



First report of phthalate acid esters (plasticizers) in blubber of southern right whales (*Eubalaena australis*) from Península Valdés, Argentina

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Abstract

Phthalate acid esters (PAEs) are widely used plastic additives that have become ubiquitous contaminants in marine environments. Filter-feeding baleen whales may be exposed to these pollutants through water, prey or ingested plastic debris. While macroplastics have been reported in the gut of a southern right whale (SRW, *Eubalaena australis*), exposure to PAEs has not been assessed. Here, we quantified three lipophilic PAEs, dimethyl phthalate (DMP), dibutyl phthalate (DBP), and di-2-ethylhexyl phthalate (DEHP), in the blubber of 13 SRWs (three calves, three juveniles and seven adults) stranded in 2021 at Península Valdés, Argentina. More than 90% of samples contained detectable PAE levels, and 46.2% were above quantification limits. DEHP and DBP were the most prevalent compounds (61.5% and 30.8%, respectively), although the biological significance of the detected concentrations remains uncertain. This is the first report of PAEs in SRW blubber and suggests potential mother-calf transfer, highlighting exposure of Southern Hemisphere mysticetes to plastic-derived chemicals.

Keywords Dimethyl phthalate · Dibutyl phthalate · Di-2-ethylhexyl phthalate · Plastic pollution · Southwest Atlantic · Whale calf

Introduction

There is growing evidence that plastics pose significant threats to marine wildlife (Puskic et al. 2020). More than 1,400 species are known to interact with marine debris

through entanglement or ingestion (Claro et al. 2019). Cetaceans are among the most affected taxa (IWC 2019), and the effects of marine debris on these species are recognized as priority concern for international organizations such as the International Whaling Commission (IWC 2022) and

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the International Union for Conservation of Nature (IUCN 2025). However, a significant geographic imbalance exists in terms of data availability, with information being limited in the Southern Hemisphere (Fossi et al. 2018; López-Martínez et al. 2021).

Plastic additives such as phthalate acid esters (PAEs) are incorporated into polymers to enhance their flexibility and mechanical performance (Hahladakis et al. 2018). As these compounds are not chemically bound to the polymer matrix, they can gradually migrate into the environment and become bioavailable to marine animals through the water, contaminated prey, or directly via ingestion of plastic items (Baini et al. 2017; García-Garin et al. 2022). Most PAEs are hydrophobic and accumulate in lipid-rich tissues; however, they are rapidly metabolized and thus show limited potential for biomagnification within marine food webs (Zhang et al. 2021). Despite this, continuous release and chronic exposure to PAEs can adversely affect marine organisms, impacting metabolism, development, and reproductive, immune, and endocrine function (Zhang et al. 2021).

The occurrence of PAEs in mysticetes has been increasingly studied over the past decade, primarily in the Northern Hemisphere. PAEs have been reported in blubber of stranded specimens or skin biopsies, mainly from fin whales (*Balaenoptera physalus*) (Fossi et al. 2012, 2014, 2016; Baini et al. 2017; Routti et al. 2021; García-Garin et al. 2022; Galli et al. 2023), blue whales (*Balaenoptera musculus*) (Routti et al. 2021; Naz et al. 2022) and bowhead whales (*Balaena mysticetus*) (Routti et al. 2021). However, most studies have not examined the relationships between phthalate levels and key biological variables (e.g., sex, age) or trends over time, likely due to small sample sizes and/or the difficulties in obtaining data from free-living individuals (e.g., Baini et al. 2017; Fossi et al. 2012, 2014, 2016; Routti et al. 2021). Furthermore, because di-2-ethylhexyl phthalate (DEHP) is the most widely used and environmentally prevalent PAE, several studies have focused solely on this chemical and, in some cases, its primary metabolite (i.e., MEHP; Fossi et al. 2012, 2014, 2016). More recent studies have expanded the scope to include additional parent PAEs (Baini et al. 2017; Routti et al. 2021; Galli et al. 2023; García-Garin et al. 2022). While parent compounds are generally used to assess environmental exposure, metabolites may provide complementary information on internal biotransformation processes.

Studies in the North Atlantic Ocean suggest that baleen whales are particularly susceptible to microplastic ingestion leading to exposure to associated chemical additives. This vulnerability is largely attributed to their filter-feeding strategies (Kahane-Rapport et al. 2022) and their spatial overlap with oceanic microplastic accumulation zones or “hotspots” (Werth et al. 2024). García-Garin et al. (2021) estimated that

North Atlantic fin whales could ingest tens of thousands of microplastics particles daily, while Kahane-Rapport et al. (2022) suggested that krill-feeding blue whales may filter and potentially ingest 10 million microplastics per day. Together, these estimates underscore the substantial exposure of filter-feeding whale adults to both microplastics and their plastic-derived chemicals, although seawater and prey may also represent important exposure pathways for PAEs. Despite this, no studies to date have investigated PAE exposure in whale calves, even though maternal transfer may represent a route of early-life exposure (Sala et al. 2022). In humans, PAE metabolites have been detected in multiple maternal and neonatal matrices, including amniotic fluid (Jensen et al. 2012), placental tissue (Mose et al. 2007), umbilical cord blood (Lashley et al. 2004), meconium (Kato et al. 2006), and breast milk (Kim et al. 2020). Similarly, whale calves may be exposed to these lipophilic compounds through placental transfer during gestation and/or via milk during nursing, potentially compromising their health (Sala et al. 2022).

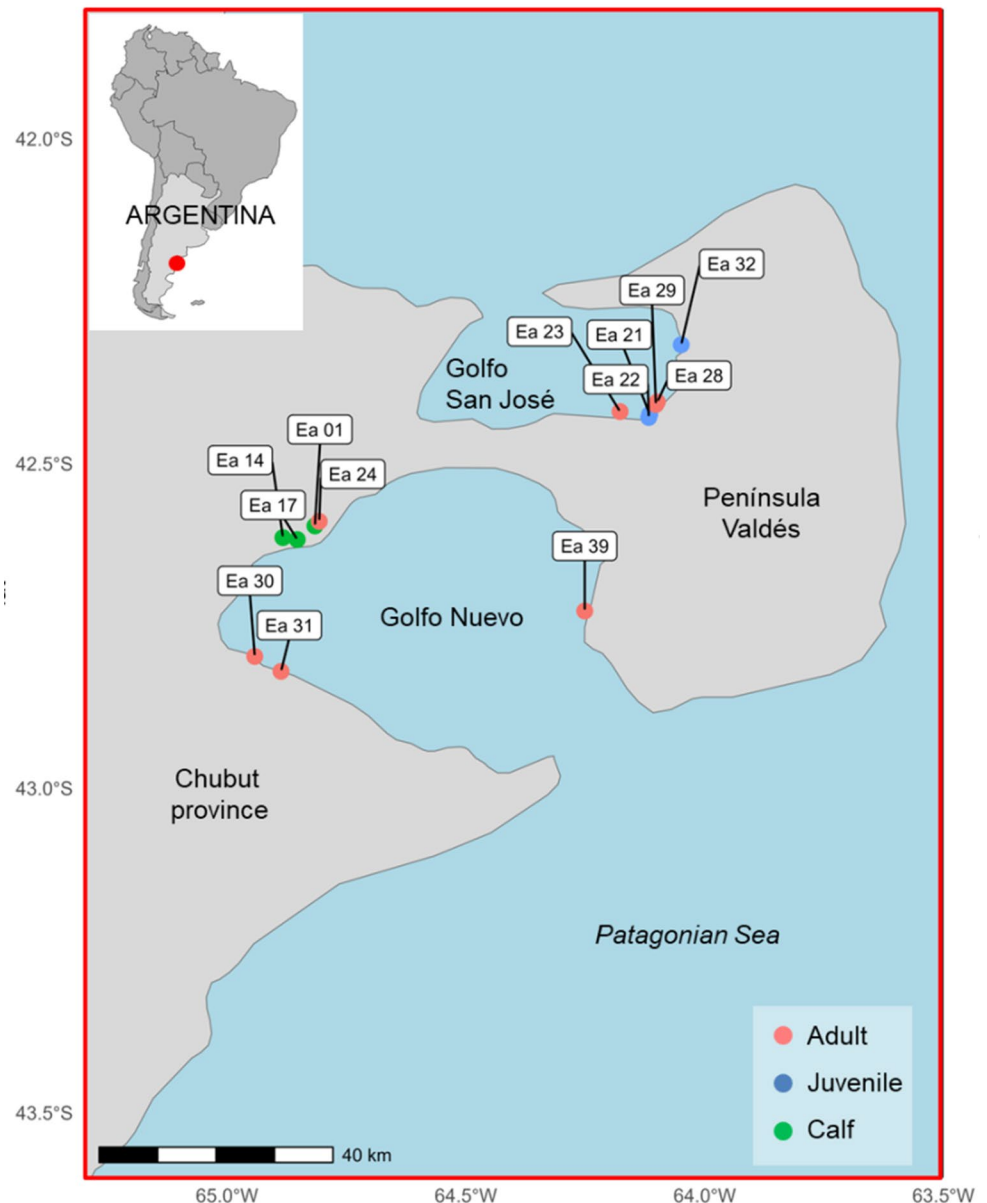
Southern right whales (SRW, *Eubalaena australis*) use the coastal waters off Peninsula Valdés, Argentina, as a winter breeding area, although feeding has been increasingly recorded in spring over the last decade (D’Agostino et al. 2023). While ingestion of macroplastics has been documented in one juvenile SRW (Alzugaray et al. 2020), no prior studies have examined PAE transfer and accumulation in this population. In this study, three PAEs, dimethyl phthalate (DMP), dibutyl phthalate (DBP), and di-2-ethylhexyl phthalate (DEHP), were quantified in the blubber of SRWs of multiple age categories that stranded dead at Península Valdés, Argentina, in 2021. These compounds were selected to represent PAEs with different molecular weights, lipophilicity profiles and toxicological relevance.

Methods

Sample collection

Samples of blubber (20 × 20 cm blocks) were collected from 13 whale carcasses (three calves, three juveniles, six adult females, and one adult male) from a total of 45 stranded in 2021 at Península Valdés, Chubut province, Argentina (Fig. 1). Records from the Southern Right Whale Health Monitoring Program show 2021 was a year of moderate mortality for this nursery ground (annual average 2003–2022, 49 strandings) (Rowntree et al. 2013; Uhart et al. 2016; McAloose et al. 2016; Sironi et al. 2023). Whales were necropsied following established standardized protocols. Carcass decomposition state was recorded following Geraci and Lounsbury (2005), and individual condition

Fig. 1 Study area in Península Valdés, Chubut, Argentina. Circles indicate the location where right whale carcasses were found stranded. Colors represent age classes (shown in legend), and the codes inside the boxes correspond to individual ID (Ea: *E. australis*)



codes are provided in Table S1. Age class of animals (i.e., calf, juvenile, adult) was estimated according to McAloose et al. (2016), based on a combination of morphological features including body length (snout to fluke notch), appearance of the umbilicus, snout-to-blowhole length as a percentage of body length, appearance of the blowhole and callosities, and the presence, color, and location of cyamids. Samples were stored in ziploc bags at -20°C until analysis. At the laboratory, subsamples of blubber from the core of the block (20 g mean weight) were extracted, wrapped in heat-treated aluminum foil (450°C for six hours) and conserved frozen at -20°C until analysis. To prevent cross-contamination between individuals, reusable utensils (i.e. knife and scalpel blades, tweezers) were washed with

detergent and MilliQ water, then rinsed sequentially with methanol, dichloromethane, and hexane before reuse.

Analytical methods

The analytical determination of phthalate acid esters (PAEs) in southern right whale blubber followed the validated protocol previously developed for seabird preen glands (Gallo et al. 2026), with specific modifications. A detailed, extended description of the standards and reagents, sample preparation, instrumental conditions and QA/QC procedures are available in the Supplementary Information. Briefly, 1 g of lyophilized blubber (dry weight) was fortified with isotopically labelled internal standards (DMP- d_4 , DBP- d_4 , DEHP- d_4 ; 1 mg/L). The extraction and cleanup

involved mechanical agitation-assisted acetonitrile extraction, dispersive solid-phase extraction (dSPE) with octadecylsilane (C18) sorbent, and a freeze-out lipid precipitation step. Final determination was performed using low-pressure gas chromatography coupled with tandem mass spectrometry (LPGC-MS/MS; Agilent 7890B–7000 C; helium carrier gas, splitless mode, 280 °C injector). The lowest calibration level was defined according to the instrument sensitivity and response characteristics, with six matrix-matched calibration points, ranging from 1.6 to 50 µg/L for DMP and from 6.3 to 200 µg/L for DBP and DEHP. Limits of detection (LOD) and limits of quantification (LOQ) were calculated from the standard deviation (SD) of the procedural calibration as: $LOD = 3 \times SD / \text{slope}$ and $LOQ = 10 \times SD / \text{slope}$ (ICH 2005). Method validation followed the SANTE/11312/2021v2 guidelines (European Commission 2024), yielding recoveries of 90–95% and LOQs of 10 ng/g dw (DMP) and 50 ng/g dw (DBP, DEHP). Procedural blanks were included in each analytical batch to verify background contamination. All glassware used was thoroughly cleaned with hot Extran® detergent (Merck Millipore) solution, rinsed with pesticide-grade petroleum ether (30–60 °C; Sintorgan, Argentina), oven-dried (250 °C, 4 h), and stored in heat-treated aluminum foil (450 °C, six hours) to prevent potential airborne contamination. Concentrations were first classified into two analytical categories: (1) detectable concentrations ($\geq LOD$) and (2) non-detects ($< LOD$, no confirmed instrumental signal). Detectable concentrations included: (1) quantifiable concentrations ($\geq LOQ$) and (2) “trace-level” concentrations (values for which an instrumental signal was detected, but that fell between the LOD and LOQ).

Statistical analyses

Detection frequency (%; no. individuals with detectable concentrations $\geq LOD$ / no. individuals evaluated), quantification frequency (%; no. individuals with concentrations $\geq LOQ$ / no. individuals evaluated), and frequency of trace levels (%; no. individuals with concentrations between LOD and LOQ / no. individuals evaluated) were calculated for each PAE. Descriptive statistics were calculated only for detectable concentrations. For this, trace-level detections were assigned a value of 50% of the LOQ for the respective analytical run, representing a central estimate within the known interval between the LOD and LOQ (Hornung and Reed 1990; Antweiler and Taylor 2008). This substitution represents a pragmatic midpoint estimate within the bounded interval of plausible concentrations and is consistent with commonly used approaches for handling left-censored chemical concentration data. Specifically, it aligns with the “middle-bound” framework proposed for censored contaminant datasets, in which concentrations below

quantification limits are assigned an intermediate estimate between lower-bound and upper-bound assumptions (EFSA 2010; USEPA 2022). Additionally, descriptive statistics were calculated separately for the subset of individuals with quantifiable concentrations (i.e. excluding those with trace levels). DMP was detected exclusively at trace levels; therefore, only detection frequency is reported.

Given the limited sample size and the high proportion of non-detects within some age classes, formal statistical comparisons among age classes were not performed. Instead, a heatmap was generated to visualize individual PAE concentration profiles and explore potential differences in exposure patterns among whales and age classes. Statistical analyses and graphics were performed with R version 4.5.1 (R Core Team 2025), using the packages “pheatmap” v1.0.13, “sf” v1.0.21, “ggplot2” v3.5.2, “rnatuarearth” v1.0.1, “ggspatial” v1.1.9.

Results

At least one PAE was detected in 92.3% of individuals (12 whales) and 46.2% of individuals (six whales) had PAEs at quantifiable levels (Table 1; see also Table S1 for individual metadata and quantification results). One calf showed simultaneous detection of all three PAEs, with DMP detected at trace levels ($< LOQ$), and DBP and DEHP detected at quantifiable concentrations. DMP was detected at trace level in six (46.2%) whales (i.e., one calf, three adult females, one adult male, and one juvenile). DBP was detected in four (30.8%) whales, including two calves, one adult female, and one juvenile. Quantifiable DBP concentrations were observed in three individuals (23.1%; two calves and one juvenile), whereas trace levels were detected in one adult female (7.7%). Median DBP concentration among quantifiable samples was 213.3 ng/g dw (range = LOQ–437.7 ng/g dw). DEHP was detected in eight (61.5%) whales, including three calves, three female adults, and two juveniles. Quantifiable DEHP concentrations were observed in six individuals (46.1%; three calves, one adult female, and two juveniles), whereas trace levels were detected in two adult females (15.4%). Median DEHP concentrations among quantifiable samples was 304.0 ng/g dw (range = LOQ–2,605.3 ng/g dw).

PAE concentrations by individual (including sex and age class) are presented in a heatmap (Fig. 2), with maximum values highlighted in red tiles. One sample (juvenile female Ea21) exhibited a DEHP concentration approximately 10 times higher than the others, reaching 2,605 ng/g. The heatmap reveals a distinct separation in the PAE concentration profiles between calves and older age classes, with the exception of sample Ea31 (adult female), which showed a similar pattern to calves.

Table 1 Phthalate acid esters (PAEs) detection and concentration (ng/g, dry weight) in the blubber of southern right whales stranded dead in 2021 at Península Valdés, Argentina. Proportion of samples (frequency) with: detectable levels (no. individuals with detectable levels $\geq$ LOD / total individuals), quantifiable levels (no. individuals with levels $\geq$ LOQ / total individuals), and trace levels (no. individuals with detectable levels $<$ LOQ / total individuals). Descriptive statistics for PAEs concentrations (mean $\pm$ SD and median) were calculated for two data subsets: (1) detectable (all detectable, including trace levels), (2) quantifiable ($>$ LOQ only, excluding trace levels). Maximum values are also provided for each PAE. Values in uppercase indicate statistics calculated excluding the outlier sample Ea21 (DEHP = 2,606.3 ng/g)

	Dimethyl phthalate	Dibutyl phthalate	Di-2-ethyl-hexyl phthalate
Frequency (positive/total) %			
Detectable	6/13 46.2%	4/13 30.8%	8/13 61.5% ^(58.3)
Quantifiable	0/13 0%	3/13 23.1%	6/13 46.2% ^(41.7)
Trace levels	6/13 46.2%	1/13 7.7%	2/13 15.4% ^(16.7)
Median			
Detectable	—	148.8	217.7 ^(134.3)
Quantifiable	—	213.3	304.0 ^(301.0)
Mean $\pm$ SD			
Detectable	—	190.1 $\pm$ 182.8	477.1 $\pm$ 869.5 (173.1 $\pm$ 139.5)
Quantifiable	—	245.1 $\pm$ 178.8	627.8 $\pm$ 974.5 (232.3 $\pm$ 118.0)
Maximum	5.0	437.7	2,605.3 ^(341.3)

Notes: For samples with detectable PAE concentrations $<$ LOQ, 50% of the LOQ value was used for statistical analysis

Discussion

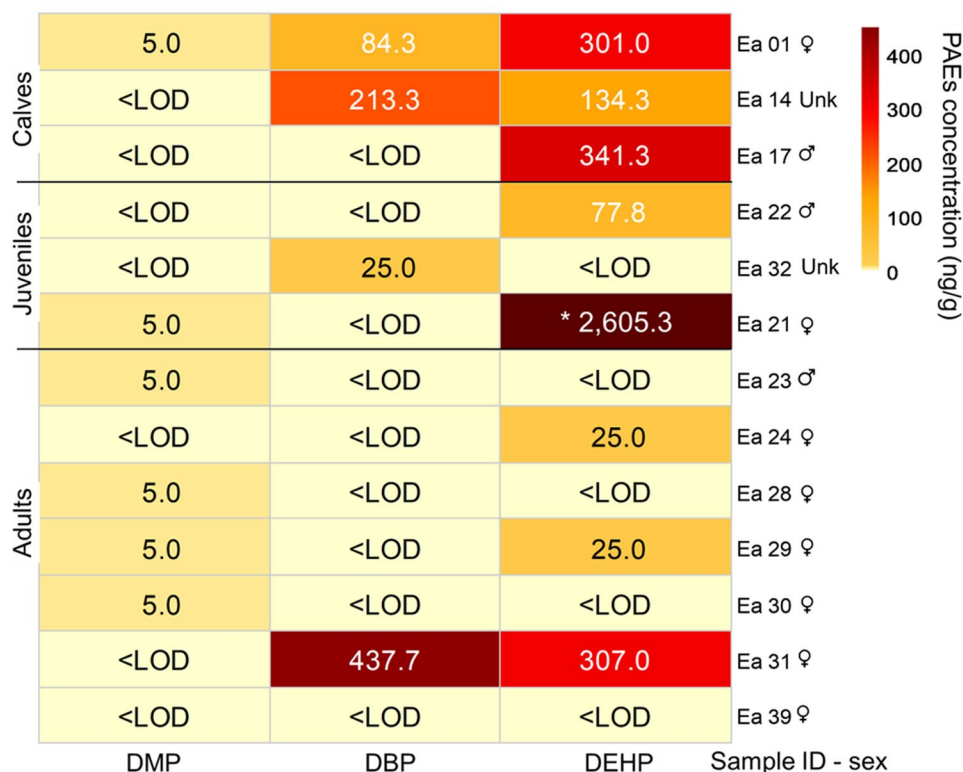
Our findings represent the first evidence of PAEs exposure in SRW from Península Valdés, and the first worldwide documentation of their accumulation in whale calves. The detection of at least one PAE in 92.3% of whales, with nearly half (46.2%) showing quantifiable levels, indicates that exposure to plastic-derived chemicals is widespread in this population. These results provide new data on PAE occurrence in mysticetes in the Southern Hemisphere and support the need to better assess the potential health effects on whales.

Among the compounds analyzed, DEHP showed the highest occurrence and concentrations, followed by DBP, whereas DMP was detected only at trace levels. Median concentrations of DBP and DEHP were similar or lower than levels previously reported in mysticetes from the Northern Hemisphere, including fin whales (Table S2; see Baini et al. 2017; Routti et al. 2021; García-Garin et al. 2022; Galli et al. 2023). Conversely, earlier studies on Mediterranean and Cortez Sea fin whales analyzing blubber from stranded dead and biopsies from live animals, did not

detect DEHP; instead, its primary metabolite (i.e., MEHP), was proposed as a marker of DEHP exposure (Fossi et al. 2012, 2014, 2016). DMP has only been measured in mysticetes by García-Garin et al. (2022), who reported median concentrations (8 ng/g dw) in fin whale muscle tissue. In our study, DMP was detected exclusively at trace levels. Overall, this pattern possibly reflects greater exposure to plastic additives in northern oceanic feeding grounds (Baini et al. 2017; Routti et al. 2021; Galli et al. 2023; García-Garin et al. 2022). Notwithstanding, comparisons between studies should be interpreted with caution due to differences in the tissues (e.g., blubber or muscle) and animals (e.g., live or dead, mostly adults) analyzed, analytical methods employed, measurement units adopted (ng/g wet, dry or lipid weight), and detection and quantification thresholds used for reporting results. In particular, reported mean or median values often include individuals with concentrations below the limit of detection (LOD), for which different substitution approaches are applied across studies (Table S2; e.g., assigning the value of the LOD, 50% of the LOD, or zero). In addition, although some studies explicitly report how values below the limit of quantification (LOQ) are handled, the approaches vary across studies (e.g. assigning the value of the LOQ or 50% of the LOQ). Such methodological variability can significantly influence reported concentrations, especially in datasets with low detection frequencies. Considering these methodological differences among studies, median concentrations of DEHP and DBP in this study were within the range previously reported in mysticetes (Table S2). Additionally, because most analyzed samples were from moderately to highly decomposed carcasses, decomposition-related effects on measured PAE concentrations cannot be excluded. Borrell and Aguilar (1990) reported losses of lipophilic contaminants such as POPs, in tissues of stranded dolphins, suggesting that decomposition-related lipid loss may contribute to underestimation of lipophilic compounds in blubber, particularly on a wet-weight basis. In this study, concentrations were expressed on a dry-weight basis, which may reduce some variability. Importantly, studies on southern right whale calves found no significant changes in blubber lipid content with carcass decomposition state (Marón et al. 2018). However, to our knowledge, no studies have evaluated the effects of decomposition on phthalate concentrations in marine mammal blubber, making this an important topic for future research.

Experimental studies on animals and clinical studies in humans have shown that several PAEs, including DEHP and DBP, can impair development, reproduction, metabolism, immune function, and endocrine regulation, as well as induce neurotoxicity and carcinogenicity (Benjamin et al. 2017; Zhang et al. 2021). Moreover, studies examining co-exposure scenarios have revealed that PAEs can interact with

Fig. 2 Heatmap for the concentrations of the three PAEs (DMP=dimethyl phthalate, DBP=dibutyl phthalate, DEHP=di-2-ethylhexyl phthalate) analyzed in the blubber of southern right whale carcasses ($n=13$) stranded at Península Valdés, Argentina, in 2021. Each row represents an individual whale identified by sample code and sex (♀ = female, ♂ = male, Unk=sex undetermined), grouped according to age class (calf, juvenile, adult). Values between the limits of detection (LOD) and quantification (LOQ) were classified as “trace level” concentrations, and assigned a value of 50% of LOQ (i.e., DMP=5ng/g, DBP and DEHP=25ng/g). Non-detected values are labeled as <LOD (i.e., DMP=<2 ng/g, DBP and DEHP=<10 ng/g). The outlier DEHP concentration in sample Ea 21 (2,605.3 ng/g) exceeds the color scale range and is shown manually with an asterisk (*)



other environmental contaminants, producing either synergistic or antagonistic toxic responses (Xu et al. 2025). More recently, in vitro gene assays using blubber tissue from fin whales demonstrated that DEHP has the potential to modulate the transcriptional activity of thyroid hormone receptor beta at experimental concentrations of 25 and 50 μ M (Routti et al. 2021). DEHP concentrations detected in the present study were comparable to those reported for fin whales from the Norwegian Arctic in the same study. Routti et al. (2021) concluded that DEHP concentrations detected in Norwegian Arctic fin whales were below the experimental thresholds associated with receptor modulation. Therefore, although direct comparisons between experimental and field-derived tissue concentrations should be interpreted cautiously due to differences in reporting units, our results suggest that DEHP levels detected in SRWs were also below these experimental effect levels (Routti et al. 2021). Nevertheless, biologically meaningful toxicity thresholds for PAEs have not been established for SRWs or other baleen whales under natural exposure conditions, and the health significance of the concentrations detected here remains uncertain. However, as previous studies have shown that long-term PAE exposure can induce toxicity in aquatic organisms even at μ g/L levels (Xu et al. 2025), further research is needed to assess potential health impacts of persistent low-level exposures on SRW and other long-lived mysticetes. Several authors have suggested incorporating “omics” approaches (e.g.,

genomics, transcriptomics, metabolomics) to elucidate the alterations occurring in metabolic and gene expression pathways in marine organisms exposed to environmental contaminants (de Lima et al. 2025).

Microplastics are proposed as a major source of PAEs in aquatic environments (Cao et al. 2022). Mysticetes can ingest large quantities of microplastics while filter-feeding (Kahane-Rapport et al. 2022). Nevertheless, exposure through both seawater and prey (e.g. plankton) may also represent additional pathways for PAE uptake in SRW and other mysticetes. Several studies conducted in the Mediterranean Sea have reported high concentrations of PAEs (including DEHP and its primary metabolite MEHP) in superficial neustonic/planktonic samples collected using a manta trawl (Fossi et al. 2012; Baini et al. 2017). These samples typically contain microplastics and zooplankton, to which fin whales are commonly exposed while filter-feeding. SRWs use the coastal waters off Península Valdés as a winter breeding and calving area, although feeding has been increasingly recorded in spring over the last decade (D’Agostino et al. 2023). Moreover, PAEs have been reported in seawater, snow, and krill samples from Antarctica (Cincinelli et al. 2005; Han and Liu 2017; Zhang et al. 2018; Motas et al. 2025), which is an important feeding area for the SRW population from Península Valdés (Valenzuela et al. 2018; Agrelo et al. 2021). Future research should

assess the presence and concentrations of PAEs in seawater and prey from key areas in SRW life cycle.

Most studies reporting PAE concentrations in mysticetes do not explore associations with biological variables such as age or sex, likely due to limited sample number of available samples and the challenges in obtaining such information when sampling free-ranging animals. For instance, García-Garin et al. (2022) reported no significant association between PAEs levels and age or sex in North Atlantic fin whales ranging from 7 to 41 years old. Despite the small sample size, the heatmap visualization suggests heterogeneous patterns of PAE exposure and/or accumulation among whales and ages classes in this study. Moreover, this study represents the first report of PAEs in whale calves worldwide. With the exception of one adult female (Ea31) likely in a resting year and thus not transferring contaminants during gestation and/or nursing, calves tended to show higher concentrations of PAEs than older age classes, primarily driven by DEHP levels. This, together with differences between individual calves, suggests likely maternal transfer of plasticizers, which could impair calf health during early life stages; a critical period characterized by high mortality rates among perinates and calves-of-the-year at Península Valdés (Rowntree et al. 2013; McAloose et al. 2016). Future studies including larger sample sizes and broader demographic representation will be necessary to robustly evaluate age-related patterns of PAE exposure and accumulation in SRWs.

The detection of PAEs in SRWs across all demographic categories underscores the need for continued monitoring of exposure to plastics and plastic additives in this population. The Southern Right Whale Health Monitoring Program has been collecting tissue samples from SRW strandings at Península Valdés since 2003, providing a valuable long-term dataset to assess temporal trends, potential maternal transfer of PAEs (through lactation, and/or placenta), age- and sex-related differences in accumulation and elimination or excretion, and other relevant questions. Forthcoming studies will allow exploration of outstanding questions as well as insights on the health effects of early-life and chronic exposure to plastic additives in baleen whales.

SRWs and other marine megafauna play important ecological roles and are charismatic and iconic species. They can therefore act as sentinels of ocean health, raising awareness among scientists, policymakers, and local communities, and promoting collective actions to mitigate marine plastic pollution (IWC 2019). This study provides scientific evidence of the ubiquity of plastic-derived contaminants, including their presence in a socially and economically-relevant marine species like the SRW that is protected in a UNESCO World Heritage Site (UNESCO 1999). Regulating these chemicals of concern is a necessary step to

safeguard public and environmental health and ensure long-term benefits for both ecosystems and society (Farrelly et al. 2025). Global actions and consumer behavioral change are urgently needed to halt marine plastic pollution.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00300-026-03508-8>.

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Data availability All data supporting the findings of this study are available within the paper and its Supplementary Information.

Declarations

Ethical approval Not applicable.

Competing interests The authors declare no competing interests.

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