SUPPORTING INFORMATION

Microplastic effect thresholds for freshwater benthic macroinvertebrates

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19 pages

3 tables

7 figures

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Table S1. Calculation of the weighted average density for environmental microplastics based on data provided by Andrady *et al.,* 2011[1].

A) Calculation assuming a density of 1 g/cm^3 for the rest fraction of 6%.

Plastic type	**Density** (g/cm^3)[a]	**Fraction Produced**[a]	**Weighted** (g/cm^3)
LDPE	0.92	0.21	0.1932
HDPE	0.94	0.17	0.1598
PP	0.84	0.24	0.2016
PS	1.05	0.06	0.063
PET	1.37	0.07	0.0959
PVC	1.38	0.19	0.2622
REST[b]	1	0.06	0.06
WEIGHTED AVERAGE DENSITY[c]			**1.0357**

[a] according to Table 1 in Andrady et al (2011)[1]

[b] for the REST fraction of 6% a density of 1 g/cm^3 was assumed

[c] the weighted average density is the average density of environmental microplastic, assuming all produced plastic types contribute to microplastic with weights equal to the their production fraction.

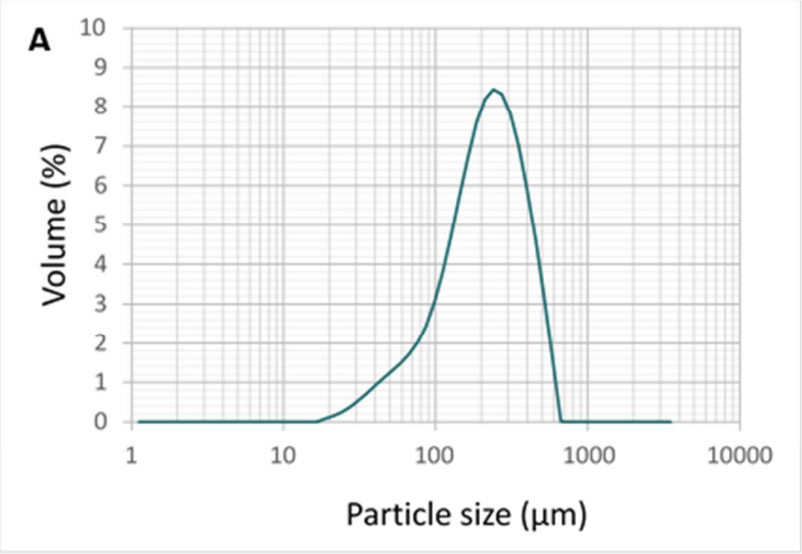
Table S1, continued

B) Calculation neglecting the unknown rest fraction by scaling the sum of the fractions for the known polymers to 100%

Plastic type	**Density** (g/cm^3)[a]	**Fraction Produced**[a]	**Weighted** (g/cm^3)
LDPE	0.92	0.21	0.1932
HDPE	0.94	0.17	0.1598
PP	0.84	0.24	0.2016
PS	1.05	0.06	0.063
PET	1.37	0.07	0.0959
PVC	1.38	0.19	0.2622
WEIGHTED AVERAGE DENSITY [b]			**1.0380**

[a] according to Table 1 in Andrady et al (2011)[1]

[b] the weighted average density is the average density of environmental microplastic, assuming all produced plastic types contribute to microplastic with weights equal to the their production fraction.



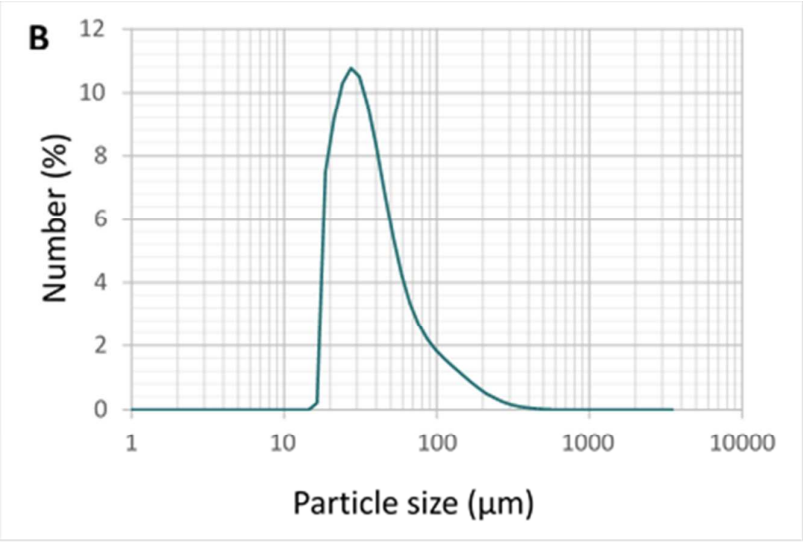


Figure S1. Particle Size Distribution (n=3) of the original microplastic mixture in: A) Volume %; and B) Number %.

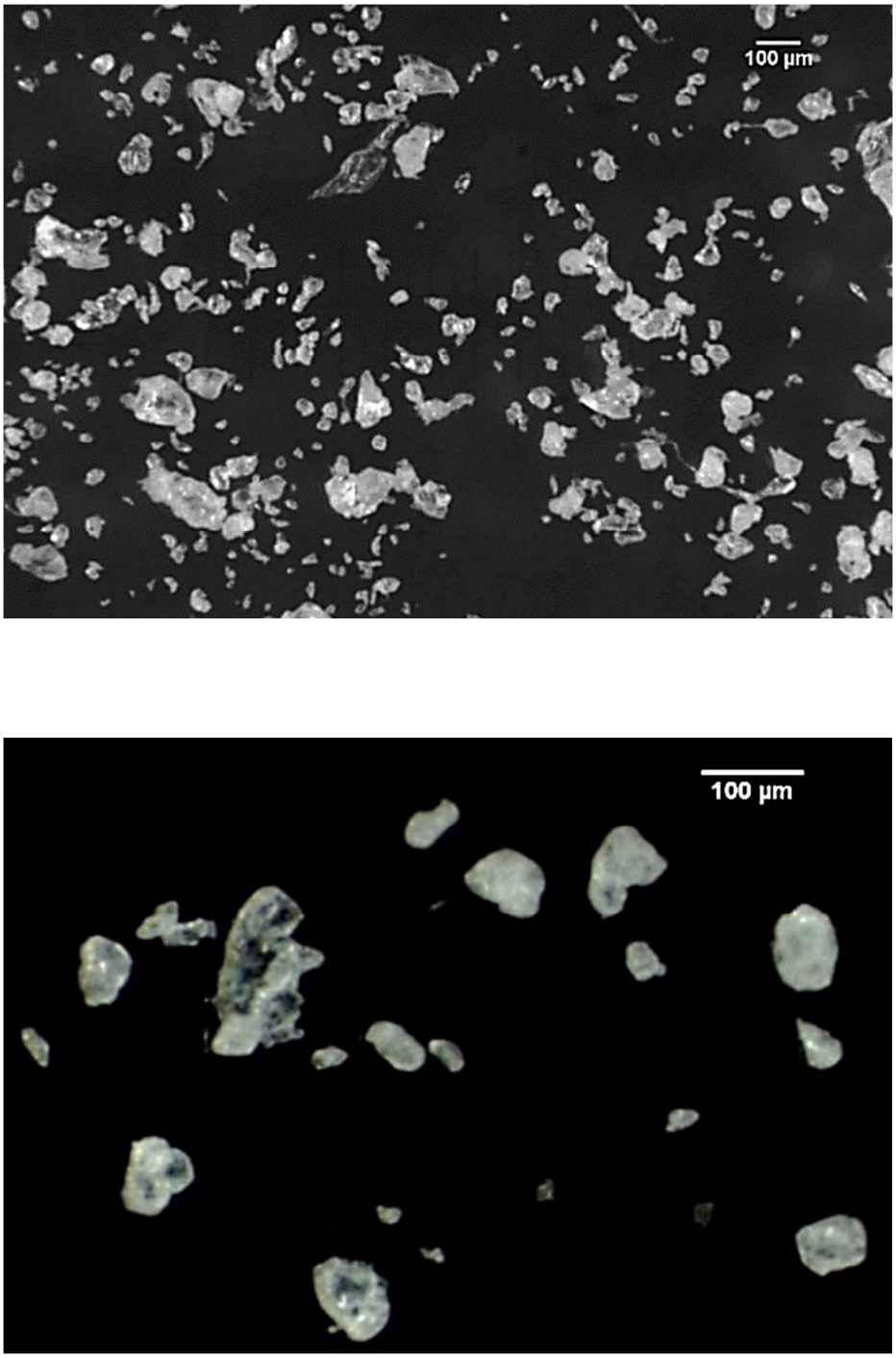


Figure S2. Light microscope pictures of the irregularly shaped particles (Olympus SZX10 Stereomicroscope).

Table S2. Background elemental concentrations of Zn, Cd, Cr, Cu, Ni and Pb in the Veenkampen sediment using A) Extraction with HNO_3-HCl; B) Extraction with 0.01M $CaCl_2$, compared to the Dutch Sediment Quality Criteria (SQC) based on Target values.

A)

	Zn [mg/kg]	**Cd [mg/kg]**	**Cr [mg/kg]**	**Cu [mg/kg]**	**Ni [mg/kg]**	**Pb [mg/kg]**
Detection limit	5	0.05	0.8	3	1.6	0.3
Veenkampen sediment	75	0.47	47.5	26	31.7	31.9
Dutch SQC [a]	140	0.8	100	36	35	85

B)

	Zn [mg/kg]	**Cd [mg/kg]**	**Cr [mg/kg]**	**Cu [mg/kg]**	**Ni [mg/kg]**	**Pb [mg/kg]**
Detection limit	300	3	5	400	6	20
Veenkampen sediment	11	1	14	6	16	0
Dutch SQC [a]	140	0.8	100	36	35	85

[a] Values giving an indication of the benchmark for environmental quality in the long term on the assumption of negligible risks to the ecosystem (background concentration of metals (Cb) presented in Table 6.2 in Lijzen *et al.*, 2001).[2]

Table S3. Water Quality Parameters (Mean±s.d.)

Species	**pH**	**O_2 [mg/L]**	**EC [µS/m]**	**T [^oC]**	**NH_3 [mgN/L]**
G. pulex	7.7±0.1	8.8±0.1	474±17	16.2±0.06	0.04±0.01
H. azteca	7.4±0.1	9.2±0.2	409±15	16.0±0.04	0.04±0.01
A. aquaticus	7.3±0.1	9.0±0.1	562±110	15.8±0.09	n.a.
S. corneum	7.3±0.2	8.8±0.2	473±16	16.7±0.08	0.02±0.003
L. variegatus	7.2±0.1	8.7±0.2	478±28	15.8±0.04	n.a.
Tubifex spp.	7.1±0.1	8.8 ±0.2	466±25	15.9±0.04	n.a.

n.a.= not analysed

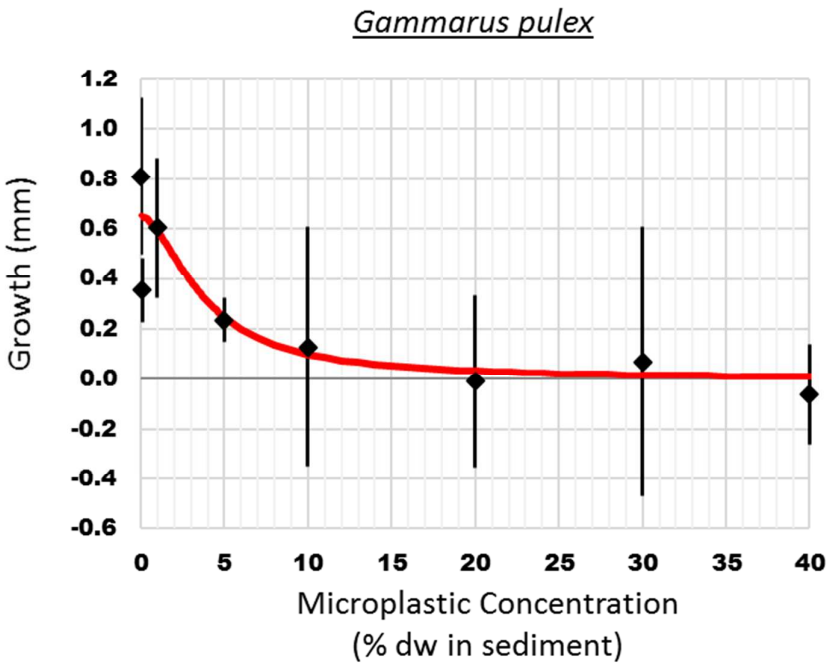


Figure S3. Growth of *G.pulex* as a function of polystyrene microplastic dose. The red curve relates to the best fit of the log-logistic response model (Eq. 2 in the main manuscript). The 50 % effect (EC_{50}) is fitted at a dose of 3.57% dw. The EC_{10} was obtained by solving Eq 2 for the dose at 10% of the observed effect (1.07 % dw).

A) *Gammarus pulex*

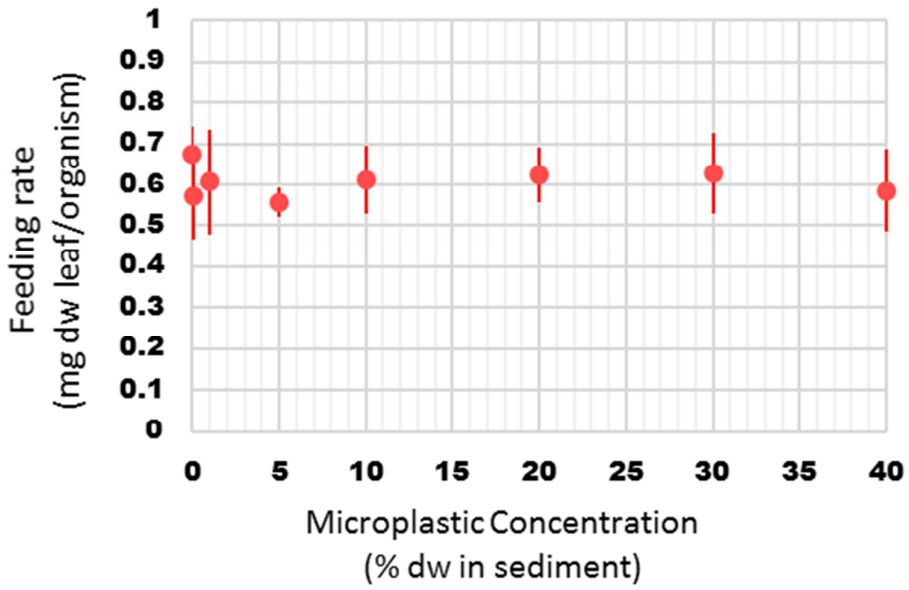


Figure S4A. Mean feeding rate (±s.d.) as mg dw of *Populus spp*. leaves consumed per organism of *G. pulex* per day during the 28-d exposure to PS microplastic concentrations ranging from 0 to 40 % in sediment (dw).

B) *Hyalella azteca*

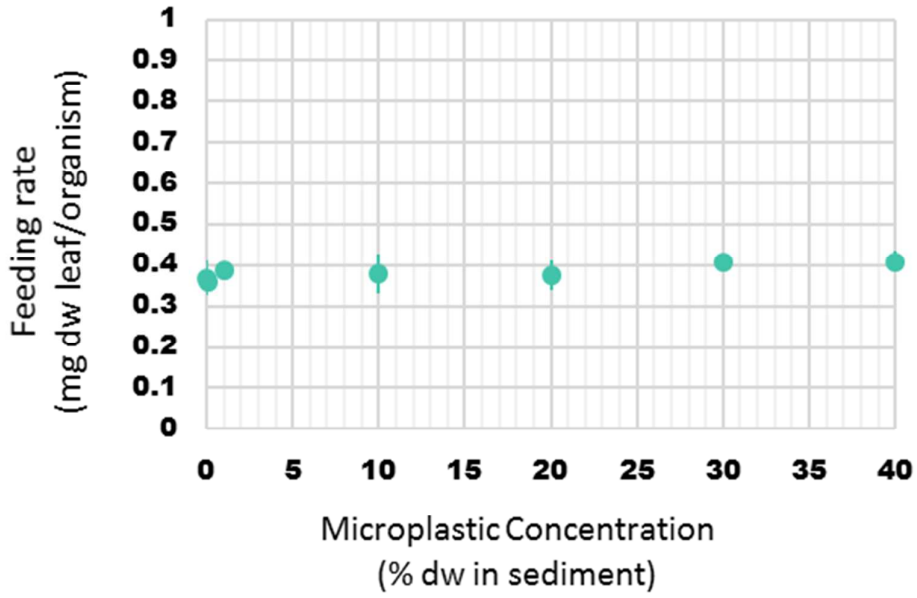


Figure S4B. Mean feeding rate (±s.d.) as mg dw of *Populus spp.* leaves consumed per organism of *H. azteca* per day during the 28-d exposure to PS microplastic concentrations ranging from 0 to 40 % in sediment (dw).

C) *Asellus aquaticus*

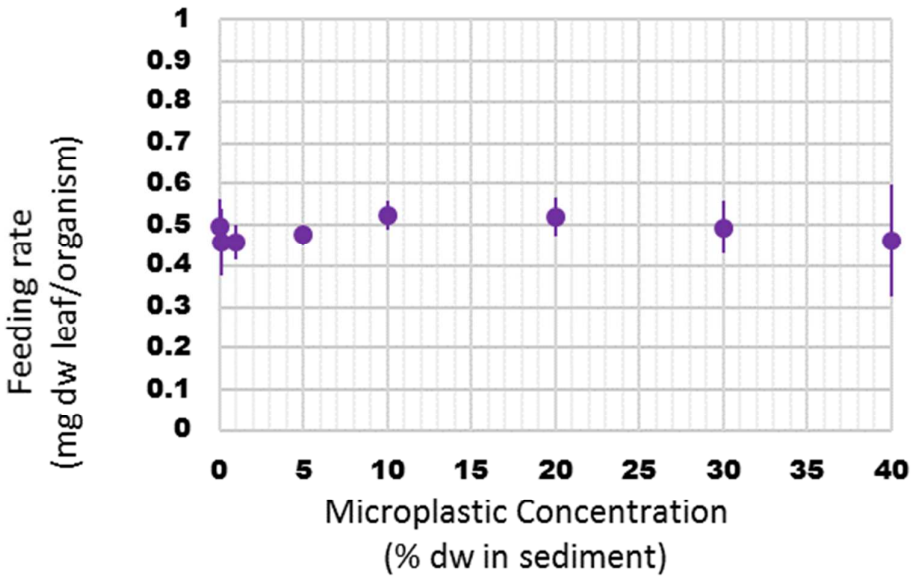


Figure S4C. Mean feeding rate (±s.d.) as mg dw of *Populus spp*. leaves consumed per organism of *A. aquaticus* per day during the 28-d exposure to PS microplastic concentrations ranging from 0 to 40 % in sediment (dw).

A) Tubifex spp.

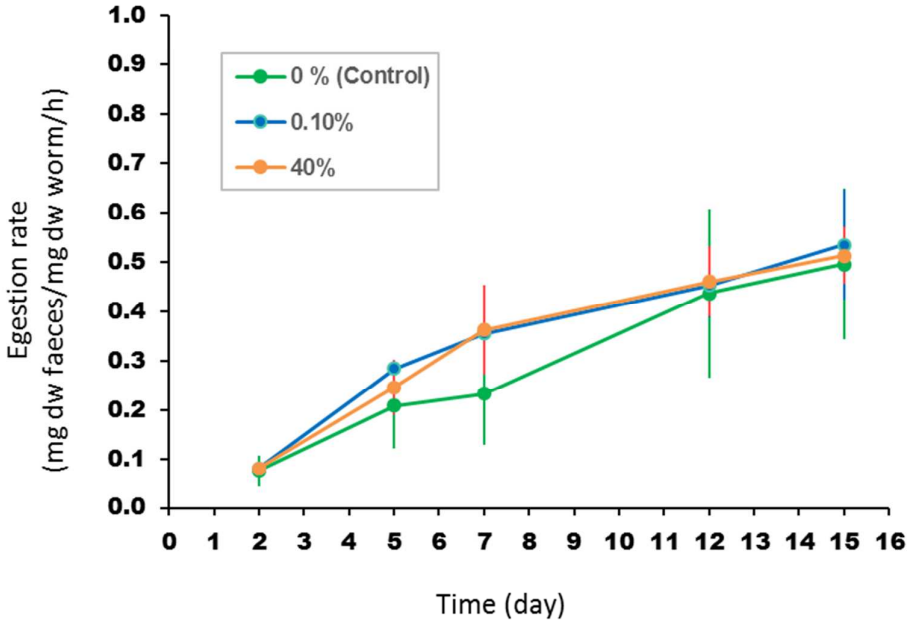


Figure S5A. Mean egestion rate (±SD) as mg dw of faeces egested per organism per day for *Tubifex spp.* (A) during a 15-d exposure to PS microplastic concentrations ranging from 0 to 40 % in sediment (dw).

B) Lumbriculus variegatus

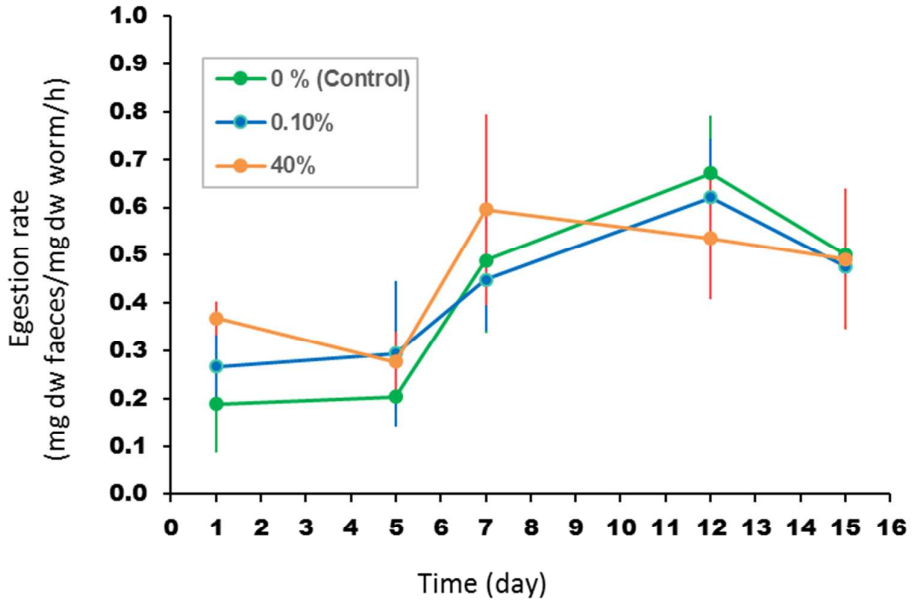


Figure S5B. Mean egestion rate (±SD) as mg dw of faeces egested per organism per day for *L. variegatus* (B) during a 15-d exposure to PS microplastic concentrations ranging from 0 to 40 % in sediment (dw).

A)

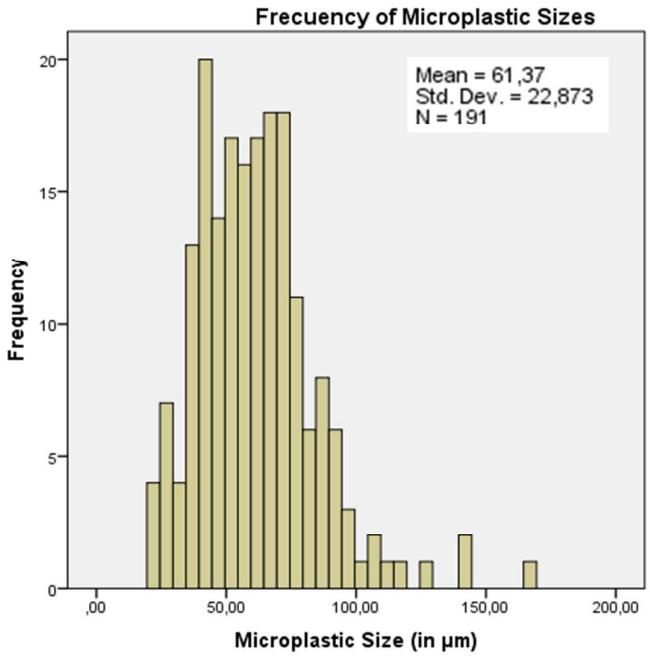


Figure S6A. Size Frequency of Retained Microplastics.

B)

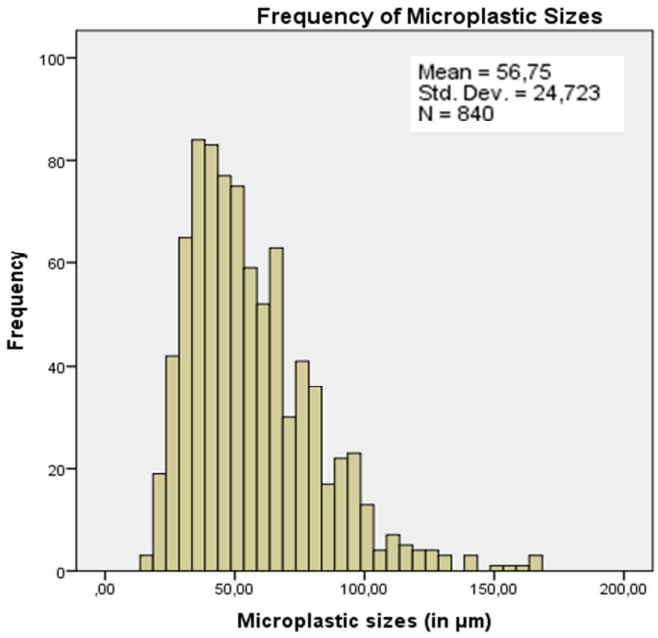


Figure S6B. Size Frequency of Egested Microplastics.

C)

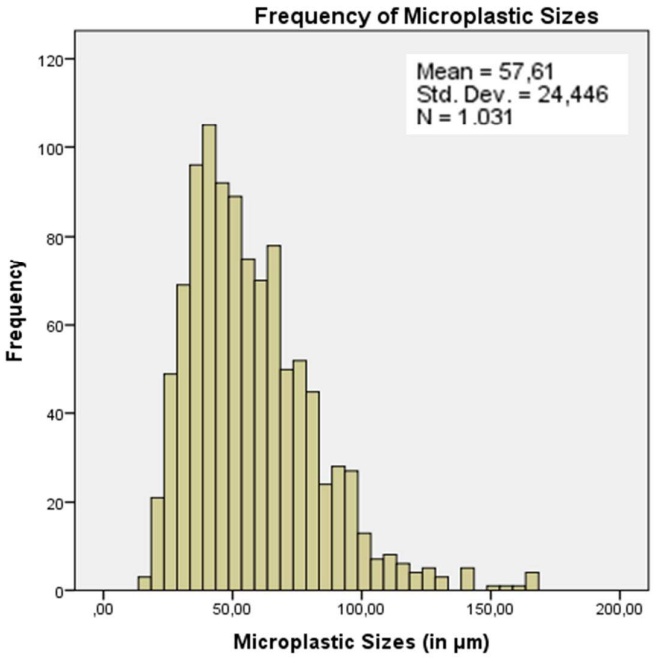


Figure S6C. Size Frequency of Total Ingested Microplastics (sum of A and B).

A)

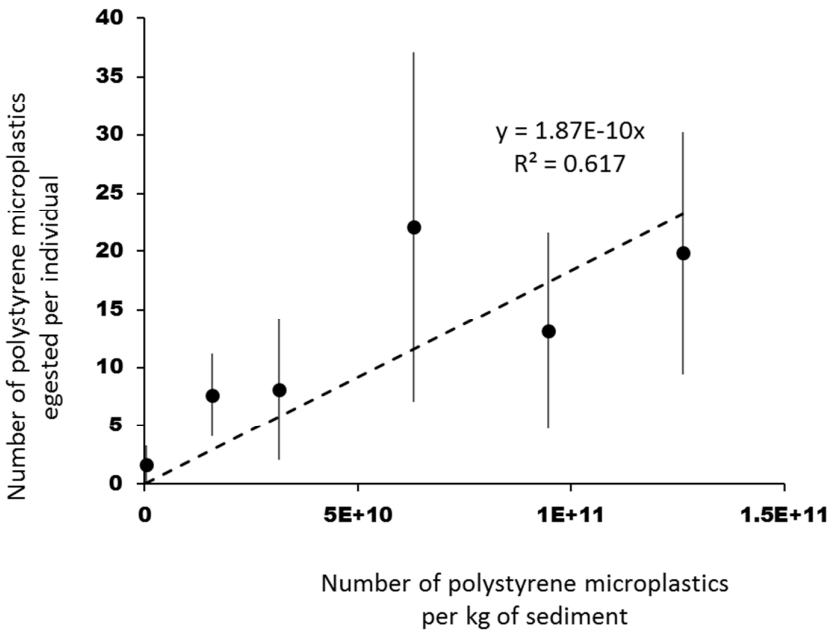


Figure S7A. Mean microplastic concentration (n=4) per individual of *G. pulex* (±s.d) at increasing microplastic concentrations in sediment as number of microplastics egested per organism by number of microplastics per kg of sediment (dw).

B)

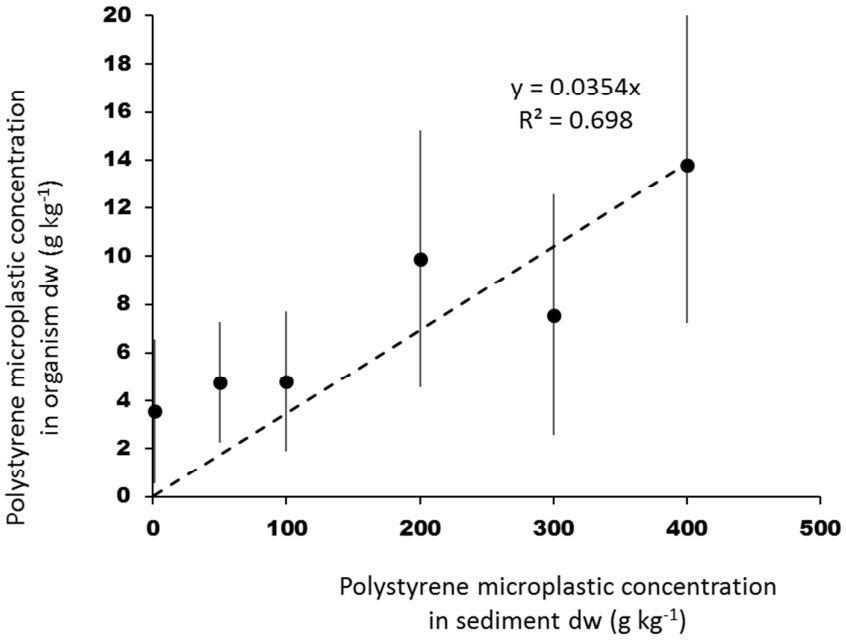


Figure S7. Mean microplastic concentration (n=4) per individual of *G. pulex* (±s.d) at increasing microplastic concentrations in sediment as: g kg^{-1} of microplastics egested per organism dw by g kg^{-1} of microplastics per sediment (dw).

Literature

(1) Andrady, A. L. Microplastics in the marine environment. *Mar. Pollut. Bull.* **2011**, *62* (8), 1596–1605.

(2) Lijzen, J. P. A.; Baars, A. J.; Otte, P. F.; Rikken, M. G. J.; Swartjes, F. A.; Verbruggen, E. M. J.; Van Wezel, A. P. *RIVM report 711701 023. Technical evaluation of the Intervention Values for Soil/sediment and Groundwater Human and ecotoxicological risk assessment and derivation of risk limits for soil, aquatic sediment and groundwater*; 2001.