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Supplementary Materials

Bioaccumulation, transfer, and impacts of microplastics in aquatic food chains

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Plastic Morphology Metric Calculations

To calculate the total volume of plastic in each treatment, we combined 0.1 g (HDPE = 0.95 g/cm^3) of plastic with 49.9 g of distilled water. This resulted in 0.095 cm^3 of plastic in 50 mL of solution. This number was then divided by the total volume estimated for a single plastic particle given its size ($V = 4/3 \times \pi \times r^3$), where r = 12.5, 35, and 275 µm for the tween, small, and large spheres, respectively. This provided the total number of each type of microplastic sphere present in our stock solutions.

$$0.095 / (4/3 \text{ x } \pi \text{ x } r^3) \quad (1)$$

We multiplied the number of spheres in 0.1 g in 50 mL solution by their surface area ($4 \times \pi \times r^2$) to estimate the plastic surface area in each stock solution. Stock solutions were then used to create experimental stocks, that would be added to our jars and tanks, depending on the experiment. We diluted 2% of the stock liquid (1 mL) to 10 mL (9 mL of distilled water). These experimental solutions were then added to experimental systems - for trials carried out in algal/copepod trials, we added 1.5 mL of experimental

solution to 148.5 mL of distilled or seawater (150 mL total volume); and for fish trials, we added 10 mL of experimental solution to 1.99 L of freshwater.

With these estimates of plastic abundance, volume, and surface area, we could then determine the concentration and total surface areas that were contained in each experimental replicate. To estimate the total number of plastics in a jar, we divide the plastics in the 10 mL experimental stock (Equation 1) by 10 and multiply by 1.5. This number is then multiplied by the surface area of a particle to derive the surface area in a jar. To estimate the total number of plastic particles and their surface area in tanks, we divided the numbers present in the experimental solutions (10 mL) by 2000.

For jars with large spheres:
Diameter = 0.055 cm; radius = 0.028 cm; surface area of 1 particle = 0.009 cm^2, volume of 1 particle = 0.00009 cm^3; plastics in a jar 16.36 particles and 0.155 cm^2, plastics in a tank 109 particles and 1.03 cm^2.

For jars with small spheres:
Diameter = 0.007 cm; radius = 0.035 cm; surface area of 1 particle = 0.0002 cm^2, volume of 1 particle = 1.8×10^{-7} cm^3; plastics in a jar 7,939 particles and 1.22 cm^2, plastics in a tank 52,924 particles and 8.14 cm^2.

Tween Toxicity Trials

We used fluorescently labeled high-density polyethylene microspheres ("polyspheres," Cospheric, Santa Barbara, CA, USA) with consistent diameters and density in our exposure trials of algae, copepod consumers, and fish second-order consumers. In accordance with manufacturer recommendations[1], we understood that a surfactant would promote the suspension of the microspheres in seawater and freshwater media rather than accumulating on the liquid's surface. Surfactants occur naturally in the environment (Alkyl sulfate, Alcohol ethoxylate, Benzalkonium chloride, *etc*.), and undoubtedly perform the same function in suspending materials in water where the density is similar to the aqueous solution in which they are mixed[2,3]. The recommended surfactant for this application was Tween 80[1], and before assessing the potential toxicity of plastics in food webs, we first needed to confirm that Tween80

would not impact algal productivity or copepod survival.

To assess for potential of Tween80 toxicity, we performed exposures of algae and copepods to four experimental treatments: algae grown with the addition of Tween80 (*T*: Tween80 in seawater), microplastics without surfactant (*M*: polyspheres in seawater), Tween & microplastics (*T&M*: Tween80, and polyspheres in seawater), and controls (*C*: seawater only). We used small-bodied marine copepods (*Apocyclops panamensis*) as the primary consumers and the smallest size class of polyspheres (25 µm diameter - largest abundance and SA:Vol). We adjusted the water salinity twice a week to ensure it remained within the optimum range of the copepod consumer (25-30 psu[4]).

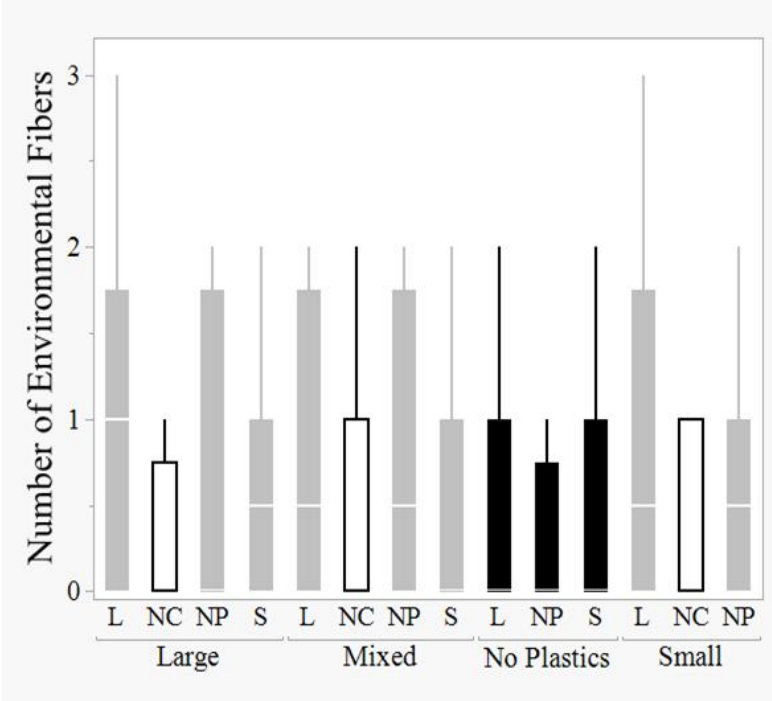
We suspended fluorescently labeled polyspheres in a concentrated stock solution of: 0.1 g of microplastic in 50 g of water. Tween treatment (*T*) stock solutions were comprised of 0.1 g of Tween80 in 50 g of water. Finally, a concentrated stock solution of plastic and Tween (T&M) contained 0.1 g of microplastics and 0.1 g of Tween in 50 g of water. All stock solutions were maintained in 50 mL centrifuge tubes away from ambient light to reduce the likelihood of algal growth and light exhaustion of the fluorescent additives.

Tween 80 exposures did not impact Chlorophyll *a* concentrations ($P = 0.519$, $R^2 = 0.013$) or survivorship of, *Apocyclops panamensis* ($P = 0.443$, $R^2 = 0.018$). We therefore proceeded with all subsequent copepod and fish feeding trials with Tween80 in all exposure solutions. The use of surfactant in this study did not impact algal growth or copepod survivorship, so we found that Tween80 was a safe means to assess for impacts of plastic exposure to algae and copepods.

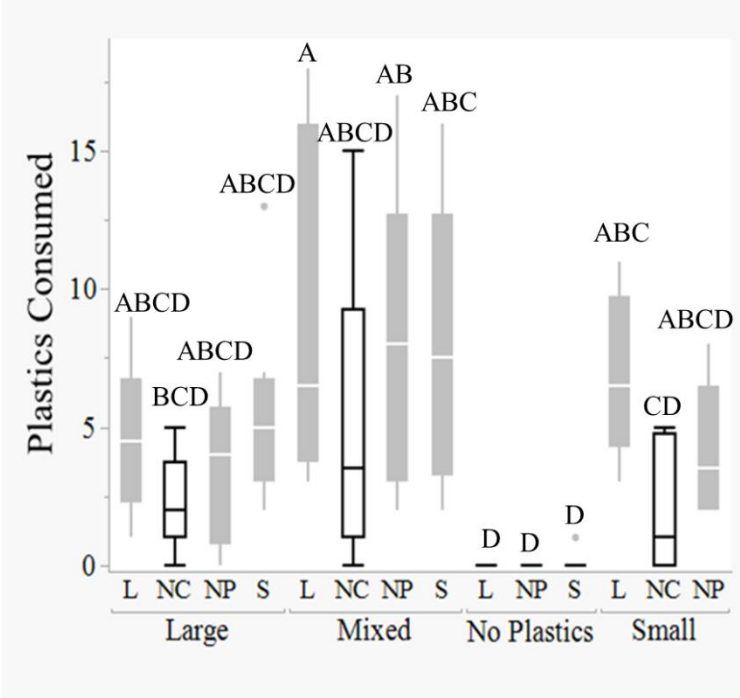
Environmental Fibers

In our experimental systems, we observed microfibers of unknown origin in addition to the polyspheres and plastic microfibers that we introduced. This was not unexpected, as environmental fibers are often present in natural systems[5,6]. However, we were able to distinguish them from our experimental plastics based on their shape and lack of fluorescence excitation response. Although we took precautions to prevent cross-contamination, we could not exclude the possibility of airborne or waterborne

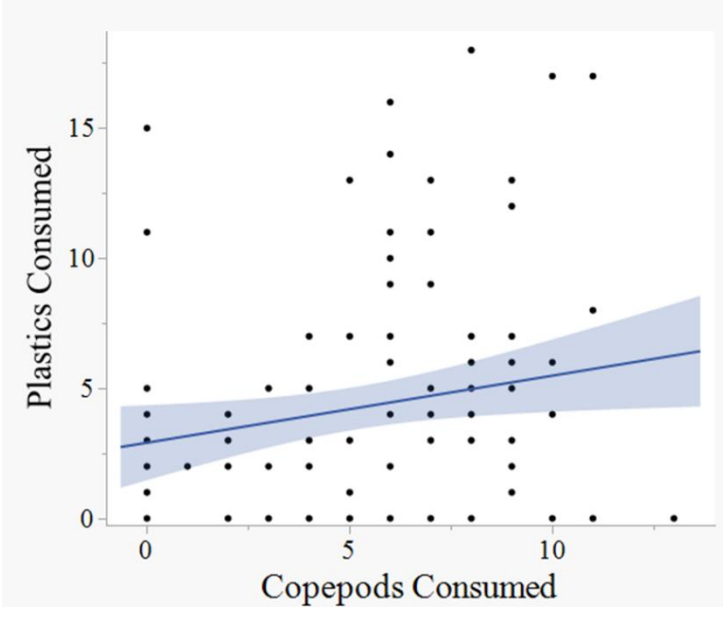
contaminants in our facilities. These fibers were consistent across all treatments and did not appear to be more prevalent in any specific situation [Supplementary Figure 1]. As a result, we did not include them in our analysis of plastic contaminants. It is worth noting that such contaminants are commonly reported in natural systems[7,8].



Supplementary Figure 1. The number of environmental fibers found during fish dissections did not vary across trial types. Feeding trials included various types of plastic exposure to both the fish and the copepods they were consuming. There were three different types of copepod exposure: large polyspheres (L), small polyspheres (S), and no plastics (NP). Several trials did not contain copepods (NC). There were also four types of fish exposure: large polyspheres, large and small polyspheres (Mixed), no plastics, and small polyspheres. Combinations of exposures resulted in 14 trial types. Dark filled boxes represent trials where only copepods were available to the fish, light filled boxes represent trials where fish had both copepods and polyspheres, and open boxes represent trials where only plastics were given to fish.



Supplementary Figure 2. There is no discernible trend for the number of plastics consumed across the fish feeding trials regardless of the presence of plastics (large [L], small [S], mixed) in copepod cultures or offered as food and copepods without any plastics exposed (NP). Lettering above the boxes indicates significant differences based on an alpha value of 0.05 using Tukey's HSD pairwise post hoc analyses.



Supplementary Figure 3. The number of experimental plastics (large and small HDPE microspheres combined) found in the gut of the fish during dissections increased as more copepods were consumed ($P = 0.0291$, $R^2 = 0.043$).

Estimations for the Preferential Feeding of Fish

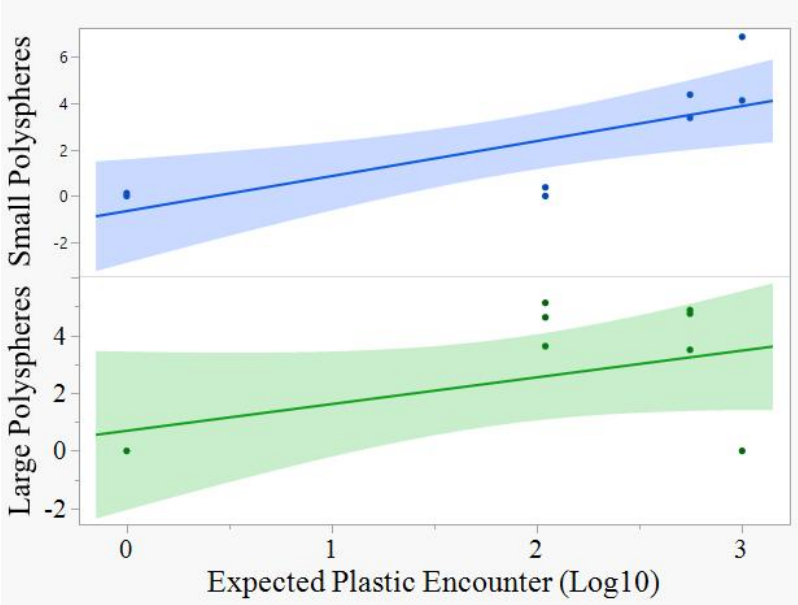
In an effort to understand the relative feeding preferences of blacknose dace for copepods or plastics, we conducted a series of trials in which we calculated the expected frequency of encountering each food option. We based our calculations on estimations of the oxygen concentration in the tanks and the respiration rate of the fish. While we acknowledge the roughness of our estimates, we believe they are informative in demonstrating the magnitude of the impact of microplastics on fish feeding behavior. Based on our calculations, we estimate that the oxygen concentration in the tanks was around 8.1 ppm (mg/L) in water[9]. This indicates that there are approximately 16.2 mg O_2 in the 2L of water in our experimental feeding tanks. According to the general respiration rate of fishes[10], a fish weighing 5g (0.005 kg, which we used as an approximation for our sample fish population) would require 0.4285 mL O_2 per hour and 0.214 mL O_2 during our 30 min trials. Using the dissolved state at 8.1 mg/L, we estimated that 37.85 mL of water would need to be passed over the gills to acquire the required oxygen.

If fish consumed plastics at a rate that was close to random encounter, and assuming that the fish and plastics maintained their position in the tank (which we acknowledge is an unrealistic assumption), we would expect that only 1,001 of the 52,900 polyspheres in the tank would be present in the gut of the fish for small plastics (70 mm diameter). Similarly, we would expect fewer only one of the 109 large (550 mm) plastics to be consumed if they were ingested consistent with their encounter rate.

Lastly, we can estimate the random encounter rate for a copepod with a fish predator by dividing the number of invertebrates (n = 15) by the tank volume (2,000 mL) and the proportion of the tank that the fish will breathe (37.85/2,000 mL). Based on this calculation, we can expect fewer than 1 copepod will encounter a fish in each 30 min trial. While these estimates are conservative in nature given the ability of copepods to move in the water and likely movement of fish in the tank, they allow for a baseline null with which to compare our experimental data.

The consumption rates of plastics were lower than expected for small (70 mm) polyspheres (95% CI = 5.54-2.76, DF = 1, Chi = 2,523, $P < 0.01$) but not for large (550

mm) polyspheres (95% CI = 1.72-3.10, DF = 1, Chi = 1.60, $P > 0.05$). The consumption rates of plastics [Supplementary Figure 4] were lower than expected for small (70 mm) polyspheres (95% CI = 5.54-2.76, DF = 1, Chi = 2,523, $P < 0.01$) but not for large (550 mm) polyspheres (95% CI = 1.72-3.10, DF = 1, Chi = 1.60, $P > 0.05$). The consumption rates for copepods was greater than expected (95% CI = 5.95-7.07, DF = 2, Chi = 14.66, P <0.01).



Supplementary Figure 4. The number of small plastics (70mm, top) consumed by fish in feeding trials increased as the density of plastics increased; however, the rate was still lower than expected by chance (F = 65.3, $R^2 = 0.879$, estimate = 0.006, $P <$ 0.0001). The number of large (550 mm, bottom) also consistently increased with the frequency of polyspheres available for consumption (F = 934.1, $R^2 = 0.791$, estimate = 0.044, $P = 0.000$).

REFERENCES

1. Lipovetskaya Y. Suspension of hydrophobic particles in aqueous solution. *Cospheric* 2017.[DOI:10.13140/RG.2.2.16349.87521]

2. Britton L.N. Surfactants and the Environment. *J Surfactants and Deterg* 1998; 1:109-117.[DOI:10.1007/s11743-998-0014-6]

3. Ivankovic T., Hrenovic J. Surfactants in the Environment. *Arh Hig Rada Toksikol* 2009; 65:95-110.[DOI:10.1016/j.marenvres.2014.02.002]

4. Cruz-Rosado L., Contreras-Sanchez W.M., Hernandez-Vidal U., Perez-Urbiola J.C.,

179 Contreras-Garcia M.J. Population growth of a generational cohort of the copepod
180 *Apocyclops panamensis* (Marsh, 1913) under different temperatures and salinities.
181 *Ecosist. Recur. Agropec.* 2020;7:e2505.[DOI:10.19136/era.a7n2.2505]
182 5. Rebolledo E.L.B., Van Franeker J.A., Jansen O.E., Brasseur S.M.J.M. Plastic
183 ingestion by harbour seals (*Phoca vitulina*) in the Netherlands. *Mar. Pollut. Bull.* 2013;
184 67:200-202.[DOI:10.1016/j.marpolbul.2012.11.035]
185 6. Horn D., Miller M., Anderson S., Steele C. Microplastics are ubiquitous on
186 California beaches and enter the coastal food web through consumption by Pacific
187 mole crabs. *Mar. Pollut. Bull.* 2019;
188 139:231-237.[DOI:10.1016/J.MARPOLBUL.2018.12.039]
189 7. Nelms S.E., Barnett J., Brownlow A., et al. Microplastics in marine mammals
190 stranded around the British coast: Ubiquitous but transitory? *Sci Rep* 2019;
191 9:1075.[DOI:10.1038/s41598-018-37428-3]
192 8. Avery-Gomm S., O'Hara P.D., Kleine L., Bowes V., Wilson L.K., Barry K.L.
193 Northern fulmars as biological monitors of trends of plastic pollution in the eastern
194 Northern *Pacific. Mar. Pollut. Bull.* 2012;
195 64:1776-1781.[DOI:10.1016/j.marpolbul.2012.04.017]
196 9. Wetzel, R.G., Likens, G.E. Dissolved Oxygen. In: Limnological Analyses. 2000.
197 Springer, New York, NY. [DOI:10.1007/978-1-4757-3250-4_6]
198 10. Johnston I.A., Bernard L.M. Aquatic and aerial respiration rates, muscle capillary
199 supply and mitochondrial volume density in the air-breathing catfish (*Clarias*
200 *mossambiques*) acclimated to either aerated or hypoxic water. *J. Exp. Biol.* 1983;
201 105:317-338.