

Supplementary information

Search method and the hit score system

The research articles included in this review were identified through an extensive literature search utilizing multiple databases, including Web of Science, Google Scholar, PubMed, and Scopus. The search was conducted using specific keywords, namely “transcriptomics + nanoplastics,” “proteomics + nanoplastics,” and “metabolomics + nanoplastics.” The search results were subsequently and manually filtered based on species and publication year, with a specific focus on research articles on aquatic species published between 2013 and 2023. Following this, the search results underwent individual scrutiny to select studies that align with the scope of this review. Data extraction was carried out for each of the 37 Omics studies solely on aquatic species, comprising 22 transcriptomic studies, six proteomic studies, and nine metabolomic studies. Detailed data were systematically tabulated to provide an analytical summary of the species investigated, the type, size, and concentration of nanoplastics utilized, the primary toxicity pathways examined, as well as the underlying mechanisms of toxicity (Tables S2, S4, and S6). The hit score system operates akin to a counting metric. Each time a disrupted pathway is identified, it is tallied as the first hit and subsequently for all reported pathways under a specific nanoplastic treatment condition (Tables S1, S3, S5, and S7).

Table S1 Omics hit score system for affected pathways of aquatic nanoplastics toxicity.

Affected pathways	Transcriptomics	Metabolomics	Proteomics	Total
Common				
Oxidative stress	5		4	9
Lipid metabolism	4	4	1	9
Energy metabolism	2	1	4	7
Carbon metabolism	2	1	3	6
Metabolism	5			5
Metabolism of xenobiotics by cytochrome P450	5			5
amino acid metabolisms	2	3		5
Photosynthesis	2		3	5
Citrate cycle (TCA cycle).	1	3	1	5
Fatty acid metabolism	1	4		5
Oxidative phosphorylation	2		2	4
Purine metabolism	1	3		4
ABC transporters	3			3
Amino acid synthesis & proline metabolism	3			3
Lysosome pathway	3			3
Cardiac Development & Hypertrophy	2		1	3
Glutathione metabolism	2		1	3
Aminoacyl-tRNA biosynthesis	2			2
Aminoacyl-tRNA ligases	2			2
antioxidant activity	2			2
Cell cycle dysfunction	2			2
DNA repair and growth arrest	2			2
Drug metabolism	2			2
Iron ion binding	2			2
Nervous system development	2			2
NOD-like receptor signaling pathway	2			2
Ppara regulation	2			2
Starch and sucrose metabolism	2			2
Translation process	2			2
Protein synthesis	1	1		2
Omics specific				
Transcriptomics				
Apoptosis	1			1
Cancer-related pathways	1			1
Carnitine biosynthetic pathway.	1			1
DNA synthesis	1			1
Glycerolipids metabolism	1			1
Glycolipid metabolism	1			1

Glycolysis/gluconeogenesis	1		1
Glyoxylate and dicarboxylate metabolism	1		1
Gonad development	1		1
Heme binding	1		1
Hippo and wnt signaling pathways.	1		1
Homologous recombination	1		1
Lipid storage	1		1
Lipid synthesis	1		1
MAPK signaling pathway	1		1
neuroactive ligand-receptor interaction	1		1
Primary bile acid biosynthesis	1		1
Proteolysis	1		1
Pyruvate metabolism	1		1
Saccharide metabolism	1		1
Serotonergic synapse	1		1
Sphingolipid signaling	1		1
Steroid biosynthesis	1		1
Sugar metabolism and synthesis	1		1
Sulfur compound metabolism	1		1
Transcription	1		1
Unsaturated fatty acid biosynthesis	1		1
Vitellogenesis	1		1
Voltage-gated potassium channel complexes	1		1
Metabolomics			
Folate biosynthesis		1	1
Pentose phosphate metabolism		1	1
Taurine hypotaurine metabolism		1	1
Nicotinate nicotinamide metabolism		1	1
Proteomics			
Signal transduction			1 1
Protein metabolism			1 1
Carbohydrate metabolism			1 1
Nucleic acid metabolism			1 1
Calcium metabolism			1 1

Table S2 Summary of transcriptomic studies of nanoplastic toxicity in different aquatic species.

Animal	Sequencing technology	Type & dose	Exposure regime	Toxicological response	Reference
<i>Brachionus plicatilis</i>	Illumina sequencing	Polystyrene microbeads 10 mg/L with diameters of 0.05 and 6 µm.	Rotifer - 24 hrs	Metabolism deficiency and oxidative stress	(Shin and Jeong, 2022)
<i>Cherax quadricarinatus</i>	Illumina sequencing	Polystyrene, 0.025, 0.25, and 2.5 mg/L of NPs on red crayfish	Adult crayfish - 14 days	Antioxidant activity, metabolism, and immune defects	(Cheng et al., 2022)
<i>Danio rerio</i>	Illumina sequencing	0.02, 0.2 mg/L, 100 nm polystyrene nanoplastics (PS-NPs) on zebrafish intestinal cells	Adult zebrafish-21 days	Change in macrophages immunological recognition and apoptosis processes. Affects enterocytes & and alters oxidative stress and abnormality of lipid metabolism.	(Yu et al., 2022)
<i>Isognomon alatus</i>	Illumina sequencing	0.0075 and 0.015 mg/L NP-PS and NP-G (naturally collected marine NPs). NP-G is a mix of plastics, mainly polyethylene and polypropylene. Sizes are 260 nm for NP-PS and 280 nm for NP-G	Oyster - 7 days	Nanoplastics impaired mitochondrial metabolism, oxidative stress, and apoptotic response in oysters.	(Arini et al., 2022)
<i>Streptomyces coelicolor</i>	Illumina sequencing	Polystyrene (PS), M145 was treated with 20 nm, 100 nm, and 1 µm particles at the concentration of 10 mg/L	Gram +ve bacteria, 30 °C for 24 hrs.	Inhibit the transport capacity, primary metabolism, and oxidative phosphorylation of M145; the inhibition extension was negatively related to the particle size. The toxicity of microplastics to M145 was significantly less than that of nanoplastics.	(Liu et al., 2021a)
<i>Chlorella pyrenoidosa</i>	Illumina sequencing	Polystyrene nanoplastics (80 nm in size) 0, 5, 10, 20, 30, 40, and 50 mg/L	Freshwater green algae, 96 hrs	Blockage of the gene expression of aminoacyl tRNA synthetase and the synthesis of related enzymes and proteins. Affected DNA damage repair and hindered photosynthesis.	(Yang et al., 2021a)
<i>Procambarus clarkii</i>	Illumina sequencing	100 nm carboxylated polystyrene nanoparticles (PS NPs). 100 µg PS NPs in food supplement.	Adult red swamp crayfish. through diet in a 72 hrs acute toxicity test	Altered immune response, oxidative stress, gene transcription and translation, protein degradation, lipid metabolism, oxygen demand, and reproduction.	(Capanni et al., 2021)
<i>Karenia mikimotoi</i>	Illumina sequencing	micro/nanoplastics (MPs/NPs; polystyrene and polymethyl methacrylate), 10 mg/L, 65 nm, 100 nm, and 1 µm	Marine dinoflagellates, for 72 hrs	Toxicity response and metabolism	(Zhao et al., 2022)
<i>Oreochromis mossambicus</i>	Illumina sequencing	Polystyrene nanoparticles 100 nm, 20 mg/L.	larval tilapia, for seven days and then returned to freshwater	Abnormal metabolism of glycolipids, energy, and amino acids.	(Pang et al., 2021)

			without Polystyrene nanoparticles for another seven days	Induction of inflammation.	
<i>Sinonovacula constricta</i>	Illumina sequencing	0.005, 0.05, 0.5 and 50 mg/L of 75 nm polystyrene nanoplastics (PS-NPs).	Razor clam, 48 hrs		(Jiang and Zhang, 2021)
<i>Daphnia pulex</i>	Illumina sequencing	Polystyrene nanoplastic, 71 nm, 1 mg/L	96 hrs	Oxidative stress, immune defense, and glycometabolism.	(Liu et al., 2021b)
<i>Brachionus koreanus</i>	Illumina sequencing	Nano-sized microplastic (0.05 µm), 0.0001 mg/L and 0.001 mg/L	7 days, transgenerational effect	Cell cycle dysfunction, and ppara regulation	(Jeong et al., 2021)
<i>Symphysodon aequifasciatus</i>	Illumina sequencing	900 µm, fiber, MFs) or nanoplastics (~88 nm, bead, NPs) with three concentrations (0, 0.02 and 0.2 mg/L).	Juvenile discus fish, 96 hrs	Neuroactive ligand-receptor interaction and serotonergic synapse. Dopaminergic synapse pathway.	(Huang et al., 2022)
<i>Ictalurus punctatus</i>	Illumina sequencing	(0, 5, 10, 25, and 50 mg/L) of 75-nm polystyrene nanoplastics.	larval channel catfish, 24 hrs or 48 hrs	Protein digestion and absorption pathway. PPAR signaling pathway. Biosynthesis of unsaturated fatty acid. Glycolysis pathway Altered arachidonic acid metabolism pathway. Altered proteasome pathway. The pathway to synthesize acetoacetate from tyrosine was affected. Inhibition of xenobiotics metabolism.	(Jiang et al., 2022)
<i>Danio rerio</i>	Illumina sequencing	700 nm, polystyrene	Inject zebrafish larvae. Based on the biodistribution data, injection at the long-pec stage (2 dpf) was chosen for RNAseq.	Altered immune complement pathway.	(Veneman et al., 2017)
<i>Mytilus coruscus</i>	Illumina sequencing	100 nm, 20 mg/L, polystyrene	Adult mussel, 48 hrs.	Xenobiotics: cytochrome P450 exercised detoxification. Lysosome mediated phagocytosis and autophagy Immune signaling pathways represented innate defense mechanisms.	(Qi et al., 2023)
<i>Microcystis aeruginosa</i>	Illumina sequencing	50 nm, polystyrene nanoplastics	freshwater algae, 15-day exposure	Antioxidant defense system Photosynthesis	(Zhang et al., 2022)
<i>Danio rerio</i>	Illumina sequencing	Polystyrene, 200 nm. 100, and 1000 ppb concentrations were analyzed for transcriptomic effects	Zebrafish larvae, 120 dpf	Skeletal/muscular and nervous system development and function pathways. Dysregulated metabolic, cardiac, and hepatic pathways. Metabolic dysfunction. Epigenetic modification.	(Pedersen et al., 2020)





Table S4 Summary of proteomic studies of nanoplastic toxicity in different aquatic species.

Animal	Proteomics approach	Particle & dose	Exposure	Toxicological response	Reference
<i>Daphnia pulex</i>	HPLC fractionation and LC-MS/MS analysis	Spherical polystyrene nanoplastic, the average size of polystyrene is 71.18 nm	21 days	Induces oxidative stress, affects signaling transduction, energy production, protein and lipid metabolism, and cuticle and chitinase	(Liu et al., 2021b)
<i>Microcystis aeruginosa</i>	LC-MS/MS	50 nm amino-modified polystyrene nano-spheres (PS-NH ₂) particles	Acute (2 days) and long-term (10 days)	Impaired cell membrane integrity and inhibited photosystem efficiency	(Feng et al., 2020)
<i>Emiliania huxleyi</i>	nanoLC-ESI-MS/MS analysis	50 nm polystyrene nanoparticles (PS-NPs), 0.001% w/v	Phytoplankton, 72 hrs	Reduction in photosynthetic efficiency	(Dedman et al., 2022)
<i>Anabaena sp</i>	Pierce High pH Reversed-Phase Peptide Fractionation RP-LC-MS/MS	30 nm polystyrene nanoparticles (PS-NPs), 1 to 200 mg/L	Filamentous cyanobacteria, 72 hrs	Oxidative stress and photosynthetic activity deficiency	(Tamayo-Belda et al., 2021)
<i>Zebrafish</i>	Peptide purification by reversed-phase chromatography. High-resolution mass spectrometry analysis (nLC-MSMS)	500 nm polystyrene nanoparticles, 200 µg/L	Zebrafish larvae, 7 days	Nanoplastics impacted zebrafish behavior and swimming patterns. Dysregulated proteins are associated with genetic processes, immune response, cytoskeleton, and molecule transport.	(Parenti et al., 2021)
<i>Zebrafish</i>	nanoLC-MS/MS	50 nm Amine-functionalized polystyrene nanoplastics (PS-NH ₂ NPs), 0.1 mg/L	Zebrafish larvae exposed for 72 hrs	Induction of oxidative stress and the oxidative phosphorylation rate in the mitochondria	(Duan et al., 2023)

Table S5 Proteome hit score system for dysregulated pathways and mechanisms of nanoplastics toxicity in different aquatic species.

Altered pathways	<i>Daphnia pulex</i>	<i>Daphnia magna</i>	<i>Microcystis aeruginosa</i>	<i>Danio rerio</i>	<i>Danio rerio</i>	<i>Anabaena sp</i>	<i>Emiliana huxleyi</i>
Oxidative stress	■		■		■		
Carbohydrate metabolism	■		■				
Protein metabolism	■						
Energy metabolism					■		
Lipid metabolism	■						
ABC transporter			■				
Metabolism		■					
Photosynthesis			■			■	
Signaling transduction	■						
Genetic processes				■			
Cytoskeleton				■			
Organo-nitrogen metabolism						■	
Nucleic acid metabolism						■	
Cuticle and chitinase	■						
Surface location						■	
phototrophic metabolism							
Catalytic				■			

Table S6 Summary of metabolomic studies of nanoplastic toxicity in different aquatic species.

Animal	Metabolomics approach	Particle & dose	Exposure	Toxicological response	Reference
<i>Euglena gracili</i>	UHPLC-MS	Polystyrene-nanoplastics, 100 nm, 50 µg/L (1.1 × 10 ¹⁰ particles/L).	Fresh water algae, 96 hrs	Metabolic disruption of purine and carbohydrate	(Cao et al., 2022)
<i>Microcystis aeruginosa</i>	GC-MS	Polystyrene-nanoplastics, 30 nm with and without sulphonic acid modification, 10 mg/L, 50 mg/L, and 100 mg/L.	Fresh water cyanobacteria, 96 hrs	Fatty acid metabolism Oxidative stress.	(Wang et al., 2023)
<i>Phaeodactylum tricornutum</i>	GC-MS	Polystyrene-nanoplastics, 80-100 nm, 0.01–10 mg/L.	Diatom, 120 hrs	Fatty acid, amino acid, energy, and carbohydrate metabolism. Fatty acid biosynthesis Biosynthesis of phenylalanine, tyrosine, and tryptophan Antioxidant system	(Yao et al., 2023)
<i>Sinonovacula constricta</i>	UHPLC-TripleT OF	Polystyrene-nanoplastics, 75 nm, 0.005, 0.05, 0.5 and 50 mg/L.	Adult, 48 hrs	Purine metabolism.	(Jiang and Zhang, 2021)
<i>cyanobacterium Synechocystis</i>	UHPLC-MS	Polystyrene-nanoplastics, 0.05 µm, 50 mg/L.	Cyanobacteria, 96 hrs	Oxidative stress TCA cycle and glycerophospholipids metabolism	(You et al., 2021)
<i>Danio rerio</i>	UHPLC-MRM-MS/MS	Polystyrene-nanoplastics, 44 nm, 1, 10, and 100 µg/L.	Adult, 30 days	Altered neurotransmitter metabolites and gut microbiota.	(Teng et al., 2022)
<i>Oreochromis mossambicus</i>	UPLC-Q-TOF-MS	Polysyrene-nanoplastics, 100 nm, 20 mg/L.	Larvae, 7 days	Metabolism of glycolipids, energy, and amino acids.	(Pang et al., 2021)
<i>Danio rerio</i>	GC-TOF MS	Polystyrene-nanoplastics, 100 nm, 2 × 10 ⁴ items of n-PS suspended in 50 mL.	Larvae, 72 hrs	Biosynthesis of unsaturated fatty acid and linoleic acid metabolism, glutamate, taurine and hypotaurine Antioxidant system.	(Duan et al., 2020)
<i>Danio rerio</i>	LC-QTOF	Polystyrene-nanoplastics, 20 nm, 3 nL of polystyrene nanoplastic stock solution.	Larvae, 120 hpf	Oxidative stress Lipid accumulation	(Sulukan et al., 2022)
<i>Brachionus plicatilis</i>	UHPLC-Q	Microplastic/Nanoplastic (70 nm, 500 nm, and 2 µm), 0, 20, 200, and 2000 µg/L.	euryhaline rotifer, 48 hrs	Disruptions of purine-pyrimidine metabolism, TCA cycle, and protein synthesis.	(Li et al., 2023)

Table S7 Metabolome hit score system for dysregulated pathways and mechanisms of nanoplastics toxicity in different aquatic species.

Altered pathways	<i>Euglena gracili</i>	<i>Microcystis aeruginosa</i>	Diatoms	Zebrafish	Zebrafish	Tilapia	Razor clam	<i>Synechocystis</i>	<i>Brachionus plicatilis</i>
Lipid metabolism	■			■			■		
Fatty acid metabolism		■	■		■	■			
Amino acid metabolism	■		■		■				
Tricarboxylic acid cycle (TCA)		■						■	■
Purine-pyrimidine metabolism	■						■		■
Pentose phosphate metabolism				■					
Taurine and hypotaurine metabolism						■			
Nicotinate and nicotinamide metabolism						■			
Protein synthesis pathway									■
Carbohydrate metabolism	■								
Energy metabolism							■		
Folate biosynthesis					■				

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