

Supplement: Polystyrene Plastic Particles Result in Adverse Outcomes for *Hyaella azteca* When Exposed at Elevated Temperatures

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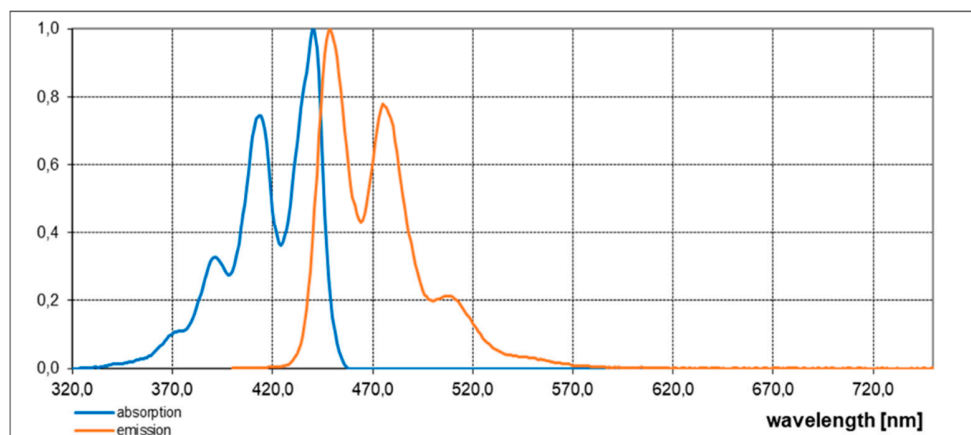


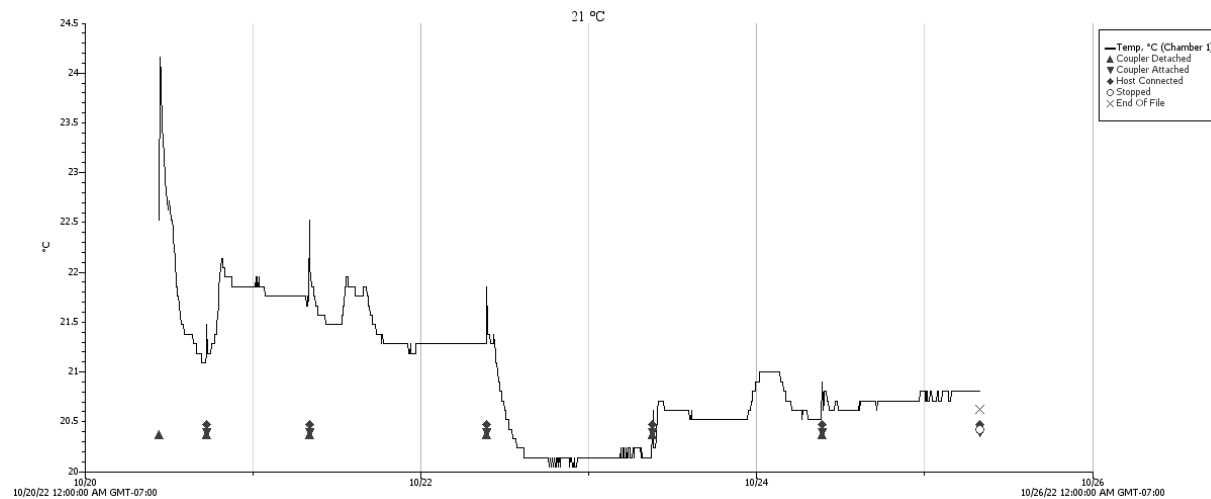
Figure S1. Spectral absorption and emission graph of polystyrene beads. Blue fluorescent beads with a mean diameter of 519 and 1294 nm were ordered from Applied Microspheres GmbH (formerly BS-Partikel GmbH, Mainz, Germany) at a concentration of 5 % m/m (Surface: not-modified polystyrol, no surface functionalization, sulfonic acid end group).

Table S1. Tested polystyrene concentrations in the range finder study (*). “Conc. 5” was used in the main exposure study.

		Original solution	Stock solution (1:10000)	Conc. 1	Conc. 2	Conc. 3	Conc. 4	Conc. 5
	% wt	5	0.0005					
	g/mL	0.05	0.000005					
	mg/L	50,000	5	0.00000431	0.0000431	0.000431	0.0431	0.431
	ng/mL	50,000,000	5000	0.00431	0.0431	0.431	43.1	431
500 nm	p/mL			640	6400	64,000	6,400,000	64,000,000
1000 nm	p/mL			80	800	8000	800,000	8,000,000

* A range finder conducted without the presence of food particles, aimed to evaluate the effects of a wide range of concentrations on survival, growth, swimming behavior, and confocal imaging outcomes. The goal was to determine a concentration that leads to behavioral differences compared to controls and shows particle uptake clearly. The survival rates, total length, and raw data of TDM were assessed at five concentrations at the medium temperature of 24 °C over a period of 96 hours. No significant differences were observed. The TDM of the control group (N = 6) had a mean of 2171.3 ± 377.4 mm, the 500 nm treatment group (N = 30) a mean of 1847.4 ± 413.4 mm, and the 1000 nm treatment group (N = 30) a mean of 2293.0 ± 628.7 mm. The study aimed to distinguish clear differences in

fluorescent levels between the noise of control specimens and those exposed. To deepen understanding of fluctuations in particle uptake across different temperatures and the potential particle mobility, the primary investigation utilized a concentration of 0.43 mg/l of fluorescent PS beads. While this concentration lacks ecological significance, it facilitated a detailed examination of mechanistic relationships among endpoints and exhibited uptake differences from controls compared to lower concentrations. Additionally, we added food particles in the primary investigation to increase uptake. This study prioritized elucidating the interconnectedness of various factors over mimicking environmental conditions with concentrations.



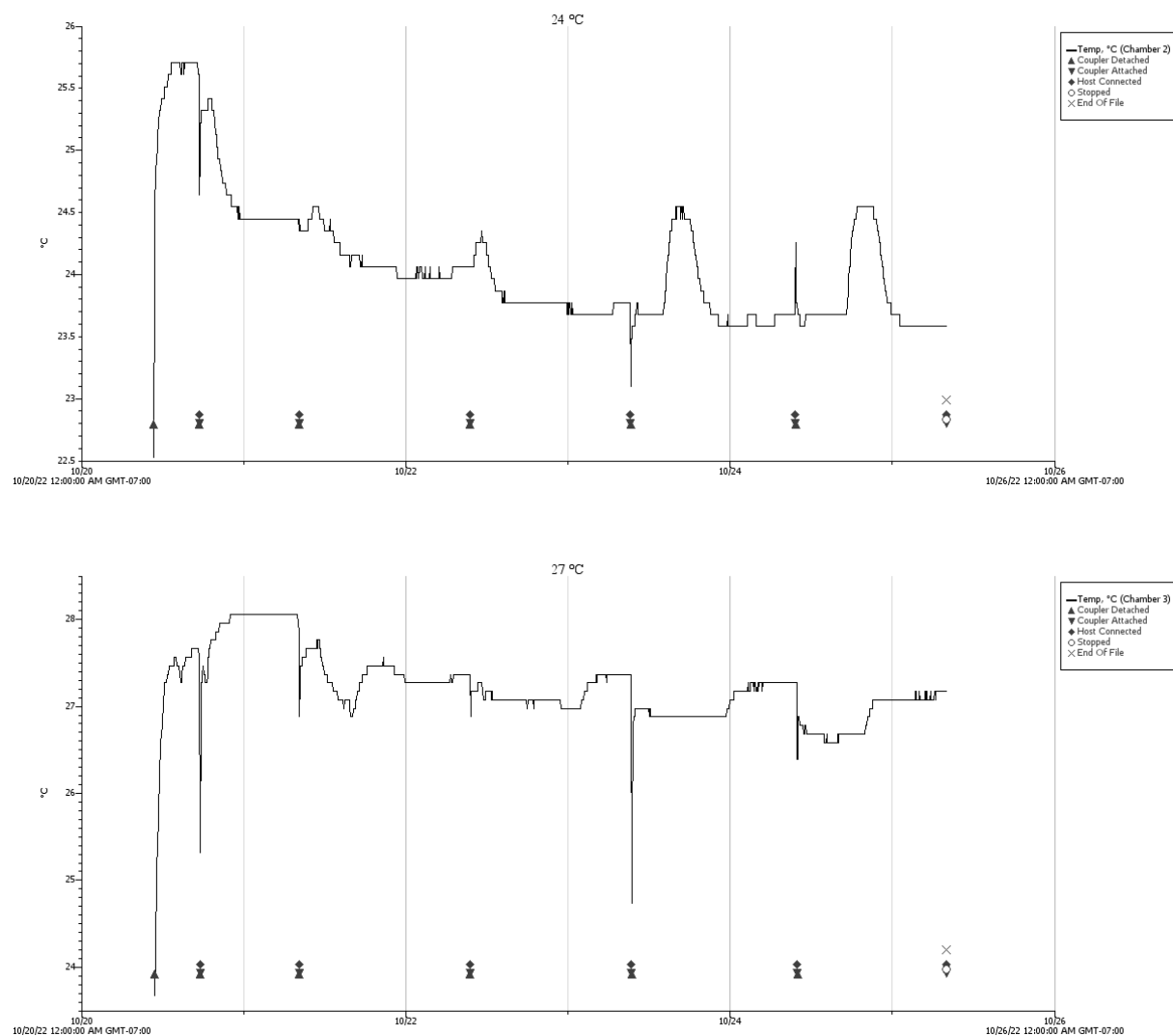


Figure S2. Temperature logger data from each climate chamber set to 21, 24, and 27 °C during the 96-h exposure starting on 10/21.

Table S2. Water parameters were measured via YSI during the 96-h exposure of *H. azteca* to polystyrene particles at three temperatures (21 °C, 24 °C, 27 °C). The three temperatures were regulated in climate chambers (Ch1-3).

Date	Chamber	Temperature (°C)	p.H. (S.U.)	D.O. (mg/L)	D. O. (%)	Salinity (ppt)	Conductivity (λ)
0h	Ch1	21.2	8.5	8.6	97.5	0.2	543
	Ch2	23.1	8.5	8.6	98.7	0.2	570
	Ch3	25.5	8.5	9.1	104.5	0.2	553
	100ml unloaded water		8.5		93.0	0.2	
24h	Ch1	21.4	8.5	8.6	98.3	0.2	490
	Ch2	23.8	8.5	8.6	99.7	0.2	495
	Ch3	26.6	8.6	8.9	103.1	0.2	503
48h	Ch1	20.1	8.5	8.8	97.2	0.2	499
	Ch2	23.6	8.6	8.8	100.7	0.2	501
	Ch3	26.4	8.6	8.7	101.9	0.2	503
72h	Ch1	20.9	8.6	8.2	94.1	0.2	515
	Ch2	23.9	8.6	8.4	98.2	0.2	514
	Ch3	26.4	8.6	8.8	101.7	0.2	520
96h	Ch1	20.9	8.6	8.8	98.0	0.2	530
	Ch2	23.3	8.6	9	101.4	0.2	548
	Ch3	25.9	8.6	9.2	105.3	0.2	550
Mean	Ch1	20.8	8.6	8.6	96.9	0.2	508.5
	Ch2	23.7	8.6	8.7	100.0	0.2	514.5
	Ch3	26.3	8.6	8.9	103.0	0.2	519.0
SEM	Ch1	0.3	0.0	0.1	1.0	0.0	8.8
	Ch2	0.1	0.0	0.1	0.7	0.0	11.8
	Ch3	0.1	0.0	0.1	0.8	0.0	11.1

Table S3. Correlation between *H. azteca* behavioral parameters (either TDM or thigmotaxis) and the polystyrene particle treatment (control, 500 nm, 1000 nm), or the temperature (21 °C, 24 °C, 27 °C) in the dark and light period. Spearman correlation matrix: * $p < 0.05$ are marked in red.

Spearman Correlation	Condition	Periods			
		Dark		Light	
		<i>rs</i>	<i>p</i>	<i>rs</i>	<i>p</i>
Treatment vs. TDM	21 °C	-0.15	0.05	-0.15	0.06
	24 °C	0.06	0.42	0.04	0.58
	27 °C	0.09	0.25	0.03	0.71
Treatment vs. Thigmotaxis	21 °C	0.04	0.62	0.09	0.24
	24 °C	-0.04	0.61	-0.07	0.40
	27 °C	0.00	0.99	0.01	0.86
Treatment vs. Velocity	21 °C	-0.15	0.05	-0.15	0.06
	24 °C	0.06	0.43	0.04	0.58
	27 °C	0.09	0.24	0.03	0.71
Temperature vs. TDM	Control	-0.22	0.01	-0.17	0.03
	500nm	0.13	0.10	0.14	0.07
	1000nm	0.02	0.81	0.00	0.99
Temperature vs. Thigmotaxis	Control	0.09	0.26	0.13	0.10
	500nm	-0.03	0.75	-0.13	0.10
	1000nm	0.06	0.48	0.03	0.69
Temperature vs. Velocity	Control	-0.22	0.01	-0.17	0.03
	500nm	0.13	0.10	0.14	0.07
	1000nm	0.02	0.82	0.00	1.00

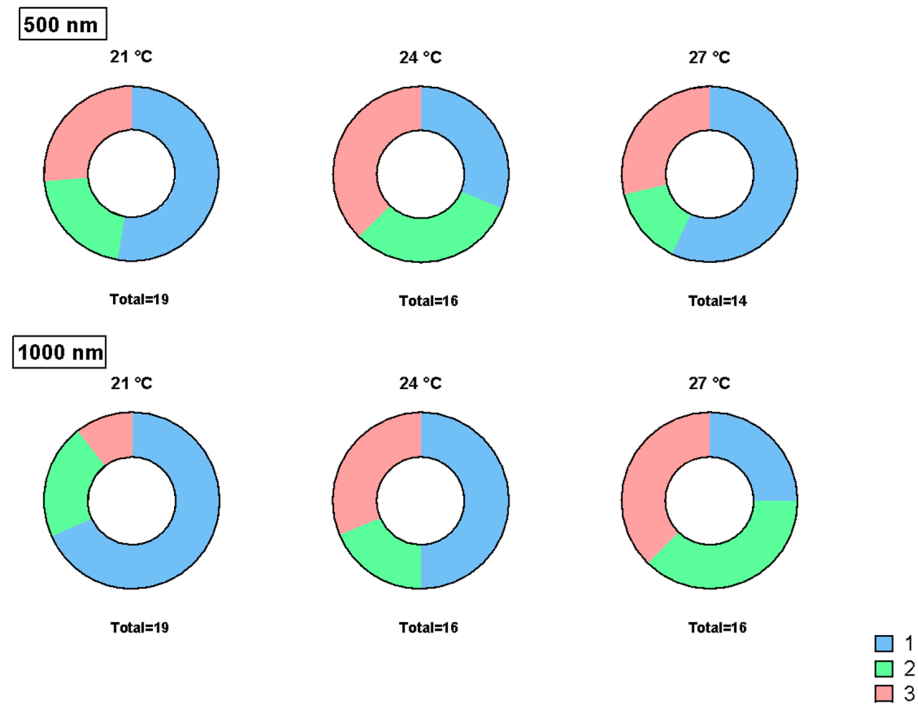


Figure S3. Uptake across particle (500 nm or 1000 nm sized polystyrene particles) and temperature treatments of pooled *H. azteca* (N = 14-19) by ranking: 1 = minor fluorescence (only stomach and mouth parts), 2 = fluorescence additionally in parts of the guts, 3 = fluorescence in the majority of the gut.

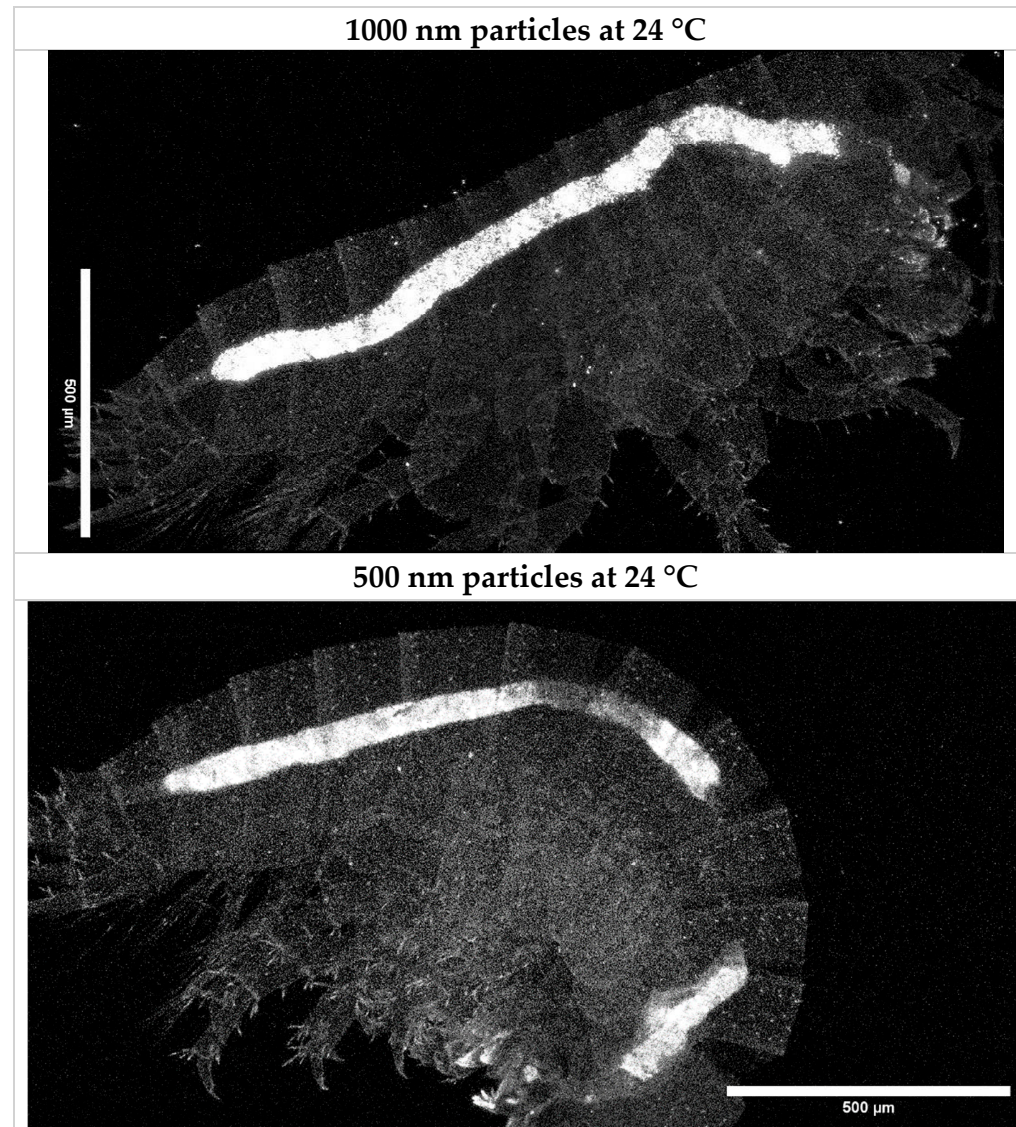


Figure S4. Examples of confocal images for quantifying fluorescent 500 nm or 1000 nm polystyrene particle uptake by *H. azteca* at 24 °C: Maximal intensity of z-stack layers.

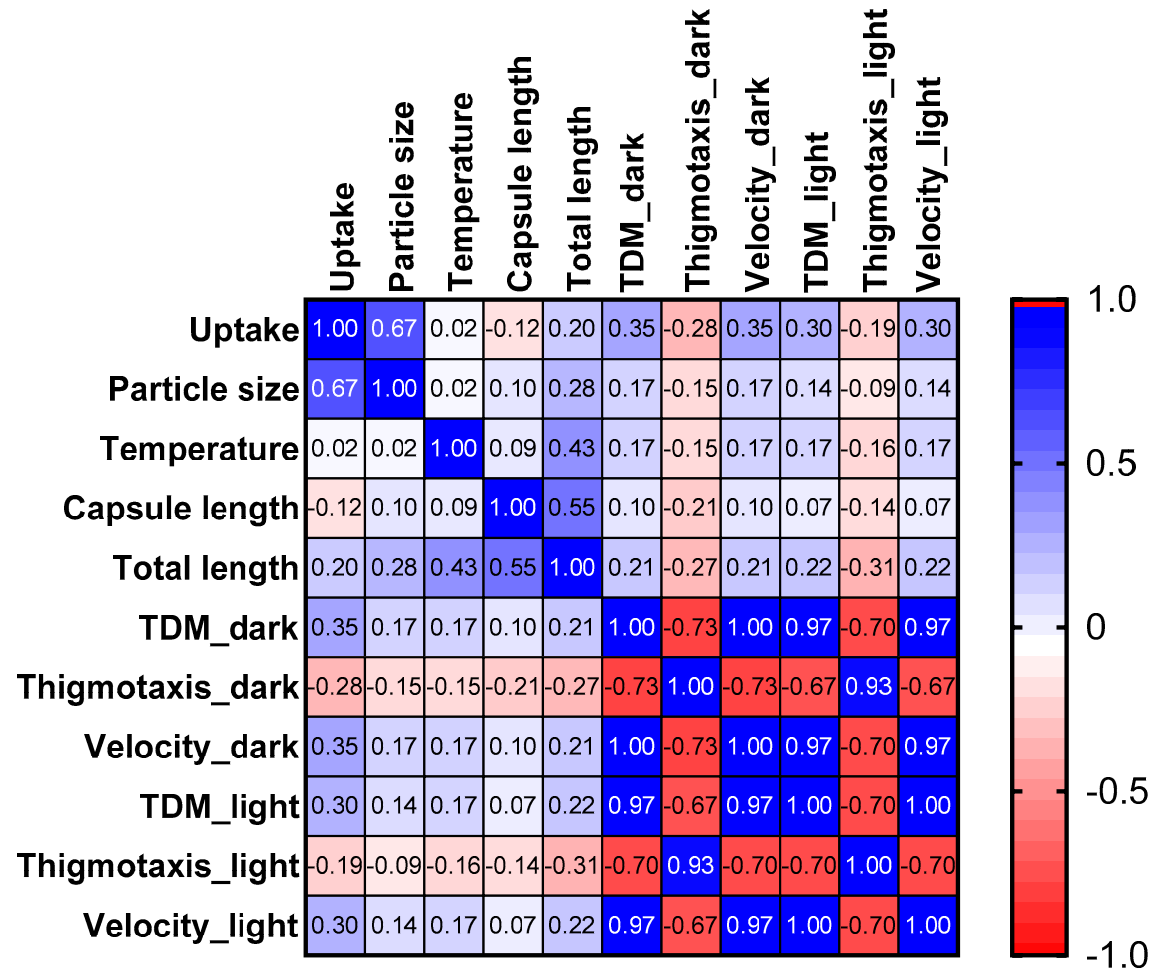


Figure S5. Correlation between PS uptake and endpoints: Spearman correlation (r_s values) between *H. azteca* (N = 52) uptake and further endpoints. Strongest correlation: Uptake vs. particle size ($r_s = 0.67$; $p = <0.001$). The r values of 0-0.19 are commonly regarded as very weak, 0.2-0.39 as weak, 0.40-0.59 as moderate, 0.6-0.79 as strong and 0.8-1 as very strong.