fishes

MDPI

Review

Polystyrene Micro- and Nanoplastics (PS MNPs): A Review of Recent Advances in the Use of -Omics in PS MNP Toxicity Studies on Aquatic Organisms

Maria Concetta Eliso, Barbara Billè, Tiziana Cappello * and Maria Maisano

Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, 98166 Messina, Italy; maeliso@unime.it (M.C.E.); barbara.bille@studenti.unime.it (B.B.); mmaisano@unime.it (M.M.)

* Correspondence: tcappello@unime.it; Tel.: +39-090-6765741

Abstract: In recent years, micro- and nanoplastics (MNPs) have represented an emerging threat for the aquatic environment due to their persistence and widespread distribution. Indeed, their small size and increased surface area lead to a high biological reactivity, which can be crucial for the health status of biota. To date, several studies have investigated lethal and sublethal endpoints of MNPs, while one of the main challenges is to understand their mechanisms of toxicity in a comprehensive manner. With this aim, the field of aquatic ecotoxicology can now benefit from the -omics approaches. This review focuses on the recent advances related to the use of transcriptomics, proteomics, and metabolomics to deeply investigate the molecular and biochemical responses of aquatic organisms, both fishes and aquatic invertebrates, to pristine polystyrene (PS) MNPs. The literature reviewed revealed that transcriptomics and metabolomics are the most frequently used -omic approaches. Overall, the studies taken into consideration shed light on the events triggered by PS MNPs at molecular and cellular levels, identifying as mechanisms of toxicity the pathways involved in oxidative stress, energy metabolism, immune response, and the nervous system. Future studies should therefore focus on the use and integration of multi-omics approaches for a more comprehensive understanding of the mechanisms involved in MNPs toxicity.

Keywords: microplastics; nanoplastics; polystyrene; -omics approaches; transcriptomics; proteomics; metabolomics; mechanism of toxicity; fish; aquatic invertebrates

Key Contribution: The investigation of the toxicity mechanism of micro- and nanoplastics (MNPs) is a topic of major concern for the scientific community. The use of transcriptomics, proteomics, and metabolomics has suggested that the main pathways affected by polystyrene (PS) MNPs are related to energy metabolism, oxidative stress, immune response, and the nervous system, both in fishes and aquatic invertebrates. The analyses performed in this review offer a comprehensive overview of the molecular and biochemical responses triggered by PS MNPs, allowing the identification of some gaps and the importance in the use of multi-omic approaches in future studies.

check for updates

Citation: Eliso, M.C.; Billè, B.; Cappello, T.; Maisano, M. Polystyrene Micro- and Nanoplastics (PS MNPs): A Review of Recent Advances in the Use of -Omics in PS MNP Toxicity Studies on Aquatic Organisms. *Fishes* **2024**, *9*, 98. https://doi.org/10.3390/fishes9030098

Academic Editor: Shosaku Kashiwada

Received: 31 January 2024
Revised: 27 February 2024
Accepted: 2 March 2024
Published: 5 March 2024

1. Introduction

Plastic pollution is a fast-rising environmental threat. Due to plastics' low cost, durability, and flexibility, their use has increased worldwide, leading to an augmentation of their release into the marine environment. Most of the plastic debris found in the seas originates from land-based sources [1]. Once in the natural environment, plastic can be degraded into micro- (MPs, <5 mm) and nanoscale sizes (NPs, <1 µm) [2,3] by weathering, sunlight radiation, and biodegradation processes [4–8]. MPs and NPs (MNPs) can be also classified into primary and secondary based on their sources. Primary MNPs are those that enter the environment in their original small size associated with specific applications and consumer products (e.g., cosmetics, clothing fibers, drug delivery, ink for 3D printers), whilst

secondary MNPs are a consequence of macro/microplastics degradation [4,9–11]. The formation of smaller particles leads to alterations in the physical-chemical proprieties, surface area, and size of MNPs, wherein, once the nanoscale is reached, the strength, conductivity, and reactivity will differ substantially from those of macro-/micro-sized particles [12–15]. Obviously, as the size of the plastic particle decreases, biological reactivity, on the other hand, increases. Thus, it is crucial to comprehend the burden of MNPs' availability and their biological impact on aquatic biota [14,16].

Up to now, polystyrene (PS) ha sbeen chosen as a proxy for MNPs due to the fact that it is one of the most largely used non-biodegradable plastics worldwide and, unlike other polymers, it shows a greater stability in sea water suspension with low styrene release [17]. Several studies have been conducted to evaluate the lethal and sublethal effects of PS MNPs on aquatic biota, reporting fertility, growth, and reproduction abnormalities [18–28], as well as metabolism disorders, oxidative stress, and immune and nervous system dysfunction [13,29–36]. Consequently, one of the main challenges today is to understand the mechanism of the toxicity of MNPs correlated to the lethal/sublethal effects studied so far. With this aim, the aquatic ecotoxicology field can benefit significantly from using the -omics approaches, which are emerging systemic and holistic tools for the global identification of the processes and pathways involved in the normal and abnormal physiological states, that allow not only the study of the mode of action of chemicals, but also the prediction of their toxicological effects on a given biological system [37]. -Omics approaches permit the production of large-scale datasets, measuring simultaneously the changes in gene expressions, proteins, and metabolites (by application of transcriptomics, proteomics, and metabolomics, respectively) occurring at the molecular, cell, tissue, and whole-organism levels [38,39]. These approaches allow the characterization of complex signal pathways and correlation of gene/protein expression, rather than focusing on the modulation of individual genes/proteins. Among others, -omics technologies include: (i) transcriptomics, which is used to study the whole set of RNA transcripts and to identify general and specific transcript biomarkers as transcriptional consequences related to natural environmental factors or the mode of action of environmental pollutants in an organism [38,40]; (ii) proteomics, which is used to study the whole set of proteins in order to evaluate any alterations in their function and/or structure in an organism after changes in the environmental conditions [41]; and (iii) metabolomics, which is used to study the whole set of cell metabolites, and has been employed in the past several years with the purpose of unveiling the molecular and biochemical mechanisms underlying the response, sensitivity, tolerance, and adaptation of aquatic organisms to environmental challenges or pollution [42,43]. Transcriptomic studies dominated until 2016, whereas a shift towards proteomics, and mostly metabolomics, including multi-omics studies, is now apparent [44].

While many review articles have previously summarized the impact of PS MNPs on aquatic organisms, addressing the attention on their lethal/sublethal effects, few of them have exclusively focused on the studies dealing with -omics approaches. Therefore, the present review aims to discuss the latest findings from the various -omics studies for a better elucidation of the effects of pristine PS MNPs on aquatic organisms, both freshwater and marine fishes and invertebrates, as well as to provide a holistic understanding of the mechanism of toxicity of PS MNPs.

2. -Omics Approaches in Studies of PS MNP Toxicity

A survey of the available published peer-reviewed literature was conducted until September 2023, through a bibliographic study using Thompson Reuters database ISI Web of Science and Scopus database. A combination of keywords was used as criteria, including "omic*", "transcriptom*", "proteom*", "metabolom*", "polystyrene", "microplastic*", "nanoplastic*", "fish*", "aquatic AND invertebrates". After carefully reading all the abstracts of candidate publications, a total number of 56 articles was taken into consideration for this review, with exclusive selection of those studies related to the effects induced by exposure to pristine PS MNPs on fresh- and seawater fishes and invertebrates using

transcriptomics, proteomics, and metabolomics approaches. As shown in Figure 1, the number of transcriptomic studies remained more or less constant across the years, confirming it as the most widely used -omic approach. This is probably due to the fact that, up to now, the techniques for mRNA quantification and data interpretation are more developed, compared to proteomics or metabolomics. The studies taken into consideration in this review showed that proteomics is the least used approach, likely because of its limitations, which are mainly related to poor genomic annotation [45], while studies concerning the effects of PS MNPs evaluated by metabolomics have increased in recent years. In fact, today, metabolomics is a well-established scientific field in the systems biology, representing the most functional measure of an organism's physiology and response to toxic stress [43,46].

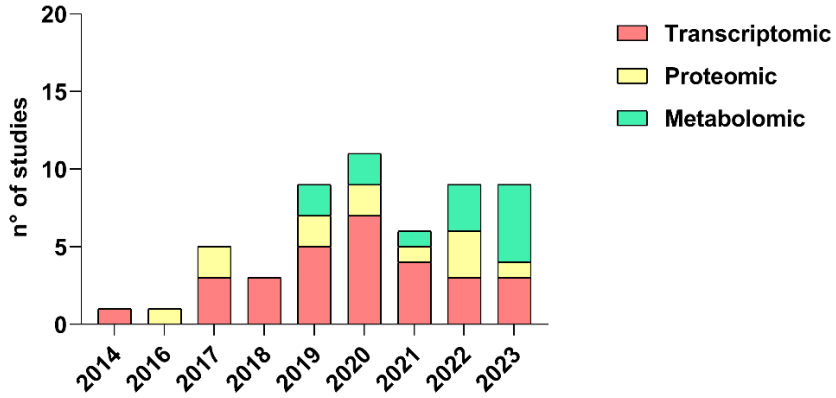

Figure 1. Number of transcriptomic, proteomic, and metabolomic studies performed in fishes (a total of 24 studies) and aquatic invertebrates (a total of 32 studies).

As showed in Figure 2, from the literature reviewed it was found that a total of 24 studies used -omics to investigate the effects of PS MNPs in fishes (45.83% transcriptomics, 8.33% proteomics, and 45.83% metabolomics), while the remaining part (a total of 32 studies) dealt with the impact of PS MNPs by applying the -omic approaches in aquatic invertebrates (56.25% transcriptomics, 31.25% proteomics, and 12.50% metabolomics). Interestingly, it was found that the aquatic organisms used for the investigation of PS MNP toxicity were more likely to be invertebrates than fishes (32 vs. 24 studies). This is probably due to the high sensitivity, inexpensive management, and ease of running experimental tests with invertebrates compared to vertebrates, besides the ethical rationale for animal welfare [47]. Moreover, transcriptomics and metabolomics appeared to be the approaches used mostly for fishes (a total of 11 out of 24 studies used transcriptomics and a total of 11 out of 24 studies used metabolomics in fish), while in the case of aquatic invertebrates, the most common -omic approach used was found to be the transcriptomics (a total of 18 out of 32 studies).

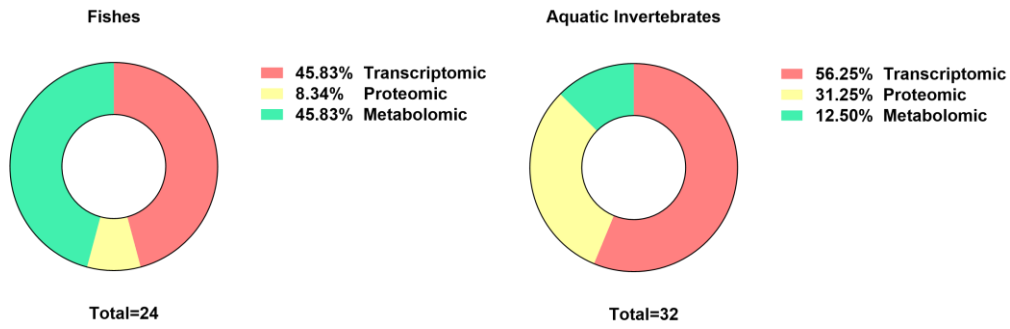

Figure 2. Percentage of transcriptomics, proteomics, and metabolomics studies performed in fishes (a total of 24 studies) and aquatic invertebrates (a total of 32 studies).

2.1. Transcriptomics

Over the past decades, transcriptomics has predominantly been applied for environmental risk assessment by evaluation of the health status of aquatic animals [48]. It determines the changes in gene expression by measuring the level of mRNA after studying the whole set of transcripts, also named the transcriptome, present in an organism. Indeed, the quantitative real-time polymerase chain reaction (qRT-PCR) is the simplest and most widely used technique to conduct a transcriptomic analysis. Although the relative expression of selected genes is easy to undertake, as the amount of the gene studied is compared to the amount of a control reference gene, qRT-PCR can quantify only a limited number of genes, with the requirement for prior knowledge of target genes. To cope with these limitations and to target thousands of single mRNAs in a single run, microarrays and RNA-sequencing (RNA-seq) have therefore been used lately. In particular, the last mentioned technique uses high-throughput sequencing methodologies to detect the presence and quantity of RNA in a biological sample with the aim of analyzing the whole cellular transcriptome. In brief, the method consists of isolating total RNAs from biological samples and then performing its reverse transcription to obtain double-stranded cDNA. After that, cDNAs are sequenced as short reads, aligned, and mapped against a known genomic reference sequence. In recent years, RNA-seq has been successfully used to assess differential responses in a variety of aquatic species since it is effectively able to analyse whole transcriptomes, generating data on more differentially expressed genes (DEGs), which, through bioinformatics, will give information about the major pathways affected following a stress condition [44]. A description of the effects of PS MNPs at the transcription level in fishes and aquatic invertebrates is reported in Table 1.

2.1.1. Effects of PS MNPs on the Transcriptome of Fishes

As reported in Figure 2, 45.83% of the selected studies concern the use of transcriptomics to investigate the mechanism of toxicity of PS MNPs in fishes. Many of these studies used the zebrafish *Danio rerio* since it has many advantages as a model organism, such as small size, ex utero development of the embryo, short reproductive cycle, and transparent embryos. Moreover, its genome is very well characterized and it shares a high degree of homology with the human genome [72]. Several studies performed on zebrafish embryos showed that PS MNPs (see Table 1) can alter the expression of genes involved in the stress response and cellular detoxification, apoptotic genes, immune response, and genes involved in neurotransmission, leading to developmental delay, hatching problems, and impaired swimming behaviour [35,49–51]. The same model organism was used to analyze the full transcriptome of embryos exposed to PS NPs (50–200 nm, 100–1000 ppb). Also in this case, results suggested alterations in the nervous system development and function pathways at both high and low concentrations, coinciding with an altered behavioral response, as evidenced through swimming hyperactivity, thus resulting in consequences for the organism's fitness [54]. The alterations in similar pathways have also been found in marine medaka larvae (*Oryzias melastigma*). In particular, the transcriptional expression levels of inflammatory response genes decreased after the exposure to 0.02 mg/L in each of the the three tested PS NPs, namely plain PS, carboxylated PS (PS-COOH), and aminated PS ($PS\text{-}NH_2$). Moreover, $PS\text{-}NH_2$ could lower the detoxification capacity of the larvae, while the downregulation of ATP-ase in PS-COOH might induce a disorder in osmoregulation, heart development, and nervous system function in the larvae [57]. Additionally, the transcriptome analysis of *O. melastigma* larvae exposed to PS MNPs of different sizes (0.05, 0.50, and 6.00 µm) showed a significative enrichment in pathways involved in immune and inflammatory responses, while the results obtained from mRNA expression levels showed upregulation of genes involved in heart formation and hatching, underlying developmental defects as observed [58].

Table 1. Table summarizing the effects of PS MNPs at transcript level evaluated by transcriptomics in fishes and aquatic invertebrates.

FISHES								
Method Used	**Organism Tested**	**PS MNPs Size**	**Concentration Tested**	**Time of Exposure**	**Organ/Tissue Target**	**Life Stage**	**Effect**	**References**
qRT-PCR	*Danio rerio* (zebrafish)	10 µm	200 particles/mL	120 hpf	Whole organism	Larvae	↑ *sod1*, *sod2*, *cat*, *gst* and *cyp*	[35]
qRT-PCR	*Danio rerio* (zebrafish)	500 nm	0.1, 1 and 10 ppm	96 hpf	Whole organism	Larvae	↑ *p53*, *cas-3* and *cas-9*; ↓ *bcl* and bdnf	[49]
qRT-PCR	*Danio rerio* (zebrafish)	30 nm	0.1, 0.5 and 3 ppm	120 hpf	Whole organism	Larvae	↑ *sod1*, *sod2*, *cas-1*, *cas-8*, and *il1β*; ↓ *hsp70*, *bcl-2*, *ache*, DNA repair genes *gadd45α* and *rad51*	[50]
qRT-PCR	*Danio rerio* (zebrafish)	1 µm	1000 µg/L (around 1.91×10^7 particles/L)	96 hpf	Whole organism	Larvae	↑ *il1β*; ↓ *cat*	[51]
qRT-PCR and RNA-seq	*Danio rerio* (zebrafish)	5 µm	20–100 µg/L	21 d	Liver	Adult	↑ *aco* and *fabp6*; ↓ *cpt1*, *ppar-α*, *acc1*, *fas*, *srebp1α*, *ppar-α*. KEGG pathways analysis revealed carbon, lipid and amino acid metabolism effect	[52]
RNA-seq	*Danio rerio* (zebrafish)	size ranging from 25 to 90 µm	100–1000 µg/L	20 d	Liver	Adult	Alteration in pathways related to immune response and lipid metabolism, i.e., sterol biosynthetic process, steroid metabolic process and fatty acid metabolic process	[53]
RNA-seq	*Danio rerio* (zebrafish)	50 and 200 nm,	100–1000 ppb	5 d	Whole organism	Larvae	Nervous system development and function pathways	[54]
qRT-PCR	*Oncorhynchus mykiss* (rainbow trout)	100–400 µm	500–2411 particles/fish/day	4 w	Intestine	Adult	No change in immune response related genes	[55]
qRT-PCR	*Oncorhynchus mykiss* (rainbow trout)	0.2, 1, 20, 40 and 90 µm	2×10^5 particles/L	2 h	Gills	Adult	↑ *ifnγ* gene exposed to 0.2 and 40 µm beads; ↓ *il1β* (bead size 1 µm), *s100a* (bead size 40 µm) and *saa* (1, 40 and 90 µm)	[56]
qRT-PCR	*Oryzias melastigma* (marine medaka)	plain PS, carboxylated PS: PS-COOH and aminated PS: PS-NH_2 with a size of 1 µm	0.02 mg/L	7 d	Whole organism	Larvae	↓ *cox1* and *cox2* by PS, PS-COOH and PS-NH_2; ↓ *cyp1a*, multifunction gene (ATPase) by PS-NH_2 and PS-COOH, respectively. No impairment of oxidative stress genes in all the treatments	[57]
RNA-seq and qRT-PCR	*Oryzias melastigma* (marine medaka)	0.05, 0.50, and 6.00 µm	0.1; 1×10^3; 1×10^6 particles/mL	19 d	Whole organism	Larvae	↓ inflammatory and immune-related signaling pathways (Hippo, B cell receptor, RIG-I-like receptor, and inflammatory mediator regulation of the TRP-channels-signaling pathway); heart development (↓ *gata4 and nkx2.5*, and ↑ bmp4) hatching enzyme (*hce* and *lce*)	[58]

Table 1. *Cont.*

AQUATIC INVERTEBRATES								
Method Used	**Organism Tested**	**PS MNPs Size**	**Concentration Tested**	**Time of Exposure**	**Organ/Tissue Target**	**Life Stage**	**Effect**	**References**
qRT-PCR	*Artemia franciscana* (brine shrimp)	50 nm PS-NH_2	1 µg/mL	14 d	Whole organism	Adult	↑ *clap* and *cstb* genes	[59]
RNA-seq	*Artemia franciscana* (brine shrimp)	5 µm	1 mg/L	14 d	Whole organism	Adult	KEGG enrichment analysis mapped into arrhythmogenic right ventricular cardiomyopathy, viral myocarditis, hypertrophic cardiomyopathy, phagosome, fluid shear stress, atherosclerosis and regulations of actin cytoskeleton, with most of the DEGs correlated with ROS activity and apoptosis activity	[60]
qRT-PCR	*Artemia franciscana* (brine shrimp)	50 nm PS-NH_2	1 µg/mL	48 h and 14 d	Whole organism	Neonates and adult	Time-dependent ↑ *clap* and *cstb* genes and *hsp60* and *hsp70*	[61]
RNA-seq	*Ciona robusta* (ascidian)	50 nm PS-NH_2	10 and 15 µg/mL	22 hpf	Whole organism	Embryos	↓ genes involved in glutathione metabolism (*glutamate--cysteine ligase catalytic subunit-like transcript variant X1 and X2; glutathione S-transferase omega-1-like*), immune defense (*integumentary mucin C.1-like transcript variant; mucin-5AC transcript variant; interferon-induced protein 44-like; plasminogen-like*), nervous system (*acetylcholinesterase-like; sco-spondin*), transport by aquaporins (*aquaporin-like*) and energy metabolism (*succinate--CoA ligase [ADP/GDP-forming] subunit alpha mitochondrial-like; 6-phosphofructo-2-kinase/fructose-26-bisphosphatase 1 transcript variant; glycoside hydrolase transcript variant*)	[24]
qRT-PCR	*Daphnia magna* (water flea)	50 nm	0.05, 0.5 µg/L	21 d	Whole organism	Adult	↓ *cat* after exposure of 21 d to 0.5 µg/mL	[62]
qRT-PCR	*Daphnia pulex* (water flea)	75 nm	0.1, 0.5, 1, 2 mg/L	21 d	Whole organism	Adult	*Sod*, *gst*, *gpx* and *cat* initially ↑ and then inhibited. ↑ *hsp* in all the treatment groups	[63]

Table 1. *Cont.*

AQUATIC INVERTEBRATES								
Method Used	**Organism Tested**	**PS MNPs Size**	**Concentration Tested**	**Time of Exposure**	**Organ/Tissue Target**	**Life Stage**	**Effect**	**References**
RNA-seq	*Daphnia pulex* (water flea)	~70 nm	1 mg/L (5.32×10^8 particles/mL)	96 h	Whole organism	Neonates	Alterations in oxidative stress (arachidonic acid metabolism, glutathione metabolism, and porphyrin and chlorophyll metabolism), immune response (drug metabolism–cyp450 and other enzymes, metabolism of xenobiotics by cyp450, glutathione metabolism, hippo signaling pathway, and adherens junction) and energy metabolism pathways (starch and sucrose metabolism, pentose and glucuronate interconversions, galactose metabolism, fructose and mannose metabolism, carbohydrate digestion and absorption, and glycolysis/gluconeogenesis)	[64]
RNA-seq	*Daphnia pulex* (water flea)	75 nm	1 mg/L	21 d	Whole organism	Adult	Alteration in genes involved in chitin metabolism, trehalose transport and metabolism, growth-related genes, long-chain fatty acids metabolism, defense mechanisms, and sex differentiation	[65]
qRT-PCR	*Litopenaeus vannamei* (whiteleg shrimp)	100 nm	200 and 2000 mg/kg	14 and 28 d	Hepatopancreas	Adult	↑ Beta-glucan binding protein, *LPS*/β-glucan binding protein, and *hsp90* genes. ↑ *TLR* gene	[66]
RNA-seq	*Meretrix meretrix* (marine clam)	100 nm PS-NH_2 200 nm PS-COOH	2 mg/L	7 d	Digestive gland	Adult	↑ energy homeostasis imbalance (e.g., lipid metabolism, PPAR signaling pathway, protein digestion and absorption, pyruvate metabolism, and glycolysis; Impairment of immune system (e.g., NLRs, NF-κB signaling pathway, TLR signaling pathway, phagosome, lysosome, and apoptosis)	[67]
qRT-PCR	*Mytilus galloprovincialis* (Mediterranean mussel)	50 nm PS-NH_2	0.150 mg/L	24 and 48 hpf	Whole organism	Embryos	↓ cs, ca, and ep genes	[25]
qRT-PCR	*Mytilus galloprovincialis* (Mediterranean mussel)	3 µm	50–500 particles/mL	24 and 48 hpf	Whole organism	Embryos	↑ *ep*, *ca*, and *cs* genes; ↑ *mytc* and *mytb* genes; ↓ *gusb*, *hex*, *ctsl* genes	[68]
qRT-PCR	*Mytilus galloprovincialis* (Mediterranean mussel)	50 nm PS-NH_2	10 µg/L	First exposure 24 h, rest period 72 h, second exposure 24 h	Hemocytes	Adult	↑ *epp*, *lyso*, *amps*, *mytb*, *mytc*, *frep*	[69]

Table 1. *Cont.*

AQUATIC INVERTEBRATES								
Method Used	**Organism Tested**	**PS MNPs Size**	**Concentration Tested**	**Time of Exposure**	**Organ/Tissue Target**	**Life Stage**	**Effect**	**References**
qRT-PCR	*Paracentrotus lividus* (sea urchin)	50 nm PS-NH_2	3 µg/mL	24 and 48 hpf	Whole organism	Embryos	↑ *cas8*	[19]
qRT-PCR	*Paracentrotus lividus* (sea urchin)	50 nm PS-NH_2	3 and 4 µg/mL	24 and 48 hpf	Whole organism	Embryos	↑ *hsp70*, *p38 Mapk*, univin and *cas8*	[20]
RNA-seq	*Pinctada margaritifera* (black-lip pearl oyster)	6 and 10 µm	0.25–2.5–25 µg/L	2 months	Mantle	Adult	Alteration in energy, stress, and immune-related genes. ↓ *cyp2d11*, *gst1*, *sult1c4* and *abcb1*, *cel* and *actin* gene	[70]
RNA-seq	*Procambarus clarkia* (red swamp crayfish)	0.10 µm	1.4×10^{11} particles/L	72 h	Hemocytes and hepatopancreas	Adult	In hemocytes, ↑ 8 DEGs involved in gene transcription and translation. In hepatopancreas, differential expression of only 3 genes (*cyp49a1* and two unknown genes)	[71]
qRT-PCR	*Sterechinus neumayeri* (Antarctic sea urchin)	40 nm PS-COOH 50 nm PS-NH_2	1 and 5 µg/mL	6 and 24 h	Coelomocytes	Adult	↑ antioxidant genes at 1 µg/mL PS-COOH. ↓ *sod* at 1 µg/mL PS-NH_2, ↑ *mt* at both the concentrations tested PS-NH_2. ↑ the immune-related gene *NF-kB* and *LBP/BPI* by PS-NH_2	[30]

↑: increase; ↓: reduction; qRT-PCR: quantitative real-time polymerase chain reaction; RNA-seq: RNA-sequencing; hpf: hours post-fertilization; d: days; w: weeks; ppm: parts per million; ppb: part per billion; sod: superoxide dismutase; *cat*: catalase; *gst*: glutathione S-transferase; *cyp*: cytochrome P450; *p53*: tumor protein p53; *cas*: caspase; *bcl-2*: B-cell lymphoma 2; *bdnf*: brain-derived neurotrophic factor; *il1β*: interleukine 1-β; *aco*: acyl-CoA oxidase; *fabp6*: fatty acid binding protein 6; *cpt1*: carnitine palmitoyltransferase 1; *ppar-α*: peroxisome proliferator-activated receptor-α; *acc1*: acetyl-CoA carboxylase 1; *fas*: fatty acid synthase; *srebp1α*: sterol regulatory element binding protein 1α; *ppar-γ*: peroxisome proliferator-activated receptor-γ; *cox*: cyclooxygenase; *hsp*: heat shock protein; *ache*: acetylcholinesterase; *gadd45α*: growth arrest and dna damage inducible alpha; *rad51*: rad51 recombinase; *ifnγ*: interferon-gamma; *s100a*: s100 calcium binding protein a1; *saa*: serum amyloid A; *RIG-I*: retinoic acid-inducible gene I; *TRP*: transient receptor potential; *bmp4*: bone morphogenetic protein 4; *hce:* high choriolytic enzyme; *lce:* low high choriolytic enzyme; *gpx*: glutathione peroxidase; *NLRs*: NOD-like receptor signaling pathway; *cs*: chitin synthase; *ca*: carbonic anhydrase; *ep*: extrapallial protein; *mytc*: myticin C; *mytb*: mytilin B; *gusb*: β glucorinidase; *hex*: hexosaminidase; *ctsl*: catepsine L; *p38 Mapk*: mitogen-activated protein kinase; *epp*: extrapallial protein precursor; *lys*: lysozime; *amps*: antimicrobial peptides; *frep*: fibrinogen-related proteins; *sult1c4*: sulfotransferase 1C4; *abcb1*: ATP binding cassette subfamily B member 1, *cel*: bile salt-activated lipase; *mt*: metallothioneine; *NF-kB*: nuclear factor kappa-light-chain-enhancer of activated B cells; *LBP/BPI*: lipopolysaccharide-binding protein and bactericidal/permeability-increasing protein; *LPS*: lipopolysaccharide; *TLR*: toll like receptor.

In adult fishes, analysis at the transcriptomic level related to the effects of PS MNPs was mainly conducted on the digestive tract. This is probably due to the fact that the principal route of accumulation and absorption of plastics is ingestion [15,73]. In particular, qRT-PCR and transcriptome analysis revealed the effect on genes and pathways related to immune response and energy metabolism (e.g., glycolipid, carbon, lipid, and amino acid metabolism) in the liver of *D. rerio*, assuming that PS MNPs could potentially affect the behavior and physiology of the organism, potentially decreasing its fitness [52,53]. In rainbow trout (*Oncorhynchus mykiss*) exposed to 100–400 µm PS MPs, no effects were found for genes involved in cytokines and in the structural integrity of intestinal tissue, probably because of the capability of the epithelial barrier and the mucosal immune system of the intestine to cope with stressors [55]. The effects of PS MPs of different sizes were also investigated on the transcriptome of the gills of the same organism, suggesting an alteration in the genes involved in inflammatory and acute immune response, thus leading to potential distruction of the tissue that plays an essential role in immunity [56].

2.1.2. Effects of PS MNPs on the Transcriptome of Aquatic Invertebrates

Several studies of PS MNPs and their effects at the transcriptomic level have been performed on aquatic invertebrates (56.25%, see Figure 2), since they offer ethical, cost-effective, and repeatable testing [74]. One of the most widely used invertebrate for these studies is the freshwater crustacean *Daphnia* spp. Species of the genus *Daphnia* provide an important link between aquatic primary producers and consumers of higher trophic levels. Furthermore, the high sensitivity to several pollutants combined with their biological characteristics (e.g., short life cycles, rapid reproduction, and transparent exoskeleton), make them an ideal model system for ecotoxicological studies [75]. The chronic exposure of water fleas *D. pulex* and *D. magna* to environmental relevant concentrations of PS NPs (50 and 75 nm) was shown to alter the expression of stress defense genes and heat shock proteins, resulting in an impact on the antioxidant system, thus causing damage to the organism [62,63]. The analysis of the whole transcriptome of *D. pulex* exposed to 75 nm PS NPs after 21 days showed the alteration of the genes involved in chitin metabolism, trehalose transport and metabolism, growth-related genes, long-chain fatty acid metabolism, defense mechanisms, and sex differentiation, leading to delayed growth and reproduction, aging, and changes in the reproductive pattern and population sex ratio [65]. The same techniques have been also applied to quantitatively measure the expression level of transcripts and to identify the pathways affected in *D. pulex* neonates after the exposure to 1 mg/L of 70 nm PS NPs (5.32×10^8 particles/mL). The authors demonstrated that more than 200 transcripts were differentially expressed, showing alterations in oxidative stress immune response and energy metabolism pathways, thereby identifying the elements of the oxidative stress defense system as possible biomarkers for nanoplastic contaminants [64].

In marine invertebrates, most studies have focused on embryo development since the early developmental stages are known to be the most sensitive to environmental perturbations [76]. Amino-modified PS NPs (50 nm PS-NH_2; 0.150 mg/L) and PS MPs (3 µm; 50–500 MP/mL) are able to negatively influence the development of *Mytilus galloprovincialis,* altering the expression of genes involved in shell formation and immunomodulation, and genes coding for lysosomal enzymes [25,68]. In the sea urchin *Paracentrotus lividus*, PS-NH_2 can induce a diverse modulation of genes involved in stress response, apoptosis, and development during the embryo development [19,20]. More recently, whole transcriptome analysis of the exposure to PS-NH_2 during the embryo development of the ascidian *Ciona robusta* revealed the alteration in the expression level of genes related to glutathione metabolism, immune defense, the nervous system, transport by aquaporins, and energy metabolism, thus affecting the correct embryonic development, which is essential to ensure a subsequent proper life cycle [24]. Bivalves and sea urchins have also been used to investigate the effects of PS MPs on their immune system. As an example, in mussel *M. galloprovincialis* hemocytes, the acute exposure to 10 µg/L of 50 nm PS-NH_2 caused the upregulation of six genes involved in the immune response [69]. Conversely, in the Antarctic sea urchin *Sterechinus neumayeri* coelomocytes, 40 nm PS-COOH modulated the expression of genes related to external challenges, antioxidant responses, and cell protection against stress and apoptosis, while 50 nm PS-NH_2 induced a low gene modulation, suggesting a threshold in coelomocytes' defence ability against PS-NH_2 [30].

In the aquatic environment, zooplankton play an important role in the food chain as a primary consumer and a major food source for higher trophic organisms [77–79]. Thus, several studies investigated the impact of PS MNPs on zooplankton since they could represent a threat to the aquatic ecosystem [80]. In the brine shrimp *Artemia franciscana*, 50 nm PS NH_2 (1 µg/mL) significantly induced *clap* and *cstb* genes, which have a role in larval growth and molting, and *hsp70* and *hsp26* genes involved in cytoprotection against oxidative stress [59,61]. The same model organism was used to evaluate the impact of 5 µm PS MPs on the whole transcriptome [60]. The reported results demonstrated that chronic exposure to these PS MPs can alter the pathways of ventricular cardiomyopathy, viral myocarditis, hypertrophic cardiomyopathy, phagosome, fluid shear stress, atherosclerosis, and regulation of actin cytoskeleton. Moreover, seven key genes were differentially regu-

lated, resulting in the impairment of the immune response, oxidative stress, and apoptosis pathways [60]. In the hepatopancreas of the Pacific white shrimp *Litopenaeus vannamei*, the exposure to 100 nm PS NPs induced an upregulation of genes involved in immune response, and genes involved in the antioxidant response and cellular homeostasis, resulting in the inhibition of the growth due to the ingestion of plastics [66].

Other marine organisms used to evaluate the impact of PS MPs at the transcriptomic level were the pearl oyster *Pinctada margaritifera*, the marine clams *Meretrix meretrix*, and the red swamp crayfish *Procambarus clarkii*. Gardon and colleagues [70] used a genome-wide transcriptomic approach on the mantle tissue of *P. margaritifera* to understand the effect of 6 and 10 µm PS MPs. Transcriptomic analysis revealed that energy-, stress-, and immune-related genes appear to be profoundly impacted by PS MPs, indicating that the reduction in energetic budget could lower Darwinian fitness [70]. Another interesting work evaluated the effect of 100 nm PS-NH_2 and 200 nm PS-COOH (2 mg/L) on the digestive gland of the marine clams *M. meretrix*. RNA-seq results and the pathway enrichment analyses showed the impairment of the energy metabolism and immunomodulation, highlighting the potential risks of PS NPs in the stability and function of marine benthic ecosystems [67]. In the red swamp crayfish, RNA-seq analysis was performed on hemocytes and hepatopancreas [71]. Results showed that eight genes involved in gene transcription and translation were upregulated. Furthermore, two cytoskeleton-associated genes were also identified. Conversely, in hepatopancreas, only three genes were differentially expressed, namely, *cyp49a1* and two unknown genes. The overall data revealed the altered expression of a few genes involved in immune response, oxidative stress, gene transcription and translation, protein degradation, lipid metabolism, oxygen demand, and reproduction after PS NP exposure, suggesting that, even at low concentration, PS NPs may induce mild stress in crayfish with a potential impact also at the population level [71].

2.2. Proteomics

Proteomics is used to analyse translational and post-translational variations in proteins. The main analytical strategies for separation and identification of the proteome are based on two different approaches: the top-down and the bottom-up approach. Top-down approaches rely on the use of intact proteins for direct separation and identification, typically performed by liquid chromatography–tandem mass spectrometry (LC-MS/MS). These two strategies present different advantages for the complete analysis of proteoforms. However, challenges regarding solubility, separation, and ionization efficiency still remain to be addressed. In contrast, proteomic approaches that use a bottom-up workflow minimize problems by analyzing peptides prepared by enzymatic proteolysis. In this strategy, however, greater sample separation power is required [81,82]. The main proteomic approach that has been established in recent years consists of protein separation in gels and mass spectrometry analysis. Gel separation of proteins is used for the separation and global quantification of proteins. It commonly relies on using an older technique of two-dimensional gel electrophoresis (2D-GE). However, the loss of all membrane proteins, the appearance of multiple proteins in a single spot, and the presence of a single protein in multiple spots constitute some important limitations in the use of this technique. As a result, the most frequently used method for measuring cellular proteins has become liquid chromatography coupled with mass spectrometry (LC-MS or LC-MS/MS), because of its sensitivity, selectivity, accuracy, speed, and throughput [83–86]. Hence, proteomics has recently been applied to study the metabolic pathways' modifications, biodistribution, and bioaccumulation caused by plastic molecules in aquatic environments, showing huge potential [87]. A description of the effects of PS MNPs at proteomic level in fishes and aquatic invertebrates is reported in Table 2.

2.2.1. Effects of PS MNPs on the Proteome of Fishes

At present, there are very few works (8.33%, see Figure 2) related to proteomics applied to fishes to unveil the impact of PS MNPs on specific proteins, and many of them have used

traditional biochemical methods, such as Western blot or ELISA. In a recent research study, a reduction in cytokines and fin regeneration proteins was found in *D. rerio* larvae exposed to 50 and 500 nm PS NPs after fin amputation at 72 hpf. Therefore, these data suggest an inhibitory effect induced by PS MNPs on the regenerative capacity of injured fish, further supported by the observed alteration in ROS signaling and immune response, both of which are essential for tissue repair and regeneration [88]. Moreover, an increase in the protein levels of NF-kB and iNOS, which play an important role in the inflammatory and immune responses, was also reported in zebrafish *D. rerio* larvae exposed to 0.01 mg/L PS MPs for 7 days. These findings, therefore, indicate the induction of a potential inflammatory response mechanism in larval zebrafish triggered by PS MP exposure [89].

Table 2. Table summarizing the effects of PS MNPs at protein level evaluated by proteomics in fishes and aquatic invertebrates.

FISHES								
Method Used	**Organism Tested**	**PS MNPs Size**	**Concentration Tested**	**Time of Exposure**	**Organ/Tissue Target**	**Life Stage**	**Effect**	**References**
ELISA	*Danio rerio* (zebrafish)	50 and 500 nm	0.1, 1, 10 mg/L	12 and 72 hpa	Whole organism	Larvae	Slight ↑ Il-10, Tnf-α and Nf-κB at the lowest dose. ↓ Il-10, Tnf-α, FgF20a and Nf-κB at the higher dose	[88]
WB	*Danio rerio* (zebrafish)	5 µm	50 ng/mL	7 d	Whole organism	Larvae	↑ iNOS and Nf-κB	[89]
AQUATIC INVERTEBRATES								
WB	*Brachionus koreanus* (marine rotifer)	0.05 and 0.5 µm	10 µg/mL	24 h	Whole organism	Neonates	↑ phosphorylation of JNK and p38 Mapk related to ↑ ROS level	[90]
LC-MS/MS	*Crassostrea gigas* (Pacific oyster)	2 and 6 µm	0.023 mg/L	60 d	Oocytes	Gamets	↓ arginine kinase and ↑ severin	[18]
LC-MS/MS	*Daphnia magna* (water flea)	Mean particle size 13.03 ± 7.75 µm	101.6 mg/L	19 d	Whole organism	Adult	Changes in 41 proteins, mostly those related to sulfation, chitin-binding and cuticle's structural integrity. The less abundant proteins are related to pigment binding, response to stimuli, response to ROS, response to oxidative stress, and response to oxygen-containing compound	[91]
RPLC/MS	*Daphnia pulex* (water flea)	500 nm	1 mg/L	14 d	Whole organism	Adult	Changes in 89 proteins, including those involved in P-body assembly, nuclear-transcribed mRNA catabolic process, ATP-dependent chromatin remodeling, energy metabolism and unfolded protein responses	[92]
LC-MS/MS	*Daphnia pulex* (water flea)	Mean particle size 71.18 nm	0.5–2 mg/L	21 d	Whole organism	Adult	327 proteins ↓, including those involved in cell signaling, immune function, detoxification, energy metabolism, ECM-receptor interaction pathways, and glutathione metabolism	[93]

Table 2. *Cont.*

AQUATIC INVERTEBRATES								
Method Used	**Organism Tested**	**PS MNPs Size**	**Concentration Tested**	**Time of Exposure**	**Organ/Tissue Target**	**Life Stage**	**Effect**	**References**
nLC-MS/MS	*Dreissena polymorpha* (Zebra mussel)	1 and 10 µm size	4×10^6 particles/L mixtures	6 d	Gills	Adult	78 proteins ↓ and 18 proteins not expressed. Effect of catalytic activity (27%), nucleotide binding, proteins involved in structural molecule activity (12%) and protein binding (11%), proteins related to RNA (5%) and metal ion (4%) bindings	[94]
LC-MS/MS	*Litopenaeus vannamei* (Pacific white shirmp)	100–200 µm	1 mg/L	14 d	Haemolymph	Adult	47 proteins ↓, including those belonging to extracellular, plasma membrane and lysosomal localization, and related to T cell receptor signaling pathway, epithelial cell signaling in *Helicobacter pylori* infection, and phospholipase D signaling pathway	[95]
Nano HPLC MS/MS	*Paracentrotus lividus* (sea urchin)	45 µm	10, 50, 10^3, 10^4 particles/L	72 h	Coelomocytes	Adult	↑ proteins involved in endosome transport via multivesicular body sorting pathway and in establishment of protein localisation, and proteins involved in catabolic processes	[34]
WB	*Paracyclopina nana* (marine copepod)	0.05 µm	10 µg/mL	24 h	Whole organism	Neonates	Oxidative stress induction (↑ ROS level) and ↑ phosphorilation of the proteins p38 Mapk, ERK and Nrf2	[96]
LC-MS/MS	*Tigriopus japonicus* (marine copepod)	6 µm	0.23 mg/L	Two generations (F1 and F2)	Whole organism	Adult	↑ proteins involved in several cellular biosynthesis and ↓ cellular energy storage in F1 generation. Transgenerational proteome plasticity in F2 generation with elevated energy metabolism and stress related defense	[97]

↑: increase; ↓: reduction; ELISA: Enzyme-Linked Immunoassay; WB: Western Blot; LC-MS/MS: Liquid Chromatography–Tandem Mass Spectrometry; RPLC/MS: Reversed-Phase Liquid Chromatography Tandem Mass Spectrometry; nLC-MS/MS: nano-Liquid Chromatography Mass Spectrometry; Nano HPLC MS/MS: nano High-Performance Liquid Chromatography and Mass Spectrometry; hpa: hours post amputation; hpf: hours post-fertilization; d: days; Il-10: cytokines Il-10; Tnf-α: tumor necrosis factor; Nf-kB: nuclear factor kappa-light-chain-enhancer of activated B cells; FgF20a: fibroblast growth factor 20a; iNOS: nitric oxide synthase; ROS: reactive oxygen species; ECM: extracellular matrix; p38 Mapk: mitogen-activated protein kinases; ERK: extracellular signal-regulated kinases; Nrf2: nuclear factor erythroid 2 related factor 2; JNK: jun n-terminal kinases.

2.2.2. Effects of PS MNPs on the Proteome of Aquatic Invertebrates

The most recent proteomic studies of PS MNPs on aquatic invertebrates (as reported in Figure 2, 31.25%) have mainly focused on copepods, rotifers, shrimps, bivalves, sea urchins, and daphnids. In *Paracyclopina nana* and *Brachionus koreanus*, 0.05 and 5 µm PS MPs resulted in an alteration in the signaling pathways involved in cell survival and proliferation in response to oxidative stress, and in cell death processes. Specifically, increased phosphorylation of c-Jun N-terminal kinase (p-JNK), extracellular signal-regulated kinase (p-ERK), and mitogen-activated protein kinases (p38 Mapk) with Nrf2 was found, indicating a positive correlation with intracellular ROS levels [90,96]. The analysis of the entire proteome of the haemolymph of the shrimp *L. vannamei* exposed to PS MPs (100–200 µm size; 1 mg/L) revealed the upregulation and downregulation of 20 and 27 proteins, respectively. Among

these, proteins with extracellular, plasma membrane, and lysosomal localization were found, as well as those related to the "T cell receptor signalling pathway", "epithelial cell signaling in *Helicobacter pylori* infection", and the "phospholipase D signaling pathway". Thus, all these proteins could play an important role in the detoxification process to cope with the stress induced by PS MPs [95]. Furthermore, the proteomic profile also revealed the effect of several proteins in the coelomocytes of the sea urchin *P. lividus* in a dose-response manner. In fact, at the lowest concentration tested (50 PS MPs/L), there is an enrichment of proteins related to transport and vacuoles for ubiquitin-dependent catabolic processes, as well as proteins involved in cell aerobic respiration, while at the highest concentrations (10^3 and 10^4 PS MPs/L), an enrichment of proteins involved in cytoskeletal regulation, cadherin signaling, and the integrin pathway became evident. It is likely that these findings depend on the fact that, to counteract external factors such as MPs, an immune defense action is necessary, which involves cellular detoxification processes that have a significant metabolic cost [34]. PS MPs can also cause reproductive disruption in oysters, with significant impacts on offspring. In fact, the proteome of oocytes from adult *Crassostrea gigas* exposed to PS MPs (2 and 6 µm, 0.023 mg/L) for 60 days revealed the alteration in arginine kinase (downregulated) and severin (upregulated), which are related to ATP metabolism and cytoskeletal dynamics, suggesting deleterious effects of PS MPs on oocyte maturation, fertilization, and embryo development [18]. Another work related to proteomics and PS MPs was performed on the marine copepod *Tigriopus japonicus*. Proteomic analysis, conducted for the generations F1 and F2 exposed to 0.23 mg/L of 6 µm PS MPs, showed an increasing level of proteins involved in several cellular biosynthesis processes and, in turn, reduced energy storage (e.g., ribosome, peptide biosynthetic process, GTPase activity, protein translation, gene expression, galactose oxidase, and c-type lectin-like/link domain) due to the trade-off, hence compromising survival and reproduction of the treated copepods in F1. Interestingly, the proteome analysis of the recovery generation F2 showed an increased energy metabolism and stress-related defense pathway (e.g., structural constituent of cuticle, carbohydrate metabolic process, lysosome, cell redox homeostasis, proteolysis, antioxidant activity, and HSP20-like chaperone) to regain the compromised phenotypic traits during the recovery (the so-called transgenerational proteome plasticity) [97].

Furthermore, the use of more sophisticated proteomics techniques allowed the identification and quantification of the proteins affected by the chronic exposure of water flea *D. pulex* neonates to 1 mg/L of 500 nm PS MPs, showing an activation of DNA repair and transcription (upregulated proteins: U6 snRNA-associated Sm-like protein LSm3 and LSm4, helicase SWR1, and DNA excision repair protein ERCC6), and an inhibition of lipid metabolism and response to unfolded or misfolded proteins (e.g., regulation of glucose metabolic process, very long-chain fatty acid catabolic process, positive regulation of fatty acid oxidation, IRE1-mediated unfolded protein response, and protein quality control for misfolded or incompletely synthesized proteins) [92]. The same model organism was used to investigate the effects of PS NPs (1 mg/L) in the nano-size range (average size 70 nm) after 21 days of exposure. Even in this case, proteomics analysis showed a significative differential expression of proteins involved in the pathways mentioned above, such as energy metabolism (e.g., glycine, serine, threonine, arachidonic acid, d-glutamine, d-glutamate, butanoate, and nitrogen metabolism), signaling transduction (e.g., mTOR, FoxO, and Jak-STAT signaling pathways), and oxidative stress (glutathione metabolism) and extracellular matrix (ECM)-receptor interaction pathways [93]. A recent work on *D. magna* daphnids exposed to PS MPs (mean particle size of 13.03 ± 7.75 µm) showed the alteration in 41 proteins, with an increase in several sulfotransferases involved in basic biochemical pathways, and GABA transaminase catalyzing the degradation of the neurotransmitter GABA and cuticula building proteins, while proteins such as the DNA-directed RNA polymerase subunit and other proteins connected to biotic and inorganic stress and reproduction were decreased. This suggests the onset of transcription stress associated with serious consequences for the entire organism. Furthermore, the reduced abundance of proteins linked to the transport of harmful compounds suggests a disturbed defense mechanism,

probably caused by the ingestion of PS-MPs [91]. The overall data seem to suggest that PS MNPs can alter the antioxidant capacity and the energy metabolism, leading to a potential trade-off among life-history traits to maintain fecundity at the cost of self-maintenance of the two cladocerans. Interestingly, Magni and collaborators [94] found a similar result in the proteome profiling of the gills of the freshwater zebra mussel *Dreissena polymorpha* exposed to a mixture of PS MPs (1 and 10 µm size; 4×10^6 PS MPs/L mixtures). In fact, the altered proteins belong to pathways involved in catalytic activity (27%), nucleotide binding, proteins involved in the structural molecule activity (12%) and protein binding (11%), and proteins related to RNA (5%) and metal ion bindings (4%) [94]. Overall, all these studies indicate the potential risks for aquatic invertebrates provoked by PS MNP exposure at the protein level.

2.3. Metabolomics

Metabolomics is the most recent discipline among the -omic sciences. It studies the metabolome, a term coined by Oliver in 1998 [98], indicating the entire set of metabolites present in a biological system. Metabolomics is a powerful bioanalytical technique that allows the systematic identification and quantification of all endogenous metabolites with low molecular weight (<1500 Da), which may vary according to the physiological state, developmental stage, or pathological condition of cells, tissues, or organs, or of the whole organism under examination [99]. Therefore, analysis of complex metabolite data along with uni- and multivariate statistics, as well as mapping of altered pathways, enables the mechanistic understanding of biological phenotypes and discovery of biomarkers or drug targets for a variety of conditions [100,101]. To obtain the global metabolic profiling of a given organism or biological sample in relation to external stimuli, two metabolomic analytical platforms, namely, nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS), are commonly employed. Although NMR is relatively less sensitive than MS, it offers numerous benefits to the metabolomics field since it is highly reproducible and quantitative, non-selective and non-destructive, does not require sample preparation or separation, and enables the identification of unknown metabolites in complex biological mixtures [101]. On the contrary, MS is a highly sensitive technique able to detect thousands of metabolites, prior to separation using liquid or gas chromatography (LC or GC, respectively) [102]. Overall, both of the two metabolomics platforms, in combination with powerful chemometric software for multivariate data analysis, which is necessary to deconvolute the huge amount of data produced following a metabolomic experiment [100,103], allow the simultaneous determination and comparison of a wide range of metabolites, offering numerous advantages to clarify the organism–environment interactions [35,36,43,104–113]. A description of the effects of PS MNPs at metabolomic level in fishes and aquatic invertebrates is reported in Table 3.

Table 3. Table summarizing the effects of PS MNPs at metabolite level evaluated by metabolomics in fishes and aquatic invertebrates.

FISHES								
Method Used	**Organism Tested**	**PS MNPs Size**	**Concentration Tested**	**Time of Exposure**	**Organ/Tissue Target**	**Life Stage**	**Effect**	**References**
^{1}H NMR	*Danio rerio* (zebrafish)	5 µm	50 and 500 µg/L	21 d	Intestine	Adult (5 months)	Changes in 36 metabolites. ↑ proline, propylene glycol, alanine, glutamine, leucine, ornithine, carnitine, threonine, TMAO; ↓ lysine, phenylalanine, tyrosine, linoleic acid, palmitic acid, triglycerides.	[31]

Table 3. *Cont.*

FISHES								
Method Used	**Organism Tested**	**PS MNPs Size**	**Concentration Tested**	**Time of Exposure**	**Organ/Tissue Target**	**Life Stage**	**Effect**	**References**
GC-MS	*Danio rerio* (zebrafish)	100 nm	250 and 2×10^4 items of PS MNPs in 50 mL	72 hpf	Whole organism	Embryo	Changes in 508 metabolites. Disorders in unsaturated fatty acids, linoleic acid, taurine, hypotaurine, nicotinate, nicotinamide, alanine, aspartate, glutamate.	[22]
GC-MS	*Danio rerio* (zebrafish)	5–50 µm	100 and 1000 µg/L	7 d	Whole organism	Embryo	Changes in 78 (5 µm) and 121 (50 µm) metabolites. Disorders in carbohydrates, fatty acids, amino acids, nucleic acids and others.	[114]
LC-MS/MS	*Danio rerio* (zebrafish) *Perca fluviatilis* (perch)	5–12 µm	1, 50 e 100 mg	21 d	Gills and liver	Adult	Changes in 33 metabolites. Zebrafish gills: ↓ phenylalanine, carnitine, proline, salicylic and lactic acid, choline. Perch gills: ↑ phenylalanine, salicylic acid; ↓ acetyl-carnitine, alanine, glutamic and pyruvic acid. Zebrafish liver: ↑ adenine, adenosine, glutamine; ↓ hypoxanthine, uridine, deoxyadenosine, valine, arginine, phenylalanine, asparagine, proline. Perch liver: ↑ arginine, succinic acid, adenosine; ↓ hypoxanthine, oxoglutaric acid, citrulline, creatinine, adenine	[115]
LC-MS	*Gobiocypris rarus* (rare minnow)	1 µm	200 µg/L	4 w	Liver	Subadult (3 months)	Changes in 41 metabolites. ↑ glyceraldehyde; cytosine, glucose, fructose, mannose; ↓ mannitol 1-phosphate, acetyl-phenylalanine, mannonate	[116]
UPLC-Q-TOF-MS	*Oreochromis mossambicus* (tilapia)	100 nm	20 mg/L and recovery	7 d	Whole organism	Larvae	Changes in 203 metabolites. Disorders in fatty acyls, carboxylic acids and their derivatives, organooxygen compounds, keto acids and their derivatives.	[117]
LC-MS	*Oreochromis niloticus* (red tilapia)	0.3, 5 and 70–90 µm	100 µg/L	14 d	Liver	Adult	Changes in 31 (0.3 µm), 40 (5 µm) and 23 (70–90 µm) metabolites. Disorders in amino acids, fatty acids, glycerophospho-ethanolamines, glycerophosphoserines, glycerophospho-cholines, purine nucleosides, eicosanoids.	[118]

Table 3. *Cont.*

Method Used	Organism Tested	PS MNPs Size	Concentration Tested	Time of Exposure	Organ/Tissue Target	Life Stage	Effect	References
FISHES								
^{1}H NMR	*Oryzias javanicus* (Javanese medaka)	5 µm	100, 500 and 1000 µg/L	21 d	Gut	Adult	Changes in 9 metabolites. ↑ glucose, lactate, alanine, glutamate, glucoronate, valine, anserine, 2-hydroxyvalerate, creatine.	[119]
GC-MS	*Oryzias melastigmas* (marine medaka)	10 and 200 µm	10 mg/L	60 d	Liver	Adult (8 months)	Changes in 83 metabolites. ↑ disaccharides, trisaccharides, fatty acids, fatty acid methyl and ethyl esters; ↓ monosaccharides, organic acids, amino acid.	[120]
LC-MS/MS	*Sebastes Schlegelii* (marine jacopever)	5 µm and 100 nm	0.23 mg/L	15 d	Liver	Juvenile	Changes in 345 metabolites. Disorders in essential amino acids, omega-3 fatty acids, intermediate products of glucose metabolism and TCA intermediates. ↓ gluconic acid, cis-aconitate, malic acid, tyrosine, targinine, glycerol phospholipid	[121]
LC-MS	*Xiphophorus helleri* (swordtail fish)	1 µm	1×10^{6} microspheres/L (B) and 1×10^{7} microspheres/L (C)	72 h	Liver	Adult (3 months)	Changes in 37 (B) and 103 (C) metabolites. ↑ 3-hydroxyanthranilic acid, histidine, citrulline, linoleic acid, pantothenate, xanthine.	[122]
AQUATIC INVERTEBRATES								
LC-MS/MS	*Crassostrea gigas* (Pacific oyster)	6 and 50/60 µm	1×10^{4} particles/L	14 d	Gills	Adult	Changes in 22 metabolites. ↑ asparagine, phenylalanine, glutathione, glucose-6-phosphate, carbohydrates, lactose, mannose; ↓ N-palmitoyl taurine, fatty acids.	[123]
^{1}H NMR	*Daphnia magna* (water flea)	53 nm ($PS-NH_2$), 62 nm (PS-COOH)	3.2 µg/L	37 d	Whole organism	Adult	Changes in 15 metabolites. ↑ alanine, asparagine, glutamate, glutamine, isoleucine, leucine, lysine, phenyl alanine, tyrosine, valine, lactate, methionine sulfoxide; ↓ glucose, glycogen, nucleic acids, isopropanol.	[124]
LC-MS/MS	*Litopenaeus vannamei* (whiteleg shrimp)	2 µm	0.02 to 1 mg/L	72 h	Hepatopancreas	Post-larvae	Changes in 119 metabolites. ↑ amino acids and dipeptides, e.g., taurine, aspartic acid and alanine; ↓ glyceraldehyde and fatty acids, e.g., 3-methyladipic acid and leucinic acid.	[125]

Table 3. *Cont.*

AQUATIC INVERTEBRATES								
Method Used	**Organism Tested**	**PS MNPs Size**	**Concentration Tested**	**Time of Exposure**	**Organ/Tissue Target**	**Life Stage**	**Effect**	**References**
^{1}H NMR	*Mytilus gallo-provincialis* (Mediterranean mussel)	3 µm	50 particles/mL	72 h	Gills and hepatopancreas		Changes in 10 (in gills) and 18 (in hepotopancreas) metabolites. Gills: ↑acetoacetate, ATP/ADP, mytilitol, betaine, taurine, homarine; ↓ alanine, glycine, succinate, acetylcholine. Hepatopancreas: ↑ isoleucine, leucine, valine, alanine, dimethylglycine, tyrosine, lactate, glycogen, glucose, betaine, taurine, homarine, glutathione; ↓ glycine, acetoacetate, succinate, malonate, hypotaurine.	[32,36]

↑: increase; ↓: reduction; hpf: hours post-fertilization; h: hours; d: days; w: weeks; ^{1}H NMR: proton Nuclear Magnetic Resonance; GC-MS: Gas Chromatography-Mass Spectrometry; LC-MS/MS: Liquid Chromatography-Tandem Mass Spectrometry; UPLC-Q-TOF: Ultra-high Performance Liquid Chromatography with Quadrupole Time-of-Flight; TMAO: trimethylamine N-oxide; TCA: tricarboxylic acids.

2.3.1. Effects of PS MNPs on the Metabolome of Fishes

As depicted in Figure 2, it is interesting to note that 45.83% of the selected studies concern the use of metabolomic to evaluate the mechanism of toxicity of PS MNPs in fishes. Many of these studies have examined metabolite changes following exposure to PS MNPs using the zebrafish as a model organism due to its small size, high fecundity, and well-annotated genome [72]. The adult zebrafish *Danio rerio* experimentally exposed to 50 µg/L and 500 µg/L of 5 µm PS MPs for 21 days exhibited gut damage with important alterations related to metabolites involved in oxidative stress, inflammation, and lipid metabolism [31]. Kaloyianni and colleagues [115] also observed significant metabolic alterations in the gills and liver of adult zebrafish *D. rerio* and perch *Perca fluviatilis* exposed to PS MPs (5–12 µm in size) for 21 days. The interaction between PS MPs and cellular components caused changes mainly in amino acid, nitrogen, and energy metabolism, highlighting species- and tissue-specific toxicity, with the perch more sensitive to PS MPs than *D. rerio*, and gills more liable to respond to MP insult than liver [115]. Notably, differential metabolic pathways seem to be altered by PS MNP challenge more in fish embryo-larval stages than adult. Duan and collaborators [22] studied the effects of PS MNPs on the chorion of *D. rerio* embryos using GC-MS, which was able to identify a total of 508 metabolites. This study confirmed the high affinity of embryonic chorions to PS particles, which induced development toxicity via alteration in the metabolic pathways related to the antioxidant system and to biosynthesis of unsaturated fatty acid, linoleic acid metabolism, and alanine, as well as aspartate and glutamate metabolism [22]. Thus, it was demonstrated that although embryonic chorions may efficiently block the entry of MNPs, these can still affect the early development of aquatic organisms. Similarly, Wan and colleagues [114] observed the alteration of 121 metabolites involved in differential metabolic pathways in *D. rerio* larvae exposed to 100 and 1000 µg/L fluorescent and virgin PS MPs having diameters of 5 and 50 µm [114]. Specifically, it was documented that PS MPs provoked damage in the main energy-related metabolic processes, resulting in depletion of energy reserves and locomotor defects, as well as disorders in the metabolism of nucleic acids and glycolipid metabolism, and induction of oxidative stress and inflammatory and neurotoxic responses, thus suggesting that the potential risks of PS MNPs to aquatic organisms should not be ignored.

A more recent study of rare minnows (*Gobiocypris rarus*) exposed to 1 µm PS MPs at a concentration of 200 µg/L documented various sublethal effects at hepatic levels after four weeks of exposure. In particular, metabolomic analyses revealed alterations in pathways involved in the metabolism of glycolipids, amino acids, and nucleotides, demonstrating that the exposure to PS MPs can induce immune reaction, oxidative stress, and disorders in energy and glycolipid metabolism [116].

Besides the evaluation of the effects of PS MNPs, a recent study conducted on the tilapia *Oreochromis niloticus* also aimed to assess the recovery ability of fish by application of metabolomics. In detail, in the study conducted by Pang et al. [117], tilapia larvae were first exposed to 100 nm PS NPs (20 mg/L) for seven days and then returned to freshwater without PS NPs for an additional seven days to establish the toxic impact of PS NPs at the metabolite level. A total of 203 significantly changed metabolites were identified between the control, PS NP treatment, and recovery groups, indicating that PS NPs triggered disorders in glycolipid, energy, and amino acid metabolism in tilapia larvae after short-term exposure. Notably, metabolomic results also revealed a persistent impact of PS NPs in tilapia that can hardly be eliminated though the recovery period. The adult red tilapia was selected as a model organism by Ding and colleagues [118] to assess the hazardous effects of PS MNPs of three sizes (0.3, 5, and 70–90 µm) at the hepatic level. After 14 days of exposure, PS MNPs produced 31, 40, and 23 significantly differentially expressed metabolites, respectively, in which the tyrosine metabolism pathway was significantly affected by all three sizes [118], therefore revealing that toxicity of PS MNPs may not exhibit a simply negative correlation with their sizes. Similar effects were also recorded in the liver of another vertebrate model organism, the swordtail fish *Xiphophorus helleri*, after acute exposure to 1 µm PS MPs for 72 h. Specifically, using LC-MS metabolomics, important alterations were observed at the level of beta-alanine metabolism, amino acid biosynthesis, linoleic acid metabolism, and aminoacyl-tRNA biosynthesis. These findings revealed disorders in differential metabolic pathways, including oxidative stress, immune function, energy metabolism, sugar metabolism, lipid metabolism, molecule transport, and animal growth [122], further highlighting the urgent need to pay attention to the potential risks of MNPs on aquatic organisms. Similar findings were also reported at the fish hepatic level by Sun and colleagues following exposure of the marine jacopever to PS MNPs at 0.23 mg/L [121]. Significant changes were found in 345 metabolites by application of LC-MS/MS, including essential amino acids, omega-3 fatty acids, intermediate products of glucose metabolism, and the tricarboxylic acid (TCA) cycle, therefore interfering with liver metabolism of proteins, fatty acids, glycerol phospholipids, and carbohydrates, with serious implications also for the quality and safety of seafood [121].

Another species commonly used as a model organism is the marine medaka *O. melastigma*. The metabolomic approach based on GC/MS was recently applied to this fish species to evaluate the metabolic disorders triggered in liver by exposure to 10 µm and/or 200 µm PS MPs [120]. Indeed, Ye and collaborators documented that PS MPs provoked the alteration of 83 metabolites, resulting in the inhibition of monosaccharide metabolism, TCA cycle, glycolysis, pentose phosphate pathway, and amino acid metabolism, as well as the accumulation of fatty acids, and fatty acid methyl and ethyl esters. Furthermore, different effects on the hepatic metabolism were observed depending on the size of MPs, with the greater impact induced by the smaller PS MP size than 200 µm PS MPs [120]. Similar effects were also reported in the gut of the Javanese medaka *O. javanicus* exposed to 5 µm beads of PS MP suspensions at concentrations of 100 µg/L, 500 µg/L, and 1000 µg/L for a period of 21 days. In fact, the intestinal metabolic profile revealed an upregulation of nine metabolites related to energy metabolism, via the TCA cycle, creatine pathway, and urea cycle, therefore highlighting the potential health risks of MP toxicity for animals and, possibly, humans [119].

2.3.2. Effects of PS MNPs on the Metabolome of Aquatic Invertebrates

In the field of ecotoxicology, invertebrate organisms such as the bivalve *M. galloprovincialis* have proven to be the most suitable organisms for biomonitoring of aquatic environments, becoming good indicators for their natural habitat. In fact, mussels have also been used as promising model species for the assessment of the risks of a short-term exposure (up to 72 h) to 3 μm red PS MPs (50 particles/mL), owing to their ability to ingest MPs by filter feeding and to their commercial relevance, using ^{1}H NMR-based metabolomics [32,36]. In two distinct mussel organs, namely the hepatopancreas [32] and gills [36], metabolomic data highlighted that PS MPs caused fluctuations in metabolites associated with disorders in amino acid metabolism and disturbances in osmoregulatory processes. Moreover, specific alterations in the cholinergic nervous system were also observed exclusively in the gills, in addition to the onset of oxidative stress and alterations in energy metabolism [36]. These findings therefore highlight the early time- and tissue-dependent mechanisms of toxicity of PS MPs in mussels, underlining the potential risks for the environment and human health posed by MP contamination.

The effects of PS MPs were also evaluated on another invertebrate organism, the shrimp *L. vannamei*, exposed to a range of microplastics from 0.02 to 1 mg/L, using the LC-MS/MS metabolomic approach [125]. Exposure to PS MPs caused evident metabolic alterations in the hepatopancreas of *L. vannamei*, depending on PS MP concentrations. Overall, metabolic pathway analysis conducted in this study revelaed that PS MP exposure interfered with hepatopancreatic metabolism of crustaceans, even at a very low exposure level, mainly affecting glycolysis, lipolysis, and amino acid metabolism [125]. Furthermore, evidence was reported about the potential of PS MPs to alter the energy metabolism and osmotic adjustment pathway in the gills of Pacific oyster *C. gigas* exposed to 1×10^{4}/L spherical PS MPs of different sizes (small PS MPs 6 μm, large PS MPs 0–60 μm) under three salinity levels (21, 26, and 31 practical salinity units (psu)) for 14 days [123]. In detail, it was reported that oysters exposed to MPs were not able to effectively counteract oxidative stress, and overall they exhibited a higher resistance to combined exposure of low salinity and MPs. Therefore, these findings highlight the necessity to take into consideration the potential of environmental factors to influence the toxic effects of MPs on aquatic organisms.

To the best of our knowledge, only one study has dealt with the effects of PS MNPs at the metabolic level in a freshwater invertebrate to date. Kelpsiene and collaborators [124] applied metabolomics using ^{1}H NMR to characterize the metabolic changes in *D. magna* following long-term (37 days) chronic exposure to low concentrations of positively (PS-NH_2) and negatively (PS-COOH) charged PS NPs up to concentrations of 3.2 μg/L. Surprisingly, the positively and negatively charged particles induced similar metabolic changes, which were also largely independent of particle concentration, resulting mainly in alterations in amino acids and the isopropanol metabolite [124]. Therefore, these data suggest that surface charges of PS NPs do not affect the metabolome differently, which is important given that PS-COOH NPs were shown to be non-toxic after acute exposure [121]. Interestingly, this study also reported that daphnid metabolism was affected by PS NPs after two days of exposure, and that the effects remained throughout the entire chronic exposure, an issue that surely deserves to be addressed for a broader understanding of PS MNP toxicity to aquatic organisms.

3. Conclusions and Future Perspectives

In recent years, a wide interest has arisen within the scientific community regarding MNPs and the mechanism of toxicity responsible for their extensively studied sublethal effects on aquatic organisms. With this aim, -omics approaches have facilitated this work, providing an important contribution to the aquatic ecotoxicologists to understand the molecular and biochemical responses of aquatic organisms exposed to MNPs. This review focuses on the recent advances related to the use of transcriptomics, proteomics, and metabolomics to unveil the mechanisms of toxicity of pristine PS MNPs on aquatic organisms, both freshwater and marine fishes and invertebrates. All the studies taken

into consideration revealed that PS MNPs of different sizes can alter similar pathways related to energy metabolism (e.g., lipid, carbon, and amino acid metabolism), oxidative stress, immune response, and the nervous system both in fishes and aquatic invertebrates, and that these can be linked to the alteration in widely studied lethal/sublethal endpoints (e.g., reproduction, development, growth, mortality, nervous system, and metabolism disorders) at population level. Despite the validity and utility of the -omics techniques, the use of a single holistic approach to study the effects of PS MNPs can have some limitations since only one level of the biological organization will be taken into consideration. In particular, transcriptomics, which is the most widely used, can reveal the pathways that are disturbed by PS MNPs, but if performed solely, it can be difficult to interpret the meaning of the obtained results since the change in gene expression may not always reflect a physiological or morphological effect. Proteomic studies are useful to gain a complete understanding of the effect on contaminants at the molecular level, but the identification of proteins remains difficult in a species without a sequenced genome. This contrasts with the case of metabolomics, which gives information about the actual occurrence of a cellular process since it is the result of transcriptional and translational events. Information from this -omic technique can therefore report the actual functional status of the organisms, which could be more easily related to the phenotype. It is necessary to state that, although there are numerous advantages in using these holistic techniques for research purposes, there are also some limitations that are mainly related to the costs of equipment and to the fact that these approaches may be considered relatively time consuming in respect to traditional methods. However, it is evident that high-throughput -omics approaches enable the measurement of molecular changes at different levels of an organism's biological organization in a very comprehensive manner, and thus provide a unique and precious contribution in the field of ecotoxicology to comprehend globally the impact of stressors on aquatic biota. Overall, future studies should therefore take into consideration the use of a multi-omics approach, since only the integration of holistic methods can significantly deepen the understanding of the mechanisms involved in the toxicity of stressors, including MNPs.

Author Contributions: Conceptualization, T.C. and M.M.; data curation, M.C.E., B.B. and T.C.; writing—original draft preparation, M.C.E. and B.B.; writing—review and editing, T.C. and M.M.; visualization, M.C.E. and B.B.; supervision, T.C. and M.M. All authors have read and agreed to the published version of the manuscript.

Funding: M.C.E. received a research post-doctoral fellowship within the National Interest Research Project PRIN 2020, prot. 20204YRYS5_003.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The data presented in this study are avalable from the corresponding author.

Acknowledgments: Parts of the figures within the Graphical Abstract were drawn by using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0, accessed on 1 March 2024).

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Jambeck, J.R.; Geyer, R.; Wilcox, C.; Siegler, T.R.; Perryman, M.; Andrady, A.; Narayan, R.; Law, K.L. Plastic Waste Inputs from Land into the Ocean. *Science* **2015**, *347*, 768–771. [CrossRef]
2. Gigault, J.; Ter Halle, A.; Baudrimont, M.; Pascal, P.-Y.; Gauffre, F.; Phi, T.-L.; El Hadri, H.; Grassl, B.; Reynaud, S. Current Opinion: What Is a Nanoplastic? *Environ. Pollut.* **2018**, *235*, 1030–1034. [CrossRef]
3. Missawi, O.; Bousserrhine, N.; Zitouni, N.; Maisano, M.; Boughattas, I.; De Marco, G.; Cappello, T.; Belbekhouche, S.; Belbekhouche, S.; Guerrouache, M.; et al. Uptake, Accumulation and Associated Cellular Alterations of Environmental Samples of Microplastics in the Seaworm *Hediste diversicolor*. *J. Hazard. Mater.* **2021**, *406*, 124287. [CrossRef]
4. Andrady, A.L. Microplastics in the Marine Environment. *Mar. Pollut. Bull.* **2011**, *62*, 1596–1605. [CrossRef]

5. Dawson, A.L.; Kawaguchi, S.; King, C.K.; Townsend, K.A.; King, R.; Huston, W.M.; Bengtson Nash, S.M. Turning Microplastics into Nanoplastics through Digestive Fragmentation by Antarctic Krill. *Nat. Commun.* **2018**, *9*, 1001. [CrossRef] [PubMed]
6. Ekvall, M.T.; Lundqvist, M.; Kelpsiene, E.; Šileikis, E.; Gunnarsson, S.B.; Cedervall, T. Nanoplastics Formed during the Mechanical Breakdown of Daily-Use Polystyrene Products. *Nanoscale Adv.* **2019**, *1*, 1055–1061. [CrossRef]
7. Gigault, J.; El Hadri, H.; Reynaud, S.; Deniau, E.; Grassl, B. Asymmetrical Flow Field Flow Fractionation Methods to Characterize Submicron Particles: Application to Carbon-Based Aggregates and Nanoplastics. *Anal. Bioanal. Chem.* **2017**, *409*, 6761–6769. [CrossRef] [PubMed]
8. Lambert, S.; Wagner, M. Characterisation of Nanoplastics during the Degradation of Polystyrene. *Chemosphere* **2016**, *145*, 265–268. [CrossRef] [PubMed]
9. Cole, M.; Lindeque, P.; Halsband, C.; Galloway, T.S. Microplastics as Contaminants in the Marine Environment: A Review. *Mar. Pollut. Bull.* **2011**, *62*, 2588–2597. [CrossRef]
10. Paul, M.B.; Stock, V.; Cara-Carmona, J.; Lisicki, E.; Shopova, S.; Fessard, V.; Braeuning, A.; Sieg, H.; Böhmert, L. Micro- and Nanoplastics—Current State of Knowledge with the Focus on Oral Uptake and Toxicity. *Nanoscale Adv.* **2020**, *2*, 4350–4367. [CrossRef]
11. Gaylarde, C.C.; Baptista Neto, J.A.; Da Fonseca, E.M. Nanoplastics in Aquatic Systems—Are They More Hazardous than Microplastics? *Environ. Pollut.* **2021**, *272*, 115950. [CrossRef]
12. Klaine, S.J.; Koelmans, A.A.; Horne, N.; Carley, S.; Handy, R.D.; Kapustka, L.; Nowack, B.; Von Der Kammer, F. Paradigms to Assess the Environmental Impact of Manufactured Nanomaterials. *Environ. Toxicol. Chem.* **2012**, *31*, 3–14. [CrossRef]
13. Mattsson, K.; Ekvall, M.T.; Hansson, L.-A.; Linse, S.; Malmendal, A.; Cedervall, T. Altered Behavior, Physiology, and Metabolism in Fish Exposed to Polystyrene Nanoparticles. *Environ. Sci. Technol.* **2015**, *49*, 553–561. [CrossRef]
14. Mattsson, K.; Jocic, S.; Doverbratt, I.; Hansson, L.-A. Nanoplastics in the Aquatic Environment. In *Microplastic Contamination in Aquatic Environments*; Elsevier: Amsterdam, The Netherlands, 2018; pp. 379–399, ISBN 978-0-12-813747-5.
15. Shi, C.; Liu, Z.; Yu, B.; Zhang, Y.; Yang, H.; Han, Y.; Wang, B.; Liu, Z.; Zhang, H. Emergence of Nanoplastics in the Aquatic Environment and Possible Impacts on Aquatic Organisms. *Sci. Total Environ.* **2024**, *906*, 167404. [CrossRef]
16. Ferreira, I.; Venâncio, C.; Lopes, I.; Oliveira, M. Nanoplastics and Marine Organisms: What Has Been Studied? *Environ. Toxicol. Pharmacol.* **2019**, *67*, 1–7. [CrossRef] [PubMed]
17. Moore, C.J. Synthetic Polymers in the Marine Environment: A Rapidly Increasing, Long-Term Threat. *Environ. Res.* **2008**, *108*, 131–139. [CrossRef] [PubMed]
18. Sussarellu, R.; Suquet, M.; Thomas, Y.; Lambert, C.; Fabioux, C.; Pernet, M.E.J.; Le Goïc, N.; Quillien, V.; Mingant, C.; Epelboin, Y.; et al. Oyster Reproduction Is Affected by Exposure to Polystyrene Microplastics. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 2430–2435. [CrossRef] [PubMed]
19. Della Torre, C.; Bergami, E.; Salvati, A.; Faleri, C.; Cirino, P.; Dawson, K.A.; Corsi, I. Accumulation and Embryotoxicity of Polystyrene Nanoparticles at Early Stage of Development of Sea Urchin Embryos *Paracentrotus lividus*. *Environ. Sci. Technol.* **2014**, *48*, 12302–12311. [CrossRef] [PubMed]
20. Pinsino, A.; Bergami, E.; Della Torre, C.; Vannuccini, M.L.; Addis, P.; Secci, M.; Dawson, K.A.; Matranga, V.; Corsi, I. Amino-Modified Polystyrene Nanoparticles Affect Signalling Pathways of the Sea Urchin (*Paracentrotus lividus*) Embryos. *Nanotoxicology* **2017**, *11*, 201–209. [CrossRef]
21. Pitt, J.A.; Kozal, J.S.; Jayasundara, N.; Massarsky, A.; Trevisan, R.; Geitner, N.; Wiesner, M.; Levin, E.D.; Di Giulio, R.T. Uptake, Tissue Distribution, and Toxicity of Polystyrene Nanoparticles in Developing Zebrafish (*Danio rerio*). *Aquat. Toxicol. Amst. Neth.* **2018**, *194*, 185–194. [CrossRef]
22. Duan, Z.; Duan, X.; Zhao, S.; Wang, X.; Wang, J.; Liu, Y.; Peng, Y.; Gong, Z.; Wang, L. Barrier Function of Zebrafish Embryonic Chorions against Microplastics and Nanoplastics and Its Impact on Embryo Development. *J. Hazard. Mater.* **2020**, *395*, 122621. [CrossRef]
23. Eliso, M.C.; Bergami, E.; Manfra, L.; Spagnuolo, A.; Corsi, I. Toxicity of Nanoplastics during the Embryogenesis of the Ascidian *Ciona robusta* (Phylum Chordata). *Nanotoxicology* **2020**, *14*, 1415–1431. [CrossRef] [PubMed]
24. Eliso, M.C.; Bergami, E.; Bonciani, L.; Riccio, R.; Belli, G.; Belli, M.; Corsi, I.; Spagnuolo, A. Application of Transcriptome Profiling to Inquire into the Mechanism of Nanoplastics Toxicity during *Ciona robusta* Embryogenesis. *Environ. Pollut.* **2023**, *318*, 120892. [CrossRef] [PubMed]
25. Balbi, T.; Camisassi, G.; Montagna, M.; Fabbri, R.; Franzellitti, S.; Carbone, C.; Dawson, K.; Canesi, L. Impact of Cationic Polystyrene Nanoparticles (PS-NH_2) on Early Embryo Development of *Mytilus galloprovincialis*: Effects on Shell Formation. *Chemosphere* **2017**, *186*, 1–9. [CrossRef] [PubMed]
26. Tallec, K.; Huvet, A.; Di Poi, C.; González-Fernández, C.; Lambert, C.; Petton, B.; Le Goïc, N.; Berchel, M.; Soudant, P.; Paul-Pont, I. Nanoplastics Impaired Oyster Free Living Stages, Gametes and Embryos. *Environ. Pollut.* **2018**, *242*, 1226–1235. [CrossRef] [PubMed]
27. Li, Y.; Liu, Z.; Jiang, Q.; Ye, Y.; Zhao, Y. Effects of Nanoplastic on Cell Apoptosis and Ion Regulation in the Gills of *Macrobrachium nipponense*. *Environ. Pollut.* **2022**, *300*, 118989. [CrossRef] [PubMed]
28. Liu, Z.; Zhang, Y.; Zheng, Y.; Feng, Y.; Zhang, W.; Gong, S.; Lin, H.; Gao, P.; Zhang, H. Genome-Wide Identification Glutathione-S-Transferase Gene Superfamily in *Daphnia pulex* and Its Transcriptional Response to Nanoplastics. *Int. J. Biol. Macromol.* **2023**, *230*, 123112. [CrossRef] [PubMed]

29. Gambardella, C.; Morgana, S.; Ferrando, S.; Bramini, M.; Piazza, V.; Costa, E.; Garaventa, F.; Faimali, M. Effects of Polystyrene Microbeads in Marine Planktonic Crustaceans. *Ecotoxicol. Environ. Saf.* **2017**, *145*, 250–257. [CrossRef] [PubMed]
30. Bergami, E.; Krupinski Emerenciano, A.; González-Aravena, M.; Cárdenas, C.A.; Hernández, P.; Silva, J.R.M.C.; Corsi, I. Polystyrene Nanoparticles Affect the Innate Immune System of the Antarctic Sea Urchin Sterechinus Neumayeri. *Polar Biol.* **2019**, *42*, 743–757. [CrossRef]
31. Qiao, R.; Sheng, C.; Lu, Y.; Zhang, Y.; Ren, H.; Lemos, B. Microplastics Induce Intestinal Inflammation, Oxidative Stress, and Disorders of Metabolome and Microbiome in Zebrafish. *Sci. Total Environ.* **2019**, *662*, 246–253. [CrossRef]
32. Cappello, T.; De Marco, G.; Oliveri Conti, G.; Giannetto, A.; Ferrante, M.; Mauceri, A.; Maisano, M. Time-Dependent Metabolic Disorders Induced by Short-Term Exposure to Polystyrene Microplastics in the Mediterranean Mussel *Mytilus galloprovincialis*. *Ecotoxicol. Environ. Saf.* **2021**, *209*, 111780. [CrossRef] [PubMed]
33. Murano, C.; Bergami, E.; Liberatori, G.; Palumbo, A.; Corsi, I. Interplay between Nanoplastics and the Immune System of the Mediterranean Sea Urchin *Paracentrotus lividus*. *Front. Mar. Sci.* **2021**, *8*, 647394. [CrossRef]
34. Murano, C.; Nonnis, S.; Scalvini, F.G.; Maffioli, E.; Corsi, I.; Tedeschi, G.; Palumbo, A. Response to Microplastic Exposure: An Exploration into the Sea Urchin Immune Cell Proteome. *Environ. Pollut.* **2023**, *320*, 121062. [CrossRef] [PubMed]
35. De Marco, G.; Conti, G.O.; Giannetto, A.; Cappello, T.; Galati, M.; Iaria, C.; Pulvirenti, E.; Capparucci, F.; Mauceri, A.; Ferrante, M.; et al. Embryotoxicity of Polystyrene Microplastics in Zebrafish *Danio rerio*. *Environ. Res.* **2022**, *208*, 112552. [CrossRef]
36. De Marco, G.; Eliso, M.C.; Conti, G.O.; Galati, M.; Billè, B.; Maisano, M.; Ferrante, M.; Cappello, T. Short-Term Exposure to Polystyrene Microplastics Hampers the Cellular Function of Gills in the Mediterranean Mussel *Mytilus galloprovincialis*. *Aquat. Toxicol.* **2023**, *264*, 106736. [CrossRef]
37. Portugal, J.; Mansilla, S.; Piña, B. Perspectives on the Use of Toxicogenomics to Assess Environmental Risk. *Front. Biosci.-Landmark* **2022**, *27*, 294. [CrossRef]
38. Brockmeier, E.K.; Hodges, G.; Hutchinson, T.H.; Butler, E.; Hecker, M.; Tollefsen, K.E.; Garcia-Reyero, N.; Kille, P.; Becker, D.; Chipman, K.; et al. The Role of Omics in the Application of Adverse Outcome Pathways for Chemical Risk Assessment. *Toxicol. Sci.* **2017**, *158*, 252–262. [CrossRef]
39. Zhang, X.; Xia, P.; Wang, P.; Yang, J.; Baird, D.J. Omics Advances in Ecotoxicology. *Environ. Sci. Technol.* **2018**, *52*, 3842–3851. [CrossRef]
40. Sauer, U.G.; Deferme, L.; Gribaldo, L.; Hackermüller, J.; Tralau, T.; Van Ravenzwaay, B.; Yauk, C.; Poole, A.; Tong, W.; Gant, T.W. The Challenge of the Application of 'omics Technologies in Chemicals Risk Assessment: Background and Outlook. *Regul. Toxicol. Pharmacol.* **2017**, *91*, S14–S26. [CrossRef]
41. Cui, M.; Cheng, C.; Zhang, L. High-Throughput Proteomics: A Methodological Mini-Review. *Lab. Investig.* **2022**, *102*, 1170–1181. [CrossRef]
42. Wu, Y.; Zeng, J.; Zhang, F.; Zhu, Z.; Qi, T.; Zheng, Z.; Lloyd-Jones, L.R.; Marioni, R.E.; Martin, N.G.; Montgomery, G.W.; et al. Integrative Analysis of Omics Summary Data Reveals Putative Mechanisms Underlying Complex Traits. *Nat. Commun.* **2018**, *9*, 918. [CrossRef]
43. Cappello, T. NMR-Based Metabolomics of Aquatic Organisms. *eMagRes* **2020**, *9*, 81–100. [CrossRef]
44. Ebner, J.N. Trends in the Application of "Omics" to Ecotoxicology and Stress Ecology. *Genes* **2021**, *12*, 1481. [CrossRef] [PubMed]
45. Madeira, C.; Costa, P.M. Proteomics in Systems Toxicology. In *Advances in Protein Chemistry and Structural Biology*; Elsevier: Amsterdam, The Netherlands, 2021; Volume 127, pp. 55–91, ISBN 978-0-323-85319-4.
46. Taylor, N.; Gavin, A.; Viant, M. Metabolomics Discovers Early-Response Metabolic Biomarkers That Can Predict Chronic Reproductive Fitness in Individual *Daphnia magna*. *Metabolites* **2018**, *8*, 42. [CrossRef] [PubMed]
47. Aydin, M.E.; Aydin, S.; Tongur, S.; Kara, G.; Kolb, M.; Bahadir, M. Application of Simple and Low-Cost Toxicity Tests for Ecotoxicological Assessment of Industrial Wastewaters. *Environ. Technol.* **2015**, *36*, 2825–2834. [CrossRef] [PubMed]
48. Snape, J.R.; Maund, S.J.; Pickford, D.B.; Hutchinson, T.H. Ecotoxicogenomics: The Challenge of Integrating Genomics into Aquatic and Terrestrial Ecotoxicology. *Aquat. Toxicol.* **2004**, *67*, 143–154. [CrossRef] [PubMed]
49. Suman, A.; Mahapatra, A.; Gupta, P.; Ray, S.S.; Singh, R.K. Polystyrene Microplastics Modulated Bdnf Expression Triggering Neurotoxicity via Apoptotic Pathway in Zebrafish Embryos. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2023**, *271*, 109699. [CrossRef]
50. Martin-Folgar, R.; Torres-Ruiz, M.; De Alba, M.; Cañas-Portilla, A.I.; González, M.C.; Morales, M. Molecular Effects of Polystyrene Nanoplastics Toxicity in Zebrafish Embryos (*Danio rerio*). *Chemosphere* **2023**, *312*, 137077. [CrossRef]
51. Qiang, L.; Cheng, J. Exposure to Microplastics Decreases Swimming Competence in Larval Zebrafish (*Danio rerio*). *Ecotoxicol. Environ. Saf.* **2019**, *176*, 226–233. [CrossRef] [PubMed]
52. Zhao, Y.; Bao, Z.; Wan, Z.; Fu, Z.; Jin, Y. Polystyrene Microplastic Exposure Disturbs Hepatic Glycolipid Metabolism at the Physiological, Biochemical, and Transcriptomic Levels in Adult Zebrafish. *Sci. Total Environ.* **2020**, *710*, 136279. [CrossRef]
53. Limonta, G.; Mancia, A.; Benkhalqui, A.; Bertolucci, C.; Abelli, L.; Fossi, M.C.; Panti, C. Microplastics Induce Transcriptional Changes, Immune Response and Behavioral Alterations in Adult Zebrafish. *Sci. Rep.* **2019**, *9*, 15775. [CrossRef]
54. Pedersen, A.F.; Meyer, D.N.; Petriv, A.-M.V.; Soto, A.L.; Shields, J.N.; Akemann, C.; Baker, B.B.; Tsou, W.-L.; Zhang, Y.; Baker, T.R. Nanoplastics Impact the Zebrafish (*Danio rerio*) Transcriptome: Associated Developmental and Neurobehavioral Consequences. *Environ. Pollut. Barking Essex* **2020**, *266*, 115090. [CrossRef]

55. Ašmonaitė, G.; Sundh, H.; Asker, N.; Carney Almroth, B. Rainbow Trout Maintain Intestinal Transport and Barrier Functions Following Exposure to Polystyrene Microplastics. *Environ. Sci. Technol.* **2018**, *52*, 14392–14401. [CrossRef]
56. Lu, C.; Kania, P.W.; Buchmann, K. Particle Effects on Fish Gills: An Immunogenetic Approach for Rainbow Trout and Zebrafish. *Aquaculture* **2018**, *484*, 98–104. [CrossRef]
57. Zhang, Y.T.; Chen, M.; He, S.; Fang, C.; Chen, M.; Li, D.; Wu, D.; Chernick, M.; Hinton, D.E.; Bo, J.; et al. Microplastics Decrease the Toxicity of Triphenyl Phosphate (TPhP) in the Marine Medaka (*Oryzias melastigma*) Larvae. *Sci. Total Environ.* **2021**, *763*, 143040. [CrossRef]
58. Chen, J.-C.; Chen, M.-Y.; Fang, C.; Zheng, R.-H.; Jiang, Y.-L.; Zhang, Y.-S.; Wang, K.-J.; Bailey, C.; Segner, H.; Bo, J. Microplastics Negatively Impact Embryogenesis and Modulate the Immune Response of the Marine Medaka *Oryzias melastigma*. *Mar. Pollut. Bull.* **2020**, *158*, 111349. [CrossRef]
59. Varó, I.; Perini, A.; Torreblanca, A.; Garcia, Y.; Bergami, E.; Vannuccini, M.L.; Corsi, I. Time-Dependent Effects of Polystyrene Nanoparticles in Brine Shrimp *Artemia franciscana* at Physiological, Biochemical and Molecular Levels. *Sci. Total Environ.* **2019**, *675*, 570–580. [CrossRef]
60. Suman, T.Y.; Jia, P.-P.; Li, W.-G.; Junaid, M.; Xin, G.-Y.; Wang, Y.; Pei, D.-S. Acute and Chronic Effects of Polystyrene Microplastics on Brine Shrimp: First Evidence Highlighting the Molecular Mechanism through Transcriptome Analysis. *J. Hazard. Mater.* **2020**, *400*, 123220. [CrossRef]
61. Bergami, E.; Pugnalini, S.; Vannuccini, M.L.; Manfra, L.; Faleri, C.; Savorelli, F.; Dawson, K.A.; Corsi, I. Long-Term Toxicity of Surface-Charged Polystyrene Nanoplastics to Marine Planktonic Species *Dunaliella tertiolecta* and *Artemia franciscana*. *Aquat. Toxicol. Amst. Neth.* **2017**, *189*, 159–169. [CrossRef]
62. De Felice, B.; Sugni, M.; Casati, L.; Parolini, M. Molecular, Biochemical and Behavioral Responses of *Daphnia magna* under Long-Term Exposure to Polystyrene Nanoplastics. *Environ. Int.* **2022**, *164*, 107264. [CrossRef]
63. Liu, Z.; Yu, P.; Cai, M.; Wu, D.; Zhang, M.; Huang, Y.; Zhao, Y. Polystyrene Nanoplastic Exposure Induces Immobilization, Reproduction, and Stress Defense in the Freshwater Cladoceran *Daphnia pulex*. *Chemosphere* **2019**, *215*, 74–81. [CrossRef]
64. Liu, Z.; Li, Y.; Pérez, E.; Jiang, Q.; Chen, Q.; Jiao, Y.; Huang, Y.; Yang, Y.; Zhao, Y. Polystyrene Nanoplastic Induces Oxidative Stress, Immune Defense, and Glycometabolism Change in *Daphnia pulex*: Application of Transcriptome Profiling in Risk Assessment of Nanoplastics. *J. Hazard. Mater.* **2021**, *402*, 123778. [CrossRef]
65. Zhang, W.; Liu, Z.; Tang, S.; Li, D.; Jiang, Q.; Zhang, T. Transcriptional Response Provides Insights into the Effect of Chronic Polystyrene Nanoplastic Exposure on *Daphnia pulex*. *Chemosphere* **2020**, *238*, 124563. [CrossRef]
66. Zhu, X.; Teng, J.; Xu, E.G.; Zhao, J.; Shan, E.; Sun, C.; Wang, Q. Toxicokinetics and Toxicodynamics of Plastic and Metallic Nanoparticles: A Comparative Study in Shrimp. *Environ. Pollut.* **2022**, *312*, 120069. [CrossRef]
67. Liu, L.; Zheng, H.; Luan, L.; Luo, X.; Wang, X.; Lu, H.; Li, Y.; Wen, L.; Li, F.; Zhao, J. Functionalized Polystyrene Nanoplastic-Induced Energy Homeostasis Imbalance and the Immunomodulation Dysfunction of Marine Clams (*Meretrix Meretrix*) at Environmentally Relevant Concentrations. *Environ. Sci. Nano* **2021**, *8*, 2030–2048. [CrossRef]
68. Capolupo, M.; Franzellitti, S.; Valbonesi, P.; Lanzas, C.S.; Fabbri, E. Uptake and Transcriptional Effects of Polystyrene Microplastics in Larval Stages of the Mediterranean Mussel *Mytilus galloprovincialis*. *Environ. Pollut.* **2018**, *241*, 1038–1047. [CrossRef]
69. Auguste, M.; Lasa, A.; Balbi, T.; Pallavicini, A.; Vezzulli, L.; Canesi, L. Impact of Nanoplastics on Hemolymph Immune Parameters and Microbiota Composition in *Mytilus galloprovincialis*. *Mar. Environ. Res.* **2020**, *159*, 105017. [CrossRef]
70. Gardon, T.; Morvan, L.; Huvet, A.; Quillien, V.; Soyez, C.; Le Moullac, G.; Le Luyer, J. Microplastics Induce Dose-Specific Transcriptomic Disruptions in Energy Metabolism and Immunity of the Pearl Oyster Pinctada Margaritifera. *Environ. Pollut.* **2020**, *266*, 115180. [CrossRef]
71. Capanni, F.; Greco, S.; Tomasi, N.; Giulianini, P.G.; Manfrin, C. Orally Administered Nano-Polystyrene Caused Vitellogenin Alteration and Oxidative Stress in the Red Swamp Crayfish (*Procambarus clarkii*). *Sci. Total Environ.* **2021**, *791*, 147984. [CrossRef]
72. Magyary, I. Recent Advances and Future Trends in Zebrafish Bioassays for Aquatic Ecotoxicology. *Ecocycles* **2018**, *4*, 12–18. [CrossRef]
73. Benson, N.U.; Agboola, O.D.; Fred-Ahmadu, O.H.; De-la-Torre, G.E.; Oluwalana, A.; Williams, A.B. Micro(Nano)Plastics Prevalence, Food Web Interactions, and Toxicity Assessment in Aquatic Organisms: A Review. *Front. Mar. Sci.* **2022**, *9*, 851281. [CrossRef]
74. Rosner, A.; Ballarin, L.; Barnay-Verdier, S.; Borisenko, I.; Drago, L.; Drobne, D.; Concetta Eliso, M.; Harbuzov, Z.; Grimaldi, A.; Guy-Haim, T.; et al. A Broad-taxa Approach as an Important Concept in Ecotoxicological Studies and Pollution Monitoring. *Biol. Rev.* **2023**, *99*, 131–176. [CrossRef]
75. Yin, J.; Long, Y.; Xiao, W.; Liu, D.; Tian, Q.; Li, Y.; Liu, C.; Chen, L.; Pan, Y. Ecotoxicology of Microplastics in *Daphnia*: A Review Focusing on Microplastic Properties and Multiscale Attributes of *Daphnia*. *Ecotoxicol. Environ. Saf.* **2023**, *249*, 114433. [CrossRef]
76. Corsi, I.; Bellingeri, A.; Eliso, M.C.; Grassi, G.; Liberatori, G.; Murano, C.; Sturba, L.; Vannuccini, M.L.; Bergami, E. Eco-Interactions of Engineered Nanomaterials in the Marine Environment: Towards an Eco-Design Framework. *Nanomaterials* **2021**, *11*, 1903. [CrossRef]
77. Lee, K.W.; Park, H.G.; Lee, S.-M.; Kang, H.-K. Effects of Diets on the Growth of the Brackish Water Cyclopoid Copepod *Paracyclopina nana Smirnov*. *Aquaculture* **2006**, *256*, 346–353. [CrossRef]
78. Pinto, C.S.C.; Souza-Santos, L.P.; Santos, P.J.P. Development and Population Dynamics of *Tisbe biminiensis* (Copepoda: Harpacticoida) Reared on Different Diets. *Aquaculture* **2001**, *198*, 253–267. [CrossRef]

79. Sun, B.; Fleeger, J.W. Sustained Mass Culture of *Amphiascoides atopus* a Marine Harpacticoid Copepod in a Recirculating System. *Aquaculture* **1995**, *136*, 313–321. [CrossRef]
80. Botterell, Z.L.R.; Beaumont, N.; Dorrington, T.; Steinke, M.; Thompson, R.C.; Lindeque, P.K. Bioavailability and Effects of Microplastics on Marine Zooplankton: A Review. *Environ. Pollut.* **2019**, *245*, 98–110. [CrossRef]
81. Dupree, E.J.; Jayathirtha, M.; Yorkey, H.; Mihasan, M.; Petre, B.A.; Darie, C.C. A Critical Review of Bottom-Up Proteomics: The Good, the Bad, and the Future of This Field. *Proteomes* **2020**, *8*, 14. [CrossRef]
82. Duong, V.-A.; Park, J.-M.; Lee, H. Review of Three-Dimensional Liquid Chromatography Platforms for Bottom-Up Proteomics. *Int. J. Mol. Sci.* **2020**, *21*, 1524. [CrossRef]
83. Chen, G.; Pramanik, B.N. LC-MS for Protein Characterization: Current Capabilities and Future Trends. *Expert Rev. Proteom.* **2008**, *5*, 435–444. [CrossRef] [PubMed]
84. Chen, G.; Liu, Y.; Pramanik, B.N. LC/MS Analysis of Proteins and Peptides in Drug Discovery. In *HPLC for Pharmaceutical Scientists*; Kazakevich, Y., LoBrutto, R., Eds.; Wiley: Hoboken, NJ, USA, 2007; pp. 837–899, ISBN 978-0-471-68162-5.
85. Chen, G.; Pramanik, B.N.; Liu, Y.; Mirza, U.A. Applications of LC/MS in Structure Identifications of Small Molecules and Proteins in Drug Discovery. *J. Mass Spectrom.* **2007**, *42*, 279–287. [CrossRef] [PubMed]
86. Chen, G.; Pramanik, B.N. Application of LC/MS to Proteomics Studies: Current Status and Future Prospects. *Drug Discov. Today* **2009**, *14*, 465–471. [CrossRef] [PubMed]
87. Gajahin Gamage, N.T.; Miyashita, R.; Takahashi, K.; Asakawa, S.; Senevirathna, J.D.M. Proteomic Applications in Aquatic Environment Studies. *Proteomes* **2022**, *10*, 32. [CrossRef] [PubMed]
88. Gu, L.; Tian, L.; Gao, G.; Peng, S.; Zhang, J.; Wu, D.; Huang, J.; Hua, Q.; Lu, T.; Zhong, L.; et al. Inhibitory Effects of Polystyrene Microplastics on Caudal Fin Regeneration in Zebrafish Larvae. *Environ. Pollut.* **2020**, *266*, 114664. [CrossRef] [PubMed]
89. Yang, H.; Lai, H.; Huang, J.; Sun, L.; Mennigen, J.A.; Wang, Q.; Liu, Y.; Jin, Y.; Tu, W. Polystyrene Microplastics Decrease F–53B Bioaccumulation but Induce Inflammatory Stress in Larval Zebrafish. *Chemosphere* **2020**, *255*, 127040. [CrossRef]
90. Jeong, C.-B.; Won, E.-J.; Kang, H.-M.; Lee, M.-C.; Hwang, D.-S.; Hwang, U.-K.; Zhou, B.; Souissi, S.; Lee, S.-J.; Lee, J.-S. Microplastic Size-Dependent Toxicity, Oxidative Stress Induction, and p-JNK and p-P38 Activation in the Monogonont Rotifer (*Brachionus koreanus*). *Environ. Sci. Technol.* **2016**, *50*, 8849–8857. [CrossRef]
91. Trotter, B.; Wilde, M.V.; Brehm, J.; Dafni, E.; Aliu, A.; Arnold, G.J.; Fröhlich, T.; Laforsch, C. Long-Term Exposure of *Daphnia magna* to Polystyrene Microplastic (PS-MP) Leads to Alterations of the Proteome, Morphology and Life-History. *Sci. Total Environ.* **2021**, *795*, 148822. [CrossRef]
92. Zhu, C.; Zhang, T.; Liu, X.; Gu, X.; Li, D.; Yin, J.; Jiang, Q.; Zhang, W. Changes in Life-History Traits, Antioxidant Defense, Energy Metabolism and Molecular Outcomes in the Cladoceran *Daphnia pulex* after Exposure to Polystyrene Microplastics. *Chemosphere* **2022**, *308*, 136066. [CrossRef]
93. Liu, Z.; Li, Y.; Sepúlveda, M.S.; Jiang, Q.; Jiao, Y.; Chen, Q.; Huang, Y.; Tian, J.; Zhao, Y. Development of an Adverse Outcome Pathway for Nanoplastic Toxicity in *Daphnia pulex* Using Proteomics. *Sci. Total Environ.* **2021**, *766*, 144249. [CrossRef] [PubMed]
94. Magni, S.; Della Torre, C.; Garrone, G.; D'Amato, A.; Parenti, C.C.; Binelli, A. First Evidence of Protein Modulation by Polystyrene Microplastics in a Freshwater Biological Model. *Environ. Pollut.* **2019**, *250*, 407–415. [CrossRef]
95. Duan, Y.; Xiong, D.; Wang, Y.; Zhang, Z.; Li, H.; Dong, H.; Zhang, J. Toxicological Effects of Microplastics in *Litopenaeus vannamei* as Indicated by an Integrated Microbiome, Proteomic and Metabolomic Approach. *Sci. Total Environ.* **2021**, *761*, 143311. [CrossRef]
96. Jeong, C.-B.; Kang, H.-M.; Lee, M.-C.; Kim, D.-H.; Han, J.; Hwang, D.-S.; Souissi, S.; Lee, S.-J.; Shin, K.-H.; Park, H.G.; et al. Adverse Effects of Microplastics and Oxidative Stress-Induced MAPK/Nrf2 Pathway-Mediated Defense Mechanisms in the Marine Copepod *Paracyclopina nana*. *Sci. Rep.* **2017**, *7*, 41323. [CrossRef]
97. Zhang, C.; Jeong, C.-B.; Lee, J.-S.; Wang, D.; Wang, M. Transgenerational Proteome Plasticity in Resilience of a Marine Copepod in Response to Environmentally Relevant Concentrations of Microplastics. *Environ. Sci. Technol.* **2019**, *53*, 8426–8436. [CrossRef]
98. Oliver, S. Systematic Functional Analysis of the Yeast Genome. *Trends Biotechnol.* **1998**, *16*, 373–378. [CrossRef] [PubMed]
99. Lin, C.Y.; Viant, M.R.; Tjeerdema, R.S. Metabolomics: Methodologies and Applications in the Environmental Sciences. *J. Pestic. Sci.* **2006**, *31*, 245–251. [CrossRef]
100. Johnson, C.H.; Ivanisevic, J.; Siuzdak, G. Metabolomics: Beyond Biomarkers and towards Mechanisms. *Nat. Rev. Mol. Cell Biol.* **2016**, *17*, 451–459. [CrossRef] [PubMed]
101. Nagana Gowda, G.A.; Raftery, D. NMR-Based Metabolomics. In *Cancer Metabolomics*; Hu, S., Ed.; Advances in Experimental Medicine and Biology; Springer International Publishing: Cham, Switzerland, 2021; Volume 1280, pp. 19–37, ISBN 978-3-030-51651-2.
102. Gowda, G.A.N.; Djukovic, D. Overview of Mass Spectrometry-Based Metabolomics: Opportunities and Challenges. In *Mass Spectrometry in Metabolomics*; Raftery, D., Ed.; Methods in Molecular Biology; Springer: New York, NY, USA, 2014; Volume 1198, pp. 3–12, ISBN 978-1-4939-1257-5.
103. Idle, J.R.; Gonzalez, F.J. Metabolomics. *Cell Metab.* **2007**, *6*, 348–351. [CrossRef] [PubMed]
104. De Marco, G.; Billè, B.; Brandão, F.; Galati, M.; Pereira, P.; Cappello, T.; Pacheco, M. Differential Cell Metabolic Pathways in Gills and Liver of Fish (White Seabream *Diplodus sargus*) Coping with Dietary Methylmercury Exposure. *Toxics* **2023**, *11*, 181. [CrossRef]

105. Brandão, F.; Cappello, T.; Raimundo, J.; Santos, M.A.; Maisano, M.; Mauceri, A.; Pacheco, M.; Pereira, P. Unravelling the Mechanisms of Mercury Hepatotoxicity in Wild Fish (*Liza aurata*) through a Triad Approach: Bioaccumulation, Metabolomic Profiles and Oxidative Stress. *Metallomics* **2015**, *7*, 1352–1363. [CrossRef]
106. Cappello, T.; Maisano, M.; Giannetto, A.; Parrino, V.; Mauceri, A.; Fasulo, S. Neurotoxicological Effects on Marine Mussel *Mytilus galloprovincialis* Caged at Petrochemical Contaminated Areas (Eastern Sicily, Italy): 1H NMR and Immunohistochemical Assays. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2015**, *169*, 7–15. [CrossRef] [PubMed]
107. Cappello, T.; Brandão, F.; Guilherme, S.; Santos, M.A.; Maisano, M.; Mauceri, A.; Canário, J.; Pacheco, M.; Pereira, P. Insights into the Mechanisms Underlying Mercury-Induced Oxidative Stress in Gills of Wild Fish (*Liza aurata*) Combining 1 H NMR Metabolomics and Conventional Biochemical Assays. *Sci. Total Environ.* **2016**, *548–549*, 13–24. [CrossRef] [PubMed]
108. Cappello, T.; Maisano, M.; Mauceri, A.; Fasulo, S. 1 H NMR-Based Metabolomics Investigation on the Effects of Petrochemical Contamination in Posterior Adductor Muscles of Caged Mussel *Mytilus galloprovincialis*. *Ecotoxicol. Environ. Saf.* **2017**, *142*, 417–422. [CrossRef] [PubMed]
109. Cappello, T.; Giannetto, A.; Parrino, V.; De Marco, G.; Mauceri, A.; Maisano, M. Food Safety Using NMR-Based Metabolomics: Assessment of the Atlantic Bluefin Tuna, *Thunnus thynnus*, from the Mediterranean Sea. *Food Chem. Toxicol.* **2018**, *115*, 391–397. [CrossRef] [PubMed]
110. Missawi, O.; Venditti, M.; Cappello, T.; Zitouni, N.; Marco, G.D.; Boughattas, I.; Bousserrhine, N.; Belbekhouche, S.; Minucci, S.; Maisano, M.; et al. Autophagic Event and Metabolomic Disorders Unveil Cellular Toxicity of Environmental Microplastics on Marine Polychaete Hediste Diversicolor. *Environ. Pollut.* **2022**, *302*, 119106. [CrossRef] [PubMed]
111. Vignet, C.; Cappello, T.; Fu, Q.; Lajoie, K.; De Marco, G.; Clérandeau, C.; Mottaz, H.; Maisano, M.; Hollender, J.; Schirmer, K.; et al. Imidacloprid Induces Adverse Effects on Fish Early Life Stages That Are More Severe in Japanese Medaka (*Oryzias latipes*) than in Zebrafish (*Danio rerio*). *Chemosphere* **2019**, *225*, 470–478. [CrossRef]
112. Zitouni, N.; Cappello, T.; Missawi, O.; Boughattas, I.; De Marco, G.; Belbekhouche, S.; Mokni, M.; Alphonse, V.; Guerbej, H.; Bousserrhine, N.; et al. Metabolomic Disorders Unveil Hepatotoxicity of Environmental Microplastics in Wild Fish Serranus Scriba (Linnaeus 1758). *Sci. Total Environ.* **2022**, *838*, 155872. [CrossRef] [PubMed]
113. Xu, H.-D.; Wang, J.-S.; Li, M.-H.; Liu, Y.; Chen, T.; Jia, A.-Q. 1H NMR Based Metabolomics Approach to Study the Toxic Effects of Herbicide Butachlor on Goldfish (*Carassius auratus*). *Aquat. Toxicol.* **2015**, *159*, 69–80. [CrossRef]
114. Wan, Z.; Wang, C.; Zhou, J.; Shen, M.; Wang, X.; Fu, Z.; Jin, Y. Effects of Polystyrene Microplastics on the Composition of the Microbiome and Metabolism in Larval Zebrafish. *Chemosphere* **2019**, *217*, 646–658. [CrossRef]
115. Kaloyianni, M.; Bobori, D.C.; Xanthopoulou, D.; Malioufa, G.; Sampsonidis, I.; Kalogiannis, S.; Feidantsis, K.; Kastrinaki, G.; Dimitriadi, A.; Koumoundouros, G.; et al. Toxicity and Functional Tissue Responses of Two Freshwater Fish after Exposure to Polystyrene Microplastics. *Toxics* **2021**, *9*, 289. [CrossRef]
116. Wang, C.; Hou, M.; Shang, K.; Wang, H.; Wang, J. Microplastics (Polystyrene) Exposure Induces Metabolic Changes in the Liver of Rare Minnow (*Gobiocypris rarus*). *Molecules* **2022**, *27*, 584. [CrossRef]
117. Pang, M.; Wang, Y.; Tang, Y.; Dai, J.; Tong, J.; Jin, G. Transcriptome Sequencing and Metabolite Analysis Reveal the Toxic Effects of Nanoplastics on Tilapia after Exposure to Polystyrene. *Environ. Pollut.* **2021**, *277*, 116860. [CrossRef]
118. Ding, J.; Huang, Y.; Liu, S.; Zhang, S.; Zou, H.; Wang, Z.; Zhu, W.; Geng, J. Toxicological Effects of Nano- and Micro-Polystyrene Plastics on Red Tilapia: Are Larger Plastic Particles More Harmless? *J. Hazard. Mater.* **2020**, *396*, 122693. [CrossRef]
119. Usman, S.; Razis, A.F.A.; Shaari, K.; Azmai, M.N.A.; Saad, M.Z.; Isa, N.M.; Nazarudin, M.F. Polystyrene Microplastics Induce Gut Microbiome and Metabolome Changes in Javanese Medaka Fish (*Oryzias javanicus* Bleeker, 1854). *Toxicol. Rep.* **2022**, *9*, 1369–1379. [CrossRef]
120. Ye, G.; Zhang, X.; Liu, X.; Liao, X.; Zhang, H.; Yan, C.; Lin, Y.; Huang, Q. Polystyrene Microplastics Induce Metabolic Disturbances in Marine Medaka (*Oryzias melastigmas*) Liver. *Sci. Total Environ.* **2021**, *782*, 146885. [CrossRef]
121. Sun, X.; Wang, X.; Booth, A.M.; Zhu, L.; Sui, Q.; Chen, B.; Qu, K.; Xia, B. New Insights into the Impact of Polystyrene Micro/Nanoplastics on the Nutritional Quality of Marine Jacopever (*Sebastes schlegelii*). *Sci. Total Environ.* **2023**, *903*, 166560. [CrossRef] [PubMed]
122. Zhang, Y.-K.; Yang, B.-K.; Zhang, C.-N.; Xu, S.-X.; Sun, P. Effects of Polystyrene Microplastics Acute Exposure in the Liver of Swordtail Fish (*Xiphophorus helleri*) Revealed by LC-MS Metabolomics. *Sci. Total Environ.* **2022**, *850*, 157772. [CrossRef] [PubMed]
123. Du, Y.; Zhao, J.; Teng, J.; Ren, J.; Shan, E.; Zhu, X.; Zhang, W.; Wang, L.; Hou, C.; Wang, Q. Combined Effects of Salinity and Polystyrene Microplastics Exposure on the Pacific Oysters Crassostrea Gigas: Oxidative Stress and Energy Metabolism. *Mar. Pollut. Bull.* **2023**, *193*, 115153. [CrossRef] [PubMed]
124. Kelpsiene, E.; Cedervall, T.; Malmendal, A. Metabolomics-Based Analysis in *Daphnia magna* after Exposure to Low Environmental Concentrations of Polystyrene Nanoparticles. *Environ. Sci. Nano* **2023**, *10*, 1858–1866. [CrossRef]
125. Zeng, Y.; Deng, B.; Kang, Z.; Araujo, P.; Mjøs, S.A.; Liu, R.; Lin, J.; Yang, T.; Qu, Y. Tissue Accumulation of Polystyrene Microplastics Causes Oxidative Stress, Hepatopancreatic Injury and Metabolome Alterations in *Litopenaeus vannamei*. *Ecotoxicol. Environ. Saf.* **2023**, *256*, 114871. [CrossRef] [PubMed]