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Investigation of plastic debris ingestion by four species of sea turtles collected as bycatch in pelagic Pacific longline fisheries

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ABSTRACT

Ingestion of marine debris is an established threat to sea turtles. The amount, type, color and location of ingested plastics in the gastrointestinal tracts of 55 sea turtles from Pacific longline fisheries from 2012 to 2016 were quantified, and compared across species, turtle length, body condition, sex, capture location, season and year. Six approaches for quantifying amounts of ingested plastic strongly correlated with one another and included: number of pieces, mass, volume and surface area of plastics, ratio of plastic mass to body mass, and percentage of the mass of gut contents consisting of plastic. All olive ridley (n = 37), 90% of green (n = 10), 80% of loggerhead (n = 5) and 0% of leatherback (n = 3) turtles had ingested plastic; green turtles ingested significantly more than olive ridleys. Most debris was in the large intestines. No adverse health impacts (intestinal lesions, blockage, or poor body condition) due directly to plastic ingestion were noted.

1. Introduction

In 2010, an estimated 4.8 to 12.7 million metric tons of plastic waste was dumped in the ocean by 192 countries, and the amount of plastic entering the ocean is projected to increase by one order of magnitude by 2025 (Jambeck et al., 2015). The durability and lightweight nature of plastic means that it can be found in all the world's oceans (Barnes et al., 2009), far from its original source (Baztan et al., 2014). The highest concentrations of marine plastic debris are observed in subtropical latitudes and associated with large-scale convergence zones (Law et al., 2010).

To date, 557 species of marine organisms have either been entangled in or are known to ingest marine debris (Kühn et al., 2015). Ingestion of plastics is well documented in seabirds, sea turtles, and marine mammals and has been associated with malnutrition, because dietary nutrients can be diluted by consumed debris (dietary dilution), and mortality from gastrointestinal (GI) blockages or perforations (Kühn et al., 2015; McCauley and Bjorndal, 1999; Nelms et al., 2015; Santos et al., 2015). Quantifying the impact of plastic ingestion at the population level is difficult in marine species but is identified as a research priority (Vegter et al., 2014).

Globally, there are seven species of sea turtles; six are listed from vulnerable to critically endangered and one as data deficient on the International Union on the Conservation of Nature Red List (IUCN, 2017). All seven species have been documented to ingest plastic debris (Kühn et al., 2015). As a result, evaluating the impact of marine debris on sea turtle development, survivorship, health, and reproduction is a global research priority (Hamann et al., 2010; Nelms et al., 2015).

Assessing plastic ingestion in live turtles by lavage or through feces is difficult and can underestimate ingestion rates (Hoarau et al., 2014; Schuyler et al., 2014a; Seminoff et al., 2002). Necropsy is the most direct method to measure debris ingestion, but there can be biases between stranded dead animals and bycatch in fisheries (Casale et al., 2016). A proportion of stranded turtles are often diseased (Chaloupka et al., 2008), which makes it difficult to isolate the health effects of ingested plastic. Sea turtles that die after incidental capture and drowning in Pacific fisheries offer a less biased source to assess marine debris ingestion (Parker et al., 2005; Parker et al., 2011; Wedemeyer-Strombel et al., 2015; Work and Balazs, 2002) because these animals are presumably healthy. Some of the highest frequencies of debris ingestion for turtles to date were reported in sea turtle bycatch from Pacific longline fisheries (Wedemeyer-Strombel et al., 2015).

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Typical sea turtle bycatch in Pacific longline fisheries include olive ridley (*Lepidochelys olivacea*), green (*Chelonia mydas*), loggerhead (*Caretta caretta*) and leatherback (*Dermochelys coriacea*) sea turtles. Biological characteristics of these species, such as nesting origin and life stage, influence their migration, diet and amount of plastic ingested. Some of these characteristics have been studied in these pelagic turtles. Olive ridley turtles are known to spend the majority of their life cycle in the pelagic ocean (Bolten, 2003). They are considered to have the highest risk of ingesting plastics due to foraging on gelatinous zooplankton and fish, often in convergence zones which entrain floating plastics (Schuyler et al., 2016; Wedemeyer-Strombel et al., 2015). Ten olive ridley turtles captured in the Pacific longline fisheries were from nesting beaches in the East Pacific (67%) and West Pacific (33%), remained in the central pelagic Pacific, and made dives deeper than 150 m (Polovina et al., 2004). Green sea turtles are well known for their ontogenetic shift from omnivorous pelagic juveniles to primarily herbivorous benthic older juveniles (Bjorndal, 1997). These fisheries capture only immature green turtles (Parker et al., 2011; Work and Balazs, 2010) that are mainly carnivorous, feeding in the top 100 m, and have natal origins of the Hawaiian Islands (when captured north of Hawaii) or East Pacific (when captured south of Hawaii) (Parker et al., 2011). North Pacific loggerheads migrate from nesting beaches in Japan to foraging habitats in the Central North Pacific and/or eastern Pacific (Bowen et al., 1995; Briscoe et al., 2016; Peckham et al., 2011). Loggerhead turtles specifically captured in pelagic Pacific fisheries forage carnivorously close to the surface, primarily above 40 m, and are predominately from Japanese nesting beaches (Parker et al., 2005; Polovina et al., 2004). Leatherback turtles feed exclusively on jellyfish and other gelatinous organisms (Bjorndal, 1997). Two leatherbacks captured by the longline fisheries were genetically identified as coming from two disparate nesting regions in Indonesia and East Pacific (Dutton et al., 1998). Migrations of immature leatherbacks, like those sampled in the current study, are not known in the Pacific Ocean. However, satellite tracks of adults indicate that those nesting in the West Pacific forage in a variety of areas in the West Pacific or make trans-Pacific migrations to the west coast of North America (Bailey et al., 2012). In contrast, those nesting in the East Pacific head southwest but primarily stay in the East Pacific.

Types and amounts of plastic debris ingested by sea turtles are affected by species, life-history stage, and diet (Nelms et al., 2015; Schuyler et al., 2014a; Schuyler et al., 2016). Species differences are evident in studies assessing plastic ingestion by turtles incidentally captured in the Pacific longline fisheries. Green turtles (70% to 91%) and olive ridley turtles (82%) have the highest frequencies (Parker et al., 2011; Wedemeyer-Strombel et al., 2015) compared with loggerhead turtles (34.6%; Parker et al. (2005)) and only two leatherbacks assessed (0%, Wedemeyer-Strombel et al. (2015)). Elsewhere where larger sample sizes of leatherback turtles have been assessed, plastic ingestion frequencies range from 12% to 55% (Mediterranean Sea and Atlantic Ocean; reviewed by Nelms et al. (2015)).

Even though the understanding of plastic ingestion in sea turtles has advanced markedly in the past decade, all risk assessments and review articles on this topic are limited to frequency of occurrence (presence/absence data) instead of quantified amounts of ingested debris (Nelms et al., 2015; Schuyler et al., 2014a; Schuyler et al., 2016). Quantity is important, because a population with 100% ingestion of negligible amounts could be at less risk than a population with 20% ingestion of much larger amounts. Standardization of how to quantify ingested plastics is lacking (Casale et al., 2016).

Our goal was to expand on a prior study by Wedemeyer-Strombel et al. (2015) that used pelagic sea turtles captured as bycatch in the Hawaiian and American Samoan longline fisheries in order to: 1) quantify the amount of plastic debris pelagic Pacific sea turtles ingested from 2012 to 2016 using six different approaches (total number of pieces, total mass, volume and surface area, ratio of total plastics mass to body mass, and percentage of gut contents mass consisting of plastics); 2) assess types, colors, and locations of debris in the gastrointestinal (GI) tract; and, 3) test if amounts, types, colors, and location of debris in the GI tract vary by species, capture location, season, year, turtle length, sex, and body condition. The current study is novel, globally for sea turtles, in its comparison of six different approaches for quantifying plastic ingestion, which will encourage standardization of methods. Another novelty is the inclusion of correlations with body condition indices to begin to investigate malnutrition as a possible sublethal health impact. Finally, this study is novel in the Pacific Ocean for reporting the location of debris in the GI tract, which has been reported elsewhere from Florida, Brazil, and Sardinia (Bjorndal et al., 1994; Camedda et al., 2014; Jerdy et al., 2017) and can help to estimate the of timing of and migration distance since plastic ingestion (Camedda et al., 2014).

2. Methods

2.1. Sample and data collection

The U.S. National Oceanic and Atmospheric Administration (NOAA) Pacific Islands Regional Office (PIRO) uses observers on the Hawaiian and American Samoan longline fisheries. Bycatch from these fisheries between June 2012 and Feb 2016 included the 55 sea turtles (3 leatherback, 5 loggerhead, 10 green and 37 olive ridley sea turtles) sampled for this study. All were collected in the geographic area bounded by latitudes 16°S and 30°N, longitudes 138°W and 171°W (Fig. 1) and determined to be dead by specific criteria (Balazs et al., 1995). Loggerhead turtles were captured significantly further north and farther from the equator than olive ridley and leatherback turtles (Kruskal-Wallis with Wilcoxon multiple comparison tests, $p < 0.05$). Olive ridleys were captured further north and farther from the equator than leatherback turtles ($p < 0.05$), and green turtle capture latitudes were not significantly different than the other three species. Turtle carcasses were stored frozen and shipped to the NOAA Pacific Islands Fisheries Science Center in Honolulu, Hawaii. At necropsy, weight (kg) and straight carapace length (SCL in cm) were recorded. Body condition was classified by the attending pathologist as either poor, fair, good, or excellent based on the appearance of muscle and fat tissue in the inguinal region and under the plastron (Work, 2000). In addition, body condition index (BCI) was calculated as turtle mass (in kg) divided by the cube of SCL (in cm) and multiplied by 100,000 [body condition = -mass / (SCL^3) × 100,000] as described by Keller et al. (2004). The sex and size class of turtles were determined by visual examination of gross gonadal morphology and by SCL (See Supplemental material Table S1 for individual turtle measurements and body condition). Comprehensive necropsies entailed a complete external and internal exam of all organ systems, including histology of most organs, though not the tissues of the GI system, and tissue sampling for the Biological and Environmental Monitoring and Archival of Sea Turtle Tissues (BEMAST) project of the U.S. National Institute of Standards and Technology (NIST) Marine Environmental Specimen Bank (Keller et al., 2014).

The longline hook and any attached monofilament line were not included as marine debris in this study. Each section of the entire GI system from esophagus to rectum was opened sequentially and visually assessed and sampled for debris using methods described in Keller et al. (2014). The entire wet contents of the GI tract was carefully retained and weighed to the nearest g. Plastics were collected with hexane-rinsed forceps from each section of the GI tract, rinsed with MilliQ water, dried on aluminum foil overnight at room temperature, and total ingested plastics per turtle were weighed to the nearest 0.00001 g. Each plastic fragment was classified by color and consistency as hard plastic fragments, flexible sheet, flexible line/rope, net, nurdle or pellet, fabric, or foam. Measurements to the nearest mm of average length, width, and depth of each piece were used to estimate surface area using the equation for a rectangular box. Surface area estimations of all pieces consumed were summed per turtle. The location where the debris was

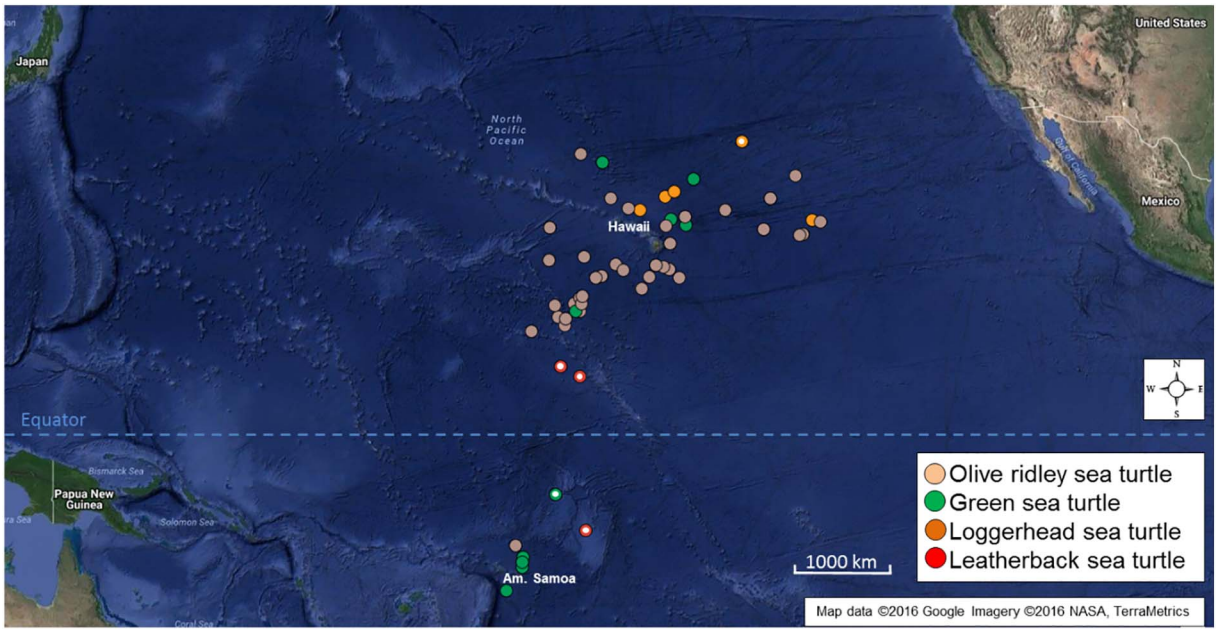

Fig. 1. Pacific pelagic longline capture locations of sea turtles sampled in this study. Olive ridley turtles (brown, n = 37), green turtles (green, n = 10), loggerhead turtles (orange, n = 5) and leatherback turtles (red, n = 3). Capture locations of turtles that had no ingested plastic are indicated with inner white circles. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

collected within the GI tract (esophagus, stomach, small and large intestines identified as described in Wyneken (2001)) was also recorded. Additionally, volume of total ingested debris was measured using water displacement in graduated cylinders with 1 mL or 2 mL increments.

2.2. Statistical analysis

For each turtle, we quantified number of pieces, mass, volume, and surface area of ingested debris along with grams of debris/kg of turtle (or body burden) and percent of wet mass gut contents that were debris. In addition, the percentages of each debris type and color were calculated for each turtle (Schuyler et al., 2012) as well as the percentage of occurrence in the esophagus, stomach, small intestine, and large intestine.

Prior to analysis, we tested variables for normality using the Shapiro-Wilk test. Because most data were not normally distributed, even with transformations, we used non-parametric statistical tests. Specifically, we used pairwise Spearman correlations to compare the six approaches to quantify ingested debris amounts and Kruskal-Wallis tests followed by Wilcoxon multiple-comparison tests to compare species for amounts and percentages of types, colors, and GI location of ingested plastic.

Because of species differences, additional statistical tests were performed separately on olive ridley and green turtles. For tests with olive ridleys, we excluded one exceptionally small, poor body condition turtle and used Spearman correlations to assess relationships between the plastic data (amounts and percentages of types, colors and GI locations) and turtle length (SCL), body condition index (BCI), latitude, absolute value of latitude as a measure of distance from the equator, longitude, and year. Likewise, we used Kruskal-Wallis tests to assess differences in plastic data between olive ridley size classes and sexes and among body condition categories and seasons. Capture location units were in decimal degrees. Seasons were defined as the Northern Hemisphere winter (December–February), spring (March–May), summer (June–August), and fall (September–November) for consistency with other studies (Wedemeyer-Strombel et al. (2015) and because all but one olive ridley turtle, the only species tested for seasonal differences, was captured in the Northern Hemisphere.

For green turtles, some of the plastic data were normally distributed, so we used Pearson correlations to assess relationships between the plastic amounts and green turtle SCL, BCI, latitude, absolute value of latitude, longitude, and year. We used Spearman correlations to assess relationships between the percentages of plastic types, colors, and GI location and these variables and Kruskal-Wallis tests or analysis of variance (ANOVA), depending upon assumptions of normality or homoscedasticity (tested by Shapiro-Wilk and O'Brien tests, respectively), to assess differences between sexes or body condition categories. The sample size of green turtles was too small to assess differences between size classes and seasons since all were immature with one individual captured in summer and two in spring. All analyses were carried out using JMP 11.0.0 (SAS Institute; Cary, NC) with a $p \leq 0.05$ considered significant. Results are presented as means ± one standard deviation (SD), unless otherwise noted.

3. Results and discussion

3.1. Turtle morphometrics

Olive ridley turtles (n = 37) averaged 57.8 ± 5.8 cm SCL and 27.5 ± 6.1 kg with 59% being adults. Green turtles (n = 10) averaged 43.9 ± 5.2 cm SCL and 12.0 ± 3.8 kg with all being pelagic juveniles. Two of the ten were the eastern Pacific green turtle morphotype (or black turtles). Loggerheads (n = 5) averaged 68.9 ± 3.8 cm SCL and 48.2 ± 9.6 kg, with two being adults, two subadults, and one of unknown size class. Finally, the leatherback turtles (n = 3) averaged 74.6 ± 13.3 cm SCL and 52.8 ± 23.8 kg, with all being immature.

All turtles were in good or excellent body condition, except for two green turtles in fair condition and one very small olive ridley in poor condition. The residuals of the regression of BCI to SCL were not correlated with SCL for either olive ridley or green turtles, so BCI was insensitive to turtle length. The use of BCI (similar to body mass index used in human healthcare) to quantify the nutritional health or quantity of fat reserves in sea turtles has been validated in loggerhead sea turtles (Barco et al., 2016). Body condition indices averaged 14.0 ± 1.0, 13.9 ± 1.0, 14.6 ± 0.9, and 12.0 ± 0.4 for olive ridley, green, loggerhead and leatherback turtles, respectively. Few studies are available to compare these body condition indices to those of other

Pacific sea turtles. Where comparisons can be made, these values are most similar to sea turtles that were found to be the most robust seasonally or that died acute deaths rather than from chronic disease. For example, the pelagic green turtles were more similar to the East Pacific green turtles from Baja California in the summer (13.7 ± 1.3) than those in winter (13.0 ± 1.1) (Koch et al., 2007). The pelagic green turtles were more similar to stranded Hawaiian green turtles that died from fishing-induced or boat strike trauma (12.9 ± 1.9) than infectious/inflammatory diseases (11.5 ± 1.7) (Work et al., 2015) or those with fibropapilloma tumors (means of 10.0, 10.7, and 11.3) (Work et al., 2004). The BCI of the pelagic loggerhead turtles were similar to apparently healthy loggerhead turtles from North Carolina (14.8 ± 1.5) (Keller et al., 2004) and to loggerheads that died from acute vessel or fishery interaction in Virginia (15.8 ± 1.9) (Barco et al., 2016). These comparisons suggest that the BCIs of pelagic turtles represent those of turtles with good to excellent body condition (as visually observed) and healthy animals elsewhere.

3.2. Ingested debris amounts

Ingested plastic was found in 50 of the 55 turtles evaluated (90.9%). All olive ridley turtles ingested plastic, followed by 90% of greens, 80% of loggerheads, and none of the immature leatherback turtles. These plastic ingestion frequencies were generally similar to or higher than those observed previously in pelagic Pacific sea turtles (see Table 1) (Parker et al., 2005; Parker et al., 2011; Wedemeyer-Strombel et al., 2015). Globally, few olive ridley turtles have been assessed, only in this region and off the coast of Brazil, and have consistently very high frequencies (≈ 100%) of plastic ingestion (Table 1). Green and loggerhead turtles from the pelagic Pacific have high plastic ingestion frequencies (35% to 100%) compared to more neritic aggregations across the Pacific Ocean (Table 1). Immature leatherback sea turtles (< 100 cm) are seldom encountered in the wild (Eckert, 2002) and plastic ingestion data on this size class are limited to only five individuals, including three from this study and two from Wedemeyer-Strombel et al. (2015) (Table 1). All immature leatherbacks incidentally taken in Pacific longline fisheries have had no evidence of ingested debris. Comparing the findings of the current and past studies (Table 1) to plastic ingestion assessed in future bycatch of pelagic Pacific sea turtles will help better address long-term time trends.

We found a total of 2880 pieces of debris across all turtles with an average of 52 ± 67 ingested pieces of debris per turtle (range: 0–314 pieces) (Supplemental material Table S1; Figs. S1–S3). The total mass was 645 g with a mean mass of 11.7 ± 17.4 g ingested debris per turtle (range: 0–71.4 g), a mean volume of 12.4 ± 17.8 mL per turtle (range: 0–83 mL), and a mean surface area of 420 ± 946 cm^2 per turtle (range: 0–6300 cm^2). Body burden (g plastic/kg turtle mass) averaged 0.659 ± 1.24 g/kg (range: 0–5.4 g/kg). The percent of total wet gut contents that consisted of plastic averaged 1.26 ± 2.00% (range: 0–8.16%).

Summary statistics of the six approaches to quantify plastic ingestion separated by species are shown in Table 2. It was difficult to compare these values to other published studies because 1) few studies quantified amounts beyond presence/absence, 2) studies used a variety of approaches, many of which could not be compared directly to any one of our six approaches, and 3) many studies inappropriately excluded turtles that had not ingested plastic, which greatly overestimates the population means. The few possible comparisons indicated that the pelagic Pacific olive ridley, green and loggerhead sea turtles ingest larger amounts than these species in other locations. For example, one olive ridley and one green turtle in Brazil ingested approximately four-times fewer pieces (10 and 20, respectively) (Mascarenhas et al., 2004) than the means in our study (41.5 and 93.8, respectively). Greens in Brazil ingested seven-times less mass of debris (mean 2.8 g) (Santos et al., 2015) than those in the pelagic Pacific (19.5 g). Greens in Florida ingested five-times less debris on a percentage of wet mass gut contents (0.52%) (Bjorndal, 1997) compared to the mean of pelagic Pacific greens (2.44%). Loggerheads, that had ingested plastic, in the Mediterranean Sea ingested 4–14 times fewer pieces (6 to 20 pieces) or 40–130 fold less mass (0.2–0.7 g) (Camedda et al., 2014; Casale et al., 2016) than the mean in our study (29.4 g) which included turtles that did not ingest plastic. All six approaches for quantifying plastic ingestion were correlated (Table 3; Supplemental material Fig. S4), suggesting all methods reasonably predict similar ingestion amounts per turtle. This is supported by a previous study in which ingested plastic mass and volume in green

Table 1
Frequency of occurrence of ingested plastic across selected sea turtle studies with a focus on the Pacific Ocean. Size classes are immature (I), juvenile (J), adult (A) or not available (N/A).

Species	Location	Ocean/water body	Sampling type	Size class	% occurrence	n	Reference
Olive ridley	Pelagic Pacific	N Central Pacific	Bycatch, necropsy	I & A	100	37	This study
Olive ridley	Pelagic Pacific	N Central Pacific	Bycatch, necropsy	N/A	82	45	(Wedemeyer-Strombel et al., 2015)
Olive ridley	Brazil	SW Atlantic	Stranding, necropsy	A	100	1	(Mascarenhas et al., 2004)
Olive ridley	Brazil	SW Atlantic	Stranding, necropsy	I & A	100	2	(Poli et al., 2014)
Green	Pelagic Pacific	N & S Central Pacific	Bycatch, necropsy	Pelagic J	90	10	This study
Green	Pelagic Pacific	N & S Central Pacific	Bycatch, necropsy	Pelagic J	91	22	(Wedemeyer-Strombel et al., 2015)
Green	Pelagic Pacific	N Pacific	Bycatch, necropsy	I & A	70	10	(Parker et al., 2011)
Green	Australia	SW Pacific	Stranding or found in fish stomach, necropsy	Post-hatchlings	66	47	(Boyle and Limpus, 2008)
Green	Peru	SE Pacific	Bycatch, necropsy	N/A	41.7	192	(Quinones et al., 2010)
Green	Baja California	Gulf of California	Stranding, necropsy	N/A	29	7	(Seminoff et al., 2002)
Loggerhead	Pelagic Pacific	N Central Pacific	Bycatch, necropsy	I & A	80	5	This study
Loggerhead	Pelagic Pacific	N Central Pacific	Bycatch, necropsy	I	100	2	(Wedemeyer-Strombel et al., 2015)
Loggerhead	Pelagic Pacific	N Central Pacific	Bycatch, necropsy	I & A	34.6	52	(Parker et al., 2005)
Loggerhead	Australia	SW Pacific	Stranding, necropsy	Pelagic J	57	7	(Boyle and Limpus, 2008)
Loggerhead	Baja California	NE Pacific	Stranding, necropsy	N/A	0	89	(Peckham et al., 2011)
Leatherback	Pelagic Pacific	N & S Central Pacific	Stranding, necropsy	I	0	3	This study
Leatherback	Pelagic Pacific	N Central Pacific	Stranding, necropsy	I	0	2	(Wedemeyer-Strombel et al., 2015)
Leatherback	Pacific Region (1968–2006)[a]	Various Pacific locations	Stranding and bycatch, necropsy	N/A	20[a]	10	(Mrosovsky et al., 2009)

[a] Using the table in Supplemental material of Mrosovsky et al. (2009) we calculated the % occurrence for 1968–2006 by tallying turtles reported during this time for any country that has a Pacific coastline.

Table 2
Amounts of plastic marine debris ingested by pelagic Pacific sea turtles. (SD = standard deviation; ND = not detected).

Species	Statistic	Pieces/turtle	Mass (g)/turtle	Volume (mL)/turtle	g plastic/kg turtle	% of wet gut contents mass	Surface area (cm^2)/turtle
Olive ridley (N = 37)	Mean ± SD	41.5 ± 56.0	6.80 ± 7.89	7.66 ± 7.24	0.293 ± 0.452	0.760 ± 1.40	170 ± 181
	Range	1–314	0–36.9	0.5–28.0	0–2.50	0–6.80	0.362–619
Green (N = 10)	Mean ± SD	93.8 ± 83.6	19.5 ± 17.2	37.4 ± 29.3	1.74 ± 1.75	2.44 ± 2.13	1386 ± 1909
	Range	0–248	0–44.7	0–83.0	0–4.97	0–6.58	0–6303
Loggerhead (N = 5)	Mean ± SD	81.6 ± 92.7	29.4 ± 33.8	6.67 ± 7.02	0.675 ± 0.803	2.28 ± 2.73	630 ± 900
	Range	0–201	0–69.3	0–14.0	0–1.67	0–6.12	0–2144
Leatherback (N = 3)	Mean ± SD	ND	ND	ND	ND	ND	ND
	Range	ND	ND	ND	ND	ND	ND

turtles were correlated (Santos et al., 2015). To help standardize methods, we encourage the collection of the number of debris pieces per turtle and body burden, because they are informative in different ways and are the easiest to measure and explain. These two approaches were the focus of the remaining statistical results in this study.

Considering number of debris pieces and body burden, immature green turtles ingested significantly more than olive ridleys and leatherbacks, and olive ridleys ingested significantly more than leatherbacks (Kruskal-Wallis Chi square > 11, df = 3, $p < 0.011$; Fig. 2). This finding suggests that immature green turtle foraging habitat or behavior places them at higher risk for eating marine plastic debris. The amounts ingested by loggerheads did not differ from the other three species, probably due to small sample size and large variability. We would expect loggerheads to ingest more plastic than olive ridleys, because loggerheads are known to associate with fronts and eddies, dive shallower, and spend less time at depth than olive ridleys (Polovina et al., 2004), putting them in closer proximity to floating plastic debris. A larger sample size of loggerheads may be needed to reveal significant species differences.

Because of species differences, we analyzed olive ridley and green turtles separately. For olive ridleys, no differences were seen between sexes or among size classes, but turtle length was negatively related to amounts of ingested plastic (Fig. 3). Correlations with sea turtle length have been inconsistent in the literature with the opposite trend seen for olive ridleys from the same capture region and technique (Wedemeyer-Strombel et al., 2015). The reason for the differences between studies is unclear, but the current sample set of turtles is 4 cm longer on average (58.5 cm excluding the smallest emaciated turtle) and more variable in length (3.4 cm standard deviation), which might influence the outcomes. Based on the negative correlation in the current study, we hypothesize that longer, more likely older, olive ridleys can dive deeper for longer time periods, which has been shown in other species of sea turtles (Salmon et al., 2004), and may reduce the amount of time they interact with floating plastic at the surface. The correlation may indicate an ontogenetic shift in diving and foraging behaviors between juvenile and adult olive ridley turtles.

The lack of relationship with BCI (Spearman rho < 0.03, $p > 0.8$) suggests minimal to no effect of ingested plastic on the nutritional status of these olive ridley turtles. Some could argue that this sampling and statistical approach is flawed because the possible cause and effect were measured at the same time. However, because most of the plastic was found within the large intestine (see results below), we estimate that these turtles ingested plastics several weeks prior to capture. We suspect that weight loss, if the plastics were to cause it, would have been detectable within this time frame, making it possible to see a correlation in this case.

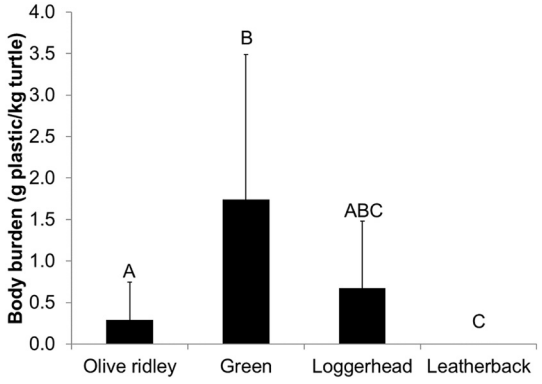

Fig. 2. Mean and standard deviation of plastic ingestion by species of pelagic Pacific sea turtles. Leatherback turtles had no plastic in their gastrointestinal tracts. Different letters above bars indicate significant differences between species (Wilcoxon each pair tests, $p < 0.05$).

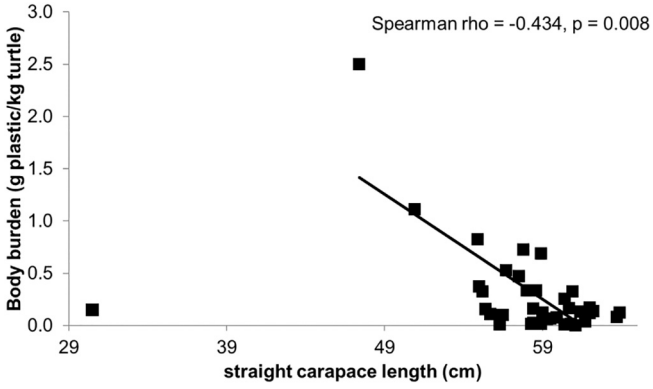

Fig. 3. Correlations between amounts of plastic debris ingested by pelagic Pacific olive ridley sea turtles and turtle length. Statistics are from a Spearman correlation and exclude the smallest, poor body condition outlier.

Within olive ridleys, we did not find seasonal differences, which have been observed previously (Wedemeyer-Strombel et al., 2015). No correlations were seen with capture latitude; however, two of the measures of debris ingestion amounts (percent of GI contents consisting

Table 3
Spearman correlations (rho, p-value) between six methods of measuring ingested plastic debris in pelagic Pacific sea turtles.

	Pieces	Mass (g)	Volume (mL)	Body burden (g/kg)	% of GI contents
Mass (g)	0.8589, < 0.0001				
Volume (mL)	0.8420, < 0.0001	0.9754, < 0.0001			
Body burden (g/kg)	0.8767, < 0.0001	0.9657, < 0.0001	0.9681, < 0.0001		
% of GI contents	0.8639, < 0.0001	0.9438, < 0.0001	0.9379, < 0.0001	0.9504, < 0.0001	
Surface area (cm^2)	0.8781, < 0.0001	0.8900, < 0.0001	0.8969, < 0.0001	0.9154, < 0.0001	0.8975, < 0.0001

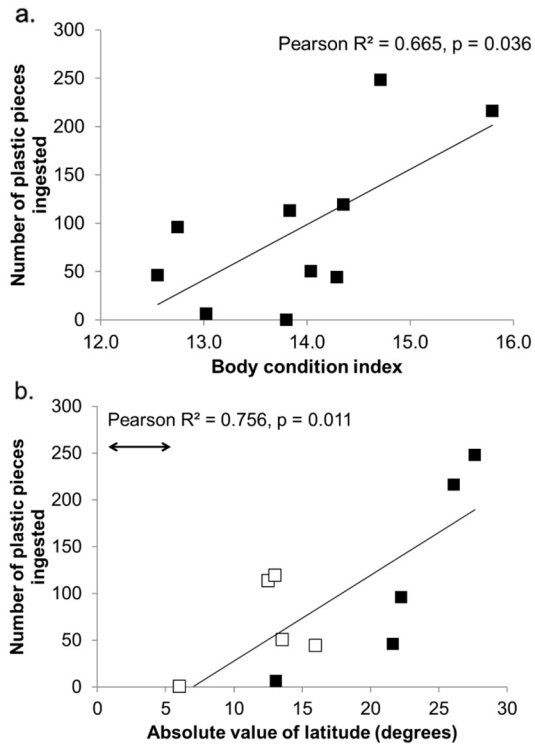

Fig. 4. Correlations of amounts of plastic debris ingested by Pacific pelagic immature green sea turtles with a.) body condition and b.) distance from the equator (empty points = Southern Hemisphere; filled points = Northern Hemisphere). Double arrow indicates the scale of 4.5° of latitude, which is the distance green turtles may travel over an estimated three weeks between plastic ingestion and capture.

of debris and surface area) negatively correlated with capture longitude, indicating turtles captured farther West ingested more plastic debris. Ingestion rates held steady from 2012 to 2016, as was seen during the 18 years prior to the current study (Wedemeyer-Strombel et al., 2015).

Within green turtles, the absolute value of latitude and BCI positively correlated with plastic ingestion amounts (Fig. 4). Green turtles captured further away from the equator (higher absolute value of latitude) ingested more plastic pieces (Pearson $r^2 = 0.756$, $p = 0.011$), which could be due to proximity to subtropical convergence zones in both hemispheres where debris is highly concentrated (Eriksen et al., 2013; Pichel et al., 2007). Why a similar result was not observed in olive ridley turtles is unknown, but may be due to the relatively narrower range of latitude and wider range of longitude in the olive ridley capture locations than the green turtles. Green turtles that ingested more plastic had higher BCIs (Pearson $r^2 = 0.442$, $p = 0.036$). This is contrary to what was expected, that ingested plastics may dilute their caloric intake from food (McCauley and Bjorndal, 1999) and possibly lead to weight loss. In Brazil, green turtles that died from fisheries' interactions had a higher BCI (10.9) compared to turtles that died from debris ingestion (8.2) (Santos et al., 2015). The reason for the unexpected correlation in this study may be that more robust turtles have a tendency to eat more, whether it is plastic or prey. Similar to the olive ridley turtle, our results suggest that ingested plastic had not detrimentally affected the body condition of these green turtles.

3.3. Ingested debris types

Across all turtles, plastic fragments accounted for the majority (79.5%) of debris items ingested, followed by sheets (12.5%), line/rope (6.1%), foam (1.7%), net, nurdles and fabric each accounting for < 0.2%. Composition of ingested debris differed among species (Fig. 5) with green turtles ingesting proportionally less fragments and more

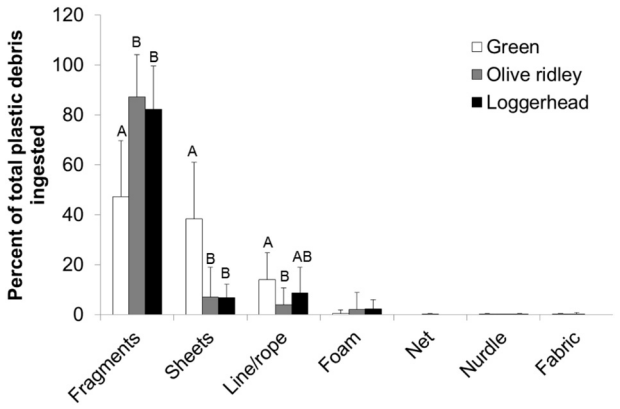

Fig. 5. Debris types ingested by three species of pelagic Pacific sea turtles. Data are the percentage of total plastic pieces consisting of each particular type ingested by each turtle, and shown as mean and standard deviation across turtles of each species. Turtles that did not consume plastic were excluded from this analysis. Different letters above bars indicate significant differences between species for that debris type (Wilcoxon each pair tests, $p < 0.05$).

sheets than olive ridleys and loggerhead turtles. Green turtles also ingested proportionally more line/rope than olive ridley turtles. The reasons for these species differences in composition are unknown but spatial differences or differences in foraging strategies could play roles. Half of the green turtles, compared to only one olive ridley, were captured in the Southern Hemisphere. It is possible this spatial separation exposed the green turtles to a different composition of available marine debris. In addition, young green turtles are known to feed shallower than olive ridleys in the pelagic environment (Parker et al., 2011; Polovina et al., 2004), so they may encounter or select different types of plastic because of availability at that depth or attraction to different types.

For olive ridley turtles, percentage of fragments and foam increased over time and sheets and line/rope decreased over time (the Spearman rho values were greater or equal to the absolute value of 0.330, $p < 0.05$). Among green turtles, debris types were correlated with latitude and longitude such that turtles captured further east, further north, and further from the equator ingested proportionally more fragments and less sheets (Spearman |rho0.667 ≤ |, $p < 0.05$). These relationships suggest that spatiotemporal differences in plastic types polluting different regions of the Pacific Ocean may exist, but to our knowledge, no marine debris study has specifically assessed the spatial distribution of different debris types across the Pacific Ocean.

3.4. Ingested debris colors

All sea turtles are primarily visual feeders with the ability to see color (Fritsches and Warrant, 2013) and are likely selective in what they consume (Schuyler et al., 2014b); however, whether color preference plays a role in plastic ingestion is controversial for sea turtles (Kühn et al., 2015). Benthic turtles have been observed to have a strong selectivity for clear, soft plastic, while pelagic turtles were much less selective (Schuyler et al., 2012). Captive green turtles were found to consume a variety of colors of latex but avoided clear pieces (Lutz, 1990).

For all turtles in this study, white plastic was the most abundant color ingested (59%) followed by blue, black, and clear pieces, each representing < 10% (Fig. 6). Previous studies have documented that light colored and clear plastic pieces dominate floating marine debris; thus, simply abundance and availability of particular colors may be more important than color selectivity by turtles (Kühn et al., 2015). White fragments also dominate beach debris surveys, and white and clear plastic were the most frequently ingested debris color by sea turtles near Queensland, Australia (Schuyler et al., 2012). Color

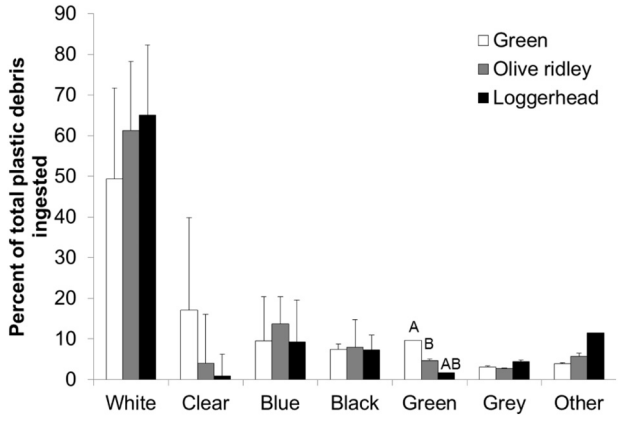

Fig. 6. Debris colors ingested by three species of pelagic Pacific sea turtles. Data are the percentage of total plastic pieces consisting of each particular color ingested by each turtle, and shown as mean and standard deviation across turtles of each species. "Other" colors include pink, orange, red and silver. Turtles that did not consume plastic were excluded from this analysis. Different letters above bars indicate significant differences between species for that debris color (Wilcoxon each pair tests, $p < 0.05$).

proportions were similar among species, except green turtles ingested proportionally more green pieces than olive ridleys (Fig. 6). Additional, less biologically relevant statistical findings ($p < 0.05$) are discussed in Supplemental material.

3.5. Debris location within the gastrointestinal tract

Ingested marine debris was predominantly found within the large intestine (75%) for all turtles and was significantly greater in the large intestine compared to the small intestine (14%) and stomach (10%) for all species (Fig. 7). Debris was found in the esophagus of only one turtles, so this portion of the GI tract was not included in statistical tests. Green turtles had a higher percentage of marine debris in the small intestine than olive ridleys (Fig. 7). Our findings are some of the first to document where anthropogenic debris is found within the gut. All four studies on this topic indicate that the majority of debris is found in the intestines and suggest that studies assessing only one portion of the GI system underestimate the amount of plastic ingestion (Bjorndal et al., 1994; Camedda et al., 2014; Jerdy et al., 2017). For example, previously only the stomach was assessed in 56% of the Pacific longline-captured olive ridley turtles (Wedemeyer-Strombel et al. (2015). We found no debris in the stomachs of 54% of the olive ridley turtles that had debris further down the intestines, which could explain the difference in the plastic ingestion frequency of olive ridley turtles between the previous (82%) and current (100%) studies (Wedemeyer-Strombel et al., 2015). Furthermore, relying only on stomach contents may grossly underestimate plastic ingestion in sea turtles. Future studies should examine the entire GI tract and record the location of plastics to better understand plastic ingestion and possibly timing of ingestion.

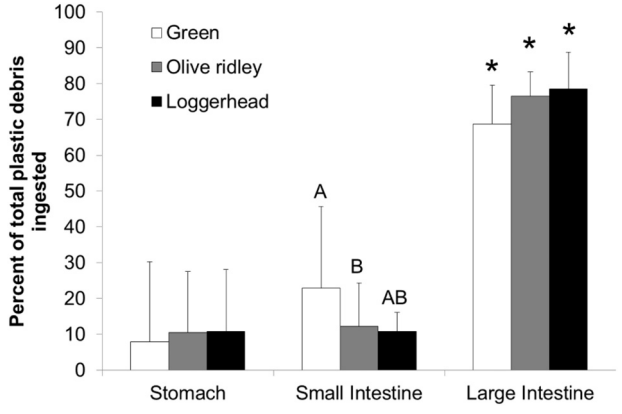

Fig. 7. Locations within the gut where ingested plastic pieces were found in three species of pelagic Pacific sea turtles. Data are the percentage of total plastic pieces found in each particular section of the gut for each turtle, and shown as mean and standard deviation across turtles of each species. Turtles that did not consume plastic were excluded from this analysis. Different letters above bars indicate significant differences between species for that GI location (Wilcoxon each pair tests, $p < 0.05$). Asterisks indicate significant differences from the other GI locations by species (Matched pairs t-tests or Wilcoxon, $p < 0.05$).

Few studies have measured transit time of ingested plastics in sea turtle guts. Wild green turtles held in an enclosure took an average of 23 ± 7 days to pass 3 mm × 1 mm plastic beads, which were meant to simulate the passage time of their natural prey (Amorocho and Reina, 2008). In captive green turtles, gut passage time of small Teflon markers (simulating natural prey) was found to be (10 to 13) days, compared to up to four months to pass (1 to 10) cm^2 latex plastic sheets (Lutz, 1990). The plastic sheets were occasionally found bound together in the feces (Lutz, 1990). These findings suggest that passage of debris with larger surface area through a sea turtle gut takes longer than that of digestible prey tissues, perhaps by an extra four or five weeks. This time estimate is consistent with the time to defecation of plastic debris from loggerhead turtles brought into rehabilitation: (6 to 41) days (Hoarau et al., 2014), up to 129 days with a simulated peak at 30 days (Casale et al., 2016), and most within 14 days but some after 30 days (Camedda et al., 2014). Size of the plastics likely also effects passage time with larger pieces taking longer to pass, which has been documented for gastric passage in dogs and reticulorumen passage in sheep (Itoh et al., 1986; Kaske and Vonengelhardt, 1990). We speculate that this effect could explain why green turtles in the current study, which ingested proportionally more slower-moving sheets, had proportionally more debris in their small intestines than olive ridley turtles.

The high percentage of debris found in the large intestine suggests that most turtles consumed debris at least a few weeks to months prior to capture. Furthermore, some turtles had debris only in their large intestines (41% of the olive ridley, 20% of the loggerhead and none of the green turtles). Since pelagic sea turtles are highly migratory and some time had passed between ingestion and capture, capture location may not reliably predict spatial plastic pollution. Researchers, therefore, should consider the spatial scale of turtle migration over time as was done in Camedda et al. (2014). The mean swimming speed of three satellite tagged green sea turtles incidentally captured in the Pacific longline fishery ranged from 0.4 km/h to 1.5 km/h with an average of 1 km/h (Parker and Balazs, personal communication, unpublished mapping data). If we assume the green turtles in the current study ate plastics three weeks before capture, they could have traveled on average approximately 500 km, equivalent to 4.5° of latitude in scale. Studies should consider the impacts of the scale of migration when doing spatial analyses like those shown in Fig. 4b.

3.6. Health impacts

All turtles from this study died by forced submergence in longline fisheries (Work and Balazs, 2002, 2010), and most were in good body condition at time of death. The lack of negative relationships between amounts of plastic ingested and BCI suggests that dietary dilution from eating the observed plastics had not yet led to detectable malnutrition. Likewise, the lack of impaction, ulceration, inflammation, perforation, or other gross lesions suggestive of pathology associated with foreign body ingestion also argues against plastics having a detrimental effect on the gastrointestinal tract of these sea turtles. This is substantially different than the findings of Santos et al. (2015) who reported that debris ingestion of < 2.5 g, or even 0.5 g, could lead to digestive tract blockage and death in stranded green turtles in Brazil. The curved carapace lengths (CCL) of the Brazilian green turtles were 38.0 ± 6.6 cm, which is 8.6 cm smaller than the pelagic Pacific green turtles (46.6 ± 5.3 cm CCL). The Pacific green turtles ingested

approximately eight-times more plastics (19.5 g) per turtle, but showed no visual signs of impacted health from ingested plastics. Two of these green turtles had fair body condition and had ingested 18.5 g and 4.1 g of debris, comprising 1.56% and 0.45% of their gut contents, without showing evidence of GI blockage or lesions. The turtle with the worst body condition was the smallest olive ridley with 1.04 g of ingested plastic or 0.350% of the gut contents with no GI tract pathology.

The methods used to assess the health of turtles in the current study have drawbacks that may have limited our ability to detect health effects. We did not assess biochemical or molecular sublethal health effects, nor could we follow the future survival or health outcomes caused by the ingested plastics in these turtles. Moreover, the GI tissues were not available for histology. For these reasons, we cannot rule out the possibility that the ingested plastics were in the process of causing health effects that went undocumented.

Chronic or sublethal health impacts of anthropogenic debris are poorly understood for sea turtles. We do know that post-hatchling loggerhead turtles do not compensate for lost nutrition from diluted foods by eating more (McCauley and Bjorndal, 1999), and ingestion of very small pieces of latex can interfere with blood glucose levels (Lutz, 1990). Furthermore, plastic marine debris is known to concentrate persistent organic pollutants from surrounding seawater (Mato et al., 2001). Thus, ingesting plastic may be an additional route of exposure to these toxic compounds (Clukey, 2016).

4. Conclusion

As marine plastic pollution increases and persists in the environment, understanding its effects on sea turtle populations is a high priority. Here, we expanded the sample size of pelagic Pacific sea turtles assessed for debris ingestion and found congruency among six different approaches to help standardize methods for quantifying ingested plastic amounts. Based on our results and ease of data collection, we encourage future studies to count the number of pieces ingested as well as calculate body burden. Because different sections of the GI tract contained different plastic amounts and knowing the GI location may help estimate when the turtle consumed the plastic, we highly suggest assessing the entire GI tract and recording the locations where debris are found. Comparing the four species, we found that pelagic juvenile green turtles ate the greatest amounts of plastic and proportionally more sheets and line. Conservation managers might be most interested in the threat of plastic ingestion on critically endangered Pacific leatherbacks. Of the 15 Pacific leatherbacks ever assessed in the literature (Mrosovsky et al., 2009; Wedemeyer-Strombel et al., 2015), none of the five immatures captured by longline fisheries ingested plastics. Although we observed a high percentage of plastic ingestion in this study (91% overall), we did not observe any adverse effect of debris ingestion.

Disclaimer

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.marpolbul.2017.04.064.

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