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

PIs: Zhanfei Liu, The University of Texas at Austin; Wei Xu, Texas A&M University Corpus Christi

Period: September 1st, 2023, to December 31st, 2023

During the last quarter, September to December 2023, we worked mainly on characterizing weathered nurdles and toxicity assessment of polycarbonate (PC) and PE leachates on medaka embryos.

Characterization of weathered nurdles

Nurdles from both light and dark treatments underwent comprehensive analyses from analytical angles. Parameters such as weight loss and color changes were documented. Based on the weight results of nurdles before and after treatment, the weight loss of both PE and PC nurdles were not significant. The color of PE nurdles did not change after the light treatment, whereas the PC nurdles became yellower (Figure 1), which reflected the impact of photochemical reactions during the light treatment process.

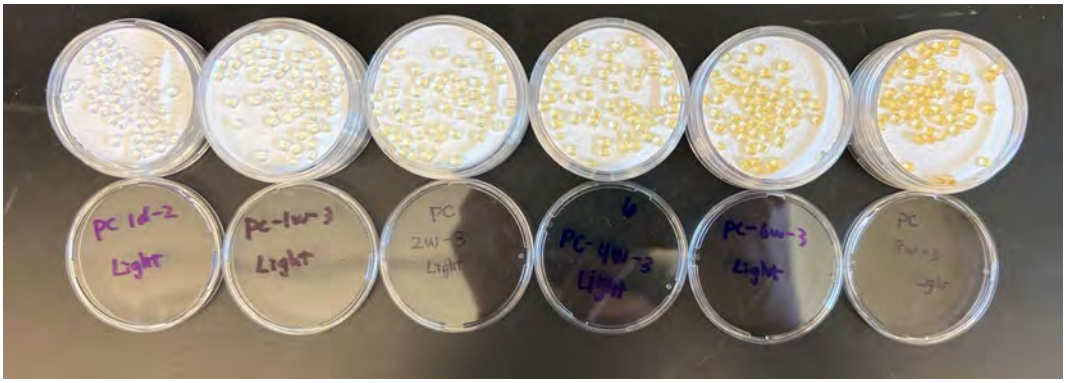

Figure 1. PC nurdles sampled at 1d, 1w, 2w, 4w, 6w, and 8w.

The nurdles were measured by Fourier Transform Infrared spectroscopy (FTIR) to examine changes of the functional groups on plastics during weathering process (Figure 2). The FTIR spectra were recorded in the range of 4000 to 700 cm^{-1} at a resolution of 8 cm^{-1}, averaging 32 scans. Newly formed functional groups on plastic surface, including hydroxyls (3300-3400 cm^{-1}), carbonyls (1690-1730 cm^{-1}), vinyl (alkenes, 1620-1650 cm^{-1}), and ethers (1100-1200 cm^{-1}), were identified and used for characterizing the weathering degree of the nurdles.

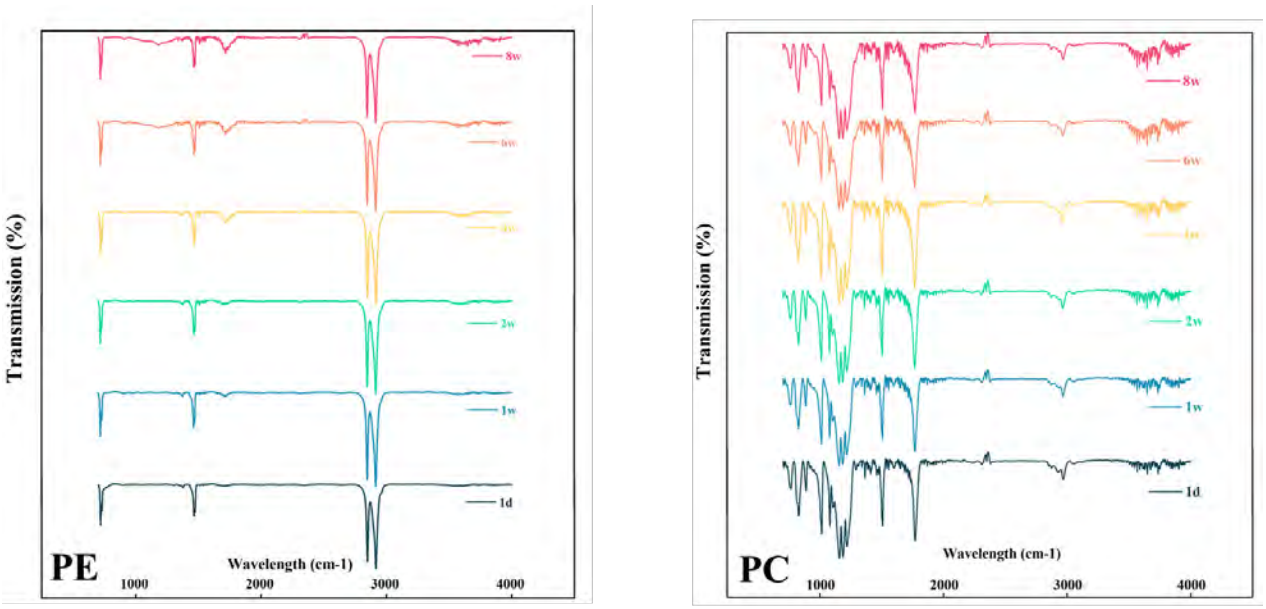

Figure 2. FTIR spectra of PE and PC nurdles sampled at 1d, 1w, 2w, 4w, 6w, and 8w.

An oxidation index was calculated to assess the surface oxidation of the nurdles (Figure 3). The oxidation index for each polymer was determined by summing four individual bonds: R-OH (alcohol), C-O (ether), C=O (ketone), and C=C (vinyl). The indices were calculated by comparing the maximum absorbance value of the corresponding peak to the value of a reference peak specific to each polymer. The oxidation index for both nurdles exhibited a consistent increase with prolonged weathering time, reaching a significant rise at 6 and 8 weeks. In the case of PE nurdles, the oxidation index was initially influenced predominantly by the C=O bond before 4 weeks, subsequently shifting to the C-O bond after this period. This transition is associated with the presence of carbonyl groups and ether groups, respectively. Conversely, for PC nurdles, the oxidation index was primarily dominated by the C-O bond due to its inherent backbone structure, with a notable increase in the C=O bond at later stages (after 4 weeks). These findings suggest distinct photo-oxidation reactions occurring at different weathering times. Notably, the prevalence of the C=O bond in the oxidation index signifies the predominance of

Norrish-type reactions, leading to the formation of carbonyl groups along the plastic chain.

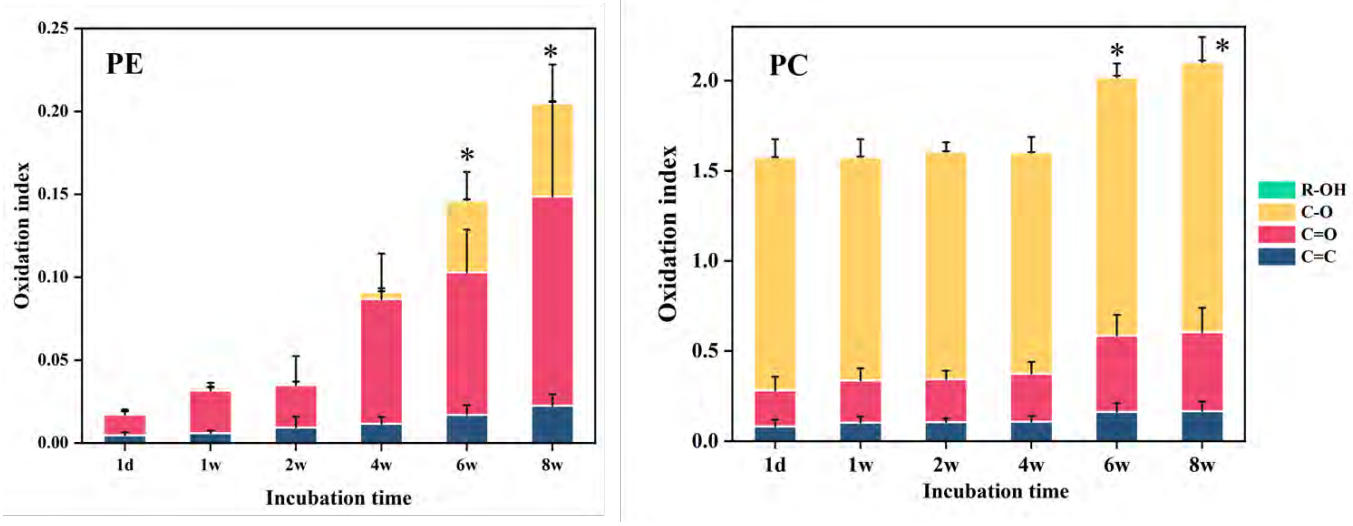

Figure 3. Oxidation indices of PE and PC nurdles sampled at 1d, 1w, 2w, 4w, 6w, and 8w.

Toxicity assessment on PC and PE leachates in medaka embryos

All fish embryos were kept in dark to avoid further photodegradation of plastic particles in water. Embryos were examined daily under an optical microscope and checked for mortalities, developmental stages, and maldevelopment of the embryos. For staging, we compared developmental stages to published standards (Murata et al., 2019). The abnormalities, fungi, or bacterial infection, and developmental stages, were all logged. In addition, the hatched embryos were also recorded, and the hatching rate of each treatment group was calculated by the end. The parameters used for maldevelopment include the size of the embryo, body morphology, and dislocation of organs in the embryonic cavities. According to the results, we found that:

1. High concentration of photodegraded PE resulted in high mortality of medaka embryos

Mortalities were significantly increased for embryos treated with 8-week photodegraded PE at 5 ppm (Figure 4). 40% mortality was observed in this group by day 20 of the treatment compared to less than 20% mortality in the control group. A slight increase in mortalities for the 0.1 ppm group (25%) but there was no statistical difference between the 0.1 ppm degraded PE treated group and the negative control. Similarly, photodegraded PC at 0.1 and 0.5 ppm showed minimum lethal effects during the development of medaka embryos. Less than 20% mortality rates were observed in the two treatment groups. However, a higher concentration of degraded PC was not assessed due to the limit of water samples. Therefore, the toxicities of photodegraded PE and PC at 5 ppm levels cannot be compared at this point.

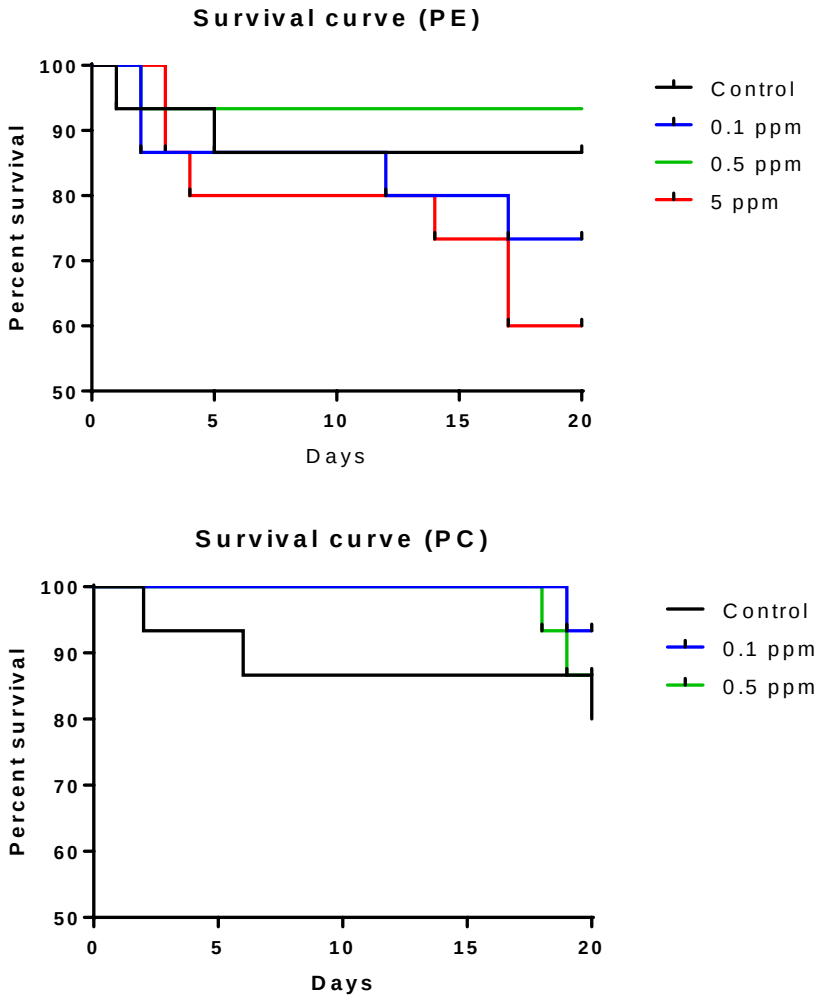

Figure 4. Survival curves of medaka embryos treated with PE and PC with various concentrations.

2. No effects of photodegraded PE and PC on developing rates and hatching rates of medaka embryos were observed.

While no statistically significant differences were observed between groups regarding developmental stages for either sample (Figure 5, *PE data not shown*), we observed differences in average sizes and/or morphologies. For PC exposed animals, abnormalities were noted in 50 % of 0.5 ppm treated animals, and 58.3 % of 0.1 ppm treated animals. Abnormalities included twitching (1 out of 15), small size (11 out of 15), and organs forming outside of the abdominal cavity (1 out of 15). Due to the relatively small sample sizes, comparisons between treatments and the control and statistical analyses were not performed. In contrast, photodegraded PE showed minimum effects on the development of embryos.

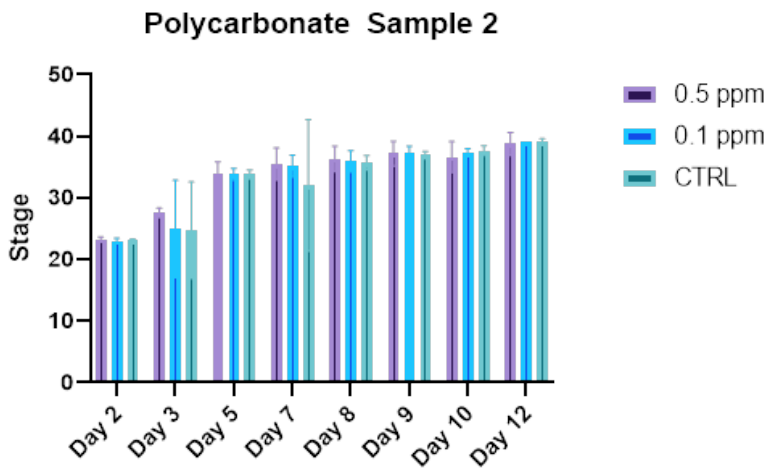

Figure 5. Staging of embryos exposed to 8-week photodegraded

Hatching rates, while not statistically significantly different between groups, tended to range from 11 to 25 days (Figures 6 & 7). A clear difference in hatching trends is also apparent between PC and PE-exposed animals when comparing Figures 4 with 5. Specifically, 5 ppm of PD PE seems to have produced earlier hatching rates than any other group, while 0.1 ppm of the PD PC appears to have condensed the emergence window of treated groups.

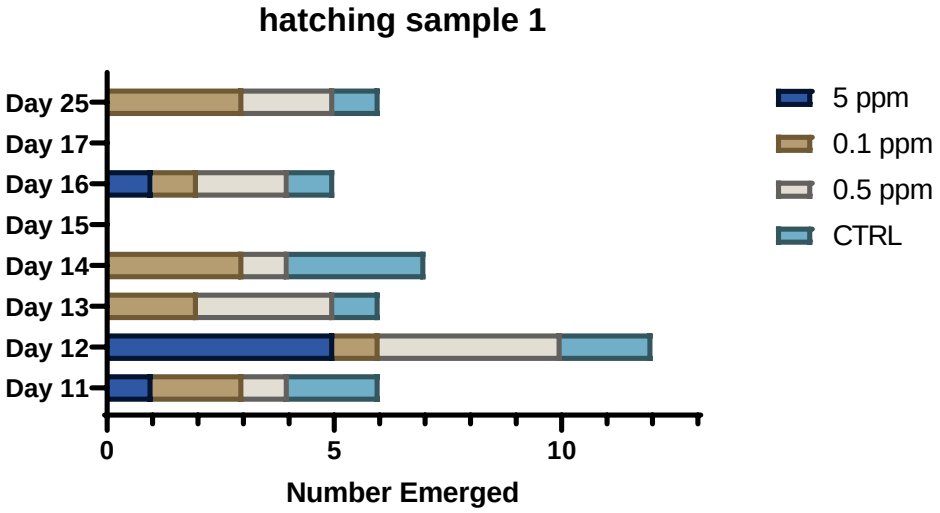

Figure 6. Hatching data for embryos exposed to PE photodegraded for 8 weeks.

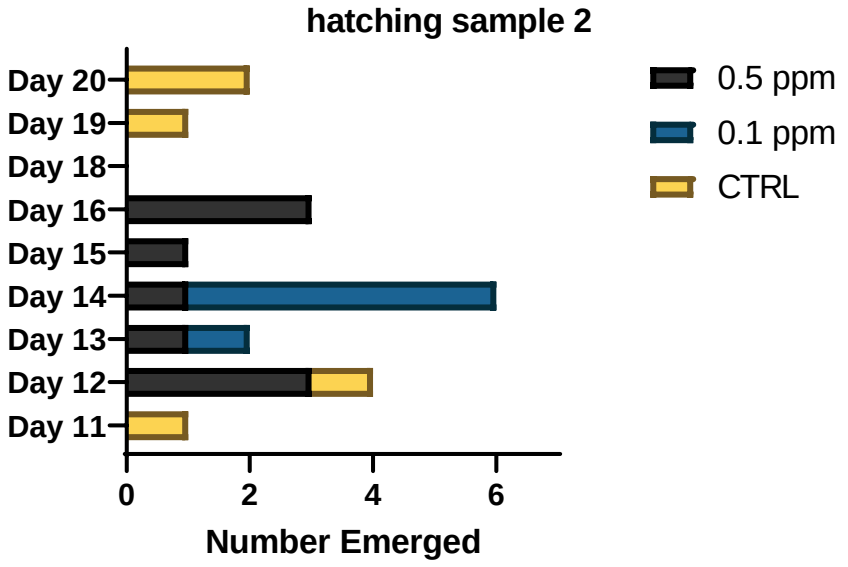

Figure 7. Hatching data for embryos exposed to PC photodegraded for 8 weeks.

Experimental plan for next step

1. Characterization of plastic leachate

The remaining incubation solutions of different treatments after TOC analysis (last quarterly report) will be solid phase extracted using PPL cartridge. The extraction efficiencies will be calculated. Molecular level information of the plastic leachate will be acquired using an Ion Mobility Quadrupole Time of Flight Liquid Chromatography Mass Spectrometer (IM Q-TOF LC/MS, Agilent 6560) with an orthogonal electrospray ionization (ESI) source. Both ESI- and ESI+ modes will be applied. Molecular formulas will be assigned to detected masses based on published rules.

2. Modification of treatment procedure

While we seem to be observing a concentration-dependent effect on mortality in the PE group, at the highest concentration of 5 ppm, we do not see any corresponding teratogenicity evidenced by malformations, small size, or abnormal movement at any of the concentrations investigated. For the PC group, we do observe some abnormal developmental effects beginning around day 7 and persisting through hatching. These abnormalities include twitching, small size, and organs developed outside of the abdominal cavity and are apparent in both treatment groups investigated (0.5 ppm and 0.1 ppm). However, PC does not appear to affect mortality rates at any of the concentrations investigated. We would like to use a higher concentration of photodegraded PC to allow us to compare the toxic effects of degraded PC and PE at the 5 ppm level. Additionally, more biological replicates will be needed to confirm the findings of the experiments.

We also observed the unusually slow hatching rates of all medaka groups including the negative control. This was possibly caused by the lack of light exposure and isolated culture method. Hatching rates are affected by the Medaka hatching enzyme which is secreted by the embryo from a gland in the oral cavity. This enzyme decomposes the egg envelope and diffuses into the medium, acting on other embryos in the clutch. Therefore, keeping the embryos as singlets in 96-well plates may also be a confounding variable that is affecting hatching rates. It was also reported that light activation is required for the enzymes to assist the embryos in breaking the chorions. In future experiments, we will culture the medaka embryos in a group manner (5-6 per well) and with a light: dark cycle at 12h:12h. Since the photodegradation of plastics was performed with strong UV to simulate the natural photodegradation for several years, normal light exposure (full spectrum lamp) for less than two weeks is not expected to significantly further degrade the plastic particles in water.

3. Toxicity assessment on photodegraded PC and PE at the molecular level in medaka embryos

To further confirm the results from the observation, we will be using the molecular method for the analyses. We will focus on the expression levels of several genetic markers that are commonly used to assess the stress levels of fish. We are also interested in the expression of some growth hormone-encoding genes that are directly related to embryonic development. Additionally, the activities of enzymes related to embryonic metabolism will be also investigated. Combined with direct observation, these data are expected to show evidence of the toxic effects of the photodegradated plastic particles.