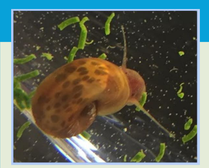

Impact of Microplastic Fiber Pollution on Ramshorn Snail (*Planorbella campanulata*) Fecundity and Mortality

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Introduction

Plastic pollution in bodies of water is an emerging issue. Plastic microfiber pollution (synthetic fibers <5mm shed notably from clothing and carpeting) is a particular problem because of its potential impact on marine life. Microfibers are increasingly being found and measured in aquatic environments causing great concerns for how they may be affecting the biota (Anderson et al. 2016).

Research has shown that microplastic can have negative effects on aquatic organisms including impacts on shell formation in mud snails (*Potamopyrgus antipodarum)*(Imhof and Laforsch 2016), mortality in water fleas (*Daphnia magna*)(Jemec et al 2016), and decreased energy reserves in a marine worm (*Arenicola marina*)(Wright et al 2013). Our study examined the potential effects on survival and reproduction of exposure to plastic microfibers on the freshwater snail *Planorbella campanulata*. The snails were exposed to polyester microfibers from a blue Dacron fleece jacket in laboratory mesocosms for six weeks. Plastic microfibers from clothing are likely to enter aquatic systems through wastewater effluent and are known to occur in tributary streams and lakes in Indiana (Peller et al. 2019). Understanding the impacts of exposure on common aquatic organisms is essential to a better understanding of the impacts on the entire aquatic ecosystem of plastic microfiber pollution.

Research Question:
How does exposure to plastic microfibers affect fecundity & mortality of Ramshorn Snails?

Figure 1: Source of plastic microfibers. Left: Polyester Blue Dye Fleece/ Right: Cut up Polyester Microfiber plastic strands used in the experiment.

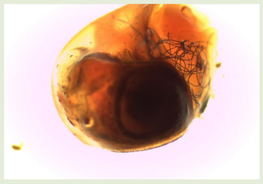

Figure 2: Enlarged image of exposed Ramshorn snail showing accumulation of fibers near the snail's mouth

Materials & Methods

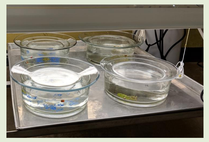

Figure 3. Experimental Mesocosms = 1.2 L Great Value spring water+ 2-4 Adult snails+ 5cm *Anacharis* plant+ .05 g Microfibers (Experimental) or no Microfibers (control).).

Two rounds of the experiment were completed, one with four adult snails per mesocosm, 12 control and 12 experimental replicates. and a second with two adult snails per mesocosm and 11 replicates/treatment

Figure 4: Laboratory set up. Mesocosms were provided a 12hr light/dark cycle. 22 C, and new water to replace evaporation. They were fed once every 3 weeks (0.015 g Wardly Weekend Fish Feeder with Calcium Sulfate matrix).

Figure 5: Adult snail marking. Snails were marked to identify individuals throughout the experiment with white and Pink nail polish.

Figure 6: Data collection. Each mesocosm was assessed once per week for adult mortality, egg sacs, total eggs production, and juvenile snails over a 6-week Period.

Results

We found no significant differences in the two rounds of the experiment, one with two starting adults and one with four initial adults, thus we combined all data for analysis using a linear mixed effects model in R.

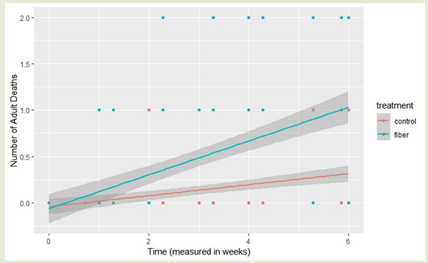

Figure 7. Adult Mortality was higher in snails exposed to plastic microfibers**.** Adult deaths in each control mesocosm is predicted to increase 0.06 each week, while 0.18 each week in experimental mesocosms (t = 6.336, p = 7.93 x 10^{-10}).

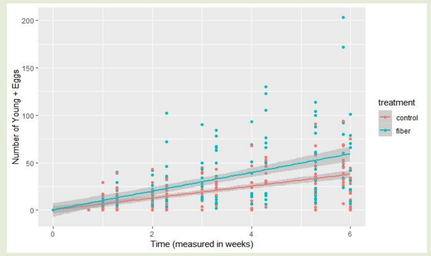

Figure 8. Total offspring as measured by number of eggs plus number of young each week was higher per adult snail in experimental mesocosms. Control mesocosms were predicted to increase in total offspring by around 5 per week while the experimental mesocosms were predicted to increase in total offspring by around 7.5 per week. (t = 2.149, p = 0.033).

Overall conclusions:

- **Snails exposed to plastic microfibers experienced higher mortality.**
- **Adult snails produced more offspring per snail in mesocosms with higher adult mortality.**
- **Exposure to plastic microfibers resulted in more total offspring compared to the control treatment over the course of the six week experiment.**

Conclusion/ Remaining Questions

- The increased reproduction we observed in the experimental treatment may be caused by blue dye or other chemicals leaching out of the fibers themselves. One study in particular looked at the snail species *Potamopyrgus antipodarum*, that were bred in glass and PET (polyethylene terephthalate) bottles. The results showed that there was an increase in snail reproduction in the PET bottles possibly due to estrogenic effects (Wagner et al., 2009) and a similar phenomenon could be happening in our experimental system.
- Mortality and reproduction could be linked in snails as it is in other animals (Tarwater and Arcese 2017), where senescing individuals pump out one last pulse of offspring before dying (called terminal allocation hypothesis). If exposure to plastic leads to higher mortality, then a concomitant pulse of offspring might be expected at the end of life.
- We exposed snails to large amounts of polyester microfibers such as would occur if cast-off clothing becomes pollution in an aquatic system. However, little is known about actual levels of polyester microfibers through aquatic ecosystems, thus our results must be viewed with caution.

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