

Polymer Identification of Plastic Debris Ingested by Pelagic-Phase Sea Turtles in the Central Pacific

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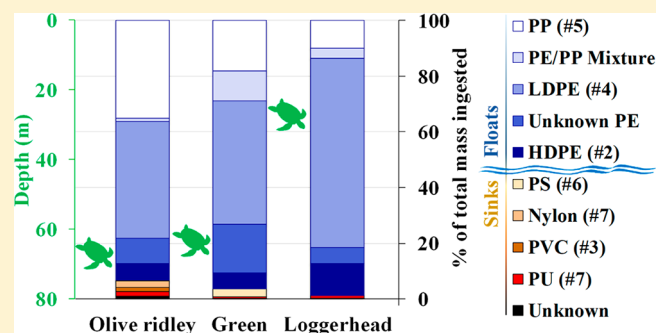
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Supporting Information

ABSTRACT: Pelagic Pacific sea turtles eat relatively large quantities of plastic (median 5 g in gut). Using Fourier transform infrared spectroscopy, we identified the polymers ingested by 37 olive ridley, 9 green, and 4 loggerhead turtles caught as bycatch in Hawaii- and American Samoa-based longline fisheries. Unidentifiable samples were analyzed using high-temperature size exclusion chromatography with multiple detectors and/or X-ray photoelectron spectroscopy. Regardless of species differences in dive depths and foraging strategies, ingested plastics were primarily low-density, floating polymers (51% low-density polyethylene (LDPE), 26% polypropylene (PP), 10% unknown polyethylene (PE), and 5% high-density PE collectively). Albeit not statistically significant, deeper diving and deeper captured olive ridley turtles ate proportionally more plastics expected to sink (3.9%) than intermediate-diving green (1.2%) and shallow-diving loggerhead (0.3%) turtles. Spatial, but no sex, size, year, or hook depth differences were observed in polymer composition. LDPE and PP, some of the most produced and least recycled polymers worldwide, account for the largest percentage of plastic eaten by sea turtles in this region. These novel data inform managers about the threat of plastic ingestion to sea turtles and may motivate development of more environmentally friendly practices for plastic production, use, and waste management.



INTRODUCTION

Annual plastic production grew from 2 million metric tons (Mt) in 1950 to 380 Mt in 2015, with \approx 8300 Mt produced to date,¹ leading to an increase in plastic debris on land and in the ocean.² As plastics weather, they fragment into persistent secondary microplastics that are now considered an emerging contaminant requiring immediate attention.^{3–6} The wide range of marine plastic debris sizes makes them easily available for ingestion by various organisms leading to an estimated 331 species being affected worldwide.⁷ However, the polymer compositions making up ingested debris in different species, each with varying foraging patterns, are largely unknown.⁸

All seven species of sea turtles, most of which are listed from Vulnerable to Critically Endangered on the International Union of Conservation of Nature (IUCN) Red List, were documented to have ingested plastic debris.^{9–12} Since the

earliest account in the 1950s,⁹ research on plastic ingestion by sea turtles has increased.¹⁰ Across the globe, pelagic-phase Pacific olive ridley, green, and loggerhead turtles ingest greater than 4-fold more debris quantities than in other locations.¹³

Many studies on plastic ingestion by sea turtles have described the physical characteristics of ingested items, such as color, size, and type (foam, line, fragment, nurdle, sheet).^{13–19} Hard fragments (79.5% of debris items) and white (59%) were the most common plastic type and color ingested by pelagic-phase olive ridley, loggerhead, and green sea turtles in the central Pacific Ocean.¹³ Describing debris characteristics helps

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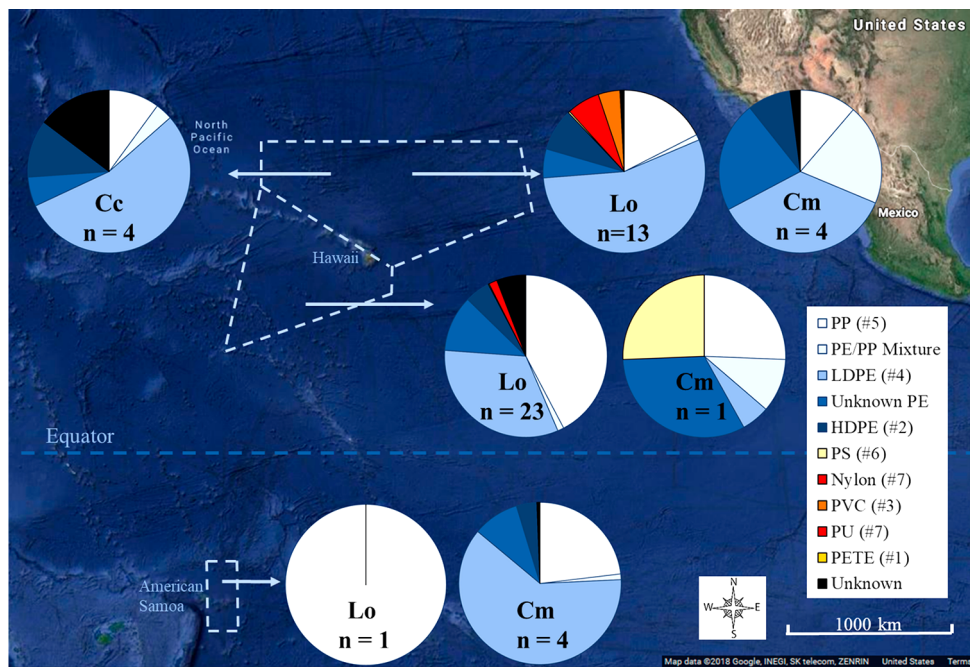


Figure 1. Polymer composition of ingested plastic debris mass from olive ridley (Lo), green (Cm), and loggerhead (Cc) sea turtles caught as bycatch by the Hawaii- and American Samoa-based longline fisheries. Capture regions are delineated by dashed lines. Abbreviations: polypropylene (PP), low-density polyethylene (LDPE), high-density polyethylene (HDPE), polystyrene (PS), polyvinyl chloride (PVC), polyurethane (PU), and polyethylene terephthalate (PETE).

us understand its sources and factors that make it favorable for ingestion.¹⁴ This information, in turn, can help prioritize the most useful management options to mitigate plastic ingestion by sea turtles.¹⁰

Beyond these characteristics, identifying the polymer composition of marine debris can be a powerful tool to provide additional source information and help focus efforts to reduce particular types of plastics entering the oceans.²⁰ Moreover, knowing how much particular polymers are discharged in the ocean could help evaluate the efficacy of recycling programs or shift production priorities, especially for single-use products. Identifying ingested polymers is important for another reason; different polymers release different potentially hazardous chemicals or sorb persistent organic pollutants (POPs) at different rates and concentrations.^{21–25} Thus, certain polymers may be better vectors for this potential threat from plastic ingestion, and understanding the polymeric composition can help model chemical exposure routes.²⁶ POP concentrations were measured in fat samples from the same turtles used in this study.²⁷ Results suggested that POP accumulation was mainly through prey, but transfer from ingested plastic remains a possibility.²⁸

Each plastic polymer has a specific chemical composition and molecular architecture that influence the physical and chemical properties of the material. For instance, each type of polymer has a unique density range that affects its distribution in the water column, which may influence the encounter rates for marine organisms that use different marine habitats.^{3,29,30} Floating plastic debris is predominantly composed of polypropylene (PP), low-density polyethylene (LDPE), and high-density polyethylene (HDPE) due to their low densities.³ Polyethylene terephthalate (PETE) and polyvinyl chloride (PVC) are denser than seawater and sink in the water column.^{30,31} Polystyrene (PS) exceeds the density of seawater

only slightly but will float along with other dense plastics if the object contains entrapped air.²⁹

Raw material density alone does not determine the stratification of plastic marine debris in the water column. Biofouling or entanglement with other debris can increase the density of buoyant polymers causing them to sink.^{3,32} Sinking fecal matter can also facilitate the transport of plastic debris through the water column.³³ Upward transport is also possible if the buoyancy of an item increases due to biofouling.³⁴ In addition, many manufacturing practices can alter the effective densities of plastic parts. For example, a large percentage of commercial plastics contain additives or consist of copolymers or blends of different polymers, making predictions of their density, and their stratification in the water column, challenging. However, density of the bulk polymer is a strong factor influencing the transport and buoyancy of plastic marine debris.

Different sea turtle species forage at different depths.³⁵ Our study focused solely on Pacific sea turtles foraging in pelagic waters where the seafloor (>1000 m) exceeds their maximum diving depths.²⁷ Olive ridley turtles (*Lepidochelys olivacea*) in this region perform deep dives, often >40 m, with the capability of diving below 150 m and consume mainly subsurface gelatinous zooplankton.³⁵ Juvenile pelagic-phase green turtles (*Chelonia mydas*) forage on invertebrates at variable depths within the first 100 m of the water column,³⁶ and adults are known to dive deeper than 135 m.³⁷ Pelagic loggerhead (*Caretta caretta*) turtles in the Central Pacific spend most of their time at the surface or performing shallow dives (<40 m), feeding predominantly on neustonic species and only rarely on deeper-water prey.^{35,38,39} These differences in feeding ecology and habitat use may influence the polymer composition of the plastics ingested by these species in the Central Pacific.

Only four studies have documented polymer types ingested by sea turtles.^{40–43} This is the first study to determine polymer composition ingested by sea turtles in the Central Pacific Ocean and to evaluate the possible influences of sea turtle species, sex, size class, year, foraging depth, or geography on the composition of ingested plastics. We expected species differences related to their diving habits, with more higher density polymers (PETE, PVC, and PS) ingested by deeper diving species. We also calculated the depths at which each individual turtle was hooked in the mouth,⁴⁴ a novel measurement of sea turtle behavior, and examined correlations with polymer composition. By comparing differences among species, we can start to understand whether specific polymers represent a disproportionate threat to specific species and inform waste minimization strategies and disposal management. This information may spur development of next-generation materials where end-of-life considerations such as biodegradability are designed into the polymer structure, mitigating the risk of environmental threats to marine species.

MATERIALS AND METHODS

Sample Collection. All sea turtles included in this study (37 olive ridley, 9 green, 4 loggerhead) had ingested plastics and were caught as bycatch by the Hawaii- and American Samoa-based longline fisheries (Figure 1) between June 2012 and February 2016. Information about these turtles has been previously described, including capture locations, quantities and physical characteristics of ingested plastics, and sex and age classes, as determined by gross gonadal morphology and straight carapace length (SCL).¹³ The ingested plastics and tissues from these turtles remain cryogenically archived.⁴⁵

The entire gastrointestinal (GI) tract from esophagus to colon was examined for plastic.¹³ Each individual piece was rinsed with Millipore water from a polytetrafluoroethylene (PTFE) squirt bottle, blotted or gently wiped with cleanroom wipers (100% cotton, ITW Texwipe, Kernersville, NC), and measured using a ruler for length (cm), width (cm), and depth (mm). Ingested plastic from each turtle was combined in hexane-rinsed foil, dried overnight in a fume hood, and weighed. The foil packet was placed in a fluorinated ethylene propylene (FEP) bag and stored at $-80\text{ }^{\circ}\text{C}$ or below. This handling and storage protocol was designed for future measurements of sorbed organic pollutant concentrations.

FT-IR Polymer Identification. Plastics from each turtle were thawed and sorted with hexane-rinsed forceps on cleanroom wipers into obviously similar groups based on color, thickness, and texture (Figure S1). We assumed all pieces from each group were smaller fragments broken from the same piece of debris. One piece from each group was chosen for polymer identification to minimize analytical labor while obtaining complete polymer composition of all plastics ingested by each turtle. Replicate pieces from several different groups resulted in the same polymer identification, confirming our ability to accurately group the pieces. Any piece that was questionably different than others was analyzed individually. The mass (to the nearest 0.00001 g) of each chosen piece was recorded along with color and type (fragment, sheet, line, foam, nurdle). Remaining pieces (not selected) in each group were weighed together to the nearest 0.00001 g, and the number of pieces per group was noted. Represented plastics were frozen in original foil and bags.

Each selected plastic piece ($n = 828$) was cleaned and analyzed using a PerkinElmer attenuated total reflectance

Fourier transform infrared (ATR FT-IR) Spectrometer Spectrum Two (Waltham, MA) according to Jung et al.²⁰ The ATR FT-IR crystal was cleaned with isopropanol, and a background spectrum was run before each sample. Samples were applied to the crystal with a force between 80 and 100 N. When a usable spectrum was produced, identifiable absorption bands were labeled via the instrument's software (Spectrum 10; PerkinElmer). Spectra were analyzed manually, and a minimum of four matching absorption bands were required for polymer identification.²⁰ LDPE was differentiated from HDPE by the presence of a small absorption band at 1377 cm^{-1} , which was determined in our previous study to be accurate 85% of the time.²⁰ No reference library was used.

HT-SEC Polymer Identification. Selected ingested pieces were also characterized by high temperature size exclusion chromatography (HT-SEC) with differential refractive index, infrared, and multiangle light scattering detection. Twenty-seven of the 30 pieces with unknown polymer identity by FT-IR were analyzed with HT-SEC. The three remaining unknown pieces did not have enough mass for HT-SEC methods. Of the 40 pieces identified as polyethylene (PE)/PP mixtures by FT-IR due to the presence of specific absorption bands characteristic of both PE and PP,²⁰ 25 were analyzed by HT-SEC for confirmation. Sample preparation and analysis conditions were identical to methods described in our previous study.²⁰

Several qualitative and quantitative pieces of information were used to identify the polymers analyzed by HT-SEC, including the magnitude (positive or negative) of the differential refractive index (RI) detector peak and branching content averaged across the molar mass distribution for each sample (denoted as average methyl content per 1000 total carbons or $\text{CH}_3/1000\text{ total C}$) as previously described.²⁰ Samples were identified as HDPE when the average branching content was 10 $\text{CH}_3/1000\text{ total C}$ or less, as PP for polymers with an average methyl content of 330 $\text{CH}_3/1000\text{ total C}$ (± 33), and as LDPE when average branching content was between HDPE and PP or 10 to 300 $\text{CH}_3/1000\text{ total C}$.²⁰ For samples identified as possible PE/PP mixtures, branching content was also assessed across the molar mass distribution to see how branching content varied as a function of polymer molar mass, using the same ranges just described. There are numerous formulations of PE, PP, LDPE, and linear LDPE (LLDPE) commercially produced including blending of homopolymers, copolymerization, and chain catalysis that can produce polyolefins with varied branch length and total branching content across the molar mass distribution. Extensive reverse engineering measurements would need to be conducted in addition to HT-SEC to quantitatively assess which homopolymers and copolymers are present, including differential scanning calorimetry, rheological measurements, and intrinsic viscosity measurements, among others. Representative molar mass distributions of PP, LDPE, and PP/PE mixtures are shown in Figure 2. Samples that had high branching content, greater than 300 $\text{CH}_3/1000\text{ total C}$, must contain PP based on theoretical calculations and were identified as PP/PE mixtures. No further attempts were made in this study to discern LDPE from LLDPE.

Samples that were insoluble in the mobile phase (1,2,4-trichlorobenzene, $160\text{ }^{\circ}\text{C}$) or samples that were only minimally soluble, resulting in inconclusive HT-SEC data, were measured by X-ray photoelectron spectroscopy (XPS) to determine chemical composition. Samples were analyzed using a Kratos

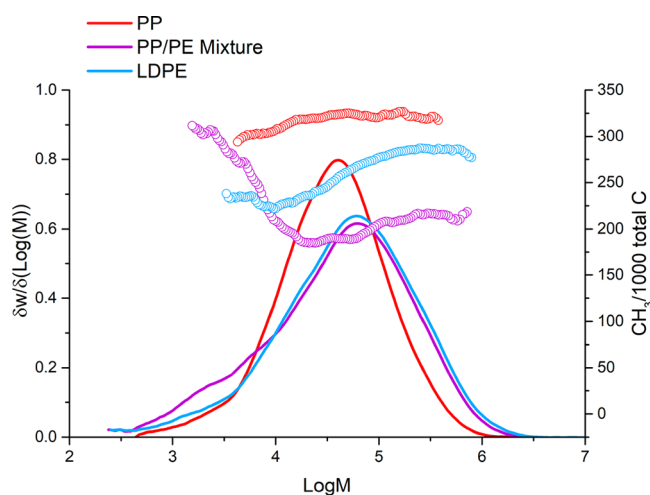


Figure 2. Differential weight fraction ($\delta w/\delta \log(M)$, left axis, line) and branching content ($\text{CH}_3/1000$ total C, right axis, open symbols) versus log of molar mass ($\log M$) (from g/mol) of three representative ingested plastic samples. The samples were identified as PP (red traces), LDPE (blue traces), and PE/PP mixture (purple traces) based on the branching content as a function of $\log M$.

Ultra DLD spectrometer using an Al $K\alpha$ monochromic source (1486.6 eV) operated at 140 W. Measurements were performed at a takeoff angle of 0° (normal to the surface), resulting in a depth sensitivity of 5 to 10 nm. Survey (0 to 1200 eV binding energies) scans and C 1s region spectra were taken on at least three spots per sample and three scans per spot to accurately sample the film. Quantification of the carbon environments was determined by integration of fitted carbon regions by CasaXPS, and compared to database spectra of pure polymers to compare atomic percentages between measured samples and identify the material. Samples that matched the polymer database for carbon, nitrogen, oxygen, and chlorine content within 5% of theoretical values were identified as that polymer. Remaining samples that did not match database entries were labeled as unknown.

Polymer Composition Calculations. The percentage of all plastic ingested per turtle consisting of each polymer type was calculated by mass. For example, masses of all pieces of PP from a turtle were summed, including pieces selected for analysis and those of the represented groups. That value was divided by the total mass of ingested plastics and multiplied by 100.

Capture Depths. Method 1 described in Bigelow et al.⁴⁴ was used to estimate hook depths (m) corrected for shoaling of the longline gear. Observers recorded the specific location of the hook that caught each sea turtle along the main line between floats as well as several other gear dimensions, allowing for this calculation. All turtles were hooked in the mouth and most had ingested bait fish, indicating that they were actively foraging at the hook depth. All longline sets for this study were deepset. An easy-to-follow protocol explaining calculations, the R code (version 3.4.0) for calculating catenary angles, and estimations used for missing data to estimate hook depth is available in [Hook Depths Protocol and Table S1](#).

Statistical Analysis. Mean, median, range, standard deviations, and percent occurrence were calculated for the mass and % mass of each polymer within all three species. Nondetects were set to a value of zero. All variables were tested for normality using the Shapiro-Wilk test in JMP 12.10 (SAS

Institute Inc., Cary, NC), using $\alpha = 0.05$ to test for significance. We used PC-ORD 6.08 (Gleneden Beach, Oregon), multiresponse permutation procedures (MRPP) along with indicator species analyses (ISA), to determine differences in polymer composition between species (37 olive ridley turtles, 9 green turtles, 4 loggerhead turtles).^{46,47} A similar analysis was done with polymers grouped as floating (PP, PE/PP mixture, LDPE, unknown PE, and HDPE) and sinking (PS, nylon, PVC, and polyurethane (PU)) based solely on their chemical density. MRPP with ISA was also used for olive ridley turtles to evaluate effects of sex (30 females vs 7 males), size class (8 immature/subadults vs 22 adults), and year caught (7 in 2012, 10 in 2013, 5 in 2014, 11 in 2015, and 4 in 2016) on polymer types ingested (information available in ref 13). Using JMP, Spearman's Rank Order correlations were performed for each polymer identified to determine if ingested polymer composition varied across capture latitude, longitude, or SCL of olive ridley or green turtles, separately. Spearman's Rank Order correlations were also performed between polymer composition and hook depth, with all turtles combined. For these correlations, a Bonferroni correction was applied (significance defined as $p \leq 0.005$). A Kruskal–Wallis test followed by Wilcoxon rank-sum post hoc tests was used to evaluate if olive ridley, loggerhead, and green turtles were caught at significantly different depths.

RESULTS AND DISCUSSION

Size of Turtles and Ingested Plastic Debris. Turtles in this study ranged from 29.9 to 63.9, 37.9 to 54.9, and 64.7 to 72.9 cm SCL for olive ridley, green, and loggerhead turtles, respectively, as described previously.¹³ All ingested plastic items ($n = 4045$) measured to date from 64 turtles captured in the central Pacific and sampled for the NIST biorepository (BEMAST) represent a wide size range, with lengths of the longest dimension between 0.1 and 60 cm, a mean of 2 cm and standard deviation of 3 cm, and a median of 1 cm. Twenty percent of the pieces are considered microplastics (length of 1–5 mm).⁴⁸ The mass of individual plastic pieces analyzed for this study ($n = 828$) ranged from 0.00097 to 13.41106 g, and dimensions ranged from a fragment of 2 mm \times 2 mm \times 0.5 mm to a full snack mix bag of 205 mm \times 104 mm \times 0.5 mm. The FT-IR was able to identify polymers on this wide range of debris sizes.²⁰

Overall Polymer Composition. Identifying the plastic polymers helps address a variety of questions that otherwise cannot be answered. Different polymers are used to produce different consumer goods.⁴⁹ For example, PETE, PP, and LDPE are mainly used for single-use drink bottles, food packaging, and bags, respectively, while PVC is used primarily in construction materials.⁴⁹ Knowing the polymer composition can help determine the potential original use of fragments ingested by sea turtles. Polymer identification also helps determine the fate and transport of debris.²⁰ Floating polymers will travel much farther distances on the sea surface and be exposed to harsher weathering conditions, while sinking polymers will persist closer to their source on the seafloor. Finally, polymers vary in their capacity to release hazardous molecules and to sorb pollutants from the environment.^{23–25} For example, polyethylene is known to sorb and accumulate more pollutants than other polymers,^{22,25} increasing the potential of POP exposure from specific polymers after ingestion to marine organisms.

ATR FT-IR spectral data from all analyzed ingested debris pieces are included in the [Supporting Information](#). Summary statistics on polymer composition by species are shown in [Tables S2 and S3](#). LDPE was the predominant polymer ingested by all three species and was found in 92% of olive ridley, 100% of loggerhead, and 100% of green sea turtles. LDPE, HDPE, PP, and unknown PE were within the top five polymers ingested for each species ([Figure 3](#)). Overall, the

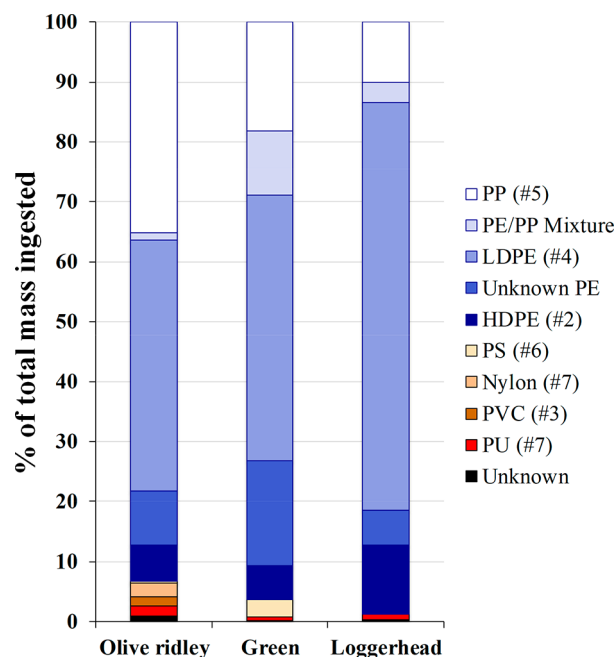


Figure 3. Average polymer composition of ingested plastic debris mass from olive ridley ($n = 37$), green ($n = 9$), and loggerhead ($n = 4$) turtles. Abbreviations: polypropylene (PP), low-density polyethylene (LDPE), high-density polyethylene (HDPE), polystyrene (PS), polyvinyl chloride (PVC), and polyurethane (PU). Blue shaded polymers float in seawater based solely on chemical density, while warmer toned polymers sink.

composition of ingested plastics by mass were 51.2% LDPE (resin code #4), 25.7% PP (#5), 9.95% unknown PE (#2 or #4), 5.02% HDPE (#2), 5.25% PE/PP mixture, 1.51% PU (#7), 0.28% PVC (#3), 0.17% PS (#6), and 0.16% nylon (#7). Polymers which we had in our in-house spectral library but were not detected were PETE (#1) and several resin code #7 polymers: acrylonitrile butadiene styrene (ABS), cellulose acetate (CA), ethylene vinyl acetate (EVA), latex, nitrile, polycarbonate (PC), poly(methyl methacrylate) (PMMA or acrylic), PTFE, and fluorinated ethylene propylene (FEP), which would all be expected to sink in seawater. Less than one percent (0.79%) could not be identified and was labeled as unknown.

The majority of plastic ingested by pelagic-phase Pacific sea turtles was made of buoyant PE and PP (97.1%), a similar composition to that of beach plastics from Kauai, one of the Main Hawaiian Islands,⁵⁰ sea surface waters from the North Pacific garbage patch,⁵¹ and along the Adriatic Sea.⁵² Floating microplastics off the coast of Indonesia were also comprised mainly of PP, PE, LDPE, and PS⁵³ which is similar to microplastics collected 5 m below the surface in the Ross Sea in Antarctica.⁵⁴ Velez-Rubio et al.⁵⁵ identified all plastics ingested by 96 green sea turtles off the Uruguayan coast from 2005 to 2013 as buoyant items. These studies together suggest

that sea turtles mainly ingest the most abundant polymers documented at or near the sea surface.

The dominance of PE and PP as the main polymers recovered in marine environments around the world might be expected because they comprise most of the annual plastic demand (14 Mt of PE and 9.1 Mt of PP in Europe alone) and the highest percentages in U.S. municipal solid waste.^{29,49} PE and PP are used commonly in single-use items such as food packaging⁴⁹ but also heavily in materials used in the fishing, aquaculture, and shipping industries, which represent the majority of debris that washes ashore on Hawaiian Island beaches.^{56,57} The percent of ingested PE (66.2%) and PP (25.7%) in the sea turtles exceeded their percentages in market demand (28% and 19%, respectively) and solid waste (37% and 14%, respectively), suggesting that inputs and fate of different polymers in the Pacific sea surface are disproportionate to use and disposal from other regions ([Figure 4](#)). The

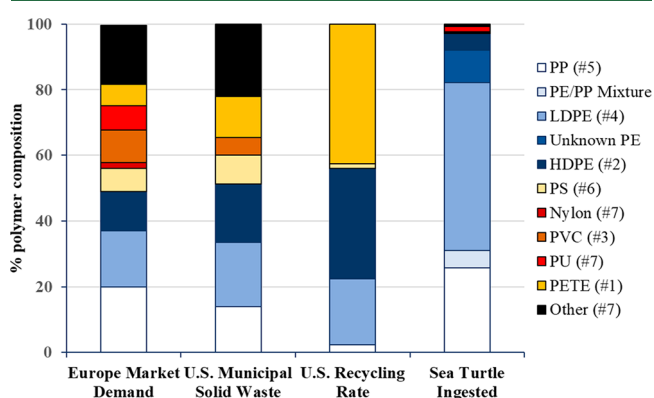


Figure 4. Comparison of polymer composition among Pacific sea turtle ingested plastic debris, plastic market demand in Europe,⁴⁹ U.S. total plastic municipal solid waste,²⁹ and U.S. recycled plastics (calculated from the total plastic municipal solid waste quantities).²⁹ Abbreviations: polypropylene (PP), low-density polyethylene (LDPE), high-density polyethylene (HDPE), polystyrene (PS), polyvinyl chloride (PVC), polyurethane (PU), and polyethylene terephthalate (PETE). Blue shaded polymers float in seawater based solely on chemical density, while warmer toned polymers sink.

difference stems from two likely reasons. First, the fishing, aquaculture, and shipping industries, which are responsible for the majority of marine debris in the North Central Pacific,^{56,57} might use and release a greater proportion of PE and PP (i.e., nets, rope, crates, trays, buckets, other fishing gear, packaging materials) than activities using plastics on land. Second, high-density plastics released into the ocean are expected to sink. PU (1.51%), PVC (0.28%), PS (0.17%), and PETE (0%) were ingested at much lower percentages than used in the commercial marketplace (7%, 10%, 7%, and 6%, respectively; [Figure 4](#)). This provides evidence that if high-density plastics are released into the ocean, they will sink and become unavailable to pelagic-phase turtles inhabiting regions that are too deep for them to reach and forage on the seafloor.

Comparing ingested polymer composition to recycling rates might help monitor the success of waste management strategies. The two predominant polymers ingested by the sea turtles (LDPE and PP) are not commonly recycled in the U.S., whereas the two most commonly recycled polymers (PETE and HDPE) were absent or found at lower percentages in the turtles ([Figure 4](#)).²⁹ If recycling programs or economic incentives became widely available for the other polymers,

especially LDPE and PP, this might reduce abundance of these plastics ingested by sea turtles.

Our results are similar to the composition of plastics identified in sea turtle GI tracts elsewhere. Plastic pieces from 20 oceanic-stage juvenile loggerhead turtles off the North Atlantic Subtropical Gyre consisted of 60% PE and 20% PP.⁴¹ Mesoplastic pieces from 13 mostly posthatchling Eastern Florida stranded sea turtles consisted of 54% PE and 24% PP.⁴² PE/PP mixtures and high-density, sinking polymers (rayon, PVC, polyvinyl acetate, nylon, PETE, and PS) were also identified but in much lower percentages.^{41,42} Four plastic fragments from one loggerhead turtle from the Tyrrhenian Sea, off the coast of Italy, were identified as a poly(ethylene oxide) (PEO; also known as polyethylene glycol; $n = 1$) and as a copolymer of low-density PETE (LDPETE) and PEO ($n = 3$).⁴⁰ We believe PEO was an incorrect identification. Relying on automated spectral library searches alone without consideration of the physical and chemical properties of polymers can lead to misidentifications. Some PEO materials are liquids at environmental temperatures, and PEO is more often used as an additive rather than the main polymer;⁵⁸ therefore, it is more likely that those fragments identified as PEO were actually weathered or oxidized PE which would be more in line with recent studies.⁴¹ Alternatively, it is possible, but unlikely for 3 of 4 of the pieces they analyzed, that a large concentration of PEO additive in the analyzed plastic debris items could dominate the spectra, prohibiting the identification of the bulk polymer. Finally, two benthic-feeding green turtles stranded in Australia ingested microplastics consisting of a variety of polymers, many of which would sink (i.e., polyvinyl acrylic, nylon, and fabric fibers).⁴³

Published reports of polymer identification of ingested debris in other marine species are sparse but increasing. Demersal and pelagic fish from the North Sea and Baltic Sea showed a similar composition of ingested plastics to that of sea turtles with ingested particles containing a large proportion of low-density floating polymers (40% PE and 13% PP) and some high-density, sinking polymers (22% nylon, 0.1% PS, 0.05% PETE, 0.05% polyester, and 0.05% PU).⁵⁹ However, studies on demersal and pelagic fish from the English Channel,⁶⁰ benthic-foraging fish species in Sydney Harbour, Australia,⁶¹ pelagic-neritic Sprat (*Sprattus sprattus*) in the North Sea,⁶² and a deep diving True's beaked whale (*Mesoplodon mirus*) stranded off the coast of Ireland⁶³ showed ingested compositions of mainly high-density, sinking plastics with low-density floating polymers being less abundant. Nilsen et al.⁶⁴ used gas chromatography–mass spectroscopy (GC-MS) to identify ingested plastic debris from boluses of Laysan albatross (*Phoebastria immutabilis*) from Kure Atoll, Hawai'i. Similar to our findings, PP comprised a large proportion (68.4%). However, the proportions of PVC, PETE, PS, and HDPE (20.5%, 8.5%, 6.8%, and 0.8%, respectively) found in sea birds differed greatly from sea turtles where virtually no PVC, PETE, or PS was found ingested and a large proportion of HDPE was present. The differences in results between these studies suggest that influences from different chemical identification methods and different species foraging at different depths on different prey and in different geographic regions, along with different sources, degradation, and transport processes of plastic debris, affect what polymer types are available for ingestion.

Species Comparison for Polymer Composition. MRPP tests showed a significant difference ($T = -3.28$, $p < 0.01$) in

the polymer composition ingested by olive ridley and green turtles. Using ISA, green turtles ingested significantly more percent mass of unknown PE ($p < 0.03$) and PE/PP mixture ($p < 0.04$) than olive ridley turtles. In addition, green turtles ingested significantly more line than olive ridley turtles,¹³ and line was found to be composed primarily of the PE/PP mixture (Figure 5). Green turtles ingesting significantly greater

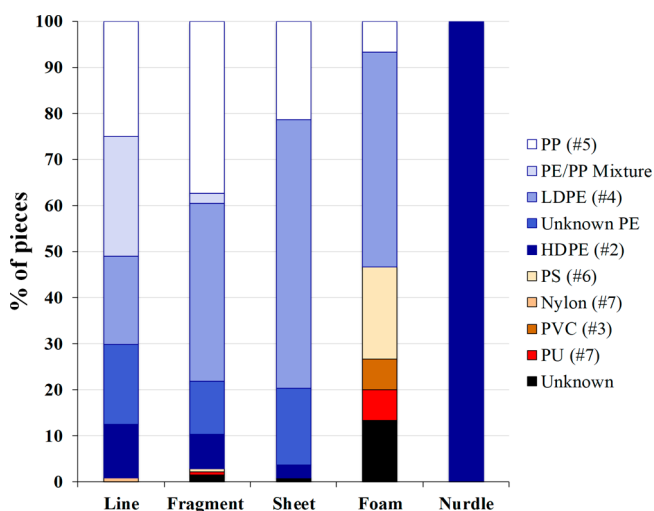


Figure 5. Polymer composition of different types of debris ingested by olive ridley ($n = 37$), green ($n = 9$), and loggerhead ($n = 4$) turtles. Abbreviations: polypropylene (PP), low-density polyethylene (LDPE), high-density polyethylene (HDPE), polystyrene (PS), polyvinyl chloride (PVC), and polyurethane (PU). Blue shaded polymers float in seawater based solely on chemical density, while warmer toned polymers sink.

proportions of low-density polymer types (PE and PP) supports the idea that species foraging at shallower depths are more likely to ingest lower-density floating plastics. The small sample size of loggerhead turtles ($n = 4$) may explain why no difference was observed between the surface foraging loggerhead and deeper diving olive ridley turtles. Monitoring ingested polymer composition should continue as more individual loggerhead turtles become available.

While no other significant differences were found among species in regard to polymer composition, even when simply grouped as floating or sinking, the number of different high-density polymer types ingested varied. Four high-density polymer types were ingested by deep diving olive ridley turtles (5 pieces of PS, 1 PVC, 1 nylon, 7 PU), and two high-density polymers were ingested by intermediate green turtles (2 PS, 3 PU) while only one was ingested by surface foraging loggerhead turtles (4 PU pieces; Figure 3). The percentage of high-density polymers ingested by deep diving olive ridley turtles was higher (3.9%), but not significantly so, than green turtles (1.2%) and loggerhead turtles (0.3%; Figure 3). PS can exist as hard plastic or in the form of expanded PS foam.^{3,29} Both PS pieces found in green turtles were in the form of hard fragments, while the five found in olive ridley turtles were hard fragments ($n = 2$) or foam ($n = 3$). Similar to PS, PU is often used in foam for home furnishings. The one PU found in a loggerhead turtle was foam while the one found in a green turtle was a fragment. The three PU pieces found in olive ridley turtles were identified as 2 fragments and 1 foam. Even though the average densities of PS (1.04–1.08 g/cm³)⁶⁵ and PU (1.23 g/cm³)⁶⁶ exceed that of seawater, air blown foam

pieces can float on the surface.^{3,29} Furthermore, high-density polymers can be suspended in the water column by turbulence, by biofilms that increase buoyancy, or at a stratified layer of higher density seawater.^{31,34,67} Therefore, the PS hard fragments ingested by green and olive ridley turtles and the PU hard fragments ingested by all three species could have been consumed either at the surface or within the water column. Plastics settled on the seafloor were not available for the assessed turtles to ingest, because the water column was too deep at their capture locations.²⁷ Both PVC and nylon, also found in olive ridley turtles, would sink through the water column and eventually reach the seafloor.^{29,31} These results indicate that turtles foraging at the surface or midwater (unable to reach the seafloor) are interacting with a small proportion of high-density plastics, as they are either sinking through the water column or held at the surface by some other mechanism (e.g., turbulence,³¹ containing trapped air, or rafted with floating prey).

Polymer Composition and Hook Depths. The estimated depths of hooking individual turtles are available in Table S4. The average hook depths for olive ridley ($n = 35$), green ($n = 9$), and loggerhead ($n = 4$) turtles were 68.4 ± 52.2 , 65.5 ± 28.1 , and 29.3 ± 1.6 m, respectively (Figure S2). These results fall within the dive and foraging depths reported for olive ridley (>40 m),³⁵ green (100 m or shallower),³⁶ and loggerhead (<40 m)³⁵ turtles in this region. A significant species difference was found for hook depth (Kruskal–Wallis; $H(2) = 6.84$, $p = 0.033$). Olive ridley and green turtles were both caught significantly deeper (Wilcoxon $W_s = 2.25$, $p = 0.025$ and $W_s = 2.70$, $p = 0.007$, respectively) than loggerhead turtles (Figure S2). For all species combined, 83% of hookings were above 100 m when corrected for shoaling (Figure S3). Two olive ridley turtles were removed due to insufficient data to calculate hook depth. Because the estimated hook depths are congruent with previous foraging depth results using diet analysis or depth recorders, estimating individual turtle foraging depths using the hook depth calculation has many applications beyond this study.

No correlation was seen between hook depth and any individual polymer composition (Figure S4). These results demonstrate that olive ridley and green turtles are being hooked at different depths in the water column, but the prevalence of buoyant plastics in the GI tracts of all three species, even the deeper diving olive ridley turtles, indicates that the pelagic-phase of the three species are ingesting plastics primarily at or near the surface.

Sex, Age Class, and Year Caught. Within olive ridley turtles, polymer composition did not differ between sexes (Figure S5), age classes (Figure S6), or years caught (Figure S7). Future studies should continue to monitor the polymer composition of ingested marine debris in coming years to track changes in production or waste management strategies. No significant correlations were observed between SCL and individual polymer percentages (Figures S8 and S9) for olive ridley or green turtles.

Geographical Differences. Significant geographical differences were seen among individual polymer percentages. Olive ridley turtles captured farther north (Spearman's $\rho = -0.518$, $p = 0.001$; Figure S10D) and east (Spearman's $\rho = -0.464$, $p = 0.004$; Figure S11D) had ingested significantly lower percentages of PP (Figure 1). Relationships that were not significant between latitude and longitude and other ingested polymers for olive ridley turtles are available in Figures S10 and

S11, respectively. The results of these correlations suggest that turtles foraging closer to a hot spot of marine debris in the North Pacific Subtropical Gyre, estimated at 35° N, 140° W,⁶⁸ encounter less PP and more of other polymers. Only one study to our knowledge has examined the polymer composition of sea surface debris in the pelagic Pacific Ocean.⁵¹ PE (64% of pieces) and PP (20%) dominated the plastic debris pieces found in the North Pacific Subtropical Gyre where immense and increasing amounts of plastic congregate.^{51,68} Polymer compositions in southern regions remain unknown.

Differences were also found in green turtles with turtles captured farther east ingesting significantly higher percentages of the PE/PP mixture (Spearman's $\rho = 0.879$, $p = 0.002$; Figure S13E). MRPP showed a significant difference ($p < 0.001$) in polymer composition between green turtles captured north and south of the equator. ISA identified that green turtles south of the equator ingested more LDPE ($p < 0.03$) while green turtles north of the equator ingested more PE/PP mixture ($p < 0.01$) (Figure 6). The geographic differences

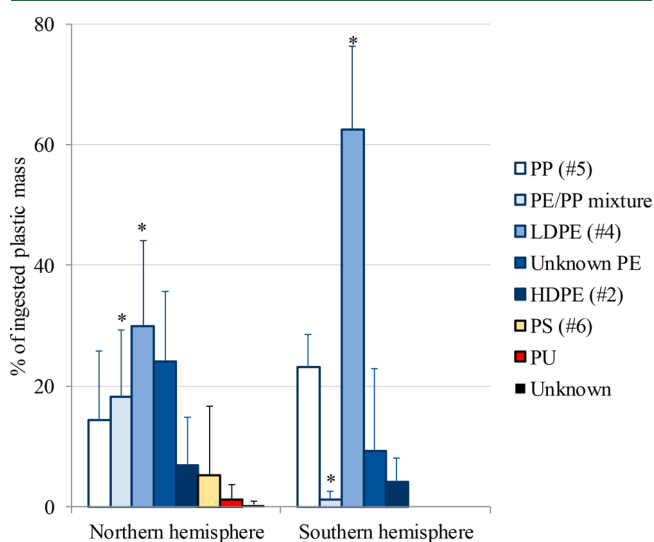


Figure 6. Mean and standard deviation of the polymer composition of plastic debris ingested by green turtles north ($n = 5$) and south ($n = 4$) of the equator. Abbreviations: polypropylene (PP), low-density polyethylene (LDPE), high-density polyethylene (HDPE), polystyrene (PS), and polyurethane (PU). Blue shaded polymers float in seawater based solely on chemical density, while warmer toned polymers sink. Asterisks above the bars indicate significant differences between percent composition of a polymer between north and south ($p < 0.05$).

observed in green turtles are not likely due to different life history stages, because turtles from both hemispheres were similar sizes (mean SCL was 45.1 cm in north and 43.8 cm in south). Relationships that were not significant between latitude and longitude and other polymers for green turtles are available in Figures S12 and S13, respectively. The reasons behind these geographical trends in polymeric compositions are still unknown and deserve future research.

The access to an ATR FT-IR and a specimen bank of plastics ingested by pelagic-phase Pacific sea turtles allowed us to conduct the largest study of polymer types ingested by any marine species and to compare among three species with different diving behaviors. PE of all types (LDPE, HDPE, and unknown PE), PP, and PE/PP mixtures comprised more than 90% of plastic debris ingested by these three species in the

Central Pacific indicating that they are interacting mainly with floating, lower density polymers. To reduce incidence of ingested plastics in sea turtles, mitigation should focus on these specific polymers. The success of mitigation measures (e.g., increased recycling programs for LDPE and PP) could be tracked over time with FT-IR polymer identification of marine debris. Accurate measurements of marine debris availability and bioaccumulation, including stratification within the ocean and geographic locations might improve knowledge of localized plastic debris types to target remediation and preventative strategies moving forward. These efforts will require interdisciplinary collaboration between oceanographers, meteorologists, marine biologists, materials scientists, and engineers to develop comprehensive understanding of how debris moves and settles in ocean environments, the lifetime of existing marine debris, and the complex impact of effects on the marine ecosystem.

■ ASSOCIATED CONTENT

● Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.est.8b03118](https://doi.org/10.1021/acs.est.8b03118).

Hook depths protocol, sag ratios and catenary angles; summary statistics of the mass and the composition of ingested polymers; hook depths of individual turtles and summary graphs; photo of grouped ingested plastic; correlations of % polymers versus hook depth; correlations of % polymers in olive ridleys versus sex, age class, and year; correlations of % polymers in olive ridley and green turtles separately versus SCL, latitude, and longitude. (PDF)

FT-IR spectral data (XLSX)

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Notes

Certain commercial equipment, instruments, or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose. Mention of products and trade names do not imply endorsement by the U.S. Government.

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