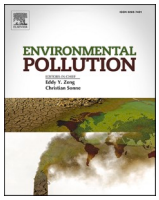
Environmental Pollution 356 (2024) 124340

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol



Ingestion of polyethylene terephthalate microplastic water contaminants by *Xenopus laevis* tadpoles negatively affects their resistance to ranavirus infection and antiviral immunity☆,☆☆

Binghong Cai [a,c], Francisco De Jesus Andino [a], James L. McGrath [c], Samantha S. Romanick [c], Jacques Robert [a,b,*]

[a] University of Rochester Department of Microbiology and Immunology, USA
[b] University of Rochester Department Environmental Medicine, USA
[c] University of Rochester Biomedical Engineering, USA

ARTICLE INFO

Keywords:
Microplastics
Xenopus laevis
Bioaccumulation
Growth delay
Frog virus 3
Immune response

ABSTRACT

Small plastic debris (0.1 μm–5 mm) or microplastics (MPs) have become major pollutants of aquatic ecosystems worldwide and studies suggest that MPs exposure can pose serious threats to human and wildlife health. However, to date the potential biological impacts of MPs accumulating in low amount in tissues during early life remains unclear. Here, for a more realistic assessment, we have used environmentally representative, mildly weathered, polyethylene terephthalate microplastics (PET MPs), cryomilled (1–100 μm) and fluorescently labelled. We leveraged the amphibian *Xenopus laevis* tadpoles as an animal model to define the biodistribution of PET MPs and determine whether exposure to PET MPs induce perturbations of antiviral immunity. Exposure to PET MPs for 1–14 days resulted in detectable PET MPs biodistribution in intestine, gills, liver, and kidney as determined by fluorescence microscopy on whole mount tissues. PET MPs accumulation rate in tissues was further evaluated via a novel *in situ* enzymatic digestion and subsequent filtration using silicon nanomembranes, which shows that PET MPs rapidly accumulate in tadpole intestine, liver and kidneys and persist over a week. Longer exposure (1 month) of tadpoles to relatively low concentration of PET MPs (25 μg/ml) significantly increased susceptibility to viral infection and altered innate antiviral immunity without inducing overt inflammation. This study provides evidence that exposure to MPs negatively impact immune defenses of aquatic vertebrates.

1. Introduction

Microplastics (MPs), technically classified as plastic particles with a size ranging from 0.1 μm to 5 mm, have become major environmental and public health concerns. Owing to their widespread occurrence in most ecosystems and organisms it is imperative to investigate their potential ecological and public health impacts (Thompson et al., 2004). Studies have reported the presence of MPs in environments as varied as urban landscapes, remote terrestrial regions, freshwater ecosystems, marine environments, and even in pristine remote expanses, such as Arctic Sea ice (Eriksen Lebreton et al., 2014, Obbard et al., 2014; Horton et al., 2017). MPs originate from a plethora of sources, including but not limited to fragmentation of larger plastic materials, byproducts of various industrial processes, and even direct release from personal care products (Andrady, 2011). In the aquatic environment, MPs are consumed by a wide variety of organisms from invertebrates (molluscs, crustaceans) to vertebrates such as fish, amphibians and ultimately humans. In fish, both experimental and field studies indicate that MPs preferentially accumulate in the gills, intestine, and liver (Hamlin et al., 2015; Lönnstedt and Eklöv, 2016, Rummel et al., 2016; Naidoo and Glassom, 2019). Although their biodistribution in human remains to be fully characterized, MPs have been detected in human heart, intestine,

☆ This paper has been recommended for acceptance by Michael Bank. ☆☆ This study has been funded by the National Institute of Allergy and Infectious Diseases at the National Institutes of Health (R24-AI059830 – R21-AI139718) and the NSF (1754274), a Pilot Project Grant from the Rochester Environmental Health Sciences Center (P30-ES001247) and a research program project from the National Institute of Environmental Health Sciences P01ES035526

* Corresponding author.Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, NY 14642, USA.
E-mail address: Jacques_Robert@urmc.rochester.edu (J. Robert).

https://doi.org/10.1016/j.envpol.2024.124340
Received 23 March 2024; Received in revised form 30 May 2024; Accepted 6 June 2024
Available online 6 June 2024

stools, placenta, and breast milk (Schwabl et al., 2019; Ragusa et al., 2021). Studies suggest that the ingestion of these minute particles by organisms across the taxonomical spectrum can lead to a host of biological impacts that are only beginning to be elucidated (Wright and Kelly, 2017).

Some evidence indicates that MPs accumulation can result in developmental and reproductive defects in fish and amphibians (Lönnstedt and Eklöv, 2016; Conlin et al., 2018; Mak, Ching-Fong Yeung et al., 2019; Naidoo and Glassom, 2019). *In vitro* studies also suggest that MPs can be taken up by phagocytic cells such as neutrophils and macrophages (Espinosa, Garcia Beltran et al., 2018, Hwang et al., 2019); and induce inflammation (Jin, Xia et al., 2018). Additionally, MPs can affect fish leukocytes function *in vitro* such as oxidative stress (Espinosa, Garcia Beltran et al., 2018). Furthermore, effects on innate immunity have been reported in invertebrates (Green et al., 2019; Liu et al., 2019), and fish (Greven, Merk et al., 2016, Jinhui et al., 2019). In murine models, exposure to MPs has been linked with alterations of immune responses, including inflammatory reactions and shifts in cytokine expression profiles (Qiu, Qi et al., 2022). Recently, ingestion of manufactured polystyrene microspheres by tadpoles of the midwife toads has been reported to increase the burden of infection by chytrid fungus, a major amphibian pathogen, although no immune mechanism was proposed (Bosch et al., 2021).

Whilst the implications of MP exposure on human health remain to be fully assessed, the immune perturbations observed in other organisms' post-MP exposure suggest that similar effects can occur in humans and underscore the importance to continue rigorous comparative investigations. The significance of this issue is further magnified considering recent estimates of MP ingestion rates in humans, indicating a potential for chronic accumulation with undefined health risks (Yee et al., 2021).

In this context, the amphibian *Xenopus laevis*, serves as an attractive model organism for studying the impacts of MPs on the immune system. Given their aquatic lifestyle and external development free of maternal influence, *X. laevis* are ideally suited for MP studies, offering valuable insights into the environmental changes induced by MPs (Blaustein & Kiesecker, 2002). *X. leavis* embryology, developmental processes and physiology are very similar to mammals, which has led to fundamental discoveries about pathophysiology, developmental defects, and medical immunology (LaBonne and Zorn, 2015; Robert, 2020; Tandon et al., 2017). Furthermore, the remarkable conservation between the *X. laevis* and human immune systems allows for insightful comparative analysis of human immune responses (Du Pasquier et al., 1989; Ohta, 2009).

To date, studies on MPs have mainly involved sterile manufactured polystyrene or polyethylene microspheres as reference particles with exposure at often very high (mg/ml) concentration (da Costa Araújo et al., 2020). As such, it is currently unclear whether MPs induce effects that are more subtle than mechanical occlusion or acute inflammation of the digestive track. In this study, we have focused on environmentally representative, mildly weathered, Polyethylene terephthalate (PET). PET is extensively used, especially in the packaging industry, and is a significant contributor to environmental plastic pollution (Hopewell, Dvorak & Kosior, 2009). In addition, the environmental impact of PET is under-investigated (Eerkes-Medrano, Thompson, & Aldridge, 2015). Consequently, we have investigated the biodistribution and biological impacts of PET microplastics (PET MPs) in *X. laevis* tadpoles. Our results show that upon exposure PET MPs rapidly accumulate in tadpole intestine, liver and kidneys and persist over a week, which compromise antiviral immunity and weaken resistance to viral infection.

2. Materials & methods

2.1. Animals

Tadpoles between stages 54 and 56 (three-week old, 1.5 cm long (Zahn et al., 2022);) were sourced from the *X. laevis* research resource for immunology at the University of Rochester Medical Center (https://www.urmc.rochester.edu/microbiology-immunology/research/xenopus-laevis.aspx). All procedures involving the animals were strictly adhered to the laboratory and University Committee on Animal Resources regulations (approval number 100577/2003-151).

2.2. Preparation of PET MPs

Samples of PET MPs were kindly gifted to us by Dr. Kim Rogers at the EPA. Mildly weathered PET plastic (Supplementary Fig. S1) was collected from the Pacific Ocean near Hawaii by Dr. Jennifer Lynch with the National Institute of Standards and Technology (NIST) and Hawaii Pacific University (Brignac et al., 2019). The plastics were used as feedstocks for milling to obtain a size distribution of particles between 1 μm and 100 μm (average diameter 80 μm) using a Retsch ball cryomill in 5 grind cycles (25 hz for 2 min/cycle with a 30 s intermittent cooling cycle at 5 hz). The resulting milled powder was dispersed in water with (0.5% v/v) surfactant (FL-70; Fisher Scientific, SF105-1) into a glass vial then bath sonicated for 1 h prior to vacuum filtration through a 1 μm PTFE filter. PET MPs was fluorescently labelled with Nile Red dye ((50 g in 10 mL of 10 μg/mL Nile Red dye; Abcam ab228553) (Maes et al., 2017)) for 1 h, collected by sedimentation via centrifugation at 1000 rpm (g = 113), washed and resuspended in RNAse/DNAse free H2O (1% w/v). Before use, stained PET MPs were diluted in RNAse/DNAse-free H_2O and sonicated for 1 min. Successful staining was confirmed by fluorescent microscopy (Invitrogen™ EVOS™ FL Digital Inverted Fluorescence Microscope), and a standard curve of serial PET MP concentrations was established to show the dose dependence in the fluorescence metric (Supplementary Fig. S2). The fluorescent signal remained stable upon treatment at different pH (4–8) and temperature (boiling) as well as over 4-month time in water sediment. (Supplementary Fig. S1).

2.3. Exposure and infection with FV3

Two different tadpole MPs exposure methods were performed: (1) *Individual exposure* to determine PET MPs biodistribution, and (2) *Bulk exposure* to assess biological effects of exposure to PET MPs. No surfactant was used in the aquatic environment during exposure and incubation.

(1) For individual exposures, tadpoles were exposed individually in 50 ml centrifuge tubes filled with 25 ml water containing 100 mg/L (1.8×10^4 particles/ml) PET MPs s for 24–72 h and euthanized in 1 g/L tricaine methanesulfonate (TMS-222) solutions. To evaluate transit and accumulation, tadpoles exposed individually for 24 h in 25 ml volume with 100 mg/L PET MPs, were transferred into a new tube with 25 ml of clean water for 24 h, then transferred again into a new tube for 24 h. This was repeated 3 times. The initial number of 100 mg/L of PET MPs s in 25 ml as well as number left after 24 h exposure and number released at each 24 h transfer was determined as follow: each tube containing PET MPs s was centrifuged at 1000 rpm (g = 113) for 15 min, and the pellets were resuspended in 1 mL of distilled water. Then, 10 μl of each suspension was loaded on a hematocytometer to count PET MPs s under a fluorescent microscope. This experiment was repeated 3 times independently.

(2) For bulk exposures, a group of 15 tadpoles were exposed for 1 month at different doses (25–500 mg/L or 0.45×10^4 - 9.2×10^4) in 4 L total volume. These concentrations were chosen based on other studies with frogs including *Xenopus* (De Felice et al., 2018; da Costa Araújo et al., 2020; Ruthsatz et al., 2023). Because the dense PET MPs sediment rapidly in the bottom of the tanks, water was stirred once a day. Tadpoles were transferred into new recipient with fresh water and new PET MPs s once a week. Survival was monitored every day.

In two independent experiments, tadpoles were exposed for 1 month to 100 or 25 mg/L (but not to 500 mg/ml because of its toxicity), then randomly selected (6 per group) and infected with the ranavirus FV3 by intraperitoneal (i.p.) injection of 10,000 plaque forming units (PFUs) in a volume of 5 μl of amphibian phosphate-buffered saline (APBS). Uninfected control animals were mock-infected with an equivalent volume of APBS.

2.4. Tissue lysis and filtration on silicon nanomembranes

Tadpole tissues were enzymatically digested overnight at 55 °C in a lysis buffer (50 mM Tris pH 7.5, 5 mM Ethylenediaminetetraacetic acid, 50 mM NaCl, 0.5% Sodium Dodecyl Sulfate) containing proteinase K (0.25 mg/ml; Thermo Fisher Scientific, 25530015) and collagenase type I (0.1 mg/ml; Sigma Aldrich, C0130). Lysates were then filtered through a size selective silicone nanomembrane with microslits measuring 2 μm × 50 μm (SiMPore Inc., West Henrietta, NY, SC400524) assembled onto the bottom of a SepCon vial, housed inside of a 1.5 ml Eppendorf tube, specifically designed to fit these membranes. SepCon Vials were assembled and disassembled as described (https://nanomembranes.org/sepcon-assembly-written-description/). Fluorescent PET MPs retained on the membrane were then imaged via fluorescent microscopy at excitation wavelength of 595 nm. To quantify retained PET MPs, the overall surface area of fluorescent PET MPs on each nanomembrane was calculated using Image J. The PET MPs retained on the nanomembrane were then resuspended in water, sonicated for 1 min, and then counted on a hematocytometer under a fluorescent microscope.

2.5. Viral production and viral load

FV3 was grown using a single passage through baby hamster kidney cells (BHK-21; ATCC CCL-10), subsequently purified by ultracentrifugation on a 30% sucrose cushion and quantified by plaque assay on BHK-21 monolayer under an overlay of 1% methylcellulose. Viral loads were determined by qPCR on DNA (125 ng) extracted from spleen, liver, kidney, and intestines using TRIzol reagent according to the manufacturer's protocol (ThermoFisher/Invitrogen). Signal levels were compared to a serially diluted standard curve of an FV3 DNA Polymerase II PCR fragment cloned into the pGEM-T Easy vector (Promega, Madison, WI, USA). This construct was quantified and serially diluted to yield 10^{10} to 10^{1} FV3 DNA polymerase II plasmid copies. These dilutions were employed as a standard curve in subsequent absolute qPCR experiments to derive the viral genome copy numbers relative to this standard curve.

2.6. MP quantification on whole mount organs

Tadpole's intestines, livers, kidneys, and gills were isolated and placed on microscope slides with PBS for viewing under a fluorescence microscope.

2.7. Quantitative gene expression analyses

Total RNA was extracted from the different tadpole organs using TRIZOL reagent according to manufacturer's protocol (Invitrogen). RNA (0.5 μg) was reverse transcribed into complementary DNA (cDNA) with M-MLV reverse transcriptase (Invitrogen) and a mixture of oligo(dT) primer (Invitrogen). The relative gene expression was determined for 125 ng of cDNA with ABI 7300 Real-Time PCR System and PerfeCTa SYBR Green FastMix ROX. The expression levels were normalized to that of an endogenous housekeeping gene, GAPDH, then further normalized against the lowest observed expression. All the primers used in the study are listed in Supplementary Table S1.

2.8. Statistical analysis

Normality of all the data was assessed using both the shapiro-wilk test and q-q plot set a at a p value of 0.01 or lower. (GraphPad Prism 9; GraphPad, San Diego, CA, USA). One-way analysis of variance (ANOVA) followed by Tukey's multiple-comparison test was used for statistical analysis of gene expression. Analyses were performed using a VassarStats online resource (http://vassarstats.net/utest.html). Statistical analysis of survival data was performed using a log rank test (GraphPad Prism 9; GraphPad, San Diego, CA, USA). A probability (P) value of 0.05 was used in all analyses to indicate significance. Error bars on all graphs represent the standard errors of the means (SEM).

3. Results

3.1. Toxicity, biodistribution and persistence of PET MPs

To study the biological activity of microplastics more relevant to human health than sterile manufactures microbeads, we choose PET, which is a significant contributor to environmental plastic pollution. Environmentally representative mildly weathered samples of PET (Supplementary Fig. S1), cryomilled to size ranging from 1 to 100 μm and stained with Nile red fluorescent dye were first tested for toxicity. Three week-old *X. laevis* tadpoles (developmental stage 55) were exposed to different concentrations of PET MPs (25–500 mg/L) corresponding to 4.5×10^{7} particles/L and 9×10^{7} particles/L, respectively, and monitored survival for 1 month. Significant mortality occurred at 500 mg/L and to a lesser extent at 100 mg/L, but not 25 mg/L PET MPs exposure (Fig. 1). In addition, at 25 mg/L PET MPs did not cause detectable side effects on the survival, growth and swimming activity, and no obvious organ damage were detected. At higher PET MPs concentration (500 mg/L), we observed morphological defects of the large intestine in some tadpoles (data not shown).

In parallel, we examined PET MPs biodistribution in tadpoles' organs. Exposure to high PET MPs concentration (500 mg/L) for 24 h resulted in high Nile Red fluorescent signal detected in whole preparation of tadpole intestine, indicative of large amount of ingested PET MPs (Fig. 2A). In addition, numerous red fluorescent particles were detected in tadpole organs in the liver and to a lesser extent in kidneys (Fig. 2B and C). Some PET MPs were also detected in the gills, but not in other organs including spleen and lung (Supplementary Fig. S3). Nile red stained PET MPs particles detected in wholemount organs only emitted red fluorescent signals, distinguishable from other pigments or autofluorescent signals that emitted both red and green signals. As shown in Fig. 2D and E, ingestion of PET MPs resulted in strong red fluorescent signal in tadpole stomach but no green fluorescent signal. Comparable biodistribution of Nile Red fluorescent PET MPs was observed in the tadpole after 24 h exposure at a lower concentration of 100 mg/ml PET MP, including the stomach (Fig. 2E), liver (Fig. 2E) and kidneys (data not shown). Fluorescent PET MPs were also detected in tadpole organs after a longer exposure from 3 to 14 days (Fig. 2C and D). Interestingly, the amount of PET MPs fluorescent signal detected in intestine decreased after 24 h exposure, whereas PET MPs signal detected in tadpole organs such as liver and kidneys did not markedly change. In addition, the PET MPs detected in tissues were smaller (~10 μm) compared to the range of different sizes tadpoles were exposed, suggesting that PET MPs >10 μm cannot easily pass the intestinal epithelium and thus is excreted from the organism.

To further evaluate the bioaccumulation, transit, and excretion of PET MPs, we determined the amount of material ingested and excreted per single tadpole over 7 days. We individually exposed tadpoles for 24 h to PET MPs (100 mg/L in a volume of 25 ml equivalent a total of 4.5×10^{5} PET particles). We then collected PET MPs excreted each day for 7 days by transferring animals into new recipient with pure water. Excreted PET MPs were collected by centrifugation and enumerated under fluorescent microscopy (Fig. 3). Interestingly, comparable amounts of PET MPs were excreted each day, with a slight increase at day 3. By comparing the input with the excreted PET MPs collected for 7 days, we were able to estimate the amount of PET MPs ingested and

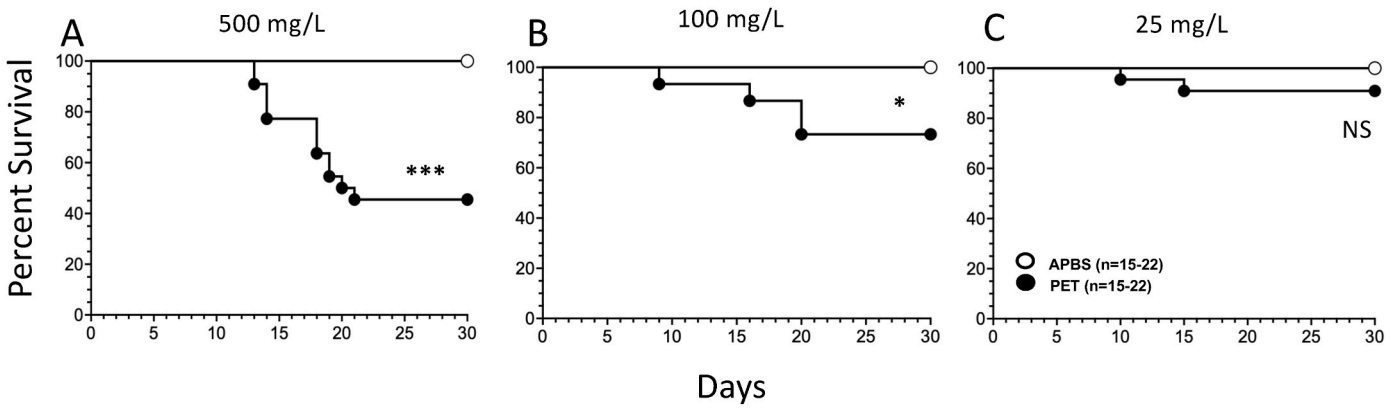


Fig. 1. Survival of tadpoles exposed to different dose of PET MPs s. Tadpoles were exposed to (A) 500 mg/L (22 individuals), (B) 100 mg/L (22 individuals) or (C) 25 mg/L (15 individuals) PET MPs s, or no PET MPs s (only APBS added, 22 or 15 individuals). Statistical analysis of survival data was performed using a Kaplan-Meier Log-Rank (Mentel-Cox) Test (GraphPad Prism 9, San Diego, CA, USA). ***$p < 0.0001$; p* <0.05.

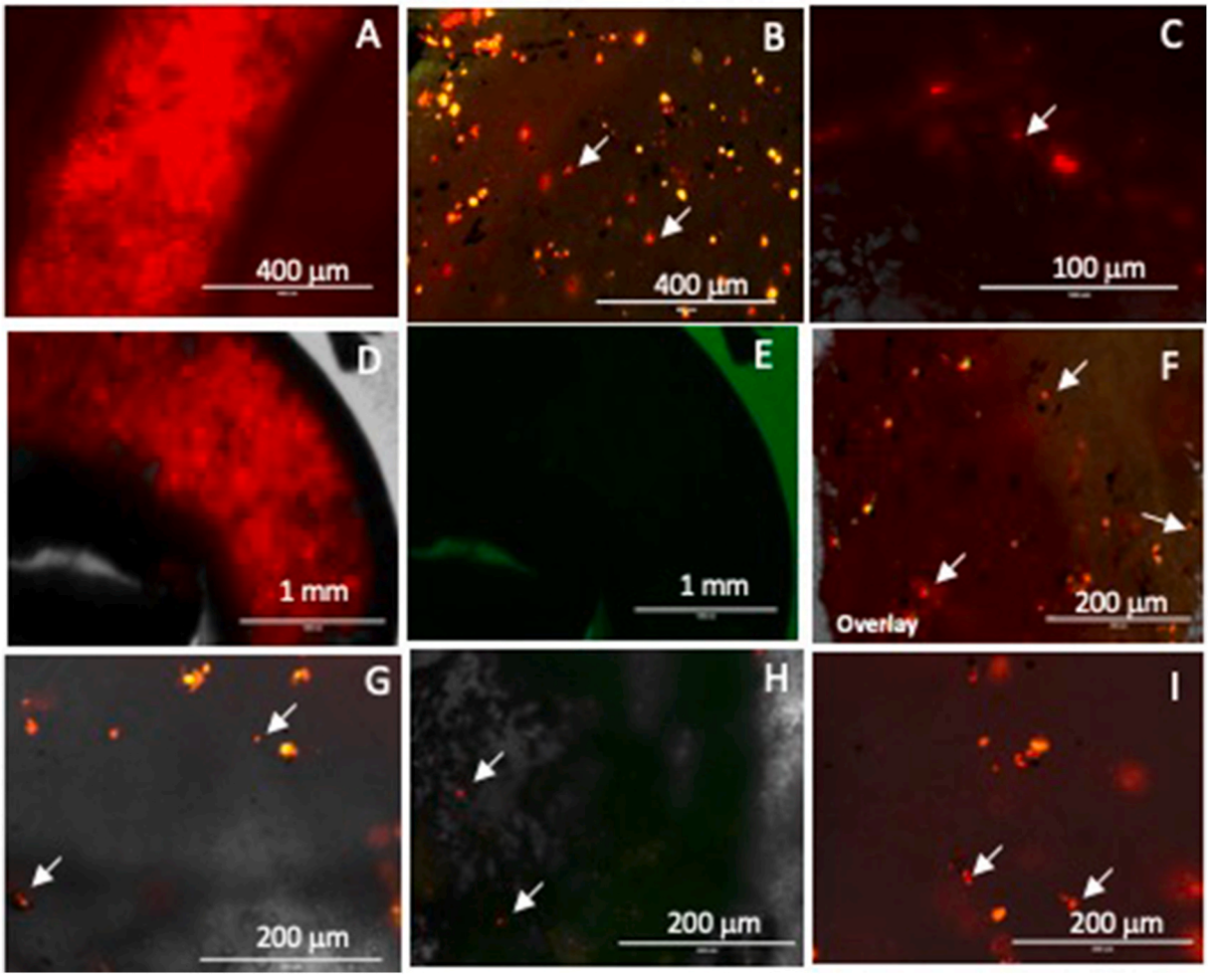


Fig. 2. Detection of PET MPs s in tadpoles exposed for 1 to 7 days on whole mount organ preparation by fluorescence microscopy. *Upper panel*: Tadpoles exposed 24 h to 500 mg/L PET MPs: (A) Intestine; (B) liver; and (C) kidney. *Middle panel:* Tadpoles exposed 24 h to 100 mg/L PET MPs: (D) Red fluorescent signal in stomach; (E) Green fluorescent signal of the same stomach preparation; (F) liver. *Lower panel*: Tadpoles exposed to 100 mg/L PET MPs for 7 days: (G) liver; (H) kidney (H), and (I) liver of tadpoles exposed 14 days. All pictures except E are overlays of the green and red fluorescent emission. Arrows in overlay picture indicate PET MPs s that are not autofluorescent (no green fluorescent signal). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

remaining in each tadpole (Table 1). On average, almost 2 mg of PET MPs was ingested within 24 h exposure, which represent 0.6% of the tadpole weight. Based on the signal intensity detected by fluorescent microscopy, a large fraction of this ingested material is likely to reside in the digestive track. After 7 days, we estimated that on average 5.2×10^4 PET MPs, which is equivalent to 0.29 mg, were still retained in each

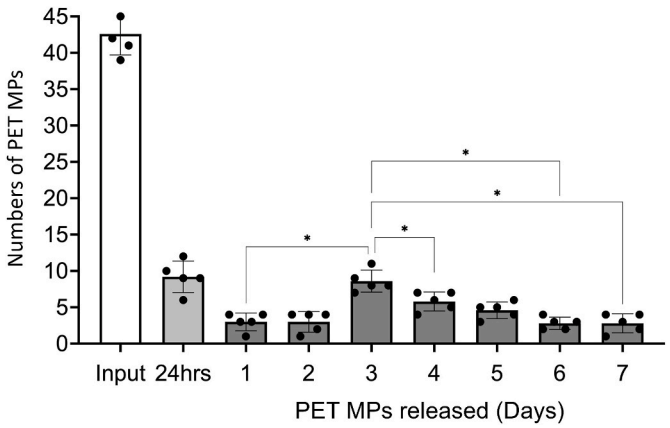


Fig. 3. Number of PET MPs excreted daily per tadpole following ingestion during a 24 h exposure period. Representative of 3 independent experiments where tadpoles were individually exposed to 100 mg/L PET MPs s for 24 h and then transferred into clean water each 24 h for 7 days. (*Input)*: total numbers of PET MPs s in 25 ml volume used to expose each tadpole; (24hrs): numbers of PET MPs s remaining after 24 h exposure; (1–7) numbers of PET MPs s released for each day for each tadpole. Values were determined by counting PET MPs under fluorescent microscope after concentration by centrifugation. All data were analysed using a 1-way ANOVA and Tukey's post-hoc test. N = 5 individuals, *p ≤ 0.0119.

tadpole 7 days post-exposure.

To substantiate these results, organs from tadpoles exposed to PET MPs (100 μg/ml) for 1–7 days were enzymatically digested and PET MPs were recovered by filtration through a silicon nanomembrane and enumerated under fluorescence microscopy (Fig. 4). Some issues we encountered was incomplete tissue digestion, which resulted in the capture of tissue debris, as well as PET MP aggregation. However, we were able to circumvent this by examining the total surface area of fluorescently labelled PET MPs retained on the filter (Fig. 5A) and subsequent particle enumeration (Fig. 5B). Consistent with microscopic examination of whole mount organs, intestine contained higher amounts of PET MPs indicated by higher overall red fluorescent surface area at 24 h exposure but showed rapid decrease at later time points of exposure (Fig. 5A). In the liver, similar PET MPs surface area was detected at all time points with an apparent increase after 3 days of exposure that did not reach statistical significance. In contrast, kidney lysate exhibited a significant increase of PET MPs surface area at later time of exposure (day 7). Relatively high levels of PET MPs were detected in the gills across the entire exposure period. An alternative method to analyze these data, and to disrupt PET MP aggregation, the retained particles on silicon nanomembranes were resuspended in pure water, sonicated, and counted using a hemacytometer (Fig. 5B). Overall, the data obtained were comparable to the surface area results. High number of PET MPs were counted in the intestine at day 1 of exposure, while their numbers significantly decreased from day 3 onward. Conversely, the number of PET MPs significantly increased after 3 days of exposure in the liver and remained at a consistent number at later time points, while PET MPs accumulation in kidneys was delayed compared to the liver.

3.2. Effects of PET MPs on tadpole resistance to viral pathogens

To examine whether the relatively low level of PET MPs accumulation persisting in tadpoles' organs upon exposure impacts their antiviral immune response, we conducted long-term exposure experiments where tadpoles were raised in water containing PET MPs for 1 month. Because of its toxicity, the 500 mg/L concentration was not assayed. Following 1 month exposure to either 100 mg/L or 25 mg/L of PET MPs, tadpoles were inoculated by intraperitoneal injection with the ranavirus FV3, a major amphibian pathogen, and viral loads were determined 6 days post-infection via qPCR. Both active viral replication and host immune response occurs at this time point and have been extensively characterized in *X. laevis* (De Jesús Andino et al., 2012; Robert et al., 2019). Significantly higher FV3 genome copy numbers were detected in the kidney of 100 mg/L PET MPs-exposed groups, indicating a higher susceptibility to viral infection (Fig. 6A). Significant increase of viral loads was also detected for tadpoles exposed to lower PET MPs concentration (25 mg/L) (Fig. 6B). In addition to the kidneys that are the main site of FV3 replication, significant higher viral loads were also found in intestine and liver indicating that exposure to PET MPs increased dissemination of viral infection in exposed tadpoles. To obtain further evidence that PET MPs exposure impaired the tadpole control of FV3 replication, we determined the number of infectious particles produced in tadpole kidneys by plaque assay and found a significant increase in pfu (almost 2-fold more plaques) in PET MPs exposed tadpoles (Fig. 6C). A second independent experiment with tadpole exposed to 25 μg/ml PET MPs resulted in similar increased viral loads (Fig. S4).

3.3. Effects of PET MPs on tadpole innate antiviral immune responses

To assess the effects of PET MPs exposure on immune function at steady state and following FV3 infection, relative expression of several key innate antiviral immune genes was determined by qPCR. Contrary to expectation, PET MPs exposure did not induce a marked increase of inflammatory response as shown by the modest changes in relative expression of TNF-α and IL-1β genes between PET MPs exposed (both 25 and 100 mg/L) and control animals (Fig. 7). In fact, unlike APBS exposed tadpoles, *tnfa* and *il1b* transcript levels did not significantly increase upon FV3 infection in kidneys and liver of tadpoles exposed to either 100 or 25 mg/L PET MPs, suggesting a slightly weaker innate immune response. While no consistent difference in inf*1* (Fig. 7) and *inos* (Fig. 8) gene expression response was observed following FV3 infection, transcript levels of macrophage (*csf1r*) and granulocyte (*csf3r*) receptors were significantly decreased, which suggests some impairment of leukocyte antiviral function (Fig. 8).

4. Discussions

While small plastic debris or microplastics have become major

Table 1
Estimation of PET MPs retainment and excretion, per day for 7 days, in tadpole after 24hrs exposure.

	Exposure	Average PET MPs retained per day per tadpole (N = 5)						
Per tadpole	Non-ingested	1	2	3	4	5	6	7
Number of PET MPs [x10^4]	9.20 ±2.17	4.80 ±2.95	3.00 ±1.41	7.40 ±2.30	5.00 ±1.22	4.60 ± 2.70	3.00 ±2.12	2.80 ±1.30
Calculated mass [mg]	0.51 ±0.12	0.27 ±0.16	0.17 ±0.08	0.41 ±0.13	0.28 ± 0.07	0.25 ±0.15	0.17 ±0.12	0.16 ±0.07
Estimated number of PET MPs ingested [x10^4]	35.80 ±2.17	31.00 ±4.18	28.00 ±3.87	20.60 ±3.27	15.60 ± 2.70	11.00 ±5.10	8.00 ±7.04	5.20 ±7.95
Estimated mass of PET MPs ingested [mg]	1.99 ±0.12	1.72 ±0.23	1.56 ±0.22	1.14 ±0.18	0.87 ± 0.15	0.61 ±0.28	0.44 ±0.39	0.29 ±0.44

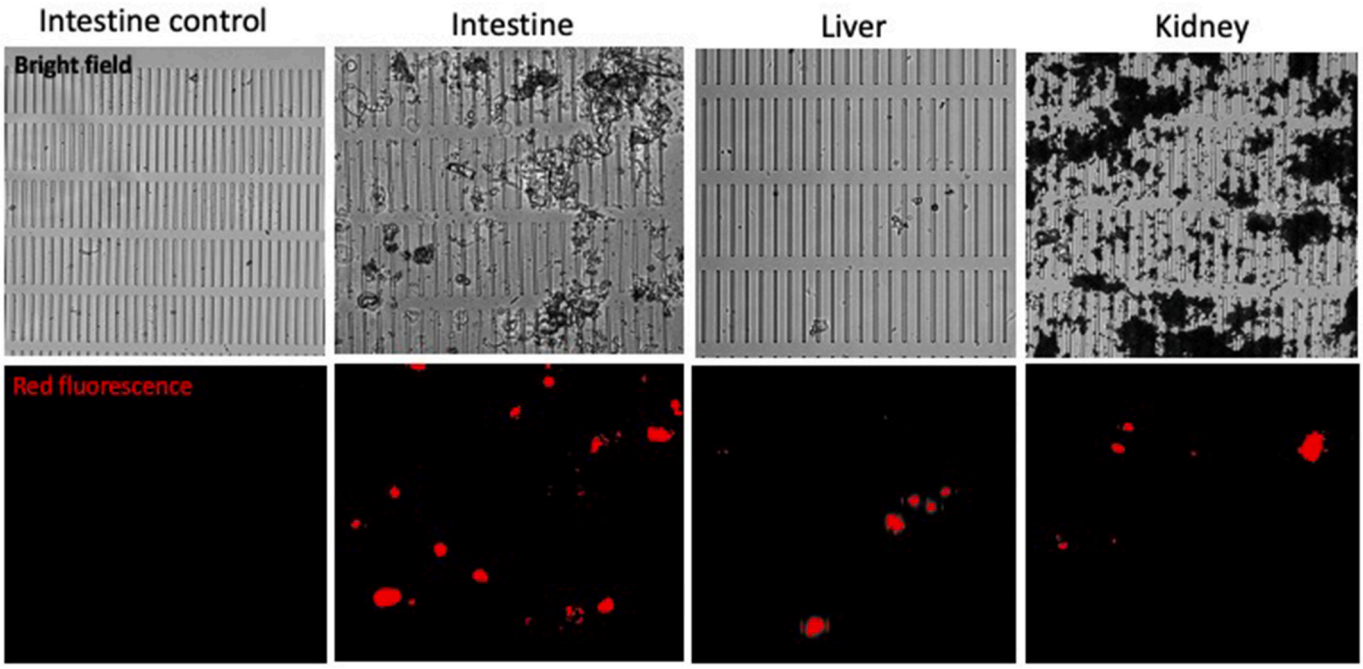


Fig. 4. PET MPs s detection in organ lysates of tadpoles exposed for 7 days. Following 7 days exposure to 100 mg/L of PET MPs, tadpole intestine, liver and kidney were harvested and enzymatically digested. PET MPs recovered by filtration on a nanomembrane with 2 μm × 50 μm microslits were detected under bright field (upper panels) and fluorescence microscopy (lower panels). Intestine control: lysate of tadpoles' intestine in water without PET MP exposure for 7 days.

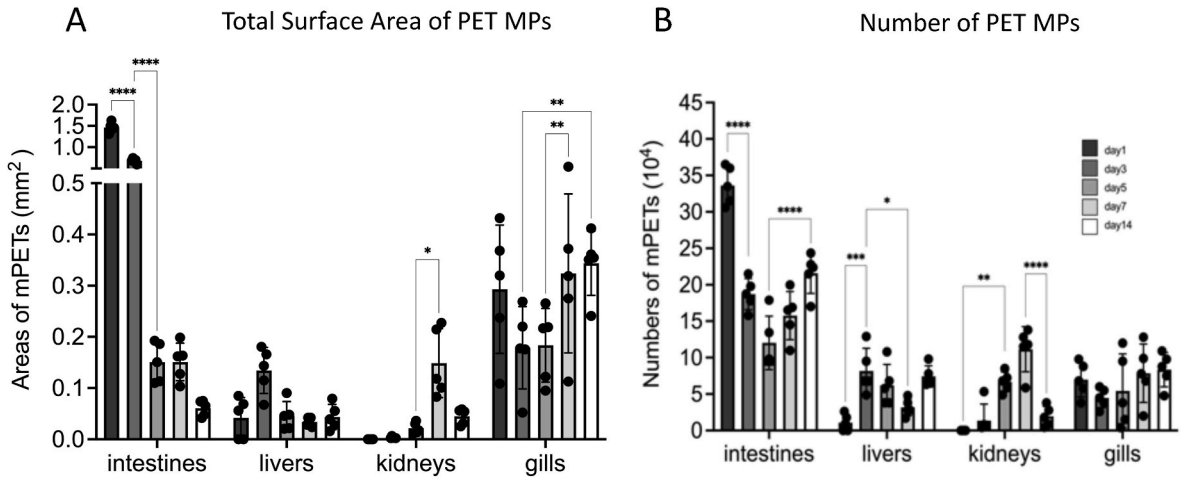


Fig. 5. Quantification of PET MPs s recovered from organ lysates of tadpoles exposed for 1 to 14 days. Tadpoles were exposed to 100 mg/L PET MPs from day 1 to day 14. **(A)** The overall surface area of PET MPs recovered from organ lysates was determined via ImageJ. (B) PET MPs retained on silicon nanomembranes were subsequently resuspended and counted. All data were analysed using a Two-way ANOVA and Tukey's post-hoc test. ***p ≤ 0.0005 **p ≤ 0.005 * p ≤ 0.01. N = 5.

pollutants of aquatic ecosystems and are widely found in tissues of animals and humans, their biological impacts on health and particularly on host resistance to pathogens is still poorly understood. Here, leveraging the amphibian *Xenopus* tadpoles and using mildly weathered post-consumer PET MPs collected from the Pacific Ocean, our study provides novel relevant *in vivo* evidence that these water contaminants are rapidly ingested by tadpoles and that a fraction of them can accumulate and persist in the liver and kidney. Furthermore, PET MPs ingestion and accumulation in tadpole organs results in increased susceptibility to viral infection.

It is noteworthy that besides being a representative ectothermic vertebrate, *X. laevis* embryology, developmental processes and physiology are remarkably similar to mammals, which has led to fundamental discoveries about development, and immunology (LaBonne and Zorn, 2015; Tandon et al., 2017; Robert, 2020).Thus, the data obtained here have relevance for both aquatic vertebrates and human health. Furthermore, the type of plastic, PET, used in our study is representative of a large fraction of plastic pollutant found in the environment, and the aging process followed by cryomilling results in MPs that are more heterogeneous in size and shape than manufactured polystyrene microbeads. This is an important distinction because to date most studies have used sterile homogeneous manufactured polystyrene microbeads as model for biodistribution and biological assessments (Gouin et al., 2022).

MPs concentration found in marine and aquatic environment has been reported to generally range from 2 to 5 particles/m^3 (Buckingham et al., 2022). Higher concentrations have been found for example, in urbanized North Shore Channel of Chicago where the concentrations

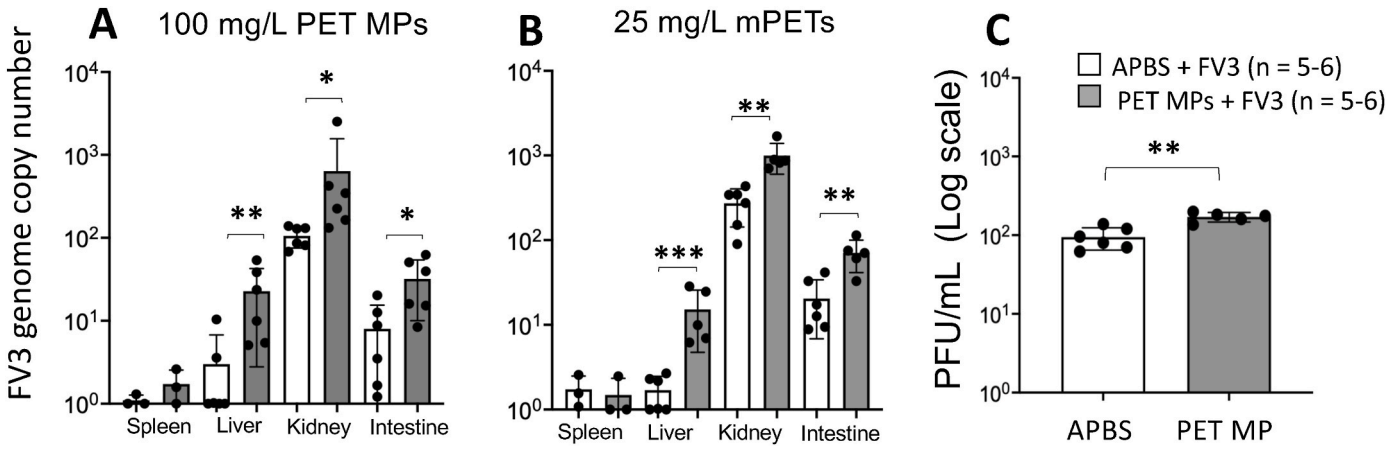


Fig. 6. Effects of PET MPs s exposure on tadpole susceptibility to viral (FV3) infection. Tadpoles (N = 6 per groups) exposed to 100 (A) or 25 mg/L (B, C) PET MPs s or water spiked with APBS as control were intraperitoneally infected with FV3 (1×10^4 PFU) and viral load (FV3 genome copy number) was determined 6 days post-infection by qPCR. All data were analysed using a Two-way ANOVA and Tukey's post hoc test***$p < 0.0005$ **$p < 0.005$ *$p < 0.05$.

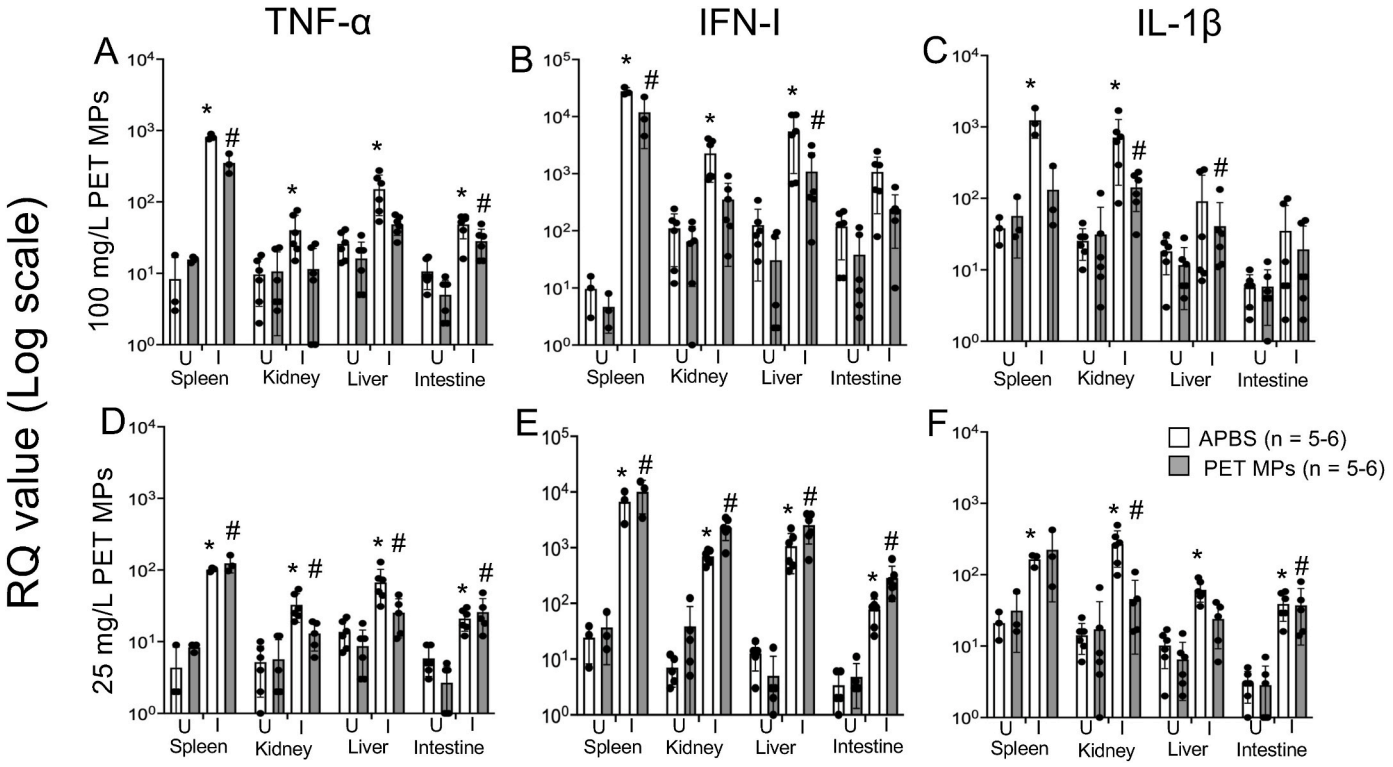


Fig. 7. Effects of PET MPs s exposure on antiviral inflammatory innate immune response. Tadpoles exposed to 100 (A, B, C) or 25 mg/L (D, E, F) PET MPs s (grey) or water (blank) spiked with APBS as control were intraperitoneally injected with 5 μL APBS as control (U) or with 1×10^4 PFU (5 μL) of FV3 (I) for 6 days. Relative gene expression was determined by qPCR for non-infected and infected tadpole spleen, kidneys, liver, and intestine for each gene relative to GAPDH.S. All data were analysed using a Two-way ANOVA and Tukey's post-hoc. N = 5–6. (*) Indicate significant ($p < 0.05$) differences between uninfected and infected groups; (#) significant difference between APBS and PET MPs exposed groups. All qPCR primers used are indicated in Supplementary Table S1.

vary from 1.9 to 17.9 particles/m^3 (McCormick et al., 2014), in the Great Lakes tributaries between 0.05 and 32 particles/m^3 (Baldwin et al., 2016) and in the ocean's upper, well-mixed, layer with up to 390 particles/m^3 (Tanhua et al., 2020). Thus, we realize that the concentration of PET MPs used for investigating immune effects (25 mg/L corresponding to 1.72×10^{10} pieces/m^3) far exceed that found in aquatic environment. However, there are several points to consider. First, the production and consummation of plastics continues to grow, which increase amounts of MPs in water systems. Second, MPs tend to significantly concentrate in sediments were some aquatic animals leave and feed. Third, MPs accumulation and persistence over time in organisms is difficult to evaluate, but MPs concentration as high as 14 μg/ml has been detected in human blood (Leslie et al., 2022) and up to 10 μg/g of fish tissues (Haave et al., 2021). A last more technical point is that was critical to use a concentration of PET MPs sufficient for detection in tadpole tissues without causing side effects on the survival, growth, and swimming activity of the tadpoles. It is noteworthy that our concentration for long term exposure remained relatively low compared to other studies in zebrafish (Wang et al., 2024) and frogs (da Costa Araújo et al., 2020). Indeed, we used concentration previously used in *Xenopus*

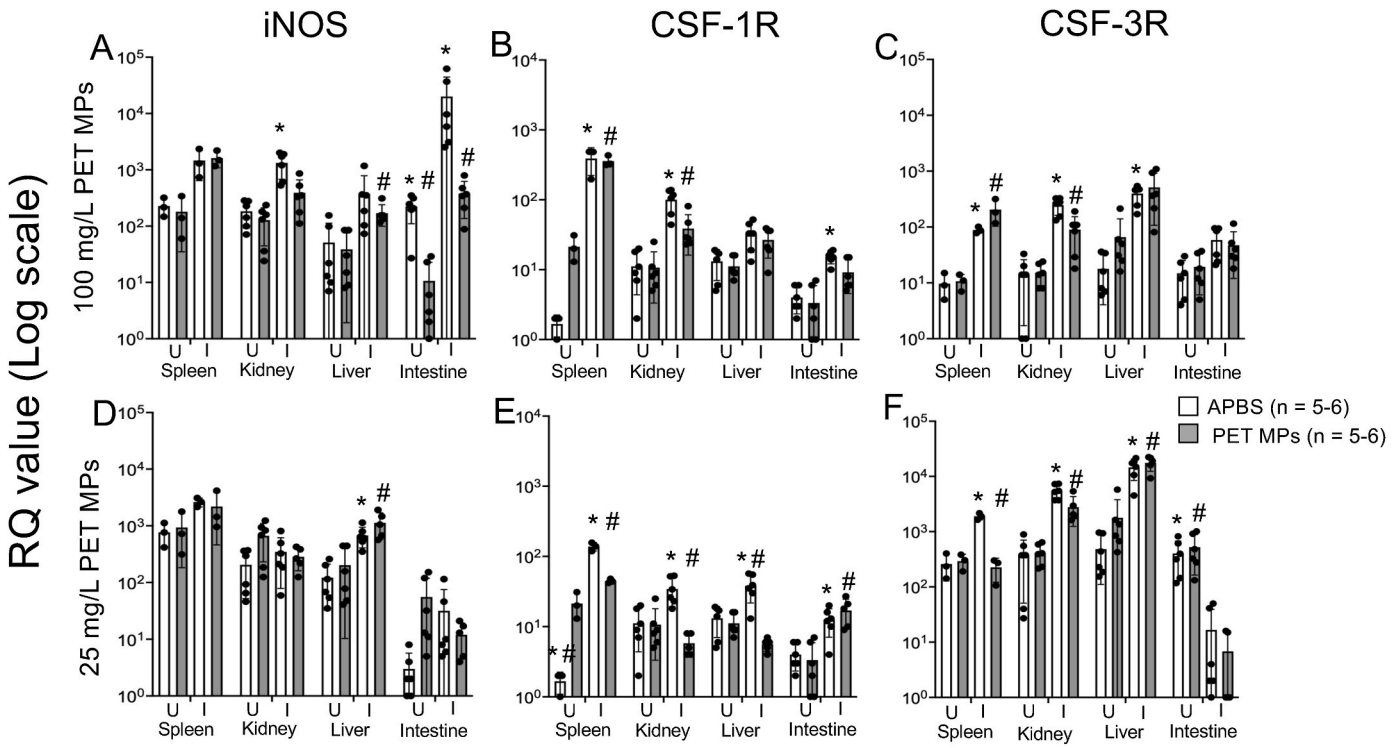


Fig. 8. Effects of PET MPs exposure on antiviral innate immune response. Tadpoles exposed to 100 (A, B, C) or 25 mg/L (D, E, F) PET MPs s or water spiked with APBS as control were intraperitoneally injected with APBS as control (U) or with 1×10^4 PFU of FV3 (I) for 6 days. Relative gene expression was determined by qPCR for non-infected and infected tadpole spleen, kidneys, liver, and intestine for each gene relative to gapdh.S. All data were analysed using a 2-way ANOVA and Tukey's post-hoc. N = 6. Significant difference ($p > 0.05$) between PET MPs and nonPET groups; (#) Significant difference ($p > 0.05$) between non-infected and infected groups.

(De Felice et al., 2018; Ruthsatz et al., 2023). Even after one month exposure no obvious organ damage were detected and very little inflammation was observed. Importantly, the higher concentration did allow us to follow the biodistribution of ingested MPs in a robust manner that would have been far more challenging at lower doses. Thus, our data will provide a useful baseline to further assess potential immunological impact of exposure to PET MPs at lower concentration.

An aspect revealed by this study is the rapid transit of PET MPs from the intestine to the liver. It is not clear the mechanism by which PET MPs pass the intestine epithelium layer to end up in the liver. Our observation indicates that most of these PET MPs are extracellular and most likely reside in hepatic sinusoids. Similarly, experiments have shown that after mice were orally administered MPs, MPs appeared in the liver and promoted liver fibrosis (Djouina et al., 2023). However, MPs up to 600 μm have been found in fish liver (Collard et al., 2017; Collard et al., 2018). We speculate that some of these PET MPs should cross the intestine epithelium into the blood circulation and become trapped in hepatic sinusoids and then at later points in the kidneys. Transepithelial penetration of polystyrene spheres (10 μm) has been observed on human intestinal explants *in vitro* (Donkers et al., 2022). In this regard, it is notable that PET MPs detected in tadpole tissues were small in size (10 μm), which suggest that larger particles could not efficiently pass the intestinal epithelium, except perhaps by damaging the tissue. Collectively data from our study are consistent with a kinetic process where PET MPs are first ingested and accumulate in the digestive track where the MPs of smaller size can pass the intestine epithelium to be trapped first in the liver then later in in the kidney. More studies will be needed to determine mechanisms allowing these PET MPs to cross the intestine epithelium.

Our estimate of PET MPs retention in tadpoles after 1 week exposure range around 0.2–0.3 mg equivalent to 5×10^4 plastic particles per tadpoles. With an average weight 300 mg per tadpole, this gives approximately 0.5–1 mg/g of tissue. This estimate was done in absence of reingestion or continual exposure to PET MPs, which may result higher uptake and persistence. However, it is interesting to note that the amount of PET MPs recovered by filtration from organ lysates of tadpoles exposed for longer (up to 14 days), did not markedly increase. These results suggest that only a fraction of PET MPs ingested and accumulating in internal tadpole organs are retained. The accumulation in human children has been estimated at 8×10^3 particles/capita or 6.4 ng/capita (Mohamed Nor et al., 2021). Mice fed with 20 μm polystyrene spheres showed increasing accumulation of MPs in their bodies until 14 days when the cumulative content plateaued reaching 0.78 mg/g or 1.78×10^5 items/g (Deng et al., 2017). These values are consistent with our data, which suggests that PET MPs concentrations used for exposure, while higher than that usually found in aquatic environment, remained in physiological range, and has relevance for investigating PET MPs impacts on viral immunity.

At high concentrations PET MPs s exposure leads to significant tadpole death presumably due to physical damages in the digestive track such as bowel obstructions and wounding by sharp plastic particles as reported for MP fibres (Bacchetta et al., 2021). Importantly, our data reveal that although not overtly toxic, exposure to lower concentration of PET MPs still induce notable immunological impacts in tadpoles such as increased susceptibility to viral infection. A previous study reported that exposure of frogs to manufactured polystyrene microbeads resulted in increased susceptibility to infection by chytrid fungus (Bosch et al., 2021). Our study support and extend this work to another major promiscuous pathogens, the ranavirus FV3, infecting a wide variety of aquatic vertebrates (reviewed in (Gray and Chinchar, 2015)). Furthermore, our data provide evidence using an environmentally relevant plastic water contaminant. Importantly, these data suggest that the increase in susceptibility to viral pathogens is in part due to a compromised antiviral innate immune response resulting from PET MPs

ingestion. However, the immune alteration induced by exposure does not appear to result from an exacerbated inflammation (even at higher PET MPs concentration) but rather some disfunction of the cell of myeloid lineage. Indeed, the relative expression of genes encoding two well-known pro-inflammatory cytokines, TNF-α and IL-1β was significantly decreased at the main site of infection (kidney) and transcript levels of another inflammatory gene iNOS were not perturbed by PET MPs exposure. These data suggest that the concentration of PET MPs used for exposure was not excessive. Instead of exacerbated inflammation, the significant decreased expression of genes encoding the macrophage and granulocyte receptors in FV3 infected kidneys and liver suggest a less active involvement of these cells in these infected organs, which together with the lower levels of TNF-α and IL-1β transcripts usually produced by these cells, is consistent with a PET MPs-mediated weakened response of innate leukocytes. More detailed transcriptomic analyses will be needed to determine the PET MPs mechanism of action on immune cells. We have previously shown that *X. laevis* immune responses to FV3 exhibit many similarities to antiviral immune response in human including TNFα and type I interferon (Chen and Robert, 2011). Notably, we have provided evidence of the critical role of macrophages in host defences against this large DNA virus reminiscent of poxvirus like vaccinia (Grayfer and Robert, 2013; Grayfer and Robert, 2014, Samanta et al., 2021). Therefore, the potential perturbation of antiviral macrophage function resulting from PET MP exposure is of relevance beyond *Xenopus*. It will be of interest to investigate more specifically the effect of PET MP exposure on macrophage antiviral function *in vitro* and *in vivo*.

Our study also provides evidence of the usefulness of silicon nanomembranes (Carter et al., 2023) to isolate and quantify PET MPs (or other MPs) from organ lysates. Specifically, we show that: 1) the chemical durability of the membranes make it possible to digest isolated organs directly on top of the membranes, resulting in the capture of ingested particles in the membrane microslits, and 2) the outstanding optical properties of the membranes allow us to directly perform bright field and fluorescence microscopy on the membranes immediate after capture (Madejski et al., 2020). To circumvent aggregate formation on the filter, we used two methods: determination of the total surface area of fluorescently labelled PET MPs retained on the filter and enumeration of resuspended particle on a hemacytometer. Comparable results were obtained by these two methods, which allowed us to reliably estimate the amount of PET MPs isolated from the organ lysate. Notably, while a large amount of PET MPs accumulates in the intestine during the first 24 h of exposure, there was a rapid decrease in the following days despite the continual presence of PET MPs in the water. This decrease was also observed in liver but not in the gills. This suggests that tadpoles try to avoid ingesting PET MPs over time but cannot prevent PET MPs in the gills with oxygenated water. It would be interesting to conduct a behavioural experiment to assess tadpole ability to detect and avoid plastic ingestion.

5. Conclusion

Results obtained using the amphibian *Xenopus* as a relevant model for human health show that following exposure, environmentally representative mildly weathered post-consumer PET MPs rapidly accumulate in tadpole intestine, liver and kidneys and persist over a week, which compromise antiviral immunity and weaken resistance to viral infection. These data are significant and raise concern not only for aquatic vertebrates and but also for human health.

Funding

This research was supported by R24AI059830 from the National Institute of Allergy and Infectious Diseases (NIH/NIAID), as well as a Research Program Project P01ES035526 from the National Institute of Environmental Health Sciences,and a Pilot Project Grant from the Rochester Environmental Health Sciences Center (P30-ES01247). Contributions from JLM and SSR were supported by NIH R44 ES031036 and grant from theFoundation for Chemistry Research & Initiatives.

CRediT authorship contribution statement

Binghong Cai: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Francisco De Jesus Andino:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation. **James L. McGrath:** Writing – review & editing, Resources, Methodology, Investigation, Conceptualization. **Samantha S. Romanick:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis. **Jacques Robert:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

James L. McGrath reports a relationship with SiMPore, Inc that includes: board membership and equity or stocks. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We would like to thank Tina Martin for the expert animal husbandry. We would like also to acknowledge Dr. Kim Rogers and Dr. Mark C. Surette at the USEPA Office of Research and Development Center for Environmental Measurement and Modeling for generously gifting cryomilled MPs to this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.124340.

References

Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62 (8), 1596–1605.
Bacchetta, R., Winkler, A., Santo, N., Tremolada, P., 2021. The toxicity of polyester fibers in xenopuslaevis. Water 13 (23), 3446.
Baldwin, A.K., Corsi, S.R., Mason, S.A., 2016. Plastic debris in 29 Great Lakes tributaries: relations to watershed attributes and hydrology. Environ. Sci. Technol. 50 (19), 10377–10385.
Bosch, J., Thumsová, B., López-Rojo, N., Pérez, J., Alonso, A., Fisher, M.C., Boyero, L., 2021. Microplastics increase susceptibility of amphibian larvae to the chytrid fungus Batrachochytrium dendrobatidis. Sci. Rep. 11 (1), 22438.
Brignac, K.C., Jung, M.R., King, C., Royer, S.-J., Blickley, L., Lamson, M.R., Potemra, J.T., Lynch, J.M., 2019. Marine debris polymers on main Hawaiian island beaches, sea surface, and seafloor. Environ. Sci. Technol. 53 (21), 12218–12226.
Buckingham, J.W., Manno, C., Waluda, C.M., Waller, C.L., 2022. A record of microplastic in the marine nearshore waters of South Georgia. Environ. Pollut. 306, 119379.
Carter, J., Horan, T., Miller, J., Madejski, G., Butler, E., Amato, C., Roussie, J., 2023. Comparative evaluation of filtration and imaging properties of analytical filters for microplastic capture and analysis. Chemosphere 332, 138811.
Chen, G., Robert, J., 2011. Antiviral immunity in amphibians. Viruses 3 (11), 2065–2086.
Collard, F., Gasperi, J., Gilbert, B., Eppe, G., Azimi, S., Rocher, V., Tassin, B., 2018. Anthropogenic particles in the stomach contents and liver of the freshwater fish Squalius cephalus. Sci. Total Environ. 643, 1257–1264.
Collard, F., Gilbert, B., Compère, P., Eppe, G., Das, K., Jauniaux, T., Parmentier, E., 2017. Microplastics in livers of European anchovies (Engraulis encrasicolus, L.). Environ. Pollut. 229, 1000–1005.

Conlin, S.M., Tudor, M.S., Shim, J., Gosse, J.A., Neilson, A., Hamlin, H.J., 2018. Elevated nitrate alters the metabolic activity of embryonic zebrafish. Environ. Pollut. 235, 180–185.
da Costa Araújo, A.P., de Melo, N.F.S., de Oliveira Junior, A.G., Rodrigues, F.P., Fernandes, T., de Andrade Vieira, J.E., Rocha, T.L., Malafaia, G., 2020. How much are microplastics harmful to the health of amphibians? A study with pristine polyethylene microplastics and Physalaemus cuvieri. J. Hazard Mater. 382, 121066.
De Felice, B., Bacchetta, R., Santo, N., Tremolada, P., Parolini, M., 2018. Polystyrene microplastics did not affect body growth and swimming activity in Xenopus laevis tadpoles. Environ. Sci. Pollut. Res. Int. 25 (34), 34644–34651.
De Jesús Andino, F., Chen, G., Li, Z., Grayfer, L., Robert, J., 2012. Susceptibility of Xenopus laevis tadpoles to infection by the ranavirus Frog-Virus 3 correlates with a reduced and delayed innate immune response in comparison with adult frogs. Virology (in press).
Deng, Y., Zhang, Y., Lemos, B., Ren, H., 2017. Tissue accumulation of microplastics in mice and biomarker responses suggest widespread health risks of exposure. Sci. Rep. 7, 46687.
Djouina, M., Waxin, C., Dubuquoy, L., Launay, D., Vignal, C., Body-Malapel, M., 2023. Oral exposure to polyethylene microplastics induces inflammatory and metabolic changes and promotes fibrosis in mouse liver. Ecotoxicol. Environ. Saf. 264, 115417.
Donkers, J.M., Höppener, E.M., Grigoriev, I., Will, L., Melgert, B.N., van der Zaan, B., van de Steeg, E., Kooter, I.M., 2022. Advanced epithelial lung and gut barrier models demonstrate passage of microplastic particles. Microplastics and Nanoplastics 2 (1), 6.
Eriksen, M., Lebreton, L.C., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan, P.G., Reisser, J., 2014. Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PLoS One 9 (12), e111913.
Espinosa, C., Garcia Beltran, J.M., Esteban, M.A., Cuesta, A., 2018. In vitro effects of virgin microplastics on fish head-kidney leucocyte activities. Environ. Pollut. 235, 30–38.
Gouin, T., Ellis-Hutchings, R., Thornton Hampton, L.M., Lemieux, C.L., Wright, S.L., 2022. Screening and prioritization of nano- and microplastic particle toxicity studies for evaluating human health risks - development and application of a toxicity study assessment tool. Microplast nanoplast 2 (1), 2.
Gray, M.J., Chinchar, V.J., 2015. Ranaviruses: Lethal Pathogens of Ectothermic Vertebrates. Springer Open, Heidelberg, New York, Dordrecht, London.
Grayfer, L., Robert, J., 2013. Colony-stimulating factor-1-responsive macrophage precursors reside in the amphibian (Xenopus laevis) bone marrow rather than the hematopoietic subcapsular liver. J. Innate Immun. 5 (6), 531–542.
Grayfer, L., Robert, J., 2014. Divergent antiviral roles of amphibian (Xenopus laevis) macrophages elicited by colony-stimulating factor-1 and interleukin-34. J. Leukoc. Biol. 96 (6), 1143–1153.
Green, D.S., Colgan, T.J., Thompson, R.C., Carolan, J.C., 2019. Exposure to microplastics reduces attachment strength and alters the haemolymph proteome of blue mussels (Mytilus edulis). Environ. Pollut. 246, 423–434.
Greven, A.C., Merk, T., Karagoz, F., Mohr, K., Klapper, M., Jovanovic, B., Palic, D., 2016. Polycarbonate and polystyrene nanoplastic particles act as stressors to the innate immune system of fathead minnow (Pimephales promelas). Environ. Toxicol. Chem. 35 (12), 3093–3100.
Haave, M., Gomiero, A., Schönheit, J., Nilsen, H., Olsen, A.B., 2021. Documentation of microplastics in tissues of wild coastal animals. Front. Environ. Sci. 9.
Hamlin, H.J., Marciano, K., Downs, C.A., 2015. Migration of nonylphenol from food-grade plastic is toxic to the coral reef fish species Pseudochromis fridmani. Chemosphere 139, 223–228.
Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E., Svendsen, C., 2017. Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities. Sci. Total Environ. 586, 127–141.
Hwang, J., Choi, D., Han, S., Choi, J., Hong, J., 2019. An assessment of the toxicity of polypropylene microplastics in human derived cells. Sci. Total Environ. 684, 657–669.
Jin, Y., Xia, J., Pan, Z., Yang, J., Wang, W., Fu, Z., 2018. Polystyrene microplastics induce microbiota dysbiosis and inflammation in the gut of adult zebrafish. Environ. Pollut. 235, 322–329.
Jinhui, S., Sudong, X., Yan, N., Xia, P., Jiahao, Q., Yongjian, X., 2019. Effects of microplastics and attached heavy metals on growth, immunity, and heavy metal accumulation in the yellow seahorse, Hippocampus kuda Bleeker. Mar. Pollut. Bull. 149, 110510.
LaBonne, C., Zorn, A.M., 2015. "Modeling human development and disease in Xenopus. Preface." Dev Biol 408 (2), 179.
Leslie, H.A., van Velzen, M.J.M., Brandsma, S.H., Vethaak, A.D., Garcia-Vallejo, J.J., Lamoree, M.H., 2022. Discovery and quantification of plastic particle pollution in human blood. Environ. Int. 163, 107199.
Liu, Z., Yu, P., Cai, M., Wu, D., Zhang, M., Chen, M., Zhao, Y., 2019. Effects of microplastics on the innate immunity and intestinal microflora of juvenile Eriocheir sinensis. Sci. Total Environ. 685, 836–846.
Lönnstedt, O.M., Eklöv, P., 2016. Environmentally relevant concentrations of microplastic particles influence larval fish ecology. Science 352, 1213–1216.
Madejski, G.R., Ahmad, S.D., Musgrave, J., Flax, J., Madejski, J.G., Rowley, D.A., DeLouise, L.A., Berger, A.J., Knox, W.H., McGrath, J.L., 2020. Silicon nanomembrane filtration and imaging for the evaluation of microplastic entrainment along a municipal water delivery route. Sustainability 12 (24).
Maes, T., Jessop, R., Wellner, N., Haupt, K., Mayes, A.G., 2017. A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. Sci. Rep. 7, 44501.
Mak, C.W., Ching-Fong Yeung, K., Chan, K.M., 2019. Acute toxic effects of polyethylene microplastic on adult zebrafish. Ecotoxicol. Environ. Saf. 182, 109442.
McCormick, A., Hoellein, T.J., Mason, S.A., Schluep, J., Kelly, J.J., 2014. Microplastic is an abundant and distinct microbial habitat in an urban river. Environ. Sci. Technol. 48 (20), 11863–11871.
Mohamed Nor, N.H., Kooi, M., Diepens, N.J., Koelmans, A.A., 2021. Lifetime accumulation of microplastic in children and adults. Environ. Sci. Technol. 55 (8), 5084–5096.
Naidoo, T., Glassom, D., 2019. Decreased growth and survival in small juvenile fish, after chronic exposure to environmentally relevant concentrations of microplastic. Mar. Pollut. Bull. 145, 254–259.
Obbard, R.W., Sadri, S., Wong, Y.Q., Khitun, A.A., Baker, I., Thompson, R.F., 2014. Global warming releases microplastic legacy frozen in Arctic Sea ice. Earth's Future 2 (6), 315–320.
Qiu, X., Qi, Z., Ouyang, Z., Liu, P., Guo, X., 2022. Interactions between microplastics and microorganisms in the environment: modes of action and influencing factors. Gondwana Res. 108, 102–119.
Ragusa, A., Svelato, A., Santacroce, C., Catalano, P., Notarstefano, V., Carnevali, O., Papa, F., Rongioletti, M.C.A., Baiocco, F., Draghi, S., D'Amore, E., Rinaldo, D., Matta, M., Giorgini, E., 2021. Plasticenta: first evidence of microplastics in human placenta. Environ. Int. 146, 106274.
Robert, J., 2020. Experimental platform using the Amphibian Xenopus laevis for research in fundamental and medical immunology. Cold Spring Harb. Protoc. 2020 (7), 106625.
Robert, J., McGuire, C.C., Nagel, S., Lawrence, B.P., Andino, F.J., 2019. Developmental exposure to chemicals associated with unconventional oil and gas extraction alters immune homeostasis and viral immunity of the amphibian Xenopus. Sci. Total Environ. 671, 644–654.
Rummel, C.D., Loder, M.G., Fricke, N.F., Lang, T., Griebeler, E.M., Janke, M., Gerdts, G., 2016. Plastic ingestion by pelagic and demersal fish from the North sea and baltic sea. Mar. Pollut. Bull. 102 (1), 134–141.
Ruthsatz, K., Schwarz, A., Gomez-Mestre, I., Meyer, R., Domscheit, M., Bartels, F., Schaeffer, S.M., Engelkes, K., 2023. Life in plastic, it's not fantastic: sublethal effects of polyethylene microplastics ingestion throughout amphibian metamorphosis. Sci. Total Environ. 885, 163779.
Samanta, M., Yim, J., De Jesús Andino, F., Paiola, M., Robert, J., 2021. TLR5-Mediated reactivation of quiescent ranavirus FV3 in Xenopus peritoneal macrophages. J. Virol. 95 (12).
Schwabl, P., Köppel, S., Königshofer, P., Bucsics, T., Trauner, M., Reiberger, T., Liebmann, B., 2019. Detection of various microplastics in human stool: a prospective case series. Ann. Intern. Med. 171 (7), 453–457.
Tandon, P., Conlon, F., Furlow, J.D., Horb, M.E., 2017. Expanding the genetic toolkit in Xenopus: approaches and opportunities for human disease modeling. Dev. Biol. 426 (2), 325–335.
Tanhua, T., Gutekunst, S.B., Biastoch, A., 2020. A near-synoptic survey of ocean microplastic concentration along an around-the-world sailing race. PLoS One 15 (12), e0243203.
Thompson, R.F., Olsen, Y.S., Mitchell, R.N., Davis, A.P., Rowland, S.J., John, A.W., McGonigle, D.F., Russell, A.E., 2004. Lost at sea: where is all the plastic? Science 304 (5672), 838.
Wang, L., Pei, W., Li, J., Feng, Y., Gao, X., Jiang, P., Wu, Q., Li, L., 2024. Microplastics induced apoptosis in macrophages by promoting ROS generation and altering metabolic profiles. Ecotoxicol. Environ. Saf. 271, 115970.
Wright, S.L., Kelly, F.J., 2017. Plastic and human health: a micro issue? Environ. Sci. Technol. 51 (12), 6634–6647.
Zahn, N., James-Zorn, C., Ponferrada, V.G., Adams, D.S., Grzymkowski, J., Buchholz, D. R., Nascone-Yoder, N.M., Horb, M., Moody, S.A., Vize, P.D., Zorn, A.M., 2022. Normal Table of Xenopus development: a new graphical resource. Development 149 (14).
Robert, J., Ohta, Y., 2009. Comparative and developmental study of the immune system in Xenopus. Dev. Dyn. PMID: 19253402; PMCID:PMC2892269.