



Fourier transform infrared (FTIR) analysis identifies microplastics in stranded common dolphins (*Delphinus delphis*) from New Zealand waters

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ARTICLE INFO

Keywords:

Marine debris
Litter
Microplastics
Fragments
Marine mammals

ABSTRACT

Here we provide a first assessment of microplastics (MPs) in stomach contents of 15 common dolphins (*Delphinus delphis*) from both single and mass stranding events along the New Zealand coast between 2019 and 2020. MPs were observed in all examined individuals, with an average of 7.8 pieces per stomach. Most MPs were fragments (77%, $n = 90$) as opposed to fibres (23%, $n = 27$), with translucent/clear (46%) the most prevalent colour. Fourier transform infrared (FTIR) spectroscopy revealed polyethylene terephthalate (65%) as the most predominant polymer in fibres, whereas polypropylene (31%) and acrylonitrile butadiene styrene (20%) were more frequently recorded as fragments. Mean fragment and fibre size was 584 μm and 1567 μm , respectively. No correlation between total number of MPs and biological parameters (total body length, age, sexual maturity, axillary girth, or blubber thickness) was observed, with similar levels of MPs observed between each of the mass stranding events. Considering MPs are being increasingly linked to a wide range of deleterious effects across taxa, these findings in a typically pelagic marine sentinel species warrants further investigation.

1. Introduction

Plastics are highly pervasive synthetic organic polymers currently recognized as a major environmental threat to aquatic and terrestrial wildlife worldwide (Jambeck et al., 2015; UNEP, 2011; Wagner and Lambert, 2018; Santos et al., 2021). They exist in different sizes including macroplastics (>200 mm), mesoplastics (5–200 mm) and microplastics (<5 mm, hereafter MPs) (Derraik, 2002; Germanov et al., 2018). Considered as a major threat to marine environments, MPs are small plastic particles that have originated either from primary or secondary sources (Cole et al., 2011; Avio et al., 2017). Primary MPs mostly occur in personal care and cosmetic products (e.g., shower gel, toothpaste and facial cleaner), whereas secondary plastics are either created by the environmental breakdown of large plastic items (including fishing gear and food/beverage packaging), or are present in the effluent from domestic wastewater containing microfibrils from washing of synthetic textiles, films and microbeads from cosmetics (Acharya et al.,

2021; Bayo et al., 2020; Browne et al., 2011; Mateos-Cárdenas et al., 2020; Napper et al., 2015; Napper and Thompson, 2016; Ziajahromi et al., 2016).

Composed of a wide range of particle sizes (<5 mm), shapes, colours, and polymers due to their difference in origin and function, MPs exist in many forms. MPs which become bioavailable through trophic levels, are considered as vectors (Arienzo et al., 2021; Lohmann, 2017) for persistent organic pollutants (POPs) and may have fitness consequences for both individuals and associated populations accordingly (Teuten et al., 2009). For example, mass produced plastics in the form of polyethylene (PE), polypropylene (PP), and expanded polystyrene (PS) are available for transfer to higher trophic levels, not only to various marine species (Bellas et al., 2016; Gomez et al., 2020; Murphy et al., 2017) but also ultimately to complex mammals (Cole et al., 2011; Fossi et al., 2014). Additionally, plastics contain additives to facilitate the manufacture, performance and prolong the lifespan of the product (Hahladakis et al., 2018). Many of these additives are known toxicants,

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<https://doi.org/10.1016/j.marpolbul.2021.113084>

Received 6 July 2021; Received in revised form 17 October 2021; Accepted 17 October 2021

Available online 11 November 2021

0025-326X/© 2021 The Authors.

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including types of plasticizer, flame retardant, stabilizer, pigment, and antimicrobials, which are not chemically bound to the polymer matrix and therefore can leach from plastic. Despite this, our understanding on the potential fitness consequences of MP ingestion in both humans and wildlife remains limited (Carbery et al., 2018; Machovsky-Capuska et al., 2019).

There is a growing consensus in the literature that recognizes the interconnectedness of humans, wildlife, and their environments, via the One Health paradigm (Gibbs, 2014). While originally proposed as a systems approach, recent criticism highlights the lack of inclusion of environmental concepts while exploring health issues in humans and wildlife in isolation (Khan et al., 2018). Thus, animal sentinel species and their populations are vital to obtain a powerful transdisciplinary context to assess the health of ecosystems and humans worldwide (Destoumieux-Garzón et al., 2018). One Health's vision is particularly

relevant to the impacts of microplastic pollution worldwide, with considerable potential ramifications to wildlife and human health and their environments (Rabinowitz et al., 2018).

Marine mammals are characterized by long lifespans combined with the ability to control or mitigate toxic effects of anthropogenic pollutants (e.g., heavy metals, organochlorine compounds), making them a valuable biomarker for the health of aquatic environments (Bossart, 2011; Würsig et al., 2018). Cetaceans especially, exhibit complex and heterogeneous distributions throughout marine and freshwater habitats, often exploiting identical if not comparable food sources to humans (Young et al., 2015). Common dolphins (*Delphinus delphis*) are small cetaceans that have been considered as sentinel species for monitoring heavy metal concentrations (Das et al., 2003; Law, 1994; Lavery et al., 2008; Machovsky-Capuska et al., 2020; Stockin et al., 2007), persistent organic pollutants (POPs, Stockin et al., 2007), per- and polyfluoroalkyl

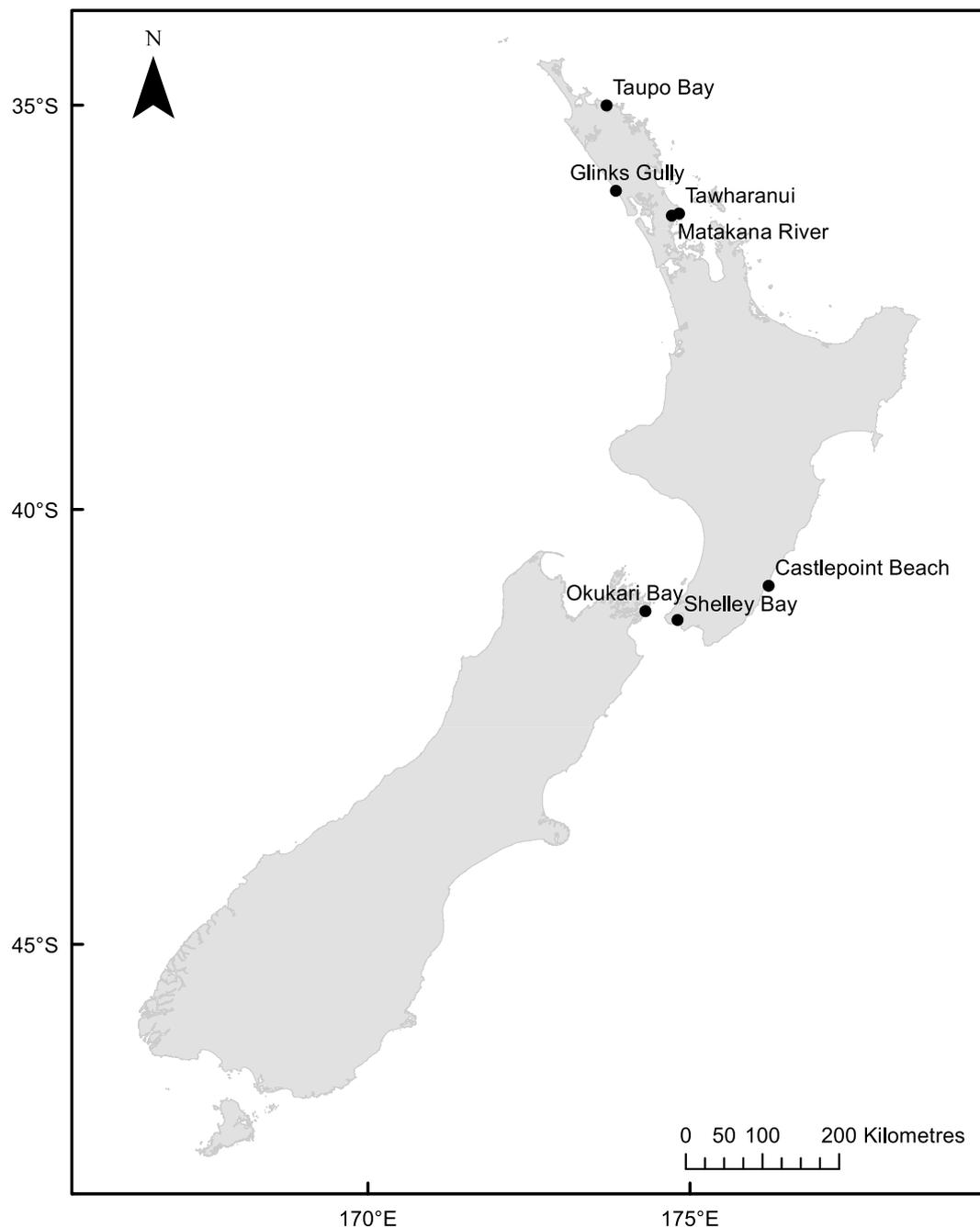


Fig. 1. Locations of common dolphins (*Delphinus delphis*; n = 15) stranded on the New Zealand coast (2019–2020) and subsequently assessed for microplastics.

substances (PFAs, Stockin et al., 2021) worldwide. Despite previous evidence on the ingestion of plastics for this species around the Galician (Hernandez-Gonzalez et al., 2018), Irish (Lusher et al., 2018) and British coasts (Nelms et al., 2018, 2019a, 2019b), the presence of MPs within any marine mammal taxa including common dolphin, is yet to be investigated within the South Pacific. Here we evaluate the presence of MPs in the stomach contents of common dolphins that stranded around the New Zealand coastline. We examine the general abundance, type, size of MPs ingested and characterize the polymers present. Specifically, the aims of this study are to; (a) describe, measure and quantify the number of MPs in the stomach contents (b) assess the relationship between body length and age and proxies of health (blubber thickness and axillary girth) with respect to MP burden, and (c) compare the occurrence, size, number and polymer type of MPs with respect to single vs mass stranded individuals.

2. Materials and methods

2.1. Sample collection

Fifteen common dolphins were recovered and sampled post-mortem for MPs (Fig. 1). Most individuals (n = 10) originated from two independent live mass stranding events at:

1. Okukari Bay (41° 20'S, 174° 31'E) Marlborough Sounds, South Island New Zealand on 27 February 2019 (n = 5), and
2. Taupo Bay (34° 99'S, 173° 71'E) Northland, North Island New Zealand on 13 April 2019 (n = 5).

The remaining dolphins (n = 5) originated from single stranding events around North Island, New Zealand between 9 April 2019 and 4 January 2020 (Table 1). Most carcasses were frozen to -20 °C within 4 h of death and transferred (via refrigerated transportation) to a post-mortem laboratory for subsequent examination under controlled conditions as per Stockin et al. (2007).

2.2. Age, sexual maturity and body condition

Teeth were used to age individual dolphins via growth layer groups (GLGs) following Stockin et al. (2021). In summary, up to three teeth of the least worn/curved teeth were extracted post-mortem from each animal and stored in 70% ethanol for subsequent aging. Teeth were then fixed, decalcified, thin sectioned (12–18 µm), stained and mounted using methods that are further detailed in Stockin et al. (2021). All age estimates were initially made 'blind' (i.e., with the reader having no biological information on the animal). The most central sections were

examined twice and if there was a discrepancy between the duplicate readings, the tooth was re-examined, with knowledge of the prior estimates, until a final estimate was determined (after Westgate and Read, 2007). On occasions where ages could not be determined (i.e., in older teeth), another tooth was sectioned and read. One GLG in common dolphins is considered to represent one year of life (Gurevich et al., 1980).

Sexual maturity status of females was defined from gross examination of ovaries (Murphy et al., 2009). Females were classified as immature if they had no corpora (scars of ovulation and pregnancy) on either ovary. Females were considered mature if the ovaries contained at least one *corpus albicans* or *corpus luteum* and/or they were pregnant or lactating. Males were classified as immature or mature based on testis morphology and histology (Westgate and Read, 2007). Immature males were defined as those with no spermatogenic activity in the seminiferous tubules, no sperm present in the epididymides, and small (<80 µm) seminiferous tubule diameters. Mature males were defined as those with evidence of spermatogenesis, including spermatozoa in the tubules and/or mature sperm present in the epididymides, and with larger (>100 µm) tubule diameters.

Body condition (Table 1) was defined as per Stockin et al. (2007) and included three (dorsal, lateral and ventral) blubber depth measures in conjunction with the axillary girth to control for decomposition distention.

2.3. Contamination control

Extensive measures were taken to minimize the risk of contamination of samples by airborne MPs or from use of equipment. During post-mortem examination, all personnel wore bright blue cotton-based overalls (to identify any contamination from the post-mortem team) and nitrile gloves until the stomach was excised out of the abdomen. Upon dissection into the abdominal cavity, the stomach was immediately isolated at the oesophageal and duodenal sphincters using cotton twine prior to ligation and intact removal. The unopened stomach was subsequently double wrapped in aluminium foil and deposited into inverted sealed aluminium foil trays prior to freezing to -20 °C in preparation for transportation. All subsequent dissection and examination of stomach contents was carried out at an independent forensics laboratory within a Class II biological safety cabinet. All equipment was covered with aluminium foil when not in use, and 100 µm steel mesh lids were placed over filter funnels during vacuum filtering. Natural clothing was worn as much as possible, and orange lab coats, nitrile gloves and PVC sleeve covers were used. Sleeve covers and gloves were washed down with reagent grade ethanol diluted to 70% and MilliQ water between samples.

Table 1

Biological parameters and the stranded locations of common dolphins (*Delphinus delphis*) stranded on the New Zealand coast 2019–2020 and subsequently assessed for microplastics (n = 15). Note: TBL = total body length.

Code	Location	Date	Sex	TBL	Age	Sexual maturity	Decomposition	Body condition
KS19-10Dd	Okukari Bay, Marlborough Sounds	27 Feb 2019	F	182	7	Immature	Mild	Good
KS19-11Dd	Okukari Bay, Marlborough Sounds	27 Feb 2019	F	174	4	Immature	Moderate	Moderate
KS19-12Dd	Okukari Bay, Marlborough Sounds	27 Feb 2019	M	201	9	Immature ^a	Mild	Good
KS19-13Dd	Okukari Bay, Marlborough Sounds	27 Feb 2019	F	189	5.5	Immature	Mild	Good
KS19-14Dd	Okukari Bay, Marlborough Sounds	27 Feb 2019	F	198	10	Mature	Mild	Good
KS19-16Dd	Glinks Gully, Northland	9 April 2019	F	173	9	Immature	Mild	Good
KS19-17Dd	Taupo Bay, Northland	13 Apr 2019	F	193	12	Mature	Fresh	Moderate
KS19-19Dd	Taupo Bay, Northland	13 Apr 2019	F	192	11	Mature	Fresh	Moderate
KS19-20Dd	Taupo Bay, Northland	13 Apr 2019	F	189	12	Mature	Fresh	Good
KS19-22Dd	Taupo Bay, Northland	13 Apr 2019	F	197	18	Mature	Fresh	Good
KS19-23Dd	Taupo Bay, Northland	13 Apr 2019	F	191	7	Mature	Fresh	Moderate
KS19-26Dd	Castlepoint Beach, Wairarapa	22 Feb 2019	F	205	15	Mature	Moderate	Good
KS19-38Dd	Tawharanui, Auckland	15 Dec 2019	F	82	0	Immature	Fresh	Good
KS19-39Dd	Matakana River, Auckland	19 Dec 2019	F	183.5	8	Mature	Mild	Poor
KS20-05Dd	Shelley Bay, Wellington	4 Jan 2020	M	210	9.5	Mature	Moderate	Good

^a Pubertal.

Metal or glass equipment was used where possible. Where an alternative could not be sourced, a sample of plastic was taken from the item and analysed by Fourier transform infrared (FTIR) spectroscopy for future reference. Plastic pouring rings were removed from all glass Schott™ bottles and new lids were used to avoid the possibility of fragments from aged lids. All equipment was washed with acetone and MilliQ water before use, and all surfaces were wiped down with reagent grade diluted to 70% ethanol between samples. Stainless steel mesh filters (300 and 100 µm) were muffle furnace (550°C overnight) to remove any potential carbon contamination.

2.4. Stomach content extraction

Each stomach was thawed overnight at 4 °C in a cleaned stainless-steel tray. The string ties were removed, and the stomach rinsed thoroughly on the outside with MilliQ water to remove any exogenous MP contamination. The stomach was then transferred to a fresh stainless steel tray. Using stainless steel surgical scissors, the stomach was dissected open longitudinally from the oesophageal entrance to the pyloric termination. Each of the three stomach chambers were inverted and carefully rinsed out into the tray with MilliQ water. Stomach contents including sand and large prey items (partially intact partially soft tissue, intact undigested squid beaks), were carefully rinsed with MilliQ water and removed. The resulting wash solution was transferred to a glass beaker, and the tray rinsed into the beaker with MilliQ water.

2.5. Digestion and isolation of microplastics

Using a glass filter funnel with stacked 300 and 100 µm stainless steel mesh filters (15 mm separation) to prevent blockage, the solution was filtered, under vacuum. Both filters were then removed using metal forceps and placed into a 1 litre Schott™ bottle with 100 ml of 0.3125% trypsin (Gibco™) solution for enzymatic digestion as per Courteney-Jones et al. (2017) and placed in a shaking incubator (MaxQ™ 4000, ThermoFisher Scientific™) at 40°C, 180 rpm, for 48 h. The trypsin was replenished after 24 h with an addition 20 ml. Following incubation, the bottles were sonicated for 6 min (35 kHz) (Bandelin Sonorex RK100H) to separate any remaining sand. Both mesh filters were turned over with forceps after 3 min. The filters were rinsed with MilliQ water (as opposed to deionised water as used Courteney-Jones et al., 2017) by directly into the bottle as they were removed, and the resulting solution filtered, under vacuum, onto a new 100 µm mesh filter. The Schott™ bottle was rinsed with MilliQ, which was then added onto the mesh filter. Where a filter became blocked, the digestion solution was split over multiple mesh filters. The resulting filters were placed in 100 ml Schott™ bottle with 30 ml sodium iodide (14.98 mg ml⁻¹ NaI; ECP Ltd) and sonicated for 6 min as described previously. The filter was rinsed with fresh NaI (10 ml) directly into the bottle, and the resulting solution transferred to a 50 ml Falcon tube (Cellstar™) and centrifuged for 2 min, 4500 ×g to separate any ingested sand in the case of live stranded animals. The supernatant was then filtered, under vacuum, onto a PCTE filter membrane (Whatman™ Nuclepore™ Track-Etched Polycarbonate, 4.7 cm, 10 µm). The remaining pellet was resuspended in MilliQ water and filtered onto a fresh PCTE filter. Filters were placed in individual glass Petri dishes with lids and dried at 40 °C for 48 h.

2.6. Contamination and isolation efficiency

To test and account for airborne contamination of MP particles within the post-mortem facility, blanks were used. Filter papers moistened with MilliQ water were placed in Petri dishes close to the area of work, and lids removed during times when work was being conducted to replicate the levels of exposure experienced by the samples. Procedural blanks were also carried out with each batch. MilliQ water approximating the volume used to wash the stomachs, was poured into the metal trays and swilled around before being poured into a beaker. This

was subsequently processed identically to the stomach wash solutions. A total of six procedural blanks were conducted during the processing of all 15 stomachs.

To determine the efficiency of isolation of MPs from the stomach samples, we further spiked five of the rinsed stomach contents and the six procedural blanks with bespoke reference MPs that spanned a range of sizes and densities. The spikes included particles of polyamide, expanded polystyrene (PS), polypropylene (PP) and high-density polyethylene (PE) in the following size ranges: 100–300 µm, 300–500 µm and 500–1000 µm. A total of 11 replicates were completed for each of the three size ranges, with an average of 77%, 85% and 92% isolation efficiency for each size for all polymer types, respectively.

2.7. Microplastic identification and characterization

Dried filters were examined under a Leica M125 microscope (magnification 8–100×), and all suspected MP particles (Norén, 2007; Hidalgo-Ruz et al., 2012) were photographed in situ with a mounted Leica MC170 digital camera, measured and characterized based on morphology (fibre, fragment, or film). Subsequently, all natural and synthetic particles (n = 1061) were transferred to a diamond compression cell (Almax EasyLab) for analysis by micro-Fourier transform infrared spectroscopy (µFTIR; PerkinElmer Spectrum 2, with Spotlight 200i microscope, Spectrum software v10.5.2.636). Particles were scanned at a resolution of 4 cm⁻¹, a scan area of 1000 × 1000 µm, and a spectral wavelength range of 4000–1000 cm⁻¹. The resulting spectra were compared against a series of pre-loaded polymer spectral reference libraries (Supplementary Information) to identify the plastic polymer type. Criteria for a match was a score greater than 75%, coupled with a manual check of characteristic peaks of each polymer from the pre-loaded reference libraries (Kroon et al., 2018). Upon FTIR identification, all MPs were subsequently photographed (Fig. 2).

2.8. Data analysis

We considered the data to originate from 3 independent groups, including two mass stranding events at: (1) Okukari Bay, South Island (MSE1), and (2) Taupo Bay, North Island (MSE2); and additionally, (3) singleton (SINGLE) stranded individuals from around North Island, New Zealand. Counts of MPs (adjusted for procedural controls) from individual dolphins were categorised by morphotype (fibres or fragments)

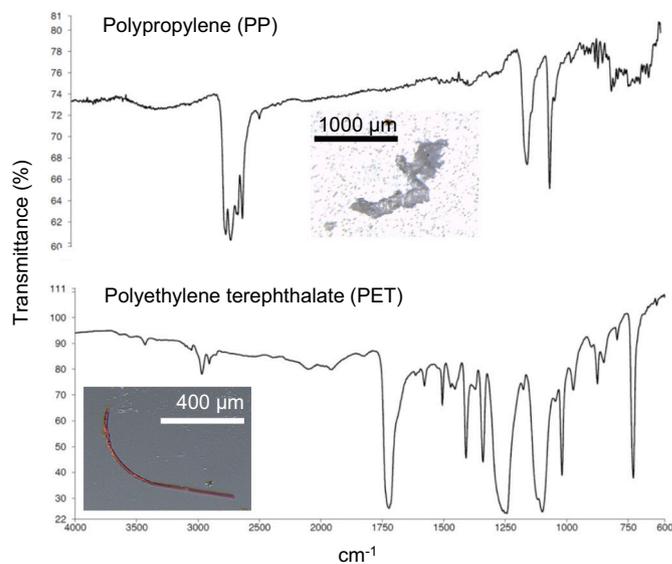


Fig. 2. Two examples of Spectra FTIR analysis and their associated images of microplastics recovered from stomachs of common dolphins (*Delphinus delphis*, n = 15) stranded on the New Zealand coast 2019–2020.

and further classified by polymer type. Fibres were classified as one of five polymer categories (PA, PE, PET, PP, PU) as determined using FTIR. Fragments were additionally subclassified as one of nine polymer types (ABS, PA, PBA, PE, PET, PP, PS, PU or Other).

2.8.1. Multivariate analyses

We considered three hypotheses examining the multivariate distribution of the 14 polymer/morphotype combinations (as defined in Section 2.8).

1. Differences in (multivariate) abundances of MP polymers between the three stranding groups (MSE1, MSE2 and SINGLE) were analysed using a Permutational Analysis of Variance with 4999 permutations (PERMANOVA) (Anderson, 2001). The dispersions of each group were formally compared using a permutation test of dispersion with 4999 permutations (PERMDISP, Anderson, 2006).
2. We examined whether individuals from the same mass stranding event had similar levels of MP types compared to individuals from the other groups (i.e. MSE1, MSE and SINGLE). Accordingly, we analysed the joint distribution of MPs using a canonical analysis of principal co-ordinates (CAP) (Anderson and Willis, 2003). The CAP analysis visualized the data by creating axes through the joint distribution that attempted to separate the three groups. The CAP also measured the model's ability to classify individuals using leave-one-out misclassification success, i.e., it removed an individual's datapoint, ran the CAP model on the remaining data, and classified the removed data point—this was repeated for each individual.
3. We examined whether the distribution of MPs had a linear relationship with age and or total body length. This was tested using a distance-based linear model (DISTLM, Legendre and Anderson, 1999; McArdle and Anderson, 2001) with 4999 permutations for the test.

All multivariate analyses of standardized MP counts were completed using the PRIMER v7 computer program (Clarke and Gorley, 2006) with the PERMANOVA+ add-on package (Anderson and Gorley, 2008). All multivariate analyses conducted were based on Euclidean distances calculated on the MP counts of the 14 polymer/morphotype variables after mean centring and standardization (i.e., units were measured in standard deviations) to account for differences in scale.

2.8.2. Univariate analyses

Generalized linear models (GLM) were used to examine differences in microplastic abundance between stranding groups. Aggregated (standardized count) of (i) all MPs, (ii) MP fibres and (iii) MP fragments were modelled separately, with total body length, age, sexual maturity, blubber thickness and axillary girth all considered as potential covariates. A gaussian error family with a log-link function were chosen because the response variables were not continuous after accounting for contamination (which subtracted the MP mean from concomitant procedural blanks) and because of differences in variability between the groups. Collinearity between variables was checked by examining variance inflation factors and variables were selected using backwards selection. Univariate analyses were performed in the statistical programming environment R 3.6.3 (R Development Core Team, 2020).

3. Results

3.1. Samples

A total of two males and 13 females were examined for MPs, with total body length and age ranging from 82 to 210 cm (mean = 184 ± 29 cm) and <1 to 18 years, respectively. The specimens examined from the Taupo Bay mass stranding event involved only mature females (n = 5). An additional mature female (KS19-21Dd, not reported here) and one male (KS19-18Dd, confirmed as a yearling calf at

post-mortem, not reported here), suggests a nursery pod (as defined in Stockin et al., 2007; Stockin et al., 2021). The specimens examined from the Okukari Bay mass stranding comprised a mixture of mature (n = 1) and immature (n = 3) females and a single immature (pubescent) male, while the singleton strandings (n = 5) were comprised of two mature females, two immature females and a mature male (Table 1). All examined carcasses were mostly fresh (n = 6) or mild (n = 6) in decomposition score, while body condition was predominantly either good (n = 10) or moderate (n = 4; Fig. 1).

3.2. Contamination control efficiency

Procedural blanks (n = 6) revealed a total of 20 MPs (fibres and fragments combined), demonstrating a mean contamination of 3.33 ± 2.62 particles per blank. Therefore, contamination was reported at 16% of the total MPs found in the stomachs (uncorrected number). Notably however, most of the contamination was by fibres (12.8%, of uncorrected total number of MPs in stomachs), whereas fragments were the predominant particle type represented in the stomach contents themselves. Specifically, fragments made up only 3.2% of uncorrected total number of MPs in stomach. Particle counts of MPs reported in this study have been subsequently adjusted to account for possible contamination by subtracting the mean of MPs identified in the procedural blanks of the same colour, morphology, and polymer type.

3.3. Microplastic characteristics

Microplastics were ubiquitously recorded in the stomach contents of all 15 dolphins examined. The ingestion of MPs in individuals ranged from 1 to 21 (mean = 7.8 ± 1.4 SE) particles. From a total of 117 MP pieces recovered from the stomachs and identified by FTIR, fragments (77%, n = 90) as opposed to fibres (23%, n = 27; Fig. 3a) were the most prevalent MP. Polymer morphotype varied between fragments and fibres, with polypropylene (PE, 31%), acrylonitrile butadiene styrene (ABS, 20%) and polyethylene terephthalate (PET, 15%) the most frequently recorded in fragments, while polyethylene terephthalate (PET, 65%) and polypropylene (PP, 13%) were the most prevalent in recovered fibres (Fig. 3a). Six colour categories were recorded, with translucent/clear (46%), black (10%), orange (10%) and multicoloured (10%), most prevalent (Fig. 3b). Fragment size ranged from 44 to 4361 μm (mean = 584 ± 925 μm), whereas fibres ranged from 198 to 10,032 μm (mean = 1567 ± 1969 μm ; Fig. 4).

3.4. Factors affecting microplastic burden

We found no evidence of a difference in MP types (PERMANOVA, $p = 0.12$) or dispersion ($p = 0.79$, PERMDISP) between stranding groups (MSE1, MSE, SINGLE). There was also no evidence of a relationship between the distribution of MPs with either age ($p = 0.219$, DISTLM), total body length ($p = 0.126$, DISTLM), blubber thickness ($p = 0.092$, DISTLM) or axillary girth ($p = 0.131$, DISTLM).

There was preliminary evidence that dolphins within each mass stranding event had comparatively similar MP burdens (Fig. 5), although our limited sample size means this observations should be considered exploratory. The CAP analysis correctly matched two of the five individuals, MSE2 four out of five, whereas singles were only correctly classified one of the five individuals (Supplementary Info). The average number of all MPs for each stranding group is shown in Fig. 6. The GLMs revealed no evidence of a difference in either the total number of MPs ($p = 0.507$), the number of fragments ($p = 0.598$) or fibres ($p = 0.135$) between stranding groups (Fig. 6). After Bonferroni adjustments (to account for tests on multiple covariates), there was no evidence ($p > 0.1$ for all variables) to indicate that either sexual maturity, body length, girth-at-flipper, or naval blubber thickness affect the number of MPs (total), MP fibres or MP fragments.

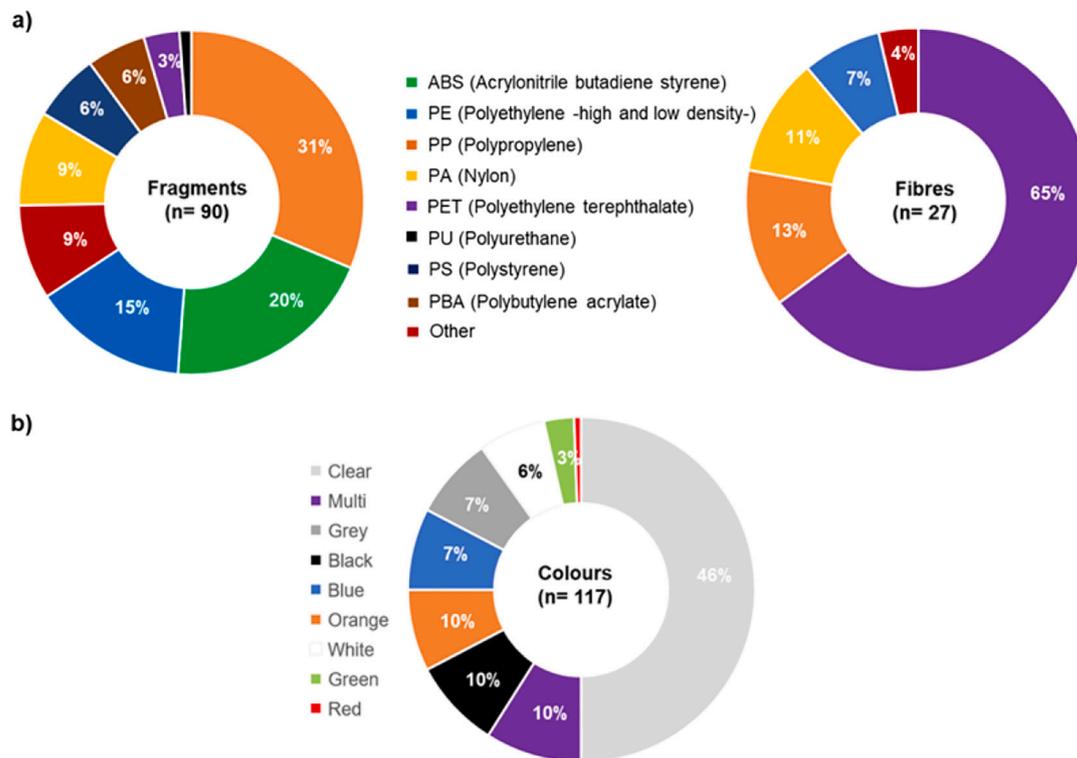


Fig. 3. Percentage of microplastics recovered from stomachs of common dolphins (*Delphinus delphis*, n=15) stranded on the New Zealand coast 2019–2020. Microplastics coded by (a) polymer and (b) colour composition. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

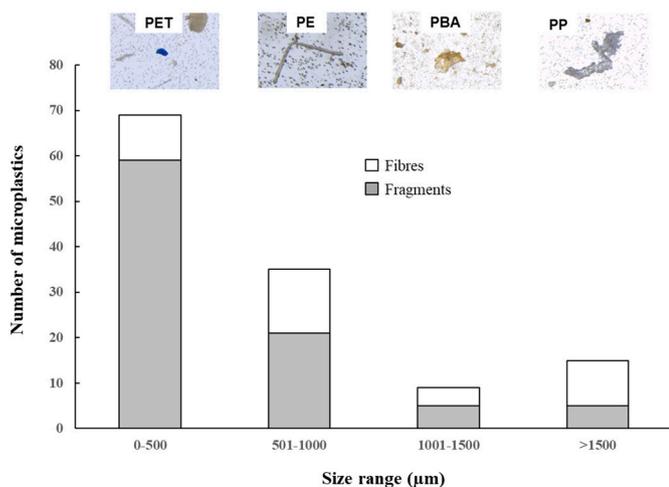


Fig. 4. Size distribution and representative photos of fibres and fragments found in the stomachs of common dolphins that stranded 2019–2020 along the New Zealand coast.

4. Discussion

Over the past decade there has been increasing scientific, public, and regulatory interest in the occurrence and impacts of plastics in the marine environment. Annually, it is estimated that poor waste management resulted in up to 23 million metric tonnes of plastics being released into the ocean (Borrelle et al., 2020). Plastic pollution is documented to affect marine mammals in a variety of manners including entanglement (Gall and Thompson, 2015; Lusher et al., 2018) and ingestion and digestive disruption (Denuncio et al., 2011, 2017; Fossi et al., 2014). Indeed, in a recent global review of horizon threats facing marine

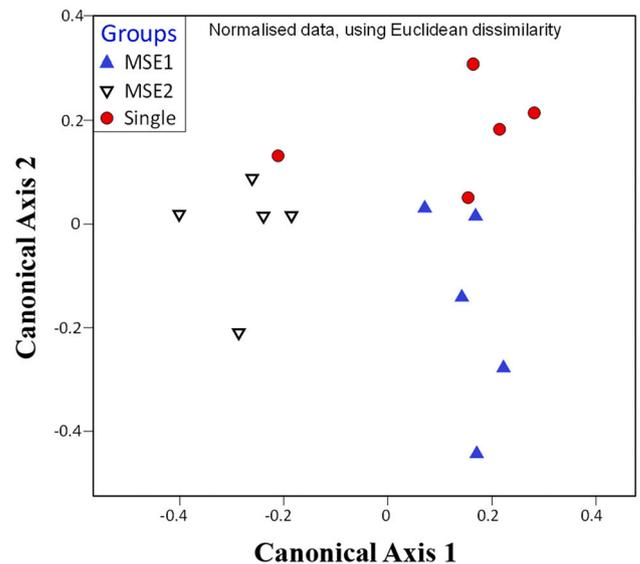


Fig. 5. Canonical analysis of principal co-ordinates visualizing differences of stomach content MPs between the three groups (MSE1, MSE2, Single) of common dolphins (*Delphinus delphis*, n=15) that stranded 2019–2020 along the New Zealand coast.

mammals, marine debris and in particular MPs were identified as one of the key significant knowledge gaps (Nelms et al., 2021). Regarded as a major pollutant, MPs have been discussed in both the context of human (Carbery et al., 2018; Smith et al., 2018) and wildlife health (Bucci et al., 2020; Machovsky-Capuska et al., 2019), receiving increasing scientific and societal focus accordingly. However, with the growth of research in this scientific field, there is a consensus on the need for standardization

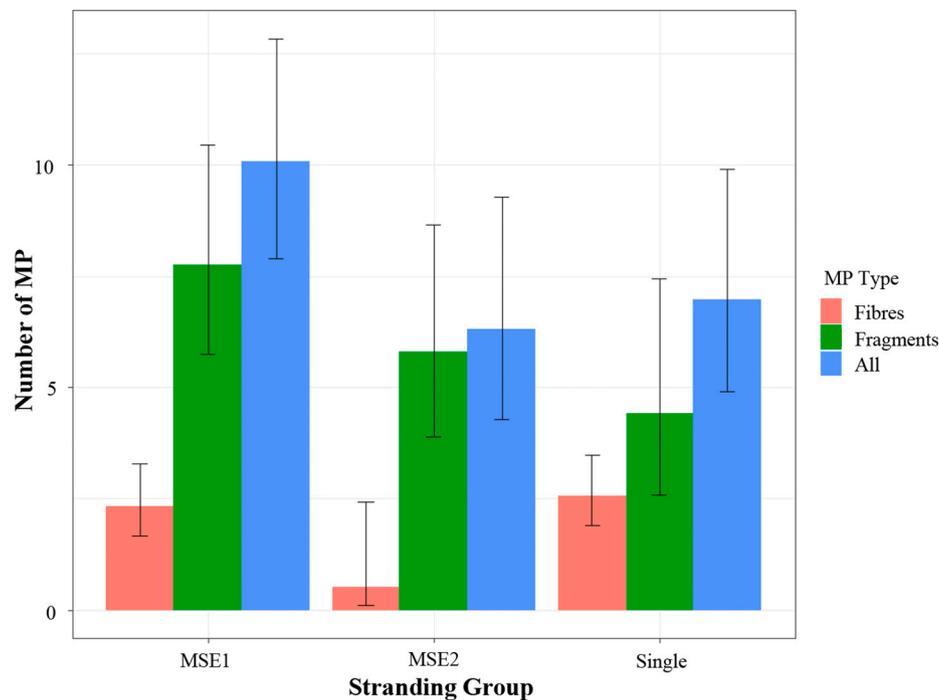


Fig. 6. The mean number of MPs for the three common dolphins (*Delphinus delphis*) groups (MSE1, MSE2, Single) that stranded 2019–2020 within New Zealand waters. Error bars are back-transformed standard errors from the GLMs (using log-link).

of methodological approaches, MP identification protocols and the prevention of contamination (Provencher et al., 2017, 2020; Kühn et al., 2021; Zantis et al., 2020; Meaza et al., 2021). Our study provides first, exploratory insights into potential MP loads for marine mammals in the South Pacific by examining post-mortem, single and mass stranded common dolphins from New Zealand waters. To do this, we examined morphotype, size and occurrence of MP polymers using strict contamination controls and FTIR identification methodologies.

Polypropylene, acrylonitrile butadiene styrene and polyethylene were the predominant MPs observed in the stomach contents of examined dolphins. Additionally, most MPs detected were translucent/colourless, which is consistent with what has been reported for MPs detected in beach sediments within Auckland, New Zealand (Bridson et al., 2020) and also scats of Northern (*Callorhinus ursinus*) and Southern fur seals (*Arctocephalus gazella*) (Eriksson and Burton, 2003; Donohue et al., 2019) and fish (Choy and Drazen, 2013; Tanaka and Takada, 2016), although differs to other stomach content studies on marine mammals that reported a predominance of blue/black MPs (Hernandez-Gonzalez et al., 2019; Lusher et al., 2018; Nelms et al., 2019a, 2019b; Novillo et al., 2020; Xiong et al., 2018; Zhu et al., 2019). To explore this further, several non-exclusive explanations are suggested. Firstly, New Zealand translucent PP and PET are commonly used for medicine containers, bottle caps and beverage packaging, respectively. Thus, the broad use of these translucent MPs within New Zealand, as also reflected in the MPs sampled on New Zealand beaches (Bridson et al., 2020) could explain their increased availability in the environment. Second, the harsh enzyme activity in the gastrointestinal tracks of animals has been suggested to influence the MPs observed (Donohue et al., 2019). This affect could potentially be amplified if MPs are transferred across trophic levels as a consequence of secondary ingestion. Finally, MPs are subject to environmental conditions including but not limited to photochemical processes, that may lead to colour degradation (Andrady, 2011; Liu et al., 2020). This is perhaps more pertinent to MPs transiting from land to sea via shallow waterways such as streams, and especially so in countries like New Zealand, where diminished ozone and relatively high UV levels are reported (Mckenzie et al., 1999, 2003). While we note colour bleaching during sample

processing can occur from harsh chemicals such as hydrogen peroxide (H_2O_2) (Donohue et al., 2019), our selection of Trypsin and Sodium Iodine within the methods for digestion and separation processes was deliberate, since neither chemicals are known to induce microplastic degradation (Cutroneo et al., 2021; Hurley et al., 2018). As such, we believe the methods themselves unlikely influenced our findings, and especially since overall, 54% of MPs were still of various colour in our study.

MPs were present across all examined animals in our study, with most of the detected polymers represented via fragments. Although the presence of urban settlements and usage trends could provide a plausible explanation for the availability of fragments in New Zealand waters and species living within them, no clear patterns emerged from comprehensive spatial studies on the New Zealand green lipped mussel (*Perna canaliculus*) (Webb et al., 2019) and a wide range of coastal and pelagic fish species (Horn, 2021). However, fragments were nonetheless the most prevalent form (78%) of MPs recorded in New Zealand stream sediments (Bridson et al., 2020) which aligns with the findings of our study. While the predominance of fragments has also been previously reported in pinniped scats (reviewed in Meaza et al., 2021) and the GI of fish (Boerger et al., 2010; Rochman et al., 2015) and beluga whales (*Delphinapterus leucas*) (Moore et al., 2020), other studies have revealed fibres as the more predominant morphotype in GI for different species of marine mammals (Battaglia et al., 2020; Hernandez-Gonzalez et al., 2018; Novillo et al., 2020; reviewed in Zantis et al., 2020). While fibres can result from airborne and wider contamination (Besseling et al., 2015; Rebolledo et al., 2013), which may pose challenges to studies which have not had the benefit of forensic conditions (Liu et al., 2020), it remains unclear if or how this bears resemblance to disparities noted in our study. As fragments remained the predominant morphotype in both raw as well as the control blank corrected datasets in our study, this suggests our finding was not solely driven by reduced contamination probability of fibres. Instead, to what extent the specific region of the gut examined may have affected the proportion of fibres vs fragments we observed is perhaps an alternative suggestion, since our study focused on the mid-GI tract only. Indeed, whether this better explains differences observed between this and other studies which have examined different

or indeed wider regions of the GI tract, warrants further investigation.

It is known that the origin and volume of the gut content analysed can affect the abundance of MPs detected due to their uneven distribution and/or sampling variation (Moore et al., 2020; Nelms et al., 2019b). However, it is less clear whether the proportion of fibres would have increased if the hindgut had been included in our study, since anatomical variation has formerly been suggested to favour the retention of fibres (Lusher et al., 2015; Welden and Cowie, 2016). Although, variability within the stomach contents of animals may further influence the results presented (fibres are more likely to get lost in filters/extracted sand than fragments), our origin of sampling (midgut) along with species (common dolphin) was standardized, allowing for consistent reporting between animals within our study. While some degree of stomach size variability by age class is inevitable, most animals examined were physically (even if not sexually) mature.

The inclusion of two mass strandings within our study is a clear point of difference to other studies that have reported MPs in marine mammals. While this opportunity offered a unique and somewhat unapparelled possibility to examine the extent of MP ingestion across several individual animals in spatial and temporally comparable settings, it may further explain how trends in either polymer type, shape or colour may have been magnified across individual dolphins feeding on the same prey with the same regions simultaneously. Indeed, the inclusion of single stranded animals in our study did allow for that comparison and while no difference between mass and single stranding MPs was found, we acknowledge the preliminary nature of this finding and need to increase sample sizes from future events.

In our study, the ingestion of MPs was evident across all animals, with no correlation found to exist between total microplastic burden and any of the biological or health parameters explored, including age, body length, auxiliary girth or blubber thickness. We further found no evidence to suggest differences between the three stranding groups, even though variability in feeding strategies and diets of common dolphins within New Zealand are documented (Meynier et al., 2008b; Stockin et al., 2009; Peters et al., 2020). Nonetheless, these findings should be considered exploratory given the limited sample size available for this study.

While the bioavailability of MPs and potential impacts to aquatic environments and organisms are debated by some to be less impactful than first believed (Ivar do Sul and Costa, 2014), recently it has been suggested that fibres may have the potential to cause inflammatory damage to the GI tract, reducing food intake and/or alter the gut microbiome (Issac and Kandasubramanian, 2021; Parton et al., 2020; Qiao et al., 2019). Concern has also been raised about potential negative changes in body condition, reduced growth and even mortality of taxa from lower trophic levels and their potential disruption on the functioning of aquatic environments (Rebelein et al., 2021). The presence of MPs and the threat they pose remains unclear, especially where the detrimental long-term toxicological effects and nutritional deficiencies may easily go undetected (Zhang et al., 2021). For example, Nelms et al. (2019a, 2019b) demonstrated how animals that died due to infectious diseases also exhibited marginally higher numbers of MPs than those that died of trauma and other drivers of mortality, indicating a possible relationship between the cause of death and MP abundance.

While full pathological screening of animals did not occur in our study, blubber thickness and axillary girth (when controlled for decomposition) did offer a rudimentary means to assess the MPs burden in the context of individual health antemortem. While neither measure was found to correlate with MP burden in our study, that does not negate the possibility that observed MP burdens are still associated with health. Notably, the absence of systematic pathological screening for infectious disease of most stranded cetaceans in New Zealand further negates conclusions being drawn in this context. As health impacts of MPs remain an emerging topic across many species worldwide, future research should seek to not only increase the exploratory sample sizes presented here but additionally include pathological screening of

animals who undergo MPs assessment.

Contextualizing MPs detected in New Zealand and wider South Pacific marine mammals is generally difficult, in part due to the lack of studies examining marine mammal MPs in the southern hemisphere (Zantis et al., 2020). However, within European North Atlantic waters, Hernandez-Gonzalez et al. (2018), Nelms et al. (2019a, 2019b) and Puig-Lozano et al. (2018) provide information on the occurrence of plastics (both macro and microplastics) in the stomach contents of common dolphins stranded on the Galician coasts of Spain, on the presence of plastics in marine mammals stranded around the British coast and finally, and concerning pathology associated with the presence of foreign bodies in stranded cetaceans in the Canary Islands, respectively. These studies collectively provide compelling evidence that MPs are ubiquitous in smaller odontocete species. Where possible, future studies should further elucidate links between habitat and the exposure of prey to MPs (Burkhardt-Holm and N'Guyen, 2019; Fossi et al., 2017a, 2017b; Jawad et al., 2021).

As with former studies, determining source of MPs reported in this study is not without complication. As discussed by several authors, MPs can be a consequence of secondary ingestion (i.e., transferred from prey to predator) rather than primary intake. The omnipresence of plastics throughout the different environments has recently led to the suggestion that micro- and macro-plastics form part of the definition of foods that animals consume (Machovsky-Capuska et al., 2019). Therefore, prey are known as the main nutritional, energy, and pollutant source for marine predators (Machovsky-Capuska and Raubenheimer, 2020). Common dolphins within New Zealand waters predominantly consume arrow squid (*Nototodarus* spp.), jack mackerel (*Trachurus declivis*) and anchovies (*Engraulis australis*) (Meynier et al., 2008a). In support of a potential secondary ingestion pathway, microplastics have been documented in jack mackerel within New Zealand waters (Horn, 2021; Jawad et al., 2021; Markic et al., 2018) and in squid within the Pacific Ocean (Daniel et al., 2021; Laist, 1997). While the potential consequences of ingesting MPs are not clear for common dolphins, an important area of future research could be to explore the interplay between nutrition and plastic ingestion to understand how individuals, their populations and trophic relation interactions respond to them (Machovsky-Capuska et al., 2019). However, such studies would need to be conducted with standardization and quality control at the forefront of any experimental design, as first suggested by Provencher et al. (2017).

Our study employed FTIR analysis to identify subsampled putative MP polymers. While spectra match confidence thresholds of the MPs minimized the potential for misclassification (Kuhn et al., 2020), contamination still exceeded the 10% sample count level recommended by Provencher et al. (2017). However, corrected counts in addition to the higher prevalence of fibres (when stomachs predominately contained fragments) permit confidence in the final counts reported here. The correction of raw data is particularly important when dealing with emerging contaminants which have the potential to induce health implications in the future.

Our findings highlight increased need for systematic pathological screening of carcasses to investigate specifically, the role MPs play in cetacean morbidity and mortality. Santos et al. (2021) proposed the use of the three functional traits (food selection, nutritional state, level of prey resemblance with plastics) combined with an assessment of plastic availability to develop a comprehensive risk assessment for plastic ingestion in wildlife. Moving forward, prospective study designs with long-term sampling alongside necessary tissue archiving, are the foundation to documenting the expected increase in MPs in the environment and their risks to marine organisms.

5. Conclusion

We present first insights to the MP burden of cetaceans inhabiting South Pacific waters. Our findings demonstrate the increasing importance of stranding investigations to monitor MPs in marine mammals.

Specifically, our findings call for a comprehensive transdisciplinary approach which includes nutritional ecologists, toxicologists, wildlife pathologists and biologists, accordingly. Only via transdisciplinary standardized studies and larger sample sizes, will the full extent of MP burden on long-lived, marine sentinel species become apparent.

CRedit authorship contribution statement

Karen A. Stockin: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Writing – original draft, Writing – review & editing. **Emma L. Betty:** Data curation, Funding acquisition, Writing – review & editing. **Emily I. Palmer:** Data curation, Project administration, Writing – review & editing. **Matthew R. Perrott:** Formal analysis, Funding acquisition, Writing – review & editing. **Gabriel E. Machovsky-Capuska:** Formal analysis, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

KAS is supported by a Royal Society Te Apārangi Royal Society Fellowship (2019–2023). ELB is supported by a Kate Edger Educational Charitable Trust Postdoctoral Award (2021). This research was funded by a Massey University Research Fund (MURF) grant awarded to KAS and ELB (RM22283) as part of the Aotearoa Impacts and Mitigation of Microplastics (AIM2) project, in receipt of funds from a New Zealand Ministry of Business, Innovation and Employment (MBIE) Endeavour Fund Grant (C03X1802). Research was conducted under a research permit issued to Massey University by the New Zealand Department of Conservation (39239-MAR). Access to carcasses was kindly supported by Mana Whenua and facilitated via the New Zealand Department of Conservation. Special thanks to Amai Thompson and Harvey Ruru (Te Atiawa Trust), Ngāti Kahu, Ngāpuhi and Hannah Hendriks, Irene Petrove, Catherine Peters, Aubrey Tai and Tansy Bliss (Department of Conservation). We acknowledge Odette Howarth, Rebecca Boys, Beth Hinton and Deborah Casano-Bally (Massey University) for post-mortem laboratory support. We would like to thank Jamie Bridson (Scion) who as part of the AIM2 project, produced the microplastic spike samples for determination of MP recovery efficiency. The authors additionally thank Louis Tremblay, Grant Northcott and ESR laboratories for their support of this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2021.113084>.

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