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The deep dive of organohalogen compounds: Bioaccumulation in the top predators of mesopelagic trophic webs, pygmy and dwarf sperm whales, from the Southwestern Atlantic ocean

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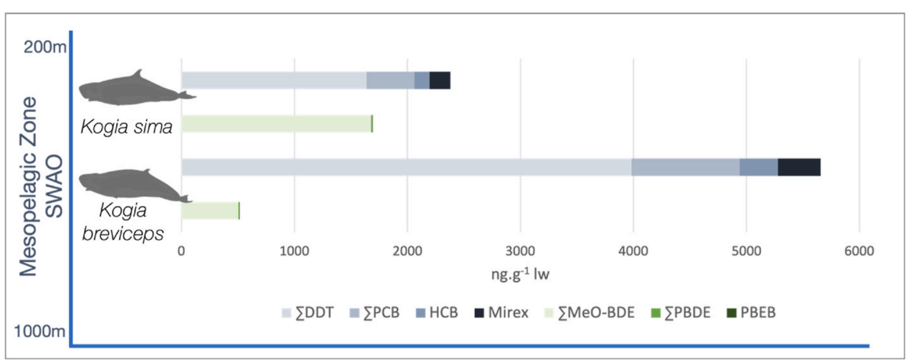
HIGHLIGHTS

- The mesopelagic *Kogia sima* and *K. breviceps* can be sentinels of deep-water habitats.
- DDTs were the main group of organochlorines and MeO-BDEs of organobromines detected.
- PBEB, an emergent pollutant, was detected in these mesopelagic predators.
- *K. sima* presents higher concentration of natural and *K. breviceps* of synthetic compounds.
- Differences in the profile suggest niche segregation in these sympatric species.

GRAPHICAL ABSTRACT



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ABSTRACT

Kogia sima and *Kogia breviceps* are apex predators of mesopelagic trophic webs being far from most anthropogenic threats. However, chemical pollutants and naturally synthesized compounds may travel long distances. This study aimed to use kogiid whales as sentinels of mesopelagic trophic webs in the Southwestern Atlantic Ocean. Persistent organic pollutants (POPs), e.g., polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT) and metabolites, mirex, hexachlorobenzene (HCB), polybrominated diphenylethers (PBDEs), pentabromoethylbenzene (PBEB) and hexabromobenzene (HBB), and the naturally produced methoxylated BDE (MeO-BDEs) were determined in the blubber of 16 *K. sima* and 15 *K. breviceps*. Among the organochlorine compounds, DDTs were the main group found in *K. sima* and in *K. breviceps* (1636.6 and 3983.3 ng g^{-1} lw, respective medians), followed by PCBs (425.9 and 956.1 ng g^{-1} lw, respectively), mirex (184.1 and 375.6 ng g^{-1} lw, respectively), and HCB (132.4 and 340.3 ng g^{-1} lw, respectively). As for the organobromine, the natural MeO-BDEs were predominant (1676.7 and 501.6 ng g^{-1} lw, respectively), followed by PBDEs (13.6 and 10.3 ng g^{-1} lw, respectively) and PBEB (2.2 and 2.9 ng g^{-1} lw, respectively). In general, POPs concentration was higher in *K. breviceps* than in *K. sima*. Conversely, MeO-BDEs concentration was higher in *K. sima* than in *K. breviceps*. Differences in concentrations in these sympatric odontocetes were attributed to distinct species, sampling sites, and biological parameters and suggest some level of niche segregation. It is noteworthy the long-range reach and bioaccumulation of these synthetic compounds in an unexplored habitat, that present an increasing economic interest.

1. Introduction

The mesopelagic zone, between 200 and 1000 m deep, presents cold and nutritive waters with biomass relying greatly on the dynamics of water masses and encompasses several species that perform diel migrations (Siegelman-Charbit and Planque, 2016; Sutton et al., 2017). The diversity and main threats of the mesopelagic zone remain mostly unknown given the hardship of accessing it. Still, there is an increasing interest in understanding the feasibility of mesopelagic fisheries as a source of protein and oil for human and animal consumption posing an alternative to epipelagic trawling which borders on unsustainability (Dowd et al., 2022; Prellezo, 2019). Hence, increasing the relevance of comprehending how anthropogenic threats can affect it (Romero et al., 2018).

The presence of persistent organic pollutants (POPs) is a potential problem for the exploitation of mesopelagic biota as food sources not yet addressed extensively. Organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), and brominated flame retardants (BFRs) were indiscriminately applied for many years until their toxic effects started to be described (Diamond et al., 2010; Jepson and Law, 2016; Van Den Berg et al., 2012). A few decades ago, the Stockholm Convention on POPs placed regulation policies concerning the production, application, and discard of legacy and emerging POPs in an attempt to decrease the environmental levels of these compounds, and their deleterious consequences for the exposed organisms (UNEP, 2017). These lipophilic compounds are easily susceptible to atmospheric transport and have already been reported in remote locations far from their emission sources, including the poles (Borgå et al., 2004; Nöel et al., 2018) and oceanic trenches (Jamieson et al., 2017). Moreover, these pollutants can undergo biomagnification in trophic webs, reaching elevated concentrations in top predators (Gray, 2002; Losada et al., 2009), and pose a serious risk to odontocete populations which have been predicted to decline due to their high bioaccumulation (Desforges et al., 2018; Oliveira-Ferreira et al., 2021).

Conversely, naturally synthesized organobromine compounds like the methoxylated brominated diphenyl ethers (MeO-BDEs) fit as indicators of environmental conditions, being produced by algae, sponges, and associated organisms (Barón et al., 2015; Vetter et al., 2002). The ecosystem community composition is reflected directly in the profile found in apex predators (e.g., Oliveira-Ferreira et al., 2022), and cetaceans, particularly those close to coral reef systems, tend to accumulate elevated concentrations of these natural compounds around the world (Alonso et al., 2014).

Cetaceans are important marine sentinels due to intrinsic characteristics, like longevity, elevated trophic position, and the presence of a thick blubber layer that favors the bioaccumulation of lipophilic compounds (Bossart, 2011; Ross, 2000; Schwacke et al., 2013). Hence, odontocetes with mesopelagic foraging habits can be used to track the vertical reach of POPs and the presence of biogenic compounds in deep waters and can help shed some light on their transport and partitioning in oceanic food webs.

The pygmy, *Kogia breviceps*, and dwarf, *K. sima*, sperm whales are the two species of the Kogiidae family. These odontocetes are distributed in the deep tropical to temperate waters of the Atlantic, Pacific, and Indian Oceans (Jefferson et al., 2015). The two species present a certain overlap in distribution, but there is a difference in latitudinal habitat preference in the Southwestern Atlantic Ocean (SWAO) since *K. sima* seems to prefer warmer waters whereas *K. breviceps* is more likely to be found in colder and highly productive environments (Bearzi, 2005; Bloodworth and Odell, 2008; McAlpine, 2018; Moura et al., 2016). Both species feed mainly on offshore cephalopods that occupy mesopelagic and bathypelagic zones (dos Santos and Haimovici, 2001), as well as on deep-water shrimp and fish species (Gurjão et al., 2003; Pinedo, 1987; Secchi et al., 1994), supporting their presence beyond the continental shelf and slope.

These semi-cryptic species are rarely observed in their natural habitat and are hard to differentiate in the field because of morphological similarities, like body shape, color, and intersecting size range (Jefferson et al., 2015; McAlpine, 2018). Hence, some biological and ecological features of *Kogia* species, e.g., habitat use, foraging habits, population structure, and anthropogenic threats are still a mystery. Thus, the assessment of POPs and natural organohalogen compound concentrations in both *Kogia* species can provide valuable information, such as differences in the ecological niche of those sympatric species along their distribution in Brazilian waters, where there is extremely limited data available.

Hence, this study aims to address the bioaccumulation of POPs and contaminants of emerging concern in the SWAO, as well as the presence of naturally produced organobromine compounds in mesopelagic trophic webs using *K. breviceps* and *K. sima* as sentinels. This study also seeks to investigate differences between the species and the influence of biological parameters in these deep-water apex predators.

2. Materials & methods

2.1. Study site

The 8000 km of the Brazilian coastline, ranging from 4°N to 34°S in the Atlantic Ocean, comprises an important habitat for several cetacean species (Di Tullio et al., 2016; Meirelles et al., 2009; Rossi-Santos et al., 2006). Different and complex oceanographic processes, mainly driven by the Northern Brazil Current, the Brazil Current, the upwelling systems of the South Brazil Bight, and the Malvinas/Falklands Current, contribute to the diverse types of habitats in the continental shelf and slope that vary from warm and oligotrophic, to highly productive and cold waters (Castro et al., 2005; Silveira et al., 2000).

A biogeographic pattern of mesopelagic zones also separates Brazilian waters roughly into north and south of Cape São Tomé, based on biotic and abiotic regional information (Fig. 1) (Sutton et al., 2017). The unstable meanders around the region of Cape São Tomé, in Rio de Janeiro state (RJ), favor the upwellings of deep-waters and pose an important oceanographic feature that influences the areas north and south of the cape differently (Mill et al., 2015; Palóczy et al., 2014; Sutton et al., 2017).

The multifaceted oceanographic processes influencing the continental shelf and slope of Brazilian waters have implications for the different latitudinal distribution patterns of the two kogiid species in the Southwestern Atlantic Ocean (Moura et al., 2016). These may also contribute to the dynamic of contaminants from this area. Agricultural processes, wood conservation processes, and control of vector-borne diseases were important sources of legacy OCPs in the environment (Kaiser, 1978; Van Den Berg et al., 2012). Additionally, the industrialization and heavy urbanization of coastal areas resulted in the input of organohalogen contaminants such as PCBs and BFRs (De Wit, 2002; Diamond et al., 2010). These pollutants can leachate from soil and landfills and/or undergo atmospheric transport to oceanic zones, thus entering oceanic trophic webs far from their original emission sources (Jamieson et al., 2017).

2.2. Sampling

Samples of *K. breviceps* (n = 15) and *K. sima* (n = 16) were obtained from carcasses stranded along the Brazilian coast, in the states of Ceará (n = 3 *K. breviceps* and n = 12 *K. sima*), Bahia (n = 2 *K. breviceps*), and Espírito Santo (n = 1 *K. sima*), north of Cape São Tomé, and Rio de Janeiro (n = 1 *K. breviceps* and n = *1 K. sima*), Santa Catarina (n = 6 *K. breviceps* and n = 2 *K. sima*), and Rio Grande do Sul (n = 3 *K. breviceps*), in Southern Brazil, totaling 31 specimens between the years of 2005 and 2022 (Table S1, Supporting Information). Necropsy of individuals followed a standard methodology (Geraci and Lounsbury, 2005). Blubber samples were collected, and frozen at −20 °C until analysis. Only fresh carcasses (classified as codes 2 and 3; Geraci and Lounsbury, 2005) were included in the present study. Biological information such as sex, total length (TL), and decomposition code were obtained during the necropsy. Sexual maturity was determined based on individuals' TL information available in the literature for other populations (Plön, 2004) and/or macroscopic evidence observed during the necropsy (e.g., pregnancy and lactation).

2.3. Organohalogen compounds analyses

The methodology for analyses of organohalogen compounds was based on Oliveira-Ferreira et al. (2022). Approximately 0.5 g of blubber samples were homogenized with anhydrous Na_2SO_4, spiked with chlorine and bromine internal standards (PCB 103 + PCB 198 and PBDE 181, respectively), and extracted via Soxhlet for 8 h on dichloromethane and *n*-hexane (1:1). An aliquot of the extract was used for gravimetric determination of lipid content. The extract purification followed an acidic attack with H_2SO_4 and a two-step elution in Al_2O_3 cartridge with dichloromethane and *n*-hexane (2:1) and dichloromethane and methanol (9:1). The purified extract volume was reduced in nitrogen flow. Analyses were performed in an *Agilent Technologies 7890* Gas Chromatographer equipped with a silica capillary column *HP-5MS* using ultrapure helium (99,999% pure) as the carrier gas, coupled to an *Agilent*

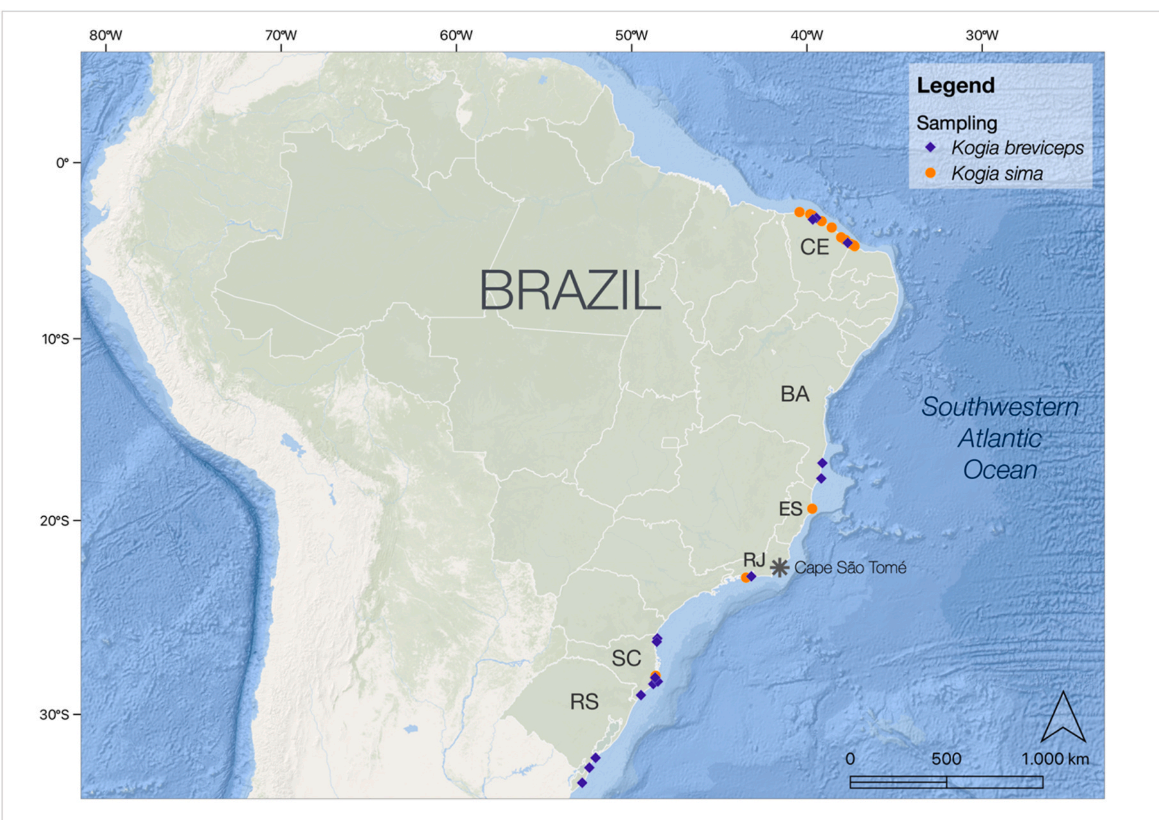

Fig. 1. Location of strandings of Kogiidae individuals sampled along the Brazilian coast, including the states of Ceará (CE), Bahia (BA), Espírito Santo (ES) - north of Cape São Tomé, and Rio de Janeiro (RJ), Santa Catarina (SC), and Rio Grande do Sul (RS) - south of Cape São Tomé.

*Technologies 597*5 Mass Spectrometer (GC/MS).

An aliquot of 2 μL of the purified sample was injected at 280 °C and 13psi by *Agilent Technologies 7683B* Automatic Injector and the chromatographic run for organochlorine compounds analyses lasted 67 min. The temperature slope started at 70 °C for 1 min, and increased 40 °C/min until 170 °C, 1.5 °C/min until 240 °C and, finally, 15 °C/min until 300 °C. The GC/MS operated on Electron Impact (EI) and Selected Ion Monitoring (SIM) mode. In this methodology were analyzed 28 PCBs congener numbers: 8, 28, 31, 44, 49, 52, 70, 74, 97, 99, 101, 105, 118, 132, 138, 141, 151, 153, 158, 169, 170, 177, 180, 183, 187, 194, 195, and 206 (ΣPCB); and the OCPs: the *p.p'* isomer of dichlorodiphenyltrichloroethane (*p.p'*-DDT) and its metabolites dichlorodiphenyldichloroethylene (*p.p'*-DDE) and dichlorodiphenyldichloroethane (*p.p'*-DDD) (ΣDDT); hexachlorobenzene (HCB); and mirex.

The injection for organobromine compounds analyses followed the parameters as described for organochlorine. The chromatographic run lasted for, approximately, 82 min, starting at 110 °C for 1 min, 8 °C/min until 180 °C and 2 °C/min until 300 °C. The GC/MS operated in a Negative Chemical Ionization source (NCI) using ammonia as ionizing gas, also on SIM mode. In this study, seven polybrominated diphenyl ethers (PBDE) of primary interest congener numbers: 28, 47, 99, 100, 153, 154, and 183 (ΣPBDE); pentabromoethylbenzene (PBEB); hexabromobenzene (HBB); and eight naturally-produced methoxylated BDEs (MeO-BDE): 6-MeO-BDE 47, 5-MeO-BDE 47, 4′-MeO-BDE 49, 2′-MeO-BDE 68, 5′-MeO-BDE 99, 5′-MeO-BDE 100, 4′-MeO-BDE 101, and 4′-MeO-BDE 103 (ΣMeO-BDE) were contemplated.

Data integration was performed in the *Agilent Technologies* software *Enhanced ChemStation* and compounds concentrations were calculated on an individual lipid basis (ng.g^{-1} lw).

2.4. Quality control/quality assurance (QC/QA)

QC/QA approaches for this study included the use of organohalogen-certified standards for calibration curves, recovery calculation of internal standards, sample fortification, analysis of reference material, and use of procedural blanks in every analysis batch. Organochlorine standards and PBDEs of primary interest were purchased from *AccuStandard Laboratories*, PBEB and HBB standards from *Cambridge Isotope Laboratories*, and MeO-BDEs from *Wellington Laboratories*. A ±30% variation was accepted for internal standards recovery. For organochlorine, the ISTD recovery was on average 90.4% ± 16.5% (mean ± standard deviation), whereas for organobromine was 94.6% ± 15.9% (mean ± standard deviation). Furthermore, Standard Reference Material® 1945 from the National Institute of Standards and Technologies (NIST), consisting of pilot-whale blubber was analyzed. For all analytes, the mean recovery of the SRM®1945 was 82.4% ± 11.0%. In addition, sample fortification of randomly assigned *Kogia* individuals were spiked with POPs and MeO-BDEs standards solution for peak confirmation.

Limit of detection (LOD) was considered three times the standard deviation of five multiple injections and is expressed in ng.mL^{-1}, while limit of quantification (LOQ) was considered the LOD by the average mass of samples and it is expressed in ng.g^{-1} lw (Tables S2 and S3, Supporting Information; Long and Winefordner, 1983). Compounds found below these values were discarded from statistical treatment of data. Analytical blanks were used to detect contamination during the procedure and no compounds were detected above LOD.

2.5. Data analyses

Data analyses were performed in the software STATISTICA 10.0®. Descriptive statistical analyses and normality test (Kolmogorov-Smirnov test) were used to understand the data distribution and the variance around central values. A Mann-Whitney test was applied to investigate the differences in the organohalogen compounds concentrations between the species. Because statistical differences were detected between species (see section *3.2 Organohalogen compounds in Kogiidae from the SWAO*), further statistical treatment was performed for each species separately. To investigate the influence of biological parameters (sex, sexual maturity, and total length) on the concentrations of compounds, a Kruskal-Wallis test paired with a test for multiple comparisons and a Spearman correlation were performed. The Kruskal-Wallis test was used to explore differences between mature males, mature females, and immatures, while the Spearman correlation was used to assess the relationship between the total length and the concentrations in immature and mature females, and in immature and mature males. Furthermore, differences between north and south of Cape São Tomé were investigated with a Mann-Whitney test for each species.

3. Results & discussion

3.1. The deep dive of organohalogen compounds

The mesopelagic zone is a vast and diverse habitat and the arrival of organohalogen compounds to this environment is perceived by sentinel species, such as the *Kogia* species. The accumulation profile detected in *K. sima* was ΣMeO-BDE > ΣDDT > ΣPCB > mirex > HCB > ΣPBDE > PBEB, whereas for *K. breviceps* it consisted of ΣDDT > ΣPCB > ΣMeO-BDE > HCB > mirex > ΣPBDE > PBEB (Fig. 2, Table 1), differing among the species. HBB was not detected above the LOD in any specimens (Table 1; Tables S5–S8 in Supporting Information). The general profile highlights the increased contribution and elevated concentrations of the natural organobromine compounds in *K. sima*, in contrast to the predominance of POPs, e.g., DDTs and PCBs, in *K. breviceps*.

Regarding the anthropogenic compounds, the dominance of DDTs in the profile differs from what is currently observed for coastal cetacean species in the SWAO, in which PCBs are predominant (Durante et al., 2016; Lailson-Brito et al., 2012; Oliveira-Ferreira et al., 2021; Santos-Neto et al., 2014), except when an unusual event is responsible for the availability of previously immobilized OCPs (Oliveira-Ferreira et al., 2022).

Both PCBs and DDTs are transported down along with sinking particles, but DDTs are largely adsorbed to suspended matter (Tanabe and Tatsukawa, 1983), promoting a rapid downward movement. Moreover, the long-range transport of DDT in air and water also seems to be slightly facilitated comparatively to some PCB congeners (Beyer et al., 2000). This scenario could ultimately influence the efficient transfer of DDTs to mesopelagic food webs and drive their dominance in oceanic and mesopelagic species. Hence, incidents that are responsible for the remobilization or input of DDTs in the marine environment may potentially increase the availability of this pesticide far from emission sources (Lippold et al., 2019; Oliveira-Ferreira et al., 2022). For example, the collapse of a mining dam in SE Brazil increased OCPs concentrations, such as DDTs, mirex, and HCB, in the coastal Franciscana dolphins (*Pontoporia blainvillei*) (Oliveira-Ferreira et al., 2022), which could be of particular concern for mesopelagic trophic webs. Although the dataset is small to draw conclusions, the concentration of ΣDDT in Ks #13 and Kb #5 sampled in the area impacted by this incident, are among the highest in the present study, underscoring the effect that a coastal impact may have on deep-water fauna.

Continental shelf/oceanic marine mammals stranded on the northeastern Brazilian coast, e.g., Fraser's dolphins (*Lagenodelphis hosei*), Atlantic-spotted-dolphin (*Stenella frontalis*), and striped dolphins (*S. coeruleoalba*) also presented a DDT dominance over PCB (Santos-Neto et al., 2014). Worldwide, a similar pattern can be perceived for marine mammal species foraging on mesopelagic prey, such as oceanic cephalopods and myctophids. Fraser's dolphins from Argentina (Durante et al., 2016), long-finned pilot whales (*Globicephala melas*) from Chile (Garcia-Cegarra et al., 2021), as well as the mesopelagic northern elephant seals (*Mirounga angustirostris*) from the North Pacific (Peterson et al., 2015) also presented DDT dominance.

Among the DDTs, *p.p'*-DDE was the main one detected in the blubber of both species, comprising 45% of the profile in *K. sima* and 47% in

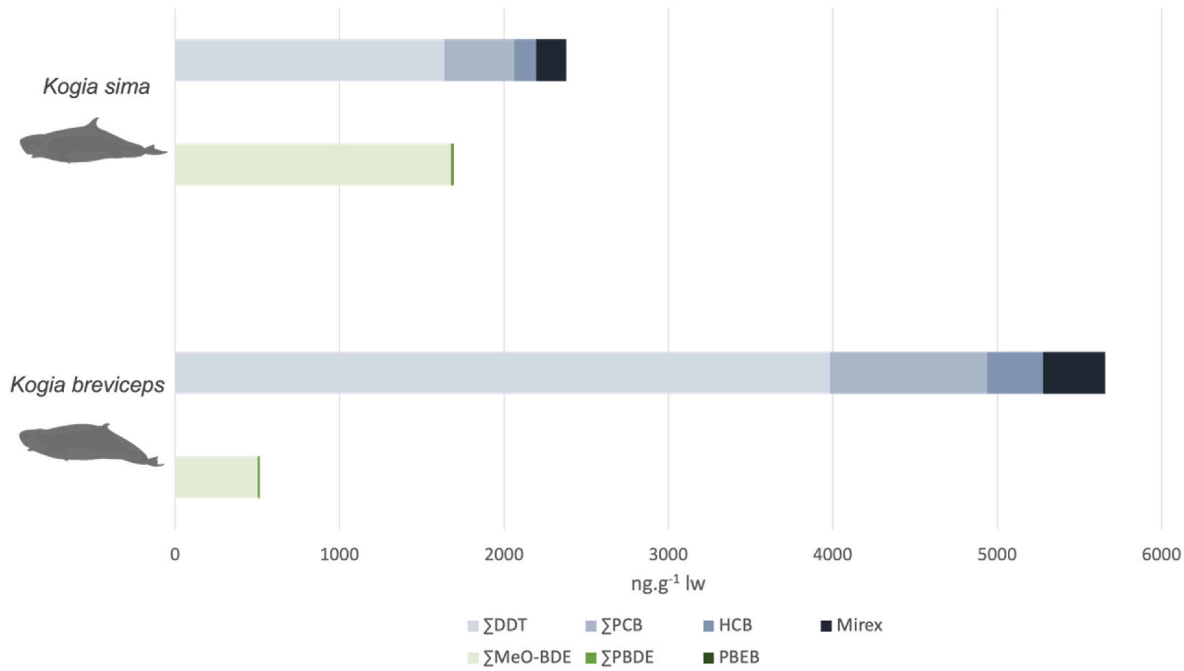

Fig. 2. Organochlorine (ΣDDT, ΣPCB, HCB, and mirex) and organobromine (ΣMeO-BDE, ΣPBDE, and PBEB) median concentrations, expressed in ng.g^{-1} lipid weight, and profile in *Kogia sima* and *Kogia breviceps* from the Southwestern Atlantic Ocean.

Table 1
Concentrations (expressed in ng.g^{-1} lw) of the synthetic ΣDDT, ΣPCB, HCB, mirex, ΣPBDE, and PBEB and the natural ΣMeO-BDE in *K. sima* and *K. breviceps* from the Southwestern Atlantic Ocean (SWAO). The total length (TL) of the individuals is expressed in centimeters (cm) and lipid content in percentage (%).

	Total length	Lipid content	ΣDDT	ΣPCB	HCB	Mirex	ΣPBDE	PBEB	ΣMeO-BDE
Kogia sima (n = 16)									
Median	217	60.2	1636.6	425.88	132.4	184.1	13.6	2.2	1676.7
Mean ± SD	209 ± 46	56.1 ± 12.9	3272.7 ± 3974.8	1195.3 ± 1524.9	244.1 ± 249.9	573.4 ± 710.2	25.8 ± 31.2	2.2 ± 2.3	3536.4 ± 4686.7
Min - Max	110–289	32.8–72.7	304.48–14928	18.950–5134.2	39.48–933.7	<3.5–2035	<0.1–95.6	<0.4–3.9	346.31–16058
Kogia breviceps (n = 15)									
Median	253	43.4	3983.3	956.06	340.3	375.6	10.3	2.9	501.57
Mean ± SD	246 ± 47	42.8 ± 17.8	5530.2 ± 4188.0	1808.6 ± 2058.2	386.2 ± 191.8	697.7 ± 728.1	22.2 ± 25.6	2.9 ± 1.5	1430.0 ± 1985.4
Min - Max	145–303	20.1–82.3	1892.3–15924	293.89–7739.0	101.0–728.0	58.66–2789	<0.2–77.1	<0.4–5.4	42.490–6480.7

K. breviceps. This is often indicative of historical contamination by DDTs, considering the original formulations of DDT used as a pesticide in agriculture and as vector control, as well as its environmental degradation (Aguilar, 1984). *p.p'*-DDD was the second most important compound for *K. sima* (31%), in particular for the specimens sampled north of Cape São Tomé, and *p.p'*-DDT was the third (24%). Conversely, *p.p'*-DDT was slightly more representative for *K. breviceps* (28%) than *p.p'*-DDD (25%).

For PCBs, hexa-, hepta-, and penta-chlorinated congeners were the most abundant. This is a common profile for cetaceans in SWAO as the formulations imported mainly from Germany and the United States of America (USA) were rich in these recalcitrant congeners (Lailson-Brito et al., 2012; Oliveira-Ferreira et al., 2021; Santos-Neto et al., 2014). However, for oceanic cetaceans, the atmospheric, oceanographic, and biological transport also pose a major influence on this profile. Interestingly, a few individuals of *K. sima* presented the contribution of the lighter tetra-chlorinated PCBs, like PCB 52, which were not detected in *K. breviceps* (Table S6, Supporting Information). The transport of heavier congeners adhered to organic matter, resulting in a vertical partitioning of congeners, can influence these discrete differences in the profiles (Dachs et al., 1997), and may start to suggest differences in trophic webs between the species or between prey species. The atmospheric transport of the lighter congeners further from the coast may also impact this profile as observed in other oceanic marine mammals (Megson et al., 2022).

For BFRs, BDE 47 was the main compound detected (32% and 43% in *K. sima* and *K. breviceps*, respectively), which also goes accordingly to profiles described for odontocetes from Brazil along the coast-ocean gradient (Alonso et al., 2014; Dorneles et al., 2010; Oliveira-Ferreira et al., 2022, 2023). Like in the PCB profile, *K. sima* presented an increased contribution of lower molecular weight BDE 28 (29%), whereas the heavier BDE 153 was more representative in *K. breviceps* (27%). This may also be a consequence of their vertical transport adhered to organic matter (Dachs et al., 1997) in distinct localities or differences in prey selection.

It is important to note that PBEB, a BFR of emergent concern in the SWAO, was detected in both species. Almost half of the *K. breviceps* individuals herein analyzed presented this emergent contaminant and two individuals of *K. sima* presented PBEB in detectable concentrations in their blubber. PBEB was considerably less used than conventional BFRs, such as PBDEs (Covaci et al., 2011), with an estimated production between 5 and 450 tons per year between 1970 and 1980 (Hoh et al., 2005), whereas PBDEs have an estimated production of approximately 1800 kilotons globally (Abbasi et al., 2019). Hence, the occurrence of PBEB in the mesopelagic zone raises a red flag since it indicates its vertical range in the water column and availability for mesopelagic fauna.

As for the profile of natural compounds, it was dominated by 2'-MeO-

BDE 68 (64% and 61% in *K. sima* and *K. breviceps*, respectively), followed by 6-MeO-BDE 47 (36% and 39% in *K. sima* and *K. breviceps*, respectively). *K. sima* also presented lower concentrations of 5-MeO-BDE 47, 4-MeO-BDE 49, and 5-MeO-BDE 100, which were not detected in *K. breviceps*. While there is not enough information available regarding the production of these other methoxylated compounds, it is interesting to note that the *K. sima* individuals from the present study were mostly sampled north of Cape São Tomé, where there may be a strong influence of coral reef systems responsible for their synthesis. On the other hand, these compounds were not detected in *K. breviceps.* This may be an influence of the sampling site but can also be a result of the biotransformation and excretion of those, not necessarily meaning that they are not bioavailable for incorporation. 2′-MeO-BDE 68 and 6-MeO-BDE 47 are the main natural brominated products found in cetaceans in all oceans and their contribution varies according to the local features. In general, the synthesis of 2′-MeO-BDE 68 is strongly linked to the presence of sponges and associated organisms, e.g., cyanobacteria, while 6-MeO-BDE 47 is mostly related to the presence of algae (Vetter, 2006; Vetter et al., 2001, 2002). Thus, the increased contribution of natural compounds produced by organisms that depend less on the euphotic zone, like the 2′-MeO-BDE 68, for these mesopelagic feeders was expected. Furthermore, sites that are strongly influenced by coral formations present high concentrations of these biogenic compounds (Alonso et al., 2014; Mwevura et al., 2010; Oliveira-Ferreira et al., 2022; Vetter et al., 2002).

Altogether, the differences in the accumulation of these compounds in *Kogia* bring to light some important aspects of the transport of chemical pollutants to mesopelagic trophic webs, as well as of these species in the SWAO. The detection of POPs in all individuals analyzed in the SWAO shows an efficient transport of these micropollutants down the water column, characterizing the mesopelagic zone as a relevant reservoir. Given the distinct accumulation profiles, it is likely that the species share a few dimensions of the ecologic niche, but not completely. The higher concentrations of POPs in *K. breviceps* may be indicative of preying in distinct ontogenetic stages than *K. sima*, and/or in distinct trophic webs/locations. Furthermore, the increased contribution of natural compounds to *K. sima* combined with the fact that most of the individuals of this species were sampled north of Cape São Tomé, suggests an increased synthesis of MeO-BDEs by local coral reef formations (see section *3.2 Organohalogen compounds in Kogiidae from SWAO*).

Since there is an increasing interest in the forthcoming exploitation of the mesopelagic zone for food consumption and fish oil (Dowd et al., 2022), it is important to highlight the importance of kogiids as sentinels. They provide an integrated contamination scenario of both biological and physical-chemical transport of chemical pollutants to deep waters. *Kogia* prey mainly on cephalopods with a short life cycle, or fish and shrimp species that perform vertical migrations (Haimovici et al., 2007), optimizing the biological transport of organohalogen compounds down the water column. This highlights the availability in deep-water environments and their bioaccumulation potential in other mesopelagic predators. The use of sentinel species for the mesopelagic zone, particularly for the South Altlantic, is called for as other micropollutants, e.g., mercury, present higher levels in the Atlantic than in the Pacific (Furtado et al., 2021) and mesopelagic fish species are potential vectors of POPs to deeper layers of the ocean (Justino et al., 2022; Rochman et al., 2014). Ultimately, it is important to explore the multiple stressors that may be affecting this environment and understand how the mesopelagic trophic webs are essentially entangled with epipelagic webs, as it is a key mechanism for the sinking of these compounds.

3.2. Organohalogen compounds in Kogiidae from SWAO

Given the difficulties of obtaining information regarding the *Kogia* species that are inherent to their behavior and oceanic habit, few ecotoxicological assessments have been conducted to date (see Tables S8 and S9 in Supporting Information). To the best of our knowledge, eight previous studies investigated organohalogen compounds in this family, and seven of them obtained only one or two samples of each species. This is the most comprehensive evaluation of the accumulation of organohalogen compounds in *K. breviceps* and *K. sima* and the first one in the SWAO.

Concentrations of the natural methoxylated compounds, 2′-MeO-BDE 68, 6-MeO-BDE 47, and ΣMeO-BDE, as well as of the pesticides HCB, p.p'-DDE, p.p'-DDT, and ΣDDT in their blubber were species-specific (Mann-Whitney test, $p < 0.05$; Fig. 3; and see Table S11 in Supporting Information). Some organohalogen concentrations were influenced by the sampling site (north and south of Cape São Tomé, Mann-Whitnet test, $p < 0.05$; and see Tables S12 and S13 in Supporting Information) and by biological parameters, like total length (Spearman correlation, $p < 0.05$; see Tables S14 and S15 in Supporting Information), sex and maturity stages (mature males, mature females, and immature, Kruskal-Wallis test, $p < 0.05$; see Table S16 in Supporting Information). Mirex, PCBs, and PBDEs ($p > 0.05$), were not influenced by species.

The differences in concentrations of these compounds between species are likely a consequence of multiple factors. First, *K. breviceps* is larger than *K. sima* (Jefferson et al., 2015), which may impact their feeding habits and, ultimately, the bioaccumulation of these compounds. Across their distribution, there is some level of overlap in trophic niche and prey species, despite differences in prey size (dos Santos and Haimovici, 2001; Staudinger et al., 2014). Both *Kogia* are essentially teuthophagous (while preying on other groups may be underestimated), foraging on the same families of cephalopods, including in the SWAO (dos Santos and Haimovici, 2001; Staudinger et al., 2014; West et al., 2009). However, *K. breviceps* was observed to feed on slightly larger prey than *K. sima* (Staudinger et al., 2014) and the larger size of *K. breviceps* may also reflect on differences in nutritional requirement, demanding a greater amount of prey intake.

Second, while there is this overlap in the species distribution in the SWAO, *K. sima* tends to be found in the warmer waters north of Cape São Tomé whereas *K. breviceps* in the colder waters south of the Cape, influencing their stranding events along the coast (Moura et al., 2016) and, consequently, in the sample set of the present study. Most *K. sima* individuals were sampled in the north of Cape São Tomé (75%) and most *K. breviceps* were collected in the south (67%). The Cape marks, roughly, a biogeographic classification of mesopelagic fauna in the SWAO (Sutton et al., 2017). The region found north of Cape São Tomé is strongly influenced by the large-scale formation of Royal Charlotte and Abrolhos Bank and the Amazon reef system (Carneiro et al., 2022), hotspots for biodiversity in the Brazilian coast that tend to influence the concentrations of biosynthesized organobromine compounds in cetaceans (Oliveira-Ferreira et al., 2022). Since most *K. sima* were stranded in the NE region, it suggests that their trophic web is greatly influenced by these natural formations and explains why the concentration of methoxylated compounds is higher in this species as it is their contribution to the organohalogen profile. Conversely, the SE and S regions – south of the Cape - are highly urbanized which increases the emissions of POPs (Oliveira-Ferreira et al., 2021), resulting in the elevated concentrations of anthropogenic compounds in the blubber of *K. breviceps*. The continuous monitoring by a stranding network could provide a better resolution of how the SWAO is used by these species. In addition, the preference for colder waters may also increase the nutritional demands of *K. breviceps* (Costa and Maresh, 2018).

When taking a closer look at the organohalogen profile found in *K. sima* across the species distribution, some interesting features appeared. The correlation between the total length and the concentrations of BDE 47, ΣPBDE, HCB, *p.p'*-DDE, *p.p'*-DDD, *p.p'*-DDT, ΣDDT, PCB 138, PCB 151, PCB153, PCB 180, and ΣPCB when mature males and immature individuals are in accordance with chronic exposure to these recalcitrant compounds throughout their life cycle (Spearman correlation, $p < 0.05$; Supplementary Information). Interestingly, ΣDDT and mirex also presented positive correlations when mature females and

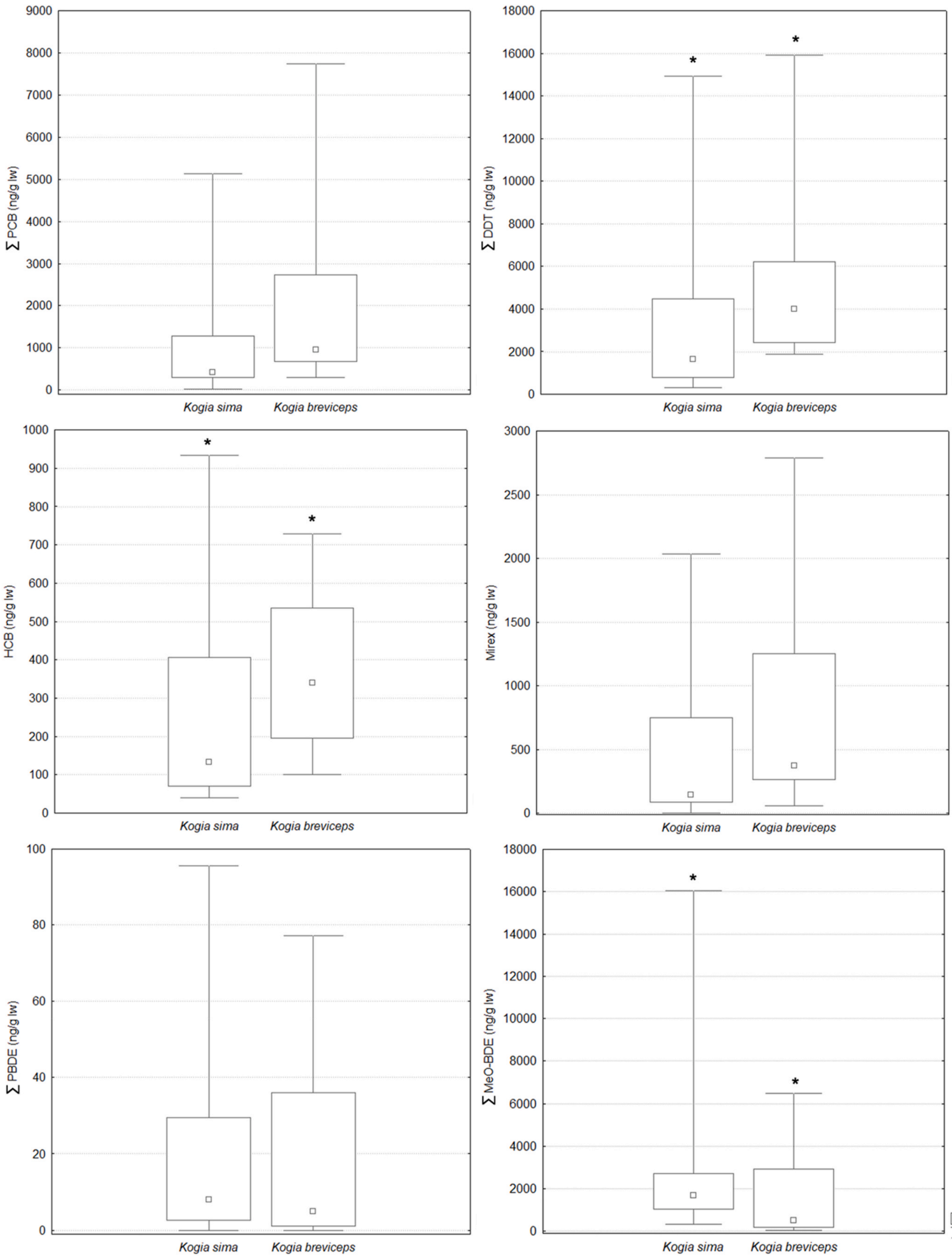

Fig. 3. Organohalogen concentrations (ΣDDT, ΣPCB, HCB, mirex, ΣPBDE, and ΣMeO-BDE), expressed in ng.g^{-1} lipid weight, in *Kogia sima* and *Kogia breviceps* from the Southwestern Atlantic Ocean.
Legend: (□) Median; (▭) 25%–75%; (⊥) Minimum – Maximum; (*) $p < 0.05$ in a Mann-Whitney test.

immature were grouped (Spearman correlation, $p < 0.05$; Supplementary Information). The maternal transference of compounds via placenta and lactation can reflect an increased concentration in immatures and lower concentrations in the offloaded mothers. But mirex is a heavy-weight pesticide with high K_{ow} (Zitko, 2003), difficulting their transference from mother to their offspring and increasing throughout their lifetime. For DDTs, this increase in concentration with length is also interesting and may be due to their relevant contribution to the profile of these mesopelagic sentinels. Despite being transferred to the offspring, they are still found in high concentration in mature individuals. The maternal transference of organic compounds was well represented in the pair mother and pup, Ks #9 and Ks #1 (Table 1; Tables S5–S8, Supplementary Information).

Furthermore, differences in mature males, mature females, and immatures were also detected in a few pesticides for *K. sima* (Kruskal-Wallis test, $p < 0.05$; Supplementary Information). HCB concentrations were higher in mature males than in mature females, which is probably due to HCB being a lightweight organohalogen and easily transferred to the offspring (Starek-Świechowicz et al., 2017). Additionally, ***p.p'***-DDE, ***p.p'***-DDD, and ΣDDT concentrations were higher in mature males than in mature females, supporting the bioaccumulation throughout their lifetime, chronic exposure, and low metabolic rate (Aguilar et al., 1999).

The specimens found north of Cape São Tomé present a rather distinct contribution of ***p.p'***-DDD as well as of light-weight PCBs and PBDEs (Tables S5–S8, Supporting Information). ***p.p'***-DDD is often a product of the anaerobic metabolism of ***p.p'***-DDT (D'Amato et al., 2002) and its increased presence in the northern area may be indicative of the influence of warmer and hypoxic waters on their trophic web. Moreover, areas with boosted productivity tend to present increased concentrations of particulate matter to which organohalogen compounds, particularly heavy-weight compounds, may adhere to and sink to mesopelagic waters (Dachs et al., 1997). This, in addition to the facilitated atmospheric transport (Megson et al., 2022), would explain the presence of the lightweight PCB 52 and PBDE 28 in *K. sima* from the north of Cape São Tomé, and the highest concentrations of PCBs, OCPs, and BFRs in *K. sima* south of the Cape. While it would be interesting to perform a similar assessment for *K. breviceps*, this subset is biased by the sexually mature females, and the maternal transference of compounds during reproduction is an important biological feature that affects the profile in cetaceans (Kajiwara et al., 2008).

For kogiids, in general, once the organochlorine compounds are incorporated the effect of biological parameters follows the dynamic of what is often observed in other cetacean species (Aguilar et al., 1999; Marsili et al., 2019). For the organobromine compounds, both from natural and anthropogenic sources, the influence of biological parameters is not as clear as for organochlorines (Oliveira-Ferreira et al., 2022), and it could be related to the metabolism of these compounds and their debromination in the medium and in vertebrates (Roberts et al., 2011; Stapleton et al., 2004).

Thus, the accumulation profile of the anthropogenic contaminants in these species reflects the abundant use of these compounds on the coast, the individuals' biological traits, and the complexity of their physical and biological transport, media partitioning, and biogeochemical cycle that will result in their bioavailability to mesopelagic food webs. Conversely, the profile and concentrations of biogenic compounds are widely dependent on conditions that will influence the local biodiversity for their synthesis and transport, such as light and oxygen availability. But while the general profile of accumulation differed between the species, the congeners/isomers contribution within groups, i.e., the *p.p'*-isomers of DDE, DDD, and DDT in the ΣDDT, the chlorination level of PCB congeners in the ΣPCB, and so on, was mostly similar among them, evidencing that the physical-chemical properties of these compounds are of extreme importance to their long-range transport.

The concentrations reported do not exceed the threshold for deleterious effects known for Delphinidae (Kannan et al., 2000). However, Kogiidae presents a distinct evolutionary history and there are no specific studies related to this family. The trophic history is a driver of the metabolic potential of distinct groups (Bartrons et al., 2012) and the threshold interpolation may not be valid among these families. Negative health effects were already associated with exposure to other pollutants in *K. breviceps*, with the obstruction of the gastrointestinal tract by plastic ingestion (Brentano and Petry, 2020) and cardiomyopathy induced by trace-elements contamination (Bryan et al., 2012). Furthermore, some of the individuals analyzed in the present study were diagnosed with infections (e.g., Kb #4 whose cause of death was attributed to sepsis), and the presence of POPs may trigger immunosuppression in marine mammals (Desforges et al., 2016, 2017), hence the possibility of chronic effects in these deep-diving sentinels should not be discarded.

Ultimately, the bioaccumulation of POPs and contaminants of emergent concern in oceanic species shows the long-range these micropollutants travel and their persistence in the environment, posing a threat even for species that inhabit areas far from emission sources, and at critical developmental stages, such as the pregnant and lactating females exposing the offspring. Moreover, it is also observed an accumulation of the natural compounds essentially produced by sponges and associated organisms, that may rely less on the euphotic zone.

While it is important to highlight the relevance of information herein discussed, considering the rarity of these samples and the valuable data it holds regarding the mesopelagic zone, the sample set also present caveats that may impact the data treatment. For example, the sample set is affected by too many variables, and when all of those are considered, the sample size turns small for statistical treatment. Furthermore, when performing an ecotoxicological evaluation of marine mammals, the study of carcasses may be biased by the stranding of unhealthy individuals or carcass drift. Still, this study marks differences between the *Kogia* species that were not previously accessed as well as a contamination profile of the mesopelagic zone of the SWAO, and may be a starting point into investigating this environment.

3.3. Comparison with Kogia around the world

Considering *Kogia* as indicators of the transference of organohalogen compounds to mesopelagic food webs across oceans, it is important to situate the contamination status of this mesopelagic ecoregion in SWAO in comparison to other locations (Sutton et al., 2017), especially because these concentrations were scarcely assessed (Tables S9 and S10, Supporting Information). However, caution is warranted in these comparisons given the small number of samples in previous work and the sampling date, distinct tissue, and quantification based on wet or lipid weight, which may differ from the present study. As aforementioned, most assessments conducted to date only accounted for one or two individuals of each species, except for de Kock et al. (1994) which included five *K. breviceps* and four *K. sima*.

As for POPs, previous reports from the west coast of South Africa (de Kock et al., 1994) and northern Pacific Islands (Bachman et al., 2014) shown a predominance of DDT in the blubber of *K. sima* and *K. breviceps*, as herein reported. On the other hand, two records from the North Atlantic Ocean show an equivalent contribution of both DDTs and PCBs in the liver of *K. breviceps* from the USA (Watanabe et al., 2000) and in the blubber of *K. breviceps* from the United Kingdom (UK) (Law et al., 2006). It is noteworthy that PCBs were extensively applied in the Northern Hemisphere for many decades, facing a global ban in the late 1970s. Thus, during the sampling year of the assessments in the USA and UK (between 1989 and 2002), these were still highly detected in environmental samples because of the persistence, widespread use and production, transport, and recent prohibition (Jepson and Law, 2016).

Concerning the DDTs, average concentrations for *K. breviceps* (8130 ng g^{-1} lw) and *K. sima* (3030 ng g^{-1} lw) from the Hawaiian Islands (Bachman et al., 2014) were in the same order of magnitude as in the SWAO (see Table 1), and concentrations were also higher in *K. breviceps* than in *K. sima*.

For PCBs, *K. breviceps* from Taiwan waters (14800 ng g^{-1} lw) presented a concentration ten times higher than median concentrations in the SWAO – but this was investigated in a single individual (Chou et al., 2004). In the North Pacific, PCBs concentration (5310 ng g^{-1} lw; Bachman et al., 2014) was nearly three times higher than in individuals from the SWAO.

Mirex and HCB were also assessed and detected at high concentrations in kogiids from the Northern and Southern Hemispheres (Bachman et al., 2014; de Kock et al., 1994; Law et al., 2006; Watanabe et al., 2000). In the North Pacific, where concentrations are comparable, HCB was half of the median concentration detected in Brazil for both species (79.8 and 227.5 ng g^{-1} lw in *K. sima* and *K. breviceps*, respectively; Bachman et al., 2014). Mirex also presented increased concentrations in *Kogia* from the SWAO than in North Pacific individuals (106 and 113.5 ng g^{-1} lw in *K. sima* and *K. breviceps*, respectively; Bachman et al., 2014), altogether suggesting an extensive application in agricultural activities and low environmental degradation in the SWAO.

As for BFRs, PBDEs detected in kogiids from the North Atlantic were 2- to 3-fold what was observed in the individuals herein analyzed (Bachman et al., 2014; Law et al., 2005), and the present study is the first to describe the presence of PBEB in the mesopelagic kogiids.

Regarding the natural compounds, Vetter et al. (2002) described the presence of 2′-MeO-BDE 68, synthesized by sponges in *K. breviceps* from Australia, influenced by the Great Barrier Reef (Vetter et al., 2002), within the range reported for SWAO samples.

In summary, OCPs concentrations herein reported for *K. breviceps* and *K. sima* from the SWAO are in the same order of magnitude or higher than the specimens sampled in the North Pacific (Bachman et al., 2014). On the other hand, PCB concentrations were one-fold lower in the SWAO (Bachman et al., 2014). The BFRs PBDEs are lower but within the range of concentrations detected in the North Pacific (Bachman et al., 2014; Law et al., 2005) and the biosynthesized halogenated compounds were also similar to what was detected in *K. breviceps* from Australia (Vetter et al., 2002).

4. Conclusion

This is the first record of the accumulation of POPs, emergent pollutants, and organohalogen natural products in mesopelagic fauna from the SWAO. Given the analyses of the unprecedented and large sampling size of *Kogia*, some ecological aspects of the species, such as potential niche differences in areas where sympatry is likely to occur in SWAO, started to be unraveled. Mesopelagic kogiids inhabit locations far from POPs emission sources, but the results showed that the long-range vertical transport of these lipophilic compounds is highly efficient, affecting the bioaccumulation in these species.

Concentrations of anthropogenic compounds were higher in K. *breviceps* than in *K. sima*. On the other hand, natural compounds were higher in *K. sima*. This appears to be related to the higher trophic level of *K. breviceps*, increased body size compared to *K. sima*, and the distinct habitat preference of the species. In addition, variables such as sex and maturity stages were also driving the accumulation pattern in these species, in particular for organochlorine compounds.

Differences in the organohalogen profile of sympatric *K. sima* and *K. breviceps* sampled north of Cape São Tomé suggest distinct foraging habits or, at least, predation in distinct ontogenetic stages. Given the larger size of *K. brevi*ceps and its higher POPs concentration, it is likely that the species feeds on bigger prey.

Differences in the profile of *K. sima* sampled north and south of Cape São Tomé also suggest some level of niche partitioning within the species along the SWAO, which needs to be further investigated.

Thus, the use of kogiids as sentinels of the mesopelagic zone provides a novel and integrated scenario of the biological and physical-chemical transport of pollutants to a deeper layer of the oceans that is in the prospect of being exploited and characterizes the mesopelagic zone of the SWAO as an important reservoir of POPs.

Authorship statements

Nara de Oliveira-Ferreira: Conceptualization; Data curation; Methodology; Validation; Formal analysis; Investigation; Visualization; Writing – original draft; Writing – review & editing. Elitieri Santos-Neto: Conceptualization; Data curation; Methodology; Validation; Investigation; Visualization; Writing – review & editing. Bárbara M. R. Manhães: Conceptualization; Data curation; Methodology; Validation; Investigation; Visualization; Writing – review & editing. Vitor Luz Carvalho: Resources; Writing – review & editing. Letícia Gonçalves: Resources; Writing – review & editing. Pedro Volkmer Castilho: Resources; Writing – review & editing. Camila Domit: Resources; Writing – review & editing. Eduardo Resende Secchi: Resources; Writing – review & editing. Silvina Botta: Resources; Writing – review & editing. Milton Marcondes: Resources; Writing – review & editing. Adriana C. Colósio: Resources; Writing – review & editing. Marta Jussara Cremer: Resources; Writing – review & editing. Haydée Andrade Cunha: Resources; Funding acquisition; Writing – review & editing. Alexandre de Freitas Azevedo: Resources; Funding acquisition; Writing – review & editing. Tatiana Lemos Bisi: Resources; Funding acquisition; Writing – review & editing. José Lailson-Brito: Conceptualization; Resources; Visualization; Supervision; Funding acquisition; Writing – review & editing.

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Declaration of competing interest

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

Data availability

I have shared the data used in this article in the Supplementary Material

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2023.140456.

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