# ASSESSMENT OF PLASTIC INGESTION AND PERSISTENT ORGANIC POLLUTANTS IN SEA TURTLES ACROSS THE PACIFIC OCEAN

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**Abstract**

Plastic debris is a growing concern for many marine organisms due to entanglement, ingestion, and exposure to toxic chemicals. The increasing presence of micro and macro plastics in our environment threatens marine animals, especially sea turtles, as their tendency to investigate, pry into, or eat floating debris is substantial. I examined plastic ingestion rates and frequency of ingestion of 38 sea turtles [3 leatherback (*Dermochelys coriacea*), 3 loggerhead (*Caretta caretta*), 6 green (*Chelonia mydas*) and 26 olive ridley (*Lepidochelys olivacea*) sea turtles] that were incidentally captured in Hawaiian and American Samoan longline fisheries and quantified the amounts, types, sizes, and colors of ingested plastics in their gastrointestinal tracts. Additionally, I hypothesized that ingestion of plastic debris is a potential source of exposure of persistent organic pollutants (POP) to threatened pelagic sea turtles of the Pacific Ocean, by first providing baseline POP contaminant data for pelagic Pacific sea turtles and then correlating these data with plastic ingestion amounts. Ingested plastic was found in 87% (n = 33) of the turtles, with no plastic found in the 3 leatherback turtles, in 1 adult loggerhead, and in 1 juvenile green turtle. Mean dry mass of ingested plastic in all turtles sampled was 9.68 g with a range of 0.0185 g to 64.2 g amongst turtles that ingested plastic. The percentage of individual total gut contents comprised of plastic ranged from 0.00113% to 8.16% amongst turtles with ingested plastic and a mean of 1.01% in all turtles sampled. Juvenile green turtles ingested significantly more plastic than other species. Additionally, adipose samples from 25 of the turtles (2 loggerhead, 6 green, 17 olive ridley) were analyzed by gas chromatography/mass spectrometry for 83

polychlorinated biphenyls (PCBs), 20 organochlorine pesticides, 32 brominated flame-retardants and by liquid chromatography tandem mass spectrometry for hexabromocyclododecane (HBCD). I analyzed differences among species, sex, and correlations with turtle length and capture locations. Total dichlorodiphenyltrichloroethanes (DDTs) were the predominant POP in both loggerhead (mean = 18.3 ng/g wet mass) and olive ridley (15.8 ng/g wet mass) turtles, and the second highest POP class in green turtles (1.80 ng/g wet mass). Total PCBs were the predominant POP in green turtles (2.71 ng/g wet mass), yet they had lower total PCB concentrations than loggerhead (4.92 ng/g wet mass) and olive ridley (3.95 ng/g wet mass) turtles. Green turtles had the highest concentrations of α-HBCD (1.46 ng/g wet mass), which was the only detected HBCD isomer. Among olive ridley turtles, few sex differences were seen in POP concentrations, likely because sampled turtles were mainly juvenile. Concentrations of several POPs increased with straight carapace length of olive ridleys, suggesting bioaccumulation through age. A geographic gradient was observed with concentrations of several POPs increasing with capture latitude. Plastic ingestion is extremely common in sea turtles and effects of toxic chemicals could have detrimental effects on their health and survival. Amounts of ingested plastic were unrelated to POP concentrations, suggesting that sea turtle exposure to POPs is predominately through their natural food chain rather than from ingested plastics.

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# CHAPTER 1

## Introduction

Pollution in the environment is a growing concern on both land and at sea. Contamination of the ocean has accelerated dramatically in the past three centuries as industrial discharge and runoff from farms and coastal cities has increased. Many man-made pollutants such as pesticides, herbicides, chemical fertilizers, detergents, oil, sewage, plastics, and other solids collect in the ocean water (Ross and Birnbuam 2003; Gouin et al. 2004; Wania and Mackay 1995) and are transferred to marine organisms by many methods, predominately through the food chain. Man-made toxic chemicals have been linked to population declines and ecosystem imbalances (Fox 2001; Guillette et al. 1994).

Chemicals that persist for long periods in the environment have the ability to bioaccumulate in animal tissues causing toxic effects. Chemical contaminants with these characteristics that are also organic in structure (i.e. consist of a carbon backbone) have been termed persistent organic pollutants (POPs; United Nations Environmental Programme 2001). POPs are commonly found in environments far from their original source, with transport occurring via agricultural runoff (Ross and Birnbuam 2003), atmospheric circulation (Gouin et al. 2004), and ocean circulation (Wania and Mackay 1995). POPs have been associated with declines of a number of wildlife species (Fox 2001; Guillette et al. 1994), including Rachel Carson's famous description in her 1962 book *Silent Spring* (Carson, 1962), of highly toxic organochlorine insecticides, like dichlorodiphenyltrichloroethane (DDT), responsible for the thinning of bird eggs and contamination of human health. Her book and description of the toxic effects of these chemicals prompted the United States and some other countries to ban certain POPs in the 1970s and 1980s. In 2001 an international treaty known as the United Nations Stockholm

Convention on Persistent Organic Pollutants was signed naming 12 chemical classes as POPs considered to be too persistent, too bioaccumulative, and too toxic for continued widespread use; these including: aldrin, chlordane, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, toxaphene, PCBs, DDT, and polychlorinated dibenzofurans. Because of their persistent and bioacculuative nature, even with the ban or restriction of these chemicals in certain countries, wildlife species worldwide are still to this day found to have these chemicals in their tissues.

The world's oceans are home to seven species of sea turtles: flatback (*Natator depressus*), green (*Chelonia mydas*), hawksbill (*Eretmochelys imbricata*), Kemp's ridley (*Lepidochelys kempii*), leatherback (*Dermochelys coriacea*), loggerhead (*Caretta caretta*) and olive ridley (*Lepidochelys olivacea*). Every species, except for the flatback, inhabits U.S. waters and was listed as threatened or endangered under the Endangered Species Act (ESA) more than 40 years ago (US EPA 1974). Despite associated protection measures, no sea turtle species has recovered enough to be removed from the ESA and some populations continue to decline due to exploitation of their meat and eggs, habitat destruction (ba), interactions with fisheries (Lewison 2004), pollution and environmental contaminants (Keller et al. 2006). While no studies have directly investigated the effects of environmental pollutants on sea turtles at the population level, many scientists suspect that contaminants contribute to health problems, disease prevalence (Aguirre et al. 1994; Herbst and Klein 1995), altered embryonic growth (van de Merwe et al. 2010b), mortality or reduced reproductive success of sea turtles (Keller et al. 2004a;). While a handful of studies have provided correlative evidence that chemical pollutants may affect the health, survival, or reproduction of sea turtles, much more research and a weight of evidence

approach is needed to better understand the toxic effects and more importantly to provide resource managers information to determine mortality risk due to this threat (Keller 2013). Currently, numerous data gaps exist for baseline POP concentration levels on many sea turtle species, especially in the pelagic Pacific Ocean.

Recently, plastic has become an increasing threat that is now ubiquitous in the marine environment, affecting a wide range of taxa, from microscopic zooplankton to large vertebrates (Laist 1987). Plastic is a synthetic substance made of a range of organic polymers and because most forms of plastics are recalcitrant they often remain persistent in the environment, even though they are broken down by photo-degradation into smaller and smaller pieces that will never fully degrade (Jones 1974). Furthermore, plastic's lightweight constitution and widespread application in modern society means that plastic can be found in every continent and ocean on Earth (Barnes et al. 2009). Over the past 30 years the production of plastics has quadrupled, leading to a measurable increase in plastic pollution in the ocean (Barnes et al. 2009).

The risk of exposure to toxic chemicals through plastic ingestion is potentially dangerous to the health of an organism. Hazardous chemicals and POPs in the environment can adhere to the surface of the plastic (Mato et al. 2001). Specifically, the hydrophobic character of plastic fragments attracts POPs found in the ocean, resulting in higher concentration of POPs on the plastic fragments relative to the seawater (Andrady 2011). Plastic resin pellets found in the marine environment contain polycholorinated biophenyl (PCB), dichlorodiphenyldichloroethylene (DDE), pesticides, and polycyclic aromatic hydrocarbons (PAH) absorbed from ambient seawater (Carpenter et al. 1972; Mato 2001; Rios et al. 2010). Plastic's persistence and dispersal throughout marine

ecosystems has meant that the threat is growing, particularly for species of conservation concern, such as sea turtles.

Sea turtles have a variety of habitats, migratory behaviors, and complex life histories that place them at high exposure to environmental contaminates and marine plastic pollution. Sea turtles have been documented to commonly ingest marine debris, affecting up to 90% of individuals investigated (Balazs 1985; Laist 1997; Schuyler et al. 2014; Santos et al. 2015; Wedemeyer-Strombel et al. 2015). At various stages throughout sea turtles' lives, they may live and feed primarily in the open ocean, predominantly in neritic areas, or they may switch back and forth (Walker and Parmenter 1990; Bolten 2003; Godley et al. 2008; Rees et al. 2012). Turtles living in oceanic or coastal environments and feeding pelagically or benthically may encounter very different densities and types of marine debris and may therefore have different probabilities of debris ingestion (Schuyler et al. 2014).

Sea turtles in Hawaii and American Samoan are occasionally caught in the long-line fisheries and provide an opportunity to sample for contaminants. Species typically encountered in Pacific pelagic longline fisheries include olive ridley, green, loggerhead, and leatherback sea turtles. Of these species, olive ridley and leatherback spend their entire life cycle in the pelagic ocean (Carr 1987b; Plotkin 1995; Bolten 2003; Plotkin 2010). All species encountered in Pacific longline fisheries have a high chance for contact with marine debris due to their long distance migrations. Genetic analysis of Pacific longline caught olive ridleys has shown nesting populations from both western and eastern Pacific forage in the central North Pacific (Dutton et al. 1999). Loggerheads make Pacific oceanic migrations between their nesting beaches in Japan and Australia

and their foraging habitat in the eastern Pacific (Bowen et al. 1995). Green sea turtles exhibit a pelagic developmental stage where they spend time foraging in the open ocean as juveniles and subadults (Carr 1987b; Bjorndal 1997; Bjorndal 1999). Pelagic green turtles (30 cm to 70 cm curved carapace length) captured in Pacific longline fisheries consisted of two distinct morphotypes corresponding to the central Pacific and the eastern Pacific green turtle populations (Parker et al. 2011).

The highly migratory patterns for all sea turtle species encountered in Pacific long-line fisheries places them at high risk of exposure to a variety of toxic chemicals and anthropogenic debris. Two highly concentrated agglomerations of marine debris have accumulated in convergence zones in the Pacific Ocean, the Western Garbage Patch that occurs off Japan and the Eastern Garbage Patch that resides between Hawaii and California (NOAA 2012). The Eastern patch corresponds to locations of two sub-gyres within the North Pacific Gyre connected by a narrower band of marine debris north of the Hawaiian archipelago (NOAA 2012). Ingestion of plastic debris, most commonly in the form of fragments derived from larger plastic items, such as plastic bags and bottles, is thought to occur when marine debris is mistaken for prey or inadvertently through consuming other prey. In fact, 96% of the plastic found within the North Pacific were pieces smaller than 25 mm (Robards et al. 1997).

Sea turtles are primarily visual feeders, relying on visual cues over chemical cues when foraging (Southwood et al. 2007; Constantino and Salmon 2003). Leatherback turtles are the only species to feed exclusively on jellyfish and other gelatinous organisms (Shaver 1991; Bjorndal 1997). Clear plastic pieces can easily be mistaken for gelatinous prey, such that leatherbacks have been found more likely to ingest debris over the

carnivorous loggerheads and Kemp's ridleys (Schuyler et al. 2014). Globally, hawksbill turtles are most likely to ingest debris, followed green turtles (Schuyler et al. 2014). Juvenile, pelagic green turtles are mostly opportunistic, mainly carnivorous feeding at or near the surface, where most plastic floats (Parker et al. 2011). Olive ridley turtles captured in American Samoa and Hawaii pelagic longline fisheries were found to be opportunistic generalists consuming gelatinous zooplankton and fish and were found to ingest a high frequency of anthropogenic debris (Wedemeyer-Stombel et al. 2015).

Each sea turtle species plays an important role in ocean ecosystems by maintaining healthy seagrass beds and coral reefs, providing key habitat for other marine life, helping to balance marine food webs, and facilitating nutrient cycling from water to land (Bjorndal 1997). The conservation of sea turtle species is thus critical for the health of our oceans. A better understanding of pollution sources, factors influencing pollutant dispersal, toxicology, quantitative links between pollutant and impact, and how to evaluate the use of incentives to minimize pollution is of particular relevance (Hamann et al. 2010). The effects of small plastic debris on marine animals, including toxicity of pellets and fragments throughout the Hawaiian Archipelago, remain unknown (McDermid 2004). Data are needed to assess trophic dynamics of POPs via plastic throughout the marine food web. Currently, there is only a limited amount of data on POP concentrations of pacific sea turtles and fewer data on the transfer of POPs from plastic marine debris to higher order marine organisms, such as sea turtles.

Here, I aim to provide plastic ingestion rates and frequency of ingestion of four different species of pelagic Pacific sea turtles. I quantify the amounts, types, sizes, and colors of ingested plastics in the gastrointestinal tracts of 38 individual sea turtles.

Additionally, I hypothesize that ingestion of plastic debris is a potential source of exposure of persistent organic pollutants (POP) to threatened pelagic sea turtles, by first providing baseline POP contaminant data for pelagic Pacific sea turtles and then correlating these data with plastic ingestion amounts. I use these novel baseline POP contaminant data to provide spatial trends of contaminant levels in three species of sea turtles inhabiting the Pacific Ocean, by species, age class and sex.

# CHAPTER 2

## Plastic Ingestion by Sea Turtles Across the Pacific Ocean

Abstract

Plastic debris is a growing concern for many marine organisms due to entanglement, ingestion, and exposure to toxic chemicals, and recent studies have shown that debris ingestion by marine turtles is rising. My goal was to quantify the amounts, types, and colors of ingested plastics in the gastrointestinal tracts of 38 Pacific pelagic sea turtles [3 leatherback (*Dermochelys coriacea*), 3 loggerhead (*Caretta caretta*), 6 green (*Chelonia mydas*) and 26 olive ridley (*Lepidochelys olivacea*) sea turtles] that were incidentally captured in Hawaiian and American Samoan longline fisheries. Ingested plastic was found in 87 % (n = 33) of the turtles, with no plastic found in the 3 leatherbacks, 1 adult loggerhead, and 1 juvenile green turtle. I measured total mass of ingested plastic, volume of ingested plastic, total pieces of plastic debris ingested, the mass of ingested plastic relative to turtle body mass, and the percentage of total wet gut contents mass comprised of plastic. White and clear plastic fragments and sheets were most commonly ingested, with the majority of debris found within the lower intestines of all species sampled. The percentage of total gut contents comprised of plastic ranged from 0.00113% to 8.16% amongst turtles with ingested plastic and a mean of 1.01% in all turtles sampled. Amounts of ingested plastic were not correlated with capture location. Plastic ingestion rates differed among species, with green turtles ingesting significantly more plastic than other species. Plastic ingestion is extremely common in sea turtles and the need for global research of pollution impacts on marine wildlife is of high priority as the threat of plastic pollution on the marine environment is growing.

Introduction

In the year 2010, it was estimated that 4.8 to 12.7 million metric tons of plastic waste generated by 192 coastal countries entered the ocean, representing 1.5 % to 4.5 % of the world's total annual plastic production (Jambeck et al., 2015). Furthermore, it is predicted that plastic waste available to enter the ocean will increase by an order of magnitude by 2025 (Jambeck et al., 2015). The high 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). Recent estimates found that 557 species had either been entangled in or ingested marine debris (Kühn et al., 2015). Ingestion of plastics mistaken for food is well documented in seabirds, sea turtles, and marine mammals and in certain cases has been associated with mortality (Pierce et al., 2004; Jacobsen et al., 2010; Santos et al., 2015). The impact plastic ingestion has on the population level is difficult to quantify as it is impossible to accurately document each case of death caused by marine debris as animals can quickly drown at sea or be an easy target for predators. The need for global research of pollution impacts on marine wildlife is of high priority as the threat of plastic pollution on the marine environment is increasing (Vegter et al., 2014).

All six species of sea turtles listed on the IUCN Red list have been documented to ingest anthropogenic debris (Balazs, 1985; Laist, 1997; Schuyler et al., 2014; Wedemeyer-Strombel et al., 2015) and evaluating the impact of marine debris on their development, survivorship, health, and reproduction is a global research priority (Hamann et al., 2010; Nelms et al., 2015). Assessing plastic ingestion of live turtles is

difficult and often underestimates debris ingestion (Seminoff et al., 2002). As a result necropsy is the most effective method in measuring debris ingestion, but sample sizes are often limited to stranded dead turtles, which could be a biased sample. Sea turtles incidentally captured and drowned in Pacific longline fisheries offer an opportunity to opportunistically assess marine debris ingestion of these threatened species (Schuyler et al., 2015; Wedemeyer-Strombel et al., 2015). Wedemeyer-Strombel (2015) found some of the highest anthropogenic debris ingestion rates for pelagic Pacific sea turtles to date, however they had a mixture of turtle stomachs only and entire GI tracts, which underestimates total plastic ingestion.

Species typically encountered in Pacific pelagic longline fisheries include olive ridley (*Lepidochelys olivacea*), green (*Chelonia mydas*), loggerhead (*Caretta caretta*) and leatherback (*Dermochelys coriacea*) sea turtles. Of these species, olive ridley and leatherback turtles spend the majority of their life cycle in the pelagic ocean (Carr, 1987; Plotkin, 1995; Bolten, 2003; Plotkin, 2010). Loggerheads make Pacific oceanic migrations between their nesting beaches in Japan and Australia and their foraging habitat in the eastern Pacific (Bowen et al., 1995). Green sea turtles exhibit a pelagic developmental stage where they spend time foraging as mainly carnivorous juveniles and subadults in the open ocean until their ontogenetic shift to nearshore habitats and become herbivorous (Carr, 1987; Davenport and Balazs, 1991; Bjorndal, 1997; Bjorndal, 1999). Pelagic green turtles (30 cm to 70 cm curved carapace length) captured in Pacific longline fisheries consist of two distinct morphotypes corresponding to the central Pacific and the eastern Pacific green turtle populations (Parker et al. 2011).

At various stages throughout their lives, sea turtles may live and feed primarily in

the open ocean, predominantly in neritic areas, or they may switch back and forth (Walker & Parmenter, 1990; Bolten, 2003; Godley et al., 2008; Rees et al., 2012). There would be an assumption that sea turtles living in or near oceanic and coastal environments associated with high accumulation of anthropogenic debris would have a higher frequency of ingestion. However, research has found that ingestion of debris is more related to species life-history stage and diet (Balazs, 1985; Plotkin and Amos, 1990; Schuyler et al., 2012). Green turtles around the world are found with high frequency of ingested debris (Balazs, 1985; Schuyler et al., 2014; Santos et al., 2015; Wedemeyer-Strombel et al., 2015). As pelagic juveniles, green turtles are opportunistic, mainly carnivorous, feeding at or near the surface (Parker et al., 2011). Throughout their life, olive ridley turtles feed as opportunistic generalists, consuming gelatinous zooplankton and fish (Wedemeyer- Strombel et al., 2015). Additionally, pelagic Pacific olive ridleys incidentally caught on longlines have been found with a high frequency of anthropogenic debris ingestion (Wedemeyer-Strombel et al., 2015). Pelagic Pacific loggerheads are mainly carnivorous feeding at the surface (Parker et al., 2005) but will actively forage at deeper depths if high densities of prey are available (Polovina et al., 2003). Globally, loggerheads have been found with a lower frequency of anthropogenic debris ingestion compared to green and olive ridley turtles (Schuyler et al., 2014; Wedemeyer-Strombel et al., 2015). Leatherback turtles are the only species to feed exclusively on jellyfish and other gelatinous organisms (Bjorndal, 1997). In debris ingestion studies reviewed by Nelms et al. (2015), 12-100 % of leatherbacks sampled per study ingested plastic, which is thought to be due to similarities to prey items. However, juvenile Pacific leatherbacks had a low frequency of anthropogenic debris ingestion (Wedemeyer-Strombel et al.,

2015).

Sea turtles are primarily visual feeders. Turtles have a well-developed visual system with at least three different photopigments, indicating the ability to see color (Fritsches and Warrant 2013). Their ability to find food is based more on visual cues rather than chemical cues (Southwood et al., 2007; Constantino and Salmon, 2003). Sea turtle visual pigments are slightly shifted towards shorter wavelengths, in response to the clear, oceanic waters in which they live (Lythgoe, 1979). Investigation of debris selectivity of sea turtles in both benthic and pelagic habitats revealed benthic turtles had a strong selectivity for soft, clear plastic, which has a resemblance to their natural gelatinous prey, while pelagic turtles were much less selective (Schuyler et al., 2012). Debris color preference leaned towards white and clear/translucent particles, however nearby beach surveys of marine debris revealed white plastic as the most abundant debris color. Blue plastic was the second most abundant color found in beach surveys, yet the fact that both benthic and pelagic turtles select against blue could indicate that blue plastics are less visible against the blue background (Schuyler et al., 2012).

In this study, I aimed to (i) quantify amounts of ingested debris in pelagic sea turtles inhabiting the Pacific Ocean surrounding Hawaiian and American Samoan waters using old and new methods of measuring ingested marine debris (mass (g) of ingested plastic per mass (kg) turtle and plastic mass per mass of total wet GI contents) as a way to assist with long term data collection of debris ingestion rates of marine life; (ii) assess types, colors and locations in the GI of debris; (iii) identify which sea turtle species ingests more marine debris in the pelagic Pacific Ocean; (iv) determine geographical trends in ingested debris amounts; and, (v) determine if ingested plastic amounts affect

turtle body condition. There is a great importance to continue collecting marine debris ingestion rates to understand its impact over time as the threat of marine debris is expected to increase.

## Methods

### *Sample collection*

The U.S. National Oceanic and Atmospheric Administration (NOAA) Pacific Islands Regional Office (PIRO) uses 100 % observer coverage on the Hawaiian and American Samoan longline fisheries to collect fisheries catch and bycatch data. Between June 2012 and May 2015, 38 marine turtles (3 leatherback, 3 loggerhead, 6 green and 26 olive ridley sea turtles) incidentally taken as bycatch in these fisheries and determined dead by specific criteria (Balaz et al., 1995) were used for this study. Observers recorded the capture latitude and longitude as well as straight carapace length (SCL). Turtles were stored frozen and sent to NOAA Pacific Islands Fisheries Science Center in Honolulu, Hawaii for necropsy. Turtle weight (kg) and additional length measurements were taken and necropsies were performed. Body condition at necropsy was classified as either poor, fair, good, or excellent based on the appearance of muscle and fat tissue in the inguinal region and under the plastron. Body condition index was calculated as weight (kilograms) divided by the cube of SCL (centimeters) and multiplied by 100,000 [body condition = $\text{weight}/(\text{SCL}^3) \times 100{,}000$] as described by Bjorndal et al. (2000). The sex and age class of turtles was determined by visual examination of gross gonadal morphology. Gross necropsies entailed a complete external and internal exam of all organ systems, including histology of most organs, 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 entire gastrointestinal (GI) system was analyzed from esophagus to colon for presence of anthropogenic objects using methods described in Keller et al. (2014). The GI tract was opened over a plastic bin to allow all gut contents to fall into and be collected and weighed (to the nearest gram). Subsamples of prey and digesta were collected in aluminum foil and glass jars (respectively) then weighed wet (to the nearest gram) and archived by BEMAST. As anthropogenic debris was encountered, it was removed with hexane-rinsed forceps and rinsed with MilliQ water. Each separate piece of debris was tallied and classified by size (cm), color, type: hard plastic fragment (derived from larger plastic debris); flexible plastic sheet; flexible plastic line; nurdle or smooth hard plastic pellet; fabric; or foam. The location where the debris was collected within the GI tract was recorded. Plastics were left to dry in aluminum foil overnight at room temperature before being weighed to the nearest 0.0001 g. Additionally, using water displacement in graduated cylinders with 1 mL or 2 mL increments, volume of total ingested debris was measured.

*Statistical Analysis*

The relative percent abundance of debris type and color ingested by each turtle was calculated using methods outlined by Schuyler et al. (2012). The total percentage of occurrence in the stomach, upper intestine, and lower intestine was calculated for each turtle and the percentage of occurrence was averaged using methods outlined by Schuyler et al. (2012). Using total mass of ingested debris for each turtle, grams of ingested plastic

per kilogram turtle was calculated. The percentage of total GI contents (total wet mass) that consisted of plastic (dry mass) was determined.

All five methods of measuring ingested debris (total number of debris pieces ingested, mass of ingested debris, volume of ingested debris, grams of ingested plastic per kilogram turtle and the percentage of total GI contents that consisted of plastic) were square root transformed and compared using pairwise correlations.

I used grams plastic per kilogram turtle mass and the percentage of the total GI wet contents that consisted of plastic as the most representative measures of plastic ingestion related to species differences, animal capture location and body condition index. These methods of measurement could provide more of an insight into the burden ingested debris has on an animal by taking into account the animal mass and percentage of the total gut fill.

I tested for differences in the amounts of ingested debris (grams plastic per kilogram turtle and the percentage of the GI that consisted of plastic) using ANOVA with a Tukey-Kramer post-hoc comparison. I did not test for sex or age class differences because most species categories are represented by just one sex or age category. Spearmans correlations were performed between animal capture latitude (decimal degrees), longitude, or turtle BC Index and both grams plastic per kilogram turtle and the percentage of total GI contents that consisted of plastic. All analyses were carried out using JMP Pro 12 (http://www.jmp.com/en_us/software/jmp-pro.html; Cary, NC) with a $p \leq 0.05$ considered significant. Results are presented as means ± SD, unless otherwise noted.

## Results and Discussion

### *Sample collection*

The turtles were captured between latitude 13.5 °S and 29.6 °N and longitude 140 °W and170 °W (Figure 2.1). Olive ridley turtles averaged 57.7 ± 6.34 cm SCL and 27.6 ± 6.24 kg with 70% of the turtles classified as adults. All six green turtles in the study were classified as immature with a mean SCL of 41.4 ± 3.14 cm and weight of 10.3 ± 2.12 kg. Two of the loggerhead turtles were classified as adults and one was listed as unknown, loggerheads averaged 70.6 ± 3.96 cm SCL and 51.8 ± 11.6 kg. The three leatherback turtles were all immature and averaged 84.1 ± 13.3 cm SCL and 52.8 ± 23.8 kg (Table 2.1).

Of the 38 turtles in this study, only five did not ingest any noticeable anthropogenic debris. Olive ridley turtles had a 100 % occurrence of ingestion of anthropogenic debris, followed by 85 % occurrence in greens, 67 % occurrence in loggerheads, and 0 % in the immature leatherback turtles. High ingestion rates in marine turtles was expected, however this study revealed ingestion rates slightly higher in both olive ridley and green sea turtles from the Pacific Ocean (Parker et al., 2011; Schuyler et al., 2014; Wedemeyer-Strombel et al., 2015). Immature leatherback sea turtles (<100 cm) are seldom encountered in the wild (Eckert, 2002) and plastic ingestion data on this age class of leatherback turtle is limited (Nelms et al., 2015). Additionally, two immature leatherbacks from Pacific longline fisheries were also found with no anthropogenic debris (Wedemeyer-Strombel et al., 2015).

### *Debris types*

When all turtles were combined, plastic fragments accounted for the majority (79 %) of debris items ingested, followed by plastic sheets (14 %), plastic line (6 %) and foam, nurdles, and fabric pieces each accounting for less than 1 %. Olive ridleys ingested the highest percentage of plastic fragments (87 %) followed by loggerhead turtles (73 %). Green turtles ingested the highest percentage of plastic sheets (37 %), while both olive ridley and loggerhead turtles ingested less than 12 % (Figure 2.2). No significant differences were observed.

*Debris colors*

White plastic pieces were the most abundant color ingested by all turtles (62 %), followed by blue and then clear pieces, with both representing <10 % for all turtles combined. White pieces were the highest percent of total plastic ingested for all three species with visually few differences among the groups. Clear plastic and blue plastic were the next most abundant debris colors by all turtles, with green turtles ingesting a higher percentage of clear plastic than the other two species (Figure 3.2). White plastic has also been identified as the most frequently ingested debris color by marine turtles near Queensland, Australia. However, it is difficult to conclude if sea turtles are preferentially selecting white plastic, because both selectivity by sea turtles in other studies and predominance of white plastic fragments found in beach debris surveys have been found (Schuyler et al., 2012). Other studies have shown a lower frequency of ingested blue plastic in pelagic sea turtles (<5 %; Schuyler et al., 2012), whereas this study of pelagic longline caught sea turtles shows a slightly higher frequency of ingestion (3 % to 16 %) for this color.

*Debris location within the GI*

Ingested marine debris was predominantly found within the lower intestine for all turtles in this study, with 81 % found in the lower intestine of all turtles compared to 9.28 % in the upper intestine and 9.77 % in the stomach. Loggerhead turtles had the highest percentage of marine debris in the lower intestine (90.4 %), whereas olive ridleys averaged 82.2 % of occurrence of debris in the lower intestine and green turtles 70.9 % (Figure 2.4). No anthropogenic debris was found in the upper intestine of the three loggerhead turtles examined. This is the first study to document the location within the GI anthropogenic debris is found. Amorocho and Reina (2008) documented an average of 23 days for green turtles to pass food through their guts. Even though is difficult to determine how quickly or smoothly anthropogenic debris passes through the GI tract of sea turtles, the higher percentage of debris found in the lower intestine of all species could imply that the turtles have a difficult time eliminating debris. For normally feeding green turtles, retention of plastic in the GI tract could last for nearly four months (Lutz, 1990). Wedemeyer-Strombel et al. (2015) documented turtles of the same species, age class and capture ranges to also be found with high occurrence of anthropogenic debris, but they used a mixture of only stomachs and entire GI tracts which could be underestimating plastic ingestion because in this study we report highest occurrence of debris located within the lower intestine. There seems to be a great importance for future studies to collect the entire GI tract and record location of where plastic are found in order to get a better representation of plastic ingestion rates for marine species.

*Methods of measurement*

Pairwise correlations between all measurements of plastic ingestion were found to be correlated (Table 2.2). Combined, turtles in this study ingested a total of 1,795 pieces of debris and averaged 47 ± 56.3 ingested pieces of debris per turtle. Total debris items collected accounted for 368 g among the 38 turtles analyzed with a mean weight of 9.68 ± 14.0 g ingested debris per turtle (range: 0.00 – 64.2 g). Volume of ingested debris average 12.7 ± 17.9 mL (range: 0.00 – 83 mL). Grams plastic per kilogram turtle mass averaged 0.657 ± 1.39 (range: 0.00 – 5.4). The percentage of the total GI wet contents that consisted of plastic averaged 1.01 ± 1.73 % per turtle (range: 0 – 8.16).

*Species differences*

Juvenile green turtles ingested significantly more grams plastic per kilogram turtle than olive ridley, loggerhead, and leatherback sea turtles ($p < 0.005$). Juvenile green turtles also had significantly higher percentage of the total GI contents comprised of plastic than the other three species ($p < 0.005$; Figure 2.5). No significant differences were seen among the other species. Other studies have found similar results in comparisons of debris ingestion between species with green and olive ridley turtles ingesting high amounts of debris (Wedemeyer-Strombel et al., 2015). However it seems that green turtles are less selective in their feeding than olive ridley turtles or are located in areas with higher plastic debris exposure.

*Capture location*

Turtles in this study captured further north are in closer proximity to known areas of high debris concentration in the subtropical convergence zone in the Pacific Ocean (NOAA, 2015). However, our data analysis did not reveal any significant relationships between turtle capture latitude and mass or volume of ingested debris. Rather, we saw a negative relationship in both grams plastic per kilogram turtle ($r = -0.008$) and percentage of the total GI contents that consisted of plastic (-0.04) most likely influenced by four green turtles captured in southerly American Samoan fisheries. Statistical analysis has already revealed that green turtles ingested more plastic than other pelagic species in this study, therefore slightly influencing the correlation between amounts of ingested debris and capture location.

*Body condition index*

No significant relationships were found between ingested plastic (grams plastic per kilogram turtle mass and percentage of the total GI contents that consisted of plastic) and body condition index among each species. Turtles from this study all died incidentally in longline fisheries and were in good body condition at time of death. The lack of relationships suggests that the ingested plastics were not having sublethal effects, like dietary dilution leading to malnutrition. I also did not observe blockage of the GI tract or lesions associated with plastic ingestion in this turtles. Sublethal effects of anthropogenic debris are not well-known, but recently it has been hypothesized that the fatal effects of debris ingestion are often not seen before death in fisheries bycatch (Santos et al., 2015). Santos et al. (2015) found that nearly half of the stranded green turtles in Brazil died as a direct result of marine debris ingestion. A mass of ingestion of

marine debris less than 2.5 g, with a critical amount of only 0.5 g, was reported to cause death by digestive tract blockage in that study. Whereas, equally sized green turtles in our study ingested on average 30.3 g of debris and showed no visual signs of decreased health. Only one green turtle was described as "fair" body condition, but its BC index of 13.8 was only slightly lower than the average green turtle BC Index of 14.4. Besides for eosinophilic material and clumps of edicular cells in the lungs, all other organs showed no remarkable lesions indicating decreased health in this turtle. This particular turtle ingested 18.5 g of debris comprising 1.56 % of the turtle's total GI contents without showing evidence of GI blockage or lesions.

## Conclusion

Plastic ingestion is a serious threat to sea turtles as sea turtles are one of the species most commonly affected by marine debris (Laist, 1997). As marine pollution increases and persists in the environment, understanding its effects on populations is of high priority. I provide important debris ingestion rates for several species of marine turtles across the Pacific Ocean showing a high percentage of occurrence (87 %) in pelagic Pacific sea turtles. I provide important ingestion data on amounts and occurrence of types and colors of anthropogenic debris in sea turtles to aid in future research and conservation of these protected species. I found that pelagic juvenile green turtles are most vulnerable to the threat of marine debris ingestion although it is unclear the effects that this will have on the fitness of individuals or on the population level. There clearly is a great importance to continue monitoring marine species interactions with anthropogenic

debris with standardized methods and I determined five methods (one being novel to the field) are all correlated to each other.

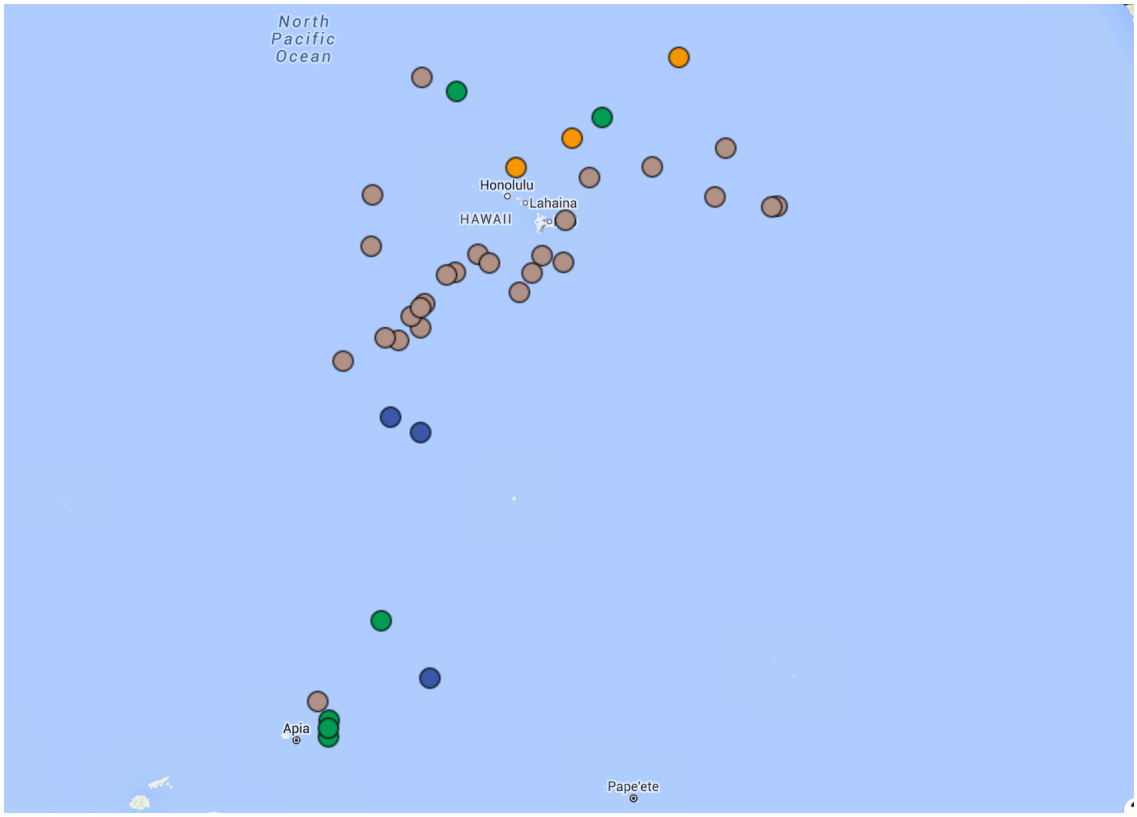
Table 2.1. Information about sea turtles caught in the Hawaiian and American Samoan pelagic Pacific longline fisheries. Ages are immature (I) and adult (A) or unknown (U). Body condition (BC) is classified as poor (P), fair (F), good (G) or excellent (E)

| ID | Species | Capture Date | Mass (kg) | SCL (cm) | Age | Sex | BC | BC Index | Total Pieces of Plastic | Mass of ingested plastic (g) | Volume of ingested plastic (mL) | g plastic/ kg turtle | Percent GI contents plastic |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LL456601 | *C. caretta* | 3/11/13 | 60.8 | 72.9 | A | F | E | 15.7 | 26 | 11.3 | 14.0 | 0.186 | 0.871 |
| LL475601 | *C. caretta* | 10/27/13 | 55.9 | 72.8 | A | M | E | 14.5 | no plastic | no plastic | no plastic | no plastic | no plastic |
| LL520119 | *C. caretta* | 2/18/15 | 38.8 | 66 | U | M | G | 13.5 | 19 | 4.91 | 6 | 0.127 | 0.299 |
| LL476104 | *C. mydas* | 11/1/13 | 8.60 | 37.9 | I | M | E | 15.8 | 216 | 41.6 | 50.0 | 4.83 | 3.81 |
| LL480011 | *C. mydas* | 12/28/13 | 9.00 | 39.4 | I | F | E | 14.7 | 248 | 47.5 | 60.0 | 5.28 | 4.62 |
| AS015728 | *C. mydas* | 12/9/13 | 13.4 | 45.7 | I | F | G | 14.0 | 50 | 10.2 | 14.0 | 0.763 | 5.35 |
| AS016421 | *C. mydas* | 3/22/14 | 8.00 | 38.7 | I | M | G | 13.8 | no plastic | no plastic | no plastic | no plastic | no plastic |
| AS015316 | *C. mydas* | 8/5/13 | 11.0 | 43.0 | I | M | F | 13.8 | 113 | 18.5 | 36.0 | 1.68 | 1.56 |
| AS015808 | *C. mydas* | 10/27/13 | 11.9 | 43.6 | I | F | G | 14.4 | 119 | 64.2 | 83.0 | 5.40 | 8.16 |
| LL501901 | *D. coriacea* | 7/21/14 | 26.1 | 59.4 | I | U | G | 12.4 | no plastic | no plastic | no plastic | no plastic | no plastic |
| AS014925 | *D. coriacea* | 5/5/13 | 71.8 | 84.1 | I | F | G | 12.1 | no plastic | no plastic | no plastic | no plastic | no plastic |
| LL529603 | *D. coriacea* | 4/22/15 | 60.4 | 80.4 | I | F | G | 11.6 | no plastic | no plastic | no plastic | no plastic | no plastic |
| LL445715 | *L. olivacea* | 12/7/12 | 3.90 | 29.9 | I | F | P | 14.8 | 21 | 1.04 | 2.00 | 0.265 | 0.350 |
| LL444515 | *L. olivacea* | 12/2/12 | 26.9 | 58.0 | A | F | G | 13.8 | 45 | 9.61 | 12.0 | 0.357 | 0.579 |
| LL431606 | *L. olivacea* | 6/13/12 | 24.8 | 55.0 | A | F | E | 14.9 | 36 | 8.07 | 12.0 | 0.325 | 0.485 |
| LL431609 | *L. olivacea* | 6/16/12 | 31.6 | 58.4 | A | F | G | 15.9 | 5 | 0.790 | 2.00 | 0.0250 | 0.0423 |
| LL445510 | *L. olivacea* | 11/28/12 | 35.0 | 62.2 | A | F | E | 14.5 | 18 | 4.72 | 7.00 | 0.135 | 0.352 |
| LL441507 | *L. olivacea* | 10/14/12 | 29.3 | 58.9 | A | F | G | 14.3 | 46 | 20.3 | 28.0 | 0.693 | 1.62 |
| LL450502 | *L. olivacea* | 1/18/13 | 27.2 | 60.4 | A | F | G | 12.3 | 51 | 6.89 | 10.0 | 0.253 | 0.570 |
| LL452515 | *L. olivacea* | 2/11/13 | 33.0 | 62.0 | A | F | G | 13.8 | 32 | 5.75 | 8.00 | 0.174 | 0.480 |
| AS013413 | *L. olivacea* | 10/20/12 | 28.6 | 61.1 | A | F | G | 12.5 | 1 | 0.019 | <1 | 0.000647 | 0.00113 |
| LL458504 | *L. olivacea* | 4/6/13 | 24.9 | 55.2 | I | M | E | 14.8 | 21 | 8.61 | 12.0 | 0.346 | 0.403 |
| LL461308 | *L. olivacea* | 5/12/13 | 22.2 | 56.5 | I | M | E | 12.3 | 15 | 2.23 | 3.00 | 0.101 | 0.104 |
| LL460203 | *L. olivacea* | 4/22/13 | 30.2 | 59.3 | A | F | E | 14.5 | 10 | 1.63 | 2.00 | 0.0539 | 0.107 |
| LL474511 | *L. olivacea* | 10/24/13 | 29.7 | 59.9 | A | F | G | 13.8 | 10 | 2.22 | 3.00 | 0.0747 | 0.138 |
| LL468213 | *L. olivacea* | 8/12/13 | 28.6 | 61.7 | A | M | G | 12.2 | 5 | 1.11 | 1.00 | 0.0387 | 0.0675 |
| LL469204 | *L. olivacea* | 8/8/13 | 30.8 | 60.8 | A | F | G | 13.7 | 94 | 0.360 | 1.00 | 0.0117 | 0.0280 |
| LL477006 | *L. olivacea* | 11/23/13 | 25.5 | 57.5 | A | F | E | 13.4 | 97 | 11.2 | 14.0 | 0.437 | 1.07 |
| LL481001 | *L. olivacea* | 12/22/13 | 26.3 | 57.8 | A | F | E | 13.6 | 91 | 20.5 | 21.0 | 0.779 | 1.23 |
| LL517203 | *L. olivacea* | 1/2/15 | 25.9 | 55.7 | U | F | G | 15.0 | 34 | 1.45 | 3 | 0.0562 | 0.173 |
| LL519305 | *L. olivacea* | 1/25/15 | 19.1 | 50.9 | I | F | G | 14.5 | 81 | 21.5 | 26 | 1.12 | 2.36 |
| LL525509 | *L. olivacea* | 3/15/15 | 30.7 | 59.5 | I | F | G | 14.6 | 49 | 1.19 | 3 | 0.0389 | 0.220 |
| LL527602 | *L. olivacea* | 3/23/15 | 32.3 | 62 | A | F | G | 13.6 | 22 | 4.98 | 5 | 0.154 | 0.454 |
| LL528412 | *L. olivacea* | 4/15/15 | 30.5 | 58.4 | U | M | G | 15.3 | 32 | 2.07 | 5 | 0.0679 | 0.064 |
| LL530504 | *L. olivacea* | 4/22/15 | 28.6 | 58.6 | A | F | G | 14.2 | 43 | 8.35 | 12 | 0.292 | 0.292 |
| LL531413 | *L. olivacea* | 5/14/15 | 34.9 | 63.9 | A | F | G | 13.4 | 14 | 1.48 | 5 | 0.0423 | 0.180 |
| LL531416 | *L. olivacea* | 5/18/15 | 34.0 | 60.9 | A | F | G | 15.1 | 16 | 11.4 | 5 | 0.336 | 1.07 |
| LL532410 | *L. olivacea* | 5/24/15 | 22.8 | 56.7 | U | M | G | 12.5 | 115 | 12.1 | 16 | 0.533 | 1.19 |

Table 2.2. Spearman's pairwise correlations between all units of measure for ingested plastic debris by pelagic Pacific longline caught sea turtles

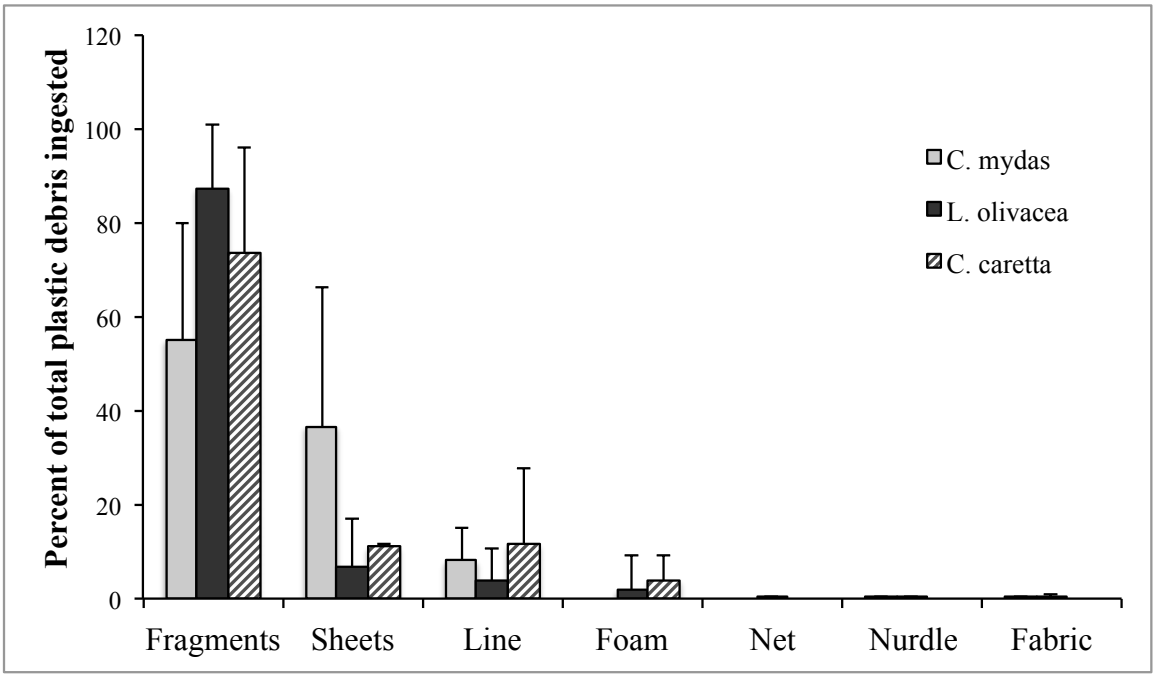
| Variable | by Variable | rho | p-value |
|---|---|---|---|
| Volume of ingested plastic (mL) | Mass of ingested plastic (g) | 0.987 | <.0001 |
| Grams plastic per kilogram turtle | Mass of ingested plastic (g) | 0.951 | <.0001 |
| Grams plastic per kilogram turtle | Volume of ingested plastic (mL) | 0.945 | <.0001 |
| Percent of GI contents comprised of plastic | Mass of ingested plastic (g) | 0.890 | <.0001 |
| Percent of GI contents comprised of plastic | Volume of ingested plastic (mL) | 0.884 | <.0001 |
| Percent of GI contents comprised of plastic | Grams plastic per kilogram turtle | 0.854 | <.0001 |
| Grams plastic per kilogram turtle | Total pieces of plastic of ingested | 0.843 | <.0001 |
| Volume of ingested plastic (mL) | Total pieces of plastic of ingested | 0.803 | <.0001 |
| Mass of ingested plastic (g) | Total pieces of plastic of ingested | 0.802 | <.0001 |
| Percent of GI contents comprised of plastic | Total pieces of plastic of ingested | 0.662 | <.0001 |

Figure 2.1.



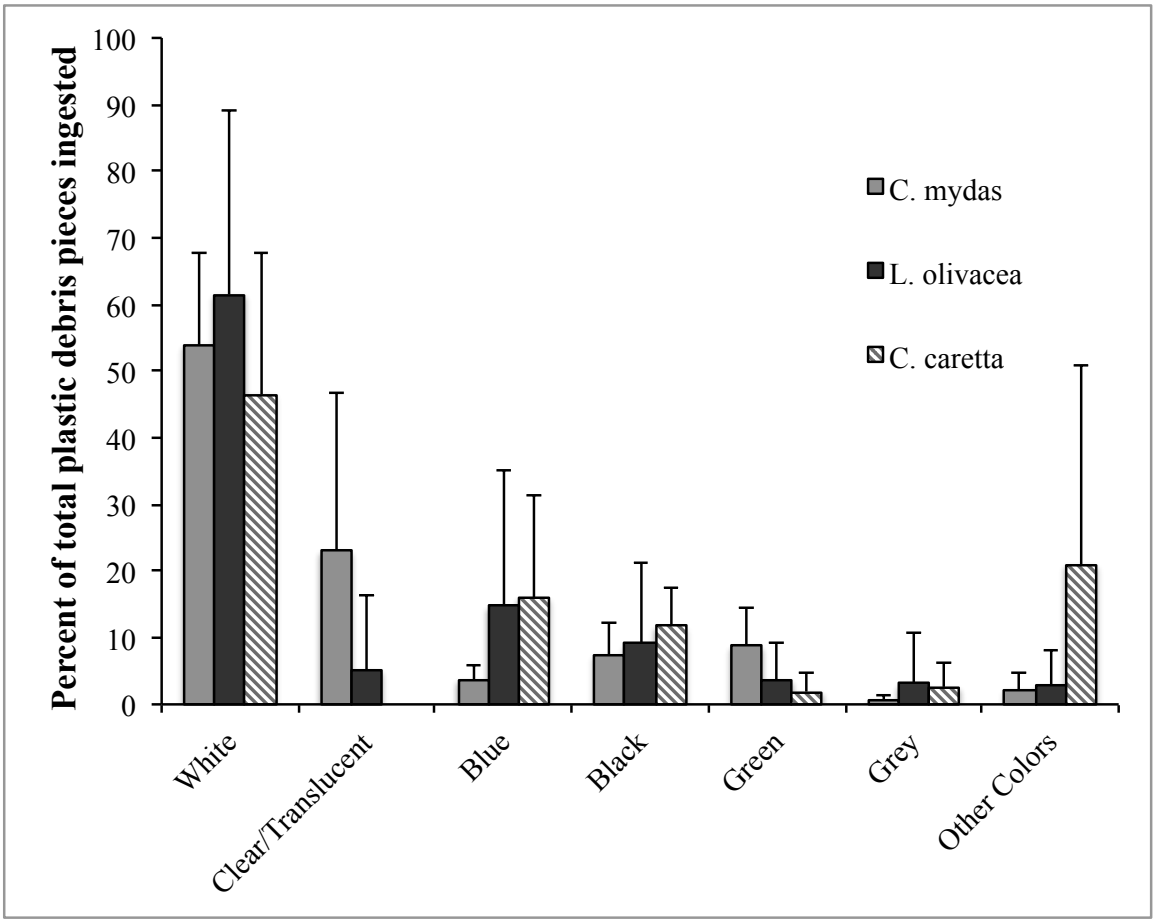

Pacific pelagic longline capture locations of sea turtles sampled in this study. Olive ridle turtles (brown, n=26), green turtles (green, n=6), loggerhead turtles (orange, n=3) and leatherback (blue, n=3).

Figure 2.2.



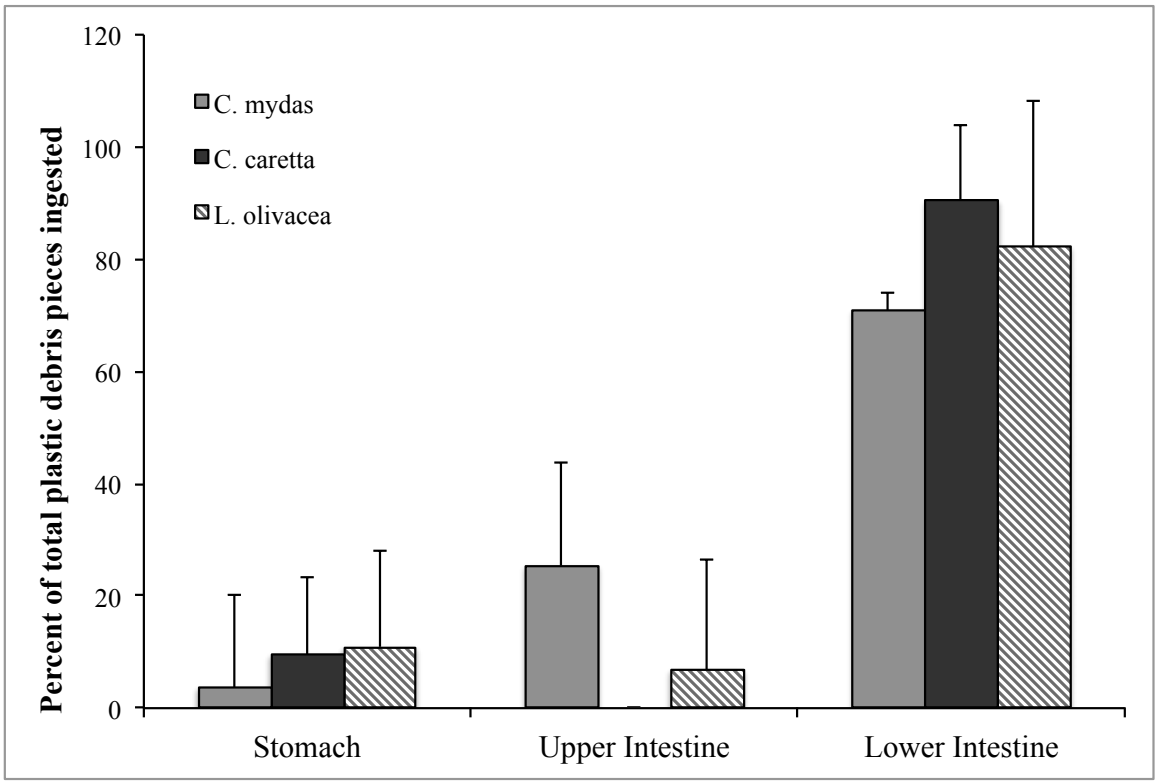

Selection of plastic types ingested by difference species of pelagic Pacific sea turtles. Data are the percentage of total plastic pieces consisting of each particular type ingested by each turtle, and shown as averages and standard deviation across turtles of each species. Turtles that did not consume plastic were excluded from this analysis.

Figure 2.3.



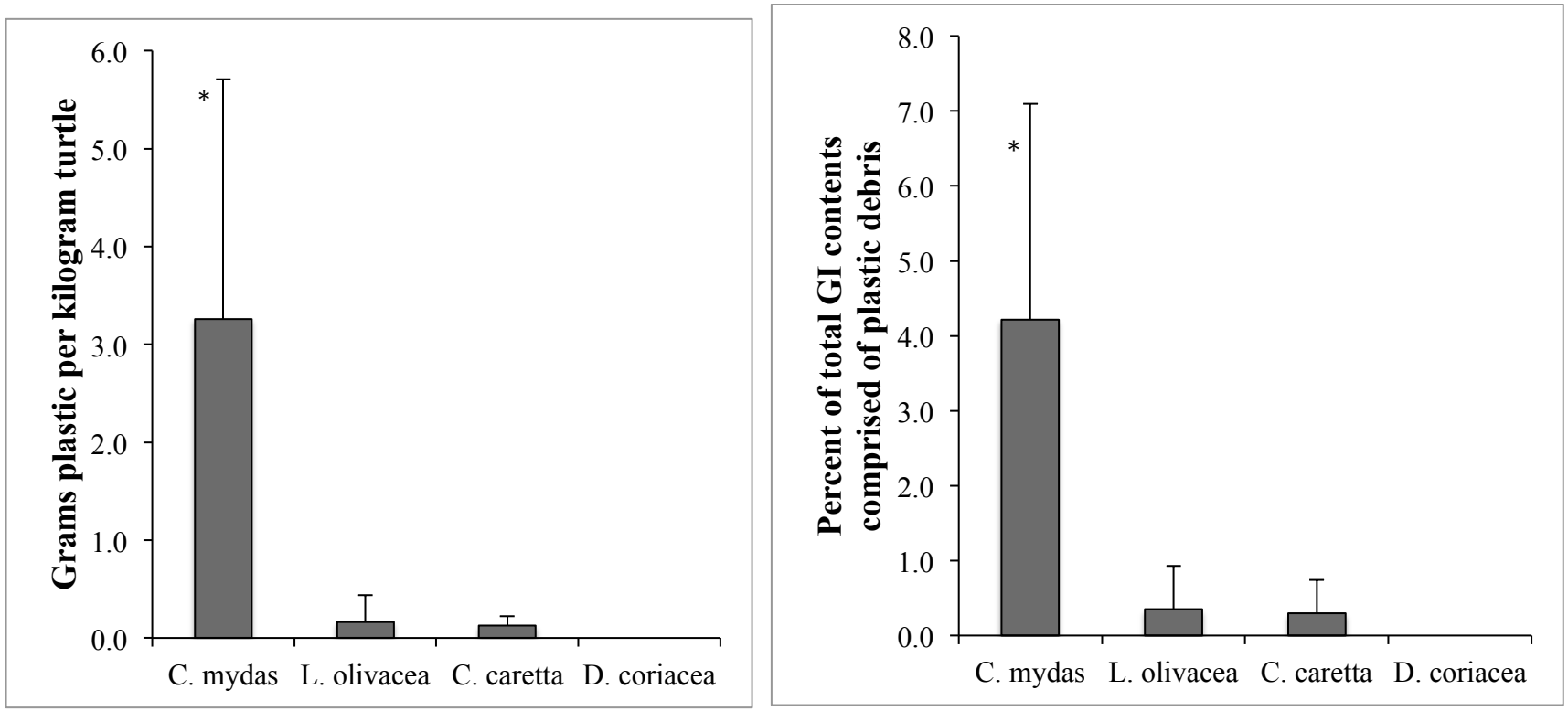

Selection of different plastic colors ingested by difference species of pelagic Pacific sea turtles. Data are the percentage of total plastic pieces consisting of each particular color ingested by each turtle, and shown as averages and standard deviation across turtles of each species. Other colors include pink, orange, red and silver. Turtles that did not consume plastic were excluded from this analysis.

Figure 2.4.




Locations within the gut where ingested plastic pieces were found in difference 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 averages and standard deviation across turtles of each species. Turtles that did not consume plastic were excluded from this analysis.

Figure 2.5.

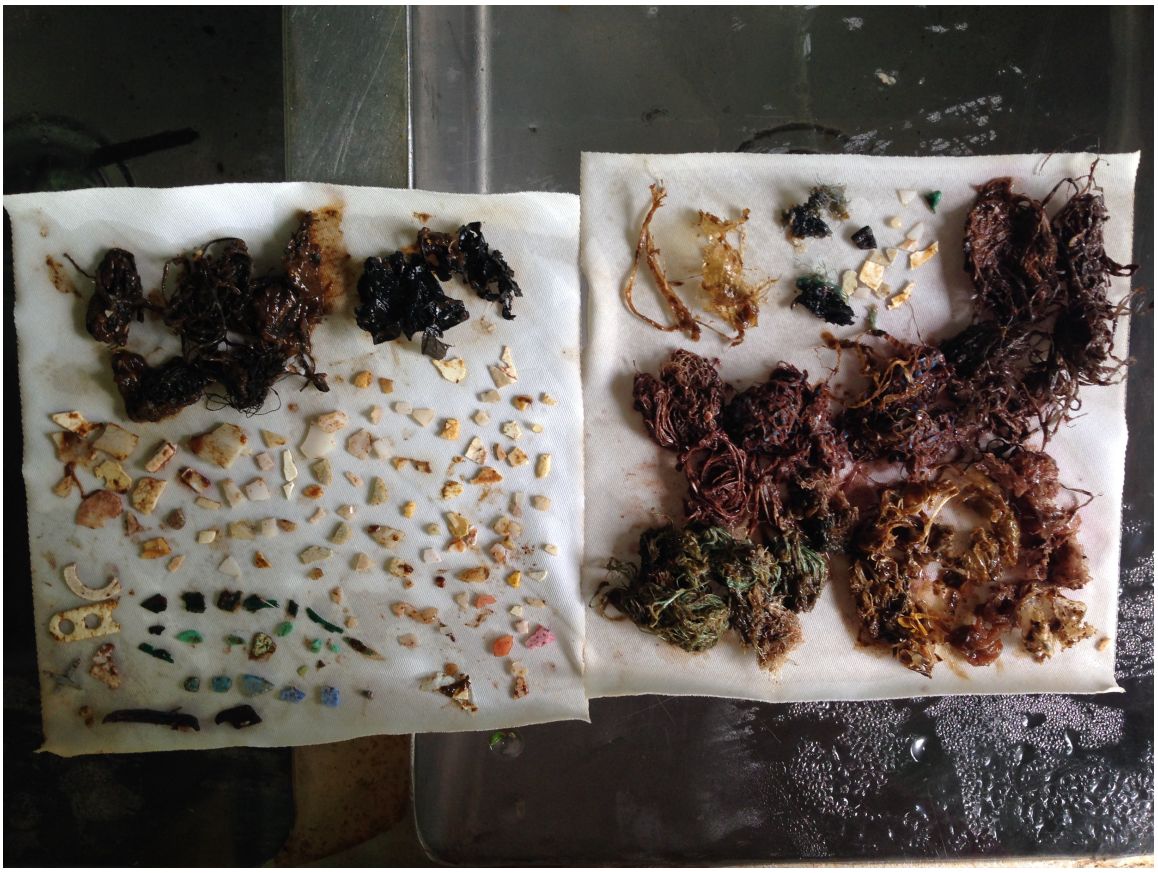



Amounts of anthropogenic debris ingested by difference species of pelagic Pacific sea turtles. Data are means and standard deviations for two methods of assessing ingested plastic. Leatherback turtles did not consume plastic. An asterisk indicates a significant difference from all other species.

Figure 2.6.

Anthropogenic debris ingested by one olive ridley (*Lepidochelys olivacea*). Picture show one 9 × 9 inch Cleanroom wiper with ingested debris mass totaling 6.89 g and comprising 0.570 % of the turtle's total GI contents.

Figure 2.7.



Anthropogenic debris ingested by one green turtle (*Chelonia mydas*). Picture shows two 9x9 inch Cleanroom wipers with ingested debris mass totaling 41.6 g and comprising 3.81 % of the turtle's total GI contents.

# CHAPTER 3

## Persistent Organic Pollutants in Adipose of *Caretta caretta*, *Chelonia mydas,* and *Lepidochelys olivacea* Sea Turtles from the Pacific Ocean

Abstract

Persistent organic pollutants (POPs) are synthetic chemicals that are recalcitrant, lipophilic, and biomagnify in food webs, leading to marine pollution and possible population declines of sea turtles. To analyze for POP concentrations, patterns and profiles of pelagic sea turtles, I necropsied 25 sea turtles [2 loggerhead (*Caretta caretta*), 6 green (*Chelonia mydas*) and 17 olive ridley (*Lepidochelys olivacea*) sea turtles] that were incidentally captured in Hawaiian and American Samoan longline fisheries. Adipose samples were analyzed by gas chromatography/mass spectrometry for 83 polychlorinated biphenyls (PCBs), 20 organochlorine pesticides, 32 brominated flame-retardants and by liquid chromatography tandem mass spectrometry for hexabromocyclododecane (HBCD). I analyzed differences among species, sex, and correlations with turtle length and capture locations. Total dichlorodiphenyltrichloroethanes (DDTs) were the predominant POP in both loggerhead (mean = 18.3 ng/g wet mass) and olive ridley (15.8 ng/g wet mass) turtles, and the second highest POP class in green turtles (1.80 ng/g wet mass). Total PCBs were the predominant POP in green turtles (2.71 ng/g wet mass), yet they had lower total PCB concentrations than loggerhead (4.92 ng/g wet mass) and olive ridley (3.95 ng/g wet mass) turtles. Green turtles had the highest concentrations of α-HBCD (1.46 ng/g wet mass), which was the only detected HBCD isomer. Total PCBs in pelagic green turtles were seven times higher than the average Hawaiian green turtle foraging near Kailua, Oahu. Among olive ridley turtles, no sex differences were seen in POP concentrations, likely because sampled turtles were mainly juvenile. Concentrations of several POPs increased with straight carapace length of olive ridleys, suggesting bioaccumulation

through age. A geographic gradient was observed with concentrations of several POPs increasing with capture latitude. Adipose POP concentrations were not correlated with amounts of ingested plastic marine debris, suggesting that sea turtle exposure to POPs is predominately through their natural food chain. This data provide important baseline POP concentrations for Pacific sea turtles, as this area has not been extensively monitored.

Introduction

Persistent organic pollutants (POPs) are man-made chemicals that are extremely persistent, lipophilic, and biomagnify in food webs. POPs include a variety of industrial compounds, including polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and organochlorine pesticides (OCPs). POP uses consist of pesticides, flame retardants and other various household and industrial items (United Nations Environmental Programme, 2001). POPs are very often found in environments far from their original source, with transport occurring via agricultural runoff (Ross and Birnbuam, 2003), atmospheric circulation (Gouin et al., 2004), and ocean circulation (Wania and Mackay, 1995). Plastic's hydrophobic nature attracts chemicals to its surface and recently, POPs have been found in plastic debris collected from beaches around the world (Mato et al., 2001; Rios et al., 2007). Therefore, plastic could be an additional transportation mechanism for POPs and exposure to species that incidentally eat marine debris.

POPs have contributed to population declines of several species of wildlife, including alligators and many species of birds (Carson, 1962; Fox, 2001; Guillette et al., 1994). In sea turtles, effects of environmental contaminants are still poorly understood (Keller, 2013). The IUCN (2015) lists five of the six extant sea turtle species found in the Pacific Ocean as critically endangered, endangered, or vulnerable and includes leatherback (*Dermochelys coriacea*), hawksbill (*Eretmochelys imbricata*), olive ridley (*Lepidochelys olivacea*), green (*Chelonia mydas*), and loggerhead (*Caretta caretta*). In addition to anthropogenic chemical contamination sea turtles have a long history of human impacts effecting their survival (Carr 1987a). Although POP concentrations in

sea turtles are low relative to other wildlife that feed at higher trophic levels, concentrations have been significantly correlated with several health indicators, including white blood cell counts and some plasma chemistry measurements (Keller et al., 2004a and 2006).

The East Coast of the United States has been the region most extensively studied for POPs in sea turtles (see Keller 2013 for review), while there are limited data within the literature for POP concentrations in sea turtle species inhabiting the Pacific Ocean. Knowledge of POP concentrations in sea turtles surrounding Hawaiian Islands are even more limited, with only three published studies (Aguirre et al., 1994; Miao et al., 2001, Keller et al., 2014a), but only two of these studies used methods that could detect target compounds. Recently, baseline data have been provided in areas such as Australia, Japan, Baja California, and Malaysia (Hermanussen et al., 2008; van de Merwe et al., 2009a, 2009b, 2010a, 2010b; Richardson et al., 2010; Labrada-Martagon et al., 2011), but data are still lacking in pelagic areas. Additionally, most of these studies focused on green sea turtles with only one olive ridley and one loggerhead having been measured for POPs (Gardener, 2003) and four olive ridleys and four loggerheads for PCBs (Richardson et al., 2010). A spatiotemporal analysis of POP exposure in vast regions inhabited by sea turtle species of all age classes is important for better understanding of global contaminant levels.

POP exposure occurs mostly through the food chain and broad comparisons across all studies support the general conclusion that POP concentrations follow trophic status and are highest in Kemp's ridley sea turtles, followed by loggerhead, leatherback and finally green turtles (Keller, 2013). Data on olive ridleys has been too limited to

place this species among other sea turtles in relation to POP concentrations. In an 18-year study of diet content analysis of sea turtles captured in American Samoa and Hawaii pelagic longline fisheries, olive ridleys were found to be opportunistic generalists consuming gelatinous zooplankton and fish and were found to ingest a high frequency of anthropogenic debris (Wedemeyer-Stombel et al., 2015) and often would often graze from longline hooks (Work and Balazs, 2002). Juvenile pelagic green turtles captured in Pacific longlines were found to be opportunistic, mainly carnivorous, feeding at or near the surface (Parker et al., 2011). Pacific loggerheads fed primarily at the surface with few deep water prey including molluscks, snails, hydozoans and pyrosomes (Parker et al., 2005). Given these feeding habits suggests that olive ridleys would place somewhere between Kemp's ridleys and loggerheads for POP concentration levels based on their trophic status, and that the pelagic, younger, carnivorous life stage of green turtles would place them higher than other previously analyzed herbivorous age classes of green turtles.

Additionally, ingestion of contaminated debris could be an added source of exposure to POPs for sea turtles. There have been several reports of PCBs, OCPs, including dichlorodiphenyltrichloroethane (DDTs), in plastic pieces collected from beaches around the world (Mato et al., 2001; Endo et al., 2005; Ogata et al., 2009). Furthermore, correlations of chemicals found in ingested plastics by seabirds and those chemicals accumulated in their fat have suggested that plastic may be an additional source of exposure to these classes of POPs (Colabuono et al., 2010; Tanaka et al., 2013). Specifically, higher brominated PBDE congeners, which are not present in the natural prey, but are applied to plastics and to textiles as flame-retardants have been found in seabird tissues (Tanaka et al., 2013).

Given our limited knowledge about contaminants in the Pacific pelagic zone, my goal is to provide baseline contaminant levels of three species of pelagic Pacific sea turtles and use the data to provide spatial trends of contaminant levels in sea turtles inhabiting the Pacific Ocean. Providing baseline concentrations is important for all three species, but especially for olive ridley turtles since only five have ever been measured for POPs. POPs are known to accumulate through the life of animals, increasing with age for males and increasing until reproductive maturity for females, when a significant portion of the POP burden is offloaded into their eggs (Stewart et al., 2011). My goal was to examine POP concentrations among sex and size relationships of Pacific olive ridley turtles. Additionally, I hypothesis that the amount of plastic ingested by pelagic Pacific sea turtles (Chapter 2) is correlated with the concentrations of POPs accumulated in fat.

## Methods

### *Sample collection*

The U.S. National Oceanic and Atmospheric Administration (NOAA) Pacific Islands Regional Office (PIRO) uses 100% observer coverage on the Hawaiian and American Samoan longline fisheries to collect fisheries catch and bycatch data. Between June 2012 and December 2013, 25 sea turtles (two loggerhead, six Pacific green, and 17 olive ridley turtles), incidentally taken as bycatch in these fisheries and determined dead (Balaz et al., 1995). Observers recorded the capture latitude and longitude as well as straight carapace length (SCL) of these 25 turtles which were then stored frozen and sent to NOAA, National Marine Fisheries Service, Pacific Islands Fisheries Science Center in

Honolulu, Hawaii, for necropsy. Turtle wet weight (kg) and additional length measurements were taken and necropsies performed on all individuals. Body condition at necropsy was classified as poor, fair, good, or excellent based on the appearance of muscle and fat tissue in the inguinal region and under the plastron. The sex and age class of turtles was determined by visual examination of gross gonadal morphology. Gross necropsies entailed a complete external and internal exam of all organ systems, including histology of most organs. The cause of death determined for all turtles was forced submergence. Body condition index was calculated as body mass (kilograms) divided by the cube of SCL (centimeters) and multiplied by 100,000 [body condition = mass/($SCL^3$) × 100,000; Bjorndal et al. 2000]. Adipose samples were taken from the left inguinal region from each turtle using hexane-rinsed stainless steel scalpel blade and forceps and stored in a 15 mL Teflon jar. Fat samples were shipped in liquid nitrogen dry vapor shippers (-150 °C) to the National Institute of Standards and Technology (NIST), Hollings Marine Laboratory, Charleston, South Carolina, to be stored in the Biological and Environmental Monitoring and Archival of Sea Turtle Tissues (BEMAST) project of the Marine Environmental Specimen Bank (Keller et al., 2014b) until cryo-homogenized at liquid nitrogen vapor temperatures and analyzed for POPs. The gastrointestinal (GI) system was removed and stored at -80 °C until analyzed for presence of anthropogenic objects as described in Chapter 2.

*Persistent organic pollutants*

*Sample preparation, extraction and cleanup*

Fat samples were cryo-homogenized using the Retsch Cryomill (Haan, near

Düsseldorf, Germany) machine at 25 Hz for 5 min. Fat subsamples (≈ 1 g) were combined with sodium sulfate, transferred to pressurized fluid extraction (PFE) cells and spiked gravimetrically with internal standard solution. The internal standard solution contained $^{13}$C-labeled PCB congeners (28, 52, 77, 126, 169, 118, 153, 180, 194, 206), 6-F-PBDE 47, PBDE 104, 4′ -F-PBDE 160, 4′ -F-PBDE 208, $^{13}$C -labeled PBDE 209, $^{13}$C -labeled pesticides (hexachlorobenzene (HCB), trans-chlordane, trans-nonachlor, oxychlordane, 4,4′ -DDE, 4,4′ -DDD, 4,4′ -DDT), $^{13}$C -labeled methyl-triclosan and $^{13}$C -labeled $\alpha$ -, $\beta$ -and $\gamma$ -hexabromocyclododecanes (HBCDs). POPs were extracted using PFE with 3 cycles of dichloromethane (DCM) at 100 $^{o}$C and 13.8 MPa. Total extractable organic (TEO) content (or lipid content) was determined by removing 12 % of the extract gravimetrically, allowing it to dry in a tared aluminum pan, and weighing dried residue to 0.01 mg approximately 24 h later. Remaining extracts were cleaned up using size exclusion chromatography (SEC) with 10 mL/min DCM on a PLGel (600 mm × 25 mm, 10 $\mu$ m particle size with 100 Å diameter pores, Polymer Labs, Amherst, MA). Additional clean-up and fractionation of the samples was carried out using an automated solid phase extraction system (SPE, Rapid Trace Workstation, Biotage, Charlotte, NC) with acidified silica columns as described in Keller et al. (2009). Fraction One (F1) extracts were solvent exchanged to *iso*-octane, evaporated, and transferred to autosampler vial (ASV) inserts with a final volume of 0.2 mL. Fraction Two (F2) extractions were solvent exchanged to methanol, evaporated, and transferred to ASV inserts with a final volume of 0.2 mL. FI extracts were destined for injection by gas chromatography mass spectrometer (GC/MS) for nearly all compounds, and F2 extracts were destined for injection by liquid chromatography tandem mass spectrometry (LC/MS/MS) for HBCDs.

*GC/MS analysis*

Each sample (F1) was injected onto a GC/MS two different times for different target constituents. The first injection was performed with an electron impact (EI) source and a programmable temperature vaporization (PTV) inlet operated in the solvent vent mode onto a 5 m × 0.25 mm Restek Siltek guard column connected to a 0.180 mm × 30 m × 0.18 µm film thickness Agilent DB-5MS capillary column. PCBs, selected PBDEs, selected pesticides, and selected additional BFRs were quantified from this injection. PBDEs and selected additional BFRs were quantified from the second injection which was performed with a negative chemical ionization (NCI) source with an injection of 2 µL cool on-column onto a 5 m × 0.25 mm Restek Siltek guard column connected to a 0.18 mm × 10 m × 0.18 µm film thickness DB-5MS Agilent analytical column.

*LC/MS/MS analysis*

HBCDs were quantified using 20 µL injections of F2 extracts as described in Bachman et al (2014). An Agilent Eclipse Plus XDB-C18 (3.0 mm x 150 mm x 3.5 µm) column on an Agilent 1100-series LC was connected to an electrospray ionization source on an API 4000 MS/MS (Applied Biosystems, Foster City, CA).

*QA/QC and quantification*

Three replicates of NIST Standard Reference Material (SRM) 1945 Organics in Whale Blubber were analyzed as controls. These samples, laboratory procedural blanks and calibration solutions were extracted, processed and analyzed concurrently with the

sample set. Six-point calibration curves ranged from 0.06 ng to 300 ng of compounds found in the following solutions: SRM 2261 Chlorinated Pesticides in Hexane, SRM 2262 Chlorinated Biphenyl Congeners in Isooctane, SRM 2274 PCB Congener Solution-II in Isooctane, SRM 2275 Chlorinated Pesticide Solution-II in Isooctane, additional solutions containing 46 PCB and 28 PBDE congeners, the following from Accustandard (New Haven, CT, USA): octachlorostyrene, $\alpha$-, $\beta$-, $\gamma$-HBCDs, pentachlorobenzene, and the following from Wellington Laboratories (Guelph, Ontario, Canada): 1,2-bis(246-tribromophenoxy) ethane (BTBPE), hexabromobenzene (HBB), pentabromoethylbenzene (PBEB), decabromodiphenylethane (DBDPE), 4-methoxy-2,3,3',4',5-pentachlorobiphenyl (4-methoxy PCB 107), 4-methoxy-2,2',3,4',5,5'-hexachlorobiphenyl (4-methoxy PCB 146), 4-methoxy-2,2',3,4',5,5',6-heptachlorobiphenyl (4-methoxy PCB 187), 6-methoxy-2,2',4,4'-tetrabromodiphenyl ether(6-methoxy PBDE 47).

The internal standard approach was used to quantify each compound amount. Amounts of each analyte were calculated using the slope and y-intercept of at least a three point calibration curve that bracketed the peak area ratios observed in the samples. Concentrations were determined by dividing the calculated analyte mass by the extracted sample mass. The reporting limit (RL) was determined as per Ragland et al. (2011).

*Statistical analysis*

All statistical analyses were conducted in program R (R Development Core Team, 2005). Mean, median and standard deviations were calculated using R NADA package through Kaplan–Meier, maximum likelihood estimation (MLE) or regression on order statistical (ROS) models as recommended for left censored data (Helsel, 2005). All data

were wet mass (ng/g wet mass) and summed for the following compound classes: Σ PCBs (polychlorinated biphenyls 1, 8, 18, 28, 29, 31, 44, 45, 49, 50, 52, 56, 63, 66, 70, 74, 77, 79, 82, 87, 92, 95, 99, 101, 104, 105, 106, 107, 110, 112, 114, 118, 119, 121, 126, 127, 128, 130, 132, 137, 138, 146, 149, 151, 153, 154, 156, 157, 158, 159, 163, 165, 166, 167, 169, 170, 172, 174, 175, 176, 177, 178, 180, 183, 185, 187, 188, 189, 191, 193, 194, 195, 196, 197, 199, 200, 201, 202, 203, 205, 206, 207, 208, 209), Σ PBDEs (PBDE 17, 25, 28, 30, 33, 47, 49, 66, 71, 75, 85, 99, 100, 116, 119, 138, 153, 154, 155, 156, 181, 183, 190, 191, 203, 205, 206, 209), Σ DDTs (2,4'- and 4,4′ -DDE, 2,4'- and 4,4'-DDD, 2,4'- and 4,4'-DDT), Σ CHLs (cis-chlordane, cis-nonachlor, heptachlor, oxychlordane, trans-chlordane, trans-nonachlor), Σ HBCDs ($\alpha$-, $\beta$- and $\gamma$-isomers), Σ HCHs ($\alpha$-, $\beta$- and $\gamma$-hexachlorocyclohexanes), mirex, HCB, octachlorostyrene (OCS), and pentachlorobenzene (PeCB).

Normality and homoskedasticity of raw and then log-transformed data were tested using Shapiro-Wilk and Bartlett tests, respectively. R's NADA package was used to perform either a parametric (regression by maximum likelihood estimation for left-censored data using the function cenmle) or nonparametric (test censored empirical cumulative distribution function differences for left-censored data using the function cendiff) of comparisons between species and comparisons between male and female olive ridley sea turtles. Cenmle was only used for comparisons between male and female olive ridleys for mirex and PBDE 47. For analysis of size of straight carapace length (SCL), amounts of plastic ingested including ingested plastic mass, grams of ingested plastic per kilogram mass of turtle and percent of total gut content mass comprised of plastic, and spatial trends based on capture location, R's NADA package was used to perform either a

parametric (regression equation and the likelihood correlation coefficient for left-censored data using the function cenreg) or nonparametric (Kendall's tau correlation coefficient and associated line for left-censored data using the function cenken). For analysis of capture location, all latitude capture locations south of the equator were changed to the absolute value. Cenreg was used for analysis of olive ridley SCL in PCBs 99, 105, 118, 128, 138, 146, 149, 153+132, 158, 163, 170, 180+193, and 183. As well as for analysis of capture location with PCB 99, PCB 105 and PBDE 47 and additionally PBDE 47 for mass of ingested plastic, percent GI contents comprised of plastic and volume of ingested plastic. We examined sex and age class relationships among only olive ridley turtles, excluding loggerheads since the sample size is small and green turtles because they were all immature. All tests for significance used a p-value of less than 0.05.

## Results and Discussion

### *Sampling*

Specimens were collected between latitude 13.5 °S and 29.6 °N and longitude 140 °W and 170 °W (Figure 3.1). Turtles ranged from 3.9 kg to 60.8 kg and 29.9 cm SCL to 72.9 SCL (Table 3.1). Both loggerheads were adults (Chapter 2), but only one was found with ingested plastic, which totaled 11.3 g. Only 3 of the 17 olive ridleys were classified as immature, with 100 % occurrence of plastic debris found within the GIs of the olive ridleys with a mean mass of ingested plastic of 4.89 g (Chapter 2). All six green turtles were immature and were the species to ingest the most amount of plastic, averaging 36.4 g per juvenile green turtle. Only one green turtle in this study was not found with any

noticeable ingested debris (Chapter 2). All but two turtles were in either good or excellent body condition with good overall gut fill. One immature female olive ridley was in poor body condition. I acknowledge the complexity of this data set originating from the opportunistic capture of diverse species and age classes.

*POP concentrations*

The mass fractions of POPs in the replicates of SRM 1945 were on average within 12 % of the certified values, providing good confidence in the data from the sea turtle samples. Of all the chemical classes tested, 29 individual congers were above reporting limit for the turtles in this study (Table S3.1, Table 3.2). Total DDTs were the predominant POP. One olive ridley turtle had an order of magnitude greater ΣDDT accumulation (159 ng/g wet mass) than the rest, which ranged from below reporting limit to 14.3 ng/g wet mass ΣDDT concentration. Only one green turtle and one olive ridley turtle were below reporting limits for ΣDDT. ΣPCBs were the second highest contaminant class followed by ΣCHLs, with loggerheads showing the highest mean concentration for both classes of compounds. The only detected HBCD isomer was $\alpha$-HBCD with eight turtles having concentrations above the reporting limit and green turtles having the highest mean concentration of 1.46 ng/g wet mass. The lowest detected concentration contaminant class was ΣPBDEs, with 19 of the 25 turtles showing detectable levels. Specifically, mirex and hexachlorobenzene were detected in all species, while 6-methoxy PBDE 47 was detected only in olive ridley turtles (n = 3) and juvenile green turtles (n = 2). DBDPE was only detected in one adult male olive ridley turtles with 1.17 ng/g wet mass. Pentachlorobenzene was only detected in the two

loggerhead turtles with average mass faction of 0.699 ng/g wet mass. Octachlorostyrene, BTBPE, HBB, PBEB, 4-methoxy PCB 107, 4-methoxy PCB 146 and 4-methoxy PCB 187 were not detected in any of the sea turtles.

ΣPCBs were the highest of the POP classes in pelagic juvenile green turtle fat samples and averaged 2.71 ng/g wet mass. To compare this concentration to older, herbivorous green turtles foraging around coastal Hawaii (Keller et al., 2014a), we converted the fat concentrations in the current study to estimated plasma concentrations. Using linear equations for the conversion of fat to plasma PCB concentrations determined for loggerhead turtles by Keller et al. (2004b) the pelagic green turtles would have approximately 243 ng/g lipid ΣPCBs in their plasma, or 826 pg/g wet mass if we assume they have similar average TEO of green turtles foraging near Kailua, Oahu, Hawaii (Keller et al., 2014a). This concentration is seven times higher than the average Hawaiian green turtle foraging near Kailua, Oahu (114 pg/g wet mass; Keller et al., 2014a). While the Kailua turtles are feeding in much closer proximity to a coastal developed areas than the pelagic green turtles of the current study, they are herbivorous (having undergone their ontogenetic switch), so they are feeding on a lower trophic level and may be experiencing growth dilution as seen in other neritic sea turtles (Keller et al., 2004b).

All sea turtles species in this study had much lower fat concentrations of ΣDDTs, ΣPCBs, and ΣCHLs than sea turtles along Baja California (Gardner et al., 2003; Table 3.2). Sea turtles along the coast of Baja California are utilizing habitats in closer proximity to coastal developed areas and therefore could be exposed to higher contamination. ΣPBDEs measured in an adult female green off the coast of Queensland,

Australia were found with nearly double the mean ΣPBDEs for the juvenile green turtles in this study. An adult green turtle is able to accumulate more toxins throughout its lifespan. However, as noted in the Kailua, Hawaii adult green turtles that have undergone their ontogenetic switch and are feeding on a lower trophic level, growth dilution can affect concentration levels. Sea turtles off the coast of Queensland, Australia may be exposed to higher contamination and use of PBDEs.

In the Southeastern US coast sea turtles have ΣPCBs as the predominant POPs contaminant class followed by concentrations of ΣDDTs (Rybitski et al., 1995; Keller et al., 2004b). However, both contaminant classes were higher compared to the pelagic Pacific turtles from our study. These differences suggest that sea turtles along the SE U.S. coasts are exposed to much higher contamination than pelagic turtles (Rybitski et al., 1995; Keller et al., 2004b).

POP concentrations in sea turtles are generally lower relative to other wildlife that feed at higher tropic levels. For example, the highest trophic level cetacean around the Hawaiian Islands, the false killer whale (*Pseudorca crassidens*) averaged 63,000 ng/g lipid (SD: 28,000) ΣDDT in adult males and 20,000 ng/g lipid (SD: 4,900) ΣDDT in both male and female subadults (Ylitalo et al., 2008). Average ΣDDT blubber concentrations of cetaceans around the Hawaiian Islands were 9,650 ng/g lipid (Bachman et al., 2014). Lower trophic level Hawaiian monk seals (*Monachus schauinslandi*) from the main Hawaiian Islands averaged 690 ng/g lipid ΣDDT in adult males, 390 ng/g lipid ΣDDT in subadults, and 190 ng/g lipid ΣDDT in adult females (Lopez et al., 2012). Sea turtles in this study averaged 51.9 ng/g lipid or 38.9 ng/g wet mass ΣDDT. While other species have higher POP concentrations, toxic and sublethal effects of POPs on sea turtles is still

not completely understood.

*POP profiles*

ΣDDTs were the predominant POP followed by ΣPCBs, ΣCHLs, and ΣPBDEs, respectively, a pattern observed in other Pacific wildlife (Ylitalo et al., 2008; Bachman et al., 2014). POPs patterns in sea turtles in the Atlantic Ocean and along the coast of Baja California found that ΣPCBs were the predominant POP followed by ΣDDTs (Garder et al., 2003; Keller et al., 2004a and 2004b; Stewart et al., 2011; Ragland et al., 2011). Although it is difficult to accurately determine sources of contamination for these pelagic turtles, the switch we see of ΣDDTs as the predominant contaminant class in pelagic turtles versus ΣPCBs being predominant along the coasts of the US, can perhaps be assumed that there is a more continued use of DDT and less PCB use in some Pacific Islands and Pacific Ocean countries compared to the US.

In comparison of contaminant composition profiles within four chemical classes among the three species in this study, visually they are generally similar (Figure 3.2) with only green turtles having a visually higher proportion of *trans*-nonachlor than the other two species. For all species, 4,4'-DDE consisted of 90 % to 94 % of ΣDDTs with only 4,4'-DDT being the only other DDT congener detected. 4,4'-DDE is the metabolite of its parent congener DDT indicating that Pacific pelagic sea turtles are exposed to older, rather than recent, usage of 4,4'-DDT (Aguilar ,1984).

Visually, higher proportions of PCB 138, PCB 146, PCB 153 + 132, and PCB 180 +193 were seen in all three species, but there were no significant differences. This is similar to sea turtles in the Atlantic except with pelagic Pacific turtles having higher

proportions of PCB 146 and lower proportions of PCB 187 and PCB 199 (Ragland et al., 2011). Globally, PBDE 47 is the PBDE congener in highest concentration in most wildlife (Hites, 2004). PBDE 47 was detected between 40 % and 50 % of total PBDEs in all three species. Only one immature male olive ridley turtle that had concentrations of PBDE 153 and 154 above reporting limits with PBDE 47 being below the limit of detection.

*Species differences*

Though loggerhead turtles had higher concentrations of ΣDDTs, ΣPCBs, and ΣCHLs than the other species, sample size (n = 2) prevented comparisons. Statistically significant differences in concentrations for some compounds were observed between olive ridley and green turtles (Table 3.3). Olive ridleys showed significantly higher levels of PCB 149 ($\chi$ = 18.5; df = 1, $p < 0.001$ but not ΣPCBs), 4,4'-DDE ($\chi$ = 17.9, df = 1, p = 0.008) and total DDTs ($\chi$ = 3.7; df = 1, p = 0.008) whereas green turtles showed significantly higher levels of mirex ($\chi$ = 15.3; df = 1, $p < 0.001$) and $\alpha$-HBCD ($\chi$ = 6.6; df = 1; p = 0.022). Of the 17 olive ridley turtles analyzed, only three were classified as immature while all six green turtles were immature. Although these pelagic juvenile green turtles are feeding opportunistically and omnivorously, they are too young to have accumulated as much POPs as adult olive ridleys.

In Baja California ΣPCBs concentrations were highest in loggerheads followed by olive ridley and then green turtles (Richardson et al., 2010). Loggerheads feed at a slightly higher trophic level than the other two species and further analysis of POP concentrations in all sea turtle species spanning the Pacific Ocean is required to make a

more accurate comparison of species contaminant levels.

*Sex/age class relationships*

No differences for any POP existed between the sexes in olive ridleys (14 females, 3 males) ($p>0.05$). However, positive correlations between SCL and POP concentrations were seen for PCB 153+132, PCB 180+193, PCB 187, total PCBs, *cis*-chlordane, *trans*-nonachlor, oxychlordane and ΣCHLs (Figure 3.3) suggesting bioaccumulation through age. Total PCBs showed a linear fit with SCL of $y = 0.261x – 11.1$ ($R^2 = 0.062$; tau = 0.404; $p = 0.0247$). Total CHLs showed a linear increase of $y = 0.0586x – 2.26$ ($R^2 = 0.139$; tau = 0.485; $p = 0.00742$). Removing the turtle with the smallest SCL, ΣPCBs showed a linear fit of $y = 1.48x – 83.2$ ($R^2 = 0.181$) and ΣCHLs a linear fit of $y = 0.245x – 13.3$ ($R^2 = 0.231$).

*Geographic comparison of POP concentrations*

POP concentrations were not correlated with longitude ($p > 0.05$), but were positively correlated with latitude. Specifically, positive correlations were found with PCB 99, PCB 138, PCB 153+132, PCB 180+193, PCB 187, ΣPCBs, 4,4'-DDE, ΣDDTs, *trans*-nonachlor and ΣCHLs when all turtles were included (Figure 3.4). The greatest correlation was seen in ΣDDTs ($y = 0.0135x^2 + 0.0466x$; $R^2 = 0.3034$; $p = 0.0133$), followed by ΣPCBs ($y = 0.0041x^2 + 0.085x$; $R^2 = 0.035$; $p = 0.0328$) and then ΣCHLs ($y = 0.0015x^2 + 0.0213x$; $R^2 = 0.0478$; $p = 0.0122$).

Similar increases in sea turtle POP concentrations moving away from the equator have been observed along the east coast of the US and are thought to be due to greater

human density and use of compounds farther north (O'Connell et al., 2010; Alava et al., 2011; Ragland et al., 2011). This trend in the Pacific Ocean is most likely related to global distillation, the geochemical process by which certain chemicals, most commonly POPs, which are semi-volatile are transported from warmer regions to colder regions through evaporation and condensation (Simonich and Hites, 1995; Fernandez and Grimalt, 2003). The process is repeated in "hops" with latitude, giving it the name "grasshopper effect," carrying chemicals thousands of kilometers in a matter of days towards the poles (Gouin et al. 2004).

*Correlations with plastic ingestion*

The hypothesis that ingested plastic can increase levels of POP concentrations was not supported by the documented data. No correlations were observed between POP concentrations and four different methods of measuring ingested plastics: mass of ingested plastic, total number of debris pieces ingested, mass of plastic ingested per kilogram of turtle, and the percentage of total GI contents that consisted of plastic. Accumulation of POPs is mostly through ingestion of contaminated food and although we see mass fractions of POPs in sea turtles much lower than other species that feed at higher trophic levels, sea turtles are known to ingest high amounts of anthropogenic debris (Balazs, 1985; Laist, 1997; Santos et al., 2015; Wedemeyer-Strombel et al., 2015). Although these pelagic turtles are often feeding near the surface, POPs measured in deep-sea fishes in the Western North Pacific Ocean show similar POPs profiles (Takahashi et al., 2010) whereas POPs measured in plastic resin pellets from near Hawaii have ΣPCBs as the predominant POP contaminant class (Heskett et al., 2012).

Green turtles did have ΣPCBs as the predominant POP contaminant class and ingested the highest amounts on plastic (Chapter 2); however, no significant correlations were found. Also, green turtles on average had the highest concentration of $\alpha$-HBCD compared to the other two species. HBCD is a widely used brominated flame retardant applied to plastics, textiles, and electronics (United Nations Environmental Programme, 2001). However again, no correlations were found between HBCD and the amounts of plastic ingested. Taken together, this information and the lack of correlations suggest that the POPs are most likely coming from the natural prey of the sea turtles, not from ingested plastics. Perhaps more investigation is needed for green turtles and the transfer of POPs from ingested plastic since this study only used a sample size of 6 green turtles.

Conclusion

The Hawaiian and American Samoan longline caught sea turtles provides the largest POPs evaluation for three species of pelagic Pacific sea turtles, which previously had very limited data. Despite sample size limitations, we are still able to demonstrate trends in POP concentrations among various pelagic sea turtle species and geographic locations. Importantly, we established initial concentration values for POPs in the adipose of several olive ridley sea turtles that varied in both sex and age class. These data will aid scientists and managers in addressing environmental health, global monitoring of POPs and in conservation and management strategies of sea turtles on a greater scale.

The hypothesis that ingested plastic can increase levels of POP concentrations was not supported by the documented data. POPs detected in these pelagic Pacific sea

turtles are most likely coming from their natural prey. However, the issue of contamination transfer from marine debris should not be disregarded and should be investigated further. The fact that these pelagic turtles had such a high frequency of debris ingestion should highlight a greater concern for all ocean conservation.

Table 3.1.

Summary statistics for necropsied turtles caught by the Hawaii and American Samoan based pelagic longline fisheries. Age are immature (I) and adult (A). Body condition (BC) are classified as poor (P), fair (F), good (G) and excellent (E). Body condition index was calculated as body mass (kg) divided by the cube of SCL (cm) and multiplied by 100,000 [body condition = mass/($SCL^3$) × 100,000] as described by Bjorndal et al. (2000).

| Species | Capture Date | Turtle ID | Mass (kg) | SCL (cm) | Age | Sex | BC Index | BC |
|---|---|---|---|---|---|---|---|---|
| *C. caretta* | 3/11/13 | LL456601 | 60.8 | 72.9 | A | F | 15.7 | E |
| | 10/27/13 | LL475601 | 55.9 | 72.8 | A | M | 14.5 | E |
| *C. mydas* | 11/1/13 | LL476104 | 8.60 | 37.9 | I | M | 15.8 | E |
| | 12/28/13 | LL480011 | 9.00 | 39.4 | I | F | 14.7 | E |
| | 12/9/13 | AS015728 | 13.4 | 45.7 | I | F | 14.0 | G |
| | 3/22/14 | AS016421 | 8.00 | 38.7 | I | M | 13.8 | G |
| | 8/5/13 | AS015316 | 11.0 | 43.0 | I | M | 13.8 | F |
| | 10/27/13 | AS015808 | 11.9 | 43.6 | I | F | 14.4 | G |
| *L. olivacea* | 12/7/12 | LL445715 | 3.90 | 29.9 | I | F | 14.6 | P |
| | 12/2/12 | LL444515 | 26.9 | 58.0 | A | F | 13.8 | G |
| | 6/13/12 | LL431606 | 24.8 | 55.0 | A | F | 14.9 | E |
| | 6/16/12 | LL431609 | 31.6 | 58.4 | A | F | 15.9 | G |
| | 11/28/12 | LL445510 | 35.0 | 62.2 | A | F | 14.5 | E |
| | 10/14/12 | LL441507 | 29.3 | 58.9 | A | F | 14.3 | G |
| | 1/18/13 | LL450502 | 27.2 | 60.4 | A | F | 12.3 | G |
| | 2/11/13 | LL452515 | 33.0 | 62.0 | A | F | 13.8 | G |
| | 10/20/12 | AS013413 | 28.6 | 61.1 | A | F | 12.5 | G |
| | 4/6/13 | LL458504 | 24.9 | 55.2 | I | M | 14.8 | E |
| | 5/12/13 | LL461308 | 22.2 | 56.5 | I | M | 12.3 | E |
| | 4/22/13 | LL460203 | 30.2 | 59.3 | A | F | 14.5 | E |
| | 10/24/13 | LL474511 | 29.7 | 59.9 | A | F | 13.8 | G |
| | 8/12/13 | LL468213 | 28.6 | 61.7 | A | M | 12.2 | G |
| | 8/8/13 | LL469204 | 30.8 | 60.8 | A | F | 13.7 | G |

Table 3.2. Persistent organic pollutant levels in fat of sea turtles from selected studies. Mean (sd) or range in ng/g wet mass. Ranked generally from highest to lowest by species and location. Loggerhead (Cc), green (Cm), olive ridley (Lo) sea turtles. Juvenile (J), adult (A), male (M), female (F). Dichlorodiphenyldichloroethylene (DDE), dichlorodiphenyltrichloroethanes (DDT), polychlorinated biphenyls (PCBs), chlordanes (CHLs), polybrominated diphenylethers (PBDEs), hexachlorobenzene (HCB). Below reporting limit (<RL).

| Species | Stage/ Sex | Location | Year | Tissue | N | 4,4'-DDE | Σ DDTs | Σ PCBs | Σ CHLs | Σ PBDEs | Mirex | HCB | % Lipid | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cc | JAMF | Virginia - North Carolina | 1991 - 1992 | Fat | 20 | 195 (266) | | 551 (473) | | | | | | Rybitski et al. (1995) |
| Cc | JMF | Core Sound, North Carolina | 2000-2001 | Fat | 44 | 64.9 (64.3) | | 256 (269) | 26.9 (21.3) | | 4.52 (4.06) | 1.13 (2.38) | 26.3 (20.6) | Keller et al. (2004b) |
| Cm | JMF | Baja California | NR | Adipose | 7 | | (<RL - 12.2) | (<RL - 49.5) | (<RL - 65.1) | | | <RL | | Gardner et al. (2003) |
| Lo | NR | Baja California | NR | Adipose | 1 | | 5.1 | 18.4 | 8.1 | | | <RL | | Gardner et al. (2003) |
| Cc | NR | Baja California | NR | Adipose | 1 | | <RL | <RL | <RL | | | <RL | | Gardner et al. (2003) |
| Cc | AMF | Pacific Pelagic | 2012-2013 | Adipose | 2 | (14.0 - 22.0) | (14.1 - 22.5) | (4.54 -5.30) | (2.54 - 2.70) | (0.211 - 0.227) | (0.139 - 0.157) | (1.88 - 2.46) | (84.5 - 86.5) | This study |
| Lo | JAMF | Pacific | 2012-2013 | Adipose | 17 | 15.5 (36.9) | 15.8 (37.1) | 3.95 (7.79) | 1.13 (1.15) | 0.173 (0.0821) | 0.0714 (0.158) | <RL | 64.5 (21.6) | This study |
| Cm | AF | Queensland, Australia Pelagic | 2004-2006 | Adipose | 1 | | | | | 0.2574 | | | 78 | Hermannusen et al. (2008) |
| Cm | JMF | Pacific | 2012-2013 | Adipose | 6 | 1.68 (1.41) | 1.80 (1.37) | 2.71 (2.80) | 0.554 (0.512) | 0.150 (0.0315) | 0.611 (0.689) | 0.251 (0.270) | 69.3 (12.7) | This study |
| Cm | AMF | Kailua Bay, Oahu | 2011- 2012 | Blood (Estimated Fat Concentration)* | 13 | 5.85 | | 0.725 | 0.156 | <RL | | | | Keller et al. (2014) |

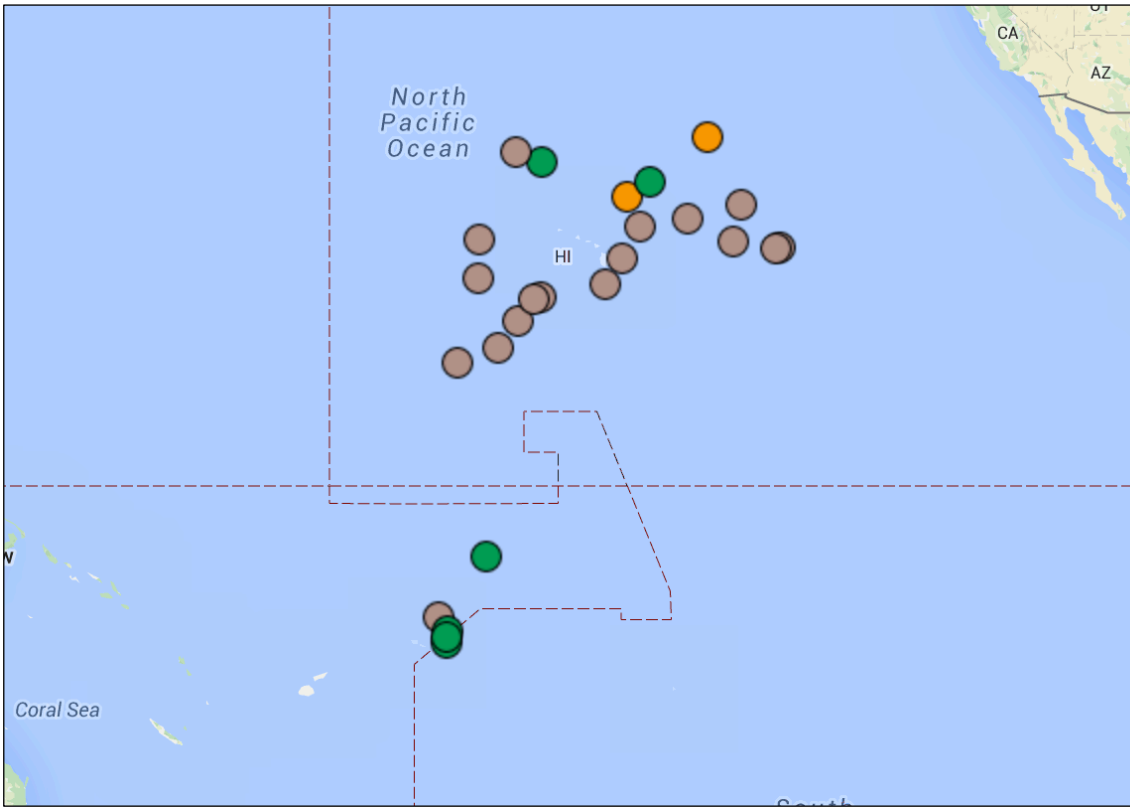
*Summary statistics recalculated using original data in pg/g wet mass plasma using backwards linear equations for the conversion of fat to plasma DDT, PCB and CHL concentrations determined for loggerhead turtles by Keller et al. 2004b.

Table 3.3. Concentrations of persistent organic pollutants (ng/g wet mass) in fat of three pelagic Pacific sea turtle species incidentally captured in longline fisheries. Dichlorodiphenyldichloroethylene (DDE), dichlorodiphenyltrichloroethanes (DDT), polychlorinated biphenyls (PCBs), chlordanes (CHLs), polybrominated diphenylethers (PBDEs), hexabromocyclododecane (HBCD), pentachlorobenzene (PeCB), hexachlorobenzene (HCB). Below reporting limit (<RL). Standard Deviation (SD)

| | *L. olivacea (n=17)* | | | | *C. mydas (n=6)* | | | | *C. caretta (n=2)* | |
|---|---|---|---|---|---|---|---|---|---|---|
| Compound | Detection frequency (%) | Median | Mean ± SD | Range | Detection frequency (%) | Median | Mean ± SD | Range | Detection frequency (%) | Range |
| 4,4'-DDE | 94 | 5.83 | 15.5 ± 36.9* | (<0.593 - 157) | 83 | 0.886 | 1.68 ± 1.41 | (<0.584 - 4.27) | 100 | (14.0 - 22.0) |
| Σ DDTs | 94 | 6.12 | 15.8 ± 37.1* | (<RL- 159) | 83 | 1.02 | 1.80 ± 1.37 | (<RL - 4.27) | 100 | (14.1 - 22.5) |
| PCB 99 | 82 | 0.128 | 0.317 ± 0.623 | (<0.0595 - 2.68) | 100 | 0.1 | 0.229 ± 0.220 | (0.0652 - 0.604) | 100 | (0.391 - 0.459) |
| PCB 118 | 65 | 0.138 | 0.287 ± 0.475 | (<0.0956 - 2.06) | 83 | 0.138 | 0.255 ± 0.212 | <0.0943 - 0.574) | 100 | (0.418 - 0.436) |
| PCB 138 | 65 | 0.417 | 0.917 ± 1.638 | (<0.281 - 6.85) | 50 | 0.317 | 0.707 ± 0.622 | (<0.277 - 1.70) | 100 | (0.916 - 1.25) |
| PCB 149 | 12 | 0.00237 | 0.0221 ± 0.0653* | <0.0591 - 0.267) | 83 | 0.114 | 0.113 ± 0.0448 | <0.0582 - 0.159) | 100 | (0.0814 - 0.0955) |
| PCB 153+132 | 76 | 0.770 | 1.42 ± 2.33 | (<0.356 - 9.62) | 100 | 0.576 | 1.22 ± 0.993 | (0.465 - 2.58) | 100 | (1.786 - 2.02) |
| PCB 180+193 | 59 | 0.391 | 0.710 ± 0.815 | (<0.294 - 3.46) | 33 | 0.640 | 0.673 ± 0.0530 | (<0.289 - 0.757) | 100 | (0.616 - 0.705) |
| PCB 187 | 76 | 0.148 | 0.315 ± 0.529 | (<0.0751 - 2.17) | 67 | 0.183 | 0.255 ± 0.117 | <0.0740 - 0.451) | 100 | (0.616 - 0.705) |
| Σ PCBs | 82 | 1.96 | 3.95 ± 7.79 | (<RL- 32.3) | 100 | 1.11 | 2.71 ± 2.80 | (0.548 - 6.79) | 100 | (4.54 -5.30) |
| *trans*-nonachlor | 94 | 0.764 | 0.952 ± 0.909 | (<0.175 - 4.32) | 50 | 0.226 | 0.494 ± 0.421 | (<0.172 - 1.06) | 100 | (2.13 - 2.32) |
| oxychlordane | 59 | 0.153 | 0.198 ± 0.110 | (<0.212 - 0.565) | 67 | 0.137 | 0.137 ± 0.00258 | (<0.209 - 0.139) | 100 | (0.370 - 0.410) |
| Σ CHLs | 94 | 0.927 | 1.13 ± 1.15 | (<RL - 5.34) | 50 | 0.228) | 0.554 ± 0.512 | (<RL - 1.23) | 100 | (2.54 - 2.70) |
| PBDE 47 | 59 | 0.148 | 0.153 ± 0.0302 | (<0.390 - 0.212) | 100 | 0.140 | 0.150 ± 0.0315 | (0.102 - 0.185) | 100 | (0.211 - 0.227) |
| Σ PBDEs | 65 | 0.152 | 0.173 ± 0.0821 | (<RL - 0.465) | 100 | 0.140 | 0.150 ± 0.0315 | (0.102 - 0.185) | 100 | (0.211 - 0.227) |
| a-HBCD | 24 | 0.0147 | 0.264 ± 0.578* | (<0.100 - 1.72) | 67 | 0.938 | 1.46 ± 1.04 | (<0.0985 - 3.41) | 0 | <0.106 |
| 6-methoxy PBDE 47 | 18 | 0.22 | 0.267 ± 0.102 | (<0.144 - 0.539) | 33 | 0.0961 | 0.181 ± 0.191 | (<0.142 - 0.555) | 0 | <0.153 |
| Mirex | 18 | 0.011 | 0.0714 ± 0.158* | (<0.106 - 0.619) | 83 | 0.23 | 0.611 ± 0.689 | (<0.105 - 1.92) | 100 | (0.139 - 0.157) |
| PeCB | 12 | <RL | <RL | (<RL - 0.166) | 0 | <RL | <RL | <RL | 100 | (0.689 - 0.709) |
| HCB | 0 | <RL | <RL | (<0.129 - 0.588) | 33 | 0.0897 | 0.251 ± 0.270 | (<0.127 - 0.736) | 100 | (1.88 - 2.46) |
| Total extractable organics (% lipid) | | 71.2 | 64.5 ± 21.6 | (0.120- 81.9) | | 70.4 | 69.3 ± 12.7 | (49.0 - 83.7) | | (84.5 - 86.5) |

* Indicates significant differences in concentrations among olive ridley and green sea turtles speci

Figure 3.1.




Capture locations of all sea turtles sampled in this study. Olive ridley turtles (brown), green turtles (green), and loggerhead turtles (orange).

Figure 3.2. Contaminant composition profiles among three species of sea turtles caught in pelagic Pacific Ocean longline fisheries. A) Profile of polychlorinated biphenyls (PCBs); B) Profile of dichlorodiphenyltrichloroethanes (DDT); C) Profile of chlordanes (CHLs); D) Profile of polybrominated diphenylethers (PBDEs).

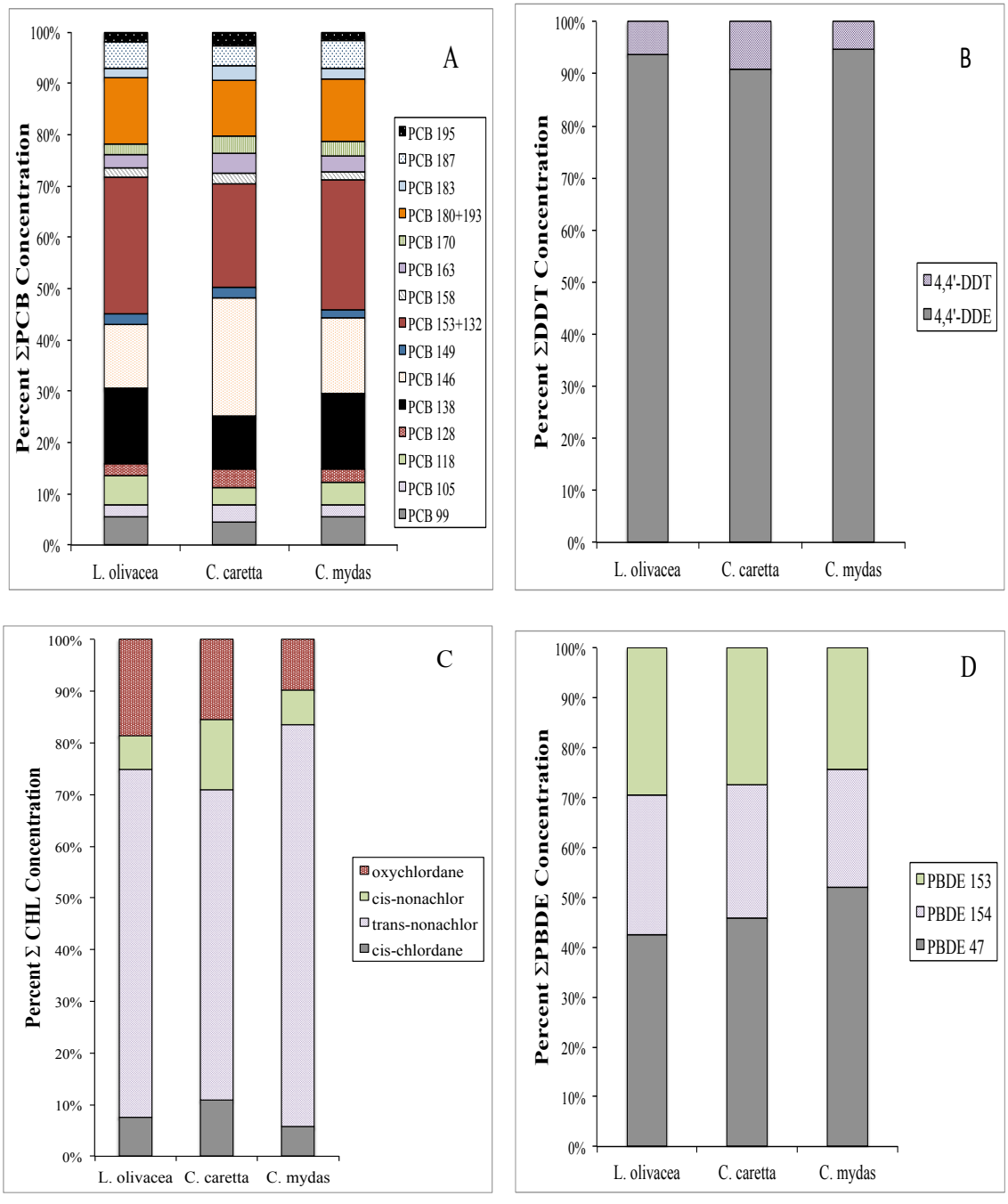
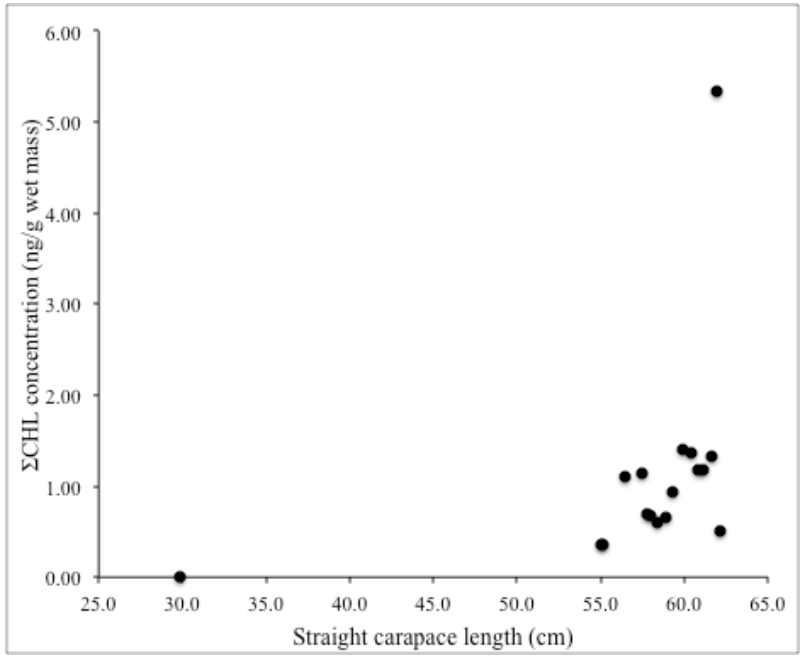


Figure 3.3. Correlation with total chlordanes (CHLs) and straight carapace length and correlation with total polychlorinated biphenyls (PCBs) and straight carapace length in all olive ridley sea turtles.



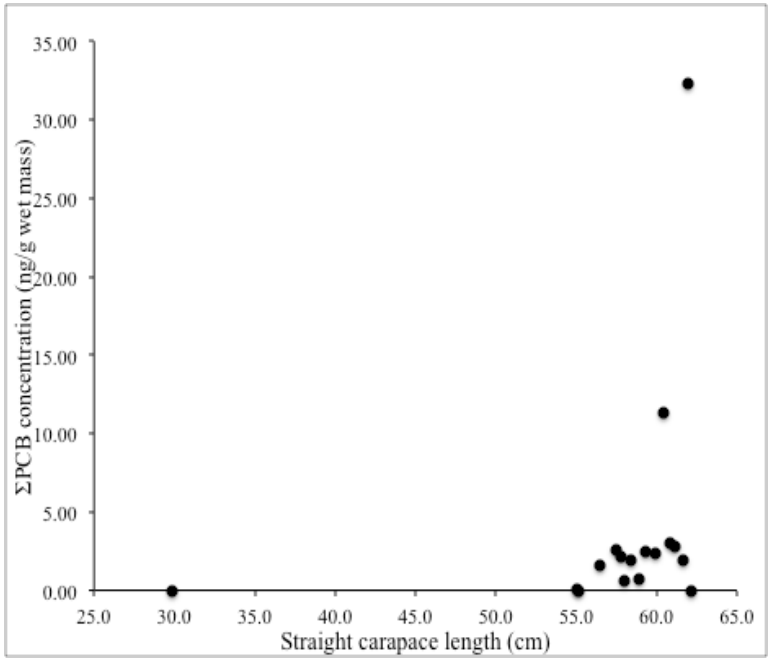
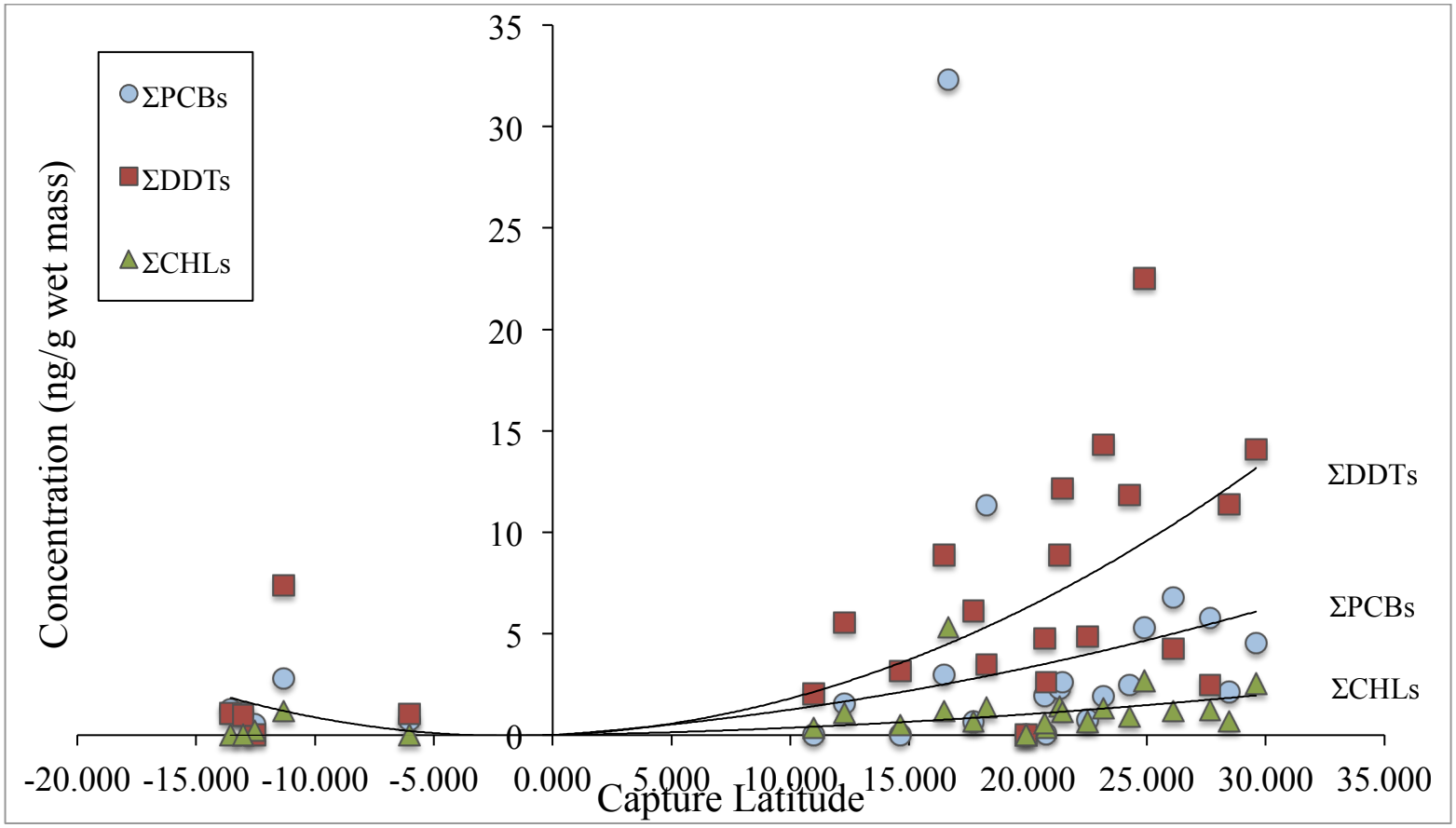



Figure 3.4. Correlations in capture latitude with total polychlorinated biphenyls (PCBs), total dichlorodiphenyltrichloroethanes (DDT), and total chlordanes (CHLs) for all species of sea turtles captured in this study. Removed total DDT concentration for the one adult female olive ridley that was an outlier for total DDTs, but included this turtle's concentrations of total PCBs and total CHLs.



# CHAPTER 4

# Conclusions

Ocean pollution is a serious concern for marine organisms and ecosystems. Here, I quantified a high frequency of debris ingestion (87 %) by pelagic Pacific sea turtles, with a 67 % ingestion rate by loggerheads, 83 % in greens, and 100 % in olive ridleys. These data are consistent with other sea turtle plastic ingestion studies (Laist 1997; Santos et al. 2015; Schuyler et al. 2015; Wedemeyer-Strombel et al. 2015) and further highlights the greater concern for ocean conservation, especially for species of high conservation priority.

Although nearly all sea turtles investigated were in either good or excellent body condition at time of necropsy, effects of such high frequency of debris ingestion should not be overlooked. A recent study suggested that death by anthropogenic debris ingestion is potentially underestimated (Santos et al. 2015). Santos et al. (2015) hypothesized that more immediate causes of death, like fisheries bycatch, may occur before the fatal effects of anthropogenic debris ingestion take their toll. Nearly half of the juvenile green turtles in the study by Santos et al. (2015) died as a direct result of anthropogenic debris ingestion of less than 2.5 g of debris, with a critical amount of only 0.5 g enough to cause death by digestive tract blockage. Equally sized green turtles in this study had a mean mass of ingested debris of 31.2 g, 62.4 times greater than the critical amount of ingested debris determined to cause death. One juvenile green turtle ingested 64.2 g of anthropogenic debris. Olive ridleys had a mean mass of 6.52 g of ingested debris, 13.0 times greater than the critical amount.

Debris ingestion rates were not correlated with capture locations near oceanic convergence zones where anthropogenic debris accumulates. Rather, I found significant differences among species, suggesting that debris ingestion is more related to a species

diet and life cycle. White and clear plastic fragments and sheets are most commonly ingested and most debris was found in the lower intestines, highlighting the importance of collecting the entire GI to accurately determine sea turtle debris ingestion rates.

Additionally, this sample set of Hawaiian and American Samoan longline caught sea turtles provides the largest POPs evaluation for three species of pelagic Pacific sea turtles, which previously had very limited data. These baseline data demonstrate trends in POP concentrations among various pelagic sea turtle species, age classes and geographic locations. As expected, differences in POP concentrations were observed among species as POP concentrations most commonly reflect species trophic status. Additionally, it was expected that POP concentrations should increase with age, as POPs bioaccumulate in organisms.

These baseline contaminant data can aid others in addressing environmental health issues and the global monitoring of POPs. In these pelagic Pacific sea turtles I saw lower POP concentrations than POP concentrations in sea turtles near coastal cities, which would be expected as areas of higher population have higher contamination. However, I found a different trend with ΣDDTs being the predominant POP class rather than ΣPCBs in both olive ridley and loggerhead sea turtles, perhaps implicating the continued use of DDTs in Pacific island regions.

Amounts of ingested plastic were not correlated with POP concentrations in these Pacific sea turtles suggesting contamination is most likely coming from their natural prey. POPs measured in deep-sea fishes in the Western North Pacific Ocean show similar POPs profiles to what was observed in both olive ridley and loggerhead sea turtles (Takahashi et al. 2010). POPs measured in plastic resin pellets from near Hawaii have Σ

PCBs as the predominant POP contaminant class (Heskett et al. 2012), similar to what was observed in these juvenile green turtles. Although ingested plastic was not correlated with POP concentrations, the transfer of contamination from marine debris should not be disregarded and should be investigated further; especially in juvenile green turtles that ingest significantly greater amounts of debris than other sea turtle species.

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**Supplementary Tables:**

Supplementary Table S3.1. Concentrations of all detectable persistent organic pollutants (ng/g wet mass) in fat samples of all individual pelagic Pacific sea turtles. Polychlorinated biphenyls (PCB), dichlorodiphenyldichloroethylene (DDE), dichlorodiphenyltrichloroethanes (DDT), chlordanes (CHL), pentachlorobenzene (PeCB), hexachlorobenzene (HCB). polybrominated diphenylethers (PBDE), hexabromocyclododecane (HBCD). Below reporting limit (<RL).

| Species | *C. caretta* | *C. caretta* | *L. olivacea* | *L. olivacea* | *L. olivacea* | *L. olivacea* | *L. olivacea* | *L. olivacea* | *L. olivacea* | *L. olivacea* | *L. olivacea* | *L. olivacea* | *L. olivacea* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Turtle ID | LL475601 | LL456601 | LL445715 | LL444515 | LL431606 | LL431609 | LL445510 | LL441507 | LL450502 | LL452515 | AS013413 | LL458504 | LL461308 |
| PCB 99 | 0.391 | 0.459 | <RL | 0.113 | 0.078 | 0.155 | <RL | 0.109 | 0.538 | 2.68 | 0.187 | <RL | 0.168 |
| PCB 105 | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | 0.380 | <RL | <RL | <RL |
| PCB 118 | 0.418 | 0.436 | <RL | <RL | <RL | 0.138 | <RL | <RL | 0.534 | 2.06 | 0.178 | <RL | 0.152 |
| PCB 128 | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | 0.694 | <RL | <RL | <RL |
| PCB 138 | 0.916 | 1.25 | <RL | <RL | <RL | 0.417 | <RL | <RL | 2.48 | 6.85 | 0.452 | <RL | 0.415 |
| PCB 146 | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | 1.05 | <RL | <RL | <RL |
| PCB 149 | 0.095 | 0.081 | <RL | <RL | <RL | <RL | <RL | <RL | <RL | 0.267 | <RL | <RL | <RL |
| PCB 153+132 | 1.79 | 2.02 | <RL | 0.446 | <RL | 0.770 | <RL | 0.514 | 4.38 | 9.62 | 0.881 | <RL | 0.676 |
| PCB 158 | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | 0.510 | <RL | <RL | <RL |
| PCB 163 | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | 1.19 | <RL | <RL | <RL |
| PCB 170 | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | 0.25 | 0.76 | <RL | <RL | <RL |
| PCB 180+193 | 0.616 | 0.705 | <RL | <RL | <RL | 0.37 | <RL | <RL | 1.90 | 3.46 | 0.625 | <RL | <RL |
| PCB 183 | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | 0.21 | 0.623 | <RL | <RL | <RL |
| PCB 187 | 0.317 | 0.344 | <RL | 0.09 | <RL | 0.11 | <RL | 0.12 | 1.011 | 2.17 | 0.220 | <RL | 0.148 |
| PCB 195 | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | 0.245 | <RL | <RL |
| total PCBs | 4.54 | 5.30 | <RL | 0.647 | 0.078 | 1.96 | <RL | 0.746 | 11.3 | 32.3 | 2.79 | <RL | 1.56 |
| 44DDE | 14.0 | 22.0 | <RL | 5.83 | 2.39 | 4.51 | 2.99 | 4.64 | 3.36 | 158 | 7.11 | 1.86 | 5.28 |
| 44DDT | 0.130 | 0.474 | <RL | 0.295 | 0.237 | 0.249 | 0.154 | 0.221 | 0.112 | 0.897 | 0.284 | 0.187 | 0.271 |
| total DDTs | 14.1 | 22.5 | <RL | 6.12 | 2.63 | 4.76 | 3.15 | 4.86 | 3.47 | 159 | 7.40 | 2.05 | 5.55 |
| cis-chlordane | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | 0.078 | 0.167 | <RL | <RL | <RL |
| trans-nonachlor | 2.13 | 2.32 | <RL | 0.677 | 0.351 | 0.606 | 0.501 | 0.662 | 1.13 | 4.32 | 0.966 | 0.354 | 0.930 |
| cis-nonachlor | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | 0.288 | <RL | <RL | <RL |
| oxychlordane | 0.410 | 0.371 | <RL | <RL | <RL | <RL | <RL | <RL | 0.153 | 0.565 | 0.213 | <RL | 0.167 |
| total CHLs | 2.54 | 2.70 | <RL | 0.677 | 0.351 | 0.606 | 0.501 | 0.662 | 1.37 | 5.34 | 1.18 | 0.354 | 1.10 |
| PeCB | 0.689 | 0.709 | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL |
| HCB | 1.88 | 2.46 | <RL | <RL | <RL | <RL | <RL | <RL | <RL | 0.588 | <RL | <RL | <RL |
| mirex | 0.139 | 0.157 | <RL | <RL | <RL | <RL | <RL | <RL | 0.254 | 0.619 | 0.187 | <RL | <RL |
| PBDE 47 | 0.227 | 0.211 | <RL | 0.154 | 0.202 | <RL | 0.203 | 0.129 | 0.177 | <RL | 0.164 | 0.212 | <RL |
| PBDE 154 | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | 0.205 |
| PBDE 153 | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | 0.261 |
| total PBDEs | 0.227 | 0.211 | <RL | 0.154 | 0.202 | <RL | 0.203 | 0.129 | 0.177 | <RL | 0.164 | 0.212 | 0.465 |
| a-HBCD | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | 0.111 | <RL | <RL | 1.722 |
| 6-methoxy PBDE 47 | <RL | <RL | <RL | <RL | 0.495 | <RL | <RL | <RL | <RL | <RL | 0.383 | <RL | <RL |
| TEO (%) | 84.5 | 86.5 | 0.120 | 62.5 | 80.1 | 59.9 | 81.9 | 79.1 | 71.2 | 62.3 | 62.1 | 75.8 | 70.4 |

| Species | *L. olivacea* | *L. olivacea* | *L. olivacea* | *L. olivacea* | *L. olivacea* | *L. olivacea* | *C. mydas* | *C. mydas* | *C. mydas* | *C. mydas* | *C. mydas* | *C. mydas* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Turtle ID | LL460203 | LL474511 | LL468213 | LL469204 | LL481001 | LL477006 | LL480011 | LL476104 | AS015728 | AS016421 | AS015316 | AS015808 |
| PCB 99 | 0.197 | 0.168 | 0.116 | 0.198 | 0.221 | 0.232 | 0.393 | 0.604 | 0.097 | 0.100 | 0.065 | 0.115 |
| PCB 105 | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL |
| PCB 118 | 0.175 | 0.151 | 0.138 | 0.218 | 0.136 | 0.179 | 0.465 | 0.574 | 0.138 | 0.117 | <RL | 0.114 |
| PCB 128 | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL |
| PCB 138 | 0.481 | 0.444 | 0.341 | 0.589 | 0.470 | 0.596 | 1.28 | 1.70 | 0.306 | <RL | <RL | <RL |
| PCB 146 | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL |
| PCB 149 | <RL | <RL | <RL | 0.071 | <RL | <RL | 0.114 | 0.159 | 0.147 | 0.072 | <RL | 0.071 |
| PCB 153+132 | 0.812 | 0.825 | 0.703 | 1.01 | 0.770 | 0.924 | 2.40 | 2.58 | 0.576 | 0.465 | 0.483 | 0.810 |
| PCB 158 | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL |
| PCB 163 | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL |
| PCB 170 | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL |
| PCB 180+193 | 0.582 | 0.543 | 0.472 | 0.663 | 0.391 | 0.469 | 0.757 | 0.723 | <RL | <RL | <RL | <RL |
| PCB 183 | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL |
| PCB 187 | 0.222 | 0.169 | 0.162 | 0.222 | 0.146 | 0.214 | 0.351 | 0.451 | <RL | <RL | <RL | <RL |
| PCB 195 | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL |
| total PCBs | 2.47 | 2.30 | 1.93 | 2.97 | 2.13 | 2.61 | 5.76 | 6.79 | 1.26 | 0.754 | 0.548 | 1.11 |
| 44DDE | 11.5 | 8.89 | 14.3 | 8.66 | 11.2 | 11.9 | 2.28 | 4.27 | 0.886 | 0.905 | <RL | 0.879 |
| 44DDT | 0.300 | <RL | <RL | 0.216 | 0.202 | 0.227 | 0.198 | <RL | 0.176 | 0.118 | <RL | 0.105 |
| total DDTs | 11.8 | 8.89 | 14.3 | 8.87 | 11.4 | 12.1 | 2.48 | 4.27 | 1.06 | 1.02 | <RL | 0.983 |
| cis-chlordane | <RL | <RL | <RL | <RL | <RL | <RL | 0.0829 | <RL | <RL | <RL | <RL | <RL |
| trans-nonachlor | 0.764 | 1.11 | 1.04 | 0.950 | 0.548 | 0.920 | 1.02 | 1.06 | <RL | <RL | 0.211 | <RL |
| cis-nonachlor | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL |
| oxychlordane | 0.163 | 0.293 | 0.277 | 0.222 | 0.138 | 0.215 | 0.135 | 0.139 | <RL | <RL | <RL | <RL |
| total CHLs | 0.927 | 1.41 | 1.32 | 1.17 | 0.687 | 1.13 | 1.23 | 1.20 | <RL | <RL | 0.211 | <RL |
| PeCB | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL |
| HCB | <RL | <RL | <RL | 0.201 | <RL | <RL | 0.736 | 0.412 | <RL | <RL | <RL | <RL |
| mirex | <RL | <RL | <RL | <RL | <RL | <RL | 0.494 | 1.92 | <RL | 0.145 | 0.230 | 0.691 |
| PBDE 47 | 0.164 | <RL | <RL | 0.148 | 0.152 | <RL | 0.130 | 0.164 | 0.185 | 0.177 | 0.102 | 0.141 |
| PBDE 154 | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL |
| PBDE 153 | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL |
| total PBDEs | 0.164 | <RL | <RL | 0.148 | 0.152 | <RL | 0.130 | 0.164 | 0.185 | 0.177 | 0.102 | 0.141 |
| a-HBCD | 1.719 | <RL | <RL | <RL | 0.766 | <RL | 1.53 | 3.41 | 0.981 | 0.938 | <RL | <RL |
| 6-methoxy PBDE 47 | 0.539 | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | <RL | 0.555 | 0.212 |
| TEQ (%) | 75.7 | 81.1 | 22.7 | 75.3 | 71.9 | 64.9 | 83.7 | 80.5 | 49.0 | 71.3 | 69.6 | 61.6 |