QUANTIFYING DIRECT AND INDIRECT EVIDENCE OF PLASTIC INGESTION BY THE CRITICALLY ENDANGERED NEWELL'S SHEARWATER(*PUFFINUS NEWELLI*) IN THE HAWAIIAN ISLANDS

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ABSTRACT

Marine plastic debris is a pervasive pollutant found throughout the global ocean. Due to the wide range of plastic pollution in the ocean, hundreds of marine species are impacted via entanglement, ingestion, or both. The Newell's shearwater (*Puffinus newelli*) is a critically endangered species endemic to the Hawaiian Islands, which has yet to be assessed for plastic impacts, despite ingesting this pollutant. This thesis updates plastic ingestion rates (incidence and loads) for this species and investigated potential impacts in integrating four novel approaches: (i) plastic identification via attenuated total reflectance Fourier-transform infrared spectroscopy; (ii) weighing and analyzing heart and spleens in relation to plastic loads; (iii) generalized linear model analysis of plastic ingestion drivers; and (iv) gas-chromatography mass spectrometry of preen oil for contaminants. Moreover, this research explores the potential use of a closely-related species (the wedge-tailed shearwater *Ardenna pacifica*) as an indicator of plastic pollution in the Newell's shearwater via the inter-specific comparison of plastic ingestion drivers and the analysis of preen gland oil. This thesis is the first to document plastic ingestion in adult Newell's, and the presence of PCBs, DDE, and BUVs in their preen oil. On a qualitative level via niche comparison and a quantitative level via statistical modeling, wedge-tailed shearwaters can be used to indicate plastic ingestion rates and impacts in Newell's shearwaters. USFWS acknowledges the need for Newell's shearwater protection and conservation efforts, but fails to recognize the impacts of plastic and persistent organic pollutants (POPs) on the species. Thus, it is my hope that this study will motivate an update in the UFSWS recovery plan for the Newell's shearwater that address plastic ingestion, POPs contamination and their sublethal impacts. Ignorance cannot lead to negligence for the conservation of this critically endangered species.

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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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Introduction

Plastic is a pervasive pollutant in surface waters throughout the global ocean, accumulating in a variety of oceanographic features, ranging from large-scale subtropical gyres (1,2,3,4) to small-scale convergence zones (4,5,6) and slicks (7). Marine plastic debris (MPD) can persist for years or even decades in the ocean (5) with microbes and invertebrates using it as substrate, a process called biofouling (1,8). As larger items break down into smaller particles, they become bio-available to a wide range of organisms from urchins to fish to seabirds to marine mammals (9,10). As a result, a growing number of monitoring programs and studies use ingestion by marine organisms as metrics of plastic abundance and trends (10,11,12). In particular, epi- and mesopelagic fish and seabirds are common MPD bioindicators for tracking changes in the amounts and types of plastic pollution over time (12,13,14,15).

The ingestion of plastic by seabirds is widespread, with 44.0% of seabird species globally (16) and 64% of North Pacific seabird species ingesting MPD (12). The number of exposed species increases with every global and regional review (17), since the first reported case of Laysan Albatrosses (*Phoebastria immutabilis*) from the Northwest Hawaiian Islands in 1969 (18,19,20,21,22). By 2050, 99.8% (95% CI, 96.6–100.0%) of all seabird species will ingest plastic debris (21). Due to their global ingestion of marine debris, seabirds are often the focus of ingestion studies. Since seabirds have wide foraging distributions and nest in large colonies, studying their MPD ingestion rates and loads provides insights into the distribution and trends of plastic in the marine environment (13,20,22,23,24). Seabirds are especially valuable when their foraging ranges overlap greatly with plastic pollution (6,20,21). This approach is supported by the findings of Wilcox et al. (2015), who documented a significant relationship between species-specific plastic ingestion rates and availability within their foraging ranges. In other words, as MPD pollution increases, seabirds are expected to ingest more plastic (20,21).

Previous multi-species surveys suggest that Procellariiformes (tubenoses) are the seabirds most affected by MPD due to their vast foraging ranges, broad diets, and unique stomach anatomy, which retains and breaks down ingested MPD and squid beaks (13,16,19,20,25,26,27). Taxonomic classification is the best predictor for MPD ingestion and loads, with tubenoses (consisting of albatrosses, storm-petrels, fulmars, petrels, diving petrels, and shearwaters) consistently having the highest MPD loads (19,20,22,28). The meta-analysis of Kühn and van Franeker (2020) reported that 91 out of 144 (63.2%) Procellariiform seabirds ingested plastic, the largest frequency of any seabird group. Furthermore, due to long retention times of ingested MPD (29,30,31) and extreme longevity (32), Procellariiformes is the ideal focal group to investigate sublethal effects from plastic ingestion. Savoca et al. (2022) completed an extensive study aiming to identify key bioindicator species using a scoring system ranking exposure, sampling availability, distribution, and frequency of plastic ingestion (12). The seabird species that scored highest were (in decreasing order): the northern fulmar (*Fulmarus glacialis*); Leach’s storm petrel (*Oceanodroma leucorhoa*); the Laysan albatross (*Phoebastria immutabilis*; and the black-footed albatross (*P. nigripes*). Since all of these species are Procellariiform seabirds, this ranking highlights the benefits of using Procellariiformes as bioindicators. Furthermore, tubenoses are the most endangered seabird group globally due to impacts from fisheries bycatch and introduced predators into nesting islands, and as a result plastic ingestion can contribute to population level impacts (33).

The impacts of plastic ingestion by seabirds must be investigated, as the extensive list of impacted species grows and includes endangered and critically endangered species. To effectively monitor the diverse impacts of MPD in critically endangered or new species, a comprehensive review of the evidence and approaches is needed. This review synthesizes the aforementioned comprehensive review by breaking down MPD impacts into acute and chronic forms on Procellariiformes, due to their disproportionately high exposure (Fig. 1.1). I survey an extensive list of potential impacts ranging from slower growth rates to starvation and methodologies to ultimately identify effects of plastic ingestion based on prevalence and lethality in the group.

1. Exposure

Exposure involves the potential for an individual to interact with marine debris at any point during their life and is correlated to ingestion rates of marine debris (20). The more regularly an individual interacts with marine plastic debris, the more likely it is to experience ingestion, thus leading to higher loads of ingested plastic (14,20). For instance, species that forage in regions with high densities of plastic debris consume more plastic items on average than species that forage in low-density regions (20,34). Roman et al. (2019a) highlighted that Laysan and black-footed albatrosses from the Hawaiian Islands (a region where marine debris concentrates) experience higher ingestion rates and have higher plastic loads than albatrosses that forage around Australia, including antipodean (*Diomedea antipodensis*), Buller's (*Thalassarche bulleri*), grey-headed (*T. chrysostoma*), and wandering (*D. exulans*) albatrosses (20). van Franeker and Bell (2015) concluded that the majority of cape petrels (*Daption capense*) ingest more plastic in their winter feeding grounds than in their summer feeding grounds around

Antarctica, which reportedly have less plastic (34). van Franeker and Bell (2015) also provided evidence that species that undergo more extensive migrations into temperate regions, such as the Wilson's storm petrel (*Oceanites oceanicus*) and the cape petrel, ingest plastic more often than those with less drastic seasonal migrations, such as the snow petrel (*Pagodroma nivea*) and the Antarctic petrel (*Thalassoica antarctica*) (34). In other words, species that seasonally migrate into regions with high MPD density consume more plastic than those that remain in areas with little plastic year round.

Differences in exposure potential can lead to inter- and intraspecific variations in ingestion rates. Bycaught individuals of Laysan (*Phoebastria immutabilis*) and black-footed albatrosses (*P. nigripes*) had ingestion rates of 83.3% and 51.7% (respectively) near the Hawaiian Islands (35). However, 71.8% and 31.8% of Laysan and black-footed albatrosses (respectively) caught by longline fisheries near Japan ingested marine debris (36), underscoring the regional differences in ingestion. Additionally, because the amount of plastic ingested by the northern fulmar depended on the foraging locations of individual birds, this variability emphasizes how marine debris loads reflect local plastic distributions (37). Exposure potential also varies spatially across colonies (21). Young et al. (2009) reported differences in ingested plastic quantities between Laysan albatross on Kure Atoll (38.03 ± 5.32 g and70.66 ± 11.5 items) compared to on O'ahu (4.37 ± 2.10 g and17.4 ± 5.5) (22). They attributed the significant differences mass ($p = 0.0001$) and count ($p = 0.0004$) to Kure Laysans spending more time foraging in the Western Pacific Garbage Patch and the O'ahu Laysans spending less time foraging in the Eastern Pacific Garbage Patch.

As a result of the differences in exposure potential across species and colonies, the impacts of plastic ingestion on seabirds can vary taxonomically and regionally. For example, Ito et al. (2013) measured persistent organic pollutants (POPs) in the preen gland oil of streaked shearwaters (*Calonectris leucomelas*) and determined that the types and concentration depend on the foraging areas of the birds, as evident from GPS tracking data (13). While this review focuses on the impacts of ingesting plastic debris, the observed variability of exposure potential across regions should be taken into account when scaling the species-specific severity of these impacts and when estimating regional distributions of marine plastic debris.

2. The Litter-EcoQO Manual

The primary method for quantifying plastic exposure and its associated impacts—both chronic and acute—is via necropsy, which allows the analysis of stomach contents and individual body condition (16,38). While necropsy is foundational in MPD ingestion analysis with the scientific field dating back to the 1960's, a standardized procedure for necropsy of seabirds was generated only in 2004: Save the North Sea Fulmar — Litter-EcoQO Manual (39). This manual created by van Franeker outlines necropsy procedures aimed at international use for monitoring the impacts of MPD. Adopting this manual is highly recommended (38) to allow for maximum comparability and meta-analysis. Necropsy allows for examination of the gastrointestinal tract and collection of diet samples for later MPD analysis. These lethal and sublethal impacts cannot be identified without looking inside the seabird, so either necropsy or surgical means are required. However, determining cause of death of an individual is quite difficult, as obvious injuries may not necessarily be the cause of death (40,41,42).

3. Identification of Plastic Items

For the purpose of this review, plastic items are categorized into the following size classes, based on their longest dimension: microplastics (1-5 mm), mesoplastics (5-25 mm), and macroplastics (>25 mm) (43). Identifying ingested marine debris plastic is critical to understand before analyzing the impacts of plastic ingestion on Procellariiformes. Researchers must confidently and accurately identify the items as plastic to potentially connect any adverse health impacts on the individual seabird to plastic, rather than disease or environmental conditions.

The traditional method of identifying ingested MPD is via dyes that adhere to plastic (Nile Red (44), Rhodamine B (45), etc.) or to organic matter (rose Bengal (46,47)) (48). Nile Red does stain plastics very well, but also has a tendency to stain organic compounds, leading to false positives (48). Additionally, there is no standard for identification of plastics based on fluorescence of the Nile Red, making plastic identification more qualitative than is preferred (48). In comparison, rose bengal does not stain MPD (instead staining organic material (46)) and has been used to differentiate plastic from organic material, like bone (47). However, there is again potential for false positives, as some naturally occurring (chitin and $CaCO_3$) and inorganic (rocks, sand) materials are unaffected by rose bengal. While burning the unknown material can aid in identification, with the melting and smell being indicative of plastic, this is an approach of last resort, as the sample is destroyed in the process (19). While both dyes do work, they are not the most efficient method.

Starting in 2004 (49), attenuated total reflectance Fourier transform infrared spectroscopy (ATR FT-IR) has become the standard method for plastic identification, due to its highly specific identification capacity (50). ATR FT-IR can not only differentiate between natural and anthropogenic compounds, but also identifies MPD polymer composition via differences in absorption bands. Due to the highly specific identification capacity, ATR FT-IR is becoming the new standard for MPD identification.

4. Analysis of Plastic Items

Three metrics are regularly used to quantify plastic debris ingestion: frequency of occurrence (% FO), number/quantity, and mass (51). Although the frequency of occurrence is very important to note (52), the most appropriate metrics for quantifying impacts and relationships are count and mass. However, which metric is preferred and more effective at determining relationships is heavily debated in the literature (38,52). Plastic counts are simpler to obtain and more feasible in the field, whereas plastic masses can be more descriptive and are essential for toxicology analyses (38). Ultimately, the preferred metric for analysis depends on the goal of the study, but using all three metrics (% FO, count, and mass) is recommended to enhance the comparability and application of the results (38). Additionally, mass and number are related when species eat items of a single size (53).

As with exposure, the identification and analysis of plastic items is not directly related to the purpose of this literature review, but a foundational understanding of how MPD is measured in the following studies is central to critically analyzing the impacts of MPD in Procellariiformes.

5. Defining and Quantifying Acute Impacts of Plastic Ingestion

For the purpose of this review, acute impacts are lethal and sublethal effects of ingesting plastic that immediately affect the health or survivorship of the individual bird. These acute impacts are ascribed to specific events: the ingestion of particularly damaging items (large or sharp fragments), obstructing items (bags or sheets), or large volumes of MPD. Granted, the impacts of this single event may be lagged, but these impacts still date back to that one event, rather than long term exposure.

5 a. Lethal Acute Impacts: Obstruction and Perforation

The most obvious example of acute impacts of plastic ingestion is obstruction of the digestive tract by plastic items. Obstruction prevents food from passing through either stomach chamber into the intestines for absorption of nutrients, ultimately causing starvation of the individual (31,40,42,54,55,56). Pierce et al. (2004) is the most thorough case study of lethal obstruction, whereby a greater shearwater (*Puffinus gravis*) died of starvation due to obstruction of the gastrointestinal tract by ingested MPD (55, Fig. 1.2). A necropsy revealed that the shearwater ingested a large (1.4 cm by 0.8 cm) fragment of MPD blocking the junction between the ventriculus and the small intestine (the pylorus). A necropsy revealed that all food items fed to the shearwater were found in the proventriculus and esophagus, and no feces were passed by the bird in the rehabilitation facility. Thus, Pierce et al. (2004) ascribed the cause of death to be starvation due to the impaction. The case study highlights the inherent difficulty in studying lethal impacts, due to the inability to confidently determine the cause of death unless the specimens have been observed before and after death. While most birds are opportunistically sampled, often salvaged from bycatch or beach-cast, the attempted rehabilitation of this bird facilitated the evidence of plastic-related mortality. Another instance of obstruction due to plastic ingestion was documented by Pettit et al. (1981) in a naturally-deceased juvenile Laysan Albatross opportunistically sampled in the field. Because this individual contained a “high quantity” of MPD in the proventriculus, and stomach oil occurred only in the proventriculus. The evidence from the necropsy lead researchers to the conclusion that obstruction had led to death by starvation. Nevertheless, the cause of death is often inconclusive with varying rates of occurrence and likely caused by multiple interacting drivers (18,29,40,41). For example, the proportion of inconclusive deaths can ranges widely from 5.8% (57) to 26.3% (40) to 73.3% (58) in the literature cited in this review. In studies where the causes of death are undetermined, researchers often invoke obstruction whenever large amounts go MPD were found in the proventriculus or ventriculus (40). However, this assessment can be based on different metrics, namely the number of ingested items or their mass, with clearcut LD-50 estimates still being refined (40). For instance, while an individual has a 20.4% chance of dying from one single piece of plastic, the chance increases to a 50% chance after ingesting 9 pieces and then to a 100% chance after ingesting 93 pieces, presumably due to the sheer quantity of items ingested (40). However, the LD-50 estimate was solely based on number of plastic items, which as was discussed before, the estimate must also include mass and type of plastic. Including these parameters would better represent the LD-50 of plastic ingestion, rather than plastic counts alone. This LD-50 estimate provides no differentiation between eating a single small piece of plastic versus one large plastic, where the larger piece is potentially more damaging. Furthermore, the LD-50 estimate is independent of taxonomy, the strongest driver of plastic ingestion rates and quantities (20).

However, whether obstruction is documented or inferred, obstruction remains an infrequent cause of death, both in studies quantifying MPD ingestion or investigating the impacts and lethality of such ingestion (40,41,52). For instance, out of 1733 Procellariiform individuals opportunistically necropsied by Roman et al. (2019 b), 557 (32.1%) contained MPD (40). Of those 557 individuals, 13 (2.33%) conclusively died due to obstruction by marine debris, with an additional 9 (1.61%) potentially dying from obstruction. Ryan and Jackson (1987) artificially fed 7 white-chinned petrels (*Procellaria aequinoctialis*) plastic items and necropsied an additional 400 deceased birds of various species (59). Neither sample yielded evidence of MPD obstruction, underscoring the rarity of obstruction of the gastrointestinal tract by plastic. Although, there may be potential biases in the reporting of obstruction towards larger birds (e.g., surface foreign albatrosses), either because they are logistically easier to study and readily available from fisheries bycatch or because obstruction is easier to identify due to the larger size of the items ingested by these species.

The other observed lethal acute impact of MPD is perforation of the gastrointestinal tract. Perforation, which occurs when plastic debris physically breaks the tissue lining the digestive system, occurs even less frequently than obstruction. Carey (2011) reported two cases in a sample of 67 in short-tailed shearwaters (*Ardenna tenuirostris*) (60), and Roman et al. (2019 b) reported one case in an unspecified Procellariiform (40). Even though obstruction and perforation are the most obvious causes of death induced by MPD ingestion, they are rarely observed, suggesting that the majority of MPD impacts are sublethal acute impacts or chronic.

5 b. Sublethal Acute Impacts: Ulcers

The infrequency of lethal impacts suggests that the majority of the acute impacts are sublethal. These sublethal impacts often involve plastic debris physically injuring the gastrointestinal tract via ulcers and other stomach lesions (54). However, the presence of plastic debris does not guarantee the development of an ulcer (57). Once again the greater shearwater case study documented by Pierce et al. (2004) is the only case study that explicitly described ulcerations by ingested plastic due to the thorough measurements and provided images (55), whereby the obstructing MPD aligned with lesions in the ventriculus, thus providing conclusive evidence that plastic can cause ulcerations and lesions in the digestive tract of seabirds. While the Pierce et al. (2004) incidence occurs in the ventriculus, ulcerations, and other lesions are more common in the proventriculus (29,59), especially with large MPD items (41). Ulcers are documented opportunistically because studies do not focus on assessing sublethal acute impacts, such as stomach lesions (29,31,40,41). Additionally, ulcers can become infected and lead to disease and death indirectly (29). Signs of local infection by lesions in the proventriculus were commonly found by Roman et al. (2019 b) in individuals with blockage and obstruction of the digestive tract (40). Ulcerations and other forms of stomach lesions are more common than obstruction, but these do not fully impact the health of the individual birds beyond local infection, suggesting that the most severe impacts are due to chronic exposure of MPD, which will be expanded on later.

6. Defining and Quantifying Chronic Impacts of Plastic Ingestion

For the purpose of this review, any lethal and sublethal impacts that are a result of prolonged plastic ingestion or retention are considered chronic impacts. These chronic impacts are not ascribed to the ingestion of specific items or large volumes of MPD, but may be cumulative. In comparison to acute impacts, which are the result of a single event, chronic impacts are the result of multiple events over a long period of time.

6 a. Lethal Chronic Impacts: Starvation and Dehydration

Chronic plastic ingestion is often cited as the ultimate cause of death via starvation and dehydration (31,40,52,55). While the most dramatic events of starvation are a result of acute ingestion of a few items, there may be more subtle long term effects of starvation and dehydration. Starvation and dehydration are the most common causes of death ranging from 56.6% (57) to 69.7% (58) of deaths in the literature, where cause was identified and obstruction as separate cause of death. However, these statistics should be approached with caution because neither study attributed their deaths due to starvation to dehydration to the chicks ingesting plastic, as some other studies have proposed (41,55). These apparent contradictions caused by the difficulty connecting plastic ingestion to dehydration and death can be reconciled given their different methods and assumptions. While these deaths could be attributed to ingested plastic after the fact, using our improved understanding of plastic impacts, these studies did not consider these deaths to plastic ingestion, therefore the interpretation of these studies for assessing the influence of starvation and dehydration should be done with caution. In general, studying starvation and dehydration is very challenging, as we need live seabirds to observe behavioral responses to ingesting plastic. Therefore, there is little empirical evidence of starvation and dehydration as a result of plastic ingestion, even though they are still very likely to occur.

Additionally, suppressed appetite is a related impact of plastic ingestion (54). The loss of appetite (and begging in chicks) comes from a feeling of satiation due to the large quantity of plastic remaining in the digestive tract, but also potentially due to a distended proventriculus. As concisely explained by Day et al. (1985), appetite is stimulated by an empty stomach, and satiety can occur with an empty stomach from dehydration and distention of the gastrointestinal tract (54). In relation to plastic ingestion, if plastic remains in the proventriculus and/or ventriculus for a long period of time, the bird will consistently feel “full” and not eat, thus leading to starvation and dehydration.

The lack of explicit evidence implicating the causal link between starvation and dehydration and plastic ingestion suggests that most impacts of long-term plastic ingestion and exposure are sublethal or possess unknown lethality. Sublethal chronic impacts are much more difficult to detect and quantify, as discussed in the next section.

6 b. Sublethal Chronic Impacts: Whole Body, Organ, Tissue, and Cellular Levels

The strongest sublethal impact of plastic ingestion is impaired health and body condition of seabirds with high levels of plastic ingestion (28,57,58,61). A significant negative relationship exists between the quantity of ingested MPD and the body mass of the individual bird (28). As discussed in Ryan (1987 b), the negative correlation between plastic load and condition could be the result of birds in poor health eating more plastic (62), since birds in poor body condition eat higher quantities of inorganic material (64). However, Spear et al. (1995) found a positive relationship ($p < 0.001$, $B = 0.00685$) between plastic incidence and body weight, leading to the assumption that heavier (and therefore healthier) birds are feeding more often, and therefore have a higher probability of ingesting plastic (28). This finding was followed by the negative relationship ($p = 0.0111$, $B = -0.00461$) between body weight and plastic number causing the authors to suggest that individuals with higher quantities of MPD have less body weight. When combining the results of various papers (28,57,58,61,62), healthier birds seem to be more likely to consume plastic, and as a result, their health suffers due to chronic sublethal effects of ingesting MPD. Granted, this pattern is likely more complicated than Spear et al. (1995) implied due to interactions between differences in age, taxonomy, and season, but their work generated the foundation for future studies on sublethal chronic impacts.

As part of the impaired health and body condition, researchers have documented significant relationships between ingested MPD loads and the health code and size of various organs. For instance, the relationship between spleen mass ($p = 0.030$, $r = 0.427$) and liver mass ($p = 0.039$, $r = 0.407$) with MPD mass in the proventriculus and the relationship between liver mass ($p = 0.020$, $r = 0.424$) and heart mass ($p = 0.021$, 0.421) with MPD mass in the gizzard found by Rapp (2005) in opportunistically sampled Laysan albatross chicks suggests that these organs increase in size in response to higher concentrations of MPD (63). Additionally, Chamberlain (2019) documented a positive relationship between MPD and spleen mass ($p = 0.022$, $r = 0.362$) in Bonin petrel (*Pterodroma hypoleuca*) chicks opportunistically sampled (64). Enlarged spleens are correlated with stressors (64), but also with better body condition (63,64,65,66,67). As pointed out by both Rapp (2005) and Chamberlain (2019), the relationship with body condition suggests simply that these individuals are fed more, and therefore have higher exposures to plastic (63,64). However, the inconsistencies between body condition and spleen mass versus stressors and spleen mass suggest that further research into the impacts and potential causes of enlarged spleens is needed. The relationship with MPD and enlarged livers could also be derived due to higher feeding rates, and subsequent higher plastic exposure. Furthermore, the relationship between heart mass and MPD suggests a physiological response in the heart to the MPD in Procellariiformes. The only other study reporting the potential connection between heart size and pollutants in seabirds used guillemots (a member of Alcidae) in relation to polyisobutylene (PIB) pollution (68). The lack of published evidence demonstrates the lack of understanding of this sublethal impact of MPD in seabirds in general, but especially in Procellariiformes.

Health impacts of MPD on Procellariiformes can also be evident a tissue-specific basis. Decreased adipose tissue reserves are often correlated with increased ingested marine debris (41,63,64). This relationship occurs for both subcutaneous ($p = 0.003$, $r = -0.524$) and intestinal ($p = 0.001$, $r = -0.560$) fat (63), potentially due to a lack of consumed food, as discussed earlier. The reduced fat impacts the survival of an individual due to the lack of energy reserves available during periods of hardship or high energetic demands. For instance, reduced fat reserves can hinder the development and survivorship of chicks, which rely on adipose tissue to supply nutrients for feather growth (69). Another tissue affected by MPD ingestion is blood. In seabirds, decreased food intake decreases the red blood cell (RBC) density and plasma (70), so seabirds with high plastic loads (who subsequently consume less food) potentially have decreased RBC counts and less plasma (71). Additionally, ingested MPD alters the blood chemistry of seabirds. This relationship is illustrated in Lavers et al. (2019) who documented the following relationships between blood chemical parameters and MPD in flesh-footed shearwaters (*Ardenna carneipes*),: blood calcium and presence ($p = 0.022$, $\beta = -0.133 \pm 0.056$, $F = 5.62$), number ($p = 0.029$, $\beta = -0.001 \pm 0.001$, $F = 5.06$), and mass ($p = 0.048$, $\beta = -0.007 \pm 0.004$, $F = 4.096$); blood cholesterol and presence ($p = 0.036$, $\beta = 0.276 \pm 0.128$, $F = 4.66$); amylase concentration and mass ($p = 0.018$, $\beta = 5.190 \pm 2.112$, $F = 56.037$); and uric acid concentration and number ($p = 0.05$, $\beta = 0.006 \pm 0.003$, $F = 4.05$) (72). The reduced blood calcium was attributed to poor fat reserves—as cholesterol and adipose tissue have a direct relationship in seabirds (73)—combined with exposure to plastic additives. Another potential explanation would be cellular-level damage to tubular glands, which will be expanded on shortly (74). High cholesterol occurs during periods of stress, including starvation and periods of fasting, but the levels reported by Lavers et al. (2019) are much higher than those in fasting individuals (72) implicating MPD as the stressor (72). High uric acid and amylase concentrations can indicate starvation and renal failure and cause kidney stones and diabetes, which suggests that MPD can lead to long-term, kidney-related health conditions in seabirds (72). While alterations in blood chemistry are not necessarily fatal, prolonged exposure to MPD can lead to health conditions that impair the bird's quality of life and reduce survivorship.

In a histopathological approach, a chronic impact that was recently discovered is a plastic-induced fibrotic disease termed "plasticosis" (74,75, Fig. 1.3). Plasticosis is defined as plastic-induced inflammation and fibrosis (excessive scar-tissue development) in the digestive tract that only recently was observed in free-living organisms (75). Rivers-Auty et al. (2023) not only found micro- and nano-plastics embedded in the proventriculus, spleen, and kidneys of flesh-footed shearwater chicks, but also found a direct connection between ingested MPD and cellular-level inflammation (74). Naturally occurring debris, such as pumice and beaks, did not cause any inflammation indicating plastic as the cause. This conclusion was supported by the findings of Charlton-Howard et al. (2023) who found the same significant relationship ($p = 0.037$, $r = 0.387$) with the remaining variation left unexplained by pumice ($p = 0.975$). As part of plasticosis, ingested plastic causes substantial losses of rugae throughout the entire proventriculus ($p<0.001$), which impairs digestion and nutrient absorption (74). Additionally, tubular glands (which aid in secreting protective mucus in the proventriculus) were significantly damaged in the superior ($p = 0.002$, $r = -0.520$) and inferior ($p = 0.032$, $r = -0.412$) proventriculus in proportion to MPD mass (74). Not only does plastic hinder digestive efforts within these seabirds, but it also damages the protective gland creating a positive feedback loop leading to more tissue damage (74,75). This proventriculus damage was also connected to kidney tissue damage suggesting physiological stress due to plasticosis (74). Overall, plasticosis indicates cellular-level damage of ingesting MPD with cascading effects spreading to multiple organ systems (75).

6 c. Chronic Impacts with Unknown Lethality: Heavy Metals, POPs, and MPD Additives

Since plastics tend to adsorb heavy metals, namely cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), nickel (Ni), and zinc (Zn), from the marine environment (76,77), the potential for MPD to be a mechanism for transporting high concentrations to seabirds after ingestion is of great concern. Some metals are "essential" (Cr, Co, Cu, Fe, Ni, and Zn), while others are "non-essential" (Cd, Pb), so plastic could introduce non-essential metals into seabirds and potentially increase essential metals to toxic levels (73). Above normal traces of both essential and non-essential heavy metals have been found in several studies. Lavers et al. (2014) found significantly heightened levels of Cr ($\beta = 0.99$, $r^2 = 0.40$, $p = 0.04$) in breast feathers of flesh-footed shearwaters in relation to increasing plastic mass (78); Lavers & Bond found significant relationships between plastic mass and concentrations of Fe ($\beta = 3637 \pm 819$ μg/g plastic^{-1}, $r^2 = 0.75$, $p < 0.01$), Mn ($\beta = 71.35 \pm 5.97$ μg/g plastic^{-1}, $r^2 = 0.95$, $p < 0.01$), and Pb ($\beta = 16.44 \pm 5.21$ μg/g plastic^{-1}, $r^2 = 0.60$, $p = 0.03$) in Bonin petrels (79). However, heightened heavy metal concentrations are not a uniform impact since some studies reported no relationships between heavy metals and MPD mass. Roman et al. (2020) reported no significant trends between plastic mass and Mn, Fe, Co, or Zn in slender-billed prions (*Pachyptila belcheri*) (71); Puskic et al. (2020) found no relationship between plastic mass and trace elements Cd, Cu, and Zn (80). Due to the inconclusive results, relationships between plastic mass and trace element concentration are presumably extremely species-dependent and element dependent (71). Therefore the relationship between MPD and metals is poorly defined, and the implications for lethality remain undetermined.

MPD also correlates to persistent organic pollutants (POPs), but the impacts of ingesting MPD with adsorbed POPs and lethality are still undefined. POPs biomagnify in marine ecosystems (13), and MPD can sorb POPs and potentially serve as a vector (31,52,81,82). As mentioned previously, preen gland oil sampled from streaked shearwaters shows regional and short-term variations in marine POPs showing the benefits of using seabirds as bioindicators (13). Three POPs were found with significant regional variation in concentration: polychlorinated biphenyls (PCBs, $F_{(4,31)} = 25.091$, $p < 0.001$), dichlorodiphenyltrichloroethane (DDT, $F_{(4,31)} = 19.375$, $p < 0.001$), and hexachlorocyclohexanes (HCHs, $F_{(4,31)} = 11.795$, $p < 0.001$). Although plastic ingestion was not involved in this study, MPD does serve as a vector for many POPs, suggesting that the POPs found in the streaked shearwaters could either be from their prey or ingested MPD (81,82). PCBs and DDT have been found in plastic ingested by 97 different Procellariiform birds representing 8 species, but the investigation into presence within the tissues was not conducted (83). Relationships between higher-chlorinated PCBs and ingested marine debris has yet to be statistically significant (81,82,84), but a correlation with lower-chlorinated PCBs found in adipose tissue of short-tailed shearwaters ($r = 0.63$, $p = 0.03$) (84). The difference was attributed to higher-chlorinated congeners using prey as a vector, and lower-chlorinated PCB congeners using plastic as a vector (84). However, the adsorption of PCBs and other POPs by plastic is highly dependent on the plastic compound, weathering, residence time in the ocean, and environmental conditions (such as pH and temperature) (85). PCB concentration decreases fat reserves in seabirds ($r =-0.439$, $n = 18$, one-tailed $p < 0.05$) (82), which drastically impacts their survival, as discussed before. Additionally, exposure to PCBs initiates an immune response causing an increase in lymphocyte proliferation ($r = 0.548$, $p = 0.033$, $n = 15$) implying misregulation of the immune system and heightened stress (86). Even though relationships between plastic ingestion and PCBs and other POPs are quite vague, the connection should still be monitored to better understand the role of MPD as a driving factor of POPs accumulation in seabirds.

The pollutants found in the tissues of seabirds with the most direct relationship to ingesting MPD are, of course, plastic additives. Since plastic additives biomagnify less than PCBs and other POPs, likely due to their high molecular weight (87,88,89), Tanaka et al. (2013) suggest that the role of ingested MPD is clearer due to less exposure of seabirds from prey (88). While analyzing adipose tissue reserves in short-tailed shearwaters, Tanaka et al. (2013) determined that ingested MPD functioned as a vector for polybrominated diphenyl ethers (PBDE), a flame retardant plastic additive. Although the correlation between PBDE concentration and plastic load was not assessed, the congeners of PBDE found in adipose tissues matched the PBDE congeners found in ingested MPD on an individual basis implying a direct transfer from MPD to seabird. The relationship between plastic additives and ingested MPD was expanded on by Tanaka et al. (2020) in which they artificially fed short-tailed shearwater chicks plastic, and then euthanized and necropsied them to sample and analyze adipose tissue, liver, and preen oil for PBDE (BDE209) as well as one benzophenone (BP-12) and three benzotriazole UV stabilizers (UV-326, 327, and 328) (90). All stabilizers were in higher concentrations in comparison to the control group. Leeching of the additives was higher in the ingested plastics than in plastics in water, likely due to the influence of stomach oil on the plastic pellets. Additionally, additive concentrations in the pellets decreased at the same rate as concentrations in the seabirds increased, creating direct evidence for the transfer of plastic stabilizers into seabird tissues via MPD ingestion. Livers from several albatross species have tested positive for various perfluorinated alkyl acids (PFAAs), a group of carcinogenic and highly toxic plastic additives, including perfluorooctanesulfonate (PFOS), perfluorooctanoic acid (PFOA), and several long-chain perfluorocarboxylates (PFCAs) (91,92). However, the relationship to ingested plastic needs to be investigated further to see if these PFAAs are a result of biomagnification or MPD ingestion. In Hardesty et al. (2015), short-tailed and wedge-tailed shearwaters contained plastics with dibutyl phthalate (DBP) and bis(2-ethylhexyl)-phthalate (DEHP) as additives in their gastrointestinal tract and tested positive for DBP and DEHP in their preen oil (93). Plastic load correlated strongly with additive concentration in the preen oil for both DBP ($r^2 = 0.48$) and DEHP ($r^2 = 0.75$) indicating a direct transfer of plastic additive from ingested MPD to preen oil (91). Although small concentrations of plastic additives can be detected in samples from birds lacking ingested MPD (likely due to the small level of biomagnification that occurs), high concentrations occur only in species with high MPD ingestion rates (89). For instance, a Hawaiian petrel (*Pterodroma sandwichensis*) had the highest concentration of decabromo diphenyl ether (BDE209), a brominated flame retardant in plastics, of 83ng per g of oil also contained the largest plastic load of 12 pieces in Yamashita et al (2021) (89). Furthermore, preen oil analysis reflects the distribution of plastic additives in MPD, with concentrations matching those in MPD sampling (89). Preen oil analysis allows for the sampling of live seabirds (13), and the ratio between plastic additive concentration and ingested MPD load (89,90,93) indicates the potential to sample and quantify MPD load in living seabirds.

7. Intergenerational Transfer

In conjunction with the weakened health of adults via MPD ingestion, which reduces adult survival, seabird parents run the risk of transferring their plastic load to their chicks. First proposed by Ryan (1988) on blue petrels (*Halobaena caerulea*) (94), intergenerational transfer of MPD occurs when parents inadvertently pass ingested MPD to their chicks while regurgitating food (28,30,57,59,94). Since chicks are unable to leave the nest for the first few months of their lives, the only source of plastic would be regurgitation from the parents while feeding (60). On several occasions, chicks were found to have different quantities of plastic than adults: in northern fulmars (24); in black-footed albatrosses and in Laysan albatrosses (63); in Bonin petrels (64); in blue petrels, in white-chinned petrels, and in Kerguelen petrels (*Amphodroma brevirostris*) (94); in Cory's shearwaters (*Calonectris diomedea*) (95); and in flesh-footed shearwaters and in wedge-tailed shearwaters (*Ardenna pacifica*) (96). In the literature used in this literature review where both adults and chicks were reported, plastic incidence in chicks ranged from 30.8% to 100.0% and on average (71.1 ± 28.3)% whereas in adults, plastic incidence ranged from 8.7% to 100% and on average (58.3 ± 32.8)% (24,63,64,94,95,96). Generally, chicks had higher reported plastic incidences than adults occurring in 6 out of 9 species (24,63,64,94,95,96). Although there are no direct observations (ie endoscopy before and after feedings), intergenerational transfer is supported by seasonal variations in MPD rates that track the breeding seasons, such that adults sampled later in the breeding season—after chick provisioning has started—possess less plastic than adults sampled earlier in the breeding season —before chick provisioning—(28,97).

The study with the most direct results connecting chick growth rates and MPD ingestion is Sievert & Sileo (1993) (58). In this study, Laysan albatross chicks with high volumes

(>22cm3) of ingested MPD had significantly smaller fledging mass and reached peak mass earlier ($p = 0.002$ and $p = 0.018$, respectively) than chicks with low volumes (<22cm3) of ingested plastic. Additionally, MPD impacted wing cord length ($\beta = -1.42$, $r^2 = 0.24$, $p < 0.01$) and head length ($\beta = -0.11$, $r^2 = 0.13$, $p = 0.02$) of flesh-footed shearwaters (78). The species impacts of intergenerational transfer (94) are extremely important to consider when investigating various impacts of MPD ingestion, as exemplified by Sievert & Sileo (1993). Intergenerational transfer appears to be a severely overlooked chronic impact of plastic ingestion, as people primarily focus on the impacts to the chicks or adults alone, rather than analyzing them in parallel. While the impacts of intergenerational transfer do not negatively affect the health of the individual (parent) with the plastic (in fact, it may lessen the impacts), the transfer of plastic from parent to chick affects the health of the chick secondarily, as chicks with MPD from their parent(s) have impaired growth rates and reduced survivability (58,78).

Recommendations

Although the main goal of this review was to synthesize the impacts of ingesting MPD, I also aimed to generate a ranking system for MPD impacts based on prevalence and lethality, for studying rare or critically endangered species. With how (unfortunately) prevalent MPD is in the ocean, simply identifying plastic ingestion rates and quantities is frankly not enough anymore. The lethal impacts of MPD, like starvation and obstruction, are the most direct impacts of plastic ingestion, and in some situations (e.g. perforation) easier to detect. However, as described above, the incidence rate of these impacts is very low relative to sublethal impacts. While important to note and include in population-level studies, these impacts should not be the focus of the research, especially when studying and analyzing impacts on rare species with inherently small sample sizes. The targets of studies should be sublethal impacts, since sublethal impacts are less clear and understood than lethal impacts, and there are many impacts of MPD that are still being discovered (e.g. plasticosis, relationship with plastic additives and trace metals). Impacts with chronic effects and unknown lethality are more likely to affect survival of a species, especially via intergenerational transfer, because they affect individuals over long time scales. Therefore, snapshot observational studies describing MPD rates in one species over a short time period cannot appropriately report on long-term trends and impacts. Instead, longitudinal studies that follow individual birds and their offspring over multiple generations would be needed. Simply put, when studying a critically endangered or rarely studied species, focus on studying chronic, sublethal impacts because those impacts demand larger sample sizes covering an extensive time period to observe any trends.

Unresolved Issues

The main limitation in studying MPD effects in seabirds, as touched on before, is the lack of long term standardized metrics and trends. Namely, many published studies have limited sample sizes, obtained by combining specimens spanning several years. The literature recommendations of yearly sample size range from 20 individuals (98) to 40 individuals (12,99). Recommended sample size varies based on study methods and statistical analysis, but for the purpose of this review, a strong sample size is considered any sample $n \geq 20$ per species per year. Overall, of the 55 studies observing plastic ingestion rates referred to in this literature review, 6 studies had insufficient sample size. Of the 44 remaining, 37 (67.3%) had adequate sample size for at least one species studied, but did not have enough to apply to yearly trends. In some cases, researchers with large samples fail to provide information on overall or yearly sample sizes. For instance, Roman et al. (2019 b) analyzed 1733 specimens from 51 species, but failed to mention any sampling year(s) or trends over time (40). I understand studies analyzing long term trends are difficult to implement, but they are vital to fully understanding how MPD affects seabirds, not just Procellariiformes. Frankly speaking, excluding the influence of time hinders future research efforts. Of the 44 aforementioned studies, only 7 (15.9%) had adequate sample sizes for generating time-based trends. For instance, Kain et al. (2016) analyzed 30 Newell's shearwater (*Puffinus newelli*) covering seven years (2007-2013) (100). While 30 individuals is a very strong sample size for such a critically endangered species, analyzing long term trends, we need these sample sizes to be replicated for each year to confidently identify trends of MPD ingestion. Additionally, Yamashita et al. (2021) analyzed 145 seabirds covering nine years (2008-2016), but their sample size included 32 species (89). Simply put, they did not have enough individuals from a given species to generate a trend over time. In contrast, many studies have excellent sample sizes, but lack the yearly samples for quantifying trends over time. Including sampling year(s) allows for comparability between studies and enables meta-analyses of long term trends. Therefore, having a large enough sample size per year (not just the overall study) is imperative for analyzing global impacts of plastic ingestion.

For maximum impact, studies need to sample multiple age classes in their analysis (28, 100). As discussed before, adults and chicks have different MPD rates, so plastic analysis should be conducted on both age classes whenever feasible. In the studies used in this literature review, 65.0% analyzed only one age class (30.0% chicks or fledglings and 35.1% adults only), 22.8% studies analyzed both age classes, and 0.4% didn't report the age classes. Potentially, the reason for only using one age class could be due to sampling methods. Specimens from bycatch are after-hatch year birds, and specimens from colonies are usually hatch year birds. However, both age classes should be sampled whenever possible (12). Since most studies only analyze one age class, there is potential for a gap in our knowledge about plastic incidence and quantities in the age classes that are poorly studied, which is very species dependent.

The primary gaps in our knowledge are the chronic impacts with unknown lethality: relationships to heavy metals, POPs, and MPD additives. These are the impacts that research efforts must prioritize, especially in analyzing critically endangered species. Comparable analyses and standardized reporting of results will advance this research arena, by allowing intra-specific and inter-specific comparisons. Standardization of these impacts will facilitate interspecies analyses.

Conclusions

In this literature review, I compile and synthesize thorough lists of all known and potential impacts of marine plastic debris on Procellariiformes, and through this information, I generate a basic ranking system of these impacts based on prevalence and lethality with an emphasis on application for rare and critically endangered species. While the ranking system is very qualitative, I generate a foundation for a more quantitative ranking system. Ultimately, the field is shifting away from a focus on merely reporting plastic ingestion rates, and embracing a more cumulative approach to quantify and understand all MPD ingestion impacts. The literature discussed both acute and chronic impacts ranging from lethal to sublethal to unknown lethality, with an emphasis on Procellariiformes. Although Procellariiformes are the most impacted order, all other orders ingest MPD, emphasizing the global nature of plastic pollution (20,21,25,27). Therefore, understanding MPD impacts will require considering the specific life-history and population ecology of seabirds from all families and marine ecosystems.

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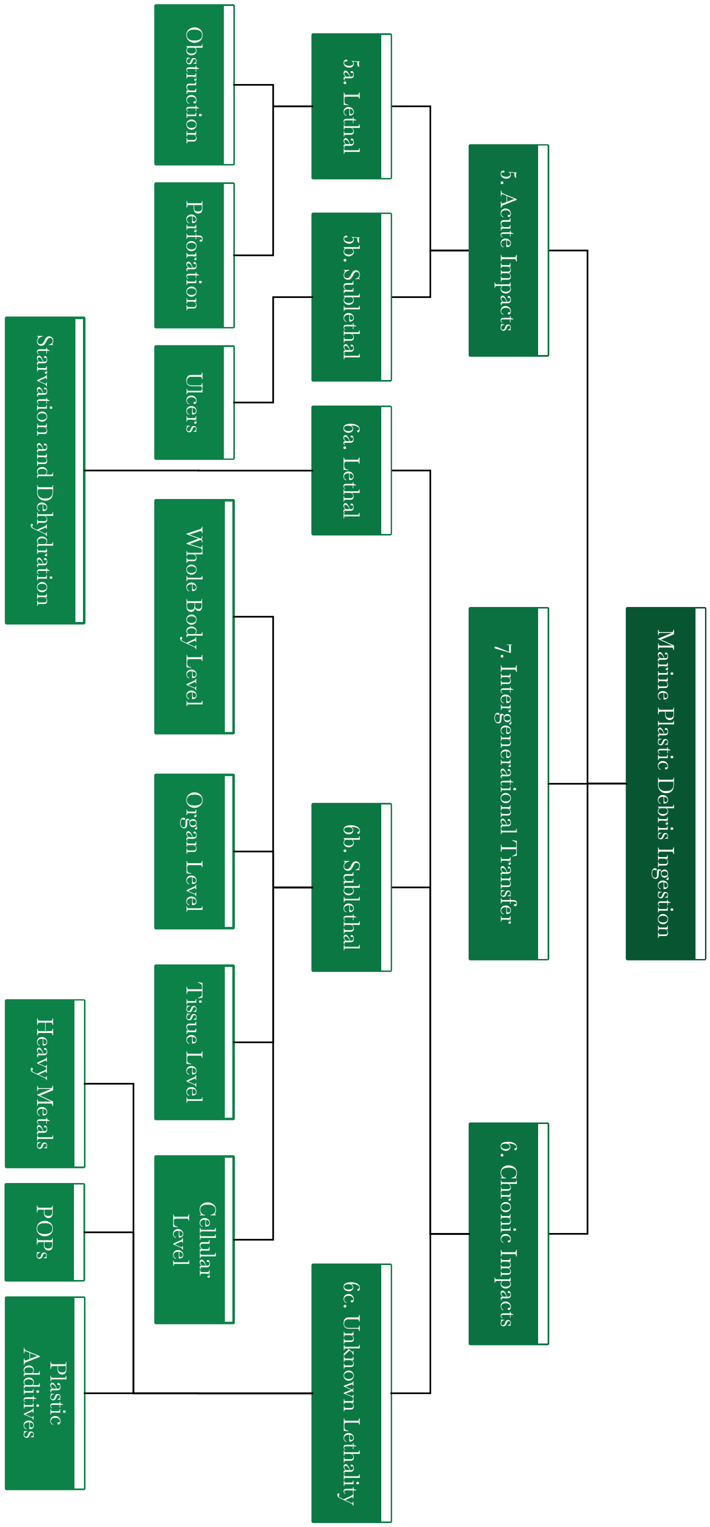

Figure 1.1. Graphical abstract of MPD impacts reviewed in this literature review.

Figure 1.2. Image from Pierce et al. 2014. Left: (a) Gizzard of the Greater Shearwater (*Puffinus* gravis) and (b) the fragment of red user plastic that obstructed the pylorus in (a). Right: (c) Capelin in upper digestive tract obstructed by (b). (55)

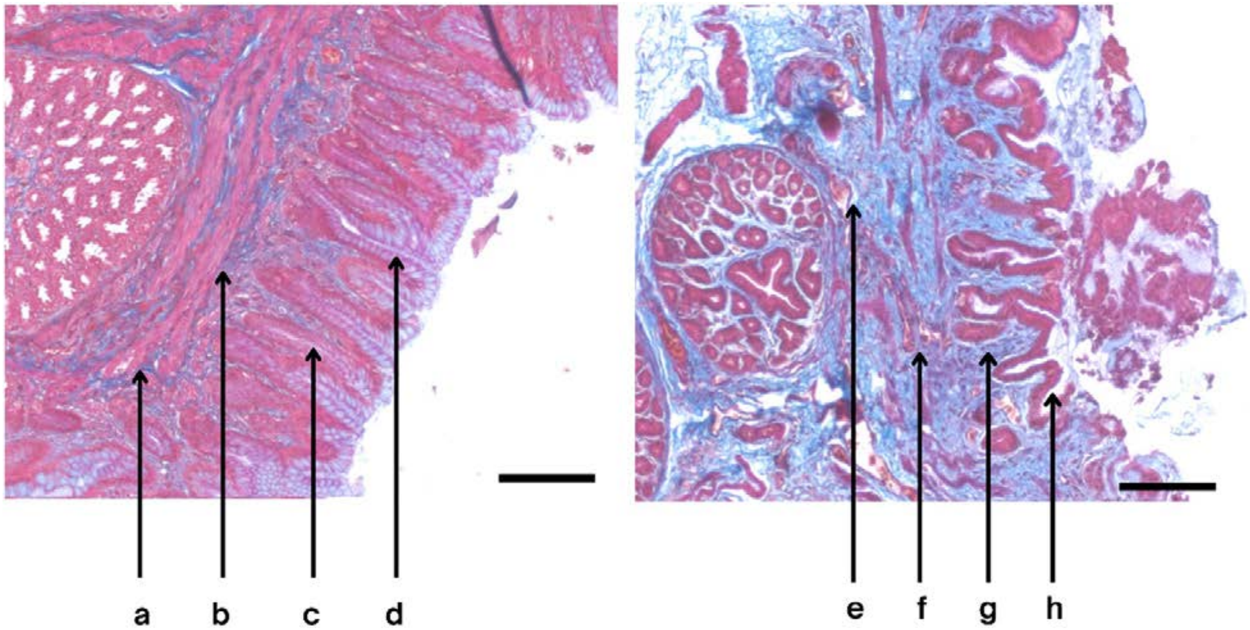

Figure 1.3. Figure from Charlton-Howard et al. 2023. Left: Grade 0 proventriculus (no plastic impact), Right: Grade 5 proventriculus (high plastic impact). Healthy proventriculus tissue characterized by (a) organized submucosa, (b) minimal collagenous deposition within the submucosa, (c) minimal collagenous thickening of the lamina propria within the tubular glands, (d) long, uniformly shaped tubular glands. Signs of a proventriculus suffering from plasticosis were (e) disorganized submucosa, (f) extensive collagen deposition within the submucosa, (g) collagenous thickening of the lamina propria within the tubular glands, (h) and the loss of tubular gland structure. The Images taken at 20x magnification, scale bar = 100 µm. (75)

CHAPTER 2: CHARACTERIZING INGESTED MARINE PLASTIC DEBRIS AND IMPACTS IN THE CRITICALLY ENDANGERED NEWELL'S SHEARWATER (*PUFFINUS NEWELLI*)

INTRODUCTION

Marine plastic debris (MPD) is a pervasive pollutant found throughout the global ocean. In 2019, 82-358 trillion plastic pieces weighing 1.1 - 4.9 million tonnes were estimated to be floating around the ocean (Eriksen et al. 2023). This MPD accumulate in large scale and small scale ocean features, ranging from subtropical convergence zones (Howell et al. 2012) to slicks (Gove et al. 2019). Notably, MPD accumulates in the North Pacific Subtropical Gyre (NPSTG), due to its clockwise rotation, and amplified the seasonal "dead zone" of surface currents around 140˚W and 35˚N (Howell et al. 2012). On a large (1000s km) scale, concentration and retention of MPD in the NPSTG causes the infamous Great Pacific Garbage Patch (Howell et al. 2012), yet on smaller scales (10s-100s km) processes repel matter from the NPSTG, given the proper rotation, and can transport MPD towards the Hawaiian Islands. Another transport mechanism for MPD to the Hawaiian Islands involves the seasonal latitudinal shift of the North Pacific Subtropical Convergence Zone (NPSTCZ) (Pichel et al. 2007). MPD converges in the NPSTCZ due to Ekman transport along the high pressure ridge of westerly and easterly trade winds. Similar to the NPSTG, eddies can transport plastic towards the Hawaiian Islands, specially in winter when this feature is located further south (Howell et al. 2012).

These two large oceanographic features in the North Pacific are the main source of plastic debris to the Hawaiian Islands (Morishige et al. 2007; Augustin et al. 2015). The movement of MPD threatens the marine life around the Hawaiian islands, which tend to forage in the same regions were MPD accumulates and consequently interact with MPD (Pichel et al. 2007). This interaction involves ingestion and entanglement, first documented in the 1960s (Kenyon & Kriddler 1969) and the 1980s (Conant 1984), respectively. Entanglement and ingestion threaten native marine life around the Hawaiian Islands ranging from sea turtles to whales to seabirds (Kühn & van Franeker 2022). While entanglement is a more conspicuous and dangerous interaction, ingestion is more common issue, yet its impacts are more difficult to document. For instance, while 27.4% of all seabird species suffer from entanglement globally, 44.0% suffer from MPD ingestion (Kühn & van Franeker 2022). Although many Hawaiian seabird species die after becoming entangled in marine debris and derelict fishing gear (Hyrenbach et al. 2020), the effects of MPD ingestion on an individual level are still being investigated and evaluated.

In particular, seabirds are the focus of MPD ingestion analyses due to their wide foraging distributions and their ease of sampling due to their reliance on land for nesting (Piatt and Sydeman 2007; Savoca et al. 2022). Within seabirds, tubenoses (order Procellariiformes) is the most impacted group of seabirds, with 63.2% of the species ingesting plastic (Kühn & van Franeker 2022). This high incidence rate has been attributed to their two-chambered stomach, with an anterior proventriculus and a posterior gizzard separated by a narrow isthmus. MPD, squid beaks, and fish bones collect in the gizzard, where they remain for weeks to months until they are grounded to a size small enough to pass into the intestine (Terepoki et al. 2017). These potentially long retention times of ingested MPD and their extreme longevity (Tacutu et al. 2018) make Procellariiformes the ideal focal group to investigate sublethal effects from plastic ingestion. Since the 1980s, plastic ingestion has been documented extensive in five species of Hawaiian tubenoses: black-footed albatross (*Phoebastria nigripes*), Bonin petrel (*Pterodroma hypoleuca*), Laysan albatross (*Phoebastria immutabilis*), Tristram's storm-petrel (*Oceanodroma *tristrami*), and wedge-tailed shearwater (*Ardenna pacifica*) (Sileo et al. 1990, Rapp et al. 2017, Yamashita et al. 2021, Savoca et al. 2022). Despite its critical conservation status, plastic ingestion by the Newell's shearwater (*Puffinus newelli*, NESH) has been poorly studied, with only two other studies documenting plastic ingestion: 11% in 1987 (Sileo et al. 1990) and 50% from 2007 to 2013 (Kain et al. 2016), both from accidentally grounded specimens.

As part of the investigation into NESH, this study will update the ingestion rates published by Kain et al. (2016) a decade ago, and will investigate potential impacts of plastic ingestion in NESH using novel approaches. Although this exploratory study quantifies correlational relationships, it addresses two hypotheses: (i) ingestion trends: because the amount of plastic ingested by seabirds reflects the quantities available in their foraging habitat, Newell's shearwaters sampled over time (2013-2021) will have higher ingested MPD incidence (frequency of occurrence) and loads (count and mass), reflecting increasing plastic pollution in the marine environment; and (ii) seabird response: because ingested plastic is correlated with the mass of various organs in other Procellariiform seabirds, there will be positive relationships between ingested plastic mass and organ (heart and spleen) masses in Newell's shearwaters.

METHODS

Specimen Collection

This study relied on naturally-deceased specimens sampled opportunistically. NESH were provided by the Save Our Shearwaters rehabilitation program, a non-profit organization located on Kaua'i. These specimens became grounded due to attraction to artificial lights and collisions with anthropogenic structures or were attacked by terrestrial predators. Some died on arrival, others died in care of unknown causes, and the remaining individuals were humanely euthanized.

The specimens, were stored frozen at -20˚C until they were necropsied following standardized procedures previously modified for Hawaiian seabirds (van Franeker 2004; Rapp et al. 2017).

Due to the opportunistic nature of our sampling, sample sizes varied amongst years and between age groups. Based on recommended sample sizes of 40 and 20 birds (van Franeker and Meijborn 2002; National Research Council 2009), our target sample size would be a minimum of 440 specimens over the study period. However, we sampled 108 specimens, with at most 15 hatch year birds (HY, which includes chicks and fledglings) and 9 after-hatch year (AHY, which includes pre-breeding and breeding adults) in a year (Table 1).

Stomach contents were taken from both the proventriculus and ventriculus (hereafter gizzard) using standardized procedures (van Franeker 2004), because plastic incidence rates in NESH fledglings differed these two chambers (Kain et al. 2016). The samples were stored in 70% ethanol until they were manually sorted into four broad categories: food items (squid beaks, fish eye lenses, fish bones), natural non-food items (plant matter, sand, pumice, endoparasitic worms), non-natural, non-food items (plastic, glass), and unidentified items using 10x magnification via a binocular dissecting microscope, congruent with other plastic-ingestion studies (van Franeker et al. 2011; Rapp et al. 2017). Following these standardized protocols, plastic items were categorized into four types: fragments, line, foam, and sheet. In other words, each specimen yielded 8 mass measurements per chamber: 4 diet categories and 4 plastic types.

Each diet category and plastic type was weighed twice using a Mettler Toledo Model MS104S scale, with a resolution of 0.0001 g. Half of the minimum detection threshold (0.00005 g) was recorded when the scale yielded 0.0000 g while weighing a sample. Since each sample was weighed twice, the root mean squared error (RMSE) was used to determine the variation between replicate mass measurements across all samples (Armstrong & Collopy 1992; Rapp et al. 2017). RMSE was calculated with R's rmse command in the Metrics package.

To ensure that all plastic items were collected and analyzed, all putative MD and unknown items in the diet were isolated, weighed, and analyzed with attenuated total reflectance Fourier transform infrared spectroscopy (ATR FT-IR), which identifies plastic compounds by matching well-known infrared absorption bands representing distinct chemical functionalities found in plastic with the item in question (Provencher et al. 2017; Jung et al. 2018). Because small and rigid plastic fragments can be crushed during scanning by the diamond tip of the instrument, each item was weighed individually before ATR FT-IR. This measure was to ensure that should an item not be plastic, it could be removed from the total plastic weight.

Attenuated Total Reflectance Infrared Spectroscopy Analysis

Using a Thermo Fisher Scientific Nicolet iS5 ATR-FTIR spectrometer, IR spectra were collected from all putative plastic items and unknown items. Following the methods of Corniuk et al (2023), spectra were generated from 16 scans each with resolution at 4 cm^{-1} and a data interval of 1 cm^{-1}. The section from 4000 cm^{-1} to 500 cm^{-1} were compared against the standardized polymer spectra provided in Jung et al. (2018). Two steps were taken to ensure maximum clarity of the spectra: (i) the samples and the instrument's diamond crystal were cleaned with a kim wipe and 70% isopropanol before each scan, and (ii) a background (control) scene was taken between each sample.

Plastic Characterization

Following the characterization of plastic in Donnelly-Greenan et al. (2018), plastic samples were scanned using the Epson Perfection V37 digital scanner at 500 dpi with a custom cover to ensure consistent lighting. Images were subsequently analyzed with Fiji's Image J open-source software (Schindelin et al. 2012) to measure the volume of the plastic item. Items were manually outlined and area, solidity, roundness, and aspect ratio were calculated. HSB values of the center of each plastic item were measured, using the center coordinates provided by Image J.

Statistical Analysis

All statistical analyses were performed with R using the commands and {packages} listed below. Gizzard and proventriculus samples from each bird were analyzed in parallel using various regressions to determine if the occurrence (presence/absence) and the quantities (number and mass of items) varied with respect to three driver variables: (i) squid beak count, (ii) ENSO phase (via the Multivariate El Niño Index, MEI.v2 value), and (iii) year.

Because the plastic count data followed a negative binomial distribution, as revealed by the descdist and fit.dist {fitdistrplus}. Several generalized linear models (GLMs) with multiple deriving factors were run via glm.nb {MASS} to determine the influence and interaction between two or more predictor variables. Since negative binomial distributions suffer from zero-inflation, all models were checked via check_zeroinflation {performance}. In regards to the plastic mass data, a tweedie distribution was used, and GLMs were run via cpglm {cplm} following the same process as the count data.

Since there are so many models, the ability of each model to explain the observed variability was determined by ranking the model via the corrected Akaike information criterion

(AICc). Due to the small sample size of individual birds collected each year, AICc was used to prevent overfitting by AIC due to small sample sizes (Hurvich & Ling 1989). The relative likelihood of each model was quantified by calculating the Akaike weight with model.avg {MuMIn} to prevent overfitting, with the “best” model(s) having a total weight ≥80% (Michael et al. 2014). The most important variables in the models were identified using a scaled variable weight to determine the influence each variable on the weights of the models. Then, the model weights were averaged to assess the importance of each parameter across all models (Michael et al. 2014). A value greater than 1.0 for a parameter means that the models including said parameter performed better than the average model. To represent the temporal trends accounting for the ENSO cycle, modeled plastic loads were generated by setting MEI to zero (thereby representing a neutral ENSO) and using the predict {stats} function in R. This modeling was only done for HY due to the limited sample size of AHY.

Additionally, to compare with historical reports of plastic ingestion in NESH (Sileo et al. 1990, Kain et al. 2016), χ^2 tests for independence were run comparing incidence rates, and due to the non-normal distributions of the plastic data, one-sample Wilcoxon tests were run against previous point estimates of plastic loads.

Morphometrics

To determine potential relationships between plastic ingestion and body size, seven morphometrics were taken: culmen length, bill depth at gonys, bill depth at base, tarsus length (between indents of joints), head length, wing length, and mass. Measurements were taken with calipers (0.1 mm resolution), wing length with wing ruler (1 mm), and mass with a hanging scale (1 g). A principal component analysis was used to develop a body condition index (body mass as a function of size) and to analyze relationships between the resulting PC axes for size and body condition and plastic abundance (Rapp 2005). Pair-wise correlations were run between the plastic abundance (count and mass) of individual birds and their coordinates along the body size and body condition PCA axes.

Organ Mass Sampling

To determine the potential physiological impact of plastic ingestion on NESH, the mass of the spleens and hearts of the specimens representing 2018-2023 were weighed (0.0001 g resolution) following the same procedure as the diet samples. The organs from those specimens necropsied prior to the beginning of this project (2013-2017) or with severe internal damage due to collisions were not weighed.

Following the procedures of Rapp (2005) and Chamberlain (2019), the heart was removed from the pericardial sac, and all arteries and veins entering and leaving the heart were cut flush with the main atria and ventricles. Unlike the diet samples, the heart and spleens were weighed wet, and only one weight was taken on account of evaporation underestimating the second weight.

Pearson and Spearman Rank correlations between plastic loads (count and mass) and the organ mass were used to identify any relationships. Furthermore, a PCA was run using the morphometrics of the birds to characterize the relationship with bird size and/or body condition. Once again Pearson and Spearman Rank correlation tests were run between organ weight and the coordinates of birds along the PCA body size and body condition axes.

RESULTS

Plastic Occurrence

Out of a total of 108 (85 + 23 = 108) NESH specimens sampled between 2013 and 2023, 85 (78.7%) were HY birds and 23 (21.3%) were AHY (Table 2.1). Overall, 23.5% (20 of 85) of the HY birds contained ingested MPD compared to 17.4% (4 of 23) of AHY birds. A χ^2 test of presence/absence between HY and AHY was not significant (χ^2 = 0.499, df = 1, p = 0.48).

Plastic Loads

Plastic loads were quantified by bird and by stomach chamber, using two significantly cross-correlated metrics: the number of items and their mass. Plastic counts and masses were significantly correlated with each other (Table 2.2). HY NESH (n = 85) contained, on average 0.441 ± 0.923 pieces of plastic (median: 0, max: 6) with an average weight of 0.0014 ± 0.0050 g of plastic (median: 0.000 g, max: 0.0408 g) (Figure 2.1 & 2.2) . Of the HY with plastic (n = 23), on average there were 1.61 ± 1.12 pieces of plastic (median: 1, max: 6) with an average weight of 0.0050 ± .0086 g (median: 0.0017 g, max: 0.0408 g). AHY NESH (n = 23) contained on average 0.261 ± 0.689 pieces of plastic (median: 0, max: 3) with an average weight of 0.0002 ± 0.0006 g of plastic (median: 0.000 g, max: 0.0028 g) (Figure 2.3 & 2.4). Of the AHY with plastic (n = 4), on average there were 1.50 ± 1.00 pieces of plastic (median: 1, max: 3) with an average weight of 0.0014 ± .0012 g (median: 0.0014 g, max: 0.003 g). However, like with incidence, the differences in loads between AHY and HY were not significant for neither count (W = 866, p = 0.321) nor mass (W = 856.5, p = 0.279); when you consider only the individuals with plastic, the relationship was also not significant for neither count (W = 42.5, p = 0.811) nor mass (W = 33, p = 0.391).

The RMSE of the weights of all plastic items was 6.380775 x10-5 and the high correlation between the replicate weights of the same plastic items ($S = 44.407$, $\varrho = 0.994$, $p < 2.2e-16$) suggest that our measurements were highly precise.

Historical Comparison

There were two previous studies analyzing plastic ingestion in NESH (HY only). Sileo et al. (1990) had an 11% frequency of occurrence. While the incidence was 24% for this study, the difference against Sileo et al. (1990) was not significant ($\chi^2 = 1.33$, $df = 1$, $p = 0.248$). The other NESH plastic ingestion study by Kain et al. (2016) had a 50% frequency, but the difference in incidence was significant this time ($\chi^2 = 4.12$, $df = 1$, $p = 0.042$). After performing a one-sample Wilcoxon test, the differences in HY plastic loads between this study and Kain et al. (2016) was significant for both plastic counts ($V = 293$, $p = 2.68e-12$) and mass ($V = 90$, p-value = 2.025e-15).

Models of Ingested Plastic

Plastic load was calculated by bird (considering the content of each individual's proventriculus and gizzard together) and by stomach chamber, considering the proventriculus and gizzard separately, since plastic occurrence was higher in the gizzard (HY: 24%, AHY: 13%) than in the proventriculus (HY: 4%, AHY: 4%). Furthermore, this difference was significant for HY ($W = 4244.5$, $p = 0.0002$), but not AHY ($W = 288$, $p = 0.301$). The two response variables (mass and number) were analyzed in four ways as a result: by bird as a whole, by proventriculus, by gizzard, and another series of models accounting for the differences between the organ chambers, causing a total of 8 results per age class. However, for simplicity, we will break down models based on (i) age class and (ii) response variable.

HY Modeling — by number

The results in regards of the count models were generally inconclusive, although none of the models had zero-inflation (Sup. Table 2.1). For the proventriculus, no best model was selected due to low model weights and small (<2) ΔAICc values. Additionally, no proventriculus GLM models had significant Pr(>|z|) scores. Moreover, no best predictors were identified due to all scaled parameter weights being smaller than 1.0. Gizzard models yielded similar results with many small ΔAICc values, low model weights, and no significant Pr(>|z|) scores, yet the models with squid beaks performed better than the average model, with beaks having a scaled parameter weight of 1.21. Models analyzing the bird as a whole (using total plastic quantities), also yielded the same inconclusive results, with no best parameter either. When isolating the impacts of the different stomach chambers, there was no best model, but squid beaks were identified as an important predictor with a scaled parameter weight of 1.17. The predicted plastic counts across time were negative, indicating that the temporal trends had a marginally small effect compared to the relatively stronger influence of ENSO (Figure 2.5).

HY Modeling — by mass

Mass models yielded conclusive results (Sup. Table 2.2). The proventriculus model with the largest model weight (75.1%) was plastic ~ year, with that predictor having a scaled parameter weight of 1.74. The gizzard model with the largest model weight (83.3%) was plastic ~ beaks + year. Both predictors had weights larger than the average model: squid beaks (1.75) and year (1.68). When considering the total plastic per bird, beaks (1.57) and MEI (1.63) were the most influential predictors, yet no best model or model pair was identified. When accounting for the difference in plastic loads between the stomach chambers, the best models were plastic ~ beaks + year + MEI + organ (54.9%) and plastic ~ beaks + organ + year (45.0%), and as a result, all parameters contained >1 weights. The predicted plastic masses based on temporal influence alone indicated a decreasing trend, implying that the mass of items were decreasing over the course of the study period (Figure 2.6).

AHY Modeling — by number

In regards to the count models, the results were inconclusive, although none of the models had zero-inflation (Sup. Table 2.3). For the gizzard and total plastic models, no model or model group was selected to be the best model, due to low model weights and many small (<2) ΔAICc values. Additionally, no proventriculus GLM models had significant Pr(>|z|) scores. No best predictors were identified with all scaled weights being smaller than 1.0. Although there was not clear best performing model for proventriculus plastic either, those including MEI (1.16) performed better than the average model. When isolating the impacts of the different stomach chambers, there was neither a best model nor an influential parameter.

AHY Modeling — by mass

As with the HY, the AHY mass models yielded more conclusive results than the count models (Sup. Table 2.4). For the proventriculus, the model with year as the only predictor (plastic ~ year) was the best model (99.7%) with year being the most influential parameter with a scaled parameter weight of 1.75. Although there was no best model (or group of models) for gizzard, both year (1.50) and MEI (1.08) were very influential parameters. Total plastic in the bird lacked a clear best model as well, although models with year (1.62) performed better on average. When including the differences between the organs, the two best models were plastic ~ organ + year + MEI (57.1%) and plastic ~ organ + year (33.6%), and both year (1.81) and MEI (1.24) were very influential parameters again.

Plastic Identification

A total of 44 putative plastic items identified during sorting were scanned with FT-IR: 92.9% of the fragments (26 of 28), 87.5% of the line (7 of 8) and 37.5% (3 of 8) of the unknown items were confirmed as plastic with FT-IR. No foam was found inside of the shearwater stomachs.

All remaining 36 plastic items were matched to a polymer with high degree of certainty (match > 80%): the majority (83%) were polyethylene (PE), 11% were polypropylene (PP), 3% polyvinyl chloride (PVC), and 3% ethylene-vinyl acetate (EVA). Additionally, one individual contained glass (26 pieces weighing 0.1631g) in the gizzard, which was also confirmed with ATR FT-IR. Although anthropogenic in origin, these glass items were not included in the statistical analysis.

Plastic Characterization

Out of 44 plastic items from 27 birds, 23 plastic items were scanned and analyzed through Fiji's Image J. Majority of items were essentially two-dimensional due to small depths (<1 mm), with the exception of item that was conical in shape. Plastic fragments had a volume of 9.398 ± 8.347 mm^3 on average (median: 5.838 mm^3, range 0.555–33.14 mm^3). The shape of the plastic fragments varied greatly, with roundness ranging from 0.243 to 0.936; solidity ranging from 0.624 to 0.999; and aspect ratio ranging from 1.068 to 4.113. Colors of the plastic items (both fragments and line) ranged from red hues (12˚) to cyan hues (203˚) with majority being yellowish hues (median: 76˚, mean: 85.8˚). Plastic items were generally pale with saturation being on average 23.61 ± 18.05 % (median: 18.00%, range: 2–65%), but they encompassed the entire greyscale, with brightness ranging 13–100% (median: 70%, mean: 61.61%).

Morphometrics

A subset of 71 HY birds were included in the morphometric analysis, after excluding 14 specimens with missing values for certain measurements (e.g., some birds lacked a culmen length due to having broken beaks). The PCA yielded two clear axes, which together captured 58.4% of the variability in the dataset: while all the variables loaded positively onto the size axis (PC1, 39.5%), only weight and wing chord were strongly related to the body condition axis (PC 2, 18.9%) (Table 2.3, Figure 2.7). This interpretation is supported by the results of morphometric cross-correlations: wing length and mass were the most strongly correlated variables ($t = 3.599$, $df = 69$, $r = 0.398$, $p < 0.001$). Due to the non-normality of the plastic data, Spearman rank test were used to correlate plastic loads in a given bird to that specimen's coordinate in axes 1 and 2. None of the correlations between either component and plastic load (neither count nor mass) were significant.

A subset of 21 AHY birds were included in the morphometric analysis after excluding two specimens missing measurements. PC2 had the best relationship with bird weight and bill depth at base; PC1 correlated most strongly with the remaining morphometrics (Table 2.4, Figure 2.8). As a result, these two axes, which together accounted for 51.8% of the variance, captured gradients in specimen size (PC1, 27.6%) and body condition (PC2, 24.1%). This interpretation was supported by the morphometric cross-correlations: bill depth at base was significantly correlated with weight ($t = 3.02$, $df = 15$, $r = 0.615$, $p = 0.009$). To ensure the PCA results were not influenced by potential sexual size dimorphism, commonly represented in bill measurements in other petrels (Navarro et al. 2009), the morphometric measurements were compared between sexes using t-tests, but there were no significant differences (Table 2.5). Finally, four Spearman rank correlations between the plastic loads (count and mass) of individual specimens and their coordinates along the two PC axes were performed, and none were significant.

Organ Weight Analysis

Spleen Analysis

Spleen weights of 50 HY birds were collected averaging 0.1092 ± 0.1226 g (range: 0.0139–0.7770 g, median: 0.0677 g) for HY birds. Due to the non-normality of the spleen weights, Spearman rank correlations were used to relate spleen mass to seven morphometric variables, and only bird mass was significantly correlated ($S = 10029$, $\varrho = 0.518$, $p = 0.0001$). There were no significant relationships with plastic quantities. Spleen weights of 4 AHY birds were collected averaging 0.0473 ± 0.0282 g (max: 0.0849 g). No correlations were significant. Due to limited sample size, leading to violation of assumptions, PCA was not conducted for the AHY spleen weights.

Heart Analysis

Heart weights of 47 HY birds were collected averaging (3.3243 ± 1.1167)g (range: 0.3066–6.4601 g, median: 3.2718 g). HY heart weights were normally distributed, so Pearson correlation results were prioritized. Heart weight was significantly correlated with proventriculus plastic for both count ($t = 2.46$, $df = 45$, $p = 0.018$, $r = 0.345$) and mass ($t = 2.69$, $df = 45$, $r = 0.372$, $p = 0.010$). Further exploration yielded a significant correlation between bird mass and heart weight ($t = 3.474$, $df = 45$, , $r = 0.460$, $p = 0.001$). Since heart mass showed a relationship between bird mass and proventriculus plastic load, a correlation was run between the two to see if there is a relationship. Neither plastic count ($S = 13876$, $\varrho = 0.198$, $p = 0.183$) nor mass ($S = 13997$, $\varrho = 0.191$, p-value = 0.199) had a relationship with bird mass. Heart weights of 4 AHY birds were collected averaging (3.2697 ± 0.6296)g (max: 4.0828 g). No correlations with plastic were significant.

Organ PCA

39 HY birds were analyzed in the PCA. Principal component 2 had the best relationship with bird mass, spleen weight, and heart weight; PC 1 correlated best with the remaining morphometrics (Table 2.6, Figure 2.9). As a result, PC 2 represents body condition, and PC 1 bird size, representing 47.2% of variance (PC1 28.3%, PC2 18.9%). Similar to the morphometric analysis, PC 1 represented bird size, and PC 2 represented body condition of the bird. The direction of the arrows representing the organ weights implies the sizes of the organs are tied to the body condition of the bird. Accounting for the differences in organ weight based on bird health, there were no significant relationships between plastic loads and the body condition axis.

DISCUSSION

Comparison of Previous NESH Plastic Literature

The goals of this study were to update plastic ingestion occurrence and loads (mass and number) in the Newell's shearwater (*Puffinus newelli*), building upon the efforts of Sileo et al. (1990) and Kain et al. (2016). HY incidence (24%) was intermediate to those of Sileo et al. (1990) (11%) and Kain et al. (2016) (50%). As a result, two separate analyses were conducted, one for each study. Plastic incidence in our 2013-2023 sample was two-fold higher compared to the results of Sileo et al. (1990), who analyzed 18 birds in 1987. However, even though the incidence differed, the results of the χ^2 test with Sileo et al. (1990) was not significant ($\chi^2 = 1.33$, df = 1, p = 0.248). In contrast, we documented a lower plastic ingestion rate than the one documented by Kain et al. (2016), who analyzed 30 birds from 2007 to 2013 with a 50% incidence. The results of the χ^2 test was not significant ($\chi^2 = 1.33$, df = 1, p = 0.248), once again suggesting that there has been no change in the number of birds ingesting plastic. This is contradictory to the global trend found in other seabird MPD studies which document increasing plastic incidence interspecially (more species are eating plastic) and intraspecially (more birds in a species are eating plastic) as time passes (Robards et al. 1995; Spear et al. 1995; Wilcox et al. 2015). Although, the one-sample Wilcoxon tests (count: V = 293, p = 2.68e-12; mass: V = 90, p-value = 2.025e-15) were both significant, suggesting that while plastic incidence has remained essentially constant over time, the quantity of plastic ingested is changing over time. Since this study found higher plastic loads in HY NESH than Kain et al. (2016), plastic loads are likely increasing over time, a trend previously documented in the literature (Robards et al. 1995). However, this result (higher plastic loads compared to Kain et al. (2016)) is complicated by the fact that Kain et al. (2016) sampled from weak La Niña and neutral ENSO years, which we found to cause lower plastic ingestion rates in NESH. The small ENSO range could lead to an underestimate of how much plastic seabirds are ingesting for the average year.

Additionally, this was the first study to document plastic ingestion in AHY NESH (Sp-FO: 17%). Despite the difference frequencies of plastic occurrence between age classes, this was not a significant difference based on a χ^2 test for independence for incidence ($\chi^2 = 0.499$, df = 1, p = 0.48), count (t = -0.868, df = 105, p-value = 0.387), or mass (t = -1.07, df = 105, p-value = 0.285), suggesting that we can observe plastic trends in NESH irrespective of the age class being sampled. Although not done for this study, future studies could combine AHY and HY into one larger sample size, yet a χ^2 test for independence should be run each time to ensure that one age class does not begin to deviate from the other as a general precaution. AHY and HY age classes tend to have different plastic ingestion rates and loads (Kühn and van Franeker 2020), although this difference may not be statistically significant, as was the case in this study.

Debris Characterization

The range of colors of plastic (represented by HSB values) are consistent with other studies investigating MPD ingested by seabirds (Donnelly-Greenan et al. 2018), the colors observed in NESH previously (Kain et al. 2016), and the colors of MPD found in the open ocean (Martí et al. 2020). The roundness (0.243–0.936) and aspect ratio (1.068–4.113) of the ingested plastic items are similar to those found in the open ocean (0.11–1.0 and 1.02–7.60, respectively) (Serranti et al. 2018). Additionally, polymers in the ocean primarily consist of polyethylene (73%) and polypropylene (21%), consistent to the polymer composition of the ingested items found in NESH stomachs. All of these suggest that the plastic ingested by both AHY and HY comes from the ocean, likely obtained by foraging AHY and subsequently transferred to HY birds at the colony (Carey et al. 2011; Rodríguez et al. 2012).

While not as prevalent, ingestion of glass by seabirds is not uncommon, having been documented in various species from multiple taxonomic groups (Roman et al. 2016) and representing the second most common anthropogenic material ingested some opportunistic species, like the kelp gull (*Larus dominicanus*) (Lenzi et al. 2016).

Generalized Linear Model Outcomes

The results were quite inconclusive, with few models performing well (Sup. Tables 2.1-2.4), likely due to the relatively low frequency of plastic items in NESH, although no models were found to have zero-inflation. Despite none of the models having zero-inflation, there were no clear best models. Thus, determining the drivers of plastic ingestion lies on the scaled parameter weights.

HY models with squid beaks as the predictor often performed better than the average model. In other words, birds with more squid beaks tended to have more plastic, although there often was no significant relationship between squid beaks and plastic loads (Sup. Tables 2.1 & 2.2). Kain et al. (2016). Kain et al. (2016) hypothesized that NESH had relatively higher quantities of line due to transfer from its primary prey species, the purple-back flying squid (*Sthenoteuthis oualaniensis*) (Ainely et al. 2014). Kain et al. (2016) stated that since purple-back flying squid ingest myctophids, which ingest plastic, they potentially transfer this material up the food chain to NESH. Although purple-back flying squid have yet to be documented ingesting plastic, the orange-back flying squid (*Sthenoteuthis pteropus*), another species in the same genus which also ingests myctophids, was recently was found to ingest MPD (Sambolino et al 2023). These two species are comparable in size (550-650 mm) and foraging range (Jereb & Roper

2010), so there is potential for NESH to ingest plastic via their Ommastrephidae prey. However, a plastic ingestion analysis of purple-back flying squid is required for definitive answers.

AHY mass models with year as a parameter always performed better than the average model, and models with MEI as a parameter often performed better. In fact, the negative coefficients estimates suggest that there was a negative influence of time, with plastic loads decreasing over time, and plastic loads were lower for El Niño sampling periods than La Niña. However, these conclusions should be taken with caution, as AHY yearly sample sizes were very small (n=1-3) for most years. As a result, this AHY sample size is not ideal for noting trends over time. Ideally, yearly sample sizes should be 20-40 individuals to best represent trends (van Franeker & Meijborn 2002; National Research Council 2009; Savoca et al. 2022). However, due to the small population of NESH (Ainley et al. 2001; Raine et al. 2017), this sample size is unfeasible. Fortunately, new methodologies allow for the testing of live birds for plastic ingestion, such as preen oil GC-MS (Hardesty et al. 2015; Yamashita et al. 2021).

MEI was another influential parameter, yet the same issue arises. AHY MEI values ranged -2.2 to 0.5 with no sampling during strong El Niño events. As a result, MEI models are skewed towards La Niña, so the influence of MEI should also be approached with caution until a sample size with a better representation of ENSO phases is available. Although, HY MEI values ranged from -1.4 to 2.1, better capturing different ENSO phases and strengths, and MEI was only an influential parameter for total plastic mass. Therefore, MEI likely does not seem to have a strong influence on MPD ingestion by NESH outside of (potentially) influencing the availability of their squid and flying fish prey.

Morphometrics and Organ Results

Since there were no significant correlations between plastic loads either principal component, there is no relationship between size of the bird or body condition (irrespective of size) and the plastic loads in the bird. Similarly, after subsetting for the individuals with spleen data, there was no relationship between spleen mass and size of the bird or body condition.

Investigation into potential impacts of MPD ingestion in NESH involved correlations between plastic loads and organ mass. Namely, the mass of the heart and spleen, have correlated with ingested MPD quantities (Rapp 2005; Chamberlain 2019). Even though there were no significant relationships between spleen weight and plastic load, the heart PCA results tell a very different story. Since PCA depicted a relationship between heart size and body condition (rather than simply bird size), NESH hearts are larger in healthier birds. Combined with the significant relationship with plastic load in the stomach as well as the nonsignificant relationship between plastic load and bird mass, this PCA result implies that there may be a cause-and-effect of heart mass with health and size of the bird, amplified by plastic ingestion.

A similar result was documented in Laysan albatross chicks ($p = 0.021$, $r = 0.421$) with plastic load in the gizzard (Rapp 2005). While in this study there was no relationship with gizzard plastic load, the connection between ingested MPD and heart size is a point of concern needing future research into the possible implications and causes. Potentially, ingesting MPD causes strain on the body causing the heart to pump harder, and therefore increase in mass. Additionally, ingested MPD alters the blood chemistry of seabirds. Lavers et al. (2019) documented variations in blood calcium, blood cholesterol, amylase concentration, and uric acid concentration with ingested MPD. The reduced blood calcium was attributed to poor fat reserves —as cholesterol and adipose tissue have a direct relationship in seabirds (Newman et al. 1997)— combined with exposure to plastic additives. High cholesterol occurs during periods of stress, including starvation and periods of fasting, but the levels reported by Lavers et al. (2019) are much higher than those in fasting individuals implicating MPD as the stressor. These alterations in blood chemistry can put strain on the heart, which may increase in size in response.

Although the consequences of the relationship between heart and MPD are still under investigation, documenting these correlations is informative, especially in such a critically endangered species. When we eventually identify the effects and consequences of enlarged hearts and spleens, we will have baseline data for NESH.

Overview and Implications

The NESH population has decreased by 30-60% from 1974 to 1994 (Ainley et al. 2001) and 94% between 1993 and 2013, with a 13% average annual decline (Raine et al. 2017). This decline is attributed to invasive, mammalian predators and habitat loss to development (Ainley et al. 2001). While these terrestrial threats are extremely important to monitor and mitigate, this critically endangered species is also susceptible to impacts from plastic ingestion and other pollutants. In addition to long-term impacts on long-living individuals, MPD can potentially scale up to population-level impacts, especially when facing declining population sizes (Senko et al. 2020). Therefore, plastic ingestion studies for critically endangered species are imperative. However, since NESH are rare and critically endangered, statistically robust studies of exposure, impacts, and trends over time are constrained by small sample sizes (van Franeker and Meijborn 2002; National Research Council 2009; Kain et al. 2016). To date, there are only two published studies reporting NESH ingestion rates dating back to the 1980s (Sileo et al. 1990) and 2010s (Kain et al. 2016). However, limited sample sizes do not excuse the lack of understanding of impacts. Rather, limited sample sizes should promote the development of novel approached for the in-depth investigation of the few specimens available.

USFWS acknowledges the need for protection and conservation efforts of NESH, but fails to recognize that NESH ingest plastic much less the potential health and population impacts of MPD ingestion (USFWS 1983; USFWS 2019). While terrestrial threats, such as invasive mammalian predators and habitat loss, are the main causes for the population decline (Ainley et al. 2001), all anthropogenic impacts on this critically endangered species should be considered, especially when MPD ingestion affects many other seabird species and appears to be worsening (Wilcox et al. 2015; Kühn & van Franeker 2020). Thus, it is my hope that this study will motivate an update in the UFSWS recovery plan for the Newell's shearwater, to address MPD ingestion, its sublethal impacts, and the investigation of the potential for biomagnification via consumption of purple-back flying squid and flyingfish.

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Table 2.1. NESH Specimens (2013-2023). HY refers to hatch-year birds (chicks and fledglings) and AHY refers to after hatch-year birds (pre-breeding and breeding adults).

	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	Total
HY	3	1	12	2	15	12	8	12	9	0	10	85
AHY	1	2	9	4	2	1	3	1	0	0	0	23
Total	4	3	21	6	17	13	11	13	9	0	10	108

Table 2.2. Spearman rank (ϱ) correlation results of plastic count vs mass.

		S	ϱ	p-value
HY	**Proventriculus**	102.8	0.9990	<2.2e-6
	Ventriculus	1818	0.9816	<2.2e-6
	Bird as a Whole	2348	0.9762	<2.2e-6
AHY	**Proventriculus**	0	1	<2.2e-6
	Ventriculus	1.459	0.9993	<2.2e-6
	Whole Bird	4.584	0.9977	<2.2e-6

Table 2.3. HY morphometric principle component PCA contributions. Strongest contributor for each component is bolded.

	PC 1	PC 2
Culmen Length	**22.2**	1.98
Bill Depth	20.2	0.286
Bill Depth at Base	18.4	6.56
Head Length	19.7	3.68
Tarsus Length	11.6	0.573
Wing Length	3.96	46.4
Weight	3.80	**40.5**

Table 2.4. AHY morphometric principle component PCA contributions. Strongest contributor for each component is bolded.

	PC 1	PC 2
Culmen Length	0.388	12.2
Bill Depth	**33.2**	6.56
Bill Depth at Base	28.7	17.2
Head Length	12.7	3.46
Tarsus Length	5.17	19.0
Wing Length	11.2	8.79
Weight	8.68	**32.8**

Table 2.5. AHY morphometric comparison between sexes (males n = 8, females n = 13). SDI: Storer's sexual dimorphism index=100×(male–female)/[(male+female)×0.5]. Lengths in mm and weight in g

	Males	Females	SDI	Test Statistic	df	p-value
Culmen	33.9 ± 1.26	33.4 ± 1.35	1.73	-1.00	15	0.330
Bill Depth	7.71 ± 0.698	8.11 ± 0.482	-5.00	1.41	15	0.186
BIll Depth at Base	11.7 ± 0.96	11.5 ± 1.11	1.76	-0.447	15	0.661
Head	78.3 ± 3.61	78.6 ± 1.64	-0.50	0.286	15	0.781
Tarsus	45.5 ± 1.19	46.6 ± 1.52	-2.34	1.81	15	0.087
Wing	228 ± 37.7	244 ± 8.25	-6.59	1.15	15	0.286
Weight	352 ± 64.5	356 ± 61.8	-1.15	0.142	15	0.889

Table 2.6. HY organ principle component PCA contributions. Strongest contributor for each component is bolded.

	PC 1	PC 2
Culmen Length	**26.5**	4.87
Bill Depth	11.5	2.25
Bill Depth at Base	11.8	9.55
Head Length	23.5	4.64
Tarsus Length	9.03	2.11
Wing Length	8.58	0.101
Bird Mass	6.40	**29.3**
Spleen Weight	0.145	18.0
Heart Weight	2.54	29.2

Figure 2.1. Plastic loads of HY NESH across time in terms of number of items. Color scale represent MEI.v2 values. Black line represents a loess curve trend of plastic ingestion, due to the nonlinear nature of the results.

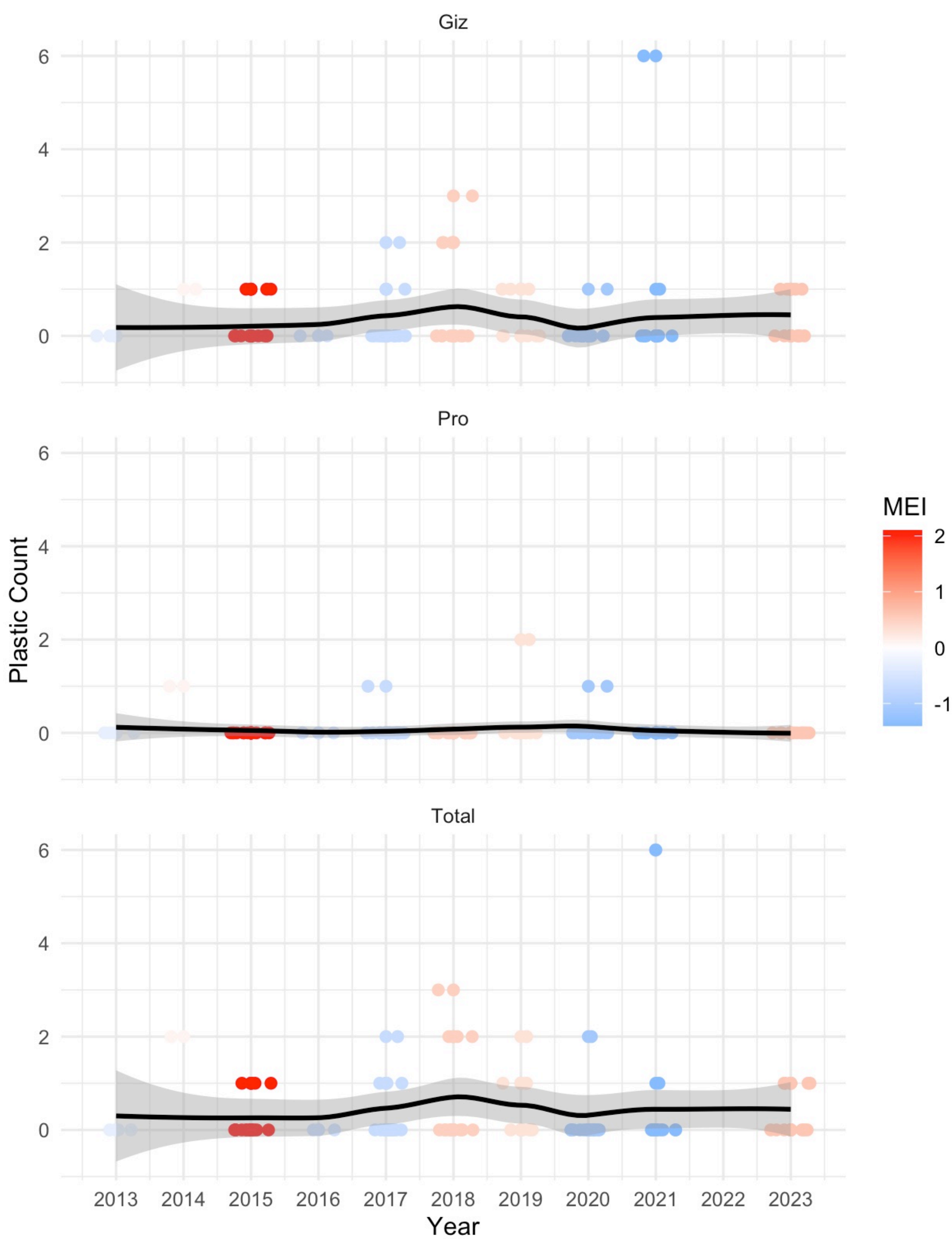


Figure 2.2. Plastic loads of HY NESH across time in terms of mass of items. Color scale represent MEI.v2 values. Black line represents a loess curve trend of plastic ingestion, due to the nonlinear nature of the results.

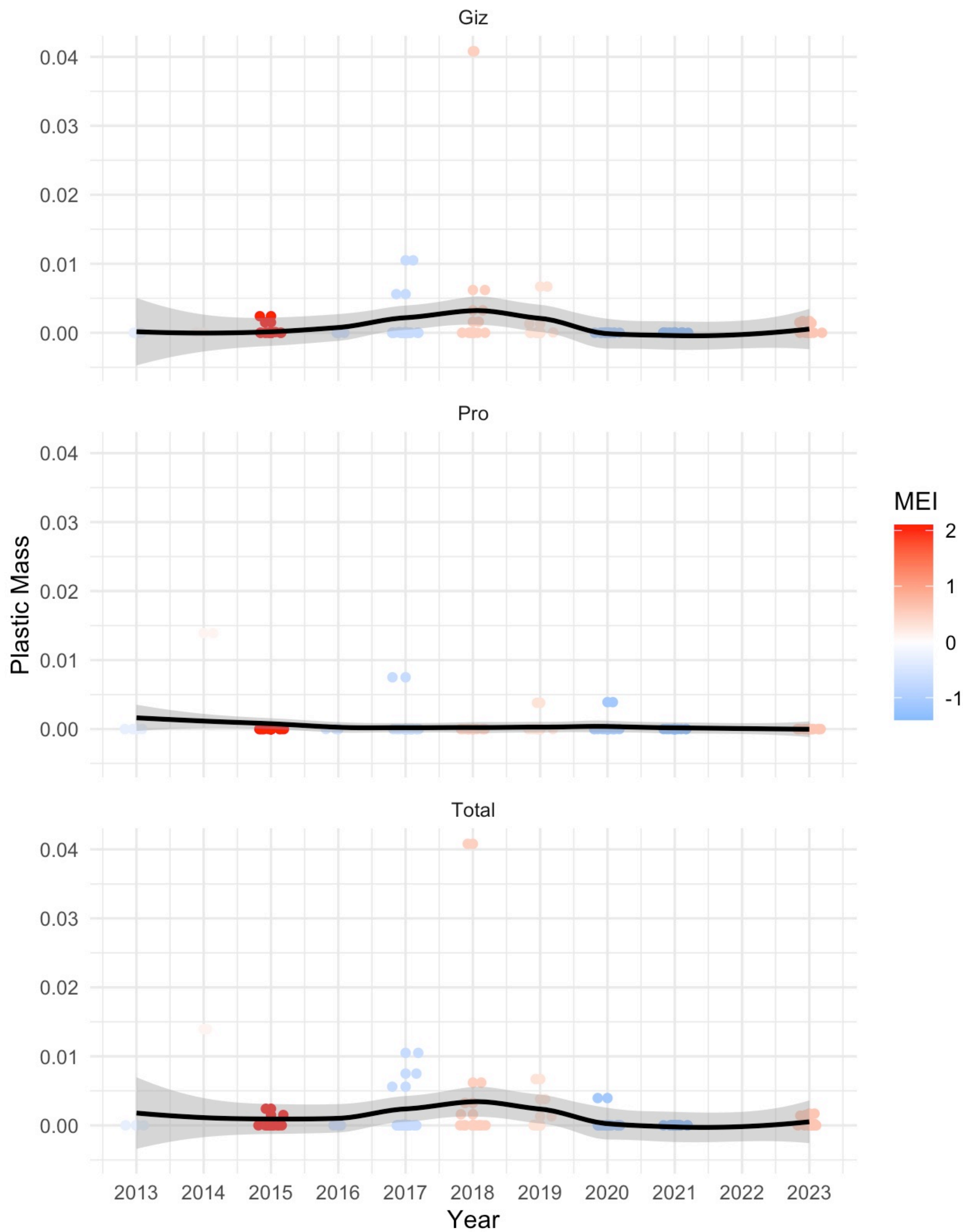


Figure 2.3. Plastic loads of AHY NESH across time in terms of number of items. Color scale represent MEI.v2 values. Black line represents a loess curve trend of plastic ingestion, due to the nonlinear nature of the results.

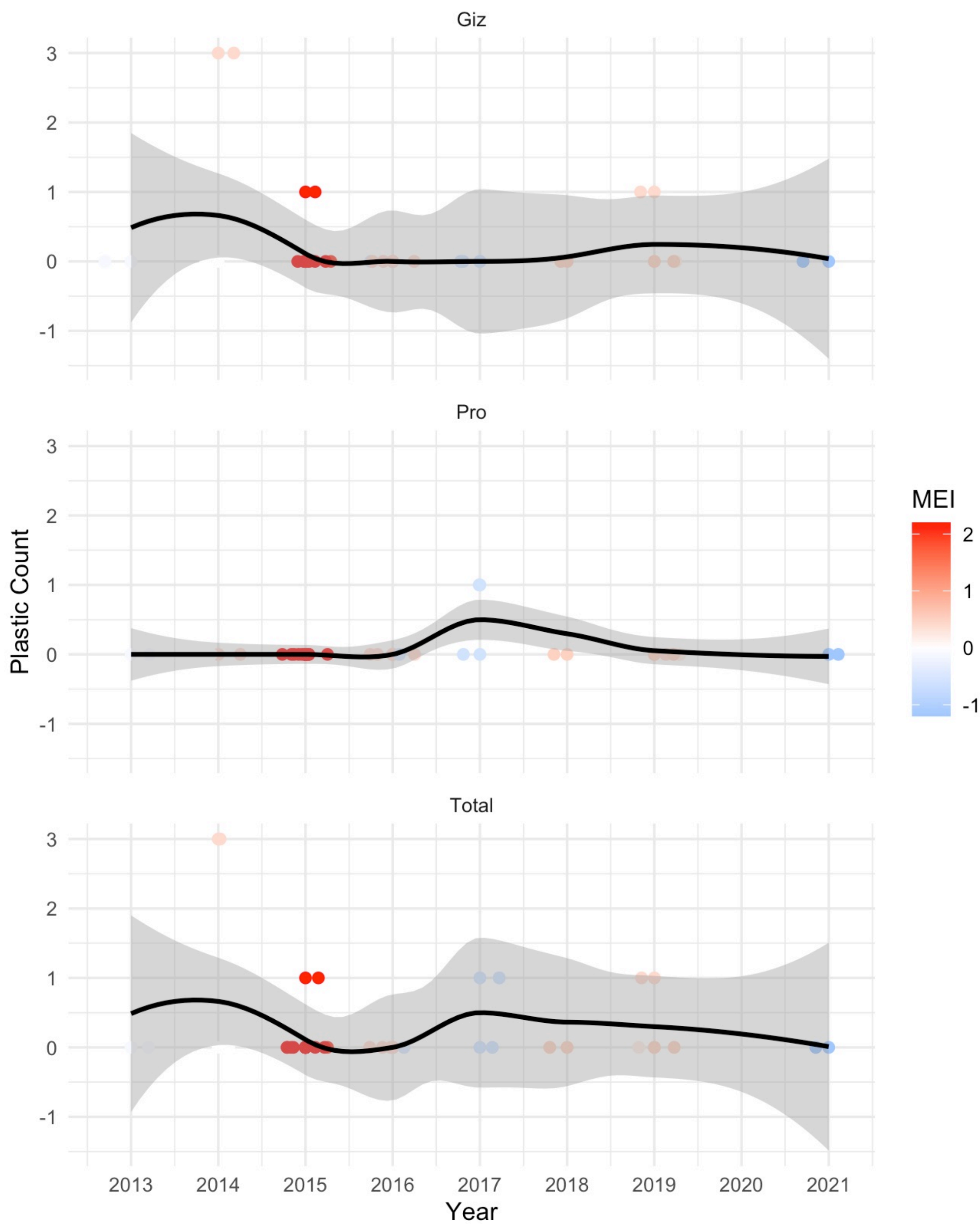


Figure 2.4. Plastic loads of AHY NESH across time in terms of mass of items. Color scale represent MEI.v2 values. Black line represents a loess curve trend of plastic ingestion, due to the nonlinear nature of the results.

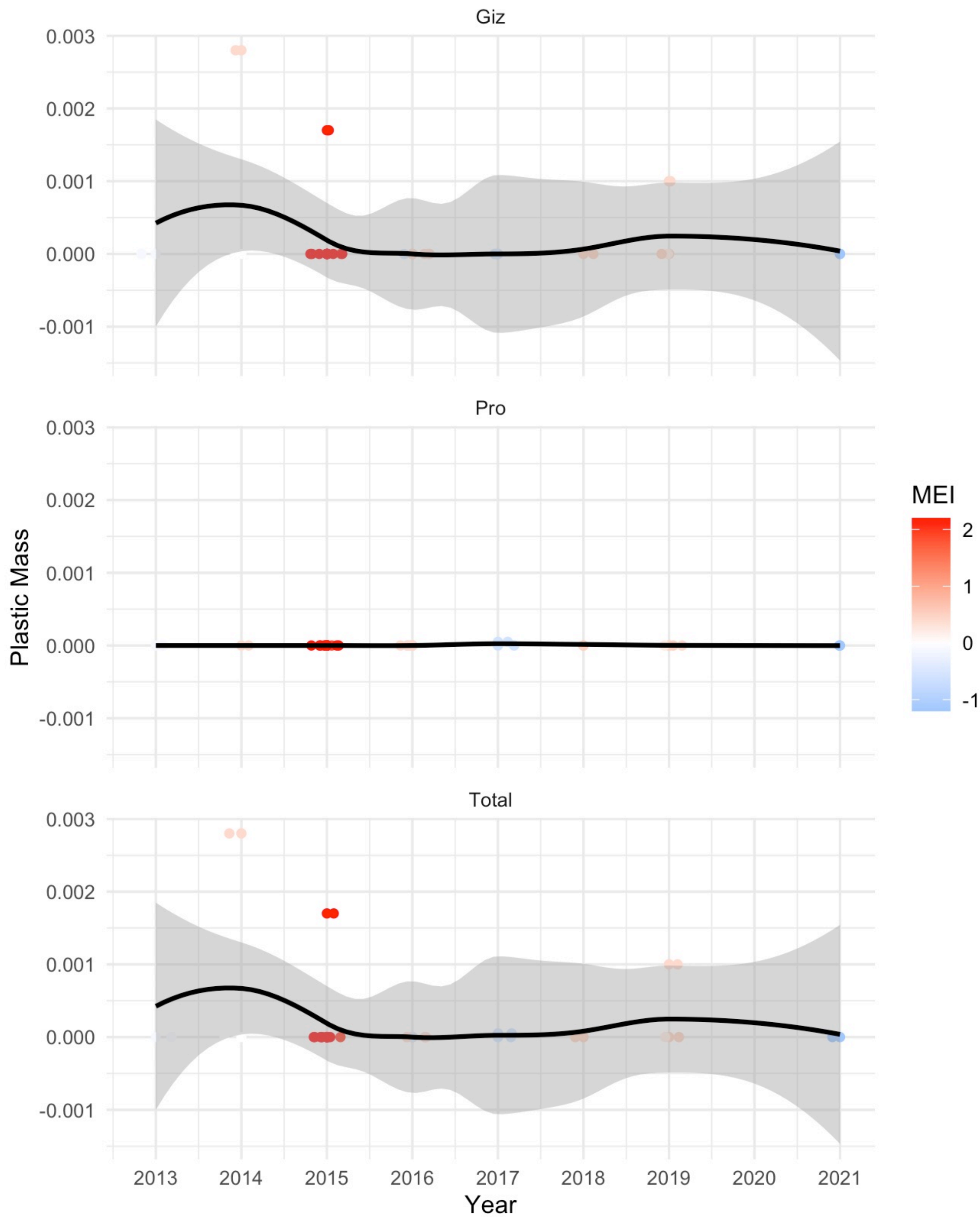


Figure 2.5. Predicted HY plastic counts based on temporal trends alone.

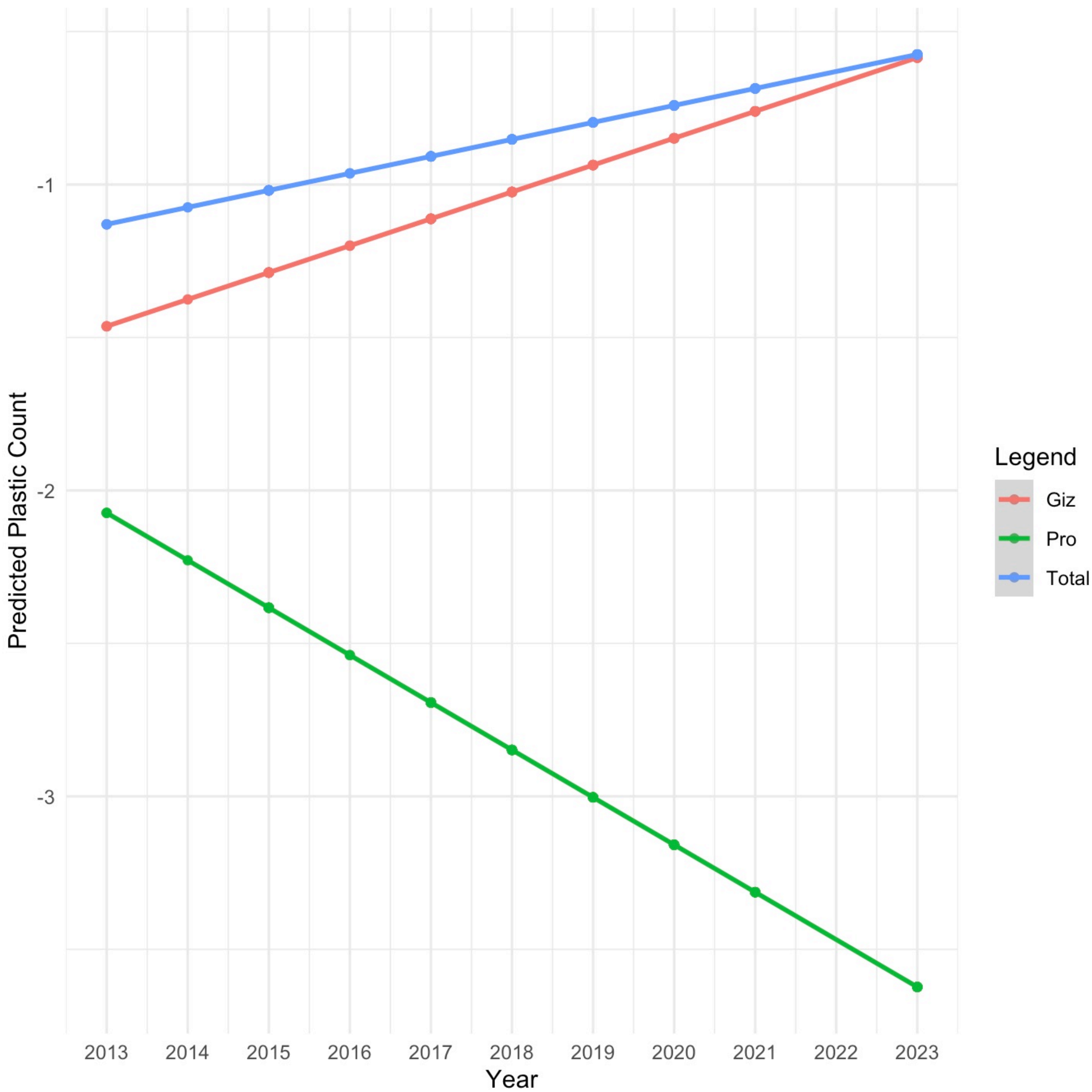


Figure 2.6. Predicted HY plastic masses based on temporal trends alone.

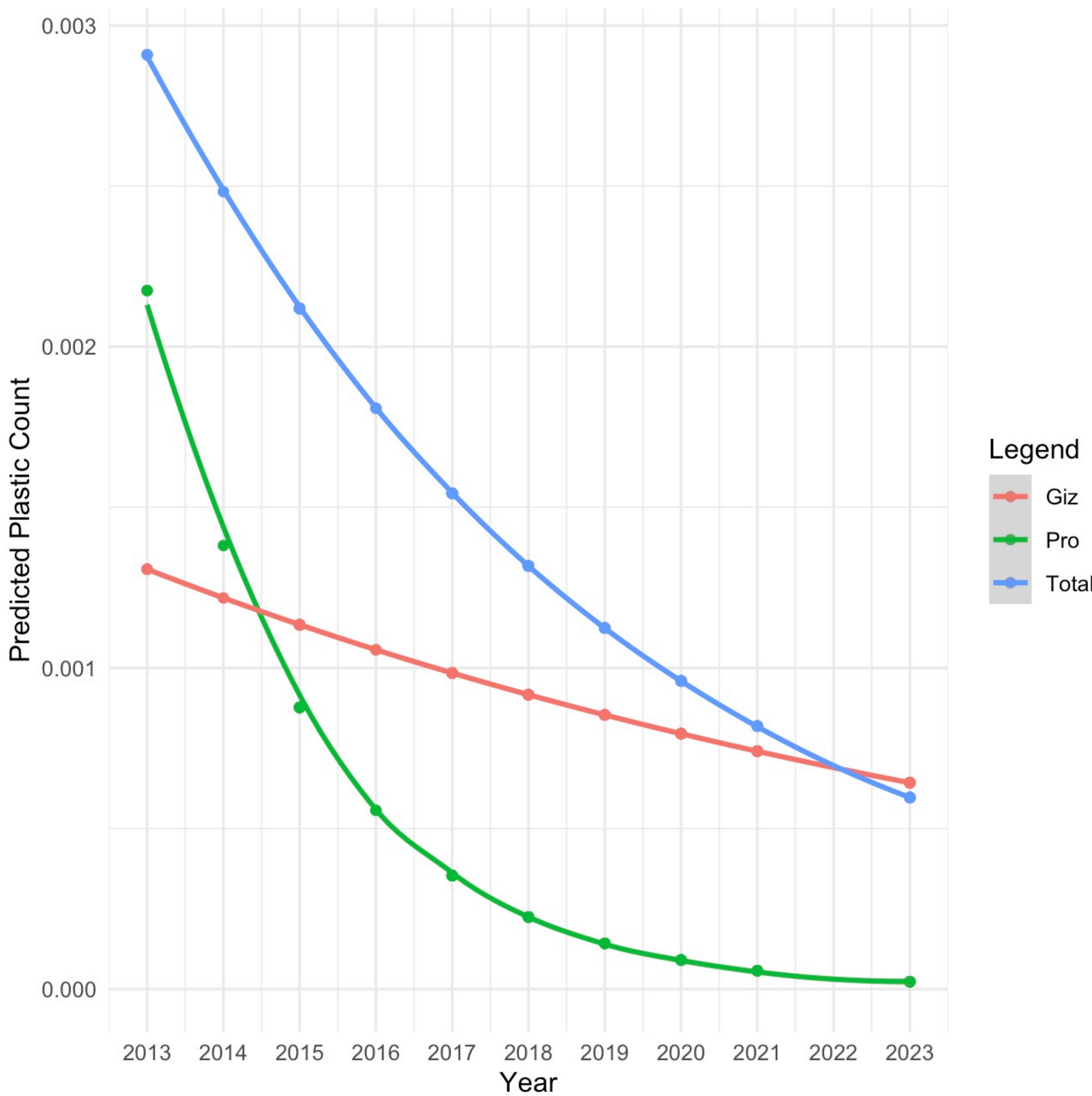


Figure 2.7. HY NESH Morphometric PCA.

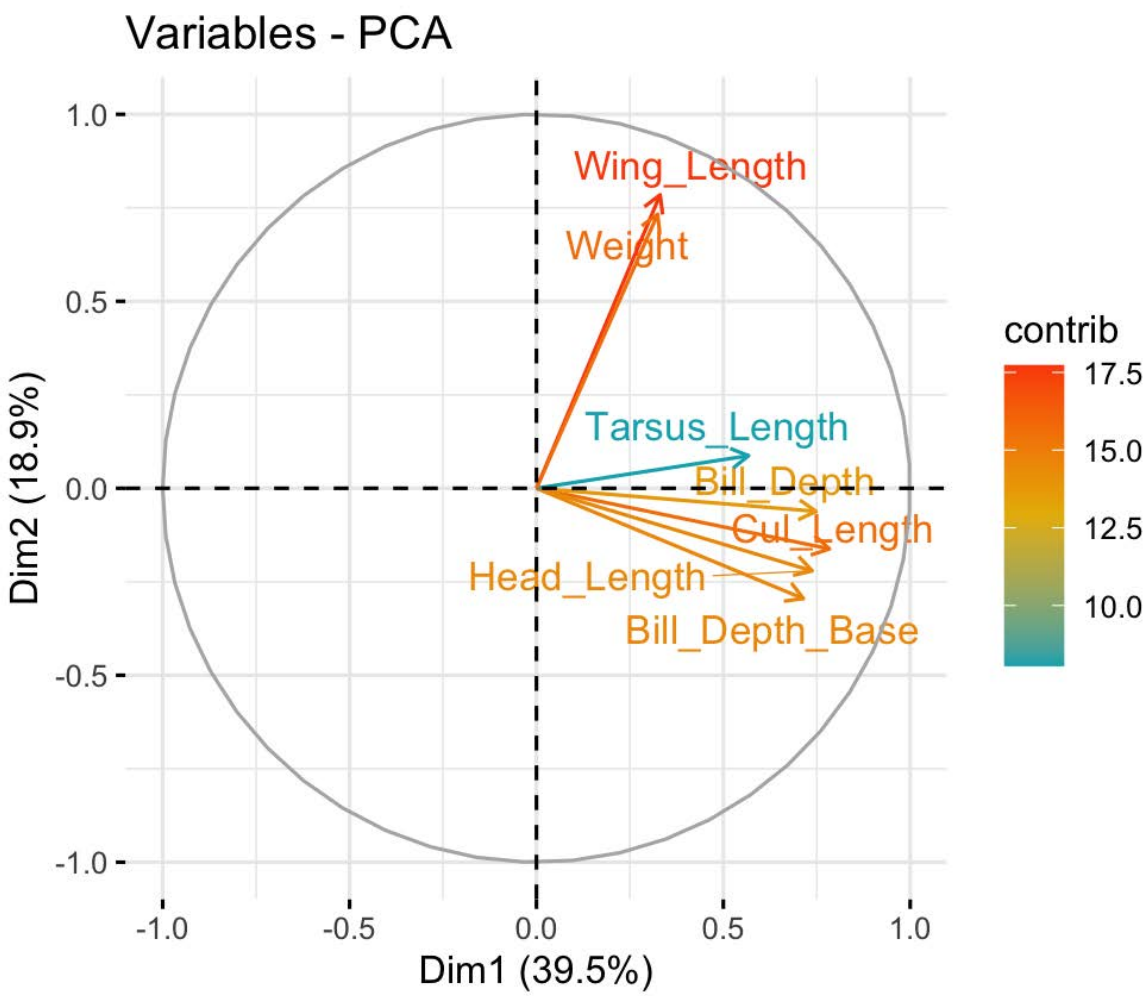


Figure 2.8. AHY NESH Morphometric PCA.

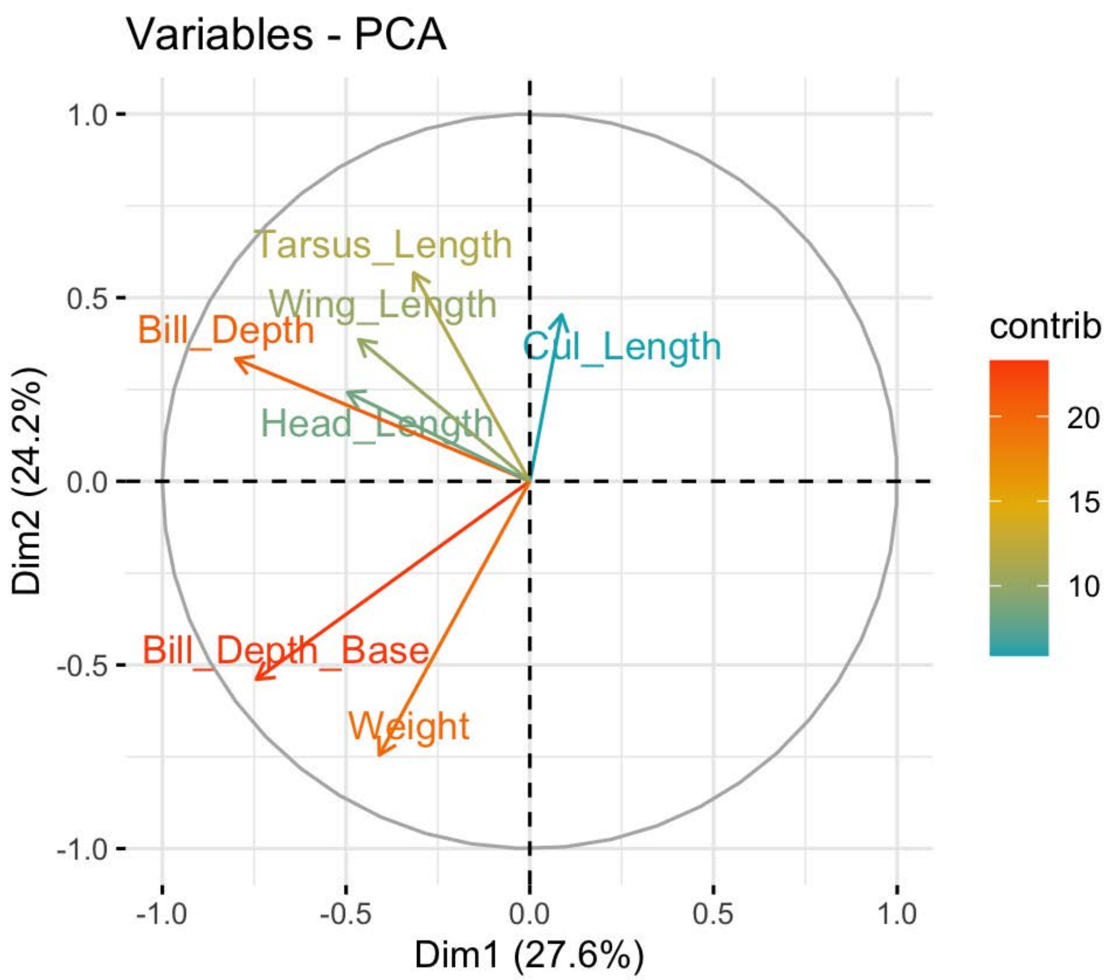
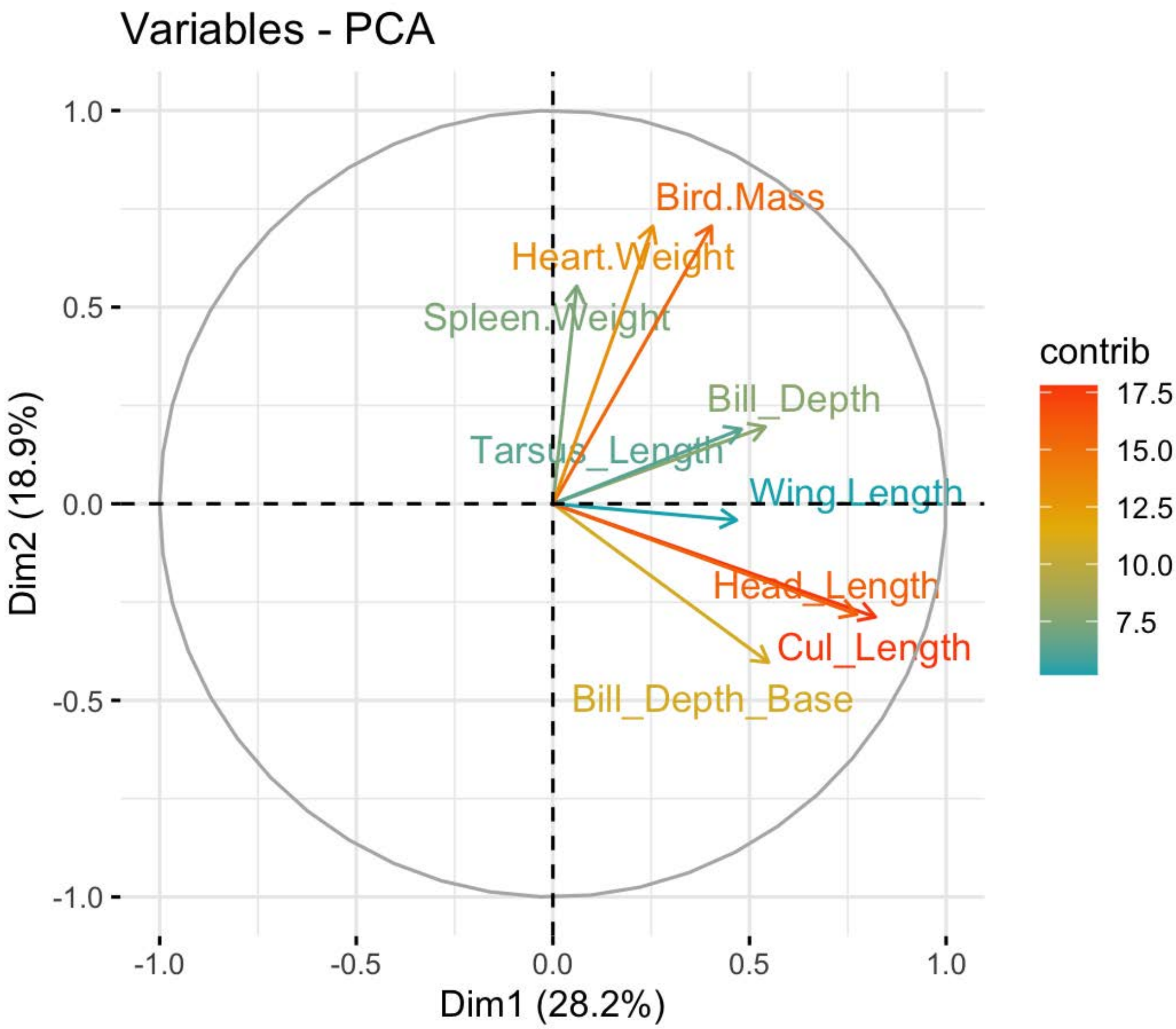


Figure 2.9. HY NESH Organ PCA.



CHAPTER 3: ANALYZING THE BIOINDICATOR POTENTIAL OF THE WEDGE-TAILED SHEARWATER (*ARDENNA PACIFICA*) FOR THE MAIN HAWAIIAN ISLANDS

INTRODUCTION

Marine plastic debris (MPD) is a pervasive pollutant found throughout the global ocean (Cózar et al. 2014; Eriksen et al. 2023). Due to the wide range of plastic pollution in the ocean, hundreds of marine species are impacted via entanglement (n = 354), ingestion (n = 701) or both (n = 914, Kühn and van Franeker 2020). Entanglement is a more visible interaction, but ingestion is a more prolific issue. For instance, 39.8% of marine mammal and 27.4% of seabird species worldwide suffer from entanglement, whereas 56.1% and 44.0% (resp.) suffer from MPD ingestion (Kühn & van Franeker 2022). Furthermore, while the impacts of entanglement are direct and clearer (ie: drowning, loss of limbs, etc.), the impacts of ingestion of marine debris are currently under investigation. While reduction of entanglement incidences are valuable to the survival of marine fauna, ingestion can lead to more chronic, life-long health effects (ie: plasticosis, reduction of fat stores, altered blood chemistry, etc.) and therefore are more pertinent to study, especially concerning long lived marine species.

Due to the prevalence of MPD around the ocean, and the heavy impacts on marine fauna, researchers regularly develop bioindicator species for specific regions of the ocean (Savoca et al. 2022). In the North Pacific, Savoca et al. (2022) completed an extensive study aiming to identify key bioindicator species using a scoring system ranking exposure, sampling availability, distribution, and frequency of plastic ingestion. The seabird species that scored highest were (in decreasing order): the northern fulmar (*Fulmarus glacialis*); the Leach’s storm petrel

(*Oceanodroma leucorhoa*); the Laysan albatross; and the black-footed albatross (*Phoebastria nigripes*). All four seabirds belong to the group Procellariiformes, which is consistently identified as the most MPD impacted seabird group due to their vast foraging ranges, broad diets, and unique stomach anatomy (Ryan 1987a; Ito et al. 2013; Rapp et al. 2017; Terepocki et al. 2017; Thiel et al. 2018; Roman et al. 2019; Kühn and van Franeker 2020). The top rankings of these four tubenose species highlights the utility of Procellariiformes as basin-wide, North Pacific bioindicators for the temperature and sub polar waters in the PICES (North Pacific Marine Science Organization) region of interest (≥30˚N in the Pacific Ocean).

While thorough, the PICES indicator species were limited to a specific geographic area, therefore its applicability to the entirety of the North Pacific is constrained. That is, species with smaller foraging ranges received smaller scores or species that rarely forage in the PICES region received smaller bioindicator scores regardless of their potential for representing the region where they forage. As a result, the PICES focal species could be augmented to develop smaller scale bioindicators. For instance, the Bonin petrel (*Pterodroma hypoleuca*) was recommended as a bioindicator species for the Northwestern Hawaiian Islands (Chamberlain 2019), but it received a score of 20/24 as a PICES bioindicator potential (Savoca et al. 2022). The Bonin petrel received perfect scores for sampling availability and plastic frequency of occurrence, but lost points due to their limited distribution. Despite not being an ideal PICES bioindicator species, this score suggests that the Bonin Petrel could be a valuable indicator of plastic within its small foraging range. This concept could be expanded on in the Main Hawaiian Islands (MHI), where a formal bioindicator species has yet to be identified. Hawai'i would benefit greatly from a bioindicator species for plastic pollution due to its close proximity to MPD aggregations at the subtropical convergence and the North Pacific Subtropical Gyre (Howell et al. 2012). The wedge-tailed shearwater (*Ardenna pacifica,* WTSH) is an abundant native species with high potential utility as a bioindicator in the MHI (Whittow 1997). WTSH received a score of 16/24 for the PICES region, only losing points for lack of foraging overlap with the PICES region, like the Bonin petrel (Savoca et al. 2022). Moreover, the abundance and accessibility of WTSH across the MHI and their restricted foraging range during the breeding season make this species the strongest region MPD bioindicator species for this archipelago.

Not only can the WTSH be a bioindicator species for plastic pollution around the MHI, but also a bioindicator species for less populous species, such as the Newell's shearwater (*Puffinus newelli*, NESH). NESH is a critically endangered tubenose species endemic to the Hawaiian Archipelago, with 90% of the population breeding in inaccessible mountainous terrain on Kaua'i (U.S. Fish and Wildlife Service 2019). NESH is part of the tuna-bird foraging guild: diving down to 50 m in pursuit of prey and primarily feeding on flying squid (Ommastrephidae) and flying fish (Exocoetidae) (Ainley et al. 2014; Kain et al. 2016) in association with skipjack tuna (*Katsuwonus pelamis)* (Hebshi et al. 2008). The NESH population has decreased by 30-60% decline from 1974 to 1994 (Ainley et al. 2001) and 94% between 1993 and 2013 with a 13% average annual decline (Raine et al. 2017). Majority of specimens in MPD studies for the NESH come from fallout victims, since 90% of the population breeds in inaccessible mountainous terrain on Kaua'i (USFWS 1983; USFWS 2019). Because it is very difficult to monitor NESH, researchers are seeking equivalent bioindicator species as proxies for studying NESH foraging ecology and plastic ingestion (Raine et al. 2017; Young et al. 2019). In a similar situation, Suryan & Kuletz (2018) discuss the potential for the black-footed albatross (*Phoebastria nigripes*) as a bioindicator for the critically endangered short-tailed albatross (*Phoebastria. albatrus*).

WTSH are an ideal NESH proxy for a comparative study, since they experience the same anthropogenic stressors: invasive predators, artificial light attraction, and collisions with anthropogenic structures (VanderWerf et al. 2014; Friswold et al. 2020; Hyrenbach et al. 2022). WTSH also ingest MPD (Fry et al. 1987; Kain et al. 2016) and forage alongside skipjack tuna and pursue prey under water at 14 m on average, reaching depths up to 66 m (Burger 2001; Hebshi et al. 2008; Hyrenbach et al. 2014). Since WTSH are more abundant than NESH and have very similar niches and foraging ecologies, I contend that WTSH can be used as a proxy to study the potential drivers of MPD ingestion in the closely-related NESH. Plastic ingestion rates in WTSH have been compared to those of NESH (Kain et al. 2016), but the potential of WTSH as a bioindicator for NESH has yet to be thoroughly analyzed. This study will conduct a statistical analysis of MPD drivers in both NESH and WTSH. If WTSH are an appropriate bioindicator species for NESH, then the two species will have the same statistical drivers of MPD ingestion (regardless of physical MPD loads). If this pattern is found, then WTSH can be used to represent the MPD around the MHI.

METHODS

Specimen Collection

Naturally-deceased NESH were provided by Save Our Shearwaters, a non-profit organization located on Kaua’i. WTSH were provided by Feather and Fur, a local seabird hospital, and Sea Life Park, a local seabird rehabilitation center, both on the windward shore of O’ahu. All individuals of both species were victims of fallout due to attraction to artificial lights and collisions with obstacles. Some were found dead on arrival, others died in care of unknown causes, and the remaining individuals were humanely euthanized. The specimens, were stored frozen at -20˚C until they were necropsied following standardized procedures previously modified for Hawaiian seabirds (van Franeker 2004; Rapp et al. 2017).

Due to the opportunistic nature of our sampling, sample sizes varied amongst years and between age groups. Based on recommended sample sizes of 40 and 20 birds (van Franeker and Meijborn 2002; National Research Council 2009), our target sample size would be a minimum of 440 specimens over the study period. However, we sampled 107 NESH, with at most 15 hatch year birds (HY, which includes chicks and fledglings) and 9 after-hatch year (AHY, which includes pre-breeding and breeding adults) in a year. Furthermore, we sampled 305 WTSH, with at most 25 HY and 18 AHY in a year.

Diet samples were taken from both the proventriculus (stomach) and ventriculus (hereafter gizzard) using standardized procedures (van Franeker 2004), as a significant difference in plastic incidence rates between these two chambers were previously observed in NESH and WTSH fledglings (Kain et al. 2016). The samples were stored in 70% ethanol until manual sorting. Diet samples were sorted into three broad categories: food items (squid beaks, fish eye lenses, fish bones, etc.), natural non-food items (plant matter, sand, pumice, endoparasitic worms), and non-natural, non-food items (plastic items, glass) using 10x magnification via a binocular dissecting microscope, congruent with other plastic-ingestion studies (van Franeker et al. 2011; Rapp et al. 2017). Following the same standardized protocols, plastic items were categorized into four types: fragments, line, foam, and sheet. In other words, each specimen had generated 8 mass measurements per chamber: 4 diet categories and 4 plastic types.

Each diet category and plastic type was weighed to a resolution of 0.0001 grams using a Mettler Toledo Scale, Model MS104S. Half of the minimum detection threshold (0.00005 g) was recorded when the scale yielded 0.0000, but there was a sample present. Since each sample provides two masses, the root mean squared error (RMSE) was calculated of the masses, which is the square-root of the sum of squares divided by the sample size ($[SS/n]^{1/2}$), to determine the variation between the two mass measurements across all samples (Armstrong & Collopy 1992; Rapp et al. 2017).

To ensure that all plastic items were collected and analyzed, all putative MD and unknown items in the diet were isolated, weighed, and analyzed with attenuated total reflectance Fourier transform infrared spectroscopy (ATR FT-IR), which identifies plastic compounds by matching well-known infrared absorption bands representing distinct chemical functionalities found in plastic with the item in question (Provencher et al. 2017; Jung et al. 2018). Because small and rigid plastic fragments can be crushed during scanning by the diamond tip of the instrument, each item was weighed individually before ATR FT-IR. This measure was to ensure that should an item not be plastic, it could be removed from the total plastic weight.

Attenuated Total Reflectance Infrared Spectroscopy Analysis

Using a Thermo Fisher Scientific Nicolet iS5 ATR-FTIR spectrometer, IR spectra were collected on all plastic samples and unknown items. Following the methods of Corniuk et al (2023), spectra were generated from 16 scans each with resolution at 4 cm^{-1} and a data interval of 1 cm^{-1}, and the section from 4000 cm^{-1} to 500 cm^{-1} were compared against the standardized polymer spectra provided in Jung et al. (2018). Two steps were taken to ensure maximum clarity of the spectra: (i) the samples and the instrument's diamond crystal were cleaned with a kim wipe and 70% isopropanol before each scan, and (ii) a background (control) scene was taken between each sample.

Statistical Analysis

All statistical analyses were performed with R using the commands and {packages} listed below. Gizzard and proventriculus samples from each bird were analyzed in parallel using various regressions to determine if the occurrence (presence/absence) and the quantities (number and mass of items) varied with respect to three driver variables: (i) squid beak count, (ii) ENSO phase (via the Multivariate El Niño Index, MEI.v2 value), and (iii) year.

Because the plastic count data followed a negative binomial distribution, as revealed by the descdist and fit.dist {fitdistrplus}. Several generalized linear models (GLMs) with multiple deriving factors were run via glm.nb {MASS} to determine the influence and interaction between two or more predictor variables. Since negative binomial distributions suffer from zero-inflation, all models were checked via check_zeroinflation {performance}. In regards to the plastic mass data, a tweedie distribution was used, and GLMs were run via cpglm {cplm} for the proventriculus and gizzard data and via glmmTMB {glmmTMB} for the whole bird and organ data. The switch from cpglm to glmmTMB for whole bird and organ data was to treat likely zero-inflation in the beak mass models of these two datasets.

Since there are so many models, the ability of each model to explain the observed variability was determined by ranking the model via the corrected Akaike information criterion (AICc). Due to the small sample size of individual birds collected each year, AICc was used to prevent overfitting by AIC due to small sample sizes (Hurvich & Ling 1989). The relative likelihood of each model was quantified by calculating the Akaike weight with model.avg {MuMIn} to prevent overfitting, with the “best” model(s) having a total weight ≥80% (Michael et al. 2014). The most important variables in the models were identified using a scaled variable weight to determine the influence each variable on the weights of the models. Then, the model weights were averaged to assess the importance of each parameter across all models (Michael et al. 2014). A value greater than 1.0 for a parameter means that the models including said parameter performed better than the average model. To represent the temporal trends accounting for the ENSO cycle, modeled plastic loads were generated by setting MEI to zero (thereby representing a neutral ENSO) and using the predict {stats} function in R. This modeling was only done for HY to align with NESH modeling.

Development of a Bioindicator

If WTSH can represent NESH in terms of plastic ingestion, both species should have the same statistical drivers. To quantify this comparison, paired Wilcoxon tests were run comparing the scaled parameter weights between the species (for each parameter) to determine if the weights can be treated as essentially the same. If the weights are essentially the same, then this would suggest that the species respond to the drivers the same. This analysis is mostly exploratory to see if development of a bioindicator species can be statistically quantified, rather than only subjectively compared. Additionally, to determine if there was a 1:1 relationship (or similar) between the NESH and WTSH scaled parameter weights, correlations were run per parameter (e.g. NESH weights for MEI were run against WTSH weights for MEI).

RESULTS

For NESH results please refer to chapter 2

Plastic Occurrence

Out of a total of 305 WTSH specimens sampled between 2013 and 2023, 217 (71.1%) were HY birds and 88 (28.9%) were designated as AHY (Table 3.1). Overall, 57.1% (124 of 217) of HY ingested MPD (pro: 29.5%, giz: 48.4%), and similarly 56.8% (50 of 88) of AHY ingested MPD (pro: 26.1%, giz: 50.0%). A $\chi 2$ test of presence/absence of plastic between HY and AHY was not significant ($\chi 2 = 0.0865$, df = 1, p = 0.769).

Plastic Loads

Plastic loads were quantified by bird and by stomach chamber, using two significantly cross-correlated metrics: the number of items and their mass. Plastic counts and masses were significantly correlated with each other (Table 3.2). HY WTSH (n = 217) contained on average 1.80 ± 2.46 pieces of plastic (median: 1, max: 15) (Figure 3.1) with an average weight of 0.0182 ± 0.0365 g of plastic (median: .0040 g, max: 0.2805 g) (Figure 3.2). HY with plastic (n = 124), contained on average 3.03 ± 2.54 pieces of plastic (median: 2, max: 15) with an average weight of 0.0306 ± .0432 g of plastic (median: 0.0167 g, max: 0.2805 g).

AHY WTSH (n = 88) contained on average 3.61 ± 6.34 pieces of plastic (median: 1, max: 32) with an average weight of 0.0693 ± 0.1664 g of plastic (median: 0.0038 g, max: 1.0136 g). AHY with plastic (n = 50) contained on average 6.36 ± 7.31 pieces of plastic (median: 3, max: 32) (Figure 3.3) with an average weight of 0.1220 ± 0.2063 g of plastic (median: 0.0443 g, max: 1.0136 g) (Figure 3.4). Plastic counts and mass significantly correlated with each other (Table 3.2), and plastic loads differed significantly between the proventriculus and gizzard for HY WTSH (W = 28654, p = 7.57e-6) and AHY WTSH (W = 4790, p = 1.83e-3). However, like with incidence, the differences in loads between AHY and HY were not significant for neither count

(W = 866, $p = 0.321$) nor mass (W = 10419, $p = 0.195$); when you consider only the individuals with plastic, the relationship was also not significant for count (W = 3806.5, p-value = 0.0552) but was significant for mass (W = 4328, $p = 3.932e-4$).

The RMSE of the weights of all plastic items was 0.2560796, and the high correlation between the replicate weights of the same plastic items (S = 272376, ϱ = 0.990, $p < 2.2e-16$) suggest that our measurements were highly precise.

Models of Ingested Plastic

Plastic load was calculated by bird (considering the content of each individual's proventriculus and gizzard together) and by stomach chamber, considering the proventriculus and gizzard separately, since plastic occurrence was higher in the gizzard (HY: 48.4%, AHY: 50.0%) than in the proventriculus (HY: 29.5%, AHY: 26.1%). Furthermore, this difference in loads was significant for both HY (W = 28654, $p = 7.57e-6$) and AHY (W = 4790, $p = 1.83e-3$). The two response variables (mass and number) were analyzed in four ways as a result: by bird as a whole, by proventriculus, by gizzard, and another series of models accounting for the differences between the organ chambers, causing a total of 8 results per age class. However, for simplicity, we will break down models based on (i) age class and (ii) response variable.

HY Modeling — by number

In regards to the WTSH count models, the results varied depending on the data analyzed (Sup. Table 3.1). For the proventriculus, the two models performing the best were plastic ~ beaks + year + MEI (54.1%) and plastic ~ beak + year (43.0%), with beak and year being influential parameters (1.75 and 1.70 respectively). In respect to the gizzard GLMs, there was no "best" model(s), but models with squid beaks performed better than the average model (parameter weight 1.73). Similarly, when looking at total plastic in the bird, there was no “best” model, and all parameters were influential with all having parameter weights >1. Accounting for variation between stomach chambers, the model with the largest weight was plastic ~ beaks + year + MEI + organ (72.2%), and as a result, all driving variables had parameter weights >1. The predicted plastic counts based on temporal influence alone indicated an increasing trend, implying that the number of ingested plastic items were increasing over the course of the study period (Figure 3.5).

HY Modeling — by mass

The WTSH mass models yielded very similar results (Sup. Table 3.2). For the proventriculus, the best model was plastic ~ beaks + MEI (89.9%), with beaks (1.75) and MEI (1.69) being quite influential. Similar to the count GLMs, the gizzard plastic mass GLMs weren’t insightful with no best performing model, especially because all parameters were influential: beaks (1.18), year (1.44) and MEI (1.41). However, when considering total plastic mass in the bird, there was a clear best model: plastic ~ beaks + year + MEI (100%) causing all parameters to have equal influence (weights of 1.75). Again, when accounting for the difference in plastic loads between the two stomach chambers, plastic ~ organ + year + beaks + MEI was clearly the best model (94.8%), which caused all scaled parameter weights to be >1. The predicted plastic masses based on temporal influence alone indicated an increasing trend as well, implying that the mass of ingested plastic items were increasing over the course of the study period, congruent with the HY plastic count model (Figure 3.6).

AHY Modeling — by number

The AHY WTSH count models were inconclusive (Sup. Table 3.3). When considering plastic found only in the proventriculus, there was no best model, but models with year (1.01) as a parameter performed better than the average model. This situation occurs in the gizzard count models, but instead models with squid beaks (1.37) performed better than average. Total plastic load GLMs required multiple models to reach the 80% threshold because multiple models had ΔAICc < 2, but those with squid beaks (1.74) once again performed better. However, when accounting for the difference between the organ chambers, no model stood out, nor was there a particularly influential parameter (weights < 1).

AHY Modeling — by mass

The AHY mass model results consistently identified year as being important for the models, with other parameters being influential in certain data sets (Sup. Table 3.4). When analyzing proventriculus plastic weights, the two best models were plastic ~ beaks + year (59.7%) and plastic ~ year (19.6%), with both beaks (1.25) and year (1.56) being influential parameters for the models. The only data set where year was not influential was the gizzard with the two best performing models being plastic ~ beaks + MEI (46.3%) and plastic ~ beaks + year + MEI (43.7%), with beaks (1.75) and MEI (1.57) being the most influential parameters. When analyzing the mass of plastic found in the whole bird, there was no best model, although models with year (1.08) as a driver performed better than average. Finally, when accounting for the differences in plastic loads between the two organ chambers, there was no best model due to 6 of

8 models having ΔAICc < 2, yet once again models with year (1.06) performed better than average.

Plastic Identification

568 items found in WTSH stomach samples were run through ATR FT-IR. Of the 539 items identified as plastic fragments during sorting, 529 (98.1%) were indeed plastic, and of the 18 line items identified during sorting, 17 (94.4%) were indeed plastic. However, I would like to note that the one line item that was not plastic was identified as polyester, also anthropogenic in source. Furthermore, there was a glass item found during sorting (confirmed by FTIR). Due to the non-plastic nature of these two compounds, they were excluded from plastic modeling and analysis. Additionally, 10 items could not be identified as plastic or natural during sorting, and 5 (50.0%) of these items turned out to be plastic. 2 of the unknown items could not identified, likely due to biofouling, so they were excluded from further analysis. In the end, 551 plastic items were identified during FTIR. 467 items (84.8%) were polyethylene, 72 (13.1%) were polypropylene, 6 items (1.1%) were considered a mix of either polyethylene or polypropylene, and 1 item (0.2%) was polystyrene. 4 items (0.7%) were considered “additive masked”, as FTIR labeled the items as perhydrofluorene. Although composition couldn’t be identified, these items were still included in the analysis because they were lime green, and thus almost certainly plastic (Corniuk et al. 2023). Finally, 1 item (0.2%) was confirmed as plastic due to a non-organic spectra, but the plastic polymer could not be identified, presumably due a complicated mixture of polymers which could not be isolated and identified.

Interspecies Scaled Parameter Weight Analysis

None of the correlations were significant when comparing the parameters, likely due to the extreme interspecies variation in the parameter weights and potential limitation from small sample sizes. However, the results of the Wilcoxon tests regularly showed similarities between the two species (Table 3.3). When considering the HY scaled parameter weights, the count and mass data yielded different results (Fig. 3.7 & 3.8). For counts, the differences between the weights was significant for squid beaks ($W = 0$, $p = 0.0294$) and for MEI ($W = 0$, $p = 0.0286$). However, year was not significantly different between the two species ($W = 4$, $p = 0.323$) implying that the number of plastic items found in NESH are comparable to those found in WTSH. For the mass data, all parameters were not significant suggesting that the mass of plastic varies statistically the same in both species in response to all parameters. The same is true for AHY (Fig. 3.9 & 3.10): both counts and masses of plastic vary essentially the same between the two species when the parameters change.

DISCUSSION

WTSH Generalized Linear Model Outcomes

The results of the GLMs were inconsistent, as regularly there were no best model identified due so many models having $\Delta AICc < 2$ (and therefore being treated as essentially the same model) (Sup. Tables 1-4). Similar to the NESH GLMs, determining the main drivers of plastic ingestion lies on the scaled parameter weights (Tables 3.4 & 3.5).

HY models with squid beaks often performed better than the average model (Table 3.4, Fig. 3.1 & 3.2), and models only had positive coefficients for beaks. This result is not surprising, since the quantity of squid beaks often correlated with plastic load for HY (Table 3.6). Of the significant relationships, the correlations were positive, implying that with increasing quantities of squid beaks, plastic loads are higher in HY. This outcome likely comes from parental foraging. HY birds receive all plastic from their parents (Carey 2011; Rodríguez et al. 2012). As parents provide more food for their chicks, which increases the amount of squid beaks found in the digestive tract, they are inadvertently transferring more plastic items in the process. Spear et al. (1995) found that healthier AHY ingest more plastic because they are foraging more regularly and therefore exposed to more MPD. When HY are provided by healthy AHY, they subsequently receive more MPD, which helps to explain the large (>1) scaled parameter weights of beaks in WTSH. Although this study did not find any significant relationships between plastic load and body condition, this finding does explain the consistency of large weights for squid beaks. In comparison, the AHY models, which also regularly had beak as an influential parameter (Table 3.5, Fig. 3.3 & 3.4), had negative coefficients. This implies that as AHY eat more squid, they ingest less MPD, or (more likely) when they have higher loads of MPD they do not have enough room for squid. This finding is potentially due to birds consistently feeling "full" and not eat, should MPD remain in the digestive tract for long periods of time, thus leading to starvation and dehydration.

In HY models, MEI was also consistently influential (Table 3.4, Fig. 3.1 & 3.2), having only positive coefficients. Therefore, HY WTSH ingest more MPD during El Niño. ENSO-related patterns of plastic deposition around the Hawaiian Islands indicate higher MPD around the islands during El Niño phases (Morishige et al. 2007; Agustin et al. 2015; Berg et al. 2023). During the breeding season (August to early November), adults forage close to the islands to provide food for their chicks (Felis et al. 2019; Adams et al. 2020). As a result, WTSH chicks are useful bioindicators for plastic abundance around the MHI because they collect the material from within the parental foraging range and during the chick growth period. HY WTSH likely reflect shifts in plastic distribution due to MEI, leading to the large scaled parameter weights. However, this relationship was not observed for AHY (Table 3.5, Fig. 3.3 & 3.4), likely because adults travel throughout the North Pacific Ocean ranging from California to Japan collecting plastic from all regions (Hebshi et al. 2008; Theil et al. 2018; Whittow et al. 2020).

In both HY (Table 3.5, Fig. 3.1 & 3.2) and AHY (Table 3.6, Fig. 3.3 & 3.4), year was also very influential, with positive coefficients. These models suggest that over time, the quantities of ingested plastic are increasing. Our finding is consistent with the global trend, which suggests increasing plastic incidence with higher loads over time (Robards et al 1995). The more regularly an individual interacts with marine plastic debris, the more likely it is to experience ingestion, thus leading to higher loads of ingested plastic (Roman et al. 2019; Nishizawa et al. 2021). With increasing densities of MPD in the ocean, AHY WTSH are likely encountering more plastic on their foraging trips, ingesting more plastic, and subsequently providing more plastic to their chicks.

WTSH as a Bioindicator for NESH

It is very difficult to monitor the NESH population because 90% breeds in inaccessible mountainous terrain on Kaua'i (USFWS 1983; USFWS 2019). In particular, relying on opportunistic sampling of naturally-deceased specimens to quantify pollutant loads and MPD ingestion studies is inherently problematic because of the variable sample sizes, and the potential sampling biases. Thus, many researchers are seeking alternatives to sampling NESH specimens (Raine et al. 2017; Young et al. 2019). This study investigated the potential for WTSH to serve as a surrogate NESH and as a bioindicator for plastic exposure in Hawaiian shearwaters. Below, I discuss the qualitative arguments for the using WTSH, and provide a quantitative assessment of the suitability of this approach.

Qualitative Analysis

As discussed previously, NESH and WTSH have very similar ecological niches and suffer from the same anthropogenic stressors (VanderWerf et al. 2014; Friswold et al. 2020; Hyrenbach et al. 2022). Both species belong to the tuna-foraging guild (Hebshi et al. 2008) diving to similar depths of up to 50 m (NESH, Kain et al. 2016) and 25 m (WTSH, Hyrenbach et al. 2014), and they prey on very similar species. NESH prey primarily on flying squid (family Ommastrephidae) and secondarily on flying fish (family Exocoetidae), with these families occurring in 100.0% and 26.3%, respectively, of the specimens (n = 19) in 1993 and 1994, and occurring in 97.5% and 5.0%, respectively, of the specimens (n = 79) sampled from 2001 to 2009 (Ainley et al. 2014). Specifically NESH consumes species in the genus *Sthenoteuthis* (occurring in 100% (n = 19) of 1993-94 samples and 79.8% (n = 79) of 2001-09 samples) and the genus *Exocoetus* (occurring in 10.5% (n = 19) of 1993-94 samples) (Ainley et al. 2014). Although there are no recent WTSH diet studies in Hawai'i, in Japan they consume primarily *Chelipogon* members of the Exocoetidae family (found in 54.95% of samples, n = 96) and *Benthosema* members of the Myctophidae family (38.46%), but also forage regularly on *Stenoteuthis* members (47.25%) during the breeding season (Komura et al. 2018). Even if NESH don't feed on myctophids, their *Stenoteuthis* sp. prey do (Shchetinnikov 1992), creating a large overlap in their diet, and thus a large overlap in potential sources of secondary (or tertiary) MPD ingestion (Kain et al. 2016).

Furthermore, WTSH breeding season, which is primarily when AHY are around the Hawaiian Islands, overlaps with the NESH breeding season. AHY WTSH return to the Hawaiian Archipelago from their pre-lay exodus to lay their eggs in early to mid-June; chicks hatch late July to late August; and chicks fledge mid- to late November (Whittow 1997). AHY NESH return from their pre-lay exodus to lay their eggs in mid- to late May; chicks hatch mid- to late July; and chicks fledge early to late October (Raine et al. 2023). When around the Hawaiian Islands, AHY WTSH forage north of the archipelago (Felis et al. 2019; Adams et al. 2020), and (although yet to be formally studied), the current hypothesis for NESH is they also forage north of the archipelago. As a result, WTSH and NESH forage in approximately the same areas during very similar time periods.

All of these similarities (anthropogenic threats, foraging ecology, and diet) between the two species are why WTSH was proposed to become a bioindicator species for NESH. Since WTSH are more common, and therefore more thoroughly studied, the impacts and trends of MPD ingestion are better understood. Since these two species have such strong overlap in their niches, we propose that WTSH can be used to represent NESH.

Quantitative Analysis

Although the niches of WTSH and NESH are very similar, there can still be differences in plastic ingestion between these two species. In a similar situation, plastic ingestion of flesh-footed shearwaters (*Ardenna carneipes*, FFSH) was compared to that of WTSH found on Lord Howe Island, Australia (Hutton et al. 2008). FFSH have overlapping niches with WTSH in this study in that they breed in the close proximity to each other, at the exact same time, and both are diving, tuna birds (Spear et al. 2007; Hutton et al. 2008). However, these two species have significantly different plastic ingestion incidences and loads (ANOVA, $F_{1, 53} = 4.541$, $p = 0.038$; FFSH: 79%, n = 56, mean volume 2.6 ± 3.6 cm^3; WTSH: 43%, n = 30, mean volume 0.7 ± 0.9 cm^3), potentially due to WTSH foraging shallower than FFSH. This difference implies that finding surrogate species for MPD studies is difficult and needs to consider many ecological drivers.

As part of an exploratory analysis to quantify the similarities in MPD ingestion between WTSH and NESH, this study generated several dozen generalized linear models and scaled parameter weights per species for three predictors (squid beaks representing food, year representing temporal trends, and MEI.v2 representing ENSO shifts) and our response variables (one for each stomach chamber, proventriculus and gizzard; one for the bird as a whole; and one accounting for the differences in MPD rates and loads between the two stomach chambers). Many GLMs did not provide significant results (p-values), with multiple yielding ΔAICc values < 2, thus preventing identification of a single model that best represents the observed pattern.

However, the two species exhibited similar qualitative patterns. For instance, no best model was found for the AHY whole bird plastic mass, for WTSH and NESH, yet those with year as a parameter explained the observed variance better than the average model (WTSH: 1.06, NESH: 1.62). Similarly, in both WTSH and NESH HY organ mass models, the best model was plastic ~ organ + beaks + year + MEI (WTSH: 94.8%, NESH: 54.9%) causing all scaled parameters weights to be meaningful (>1).

To further quantify the similarities of the models, paired Wilcoxon tests were used to determine if the same parameters were meaningful (had similar weights) for both species. The Wilcoxon tests were run on a predictor basis, separating counts from masses and AHY from HY, for a total of 12 tests. The scaled parameter weights (Table 3.3) were similar for both species in all but two parameters (HY beaks and MEI in plastic count), suggesting that both age classes of WTSH and NESH were influenced by the parameters in similar ways. Due to the nonsignificant results of the paired Wilcoxon tests, we cannot confidently say that the scaled parameter weights differed between the two species, even though the point estimates (the actual weights) differed.

Although, the two species could react in opposite ways (with the same magnitude), so we needed to consider the coefficients of the GLMs to understand how each species responds to the parameters. For both species, the majority of HY GLM models indicated a positive relationship between plastic and squid beaks. In contrast, the majority AHY GLMs indicated a negative relationship between plastic and beaks for both species. However, none of the other predictors created parallel trends across the species. For example, the majority of NESH HY GLMs generated a negative relationship between plastic load and year, whereas the majority of WTSH HY GLMs generated a positive relationship between plastic load and year. Although the variation between the models does not invalidate WTSH as a bioindicator necessarily. The Wilcoxon tests of the scaled parameter weights indicate WTSH and NESH respond to the environmental drivers to the same magnitude, but with the coefficients of the models we know they respond in opposite ways. For instance, NESH AHY had a positive relationship with MEI (meaning higher plastic ingestion during El Niño), whereas WTSH AHY had a negative relationship with MEI (meaning lower plastic during El Niño). These results indicate that when we document a decline in plastic in WTSH due to a shift in ENSO phase towards El Niño, we can also expect an increase in plastic in NESH at the same rate. While the species-specific response to the same ecological driver is different, the magnitude of change is comparable between WTSH and NESH, allowing one species to serve as a bioindicator of the other.

Overview and Implications

The primary goal of this study was to document the potential of the Wedge-tailed Shearwater to serve as a plastic pollution bioindicator species for the Main Hawaiian Islands, an approach implemented for other regions of the Pacific (Chamberlain 2019; Savoca et al. 2022). Since we know where these birds forage when they are around the islands (Felis et al. 2019; Adams et al. 2020), specimens collected during specific periods can track changes in MPD around Hawai'i. Moreover, this study expanded this approach by investigating the potential of WTSH as a bioindicator for Newell's shearwaters, a closely-related, critically endangered species endemic to the Hawaiian Islands (USFWS 1983; USFWS 2019). Due to its status, there is little knowledge about the trends and impacts of MPD ingestion on this species (Sileo et al. 1990; Kain et al. 2016).

When comparing the observed niches of the two species around the Hawaiian Islands, WTSH and NESH greatly overlap consuming the same prey, in similar areas, during the similar times of the year (Whittow 1997; Ainley et al. 2014; Komura et al. 2018; Felis et al. 2019; Adams et al. 2020; Raine et al. 2023). On a subjective and qualitative level, WTSH should easily represent MPD rates (but not necessarily loads) in NESH. However, the frequency of occurrence between the two species differs drastically with 22.2% of NESH ingesting plastic and 57.0% of WTSH ingesting plastic, a difference documented previously (Kain et al. 2016).

As a result, this study attempted to take this comparison to a more objective and quantitative level, due to the differences in MPD ingestion rates and loads. After analyzing dozens of GLMs, we can confidently say that WTSH can be used to represent NESH with regards to trends in plastic ingestion. Although they may respond to certain environmental parameters differently (namely MEI), the magnitude of their responses are statistically similar in magnitude, as evidenced by their scaled parameter weights not being statistically significant.

The next steps of this understanding would be to attempt to predict plastic ingestion in NESH using incidentally sampled WTSH. We hope that this study will establish a foundation for future analyses of MPD impacts in NESH, but also set an example for other critically endangered species where a close relative species can be used as a surrogate.

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Age Class	Metric	Data Set	S	ϱ	p-value
HY	**Count**	**Proventriculus**	974554	0.428	**4.58e-11**
		Ventriculus	1444741	0.152	**0.0255**
		Whole Bird	1321966	0.224	**9.03e-4**
	Mass	**Proventriculus**	995268	0.416	**1.80e-10**
		Ventriculus	1502443	0.118	0.0835
		Whole Bird	1420472	0.166	**0.0144**
AHY	**Count**	**Proventriculus**	84531	0.256	**0.0162**
		Ventriculus	126394	-0.113	0.295
		Whole Bird	134501	-0.184	0.0855
	Mass	**Proventriculus**	83208	0.267	**0.0118**
		Ventriculus	135807	-0.196	0.0674
		Whole Bird	134663	-0.186	0.0831

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Table 3.1. NESH and WTSH Specimens (2013-2023). HY refers to hatch-year birds (chicks and fledglings) and AHY refers to after hatch-year birds (pre-breeding and breeding adults).

		2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	Total
NESH	HY	3	1	12	2	15	12	8	12	9	0	10	85
	AHY	1	2	9	4	2	1	3	1	0	0	0	23
	Total	4	3	21	6	17	13	11	13	9	0	10	**107**
WTSH	HY	25	25	25	16	23	1	22	27	25	4	24	217
	AHY	0	0	3	10	12	5	11	18	14	15	0	88
	Total	25	25	28	26	35	6	33	45	39	19	24	**305**
Cumulative		29	28	49	32	52	19	44	58	48	19	34	**412**

Table 3.2. WTSH Spearman rank (ϱ) correlation results of plastic count vs mass.

			S	ϱ	p-value
NESH	HY	**Proventriculus**	102.8	0.9990	<2.2e-6
		Ventriculus	1818	0.9816	<2.2e-6
		Whole Bird	2348	0.9762	<2.2e-6
	AHY	**Proventriculus**	0	1	<2.2e-6
		Ventriculus	1.459	0.9993	<2.2e-6
		Whole Bird	4.584	0.9977	<2.2e-6
WTSH	HY	**Proventriculus**	16418	0.9904	<2.2e-16
		Ventriculus	84278	0.9505	<2.2e-16
		Whole Bird	112918	0.9337	<2.2e-16
	AHY	**Proventriculus**	945.7	0.9917	<2.2e-16
		Ventriculus	3182.2	0.9720	<2.2e-16
		Whole Bird	2693.5	0.9763	<2.2e-16

Table 3.3. Wilcoxon test results for scaled parameter weights between NESH and WTSH. To emphasize the interspecies relationship, *non*-significant results are in bold.

Age Class	Metric	Parameter	W	p-value
HY	Counts	**Beaks**	0	0.0294
		Year	4	**0.323**
		MEI	0	0.0286
	Mass	**Beaks**	6.5	**0.766**
		Year	9	**0.886**
		MEI	1	**0.0571**
AHY	Counts	**Beaks**	0	**0.0286**
		Year	9	**0.886**
		MEI	11	**0.486**
	Mass	**Beaks**	9	**0.886**
		Year	15	**0.0571**
		MEI	9	**0.886**

Table 3.4. Scaled parameter weights between HY NESH and WTSH. Influential parameters are in italics. Influential parameters that are the same in both NESH and WTSH are in bold.

		NESH				WTSH			
		Stomach	Gizzard	Whole Bird	Organ	Stomach	Gizzard	Whole Bird	Organ
Count	**Beak**	0.85	***1.21***	0.93	***1.17***	*1.75*	***1.73***	*1.75*	***2.00***
	Year	0.77	0.72	0.87	0.55	*1.70*	0.54	*1.09*	*1.72*
	MEI	0.76	0.58	0.65	0.52	0.97	1.00	*1.18*	*1.64*
Mass	**Beak**	0.23	***1.75***	***1.57***	***2.00***	*1.75*	***1.18***	***1.75***	***2.00***
	Year	*1.74*	***1.68***	0.98	***2.00***	0.18	***1.44***	*1.75*	***1.90***
	MEI	0.21	0.23	***1.63***	***1.10***	*1.69*	*1.41*	***1.75***	***2.00***

Table 3.5. Scaled parameter weights between AHY NESH and WTSH. Influential parameters are in italics. Influential parameters that are the same in both NESH and WTSH are in bold.

		NESH				WTSH			
		Stomach	Gizzard	Whole Bird	Organ	Stomach	Gizzard	Whole Bird	Organ
Count	Beak	0.54	0.67	0.70	0.46	0.72	*1.37*	*1.74*	0.78
	Year	0.60	0.81	0.75	0.50	*1.01*	0.56	0.46	0.73
	MEI	*1.16*	0.68	0.75	0.60	0.92	0.56	0.47	0.70
Mass	Beak	0.00	0.66	0.17	0.00	*1.25*	*1.75*	0.93	0.56
	Year	***1.75***	*1.50*	***1.62***	*1.81*	***1.56***	0.94	***1.08***	***1.06***
	MEI	0.00	***1.08***	0.97	*1.24*	0.36	***1.57***	0.75	0.66

Table 3.6. Spearman rank (ϱ) correlation results of plastic loads vs beak load. Significant results are in bold.

Figure 3.1. Plastic loads of HY WTSH across time in terms of number of items. Color scale represent MEI.v2 values. Black line represents a loess curve trend of plastic ingestion, due to the nonlinear nature of the results.

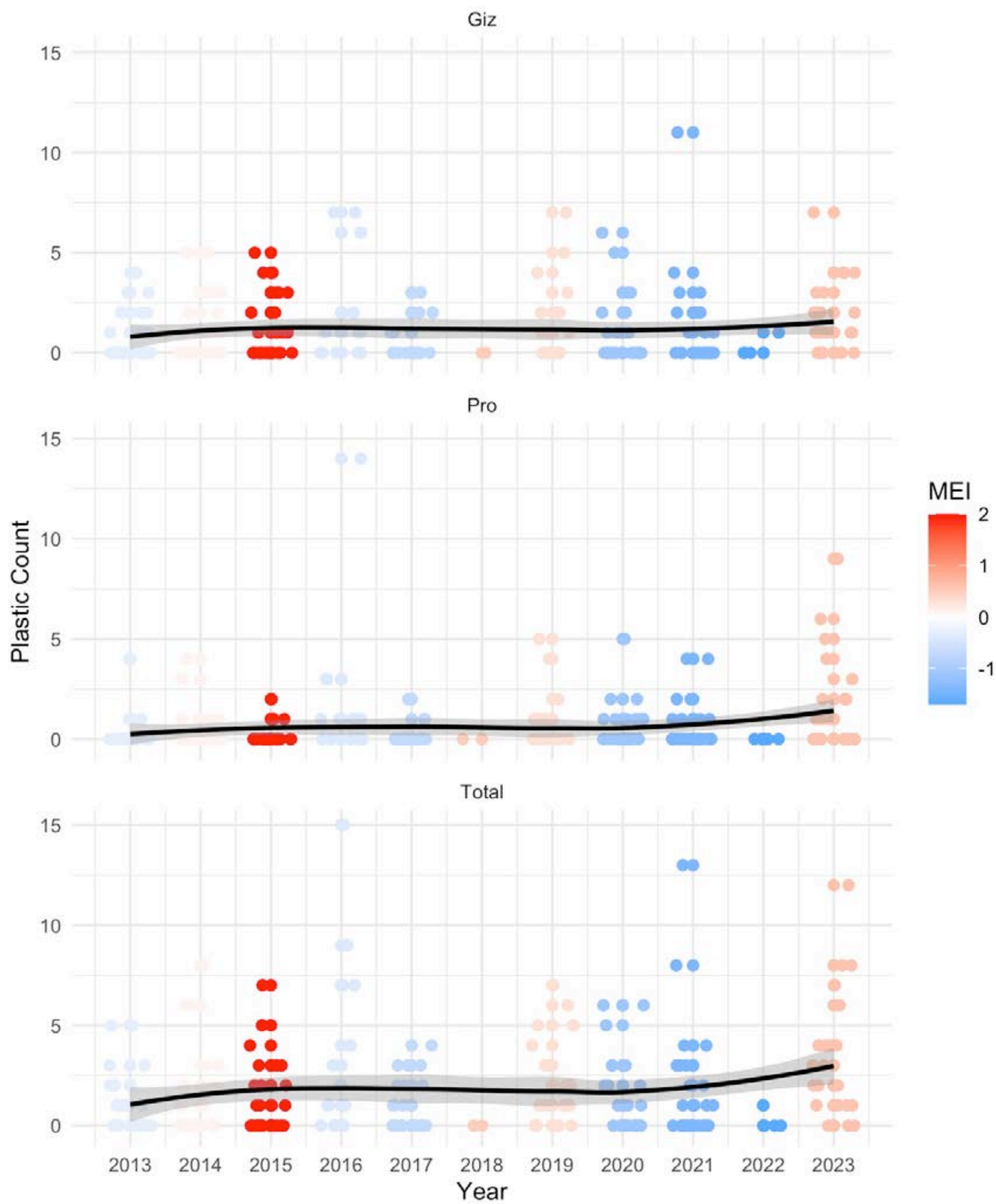


Figure 3.2. Plastic loads of HY WTSH across time in terms of mass of items. Color scale represent MEI.v2 values. Black line represents a loess curve trend of plastic ingestion, due to the nonlinear nature of the results.

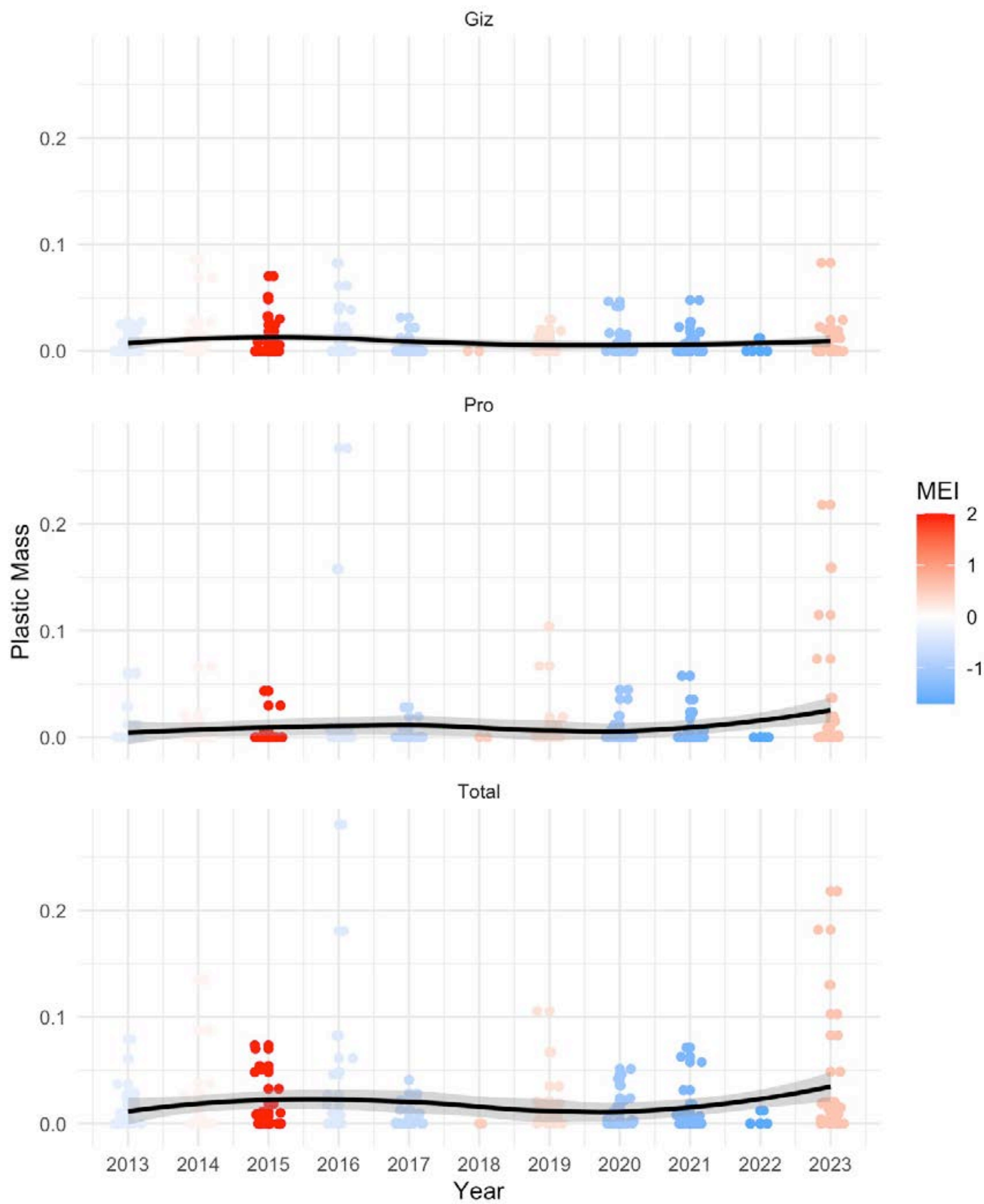


Figure 3.3. Plastic loads of AHY WTSH across time in terms of number of items. Color scale represent MEI.v2 values. Black line represents a loess curve trend of plastic ingestion, due to the nonlinear nature of the results.

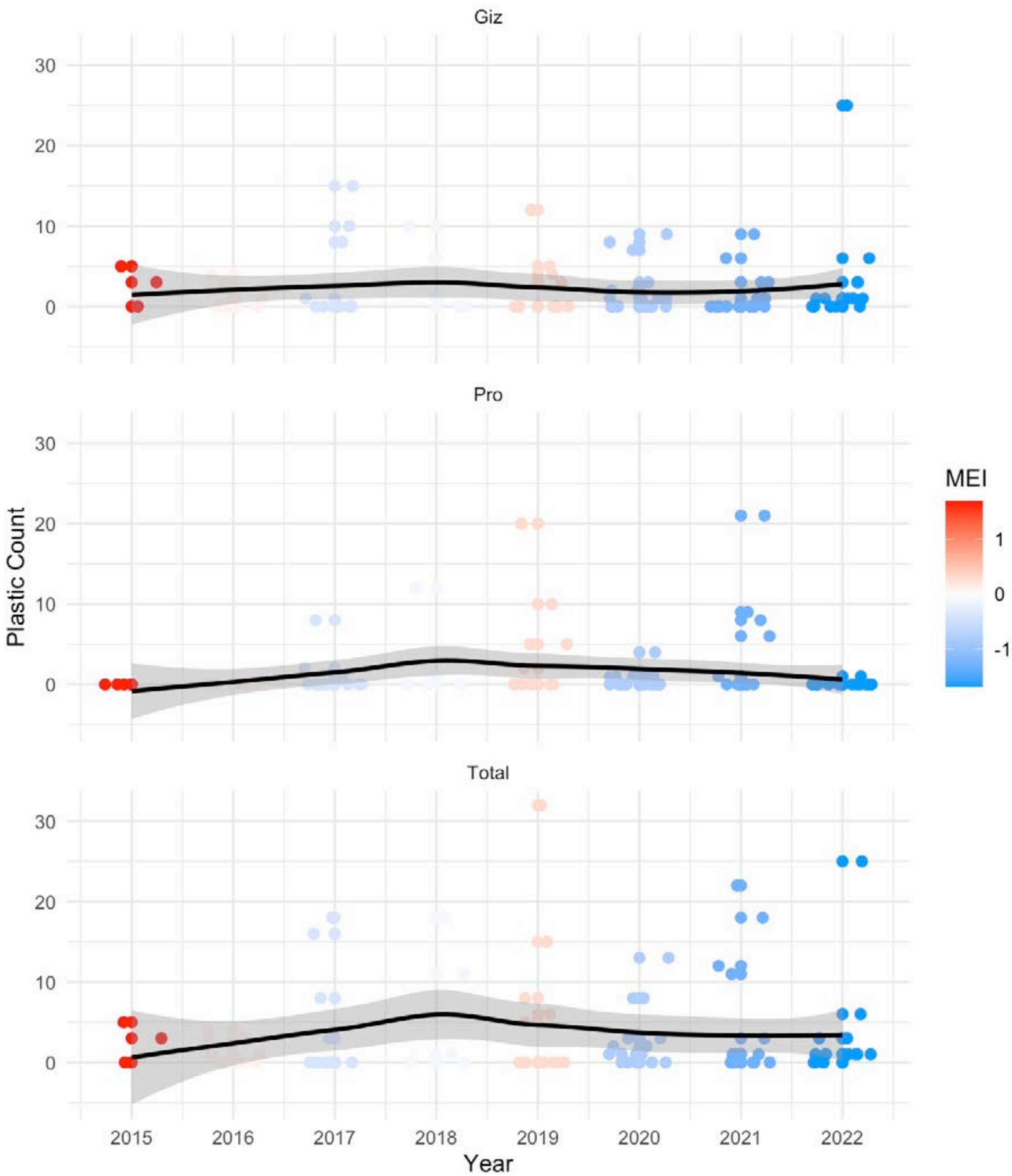


Figure 3.4. Plastic loads of AHY WTSH across time in terms of mass of items. Color scale represent MEI.v2 values. Black line represents a loess curve trend of plastic ingestion, due to the nonlinear nature of the results.

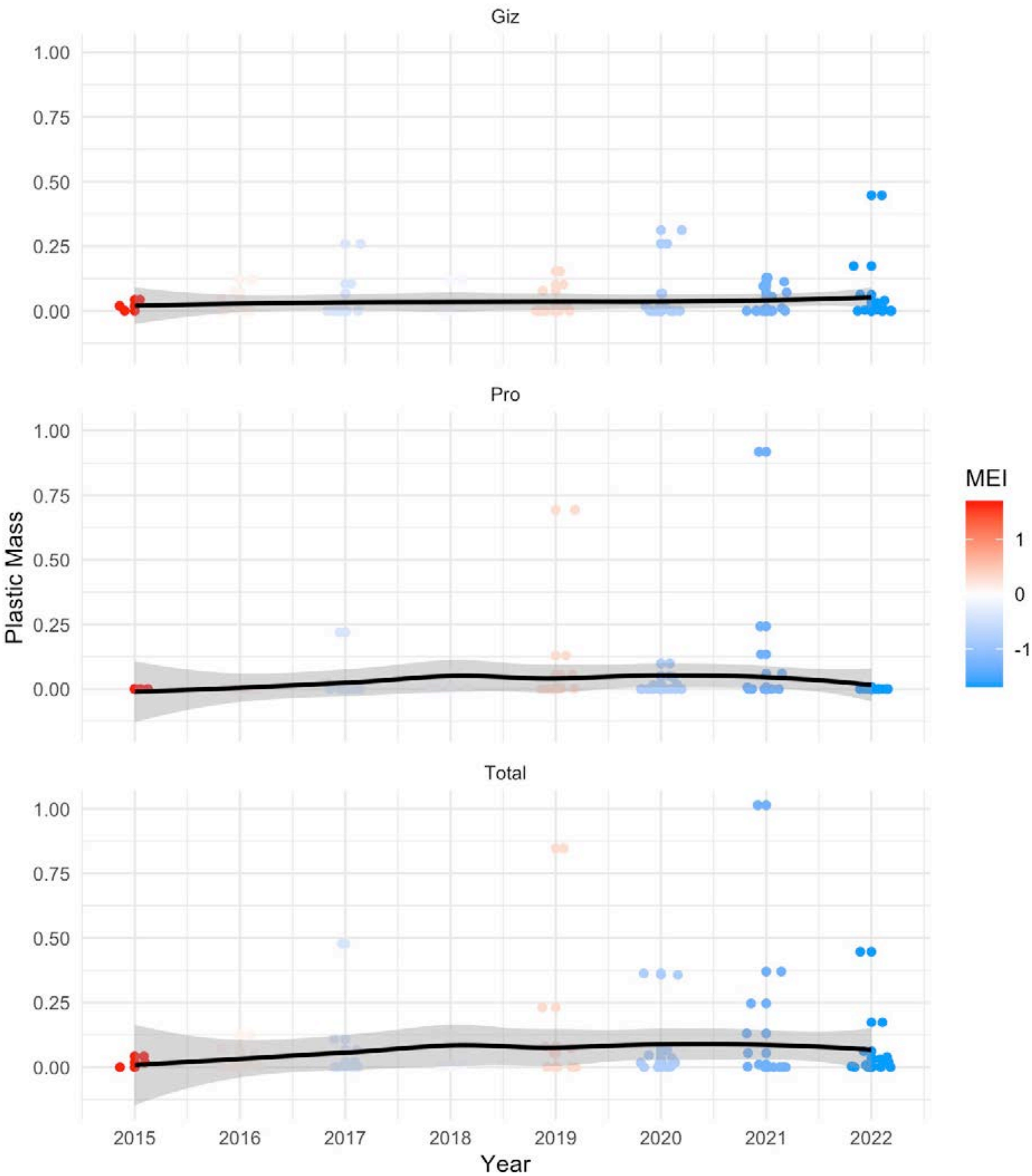


Figure 3.5. Predicted WTSH HY plastic masses based on temporal trends alone.

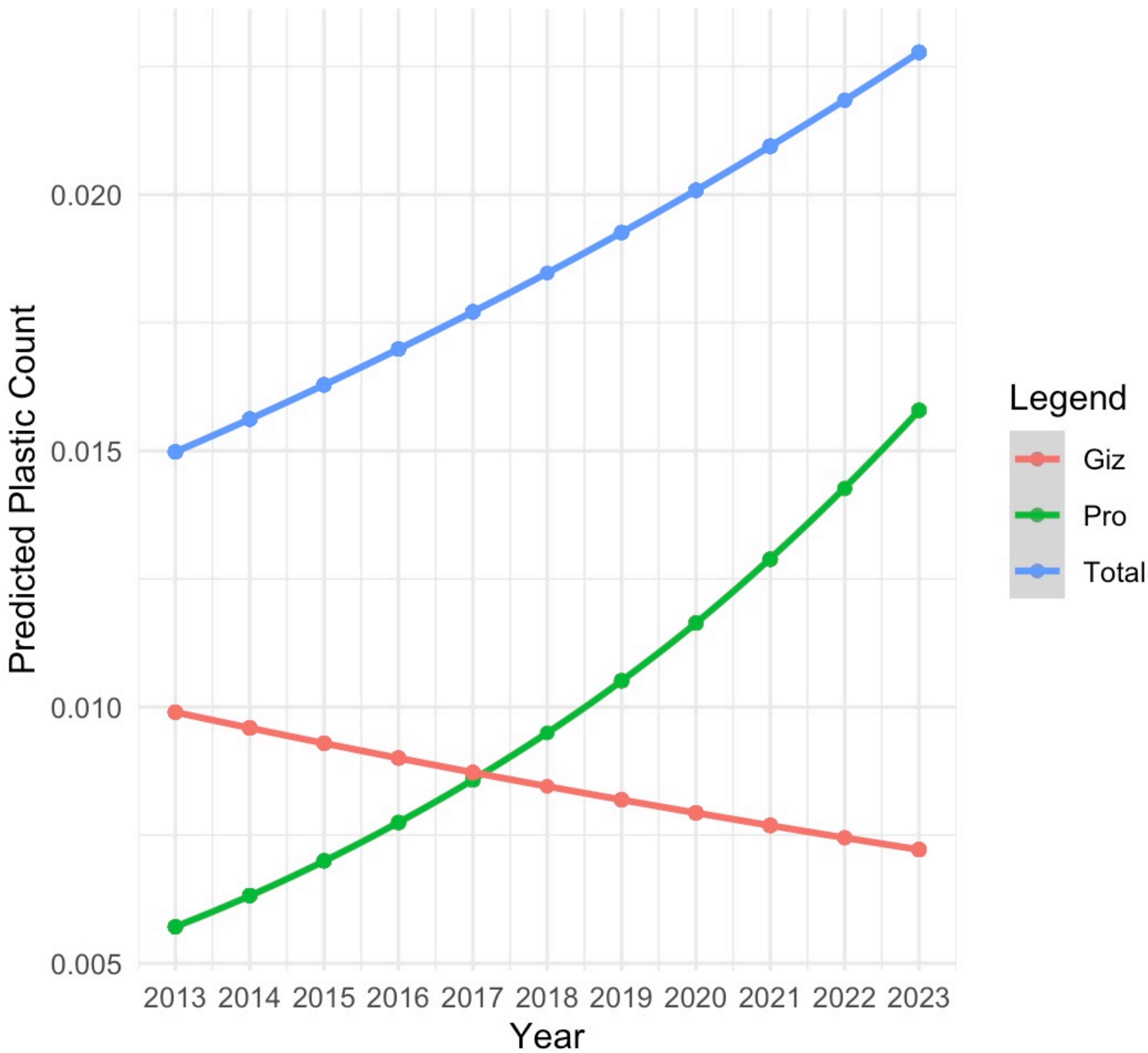


Figure 3.6. Predicted WTSH HY plastic masses based on temporal trends alone.

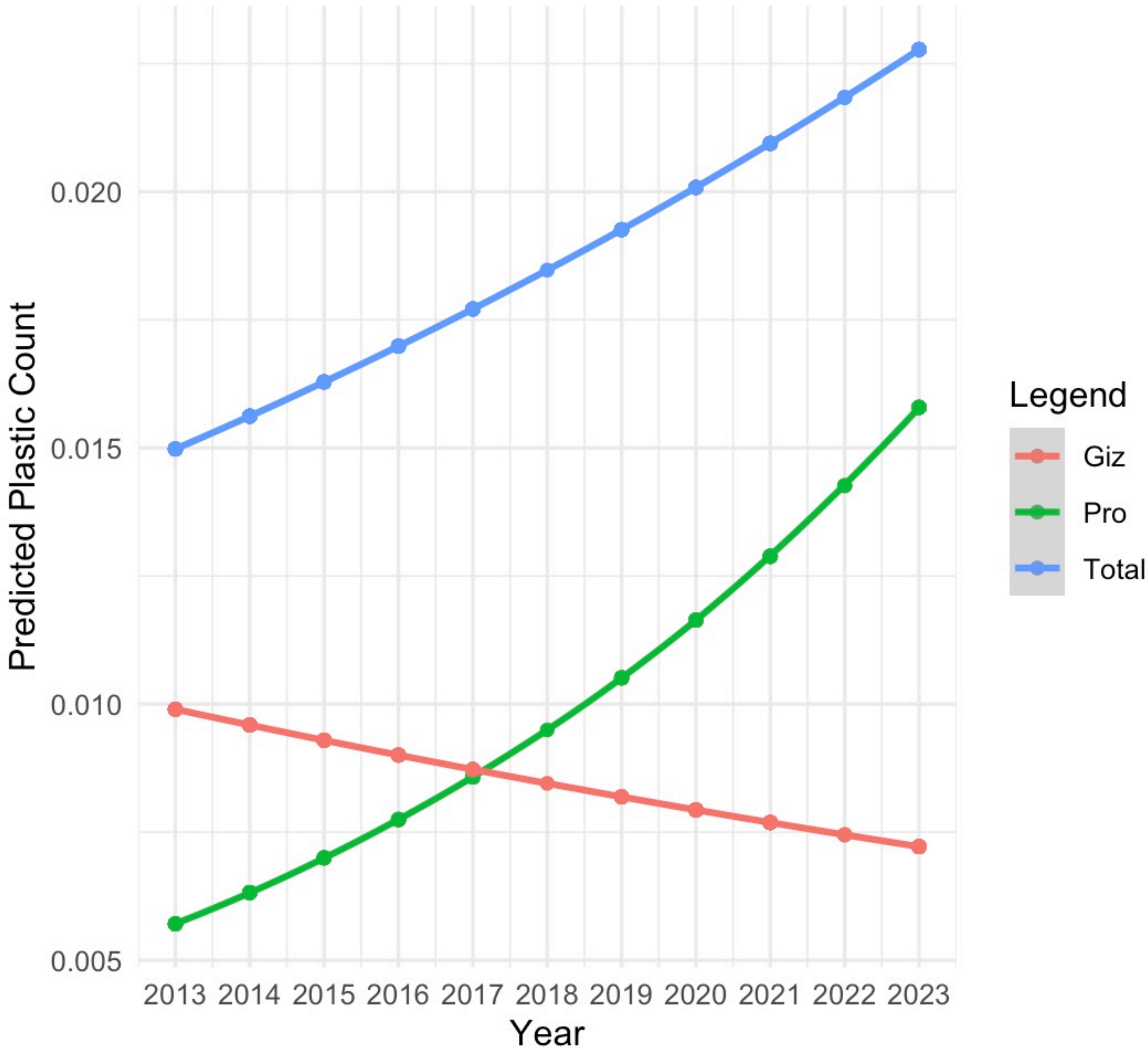


Figure 3.7. HY scaled parameter weights for count data. Colors vary based on the GLM group, and the dashed line represents the 1.0 weight threshold.

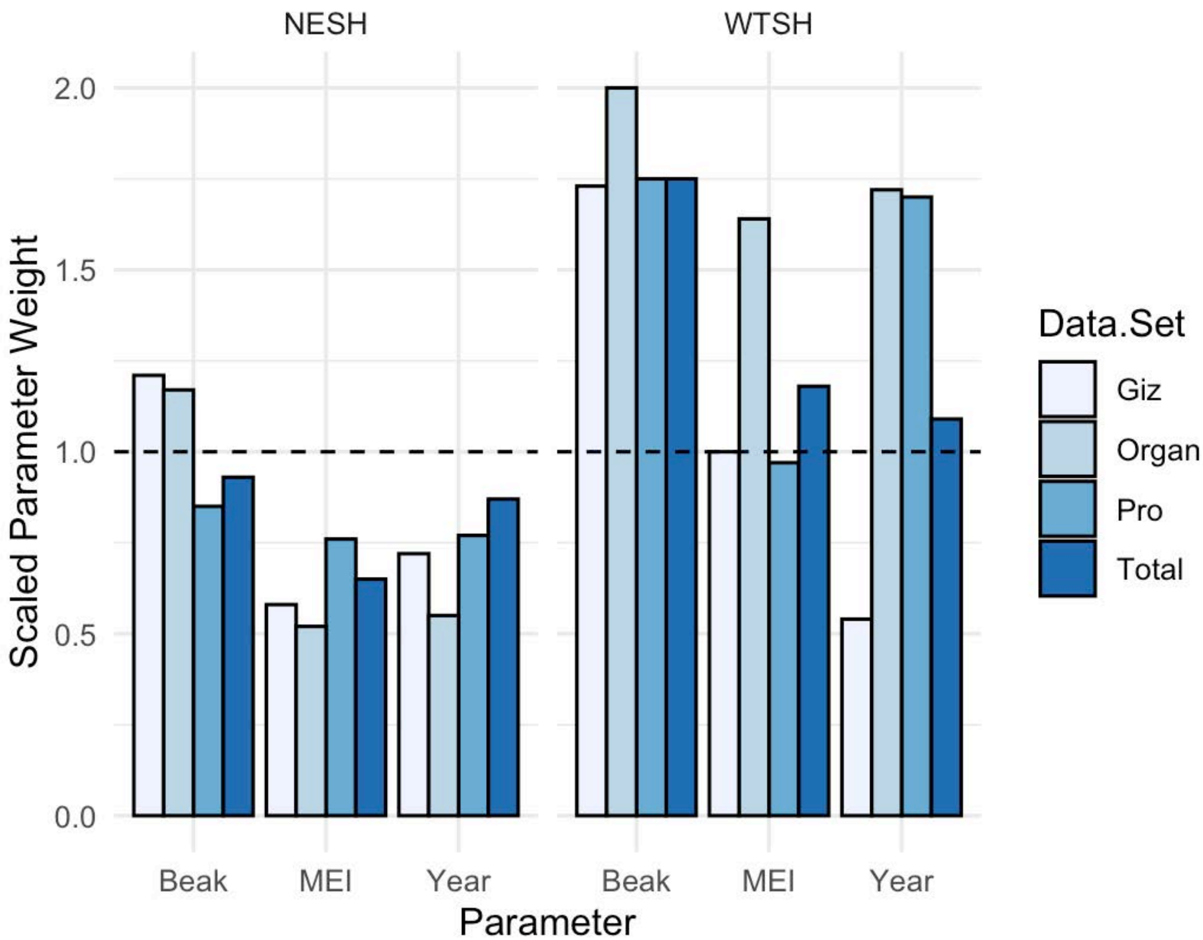


Figure 3.8. HY scaled parameter weights for mass data. Colors vary based on the GLM group, and the dashed line represents the 1.0 weight threshold.

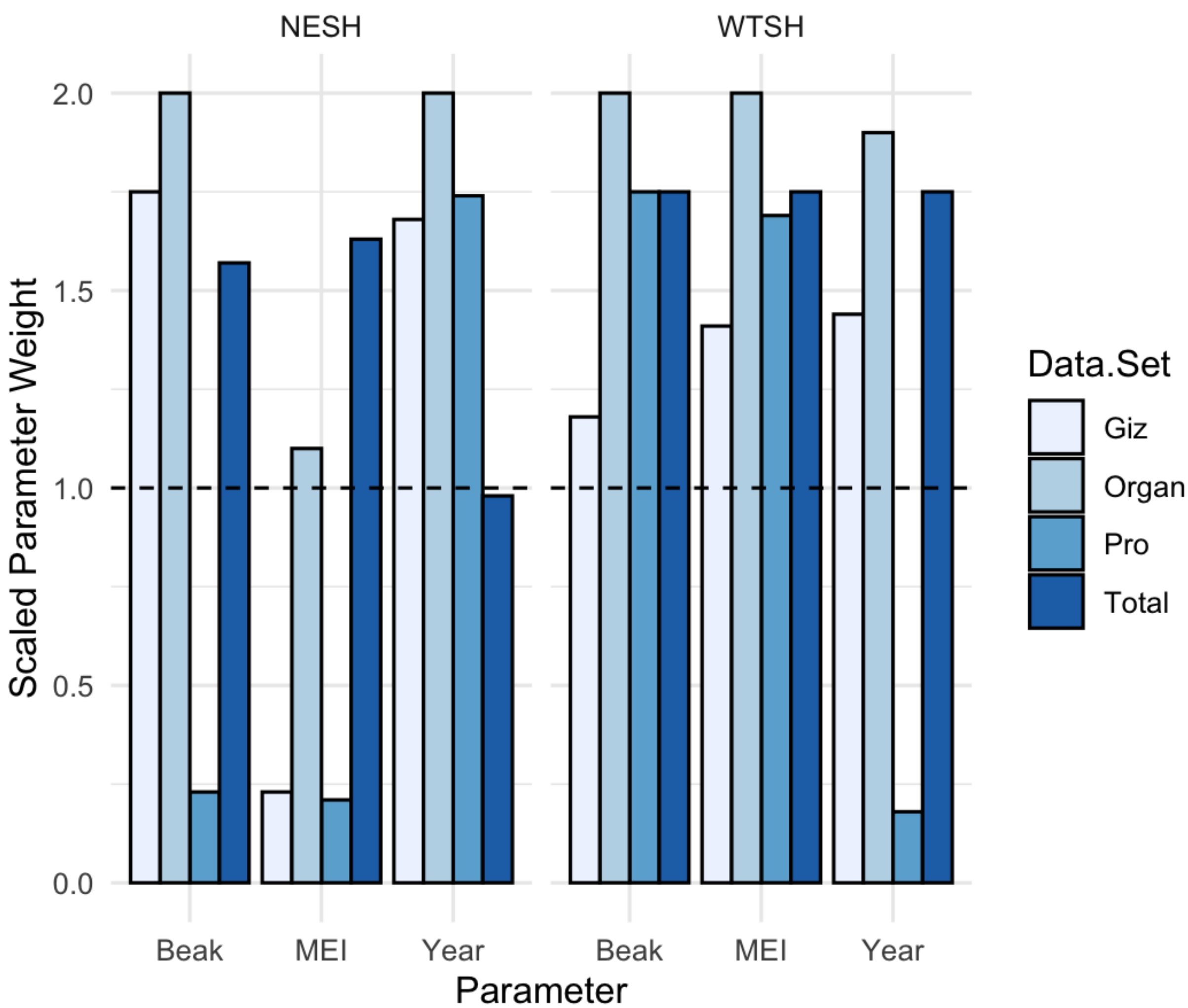


Figure 3.9. AHY scaled parameter weights for count data. Colors vary based on the GLM group, and the dashed line represents the 1.0 weight threshold.

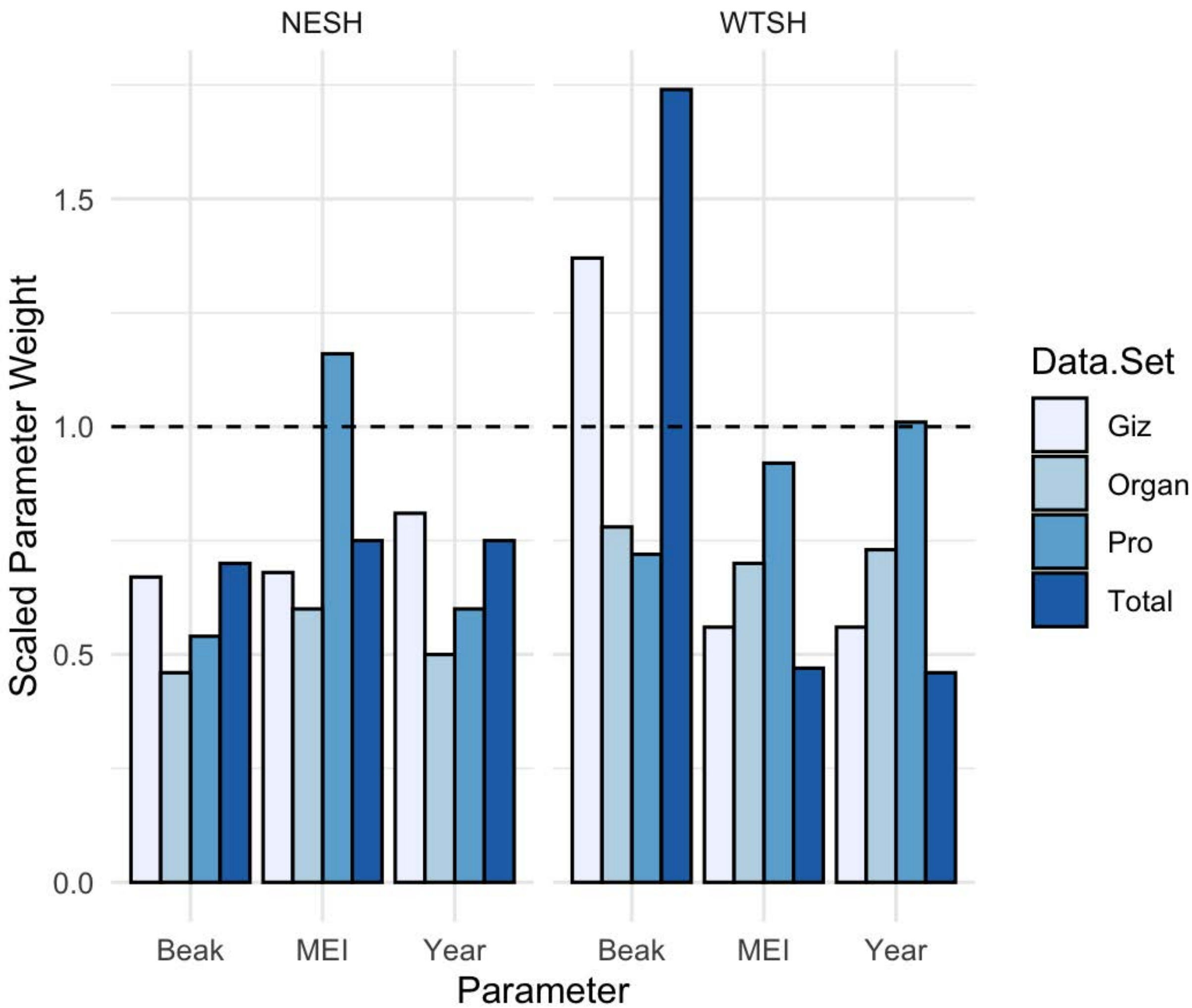
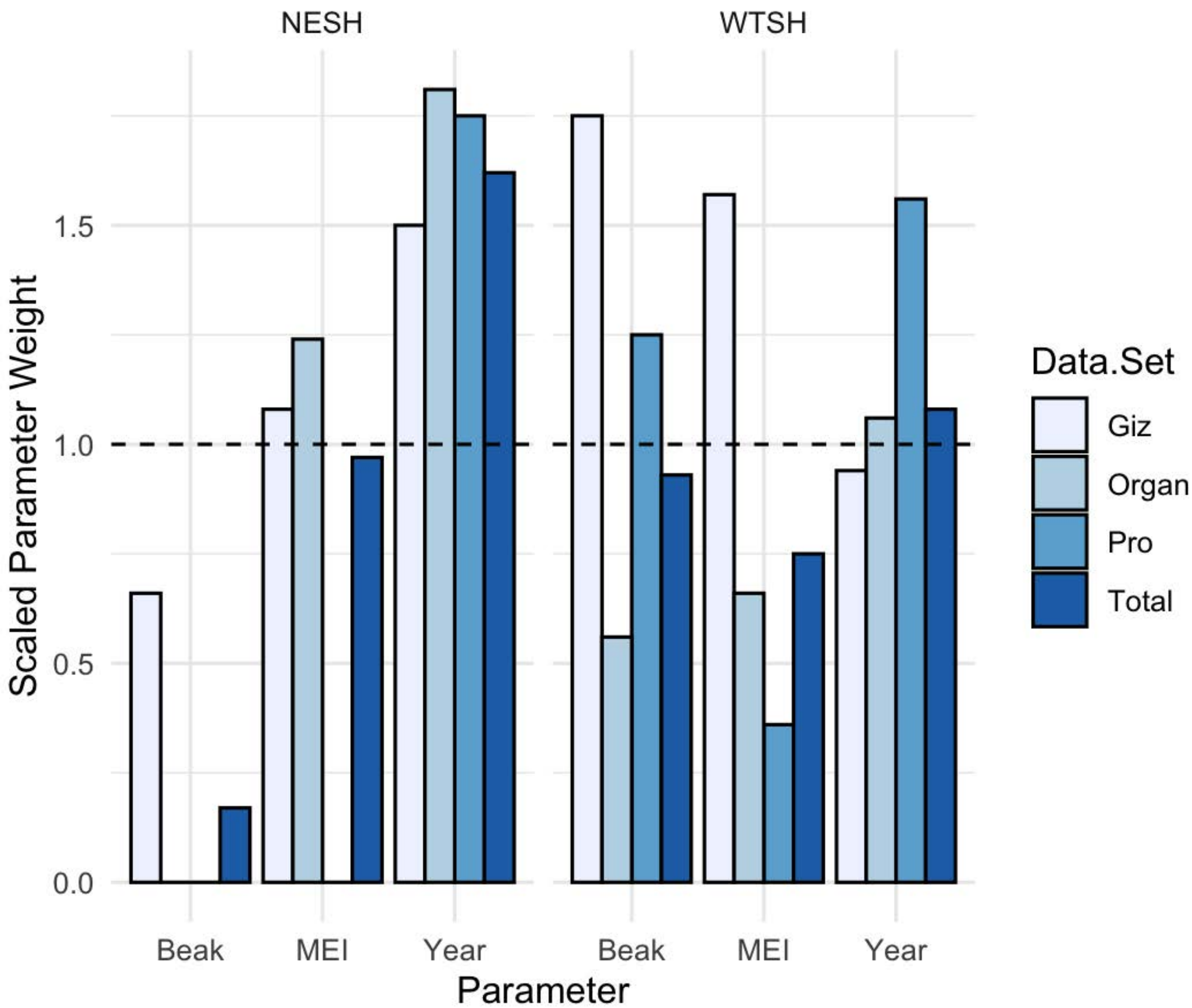


Figure 3.10. AHY scaled parameter weights for mass data. Colors vary based on the GLM group, and the dashed line represents the 1.0 weight threshold.



CHAPTER 4: PLASTIC ADDITIVES AND PERSISTENT ORGANIC POLLUTANTS IN THE PREEN GLAND OIL OF NEWELL'S (*PUFFINUS NEWELLI*) AND WEDGE-TAILED (*ARDENNA PACIFICA*) SHEARWATERS IN HAWAI'I.

INTRODUCTION

Marine plastic debris (MPD) is a pervasive pollutant found throughout the global ocean (Cózar et al. 2014; Eriksen et al. 2023). Due to the wide range of plastic pollution in the ocean, hundreds of marine species are impacted via entanglement (n = 354), ingestion (n = 701) or both (n = 914, Kühn and van Franeker 2020). Entanglement is a more visible interaction, but ingestion is a more prolific issue. For instance, 39.8% of marine mammal and 27.4% of seabird species worldwide suffer from entanglement, whereas 56.1% and 44.0% (resp.) suffer from MPD ingestion (Kühn & van Franeker 2022). The impacts of entanglement are direct and clear (ie: drowning, loss of limbs, etc.), the impacts of ingestion of marine debris are currently under investigation. While reduction of entanglements enhances to the survival of marine fauna, ingestion can lead to more chronic, life-long health effects (ie: plasticosis, reduction of fat stores, altered blood chemistry, etc.) and therefore are more pertinent to study, especially concerning long lived marine species.

The main limitation of studying MPD effects in seabirds is the lack of long term standardized metrics and trends. Namely, many published studies have limited sample sizes, obtained by combining specimens spanning several years. The literature recommendations of yearly sample size range from 20 individuals (National Research Council 2009) to 40 individuals (van Franeker & Meijborn 2002; Savoca et al 2022). MPD studies have a never ending battle with limited sample size, especially with rare species (Sileo et al. 1990) or expensive research methods (Ito et al. 2013; Lavers & Bond 2016; Nishizawa et al. 2021). In contrast, many studies have excellent sample sizes, but lack the yearly samples for quantifying trends over time (Kain et al. 2016; Roman et al. 2019; Yamashita et al. 2021). Including sampling year(s) allows for comparability between studies and enables meta-analyses of long term trends. Long term trends are difficult to implement, but they are vital to fully understand how MPD affects seabirds. For instance, Kain et al. (2016) analyzed 30 Newell's shearwater (*Puffinus newelli*) covering seven years (2007-2013) (100). While 30 individuals is a very strong sample size for such a critically endangered species, analysis of long term trends identify patterns of MPD ingestion, which requires these samples sizes to be replicated over many years

To mitigate the limitations from sample size, researchers are developing novel technologies and methodologies that can be applied to living specimens, since a primary constraint in MPD studies is the heavy reliance on deceased specimens. However implementation of these methodologies may be insufficient. For instance, stomach lavage (or pumping) can collect plastic in the digestive tracts of seabirds. While less invasive, lavages may not collect all MPD in specimens that are very full or, like Procellariiforms, have a gizzard stomach chamber that lavage does not reach (Bond & Lavers 2013; Rapp et al. 2017; Provencher et al. 2019). A promising, minimally invasive technique that can be applied on living seabirds, and validated through deceased specimens, is the analysis of preen gland oil (Hardesty et al. 2015). Not only can preen gland oil be used on living specimens, but it can be applied to the same individuals repeatedly over a long period of time to evaluate trends over time and specially (Ito et al. 2013). Additionally, the concentrations of plastic additives and POPs in the preen gland oil vary in proportion to the quantity of ingested MPD (Yamashita et al. 2021), they can indicate the variation in exposure of plastic ingestion intra- and interspecially.

This study applies preen gland oil analysis via gas chromatography-mass spectrometry to two new groups: Newell's shearwaters (NESH, *Puffinus newelli*) and wedge-tailed shearwaters (WTSH, *Ardenna pacifica*) in Hawaiian colonies. Newell's shearwaters were selected due to their critically endangered status and large knowledge gaps in the effects of MPD (Chapter 2; USFWS 1983; Raine et al. 2017; USFWS 2019). Although preen gland oil was studied in Australian colonies for different contaminants (Hardesty et al. 2015), wedge-tailed shearwaters were included because they are culturally significant for Hawaiian fishers (Harrison 1990) and are useful bioindicators for MPD abundance around the Main Hawaiian Islands (Chapter 3). Since additives and POPs found in preen gland oil are related to diet (Yamashita et al. 2021), WTSH and NESH were expected to have high concentrations (>100 ng/g-lipid) of PCBs, DDE (substituting for DDT), UVP, and UV329, like other seabird species that that mainly consume squid and fish.

METHODS

Specimen Collection

Naturally-deceased NESH were provided by Save Our Shearwaters, a non-profit organization located on Kaua'i. WTSH were provided by Feather and Fur, a local seabird hospital, and Sea Life Park, a local seabird rehabilitation center, both on the windward shore of O'ahu. All individuals of both species were victims of fallout due to attraction to artificial lights and collisions with obstacles. Some were found dead on arrival, others died in care of unknown causes, and the remaining individuals were humanely euthanized. The specimens, were stored frozen at -20˚C until they were necropsied following standardized procedures previously modified for Hawaiian seabirds (van Franeker 2004; Rapp et al. 2017).

Stomach contents were taken from both the proventriculus and ventriculus (hereafter gizzard) using standardized procedures (van Franeker 2004), because plastic incidence rates in NESH and WTSH fledglings differed these two chambers (Kain et al. 2016). The samples were stored in 70% ethanol until they were manually sorted into four broad categories: food items (squid beaks, fish eye lenses, fish bones), natural non-food items (plant matter, sand, pumice, endoparasitic worms), non-natural, non-food items (plastic, glass), and unidentified items using 10x magnification via a binocular dissecting microscope, congruent with other plastic-ingestion studies (van Franeker et al. 2011; Rapp et al. 2017). Following these standardized protocols, plastic items were categorized into four types: fragments, line, foam, and sheet. In total, each specimen yielded 8 mass measurements per chamber: 4 diet categories and 4 plastic types.

Each diet category and plastic type was weighed twice using a Mettler Toledo Model MS104S scale, with a resolution of 0.0001 g. Half of the minimum detection threshold (0.00005 g) was recorded when the scale yielded 0.0000 g while weighing a sample. However, this rarely happened, only occurring in 3.13% of 1,151 sample weights. Since each sample was weighed twice, the root mean squared error (RMSE) was used to determine the variation between replicate mass measurements across all samples (Armstrong & Collopy 1992; Rapp et al. 2017). RMSE was calculated with R’s rmse command in the Metrics package.

To ensure that all plastic items were collected and analyzed, all putative MD and unknown items in the diet were isolated, weighed, and analyzed with attenuated total reflectance Fourier transform infrared spectroscopy (ATR FT-IR), which identifies plastic compounds by matching well-known infrared absorption bands representing distinct chemical functionalities found in plastic with the item in question (Provencher et al. 2017; Jung et al. 2018). Because small and rigid plastic fragments can be crushed during scanning by the diamond tip of the instrument, each item was weighed individually before ATR FT-IR. This measure was to ensure that should an item not be plastic, it could be removed from the total plastic weight.

Attenuated Total Reflectance Infrared Spectroscopy Analysis

Using a Thermo Fisher Scientific Nicolet iS5 ATR-FTIR spectrometer, IR spectra were collected from all putative plastic items and unknown items. Following the methods of Corniuk et al (2023), spectra were generated from 16 scans each with resolution at 4 cm^{-1} and a data interval of 1 cm^{-1}. The section from 4000 cm^{-1} to 500 cm^{-1} were compared against the standardized polymer spectra provided in Jung et al. (2018). Two steps were taken to ensure maximum clarity of the spectra: (i) the samples and the instrument's diamond crystal were cleaned with a kim wipe and 70% isopropanol before each scan, and (ii) a background (control) scene was taken between each sample.

Preen Gland Oil Sampling and Analysis

Preen oil samples were taken with autoclaved, glass-fiber filters and wrapped in in aluminum foil before and after sampling. The filter was folded in half, placed on the preen gland, and the gland was squeezed to obtain as much preen oil as was available. Samples were stored at -20˚C until analyzed via gas chromatography mass spectrometry following the methods of Yamashita et al. (2021).

Preen gland oil samples were taken from individuals of both species, and stratified by age class (HY: hatch year, and AHY: after-hatch year), good/poor body condition, and presence/ absence of ingested MPD (Table 4.1). Good body condition represented a healthy bird with a body condition index (BCI) ≥ 6, and poor body condition represented a starving or emaciated bird with a BCI < 6. BCI = 6 was the threshold because individuals with low muscle mass may be considered healthy due to having a lot of fat, but little muscle, presumably due to lack of activity prior to fledging, and would receive a score of 6. Due to cost of sample analysis limiting the available sampling (n = 20), more NESH specimens were sampled than WTSH, due to the rarity of NESH. Additionally, due to limited availability of AHY, the majority of preen gland oil samples were from HY birds. To reduce compounding factors of influence of MPD ingestion on body condition, individuals with MPD with good BCI scores were prioritized over individuals with both poor BCI and MPD.

Statistical Analysis

To determine if there is a difference in plastic additives and POPs between each category (e.g. good BCI vs poor BCI), paired Wilcoxon tests were run between each category as well as between species (e.g. WTSH HY vs NESH AHY). To detect any relationship between concentrations of ingested MPD and plastic additives/POPs, two-tailed spearman rank correlations were run. Furthermore, due to the potential biomagnification of POPs and plastic additives, two-tailed spearman rank correlations were used to compare food in the stomach contents against all the pollutants. Although these correlations were primarily exploratory, the total food count model (combining the contents of the proventriculus and the gizzard) was expected to generate the strongest relationship, since it incorporates prey items ingested over a longer period of time, and squid beaks retain in the digestive tract longer than other prey items (like fish bones and otoliths).

Since the blank values varied greatly between GC-MS runs, and in an effort to generate a more conservative analysis, only BUV concentrations greater than the 95% CI upper limit of the blank concentrations were considered meaningful for analysis. After filtering the data, the concentration of benzotriazole UV stabilizers (BUVs), polychlorinated biphenyls (PCBs), and dichlorodiphenyldichloroethylene (DDE) were analyzed with a Principal Component Analysis (PCA) to analyze any patterns of co-variability and to develop a pollution index based on correlated the resulting PC axis(es) with ingested MPD loads. Coordinates for PCs were isolated, and Spearman rank correlations were used to compare the sampled specimens in terms of their coordinates and their plastic loads, which were not normally distributed. To validate the PCA ordination, a a linear discriminant analysis (LDA) was used to assign WTSH and NESH to distinct groups, tased on their BUVs and POPs concentrations using linear discriminant analysis (lda) in R package {MASS}.

RESULTS

A total of 20 preen oil samples were analyzed through GC-MS (Table 4.1). However, only 19 were included in the analyses due to contamination of one sample (a NESH HY with no plastic). Out of 10 BUVs investigated, only 4 (UVP, UV326, UV328, and UV9) were found in NESH, and 5 (UVP, UV326, UV328, UV329, and UV9) were found in WTSH. PCBs and DDE were found in every preen oil sample. The concentrations of total PCBs correlated significantly with the concentrations of DDE for the entire sample set ($S = 82.536$, $\varrho = 0.928$, $p = 1.10e\text{-}8$) and for the NESH samples specifically ($S = 16.515$, $\varrho = 0.971$, $p = 2.07e\text{-}9$) (Fig. 4.1). However, there were no significant relationships between the POPs and BUVs (PCBs: $S = 1188$, $\varrho = -0.0421$, $p = 0.866$; DDE: $S = 1405.1$, $\varrho = -0.233$, $p = 0.338$). Finally, the LDA did not yield meaningful results, as the model could not accurately predict the species of a sample. Since there were only two species, only one linear discriminant function was calculated with the following coefficients: BUV = -0.00520, PCB = -0.0586, and DDE = 0.0411. The LDA had a 78.9% accuracy rate, but failed to discriminate between the two species. Instead, the LDA labeled all samples as NESH, the most numerous species of which 78.9% of preen oil samples were in fact NESH.

PCBs

All WTSH and NESH preen oil samples contained at least one PCB congener, and one NESH sample contained all congeners with an average concentration of 22.62 ± 23.98 ng per g of lipid (range: 1.43-95.46 ng/g-lipid, median: 14.82 ng/g-lipid). 18 PCB congeners were detected with pentachlorobiphenyl (IUPAC number 118) occurring in all samples. Nonachlorobiphenyl (IUPAC 206) was occurred the least common congener, occurring in only 7 samples (36.8%). The statistical analysis rarely yielded significant results, underscoring the interspecific similarities. Wilcoxon tests indicated that total PCB concentration did not vary between species ($W = 32$, $p = 0.885$). Moreover, there was no difference between age classes for NESH ($W = 30$, $p = 0.101$). No age class analysis was conducted for WTSH since all samples were HY. After separating between the species (due to differences in sample size potentially influencing results), no relationships with plastic were significant neither in terms of presence absence (NESH: $W = 34$, $p = 0.536$) nor for any correlations (Table 4.2). Similarly, there were no significant relationships with food loads. However, PCB concentration differed significantly between good and poor body condition ($W = 2$, $p = 1.24e-3$) with a negative relationship ($S = 1011.1$, $\varrho = -0.805$, $p = 2.90e-4$) for NESH, but not for WTSH ($W = 0$, $p = 0.5$) (Fig 4.2).

DDE

All WTSH and NESH preen oil samples contained DDE with an average concentration of 10.75 ± 15.51 ng/g-lipid (range: 0.51-64.02 ng/g-lipid, median: 4.42 ng/g-lipid). Statistical analysis rarely again yielded significant results. Wilcoxon tests indicated that total DDE concentration did not vary between species ($W = 24$, $p = 0.582$). There was no difference between age classes for NESH ($W = 30$, $p = 0.0966$). After separating between the species (due to differences in sample size potentially influencing results), no relationships with plastic were significant neither in terms of presence absence (NESH: $W = 38$, $p = 0.271$) nor for any correlations (Table 4.3). Again, there were no significant relationships with food loads. However, PCB concentration differed significantly between good and poor body condition ($W = 0$, $p = 1.45e\text{-}3$) with a negative relationship ($S = 1046$, $\varrho = -0.868$, $p = 2.75e\text{-}5$) for NESH, but not for WTSH ($W = 1$, $p = 1$) (Fig. 4.2).

BUVs

After filtering for meaningful values, all WTSH and NESH preen oil samples contained meaningful BUV concentrations, although concentration varied greatly with an average concentration of 91.16 ± 106.84 ng/g-lipid (range: 8.54-409.56 ng/g-lipid, median: 48.94 ng/g-lipid). Wilcoxon tests indicated that total BUV concentration did not vary between species ($W = 32$, $p = 0.885$). There was no difference between age classes for NESH ($W = 24$, $p = 0.448$). After separating between the species (due to differences in sample size potentially influencing results), no relationships with plastic were significant neither in terms of presence absence (NESH: $W = 15$, $p = 0.152$) nor for any correlations (Table 4.4). There were no significant relationships with food loads, nor with body condition for either species.

Pollutant Principal Component Analyses

Since BUVs and POPs did not significantly differ between species (using the results of the Wilcoxon tests and the LDA), all 19 samples were run together through a PCA irrespective of species. Principal component 1 had the best relationship with both POPs; PC 2 correlated best with total BUV values (Table 4.5, Fig 4.3). As a result, PC 1 represents pollutants, and PC 2 plastic additives, representing a cumulative of 97.5% of variance (PC1 66.3%, PC2 31.1%). After isolating the coordinates, no relationships were significant with plastic loads for either dimension. Since concentrations of individual BUVs varied greatly depending on the compound and the previous PCA had no significant relationships, another PCA was run splitting the BUVs. Principal component 1 again had the best relationship with POPs, and PC 2 with all BUVs (Table 4.6, Fig 4.4). PC 1 represents pollutants, and PC 2 plastic additives, representing a cumulative of 51.6% of variance (PC1 30.4%, PC2 21.2%). After isolating the coordinates, no relationships were significant with plastic loads for either dimension.

DISCUSSION

Interspecies Comparison

As expected, the concentrations of additives and POPs did not significantly differ between the two species. Not only were Wilcoxon tests not significant for any contaminants, but the LDA failed to differentiate between NESH and WTSH. As discussed in Chapter 3, the two species have extremely similar ecological niches. WTSH and NESH suffer from the same anthropogenic stressors (VanderWerf et al. 2014; Friswold et al. 2020; Hyrenbach et al. 2022). Additionally, both species belong to the tuna-foraging guild (Hebshi et al. 2008) diving to similar depths of up to 50 m (NESH, Kain et al. 2016) and 25 m (WTSH, Hyrenbach et al. 2014), and they prey on very similar species (Ainley et al. 2014; Komura et al. 2018), and thus overlap in potential sources of secondary (or tertiary) MPD ingestion (Kain et al. 2016). Around the Hawaiian Islands, AHY WTSH forage north of the archipelago (Felis et al. 2019; Adams et al. 2020), and the NESH are also believed to forage north of the archipelago. The WTSH breeding season when AHY are around the Hawaiian Islands, overlaps with the NESH breeding season. As a result, WTSH and NESH forage in approximately the same areas during similar time periods. Since these two species have such similar ecological niches, the similarities between concentrations of additives and POPs were not surprising. This result builds on the modeling efforts from chapter 3 in that we provide more quantitative evidence for the interspecies comparisons. Statistically speaking, the incidence and concentrations between the contaminants were indistinguishable, so we can understand trends in NESH by sampling Hawaiian WTSH. However, this result should be approached with caution due to the limited sample size, especially with regards to WTSH (van Franeker and Meijborn 2002; National Research Council 2009).

POP Impacts on Body Condition

Both POPs were significantly correlated with body condition with negative relationships (PCB: $S = 1011.1$, $\varrho = -0.805$, $p = 2.90e\text{-}4$; DDE: $S = 1046$, $\varrho = -0.868$, $p = 2.75e\text{-}5$). This result implies that individuals with less fat have higher concentrations of POPs, yet there were no relationships with BUVs. A similar result was found previously with Ryan et al. (1988) finding a negative relationship with PCBs and DDE and the amount of adipose tissue in great shearwaters (*Puffinus gravis*). The negative relationship has two main implications: (i) birds have the same level of POPs, but those with less fat have less tissue to dilute the POPs, or (ii) POP concentrations influence the amount of adipose tissue present. However, further testing is required to differentiate which option is the case. If the same analysis was run using only the GC-MS peaks (rather than the concentrations), and the results were not significantly different between the two groups, then hypothesis (i) would be supported. The non-significant result would imply that the amount of POP found in the tissue was constant, and the amount of fat is what caused the difference. However, if the results are still significantly different, then hypothesis (ii) would be supported, as it would imply that the levels of POPs vary between body condition, irrespective of the fat.

Relationships (or Lack Thereof) with Plastic

Unfortunately, we did not find any significant relationships between any contaminants and plastic loads in either species. BUVs were found in all individuals regardless of the amount of ingested MPD. While initially this may imply there is not correlation between contaminants and ingested MPD (ie: contaminants are so pervasive they will be found regardless), this result instead suggests a more complicated situation than initially hypothesized. There are several factors which we do not fully comprehend which would obscure the nature of this relationship. For instance, PCB presence in preen oil tends to be an underestimate in comparison to the levels in adipose tissue (Yamashita et al. 2007), so we may not be finding relationships relying on preen gland oil alone. If we analyzed fat samples from the same individuals, then we may observe significant relationships.

Furthermore, the residence time of ingested MPD is still greatly debated and unknown. Estimates of retention times range from around 1.5 months (van Franeker & Law 2015) to up to 6 months (Day et al. 1985). While we do have definitive evidence that seabirds break down plastic in their stomachs (Tanaka et al. 2020; Nania & Shugart 2021), it is a very slow process, as shown in Tanaka et al. (2020) who observed a 0.44% decrease in mass over a month in plastic items fed to streaked shearwaters (*Calonectris leucomelas*) chicks. Residence time is complicated by differences in digestive tracts; size of the individual bird; minimum size for particles to pass into the intestine; initial size of the particle; and regurgitation (Ryan 2015). Another complicating factor is our limited understanding of the generation time of preen oil, as well as the influence of external factors such as diet, stress, age, environmental shifts, genetics, and intraspecies interactions (Pacyna-Kuchta 2023; Whittaker et al. 2023). Simply put, we do not know the time delay between plastics found in the digestive tract and appearance in the preen oil. Our preen oil samples may not fully represent the plastic found in the stomach in that it may still have traces of older items that have already passed into the intestines.

To circumvent this issue of retention time, we need to conduct a more thorough and long term analysis of preen oil in reoccurring individuals similar to Ito et al. (2013), who sampled preen oil of streaked shearwater individuals before and after foraging trips. Their study showed that preen oil reflects short term exposure to POPs, but also significant regional differences. If we conduct similar studies with WTSH and NESH, but in regards to plastic additives, we can better understand how preen oil samples represent ingested MPD. Additionally, in case of biomagnification (like with POPs) we should analyze the prey species of NESH and WTSH for BUVs and POPs. If the preen oil doesn’t represent ingested MPD, then their prey may be the source of these contaminants instead. More likely, preen oil represents a combination of the two, so to fully understand the relationship preen oil has with contaminants in NESH and WTSH, we should pursue both options.

Overview and Implications

This is the first study reporting and analyzing the presence and concentration of PCBs, DDEs, and BUVs in the preen oil in Newell's and wedge-tailed shearwaters. Although WTSH preen oil has been analyzed before (Hardesty et al. 2015), different contaminants were analyzed. Our original hypothesis was that WTSH and NESH were expected to have high concentrations (>100 ng/g-lipid) of PCBs, DDE (substituting for DDT), UVP, and UV329, like other seabird species that that mainly consume squid and fish. Although, high concentrations were only found in UV326, we found meaningful concentrations of UVP, UV326, UV328, UV329, UV9, PCBs, and DDE, congruent with previous results of seabirds with similar diets (Yamashita et al. 2021). While we found few statistically significant relationships with conditions of the bird and none with plastic loads, these values are still important to report, and this study creates a foundation for future monitoring efforts. These compounds were found in individuals regardless of MPD loads implying that these contaminants are more ubiquitous and pervasive than individual pieces of MPD for NESH and WTSH.

Future efforts should include analysis of WTSH and NESH prey species for contaminants as well as long term, location-tracked preen oil studies to better understand the involvement of the food web and trends over time of contaminants in the preen oil of these species. To mitigate the limitation in research from sample size, researchers are aiming to develop technologies and methodologies that can be applied to living specimens, as a primary constraint in MPD studies is the heavy reliance on deceased specimens. Although a newer methodology, preen oil is a promising method for monitoring contaminants in NESH and WTSH. Samples can easily be taken by trained individuals at rehabilitation facilities and sent in for analysis, thus enabling long term observation with relatively minimal effort.

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Table 4.1. Preen gland oil sample groups. Each bird (n= 20) falls into multiple categories to maximize comparisons. Good body condition meaning BCI ≥ 6, and poor body condition meaning BCI <6.

	No MPD	Yes MPD	HY	AHY	Good Body Condition	Poor Body Condition
WTSH	0	4	5	0	3	1
NESH	9	7	13	3	9	7
Totals	9	11	18	3	12	8

Table 4.2. Spearman rank (ϱ) correlation results of plastic loads against total PCB concentration.

Group	Metric	Data Set	S	ϱ	p-value
NESH	**Count**	**Proventriculus**	767.85	-0.371	0.173
NESH	**Count**	**Ventriculus**	479.31	0.144	0.608
NESH	**Count**	**Whole Bird**	581.25	-0.0379	0.893
NESH	**Mass**	**Proventriculus**	767.85	-0.371	.173
NESH	**Mass**	**Ventriculus**	512.28	0.0852	0.763
NESH	**Mass**	**Whole Bird**	610.6	-0.0904	0.749
WTSH	**Count**	**Proventriculus**	17.746	-0.775	0.225
WTSH	**Count**	**Ventriculus**	0	1	0.0833
WTSH	**Count**	**Whole Bird**	0	1	0.0833
WTSH	**Mass**	**Proventriculus**	12	-0.2	0.917
WTSH	**Mass**	**Ventriculus**	0	1	0.0833
WTSH	**Mass**	**Whole Bird**	0	1	0.0833

Table 4.3. Spearman rank (ϱ) correlation results of plastic loads against total DDE concentration.

Group	Metric	Data Set	S	ϱ	p-value
NESH	Count	Proventriculus	785.37	-0.402	0.137
		Ventriculus	507.07	0.0945	0.738
		Whole Bird	614.24	-0.0969	0.731
	Mass	Proventriculus	785.37	-0.402	0.137
		Ventriculus	564.89	-0.00872	0.975
		Whole Bird	673.94	-0.203	0.467
WTSH	Count	Proventriculus	12.582	-0.258	0.742
		Ventriculus	2	0.8	0.333
		Whole Bird	2	0.8	0.333
	Mass	Proventriculus	6	0.4	0.75
		Ventriculus	2	0.8	0.333
		Whole Bird	2	0.8	0.333

Table 4.4. Spearman rank (ϱ) correlation results of plastic loads against total BUV concentration.

Group	Metric	Data Set	S	ϱ	p-value
NESH	**Count**	**Proventriculus**	560	0	1
		Ventriculus	512.02	0.0857	0.761
		Whole Bird	521.75	0.0683	0.809
	Mass	**Proventriculus**	560	0	1
		Ventriculus	388.62	0.306	0.267
		Whole Bird	374.48	0.331	0.228
WTSH	**Count**	**Proventriculus**	17.746	-0.775	0.225
		Ventriculus	0	1	0.0833
		Whole Bird	0	1	0.0833
	Mass	**Proventriculus**	12	-0.2	0.917
		Ventriculus	0	1	0.0833
		Whole Bird	0	1	0.0833

Table 4.5. Preen gland oil pollutant principle component PCA contributions. Strongest contributor for each component is bolded.

	PC 1	PC 2
Total [BUV]	6.86	**92.4**
Total [PCB]	45.6	6.09
[DDE]	**47.6**	1.53

Table 4.6. Preen gland oil pollutant principle component PCA contributions with individual BUVs. Strongest contributor for each component is bolded.

	PC 1	PC 2
[UVP]	5.97	11.2
[UV326]	14.0	**32.7**
[UV329]	0.0195	14.8
[UV328]	0.327	2.22
[UV9]	6.84	16.8
Total [PCB]	34.3	12.3
[DDE]	**38.6**	8.97

Figure 4.1. Correlation of PCBs against DDE for both WTSH and NESH. Units are of pollutants in ng/g-lipid. Blue line represents the least squares regression line with a smooth outline.

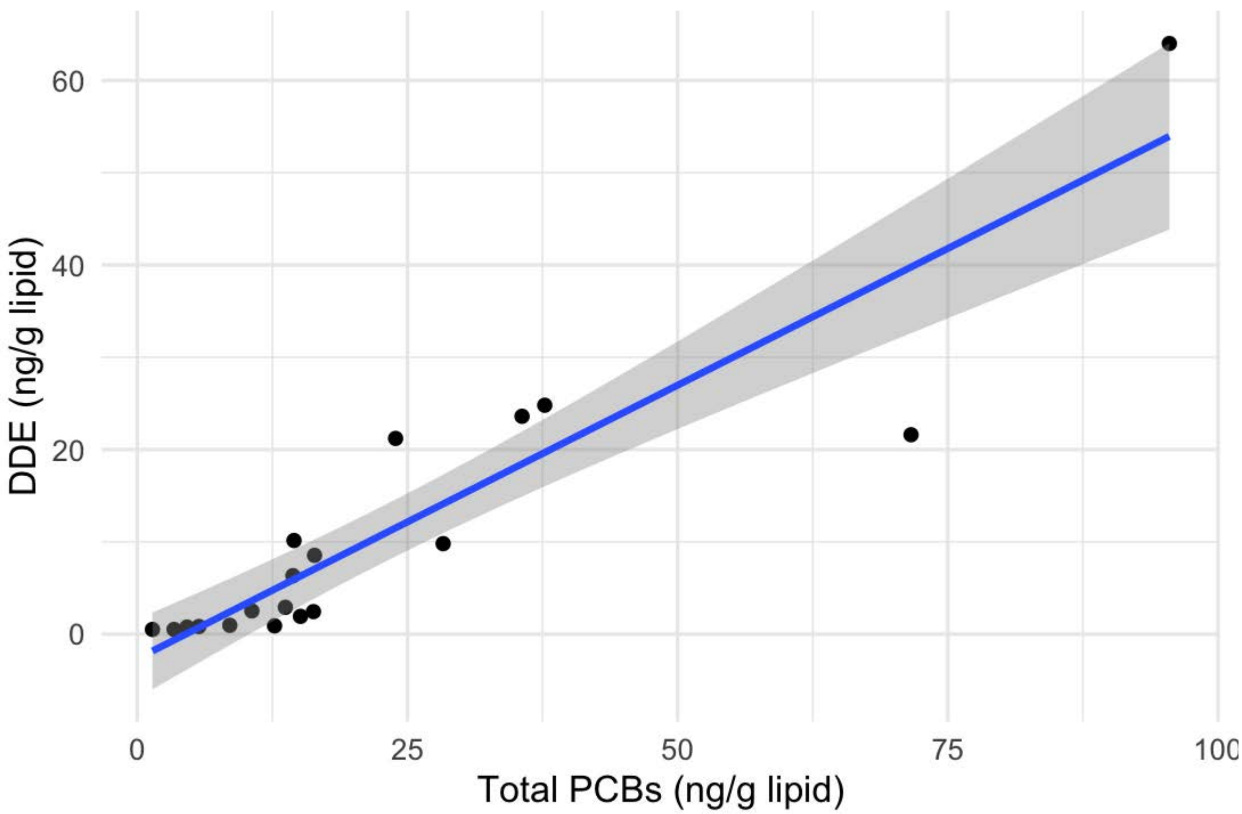


Figure 4.2. Correlation of POPs against body condition of the NESH. Units are of pollutants in ng/g-lipid. Red represents PCBs, and blue represents DDE.

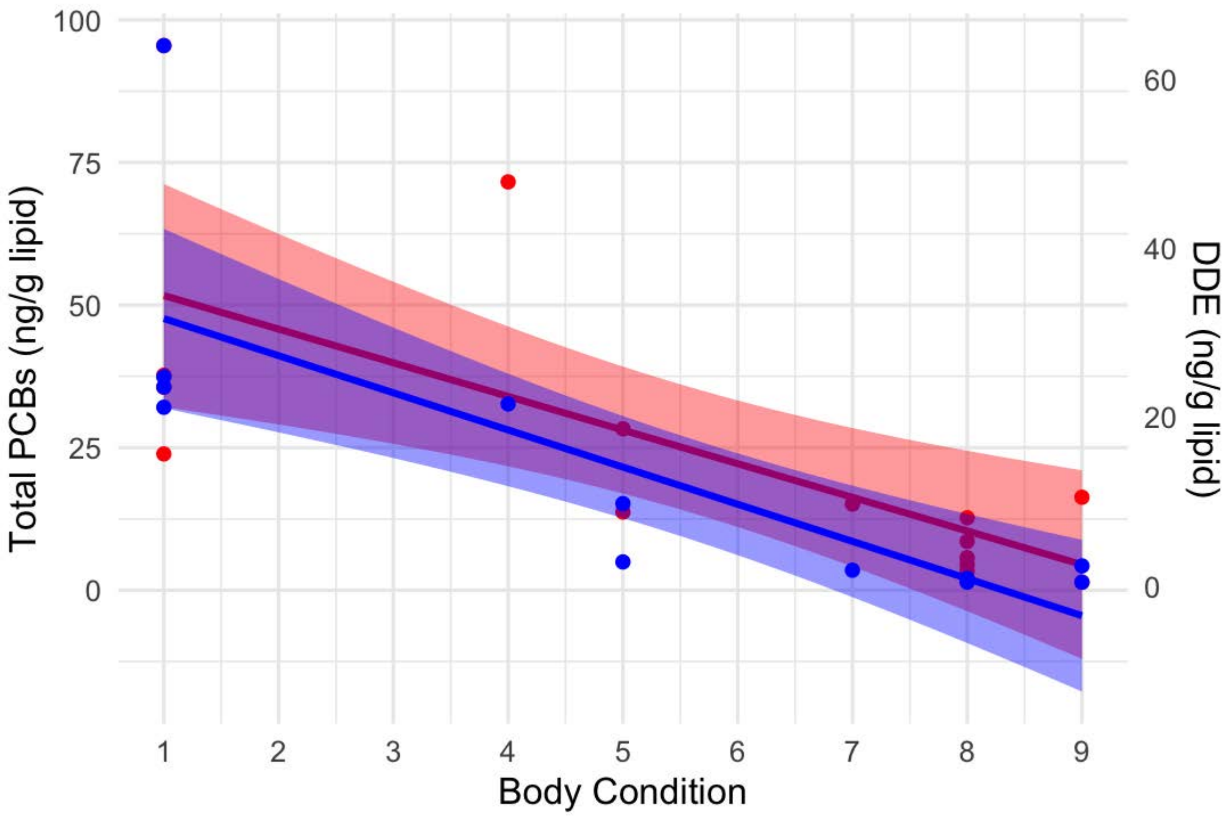


Figure 4.3. Principal component analysis of pollutants in preen gland oil. Dimensions include GC-MS for both WTSH and NESH. Units are of pollutants in ng/g-lipid.

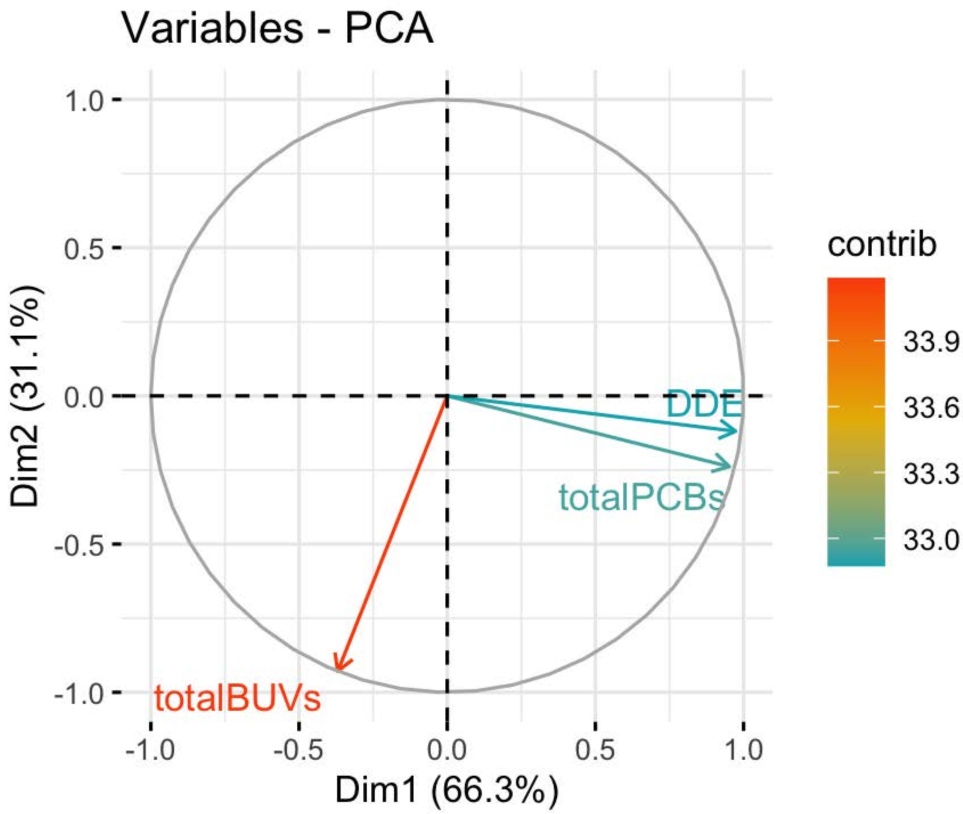
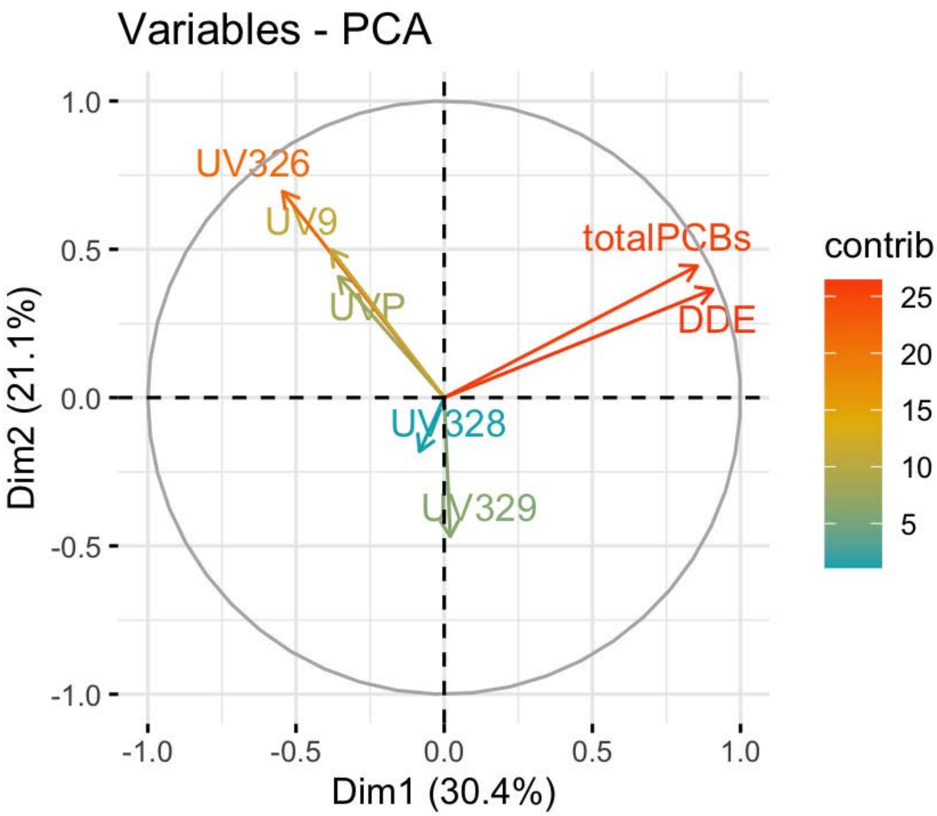


Figure 4.4. Principal component analysis of pollutants in preen gland oil with BUV breakdown. Dimensions include GC-MS for both WTSH and NESH. Units of pollutants are in ng/g-lipid.



THESIS CONCLUSION

This study aimed not only to update plastic ingestion rates and quantities, but also to apply a deeper look into the impacts of plastic ingestion in NESH. This study showed the potential for a causal relationship between plastic and heart mass, which has unknown health implications for the birds. As stated previously, plastic causes long-term impacts on long-living individuals, exemplified by the significant relationships with heart mass. To explore impacts of MPD with unknown lethality, this study analyzed preen oil for contaminants (BUVs, PCBs, and DDE), being the first and only study to do so for NESH. Since all contaminants were detected in every individual sampled irrespective of MPD load, these compounds are more pervasive than previously believed. Furthermore, technological advancements in plastic technology refined Sp-FO estimates, with this study being the first to document plastic ingestion in adult NESH. MPD can potentially scale up to population-level impacts when both AHY and HY birds ingest high quantities of MPD. Therefore, plastic ingestion studies for critically endangered species are imperative.

Another primary goal this study was to document the potential of wedge-tailed shearwaters to be a bioindicator species for the Main Hawaiian Islands and for Newell's shearwaters, a closely-related, critically endangered species endemic to the Hawaiian Islands. When comparing the observed niches of the two species around the Hawaiian Islands, WTSH and NESH greatly overlap consuming the same prey, in similar areas, during the similar times of the year. This study attempted to take this comparison to a more objective and quantitative level, due to the differences in MPD ingestion rates and loads. After analyzing dozens of GLMs, we can confidently say that WTSH can be used to represent NESH in regards to trends in plastic ingestion. This representation is exemplified by the analysis of preen oil. There were no significant differences in concentrations of contaminants in the preen oil between the two species, therefore there is potential for observation of trends in NESH by sampling Hawaiian WTSH, and visa versa.

USFWS acknowledges the need for protection and conservation efforts of NESH, but fails to recognize that NESH ingest plastic, much less POP contaminations and the potential health and population impacts of MPD and POP ingestion. All anthropogenic impacts on this critically endangered species should be considered, not just the most visual. Thus, it is my hope that this study will motivate an update in the UFSWS recovery plan for the Newell's shearwater, to address MPD ingestion and POP contamination, their sublethal impacts, and the investigation of the potential for biomagnification, potentially using WTSH as a bioindicator. Ignorance of these anthropogenic threats against NESH should not lead to negligence of conservation efforts.

APPENDIX: SUPPLEMENTARY TABLES AND FIGURES

Supplementary Table 2.1. GLM model results for HY NESH plastic count data. Proventriculus models were compared against each other; ventriculus models against each other; whole bird models against each other; organ models against each other.

	Proventriculus			Ventriculus			Whole Bird		
Drivers	**Pr(>|z|)**	**ΔAICc**	**Weight**	**Pr(>|z|)**	**ΔAICc**	**Weight**	**Pr(>|z|)**	**ΔAICc**	**Weight**
Beaks	0.355	0.00	25.1%	0.0873	0.00	36.3%	0.421	0.00	25.8%
Year	0.636	0.318	21.4%	0.403	1.79	14.8%	0.536	0.135	24.1%
MEI	0.622	0.316	21.4%	1.00	2.44	10.7%	0.888	0.472	20.3%
Organ	--	--	--	--	--	--	0.0005	0.730	21.9%
Beaks + Year	0.281, 0.553	1.88	9.8%	0.101, 0.470	1.69	15.6%	0.459, 0.580	1.91	20.3%
Beaks + MEI	0.338, 0.621	1.99	9.3%	0.0868, 0.938	2.20	12.1%	0.420, 0.876	2.18	9.9%
Organ + Beaks	--	--	--	--	--	--	0.066, 0.064	0.00	31.5%
Year + MEI	0.509, 0.487	2.09	8.8%	0.346, 0.715	3.87	5.3%	0.533, 0.915	2.33	8.0%
Organ + Year	--	--	--	--	--	--	0.0005, 0.620	2.59	8.6%
Organ +MEI	--	--	--	--	--	--	0.0005, 0.830	2.78	7.8%
Beaks + Year + MEI	0.189, 0.410, 0.441	3.62	4.1%	0.104, 0.440, 0.809	3.90	5.2%	0.462, 0.586, 0.946	4.17	3.2%
Organ + Beaks + Year	--	--	--	--	--	--	0.0670, 0.0704, 0.694	1.97	11.8%
Organ + Beaks + MEI	--	--	--	--	--	--	0.0670, 0.0631, 0.780	2.05	11.3%
Organ + Year + MEI	--	--	--	--	--	--	0.0005, 0.649, 0.980	4.72	3.0%
Organ + Beaks + Year + MEI	--	--	--	--	--	--	0.0673, 0.0691, 0.755, 0.894	4.11	4.0%

Supplementary Table 2.2. GLM model results for HY NESH plastic mass data. Proventriculus models were compared against each other; ventriculus models against each other; whole bird models against each other; organ models against each other.

	Proventriculus			Ventriculus			Whole Bird		
Drivers	**Pr(>|z|)**	**ΔAICc**	**Weight**	**Pr(>|z|)**	**ΔAICc**	**Weight**	**Pr(>|z|)**	**ΔAICc**	**Weight**
Beaks	0.778	10.9	0.3%	0.319	4.26	3.4%	0.594	3.66	2.0%
Year	0.078	0.00	75.1%	0.594	17.8	0.1%	0.257	6.40	0.5%
MEI	0.629	17.7	0.0%	0.231	12.45	0.0%	0.479	1.67	5.4%
Organ	--	--	--	--	--	--	0.162	42.53	0.0%
Beaks + Year	0.564, 0.0783	5.74	12.5%	0.238, 0.384	0.00	83.3%	0.577, 0.248	4.13	4.6%
Beaks + MEI	0.761, 0.606	16.57	0.1%	0.429, 0.325	9.32	0.8%	0.554, 0.454	0.00	36.6%
Organ + Beaks	--	--	--	--	--	--	0.465, 0.492	38.03	0.0%
Year + MEI	0.0154, 0.149	5.82	11.9%	0.716, 0.292	12.98	0.1%	0.324, 0.673	4.30	4.2%
Organ + Year	--	--	--	--	--	--	0.0619, 0.0853	13.78	0.0%
Organ +MEI	--	--	--	--	--	--	0.177, 0.713	38.85	0.0%
Beaks + Year + MEI	0.386, 0.0174, 0.129	26.59	0.0%	0.337, 0.524, 0.413	6.03	12.3%	0.550, 0.320, 0.639	1.73	46.5%
Organ + Beaks + Year	--	--	--	--	--	--	0.290, 0.246, 0.0562	0.40	45.0%
Organ + Beaks + MEI	--	--	--	--	--	--	0.483, 0.510, 0.747	35.33	0.0%
Organ + Year + MEI	--	--	--	--	--	--	0.0577, 0.112, 0.876	14.24	0.0%
Organ + Beaks + Year + MEI	--	--	--	--	--	--	0.272, 0.241, 0.0747, 0.795	0.00	54.9%

Supplementary Table 2.3. GLM model results for AHY NESH plastic count data. Proventriculus models were compared against each other; ventriculus models against each other; whole bird models against each other; organ models against each other.

	Proventriculus			Ventriculus			Whole Bird		
Drivers	**Pr(>|z|)**	**ΔAICc**	**Weight**	**Pr(>|z|)**	**ΔAICc**	**Weight**	**Pr(>|z|)**	**ΔAICc**	**Weight**
Beaks	0.879	2.104	14.5%	0.849	0.454	24.1%	0.525	0.097	24.6%
Year	0.639	1.928	15.8%	0.547	0.000	30.2%	0.600	0.021	25.5%
MEI	0.254	0.000	41.4%	0.850	0.448	24.2%	0.524	0.000	25.8%
Organ	--	--	--	--	--	--	0.210	0.00	40.7%
Beaks + Year	0.891, 0.645	4.868	3.6%	0.762, 0.546	2.910	7.1%	0.437, 0.570	2.630	6.9%
Beaks + MEI	0.785, 0.224	2.847	10.0%	0.802, 0.817	3.378	5.6%	0.525, 0.535	2.732	6.6%
Organ + Beaks	--	--	--	--	--	--	0.296, 0.872	2.39	12.3%
Year + MEI	0.631, 0.554	2.465	12.1%	0.486, 0.661	2.810	7.4%	0.386, 0.347	2.199	8.6%
Organ + Year	--	--	--	--	--	--	0.220, 0.701	2.24	13.3%
Organ +MEI	--	--	--	--	--	--	0.179, 0.372	1.75	17.0%
Beaks + Year + MEI	0.687, 0.576, 0.513	5.469	2.7%	0.661, 0.481, 0.615	6.028	1.5%	0.411, 0.384, 0.349	5.152	2.0%
Organ + Beaks + Year	--	--	--	--	--	--	0.320, 0.832, 0.695	4.74	3.8%
Organ + Beaks + MEI	--	--	--	--	--	--	0.296, 0.682, 0.344	4.21	5.0%
Organ + Year + MEI	--	--	--	--	--	--	0.193, 0.460, 0.289	3.74	6.3%
Organ + Beaks + Year + MEI	--	--	--	--	--	--	0.347, 0.568, 0.452, 0.259	6.28	1.8%

Supplementary Table 2.4. GLM model results for AHY NESH plastic mass data. Proventriculus models were compared against each other; ventriculus models against each other; whole bird models against each other; organ models against each other.

	Proventriculus			Ventriculus			Whole Bird		
Drivers	**Pr(>|z|)**	**ΔAICc**	**Weight**	**Pr(>|z|)**	**ΔAICc**	**Weight**	**Pr(>|z|)**	**ΔAICc**	**Weight**
Beaks	0.301	162	0.0%	0.381	4.95	2.4%	0.432	8.98	0.5%
Year	0.630	0.00	99.7%	0.4460	0.25	25.0%	0.473	0.16	40.1%
MEI	0.0577	11.9	0.3%	0.880	2.31	8.9%	0.895	3.78	6.6%
Organ	--	--	--	--	--	--	0.0061	5.12	4.4%
Beaks + Year	0.239, 0.445	169	0.0%	0.332, 0.428	1.94	10.7%	0.461, 0.620	4.71	4.1%
Beaks + MEI	< 2e-16, 4.43e-16	220.	0.0%	0.392, 0.931	4.71	2.7%	0.312, 0.488	9.14	0.5%
Organ + Beaks	--	--	--	--	--	--	0.0058, 0.0955	43.83	0.0%
Year + MEI	0.352, 0.254	77.3	0.0%	0.438, 0.885	0.00	28.3%	0.486, 0.889	0.00	43.6%
Organ + Year	--	--	--	--	--	--	0.0055, 0.413	1.06	33.6%
Organ +MEI	--	--	--	--	--	--	6.39e-3, 0.936	4.89	4.9%
Beaks + Year + MEI	< 2e-16, 5.29e-3, 0.0210	229	0.0%	0.290, 0.335, 0.624	0.51	21.9%	0.266, 0.506, 0.354	4.48	4.6%
Organ + Beaks + Year	--	--	--	--	--	--	3.78e-3, 0.0635, 0.359	36.07	0.0%
Organ + Beaks + MEI	--	--	--	--	--	--	2.88e-3, 0.0488, 0.398	43.31	0.0%
Organ + Year + MEI	--	--	--	--	--	--	4.97e-3, 0.370, 0.681	0.00	57.1%
Organ + Beaks + Year + MEI	--	--	--	--	--	--	9.81e-4, 0.0129, 0.163, 0.140	33.99	0.0%

SupplementaryTable 3.1. GLM model results for HY WTSH plastic count data. Proventriculus models were compared against each other; ventriculus models against each other; whole bird models against each other; organ models against each other.

	Proventriculus			Ventriculus			Whole Bird		
Drivers	**Pr(>|z|)**	**ΔAICc**	**Weight**	**Pr(>|z|)**	**ΔAICc**	**Weight**	**Pr(>|z|)**	**ΔAICc**	**Weight**
Beaks	3.89e-9	6.87	1.7%	0.0020	0.449	30.4%	1.92e-5	2.20	15.1%
Year	0.0161	30.9	0.0%	0.342	9.16	0.4%	0.0391	15.0	0.0%
MEI	0.9113	36.9	0.0%	0.319	9.12	0.4%	0.404	18.7	0.0%
Organ	--	--	--	--	--	--	4.15e-4	34.5	0.0%
Beaks + Year	4.73e-9, 0.00571	0.46	43.0%	2.81e-3, 0.673	2.34	11.8%	6.67e-05, 0.138	1.92	17.3%
Beaks + MEI	2.43e-9, 0.201	7.60	1.2%	6.98e-4, 0.0979	0.00	38.1%	4.54e-6, 0.0691	1.41	22.4%
Organ + Beaks	--	--	--	--	--	--	0.678, 4.87e-8	5.56	4.5%
Year + MEI	0.0190, 0.720	32.9	0.0%	0.215, 0.197	9.65	0.3%	0.0254, 0.197	15.5	0.0%
Organ + Year	--	--	--	--	--	--	1.66e-4, 0.0116	30.2	0.0%
Organ +MEI	--	--	--	--	--	--	4.4e-4, 0.429	36.0	0.0%
Beaks + Year + MEI	1.52e-9, 3.20e-3, 0.0877	0.00	54.1%	1.12e-3, 0.423, 0.0744	1.43	18.6%	1.37e-5, 0.0731, 0.0347	0.00	45.2%
Organ + Beaks + Year	--	--	--	--	--	--	0.499, 2.02e-7, 0.0456	3.35	13.6%
Organ + Beaks + MEI	--	--	--	--	--	--	0.839, 7.02e-9, 0.0411	4.01	9.8%
Organ + Year + MEI	--	--	--	--	--	--	1.72e-4, 7.29e-3, 0.180	30.5	0.0%
Organ + Beaks + Year + MEI	--	--	--	--	--	--	0.640, 2.15e-8, 0.0184, 0.0142	0.00	72.2%

Supplementary Table 3.2. GLM model results for HY WTSH plastic mass data. Proventriculus models were compared against each other; ventriculus models against each other; whole bird models against each other; organ models against each other.

	Proventriculus			Ventriculus			Whole Bird		
Drivers	**Pr(>|z|)**	**ΔAICc**	**Weight**	**Pr(>|z|)**	**ΔAICc**	**Weight**	**Pr(>|z|)**	**ΔAICc**	**Weight**
Beaks	6.54E-06	20.1	0.0%	0.274	7.26	1.3%	1.45E-03	37.43	0.0%
Year	0.153	71.7	0.0%	0.203	3.795	7.4%	0.432	60.37	0.0%
MEI	0.474	56.0	0.0%	0.047	3.21	9.9%	0.130	61.69	0.0%
Organ	--	--	--	--	--	--	0.670	99.67	0.0%
Beaks + Year	1.69e-6, 0.0466	6.46	3.6%	0.150, 0.117	3.08	10.6%	7.51e-4, 0.298	19.85	0.0%
Beaks + MEI	6.32e-7, 0.0675	0.00	89.9%	0.124, 0.0222	4.13	6.4%	9.6e-5, 8.36e-3	16.57	0.0%
Organ + Beaks	--	--	--	--	--	--	0.402, 1.79e-6	39.46	0.0%
Year + MEI	0.168, 0.490	79.3	0.0%	0.426, 0.0947	2.39	15.0%	0.332, 0.108	63.24	0.0%
Organ + Year	--	--	--	--	--	--	0.762, 0.433	98.58	0.0%
Organ +MEI	--	--	--	--	--	--	0.633, 0.105	93.80	0.0%
Beaks + Year + MEI	4.30e-7, 0.0418, 0.0621	7.31	6.6%	0.0835, 0.285, 0.0525	0.00	48.4%	2.84e-5, 0.140, 3.85e-3	0.00	100.0%
Organ + Beaks + Year	--	--	--	--	--	--	0.494, 2.01e-6, 0.417	28.46	0.0%
Organ + Beaks + MEI	--	--	--	--	--	--	0.325, 4.85e-8, 4.57e-3	5.82	5.2%
Organ + Year + MEI	--	--	--	--	--	--	0.739, 0.325, 0.0879	101.48	0.0%
Organ + Beaks + Year + MEI	--	--	--	--	--	--	6.12e-8, 0.222, 3.17e-3	0.00	94.8%

Supplementary Table 3.3. GLM model results for AHY WTSH plastic count data. Proventriculus models were compared against each other; ventriculus models against each other; whole bird models against each other; organ models against each other.

	Proventriculus			Ventriculus			Whole Bird		
Drivers	**Pr(>|z|)**	**ΔAICc**	**Weight**	**Pr(>|z|)**	**ΔAICc**	**Weight**	**Pr(>|z|)**	**ΔAICc**	**Weight**
Beaks	0.5590	0.260	18.4%	0.0881	0.000	44.0%	0.0020	0.00	54.4%
Year	0.2900	0.059	20.4%	0.880	3.124	9.2%	0.614	10.15	0.3%
MEI	0.5970	0.377	17.4%	0.904	3.131	9.2%	0.852	10.32	0.3%
Organ	--	--	--	--	--	--	0.168	0.00	27.8%
Beaks + Year	0.462, 0.236	1.767	8.7%	0.0842, 0.878	2.172	14.9%	2.08e-3, 0.940	2.19	18.2%
Beaks + MEI	0.624, 0.684	2.333	6.5%	0.0870, 0.951	2.192	14.7%	0.002, 0.765	2.10	19.0%
Organ + Beaks	--	--	--	--	--	--	0.109, 0.285	0.83	18.3%
Year + MEI	0.0979, 0.161	0.000	21.0%	0.931, 0.992	5.320	3.1%	0.301, 0.365	11.42	0.2%
Organ + Year	--	--	--	--	--	--	0.153, 0.478	1.71	11.8%
Organ +MEI	--	--	--	--	--	--	0.161, 0.747	2.00	10.2%
Beaks + Year + MEI	0.644, 0.102, 0.183	2.020	7.6%	0.0826, 0.833, 0.887	4.402	4.9%	2.48e-3, 0.497, 0.479	3.95	7.6%
Organ + Beaks + Year	--	--	--	--	--	--	0.109, 0.315, 0.612	2.76	7.0%
Organ + Beaks + MEI	--	--	--	--	--	--	0.0982, 0.265, 0.647	2.76	7.0%
Organ + Year + MEI	--	--	--	--	--	--	0.0821, 0.126, 0.173	1.81	11.2%
Organ + Beaks + Year + MEI	--	--	--	--	--	--	0.0625, 0.310, 0.145, 0.167	2.89	6.6%

Supplementary Table 3.4. GLM model results for AHY WTSH plastic mass data. Proventriculus models were compared against each other; ventriculus models against each other; whole bird models against each other; organ models against each other.

	Proventriculus			Ventriculus			Whole Bird		
Drivers	**Pr(>|z|)**	**ΔAICc**	**Weight**	**Pr(>|z|)**	**ΔAICc**	**Weight**	**Pr(>|z|)**	**ΔAICc**	**Weight**
Beaks	0.301	162	0.0%	0.381	4.95	2.4%	0.273	0.44	19.1%
Year	0.630	0.00	99.7%	0.4460	0.25	25.0%	0.203	0.00	23.8%
MEI	0.0577	11.9	0.3%	0.880	2.31	8.9%	0.575	1.27	12.6%
Organ	--	--	--	--	--	--	0.717	0.05	23.6%
Beaks + Year	0.239, 0.445	169	0.0%	0.332, 0.428	1.94	10.7%	0.255, 0.191	1.01	14.3%
Beaks + MEI	< 2e-16, 4.43e-16	220.	0.0%	0.392, 0.931	4.71	2.7%	0.326, 0.755	2.59	6.5%
Organ + Beaks	--	--	--	--	--	--	0.700, 0.618	1.91	9.3%
Year + MEI	0.352, 0.254	77.3	0.0%	0.438, 0.885	0.00	28.3%	0.158, 0.423	1.60	10.7%
Organ + Year	--	--	--	--	--	--	0.653, 0.139	0.00	24.2%
Organ +MEI	--	--	--	--	--	--	0.744, 0.530	1.77	10.0%
Beaks + Year + MEI	< 2e-16, 0.00529, 0.02100	229	0.0%	0.290, 0.335, 0.624	0.51	21.9%	0.0945, 0.0502, 0.147	1.21	13.0%
Organ + Beaks + Year	--	--	--	--	--	--	0.626, 0.572, 0.132	1.82	9.7%
Organ + Beaks + MEI	--	--	--	--	--	--	0.714, 0.498, 0.434	3.45	4.3%
Organ + Year + MEI	--	--	--	--	--	--	0.529, 0.0879, 0.305	1.08	14.1%
Organ + Beaks + Year + MEI	--	--	--	--	--	--	0.544, 0.847, 0.118, 0.381	3.21	4.8%