Microplastics at environmentally relevant concentrations had minimal impacts on pelagic zooplankton communities in a large in-lake mesocosm experiment

Desiree Langenfeld[1,2], Kennedy Bucci[3], Cody Veneruzzo[4], Rachel McNamee[5], Gloria Gao[3], Chelsea M. Rochman[3], Michael D. Rennie[4], Matthew J. Hoffman[6], Diane M. Orihel[7], Jennifer F. Provencher[8], Scott N. Higgins[1], Michael J. Paterson[1,2].*

[1]International Institute for Sustainable Development Experimental Lakes Area, Winnipeg, Manitoba, R3B 0T4, Canada

[2]Department of Entomology, University of Manitoba, Winnipeg, Manitoba, R3T 2N2, Canada

[3]Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Ontario, M5S 3B2, Canada

[4]Department of Biology, Lakehead University, Thunder Bay, Ontario, P7B 5E1, Canada (ORCID: 0000-0001-7533-4759)

[5]Department of Biology, University of Waterloo, Waterloo, Ontario, N2L 3G1, Canada

[6]School of Mathematics and Statistics, Rochester Institute of Technology, Rochester, New York, 14623, USA

[7]Department of Biology and School of Environmental Studies, Queen's University, Kingston, Ontario, K7L 3N6, Canada (ORCID: 0000-0002-6933-3650)

[8]Ecotoxicology and Wildlife Health Division, Environment and Climate Change Canada, Ottawa, K1S 5B6, Canada (ORCID: 0000-0002-4972-2034)

[*]Email: dlangenfeld@iisd-ela.org

Table of Contents Summary:

This SI is 16 pages, and includes 2 tables, and 14 figures.

Supplementary Tables

Table S1. Polymer summary for microplastics used in the mesocosm experiment.

	Polyethylene Terephthalate (PET)	**Polystyrene (PS)**	**Linear Low-Density Polyethylene (LLDPE)**
Colour	Blue	Pink	Yellow
Chemical additives	Blue pigment	Red pigment, Titanium dioxide (0.15%), Irgafos 126 Antioxidant (0.1%), N,N Ethylene Bis-stearamide (0.01%)	Yellow pigment, Chimassorb 944 HALS UV (0.05%), Tinuvin 622 HALS UV (0.05%), Irganox B215 Antioxidant (0.025%), Irganoz 168 Antioxidant (0.025%), Benzotriazole-Acetostab 236 (0.05%)
Polymer buoyancy	1.4 g/cm3	1 g/cm3	0.93 g/cm3
Size range (µm)	52-1408	48-1408	37-1086

Table S2. Final yellow perch densities and by-catch retrieved from mesocosms at the end of the experiment.

Nominal MP Treatment (particles/L)	**Final yellow perch densities**	**By-catch**
Control 1	4	
Control 2	17	
6	9	1 spottail shiner
24	NA	NA
100	12	1 spottail shiner
414	10	1 spottail shiner
1710	10	1 spottail shiner, 1 burbot
7071	9	4 spottail shiner
29,240	9	1 spottail shiner

Supplementary Figures

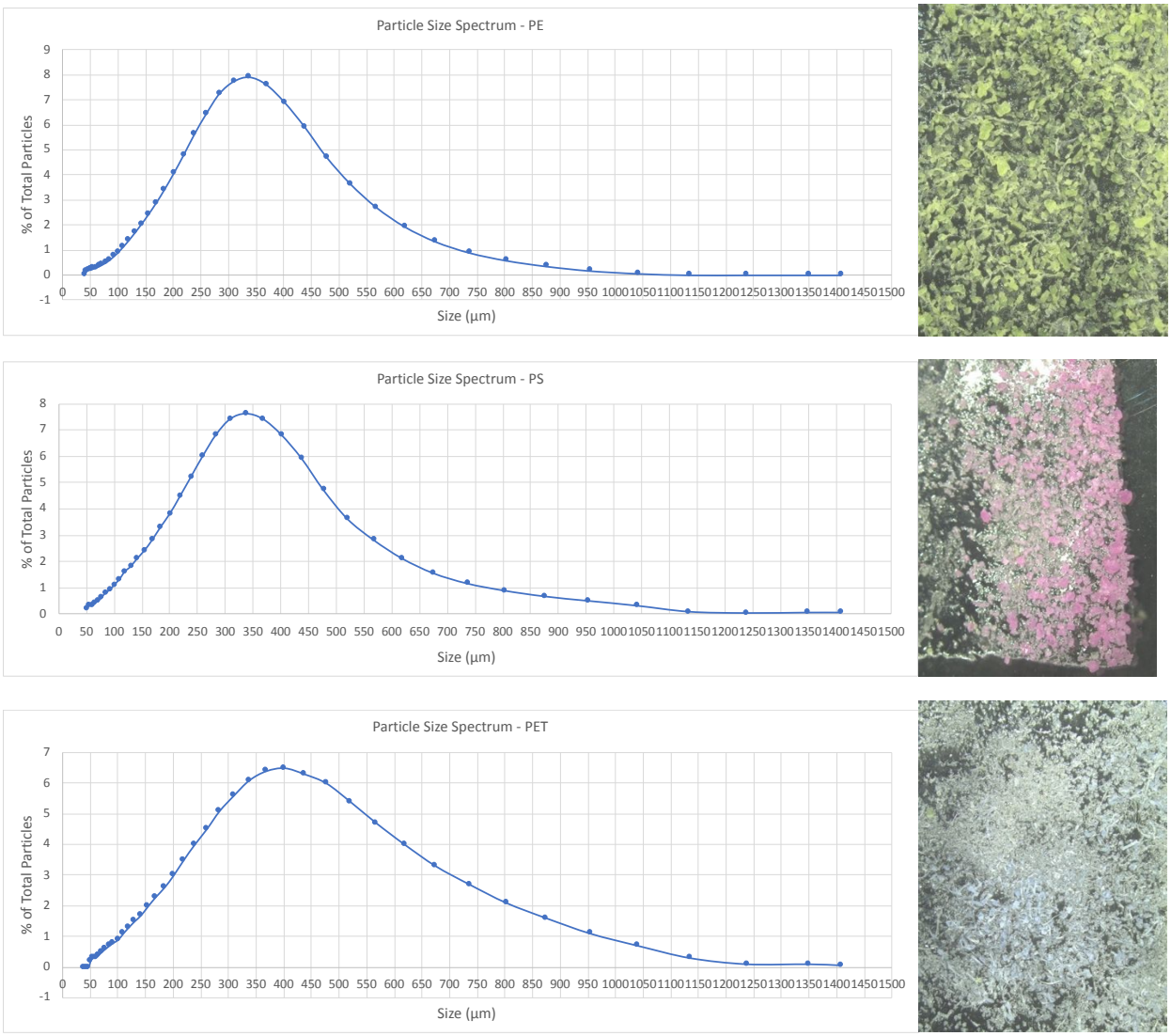

Figure S1. Size distributions of particles for each polymer as measured by Techmer (using stacked sieves) and images of PE (top), PS (middle), and PET (bottom) particles (from Rochman et al., 2024).

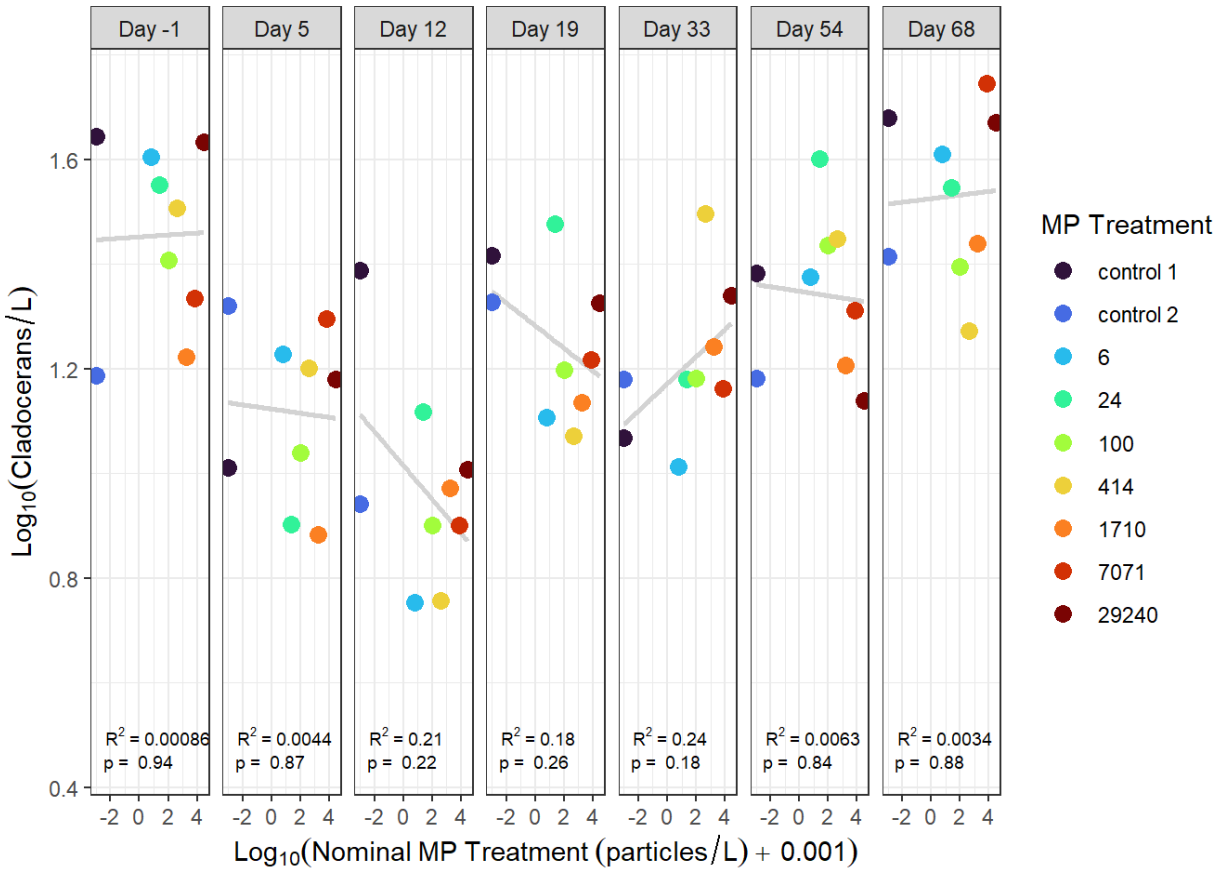

Figure S2. Cladoceran abundance versus microplastic treatment for 7 time points during the experiment. Light grey lines indicate non-significant regressions.

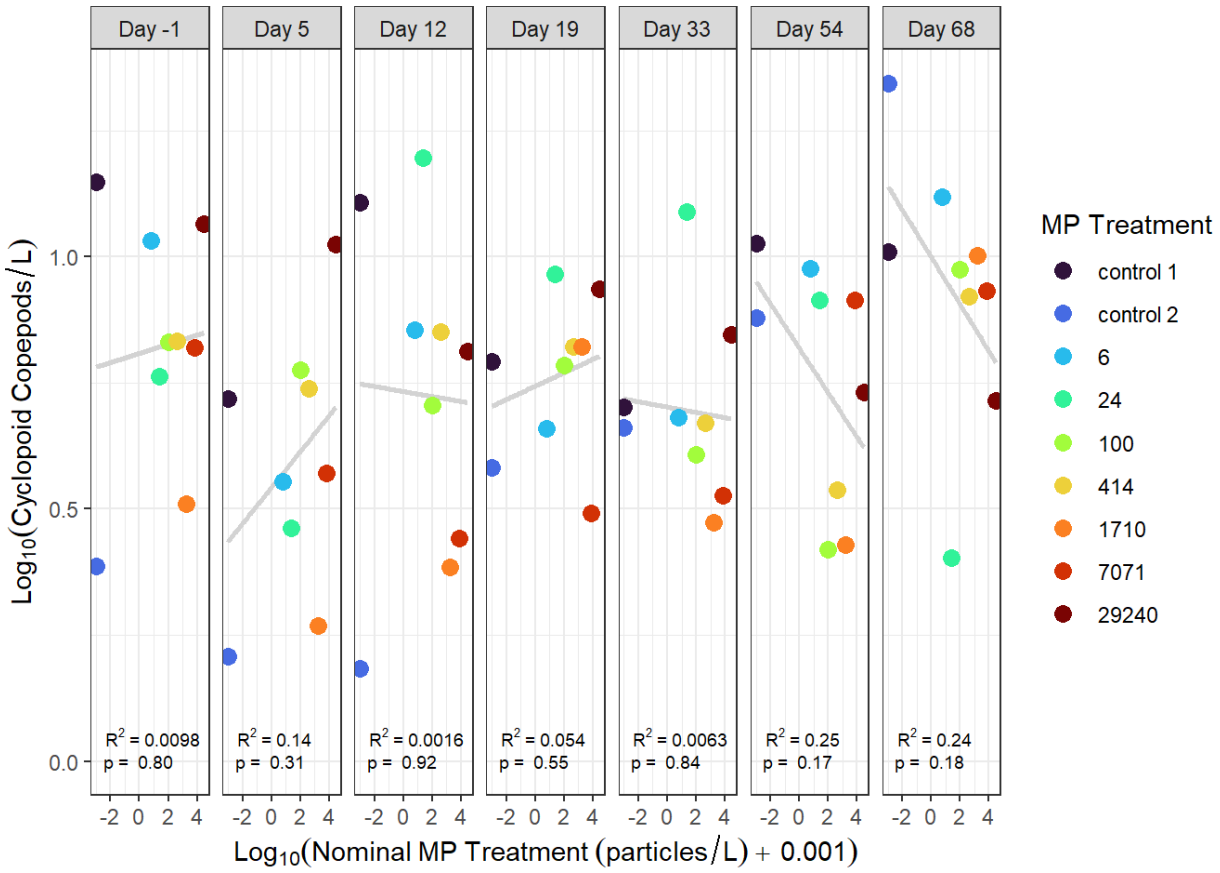

Figure S3. Cyclopoid copepod abundance versus microplastic treatment for 7 time points during the experiment. Light grey lines indicate non-significant regressions.

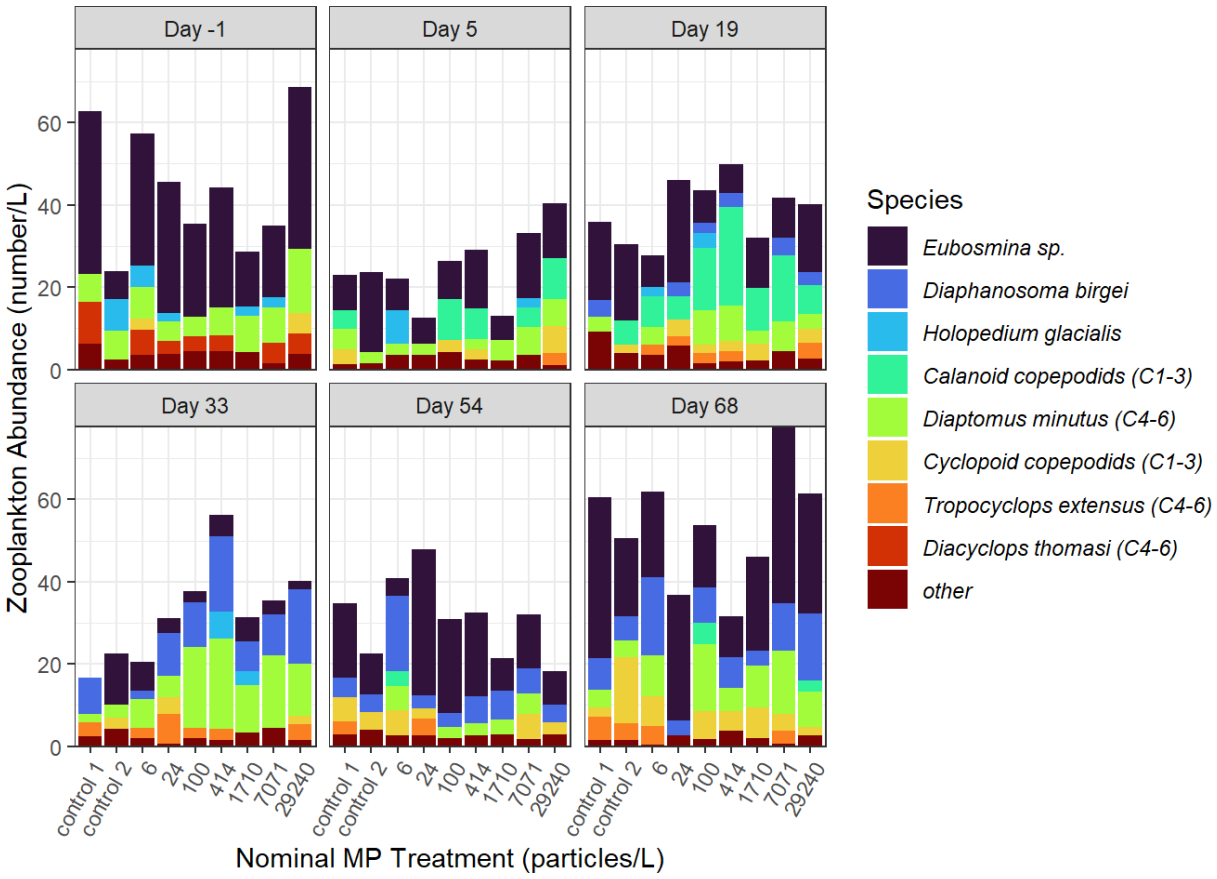

Figure S4. Zooplankton species composition for each nominal microplastic treatment on different days of the experiment.

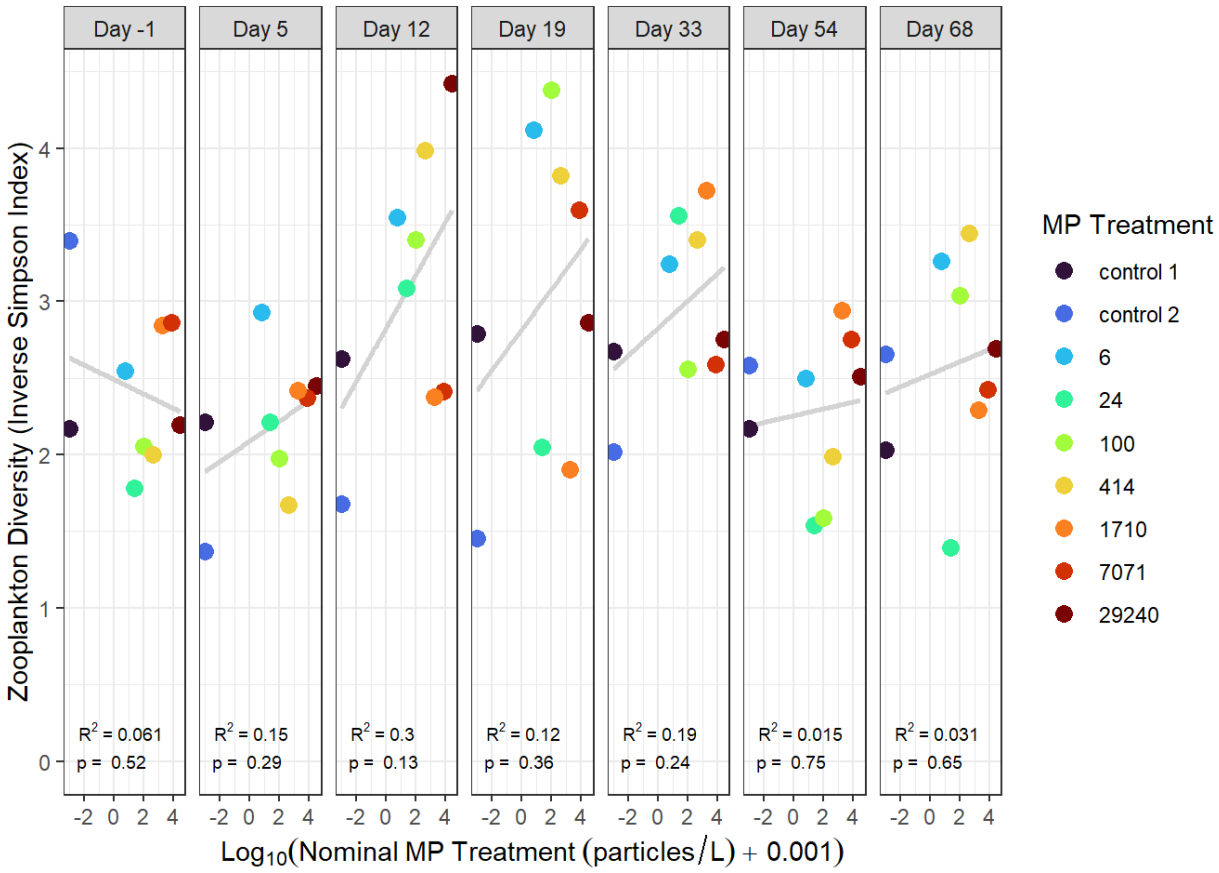

Figure S5. Zooplankton community diversity, measured by the Inverse Simpson Index, versus nominal microplastic treatment concentration during the experiment. Light grey lines indicate non-significant regressions.

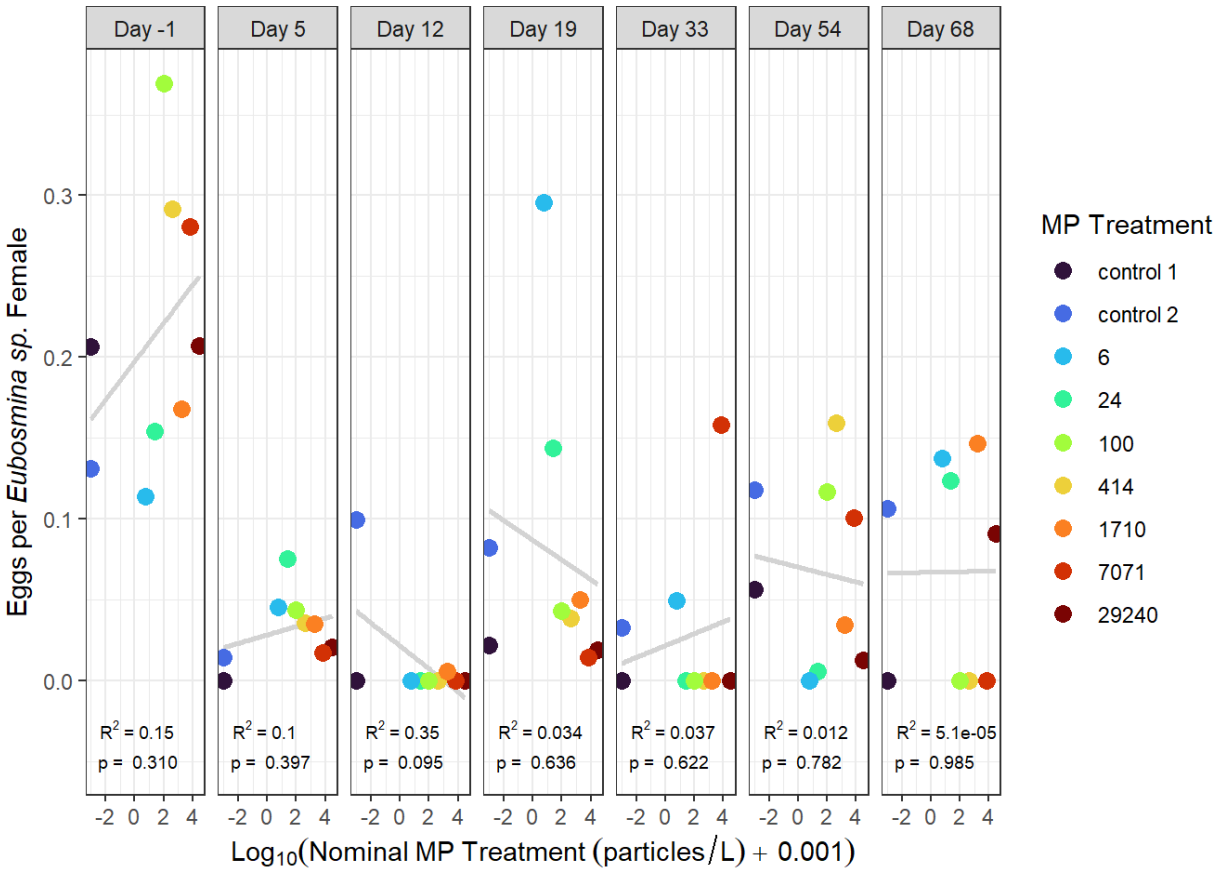

Figure S6. Egg ratios (eggs/female) for the cladoceran *Eubosmina sp*. regressed against nominal microplastic treatment concentration for various days of the study. Light grey lines indicate non-significant regressions.

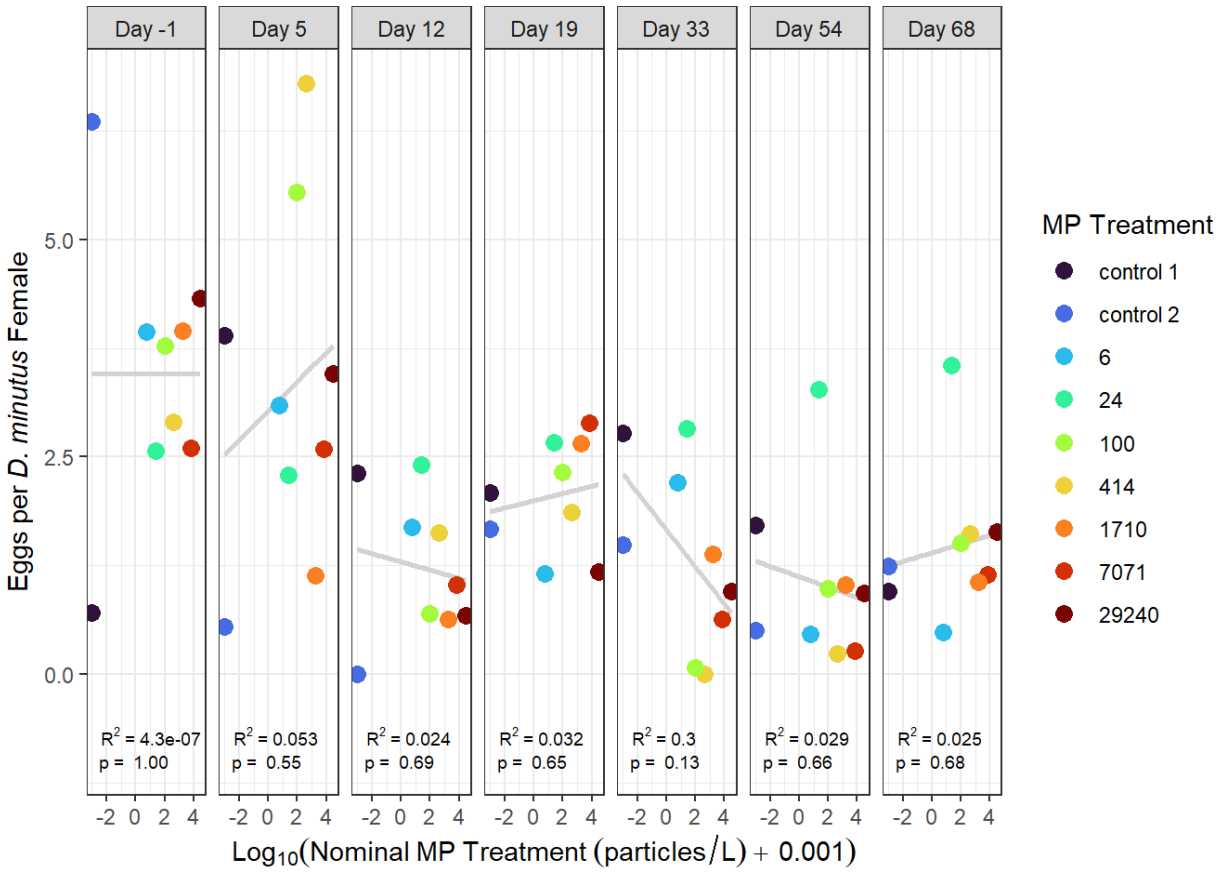

Figure S7. Egg ratios (eggs/female) for the calanoid copepod *Diaptomus minutus* regressed against nominal microplastic treatment concentration for various days of the study. Light grey lines indicate non-significant regressions.

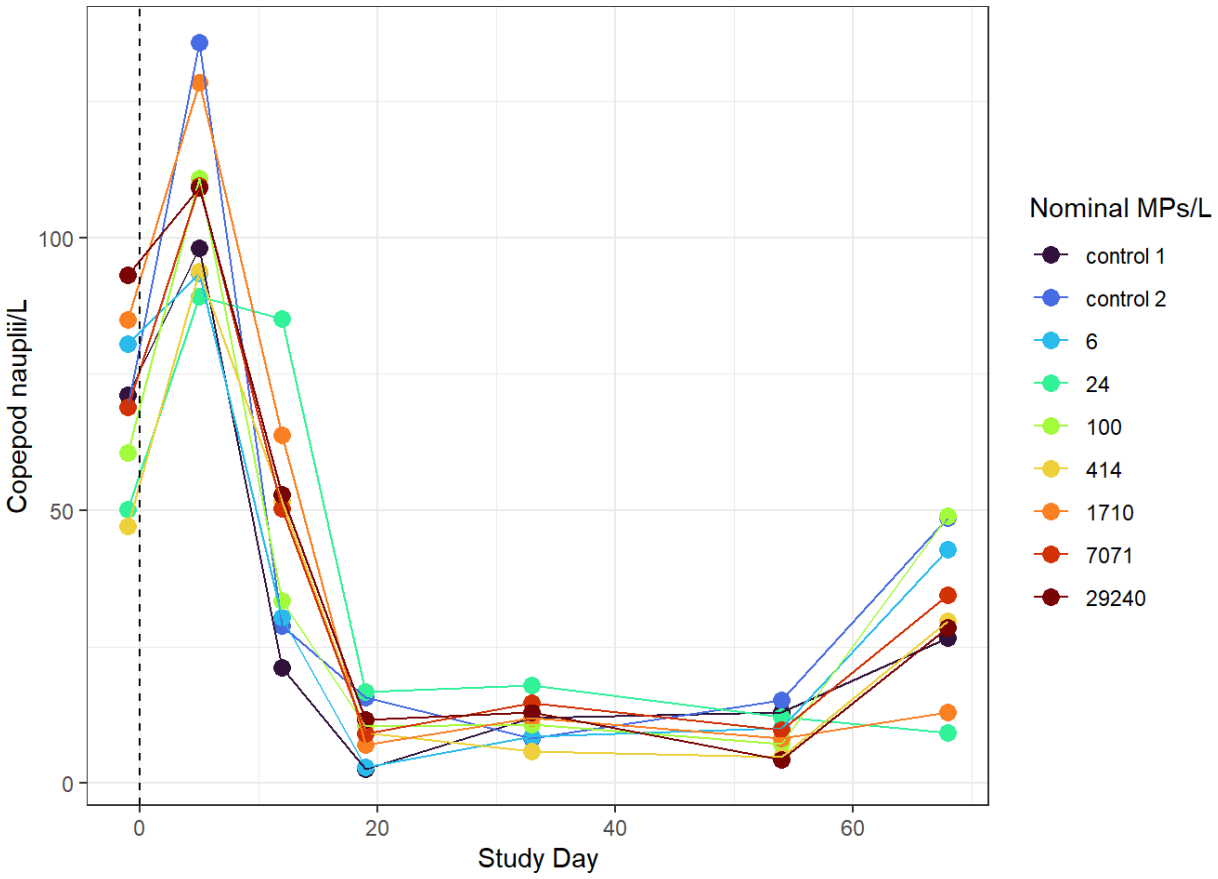

Figure S8. Copepod nauplii abundance throughout the study for each nominal microplastic treatment.

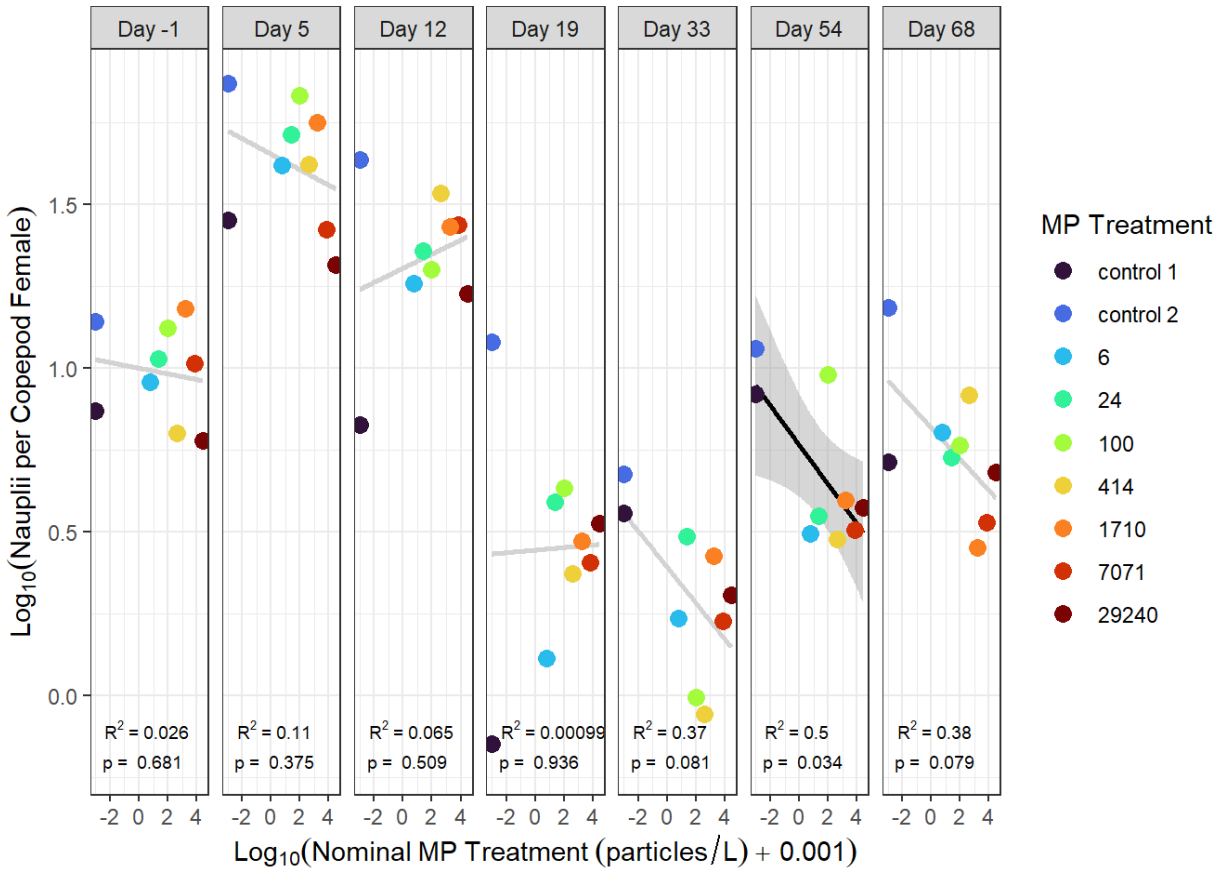

Figure S9. Nauplii per copepod female versus nominal microplastic treatment for 7 time points during the mesocosm experiment. Significant regressions are indicated by black lines and light grey lines indicate a non-significant regression. 95% confidence intervals are indicated by shaded regions.

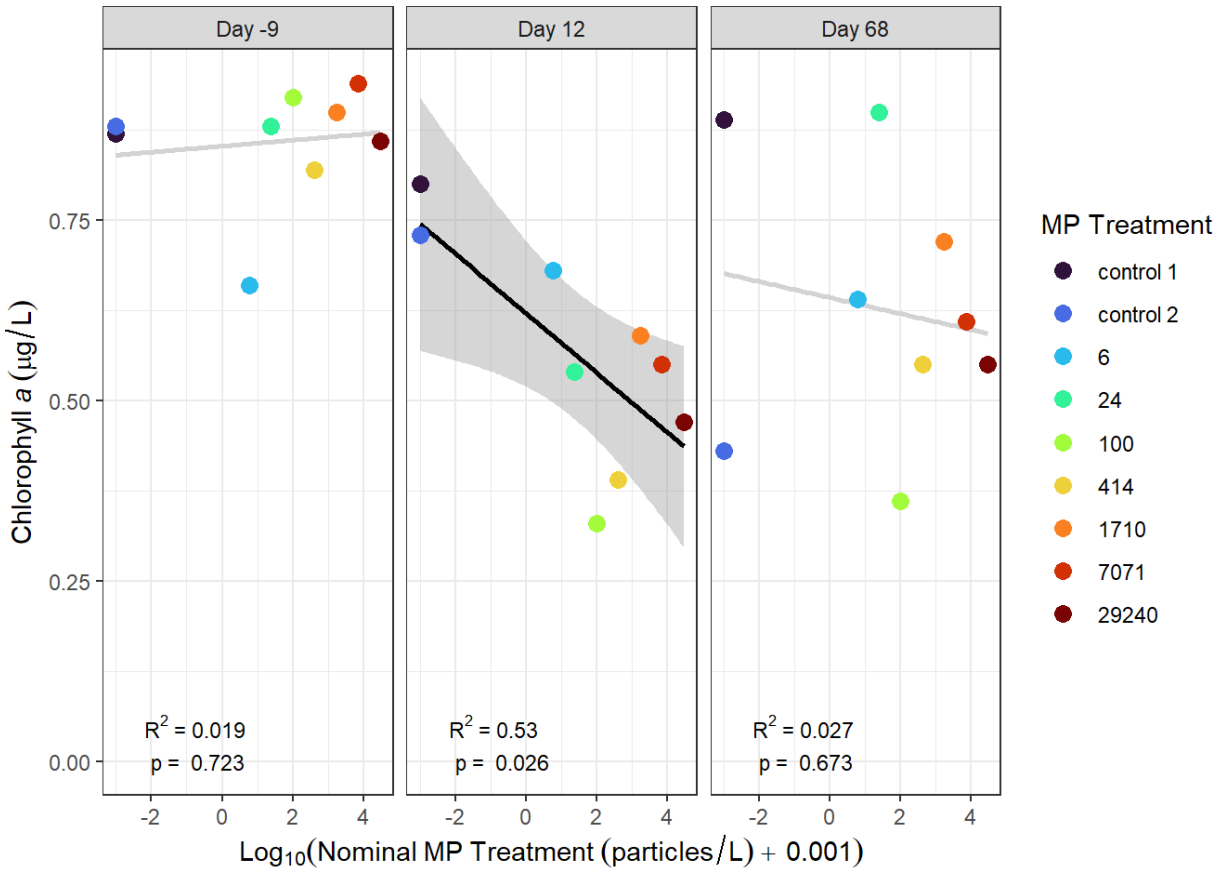

Figure S10. Chlorophyll *a,* linearly regressed against $\log_{10}$ nominal microplastic treatment concentration, for three different time points throughout the experiment. Significant regressions are indicated by black lines and light grey lines indicate a non-significant regression. 95% confidence intervals are indicated by shaded regions.

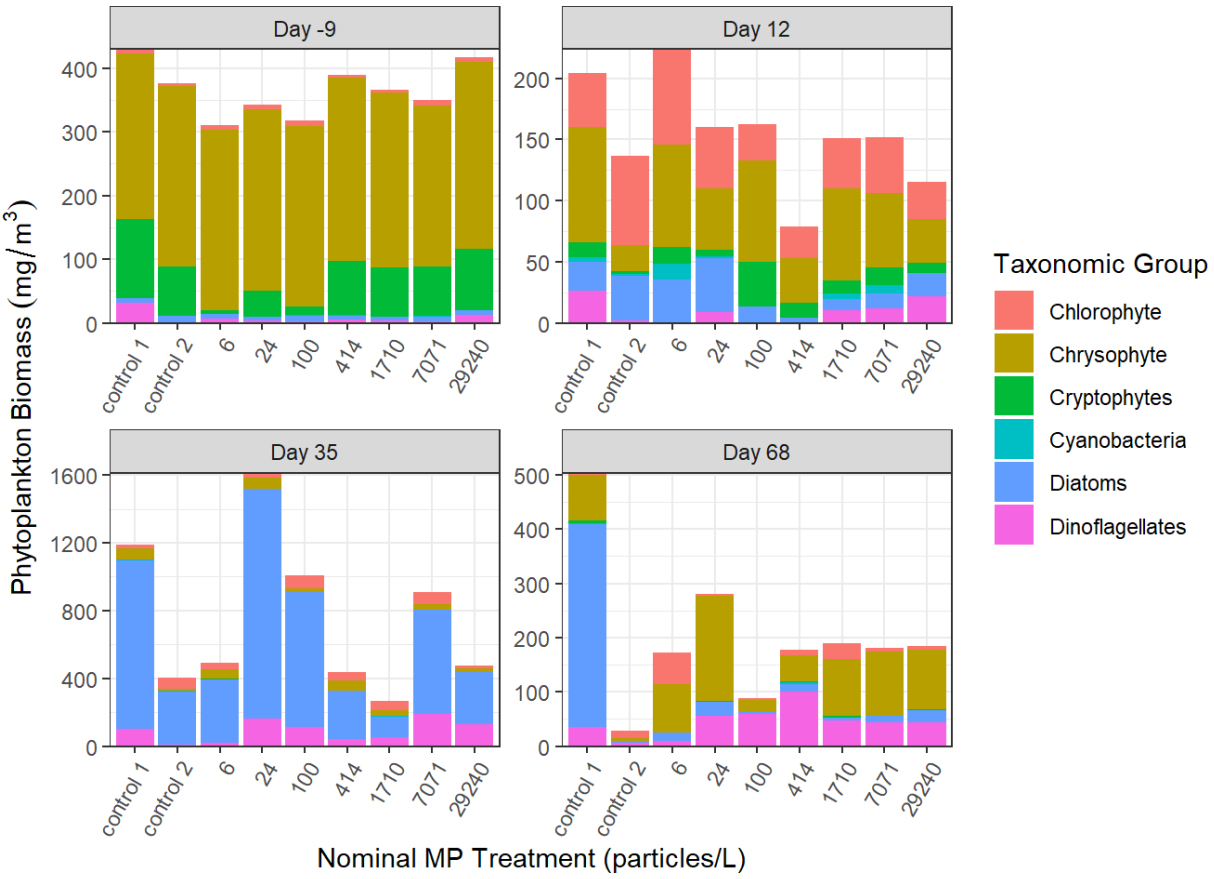

Figure S11. Biomass-based phytoplankton community composition (wet weight) for each microplastic treatment. Note the different scales for each plot.

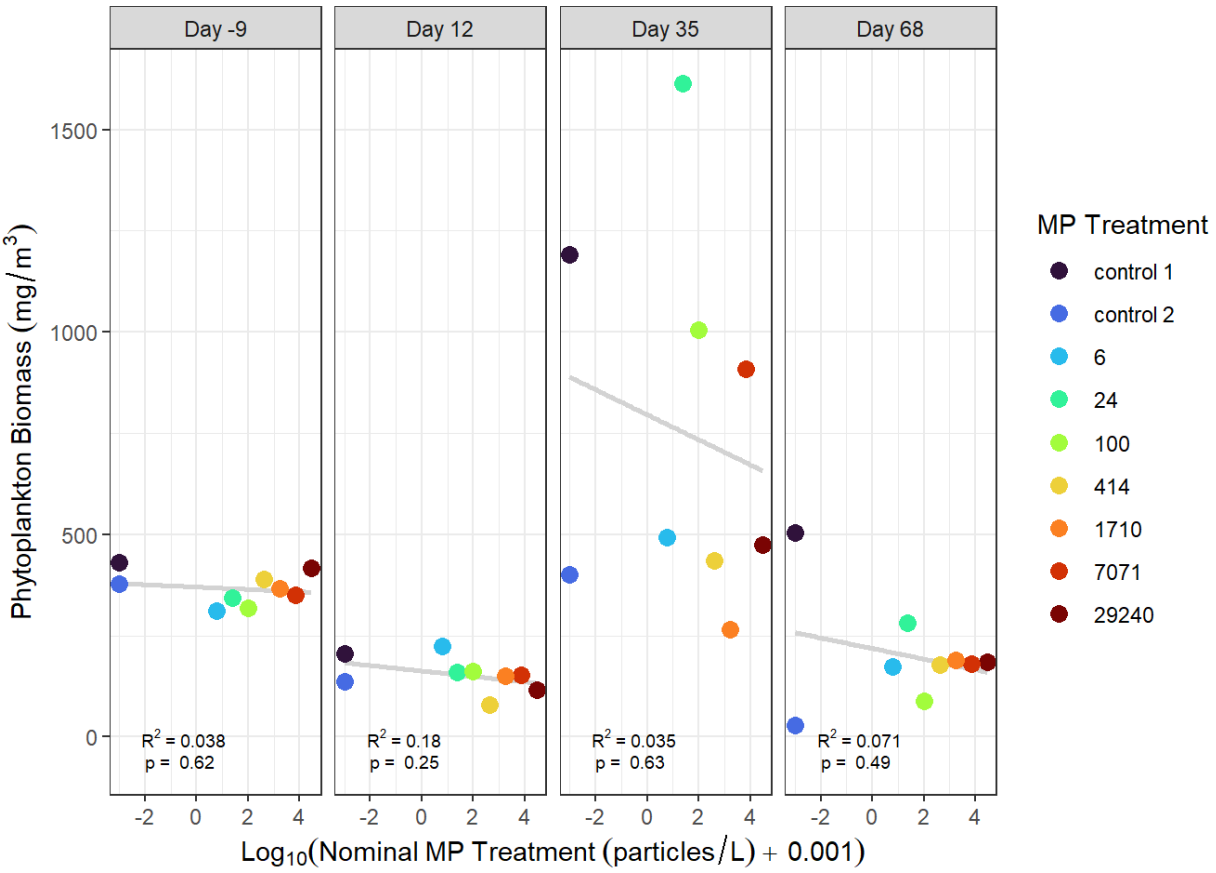

Figure S12. Total phytoplankton biomass linearly regressed against $\log_{10}$ microplastic treatment concentration for 4 different days of the experiment. Light grey lines indicate non-significant regressions.

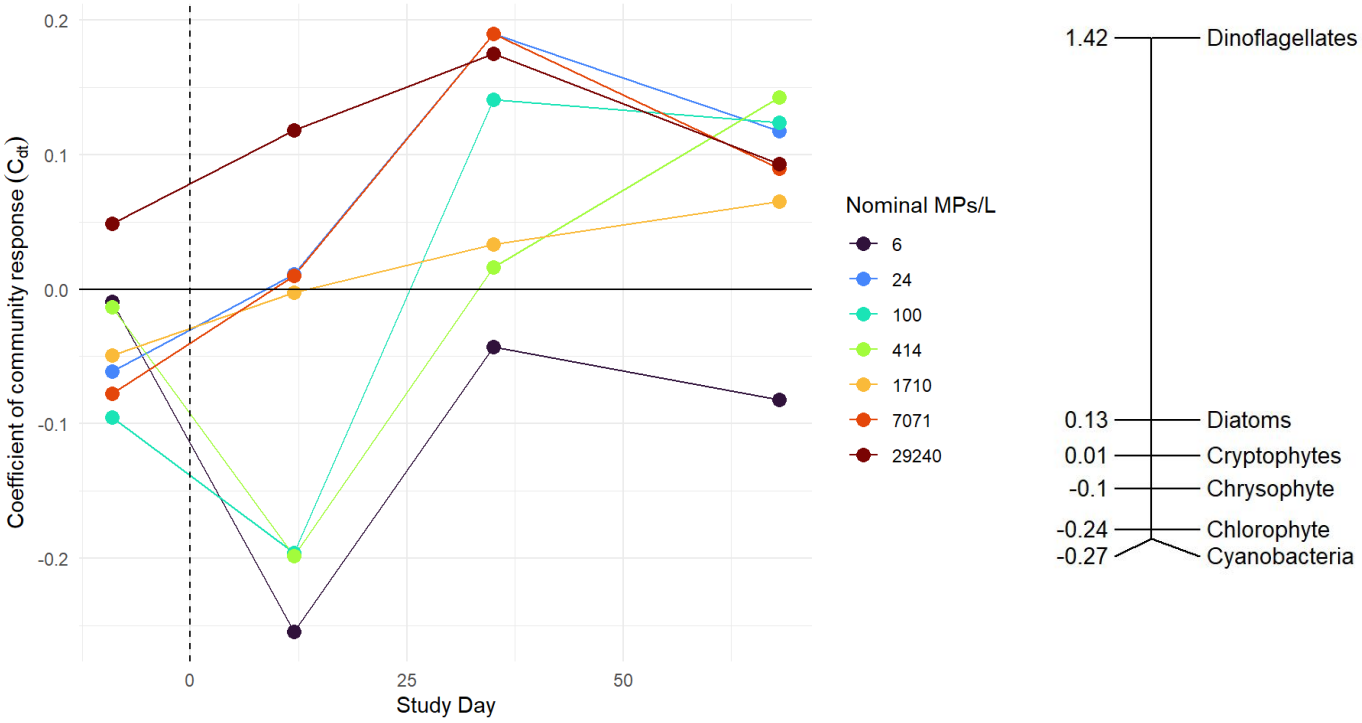

Figure S13. Principal response curve (PRC) for biomass-based phytoplankton community composition. Phytoplankton biomass was $\log_{10}$ transformed prior to analysis. The community response (C_{dt}) for each microplastic treatment is displayed on the left side of the y axis and species weights (b_k) are displayed on the right. The solid black horizontal line represents the average of the control treatments (where $C_{dt} = 0$), and the dashed vertical line represents the day of microplastic addition (day 0). The PRC axis was not significant ($p = 1$).

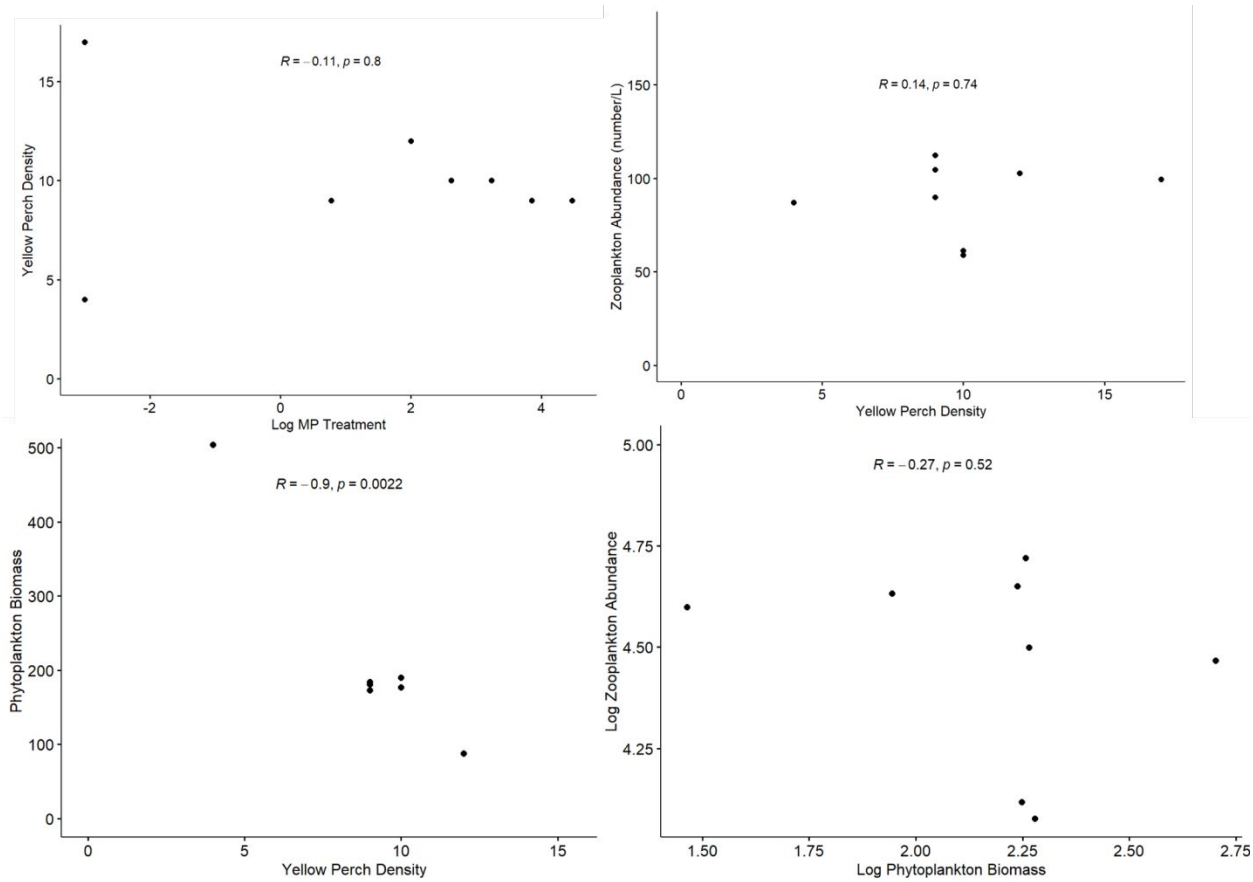

Figure S14. Correlations between confounding variables on day 68 of the mesocosm experiment including yellow perch density versus nominal microplastic treatment (top left), zooplankton abundance versus yellow perch density (top right), phytoplankton biomass versus yellow perch density (bottom left) and zooplankton abundance versus phytoplankton biomass (bottom right).

References

Rochman, C. M.; Bucci, K.; Langenfeld, D.; McNamee, R.; Veneruzzo, C.; Covernton, G. A.; Gao, G. H. Y.; Ghosh, M.; Cable, R. N.; Hermabessiere, L.; Lazcano, R.; Paterson, M. J.; Rennie, M. D.; Rooney, R. C.; Helm, P.; Duhaime, M. B.; Hoellein, T.; Jeffries, K. M.; Hoffman, M. J.; Orihel, D. M.; Provencher, J. F. Informing the Exposure Landscape: The Fate of Microplastics in a Large Pelagic In-Lake Mesocosm Experiment. *Environ. Sci. Technol*. **2024**. https://doi.org/10.1021/acs.est.3c08990.