms experience. Ubhi and Matthews (2017) subject dragonfly nymphs to increased hypoxia and observed increased average ventilation rates. They concluded that ventilation frequency was increased to maintain internal homeostasis under oxidative stress (Ubhi & Matthews, 2017). Therefore, it was surprising that the experimental conditions presented here did not elicit a negative effect. Future studies should study the impact of higher concentrations or longer exposure, which may further reduce water quality and increase stress on the organism.

Future studies should also emphasize environmentally relevant MP concentrations (Varg et al., 2022). However, the concentration of MP in freshwater systems is not well known. Eriksen et al. (2013) found an average of 43,157 microplastics/km^2 in 21 samples from the Great Lakes. Typically, studies exploring the impact of MP on organisms report microplastics per volume (as done here). So, more studies are needed to better quantify the number of microplastics per volume in nature.

Conclusion

Varg et al. (2022) found that MP can be transferred through multiple trophic levels. Daphnids were directly exposed to MP, then the daphnids were fed to damselfly nymphs, which were subsequently fed to dragonfly nymphs. The MP that were exposed to the daphnids were transferred to dragonfly nymphs and reduced the microbiome diversity and abundance within the dragonfly nymph (Varg et al., 2022). Based on the trophic transfer of microplastic effects observed by Varg et al. (2022) from daphnids to dragonfly nymphs, terrestrial predators of dragonfly nymphs (e.g. birds) may also be at risk of MP exposure (Kennedy et al., 2019). Al-Jaibachi et al. (2019) explained that dragonflies have a significant role in trophic transfer between aquatic and terrestrial environments. Therefore the health and safety of the dragonflies is important to protect other organisms within the community. After 14 days of 15,000 microplastics/mL concentration exposure, dragonfly nymph respiration rates were unaffected, however MP were observed on the external and internal structures of the abdominal and thoracic cavities. Birds, fish, and amphibian populations that feed on contaminated dragonfly nymphs will then be at risk of consuming MP. Bhatt & Chauhan (2023) reviewed the ecological impact of MP transfer within aquatic and freshwater ecosystems and found that bioaccumulation, trophic transfer, and biomagnification are likely, however further research is needed to develop a multidimensional understanding of MP exposure through trophic transfer (Bhatt & Chauhan, 2023).

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