Effects of Environmentally-Realistic Concentrations of Microplastics on *Daphnia magna* Growth and Development

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INTRODUCTION

- Microplastics have been identified ubiquitously in the environment, and the prevalence of microplastics in freshwater and marine ecosystems has been of particular concern since contamination in these water sources would have a global impact and affect virtually all organisms (Eerkes-Medrano *et al.* 2015).
- Studies on the effects of microplastics on aquatic life have found that microplastics exposure reduced fertility and growth in organisms such as *Daphnia magna* (Cauwenberghe and Janssen 2014, Sussarellu *et al.* 2016, Bessling *et al.* 2014).
- However, researchers have criticized those studies for using experimental concentrations of microplastics that were orders of magnitude higher than environmentally observed concentrations and particles that were orders of magnitude smaller than what has been observed in the wild (Lenz *et al.* 2016).
- For this experiment, we aimed to observe the effects of microplastics on the population, growth of specific features, and fertility of *Daphnia magna* over the course of three weeks (considerably longer than average gestation of daphnia, allowing for observation of offspring) and compare them to a negative control.

QUESTION

Does environmentally-realistic concentration of microplastics have a biological effect on aquatic life?

HYPOTHESIS

If microplastics at environmentally-realistic concentrations do have a biological effect on *Daphnia magna*, then we expect to see statistically significant differences in growth and development between the experimental group and the control.

METHODS

- The experimental group was treated with pristine polyethylene beads (10^2 particles/mL, d=7 µm) that were more similar in concentration and size to microplastics detected in the wild. Both groups were in spring water.
- We taped a paper ruler to a glass slide and measured the base length (eye to the base of the tail), antenna length, tail length, and girth (along the widest point in the body) of each *Daphnia.*
- We also took note of the population size and number of pregnant *Daphnia* in each group. To start, there were 16 *Daphnia* in each group. None were pregnant.
- All statistical analyses were performed with R Studio software.

STATISTICAL ANALYSIS AND RESULTS

- When excluding the baseline from the Week x Condition 2-way ANOVA, there is only a statistically significant main effect of week for tail length ($p < .001$). In other words, base length and girth do not vary as a function of week after the initial time point.
- The homogeneity of variances assumption was met for all measurements except the antenna length. Antenna length had also violated the normality assumption. Therefore, measurements of antenna length were excluded from the analysis.

Tail Length Longer at Week 2

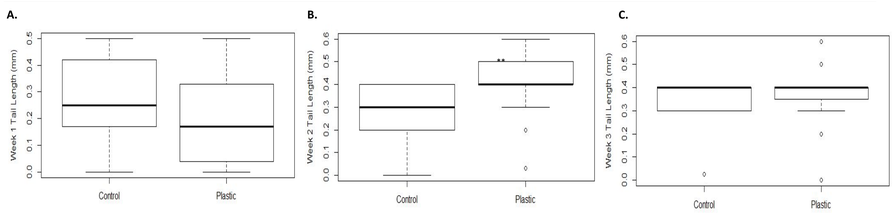

Figure 1. Tail length measurements (mm) for *Daphnia* in control and experimental ("plastic") groups at A. Week 1 B. Week 2 C. and Week 3. Performing a independent samples t-test, we observed that tail length measurements are statistically significantly higher in the experimental group only at week 2 (** = $p < .01$). Although longer tail lengths have been associated with stress response in *Daphnia*, it remains unknown the difference in tail length between the two groups would not continue onto week 3.

Pregnancy Cycle Altered

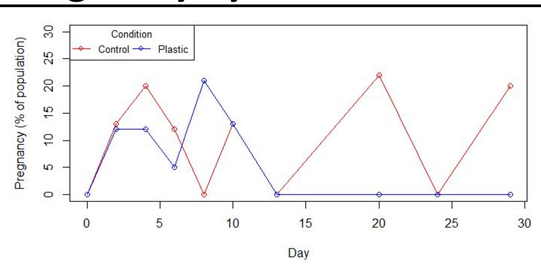

Figure 2. Percentage of Daphnia Population Pregnant in Control or Plastic Conditions. Performing a two-way ANOVA (Day x Condition) for population and pregnancy data (excluding Day 29 from the dataset since it likely violates an assumption), we observe no statistically significant effect of time or condition on pregnancy. However, there is a statistically significant main effect of condition on population. In other words, the population does not vary as a function of time, but of condition. In fact, according to the partial eta squared, 26.3% of the variance in population size can be explained by the condition after controlling for time.

CONCLUSIONS

- Given limitations in sample size, we were unable to corroborate previous findings about the effect of microplastics on *Daphnia* growth and development
- At environmentally-realistic concentrations there is evidence to suggest that microplastics have a biological effect on *Daphnia.*
- However, the biological effect of microplastics on *Daphnia* growth seems to exhibit fewer macro-level manifestations. And although longer tail lengths at Week 2 may suggest increased stress, there is not enough data to make this conclusion definitively.
- The alterations in fertility cycle induced by the presence of microplastics, thus far, are the most suggestive of a negative biological effect resulting from even relatively small environmentally-realistic concentrations of microplastics.

FUTURE DIRECTIONS

- To run this experiment with a larger sample size (n=80).
- Omit measurements of girth and antenna length, both of which yielded little useful information.
- Consistently measure pH and temperature of the tanks.
- Take measurements at consistent time intervals.

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