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A multilevel risk assessment framework for nanoplastics in aquatic ecosystems

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Table S1: Listing of research works focused on the effects of nanoplastics on **freshwater** biota, where the target was to assess or deliver median lethal and sublethal effective concentrations (**LC₅₀** and **EC₅₀**). For comparison purposes, from those same articles also non-observed effective concentrations (**NOEC**), lowest observed effective concentrations (**LOEC**), or other effective concentrations causing *X*% of effect (**LC_x** or **EC_x**) were also enlisted. Other abbreviations are as follows: AFM – Atomic Force Microscopy; CAT – catalase; Chl a – chlorophyll a; d – days; DLS – Dynamic Light Scattering; ELS – Electrophoretic Light Scattering; EPS – exopolysaccharides; FTIR– Fourier transform infrared spectroscopy; GSH–glutathione; h – hours; hpf – hours post-fertilization; H₂O₂ – hydrogen peroxide; LDPE– low-density polyethylene; LPO – lipid peroxidation; MDA – malondialdehyde; (N)SW – natural seawater; PDI – polydispersity index; PLA–polylactic acid; PMMA – polymethylmethacrylate; PP – polypropylene; PS – polystyrene; PS-COOH – polystyrene carboxyl-functionalized; PSII – photosystem II; PS-NH₂ – polystyrene amino-functionalized; ROS – reactive oxygen species; SEM – scanning electron microscopy; SOD – superoxide dismutase; TEM – transmission electron microscopy; ZP – zeta potential.

Species Filo/Order	Polymer (type, size, and shape) Origin	Characterization (technique, matrix, concentration, and time point)	Concentration range mg/L	Age/Animal		Exposure duration	Assessed endpoints	L(E) ₅₀ mg/L	NOEC, LOEC, other mg/L
				Developmental stage					
				Replicates/ Orgs/replicate					
<i>Chlamydomonas reinhardtii</i> Chlorophyta Chlamydomonadales	PS 102 nm Spherical Commercial	DLS, ZP Culture medium 0h, no indication of the concentration	0, 50, 250, 500	Exponential phase culture 3 replicates 1 x 10 ⁶ cells/mL/rep	growth	96h	Growth rate Chl a EPS Antioxidant enzymes	300.35	NOEC, LOEC (Chl a) = 50 and 250 NOEC, LOEC (EPS) = 250 and 500 LOEC (SOD, CAT): 50 LOEC (MDA, membrane permeability) = 50 and 250
<i>Chlorella vulgaris</i> Chlorophyta Chlorellales	PS 50 and 70 nm Spherical Commercial	SEM, FTIR, DLS, PdI BG-11 culture medium 3, 24, 72h, concentration of 10 mg/L	0, 0.5, 2.5, 5, 10, 20, 50	Exponential phase culture 3 replicates 1 x 10 ⁶ cells/mL/rep	growth	72h	Growth rate Chl a Oxidative stress Esterase activity Cell morphology Membrane integrity	19.89 (growth rate, 50 nm) 17.52 (Chl a, 50 nm)	NOEC, LOEC (growth rate, 50 nm, 70 nm) = 2.5 and 5 NOEC, LOEC (Chl a, 50 nm, 70 nm) = 5 and 10 NOEC, LOEC (ROS, 50 nm) = 5 and 10 NOEC, LOEC (ROS, 70 nm) = 2.5 and 5 NOEC, LOEC (CAT, 50 nm) = 5 and 10 NOEC, LOEC (CAT, 70 nm) = 2.5 and 5 NOEC, LOEC (MDA, 50 nm, 70 nm) = 5 and 10

<i>Cylindrotheca closterium</i>	PS (carboxyl) 49 nm	DLS, ELS, AFM	1.5, 2.5, 3.5, 5 (PS-NH ₂)	Exponential phase culture	growth	72h 14d	Growth rate ROS	3.88 (PS-NH ₂) 185 (PS-COOH)	LOEC (growth, PS-NH ₂) = 1.5
Bacillariophyta Bacillariales	PS (amino) 51 nm	MiliQ and natural seawater	10, 50, 100, 200 (PS-COOH)	2 replicates 10 ⁵ cells/mL/rep			MDA		LOEC (growth, PS-COOH) = 10
	Spherical	0 and 24h, concentration of 50 µg/mL							
	Commercial								
<i>Synechococcus elongatus</i>	PS (amino) 50 nm	TEM, DLS	0, 2.5, 5, 7, 9	Exponential phase culture	growth	48h	Growth rate Antioxidant enzymes	3.81 (growth rate)	LOEC (ROS, PS-NH ₂) = 2.5
Cyanobacteria Synechococcales	Commercial	Deionized water, BG- 11 culture medium		5 replicates initial optical density (λ=730) of 0.03/rep			Membrane integrity		(membrane permeability, PS-NH ₂) = 2.5
	Spherical	48h, no indication of the concentration							
<i>Anabaena</i> sp.	PS	DLS, ZP	0, 1 to 200	Exponential phase culture	growth	72h	Growth rate	64.4 (growth rate)	EC₁₀ , EC₉₀ = 46.12 and
Cyanobacteria	24 nm		(no dilution factor disclosed)				Chl a	59.35 (Chl a)	81.26
Nostocales		Culture medium					Phycocyanin	59.47 (Phycocyanin)	EC₁₀ , EC₉₀ = 40.51 and
	Spherical			3 replicates					75.71
		0h, concentration of		Not disclosed					EC₁₀ , EC₉₀ = 40.03 and
	Commercial	100 mg/L							76.53
<i>Anabaena</i> sp.	PS	DLS, FTIR, ZP	0, 12.5, 25, 50,	Exponential phase culture	growth	24h	Bioluminescence	58.3	LOEC = 12.5
Cyanobacteria Nostocales	30 nm		100, 200						

	Spherical	MiliQ water and culture media		3 replicates					
	Commercial			Not disclosed					
		0h, concentration of 200 mg/L							
<i>Spirodela polyrhiza</i> (Tracheophytes)	PS	DLS, FTIR, ZP	0, 12.5, 25, 50, 100, 200	Turions	72h	Growth rate	170		NOEC, LOEC = 50 and 100
Alismatales	30 nm								
	Spherical	MiliQ water and culture media		3 replicates					
				1 turion/rep					
	Commercial	0h, concentration of 200 mg/L							
<i>Daphnia magna</i>	PMMA	TEM, DLS	0, 0.01, 0.1, 1, 500, 1000	< 24h-old	48h	Mortality	1550 (24h)		NOEC (24h) = 524
Arthropoda	(hydrophobic)						879 (48h)		NOEC (48h) =524
Branchiopoda	86 nm			4 replicates					
				5 org/rep					
	Spherical								
	Commercial								
<i>D. magna</i>	PS	TEM	0, 2.5, 5, 8.5, 11, 14.5	< 24h-old	48h	Mortality	15.13		
Arthropoda	50 nm								
Branchiopoda				4 replicates					
	Spherical			5 org/rep					
	Commercial								

<i>D. magna</i>	PS	Not disclosed	0, 1, 5, 10, 20,	< 24h-old	48h	Mortality	5	
Arthropoda	100 nm		50, 75					
Branchiopoda				4 replicates				
	Spherical			5 org/rep				
	Commercial							
<i>D. magna</i>	PS	DLS, Pdl	0, 10, 20, 30, 40,	< 24h-old	24h	Mortality	86.31 (mortality)	
Arthropoda	100 nm		50, 60, 70, 80,			Internalization		
Branchiopoda		Deionized water and	90, 100, 125,	3 replicates				
	Spherical	Elendt M4 medium	150, 200	12 org/rep				
	Commercial	0 and 24h, 50 µg/mL						
<i>D. magna</i>	PS (amino)	DLS, ZP	0, 5, 10, 15, 20,	< 24h-old	48h	Immobilization	~9 (100 nm)	
Arthropoda	20, 40, 60, and		25, 30				~3 (60 nm)	
Branchiopoda	100 nm	MiliQ water and		4 replicates			~1 (40 nm)	
		culture medium		5 org/rep			~1 (20 nm)	
	Spherical							
	Commercial	0h, concentration of						
		50 mg/L						
<i>D. magna</i>	PS	TEM, ZP, Pdl	0, 15.6, 31.2	< 24h-old	48h	Mortality	244 (mortality)	NOEC, LOEC (swimmed
Arthropoda	73 nm		62.5 125, 250,			ROS		distance) = 62.5 and 125
Branchiopoda		MiliQ water and	500	4 replicates	21d	Generational		NOEC, LOEC (ROS) = 31.2
	Spherical	culture media		5 org/rep		exposure		and 62.5

	Commercial	0h, concentration of 50 mg/L		10 replicates 1 org/rep		Swimming behaviour		
<i>D. magna</i>	PS	DLS, ZP, FTIR	0, 12.5, 25, 50, 100, 200	< 24h-old	48h	Immobilization	32.4	NOEC, LOEC = 50 and 100
Arthropoda	30 nm							
Branchiopoda		MiliQ water and Spherical culture media		4 replicates 5 org/rep				
	Commercial	0h, concentration of 200 mg/L						
<i>D. magna</i>	PS (amino)	SEM, DLS, ZP	0, 5, 10, 15, 20	< 24h-old	48h	Mortality	12.36 (100 nm)	
Arthropoda	20, 40, 60, and					Uptake and	7.62 (60 nm)	
Branchiopoda	100 nm	MiliQ water, Evian bottled water, natural		4 replicates 5 org/rep		adsorption	2.45 (40 nm) 2.45 (20 nm)	
	Spherical	lake water						
	Commercial	0 h, 0.5, 1, 2, 4, 8, 24, 48 h, concentration of 20 mg/L						
<i>Daphnia pulex</i>	PS	DLS, FTIR	0, 10, 50, 100, 150, 200, 400	< 24h-old	48h	Mortality	76.69 (mortality)	LOEC (body length, 21d) = 0.1
Arthropoda	75 nm							
Branchiopoda		MiliQ water		5 replicates		Reproduction		LOEC (total offspring/female, 21d) = 0.1
	Spherical		0, 0.1, 0.5, 1, 2	5 org/rep		Biochemistry		
		Not disclosed						
	Commercial							NOEC, LOEC (growth rate, 21d) =1 and 2

								LOEC (n° broods, 21d) = 0.1
<i>D. pulex</i>	PS	TEM, DLS, ZP	0, 10, 50, 100,	newly-born (1 day)	48h	Immobilization	80 (1d-old)	
Arthropoda	75 nm		150, 200, 400	juveniles (4 days)			110 (4d-old)	
Branchiopoda		Freshwater		mature adults (7 days)			105 (7d-old)	
	Spherical			reproductive adults (14 days)			140 (14d-old)	
		0h, concentration not disclosed		senescent adults (21 d)			85 (21d-old)	
	Commercial							
				5 replicates				
				5 org/rep				
<i>Daphnia longispina</i>	PS	SEM, DLS	0, 0.01, 0.10, 1,	Juveniles (5-d-old)	48h	Mortality	2.49 (50 nm)	EC₁₀ (48h, 50 nm) = 0.28
	50 and 100 nm		2, 10, 20, 100				59.39 (100 nm)	EC₁₀ (48h, 100 nm) = 47.5
Arthropoda		Z8 and FW04 culture		4 replicates			(average of 3 clones)	(average of 3 clones)
Branchiopoda	Spherical	media, distilled water		5 org/rep				
	Commercial	0h, concentration of 10 mg/mL						
<i>Macrobrachium nipponense</i>	PS	TEM, DLS, ZP	0, 235, 372, 469,	Juveniles	96h	Mortality and moulting	396.4 (mortality)	LOEC (moulting) = 235
	75 nm		545, 606					LOEC (SOD, 28d) = 5
Arthropoda				3 replicates				NOEC, LOEC (CAT, 28d) =
Decapoda	Spherical		0, 5, 10, 20, 40	10 org/rep				10 and 20
					28d	Biochemical analysis		LOEC (GSH, 28d) = 5
	Commercial			3 replicates				NOEC, LOEC (H ₂ O ₂ content, 28d) = 5 and 10
				40 org/rep				

								LOEC (MDA, 28d) = 5
								LOEC (LPO, 28d) = 5
<i>Corophium volutator</i>	PMMA (hydrophobic)	TEM, DLS	0, 0.01, 0.1, 1, 500 mg/l	Adults	10d	Mortality		NOEC (mortality) = 500
Arthropoda	86 nm			5 replicates		Reburial		NOEC (reburial) = 500
Amphipoda				20 org/rep				
	Spherical							
	Synthesized by the research group							
<i>Gammarus fossarum</i>	PS (amino) 20, 40, 60, and 100 nm	SEM, DLS, ZP	0, 5, 10, 15, 20	< 24h-old	48h	Mortality	13.86 (100 nm)	
Arthropoda						Uptake	and 16.35 (60 nm)	
Amphipoda		MiliQ water, Evian bottled water, natural lake water		4 replicates		absorption	4.07 (40 nm)	
				5 org/rep			14.24 (20 nm)	
	Spherical							
	Commercial	0 h, 0.5, 1, 2, 4, 8, 24, 48 h, concentration of 20 mg/L						
<i>Hydra viridissima</i>	PMMA	DLS	0, 1, 5, 10, 20,	Hydras without buds	96h	Mortality	84 (mortality)	EC ₂₀ (mortality) = 76.5
Cnidaria	40 nm		40, 80, 10, 320,			Regeneration	52.3 (regeneration)	EC ₂₀ (regeneration) = 8.40
Anthoathecata		MiliQ water and	640	6 replicates		Post-exposure		LC ₁₀ (mortality) = 73
	Spherical	culture media		1 org/rep		feeding		LC ₁₀ (regeneration) = 3.23

	Synthesized by the research group	0, 24, 96h for 1, 10, 40 mg/L						NOEC, LOEC (mortality) = 80 and 160 NOEC, LOEC (morphology) = 40 and 80
<i>H. viridissima</i>	PLA	DLS, ZP	0, 0.001, 0.01, 0.1, 1, 10, 100	Hydras without buds	96h	Mortality	109 (LDPE, mortality)	LC₂₀ (LDPE) = 92.1
Cnidaria	78 nm					Regeneration	2.40 (LDPE, feeding)	NOEC, LOEC (mortality, malformations, LDPE) = 10 and 100
Anthoathecata	LDPE 91 nm	MiliQ water and culture medium		6 replicates		Post-exposure feeding		NOEC, LOEC (regeneration, LDPE) = 10 and 100
	Irregularly shaped	0, 24, 48, 72, 96h, concentration of 10 mg/L		1 org/rep				
	Mechanical grinding (lab)							
<i>Hydra attenuata</i>	PS	DLS	0, 1.25, 2.5, 5, 10, 20, 40, 80	Hydras without buds	96h	Mortality	3.6 (morphology, 50 nm)	NOEC, LOEC (lipids, 50 nm) = 2.5 and 5
Cnidaria	50 and 100 nm					Morphological alterations	18.4 (morphology, 100 nm)	NOEC, LOEC (lipids, 100 nm) = 5 and 10
Anthoathecata	Spherical	MiliQ, culture media		3 replicates		Biomass	14 (biomass, 50 nm) 28 (biomass, 100 nm)	LOEC (oxidative stress, 50 nm) = 1.25 NOEC, LOEC (oxidative stress, 100 nm) = 1.25 and 2.5
	Commercial	0h, concentration of 500 mg/L		3 hydras/rep				
<i>Danio rerio</i>	PS	SEM, DLS, ZP	0, 100, 200, 400	24 hpf	72h	Mortality	431.1 (mortality)	LOEC (heart rate) = 100
Cypriniforms	106 nm					Hatching		

Chordata		Deionized water	3 replicates			Body length	NOEC, LOEC (body length)
	Spherical		100 embryos/rep			Behaviour	= 200 and 400
		Not disclosed				Biochemistry	NOEC, LOEC (SOD) =200 and 400
	Commercial						LOEC (CAT) = 100
<i>D. rerio</i>	PLA	DLS, ZP	0, 0.001, 0.01,	3 hpf	96h	Mortality	NOEC, LOEC (heart rate,
Cypriniforms	78 nm		0.1, 1, 10, 100			Heartbeat rate	PLA) = 0.1 and 1
Chordata		MiliQ water, carbon-	10 replicates		120h	Swimming	NOEC, LOEC (swimming
	LDPE	filtered water	1 embryo/rep		(swimming		speed, PLA) = 0.1 and 1
	91 nm				behaviour)		NOEC, LOEC (swimming
		0, 24, 48, 72, 96h,					distance, PLA) = 0.01 and
	Irregularly shaped	concentration of 10 mg/L					0.1
	Mechanical grinding (lab)						NOEC, LOEC (swimming time, PLA) = 0.01 and 0.1
							LOEC (swimming speed, LDPE) = 0.001
							NOEC, LOEC (swimming distance, LDPE) = 0.01 and 0.1
							NOEC, LOEC (swimming time, LDPE) = 0.01 and 0.1

Table S2: Listing of research works focused on the effects of nanoplastics on **marine and/or estuarine biota**, in which the target was to assess or deliver median lethal and sublethal effective concentrations (**LC₅₀**, **EC₅₀**). For comparison purposes, from those same articles also non-observed effective concentrations (**NOEC**), lowest observed effective concentrations (**LOEC**), or other effective concentrations causing X% of effect (**LC_x** or **EC_x**) were also enlisted. Other abbreviations are as follows: Chl a – chlorophyll a; d – days; DLS – dynamic light scattering; FTIR– Fourier transform infrared spectroscopy; F1 – generation 1; h–h; LPO – lipid peroxidation; (N)SW – natural seawater; PdI – polydispersity index; PMMA – polymethylmethacrylate; PP – polypropylene; PS – polystyrene; PS-COOH – polystyrene carboxyl-functionalized; PSII – photosystem II; PS-NH₂ – polystyrene amino-functionalized; ROS – reactive oxygen species; SEM – scanning electron microscopy; TEM – transmission electron microscopy; ZP – zeta potential.

Reference	Species Filo/Order	Polymer (type, size, and shape)	Characterization (technique, matrix, concentration, and time point)	Concentration range mg/L		Age/Animal Developmental stage		Exposure duration	Assessed endpoints	L(E) ₅₀ mg/L	NOEC, LOEC, other mg/L
		Origin				Replicates/ Orgs/replicate					
[21]	<i>Dunaliella tertiolecta</i>	PS	DLS, ZP, PdI	0, 0.5, 1, 5, 10,		Exponential growth		72h	Growth rate	12.97 (PS-NH ₂)	
	Chlorophyta	40 nm (carboxyl)		25, 50		phase culture					
	Chlamydomonadales	50 nm (amino)	MiliQ water, (N)SW								
						3 replicates					
		Spherical	0h, concentrations of 50 and 100 mg/ml			7 x 10 ⁵ cells/mL/rep					
[22]	<i>Tetraselmis chuii</i>	PMMA	SEM, DLS	0, 150.0,		Exponential growth		96h	Growth rate	132.5	EC₂₀ = 117.4 LOEC = 150
	Chlorophyta	40 nm		168.8, 189.8,		phase culture					
	Chlorodendrales		MiliQ water, SW	213.6, 240.3,							
		Spherical		270.3, 304.1		3 replicates					
			0h, concentration of 75 mg/L			1 x 10 ⁵ cells/mL/rep					

Synthetized by the research group											
[23]	<i>Rhodomonas baltica</i> Cryptophyta Pyrenomonadales	PMMA (plain, carboxyl) 50 nm Spherical Commercial	DLS, ZP, Pdl MiliQ water, ISO 14669 medium 72h, concentration of 50 µg/mL	0, 0.5, 1, 5, 10, 25, 50, 100	Exponential growth phase culture 3 replicates 10 x 10 ³ cells/mL/rep	72h	Cell number Chl a Cell size, complexity, and viability Cell metabolic activity ROS, LPO Mitochondrial membrane potential	78.5 (PS-COOH)	LOEC (growth, PMMA) = 0.5 NOEC, LOEC (growth, PMMA-COOH) = 0.5 and 1 NOEC, LOEC (Chl a, PMMA) = 1 and 5 LOEC (Chl a, PMMA-COOH) = 0.5 NOEC, LOEC (cell size, PMMA) = 1 and 5 NOEC, LOEC (cell size, PMMA-COOH) = 0.5 and 1		
[22]	<i>Nannochloropsis gaditana</i> Gyrista Eustigmatales	PMMA 40 nm Spherical	SEM, DLS MiliQ water, SW 0h, concentration of Synthetized by the research group	0, 150.0, 168.8, 189.8, 213.6, 240.3, 270.3, 304.1	Exponential growth phase culture 3 replicates 1 x 10 ⁵ cells/mL/rep	96h	Growth rate	116.5	LOEC = 150		

[22]	<i>Isochrysis galbana</i> (Haptophyta) Isochrysidales	PMMA 40 nm Spherical	SEM, DLS MiliQ water, SW 0h, concentration of 75 mg/L	0, 150.0, 168.8, 189.8, 213.6, 240.3, 270.3, 304.1	Exponential growth phase culture 3 replicates 1 x 10 ⁵ cells/mL/rep	96h	Growth rate	123.8	NOEC, LOEC = 213.6 and 240.3 EC₂₀ = 106.3
[22]	<i>Thalassiosira weissflogii</i> (Heterokonta) Thalassiosirales	PMMA 40 nm Spherical	SEM, DLS MiliQ water, SW 0h, concentration of Synthetized by the research group	0, 75.0, 94.1, 118.1, 148.3, 186.1, 233.5, 293.0	Exponential growth phase culture 3 replicates 1 x 10 ⁵ cells/mL/rep	96h	Growth rate	83.4	LOEC = 75 EC₂₀ = 48.9
[24]	<i>Phaeodactylum tricornutum</i> (Heterokonta) Bacillariales	PS 50 and 100 nm Spherical	SEM, TEM, FTIR, Pdl, DLS MiliQ water, (N)SW Commercial 0, 1, 3, 24, and 72h, concentration of 26 mg/L	0, 0.1, 1, 5, 10, 20, 50	Exponential growth phase culture 3 replicates 10 ⁵ cells/mL/rep	72h	Growth rate inhibition Cell complexity Cell size Chl a	1.1 and 1.3 (cell complexity, 50 and 100 nm) 44.9 and 11.5 (Chl a, 50 and 100 nm) 8.7 and 18.5 (cell size, 50 and 100 nm)	LOEC (growth rate, 50 and 100 nm) = 0.1 and 0.1 NOEC, LOEC (cell complexity, 50 nm) = 10 and 20 NOEC, LOEC (cell complexity, 100 nm) = 5 and 10 NOEC, LOEC (PSII, 50 nm) = 0.1 and 1 NOEC, LOEC (PSII, 100 nm) = 20 and 50

										32.6 and 3.8 (PSII, for 50 and 100 nm)
										31.9 and 5.5 (electron transport rate, for 50 and 100 nm)
[25]	Aliivibrio fischeri	PS	DLS, ZP	0, 0.00045,	Exponential growth	5, 15, and 30 min	Bioluminescence	0.217 (5 min exposure)		
	Pseudomonadota	50 nm		0.00091,	phase culture					
	Vibrionales		MiliQ, SW	0.0018,						
		Spherical		0.0036,	3 replicates			0.196 (15 min exposure)		
			2 and 12h,	0.0073,						
		Commercial	concentration of 10 µg/L	0.0145,				0.180 (30 min exposure)		
				0.0291,						
				0.0582, 0.116,						
				0.233, 0.466,						
				0.931, 1.862,						
				3.724						
[21]	Artemia franciscana	PS	DLS, ZP, Pdl	0, 0.1, 1	Newborns (instar I)	48h	Mortality	0.83 (PS-NH ₂ , 14 d, mortality)	NOEC, LOEC (gene expression) = 0.1 and 1	
	Arthropoda	40 nm (carboxyl)				14d	Gene expression			
	Anostraca	50 nm (amino)	MiliQ water, (N)SW		3 replicates					
					200 org/rep					
		Spherical	0h, concentration of 50 and 100 mg/ml of							
		Commercial	PS-COOH and PS-NH ₂ , respectively							

[26]	<i>Tigriopus japonicus</i>	PS	Not disclosed	0, 0.125, 1.25,	nauplii (<24 h old)	14d	Immobilization	2.15	(14d,	NOEC, LOEC = 0.125
	Arthropoda	50 nm		12.5, 25			Generational	mortality)		and 1.25
	Harpacticoida				3 replicates			0.16	(F1, 14d,	NOEC, LOEC (F1
		Spherical			10 org/rep			mortality)		mortality) = 0.125 and 1.25
Commercial										
[27]	<i>Brachionus plicatilis</i>	PS (carboxyl)	DLS, ZP, Pdl	0, 0.5, 1, 5, 10,	< 24h-old	48h	Mortality	13.04	(PS-NH ₂ ,	
	Rotifera	40 nm		25, 50				24h)		
	Ploima		MiliQ water, SW		3 replicates			6.62	(PS-NH ₂ , 48h)	
		PS (amino)			10 nauplii/rep					
		50 nm	48h, concentration of 10 µg/ml							
Spherical										
Commercial										
[22]	<i>Brachionus plicatilis</i>	PMMA	DLS, SEM	0, 4.7, 9.4,	neonates (<24h-old)	48h	Mortality	26.7	(average	LC₂₀ (average three
	Rotifera	40 nm		18.9, 37.5,				three sizes)		sizes) = 8.1
	Ploima		MiliQ water, SW	75.0	4 replicates					
		Spherical			5 org/rep					
			0h, concentration of							
Synthetized by the research group										
[28]	<i>Pinnigorgia flava</i>	PP	DLS, SEM	0, 0.001, 0.1,	3 cm, 2.5 g, average of	72h	Mortality			NOEC (all endpoints)
	Cnidaria	100 nm		1, 10	32 polyps/fragment		Polyp retraction			= 0.1
	Malacalcyonacea		Culture media				Mucus production			LOEC (all endpoints)
		Spherical			6 replicates		Tissue bleaching			= 1
					1 fragment/rep		Tissue swelling			

Synthetized by 25 min frame, the research concentration of 10 group mg/L										
[29]	<i>Aurelia</i> sp. Cnidaria Semaestomeae	PS 100 nm Spherical Commercial	Not disclosed	0, 0.1, 1, 10	Ephyrae 24h-old 3 replicates 8 ephyrae/rep	7d	Immobilization Behaviour (frequency of pulsation)	7.20 (pulsation)	NOEC, (pulsation) = 1 and 10	LOEC
[30]	<i>Paracentrotus lividus</i> Echinodermata Camarodonta	PS 40 nm (carboxyl) 50 nm (amino) Spherical Commercial	TEM, DLS, ZP SW	0, 2.5, 5, 10, 25, 50 (PS-COOH)	Embryos (30-min) 3 replicates 250 embryos/mL/rep	48h	Embryotoxicity	3.82 (PS-NH ₂ , 24h) 2.61 (PS-NH ₂ , 48h)		
[31]	<i>Mytilus galloprovincialis</i> Mollusca Mytilida	PS (amino) 50 nm Spherical Commercial	DLS, ZP, SEM, Pdl SW 0h, concentration of 50 µg/ml	0, 0.001, 0.01, 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5, 10, 20	fertilized eggs (30-48h min) 4 replicates 60 embryos/rep		Embryotoxicity	0.142 (D-shape larvae)	NOEC, LOEC = 0.001 and 0.01	
[32]	<i>Crassostrea gigas</i> Mollusca Ostreida	PS (plain, carboxyl, and amine-modified) 50 nm	DLS, ZP, Pdl MiliQ water, SW	0, 0.1, 1, 10, 25	adult males and females 5 replicates 1000 oocytes/mL, 100: 1	1.5h	Gamete assay/fertilization	4.9 (gamete assay, PS-NH ₂)	NOEC, (fertilization, all PS = 0.1 and 1 NOEC, LOEC (larval assay, PS, PS-COOH)	LOEC

			0 and 24h, concentration of 100 µg/mL		spermatozoa:oocyte ratio	36h	Embryo-larval assay	0.15 (embryo-larval assay, PS-NH ₂) = 0.1	= 1 and 10 LOEC	
		Commercial			Embryos 5 replicates 60 embryos/rep	24h	Metamorphosis assay			
					pediveliger larvae 6 replicates 10 larvae/rep					
[33]	<i>Ciona robusta</i>	PS (amino)	DLS, ZP, Pdl	0, 5, 25, 100	Two-cell stage (1-hour	22h (until free	Embryotoxicity	7.52 (morphology,	NOEC,	LOEC
	Chordata	50 nm		(PS-COOH)	post-fertilization)	swimming	Swimming	PS-NH ₂)	(morphology,	PS-
	Phlebobranchia		MiliQ water, (N)SW			larva stage)	behaviour		NH ₂) = 5 and 7.5	
		PS (carboxyl)		0, 2, 5, 7.5, 10,	6 replicates		Neurotoxicity		NOEC,	LOEC
		60 nm	0, 6, and 22h, concentration of 20	15 (PS-NH ₂)	10 embryos/rep		ROS		(Abnormalities, PS-NH ₂) = 7.5 and 10	
		Spherical	µg/ml						NOEC, LOEC	(ROS, PS-NH ₂) = 7.5 and 10
		Commercial								

Table S3: Resumes of studies, site locations, and sampled matrices in freshwater environments reporting nanoplastic concentrations. Owing to the difficulty of gathering information on environmental concentrations, the upper size limit defined here for NPLs was 450 nm. Abbreviations: LOD, limit of detection; PE, polyethylene; PET - Polyethylene terephthalate; PMMA, polymethylmethacrylate; PP, polypropylene; PS, polystyrene; PVC, polyvinyl chloride.

Location	Matrice	Polymer type (when information available)	Size range nm	Concentration range (if available by polymer) (µg/L)	Reference
China, Fuhe river	surface and ground waters	PP	< 200	0.283–0.793	[34]
		PE		0.088–0.242	
		PET		0.011–0.079	
		PS		0.007 – 0.058	
		PVC		0.006 – 0.057	
		PMMA		0 – 0.046	
Denmark, Greenland	sea ice cores	PE	< 200	6.5 (average)	[35]
		PET		2.7	
		PP		0.57	
		PS		0.11	
		PVC		0.11	
Sweden, Stora Bjurevatten	stream water	PVC	< 200	122	[36]
		PE		276	
		PET		61	
Russia, Kytalyk	central ice core, 14-m deep	PVC	< 200	109	[36]
		PS		15	
UK, Tawe River	superficial water samples	PS	< 450	241.8	[37]
		PP		Below LOD	
		PVC		Below LOD	
Austria, Sonnblick	surface snow	PET	< 200	15.1	[38]
		PP		29.5	
		PE		25	
		PS		5	
Tahiland, Chi river	water samples	PS	20 < x < 450	189 - 488	[39]
		PVC			
		PET			
		PE			
		PP			

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