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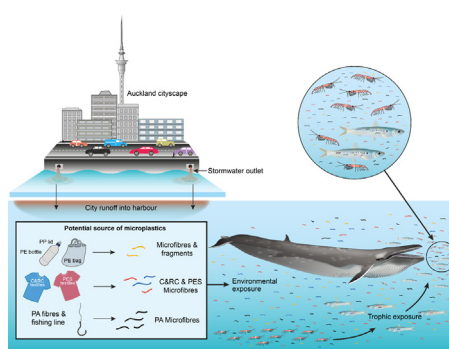
Assessing microplastic exposure of large marine filter-feeders

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HIGHLIGHTS

- Marine filter-feeders are continuously sampling the environment for plastics.
- We determined microplastic exposure from prey and scats of whales in New Zealand using novel stochastic simulation model.
- Whales had an estimated daily ingestion of around three million microplastics.
- Trophic transfer is considered the major path of microplastic exposure for whales.
- Measuring microplastic from water underestimates exposure by 4 orders of magnitude.

GRAPHICAL ABSTRACT



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ABSTRACT

Large filter-feeding animals are potential sentinels for understanding the extent of microplastic pollution, as their mode of foraging and prey mean they are continuously sampling the environment. However, there is considerable uncertainty about the total and mode of exposure (environmental vs trophic). Here, we explore microplastic exposure and ingestion by baleen whales feeding year-round in coastal Auckland waters, New Zealand. Plastic and DNA were extracted concurrently from whale scat, with 32 ± 24 (mean \pm SD, $n = 21$) microplastics per 6 g scat sample detected. Using a novel stochastic simulation modeling incorporating new and previously published DNA diet information, we extrapolate this to total microplastic exposure levels of 24,028 (95% CI: 2119, 69,270) microplastics per mouthful of prey, or 3,408,002 microplastics (95% CI: 295,810, 10,031,370) per day, substantially higher than previous estimates for large filter-feeding animals. Critically, we find that the total exposure is four orders of magnitude more than expected from microplastic measurements of local coastal surface waters. This suggests that trophic transfer, rather than environmental exposure, is the predominant mode of exposure of large filter feeders for microplastic pollution. Measuring plastic concentration from the environment alone significantly underestimates exposure levels, an important consideration for future risk assessment studies.

1. Introduction

Plastic pollution is a prominent threat to marine environments and is receiving growing societal and scientific attention due to the unprecedented

scale at which it is accumulating (Barboza et al., 2018; Galloway and Lewis, 2016). Of particular concern are microplastics (<5 mm), which have been detected in every marine environment sampled (Bergmann et al., 2019; Shim and Thomposon, 2015; Van Cauwenbergh et al.,

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2013), from polar seas (Bergmann et al., 2019; Lusher, 2015) to inshore habitats near urban areas (Dris et al., 2018). There is a positive correlation between urban density and microplastic abundance (Eriksen et al., 2013; Zhao et al., 2015), therefore, marine organisms inhabiting these cosmopolitan waters are at risk of microplastic ingestion (Au et al., 2017).

Microplastics are ingested by marine animals in three ways; i) microplastics may be actively selected because they are mistaken for food, especially by small animals (Eriksen et al., 2014), ii) they may move up the food web via trophic transfer (trophic exposure (Au et al., 2017; Ivar Do Sul and Costa, 2014; Nelms et al., 2018)) and, iii) they may be directly consumed from the water by accident by animals with bulk foraging strategies, such as filter-feeders (environmental exposure (Besseling et al., 2015; Germanov et al., 2018)). This last route is thought to be especially relevant to large filter-feeders, such as manta rays (*Mobula* spp.), basking sharks (*Cetorhinus maximus*), whale sharks (*Rhincodon typus*) and baleen whales as they filter large volumes of seawater to extract prey (Germanov et al., 2018; Guerrini et al., 2019).

Large filter-feeders have long been acknowledged as ecosystem indicator species (Burek et al., 2008). Their mode of feeding potentially turns large filter-feeders into bellwethers of microplastic exposure in marine ecosystems (Fossi et al., 2014). Essentially, they are continuously sampling their environment and the food-web concurrently as a biological autonomous sampling system (Boehlert et al., 2001). If estimates of microplastic incidence in the environment is known, then the relative contribution of trophic exposure can be inferred. Despite this, the exposure levels of large filter-feeders to plastic pollution are poorly understood (Lusher, 2015; Germanov et al., 2018; Lusher et al., 2018; Zantis et al., 2020). So far only a handful of studies have estimated exposure of baleen whales to microplastics. Only two studies determined levels of microplastics directly from the gut content of a single humpback whale (*Megaptera novaeangliae*) (Besseling et al., 2015) and several fin whales (*Balaenoptera physalus*) (Garcia-Garin et al., 2021). Four studies estimated exposure via trophic transfer (Burkhardt-Holm and N'Guyen, 2019; Desforges et al., 2015) or environmental exposure (Germanov et al., 2018; Guerrini et al., 2019) in several species of baleen whales. Moreover, differences in diet and variability of microplastic pollution in different locations may play an important role in plastic exposure (Burkhardt-Holm and N'Guyen, 2019). Given their important role in ecosystems, there is an urgent need to better estimate exposure levels in baleen whales (Garcia-Garin et al., 2021).

Due to their large size and conservation status, determining microplastic exposure has proven difficult for baleen whales. A promising novel tool to estimate exposure is faecal analysis, which enables us to simultaneously characterise diet (Carroll et al., 2019) and quantify microplastic exposure (Nelms et al., 2019). It has been successfully used for other species, such as pinnipeds, as it is non-invasive, relatively simple to collect for aquatic and semi-aquatic species and can be used for different research purposes (e.g. diet analyses (Pompanon et al., 2012)). For example, Nelms et al. (2019) combined a microplastic isolation method with a diet analysis from faeces to investigate the diet composition and microplastic abundance in grey seals (*Halichoerus grypus*). As this is a novel tool for whales, here we present a case study of two large filter-feeders, the Bryde's whale (*Balaenoptera edeni brydei*) and the sei whale (*Balaenoptera borealis*), estimating their exposure to microplastics in the Hauraki Gulf (Tikapa Moana – Te Moananui-ā-Toi), the waters adjacent to Auckland, Aotearoa – New Zealand's largest city. The productive Gulf waters are a critical habitat for a resident population of Bryde's whales, with occasional sightings of sei whales (Gostischa et al., 2021). The whales are likely exposed to microplastics from a combination of environmental (from seawater) and trophic (from prey) exposure, amplified by living in close proximity to a large city (Fig. 1). Here, we quantify the number of putative microplastics in whale scat and estimate total exposure levels with a novel stochastic simulation framework. We also discuss recommendations for future work aimed at identifying the exposure pathways for these, and other large filter-feeder marine species. This information will be key for estimating the total exposure levels of marine organisms, which is critical to the development of robust risk assessments for microplastic pollution.

2. Materials and methods

2.1. Sample collection and preparation

Data were collected opportunistically from a University research vessel and a commercial whale-watch vessel from 12 October 2014 to 19 January 2019 (Table S1). Scats were collected with a 150 µm mesh (Clear Edge Monodur™). The mesh was cleaned with a hose to remove particulate matter after sampling and rinsed before and after usage with distilled water. In total, three sei whale and 18 Bryde's whale scats were collected (Table S1). Samples from the water used to rinse the net were taken as an environmental control. Moreover, samples of the mesh, clothing and boat furniture (e.g. hose, carpet) were taken as control samples to compare these to the putative microplastics from scat samples.

Methods and techniques for microplastic analyses were based on Nelms et al. (2019), with some modifications and adaptations to accommodate working with whale scat (Fig. 2). Each scat sample was agitated to avoid the accumulation of particles on the surface or bottom of the sample. For each sample ($n = 21$), three sub-samples of 2 g each were taken for analysis (a total of 6 g per scat). This involved centrifugation of 15 mL scat with 30 mL 100% ethanol (5 min at 2500 x g). Supernatant was removed and stored for analysis of residual microplastic. Excess scat was used to determine the dry weight of each sample via incubation at 55 °C for ~34 h. Blanks were included with every sample using 2 g Milli-Q water instead of scat. To each tube, 7.5 mL of homogenising solution (1 M Tris-HCl pH 8, 0.5 EDTA, 5 M NaCl, 10% SDS) and 70 µL at 200 µg/mL of Proteinase K (Sigma) were added, and samples incubated at 55 °C for 30 min. Next, 2.14 mL of 200 µg/mL sodium perchlorate was added, the sample was shaken for 20 min and incubated at 65 °C for another 20 min.

2.2. Laboratory analysis: microplastics

2.2.1. Microplastic recovery

Prior to filtration, filter papers were inspected under a microscope for microplastics (Mag x25, Wild Heerbrugg M3C Stereo Zoom Microscope) then kept in sterilised petri dishes. Digested samples and supernatant were filtered through an 8 µm MF-Millipore™ Membrane Filter (47 mm diameter). Following each filtration, the unit was flushed with Milli-Q water to ensure all plastic had been collected. The filter paper was removed using tweezers and placed in a petri dish (90 × 14 mm, Techno-Plas), sealed and stored at 4 °C until further analysis under the microscope. This process was repeated for all sub-samples and blanks.

2.2.2. Microplastic identification

The filter papers were examined under a microscope (Mag x40, Wild Heerbrugg M3C Stereo Zoom Microscope) to visually identify microplastics in the scat. Each potential microplastic was photographed (microscope with a JENOPTIK GRYPHAX® NAOS camera) and type (fragment or fibre), colour, shape and size were recorded (Barrows et al., 2017). Particles were defined as plastic following the criteria from Norén (2007), such as no organic or cellular structures, visible on the particles, fibres should be consistently thick, have a three-dimensional bending, and be clearly coloured.

To confirm polymer types, further analysis was carried out by using Fourier transform infrared (FTIR) spectroscopy (Bruker Tensor27 attached to a Bruker Hyperion 3000 with MCT detector, Scion, New Zealand). Selected plastic particles ($n = 40$, 6% of overall particles count) were analysed following Norén (2007). Each potential microplastic was scanned at a resolution of 6 per cm (wavelength range = 6000–600 per cm). Spectra were processed and compared to a reference database (Primpke et al., 2018). Only FTIR readings with reliable spectra matches and a search score confidence of 70% or higher were accepted during the output analysis. While we acknowledge that we can only confirm that a subset of the particles found is synthetic, we will use the term putative microplastics to describe the particles in general as they conformed to descriptive criteria used to define synthetic particles (Norén, 2007).

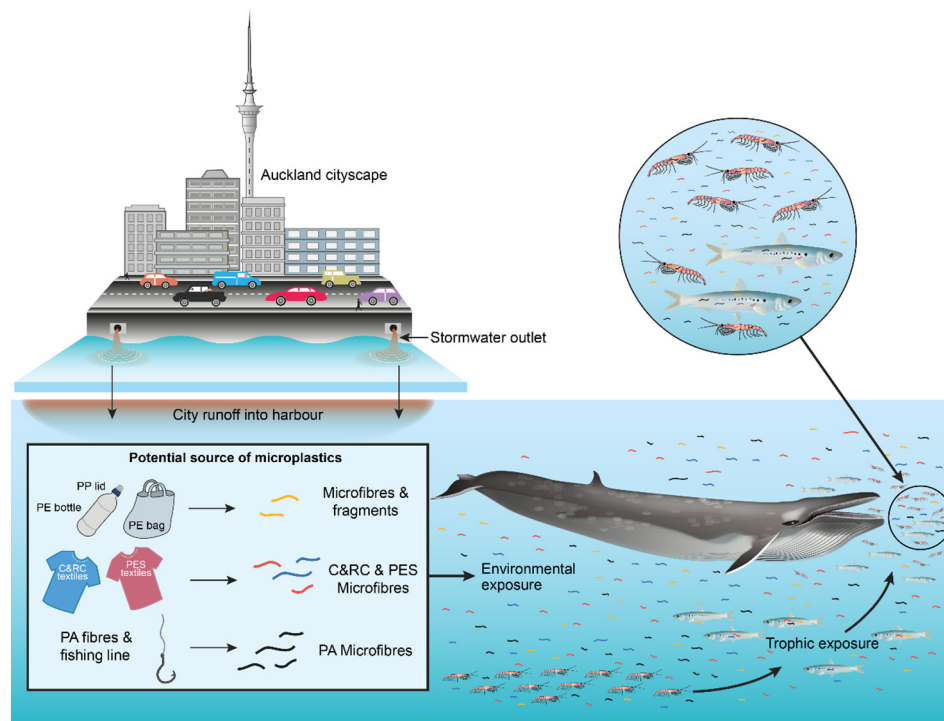


Fig. 1. Theoretical exposure pathways (Gago et al., 2018) of baleen whales to microplastics in the Hauraki Gulf, indicating the potential sources of microplastics, their presence in the water column (environmental exposure) and uptake in prey species (trophic exposure), which combined cause exposure in whales. Animals and cars not to scale. Abbreviations as follows: C&RC: cellulose and regenerated cellulose; PE: polyethylene; PA: polyamide; PES: polyester; PP: polypropylene.

2.3. Laboratory analysis: DNA diet work

2.3.1. DNA extraction and amplicon library preparation

Scat samples were subsampled in 1 g triplicates and centrifuged (5 min at 4000 xg) prior to the DNA extraction. DNA was extracted using the manufacturer's protocol for the QIAamp DNA stool Mini Kit (QIAGEN) with the following modifications: incubation overnight at 55 °C, 90 µL of elution buffer instead of 200 µL. Diet was assessed using targeted metabarcoding of the whale's preferred prey groups: euphausiids (Jarman et al., 2006a), copepods (Bissett et al., 2005), fish (Jarman et al., 2006b) and salps (Chow et al., 2009). Amplicons were cleaned using AMPURE XP magnetic beads and pooled per subsample, before being quantified with Qubit dsDNA HS assay kit. Next-generation sequencing was performed on the Illumina MiSeq platform at New Zealand Genomics.

2.4. Contamination control

Strict protocols were followed to avoid DNA and microplastic contamination (see Supplementary Material 1). Briefly, blanks served as contamination controls for both DNA and microplastics, work was conducted inside flow hoods that were thoroughly washed with bleach and MilliQ water at first use and in between samples; and non-plastic equipment was used where possible (e.g., cotton lab coat, nitrile gloves). Control samples ($n = 10$) from lab equipment, such as pipettes tips or lids, and environmental, e.g., clothing, were also tested under the FTIR.

2.5. Data analysis

2.5.1. Microplastics

After subtracting microplastics that were likely due to contamination (see Supplementary Material 1 and Dataset S1) from the results, the mean and standard deviation of number of microplastics found per sample were calculated; the mean and standard deviation of microplastic size found across all scats were also calculated.

2.5.2. Bioinformatic analyses

Bioinformatic pipeline of DNA sequences is available in Supplementary Material (Tables S2 and S3) and, briefly: we used basic bash functions, PEAR for alignment (Zhang et al., 2014), and USEARCH/UNOISE2 for quality control and designation of zero-radius operational taxonomic unit (operational definition used to classify groups of closely related individuals; ZOTUs (Edgar, 2016)). Taxonomy was assigned to amplicons using a naïve Bayesian classifier (Wang et al., 2007) against a reference library constructed using fuzzy primer searches with *tre-agrep* (<https://laurikari.net/tre/tre-0-8-0-released/>) against the GenBank database (accessed: April 2020) and taxid associations using the *obiannotate* command (Boyer et al., 2016).

2.5.3. Calculation of microplastic exposure

As microplastics in whale scat reflect both environmental and trophic exposure routes, we used our estimates to infer total microplastic exposure of large filter feeders in the Gulf. This was a four-step process implemented as a stochastic simulation in the statistical language R (Table 1). First, we estimated the ratio of grams of scat to grams of prey. To do this we estimated the dry weight of scat to be $6 \pm 4\%$ ($scat_{dw}$, mean \pm SD, as a % of wet weight) based on analysis of 20 scats as described above (for one scat there was not sufficient material to estimate dry weight). We also used the literature to source comparable estimates of prey dry weight ($prey_{dw} = 19.71 \pm 3.90\%$ (Kjørboe, 2013)).

We then converted between whale scat and whale prey using published information on micronutrients. Baleen whales do not uptake micronutrients such as manganese or iron from their prey, as they cannot excrete it and instead recycle it through internal biochemical pathways (Ratnarajah et al., 2014; Ratnarajah et al., 2017). These micronutrients are defecated at orders of magnitude higher concentration than that of seawater, increasing the productivity of the Southern Ocean (1000 to 30 million times higher concentration (Ratnarajah et al., 2014; Ratnarajah et al., 2017)). Here, we assumed that the ratio of micronutrients in whale scat and whale prey is indicative of the biomass of prey consumed since these

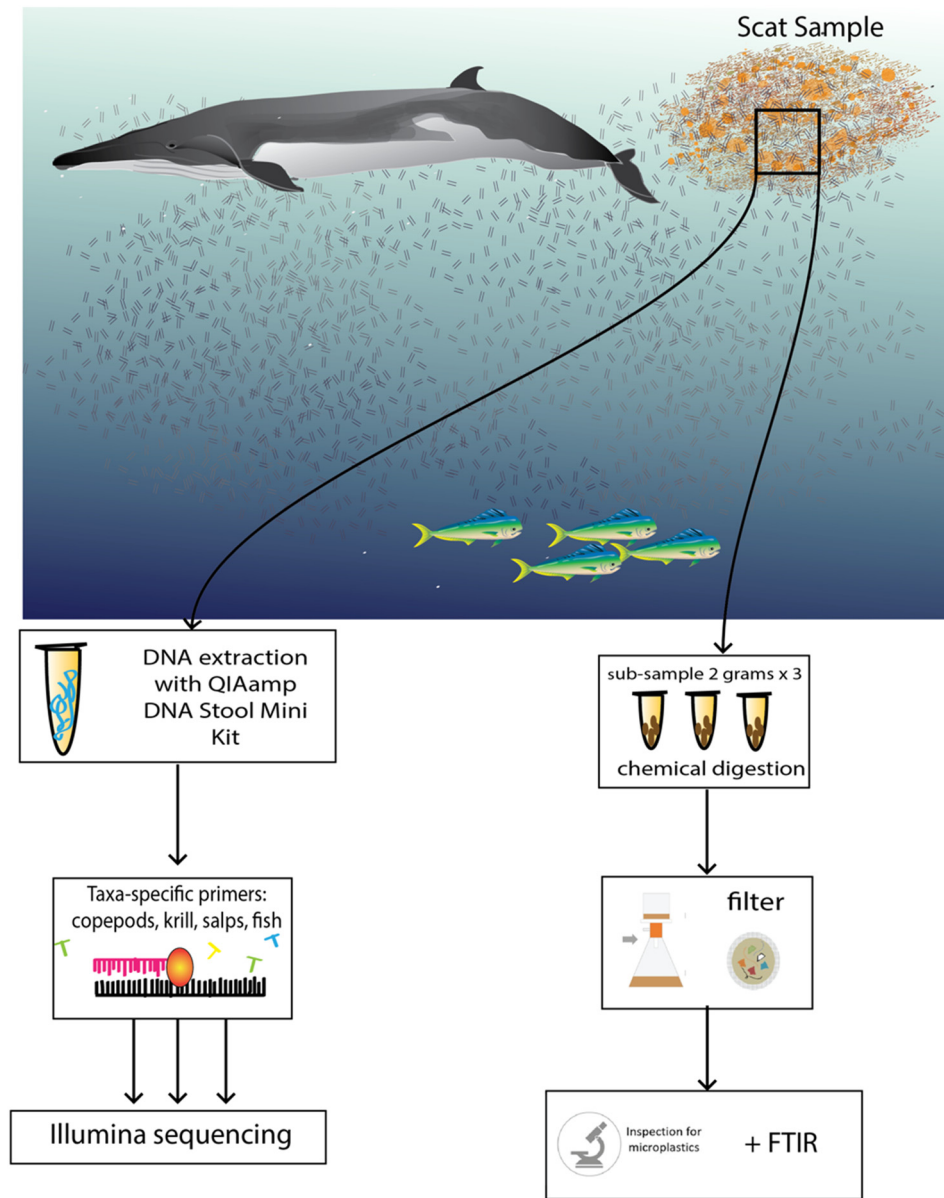


Fig. 2. Methods used in this study to concurrently extract microplastic particles and prey DNA from whale scat of Bryde's whale and sei whale.

micronutrients cannot come from the whale itself and are at very low levels in the environment. To do this, we used published values of the amount of trace elements in scat and whole prey to estimate the ratio of scat dry weight to prey dry weight ($prey_{conv}$, 4.44 ± 4.38 g of prey/g of scat (Ratnarajah et al., 2014)). The scat was converted to prey biomass by multiplying the dry weight of the scat by this ratio and then converting back to wet weight (using the term $100/prey_{dw}$): $ratio_{sp} = scat_{dw} * prey_{conv} * (100/prey_{dw})$.

The second step estimated the amount of prey (grams) in the 15 m^3 of water engulfed per mouthful by the typical 14 m Bryde's or sei whale (Kahane-Rapport and Goldbogen, 2018). To do this we used a uniform distribution of 150 g to 1000 g per m^3 ($mouth_{prey} = U[2250, 15,000]$) based on minimum values used in energetic studies (Goldbogen et al., 2011) and a maximum from zooplankton surveys in the Gulf (Zeldis and Willis, 2015). Third, we estimated the number of microplastics per whale mouthful ($mouth_{mp}$) using the mean number of microplastics per six gram scat found in this study ($scat_{mp} = 32 \pm 24$ (mean \pm SD)) and the $ratio_{sp}$. Essentially, we sampled the prey biomass (grams) from the $mouth_{prey}$ distribution and then divided this by a value from the $ratio_{sp}$ distribution to convert it to

equivalent amount of scat in grams. We then divided it by six to account for the fact that $scat_{mp}$ was based on six grams of scat and then multiplied it by this a value of sampled from the $scat_{mp}$ distribution: $mouth_{mp} = ((mouth_{prey}) / (ratio_{sp})/6) * scat_{mp}$.

Finally, extrapolation to daily exposure was conducted using published information on the foraging behaviour of Bryde's whales in the Hauraki Gulf to estimate the number of mouthfuls per day (Izadi et al., 2018). We simulated foraging sessions by sampling from the distribution of mouthfuls per session (5.5 ± 6.1 , minimum 2 mouthfuls, 30 s each) and the distribution of intervals between mouthfuls (107.16 ± 75.36 s). As Bryde's whales primarily forage during daylight hours, we assumed 12 h foraging per day, and continued to simulate foraging sessions, with foraging session intervals sampled from the published distribution (1262.8 ± 1179.8 s), until the 12 h time threshold had been reached. We did this 1000 times to estimate the mean and 95% confidence intervals of the number of mouthfuls per day (day_{mouth}). We used the estimated distributions of day_{mouth} and $mouth_{mp}$ to estimate total daily exposure to microplastics (mean and 95% confidence intervals). To explore the robustness of our results to the model parameters, we undertook sensitivity analyses for key model parameters by: (1) varying

Table 1

Overview of steps used in the stochastic simulation model used to estimate total daily exposure of filter feeding whales to microplastics in the Hauraki Gulf. Values are presented with their mathematical distribution, mean (μ) and standard deviations (SD), or range, where parameter is used as a model input; Simulated output with mean and 95% CI are shown when it is an output. Additional details, including details on truncated normal distributions to prevent negative value sampling, are provided in the Supplementary Material (Table S4 and S5) and in the simulation code <https://github.com/emmcarr/PlasticWhale>.

Parameter	Description	Method	Value	Citation
1. Estimate ratio of grams of scat to grams of prey (wet weight)				
$scat_{dw}$	Percentage dry weight for whale scat	Sub-sample of each whale scat was oven-dried for 48 h	Normal distribution, $\mu = 6$, $SD = 4$, unit = % dry weight	This study, Table S4
$prey_{conv}$	Ratio scat to prey dry weight	Published values of trace elements in whale scat and prey species	Normal distribution, $\mu = 4.44$, $SD = 4.38$, unit = grams of prey dry weight / grams of scat dry weight	Ratnarajah et al. (2014), summarised in Table S5
$prey_{dw}$	Percentage dry weight for prey species	Published dry weight percentage for euphausiids and copepods	Normal distribution, $\mu = 19.71$, $SD = 3.90$, unit = % dry weight	Kjørboe (2013)
$ratio_{sp}$	$scat_{dw}^* / (prey_{conv}^* (100 / prey_{dw}))$	Sampled 1000 times from distribution of each parameter in calculation	Simulated output, $\mu = 2.23$, $SD = 1.85$, unit = grams of prey wet weight / grams of scat wet weight	This study
2. Estimate of grams of prey per mouthful				
$mouth_{prey}$	Grams of prey per mouthful	Lower and upper bounds defined using published estimates of prey density and published estimate of 15m ³ volume for typical 14 m whale	Uniform distribution, range = [2250, 15,000], units = grams of prey	Goldbogen et al. (2011); Zeldis and Willis (2015); Kahane-Rapport and Goldbogen (2018)
3. Estimate number of microplastics per prey mouthful				
$scat_{mp}$	Microplastics per 6 g scat	Count of microplastics found in 3 × 2 g of whale scat	Normal distribution, $\mu = 32$, $SD = 24$, units = microplastics found in 6 g scat	This study
$mouth_{mp}$	$((mouth_{prey}) / (ratio_{sp} / 6)) * scat_{mp}$	Sampled 1000 times from distribution of each parameter in calculation to estimate mean and CI	Simulated output, 24,028 (95% CI: 2119, 69,270), units = microplastics per mouthful	This study
4. Estimate total daily exposure of filter feeding whales to microplastics				
day_{mouth}	Estimate number of mouthfuls per day	Simulated foraging sessions of Bryde's using published information on duration and number of lunges (mouthfuls) per feeding session, and interval between feeding sessions	Simulated output: 141 (95% CI: 115, 168), unit = mouthfuls per day	Izadi et al. (2018)
day_{mp}	Total daily microplastic exposure: $mouth_{mp} * day_{mouth}$	Sampled 1000 times from distribution of each parameter in calculation to estimate mean and CI	Simulated output: 3,408,002 (95% CI: 295,810, 10,031,370), unit = microplastics ingested per day	This study

$scat_{mp}$ by increasing or decreasing the mean number of microplastics per six grams of scat by 50% to represent variation in exposure; (2) varying $prey_{dw}$ to compare the impact of different types of prey; euphausiids (dry weight $22.8 \pm 1.4\%$ (Kjørboe, 2013)) or copepods (dry weight $16.2 \pm 2.4\%$ (Kjørboe, 2013)); (3) varying feeding duration to assess the impact of daylength variation observed in the Hauraki Gulf: approximately 10 h to 16 h.

3. Results and discussion

3.1. Putative microplastic abundance

Here we reveal the level of exposure of two large filter-feeders to microplastic pollution. All 21 scats examined contained synthetic particles. A total of 672 putative microplastics >150 μ m in length were found in Bryde's whale ($n = 18$) and sei whale samples ($n = 3$) after accounting for suspected sampling and laboratory-based contamination (see Dataset 1). Each scat sample was of different size, so we standardised the sample analysis by taking 3 × 2 g replicates (Fig. 3). When putative microplastic incidence was summed across replicates, we found an average of 32 ± 24 (mean \pm SD, range 5–118) putative microplastics per 6 g of scat. This represents approximately five putative microplastics per gram of whale scat.

The majority of the 672 putative microplastics were blue or black fibres (83%), followed by red (9%), clear (3%), green (2%), brown (2%) and purple (1%). More than 99% of the putative microplastics identified were

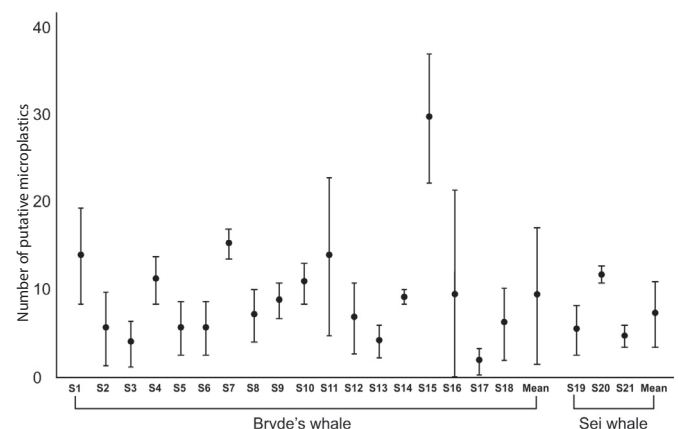


Fig. 3. Mean and standard deviation of the number of putative microplastics found in 2 g sub-samples ($n = 3$) of Bryde's and sei whale scat, with pooled mean and variance for each species also displayed.

fibres, with only four identified as fragments or films. A high proportion of blue fibres has been documented in studies on marine mammal exposure to microplastics (Zantis et al., 2020), which suggests these fibres may be more frequently found in the environment and/or ingested by small prey (Dris et al., 2018; Napper and Thompson, 2016). A subset ($n = 40$) of these putative microplastics were analysed with FTIR and were found to be anthropogenic in origin, e.g. brightly coloured cellulose and regenerated cellulose of uniform thickness with no visible cellular or organic structure (84%), and plastic (polyester; 4%; see Dataset 1). Examples are shown in Fig. 4. Anthropogenically altered cellulose and regenerated cellulose are most commonly found in textiles (e.g., jeans, fleece) and rayon (e.g., cigarette filters, tampons) (Reddy and Yang, 2015).

Although not measured in the current study, there are similarities between the microplastics found in whale scats and those found in Auckland beach sediments (Bridson et al., 2020) and surface waters (Shetty, 2021). Microfibres were the most commonly found microplastics (99% in scat; 88% in beach sediments (Bridson et al., 2020); 89% in surface waters (Shetty, 2021)), and regenerated cellulose was the most common material (84% in scat; 34% in beach sediments (Bridson et al., 2020); no comparative analysis of surface waters). Like our findings, Digka et al. (2018) found that the same shape and type of microplastics was present in surface seawater, sandy beaches and fish and mussels of the Mediterranean. This study highlights that the microplastics found in organisms, sediments and waters are similar, which potentially shows the transport of microplastics from terrestrial to marine environments, and thus the uptake by marine organisms.

3.2. DNA diet analysis

The DNA metabarcoding analysis showed that the primary components of the diet were copepods and euphausiids (Tables S2 and S3; raw data available at the NCBI Shortread Archive, Bioproject ID: PRJNA699299). This confirms a previous DNA diet study of Bryde's whales in the Gulf that found prey preferences of these whales were constant year-round, with their diet comprising euphausiids, copepods, and salps, despite seasonal changes in zooplankton communities (Carroll et al., 2019). Furthermore, our analysis did not detect schooling fish, which is consistent with previous DNA diet analyses and observational studies suggesting that fish has decreased as a dietary component in Bryde's whales in the Gulf over the last 20 years (Carroll et al., 2019; Gostischa et al., 2021; Jarman et al., 2006b).

Given the challenges in collection of scat samples from elusive, and in this case, endangered marine mammals, we had an opportunistic, citizen science approach to sampling conducted in collaboration with a commercial whale watch operator. The sample size of 21 scats collected over five years represents one of the largest datasets of its type known to the authors, and shows how successful such collaborations can be. In fact, this is likely the first study using baleen whale scat to concurrently investigate microplastic exposure and diet, and previous diet studies have had sample

sizes ranging from one (Jarman et al., 2002) to 34 (de Vos et al., 2018). Our approach can also mean that there is a risk of contamination as it is difficult to use optimal procedures like concentrated bleach to clean equipment in between sampling. However, this is low risk as this study did not sample water, as is done in an eDNA or microbiome study that are prone to contamination, but rather sampled bulky scat material. Furthermore, we employed strict protocols during DNA extraction to minimise cross-contamination or contamination from other potential DNA sources (See Supplementary Materials), and previous investigation of contamination using methods employed here did not reveal any issues (Carroll et al., 2019).

3.3. Microplastic exposure

Using our stochastic simulation model, we estimated total microplastic exposure risk (environmental and trophic) directly from scat samples. We estimate an uptake of 24,028 (95% CI: 2119, 69,270) microplastics ($>150 \mu\text{m}$) per mouthful when feeding (Table 1). Using empirical information on the number of mouthfuls per foraging session (Izadi et al., 2018), and the inter-session intervals, we estimated that whales take 141 (95% CI: 115, 168) mouthfuls per day (Table 1). Using an approach designed to account for uncertainty in these values, we estimate that the mean total daily exposure for a large filter-feeding whale in the Gulf is 3,408,002 (95% CI: 295,810, 10,031,370) microplastics (Table 1).

Although this estimate has considerable uncertainty, the point estimate and lower 95% confidence estimate are substantially higher than several previous estimates. For example, two studies estimated microplastic uptake by Mediterranean fin whales based on microplastic levels in the water column, and estimated total exposure to be 3653 particles per day (Fossi et al., 2014) and “thousands of particles” per day (Fossi et al., 2016). Fossi et al. (2014) also estimated the microplastic uptake of basking sharks to be 13,110 particles per day. Based on the environmental abundance of plastics (up to 200 mm in length) and estimated water filtration rates, theoretical plastic exposure rates were calculated for manta rays and whale sharks in Indonesia with estimates of up to 62.7 pieces per hour for manta rays and 137 pieces per hour for whale sharks (Germanov et al., 2019). Furthermore, Fossi et al. (2017) estimated that whale sharks are ingesting 171 microplastic particles every day near a highly populated city La Paz Bay (Mexico), which is lower in comparison to our results. We hypothesise that our estimate is higher than these because we aimed to infer total daily exposure through both trophic and environmental routes, whereas these estimates derived from microplastic levels in the water column are primarily focusing on the environmental component of exposure. In contrast, Desforges et al. (2015) took a different approach and used levels of microplastics in two zooplankton species to extrapolate total exposure of humpback whales, which was estimated at 300,000 microplastics per day. This trophic transfer-based approach was more similar to our estimates.

Our sensitivity analyses also highlight what additional processes could contribute to differences between studies and factors that would be

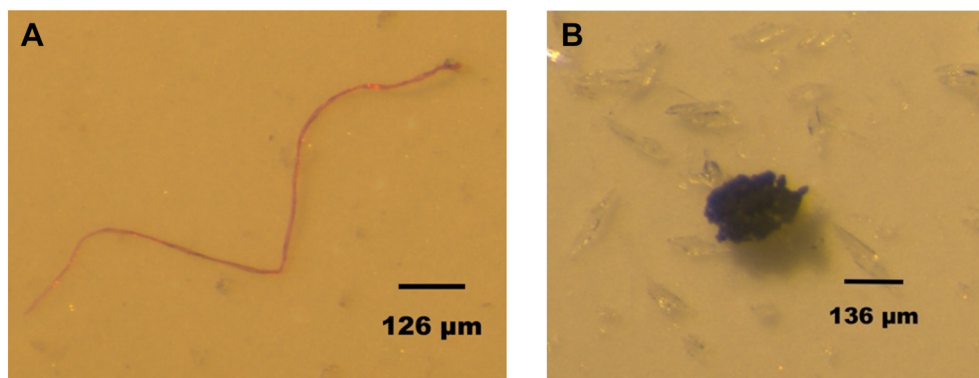


Fig. 4. Examples of two types of microplastics found in Bryde's whale scats and identified by FTIR. A) Red fibre corresponding to cotton, B) blue fragment identified as polyethylene.

Table 2

Estimates of exposure of filter feeding baleen whales to microplastics in the Hauraki Gulf. Presented are the base case scenario, as well as sensitivity analyses which decrease (1A: low microplastics) or increase (1B: high microplastics) amount of microplastics in scat by 50%; (2) use prey dry weight values specific for euphausiids (2A: euphausiid) or copepods (2B: copepods); and vary feeding duration with the seasonal day-lengths found at the study site to (3A: short day) 10 h or (3B: long day) 16 h. Means, and 95% CIs in brackets, are reported.

	Base case	1A: low microplastics	1B: high microplastics	2A: euphausiids	2B: copepods	3A short day	3B. long day
Microplastics per mouthful, $mouth_{mp}$	24,028 (2119, 69,270)	17,165 (1187, 52,882)	34,112 (3916, 97,167)	26,350 (2638, 73,466)	21,177 (2167, 64,660)	25,246 (2290, 75,244)	25,851 (2771, 74,260)
Total daily exposure, day_{mp}	3,408,002 (295,810, 10,031,370)	2,450,935 (170,519, 7,528,059)	4,902,934 (555,863, 14,093,714)	3,738,619 (350,976, 10,478,960)	2,995,386 (315,371, 9,194,921)	2,991,609 (260,620, 8,860,490)	4,858,748 (529,761, 13,687,078)

important for future studies (Table 2). We found, unsurprisingly, that a proxy for exposure, variation in the number of microplastics per gram of scat (50% increase/decrease), had a strong impact on our findings; as it was a multiplicative parameter in the model it has a corresponding impact on total daily exposure. It is likely that contamination would have a lower impact ($\pm 10\%$, Supplementary Material 1), but both are important factors to consider in future research and will contribute to differences between studies. Another key process to consider is the foraging strategy of the study animal. Bryde's whales forage almost continuously during daylight hours, which vary seasonally across the year in Auckland (Izadi et al., 2018). The number of hours impacts the number of mouthfuls taken by the whales (16 h: 188, 95% CI: 159, 218; 10 h: 118, 95% CI: 95, 144), which then will impact total daily exposure. In contrast, our results were fairly robust to variation in prey dry weight (Table 2), with $\sim 10\%$ change in the statistics of interest when using values specific to different prey types. However, we were not able to simulate differences in microplastic uptake between prey types (Burkhardt-Holm and N'Guyen, 2019; Botterell et al., 2019), which could be an important factor to consider in future studies.

3.4. Microplastic size relative to prey size and potential route of transfer

Our results are consistent with trophic transfer as a likely pathway of ingestion as the mean length of the microplastics was $1085 \pm 1395 \mu\text{m}$

(mean \pm SD, range 152–26,290 μm), which is smaller than the size of the whales' preferred prey species of copepods, krill and salps (Carroll et al., 2019). DNA metabarcoding of the scat samples identified *Nyctiphanes australis* as the primary euphausiid prey for the Bryde's whales and *Paracalanus* spp. and *Ditrichocorycaeus anglicus* as the primary copepod prey for the sei whale, with sizes shown graphically in Fig. 5 (for more details see Tables S2 and S3). Euphausiids and other zooplankton species have been found to ingest microplastics up to 2000 μm in size (Desforges et al., 2015). The majority of microplastics found in our study are within this size range, which could potentially suggest that these species are enabling trophic transfer.

However, a current estimation of environmental exposure in the surface waters of the Gulf, where the whales concentrate their foraging effort (Izadi et al., 2018; Izadi, 2018), was measured to be on average 0.23 ± 0.03 pieces of microplastics (Shetty, 2021) per m^3 , levels which are comparable to other studies (Lusher, 2015). Assuming 15 m^3 intake of water and prey per Bryde's whale mouthful, only 3.45 of the estimated 24,028 microplastics engulfed would be from the environment; a difference of four orders of magnitude. Due to the size range of microplastics in the whale's scat being compatible with trophic transfer, and the low estimated amount of microplastics from the surface waters, we hypothesise that the majority of microplastic exposure of this baleen whale species in this Gulf is from trophic transfer rather than environmental exposure. Future studies could ground-truth this hypothesis by simultaneously sampling microplastics in seawater, prey and filter-feeder scat.

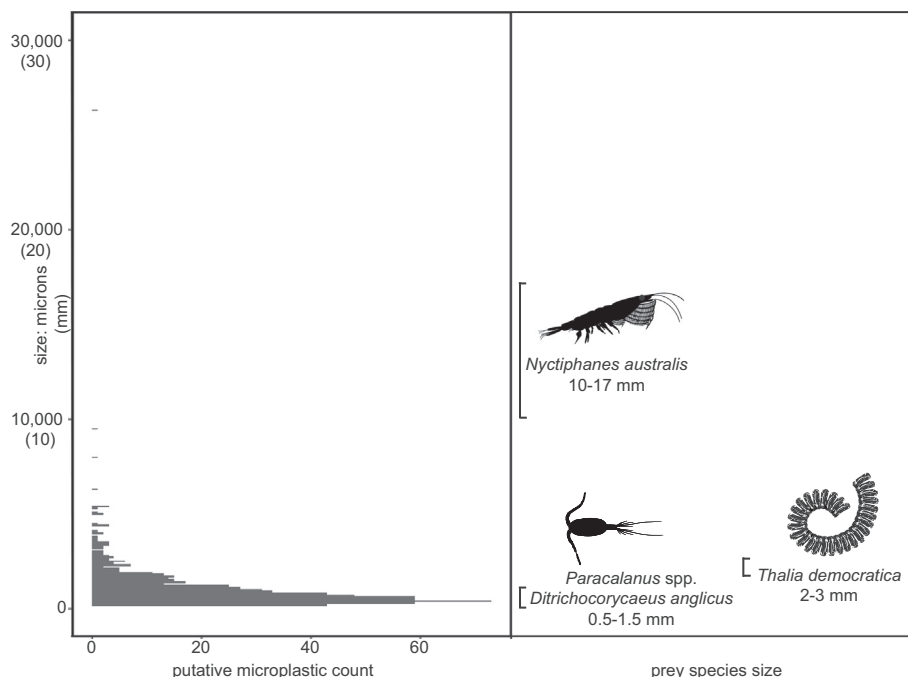


Fig. 5. Size distribution of putative microplastics found in whale scat, compared with size of prey species identified using DNA metabarcoding (size shown in μm and mm in parentheses; 1000 μm = 1 mm). Note that the size range of plastics identified in the scat was up to 28 mm.

Additionally, research is urgently needed on the toxicological effects of microplastics, and also the potential long-term consequences associated with chronic exposure to microplastics. These issues remain unknown for all species and ecosystems. Large marine animals are facing multiple anthropogenic stressors such as direct hunting, fisheries interactions, climate change and other unsustainable forms of habitat degradation (e.g. Pacoureau et al., 2021). It is important that we understand the effects of the high levels of microplastic ingestion when developing risk assessments as this potential threat will only continue to increase.

CRedit authorship contribution statement

All authors conceived and designed the study, LJZ and FL did the lab work, LJZ, FLC, ROR and ELC did the analysis, LJZ, ELC, TB, SN, RC and ROR led the manuscript and all authors contributed to development of ideas and editing the manuscript.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2021.151815>.

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