Science Advances
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Supplementary Materials for

Environmental exposure enhances the internalization of microplastic particles into cells

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Published 9 December 2020, *Sci. Adv.* **6**, eabd1211 (2020)
DOI: 10.1126/sciadv.abd1211

This PDF file includes:

Supplementary Figures and Tables

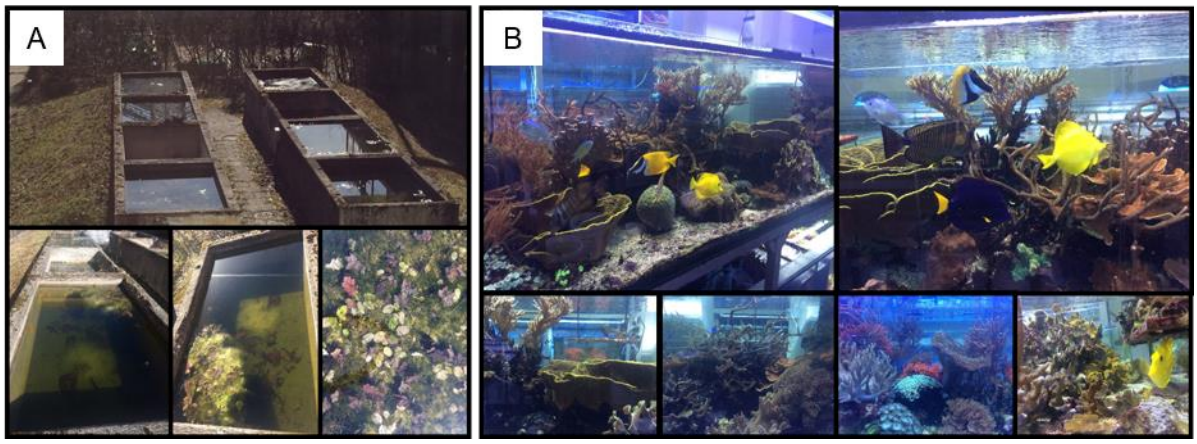

Fig. S1. Images of the sampling sites for the incubation waters. A) Freshwater was obtained from an artificial pond and B) saltwater was obtained from a marine aquaria facility, both inhabiting a rich floral and faunal population. Photo Credit: Anja F.R.M. Ramsperger, University of Bayreuth.

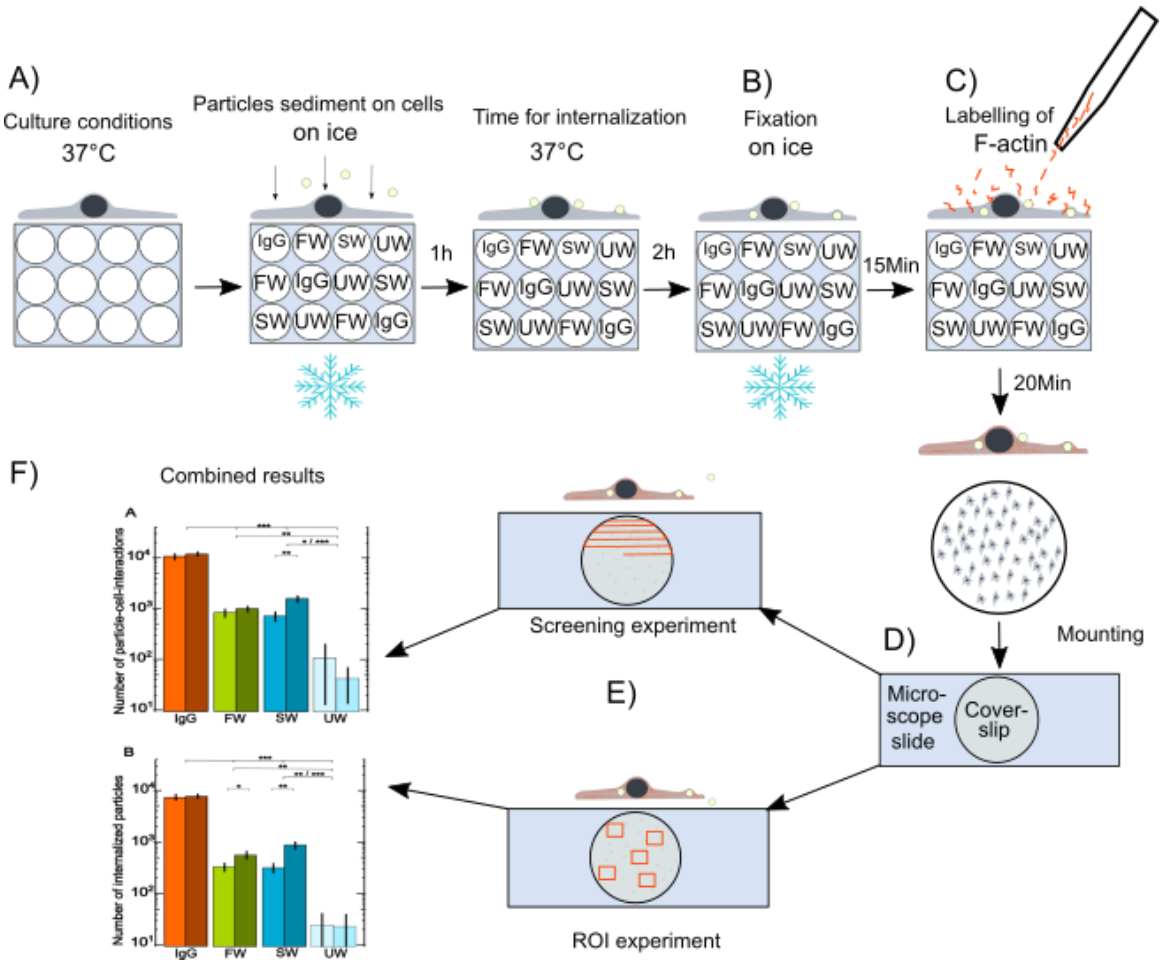

Fig. S2. Scheme of experimental procedure for the cell experiments. A) Cells were cultured under standard culture conditions and then transferred on ice to pause cellular activity. Corresponding treatments were randomly added to coverslips within a well plate and microplastic particles sedimented onto coverslips for one hour. Afterwards, the well plates were transferred to 37 °C culture condition to allow internalization of microplastic particles for all cells at the same time. B) After two hours of internalization time, coverslips were washed with PBS buffer to remove unattached microplastic particles and cells were fixed with 4 % paraformaldehyde for 15 minutes on ice to stop cellular activity for all cells simultaneously. C) Coverslips were again washed and filamentous actin was fluorescently labelled within 20 minutes at room temperature. D) Finally, coverslips were mounted on glass objective holders and stored at 4 °C until microscopic analysis. E) From each coverslip region of interest (ROI) experiments were conducted to evaluate the number of particle–cell-interactions and to calculate the area covered with cells on a whole coverslip using maximum intensity projected images of confocal stacks. On the same coverslip, single cells were observed to evaluate the number of internalized microplastic particles using single differential interference contrast images and confocal stacks of fluorescently labelled cells (screening experiment). F) Results were combined to compare treatments.

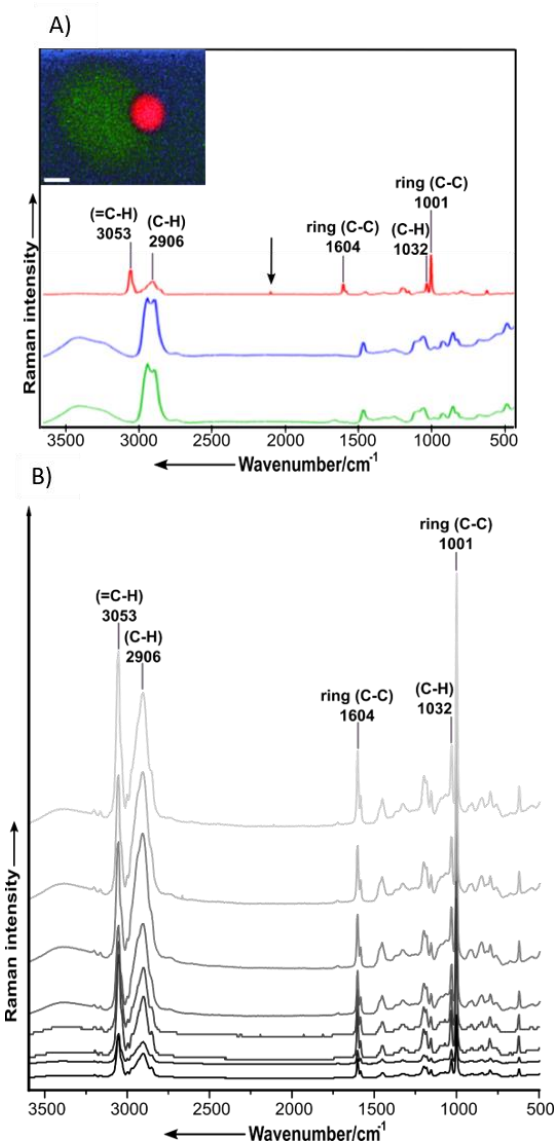

Fig. S3. Raman spectral analysis of a particle and a cell to investigate whether the particle is one of the utilized polystyrene microplastic particles. A) False color Raman image of a cell-particle-interaction (in this case, the particle was exposed to freshwater, scale bar: 2 µm). Colors from Raman image match colors in corresponding spectra. The red spectrum shows peaks specific for polystyrene, the blue spectrum shows peaks corresponding to the mounting media and the green spectrum shows peaks corresponding to cells. Arrow in the red spectrum highlights specific peak for thiocyanate, which is a possible component of the eco-corona on microplastic particles exposed to freshwater as the same peak was found in the eco-corona analysis (Fig. 4 main text). B) Each spectrum represents a mean spectrum acquired from ten particles measured from each of the eight treatments (IgG, freshwater, saltwater and ultrapure water two and four weeks, respectively). All spectra have been vector normalized and offset for the ease of representation and show Raman signatures specific to polystyrene.

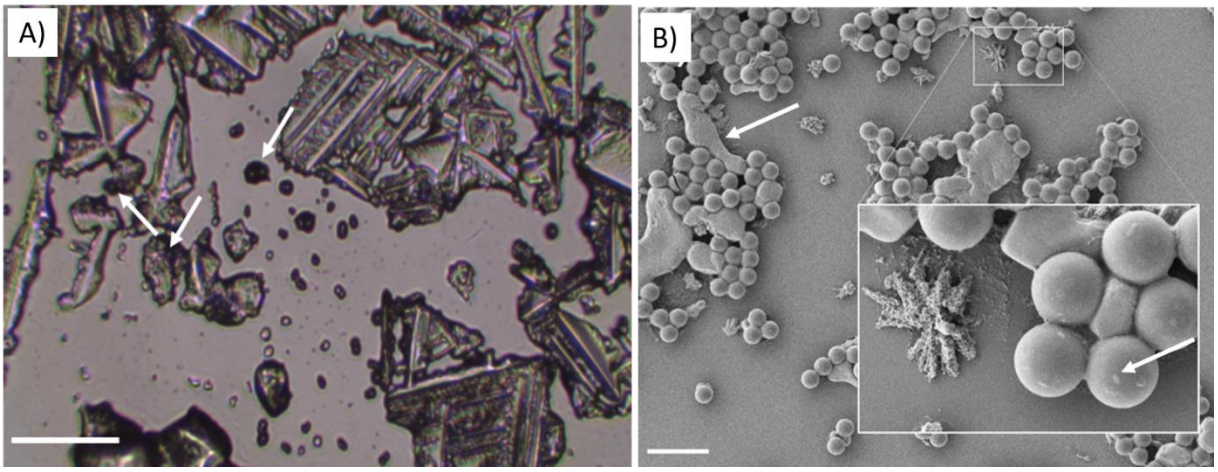

Fig. S4. Bright field image and SEM-image of microplastic particles exposed to saltwater. (A) Bright field image with arrows highlighting the position of microplastic particles which are embedded within salt crystals; scale bar: 100 µm. (B) SEM image with magnified area shows that microplastic particles are embedded in larger salt crystals (arrow) and show circular layers on microplastic particles in magnified view (arrow); scale bar: 10 µm.

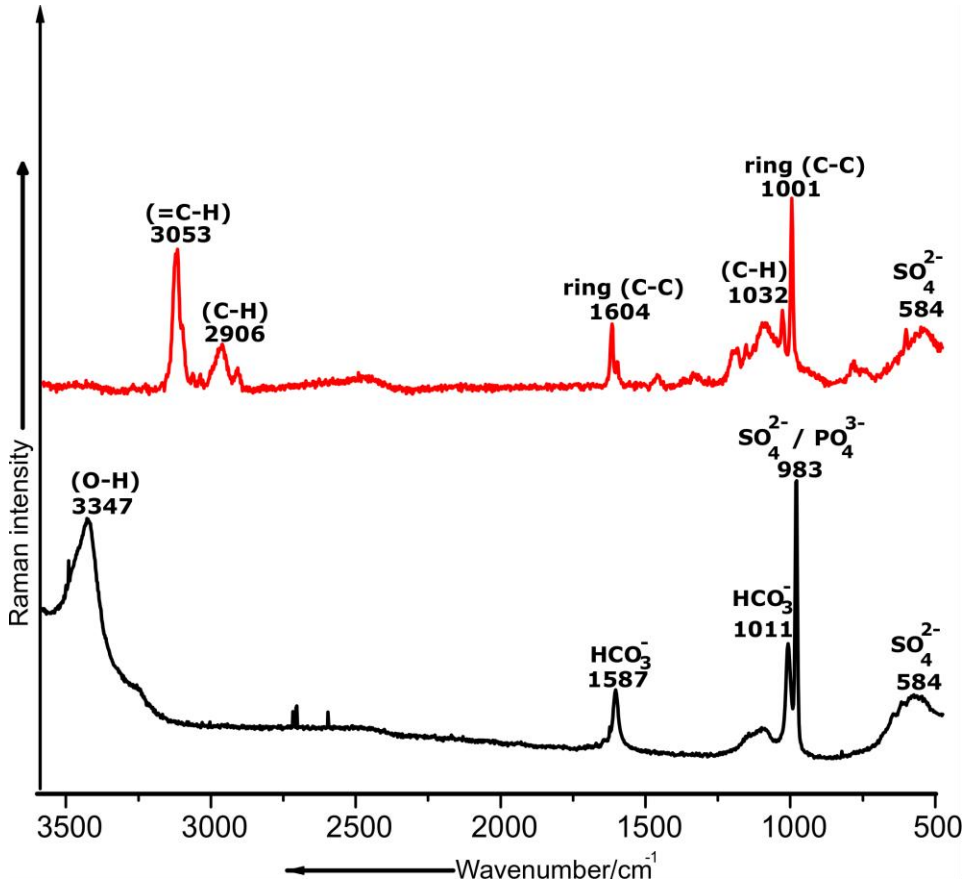

Fig. S5. Raman spectrum of microplastic particles exposed to saltwater (red) and spectrum of the saltwater itself (black). Spectral signatures such as =C-H stretching mode (3053 cm^{-1}), the C-H bending mode (2906 cm^{-1}), the ring C-C skeletal stretching mode (1604 cm^{-1}), the C-H bending mode (1032 cm^{-1}) and C-C ring stretching mode (1001 cm^{-1}) correspond to the polystyrene microplastic particle from saltwater in the red spectrum. Likewise, signatures corresponding to the salts such as sulphates, bicarbonate and phosphates are observed in the black spectrum. A comparison of the two spectra indicates that the Raman signatures of PS microplastic particles incubated in saltwater are likely to have contribution from the sulphates, bicarbonate and phosphates-based salts whose Raman bands appear to coincide with the bands of the PS microplastic particle.

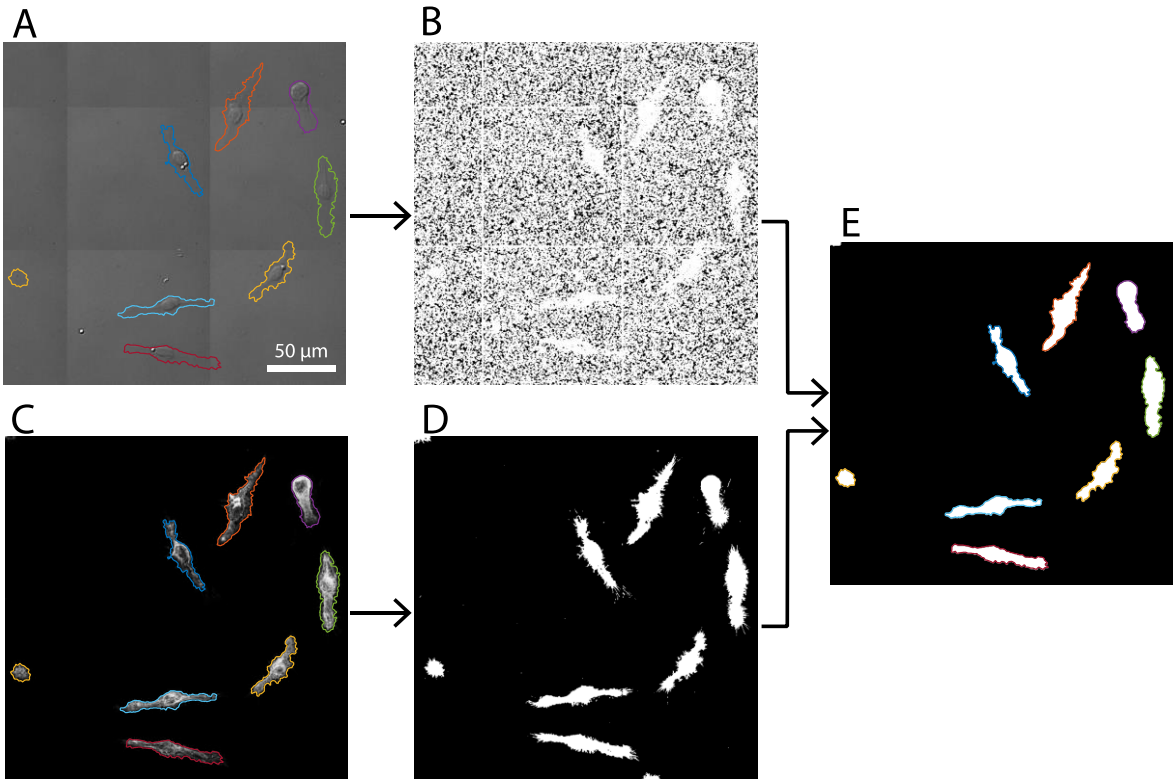

Fig. S6. Illustration of the cell detection algorithm used in the ROI- experiments. A local contrast filter was applied to DIC images (A) to achieve a rough mask of the regions covered by the macrophages (B). To refine the mask, we applied a threshold to the fluorescence images (C) to get a mask depicting the locations of the cells in the fluorescence channel. Both masks were combined by an 'AND'-operation, small, remaining objects were removed and the resulting mask was filtered by a gaussian filter with a size of 3 px to achieve a smooth and robust final cell mask (E). The outlines shown in (A) and (C) are identical to the outlines of the final mask (E). The algorithm was implemented in Matlab 2017b (The MathWorks, Inc.).

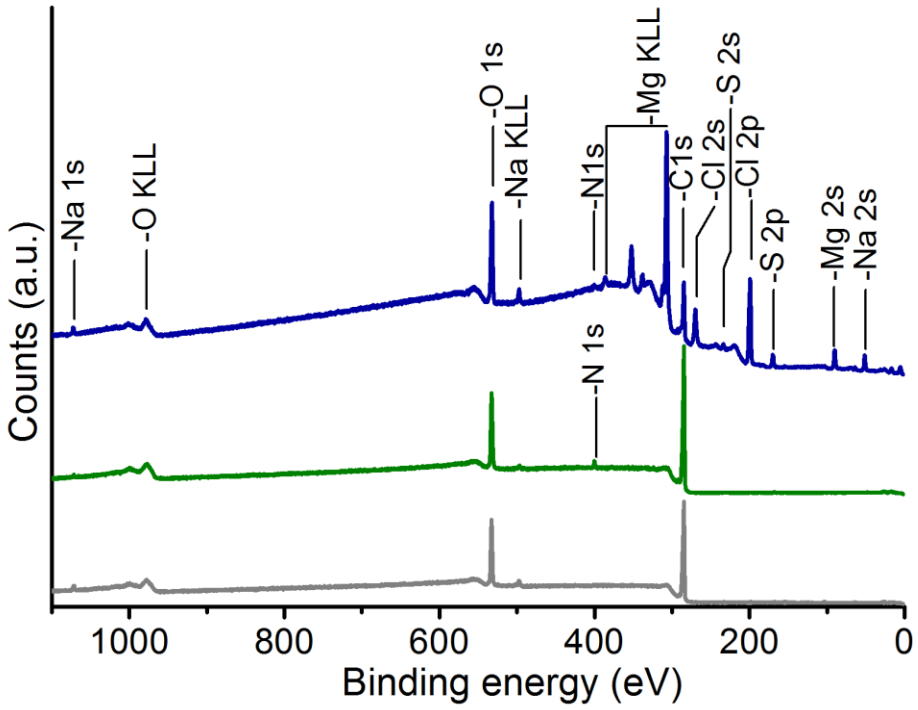

Fig. S7. XPS survey spectra of microplastic particles incubated in different water samples. The survey spectra of dried ultrapure water (gray), fresh- (green) and saltwater (blue) samples showing prominent signals correspond to carbon (C 1s, 284.8 eV), oxygen (O 1s and O KLL, ca. 532. 5 eV and 979.1 eV, respectively), nitrogen (N 1s, 400.1 eV), sodium (Na 1s and 2s, ca. 1072 and 51.5 eV, respectively), magnesium (Mg 2s and Mg KLL, 90.1 eV and ca. 307-385 eV), chlorine (Cl 2p and 2s, 199.1 eV and 270.1 eV) and sulphur (S 2p, 169.7 eV) elements.

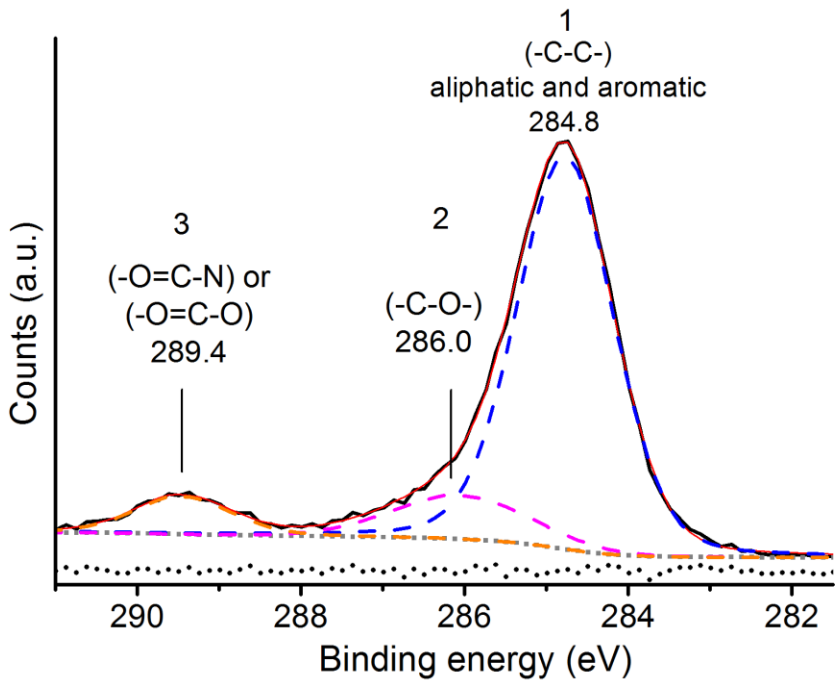

Fig. S8. The deconvolved C 1s core level spectrum of microplastic particles incubated in saltwater. Color code: original spectrum (black); composite spectrum (red); fitted band 1 (blue dashed line) with the peak maximum at 284.8 eV, corresponding to the carbon from –$C_{\text{aliphatic and aromatic}}$ units; fitted band 2 (magenta dashed line) with the peak maximum at 286.0 eV corresponds to the carbon from a -C-O- group; fitted band 3 (magenta dashed line) with the peak maximum at 289.4 eV (orange dotted line) attributed to the carbon from either -O=C-N- or -O=C-O- groups; background (gray dashed line) and fit residual (black dotted line).

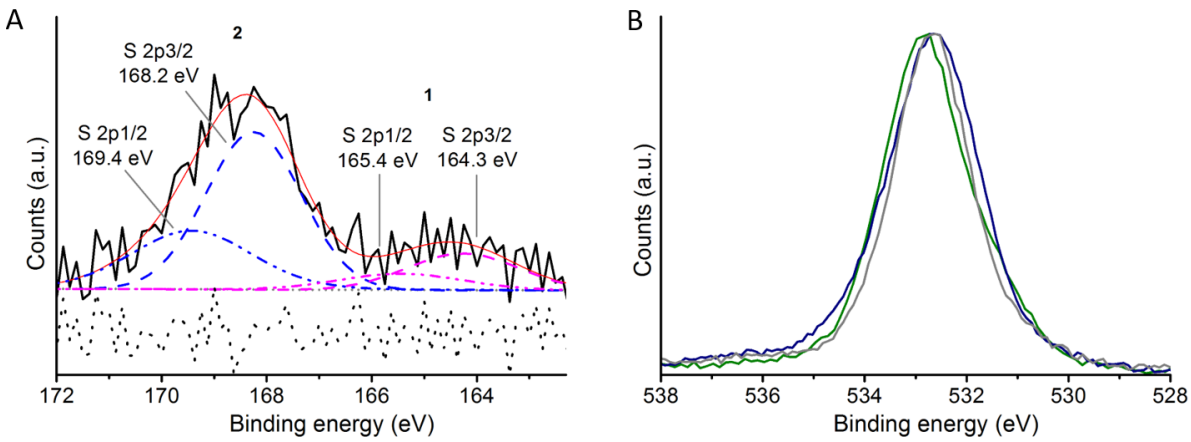

Fig. S9. The deconvolved S 2p and O 1s core level spectra of microplastic particles incubated in freshwater. (A) The deconvolved S 2p spectrum of microplastic particles incubated in freshwater. Color code: original spectrum (black); composite spectrum (red); band 1 – multiplet splitting of the S 2p band centered at 164.5 eV to S 2p3/2 (164.3 eV) and S 2p1/2 (165.4 eV), corresponding to the sulphur from thiophenic, thiols and thioether functional groups; band 2 - multiplet splitting of the S 2p band centered at 168.5 eV to S 2p3/2 (168.2 eV) and S 2p1/2 (169.4 eV), originating from the sulphate groups (*42*). (B) O 1s core level spectra of microplastic particles incubated in ultrapure water (gray), fresh- (green) and saltwater (blue) centered at ca. 532.5 eV with the full-width-half-maximum (FWHM) of 1.74 eV, 2.04 eV and 2.1 eV, respectively. In line with the indication for the presence of –C-O-, -O=C-N- and sulphate functional groups on microplastic particles incubated in fresh- and saltwater, their O1s core level peaks FWHM (ca. 2.0 eV) is slightly higher than that of the microplastic particles incubated in ultrapure water (1.74 eV). In addition, the O/C atomic ratio calculated from the area under the corresponding O 1s and C 1s peaks increases from microplastic particles incubated in ultrapure water (0.17) to freshwater (0.18) and saltwater(0.75), which corroborates the highly functionalized surfaces of microplastic particles incubated in fresh- and saltwater.

Table S1. Summary of the mean numbers of particle-cell-interactions ("PCI") and the numbers of microplastic particles internalized by cells ("Internalized") for all treatments and incubation times and the corresponding standard errors of mean ("s.e."). Significant differences (Kruskal-Wallis rank sum test) are highlighted in grey. IgG = IgG-opsonized, FW = freshwater-, SW = saltwater-, UW= ultrapure water-exposed microplastic particles, respectively.

Category	Incubation time [weeks]	N per treatment	Treatment	Mean	± se	Kruskal- Wallis rank sum test		
						Chi-squared	Df	p
PCI	2	10	IgG	10,504.36	1,494.40	30.606	3	$1.029e^{-06}$
			FW	829.14	155.23			
			SW	710.57	155.49			
			UW	106.66	93.89			
PCI	4	10	IgG	11,872.38	1,261.52	34.429	3	$1.608e^{-07}$
			FW	992.45	134.47			
			SW	1,555.79	244.01			
			UW	41.36	27.90			
Internalized	2	10	IgG	7,457.40	1,046.95	32.128	3	$4.919e^{-07}$
			FW	329.60	63.48			
			SW	319.68	65.19			
			UW	24.77	17.25			
Internalized	4	10	IgG	7,803.31	849.56	34.043	3	$1.94e^{-07}$
			FW	566.13	101.30			
			SW	876.88	150.36			
			UW	20.19	20.19			

Table S2: Summary statistical analysis for differences between treatments and incubation time. Subsequent to the non-parametric Kruskal Wallis test to investigate differences between all tested treatments a Games-Howell-post hoc test was conducted. The Games-Howell post hoc test analyzes each treatment and incubation time combination (e.g. IgG 2 weeks vs. FW 2 weeks, IgG 2 weeks vs. SW 2 weeks, etc.) to compare the differences in the particle-cell-interactions (PCI) and number of internalized particles. For pairwise comparisons of the incubation times within one treatment a non-parametric Mann-Whitney U test was performed. Grey color highlights statistically significant values. IgG= IgG-opsonized, FW = freshwater, SW = saltwater, UW= ultrapure water.

Category	Incubation time [weeks]	Groups tested		Mean difference	s.e.	t	df	p	Upper limit	Lower limit	Mann-Whitney U			
											Groups tested [weeks]		W	P
PCI	2	IgG	FW	-9,575.21	1,062.39	6.44	9.19	0.001	-5,004.93	-14,345.50	IgG 2	IgG 4	41	0.528
			SW	-9,793.79	1,062,33	6.52	9.19	0.000	-5,123.56	-14,464.02				
			UW	-10,397.69	1,058,78	6.94	9.07	0.000	-5,730.70	-15,064.69				
		FW	SW	-118.58	154,86	0.54	18.00	0.948	500.404	-737.56	FW 2	FW 4	40	0.481
			UW	-722.48	128,28	3.98	14.807	0.006	-198.81	-1,246.15				
		SW	UW	-603.90	127,83	3.34	14.850	0.021	-92.24	-1,125.57				
PCI	4	IgG	FW	-10,879.93	897.08	8.58	9.2	0.000	-6,937.19	-14,822.66	SW 2	SW 4	15	0.007
			SW	-10,316.59	908.56	8.03	9.67	0.000	-6,361.40	-14,271.78				
			UW	-11,831.02	892.25	9.38	9.01	0.000	-7,892.62	-15,769.41				
		FW	SW	563.34	197.01	2.02	14.01	0.226	1,373.10	-246.43	UW 2	UW 4	50	1
			UW	-951.09	97.11	6.93	9.77	0.000	-529.16	-1,373.02				
		SW	UW	-1,514.43	173.67	6.17	9.24	0.001	-751.66	-2,277.20				
Internalized	2	IgG	FW	-7,127.91	741.66	6.80	9.07	0.000	-3,858.27	-10,397.32	IgG 2	IgG 4	40	0.481
			SW	-7,137.71	741.74	6.80	9.07	0.000	-3,868.12	-10,407.30				
			UW	-7,432.63	740.41	7.10	9.01	0.000	-4,164.18	-10,701.08				
		FW	SW	-9.92	64.34	0.11	17.99	1.000	247.25	-267.10	FW 2	FW 4	23	0.043
			UW	-304.84	46.51	4.63	10.32	0.004	-104.74	-504.95				
		SW	UW	-294.92	47.68	4.37	10.26	0.006	-89.54	-500.30				
Internalized	4	IgG	FW	-7,237.17	604.99	8.48	9.26	0.000	-4,581.14	-9,893.20	SW 2	SW 4	12	0.003
			SW	-6,926.42	610.07	8.03	9.56	0.000	-4,264.96	-9,587.89				
			UW	-7,783.11	600.90	9.16	9.01	0.000	-5,130.81	-10,435.42				
		FW	SW	310.75	128.20	1.71	15.77	0.349	830.26	-208.76	UW 2	UW 4	54	0.670
			UW	-545.94	73.04	5.29	9.71	0.002	-228.25	-863.63				
		SW	UW	-856.69	107.27	5.65	9.33	0.001	-386.41	-1,326.96				