10.1071/EN23131
Environmental Chemistry

Supplementary Material

Daphnia reproductive impacts following chronic exposure to micro- and nano-scale particles from three types of rubber

Brittany E. Cunningham[A], *Bryan J. Harper*[A], *Susanne M. Brander*[B] and *Stacey L. Harper*[A,C,*]

[A]Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR, USA

[B]Coastal Oregon Marine Experiment Station, Department of Fisheries, Wildlife, and Conservation Sciences, Oregon State University, Newport, OR, USA

[C]School of Chemical, Biological and Environmental Engineering, Oregon State University, Corvallis, OR, USA

*Correspondence to: Email: stacey.harper@oregonstate.edu

Supplemental Material

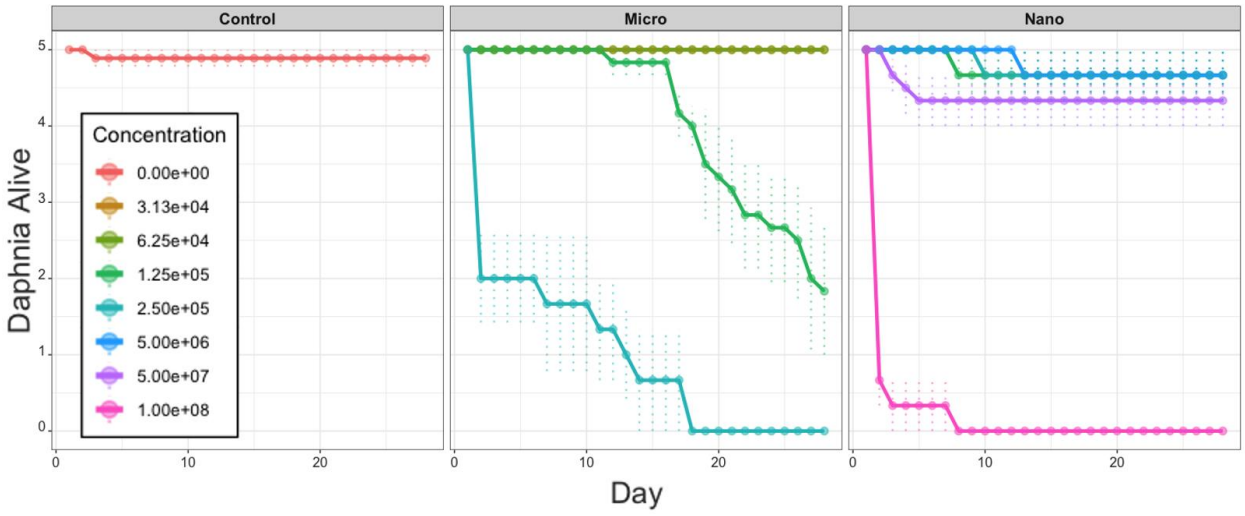

Figure S1. *Daphnia* survival during preliminary chronic exposure of TPs in NOM over 28 days to identify sublethal exposure concentrations.

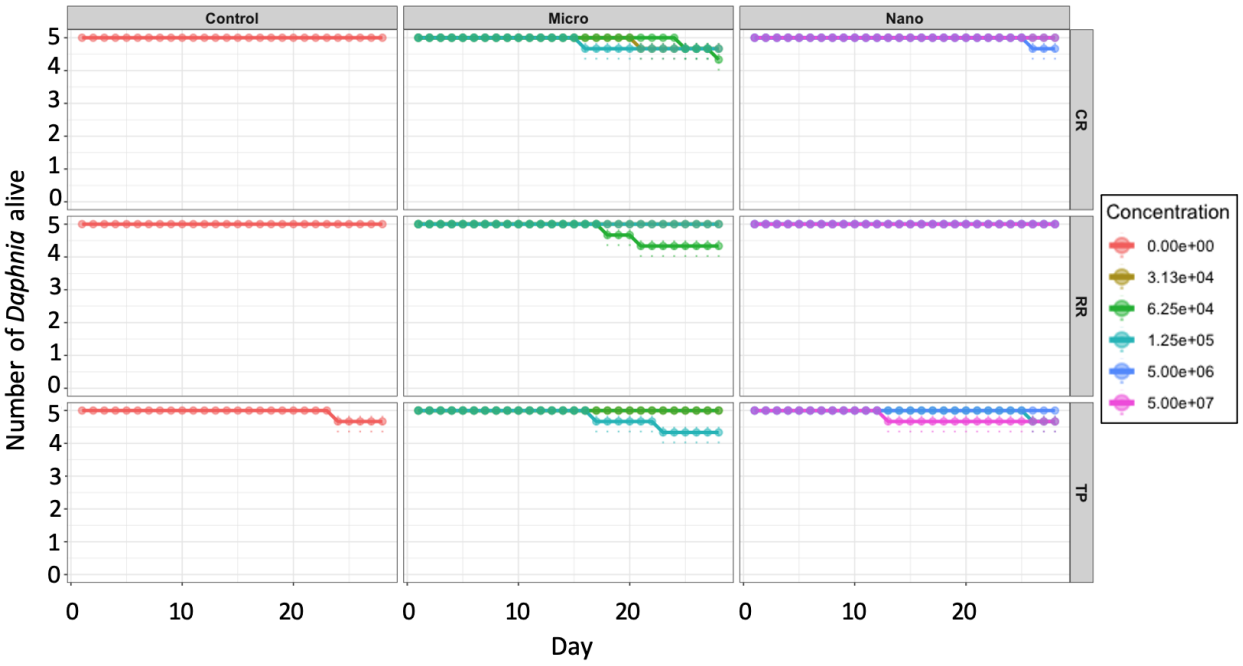

Figure S2. Number of *Daphnia* alive in each exposure group over the course of the 28-day, chronic experiment. All exposures started with five *Daphnia* on Day 1, points on the figure are an average of n=3 with standard error bars. Concentrations are in particles/mL. CR = crumb rubber, RR = recycled rubber, TP = tire particles

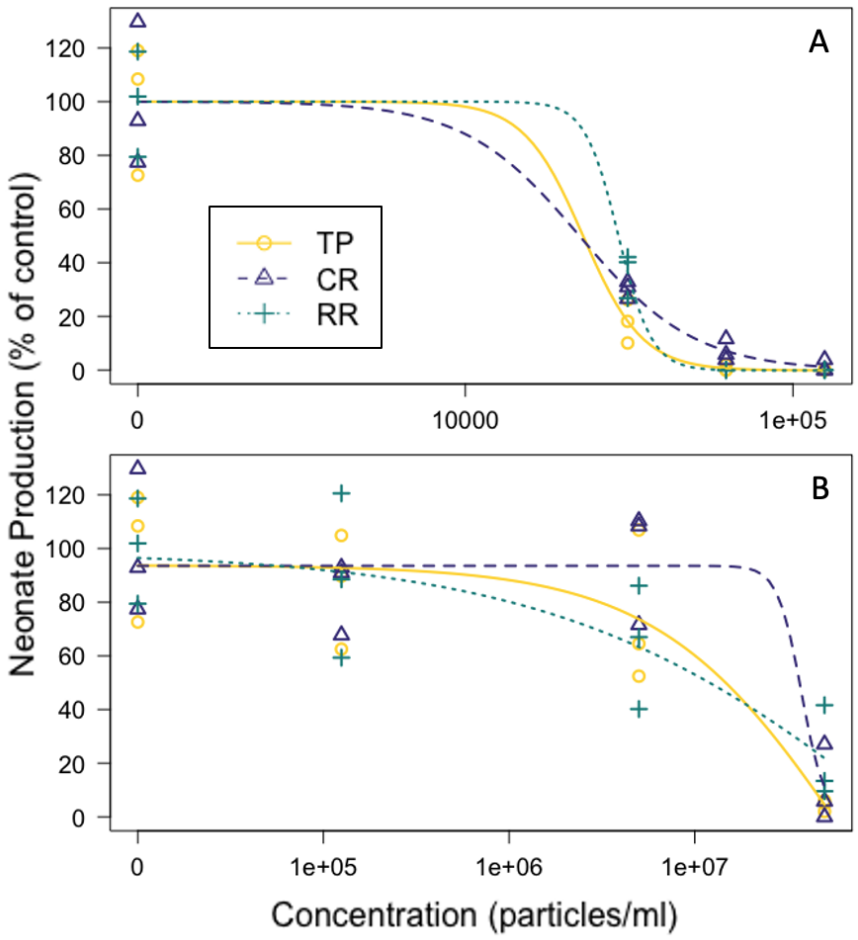

Figure S3. Concentration-response curves for *Daphnia* neonate production in neonates/daphnia/day as percent of control for the (A) Micro and (B) Nano rubber exposures.

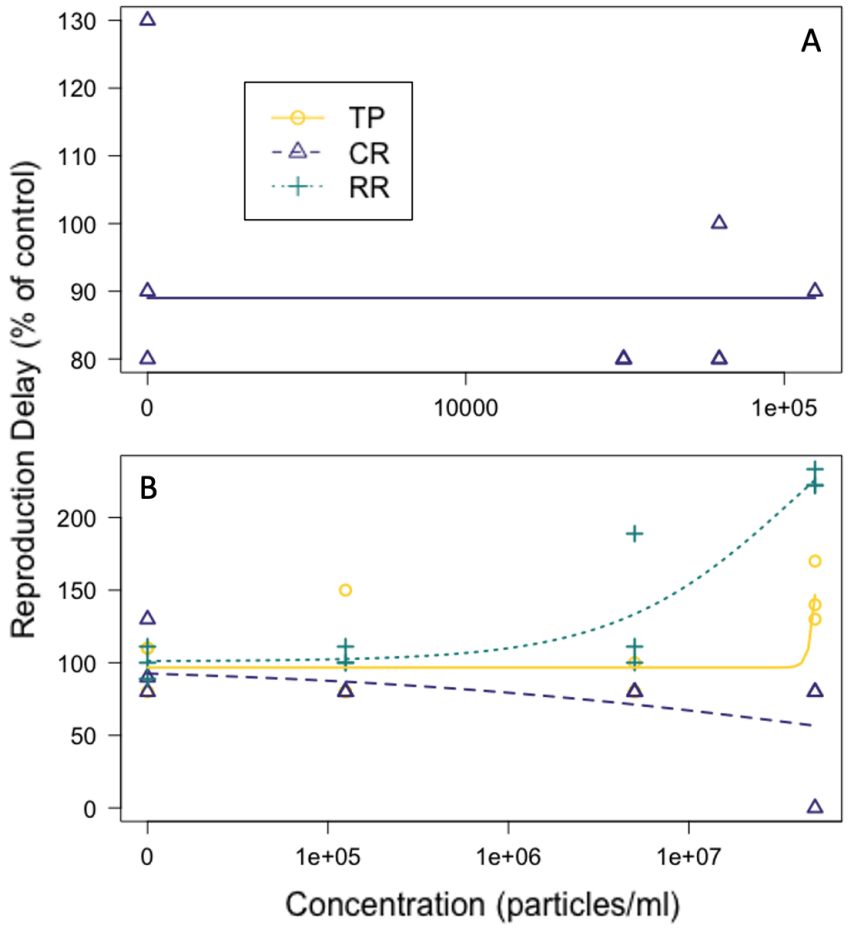

Figure S4. Concentration-response curves for *Daphnia* reproduction delay in time to first reproduction as percent of control for the (A) Micro and (B) Nano rubber exposures.

Table S1. Final growth measurements of *Daphnia* following chronic exposure to micro and nano rubber particles. CR = crumb rubber, RR = recycled rubber, TP = tire particles

Rubber Type	Group	Concentration (particles/mL)	Length (µm)	± SE (µm)
TP	Control	0	2897	41
	Micro	3.13E+04	3060	19
		6.25E+04	2968	37
		1.25E+05	2838	54
	Nano	1.25E+05	2855	34
		5.00E+06	2851	37
		5.00E+07	3024	45
RR	Control	0	2973	43
	Micro	3.13E+04	2817	167
		6.25E+04	3038	20
		1.25E+05	3093	14
	Nano	1.25E+05	2950	23
		5.00E+06	3018	16
		5.00E+07	3004	38
CR	Control	0	2908	42
	Micro	3.13E+04	2808	4
		6.25E+04	2774	17
		1.25E+05	2806	21
	Nano	1.25E+05	2799	4
		5.00E+06	2807	12
		5.00E+07	2839	11

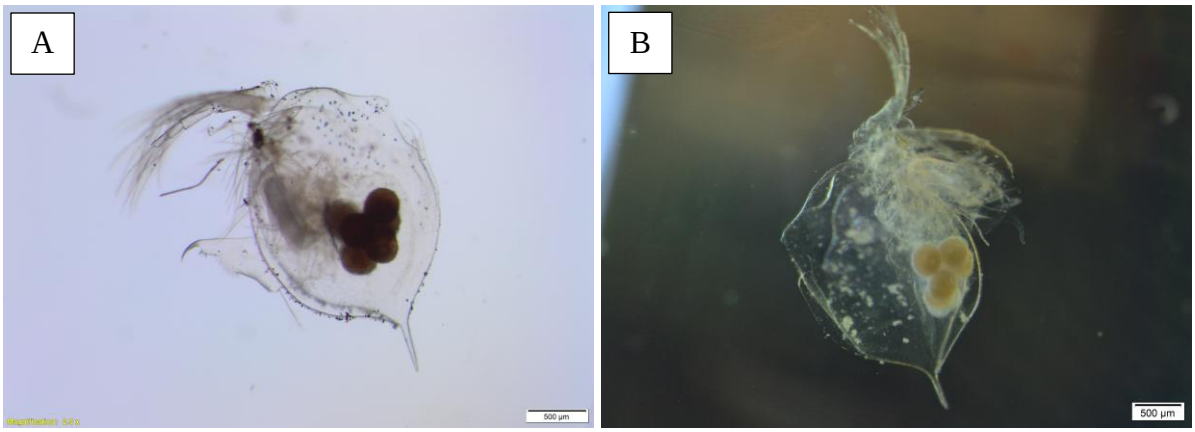

Figure S5. Examples of Daphnia molt with undeveloped eggs from group exposed to (A) micro crumb rubber (CR) at 1.25 x 10^5 particles/mL and (B) micro tire particles (TP) at 3.13 x 10^4 particles/mL.

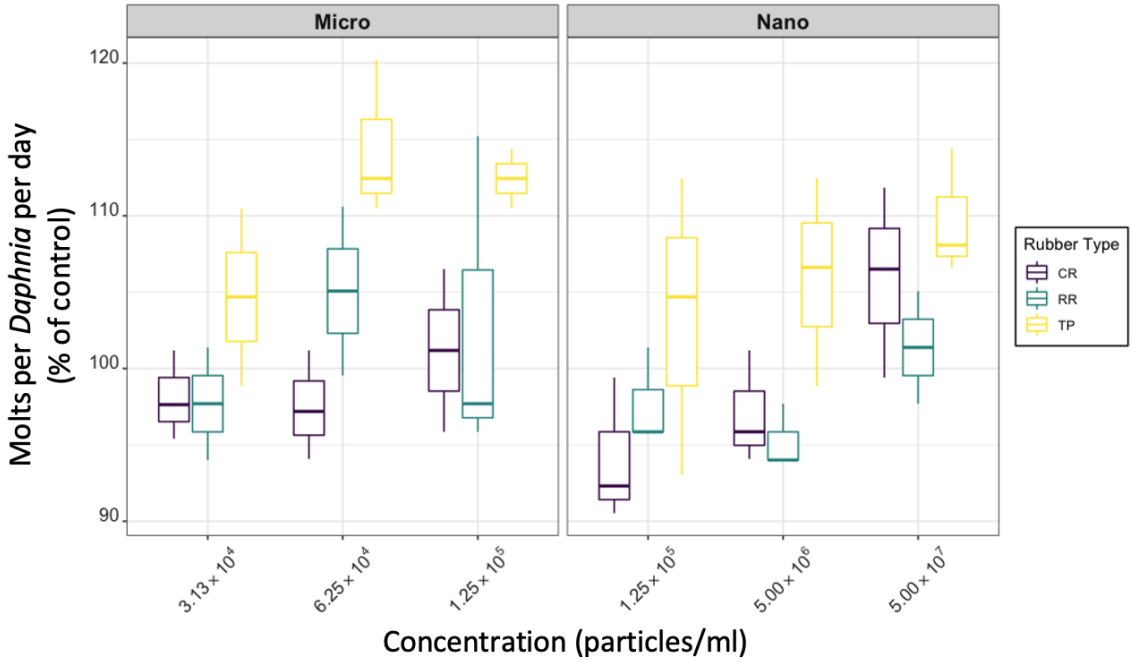

Figure S6. The number of molts per *Daphnia* per day normalized as a percentage of the control values over the 28-day chronic micro- and nano-sized rubber exposure. CR = crumb rubber, RR = recycled rubber, TP = tire particles. Non-normalized figures with control bars in supplemental material (Figure S8).

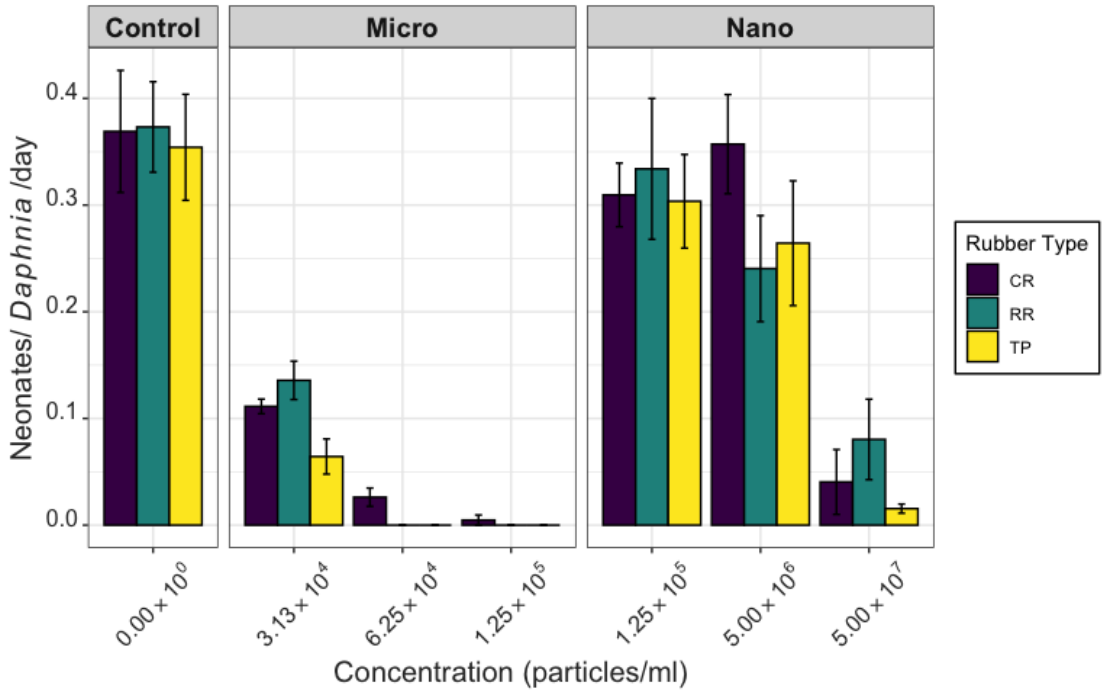

Figure S7. The parental generation number of neonates produced per daphnia per day over the 28-day chronic micro- and nano-sized rubber exposure. CR = crumb rubber, RR = recycled rubber, TP = tire particles

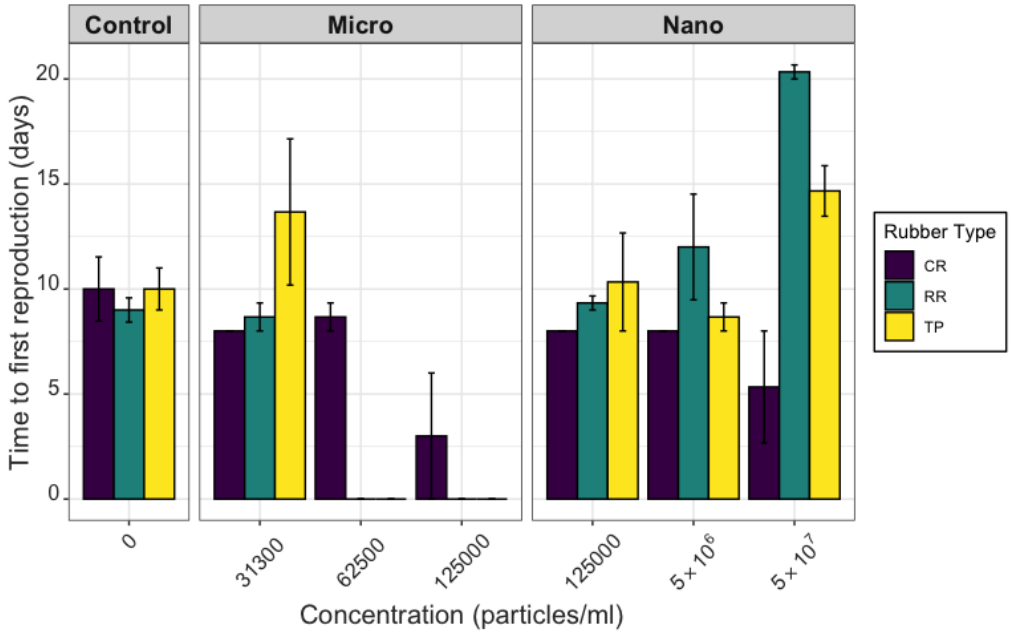

Figure S8. The parental generation length of time in days that it took for *Daphnia* to first produce neonates. CR = crumb rubber, RR = recycled rubber, TP = tire particles

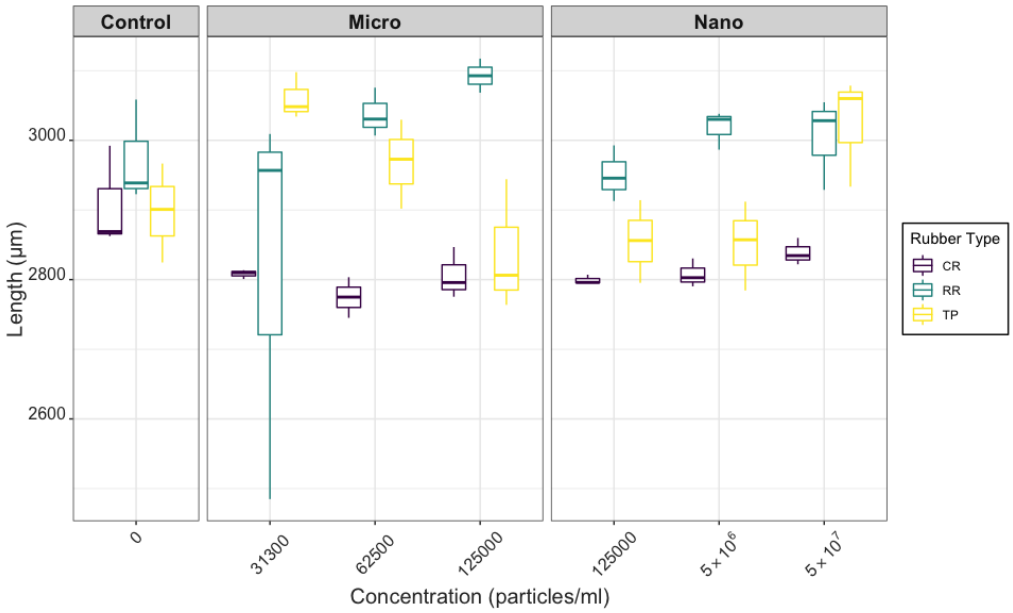

Figure S9. The parental generation final length of the surviving *Daphnia* on Day 28 of the exposure to chronic micro- and nano-sized rubber.

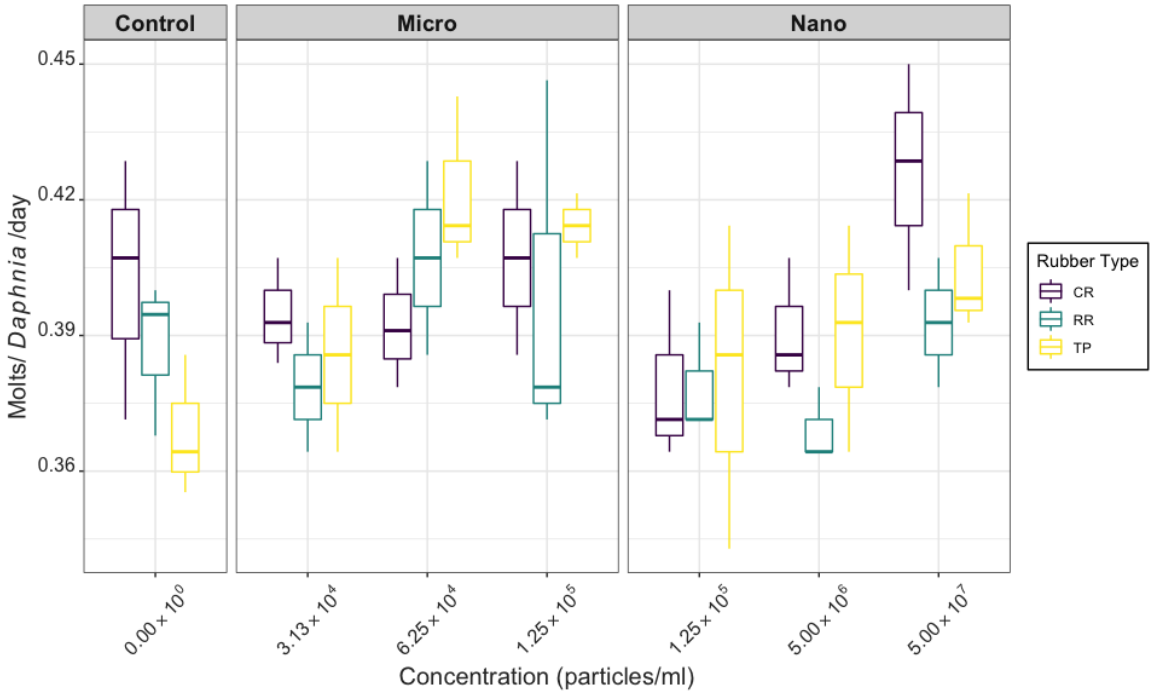

Figure S10. The parental generation number of molts per *Daphnia* per day over the 28-day chronic micro- and nano-sized rubber exposure. CR = crumb rubber, RR = recycled rubber, TP = tire particles.

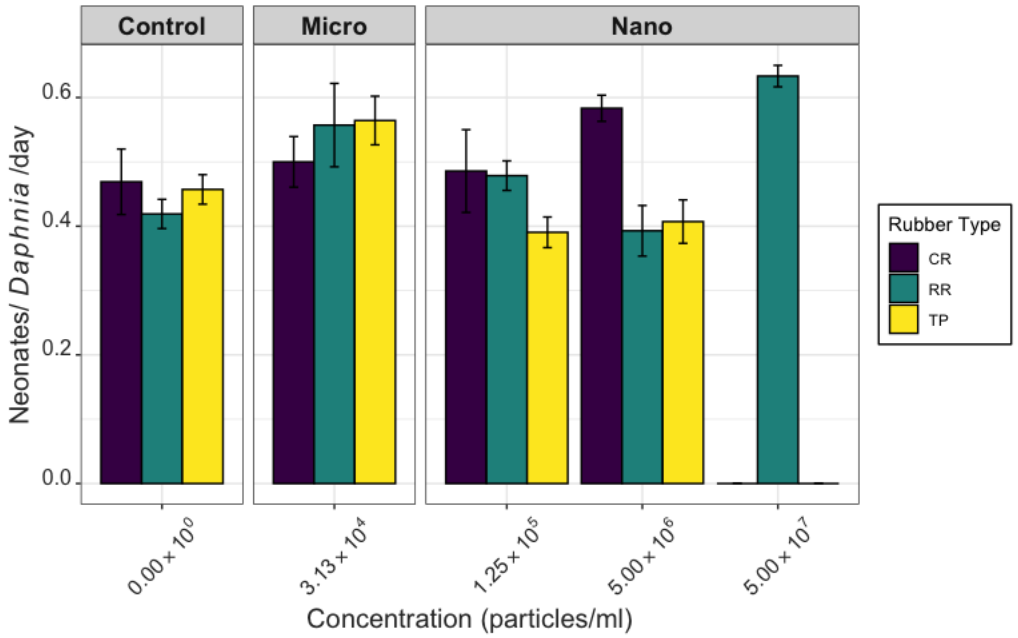

Figure S11. The F_1 generation number of neonates produced per *Daphnia* per day over the 28-day grow-out. CR = crumb rubber, RR = recycled rubber, TP = tire particles

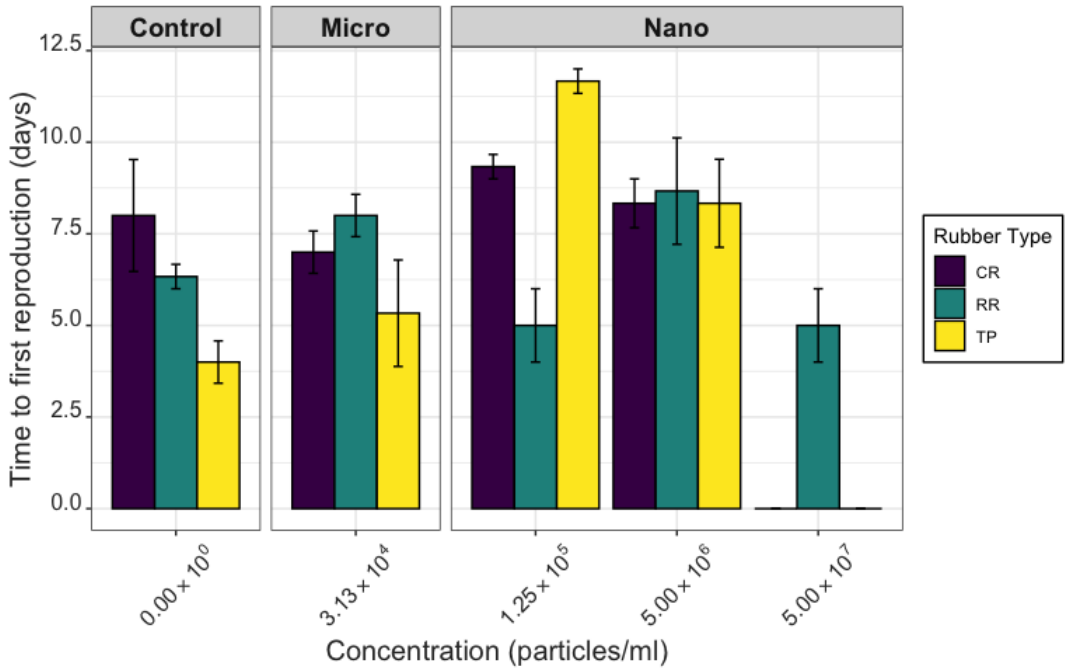

Figure S12. The F_1 generation length of time in days that it took for *Daphnia* to first produce neonates. CR = crumb rubber, RR = recycled rubber, TP = tire particles

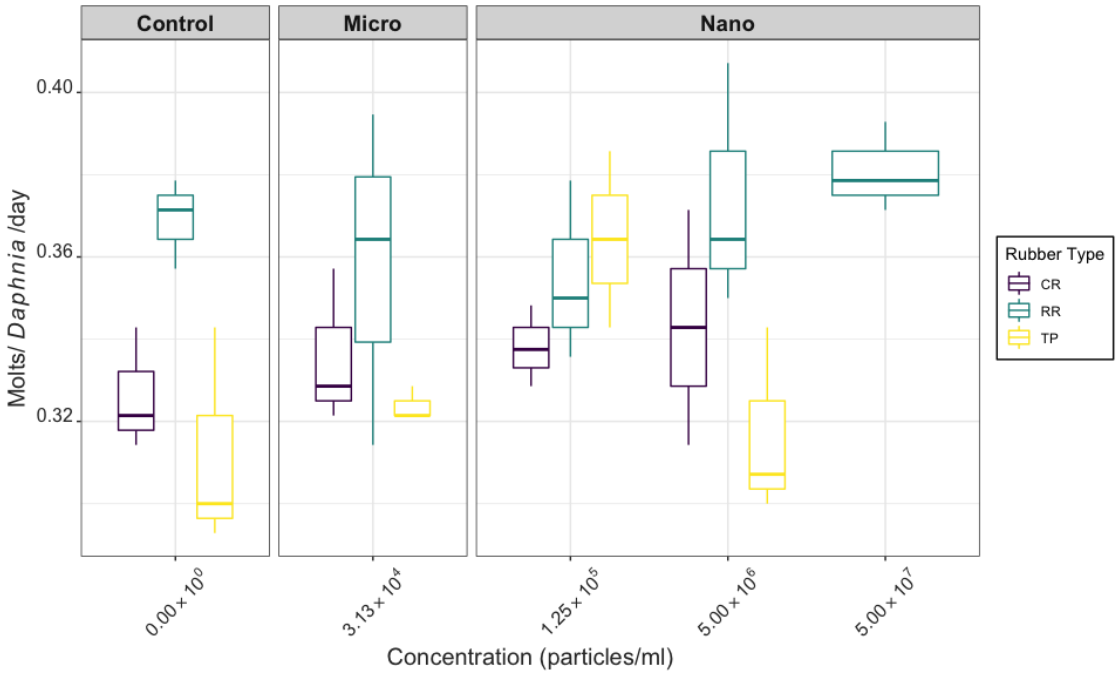

Figure S13. The F_1 generation number of molts per *Daphnia* per day over the 28-day grow-out. No significant differences in growth identified (two-way ANOVA with Tukey HSD, $p > 0.05$). CR = crumb rubber, RR = recycled rubber, TP = tire particles

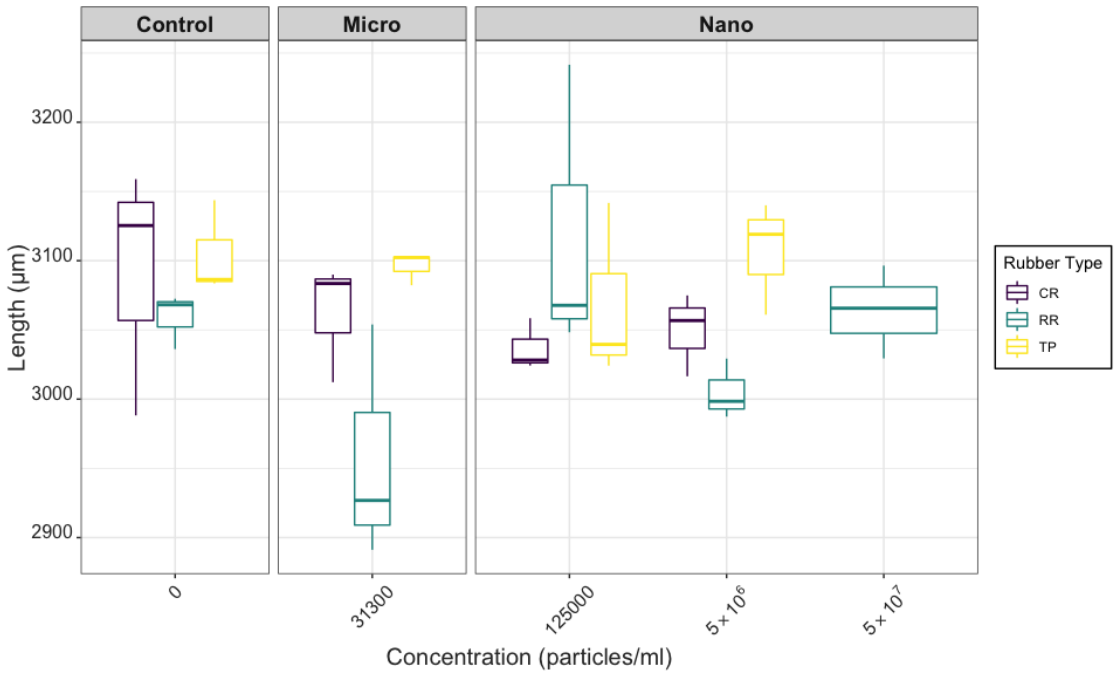

Figure S14. The final length of the surviving Daphnia on Day 28 of the grow-out. No significant differences in growth identified (two-way ANOVA with Tukey HSD, $p > 0.05$). CR = crumb rubber, RR = recycled rubber, TP = tire particles