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Persistent organic pollutants in plasma and stable isotopes in red blood cells of *Caretta caretta*, *Chelonia mydas* and *Lepidochelys olivacea* sea turtles that nest in Brazil

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ARTICLE INFO

Keywords: Polychlorinated biphenyls Organochlorinated pesticides Green turtle Loggerhead Olive ridley Marine turtles

ABSTRACT

Studies of persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), organochlorinated pesticides (OCPs), and polybrominated diphenyl ethers (PBDEs), in sea turtles are reported, but there are still spatial data gaps worldwide. POP contamination of live female blood plasma from *Caretta caretta* (n = 28), *Chelonia mydas* (n = 31) and *Lepidochelys olivacea* (n = 19), which nest in Brazil and feed along the South Atlantic Ocean, was investigated. Carbon and nitrogen stable isotopes from red blood cells (RBC) were also evaluated to obtain information about trophic ecology. *C. caretta* had the highest POP concentrations, followed by *L. olivacea* and *C. mydas*. PCBs predominated in all species, and the major OCPs were the DDTs (dichlorodiphenyltrichloroethane and derivatives) and Lindane. POPs and stable isotopes revealed intra- and interspecific variations, which reflect the high plasticity in the use of habitat and food resources, making individuals within the same population susceptible to different exposures to pollutants.

1. Introduction

Anthropic pollution is a threat to sea turtles, and it has been considered a global research priority for the conservation of these animals (Hamann et al., 2010). Among the hazards, persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and polybrominated diphenyl ethers (PBDEs) have been reported in sea turtle populations (Keller, 2013). After the Second World War, countries produced and imported these synthetic compounds which were used as pesticides, electrical insulation, and flame retardants, and were unintentionally produced industrially. POPs have high stability and toxicity, as well as low degradability and lip-ophilicity, that cause them to remain in the environment, bioaccumulate in organisms, and undergo biomagnification along the food chain such that the tissues of species at upper trophic levels have the highest concentrations (Kodavanti et al., 2014).

These chemicals can cause deleterious effects in wildlife, including alterations in neurological and immune function, growth, and reproduction, and act as endocrine disruptors (Fox, 2001). Despite not knowing the toxicological effects and detrimental threshold for marine

reptiles (Guirlet et al., 2010), these pollutants affected the immune system of *C. caretta* and *L. kempii* (Keller et al., 2006; Swarthout et al., 2010). Keller et al. (2004c) reported that POPs could probably cause anemia, hepatocellular damage and immunomodulation in *C. caretta*. The possible reduction of hatching success of *C. mydas* and *D. coriacea* caused by those compounds was discussed by Van de Merwe et al. (2010b) and De Andrés et al. (2016). Thus, it is crucial to monitor POPs in wildlife, especially for species that are threatened, as these compounds could cause harmful health effects and contribute to population decline (Keller et al., 2004a). All seven sea turtles species are included in the IUCN Red List of Threatened Species, classified as Vulnerable (*Caretta caretta; Dermochelys coriacea; Lepidochelys olivacea*), Endangered (*Chelonia mydas*), Critically endangered (*Eretmochelys imbricata; Lepidochelys kempii*) and Data deficient (*Natator depressus*) (IUCN, 2020).

Some studies reported POP for marine turtle species distributed in oceans and coasts of America (Caribbean Sea, Pacific Ocean), Europe (Adriatic Sea), and Africa (Atlantic Ocean). Brazil acts as a foraging and spawning area for five marine turtles - *C. mydas, C. caretta, D. coriacea, E. imbricata*, and *L. olivacea* – in the Southwest Atlantic Ocean (Marcovaldi et al., 2011). Despite that, only a few studies have been conducted,

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https://doi.org/10.1016/j.marpolbul.2021.112283

Received 6 October 2020; Received in revised form 11 March 2021; Accepted 13 March 2021 0025-326X/@ 2021 Elsevier Ltd. All rights reserved.

such as an analysis of OCPs and PCBs in unhatched eggs of *C. caretta* (Baldassin et al., 2002) and in carcasses of juvenile *C. mydas* (Da Silva et al., 2016; Sánchez-Sarmiento et al., 2017), and an analysis of PCBs in the blood of juveniles and one adult female of *C. mydas* (Rossi, 2014).

Across the globe, the studies of POPs in sea turtles mainly used tissues collected from stranded carcasses, such as fat, liver, muscle, and kidney (Clukey et al., 2018), as well as fresh and unhatched eggs (Salvarani et al., 2019). However, to assess POPs in live sea turtles, researchers are analysing blood or plasma, as these matrices are noninvasive, easier to collect and enable recollection (Keller et al., 2004b; Swarthout et al., 2010; Barraza et al., 2020). Despite these benefits, the analysis of these matrices requires the use of sensitive equipment to detect POP, since they have lower concentrations than solid tissues (Keller, 2013). In general, the concentrations of POPs in solid tissues of sea turtles in Brazil are among the lowest ever detected.

As food is the main route of contamination by POPs (IARC, 2016), stable isotopes can help to obtain information about dietary habits and habitat. The values of the isotopic carbon ratio $({}^{13}C/{}^{12}C = \delta^{13}C)$ show low enrichment, ranging from 0 to 1‰ per trophic level (DeNiro and Epstein, 1978). On the other hand, the isotopic nitrogen ratio $({}^{15}N/{}^{14}N = \delta^{15}N)$ of an animal is higher than that of its food, with an increase of 3 to 4‰ for each successive trophic level (Minagawa and Wada, 1984). Thus, $\delta^{13}C$ data can be used to distinguish oceanic from neritic area, and $\delta^{15}N$ can be used to identify the organism's trophic position (Reich et al., 2007). The tissues have different turnover. For sea turtles, the turnover of red blood cells (RBC) is estimated to reflect the foraging habits of the previous 4–7 months (Ceriani et al., 2012), representing the stable isotopes incorporated in the foraging area used by the female before migration to the nesting area.

This study aimed to assess POP contamination in plasma of female *C. mydas, C. caretta* and *L. olivacea* sea turtles that nest in Brazil and feed along the Atlantic Southeast Ocean. Furthermore, carbon and nitrogen stable isotopes in RBC were used to evaluate the influence of dietary habits in the exposure to these pollutants.

2. Materials and methods

2.1. Study sites and sample collection

The study comprised different areas of northeastern Brazil. The 31 specimens of *C. mydas* were sampled in the Atol das Rocas Biological Reserve – Rebio Atol das Rocas (Rio Grande do Norte state), between

February and May of 2017. This conservation unit is the only atoll in the South Atlantic Ocean $(03^{\circ} 45/03^{\circ} 56 \text{ S} \text{ and } 33^{\circ} 37/33^{\circ} 56 \text{ W})$ (ICMBIO, 2007), and it is the second-largest nesting site for green sea turtles in the Western South Atlantic (Bellini et al., 1996).

For *C. caretta* and *L. olivacea*, the samples were collected at the north coast of Bahia state, an important Brazilian nesting area for these species (Marcovaldi and Laurent, 1996). Between November and December of 2016 and 2017, 28 female *C. caretta* were sampled at Busca Vida Beach (12° 52' 27.5"S, 38° 16'08.1"W) and Praia do Forte Beach (12° 34'40.8"S, 38° 00'07.8"W). In the same period of the last year, 19 turtles of *L. olivacea* were collected in Mangue Seco (11° 41'29.2"S, 37° 23'09.8"W) and Coqueiro (11° 29'57.1"S, 37° 23'09.8"W) (Fig. 1).

In night patrols, during oviposition, approximately 10 mL of blood was collected from the dorsocervical sinus (Owens and Ruiz, 1980) of each nesting female and transferred to glass vials containing sodium heparin. Prior and after blood collection, the venipuncture site was swabbed with alcohol. The curve carapace length (CCL) and width (CCW) was measured with a flexible tape (Bolten, 1999). *C. caretta* and *L. olivacea* were identified through Inconel flipper tags (#681 National Band and Tag Company), and *C. mydas* was identified with a microchip (PIT tag). The blood samples were centrifuged for 10 min at 3000 rpm to separate plasma from RBC and other cellular material (Keller et al., 2014). Each plasma sample was transferred to another glass vial using a Pasteur pipette. All the glassware used were previously baked at 450 °C. The samples were kept frozen at -20 °C until analysis.

2.2. POP analysis

The method used to analyse the organic compounds was based on liquid-liquid extraction described by Keller et al. (2009), with some modifications.

Briefly, 2 g of defrosted plasma was amended with 100 μ L of internal standard (PCB 103 and PCB 198-10 g μ L⁻¹) and mixed using a vortex. Formic acid was added (2.5 mL), followed by 6 mL of the extraction solvents hexane and dichloromethane (4:1, v:v). The sample was vortexed for 1 min and then centrifuged for 20 min at 2500 rpm; the organic phase was transferred to another glass vial. The TEO (total extractable organic matter) was used as a proxy for lipid content (Keller et al., 2014). It was determined gravimetrically from a subsample of the extract. The extract was concentrated to 0.5 mL and cleaned up using 1 g acidified silica column (SiO₂: H₂SO₄, 2:1) in a chromatographic column. POPs were eluted with 4 mL of solvent hexane and dichloromethane



Fig. 1. Study areas where the female sea turtles were sampled in northeastern Brazil.

(1:1, v:v). After that, samples were solvent-exchanged to iso-octane and concentrated using a rotary-evaporator followed by nitrogen evaporation. Then, 9 μ L of secondary standard (TCMX – Tetrachlorometaxylene - 100 pg μ L⁻¹) was added, achieving a final volume of 90 μ L, before the gas chromatographic analysis. A procedural blank was run for every set of nine samples.

POP analyses were performed in an Agilent Technologies 7890B gas chromatograph with a 7010B triple quadrupole mass spectrometer (GC/MS/MS) using a 30 m × 0.25 mm i.d. capillary column coated with a 5% phenyl-substituted dimethylpolysiloxane phase, with a film thickness of 0.25 μ m. For each sample, 1 μ L was injected in pulsed splitless mode. The carrier gas used was helium, with a constant flow of 1.2 mL min⁻¹. The temperature of the injector, interface and source was 300 °C, while the quadrupoles were kept at 150 °C. The oven temperature program was 50 °C for 1 min, followed by an increase at 20 °C min⁻¹ to 200 °C and an increase at a rate of 10 °C min⁻¹ until it reached 300 °C, remaining constant for 5 min.

To ensure the reliability of the method, quality control was carried out using replicates and certified reference material. The Standard Reference Material (SRM) used was SRM 1958 - *Organic contaminants in fortified human serum*, purchased from the National Institute of Standards and Technology (NIST, USA). The recovery of analytes in spiked blanks was between 65.6 and 117% and for matrices was between 62.5 and 120%, within established limits (50–120%). Analytes found in laboratory blanks were subtracted from the samples. The results were expressed as ng g⁻¹ ww (wet weight). The limit of quantification (LOQ) for PCBs was 0.0025 ng g⁻¹ and 0.0050 ng g⁻¹ ww for OCPs and PBDEs. The quantification of analytes was performed using a twelve-level curve and followed the internal standard procedure. Internal standard recoveries ranged from 53.6 to 90.8%.

Fifty-one PCB congeners were analysed, including the following IUPAC numbers: 8, 18, 28, 31, 33, 44, 49, 52, 56, 60, 66, 70, 74, 77, 81, 87, 95, 97, 99, 101, 105, 110, 114, 118, 123, 126, 128, 132, 138, 141, 149, 151, 153, 156, 157, 158, 167, 169, 170, 174, 177, 180, 183, 187, 189, 194, 195, 199, 203, 206 and 209. For OCPs, the fifteen investigated compounds were dichlorodiphenyltrichloroethane and derivatives (o,p'-DDT, p,p'-DDT, o,p'-DDD, p,p'-DDD, o,p'-DDE and p,p'-DDE), hexa-chlorocyclohexanes (α , β -, δ - and γ -HCH), hexachlorobenzene (HCB), chlordane (*cis, trans* and oxi-chlordane) and mirex. For PBDE, the evaluated congeners IUPAC numbers were 28, 47, 99, 100, 153, 154 and 183.

2.3. Stable isotope analysis

The analyses were based on the methodology described in Colabuono et al. (2014), with some modifications. First, the RBCs were freeze-dried (Thermo Savant ModulyoD) for 48 h and subsequently powdered using a mortar and pestle. The samples were not submitted to lipid extraction. Approximately 0.60 mg of each sample was weighed on a precision analytical micro-scale and placed in a tin capsule.

The samples were analysed in an elemental analyser (Costech Instruments Elemental Combustion System - ECS 4010) coupled with an isotopic ratio mass spectrometer (IRMS Thermo Scientific Delta V Advantage). Stable isotope ratios were δ notation as parts per thousand (‰) deviating from the international standards Pee Dee Belemnite limestone (carbon) and atmospheric air (nitrogen) using Eq. (1):

$$\delta X(\%_0) = \left(\frac{R \ sample}{R \ standard}\right) \tag{1}$$

where X is ¹³C or ¹⁵N and R the corresponding ratio ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) of the sample (R_{sample}) and the standard ($R_{standard}$).

2.4. Statistical analysis

The Shapiro-Wilk test confirmed the absence of normal distribution

in POPs, biometric and stable isotope data. Therefore, the statistics were based on non-parametric analyses. Except for PCB, which all concentrations were above the limit of quantification (LOQ), for the other POPs, the data <LOQ were substituted by the half of the LOQ. After that, medians were calculated, and boxplot graphs were constructed. To statistical analysis, the outliers were not included. The graphs of PCB congeners, DDT metabolites, HCH isomers and Chlordane compounds comprised only the compounds that had at least one data >LOQ.

The Kruskal-Wallis test, followed by Mann-Whitney pairwise tests, was used to evaluate the occurrence of significant differences in the medians of POPs and in the medians of stable isotopes between the three species. The Spearman coefficient correlation test (r_s) assessed the relationship between POPs and TEO; POPs and biometry; and POPs and stable isotopes. Statistical analyses were performed using the software PAST 3.16 (Paleontological Statistics software package for education and data analysis – Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2017), with a significance level of 5% (p = 0.05).

3. Results

3.1. Persistent organic pollutants

POP median and range concentrations in the female sea turtle plasma, as well the number and percentage of samples above LOQ, are shown in Table 1. POPs were detected in all sea turtle plasma samples, with a predominance of PCB. *C. caretta* had the highest concentrations of POPs, followed by *L. olivacea* and then *C. mydas*. Approximately 92% of the females showed values >LOQ for OCPs. For *C. caretta* and *L. olivacea*, Σ DDT was the second prevailing group, followed by Σ HCH. On the other hand, for *C. mydas*, Σ HCH dominated the OCPs, followed by Σ Chlordane and Σ DDT. The POP concentrations and TEO for each female analysed are shown in Supplementary data (Tables S1, S2 and S3).

POP concentrations showed intra- and interspecific variation, with a greater extent in *C. caretta* and then *C. mydas. L. olivacea* had the lowest deviations. According to statistical analyses, the three species were significantly different for Σ PCB and Σ DDT medians, but not for Σ HCH, HCB, and Σ PBDE. For Σ Chlordane, *L. olivacea* showed significant differences from the other species. The concentrations of the pollutant groups are shown in Fig. 2.

C. caretta showed the highest median for Σ PCB (1.02 ng g⁻¹), followed by *L. olivacea* (0.441 ng g⁻¹). Based on the sum of congeners of the same chlorination, for both species, compounds with six, seven and five chlorine atoms predominated. On the other hand, *C. mydas* had the lowest concentrations (median = 0.171 ng g⁻¹), and the sum of congeners with five, six and four chlorine atoms prevailed (Fig. 3).

Among 51 PCB congeners analysed, 39 were detected in *C. mydas*, 46 in *L. olivacea* and 48 in *C. caretta* (Fig. 4). PCBs 138 and 153 were among the most relevant congeners for the three species. Based on the median concentration, the principal congeners for each species were as follows: 138 > 101 > 153 > 110 > 95 (*C. mydas*), 138 > 153 > 180 > 170 > 118 (*C. caretta*) and 138 > 153 > 180 > 187 > 170 (*L. olivacea*). The following dioxin-like PCBs were detected in females: 77, 81, 105, 114, 123, 126, 156, 157 and 189. The sum of these congeners was >LOQ in approximately 80% of *C. mydas* and more than 90% of *C. caretta* and *L. olivacea*.

Values >LOQ for OCPs were present in all females of *C. caretta*, in 94.7% of *L. olivacea* and in 83.9% of *C. mydas*. The metabolites of DDT, isomers of HCH and compounds of Chlordane > LOQ in the three species are shown in Fig. 5.

The Σ DDT, composed only of p,p'-DDE, was the OCP with the larger contribution in *C. caretta* and *L. olivacea* plasma and was detected in 100% and 95% of the turtles, respectively. DDT concentrations ranged from 0.007 to 0.396 ng g⁻¹ for *C. caretta* and <LOQ to 0.560 ng g⁻¹ for *L. olivacea*. On the other hand, this group was found in just 29% of *C. mydas*, and although the p,p'-DDE predominated (<LOQ - 0.016 ng g⁻¹), o,p'-DDT was detected in two turtles (<LOQ - 0.007 ng g⁻¹).

Table 1

Number and percentage of samples above the limit of quantification (>LOQ), median and range levels of persistent organic pollutants (ng g⁻¹ ww) analysed in plasma samples from *C. mydas*, *C. caretta* and *L. olivacea*. $\Sigma PCB = sum$ of 51 congeners; $\Sigma DDT = sum$ of six metabolites; $\Sigma HCH = sum$ of four isomers; Chlordane = sum of three compounds; $\Sigma PBDE = sum$ of 7 congeners.

Compounds		Che	elonia mydas	;	Caretta caretta				Lepidochelys olivacea						
	(n = 31)					(n = 28)					(<i>n</i> = 19)				
	n > LOQ	n > LOQ (%)	Median	Min	Max	n > LOQ	n > LOQ (%)	Median	Min	Max	n > LOQ	n > LOQ (%)	Median	Min	Max
ΣΡCΒ	31	100	0.171	0.039	0.951	28	100	1.02	0.018	2.40	19	100	0.441	0.088	3.04
ΣDDT	9	29	0.003	<loq< td=""><td>0.022</td><td>28</td><td>100</td><td>0.102</td><td>0.007</td><td>0.396</td><td>18</td><td>95</td><td>0.031</td><td><loq< td=""><td>0.560</td></loq<></td></loq<>	0.022	28	100	0.102	0.007	0.396	18	95	0.031	<loq< td=""><td>0.560</td></loq<>	0.560
ΣΗCΗ	24	77	0.018	<loq< td=""><td>0.107</td><td>19</td><td>68</td><td>0.038</td><td><loq< td=""><td>0.195</td><td>11</td><td>58</td><td>0.010</td><td><loq< td=""><td>0.069</td></loq<></td></loq<></td></loq<>	0.107	19	68	0.038	<loq< td=""><td>0.195</td><td>11</td><td>58</td><td>0.010</td><td><loq< td=""><td>0.069</td></loq<></td></loq<>	0.195	11	58	0.010	<loq< td=""><td>0.069</td></loq<>	0.069
ΣChlordane	9	29	0.003	<loq< td=""><td>0.022</td><td>17</td><td>61</td><td>0.006</td><td><loq< td=""><td>0.082</td><td>0</td><td>0</td><td><loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<></td></loq<></td></loq<>	0.022	17	61	0.006	<loq< td=""><td>0.082</td><td>0</td><td>0</td><td><loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<></td></loq<>	0.082	0	0	<loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""></loq<></td></loq<>	<loq< td=""></loq<>
HCB	1	3	0.003	<loq< td=""><td>0.006</td><td>7</td><td>25</td><td>0.003</td><td><loq< td=""><td>0.010</td><td>2</td><td>11</td><td>0.003</td><td><loq< td=""><td>0.045</td></loq<></td></loq<></td></loq<>	0.006	7	25	0.003	<loq< td=""><td>0.010</td><td>2</td><td>11</td><td>0.003</td><td><loq< td=""><td>0.045</td></loq<></td></loq<>	0.010	2	11	0.003	<loq< td=""><td>0.045</td></loq<>	0.045
Mirex	0	0	<loq< td=""><td><loq< td=""><td><loq< td=""><td>0</td><td>0</td><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>1</td><td>5</td><td>0.003</td><td><loq< td=""><td>0.019</td></loq<></td></loq<></td></loq<></td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td>0</td><td>0</td><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>1</td><td>5</td><td>0.003</td><td><loq< td=""><td>0.019</td></loq<></td></loq<></td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td>0</td><td>0</td><td><loq< td=""><td><loq< td=""><td><loq< td=""><td>1</td><td>5</td><td>0.003</td><td><loq< td=""><td>0.019</td></loq<></td></loq<></td></loq<></td></loq<></td></loq<>	0	0	<loq< td=""><td><loq< td=""><td><loq< td=""><td>1</td><td>5</td><td>0.003</td><td><loq< td=""><td>0.019</td></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td>1</td><td>5</td><td>0.003</td><td><loq< td=""><td>0.019</td></loq<></td></loq<></td></loq<>	<loq< td=""><td>1</td><td>5</td><td>0.003</td><td><loq< td=""><td>0.019</td></loq<></td></loq<>	1	5	0.003	<loq< td=""><td>0.019</td></loq<>	0.019
ΣPBDE	2	6	0.003	<loq< td=""><td>0.007</td><td>1</td><td>4</td><td>0.003</td><td><loq< td=""><td>0.006</td><td>2</td><td>11</td><td>0.003</td><td><loq< td=""><td>0.021</td></loq<></td></loq<></td></loq<>	0.007	1	4	0.003	<loq< td=""><td>0.006</td><td>2</td><td>11</td><td>0.003</td><td><loq< td=""><td>0.021</td></loq<></td></loq<>	0.006	2	11	0.003	<loq< td=""><td>0.021</td></loq<>	0.021

Notes: n = number of samples; OCPs and PBDEs: LOQ = 0.0050 ng g⁻¹; PCBs: LOQ = 0.0025 ng g⁻¹.



Fig. 2. Persistent organic concentrations (ng g^{-1} ww) in plasma of *C. mydas, C. caretta* and *L. olivacea*. The inner line of the box corresponds to the median, while the lower and upper line are the quartiles (25 and 75%). The bars represent extreme values, and the circles are outliers. Equal letters (A, B or C) indicate no statistically significant difference among POP median concentration (p > 0.05) in Kruskal-Wallis/Mann-Whitney.

The Σ HCH was the most frequent OCP for C. mydas and was detected in 77% of females (median = 0.018 ng g⁻¹), while for C. caretta (median = 0.038 ng g⁻¹) and L. olivacea (median = 0.010 ng g⁻¹) it was present in 68% and 58% of females. Except for one L. olivacea, where β -HCH was present, in the other females, independent of the species, Υ -HCH (Lindane) was the only isomer detected.

 Σ Chlordane was detected in 61% of the *C. caretta* and in 29% of *C. mydas* but was <LOQ in all females of *L. olivacea*. Υ -Chlordane (transchlordane) represented all Σ Chlordane in *C. mydas* (median = 0.0036)

ng g⁻¹). For *C. caretta*, Σ Chlordane (median = 0.006 ng g⁻¹) was composed by Υ -Chlordane and Oxychlordane.

HCB was >LOQ in one *C. mydas*, seven *C. caretta*, and two *L. olivacea*, with low median concentrations of 0.003 ng g^{-1} for all species. Mirex was detected in only one female of *L. olivacea* (0.019 ng g^{-1}).

Among the seven analysed PBDE congeners, four were >LOQ (99, 100, 154 and 183). Σ PBDE was present in two *C. mydas*, two *L. olivacea* and one *C. caretta*, and the median concentration for all the three species were 0.003 ng g⁻¹.



Fig. 3. Chlorine percentage of the PCBs identified in the plasma of female *C. mydas, C. caretta* and *L. olivacea*.

No correlation was found among POPs and sea turtle size (length). For POPs and TEO, weak to moderate significant correlation was observed (p < 0.05), except for *C. caretta*. In *C. mydas*, positive correlations for Σ HCH (0.59), Σ DDT (0.37) and PCB (0.58) were obtained, while a negative correlation was found for PCB (-0.59) in *L. olivacea*.

3.2. Stable isotopes

Similar to POPs, the most significant variations in isotopic ratios were found in *C. caretta*, followed by *C. mydas* and then by *L. olivacea* (Table 2). For δ^{13} C, *C. caretta* had higher values than *L. olivacea* and *C. mydas*, and the Kruskal-Wallis/Mann-Whitney showed that the first species was significantly different from the others (p < 0.05). For δ^{15} N, *L. olivacea* had the highest value, followed by *C. caretta* and then by *C. mydas*; all three species were statistically significantly different (p < 0.05). The values of isotopic ratios for each female were presented in Supplementary data Tables S4, S5 and S6.

Approximately 70% of *C. mydas* showed δ^{13} C values between -18 to -20% and δ^{15} N between 4 and 7‰. For *C. caretta*, approximately 68% of females had δ^{13} C between -15 and -17% and δ^{15} N between 4 and 9‰. For *L. olivacea*, nearly 80% of the females had δ^{13} C values between -18 and -20% and for δ^{15} N values between 7 and 10% (Fig. 6).

3.3. Stable isotopes and persistent organic pollutants

The Spearman's correlation coefficient (r_s) was used to evaluate the correlation among the different POPs and the values of δ^{13} C and δ^{15} N, except for Mirex that showed only one value >LOQ. The results are shown in Table 3.

C. caretta showed moderate positive correlations ($r_s = 0.45$ to 0.55) between $\delta^{15}N$ and Σ PCB, PCB 138, PCB 153 and Σ DDT. Females of *C. mydas* had positive moderate correlation only between $\delta^{15}N$ and PCB 138. On the other hand, for *L. olivacea*, no significant correlation was found between stable isotope and Σ PCB, PCB 138, PCB 153, Σ DDT and Σ HCH. For all the three species, there was no correlation with PBDE, as well as with HCB, except among $\delta^{13}C$ and HCB ($r_s = 0.43$) in *C. caretta*.

4. Discussion

4.1. Persistent organic pollutants

The prevalence of Σ PCB and Σ DDT in the species of the present study are in accordance with other reports that analysed sea turtles tissues, such as plasma, blood, eggs, liver and fat. A review of studies of POPs in those tissues is available in Keller (2013). As it is shown in Table 4, despite the predominance of the same POPs, the concentrations found in this study are lower than other studies from the Northern hemisphere - USA, Mexico, Eastern Atlantic Ocean, Adriatic Sea and Malaysia. Although most of the works did not detect HCH, in the analysed samples, this group was one of the main pesticides detected and agrees with the literature review of Barra et al. (2006), which showed that PCBs, followed by DDT and HCH, are the principal POPs that occur in aquatic organisms in South America.

Aquatic air breathing organisms such as sea turtles can achieve relatively high concentrations of POPs in the environment they live through their contact with water, sediment and air. Furthermore, POPs can enter into biota through feeding and transfer along the food chain (Voutsas et al., 2002), since the feeding is the main route of contamination by those compounds (IARC, 2016).

The differences between the number of POPs >LOQ and among the concentrations found in the three studied species are probably related to their eating habits. *C. caretta* and *L. olivacea* had higher concentrations, since they are primarily carnivorous. On the other hand, the lowest concentrations occurred in *C. mydas*, which is mainly herbivorous during adulthood.

In addition to the trophic level in the food chain, the feeding area and habits of each individual also play a role. Information about the foraging area of sea turtles that nest in Brazil is scarce, but studies showed that the analysed species forage within the South Atlantic Ocean. Genetic studies suggested that nesting females of C. mydas from Rebio Atol das Rocas feed uniformly along the Southwest Atlantic Ocean (Naro-Maciel et al., 2012). For C. caretta, satellite telemetry in nesting females of the north of Bahia revealed that these turtles moved along the Brazilian continental shelf after the reproduction period until reaching their foraging areas in the states of Ceará, Pará and Maranhão (Marcovaldi et al., 2010). Furthermore, carcass analyses suggested that the south of Brazil could be a feeding area for the females that nest in the states of Rio de Janeiro, Espírito Santo, Bahia and Sergipe (Marcovaldi and Chaloupka, 2007; Monteiro et al., 2016). Satellite telemetry placed in L. olivacea females that nest in Sergipe identified feeding areas in Brazilian states and beyond its geographical boundary - the continental shelf of the states of Espírito Santo, Alagoas, Pernambuco, Rio Grande do Norte, Pará, Rio de Janeiro, São Paulo, Paraná and Santa Catarina, as well as French Guiana and ocean waters near northwest Africa (Da Silva et al., 2011; Dos Santos et al., 2016).

Studies in different countries using satellite telemetry showed that species that were previously considered to use only neritic areas in adulthood could use oceanic regions to feed. Adult females of C. caretta in Japan and Cape Verde Island demonstrated dichotomy in postbreeding behaviour. Some turtles migrated to neritic areas and possibly fed on benthic organisms, while others moved to the oceanic region and ingested epipelagic/planktonic organisms (Hawkes et al., 2006; Hatase et al., 2002). In the Sultanate of Oman, C. caretta females alternated among oceanic and neritic areas (Rees et al., 2010). Females of L. olivacea from Australia presented three different foraging ecologies after the post-nesting period: shallow near shore; continental shelf; or continental slope shelf (McMahon et al., 2007; Whiting et al., 2007). For females of C. mydas from Japan, after the reproduction period, although the majority of turtles moved to neritic foraging grounds and were herbivorous, approximately 30% of the females migrated to oceanic feeding areas and ingested planktonic organisms. According to these findings, the authors proposed that the ontogenetic shift from omnivorous oceanic to herbivorous neritic could be facultative and not obligatory, as was supposed (Hatase et al., 2006). Nesting C. mydas of Galapagos used different foraging grounds: neritic, near to nest areas, and oceanic (Seminoff et al., 2008). Based on the information shared and according to Godley et al. (2008), individuals of a sea turtle population can use different strategies of post-nesting movements, and there is more behavioural plasticity than previously proposed. Thus, perhaps this occurs in the adult females that nest in Brazil, resulting in wide intraspecific variations. Future studies in this field would help to evaluate the behaviour and plasticity in the resources use by these turtles.

The mixture of compounds found in water and organisms differs



Fig. 4. PCB median concentrations (ng g^{-1} ww) of the congeners found in the plasma of *C. mydas, C. caretta* and *L. olivacea* females.



Fig. 5. Median concentrations (ng g⁻¹ ww) of DDT metabolites, HCH isomers and Chlordane compounds detected in females of *C. mydas*, *C. caretta* and *L. olivacea*.

Table 2

Carbon and nitrogen isotopic ratio median values (amplitude) from red blood cells (RBC) of *C. mydas, C. caretta* and *L. olivacea*. The letters a, b and c were used to represent Kruskal-Wallis/Mann-Whitney analysis. Different letters indicate a significant difference between the species (p < 0.05), while the same letters denote no distinction.

Sea turtle	$N^{\circ}\xspace$ samples	RBC					
		δ ¹³ C (‰)	δ ¹⁵ N (‰)				
		Median (amplitude)	Median (amplitude)				
C. mydas	31	-18.90 (-20.48 to -15.78) ^a	5.81 (3.30 to 10.38) ^a				
C. caretta	28	$-16.27 (-19.41 \text{ to } -14.54)^{\text{b}}$	7.87 (4.52 to 15.17) ^b				
L. olivacea	19	-18.86 (-19.81 to -16.35) ^a	8.94 (4.83 to 12.05) ^c				



Fig. 6. δ^{15} N and δ^{13} C values from RBC of C. mydas, C. caretta and L. olivacea.

from technical products since each congener in the environment is controlled by physical-chemical and biological processes (Richardson et al., 2010). The PCB congeners with higher chlorination are generally associated with sediments (Duinker et al., 1983), becoming available to benthic organisms (Penteado and Vaz, 2001). *C. caretta* and *L. olivacea* can feed on benthic animals (e.g., molluscs and crustaceans) and other organisms (Jones and Seminoff, 2013), and these species ingest these compounds indirectly through the consumption of those organisms. On the other hand, *C. mydas* changes their diet habit during their life cycle from omnivorous to mostly herbivorous, eating algae and sea grass (Bjorndal, 1997). Studies of estuarine vegetation (*Spartina alterniflora*) verified the accumulation of lighter PCBs (Mrozek and Leidy, 1981), and when the macroalgae (*Cladophora glomerata*) was exposed to the commercial product, it accumulated the same PCBs available in water and the same proportions (Larsson, 1987). Thus, considering that sea grass and other algae follow the same accumulation pattern, in the period when *C. mydas* are omnivorous, they are likely to accumulate intermediate (with 5 to 6 chlorine) and heavier PCBs, but as they grow, PCBs dilute in their body (Pugh and Becker, 2001), and with the change in diet, they ingest foods with lighter PCBs.

The main individual congeners found in the plasma of *C. mydas* and *C. caretta* in this study were present in previous reports. Although the sum of pentachlorines predominated in *C. mydas*, the individual congeners – PCB138 and PCB153 – had the highest median concentration in the present study, as well as in *C. mydas* from Australia and the United States of America (USA) (Van de Merwe et al., 2010a; Barraza et al., 2020). For *C. caretta*, in addition to these congeners, PCBs 118 and 180 were among the most frequent in plasma from Africa and Spain (Camacho et al., 2013; Bucchia et al., 2015). As there is no information for POPs in blood or plasma of *L. olivacea*, a comparison has been done with *Lepidochelys kempii* from the USA that showed a similarity of the main congeners – PCBs 138, 153, 180 and 187 (Keller et al., 2004b; Swarthout et al., 2010).

Metabolising enzymes – such as the cytochrome system (P450) – biotransform xenobiotic compounds, such as PCBs, to increase their hydrophilicity and allow their excretion (Richardson et al., 2010). Data regarding the ability of biotransformation and metabolism by reptiles are scarce, but some studies suggested that sea turtles use P4502B but have a weak or absent P4501A activity (Keller et al., 2004b; Richardson et al., 2010; Richardson and Schlenk, 2011). Based on the information above and in the classification of organisms' biotransformation capacity proposed by Kannan et al. (1995), the congeners 153 and 180 were not metabolisable in other animals, while PCBs 118, 138 and 170 accumulated in sea turtles due to a lack of P4501A activity. In addition, other frequent PCBs found in the plasma samples were also major compounds in commercial mixtures used in Brazil, such as Aroclor 1260 - PCBs 153, 180, 101, 138, 110, and Aroclor 1254 - PCBs 110, 118, 101, 138 and 153 (IPCS, 1993).

The presence of DDTs as the most frequent pesticide group in this study is related to the intensive usage in South America and Africa during the sixties and seventies to combat disease vectors (e.g., Malaria) and for agriculture (UNEP, 2002). Previous reports also described p,p'-DDE as the major metabolite found in sea turtles and, in most cases, was the only DDT derivative detected (Keller et al., 2004b; Komoroske et al., 2011; Camacho et al., 2013; Bucchia et al., 2015). DDD is an unstable metabolite and becomes DDA, which is soluble and excretable, while DDE is stable and tends to persist in the organism (Pugh and Becker,

Table	3
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Sea turtles	Isotope (‰)	ΣΡCB	PCB 138	PCB 153	ΣDDT	ΣΗCΗ	ΣChlordane	HCB	PBDE
C. mydas	$\delta^{13}C$	n.s.c.	n.s.c.	n.s.c.	n.s.c.	n.s.c.	n.s.c.	n.c.	n.c.
	$\delta^{15}N$	n.s.c.	0.48	n.s.c.	n.s.c.	n.s.c.	n.s.c.	n.c.	n.c.
C. caretta	δ ¹³ C	n.s.c.	n.s.c.	n.s.c.	n.s.c.	n.s.c.	n.s.c.	0.43	n.c.
	$\delta^{15}N$	0.47	0.55	0.45	0.49	n.s.c.	n.s.c.	n.s.c.	n.c.
L. olivacea	$\delta^{13}C$	n.s.c.	n.s.c.	n.s.c.	n.s.c.	n.s.c.	n.c.	n.c.	n.c.
	$\delta^{15}N$	n.s.c.	n.s.c.	n.s.c.	n.s.c.	n.s.c.	n.c.	n.c.	n.c.

Spearman's correlation coefficient (r_s) obtained for the concentrations of POP in plasma, δ^{13} C and δ^{15} N in RBC of *C. mydas, C. caretta* and *L. olivacea*.

Notes:

n.s.c.: no significant correlation (rs \neq 0; p > 0.05).

n.c.: no correlation (rs = 0; p = 0).

2001).

The detection of HCH in the females studied is related to the physicochemical characteristics of these groups and their use. HCH residues are among the most widely distributed organochlorine contaminants and are often detected in the environment, as they are relatively soluble in water and have a moderately high vapour pressure (Walker et al., 1999). Thus, these compounds can be transported over long distances from their application area and undergo dissolution in the aquatic environment (Vijgen et al., 2011). The exclusive presence of Y-HCH (Lindane) in the females - except for one L. olivacea that contained β -HCH – is related to the intensive use of this pesticide in countries from the southern hemisphere (MMA, 2015). Brazil produced and imported Lindane (MMA, 2015), and there are still some contaminated areas with residues of this pesticide (UNEP, 2002). Lindane was detected in C. mydas females from Malaysia and Mexico and juveniles and adults from the USA (Van de Merwe et al., 2010a; Komoroske et al., 2011; García-Besné et al., 2015). C. caretta juveniles and adult females from the Canary Islands (Spain) and Cape Verde had only β-HCH (Camacho et al., 2013).

The low concentrations of chlordane in the females studied probably reflect the straight usage of these compounds in South America (UNEP, 2002; Barra et al., 2006). When the compounds α -Chlordane (*cis*) and Y-Chlordane (*trans*) are metabolised, they produce oxychlordane (Pugh and Becker, 2001). Thus, the lack of detection of α -Chlordane could be related to the faster metabolism of this compound or to low concentrations that could not be detected. Other studies have also found that Y-Chlordane and oxychlordane are common (Keller et al., 2004a,b, 2014; Labrada-Martagón et al., 2011), but α -Chlordane was detected only in one study of Californian *C. mydas* (Komoroske et al., 2011).

HCB was detected in a minority of females. This compound is produced unintentionally as an industrial by-product and as a pesticide (MMA, 2015), being widely distributed in the environment. Its high mobility enables atmospheric transport, with preferential deposition and accumulation in the polar regions (Wania and Mackay, 1996). HCB was also detected in *C. mydas* and *C. caretta* in juveniles, sub-adults and adult females in California - USA (Labrada-Martagón et al., 2011), Canary Islands - Spain and Cape Verde - Africa (Camacho et al., 2013) and in the Adriatic Sea - Europe (Bucchia et al., 2015).

The detection of Mirex in only one turtle could be explained by its low use and its use in restricted areas of South America (UNEP, 2002); its low mobility (Wania and Mackay, 1996); and/or because of its high lipophilicity (Geyer et al., 2000). Most likely, this pesticide - used on control of ants, as well as fire retardant - remains more associated with fatty tissues than plasma, which is an aqueous matrix. Mirex was not detected in samples of air, soil, and biota from South America, but in addition to the low concentrations, this compound was >LOQ in sediments and water (Barra et al., 2006). Other previous reports had also not detected this pollutant in the blood and plasma of juveniles, sub-adults and adult females of *C. mydas* and *C. caretta* from the USA, Africa and Spain (Komoroske et al., 2011; Camacho et al., 2013). In other cases, Mirex was found in the species above, as well as *L. kempii*, in the USA, Australia, Malaysia and Mexico (Keller et al., 2004b; Swarthout et al., 2010; Van de Merwe et al., 2010a; García-Besné et al., 2015). The PBDE congeners found in the females are related to the main composition of the commercial mixtures - c-Penta-BDE and c-Octa-BDE (McDonald, 2002). Although Brazil did not produce PBDEs, the country imported products with these compounds (e.g., electronics) and possibly used the commercial products (MMA, 2015). The low frequency in the number of samples that remained > LOQ for this group of pollutants also occurred in *C. mydas* in California (Komoroske et al., 2011). Other sea turtle reports showed the same congeners, except 183. Instead, PBDE 47 is commonly found (Keller et al., 2014; Hermanussen et al., 2008; Van de Merwe et al., 2010a), but it was not the case in the Brazilian adult females.

4.2. Stable isotopes

The interspecific differences in stable isotopes among the three species, except for some overlaps, suggest the use of different foraging ecology, while the intraspecific differences could be due to the use of different foraging areas (with distinct isotopic baselines), different feed and intraspecific isotopic variations of their prey (Vander Zanden et al., 2012). Thus, individuals with the same diet but with different foraging areas can have distinct isotopic values (Vander Zanden et al., 2013). In addition, some studies have shown that, although a population is generalist - consuming a wide array of food - its individuals can be specialists - with ingestion of restricted food items among the possible range of the species. Vander Zanden et al. (2010) proposed three models to characterise a population, according to the use of the resources of their individuals: 1. Specialist population; 2. Generalist population with generalist individuals; and 3. Generalist population with specialist individuals. In all three, isotopic signatures can be influenced by the diet, habitat and geographic location. In the first model, individuals and the population show narrow isotopic niche widths. In the second, generalist individuals have broad variation in the use of resources, leading to changes in the isotopic records over time, and both the individual and the population occupy a broad isotopic niche. In the last model, individuals are specialists and the maintenance of the use of resources is reflected by a restricted isotopic niche, but the variation among the individuals cause breadth in the population's isotopic niche. Analysis of stable isotopes in the carapace of C. mydas in the Caribbean revealed that the turtles are specialists but are part of a generalist population (Vander Zanden et al., 2013). Likewise, another study with females of C. caretta from Florida also found the same pattern (Vander Zanden et al., 2010). Based on the data presented above, a possibility for the wide isotopic niche widths among the individuals of the same species could be due to the generalist population.

Variations in δ^{13} C values are caused by temperature, carbon dioxide concentration in surface water and the differences in plankton synthesis or metabolism (Rubenstein and Hobson, 2004). The δ^{13} C values of primary producers vary between ocean basins, becoming higher in the ocean region towards the coast (Clementz and Koch, 2001). In addition, pelagic environments, which have phytoplankton as the base of the food chain, are more carbon depleted than neritic regions (Ceriani et al., 2012). On the other hand, differences in δ^{15} N could be due to the baseline of primary producers in each area or different trophic levels

Table 4		
Summary of POPs measured in blood and plasma of sea turtles reported in the literatur	e. Values presented are means (SD) in ng g $^-$	¹ wet mass, unless otherwise noted.

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Specie	Stage/ sex	Tissue	Ν	Year	Location	ΣΡCΒ	ΣDDT	ΣΗCΗ	Σchlordane	Mirex	HCB	ΣPBDE	Reference
Chelonia mydas	AF	Р	31	2017	Atol das Rocas Biological Reserve, Brazil	0.275	0.005	0.027	0.007	ND	0.003	0.003	Present study
Chelonia mydas	JMF	WB	9	2001-2002	Gulf of Mexico, USA	0.534	0.128	NE	0.011	0.015	NE	0.158	Swarthout et al. (2010)
Chelonia mydas	AF	WB	11	2004	Terrengganu, Malaysia	0.579	(0.114) NA	0.501	(0.0203) NA	0.161	NA	0.121	Van de Merwe et al.
Chelonia mydas	JMF	WB	16	2006-2007	Queensland, Australia	(0.086D) 0.684	ND	(0.060D) NA	ND	(0.043D) 0.043	ND	(0.014b) 0.079	Van de Merwe et al.
Chelonia mydas	JMF	Р	16	2015-2017	Seal Beach National Refuge -	0.85 (0.33)	0.05	NE	NA	NE	NE	ND	(2010a) Barraza et al. (2020)
Chelonia mydas	AMF	Р	23	2015-2017	San Diego Bay - California, USA	8.31 (7.25)	0.02	NE	NA	NE	NE	ND	Barraza et al. (2020)
Chelonia mydas	JAMF	Р	31	2007-2009	San Diego Bay, USA	ND	0.736	0.915	NA	ND	ND	NA	Komoroske et al.
Chelonia mydas	JA	WB	42	2005-2007	Punta Abreojos - California, USA	NE	(0.097) 0.745* ^a	NA	0.23* ^a	NE	0.387* ^a	NE	Labrada-Martagón
Chelonia mydas	JA	WB	14	2005-2007	Bahía Magdalena - California,	NE	ND	NA	0.313* ^a	NE	0.960* ^a	NE	Labrada-Martagón
Chelonia mydas	AJMF	WB	7	2004–2008	Queensland, Australia	NE	NE	NE	NE	NE	NE	0.004	Hermanussen et al.
Caretta caretta	AF	Р	28	2016-2017	North coast of Bahia, Brazil	1.16	0.113	0.048	0.015	ND	0.004	0.003	Present study
Caretta caretta	AF	Р	197	2009–2010	Cape Verde, Eastern Atlantic	0.08*	0.21*	0.02*	ND	ND	0.24*	NE	Camacho et al. (2013)
Caretta caretta	J	Р	30	2011-2012	Canary Island, Eastern Atlantic Ocean	$1.12^{*^{a}}$	0.17*	ND	ND	ND	0.03*	NE	Bucchia et al. (2015)
Caretta caretta	J	Р	162	2007-2010	Canary Island, Eastern Atlantic Ocean	1.92*	0.78*	0.02* (0.54)	ND	ND	0.41*	NE	Camacho et al. (2013)
Caretta caretta	J	Р	29	2003	South Carolina, Georgia, northeastern Florida, USA	2.53	NA	NA	NA	NA	NA	0.131	Keller et al. (2005)
Caretta caretta	J	WB	5	1998-2001	North Carolina, USA	5.14 (3.95)	0.583 (0.307)	ND	0.260 (0.182)	0.056 (0.075)	ND	NE	Keller et al. (2004a)
Caretta caretta	AM	Р	9	2006–2007	Atlantic coast USA (resident group)	5.37 (2.25)	0.092 (0.032)	NA	NA	0.023 (0.016)	NA	NA	Ragland et al. (2011)
Caretta caretta	JMF	WB	48	2000-2001	North Carolina, USA	5.56 (5.28)	0.649 (0.685)	NE	0.225 (0.201)	0.044 (0.071)	NE	NE	Keller et al. (2004c)
Caretta caretta	J	Р	5	1998–2001	North Carolina, USA	7.13 (4.94)	0.578 (0.294)	ND	0.238 (0.155)	0.036 (0.053)	ND	NE	Keller et al. (2004a)
Caretta caretta	AM	Р	10	2006–2007	Cape Canaveral, USA (transient group)	13.10 (17.24)	1.56 (2.65)	NA	NA	0.078 (0.062)	NA	0.088 (0.096)	Ragland et al. (2011)
Caretta caretta	JA	Р	35	2011-2012	Adriatic Sea, Mediterranean Basin	28.45* ^a	1.15* (0.96)	ND	ND	ND	0.02* (0.04)	NE	Bucchia et al. (2015)
Lepidochelys olivacea	AF	Р	19	2017	North coast of Bahia, Brazil	0.814 (0.851)	0.084 (0.133)	0.016 (0.017)	ND	0.003 (0.004)	0.005 (0.010)	0.004 (0.005)	Present study
Lepidochelys kempii	JMF	WB	46	2001-2002	Gulf of Mexico, USA	4.27 (3.62)	0.686 (0.656)	NE	0.113 (0.100)	0.015 (0.014)	NE	0.230 (0.273)	Swarthout et al. (2010)
Lepidochelys kempii	JMF	WB	3	2001-2002	Southeast, USA	10.70 (12.20)	1.49 (1.79)	NE	1.22 (1.49)	0.009 (0.0005)	NE	0.148 (0.141)	Swarthout et al. (2010)
Eretmochelys imbricata	AF	WB	60	2014–2015	Campeche, Mexico	NE	0.95 (0.33)	1.09 (0.48)	0.30 (0.11)	NE	NE	NE	Salvarani et al. (2018)
Eretmochelys imbricata	JF	WB	1	2004–2008	Queensland, Australia	NE	NE	NE	NE	NE	NE	0.013	Hermanussen et al. (2008)
Dermochelys coriacea	AF	WB	38	2006	Yalimapo, French Guiana	1.26* (0.71)	0.31* (0.22)	1.44* (1.26)	NE	NE	NE	NE	Guirlet et al. (2010)
Dermochelys coriacea	AF	WB	6	2003	Juno Beach - Florida, USA	2.5 (2.27)	0.424 (0.235)	NE	0.328 (0.158)	ND	0.054 (0.042)	0.198 (0.190)	Stewart et al. (2011)
Natator depressus	AF	WB	1	2004–2008	Queensland, Australia	NE	NE	NE	NE	NE	NE	0.006	Hermanussen et al. (2008)

Notes: A = adult, J = juvenile, M = male, F = female, WB = whole blood, P = plasma, N = number of samples, NE = not evaluated, NA = not available, ND = not detected, $* = ng mL^{-1} blood$, a = median, b = standard error (SE).

(Ceriani et al., 2012).

For *C. mydas*, the most negative values of δ^{13} C can be related to the main intake of algae (Bezerra et al., 2015) or the use of oceanic feeding areas, while the most enriched values could come from the consumption of marine grasses (Ciotti, 2012), mangrove leaves and fruits and invertebrates (Jones and Seminoff, 2013). The lower δ^{15} N value, compared to the other species, is related to their diet.

As cited above, *C. caretta* ingests mainly benthic organisms, which have more enriched δ^{13} C values than other turtles. A hypothesis for females with higher values of δ^{15} N than the majority is the ingestion of fish from fishing discards, as described in previous studies (Barros et al., 2009; Bugoni et al., 2003). Two turtles overlap with *L. olivacea* values, showing that they probably use the same area or eat similar prey.

Studies have shown that *L. olivacea* could use two distinct foraging areas: neritic regions – feeding on benthic organisms and fish; and oceanic regions – feeding on thaliaceans, gastropods and fish (Jones and Seminoff, 2013; Dos Santos et al., 2016; Petitet and Bugoni, 2017). Furthermore, the isotopic values found in this study are similar to a study with nesting females of these species, whose authors concluded that the majority of turtles migrated to ocean areas (Petitet and Bugoni, 2017). Therefore, in the same way, the majority of females of this work seem to move to oceanic foraging ground areas.

4.3. Stable isotopes and persistent organic pollutants

As the δ^{15} N is used to evaluate trophic position, the positive correlation found with PCB congeners in *C. caretta* and *C. mydas* can be related to the persistence of PCB 138 and PCB 153, which are respectively poorly and not metabolised, transferred along the food chain and accumulated in sea turtle (Kannan et al., 1995; Pugh and Becker, 2001; Keller et al., 2004b; Richardson et al., 2010).

The lack of some correlations among stable isotopes and POPs is possibly due to differences in metabolism between species and a reduction in the level of contamination of the female due to the maternal transfer to the eggs (Guirlet et al., 2010; Alava et al., 2011) and the number of nesting seasons in which she participated.

Studies that associate POPs and stable isotopes have focused on other organisms, with variable results (e.g., Colabuono et al., 2014). Only one study was found for sea turtles, and there was a negative correlation between the organochlorine pesticides in the blood of *C. mydas* and the δ^{13} C of the shell (Monzón-Argüello et al., 2018). Thus, further studies are needed to understand what causes this variation between species and the lack of correlation between some pollutants and isotopic ratios.

5. Conclusions

Although POPs have been banned and/or restricted, females of *C. mydas, C. caretta* and *L. olivacea* that nest in Brazil are still exposed to these compounds. The concentrations found were, in general, smaller than other studies made in the Northern hemisphere. Furthermore, POPs and stable isotopes revealed intraspecific and interspecific variations, which reflect the high plasticity in the use of habitat and food resources, making individuals within the same population susceptible to exposure to different contaminants. This study contributed to the knowledge of the POP contamination that these animals are exposed to and provided references for future research.

CRediT authorship contribution statement

Luciana S. Filippos: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. Satie Taniguchi: Conceptualization, Methodology, Writing – review & editing. Paula Baldassin: Conceptualization, Resources, Writing – review & editing. Thaís Pires: Resources, Writing – review & editing. Rosalinda C. Montone: Conceptualization, Resources, Writing – review & editing, Supervision, Funding acquisition.

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.

Acknowledgements

The authors would like to thank Fundação Projeto Tamar and Atol das Rocas Biological Reserve for the fieldwork assistance and logistical support for sample collection; BW Consultoria Veterinária and IGUI Ecologia for financing the Sea Turtle Microchipping Project in the Atol das Rocas Biological Reserve and acquiring the PIT tags; Fundação Mamíferos Aquáticos for the storage of samples; and the Foundation for Research Support of the State of São Paulo (Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP; Process 2016/18348-1) for the use of the gas chromatograph with triple quadrupole mass spectrometer. We also thank the reviewers that helped to improve the manuscript. Additionally, this study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001, with the granting of the doctor's scholarship to the principal author.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2021.112283.

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