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Assessment of plastic ingestion by pole-caught pelagic predatory fish from O'ahu, Hawai'i

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Abstract

1. Although the frequency of occurrence of plastic ingestion in the large-sized dolphinfish and tunas taken by the Hawai'i longline fishery is very low (frequency of occurrence < 5% of sampled individuals), the ingestion of plastic in smaller-sized specimens caught with pole-and-line gear by commercial and recreational fishers has not been investigated.
2. This study examined ingestion of >0.25 mm marine plastic debris (MPD) by four predatory fish species caught by commercial fishers around the Main Hawaiian Islands, and documented ingestion in three species: 85.7% of albacore tuna (n = 7), 40.0% of skipjack tuna (n = 10) and 12.5% of dolphinfish (n = 8).
3. Yellowfin tuna (n = 10) did not contain any MPD, probably owing to the high proportion of empty stomachs (60%).
4. For skipjack tuna, the frequency of occurrence of MPD ingestion was significantly higher for the smaller-sized specimens caught with pole-and-line (40%), compared with the larger-sized specimens caught with longlines (0%).
5. For dolphinfish, the frequency of occurrence of MPD ingestion was similar for the similar-sized specimens caught with pole-and-line and with longlines.
6. The ingested MPD items were micro–meso plastics, between 1 and 25 mm. While most ingested items were fragments, albacore also ingested line and skipjack also ingested sheets.
7. The predatory fishes ingested light MPD items that float in sea water, but there were species-specific differences in their polymer composition: albacore contained more polypropylene and polyethylene, and skipjack contained more elastomers, characterized by a high percentage of ester plasticizers.
8. Altogether, these results suggest that albacore and skipjack tunas ingest plastic of different types and polymers. Yet more research is needed to understand how differences in vertical distribution, foraging ecology and diet influence the MPD sampled by these predatory fish species.

KEYWORDS
albacore, Fourier transform infrared spectroscopy, FT-IR, Hawai'i, *Katsuwonus pelamis*, marine plastic debris, plastic ingestion, predatory fish, skipjack, *Thunnus alalunga*

1 | INTRODUCTION

The ingestion of marine plastic debris (MPD) is a widespread and pervasive phenomenon globally (Cole, Lindeque, Halsband, & Galloway, 2011; Gall & Thompson, 2015; Gouin, 2020; Markic, Gaertner, Gaertner-Mazouni, & Koelmans, 2020), which has been documented in an increasing number of marine species, ranging from lower-trophic-consumers (e.g. gelatinous zooplankton; Macali et al., 2018) to upper-trophic predators (e.g. cetaceans; Lusher et al., 2018). Multispecies assessments have documented MPD ingestion in commercially valuable fish species and their prey (Gouin, 2020; Markic et al., 2020). Yet, despite some recent comparative studies (Forrest & Hindell, 2018; Markic et al., 2018; Thiel et al., 2018), understanding of how plastic contamination of commercial fish species differs across the Pacific Ocean is inhibited by a lack of data for many regions and archipelagos.

Plastic ingestion by predatory fish and their prey is of particular concern for the potential transfer of marine debris through food webs, leading to human consumption (Chagnon et al., 2018; Forrest & Hindell, 2018; Gove et al., 2019; Markic et al., 2018). In particular, fish are an especially important food resource for oceanic islands, where commercial, consumption and recreational fishers target a wide array of pelagic and demersal species. For instance, a recent broad-scale assessment of plastic ingestion by 34 fish species common in the diet of South Pacific island inhabitants revealed pervasive MPD in 33 species. Moreover, the incidence rate was significantly higher in Rapa Nui (Easter Island), compared with the other three study locations (New Zealand, Samoa, Tahiti; Markic et al., 2018). Plastic ingestion by marine predators is pervasive in Rapa Nui, which is located within a MPD aggregation zone associated with the South Pacific Subtropical Gyre (Thiel et al., 2018).

In the northern hemisphere, MPD concentrates within the North Pacific subtropical gyre, a vast surface convergence zone characterized by dense floating plastic aggregations, and where epipelagic and mesopelagic fish species have been documented ingesting plastic (Boerger, Lattin, Moore, & Moore, 2010; Davison & Asch, 2011; Titmus & Hyrenbach, 2011). MPD is also pervasive throughout the Hawaiian archipelago, where tons of floating plastic wash ashore every year, driven by large-scale oceanographic processes (Howell, Bograd, Morishige, Seki, & Polovina, 2012; Morishige, Donohue, Flint, Swenson, & Woolaway, 2007; Pichel et al., 2007). The Hawai'i longline fishery operates across a vast expanse of the Central Pacific Ocean (5° S to 50° N, 170° E to 125° W), and targets tunas and swordfish along the same frontal systems that aggregate derelict fishing gear (Bigelow, Boggs, & He, 1999; Choy & Drazen, 2013; Uhrin, Walsh, & Brodziak, 2020).

Choy and Drazen (2013) analysed the stomach contents of 595 predatory fish caught by the longline fishery and landed in Hawai'i, and documented widespread MPD ingestion, with seven of the 10 species (70%) examined containing plastic. In particular, three species had the highest frequency of occurrence (FO): the small-eye moonfish (*Lampris* sp.; FO = 58%, *n* = 24), the big-eye moonfish (*Lampris* sp.; 43%, *n* = 115) and the longnosed lancetfish (*Alepisaurus ferox*; FO = 30%, *n* = 144). Interestingly, the incidence of MPD ingestion was very low in the large-bodied tuna specimens targeted by the longline fishery. In fact, only bigeye tuna (*Thunnus obesus*) contained plastic (FO = 3%, *n* = 35), with no evidence of ingestion in skipjack tuna (*Katsuwonus pelamis*, *n* = 29) or yellowfin tuna (*Thunnus albacares*, *n* = 26). Moreover, only 2% of dolphinfish (*Coryphaena hippurus*, *n = 42*) and 1% of broadbill swordfish (*Xiphias gladius*, *n* = 31) had ingested MPD. While they are taken in low numbers by the Hawai'i longline fishery, no albacore tuna (*Thunnus alalunga*) were sampled by Choy and Drazen (2013).

Even though the longline fishery is the largest and most valuable in Hawai'i, with the catch being sold for local and international consumption, commercial and recreational fishers target a variety of pelagic fish in the waters surrounding the Main Hawaiian Islands (MHI) (Graham, Grubbs, Holland, & Popp, 2007; Hebshi, Duffy, & Hyrenbach, 2008; NMFS, 2017). Choy and Drazen (2013) documented very low MPD incidence (FO < 5% of sampled individuals), in the large-sized tuna and dolphinfish caught by the Hawai'i longline fishery, but plastic ingestion for the smaller-sized specimens caught with pole-and-line gear around the MHI has not been investigated, despite their importance for island-based subsistence, commercial and recreational fishers (Loke, Geslani, Takenaka, & Leung, 2012; NMFS, 2017).

To complement the previous studies in Hawai'i, involving the analyses of longline-caught fish (Choy & Drazen, 2013) and larvae sampled from nearshore nurseries (Gove et al., 2019), MPD ingestion by four pelagic predatory fish species (albacore, skipjack, yellowfin and dolphinfish) caught with pole-and-line fishing gear around the MHI during one winter season (October 2012 to March 2013) was examined. The primary goal of this snapshot assessment was to determine if the incidence of ingested MPD in the smaller-sized tuna and dolphinfish caught with pole-and-line gear differs from that reported for the larger-sized specimens caught by the longline fishery. The secondary goal was to augment the existing database of MPD ingestion (occurrence and loads) in Hawai'i, to facilitate comparisons with other Pacific Ocean archipelagos.

2 | METHODS

2.1 | Fish sampling

A commercial fisher (Captain Michael Diamond) captured the fish with conventional pole-and-line fishing gear using artificial lures, within one day's journey (<90 km) from the Island of O'ahu. While we sought a sample size of 10 specimens per species, only 35 fish were caught during the study period: seven albacore tuna (AT), eight dolphinfish (DF), 10 skipjack tuna (ST) and 10 yellowfin tuna (YT). Upon capture, the fork length (FL) was recorded to the closest 0.25 inch (0.625 cm) and the stomachs were extracted whole, stored in sample bags and transported frozen to the Oceanic Institute, in O'ahu.

2.2 | Stomach content analysis

The dissection of the stomachs, sorting of stomach contents and plastic quantification were performed by the same person (ZM), following standardized protocols. The first step entailed removing the stomach from the other organs. Owing to the unique anatomy of the ST, their stomach and duodenum (often nearly the same size of the stomach) were sampled (Magnuson, 1969). Only the contents of the stomach were analysed for the other species. While ingested MPD can travel through the entire gastrointestinal system of the fish, the contents of the intestines were not analysed, to facilitate the comparison of our results with those previously published from the Hawai'i longline fishery (Choy & Drazen, 2013; Jantz, Morishige, Bruland, & Lepczyk, 2013).

To ensure all the stomach contents were collected, the stomach lining was rinsed with fresh water to remove all of the solid items and fluids, and sieved through a 250 μm mesh sieve to retain the ingested prey and MPD. For each predator species, the proportion of fish (mean ± SD) with empty stomachs (Vinson & Andradi, 2011) was calculated using binomial probability (Zar, 1984; Table 1).

2.3 | Plastic sorting and quantification

Potential plastic items in the stomach contents were sorted using light (2×, 5×) and higher (10–40×) magnification under a binocular dissecting microscope (Motic Digital), aided by the use of Rose Bengal (1:100 dilution in 70% EtOH; Davison & Asch, 2011). This dye, which colours prey remains (otoliths, scales, bone fragments) pink but leaves plastic and inorganic items (sand) unstained, facilitates the visual identification of MPD. Initially, 28 potential plastic items (six line pieces, three line clumps, 17 fragments and two sheets) were examined, but only 22 were validated using Rose Bengal (six line pieces, three line clumps, 11 fragments and two sheets). For each predator species, the frequency of occurrence (mean ± SD) of ingested plastic, defined as the proportion of stomachs that contained any MPD, was calculated (Table 1). Throughout this paper, the terms 'incidence' and 'loads' refer to the FO and the mass of ingested MPD, respectively.

Following standardized protocols, the MPD items were classified into four categories, defined by their shape, compressibility and flexibility: line, sheets, fragments and foam (Rapp, Youngren, Hartzell, & Hyrenbach, 2017; van Franeker et al., 2011). The 22 MPD items were scanned at high resolution (1,200 dpi) using a digital scanner (Epson Perfection V37) with a custom-made cover to ensure consistent lighting and analysed using Fiji's Image J open-source software (Schindelin et al., 2012) by the same person (DR). For each MPD item, the size of the longest dimension (length) was measured with 0.1 mm resolution, and the colour was recorded as white, high visibility (orange, yellow, red) or low visibility (green, brown, blue, black) (Titmus & Hyrenbach, 2011).

The MPD items were dried under a fume hood for 1–2 days in a temperature-controlled laboratory and weighed in a foil package using a Mettler Toledo NewClassic MS analytical balance, equipped with a

TABLE 1 Summary of plastic ingestion by predatory fish species sampled with pole-and-line gear in the Main Hawaiian Islands

	Albacore tuna (AT)	Dolphinfish (DF)	Skipjack tuna (ST)	Yellowfin tuna (YT)
Sample size (no. fish sampled)	7	8	10	10
Fork length, mean ± SD (max–min)	98.2 ± 3.1 (105.0–95.0)	101.6 ± 19.5 (130.0–75.0)	45.9 ± 1.9 (50.0–43.7)	61.0 ± 1.3 (62.5–60.0)
Empty stomachs (%) Mean ± SD	0.0 ± 0.0	25.0 ± 16.7	0.0 ± 0.0	60.0 ± 16.4
Plastic incidence (%) Mean ± SD (no. fish with plastic)	85.7 ± 14.3 (6)	12.5 ± 12.5 (1)	40.0 ± 16.3 (4)	0.0 ± 0.0 (0)
No. plastic items per fish sampled, mean ± SD (max–min)	2.0 ± 1.3 (4–0)	0.1 ± 0.3 (1–0)	0.7 ± 0.9 (2–0)	NA
No. plastic items per fish with plastic, mean ± SD (max–min)	2.3 ± 1.0 (4–1)	1.0 ± 0 (NA)	1.8 ± 0.5 (2–1)	NA
Plastic weight (g) per fish sampled, mean ± SD (max–min)	0.0322 ± 0.0333 (0.0870–0)	0.0002 ± 0.0005 (0.0016–0)	0.0023 ± 0.0062 (0.0198–0)	NA
Plastic weight (g) per fish with plastic, mean ± SD (max–min)	0.0376 ± 0.0330 (0.0870–0.0004)	0.0016 (NA)	0.0057 ± 0.0095 (0.0198–0.0001)	NA
No. plastic items (fragments, line, sheet)	14 (7, 7, 0)	1 (1, 0, 0)	7 (5, 0, 2)	0 (0, 0, 0)
Fragment length (mm), mean ± SD (median, max–min)	9.79 ± 4.21 (15.71–5.53)	2.90 (NA)	4.09 ± 1.60 (5.78–1.55)	NA
Line length (mm), mean ± SD (median, max–min)	18.05 ± 6.69 (17.69, 24.79–8.07)	NA	NA	NA
Sheet length (mm), mean ± SD (median, max–min)	NA	NA	2.95 ± 1.24 (2.95, 3.82–2.07)	NA

Note: While the number and mass of ingested items involves all three plastic types combined, the sizes of fragments, line and sheet are reported separately.

draft shield (120 g capacity and 0.0001 g resolution). Following the 'good weighing practice' recommendations of the manufacturer, each package was weighed four times in close succession: twice empty (tare measurements) and twice containing 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, the sample was reweighed. Additionally, a test weight was used after each sample, and the scale was recalibrated as necessary (Mettler Toledo, 2012). 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, were used to quantify the precision of the mass measurements (Rapp et al., 2017).

The descriptive statistics of the mass and the number of ingested MPD items were calculated twice: (i) considering all of the sampled fish (10 AT, 8 DF, 10 ST, 7 YT) to provide a population-level metric of exposure; and (ii) considering only those fish that contained plastic (6 AT, 1 DF, 4 ST, 0 YT), to provide an individual-level metric of exposure (Table 1).

2.4 | Comparing specimens caught with pole-and-line and longline gear

For three species that were sampled with both pole-and-line and longlines, we compared the size (fork length) and MPD incidence (FO) from our specimens with those examined by Choy and Drazen (2013). One-sample t-tests were used to compare the mean (± SD) of the fork lengths (Table 2) and Fisher exact tests were used to compare the proportion of examined fish that had ingested MPD (Table 3). All statistical tests were performed using R (R Core Team, 2018).

2.5 | FT-IR identification of ingested plastics

A Perkin Elmer Spectrum Two FT-IR Spectrometer with a diamond universal attenuated total reflectance accessory was used to characterize the polymer composition of ingested MPD, by comparing major absorption bands in the spectra with raw polymer standards (Jung, Balazs, et al., 2018; Jung, Horgen, et al., 2018). All measurements were made by the same person (KP), by placing the individual MPD samples against the universal attenuated total reflectance crystal, using a standardized amount of pressure (80–100 units), as determined by the instrument's force gauge. For each MPD item, the spectra spanning the wavenumber (spatial frequency measured in cycles per unit distance) range of 4,000–450 cm^{-1}, were recorded with a spatial resolution of 4 cm^{-1}, by averaging four scans. To ensure data quality, each sample scan was followed by a background scan devoid of a sample.

Twenty-two MPD items were measured repeatedly to determine whether sample cleaning affected FT-IR performance by scanning every item twice: (i) without any cleaning; and (ii) after cleaning using physical abrasion with a disposable wipe saturated in 2-propanol. Additionally, for improved signal-to-noise, the more durable fragments were cleaned mechanically to expose the unweathered surface of the material, by sanding them with a 4,000-grit sandpaper or by cutting them with a razor blade (Supporting Information, Figure S1).

The polymer types were identified by comparing the absorption bands of the MPD items with spectra from standard plastic samples recorded on the same instrument (Jung, Horgen, et al., 2018). Those items with absorption bands characteristic of both polyethylene (PE) and polypropylene (PP) were classified as a 'PE–PP' mixture. Moreover, the PE items were considered high density (HDPE, 0.93–0.97 g ml^{-1}) or low density (LDPE, 0.91–0.94 g ml^{-1}), using the

TABLE 2 Comparison of the size (fork length, cm) of specimens of three species taken with pole-and-line gear and longlines

	Pole-and-line (this study)			Longline (Choy & Drazen, 2013)			One-sample *t* test		
Fish species	Mean (SD)	95% CI	*n*	Mean (SD)	95% CI	*n*	*t*	d.f.	*p*
DF	101.6 (19.5)	88.1–115.1	8	90.9 (12.7)	87.0–94.7	42	+1.547	7	0.166
ST	45.9 (1.9)	47.1–44.7	10	71.5 (8.3)	68.5–74.5	29	−43.379	9	**<0.001**
YT	61.0 (1.3)	60.2–61.8	10	99.6 (26.9)	89.2–109.9	26	−94.550	9	**<0.001**

Note: The size distributions of the pole-and-line samples were compared to the means from the longline samples. Significant results are highlighted in bold.

TABLE 3 Comparison of the incidence of marine plastic debris (MPD) ingestion (mean ± SD) in specimens of three species taken with pole-and-line gear and longlines

	Pole-and-line (this study)			Longline (Choy & Drazen, 2013)			Fisher's exact test		
Fish species	Incidence (%)	SD	*n*	Incidence (%)	SD	*n*	Chi-squared	d.f.	*p*
DF	12.5	12.5	8	2.4	2.4	42	0.5	1	0.297
ST	40.0	16.3	10	0	–	29	12.9	1	**0.002**
YT	0	–	10	0	–	26	0.0	1	1.000

Note: The number of fish with and without MPD were compared with Fisher's exact tests. Significant results are highlighted in bold.

FT-IR spectra and density measurements (Jung, Horgen, et al., 2018). Each PE item was placed in various aqueous solutions of ethanol (200 proof, A.C.S reagent grade, Acros Organics, Fair Lawn, NJ, USA) using Type 1 water. After volumetrically preparing the solutions, their density was measured by weighing 25 ml of solution in a 25 ml graduated cylinder to the closest 0.0001 g. The polymer densities were estimated based on the floating or sinking behaviour of the MPD items, in a series of solutions ranging from 22 to 42% ethanol in ~2% increments.

2.6 | Species-specific differences in MPD types and polymer composition

Species-specific comparisons focused on the two species which had ingested multiple MPD items: albacore tuna (AT) and skipjack tuna (ST). For this analysis, we considered four plastic types (line, fragment, sheet, foam) and five polymer types (HDPE, LDPE, PP, PP–PE and elastomers). Because the mass of the individual MPD items varied widely, from 0.1045 g to 0.0001 g, we did not quantify the relative importance of different plastic types and polymers using the number of ingested items. Rather, we compared the relative mass of each plastic type and plastic polymer retrieved from AT and ST. Moreover, owing to the non-normal data distributions, Spearman correlations were used to compare the ranks of these proportions (Zar, 1984).

3 | RESULTS

3.1 | Marine plastic debris incidence

Overall, 11 of the 35 (31.4%) predatory fish we sampled, belonging to three of the four focal species, contained MPD. Albacore tuna (AT) had the highest ingestion incidence (FO = 85.7% ± 14.3 SD), with six of the seven examined individuals containing plastic. Skipjack tuna (ST) had an intermediate ingestion incidence (FO = 40.0% ± 16.3 SD), involving four of the 10 individuals sampled, and dolphinfish (DF) had the lowest ingestion incidence (FO = 12.5% ± 12.5 SD) with a single fish containing plastic. Finally, the yellowfin tuna (YT) did not contain any MPD. However, because 60.0% of the YT were devoid of any stomach contents, this frequency of occurrence needs to be considered cautiously (Table 1).

3.2 | Comparing specimens caught with pole-and-line and longline gear

When the size (fork length) and MPD incidence (percentage of fish examined with plastic ingestion) for three species that were sampled with both pole-and-line and longlines were compared, species-specific differences were found. While the tuna (ST and YF) caught with pole-and-line gear were significantly smaller than the specimens caught with longlines, the size of the dolphinfish (DF) caught with both fishing gears was highly variable and indistinguishable (Figure 1a, Table 2). Two of the three species (DF and YT) had similar MPD incidence when specimens caught with pole-and-line and longline gear were compared: DF had low MPD incidence (12.5% vs. 2.4%), and no plastic was found in the stomach of YT. On the other hand, ST caught with pole-and-line gear had a significantly higher MPD incidence (40.0% vs. 0.0%) than the longline-caught specimens (Figure 1b, Table 3).

3.3 | Characterization of ingested plastics

We documented three types of ingested MPD items (fragment, line, sheet), and did not find any foam in the stomachs of the predatory fish. Fragments were the most common type and accounted for 71.4% (ST) and 50.0% (AT) of the ingested items, and 89.0% (ST) and 93.9% (AT) of the ingested mass. Moreover, we retrieved a single fragment from a DF. Only AT ingested line, which accounted for 50.0% of the items and 6.1% of the mass, respectively. Sheets were the least common plastic type, occurring only in ST, and accounting

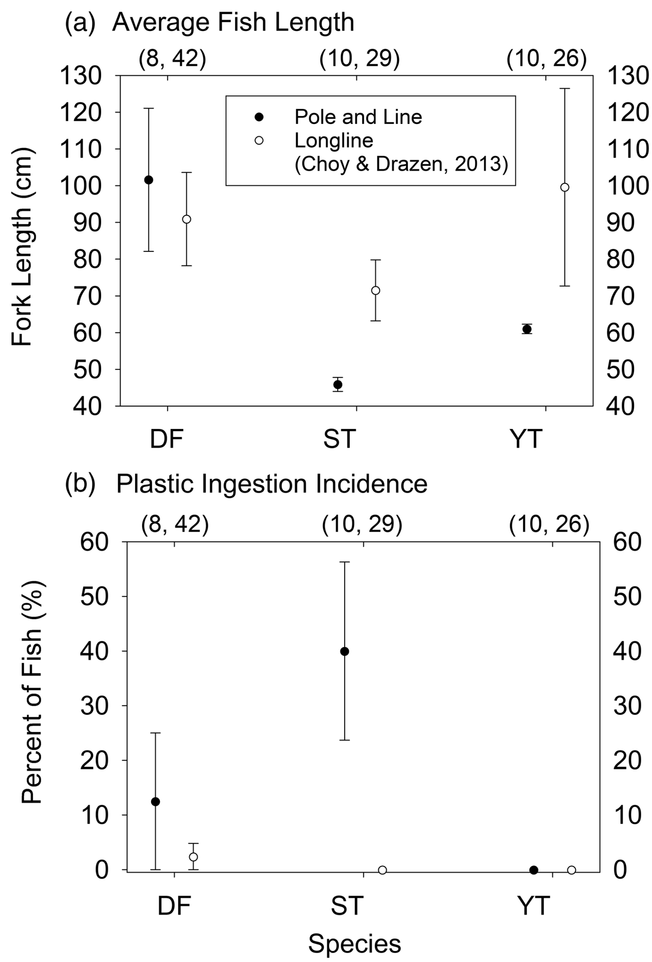

FIGURE 1 Comparison of the predatory fish sampled with pole-and-line (this study) and longlines (Choy & Drazen, 2013). (a) Average (± SD) fork length. (b) Average (± SD) frequency of plastic occurrence. The numbers in brackets indicate the sample sizes

for 28.6% of the items and 11.0% of the MPD mass ingested by this species (Table 1).

The ingested items were micro–meso MPD, ranging in length between 1.5 and 24.8 mm (mean = 10.18 mm ± 7.45 SD, median = 7.28, n = 22). While the mass of the ingested items also ranged widely (mean = 0.0148 g ± 0.0288 SD, median = 0.0023, range = 0.0001–0.1045, n = 22), the replicate weight measurements were highly precise (RMSE = 0.0002; Pearson correlation, r = +0.999, n = 22).

Finally, all of the ingested MPD was either white, or low-visibility colours (blue, black), with no high-visibility colours (orange, yellow, red) or transparent items. Although the DF contained a single blue fragment, white was the dominant colour of the MPD ingested by the AT and ST, accounting for 64.3 and 42.8% of the items, respectively. The other ingested MPD items were either blue (28.6% for both AT and ST) or black (7.1% for AT and 28.6% for ST).

3.4 | FT-IR identification of ingested plastic polymers

Cleaning of the plastic fragments was critical for obtaining a high quality and precise spectra of the underlying polymers. The spectra from uncleaned plastic items changed after wiping the surface with a lint-free disposable wipe, followed by light sanding with 4,000-grit sandpaper to expose uncontaminated polymer. These steps yielded progressively cleaner spectra, by reducing the peaks associated with the stomach digestate, and allowing us to measure the known polymer peaks with high signal-to-noise (Supporting Information, Figure S1).

Altogether, FT-IR classified 91% (20 of 22) of the MPD items retrieved from predatory fish stomachs and identified three polymer types, which together accounted for 86.4% of all the ingested items: HDPE (36.4%), LDPE (31.8%) and PP (18.2%). Additionally, one item with a mixed polymer composition (PP–PE) was identified. Finally, two elastic and compressible items of an unknown polymer with a high percentage of phthalate additive were classified as 'elastomers', owing to their FT-IR spectra characterized by dominant peaks consistent with ester plasticizers. Furthermore, HDPE and LDPE items were differentiated using the absorption bands between 1,400 and 1,330 cm^{-1} and confirmed by measuring their density with a float/sink test in aqueous ethanolic solutions (Supporting Information, Table S1).

3.5 | Species-specific differences in MPD type and polymer composition

The types of MPD ingested by AT and ST were not significantly different (Spearman rank correlation, r_s = +0.50, n = 3, p = 1.00). While both species mostly ingested fragments (89.0% for ST and 93.9% for AT, by mass), ST also ingested sheets (11.0%) and AT also ingested line (6.1%) (Figure 2a). On the other hand, the MPD polymers ingested

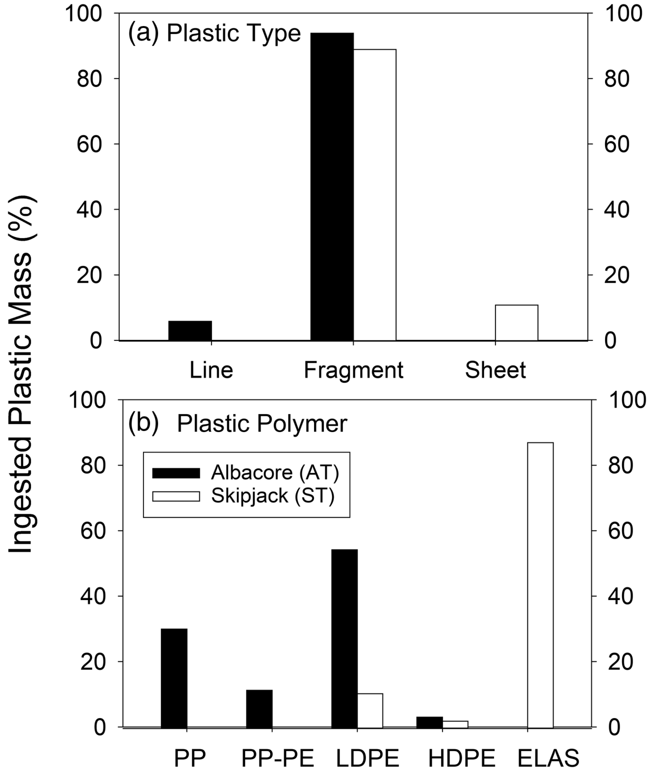

FIGURE 2 Characterization of plastic items ingested by albacore tuna (AT) and skipjack tuna (ST). (a) Three plastic types are considered: fragments, line, and sheet. (b) Four polymer types are considered: polyethylene (PE), polypropylene (PP), high-density polyethylene (HDPE) and low-density polyethylene (LDPE). PP–PE indicates mixed polymer items, and ELAS indicates elastomers with a high percentage of ester plasticizers

by these two predatory fishes did vary significantly (Spearman rank correlation, r_s = –0.89, n = 5, p = 0.04). Both species ingested HDPE and LDPE. However, albacore also contained PP and PP–PE, and ST contained elastomers with high phthalate additives (Figure 2b).

4 | DISCUSSION

We investigated the plastic ingestion of four predatory fishes caught by commercial fishers with pole-and-line gear in the MHI. In spite of our small sample sizes, we documented MPD ingestion by three species: albacore tuna (AT), dolphinfish (DF) and skipjack tuna (ST).

AT had the highest incidence of plastic ingestion of the three species we examined, with 85.7 ± 14.3% SD of the specimens containing MPD (Table 1). While no AT from the Hawai'i longline fishery (Choy & Drazen, 2013) or from larval nursery grounds (Gove et al., 2019) were analysed, a previous study documented plastic in the gut of this species in the Central Mediterranean Sea, where 12.9% of the fish analysed (four of 31) had ingested fragments (1–5 mm in length), and contained, on average, one plastic item (Romeo et al., 2015). Additionally, Rochman et al. (2015) analysed the gastrointestinal tract of two more specimens from the NE Pacific Ocean and failed to detect any ingested plastic. Thus, this is the first record of AT MPD ingestion in the Pacific Ocean, which represents the highest documented incidence and loads for this species (Tables 4 and 5).

North Pacific AT inhabit the transition domain and the subtropical water mass, with most catches concentrated on the clear (chlorophyll-*a* concentration < 0.2 mg m^{-3}) and warm (SST > 18°C) side of the subtropical frontal zone (Polovina, Howell, Kobayashi, & Seki, 2001). This species migrates across the North Pacific, from Japan to North America, with catches from the troll fishery progressing westwards through the late spring and early summer (Kimura, Nakai, & Sugimoto, 1997; Laurs & Lynn, 1991). Thus, is it likely that migrating AT enter the MHI fishing grounds during winter, as oceanographic fronts reach their southernmost seasonal position. In fact, all of the AT we sampled were caught in January, when the TCFZ is located farthest south along the central North Pacific (150–180°W; Bograd et al., 2004; Polovina et al., 2001).

Small-sized ST taken by pole-and-line gear had the second highest plastic incidence (FO = 40.0% ± 16.33 SD, *n* = 10) of the three species we examined, and the highest published to date for this species (Tables 4 and 5). This high frequency of occurrence contrasts with the lack of ingested MPD in the 29 larger-sized longline-caught fish (Tables 2 and 3). For DF, the incidence of plastic ingestion for pole-caught fish was higher (FO = 12.50 ± 13.36% SD, *n* = 8), but not

TABLE 4 Published studies of MPD ingestion by the four focal fish species in the Pacific Ocean, showing the sample locations, the region, the sample years, and the source of the samples

Species (record no.)	Sample location	Region (hemisphere)	Sample years	Sample source	Reference
AT (1)	O'ahu, Main Hawaiian Islands	Central (N)	2012–2013	Small-scale fisheries	This study
AT (2)	Half Moon Bay, California	East (N)	2014	Market purchase	Rochman et al. (2015)
DF (1)	Kona, Main Hawaiian Islands	Central (N)	2006–2018	Larvae sampled with neuston nets	Gove et al. (2019)
DF (2)	Central North Pacific Ocean	Central- east (N)	2007–2012	Large-scale fishery observers	Choy & Drazen (2013)
DF (3)	O'ahu, Main Hawaiian Islands	Central (N)	2012–2013	Small-scale fisheries	This study
DF (4)	Gulf of Tehuantepec, Mexico	Eastern (N)	2017–2018	Small-scale fisheries	Del Carmen Alejo-Plata et al., (2019)
DF (5)	Tahiti, French Polynesia	Central (S)	2015–2016	Local markets *or* small-scale fisheries	Markic et al. (2018)
DF (6)	Various South Pacific islands	Central-west (S)	2015–2016	Small-scale fisheries	Forrest & Hindell (2018)
ST (1)	Central North Pacific Ocean	Central-east (N)	2007–12	Large-scale fishery observers	Choy & Drazen (2013)
ST (2)	O'ahu, Main Hawaiian Islands	Central (N)	2012–13	Small-scale fisheries	This study
ST (3)	Makassar, Sulawesi, Indonesia	Western (N)	2014	Market purchase	Rochman et al. (2015)
ST (4)	Coastal Australia	Western (S)	2010–2015	Captured for study *or* market purchase	Cannon et al. (2016)
ST (5)	Samoa	Central (S)	2015–2016	Local markets *or* small-scale fisheries	Markic et al. (2018)
YT (1)	Central North Pacific Ocean	Central-east (N)	2007–2012	Large-scale fishery observers	Choy & Drazen (2013)
YT (2)	O'ahu, Main Hawaiian Islands	Central (N)	2012–2013	Small-scale fisheries	This study
YT (3)	Rapa Nui	East (South)	2015–2016	Small-scale fisheries	Chagnon et al. (2018)
YT (4)	Samoa	Central (South)	2015–2016	Local markets *or* small-scale fisheries	Markic et al. (2018)
YT (5)	Tahiti, French Polynesia	Central (South)	2015–2016	Local markets *or* small-scale fisheries	Markic et al. (2018)
YT (6)	Rapa Nui	East (South)	2015–2016	Local markets *or* small-scale fisheries	Markic et al. (2018)

Note: Table modified from Gouin (2020) and Markic et al. (2020).

TABLE 5 Published studies of MPD ingestion by the four focal fish species in the Pacific Ocean, showing the methods used to characterize MPD ingestion and the incidence, loads and types of ingested plastic

Species (record no.)	Sample (G, GI)	Method (1–3)	*N* (no. fish sampled)	*n* (no. fish with MPD)	FO (%)	PL (no. /*n*)	Plastic type	Plastic size (mm)	Reference
AT (1)	G	2	7	6	85.7	2.3	FR, LI	>5	This study
AT (2)	GI	3	2	0	0.0	0.0	—	—	Rochman et al. (2015)
DF (1)	G	2	27	0	0.0	0.0	—	—	Gove et al. (2019)
DF (2)	G	1	42	1	2.4	1.0	FR	>5	Choy & Drazen (2013)
DF (3)	G	2	8	1	12.5	1.0	FR	1–5	This study
DF (4)	G	2	32	7	21.9	62.0	FI	<1	Del Carmen Alejo-Plata et al. (2019)
DF (5)	GI	3	10	2	20.0	2.0	FI		Markic et al. (2018)
DF (6)	GI	1	1	0	0.0	0.0	—	—	Forrest & Hindell (2018)
ST (1)	G	1	29	0	0.0	0.0	—	—	Choy & Drazen (2013)
ST (2)	G	2	10	4	40.0	1.8	FR, SH	1–5	This study
ST (3)	GI	3	9	0	0.0	0.0	—	—	Rochman et al. (2015)
ST (4)	G, GI	2	1	0	0.0	0.0	—	—	Cannon et al. (2016)
ST (5)	GI	3	26	6	23.1	1.5	FR	<1	Markic et al. (2018)
YT (1)	G	1	26	0	0.0	—	—	—	Choy & Drazen (2013)
YT (2)	G	2	10	0	0.0	0.0	—	—	This study
YT (3)	G, GI	2	50	1	2.0	5.0	FR	NA	Chagnon et al. (2018)
YT (4)	GI	3	25	6	24.0	2.2	FR	<1	Markic et al. (2018)
YT (5)	GI	3	33	5	15.2	2.2	FR	<1	Markic et al. (2018)
YT (6)	GI	3	10	7	70.0	2.2	FR	<1	Markic et al. (2018)

Note: Sample involves the gastric (G) or gastrointestinal (GI) tract. Methods involve: (1) visual examination of the gut content by naked eye; (2) visual examination of the gut content with optical microscope; and (3) digestion of gut content with subsequent filtration and microscopic analysis. FO, Frequency of occurrence; PL, plastic load (number of items per individual, including only those individuals with MPD). Plastic types involve: fibers (FI), fragments (FR), line (LI) and sheet (SH). Table modified from Gouin (2020) and Markic et al. (2020).

significantly different from the incidence in longline-caught fish (Table 3). Interestingly, the fork lengths of the specimens caught with pole-and-line and longlines were not significantly different (Table 2), suggesting the two fishing gears sampled the same component of the population.

Finally, we did not record any plastic ingestion in YT, reinforcing previous results from the Hawai'i longline fishery (Choy & Drazen, 2013). Overall, none of the 10 pole-caught fish we analysed, and none of the 29 longline-caught fish had ingested plastic (Table 3). Yet all of these fish were larger than the threshold (between 45 and 50 cm FL) of the ontogenetic dietary shift documented in the MHI, whereby smaller individuals feed on planktonic mixed-layer organisms (e.g. larval stomatopod and decapod crustaceans) and larger individuals feed on fish and vertically migrating mesopelagic shrimp (e.g. *Oplophorus gracilirostris*) (Graham et al., 2007). Thus, additional smaller-sized (FL < 45 cm) specimens would be needed to fully document MPD ingestion by YT in the MHI.

4.1 | Ingested plastic types and polymers

When the two species with the highest incidence of MPD ingestion (AT and ST) were compared, fragments were the most commonly ingested plastic type (>89% by mass; Figure 2a). Moreover, by comparing the spectra of MPD with raw polymer standard resin codes, we identified 91% of the ingested plastic items with a high degree of certainty (Supporting Information, Table S1). This analysis revealed that AT and ST ingested different polymers, with the former ingesting more PP and PE and the latter ingesting more elastomers (Figure 2b). Yet this result needs to be considered with caution, owing to our small sample sizes involving 21 MPD items (14 for AT and 7 for ST). Nevertheless, our results suggest that these two tuna species are not ingesting heavy polymers, like polystyrene, polyvinyl chloride or nylon. The preponderance of the light polymers mirrors previous ATR FT-IR analyses of MPD ingested by mid-level and upper-trophic pelagic fishes, which is dominated by PE (e.g. Chagnon et al., 2018; Ory, Sobral, Ferreira, & Theil, 2017; Rummel et al., 2016).

Owing to the opportunistic nature of the sampling and the variable incidence of plastic ingestion, multispecies assessments of MPD ingestion are characterized by unequal sample sizes, with the number of fish sampled per species ranging widely. For instance, Romeo et al. (2015) analysed a total of 22 fish from three species (median fish per species = 7, range = 4–11) and Rummel et al. (2016) analysed a total of 16 fish from four species (median fish per species = 3, range = 1–9). Thus, the numbers of fish with ingested MPD that were analysed (1 DF, 6 AT, 4 ST) are comparable with the sample sizes of previously published studies of MPD ingestion and polymer characterization in pelagic fishes. Nevertheless, additional analyses of larger numbers of fish and ingested MPD items are needed to fully characterize the incidence, loads and types of plastic ingested by predatory fishes in the MHI.

4.2 | Developing bioindicators of marine plastic pollution for the MHI

Although floating MPD is regularly sampled using neuston nets and visual surveys, much less is known about plastic distributions in the mixed layer and at the thermocline (e.g. Doyle, Watson, Bowlin, & Sheavly, 2011; Titmus & Hyrenbach, 2011). Yet there is empirical evidence that strong wind mixes buoyant millimetre-sized particles throughout the upper mixed layer (Kukulka et al., 2012) and that non-migrating mid-water fishes ingest MPD (Davison & Asch, 2011). Predatory fishes caught by commercial and recreational fishers can help fill in these knowledge gaps, via the analysis of ingested MPD and prey remains (e.g. Chagnon et al., 2018; Ory et al., 2017).

The vertical distribution of pelagic fishes probably influences their trophic ecology, their pollutant loads and their utility as biological samplers of MPD (Brill & Lutcavage, 2001; Choy & Drazen, 2013; Choy, Popp, Kanekoc, & Drazen, 2009). For instance, the longnosed lancetfish, an upper mesopelagic resident taken incidentally by the Hawai'i longline fishery, is being used to sample MPD in the water column (from the surface to 1,000 m; Choy et al., 2009; Jantz et al., 2013). Additional species capable of sampling specific layers of the water column (e.g. surface, mixed-layer and thermocline) are needed to fully monitor plastic pollution trends in oceanic ecosystems (e.g. Ueno et al., 2003; van Franeker et al., 2011).

Our snapshot assessment documented MPD ingestion in three predatory fish species caught in the MHI. Moreover, our results underscore the importance of considering the source (e.g. fishing gear) and the size (e.g. fork length) of the specimens used to estimate MPD ingestion by pelagic fishes. In particular, commercial and recreational fishers in the MHI sample some species (AT) and size-classes (smaller-sized ST) different from those targeted by the Hawai'i longline fishery operating in the Central Pacific.

Previously, the effectiveness of using fish species as MPD biomonitors was assessed using five criteria: the distribution on a global scale, the length of the gastrointestinal tract, the commercial value, the home range and vagility, and the frequency of occurrence of plastic ingestion reported in the literature (Bray et al., 2019; Gouin, 2020). Based on these criteria, tuna and dolphinfish species would be expected to rank highly as MPD bioindicators, owing to their large distributions and high commercial value. Conversely, their high vagility and seasonal migrations would inhibit their use as bioindicators of regional pollution, but would facilitate the sampling of large biogeographic zones (e.g. albacore trans-Pacific migration; Polovina et al., 2001). Our finding of higher MPD frequency of occurrence in the smaller-sized specimens of these species will increase their rank as potential bioindicators. Based on two recently published reviews (Gouin, 2020; Markic et al., 2020) and our findings, we propose the use of three of the four species we sampled for monitoring MPD in Hawai'i and throughout the tropical/subtropical Pacific Ocean.

We contend that ST is an ideal MPD bioindicator in the mixed layer of the tropical North and South Pacific, where they are caught by recreational and commercial fishers, owing to their near-surface foraging and unique stomach anatomy, which probably retains plastic in their large duodenum (Magnuson, 1969). In the central Pacific, the larger longline-caught fish forage over a wider depth range (0–300 m) and their diet consists of epipelagic (~50% by mass) and vertically migrating mesopelagic prey (~45% by mass) (Choy et al., 2009). The smaller-sized fish caught by pole-and-line gear often forage with seabirds at the surface (Hebshi et al., 2008), and have a significantly higher incidence of MPD ingestion than the larger-sized fish taken by longlines. Additionally, these fish are taken by purse seines in the equatorial Pacific, by longlines on either side of the equator and by island-associated commercial and recreational fishers (Allain, 2003; Choy & Drazen, 2013; Hebshi et al., 2008).

DF could also serve as a bioindicator of floating and near-surface MPD in tropical waters, as evidenced by their diet, which is dominated by epipelagic prey living within the upper 50 m of the water column (Allain, 2003; Choy et al., 2009). Moreover, their broad diet, their ability to ingest large prey (and MPD items) and their tendency to accumulate the hard parts of digested cephalopods (beaks and gladius; Allain, 2003) and flying fish (fin rays; Manooch & Mason, 1984) make this species an ideal bioindicator of epipelagic mesoplastics. Accordingly, DF was proposed as an 'indicator of environmental conditions of offshore waters' along the south-eastern US and the Gulf of Mexico, owing to its tendency to ingest tar balls and a wide range of plastics (Manooch & Mason, 1984). Similarly, this species could be used in the Pacific Ocean, where it is taken with a variety of fishing gear, both by large-scale high-seas fisheries and by smaller-scale recreational and commercial fishers operating around oceanic islands (Allain, 2003; Choy & Drazen, 2013; Hebshi et al., 2008). While there is evidence of low levels of MPD ingestion in longline-caught fish from the central North Pacific (Choy & Drazen, 2013) and in pole-caught fish from the MHI (this study), there is circumstantial evidence of MPD ingestion by this species in the South Pacific, via dietary studies of longline and purse-seine caught fish (e.g. Allain, 2003) and from accounts from fishers in the Tuamutu archipelago, French Polynesia (e.g. Forrest & Hindell, 2018).

Owing to their biogeography and seasonal migrations, AT are a transition domain/subtropical species with an affinity for cooler waters north of Hawai'i (Kimura et al., 1997; Laurs & Lynn, 1991). Thus, the high plastic incidence and loads in these fish are probably indicative of plastic concentrations at the frontal zones associated with the subtropical convergence, rather than of MPD levels in the tropical–subtropical waters of the MHI (Howell et al., 2012; Pichel et al., 2007). Furthermore, this species is only marginally captured by island-associated fisheries, as evidenced by the low commercial catch records (State of Hawai'i Department of Aquatic Resources, http://dlnr.Hawaii.gov/dar/fishing/commercial-fishing/; 18 May 2020). In fact, the relative abundance (annual catch per unit of effort) of this species (by number) in the core area of the deep-set Hawai'i longline fishery (between 12 and 27° N latitude), has decreased by 9.1% annually over the last decade, from 15% (in 1996) to 2% (in 2006) (Polovina, Abecassis, Howell, & Woolworth, 2009). Accordingly, no AT from the Hawai'i longline fishery were analysed for plastic ingestion between 2007 and 2012 by Choy and Drazen (2013). Thus, owing to its seasonal occurrence and decreasing catches, this species is likely not a useful MPD bioindicator for the MHI. Instead, it could be used to monitor the North Pacific subtropical convergence zone, an area of known MPD aggregation (Pichel et al., 2007; Polovina et al., 2001). While this species is also taken by longlines in the southern hemisphere (e.g. Allain, 2003), currently, there are no published southern hemisphere studies of MPD ingestion by AT (Gouin, 2020; Markic et al., 2020).

Finally, despite the lack of ingested plastic in YT caught with longlines (Choy & Drazen, 2013) and pole-and-line (this study), additional research of the smaller-sized fish is warranted owing to their foraging habits, similar to those of ST. The larger longline-caught fish forage within the top 100 m of the ocean during the day, as evidenced by a diet dominated (>95% by mass) by epipelagic species, with instances of lower mesopelagic migrators (Brill et al., 1999; Choy et al., 2009). However, there is evidence of size-specific feeding segregation owing to an ontogenetic shift in foraging depth, whereby only the larger fish (>55 cm FL) have sufficient endothermic capabilities for accessing prey in deeper, colder water (Graham et al., 2007). The large-sized YT feed deeper in the water column, away from the surface and the mixed-layer, and the smaller-sized YT feed on planktonic organisms in the mixed layer. However, our study failed to detect MPD ingestion in this species, probably owing to our small sample size and the high proportion of empty stomachs in our sample. Thus, we contend that more research is needed before plastic ingestion by North Pacific YT can be ruled out. Interestingly, small-sized (FL = 70 cm ± 13 SD, n = 50) YT from Rapa Nui (Easter Island), in the South Pacific subtropical gyre, have 8.0% incidence of MPD ingestion (Chagnon et al., 2018).

4.3 | Towards an ecosystem-wide assessment of MPD in Hawai'i and the Pacific Ocean

Despite the high per capita seafood consumption and the economic and subsistence importance of fisheries in the state (Loke et al., 2012; NMFS, 2017), no comprehensive assessment of MPD ingestion by fish has been completed in the MHI. To date, the two assessments for the Hawaiian Islands are based on records from large-sized specimens taken by large-scale longline fisheries operating across the central North Pacific (Choy & Drazen, 2013), or from the larvae captured with neuston nets in near-shore nursery areas (Gove et al., 2019).

Our results support the potential use of commercially valuable predatory fishes as bioindicators of MPD in the central North Pacific, and provide a baseline for monitoring the incidence and loads of MPD ingested by DF and ST in the MHI.

Globally, there is mounting evidence of widespread plastic ingestion in pelagic fishes, with data from multispecies assessments being integrated into global reviews (Gouin, 2020; Markic et al., 2020). Yet comparisons of the levels of plastic contamination of commercial fish species across the Pacific Ocean are inhibited by a general lack of data, and by methodological differences (Table 3). For instance, a review of the literature reveals that, to date, there have been eight published papers investigating MPD ingestion in the four focal species we examined in the MHI: AT (one record with two fish), DF (five records with 112 fish), ST (four records with 65 fish) and YT (three records with 144 fish) (Table 5). Thus, additional studies of these focal species are needed, using standardized methods and large sample sizes (Gouin, 2020; Markic et al., 2020).

We advocate developing a portfolio of bioindicator species with different vertical distributions and trophic ecologies, to sample different layers of the water column and a variety of MPD size classes. In particular, a dual approachs should be used, whereby large-scale (high-seas) fisheries are used to sample cosmopolitan and highly-migratory species across vast geographic areas, and localized (small-scale) commercial/recreational/subsistence fisheries are used to sample the same species from specific island archipelagos and continental shelves (Table 4). Purse seine and longline fisheries have been used to conduct broad-based dietary and pollution studies of predatory fishes (Allain, 2003; Choy et al., 2009) and to assess MPD ingestion in entire species assemblages and bioindicator taxa (Choy & Drazen, 2013; Jantz et al., 2013). Yet comparative studies will need to consider the sizes and gut fullness of the specimens sampled by these different gears (Allain, 2003).

Moreover, owing to the selectivity of different fishing gears for specific size classes, multiple sampling methods may be required to sample a wide size range of MPD bioindicators. While studies have largely focused on fisheries, owing to the links to human consumption and the logistical ease of collection, other methods may be needed to sample size classes not targeted by commercial/recreational fishers, like larvae and juveniles. This comprehensive perspective will require an integrated sampling approach, including the use of marine predators as bioindicators (Maximenko et al., 2019).

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DISCLAIMER

Certain commercial equipment, instruments, or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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Information, Table 1. Summary of ingested MPD characterization, showing the type and polymer, the wavelength of the resulting FT-IR major and secondary peaks, and the estimated density from float-sink tests of the items ingested by three predatory fish species: albacore tuna (AT), dolphinfish (DF), and skipjack tuna (ST).

Fish Species	MPD Item	Plastic Type	Polymer Type	Major Peaks (wave number, cm $^{-1}$)	Other Peaks (wave number, cm $^{-1}$)	Density (g mL^{-1})
AT	1	Fragment	HDPE	2915, 2848, 1714, 1473, 730, 719	1707	> 0.94
AT	2	Line	HDPE	2914, 2848, 1714, 1472, 730, 718		> 0.94
AT	3	Line	HDPE	2915, 2848, 1473, 1463, 730, 719	-	> 0.94
AT	4	Line	HDPE	2915, 2848, 1472, 1463, 730, 719	-	> 0.94
AT	5	Fragment	LDPE	2915, 2848, 1472, 730, 717	-	0.94
AT	6	Fragment	LDPE	2914, 2848, 1472, 718	1640, 1542, 1084	< 0.92
AT	7	Fragment	LDPE	2916, 2848, 1468, 1239, 1019, 718	1741,1636, 1535	0.92 - 0.93
AT	8	Fragment	LDPE	2916, 2848, 1472, 717	-	0.92 - 0.93
AT	9	Fragment	LDPE	2914, 2848, 1715, 1463, 730, 718	-	0.92 - 0.93

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AT	10	Line	LDPE	2915, 2848, 1472, 717	-	0.93 - 0.94
AT	11	Line	PP	2950, 2917, 2838, 1456, 1376, 973	2870, 1167, 998, 972, 874, 837, 807, 716	-
AT	12	Line	PP	2950, 2917, 2838, 1455, 1376, 998, 841	2870, 1643, 1167, 998, 973, 807	-
AT	13	Line	PP	2950, 2917, 2838, 2646, 1454, 1376, 998	3295, 2870, 1640, 1546, 1253, 1167, 975, 840, 807	-
AT	14	Fragment	PP - PE	2952, 2850, 1456, 1376, 997, 717	2915, 1640, 1167, 972, 840	0.92 - 0.93
DF	15	Fragment	PP	2950, 2838, 1456, 1376, 973	2874, 1167, 998, 897, 840, 807	-
ST	16	Fragment	Elastomer	3294, 2921, 1717, 1012	2855, 1542, 874	> 0.94
ST	17	Fragment	Elastomer	3284, 2922, 1728, 1417, 1257, 1017, 875	2852, 1538, 1174, 1118, 1065,	> 0.94
ST	18	Fragment	HDPE	2914, 2848, 1472, 718		> 0.94
ST	19	Fragment	HDPE	2915, 2848, 1473, 1462, 1050, 730, 719	1715	> 0.94
ST	20	Fragment	HDPE	2915, 2848, 1714, 1463, 730, 718	1474	> 0.94
ST	21	Sheet	HDPE	2916, 2848, 1472, 730, 718	1459	> 0.94
ST	22	Sheet	LDPE	2915, 2848, 1713, 1463, 1077, 730	716	0.92 - 0.93

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Supporting Information, Figure 1. Normalized FT-IR (UATR) spectra of ingested MPD item (#19) from a ST (#10). The plastic fragment was measured without cleaning (green trace), after wiping with lint-free disposable wipe (blue trace), and after sanding lightly (red trace) with 4000 grit sandpaper (Micro Mesh® 4000, Scientific Instrument Services). The non-normalized spectrum of dried digestate from ST stomach is offset above the plastic fragment spectra (black trace).