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Baseline

Occurrence of organochlorines in the green sea turtle (*Chelonia mydas*) on the northern coast of the state of São Paulo, Brazil

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ABSTRACT

Organochlorines (OCs), such as pesticides and polychlorinated biphenyls (PCBs), are persistent, toxic and widely distributed through atmospheric transport and ocean currents. Few studies have been conducted on OCs in sea turtles, especially on the coast of Brazil. *Chelonia mydas* is the largest hard-shell sea turtle and is found tropical and subtropical regions in all oceans. The aim of the present study was to determine the occurrence of OCs in the green sea turtle (*C. mydas*). Fat, liver, kidney and muscle samples were collected from 27 juveniles found on the beach of the city of Ubatuba on the northern coast of the state of São Paulo, Brazil. OCs were extracted with organic solvents and the extract was purified with concentrated acid. Gas chromatography-mass spectrometry and electron capture detection were used for the identification and quantification of PCBs and pesticides, respectively. No organochlorine pesticides were detected in any of the samples. Concentrations of total PCBs in wet weight were <1.6 to 48.9 ng/g in fat tissue, <1.6 to 17.4 ng/g in liver tissue and <1.6 to 37.7 ng/g in kidney tissue. The low levels found are mainly related to diet, as the green sea turtle is basically herbivorous and lower PCB contamination compared to other regions.

Due to their physicochemical characteristics, organochlorines are persistent, toxic compounds that are widely distributed through atmospheric transport and ocean currents. Moreover, these compounds bioaccumulate and biomagnify in the food chain. Organochlorines form the first generation of pesticides employed on a large scale, used extensively throughout the world in public health campaigns and to defend agricultural crops, especially after the onset of World War II (Yogui, 2002).

Polychlorinated biphenyls (PCBs) were first synthesized in 1929 in the United States of America by the Monsanto Company. In 1966, these compounds were recognized as environmental contaminants when detected in tissue samples from wild animals (Salgado, 2002). PCBs are flame resistant and have low electrical conductivity, high thermal conductivity and a low degree of solubility. These compounds were used as refrigerating fluids in transformers and capacitors, plasticizers in PVC, heat transfer fluid in machinery as well as for the purposes of water-proofing and other applications (Baird, 2002). Some organochlorines can imitate natural chemical substances, such as hormones, and can disrupt the chemical processes of living organisms, debilitate the immune system, affect organ development and cause cancer (Santamarta, 2001).

Together with their hydrophobicity, the lack of an efficient pathway for the degradation of organochlorines has led to the accumulation of these compounds in sea birds, fish and other organisms (Baird, 2002), with the occurrence of bioaccumulation and biomagnification in the food chain. Sea turtles are also susceptible to bioaccumulation of these contaminants. *C. mydas* is widely distributed in coastal waters of tropical and subtropical seas and is less frequent in temperate waters (FAO Species Catalogue, 1990). In Brazil, *C. mydas* spawns mainly on oceanic islands, such as Trindade, Rocas Atoll and the Fernando de Noronha archipelago (Moreira et al., 1995; Bellini et al., 1996; Bellini & Sanches, 1996). Juveniles feed along the coast of Brazil and around oceanic islands. In the early years of life, *C. mydas* is considered omnivorous, with a tendency toward carnivorous behavior (Bjorndal, 1997). After the pelagic phase, when shell length is approximately 20 cm (Bjorndal, 1980), *C. mydas* becomes herbivorous, feeding mainly on macroalgae and phanerogams (Mortimer, 1981). In the breeding season, adult females leave the feeding grounds and migrate to the region in which they were born to spawn and subsequently return to the feeding grounds, travelling long distances in the process (FAO Species Catalogue, 1990). Carr (1987) suggests that newborns perform pelagic migrations passively, associated with free-floating brown algae

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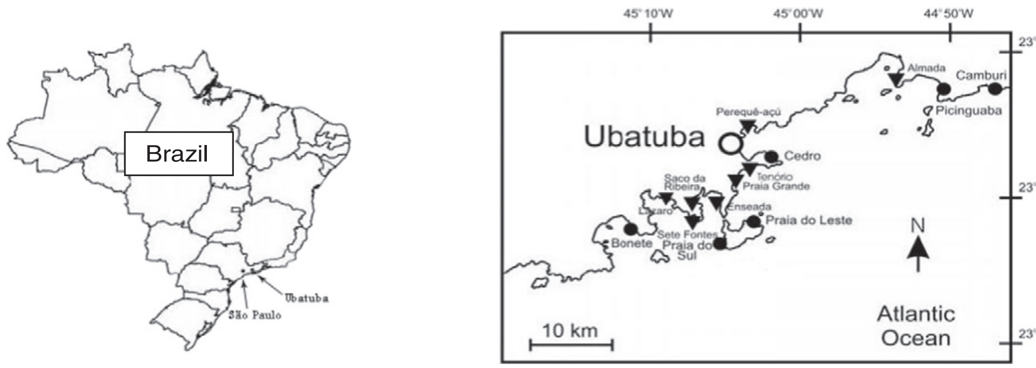

Fig. 1. Map of Ubatuba region, São Paulo, Brazil.
(adapted from Gallo et al., 2006).

(*Sargassum*) in the convergence zones of ocean currents. Juveniles and adults of many populations reside in feeding grounds (FAO Species Catalogue, 1990).

Due to their long lifespan, behavioral and morphological aspects of sea turtles can be altered by long-term exposure to low concentrations of organochlorines, which may make them more susceptible to predation and reduce both survival and reproduction rates, as occurs with birds (Fry, 1995). Chemical pollutants may also diminish the resistance of sea turtles to different viruses, as the stress caused by these compounds may result in a reduction of energy available for basic physiological processes, causing changes in cell immunity and increasing susceptibility to infectious agents (Aguirre et al., 1994).

The aim of the present study was to determine the occurrence of organochlorines in juveniles of the species *C. mydas* found on the northern coast of the state of São Paulo, Brazil.

Samples were collected from 27 *C. mydas* juveniles (21 females and 6 males) between June and December 2007. The turtles were either found dead on the beach or were captured incidentally in fishing nets in the city of Ubatuba on the northern coast of the state of São Paulo [southern limit of Figueira Beach (23° 35′17″ S and 45° 16′38″ W) and northern limit of Camburi Beach (23° 22′17″ S and 44° 47′02″ W)] (Fig. 1). This region has rocky outcrops that serve as shelter and foraging grounds for the species. Fat, muscle, kidney and liver samples were collected for analysis. All samples were placed separately in aluminum foil, identified and frozen.

The tissue samples were macerated with approximately 6 g of anhydrous sodium sulfate. One hundred microliters of each internal standard (PCB 103 and PCB 198) were added to the samples and blanks. In the fortified matrix and fortified blank, 50 μL of each mixture containing the compounds analyzed (PCB 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, 141, 149, 151, 153, 156, 157, 158, 167, 169, 170, 174, 177, 180, 183, 187, 189, 194, 195, 199, 203, 206, 209, α-HCH, β-HCH, γ-HCH, δ-HCH, heptachloride, oxychlordane, γ-chlordane, op'-DDE, pp'-DDE, op'-DDD, pp'-DDD, op'-DDT, pp'-DDT, mirex) were also added at a concentration of 1 ng/μL. For the detection limit, 100 μL of each mixture containing the compounds analyzed were added at a concentration of 20 pg/μL, the limit of detection ranged from 1.65 to 6.01 ng g-1 for PCBs, pesticides and ranged from 2.01 to 10.22 ng g^{-1}. The samples were extracted in a Soxhlet extractor for 8 h with 80 mL of n-hexane and dichloromethane (1:1, v/v). The extract was concentrated to 1.0 mL, from which 0.1 mL was withdrawn for the determination of lipids. An acid treatment was employed for the purification of the fatty extracts.

For the analysis of PCBs, the sample extracts were injected into a gas chromatograph coupled to a mass spectrometer (GC/MS) (5973N, Agilent Technologies) with electron impact operating in selective ion monitoring mode (70 eV). The carrier gas was helium at a constant flow of 1.1 mL/min. The injection volume was 1 μL in splitless mode. The analysis of pesticides was performed using a gas chromatograph coupled to an electron capture detector (GC-ECD) (6890, Agilent Technologies). The carrier gas was hydrogen with constant compression at 13 psi. The injection volume was 2 μL in splitless mode. The column used for both GC/MS and GC-ECD was HP 5MS (Agilent Technologies) with a length of 30 m, inner diameter of 0.25 mm and 5% phenylmethyl siloxane film thickness of 0.25 μm.

Standard Reference Material (SRM) 1945 (Organics in Whale Blubber) was used for the evaluation of the method employed in the present study. SRM 1945 is homogenized subcutaneous fat from a female pilot whale found beached in Cape Cod, Massachusetts (USA), in September 1991. The SRM employed herein (0.25 g) was obtained from the US National Institute of Standards and Technology. All sampling was authorized by a Brazilian federal license for scientific activities (Sisbio number 12072-1).

To check whether there was a statistical difference in the PCB concentrations to different tissues, the data were first evaluated for normality evaluated using the Q-Q plot. As these non-normal distribution, we opted for a non-parametric Kruskal-Wallis test with 95% significance level.

Table 1
Characteristics of samples, percentage of lipids and levels of PCBs and in *Chelonia mydas* juveniles from the northern coast of the state of São Paulo, Brazil.

Sex	CCL[a] (cm)	Weight (kg)	Tissues	∑ PCBs (SD) (ng/g)	Lipids (SD) (%)
Male	35 to 42	4 to 7.5	Fat	<1.6 to 26 (5 ± 10)	57 (±22)
			Liver	<1.6	37 (±6)
			Kidney	<1.6	29 (±5)
Female	36.5 to 58	4 to 22	Fat	<1.6 to 48.9 (11 ± 16)	54 (±27)
			Liver	<1.6 to 17.4 (4 ± 6)	28 (±8)
			Kidney	<1.6 to 37.7 (3 ± 10)	23 (±9)

[a] Curvilinear carapace length.

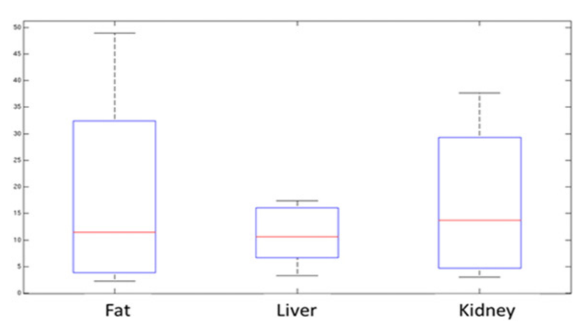

Fig. 2. Box plot of the concentration of PCBs in fat, liver and kidney tissues of *Chelonia mydas*.

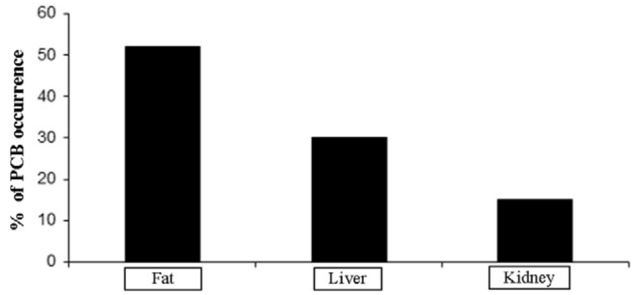

Fig. 3. Average concentrations of PCBs in fat, liver and kidney tissues in *Chelonia mydas* juveniles from the northern coast of the state of São Paulo, Brazil.

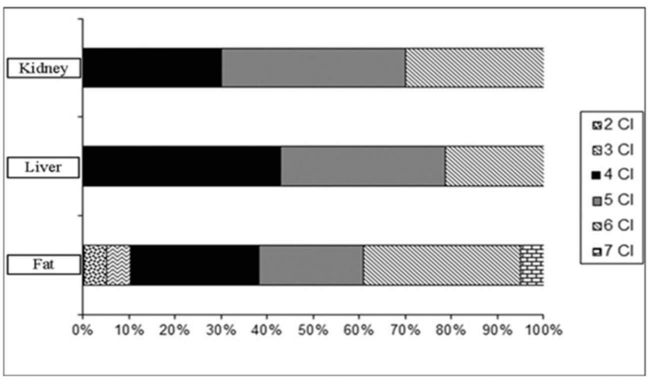

Fig. 4. PCB chlorination levels in fat, liver and kidney.

PCBs were the only compounds detected in the tissue samples of the 27 *C. mydas* juveniles analyzed. Total PCB concentration in wet weight (sum of 50 congeners analyzed) was <1.6 to 48.9 ng/g in fat tissue, <1.6 to 17.4 ng/g in liver tissue and <1.6 to 37.7 ng/g in kidney tissue. PCB concentrations ranged from <1.6 to 26.0 ng/g in males and <1.6 to 48.9 ng/g in females (Table 1).

The bioacummulation of organochlorines is generally influenced by the sex of the organism, especially in the adult phase, when females excrete these compounds through eggs in the breeding season (Matthews and Dedrick, 1984). However, in this study, because of the small number of samples that refer to juveniles, it was decided to group males and females in one group for future comparisons between tissues and different studies.

They were not statistically significant differences in PCB concentrations between fat, liver and kidney were found. All samples are within the same concentration range (Fig. 2).

Lipophilic contaminants tend to preferentially bioaccumulate in lipid rich tissues, which is a common characteristic in the majority of organisms. The accumulation of PCBs in the sea turtles analyzed in the present investigation follows a tendency described in previous studies (STORELLI ET AL., 2007; MCKENZIE ET AL., 1999; CORSOLINI ET AL., 2000), with a greater occurrence of analytes in tissues with a greater concentration of lipids: fat > liver > kidney (Fig. 3). The concentration of contaminants was below the detection limit in the muscle samples.

Each tissue analyzed presented the predominance of tetra-, penta- and hexachlorobiphenyls (Fig. 4). In fat samples it was possible to observe the presence of lighter congeners (di and trichlorobiphenyls) and heavier compounds such as heptachlorobiphenyls, due to higher content of lipids and consequently higher concentrations of PCBs. The predominant congeners in all samples were PCB 52 and 44 (tetrachlorobiphenyls), PCB 99 and 101 (pentachlorobiphenyls) and PCB 118, 153, 132 and 138 (hexachlorobiphenyls) (Fig. 5).

In the comparison of studies involving different species (Camacho et al., 2014; García-Besné et al., 2015; Storelli et al., 2007; Mckenzie et al., 1999; Gardner et al., 2003; Rybitski et al., 1995; Corsolini et al., 2000; Keller et al., 2004; Aguirre et al., 1994), the following tendency is found with regard to the concentration of contaminants: *Lepidochelys olivacea* > *Caretta caretta* > *Chelonia mydas* = *Dermochelys coriacea*. This tendency is likely based on differences in eating habits: *Lepidochelys olivacea* feeds on fish and bryozoans; *Caretta caretta* feeds on crustaceans, mollusks and hydrozoans; *Dermochelys coriacea* feeds on jellyfish; and *C. mydas* is herbivorous in adulthood (Bjorndal, 1997).

Analyzing liver samples from *Lepidochelys kempii*, Lake et al. (1994) found that the concentrations of PCBs and *p,p*'DDE in juveniles are generally higher, in comparison to adults of other species, which may be due to variations in species, age, metabolism or to levels in the food. There are no studies on this aspect of PCB concentration in *C. mydas* juveniles and adults. However, ontogenic differences suggest a similarity with the data reported for *L. kempi*, as *C. mydas* becomes herbivorous in the juvenile phase and is no longer exposed to these compounds (or is exposed to a lower concentration).

The specimens of *C. mydas* analyzed in the present investigation had lower levels of organochlorines in comparison to concentrations described in previous studies conducted in other regions, such as the Mediterranean Sea (39.0 to 261.0 ng/g) and Hawaii (44.7 to 73.1 ng/g) (Mckenzie et al., 1999; Miao et al., 2001), except Aguirre et al., 1994 work which was not detected PCBs.

The low levels of contamination by organochlorines in *C. mydas* in southeastern Brazil is likely related to diet, as the green sea turtle is basically herbivorous, and the fact that all specimens examined were juveniles. The presence of PCBs in *C. mydas* may be related to a local source of contaminants as well as the atmospheric transport of these compounds. Moreover, the fact that the migratory routes of these turtles have not yet been fully detailed or understood underscores the need for further studies to determine the association between contaminants and potential sources.

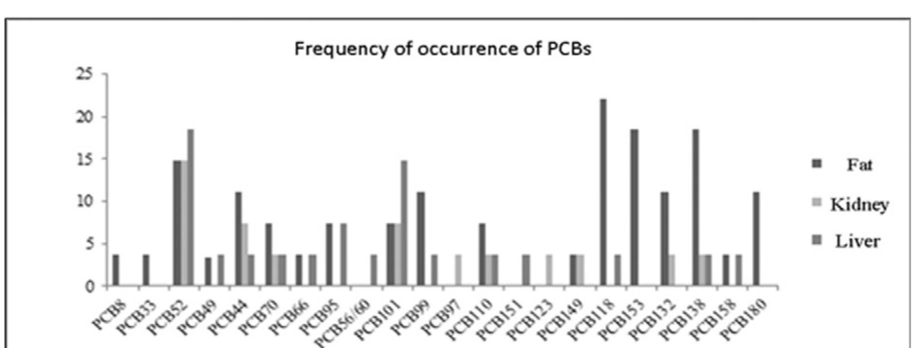

Fig. 5. PCB congeners distribution in fat, liver and kidney samples.

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