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Review

Marine mammals and microplastics: A systematic review and call for standardisation☆

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ABSTRACT

Microplastics receive significant societal and scientific attention due to increasing concerns about their impact on the environment and human health. Marine mammals are considered indicators for marine ecosystem health and many species are of conservation concern due to a multitude of anthropogenic stressors. Marine mammals may be vulnerable to microplastic exposure from the environment, via direct ingestion from sea water, and indirect uptake from their prey. Here we present the first systematic review of literature on microplastics and marine mammals, composing of 30 studies in total. The majority of studies examined the gastrointestinal tracts of beached, bycaught or hunted cetaceans and pinnipeds, and found that microplastics were present in all but one study, and the abundance varied between 0 and 88 particles per animal. Additionally, microplastics in pinniped scats (faeces) were detected in eight out of ten studies, with incidences ranging from 0% of animals to 100%. Our review highlights considerable methodological and reporting deficiencies and differences among papers, making comparisons and extrapolation across studies difficult. We suggest best practices to avoid these issues in future studies. In addition to empirical studies that quantified microplastics in animals and scat, ten studies out of 30 (all focussing on cetaceans) tried to estimate the risk of exposure using two main approaches; i) overlaying microplastic in the environment (water or prey) with cetacean habitat or ii) proposing biological or chemical biomarkers of exposure. We discuss advice and best practices on research into the exposure and impact of microplastics in marine mammals. This work on marine ecosystem health indicator species will provide valuable and comparable information in the future.

1. Introduction

Marine mammals play key roles in influencing the structure and function of the marine environment and are sentinels for ecosystem health (Burek et al., 2008; Moore, 2008). However, due to an increase in anthropogenic activities, including fishing (Barcenas-De la Cruz et al., 2018; Ocampo Reinaldo et al., 2016), shipping (Halliday et al., 2017; Riley and Hollich, 2018), pollution (Brown et al., 2018; Frouin et al., 2012) and climate change (Albouy et al., 2020; Sanderson and Alexander, 2020), many marine mammals species are of conservation concern (Nelms et al. In prep; Davidson et al., 2012; Pompa et al., 2011).

Plastic pollution is known to affect marine mammals, through entanglement (Kraus, 2018), ingestion (Alexiadou et al., 2019; De Stephanis et al., 2013; Unger et al., 2016) and potential habitat degradation (Gall and Thompson, 2015; Pawar et al., 2016). One area of specific concern is the exposure of marine mammals to microplastics. These small (<5 mm), pervasive and persistent synthetic particles (Moore, 2008) are bioavailable to marine organisms, through direct ingestion and/or via trophic transfer (Cole et al., 2011; Eriksson and Burton, 2003; Nelms et al., 2019a). Mysticetes (baleen-whales), for example, are megafilter feeders that engulf large volumes of water alongside their prey, and are

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potentially exposed to microplastics via both pathways; direct uptake of microplastics from the environment (environmental exposure, e.g. Germanov et al., 2018; Guerrini et al., 2019), and indirect ingestion, from consuming contaminated prey (trophic transfer exposure, e.g. Burkhardt-Holm & N'Guyen, 2019; Desforges et al., 2015). In comparison, odontocetes (toothed-whales) and pinnipeds (seals, sea lions and walruses) are most likely to be exposed through trophic transfer (Au et al., 2017; Ivar Do Sul and Costa, 2014; Nelms et al., 2018; Perez-Venegas et al., 2018). Studies on other taxa indicate that microplastics may present a number of potential impacts, acting as a vector for pathogens or chemical contaminants (Prinz and Korez, 2020).

Though the impact of microplastics on marine mammals is relatively understudied compared to other taxa, research on the uptake and exposure of marine mammals to microplastics has increased in recent years. Studies have investigated microplastic abundance and exposure risk in marine mammals using gut content analysis (e.g. Lusher et al., 2015; Nelms et al., 2019b), faecal analysis (e.g. Hudak and Sette, 2019; Nelms et al., 2018; Ryan et al., 2016) as well as indirectly by measuring levels of chemical biomarkers, such as phthalates (e.g. Baini et al., 2017; Fossi et al., 2014). Importantly, a wide range of microplastic identification and contamination prevention methods are used within these studies, highlighting the need for standardized protocols for robust and comparable microplastic analysis (Panti et al., 2019; Stock et al., 2019).

Reviews on plastic ingestion and entanglement by marine mammals (e.g. Baulch and Perry, 2014; Simmonds, 2012) have highlighted the abundance of interactions of marine mammals with plastic debris. Given the growing interest in this field, the objective of this study was to conduct the first systematic literature review on microplastics and marine mammals. We sought to synthesize and summarize the existing literature on the topic, highlight knowledge gaps and recommend avenues for future research, and suggest best practices to move the field forward.

2. Materials and methods

2.1. Literature search parameters

The design of this systematic literature review follows the guidelines of Siddaway et al. (2019). The main search for literature was conducted in September 2019, and an update was made in May 29, 2020. Searches for relevant peer-reviewed literature were made using two online publication databases; Web of Science and PubMed. The selection process of articles is summarized according to the PRISMA approach (Moher et al., 2009; Fig. S1). The bibliographies of peer-reviewed publications were also explored, and potentially relevant studies not found in online databases were recorded.

The following search terms were utilised during a first scoping exercise and resulted in a selection of relevant articles:

- *Subject:* Microplastic*, "Plastic particle*", "Marine Debris*"
- *Target:* Whale*, Cetacean*, Dolphin*, Delphinid*, Mysticete*, Odontocete*, Porpoise*, Phocid*, Otariid*, Pinniped*, Seal*, "Sea lion*", Manatee*, "Polar bear*".

The terms within each category ("subject" and "target") were combined using the Boolean operator "OR". The two categories were then combined using the Boolean operator "AND". An Asterix (*) is a wildcard that represents any group of characters, including no characters. The full search string thus reads as follows:

> (Microplastic* OR "Plastic particle*" OR "Marine Debris*") AND (Whale* OR Cetacean* OR Dolphin* OR Delphinid* OR Pinniped* OR Seal* OR Manatee* OR "Polar bear*" OR Mysticete* OR Odontocete* OR Porpoise* OR Phocid* OR Otariid* OR "Sea lion*")

2.2. Screening process

Articles found during the searches were assessed for inclusion using a two-step screening process:

Step 1: Study inclusion criteria

The title and abstract of each publication were evaluated for relevance using a number of inclusion criteria;

- *Subject:* Discusses link between microplastic pollution and marine mammals, including pinnipeds, cetaceans, manatees or polar bears.
- *Results:* Presents information on the interaction between marine mammals and microplastic. For a detailed list of variables, we searched for and minimum requirements see Table S1.
- *Type of study:* Empirical study published in a peer−reviewed journal

Step 2: Data extraction and presentation

Potentially relevant papers were read in full, and information and data which were relevant for this review were extracted from the eligible papers. When available, information on study type, target species, study location, method, abundance of microplastics, polymer identification protocol, polymer characteristics and contamination identification protocol were collected (See Table S1 for extracted information).

In the results we summarize and discuss the results focussing on digestive tracts (section 3.1) and scat samples (section 3.2). Next, we summarize and discuss methodological differences (section 3.3) followed by suggestion on best practices (section 3.4). In section 3.5, we will discuss inferential studies in which biomarkers or levels of microplastics in prey are linked to risk of exposure.

3. Results and discussion

Searches with the main search terms in two databases returned a total of 297 articles. Three additional articles were found through other sources. After removing duplicates, 219 articles were left. Title and abstract screening further excluded 156 articles. A remaining 63 publications were then screened based on their full text, resulting in 30 articles, which were finally included in this review (Table S2).

Most of the scat and gut studies on microplastics and marine mammals were conducted in Europe (47%; $n = 10$) − mostly in the United Kingdom and in Italy, followed by North America (19%; $n = 4$), Sub Antarctic and Antarctica (14%; $n = 3$ pinniped studies), Latin America (10%; $n = 2$) and Asia (10%; $n = 2$; Fig. 1).

The majority of papers on gut content analyses focussed on cetaceans, particularly odontocetes (Fig. S2). In contrast, all studies on microplastics in faeces used scat from pinnipeds, mostly otariids (eared seals). No studies on sirenians and polar bears were identified (Fig. S2).

3.1. Microplastics in digestive tracts

In total, 12 publications were identified that examined digestive tracts for microplastics using samples from beached ($n = 8$

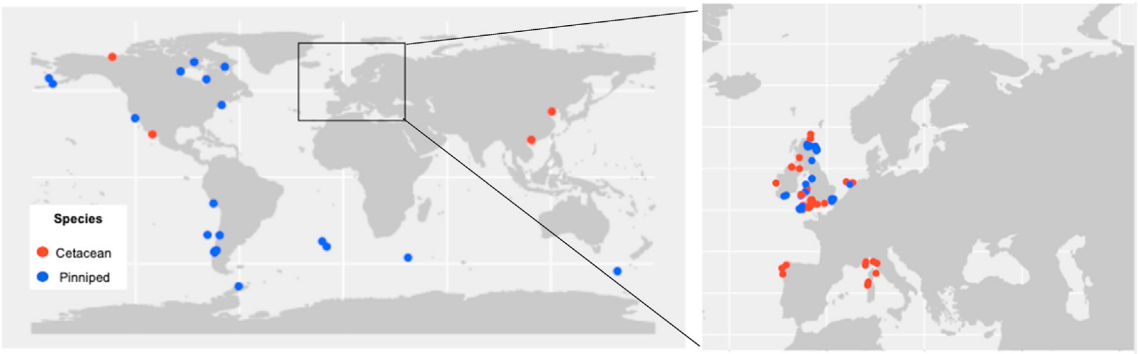

Fig. 1. The global distribution and focus of studies on microplastics and marine mammals. Note: modelling studies were not included.

publications), by-caught ($n = 2$) or hunted ($n = 2$) marine mammals (Table 1; Fig. S2).

All of the studies found suspected microplastics in at least one animal examined (Table 1), with the exception of Bourdages et al. (2020), who reported none in the stomach contents of 142 hunted arctic seals (ringed seals; *Phoca hispida*; $n = 135$, bearded seals; *Erignathus barbatus*; $n = 6$, and one harbour seal; *Phoca vitualina*; $n = 1$). Drawing direct comparisons among studies is challenging due to differences in the amount of digestive tract content analysed, and the lack of information provided about the analysed amount. For example, some studies examined all content from the whole digestive tract and reported the number of suspected microplastics per animal (Lusher et al., 2015, 2018; Nelms et al., 2019b). This ranged from three in a white-beaked dolphin (Nelms et al., 2019b) to 88 in a True's beaked whale (*Mesoplodon mirus*) (Lusher et al., 2015, Table 1). This information on microplastic abundance per animal, coupled with information on animal size, age-class, sex and species, allows for further investigation into potential drivers any observed trends in microplastic load.

Where sub-samples were taken from the digestive tract, some studies report the number of microplastics per animal without reporting the volume of content examined, making it impossible to calculate total microplastic load. Another approach involved extrapolating the number of microplastics found within sub-samples, to estimate the microplastic abundance range for the whole animal. For example, Moore et al. (2020) found 81 microplastics in digestive tract sub-samples of seven Beluga whales (*Delphinapterus leucas*) and estimated that each whale contained 18 to 147 microplastics (average of 97 ± 42 per individual) by estimating the intestinal length and calculating the potential microplastic abundance throughout. Though this approach is useful where no other means of garnering such information exist, it should be used with caution.

Fibres were the predominant particle shape for the majority of studies (Table S2). However, Moore et al. (2020) found that approximately half of microplastics in Beluga whales were fragments and half were fibres (51% and 49%, respectively; Table S2). In addition, three studies also reported foam, sheet and bead-shaped particles (Besseling et al., 2015; Hernandez-Gonzalez et al., 2018; Xiong et al., 2018). Due to concerns regarding air-borne contamination, some studies did not seek to extract microfibres or excluded them, or particles below a certain size limit, from their results (Besseling et al., 2015; Bourdages et al., 2020; Hernandez-Milian et al., 2019; van Franeker et al., 2018). Only five studies presented information on the colour of particles detected, of which blue and black were the most common (Table S2).

Of the 11 studies that report the presence of suspected microplastics in digestive tracts, seven presented information on polymer type for all, or a sub-sample of, particles using analytical polymer characterisation techniques, such as Fourier-transform spectroscopy (FTIR) or Near Infrared Spectroscopy (NIR; Table S3). The proportion of suspected microplastics analysed for polymer type varied from 19%–100% among studies and of those particles analysed, the proportion that were confirmed as synthetic ranged from 16% to 77% per study. The remaining particles were either natural, semi-synthetic or too degraded/dirty to obtain reliable spectra matches. Of the confirmed microplastics, sixteen main polymer types were reported, but the composition varied considerably among studies (Table S2). This variation is likely due to the heterogeneity of plastic pollution sources as well as lack of uniformity in polymer analysis techniques and equipment (e.g. polymer libraries, interpretation of spectral matches, confidence criteria). For example, four of the studies accepted FTIR spectra matches with confidence levels of between 70% and 80% but the remaining three studies do not specify their accepted confidence thresholds.

3.2. Microplastics in scat samples

In total, nine peer-reviewed papers have analysed marine mammal scats for the presence of microplastics (Table 2; Fig. S2). All of these examined scats originate from pinnipeds, likely because of i) ease of collection compared with cetaceans due to use of terrestrial habitats (e.g. haul out sites) and ii) access to long-term datasets where scat was collected for other purposes (e.g. diet analyses).

In the six studies for which microplastics in scat were reported, the occurrence varied from 1% in scats collected in 2016/2017 from grey seals (*Halichoerus grypus atlantica*) on the Atlantic coast of the USA ($n = 129$, Hudak and Sette, 2019) to 100% in scats collected in 1996/1997 from Sub Antarctic and Antarctic fur seals (*Arctocephalus tropicalis*; *A. gazella*) on Marion Islands ($n = 100$, Eriksson and Burton, 2003, Table 2). The reporting of microplastic load varied, as some studies reported it as a mean or incidence for all scats analysed (*all*), while some reported statistics only for those scats in which microplastics were detected (*positives*). This also could have contributed to increased variance, ranging from a mean of 0.87 ± 1.09 in 31 grey seal scats collected from captive animals (Nelms et al., 2018: all scats) to a mean of 37.3 ± 38.1 per positive scat in the 34 scats found to have microplastics in Perez-Venegas et al. (2018) (Table 2). The numbers reported by Perez-Venegas et al. (2018) need to be interpreted with caution, as several mistakes were found in their supplementary information. The authors have been notified, and will produce a correction, but this was not available at the time of printing of this article.

The route of exposure was also examined, with the study by Nelms et al. (2018) being a key paper as this is the only controlled

Table 1
Summary of results of studies investigating microplastic (MPs) in the gastrointestinal track of bycaught, hunted or beached marine mammals. N/R means not recorded within the study.

Species	Sample origin	Sample size	Number of particles (confirmed or suspected microplastics)				Size of particles		Source
			Total MPs [a]	% samples with MPs	"All" mean MPs per animal	Range MPs per animal	Mean size (±SD) (mm)	Size range (mm)	
Mysticete									
Humpback whale	Part of GIT	1	16	100%	16	16	N/R	1.1−4.7 x 0.4− 2.4	Besseling et al. 2015
Odontocete									
Atlantic white-sided dolphin	GIT	1	8	100%	5.5 ± 2.7[b]	3-12[b]	Fib: 2.0±2.3 Frag: 0.9±1.1[b]	Fib: 0.1- 20, Frag: 0.1-4[b]	Nelms et al. 2019b
Beluga whale	GIT	7	81	100%	11.6 ± 6.6	3-24	<1mm (87%), 1-2mm (20%)	N/R	Moore et al. 2020
Bottlenose dolphin	GIT	1	6	100%	5.5 ± 2.7[b]	3-12[b]	Fib: 2.0±2.3 Frag: 0.9±1.1[b]	Fib: 0.1- 20, Frag: 0.1-4[b]	Nelms et al. 2019b
	GIT	2	39	100%	25.5[b]	1-88[b]	N/R	0.3 - 16.7[b]	Lusher et al. 2018
Common dolphin	GIT	16	91	100%	5.5 ± 2.7[b]	3-12[b]	Fib: 2.0±2.3 Frag: 0.9±1.1[b]	Fib: 0.1- 20, Frag: 0.1-4[b]	Nelms et al. 2019b
	Stomach	35	411	94%	12 ± 8	3-41	Fib: 2.11±1.26, Frag: 1.29±0.93	Fib: 0.29-4.92 Frag: 0.49-4.07 Bead: 0.95	Hernandez Gonzalez et al. 2018
	GIT	9	187	100%	25.5[b]	1-88[b]	N/R	0.3 - 16.7[b]	Lusher et al. 2018
Cuvier's beaked whale	GIT	1	53	100%	25.5[b]	1-88[b]	N/R	0.3 - 16.7[b]	Lusher et al. 2018
	Intestine	7	134	100%	19.1 ± 7.2	10-32	N/R	N/R	Xiong et al. 2018
Finless porpoise	GIT	21	110	100%	5.5 ± 2.7[b]	3-12[b]	Fib: 2.0±2.3 Frag: 0.9±1.1[b]	Fib: 0.1- 20, Frag: 0.1-4[b]	Nelms et al. 2019b
Harbour porpoise	Stomach	654	71	7%	0.11 ± 0.02	1-5	0.009 ± 0.004	0.2-2.6g	van Franeker et al., 2018
	GIT	5	103	100%	25.5[b]	1-88[b]	N/R	0.3 - 16.7[b]	Lusher et al. 2018
Indo-Pacific humpbacked dolphin	Intestine	3	77	100%	0.2-0.6 items/g	2-45	2.2± 0.4	0.1-4.8	Zhu et al. 2019
	GIT	1	39	100%	25.5[b]	1-88[b]	N/R	0.3 - 16.7[b]	Lusher et al. 2018
Killer whale	GIT	1	4	N/R	5.5 ± 2.7[b]	3-12[b]	Fib: 2.0±2.3 Frag: 0.9±1.1[b]	Fib: 0.1- 20, Frag: 0.1-4[b]	Nelms et al. 2019b
Pygmy sperm whale	GIT	1	9	N/R	5.5 ± 2.7[b]	3-12[b]	Fib: 2.0±2.3 Frag: 0.9±1.1[b]	Fib: 0.1- 20, Frag: 0.1-4[b]	Nelms et al. 2019b
Risso's dolphin	GIT	1	7	N/R	5.5 ± 2.7[b]	3-12[b]	Fib: 2.0±2.3 Frag: 0.9±1.1[b]	Fib: 0.1- 20, Frag: 0.1-4[b]	Nelms et al. 2019b
Striped dolphin	GIT	1	88	100%	N/R	88	2.2±1.4	0.3 − 7	Lusher et al. 2015, Lusher et al. 2018
True's beaked whale	GIT	1	3	100%	5.5 ± 2.7[b]	3-12[b]	Fib: 2.0±2.3 Frag: 0.9±1.1[b]	Fib: 0.1- 20, Frag: 0.1-4[b]	Nelms et al. 2019b
White-beaked dolphin									
Phocidae									
Bearded seals	Stomach	6	0	0	0	0	0	0	Bourdages et al. 2020
Grey seal	Intestine	13	363	100%	27.9 ± 14.7	13-71	N/R	N/R	Hernandez-Milian et al. 2019
	GIT	3	18	100%	5.5 ± 2.7[b]	3-12[b]	Fib: 2.0±2.3 Frag: 0.9±1.1[b]	Fib: 0.1- 20, Frag: 0.1-4[b]	Nelms et al. 2019b
Harbour seal	Stomach	1	0	0	0	0	0	0	Bourdages et al. 2020
	GIT	4	17	100%	5.5 ± 2.7[b]	3-12[b]	Fib: 2.0±2.3 Frag: 0.9±1.1[b]	Fib: 0.1- 20, Frag: 0.1-4[b]	Nelms et al. 2019b
	Stomach and Intestine	Stom: 107, Int: 100	Stom: 28, Int: 7	Stom: 11.2% Int: 1%	Stom: 0.26 Int: 0.07	0-8	N/R	N/R	Bravo Rebolledo et al. 2013
Ringed seals	Stomach	135	0	0	0	0	0	0	Bourdages et al. 2020

[a] # all suspected microplatics: some studies did not confirm whether observed particles were actual plastic polymers, or analyzed a subset
[b] average within study including multiple species

study on microplastic and marine mammals to date. In this study, the microplastic load of both prey and scat was directly measured, and a similar incidence, type and colour of microplastic was found in the fish used to feed captive grey seals and their scat. These results support the hypothesis of trophic transfer. In field experiments, the authors typically either did not specifically hypothesise about the route of exposure (Donohue et al., 2019; Hudak and Sette, 2019; Perez-Venegas et al., 2018) or suggested trophic transfer

Table 2
Summary of results of studies investigating microplastics (MPs) in scat of pinnipeds. N/R means not recorded within the study.

Species	Sample size	Number of particles				Size of particles		Author
		Total MP[a]	% samples with MPs	"All" mean MPs per scat±SD	Range MPs per scat	Mean size (mm)	Size range (mm)	
Otariidae								
Antarctic fur seal	145	164[c]	100%	1.13 ± 0.43[c]	1–4[c]	4.1 × 1.9[c]	89%: 2–5[c]	Eriksson and Burton (2003)
	42	0	0	0	0	0	0	Garcia Garin et al. (2020)
Juan Fernández fur seal	40	Unknown[b]	Fib: 62.5%; Frag: 12%	Fib: 30; Frag: 2	Fib: 0–200; Frag: 0-30	N/R	N/R	Perez-Venegas et al., (2020)[¥]
Northern fur seals	44	584	Frag: 55%; Fib: 41%	Frag: 16.6 ± 19.1, Fib: 3.8 ± 3.4	Frag: 1–86; Fib: 1-18	N/R	Frag: 82%: <1, Fib: 70%: <2, 28%: 2-10	Donohue et al. (2019)
Sub Antarctic fur seals	4905	0	0	0	0	0	0	Ryan et al. (2016)
	145	164[c]	100%	1.13 ± 0.43[c]	1–4[c]	4.1 × 1.9[c]	89%: 2–5[c]	Eriksson and Burton (2003)
South American fur seal	79	Unknown[b]	Fib: 65%; Frag: 6%	Fib: 16.5; Frag: 1	Fib: 0–182; Frag: 0-32	N/R	N/R	Perez-Venegas et al., (2020)
	51	1268[c]	67%	37.26 ± 38.08	3–182	N/R	Fibres: 67% > 0.1	Perez-Venegas et al. (2018)
South American sea lion	36	Unknown[b]	Fib: 86%; Frag: 11%	Fib: 43; Frag: 1	Fib: 0–267; Frag: 0-18	N/R	N/R	Perez-Venegas et al., (2020)
Phocidae								
Grey seals	129	2	1%	0.02 ± 0.12	0–1	N/R	1.9 × 0.8-2.6 × 1.1	Hudak and Sette (2019)
	31	Prey: 18, seal scat: 26	48%	0.87 ± 1.09	0–4	1.5 ± 1.2	Scat: Frag: 0.4–5.5, Fib: 0.6–3.5.	Nelms et al. (2018)
Harbor seal	32	2	6%	0.06 ± 0.25	0–1	N/R	1.19 × 0.58 - 3.45 × 1.81	Hudak and Sette (2019)
	125	0	0	0	0	0	0	Bravo Rebolledo et al. (2013)

[a] All suspected microplastics: some studies did not confirm whether observed particles were actual plastic polymers, or analysed a subset.
[b] Authors classify all particles found as MPs but state they only tested the contents of 6 scats for each seal population (number of particles unknown). Of the particles tested 30% were confirmed as polymers (PET and Nylon).
[c] Average within study including multiple species

rather than environmental exposure (Eriksson and Burton, 2003; Perez-Venegas et al., 2020).

The majority of studies reported fragments as the most dominant particle shape (Table S4). However, two studies only found fibres in scat samples (Table S4; Perez-Venegas et al., 2018, 2020). Most studies presented information on the colour of particles detected, of which white, blue and black were the most common (Table S4) (Donohue et al., 2019; Eriksson and Burton, 2003; Nelms et al., 2018; Perez-Venegas et al., 2018, 2020). However, Hudak & Sette (2019) mostly observed red and purple fragments in their study on grey seals. Of the six studies that report the presence of suspected microplastics, five presented information on polymer type for all, or a sub-sample of, particles using analytical polymer characterisation techniques, such as Fourier-transform spectroscopy (FTIR; Table S3). Of the confirmed microplastics, five main polymer types were reported (polyethylene, nylon/polyamide, polypropylene, phenoxy resin and rubber; Table S4). One study also identified semi-synthetic particles, such as cellophane (Hudak and Sette, 2019).

3.3. Differences in methodological approaches

There are three key steps in the determination of microplastic in scat and digestive tracts: 1) collection, 2) extraction and 3) identification. In addition, the prevention of contamination is a key part of determining microplastics levels. However, there are considerable methodological differences across studies, preventing comparisons among studies.

3.3.1. Collection of samples

The amount and origin of the *gut content* differed significantly among studies (Table S3). For example, some studies inspected whole, or sub-samples of, single digestive tract sections (e.g. stomach or intestines only; Bourdages et al., 2020; Hernandez-Gonzalez et al., 2018; Hernandez-Milian et al., 2019; van Franeker et al., 2018; Xiong et al., 2018; Zhu et al., 2019). Others examined all, or sub-samples of, the whole digestive tract (Bravo Rebolledo et al., 2013; Lusher et al., 2015, 2018; Moore et al., 2020; Nelms et al., 2019b). The volume and origin of gut content analysed is likely to affect the abundance of microplastics detected due to variation in sampling effort and the uneven distribution of microplastics throughout the digestive tract (Lusher et al., 2015; Moore et al., 2020; Nelms et al., 2019b).

There was limited variation in collection of *scat samples*, as they were all taken from haul out sites, although these did vary between coastal and offshore locations. The amount of scat analysed varied among studies and was often not reported. The impact of the age (i.e. time since deposition) of the scats was investigated in one study, but no statistically significant difference in microplastic load between fresh or aged scats was found (Perez Venegas et al., 2018).

3.3.2. Extraction protocols

Once the *gut content* was extracted, potential microplastics were isolated from organic material using a range of techniques, including physical separation (e.g. sieving and/or filtering), digestion (e.g. using chemicals or enzymes), or a combination of both (Table S3). Potassium hydroxide (KOH; usually a 10% concentration applied for a range of durations) was the most commonly used chemical digestion technique (Table S3), while Nelms et al. (2019b) used enzymatic digestion with Proteinase K. Finally, the range of filter and sieve mesh sizes (20 μm–1000 μm) used to extract microplastics also varied considerably (Table S3). This likely

affected the number and sizes of particles detected in each study (Lindeque et al., 2020).

Similarly, for the *scat samples*, the digestion and filtration steps differed significantly among studies (Table S3). Three studies did not use or specifically detail a digestion step (e.g., Eriksson and Burton, 2003; Hudak and Sette, 2019; Ryan et al., 2016), one paper physically degraded scat samples via homogenization (Donohue et al., 2019), while the remaining four studies used chemical digestion with KOH (Garcia-Garin et al., 2020; Perez-Venegas et al., 2018, 2020) or enzymatic digestion with proteinase K (Nelms et al., 2019a) (Table S3). The remaining paper used an alternative enzymatic digestion approach where scats were machine-washed in fine-mesh laundry bags with washing detergent (Bravo Rebolledo et al., 2013). The size of the mesh used during the filtration step likely influences the findings, as highlighted in the previous section. For example, Perez-Venegas et al. (2018) used fine mesh (0.7 μm) which was several orders of magnitude finer than that used by Ryan et al. (2016; 0.5 mm). The ability to detect smaller microplastics will likely increase the detectable amount in the scat (Huvet et al., 2016; Lenz et al., 2016).

3.3.3. Identification of potential microplastics

There is a wide range of approaches used to identify potential microplastics extracted from samples (Table S3). The simplest and cheapest form is visual identification of potential microplastics, however, it is important to note that this method could give high error rates of up to 70% (Hidalgo-Ruz et al., 2012). Therefore it is highly recommended for microplastics to undergo further analysis and identification (Dekiff et al., 2014). A variety of more precise methods are available to characterise the microplastic polymer, ranging from thermal analysis to spectroscopy (Hidalgo-Ruz et al., 2012; Shim et al., 2017). Additional analysis is important as it gives more information on whether a particle is an actual microplastic, while providing additional information on the type of plastic and, potentially, its origin and source (Dioses-Salinas et al., 2020; Schwarz et al., 2019).

Of the studies that directly measured microplastics from scat or inside organisms (n = 20), four studies used visual identification under a microscope only (Table S3). As indicated above, these results need to be treated with caution due to potential high error rates in the identification process (Lusher et al., 2020). The majority of studies did perform further analyses to characterise the type of polymer found, with 12 using (micro-)Fourier transform infrared (FTIR) analysis, one Raman Spectroscopy and one a Phazir (NIR) to characterise the type of polymers found (Table S3). In addition, three studies did not use or define any methods to confirm that the particles found were microplastics (Table S3).

Encouragingly, more recent studies (i.e., publication from 2019 to 2020) are more likely to use FTIR spectrometry to identify polymer types. However, FTIR identification is an expensive process, and most studies only analyse a subset of their suspected particles. Importantly, when using techniques such as FTIR it is key to have clear QA/QC protocols in place, for example a threshold for matching, to minimize misclassification (Kühn et al., 2020). Furthermore, terminology varies significantly among studies and if polymer types are not confirmed, terminology needs to include caveat, e.g. “suspected”, “putative” or “potential” microplastics. Determining the colour of a potential microplastic can be very subjective, depending on the viewer’s perception of a colour and can be influenced by background colour of the filter or light used during microscopic analysis for example.

3.3.4. Contamination prevention

The contamination of samples with microplastics during collection, preparation and analysis, can alter the results of a study. Therefore measures to limit and account for contamination are necessary for obtaining accurate estimates of microplastics (Hidalgo-Ruz et al., 2012). Out of the 20 studies we reviewed that quantified microplastics in scat or gut content, there was a wide range of contamination prevention protocols, ranging from absent to extensive (Table S5). Five papers did not describe a contamination protocol, and we assume they did not have any methods to limit or control for contamination in place (Table S5). However, three of these five studies did not include fibres as they were seen as a potential contamination source (Besseling et al., 2015; Bravo Rebolledo et al., 2013; van Franeker et al., 2018).

During sample preparation and analysis, the most common methods used to prevent contamination were to cover samples when not used ($n = 14$ publications), the use of clean equipment (e.g. wiped with ethanol and Milli-Q water; $n = 12$), to work under appropriate conditions that minimize environmental contamination in the laboratory (e.g. positive pressure laminar flow hood; $n = 6$) and to wear non-synthetic clothing (e.g. cotton lab coats; $n = 7$). Finally, to account for possible airborne contamination some studies ($n = 5$ publications) exposed a wet filter in a Petri-dish to the same conditions as the samples and examined them for particles. Negative controls or blanks were also used to determine any background contamination ($n = 11$ publications). Four studies also sampled equipment for further analysis to compare with their findings, three sampled plastic equipment used in the laboratory (Donohue et al., 2019; Hudak and Sette, 2019; Nelms et al., 2019a) and one took clothing samples during sample collection (Moore et al., 2020).

Of the 16 papers with contamination control measures in place, only four had a very detailed protocol, which accounted for contamination during all stages of sample processing, from collection to analysis. In these studies, control samples from clothing were taken during animal sample collection and blanks were used during the microplastic analysis to monitor potential contamination. In addition, the analysis was done inside a positive pressure laminar flow hood, equipment was cleaned in advanced, if possible plastic material was avoided and cotton lab coats and gloves were worn (Nelms et al., 2018, 2019b; Donohue et al., 2019; Moore et al., 2020). However, most papers had a much less elaborate protocol, and often only checked for a limited number of contamination sources (Table S5). Moreover, some contamination protocols might not be very effective, or could actually introduce microplastics (for example, rinsing with tap water without collecting the residues, Bourdages et al., 2020). Importantly, as some studies had no or limited measures in place, it is difficult to be confident that the suspected microplastics are actual microplastics from collected samples. Several studies without a protocol to determine air contamination excluded microfibres from their results and considered them all as airborne contamination (Table S5). This method, however, might underestimate the presence of microplastic in animals, as the majority of microplastic detected in samples are microfibres (see Tables S2 and S4).

Several of the more recent papers had more detailed and elaborate protocols for contamination prevention compared to papers which were published 3–15 years ago (Table S5), highlighting the increased awareness among scientists about the risk of contamination (Hidalgo-Ruz et al., 2012; Löder and Gerdts, 2015; Norén, 2007).

3.4. Best practices for future studies

As highlighted in previous sections, the differences in contamination protocols among studies make comparing results across species difficult. In order to facilitate harmonisation across studies,

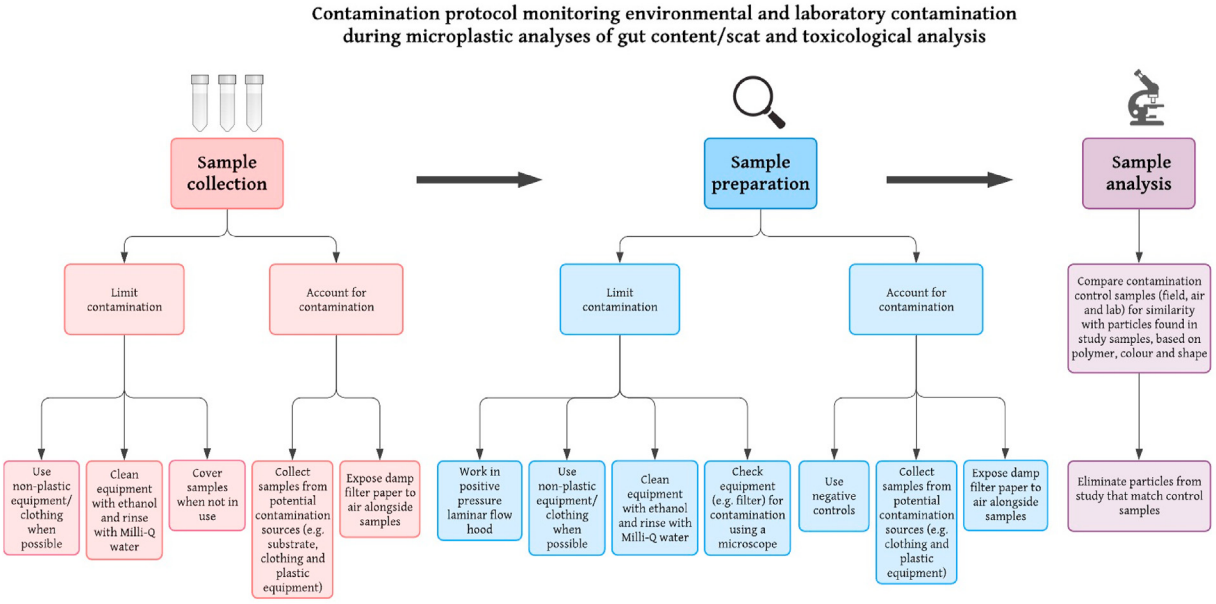

Fig. 2. Recommended standardised protocol for limiting and accounting for potential environmental and laboratory contamination during microplastic analyses of gut content and scat analyses of marine mammals.

we have developed a standardized protocol to limit and account for potential contamination sources in different key steps of the collection and extraction process (Fig. 2). By using this proposed standardized protocol, we can improve comparability, reproducibility and transparency across studies.

In addition, there is a wide range in reporting of results (Table S3). In order to facilitate meaningful comparisons across studies, we have also developed guidelines for the collection and reporting of qualitative and quantitative metrics during microplastic studies (Fig. 3). We also recommend defining colour categories (e.g. making "orange, yellow, gold" one category) to make results more consistent (Gauci et al., 2019; Wright et al., 2013, Fig. 3). Adoption of these guidelines will enable future work to be synthesised to facilitate comparisons across studies, comparisons by taxa, and to identify species or regions with highest levels of exposure. Moreover, to ensure transparency and reproducibility in science, raw data per sample should be made available as supplementary material or as online dataset (see https://www.nature.com/sdata/policies/repositories#other for suggested databases).

To allow for better comparison across studies, we suggest reporting i) total number of microplastics found and total number of samples (scat or GIT) analysed; ii) proportion of samples which had at least one microplastic, and iii) the microplastic load on a per gram basis, clearly stated as wet or dry weight. In addition to reporting, the identification of prey species or trophic level of the prey species within a study would be a major step towards understanding microplastic exposure from trophic transfer. However, most studies did not determine prey species or trophic level in their studies, even though well-developed protocols are available. In pinnipeds, identification of otoliths or other hard parts in scat has been a common method of assessing diet for decades (Bowen and Iverson, 2012; Tollit et al., 2009). DNA diet methods are also becoming more common and affordable (Pompanon et al., 2012), and have been used in both cetaceans (Carroll et al., 2019; de Vos et al., 2018; Jarman et al., 2002) and pinnipeds (Casper et al., 2007; Deagle et al., 2009; Hardy et al., 2017). Concurrent assessment of diet and microplastic load per scat/GIT sample should be encouraged in future studies to start building a picture of exposure from environmental and trophic transfer routes (Nelms et al., 2019a).

3.5. Microplastics exposure assessment

Aside from quantifying levels of microplastics in organisms and scat, a total of ten studies attempted to infer exposure (and sometimes risk) levels of microplastics to marine mammals, all focussing on cetaceans (Table S6). Six of these studies linked habitat or prey species to exposure risk, while four studies attempted to use chemical and biological markers to assess exposure levels.

3.5.1. Linking habitat and prey to exposure risk

The linking of habitat and prey to exposure risk has been done, both on a global scale (Germanov et al., 2018; Burkhardt-Holm & N'Guyen, 2019), as well as a more regional scale (Fossi et al., 2017; Guerrini et al., 2019). A broad scale study was conducted by Germanov et al. (2018) in which baleen whale distribution was combined with recognized microplastic hot-spots. Not only did the paper provide some insight into the overlap between whale habitat and microplastic hotspots, it also highlights how the biology of individual species needs to be adequately accounted for in broad-scale assessments and modelling exercises. For instance, humpback whales were considered to have a presence in all key buoyant microplastic pollution hotspots bar one (Mediterranean Sea) by Germanov et al. (2018). However, exposure risk might not be high in each of the microplastics hotspots. For example, satellite telemetry work in the South Atlantic shows that humpback whales migrate through the South Atlantic gyre, likely with minimal feeding (Zerbini et al., 2006, 2011), and therefore the actual exposure is most probably minimal as foraging is unlikely to occur here. The approach by Burkhardt-Holm & N'Guyen (2019) did include the feeding biology of whales, and this approach is therefore, in our opinion, a better approach to estimate levels of exposure. However, uptake via seawater was not included in the assessment, even though that is a likely important source for mega-filter feeders (Burkhardt-Holm & N'Guyen, 2019).

In contrast to the previous two studies, more detailed and complex modelling studies were conducted by Fossi et al. (2017) and Guerrini et al. (2019). Fossi et al. (2017) conducted a study in

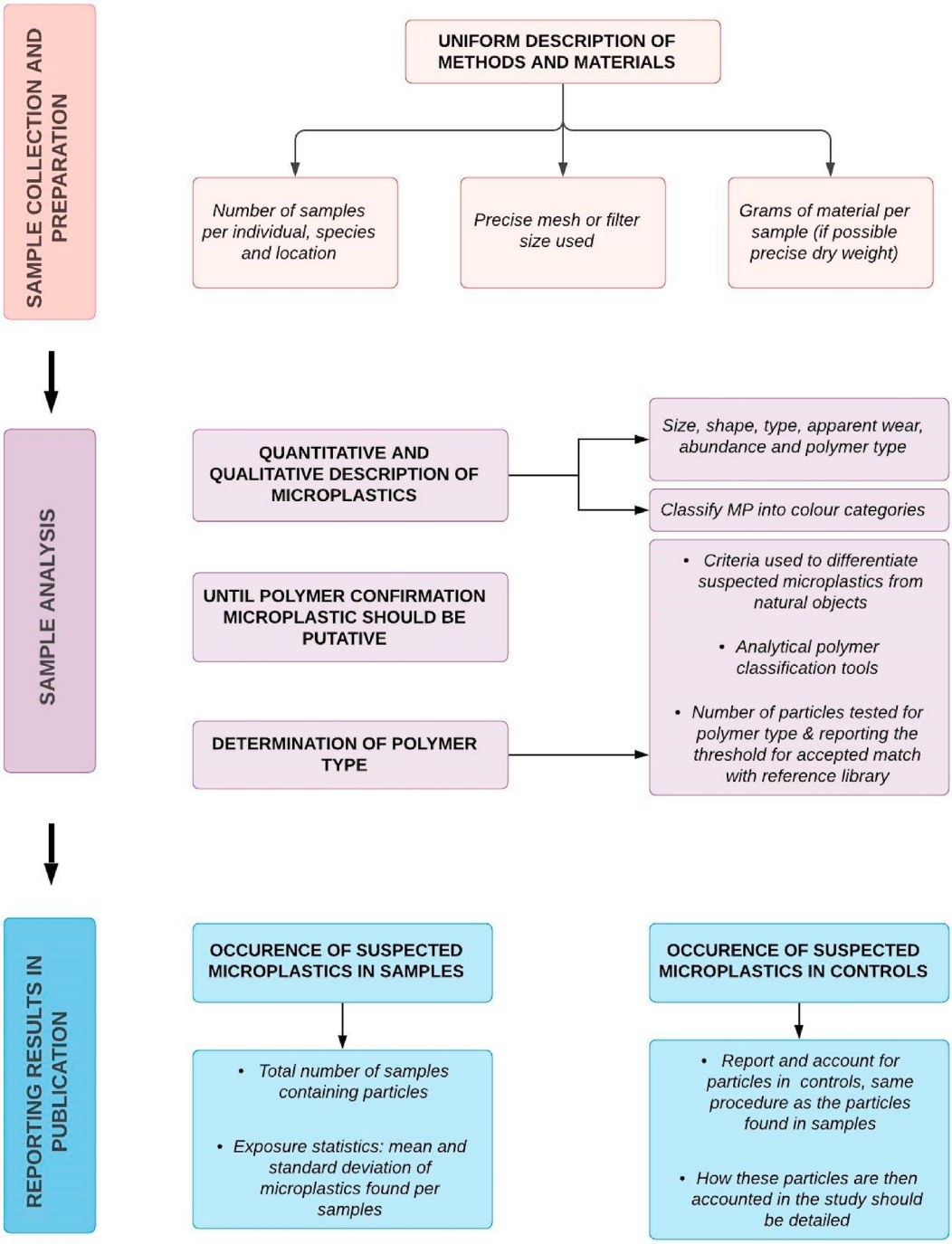

Fig. 3. Key information to report in any marine mammal study on microplastics.

which field measurements of zooplankton, microplastic abundance and cetacean survey data were combined with models on ocean circulation and potential fin whale habitat. This resulted in a preliminary risk assessment for whales, highlighting that areas with high levels of microplastic overlap with fin cetacean habitat and several sightings (Fossi et al., 2017). Guerrini et al. (2019) used a model to track particles from release points (sources) to estimate the hazard. This approach does allow for identifying areas where exposure might be relatively high. However, there currently is limited data on the contribution of microplastics from different sources, and this data is needed to improve the accuracy of the model.

Importantly, both Fossi et al. (2017) and Guerrini et al. (2019) highlight that their approach could be used in risk *assessment*. However, in our opinion it provides a confirmation that there is *risk of exposure* of fin whales within the area but falls short of a risk assessment. In *risk assessment* there is a need to determine the severity and the probability of adverse effects (Suter II, 2016), not just exposure to a contaminant. In both cases the adverse effects of microplastics on whales were not assessed, only the likelihood of exposure. Additionally, to conduct a risk assessment future research should focus on i) how long microplastics remain inside

the digestive tract and whether there is transfer to the tissue of marine mammals (Perez-Venegas et al., 2018) and ii) whether microplastic exposure results in any effects on animal health (Claro et al., 2019; Panti et al., 2019).

3.5.2. Phthalates and other persistent contaminants as biomarkers

Four papers that investigated the use of biomarkers to predict marine mammal exposure to microplastics. The studies focus on phthalate levels [predominantly mono(2-ethylhexyl) phthalate (MEHP) and bis(2-ethylhexyl) phthalate (DEHP)], within the environment, in zooplankton and/or in whale blubber. Phthalates are added to plastics to increase plasticity and can leach from plastic into the environment (Hermabessiere et al., 2017; Teuten et al., 2009). In addition, phthalates can bioaccumulate in organisms, and can cause potential adverse effects, including effects on embryo development and reproduction, and the disruption of endocrine functioning (Gunaalan et al., 2020; Hermabessiere et al., 2017).

However, we want to highlight several issues with these studies which need to be addressed before this approach can be used to determine exposure levels. First of all, in all these studies the variance was often (very) high making meaningful statistics difficult to perform. In many cases the coefficient of variance (CoV; standard deviation/mean x 100%) exceeded 100% for key measurements (e.g. microplastic levels and DEHP and MEHP levels in zooplankton and whale blubber). Secondly, phthalates (including MEHP) are used in a range of different products and industrial processes, and therefore can enters the environment from different sources, including wastewater (Jiang et al., 2018). This makes the direct linkage between MEHP levels in organisms and microplastic exposure difficult to establish. Finally, these studies had low sample sizes (for example Baini et al. (2017) sampled between $n = 1$ and $n = 3$ animals per species), and therefore can only be used as preliminary studies (which was also highlighted by the authors). For these reasons, significant further work is needed to validate and optimize this approach.

In addition, the level of other organochlorine contaminants (HCB, DDT and its metabolites and PCBs) were determined in Fossi et al. (2016), as well as certain biomarkers, including CYP1a and CYP2b (CYP family of enzymes, responsible for the metabolism of organic contaminants) and lipid peroxidation (LPO: indicator of oxidative stress). The organochlorine contaminants were included based on the Trojan Horse hypothesis, which is centred around the idea that microplastic can be a vehicle for the transfer of other organic contaminants into organisms (Burns and Boxall, 2018). However, this hypothesis is widely debated (Burns and Boxall, 2018), and there is no consensus in the scientific community that microplastics are a major source of transfer of organic contaminants into organisms (Bakir et al., 2016; Burns and Boxall, 2018; Lohmann, 2017). Therefore, this approach should also be used with caution.

3.5.3. Total exposure

Though the papers above attempt to determine risk of exposure and identify markers of exposure, only very few studies have attempted to quantify total exposure levels. A first attempt was made by Desforges et al. (2015) which estimated levels of microplastics in two foundation zooplankton prey species (*Neocalanus cristatus* and *Euphausia pacifia*) in the Northeast Pacific. They encountered microplastics in 2.9% and 5.9% in *N. cristatus* and *E. pacifia*, respectively. Using these results, the authors estimated that a humpback whale in coastal British Columbia is exposed to 300 000 microplastics d^{-1} (assuming it consumes 1.5% of its body weight in krill and zooplankton every day). In a similar way, Lusher et al. (2016) attempted to determine microplastic exposure of striped dolphins through trophic transfer. Levels of microplastic in mesopelagic fish were determined within the North Atlantic, and these levels were linked to dietary composition. Lusher et al. (2016) estimated that a single individual could be exposed to 1.3 million particles day^{-1}, or 463 million particles $year^{-1}$. As far as we are aware, these are the only studies that attempt to quantify uptake through trophic transfer in wild marine mammals. In addition, two studies (Fossi et al., 2014, 2016) attempted to quantify the levels of microplastic taken up by fin whales, based on microplastic abundances recorded for seawater and the whales' filtering capacity. Uptake was estimated to be 3653 particles day^{-1} (Fossi et al., 2014) and "thousands of particles" per day (Fossi et al., 2016).

Although this could be an interesting and illustrative approach to quantify uptake of microplastics from the water column, it is over-simplified and significant improvements are needed. We highlight this point, as an extreme example, by taking the blue whale (*Balaenoptera musculus*) feeding of the Coast of British Columbia in the Northeast Pacific Ocean. The blue whale can engulf 83 m^3 of sea water per mouthful (Goldbogen et al., 2011). Desforges et al. (2014) conducted a study on microplastics in the size range 62–5000 μm and found an average level of 279 particles m^{-3}, but a range from 8 to 9200 particles m^{-3}. This means that, based on this reported range, a blue whale feeding of the coast of British Columbia could engulf anywhere between 663 and 763600 particles per mouthful. However, there is considerable uncertainty about levels of microplastics in surface waters, especially at lower size ranges of plastics (Huvet et al., 2016; Lenz et al., 2016). A recent study of the coast of British Columbia using advanced quantification techniques to detect particles as small as 5 μm estimated average levels of ~4 million microplastic m^{-3} in the open ocean and 15 million microplastic m^{-3} in coastal waters (empirical findings; Brandon et al., 2020). Using this range, it can best estimated that blue whales could be exposed to between 332 and 1245 million microplastics per mouthful. Clearly, given this range between studies, significant work needs to be done to estimate exposure levels of marine mammals to microplastics.

4. Conclusion

Charismatic megafauna such as marine mammals can help bring the public's attention to anthropogenic impacts. However, to fully assess risk of exposure to threats, and how they vary across species and ecosystems, standardised analysis and reporting protocols are required. Therefore, a key output of this paper is a framework to improve consistency across studies that examine the incidence of microplastics in marine mammal gut and scat. We strongly urge scientists working in this field to adopt our protocols where possible. However, if not possible, for example due to financial or technical constrains, transparency about study constraints is essential. Alternatively, increased collaborations between partners and institutions with access to advanced equipment would help optimize the quality of reported data. In addition, a continuous search to develop improved and more affordable technology to extract and identify microplastics is needed, but this is important for all studies focussing on microplastic pollution, as this research field seems likely to continue to burgeon in the future.

Overall, it is encouraging to see the marine mammal community produce a rapidly growing body of work on the exposure of these taxa to microplastics. Microplastics were detected in most marine mammals samples analysed, with large variation among samples, even within studies. A key next step is to try and understand impacts of microplastics on marine mammal health, for example by using marine mammal cell lines linked directly to empirical measurements of microplastic exposure. The use of biological or chemical markers was suggested in several preliminary studies, but significant work is needed to confirm that these markers can be

effectively linked to microplastic exposure. Overlaying levels of microplastics in prey and the water column with the feeding biology of marine mammals is likely a more promising avenue to estimate total exposure, but more research on this is needed to understand the variation in microplastic exposure by region, season and ocean depth, as well as trophic transfer mechanisms.

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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

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