Does susceptibility to PET-NP exposure vary among *Daphnia magna* clones?

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Introduction

Nanoplastics (NPs) are emerging aquatic pollutants that may affect organismal health. *Daphnia magna*, a key ecotoxicological model, may exhibit clone-specific responses to contaminants, but the role of genetic variation in NP toxicity remains poorly understood. Existing studies of genotype-by-environment (G × E) interactions have focused on larger microplastics rather than NPs. Here, we investigated clonal variation in life-history responses to bottom-up synthesised PET-NPs (80 nm). To our knowledge, this is the first study to examine clonal variation in *D. magna* responses to PET-NPs. Understanding such variation can help improve predictions of ecological risk and inform assessments of species' responses to environmental change and pollution.



Fig.1: *Daphnia magna*

Objectives

- **Determine whether genetic variation affects *D. magna* life history traits.**
- **Determine whether PET-NP exposure affects *D. magna* life history traits.**
- **Test whether clones differ in their response to PET-NPs (G × E interaction).**

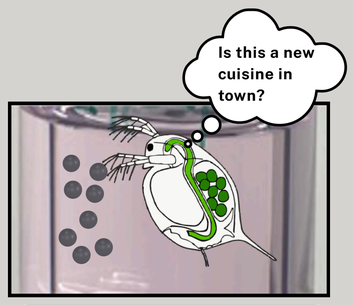


Fig.2: Pink solution represents NP suspension; grey spheres depict NPs; schematic, not to scale

Methodology

- Nanoplastics: synthesised by nanoprecipitation; DLS and SEM for characterisation
- Design: 3 *D. magna* clones × 2 treatments (Control, 3 mg/L PET-NPs), n = 10 each, high food (*C. vulgaris*)
- Experiment duration: 3 weeks or until 3rd clutch is dropped.
- Analysis: 8 life history traits; PCA, PERMANOVA and two-way ANOVA (Tukey's HSD).

Results-NP characterization

- DLS analysis revealed particle sizes in the range of **68nm-80nm.**
- Surface morphology analysis using SEM revealed a **spherical shape** of the synthesised NPs.

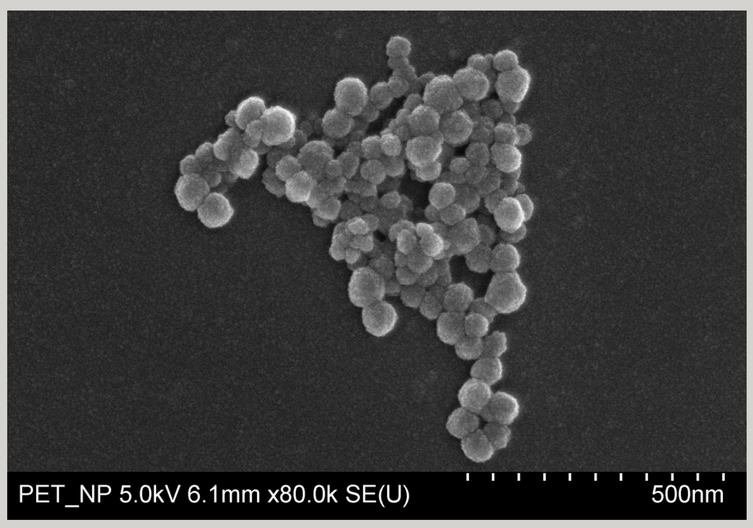


Fig.3: SEM micrograph of PET-NPs

Results- Life history traits

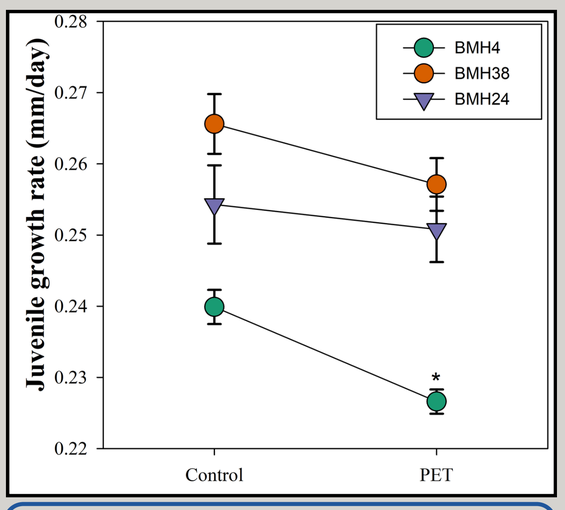


Fig.4: Reaction norms of juvenile growth rate (mean ± SE). Two-way ANOVA: **Clone (p < 0.001)**, **Treatment (p = 0.012)**, Clone × Treatment (p = 0.469). * indicates a significant difference between Control and PET within clone BMH4 **(p=0.021)**; no symbol indicates no significant difference.

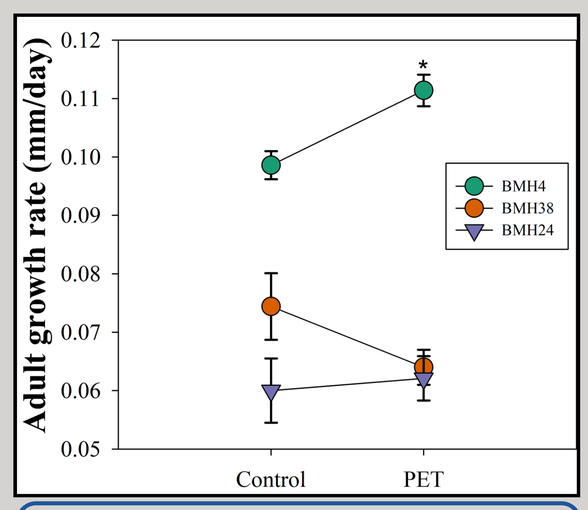


Fig.5: Reaction norms of adult growth rate (mean ± SE). Two-way ANOVA: **Clone (p < 0.001)**, Treatment (p = 0.383), **Clone × Treatment (p = 0.088)**. * indicates a significant difference between Control and PET within clone BMH4 **(p=0.032)**; no symbol indicates no significant difference.

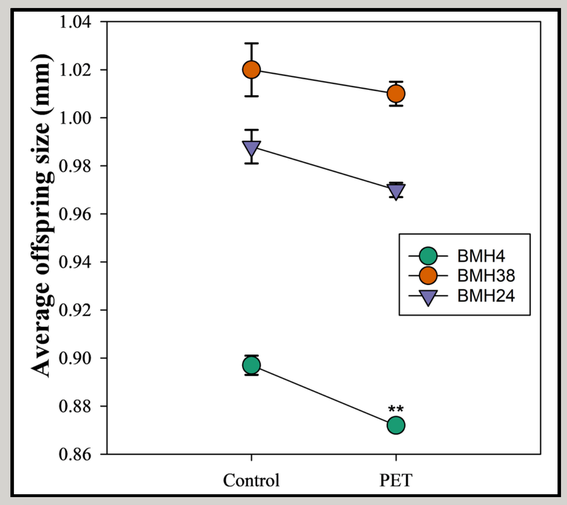


Fig.6: Reaction norms of average offspring size (mean ± SE). Two-way ANOVA: **Clone (p < 0.001)**, **Treatment (p = 0.004)**, Clone × Treatment (p = 0.425). ** indicates a significant difference between Control and PET within clone BMH4 **(p=0.007)**; no symbol indicates no significant difference.

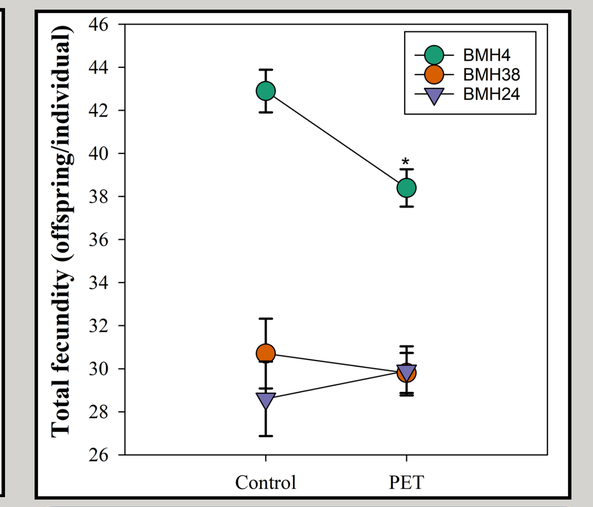


Fig.7: Reaction norms of total fecundity (mean ± SE). Two-way ANOVA: **Clone (p < 0.001)**, Treatment (p = 0.199), **Clone × Treatment (p = 0.072)**. * indicates a significant difference between Control and PET within clone BMH4 **(p=0.014)**; no symbol indicates no significant difference.

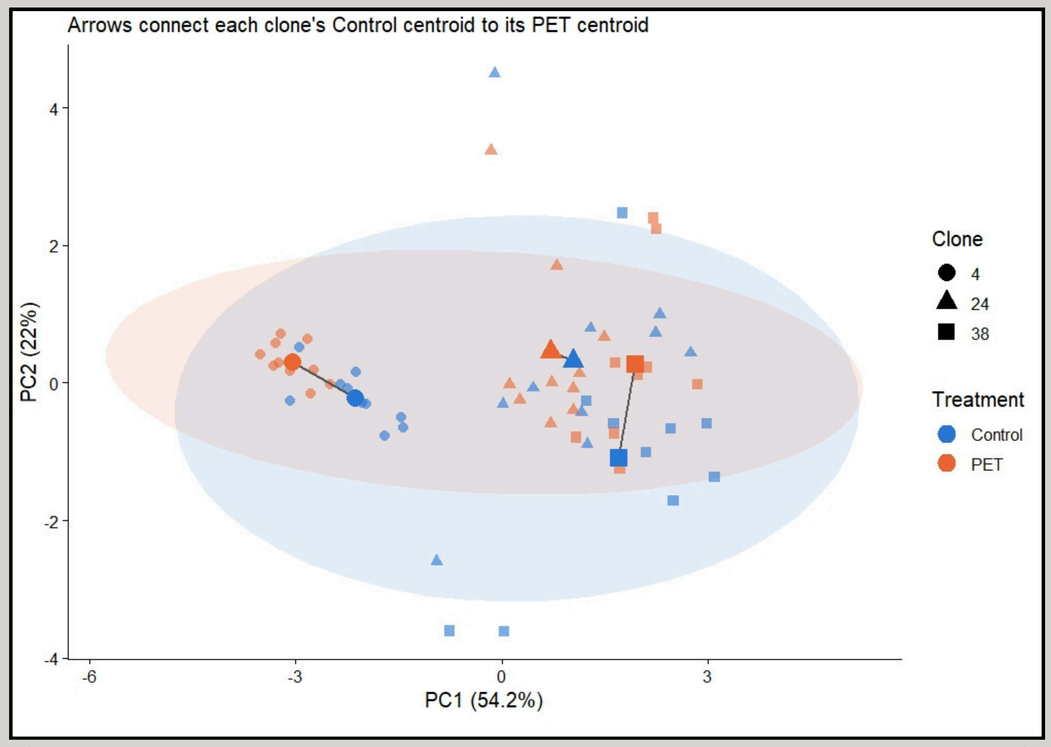


Fig.8: PCA of eight life history traits. Large symbols are group centroids, and arrows run from Control to PET for each clone. Clone explained 52% of multivariate variation. PERMANOVA: Clone R^2 = 52%, $p < 0.001$; PET R^2 = 2%, $p \approx 0.05$; Clone × PET R^2 = 3%, p = 0.118.

Conclusion and future directions

- **Clone identity was the main driver of life history variation**. It explained 52% of multivariate variation **(PERMANOVA, p < 0.001)** and significantly affected six of eight traits.
- **PET had a small, marginal overall effect** (R^2 = 2%, $p \approx 0.05$). It reduced offspring size and juvenile growth rate across clones
- **The clone x PET interaction was not significant** among clones.
- **Next steps: test clones under low food and higher temperature.**

Acknowledgements

Picture credits: Dr. Stewart Plaistow for the *D. magna* light microscope image and cartoon sketch; Ellie Wilson for the NP suspension image.

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