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Introduction

The Lahille's bottlenose dolphin (*Tursiops gephyreus*) is an endemic coastal cetacean of the western South Atlantic (wSA), occurring along the coastal habitats of Argentina, Uruguay and southern Brazil (Fruet et al., 2015, 2014; Genoves et al., 2020). The low abundance of this subspecies leads to the classification as "vulnerable" in the last IUCN Red List assessment and as Endangered in the National Red List of Threathened Species in Brazil (MMA 148, 2022). Due to their low numbers, high site fidelity and restricted coastal distribution, Lahille's bottlenose dolphins are particularly sensitive to local anthropogenic impacts.

The Patos Lagoon estuary (PLE), southern Brazil, represents an important area for Lahille's bottlenose dolphins, in which some individuals shows strong site fidelity and residence pattern (Fruet et al., 2015, 2014; Genoves et al., 2020). The local population is composed by resident and semi-resident dolphins that uses the estuarine habitat and adjacent coastal areas. Total abundance is estimated to be about 163 individuals (see Fruet et al. 2023). Apart from bycatch, dolphins from PLE population are exposed to other stressors that can also act as a threat to their conservation, such as habitat degradation and chemical pollution (Righetti et al., 2019). The PLE has experienced increasing urban and industrial development over the last three decades. Human activities are intense, as the region is an important fishing ground and host one of the busiest ports and the largest port in the country. Chemical industries are installed in the margins of the estuary, which also receives untreated domestic, agricultural and industrial sewage disposals produced by the population living along the margins of the lagoon

Persistent organic pollutants (POPs) are ubiquitous in the ecosystems (Aguilar and Borrell, 1994; Alava et al., 2017) and bioaccumulate in marine biota and undergo biomagnification throughout trophic webs, resulting in higher concentrations in top predators, such as Lahille's bottlenose dolphins (Pereira et al., 2020). POPs are endocrine disruptors and immune suppressors in marine mammal organisms (Desforges et al., 2016; Galligan et al., 2019; Kannan et al., 2000). Their bioavailability depends on physicochemical properties of the environment, and in biota, their bioaccumulation is highly influenced by sex, age, and other parameters(Aguilar and Borrell, 1988; Oliveira-Ferreira et al., 2021) In addition, POPs can be transferred from mothers to their offspring, especially throughout lactation (Garcia-Cegarra et al., 2021; Ylitalo et al., 2001). Therefore, to investigate changes in POPs'bioccumulation over the years, males can

be used as target individuals, since bioaccumulation occurs throughout their lives (Durante et al., 2016; Yordy et al., 2010). Hence, this study aimed to characterize organochlorine compounds' bioaccumulation-polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), and mirex in the blubber of males Lahille's bottlenose dolphins from Patos Lagoon Estuary (PLE), southern Brazil, and to the investigate temporal trends of these compounds.

Material and Methods

Sampling

Blubber biopsy samples from known adult males of Lahille's bottlenose dolphins (n=28) were collected from living individuals between 2010 and 2020 (see TableS1), during boat surveys conducted along the Patos Lagoon Estuary and surrounding coastal areas (PLE) (30°30′S and 32°12′S). The area sampled was 85 km^2, considering the southernmost portion of the PLE (40 km^2) and adjacent marine coastal areas (45 km^2), which is the preferred used area by Lahille's bottlenose dolphins in the region (Fruet et al., 2015; Genoves et al., 2018; Mattos et al., 2007). Detailed methodology for sampling biopsies is described elsewhere (Fruet et al., 2017; Genoves et al., 2020) but important to highlight that simultaneous photoidentification of biopsies attempts avoided duplicates. Sampled dolphins were aged using the available long-term photoidentification database that is updated and maintained since 1974 by the Universidade Federal do Rio Grande – FURG.

Analyses of Organochlorine Compounds

The methodology was adapted from the literature (Lailson-Brito et al., 2012; Oliveira-Ferreira et al., 2021; Santos-Neto et al., 2014). Briefly, aliquots of 0.1 to 0.5 g of blubber were homogenized with anhydrous sodium sulfate (1:5), an internal standard (PCB 103 and PCB 198) was added, and extracted by continuous soxhlet apparatus with 100 mL of a mixture of hexane: dichloromethane (1:1) for 8 hours. The resultant extract was reduced to approximately 5 ml and then mixed with sulphuric acid for the clean-up, followed by purification in an aluminum oxide column, eluted in dichloromethane and n-hexane (2:1) and in dichloromethane and methanol (9:1). The final extract volume was reduced in nitrogen flow and lipid content was measured gravimetrically. The analysis was performed in a gas chromatographer (*Agilent Technologies*, 7890) coupled to a mass spectrometer detector (GC-MS, *Agilent Technologies*, 5975). The GC-MS was operating with an Electron Impact (EI) source in Selected Ion Monitoring (SIM) mode. Integration and data acquisition were performed in Enhanced ChemStation (*Agilent Technologies*, MSD ChemStation E.02.02.1431) for GC-MS. Organochlorine concentrations are expressed in μg. g^{-1} lipid weight (lw).

Quality control

The organochlorine compounds analyzed in the present study were PCBs and pesticides. Pesticides were expressed as the sum of DDT and metabolites (p.p'-DDE, p.p'-DDD), besides mirex. The sum of PCBs was represented by 27 congeners (PCB 8, PCB 31, PCB 28, PCB 52, PCB 49, PCB 44, PCB 74, PCB 70, PCB 101, PCB 151, PCB 99, PCB 97, PCB 118, PCB 153, PCB 105, PCB 141, PCB 138, PCB 158, PCB 187, PCB 183, PCB 177, PCB 180, PCB 170, PCB 203, PCB 195, PCB 194, PCB 206). The recovery of the samples was considered satisfactory when ranging from 70 to 130% (Wade and Cantillo, 1994) (Mean± SD: 102.8 ± 17 %). The standard solutions used were the PCB Mix for West Coast Fish Studies and the WHO/NIST/NOAA Congener List for PCBs and Pesticide Mix for Pesticide, all from AccuStandard® Laboratory. The analytical method was validated using two certificated materials (Pilot Whale Adipose Tissue -SRM-1945) from the National Institute of Standards and Technology- NIST). Procedural blanks were carried out in every batch and analytes were not found above detection limits. For PCBs, the limit of detection (LOD) varied from 0.001 and 0.008 μg/mL and the limit of quantification (LOQ) varied from 0.01 to 0.08 μg. g^{-1} lw. For pesticides, the LOD varied between 0.001 and 0.002 μg mL^{-1}, whereas the LOQ varied between 0.01 and 0.02 μg g^{-1} lw.

Data Analyses

The statistical treatment was performed using the software R (R Core Team, 2019). Only the organochlorine compounds identified in more than 50% of the samples were considered in the analyses. The temporal trends of organochlorine compounds detected in the blubber of Lahille's bottlenose dolphins

between 2010 and 2020 were investigated using a generalized additive model (GAM).The model was performed in R using the "*mgcv"* package with a *gamma* distribution. The gamma value was set at 1.4, and a cubic spline was chosen to smooth the data (Jepson and Law, 2016). Several model combinations were performed and the one with lower Akaike's Information Criterion (AIC) was chosen for each group of contaminant/congener investigated."Year of Sampling" "Lipidic Content" and "Age of Individuals" were the available covariables used in the model for the analyzed compounds. The compounds/congeners selected for these analyses were chosen based on their representation in the profile of each group of compounds (PCBs or pesticides); therefore, the most abundant were included in the models.

Results

General profile

POPs concentrations are presented in Table 1. PCBs presented higher concentrations, followed by DDTs and mirex. For PCBs, the most representative congeners were hexa- and hepta- chlorinated congeners, such as PCB 153, followed by PCB 138 and PCB 180; for DDTs, *p.p'*-DDE was the most abundant metabolite, followed by p.p'-DDD and p.p'-DDT.

Table 1: Median, mean, standard deviation (SD), minimum (Min), and maximum (Max) of age and organochlorine concentrations found in the blubber of adult males of Lahille's bottlenose dolphins (n=28). Age is expressed in years and concentrations in µg. g^{-1} lipidic weight (lw).

	Age	**∑PCB**	**∑DDT**	**Mirex**
Mean ± SD	12 ± 5	131 ± 132	24 ± 18	3.3 ± 1.8
Median	12	79	20	2.8
Min- Max	7-28	20- 713	1.9- 75	0.5- 8

Temporal trends

Temporal trends were found for PCBs and pesticides (Figure 1; Table 2). Year of Sampling" "Lipidic Content" and "Age of Individuals" were the suitable covariables fitting in the model for PCBs, whereas for pesticides these were "Year of Sampling" and "Lipidic Content". In fact, the year of sampling was the investigated covariable, since compounds' concentrations showed alterations along the years. Lipid content corresponds to the percentage of lipids in biopsy samples and was a probable variable, since compounds concentrations are expressed on that basis. In addition, the age of individuals is a parameter that influence POPs bioaccumulation, and these result was expected.

Among the analysed congeners, ∑PCB and PCB 138 showed an increase over the years, and "Age of Individuals (Age)" and "Lipidic Content (Lip)" were also significant covariables (For ∑PCB: p=0.01; Year: p=0.049; Age: p= 0.0038), and for PCB 138 (Year: p=0.00001) Among pesticides, significant temporal trends were observed for ∑DDT and *p.p*'-DDE, with a decline from 2010 to 2015, followed by an increase. "Lipidic Content" also influenced this result (For ∑DDT: p=0.01; Year=0.03; Lip= 0.007) and for *p.p*'-DDE: p=0.01; Year=0.03; Lip= 0.007). Mirex did not present significant temporal trend.

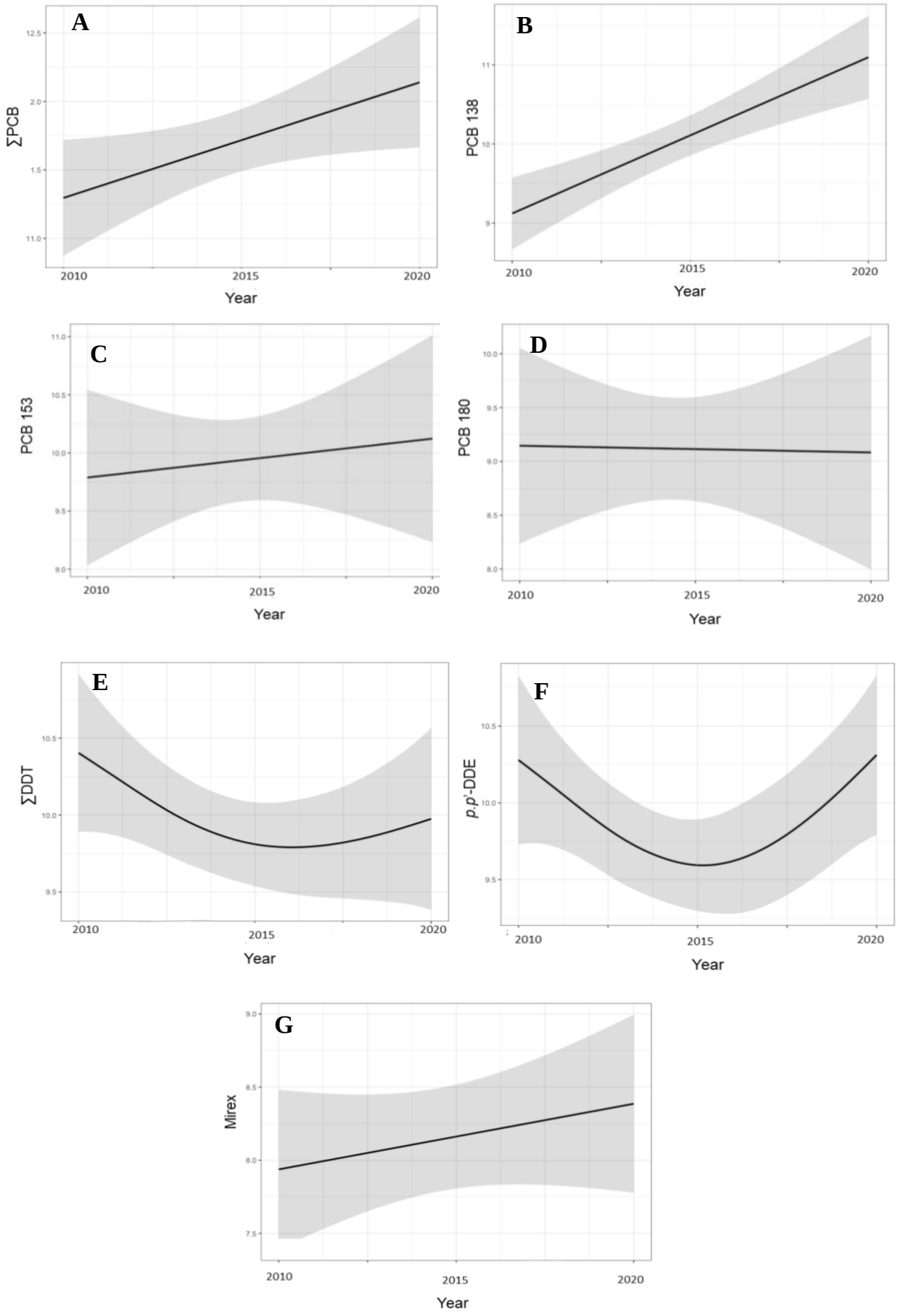

Figure 1: Temporal trend for organochlorine compounds in Lahille's bottlenose dolphin *(Tursiops gephyreus*) collected between 2010 and 2020. Natural log-transformed concentrations of (A) ΣPCB; (B) PCB 138; (C) PCB 153Σ; (D) PCB 180; (E) ΣDDTs; (F) *p.p'*-DDE; (G) Mirex across smoothed sampling period. The line represents the smoothed trend, and the shaded grey area represents the 95% confidence interval as estimated by fitting the data in a Generalized Additive Model (GAM).

Table 2: F and p-values for Generalized Additive Model (GAM) of organochlorine compounds in Lahille's bottlenose dolphin (*Tursiops gephyreus*) collected between 2010 and 2020. (*) indicates significative results at $p \leq 0.05$; (**) indicates significative results at $p \leq 0.01$; (***) indicates significative results at $p \leq 0.0001$.Year, Age of individuals and Lipidic Content were smoothed variables (s).

	Year (s)		Age (s)		Lipidic Content (s)	
	F	p-values	F	p-values	F	p-values
PCBs						
PCB 153	0.195	0.6623	3.737	0.0646	-	-
PCB 138	10.294	0.00376**	0.171	0.79298	0.797	0.33031
PCB 180	0.005	0.945	1.551	0.181	-	-
∑PCB	4.308	0.0484*	4.765	0.0386*	-	-
Pesticides						
p.p'-DDE	3.559	0.03461*	-	-	8.490	0.00761**
∑DDT	-	-	-	-	2.972	0.03*
Mirex	0.933	0.344	-	-	33.420	<2e-16***

Discussion

PCBs are known for their wide use and persistence in the environment especially due to their lipophilicity and ability to bioaccumulate and biomagnify in trophic webs (Aguilar and Borrell, 1994; Lailson-Brito et al., 2012). The most representative congeners herein found are due to their high degree of chlorine substitution and high molecular weights, which results in low degradation in the environment, besides their extensive use in commercial mixtures (Alava et al., 2020; Santos-Neto et al., 2014). This result is also corroborated by other studies on the Brazilian coast (Lailson-Brito et al., 2010; Oliveira-Ferreira et al., 2021).

The high ΣPCB concentrations found in the present study (median: 79 $\mu g.g^{-1}$ lw) corroborated the preliminary results reported in biopsy samples collected from living males of the same species during 2015-2016 (Minimum- Maximum: 23- 51 $\mu g.g^{-1}$ lw) (Righetti et al., 2019). ΣPCB mean and median concentrations in Lahille's bottlenose dolphins (Table 1) are much above the thresholds established in the literature regarding PCB toxicity, which are: 1.3 $\mu g.g^{-1}$ lw for endocrine disruption (Mos et al., 2010), 10 $\mu g.g^{-1}$ lw for risk of decline in population growth rates (Hall et al., 2006), and 17 $\mu g.g^{-1}$ lw in cetaceans' blubber for physiological effects(Kannan et al., 2000).

Among pesticides, DDT's profile reflects the historical use of DDT in PLE related to agricultural activities (Niencheski et al., 2014) as well as other regions along the Brazilian coast (Oliveira-Ferreira et al., 2021; Yogui et al., 2010). DDT can act as an endocrine disruptor and reproductive impairment, as already reported in marine mammals (Galligan et al., 2019). Pollutant synergy is also an important characteristic influencing reproductive success and affects the individuals' immunologic system (Desforges et al., 2016; Reddy et al., 2001). Median ΣDDT concentrations found in this study are similar to the highest concentration reported in an adult male of Lahille's bottlenose dolphins (maximum: 5 $\mu g.g^{-1}$ lw) (Righetti et al., 2019). The mean mirex concentration was an order of magnitude higher than a previous study in PLE (mean: 0.3 ± 0.1 $\mu g.g^{-1}$ lw) (Righetti et al., 2019).

PCBs showed an increase trend over the years (from 2010 to 2020). Such pattern is in contradiction with that reported for the Northern Hemisphere, where it was detected a decreasing trend in PCBs concentrations over the years, reflecting their prohibition (Kajiwara et al., 2008). The pattern observed in the present study also differs from the temporal trends observed for other cetaceans in the Southern Hemisphere, which showed a steady trend (Aono et al., 1997; Law, 2014; Oliveira-Ferreira et al., 2022). The exact causes for this temporal increase in PCBs on the blubber of Lahille's dolphins from PLE is unknown and should be carefully investigated, as POPs' concentrations in the environament can be related to diffuse sources, including forces that combines natural and anthropogenic activities (Barletta et al., 2019; Niencheski et al., 2014). The Patos Lagoon Estuary hosts a large port and many industries installed on their margin, making them subject to intense human interference such as fisheries activities, dredging

operations and intense commercial maritime traffic. Fine sediment is brought into the Patos Lagoon by the rivers and its deposits are likely to have long residence times (Calliari *et al*. 2008). Port activities, generated waves and water circulation by fresh water runoff can power remobilization of deeper sediments to the water column, enhancing pollutants' bioavailability and, consequently, their bioaccumulation in marine biota (Martelo et al., 2019; Mirlean et al., 2020). Indeed, high POPs concentrations were detected in sediment from PLE (Santos et al., 2020). The continuation of PCBs input to the environment should not be discarded and must be also investigated.

Regarding pesticides temporal trends, a different pattern from PCBs was observed. DDT concentrations decreased from 2010 to 2015, and after that, an increase was observed. This decline is probably associated with prohibition usage after 2009 (UNEP, 2017) resulting in low bioavailability in the environment and, thereafter, lower bioaccumulation. The observed increase trend after 2015 is a source of concern and needs further clarification. Apart from the discussed above, agricultural activities also can evoke an input of pesticides to the drainage basin and can be another source of these pollutants to PLE (Niencheski et al., 2014).

In addition to the influence of the time, the model indicates the importance of other variables, such as "Lipidic Content" and "Age of Individuals", as significant co-variants. The higher concentrations of ∑PCB and ∑DDT were observed in the oldest male (with 28 years), which was sampled in 2020. In fact, biological parameters also influenced POPs concentrations, as well as have been reported in the literature (Oliveira-Ferreira et al., 2022; Wells et al., 2005). Thus, the pattern described here would benefit from further analysis including a large and more balanced sample size in term of dolphin's age and time of sampling.

In conclusion, the high POPs concentrations found in Lahille's bottlenose dolphins from PLE corroborate a preliminary study performed in the same area. The higher number of samples, associated with temporal trends, reflects a major concern regarding this population, especially when considering the pollutant's effects on reproduction and immunity. In that sense, POPs also highlight another stressor for the species, already threatened with extinction.

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Table S1: Identification and Sampling Year of biopsies of adult males of Lahille's bottlenose dolphins (*Tursiops gephyreus*) (n=28).

Identification	Sampling Year
PLE 1	2010
PLE 2	2011
PLE 3	2011
PLE 4	2011
PLE 5	2011
PLE 6	2011
PLE 7	2011
PLE 8	2013
PLE 9	2013
PLE 10	2013
PLE 11	2014
PLE 12	2014
PLE 13	2014
PLE 14	2014
PLE 15	2014
PLE 16	2015
PLE 17	2015
PLE 18	2015
PLE 19	2015
PLE 20	2015
PLE 21	2015
PLE 22	2016
PLE 23	2016
PLE 24	2019
PLE 25	2020
PLE 26	2020
PLE 27	2020
PLE 28	2020