Published in final edited form as:

Sci Total Environ. 2018 December 01; 643: 324–334. doi:10.1016/j.scitotenv.2018.06.186.

Maternal transfer of nanoplastics to offspring in zebrafish (*Danio rerio*): a case study with nanopolystyrene

Jordan A. Pitt^{a,b}, Rafael Trevisan^{a,*}, Andrey Massarsky^a, Jordan S. Kozal^a, Edward D. Levin^c, Richard T. Di Giulio^a

^aNicholas School of the Environment, Duke University, Durham, NC 27708, USA.

^bState University of New York, College of Environmental Science and Forestry, Syracuse, NY, 13210, USA.

^cDepartment of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, NC 27710, USA

Abstract

Plastics are ubiquitous anthropogenic contaminants that are a growing concern in aquatic environments. The ecological implications of macroplastics pollution are well documented, but less is known about nanoplastics. The current study investigates the potential adverse effects of nanoplastics, which likely contribute to the ecological burden of plastic pollution. To this end, we examined whether a dietary exposure of adult zebrafish (*Danio rerio*) to polystyrene nanoparticles (PS NPs) could lead to the transfer of nanoplastics to the offspring, and whether nanoplastics exposure affects zebrafish physiology. Specifically, adult female and male zebrafish (F0 generation) were exposed to PS NPs via diet for one week and bred to produce the F1 generation. Four F1 groups were generated: control (unexposed females and males), maternal (exposed females), paternal (exposed males), and co-parental (exposed males and females). Parental PS NP exposure did not significantly affect reproductive success. Assessment of tissues from F0 fish revealed that exposure to PS NPs significantly reduced glutathione reductase activity in brain, muscle, and testes, but did not affect mitochondrial function parameters in heart or gonads. Assessment of F1 embryos and larvae revealed that PS NPs were present in the yolk sac, gastrointestinal tract, liver, and pancreas of the maternally and co-parentally exposed F1 embryos/larvae. Bradycardia was also observed in embryos from maternal and co-parental exposure groups. In addition, the activity of glutathione reductase and the levels of thiols were reduced in F1 embryos/larvae from maternal and/or co-parental exposure groups. Mitochondrial function and locomotor activity were not affected in F1 larvae. This study demonstrates that (i) PS NPs are transferred from mothers to offspring, and (ii) exposure to PS NPs modifies the antioxidant system

*Corresponding author: rafael.trevisan@duke.edu.

Author Contributions Statement

JP and RT designed the experiment and wrote the main manuscript text. J.P, R.T, A.M and J.S.K performed the experiments. R.T.D.G and E.D.L. contributed with reagents, materials and analysis tools. All authors reviewed the manuscript.

Declaration of Interests

The authors declare no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2018.06.186>.

in adult tissues and F1 larvae. We conclude that PS NPs could bioaccumulate and be passed on to the offspring, but this does not lead to major physiological disturbances.

Keywords

Plastics; Zebrafish; Cross-generational; Diet exposure; Maternal transfer

1 Introduction

Plastic pollution is a rapidly developing research field. The abundance of plastic in the oceans is estimated at 5.25 trillion particles and the number of particles continues to increase (Eriksen et al., 2014); however, the potential adverse ecological implications of micro and nanoplastics (plastic particles with at least 1 dimension ≥ 100 nm) are largely unknown (Andrady, 2011). Similarly, the ecological implications of micro and nanoplastics in fresh water ecosystems are also poorly understood (Anderson et al., 2016).

Some information is available for microplastics. As reviewed by Anderson et al. (2016), microplastics are ubiquitous in freshwater and marine environments and are regarded as contaminants of emerging concern. Microplastics are present in several personal care products for product stabilization, viscosity regulation, and skin conditioning and some of these particles reach the aquatic environment via sewage effluents. They can also form through degradation of larger pieces, and their fate and behavior depend on their composition – low-density plastics are typically buoyant, whereas high-density plastics are more likely to sink and accumulate in sediment (Anderson et al., 2016). Concentration of microplastics in aquatic environments is variable at both temporal and spatial scales, but usually ranges from as low as few particles to dozens of thousands particles per cubic meter of surface water (Li et al., 2016). The ingestion of microplastics by invertebrates and fish has been shown in different species and areas of the globe (Wesch et al., 2016), at concentrations as low as <1 to hundreds of particles per organism (Vandermeersch et al., 2015). For instance, 27% of red mullet (*Mullus surmuletus*) caught near Balearic Islands and 15% of European pilchards (*Sardina pilchardus*) and anchovies (*Engraulis encrasicolus*) near Spanish Mediterranean coast had measureable quantities of microplastics in the gastrointestinal tract (up to 1 particle/individual) (Alomar et al., 2017; Compa et al., 2018). Their toxicity is thought to be linked to stress of ingestion (blockage of digestive tract), leakage of additives within plastics, and presence of adsorbed organic pollutants on the surface of microplastics (Anderson et al., 2016). Due to the potential environmental risks of microplastics several European countries and a few of the states within US banned microplastics in consumer products (Anderson et al., 2016).

Much less is known about nanoplastics, which are predicted to be present in the aquatic environment (Cozar et al., 2014), and are thought to form primarily via photo- and physical degradation of larger plastic particles (Andrady, 2011). The presence of nanoplastics is not yet quantified in the aquatic environment and biota due to limitations in analytical methods (Koelmans et al., 2015). Recent studies suggest that plastic nanoparticles accumulate in various aquatic invertebrates, which could lead to their accumulation within the food web

(Bergami et al., 2016; Della Torre et al., 2014; Mattsson et al., 2015; von Moos et al., 2012). Moreover, several studies document that plastic nanoparticles not only accumulate in various fish species, but also lead to physiological alterations when using polystyrene nanoparticles (PS NPs) as a model. For example, PS NPs have been shown to accumulate in the brain of adult Crucian carp (*Carassius carassius*; dietary exposure of ~130 mg of particles per feeding), leading to morphological changes in the brain and behavioral changes (lower activity and longer feeding time) (Mattsson et al., 2017). Accumulation of PS NPs (1–50 mg/L) in zebrafish (*Danio rerio*) embryos/larvae has also been reported (Chen et al., 2017; van Pomerén et al., 2017), which was associated with behavioral alterations, oxidative stress, and a reduction in acetylcholinesterase activity (Chen et al., 2017).

Our previous study independently corroborated the ability of PS NPs to penetrate the zebrafish chorion and accumulate in the yolk sac and other regions upon a waterborne exposure (Pitt et al., 2018). We also showed several effects on embryos/larvae (e.g. bradycardia and locomotor hypoactivity). Notably, accumulation of PS NPs in the embryo yolk sac suggests that egg yolk is a potential target for PS NPs accumulation in adult female fish. Such accumulation of PS NPs in maternal gametes could transfer to the offspring, potentially altering physiology and development. While the cross-generational transfer of PS NPs has been already documented in invertebrate models (e.g. Brun et al., 2017; Cui et al., 2017; Lee et al., 2013; Zhao et al., 2017), it has not yet been examined in a vertebrate model. The current study used a dietary exposure to PS NPs as an ecologically-relevant route of exposure to assess the potential transfer of nanoplastics to the offspring. Taking into account that nanoplastics were shown to induce developmental issues, changes on locomotor activity and oxidative stress in zebrafish (Chen et al., 2017; van Pomerén et al., 2017; Pitt et al., 2018), the adverse effects of PS NPs were also evaluated in both the F0 and F1 generation using a set of biomarkers to investigate if these effects persist after a parental exposure.

2 Materials and methods

2.1 Materials

Fluorescent and non-fluorescent PS NPs (cat. #FSDG001 and #PS02002, respectively) were purchased from Bangs Laboratories, Inc. (Fishers, IN, USA). The fluorescent stock solution contained 1% (internally labeled with Dragon Green; ex./em. 480/520) PS NPs with a nominal mean diameter of 42 nm. The non-fluorescent stock solution contained 10% PS NPs also with a nominal mean diameter of 42 nm. Additionally, the stock solutions contained 0.1% sodium dodecyl sulfate (SDS; surfactant to prevent particle aggregation) and 0.05–0.09% sodium azide (bacteriostatic preservative). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise specified.

2.2 PS NPs characterization and preparation

Non-fluorescent PS NPs were characterized using a dynamic light scattering (DLS) (Zetasizer Nano, Malvern Instruments Ltd., Malvern, UK). The hydrodynamic diameter and zeta potential of 5 mg/L PS NPs were assessed in 0.065‰ artificial seawater (ASW; Instance Ocean, Blacksburg, VA, USA). The particle size characterization of fluorescent PS NPs was assessed in our previous study in 0.065‰ ASW (Pitt et al., 2018).

Prior to addition of PS NPs to zebrafish food (see section 2.3), the particles were centrifuged using a Vivaspin® 2mL Ultrafiltration Device (300,000 molecular weight cut-off, cat. #AA022) (Bangs Laboratories, Inc., Fishers, IN, USA) at 4,000 *g* for 10 min intervals to remove the sodium azide and SDS present within the solution. The PS NPs solution was then washed three times in deionized (DI) water and filtered in the same manner. The PS NPs were then brought up to a final volume to reach 5% of the total solution by mass in DI water.

2.3 Diet preparation

Two diets were prepared: a control diet and PS NPs diet with fluorescent or non-fluorescent particles, depending on the experiment. To prepare the control diet, crushed Zeigler's Adult Zebrafish Complete Diet (Aquatic Habitats, Inc., Gardners, PA, USA), decapsulated brine shrimp egg, and gelatin (Carolina Biological Supply Company, Burlington, NC, USA) were mixed in DI water, such that the final concentrations of the aforementioned components were 90, 45, and 120 mg/mL, respectively. (Bisesi et al., 2015; Blickley et al., 2014). For the treated diet, PS NPs were added such that the final concentration of the particles was approximately 10% of the food by mass (the gelatin content was not considered part of the diet). This concentration was chosen based on a previous study with medaka and low-density polyethylene microplastics (Rochman et al., 2013). This mixture was heated to 60°C and then vortexed. Enough food was prepared for a week of feeding based upon the average fish mass of each tank. Food was transferred to vials in aliquots of the daily amount of food required for an average 1% of the total fish mass of each individual tank twice a day. The aliquots were stored in capped vials at 4°C and a representative picture of the diet is shown in Figure S1. Assuming that animals consumed 100% of the food, each individual was exposed to approximately 0.3 mg per feeding (about 1 mg of PS NPs per gram of fish). This value is considerably lower than other studies with dietary exposure to PS NPs (9–17 mg of PS NPs per gram of fish, Mattsson et al. 2015).

2.4 Zebrafish husbandry

Laboratory reared wild-type (EkkWill Waterlife Resources; Ruskin, FL, USA) *D. rerio* were maintained in a recirculating AHAB system (Aquatic Habitats, Inc., Apopka, FL, USA) with a 14:10 h light/dark cycle. The water quality was sustained at 27–29°C and pH 7.0–8.0 in 0.065‰ ASW. Prior to initiation of the experiments, the fish were fed twice daily with brine shrimp (INVE aquaculture, Inc., Salt Lake City, UT, USA) in the morning and Zeigler's Adult Zebrafish Complete Diet in the afternoon. All zebrafish care and husbandry procedures were approved by the Duke University Institutional Animal Care and Use Committee (protocol number A139–16-06).

2.5 Experimental set-up

Sexually mature zebrafish (3–4 months with an average mass of 300 mg) were selected for the study. The fish were acclimated to the control diet for a week prior to the exposure. Further, the fish were bred twice during the week prior to the start of exposure, in order to familiarize the fish with the breeding set-up. Additionally, the fish were acclimated to the exposure tanks for two days. The fish were kept at a density of 6 fish of the same sex per 6 L of 0.065‰ ASW with 2 tanks per exposure group for each sex. The tanks were kept at 28°C

under constant aeration and physical and biological water filtration. The filters were turned off for 2 h during the feeding periods to prevent the loss of food particles. The day prior to the start of the exposure, the fish were bred (2 females: 2 males) to remove existing mature oocytes. During the 7-day exposure period, the fish were fed with the respective food diet (see section 2.3) twice per day (corresponding to 1% fish mass each time), once in the morning and once in the afternoon. The water was partially (50%) refreshed every other day.

At the end of the exposure, the parental fish (F0) were bred between the different exposure conditions in order to obtain control (non-exposed females and males), maternally- (exposed females), paternally- (exposed males), and co-parentally- (exposed females and males) exposed F1 embryos. Breeding crosses (2 females: 2 males) were set at 17:00, and the following morning the embryos were collected within 3 h of spawning between 9:00 and 12:00. A total of 6 breeding pairs were used per experiment/cohort with non-fluorescent PS NPs. A total of 2 breeding pairs were used per experiment/cohort with fluorescent PS NPs. Following the breeding event, the fish were euthanized and the liver, gonads, muscle, and brain were dissected, collected into 1.5 mL microcentrifuge, and portions were flash frozen in liquid nitrogen, and stored at -80°C . The mitochondrial function of the heart and gonads was assessed with freshly dissected tissue samples (see section 2.6.3). The experiment was repeated three times with the non-fluorescent PS NPs (total of 72 animals, 6 animals per exposure condition per experiment) and twice with the fluorescent PS NPs (total of 28 animals, 3–4 animals per exposure condition per experiment).

The collected F1 eggs were kept in 30% Danieau's medium (in mM: 58 NaCl, 0.7 KCl, 0.4 MgSO_4 , 0.6 $\text{Ca}(\text{NO}_3)_2$, 5 HEPES) at 28°C , and screened at 6 h post-fertilization (hpf). At random 60 embryos per group (control, maternal, paternal, and co-parental) were transferred into two glass Petri dishes (30 embryos/dish) at a density of 1 embryo/mL in 30% Danieau's for observations.

2.6 Assessments in F0 adults

2.6.1 Reproductive success—The effect of dietary PS NPs exposure on reproductive success was estimated by counting the total number of eggs produced and calculating the percentage of fertilized eggs after each breeding event.

2.6.2 Antioxidant enzymes—The activities of antioxidant enzymes glutathione reductase (GR; EC 1.8.1.7), glutathione peroxidase (GPx; EC 1.11.1.9), and catalase (CAT; EC 1.11.1.6) were assessed in brain, liver, muscle, ovaries, and testes of adult F0 zebrafish ($n=18$ over 3 cohorts). Brain, liver and muscle were shown to contain PS NPs in zebrafish larvae after waterborne exposure (Pitt et al., 2018), while gonad tissues were analyzed as a possible PS NPs target during parental exposure. The tissues were homogenized in ice-cold 50 mM potassium phosphate buffer pH 7.0 containing 0.5 mM EDTA and protease inhibitor cocktail, using a probe sonicator. The homogenates were centrifuged at 3000 g for 15 min, and the resulting supernatants were transferred to fresh 0.5 mL microtubes. GR activity was measured by following the consumption of NADPH (0.2 mM) at 340 nm in the presence of oxidized glutathione (GSSG, 1 mM) (Carlberg and Mannervik, 1985). GPx activity was measured by following the consumption of NADPH (0.2 mM) at 340 nm, but in presence of

1 mM cumene hydroperoxide, 1 mM reduced glutathione (GSH), and 0.2 U/mL baker's yeast GR (Wendel, 1981). CAT activity was measured by following the degradation of 10 mM H₂O₂ at 240 nm in presence of 0.01% Triton X-100 (Aebi, 1984). Spectramax M5 plate reader (Molecular Devices, San Jose, CA, USA) was used to measure the absorbance at aforementioned wavelengths. Enzyme activities were normalized to protein concentration in the supernatant, which was measured using the Pierce BCA protein content kit (Thermo Fisher Scientific, Waltham, MA, USA). The enzyme activities were normalized to the control and expressed as fold change.

2.6.3 Mitochondrial function—The oxygen consumption rate (OCR) in heart and gonads of F0 adult fish was assessed *ex vivo* using the XFe24 Extracellular Flux Analyzer (Agilent Instruments, Santa Clara, CA, USA) according to previously methods (Jayasundara et al., 2015, Kozal et al., 2018). The fish were euthanized in ice-cold water, and the heart and gonads were extracted and rinsed with Ringer's solution. For the ovaries only about 10 mg of tissue were used, while the other tissues were used in full (~0.6 mg heart tissue and ~0.9 mg testes tissue). OCR values were measured in the presence or absence of pharmacological agents specifically targeting electron transfer chain components (4 blanks were used per plate with 2–4 tissues each type/group/plate over 2 cohorts). Additional details about the assay are described in the supplementary material.

2.7 Assessments in F1 embryos/larvae

2.7.1 General physiology—The F1 embryos and larvae parentally-exposed to non-fluorescent PS NPs were screened daily for mortality until 96 hpf, with dead individuals being removed (n=4 plates, with 2 plates of 30 embryos per cohort and 2 cohorts). Heart rate was measured at 48 hpf in 10 randomly selected larvae per exposure condition (n=20 embryos over 2 cohorts). Embryos/larvae were monitored daily using a dissecting microscope to assess the overall development and potential presence of deformities (pericardial edema, yolk sac edema, curved notochord or tail, and truncated jaw according to Panzica-Kelly et al., 2010) (n=4 plates, with 2 plates of 30 embryos per cohort and 2 cohorts).

2.7.2 Distribution of PS NPs—The F1 embryos and larvae parentally-exposed to fluorescent PS NPs were imaged using a Zeiss Axioskop (Carl Zeiss, Oberkochen, Germany) fluorescence microscope at 24 h intervals from 24 hpf to 144 hpf. At each time point, 3 randomly selected individuals from each group were imaged (n=6 embryos or larvae over two cohorts). At 24 hpf the zebrafish were imaged with intact chorions, and at 48 hpf larvae that had already hatched were selected for imaging. Fluorescence images were captured using a 75W/2 xenon short-arc lamp, a Photometric CoolSNAPfx monochrome CCD camera (Roper Scientific, Tucson, AR, USA), 480/30 nm (em) and 535/40 nm (ex) filters with a 1 s camera exposure. Fluorescence intensity was analyzed using ImageJ software and the values were normalized to the control fluorescence and expressed as fold change.

2.7.3 Larval behavior—At 96 hpf, F1 zebrafish larvae were transferred into beakers with 200 mL of 30% Danieau's. The larvae were then transported to the behavioral facility,

where they were kept on a 14:10 h light/dark cycle at 28°C. At 144 hpf the larvae were individually transferred into a 96-well plate (n=36–48 larvae over 2 cohorts, with 12–24 larvae per cohort). The larvae were allowed to acclimate in the light for 1 h prior to being transferred to a DanioVision™ observation chamber (Noldus Inc., Wageningen, The Netherlands) for an alternating light/dark test where larval locomotor activity was recorded (Bailey et al., 2016; Massarsky et al., 2015). Additional details are described in the supplementary material.

2.7.4 Oxidative stress markers

2.7.4.1 Thiol levels: Analysis of total thiol levels was assessed *in vivo* following a protocol using *Arabidopsis* cell suspensions (Meyer et al., 2001) with minor modifications. F1 zebrafish larvae (96 hpf) were transferred into a 24-well plate and incubated in presence of 300 µM monobromobimane (mBrB) in the dark at room temperature for 4 h (n=16 larvae across 2 cohorts, 100 µL of mBrB per larva). After incubation, the larvae were rinsed three times with fresh 30% Danieau's, and individually transferred into a 384-well plate with 100 µL of Danieau's/well. The fluorescence levels were measured using a Spectramax M5 plate reader (Molecular Devices, San Jose, CA, USA) with 340/10 nm (ex) and 520 nm (em) filters. The fluorescence levels were subtracted from a blank (Danieau's only), normalized to the control, and expressed as fold change.

2.7.4.2 Redox state: The cellular redox state was assessed following a protocol using mammalian cell cultures (Farcal et al., 2015) with minor modifications. F1 zebrafish larvae (96 hpf) were transferred into a 24-well plate and incubated in presence of 200 µg/mL resazurin in the dark at room temperature for 4 h (n=14–16 larvae across 2 cohorts, 100 µL resazurin per larva). After incubation, the larvae were rinsed three times with fresh 30% Danieau's, and individually transferred into a 384-well solid plate with 100 µL of pure methanol for resorufin extraction. The larvae were incubated in methanol for 30 min. Subsequently, fluorescence was measured using a Spectramax M5 plate reader with 550/10 nm (ex) and 590 nm (em) filters. The fluorescence levels were subtracted from a blank (Danieau's only), normalized to the control, and expressed as fold change.

2.7.4.3 Antioxidant enzymes: The activities of antioxidant enzymes GR, GPx, and CAT were assessed in 96 hpf zebrafish larvae. The larvae were homogenized in ice-cold 50 mM potassium phosphate buffer (pH 7.0) containing 0.5 mM EDTA and protease inhibitor cocktail. The homogenates were centrifuged, and the resulting supernatants were used to assess enzyme activities following the methods described above.

2.7.5 Mitochondrial function—The OCR was assessed in 24 hpf F1 zebrafish embryos using the XFe24 Extracellular Flux Analyzer (Agilent Instruments, Santa Clara, CA, USA) according to a previously established protocol (Stackley et al., 2011). For this assessment, embryos were staged as two embryos per well in a 24-well plate with 700 µL of 0.065‰ ASW (5 wells per group with 4 blank wells over 2 cohorts). Additional details about the assay are described in the supplementary material.

2.8 Statistical analysis

The results are presented as mean $\pm$ standard error of the mean. All data were analyzed for normal distribution (Shapiro-Wilk test) and homoscedasticity. For F1 embryo and larval endpoints, one-way Analysis of Variance (ANOVA) with a post hoc Tukey method (parametric) or Kruskal-Wallis followed by Dunn's posthoc (non-parametric) were used to assess statistical differences between treatment groups. Two-way ANOVA with a post hoc Tukey method was used to analyze cohort effect and locomotor activity. The F0 adult enzyme analysis was completed with an unpaired t-test (parametric) or Mann-Whitney U test (nonparametric). These analyses were conducted using Graph Pad Prism 6.0 (San Diego, CA, USA). For all endpoints, p -value <0.05 was considered statistically significant.

3 Results

3.1 PS NPs characterization

The non-fluorescent PS NPs (5 mg/L in ASW medium) had a mean hydrodynamic diameter of 30.67 ± 8.97 nm and a zeta potential of -24.7 ± 2.93 mV. The fluorescent PS NPs (5 mg/L in ASW medium) had a mean hydrodynamic diameter of 34.5 ± 10.8 nm and a zeta potential of -21.1 ± 2.47 mV. Both values indicate a low aggregation behavior for both types of PS NPs.

3.2 Assessments in F0 adults

3.2.1 Reproductive success—There was no statistically significant effect of PS NPs exposure on the number of eggs spawned (Fig. 1A). The fertilization rate was also not significantly different (Fig. 1B).

3.2.2 Antioxidant enzymes—GR activity was significantly lower in brain and muscle of exposed females and muscle of exposed males (Fig. 2A and B), as well as gonads of exposed males (Fig. 2B). GPx activity was elevated in the brain of exposed females, but no changes were detected in males (Fig. 2C and D). CAT activity was not significantly affected by the dietary exposure to PS NPs in any of the tissues in females or males (Fig. 2E and F).

3.2.3 Mitochondrial function—The *ex vivo* OCR analysis of heart and gonad tissues of F0 adults did not reveal statistically significant changes in any of the analyzed parameters (Fig. 3). However, a marginal effect of PS NPs was observed in female gonads, such that all OCR values (basal, maximum, non-mitochondrial, basal mitochondrial, and mitochondrial spare capacity) were slightly higher than the control group ($p = 0.08$ – 0.12 , Fig. 3).

3.3 Assessments in F1 embryos/larvae

3.3.1 General physiology—Mortality was not significantly different between the different exposure groups in the F1 zebrafish throughout embryonic and larval development at any of the measured time points (24–96 hpf) (Fig. 4A). Heart rate significantly decreased in F1 larvae from the co-parental exposure groups with the maternal and paternal groups trending relative to the control (Fig. 4B). No deformities were noted in the embryos/larvae; however, the swim bladders of larvae that were exposed to PS NPs co-parentally were uninflated at 144 hpf (Fig. 4C and D).

3.3.2 Distribution of PS NPs—Green fluorescence was used to semi-quantitatively evaluate distribution of PS NPs in F1 zebrafish embryos and larvae from 24 hpf to 120 hpf. At 24 hpf, fluorescence was observed in the yolk sac of F1 embryos (Fig. 5). The observed fluorescence was significantly higher in the co-parental exposure group (~7 fold) and elevated but not significantly in the maternal exposure group (~4 fold) relative to the controls (Fig. 5). At 48 and 72 hpf, the same fluorescence pattern was observed in the yolk sac, except for the maternal exposure which significantly increased the yolk sac fluorescence (~4 fold) at 72 hpf (Fig. 5).

By 120 hpf, the larvae have a fully functional and developed GI tract, liver, pancreas, and gall bladder. The co-parentally exposed F1 larvae at this time point had a significant amount of fluorescence in the liver and GI tract (Fig. 6). Additionally, the maternally exposed F1 larvae had a significant amount of fluorescence in the GI tract and pancreas (Fig. 6). The gall bladder, while not significant, trended to increased fluorescence in the co-parental and maternal exposure groups (Fig. 6).

3.3.3 Larval behavior—Larval locomotor activity was monitored over the course of an alternating light/dark test at 144 hpf. No significant effect on larval locomotor activity were detected for any of the F1 larvae (Fig. S2).

3.3.4 Oxidative stress markers—Fluorescence of mBrB was used to qualitatively assess the differences in total thiol levels (reduced forms of protein and non-protein thiols) in F1 96 hpf larval zebrafish relative to controls. The fluorescence was significantly reduced in both the maternal and co-parental exposure groups (Fig. 7A).

The redox sensor resazurin was used to quantify the overall cellular redox state of the F1 96 hpf larvae. There was no indication of major oxidation events in the larvae due to no change in fluorescence relative to the controls for any of the exposure groups (Fig. 7B).

Activities of three antioxidant enzymes were quantified in the F1 96 hpf larvae. GR activity was significantly reduced in the F1 co-parental exposure group compared to the control (Fig. 7C). Activities of GPx and CAT were not different in any of the exposure groups for the F1 generation (Fig. 7D and E).

3.3.5 Mitochondrial function—*In vivo* OCR analysis in F1 24 hpf embryos revealed no statistically significant differences in any of the OCR parameters (Fig. S2).

4 Discussion

The main aim of this study was to investigate whether dietary exposure to PS NPs results in physiological alterations in adult fish and their offspring. We demonstrate that dietary exposure did not affect the reproductive success of adult fish, since the total number of eggs and the percentage of fertilized eggs were similar across groups. Interestingly, PS NPs were present in the yolk sac of embryos maternally or co-parentally exposed F1 embryos/larvae. The PS NPs were observed to accumulate first in the yolk sac as early as 24 hpf. The accumulation and distribution of PS NPs that was observed in the current study were similar to those reported in our waterborne exposure study (Pitt et al., 2018). These results also

agree with a previous study that reported that PS NPs were passed down through generations in crustaceans with the PS NPs binding to the lipids in both F0 and F1 generations (Cui et al., 2017). It is unclear how maternal transfer of PS NPs is mediated, but certain nanoparticles appear to have high affinity for plasma proteins, including lipid transport proteins, vitellogenin, and zona pellucada (Gao et al., 2017), and PS NPs have previously been shown to interact with vitellogenin (Rossi et al., 2014). Vitellogenin could facilitate the transfer of PS NPs to the oocytes, and ultimately, the embryo yolk sac. This could explain why only maternally-exposed and co-parentally-exposed, but not paternally-exposed embryos, showed presence of PS NPs in the yolk sac at 24 hpf. The transfer of PS NPs between generations in a vertebrate species has implications for both environmental and human health.

After the initial localization to the yolk sac, the PS NPs translocated to the gastrointestinal tract, liver, and pancreas. These results are consistent with previous studies that exposed zebrafish to PS NPs in the embryo medium (Pitt et al., 2018; van Pomeran et al., 2017). Despite the visible presence of PS NPs in the yolk sac, the survival and incidence of deformities were not significantly different across groups. However, the maternally and co-parentally exposed larvae exhibited bradycardia. This result is congruous with the bradycardia observed in our waterborne exposure study (Pitt et al., 2018). The observed bradycardia could be a result of the PS NPs interacting with the cardiac sarcomeres from their localization into cells yielding a change in heart function (Geiser et al., 2005). It should be noted that the pericardium did not have a significantly increased amount of fluorescence in the current study.

The swim bladder of zebrafish typically inflates at 72–96 hpf (Robertson et al., 2007); however, 100% of the co-parentally exposed larvae had uninflated swim bladders at 144 hpf. Swim bladder inflation is important as it provides stability for the vertical orientation and migration of the fish, which is critical for predator avoidance as zebrafish engage in diurnal migration (Robertson et al., 2008; Robertson et al., 2007). Further, the lack of inflation of the swim bladder can indicate a decrease in feeding efficiency, which is critical as the larva develops and the nutrient-filled yolk sac is resorbed (Goolish and Okutake, 1999; Tait, 1960). It was also reported that the lack of swim bladder inflation could lead to the development of spinal curvature in the developing larvae (Goolish and Okutake, 1999). Overall, this developmental delay as well as the aforementioned bradycardia indicates that co-parental exposure to PS NPs could reduce organismal fitness.

Interestingly, the larval locomotor activity was not affected by parental exposure to PS NPs, even in the co-parentally exposed larvae that displayed an uninflated swim bladder [larvae with uninflated swim bladders are typically hypoactive as they incur a greater metabolic cost for maintaining their position in the water column (Robertson et al., 2007)]. The lack of an effect on locomotor activity contrasts the larval hypoactivity that was reported in previous waterborne exposure studies (Chen et al., 2017; Pitt et al., 2018), which could be related to the extent of PS NPs uptake. It is likely that the larval PS NPs body burden is different between waterborne and parental exposures. Specifically, in the current study co-parentally-exposed embryos had ~7 fold higher fluorescence levels in the yolk sac at 24 hpf relative to

the control group, whereas fluorescence increases of up to 100 fold in the yolk sac at 24 hpf relative to the control group were reported upon a waterborne exposure (Pitt et al., 2018).

We also aimed to establish whether oxidative stress could be involved in the organismal response to PS NPs exposure and whether alterations in the antioxidant system occur in adult tissues and larval fish. We demonstrate that dietary exposure to PS NPs modulated the antioxidant system in the F0 generation. This was evidenced by the reduction of GR activity in the brain of females, gonads of males, and muscle of both females and males, in addition to the increased GPx activity in the brain of females. Both GR and GPx are related to glutathione metabolism, which is an important cellular process responsible, in part, for the detoxification of peroxides and electrophilic agents. The imbalance of reactive oxygen species (ROS) production and detoxification in favor of the former can result in higher concentrations of ROS, such as hydrogen peroxide and organic peroxides, leading to oxidative stress and potentially cellular damage, and apoptosis (Sies et al., 2017). The increased GPx activity and decreased GR activity in the brain of the females is similar to one seen in hypoxia conditions. In that case the GR activity is decreased as GR is inactivated by an oxidant, and GPx activity is increased in the brain in order to counteract signs of oxidative stress in the organism (Lushchak et al., 2001; Lushchak et al., 2005). The differing responses in the brain between the F0 males and females could be due to the male brain receiving a larger dose of PS NPs as the females off-loaded some of their dose into the F1 generation. As such, the males experienced a greater rate of oxidative stress causing more cellular damage leading to loss of GPx activity when compared with the females (Zhang et al., 2003). The interpretation of oxidative stress based upon antioxidant activities data alone provides a limited insight about the oxidative potential of PS NPs, and additional data regarding gene expression, cellular damage, and other antioxidant molecules are necessary to provide a mechanistic point of view. Nonetheless, the effects on GR and GPx activities in the brain are in agreement with studies that have reported accumulation of nanoplastics in brain of adult crucian carp (Mattsson et al., 2017). In addition, micro- and nano-sized polystyrene particles were shown to have pro-oxidant properties, leading to decreased levels of reduced glutathione in zebrafish larvae, and increased ROS levels and antioxidant enzyme activities in copepods *Paracyclopsina nana* (Chen et al., 2017; Jeong et al., 2017; Jeong et al., 2016). The current results provide further support that PS NPs potentially interfere with the ROS metabolism and antioxidant system of aquatic species, which can potentially lead to further cellular and physiological impairments and reduced capability to respond to additional environmental stressors.

Moreover, ROS production and impairment of the antioxidant system could lead to mitochondrial dysfunction, which has been reported for several environmental toxicants (Dranka et al., 2011; Jayasundara, 2017). In this study, the mitochondrial metabolic profile was not altered in the heart or gonads of F0 adults. However, the ovary tissues were trending towards having increased OCR for all parameters except for the mitochondrial reserve capacity. It is still unknown whether nano or microplastics are able to cause significant changes in mitochondrial function, and future studies should examine in more detail the potential pro-oxidant properties of PS NPs and the potential adverse effects on mitochondrial function.

Oxidative stress markers were also examined in F1 larvae. Similarly to the response in brain and muscle of F0 adults, GR activity was decreased in 96 hpf maternally- or co-parentally-exposed larvae. In addition, the levels of reduced thiols (both protein and non-protein) were decreased in these same groups. Together, these changes indicate that co-parental exposure to PS NPs could increase offspring's susceptibility to oxidative stress, since there is a decrease in reduced thiol levels and a potential inhibition of GR. However, the reduced thiol levels could also be due to thiol consumption during PS NPs detoxification. Styrene is first metabolized to styrene oxide and can be further detoxified by phase II reactions such as glutathione conjugation via GST (Zitting et al., 1980), leading to GSH depletion. Currently, it is unknown whether PS NPs can inhibit GR, but such inhibition can have adverse effects, including impaired immune function, lower capacity to detoxify peroxides, oxidative damage, and even lower survival, as reported in bivalves (Franco et al., 2006; Mello et al., 2015; Trevisan et al., 2014). The concentrations of GSH and GSSG, as well as the expression of genes associated with GSH/GSSG metabolism are tightly regulated during zebrafish early development (Timme-Laragy et al., 2013). It was shown that GR may play a major role during early development, especially after 18 hpf when GSH recycling is increased. Therefore, alterations of GR activity during early development could lead to long-term physiological effects as the larva matures. In addition, reduced GR activity could increase the organism's susceptibility to additional environmental stressors, including contaminants commonly adsorbed to plastics, such as polychlorinated biphenyls, polycyclic aromatic hydrocarbons (PAHs), dichloro-diphenyl-trichloroethane and its metabolites, polybrominated diphenyl ethers, alkylphenols, and bisphenol A (Hirai et al., 2011).

Although some effects were noted within the antioxidant system, embryonic mitochondrial function was not affected by cross-generational exposure in 24 hpf embryos, similarly to our earlier study (Pitt et al., 2018). As mentioned earlier, oxidative stress could lead to mitochondrial dysfunction. However, it should be noted that mitochondrial function analysis was performed in 24 hpf embryos, whereas oxidative stress parameters were assessed at 96 hpf. Therefore, the lack of effects on mitochondrial function should be interpreted cautiously, since the possibility of long-term impacts on mitochondrial function cannot be excluded. Further, the potential for toxicants known to induce mitochondrial dysfunction, such as PAHs, to adsorb to the surface of nanoplastics in the environment and then accumulate in an organism leading to changes in mitochondrial function should be further investigated (Hartmann et al., 2017; Liu et al., 2016; Meyer et al., 2013). Future studies should examine in more details the potential effects of various plastic types on mitochondrial function throughout development.

5. Conclusions

In summary, our study indicates that dietary exposure of adult zebrafish to PS NPs could affect the physiology of the offspring. More specifically, our data suggest that PS NPs are maternally transferred to the offspring via accumulation in the eggs of exposed females. This is probably due to interactions of PS NPs with plasma proteins related to oocytes, facilitating their transport to the gametes. Although PS NPs did not exert overt toxicity to either F0 adults or the F1 embryos/larvae, several changes were noted. In adult female brain as well as both female and male muscle tissues, PS NPs were able to modulate the antioxidant system

by decreasing the activity of GR. Further, it should be noted that the F0 females displayed similar changes in antioxidant enzyme activity as the F1 larvae. This suggests that the PS NPs could be affect the organism in similar ways at different points in the fish's life cycle. In embryos/larvae, bradycardia, uninflated swim bladders, and alterations of antioxidant system were noted in maternally- and/or co-parentally-exposed fish. Since PS NPs were also able to modulate the antioxidant system of both F0 adults and F1 larvae by decreasing GR activity and thiol levels, glutathione metabolism could be one potential target of PS NPs. Compromised antioxidant system could impair other physiological processes and increase the susceptibility to other environmental stressors, especially chemicals that can adsorb to plastics and be transferred together with nanoplastics within the food chain and/or maternally-transferred to offspring. Although mitochondrial function in adult tissues and embryos, as well as larval locomotor activity were not affected in this study, more detailed examination of these and other physiological endpoints is warranted in order to establish the extent of risk that nanoplastics pose to aquatic environment. Taken together, these data suggest that PS NPs are capable of affecting developing zebrafish through a maternal transfer of particles, and some of the observed effects were similar to those reported during a waterborne exposure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank all members of the Di Giulio laboratory for help with zebrafish husbandry. We highly appreciate the technical assistance of Drs. Nick Geitner and Mark Wiesner during nanoparticle characterization. Research was supported by Duke's Superfund Research Center (NIEHS P42-ES010356), Duke's Program in Environmental Health (ITEHP) Training Grant (NIEHS T32-ES021432), and Duke's Center for the Environmental Implications of Nanotechnology (NSF 3331894).

References

- Aebi H. Catalase in vitro. *Methods Enzymol.*, 105 (1984), pp. 121–126 [PubMed: 6727660]
- Alomar C, Sureba A, Capó X, Guijarro B, Tejada S, Deudero S. Microplastic ingestion by *Mullus surmuletus* Linnaeus, 1758 fish and its potential for causing oxidative stress. *Environ. Res.*, 159 (2017), pp. 135–142 [PubMed: 28800471]
- Anderson JC, Park BJ, Palace V. Microplastics in aquatic environments: implications for Canadian ecosystems. *Environ. Pollut.*, 218 (2016), pp. 269–280 [PubMed: 27431693]
- Andrady AL. Microplastics in the marine environment. *Mar. Pollut. Bull.*, 62 (2011), pp. 1596–1605 [PubMed: 21742351]
- Bailey JM, Oliveri AN, Karbhari N, Brooks RA, De La Rocha AJ, Janardhan S, et al. Persistent behavioral effects following early life exposure to retinoic acid or valproic acid in zebrafish. *Neurotoxicology*, 52 (2016), pp. 23–33 [PubMed: 26439099]
- Bergami E, Bocci E, Vannuccini ML, Monopoli M, Salvati A, Dawson KA, et al. Nano-sized polystyrene affects feeding, behavior and physiology of brine shrimp *Artemia franciscana* larvae. *Ecotoxicol. Environ. Saf.*, 123 (2016), pp. 18–25 [PubMed: 26422775]
- Bisesi JH, Ngo T, Ponnayolu S, Liu K, Lavelle CM, Afrooz AR, et al. Examination of single-walled carbon nanotubes uptake and toxicity from dietary exposure: tracking movement and impacts in the gastrointestinal system. *Nanomaterials (Basel)*, 5 (2015), pp. 1066–1086 [PubMed: 28347052]

- Blickley TM, Matson CW, Vreeland WN, Rittschof D, Di Giulio RT, McClellan-Green PD. Dietary CdSe/ZnS quantum dot exposure in estuarine fish: bioavailability, oxidative stress responses, reproduction, and maternal transfer. *Aquat. Toxicol*, 148 (2014), pp. 27–39 [PubMed: 24440963]
- Brun NR, Beenakker MMT, Hunting ER, Ebert D, Vijver MG. Brood pouch-mediated polystyrene nanoparticle uptake during *Daphnia magna* embryogenesis. *Nanotoxicology*, 11 (2017), pp. 1059–1069 [PubMed: 29083253]
- Carlberg I, Mannervik B. Glutathione reductase. *Methods Enzymol*, 113 (1985), pp. 484–490 [PubMed: 3003504]
- Chen Q, Gundlach M, Yang S, Jiang J, Velki M, Yin D, et al. Quantitative investigation of the mechanisms of microplastics and nanoplastics toward zebrafish larvae locomotor activity. *Sci. Total Environ*, 584–585 (2017), pp. 1022–1031
- Compa M, Ventero A, Iglesias M, Deudero S. Ingestion of microplastics and natural fibres in *Sardina pilchardus* (Walbaum, 1792) and *Engraulis encrasicolus* (Linnaeus, 1758) along the Spanish Mediterranean coast. *Mar. Pollut. Bull*, 128 (2018), pp. 89–96 [PubMed: 29571417]
- Cozar A, Echevarria F, Gonzalez-Gordillo JJ, Irigoien X, Ubeda B, Hernandez-Leon S, et al. Plastic debris in the open ocean. *Proc. Natl. Acad. Sci. U. S. A.*, 111 (2014), pp. 10239–10244
- Cui R, Kim SW, An YJ. Polystyrene nanoplastics inhibit reproduction and induce abnormal embryonic development in the freshwater crustacean *Daphnia galeata*. *Sci. Rep*, 7 (2017), Article 12095
- Della Torre C, Bergami E, Salvati A, Faleri C, Cirino P, Dawson KA, et al. Accumulation and embryotoxicity of polystyrene nanoparticles at early stage of development of sea urchin embryos *Paracentrotus lividus*. *Environ. Sci. Technol*, 48 (2014), pp. 12302–12311
- Dranka BP, Benavides GA, Diers AR, Giordano S, Zelickson BR, Reily C, et al. Assessing bioenergetic function in response to oxidative stress by metabolic profiling. *Free Radic. Biol. Med*, 51 (2011), pp. 1621–1635 [PubMed: 21872656]
- PLoS One, 9 (2014), Article e111913
- Farcail L, Torres Andon F, Di Cristo L, Rotoli BM, Bussolati O, Bergamaschi E, et al. Comprehensive in vitro toxicity testing of a panel of representative oxide nanomaterials: first steps towards an intelligent testing strategy. *PLoS One*, 10 (2015), Article e0127174
- Franco JL, Trivella DB, Trevisan R, Dinslaken DF, Marques MR, Bainy AC, et al. Antioxidant status and stress proteins in the gills of the brown mussel *Perna perna* exposed to zinc. *Chem. Biol. Interact*, 160 (2006), pp. 232–240 [PubMed: 16564039]
- Gao J, Lin L, Wei A, Sepúlveda MS. Protein corona analysis of silver nanoparticles exposed to fish plasma. *Environ. Sci. Technol. Lett*, 4 (2017), pp. 174–179 [PubMed: 31531386]
- Geiser M, Rothen-Rutishauser B, Kapp N, Schurch S, Kreyling W, Schulz H, et al. Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells. *Environ. Health Perspect*, 113 (2005), pp. 1555–1560 [PubMed: 16263511]
- Goolish EM, Okutake K. Lack of gas bladder inflation by the larvae of zebrafish in the absence of an air-water interface. *J. Fish Biol*, 55 (1999), pp. 1054–1063
- Hartmann NB, Rist S, Bodin J, Jensen LH, Schmidt SN, Mayer P, et al. Microplastics as vectors for environmental contaminants: exploring sorption, desorption, and transfer to biota. *Integr. Environ. Assess. Manag*, 13 (2017), pp. 488–493 [PubMed: 28440931]
- Hirai H, Takada H, Ogata Y, Yamashita R, Mizukawa K, Saha M, et al. Organic micropollutants in marine plastics debris from the open ocean and remote and urban beaches. *Mar. Pollut. Bull*, 62 (2011), pp. 1683–1692 [PubMed: 21719036]
- Jayasundara N. Ecological significance of mitochondrial toxicants. *Toxicology*, 391 (2017), pp. 64–74 [PubMed: 28778750]
- Jayasundara N, Kozal JS, Arnold MC, Chan SS, Di Giulio RT. High-throughput tissue bioenergetics analysis reveals identical metabolic Allometric scaling for teleost hearts and whole organisms. *PLoS One*, 10 (2015), Article e0137710
- Jeong CB, Won EJ, Kang HM, Lee MC, Hwang DS, Hwang UK, et al. Microplastic size-dependent toxicity, oxidative stress induction, and p-JNK and p-p38 activation in the Monogonont rotifer (*Brachionus koreanus*). *Environ. Sci. Technol*, 50 (2016), pp. 8849–8857 [PubMed: 27438693]

- Jeong CB, Kang HM, Lee MC, Kim DH, Han J, Hwang DS, et al. Adverse effects of microplastics and oxidative stress-induced MAPK/Nrf2 pathway-mediated defense mechanisms in the marine copepod *Paracyclopsina nana*. *Sci. Rep.* 7 (2017), Article 41323
- Koelmans AA, Besseling E, Shim WJ. Nanoplastics in the aquatic environment. Critical review. Bergmann M, Gutow L, Klages M (Eds.), *Marine Anthropogenic Litter*, 1, Springer, Berlin (2015), pp. 325–340
- Kozal JS, Jayasundara N, Lindberg CD, Massarsky A, Oliveri AN, Levin ED, Meyer JN, Di Giulio RT. Mitochondrial Dysfunction and Oxidative Stress Contribute to Cross-generational Toxicity of Benzo(a)pyrene in *Danio rerio*. Unpublished results (2018)
- Lee KW, Shim WJ, Kwon OY, Kang JH. Size-dependent effects of micro polystyrene particles in the marine copepod *Tigriopus japonicus*. *Environ. Sci. Technol.* 47 (2013), pp. 11278–11283
- Li WC, Tse HF, Fok L. Plastic waste in the marine environment: a review of sources, occurrence and effects. *Sci. Total Environ.* 566–567 (2016), pp. 333–349
- Liu L, Fokkink R, Koelmans AA. Sorption of polycyclic aromatic hydrocarbons to polystyrene nanoplastic. *Environ. Toxicol. Chem.* 35 (2016), pp. 1650–1655 [PubMed: 26588181]
- Lushchak VI, Lushchak LP, Mota AA, Hermes-Lima M. Oxidative stress and antioxidant defenses in goldfish *Carassius auratus* during anoxia and reoxygenation. *Am. J. Phys. Regul. Integr. Comp. Phys.* 280 (1) (2001), pp. R100–R107
- Lushchak VI, Bagnyukova TV, Lushchak V, Storey JM, Storey KB. Hypoxia and recovery perturb free radical processes and antioxidant potential in common carp (*Cyprinus carpio*) tissues. *Int. J. Biochem. Cell Biol.* 37 (6) (2005), pp. 1319–1330 [PubMed: 15778094]
- Massarsky A, Jayasundara N, Bailey JM, Oliveri AN, Levin ED, Prasad GL, et al. Teratogenic, bioenergetic, and behavioral effects of exposure to total particulate matter on early development of zebrafish (*Danio rerio*) are not mimicked by nicotine. *Neurotoxicol. Teratol.* 51 (2015), pp. 77–88 [PubMed: 26391568]
- Mattsson K, Ekvall MT, Hansson LA, Linse S, Malmendal A, Cedervall T. Altered behavior, physiology, and metabolism in fish exposed to polystyrene nanoparticles. *Environ. Sci. Technol.* 49 (2015), pp. 553–561 [PubMed: 25380515]
- Mattsson K, Johnson EV, Malmendal A, Linse S, Hansson LA, Cedervall T. Brain damage and behavioural disorders in fish induced by plastic nanoparticles delivered through the food chain. *Sci. Rep.* 7 (2017), Article 11452
- Mello DF, Arl M, Trevisan R, Dafre AL. How important are glutathione and thiol reductases to oyster hemocyte function? *Fish Shellfish Immunol.* 46 (2015), pp. 566–572 [PubMed: 26210700]
- Meyer AJ, May MJ, Fricker M. Quantitative in vivo measurement of glutathione in *Arabidopsis* cells. *Plant J.* 27 (2001), pp. 67–78 [PubMed: 11489184]
- Meyer JN, Leung MC, Rooney JP, Sendoel A, Hengartner MO, Kisby GE, et al. Mitochondria as a target of environmental toxicants. *Toxicol. Sci.* 134 (2013), pp. 1–17 [PubMed: 23629515]
- Panzica-Kelly JM, Zhang CX, Danberry TL, Flood A, DeLan JW, Brannen KC, Augustine-Rauch KA. Morphological score assignment guidelines for the Dechorionated zebrafish teratogenicity assay. *Birth Defects Res. B Dev. Reprod. Toxicol.* 86 (2010), pp. 382–395
- Pitt JA, Kozal JS, Jayasundara N, Massarsky A, Trevisan R, Geitner N, et al. Uptake, tissue distribution, and toxicity of polystyrene nanoparticles in developing zebrafish (*Danio rerio*). *Aquat. Toxicol.* 194 (2018), pp. 185–194 [PubMed: 29197232]
- Robertson GN, McGee CA, Dumbarton TC, Croll RP, Smith FM. Development of the swimbladder and its innervation in the zebrafish, *Danio rerio*. *J. Morphol.* 268 (2007), pp. 967–985
- Robertson GN, Lindsey BW, Dumbarton TC, Croll RP, Smith FM. The contribution of the swimbladder to buoyancy in the adult zebrafish (*Danio rerio*): a morphometric analysis. *J. Morphol.* 269 (2008), pp. 666–673 [PubMed: 18302241]
- Rochman CM, Hoh E, Kurobe T, The SW. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. *Sci. Rep.* 3 (2013), p. 3263 [PubMed: 24263561]
- Rossi G, Barnoud J, Monticelli L. Polystyrene nanoparticles perturb lipid membranes. *J. Phys. Chem. Lett.* 5 (2014), pp. 241–246 [PubMed: 26276207]
- Sies H, Berndt C, Jones DP. Oxidative stress. *Annu. Rev. Biochem.* 86 (2017), pp. 715–748 [PubMed: 28441057]

- Stackley KD, Beeson CC, Rahn JJ, Chan SS. Bioenergetic profiling of zebrafish embryonic development. *PLoS One*, 6 (2011), Article e25652
- Tait JS. The first filling of the swim bladder in salmonoids. *Can. J. Zool.*, 38 (1960), pp. 179–187
- Timme-Laragy AR, Goldstone JV, Imhoff BR, Stegeman JJ, Hahn ME, Hansen JM. Glutathione redox dynamics and expression of glutathione-related genes in the developing embryo. *Free Radic. Biol. Med.*, 65 (2013), pp. 89–101 [PubMed: 23770340]
- Trevisan R, Mello DF, Uliano-Silva M, Delapiedra G, Arl M, Dafre AL. The biological importance of glutathione peroxidase and peroxiredoxin backup systems in bivalves during peroxide exposure. *Mar. Environ. Res.*, 101 (2014), pp. 81–90 [PubMed: 25265592]
- van Pomeran M, Brun NR, Peijnenburg W, Vijver MG. Exploring uptake and biodistribution of polystyrene (nano)particles in zebrafish embryos at different developmental stages. *Aquat. Toxicol.*, 190 (2017), pp. 40–45 [PubMed: 28686897]
- Vandermeersch G, Van Cauwenberghe L, Janssen CR, Marques A, Kit G, Fait G, Kotterman MJJ, Diogène J, Bekaert K, Johan R, Lisa D. A critical view on microplastic quantification in aquatic organisms. *Environ. Res.*, 143 (2015), pp. 46–55 [PubMed: 26249746]
- von Moos N, Burkhardt-Holm P, Kohler A. Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. *Environ. Sci. Technol.*, 46 (2012), pp. 11327–11335
- Wendel A. Glutathione peroxidase. *Methods Enzymol.*, 77 (1981), pp. 325–333 [PubMed: 7329310]
- Wesch C, Bredimus C, Paulus M, Klein R. Towards the suitable monitoring of ingestion of microplastics by marine biota: A review. *Environ. Pollut.*, 218 (2016), pp. 1200–1208 [PubMed: 27593351]
- Zhang J, Shen H, Wang X, Wu J, Xue Y. Effects of chronic exposure of 2, 4—dichlorophenol on the antioxidant system in liver of freshwater fish *Carassius auratus*. *Chemosphere*, 55 (2) (2004), pp. 167–174 [PubMed: 14761689]
- Zhao L, Qu M, Wong G, Wang D. Transgenerational toxicity of nanopolystyrene particles in the range of $\mu\text{g/L}$ –1 in the nematode *Caenorhabditis elegans*. *Environ. Sci. Nano*, 4 (2017), pp. 2356–2366
- Zitting A, Heinonen T, Vainio H. Glutathione depletion in isolated rat hepatocytes caused by styrene and the thermal degradation products of polystyrene. *Chem. Biol. Interact.*, 31 (1980), pp. 313–318 [PubMed: 7408038]

Figure 1. Reproductive success of adult zebrafish exposed to PS NPs via diet.

Total number of eggs spawned and percentage of fertilized eggs are presented (n = 6 over 3 cohorts). Data are presented as means ± SEM. Significance was accepted if $p < 0.05$, using Kruskal-Wallis followed by Dunn's posthoc. Values that share the same letter do not differ from each other.

Figure 2. Antioxidant enzymes activity in tissues from F0 adults dietary exposed to polystyrene nanoparticles.

Activities of the antioxidant enzymes glutathione reductase, glutathione peroxidase, and catalase in tissues of F0 adults. All data are presented as fold change relative to the respective control group (mean $\pm$ SEM, $n=10-12$ across 3 cohorts). Absolute values of the control groups are shown in Table S1. Significance was accepted if $p < 0.05$, using unpaired t-test (parametric) or Mann-Whitney U test (nonparametric) for each individual tissue. An asterisk denotes statistical differences across treatments within each tissue.

Figure 3. Mitochondrial function and metabolic partitioning in tissues of F0 adult dietary exposed to polystyrene nanoparticles.

Respiration rates (oxygen consumption rate; OCR) measured *ex vivo* with F0 heart and gonad tissues exposed to PS NPs. Data are shown as $\text{mg O}_2 \text{ h}^{-1} \text{ g}^{-1}$ ($n=4-6$ across 2 cohorts). The parameters measured were: basal respiration, maximal respiration (in presence of FCCP), non-mitochondrial respiration (in presence of rotenone and antimycin), basal mitochondrial respiration (total basal – non-mitochondrial), maximal mitochondrial respiration (total maximal – non-mitochondrial), and mitochondrial reserve capacity (total maximal – total basal). Each point represents a different parameter, as shown on the bottom graph. Significance was accepted if $p < 0.05$, using unpaired t test (parametric) or Mann-Whitney U test (nonparametric).

Figure 4. Survival, heart rate, and developmental delays in F1 zebrafish embryos/larvae cross-generationally exposed to polystyrene nanoparticles.

(A) Survival of larvae exposed to PS NPs from 24–96 hpf (n=4 plates from 2 cohorts). (B) Heart rate at 48 hpf (n=20 across 2 cohorts). (C) Swim bladder inflation was evaluated at 144 hpf (n=6 plates across 2 cohorts). All data are presented as means $\pm$ SEM. Significance was accepted if $p < 0.05$, using one-way ANOVA followed by Tukey's posthoc (parametric) or Kruskal-Wallis followed by Dunn's posthoc (nonparametric). Values that share the same letter do not differ from each other.

Figure 5. Polystyrene nanoparticles (PS NPs) fluorescence and accumulation in yolk sac in 24–72 hpf F1 zebrafish embryos cross-generationally exposed to polystyrene nanoparticles.

On top, representative images (transmitted light merged to green fluorescence) of the zebrafish embryos. At 24 hpf, embryos were imaged with their chorion intact. On the bottom, fluorescence quantification in the yolk sac at each developmental stage, presented as fold change (means $\pm$ SEM, n=6 across 2 cohorts). Significance was accepted if $p < 0.05$, using one-way ANOVA followed by Tukey's post hoc (parametric) or Kruskal-Wallis followed by Dunn's posthoc (nonparametric). Values that share the same letter do not differ from each other.

Figure 6. Polystyrene nanoparticles (PS NPs) fluorescence and distribution in 120 hpf F1 zebrafish larvae cross-generationally exposed to polystyrene nanoparticles.

On top, representative images (transmitted light merged to green fluorescence) of the zebrafish larvae. The letters in the control image correspond to various organs analyzed: (GB) gall bladder, (P) pancreas, (GI) gastrointestinal tract, and (L) liver. On the bottom, fluorescence quantification in the gall bladder, pancreas, gastrointestinal tract, and liver at 120 hpf presented as fold change (mean $\pm$ SEM, $n=6$ across 2 cohorts). Significance was accepted if $p<0.05$, using one-way ANOVA followed by Tukey's post hoc (parametric) or Kruskal-Wallis (non-parametric). Values that share the same letter do not differ from each other.

Figure 7. Oxidative stress markers in F1 zebrafish larvae cross-generationally exposed to polystyrene nanoparticles.

Reduced thiol levels and cellular redox state were detected by fluorescence assays and data are presented as fold change ($n=16$ across 2 cohorts). Activities of the enzymes glutathione reductase, glutathione peroxidase, and catalase in 96 hpf larvae are also presented as fold change ($n=5-6$ across 3 cohorts). Absolute values of the control groups are shown in Table S1. All data are presented as mean $\pm$ SEM. Significance was accepted if $p < 0.05$, using one-way ANOVA followed by Tukey's post hoc (parametric) or Kruskal-Wallis followed by Dunn's posthoc (nonparametric). Values that share the same letter do not differ from each other.