Use of indicator chemicals to characterize the plastic fragments ingested by Laysan albatross

Frances Nilsen, K. David Hyrenbach, Jiasong Fang, Brenda Jensen *

Hawai'i Pacific University, 45-045 Kamehameha Highway, Kane'ohe, HI 96744, USA

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ABSTRACT

Laysan albatross (*Phoebastria immutabilis*) ingest plastic marine debris of a wide range of shape, sizes and sources. To better characterize this plastic and provide insights regarding its provenance and persistence in the environment, we developed a simple method to classify plastic fragments of unknown origin according to the resin codes used by the Society of Plastics Industry. Known plastics were analyzed by gas chromatography–mass spectroscopy (GC–MS) to identify indicator chemicals characteristic of each plastic resin. Application of this method to fragments of ingested plastic debris from boluses of Laysan albatross from Kure Atoll, Hawai'i, yielded proportions of 0.8% High Density Polyethylene, 6.8% Polystyrene, 8.5% Polyethylene Terephthalate, 20.5% Polyvinyl Chloride and 68.4% Polypropylene. Some fragments were composed of multiple resin types. These results suggest that infrequently recycled plastics are the dominant fragments ingested by albatross, and that these are the most prevalent and persistent resin types in the marine environment.

1. Introduction

Plastic pollution is a persistent problem in marine environments (Barnes et al., 2009; Ryan et al., 2009), with areas of dense aggregation of floating debris associated with areas of surface convergence (Pichel et al., 2007). In the Pacific Ocean, the prevailing wind patterns and surface currents transport and concentrate this material in a variety of oceanographic features. The clockwise flow of the North Pacific Subtropical Gyre (NPSG) results in surface convergence that concentrates and retains floating debris within an area of high atmospheric sea level pressure (Moore, 2003). Superimposed on this large-scale feature are smaller-scale hydrographic fronts between adjacent water masses, like the North Pacific Transition Zone (NPTZ), that concentrate floating debris (Howell et al., 2012).

The transient boundaries of the NPTZ consist of two large convergent fronts, the Subarctic Frontal Zone (SAFZ) and the Subtropical Frontal Zone (STFZ). In particular, the Subtropical Convergence Zone (STCZ) at the southern end of the STFZ delineates the core of a large an area of marine debris aggregation referred to as the "Pacific Garbage Patch" (Moore, 2003; Pichel et al., 2007; Howell et al., 2012).

Plastic ingestion in seabirds, first documented in the 1960s, is such a widespread and pervasive phenomenon that is used to monitor plastic debris around the world, (Kenyon and Kridler, 1969; Azzarello and Van Vleet, 1987; Colabuono et al., 2009; Nevins et al., 2005). In particular, several recent reports have noted high incidence (80–100% of all birds sampled) of plastic ingestion in surface-feeding tubenoses (order Procellariiformes) (Auman et al., 1997; Kinan and Cousins, 2000; van Franeker et al., 2004; Young et al., 2009).

The Laysan albatross (*Phoebastria immutabilis*) is a valuable bio-indicator of plastic pollution in the North Pacific because this upper trophic-level predator has a wide diet composition and forages over a vast range that overlaps known areas of plastic debris aggregation north of Hawai'i (Gould et al., 1997; Hyrenbach et al., 2002; Kappes et al., 2010). In particular, because this species forages along the NPTZ, an important migration pathway and foraging location used by many far-ranging pelagic birds and turtles (Hyrenbach et al., 2002; Polovina et al., 2001), it can provide information about the types of plastic concentrated along this ecologically important area.

During the breeding season (November–July), Laysan albatross collect pieces of floating plastic at sea, which they in turn feed to the developing chicks at the colony (Young et al., 2009; Whittow, 1993). Immediately prior to fledging, the birds regurgitate pellets of indigestible material ("boluses") containing both debris and natural food items (Azzarello and Van Vleet, 1987; Kinan and Cousins, 2000).

The Laysan albatross breeding at Kure Atoll, in the Northwestern Hawaiian Islands (NWHI), is of particular interest because

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* Corresponding author. Tel.: +1 (808) 236 3533.
E-mail addresses: frannie.nilsen@gmail.com (F. Nilsen), khyrenbach@hpu.edu (K. David Hyrenbach), jfang@hpu.edu (J. Fang), bjensen@hpu.edu (B. Jensen).

the unique foraging pattern of the birds from this colony leads to high levels of plastic ingestion by chicks, exceeding the loads of Main Hawaiian Island (MHI) chicks by approximately ten-fold. Moreover, the albatross at Kure Atoll forage in a northwesterly direction from the breeding location, with distances increasing as the breeding period progresses through the incubation, guarding and post-guarding stages (Young et al., 2009). The NWHI Laysan albatross use the colder waters surrounding Japan, the northern portion of the NPTZ, and the SAFZ (Kappes et al., 2010; Young et al., 2009). Their movements overlap with the area of marine debris accumulation known as the Western Garbage Patch (WGP) (Howell et al., 2012).

Ingested plastics are typically characterized based on their physical characteristics (elasticity, compressibility, shape) into five broad categories: foam, nurdles, sheet, fragments, or line (van Franeker et al., 2004). While fragments represent the overwhelming majority (by mass and incidence) of plastic ingested by Laysan albatross (Gray et al., 2011; Nevins et al., 2005), this heterogeneous category includes a wide variety of items that vary widely in size (e.g. 1 mm to 10 cm). These include both identifiable post-consumer items (e.g. bottle caps, cigarette lighters, toothbrushes) and broken fragments that cannot be identified by visual examination (Kinan and Cousins, 2000; Nevins et al., 2005). A more thorough understanding of the composition and provenance of the ingested plastic debris impacting marine organisms could lead to more effective mitigation measures such as enhanced waste management and recycling efforts (Ryan et al., 2009).

The purpose of this study was to develop a simple approach to further characterize ingested plastic that can be applied to small, weathered, and degraded fragments. We developed a method to classify plastic debris by the resin corresponding to the Resin Indicator Codes (RIC) used by the Society of Plastics Industry (SPI). To this end, we extracted and analyzed known coded plastics with gas chromatography–mass spectroscopy (GC–MS) to identify chemicals characteristic of each type of plastic. The methods we employed can be easily replicated using broadly available instrumentation and are applicable to plastic debris sampled from any source.

2. Methods

2.1. Resin type reference material

Commercially available household items labelled with the SPI resin type were used to develop reference materials. Three items of each resin type (1–6) were collected and screened for extractable components that appeared unique to the resin type.

2.2. Extraction and clean up

The method of extraction was modified from the EPA method 8081b (EPA, 1996) and Colabuono et al. (2010). Triplicates of the six known plastic resin types were extracted separately for 24 h in 1:1 dichloromethane: hexane. Three 1 g plastic samples of each resin type were combined and extracted using a Soxhlet apparatus to create a reference stock. (See Figures A.1–A.17 in Supplemental Materials). The extraction conditions for the reference plastics were 8 h in 1:1 dichloromethane: hexane at a mass to volume ratio of 1:40. Triplicates of the six known plastic resin types were passively extracted separately, to confirm that the resin type indicator compounds were identical across all plastic samples. The passive extraction conditions were 24 h in 1:1 dichloromethane: hexane at a mass to volume ratio of 1:40. Plastic samples that were subject to passive extraction were ground with combusted stainless steel micro-plate food graters into particles of less than 2 mm in diameter, to increase the surface area of the plastic in contact with the extraction solvent.

Plastic were not ground prior to Soxhlet extraction, since the heat and agitation support sufficient extraction. For clean up, the sample extracts were passed through a 5% water-deactivated silica columns (pore size 100–200) activated with hexane. Once the sample was added, the column was flushed with hexane and dichloromethane (1:1, then 1:3) to elute the compounds of interest. Eluates were concentrated to a volume of 50 µL or less under a gentle stream of nitrogen. The samples were resuspended with dichloromethane to a volume of approximately 500 µL.

2.3. GC–MS analysis

The oven program was modified from EPA method 8082a (EPA, 2000). All samples were analyzed on an Agilent 7890A gas chromatograph-5975C mass spectrometer with a 30 m × 0.25 mm i.d. HP-5MS capillary column (J&W Scientific). The oven temperature started at 33 °C (held for 5 min), to 230 °C (held for 12.7 min) at 3 °C/min, then ramped at 10 °C/min to 300 °C. Helium was the carrier gas (flow rate = 23.8 ml/min). The injection in full scan mode was split-less with an injection volume of 2 µl. A 7683B Series Injector (Agilent Technologies) was used.

2.4. Resin type indicator compound identification

Compounds were identified qualitatively by retention time and mass spectra. Those compounds that were present in all of the coded samples of each resin type and not present in the other resin types were considered candidates for resin type indicator compounds. Of all possible indicators that were considered, 16 candidates emerged as potential reliable means to distinguish the six SPI resin types. These mass spectra fragmentation patterns of the 16 compounds were analyzed to postulate the identity and structure of the compounds. The compound predicted by the ChemStation Mass Spectral Reference Library module (Agilent via NIST/EPA/ NIH, 2008) provided the basis for the structure of each indicator. A more detailed explanation of the individual indicator compound identification and selection is provided in the Supplemental Information.

While mass spectra were used to postulate structures of indicator chemicals using the National Institute of Standards and Technology (NIST) 2008 Mass Spectral Reference Library (NIST/EPA/ NIH, 2008), it is important to emphasize that the identity of the chemicals in this analysis are postulated only since the mass spectral library currently available does not contain a comprehensive list of plasticizers.

The ability of the resin type indicator compounds to help identify resin codes of unknown plastics was tested using a set of blind 1 g samples of plastic without a visible resin code, randomly collected from typical household products by a volunteer. The blind test samples were extracted using passive extraction method in a 1:1 dichloromethane: hexane solution for at least 24 h. The peaks observed in the chromatograms of the unknown samples were compared to those in the standard materials based on the retention times of the compound. If the retention time matched an indicator chemical, the mass spectra of both the standard material and the unknown plastic fragment were compared. If the two compounds had the same mass fragment ions (Table 1, Fig. 1) they were considered a match.

2.5. Resin type attribution

Sample extracts were categorized by resin code based on analysis of both the gas chromatogram and the mass spectra. First, the chromatogram of each plastic sample was inspected for compounds with retention times matching those of the indicator chemicals found in the standard materials. Once a matching

Table 1
SPI resin code indicator chemicals with mass fragment ions. For each indicator, the resin type, retention time, presumed molecular ion, and discriminating fragment ions are listed. Note that this table does not provide a comprehensive list of the chemicals present and their associated ion fragments, but rather identifies those that were found to be distinct and prevalent.

SPI resin – identifier #	Postulated compound	Retention time (min)	Molecular ion	Other fragment ions (m/z)			
PETE							
SPI 1-1	Unknown Isophthalic Acid Deriative	70.6	396	381	207	149[a]	91
HDPE							
SPI 2-1	Octamethyl-Cyclotetrasiloxane	19.5	284	281[a]	265	249	207
SPI 2-2	Decamethyl Cyclopentasiloxane	27.5	355	267	251	193	73[a]
PVC							
SPI 3-1	Hexadecylic acid	58.1	227	227	97	87	74[a]
SPI 3-2	Monoethylhexyl Phthalate	78.4	390	375	279	167	149[a]
LDPE							
SPI 4-1	Bis(2-ethylhexyl) Adipate	72.1	371	259	247	147	129[a]
PP							
SPI 5-1	Oligomer of Polypropylene	22.3	170.2	154.9	127	113	99
SPI 5-2	Oligomer of Polypropylene + x	33.2	212.1	169	155.2	127.1	113
SPI 5-3	Oligomer of Polypropylene + 2(x)	42.2	254.2	211.1	193.7	155	113
SPI 5-4	Oligomer of Polypropylene + 3(x)	50.5	296.1	253	239.1	211.1	197.2
SPI 5-5	Oligomer of Polypropylene + 4(x)	57.8	253.1[b]	239.2	225	211.1	197.1
SPI 5-6	Oligomer of Polypropylene + 5(x)	64.4	281.1[b]	267.1	253.1	211.2	197.1
SPI 5-7	Oligomer of Polypropylene + 6(x)	70.4	295.1[b]	281.1	267.1	253.1	197
SPI 5-8	Oligomer of Polypropylene + 7(x)	71.5	281	253.3	239.1	211.1	197.1
PS							
SPI 6-1	(2-Phenyl-3-[(phenylsulfinyl) methyl]cyclopropyl)benzene	73.1	312	207	194	117	91[a]
SPI 6-2	3-(2-Cyclopentenyl)-2-methyl-1,1-diphenyl-Propene	77	308	289	279	265	207

[a] Base peak.
[b] A compound with a molecular ion too unstable to be identified, highest mass fragment ion is listed.

retention time was found, the mass spectrum of the compound was compared to the indicator chemical's mass spectrum of the same retention time. When the mass fragment ions from the standard material (Table 1, Fig. 1) matched the fragment ion pattern found in the plastic sample, the sample was considered a match to that SPI resin type.

2.6. Collection and processing of plastic from bird boluses

Boluses cast within a known Laysan albatross colony were collected on Kure Atoll by field personnel of the State of Hawai'i Department of Fish and Wildlife (DOFAW) during the end of the breeding season (April–June) of 2005. The 24 boluses collected were sorted and 200 large (mass > 20 mg) plastic items were selected. Since Laysan albatross ingest mostly fragments, all suitable pieces of foam (*n* = 44), sheet (*n* = 6), line (*n* = 10) and nurdles (*n* = 11) were chosen, totaling 71 non-fragment samples. Additionally, 129 samples were randomly selected from the larger fragments (mass > 50 mg) to make the total number of processed plastic samples equal to 200.

The bolus subsamples were selected to maximize the representation of each debris type in an attempt to develop and apply the resin typing method. All bolus plastic samples were ground with combusted stainless steel micro-plate food graters into particles of less than 2 mm in diameter.

2.7. Extraction and clean up of bird bolus samples

Bolus plastic subsamples were extracted for 24 h in a dichloromethane in hexane (1:1) solution at a mass to volume ratio of 1:40 and processed as above.

2.8. Quality assurance/quality control

The detection limit was identified using standard solutions of phthalates (Sigma Aldrich #48231), phenols (Sigma Aldrich #48235-U), bisphenol A (BPA, Accustandard # M-1626-01S) and polychlorinated biphenyls (PCBs, Sigma Aldrich #36989). The limit of detection of these compounds in scan mode was 10 ng/μl, as determined by serial dilutions of the standards of known concentration. Some of the standard compounds used for the development of the oven temperature program are detectable at 5 ng/μl. The environmental contaminant standards listed above were included at the beginning of every GC–MS sequence. Method blanks were extracted and processed with each set of ten samples. Duplicate samples were extracted and processed with each set of ten samples to assure consistency and reproducibility.

3. Results and discussion

3.1. Method development

To improve our ability to characterize plastics of unknown origin, we developed a method for identifying small fragments of SPI type plastics and used samples from household items with SPI resin codes to develop reference materials. Compounds that were consistently present, yet unique to the resin code were identified using gas chromatography retention time and mass spectra. Overall, a total of 16 compounds (of 20 originally identified) were used to classify the six resin types. These 16 indicator compounds, with distinct retention times and major ion fragments (Table 1), were used to identify the bolus plastics.

While the utility of the method did not necessarily depend on the complete and accurate identification of the chemical structures, the identities of the indicator compounds are postulated based on the highest percentage match to the 2008 version of the NIST Mass Spectral Library (NIST/EPA/NIH, 2008). With due consideration to the proprietary nature of plastic formulations, the likelihood of the postulated compound to be found within or used in the creation of each SPI resin type was considered, but the compound structure and identity cannot be confirmed.

The method was first tested with 10 SPI type plastic fragments with no visible resin codes. These blind trials identified the correct SPI resin type in 10 out of the 10 plastic samples. Further trials of
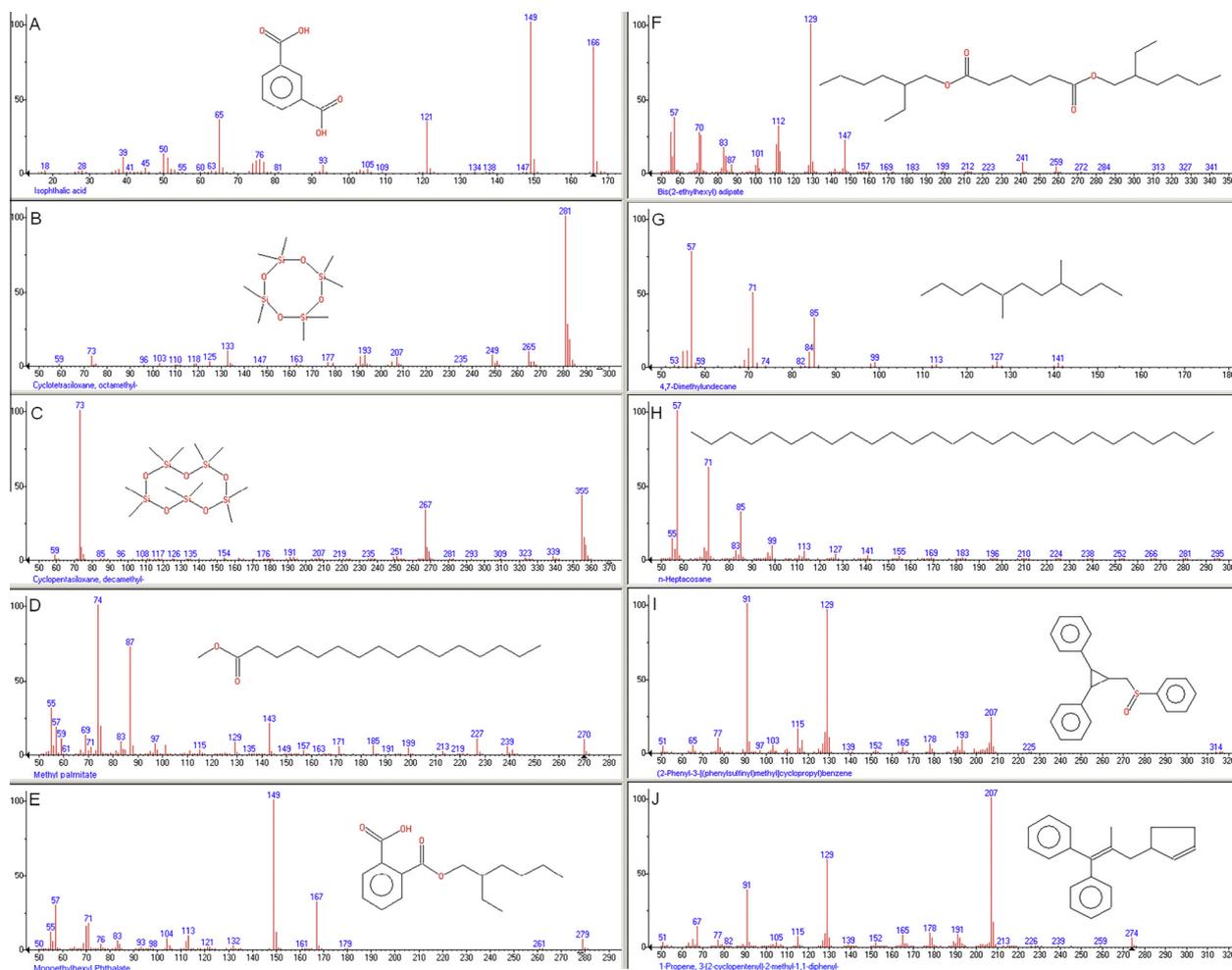
Fig. 1. Indicator chemical mass spectra, postulated compounds and percent confidence of structural identity based on fragment ion comparison to the NIST 2008 Mass Spectral Reference Library. A. PETE (SPI 1-1) – the indicator is postulated be an unknown derivative of isophthalic acid (structure shown). No percent confidence is presented because this compound is not in the NIST 2008 Library); B. HDPE (SPI 2-1) – Octamethyl-Cyclotetrasiloxane 93%; C. HDPE (SPI 2-2)- Decamethyl Cyclopentasiloxane 93%; D. PVC (SPI 3-1) – Hexadecylic acid 83%; E. PVC (SPI 3-2) – Monoethylhexyl Phthalate 21%; F. LDPE (SPI 4-1) – Bis(2-ethylhexyl) Adipate 69%; G. PP (SPI 5-1) – Oligomer of Polypropylene; H. PP (SPI-5-2) – Oligomer of Polypropylene + x; I. PS (SPI 6-1) – (2-Phenyl-3-[(phenylsulfinyl) methyl]cyclopropyl)benzene 48%; J. PS (SPI 6-2) – 3-(2-cyclopentenyl)-2-methyl-1,1-diphenyl-Propene 28%.

the method, using Soxhlet or passive solid phase extraction, showed that the indicator compounds could be identified when different solvent methods were used (Fig. 2), and both approaches have advantages. The Soxhlet extraction is very effective, but it requires far more time, specialized equipment, and chemicals compared to the passive extraction method. In applications such as this one that require analysis of hundreds of small plastic fragments, the passive extraction method is more practical. However, if time and equipment are not limiting, or if the goal is to maximize extraction efficiency, the Soxhlet extraction may be preferable.

3.2. Seabird bolus plastic analysis

We then applied this method to environmentally degraded plastics. First, the 200 plastic samples from Laysan Albatross boluses were grouped by their physical characteristics (sheet, line, nurdles, foam, fragments). Next, each plastic sample was extracted and screened for the indicator chemicals (Table 1). Indicator chemicals were identified in 176 (88%) of the 200 plastic samples processed (Table 2). While resin codes were attributed to the sample if one indicator chemical was present, not all indicator chemicals from each resin type had to be present, since resin mixtures could present different combinations of the indicator chemicals.

Indicator chemical presence was based on the mass fragmentation pattern (Table 1) and the changes in the apparent relative and dynamic abundances.

Based on identification of indicator compounds, resin codes were assigned to 88% of the plastic items ingested by albatross. Of the most common types of plastic ingested, 91% of fragments and 93% of foam were assigned resin codes. While the resin composition of the different categories of plastic debris varied widely (Fig. 3), the most prevalent type in this sample set was PP, comprising 49% of all plastics. Overall, of the 129 plastic fragments processed, the most common plastic type was PP (68.4%), followed by PVC (20.5%), PETE (8.5%), PS (6.8%) and HDPE (0.8%) (Fig. 3).

"Iconic" items such as bottle caps, fishing floats and lighters revealed various chemical signatures (Fig. 4). Thirty-one bottle caps were identified as PP (67%), PVC (17%) or PETE (3%), and five (13%) were not identifiable. Eight fishing floats were determined to be PS (44%), PP (22%), and a combination of both PETE and PS (22%). One fishing float was not attributed. Of the five sampled lighters, one (20%) was PVC and 4 (80%) were PETE. The 62 unclassified but resin type-attributed fragments were comprised of PP (64%), PVC (12%), PETE (9%), HDPE (2%) and PS (2%), with 3 samples consisting of mixtures of various resin types (4%). Resin type could not be assigned to 5 fragments in the unidentified category.
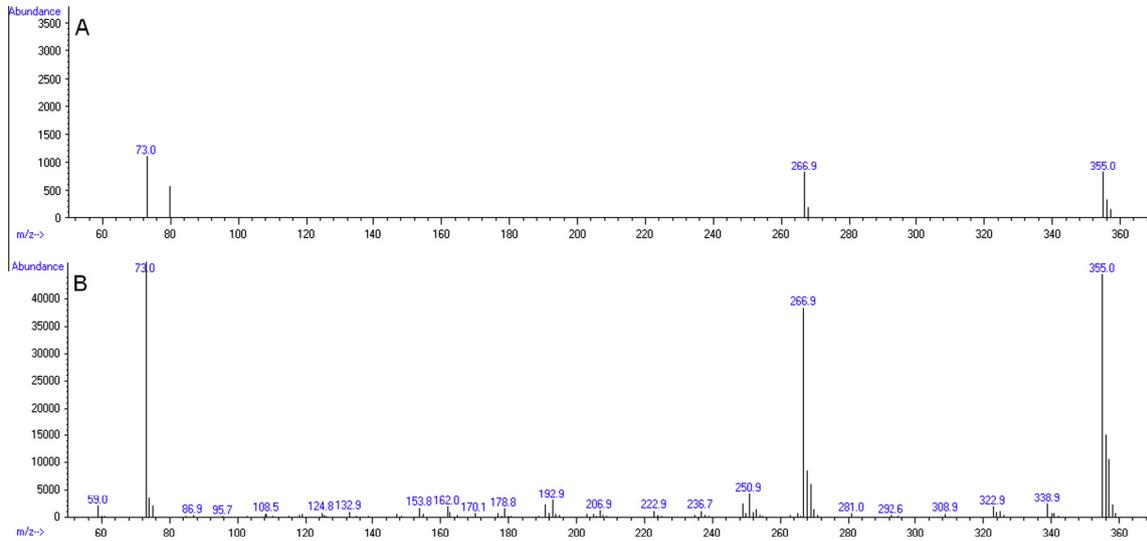
Fig. 2. Comparison of passive and Soxhlet extraction methods using identical samples of HDPE extracted using the passive and the Soxlet method. The Soxhlet method is more efficient, but identifying peaks are readily visible with the simpler passive extraction. A = passive solid phase extraction; B = Soxhlet extraction.

Table 2
Frequency of indicator chemical occurrence in ingested debris according to resin. Percentages in table correspond to the frequency that each indicator chemical contributed to the resin code attribution of plastic fragments ingested by albatross. Percentage is based on total number of ingested fragments identified to that resin type. Indicator chemicals were not found in every fragment attributed to resin type, suggesting environmental degradation occurs in ingested marine plastic.

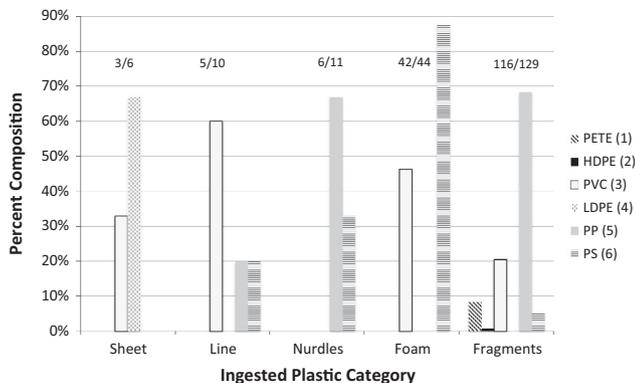| SPI resin	Indicator chemicals							
PETE (SPI 1)	SPI 1-1 100% 10 of 10							
HDPE (SPI 2)	SPI 2-1 100% 1 of 1	SPI 2-2 100% 1 of 1						
PVC (SPI 3)	SPI 3-1 55% 26 of 47	SPI 3-2 64% 30 of 47						
LDPE (SPI 4)	SPI 4-1 100% 2 of 2							
PP (SPI 5)	SPI 5-1 18% 14 of 79	SPI 5-2 27% 21 of 79	SPI 5-3 51% 40 of 79	SPI 5-4 89% 70 of 79	SPI 5-5 54% 43 of 79	SPI 5-6 58% 46 of 79	SPI 5-7 44% 35 of 79	SPI 5-8 22% 17 of 79
PS (SPI 6)	SPI 6-1 94% 45 of 48	SPI 6-2 27% 13 of 48						

Fig. 3. Summary of the chemical composition of ingested plastic identified in Laysan albatross boluses. The resin type composition for each extracted plastic category is illustrated. The numbers above the bars indicate the sample sizes of identified fragments/extracted fragments for each plastic category.

Among all plastic categories, resin types could not be assigned to a small fraction (12%) of the samples for several possible reasons. First, the samples may consist of a material impervious to the extraction solvents used (e.g., Teflon®). Alternatively, the fragments could represent a plastic formulation that was not represented in the set of indicator compounds we used (e.g. prior to SPI resin standardization). Finally, the samples could have degraded to the point that the indicator compounds were not present in high enough concentrations for detection by our method. Degradation and leaching of plasticizers from these samples could have occurred by a combination of photo-degradation as well as acidic and mechanical degradation inside the avian digestive tract (Hilton et al., 2000). In particular, the sheet samples appeared highly degraded, and were thin, faded and/or more brittle than the newer plastics used to make the standard materials. Sheets of plastic have previously been shown to degrade quickly in the presence of sunlight and acid (Zhang et al., 2004). Since plastic can persist in the ocean indefinitely, it is reasonable that some
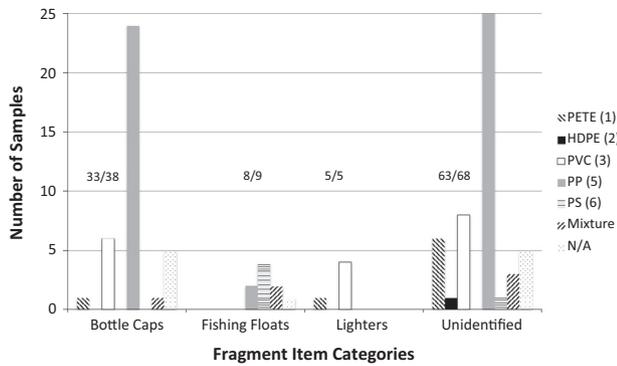
Fig. 4. Summary of the resin chemical composition within iconic and unidentified fragments in Laysan albatross boluses. The numbers above the bars indicate the sample sizes of identified fragments/extracted fragments.

fragments that are severely altered before collection may not be compatible with this analytical method.

3.3. Significance

As of this writing, no standard reference material is available for plastic types, and it is presumed that the plastics industry uses proprietary formulations that are always changing over time and with application. It is possible, but not likely, that formulation changes could prevent this method from being widely applied. Nevertheless, in light of these analytical uncertainties, it is striking that the methodology allowed successful attribution of resin codes to all of blind test plastics (representing recently manufactured plastic) and 88% of bird bolus samples (representing highly degraded plastic). Moreover, the bolus samples undoubtedly represent plastic produced over a much broader timespan prior to their introduction into the marine environment. Thus, the undefined period of their production could span decades.

Despite the logistical challenges of tracking and attributing plastic marine debris, this method offers an additional means of classifying plastic debris that complements and potentially expands the currently used methods that are based on the visual properties of the items (e.g., van Franeker et al., 2004) and infrared methods (e.g. FTIR and Raman spectroscopy) (Allen et al., 1999; Anzano et al., 2000; Gast et al., 1996). The method described here allows the resin typing of environmentally degraded plastics from extracts, with the potential for further characterization, including environmental contaminants adsorbed to the plastic's surface. Together these data can inform ongoing monitoring and risk assessment efforts. For example, Lithner et al. (2011) proposed a Hazard Ranking Model for various plastic polymers based on the components of the plastic polymers, rather than SPI resin type alone. This model characterizes polyvinyl chloride (PVC) as one of the most hazardous polymers present in the environment because of its potential to be carcinogenic, mutagenic, reproductively toxic, and persistent in the environment. Moreover, because PVC is not frequently recycled, it can enter the marine environment in large quantities (Environmental Protection Agency, 2011). For reference, approximately 25% of the samples we analyzed were identified as PVC. The majority of the identified samples (49%) were polypropylene, which falls into the least toxic Hazard Ranking framework (Lithner et al., 2011). PP is also not frequently recycled. The remaining 26% of the samples (those identified as PETE, HDPE, LDPE, and PS) fall into the acute toxicity Hazard Rating. PETE and HDPE are commonly recycled, with 29% and 28% of disposable jars recycled in 2011, respectively (Environmental Protection Agency, 2011). LDPE and PS are recycled in negligible amounts (Environmental Protection Agency, 2011).

In summary, the development and initial application of this method demonstrates that indicator chemicals can be used to classify new and environmentally degraded plastics according to their SPI resin type. While this study was designed to develop a method and conduct a test in an environmentally relevant system, these results suggest the potential value of this method toward building a risk assessment model for biological indicator species such as the albatross. Thus, this approach enhances the information that can be gained from plastic sampling in the marine environment, by providing an additional dimension for describing the plastic ingested by seabirds and investigating the risk that plastic pollution poses for seabird populations.

Acknowledgements

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.marpolbul.2014.07.055.

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