CHARACTERIZING PLASTIC INGESTION BY BONIN PETRELS (*PTERODROMA HYPOLEUCA*) FROM MIDWAY ATOLL: TOWARDS PLASTIC POLLUTION BIO-INDICATORS FOR THE NORTHWESTERN HAWAIIAN ISLANDS

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A Thesis submitted in partial satisfaction of the requirements
for the degree Master of Science

in

Marine Science

College of Natural and Computational Sciences

Hawai‘i Pacific University

Fall, 2019

Honolulu, Hawaii

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The views presented here are those of the author and are not to be construed as official or reflecting the views of Hawai‘i Pacific University

ABSTRACT

Tubenose seabirds (order Procellariformes) are effective biological indicators of marine plastic debris due to their surface-foraging behaviors, broad diets, and bi-chambered stomachs that accumulate indigestible items. In particular, we contend that the Bonin petrel (*Pterodroma hypoleuca*) is an ideal biological indicator of pelagic microplastic pollution in the Central North Pacific, due to their winter-time breeding phenology with adults provisioning chicks during winter and spring, when the subtropical convergence zone is located farther south. The goal of this study was to develop a baseline of plastic ingestion for this species, and establish necropsy and plastic quantification protocols for future monitoring. Necropsies were performed on 80 Bonin petrels collected opportunistically from Midway Atoll, to characterize (i) body condition, (ii) frequency of occurrence (FO) and loads (mass) of different plastic types, and (iii) the size and polymer composition of ingested plastic. Overall, 98% of hatch-year birds had ingested plastic (n=40), with 90% and 68% contained plastic in the gizzard and the proventriculus, respectively. Fragments (FO = 85%, average mass = 0.0099 g ± 0.0177 S.D.) and line (FO = 93%, average mass = 0.0041 g ± 0.0074 S.D.) were the most commonly ingested. Overall, 95% of after-hatch-year birds had ingested plastic (n = 40), with 93% and 25% containing plastic in the gizzard and the proventriculus, respectively. Fragments (FO = 68%, average mass = 0.0058 g ± 0.0128 S.D.) and line (FO = 83%, average mass = 0.0017 g ± 0.0017 S.D.) were the most commonly ingested plastic types. Overall, there was more plastic in the gizzard than the proventriculus as well as more plastic in hatch-year birds compared to after-hatch-year birds, expressing the need to keep these factors separate to avoid sampling bias. Fragments and line made up the majority of the ingested plastic types, with microplastics (< 5 mm) accounting for 54.6% of the ingested items. Ingested items were all low density, with two polymer types

(polyethylene and polypropylene) accounting for 93% of the plastic mass. Altogether, these results suggest Bonin petrels consume a wide range of floating microplastics and provide insights into the types of plastic within their marine foraging region in the subtropical central North Pacific.

Characterizing Plastic Ingestion by Bonin Petrels (*Pterodroma hypoleuca*) from Midway Atoll: Towards Plastic Pollution Bio-Indicators for the Northwestern Hawaiian Islands

by

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26 November 2019

This thesis is submitted in partial fulfillment of the requirements for the degree of Master of Science in Marine Science at Hawai'i Pacific University. We the undersigned have examined this document and have found that it is complete and satisfactory in all respects, and all revisions required by the final examining committee have been made.

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CHAPTER 3: Characterizing Plastic in the Central North Pacific: Using Bonin Petrels (*Pterodroma hypoleuca*) to Bio-Indicate Plastic Pollution in the North Pacific Subtropical Convergence Zone

CHAPTER 1: Plastic, Plastic Everywhere: A Review of Plastic Pollution in the Central North Pacific Ocean and Using Seabirds as Bio-Indicators

Introduction

The purpose of this literature review is to assess: (1) the accumulation and trends of marine plastic debris (MPD) in the Central North Pacific, (2) policies to address MPD, and (3) the use seabirds as biological indicators of plastic pollution to inform policy-makers and detect regional and temporal trends of MPD. To understand sources and trends in MPD, I provide a brief overview of global plastic production and consumption. From there, I analyze where MPD comes from, how it accumulates in the ocean, focusing on the North Pacific Ocean, and the composition of plastic at sea to provide insight into the sources and fates of MPD, including ingestion by wildlife and sub-lethal threats of MPD. Lastly I describe policies to reduce MPD and using seabirds as bio-indicators of MPD, specifically looking at Bonin Petrels in the Northwestern Hawaiian Islands.

1. Global Plastic Production

We live in the Plastic Age with plastic deeply imbedded in our daily lives (Yarsley and Couzens, 1945). Plastic is a term that broadly describes a wide range of materials that can be manipulated and molded for human use. Thermoplastics that are used most commonly today are primarily synthetic polymers that are manufactured for commercial use, usually with the addition of chemical additives to achieve desired properties. Plastics can be made from any feedstock that contains hydrogen and carbon, with a majority made from fossil fuels, accounting for 8% of global oil production (Andrady & Neal, 2009; Thompson et al., 2009).

The first synthetic polymer was patented in 1909, but mass production began several decades later with 1.7 million tons of plastic produced in 1950 (OSB, 2008). Plastic initially provided an affordable material for the middle class and has grown to redefine global trade and consumerism, revolutionizing modern society (Thompson et al., 2009). Plastic was a ground-breaking product due to its versatility, light weight, and durability. There are hundreds of plastic materials commercially available, but the six most commonly produced for consumer use have standard recycling resin codes based on their primary polymer, including: polyethylene terephthalate (PET, #1), high-density polyethylene (#2), polyvinyl chloride (PVC, #3), low-density polyethylene (LDPE, #4), polypropylene (PP, #5), polystyrene (PS, #6), and other plastics (acrylic, polycarbonate, polyactic fibers, nylon, fiberglass, #7) (Table 1) (Shent et al., 1998). Due to the versatility of plastics, demand has grown and global production is now 380 million tons, based on data from 2015, with PE (30%) and PP (21%) the most commonly produced (Geyer et al., 2017).

Over the past seven decades that plastics have been mass-produced, the demand has shifted towards single-use applications, such as packaging, and in developed countries the amount of plastic in municipal solid waste (MSW) has increased from 1% in 1960 to 10% as of 2005 (Jambeck et al., 2015). Furthermore, it is estimated that 70% of all plastics ever produced are no longer in use (Figure 1). Consumption of plastic is most strongly associated with

developed countries, however plastic usage is increasing in developing countries due to the associated lower costs (Andrady & Teal, 2009). Because plastics do not biodegrade, there are three fates for plastic waste: (1) recycling or repurposing, (2) thermal destruction (combustion or pyrolysis), or (3) accumulation in landfills or the natural environment (Geyer et al., 2017). While there are issues surrounding all three fates of plastic waste, the scope of this literature review focuses on plastic waste in the marine environment.

2. Plastic Pollution in the Ocean

Since this first discovery of MPD, plastic has been documented throughout the global ocean, reaching the most remote parts of the Earth (Thompson et al., 2004). To understand the mechanisms influencing MPD distribution, the sources of the plastic must first be described. The main sources of MPD are land-based waste, and maritime activity (Jambeck et al., 2015). The most data exists on land-based inputs with an estimated 4.8 – 12.7 million MT entering the ocean per year from mismanaged land-based waste. Mismanaged waste is highest in developing, middle-income countries with large populations and fast economic growth, all of which exceeds their waste management infrastructure (Table 2) (Jambeck et al., 2015).

This mismanaged waste can enter the ocean through inland waterways or outflows from wastewater along with transportation from winds and tides. Between < 1 kg to 4200 kg of plastic waste is carried through waterways every day (Carson et al., 2013; Lechner et al., 2014). Because plastics are light-weight and durable, MPD can travel long distances, floating across the ocean basins and persisting for decades (Andrady, 2003; Moore, 2009). Net and visual surveys have documented areas of high plastic concentration, associated with the high atmospheric pressure zones overlaying the subtropical gyres (Cozar et al., 2014; Law et al., 2014; Titmus & Hyrenbach, 2011).

2.1 Plastic Accumulation in the North Pacific Ocean

This literature review primarily focuses on plastic accumulation in the Central North Pacific, focusing on the North Pacific Subtropical Gyre and the North Pacific Subtropical Convergence Zone, their similarities and differences. The North Pacific Subtropical Gyre (NPSTG) accumulates large quantities of plastic debris, reaching densities of 189,800 pieces km^{-2} based on plankton net tows (Law et al., 2014).

High densities of floating plastics aggregate within the NPSTG due to the synergistic interaction of three factors: (1) This is the largest ocean circulation feature on Earth; (2) The positive atmospheric wind stress curl and resulting anticyclonic rotation leads to surface convergence, downwelling at the center and the retention of floating material within the gyre; and (3) The “dead zone”, characterized by weak winds and surface currents, associated with the center of the high atmospheric pressure cell (130°W, 25°N) accumulates floating plastic at the surface, rather than mixing this material throughout the mixed-layer (Howell et al., 2012). Altogether, floating MPD is retained in the NPSTG for an estimated 16 years (Moore, 2003).

Quantifying the abundance and trends of MPD is challenging because of the large spatial extent of the NPSTG (1000s kms), and due to the high patchiness of the MPD distributions,

resulting from the aggregation of MPD at smaller-scale oceanographic features, like fronts and eddies. These physical processes lead to highly variable localized aggregations, with MPD density estimates (number of items km $^{-2}$) ranging by up to five orders of magnitude, 0 – 15,000 pieces km^{-2} (Moore, 2003, Titmus & Hyrenbach, 2011). It is important to note that the NPSTG is characterized as oligotrophic waters with little productivity and biodiversity (Titmus & Hyrenbach, 2011).

The North Pacific Subtropical Convergence Zone (NPSTCZ), which represents the transition zone between the subarctic and subtropical regions of the North Pacific, is another persistent area of high MPD accumulation (Howell et al., 2012). The NPSTCZ is delineated by two large frontal systems, characterized by latitudinal gradients in water temperature, salinity and nutrients. Ekman transport from wind stress creates convergence at the surface layer of the NPSTCZ, with the cooler and denser subarctic water from the north sinking beneath the warmer and lighter subtropical water from the south (Howell et al., 2012; Roden, 1972).

The NPTSTCZ is also characterized by the Transition Zone Chlorophyll Front (TZCF), a sharp gradient of chlorophyll denoting the boundary between the southern low-surface chlorophyll water and the northern higher-surface chlorophyll water (Polovina et al., 2017). This frontal zone is considered a biological hotspot driven by elevated chlorophyll and low-trophic prey concentrations, which attract higher trophic predators such as Loggerhead sea turtles (*Caretta caretta*), Albacore tuna (*Thunnus alalunga*), and seabirds, including albatross and petrels (Scales et al., 2014). The same physical processes concentrating weakly-swimming prey wildlife also aggregate floating MPD, since this region is characterized by high MPD densities associated with Chlorophyll α (Chlα). The high correlation ($r = 0.75$) between foraging animals and concentrated MPD suggest marine wildlife preferentially feed in areas that also accumulate MPD (Pichel et al., 2007). Prevailing winds and currents can transport floating MPD into the NPTSTCZ region, where convergence zones increase MPD accumulation on the warmer side of the front (Franks, 1992; Howell et al., 2012; Pichel et al., 2007).

In winter, shifting atmospheric pressure systems force the Aleutian low and the subtropical high southwards, and shift the subtropical convergence and the TZCF further south to approximately 30 degrees N (Figure 2). These seasonal changes affect MPD distributions in two ways: (1) the stronger winter winds transport MPD southwards, leading to the highest densities in this region and (2) bringing this pollution closer to the Northwestern Hawaiian Islands, where winter-breeding sea birds forage offshore (Figure 3) (Howell et al., 2012; Pichel et al., 2007).

2.2 Plastic Ingestion

While the impacts of this widespread pollution in the marine environment are not fully understood, there is mounting evidence of plastic ingestion in the digestive tracts of many marine species, ranging from marine mammals, sea turtles, seabirds, fish and zooplankton (Ryan et al., 1988; Baird and Hooker, 2000; Mascarenhas et al., 2004; Jung et al., 2018; Rapp et al., 2017). In particular, growing awareness of MPD pollution in the oceanic environment has increased concern for the accumulation of this pollution in ecologically important habitats (frontal systems, eddies) and impacts on the marine vertebrates that forage and migrate through these areas (Duffy and Stachowicz, 2006; Germanov et al., 2018; Rapp et al., 2017).

Plastic ingestion has been reported in approximately 59% of seabird species, an alarming number considering seabirds are the fastest globally declining bird group, and this number is expected only to increase over time (Croxall et al., 2012; Wilcox et al., 2015). Therefore, it is critical to understand the potential individual and population-level impacts of plastic ingestion on seabirds (Lewison et al., 2012; Wilcox et al. 2015). In spite of the extensive documentation of plastic ingestion, there is little evidence of impacts, aside from a few published reports of direct death from obstruction or lacerations in the stomach (Bjorndal et al., 1994; Pierce et al., 2004). Nevertheless, the ingestion of large plastic items can fill the gut, potentially leading to reduced body condition and health (Pierce et al., 2004; Ryan, 1988). There is also growing concern about deleterious indirect effects, involving depressed immunity, hormone disruption, and failed breeding (Ryan et al., 1988).

Sub-lethal impacts of plastic can involve the assimilation of chemicals from the ingested plastics into the tissues of the birds (Ryan et al., 1988). The two types of chemicals that can penetrate into tissue from the gut involve additives (such as plasticizers) originating from the ingested plastic, and hydrophobic pollutants adsorbed from contaminated seawater (Teuten et al., 2009).

Polymers alone are porous structures so additives, including plasticizers, can be included and dispersed throughout the polymer to add desired qualities to the polymer, such as hardening or softening. The most common plasticizers are phthalates, many of which are easily released from the resin during degradation as seen in the marine environment (Teuten et al., 2009). The ability for additives to migrate from the polymer depends on many endogenous factors (including the pore size of the given polymer, the molecular size of the additives) and exogenous factors (like ambient temperature and physical processes that lead to degradation) (Grun and Blumberg, 2007). The release of additives into the environment exposes wildlife to these chemicals with reported impacts to hormone receptors (Grun and Blumberg, 2007).

Furthermore, several plastic types have an affinity to sorb organic contaminants from sea water. Once plastic with sorbed contaminants is ingested, it can expose an organism to higher amounts of harmful chemicals, such as persistent biaccumulative and toxic substances (PBTs), which can bioaccumulate in the individual (Rochman, 2013a). One such plastic known to sorb PBTs is polyethylene, which is the largest mass-produced plastic across the globe and the most commonly found in the marine environment (Andrady, 2011).

Rochman et al. (2013b) observed the complex interactions between the accumulation of plastic and PBTs in the diet of an omnivorous fish (Japanese medaka, *Oryzias latipes*), leading to a “cocktail of pollutants”. This study fed pellets of virgin and marine-treated low-density polyethylene (LDPE) and observed greater PBTs in fish fed virgin plastic pellets compared to the control, and even greater PBTs in fish fed marine-treated LDPE compared to fish fed virgin pellets. The study also observed signs of liver stress due to the chronic exposure (after 2 months) of PBTs in the fish’s diet (Rochman et al., 2013b). It is important to note that PBTs were found in all treatment groups, including the control, suggesting that fish are exposed to chemicals from their natural diet, even if they are not fed plastic pellets This result reinforces the notion that the bioaccumulation of contaminants is a complex process with plastic, pollutants in sea water and natural food items all contributing to the “cocktail” of harmful chemicals.

Never the less, different plastic polymers differ in terms of their sorption rates of contaminants: polyethylene (PE) and polypropylene (PP) have significantly higher sorption rates to contaminants such as PCBs and PAHs, compared to of polyethylene terephthalate (PETE) and polyvinyl chloride (PVC) (Rochman et al., 2013c). Because different polymers absorb persistent organic pollutants (POPs) at various rates and concentrations, characterizing the polymer composition of the ingested MPD can inform the potential impacts of these pollutants (Hermabassiere et al., 2017).

3. Composition of Plastic at Sea

Characterizing plastics at sea provides insight into the sources and fates of MPD. There are many different approaches to describe the types and sizes of MPD and a comprehensive explanation is beyond the scope of this review. Rather, I focus on describing plastics that are bio-available to foraging marine animals based on broad type categories and sizes in the micro- to meso- plastic range.

3.1 Types and Sizes of MPD

A widely-accepted method defines 5 MPD types, based on their shape, compressibility and rigidity (Table 3). First are industrial plastic pellets, which are raw, circular beads of about 4 mm in diameter that are used for industrial production of plastic. Then there are user-plastics that can be broken down into sheets, line, foam, fragment, and other (see Table 1 for definitions of each type) (Van Franecker 2004). Based on at-sea net tow surveys and MPD ingestion studies from the Central North Pacific, fragments were the most common plastic types encountered (Moore et al., 2001; Rapp et al., 2017; Youngren et al., 2018).

MPD range from large items (e.g., derelict vessels) to micro-plastics (e.g., fibers and microscopic fragments). The larger hard plastic fragments break down into smaller pieces with most particles at sea being smaller than 1 cm in diameter (Hidalgo-Ruz et al., 2012). Fragmentation is attributed to a chain-effect of photodegradation, embrittlement, and then breakage from wave-action (Andrady, 2011). This fragmentation leads to different fragment sizes ranging from microplastics to macroplastics (Table 4).

The breakdown process for plastic is very slow and variable because it depends on the properties of the items (composition, thickness) and the conditions in the marine environment (UV, water temperature) (Gregory and Andrady 2003). Thus, it is difficult to predict just how long it takes to degrade (Andrady 2003). Because MPD is slow to break down in the ocean, this material is accumulating in the marine environment (Klemchuk 1990). While it is difficult to identify plastics once they have broken down into small-sized pieces, the chemical composition of plastics can provide valuable information concerning the potential sources (Hoss and Settle, 1990).

3.2 Polymer Composition of MPD

Once plastic undergoes fragmentation, photo-degradation and biofouling at sea, it is rare to be able to identify the plastic types by their polymer resin codes. Therefore, MPD characterization relies on chemical testing (Jung et al., 2018). Recent developments in polymer analyses provide insights into key aspects concerning the identification and degradation of MPD items: (1) their potential source, transport and fate, (2) the risks surrounding their ingestion, and (3) the feasibility and success of potential waste management approaches. First, the density of individual MPD items is influenced by their polymer composition, degradation rates, and biofouling (Avio et al., 2017). Moreover, the inherent densities of the different polymers influence their distribution in the water column, and their potential interactions with marine wildlife (e.g., Jung et al., 2018). Those polymers with densities lighter than seawater (1.025 g/cm^3), typically float on the surface: high-density polyethylene (HDPE, #2), low-density polyethylene (LDPE, #4), and polypropylene (PP, #5). On the other hand, polymers heavier than seawater sink and accumulate closer to their waste input: polyethylene terephthalate (PETE, #1), polyvinyl chloride (PVC, #3), and polystyrene (PS, #6) (Brignac et al., 2019). Second, degradation, through photo- and thermal-oxidation, also changes the average molecular weight and mechanical integrity of the polymers, and affects their degradation rate (Singh and Sharma, 2008; Andraday, 2011). These changes influence their susceptibility to fragmentation, leaching plastic additives, and absorbing persistent organic pollutants (POPs), which in turn can impact ingestion in marine organisms (Teuten et al., 2009; Rochman et al., 2013). Lastly, characterizing MPD polymer composition can provide insights into the effectiveness of current management strategies and future priorities for recycling programs, waste management and polymer production industries (Jung et al., 2018).

4. Policies to Reduce MPD

As MPD input increases, social awareness of plastic pollution continues to grow with calls for effective integrated coastal management and policy solutions (Santos et al., 2005). On a global scale, many nations have recognized the need to work together to reduce plastic pollution (Lohr et al., 2017). Economically, it is estimated that the costs associated with plastic pollution in the ocean costs at least $8 billion per year (Lohr et al., 2017). Many policies are aiming to keep plastic in the economy, rather than lost in the form of waste (Cheshire et al., 2009).

The United Nations Environment Program (UNEP) have established a “Global Initiative on Marine Litter,” recognizing that MPD negatively affects societies and their economics along with marine habitats (Cheshire et al., 2009). Because of this, the UN has developed 17 Sustainable Development Goals (SDGs) that aim to prevent and reduce marine pollution (Lohr et al., 2017). Other global efforts include: the Global Partnership on Marine Litter (GPML), and the Honolulu Strategy, which outline strategies to prevent and manage MPD, and has been adapted by different regions including the U.S. and Canada. One of the main strategies focuses on the market of plastic production, specifically single-use plastic, to minimize waste using an Extended Producer Responsibility (EPR) approach. The other strategy focuses on legislative approaches to reduce MPD, such as targeted bans on certain plastic items (Cheshire et al., 2009; Xanthos & Walker, 2017).

Different parts of the world have taken measures to ban specific products to address concerns for plastic pollution. Taking a specific and focused strategy, areas such as Chicago and California banned smoking on beaches to reduce littered cigarette butts (Novotny et al., 2009). Regions including European countries, African countries, California, Hawaii, China and India have a plastic bag ban in effect and companies such as Starbucks and McDonalds plan to eliminate plastic straw distribution (Zhu, 2011; Lee, 2017).

Policies to reduce single-use plastics and MPD range vastly in their scope and effectiveness but the overall goal for marine conservation is to develop a resource-conserving society (Xanthos and Walker, 2017; Zhu, 2011). Implementation is often slow with a lag between the policy being enacted and the effects of the policy being observed. This is especially true considering the vastness of the marine environment. Because of this lag, proper monitoring is necessary to document the long-term effectiveness of management strategies (Xanthos and Walker, 2017).

5. Using Seabirds as Bio-indicators of MPD

To be an adequate bio-indicator of pollution, there are a multitude of criteria that a species must meet. First, the species has to be sensitive to changes in the environment and have a predictable response for which the cause is easily identified. Ideally, there is a high signal-to-noise ratio. The response to these changes needs to be fast; the longer the lag between the variation and the reaction the more difficult it is to locate the source of the problem. These factors will be taken into consideration when exploring if seabirds make good bio-indicators for pollution (Markert et al. 2003, Durant et al. 2009, Furness and Camphuysen 1997).

4.1 Seabirds are good bio-indicators of ocean health

Due to their trophic positioning, known migration patterns and conspicuous nature, seabirds have been identified as strong bio-indicators for ocean health. Seabirds are closely connected to the water masses and frontal systems in their distribution range. As the ocean climate shifts, avifauna are expected to migrate to new, more suitable habitats. Seabird population numbers are also shown to fluctuate in correlation with changes in water temperature (Fields et al. 1993, Hyrenbach and Veit 2003). With this predictable behavior, when seabird assemblages diverge from the observed norm it is an indication of variability in their normal distribution range.

Seabirds have a high position on the ocean's food web and signify changes that occur in the lower trophic levels. Species with known at-sea distributions are valuable for sampling pelagic pollution trends (Furness and Campyhuysen 1997, Durant et al. 2009). Furthermore, different species of seabirds hold a variety of foraging niches at sea, consisting of surface feeders, divers, and plungers. When multiple species share a breeding site but feed at different oceanic zones in the same region, information can be gathered on what is occurring across zones (Jung et al., 2018). For example, Bulwer's Petrels (*Bulweria bulweria*) are mesopelagic feeders while Cory's Shearwaters (*Calonectris diomeda*) feed in epipelagic zones. These species share breeding sites but forage in completely different niches allowing for cross-comparisons of the

two zones (Furness and Monaghan, 1987). On the other hand, populations of the same species are found throughout numerous regions across the globe.

Pragmatically, these species are comparatively easy to census and study at colonies, especially given their colonial breeding habits. Furthermore, seabirds are considered a charismatic species and generate great interest from the general public and science groups, which is valuable for funding purposes. Extensive general knowledge and historical data exists on seabirds making it easy to detect responses to changes outside of the norm and changes over time (Furness and Camphuysen 1997).

4.2 Bio-indication of plastic: Factors that affect plastic ingestion

Differences in incidence among seabird species is elicited primarily based on plastic abundance in the surrounding environment along with varying foraging strategies, search images, and stomach anatomy (Day et al., 1985; Ryan, 1987). The amount of plastic consumed is also a function of bird age (due to off-loading to chicks), time of year, and the place/year of collection (Ryan, 1987). The areas with the highest rate of plastic ingestion in seabirds are both urbanized locations and areas of oceanic convergence where plastic accumulate, including the North Pacific and portions of the South Pacific (Spear et al. 1995). In these locations, up to 80% of seabird species have ingested plastic (Ryan et al., 1990).

Plastic is most commonly found in tubenose species (order Procellariiformes) including albatross, fulmars, shearwaters and petrel species (Nevins et al., 2005; Ryan et al. 2009, Van Franeker et al., 2005). Among tubenose species, the rate of plastic ingestion is increasing rapidly. For example, Laysan Albatross had an FO of 74% in 1966 and 100% in 2012 – an increase of 26% in fifty years (Kenyon and Kridler, 1969; Lavers and Bond, 2016).

Foraging strategy plays a crucial role in the incidence of MPD ingestion. Seabirds that feed by dipping, pattering and surface foraging had the highest FO. The table below (Table 4) compares foraging strategy to plastic ingestion FO (Ryan, 1987). Surface feeding birds are most likely to interact with neustonic plastic particles that make up the majority of plastics at sea.

Diet is also largely correlated with incidence to plastic ingestion because seabirds are thought to mistake the MPD for prey (Day et al., 1985). Omnivores have been reported with the highest FO compared to diets of crustaceans, cephalopods, and fish. Omnivores are more generalists which would explain their greater FO compared to dietary specialists (Ryan, 1987). However, this assumes all plastic is mistaken for food rather than being consumed alongside prey items. This is called secondary ingestion and is exemplified in egg masses, a common food item, which adhere to plastic pieces at sea (Pettit et al., 1981; Rapp et al., 2017).

The stomach anatomy of seabirds affects the accumulation of plastic in the digestive tract. Seabirds with a simple stomach structure readily regurgitate food items, including plastic debris (Wilson, 1984). On the other hand, seabirds with a bi-chambered stomach have a constriction between the proventriculus and gizzard causing hard items and plastic pieces to accumulate (Ryan and Jackson, 1986).

4.2 Fulmar-Litter EcoQO monitoring

The North Sea Ministerial Conference was held in 2002 in an attempt to address transcontinental marine litter concerns with one of the main goals being to quantify plastic abundance. At this conference, the Fulmar-Litter-Ecological Quality Objective (EcoQO) was introduced, which aimed to measure plastic in the ocean to help implement policy. Fulmar-Litter-EcoQO monitoring analyzes plastic incidence in the stomachs of specific seabird species in a given area and uses those proportions to assess plastic debris trends for that region (Van Franeker et al. 2003). Once EcoQO monitoring was developed, the Oslo-Paris Convention met to establish specific parameters for this process and Van Franeker at al. (2003) published a list of qualifications that were needed for a seabird to be a good candidate for Litter-EcoQO monitoring:

- Abundant and found regularly in surveys, guaranteeing adequate samples for research
- Known to consume large variety of marine litter items
- Forage exclusively at sea and avoid nearshore areas
- Accumulate indigestible items in stomach rather than readily regurgitate items
- Stomach contents represent wide offshore environment, rather than one local pollution site
- Historical data is available on other locations and related species worldwide

Northern Fulmars are abundant in the North Sea and easily available to survey, making it easy for research to be conducted. These seabirds also forage exclusively at sea and have been documented thoroughly since 1982 (Van Franeker 2004, Van Franeker & Bell 1985). Lastly, this species is known to consume plastic and does not typically regurgitate marine litter, meaning that plastic will accumulate in their stomachs and give a more accurate depiction of the types of litter at sea (Van Franeker 2004). Because of this, Northern Fulmars are considered a strong indicator for pollution and became the pilot species for EcoQO Litter Monitoring. Metrics for rating the well-being of an area were established and it was decided that an acceptable EcoQO score was for less than 10% of the fulmars to have more than 0.1 g of plastic in their gut (Van Franeker et al. 2003).

With the list Van Franeker et al. created, researchers are able to take these metrics and apply it to other seabird species in various areas. For this to occur, the target amount of <10% of Fulmars having 0.1 g of plastic must be scaled according to the species being studied. In a study examining Laysan Albatross fledglings, the mean fledgling mass was calculated as 1.9 kg and compared to the Fulmar mean mass of 0.65 kg. This then scaled the 0.1 g of plastic found in Fulmars to be 0.3 g of plastic in Laysan Albatross. This species-adjusted EcoQO states that if <10% of studied Laysan Albatross fledglings have less than 0.3 g of plastic in their digestive tract, then that area being sampled gets a passing grade for their EcoQO (Van Franeker 2004, Lavers & Bond 2016).

There are several issues with the current methods used for Litter-EcoQO monitoring. First, researchers have had difficulty applying the techniques to other species and finding a good indicator species that meets the requirements laid out by Van Franeker et al. For example, in the aforementioned Laysan Albatross study, there was high variability in the amount of plastic

ingested from year-to-year. Compared to the Northern Fulmar, which had low variation between years, the Laysan Albatross was ruled out as a good indicator species even though it is considered the posterchild for plastic pollution (Fry et al. 1987, Lavers & Bond 2016, Van Franeker 2004).

Another issue with the current Litter-EcoQO monitoring system is the grading system. When areas of interest are being studied under this EcoQO, that area receives a "passing" or "failing" grade depending on whether or not >10% of the species had the species-adjusted amount of plastic in them. This was developed by OSPAR for the Ministers of the North Sea to rate countries in the North Sea on their marine plastic litter (Van Franeker 2004). However, this does not give the entire picture of what is occurring with plastic trends and it would be more beneficial to report the numbers based on a scale for long term studies.

5. Bonin Petrels and Plastic

One seabird species that has been observed ingesting plastic is the Bonin Petrel (*Pterodroma hypoleauca*) (Lavers & Bond 2016, Rapp and Hyrenbach 2015). Bonin Petrel characteristics will be examined to explore the species' suitability as bioindicators for plastic pollution.

Bonin Petrels breed exclusively in North Pacific latitudes with a majority of their population on Midway Atoll (24°N, 166°W - 28°N, 178°W). After a history of struggling to survive predation from rats introduced to Midway, Bonin Petrels are at a stable population and listed as "Least Concern" by the International Union for Conservation of Nature (IUCN) (Seto & O'Daniel, 1999; BirdLife International, 2016). This species breeds in early winter with a single attempt and when nesting has strong nest and mate fidelity and rarely disperses.

These traits have made them successful breeders on Midway, however, as sea-level is projected to rise these characteristics could harm them (Grant, 1983; Harrison, 1990; Seto, 1994; Seto & Daniel, 1999). Having a single attempt at nesting, rare dispersal and burrowing put these petrels at risk because if sea-levels rise or they experience storm flooding, they could lose their only egg for the season and their burrow is at risk of collapsing. Additionally, Bonin Petrels breed in the winter when the wave action is strongest which puts them in even greater jeopardy of a flooding event. This is a serious threat to their population because approximately 98% of the entire Bonin Petrel population inhabits Midway Atoll. If one wave-driven flooding event of 2m hits the island, 40% of the entire Bonin Petrel population could be lost (Figure 5; Reynolds et al., 2015).

Bonin Petrels feed at night predominately on lantern fishes, hatchetfishes and squid (Seto & O'Daniel, 1999). Because they feed off-shore at the surface, Bonin Petrels have a high likelihood of encountering and ingesting small neustonic plastic pieces (Harrison, 1990). Thus, the limited studies conducted on Bonin Petrels to date, revealed a high rate of incidence ranging from 75% (n = 8 chicks from Midway, NWHI) to 100% incidence (n = 5 chicks and 1 immature/adult bird from Tern Island, NWHI) (Lavers & Bond, 2012; Rapp et al., 2017).

Because Bonin Petrels are nocturnal foragers and burrow nesters, they are rather under-studied (Lavers and Bond, 2016; Reynolds et al., 2015). While there is little information about Bonin Petrel diet, health and pollutant loads to date, there are four published studies of plastic ingestion in this species (Table 5).

5.1 Bonin Petrels as Bio-indicators of Plastic

A synthesis of all the qualities needed for a species to be a good bioindicator will now be applied to what is known about Bonin Petrels on Midway Atoll. Following Van Franeker's list for qualities of a bioindicator, the first important aspect is that the species has an abundant population that can be found and surveyed easily. While Bonin Petrel populations on Midway Atoll were decimated in the early 1900s due to the introduction of rats, their numbers have stabilized since 1984 as conservation efforts have mostly eradicated black rats on the island. As of 1995, the total breeding population throughout the Hawaiian Archipelago was about 358,000 pairs and this number has been expected to increase since then (Seto, 1995).

On top of their increasing abundance, Bonin Petrels are also efficient for sampling because of the fidelity to their nest site and the density of burrows within each nest site (Harrison 1990, Seto and O'Daniel 1999). This species forages at sea and the marine range of the Hawaiian Archipelago populations is in the central Pacific Ocean to about 35-40° N between July to April (Kuroda 1991). During breeding on the Northwestern Hawaiian Islands, Bonin Petrels are observed to stay close to the nesting sites but continue to forage at sea, as opposed to near shore (Seto and O'Daniel 1999).

Bonin Petrels are surface feeders that forage at night. This species has a unique foraging niche because they consume offshore fishes and squid that are found in deep waters and surface at night (Harrison and Seki 1987). Using Bonin Petrels as bioindicators creates an opportunity to also study trends of deep sea prey items. Because they are surface feeders, it is highly likely that this species consumes plastic that floats at the surface, however there is not much data on the variety of marine litter these petrels consume. The few studies that exist report a high incidence rate of plastic in the stomachs of this species. A study by Lavers and Bond (2016) found a 75% incidence rate of plastic (n=8), while another study by Rapp and Hyrenbach (2015) found a 100% incidence rate (n=3) (Lavers and Bond 2016, Rapp and Hyrenbach 2015). In both studies, the sample sizes were too small to make any strong conclusions. The presence of a gizzard in Bonin Petrels signifies that indigestible items will most likely accumulate within the stomach rather than be readily regurgitated, which strengthens the argument towards using this species to indicate plastic trends. Compared to other seabird species, there is not a lot of historical data on Bonin Petrels. However there are numerous detailed historical accounts of other petrel species across the globe allowing for cross-species comparisons (Croxall et al. 2012, Seto and O'Daniel 1999).

6. Conclusions and Unresolved Issues

Seabirds are widely accepted as suitable bioindicators for studying wide-ranging plastic trends. Van Franeker's list of necessary qualifications for a species to be an adequate bioindicator leaves a roadmap for researchers to apply Fulmar-Litter EcoQO monitoring to a

variety of seabirds across the globe. This type of monitoring has potential to impact regional policy on anthropogenic marine debris but the methods of scoring need to move beyond a "pass/fail" approach to incorporate a larger understanding of plastic trends. From the data that exists on Bonin Petrels, this species meets many of the standards to be a strong indicator of marine plastic pollution trends. However, there is a serious lack of data on the incidence, amount and types of plastic in Bonin Petrels. In conclusion, with more research Bonin Petrels have the potential to tell a lot of information about plastic trends around Midway Atoll and throughout the Northwestern Hawaiian Islands.

References

Andraday, A.L. (ed.)2003. *Plastics and the Environment*. New York: Wiley

Andrady, A.L.; Neal, M.A. 2009. Plastics benefit mankind. *Philosophical Transactions of the Royal Society B Biological Sciences* 364:1977-1984.

Ashmole, N.P. & Ashmole, M.J. 1967. Comparative feeding ecology of sea birds of a tropical oceanic island. Peabody Museum of Natural History, Yale University, Bulletin 24. Peabody Museum of Natural history, New Haven, Connecticut, U.S.A.

Arnot, J.A. and Gobas, F.A.P.C. 2006. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. *Environmental Reviews.* 14:257-297.

Auman, H.J., Ludwig, J.P., Giesy, J.P. Colborn, T. 1997. Plastic ingestion by Laysan Albatross chicks on Sand Island, Midway Atoll, in 1994 and 1995. *Albatross Biology and Conservation*. 239-244.

Baird, R.W.; Hooker, S.K. 2000. Ingestion of plastic and unusual prey by a juvenile Harbor Porpoise. *Marine Pollution Bulletin*. 40:719-720.

Barnes, F., Galgani, R.C., Thompson, M.B. 2009. Accumulation and fragmentation of plastic debris in global environments. *Philosophical Transactions of the Royal Society of London Series B.* 364:1985-1998.

Boerger, C.M.; Lattin, G.L.; Moore, S.L.; Moore, C.J. 2010. Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. *Marine Pollution Bulletin*. 60:2275-2278.

Brignac, K.C.; Jung, M.R.; King, C.; Royer, S.J.; Blickley, L.; Lamson, M.R.; Potemra, J.T.; Lynch, J.M. 2019. Marine debris polymers on main Hawaiian Island beaches, sea surface, and seafloor. *Environmental Science Technology* 53:21.

Carson, H.S.; Lamson, M.R.; Nakashima, D.; Toloumu, D.; Hafner, J.; Maximenko, N.; McDermid, K.J. 2013. Tracking the sources and sinks of local marine debris in Hawai'i. *Marine Environmental Resources*. 84:76-83.

Cheshire, Adler, E; Barbiere, J.; R.T.; Cohen, Y.; Evans, S.; Jarayabhand, S.; Jeftic, L; Jung, R.T.; Kinsey, S.; Kusui, T.; Lavine, I.; Manyara, P.; Oosterbaan, L.; Pereira, M. Sheavley, S.; Tkalin, A.; Varadarajan, S.; Wenneker, B.; Westphalen, G. 2008. UNEP/IOC Guidelines on survey and monitoring of marine litter. *UNEP*. Book.

Clukey, K.E.; Lepczyk, C.A.; Balazs, G.H.; Work, T.M.; Li, Q.X.; Bachman, M.J.; Lynch, J.M. 2017. Persistent organic pollutants in fat of three species of Pacific pelagic longline caught sea turtles: Accumulation in relation to ingested plastic marine debris. *Science of the Total Environment*.

Cole, M.; Lindeque, P.; Fileman, E. 2013. Microplastic ingestion by zooplankton. *Science and Technology*.

Connolly, J.P.; Pederson, C.J. 1988. A thermodynamic-based evaluation of organic chemical accumulation in aquatic organisms. *Environmental Science Technology*. 22:99-103.

Croxall, J.P.; Butchart, S.H.M.; Lascelles, B.; Stattersfield, A.J.; Sullivan, B.; Symes, A.; Taylor, P. 2012. Seabird conservation status, threats and priority actions: a global assessment. *Bird Conservation International.* 22:1-34.

Derraik J.G.B. 2002. The pollution of the marine environment by plastic debris: a review. *Marine Pollution Bulletin*. 44:842-852.

Durant, J.M., Hjermann, D.O., Frederiksen, M., Charrasssin, J.B., Le Maho, Y., Sabarros, P.S., Crawford, R.J.M., Stenseth, N. Chr. 2009. Pros and cons of using seabirds as ecological indicators. *Climate Research*. 39:115-129.

Eriksen, M., Maximenko, N., Thiel, M., Cummins, A., Lattin, G., Wilson, S., Hafner, J., Zellers, A., Rifman, S., 2013. Plastic Pollution in the South Pacific subtropical gyre. *Marine Pollution Bulletin*. 68(1-2):71-76.

Eriksen, M. Lebreton, L.C.M; Carson, H.S.; Thiel, M.; Moore, C.J.; Borerro J.C.; Galgani, F.; Ryan, P.G.; Reisser, J. 2014. Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. *PLoS ONE* 9(12):e111913.

Fields, P.A., Graham, J.B., Rosenblatt, R.H., Somero, G.N., 1993. Effects of expected global climate change on marine faunas. *Trends in Ecology and Evolution*. 8:361-366

Fisk, A.T.; Hobson, K.A.; Nordstrom, R.J. 2001. Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the Northwater Polynya marine food web. *Environmental Science and Technology*. 732:35.

Fry, M.D., Fefer, S.I., Silea, L. 1987. Ingestion of plastic debris by Laysan Albatross and Wedge-Tailed Shearwaters in the Hawaiian Islands. *Marine Pollution Bulletin*. 18(6): 339-343.

Furness, B.L., Laugksch, R.C and Duffy, D.C. 1984. Cephalopod beaks and studies of seabird diets, Auk 101:619-620.

Furness, R.W. and Monaghan, P. 1987. Seabird Ecology. Blackie, Glasgow. 164 pp.

Furness, R.W. and Camphysen, C.J. 1997. Seabirds as monitors of the marine environment. *ICES Journal of Marine Science*. 54:726-737.

Gregory, M.R., Andrady, A.L., 2003. Plastics in the Marine Environment. *Plastics and the Environment*. 10: 379-402.

Gobas, F.A.P.C.; Zhang, Xin; Wells, R. 1993. Gastrointestinal magnification: the mechanism of biomagnification and food chain accumulation of organic chemicals. *Environmental Science Technology*. 27: 2855-2863.

Goldstein, M.C.; Rosenberg, M.; Cheng, L.; 2012. Increased oceanic microplastic debris enhances oviposition in an endemic pelagic insect. *Biological Letters*. 8:817-820.

Grant, G. S., J. Warham, T. N. Pettit, and G. C. Whittow. 1983. Reproductive behavior and vocalizations of the Bonin petrel. Wilson Bull. 95:522-539.

Harrison, C.S. 1990. Seabirds of Hawaii, natural history and conservation. Comstock/Cornell, Ithaca, NY. *Seabirds:Feeding Biology and Role in Marine Ecosystems*. 305-326.

Harrison, C.S., Seki, M.P. 1987. Trophic relationships among tropical seabirds at the Hawaiian Islands.

Hyrenbach, D.K., Veit, R.R. 2003. Ocean warming and seabird communities of the *southern* California Current System (1987-98): response at multiple temporal scales. *Deep-Sea Research II*. 50:2537-2565.

Kenyon, K.W., Kridler, E. 1969. Laysan Albatrosses Swallow Indigestible Matter. *The Auk*. 86(2):339-343.

Klemchuk P. P. 1990 Degradable plastics: a critical review. Polym. Degrad. Stab. **27**, 183–202.

Kuroda, N. 1991. Distributional patterns and seasonal movements of Procellariiformes in the North Pacific. *North Pacific Community Bulletin*. 53(III).

Jambeck, J.R.; Geyer, R.; Wilcox, C.; Siegler, T.R.; Perryman, M.' Andrady, A. Narayan, R., Law, K.L. 2015. Plastic waste inputs from land into the ocean. *Science* 347(6223):768-771.

Jung, M.R.; Horgen, F.D.; Orski, S.V.; Rodriguez, V.C.; Beers, K.L.; Balazs, G.H.; Jones, T.T.; Work, T.M.; Brignac, K.C.; Royer, S.J.; Hyrenbach, K.D.; Jensen, B.A.; Lynch, J.M. 2018. Validation of ATR FT-IR to identify polymers of plastic marine debris, including those ingested by marine organisms. *Marine Pollution Bulletin* 127:704-716.

Jung, M.R.; Balazs, G.H.; Work, T.M.; Jones, T.T.; Orski, S.V.; Rodriguez, V.C.; Beers, K.L.; Brignac, K.C.; Hyrenbach, K.D.; Jensen, B.A.; Lynch, J.M. 2018. Polymer identification of plastic debris ingested by pelagic-phase sea turtles in the Central Pacific. *Environmental Science and Technology* 52: 11535 – 11544.

Katija, K.; Choy, C.A.; Sherlock, R.E.; Sherman, A.D.; Robison, B.H. 2017. From the surface to the seafloor: how giant larvaceans transport microplastics into the deep sea. *Science Advances* 3:e1700715.

Lebreton, L.; Slat, B.; Ferrari, F.; Sainte-Rose, B.; Aitken, J.; Marthouse, R.; Hajbane, S.; Cunsolo, S.; Schwarz, A.; Levivier, A.; Noble, K.; Debeljak, P.; Maral, H.; Schoeneich-Argent, R.; Brambini, R.; Reisser, J. 2018. Evidence that the Great Pacific Garbage Patch is rapidly accumulating plastic. *Nature – Scientific Reports* 8:4666.

Lavers, J. L., & Bond, A. L. 2016. Ingested plastic as a route for trace metals in Laysan Albatross (Phoebastria immutabilis) and Bonin Petrel (Pterodroma hypoleuca) from Midway Atoll. *Marine Pollution Bulletin.* 110(1):493-500.

Law, K.L.; Moret-Ferguson, S.; Maximenko, N.A.; Proskurowski, G.; Peacock, E.E.; Hafner, J.; Reddy, C.M. 2010. Plastic accumulation in the North Atlantic subtropical gyre. *Science*. 2010. 329:1188-1195.

Lechner, A.; Keckeis, H.; Lumesberger-Loisl F.; Zens, B.; Krusch, R.; Tritthart, M.; Glas, M.; Schludermann, E.; 2014. The Danube so colourful: A potpourri of plastic litter outnumbers fish larvae in Europe's second largest river. *Environmental Pollution*. 188:177-181.

Lithner, D.; Nordensvan, I.; Dave, G. 2012. Comparative acute toxicity of leachates from plastic products made of polypropylene, polyethylene, PVC, acrylonitrile-butadiene-styrene, and epoxy to *Daphnia magna*. *Environmental Science Pollution*. 19:1763-1772.

Mascarenhas, R.; Santos, R.; Zeppelini, D. 2004. Plastic debris ingestion by sea turtle in Paraiba, Brazil. *Marine Pollution Bulletin*. 49:354-355.

Mato, Y., Isobe, T., Takada, H., Kanehiro, H., Ohtake, C., Kaminuma, T., 2001. Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. *Environmental Science and Technology*. 35:318-324.

Markert, B.A, Breure, A.M., Zechmeister, H.G. 2003. Bioindicators and Biomonitors. *Trace Metals and Other Contaminants in the Environment.*

Maquette. 2013. Plastics – the Facts 2013. *Plastics Europe*.

Moret-Ferguson, S.; Law, K.L.; Proskurowski, G.; Murphy, E.K.; Peacock, E.E.; Reddy, C.M. 2010. The size, mass, and composition of plastic debris in the western North Atlantic Ocean. *Marine Pollution Bulletin*. 60:1873-1878.

Morishige, C., Donohue, M.J., Flint, E., Swenson, C., Woolaway, C. 2007. Factors affecting marine debris deposition at French Frigate Shoals, Northwestern Hawaiian Islands Marine National Monument, 1990-2006. *Marine Pollution Bulletin*. 54:1162-1169.

National Research Council (U.S.) 1975. Study Panel on Assessing Potential Ocean Pollutants,*Assessing Potential Ocean Pollutants: A Report of the Study Panel on Assessing Potential Ocean Pollutants to the Ocean Affairs Board, Commission on Natural Resources, National Research Council* (National Academy of Sciences, Washington, DC, 1975).

Nevins, H.M.; Hyrenbach, D.K.; Keiper, C.; Stock, J.; Hester, M.; Harvey, J. 2005. Seabirds as indicators of plastic pollution in the North Pacific. Plastic Debris Rivers to the Sea Conference.

Ocean Studies Board. 2008. *Tackling Marine Debris in the 21st Century*. Washington, DC: The National Academic Press.

Pettit, T.N.; Grant, G.S.; Whittow, G.C. 1981. Ingestion of plastics by Laysan albatross. *Auk*. 98:839-841.

Pichel, W.G.; Churnside, J.H., Veenstra, T.S., Foley, D.G.; Friendmand, K.S.; Brainard, R.E., Nicoll, J.B.; Zheng, Q. Clemente-Colong, P. 2007. Marine debris collects within the North Pacific Subtropical Convergence Zone. *Marine Pollution Bulletin*. 54:1207-1211.

Pierce, K. E., Harris, R. J., Larned, L. S., & Pokras, M. A. 2004. Obstruction and starvation associated with plastic ingestion in a northern gannet (*Morus bassanus)* and a greater shearwater (*Puffinus gravis*). *Marine Ornithology*, 32, 187-189.

Polovina, J.J.; Kobayashi, D.R.; Parker, D.M.; Seki, M.P.; Balazs, G.H. 2000. Turtles on the edge: movement of loggerhead turtles (*Caretta caretta*) along ocean fronts, spanning longline fishing grounds in the central North Pacific, 1997-1998. *Fisheries Oceanography*. 9:71-82.

Polovina, J.J.; Howell, E.; Kobayashi, D.R.; Seki, M.P. 2001. The transition zone chlorophyll front, a dynamic global feature defining migrate and forage habitat for marine resources. *Progress in Oceanography*. 49:469-483.

Polovina, J.J.; Howell, E.A.; Kobayashi, D.R.; Seki, M.P. 2017. The Transition Zone Chlorophyll Front updated: Advances from a decade of research. *Progress in Oceanography*. 150:79-85.

Proctor, N. S. and P. J. Lynch. 1993. *Manual of Ornithology.* Yale University Press, New Haven.

Provencher, J.F.; Bond, A.L.; Avery-Gomm, S.; Borrelle, S.B.; Rebolledo, E.L.; Hammer, S.; Kuhn, S.; Lavers, J.L.; Mallory, M.L.; Trevail, A.; van Franeker, J.A. 2017. Quantifying ingested debris in marine megafauna: a review and recommendations for standardization. *Anal. Methods.* 9:1454-1469.

Rapp, D.C., Hyrenbach, K.D. 2015. Community-wide patters and impacts of plastic ingestion in the seabirds of French Frigate Shoals, Papahanaumokuakea Marine National Monument. Unpublished Thesis.

Rapp, D.C.; Youngren, S.M.; Hartzell, P.; Hyrenbach, K.D. 2017. Community-wide patterns of plastic ingestion in seabirds breeding at French Frigate Shoals, Northwestern Hawaiian Islands. *Marine Pollution Bulletin*. 123:269-278.

Ribic, C.A.; Sheavly, S.B.; Rugg, D.J.; Erdmann, E.S. 2012. Trends in marine debris along the U.S. Pacific Coast and Hawai'I 1998-2007. *Marine Pollution Bulletin*. 64:994-1004.

Rochman, C. M.; Browne, M. A.; Halpern, B. S.; Hentschel, B. T.; Hoh, E.; Karapanagioti, H. K.; Rios-Mendoza, L. M.; Takada, H.; Teh, S.; Thompson, R. C. 2013. Classify plastic waste as hazardous. *Nature* 494(7436):169−171.

Rochman, C. M.; Hoh, E.; Kurobe, T.; Teh, S. J. 2013. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. *Scientific Reports* 3:3263.

Ryan, P.G. 1987. The effects of ingested plastic on seabirds: Correlations between plastic load and body condition. *Environmental Pollution*. 46:119-125.

Ryan, P.G. 1987. The incidence and characteristics of plastic particles ingested by seabirds. *Mar. Environ. Res.* 23:175-206.

Ryan, P.G.; Jackson, S. 1987. The lifespan of ingested plastic particles in seabirds and their effect on digestive efficiency. *Marine Pollution Bulletin*. 19(3):125-128.

Ryan, P.G.; Connell, A.D.; Gardner, B.D. 1988. Plastic ingestion and PCBs in seabirds: is there a relationship? *Marine Pollution Bulletin*. 19:4:174-176.

Ryan, P.G., Connell, A.D., Gardner, B.D., 1990. Plastic ingestion and PCBs in seabirds: is there a relationship? *Marine Pollution Bulletin* .19:174–176.

Ryan, P.G.; Moore, C.J.; van Franeker, J.A.; Moloney, C.L. 2009. Monitoring the abundance of plastic debris in the marine environment. *Philosophical Transactions B*. 364:1999-2012.

Sebille, E.V., Wilcox, C., Lebreton, L., Maximenko, N., Hardesty, B.D., Van Franeker, J.A., Eriksen, M., Siegel, D., Galgani, F., and Law, K.L. 2015. A global inventory of small floating plastic debris. *Environmental Research Letters*. 10(12): 1-12.

Seto, N. 1994. The effects of rat (*Rattus rattus*) predation on the reproductive success of the Bonin Petrel (*Pterodroma hypoleuca*). Master's Thesis. University of Hawaii, Honolulu.

Seto, N. 1995. Bonin Petrel (*Pterodroma hypoleuca*). Population study, a preliminary report, Sand Island, Midway Atoll. Unpublished report to the U.S. Fish and Wildlife Service, Midway Atoll.

Seto, N. W., & O'Daniel, D. 1999. Bonin Petrel (*Pterodroma hypoleuca*). *The Birds of North America*, (385), 16.

Shent, H.; Pugh, R.J.; Forssberg, E. 1998. A review of plastics waste recycling and the flotation of plastics. *Resources, Conservation and Recycling*.

Sileo, L.; Fefer, S.I. 1987. Paint chip poisoning of Laysan albatross at Midway Atoll. *J. Wildl. Dis.* 23:432-437.

Sileo, L.; Sievert, P.R.; Samuel, M.D.; Fefer, S.I. 1990. Prevalence and characteristics of plastic ingested by Hawaiian seabirds. NOAA Tech. Memo.

Spear, L.B.; Ainley, D.G.; Ribic, C.A. 1995. Incidence of plastic in seabirds from the tropical pacific, 1984-91: Relation with distribution of species, sex, age, season, year and boyd weight. *Marine Environmental Research*. 40:123-146.

Tanaka, K.; Takada, H.; Yamashita, R.; Mizukawa, K.; Fukuwaka, M.; Watanuki, Y. 2013. Accumulation of plastic-derived chemicals in tissues of seabirds ingesting marine plastics. *Marine Pollution Bulletin*.

Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Morton, A.B., Jonsson, S., Bjorn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P.H., Tana, T.S., Prudente, M., Boonyatumanond, R., Zakaria, M.P. Akkhavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H. 2009. Transport and release of chemicals from plastics to the environment and to wildlife. *Philosophical Transactions of the Royal Society B*. 364:2027-2045.

Thompson, R.; Olsen, Y.; Mitchell, R.P.; Davis, A.; Rowland, S.J.; John, A.W.G.; McGonigle, D.' Russell, A. 2004. Lost at sea: Where is all the plastic? *Science*. 304:838.

Thompson, R.C.; Swan, S.H.; Moore, C.J.; vom Saal, F.S. 2009. Our plastic age. *Philosophical Transactions of the Royal Society B*. Online.

United Nations Environmental Programme. 2017. Listing of POPs in the Stockholm convention.

Van Cauwenberghe, L.; Vanreusel, A.; Mees, J.; Janssen, C.R. 2013. Microplastic pollution in deep-sea sediments. *Environmental Pollution*. 182:495-499.

Van Franeker J. A., Bell P. J. 1985. Plastic ingestion by petrels breeding in Antarctica. *Marine Pollution Bulletin*. 19:672–674.

Van Franeker, J.A. & Meijboom, A. 2003. Marine litter monitoring by Northern Fulmars: progress report 2003. *ALTERA-Rapport 622 (Alterra, Wageningen)*

Van Franeker, J.A., 2004. Save the North Sea Fulmar-Litter-EcoQO Manual Part 1: Collection and dissection procedures. *Wageningen, Alterra, Alterra-rapport.*

Wilcox, C.; Sebille, E.V.; Hardesty, B.D. 2015. Threat of plastic pollution to seabirds is global, pervasive and increasing. *National Academy of Sciences*. 38:1189-11904.

Xanthos, D.; Walker, T.R. 2017. International policies to reduce plastic marine pollution from single-use plastics (plastic bags and microbeads): A review. *Marine Pollution Bulletin*. Online.

Yarsley, V.E.; Couzens, E.G. 1945. *Plastics*.

Youngren, S.M.; Rapp, D.C.; Hyrenbach, K.D. Submitted. Plastic ingestion by Tristram's Storm-Petrel (*Oceanodroma tristrami*) chicks from French Frigate Shoals, Northwestern Hawaiian Islands.

Zhu, Q. 2011. An appraisal and analysis of the law of "plastic-bag ban". *Energy Procedia*. 5:2516-2521.

Tables

Table 1. Different types of common polymers and their commercial use (Shent et al. 1998)

Number	Name	Commercial Use
1	Polyethylene terephthalate (PET)	Beverage bottles, food trays
2	High-density polyethylene (HDPE)	Milk containers, cleaning bottles
3	Polyvinyl chloride (PVC)	Clear food packaging, building, material, furniture
4	Low-density polyethylene (LDPE)	Clothing, shopping bags
5	Polypropylene (PP)	Medicine bottles, straws
6	Polystyrene (PS)	Take-out trays, disposable cups, insulation, packaging

Table 2. Summary from Jambeck et al., (2015) of mismanaged waste from the top 20 contributors, considering 192 countries with coastal populations. Economic classifications: HIC – high income; UMI – upper middle income; LMI – lower middle income; LI – low income.

Rank	Country	Econ. classif.	Coastal pop. [millions]	Waste gen. rate [kg/ppd]	% plastic waste	% mismanaged waste	Mismanaged plastic waste [MMT/year]	% of total mismanaged plastic waste	Plastic marine debris [MMT/year]
1	China	UMI	262.9	1.10	11	76	8.82	27.7	1.32–3.53
2	Indonesia	LMI	187.2	0.52	11	83	3.22	10.1	0.48–1.29
3	Philippines	LMI	83.4	0.5	15	83	1.88	5.9	0.28–0.75
4	Vietnam	LMI	55.9	0.79	13	88	1.83	5.8	0.28–0.73
5	Sri Lanka	LMI	14.6	5.1	7	84	1.59	5.0	0.24–0.64
6	Thailand	UMI	26.0	1.2	12	75	1.03	3.2	0.15–0.41
7	Egypt	LMI	21.8	1.37	13	69	0.97	3.0	0.15–0.39
8	Malaysia	UMI	22.9	1.52	13	57	0.94	2.9	0.14–0.37
9	Nigeria	LMI	27.5	0.79	13	83	0.85	2.7	0.13–0.34
10	Bangladesh	LI	70.9	0.43	8	89	0.79	2.5	0.12–0.31
11	South Africa	UMI	12.9	2.0	12	56	0.63	2.0	0.09–0.25
12	India	LMI	187.5	0.34	3	87	0.60	1.9	0.09–0.24
13	Algeria	UMI	16.6	1.2	12	60	0.52	1.6	0.08–0.21
14	Turkey	UMI	34.0	1.77	12	18	0.49	1.5	0.07–0.19

Rank	Country	Econ. classif.	Coastal pop. [millions]	Waste gen. rate [kg/ppd]	% plastic waste	% mismanaged waste	Mismanaged plastic waste [MMT/year]	% of total mismanaged plastic waste	Plastic marine debris [MMT/year]
15	Pakistan	LMI	14.6	0.79	13	88	0.48	1.5	0.07–0.19
16	Brazil	UMI	74.7	1.03	16	11	0.47	1.5	0.07–0.19
17	Burma	LI	19.0	0.44	17	89	0.46	1.4	0.07–0.18
18*	Morocco	LMI	17.3	1.46	5	68	0.31	1.0	0.05–0.12
19	North Korea	LI	17.3	0.6	9	90	0.30	1.0	0.05–0.12
20	United States	HIC	112.9	2.58	13	2	0.28	0.9	0.04–0.11

*If considered collectively, coastal European Union countries (23 total) would rank eighteenth on the list

Table 2. Plastic types and their descriptions based on definitions from van Franeker (2004).

Plastic type	Description
Fragment	Hard plastic items
Sheet	Thin plastics such as plastic bags, foils; usually broken up
Foam	Foamed polystyrene, foamed cups or packaging, construction foam
Line	Remains of ropes, nets, nylon line
Pellet	Small industrial cylindrically shaped granules of around 4 mm diameter; considered raw plastic

Table 3. Distinguishing plastics into four class sizes (Eriksen et al. 2014).

Type	Size
Small microplastic	0.33 – 1.00 mm
Large microplastic	1.01 – 5.00 mm
Mesoplastic	5.01 – 200 mm
Macroplastic	>200 mm

Table 4: Foraging strategy and FO for seabird species (Ryan, 1987)

The Incidence of Ingested Plastic Particles in Seabirds in Relation to Foraging Behaviour[a]

Foraging technique	*Spp. with plastic*		*Mean frequency occurring within spp.*
	Ratio	*%*	*(%)*
All species			
Dipping and pattering	4:5	80	47
Surface seizing	25:29	86	26
Piracy	2:3	67	24
Pursuit diving	5:18	28	3
Plunge diving	0:5	0	0
Procellariiformes (excluding albatrosses and giant petrels)			
Dipping and pattering	4:5	80	47
Surface seizing	16:16	100	40
Pursuit diving	3:5	60	12

Table 5. Consolidation from four published data on plastic ingestion in Bonin Petrels

Year	Age	Location	n	FO	Mass (g)	Source
2017	Both	Midway At.	6	1.00	0.0259± 0.0217	Rapp et al., 2017
2012	Chick	Midway At.	8	0.75	0.05	Lavers & Bond, 2016
1986-1987	Both	Hawaiian Isl.	99	0.82 (G) 0.27 (P)	?	Sileo et al., 1990
1978-1981	Chick	Laysan Isl.	144	N/R	?	Harrison & Seki, 1987

Figures

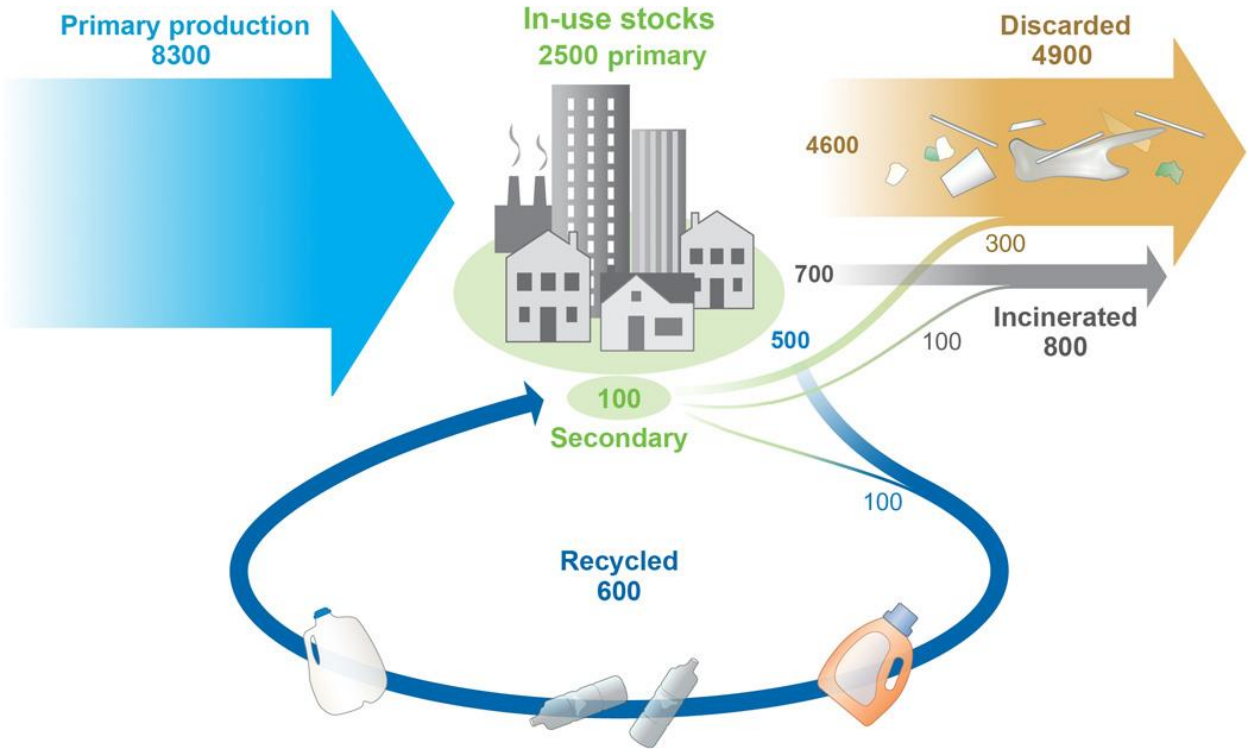

Figure 1. Figure from Geyer et al. (2017), illustrating a model of the estimated global production, use and fate of plastics produced from 1950 to 2015. The units for numbers reported are in million metric tons.

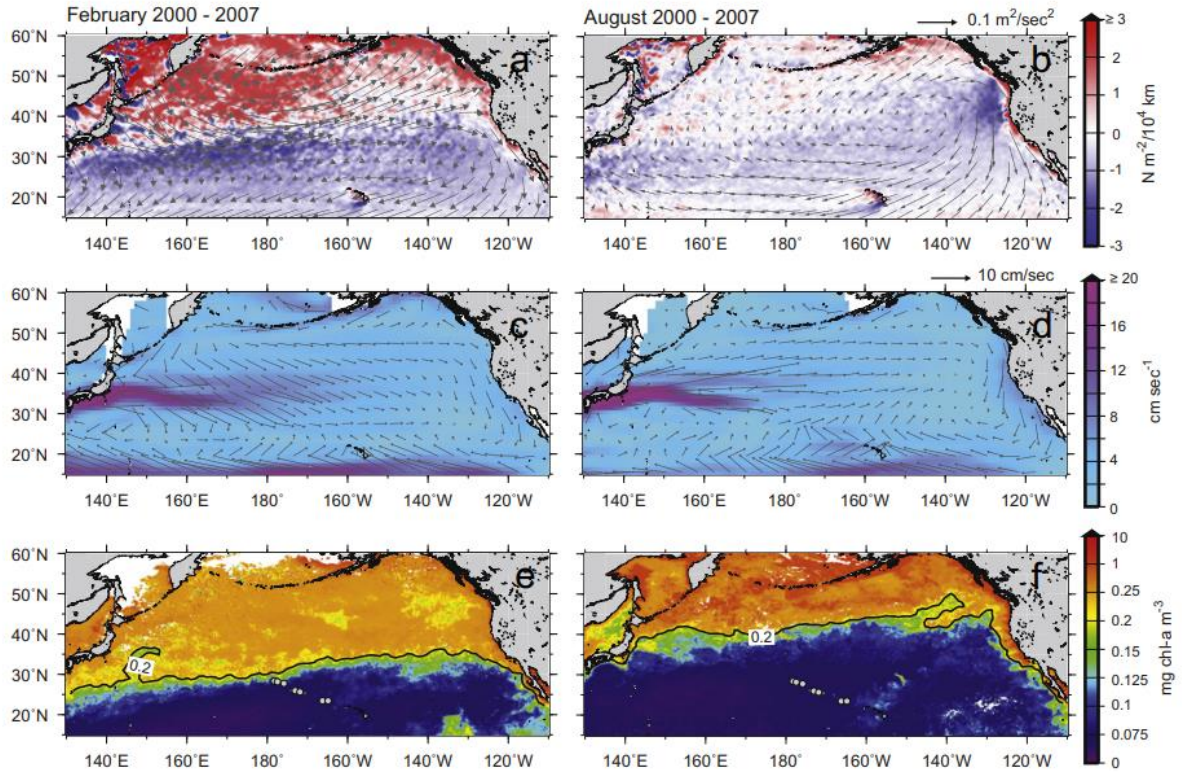

Figure 2. From Howell et al. (2012), modeling climatological averages of wind stress (gray arrows) and curl (color) (a and b), surface currents (arrows) and surface current magnitude (color) (c and d), and chlorophyll concentrations (e and f) for February (left column) and August (right column) based on data from 200 – 2007. The black line in panels (e) and (f) indicate the Transition Chlorophyll Front associated with the North Pacific Subtropical Convergence Zone.

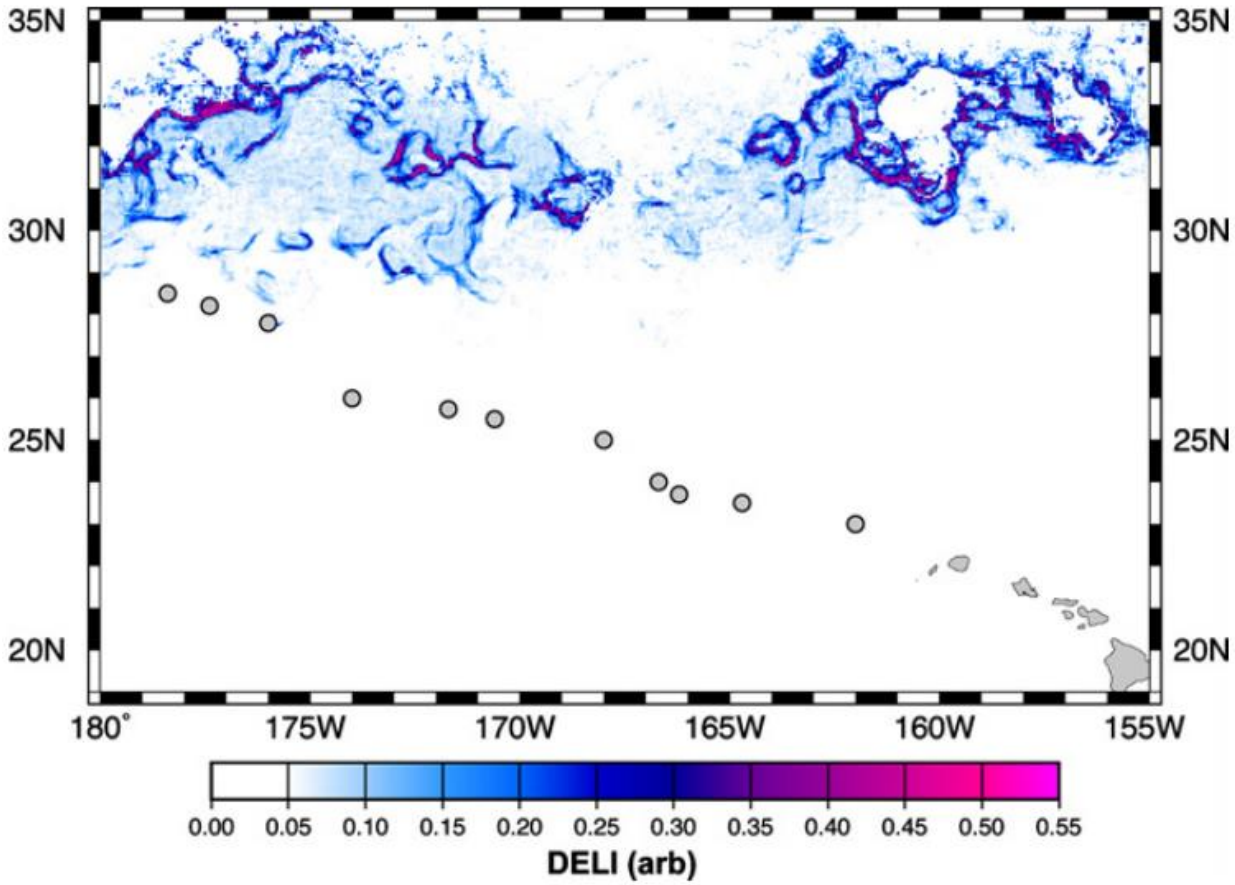

Figure 3. From Picehl et al., (2007), a Debris Estimate Likelihood Index modelling debris accumulation from aerial searches from spring – early summer

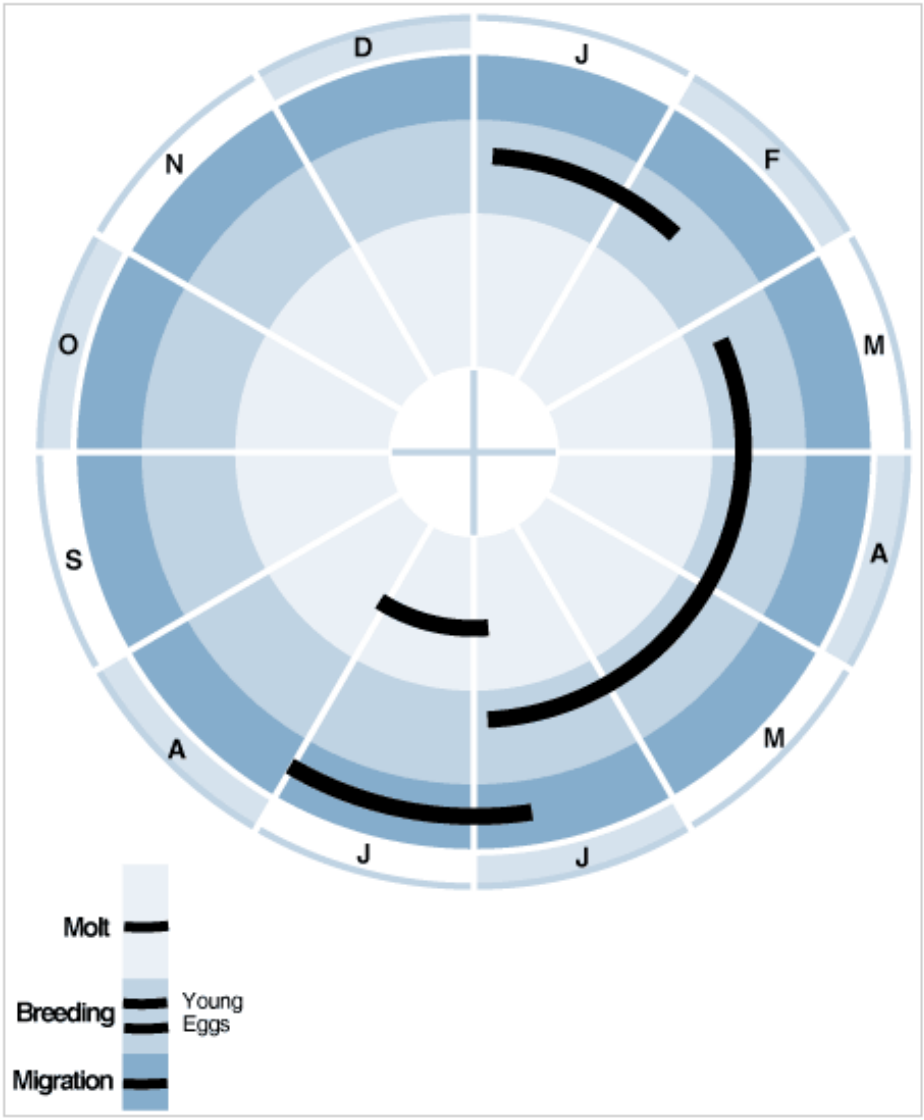

Figure 4: Annual cycle of molting, breeding and migration for Bonin Petrels (*Pterodroma hypoleuca*) on Midway Atoll.

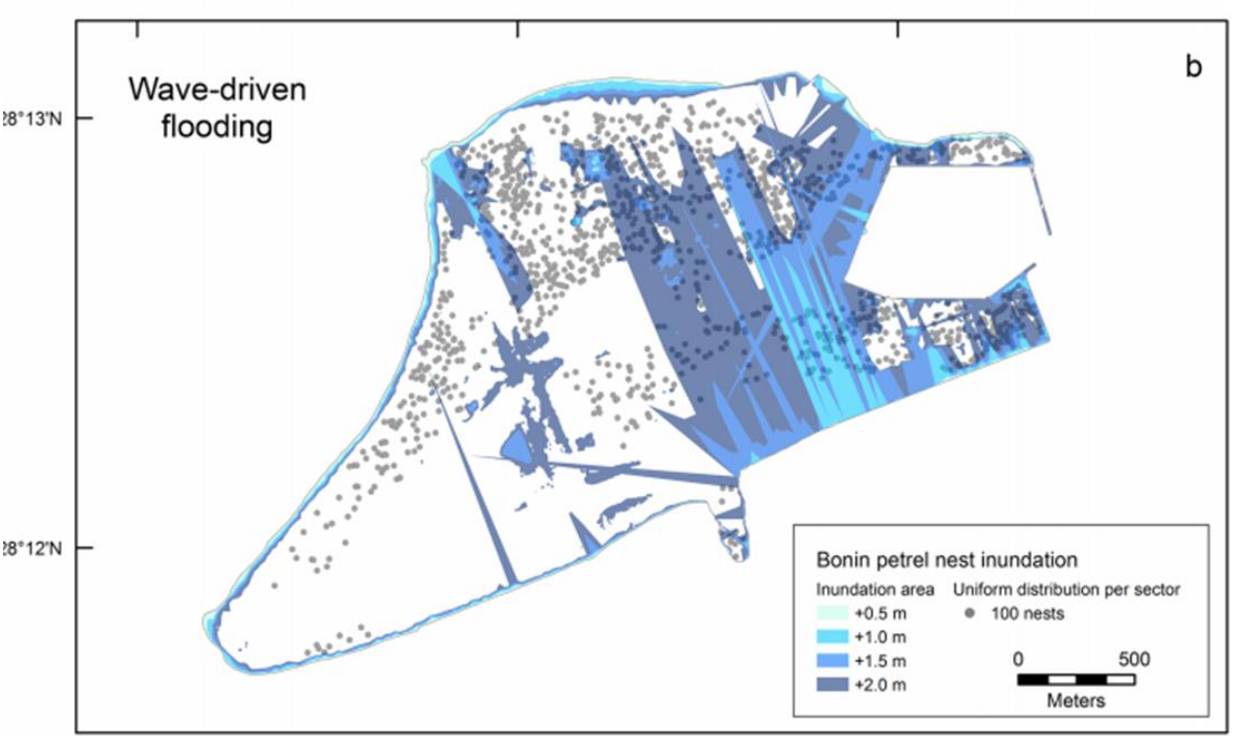

Figure 5. This figure shows projected Bonin Petrel nests inundated at sand Island, Midway Atoll. It uses four projected scenarios for sea-level rise (+0.5, 1.0, 1.5, and 2.0 m) and includes groundwater rise. (Reynolds et al. 2015).

CHAPTER 2
Plastic ingestion by Bonin Petrels (*Pterodroma hypoleuca*) from Midway Atoll: Towards plastic pollution bio-indicators for the Northwestern Hawaiian Islands

1. Introduction

Policies to reduce single-use plastics and waste mismanagement range widely in their scope and effectiveness, but their overall goal is to promote resource conservation and a sustainable society (Zhu, 2011; Xanthos and Walker, 2017). However, there is often a lag between the enactment of policies and their quantifiable benefits, mediated by their implementation and adherence. This is especially relevant when considering measures to curb marine plastic debris (MPD), given the vastness of the marine environment, the inherent difficulties associated with estimating the pool of existing MPD in the global ocean, and the large volume of MPD continuously entering the ocean (OSB, 2008). Because of these lags and uncertainties, ongoing monitoring is necessary to document the long-term effectiveness of MPD mitigation and waste management strategies (Xanthos and Walker, 2017).

Seabirds are useful biological-indicators of environmental variability because they can integrate ecological information (e.g. prey, pollutants) from the marine environment, and they can easily be sampled at colonies where large numbers aggregate every breeding season (Furness & Camphuysen, 1997; Scopel et al., 2017). Specifically, many seabird species are effective samplers of marine pollution, including MPD, due to their high-level trophic positioning and their vast foraging ranges (Nevins et al., 2005; Finkelstein et al., 2006; Hyrenbach et al., 2009). Among the many seabird species that ingest plastic, tubenoses (order Procellariiformes, involving albatross, fulmars, shearwaters, storm-petrels, diving-petrels and petrels) are characterized by the highest occurrence rates and loads of MPD (van Franeker et al., 2004; Ryan et al., 2009; Rapp et al. 2017). These species are particularly useful for sampling MPD because they are surface feeders, have broad feeding habits, and accumulate plastic in their bi-chambered stomachs, consisting of a proventriculus and a gizzard (Ryan 1987).

Seabirds have been used to track regional trends in MPD based on their particular distributions during the breeding and non-breeding seasons (Vlietstra and Parga, 2002; Young et al., 2009; van Franeker et al., 2011; Lavers and Bond, 2016). In the North Sea, for instance, the OSPAR commission (Oslo and Paris Conventions) is using Ecological Quality Objectives (EcoQOs) for MPD, defining an acceptable target as < 10% of Northern Fulmars (*Fulmaris alacialis*) having ingested > 0.1 g of MPD (van Franeker et al., 2011). In principle, use of seabirds to inform policy-makers regarding the status and trends of MPD can be expanded and adapted to diverse marine ecosystems, where suitable bio-indicator species and monitoring sites exist (Lavers and Bond, 2016; Rapp et al., 2017). Yet, to date, no seabird-based EcoQO metrics are being used to assess MPD trends in the North Pacific Ocean.

MPD ingestion by North Pacific seabirds is widespread, and has been documented across the basin, from the Eastern Tropical Pacific to Alaska, and from California to Japan (Ainley et

al., 1990; Robards et al., 1997; Rapp et al., 2017). In particular, species that forage in the central North Pacific, a region where prevailing wind and ocean currents concentrate this material are particularly susceptible to MPD ingestion (Pichel et al., 2007; Howell et al., 2012).

Bonin Petrels (*Pterodroma hypoleuca*; BOPE), have high incidence of MPD ingestion (frequency of occurrence = 0.75 -1.00) (Lavers and Bond, 2016; Rapp et al., 2017) and are ideal bio-indicators of MPD in the pelagic environment surrounding the Northwestern Hawaiian Islands due to their breeding phenology, foraging ecology and stomach anatomy. This species breeds in large numbers (350,000 pairs) on Midway Atoll during winter, with egg-laying in January and fledging in June. During this period, adults forage at the surface in the vicinity of the breeding site and return to the nest to provision to their chicks (Seto & O'Daniel, 1999). Further, BOPE have a bi-chambered stomach consisting of a proventriculus and gizzard. Food items pass through the esophagus to the proventriculus where they are chemically digested. Then, items are either regurgitated to feed the chicks, or passed to the gizzard, where indigestible items such as squid beaks, fish bones, and ingested plastic accumulate. Because parents offload food from the proventriculus, chicks are expected to accumulate more plastic than provisioning adults (van Franeker et al., 2011). Finally, large numbers of BOPE chicks and adults are accidentally killed in Midway every breeding season, due to collisions with human structures. While these salvaged specimens can be used to sample ingested plastic, it is first critical to develop a standardized sample to avoid biases due to differences in pollutant exposure in birds of different age classes and condition (Ryan, 1987; Auman et al., 1997; Stewart et al., 1997).

The goal of this study was to develop standardized protocols for using BOPE as MPD bio-indicators in the subtropical central Pacific Ocean. To this end, I quantified incidence (frequency of occurrence, F.O.) and loads (mass) of plastic ingestion by BOPE from Midway Atoll, and investigated the influence of four factors: (1) age class (comparing hatch-year and after-hatch-year birds); (2) stomach chamber (comparing the proventriculus and the gizzard); (3) cause of death (comparing birds with and without evidence of trauma); and (4) health and condition (correlating plastic loads with several visual health and body condition scores quantified during necropsies). Moreover, I developed a library of BOPE baseline data (morphometrics, body and health condition scores, diet, and ingested plastic incidence/abundance). These observations will allow resource managers to assess and interpret trends in MPD ingestion by this poorly-studied species.

2. Methods

2.1 Sample Collection

A total of 99 specimen were collected opportunistically from Sand Island (Midway Atoll) in the Northwestern Hawaiian Islands, within the Papahānaumokuākea Marine National Monument (28.2101° N, 177.3761° W), by U.S. Fish and Wildlife Service personnel in 2014 and 2015. Each bird was visually inspected for injuries and sorted by age class. In the context of this study, age class is defined as hatch-year birds that receive food from offloading, and after-hatch-year birds that forage at sea. After discarding 19 specimens (2 without a collection date, 15 young chicks without fully-developed wings, and 2 that were scavenged or not fresh) the remaining 80 specimens were necropsied. Half of these (40) were classified as hatch-year and

after-hatch-year birds, respectively. These specimens were also categorized, using their internal and external injuries, into those that died due to trauma (n = 27) and those that died from other causes (n= 53).

2.2 Specimen Necropsy

All necropsies were performed following standardized procedures, augmented to meet the objectives of this study (Work, 2000; van Franeker, 2004). All personnel was trained by Hyrenbach and all necropsies were led by Chamberlain. Only "very fresh" ("FFF" freshness code) specimens were necropsied (van Franeker, 2004). The protocol involved seven morphometric measurements, taken with calipers (0.1 mm resolution), a wing ruler (1 mm resolution), and a benchtop scale (0.1 g resolution): culmen length (mm), bill depth (mm), bill depth at base (mm), tarsus length (mm), head length (mm), wing length (mm), and body mass (g). Additionally, qualitative metrics were used to characterize molt (primaries and retrices), body condition (breast muscle, subcutaneous fat, and intestinal fat), and organ health (gut, kidney, lung, stomach) A visual body condition (VBC) index, ranging from 0 to 15, was calculated as the sum of the breast muscle score, the subcutaneous fat score, and the intestinal fat score (van Franeker, 2004; Donnelly et al., 2014).

The sex of the birds was determined by observing their reproductive organs (testes, ovaries and oviduct), and the age classes were determined using three criteria: (1) the presence of down, (2) the presence of the Bursa of Fabricius, and (3) the size and color of sex chambers (van Franeker, 2004; Donnelly et al., 2014; Rapp et al., 2017). Age classes were defined as hatch-year birds (HY) and after-hatch-year birds (AHY).

Two additional measurements were taken to validate and augment the standard necropsy procedures. First, the anatomical right side of the breast, liver and kidney were removed, weighed and frozen to be compared to the chamber scores from van Franeker (2004). Second, the heart and spleen were removed and weighed to develop additional health indices. Because previous studies have linked heart inflammation and spleen atrophy (characterized by shrinkage) to dehydration, the mass of the heart and spleen could indicate the birds' health (Work & Rameyer 1999).

2.3 Stomach Content Quantification

During necropsy, stomach contents were removed and placed in 70% ethyl alcohol for further processing in the lab by Chamberlain. Proventriculus (PRO) and gizzard (GIZ) samples were analyzed separately and these "chamber-specific" samples from the same individuals were pooled to calculate "whole stomach" incidence and mass (Youngren et al., 2018). All sample processing followed standardized protocols for cleaning, sorting and categorizing (van Franeker, 2004; van Franeker et al., 2014).

Briefly, the contents were rinsed over a 500 µm sieve to remove bile, stomach oil and highly-digested detritus. The retained items were sorted using light magnification (2x, 5 x) and high power magnification under a binocular dissecting microscope (10-40x) (Motic Digital) into 4 categories: (1) natural food (e.g. squid beaks, otoliths, fish eggs, pelagic snails, bones, eye

lenses, and flesh); (2) natural non-food (e.g., sticks, rocks, sand); (3) non-natural (e.g., plastic, glass, metal, paint chips); and (4) unidentified matter (Hyrenbach et al., 2017; Youngren et. al., 2018). Any unidentifiable items were dyed with rose Bengal disodium salt, which adheres to organic material, to determine if the objects were natural or not (Davidson & Asch, 2011). Organic items were classified as "other prey" and inorganic items were further characterized using attenuated total reflectance Fourier transform infrared spectroscopy (ATR FT-IR) (Jung et al., 2018). Once sorted, the stomach contents were dried at room temperature in the lab for a week, and the items of each category were counted and weighed (resolution = 0.0001 g) on a vibration controlled scale equipped with a draft shield (Mettler-Toledo MS104S).

Due to the highly-digested prey, the diet analysis focused on quantifying the incidence (F.O.) and abundance (mass) of five sub-categories: squid beaks, otoliths, fish eggs pelagic snails and other. Finally, highly-degraded cephalopod beaks were quantified by counting the number of rostral tips (i.e., hooks) (Youngren et al., 2018).

2.4 Ingested Plastic Quantification

Plastic items were evaluated following standardized (van Franeker et al., 2004; Rapp et al., 2017). Once sorted, plastic samples were counted and weighed (resolution = 0.0001 g) on a vibration controlled scale equipped with a draft shield (Mettler-Toledo MS104S). Each plastic type sample was weighed twice, including a tare, to quantify the repeatability through root-mean-square error (RMSE) and Pearson correlations. Replicate mass measurements for plastic were highly correlated ($r = 0.95$) and precise (RMSE = 0.003).

2.4 Statistical Analysis

R version 3.5.2 and RStudio version 3.5.2 (RStudio Team, 2018) were used to calculate descriptive statistics and to perform all statistical tests. Due to the lack of normality, summary statistics involved means ± S.D.s, medians, and ranges. Significance was assessed with alpha = 0.05.

3. Results

3.1 BOPE Morphometrics

Comparisons between sexes and age classes were performed using independent *t*-tests applied to seven measurements: culmen length (mm), bill depth (mm), bill depth at base (mm), tarsus length (mm), head length (mm), wing length (mm) and body mass (g). Overall, five of the seven morphometric measurements were significantly larger for AHY than for HY birds, except for the tarsus length ($t_{79} = 0.02$, $p = 0.982$) and body mass ($t_{79} = 1.08$, $p = 0.284$), whose means were statistically indistinguishable (Appendix 1A). Because body mass and wing length were non-normally distributed, even after a log transformation ($y' = \log(y)$), non-parametric tests were also performed. Yet, the non-parametric test results mirrored the parametric test results. (Appendix 1B). These differences in morphometric measurements between HY and AHY birds underscored the need to analyze the body condition of these two age classes separately.

To explore sexual size dimorphism, the same seven morphometric measurements were compared between both sexes (males and females) for both age classes (HY and AHY) separately (Appendix 2A and B). For those morphometric measurements where males and females were significantly different, a size dimorphism index (SDI) was used to quantify the size difference. SDI is calculated as the mean of the larger sex divided by the mean of the smaller sex, with the results arbitrarily defined as positive (minus one) and negative (plus one) when females and males are larger, respectively (Lovich and Gibbons, 1992).

Significant differences between AHY males and females were only detected for the bill depth measurements, with males being larger. On average, male bill depth was 4.9% larger, with an SDI value of 2.05 ((8.12 / 7.74) + 1). In HY birds, males and females had significantly different wing lengths and body masses, with females being larger. In particular, female wing length was 9.0% larger, with an SDI of 0.09 ((199.44 / 182.96) – 1), and female body mass was 21.5% larger with an SDI of 0.22 ((177.18 / 145.73) – 1).

3.2 Body Condition Index

A Body Condition Index (BCI) was calculated for HY and AHY birds separately using the residuals from the best-fit linear relationship between body mass (g) and bill depth at base (mm) (Brown, 1996; Jakob, 1996). Bill depth measurements were used as a proxy for body size because it was the morphometric measurement most strongly correlated with body mass for HY ($r = 0.35$, $n = 40$, $p = 0.028$) and AHY ($r = 0.61$, $n = 40$, $p < 0.001$) birds.

Four Shapiro-Wilk tests were used to quantify the normality of the BCI and VCS for both age classes separately. The BCI was not normally distributed for AHY birds ($W = 0.926$, $n = 40$, $p = 0.012$), but it was normally distributed for HY birds ($W = 0.961$, $n = 40$, $p = 0.182$). Conversely, the VCS scores were not normally distributed for HY ($W = 0.797$, $n = 40$, $p < 0.001$) or for AHY birds ($W = 0.854$, $n = 40$, $p < 0.001$). Due to the lack of normality, non-parametric Spearman rank tests were used to quantify the cross-correlations between these two body condition metrics (Table 1). For HY, the BCI and VCS were very strongly correlated ($r_s = 0.809$, $n = 40$, $p < 0.001$). For the AHY, the BCI and the VCS were also significantly cross-correlated, but the correlation was weaker ($r_s = 0.590$, $n = 40$, $p < 0.001$).

Next, the BCI metrics for the two age classes were compared as a function of the cause of death (trauma versus unknown) using non-parametric Wilcoxon rank sum tests (Figure 1.) In HY birds, there was a significant difference in body condition ($W = 94$, $n = 40$, $p = 0.048$), with the birds with evidence of trauma (median = 32.01, mean = 28.39, SD = 50.89) having higher BCI values than the birds with an unknown cause of death (median = -13.45, mean = -10.77, SD = 41.90). In AHY birds, BCI was also significantly different ($W = 119$, $n = 40$, $p = 0.044$) when birds with evidence of trauma (median = 9.24, mean = 8.02; SD = 17.82) were compared with birds with an unknown cause of death (median = -1.21, mean = -5.35, SD = 20.82). Finally, while sexual dimorphism was evident for both HY and AHY birds, there were no significant differences in body condition between sexes (Wilcoxon rank sum tests) for either HY ($W = 259$, $n = 40$, $p = 0.066$) or AHY ($W = 223$, $n = 40$, $p = 0.464$) birds (Table 1).

3.3 Diet Information

The stomach contents of the 80 BOPE individuals involved three broad categories: natural food, natural non-food and non-natural (plastic) (van Franeker, 2004). While the food (squid beaks, otoliths, fish eggs, pelagic snails, other) and natural non-food (sticks, rocks, sand) categories involved a variety of items, all non-natural items were plastic. For a summary of the non-plastic diet categories, see Appendix 3.

Diet incidence (frequency of occurrence) of the three diet categories (natural food, natural non-food, and plastic) was compared across age classes and stomach chambers using Fisher's exact tests (Table 2). Because there were significant differences in plastic incidence between age classes and stomach chambers, the plastic loads for AHY and HY birds were calculated separately for the gizzard and proventriculus.

3.4 Ingested Plastic

Considering the whole stomach, total plastic mass (g) for HY birds (mean = 0.0174 ± 0.0236 SD, median = 0.0089, range = 0.000 – 0.0945) and AHY birds (mean = 0.0093 ± 0.0196 SD, median = 0.0045, range = 0.0000 – 0.1242) varied widely (Table 3). Plastic loads (g) were compared for both age classes and stomach chambers. While none of the data were normally distributed, the two-way ANOVA tests were still performed to assess the interaction between age class and stomach chamber. Based on this two-way ANOVA, there was a significant interaction of age class and stomach chamber ($p = 0.004$) (Table 4). Yet the results need to be interpreted with caution due to the lack of normality. Thus, Wilcoxon Rank sum tests were also used to compare each factor individually (Table 5). Total plastic mass was significantly different between age classes ($W = 2404.5$, $p = 0.006$), with HY birds having more plastic than AHY birds. Furthermore, while there was a significant difference in plastic mass between the two chambers for AHY birds ($W = 1415$, $p = < 0.001$) there was no difference for HY birds ($W = 900$, $p = 0.336$) (Figure 3).

3.5 Effects of Plastic on BOPE

Plastic Loads Relative to Body Mass

On average, HY birds' body mass was 158.1 g ± 54.7 SD and mean ingested plastic was 0.017 g ± 0.024 SD. Therefore, plastic accounted for 0.11 g/kg ± 0.14 SD (0.01%) of their body mass. For AHY birds, mean body mass was 168.66 g ± 25.88 SD and mean ingested plastic was 0.0093 g ± 0.020 SD. Thus, plastic accounted for 0.06 g/kg ± 0.13 SD (or 0.006%) of their body mass. (See Appendix 1 and Table 3).

In terms of EcoQO monitoring, 0.024 g is the critical value, adjusting the goal of 0.1g in Northern fulmars to BOPE. Accordingly, 20% of HY birds (8/40 birds) and 8% of AHY birds (3/40 birds) have 0.024 g or more of plastic. HY birds are 10% over the EcoQO objective, being above the acceptable level of plastic. AHY birds are 2% under the objective, therefore are at an acceptable level of plastic according to van Franeker's metrics (van Franeker et al., 2005).

Plastic and Body Condition

An exploratory analysis of the cross-correlations of plastic loads with various factors indicative of body condition did not reveal strong relative patterns (Table 6). For HY birds, only the mass of the spleen was positively correlated with plastic loads. For AHY birds, only the pectoralis mass was negatively correlated with plastic loads.

Lastly, total plastic mass (g) was compared to cause of death (trauma vs. unknown) using a Wilcoxon rank sum test for both HY and AHY birds, separately. There were no significant differences in total plastic mass and cause of death for either HY ($W = 111$, $n = 40$, $p = 0.146$) or AHY ($W = 214.5$, $n = 40$, $p = 0.544$).

Plastic and Natural Diet

The relationship between ingested plastic mass and natural diet mass (including food and non-food items) showed differences between age classes (Table 7). In HY birds, there was a significant relationship between ingested plastic and natural diet items in both the PRO and GIZ, as well as in the whole stomach. In AHY birds, on the other hand, there was no significant relationship between ingested plastic and natural diet items, considering both stomach chambers and the whole stomach.

4. Discussion

The goal of this study was to provide a library of baseline information on Bonin Petrel morphometrics, body condition, general diet, and plastic ingestion. Furthermore, this study developed guidelines for detecting plastic ingestion by assessing biases in age classes (HY and AHY), stomach chambers (PRO and GIZ) and cause of death (birds with evidence of trauma and birds that died from unknown causes). Lastly, this study established a starting point for resource managers to measure trends in MPD ingestion in Bonin Petrels over time, with the potential of using this species as a bio-indicator of MPD around the Northwestern Hawaiian Islands.

4.1 Baseline Bird Data

This study provided detailed baseline data on Bonin Petrels that can be used to track changes in size and development of the species over time. Other studies have demonstrated changes in seabird chick growth due to changes in diet and prey availability (Abraham & Sydeman, 2004; Romano et al., 2006). It is important to have this baseline information as the study site will continue to be threatened by climate change and sea level rise with predicted changes in habitat and prey distributions (Storlazzi et al., 2015; Hazen et al., 2012).

Comparing this study to historical data, morphometric measurements appear to have remained similar over the past decades, with slight differences in mass (g), which could be due to differences in sample size (Table 8) (Harrison et al., 1983). In this study, sexual dimorphism was present in bill depth (mm) but the difference in measurements was not large enough to distinguish visually. The sexual dimorphism detected in HY birds indicated that female HY birds had larger body mass and wing length. Sexual dimorphism has been documented in seabird chicks before, however, in these studies typically there is sexual dimorphism expressed in the adults and the chicks mirror this pattern (Weimerskirch, et al., 2000; Poisbleau, 2010). This is

not exhibited in our study, where sexual dimorphism is minimal in AHY birds with males having larger bill depths, which does not explain why HY females have larger body mass and wing length. Specimens collection took place sporadically throughout May to July for both 2014 and 2015 and a Wilcoxon rank sum test comparing Julian date of collection by sex revealed no significant differences ($W = 152$, $p = 0.423$). However, females were mainly collected in the beginning of June, while males were collected consistently throughout the end of May to July, which could affect the morphometric data collected on these birds while they were developing. Because of this, it is not possible to draw conclusions about potential sexual dimorphism during chick development from this study.

Tarsus length and body mass were similar between HY and AHY birds because the HY birds analyzed were later in development. In other species, tarsus length develops faster than other morphometric measurements and this could potentially be true for Bonin Petrels as well, explaining the similar tarsus lengths for HY and AHY birds (Cuthbert, 2016). In most seabird species, the chick growth rate for body mass typically follows a bell curve pattern while all other growth rates are linear. Because the HY birds we analyzed were later in development (late April – early July), they had body masses (158.31 g ± 54.67 S.D.) similar to AHY birds and small wing lengths (189.55 mm ± 23.88 S.D.) compared to AHY birds (body mass: 168.66 g ± 25.88 S.D.; wing length: 226.90 mm ± 4.33 S.D.). This is because HY birds were putting on fat to assist with development (Seto & O'Daniel, 1999; Cuthbert, 2016). This study establishes baseline growth data for late-stage HY birds but there needs to be more research on early stage HY birds, with more consistent sampling over time, to truly understand chick development in this species, with particular emphasis on sexual dimorphism during development.

BOPE Diet

Due to differences in sampling methods, diet results are not comparable between this study and Harrison et al. (1983). Harrison et al. used an unidentified combination of lavage and necropsy in their analysis while this study focused on necropsied birds, analyzing both stomach chambers (Table 9). However, the two studies do have similarities. First, in both studies diet samples were highly digested and largely indistinguishable. Squid and fish are two of the most common ingested prey items. In this study, pelagic snails accounted for 32% of BOPE natural food but this may be overestimated because there were not many pelagic snails detected (mean AHY count = 0.063 ± 0.24) but when snails were present they had relatively large mass (Appendix 3). Squid, on the other hand, may be underestimated because most of their mass was digested and all that was left was their squid beaks (based on rostral tip, mean AHY count = 2.84 ± 3.94 S.D). Harrison et al. also reported 7% of AHY birds ingested crustaceans, which were not observed in our study. Furthermore, Harrison et al. never reported evidence of pelagic snails, which were evident throughout our diet sampling. This discrepancy could be explained by differences in sampling; eight of the nine mollusks observed were found in the gizzards and because Harrison et al. largely did lavage sampling they couldn't capture patterns from the gizzard. This further emphasizes the importance of sampling both stomach chambers.

Understanding Bonin Petrel diet can help explain how this species forages and collects plastic at sea. A detailed analysis and genetic testing of diet was not possible due to the high digestion of prey items. However, the variety of prey items distinguishable were primarily

neustonic, such as fish eggs. Based on Harrison et al. (1983), BOPE are recorded largely consuming vertical migrating fish, such as Midwater lanternfishes (*Hygophum* sp. and *Myctophum* sp.), and hatchetfishes (*Argyropelecus* sp.) (Harrison et al., 1983). Published data on Bonin Petrel foraging behavior is limited, however, they are known to forage nocturnally at the surface, which seems in line with the diet samples we observed (Seto & O'Daniel, 1999). Due to their nocturnal foraging, it is likely that Bonin Petrels rely on olfactory cues, which could attract them to bio-fouled plastic that produce the same or similar infochemicals as their prey (Savoca et al., 2016).

4.2 Ingested Plastic

Bonin Petrels had high incidence of plastic for both HY (n = 40, 98% ± 2.5 S.D.) and AHY (n = 40, 95% ± 3.49 S.D.) birds, with distinct differences by stomach chambers. There were significant differences in the incidence of whole stomachs for HY and AHY birds, as well as significant differences between PRO and GIZ for both age classes, with higher incidence in the gizzards. The likelihood of detecting plastic is significantly higher in GIZ than PRO. Similar to incidence, there were significant differences in plastic loads between the whole stomachs of HY and AHY birds, with higher loads in HY birds (based on Wilcoxon Rank Sum results). Loads in the PRO and GIZ of HY birds were indistinguishable, while in AHY birds there were significant differences between stomach chambers. The patterns in both plastic incidence and loads emphasize the importance of sampling both age classes as well as both stomach chambers to avoid sampling biases. To this end, HY and AHY birds as well as PRO and GIZ represent distinct groups that should be analyzed separately.

Due to the limited studies of plastic ingestion in Bonin Petrels, it is difficult to compare to past research to assess trends in ingestion over time. Historical data exists from 1986 – 1987 but it is limited to incidence reports, combing chicks and adults (Table 9). Based on this study, 27% ± 4.5 S.D. had plastic in the PRO and 81.82% ± 3.90 S.D. in the GIZ (n = 99) (Sileo et al., 1989). If we combine HY and AHY birds to compare to this study, 44% ± 5.6 S.D. had plastic in the PRO and 91% ± 3.2 S.D. in the GIZ (n = 80). A chi-square analysis revealed a significant difference in the incidence of both studies for PRO ($\chi 2 = 7.79$, $p = 0.006$) and GIZ ($\chi 2 = 6.78$, $p = 0.035$). Based on this, plastic incidence has increased by 16.5% in PRO and 9.4% in the GIZ between 1987 and 2015, strengthening evidence that MPD is increasing (Robards et al., 1997). However, because HY and AHY birds are distinct groups, this should be interpreted with caution.

Comparisons to current data are limited due to the small sample sizes of other studies (Table 9). One study on BOPE from 2010 – 2012 reported 100% plastic incidence for both HY (n = 5) and AHY (n=1) birds, which is 2% and 5% higher than this study respectively (Rapp et al., 2017). Another study from the same time, 2012, reported 75% incidence and mean mass of 0.05 g ± 0.07 S.D. (n = 8) in HY birds, compared to our study with 98% incidence and mean mass of 0.0174 g ± 0.0236 S.D. (n = 40) for HY birds (Lavers & Bond, 2016). The discrepancy between Rapp et al. and Lavers & Bond is due to small sample sizes considering their field collection occurred around the same time. This emphasizes the importance of having a robust sample size.

4.3 Impacts of Plastic on BOPE

HY and AHY birds had varying relationships with body condition. First, there was no relationship between BCI and plastic loads for either age class, indicating that plastic loads do not affect BOPE body mass given their size. Secondly, plastic loads were not correlated with cause of death for HY and AHY birds, signifying that birds who died from trauma and unknown causes were not directly affected by plastic loads.

HY birds did show a significant, positive relationship between plastic loads and spleen mass (g). The spleen is a key immune defense organ and other studies have found spleen mass positively correlated with stressors, such as negative effects from climatic conditions. Furthermore, birds with large spleens have been found to suffer more from parasite-induced mortality (Møller & Erritzøe, 2002). However, spleens are also associated with better body condition and overall higher body mass (Møller & Erritzøe, 2003). To test this relationship further in HY BOPE, spleens were positively correlated with BCI ($r_s = 0.69$, $p = < 0.001$), body mass ($r_s = 0.77$, $p = < 0.001$), and natural food mass ($r_s = 0.46$, $p = < 0.001$). Furthermore, natural food mass and plastic mass were positively related ($r_s = 0.63$, $p = < 0.001$). Due to these positive relationships between body condition, spleen mass, natural food and plastic mass, it can be inferred that HY birds in better body condition have larger mass, and subsequently larger spleens, because they ingest more food, making them more likely to encounter plastic.

In AHY birds, the negative relationship between breast muscle mass and plastic mass ($r_s = -0.363$, $p = 0.022$) indicates potential negative impacts of plastic on AHY birds. This pattern has been observed in other birds as well (Ryan, 1987). To explore this further, AHY breast mass was compared to body mass, BCI and natural food mass with no significant relationships with any of the metrics. This could mean that breast mass responds independently from other body condition metrics and has a unique interaction with plastic loads that should be further investigated. Because this pattern is only observed in AHY birds, it is more likely to be a response from long-term exposure to MPD. It is important to note that correlations do not indicate causality so these relationships should be interpreted conservatively.

4.4 BOPE as Bio-indicators

The Northwestern Hawaiian Islands do not have a bio-indicator species for MPD. Due to the large annual variability in plastic ingestion and vast foraging ranges, other studies have concluded Laysan Albatross would not make a good bio-indicator species (Lavers & Bond, 2016). The next option is to consider Bonin Petrels because of their breeding phenology, foraging ecology and stomach anatomy (Seto & O'Daniel, 1999; van Franeker et al., 2011). Scaling van Franeker's EcoQO metrics to Bonin Petrels, the EcoQO target for this species would be < 10% of birds with > 0.024 g of ingested plastic, assuming a 650 g fulmar and 165 g petrel. Based off this study, 20% of HY (n = 40) and 8% of AHY (n = 40) had more than 0.024 g of plastic. Fortunately, Bonin Petrels are closer to the target than other species; 100% of Laysan Albatross had more plastic than their EcoQO target (van Franeker et al., 2005; Lavers & Bond, 2016). Between recent efforts to reduce plastic in the consumer market, through policy and more sustainable product options, and the predictive models of increased marine plastic pollution, it is important to establish regional bio-indicator species for plastic pollution to track MPD changes over time (Jambeck et al., 2015; Xanthos & Walker, 2017). The NWHI is a hotspot for plastic

accumulation and further research to understand the overlap between plastic in this region and Bonin Petrel foraging ranges will determine the usability of this species as a bio-indicator of MPD.

References

Abraham, C.L.; Sydeman, W.J. 2004. Ocean climate, euphausiids and auklet nesting: inter-annual trends and variation in phenology, diet and growth of a planktivorous seabird, *Ptychoramphus aleuticus*. *Marine Ecology Progress Series*. 274:235-250.

Ainley, D.G.; Spear, L.B.; Ribic, C.A.; 1990. The incidence of plastic in the diets of pelagic seabirds in the eastern equatorial Pacific region. Proceedings of the Second International Conference on Marine Debris. NOAA Tech. Memo. NMFS, NOAA-TM-SWFSC-154; pp. 653 – 664.

Auman, H.J.; Ludwig, J.P.; Giesy, J.P.; Colborn, T. 1997. Plastic ingestion by Laysan Albatross chicks on Sand Island, Midway Atoll, in 1994 and 1995. *Albatross Biology and Conservation* pp. 239-244.

Brown, M.E. 1996. Assessing body condition in birds. *Current Ornithology*, pp. 67 – 135.

Donnelly-Greenan, E.L.; Harvey, J.t.; Nevins, H.M.; Hester, M.M.; Walker, W.A. 2014. Prey and plastic ingestion of Pacific Northern Fulmars (*Fulmarus glacialis rogersii*) from Monterey Bay, California. *Marine Pollution Bulletin*. 85:214-224.

Finkelstein, M.; Bradford, S.K.; Croll, D.A.; Tershy, B.; Jarman, W.M.; Rodriguez-Pastor, S.; Anderson, D.J.; Sievert, P.R.; Smith, D.R. 2006. Albatross species demonstrate regional differences in North Pacific marine contamination. *Ecological Applications*. 16(2): 678-686.

Franks, P.J.S. 1992. Sink or swim: accumulation of biomass at fronts. *Marine Ecology Progress Series.* 82:1-12.

Furness, R.W. and Camphuysen, C.J. 1997. Seabirds as monitors of the marine environment. *ICES Journal of Marine Sciences*. 54: 726-737.

Harrison, C.S.; Hida, T.S.; Seki, M.P. 1983. Hawaiian seabird feeding ecology. *Wildlife Monographs. Wildlife Society, Wiley*. 85:3-71.

Hazen, E.L.; Jorgensen, S.; Rykaczewski, R.R.; Bograd, S.J.; Foley, D.G.; Jonsen, I.D.; Shaffer, S.A.; Dunne, J.P.; Costa, D.P.; Crowder, L.B.; Block, B.A. 2012. Predicted habitat shifts of Pacific top predators in a changing climate. *Nature Climate Change*. Web.

Howell, E.A.; Bograd, S.J.; Morishige, C.; Seki, M.P.; Polovina, J.J. 2012. On North Pacific circulation and associated marine debris concentration. *Marine Pollution Bulletin*. 65:16-22.

Hyrenbach, K.D.; Nevins, H.; Hester, M.; Keiper, C.; Webb, S.; Harvey, J. 2009. Seabirds indicate plastic pollution in the marine environment: quantifying spatial patterns and trends in Alaska. Pp.57-61.

Hyrenbach, K.D.; Hester, M.M.; Johnson, J.A.; Lyday, S.; Bingham, S.; Pawloski, J. 2013. First Evidence of plastic ingestion by white-tailed tropicbirds from O'ahu, Hawai'i. *Marine Ornithology*. 41:167-169.

Jakob, E.M., Marshall, S.D., Uetz, G.W. 1996. Estimating fitness: a comparison of body condition indices. *Oikos*. 77:61-67.

Jambeck, J.R.; Geyer, R.; Wilcox, C.; Siegler, T.R.; Perryman, M.; Andrady, A.; Narayan, R.; Law, K.L. 2015. Plastic waste inputs from land into the ocean. *Science*. 347:6223:768 – 771.

Lavers, J. L., & Bond, A. L. 2016. Ingested plastic as a route for trace metals in Laysan Albatross (*Phoebastria immutabilis*) and Bonin Petrel (*Pterodroma hypoleuca*) from Midway Atoll. *Marine Pollution Bulletin.* 110(1):493-500.

Møller, A.P.; Erritzøe, J. 2002. Coevolution of host immune defence and parasite-mediated mortality: relative spleen size and mortality in altricial birds. *Oikos* 99:95 – 100.

Møller, A.P.; Erritzøe, J. 2003. Climate, body condition and spleen size in birds. *Oecologia*. 137:4:621-626.

Nevins, H.M.; Hyrenbach, D.K.; Keiper, C.; Stock, J.; Hester, M.; Harvey, J. 2005. Seabirds as indicators of plastic pollution in the North Pacific. Plastic Debris Rivers to the Sea Conference.

Ocean Studies Board. 2008. *Tackling Marine Debris in the 21st Century*. Washington, DC: The National Academic Press.

Pichel, W.G.; Churnside, J.H., Veenstra, T.S., Foley, D.G.; Friendmand, K.S.; Brainard, R.E., Nicoll, J.B.; Zheng, Q. Clemente-Colong, P. 2007. Marine debris collects within the North Pacific Subtropical Convergence Zone. *Marine Pollution Bulletin*. 54:1207-1211.

Poisbleua, M.; Demongin, L.; van Noordwijk, H.J.; Strange, I.J., Quillfeldt, P. 2010. Sexual dimorphism and use of morphological measurements to sex adults, immatures and chicks of Rockhopper Penguins. *BioOne Complete* 98(2): 217-224.

Polovina, J.J.; Howell, E.A.; Kobayashi, D.R.; Seki, M.P. 2017. The Transition Zone Chlorophyll Front updated: Advances from a decade of research. *Progress in Oceanography*. 150:79-85.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.

Rapp, D.C.; Youngren, S.M.; Hartzell, P.; Hyrenbach, K.D. 2017. Community-wide patterns of plastic ingestion in seabirds breeding at French Frigate Shoals, Northwestern Hawaiian Islands. *Marine Pollution Bulletin*. 123:269-278

Robards, M.D., Gould, P.J., Piatt, J.F. 1997. The highest global concentrations and increased abundance of oceanic plastic debris in the North Pacific: evidence from seabirds. In: Coe, J.M.,

Rogers, D.B. (Eds.), Marine debris. Sources, Impacts, Solutions. Springer-Verlag, New York, pp. 71-80.

Roden, G.I. 1972. Temperature and salinity fronts at the boundaries of the subarctic-subtropical transition zone in the western Pacific. *Journal of Geophysical Research*.

Romano, M.D.; Piatt, J.F.; Roby, D.D. 2006. Testing the junk-food hypothesis on marine birds: effects of prey type on growth and development. *Waterbirds*. 29:4:407-524.

RStudio Team. 2018. RStudio: Integrated Development for R RStudio, Inc. Boston, MA. URL: http://www.rstudio.com/

Ryan, P.G. 1987. The effects of ingested plastic on seabirds: correlations between plastic load and body condition. *Environmental Pollution*. 46:119-125.

Ryan, P.G.; Connell, A.D.; Gardner, B.D. 1987. Plastic Ingestion and PCBs in Seabirds: Is there a relationship? *Marine Pollution Bulletin*. 19:4:174-176.

Ryan, P.G.; Moore, C.J.; van Franeker, J.A.; Moloney, C.I.; 2009. Monitoring the abundance of plastic debris in the marine environment. *Philos. Trans. R. Soc. Bio.* 364, 1999-2012.

Savoca, M.S.; Wohlfeil, M.E.; Ebeler, S.E.; Nevitt, G.A. Marine plastic debris emits a keystone infochemical for olfactory foraging seabirds. *Chemical Ecology*. 2:11.

Scales, K.L.; Miller, P.I.; Hawkes, L.A.; Ingram, S.N.; Sims, D.W.; Votier, S.C. 2014. On the front line: frontal zones as priority at-sea conservation areas for mobile marine vertebrates. *Journal of Applied Ecology*. 51:1575-1583.

Scopel, L.C.; Diamond, A.W.; Kress, S.W.; Hards, A.R.; Shannon, P. 2017. Seabird diets as bioindicators of Atlantic herring recruitment and stock size: a new tool for ecosystem-based fisheries management. *Can. Journal of Fisheries and Aquatic Science*. 75: 1215-1229.

Seto, N. W., & O'Daniel, D. 1999. Bonin Petrel (*Pterodroma hypoleuca*). *The Birds of North America*, (385), 16.

Sileo, L.; Sievert, P.R.; Samuel, M.D; Fefer, S.I. 1989. Prevalence and characteristics of plastic ingested by Hawaiian Seabirds. NOAA Memo pp. 665 – 681.

Stewart, F.M.; Phillips, R.A.; Catry, P.; Furness, R.W. 1997. Influence of species, age and diet on mercury concetrations in Shetland seabirds. *Marine Ecology Progress Series.* 151:237 – 244.

Storlazzi, C.D.; Elias, E.P.L.; Berkowitz, P. Many atolls may be uninhabitable within decades due to climate change. *Nature*. 2015.

van Franeker, J.A., 2004. Save the North Sea Fulmar-Litter-EcoQO Manual Part 1: Collection and dissection procedures. *Wageningen, Alterra, Alterra-rapport.*

Van Franeker, J.A.; Kuhn. S.; Bravo Rebolledo, E.L.; Meijboom, A. 2014. Fulmar Litter EcoQO monitoring in the Netherlands – Update 2012 and 2013. IMARES Report C122/14. IMARES, Texel. 56pp.

Vlietstra, L.S.; Parga, J.A. 2002. Long-term changes in the type, but not amount, of ingested plastic particles in short-tailed shearwaters in the southeastern Bering Sea. *Marine Pollution Bulletin*. 44: 945-955.

Weimerskirch, H.; Barbraud, C.; Lys, P. 2000. Sex differences in parental investment and chick growth in Wandering Albatrosses: fitness consequeces. *Ecology* 81:2.

Work, T.M.; Rameyer, R.A. 1999. Mass stranding of wedge-tailed shearwater chicks in Hawaii. *Journal of Wildlife Diseases*. 35.3:48-495.

Xanthos, D.; Walker, T.R. 2017. International policies to reduce plastic marine pollution from single-use plastics (plastic bags and microbeads): A review. *Marine Pollution Bulletin*. Online.

Young, L.C.; Vanderlip, C.; Duffy, D.C.; Afanasyev, V.; Shaffer, S.A. 2009. Bringing home the trash: Do colony-based differences in foraging distribution lead to increased plastic ingestion in Laysan Albatross? *PLoS ONE*. 4(10): e7623.

Youngren, S.M.; Rapp, D.C.; Hyrenbach, K.D. 2018. Plastic ingestion by Tristram's Storm-Petrel (*Oceanodroma tristrami*) chicks from French Frigate Shoals, Northwestern Hawaiian Islands. *Marine Pollution Bulletin*. 128:369-378.

Zar, J.H., 1984. Biostatistical Analysis, 2nd Edition. Prentice Hall, Englewood Cliffs, NJ, USA.

Zhu, Q. 2011. An appraisal and analysis of the law of "plastic-bag ban". *Energy Procedia*. 5:2516-2521.

Tables

Table 1. Comparison of BCI and visual condition scores, BCI to cause of death (trauma, unknown) and BCI to sex (male, female) in HY and AHY birds. Bold font indicates significant results.

Statistical Test	Comparisons	Result (p value)	Conclusion
Spearman Correlation	**HY (BCI vs. visual)**	**r_s = + 0.809 (p ≤ 0.001)**	**Positive relationship**
Spearman Correlation	**AHY (BCI vs. visual)**	**r_s = + 0.590 (p ≤ 0.001)**	**Positive relationship**
Wilcoxon Rank Sum	**HY (BCI vs. trauma)**	**W = 94 (p = 0.048)**	**Trauma > Unknown**
Wilcoxon Rank Sum	**AHY (BCI vs. trauma)**	**W = 119 (p = 0.044)**	**Trauma > Unknown**
Wilcoxon Rank Sum	HY BCI vs. sex	W = 259 (p = 0.066)	Male = Female
Wilcoxon Rank Sum	AHY BCI vs. sex	W = 223 (p = 0.464)	Male = Female

Table 2. Contingency test results comparing incidence of three broad diet categories (natural food, natural non-food, and plastic) between two age classes and two stomach chambers. For each comparison, the degrees of freedom of the Fisher's Exact Test is 1. Bold font indicates significant results.

Category	Age Class	Comparison	p Value	Odds Ratio	95% C.I.
Natural Food	HY vs. AHY	Whole Bird	1.000	0.00	0.00 - infinite
	HY	**PRO vs. GIZ**	**0.026**	**0.00**	**0.00 – 0.79**
	AHY	**PRO vs. GIZ**	**0.055**	**0.00**	**0.00 – 1.04**
Natural Non-Food	HY vs. AHY	Whole Bird	0.263	1.82	0.69 – 4.90
	HY	PRO vs. GIZ	0.818	0.81	0.30 – 2.20
	AHY	**PRO vs. GIZ**	**< 0.001**	**0.10**	**0.02 – 0.41**
Plastic	**HY vs. AHY**	**Whole Bird**	**0.004**	**2.86**	**1.37 – 6.17**
	HY	**PRO vs. GIZ**	**0.027**	**0.24**	**0.05 – 0.87**
	AHY	**PRO vs. GIZ**	**<0.001**	**0.02**	**0.01 – 0.09**

Table 3. Table of plastic loads (g), showing the median (range), mean ± standard deviation (SD), and the sample size (n). The data are summarized by age class (HY, AHY), stomach chamber (whole stomach with both chambers combined, PRO, and GIZ) and sample size (population-level and individual level). Population-level metrics include birds that did not eat plastic and individual-level metrics include only birds with ingested plastic (non-zero values).

Population - level exposure (g)

	Median (range)	Mean ± SD	n
HY Whole	0.0089 (0.0000 - 0.0945)	0.0174 ± 0.0236	40
HY PRO	0.0008 (0.0000 - 0.0902)	0.0125 ± 0.0236	40
HY GIZ	0.0039 (0.0000 - 0.0237)	0.0049 ± 0.0052	40
AHY Whole	0.0045 (0.0000 - 0.1242)	0.0093 ± 0.0196	40
AHY PRO	0.0000 (0.0000 - 0.0502)	0.0018 ± 0.0080	40
AHY GIZ	0.0034 (0.0000 - 0.0740)	0.0075 ± 0.0127	40

Individual - level exposure (g)

	Median (range)	Mean ± SD	n
HY Whole	0.0090 (0.00005 - 0.0945)	0.0179 ± 0.0238	39
HY PRO	0.0060 (0.00005 - 0.0902)	0.0186 ± 0.0267	27
HY GIZ	0.0043 (0.00005 - 0.0237)	0.0054 ± 0.0052	36
AHY Whole	0.0049 (0.00005 - 0.1242)	0.0097 ± 0.0200	38
AHY PRO	0.0016 (0.00005 - 0.0502)	0.0089 ± 0.0160	8
AHY GIZ	0.0038 (0.00005 - 0.0740)	0.0081 ± 0.0130	37

Table 4. Comparison of total plastic load (g) by age class (HY, AHY) and stomach chamber (PRO, GIZ) using a two-way ANOVA. Bold font indicates significant results.

Factor	DF	F	p - value	Conclusion
HY vs. AHY	1	3.232	0.074	HY = AHY
PRO vs. GIZ	1	0.170	0.681	PRO = GIZ
Age class * Chamber	**3**	**8.654**	**0.004**	**Age x chamber interaction**

Table 5. Comparisons of total plastic loads (g) by age class (HY, AHY) and stomach chamber (PRO, GIZ) using three Wilcoxon Rank Sum tests. Bold font indicates significant results. Sample size is 160 (40 HY PRO, 40 HY GIZ, 40 AHY PRO, 40 AHY GIZ).

Comparison	W	p Value	Conclusion
Whole HY vs. AHY	**2404.5**	**0.006**	**HY > AHY**
HY PRO vs. GIZ	900	0.336	PRO = GIZ
AHY PRO vs. GIZ	**1415**	**< 0.001**	**GIZ > PRO**

Table 6. Correlations of plastic loads (both stomach chambers combined) with six body condition metrics (BCI, right breast muscle mass (g), heart mass (g), right kidney mass (g), right liver lobe mass (g), and spleen mass (g)). Based on Shapiro-Wilk tests, all data was non-normal so Spearman Rank tests were performed. Bolded font indicates significant results.

HY Plastic and Body Condition Correlations

Relationship with plastic	r_s	p Value	Conclusion
BCI	+ 0.227	0.159	No relationship
Breast muscle	+ 0.083	0.609	No relationship
Heart	+ 0.035	0.833	No relationship
Kidney	+0.020	0.903	No relationship
Liver	+ 0.262	0.102	No relationship
Spleen	**+ 0.362**	**0.022**	**Positive relationship**

AHY Plastic Loads and Body Condition Correlations

Relationship with plastic	r_s	p - value	Conclusion
BCI	-0.146	0.369	No relationship
Breast muscle	**-0.363**	**0.022**	**Negative relationship**
Heart	+ 0.263	0.102	No relationship
Kidney	-0.059	0.728	No relationship
Liver	-0.025	0.879	No relationship
Spleen	-0.0360	0.825	No relationship

Table 7. Spearman rank correlations between the loads (g) of natural diet (food and non-food) and ingested plastic, analyzed by age class (HY, AHY) and stomach chamber (PRO, GIZ, Whole bird: both chambers). Bold font indicates significant results. Sample size is 160 (40 HY PRO, 40 HY GIZ, 40 AHY PRO, 40 AHY GIZ).

Comparison	r_s	p Value	Conclusion
HY PRO	**+ 0.764**	**< 0.001**	**Positive relationship**
HY GIZ	**+ 0.439**	**0.005**	**Positive relationship**
HY Whole	**+ 0.635**	**< 0.001**	**Positive relationship**
AHY PRO	+ 0.231	0.152	No relationship
AHY GIZ	- 0.041	0.468	No relationship
AHY Whole	- 0.032	0.847	No relationship

Table 8. Comparing available historical baseline data to current study for AHY birds. For this study, diet percentages were calculated from mean mass of population-level exposure.

	Morphometrics			**Natural Food (%)**					
n	Mean mass (g)	Culmen length (mm)	Bill depth at base (mm)	Fish	Squid	Crustacean	Pelagic Snail	Other natural food	Source
40	168.01 ± 33.73 SD	26.57 ± 1.33 SD	11.37 ± 0.76 SD	21%	9%	n/a	32%	38%	This study
168	176 ± 1 SE	26.1 ± 0.03 SE	11.1 ± 0.02 S.E.	47%	21%	7%	n/a	24%	Harrison et al., 1983

Table 9. Comparing historical data for incidence (FO) and mass of plastic ingestion in Bonin Petrels

Year	**Age**	**Location**	**n**	**FO**	**Mass (g)**	**Study**
1986-1987	Both	Hawaiian Is.	99	PRO: 27% GIZ: 82%	n/a n/a	Sileo et al., 1989
2010 - 2012	Both	Midway Atoll	6	100%	0.0259 ± 0.0217	Rapp et al., 2017
2012	Chick	Midway Atoll	8	75%	0.05	Lavers & Bond, 2016
2014 - 2015	Both	Midway Atoll	80	HY PRO: 68% HY GIZ: 90% AHY PRO: 20% AHY GIZ: 93%	HY Whole: 0.0179 ± 0.0238 AHY Whole: 0.0097 ± 0.0200 (For PRO and GIZ info see Table 3)	This study

Figures

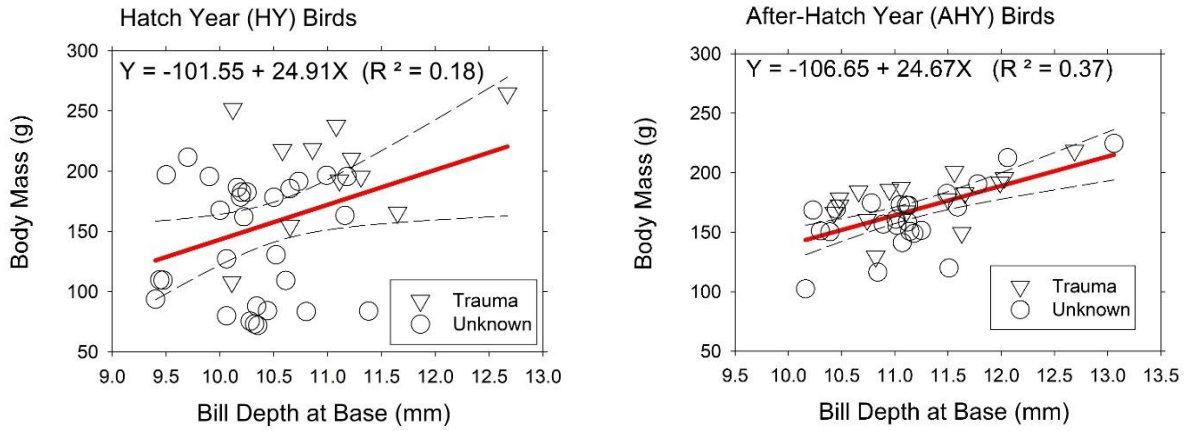

Figure 4. Body Condition Index (BCI) for hatch-year (HY) and after-hatch-year (AHY) birds. The residuals of the linear regression indicate the condition of each bird given their size with birds below (negative residual) and above (positive residual) the line having bad body condition, respectively. Individual birds are labelled according to their cause of death.

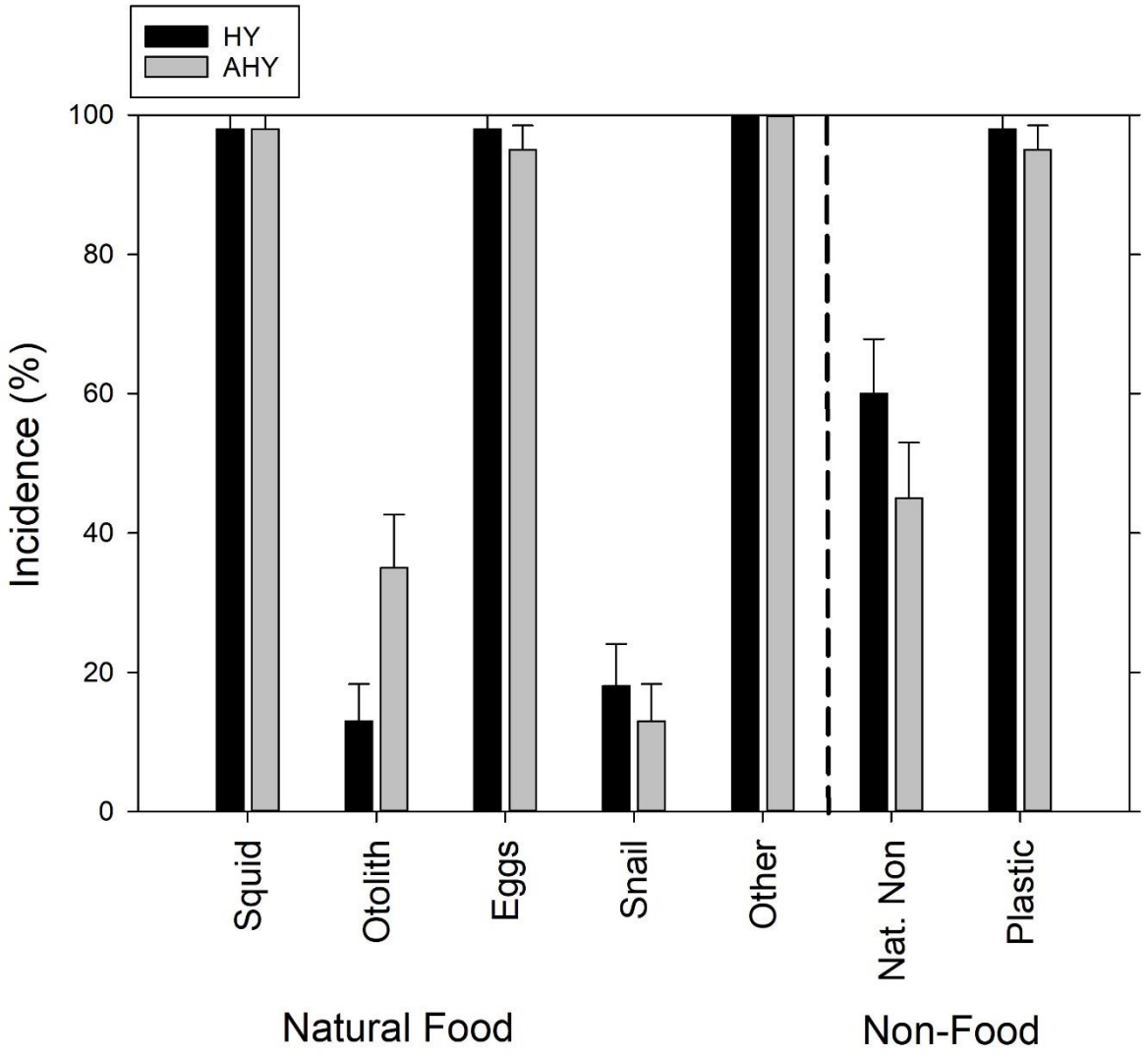

Figure 5. Incidence of diet items in HY and AHY birds, reported as mean ± 2 S.D. based on binomial probability.

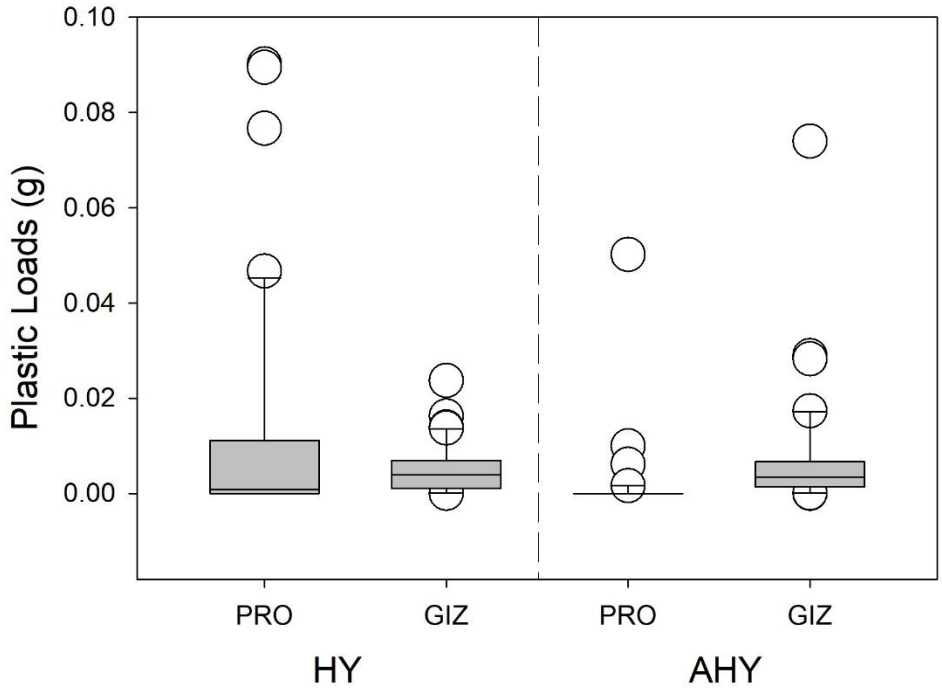

Figure 6. Boxplot of plastic loads separated by age class (HY: hatch-year, AHY: after-hatch-year) and stomach chambers (PRO: proventriculus, GIZ: gizzard). Boxplots indicate quartiles (25^{th}, 50^{th}, 75^{th} percentiles), the error bars denote the range (10^{th}, 90^{th} percentiles), and the circles indicate outliers.

Appendix

Appendix 1A. Comparison of morphometric data from after-hatch-year (AHY) and hatch-year (HY) birds, mean ± S.D., and t-test results. Bolded font highlights measurements that are significantly different.

	Morphometric Data by Age Class				
	AHY (n=40)	HY (n=40)	p Value	t	Conclusion
Bill length (mm)	**26.46 ± 1.11**	**25.55 ± 1.39**	**0.002**	**3.25**	**A>C**
Bill depth (mm)	**7.90 ± 0.43**	**7.03 ± 0.38**	**<0.001**	**9.54**	**A>C**
Bill depth at base (mm)	**11.16 ± 0.64**	**10.50 ± 0.66**	**<0.001**	**4.51**	**A>C**
Tarsus length (mm)	30.51 ± 0.89	30.50 ± 1.40	0.982	0.02	A=C
Head length (mm)	**63.71 ± 1.50**	**61.95 ± 1.74**	**<0.001**	**4.85**	**A>C**
Wing length (mm)	**226.90 ± 4.33**	**189.55 ± 23.88**	**<0.001**	**9.74**	**A>C**
Body Mass (g)	168.66 ± 25.88	158.31 ± 54.67	0.284	1.08	A=C

	Morphometric Data by Age Class				
	AHY (n=40)	HY (n=40)	p Value	W	Conclusion
Bill length (mm)	**26.54 (23.53-29.38)**	**25.37 (23.0-30.10)**	**<0.001**	**1166**	**A>C**
Bill depth (mm)	**7.96 (7.05-8.99)**	**6.97 (6.41-8.10)**	**<0.001**	**1493**	**A>C**
Bill depth at base (mm)	**11.09 (10.16-13.06)**	**10.40 (9.40-12.67)**	**<0.001**	**1240**	**A>C**
Tarsus length (mm)	30.42 (27.94-33.54)	30.67 (28.10-33.38)	0.962	806	A=C
Head length (mm)	**63.57 (60.29-67.53)**	**62.02 (57.80-65.01)**	**<0.001**	**1251**	**A>C**
Wing length (mm)	**226.00 (219.0-240.0)**	**193.50 (122.0-224.0)**	**<0.001**	**1580.5**	**A>C**
Body Mass (g)	170.85 (103.0-225.0)	173.40 (72.70-265.0)	0.855	820	A=C

Appendix 1B. Comparison of morphometric data from after-hatch-year (AHY) and hatch-year birds (HY), showing the median and range. Because body mass and wing length were non-normally distributed, a non-parametric Wilcoxon rank sum tests were performed to compare the ranked data. Bolded font indicates measurements that are significantly different.

Appendix 2A. Comparisons of morphometric data from hatch-year (HY) and after-hatch-year (AHY) males and females, showing the mean ± S.D., and t-test results. Bolded font indicates measurements that are significantly different, alpha = 0.05. Shapiro-Wilk normality tests were performed on all morphometric factors. For AHY birds, bill depth at base and tarsus measurements were non-normally distributed. For HY birds, bill length, body mass, tarsus and wing length were non-normally distributed.

	AHY Morphometric Data by Sex				
	Male (n=17)	Female (n=23)	p Value	t	Conclusion
Bill length (mm)	26.57 ± 1.33	26.38 ± 0.95	0.614	-0.51	♀=♂
Bill depth (mm)	**8.12 ± 0.41**	**7.74 ± 0.38**	**0.005**	**-3.00**	**♂>♀**
Bill depth at base (mm)	11.37 ± 0.76	11.00 ± 0.50	0.098	-1.72	♀=♂
Head length (mm)	64.24 ± 1.64	63.32 ± 1.28	0.065	-1.91	♂=♀
Body mass (g)	168.09 ± 33.73	169.08 ± 18.97	0.915	0.11	♀=♂
Tarsus length (mm)	30.68 ± 1.20	30.38 ± 0.55	0.353	-0.95	♀=♂
Wing length (mm)	227.47 ± 4.33	226.48 ± 4.38	0.481	-0.71	♀=♂

	HY Morphometric Data by Sex				
	Male (n=24)	Female (n=16)	p Value	t	Conclusion
Bill length (mm)	25.76 ± 1.50	25.24 ± 1.18	0.227	-1.23	♀=♂
Bill depth (mm)	6.99 ± 0.33	7.10 ± 0.44	0.432	0.80	♂=♀
Bill depth at base (mm)	10.55 ± 0.72	10.44 ± 0.59	0.603	-0.53	♀=♂
Head length (mm)	61.58 ± 1.90	62.52 ± 1.32	0.074	1.84	♂=♀
Body mass (g)	**145.73 ± 61.25**	**177.18 ± 37.21**	**0.051**	**2.02**	**♀>♂**
Tarsus length (mm)	30. 57± 1.53	31.01 ± 3.15	0.607	0.52	♀=♂
Wing length (mm)	**182.96 ± 26.94**	**199.44 ± 13.98**	**0.016**	**2.53**	**♀>♂**

Appendix 2B. Due to the non-normal nature of several morphometric measurements, Wilcoxon rank sum tests were performed on all morphometric data. Tests were conducted between hatch-year (HY) and after-hatch-year (AHY) males and females, including mean ± S.D., p-value, W values, and a conclusion of whether each morphometric measurement is similar or different between sexes. Bolded rows represent measurements that are significantly different, alpha = 0.05.

AHY Morphometric Data by Sex

	Male (n=17)	Female (n=23)	P Value	W	Conclusion
Bill length (mm)	26.57 ± 1.33	26.38 ± 0.95	0.575	174.5	♀=♂
Bill depth (mm)	**8.12 ± 0.41**	**7.74 ± 0.38**	**0.010**	**100.5**	**♂>♀**
Bill depth at base (mm)	11.37 ± 0.76	11.00 ± 0.50	0.090	133	♀=♂
Head length (mm)	**64.24 ± 1.64**	**63.32 ± 1.28**	**0.042**	**121**	**♂>♀**
Body Mass (g)	168.09 ± 33.73	169.08 ± 18.97	1.00	196	♀=♂
Tarsus length (mm)	30.68 ± 1.20	30.38 ± 0.55	0.245	152.5	♀=♂
Wing length (mm)	227.47 ± 4.33	226.48 ± 4.38	0.536	172.5	♀=♂

HY Morphometric Data by Sex

	Male (n=24)	Female (n=16)	p Value	W	Conclusion
Bill length (mm)	25.76 ± 1.50	25.24 ± 1.18	0.415	162	♀=♂
Bill depth (mm)	6.99 ± 0.33	7.10 ± 0.44	0.525	215.5	♂=♀
Bill depth at base (mm)	10.55 ± 0.72	10.44 ± 0.59	0.978	190.5	♀=♂
Head length (mm)	61.58 ± 1.90	62.52 ± 1.32	0.149	245	♂=♀
Body Mass (g)	**145.73 ± 61.25**	**177.18 ± 37.21**	**0.038**	**267.5**	**♀>♂**
Tarsus length (mm)	30. 57± 1.53	31.01 ± 3.15	0.847	184.5	♀=♂
Wing length (mm)	**182.96 ± 26.94**	**199.44 ± 13.98**	**0.021**	**276**	**♀>♂**

Appendix 3. Table of diet data, listing mean, standard deviation (SD), median, range, sample size (n) and frequency of occurrence (FO). Data is separated by age class (hatch-year and after-hatch-year birds), stomach chamber (whole bird (proventriculus and gizzard), proventriculus, and gizzard) and sample size (population-level and individual level). Population-level includes birds that did not eat anything and individual-level only looks at birds that ate items from each diet category. Diet categories consist of natural food, natural non-food, other (unknown items) and total natural diet (not including unnatural food such as plastic).

Hatch-Year Birds

			Population - level exposure (g)			Individual - level exposure (g)			
			Median (range)	Mean ± SD	n	Median (range)	Mean ± SD	n	FO
Whole bird	Natural Food	Squid Beaks	0.0080 (0.0000 - 0.0638)	0.0152 ± 0.0161	40	0.0081 (0.0005 - 0.0638)	0.0156 ± 0.0161	39	98%
		Otoliths	0.0000 (0.0000 - 0.0380)	0.0021 ± 0.0069	40	0.0110 (0.0060 - 0.0380)	0.0166 ± 0.0117	5	13%
		Eggs	0.0201 (0.0000 - 0.0817)	0.0249 ± 0.0185	40	0.0203 (0.0014 - 0.0817)	0.0255 ± 0.0183	39	98%
		Pelagic snail	0.0000 (0.0000 - 0.0027)	0.0002 ± 0.0006	40	0.0011 (0.0005 - 0.0027)	0.0014 ± 0.0008	7	18%
		Other natural food (bones, flesh, eye lens)	0.0169 (0.0024 - 1.0550)	0.0902 ± 0.2157	40	0.0169 (0.0024 - 1.0550)	0.0902 ± 0.2157	40	100%
		Total Natural Food	0.0537 (0.0102 - 1.1422)	0.1326 ± 0.2268	40	0.0537 (0.0102 - 1.1422)	0.1326 ± 0.2268	40	100%
	Natural Non-Food	Sand, sticks, rocks, etc.	0.0022 (0.0000 - 0.1925)	0.0123 ± 0.0329	40	0.0070 (0.00005 - 0.1925	0.0205 ± 0.0404	24	60%
	Other	Unknown	0.0000 (0.0000 - 3732)	0.0093 ± 0.0583	40	0.3732	0.3732	1	3%
	Total Natural Diet		0.0653 (0.0102 - 1.1619)	0.15542 ± 0.2363	40	0.0653 (0.0102 - 1.1619)	0.1542 ± 0.2363	40	100%
Proventriculus	Natural Food	Squid Beaks	0.00115 (0.0000 - 0.0638)	0.0087 ± 0.0148	40	0.0087 (0.0004 - 0.0638)	0.0146 ± 0.0167	24	60%
		Otoliths	0.0000 (0.0000 - 0.0060)	0.0004 ± 0.0013	40	0.0045 (0.0040 - 0.0060)	0.0048 ± 0.0009	3	8%
		Eggs	0.0013 (0.0000 - 0.0433)	0.0038 ± 0.0072	40	0.0050 (0.0007 - 0.0433)	0.0065 ± 0.0085	23	58%
		Pelagic snail	n/a	n/a	40	n/a	n/a	0	0%
		Other natural food (bones, flesh, eye lens)	0.0065 (0.0000 - 1.0377)	0.0791 ± 0.2114	40	0.0298 (0.00004 - 1.0377	0.1054 ± 0.2383	30	75%
		Total Natural Food	0.0133 (0.0000 - 1.0575)	0.0919 ± 0.2166	40	0.0244 (0.0006 - 1.0575)	0.1081 ± 0.2312	34	85%
	Natural Non-Food	Sand, sticks, rocks, etc.	0.0000 (0.0000 - 0.1891)	0.0090 ± 0.0324	40	0.0061 (0.0006 - 0.1891)	0.0257 ± 0.0506	14	35%
	Other	Unknown	0.0000 (0.0000 - 0.3732)	0.0093 ± 0.0583	40	0.3732	0.3732	1	3%
	Total Natural Diet		0.0182 (0.0000 - 1.0575)	0.1102 ± 0.2253	40	0.0203 (0.0006 - 1.0575)	0.1225 ± 0.2343	36	90%
Gizzard	Natural Food	Squid Beaks	0.0044 (0.0000 - 0.0245)	0.0065 ± 0.0054	40	0.0050 (0.0005 - 0.0245)	0.0068 ± 0.0053	38	95%
		Otoliths	0.0000 (0.0000 - 0.0380)	0.0017 ± 0.0067	40	0.0133 (0.0039 - 0.0380)	0.01713 ± 0.013	4	10%
		Eggs	0.0159 (0.0000 - 0.0765)	0.0211 ± 0.0159	40	0.0160 (0.0026 - 0.0765)	0.0222 ± 0.0156	38	95%
		Pelagic snail	0.0000 (0.0000 - 0.0027)	0.0002 ± 0.0006	40	0.0011 (0.0005 - 0.0027)	0.0014 ± 0.0008	7	17%
		Other natural food (bones, flesh, eye lens)	0.0076 (0.0000 - 0.0377)	0.0111 ± 0.0094	40	0.0083 (0.0004 - 0.0377)	0.0117 ± 0.0093	38	95%
		Total Natural Food	0.0350 (0.0015 - 0.1079)	0.0407 ± 0.0268	40	0.0350 (0.0015 - 0.1079)	0.0407 ± 0.0268	40	100%
	Natural Non-Food	Sand, sticks, rocks, etc.	0.0000 (0.0000 - 0.0313)	0.0033 ± 0.0065	40	0.0049 (0.00005 - 0.0313	0.0082 ± 0.0082	16	40%
	Other	Unknown	n/a	n/a	40	n/a	n/a	0	0
	Total Natural Diet		0.0405 (0.0052 - 0.1079)	0.0440 ± 0.0269	40	0.0405 (0.0052 - 0.1079)	0.0440 ± 0.0269	40	100%

After-Hatch-Year Birds

			Population - level exposure (g)			Individual - level exposure (g)			
			Median (range)	Mean ± SD	n	Median (range)	Mean ± SD	n	FO
Whole bird	Natural Food	Squid Beaks	0.0067 (0.0000 - 0.0288)	0.0082 ± 0.0062	40	0.0067 (0.0007 - 0.0288)	0.0084 ± 0.0062	39	98%
		Otoliths	0.0000 (0.0000 - 0.2021)	0.0100 ± 0.0330	40	0.0091 (0.0027 - 0.2021)	0.0285 ± 0.0509	14	35%
		Eggs	0.0073 (0.0000 - 0.0426)	0.0105 ± 0.0100	40	0.0079 (0.0004 - 0.0426)	0.0111 ± 0.0100	38	95%
		Pelagic Snail	0.0000 (0.0000 - 1.2327)	0.0311 ± 0.1924	40	0.0021 (0.0006 - 1.2327)	0.2485 ± 0.4921	5	13%
		Other natural food (bones, flesh, eye lens)	0.0219 (0.0023 - 0.4628)	0.0373 ± 0.0714	40	0.0219 (0.0023 - 0.4628)	0.0373 ± 0.0714	40	100%
		Total Natural Food	0.0463 (0.0098 - 1.5158)	0.0970 ± 0.2385	40	0.0463 (0.0098 - 1.5158)	0.0970 ± 0.2385	40	100%
	Natural Non-Food	Sand, sticks, rocks, etc.	0.0000 (0.0000 - 0.1750)	0.0177 ± 0.0410	40	0.0089 (0.0008 - 0.1750)	0.0393 ± 0.0538	18	45%
	Other	Unknown	0.0000 (0.0000 - 0.1902)	0.0050 ± 0.0297	40	0.0993 (0.0084 - 0.1902)	0.0993 ± 0.0909	2	5%
	Total Natural Diet		0.0583 (0.0105 - 1.5548)	0.1196 ± 0.2456	40	0.0583 (0.0105 - 1.5548)	0.1196 ± 0.2456	40	100%
Proventriculus	Natural Food	Squid Beaks	0.0000 (0.0000 - 0.0082)	0.0008 ± 0.0016	40	0.0008 (0.0003 - 0.0082)	0.0020 ± 0.0021	15	38%
		Otoliths	0.0000 (0.0000 - 0.0275)	0.0007 ± 0.0043	40	0.0142 (0.0009 - 0.0275)	0.0142 ± 0.0133	2	5%
		Eggs	0.0000 (0.0000 - 0.0005)	0.0000 ± 0.0000	40	0.0005	0.0005	1	3%
		Pelagic Snail	0.0000 (0.0000 - 1.2327)	0.0308 ± 0.1925	40	1.2327	1.2327	1	3%
		Other natural food (bones, flesh, eye lens)	0.0033 (0.0000 - 0.4187)	0.0212 ± 0.0659	40	0.0053 (0.00005 - 0.4187)	0.0265 ± 0.0727	32	80%
		Total Natural Food	0.0059 (0.0004 - 1.2602)	0.0612 ± 0.2172	40	0.0059 (0.0004 - 1.2602)	0.0612 ± 0.2172	35	88%
	Natural Non-Food	Sand, sticks, rocks, etc.	0.0000 (0.0000 - 0.1379)	0.0041 ± 0.0217	40	0.0231 (0.0010 - 0.1379)	0.0540 ± 0.0600	3	8%
	Other	Unknown	0.0000 (0.0000 - 0.1902)	0.0048 ± 0.0297	40	0.1902	0.1902	1	3%
	Total Natural Diet		0.0046 (0.0000 - 1.2602)	0.0623 ± 0.2072	40	0.0059 (0.0004 - 1.2602)	0.0712 ± 0.2201	35	88%
Gizzard	Natural Food	Squid Beaks	0.0062 (0.0000 - 0.0288)	0.0074 ± 0.0061	40	0.0064 (0.0001 - 0.0288)	0.0078 ± 0.0061	38	95%
		Otoliths	0.0000 (0.0000 - 0.1746)	0.0093 ± 0.0291	40	0.0091 (0.0022 - 0.1746)	0.0265 ± 0.0443	14	35%
		Eggs	0.0073 (0.0000 - 0.0426)	0.0105 ± 0.0100	40	0.0079 (0.0004 - 0.0426)	0.0111 ± 0.0100	38	95%
		Pelagic Snail	0.0000 (0.0000 - 0.0060)	0.0003 ± 0.0010	40	0.0017 (0.0006 - 0.0060)	0.0025 ± 0.0021	4	10%
		Other natural food (bones, flesh, eye lens)	0.0113 (0.0023 - 0.0716)	0.0161 ± 0.0147	40	0.0113 (0.0023 - 0.0716)	0.0161 ± 0.0147	40	100%
		Total Natural Food	0.0337 (0.0069 - 0.2556)	0.0435 ± 0.0410	40	0.0337 (0.0069 - 0.2556)	0.0435 ± 0.0410	40	100%
	Natural Non-Food	Sand, sticks, rocks, etc.	0.0000 (0.0000 - 0.1548)	0.0136 ± 0.0319	40	0.0089 (0.0008 - 0.1548)	0.0303 ± 0.0419	18	45%
	Other	Unknown	0.0000 (0.0000 - 0.0084)	0.0002 ± 0.0013	40	0.0084	0.0084	1	3%
	Total Natural Diet		0.0442 (0.0069 - 0.2946)	0.0573 ± 0.0529	40	0.0442 (0.0069 - 0.2946)	0.0573 ± 0.0529	40	100%

CHAPTER 3
Characterizing Plastic in the Central North Pacific: Using Bonin Petrels (*Pterodroma hypoleuca*) to bio-indicate plastic pollution in the North Pacific Subtropical Convergence Zone

1. Introduction

We currently live in the "Age of Plastic" with global production increasing from 1.5 million tons to 300 million tons in the past 60 years (Yarsley and Couzens, 1945; Derraik 2002; Lebreton et al., 2016). An estimated 4.8-12.7 million tons of plastic entered the ocean in 2010 and marine plastic debris (MPD) levels are projected to increase by an order of magnitude in the next ten years (Derraik, 2002; Jambeck et al., 2015). Understanding the inputs and trends of MPD in the marine environment are critical for assessing the effectiveness of waste management and pollution mitigation efforts.

Plastic enters the ocean through myriad pathways, involving coastal communities, river basins, and maritime activities (e.g., aquaculture, lost and discarded fishing gear), often due to waste mismanagement (Jambeck et al., 2015; Lebreton et al., 2016). Understanding the chemical composition of MPD provides insight into the sources of MPD (Jung et al., 2018). As soon as plastic enters the ocean, it is separated by density: dense polymers (e.g., polyethylene terephthalate (PETE), polyvinyl chloride (PVC), polystyrene (PS)) sink and are deposited on the benthic environment, and buoyant polymers (e.g., high-density polyethylene (HDPE), low-density polyethylene (LDPE), polypropylene (PP)) float on the sea surface or in the mixed layer, where they are transported by the wind and currents (Brignac et al., 2019). Durable MPD is degraded by a variety of mechanisms, involving UV degradation and thermal-oxidation, and mechanical fragmentation into smaller and smaller fragments (Shah et al., 2008). While MPD varies widely by size, it is characterized as microplastics (< 5 mm), mesoplastics (5 mm – 200 mm), and macroplastics (> 200 mm) (Hidalgo-Ruz et al., 2012) As plastic breaks down, it becomes bio-available for ingestion by a variety of marine organisms, ranging from upper-trophic marine predators (Odontecetes and predatory fishes), to larval fishes and larvaceans (Walker et al., 1989; Choy & Drazen, 2013; Katija et al., 2017; Steer et al., 2017).

Once MPD from the sea surface has broken down into micro- and meso-plastics, items are largely unrecognizable by their commercial use (Brignac et al., 2019). Therefore, individual plastic items are often characterized by size and categorized into five broad groups: (1) fragment – hard, rigid, unidentifiable items, (2) foam – compressible items blown with air, such as cups, packaging or construction foam, (3) line – linear and flexible items, remains of ropes, nets and nylon line, (4) pellet – small, raw industrial, cylindrically shaped granules (5) sheet – thin (2-dimensional) and flexible items such as bags (van Franeker, 2004). Long-term monitoring using surface net-tows and ingestion data from seabirds suggest that user plastics (fragments, followed by line, sheet, foam, and pellets) and industrial pellets make up the majority of MPD, globally (Vlietstra & Parga, 2002; Morét-Ferguson et al., 2010; van Franeker & Law, 2015).

Increasingly, recent developments in polymer analyses can provide insights into key aspects concerning the potential sources, transport, and fate of MPD. Polymer composition can also provide information about the potential ecological impacts associated with wildlife

ingestion, and the feasibility and success of potential waste management approaches (Teuten et al., 2009; Rochman et al., 2013; Avio et al., 2017; Jung et al., 2018).

Because plastic production and MPD are projected to increase, there are growing societal concerns and efforts to reduce the sources and pathways of plastic pollution. Many nations have taken measures to improve waste management and reduce plastic pollution. For example, European nations have developed an extended producer responsibility (EPR) model that shifts financial responsibility for waste disposal on the manufacturers, incentivizing towards a circular economy for reusing and recycling (Tibbetts, 2015; Brink et al., 2016). International initiatives are also trying to address MPD, such as the United Nations Environment Program's (UNEP) 'Global Initiative on Marine Litter' (Cheshire et al., 2009). With increasing global awareness, it is important to establish baseline information of the quantity and composition of MPD to assess future pollution trends and the successes of developing policy and management efforts.

One approach to monitoring MPD is the use of bio-indicator species, whose ingestion and loads of MPD can inform policy-makers concerning regional and temporal MPD trends (van Franeker & Law, 2015). While this approach has been adopted in the European Union (van Franeker, 2011), no such indices exist for the North Pacific, to date. The Central North Pacific is well-documented with persistent MPD accumulation and the highest global amount (mass) of microplastics (Howell et al., 2012; van Sebille et al., 2015). There is an estimated 1.7 trillion pieces, or 6.4 k tonnes of microplastics, mostly consisting of plastic types: fragment and sheet combined (581 tonnes) and line (23 tonnes), made up of buoyant polymers (PE and PP) (Lebreton et al., 2018).

The North Pacific Subtropical Convergence Zone (NPSTCZ), which is located north of the Northwestern Hawaiian Islands (NWHI), is a particular region that accumulates MPD (Pichel et al., 2007). The same physical processes that accumulate plastic in the NPSTCZ, have also created a hotspot of biodiversity of migrating and foraging species (Polovina et al., 2001; Pichel et al., 2007; Scales et al., 2014). Amongst this diverse avifauna, the Bonin Petrels (*Pterodroma hypoleuca,* BOPE) are potential MPD bio-indicators for the pelagic environment around the NWHI, due to their breeding phenology, foraging ecology and stomach anatomy. The limited knowledge of BOPE foraging behavior suggest that, during the winter chick-rearing months, they forage nocturnally at the surface, in the vicinity of their breeding sites (Seto & O'Daniel, 1999). Petrels (order Procellariformes), including BOPE, have stomachs with two distinct chambers (proventriculus (PRO) and gizzard (GIZ)) that accumulate ingested MPD and indigestible prey remains (Seto & O'Daniel, 1999). In chapter 1 of this thesis, I documented that BOPE from Midway Atoll, NWHI had high frequency of occurrence of ingested MPD (hatch-year birds: 98%, after-hatch-year birds: 95%). Furthermore, plastic accounted for 0.11 g/kg ± 0.14 S.D (hatch-year birds) and 0.06 g/kg ± 0.13 S.D. (after-hatch-year birds) of their body mass. In addition to quantifying the incidence and loads of MPD, describing the composition of ingested plastic can provide valuable insights into the sources and fate of MPD. For instance, the documented declines in the amount of industrial pellets ingested by Northern Fulmars (North Sea) and Short-tailed Shearwaters (Bering Sea) since the 1980-90s suggest a shift in the types of MPD entering the marine environment (Vlietstra & Parga, 2002; Morét-Ferguson et al., 2010; van Franeker & Law, 2015).

The objective of this study is to evaluate the potential use of BOPE as a bio-indicator of plastic pollution in the pelagic environment surrounding the NWHI. To this end, I quantified MPD ingested by BOPE using three criteria: (1) type (fragment, foam, line, pellet, and sheet), (2) size (micro-, meso-, macro- debris), and (3) polymer composition. Due to their foraging ecology and small body size, I expected that BOPE would mostly ingest microplastics (<5 mm) consisting predominately of fragment and line made of buoyant polymers (HDPE, LDPE, PP). Furthermore, to assess potential biases, I considered the influence of two ecological factors on these MPD characteristics: (1) age class (comparing hatch-year (HY) chicks and after-hatch-year (AHY) adults) and (2) stomach chambers (comparing PRO and GIZ). Altogether, this library of samples will provide resource managers with a baseline for MPD ingestion trends by this under-studied species.

2. Methods

2.1 Sample Collection

A total of 80 BOPE specimens were collected opportunistically by U.S. Fish and Wildlife Service personnel from Sand Island (Midway Atoll) in the NWHI (28.2101° N, 177.3761° W) throughout 2014-15 and 2015-16 (HY: May – July; AHY: October - May). Following chapter 1 of this thesis, specimens were necropsied and sorted into two age classes and analyzed separately: while HY birds receive food from parental offloading, AHY birds forage at sea. All personnel was trained by Hyrenbach and all necropsies were led by Chamberlain, following standardized protocols.

During necropsy, stomach contents were removed and placed in 70% ethyl alcohol for further processing by Chamberlain. PRO and GIZ samples were analyzed separately and these "chamber-specific" samples were combined to calculate "whole stomach" incidence and mass (Youngren et al., 2018). Because data chapter one of this thesis documented differences in MPD incidence and mass between the PRO and GIZ, the plastic samples from the two stomach organs were analyzed separately.

All sample processing followed standardized protocol for cleaning, sorting and categorizing (van Franeker, 2004; van Franeker, 2014). Contents were briefly rinsed over a 500 µm sieve to remove bile, stomach oil and highly-digested detritus. Remaining items were sorted into natural food (such as squid beaks, otoliths, and fish eggs), natural non-food (such as sticks, rocks, sand, etc.), plastic, and unidentified matter (Hyrenbach et al., 2017; Youngren et al., 2018). All unidentified items were dyed with rose Bengal disodium salt, which adheres to organic material, to determine if the items were natural or not (Davison & Asch, 2011). Organic items were classified as "other prey" and inorganic items were further analyzed for potential polymer analysis (Jung et al., 2018). Once sorted, the samples were dried for a week at room temperature under a fume hood for before further analysis.

2.2 Analysis of plastic by type and size

Plastic items were evaluated following standardized methods under x2 – x10 magnification, using a binocular compound microscope (van Franeker et al., 2004; Rapp et al.,

2017). All plastic and polymer analysis was conducted by Chamberlain. A total of 499 items were counted: 150 HY PRO, 143 HY GIZ, 16 AHY PRO, 162 AHY GIZ, 11 lost before analysis, and 17 identified as not plastic after ATR FT-IR analysis. Only pieces identified as plastic were enumerated in this study (n = 471). Each item was individually classified into one of five types, on the basis of their dimensionality and rigidity: (1) fragment; (2) line; (3) sheet; (4) pellet, and (5) foam. Once all items were sorted and counted, the mass of individual pieces were calculated (resolution = 0.00001 g) on a vibration controlled scale equipped with a draft shield (Mettler-Toledo MS104S). Following this, size was recorded using digital calipers (0.1 mm resolution). Considering all three dimensions, the longest length measurement was documented as length, second longest length as width, and the third dimension as height, respectively.

2.3 ATR FT-IR Polymer Identification

Methods for preparing, analyzing and interpreting polymer information followed standard protocol (Jung et al., 2018). Individual plastic pieces were cleaned using sterilized water from a polytetrafluoroethylene squirt bottle and cleanroom wipers (100% cotton, ITW Texwipe, Kernersville, NC). All pieces were analyzed using a PerkinElmer attenuated total reflectance Fourier transform infrared (ATR FT-IR) Spectrometer Spectrum Two (Waltham, MA). Before each use, the ATR FT-IR crystal was cleaned with isopropanol and cleanroom wipers. Then a background spectrum was performed before running samples. Individual samples were then placed on the crystal with a force between 80 and 100 N. Once adequate spectrums were produced, key absorption bands were identified and labelled through the ATR FT-IR's software system (Spectrum 10; PerkinElmer). Based on manual analysis of spectra, a minimum of four matching absorption bands were required for polymer identification. LDPE and HDPE were distinguished by the presence of a small absorption band at 1377 cm^{-1}, using Jung et al.'s (2018) methods for determination, reported to be accurate 85% of the time. Polymers that could not be differentiated as LDPE or HDPE were reported as PE.

2.4 Statistical Analysis

All statistical analyses were conducted using RStudio version 1.2.1335 and R version 3.5.2 with significance assessed using alpha = 0.05 (R Core Team, 2014). Shapiro-Wilk normality tests and Levene's equality of variance tests were performed on all numerical data, with data transformations implemented when necessary. Residuals were also tested with Shapiro-Wilk normality tests. Whenever normality was not achieved, non-parametric tests were reported in the body of the text and parametric tests were reported in the appendix section.

Due to the low frequency of foam (one bird ingested four foam pieces) and pellets (two birds ingested one piece each), these two plastic types were enumerated but not considered in the analyses of plastic mass, size and polymer types. Thus, these analyses considered three plastic types: fragments (n = 117), line (n = 293), and sheet (n = 55).

3. Results

3.1 Ingested Plastic by Type

Incidence of Different Plastic Types

Among the 80 BOPE specimens sampled opportunistically on Midway Atoll during the 2014 and 2015 breeding seasons, plastic was the only non-natural non-food item found in the stomach contents. Overall, the incidence of plastic ingestion in both HY (98%, n = 39 of 40) and AHY (95%, n = 38 of 40) birds was high (Figure 1). When considering both stomach chambers (whole stomach), line was the most commonly ingested plastic type (HY: 93%, n = 37 of 40; AHY: 83%, n = 33 of 40), followed by fragment (HY: 85%, n = 34 of 40; AHY: 68%, n = 27 of 40), and sheet (HY: 50%, n = 20 of 40; AHY: 30%, n = 12 of 40). Pellets (HY: 3%, n = 1 of 40; AHY: 3%, n = 1 of 40) and foam (HY: 0%, n = 0 of 40; AHY: 3%, n = 1 of 40) were rarely ingested. The original use of all plastic items was indistinguishable due to the high fragmentation, with the exception of two ingested airsoft BB pellets that were categorized as fragments.

The frequency of occurrence (FO) of the ingested types was statistically different across age classes and stomach chambers (Table 1). Due to small sample sizes within groups, a Fisher's Exact Test was performed to compare plastic incidence by age class, stomach chambers and plastic type (Table 1). Fragment incidence was significantly higher in the GIZ for both HY ($p < 0.01$) and AHY ($p < 0.01$) birds. Line incidence was significantly higher in the PRO for AHY ($p < 0.01$), but not for HY ($p = 0.24$). There were no differences in the incidence of sheets by age class or stomach chamber. Lastly, when both stomach chambers were combined and the incidence of each plastic type in the whole stomach was compared across age classes, no significant differences were found.

Plastic Loads by Type

Plastic loads for each age class (HY, AHY), stomach chamber (whole stomach, PRO, GIZ) and type (fragment, foam, line, pellet, sheet) were characterized using four descriptive statistics: median, range, mean, and standard deviation (S.D.). Ingested plastic summaries included population-level exposure, where all birds were considered, and individual-level exposure, where birds without any ingested plastics were excluded. Considering plastic loads for whole birds, HY birds (mean = 0.0174 g ± 0.0236 S.D., median = 0.0089 g, range = 0.000 – 0.0945) had higher plastic mass compared to AHY birds (mean = 0.0093 g ± 0.0198 S.D., median = 0.0045 g, range = 0.0000 – 0.1242) (Table 2). However, AHY birds had a wider range of plastic mass compared to HY birds. Overall, fragment and line were the most pervasive and abundant plastic types ingested by HY and AHY birds. When considering population-level exposure (including zero values), mean loads varied between these two types. When considering individual-level exposure (excluding zero values), mean fragment mass was higher than mean line mass for both age classes and stomach chambers.

The relative masses of each plastic type per bird was calculated using the proportion of each type's mass (g) within each individual bird, and the values were compared between PRO and GIZ for HY and AHY birds (Figure 2). Birds without plastic in each chamber were not included in the analysis. A 2-way ANOVA was then performed for each age class, with arcsine transformed data (Appendix 1). In both HY and AHY birds, there were no differences in plastic proportions by stomach chamber (HY: $F_{1, 183} = 0.09$, $p = 0.77$, AHY: $F_{1, 135} = 0.58$, $p = 0.45$). However, both age classes did show significant differences in the proportions of plastic type (HY: $F_{2, 183} = 11.76$, $p = < 0.01$; AHY: $F_{2, 135} = 8.63$, $p = < 0.01$). Lastly, proportions of plastic

type varied significantly by stomach chamber for HY birds ($F_{2, 183} = 10.54$, $p = < 0.01$) but not AHY birds ($F_{2, 135} = 2.00$, $p = 0.14$).

Because the Shapiro-Wilk and Levene's tests revealed that these data did not meet the assumptions required for performing an ANOVA, these results should be interpreted with caution. Therefore, non-parametric Kruskal-Wallis tests were performed on each age class, using the ranked data (Table 3). These tests revealed significant differences in plastic types for both HY ($p < 0.001$) and AHY ($p < 0.001$) birds, but no differences between chambers for either age class (HY: $p = 0.781$, AHY: $p = 0.369$). Bonferroni-corrected post-hoc Dunn tests were performed for to assess differences between the mass of the different plastic types (Table 4). For HY birds, the relative plastic mass of fragment and line were not different ($p = 1.000$) but there were statistical differences between fragment and sheet ($p < 0.001$) and between line and sheet ($p < 0.001$). In AHY birds, the relative mass of line was higher than fragment ($p = 0.053$) and sheet ($p < 0.001$), and fragments had higher proportions than sheet ($p = 0.030$).

3.2 Size Distribution of Plastic Items

The average heights of plastic items varied by plastic type (fragments: 0.67 mm ± 0.58; line: 0.21 mm ± 0.08; sheet: 0.14 mm ± 0.12). Combing plastic types, only 2 items were greater than 2 mm (0.4%), 2.4% (n = 11/465) were greater than 1 mm, and 9.7% (n = 45/465) were greater than 0.5 mm, indicating that most MPD were two-dimensional, flat items. Therefore, length and width were used to describe MPD size distributions.

Based on the length of the longest dimension, the percentage of items that were microplastics varied by plastic type, stomach chamber, and age class (Table 5). While the majority (> 50%) of the fragments across age classes and stomach chambers were microplastic, the majority of the line items were mesoplastics, larger than the 5 mm threshold. Sheet was highly variable, with the percentage of microplastics ranging from a low of 23% in HY PRO to a high of 67% in AHY GIZ. Overall, a general linear model analysis of the proportion of microplastic items across age classes, stomach chambers and plastic types revealed that, while age class was not significant ($t = -0.66$, $p = 0.534$), the PRO had a significantly lower proportion than the GIZ ($t = -3.15$, $p = 0.016$) (Table 6). Moreover, both line ($t = -6.29$, $p < 0.001$) and sheet ($t = -3.83$, $p = 0.006$) had significantly lower proportions of microplastic items than the fragments. The general linear model residuals were normally distributed (Shapiro-Wilk: $W = 0.973$, $p = 0.941$).

Correlations between the length (mm) and width (mm) of MPD items revealed that the dimensionality of the different plastic types varied (Figure 3). For fragments and sheet, the length and width were highly cross-correlated, suggesting that longer items were also wider. For line, on the other hand, length and width were not significantly correlated, underscoring the fact that line is inherently one-dimensional, with items having a limited width (< 0.05 mm) but widely ranging lengths (1.0 – 65.0 mm).

The mean area (length * width in mm^2) and the mean volume (length * width * height in mm^3) were calculated for each plastic type by age class and stomach chamber (Table 4). Despite line having the longest lengths of all plastic types (max length 65 mm), once width and height

were considered line had the smallest volume (HY PRO: 1.05 mm^3 ± 1.39 S.D.; HY GIZ: 0.50 mm^3 ± 0.52 S.D.; AHY PRO: 4.09 mm^3 ± 5.71 S.D.; AHY GIZ: 0.70 mm^3 ± 1.50 S.D.). Fragments, on the other hand, had the smallest average lengths of all plastic types and yet the largest volume (HY PRO: 15.71 mm^3 ± 23.11 S.D.; HY GIZ: 2.98 mm^3 ± 3.43 S.D.; AHY PRO: 4.15 mm^3 ± 5.45 S.D.; AHY GIZ: 7.87 mm^3 ± 19.40 S.D.), underscoring the importance of considering the three-dimensional shapes of the ingested MPD.

3.3 Ingested Plastic by Polymer Composition

Overall, the ATR FT-IR identified six polymers (LDPE, HDPE, PE (HDPE or LDPE), PP, PE/PP mix, and PS) with 3 items unidentified (2.2% of total mass). Combining age class, stomach chambers and plastic type, LDPE contributed the majority of the mass of ingested plastics (42.3%), followed by PP (14.4%), HDPE (14.1%), PE/PP mix (12.1%), PE (HD or LD, 10.1%), and PS (4.8%). All PS came from the one bird with ingested foam. Relative polymer composition was further computed for different age classes (HY, AHY), stomach chambers (PRO, GIZ) and plastic types (fragment, line, sheet) separately (Figure 4). PS foam was included in this because it made up a large percentage (68.1%) of the plastic mass in AHY PRO. Considering the relative mass of each polymer type (LDPE, HDPE, PE, PP, PE/PP mix, PS, and unknown), there was a strong correlation between the PRO and GIZ of HY birds, but not between the PRO and GIZ of AHY birds, and not between age classes (Table 7).

Finally, to assess potential interactions between the polymer composition and the plastic types of the MPD, the mass of all plastic items for a given plastic type (fragment, line, sheet, and foam for AHY PRO) and polymer type (LDPE, HDPE, PE, PP, PE/PP mix, PS, and unknown) were compared using four ANOVA tests for HY PRO, HY GIZ, AHY PRO, and AHY GIZ. AHY PRO and HY GIZ showed statistical differences amongst the polymers. None of these tests revealed statistical interactions between polymer composition and plastic types for any age class or stomach chamber (Appendix 2). However, because the ANOVA residuals were not normally distributed, these results should be interpreted cautiously. Therefore, eight Kruskal-Wallis tests were performed to validate these results. Only the masses of the polymers in the HY GIZ were significantly different from one another (Table 8).

4. Discussion

The goal of this study was to provide a library of ingested MPD in BOPE by characterizing plastic items by types, sizes and polymer composition. Describing ingested MPD items establishes a baseline for resource managers to measure trends in ingested MPD in BOPE over time, with the potential of using this species as a bio-indicator of MPD around the NWHI.

4.1 Ingested Plastic by Type

Insights from Distribution of Ingested Plastic Types

Similar to other ingestion studies from the Central North Pacific and at-sea net tow surveys in the subtropical gyre, fragment (FO HY: 85%, AHY: 68%) and line (FO HY: 93%, AHY: 83%) were the most common plastic types encountered (Moore et al., 2001; Rapp et al., 2017; Youngren et al., 2018).

In fact, a previous study of the plastic ingestion by petrels nesting in French Frigate Shoals (NWHI) revealed that fragments contributed the majority of the ingested plastic mass in both Bonin petrels (mean = 52.9 ± 47.5 S.D., n = 3 chicks) and Tristram storm-petrels (*Oceanodroma tristrami*) (mean = 95.9 ± 5.9 S.D., n = 57 chicks) (Rapp et al., 2017). These fragments originate from the breaking down of larger items that have persisted in the ocean for long, unknown periods of time (Barnes et al., 2009; Andrady, 2015). Therefore, the MPD accumulating in the NPSTCZ have likely travelled far distances and degraded into micro- and meso- particles (Lebreton et al., 2012; Eriksen et al., 2014). While the physical forcing leading to these MPD aggregations in the NPSTCZ are well understood, there is no information on the actual MPD composition in this region because samples are often pooled over larger geographic regions (Pichel et al., 2007; Howell et al, 2012). Nevertheless, we can compare the plastic ingested by BOPE to the MPD from the North Pacific Subtropical Gyre (NPSTG), where fragments are the most dominant plastic type (macro-plastic: 90%, micro-plastic: 58.9%) (Moore et al., 2001; Titmus & Hyrenbach, 2011). Altogether, the at-sea surveys and the seabird ingestion studies suggest that fragments are the dominant micro-plastic type in the central Pacific Ocean surface waters, likely due to the interplay of the high retention times at these convergence zones and their high durability (Moore et al., 2001; Eriksen et al., 2014; Rapp et al., 2017).

The high ingestion of line, on the other hand, is likely mediated by the high consumption of fish eggs detected in BOPE diet (FO - HY: 98%, AHY: 95%), because fish egg masses often attach to bunches of line (Rapp et al., 2017). In turn, the prevalence of line may be indirectly related to the pervasive issue of derelict fishing gear (lines and ropes) threatening the region around BOPE breeding grounds in the NWHI (Mcelwee & Morishige, 2008). The NPSTCZ, just north of the NWHI, supports large longline fisheries, as opposed to the oligotrophic waters of the NPSTG where there is no fishing activity, thus explaining the smaller quantity of line and net sampled in that region (Polovina et al., 2001; Moore et al., 2001; Titmus & Hyrenbach, 2011; Scales et al., 2014). Similar surveys of North Atlantic MPD have shown higher abundance of line in regions with larger fishing industries (Morét-Ferguson et al., 2010).

While entanglement in fishing gear around the NWHI has been widely documented threatening critical coral habitats and protected species (e.g., monk seals) fishing nets and lines also break down and become bio-available to a variety of marine predators (Bjorndal et al., 1994; Donohue et al., 2001; Wabnitz & Nichols, 2010). While ingestion of fishing line has reportedly caused the death of sea turtles through blockage, there is no evidence from any of our birds that plastic ingestion caused mortality (Bjorndal et al., 1994).

The smaller amounts of sheet ingested can most likely be explained by biofouling. Compared to fragment and line, sheet have a high surface area: volume ratio, so biofouling creates a faster change in specific density, causing sheet to sink more rapidly (Andrady, 2011; Ryan, 2015). Foam was ingested by one bird, which had four pieces from the same item broken up in the PRO. The low occurrence of foam can also be attributed to biofouling and weathering, since this type of plastic breaks down into individual beads, which sink faster once they are biofouled (Andrady, 2011; Andrady 2015).

Lastly, only two pellets were documented in this study, ingested by two different birds. Based on temporal studies of plastic trends, virgin industrial pellets used to be common in the marine environment during the 1980s, when up to 14% (1 in 7) MPD items were pellets. However, over the past thirty years there has been a 75% decrease in industrial pellets sampled in the marine environment (Morét-Ferguson et al., 2010; van Franeker & Law, 2015). This decrease can be attributed to policy changes with improved regulation of industrial waste beginning in 1991 with Operation Clean Sweep through the U.S. EPA (U.S. EPA, 1993).

Biases in detecting ingested plastic types

Understanding how different types of plastic accumulate within Bonin Petrels, based on age class and stomach chamber, is important for future sampling approaches and can also provide insight into how different types of plastic are digested. Considering the whole stomach, there were no differences in the frequency of occurrence of plastic types between HY and AHY birds, meaning the plastic ingested by birds foraging at sea was offloaded at the same rate to HY birds from their parents. By stomach chamber, there was significantly higher incidence in the GIZ compared to the PRO of fragments for HY and AHY birds, and of line for AHY birds. This underscores the importance of sampling both stomach chambers through necropsy, rather than using bolus sampling methods, which only collect items from the PRO.

When the proportions of plastic types by total plastic mass in individual birds was compared, there were no statistical differences by chamber for HY and AHY birds but there were statistical differences in proportions of each plastic type. The PRO had a greater proportion of line compared to fragment, while the GIZ had a greater proportion of fragment compared to line. This pattern was not detected in AHY birds, where fragment and line had the same proportion in the PRO and GIZ. I hypothesize that the disparity between fragment and line in the two stomach chambers has to do with the overall size and shape of the plastic types, with fragments being smaller in length (maximum length: 9 mm) and therefore easier to pass into the GIZ and accumulate. This is compared to line which can have long lengths (maximum length: 65 mm) that are more difficult to pass into the gizzard and accumulate in the PRO. This pattern was not detected in AHY birds because the sample sizes of plastic items in the PRO were too small due to AHY birds offloading proventricular contents to their chicks (fragment n = 3, line n = 5). Similar patterns have been hypothesized for Black-footed albatross (*Phoebastria nigripes*) and Laysan albatross (*Phoebastria immutabilis*) from the NWHI (Rapp et al., 2017).

4.2 Size Distribution of Plastics

Among the 467 items analyzed in this study (not including the 4 foam pieces), 54.6% of ingested plastic pieces were classified as microplastics, where the longest length was < 5 mm. There was no difference in size of plastics by age class, however, there were differences in sizes based on plastic type, with fragments having a significantly higher amount of microplastics compared to line and sheet. This is similar to other studies in the North Pacific Ocean, where line is larger in size compared to fragment and sheet, underscoring that rather than breaking into smaller pieces, line folds into clumps to become an ingestible size for marine organisms (Moore et al., 2001). Further, the PRO had larger plastic sizes compared to the GIZ for both age classes, with more microplastics accumulating in the GIZ. While the plastics in the GIZ of these birds are

smaller, it is difficult to distinguish between smaller pieces that pass more easily from the PRO into the GIZ and larger plastic pieces that breakdown from mechanical force while in the GIZ, therefore, plastic in the GIZ may not be ideal for comparing sizes to regional plastic at sea in terms of bio-indicator monitoring. Despite this, establishing a baseline of ingested plastic sizes for Bonin Petrels is important to track trends in the sizes of MPD ingested over time as the abundance of microplastics in the ocean continues to increase and are still largely unaccounted for (Eriksen et al., 2014; Thompson, 2015).

4.3 Ingested Plastic by Polymer Composition

While it has been reported that Bonin Petrels forage at the surface, they are nocturnal, feed in the off-shore pelagic environment and do not have recorded diving data (Seto & O'Daniel, 1999). Thus, it is difficult to know where in the water column these birds feed. Based on this study, Bonin Petrels only ingested buoyant polymer types, 93% consisting of PE or PP, supporting the surface foraging strategies put forth in previous studies (Seto & O'Daniel, 1999; Jung et al., 2018).

Composition of polymer types by percent of total mass were analyzed for each age class, stomach chamber and plastic type. There was a high correlation between the percent of polymer composition between HY PRO and GIZ but not for AHY PRO and GIZ, which could indicate that the plastic which passes from the PRO to the GIZ in HY birds is the same because HY birds are too young to accumulate plastic in their GIZ over time. The composition of plastic polymers in the PRO and GIZ of AHY birds, on the other hand, have no relationship potentially indicating that these birds accumulate polymer types differently in each chamber. The largest disparity was seen in PE/PP mix, where no PE/PP mix was detected in the PRO, yet it made up 30.5% of the GIZ polymer composition. A more likely explanation, however, is that the AHY PRO had very little ingested plastic (n = 16) and was unable to accurately capture patterns of polymer composition. It is important to note that there is a level of uncertainty in the ATR FTIR's ability to distinguish LDPE and HDPE, therefore further analysis with a Differential Scanning Calorimeter (DSC) of these polymer types are required.

Understanding the polymer composition of ingested plastic can help indicate the fates and sources of different polymers. The composition of polymers in this study is similar to that of other ingestion and survey studies in the Central North Pacific Ocean and beach studies around the Hawaiian Islands (Jung et al., 2018; Lebreton et al., 2018; Brignac et al., 2019). While denser polymers sink rapidly and accumulate near their source, buoyant polymers are able to travel away from their source, as indicated by the high amounts of PE and PP throughout the remote North Pacific Ocean, meaning a majority of this plastic came from far-away coastal communities (Jambeck et al., 2015; Jung et al., 2018; Brignac et al., 2019).

Knowing the composition of ingested MPD gives insight into their sources, which can be useful for enacting policy, changing the consumer market and increasing recycling efforts. PE and PP are largely used for single-use food packaging and containers, and fishing and shipping industries (Ribic, 2012; Jambeck et al., 2015). Furthermore, in the United States 42.6% (n = 11,219 / 26,282 thousands of metric tonnes) of municipal solid waste is PE (LD and HD) and 13.8% (n = 3,636 / 26,282 thousands of metric tonnes) is PP, however only 5.8% (n = 646 /

11,219 thousands of metric tonnes) of PE and 0.2% (n = 9 / 3636 thousands of metric tonnes) of PP is recovered through recycling efforts (Barnes et al., 2009). Documenting the fates of these polymers is useful to enact change in the materials used for packaging, increase recycling efforts and target waste management of fishing and shipping industries. Additionally, assessing polymer composition in ingested MPD temporally is a useful and efficient way to monitor the success of waste management strategies.

The purpose of this study was to provide baseline data on the characteristics of MPD ingestion in Bonin Petrels from Midway Atoll, including types, sizes and polymer composition of MPD. This data is useful in determining Bonin Petrels' ability to sample and bio-indicate plastic pollution in the pelagic environment surrounding the NWHI. Ingested MPD was micro- and meso- plastics, consisting predominately of fragment and line, as well as PP and PE (LDPE and/or HDPE). While there are few comprehensive studies on the characteristics of plastics in the NPSTCZ, where Bonin Petrels are assumed to forage, the types and polymers of ingested MPD largely reflects the composition of plastic sampled from at-sea surveys and other ingestion studies from the North Pacific Ocean. However, to accurately use this species as a bio-indicator of MPD trends in this region, we need tracking data to determine exactly where the birds forage and at-sea surveys within their known foraging range to compare to the ingested plastics. Regardless, the baseline data described in this study can aid resource managers in tracking trends in the characteristics of ingested plastic over time.

While this study increases our understanding of the trends and fates of plastic types in the NPSTCZ, where Bonin Petrels likely forage during the winter-spring breeding months, additional at-sea information of BOPE movements and net tows from vessels would be needed to validate the use of this species as a bio-indicator of MPD in the Central North Pacific (Shreiber & Burger, 2001; van Franeker, 2004).

References

Andrady, A.L. 2011. Microplastics in the marine environment. *Marine Pollution Bulletin* 62:1596-1605.

Andrady, A.L. 2015. Persistence of Plastic Litter in the Oceans. *Marine Anthropogenic Litter* pp. 57-72.

Avio, C.G.; Gorbi, S.; Regoli, F. 2017. Plastics and microplastics in the oceans: from emerging pollutants to emerged threat. *Marine Environmental Research* 128:2-11.

Barnes, D.K.; Galgani, F.; Thompson, R.C., Barlaz, M. 2009. Accumulation and fragmentation of plastic debris in global environments. *Philosophical Transactions of the Royal Society Series B,* 364(1526):1985–1998.

Brignac, K.C.; Jung, M.R.; King, C.; Royer, S.J.; Blickley, L.; Lamson, M.R.; Potemra, J.T.; Lynch, J.M. 2019. Marine debris polymers on main Hawaiian Island beaches, sea surface, and seafloor. *Environmental Science Technology* 53:21.

Brink, P.T.; Schweitzer, J.P.; Watkins, E.; Howe, M. 2016. Plastics marine litter and the circular economy. Briefing for: Institute for European Environmental Policy.

Bjorndal, K.A.; Bolten, A.B.; Lagueux, C.J. 1994. Ingestion of marine debris by juvenile sea turtles in coastal Florida habitats. *Marine Pollution Bulletin* 28:154-158.

Cheshire, Adler, E; Barbiere, J.; R.T.; Cohen, Y.; Evans, S.; Jarayabhand, S.; Jeftic, L; Jung, R.T.; Kinsey, S.; Kusui, T.; Lavine, I.; Manyara, P.; Oosterbaan, L.; Pereira, M. Sheavley, S.; Tkalin, A.; Varadarajan, S.; Wenneker, B.; Westphalen, G. 2008. UNEP/IOC Guidelines on survey and monitoring of marine litter. *UNEP*. Book.

Choy, C.A.; Drazen, J.C.; 2013. Plastic for dinner? Observations of frequent debris ingestion by pelagic predatory fishes from the central North Pacific. *Marine Ecology Progress Series* 485:155-163.

Davison, P., Asch, R.G., 2011. Plastic ingestion by mesopelagic fishes in the North Pacific subtropical gyre. *Marine Ecology Progress Series* 432:173–180.

Derraik J.G.B. 2002. The pollution of the marine environment by plastic debris: a review. *Marine Pollution Bulletin*. 44:842-852.

Donohue, M.J.; Boland, R.C.; Sramek, C.M.; Antonelis, G.A. 2001. Derelict fishing gear in the Northwestern Hawaiian Islands: Diving Surveys and Debris Removal in 1999 confirm threat to coral reef ecosystems. *Marine Pollution Bulletin* 42(12): 1301-1312.

Eriksen, M.; Lebreton, L.C.; Carson, H.S.; Thiel, M.; Moore, C.J.; Borerro, J.C.; Galgani, F.; Ryan, P.G.; Reisser, J. 2014. Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. *Plos One*.

Haward, M. 2018. Plastic pollution of the world's seas and oceans as a contemporary challenge in ocean governance. *Nature Communications* 9:667.

Hidalgo-Guz, V.; Gutow, L.; Thompson, R.C.; Thiel, M. 2012. Microplastics in the marine environment: a review of the methods used for identification and quantification. *Environmental Science & Technology* 46: 3060-3075.

Howell, E.A.; Bograd, S.J.; Morishige, C.; Seki, M.P.; Polovina, J.J. 2012. On North Pacific circulation and associated marine debris concentration. *Marine Pollution Bulletin*. 65:16-22.

Hyrenbach, K.D.; Hester, M.M., Adams, J., Titmus, A.J., Michael, P., Wahl, T., Chang, C.-W., Marie, A, & Vanderlip, C. 2017. Plastic ingestion by Black-footed Albatross *Phoebastria nigripe*s from Kure Atoll, Hawai'i: Linking chick diet remains and parental at-sea foraging distributions. *Marine Ornithology* 45: 225-236.

Jambeck, J.R.; Geyer, R.; Wilcox, C.; Siegler, T.R.; Perryman, M.' Andrady, A. Narayan, R., Law, K.L. 2015. Plastic waste inputs from land into the ocean. *Science* 347(6223):768-771.

Jung, M.R.; Horgen, F.D.; Orski, S.V.; Rodriguez, V.C.; Beers, K.L.; Balazs, G.H.; Jones, T.T.; Work, T.M.; Brignac, K.C.; Royer, S.J.; Hyrenbach, K.D.; Jensen, B.A.; Lynch, J.M. 2018. Validation of ATR FT-IR to identify polymers of plastic marine debris, including those ingested by marine organisms. *Marine Pollution Bulletin* 127:704-716.

Jung, M.R.; Balazs, G.H.; Work, T.M.; Jones, T.T.; Orski, S.V.; Rodriguez, V.C.; Beers, K.L.; Brignac, K.C.; Hyrenbach, K.D.; Jensen, B.A.; Lynch, J.M. 2018. Polymer identification of plastic debris ingested by pelagic-phase sea turtles in the Central Pacific. *Environmental Science and Technology* 52: 11535 – 11544.

Katija, K.; Choy, C.A.; Sherlock, R.E.; Sherman, A.D.; Robison, B.H. 2017. From the surface to the seafloor: how giant larvaceans transport microplastics into the deep sea. *Science Advances* 3:e1700715.

Lebreton, L.; Slat, B.; Ferrari, F.; Sainte-Rose, B.; Aitken, J.; Marthouse, R.; Hajbane, S.; Cunsolo, S.; Schwarz, A.; Levivier, A.; Noble, K.; Debeljak, P.; Maral, H.; Schoeneich-Argent, R.; Brambini, R.; Reisser, J. 2018. Evidence that the Great Pacific Garbage Patch is rapidly accumulating plastic. *Nature – Scientific Reports* 8:4666.

Mcelwee, K.; Morishige, C. 2008. Proceedings of the workshop on at-sea detection and removal of derelict fishing gear. December 9-10, 2008. Conference Paper.

Moore, C.J.; Moore, S.L.; Leecaster, M.K.; Weisberg, S.B. 2001. A comparison of plastic and plankton in the North Pacific central gyre. *Marine Pollution Bulletin* pp. 1-5.

Morét-Ferguson, S.; Law, K.L.; Proskurowski, G.; Murphy, E.K.; Peacock, E.E.; Reddy, C.M.; 2010. The size, mass, and composition of plastic debris in the western North Atlantic Ocean. *Marine Pollution Bulletin*. 60(10): 1873-1878.

Pichel, W.G.; Churnside, J.H., Veenstra, T.S., Foley, D.G.; Friendmand, K.S.; Brainard, R.E., Nicoll, J.B.; Zheng, Q. Clemente-Colong, P. 2007. Marine debris collects within the North Pacific Subtropical Convergence Zone. *Marine Pollution Bulletin*. 54:1207-1211.

Rapp, DC, Youngren, S.M., Hartzell, P. & Hyrenbach, K.D. 2017. Community-wide patterns of plastic ingestion in seabirds breeding at French Frigate Shoals, Northwestern Hawaiian Islands. *Marine Pollution Bulletin* 123(1–2): 269-278.

R Core Team, 2014. R: a Language and Environment for Statistical Computing. RFoundation for Statistical Computing.

Ribic, C.A.; Sheavly, S.B.; Klavitter, J. 2012. Baseline for beached marine debris on Sand Island, Midway Atoll. *Marine Pollution Bulletin* 64(8):1726-1729.

Rochman, C. M.; Browne, M. A.; Halpern, B. S.; Hentschel, B. T.; Hoh, E.; Karapanagioti, H. K.; Rios-Mendoza, L. M.; Takada, H.; Teh, S.; Thompson, R. C. 2013. Classify plastic waste as hazardous. *Nature* 494(7436):169−171.

Rochman, C. M.; Hoh, E.; Kurobe, T.; Teh, S. J. 2013. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. *Scientific Reports* 3:3263.

Ryan, P.G. 2015. Does size and buoyancy affect the long-distance transport of floating debris? *Environmental Research Letters* 10(8): 1-6.

Scales, K.L.; Miller, P.I.; Hawkes, L.A.; Ingram, S.N.; Sims, D.W.; Votier, S.C. 2014. On the front line: frontal zones as priority at-sea conservation areas for mobile marine vertebrates. *Journal of Applied Ecology* 51:1575-1583.

Seto, N. W., & O'Daniel, D. 1999. Bonin Petrel (Pterodroma hypoleuca). *The Birds of North America,* Online.

Shah, A.A.; Hasan, F.; Hameed, A.; Ahmed, S. 2008. Biological degradation of plastics: a comprehensive review. *Biotechnology Advances* 26:246 – 265.

Singh, B.; Sharma, N.; 2008. Mechanistic implications of plastic degradation. *Polymer Degradation Stability* 93:561-584.

Steer, M.; Cole, M.; Thompson, R.C.; Lindeque, P.K. 2017. Microplastic ingestion in fish larvae in the western English Channel. *Environmental Pollution.*

Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Morton, A.B., Jonsson, S., Bjorn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P.H., Tana, T.S., Prudente, M., Boonyatumanond, R., Zakaria, M.P. Akkhavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H. 2009. Transport and release of chemicals from plastics to the environment and to wildlife. *Philosophical Transactions of the Royal Society B* 364:2027-2045.

Thompson, R.C. 2015. Microplastics in the marine environment: sources, consequences and solutions. *Marine Anthropogenic Litter* pp. 185-200.

Tibbetts, J.H. 2015. Managing marine plastic pollution: policy initiatives to address wayward waste. *Environmental Health Perspectives* 123:4.

Titmus, A.J.; Hyrenbach, K.D.; 2011. Habitat associations of floating debris and marine birds in the North East Pacific Ocean at coare and meso spatial scales. *Marine Pollution Bulletin* 62 (11): 2496 – 2506.

U.S. EPA, 1993. Plastic Pellets in the Aquatic Environment - Sources and Recommendations. A Summary. United States Environmental Protection Agency EPA,842-S-93-001.

van Franeker, J.A., 2004. Save the North Sea Fulmar-Litter-EcoQO Manual Part 1: Collection and dissection procedures. *Wageningen, Alterra, Alterra-rapport.*

van Franeker, J.A. 2014. Fulmar Litter EcoQO monitoring in the Netherlands – Update 2014. IMARES Report C123/15. IMARES, Texel. Pp 55.

van Franeker, J.A.; Law, K.L. 2015. Seabirds, gyres and global trends in plastic pollution. *Environmental Pollution* 203:89-96.

van Sebille, E.; Wilcox, C.; Lebreton, L.; Maximenko, N.; Hardesty, B.D.; van Franeker, J.A.; Eriksen, M.; Siegel, D.; Galgani, F.; Law, K.L. 2015. A global inventory of small floating plastic debris. *Environmental Research Letters* 10:12.

Vauk, G.J.M; Schrey, E. 1987. Litter pollution from ships in the German Bight. *Marine Pollution Bulletin* 18(6):316-319.

Vlieststra, L.S.; Parga, J.A. 2002. Long-term changes in the type, but not the amount, of ingested plastic particles in short-tailed shearwaters in the southeastern Bering Sea. *Marine Pollution Bulletin* 44:945-955.

Wabnitz, C.; Nichols, W.J. 2010. Editorial: Plastic pollution: an ocean emergency. *Marine Turtle Newsletter*.

Yarsley, V.E.; Couzens, E.G. 1945. *Plastics.*

Youngren, S.M.; Rapp, D.C.; Hyrenbach, K.D. 2018. Plastic ingestion by Tristram's Storm-Petrel (*Oceanodroma tristrami*) chicks from French Frigate Shoals, Northwestern Hawaiian Islands.

Result Tables

Table 3. Contingency test results comparing incidence of plastic types (fragment, line, and sheet) between age classes (HY, AHY) and stomach chambers (PRO, GIZ, whole stomach). Foam and pellet were excluded from the analysis. For each comparison, the degrees of freedom of the Fisher's Exact Test is 1. Bolded values denote significant results, whereby the 95% Confidence Limit of the odds ratio does not overlap 1.

Type	Age Class	Chambers	p-value	Odds Ratio	95% C.I.
Fragment	HY vs. AHY	Whole	0.11	2.69	0.82 – 9.85
	HY	**Pro vs. Giz**	**< 0.01**	**0.21**	**0.07 – 0.58**
	AHY	**Pro vs. Giz**	**< 0.01**	**0.03**	**0.00 – 0.11**
Line	HY vs. AHY	Whole	0.31	2.59	0.54 – 16.76
	HY	Pro vs. Giz	0.24	0.52	0.18 – 1.44
	AHY	**Pro vs. Giz**	**< 0.01**	**0.03**	**0.007 – 0.12**
Sheet	HY vs. AHY	Whole	0.11	2.31	0.85 – 6.49
	HY	Pro vs. Giz	0.64	0.73	0.26 – 1.99
	AHY	Pro vs. Giz	0.14	0.34	0.07 – 1.32

Table 4. Chamber-specific (proventriculus (PRO) and gizzard (GIZ)) and whole bird (both stomach chambers combined) plastic loads calculated for the population (entire sample) and for those individuals that ingested plastic (presence-only data). Nonparametric (median, range) and parametric (mean, standard deviation (SD)) descriptive statistics, sample size (n), and frequency of occurrence (FO) are shown below.

HY Birds

		Population - level exposure (g)			Individual - level exposure (g)			
		Median (range)	Mean ± SD	n	Median (range)	Mean ± SD	n	FO
Whole	Frag	0.0038 (0.0000 - 0.0789)	0.0099 ± 0.0177	40	0.0046 (0.00005 - 0.0789)	0.0117 ± 0.0187	34	85%
	Foam	n/a	n/a	40	n/a	n/a	0	0%
	Line	0.0020 (0.0000 - 0.0452)	0.0041 ± 0.0074	40	0.0023 (0.00005 - 0.0452)	0.0044 ± 0.0076	37	93%
	Pellet	0.0000(0.0000 - 0.0039)	0.0001 ± 0.0006	40	0.0039	0.0039	1	3%
	Sheet	0.00005 (0.0000 - 0.0483)	0.0035 ± 0.0087	40	0.0025 (0.0001 - 0.0483)	0.0069 ± 0.0114	20	50%
	Total	0.0089 (0.0000 - 0.0945)	0.0174 ± 0.0236	40	0.0090 (0.00005 - 0.0945)	0.0179 ± 0.0238	39	98%
PRO	Frag	0.0000 (0.0000 - 0.0745)	0.0065 ± 0.0172	40	0.0063 (0.0002 - 0.0745)	0.0200 ± 0.0254	13	33%
	Foam	n/a	n/a	40	n/a	n/a	0	0%
	Line	0.00005 (0.0000 - 0.0451)	0.0031 ± 0.0074	40	0.0022 (0.00005-0.0451)	0.0053 ± 0.0092	23	58%
	Pellet	n/a	n/a	40	n/a	n/a	0	0%
	Sheet	0.0000 (0.0000 - 0.0483)	0.0030 ± 0.0088	40	0.0033 (0.0001 - 0.0483)	0.0091 ± 0.0135	13	33%
	Total	0.0008 (0.0000 - 0.0902)	0.0125 ± 0.0236	40	0.0060 (0.00005 - 0.0902)	0.0186 ± 0.0267	27	68%
GIZ	Frag	0.0017 (0.0000 - 0.0233)	0.0034 ± 0.0049	40	0.0036 (0.00005 - 0.0233)	0.0047 ± 0.0051	29	73%
	Foam	n/a	n/a	40	n/a	n/a	0	0%
	Line	0.0002 (0.000 - 0.0051)	0.0010 ± 0.0015	40	0.0007 (0.00005 - 0.0051)	0.0014 ± 0.0016	29	73%
	Pellet	0.0000(0.0000 - 0.0039)	0.0001 ± 0.0006	40	0.0039	0.0039	1	3%
	Sheet	0.0000 (0.0000 - 0.0048)	0.0005 ± 0.0012	40	0.0011 (0.00005 - 0.0048)	0.0016 ± 0.0017	12	30%
	Total	0.0039 (0.0000 - 0.0237)	0.0049 ± 0.0052	40	0.0043 (0.00005 - 0.0237)	0.0054 ± 0.0052	36	90%

AHY Birds

		Population - level exposure (g)			Individual - level exposure (g)			
		Median (range)	Mean ± SD	n	Median (range)	Mean ± SD	n	FO
Whole	Frag	0.0011 (0.0000 - 0.0738)	0.0058 ± 0.0128	40	0.0031 (0.0001 - 0.0738)	0.0085 ± 0.0148	27	68%
	Foam	0.0000 (0.0000 - 0.0480)	0.0012 ± 0.0075	40	0.0240 (0.00005 - 0.048)	0.0240 ± 0.0240	2	5%
	Line	0.0017 (0.0000 - 0.0072)	0.0020 ± 0.0019	40	0.0019 (0.00005 - 0.0072)	0.0024 ± 0.0018	33	83%
	Pellet	0.0000 (0.0000 - 0.0087)	0.0022 ± 0.0014	40	0.0087	0.0087	1	3%
	Sheet	0.0000 (0.0000 - 0.0036)	0.0003 ± 0.0008	40	0.0008 (0.00005 - 0.0036)	0.0012 ± 0.0011	12	30%
	Total	0.0045 (0.0000 - 0.1242)	0.0093 ± 0.0196	40	0.0049 (0.00005 - 0.1242)	0.0097 ± 0.0200	38	95%
PRO	Frag	0.0000 (0.0000 - 0.0099)	0.0003 ± 0.0015	40	0.0099	0.0099	1	3%
	Foam	0.0000 (0.0000 - 0.0480)	0.0012 ± 0.0075	40	0.0240 (0.00005 - 0.0480)	0.0240 ± 0.0240	1	3%
	Line	0.0000 (0.0000 - 0.0061)	0.0003 ± 0.0010	40	0.0016 (0.0003 - 0.0061)	0.0021 ± 0.0021	5	13%
	Pellet	0.0000 (0.0000 - 0.0087)	0.0022 ± 0.0014	40	0.0087	0.0087	1	3%
	Sheet	0.0000 (0.0000 - 0.0015)	0.0000 ± 0.0002	40	0.0008 (0.00005 - 0.0015)	0.0008 ± 0.0007	2	5%
	Total	0.0000 (0.0000 - 0.0502)	0.0018 ± 0.0080	40	0.0016 (0.00005 - 0.0502)	0.0089 ± 0.0160	10	25%
GIZ	Frag	0.0009 (0.0000 - 0.0738)	0.0055 ± 0.0128	40	0.0031 (0.0001 - 0.0738)	0.0085 ± 0.0151	26	65%
	Foam	n/a	n/a	40	n/a	n/a	0	0%
	Line	0.0014 (0.0000 - 0.0057)	0.0017 ± 0.0017	40	0.0015 (0.00005 - 0.0057)	0.0021 ± 0.0017	33	83%
	Pellet	n/a	n/a	40	n/a	n/a	0	0%
	Sheet	0.0000 (0.0000 - 0.0036)	0.0003 ± 0.0008	40	0.0008 (0.00005 - 0.0036)	0.0012 ± 0.0011	10	25%
	Total	0.0034 (0.0000 - 0.0740)	0.0075 ± 0.0127	40	0.0038 (0.00005 - 0.0740)	0.0081 ± 0.0130	37	93%

Table 3. Percentage of ingested mass by plastic type (fragment, line, sheet), calculated for each chamber (PRO, GIZ) and age class (HY, AHY), separately. The sample size involved 39 HY and 38 AHY, which had ingested plastic. Due to the lack of normality, four Kruskal-Wallis rank sum tests were performed. Bolded font indicates significant results.

Factor	DF	χ^2	p Value	Conclusion
HY Chamber	1	0.08	0.781	PRO = GIZ
HY Type	**2**	**21.11**	**< 0.001**	**Percent mass of plastic types differ**
AHY Chamber	1	0.81	0.369	PRO = GIZ
AHY Type	**2**	**24.51**	**< 0.001**	**Percent mass of plastic types differ**

Table 4. Pairwise comparisons were used to compare percentage of plastic mass by type, separated by age class (HY and AHY) and by stomach chambers (PRO + GIZ, PRO, GIZ). Due to the non-normal nature of the data, a

Dunn's test for multiple comparisons of independent samples was used with a Bonferroni adjustment. The median percentage of plastic mass was calculated for each plastic type in their respective groups to make conclusions.

	Factor	p Value	Conclusion
HY PRO + GIZ	Fragment vs. Line	1.000	Fragment = Line
	Fragment vs. Sheet	**< 0.001**	**Fragment > Sheet**
	Line vs. Sheet	**< 0.001**	**Line > Sheet**
HY PRO	**Fragment vs. Line**	**0.017**	**Line > Fragment**
	Fragment vs. Sheet	1.000	Fragment = Line
	Line vs. Sheet	**0.001**	**Line > Sheet**
HY GIZ	**Fragment vs. Line**	**0.042**	**Fragment > Line**
	Fragment vs. Sheet	**< 0.001**	**Fragment > Sheet**
	Line vs. Sheet	0.154	Line = Sheet
AHY PRO + GIZ	**Fragment vs. Line**	**0.053**	**Line > Fragment**
	Fragment vs. Sheet	**0.030**	**Fragment > Sheet**
	Line vs. Sheet	**< 0.001**	**Line > Sheet**
AHY PRO	Fragment vs. Line	0.088	Fragment = Line
	Fragment vs. Sheet	0.415	Fragment = Sheet
	Line vs. Sheet	1.00	Line = Sheet
AHY GIZ	Fragment vs. Line	0.478	Fragment = Line
	Fragment vs. Sheet	**< 0.001**	**Fragment > Sheet**
	Line vs. Sheet	**< 0.001**	**Line > Sheet**

Table 5. Summary of statistics (mean/standard deviation) for length (mm), area (length * width) (mm^2), and volume (length *width * height) (mm^3). Also included are nonparametric statistics (median, range) for length, Spearman correlation r_s and p values for length and width, and the percent of plastic that is less than the 5 mm threshold for microplastic. All information is separated by plastic type (fragment, line, and sheet), stomach chamber (PRO and GIZ) and age class (HY and AHY). Pellets were included with fragment and foam was excluded due to the low amount.

			Mean Length	Median Length (range)	Mean Area	Mean Volume	Length : Width Correlation	p Value	% < 5 mm
HY	PRO	**Frag (20)**	**4.36 ± 2.38**	**3.8 (1.3 – 9)**	**14.50 ± 15.59**	**15.71 ± 23.11**	**0.87**	**< 0.001**	**65%**
		Line (107)	14.31 ± 14.95	8.5 (2.0 – 65)	4.64 ± 5.23	1.05 ± 1.39	0.15	0.108	17%
		Sheet(23)	**10.00 ± 5.42**	**9.0 (3.0 – 6.0)**	**65.34 ± 70.87**	**10.00 ± 14.28**	**0.82**	**< 0.001**	**23%**
	GIZ	**Frag (53)**	**2.48 ± 0.99**	**2.5 (0.5 – 5.0)**	**4.70 ± 3.53**	**2.98 ± 3.43**	**0.69**	**< 0.001**	**98%**
		Line (75)	6.81 ± 5.64	5.0 (1.0 – 29)	2.22 ± 0.50	0.50 ± 0.52	0.05	0.698	50%
		Sheet (15)	**6.05 ± 5.41**	**4.0 (2.0 – 22)**	**21.62 ± 27.87**	**2.16 ± 2.79**	**0.78**	**< 0.001**	**63%**

AHY	PRO	Frag (2)	3.45 ± 0.78	3.5 (2.9 - 4.1)	6.9 ± 1.56	4.15 ± 5.45	-0.86	0.333	100%
		Line (5)	24 ± 23.9	9.5 (5.5 - 57)	10.31 ± 10.93	4.09 ± 5.71	-0.35	0.559	0%
		Sheet (4)	4.83 ± 4.15	4.2 (1.0 - 10)	14.93 ± 17.94	1.79 ± 1.62	1	0.083	50%
	GIZ	**Frag (43)**	**2.93 ± 1.13**	**3.0 (0.5 - 5.1)**	**6.67 ± 5.48**	**7.87 ± 19.40**	**0.77**	**< 0.001**	**93%**
		Line (106)	9.51 ± 9.42	6.0 (2.0 - 57)	2.96 ± 3.59	0.70 ± 1.50	0.14	0.146	38%
		Sheet(13)	**4.49 ± 3.08**	**3.9 (1.0 - 10)**	**17.78 ± 21.43**	**1.98 ± 2.14**	**0.95**	**< 0.001**	**67%**

Table 6. General Linear Model test comparing the relative (%) mass of plastic that is microplastics (< 5 mm), as a function of age class, stomach chamber, and plastic type.

Coefficient	t Value	p Value	Conclusion
Age (HY vs AHY)	-0.66	0.534	No difference
Chamber (PRO vs GIZ)	-3.15	0.016	GIZ > PRO
Type (Fragment vs Line)	-6.29	< 0.001	Fragment > Line
Type (Fragment vs Sheet)	-3.83	0.006	Fragment > Sheet

Table 7. Spearman rank correlations of the polymer types (LDPE, HDPE, PE, PP, PE/PP mix, PS, unknown), compared by percent of total plastic mass between age classes (HY and AHY) and stomach chambers (PRO and GIZ). Bold font indicates significant values.

Factor	p value	Correlation	Conclusion
HY PRO vs GIZ	**0.034**	**0.82**	**Large, positive correlation**
AHY PRO vs GIZ	0.574	-0.26	No significant correlation
(HY + AHY) PRO vs GIZ	0.662	0.21	No significant correlation
PRO: HY vs AHY	0.875	-0.07	No significant correlation
GIZ: HY vs AHY	0.088	0.71	Large, positive correlation but not significant

Table 8. Eight Kruskal-Wallis tests were performed to compare the mass of different polymers present (up to six considered: LDPE, HDPE, PE, PP, PP/PE, PS) and three plastic types (fragment, line, sheet). Bold font indicates significant results.

Group	Factor	DF	χ^2	p-value
HY PRO	Polymer	5	3.88	0.567
	Type	**2**	**6.16**	**0.046**
HY GIZ	**Polymer**	**4**	**15.53**	**0.004**
	Type	**2**	**20.58**	**< 0.001**
AHY PRO	Polymer	3	3.98	0.263
	Type	3	5.32	0.150
AHY GIZ	Polymer	5	9.02	0.108
	Type	**2**	**9.52**	**0.009**

Result Figures

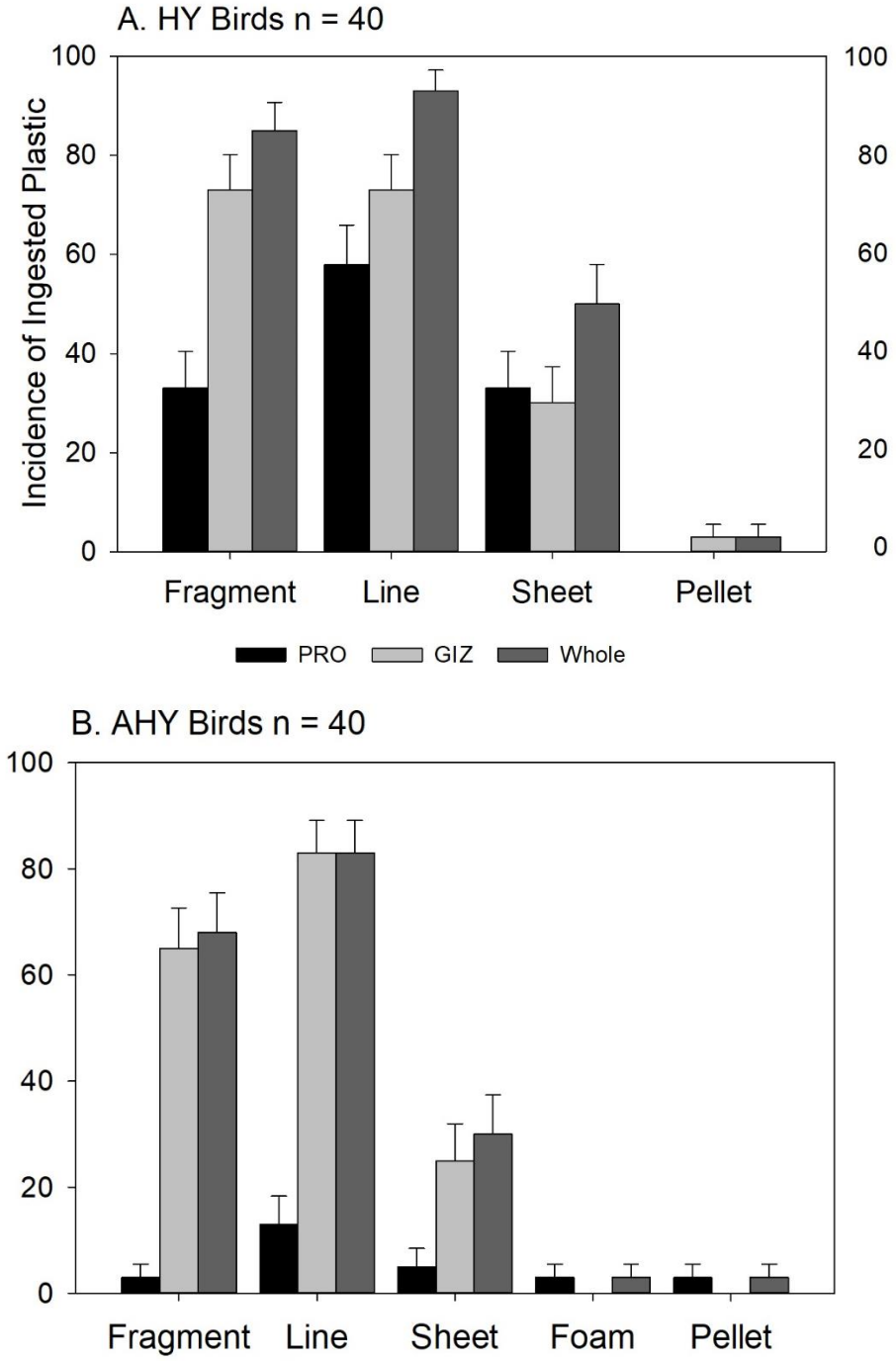

Figure 7. Incidence of ingested plastic by type (fragment, line, sheet, foam, and pellet), separated by age class (A. HY, and B. AHY), and stomach chamber (PRO, GIZ, and Whole bird).

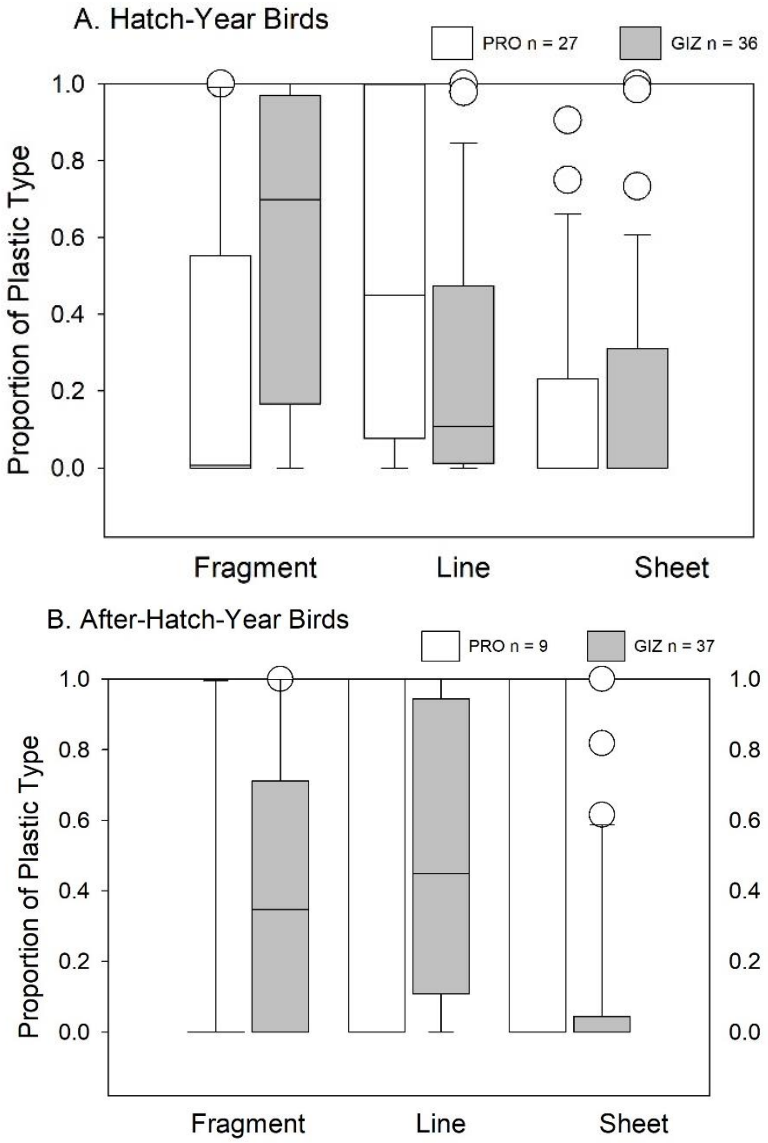

Figure 8. Proportions of mass of plastic type within individual birds, comparing PRO and GIZ for hatch-year (A) and after-hatch-year (B) birds. Box plot indicates 25th, 50th, 75th, 90th quartiles and outliers. Birds without ingested plastic were excluded. Pellet and foam data were excluded from the analysis.

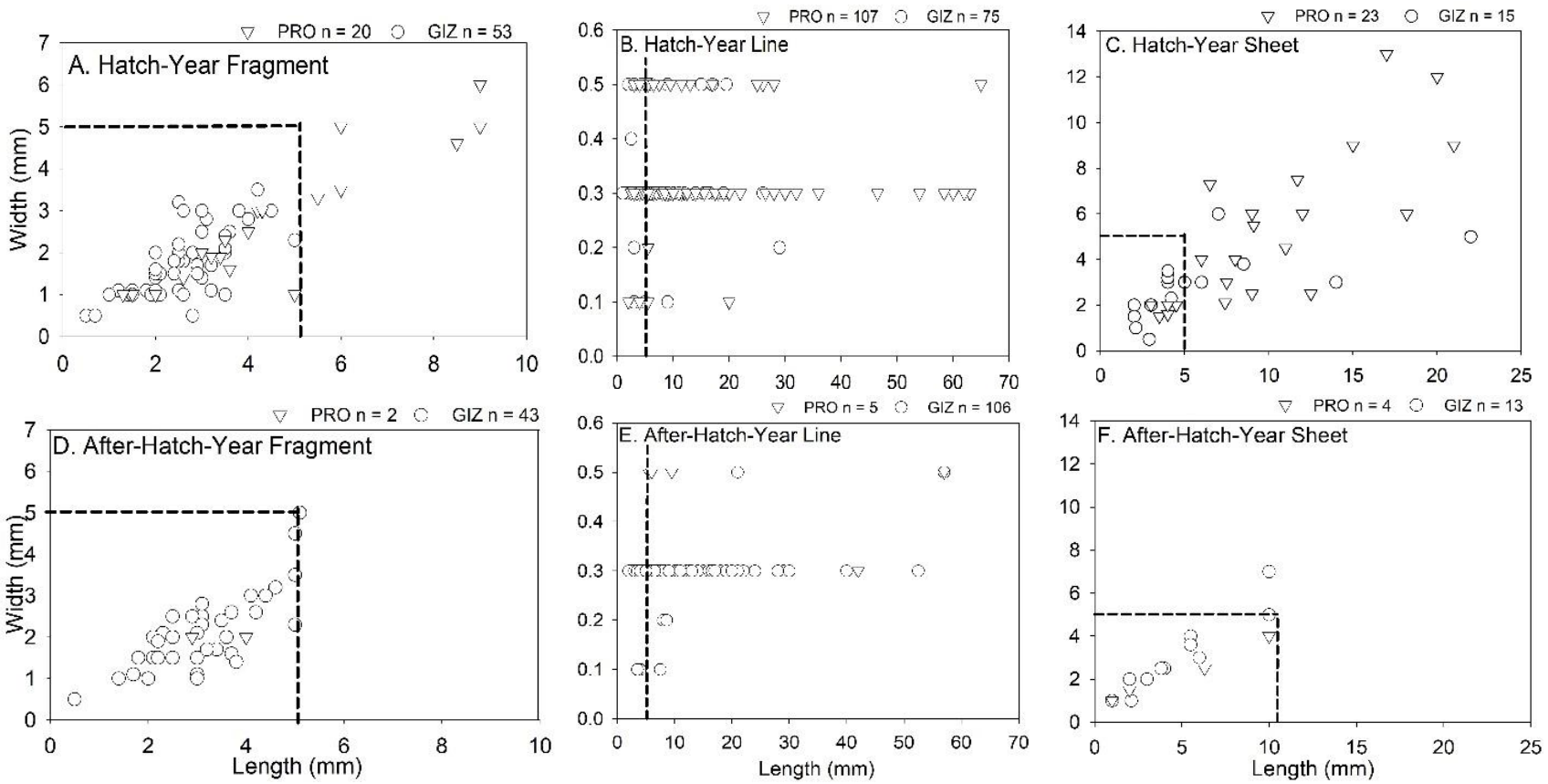

Figure 9. Correlations between the length and width of plastic items (fragment, line and sheet) from the two stomach chambers (PRO = triangle; GIZ = circle) of HY and AHY birds. The dashed lines indicate the 5 mm size threshold for microplastic.

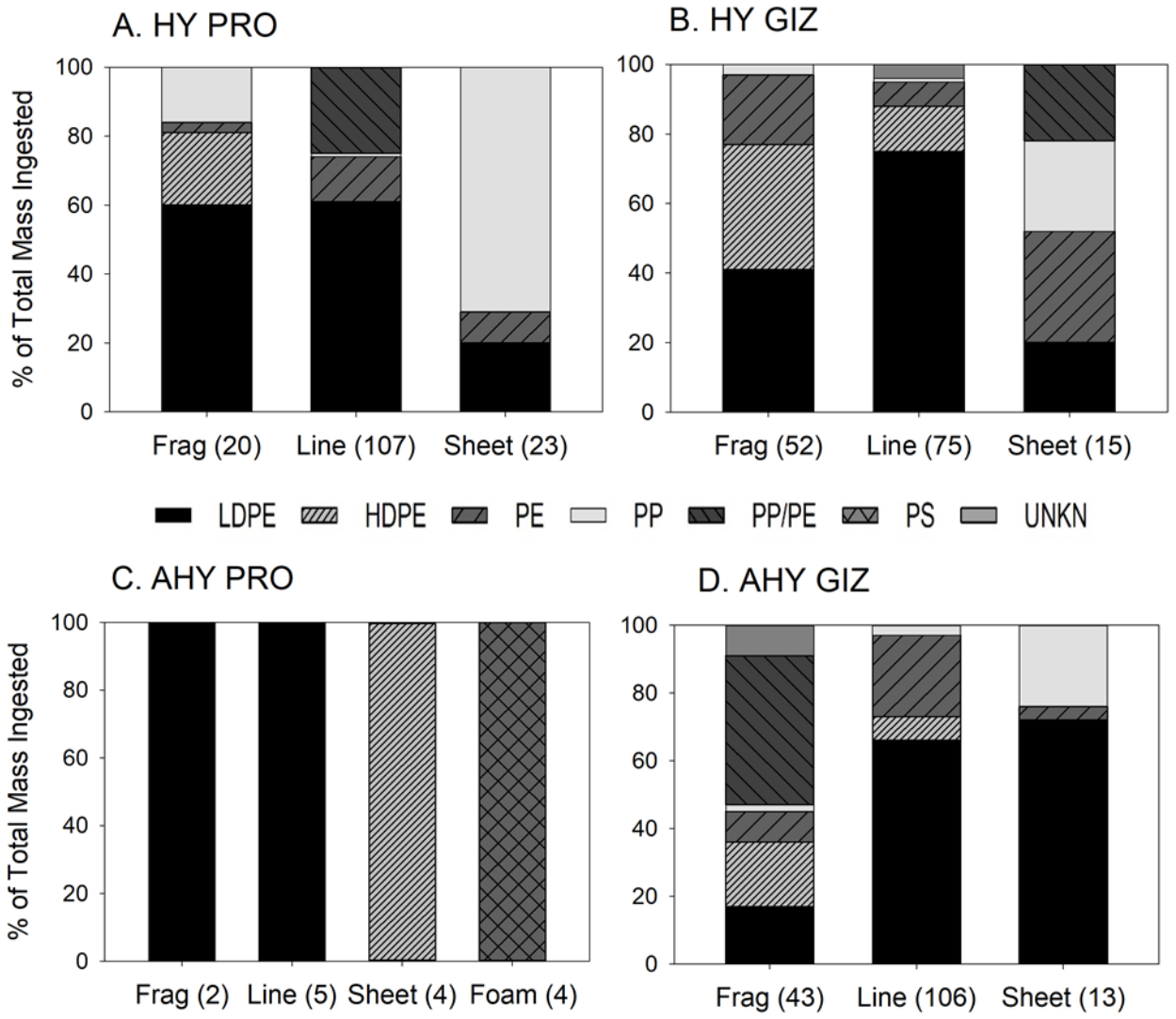

Figure 10. Total polymer composition of ingested plastic mass for each plastic type (fragment, line, and sheet), separated by age class (HY and AHY) and stomach chamber (PRO and GIZ). The one AHY PRO bird with foam was included in the table to show that all of the foam was PS. Polymer composition: low-density polyethylene (LDPE), high-density polyethylene (HDPE), high or low density polyethylene (PE), polypropylene (PP), polyethylene-polypropylene mix (PE/PP mix), polystyrene (PS) and unknown polymer (UNKN).

Appendix

Appendix 1. Percentage of plastic mass by plastic type, comparing proportions by chamber and type for each age class. Birds without ingested plastic were removed from the analysis. 2-way ANOVAs were ArcSine transformed (Shapiro-Wilk HY and AHY: p = < 0.001) Bolded font indicates significant results.

Factor	DF	F Value	p Value	Conclusion
HY Chamber	1	0.09	0.77	PRO = GIZ
HY Plastic Type	**1**	**11.76**	**< 0.01**	**Percent of plastic types are different**
HY Chamber :Type	**2**	**10.54**	**< 0.01**	**Percent of plastic is different by chamber and type**
AHY Chamber	1	0.58	0.45	PRO = GIZ
AHY Plastic Type	**1**	**8.63**	**< 0.001**	**Percent of plastic types are different**
AHY Chamber :Type	2	2.00	0.14	No interaction between chamber and plastic type

Appendix 2. Four 2-way ANOVA tests were performed comparing plastic mass by polymer composition and plastic type, separated by age classes (HY and AHY) and stomach chambers (PRO and GIZ). Because the raw data did not meet the assumptions of normality and homogeneity of variances, data was log transformed, with the Shapiro-Wilk and Levene's test p values reported. Bold font indicates significant results.

Age Class/Chamber	Factor	Sum of Squares	DF	F value	p Value	Shapiro-Wilk p Value	Levene's Test p Value
HY PRO	Polymer	11.27	5	1.02	0.418	0.336	0.787
	Type	**15.60**	**2**	**3.52**	**0.037**		
	Polymer:Type	8.61	5	0.78	0.571		
HY GIZ	**Polymer**	**30.59**	**4**	**5.32**	**< 0.001**	0.335	0.594
	Type	**36.21**	**2**	**12.60**	**< 0.001**		
	Polymer:Type	7.53	5	1.05	0.395		
AHY PRO	**Polymer**	**25.10**	**3**	**9.28**	**0.031**	0.084	0.196
	Type	**26.54**	**3**	**9.81**	**0.029**		
	Polymer:Type	n/a					
AHY GIZ	Polymer	18.44	5	1.95	0.095	0.0317	0.159
	Type	**14.56**	**2**	**3.85**	**0.025**		
	Polymer:Type	11.31	5	1.20	0.318		

CHAPTER 4 Conclusions and Future Directions

1. Summary of key findings

The overall goal of this research was to provide baseline data on Bonin Petrels (*Pterodroma hypoleuca*, BOPE) from Midway Atoll and describe plastic ingestion. To date, this is the most comprehensive report on morphometrics, body condition, and plastic ingestion for this species, including characteristics of ingested plastic: incidence, loads, type, size, and polymer composition. Secondarily, this study aimed to provide recommendations to resource managers on the most effective ways to sample plastic ingestion in BOPE by analyzing biases in detecting ingested plastic.

1.1 Morphometric Data

Baseline morphometric data was reported in Chapter 2, with a comprehensive table of average sizes for each morphometric measurement (bill depth, bill depth at base, bill length, head length, tarsus length, wing length, and body mass) to look for differences between age classes and sex. Our study focused on late-stage chicks and characterized them as hatch-year (HY) birds. Any bird that was not a chick, and forages exclusively at sea is characterized as an after-hatch-year (AHY) bird. The late-stage HY birds in this study had similar body mass and tarsus lengths, indicating that these birds develop tarsus's early and put on mass before wing development to support overall development (Seto & O'Daniel, 1999; Cuthbert, 2016). This study establishes baseline growth data for late-stage HY birds but there needs to be more research on early stage HY birds, with more consistent sampling over time, to truly understand chick development in this species, with particular emphasis on sexual dimorphism during development.

Sexual dimorphism was expressed in the bill depth between males and females, with males having 4.9 % wider bills. This is not enough to determine sex of birds unless comparing the two birds in one breeding pair. Sexual dimorphism was also expressed in HY birds but do not reflect the same sexual dimorphism as AHY birds, and therefore are more likely to be the result of inconsistent sampling.

1.2 Body Condition Index

A Body Condition Index (BCI) was established by comparing the body mass to the bill depth at base as the standard growth measurement. This was strongly correlated to visual condition scores that were observed during necropsy, indicating that our quantitative approach to assessing body condition reflects the qualitative visual condition scores that are historically used as standardized necropsy protocol (van Franeker, 2004). Furthermore, our study separated the cause of death into two groups: birds that died from visual trauma and birds that died from unknown causes. Comparing cause of death to the BCI, birds that died from trauma were in better body condition and represent a healthier population of BOPE.

1.3 Diet Information

My conclusions for BOPE natural diet are limited due to the high digestion of prey items. However, I was able to quantify diet based on several broad categories including: natural food, natural non-food, and non-natural items (plastic). Natural food items were then broken down into items that could be identified and quantified: squid beaks, otoliths, pelagic snails, and fish eggs, with squid beaks and fish eggs being the most commonly ingested.

1.4 Ingested Plastic

BOPE had high incidence for HY and AHY birds with distinct differences by stomach chambers. Therefore, it is recommended that resource managers keep HY and AHY birds separate as well as proventriculi and gizzard chambers, to get a full picture of plastic ingestion in BOPE. Based on the limited prior studies that have reported plastic ingestion in BOPE, incidence of ingestion has increased by 16.5% in the proventriculus and 9.4% in the gizzard between 1987 and 2015, indicating that MPD is increasing (Sileo et al., 1989; Robards et al., 1997).

Despite having high incidence of MPD, plastic only accounted for 0.01% of HY body mass (n = 40) and 0.006% of AHY body mass (n = 40), which is far less than the amount of plastic per body mass of other similar seabird species (Rapp et al., 2017). In terms of EcoQO monitoring 20% of HY birds (n = 8/40) and 8% of AHY birds (n = 3/40) were above the critical level (0.024 g) outlined by van Franeker et al., (2005). This is compared to Laysan Albatross from the same region where 100% were above their critical value (Lavers & Bond, 2016).

Correlations between body mass metrics and plastic loads indicated no relationships, underscoring that plastic ingestion does not directly negatively impact BOPE. The exception to this is a negative relationship between breast muscle mass and plastic loads. This is the third study to detect this relationship, and while causation cannot be based off this correlation, more research needs to be done on these dynamics (Ryan, 1987; Rapp, thesis). Overall, there was no evidence that plastic caused mortality in any specimens from this study.

In terms of characterizing ingested MPD, a majority of ingested items were fragments and line, which is similar to other ingestion studies and at-sea surveys from the Central North Pacific (Moore et al., 2001; Rapp et al., 2017; Youngren et al., 2018). However, line mass was higher in the proventriculus of HY birds, while fragment was higher in the gizzard, suggesting that these two plastic types accumulate differently in BOPE. All ingested plastic pieces were micro- and meso- plastics, with 54.6% classified as microplastics. This gives insight into the types of plastic BOPE were sampling at sea. Lastly, all ingested plastic items were comprised of

polymers with densities lighter than seawater, with 93% being PE or PP. This supports other studies reports that BOPE are surface foragers (Seto & O'Daniel, 1999; Jung et al., 2018).

2. Recommendations

2.1 Recommendations for Future Monitoring

A large focus of this study was to indicate biases in sampling to provide recommendations for future monitoring of plastic ingestion in BOPE. To that end, I explored differences in age classes, stomach chambers and cause of death in detecting plastic incidence and loads. This study found significant differences in plastic loads by age class, with HY birds ingesting more plastic than AHY birds. Due to this, it is recommended that these age classes are kept separate. Furthermore, there were large differences in incidence and loads of plastic in AHY proventriculi and gizzards. Because of this, necropsy is the best method for detecting plastic, as lavage samples only collect samples from the proventriculus (Harrison et al., 1983). Furthermore, proventriculus and gizzard samples should be kept separate rather than be combined because the two stomach chambers detect different plastic items, distinguished by type and size. Larger plastic items accumulate in the proventriculus while smaller items accumulate in the gizzard. Lastly, there were no differences in plastic ingestion based on cause of death, therefore for plastic studies it is acceptable to combine the two groups. We did, however, detect differences in body condition so any study that wants to look exclusively at a healthier population should use birds that died from trauma.

Characterizing ingested plastics are essential for future monitoring to be able to track trends in plastic, regionally and temporally (van Franeker et al., 2014). It was difficult to compare this study to others in the past because previous studies lack standardization in reporting plastic characteristics. I recommend reporting plastic types as: fragment, line, sheet, foam, and pellet, as has been put forth in other ingestion studies (van Franeker, 2004; Youngren et al., 2018). Categorizing plastic by size has inherent issues that need to be addressed. This study reported the length, width and height of all plastic types. However, there are intrinsic differences between plastic types that make them difficult to compare to one another. For example, fragments are hard, rigid items and their dimensions are absolute while being measured. Line and sheet, on the other hand, are able to fold into themselves and change dimensions, making measuring more difficult. Lastly, polymer composition is a new method for characterizing MPD that is valuable in detecting the sources, fates and potential impacts of MPD and to inform policy-makers of the issues they need to address.

2.2 Future Directions

BOPE are a relatively under-studied bird on the Northwestern Hawaiian Islands. This study provides baseline data for future monitoring of this species. However, for BOPE to be considered bio-indicators of plastic pollution, more research needs to be done. This study hypothesizes that BOPE forage within the North Pacific Subtropical Convergence Zone but before any conclusions can be made about the plastics at sea from this study, we must know where these birds actually forage (Polovina et al.,2017). There is little data on BOPE foraging ranges during their breeding months so tracking-data to quantify their at-sea foraging range is critical (Seto & O'Daniel). Furthermore, there are no studies that characterize micro-plastics within the North Pacific Subtropical Convergence Zone. So, once BOPE foraging ranges have been determined, the next step is to sample the plastics at sea with-in their foraging range. This information is essential in order to compare ingestion data and at-sea surveys because a good bio-indicator of MPD must accurately sample the types of plastic within their foraging range (van Franeker, 2003; Furness & Camphuysen, 1997) .

References

Brignac, K.C.; Jung, M.R.; King, C.; Royer, S.J.; Blickley, L.; Lamson, M.R.; Potemra, J.T.; Lynch, J.M. 2019. Marine debris polymers on main Hawaiian Island beaches, sea surface, and seafloor. *Environmental Science Technology* 53:21.

Furness, R.W. and Camphuysen, C.J. 1997. Seabirds as monitors of the marine environment. *ICES Journal of Marine Sciences*. 54: 726-737.

Harrison, C.S.; Hida, T.S.; Seki, M.P. 1983. Hawaiian seabird feeding ecology. *Wildlife Monographs. Wildlife Society, Wiley*. 85:3-71.

Jung, M.R.; Horgen, F.D.; Orski, S.V.; Rodriguez, V.C.; Beers, K.L.; Balazs, G.H.; Jones, T.T.; Work, T.M.; Brignac, K.C.; Royer, S.J.; Hyrenbach, K.D.; Jensen, B.A.; Lynch, J.M. 2018. Validation of ATR FT-IR to identify polymers of plastic marine debris, including those ingested by marine organisms. *Marine Pollution Bulletin* 127:704-716.

Lavers, J. L., & Bond, A. L. 2016. Ingested plastic as a route for trace metals in Laysan Albatross (*Phoebastria immutabilis*) and Bonin Petrel (*Pterodroma hypoleuca*) from Midway Atoll. *Marine Pollution Bulletin.* 110(1):493-500.

Polovina, J.J.; Howell, E.A.; Kobayashi, D.R.; Seki, M.P. 2017. The Transition Zone Chlorophyll Front updated: Advances from a decade of research. *Progress in Oceanography*. 150:79-85.

Rapp, D.C.; Youngren, S.M.; Hartzell, P.; Hyrenbach, K.D. 2017. Community-wide patterns of plastic ingestion in seabirds breeding at French Frigate Shoals, Northwestern Hawaiian Islands. *Marine Pollution Bulletin*. 123:269-278

Robards, M.D., Gould, P.J., Piatt, J.F. 1997. The highest global concentrations and increased abundance of oceanic plastic debris in the North Pacific: evidence from seabirds. In: Coe, J.M., Rogers, D.B. (Eds.), Marine debris. Sources, Impacts, Solutions. Springer-Verlag, New York, pp. 71-80.

Ryan, P.G. 1987. The effects of ingested plastic on seabirds: correlations between plastic load and body condition. *Environmental Pollution*. 46:119-125.

Seto, N. W., & O'Daniel, D. 1999. Bonin Petrel (*Pterodroma hypoleuca*). *The Birds of North America*, (385), 16.

Sileo, L.; Sievert, P.R.; Samuel, M.D; Fefer, S.I. 1989. Prevalence and characteristics of plastic ingested by Hawaiian Seabirds. NOAA Memo pp. 665 – 681.

van Franeker, J.A., 2004. Save the North Sea Fulmar-Litter-EcoQO Manual Part 1: Collection and dissection procedures. *Wageningen, Alterra, Alterra-rapport.*

Van Franeker, J.A.; Kuhn. S.; Bravo Rebolledo, E.L.; Meijboom, A. 2014. Fulmar Litter EcoQO monitoring in the Netherlands – Update 2012 and 2013. IMARES Report C122/14. IMARES, Texel. 56pp.

Youngren, S.M.; Rapp, D.C.; Hyrenbach, K.D. 2018. Plastic ingestion by Tristram's Storm-Petrel (*Oceanodroma tristrami*) chicks from French Frigate Shoals, Northwestern Hawaiian Islands. *Marine Pollution Bulletin*. 128:369-378.