

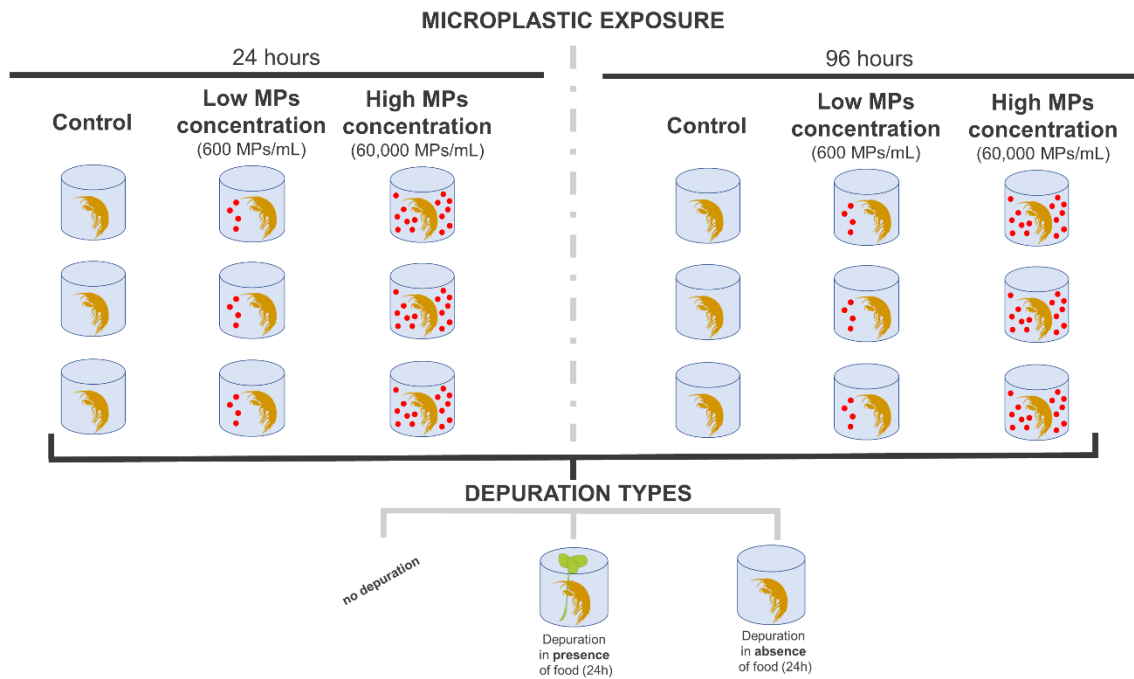
Supplementary material

Rapid fragmentation of microplastics by the freshwater amphipod

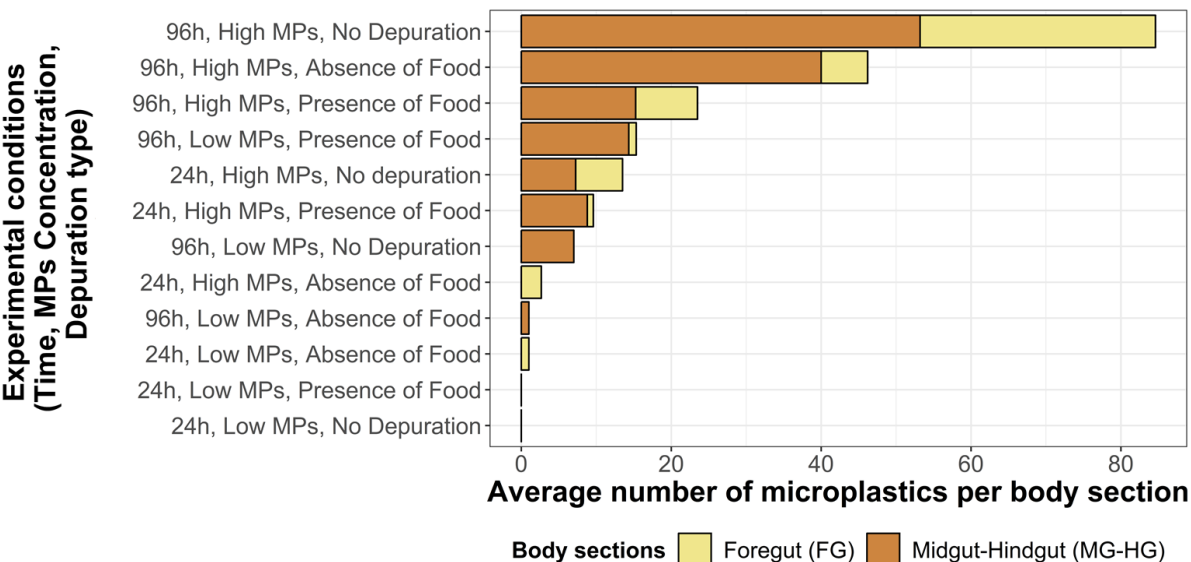
Gammarus duebeni

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2 **Supplementary Figure S1.** Experimental design showing a graphic overview of the setup for one replicate. Six
3 replicates were run. All amphipods were exposed individually. The experiment involved a total of 108 amphipods
4 exposed among two microplastic doses, two different time exposures and three depuration periods. There were N
5 = 36 control individuals (microplastic free), N = 36 individuals exposed to the low microplastic concentration and N
6 = 36 individuals exposed to the high microplastic concentration. So, at the end of the test, a total of six *G. duebeni*
7 had been individually exposed to different treatments consisting in: MPs dose/time/depuration type.
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12 **Supplementary Figure S2.** Average number of microplastics present in *G. duebeni* digestive tract sections, and
13 as a function of different experimental treatments such as: time (24h or 96h exposure to plastics), microplastic
14 concentration (Low MPs or High MPs) and depuration type (No depuration, 24h depuration in Presence or Absence
15 of Food). The figure shows the average number of microplastics for each body section ("FG" stands for "Foregut"
16 and "MG-HG" for "Midgut and Hindgut"). Here data is shown for all amphipods within each treatment. This figure
17 was produced using the "ggplot2" package in R (v3.4.3).
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19 **Supplementary Table S1.** Detailed information on the microplastic (MP) presence and numbers of microplastics
 20 per *G. duebeni* individual sample according to microplastic dose, time exposure and depuration type. Control
 21 individuals (microplastic free) are not shown. Median length (μm) is given as well as mean \pm SE length (μm).

Replicate	Sample ID	MPs dose	Time	Depuration	MP presence in amphipod	Total MPs	Median Length (μm)	Mean Length (μm)	SE (μm)
1	MP 01	Low MPs	24h	No depuration	no	0	X	X	X
2	MP 02	Low MPs	24h	No depuration	no	0	X	X	X
3	MP 03	Low MPs	24h	No depuration	no	0	X	X	X
4	MP 37	Low MPs	24h	No depuration	no	0	X	X	X
5	MP 38	Low MPs	24h	No depuration	no	0	X	X	X
6	MP 39	Low MPs	24h	No depuration	no	0	X	X	X
1	MP 04	Low MPs	24h	Presence of food	no	0	X	X	X
2	MP 05	Low MPs	24h	Presence of food	no	0	X	X	X
3	MP 06	Low MPs	24h	Presence of food	no	0	X	X	X
4	MP 40	Low MPs	24h	Presence of food	no	0	X	X	X
5	MP 41	Low MPs	24h	Presence of food	no	0	X	X	X
6	MP 42	Low MPs	24h	Presence of food	mortality	X	X	X	X
1	MP 07	Low MPs	24h	Absence of food	no	0	X	X	X
2	MP 08	Low MPs	24h	Absence of food	no	0	X	X	X
3	MP 09	Low MPs	24h	Absence of food	no	0	X	X	X
4	MP 43	Low MPs	24h	Absence of food	yes	1	1.58	1.58	0.00
5	MP 44	Low MPs	24h	Absence of food	no	0	X	X	X
6	MP 45	Low MPs	24h	Absence of food	no	0	X	X	X
1	MP 20	Low MPs	96h	No depuration	yes	1	37.83	37.83	0.00
2	MP 21	Low MPs	96h	No depuration	mortality	X	X	X	X
3	MP 55	Low MPs	96h	No depuration	no	0	X	X	X
4	MP 56	Low MPs	96h	No depuration	no	0	X	X	X
5	MP 57	Low MPs	96h	No depuration	no	0	X	X	X
6	MP 19	Low MPs	96h	No depuration	yes	13	36.18	32.04	6.27
1	MP 22	Low MPs	96h	Presence of food	yes	5	4.12	24.24	16.74
2	MP 23	Low MPs	96h	Presence of food	mortality	X	X	X	X
3	MP 24	Low MPs	96h	Presence of food	yes	1	40.99	40.99	0.00
4	MP 58	Low MPs	96h	Presence of food	no	0	X	X	X
5	MP 59	Low MPs	96h	Presence of food	yes	40	2.71	4.95	1.12
6	MP 60	Low MPs	96h	Presence of food	no	0	X	X	X
1	MP 25	Low MPs	96h	Absence of food	no	0	X	X	X
2	MP 26	Low MPs	96h	Absence of food	yes	1	45.62	45.62	0.00
3	MP 27	Low MPs	96h	Absence of food	yes	1	34.44	34.44	0.00
4	MP 61	Low MPs	96h	Absence of food	no	0	X	X	X
5	MP 62	Low MPs	96h	Absence of food	no	0	X	X	X
6	MP 63	Low MPs	96h	Absence of food	no	0	X	X	X
1	MP 10	High MPs	24h	No depuration	no	0	X	X	X
2	MP 11	High MPs	24h	No depuration	yes	29	43.10	48.62	8.20
3	MP 12	High MPs	24h	No depuration	yes	9	32.42	33.19	4.31
4	MP 46	High MPs	24h	No depuration	yes	12	5.36	12.86	3.47
5	MP 47	High MPs	24h	No depuration	yes	4	16.81	19.69	7.48
6	MP 48	High MPs	24h	No depuration	no	0	X	X	X
1	MP 13	High MPs	24h	Presence of food	no	0	X	X	X
2	MP 14	High MPs	24h	Presence of food	yes	7	33.16	35.50	1.87
3	MP 15	High MPs	24h	Presence of food	yes	14	2.77	9.39	3.83
4	MP 49	High MPs	24h	Presence of food	yes	1	42.96	42.96	0.00
5	MP 50	High MPs	24h	Presence of food	yes	22	2.17	15.16	4.50
6	MP 51	High MPs	24h	Presence of food	yes	3	43.67	47.06	4.97
1	MP 16	High MPs	24h	Absence of food	no	0	X	X	X
2	MP 17	High MPs	24h	Absence of food	yes	3	27.76	29.90	5.44
3	MP 18	High MPs	24h	Absence of food	yes	4	30.93	47.96	20.45
4	MP 52	High MPs	24h	Absence of food	yes	1	42.15	42.15	0.00
5	MP 53	High MPs	24h	Absence of food	no	0	X	X	X
6	MP 54	High MPs	24h	Absence of food	no	0	X	X	X
1	MP 34	High MPs	96h	No depuration	yes	116	38.65	37.62	0.74
2	MP 35	High MPs	96h	No depuration	yes	103	34.04	35.37	2.56
3	MP 36	High MPs	96h	No depuration	yes	24	17.27	25.01	4.50
4	MP 64	High MPs	96h	No depuration	yes	17	35.98	40.98	8.94
5	MP 65	High MPs	96h	No depuration	yes	161	38.74	38.62	2.04
6	MP 66	High MPs	96h	No depuration	mortality	X	X	X	X
1	MP 31	High MPs	96h	Presence of food	yes	2	48.02	48.02	43.68
2	MP 32	High MPs	96h	Presence of food	yes	71	14.95	21.74	2.41
3	MP 33	High MPs	96h	Presence of food	yes	16	54.71	66.11	9.26
4	MP 67	High MPs	96h	Presence of food	Yes	6	34.69	44.89	19.78
5	MP 68	High MPs	96h	Presence of food	no	0	X	X	X
6	MP 69	High MPs	96h	Presence of food	no	0	X	X	X
1	MP 28	High MPs	96h	Absence of food	yes	3	6.38	9.50	5.57
2	MP 29	High MPs	96h	Absence of food	yes	144	31.70	33.50	2.06
3	MP 30	High MPs	96h	Absence of food	yes	139	43.97	48.03	2.07
4	MP 70	High MPs	96h	Absence of food	Yes	18	45.07	49.57	5.64
5	MP 71	High MPs	96h	Absence of food	Yes	2	49.13	49.13	11.08
6	MP 72	High MPs	96h	Absence of food	no	0	X	X	X

25 **Supplementary Table S2.** Number of microplastics and their average size in μm (mean \pm standard error) according
 26 to shape type, *G. duebeni*'s body part and depuration type. Independent replicates were run (R=6) with a total of
 27 96 amphipods tested of which 36 belonged to the control (microplastic free) group. 'Total N of amphipods' in the
 28 table show the number of amphipods/body section that had accumulated microspheres and/or fragments.

NO DEPURATION										
Foregut										
		Total N of MPs among all amphipods	N amphipods with MPs in foreguts	Mean MPs/Amph	Mean MP size (μm)	$\pm\text{SE}$ (μm)		Total N of MPs per shape type		Total N of MP/foregut
Microsphere	Spherical	138	6	23.00	36.50	1.97	Microsphere	138	All MPs	181
Fragment	Small	26	6	4.33	8.89	0.18	Fragment	43		
	Flat	7	1	7.00	68.97	0.05				
	Cracked	10	3	3.33	55.17	0.06				
Midgut and hindgut										
		Total N of MPs among all amphipods	N amphipods with MPs in foreguts	Mean MPs/Amph	Mean MP size (μm)	$\pm\text{SE}$ (μm)		Total N of MPs per shape type		Total N of MP/midgut-hindgut
Microsphere	Spherical	107	6	17.83	35.47	1.52	Microsphere	107	All MPs	308
Fragment	Irregular	86	8	10.75	15.63	2.19	Fragment	201		
	Flat	12	3	4.00	104.39	11.42				
	Cracked	103	6	17.17	50.21	2.86				
TOTAL MP/all amphipods		489								

DEPURATION IN PRESENCE OF FOOD										
Foregut										
		Total N of MPs among all amphipods	N amphipods with MPs in foreguts	Mean MPs/Amph	Mean MP size (μm)	$\pm\text{SE}$ (μm)		Total N of MPs per shape type		Total N of MP/foregut
Microsphere	Spherical	10	4	2.50	39.12	4.32	Microsphere	10	All MPs	40
Fragment	Irregular	25	3	8.33	5.03	2.69	Fragment	30		
	Flat	0	0	0.00	0.00	0.00				
	Cracked	5	1	5.00	26.91	6.12				
Midgut and hindgut										
		Total N of MPs among all amphipods	N amphipods with MPs in foreguts	Mean MPs/Amph	Mean MP size (μm)	$\pm\text{SE}$ (μm)		Total N of MPs per shape type		Total N of MP/midgut-hindgut
Microsphere	Spherical	14	6	2.33	36.31	5.28	Microsphere	14	All MPs	148
Fragment	Irregular	66	6	11.00	4.19	2.49	Fragment	134		
	Flat	43	4	10.75	40.02	4.41				
	Cracked	25	8	3.13	42.37	7.13				
TOTAL MP/all amphipods		188								

DEPURATION IN ABSENCE OF FOOD										
Foregut										
		Total N of MPs among all amphipods	N amphipods with MPs in foreguts	Mean MPs/Amph	Mean MP size (μm)	$\pm\text{SE}$ (μm)		Total N of MPs per shape type		Total N of MP/foregut
Microsphere	Spherical	20	6	3.33	38.74	1.69	Microsphere	20	All MPs	40
Fragment	Irregular	7	4	1.75	9.13	4.23	Fragment	20		
	Flat	1	1	1.00	108.90	0.00				
	Cracked	12	4	3.00	59.77	8.76				
Midgut and hindgut										
		Total N of MPs among all amphipods	N amphipods with MPs in foreguts	Mean MPs/Amph	Mean MP size (μm)	$\pm\text{SE}$ (μm)		Total N of MPs per shape type		Total N of MP/midgut-hindgut
Microsphere	Spherical	52	6	8.67	34.29	1.71	Microsphere	52	All MPs	277
Fragment	Irregular	26	3	8.67	4.73	1.07	Fragment	225		
	Flat	53	2	26.50	43.56	40.40				
	Cracked	146	4	36.50	48.12	5.10				
TOTAL MP/all amphipods		317								

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 31 **Supplementary Table S3.** Most common microplastic shape type found in *G. duebeni* body sections according to
 32 depuration types. Sizes are given in mean \pm SE.

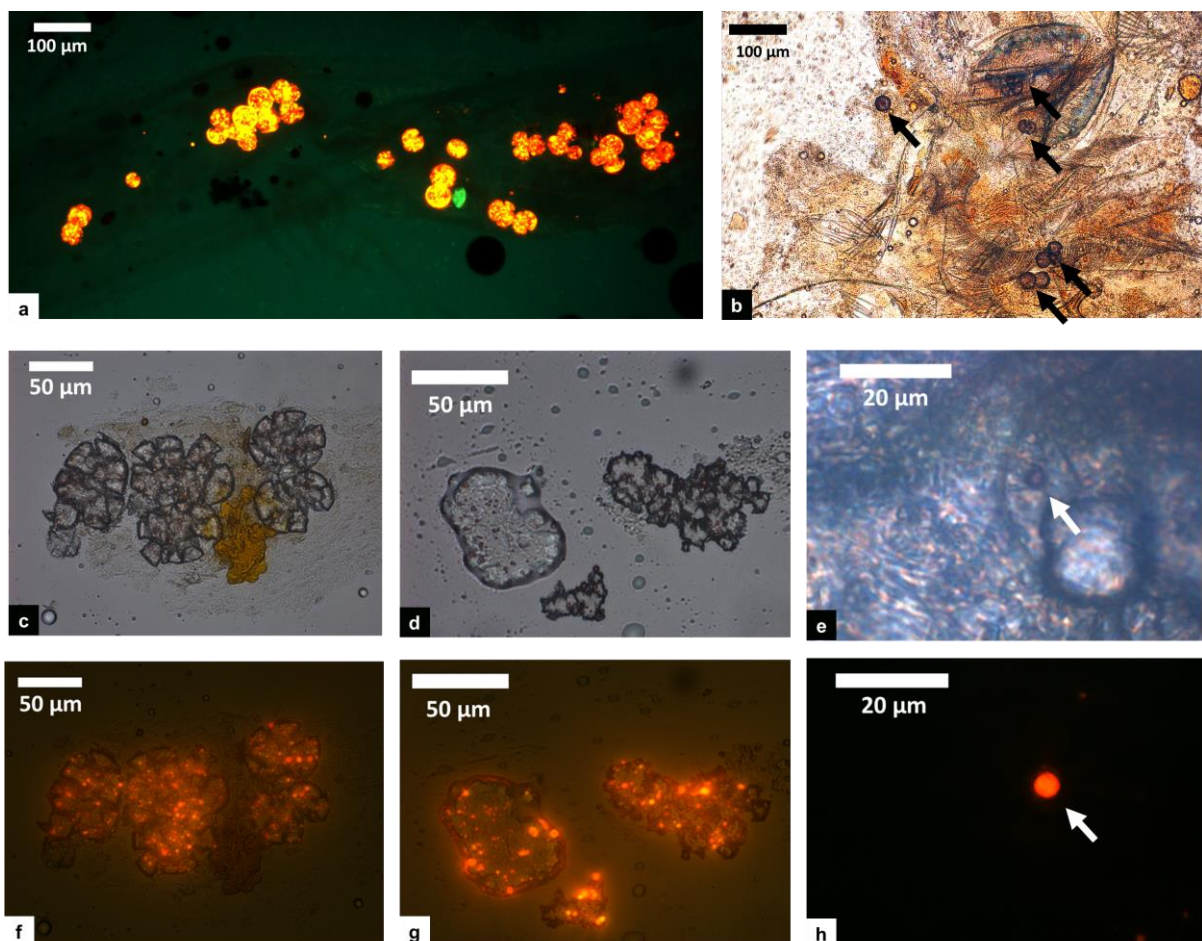
	Foreguts			Midguts-Hindguts		
	Shape type	Size	Total	Shape type	Size	Total
No depuration	Intact spherical	36.50 μm \pm 1.97	138	Intact spherical	35.47 μm \pm 1.52	107
Depuration in presence of food	Small irregular	5.03 μm \pm 2.69	25	Small irregular	4.19 μm \pm 2.49	66
Depuration in absence of food	Intact spherical	38.74 μm \pm 1.69	20	Cracked	48.12 μm \pm 5.10	146

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 35 In the absence of depuration, the most common microplastic shape in *G. duebeni* foregut sections was
 36 the intact spherical microplastic type (a total of 138 microplastics of an average size of 36.50 \pm 1.97
 37 μm , **Table S3**). After depuration in the presence of food, the most common shape in foreguts was the
 38 small irregular microplastic fragment type (25 microplastics of an average size of 5.03 \pm 2.69 μm , **Table**

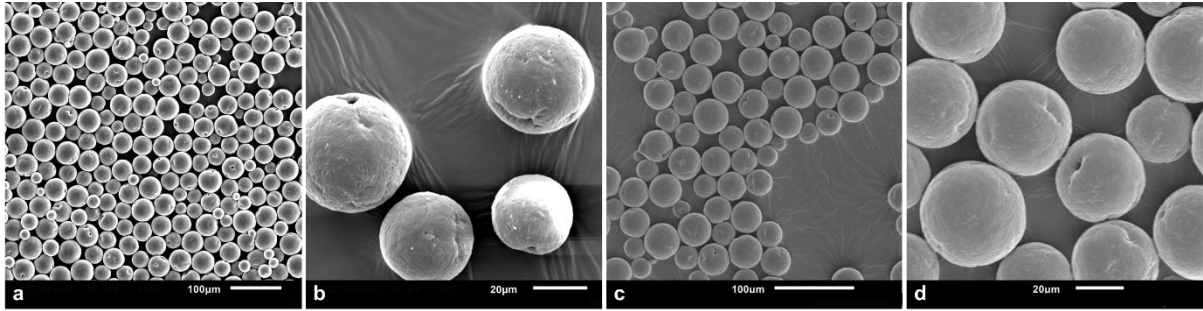
39 **S2)**. Following depuration in absence of food, the most common shape in foreguts was the whole
40 spherical microplastic (20 microplastics of an average size of $38.74 \pm 1.69 \mu\text{m}$, **Table S3**).

41 Likewise, in the absence of depuration, the most common microplastic shape in midguts-hindguts was
42 the whole spherical microplastic type (a total of 107 microplastics of an average size of 35.47 ± 1.52
43 μm , **Table S3**) though in very similar numbers to cracked fragments (a total of 103 microplastics of an
44 average size of $50.21 \pm 2.86 \mu\text{m}$). After depuration in the presence of food, the most common shape in
45 midguts-hindguts were also small irregular fragments (a total of 66) of an average size of 4.19 ± 2.49
46 μm , **Table S3**). After depuration in the absence of food, the most common shape in midguts-hindguts
47 were cracked fragments (a total of 146) of an average size of $48.12 \pm 5.10 \mu\text{m}$, **Table S3**).

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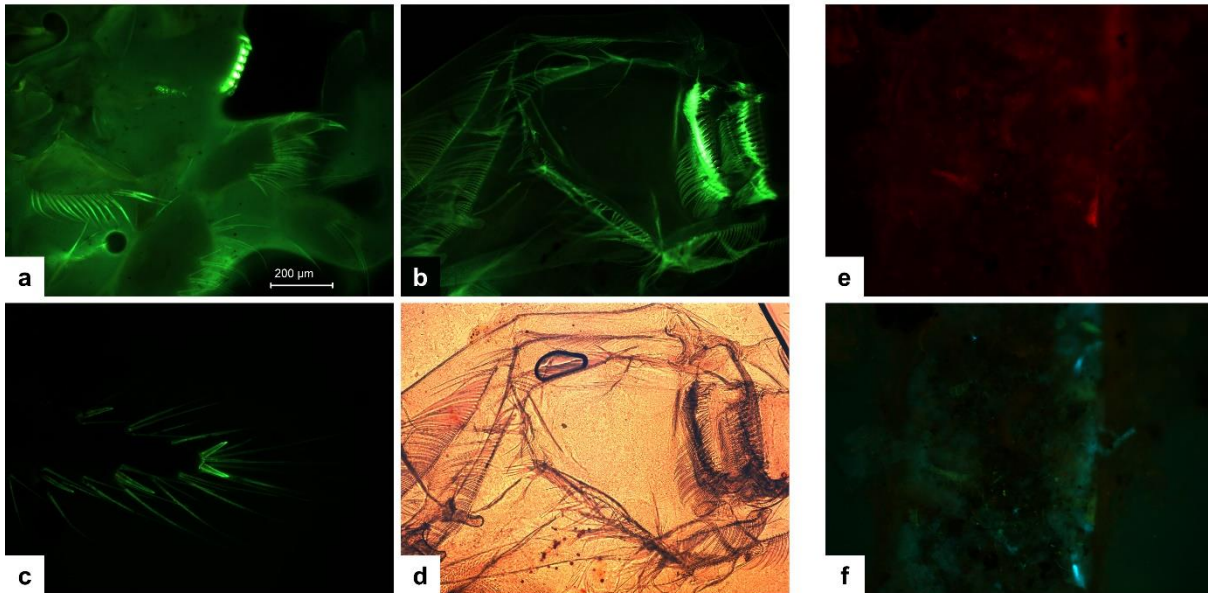
51 **Supplementary Figure S3:** Cracked and fragmented plastic particles from *G. duebeni* guts at 10x, 40x or 100x
52 magnification under fluorescence and/or light microscopy; **(a)** microplastics in midgut-hindgut under UV
53 fluorescence at 10x, **(b)** microplastics in foregut under light microscopy at 10x, **(c)** cracked microplastics under light
54 microscopy at 40x, **(d)** flat microplastics under light microscopy at 40x, **(e)** small-irregular microplastics under light
55 microscopy at 100x, **(f)** cracked microplastics under UV fluorescence at 40x, **(g)** flat microplastics under UV
56 fluorescence at 40x and **(h)** small-irregular microplastics under UV fluorescence at 100x.
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Supplementary Figure S4. Scanning electron microscopy (SEM) images of (a) microplastic stock in Tween solution as used in bioassays, (b) microplastic stock as dry powder provided by the supplier, (c) microplastics frozen at -80°C for seven days in distilled water and (d) microplastics frozen at -80°C for seven days in Tween solution.

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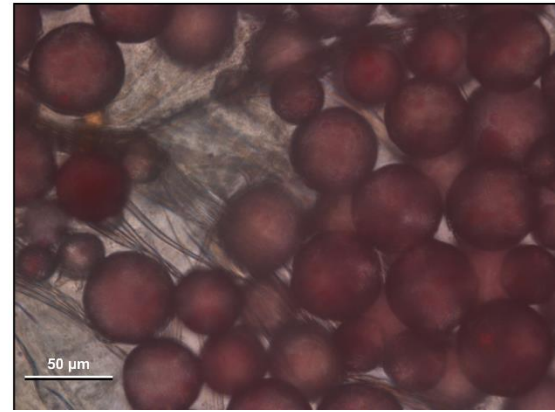


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Supplementary Figure S5. Microscope images showing the biological autofluorescence of control *G. duebeni* exoskeleton or mandible tissues (a,b,c) under fluorescence green light or brightfield (d) and *G. duebeni* gut tissues (e,f) showing biological autofluorescence under green light (e) or UV light (f). All images show tissues from control individuals, not exposed to microplastics or fluorescence dyes.



File	Edit	Font	Results				
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1	3.021	66.606	4.910	93.979	76.612	45	29.499



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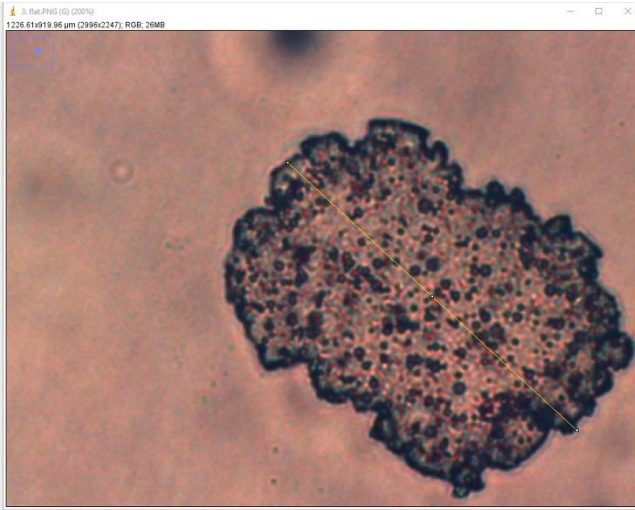


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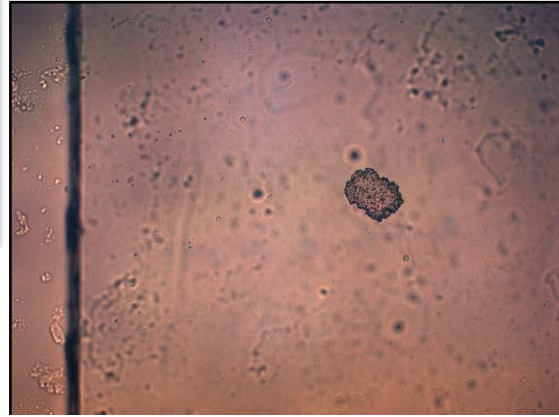


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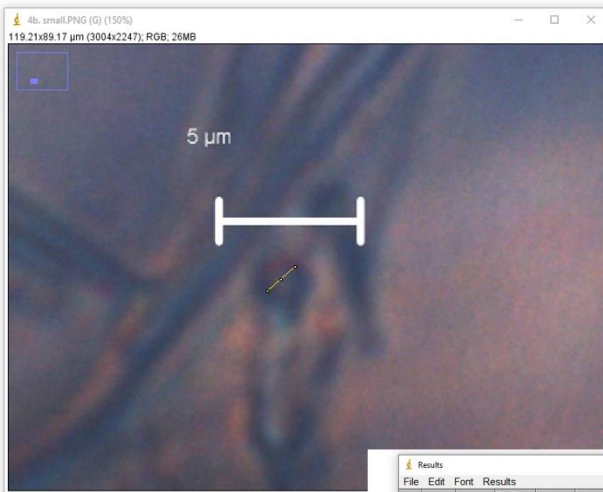


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1	52.298	97.529	30.696	30.931	163.902	-42.782	127.186

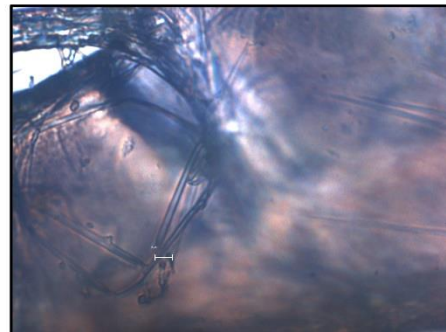
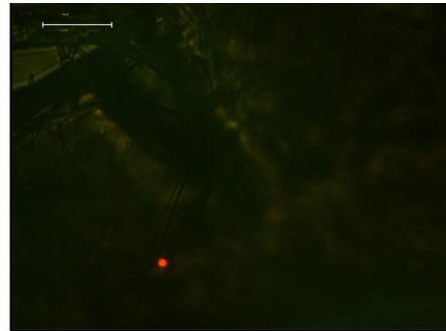


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Area	Mean	StdDev	Min	Max	Angle	Length	
1	0.050	83.369	5.447	74.281	93.336	42.397	1.236



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d

Supplementary Figure S6. ImageJ measurements of (a) an intact microsphere, (b) a cracked fragment, (c) a flat fragment and (d) a small fragment. Longest length/diameter was measured.