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# Plastic ingestion by Tristram's Storm-petrel (*Oceanodroma tristrami*) chicks from French frigate shoals, Northwestern Hawaiian Islands

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A R T I C L E I N F O

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A B S T R A C T

This study provides the first quantification of plastic ingestion in the Tristram's Storm-petrel (*Oceanodroma tristrami*) in over 20 years. We found 100% plastic incidence in 57 chicks collected opportunistically over four breeding seasons (2007, 2010, 2011, 2012), with the mass of ingested plastic per individual ranging from 0.1 to 2.8 g ($\leq$3.3% adult mass). While plastic occurred in every bird we examined, the proventriculus contained significantly more plastic, more fragments, and larger fragments than the gizzard. Most of the ingested plastic (97.5% by mass) consisted of fragments, ranging in length from 0.4 to 11.6 mm and ranging in surface area from 0.07 to 45.21 mm$^2$. While fragments were ubiquitous, occurring in every proventriculus and gizzard we analyzed, Tristram's Storm-petrels also ingested foam, line and sheets. Digital analysis of 1425 ingested plastic fragments documented a wide range of colors, involving shades of white, yellow, orange, red, blue, green, and black.

## Introduction

In 1988, the Tristram's Storm-petrel (*Oceanodroma tristrami*) was first classified as near-threatened by the International Union for the Conservation of Nature (IUCN) due to ambiguous population trends and a limited breeding range. However, "contamination of the sea with plastics and other litter" was not considered a threat to this species (BirdLife International, 2017). This listing stimulated research on Tristram's Storm-petrel population trends and threats (Marks and Leasure, 1992; McClelland et al., 2008; current study). While many studies have documented the widespread prevalence of plastic debris and other associated pollutants in seabirds (Tanaka et al., 2013; Lavers et al., 2014; Lavers and Bond, 2016), quantifying the incidence and loads of plastic ingestion for poorly-studies species remains a research priority (Lewison et al., 2012; Vegter et al., 2014; Wilcox et al., 2015).

Tristram's Storm-petrels are nocturnal offshore foragers, and feed on low trophic-level prey at the surface (Harrison et al., 1983; Keller et al., 2009; Bond et al., 2010). Parents provision their chicks intermittently with partially digested food remains, containing undigested tissue, proventricular oil, indigestible prey remains (squid beaks, fish bones and eye lenses), and non-food items (plastic, pumice) (Harrison et al., 1983; Boersma, 1986; Sileo et al., 1989). Storm-petrels and other tubenose seabirds (order Procellariiformes), have a unique stomach morphology, which retains and grinds indigestible hard parts. Their upper gastrointestinal tract consists of two discrete chambers with distinct characteristics. The esophagus and stomach make up a thin-walled and expandable compartment, hereafter referred to as the proventriculus, followed by the thick-walled and muscular (but not expandable) ventriculus, hereafter referred to as the gizzard (Place et al., 1989; Proctor and Lynch, 1998; van Franeker and Meijboom, 2002). Digestion begins in the proventriculus, which reduces ingested food to high-caloric stomach oil (Place et al., 1989; Obst and Nagy, 1993). Unregurgitated hard items pass from the proventriculus into the much smaller gizzard, where they are broken down further, before passing to the intestines.

Due to the small passageway that connects these two chambers (i.e., the gastric isthmus), material in the gizzard is unlikely to return to the proventriculus (Place et al., 1989; Proctor and Lynch, 1998; van Franeker and Meijboom, 2002). Thus, it has been assumed that the movement of plastic particles through the gastrointestinal tract is influenced by their size, with those items too large to pass into the gizzard being retained in the proventriculus, and with those items too large to pass into the intestine being retained in the gizzard (Ryan, 2008; Mallory, 2008; Terepocki et al., 2017). Because the incidence and loads of plastic in the two stomach chambers reflects the transit of this debris to downstream compartments of the gastro-intestinal tract, it is also likely affected by the amount of food ingested and by the retention of other indigestible items (Ryan and Jackson, 1987; van Franeker et al.,

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**Table 1**
Tristram's Storm-petrel plastic ingestion rates, expressed as the proportion (%) ± S.D. of individuals containing any plastic, calculated for chicks and adults separately. "Present" denotes the presence of plastic, without reporting incidence. The sample size is denoted by n.

| Location | Year(s) | Sampling method | Adults<br>% ± S.D.<br>(n) | Chicks<br>% ± S.D.<br>(n) | Citation |
|---|---|---|---|---|---|
| Laysan & Nihoa Islands, NWHI | 1979–81 | Regurgitation | Present (10) | – | Harrison et al. (1983) |
| Laysan & Nihoa Islands, NWHI | 1980–84 | Regurgitation | 42.86 ± 13.73 (14) | – | Boersma (1986) |
| Laysan Island, NWHI | 1987 | Gastric lavage | 33.33 ± 11.43 (18) | 35.29 ± 11.9 (17) | Sileo et al. (1989) |
| At Sea, Central Pacific | 1990–91 | Necropsy | 100 ± 0.00 (4) | – | Robards et al. (1997) |
| Tern Island, NWHI | 2007–12 | Necropsy | 100 ± 0.00 (1) | 100 ± 0.00 (57) | This study |

2011). When infrequent hard parts are processed in the gizzard, they do no accumulate in the proventriculus. Conversely, when abundant hard parts concentrate in the gizzard, they may block the passage of more material from the proventriculus.

Despite evidence of plastic ingestion in the Tristram's Storm-petrel dating back to the late 1970s, no assessment has been completed since the mid-1980s (Table 1). Moreover, previous studies may have underestimated the incidence of plastic ingestion due to the reliance on proventriculus samples obtained via regurgitation and lavage, because these sampling methods likely do not recover all ingested plastic (Duffy and Jackson, 1986; Sileo et al., 1989; Bond and Lavers, 2013). In fact, only a single study of necropsied adult specimens from fisheries bycatch documented 100% plastic incidence ($n = 4$) (Robards et al., 1997). Otherwise, colony-based studies using lavage and regurgitation samples revealed significantly lower incidence rates for chicks (35.29% ± 11.95 S.D., $n = 17$) and adults (33.33% ± 11.43 S.D., $n = 18$) (Sileo et al., 1989).

This study focused on plastic ingestion by naturally-deceased Tristram's Storm-petrel chicks from French Frigate Shoals (FFS), in the Northwestern Hawaiian Islands. More specifically, our goal was to provide a comprehensive description of the plastic ingested by this species. To this end, we: (1) documented current (2007–2012) plastic incidence and loads; (2) characterized the incidence and loads of different functional plastic types (fragment, line, sheet, foam); and (3) quantified the size and color of the ingested fragments. Additionally, to inform future monitoring, we addressed the incidence and loads of plastic within the two stomach chambers (proventriculus and gizzard) separately. Altogether, this paper provides the first comprehensive description of ingested plastics for this species, which can serve as a baseline for future assessments.

## 1. Materials and methods

### 1.1. Specimen Collection

We collected Tristram's Storm-petrels opportunistically from Tern Island (23°45′N, 166°10′W), FFS, within the Hawaiian Islands National Wildlife Refuge, in the Papahānaumokuākea Marine National Monument. We sampled specimens of varying freshness, ranging from recently dead "very fresh" (FFF code) to "very old" (OOO code) (coded following van Franeker, 2004), if their body wall was not perforated and the gut contents were fully encased in the carcass. We salvaged fifty-seven chicks that had died prior to departing from the colony, between February and June, over four study years: 2007 ($n = 2$), 2010 ($n = 12$), 2011 ($n = 17$), and 2012 ($n = 26$). We stored the specimens frozen and, either necropsied them in the field (in 2011), or returned them to Oahu for necropsy in the lab.

### 1.2. Specimen dissection

All specimen necropsies and collection of stomach contents followed standardized protocols (Work, 2000; van Franeker, 2004; Donnelly-Greenan et al., 2014). Trained personnel completed all the necropsies, with one of the authors in the lead: KDH lead the 2007 and 2010 necropsies in Oahu, and SMY conducted all the necropsies in 2011 (on FFS) and in 2012 (on Oahu). Once removed from the specimen, the stomach contents were stored in 70% ethyl alcohol (gizzard) or frozen (proventriculus, intestines), prior to processing.

### 1.3. Stomach content processing

SMY performed all processing (cleaning, sorting, and categorization) of stomach contents, following standardized protocols (van Franeker, 2004; Donnelly-Greenan et al., 2014; van Franeker et al., 2014). Our aim was to analyze the contents of the proventriculus and the gizzard separately, and to pool these two discrete samples to quantify plastic incidence and loads for individual birds. Because rapid post-mortem specimen decay often resulted in proventricular rupture, we rinsed and collected the spilled material from the body cavity, including the intact muscular gizzard. In 2012, we were not able to differentiate the contents of the two chambers due to decomposition in three individuals. Additionally, we did not sample the two stomach chambers separately during field necropsies in 2011 ($n = 17$). Thus, we only analyzed the two stomach chambers for 37 of the 57 necropsied specimens. For the other 20 birds, we obtained a single sample, involving the contents of both chambers (Table 2).

Hereafter 'chamber-specific' refers to the contents of the proventriculus and the gizzard sampled separately from the same specimens ($n = 37$), and "whole bird" refers to the combined chamber-specific contents for a given specimen ($n = 57$). For those specimens where the proventriculus and the gizzard contents were kept separate, we

**Table 2**
Sample sizes used to quantify plastic ingestion and diet for Tristram's Storm-petrel chicks, on a chamber-specific basis (proventriculus and gizzard separately) or for the whole bird (proventriculus and gizzard combined).

| Metric | Compartment | Sample size | | | | |
|---|---|---|---|---|---|---|
| | | 2007 | 2010 | 2011 | 2012 | Total |
| Total plastic incidence | Proventriculus | 2 | 12 | 0 | 23 | 37 |
| | Gizzard | 2 | 12 | 0 | 23 | 37 |
| | Whole bird | 2 | 12 | 17 | 26 | 57 |
| Plastic type incidence | Proventriculus | 2 | 12 | 0 | 23 | 37 |
| | Gizzard | 2 | 12 | 0 | 23 | 37 |
| | Whole bird | 2 | 12 | 17 | 26 | 57 |
| Natural food incidence | Whole bird | 2 | 12 | 0 | 23 | 37 |
| Natural non-food incidence | Whole bird | 2 | 12 | 0 | 23 | 37 |
| Total plastic mass | Proventriculus | 2 | 12 | 0 | 23 | 37 |
| | Gizzard | 2 | 12 | 0 | 23 | 37 |
| | Whole bird | 2 | 12 | 17 | 26 | 57 |
| Plastic type mass | Proventriculus | 2 | 12 | 0 | 23 | 37 |
| | Gizzard | 2 | 12 | 0 | 23 | 37 |
| | Whole bird | 2 | 12 | 17 | 26 | 57 |
| Fragment count | Proventriculus | 2 | 12 | 0 | 23 | 37 |
| | Gizzard | 2 | 12 | 0 | 23 | 37 |
| Fragment size | Proventriculus | 2 | 12 | 0 | 23 | 37 |
| | Gizzard | 2 | 12 | 0 | 23 | 37 |
| Fragment color | Whole bird | 2 | 12 | 17 | 26 | 57 |

completed all steps of cleaning, sorting, and quantification by chamber and subsequently pooled the data to calculate total values for those specimens. Finally, while we collected and analyzed the intestinal contents of 11 very fresh (freshness code = FFF) specimens, these results are not included in the 'whole bird' plastic incidence and load values.

The first step of sample processing involved rinsing the stomach lining using fresh water and collecting the ingested material (food remains, natural non-food items, and plastic). Methods for initial separation of identifiable material from the fine slurry of digested material varied slightly between specimens and chambers. In 2011, we rinsed all the material (combined chambers) through paper coffee filters in the field and returned the samples to Oahu for subsequent analysis. For sample processing in the lab, we used water pressure and a flow-through 500 μm sieve to clean the proventriculus and whole bird samples (Rapp et al., 2017). We stored the gizzard samples in 70% ethyl alcohol, to remove the bile, and sieved them through a 500 μm sieve with fresh water.

Cleaning and manipulating individual plastic items was challenging, and we took great care to avoid breaking or loosing fragments during handling (Sileo et al., 1989; van Franeker et al., 2011). We sorted the stomach contents wet in a glass petri dish with the aid of a binocular dissecting microscope (10–40×) (Motic Digital). During the sorting process, we removed all material adhered to larger items and retained all items detectable with the naked eye, including some smaller than 500 μm. We classified all items, into one of four broad categories: plastics, natural non-food, natural food, and other (Table 3). We tested suspected plastic items using Rose Bengal dye, which only stains organic material (Davison and Asch, 2011). Following standardized protocols, we classified the plastic items into four types, defined by their shape, compressibility, and flexibility: fragment, foam, line, and sheet (van Franeker et al., 2011). However, we did not attempt to differentiate between pre-production pellets and fragments of manufactured plastics as these items were functionally indistinguishable by size and shape.

### 1.4. Incidence of ingested materials and plastic types

We recorded the incidence of four plastic types (fragment, foam, line, sheet) in each chamber-specific ($n$ = 37 proventriculi and 37 gizzards) and whole bird ($n$ = 20 specimens) sample. We also quantified the incidence of total plastic, natural food and non-food items by whole bird ($n$ = 57 specimens). For all incidence values, we report the percent occurrence and the standard deviation, calculated using binomial probabilities (Zar, 1984). We used analysis of variance (ANOVA) to compare the incidence of the four plastic types between chambers, using all the specimens with discrete proventriculus and gizzard samples (n = 37 birds; 2 from 2007, 12 from 2010, and 23 in 2012).

### 1.5. Mass by plastic type

We air-dried all plastic items in a fume hood for 1–2 days in a temperature-controlled lab. Once dry, we weighed each plastic type separately in a closed foil packet, using a Mettler Toledo NewClassic MS analytical balance, equipped with a draft shield (120 g capacity and 0.0001 g resolution). We calculated chamber-specific total plastic loads by summing the masses of the four plastic types in the proventriculus and the gizzard of each bird, and calculated bird-specific total plastic loads by summing the masses in the proventriculus and gizzard of each bird.

We calculated two metrics of plastic mass, by type: (i) the mean (± S.D.) including zero values (plastic presences and absences), to describe the population level loads, and (ii) the mean (± S.D.) excluding zero values (plastic presence only), to describe the loads of those birds with plastic incidence. Due to the non-normal data, we also reported the median and the range (minimum – maximum) of these distributions.

### 1.6. Mass measurement accuracy

Following recommended practices, we weighed each foil packet 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. 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). Additionally, we repeatedly weighed a test weight throughout the weighing process and recalibrated the scale as necessary (Mettler Toledo, 2012).

The series of 212 duplicate mass measurements revealed a high degree of repeatability, except for one fragment, one sheet, and two foam samples, where one of the weights fell below the scale's resolution (0.0001 g) and were assigned half of that detection threshold (0.00005 g). Overall, the RMSE and associated Pearson correlation coefficients between the replicate masses varied by plastic type, as follows: fragment (RMSE = 0.00026 g, $r$ = 0.999; $n$ = 116), line (RMSE = 0.00019 g, r = 0.999, $n$ = 43), foam (RMSE = 0.00022 g, r = 0.999, $n$ = 44), and sheet (RMSE = 0.00017 g, $r$ = 0.983, $n$ = 9).

### 1.7. Total plastic mass by stomach chamber

We compared the total plastic mass within the proventriculus and the gizzard of the same individual birds, using the subset of 37 specimens where both chambers had been quantified separately. Before we analyzed the mass data, we used one-sample Kolmogorov-Smirnov tests to determine whether they were normally distributed. Because the plastic mass data from the proventriculus (max_diff = 0.161, $n$ = 37, $p$ = 0.016) and the gizzard (max_diff = 0.177, n = 37, $p$ = 0.005) were not normally distributed, we log-transformed the data ($y' = \log_{10} y$). After the transformation, both datasets were normally distributed: proventriculus (max_diff = 0.124, n = 37, $p$ = 0.624) and ventriculus (max_diff = 0.104, n = 37, $p$ = 0.817). We used a paired $t$-test to compare the mass of total plastic (four types combined) within the

**Table 3**
Categories used to classify stomach contents.

| Category | Type | Criteria | Includes |
|---|---|---|---|
| Plastic | Fragment | Hard and rigid | Fragments; Industrial or user pellets |
| | Line | Linear items; flexible; possibly elastic | Monofilament, rope, and net pieces |
| | Foam | Compressible; may contain visible air bubbles | High and low density |
| | Sheet | Planar or sheeted item; may be elastic; tears easily | Any thin planar and sheeted items |
| Natural non-food | Pumice seeds | Hard but porous | White to dark gray color |
| | | Vegetable material; herbaceous or woody | *Portulaca* sp.; unknown seeds |
| Natural food | – | Identifiable food items, includes indigestible parts | Squid, fish, invertebrates |
| Other | – | Any items not listed above | Sand, coral, glass, metal, rubber |

proventriculus and the gizzard from the same individual birds. This test assumed that the paired differences (proventriculus mass − gizzard mass) were normally distributed (max diff = 0.195, n = 37, $p$ = 0.119).

### 1.8. Fragment number

Using only those birds where the proventriculus and the gizzard were kept as separate samples (n = 37), we counted the plastic fragments in each stomach chamber (Mallory, 2008; Terepocki et al., 2017). The number of fragments from the proventriculus (max_diff = 0.200, n = 37, $p$ = 0.104) and the gizzard (max_diff = 0.083, n = 37, $p$ = 0.960) were normally distributed (one-sample Kolmogorov-Smirnov test). Thus, we did not perform any transformations and compared the counts using a paired t-test, assuming the paired differences (proventriculus number − gizzard number) were normally distributed (max diff = 0.170, $n$ = 37, $p$ = 0.233). We also combined these chamber-specific samples and calculated the total number of ingested fragments per whole bird, including those specimens where the two stomach chambers had not been sampled separately ($n$ = 57).

### 1.9. Fragment size

Next, we quantified the size of the ingested fragments. We first photographed the fragments on wetted white filter paper, using a Canon G12 digital camera (TV mode, shutter priority, 1/1000 s, exposure stopped to −1 2/3, ISO auto, with camera flash illumination) with a scale bar. All subsequent measurements were made using these digital photographs and the Fiji software (Image J; open-source software; Schindelin et al., 2012).

We selected a random subsample of up to 25 fragments per chamber and quantified the size of individual fragments using two metrics. We measured each fragment using the elliptical approximation, whereby the longest length was set as the major axis and the minor axis was set perpendicularly. For each ellipse, we measured the largest one-dimensional length (0.1 mm resolution) and the two-dimensional surface area (0.01 $mm^2$ resolution) using the 'measure' function.

We used two paired $t$-tests to compare the median size (length and surface area) of the plastic fragments contained within the two chambers from each individual bird (n = 37). We did not measure the fragments from those birds where the two chambers could not be compared.

### 1.10. Fragment color

We first quantified the incidence of color-specific fragment categories within a given specimen (by whole bird) using the photographs of the proventriculus and gizzard fragments. Following Sileo et al. (1989), we quantified the presence/absence of five subjective color categories: whites (includes white, yellow, tan and brown), blacks (includes gray and black), blues (includes purple and blue), greens (includes all shades of green), and red (includes pink, orange and red).

Next, we quantified the diversity of fragment colors using the Hue, Saturation, Brightness (HSB) color model. We quantified the color of a random subsample of 25 fragments per whole bird (from the two stomach chambers combined) using the ImageJ Color Inspector 3D plugin (v2.3; Barthel, 2006) in Fiji. For each chosen fragment, we averaged the color of a 0.5 mm × 0.5 mm square focal area, avoiding overly bright, shadowed, or cracked surfaces. We then quantified the averaged color using Red, Green, Blue (RGB color space) values, which we then converted into HSB values using standard algorithms in Microsoft Excel (Karcher and Richardson, 2003).

To illustrate the range of colors represented in the ingested fragments graphically, we divided the HSB values into 140 possible categories: 7 levels of hue, 5 levels of saturation, and 4 levels of brightness (Table 4). In the cylindrical three-dimensional HSB color model: Hue

**Table 4**
HSB characteristics used to characterize the color of the plastic fragments ingested by Tristram's Storm-petrel chicks.

| HSB | Categories | Explanation |
|---|---|---|
| Hue (H) | Reds | Cool magenta (285°) to warm red (29°) |
| | Orange-yellows | Orange (30°) to warm yellow (59°) |
| | Yellow | Mid yellow (60–74°) |
| | Greens | Cool yellow (75°) to cool green (134°) |
| | Cyan | Green cyan (135°) to cool cyan (209°) |
| | Blues | Blue cyan (210°) to violet (284°) |
| | Gray scale | No-hue (0° no saturation) |
| Saturation (S) | 0 | 0 is no color (gray scale) |
| | 1–25 | 1 is white, adding tint |
| | 26–50 | Adding tint |
| | 51–75 | Adding tint |
| | 76–100 | 100 is pure color |
| Brightness (B) | 76–100 | 100 has no black |
| | 51–75 | Removing black |
| | 26–50 | Removing black |
| | 0–25 | 0 is pure black |

(color) connects the red and blue ends of the visible color spectrum, depicted as a continuous 360° circle, saturation ranges from 0 (no color) to 100 (pure color), and brightness ranges from zero (white) to 100 (black). Thus, a color with a hue and saturation of zero lies on the center axis of the cylinder and ranges only in brightness (Karcher and Richardson, 2003).

### 1.11. Statistical analysis

We used SPSS to calculate descriptive statistics, and to perform $t$-tests and ANOVA tests (v21.0; IBM Corporation, 2012). Statistical significance was assessed using alpha = 0.05. All results are shown as means ± S.D.s, unless stated otherwise.

## 2. Results

### 2.1. Incidence of plastic types

Every individual we necropsied contained plastic (incidence = 100%, $n$ = 57). Moreover, all the birds where both stomach chambers were analyzed separately ($n$ = 37) contained plastic in the proventriculus and the gizzard. Fragments were the dominant type of ingested plastic, occurring in both stomach chambers of every bird. Therefore, the incidence rates of total plastic and fragments were identical, whether we considered the whole bird, or the proventriculus and the gizzard separately.

Overall, when whole birds were considered, the incidence of fragments was the highest (100%, n = 57), followed by line (89.5%, $n$ = 50 of 57) and foam (78.9%, $n$ = 45 of 57). Sheets had the lowest incidence (15.8%, $n$ = 9 of 57) (Fig. 1). Additionally, we encountered other non-natural non-food items, which were excluded from the analysis: a rubber suction cup, small pieces of glass resembling pumice, and small metal fragments.

We used the subset of birds with two discrete chambers to test for differences in the incidence of ingested plastic as a function of two interacting factors: the four different plastic types (fragment, line, foam, sheet), and the two stomach chambers (proventriculus, gizzard). This ANOVA test revealed significant differences by chamber ($F_{1,7}$ = 12.272, $p < 0.010$) and plastic type ($F_{3,7}$ = 120.133, $p < 0.001$), with a chamber*plastic type interaction ($F_{3,7}$ = 9.371, $p < 0.008$).

While the proventriculus had higher plastic incidence than the gizzard (Fig. 1), the incidence of fragments was the highest in both stomach chambers. Yet, the ANOVA also revealed significant stomach chamber * plastic type interactions: the incidence of foam and line was higher in the proventriculus, and the incidence of sheets was higher in

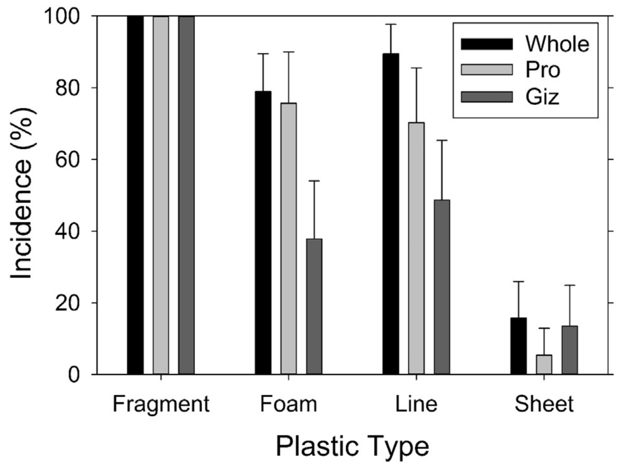


**Fig. 1.** Incidence of plastic by type in whole bird, proventriculus, and gizzard. Error bars indicate ± 2 S.D. calculated using binomial probabilities.

the gizzard (Fig. 1). Furthermore, the ANOVA residuals were normally distributed (one-sample Kolmogorov – Smirnov test, $n = 8$, max_diff = 0.166, $p = 0.956$).

Following the significant ANOVA, we used post-hoc Tukey Honest Significant Difference (HSD) tests to investigate the differences between the incidence rates of the four plastic types. The resulting pair-wise comparisons indicated that the incidence of fragments was the highest, the incidence of foam and line were intermediate and not significantly different from each other, and the incidence of sheets was the lowest.

### 2.2. Incidence of natural food and non-food items

The stomach contents of the 37 individuals necropsied in the lab consisted of brown (sometimes purplish or greyish) slurry, and often included orange proventricular oil. Because the advanced degree of digestion inhibited individual prey identification, we recorded broad prey categories using the presence and absence of the following indigestible hard parts: insects (water strider *Halobates* sp.; legs, heads, and bodies) in 34 individuals (91.9% ± 4.5 S.D.), squid (unidentified species; lenses or beaks) in 35 individuals (94.6% ± 3.8 S.D.), pelagic gastropod snails (Janthinidae family) in 14 individuals (37.8% ± 8.1%), and fish (unidentified species; bones, lenses, or otoliths) in 7 individuals (18.9% ± 6.5 S.D.) (Fig. 2). We also encountered other food items, which we did not quantify: fragments of soft bodied crustaceans, light-colored worms (likely parasitic nematodes), small orange or yellow orbs (likely *Halobates* sp. eggs), and small flakes of shiny pink and white flesh.

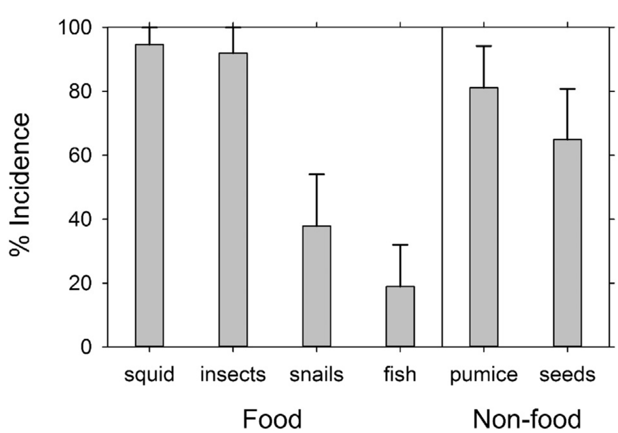


**Fig. 2.** Incidence of natural prey and non-prey items. Incidence is reported as mean ± 2 S.D. calculated using binomial probability (n = 37 birds).

Natural non-food items ingested by these 37 individuals involved seeds (*Portulaca* sp. and unidentified species) in 24 individuals (64.8% ± 7.9 S.D.), and pumice in 30 individuals (81.1% ± 6.5 S.D.) (Fig. 2). Additionally, we occasionally encountered bits of coral, sand, grass, feathers, feather barbs, and ticks.

### 2.3. Total plastic mass

Considering the 37 birds with discrete proventriculus and gizzard plastic samples, the total plastic mass (g) in the proventriculus (mean = 0.455 ± 0.467 S.D., median = 0.258, range = 0.006–2.126) and in the gizzard (mean = 0.109 g ± 0.070 S.D., median = 0.109, range = 0.030–0.288) varied widely. Nevertheless, when we compared the log-transformed total plastic masses in both stomach chambers using a paired *t*-test, there was a significantly higher mass (mean difference = 0.399, 95% C.I. = 0.216–0.583) in the proventriculus than in the gizzard of the same bird ($t_{36} = 4.409$, $p < 0.001$, effect size = 0.72).

The total plastic mass (g) by whole bird ($n = 57$), calculated by combining the mass of the proventriculus and the gizzard, ranged from 0.1145–2.8107 (mean = 0.7454 ± 0.5824 S.D., median = 0.6066). While the mass of ingested plastic in the gizzard did not exceed 0.2880 g, we documented larger plastic masses in the proventriculus, reaching up to 2.1258 g (Fig. 3). Thus, the ability to predict the total ingested plastic mass by merely sampling the contents of the proventriculus differed for birds with low and high plastic masses, below and above the median total plastic mass (0.4722 g; $n = 37$), respectively. For the birds with higher plastic loads (total mass > 0.4722 g) the proventriculus plastic masses almost perfectly correlated with their total plastic loads (Pearson correlation, $r = 0.990$, $n = 18$, $r^2 = 0.980$). This correlation was substantially weaker for the birds with low plastic loads (total mass < 0.4722 g) (Pearson correlation, $r = 0.832$, n = 18, $r^2 = 0.692$).

### 2.4. Mass of plastic types

Fragments were the most pervasive and abundant plastic type,

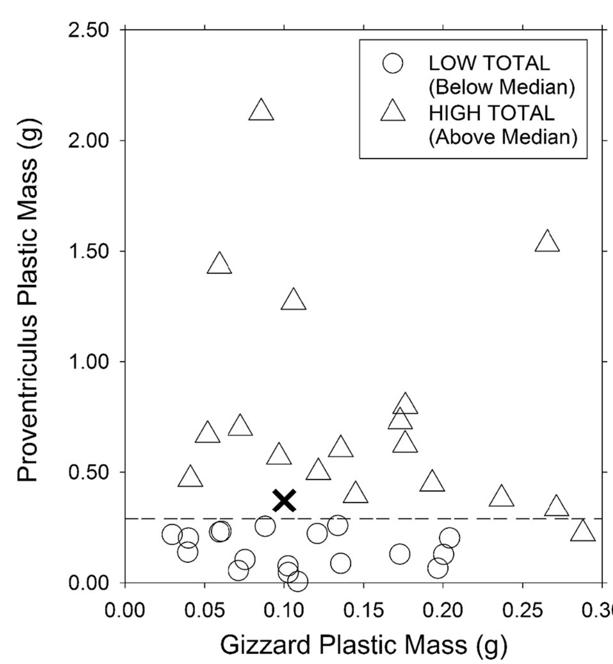


**Fig. 3.** Correlation between the plastic mass in the proventriculus and the gizzard of the same Tristram's Storm-petrel chicks (n = 37), showing those individuals with high (above) and low (below) the median plastic mass of 0.4722 g (shown by the X). The dashed line indicates the maximum plastic mass found in the gizzard (2.8107 g).

**Table 5**
Chamber-specific (proventriculus and gizzard) and whole bird plastic loads (expressed as the grams of ingested plastic), calculated for the population (including zero values) and for those individuals that ingested plastic (excluding zero values).

| | | Population – level exposure (g) | | | Individual – level exposure (g) | | |
|---|---|---|---|---|---|---|---|
| | | Median (range) | Mean ± S.D. | n | Median (range) | Mean ± S.D. | n |
| Whole bird | Fragment | 0.5877 (0.1064–2.8009) | 0.7272 ± 0.5813 | 57 | 0.5877 (0.1064–2.8009) | 0.7272 ± 0.5813 | 57 |
| | Foam | 0.0031 (0.0000–0.0314) | 0.0055 ± 0.0067 | 57 | 0.0048 (0.00005–0.0314) | 0.0069 ± 0.0068 | 45 |
| | Line | 0.0042 (0.0000–0.1171) | 0.0119 ± 0.0205 | 57 | 0.0053 (0.0003–0.1171) | 0.0135 ± 0.0213 | 50 |
| | Sheet | 0.0000 (0.0000–0.0020) | 0.0001 ± 0.0004 | 57 | 0.0007 (0.00005–0.0020) | 0.0008 ± 0.0007 | 10 |
| | Total plastic | 0.6030 (0.1145–2.8107) | 0.7447 ± 0.5827 | 57 | 0.6030 (0.1145–2.8107) | 0.7447 ± 0.5827 | 57 |
| Proventriculus | Fragment | 0.2433 (0.0059–2.1080) | 0.4372 ± 0.4655 | 37 | 0.2433 (0.0059–2.1080) | 0.4372 ± 0.4655 | 37 |
| | Foam | 0.0024 (0.0000–0.0269) | 0.0054 ± 0.0067 | 37 | 0.0054 (0.00005–0.0269) | 0.0072 ± 0.0068 | 28 |
| | Line | 0.0033 (0.0000–0.1171) | 0.0119 ± 0.0227 | 37 | 0.0080 (0.0003–0.1171) | 0.0164 ± 0.0252 | 27 |
| | Sheet | 0.0000 (0.0000–0.0010) | 0.0000 ± 0.0002 | 37 | 0.0009 (0.0008–0.0010) | 0.0009 ± 0.0001 | 2 |
| | Total plastic | 0.2579 (0.0059–2.1258) | 0.4546 ± 0.4667 | 37 | 0.2579 (0.0059–2.1258) | 0.4546 ± 0.4667 | 37 |
| Gizzard | Fragment | 0.1073 (0.0284–0.2880) | 0.1261 ± 0.0683 | 37 | 0.1073 (0.0284–0.2880) | 0.1261 ± 0.0683 | 37 |
| | Foam | 0.0000 (0.0000–0.0063) | 0.0008 ± 0.0016 | 37 | 0.0014 (0.00005–0.0063) | 0.0024 ± 0.0020 | 13 |
| | Line | 0.0000 (0.0000–0.0133) | 0.0009 ± 0.0025 | 37 | 0.0004 (0.00005–0.0133) | 0.0020 ± 0.0034 | 17 |
| | Sheet | 0.0000 (0.0000–0.0006) | 0.0000 ± 0.0001 | 37 | 0.0003 (0.00005–0.0006) | 0.0003 ± 0.0002 | 5 |
| | Total plastic | 0.1086 (0.0293–0.2880) | 0.1278 ± 0.0699 | 37 | 0.1086 (0.02930–0.2880) | 0.1278 ± 0.0699 | 37 |

occurring in all the necropsied specimens (100% incidence). Thus, the population-level and the individual-level mean plastic masses were identical, when the fragments were considered. Conversely, because the three other plastic types (line, foam, sheet) did not occur in all individuals (Fig. 1) the estimates of population-level exposure (including zero values) are lower than the estimates of individual-level loads (excluding zero values) (Table 5). Fragments accounted for most of the plastic mass ingested by every individual bird, ranging from 69.1 to 100.0% (mean = 96.4% ± 5.7 S.D., median = 98.5%). Conversely, the combined mass of the other three plastic types (foam, line, and sheet) merely accounted for 0.0–30.9% (mean 3.6% ± 5.7 S.D., median 1.5%).

### 2.5. Fragment number

We documented 11,581 fragments ingested by 57 birds. The total number of fragments per individual ranged from 32 to 615 (mean = 203.2 ± 145.6 S.D., median = 149). Considering the 37 birds with discrete proventriculus and gizzard plastic samples, the number of fragments in the proventriculus (mean = 112.1 ± 116.7 S.D., median = 65.5, range = 3–581) and in the gizzard (mean = 44.3 ± 24.3 S.D., median = 40.5, range = 11–104) varied widely. When we compared the counts using a paired *t*-test, there was a significantly higher number of fragments (mean difference = 70.027, 95% C.I. = 30.302–109.752) in the proventriculus than in the gizzard of the same bird ($t_{36}$ = 3.575, $p$ = 0.001, effect size = 0.59). Yet, the number of fragments in the two stomach chambers of the same individual was not significantly correlated ($r$ = 0.09, $n$ = 37, $p < 0.607$) and, thus, could not be used to predict each other.

### 2.6. Fragment size

For each individual bird with discrete proventriculus and gizzard samples (n = 37), we used the median maximum length (mm) and the median surface area ($mm^2$) to characterize the size of the ingested fragments in each stomach chamber. Although some stomach chambers contained fewer than 25 fragments, the number of measured fragments did not vary significantly by chamber (paired t-test, $t_{36}$ = 0.437, $p$ = 0.665, effect size = 0.07). Thus, we did not expect a bias in the median sizes calculated for the proventriculus and the gizzard due to unequal sample sizes. Nevertheless, because the summary results described below are based on a subsample ($n < 25$ fragments) of measured fragments, these analyses are subject to sampling variability.

Fragment length varied widely: ranging from 0.6 to 11.6 mm (mean = 3.0 mm ± 1.1 S.D., median 2.9 mm, $n$ = 843) for the proventriculus, and from 0.4 to 7.5 mm (mean = 2.7 mm ± 0.8 S.D., median 2.7 mm, $n$ = 786) for the gizzard. A comparison of the median fragment length from the two chambers, paired by individual bird, revealed larger fragments ($t_{36}$ = 4.018, $p < 0.001$, effect size = 0.56) in the proventriculus than in the gizzard.

Fragment surface area also varied widely: ranging from 0.23 to 45.21 $mm^2$ (mean = 5.22 mm ± 3.90 S.D., median 4.48 $mm^2$, n = 843) for the proventriculus and from 0.07 to 35.22 $mm^2$ (mean = 4.37 mm ± 2.64 S.D., median 3.92 $mm^2$, n = 786) for the gizzard. Similarly to the length results, the median fragment area was larger ($t_{36}$ = 3.027, $p < 0.005$, effect size = 0.45) in the proventriculus than in the gizzard of the same individual.

Nevertheless, the median fragment length in the proventriculus and the gizzard of the same individual ($r$ = 0.39, $n$ = 37, $p < 0.017$) and the median fragment area in the proventriculus and the gizzard of the same individual ($r$ = 0.43, n = 37, $p < 0.007$) were significantly correlated. Thus, even though the fragments in the proventriculus had a significantly larger length and surface area, the cross-correlated values within individuals suggest that some birds consistently contained longer fragments in both stomach chambers.

### 2.7. Fragment color

All the necropsied individuals contained "white" fragments (incidence = 100% ± 0 S.D.), followed by 'red' in 96% ± 2 S.D. ($n$ = 55 of 57), 'blue' in 95% ± 3 S.D. ($n$ = 54 of 57), 'black' in 89% ± 4S.D. ($n$ = 51 of 57) and 'green' in 65% ± 6 S.D. (n = 37 of 57). Overall, the incidence of these color categories varied significantly (G test, G = 33.36, df = 4, $p < 0.0005$) from the results from Sileo et al. (1989). Only the 'white' category, which was the most prevalent in both samples, did not show a significant increase. The non-overlapping S.D.s suggest the incidence of the other four color categories increased between the mid-1980s and our study (Fig. 4).

To further characterize the color of the ingested fragments, we analyzed the hue, saturation and brightness of a randomly chosen subsample of 1425 fragments (25 per bird, $n$ = 57 birds) representing 12.3% of all ingested fragments. The distribution of observed HSB values, underscored the wide range of ingested fragment colors, with large numbers in the light yellow to orange range, with blues, greens and reds, and occasionally dark colors (Fig. 5). In particular, the most frequent hue (H) categories were orange-yellow (62.5%), followed by red (12.7%), yellow (11.4%), cyan (6.6%), green (3.9%), blue (2.1%), and greyscale items (no hue) (0.9%). Moreover, over half (55.2%) of the fragments had low saturation (1–25), indicative of light hues, with

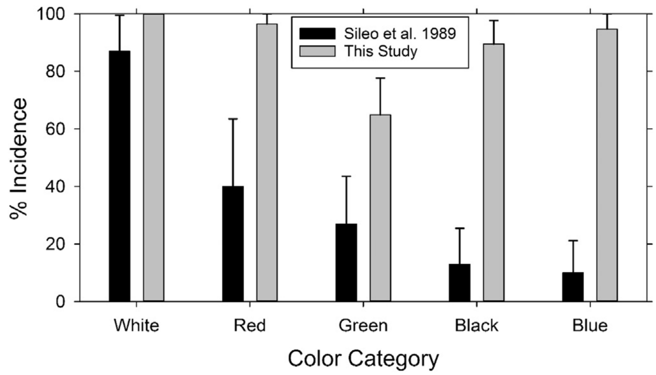


**Fig. 4.** Percent incidence of color categories comparing the current study to a past study. Incidence values ± 2 S.D. of 5 color categories: (2007–2012; necropsy whole bird; Tern Island; n = 57; chicks) and Sileo et al. (1989) (1986; stomach pumping of proventriculus; Laysan Island; $n$ = 30; adults and chicks).

decreasing frequencies for the higher saturation categories: 26–50 (31.2%), 51–75 (11.6%), and 76–100 (1.2%). The lowest category (saturation = 0), corresponding to gray scale items, was the least common (0.9%). Finally, the majority (69.0%) of the fragments were brightly colored, with brightness (B) values between 51 and 75. Brighter (76–100) and duller (26–50) fragments occurred at lower frequencies of 10.2% and 16.9%, respectively. The lowest brightness fragments (0–25) were the least common (3.9%).

## 3. Discussion

### 3.1. Plastic ingestion

Every Tristram's Storm-petrel chick we collected between 2007 and 2012 from Tern Island had ingested plastic, with an average of 0.75 g ( ± 0.58 S.D.) total plastic and 203 fragments ( ± 146 S.D.) per bird (n = 57). Yet, despite the 100% incidence, there was substantial variability in the loads of ingested plastic, both within (per stomach chamber) and across (per whole bird) individuals. Despite the ubiquitous presence of fragments in every gizzard and proventriculus we sampled, the incidence of the other plastic types (line foam, sheet) varied significantly by stomach chamber. Thus, studies that only sample the proventriculus (i.e., via regurgitation) or sample both chambers (i.e., via necropsy) will likely yield different results concerning the incidence and loads of plastic ingested by Tristram's Storm-petrels. Therefore, understanding how the different types of ingested plastic accumulate in the two stomach chambers is critical for monitoring exposure in this species, and for providing robust metrics of plastic pollution in the marine environment.

Unfortunately, the statistical analyses of trends in Tristram's Storm-petrel plastic incidence and loads over time are inhibited by the lack of observations during the last two decades (Table 1). Thus, we compared our results with the only existing published study of plastic ingestion in Tristram's Storm-petrel chicks. However, because Sileo et al. (1989) relied on gastric lavage to induce regurgitation and did not sample the contents of the gizzard, we compared the published incidence (35.3% ± 11.9 S.D.) with our estimate (100% ± 0 S.D.) based exclusively on the proventriculus samples ($n$ = 37 chicks with separate chambers). This comparison revealed a 183% increase in plastic ingestion incidence over 25 years, which, reinforces evidence of increasing plastic concentrations at-sea and ingestion by other North Pacific seabirds (Robards et al., 1997; Avery-Gomm et al., 2012; Goldstein et al., 2012).

It is conceivable that Sileo et al. (1989) underestimated Tristram's Storm-petrel plastic incidence due to the inability to retrieve ingested plastics from the gizzard via lavage (Duffy and Jackson, 1986). However, our findings suggest that the 1987 estimate was accurate for two reasons. First, Tristram's Storm-petrels ingest large numbers of small plastic fragments, many of which remain in the proventriculus and should be sampled effectively with lavage. Second, we documented the same incidence rates (100% ± 0 S.D.) for the proventriculus and the

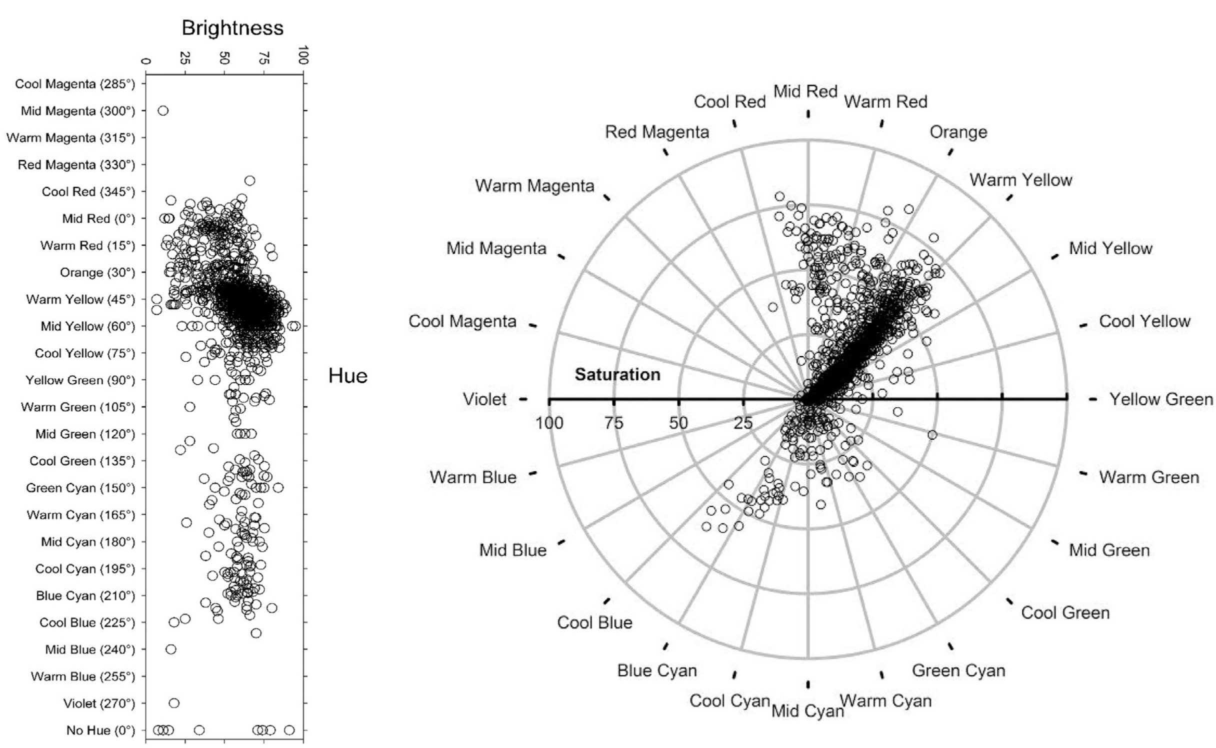


**Fig. 5.** HSB characterization of $n$ = 1425 ingested fragments in terms of their hue (color), saturation, and brightness. The polar graph shows the distribution of hue in 15° segments. Saturation describes tint and ranges from zero (white) to 100 (pure hue) along the radial axis. The scatter plot shows the distribution of brightness, with hue ranging from zero (black) to 100 (white).

gizzard, suggesting that the proventricular estimates of Sileo et al. (1989) likely reflected the overall incidence of plastic ingestion.

Furthermore, the visual categorization of the fragments in our Tristram's Storm-petrel samples ($n$ = 57 chicks) revealed a significant increase in the incidence of four of the five color categories considered (black, blue, red, green), compared to the incidence rates reported by Sileo et al. (1989) (Fig. 4). While this increase is likely caused by the increasing number of fragments ingested per bird, a statistical comparison was inhibited by the lack of historical fragment counts. Alternatively, this disparity may indicate a higher availability of newer and more colorful plastic fragments to foraging Tristram's Storm-petrels, relative to the levels of the late 1980s. Interestingly, only white fragments did not increase significantly, even though their current incidence rate reached 100%.

### 3.2. Diet and foraging ecology

Knowledge of Tristram's Storm-petrel diet and foraging ecology can help interpret the high incidence and loads of ingested plastic we documented. Our qualitative analysis revealed a diverse diet, with high incidence of neustonic invertebrates and vertically-migrating fish and squid. However, due to differences in our sampling methods, we did not compare our results with previous dietary studies (Harrison et al., 1983; Bond et al., 2010). The analysis of identifiable prey items in 10 adult regurgitations collected in 1979–1981, revealed high incidence of squid (60%), *Velella* (40%), *Halobates* sp. water striders, (30%), fish (40%), and crustaceans (10%) (Harrison et al., 1983). Notably, our necropsy samples yielded higher rates of water striders and squid, due to the retention of indigestible hard parts in the gizzard. Conversely, we did not find any gelatinous zooplankton remains, which degrade rapidly in the stomach. Nevertheless, both diet analyses are consistent with evidence of low trophic-level prey provisioned to chicks ($n$ = 14) sampled in Tern Island in 2005–2006, as revealed by the stable isotope values of their blood (Bond et al., 2010).

Overall, our results show that, despite ingesting fragments of a wide color range, Tristram's Storm-petrels contain mostly (86.54%) light shades of reds, oranges, and yellows, with low saturation values (1–25) and moderate brightness (51–75). While the abundance of different color plastic fragments within their foraging range is unknown, we hypothesize that the high frequency of reds, yellows and dark fragments suggests that Tristram's Storm-petrels are targeting plastic resembling their prey: *Halobates* (black), *Velella* and *Janthina* (blues and purples), and crustaceans (reds and oranges).

Moreover, while very little is known about the visual acuity of the Tristram's Storm-petrel, shipboard observations have documented tubenose seabirds showing a preference for red and orange (food and non-food) items, but ultimately rejecting non-food items (e.g., orange peel and colored paper) (Harper, 1979). Because Tristram's Storm-petrels are nocturnal foragers likely relying on olfactory cues, they may be ingesting non-food items due to bio-fouling by biota, which release the same keystone infochemical (i.e., dimethyl sulfide) as some of their prey (Savoca et al., 2016). Another possibility is that Tristram's Storm-petrels may be ingesting plastic items with attached prey (e.g., water strider eggs; Goldstein et al., 2012; Reisser et al., 2014). These mechanisms may explain the ingestion of many light-colored items, which make up majority of the marine debris floating at sea in the central North Pacific Ocean (89% white; Titmus and Hyrenbach, 2011).

### 3.3. Plastic impacts

Despite the high levels of plastic incidence and loads, the impacts of this ingestion on Tristram's Storm-petrels are unknown. Previous studies have documented direct mechanical damage (impaction and abrasion), and indirect impacts from leaching of toxic chemicals affecting condition, growth, and reproduction (Pierce et al., 2004; Hardesty et al., 2014; Lavers et al., 2014; Browne et al., 2015). The low incidence of line and sheet, which can cause obstructions of the gastrointestinal tract (Pierce et al., 2004), and the small size of the ingested hard fragments suggest the risks from mechanical abrasion and obstruction are minimal for the Tristram's Storm-petrel.

Nevertheless, the high plastic loads of the Tristram's Storm-petrel are particularly noteworthy, because they exceed the thresholds recommended for other larger-bodied seabird species. For instance, the Oslo/Paris Commission (OSPAR), for instance, has adopted an Ecological Quality Objective (EcoQO) goal of plastic loads of 0.1 g in < 10% of Northern Fulmar (*Fulmarus glacialis*) collected from North Sea beaches during a running 5-year period (Heslenfeld and Enserink, 2008; van Franeker et al., 2011). All individuals examined in our study exceeded the EcoQO threshold set for the larger-sized species, underscoring the pervasiveness and large magnitude of plastic ingestion in Tristram's Storm-petrels. Furthermore, while this 0.1 g plastic load represents 0.02% of an adult 650-g Northern Fulmar's body mass, it represents 0.12% of the average body mass of an adult 84-g Tristram's Storm-petrel (Harrison et al., 1983; van Franeker et al., 2011).

Tristram's Storm-petrels are likely wearing down and passing indigestible items, including plastics and hard food remains. The significantly smaller plastic fragments in the gizzard, relative to the proventriculus, are consistent with the mechanical breakdown of this material. Furthermore, the presence of smaller size (< 1 mm) fragments in 27% (3 of 11) of the intestines from fresh specimens we examined, suggest that very small plastic items can pass through the digestive tract to be excreted. This result, which reinforces previous observations of plastic in the intestine of larger-bodied albatrosses, underscores the likelihood that ingested plastics are eventually passed in many smaller-sized species equipped with a gizzard (Sileo et al., 1989).

While these results suggest that Tristram's Storm-petrels are capable of degrading and passing the ingested plastics, the larger fragment sizes and higher plastic loads found in the proventriculus suggest that the chicks were being fed plastic at a rate faster than it could be processed. The accumulation of this material demonstrates the possibility of indirect sub-lethal effects of plastic ingestion on condition, growth, and reproduction. However, because limited empirical data on retention times and passage times exist (reviewed in Ryan, 2015), estimating the exposure and flux of plastic through seabird individuals and populations remains a key question with implications for assessing potential health impacts (Ryan and Jackson, 1987; Ryan, 1988). Thus, additional studies are needed to assess the rate of degradation and passage of ingested plastics, using rehabilitated birds in captivity or temporal changes in the loads ingested by birds sampled in the wild (Terepocki et al., 2017).

In some large-bodied tubenose species (e.g., Laysan Albatross *Phoebastria immutabilis*, Young et al., 2009), chicks expel a bolus of indigestible material prior to fledging. Because we did not observe or find published accounts of bolus production in storm-petrels, we infer that all the plastic provisioned by the parents must be retained in the gizzard or broken down in the chick's gizzard and eventually passed onto the intestine towards excretion. Thus, Tristram's Storm-petrel chicks may be particularly susceptible to adverse effects from plastic ingestion (Ryan, 1988), including direct (e.g., mechanical) and indirect (e.g., chemical) impacts (Boersma, 1986; Fry et al., 1987; Lavers et al., 2014). Additionally, because it is unlikely that Tristram's Storm-petrel adults regurgitate this plastic outside of the breeding season, when adults offload this material to their chicks, such impacts are also likely to affect adults throughout their lifespan. Notably, Tristram's Storm-petrels are long-lived seabirds, with mark-recapture studies suggesting they can live for at least 14 years (Marks and Leasure, 1992). Thus, we advocate further correlational studies of Tristram's Storm-petrel growth and condition, concurrently with non-lethal sampling of pollutant loads from feathers or preen gland oil (Hardesty et al., 2014; Lavers et al., 2014).

While we did not document instances of stomach lesions or obstructions, which would suggest that the ingested plastics caused the death of the Tristram's Storm-petrel chicks, the pervasive and large plastic loads we documented are a cause of concern. Thus, further research on the potential direct and indirect impacts of this pollution on chicks and adults are needed, under the auspices of a broader population-level assessment of the species.

### 3.4. Tristram's Storm-petrels as biological indicators of plastic pollution

Seabirds are increasingly being used as biological indicators of ocean plastic pollution, by comparing the incidence and loads of ingested plastic against benchmarks of environmental quality (Heslenfeld and Enserink, 2008; van Franeker et al., 2011; Donnelly-Greenan et al., 2014).

The fragments ingested by Tristram's Storm-petrels (between 0.4 and 11.6 mm in length) overlap the microplastic (1–5 mm) and mesoplastic (5–20 mm) size ranges (Provencher et al., 2017), and fall within the most abundant size class of plastics (1.01–4.75 mm) found on the ocean's surface (Cózar et al., 2014; Eriksen et al., 2014). Thus, Tristram's Storm-petrel chicks can provide a convenient and reliable source of samples for monitoring trends in micro and mesoplastics floating at-sea. Researchers can opportunistically sample large numbers of chicks at breeding colonies, especially when they nest in accessible artificial sites used for demographic monitoring, or when they fledge. Nevertheless, the use of Tristram's Storm-petrel chicks to monitor plastic pollution in the marine environment will require additional ground-truthing, involving an understanding of the parental foraging ranges during the breeding season, and cross-correlations between the loads, sizes and colors of the ingested plastic in the chicks and floating at-sea (Durant et al., 2009).

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