



## When the small ones tease the largest: Microplastic and phthalate ester occurrence in cetaceans occasionally found in the German North Sea and Baltic Sea

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### ARTICLE INFO

#### Keywords:

Baleen whales  
Toothed whales  
FTIR  
Plastic  
Marine pollution  
PAE

### ABSTRACT

Living in potentially burdened waters and prey on loaded species, marine mammals are sentinels for microplastic (MP) pollution. Here, seven different species of cetaceans were investigated covering baleen (*Mysticeti*) and toothed (*Odontoceti*) whales stranded along the German coast between 2016 and 2021. Intestinal and faecal samples of 12 sperm whales, three minke whales, two dolphin species, one long-finned pilot whale, one fin whale and one neonate orca were analysed. The concentrations of 11 phthalates were assessed in blubber and muscle samples. MPs were detected with Nile Red staining, polymer identification was conducted using  $\mu$ FTIR spectroscopy. Four individuals showed no MP burden. An average of 4.6 MPs (Odontocetes) and 3.25 MPs (Mysticetes) were found. A share of 0 and 19 MPs was found per 10 g of faeces. Polyamide, polyolefin and polyester were detected. No significant differences between baleen or toothed whales were identified. In individuals which revealed macro debris inside their stomach also showed MPs in their faeces. Individuals with an empty stomach ( $N = 3$ ) still showed MP presence. Nine compounds of PAEs were quantified in both muscle and blubber of seven species in different concentrations. DEHP was the most abundant compound. The results cannot be linked to the burden in the North - or Baltic Sea, since not all investigated specimens are regularly occurring here. Evidence is given that both baleen and toothed whales from the northern hemisphere are similarly burdened with MPs, and that not only ingested macro-debris can be assumed as a source for MP.

### 1. Introduction

Microplastics (MPs; plastic particles smaller than 5 mm) are known to be ubiquitous in the environment. The global utilization, the increasing plastic production and the longevity of plastic in general are facilitating this fact (Barnes et al., 2009; PlasticEurope, 2023). Thus, the distribution of plastic and other anthropogenic material into the marine environment are aided by e.g., mismanaged landfills, lost fishing gear, and other local sources, like untreated wastewater (Galgani et al., 2013; Herzke et al., 2021). Especially for the marine environment the riverine input is one of the biggest carriers of MP, besides the atmospheric

transport (Goßmann et al., 2023; Strokol et al., 2023). Thus, is it not surprising to find MP ingested by organisms either by mistake or together with the original prey species. This phenomenon occurs through all levels of the food web (Setälä et al., 2014; Provencher et al., 2019; Pironti et al., 2021). Modern research now deals with three aspects: 1) data recording on which species are affected to capture the extent of the impact (Panti et al., 2019; Kühn and van Franeker, 2020); 2) to evaluate the effects the uptake might have on individual organisms (Guzzetti et al., 2018; Xu et al., 2020) and 3) to understand the potential accumulation of plastic related chemicals (Fossi et al., 2018). As top predators, marine mammals are sentinels that accumulate consumed

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MPs and pollutants (Fossi and Panti, 2017; Ribeiro et al., 2019).

In MP research specifically, the investigation of marine mammals is rather difficult, since the sample collection depends on the access to carcasses (Philipp et al., 2021) or catch-and-release approaches (Hart et al., 2022). Moreover, it can be assumed that the number of unreported cases is high due to the fact that the majority of animals being affected, die, decay and sink offshore, thus ending up being unrecorded (Williams et al., 2011; Hammer et al., 2012). Necropsies of stranded carcasses are of high value to answer questions on the health status, causes of death of individuals but also of populations (Siebert et al., 2001; Siebert et al., 2020). This includes information of entanglements in marine litter and ingested objects following an analysis of the digestive tracts of the carcasses (Unger et al., 2016, 2017; Philipp et al., 2021) but also the analysis of toxic compounds that organisms can accumulate in their tissue, particularly blubber.

Collecting samples during necropsies allows for sampling without additional effort. Nevertheless, stranding events might occur irregularly, which hampers judgements on timeframes (e.g., comparison between different seasons and temporal trends over years). Furthermore, stranding events of cetaceans are occurring tendentious in non-predictable patterns. Thus, identified health patterns can be biased if findings are only depending on stranded, by-caught or shot carcasses (Kesselring et al., 2017). In case of migratory species, those individuals help to collect information on the status of species globally, rather than being an indicator for the area where they stranded. Additionally, not all animals dying offshore are washed ashore and are available for necropsy. Thus, the animals found reflect only a small share of the animals actually being affected (Williams et al., 2011; Hammer et al., 2012).

The harbour porpoise (*Phocoena phocoena*) is the only cetacean species which is inhabiting the Baltic Sea and occurs regularly and frequently in the southern North Sea (Nachtsheim et al., 2021; Gilles et al., 2023). In the summer months, minke whales (*Balaenoptera acutorostrata*) are regularly occurring in the northern part of the North Sea (Denmark straight) and around the Doggerbank in the central North Sea (Würsig et al., 2018; Gilles et al., 2023). Stranding events of other baleen whales, such as fin whale (*Balaenoptera physalus*), sperm whales (*Physeter macrocephalus*) or other toothed whale species like killer whales (*Orcinus orca*), pilot whales (*Globicephala melas*) and dolphin species (e.g., *Delphinus delphis* and *Lagenorhynchus albirostris*), occur rarely along the German coastline covering the North Sea and the Baltic Sea (Kinze et al., 2021; Gilles et al., 2023). Based on different diving and feeding behaviour in different whale species the exposure to marine litter, including MP, can only be assumed to vary (Lusher et al., 2015; Fossi et al., 2020; López-Martínez et al., 2023). To the best knowledge of the authors, no reliable comparison with a sufficient number of individuals has been published so far.

Besides the harbour porpoises and minke whales, other cetacean species mostly enter the North Sea and Baltic Sea by mistake when following prey or water masses (Pierce et al., 2007; Gilles et al., 2023). Thus, their necropsies contribute greatly to the knowledge about the exposure of these species to MPs and to assess the potential risk in the long and global run.

Hence, those carcasses are an important source to learn more about their state of health and to contribute to gaining an overall picture of potential anthropogenic impacts, like the exposure to marine litter and MPs (Unger et al., 2016, 2017), emerging contaminants, as well as their legacy, including plastic additives (e.g. phthalate acid esters-PAEs; Bains et al., 2017). In particular, phthalates are plasticizers that are added to polymers, to increase softness and flexibility and they are widely used in the manufacture of a broad range of applications and products. Due to their use, they have become ubiquitous environmental chemicals (Net et al., 2015). The most widely used and found in the environment is phthalate di(2-ethylhexyl) phthalate (DEHP), a hormone-interfering compound (Eichler et al., 2019). However, it has recently been replaced for most applications by another high molecular weight phthalate, Diisononyl phthalate (DiNP). Both phthalates are assessed in

this study.

In the following, we present the results of 18 whales stranded on the German North Sea ( $N = 16$ ) and Baltic Sea coasts ( $N = 2$ ) as well as one fin whale found in the Danish North Sea (Jo et al., 2017). This study aims 1) to quantify MPs in intestinal and faecal samples of cetacean specimens, 2) to compare the burden in baleen and toothed whales and 3) to quantify the accumulation of phthalate esters in the blubber and muscle tissues in baleen and toothed whales. The evaluation also includes results of macro litter findings from the sperm whales stranded along the North Sea coast in 2016 and can be regarded as an addition to the results already evaluated (Unger et al., 2016). The additional findings of the presented study deliver valuable information on the occurrence of MP types and phthalate esters in seven different whale species. Moreover, based on the applied quantitative (Nile Red staining and fluorescence microscopy) and qualitative approaches ( $\mu$ FTIR spectroscopy), the findings are reliable and, thus, contribute greatly to the database on the extent of MP exposure in cetaceans on a global scale.

## 2. Material & methods

### 2.1. Sample collection and processing

As part of the monitoring program of Schleswig-Holstein, Germany, the Institute for Terrestrial and Aquatic Wildlife Research (ITAW) is regularly conducting necropsies of dead marine mammals (Siebert et al., 2001; Siebert et al., 2020). The necropsies are conducted following a strict international protocol for assessing the health status of animals (Jsseldijk et al., 2019).

Since 1990, 17 cases of baleen whales and 67 other toothed whale species other than the harbour porpoise were registered at the ITAW. Out of these 84 cases, samples of 18 individuals were available for further MP and phthalate esters investigations (Table 2, Fig. 1). Those investigated individuals comprise 10 sperm whales (*Physeter macrocephalus*), one common dolphin (*Delphinus delphis*), one white-beaked dolphin (*Lagenorhynchus albirostris*), one orca (*Orcinus orca*), one long-finned pilot whale (*Globicephala melas*), three minke whales (*Balaenoptera acutorostrata*), and one fin whale (*Balaenoptera physalus*). Thus, fourteen toothed whales (*Odontoceti*) and four baleen whales (*Mysticeti*) were considered in this study.

When being washed ashore carcasses decompose over time. The longer it takes before they are collected, the further advanced the decomposition state is. Accordingly, sampling was hampered in some cases (advanced decomposition,  $N = 3$ ) (Jsseldijk et al., 2019). In this study, besides the samples of the gastrointestinal tract (GIT), 20 subcutaneous fat samples as well as 20 muscle samples were collected for chemical contaminant analysis. Moreover, some investigated species are large in size and correspondingly, the GIT has larger dimensions and volumes, compared to harbour porpoises where intestinal samples are taken quickly out of the carcass and can easily be stored in glassware (Philipp et al., 2021). Thus, for example the faecal samples of the sperm whales were taken in situ after the whole intestine (between 135 and 165 m in length) was laid out in loops and opened, as it is a common method in cetacean necropsies (Unger et al., 2016; Jsseldijk et al., 2019). In the course of this procedure, also macro litter findings were documented and were highlighted (\*) in Table 2. To avoid a later contamination, the faecal samples were taken out of the intestine straight after opening. Following this, two different sample types were investigated: 1) faeces collected directly from the intestines ( $N = 10$ ), and 2) intestinal samples including faeces ( $N = 8$ ).

All samples were sewed into washing sachets, washed in a conventional washing machine, and further processed after Philipp et al. (2020). The add-on of enzyme detergent (Biotex®) for the washing process allows for a gentle cleaning procedure, which results in obtaining neat hard parts down to 100  $\mu$ m in size. This size fraction was retrieved, since the washing sachets were sewed out of nylon fabric, having a distinctive fibre pattern, with a mesh size of 100  $\mu$ m (Philipp

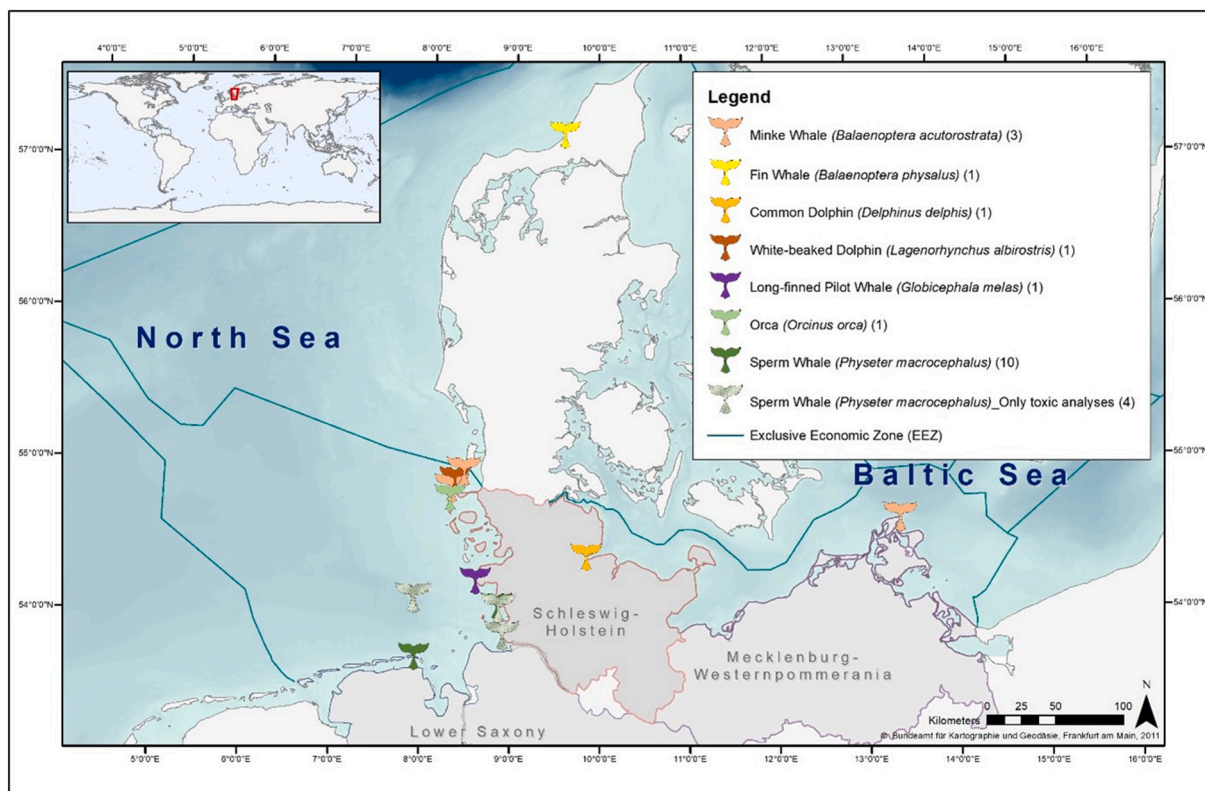


Fig. 1. Overview on the stranding locations of 18 large whales in the German North and Baltic Sea between 2016 and 2021 which were analysed for MP presence.

et al., 2020).

The inside of the washing sachet was rinsed into a saturated sodium chloride solution, enabling a density separation of the retained hard parts (Hidalgo-Ruz et al., 2012). Nevertheless, to avoid any loss of particles with a higher density, the whole sodium chloride solution was vacuum filtered onto cellulose filters (Rotilabo®, Typ11A, Ø 55 mm, pore size: 12–15 µm) in two steps: first the supernatant, subsequently the precipitate onto different filters. The filters were placed in pre-cleaned petri dishes and dried overnight. A Nile Red chloroform solution (1 mg/1 ml) was pipetted over all filter with a pre-cleaned glass pipette, to make potential MPs visible (Philipp et al., 2020). After at least 24 h, MP particles were identified via fluorescence microscopy (Kern OBN- 148 and Zeiss AX10 equipped with a TRITC HC filter set, F36–503, AHF Analysetechnik, Tuebingen, Germany) (Tamminga et al., 2017). Subsequently, those selected particles were manually transferred to an aluminum oxide (AlOx) membrane filter (Anodisc, Ø47 mm, pore size: 0.2 µm, Whatman) for µFTIR analysis as it was done in a previous study conducted by the same working group (Philipp et al., 2021).

## 2.2. QA/QC

Necropsies of those large whales are not applicable in a dissection hall or a laboratory environment. Thus, airborne contamination is likely to happen and needs to be considered, since no blank filters were taken during sample collection. Mainly due to the conditions in the field, and because the here investigated samples were partly collected years ago for other purposes, field blanks were unfortunately not considered. Thus, it was even more crucial to follow a strict protocol to avoid secondary pollution while processing and investigating the samples (Philipp et al., 2020).

Since older faecal samples were stored in polyethylene containers, sub-samples out of the middle were randomly taken with a metal spoon, those parts which were not in contact with the outer environment All intestinal samples including faeces were stored in pre-cleaned glass

containers as described in Philipp et al., 2020. All blubber and muscle tissue were first placed in aluminum foil and stored in a plastic bag.

All steps of taking the samples out of the containers, the step of sewing the samples in the pre-rinsed washing sachets, and the subsequent density separation and filtration were all conducted in a closed acrylic box, which was always cleaned prior and after work with MilliQ and ethanol.

All used instruments and materials were mainly consisting of glass or metal and were rinsed three times with MilliQ water (Millipore), and subsequently with 70 % ethanol.

To cover the potential secondary contamination, procedural blanks of all used detergents, the washing sachets and blanks of the working environment were taken into account ( $N = 10$ ). The mean number of all found fragments and fibres in the blanks were taken and the triplet standard deviation was added to calculate the limit of detection (LOD) (Horvatits et al., 2022). Using the LOD as a parameter for evaluating the amount of contamination during the sample handling prevents from overestimating the actual amount of found MP. Both, the polymer composition, and the shape of the MP (fibre or fragment) identified in those blanks, were considered for excluding MPs from the results. In total, eleven particles were agreed on to be potential MPs. Out of these particles, one fibre and two fragments could be included in the µFTIR analysis. In general, the procedural blanks showed a low contamination: 0.6 fragments and 0.5 fibres were found on average after the Nile Red investigation.

In general, the lowest size limit of investigated MPs is 100 µm, and everything smaller found in the blanks or the samples were excluded to avoid an overestimation, since their occurrence are assumed to be related to secondary contamination.

## 2.3. µFTIR analysis

In total, 77 particles were transferred onto the AlOx filters for µFTIR investigation, whereof the transfer was successful in 53 cases (from

samples: 21 fibres and 29 fragments; from blanks: 1 fibre and 2 fragments). Particles were then classified as potential MPs and non-potential MPs (mostly biogenic) using a decision tree, which takes the appearance of the particle after Nile Red staining under fluorescence light into account (Philipp et al., 2020). Furthermore, the transfer from the cellulose filter to the AlOx filter needed to be successful as well as retrievable after transfer.

Polymer analysis was conducted with a  $\mu$ FTIR (Hyperion 3000, Bruker, Ettlingen, Germany) in transmission mode in a wavenumber range of 4000–1250  $\text{cm}^{-1}$  with 32 co-added scans and a spectral resolution of 4  $\text{cm}^{-1}$ .

#### 2.4. Ecotoxicological analysis of subcutaneous blubber and muscle samples

The levels of eleven phthalate ester (PAEs) compounds (Dimethyl phthalate (DMP), Diethyl phthalate (DEP), Diallyl phthalate (DAP), Dipropyl phthalate (DPrP), Diisobutyl phthalate (DIBP), Dibutyl phthalate (DBP), Benzyl butyl phthalate (BBzP), Dicyclohexyl phthalate (DChP), Bis(2-ethylhexyl) phthalate (DEHP), Di-n-octyl phthalate (DNOP) Diisononyl phthalate (DINP)) were evaluated in the muscle and subcutaneous blubber of the 20 samples for each tissue (7 species). PAEs were analysed employing ultrasound extraction coupled with dispersive solid-phase extraction (d-SPE) as a cleanup procedure developed for biological matrices by Santini et al., 2024. Both blubber and muscle samples were freeze-dried and 0.1 g of dry muscle and 0.05 g of dry blubber were used for the extraction procedure. Samples were analysed using an Agilent 8890A series gas chromatograph equipped with a versatile HP -5MS capillary column (30 m, 0.25 mm) and coupled with an Agilent 5977B mass spectrometer.

In order to guarantee the accuracy of data quality, assurance and quality control measures were undertaken during sample preparation and analysis. A procedural blank was performed within each batch of five samples and 100  $\mu\text{L}$  of deuterated phthalates IS-MIX (DMP-d4; DEP-d4; DIBP-d4; DBP-d4; BBzP-d4; DChP-d4; DEHP-d4; DNOP-d4) were added to all samples. Limits of detection (LODs) for individual PAEs are listed in Table 1.

#### 2.5. Validation of analyses

The normal distribution was tested with the Shapiro test. For normally distributed data sets (e.g. classified by the sex), the t-test was applied (Revelle, 2023). For sample sets with non-normal distribution (e.g., classified by the suborder), the Mann-Whitney *U* test or so-called Wilcoxon-rank sum test was used. Significance was considered if the *p*-value is  $<0.05$ . All analyses and creating of plots were done in RStudio (R Core Team, 2022) with the ggplot2 package (Wickham, 2016).

Possible MPs were identified with the help of Nile-Red and Fluorescence-Microscopy. Using a decision-tree, which was established for identifying MPs based on certain fixed parameters (Philipp et al., 2020), the sensitivity of the true positive rate (TPR) by the operator was statistically verified. With a percentage of 84 % (65 out of 77) the TPR confirms the effectiveness of pre-sorting with the help of fluorescence microscopy.

The potential relationship among plastic shape categories (fragment and fibres) isolated from the GITs/faeces samples and the levels of each PAE compound detected, was tested with the Spearman's *rs* correlation test (*p*-value  $<0.05$ ).

**Table 1**

Limit of detection for each PAE compound in the muscle and blubber (ppb).

Biological tissue	DMP	DEP	DAP	DPrP	DIBP	DBP	BBzP	DChP	DEHP	DINP	DNOP
Muscle	0.34	0.34	0.86	0.34	0.34	0.34	3.43	3.43	0.86	17.14	3.43
Blubber	0.69	0.69	1.71	0.69	0.69	0.69	6.86	6.86	1.71	34.29	6.86

### 3. Results

#### 3.1. $\mu$ FTIR-analysis of MPs in faecal and intestinal samples

In total we found 149 suspected MPs larger than 100  $\mu\text{m}$  (72 fragments, 77 fibres). After the blank correction there were 77 particles left (39 fragments, 38 fibres). In total 73 % of all analysed MPs were in a size class of 100 to 300  $\mu\text{m}$ . More information of the size ranges are given in SI (Fig. S14). In the following we are referring to the blank corrected data. Out of the 77 MPs, 53 particles from samples and blanks were successfully transferred to AlOx filters for further analysis with  $\mu$ FTIR for polymer identification. In 31 cases the particle could successfully be analysed.

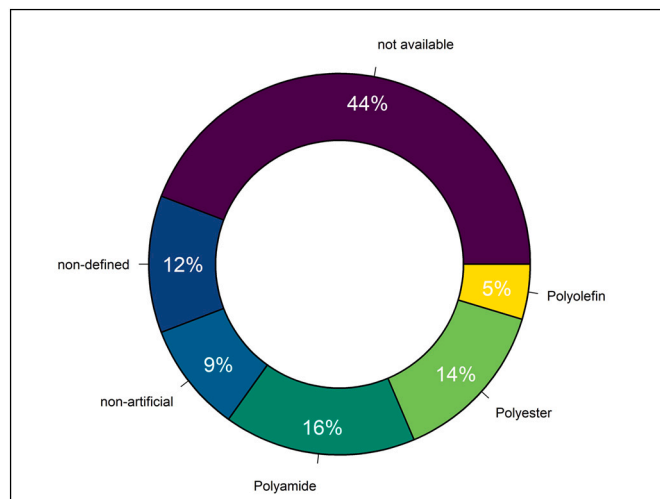
After the blank correction, 14 particles could be identified as synthetic polymers (6 fibres and 8 fragments) which consisted of three different polymer types: Polyester (4 fibres and one fragment), Polyamide (1 fibre and 6 fragments) and Polyolefin (2 fragments, incl. 1 Polyethylene) (Fig. 2). Fig. 3 shows the  $\mu$ FTIR spectra of three MPs found in this study. One polyester fibre was found in a minke whale (A), and a polyamide (B) and polyethylene fragment (C) were found in sperm whales.

In total, one polyester fibre and one polyester fragment were found in blank samples, which have been considered as secondary contamination, and were thus used for blank correction as described earlier. In addition, one silk-like fragment was detected in a blank, but not in any specimen sample, thus not further considered.

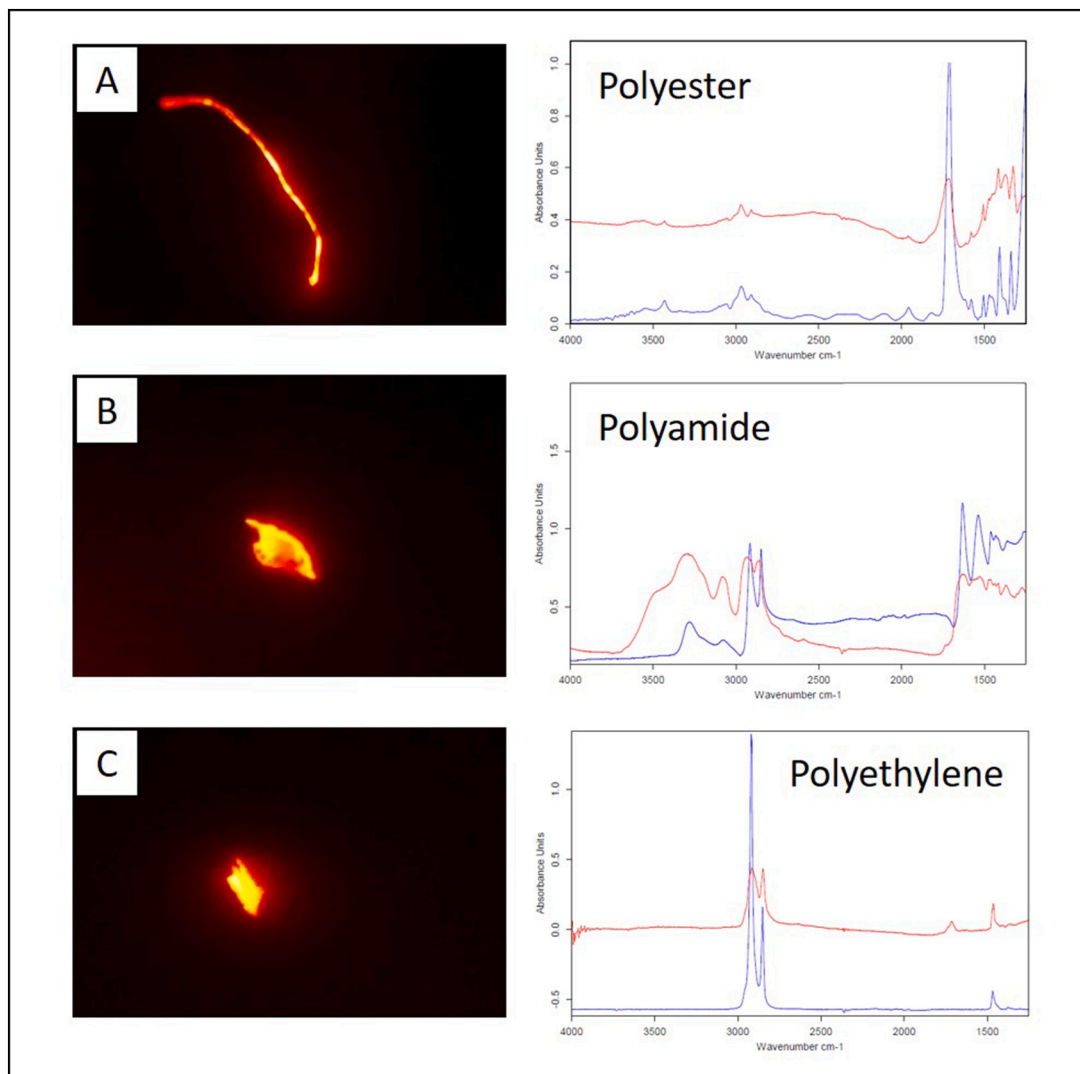
The remaining particles showed either no clear synthetic spectra, were biogenic (nonartificial), could not be identified or were assumed to be lost due to the transport to the AlOx filters and were thus not available (Fig. 2).

#### 3.2. Differences of MP loads between baleen and toothed whales

After the blank correction, four specimens showed no MP burden at all (Table 2). This includes the investigated fin whale, the white-beaked



**Fig. 2.** Results of  $\mu$ FTIR analysis. Presented are the percentages per polymer finding after blank correction and remaining objects ( $>100 \mu\text{m}$ ) which could either not be determined or identified as synthetic.



**Fig. 3.**  $\mu$ FTIR spectra of polyester (A) found in a minke whale, polyamide (B) and polyethylene (PE) MPs (both from sperm whales). The measured spectrum is depicted in red, and the blue spectrum is the reference spectrum.

dolphin, the long-finned pilot whale and one of the sperm whales. In the remaining 14 specimens, 39 fragments and 38 fibres were identified (Fig. 4). The results of two sperm whales (No. 1 and 12, see Table 2) needed to be excluded subsequently, due to different reasons: Sperm whale one showed high numbers of suspected MPs because squid beaks were mistaken for MP. No reliable intestinal or faecal sample was available for sperm whale 12, thus no MP occurrence was investigated. Nevertheless, both individuals were included in the ecotoxicological analysis of subcutaneous blubber and muscles. All results are presented in detail in Table 3 and Table 4.

In the 14 Odontocetes 64 MPs were found in total, consisting of 31 fragments and 33 fibres. A median share of 4.6 MPs per individual was found ( $M \pm SD = 4.57 \pm 4.36$ ), consisting of 2.2 fragments and 2.4 fibres on average. Whereas one sperm whale (No. 13), the white-beaked dolphin and the long-finned pilot whale showed no MP burden at all after considering the LOD. However, another sperm whale (No. 8) showed the highest MP abundance with 13 MPs. In the suborder of the Mysticetes ( $N = 4$ ) 13 MPs were found (eight fragments, five fibres). On average 3.25 MPs consisting of 2 fragments and 1.25 fibres were found per individual ( $M \pm SD = 3.25 \pm 3.3$ ). The fin whale is the only baleen whale, in which no MP could be identified after the blank correction. On the other hand, one minke whale (No. 2) showed the highest MP number (7 MPs) in this suborder. Moreover, minke whale 3, a female that was

the only cetacean found in the Baltic Sea, showed the occurrence of one fibre after considering the LOD.

Comparing the quantity of found MPs in the two different suborders, a higher share of particles was found in Odontocetes which also comes with a higher mean of MPs. No differences are detectable when taking the median into account (Fig. 5).

After having a closer look on the share of classified shapes (fibres and fragments), no clear conclusion could be drawn since the median is mainly equal. No significant differences could be determined between the two suborders after statistical investigation (Fig. 6).

Comparing the investigated species, the **sperm whales** (*Physeter macrocephalus*;  $N = 10$ ) showed the highest burden in total (45 MPs; 0–13 MPs per individual) but due to low sample sizes of the other presented species no definite conclusion could be drawn. The share of fragments was higher (0–13) than the share of fibres (0–9) which results in an average burden of 2.6 fragments and 1.9 fibres per individual. In the suborder of the Mysticetes the **minke whales** (*Balaenoptera acutorostrata*,  $N = 3$ ) showed the highest burden (1–7 MPs in total per individual, on average 2.7 fragments and 1.7 fibres on average). The other species are not considered here due to the fact that their number of specimens is too low to draw a clear conclusion.

For focussing on the MP load in faeces samples, 10 individuals (nine sperm whales and one minke whale) of which only faeces and no

**Table 2**

Overview on the animals analysed and the results of MPs over 100 µm as well as information on macro litter findings. Sperm whale 1 and 12 are listed even though no analysis could be conducted and are thus in italics. In these samples MP occurrence was not trustworthy due to the fact that they were densely assembled. The columns for muscle and blubber samples are indicating the availability of samples for the ecotoxicology analysis. NA: Not available. \*Animals with macro litter findings. All presented MP data are blank corrected.

ID	Sex	Suborder	Species	Age	Stranding area	Macro debris	MP > 100 µm			Stomach content analyses	Muscle sample	Blubber sample
							Fragments	Fibres	∑MPs			
Minke whale 1	Female	Mysticeti	<i>Balaenoptera acutorostrata</i>	Juvenile	North Sea	NA	1	4	5	Empty stomach	/	/
Minke whale 2	NA	Mysticeti	<i>Balaenoptera acutorostrata</i>	NA	North Sea	No	7	0	7	NA	x	x
Minke whale 3	Female	Mysticeti	<i>Balaenoptera acutorostrata</i>	Adult	Baltic Sea	No	0	1	1	Filled with brownish food mash	x	x
Fin whale	Male	Mysticeti	<i>Balaenoptera physalus</i>	NA	North Sea	No	0	0	0	NA	x	x
Common dolphin	Female	Odontoceti	<i>Delphinus delphis</i>	Juvenile	Baltic Sea	No	0	9	9	Empty stomach	x	x
White-beaked dolphin	Female	Odontoceti	<i>Lagenorhynchus albirostris</i>	Juvenile	North Sea	No	0	0	0	Filled stomach	x	x
Long-finned pilot whale	Male	Odontoceti	<i>Globicephala melas</i>	Juvenile	North Sea	No	0	0	0	Empty stomach	x	x
Orca	Male	Odontoceti	<i>Orcinus orca</i>	Neonate	North Sea	No	5	5	10	Breast milk remains	x	x
Sperm whale 1	Male	Odontoceti	<i>Physeter macrocephalus</i>	Juvenile	North Sea	No	NA	NA	NA	Squid beaks	x	x
Sperm whale 2*	Male	Odontoceti	<i>Physeter macrocephalus</i>	Juvenile	North Sea	Yes	7	0	7	Bucket, rope, black foil, packing tape, remains of white bag, car part, many squid beaks	x	x
Sperm whale 3	Male	Odontoceti	<i>Physeter macrocephalus</i>	Juvenile	North Sea	No	0	3	3	Many squid beaks	x	x
Sperm whale 5	Male	Odontoceti	<i>Physeter macrocephalus</i>	Juvenile	North Sea	No	1	5	6	Many squid beaks	x	x
Sperm whale 6*	Male	Odontoceti	<i>Physeter macrocephalus</i>	Juvenile	North Sea	Yes	3	0	3	Many squid beaks	x	x
Sperm whale 7	Male	Odontoceti	<i>Physeter macrocephalus</i>	Juvenile	North Sea	No	0	9	9	Many squid beaks	x	x
Sperm whale 8	Male	Odontoceti	<i>Physeter macrocephalus</i>	Juvenile	North Sea	No	13	0	13	NA	x	x
Sperm whale 9	Male	Odontoceti	<i>Physeter macrocephalus</i>	Juvenile	North Sea	Yes	0	1	1	Netting material, some squid beaks, fish remains	x	x
Sperm whale 10	Male	Odontoceti	<i>Physeter macrocephalus</i>	Juvenile	North Sea	No	2	0	2	Net, many squid beaks	x	x
Sperm whale 11*	Male	Odontoceti	<i>Physeter macrocephalus</i>	Juvenile	North Sea	Yes	0	1	1	Coffee capsule, netting material, Snickers wrapping, many squid beaks	x	x
Sperm whale 12	Male	Odontoceti	<i>Physeter macrocephalus</i>	Juvenile	North Sea	Yes	NA	NA	NA	Rope, foil and duct tape, many squid beaks	x	x
Sperm whale 13	Male	Odontoceti	<i>Physeter macrocephalus</i>	Juvenile	North Sea	No	0	0	0	Some squid beaks, fish remains	x	x

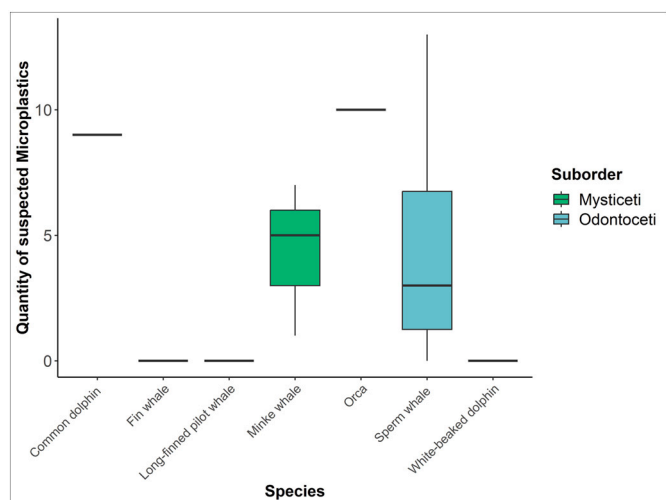
intestinal tissue were available, were here investigated. The general load of MPs in sperm whales stranded in the North Sea in 2016 was identified for  $3.22 \pm 5.81$  MPs per 10 g of faeces. This is resulting in  $3220 \pm 5810$  MPs larger than 100 µm per kilogram of faeces, if a heterogeneous MP distribution in faeces is assumed.

In general, a range between 0 and 19 particles were found per 10 g of faeces of all investigated specimens. This results in a mean of three particles larger than 100 µm ( $M \pm SD = 2.94 \pm 5.55$ ) in 10 g of faecal sample. Based on the sample size of Mysticetes and other species besides the sperm whales, this calculation is assumed to be biased by the amount

of found MPs in the sperm whales and thus not reliable enough to be further investigated.

### 3.2.1. MP occurrence in sperm whales

Four of the sperm whales dissected back in 2016 showed macro litter findings in the stomach, such as a car part or fishing gear (Unger et al., 2016). In none of the other investigated species macro litter was found in the GIT during the conducted autopsies (Table 2) (Unger et al., 2016). Nevertheless, 13 suspected MPs in total could be identified in the investigated sperm whales.



**Fig. 4.** Quantity of suspected MPs (>100 µm) in all investigated species of Odontocetes (one common dolphin, one long-finned pilot whale, one orca, ten sperm whales and one white-beaked dolphin) and Mysticetes (one fin whale and three minke whales), All presented data are blank corrected.

Sperm whale 2, in which several foreign bodies like parts of a blue plastic bucket, black agricultural foil, a white plastic bag as well as a car part were found, contained 7 MPs in the intestinal sample (7 fragments and no fibres after the blank correction).

When comparing the MP occurrence in empty versus full stomachs in all specimens it is striking that individuals with empty stomachs showed higher MP presence than individuals with full stomachs. The same is true for either only taking Odontocetes or Mysticetes into account, respectively (Table 2 and FigureSI1).

### 3.3. Differences in MP load in sexes and stranding locations

When comparing the sexes (without consideration of the suborder), the highest number of 13 MPs per individual were recorded in a male sperm whale (No. 8). The 13 males, including 10 sperm whales, one fin whale, one long-finned pilot whale and one orca, showed on average 4.2 MPs per individual (2.4 fragments, 1.8 fibres). Whereas, the investigated females (N = 4), including two minke whales, one common dolphin and one white beaked dolphin, showed on average 3.75 MPs per individual

(0.25 fragments, 3.5 fibres). Only in one female and in three males no MPs were found at all.

When it comes to the comparison of the sexes with consideration of the suborder it is striking that male Odontocetes (N = 12; on average 4.6 MPs per individual) and female Odontocetes (N = 2; on average 4.5 MPs per individual) are similarly burdened by MP occurrence (Fig. 5). Male toothed whales are particularly burdened by fragments (on average 2.4 per individual; ♀ on average 0.25 per individual), while female toothed whales showed the higher share of fibres (on average 3.5 per individual; ♂ on average 1.8 per individual). Within the suborder of Mysticetes, without a classification of the sex, a minke whale (No. 2) found in the North Sea showed the highest burden. It was highly decayed, showing no fibre burden, but a presence of 7 fragments. Normal distribution was confirmed for the data in male and female individuals, but only four females were investigated. To present reliable results, the t-test and the Wilcoxon-rank sum test were applied. The outcomes after both tests confirm a significantly higher burden in males, compared to female specimens. A detailed overview can be found in Fig. 7.

In the two individuals stranded in the Baltic Sea, the common dolphin (female, juvenile: 9 fibres found) and the minke whale (No. 3, female, adult: one fibre found) only fibres and no fragments have been identified at all after the blank correction. In all other 16 investigated specimens originating from the North Sea 2.6 fragments and 1.8 fibres per individual were found on average. Whereas a higher average of fibres (N = 6) could be confirmed in the two samples originating from the Baltic Sea, compared to those from the North Sea.

### 4. Phthalate ester concentrations in blubber and muscle samples

Nine out of 11 PAE compounds analysed were quantified both in the muscle and blubber of the seven investigated species (Table 3, Table 4), apart from DPrP and DChP that were below LOD in all the samples. DEHP and DPB are the most frequently detected compounds (80–100 % of occurrence) in all tissues. Generally, a larger number of compounds were detected in the muscle tissue rather than blubber for all the investigated species, however the levels of each compound are from 1.2-fold (DINP) to 4.3-fold (DEHP) higher in blubber than in muscle for all the specimens analysed and this also results for the sum of the total PAEs (3.5-fold).

The highest concentration of total PAEs in blubber has been detected in the minke whale 3 sample (10,589.67 ng/g w.w.), followed by sperm whales 10 and 11 (8151.93 ng/g w.w. and 6841.94 ng/g w.w.,

**Table 3**

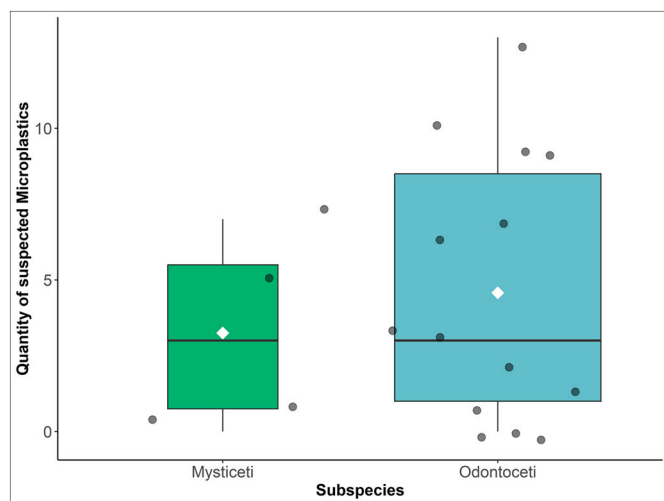
Phthalate esters levels (ng/g w.w.) in the blubber of investigated cetacean species. Values below the detection limit are reported as <LOD.

Tissue	ID sample	Sex	DMP	DEP	DAP	DPrP	DIBP	DBP	BBzP	DChP	DEHP	DINP	DNOP	∑Phthalates
BLUBBER	Minke whale 2	NA	2.46	<LOD	198.02	<LOD	55.95	280.32	650.32	<LOD	2658.15	<LOD	<LOD	3845.23
	Minke whale 3	Female	<LOD	27.22	<LOD	<LOD	229.12	110.41	<LOD	<LOD	9495.71	98.79	628.41	10,589.67
	Fin whale	Male	5.43	<LOD	<LOD	<LOD	4.64	9.45	44.22	<LOD	263.24	<LOD	770.40	1097.38
	Common dolphin	Female	<LOD	6.56	<LOD	<LOD	74.55	110.05	12.14	<LOD	109.93	<LOD	<LOD	313.22
	White-beaked Dolphin	Female	<LOD	0.84	<LOD	<LOD	<LOD	49.80	19.67	<LOD	471.10	<LOD	<LOD	541.41
	Long-finned pilot whale	Male	<LOD	27.67	<LOD	<LOD	98.18	92.12	<LOD	<LOD	1670.70	<LOD	<LOD	1888.67
	Orca	Male	<LOD	10.46	<LOD	<LOD	46.72	193.74	18.08	<LOD	3906.60	<LOD	<LOD	4175.60
	Sperm whale 1	Male	<LOD	<LOD	<LOD	<LOD	<LOD	49.19	61.42	<LOD	478.63	<LOD	188.49	777.73
	Sperm whale 2	Male	<LOD	<LOD	<LOD	<LOD	<LOD	38.39	30.42	<LOD	902.92	<LOD	221.17	1192.91
	Sperm whale 3	Male	<LOD	<LOD	3.82	<LOD	121.18	235.14	36.54	<LOD	918.49	<LOD	<LOD	1315.17
	Sperm whale 4	Male	19.54	16.33	1.51	<LOD	96.64	136.79	<LOD	<LOD	134.14	<LOD	93.83	498.77
	Sperm whale 5	Male	7.18	<LOD	2.02	<LOD	10.53	87.30	<LOD	<LOD	<LOD	<LOD	94.70	201.73
	Sperm whale 6	Male	<LOD	8.67	<LOD	<LOD	93.88	159.09	5.23	<LOD	284.14	<LOD	136.77	687.79
Sperm whale 7	Male	8.07	23.45	4.60	<LOD	146.88	169.89	<LOD	<LOD	<LOD	<LOD	161.86	514.76	
Sperm whale 8	Male	7.67	18.40	<LOD	<LOD	115.27	123.13	<LOD	<LOD	<LOD	<LOD	715.54	980.01	
Sperm whale 9	Male	4.76	29.66	<LOD	<LOD	112.79	195.55	<LOD	<LOD	86.60	<LOD	225.16	654.53	
Sperm whale 10	Male	4.73	14.19	<LOD	<LOD	155.75	286.14	8.66	<LOD	7437.64	<LOD	244.83	8151.93	
Sperm whale 11	Male	12.14	18.65	382.17	<LOD	145.00	323.48	582.48	<LOD	5378.02	<LOD	<LOD	6841.94	
Sperm whale 12	Male	9.44	13.08	<LOD	<LOD	52.43	90.29	<LOD	<LOD	1411.51	<LOD	<LOD	1576.75	
Sperm whale 13	Male	<LOD	20.95	<LOD	<LOD	117.07	72.83	31.45	<LOD	<LOD	<LOD	592.38	834.69	

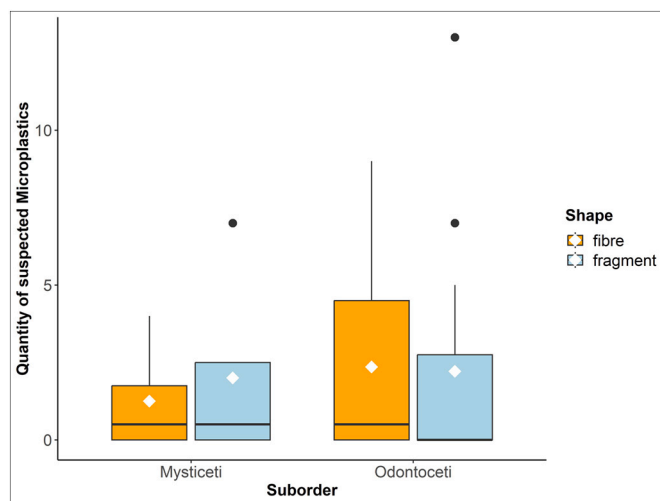
**Table 4**

Phthalate esters levels (ng/g w.w.) in the muscle of investigated cetacean species. Values below the detection limit are reported as <LOD.

Tissue	ID sample	Sex	DMP	DEP	DAP	DPrP	DIBP	DBP	BBzP	DChP	DEHP	DINP	DNOP	∑Phthalates
MUSCLE	Minke Whale 2	NA	1.75	1.96	< LOD	< LOD	12.12	34.82	143.13	< LOD	202.70	< LOD	11.61	408.09
	Minke Whale 3	Female	1.66	0.10	< LOD	< LOD	4.57	17.94	11.43	< LOD	52.80	< LOD	22.26	110.76
	Fin Whale	Male	2.15	2.38	2.46	< LOD	4.82	22.18	1.27	< LOD	41.96	< LOD	< LOD	77.22
	Common Dolphin	Female	2.11	2.06	< LOD	< LOD	36.51	63.90	5.27	< LOD	45.36	< LOD	156.90	312.11
	White Beaked Dolphin	Female	< LOD	0.01	1.80	< LOD	10.90	43.35	2.97	< LOD	57.80	< LOD	42.47	159.30
	Long Finned Pilot Whale	Male	0.51	< LOD	< LOD	< LOD	< LOD	19.73	6.00	< LOD	123.37	< LOD	282.11	431.71
	Orca	Male	0.67	0.15	1.50	< LOD	0.65	14.93	< LOD	< LOD	197.24	< LOD	3.31	218.46
	Sperm Whale 1	Male	2.61	2.47	0.60	< LOD	27.37	45.84	46.18	< LOD	930.58	< LOD	< LOD	1055.65
	Sperm Whale 2	Male	1.66	4.33	20.49	< LOD	15.97	35.96	16.51	< LOD	79.04	< LOD	< LOD	173.95
	Sperm Whale 3	Male	4.27	7.89	44.28	< LOD	147.28	41.32	167.85	< LOD	218.07	< LOD	< LOD	630.97
	Sperm Whale 4	Male	3.44	1.04	0.39	< LOD	1.31	20.38	5.43	< LOD	154.13	2.61	239.27	428.00
	Sperm Whale 5	Male	2.48	1.24	0.89	< LOD	< LOD	15.05	2.79	< LOD	184.46	< LOD	46.65	253.56
	Sperm Whale 6	Male	2.78	1.88	0.30	< LOD	3.72	34.24	13.73	< LOD	348.37	5.85	118.17	529.05
	Sperm Whale 7	Male	2.92	2.38	0.12	< LOD	< LOD	15.89	1.62	< LOD	73.04	< LOD	447.60	543.57
	Sperm Whale 8	Male	4.32	8.49	93.42	< LOD	14.28	30.23	< LOD	< LOD	249.22	32.86	< LOD	432.83
	Sperm Whale 9	Male	3.27	2.69	< LOD	< LOD	3.67	20.83	0.09	< LOD	73.39	2.02	25.14	131.09
	Sperm Whale 10	Male	3.86	2.11	0.24	< LOD	17.40	44.84	69.92	< LOD	531.63	30.90	< LOD	700.91
	Sperm Whale 11	Male	6.15	20.09	181.76	< LOD	127.48	141.65	423.64	< LOD	3935.68	< LOD	< LOD	4836.46
	Sperm Whale 12	Male	0.99	6.27	34.27	< LOD	68.13	184.25	39.28	< LOD	781.31	8.55	759.56	1882.61
	Sperm Whale 13	Male	2.38	2.10	< LOD	< LOD	2.18	21.93	27.88	< LOD	20.84	8.33	16.87	102.51



**Fig. 5.** Quantity of suspected MPs (> 100 µm) in investigated specimens shown by the suborder (Mysticeti; N = 4); Odontoceti (N = 14). All presented data are blank corrected. The white rhombus is showing the mean value of each suborder based on the found results (grey dots) and the black line is the median.



**Fig. 6.** Share of fragments and fibres (>100 µm) in both suborders. White diamond is showing the average and the black line presents the median. All presented data are blank corrected.

respectively) and the sample of the orca (4175.60 ng/g w.w.). The orca specimen was a neonate, thus we can hypothesize that PAE compounds accumulated in its blubber via maternal transfer during gestation and

lactation. Indeed, >90 % of the total PAEs in this organism is represented by DEHP (Fig. 8). The lowest total PAEs value in the blubber range is still in sperm whale 5 (201.73 ng/g w.w.) pointing out that several factors can influence PAEs accumulation in these organisms.

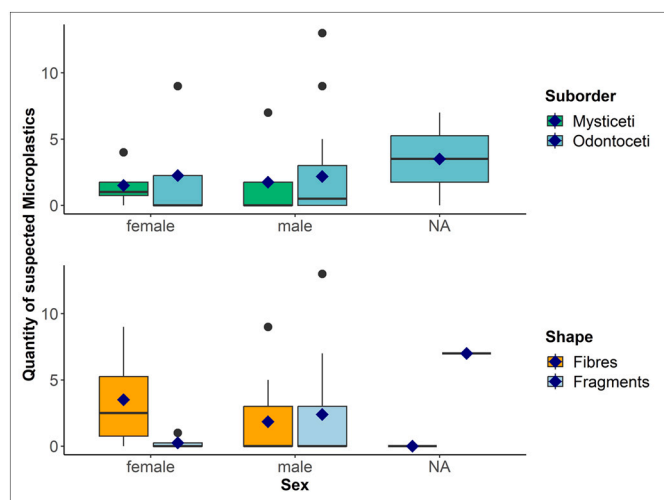


Fig. 7. Quantity of MPs (>100 μm) split by suborder and sex in the top graph. Quantity of fibres and fragments split by sex in the bottom graph. Blue rhombus is showing the mean value. Black line is showing the median. All presented data are blank corrected. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

These two individuals of sperm whales were juveniles, had both food remains and MP in their stomachs, so it is difficult to draw some conclusion on this phenomenon.

Concerning the total PAEs in the muscle and considering the six species (excluding sperm whale), the lowest value was found in the fin whale (77.22 ng/g w.w.), whereas the highest value was found in the long-finned pilot whale (431.71 ng/g w.w.), showing a lower variation compared to the blubber values. It is interesting to observe that among the 13 analysed sperm whales there is a high level of variation in the total PAE concentrations measured, as for the blubber, with an average value of  $900.09 \pm 1273.95$  ng/g w.w., ranging from 102.50 to 4836.46 ng/g w.w.

Interestingly, blubber and muscles present a different pattern of accumulation of PAE compounds (Fig. 8). This could be related to the different lipophilic nature of the PAEs, which increases with the molecular weight, and, thus, present a different affinity according to the nature of the tissues (high lipid content in the blubber elicit this tissue as a storage for apolar compounds). For all the species, except common dolphin, fin whale blubber and long-finned pilot whale muscle, DEHP is most abundant compound in both tissues (Fig. 8). However, for fin whale blubber and long-finned pilot whale and common dolphin muscle, DNOP is the most abundant compound. The common dolphin sample, instead, presents a different pattern of accumulation compared to the other species as DIBP, DBP and DEHP are the most abundant compounds with a similar concentration. These differences could be associated with the different feeding habits of the species as well as their differing habitat and feeding grounds.

### 5. Discussion

This study took a closer look at the occurrence of MPs and phthalate esters in 18 large whales which stranded along the German coastline between 2016 and 2021. All investigated species are not regularly inhabiting the North and Baltic Sea and are, therefore, only available in small sample numbers but they deliver valuable additional information on the burden of those species concerning MPs and thus contribute to a global understanding of marine pollution in whales.

In general, it is important to keep in mind that animals need to be both stranded and fresh enough to be necropsied. Thus, it is quite likely that the number of animals being affected is underestimated (Williams et al., 2011). Necropsies of stranded whales allow for an in-situ sampling with a lower risk of contamination, when compared to the collection of faeces (Torres et al., 2023). Nonetheless, collecting faeces for determining MP burden is a considered approach since the access to stranded carcasses is scarce and only little is known on the actual extent and consequences of MP occurrence. Moreover, it is also a non-invasive method, thus highly considerable as it has also successfully been used for other endangered marine mammals (Hernandez-Milian et al., 2023). Consequently, studies even with a small sample size are very

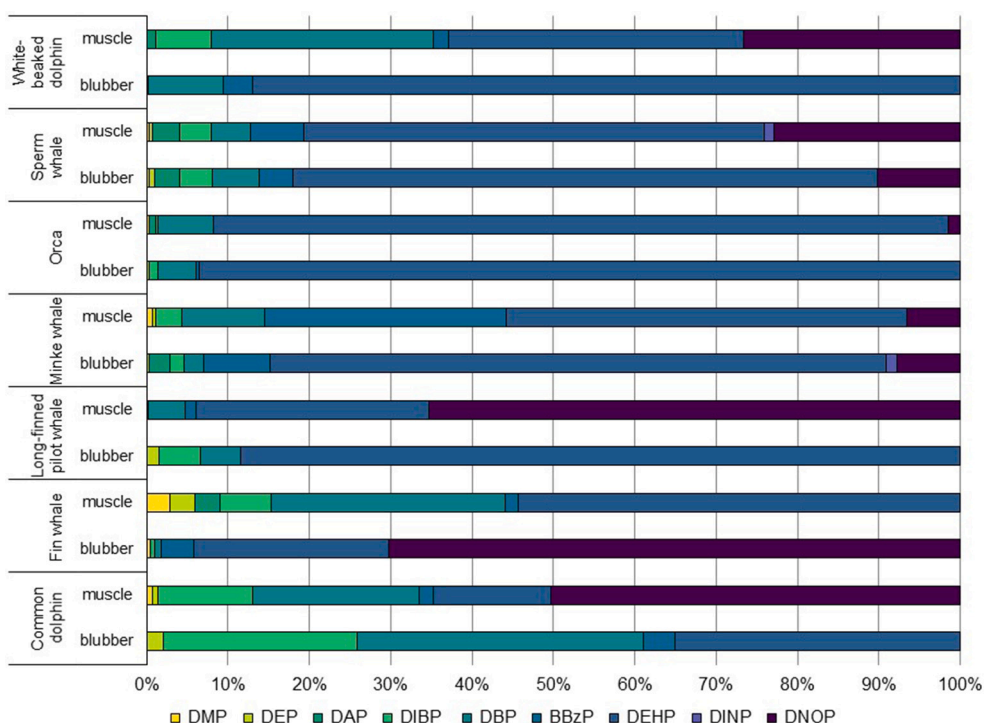


Fig. 8. Phthalate esters percentage in muscle and blubber in the species investigated.

valuable to contribute to a holistic picture as it is also shown from seven beluga whales (*Delphinapterus leucas*) investigated in the Beaufort Sea (Moore et al., 2020).

This study includes samples of the sperm whales stranded in 2016 in which 9 out of 22 dissected animals showed macro litter items in their stomach (Unger et al., 2016). With this background, this study wanted to shed light on the occurrence of the even smaller particles –MPs (<5 mm) and a potential connection between big and small plastic particles in situ.

The sperm whales showed the highest average regarding the MP burden (6.4 MPs per individual). This could have several reasons. Two presumptions are: 1) The necropsies showed that the sperm whales swallowed macro litter (9 out of 22 dissected individuals). A matching example is the sperm whale 2, which ingested several foreign bodies and showed the second highest count of MPs with 7 fragments within one faecal sample; 2) As suction feeders, they consume whatever is close to the prey they suck in. This increases the risk of MP ingestion, on the one hand by sucking in particles of that size, on the other by breaking down larger objects by chewing on them. Sperm whales were found with ingested marine debris in their stomachs as early as in the 1950s (Nemoto and Nasu, 1963). In our study, all investigated sperm whales, who showed a burden of ingested macro litter, MPs were identified. However, one of five individuals which all showed no indication of macro litter in the GIT, the highest MP burden was indicated (13 fragments, sperm whale 8). Similarities are found in the other three investigated toothed whale specimen (common dolphin, white-beaked dolphin, and long-finned pilot whale), where no macro debris in the GIT was found, and one individual showed a load of nine fibres in the faeces sample. Nevertheless, first evidence of  $3220 \pm 5810$  MPs larger than  $100 \mu\text{m}$  per kilogram of faeces is given for one individual of the here investigated sperm whales.

The MP findings do not allow any conclusion on their origin and, thus, no resulting statement about the pollution of the North Sea can be drawn. Moreover, the analysed animals did not strand in their natural habitat and are assumed to be misled into the North Sea by varying hypothesized factors (Pierce et al., 2007; Gilles et al., 2023). Thus, ingested macro- and micro debris, and potentially burdened prey can originate from other waters, than the North Sea.

The same is true for the long-finned pilot whale, which showed no MP presence. To the best of our knowledge, there is so far no publication investigating the MP occurrence in individuals from this species. Thus, no comparison can be drawn.

One of the most astonishing findings of this study is the fact that the neonate orca (which was at least three days old) (Schnitzler et al., 2019), was also found to have MPs in its faeces. The fact that the animal was obviously still being breastfed and not feeding on prey fish yet, makes this finding even more special. No other prey remains were found next to the breastmilk in the stomach (Schnitzler et al., 2019). Orca calves are nursed at least a year before consuming solid food (Ford, 2009). This raises the question how these MPs ended up in the intestine of such a young individual. There are two conceivable explanations for the amount of MPs found in this study: 1) secondary pollution caused the comparably high number of particles or 2) MPs were consumed by the calf during suckling by taking up seawater next to the milk. The uptake of MPs through breast milk is another assumption, but highly unlikely when focussing on the particle size range this study was looking at. Even when taking other size classes into account, recent studies showed the MP occurrence in human blood ( $> 700 \text{ nm}$ ) (Leslie et al., 2022) and in human placentas (5 -  $10 \mu\text{m}$ ) (Ragusa et al., 2021). Both studies are an indication that MPs can cross organic tissues. Another important evidence it is also the high level of PAEs (DEHP in particular) in the blubber of the neonate orca, which could be accumulated in the blubber and muscle tissues via maternal transfer during gestation and lactation. It has been demonstrated that phthalates can cross the placental barrier and are able to pass into breast milk, posing significant harm for the developing foetus and newborn due to undesirable effects on hormonal

levels acting as endocrine disruptors (Pocar et al., 2017).

The two investigated dolphins (common dolphin and white-beaked dolphin) showed no MP findings in one individual and 9 fibres in the other one. A comparable study conducted in the Atlantic found MP  $< 355 \mu\text{m}$  in 35 investigated stomachs of common dolphins MPs, whereof 97 % were fibres (Hernandez-Gonzalez et al., 2018). Moreover, Nelms et al. (2019) found MP larger than  $35 \mu\text{m}$  in every stomach of 50 marine mammals stranded around the British coast, whereof 43 were cetaceans. They found on average 5.5 MPs in the GIT. Thus, the found numbers of MPs in the herein presented study are fitting into the bigger picture of previous investigations worldwide.

The MP quantity is higher in Odontocetes (average 4.6 MPs) compared to Mysticetes (average 3.25 MPs), but the median values show slightly higher shares of fibres and fragments in Odontocetes. This is consistent with other studies dealing with macro litter findings in megafauna (Kühn et al., 2015; Meaza et al., 2021).

The three minke whales showed an MP share between 1 and 7 particles, thus every individual was positive for MP findings with fragments dominating. Only one other study has been published on MP presence in one minke whale stranded at the Japanese coast and reported only the presence of synthetic material within one intestinal section (Liu et al., 2023).

The studied fin whale (Mysticeti, baleen whales) did not show any MP occurrence. Whereas, former studies confirmed the presence of MP in ingested krill from stomach samples of fin whales and thus calculated a possible uptake of MPs between  $38.65 \pm 43.39$  and  $77.29 \pm 86.78$  particles per day (Garcia-Garin et al., 2021). Using DNA diet information for simulation modelling, Zantis et al. (2022) calculated an even higher MP uptake per day (3.4008 MPs; 95 % CI: 295). Moreover, the authors suggest that the high number of ingested particles was rather happening due to trophic transfer than the MP present in the environment. Furthermore, as filter feeders it is not surprising that plastic fibres were also found within the baleens of a fin whale in the Asian Sea (Im et al., 2020). Moreover, the two Mysticetes species (fin whale and minke whale) analysed in this study showed very high levels of PAEs in their blubber (average value:  $5177.43 \pm 4884.36 \text{ ng/g w.w.}$ ). The MP occurrence and its traceable substances like phthalates were already confirmed in fin whales from the Mediterranean Sea (Fossi et al., 2012; Fossi et al., 2014). Only one other study confirms plastic presence in a Mysticete found in the North Sea (Besseling et al., 2015): One humpback whale (*Megaptera novaeangliae*) stranded in 2012 in the Netherlands in which 45 suspected anthropogenic particles larger than  $500 \mu\text{m}$  were identified.

The present study supports the assumption of Nelms et al. (2019) that the stomach could be a retention site for artificial objects, since even litter was found in the stomachs of sperm whales in this study, while no MPs were found in the faeces or intestinal samples.

Even if higher numbers of MPs were found in male individuals, the median values of each sex are similar. No differences of MP presence in GITs between sexes were identified in previous studies dealing with cetaceans from different areas (Nelms et al., 2019; Novillo et al., 2020).

In addition to the 18 whales stranded in the North Sea, two additional individuals found in the Baltic Sea were investigated in this study (minke whale 3 and a common dolphin). Even if the findings in the latter mentioned one is nine fibres, no comparison is given in this study based on the small sample size. Since only two individuals could be investigated from this sea, a reliable basis for drawing conclusions on regional differences cannot be given at all. Thus, it is only given as a side note. The same is applied for the PAE levels, since the highest value was actually found in the minke whale 3 blubber which was found in the Baltic Sea, as well as different PAE fingerprints in both species. In further investigations a higher sample number from the Baltic Sea would be recommended, since the knowledge on the MP and phthalate burden in marine mammals from this sea is still scarce. Nevertheless, the presence of marine mammals, including seals and whales is generally lower in the Baltic than in the North Sea, what is additionally impeding the

acquisition of knowledge.

Originally, 53 out of 78 classified suspected MPs were manually transferred to AlOx filters for further  $\mu$ FTIR investigations. In 31 cases, the MPs could be successfully analysed. The high loss rate is caused by broken and lost particles during picking and transferring, and particles that went missing on the filter during the transport, and thus could not be found and analysed with  $\mu$ FTIR. In accordance, the here presented  $\mu$ FTIR-results are only confirming the presence of MPs in intestinal and faecal samples from the different cetaceans and are not re-presenting the whole occurrence of all potential polymer compounds. Twenty-two out of this successfully investigated pool of MPs were blank corrected resulting in 14 MPs found in the faeces and intestinal samples. The big share of the polymers PEST and PA even after considering the blank correction, is surprising since other marine mammals stranded in the North Sea and Baltic Sea showed a high presence of polyolefins (PE and PP), next to PA findings (Philipp et al., 2020; Philipp et al., 2021). PE and PP are the most widely used polymers in packaging material (PlasticEurope, 2022). On the other hand, water analysis in the transition zone from the North Sea to the Baltic Sea (Kattegat and Great Belt) showed high amounts of PEST (Lusher et al., 2021). Nevertheless, the cetacean species investigated in this study are not common in the North Sea and Baltic Sea, thus it needs to be considered that other water masses and ingested prey and items from other locations in the Atlantic can be responsible for the findings. However, in marine mammals stranded around the British coast higher amounts of PA and PEST were documented (Nelms et al., 2019), as well as within the digestive tract of the humpback whale that stranded in the Netherlands (Besseling et al., 2015). Findings of PA and PEST are well-reported around the globe as they originate from clothes, other daily life objects and fishing gear (De Falco et al., 2019; Erni-Cassola et al., 2019).

With regards to the PAEs accumulation, DBP and DEHP are among the most abundant compounds in all the species analysed. Similar data have been also reported for North Atlantic fin whales (Garcia-Garin et al., 2021), Mediterranean cetaceans (Baini et al., 2017), and other marine mammal species from the Norwegian Arctic (Routti et al., 2021). Different data, instead, was found for Odontocetes (killer whale, sperm whale, long-finned pilot whale, white-beaked dolphin, and harbour porpoise) from the Norwegian coast, where DINP was the most abundant PAE (Andvik et al., 2024). Killer whales and long-finned pilot whales from East Greenland showed no residues from DEP or DEHP in analysed blubber tissue (Pedersen et al., 2024). DEHP has high octanol-water and octanol-air partition coefficients, which could explain the high level of this compound found in the blubber tissue since its chemical properties facilitate its bioaccumulation in lipid-rich tissues.

A significant statistically positive correlation ( $p = 0.03$ ) has been found between the number of fragments and the DEHP concentrations in the muscle tissue (Table S11 and Fig. S12A) suggesting a potential release of this compound from these particles once ingested by the cetacean species. Leaching of this compound from polyolefin polymers, such as PE has been clearly demonstrated by Dhavamani et al. (2022) reporting a dissolving leachate rate ranging from  $25 \pm 2$  ng/cm<sup>2</sup> to  $79 \pm 5$  ng/cm<sup>2</sup> in seawater (varying salinity, temperature and UV exposure conditions). Moreover, its presence has been also found in several biological matrices (Di Bella et al., 2024), and marine mammal species (Baini et al., 2017; Routti et al., 2021; Sambolino et al., 2024). Indeed, DEHP is one of the most commonly used plasticizers and accounts for half of the total PE production in Western Europe (PlasticEurope, 2023). According to that, the gut surfactants, acidic conditions, and elevated temperatures (in warm-blooded animals) may facilitate its chemical desorption and accumulation in body tissues (Dhavamani et al., 2022).

A further analysis has been performed correlating the number of fragments and fibres ingested by the sperm whale with the PAE levels detected in the muscle and blubber tissues due to the larger number of organisms analysed for of this species. The strongly significant correlation observed between DAP concentration in blubber tissue and fibre content may stem from the direct release of this compound from

ingested particles. This is likely due to its role as a plasticizer and carrier, facilitating the addition of catalysts and pigments to polyester material (Tab. S13 and Fig. S13B) (Kandelbauer et al., 2013). A strong positive correlation has been found among the levels of DEHP and DBP, DIBP, DMP and DAP (Table S14 and Fig. S13A) in muscle tissue and DEHP and DBP and BBzP in blubber (Table S13 and Fig. S12B). The presence of low molecular weight phthalates like DMP, DAP, and BBzP may be due to the fact that these compounds are not deeply bound within the polymer structure but rather adhere to its surface, making them easily released into the environment or organisms upon ingestion (Liu et al., 2024). Moreover, the higher prevalence of DEHP, DBP, and DIBP is likely linked to their widespread use as plasticizers in plastic products (Beltifa et al., 2017).

It is worth highlighting that the analysis of PAEs in different tissues (as in the present study) helps to understand the load and partitioning of the different PAE compounds in the body compartments and the consequent route of exposure in these organisms. PAEs and their metabolites have been demonstrated to cause harm to organisms, including alteration of the endocrine system at different levels, also in marine mammals (Routti et al., 2021). The analysis of these compounds in cetacean species combined with MP is an important step to understand the effects of plastic and plastic additives.

## 6. Conclusion

Although it is very difficult to obtain samples from wild-ranging animals our study could integrate several species due to a well-established stranding network. Thus, our analysis delivers information to obtain a holistic picture. Nevertheless, based on the relatively small sample size per species and covered time frame of seven years, conclusions can only be tentative. The results are only giving hints about the MP and phthalate burden in Mysticetes and Odontocetes inhabiting the northern hemisphere. Nevertheless, evidence was found that both suborders are potentially burdened with artificial litter, small and large sized ones. Even though no MP was identified in the GIT, MP could still be identified in faecal samples. Thus, the transfer and excretion of MPs through the digestive tract of cetaceans is proven again. The presence of macro litter in the stomach and MP in the intestine of the same individual is not surprising, but can be seen as another hint that either 1) cetaceans are exposed to both (macro- and micro debris) and/or 2) the ingestion of macro debris results in the fragmentation of MPs, which are transferred through the intestinal system. Moreover, the presence of MP in individuals with empty stomach compartments allows for various hypotheses, e.g., 1) Did the individual ingest a larger synthetic object, but was able to break down those through activity in the gastrointestinal tract?; 2) and were able to transfer all of the particles into the following intestinal tract. In general, the following questions arise and are still unanswered: 1) Is MP persistent in the intestine, since it is hard to digest and excrete based on its small size?; and 2) How much MP can actually be egested? Those and other issues regarding the effects of plastic on the gastrointestinal tract and the whole individual itself still need to be further investigated. No strong conclusions can be drawn about the relation between ingested marine litter and a PAE occurrence in blubber and muscle tissue, since only one sperm whale showed high loads of PAE and a GIT full of macro debris. One the other hand, we have also low loads of PAEs in sperm whale individuals with ingested litter or none. Furthermore, the highest loads of PAEs were found in individuals which showed no evidence of ingested macro debris and a low amount of MPs (0 to 10 pieces) besides in the sperm whales. Thus, we need further information on 1) If ingested plastic or other marine litter objects are carrier and sources of PAEs, and 2) Does a burden in the water and of prey species play a higher role, than we know yet? Nevertheless, this study proves an association of ingested macro debris and MPs and the storage capability of PAEs in two target tissues (blubber and muscles) in marine apex species. The question of who is the main driver or carrier remains unknown: already loaded surrounding water and prey species

or ingested marine litter? Further studies are, therefore, needed to elucidate the ecotoxicological effects of both MP/marine litter and PAEs on the health of cetaceans.

### CRedit authorship contribution statement

**Bianca Unger:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Conceptualization. **Carolin Philipp:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Conceptualization. **Sonja M. Ehlers:** Writing – review & editing, Visualization, Methodology, Investigation. **Cristina Panti:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Matteo Bainsi:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Matteo Galli:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Maria Cristina Fossi:** Writing – review & editing, Writing – original draft, Supervision, Resources. **Jochen H.E. Koop:** Writing – review & editing, Writing – original draft, Resources. **Ursula Siebert:** Writing – review & editing, Writing – original draft, Supervision, Resources.

### Funding

There was no external funding used for conducting this study.

### 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.

### Acknowledgement

The authors would like to thank the seal rangers in Schleswig-Holstein (Germany) for reporting and supporting in the recovery from stranded marine mammals. Special thanks go to our colleagues in the ITAW for conducting the necropsies of especially those stranded cetaceans.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2025.118138>.

### Data availability

Data will be made available on request.

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