Contamination status by persistent organic pollutants of the Atlantic spotted dolphin (*Stenella frontalis*) at the metapopulation level

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**Abstract**

The Atlantic spotted dolphin (*Stenella frontalis*, ASD) is an endemic species of the tropical-temperate Atlantic Ocean with widespread distribution. Although this species has been the subject of a large number of studies throughout its range, it remains in the “data deficient” category of the International Union for Conservation of Nature (IUCN). Chemical pollution by persistent organic pollutants (POPs) has been listed as one of the major threats to this species, however, there is no information on a wide scale. Thus, the aim of the present study was to investigate the contamination status of spotted dolphins on the metapopulation level as well as determine spatial and temporal variations in POP concentrations and bioaccumulation. A total of 115 blubber samples collected from a large part of the Atlantic basin were analysed for PCBs, DDTs, PBDEs, chlordanes, HCB and mirex. Although PCBs and DDTs were the predominant compounds in all areas, inter-location differences in POP concentrations were observed. Dolphins found at São Paulo, southeastern coast of Brazil, had the highest PCB concentrations (median: 10.5 µg/g lw) and Canary Islands dolphins had the highest DDT concentrations (median: 5.13 µg/g lw). Differences in PCB patterns among locations were also observed. Dolphins from the Azores and São Paulo demonstrated a similar pattern, with relatively highly contributions of tetra- (6.8 and 5.2%, respectively) and penta-CBs (25.6 and 23.8%, respectively) and lower contributions of hepta-CBs (20.8 and 23.5%, respectively) in comparison to other areas. Moreover, the sex of the animals and the year in which sampling or capture occurred exerted an important influence on the majority of the POPs analysed. Comparisons with toxicity thresholds available in the literature reveal that the São Paulo and Canary Island dolphins are the most vulnerable populations and should be considered in future conservation and management programs for the Atlantic spotted dolphin.

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1. Introduction

The Atlantic spotted dolphin (*Stenella frontalis*, ASD) is an endemic species of the tropical-temperate Atlantic Ocean (Perrin et al., 1987, 1994; Moreno et al., 2005; Paro et al., 2014), primarily encountered from the continental shelf (<200 m) to the continental slope waters (200–2000 m) (Mullin and Fulling, 2003). This species has been the subject of a large number of studies throughout its...
Persistent organic pollutants (POPs) (e.g., PCBs, polychlorinated biphenyls, DDTs, dichloro diphenyl trichloroethane, PBDEs, polybrominated diphenyl ethers) merit special attention. POPs are man-made contaminants that enter aquatic environments directly (e.g., brominated diphenyl ethers) or indirectly via atmospheric transport, ocean currents (Breivik et al., 2004; Li and Macdonald, 2005) and through plastics (e.g., microplastic particles, plastic resin, etc.), where they are adsorbed and transported via ocean currents (Rios et al., 2007; Teuten et al., 2009). These pollutants are a primary concern in marine ecosystems and are cited on the OSPAR list of Chemicals for Priority Action (OSPAR, 2010). POPs are lipophilic synthetic organic compounds, which because of their chemical and physical properties, have been used in agriculture, industry and human health since the 1940s (Clark, 2001). The production and use of these compounds were restricted or banned approximately 15 years ago at the Stockholm Convention (UNEP, 2001) due to their environmental persistence and toxicity to humans and wildlife. Nevertheless, considerable amounts of these persistent compounds continue cycling in the environment.

Marine mammals bio-accumulate and bio-magnify large amounts of POPs due to the fact that they are top predators with high longevity and low biodegradation capacity (Borrel and Aguilar, 2007; Tanabe et al., 1988). Moreover, since POPs are highly lipophilic, they reach the highest concentrations in fatty tissues and particularly in the hypodermic fat or blubber of marine mammals. Several studies have shown the adverse effects of POPs on marine mammals. For instance, significant reductions in total reproductive outcome have been found to coincide with increased long-term exposure to highly biomagnified levels of PCBs in the Baltic grey seal population (Helle et al., 1976). High levels of organochlorines have been correlated with pathological uterus lesions, a disease complex including lesions on skin, claws, intestines, kidneys and adrenal glands, as well as immunosuppression (Bergman and Olsson, 1985; Jeppson et al., 2005; Kannan et al., 2000). It is also important to highlight the effect of the exposure to a complex mixture of chemical contaminants present in the environment. Even when individual stressors are present at concentrations lower than the “no-observable-effect” concentration (Brian et al., 2007; Cortenkamp, 2007; Silva et al., 2002). Therefore, marine mammals are largely considered as good sentinel organisms in aquatic and coastal environments when evaluating the presence of chemical contaminants (Reddy et al., 2001; Wells et al., 2004).

Numerous studies have shown that several ecological factors as well as biological factors, such as age and sex, affect the pollutant burden in marine mammals and have to be taken in consideration (Borrell et al., 1996; Borrell and Aguilar, 2005; Tanabe et al., 1982). These compounds enter marine mammal tissues almost exclusively through feeding and contamination levels therefore vary greatly depending on intake factors (i.e., trophic level and prey type) (Aguilar, 1989) and local environmental pollution. Each compound may have its own trophic source. Consequently, a complete POP profile may be used as a kind of fingerprint to infer dietary behaviour and feeding habitat as well as to differentiate among different species or populations (Wells et al., 1996; Borrell et al., 2006; Pierce et al., 2008; Méndez-Fernandez et al., 2017).

Thus, the aim of the present study was to assess contamination by POPs (PCBs, DDTs, PBDEs, chlorodanes, HCB and mirex) in the ASD on the metapopulation level and in light of bio-ecological factors. For such, blubber samples from a large geographic area of ASD distribution were analysed to investigate variations in POP concentrations and accumulation in this species. This paper also addresses the toxicological implications of contaminant concentrations on the health of the Atlantic spotted dolphin.

2. Material and methods

2.1. Sample collection

A total of 115 blubber samples were collected from stranded ASDs in São Paulo (n = 10, southeast Brazil), the Canary Islands (n = 11, Spain) and Guadalupe Island (n = 1, French Caribbean) between 2005 and 2015 (Table 1). An experienced stranding network personnel performed complete necropsies whenever the condition of the animal permitted. Only samples collected from fresh or moderately decomposed animals (decomposition state ≤ 3 based on the classification proposed by Kuiken and Hartmann [1991] and category 2 using the classification proposed by Geraci and Lounsbury [1993]) were used for POP analyses to prevent sampling biases associated with tissue decomposition. Biopsies of skin and blubber from São Paulo (n = 39) and the Azores (n = 54, Portugal) collected using a crossbow (Barnett 125 and 1501b) with tips specially designed for small cetaceans (dart 25 mm in length and 5 mm in diameter) were also used. Dolphins were hit below the dorsal fin when sufficiently close to the research boat. At sea, samples were stored on ice from 1 to 8 h. On land, skin and blubber were separated. Blubber samples were wrapped in aluminium foil and preserved frozen (−20°C) until analysis.

2.2. Sex determination

Molecular sexing techniques were performed for the sex determination of the specimens sampled through biopsy darting (using skin samples). The protocol proposed by Rosel (2003) was used for samples from São Paulo. Molecular sexing was performed on samples from the Azores by co-amplification of a short fragment of the male-specific SRY gene and a tetranucleotide microsatellite used as a PCR control for positive identification of females (Quéroul et al., 2010).

2.3. Persistent organic pollutant (POP) analyses

Atlantic spotted dolphins exhibit a slight stratification of POPs at different blubber depths (Méndez-Fernandez et al., 2016). Therefore, the entire dorsal blubber of stranded individuals and the more superficial blubber from the biopsy samples were used for the analyses of POPs. Approximately 0.2 g of blubber were ground with anhydrous Na2SO4 and extracted using a Soxhlet apparatus for 8 h with 80 mL of n-hexane and methylene chloride (1:1, v:v). Prior to extraction, surrogates (PCB 103 and 198) were added to samples, blanks and reference material. The extract was then concentrated by rotary evaporation to 2 mL, 200 μL of which were used to determine the amount of lipids by gravimetry. The remaining
extract was cleaned up with sulfuric acid (H₂SO₄) and centrifuged for 10 min. The supernatant was washed with pre-extracted water and all data were blank subtracted. Concentrations were quantified using a gas chromatograph coupled to a mass spectrometer (GC/MS, Agilent Technologies, model 6890 N). PCBs and PBDEs were analysed through a gas chromatographic technique (GC/ECD, Agilent Technologies, model 5973 N) in a selected ion mode (detailed target ions in Table S1). Target POPs were quantified with analytical curves generated from eight different concentrations of reference standards (SRM 1945). Blanks were included in every analytical batch (usually from 15 to 20 samples) and all data were log-transformed to account for skewness. To investigate the potential role of biological and ecological factors (i.e., geographic area, year, and sex) in the accumulation of POPs, analysis of covariance (ANCOVA) was used with sex as the covariate. For this analysis, only compounds with concentrations above the LOD were included. The one-way (Welsh) test was used to test for differences in means of log-transformed values among locations; this test is similar to an ANOVA, but does not require equal variances between groups (Rasch et al., 2011). Pairwise differences in the means of the sum of POP group concentrations followed the one-way test using a Bonferroni correction. Differences in accumulation patterns of individual compounds among areas where also investigated for PCBs, DDTs and PBDEs. The level of significance for statistical analyses was set at α = 0.05 and analyses were performed using Rstudio Team version 1.0.136 (Rstudio, 2016).

### 3. Results and discussion

#### 3.1. General trends and sex-related differences

ΣPCBs, ΣDDTs, and ΣPBDEs were the predominant POPs, respectively accounting for 64.0%, 31.2% and 2.0% of the total of POP compounds found in the ASDs. Mirex and HCB were found in much lower concentrations among areas; this test is similar to an ANOVA, but does not require equal variances between groups (Rasch et al., 2011). Pairwise differences in the means of the sum of POP group concentrations followed the one-way test using a Bonferroni correction. Differences in accumulation patterns of individual compounds among areas where also investigated for PCBs, DDTs and PBDEs. The level of significance for statistical analyses was set at α = 0.05 and analyses were performed using Rstudio Team version 1.0.136 (Rstudio, 2016).

#### 2.4. Statistical analysis

The lipid percentage of the blubber of the ASDs analysed ranged from 3.0% to 87.1%. Variations in lipid content may be due to numerous factors, such as ontogeny, nutritional status, water temperature or even the sampling technique. Thus, to facilitate comparisons among individuals and with the results of other studies, POP concentrations were lipid-normalised and expressed in μg/g lw.

Due to the great number of compounds analysed (n = 68), POP compounds were summed by groups (i.e., ΣPCBs, ΣDDTs, ΣPBDEs, HCB, ΣCHLs and mirex) and the individual from the Caribbean Sea was excluded. Data values were log (x + 1) transformed to account for skewness. To investigate the potential role of biological and ecological factors (i.e., geographic area, year, and sex) in the accumulation of POPs, analysis of covariance (ANCOVA) was used with sex as the covariate. For this analysis, only compounds with concentrations above the LOD were included. The one-way (Welsh) test was used to test for differences in means of log-transformed values among locations; this test is similar to an ANOVA, but does not require equal variances between groups (Rasch et al., 2011). Pairwise differences in the means of the sum of POP group concentrations followed the one-way test using a Bonferroni correction. Differences in accumulation patterns of individual compounds among areas where also investigated for PCBs, DDTs and PBDEs. The level of significance for statistical analyses was set at α = 0.05 and analyses were performed using Rstudio Team version 1.0.136 (Rstudio, 2016).

### Table 1

Lipid content (mean ± SD, %) and persistent organic pollutant (POP) concentrations (median and mean ± SD in brackets, in μg/g lipid weight) in blubber samples of males and females of Atlantic spotted dolphins (Stenella frontalis) by area. n = number of samples analysed. M = Male, F = Female. ND: Not determined.

<table>
<thead>
<tr>
<th>Area</th>
<th>Sex</th>
<th>n</th>
<th>Lipid</th>
<th>ΣPCBs</th>
<th>ΣDDTs</th>
<th>ΣPBDEs</th>
<th>Mirex</th>
<th>HCB</th>
<th>ΣCHLs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azores</td>
<td>M</td>
<td>34</td>
<td>11.4 ± 7.03</td>
<td>5.39</td>
<td>4.22</td>
<td>0.086</td>
<td>0.079</td>
<td>0.041</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(6.06 ± 2.51)</td>
<td>(4.45 ± 2.36)</td>
<td>(0.086 ± 0.062)</td>
<td>(0.13 ± 0.19)</td>
<td>(0.066 ± 0.099)</td>
<td>(0.033 ± 0.15)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>20</td>
<td>15.1 ± 13.9</td>
<td>3.66</td>
<td>2.94</td>
<td>0.007</td>
<td>0.07</td>
<td>0.001</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(3.54 ± 2.28)</td>
<td>(3.26 ± 1.56)</td>
<td>(0.05 ± 0.05)</td>
<td>(0.09 ± 0.17)</td>
<td>(0.05 ± 0.06)</td>
<td>(0.02 ± 0.06)</td>
<td></td>
</tr>
<tr>
<td>Canary Is</td>
<td>M</td>
<td>6</td>
<td>47.5 ± 15.2</td>
<td>12.1</td>
<td>7.3</td>
<td>0.17</td>
<td>0.18</td>
<td>0.09</td>
<td>&lt; LOD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(18.8 ± 16.7)</td>
<td>(12.5 ± 13.7)</td>
<td>(0.24 ± 0.16)</td>
<td>(0.24 ± 0.20)</td>
<td>(0.11 ± 0.07)</td>
<td>&lt; LOD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>4</td>
<td>64 ± 14.3</td>
<td>0.91</td>
<td>0.48</td>
<td>0.02</td>
<td>0.01</td>
<td>0.03</td>
<td>&lt; LOD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.98 ± 0.71)</td>
<td>(0.55 ± 0.45)</td>
<td>(0.02 ± 0.01)</td>
<td>(0.01 ± 0.006)</td>
<td>(0.07 ± 0.08)</td>
<td>&lt; LOD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>1</td>
<td>26.4</td>
<td>23.3</td>
<td>14.0</td>
<td>0.60</td>
<td>0.31</td>
<td>0.14</td>
<td>&lt; LOD</td>
</tr>
<tr>
<td>Caribbean Sea</td>
<td>F</td>
<td>1</td>
<td>13.4</td>
<td>3.09</td>
<td>1.30</td>
<td>0.16</td>
<td>0.18</td>
<td>0.03</td>
<td>&lt; LOD</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>(4.73 ± 2.36)</td>
<td>(2.73 ± 1.38)</td>
<td>(0.38 ± 0.29)</td>
<td>(0.18 ± 0.17)</td>
<td>(0.02 ± 0.04)</td>
<td>&lt; LOD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>25</td>
<td>20.4 ± 23.6</td>
<td>15.9</td>
<td>1.66</td>
<td>0.38</td>
<td>0.14</td>
<td>0.001</td>
<td>&lt; LOD</td>
</tr>
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<td></td>
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<td></td>
<td>(16.6 ± 10.3)</td>
<td>(2.85 ± 3.08)</td>
<td>(0.38 ± 0.29)</td>
<td>(0.18 ± 0.17)</td>
<td>(0.02 ± 0.04)</td>
<td>&lt; LOD</td>
<td></td>
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<tr>
<td></td>
<td>F</td>
<td>21</td>
<td>25.7 ± 23.7</td>
<td>4.8</td>
<td>0.16</td>
<td>0.16</td>
<td>0.04</td>
<td>0.001</td>
<td>&lt; LOD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(6.5 ± 6.8)</td>
<td>(1.4 ± 1.9)</td>
<td>(0.24 ± 0.28)</td>
<td>(0.10 ± 0.13)</td>
<td>(0.01 ± 0.01)</td>
<td>&lt; LOD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>3</td>
<td>21.0 ± 8.9</td>
<td>14.7</td>
<td>1.10</td>
<td>0.44</td>
<td>0.15</td>
<td>0.05</td>
<td>&lt; LOD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(14.5 ± 6.04)</td>
<td>(2.4 ± 2.3)</td>
<td>(0.40 ± 0.27)</td>
<td>(0.17 ± 0.04)</td>
<td>(0.07 ± 0.02)</td>
<td>&lt; LOD</td>
<td></td>
</tr>
</tbody>
</table>

< LOD: values below the limit of detection.


ΣDDTs = sum of op'-DDD, pp'-DDD, op'-DDD, pp'-DDD, and pp'-DDT.

ΣPBDEs = sum of PBDE 28, 47, 99, 100, 153, 154 and 183.

ΣCHLs = sum of α- and γ-chlordane.
sex, geographic area and the year when the animals were sampled or died could be used to investigate the potential role of the variables in the accumulation of POPs in spotted dolphins. All three variables demonstrated an effect on PCB and DDT concentrations (ANCOVA, p < 0.05, Table 2). Area and year exerted an effect with regard to PBDEs (ANCOVA, p < 0.05), whereas sex and year were significantly associated with concentrations of mirex (ANCOVA, p < 0.05). Only the accumulation of HCB was not significantly affected by any of these variables (ANCOVA, p > 0.05). As expected, sex was a biological factor that exerted an important effect on POP concentrations, with males exhibiting slightly higher concentrations than females for all POPs analysed, except PBDEs (Fig. 1). Conversely, higher concentrations of the lower chlorinated PCB congeners (those with five or fewer chlorine atoms) were found in females than males, contributing more to the total sum of PCB congeners (Fig. 2a). This could be the consequence of differential PCB maternal transfer during gestation and lactation, as previously demonstrated in other cetaceans (Desforges et al., 2012; Weijs et al., 2013). Concerning DDTs, males and females exhibited a very similar accumulation pattern, with slight differences (less than 20%) in the proportion of DDT analytes (Fig. 2b).

3.2. Geographic variations of POP accumulation in ASD

Differences in POP concentrations were found among the different regions of the Atlantic Ocean (Fig. 3). ΣPCBs, followed by ΣDDTs, exhibited the highest concentrations in all locations. The next most abundant compounds were ΣPBDEs in the specimens from São Paulo (southeastern Brazil) and the Canary Islands, and mirex in the Caribbean Sea dolphin.

3.2.1. Polychlorinated biphenyls (PCBs)

The highest concentrations of ΣPCBs were found in male dolphins from São Paulo and the Canary Islands (median: 15.9 and 12.1 μg/g lw, respectively) (Table 1), followed by those from the Azores (median: 5.39 μg/g lw). Moreover, the concentrations in males differed significantly between locations (pairwise test with Bonferroni correction, p < 0.01). No significant differences between locations were found for females (pairwise test with Bonferroni correction, p > 0.05), but those from São Paulo also had the highest concentrations. These results reflect the influence of the largest commercial harbour in South America (Santos harbour in the state of São Paulo) compared to less industrialised areas (i.e. Canary and Azores archipelagos and the Caribbean Sea). Moreover, this area is the most important petrochemical and metallurgical industrial centres in Brazil (Cubatao Industrial Complex) (Bícego et al., 2006).

Analysing the relative contribution of the different PCB congeners in terms of chlorination, PCBs containing five or more chlorines were predominant in dolphins from all areas. Hexa-CBs (mean: 45.2%) accounted for the highest percentage across all areas, followed by hepta-CBs (mean: 28.5%) and penta-CBs (mean: 18.2%) (Fig. 4a). These findings are related to the commercial mixture Aroclor 1254 and 1260, which were the most frequently produced and used in northern countries, such as Spain, and used in both Central and South American countries (Breivik et al., 2002; UNEP, 2002a, b). Both PCB mixtures were composed mostly of congeners with five or more chlorines (80.5 and 98.7%, respectively) (Breivik et al., 2002). However, comparing the four areas, dolphins from the Azores and São Paulo exhibited patterns with a high contribution of tetra- and penta-CBs and lower contribution of hepta-CBs than dolphins from the other two areas (Fig. 4a). Due to their chemical properties (structure, molar mass and solubility), POPs can be transported long distances from the original source of contamination (Wania and Mackay, 1996). Interestingly, hepta-CBs are rarely found far from their sources (Beyer et al., 2000) and are therefore more likely to be found in coastal dolphins. This suggests that some individuals from São Paulo with a more offshore PCB pattern may belong to the offshore ASD morphotype (i.e., found beyond the continental shelf break) (Perrin et al., 1987). The offshore morphotype was expected in the Azores, as there is no continental shelf. Indeed, a previous genetic study (Caballero et al., 2013) suggests historical or current connectivity between ASDs from the Azores and Madeira islands and those from southeast Brazil, which lends further support to our hypothesis based on PCB patterns.

3.2.2. Dichloro diphenyl trichloroethane (DDTs)

Among chlorinated pesticides, ΣDDTs had the highest concentrations in the four areas, with the highest medians found in males from the Canary Islands (7.32 μg/g lw), followed by males and females from the Azores (median: 4.22 and 2.94 μg/g lw) (Table 1). Moreover, concentrations differed significantly in males among the three areas (pairwise test with Bonferroni correction, p < 0.05). In bottlenose dolphins (Tursiops truncatus) from the Canary Islands, high concentrations of DDTs were found on the same order of magnitude as PCBs (median: 24.2 and 30.8 μg/g lw, respectively) and ranged from 0.19 to 1252 μg/g lw, with PCBs ranging from 0.24 to 1017 μg/g lw (García-Alvarez et al., 2014).

The DDT accumulation patterns, however, demonstrated subtle differences among areas, with pp’-DDE the predominant compound in all areas, followed by pp’-DDD in the Azores and Canary Islands, and pp’-DDE in the Caribbean and São Paulo dolphins (Fig. 4b). Compositions of DDT compounds are used to identify recent DDT inputs (Aguilar, 1984). Indeed, when pp’-DDE is discharged into the environment it degrades to pp’-DDE (mainly in organisms) and pp’-DDD. Therefore, the DDE/DDT ratio above 0.6 is indicative of a stable system with a lack of new DDT inputs. In the present study, the ratios ranged from 0.79 to 0.86 in the four areas, indicating that

Table 2

<table>
<thead>
<tr>
<th>POP classes</th>
<th>Explanatory variable/effects</th>
<th>Sex</th>
<th>Area</th>
<th>Year</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΣPCBs</td>
<td>&lt;0.0001 (40.544)</td>
<td>&lt;0.001 (5.323)</td>
<td>&lt;0.05 (6.351)</td>
<td>sex:area (3.379)</td>
<td></td>
</tr>
<tr>
<td>ΣDDTs</td>
<td>&lt;0.01 (11.645)</td>
<td>&lt;0.001 (5.845)</td>
<td>&lt;0.01 (10.327)</td>
<td>sex:area (7.146)</td>
<td></td>
</tr>
<tr>
<td>ΣPBDEs</td>
<td>n.s.</td>
<td>n.s.</td>
<td>&lt;0.001 (4.786)</td>
<td>sex:area (17.870)</td>
<td></td>
</tr>
<tr>
<td>Mirex</td>
<td>&lt;0.05 (5.833)</td>
<td>n.s.</td>
<td>&lt;0.05 (6.615)</td>
<td>sex:area:year (3.254)</td>
<td></td>
</tr>
<tr>
<td>HCB</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td></td>
</tr>
</tbody>
</table>


ΣDDTs = sum of op’-DDE, pp’-DDE, op’-DDD, pp’-DDD, op’-DDE, and pp’-DDT.

ΣPBDEs = sum of PBDE 28, 47, 99, 100, 153, 154 and 183.
DDT inputs in the ecosystems were not recent. The lowest ratio (0.79) was found in the Caribbean dolphins as a consequence of a high proportion of the metabolite pp'-DDT. DDTs have been largely used in the vector control programs for diseases (e.g., malaria, yellow fever, and dengue fever) (UNEP, 2002a), agricultural activities and, more recently, as an impurity of the widely used pesticide dicofol (Barra et al., 2006; Gillespie et al., 1994), which is mainly synthesised from DDT and a contributor to continued pp'-DDT and op'-DDT contamination in soil and water.

Fig. 1. Box and whisker plots of the POP concentrations in female (white box) and male (grey box) Atlantic spotted dolphins (Stenella frontalis). The bottom and top of the box are the first and third quartiles. The band inside the box is the second quartile, or the median, and the black dot the mean of the data. The ends of the whiskers represent the lowest datum of the lower quartile and the highest datum of the upper quartile. The open circles represent the outliers.
3.2.3. Polybrominated diphenyl ethers (PBDEs)

PBDEs also varied by location, with the highest and significantly different concentrations in males and females from dolphins sampled in São Paulo (median: 0.38 and 0.16 mg/g lw, respectively, \( p < 0.05 \)) (Table 1). PBDEs accounted for a higher percentage of POPs in dolphins in the northern hemisphere (<4%) than the southern hemisphere (<1%), where PBDEs are not manufactured (Fair et al., 2010; Kajiwara et al., 2006; Yogui and Sericano, 2009; Yordy et al., 2010). However, the close relationship between PBDEs and industrialised areas may explain the highest concentrations found in dolphins from the waters of São Paulo, which is one of the most industrialised areas in the southwest Atlantic. The next highest levels of PBDEs were found in dolphins from the Canary Islands and Caribbean Sea.

BDE-47 was the predominant congener in all the areas, except the Caribbean, followed by other penta-BDEs (100 and 99). Conversely, hepta-BDE 183 was not detected in the blubber of any specimen. This PBDE pattern is fairly congruent with patterns reported in previous studies on marine mammals (Fair et al., 2010; García-Alvarez et al., 2014; Isobe et al., 2009; Leonel et al., 2012;...
Yogui et al., 2010, 2011) and suggests less use of octa- and deca-BDE commercial mixtures, but high use of penta-BDE formulations in these geographic areas.

3.2.4. HCB and mirex

Among the other chlorinated pesticides, concentrations of HCB were highest in the Canary Island (Table 1) and mirex had the most similar concentrations among the areas (pairwise test with Bonferroni correction, p > 0.05). Much lower concentrations of these chlorinated pesticides were found in comparison to the other POPs. HCB concentrations were highest in ASD males and females from the northern hemisphere locations (Table 1) and significantly different between males from São Paulo and the Azores (pairwise test with Bonferroni correction, p < 0.05). These findings are in agreement with data from previous studies involving ASDs from São Paulo, reporting very low concentrations (mean: 0.02 μg/g lw; Leonel et al., 2012), as well as other cetaceans on the northern Atlantic coast of the USA, reporting higher concentrations (mean: 0.08–0.12 μg/g lw for bottlenose dolphins and 0.18 μg/g lw for the Atlantic white-sided dolphin [Lagenorhynchus acutus]) (Fair et al., 2010; Kucklick et al., 2011; Weisbrod et al., 2001). Finally, mirex had the most similar concentrations among locations (Table 1). This insecticide and flame retardant was intensively manufactured in the USA until the 1970s and used against ant species in South America, South Africa and Hawaii (Stringer and Johnston, 2001). Mirex was banned by the Stockholm convention in 2004, being part of the dirty dozen (the twelve initial POPs that have been recognized as causing adverse effects on humans and the ecosystem).

The present findings reveal that ASDs are subjected to different forms of contamination in the geographical areas studied. This was confirmed by the DDT-PCB ratios (Fig. 5), which are considered a proxy of agricultural and/or public health origins vs. industrial origins (Aguilar et al., 1999; Borrell and Aguilar, 2005). São Paulo dolphins had low and significantly different DDT-PCB ratios (0.23).
in comparison to those from the Azores and Canary Islands (0.76 and 0.65, respectively), indicating greater levels of PCBs, likely derived from highly industrialised regions, such as the city of Santos.

3.3. Temporal variations of POP accumulation in ASD

The year when the animals were sampled or found dead was the major factor affecting the accumulation POPs among the three variables tested (Table 2). A ten-year trend of the most concentrated POPs, PCBs and DDTs was investigated and the present findings demonstrate the combined effect of the geographical area and year. The most polluted individuals from the Canaries were sampled between 2008 and 2013 and the least polluted and numerous (i.e., from Azores) were sampled from 2005 to 2006. Thus, only the samples from São Paulo, which were homogeneously distributed among the different years, were used to interpret the temporal patterns (Fig. 6). The data revealed decreasing trends in PCB and DDT concentrations in the São Paulo dolphins over time. These decreasing trends were also observed when considering all the ASDs analysed, independently of geographical area. Previous studies conducted in the USA, Europe and Brazil report similar trends in marine mammal populations (Jepson et al., 2016; Law et al., 2012; Leonel et al., 2010). The sampling period (2005–2015) reflects the time after the ban of these pollutants in different regions around the world (e.g., North America, Europe and some South America countries). Thus, the observed declining trend was expected. However, some authors (e.g., Breivik et al., 2007) suggest that PCB levels in marine biota are unlikely to decline before 2040–2050, because only a small fraction (about one third) of the total PCB released into the environment has reached the oceans (Tanabe, 1988), which underscores the importance of continued temporal trend monitoring.

3.4. Toxicological implications

Comparison with established toxicity thresholds is the most common method of relating contaminant concentrations and susceptibility to disease and mortality in wildlife fauna. In the present study, a range of widely used PCB toxicity thresholds for marine mammals was considered (9 μg/g lw to 41.0 μg/g lw) based on marked reproductive impairment in ringed seals from the Baltic Sea (Helle et al., 1976). Among the individuals analysed, 33.9% exceeded the lowest PCB toxicity threshold of 9 μg/g lw. Most of these animals were from São Paulo (n = 28), followed by the Azores (n = 6) and Canary Islands (n = 5) (Fig. 7). Only one individual (a male from the Canary Islands) exceeded the highest toxicity threshold of 41 μg/g lw (Fig. 7). However, 57% and 45% of São Paulo and Canary dolphins had concentrations between these ranges. Finally, 11% of the dolphins from the Azores had concentrations below these thresholds. A total of 78.6% of São Paulo dolphins exceeding this threshold were collected near the highly industrialised area of Santos city. Conversely, the individual from the Caribbean Sea exhibited values nearly three times below the lowest PCB toxicity threshold.

To the best of our knowledge, few studies have established toxicity thresholds in marine mammals for organochlorine pesticides (i.e., DDTs) and none have established thresholds for HCB, mirex and CHLs. The only threshold for DDT concentrations in blubber associated with reproductive failure and pathological...
alterations in cetaceans was 50 μg/g lw (Wagemann and Muir, 1984). Lower concentrations in bottlenose dolphins have been related to a reduced in vitro immune response associated with levels of DDTs and PCBs in peripheral blood (Lahvis et al., 1995). However, no threshold was determined in the study cited. In the present study, no individual had concentrations above the toxicity threshold of 50 μg/g lw.

4. Conclusion

In the present study, contamination by persistent organic pollutants in the Atlantic spotted dolphin was evaluated on the metapopulation level. The results revealed clear inter-location differences in concentrations as well as accumulation patterns. In addition to sex and geographic area, the year in which the samples were collected also exerted an important influence on POP concentrations. Indeed, a decreasing trend in concentrations of PCBs and DDTs were found in São Paulo dolphins from 2005 to 2015. All POPs analysed were found in the blubber of the dolphins and 57% and 45% of São Paulo and Canary Island dolphins had concentrations between the lowest and highest toxic thresholds employed for marine mammals. Moreover, recent morphometric and genetic studies have demonstrated a discontinuous distribution of the species within the Atlantic Ocean and especially along the Atlantic Coast of South America, suggesting that spotted dolphins are distributed in several populations that should be considered different management units. If this is confirmed and in light of the POP concentrations obtained in the present study, the São Paulo and Canary Island dolphins should be considered vulnerable populations that merit special attention in future conservation and management programs. In clarify this issue better, further studies using POP concentrations as fingerprints of dietary behaviour or feeding habitat, together with other well-known ecological markers (i.e., stable isotopes, trace elements or fatty acids) are strongly encouraged to infer population structure and vulnerability.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2018.02.009.

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