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Inhibition of Xenobiotics Transporters' Efflux Ability after Nanoplastics Exposure in Larval Japanese Medaka

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Abstract: Nanoplastics can enter into the aquatic environment as primary nano-sized or fragmented from larger-sized plastic particles, and their ecological effects and environmental fate have aroused increasing public concerns. Here, we identified the disruption of ATP-binding cassette (ABC) efflux after polystyrene (PS) nanoplastics (76 ± 7 nm) exposure in larval Japanese medaka (*Oryzias latipes*). Nanoplastics (0.001–10 μg/mL) caused 3–6-fold higher lipid peroxidation in fish larvae than the control, with concomitant downregulated expression of efflux transporter-related genes (*abcb6a*, *abcc2*, *abcg2*). Two probes of rhodamine (indicative of p-glycoprotein function for parent compounds' efflux, P-gp) and fluorescein (indicative of multidrug resistance-associated protein function for metabolites' efflux, MRP) were further used to verify the inhibited ABC efflux ability, via rhodamine and fluorescein bioaccumulation results. Three-fold higher accumulation of rhodamine was observed following treatment with 10 μg/mL of nanoplastics. Excessive accumulation also occurred for fluorescein, with 1.7–1.8-fold higher concentrations than controls in larvae treated with 0.01–0.1 μg/mL of nanoplastics. Although the inhibition of ABC transporters diminished after two hours of depuration, the co-existence of nanoplastics and other contaminants still raises concerns. Collectively, this study suggests that nanoplastics can negatively impact ABC transporters' efflux ability and could cause unanticipated accumulation of co-existing organic pollutants in aquatic organisms.

Keywords: nanoplastics; lipid peroxidation; ATP-binding cassette; P-gp efflux; MRP efflux

Citation: Yu, H.; Gao, Z.; Yang, Y.; Li, M.; Chen, Q. Inhibition of Xenobiotics Transporters' Efflux Ability after Nanoplastics Exposure in Larval Japanese Medaka. *Water* **2022**, *14*, 863. https://doi.org/10.3390/w14060863

Academic Editor: Anna Barra Caracciolo

Received: 10 February 2022
Accepted: 7 March 2022
Published: 10 March 2022

1. Introduction

Nanoplastics (1 nm to 1 μm plastic particles [1]) have been observed in personal care products and environmental media, including surface waters [2,3], either as primary nanoplastics [3,4] or as degraded microplastics. Due to their small size, nanoplastics may be ingested and pose significant threats to organisms as they can potentially pass-through biological membranes. Various toxicological endpoints have been measured at multiple levels of biological organization in organisms following exposure to nanoplastics, such as reduced survival, impaired energy consumption, altered growth/feeding ability, oxidative damage, and impacts to protein synthesis [5–9]. Such effects can be further exacerbated by the interactions between nanoplastics and co-existing contaminants, which can be the adsorbents on nanoplastics or additives used in plastic manufacturing, leading to increased ecotoxicological effects on biota [10–12].

Unlike a classic, ligand-receptor binding mode of action (MoA), non-specific interactions such as oxidative damage have been hypothesized as molecular initiating events or key events for nano-sized particles [13]. Moreover, although various toxicities of nanoplastics have been demonstrated, a majority of these adverse effects can only be observed at concentrations much greater than the predicted environmental-relevant concentration (0.001 μg/mL) [14]. This suggests that a greater focus needs to be placed on exploring sensitive endpoints at environmental-relevant concentrations of nanoplastics for successful risk assessments to be conducted.

One molecular event that has been well-studied in wildlife is the inhibition of multidrug resistance (MDR), which is one of the most important biological functions of ABC proteins [15,16]. MDR-mediated ABC proteins include P-glycoprotein (P-gp), multidrug-resistance-associated protein (MRP), and breast cancer resistance protein (BCRP) [15,17]. BCRP is known to have a narrow substrate spectra mainly including physiological metabolites but not conjugated toxins [18], while the former two proteins are more involved in the defense against environmental toxicants as they are important in the elimination of numerous xenobiotics from the cell [19–21]. For example, the P-gp actively transports lipophilic and cationic amphiphilic substrates from cells to the extracellular environment, such as rhodamine [22]. In contrast, substrates of the MRP are dominated by water-soluble organic anions, such as the glutathione conjugated fluorescein, the hydrolytic metabolite of fluorescein diacetate (FDA) [23,24]. Recent results suggest that cell membrane transporter efflux ability can be inhibited by nanoplastics exposure. Both in vitro and in vivo studies reported that polystyrene (PS) nanoplastics can inhibit ABC transporter activity and subsequently increase arsenic or BDE-47 toxicity in human Caco-2 cells or the marine rotifer (*Brachionus koreanus*) [25,26]. Therefore, this suggests that nanoplastic-induced membrane disruption would cause inhibition of MDR (including P-gps and MRPs) activities [26], leading to decreases in tolerance against environmental pollutants. However, these studies were mainly carried out with cell lines or invertebrate at narrow and high exposure concentrations (1–200 or 0.1–20 μg/mL) and there is a lack of studies on vertebrates closer to humans and a wide range of concentrations, including the current environmental-relevant one (0.001 μg/mL) [14]. In our study, rhodamine was used as an indicator for P-gp efflux activity [27], and fluorescein, the hydrolysis metabolite of FDA, was used as an indicator for MRP activity [28,29].

We hypothesize that the oxidative damage of PS nanoplastics to cellular membranes may alter the function of the transporters [13,30], resulting in enhanced toxicity of pollutants on the organisms. To test this, newly hatched Japanese medaka larvae were exposed to PS nanoplastics, and gene expression of ABC transporters, lipid peroxidation, and oxidative stress in fish were measured. Fish were also exposed to fluorescent xenobiotic probes, and then fish tissue concentrations as well as ABC proteins' efflux ability were assessed. This study will provide a better understanding of the toxicity effects of nanoplastics with other persistent environmental organic pollutants.

2. Materials and Methods

2.1. Medaka Culture

Adult Japanese medaka (*Oryzias latipes*) were acclimatized in artificial freshwater (AFW, Table 1) (at 28 ± 1 °C) under a 14:10 h light:dark cycle. Feeding was fixed daily with professional fish food (PETs Family, Qimei Pet Food Co., Jinjiang, China), twice per day. A breeding stock of medaka was held in glass aquaria according to previously defined culture conditions [31,32]. Embryos were stripped from a population of sexually mature females (n = 50), totaling 2000 embryos, and collected into 500 mL glass beakers, rinsed with Milli-Q water, within 2 h post-fertilization (hpf) for experiments and kept in an incubator (28 ± 1 °C) before exposure.

Table 1. The preparation formula for artificial freshwater (AFW).

Components	Stock Solution Concentration (mg/L)	Added to Milli-Q Water (mL/L)
$CaCl_2 \cdot 2H_2O$	294	10
$MgSO_4 \cdot 7H_2O$	123.3	10
$NaHCO_3$	63	10
KCl	5.5	10

2.2. Nanoplastics Characterization and Reagents

PS nanoplastics were synthesized according to [33], in which they were prepared by mini-emulsion polymerization of styrene with sodium dodecyl sulfate as a surfactant without the addition of magnetic substances. After polymerization, PS were thoroughly washed with Milli-Q water to remove excess surfactant. The nanoplastics were characterized with a transmission electron microscope (TEM, JEM-1400, JEOL, Tokyo, Japan) at Shanghai Institute of Ceramics of the Chinese Academy of Science (SICCAS) (Figure 1). The average particle size was 76 ± 7 nm, which was quantified with Image J software (version 10; NIH, MD, USA) [34]. Analytical-grade verapamil (hydrochloride salt, ≥99.0%), indomethacin (98.5–100.5%), rhodamine B (rhodamine), fluorescein diacetate (FDA), dimethyl sulfoxide (DMSO), and n-butanol, along with all other chemicals used in the present study, were purchased from Shanghai Titan Techonl. Co., Ltd. (Shanghai, China). The FTIR spectra showed characteristic peaks of PS (Figure 1C). The two peaks at 2923 and 3027 cm^{-1} were caused by the C-H deformation of the benzene ring, and those at 1442 and 1446 cm^{-1} were attributed to the C=C stretch of the benzene ring [35]. The zeta potential of nanoplastics in water was −52.8 ± 3.2 mV, tested by dynamic light scattering with a Zetasizer Nano (Malvern Panalytical, Malvern, UK).

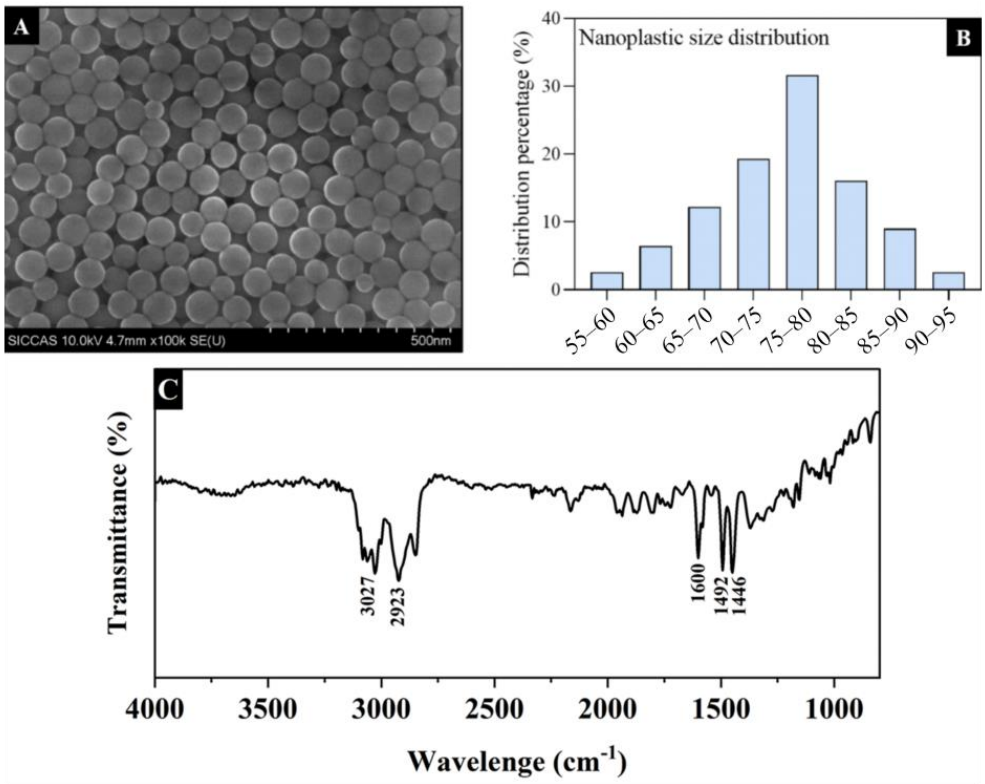

Figure 1. Transmission electron microscopy image of polystyrene (PS) nanoplastic (**A**). Particle size distribution of nanoplastics (**B**). The average nanoplastic diameter was 76 ± 7 nm (mean ± SD, *n* = 150). FTIR spectrum of PS nanoplastic (**C**).

2.3. Experimental Design

2.3.1. Experiment 1: PS Nanoplastics Exposure Test

Eleven-day post-fertilized (dpf) (the life stage was set at 11 dpf as most larvae were fully developed) medaka larvae (20 larvae per replicate and 3 replicates, total 360 larvae) were exposed to a wide range of PS nanoplastics (0.001 to 10 μg/mL) in 30 mL of AFW for 24 h [36,37] until 12 dpf. The mean values of the total length and the weight were 5.01 ± 0.03 mm and 0.67 ± 0.12 mg, respectively. For comparison with recent studies [38,39] and to assess the effects of exposure to nanoplastics, a wide range of doses from 0.001 to 10 μg/mL of nanoplastics were used in this study. Control medaka larvae were incubated

with clean AFW for 24 h. Each group was later conducted in triplicate for molecular and cellular biomarker assessment. The use of animals was approved by the National Animal Welfare Law of China.

2.3.2. Experiment 2: ABC Transporter Inhibition Test

To examine the ABC transporter activities in medaka, 11 dpf larvae (20 larvae per replicate and 3 replicates, total 360 larvae) were exposed to a series concentration of nanoplastics (0.001–10 μg/mL) together with 2 kinds of fluorescent probes of rhodamine (0.5 μM, P-gp substrate) and fluorescein diacetate (FDA, 3.6 μM, MRP substrate), and with or without inhibitors of verapamil (P-gp inhibitor) or indomethacin (MRP inhibitor). Stock rhodamine and FDA solutions were prepared in DMSO, and the final DMSO concentration in the exposure media was below 0.1%. Blank control (AFW only), solvent control (0.1% DMSO in AFW), and inhibitor controls (10 μM verapamil + 0.5 μM rhodamine for P-gp transporter inhibition assay, and 50 μM indomethacin + 3.6 μM FDA for MRP transporter inhibition assay) [28] were also tested in parallel.

To examine if the inhibitory effects could last after PS nanoplastics removal, fish larvae were also incubated in controls and PS nanoplastics (0.001–10 μg/mL) with or without inhibitors as described above, but without the addition of fluorescent probes. After 1 day of exposure, fish larvae were washed twice with AFW and then incubated in AFW in the dark at 28 °C for 2 h, together with 0.5 μM rhodamine or 3.6 μM FDA fluorescent probes.

2.4. Oxidative Stress and Lipid Peroxidation Measurements

After PS nanoplastics exposure, larvae (20 larvae per replicate and 3 replicates, total 360 larvae) were sampled and rinsed twice with AFW. Thirty larvae were pooled together as one replicate from each group, and homogenized in ice-cold, 1X phosphate-buffered saline (PBS) with a tissue homogenizer (Fisher Scientific International Inc., Hampton, NH, USA), and then centrifuged at 10,000× *g* for 3 min at 4 °C to obtain the supernatants. To reflect the total oxidative stress status in exposed larvae, the total antioxidant capacity (TAC) assay was conducted on a microplate reader (SpectraMax Plus 384, Molecular Devices, San Jose, CA, USA) at 520 nm. The free radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH), can be scavenged by fish antioxidants and the capacity was calculated as U/mg prot [40]. To determine the degree of lipid peroxidation, the amount of malondialdehyde (MDA) content, which is the secondary product of lipid peroxidation [41], was measured at 532 nm. Additionally, the content of the reduced form of GSH and the GST enzyme were also measured at 420 and 412 nm, respectively. Total protein concentrations for each sample were measured using the bicinchoninic acid (BCA) assay at 562 nm. All of the above assays were measured with commercial kits (Nanjing Jiancheng, Nanjing, China) according to the manufacturer's protocols.

2.5. ATP and Cholesterol Contents

To assess the PS nanoplastic's effects on energy supply and membrane fluidity, we further tested ATP content and cholesterol concentration in 12 dpf medaka. ATP contents in the larvae were measured by the phosphomolybdic acid colorimetry assay at 636 nm, and the cholesterol concentrations were quantified by transforming cholesterol to red quinone compounds and measured at 510 nm. Both measurements were conducted with kits (Nanjing Jiancheng, Nanjing, China), as per the manufacturer's instructions.

2.6. Measurement of Oxygen Consumption

According to a previously published method [42], fish larvae were transferred to 96-well microplastics (1 larvae/well) containing 90 μL of E3 medium of 35 μg/mL of MitoXpress Xtra (MitoXpress Xtra Reagent Pack, Agilent Technologies, Santa Clara, CA, USA) that enabled the real-time measurement of extracellular oxygen consumption in living larvae. Oxygen consumption was then measured in real time for 90 min at 28 °C in a

96-well plate using a spectrometer (Spectra Max M5, Molecular devices, Sunnyvale, CA, USA). The excitation and emission wavelengths were set at 380 and 650 nm, respectively.

2.7. Quantitative Real-Time Polymerase Chain Reaction

After the PS nanoplastics exposure, several mRNA genes related to ABC transporter functions were analyzed by the quantitative polymerase chain reaction (qPCR). Total RNA was extracted from pooled samples (20 larvae per replicate and 3 replicates, total 360 larvae) using a RNeasy Mini Kit (Qiagen, Hilden, Germany), as per the manufacturer's instructions. Following extraction, RNA was analyzed on a NanoDrop-2000 spectrophotometer (Thermo Fisher, Waltham, MA, USA) and assessed to make sure the 260/280 ratio was within 1.8–2.0, and further verified on a 1% agarose-formaldehyde gel (Figure S1). RNA was diluted to 1000 ng/μL, with 1 μg reverse transcribed to cDNA using the Reverse Transcription System kit (Promega, Madison, WI, USA), as per the manufacturer's instructions. Primers were synthesized by Integrated DNA Technologies (Coralville, IA, USA; Table 2). Each reaction had 100 ng of cDNA with specific primer pairs (10 μM). The thermal cycling conditions for qPCR underwent 2 min at 95 °C, 40 cycles of 10 s at 95 °C, 30 s at 60 °C, and then 5 s at 54 °C in a thermal cycler (CFX-6, Bio-Rad, Hercules, CA, USA). All interested genes were normalized to the housekeeping gene, β-actin. Each sample was run in triplicate and gene expression data were reported as relative fold change using the $2^{-\Delta\Delta Ct}$ method [43].

Table 2. Primer pairs used for qPCR analysis in larval medaka in the present study.

Gene	Direction	Sequence	Accession Number
β-actin	Forward	GAGGTTCCGTTGCCCAGAG	S74868
	Reverse	TGATGCTGTTGTAGGTGGTCTC	
abcb6a	Forward	GAACGTCTTCCTCCAGATGCTTG	CP020666
	Reverse	CACCATCGTCTGCATCTACGTC	
abcc2	Forward	GGCGGTCACATTAGGAGAGG	Ensembl database
	Reverse	ACGTCACACAGAACCAGCAA	
abcg2	Forward	AGGGTAAGCAGGGGATGACT	Ensembl database
	Reverse	GAGAGCTCCAACGATCAGGG	

2.8. Transporter Inhibition Measurement

After Experiment 2 exposure, fish larvae were washed twice with AFW to remove external fluorescent probes, and then immediately transferred to 2 mL centrifuge tubes without water. After adding 200 μL of n-butanol into each tube, rhodamine and fluorescein in fish tissue were extracted by 20-min sonication in an ice-water bath. Then, the sonicates were centrifuged at 13,000× *g* for 5 min at 4 °C, and the supernatants were collected and measured in black 96-well plates immediately on the microplate reader at Em/Ex of 540/580 nm for rhodamine and Em/Ex of 494/521 nm for fluorescein by using the spectrometer (Spectra Max M5, Molecular devices, San Jose, CA, USA). All the sample extraction and measurements were carried out at 25 °C in the dark at all times.

2.9. Statistics

Statistical analyses were conducted in SPSS (version 20.0). Normality and homogeneity of variance were evaluated by conducting a Kolmogorov–Smirnov one-sample test and Levene's test. A one-way analysis of variance (ANOVA) followed by a Tukey's post-hoc test was used to determine mean differences in biomarker contents or xenobiotics or metabolites' concentrations in medaka following exposure to different treatments. Statistical significance was determined at $p < 0.05$.

3. Results

3.1. Oxidative Stress after Nanoplastics Exposure

The damage of the antioxidant enzyme system in medaka larvae is shown in Figure 2. The level of MDA significantly increased 3- to 6-fold in 12 dpf medaka larvae following

exposure to 0.001 to 10 µg/mL of PS nanoplastics for 24 h (Figure 2A). Differently, there was a decreased trend in the TAC value, though it was not significantly different from controls (Figure 2B). The content of GSH was not significantly different in nanoplastics treatment groups in relation to controls (Figure 2C). Similarly, the activity of GST, an important enzyme involved in phase II biotransformation to protect against oxidative stress, was not significantly different from controls (Figure 2D).

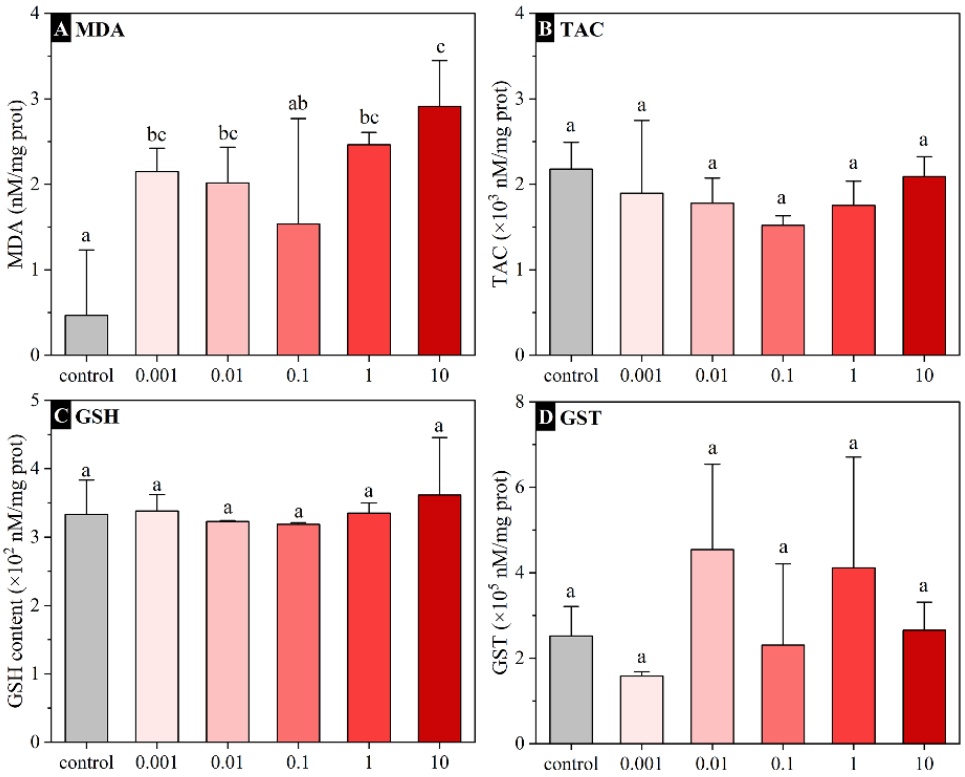

Figure 2. Nanoplastics effects on oxidative stress in larval medaka following 24 h exposure. (**A**) Malondialdehyde (MDA), (**B**) total antioxidant content (TAC), (**C**) reduced form of glutathione (GSH) level, and (**D**) glutathiones-transferase (GST) enzyme activity. Abundance values are expressed as mean ± standard deviation (n = 3 replicates per treatment). Groups sharing the same lowercase letters are statistically similar to each other (one-way ANOVA, Tukey's post hoc, $p < 0.05$).

There is a close relationship between reactive oxygen species (ROS) metabolism and cell membrane damage, both of which are regulated by the levels of ATP amount and energy charge. As shown in Figure 3, our results show that ATP levels did not significantly decrease in fish larvae after 0.001–1 µg/mL of PS nanoplastics exposure. However, at the highest exposure concentration (10 µg/mL), there was a significantly higher ATP level when compared to controls ($p = 0.030$) (Figure 3A). Similarly, nanoplastics also caused a significant increase in oxygen consumption of 124.1% ± 45.8% as compared to the control ($p = 0.047$) at the 10 µg/mL exposure level, but no significant change in the oxygen consumption after exposure to other exposure concentrations (Figure 3B). Cholesterol content in fish larvae did not significantly differ from controls, regardless of the concentration of nanoplastics (Figure 3C). There is also strong evidence to support the idea that this elevated ATP demand may serve to fuel ABC transports.

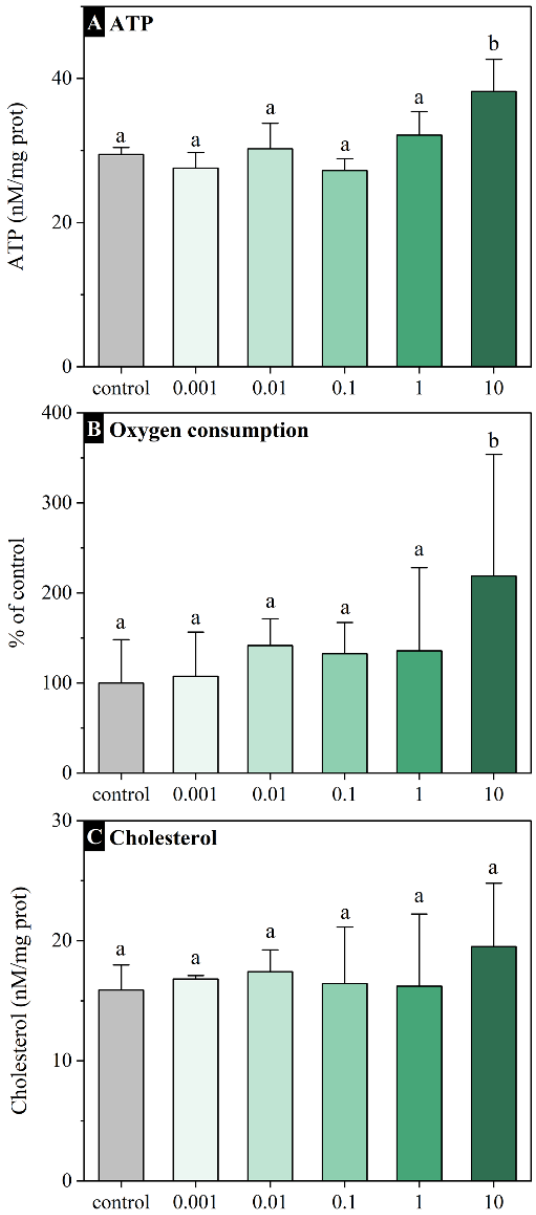

Figure 3. The energy consumption and membrane function-related biomarker changes in medaka after exposure to nanoplastics. (**A**) Adenosine 5′-triphosphate (ATP) content, (**B**) oxygen (O_2) consumption, and (**C**) cholesterol content. Abundance values are expressed as mean ± standard deviation (n = 3 replicates per treatment). Treatments sharing the same lowercase letters are statistically similar to each other (one-way ANOVA, Tukey's post hoc, $p < 0.05$).

3.2. Cellular Effects of Nanoplastics

All the sample extraction and measurements were carried out in the dark at all times. As cell membrane damage may lead to a dysregulation of ABC transmembrane transporters, we further quantified the expression of ABC transporter genes. There was a significant downregulation in the expression of the P-gp transporter gene (*abcb6a*) and both MRP transporter genes (*abcc2* and *abcg2*) in fish larvae exposed to PS nanoplastics (Figure 4). Relative to controls, *abcb6a*, *abcc2*, and *abcg2* were downregulated by 48–88%, 37–84%, and 69–87%, respectively (Figure 4A–C).

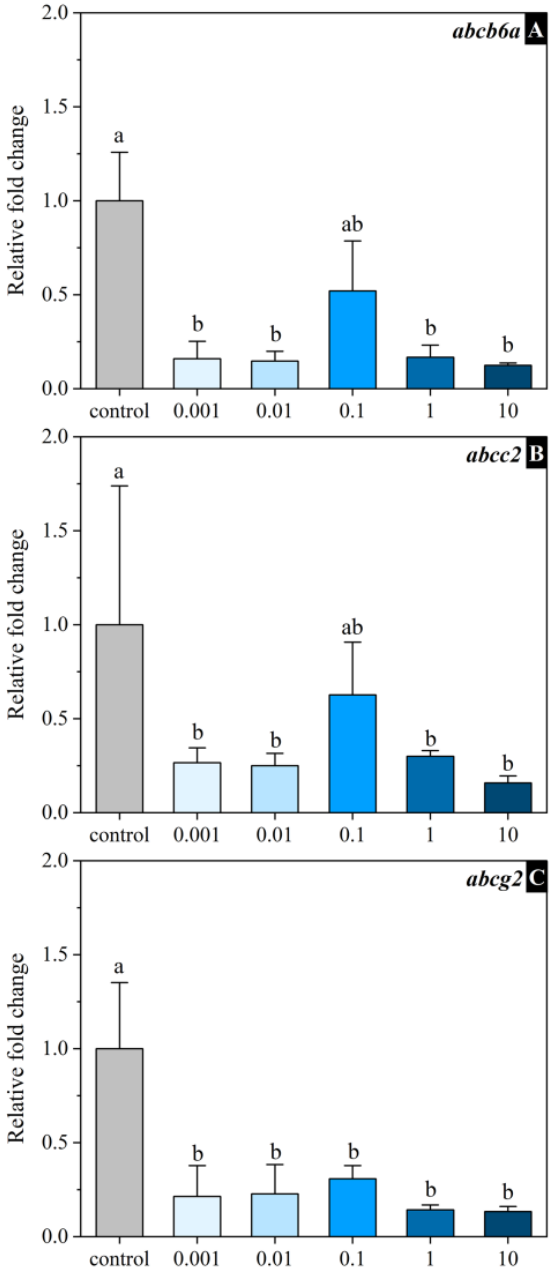

Figure 4. The relative fold change of mRNA expression of (**A**) *abcb6a*, (**B**) *abcc2*, and (**C**) *abcg2* in 12 dpf medaka larvae exposed to control and 0.001–10 μg/mL of polystyrene nanoplastics. *abcb6a* belongs to P-gp transporter genes, *abcc2* and *abcg2* belong to MRP transporter genes. Treatments sharing the same lowercase letters are statistically similar to each other (one-way ANOVA, Tukey's post hoc, $p < 0.05$).

3.3. Nanoplastics Could Lead to Higher Xenobiotics Accumulation

We found that nanoplastics can inhibit the function of ABC efflux transporters, which can increase the accumulation of the parent compound rhodamine and the hydrolytic metabolite fluorescein in fish. As for P-gp efflux, 0.001–1 μg/mL of nanoplastics did not inhibit transport activity, whereas significant inhibition was observed for rhodamine in the 10 μg/mL PS nanoplastics exposure group (2-fold higher than control, $p = 0.004$, Figure 5A). As for MRP efflux, only the lowest concentration of nanoplastics (0.001 μg/mL) did not inhibit the efflux ability. When nanoplastics concentrations reached 0.01–0.1 μg/mL, an obvious higher fluorescein accumulation was observed (166–183% more than control, $p = 0.042$ and $p = 0.018$, respectively, Figure 5B). Moreover, when the concentration

reached 1–10 μg/mL, the inhibitory effects disappeared 2 h after nanoplastics were removed (Figure 5C,D), suggesting that ABC transporters' inhibition by nanoplastics is a rapid, reversible effect.

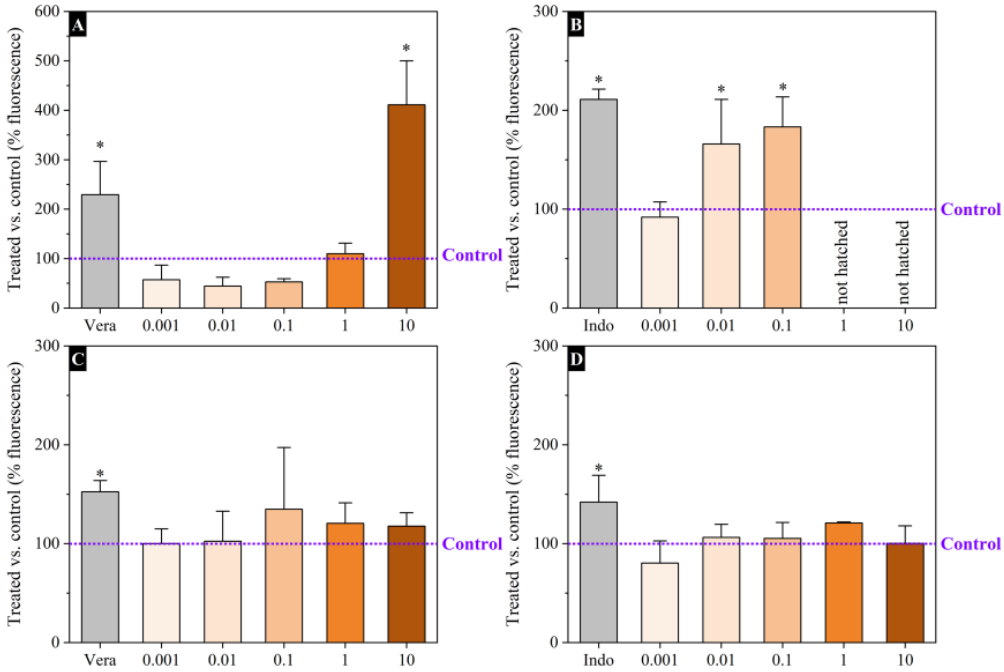

Figure 5. Inhibition of efflux transporters by nanoplastics. (**A**) Rhodamine accumulation in fish tissues together with verapamil (P-gp efflux inhibitor) and PS nanoplastics exposure for 1 day. (**B**) The hydrolytic metabolite of FDA accumulation in fish tissues together with indomethacin (MRP efflux inhibitor) and PS nanoplastics exposure for 1 day. (**C**) Rhodamine accumulation in fish tissues after removal of verapamil or PS nanoplastics exposure for 2 h. (**D**) The hydrolytic metabolite of FDA accumulation in fish tissues after removal of indomethacin and PS nanoplastics exposure for 2 h. FDA: fluorescein diacetate; P-gp: P-glycoprotein; MRP: multi-resistant protein; Vera: verapamil; Indo: indomethacin. Verapamil and indomethacin are used as positive inhibitor controls for P-gp and MRP transporters, respectively. * Indicates a significant difference compared to the control group ($p < 0.05$).

4. Discussion

4.1. Different Sensitivity

Nanoplastics exposure will have varying degrees of effects on efflux transporter ability at the molecular, cellular, tissue, and individual levels. We found that PS nanoplastics could cause membrane lipid peroxidation together with the downregulation ABC transporter-related gene expression. Subsequently, the reduced P-gp and MRP efflux ability for co-existing pollutants or metabolites was observed.

It is noteworthy that the responses at the molecular and cellular levels were relatively more sensitive than at tissue and individual levels. For example, we found significant changes in the MDA assay and gene expression alteration when the nanoplastics exposure concentration was only 0.001 μg/mL, which is an environmentally relevant concentration of nanoplastics [14], whereas obvious effects gradually appeared when the nanoplastics concentration was higher than 10 μg/mL at the tissue and individual levels. Therefore, we found some sensitive early-warning biomarkers at molecular and cellular levels, such as lipid peroxidation, which may lead to further toxicity, such as diminished ABC efflux ability.

4.2. Oxidative Stress Could Be a Molecular Initiating Event

In the present study, nanoplastics exposure can lead to oxidative stress in medaka larvae. Especially, lipid membrane peroxidation may be the initiating effect of PS nanoplastics exposure in medaka larvae. MDA, a final product of lipid peroxidation, was increased

by 3–6-fold in larvae following treatment, which suggests that PS nanoplastics may have impaired the membrane barrier protection capacity [44].

Some studies have already indicated that membrane lipid peroxidation could be induced by nanoplastics exposure [45]. Once nanoplastics are engulfed by cells through endocytosis or pinocytosis, they can not only alter the membrane structures, but also change membrane properties and lateral organization [46]. As foreign substances, nanoplastics can trigger the innate immune defense mechanisms of cells, and thus reactive oxygen species (ROS) can be generated in high quantities [45]. There is one point which needs attention: the fact that the endocytosis pathways of nanoplastics depend on particle size [47]. A previous study reported that the rate of endocytosis is related to the size of the plastic particles, and the 50 nm PS nanoplastics were endocytosed more than their other larger counterparts (100–500 nm) [48]. Microplastics > 1 μm in size cannot be endocytosed, but can be engulfed by cells via phagocytosis/macropinocytosi [48,49].

4.3. Oxidative Stress Led to Diminished ABC Transporter Efflux Function

The ABC protein superfamily is known as a fundamental first line of defense against the introduction of xenobiotic substances in biological processes. These proteins are located across the cellular membrane and pump xenobiotics and their metabolites out of cell membranes by ATP hydrolysis [15,50]. Thus, the diminished ABC transporter efflux function, which started at PS nanoplastics concentrations higher than 0.01 μg/mL (Figure 5B), may be brought about by membrane oxidative stress–lipid peroxidation that occurred at exposure concentrations higher than 0.001 μg/mL (Figure 2A).

Cell membrane oxidative stress and the changes of the ABC transporter system often occur simultaneously following exposure to nanomaterials, in terms of transporter efflux ability inhibition. For instance, a previous study reported that the dysfunction of the mitochondrial membrane potential could inhibit P-gp activity by reducing the transporter expression in human MCF-7 cells [51]. Additionally, lipid peroxidation has been reported to increase cell membrane permeability and fluidity, leading to disorders in ABC transporter function [52,53].

ROS-induced downregulation of ABC transporter function may be related to transporter expression downregulation. For instance, multi-walled carbon nanotubes (MWCNT) could reduce the ABCB1/P-gp and ABCC4/MRP4 expression in human Caco-2 cells, along with oxidative damage of cell membranes [54]. Strong evidence has been provided by scientists that this effect could be due to the increased ROS production, because H_2O_2 can decrease ABC transporters at both mRNA and protein levels, and an ROS scavenger, N-acetyl-L-cysteine, can reverse this reduction [55,56].

4.4. Nanoplastics Pre-Exposure Enhanced Co-Existing Pollutants' Accumulation

Nanoplastics exposure led to higher tissue pollutants retention in medaka (Figure 5A,B). This may be related to the disruption of PS nanoplastics on the ABC transporter systems, which can detoxicate co-existing pollutants by biotransformations mediated by ABC transporters, [57–61]. Thus, the co-existing pollutants' toxicity could be enhanced due to the higher bioaccumulation with the presence of plastic particles. Similar phenomena have also been observed in previous studies [11,62], suggesting that excessive chemical accumulation may have occurred and thus led to the subsequent toxicity.

The quick recovery of the ABC transporter efflux function (Figure 5C,D) indicated that PS nanoplastics are either eliminated quickly from the fish cells [63] or sequestered within cellular lysosomes, the endoplasmic reticulum, or the Golgi apparatus as other nanomaterials [64]. Even though this phenomenon suggests that the PS nanoplastics' effect can be reversible once the exposure is removed from the system, the ubiquitous co-existence of PS nanoplastics and other contaminants is still a non-negligible environmental concern.

4.5. Environmental Significance

Nanoplastics are one of the most frequently occurring plastic particles in the natural environments, with growing public concerns [65]. The coexistence of nanoplastics and pollutants has also become a noticeable environmental problem. However, there is still a considerable knowledge gap about the impact of nanoplastics and pollutants on human health risks [66]. The decreased efflux activities of P-gp and MRP in medaka following exposure to nanoplastics could lead to excessive accumulation of co-existing pollutants in fish. Taken together, we supposed that nanoplastics had disrupted the fluidity of cell membranes via induced oxidative damages and penetration, leading to the efflux inhibition and disruption of MRP functions, and thereby enhanced the retention of xenobiotics pollutants in aquatic organisms. This indirect effect of an increase in chemosensitivity caused by nanoplastics should be considered in evaluations of the environmental and health risks of nanoplastics and other pollutants (e.g., heavy metals and persistent organic pollutants) [26,67].

Our data indicate that nanoplastics are chemosensitizers (contaminants that can cause subversion of the multidrug resistance [68]) that can compromise the ABC transporter system by indirectly allowing normally excluded contaminants to remain in cells. Although this work was carried out with an aquatic organism, it still points to unexpected effects on human health, as ABC efflux pumps are widely distributed in mammalian tissues [68,69].

5. Conclusions

Our results indicated that nanoplastics exposure caused lipid peroxidation, disrupted the fluidity of cell membranes, inhibited the ABC transporters' efflux ability, and thereby enhanced the accumulation of co-existing pollutants in aquatic organisms. Collectively, this efflux inhibition was related to oxidative stress and transcriptional alterations. The present study represents an effective amplification pathway of nanoplastics' toxicity and provides new insights into the environmental risks of nanoplastics to aquatic organisms.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14060863/s1, Figure S1: The RNA band images.

Author Contributions: Conceptualization, H.Y. and Q.C.; Methodology, Formal Analysis, and Investigation, H.Y., Z.G., Y.Y., M.L. and Q.C.; Data Curation, Q.C.; Writing—Original Draft Preparation, H.Y. and Q.C.; Writing—Review and Editing, H.Y. and Q.C.; Visualization, all authors; Project Administration, Q.C.; Funding Acquisition, Q.C. All authors have read and agreed to the published version of the manuscript.

Funding: The authors were financially supported by the National Natural Science Foundation of China (No. 42077371) and the Joint Project of the National Natural Science Foundation of China (U19A2095).

Institutional Review Board Statement: The use of animals was approved by the National Animal Welfare Law of China.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments: We thank Fenglian Qi for providing the nanoplastics and friends at UCR for helping with the proof-reading of the original manuscript.

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

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