Marine Pollution Bulletin 206 (2024) 116788

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Persistent plastic: Insights from seawater weathering and simulated whale gut

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ARTICLE INFO

Keywords:
Plastic pollution
Marine debris
Marine mammals
Whale gut
Exposure
Tensile strength
Bisphenol A

ABSTRACT

Single-use plastics make up 60–95 % of marine plastic pollution, including common commodity films used for packaging and bags. Plastic film breaks down as a function of environmental variables like wave action, wind, temperature, and UV radiation. Here, we focus on how films degrade in cold waters across depths, time, and simulated mammal digestion. Five types of single-use film plastics (HDPE thin & thick, LDPE, PP, PE) were weathered for eight months in temperate waters at surface and depth in the Salish Sea, WA, USA, and subsequently exposed to a laboratory-simulated gray whale stomach. None of the types of plastics examined here fully degraded during the course of this 8 months study. Weathering time and depth significantly impacted many of the physical attributes of plastics, while exposure to a simulated whale gut did not. If unable to degrade plastics through digestion, whales risk long-term exposure to physical and chemical attributes of plastics.

1. Introduction

Global plastic production has grown since the late 1940s; today we produce roughly 460 million tonnes of plastic per year with over 9–14 million tonnes ending up in our oceans (Lau et al., 2020; OECD, 2022). Industries have grown dependent on plastic, especially plastic film, for its flexibility, durability, and inexpensive price (Barnes et al., 2009; Sivan, 2011; Iñiguez et al., 2018). Single-use plastic makes up 60–95 % of marine plastic pollution (Schnurr et al., 2018), including common commodity thin films used for packaging, produce wrap, and to-go bags. Unfortunately, the same elements that contribute to plastic film's popularity also contribute to its long-lasting negative environmental impacts.

In the ocean, plastic fragmentation and eventual degradation occur when plastics are exposed to physical and chemical factors like wave action, temperature, wind, UV radiation, salinity, pH, and dissolved oxygen (Hocker et al., 2014; Wadsö and Karlsson, 2013; Urbanek et al., 2018). In seawater, low temperatures and oxygen content delay the thermo-oxidative degradation of plastics (Andrady, 2011; Pegram and Andrady, 1989), increasing their longevity in marine systems. Degradation speed decreases further in habitats with lower UV and colder temperatures, low wave action, and changes in both pH and salinity (e.g. some temperate waters; Hocker et al., 2014; Wadsö and Karlsson, 2013; Zhang et al., 2021). While many of the aforementioned physical aspects leading to degradation are strongest at the ocean's surface, plastics are transported to the mid-water column or seafloor due to forces from currents and from biofouling (Fazey and Ryan, 2016; Choy et al., 2019; Harris et al., 2021), thus increasing bioavailability across depths. As a result, plastic does not readily degrade and can exist anywhere between 500 and 1000 years in our marine environment (Andrady, 2011; Mohanan et al., 2020; Chamas et al., 2020).

If ingested by marine animals, plastic debris can cause gut blockages, lacerations, ulcerations, secondary infections, starvation due to a false sense of satiation, and partial or complete digestive system blockages (Laist, 1997; Secchi and Zarzur, 1999; Derraik, 2002; Pierce et al., 2004; Stamper et al., 2006; Jacobsen et al., 2010; Stephanis et al., 2013). Potential secondary effects such as hormone disruption, reproductive impairment, immune system impairment, and other disease developments are also inherent risks of plastic ingestion (Li et al., 2016; Nabi et al., 2022). As an example, Bisphenol A (BPA) is used as a plastic

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https://doi.org/10.1016/j.marpolbul.2024.116788
Received 2 May 2024; Received in revised form 23 July 2024; Accepted 26 July 2024
Available online 9 August 2024

additive and is persistent in the environment due to its ongoing release during plastics manufacture, transport, processing, and post-consumer releases via effluent discharge (Wu and Seebacher, 2020). It is also an endocrine-disrupting compound and is known to mimic estrogen, a hormone necessary for normal vertebrate female maturity and reproduction, thus potentially interfering with those functions (Welshons et al., 2006; Flint et al., 2012; Zoeller et al., 2012; Sonavane, 2022).

The effects of marine plastic debris on large organisms, specifically marine mammals, are difficult to study due to their physiological traits and size. Filter feeding megafauna, like baleen whales (*Mysticetes)*, are especially at risk of ingesting and bioaccumulating exceedingly large quantities of plastics and additives (Simmonds, 2012; Unger et al., 2016; Nelms et al., 2018; Kühn and Andries van Franeker, 2020; Kahane-Rapport et al., 2022). Gray whales (*Eschrichtius robustus)*, a baleen whale, are estimated to ingest 6.5 to 21 million pieces of microparticle pollution per day which includes microplastics and other anthropogenic pollution (Torres et al., 2023). Several gray whale life history traits situate them to ingest large quantities of plastic over their lifespan: 1) Large geographical range, consisting of one of the longest migrations where they travel upwards of 10,000 miles (Lagerquist et al., 2019), 2) feeding on sediment on the seafloor (Jones et al., 2012), and 3) long lives, living up to 48 years (Agbayani et al., 2020). These three traits indicate gray whales have chronic exposure to marine plastics and are likely to experience physical impacts of ingestion. Ultimately, plastics that are eaten and do not break down in the digestion process can cause significant health concerns (e.g. above listed impacts; Laist, 1997; Secchi and Zarzur, 1999; Derraik, 2002; Pierce et al., 2004; Stamper et al., 2006; Jacobsen et al., 2010; Stephanis et al., 2013).

Previous studies found that commodity thin plastics films (produced in large quantities and used for a wide spectrum of applications) maintain shape and structure over several months to years in cold surface seawater (e.g. O'Brine and Thompson, 2010; Zhang et al., 2021), however, the combined effects of exposure to depth, time, and ingestion remain unclear. Plastic film breaks down as a function of environmental variables like wave action, temperature, wind, UV radiation, and salinity that are different across vertical space and time, as well as physical properties of the material (Urbanek et al., 2018; MacLeod et al., 2021). Degradation of plastic films can be identified as pitting, cracking, discoloration, erosion, or embrittlement (Iñiguez et al., 2018). In the process of degradation, mass decreases (Chamas et al., 2020), surface electrical resistance decreases (Gerritse et al., 2020), and tensile properties such as yield strain, decrease (Iñiguez et al., 2018). Assessing how plastic films break down over time and across depths in temperate cold waters can provide insights to how ingestion and digestion may impact numerous marine species.

Despite the volume of commodity plastic materials entering the marine environment and the degradation processes that result, current measures used to assess the degradation of plastic materials and certify their end-of-life performance (such as those used by ASTM to assess degradation in seawater) do not consider the impact of these materials on marine flora and fauna. In 2022, the Seattle Aquarium joined the University of Georgia New Materials Institute as one of two testing locations for the TOM FORD Plastic Innovation Prize to test the breakdown of plastics and plastic alternatives in seawater. Lonely Whale, a US-based nonprofit organization focused on preventing plastic waste from entering the ocean, partnered with TOM FORD to conduct the Plastic Innovation Prize. This unique project was designed to bring together a diverse range of stakeholders across industry, the nonprofit sector, and academia to test attributes of innovative plastic alternatives, compared to commodity plastics. The methods outlined here were developed as a function of the Aquarium's role as a testing location for the Plastic Innovation Prize and sought to incorporate potential impacts on a charismatic marine mammal, gray whale, into end-of-life performance assessments. Full details of the Plastic Innovation Prize and finalists are available online (unwrapthefuture.org).

Here, we focus on how single-use plastic films degrade in cold waters across depths, time, and simulated mammal digestion. Five types of commonly used single-use film plastics were weathered for eight months in temperate waters at surface and depth in the Salish Sea, WA, USA, and subsequently exposed to a laboratory-simulated gray whale stomach. Throughout weathering and simulated gut exposure, we visually assessed and measured four attributes of the single-use plastic films to holistically understand degradation in wide-spread environmental and digestion conditions: 1) mass, 2) electrical resistance, 3) tensile strength, and 4) BPA concentration. The tests were designed to answer the following questions: 1) How do single-use plastic films degrade across time and depths in temperate waters and 2) How do environmentally weathered single-use plastic films change when exposed to simulated whale stomachs?

2. Materials & methods

2.1. Site

This work took place at the Seattle Aquarium, centrally located on the east side of the Salish Sea in Elliott Bay, Washington State, USA (47°36′26.6"N, 122°20′38.3"W). Tidal exchanges vary 3–4 m, occurring approximately every 12.4 h. Each exchange brings in or removes up to 8 km^3 of water and causes turbulent mixing in the water column (Department of Ecology, n.d.).

2.2. Environmental set-up and weathering

Five common types of plastic film were measured for environmental degradation and subsequent whale gut simulation. All samples were cut with scissors into 10 × 10 cm squares and run in triplicate. The following materials were studied: High-density polyethylene (HDPE thin) bags, low-density polyethylene (LDPE) bags, polypropylene (PP) potato chip bags, polyethylene (PE) shipping poly-bags, and high-density polyethylene thick film (HDPE thick; FTIR spectra listed in Supplemental Fig. 1).

Samples were placed in seawater at two depths, 0.3 m and 10 m (hereafter referred to as "surface" and "deep" depths, respectively), and retrieved at 4 months and 8 months. Samples were randomly placed in blue and white nylon mesh bags divided into quarters (4 samples per bag) and lowered on a specialized rig at Pier 59 at the Seattle Aquarium (Supplemental Fig. 2). Samples were deployed in April 2022 (0 month) and retrieved in August 2022 (4 months) and December 2022 (8 months). The environmental weathering time for commodity plastic films studied here matched the methodology and testing period of 8 months used in the TOM FORD Plastic Innovation Prize for plastic alternatives.

Upon retrieval from environmental conditions, samples were cleaned of biofouling macro-organisms, but no chemical cleaning or digestion methods were used that could potentially damage the plastics. Samples were photographed (Sony NEX - 5 digital camera and SHOTBOX portable light studio) to document physical change and residual biofouling. We did not quantify biofouling as a measure of weathering or degradation, however, we noted observations. Samples were then dried for at least 24 h and weighed. Next, two 2 × 10 cm strips were cut with scissors from each of the original 10 × 10 cm samples and saved for pre-simulation tensile strength testing. The remaining 6 × 10 cm rectangle samples were re-weighed for pre whale-gut mass and underwent electrical resistance testing.

2.3. Whale gut simulation

Marine mammal stomachs have four main variables that are responsible for the breakdown of food: digestion enzymes, low pH, mixing, and elevated temperature. For this study, fundic stomach #2 was selected to simulate as this is where most plastics were found in a previous investigation of a dead whale's gut (Lusher et al., 2015).

Samples were placed individually into a flask simulating a gray whale gut. Each flask of simulated gray whale gut contained 500 ml reverse osmosis water, 1 % pepsin A to simulate mammalian digestion (Carolina Biological Supply Company, Burlington, North Carolina, CAS #9001-75-6), and a few drops of hydrochloric acid (HCl) to reach 5.25 pH and promote optimal Pepsin A activity (Jackson et al., 1987; Coffin et al., 2019; Kong and Paul Singh, 2010; Turner et al., 2001; Olsen et al., 1994). Flasks were kept at 38 °C and mixed at 300 RPM with a magnetic stir bar inside the flask (Jackson et al., 1987), on a heated stir plate to match the temperature of a whale gut for 24 h (Bakir et al., 2014).

At the end of the 24 h period, 1 ml of each solution was collected to measure BPA concentration (described below) and samples were removed from their flasks using forceps and placed on a drying rack for another 24 h. Upon drying, samples were photographed, weighed, electrical resistance measured, and subsequently cut with scissors into three 2 × 10 cm strips for post-whale gut simulation tensile strength testing.

2.4. Tests

All assays described below were carried out at three time points, 0 month (before environmental weathering), 4 months, and 8 months, at two depths, surface and deep (0.3 m and 10 m, respectively), and pre- and post- whale gut simulation. Kraft paper underwent the same experimental design as a quality control measure. All Kraft paper degraded in the temperate marine environment in <4 months (data not shown). Additionally, all pre-weathered Kraft paper samples completely degraded inside the whale gut simulation within 24 h (data not shown), unlike the plastic samples. This was much faster than it degraded in seawater, indicating that the elements present in the gut simulation are capable of accelerating the degradation of organic material.

2.4.1. Mass

Samples (6 × 10 cm) were weighed for dry weight (g) on an analytical scale (OHAUS Precision Standard, TS120).

2.4.2. Electrical resistance

Electrical resistance was measured following the standard ASTM-D257 with a digital ohmmeter (DER EE, LCR Meter Model DE-5000). Alligator clips of the LCR meter were placed 1 cm apart on the same side of the sample and set to resistance in sequence (Rs) where 1 k Hz frequency was tested in triplicate.

2.4.3. Tensile material properties

Tensile material properties of each sample were measured at Friday Harbor Laboratories, University of Washington with a tensometer (Instron model 5900, Norwood, MA, USA). Tensile material properties were measured following ASTM D3826-98 (2008) where each sample was cut to 2 × 10 cm and thickness was measured with calipers (Demes et al., 2011). The ends of each sample were placed into pneumatic grips (0.4 MPa) lined with paper to provide additional friction (Demes et al., 2011) and the sample extended length-wise at 20 mm/min until failure (Tosin et al., 2012; Suraci et al., 2020; O'Brine and Thompson, 2010; Andrady, 2011; Gerritse et al., 2020; Chamas et al., 2020). We focused on two different material properties, yield stress and yield strain (BlueHill Universal software; version 4.11).

2.4.4. BPA concentration

After whale gut simulations, 1 ml of aqueous solution was collected from each replicate and pooled into one 3 ml sample. Bisphenol A (BPA) concentration was measured only after the 24 h digestion process. Samples were frozen at −20 °C until testing occurred (all samples were processed within 4 months). BPA concentrations were measured using commercially available BPA enzyme immunoassay kits (Catalog #: BPA1, Detroit R & D, Detroit, MI, USA) per manufacturer's specifications. All assay plates included a standard curve, triplicate maximum binding wells, triplicate blank wells, and triplicate samples wells. The reported detection limit of the plate was <10 pg/ml BPA.

2.5. Data analysis

All data analyses and visualizations were performed in R v.4.1.3 (R Core Team, 2022) using the following packages: 'tidyverse' (Wickham et al., 2019), 'patchwork' (Pedersen, 2020), and 'ggbreak' (Xu et al., 2021). Level of significance was set at $\alpha < 0.05$. Each commodity plastic film type was analyzed separately. We used three-way analysis of variance (ANOVA) to test for an effect of depth, (surface v deep) time, (4 v 8 months), simulated whale gut exposure, (pre- v post-), and their interactions on response variables (mass, electrical resistance, yield strain, and yield stress) for each type of commodity plastic, separately. For significant effects, differences between treatment levels were assessed with pairwise post-hoc tests with Bonferroni corrections. Pre- weathered ($t = 0$ month) samples were removed from the dataset and evaluated separately with paired *t*-tests. BPA levels across all plastic types were below predicted no-effect concentration levels (1.5 μg; PNEC) and were thus not analyzed further.

3. Results

Two nylon bags detached during the environmental weathering and were lost. As a result, one replicate of LDPE at 8 months at deep depth and one replicate of HDPE thick film at 4 months at the surface are missing from the following results.

Generally, all plastic types weathered at the surface (except LDPE) were more visually biofouled than samples weathered at deep depth (Fig. 1). Similarly, plastics weathered for 8 months were generally more visually biofouled than samples weathered for 4 months (Fig. 1).

3.1. High density polyethylene (HDPE) thin bag

Pre-weathered HDPE thin bag mass averaged 0.01 ± 1.63 × 10^{-4} g cm^{-2} (mean ± SE; t = 0 month; Fig. 2a). Whale gut exposure did not affect mass, electrical resistance, strain, or stress in pre-weathered samples ($p > 0.1$; *t*-test). Mass was dependent on weathering depth ($p = 0.020$), where mass was 4.5 % greater at the surface than deep depth, but not weathering time, whale gut simulation, nor the interaction ($p > 0.08$; 3-way ANOVA; Table 1). Pre-weathered HDPE thin bag electrical resistance averaged 21 ± 0.43 Ohms (Fig. 2b). Electrical resistance was dependent on depth and time, where resistance at surface was 39 % and 35 % higher than deep depth at 4 months and 8 months, respectively ($p = 0.001$ and $p = 3.7 \times 10^{-5}$, respectively). However, resistance was not dependent on whale gut simulation nor the interaction ($p > 0.07$; 3-way ANOVA; Table 1). One replicate of HDPE thin film from 8 months, deep depth was too crinkled to undergo tensile tests. When examining tensile

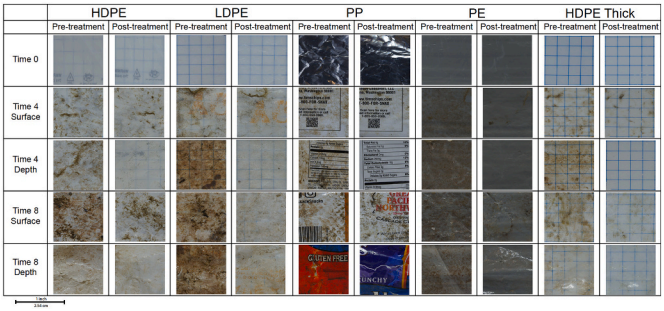

Fig. 1. Representative photographs of the five types of commodity thin film plastic weathered over 8 months at surface and deep depths and post-whale gut simulation exposure. The PP chip bag photographs from pre-weathering (0 month) are of the inside of the bag while the remaining photographs are of the outside of the bag.

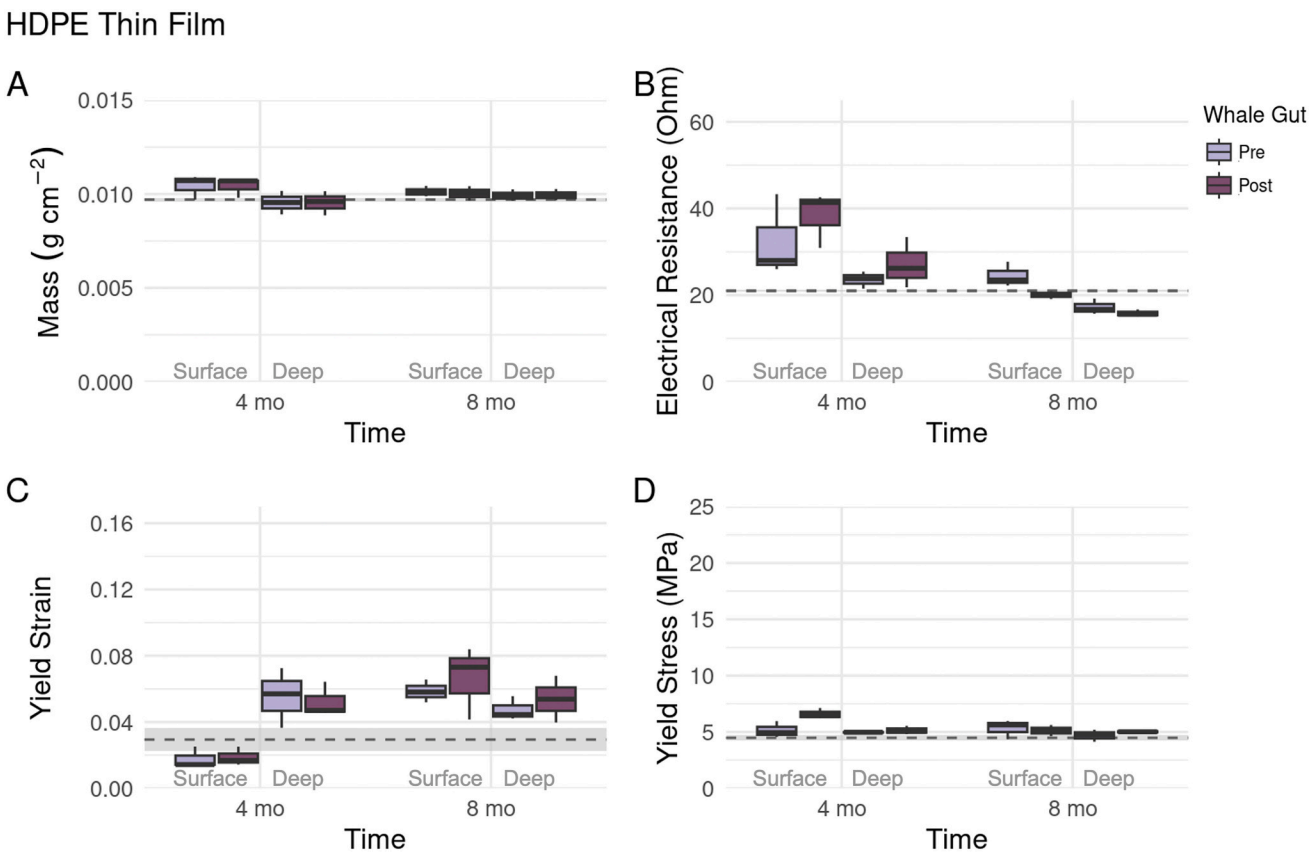

Fig. 2. High Density Polyethylene (HDPE) thin film measurement of A) mass, B) electrical resistance, C) yield strain, and D) yield stress as a function of weathering time, weathering depth and whale gut simulation. Boxes represent upper and lower quartiles, where solid lines within boxes represent median values. Dashed line represents pre- weathering and whale gut exposure samples (0 month; no statistical difference in pre- and post- whale gut samples) and gray shading represents SE.

properties of the HDPE thin bag, pre-weathered yield strain and yield stress averaged 0.017 ± 0.001 MPa and 4.12 ± 0.15 MPa, respectively (Fig. 2c,d). Yield strain was dependent on the interaction of weathering depth and time ($p = 0.001$), where strain increased 240 % at surface and decreased 15 % at deep depth from 4 months to 8 months; yield stress was dependent on depth, where the surface samples recorded 12 % greater stress, and was not dependent on time, whale gut simulation, nor the interaction ($p > 0.06$; 3-way ANOVA; Table 1).

3.2. Low density polyethylene (LDPE) bag

Pre-weathered LDPE bag mass averaged 0.005 ± 8.33 × 10^{-6} g cm^{-2} (Fig. 3a). Whale gut exposure affected electrical resistance, strain, and stress in pre-weathered samples ($p < 0.05$), but not mass ($p = 0.48$; *t*-test). LDPE bag mass was not dependent on weathering depth, time, whale gut simulation, nor the interaction ($p > 0.12$; 3-way ANOVA; Table 1). Pre-weathered LDPE bag electrical resistance averaged 10.6 ± 1.11 Ohms (Fig. 3b). Electrical resistance was dependent on the interaction of depth and whale gut simulation ($p = 0.05$; 3-way; Table 1) where resistance was highest at 4 months. One replicate of LDPE film from 4 months surface and one replicate from 8 months deep depth were too crinkled to undergo tensile tests. When examining tensile properties of the LDPE bag, pre-weathered yield strain and yield stress averaged 0.02 ± 0.003 MPa and 5.44 ± 0.61 MPa, respectively (Fig. 3c,d). Yield strain was dependent on weathering depth and time ($p < 0.05$; 3-way ANOVA; Table 1), where strain increased over time 58 % and 49 % at surface and deep depth, respectively. Yield strain was not dependent on whale gut simulation, nor the interactions ($p > 0.09$; 3-way ANOVA; Table 1). Yield stress was not dependent on weathering depth, time, whale gut simulation, nor the interaction ($p > 0.05$; 3-way ANOVA; Table 1).

3.3. Polypropylene (PP) chip bag

Pre-weathered PP chip bag mass averaged 0.001 ± 2.08 × 10^{-5} g cm^{-2} (Fig. 4a). Whale gut exposure did not affect mass, electrical resistance, strain, or stress in pre-weathered samples ($p > 0.06$; *t*-test). PP chip bag mass was dependent on weathering depth and time, where mass increased over time 5 % and 10 % at surface and deep depth, respectively. Mass was not dependent on whale gut simulation, nor the interaction ($p > 0.06$; 3-way ANOVA; Table 1). Pre-weathered PP chip bag electrical resistance averaged 20.7 ± 6.84 Ohms (Fig. 4b). Electrical resistance was dependent on weathering depth and time ($p = 2.9 \times 10^{-4}$ and $p = 8.7 \times 10^{-6}$, respectively), where resistance decreased 75 % and 52 % over time at surface and deep depth, respectively. Electrical resistance was not dependent on whale gut simulation nor the interaction ($p > 0.07$; 3-way ANOVA; Table 1). When examining tensile properties of the PP chip bag, pre-weathered yield strain and yield stress averaged 0.03 ± 0.008 MPa and 18.9 ± 0.73 MPa, respectively (Fig. 4c, d). Yield strain was dependent on weathering depth and time ($p = 8.5 \times 10^{-4}$ and $p = 6.9 \times 10^{-6}$, respectively), where over time strain increased 67 % and 41 % at surface and deep depths, respectively. Strain was not dependent on whale gut simulation or the interactions ($p > 0.05$; 3-way ANOVA; Table 1). Yield stress was dependent on weathering depth ($p = 2.6 \times 10^{-4}$), where stress was 16 % higher at the surface, but not dependent on time, whale gut simulation nor the interaction ($p > 0.09$; 3-way ANOVA; Table 1).

Table 1
Summary of all commodity plastic film 3-way ANOVAs for each physical degradation test across depth, time, and simulated gut exposure as well as the interactions. Asterisks indicate statistical significance at (*) $p < 0.05$ and (**) $p < 0.001$.

	Depth	Time	Gut	Depth * time	Depth * gut	Time * gut	Depth * time * gut
HDPE thin							
Mass	0.020*	0.898	0.891	0.084	0.858	0.968	0.912
Electrical	0.001**	0.000**	0.650	0.290	0.926	0.068	0.503
Yield strain	0.049*	0.002*	0.481	0.001*	0.784	0.477	0.907
Yield stress	0.016*	0.059	0.072	0.317	0.414	0.096	0.066
LDPE							
Mass	0.523	0.829	0.349	0.124	0.960	0.442	0.907
Electrical	0.037*	0.000**	0.160	0.090	0.049*	0.980	0.273
Yield strain	0.02*	0.000**	0.056	0.316	0.468	0.207	0.895
Yield stress	0.866	0.976	0.626	0.092	0.744	0.610	0.399
PP chip bag							
Mass	0.048*	0.043*	0.769	0.549	0.778	0.829	0.994
Electrical	0.000**	0.000**	0.302	0.071	0.570	0.455	0.099
Yield strain	0.001**	0.000**	0.148	0.375	0.052	0.095	0.162
Yield stress	0.000**	0.093	0.091	0.519	0.617	0.385	0.813
PE poly bag							
Mass	0.010*	0.007*	0.491	0.496	0.690	0.387	0.754
Electrical	0.006*	0.000**	0.008*	0.284	0.029*	0.812	0.625
Yield strain	0.000**	0.000**	0.305	0.021*	0.004**	0.162	0.196
Yield stress	0.160	0.000**	0.756	0.024*	0.164	0.848	0.116
HDPE thick							
Mass	0.142	0.543	0.883	0.837	0.886	0.969	0.983
Electrical	0.000**	0.000**	0.000**	0.000**	0.495	0.116	0.130
Yield strain	0.391	0.042*	0.496	0.752	0.807	0.091	0.614
Yield stress	0.070	0.917	0.849	0.265	0.763	0.453	0.835

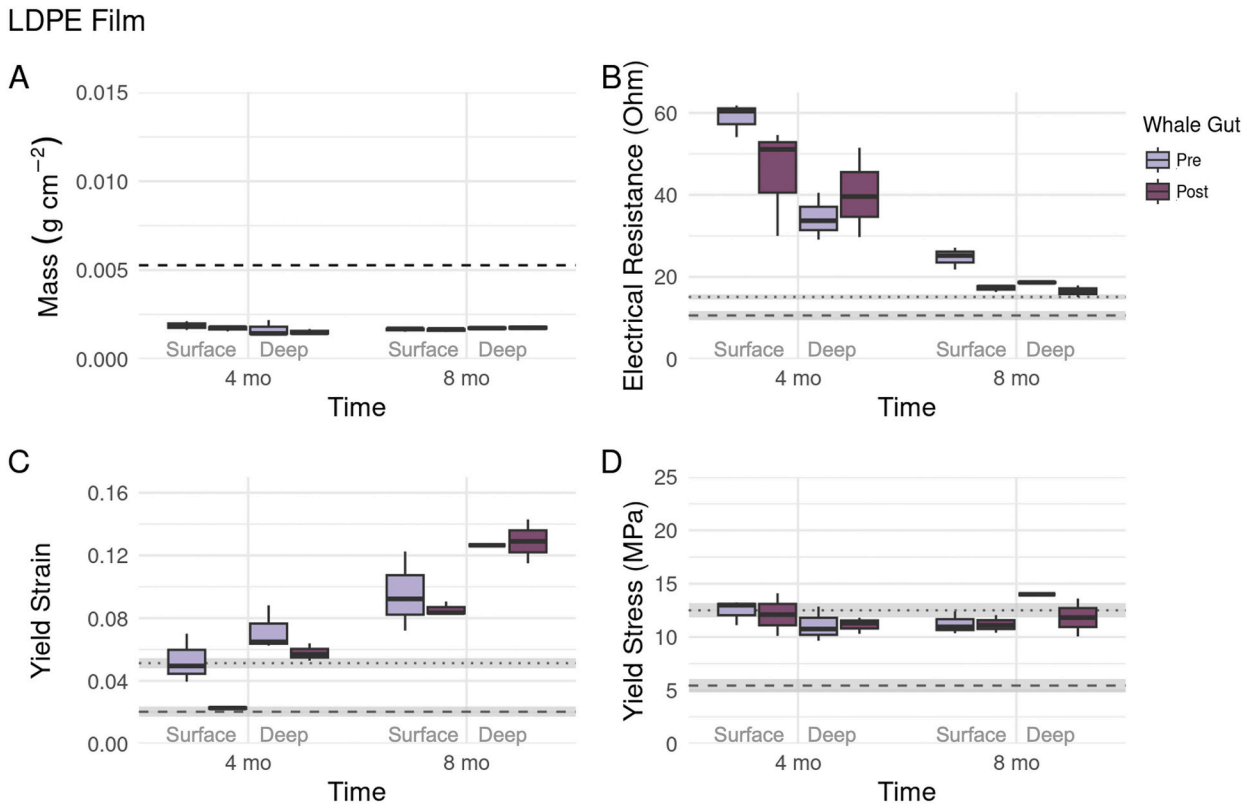

Fig. 3. Low Density Polyethylene (LDPE) film measurement averages for A) mass, B) electrical resistance, C) yield strain, and D) yield stress as a function of weathering time, weathering depth and whale gut simulation. Boxes represent upper and lower quartiles, where solid lines within boxes represent median values. Dashed line represents pre- weathering and whale gut exposure samples (0 month); Dotted line represents pre-environmental and post-whale gut exposure samples (0 month); Gray shading represents SE.

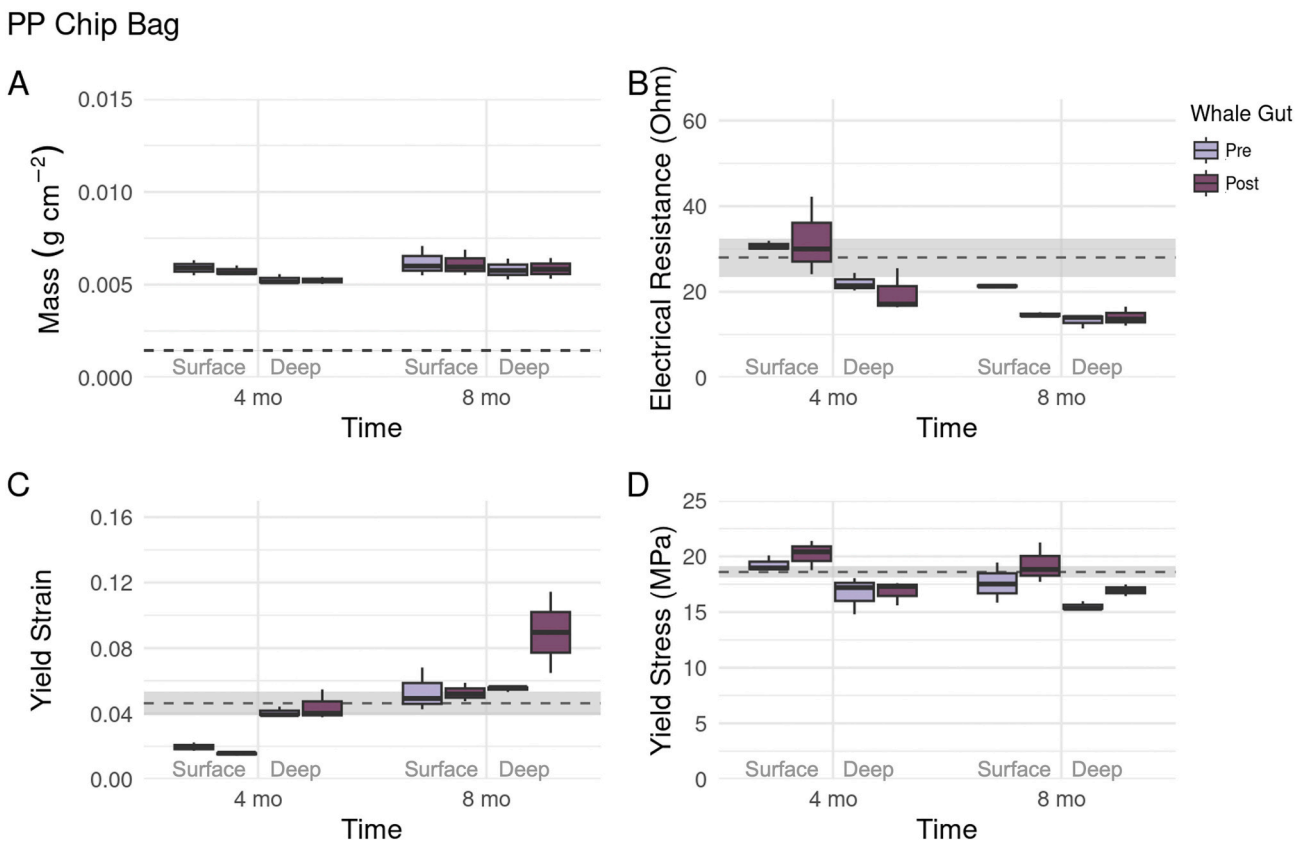

Fig. 4. Polypropylene (PP) chip bag measurement averages for A) mass, B) electrical resistance, C) yield strain, and D) yield stress as a function of weathering time, weathering depth and whale gut simulation. Boxes represent upper and lower quartiles, where solid lines within boxes represent median values. Dashed line represents pre- weathering and whale gut exposure samples (0 month; no statistical difference in pre- and post- whale gut samples) and gray shading represents SE.

3.4. Polyethylene (PE) bag

Pre-weathered PE bag mass averaged 0.006 ± 1.28 × 10^{-4} g cm^{-2} (Fig. 5a). Whale gut exposure did not affect mass, electrical resistance, strain, or stress in pre-weathered samples ($p > 0.06$; *t*-test). Mass was dependent on weathering depth and time ($p = 0.012$ and $p = 0.007$, respectively), where mass was 8 % greater at the surface and across time, but was not dependent on whale gut simulation, nor the interaction ($p > 0.38$; 3-way ANOVA; Table 1). Pre-weathered PE bag electrical resistance averaged 20 ± 1.9 Ohms (Fig. 5b). Electrical resistance was dependent on the interaction between depth and the whale gut simulation ($p = 0.008$; 3-way ANOVA; Table 1), where surface samples weathered for 8 months had highest resistance. When examining tensile properties of the PE bag, pre-weathered yield strain and yield stress averaged 0.025 ± 0.004 MPa and 4.01 ± 0.54 MPa, respectively (Fig. 5c,d). Yield strain was dependent on the interactions of weathering depth and time as well as weathering depth and whale gut simulation ($p = 0.021$ and $p = 0.004$, respectively; 3-way ANOVA; Table 1). Increased yield strain was observed between surface and deep depth samples at 4 months, but the difference dissipated at 8 months. Yield stress was dependent on the interaction of depth and time ($p = 0.0245$; 3-way ANOVA; Table 1), where samples at deep depth had higher stress at 4 months and lower stress than surface samples at 8 months.

3.5. High density polyethylene (HDPE) thick film

Pre-weathered HDPE thick film mass averaged 0.009 ± 6.82 × 10^{-5} g cm^{-2} (Fig. 6a). Whale gut exposure affected electrical resistance ($p = 0.03$) but not mass, strain, or stress in pre-weathered samples ($p < 0.1$; *t*-test). HDPE thick film mass was not dependent on weathering depth, time, whale gut simulation, nor the interaction ($p > 0.14$; 3-way ANOVA; Table 1). Pre-weathered HDPE thick film electrical resistance averaged 23.5 ± 2.54 Ohms (Fig. 6b). Electrical resistance was dependent on the interaction between weathering depth and time ($p = 5.32 \times 10^{-7}$; 3-way ANOVA; Table 1). Electrical resistance was highest in surface samples at 4 months, and decreased over time. One replicate of HDPE thick film from 4 months, surface depth was too crinkled to undergo tensile tests. When examining the tensile property of HDPE thick film, pre-weathered yield strain and yield stress was 0.014 MPa and 3.47 MPa (only 1 replicate present), respectively (Fig. 6c,d). Yield strain was dependent on weathering time ($p = 0.042$), and was not dependent on depth, whale gut simulation, nor the interactions ($p > 0.09$; 3-way ANOVA; Table 1). Yield stress was not dependent on weathering depth, time, whale gut simulation, nor the interaction ($p > 0.06$; 3-way ANOVA; Table 1).

3.6. BPA levels

BPA was detected in all aqueous solution samples at all depths except the pre-weathered PP chip bag (Supplemental Fig. 3). However, BPA levels for all samples were well below predicted no-effect concentration (PNEC) levels, thus we did not consider this variable to be significant in the final interpretation and evaluation of the materials' toxicity as they degrade.

4. Discussion

Investigating degradation of common commodity plastic films weathered in temperate marine environments is important, as ocean-bound mismanaged plastic waste is currently high and expected to increase, thus increasing the risk of ingestion by marine mammals (Eriksen et al., 2023). None of the five types of common commodity plastics

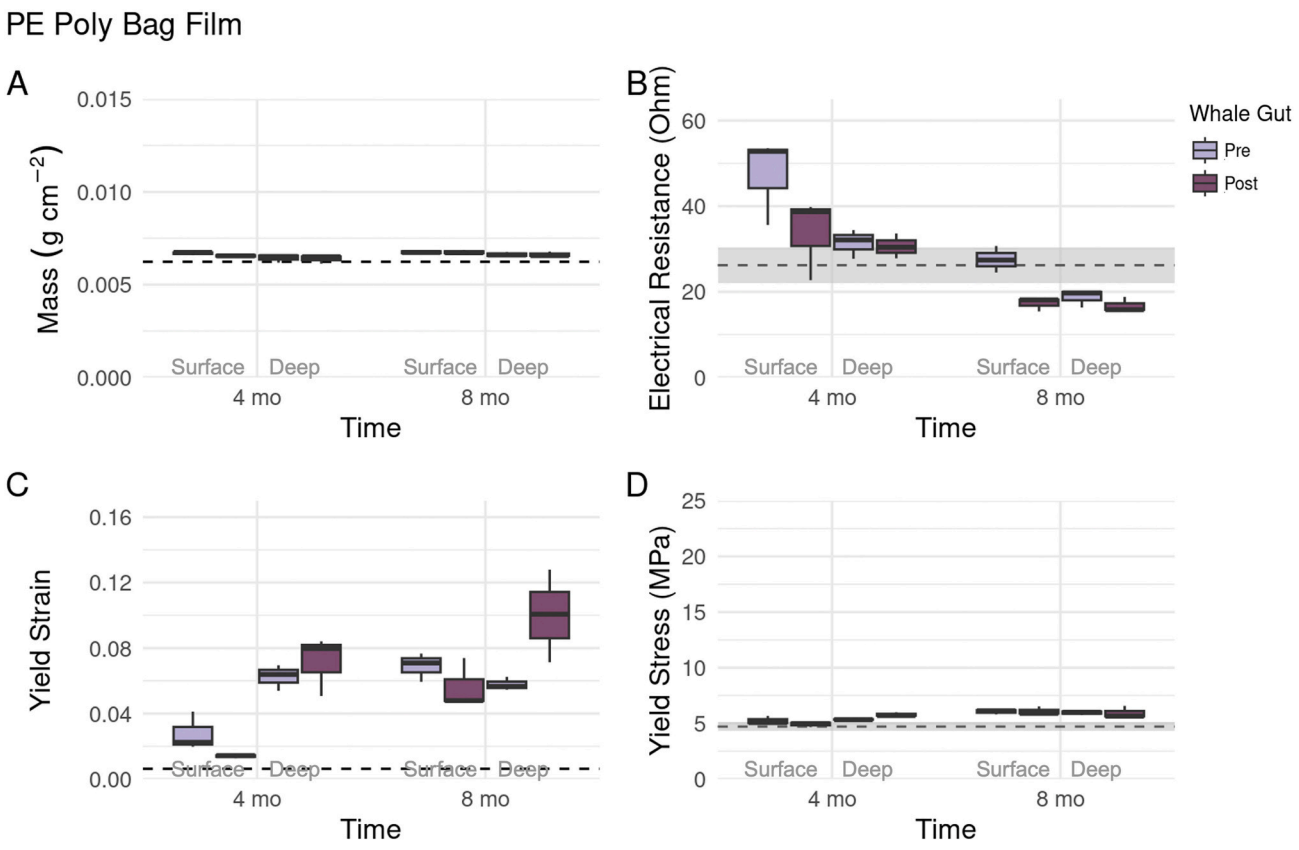

Fig. 5. Polyethylene (PE) poly bag measurement averages for A) mass, B) electrical resistance, C) yield strain, and D) yield stress as a function of weathering time, weathering depth and whale gut simulation. Boxes represent upper and lower quartiles, where solid lines within boxes represent median values. Dashed line represents pre- weathering and whale gut exposure samples (0 month; no statistical difference in pre- and post- whale gut samples) and gray shading represents SE.

studied here fully degraded over 8 months. While many physical attributes of the plastic films changed over time and across depth, few changed when exposed to a simulated whale gut.

Weathering depth was a significant factor when considering mass of HDPE thin film, PP chip bag, and PE poly bag samples, where samples weathered at the surface had greater mass at both time points than samples weathered at the deep depth. The PP chip bag gained the most mass from pre-weathering (73 %) while LDPE was the only sample to lose mass from pre-weathering (−64 %). Additionally, all plastic samples lost little to no mass ($p < 0.05$; t-test) from pre to post- whale gut simulation, indicating very little, if any, degradation occurred once ingested. These findings align with prior research that some plastics (LDPE, HDPE, PET, PE, and PP) lose minimal weight when exposed to marine conditions over the span of 6–12 months, decreasing just 0.5–2.5 % of mass (Sudhakar et al., 2007; Gerritse et al., 2020). In some cases, mass can increase in short weathering times due to oxygen incorporation and biofouling (Sudhakar et al., 2007). In the present study we removed biofouling and all macro-organisms from weathered plastics, but did not perform any chemical methods to remove residual biofouling, which likely resulted in mass increase over time (Fig. 1).

Visually, all types of plastics experienced some level of biofouling across weathering time and depths, where the extent of biofouling was variable between plastic types. In all samples, the whale gut simulation removed additional visual microorganism biofouling from the surface that we were unable to manually remove (Fig. 1; observation), indicating biologic agitation and breakdown occurred during the laboratory portion of this study (confirmed with Kraft paper control tests). Mass did not change across any of the plastic types when exposed to the whale gut exposure, indicating that while additional biofouling microorganisms were removed, the initial cleaning was consistent across samples, corroborating the measurements.

Electrical resistance significantly decreased from surface to deep depths and from 4 to 8 months across all sample types. Electrical resistance was often lowest after 8 months of weathering at deep depth (Figs. 2–6). Electrical resistance was the only response variable observed to be impacted by exposure to the gut simulation, where gut exposure generally decreased electrical resistance in PE poly bags and HDPE thick film. As plastic weathers in marine environments, pores and crevices fill with sea water and biofilm, causing the electrical resistance of the plastic to decrease (Gerritse et al., 2020). Here, we found that electrical resistance tended to decrease over time, from 4 months to 8 months, for both depths, where deep samples had generally lower resistance than surface samples (Figs. 2–6). Further, pre-weathering and pre-whale gut samples had higher electrical resistances than post-whale gut samples, indicating that both weathering as well as whale gut simulation degraded the plastics (and thus decreasing electrical resistance).

Plastics in this study, while not fully degraded, often experienced tearing or fragmentation from 8 months of weathering. These physical changes resulted in the inability to accurately test the tensile properties of some replicates. Plastics weathered for 8 months at deep depth were most affected by this, and as a result, there are fewer measurements at that time point, both pre- and post- whale gut simulation. With few replicates, we observed yield strain was dependent on depth in all sample types except HDPE thick film as well as weathering time for all sample types. Compared to pre-weathered samples, yield strain increased in samples exposed to the whale gut simulation across all types of plastic (Fig. 2–6). Conversely, once weathered for either 4 or 8 months, change in yield strain pre- to post- whale gut simulation was minimal. From 4 to 8 months of weathering, yield strain generally increased across both depths. We often observed that replicates weathered for 4 months at the deep depth were similar in strain responses to replicates weathered at 8 months at the surface (except HDPE thick

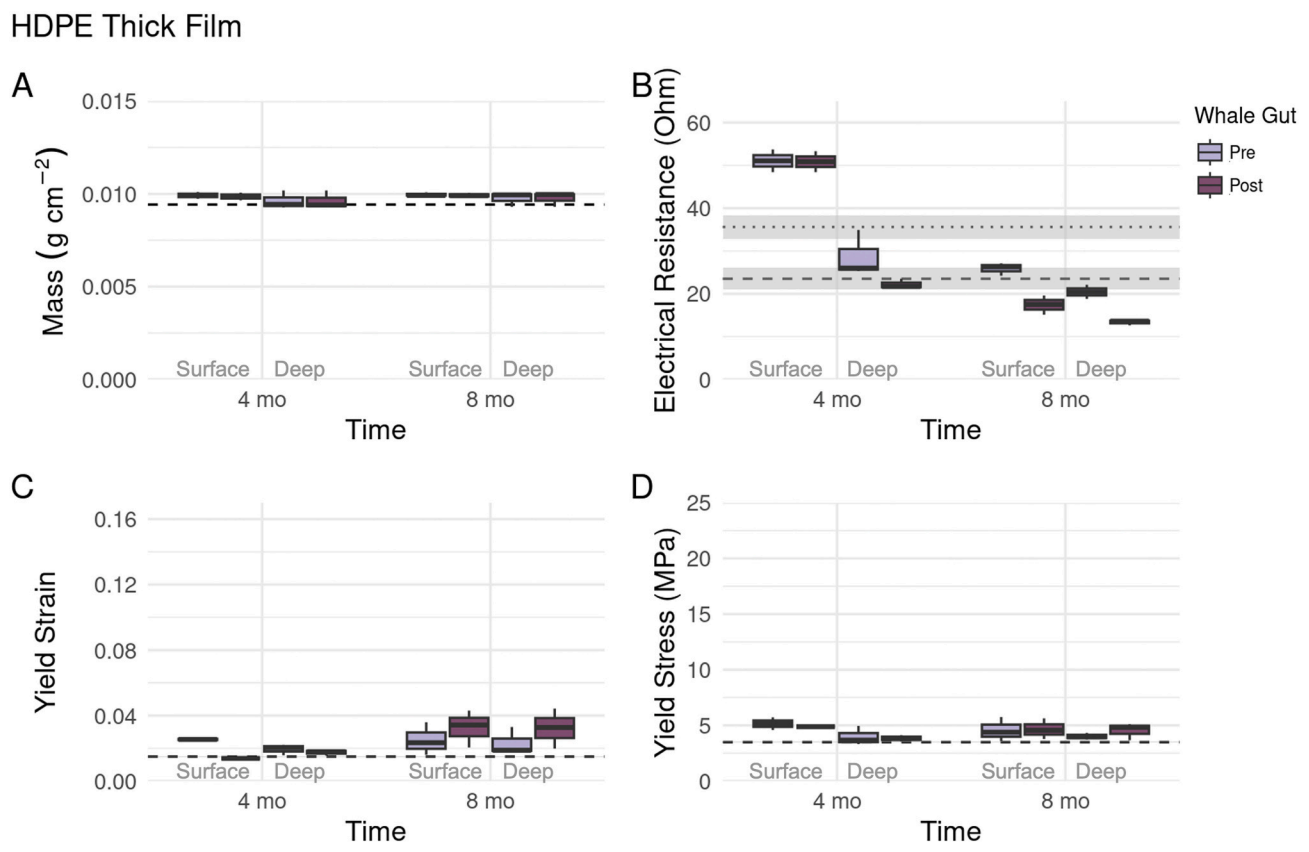

Fig. 6. High Density Polyethylene (HDPE) thick film measurement for A) mass, B) electrical resistance, C) yield strain, and D) yield stress as a function of weathering time, weathering depth and whale gut simulation. Boxes represent upper and lower quartiles, where solid lines within boxes represent median values. Dashed line represents pre- weathering and whale gut exposure samples (0 month); Dotted line represents pre-environmental and post-whale gut exposure samples (0 month); Gray shading represents SE.

film). Higher yield strain indicates the materials become more extensible during elastic deformation when exposed to environmental conditions. Yield strain and electrical resistance appear to have an inverse relationship, where yield strain increased over time and electrical resistance decreased over time. This is the opposite trend that we expected, where a decrease in strain correlates with more brittle structure (Karlsson et al., 2018). Yield stress did not significantly change when weathered plastics were exposed to the whale gut simulation. Yield stress for both HDPE (thick and thin) and PP poly-bag samples was dependent on depth, where samples weathered at the surface depth reported higher yield stresses.

Plastics weathered for 8 months had more biofouling present immediately upon extraction than those weathered for 4 months. In addition to depth and time, biofouling accumulation can be dependent on the chemical composition of film plastic (Nelson et al., 2021; Sudhakar et al., 2007). Biofouling may have delayed plastic degradation by protecting surfaces of plastics from UV radiation, wave action, and other mechanical degradation forces. Without biofouling, we would expect samples weathered at the surface to have the most exposure to mechanical degradation factors that affect physical properties (Binda et al., 2023; Fazey and Ryan, 2016; Gerritse et al., 2020; Nelson et al., 2021; O'Brine and Thompson, 2010; Tosin et al., 2012; Sudhakar et al., 2007). Biofouling organisms, however, block UV rays from reaching the film surface, therefore prolonging photodegradation that would otherwise affect films' properties (O'Brine and Thompson, 2010; Zhang et al., 2021; Binda et al., 2023).

Biofouled plastic films weathered at the surface were exposed to less UV radiation than expected, perhaps explaining why electrical resistance was often lower in samples weathered at the deep depth, indicating more degradation. Decreased electrical resistance over time could be due to an increased wettability causing more ocean water to remain in samples after the 24 h drying period (Gerritse et al., 2020). Similarly, biofouling may explain why we saw an inverse relationship with electrical resistance and yield strain, two metrics that should mirror one another. If biofouling organisms are blocking UV rays but still allowing water and biofilm to fill pores and crevices (and increasing wettability), some aspects of physical degradation would increase while others remain relatively steady. In future studies we recommend quantifying biofouling on all samples.

All types of commodity plastics studied experienced little to no measured change when exposed to a simulated whale gut (Table 1; Figs. 2–6). These findings support the multitude of stories, both popular as well as scientific, we hear about plastic filling whales' stomachs. If unable to degrade commodity plastics through digestion, whales risk long-term exposure to the physical and chemical attributes of plastics within their stomachs. Beyond the physical damage plastics cause such as gut blockage, plastic additives have the opportunity to leach chemicals into animals for longer periods of time. While the concentration of BPA was under the level of detection in this study, there is a possibility that with extended exposure, chemicals could build up to harmful concentrations. This study, in combination with ubiquitous global plastic pollution, poses an interesting thought experiment: When is the optimal time to eat plastic in the Anthropocene? For different animals and feeding strategies, we imagine the answer to vary. For whales, it appears plastics weathered at the surface for long periods of time have degraded the most and may pose the least physiological risk.

It should be noted that many elements of the experiment were designed as part of the TOM FORD Plastic Innovation Prize, with the intention of testing degradability of novel plastic alternatives submitted to the competition. Results from the materials submitted to the

competition are proprietary and could not be included in this analysis, thus common commodity plastics were included for a baseline comparison.

The TOM FORD Plastic Innovation Prize testing period lasted 8 months, and thus weathering of commodity plastics studied here followed the same methodology and weathering times. The authors believe that 8 months, while short in the lifespan of plastics, is a realistic and adequate time frame to study the initial degradation of plastic films in the environment and potential implications for marine mammals. Longer weathering experiments (over the course of several years) should be conducted to better assess the longevity of plastic and additional, long-term implications on marine systems. As part of this study, we used a simplistic version of a mammalian digestive system which successfully degraded Kraft paper, however, in future studies this set-up should be improved to more closely mimic a gray whale digestive system. Using a more accurate stomach model would more closely simulate the complicated forces present in a whale's stomach, as well as be made of a material closer in texture to that of a whale's fundic chamber (Kong and Paul Singh, 2010; Liu et al., 2021; Olsen et al., 1994). Additionally, we recommend studying stranded Cetaceans to better understand the interaction of plastics and the physicochemical conditions of the stomachs. In future studies, we recommend performing a parallel liquid or gas chromatography/mass spectrometry (LC or GC/MS) analysis on samples, another method of analysis commonly used to measure BPA concentrations (Fukata et al., 2006).

The Seattle Aquarium is committed to continuing research under our Clean Waters program to support the further development of such testing and to expand our collective understanding of the impacts of plastic pollution on the marine environment. It will take commitment from all sectors, (government, non-profit, academia, and industry), to face the threat of plastic pollution and reduce its grave effects on marine environments.

5. Conclusion

This study set out to answer two questions: 1) How do single-use plastic films degrade across time and depths in temperate waters and 2) How do environmentally weathered single-use plastic films change when exposed to simulated whale stomachs? Changes in mass, electrical resistance, yield stress, and yield strain indicate that over the course of 8 months, the five types of plastic films studied here experienced little degradation at either surface or deep depths in temperate waters. Additionally, weathered single-use plastic films had little to no measured change when exposed to a simulated whale gut.

Our results support what we have known for decades - that single-use commodity plastic films do not readily degrade in marine environments or in whale guts. None of the plastics in this study fully degraded, however, many experienced tearing or fragmentation over the weathering time, which can lead to smaller plastic particles that may be more bioavailable to other organisms. Certification standards should begin to incorporate similar test methods for evaluating the impacts of materials on marine life to support more comprehensive and holistic insights for policymakers, corporations, and the public to better understand their impacts of materials on the environment. Understanding characteristics and physical attributes of environmental plastic persistence becomes more vital as the amount of plastic pollution entering our oceans increases every year. As we seek to understand, we must simultaneously decrease the amount of plastic produced and subsequently entering the oceans.

CRediT authorship contribution statement

Jackson Fennell: Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Amy Y. Olsen:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Veronica Padula:** Writing – review & editing, Writing – original draft, Investigation. **Noah Linck:** Writing – review & editing, Investigation. **Alyssa Lind:** Writing – review & editing, Writing – original draft, Investigation. **Lael Newton:** Investigation. **Emily Carrington:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Investigation, Formal analysis. **Tim Silman:** Writing – review & editing, Writing – original draft, Resources, Project administration. **Lyda S.T. Harris:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors 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

The datasets analyzed for this study can be found in GitHub. http://github.com/greenamy808/Seattle_Aquarium_WhaleGut

Acknowledgements

We recognize this work was conducted on ceded and unceded lands and waterways of the Coast Salish peoples. This research was funded by Lonely Whale through the support of Nike and The Echo Fund. Special thanks go to the Seattle Aquarium Conservation Programs and Partnerships volunteers for assisting with data collection. Thanks to Bob Kiel for his help designing and building the rig for environmental exposure and to Shawn Larson for her help throughout the project. Thank you to Evan White at the University of Georgia New Materials Institute for the testing guidance and assistance with sample preparation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2024.116788.

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