Activity report on the project "*Evaluating photodegradation products of plastic nurdles and their toxicity in Matagorda Bay*"

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Period: November 1, 2024, to May 31, 2025

Update on the previous Part I: **Tracking the photooxidation products of primary plastic pellets (nurdles) in seawater**

This activity report that was submitted to the Trust has been just submitted to *Marine Pollution Bulletin* for peer review.

Part I: The bioavailability of photo-bleached dissolved organic carbon from environmentally prevalent plastic nurdles in coastal waters

We have submitted part of the data in a previous activity report; thus, this one is an update including refined interpretation and additional experiment.

Abstract: Microplastics pose a significant threat to marine ecosystems, but due to their small size, resistance to degradation, and vast dispersal, it is challenging to evaluate their environmental weathering and lifespan simply based on field samples. Through laboratory experiments, we assessed the photodegradation of microplastics and the bioavailability of their weathering products in seawater. Nurdles of four polymer types – HDPE, PP, PET & PS – were exposed to four and eight weeks of simulated irradiation, equating to 0.75 and 1.5 years, respectively, of natural noon summer sun in south Texas. Irradiated nurdles were analyzed for oxidation and leachate production as dissolved organic carbon (DOC). The DOC collected after irradiation was then inoculated with microbial consortium from the Gulf of Mexico coast for a 35-day period to observe bioassimilation. Overall, this work aims to provide insight into the longevity and fate of plastic-derived substances in the ocean and highlight key microorganisms with DOC degradation capabilities.

Results

1. Nurdle Photodegradation:

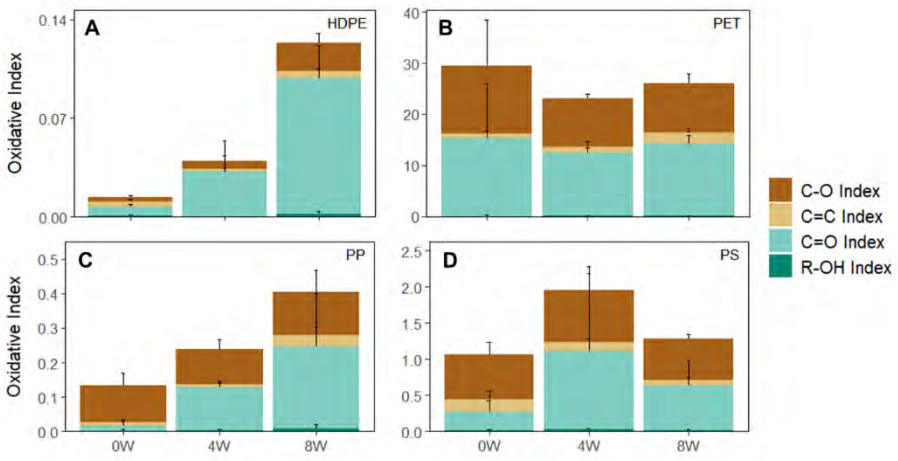

Figure 1: Oxidative indices of A) HDPE, B) PET, C) PP, and D) PS nurdles pre-irradiation and after four and eight weeks of UV exposure in the solar simulator.

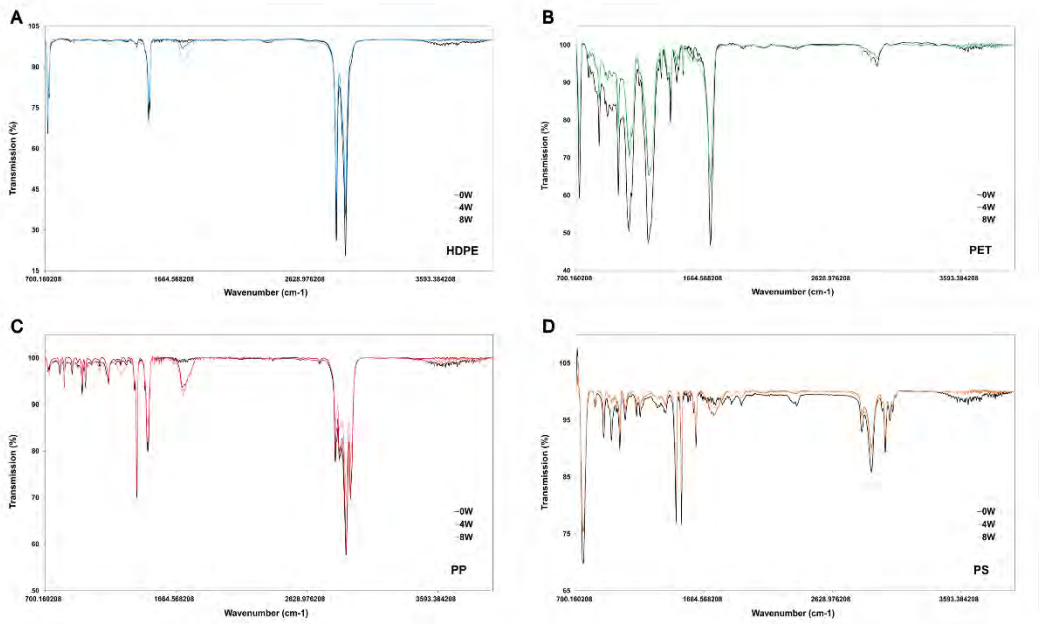

Figure 2: FT-IR spectra of A) HDPE, B) PET, C) PP, and D) PS nurdles pre-irradiation and after four and eight weeks of UV exposure in the solar simulator.

Key points of the results (Figs. 1&2):

- HDPE (high density polyethylene) exhibited the greatest photooxidation signals when analyzed with FT-IR

- PP (polypropylene) followed a similar pattern, with a continued increase in oxidation over the eight-week exposure period. PET (polyethylene terephthalate) and PS (polystyrene) had different patterns (no significant increase in oxidation)
- Of the four bonds chosen – C-O (ether), C=C (alkene, vinyl), C=O (ketone, carbonyl), and R-OH (hydroxyl) – C=O saw the most significant change overall, with the greatest change being in HDPE (0.006 ± 0.002 at zero weeks of exposure to 0.096 ± 0.023 at eight weeks). This increase of oxygen suggests oxidation of nurdles/microplastics under UV irradiation in artificial seawater
- Changes in individual indices and overall oxidation of nurdles highlights the weathering power of UV irradiation
- Decrease/increase in the % transmission of peaks in the FT-IR spectra provides further evidence of weathering and chemical changes (photodegradation and photooxidation)

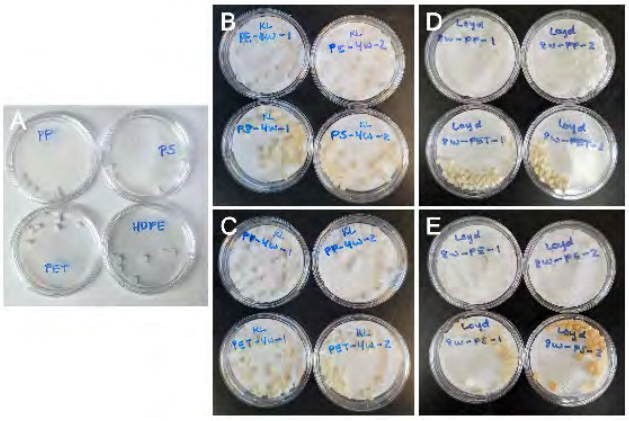

Figure 3: A) Nurdles before UV exposure. B) HDPE and PS nurdles after four weeks of irradiation. C) PP and PET nurdles after four weeks of irradiation. D) PP and PET nurdles after eight weeks of irradiation. E) HDPE and PS nurdles after eight weeks of irradiation.

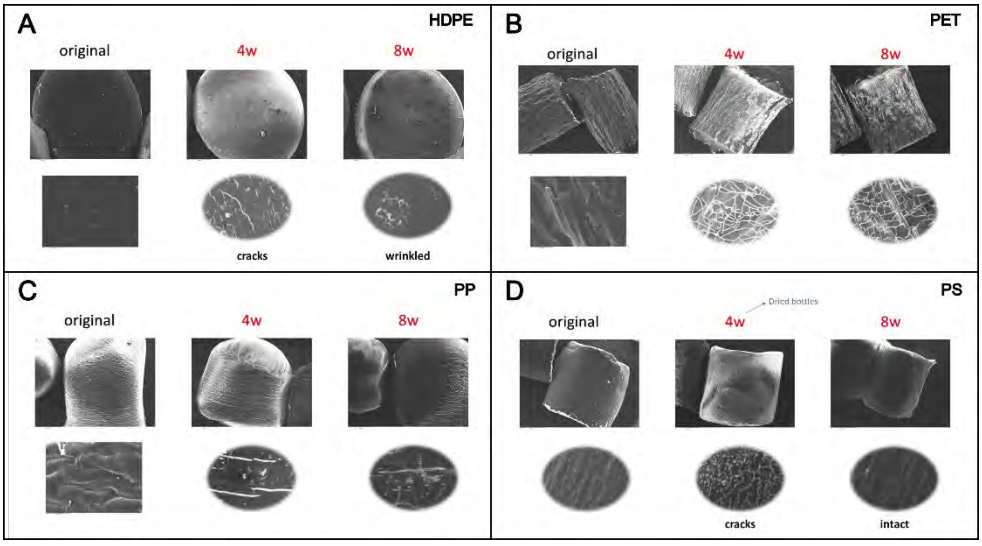

Figure 4: SEM imaging of A) HDPE, B) PET, C) PP, and D) PS nurdles pre-irradiation and after four and eight weeks of UV exposure in the solar simulator. Nurdles examined at 25X and 300X.

Key points of the results (Figs. 3&4):

- PET and PS nurdles experienced yellowing following four and eight weeks of irradiation. Irradiation is causing the formation of conjugated double bonds, chromophores, etc.
- SEM imagining revealed that irradiation led to the formation of cracks, wrinkles, and other physical alterations to the surface of all four polymers. This is a sign of photodegradation and may allow for oxygen to reach deeper into the nurdle's structure

2. Nurdle Photodegradation leads to the leaching of dissolved organic carbon (DOC):

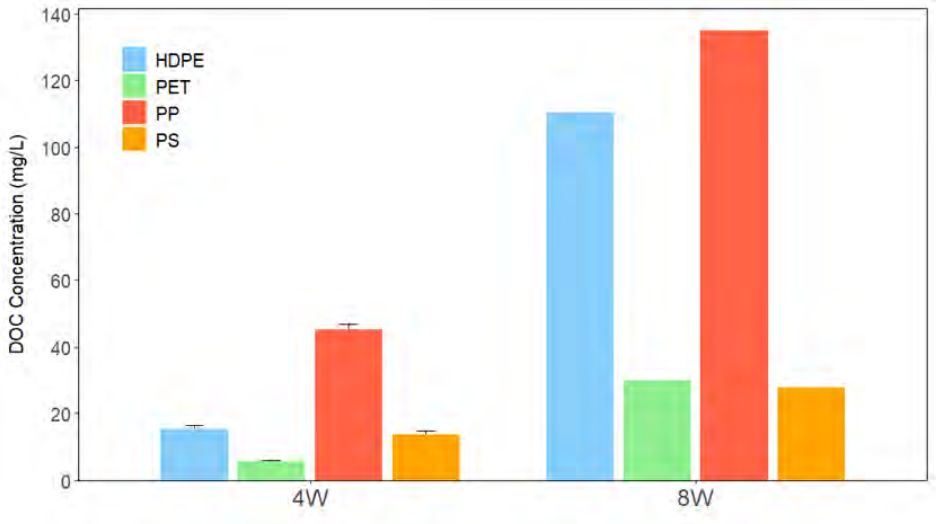

Figure 5: Concentration (mg/L) of DOC produced by nurdles of each polymer type after four and eight weeks of UV exposure in the solar simulator.

Key points of the results (Figure 5):

- All polymers produced DOC over the irradiation treatment
- DOC production by each polymer type continued to increase over the irradiation exposure time
- PP produced the most DOC, followed by HDPE – both aliphatic polymers
- The rate of DOC production also increased from the four-week to the eight-week mark, particularly for HDPE and PP, suggesting they start to leach more DOC (at a faster rate) with more sunlight exposure
- PS and PET produced less DOC than expected, perhaps due to their greater densities and oxygen limitation

Table 1: Initial nurdle mass (g), final DOC concentration (mg/L), final DOC mass produced (mg), and nurdle carbon loss (%) for each polymer type after four and eight weeks of UV exposure in the solar simulator.

Polymer	Irradiation Treatment	Initial Nurdle Mass (g)	Final DOC Concentration (mg/L)	Final DOC Mass Produced (mg)	Nurdle Carbon Loss (%)
HDPE	4W	1.003 ± 0.004	15.45 ± 1.06	1.85 ± 0.13	0.18 ± 0.01
	8W	1.001 ± 0.001	110.36	13.24	1.32
PET	4W	1.005 ± 0.001	5.80 ± 0.14	0.70 ± 0.02	0.07 ± 0.00
	8W	1.005 ± 0.001	29.94	3.59	0.36
PP	4W	1.006 ± 0.001	45.10 ± 1.70	5.41 ± 0.20	0.54 ± 0.02
	8W	1.008 ± 0.002	134.94	16.19	1.61
PS	4W	1.004 ± 0.001	13.70 ± 1.13	1.64 ± 0.14	0.16 ± 0.01
	8W	1.006 ± 0.001	27.74	3.33	0.33

Key points of the Table 1.

- Limitations: differences in buoyancy and oxygen availability to each polymer, uneven light distribution in the solar simulator, overall oxygen limitation
- Assumptions: the different oxidation patterns of PET and PS may be explained by unique internal characteristics and behaviors, such as tensile strength and UV-induced cross-linking, as well as external conditions, such as dissolved oxygen availability. Greater tensile strength may also have this effect. The different starting levels of

oxygen within the structures of the polymers (i.e., PET with initially greater transmission of C=O bonds) may influence how much oxygen can be added in during photooxidation

The main takeaways of the nurdle photodegradation and DOC leaching:

- It is necessary to utilize various techniques to get a comprehensive idea of photooxidation weathering signals. For example, PET nurdles showed limited/decreased oxidation after irradiation when measured with FT-IR but appeared to experience the greatest physical alterations with SEM imagining
- All polymers undergo chemical and physical changes due to UV irradiation, but due to internal (and perhaps external) characteristics, these changes are distinct between nurdle types
- All polymers produced DOC, with PP and PE producing high concentrations (>100 mg/L at eight weeks), yet this DOC is only <2% of the nurdle carbon mass. Assuming linear DOC loss, and with the estimate that eight weeks of irradiation in the solar simulator is equivalent to 1.5 years of noon summer sun in Port Aransas, it would take approximately 94 years for 1 gram of PP nurdles to be fully degraded into DOC under maximum UV irradiation conditions (without other weathering forces). Accounting for the average UV exposure over a 24-hour period in nature (nights, sun rising/falling, overcast, etc.), this may be closer 400 years. For the other polymer types, it would take even longer

3. DOC Biassimilation Assay: the chemical results:

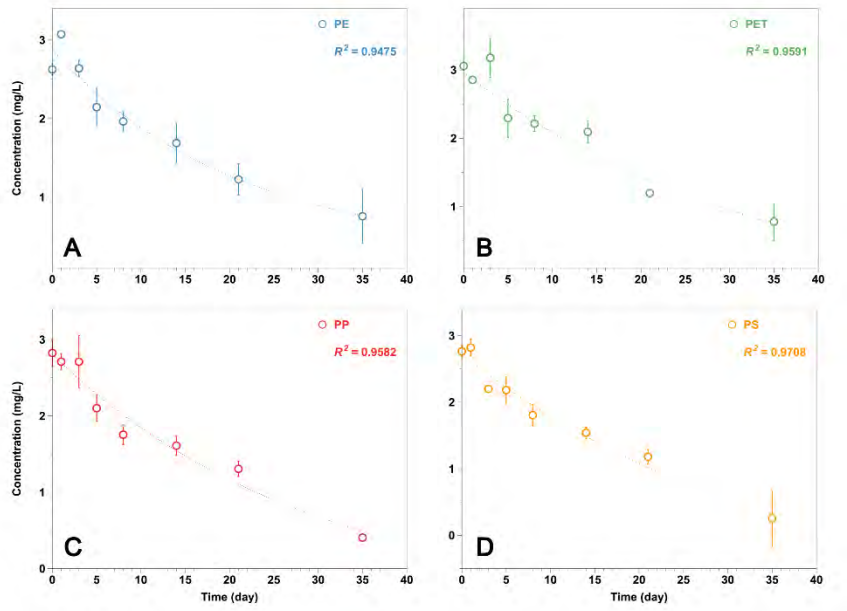

Figure 6: Acquisition of eight-week-irradiated DOC from A) HDPE, B) PET, C) PP, and D) PS by coastal microbial consortia over the 35-day incubation period.

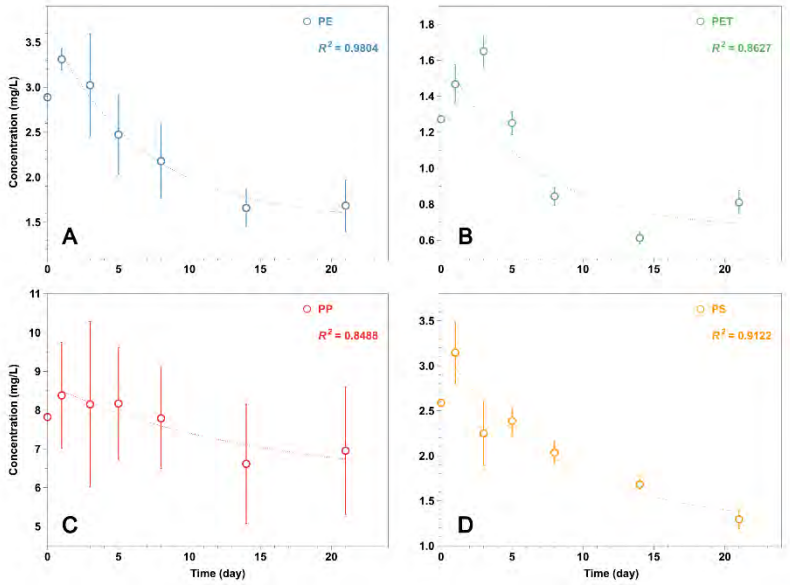

Figure 7: Acquisition of four-week-irradiated DOC from A) HDPE, B) PET, C) PP, and D) PS by coastal microbial consortia over the 21-day incubation period

Table 2: Initial and final DOC concentrations (mg/L), first order decay equation values (*A*, *B*, and *k*), bioavailability (%), and BGE (%) for eight-week irradiated leachate from HDPE, PET, PP, and PS nurdles after 35 days of laboratory incubation with microbial consortia.

$C(t) = A+B*e^{(-kt)}$

A – Refractory Concentration

B – Bioavailable Concentration

Polymer	Initial DOC Conc. (mg/L)	Final DOC Conc. (mg/L)	Refractory Conc. *A* (mg/L)	Bioavailable Conc. *B* (mg/L)	Decay Rate Constant *k*	Bioavailability (%)	BGE (%)
HDPE	2.63 ± 0.07	0.76 ± 0.34	0.29 ± 0.66	2.58 ± 0.61	0.05 ± 0.02	77.25 ± 102.67	0.29
PET	3.06 ± 0.06	0.78 ± 0.28	-0.41 ± 1.42	3.38 ± 1.42	0.03 ± 0.02	81.25 ± 32.05	3.01
PP	2.82 ± 0.17	0.41 ± 0.03	-0.52 ± 1.18	3.33 ± 1.12	0.03 ± 0.02	89.04 ± 63.68	0.85
PS	2.76 ± 0.08	0.25 ± 0.42	-1.18 ± 1.53	3.90 ± 1.47	0.03 ± 0.02	93.98 ± 34.79	0.23

Key points of the bioassay results (Figs. 6&7; Table 2):

- For eight-week irradiated leachate: Steady decrease over time, with a larger drop (in most cases) between days 21 and 35
- A significant portion of DOC was utilized by microorganisms, for all polymer leachates. The lowest % bioavailability was HDPE with 77.25%. Highest was PS with 93.98%. These values have high deviations, since they varied quite a bit between triplicate vials. Not expected that aliphatic HDPE would have the lowest bioavailability, while aromatic PS has the highest. LC-MS should provide insight into the compounds that make up the DOC (perhaps not similar to parent polymer). **Overall, the polymer type does not seem to matter, as all DOC seems to be highly bioavailable to the consortium**
- Decay rates were not significantly different. Perhaps PE DOC decaying (being used up) at a higher rate than the other plastics, which makes sense
- Comparing four- and eight-week irradiated leachate incubations: HDPE and PS starting with similar concentrations – shows similar trend of decrease and suggests that irradiation time (4w vs 8w) may not have a significant effect on the bioavailability of the DOC. 4w-irradiated PP leachate had a much higher inoculation concentration and a much lower acquisition/bioavailability %. This may be because the microorganisms have a "cap" of DOC that they utilize within a given time. **Working on carbon utilization estimates, organization of 4w-leachate incubation data, and implications of this comparison

3. DOC Biassimilation Assay: the biological results:

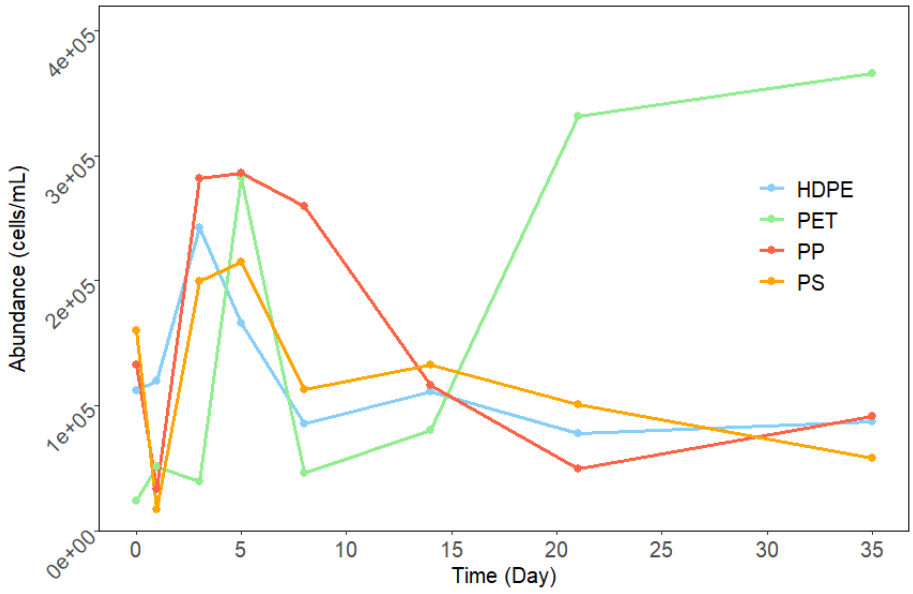

Figure 8: Average change in microbial abundance (cells/mL) over the 35-day incubation period for vials with eight-week-irradiated HDPE, PET, PP, and PS leachate.

Key point of the bacterial density (Figure 8):

- High variation among triplicate vials
- Abundance levels much lower than seen in similar experiments with natural DOM
- Population lower than the “blank” vials for three polymers (not PET) by the end. This may suggest that the plastic DOC is inhibitory or does not sustain bacterial populations as well as even a smaller amount of natural DOC
- In context with DOC: DOC concentration continued to steadily decrease over the entire incubation for all four polymer types. However, HDPE, PP, and PS vials saw a steady drop off in abundance and/or biomass (calculated from abundance w/ value of average contents of carbon per cell for coastal bacterial assemblages). High DOC acquisition and low bacterial abundance led to strikingly low BGE (%) values – see table. With the continued decrease of DOC even as the microbial populations leveled out/declined, **it can be assumed that only a small subset of the microbial population effectively mineralizes DOC.** Or, perhaps, DOC is being utilized for metabolism/maintenance and not growth or reproduction
- PET saw an increase in population towards the end of the experiment (still relatively low), but did not exhibit different DOC acquisition patterns from the other leachate vials. This may be from an opportunistic species or subset of species that was able to dominate the population with PET leachate, but not with the others. **Need to identify unique species in PET samples, focusing on changes between days 5/8 and 21/35

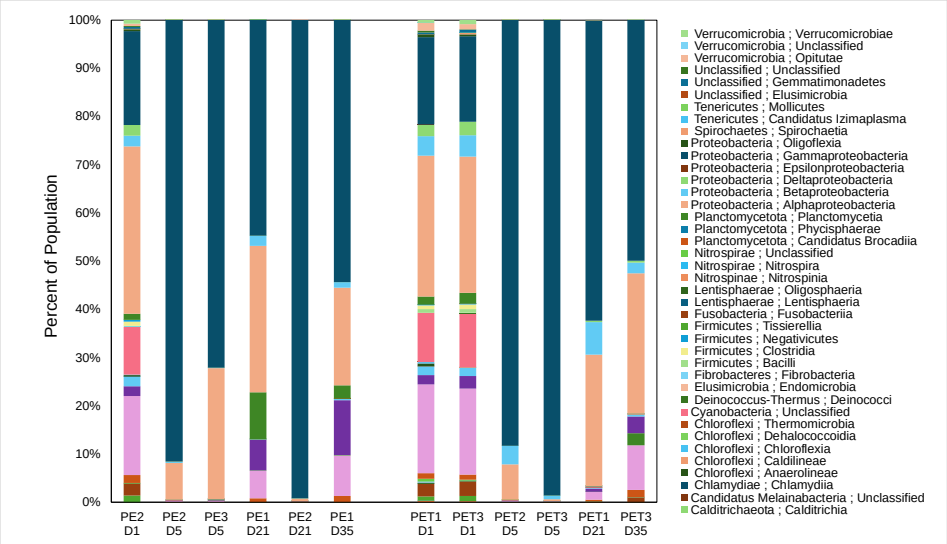

Figure 9: Class-level composition of microbial consortia (%) in HDPE and PET leachate vials, from day one to day 35 of the incubation. Samples analyzed and plotted from three triplicate vials, with temporal and spatial comparisons.

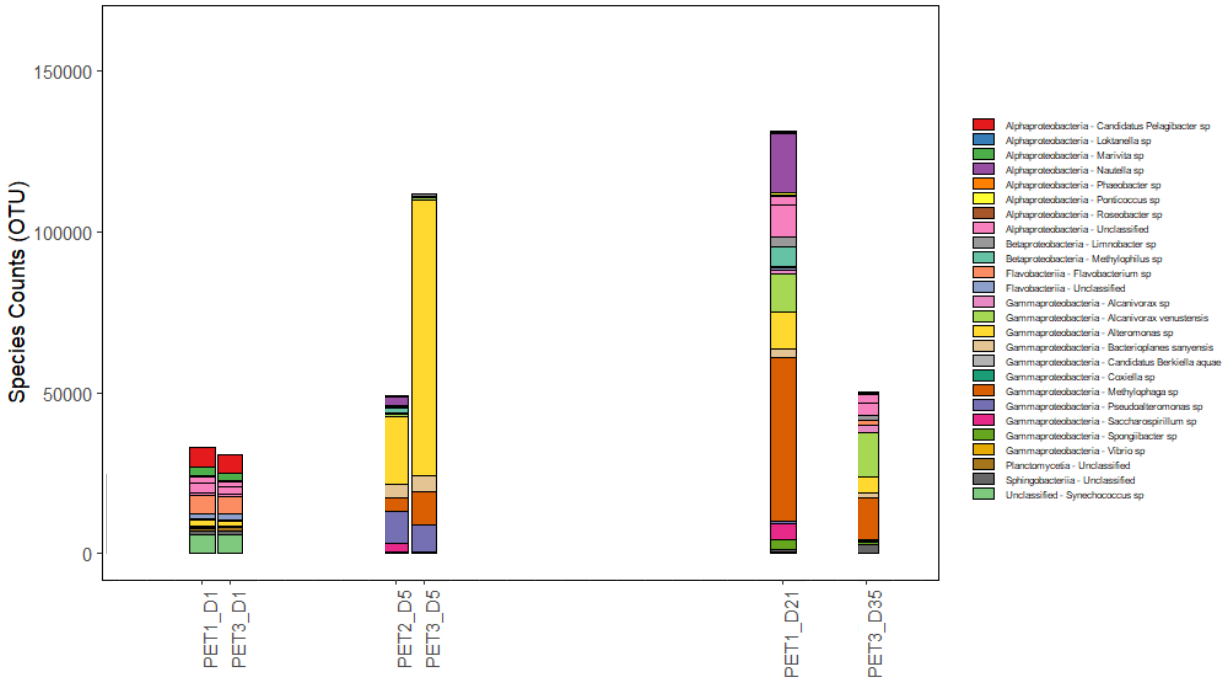

Figure 10: Preliminary counts of top 25 species within the microbial consortia for PET leachate vials, from day one to day 35 of the inoculation. Samples analyzed and plotted from three triplicate vials, with temporal and spatial comparisons.

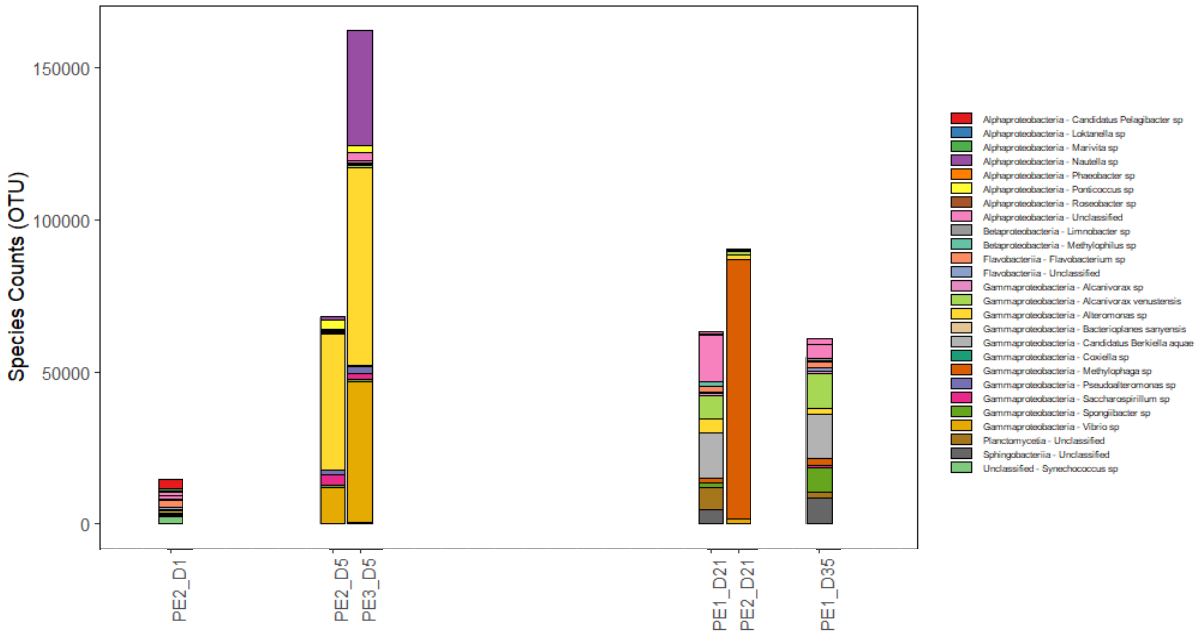

Figure 11: Preliminary counts of top 25 species within the microbial consortia for HDPE leachate vials, from day one to day 35 of the inoculation. Samples analyzed and plotted from three triplicate vials, with temporal and spatial comparisons.

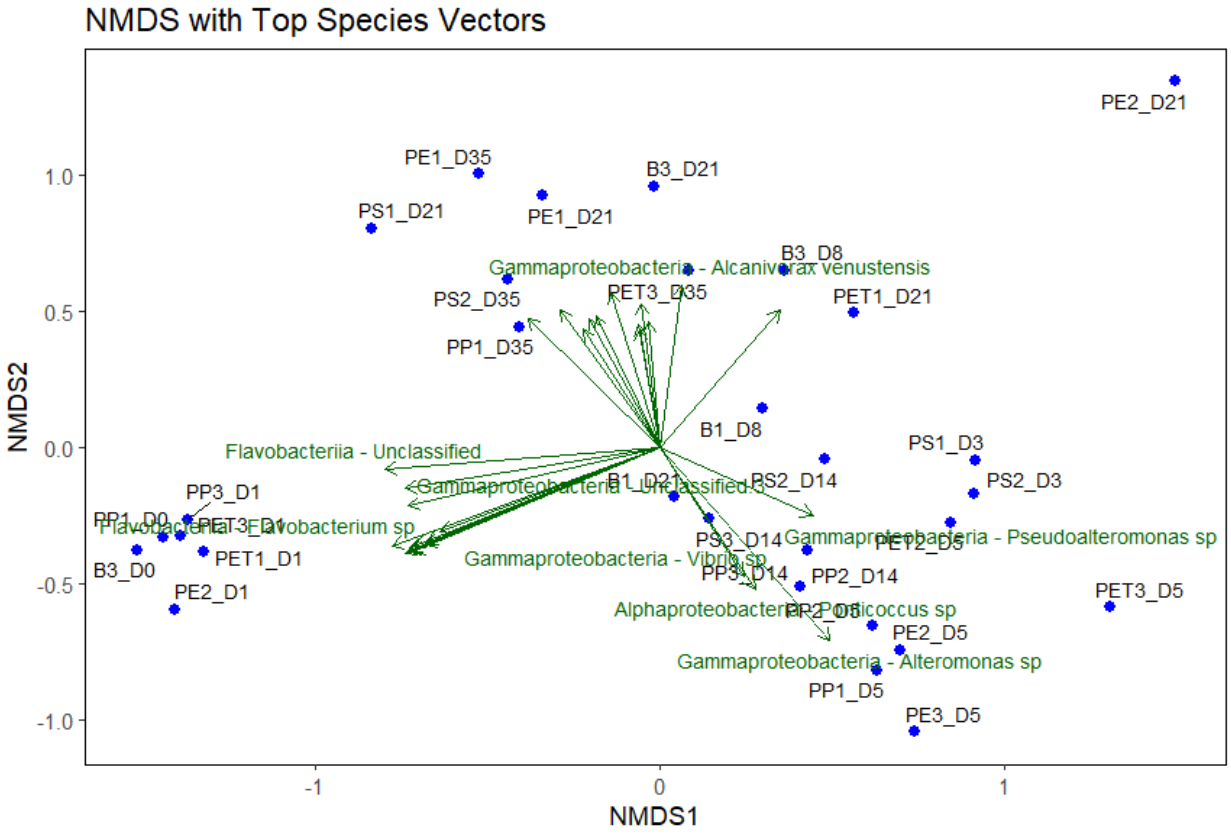

Figure 12: Preliminary NMDS of species OTU data from 30 leachate vials with 25 top species. Blue dots are the vials, while green arrows are the species vectors.

Key points of the biological community data (Figs. 9-12):

- Community composition roughly the same at the beginning of the incubation
- NMDS (preliminary figure) highlights the clear separation (of vials) based on time, not species. Consistent with the other data (not many differences between vials at any given time in terms of DOC acquisition and bacterial abundance, but changes over time)
- Species succession as time went on in the incubation. More of the generalist flavobacteria species at the beginning (D0 and D1), followed by a dominance of gamma- and alphaproteobacteria later. *Alcanivorax venustensis*, more dominant in samples from D21 and D35, is known to degrade alkanes/hydrocarbons. **Suggests that bacteria with hydrocarbon/plastic degrading capabilities became more prominent members of the consortia as the incubation went on.** **Currently looking into each of these species and their functionalities
- All samples saw a substantial DOC decrease, regardless of the species composition differences, especially at later times in the incubation period
- Not many observable differences between bacterial populations at a class level
- At a genus and species level, community differences between treatments, especially at later points of the incubation, more noticeable
- PET1 at D21 high OTU count – assuming abundance data from flow cytometry is relatively accurate, though PET3 at D35 is a bit low
- HDPE and PET both have high *Methylophaga sp.* at day 21. HDPE is lacking in other species that appear to make up half of PET1's population (this explains why PET population appears so much larger with flow cytometry data). May assume that this species is a more active participant of DOC mineralization, while the other species present (many in PET vials) are not contributing much to the acquisition
- A Canditatus berkiella aquae (intranuclear bacteria) and Unclassified sp. more dominant in HDPE leachate vials at day 35, while greater dominance of Methylophaga sp. (need methanol, evidence of plastic degradation) and Alcanivorax venustensis (known to degrade a few plastics) in PET samples. Could be random succession/dominance if DOC acquisition is not significantly different (and composition not significantly different among treatments). **Continuing to look into these species to understand why
- **Overall:** In general, it can be concluded that species composition does not greatly differ between samples, and the species present are not the primary drivers of differences between samples/treatments – time is. All DOC is similarly mineralized regardless of how communities in each vial change. Still working on making clear figures (with % instead of counts) and looking at the functions of each species in the sample.

Part II: **Toxicity Assessment of Photodegraded Plastic Particles on the Development of *Oryzias Melastigma***

Alissa Richbourg, Texas A&M University-Corpus Christi; Wei Xu, Texas A&M University

Abstract

Plastics are integral to daily life due to their unique properties, making them essential in manufacturing, packaging, and medicine. However, their widespread use has led to significant microplastic pollution in marine environments. Disposed plastic products can be degraded by harsh UV rays from the sun, causing plastics to release toxic compounds and products into the environment. The impact of these leachates on marine ecosystems remains poorly understood, particularly for photodegraded plastics. This study assessed the toxicity of photodegraded plastic leachates on the model species *Oryzias melastigma*. Nurdles of three plastic types—polycarbonate (PC), polyethylene (PE), and polyethylene terephthalate (PET)—were degraded in seawater for eight weeks, and the resulting leachate was used to treat embryos at three concentrations (0.1, 0.5, 5.0 ppm). We analyzed heart rate, developmental progression, hatching, mortality, and deformities. Heart rates were significantly lower on at least one day for all treatments (PE, PC, PET, and PS) when compared to the control groups. Developmental deformities were observed in PC and PET treatments, with PET 0.5 ppm showing significantly higher deformity rates than the control. Hatching rates were on average significantly lower than the control in the 5.0 ppm PET treatment and the 0.1 ppm PC treatment. Furthermore, all plastic types, excluding PE, showed significantly higher mortality than the control in at least one treatment group. PE leachate had minimal effects on mortality, development, and hatching. Pairwise analysis between plastic types showed that PET, PC, and PS exposure induced higher mortality rates than PE exposure. Exposure to photodegraded plastic leachate may induce oxidative stress and impair spinal development, potentially affecting locomotion and survival. Further studies will examine the mechanisms underlying these toxic effects in *O. melastigma*.

1 Introduction

Plastics have become integral to daily life due to their unique properties that make them essential in manufacturing, packaging, and medical industries. However, the rise of plastics has led to significant pollution within marine environments. Disposed plastic products can be degraded by harsh UV rays from the sun, causing plastics to release toxic compounds and chemicals into the ocean (Delre et al., 2023). The addition of these compounds, known as plastic leachates, can pose a large threat to marine environments, as it is not easy to observe, identify, or track their abundance. It is unknown how toxic plastic leachates may be, and there is a lack of knowledge surrounding the toxicity of photodegraded plastics and their potential to impair biological development at vulnerable life stages.

The use of marine medaka (*Oryzias melastigma*) as a toxicological model species has become increasingly prominent in marine research, as their small size, short generation times, distinct sexual dimorphism, and clear embryos make them ideal organisms for toxicological studies (Dong et al., 2014). Studies using marine medaka have previously focused on investigating the direct effect of microplastic beads on the early life stages and development, with an emphasis on polystyrene (PS) microplastics (Chen et al., 2022; Wang et al., 2021; Yu et al., 2023). This study will provide a holistic overview of the toxicity of photodegraded plastic leachates, including impacts on survival, development, organ structure, and genetic stress pathways. This study would be one of the first to examine the effects of photodegraded plastic leachates on marine life, as well as one of the first to focus on the impacts of polycarbonate (PC), high-density polyethylene (PE), polystyrene (PS), and polyethylene terephthalate (PET) on the early life stages of marine medaka.

2 Methods

2.1 Preparation of marine medaka (Oryzias melastigma) embryos and treatment groups

Pairs of adult marine medaka are kept in 2.5-gallon breeding tanks with approximately 15 males and 15 females and are monitored daily for embryos. Tanks are kept at a salinity of 28-30 ppt, and water

changes are performed every two weeks. Collected embryos are rinsed and cultured in glass Petri dishes with filtered artificial seawater and drops of methylene blue. Embryos are examined under a microscope to determine which embryos are 24 hours post-fertilization, using published standards of marine medaka development (Murata et al., 2019). Embryos 24 hours post-fertilization are maintained in an incubator at 28ºC under a 12:12 light-dark cycle and water changes are performed twice a week.

2.2 Experimental Procedure and Observation of Embryos

Four types of plastic nurdles (PE, PS, PET, and PC) were photodegraded in artificial seawater using a solar simulator for 8 weeks. The resulting leachate solution was diluted to three concentrations (0.1 ppm, 0.5 ppm, and 5.0 ppm) using artificial seawater to be used for embryonic exposure (Fig. 1). To ensure embryos received the proper hatching signals and were raised in an environment resembling natural conditions, the artificial seawater used in the control treatment and to dilute the leachate solutions was prepared by combining 1 part filtered seawater from the adult breeding tanks and 1 part freshly made artificial seawater (30 ppt). To determine which polymers to study in-depth, preliminary exposures were performed on groups of 15 embryos for 14 days and hatching and mortality rates were recorded. PC, PE, PET, and PS showed preliminary results indicating differences from control groups and were chosen to study in-depth based on their effects on hatching, mortality, and deformity rates.

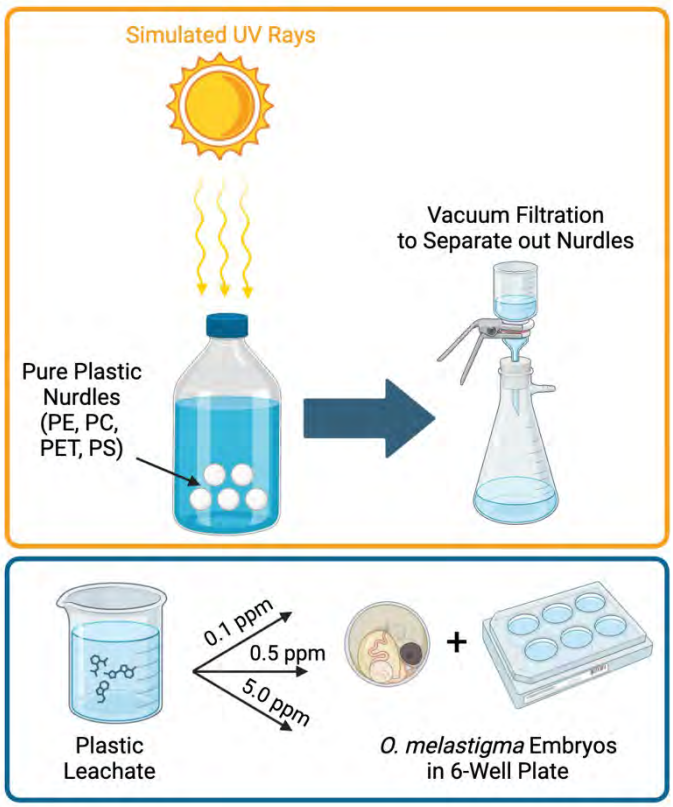

Figure 1. Graphical abstract of methods used to generate plastic leachate and setup of embryo exposures.

To assess the early development of marine medaka, developmental experiments were run with the PC, PE, PET, and PS leachates. Embryos 24 hours post-fertilization were randomly separated into the different treatment and control groups, with 5 replicates per treatment group and 5 embryos per replicate (25 embryos total). Embryo development was monitored daily for a total of 14 days, and the stage of each embryo, as well as its mortality and hatching status, was recorded. Throughout each study, the hatching rate and mortality rate were measured, and any observed deformities before and after hatching were noted and photographed. Additionally, the heartbeat rate was measured on days 4, 6, 8, and 10 by randomly selecting 5 embryos from each treatment group and counting the number of heartbeats observed in 20 seconds under a compound microscope (Huang et al., 2011).

2.3 Histological Staining and Analysis of Juveniles

Approximately ten 3-month-old marine medaka that had been exposed to PE and PC leachates for 16 days were collected from each experimental tank and fixed in paraformaldehyde for histological examinations. Samples were embedded in paraffin wax, and horizontal 4-µm sections were cut using a rotary microtome (Leica RM2125 RTS). Sections were mounted onto glass slides (VWR® Superfrost Plus) and were deparaffinized and rehydrated before being stained with hemotoxin and eosin (H&E). Coverslips were mounted over the sections using Permount (Fisher Scientific) and observed under a compound microscope. Each sample was photographed, with the observer being blind to the treatment group (Control, 0.1 PE, 0.5 PE, 0.1 PC, 0.5 PC). Photos were taken of target organs, including the gills and liver.

2.4 Statistical Analysis

Data was analyzed using R Studio and Microsoft Excel (2024). Mortality and hatching data from all developmental studies were evaluated using a Kaplan-Meier survival analysis to estimate the survival and hatching probabilities for each treatment group over time. To assess differences in survival and hatching between groups, pairwise comparisons were performed using a log-rank test. A pairwise log-rank test was

also used to compare the different treatment groups (0.1 ppm, 0.5 ppm, and 5.0 ppm) between the four plastic types.

To analyze differences in embryo heart rates across treatment groups and says, I used a linear mixed-effects model (LME) with treatment, day, and their interactions as fixed effects, and replicate group as a random effect to account for potential non-independence among embryos within the same replicate. To assess potential heteroscedasticity, a likelihood ratio test was used to compare a model that assumed equal variances across treatment groups with a model that allowed for treatments to have different variances. The model that allowed variance among treatment groups was found to be a better fit for all PE, PC, PET, and PS treatments ($p = <0.0001$, $p = 0.0374$, $p = 0.0003$, and $p = 0.0311$, respectively). Normality assumptions were verified by using a Q-Q plot and histogram, and homoscedasticity assumptions were checked by plotting residuals against fitted values. Random effects for replicate showed no clear pattern, supporting the model's assumptions of independence. The model was used to calculate estimated marginal means (EMMs) and 95% confidence intervals using the containment method for approximating degrees of freedom. Pairwise comparisons between treatments on each day were done using Tukey-adjusted contrasts to account for multiple comparisons at each time point.

Deformity data was evaluated using one-way ANOVA, followed by Tukey's HSD Test for multiple comparisons to determine significance. Deformity data is expressed as means ± standard error. For all statistical tests, differences were considered significant when $*P < 0.05$, highly significant when $**P < 0.01$, and very highly significant when $***p<0.0001$.

3 Results

3.1 PC, PET, and PS showed significant mortality compared to control groups

All plastic types, excluding PE, showed significantly higher mortality than the control in at least one treatment group (Fig. 2). For PC, the 0.1 ppm treatment group had a 28% higher mortality than the control ($p = 0.01662$; Fig. 2B). The 0.5 ppm and 5.0 ppm PC treatment groups exhibited mortality higher

than the control group as well, though not significantly so. For PET, the 5.0 ppm treatment group had a 32% higher mortality than the control group ($p = 0.0087$; Fig. 2C). For PS, the 5.0 ppm treatment group also had a 28% higher mortality than the control group ($p = 0.0100$; Fig. 2D). For both PET and PS, the 0.1 ppm and 0.5 ppm treatment groups also showed higher mortality than the control, but not significantly so. For PE, both the control and treatment groups exhibited very low mortality (Fig. 2A). Our pairwise comparison of mortality between treatment groups showed that PET induced higher mortality at all concentrations when compared to PE treatment concentrations (Fig. 3). PC induced higher mortality at 0.1 ppm and 0.5 ppm when compared to the same PE treatment concentrations, and PS induced higher mortality at 0.5 ppm when compared to the 0.5 PE treatment (Fig. 3). Additionally, PC induced higher mortality at 0.1 ppm when compared to the 0.1 ppm PS treatment (Fig. 3).

3.2 PET exposure induced significant spinal deformities

Developmental deformities were observed in all PET treatment groups. The 0.5 ppm treatment group exhibited the highest rate of deformity at 20% ± 6.32% and was significantly higher compared to the control ($p = 0.0278$, Fig. 4). Deformities consisted of spinal deformations, including curved, bent, and kinked tails that inhibited the larvae's locomotive abilities (Fig. 5).

3.3 PC exposure induced spinal and jaw deformities

Developmental deformities were observed in the 0.1 ppm and 5.0 ppm PC treatment groups, and not in the 0.5 ppm treatment group. The highest deformity rate was seen in the 0.1 ppm treatment group, with a rate of 12% ± 8.00% (Fig. 4). Deformities consisted of both spinal deformations and incomplete development of the upper jaw (Fig. 6). Spinal deformations included bent and curved tails that inhibited locomotion. Upper jaw deformities did not impact immediate survival or locomotion.

3.4 Heart rate was significantly lowered during early development for all plastic types

Heart rates were significantly lower on at least one day for all treatments (PE, PC, PET, and PS) when compared to the control groups (Fig. 7). For PE, the 5.0 ppm treatment group heart rates were on

average, 21.0 bpm lower than the control on day 6 ($p = 0.0289$), 21.6 bpm lower on day 8 ($p = 0.0233$), and 21.6 bpm lower on day 10 ($p = 0.0233$; Fig. 7A). For PC, the 5.0 ppm treatment group had heart rates that were on average 21.6 bpm lower than the control on day 6 ($p = 0.0400$; Fig. 7B). For PET, the 5.0 ppm treatment group had heart rates that were on average 31.8 bpm lower than the control on day 4 ($p = 0.0067$; Fig. 7C). On day 6, heart rates were on average 47.4 bpm lower in the 0.1 ppm group ($p = 0.0002$), 36.0 bpm lower in the 0.5 ppm group ($p = 0.0173$), and 31.2 bpm lower in the 5.0 ppm group ($p = 0.0080$). For PS, heart rates were on average 33.6 bpm lower in the 0.1 ppm group on day 4 ($p = 0.0021$) and 31.2 bpm lower in the 5.0 ppm group ($p = 0.0028$). On day 6, the 5.0 ppm group was on average 27.0 bpm lower ($p = 0.0122$). On day 8, heart rates were on average 27.0 bpm lower in the 0.5 ppm group ($p = 0.0293$) and 40.2 bpm lower in the 5.0 ppm group ($p = 0.0001$; Fig. 7D).

3.5 Treatment groups showed lower hatching rates than control groups

Hatching rates were significantly lower in the 5.0 ppm PET treatment group and 0.1 ppm PC treatment group when compared to the controls (Fig. 8). Hatching in the 5.0 ppm PET treatment group was 20% lower than the control ($p = 0.02$; Fig. 8C), as well as 20% lower in the 0.1 ppm PC treatment group ($p = 0.05$; Fig. 8B). While not significant, almost all other treatment groups had hatching rates that were 8-16% lower than the control groups.

4 Experimental plan for next steps

4.1 Primer efficiency testing for qPCR

Preliminary tests are currently underway to determine how many embryos must be pooled to obtain sufficient levels of RNA for cDNA synthesis and qPCR. Embryo pooling efficiency is being tested with 3, 5, 10, and 15 pools. In addition, primers are currently being tested to determine their efficiency.

Table 1: Chart listing forward and reverse primers used for quantitative PCR analysis of *Oryzias melastigma.*

Primer sequences are listed in Table 1. Once primers have been tested and we have chosen the most efficient number of embryos to pool, qPCR testing will begin.

Primers Used for Quantitative Real-Time PCR

Gene	Forward primer	Reverse primer
18S rRNA	5'-GACAAATCGCTCCACCAACT	5'-CCTGCGGCTTAATTTGACCC
c3	5'-GGTCAAGAGTGAATGGAATGCCTA	5'-CTAACAGAAACAAGATGGAGAGCC
sod	5'-TGTACCAGTGCGGGGCCTCACTTCA	5'-TGCGGTCACATTTCCCAGGTCCCCA
cat	5'-GCCAACTACCTGCAGATCCCCGTCA	5'-AGTTTGGAGCGCCGCCTTGGTTGT
cox-1	5'-AGTTCGACCCCACGCTGCTGTTCA	5'-AAGCTGTCGGGCATCAAAGGGTGC
cox-2	5'-CAGTGCTGACCGAGCATGGCATCA	5'-TTACGACCACCAGCAACCCGTCCT
NF-kB(p65)	5'-CATGGCTACTACGAGGCAGACC	5'-AACTCCTCCTCCCACACCTTGGAC

4.2 Evaluation of gene expression levels using qPCR

To collect embryos for qPCR, embryos will be collected 24 hours post fertilization and randomly divided into control and treatment groups. Embryos will be collected and pooled on days 4, 6, and 8 for qPCR analysis, with 3 replicates per treatment group. Collection days were chosen to align with the days heart rate was measured. Target genes include a housekeeping gene (18S), immune system response (c3 and NF-kB), oxidative stress response (sod and cat), and heart-related stress genes (cox-1 and cox-2).

5 Discussion

The release of plastic leachates from photodegraded plastic waste poses a large threat to marine ecosystems. The implications and potential toxicity of these plastic leachates remain widely unknown, making it essential for us to understand how this issue may become a larger concern as plastic pollution rates continue to rise globally. Our results indicate that exposure to PE, PC, PET, and PS plastic leachates induces significant changes in the early development of marine medaka. Plastic leachate exposure was

found to both reduce hatching rates and heart rates, as well as increase mortality and deformity rates in some treatment groups.

5.1 Effect of plastic leachates on embryo mortality

Plastic leachate exposure induced significantly higher mortality than the control in at least one treatment group for all plastic types except PE (Fig. 2). Mortality was found to be greatest in the 5.0 ppm PET treatment group, the 5.0 ppm PS treatment group, and the 0.1 ppm PC treatment group (Fig. 2). These results support that the highest concentration of plastic leachate exposure (5.0 ppm) led to higher mortality events in the PET and PS treatments. While it is unexpected to see significant mortality in the lowest PC treatment group (0.1 ppm) and not the highest, our results show that the difference in mortality rate between the two groups was only 8%, potentially suggesting that there is not a major difference in mortality rates across the different concentrations of plastic leachates. These results are supported by previous studies indicating that exposure to plastic leachates induces significant mortality in the early life stages of *Danio rerio* (Li et al., 2023). Li et al. found that plastic leachates from agricultural mulching films led to significant mortality in zebrafish embryos when exposed to high concentrations (6 g/L and 8 g/L). While these concentrations of plastic leachates are much higher than those used in the present study, their results support our findings that exposure to plastic leachates at both low and high concentrations can significantly increase mortality during early life stages. Additionally, these results are supported by previous work showing that plastics exposed to UV light induced significant mortality and toxicity in the copepod *Nitocra spinipes* (Gewert et al., 2021). However, their results comparing toxicities among different plastic types are not supported by our findings. They concluded that PS and PET showed low toxicity, while PP showed relatively high toxicity. This does not align with our conclusions that PS and PET induced significant morality, while PP was not chosen to study in depth because of preliminary findings suggesting negligible toxicity. These differences in results may be due to differences in susceptibility among copepods and marine medaka, or due to variations in the chemical compositions of the plastic pellets used. Our results that PE leachates had minimal effects on embryo mortality are

supported by previous findings indicating that zebrafish embryos experienced low mortality effects when exposed to PE microplastics (Le Dang Khoi et al., 2024).

5.2 Effect of plastic leachates on development

Developmental deformities were observed in all PET treatment groups. The 0.5 ppm treatment group exhibited the highest rate of deformity at 20% (Fig. 4). Deformities were also observed in the 0.1 ppm and 5.0 ppm PC treatment groups, and not in the 0.5 ppm treatment group (Fig. 4). It is unclear why deformities were seen in some, but not all of the treatment groups, as well as why deformity rates may be higher in lower concentration treatments rather than the higher concentration treatments. Both PET and PC leachate exposure resulted in spinal deformations that included bent, curved, or kinked tails. These deformations resulted in a partial or complete reduction in locomotive ability for the larvae, which likely has serious implications for long-term survival. Additionally, PC exposure resulted in several cases of incomplete development of the upper jaw. This deformity had no observable effects on locomotion or survival but may impact the larvae's ability to feed in long-term scenarios. Previous research has found that exposure to plastic leachates induced malformations in zebrafish larvae at high concentrations of 6 g/L and 8 g/L (Li et al., 2023). Their results did support a concentration-dependent effect, with malformations being greatest in the highest-concentration treatment group. Li et al. also examined the effects of the phenolic antioxidant 2,4-di-tert-butylphenol (2,4-DTBP) on zebrafish embryos, finding that embryo malformation rate was greatest in the 0.1 mg/L and 1.0 mg/L treatments than the 0.01 mg/L treatment. These concentrations are much closer to the concentrations used in the present study, and our results align with their findings that significant malformations may be induced at concentrations as low as 0.1 mg/L (0.1 ppm).

5.3 Effect of plastic leachates on heart rate

Heart rate was measured on days 4, 6, 8, and 10 to gain a better understanding of how the embryos were being affected during their development from fertilization to hatching. Heart rates were

significantly lower on at least one day for all plastic types (PE, PC, PET, and PS) when compared to the control groups (Fig. 7). For PE, heart rates were significantly lowered in the 5.0 ppm treatment group on days 6, 8, and 10 (Fig. 7A). PC also showed reduced heart rates in the 5.0 ppm treatment group on day 6 (Fig. 7B). PET and PS showed more pronounced differences in heart rates compared to the control groups, with PET having reduced heart rates in the 5.0 ppm treatment group on day 4, and all treatment groups having reduced heart rates on day 6 (Fig. 7C). PS showed significantly reduced heart rates on day 4 for 0.1 ppm and 5.0 ppm, on day 6 for 5.0 ppm, and on day 8 for 0.5 ppm and 5.0 ppm (Fig. 7D). Our results show that all plastic types reduced heart rates in the highest treatment concentration on day 6, which is an essential window for heart development in marine medaka (Huang et al., 2012). The heart is one of the first organs to develop and is therefore vulnerable to environmental stress, which may result in abnormal embryonic heart rates (Chen et al., 2022).

It is widely known that the cardiac development stage of fish embryos is sensitive to environmental contaminates and is used in toxicology as an indicator of health and stress (Chen et al., 2020). Our observations of decreased heart rates in marine medaka embryos exposed to plastic leachates may indicate bradycardia, which is a stress response that reflects an organism's ability to cope with environmental challenges (Alboni et al., 2011) and is a defense mechanism that allows marine medaka to survive in fluctuating conditions by optimizing oxygen use (Dong et al., 2014). Our results are consistent with other studies showing that exposure to PS microplastics and nanoplastics may illicit a bradycardia response in fish embryos. Both environmentally relevant and high concentrations of PS microplastics significantly induced bradycardia in marine medaka embryos at 8 and 9 days post-fertilization (Chen et al., 2020). Exposure to PS nanoplastics induced significant bradycardia in zebrafish embryos, even at the lowest concentrations of 0.1 ppm (Persiani et al., 2023). These strong influences on heart rate may be linked to the formation of reactive oxygen species (ROS) that are formed as plastics degrade. It has been shown that microplastics can lead to the formation of ROS, resulting in free radicals forming on the surface of plastics (Kadac-Czapska et al., 2024). ROS production has been linked to oxidative damage in fish,

initiating a toxicity response when exposed to pollutants, and it is hypothesized that increased ROS levels may lead to oxidative stress and changes in heart rate (Chowdhury and Saikia, 2020). Our results indicate that high concentrations of PE and PC may induce bradycardia during vulnerable early developmental stages and that PET and PS may induce bradycardia at plastic leachate concentrations as low as 0.1 ppm.

5.4 Effect of plastic leachates on hatching rates

Hatching rates were significantly lower in the 5.0 ppm PET treatment group and 0.1 ppm PC treatment group when compared to the controls (Fig. 8). While not significant, almost all other treatment groups had hatching rates that were 8-16% lower than the control groups. We likely saw a significant reduction in hatching in the 0.1 ppm PC treatment group because mortality was also significantly higher in that treatment group, leaving fewer embryos available to hatch. Our hatching rates did not exhibit a strong dose-dependent reaction, which differs from other studies that have shown high concentrations of plastic leachate reduced the hatching rates of zebrafish embryos in a dose-dependent manner (Li et al., 2021). However, our results more closely align with those showing that exposure to low concentrations of 2,4-DTBP did not strongly affect the hatching rates of zebrafish embryos (Li et al., 2021). These discrepancies may be a product of the vastly different concentrations of plastic leachates used. Under much higher concentrations of PE, PC, PET, and PS leachates, we would expect to see more prominent declines in hatching.

Conclusion

The current study evaluates the toxicity of PE, PC, PET, and PS plastic leachates on the early development of *Oryzias melastigma.* Our results highlight the potential of these plastic leachates to significantly impact critical early developmental stages by impacting the survival, hatching, development, and stress response of embryos. In addition to the developmental results shown here, this study will evaluate genetic pathways involved in oxidative stress response, immune system, and heart stress to better understand the underlying mechanisms of plastic leachate toxicity. Our findings suggest that PC and PET

may pose a greater threat than PE and PS to the early development of marine fish due to their effects on mortality, development, heart rate, and hatching. It is unknown why these two plastic compounds exhibited greater toxicity, but future research should investigate the degradation products of these plastics to better understand how plastic leachate toxicity varies by plastic type.

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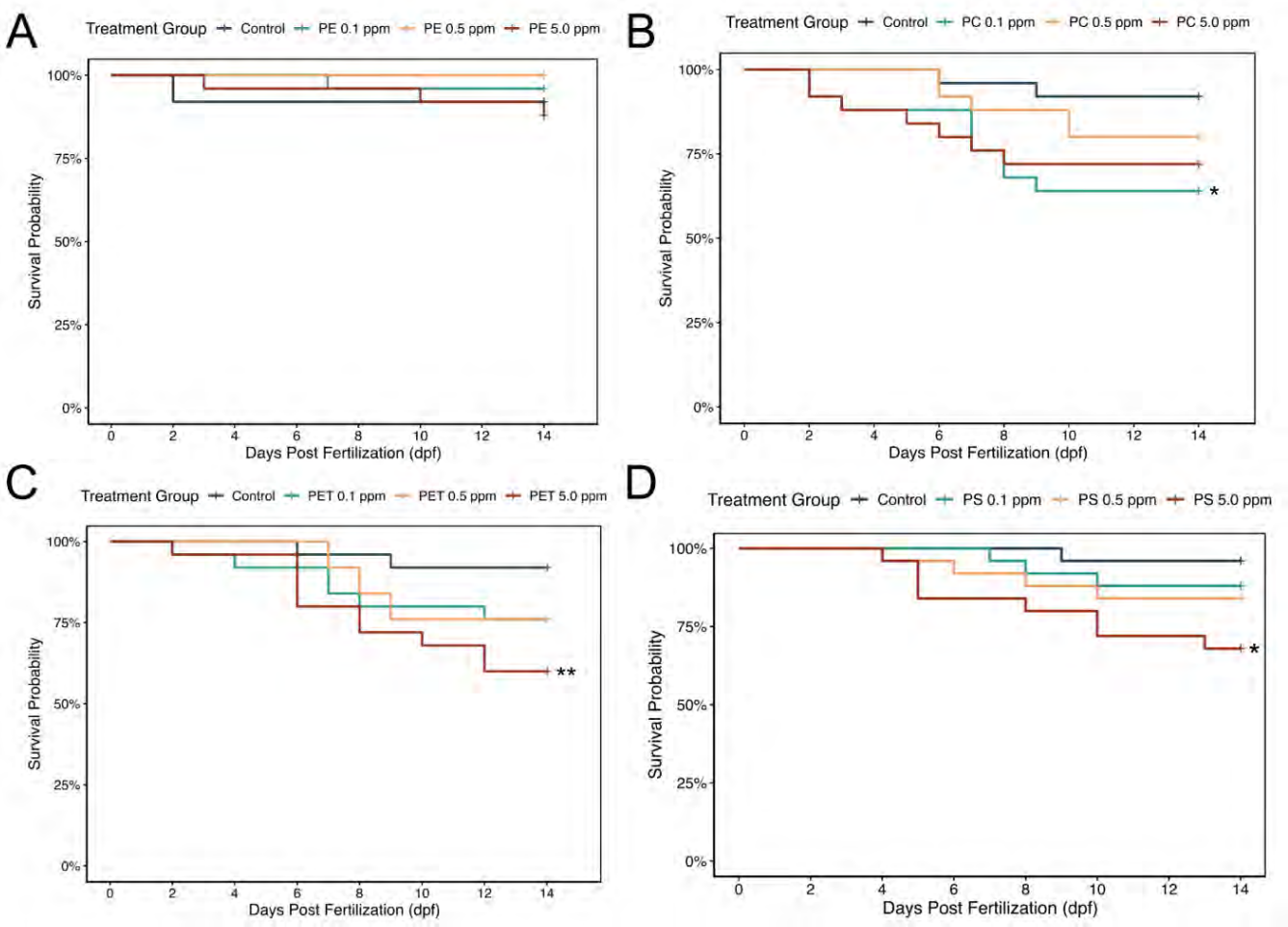

Figure 2. Percent survival of embryos exposed to PE (A), PC (B), PET (C), and PS (D) at different concentrations across a 14-day exposure (n = 25). Data expressed as a Kaplan-Meier curve, and significance was evaluated using a log-rank test at the 0.05 significance level. Asterisk (*) denotes significance compared to the control (* = 0.05, ** = 0.01, *** = <0.001).

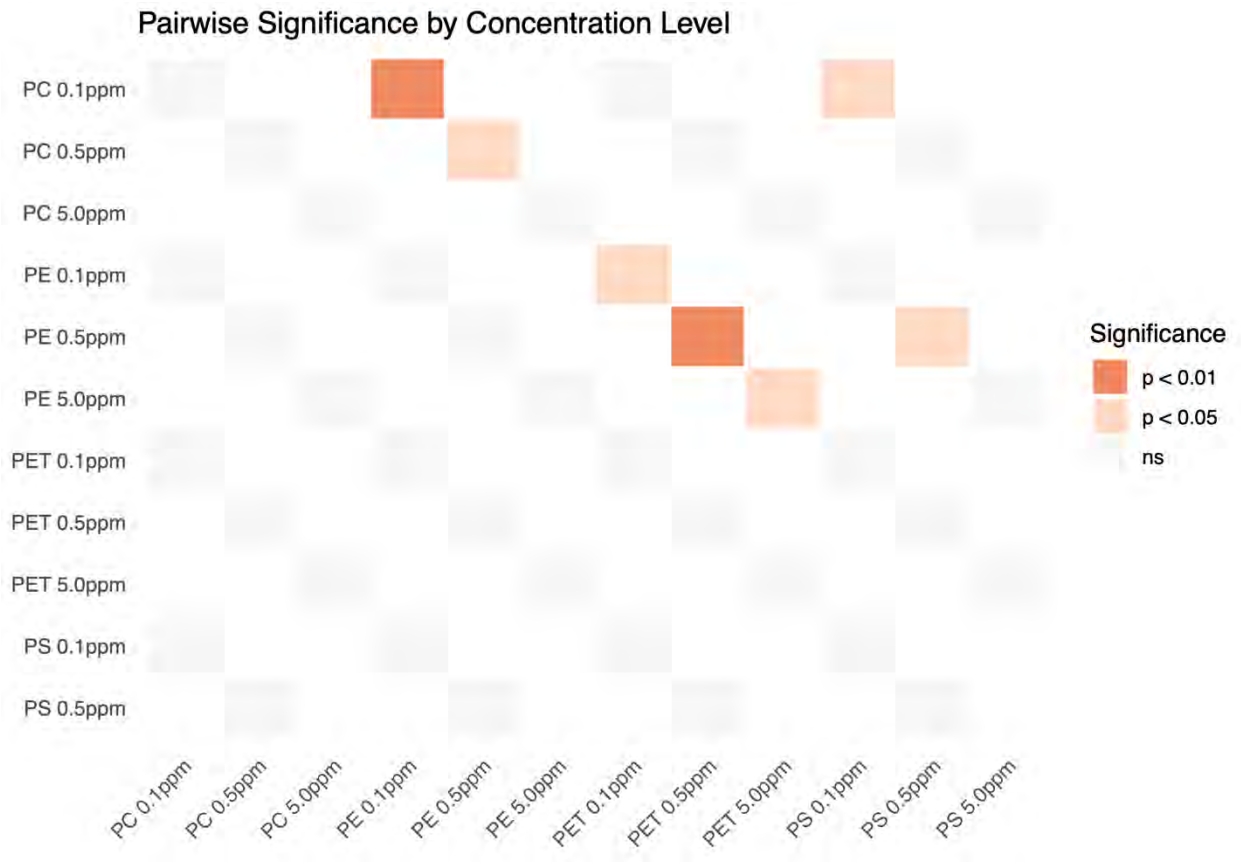

Figure 3. Pairwise significance comparison by concentration level for embryo mortality. Comparisons are between different plastic types at the same concentration, and significance is denoted by color ($p < 0.01$ is dark orange and $p < 0.05$ is light orange).

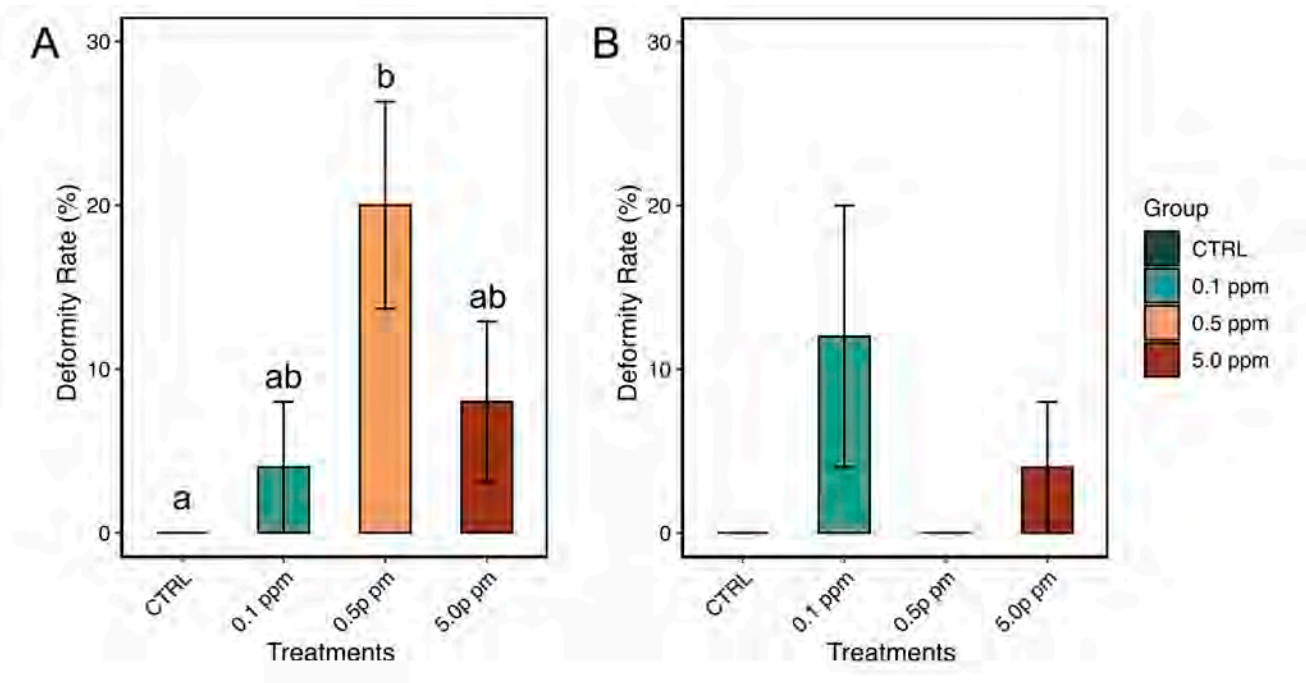

Figure 4. Effect of PET (A) and PC (B) leachates at different concentrations on embryo developmental deformity rates across 5 replicates (n = 5). Each replicate consisted of 5 embryos (a total of 25 embryos per treatment). Data expressed as mean ± S.E. Different letters denote groups that are statistically different from one another.

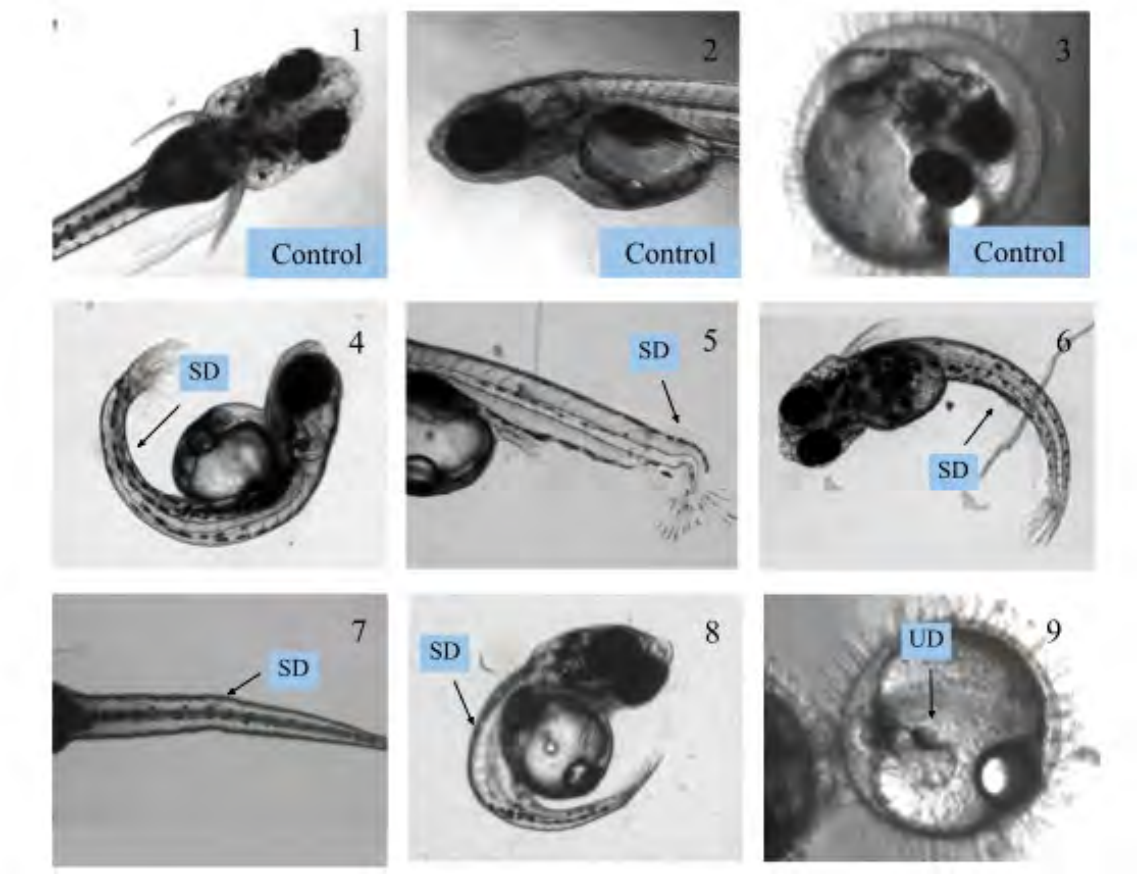

Figure 5. Morphological changes observed in *O. melastigma* larvae exposed to photodegraded polyethylene terephthalate (PET) leachate. 1-3: Control; 4: 0.1 ppm PET treatment; 5-7: 0.5 ppm PET treatment; 8-9: 5.0 ppm PET treatment. SD: Spinal deformities; UD: Underdeveloped.

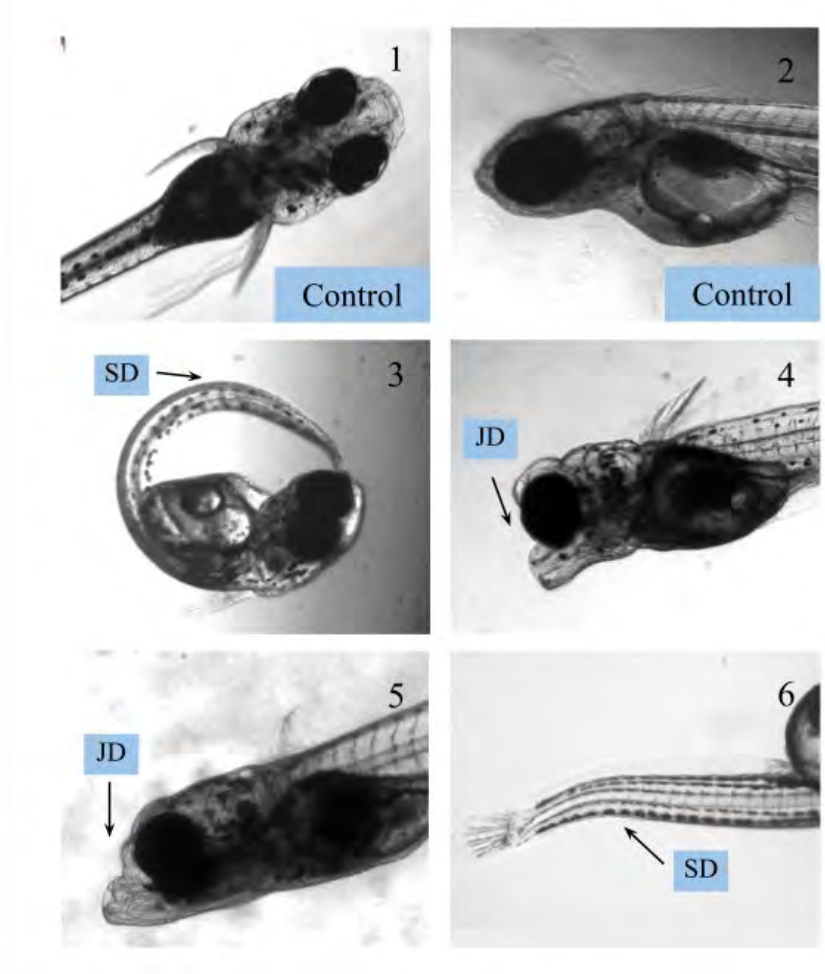

Figure 6. Morphological changes observed in *O. melastigma* larvae exposed to photodegraded polycarbonate (PC) leachate. 1-2: Control; 3-5: 0.1 ppm PC treatment; 6: 5.0 ppm PC treatment. SD: Spinal deformities; JD: Jaw deformities.

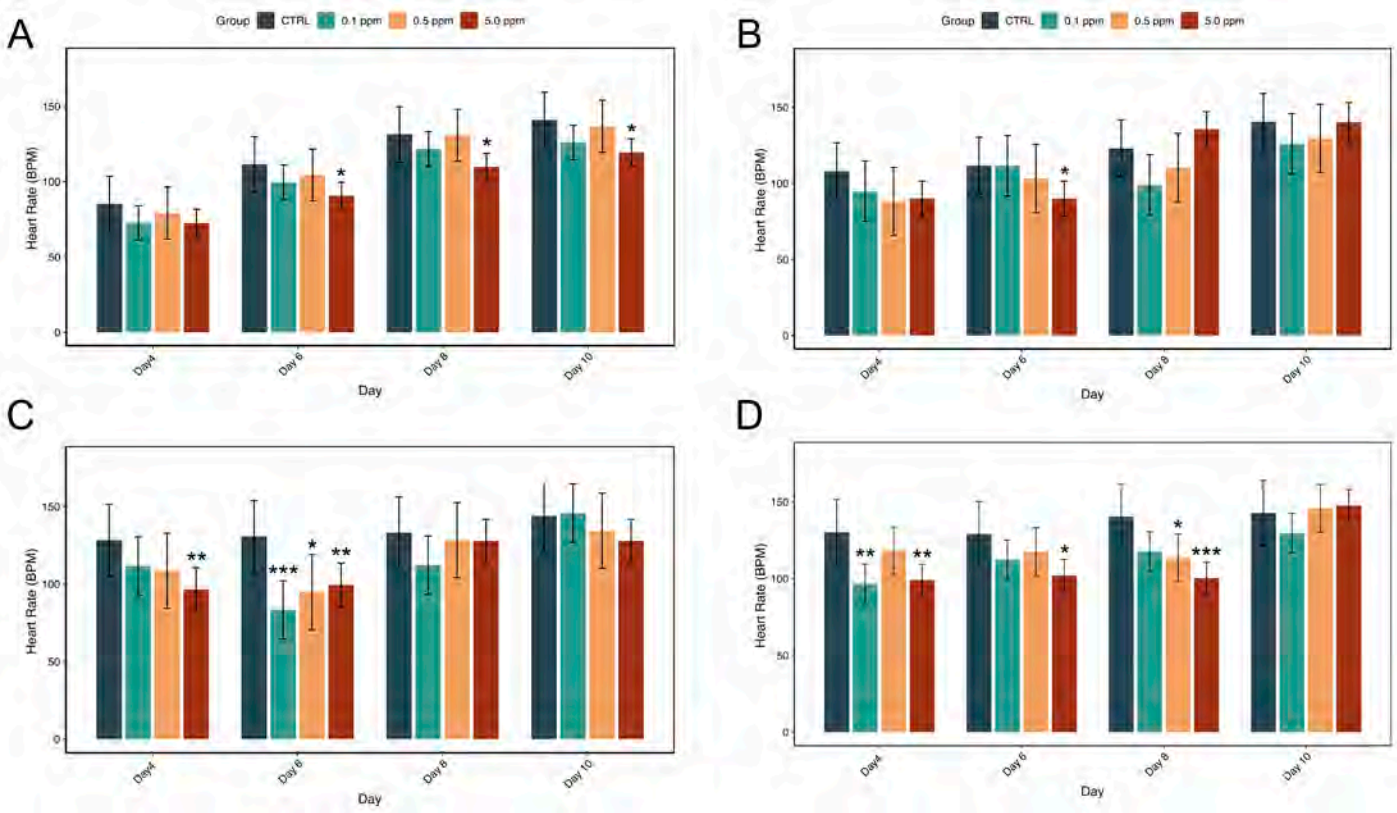

Figure 7. Effects of PE (A), PC (B), PET (C), and PS (D) leachates across 5 replicates (n = 5). Each replicate consisted of 5 embryos (a total of 25 embryos per treatment). Data expressed as estimated marginal means ± 95% CIs. Asterisk (*) denotes significance compared to the control (* = 0.05, ** = 0.01, *** = <0.001).

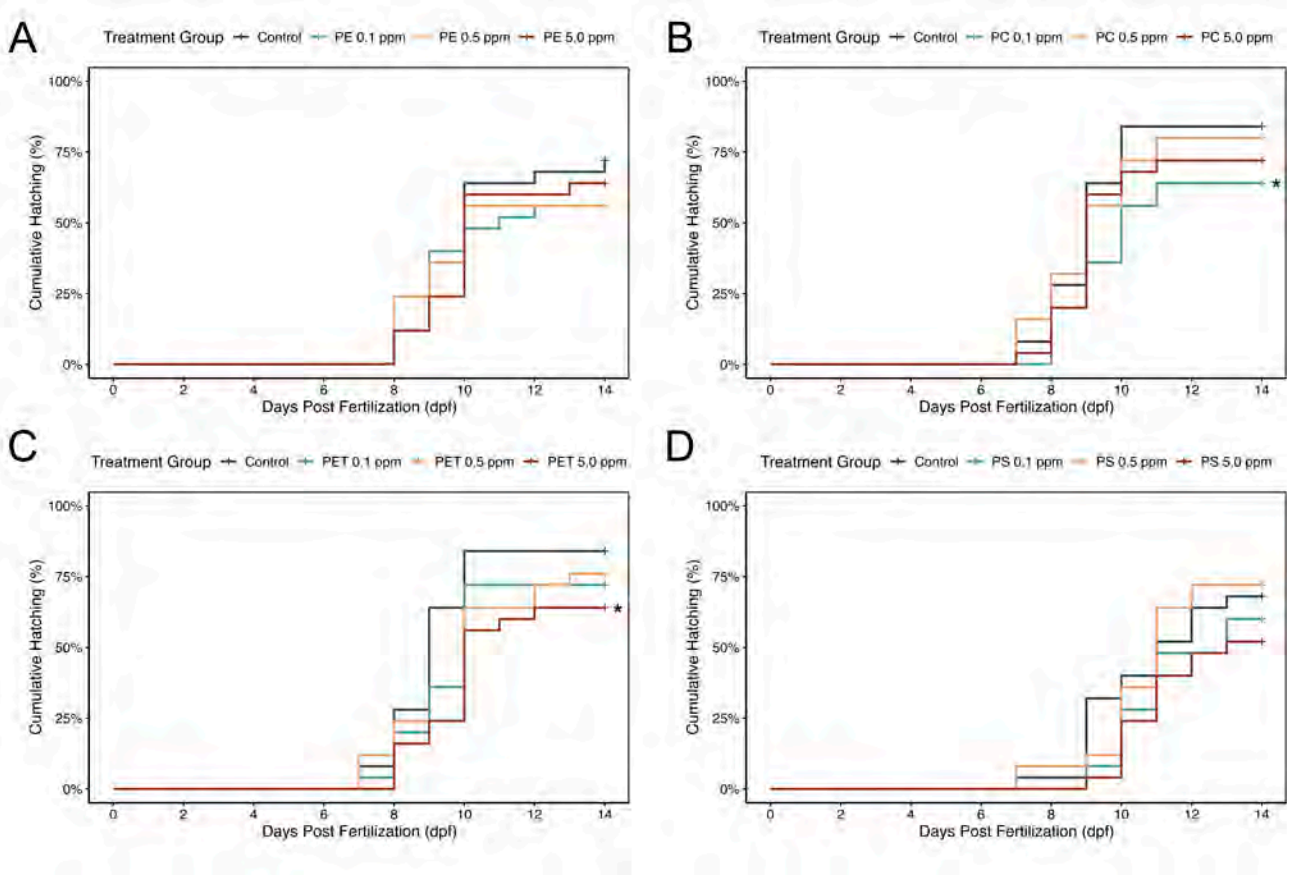

Figure 8. Cumulative hatching rate of embryos exposed to PE (A), PC (B), PET (C), and PS (D) leachates at different concentrations across a 14-day exposure (n = 25). Data expressed as a Kaplan-Meier curve, and significance was evaluated using a log-rank test at the 0.05 significance level. Asterisk (*) denotes significance compared to the control (* = 0.05, ** = 0.01, *** = <0.001).