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Plastic Debris in the Aquatic Environment

FROM MACROPLASTIC TO MICROPLASTIC: DEGRADATION OF HIGH-DENSITY POLYETHYLENE, POLYPROPYLENE, AND POLYSTYRENE IN A SALT MARSH HABITAT

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Abstract: As part of the degradation process, it is believed that most plastic debris becomes brittle over time, fragmenting into progressively smaller particles. The smallest of these particles, known as microplastics, have been receiving increased attention because of the hazards they present to wildlife. To understand the process of plastic degradation in an intertidal salt marsh habitat, strips (15.2 cm × 2.5 cm) of high-density polyethylene, polypropylene, and extruded polystyrene were field-deployed in June 2014 and monitored for biological succession, weight, surface area, ultraviolet (UV) transmittance, and fragmentation. Subsets of strips were collected after 4 wk, 8 wk, 16 wk, and 32 wk. After 4 wk, biofilm had developed on all 3 polymers with evidence of grazing periwinkles (*Littoraria irrorata*). The accreting biofilm resulted in an increased weight of the polypropylene and polystyrene strips at 32 wk by 33.5% and 167.0%, respectively, with a concomitant decrease in UV transmittance by approximately 99%. Beginning at 8 wk, microplastic fragments and fibers were produced from strips of all 3 polymers, and scanning electron microscopy revealed surface erosion of the strips characterized by extensive cracking and pitting. The results suggest that the degradation of plastic debris proceeds relatively quickly in salt marshes and that surface delamination is the primary mechanism by which microplastic particles are produced in the early stages of degradation. *Environ Toxicol Chem* 2016;35:1632–1640. © 2016 SETAC

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INTRODUCTION

Plastic debris represents one of the top anthropogenic threats to estuarine environments [1], and it is an emerging issue that may affect our ability to maintain biodiversity and community structure in these habitats. With predictions of the increased growth in coastal communities and increased production of consumer plastics in the next 10 yr, plastic debris in estuaries could increase substantially. In 2013, 29.9 million metric tons of plastic waste was generated in the United States, representing 12.8% of the total municipal solid waste; but only 9% of the total plastic generated was recovered for recycling [2]. Assuming that 2% of the municipal solid waste is mismanaged, Jambeck et al. [3] estimates that between 40 000 metric tons and 110 000 metric tons of plastic debris enters US coastal waters every year. The largest generators of this mismanaged plastic waste are believed to be urban, industrial, and recreational activities occurring in and adjacent to coastal and riparian zones [4].

Due to their chemical inertness, plastics are generally resistant to degradation, and estimates for the complete degradation of plastic debris in the environment range from decades to centuries [5]. Degradation is the result of chemical changes in the structure of the polymer that reduce its molecular weight, thereby invariably weakening the mechanical integrity of the plastic (reviewed by Singh and Sharma [6]). Plastics exposed to sunlight undergo photo-oxidation as a result of the absorbance of high-energy wavelengths of the ultraviolet (UV) spectrum by the polymers [6]. Once degradation is initiated, it can proceed through temperature-dependent thermo-oxidative reactions without further exposure to UV radiation, as long as oxygen is available [7]. Other degradative mechanisms, including hydrolysis and biodegradation, occur at rates in the environment several orders of magnitude slower than the oxidative mechanisms [7]. It has been suggested that extensively degraded plastics eventually become brittle and disintegrate, fragmenting into progressively smaller microscopic particles, known as microplastics [5,8].

Because environmental conditions such as sunlight, temperature, and oxygen play a critical role in the degradation of plastic, the rate at which it degrades will largely be dependent on its location. For example, polyethylene films and thermoplastic materials were found to degrade at slower rates when submerged in seawater compared with being in the air, probably as a result of lower sunlight exposure, lower temperatures, and lower oxygen levels [9,10]. Other location-specific factors, such as presence of mechanical forces or surface biofouling, could also alter the rate of degradation [7,8]. Mechanical forces, such as those associated with turbulence, abrasion, and wave action, could increase the susceptibility of plastics to fragmentation [8,11]. Surface biofouling, such as that associated with biofilm and colonizing invertebrates, may reduce the rate of plastic degradation by reducing the amount of UV radiation reaching the surface of the plastic, while simultaneously increasing the density of the object, causing it to sink [12].

The shallow intertidal salt marshes and associated tidal creeks of southeastern US estuarine systems are physically dynamic habitats, characterized by cyclical fluctuations in dissolved oxygen, UV radiation, temperature, and salinity largely driven by diurnal tides having a 1.5 m to 3.0 m range [13]. These habitats are considered to be primary nursery areas for many ecologically and commercially important species of fish and shellfish [14,15]. Salt marsh–tidal creek habitats serve as the hydrographic link between anthropogenic activities in the upland portion of the watershed and the adjacent estuary [16], and as such, higher levels of plastic debris have been associated with salt marshes occurring near population

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centers [17]. Although plastic debris can be found in all habitats associated with these systems, the highest abundances can be found in the high marsh associated with natural debris in strand lines known as tidal wrack [17].

Although there is a growing body of literature available on the abundance of microplastics in estuarine environments, there is little information concerning the rate of plastic degradation and the process by which microplastic particles are produced. Therefore, the main objective of the present study was to examine the overall degradation of 3 common plastic polymers (high-density polyethylene, polypropylene, and extruded polystyrene) in a typical salt marsh habitat. In a recent survey of Charleston Harbor SC, USA), it was found that these 3 polymers comprised 55% of the total plastic debris along the shoreline (H. Wertz, 2015, Master's thesis, College of Charleston, Charleston, SC, USA). A second objective was to examine how biofouling influenced the intensity of UV radiation reaching the surface of the plastic. Finally, to understand the process by which microplastics are produced, plastics were subjected to a fragmentation test and scanning electron microscopy (SEM).

MATERIALS AND METHODS

Three types of plastic strips (15.2 cm × 2.5 cm) were compared: high-density polyethylene, polypropylene, and extruded polystyrene. The high-density polyethylene and polypropylene strips were cut from PailSaver™ pail inserts (5 gallon; CDF). The average thickness of strips from both polymer types was 0.38 mm. Extruded polystyrene strips were cut from The Home Store™ Foam Plates purchased from a local discount variety store. Foam plates had a diameter of 22.5 cm and weight of 3.85 ± 0.07 g (mean ± standard deviation). The average thickness of the polystyrene strips was 2.0 mm. The high-density polyethylene and polypropylene pail inserts were uncolored (e.g., natural), and the polystyrene foam plates were white. All 3 products used US Food and Drug Administration–approved plastics, although we have no knowledge regarding the use of additives in these products.

Field-deployed plastic strips were attached to 1 of 5 sample apparatuses, each consisting of a wooden board (152.4 cm × 15.2 cm × 2.5 cm; length × width × height) with 60 perpendicularly oriented wooden dowels (1.1 cm diameter, 12.7 cm height), arranged in 2 rows of 30 dowels each. The 2 rows of dowels were each located 2.5 cm from the lengthwise edges of the board and 10.2 cm apart from each other. A plastic strip (30 total strips per apparatus, 10 of each tested polymer type) was attached at either end to a wooden dowel (1 from each row) with a finishing nail, such that plastic strips were arranged horizontally to the wooden board. In this manner, a total of 150 plastic strips (50 of each polymer) were attached. Each apparatus was buried in a shallow trench in an intertidal salt marsh in the Ashley River adjacent to The Citadel campus (Charleston, SC, USA, 32°47′45.44″N, 79°57′50.65″W), such that the plastic strips were horizontally positioned approximately 8 cm above the surface of the marsh (Figure 1). The apparatuses were deployed in close proximity to each another on 13 June 2014 in an area of the marsh devoid of shade (i.e., overhanging trees or smooth cordgrass [*Spartina alterniflora*]). During each tidal cycle, the plastic strips were submerged at high tide for approximately 6 h and exposed at low tide for approximately 6 h. Plastic strips (10 of each polymer type) were randomly removed from the apparatuses and brought back to the laboratory on 11 July 2014, 8 August 2014, 3 October 2014, and 23 January 2015, which represented 4-wk, 8-wk, 16-wk, and 32-wk exposure periods, respectively. Control strips (0 wk) were not field-deployed. Observations of the condition of the field-deployed strips were also made following 2 wk of deployment on 27 June 2014, but no strips were removed at that time.

Figure 1. Photograph of field exposure apparatus in the salt marsh 2 wk after deployment.

Following removal from the field, all 10 replicate strips for each polymer type at each sampling time were examined for biofilm development, salt marsh periwinkle (*Littoraria irrorata*) grazing, and the presence of encrusting organisms. Five of the replicate strips were then used to quantify surface area, weight, and UV transmittance, whereas 4 of the replicate strips were used to quantify fragmentation. The remaining replicate strip was either archived or used for SEM. To measure surface area, plastic strips were mounted to a camera and photographed, and the digital images were examined using ImageJ software (National Institutes of Health). To measure weight, plastic strips (with biofilm and encrusting organisms) were weighed using a Mettler-Toledo PB153-S balance. The biofilm and encrusting organisms were then removed from 1 side of the strip, and the strips were analyzed for UV transmittance (details below). The biofilm and encrusting organisms were then removed from the other side of the strip, and the strips were weighed a second time.

To determine the transmittance of UV radiation and visible light through the plastic strips together with any biofilm that had developed on the upper side of the strip, a Macam Photometrics model UV-203 IP-67 radiometer was used to measure UV-A (332–406 nm), UV-B (292–330 nm), and visible light (400–710 nm). Plastic strips were individually placed directly on top of the appropriate UV or visible light sensor with the biofilm cleaned side of the strip facing toward the sensor. Measurements were made outdoors on The Citadel campus using ambient light conditions on 9 July 2015 between 10:00 and 12:00 Eastern Daylight Time. Conditions were mostly sunny with <10% cloud cover. During this period, ambient irradiance levels were as follows: UV-A = 51.4 ± 6.6 W/m^2, UV-B = 2215 ± 416.1 mW/m^2, and visible = 452.4 ± 50.7 W/m^2.

To determine fragmentation, plastic strips (not cleaned of biofilm) were subjected to a laboratory fragmentation test. Strips were individually placed in a 4-L amber jug filled with 1 L filtered brackish seawater (salinity = 28‰) and gently rolled on a Wheaton Roller Culture Apparatus at 3 rpm for 48 h. Following 48 h, the plastic strip was removed and the seawater poured through 500-μm, 150-μm, and 63-μm sieves. Particles retained on sieves were enumerated and characterized using a dissecting scope.

To confirm the composition of microplastic particles from the laboratory fragmentation test, a subset of collected particles was identified using a Bruker ALPHA Fourier transform

infrared spectrometer. Specifically, the spectrum of selected microplastic particles ($n = 16$) was compared to that of their respective control (week 0) plastic polymer strips (polystyrene, polypropylene, and high-density polyethylene) using the Quick Compare function in OPUS Spectroscopy Software (Ver 5) to determine a percentage of correlation.

For SEM, preparations from control plastic strips and those collected at 8 wk postdeployment were coated with approximately 1.5 nm of platinum using a Denton Sputter-Edge Coater. Samples were then examined on a JEOL 5600LV SEM.

Data on effects of exposure period on surface area, weight, percentage of UV or visible light transmittance, and microplastic particle abundances, sizes, and shapes for each polymer type were compared by a repeated measures analysis of variance (ANOVA) using SAS Enterprise 4.3 (SAS Institute). Differences among exposure times for weight, surface area, light transmittance, and particle characteristics were determined using least square mean comparisons with a Bonferroni adjustment. Homogeneity of variance was examined using Bartlett's test. Percentage of UV/visible light transmission data were arcsine square root–transformed, and particle characteristics (total abundance, size fractions, and shapes) were log-transformed to meet the assumptions of normality and homogeneity of variance.

RESULTS

All 120 deployed strips were successfully recovered from the salt marsh. Biofilm was observed on all samples after 2-wk exposure. Microscopic examination of the samples collected after 4 wk found that all were coated with a biofilm, which, at least qualitatively, became thicker over time. Salt marsh periwinkles (*L. irrorata*) were observed grazing on the surface of some strips after only 2 wk of exposure. Microscopic examination of the strips revealed that periwinkle grazing trails and feces were evident on all polymer types at all exposure periods but were more frequently found on the polystyrene samples (Figure 2A). Between 37.5% and 62.5% of the polystyrene strips collected between 4 wk and 32 wk, respectively, showed evidence of periwinkle grazing, whereas <30% of the polypropylene and high-density polyethylene strips showed the same evidence. At 32 wk, oysters (*Crassostrea virginica*) and barnacles (unidentified species) had settled on 30% and 40% of the collected polystyrene strips, respectively. There was no evidence of oysters or barnacles settling on the polystyrene strips at exposure periods less than 32 wk or on any of the polypropylene and high-density polyethylene strips collected at any exposure period.

During the 32-wk exposure period, visual inspection of the strips revealed that there was no evidence of fragmentation of any of the collected strips. A repeated measures ANOVA comparing surface area over time did not produce significant models for either high-density polyethylene ($p = 0.1081$) or polystyrene ($p = 0.2139$; Figure 2B). The repeated measures ANOVA model was significant for polypropylene ($p = 0.0329$); however, least square means analysis was not able to delineate any significant change in surface area relative to that of the controls for any exposure period.

The weight of the polypropylene and polystyrene strips progressively increased over time as a result of the accretion of biofilm and, in the case of the 32-wk polystyrene strips, the encrusting organisms (polypropylene, $p = 0.00276$; polystyrene, $p < 0.0001$; Figure 2C). Significantly increased weight of the polystyrene and polypropylene strips relative to the controls was evident at 4 wk and 16 wk, respectively. At 32 wk, the weight of the polypropylene and polystyrene strips had increased by 33.5% and 167.0%, respectively. By contrast, the weight of the high-density polyethylene strips, even with the accretion of biofilm, did not increase over time ($p = 0.2201$). The weight of the cleaned high-density polyethylene and polypropylene strips did not increase over time ($p = 0.8558$ and 0.0860, respectively; Figure 2D). However, the weight of the

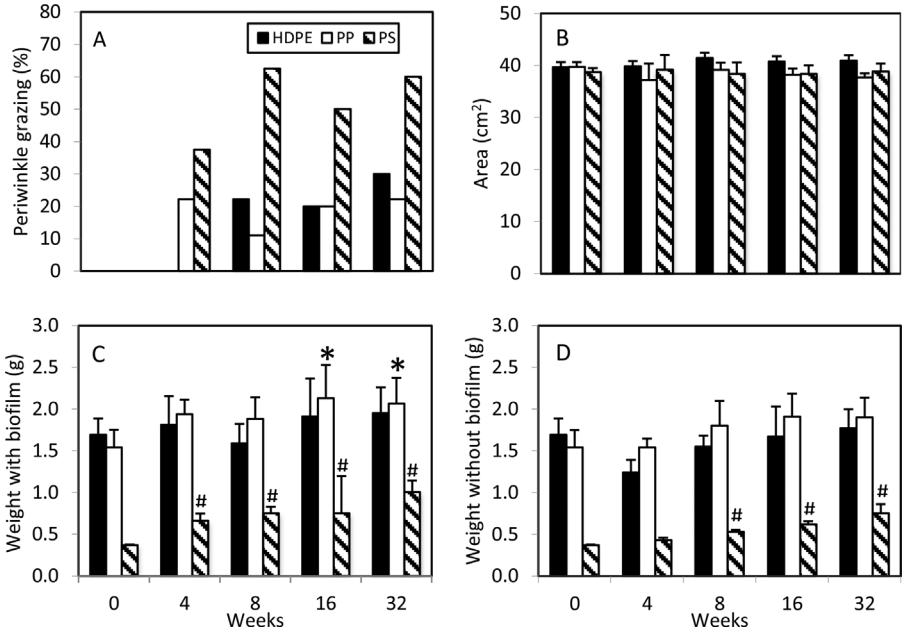

Figure 2. Percentage of strips with evidence of periwinkle grazing (**A**), surface area of strips (**B**), weight of strips with biofilm and encrusting organisms (**C**), and weight of cleaned strips (**D**) over a 32-wk exposure in a salt marsh. Significant differences in surface area and weight measurements relative to the week 0 strips (control) are denoted by * for polypropylene and # for polystyrene. There were no significant differences in any of these parameters for the high-density polyethylene strips. Mean ± standard deviation. HDPE = high-density polyethylene; PP = polypropylene; PS = polystyrene.

cleaned polystyrene strips did significantly increase with time ($p = 0.0156$), with significant increases evident at 8 wk. At 32 wk, the weight of these cleaned strips had increased 100.5% relative to that of the controls.

Transmittance of UV-A, UV-B, and visible light through the strips of all 3 polymers was negatively related to exposure period as a result of the accreting biofilm ($p < 0.0001$ for all 3 polymers; Figure 3). After only 4 wk of exposure, transmittance of both UV-A and UV-B radiation through these strips was approximately 95% less than that of the control strips. After 32 wk, transmittance of UV radiation was approximately 99% less than that of control strips. Transmittance of visible light through field-deployed strips was more variable. After 4 wk of exposure, transmittance of visible light was 76.2%, 92.0%, and 83.5% less for high-density polyethylene, polypropylene, and polystyrene, respectively. After 32 wk, transmittance of visible light was approximately 97.5% less for all 3 polymers relative to that of control strips.

The production of microplastic particles from field-collected strips in the laboratory fragmentation test was evident following 8 wk of exposure (Figure 4). The average number of particles produced by each strip in the 4-L amber jugs ranged from a low of 21.5 particles/strip for high-density polyethylene at 16 wk to a high of 75.8 particles/strip for polypropylene at 32 wk. For high-density polyethylene and polypropylene, significantly fewer particles were produced at 16 wk compared to 8 wk and 32 wk ($p = 0.0011$ and 0.0051, respectively; Table 1). The number of particles produced for polystyrene did not significantly vary among exposure periods ($p = 0.3642$). With regard to the number of particles produced at each size fraction, significantly more particles were produced in the 63-μm and 150-μm size fraction compared to that of the 500-μm size fraction for high-density polyethylene ($p = 0.0389$ and 0.0086, respectively; Figure 5 and Table 1). For polypropylene and polystyrene, the size fraction of the particles did not vary ($p = 0.7637$ and 0.7746, respectively). With regard to particle shape, high-density polyethylene and polystyrene produced significantly more fragments compared to fibers when pooled across all exposure times ($p < 0.0001$ and 0.0373, respectively; Figure 5 and Table 1). Particle shape did not significantly vary for polypropylene ($p = 0.0718$).

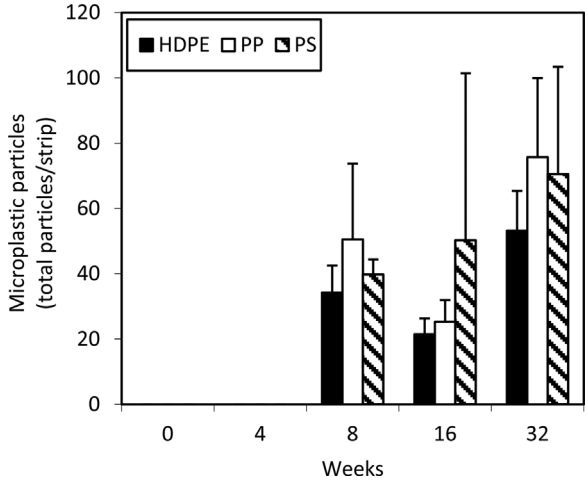

Figure 4. Abundance of microplastic particles produced in the laboratory fragmentation test of plastic strips deployed in a salt marsh over a 32-wk period. No microplastic particles were produced by the 0 wk (control) strips or the 4 wk strips. Mean ± standard deviation. HDPE = high-density polyethylene; PP = polypropylene; PS = polystyrene.

Table 1. Post hoc least square mean comparisons for microplastic particle characteristics from the laboratory fragmentation test: For each plastic type, the particles produced are compared by exposure period (8 wk, 16 wk, or 32 wk), size fraction (63 μm, 150 μm, or 500 μm), and shape (fragment or fiber)

Particle characteristic	Polymer	Comparisons
Exposure period (wk)	HDPE	16^a 8^b 32^c
	PP	16^a 8^b 32^b
	PS	8^a 16^a 32^a
Size fraction (μm)	HDPE	500^a 63^b 150^b
	PP	63^a 150^a 500^a
	PS	63^a 150^a 500^a
Shape	HDPE	$fiber^a$ $fragment^b$
	PP	$fragment^a$ $fiber^a$
	PS	$fiber^a$ $fragment^b$

Values sharing the same letter (a, b, or c) indicate that they were not significantly different ($p > 0.05$).
HDPE = high-density polyethylene; PP = polypropylene; PS = polystyrene.

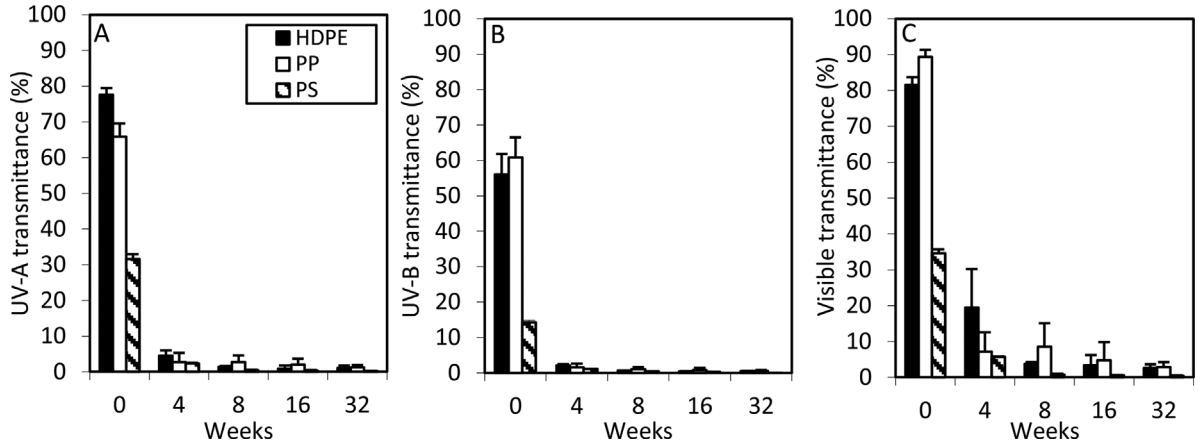

Figure 3. Percentage of transmittance of ultraviolet (UV)-A (**A**), UV-B (**B**), and visible light (**C**) through plastic strips over a 32-wk exposure in a salt marsh habitat. Percentage of transmittance was significantly reduced for all 3 plastic strips at week 4, week 8, week 16, and week 32 relative to week 0 strips (control). Mean ± standard deviation. HDPE = high-density polyethylene; PP = polypropylene; PS = polystyrene; UV = ultraviolet.

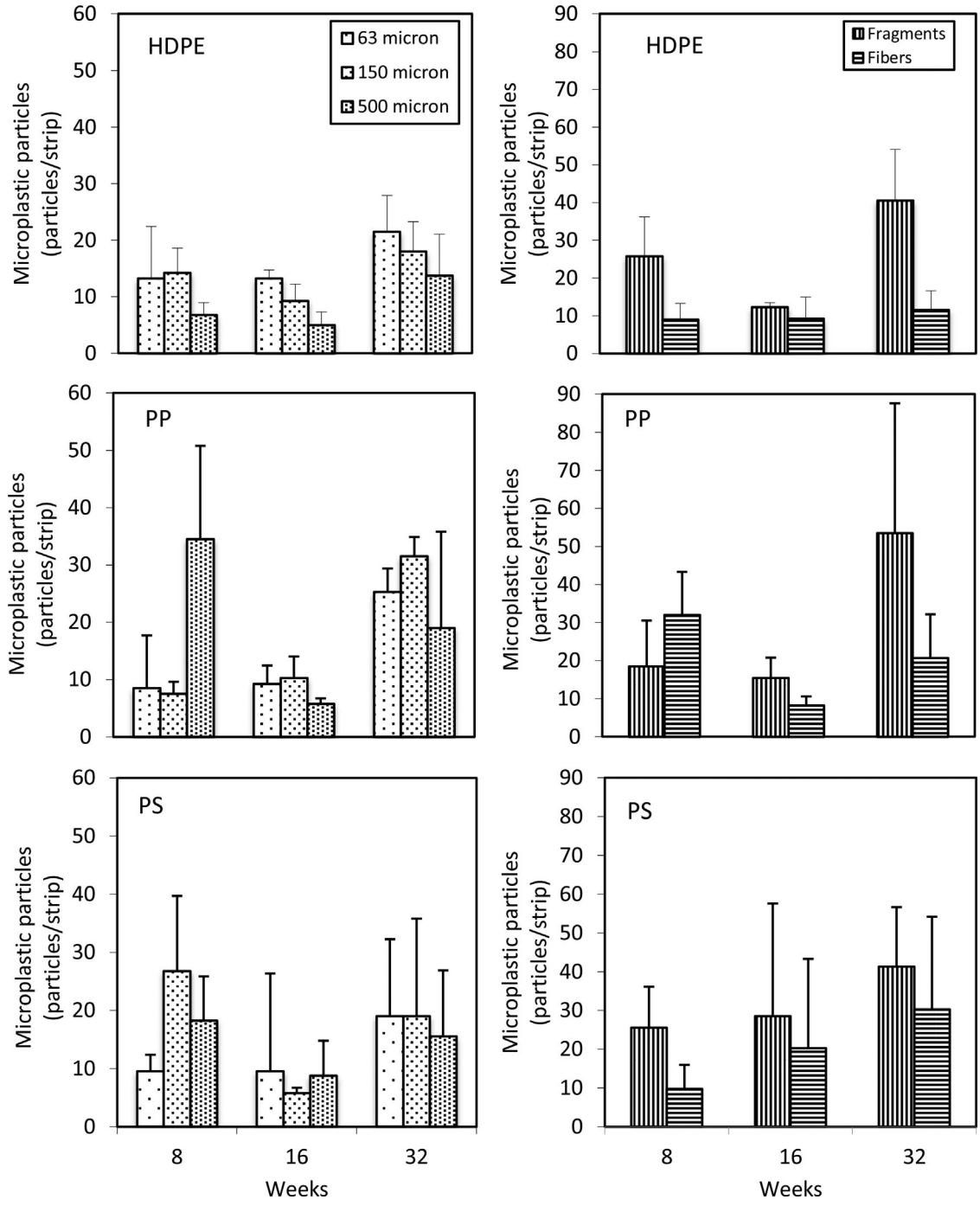

Figure 5. Microplastic particle sizes and shapes produced in the laboratory fragmentation test of plastic strips deployed in a salt marsh over a 32-wk period. No microplastic particles were produced by the 0 wk (control) strips or the 4 wk strips. Mean ± standard deviation. HDPE = high-density polyethylene; PP = polypropylene; PS = polystyrene.

There was a high correlation (>92%) between the Fourier transform infrared spectra of individual microplastic fragments collected in the laboratory from 8-wk, 16-wk, and 32-wk field-deployed plastic strips and that of their respective control polymer strips (Table 2). The average correlations for particles from the high-density polyethylene, polypropylene, and polystyrene strips were $97.34 \pm 1.76\%$, $95.82 \pm 2.65\%$, and $96.32 \pm 1.72\%$, respectively.

Examination by SEM of the surface of the control strips revealed a largely featureless and smooth surface for all 3 polymers (Figure 6A–C). Following 8 wk in the salt marsh, examination of the surface of strips from all 3 polymers revealed a dried biofilm consisting of an extracellular matrix containing numerous trapped pieces of pelagic and benthic diatoms (Figure 6D–F). When the biofilm was removed, the high-density polyethylene strip revealed a highly eroded surface with numerous pits, microcracks, and adhering material presumably associated with remnants of the biofilm (Figure 6G). The surface of the polypropylene strip exhibited numerous grooves and areas where it appeared the surface layer had been removed (Figure 6H). The surface of the polystyrene strip was also highly eroded with numerous pits and the presence of large cracks and

Table 2. Percentage correlation between the spectra of a subset of microplastic particles collected during the laboratory fragmentation test and those of their respective control strips (week 0) as determined by Fourier transform infrared analysis

Polymer	Sample number	Week collected	Fragment number	% Correlation to control
HDPE	127	8	1	97.48
	127	8	2	98.57
	127	8	3	98.49
	16	8	1	94.80
PS	41	8	1	96.04
	41	8	2	97.56
	38	8	1	96.89
	38	8	2	94.89
	38	8	3	93.34
	137	16	1	97.13
	98	32	1	98.38
PP	138	8	1	92.36
	138	8	2	98.20
	138	8	3	97.40
	78	16	1	97.55
	99	16	1	93.60

HDPE = high-density polyethylene; PP = polypropylene; PS = polystyrene.

areas where it appeared the surface layers were separating from the underlying layers (Figure 6I).

DISCUSSION

Although it is widely recognized that plastic degradation in the environment is slow and that it can take 50 yr or more for plastic debris to fully disintegrate [18,19], there is currently little information concerning when these processes become evident. We report that, at least in subtropical salt marshes, the degradation of 3 common plastic polymers begins relatively quickly, with evidence of surface erosion leading to delamination and the production of microplastic particles in as little as 8 wk. Microplastic production from the tested plastics occurred in the absence of any obvious reduction in the surface area or weight of the plastics, suggesting that visually evident fragmentation was not required for microplastic formation. Strips from each of the 3 tested polymers produced particles possessing a wide range of sizes, and each produced both fragments and fibers.

Although we confirmed that a subset of microplastic particles from the laboratory fragmentation test were the same polymer type as their respective control strips, we cannot exclude the possibility that at least some of the particles could have been from other sources. Microplastic particles from the surrounding environment or released from other strips during the exposure could have become entrained in the biofilm out in the field, then released during the laboratory fragmentation test. Previous studies have documented microplastic adherence to the polysaccharide-rich mucus produced by seaweed [20], and it is likely that the extracellular polymeric substances associated with biofilms have a similar capacity to adhere microplastics.

One unique feature of the present study is that we quantified the production of microplastic particles from field-deployed plastics as a measure of degradation. Other field studies have used mechanical properties, such as reduction in tensile strength or elongation at break, with the rationale being that a reduction in the useful properties of plastics implies that degradation has occurred [21]. These disparate methods make direct comparisons concerning the rates of plastic degradation between salt marshes and other habitats challenging. In Biscayne Bay (FL, USA), Pegram and Andrady [9] found that the extensibility of polyethylene samples floating in seawater changed little over an 8-wk period as a result of extensive fouling, whereas those exposed to air nearly embrittled over the same period. At a marina in Devon (UK), O'Brine and Thompson [22] found that the tensile strength of floating standard polyethylene plastic bags was significantly reduced following 40 wk; however, there was no significant reduction of surface area. In both studies, there was no mention of microplastic particles being produced. More recently, Welden [23] found that polyethylene, polypropylene, and nylon rope in subtidal conditions (10 m depth) of the Clyde Sea area (Scotland) exhibited a reduction in weight, averaging between 0.39% and 1.02% per month, and suggested that this weight loss was the result of the release of microplastic particles. Of interest is that Welden [23] found no correlation between the reduction in mechanical properties and the reduction in weight of the ropes. This finding suggests that microplastic particles may be produced from degrading plastic material before a significant reduction in mechanical properties. Thus, future studies of plastic degradation in the environment should consider including the quantification of microplastic production as part of a suite of degradation parameters.

Decreased production of microplastic particles was observed following 16 wk of exposure for both the high-density polyethylene and polystyrene strips, suggesting that degradation may be influenced by season. In salt marshes, seasonal variations in UV radiation, dissolved oxygen levels, temperature, and periwinkle activity have all been documented [24,25]; and it seems reasonable to suggest that biofilm assemblages associated with plastic debris may also vary with season. However, if seasonality were the only influence on the abundance of microplastics produced, it might be expected that strips collected in winter (January strips exposed for 32 wk) would have produced fewer microplastics relative to those collected either in summer (August strips collected at week 8) or in autumn (October strips collected at 16 wk). This was not the case. Certainly, the role of seasonality in the production of microplastic particles from plastic debris warrants further investigation.

The SEM images of plastic strips deployed in the salt marsh for 8 wk suggest that microplastic particles were produced as a result of delamination of the surface, as evidenced by the presence of numerous microcracks, pits, broken edges, and grooves on the surface. Surface delamination of plastic debris may be a very common process by which microplastics are produced in some habitats. Under laboratory conditions involving UV exposures, microcracks and pitting have been observed on the surface of polypropylene [26] and low-density polyethylene [27]. These same features have also been observed on weathered plastic surfaces, including polyethylene and polypropylene, from Hawaiian beaches [28]. By contrast, it has been suggested that the production of microplastic particles through weathering is less likely in submerged habitats because of the rapid attenuation of UV-B, development of a biofilm, lower temperatures, and lower oxygen concentrations [7].

Evidence in the present study suggests that a combination of abiotic and biotic factors played a role in the delamination of these plastic strips. It is generally recognized that photolytic and photo-oxidative reactions initiated by UV radiation are effective mechanisms in the degradation of plastics [7]. These reactions probably played a significant role during the first few days of

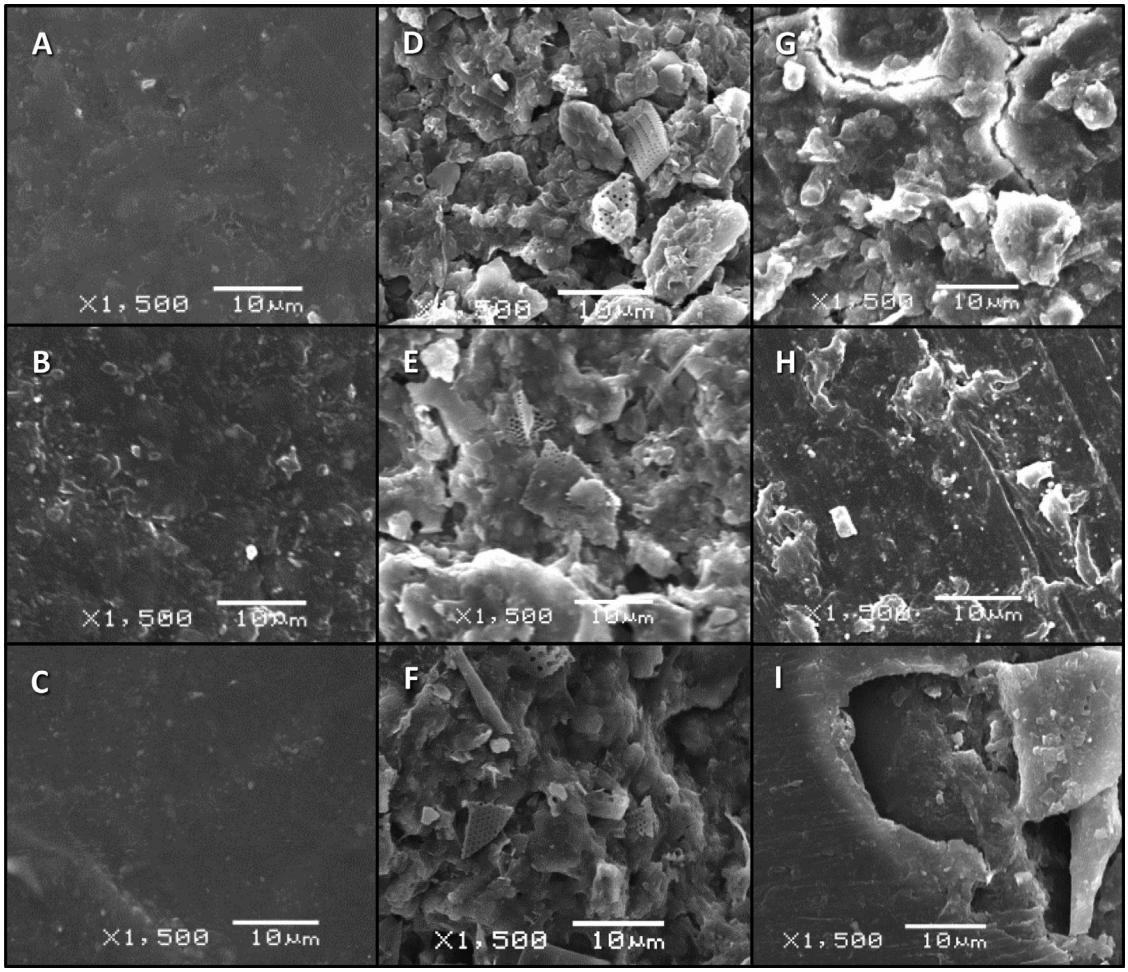

Figure 6. Scanning electron microscopic images of the surface topography of plastic strips at 0 wk and 8 wk postdeployment. Surface topography for the week 0 strips was nearly featureless for high-density polyethylene (**A**), polypropylene (**B**), and polystyrene (**C**). Surface topography of plastic strips exposed in a salt marsh for 8 wk exhibited a biofilm consisting of an extracellular matrix with benthic and pelagic diatom shells for high-density polyethylene (**D**), polypropylene (**E**), and polystyrene (**F**). Following removal of the biofilm, all 3 polymers exhibited a degraded surface (**G–I**). The high-density polyethylene strips exhibited pits, microcracks, and adhering material, presumably associated with biofilm (**G**). The polypropylene strips exhibited numerous grooves and areas where it appears that the surface had been removed (**H**). The polystyrene strips exhibited large cracks and areas where the surface layer was separating from underlying layers. All images enlarged 1500 times.

deployment, prior to the development of a biofilm. Within 4 wk of deployment, a substantial biofilm had developed on these strips, resulting in a significant reduction (∼95%) of UV radiation reaching the surface. Once the biofilm developed, processes other than photodegradation, such as thermo-oxidation, probably increased in relative contribution to the overall degradation of their surfaces. The weakened surface would have then been susceptible to microfragmentation induced by abrasion, perhaps associated with the repetitive drying and contraction of the biofilm during low tide exposures. At high tide, the surface biofilm would once again become hydrated and expand. The SEM images revealed that the accreting biofilm contained numerous silica-based shells from both pelagic and benthic diatoms, which in combination with entrained sediment particles would have created an abrasive composite moving across the surface of the plastics during biofilm contraction and expansion. Flaking of the dried portions of biofilm as the incoming tide covered the deployed strips could have also facilitated delamination. Evidence for this was provided by the observation of floating biofilm flakes containing entrained microplastic particles during the laboratory fragmentation test.

Although biodegradation is believed to be of minor importance in the overall degradation of plastic polymers in the environment (reviewed by Shah et al. [29]), we cannot completely rule out the possible role of microorganisms and macroorganisms in the relatively rapid degradation of the 3 plastic polymers deployed in the present study. Salt marshes are detritus-based ecosystems in which complex naturally occurring polymers, such as lignin and cellulose associated with the extensive stands of smooth cordgrass (*S. alterniflora*) and chitin associated with the exoskeletons of crustaceans, decompose relatively quickly. It is possible that a biofilm consisting of bacterial and fungal assemblages adapted to decomposing these complex natural polymers developed on the surface of the plastic strips deployed in the present study, then secreted enzymes that are also capable of decomposing synthetic polymers. It is interesting to note that the pitting observed on the surface of the high-density polyethylene strips in the present study was consistent with those images of surface pits associated with plastic-degrading microbial assemblages on marine plastic debris published by Zettler et al. [30]. The decomposition of smooth cordgrass (*S. alterniflora*) in southeastern US salt marshes has been attributed to 7 major

bacterial taxa (6 affiliated with the α-Proteobacteria group and 1 with Cytophagales) and 4 major fungal taxa (*Phaeosphaeria spartinicola*, *P. halima*, *Mycosphaerella* sp., and an environmental isolate, "4clt") [31]. Although these taxa have not been previously identified as having synthetic polymer-decomposing activity [29], future research should be aimed at investigating the possibility that they may degrade plastic.

Our results also suggest a possible role of salt marsh periwinkles (*L. irrorata*) in the decomposition of plastic debris. These grazing invertebrates normally feed on the microbial assemblages associated with both living and standing-dead *S. alterniflora* [32]. They also serve a role in shredding dead *Spartina* blades and conveying leaf-particulate material to the marsh sediment [33]. Observations made in the present study suggest that salt marsh periwinkles also graze on the biofilm covering the surface of plastic debris and, in the process, may ingest microplastic particles. This would both contribute to the process of delamination of the plastic surface and serve as a primary route by which microplastics enter the food chain. Salt marsh periwinkles are among the most abundant gastropods found in these habitats and serve as a major prey item for blue crabs (*Callinectes sapidus*) [34], Atlantic mud crabs (*Panopeus herbstii*), and diamondback terrapins (*Malaclemys terrapin*) [35].

Once microplastic particles are released from the surface of plastic debris, little is known concerning their environmental fate in a salt marsh. Microplastics may be ingested by invertebrates, which, if not egested, could lead to fatal injuries such as blockage of the digestive system or abrasions from sharp pieces of microplastic [36]. There is also the potential for microplastics to clog and block respiratory structures, block feeding appendages, and be passed to higher trophic levels [37]. In estuarine environments, much of the microplastic debris resides in the intertidal sediments. Recent surveys of Charleston Harbor have found microplastic particles in intertidal sediments with abundances as high as 2524 particles/m^2, with >99% of these particles being attributed to plastic degradation (i.e., secondary microplastics; H. Wertz, 2015, Master's thesis, College of Charleston, Charleston, SC, USA). Lower abundances have been recorded in the sea surface microlayer (11 particles/L) and subtidally (63 particles/kg) in the harbor (unpublished data). Based on the weight of 100 microplastic particles (8.31 mg) produced by polystyrene strips in the present study, we estimate that, if left to fully disintegrate into microplastic particles, a single polystyrene foam plate (with a 22.5 cm diameter and a 3.852 g weight) would produce 46 354 particles. This is enough to contaminate each kilometer of shoreline in Charleston Harbor (493 km) with 94 particles.

CONCLUSION

The present study's results demonstrate that the degradation of plastic debris proceeds relatively quickly in salt marshes. Degradation for all 3 tested polymers proceeded through a complex interaction between abiotic and biotic factors; however, UV radiation probably played a limited role in degradation after 4 wk because of the development of an accreting biofilm layer which reduced UV transmittance. Eventual erosion of the surface, perhaps facilitated by mechanical abrasion associated with the repeated drying and hydration of the biofilm, periwinkle grazing, and microbial degradation, led to delamination of the surface and the production of microplastic particles. In addition, microplastic particles were produced in the absence of significant reductions to the surface area or weight of the material, indicating that visually evident fragmentation of the material was not a precursor to microplastic formation. We conclude that delamination may be a common process by which microplastics are formed in salt marsh habitats. And given how quickly these events unfolded, the present study's results suggest that most plastic debris located intertidally in salt marshes are releasing microplastic particles during every tidal cycle.

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Data availability—Data, associated metadata, and calculation tools are available on request (john.weinstein@citadel.edu).

REFERENCES

1. Kennish MJ. 2002. Environmental threats and environmental future of estuaries. *Environ Conserv* 29:78–107.
2. US Environmental Protection Agency. 2015. Advancing sustainable materials management: 2013 fact sheet. EPA 530-R-15-003. Washington, DC.
3. Jambeck JR, Geyer R, Wilcox C, Siegler TR, Perryman M, Andrady A, Narayan R, Law KL. 2015. Plastic waste inputs from land into the ocean. *Science* 347:768–771.
4. Allsopp M, Walters A, Santillo D, Johnston P. 2006. Plastic debris in the world's oceans. Greenpeace, Amsterdam, The Netherlands.
5. Browne MA, Galloway T, Thompson R. 2007. Microplastic—An emerging contaminant of potential concern? *Integr Environ Assess Manage* 3:559–561.
6. Singh B, Sharma N. 2008. Mechanistic implications of plastic degradation. *Polym Degrad Stab* 93:561–584.
7. Andrady AL. 2011. Microplastics in the marine environment. *Mar Pollut Bull* 62:1596–1605.
8. Barnes DK, Galgani F, Thompson RC, Barlaz M. 2009. Accumulation and fragmentation of plastic debris in global environments. *Philos Trans R Soc Lond B Biol Sci* 364:1985–1998.
9. Pegram JE, Andrady AL. 1989. Outdoor weathering of selected polymeric materials under marine exposure conditions. *Polym Degrad Stab* 26:333–345.
10. Andrady AL, Pegram JE, Song Y. 1993. Studies on enhanced degradable plastics. II. Weathering of enhanced photodegradable polyethylenes under marine and freshwater floating exposure. *Journal of Environmental Polymer Degradation* 1:117–126.
11. Browne MA, Galloway TS, Thompson RC. 2010. Spatial patterns of plastic debris along estuarine shorelines. *Environ Sci Technol* 44: 3404–3409.
12. Kershaw P, Katsuhiko S, Lee S, Samseth J, Woodring D, Smith J. 2011. Plastic debris in the ocean. *UNEP Year Book*, 20–33.
13. Wenner EL, Holland AF, Sanger DM. 1998. Assessing short-term variability in dissolved oxygen and other water quality variables in shallow estuarine habitats. *Ocean Community Conference '98 Proceedings*, Vol 2. Marine Technology Society, Baltimore, MD, USA, pp 802–806.
14. Hettler WF. 1989. Nekton use of regularly-flooded saltmarsh cordgrass habitat in North Carolina, USA. *Mar Ecol Prog Ser* 56:111–118.
15. Ross SW. 2003. The relative value of different estuarine nursery areas in North Carolina for transient juvenile marine fishes. *Fish Bull* 101:384–404.
16. Holland AF, Sanger DM, Gawle CP, Lerberg SB, Santiago MS, Riekerk GHM, Zimmerman LE, Scott GI. 2004. Linkages between tidal creek ecosystems and the landscape and demographic attributes of their watersheds. *J Exp Mar Biol Ecol* 298:151–178.

17. Viehman S, Vander Pluym JL, Schellinger J. 2011. Characterization of marine debris in North Carolina salt marshes. *Mar Pollut Bull* 62: 2771–2779.
18. Müller RJ, Kleeberg I, Deckwer WD. 2001. Biodegradation of polyesters containing aromatic constituents. *J Biotechnol* 86:87–95.
19. Webb HK, Arnott J, Crawford RJ, Ivanova EP. 2012. Plastic degradation and its environmental implications with special reference to poly(ethylene terephthalate). *Polymer* 5:1–18.
20. Gutow L, Eckerlebe A, Giménez L, Saborowski R. 2016. Experimental evaluation of seaweeds as a vector for microplastics into marine food webs. *Environ Sci Technol* 50:915–923.
21. Andrady AL. 2003. *Plastics and the Environment*. John Wiley & Sons, Hoboken, NJ, USA.
22. O'Brine T, Thompson RC. 2010. Degradation of plastic carrier bags in the marine environment. *Mar Pollut Bull* 60:2279–2283.
23. Welden NAC. 2015. Microplastic pollution in the Clyde Sea area: A study using the indicator species *Nephrops norvegicus*. PhD thesis. University of Glasgow, Glasgow, Scotland.
24. Paul RW, Hatch WI, Jordan WP, Stein MJ. 1989. Behavior and respiration of the salt marsh periwinkle, *Littorina irrorata* (Say), during winter. *Mar Freshw Behav Physiol* 15:229–241.
25. Buzzelli C, Akman O, Buck T, Koepfler E, Morris J, Lewitus A. 2004. Relationships among water-quality parameters from the North Inlet-Winyah Bay National Estuarine Research Reserve, South Carolina. *J Coast Res* 45:59–74.
26. Yakimets I, Lai D, Guigon M. 2004. Effect of photo-oxidation cracks on behaviour of thick polypropylene samples. *Polym Degrad Stab* 86:59–67.
27. Küpper L, Gulmine JV, Janissek PR, Heise HM. 2004. Attenuated total reflection infrared spectroscopy for micro-domain analysis of polyethylene samples after accelerated ageing within weathering chambers. *Vib Spectrosc* 34:63–72.
28. Cooper DA, Corcoran PL. 2010. Effects of mechanical and chemical processes on the degradation of plastic beach debris on the island of Kauai, Hawaii. *Mar Pollut Bull* 60:650–654.
29. Shah AA, Hasan F, Hameed A, Ahmed S. 2008. Biological degradation of plastics: A comprehensive review. *Biotech Adv* 26:246–265.
30. Zettler ER, Mincer TJ, Amaral-Zettler LA. 2013. Life in the "plastisphere": Microbial communities on plastic marine debris. *Environ Sci Technol* 47:7137–7146.
31. Buchan A, Newell SY, Butler M, Biers EJ, Hollibaugh JT, Moran MA. 2003. Dynamics of bacterial and fungal communities on decaying salt marsh grass. *Appl Environ Microb* 69:6676–6687.
32. Silliman BR, Zieman JC. 2001. Top-down control of *Spartina alterniflora* production by periwinkle grazing in a Virginia salt marsh. *Ecology* 82:2830–2845.
33. Newell SY, Fallon RD, Miller JD. 1989. Decomposition and microbial dynamics for standing, naturally positioned leaves of the salt-marsh grass *Spartina alterniflora*. *Mar Biol* 101:471–481.
34. Hamilton PV. 1976. Predation on *Littorina irrorata* (Mollusca: Gastropoda) by *Callinectes sapidus* (Crustacea: Portunidae). *Bull Mar Sci* 26: 403–409.
35. Tucker AD, FitzSimmons NN, Gibbons JW. 1995. Resource partitioning by the estuarine turtle *Malaclemys terrapin*: Trophic, spatial, and temporal foraging constraints. *Herpetologica* 51:167–181.
36. Wright SL, Thompson RC, Galloway TS. 2013. The physical impacts of microplastics on marine organisms: A review. *Environ Pollut* 178:483–492.
37. Derraik JG. 2002. The pollution of the marine environment by plastic debris: A review. *Mar Pollut Bull* 44:842–852.