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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 light-weight 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 Bjørndal, 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 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 =  $\text{mass} / (\text{SCL}^3) \times 100,000$ ] 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

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