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Community-wide patterns of plastic ingestion in seabirds breeding at French Frigate Shoals, Northwestern Hawaiian Islands☆

Dan C. Rapp[a,b], Sarah M. Youngren[a,b], Paula Hartzell[c], K. David Hyrenbach[a,b,*]

[a] *Hawaii Pacific University, Marine Science Programs at Oceanic Institute, 41-202 Kalanianaole Highway, Waimanalo, HI 96795, USA*
[b] *Oikonos Ecosystem Knowledge, P.O. Box 1918, Kailua, HI 96734, USA*
[c] *U.S. Fish and Wildlife Service, 300 Ala Moana Boulevard, Honolulu, HI 96850, USA*

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ABSTRACT

Between 2006 and 2013, we salvaged and necropsied 362 seabird specimens from Tern Island, French Frigate Shoals, Northwestern Hawaiian Islands. Plastic ingestion occurred in 11 of the 16 species sampled (68.75%), representing four orders, seven families, and five foraging guilds: four plunge-divers, two albatrosses, two nocturnal-foraging petrels, two tuna-birds, and one frigatebird. Moreover, we documented the first instance of ingestion in a previously unstudied species: the Brown Booby. Plastic prevalence (percent occurrence) ranged from 0% to 100%, with no significant differences across foraging guilds. However, occurrence was significantly higher in chicks versus adult conspecifics in the Black-footed Albatross, one of the three species where multiple age classes were sampled. While seabirds ingested a variety of plastic (foam, line, sheets), fragments were the most common and numerous type. In albatrosses and storm-petrels, the plastic occurrence in the two stomach chambers (the proventriculus and the ventriculus) was not significantly different.

1. Introduction

Seabirds are valuable biological indicators of changing marine ecosystems over short and long time scales, including the spatial distributions and temporal trends in pollutants (Burger and Gochfeld, 2004; Finkelstein et al., 2006; Gaston et al., 2009; Wilcox et al., 2015). In particular, due to the broad range of trophic levels, feeding guilds, and foraging habitats, different seabird species sample distinct oceanographic domains and food web components (Day et al., 1985; Sileo et al., 1989; Hyrenbach et al., 2009; Titmus and Hyrenbach, 2011). Furthermore, because seabirds breed on land, often in large colonies, they are readily accessible for study. In particular, oceanic islands with large numbers of concurrently breeding species are ideal sites for comparative studies of trophic ecology and pollutant loads across foraging guilds (Sileo et al., 1989; Robards et al., 1995; Keller et al., 2009; Winship et al., 2016). While previous studies have documented the widespread prevalence of plastic debris and other associated pollutants in seabirds (Tanaka et al., 2013; Lavers et al., 2014; Wilcox et al., 2015; Provencher et al., 2017), quantifying the occurrence and loads of plastic ingestion by seabird populations remains a research priority, and an important step for understanding the impacts of these pollutants on marine food webs (Lewison et al., 2012; Vegter et al., 2014).

Seabirds are increasingly being used as biological sensors of the levels and trends in marine plastic pollution (Ryan et al., 2009; Galgani et al., 2013; Wilcox et al., 2015; Provencher et al., 2017). For instance, the OSPAR commission (Oslo and Paris Conventions), comprising 15 European governments and the European Union (EU), established Ecological Quality Objectives (EcoQOs) for monitoring ecosystem health in the North Sea (ICES-WGSE, 2001). These EcoQOs establish an acceptable marine plastic debris target, defined as $<$ 10% of Northern Fulmars (*Fulmarus glacialis*) having $>$ 0.1 g of plastic in their stomach contents, which is quantified using 50–100 beached birds sampled over a five-year period. While this objective has not been met in all the monitored areas of the North Sea, it has provided a metric for quantifying plastic pollution trends over time (van Franeker et al., 2011). More recently, the European Commission identified trends in the amount and composition of litter ingested by marine animals as one of the four focus areas for marine debris monitoring, under the auspices of the European Marine Strategy Framework Directive (MSFD, 2008/56/EC) (Galgani et al., 2014). This decision (2010/477/EU) sets a precedent that could be adopted in other ocean regions afflicted by plastic pollution (e.g., Lavers and Bond, 2016).

Tons of floating marine plastic debris wash ashore on the Hawaiian archipelago every year, driven by large-scale oceanographic processes (Morishige et al., 2007; Barnes et al., 2009; Ribic et al., 2011). Located

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* Corresponding author at: Hawaii Pacific University, Marine Science Programs at Oceanic Institute, 41-202 Kalanianaole Highway, Waimanalo, HI 96795, USA.
E-mail address: khyrenbach@hpu.edu (K. David Hyrenbach).

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within the North Pacific subtropical gyre, roughly equidistant between America and Asia, the waters surrounding the Hawaiian archipelago are influenced by large-scale (1000s km) and small-scale (10s km) oceanographic features, which transport and accumulate marine debris (Howell et al., 2012; van Sebille et al., 2012; Cózar et al., 2014). Recent modeling efforts estimated that the North Pacific contains the greatest number and weight of floating plastic of the world's oceans (199×10^{10} pieces; 964×10^2 tons). In fact, the magnitude of plastic deposition in the Northwest Hawaiian Islands (NWHI) is so great that the Center for Biological Diversity has nominated the region as a superfund cleanup site (Rochman et al., 2013; Eriksen et al., 2014).

The rocky islets and coral atolls in the NWHI comprise some of the most important seabird colonies in the world, providing nesting habitat for roughly 5.5 million seabirds encompassing 22 species, and supporting over 99% and 95% of the global Laysan Albatross (LAAL, *Phoebastria immutabilis*) and Black-footed Albatross (BFAL, *Phoebastria nigripes*) breeding populations, respectively (Keller et al., 2009). A previous survey of plastic ingestion by Hawaiian seabirds breeding in the NWHI completed in 1986–1987, involving 1757 samples from 16 species sampled over five sites (Midway Island, Laysan Island, Tern Island, Nihoa Island, Pearl and Hermes Reef), documented widespread exposure, with 13 species ingesting plastic (Sileo et al., 1989). Yet, despite mounting evidence of increasing marine debris in the North Pacific, there has been no systematic survey of plastic ingestion by Hawaiian seabird populations in the last three decades.

This study aimed to characterize the occurrence and loads of ingested plastic by opportunistically necropsying naturally-deceased seabirds of multiple age classes (chicks, juveniles, adults) for the species nesting in the NWHI. More specifically, the goals of this study were to: (1) document current (2006–2013) community-wide patterns of plastic ingestion in the seabird species breeding on French Frigate Shoals (FFS); (2) establish a baseline for future standardized monitoring of plastic ingestion by NWHI seabirds; and (3) whenever sample sizes and methodologies allowed, compare current ingestion levels with historical records (1980s). Additionally, to inform future monitoring, we addressed three potential biases influencing the quantification of plastic ingestion rates, relating to: (1) differences in age classes within species; (2) ingestion of different plastic types (fragment, foam, line, sheet); and (3) disparities in plastic prevalence within the two stomach chambers (proventriculus and ventriculus) of tubenose species (belonging to the order Procellariiformes).

2. Methods

2.1. Study site

FFS, located at 23.870°N 166.284°W, is the largest atoll in the NWHI, lying roughly at the midpoint of the 2575 km long Hawaiian archipelago. Thirteen small named islands exist within FFS, most of which are shifting sandy spits. Tern Island (TI) is the largest island, with an area of 105,276 m^2 (26.014 acres), and a vegetation dominated by salt-tolerant and drought-resistant plants, characteristic of beach strand and coastal scrub habitats. FFS hosts populations of 19 of the 22 seabird species that breed in the NWHI, making it an ideal location for community-wide assessments. In particular, 17 of these species breed on TI, the site with of a U.S. Fish and Wildlife Service (USFWS) field station (Keller et al., 2009).

2.2. Specimen collections and necropsy

This opportunistic study targeted all age classes (chick/immature/adult) of all locally-breeding seabird species (Table 1). Age classes were defined following the criteria previously used by Sileo et al. (1989): C = chick (pre-fledging), J = juveniles (immature birds capable of sustained flight), A = adult (mature), and B = immature or adult (unclear if I or A). Age classes were assigned using three criteria (plumage, morphometrics, development of sexual organs), on the basis of species-specific breeding phenology and life history (Pyle, 1997; Pyle, 2008; Howell, 2010). Whenever possible, USFWS banding records were used to validate the age class assignments. To facilitate broader ecological comparisons, the species were also classified into six foraging guilds, following previous categorizations (Harrison et al., 1983; Dearborn et al., 2001). For the sake of consistency, we refer to the species using the American Ornithological Union species four-letter codes throughout the text, and in all tables and figures (Table 1, Chesser et al., 2016).

A total of 362 naturally-deceased seabirds of 16 species were collected opportunistically from TI starting in 2006, with specimens from 2010 to 2012 representing the bulk (98%) of the collections. While large samples sizes (≥ 20 specimens; NRC, 2009) were sought for all age classes of the 17 locally-breeding species, the sample sizes varied greatly across different species * age groups, due to ecological and logistical limitations. Namely, because the species vary in overall abundance and breeding phenology, in relation to the field work season (November–July) (Keller et al., 2009). Moreover, chicks were much more readily available than adults, due to their higher mortality rates. Thus, while some species * age groups surpassed our target (20 specimens), others yielded smaller sample sizes (Table 1).

All specimens were coded for freshness and completeness, upon collection (van Franeker, 2004). While specimens of different freshness levels were sampled, ranging from recently dead “very fresh” (FFF code) to “very old” (OOO code), only complete specimens were included in this study. Incomplete birds with signs of scavenging by crabs or missing parts, were discarded. Complete specimens with ruptured abdomens (i.e. albatross chicks) or stomachs (i.e., Tristram's Storm-petrel, TRSP, *Oceanodroma tristrami*) were collected, as long as the ingested plastics could be recovered from the bird's esophagus or by rinsing the body cavity. Specimens were necropsied in the field or frozen and returned to the lab in Oahu. All necropsies followed standardized protocols and were completed by trained personnel, with one of the authors in the lead (Work, 2000; van Franeker, 2004).

2.3. Stomach dissection and content processing

For each specimen, the stomach was removed and dissected using standardized protocols, and the gastrointestinal tracts were stored frozen prior to sorting and quantification in the lab (van Franeker, 2004; Barrett et al., 2007). To investigate plastic retention in Procellariiform species (albatrosses, petrels, shearwaters, storm-petrels) with two distinct stomach chambers, the contents of the proventriculus and ventriculus (or gizzard) were kept separate, whenever possible, for quantification (Ryan and Jackson, 1987; Youngren et al., submitted). For all other species with non-distinct stomach chambers (frigatebirds, boobies, terns, noddies), all stomach contents were analyzed together.

Stomach contents were sieved through a series of stacked 8-in. brass sieves (ASTM E-11) with 8-in. O-ring gaskets to seal between adjacent sieves and an 8-in. plastic bucket for drain. The topmost 0.5 mm sieve served as an aerator to prevent splashing/loss of contents. Contents were placed on the second 0.5 mm sieve, and the drain bucket caught water from the stacked sieves, and directed it to a 1 mm mesh catch used to ensure that plastic contents were not being lost in the stacked sieves. A hose attached to a sink faucet provided a high-pressure water source. This protocol was modified to deal with the small fragments ingested by TRSP, whereby the stomach samples were filtered using paper filters, rinsed and sorted by SMY (Youngren et al., submitted). All other samples were cleaned and sorted by DCR.

The sieved stomach contents were placed in water for further cleaning and sorting using light magnification (2 ×, 5 ×) and high power magnification under a binocular dissecting microscope (10–40 ×) (Motic Digital). All plastics were separated from the other stomach contents, and any remaining fouling was gently wiped away from the plastics. When needed, a small jewelry cleaner (35 W; 42,000

Table 1

Plastic prevalence for sampled species * age groups, calculated as the percentage (%) of specimens that ingested plastic, with S.D.s calculated using binomial probabilities (for species * age classes where sample size ≥ 8 birds). Age classes defined following Sileo et al. (1989): C = chick, J = juvenile, A = adult, and B = unclear if juvenile or adult (J or A). Sixteen species were considered: BFAL = Black-footed Albatross (*Phoebastria nigripes*), LAAL = Laysan Albatross (*Phoebastria immutabilis*), BRBO = Brown Booby (*Sula leucogaster*), MABO = Masked Booby (*Sula dactylatra*), RFBO = Red-footed Booby (*Sula sula*), RTTR = Red-tailed Tropicbird (*Phaethon rubricauda*), GREF = Great Frigatebird (*Fregata minor*), BLNO = Black Noddy (*Anous minutus*), BRNO = Brown Noddy (*Anous stolidus*), SOTE = Sooty Tern (*Onychoprion fuscata*), WHTT = White Tern (*Gygis alba*), WTSH = Wedge-tailed Shearwater (*Ardenna pacifica*), BOPE = Bonin Petrel (*Pterodroma hypoleuca*), BUPE = Bulwer's Petrel (*Bulweria bulwerii*), TRSP = Tristram's Storm-petrel (*Oceanodroma tristrami*), GBAT = Gray-backed Tern (*Onychoprion lunatus*).

Foraging guild	Seabird species	Age class	Birds sampled	Birds with plastic	Prevalence (%)	S.D. (%)
Albatrosses	BFAL	C	28	27	96.4	3.6
		A	17	10	58.8	12.3
	LAAL	C	107	104	97.2	1.6
		A	19	17	89.5	7.2
Plunge-divers	BRBO	J	2	1	50.0	–
		B	1	0	0.0	–
	MABO	C	1	1	100.0	–
		B	1	0	0.0	–
	RFBO	C	1	0	0.0	–
		J	11	1	9.1	9.1
		A	7	0	0.0	–
	RTTR	C	3	1	33.3	–
		A	2	0	0.0	–
Frigatebirds	GREF	J	31	13	41.9	9.0
		A	8	2	25.0	16.4
Tuna-birds	BLNO	C	4	0	0.0	–
		B	3	0	0.0	–
		A	2	0	0.0	–
	BRNO	C	3	0	0.0	–
		J	14	1	7.1	7.1
		A	1	0	0.0	–
	SOTE	J	1	0	0.0	–
		B	10	0	0.0	0.0
		A	3	0	0.0	–
	WHTT	C	3	0	0.0	–
		B	1	0	0.0	–
		A	7	0	0.0	–
	WTSH	B	2	1	50.0	–
		A	2	2	100.0	–
Nocturnal-foraging petrels	BOPE	C	5	5	100.0	–
		B	1	1	100.0	–
	BUPE	A	2	0	0.0	–
	TRSP	C	57	57	100.0	0.0
		A	1	1	100.0	–
Neuston-feeding terns	GBAT	C	1	0	0.0	–
	Total birds:		362	245		

cycle energy) was used to clean heavily fouled monofilament line. Unidentifiable potential plastic pieces were dyed with rose bengal disodium salt, which adheres to organic materials, to aid identification (Davison and Asch, 2011). Additionally, potential plastic items were occasionally burned, and the distinctive smell and melting (i.e., rounded and hardened when cooled), further helped in identification.

The clean plastics were sorted into four standardized categories (van Franeker, 2004; van Franeker et al., 2014): fragment, foam, line, and sheet. All plastic items were air dried in a fume hood for 1–2 days in a temperature-controlled lab. Once dry, plastics were weighed in a foil package using a Mettler Toledo NewClassic MS analytical balance, equipped with a draft shield (120 g capacity and 0.0001 g resolution).

Following recommended practices, we weighed each foil package four times in close succession: two times empty (tare measurements) and two times with the sample (gross measurements). If the two replicate weights, defined as mass 1 (gross 1- tare 1) and mass 2 (gross 2 - tare 2) differed by 0.0010 g, we reweighed the sample. Additionally, we repeatedly weighed a test weight throughout the weighing process, and recalibrated the scale as necessary (Mettler Toledo, 2012). To quantify the precision of our mass measurements we used the Pearson correlation coefficient and the root mean squared error (RMSE), calculated as the square-root of the sum of the squared differences for each pair of replicate measurements of the same item, divided by the sample size (Armstrong and Collopy, 1992).

2.4. Community-wide assessment of plastic prevalence

The occurrence (presence/absence) of ingested plastic (from both stomach chambers combined) was used to quantify the prevalence of seabird exposure. All salvaged specimens, whether they were necropsied in the field or in the lab, were included in this analysis (Table 1). The prevalence of plastic ingestion was calculated for those specimens belonging to a given species and age group (species * age group), regardless of the sample size, as the proportion of necropsied specimens that contained any plastic. For those groups with sufficiently large sample sizes (≥ 8 specimens), the mean ($\pm$ S.D.) proportion (%) of specimens that had ingested plastic was calculated using a binomial distribution. This sample size provided a small S.D. ($\leq 20\%$), across all possible prevalence levels (from 0 to 100%). Furthermore, whenever multiple age classes of the same species were sampled in sufficient numbers ($n \geq 8$ for each), plastic occurrence was compared across age classes using a Fisher's exact test. This contingency table approach is ideal for comparing categorical data, when small sample sizes lead to the number of expected values < 5 in over 20% of the cells (Zar, 1984).

2.5. Comparison of plastic prevalence across foraging guilds

To compare plastic prevalence across foraging guilds, the overall occurrence of plastic ingestion (from both stomach chambers combined) was calculated for every species with at least two sampled specimens ($n \geq 2$), by combining the rates across age classes (Table 1). For each species, the average occurrence rate was calculated by averaging across all the sampled age classes. For instance, the overall occurrence rate for BFAL was calculated as 77.6%, by averaging the chick and adult rates (96.4% and 58.8% respectively).

An analysis of variance (ANOVA) test was used to compare species-specific prevalence rates across four foraging guilds with two or more sampled species: albatrosses (2 species), nocturnal-foraging petrels (3 species), plunge-divers (4 species), and tuna-birds (5 species). These guilds are based on the diet, the foraging methods, and the ecological associations of these tropical seabirds. For instance, those species that forage in association with predatory fishes and dolphins are classified as tuna-birds (Harrison et al., 1983; Dearborn et al., 2001).

Thus, this analysis involved 322 seabirds, 14 species and 4 guilds. In addition to comparing these four categorical groupings, this test considered two co-variates: the sample size (log of the number of birds sampled per species) and the proportion of chicks in the sample (arcsine of the square root of the proportion of chicks sampled per species). Because sample sizes varied widely across the 16 species, from 126 (LAAL) to 2 (BUPE, MABO), this potential bias was considered in the analysis. Additionally, because the proportion of chicks sampled varied from species to species, from 0% (BRBO, BUPE) to 98.3% (TRSP), this factor was considered to address age-specific disparities in plastic ingestion rates. To ensure normality, the occurrence data were arcsine transformed ($y' = \arcsin(y)^{1/2}$), prior to performing the ANOVA. All analyses were performed with the Systat software, version 11.0.

2.6. Characterization of plastic loads in species that ingest plastic

The total load of ingested plastic, combining the contents of the proventriculus and the ventriculus, was used to quantify the severity of seabird exposure. Because not all specimens contained plastic, the analyses of prevalence and loads were based on different sample sizes. While the occurrence comparisons involved all necropsied specimens, the load comparisons involved only those specimens that had ingested plastic, and excluded the absence data. Furthermore, because the stomach contents from field necropsies in the 2010–11 season could not be retrieved for analysis on the lab, not every plastic ingestion record yielded a plastic mass.

We quantified plastic loads in two ways. First, the mean ± S.D. mass (0.0001 g resolution) of the ingested plastic (from both stomach chambers combined) was calculated for each species * age group, considering only those specimens that had ingested plastic. Then, after the ingested material was sorted, the occurrence (presence/absence) and the mean ± S.D. mass (0.0001 g resolution) of each plastic type (fragment, foam, line, sheet) was calculated for each species * age group. The prevalence of the four plastic types was compared for those species * age groups with sufficiently large sample sizes ($n \geq 8$ specimens sampled) using a Fisher's Exact Test and the odds ratio. This contingency table approach is ideal for comparing categorical data, when small sample sizes lead to the number of expected values < 5 in over 20% of the cells (Zar, 1984).

2.7. Comparison of standardized plastic loads in species that ingest plastic

Finally, because adults and chicks of the species with high plastic prevalence varied greatly in body mass, their loads were standardized by dividing the total ingested plastic mass (in grams) by their body mass (in grams), after excluding the weight of the stomach contents. Thus, this dimensionless index (plastic load mass/body mass) provided a standardized metric, similar to the approach used to standardize food delivery across species of varying body sizes (Baduini and Hyrenbach, 2003).

2.8. Comparison of stomach chambers in tubenose species

For tubenose seabirds, plastic occurrence was compared between the two stomach chambers (proventriculus versus ventriculus) for individual species * age classes with sufficiently large sample sizes (≥ 8 specimens sampled) using a Fisher's exact test and the odds ratio. This contingency table approach is ideal for comparing categorical data, when small sample sizes lead to the number of expected values < 5 in over 20% of the cells (Zar, 1984).

3. Results

3.1. Community-wide assessment of plastic prevalence

Of the 362 seabird specimens sampled opportunistically between 2006 and 2013 at TI, 245 (66.68%) had ingested plastic, involving 11 of the 16 sampled species (68.75%), seven families and four orders (per the Integrated Taxonomic Information System, www.itis.gov). These species represented five of the six foraging guilds considered: plunge-divers (100%; four species), albatrosses (100%; two species), frigatebirds (100%; one species), -foraging petrels (66.67%; two of three species), and tuna-birds (40.00%; two of five species). The BRNO and the WTSH were the two tuna-birds with ingested plastic. Conversely, only one nocturnal-foraging petrel species (BUPE) did not show plastic ingestion; possibly due to the low sample size (2 adult birds). Finally, while we did not document plastic ingestion in neuston-feeding terns, this foraging guild was under-sampled, with a single examined specimen (Table 1).

Seventeen of the 35 species * age groups we sampled had ingested plastic (occurrence > 0) (Table 1). Despite varying widely in sample size (1–104 birds), tubenose seabirds were characterized by the highest plastic ingestion rates, with five species * age groups having 100% prevalence: TRSP chicks ($n = 57$), TRSP adults ($n = 1$), BOPE chicks ($n = 5$), BOPE adults/juveniles ($n = 1$), and WTSH adults ($n = 2$). Conversely, three species of terns and noddies did not show plastic ingestion, despite the large sample sizes across multiple age classes: SOTE ($n = 14$ specimens in 3 age classes), WHTT ($n = 11$ specimens in 3 age classes), and BLNO ($n = 9$ specimens in 3 age classes). Only 5.55% (1 of 18) of the BRNO sampled had ingested plastic.

Moreover, we expected differences in plastic prevalence and loads between chicks/juveniles and adults of the same species. Because foraging adults collect plastic items at-sea and deliver them to their chicks at the nest, they offload their ingested plastics alongside the food they provision their chicks (Carey, 2011; van Franeker et al., 2011). The two albatross species and the frigatebird had sufficiently large sample sizes (≥ 8 specimens sampled in both age classes) to compare the occurrence rates of plastic ingestion in chicks/juveniles versus adults. The albatross chicks had a higher probability of having ingested plastic, as evidenced by the estimated odd ratio values (> 1), indicative of how strongly age class was associated with the presence/absence of ingested plastic (Zar, 1984): 17.57 (BFAL) and 4.01 (LAAL). Yet, these results were only significant for the BFAL, where chicks had higher occurrence rates (Fisher's exact test, $p = 0.003$). On the other hand, plastic prevalence was not significantly different in LAAL chicks/adults (Fisher's exact test, $p = 0.163$). While the GREF juveniles also had a higher probability of having ingested plastics than the adults (estimated odds ratio = 2.12), this difference was not significant (Fisher's exact test, $p = 0.449$).

3.2. Comparison of plastic prevalence across foraging guilds

The comparison of the species-specific occurrence rates did not reveal significant differences across foraging guilds, ($F_{3,8} = 1.088$; $p = 0.408$), and no bias due to the varying sample sizes ($F_{1,8} = 0.166$; $p = 0.694$). Yet, the proportion of chicks/juveniles in the sample did have a significant influence on the plastic occurrence rate

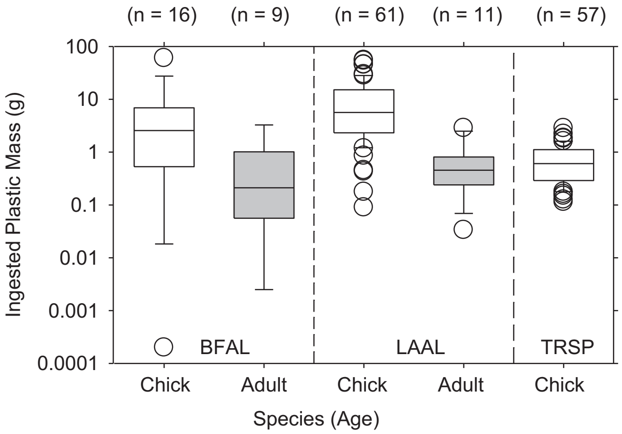

Fig. 1. Plastic mass for specimens containing plastic, belonging to species * age groups with high prevalence (> 50%) where ≥ 8 birds were sampled. The box plots indicate the quartiles (25th, 50th, 75th percentiles), the error bars depict the range (10th and 90th percentiles), and the circles denote more extreme values. Age classes defined following Sileo et al. (1989).

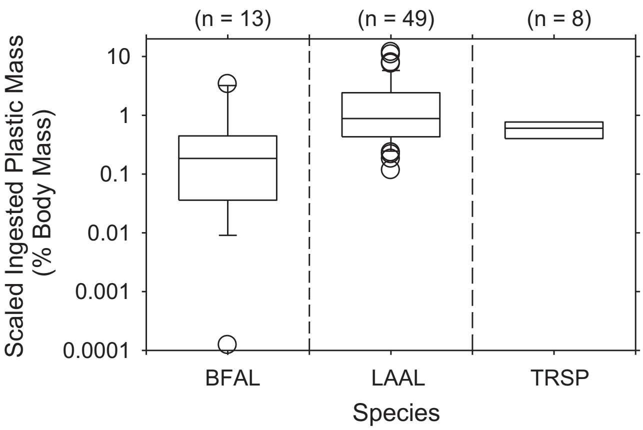

Fig. 2. Ratio of plastic mass to total body mass (%), excluding the weight of the stomach contents, for "fresh" (FFF, FF, or F codes) chicks of species with high plastic prevalence (> 50%). The box plots indicate the quartiles (25th, 50th, 75th percentiles), the error bars depict the range (10th and 90th percentiles), and the circles denote more extreme values.

($F_{3,8} = 6.146$; $p = 0.038$). Finally, the ANOVA residuals were normally distributed (One-sample Kolmogorov-Smirnov test, $n = 14$, max_diff = 0.198, $p = 0.574$). A follow-up linear regression confirmed that species-specific occurrence rates increased significantly ($F_{1,12} = 15.410$; $p = 0.002$), with a higher proportion of chicks/juveniles in the sample (slope coefficient = 1.021 ± 0.260 S.E.). This model accounted for 56.2% of the observed variance, and the regression residuals were normally distributed (One-sample Kolmogorov-Smirnov test, $n = 14$, max_diff = 0.143, $p = 0.899$).

3.3. Characterization of plastic loads in species that ingest plastic

In addition to documenting the prevalence of plastic ingestion, we quantified the ingested masses for 168 specimens from 13 species * age groups. All five species * age groups with sufficiently large sample sizes (≥ 8 specimens) had highly-skewed plastic mass distributions, with large S.D.s and outliers, indicative of some individuals having large plastic loads (Fig. 1).

When ingested plastic masses were compared between the two age groups (chicks/adults) of the two albatross species (BFAL/LAAL), chicks had the largest loads, with maximum masses of 60.3305 g (BFAL) and 55.4142 g (LAAL). Adult birds had substantially lower plastic loads, with maximum masses of 3.2639 g (BFAL) and 2.8551 g (LAAL). In fact, after excluding birds that did not contain any plastic, the log transformed plastic masses ($y' = \log(y)$) were higher in chicks than in adults ($F_{1,93} = 13.018$; $p < 0.001$), with significantly higher loads in the LAAL ($F_{1,93} = 7.485$; $p = 0.007$). Yet, there was no significant species * age class interaction ($F_{1,93} = 0.509$; $p = 0.477$). Altogether, the two-way ANOVA explained 33.1% of the observed variability in ingested plastic masses, and the residuals were normally distributed (One sample Kolmogorov – Smirnov test, $n = 97$, max_diff = 0.124, $p = 0.103$).

TRSP chicks were also characterized by high plastic loads (Fig. 1), despite their small body size (adult mass = 84 g) (Harrison et al., 1983). To facilitate interspecific comparisons with the larger-bodied albatrosses, the ingested loads were standardized as the percentage of the individual's body mass, using only recently dead and fresh specimens (FFF, FF, and F) with the stomach contents removed.

This standardized comparison highlighted the high mass-specific plastic loads in these small nocturnal-foraging petrels, compared to the larger-bodied albatrosses, with maximum scaled masses reaching 3.4% in BFAL, 11.9% in LAAL, and 1.2% in TRSP (Fig. 2). In fact, an ANOVA test of these standardized plastic loads (expressed as the % of the birds' body mass), arc-sine transformed ($y' = \arcsin(y)^{1/2}$) to achieve normality, revealed a significant difference across species ($F_{2,67} = 6.158$; $p = 0.004$). Furthermore, the ANOVA test residuals were normally distributed (One sample Kolmogorov – Smirnov test, $n = 70$, max_diff = 0.140, $p = 0.129$). The post-hoc Tukey tests revealed that LAAL chicks had significantly larger standardized loads than BFAL chicks (pairwise mean difference = 0.071, $p = 0.004$), while TRSP chick loads were not different from either albatross species: LAAL (pairwise mean difference = − 0.042, $p = 0.242$) and BFAL (pairwise mean difference = 0.029, $p = 0.616$). Altogether, these results underscored the high loads in LAAL chicks and TRSP chicks.

3.4. Characterization of plastic types in species that ingest plastic

While FFS seabirds ingested all four plastic types (fragments, foam, line, sheet), fragments were the most prevalent, in terms of occurrence and loads. In particular, fragments occurred at a high occurrence rate (> 50%) in all 7 species * age groups where plastic mass was quantified, and were the most common (highest prevalence) in 85.7% (6 of 7) of the species * age groups. Only BOPE chicks ingested line at a higher rate than fragments (Table 3).

Moreover, the quantification of the mass of ingested plastic types reinforced the importance of fragments, which accounted for the majority of the plastic ingested across the 7 species * age groups where plastic mass was quantified (Table 2). In fact, the fragments accounted for almost all (> 95%) of the plastic mass ingested by four species * age groups: LAAL chicks and adults, TRSP chicks, and GRFR immatures. Only BFAL chicks (75.1%) and adults (73.7%), and BOPE chicks (52.9%) had ingested proportionately less fragments (52.9–75.1%) by mass. Altogether, these results reinforce previous evidence that fragments are the dominant type of ingested plastic in Hawaiian seabirds (Fry et al., 1987; Sileo et al., 1989; Gray et al., 2012; Lavers and Bond, 2016). The prevalence of ingested fragments underscores the pervasiveness and durability of these items in the marine environment and in the stomachs of seabirds.

Plastic line was also frequently ingested by seabirds, occurring in all 7 species * age groups, with particularly high prevalence in BOPE chicks (100%), TRSP chicks (89.5%), BFAL chicks (81.3%), and LAAL chicks (54.1%) (Table 3). Additionally, sheet and foam were only ingested by tubenose species, and were documented in four and three species, respectively. Yet, despite the high prevalence of foam ingestion by TRSP chicks (79.0%), it occurred at low levels in the other species (prevalence < 50%). Similarly, the prevalence of plastic sheets was also low across all species (Table 3). Several processes can explain these observation: foam and line could be less pervasive or durable, likely

Table 2
Total plastic mass and plastic fragment mass, combining the contents of the proventriculus and ventriculus, ingested by different species * age groups, where stomachs were retrieved and quantified in the laboratory ($n > 2$ specimens). Age classes defined following Sileo et al. (1989): C = chick, J = juvenile, A = adult.

			Total plastic mass (g)		Plastic fragment mass (g)		Fragment proportion of total plastic mass (%)
Seabird species	Age class	Sample size	Mean ± S.D.	Range (Median)	Mean ± S.D.	Range (Median)	Mean ± S.D.
BFAL	C	16	6.8274 ± 14.7532	0.0002–60.3305 (2.5645)	5.9493 ± 14.5109	0.000–59.2082 (1.0920)	75.1 ± 34.8
	A	9	0.6781 ± 1.0467	0.0025–3.2640 (0.2113)	0.6685 ± 1.0519	0.0000–3.2640 (0.1989)	73.7 ± 45.5
LAAL	C	61	10.8377 ± 13.1191	0.0898–55.4142 (5.6423)	10.6764 ± 13.0789	0.0898–55.3713 (5.6349)	97.8 ± 6.0
	A	11	0.6781 ± 0.7769	0.0336–2.8551 (0.4539)	0.6776 ± 0.7762	0.0336–2.8525 (0.4539)	99.9 ± 0.2
GREF	J	5	0.3756 ± 0.4373	0.0041–1.0989 (0.1728)	0.3756 ± 0.4373	0.0041–1.0989 (0.1728)	99.9 ± 0.0
BOPE	C	3	0.0259 ± 0.0217	0.0018–0.0438 (0.0320)	0.0196 ± 0.0169	0.0000–0.0294 (0.0293)	52.9 ± 47.5
TRSP	C	57	0.7447 ± 0.5827	0.1145–2.8107 (0.6030)	0.7272 ± 0.5813	0.1064–2.8009 (0.5877)	95.9 ± 5.9
Total birds:		162					

being mechanically degraded rapidly in the marine environment and after ingestion.

However, all five Fisher's exact tests comparing the prevalence of all four ingested plastic types, revealed significant differences across BFAL (chicks/adults), LAAL (chicks/adults), and TRST (chicks), underscoring the higher prevalence of fragments, and the lower prevalence of line and sheet (Table 3).

3.5. Reproducibility of plastic mass measurements

Despite the large range of ingested plastic masses (60.3305–0.0002 g), replicate measurements across plastic types were highly correlated (Pearson correlation, $r = 0.999$, $n = 434$) and precise, with a RMSE = 0.0542 g.

3.6. Comparison of stomach chambers in tubenose species

Finally, we compared the prevalence of ingested plastic in five species * age groups of tubenose birds, equipped with two distinct stomach chambers: the proventriculus and the ventriculus (Table 4). All five groups we tested were characterized by high plastic occurrence prevalence (> 50%) in the proventriculus and in the ventriculus, and the occurrence rates did not vary significantly by stomach chamber.

4. Discussion

4.1. Community-wide assessment of plastic ingestion: species-specific ingestion rates

This opportunistic study underscored the pervasive nature of plastic ingestion by Hawaiian seabirds, which affects at least 11 of the 16 locally-breeding species (68.75%), belonging to five feeding guilds. Even though plastic ingestion was not documented in all species or feeding guilds (e.g., neuston-feeding terns), some species were characterized by large loads, with highly skewed distributions (mean > median), indicative of some specimens having ingested large plastic masses. Thus, additional sampling is required to quantify the individual-level exposure to plastic ingestion and to characterize the plastic loads in several species and age classes that were not adequately sampled during this study (Table 1). In particular, minimum sample sizes of 20 individuals would be desirable to place these observations in the context of recent global reviews (NRC, 2009).

Despite these small sample sizes, we documented plastic ingestion in a variety of species and age classes of plunger-divers (Table 1). Most notably, we obtained the first record of ingestion in the Brown Booby (*Sula leucogaster*, BRBO), with one of three immature birds examined having ingested plastic. While there is evidence of BRBO using marine debris as nesting material, ingestion has not been documented in this species (Lavers et al., 2013).

We also recorded plastic in three other plunge-divers, where ingestion had been previously documented in the literature: Masked Booby (*Sula dactylatra*, MABO), Red-footed Booby (*Sula sula*, RFBO), and Red-tailed Tropicbird (*Phaethon rubricauda*, RTTR) (Table 1). Despite having only examined two MABO specimens, one chick contained plastic. Previous analysis of 20 lavage samples from chicks and six necropsied adult/immature birds collected at-sea only revealed plastic ingestion in one chick from Laysan Island. Thus, while the occurrence documented here (100%) is inflated by the small sample size, it suggests a MABO prevalence higher than the historical estimates from the 1980s documented in chicks (5%, $n = 20$) and adult/immature birds (0%, $n = 6$) (Sileo et al., 1989; Spear et al., 1995). Plastic ingestion also occurred in RFBO immatures (9.1% ± 9.1 S.D., $n = 11$), even though neither the seven adults nor the single chick of this species we sampled had ingested plastic. Finally, RTTR chicks, had a plastic prevalence

Table 3
Plastic prevalence by type (fragment, foam, line, sheet), combining the contents of the proventriculus and ventriculus, ingested by different species * age groups, where stomachs were retrieved and quantified in the laboratory. Incidence is reported as the percentage (%) of specimens that ingested a particular plastic type. Fisher's Exact tests were used to compare the incidence of the four plastic types. Age classes defined following Sileo et al. (1989): C = chick, A = adult.

Seabird	Age	Sample size	Prevalence by type (%)				Fisher's exact test	
Species	Class	(Birds with plastic)	Fragment	Foam	Line	Sheet	*P* value	Result
BFAL	C	16	93.8 ± 6.3	31.3 ± 12.0	81.3 ± 10.1	37.5 ± 12.5	< 0.001	Sig.
	A	9	77.8 ± 14.7	0 ± 0	22.2 ± 14.7	22.2 ± 14.7	0.002	Sig.
LAAL	C	61	100 ± 0.0	42.6 ± 6.4	54.1 ± 6.4	19.7 ± 5.1	< 0.001	Sig.
	A	11	100 ± 0.0	0 ± 0	18.2 ± 12.2	0 ± 0	< 0.001	Sig.
TRSP	C	57	100 ± 0.0	79.0 ± 5.5	89.5 ± 4.1	15.8 ± 4.9	< 0.001	Sig.
Total birds:		162						

Table 4
Comparison of plastic prevalence in the proventriculus and the ventriculus of tubenose (procellariform) species * age groups, where stomachs were retrieved and quantified in the laboratory. Incidence is reported by stomach chamber as the percentage (%) of specimens that contained plastic. Fisher's Exact tests were used to compare the plastic incidence rates, by stomach organ. Age classes defined following Sileo et al. (1989): C = chick, A = adult.

Seabird species	Age class	Sample size (Birds with plastic)	Plastic prevalence in proventriculus (Mean ± S.D.)	Plastic prevalence in ventriculus (Mean ± S.D.)	Fisher's exact test (p value)	Result
BFAL	C	16	100 ± 0.0	81.2 ± 10.1	0.2258	Not significant (Pro > Vert)
	A	9	88.9 ± 11.1	66.7 ± 16.7	0.5765	Not significant (Pro > Vert)
LAAL	C	61	98.4 ± 1.6	98.4 ± 1.6	1.000	Not significant (Pro = Vert)
	A	11	72.7 ± 14.1	90.9 ± 9.1	0.5865	Not significant (Pro < Vert)
TRSP	C	37	100.0 ± 0.0	100.0 ± 0.0	1.000	Not significant (Pro = Vert)

(33.3% ± 33.3 S.D., $n = 3$) substantially higher than the rate derived from previous collections at FFS (14.0% ± 5.3 S.D., $n = 50$ chicks) (Sileo et al., 1989). Yet, the two sampled adults of this species had not ingested plastic.

Plastic ingestion was pervasive in tubenose species, belonging to three foraging guilds: Bonin Petrel (*Pterodroma hypoleuca*, BOPE), Tristram's Storm-petrel (*Oceanodroma tristrami*, *TRSP*), *Wedge-tailed* Shearweater (*Ardenna pacifica*, WTSH), Black-footed Albatross (*Phoebastria nigripes*, BFAL), and Laysan Albatross (*P. immutabilis*, LAAL) (Table 1). The prevalence documented for BOPE chicks (100%; $n = 5$) and adults (100%; $n = 1$) were higher than those derived from specimens previously collected at Midway Atoll: 58 adult/immature specimens necropsied in the 1986–1987 (29.3% ± 7.2 S.D.) and 8 chicks necropsied in 2012 (75.0% ± 16.4 S.D.) (Sileo et al., 1989; Lavers and Bond, 2016). While our low WTSH sample size inhibits a rigorous statistical comparison, this occurrence rate (100%; $n = 2$) is higher than what has been documented in the past. In 1984, researchers from the University of California in Davis collected 20 adult WTSH from Manana Island (O′ahu) and reported 60% ± 17.7 S.D. plastic prevalence. This rate is also higher than those resulting from the analysis of 523 adult WTSH lavage samples, collected from the NWHI sites, Johnston Atoll, and the main Hawaiian Islands, over 2 years (1986–1987): mean = 13.7%, range = 3.0–29.0%, median = 14.0% (Fry et al., 1987; Sileo et al., 1989).

In summary while we documented plastic ingestion in certain species * age groups with small sample sizes (e.g. BRBO chicks, RTTR chicks, MABO chicks, BOPE adults and chicks, TRSP adults, WTSH adults), our small sample sizes do not provide reliable occurrence rates (Table 1). For other species with larger sample sizes, we calculated robust plastic prevalence with small variability: TRSP chicks (100% ± 0 S.D., $n = 57$), LAAL chicks (97.2% ± 1.6 S.D., $n = 104$), and BFAL chicks (96.4% ± 3.6 S.D., $n = 28$) (Table 1).

In some cases, we did not document plastic ingestion, in spite of our large sample sizes. For instance, the Black Noddy (*Anous minutus*, BLNO) ($n = 9$), and the White Tern (*Gygis alba*, WHTT) ($n = 11$) did not ingest plastic, as previously reported by Sileo et al. (1989) (adults = 0%, chicks = 3%, chicks = 0%). Sooty Terns (*Onychoprion fuscata*, SOTE) ($n = 14$) also had 0% occurrence, similar to the rate from previous at-sea studies (0–2%), but lower than the results from specimens collected at Midway Atoll (8%) (Sileo et al., 1989; Ainley et al., 1990; Spear et al., 1995).

Overall, our results highlighted differences in plastic exposure across species, with species-specific rates ranging from 0% to 100%. In fact, the analysis of species-specific ingestion rates across four foraging guilds with two or more sampled species (albatrosses, nocturnal-foraging petrels, plunge-divers, tuna-birds) did not reveal significant differences, reinforcing the notion that plastic ingestion is widespread in Hawaiian seabirds, and not limited to certain foraging modes. Yet, we would expect the mechanisms by which these distinct foraging guilds collect plastic at sea to differ. While tuna-birds and plunge-divers often forage with subsurface predators and may ingest plastic via their prey, albatrosses and nocturnal-foraging petrels likely collect this material at the sea surface (Harrison et al., 1983).

The comparison of different age groups of the same species revealed significant differences in plastic exposure. In particular, occurrence rates were significantly higher in chicks/immatures than in adults of three species (BFAL, LAAL, GRFR) with sufficiently large sample sizes ($n \geq 8$ specimens per age class). Additionally, the ANOVA analysis of plastic prevalence across foraging guilds revealed that the proportion of chicks in a sample significantly influenced the overall species-specific plastic occurrence rate. These results, which underscored previous findings, suggesting that adult provisioning leads to the loading of this material in the offspring (Sileo et al., 1989; Carey, 2011; Rodríguez et al., 2012), have important implications for monitoring plastic ingestion. Namely, different age classes need to be sampled and analyzed separately to avoid biasing species-specific comparisons and temporal trends.

4.2. Trends in species-specific ingestion rates

For five species * age groups with sufficiently large sample sizes, our results suggest a worsening trend of plastic ingestion, by comparing our recent findings (2006–2013) with published historical data (1980s): TRSP chicks, GRFR immatures, GRFR adults, BRNO immatures, and RFBO immatures.

Most strikingly, the current plastic prevalence in the TRSP exceeds the rates documented in the past. In particular, a 1987 lavage study of chicks (35% ± 12 S.D., $n = 17$) and adults (33% ± 11 S.D., $n = 18$) reported occurrence rates substantially lower than those we documented, likely because only the proventriculus contents were sampled (Sileo et al., 1989). Conversely, the analysis of four adult/immature birds collected at-sea and necropsied revealed a 100% occurrence rate, likely because the proventriculus and the ventriculus were sampled (Robards et al., 1997). While differences in sampling methods inhibit rigorous comparisons over time, we documented 100% plastic occurrence in the proventriculus of the necropsied TRSP, which suggests a ~200% increase (from 35% to 100%), when compared to historical lavage samples. Nevertheless, it should be noted that potential regional differences in ingestion rates may exist, since the specimens sampled by Sileo et al. (1989) were from Laysan Island and the birds we sampled were from TI.

We also documented high levels of plastic ingestion in immature (41.9 ± 9.0 S.D., $n = 31$) and adult (25.0% ± 16.4 S.D., $n = 8$) GRFR (Table 1). Previously, a lavage study of chicks from Midway Atoll (Sileo et al., 1989) revealed plastic in 8 of the 45 specimens examined (17.8% ± 6.4 S.D.), a rate substantially lower than what we documented for adults and immatures. Brown Noddy immatures ($n = 14$) also had greater plastic prevalence (7.1% ± 7.1 S.D.) than chicks collected from FFS (0%; $n = 15$) in 1987 and from Midway Atoll in 1986–1987 (1% ± 1%; $n = 86$) (Sileo et al., 1989). Additionally, RFBO immatures ($n = 11$) had greater plastic prevalence (9.1% ± 9.1 S.D.) than chicks sampled from FFS in 1987 (0%; $n = 35$).

While plastic occurrence rates in albatrosses were consistently high (> 50%), trends were difficult to interpret given the range of sampling methods used over time, involving the analysis of chick carcasses, regurgitated boluses from chicks, and necropsies of adults and chicks (e.g., Sileo et al., 1989; Robards et al., 1997; Gray et al., 2012; Lavers and Bond, 2016). Nevertheless, occurrence rates in adult BFAL and LAAL were similar to estimates from historical and contemporary necropsy studies of specimens sampled at-sea through incidental mortality in fisheries (Robards et al., 1997; Gray et al., 2012), but higher than estimates from regurgitation samples from LAAL adults from Midway Atoll in 1986 (35.0% ± 9.0 S.D., $n = 31$) (Sileo et al., 1989). Furthermore, while we documented that LAAL adults had greater plastic prevalence than BFAL adults, this result needs to be considered cautiously due to our small sample size (Table 1).

4.3. Comparison of stomach chambers in tubenose species

When we considered the tubenose species, we did not document significant differences in the plastic occurrence in their two distinct stomach chambers. While these patterns need to be investigated further, they suggest that BFAL may be retaining relatively more ingested plastic in the proventriculus, and LAAL may be retaining relatively more ingested plastic in the ventriculus. These organ-specific differences could also be the result of a differential frequency of pellet casting, with Laysans being expected to cast their proventriculus plastics more frequently than BFALs. Moreover, while we are not aware of published reports of pellet casting by adults, this likely takes place at-sea. Conversely, TRSP had 100% plastic prevalence in both stomach chambers, suggesting this species does not cast ingested plastics (Table 4).

These disparities may be related to the higher occurrence of fragment ingestion by LAAL (100% in chicks and adults) and TRSP (100% in chicks), compared with the lower occurrence of ingested fragments in BFAL (93.8% in chicks and 77.8% in adults), and the relatively higher occurrence of ingested line (81.3% in chicks and 22.2% in adults). Thus, we hypothesize that, while fragments eventually enter the ventriculus, where they are slowly broken down mechanically, line is harder to pass and is retained in the proventriculus. Ultimately, the prevalence of ingested line also highlights differences in species-specific foraging behavior and diet. Namely, because BFAL provision their chicks with large amounts of flying fish egg masses, which are often laid upon tangles of line and other floating debris, they ingest more line than LAAL, which consume larger amounts of squid and fish (Harrison et al., 1983; Sileo et al., 1989).

4.4. Characterization of plastic loads in species that ingest plastic

Plastic loads were highly variable, with skewed distributions (mean > median) and outliers (Table 2, Fig. 1). Nevertheless, we documented larger plastic loads in albatross chicks than in adults, further suggesting that adult provisioning leads to the offloading of this material to the offspring (Sileo et al., 1989; Carey, 2011; Rodríguez et al., 2012). Nevertheless, while LAAL had larger plastic loads than BFAL, this result needs to be considered cautiously due to the small number of adult birds we sampled (Table 3).

4.5. Assessment of the status and trends in plastic ingestion: prevalence and loads

This study was motivated by the desire to provide a standardized baseline of plastic exposure in FFS seabirds, with the aim of assessing current levels of ingestion and monitoring future trends. Our results underscored the pervasive nature of plastic ingestion in Hawaiian seabirds, and for the subset of species where temporal comparisons were feasible, a worsening trend with increased plastic exposure since the 1980s. Our study also highlighted the inherent difficulties involved in quantifying the comprehensive status and trends of plastic ingestion by NHWI seabirds. While the sample sizes required to detect a minimum signal strength with a desired power level can be estimated using observed occurrence rates and loads, these data are not yet available for many species * age classes (e.g., Lavers and Bond, 2016). Yet, in principle, 10–20 specimens are needed to develop robust prevalence estimates for different species * age groups. Larger samples (50–100 specimens) may be required to quantify plastic loads, given the low ingestion rates and the non-normal distributions of ingested masses for some species * age groups.

The community-wide characterization of plastic exposure in Hawaiian seabirds was constrained by the inability to sample all species * age groups adequately to produce robust estimates (e.g., neuston-feeding terns), and by the highly variable distributions of ingested plastic loads across individuals. Thus, future studies will need to ensure large enough sample sizes to quantify plastic prevalence at the desired level of certainty, given the influence of the sample size on the S.D., and loads, given the observed highly-skewed distributions.

In addition to these inherent limitations constraining the characterization of current plastic exposure, comparisons with historical ingestion studies were further confounded by methodological differences. Namely, past assessments used necropsy and stomach pumping (lavage) as a sampling method on colonies (e.g., Sileo et al., 1989). While this non-lethal method can effectively sample stomach contents of species with a unique stomach chamber (e.g. Boobies, Noddies, Tropicbirds), it is not able to retrieve the plastic in the ventriculus of tubenose species (e.g., Bond and Lavers, 2013). Furthermore, past studies also relied on the necropsy of bycaught birds of unknown provenance and age class, which complicates the comparison with colony-based samples from known age classes (e.g., Robards et al., 1997; Gray et al., 2012).

4.6. A way forward

In spite of logistical and ecological limitations, involving the availability of specimens from all species * age classes, establishing statistically rigorous baselines of plastic prevalence and loads remains a top priority for monitoring, as described in the Papahānaumokuākea Marine National Monument management plan (PMNM, 2008) and the management needs for threatened and endangered seabird species conservation in the U.S. (USFWS, 2005; USFWS, 2011). Thus, based on sample availability and the documented rates of ingestion, we identified four specific data gaps: (1) unstudied species, (2) poorly-studied species with documented plastic ingestion, (3) poorly-studied species without documented plastic ingestion, and (4) species with high plastic ingestion rates.

Unstudied species: Two NWHI seabird species have not been studied for plastic ingestion to date: Christmas Shearwater (*Puffinus nativitatis*), and Blue-gray Noddy (*Procelsterna cerulea*). Opportunistic sampling of deceased specimens or targeted field sampling via lavage is thus a high research priority for determining whether plastic ingestion is occurring, and for documenting the prevalence and loads in these species.

Poorly-studied species with documented plastic ingestion: Additional samples are also needed for several NWHI species where plastic ingestion was documented, despite the small sample sizes: BRBO (33.3% occurrence; $n = 3$), MABO (50.0% occurrence; $n = 2$), RFBO

(5.3% occurrence; $n = 19$), RTTR (20.0% occurrence; $n = 5$), BRNO (5.5% occurrence; $n = 18$).

Furthermore, it would be desirable to sample unstudied age classes of several NWHI species with documented plastic ingestion. Plastic ingestion by multiple age classes was only investigated with sufficiently large sample sizes for three species: BFAL, LAAL, GRFR. Thus, additional sampling of multiple age classes is needed for most NWHI seabirds.

Species without documented plastic ingestion, but small sample sizes: Additional samples are also needed for five NWHI species where plastic ingestion was not documented, but may be occurring at low levels: SOTE ($n = 14$), WHTT ($n = 11$), BLNO ($n = 9$), BUPE ($n = 2$), and GBAT ($n = 1$).

Species with high plastic ingestion prevalence: Two tubenose species, where high ingestion rates were documented but sample sizes were too small to quantify the prevalence and loads, should be investigated further: WTSH (75.0% overall occurrence; 100% occurrence in adults; $n = 4$) and BOPE (100% occurrence; $n = 6$). WTSH plastic ingestion is being studied in the MHI (2009–14), where chicks have a 72% occurrence rate (Hyrenbach unpub. Data). While very little is known about plastic ingestion in BOPE, due to the restricted breeding of this species to the NWHI, a recent (2002) examination of eight fledglings necropsied in Midway Island, revealed a 75% plastic occurrence rate (Lavers and Bond, 2016).

In addition to these recommendations for monitoring seabird plastic ingestion in the Papahānaumokuākea Marine National Monument, it is imperative to undertake a similar community-wide survey for the Main Hawaiian Islands, where 20 seabird species breed, including three U.S. endangered species: the Hawaiian Petrel (*Pterodroma sandwichensis*), the Newell's Shearwater (*Puffinus newelli*), and Band-Rumped storm-petrel (*Oceanodroma castro*). Moreover, wider assessments of broadly-distributed shearwaters (Wedge-tailed), terns (Sooty, White), noddies (Black, Brown), albatrosses (Black-footed, Laysan), and boobies (Brown and Red-footed) might help assess wider regional patterns of exposure. In particular, because ingested plastic loads in far-ranging seabirds may differ across breeding colonies (e.g., Young et al., 2009), regional comparisons between the NWHI and the MHI may be particularly insightful for understanding how seabird foraging areas overlap with at-sea plastic distributions.

Finally, research is needed to understand the various mechanisms by which seabirds ingest plastic. In particular, it is unknown to what extent foraging adults mistake plastic for prey (e.g., Savoca et al., 2016), collect plastic items colonized by invertebrates (e.g., Reisser et al., 2014), or ingest this material secondarily ingestion via their prey (e.g., Ryan and Fraser, 1988). Additionally, because albatross chicks ingest non-floating material they collect around their nest (e.g., coral fragments, lead paint chips), they likely pick up plastic items in the same way. Thus, ingested plastics may reflect the material collected by foraging parents at sea and ingested by chicks at the colony. Nevertheless, it is unclear to what extent chicks from other surface-nesting species (e.g., red-tailed tropicbirds, masked boobies, brown boobies), burrowing species that nest underground (e.g., petrels, shearwaters, storm-petrels), or species that nest on the vegetation (e.g., brown noddies, red-footed boobies, greater frigatebirds) collect plastic items from the colony.

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