Supplementary Material

Effects of microplastics mixed with natural particles on *Daphnia magna* populations

Christoph Schür[a,b], Joana Beck[a], Scott Lambert[a], Christian Scherer[c], Jörg Oehlmann[a], Martin Wagner[d]*

[a] Department of Aquatic Ecotoxicology, Faculty of Biological Sciences, Goethe University, Frankfurt am Main, Max-von-Laue-Str. 13, 60438 Frankfurt am Main, Germany
[b] Department of Environmental Toxicology, Eawag, Überlandstrasse 133, 8600 Dübendorf, Switzerland
[c] Federal Institute of Hydrology, Department Biochemistry and Ecotoxicology, Am Mainzer Tor 1, 56002, Koblenz, Germany
[d] Department of Biology, Norwegian University of Science and Technology, Høgskoleringen 5, 7491 Trondheim, Norway

* Corresponding author: Martin Wagner, martin.wagner@ntnu.no, +47 73 41 30 47

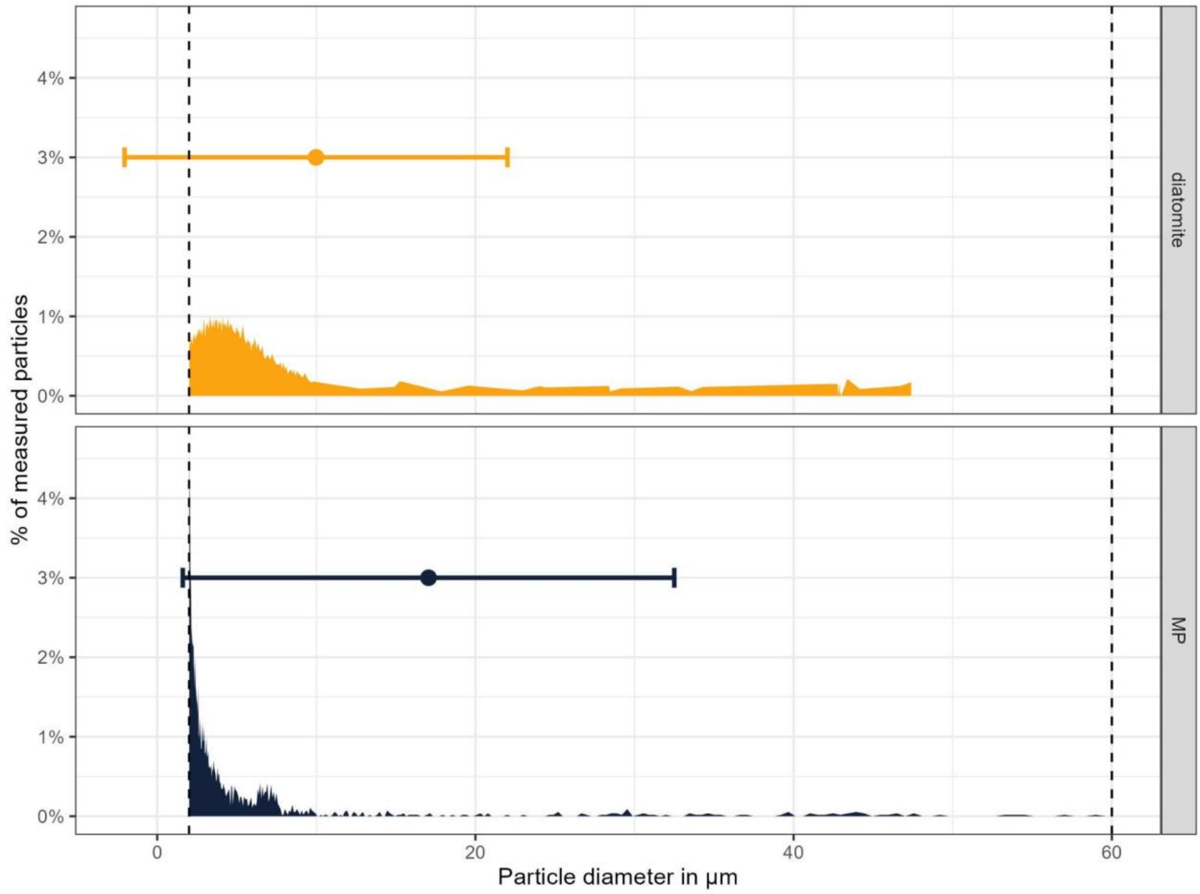

Figure S1: Particle size distributions for diatomite (top, yellow) and MP (bottom, blue), measured in a Multisizer 3 for the size range ≥ 2 µm and ≤ 60 µm (dotted vertical lines). The points and horizontal error bars at 3% indicate the mean ± standard deviation (SD) of the particle sizes, respectively.

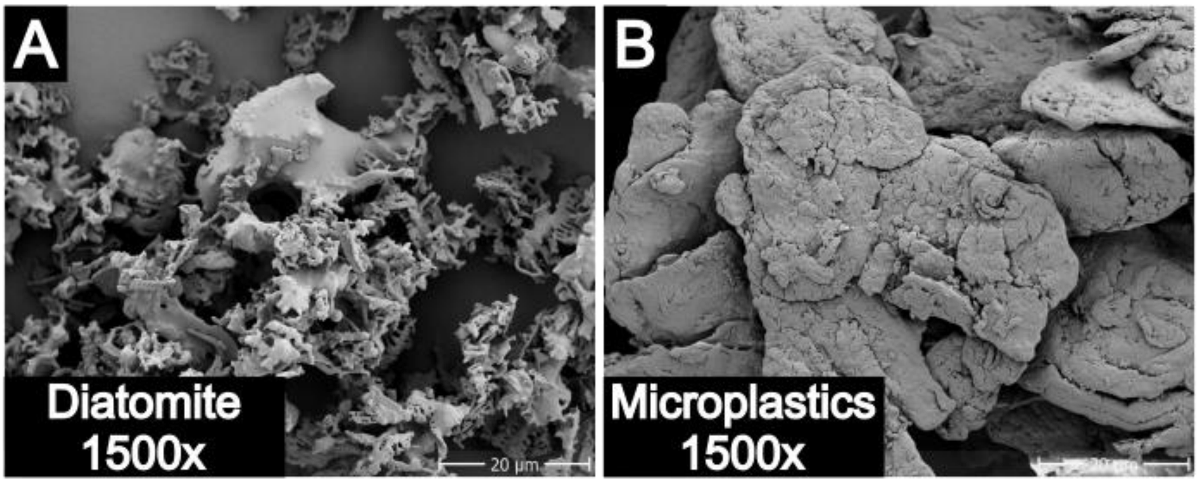

Figure S2: Scanning electron microscopy micrographs of the two particle types (A: diatomite, B: polystyrene microplastics) used throughout this study (1500× magnification). Image B was previously published as part of Figure 1 in Schür et al. (2021).

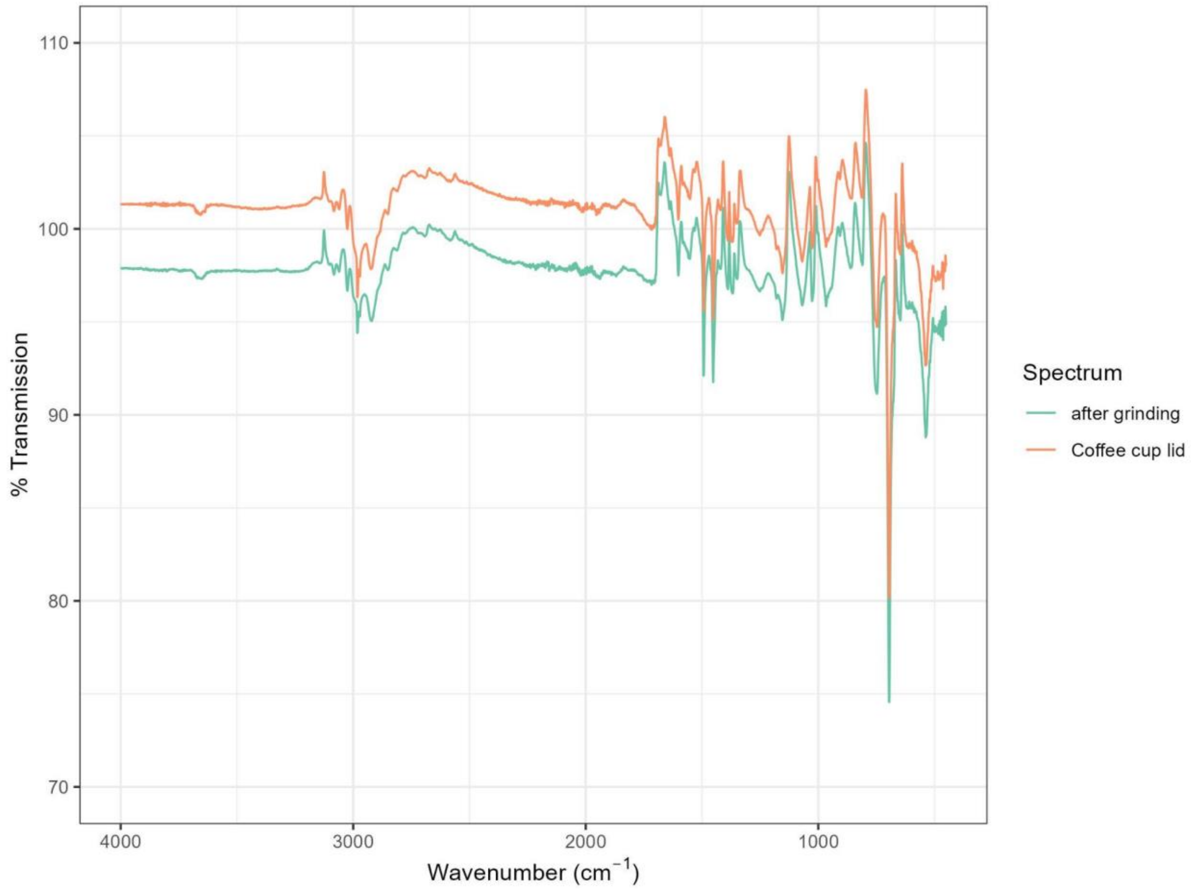

Figure S3: Fourier-transform infrared spectroscopy (FTIR) spectra of the coffee-to-go cup lids that were the raw material for the microplastics in this study and the microplastics after grinding in the ballmill (see Materials and methods in the main manuscript).

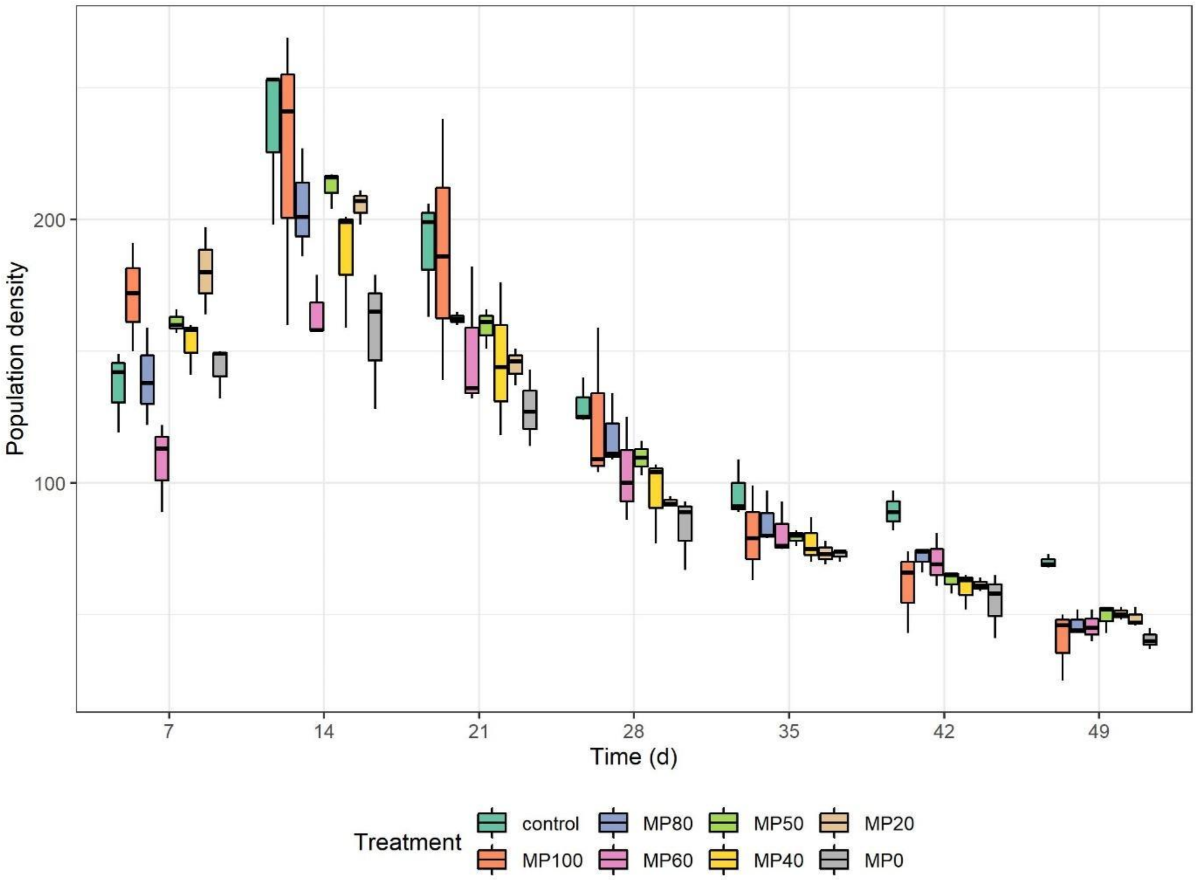

Figure S4: Box-whisker plot of population density of *Daphnia magna* exposed to polystyrene microplastics (MP100), diatomite (MP0), or their mixtures over 50 d (n = 3).

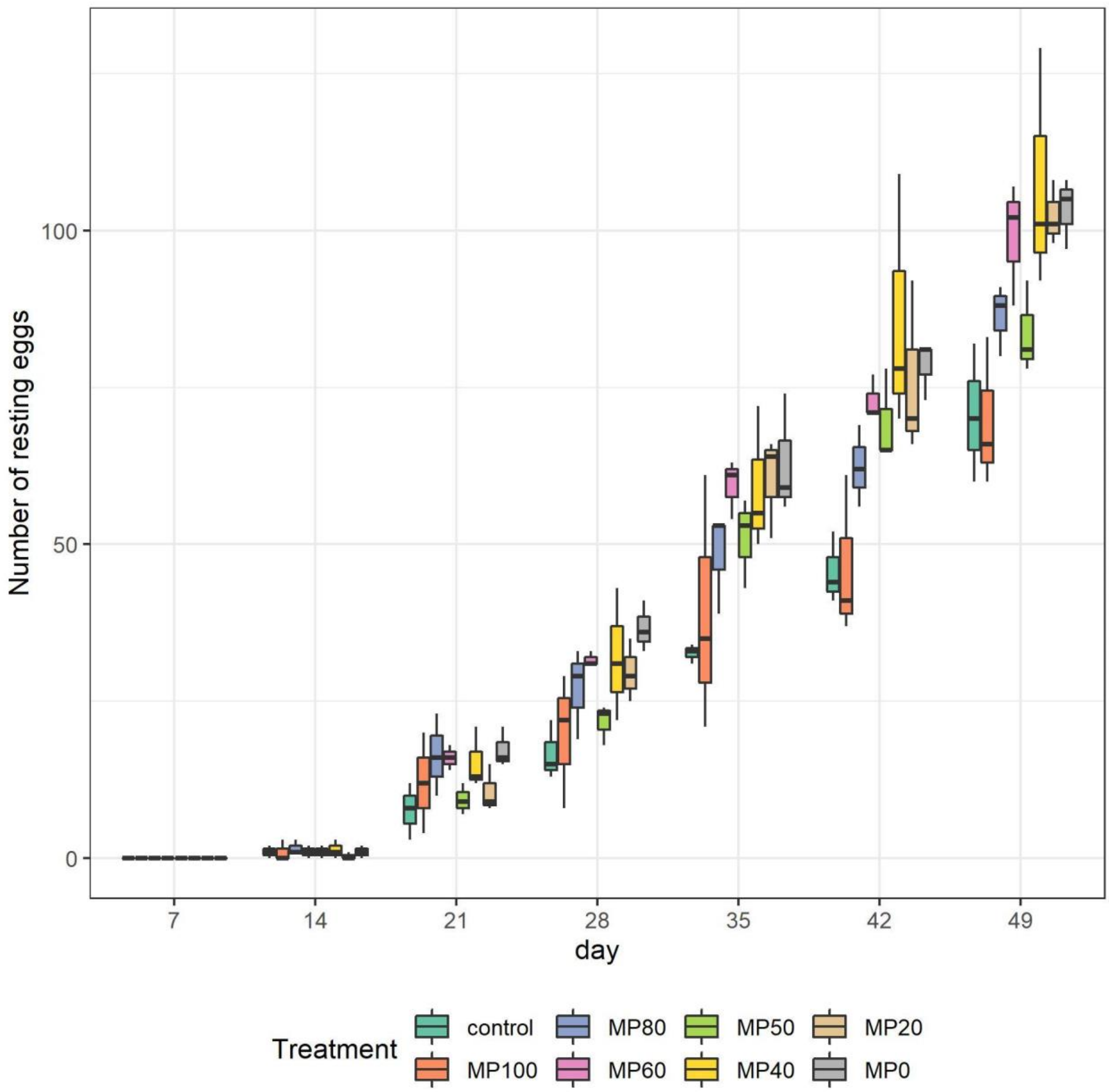

Figure S5: Number of resting eggs produced by *Daphnia magna* populations exposed to polystyrene microplastics (MP100), diatomite (MP0), or their mixtures over 50 d (n=3).

Table S1: Results of the statistical comparison of the population size of *D. magna* individuals in populations exposed to particles compared to control populations. One-way ANOVA with Holm-Šídák's multiple comparison tests for each observation time (adjusted p values).

Control vs.	d7	d14	d21	d28	d37	d42	d50
MP100	0.079	0.6232	0.9227	0.6845	0.1814	**0.0079**	**0.0029**
MP80	0.8383	0.5974	0.3956	0.6484	0.1866	**0.0426**	**0.0031**
MP60	0.1524	**0.0423**	0.2467	0.2732	0.1814	**0.0426**	**0.0029**
MP50	0.232	0.5974	0.3956	0.5013	0.1814	**0.0083**	**0.0049**
MP40	0.4994	0.2199	0.2243	0.1253	0.1408	**0.0071**	**0.0049**
MP20	**0.0207**	0.5974	0.2243	0.0938	0.0629	**0.0079**	**0.0049**
MP0	0.8383	**0.0244**	0.0535	**0.0267**	0.0617	**0.0019**	**0.0006**

Table S2: Results of the statistical comparison of the number of *D. magna* neonates, juveniles and adults in populations exposed to particles compared to control populations. One-way ANOVA with Holm-Šídák's multiple comparison tests for each size class and observation time (adjusted p values).

Control vs.	d7	d14	d21	d28	d37	d42	d50
Neonates							
MP100	0.5321	0.4257	0.5161	0.1588	0.9623	0.2818	0.3224
MP80	0.9864	0.3768	0.6793	**0.0468**	0.9623	0.2202	0.2403
MP60	0.439	**0.034**	0.2713	**0.0406**	0.672	0.2202	0.1696
MP50	0.9864	0.4257	0.5161	0.1588	0.7856	0.2202	0.4057
MP40	0.6575	0.0681	0.2713	**0.0406**	0.9623	0.2202	0.4733
MP20	0.8795	0.376	0.2713	**0.0406**	0.8379	0.1902	0.8349
MP0	0.9864	**0.0303**	0.2686	**0.0254**	0.9623	0.2202	0.8349
Juveniles							
MP100	0.9878	0.4237	0.983	0.9713	0.2534	0.1122	**0.0004**
MP80	0.9878	0.9353	0.9496	0.9713	0.3383	0.3851	**0.0028**
MP60	0.9878	0.974	0.983	0.9713	0.3383	0.3851	**0.0005**
MP50	0.3845	0.9336	0.983	0.5914	0.3383	0.1122	**0.0028**
MP40	0.9719	0.2876	0.983	0.9383	0.2534	0.0905	**0.0017**
MP20	0.1298	0.6816	0.983	0.9174	**0.0239**	0.06	**0.0005**
MP0	0.9878	0.974	0.983	0.5914	**0.0079**	**0.0303**	**0.0008**
Adults							
MP100	0.3302	0.8893	0.8835	0.9936	0.9279	0.8901	0.9163
MP80	0.5995	0.9465	0.5971	0.9936	0.9279	0.996	>0.9999
MP60	0.0807	0.3066	0.8214	0.9936	0.9432	0.8901	0.3774
MP50	0.8224	0.9451	0.8214	0.2154	0.9432	0.996	0.9163
MP40	0.8224	0.9465	0.7214	0.9936	0.9279	0.996	0.5598
MP20	0.2052	0.9465	0.9886	0.9936	0.8417	0.9382	0.4414
MP0	0.7363	0.9465	>0.9999	0.8405	0.7596	0.996	0.9163

Table S3: Mean population density as number of individuals per treatment group over time.

Treatment group	Day	Mean population density ± standard deviation
Control	7	137 ± 16
	14	235 ± 32
	21	190 ± 23
	28	130 ± 9
	37	96 ± 11
	42	89 ± 8
	50	70 ± 3
MP100	7	171 ± 21
	14	223 ± 57
	21	188 ± 50
	28	124 ± 30
	37	80 ± 18
	42	61 ± 16
	50	45 ± 16
MP80	7	140 ± 19
	14	205 ± 21
	21	162 ± 3
	28	118 ± 14
	37	85 ± 10
	42	72 ± 5
	50	47 ± 5
MP60	7	108 ± 17
	14	165 ± 12
	21	150 ± 28
	28	104 ± 20
	37	81 ± 10
	42	70 ± 10
	50	46 ± 6
MP50	7	162 ± 5
	14	212 ± 7
	21	159 ± 8
	28	110 ± 9
	37	79 ± 3
	42	63 ± 4
	50	49 ± 6
MP40	7	153 ± 11
	14	186 ± 24
	21	146 ± 29
	28	96 ± 17
	37	77 ± 9
	42	60 ± 7
	50	50 ± 3
MP20	7	180 ± 17

	14	205 ± 7
	21	145 ± 7
	28	93 ± 2
	37	73 ± 5
	42	61 ± 3
	50	49 ± 4
MP0	7	144 ± 10
	14	157 ± 26
	21	128 ± 15
	28	83 ± 14
	37	73 ± 2
	42	55 ± 12
	50	41 ± 4

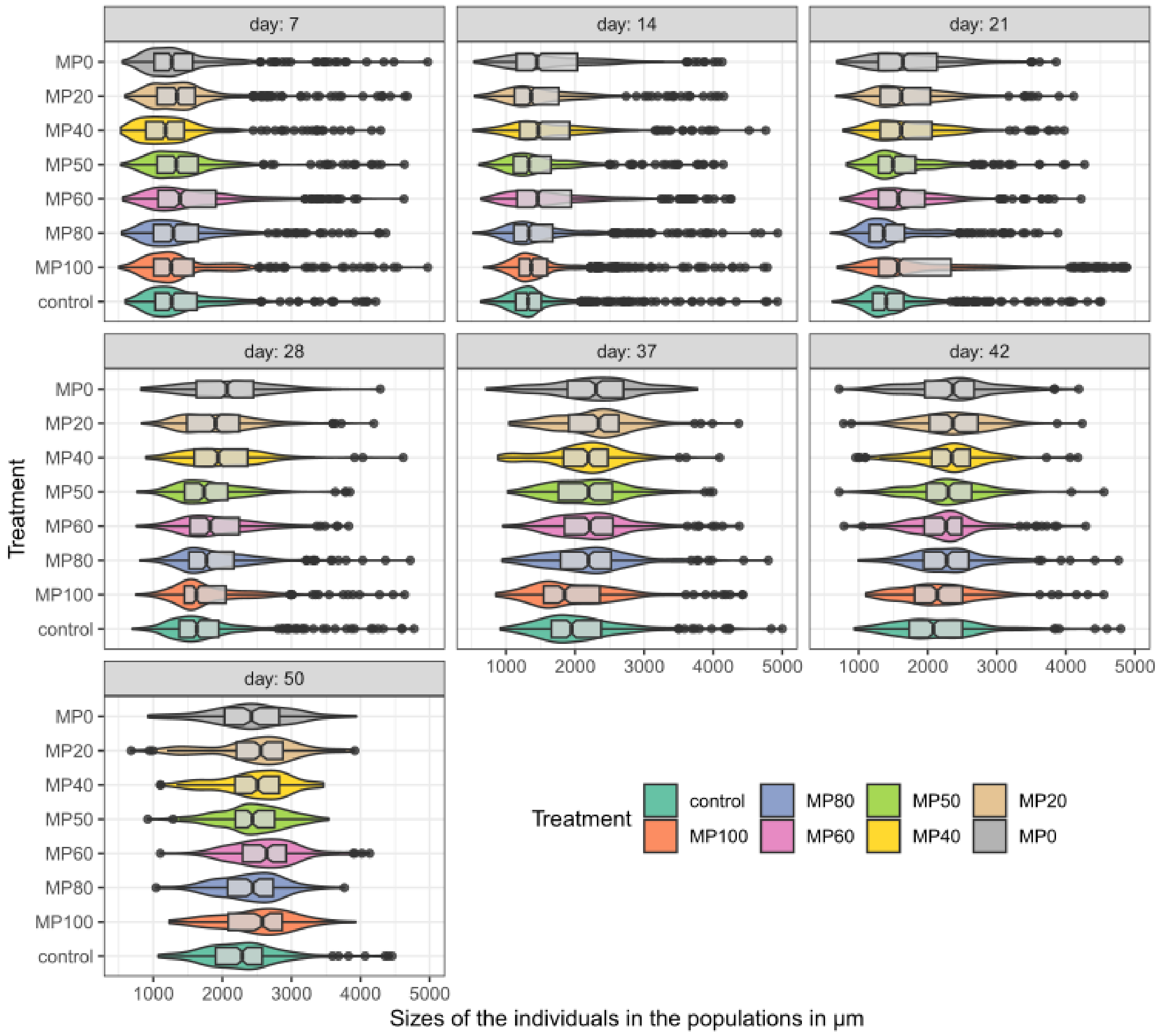

Figure S6: Distributions of the body sizes of the individuals making up each treatment group per day.

Table S4: Abundance of age groups as number of neonates, juveniles, and adults per treatment over time.

Day	Treatment	Group	Mean abundance ± standard deviation
7	Control	Adults	5.3 ± 2.1
		Juveniles	46.3 ± 4.7
		Neonates	85 ± 16.5
	MP100	Adults	8.7 ± 2.3
		Juveniles	50.7 ± 14
		Neonates	111.7 ± 16.8
	MP80	Adults	7.7 ± 1.5
		Juveniles	48 ± 7.8
		Neonates	84 ± 18.7
	MP60	Adults	10.3 ± 3.2
		Juveniles	42.7 ± 2.5
		Neonates	55 ± 18.3
	MP50	Adults	6.3 ± 1.2
		Juveniles	65 ± 7.8
		Neonates	89.7 ± 11
	MP40	Adults	6.3 ± 1.5
		Juveniles	39.7 ± 19.7
		Neonates	107 ± 20.7
	MP20	Adults	9.3 ± 1.5
		Juveniles	72 ± 19
		Neonates	99 ± 34
	MP0	Adults	7 ± 3
		Juveniles	46.7 ± 9.1
		Neonates	90 ± 11.8
14	Control	Adults	9.7 ± 2.1
		Juveniles	75.3 ± 6.4
		Neonates	149.7 ± 37.4
	MP100	Adults	12 ± 2
		Juveniles	90 ± 12.8
		Neonates	121.3 ± 45.8
	MP80	Adults	11 ± 1.7
		Juveniles	79.7 ± 7.6
		Neonates	114 ± 13.5
	MP60	Adults	15 ± 5.6
		Juveniles	74.7 ± 7.8
		Neonates	75.3 ± 16.3
	MP50	Adults	7.7 ± 3.8
		Juveniles	81 ± 6.1
		Neonates	123.7 ± 7.6
	MP40	Adults	8.3 ± 1.5
		Juveniles	92.7 ± 12.1
		Neonates	85.3 ± 32.1
	MP20	Adults	9 ± 1.7
		Juveniles	86 ± 11.8

		Neonates	110.3 ± 16.6
	MP0	Adults	11.3 ± 3.5
		Juveniles	73.7 ± 11.7
		Neonates	72.3 ± 34.9
21	Control	Adults	10.3 ± 3.1
		Juveniles	83.3 ± 15
		Neonates	95.7 ± 36.1
	MP100	Adults	37 ± 49.4
		Juveniles	85 ± 38.2
		Neonates	98.5 ± 13.4
	MP80	Adults	6.3 ± 0.6
		Juveniles	70 ± 13.5
		Neonates	86 ± 16
	MP60	Adults	7.7 ± 1.2
		Juveniles	91.7 ± 12.2
		Neonates	50.7 ± 16.8
	MP50	Adults	7.7 ± 3.8
		Juveniles	86.3 ± 4
		Neonates	65.3 ± 4.2
	MP40	Adults	7 ± 3.6
		Juveniles	92.3 ± 11.9
		Neonates	46.7 ± 30.4
	MP20	Adults	10 ± 5.3
		Juveniles	88.7 ± 9.3
		Neonates	46 ± 18.5
	MP0	Adults	10.3 ± 0.6
		Juveniles	73.7 ± 4
		Neonates	44 ± 16.7
28	Control	Adults	12 ± 3.5
		Juveniles	83.7 ± 2.5
		Neonates	34 ± 9.2
	MP100	Adults	11.3 ± 2.1
		Juveniles	89.3 ± 24
		Neonates	23.3 ± 8.5
	MP80	Adults	11 ± 6
		Juveniles	90.7 ± 13.4
		Neonates	16.3 ± 3.1
	MP60	Adults	11 ± 4.4
		Juveniles	78 ± 21.3
		Neonates	14.7 ± 4.5
	MP50	Adults	6 ± 1.4
		Juveniles	83 ± 2.8
		Neonates	20.5 ± 13.4
	MP40	Adults	10.7 ± 4.6
		Juveniles	71.7 ± 4.7
		Neonates	13.7 ± 12.5
	MP20	Adults	10.3 ± 5
		Juveniles	68.3 ± 6.7
		Neonates	14 ± 3

	MP0	Adults	16.3 ± 3.2
		Juveniles	55 ± 8.5
		Neonates	11.7 ± 5.8
37	Control	Adults	16 ± 1
		Juveniles	69.7 ± 6.7
		Neonates	10.7 ± 9
	MP100	Adults	12 ± 2.6
		Juveniles	56.3 ± 15.7
		Neonates	12 ± 7.2
	MP80	Adults	18.3 ± 8.1
		Juveniles	59.3 ± 8.1
		Neonates	7.7 ± 4
	MP60	Adults	17.3 ± 2.5
		Juveniles	61 ± 9.6
		Neonates	3 ± 1.7
	MP50	Adults	16 ± 2.6
		Juveniles	59 ± 1.7
		Neonates	4.3 ± 4.9
	MP40	Adults	12 ± 6.6
		Juveniles	56 ± 4.6
		Neonates	9.3 ± 9.7
	MP20	Adults	20.7 ± 6
		Juveniles	47.3 ± 4
		Neonates	5.3 ± 4
	MP0	Adults	21.7 ± 7.4
		Juveniles	43.3 ± 6.5
		Neonates	7.7 ± 4
42	Control	Adults	17.3 ± 6.7
		Juveniles	65 ± 10.8
		Neonates	7 ± 3
	MP100	Adults	12.7 ± 6.4
		Juveniles	43.7 ± 19.6
		Neonates	4.7 ± 3.2
	MP80	Adults	16 ± 2.6
		Juveniles	53.3 ± 6
		Neonates	2.3 ± 1.5
	MP60	Adults	13 ± 5.6
		Juveniles	54 ± 15.7
		Neonates	3.3 ± 3.2
	MP50	Adults	17 ± 3.6
		Juveniles	43.3 ± 1.5
		Neonates	2.7 ± 0.6
	MP40	Adults	16.3 ± 4.7
		Juveniles	41.3 ± 8.6
		Neonates	3.5 ± 2.1
	MP20	Adults	20.7 ± 4.9
		Juveniles	38.7 ± 4.6
		Neonates	3 ± 2.8
	MP0	Adults	16.7 ± 3.8

		Juveniles	35 ± 10.1
		Neonates	4.5 ± 0.7
50	Control	Adults	17 ± 1.7
		Juveniles	49.7 ± 4.5
		Neonates	3.3 ± 1.5
	MP100	Adults	19.3 ± 2.3
		Juveniles	20 ± 12.1
		Neonates	1.5 ± 0.7
	MP80	Adults	17 ± 3.6
		Juveniles	28.7 ± 7
		Neonates	2 ± NA
	MP60	Adults	24 ± 6.1
		Juveniles	21.3 ± 1.5
		Neonates	1 ± NA
	MP50	Adults	19 ± 4
		Juveniles	29 ± 8.7
		Neonates	2 ± 1.4
	MP40	Adults	22.3 ± 3.1
		Juveniles	26.3 ± 5.8
		Neonates	1.7 ± 0.6
	MP20	Adults	23.3 ± 6.4
		Juveniles	21.3 ± 4.9
		Neonates	4 ± 2
	MP0	Adults	14.3 ± 5
		Juveniles	23.7 ± 2.1
		Neonates	4 ± 1.4

Table S5: Number of resting eggs per replicate after 50 d of exposure.

Treatment group	Replicate	Resting eggs
Control	1	82
	2	70
	3	60
MP100	1	83
	2	66
	3	60
MP80	1	88
	2	91
	3	80
MP60	1	102
	2	107
	3	88
MP50	1	78
	2	81
	3	92
MP40	1	129
	2	101
	3	92
MP20	1	108
	2	101
	3	98
MP0	1	105
	2	97
	3	108

Table S6: Results of the statistical comparison of the body length of *D. magna* individuals in populations exposed to particles. Kruskal-Wallis tests with Dunn’s multiple comparison tests for each observation time. Δ rank indicates the difference in mean rank (negative values imply larger individuals) and p refers to the adjusted p values.

	d 7		**d 14**		**d 21**		**d 28**		**d 37**		**d 42**		**d 50**	
Control vs.	**Δ rank**	**p**	**Δ rank**	**p**	**Δ rank**	**p**	**Δ rank**	**p**	**Δ rank**	**p**	**Δ rank**	**p**	**Δ rank**	**p**
MP100	46.5	>0.9999	-271.5	**0.0018**	-26	>0.9999	-67.9	>0.9999	65.9	>0.9999	-33.9	>0.9999	-156.5	**0.0004**
MP80	2	>0.9999	-150	0.3391	68.6	>0.9999	-210.5	**0.0004**	-145.8	**0.0168**	-139.7	**0.0064**	-90.4	0.1029
MP60	-223.2	**0.0259**	-452	**<0.0001**	-363.9	**<0.0001**	-279.6	**<0.0001**	-193	**0.0005**	-101.9	0.1129	-221.6	**<0.0001**
MP50	-86.6	>0.9999	-87.5	>0.9999	-243.9	**0.0014**	-133.7	0.1807	-142.4	**0.0255**	-148	**0.005**	-112.1	**0.0144**
MP40	322	**<0.0001**	-469	**<0.0001**	-487.8	**<0.0001**	-383.6	**<0.0001**	-115.9	0.1315	-175.6	**0.0005**	-136.6	**0.0011**
MP20	-91.5	>0.9999	-196.1	0.0686	-475.6	**<0.0001**	-298.7	**<0.0001**	-260.8	**<0.0001**	-210.7	**<0.0001**	-157.5	**0.0001**
MP0	21	>0.9999	-442	**<0.0001**	-446.1	**<0.0001**	-470.2	**<0.0001**	-255.5	**<0.0001**	-183.3	**0.0004**	-94.19	0.1027