LINK BETWEEN MARINE MAMMAL EXPOSURE TO PERFLUOROOCTANE SULFONATE (PFOS) AND STABLE-CARBON ISOTOPE RATIOS IN NERITIC AND OCEANIC WATERS OFF BRAZIL

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Introduction

Since the beginning of the industrialization process marine ecosystems have become the final destination for pollutants. Perfluoroalkyl compounds (PFCs) have been used in a number of industrial and household products comprising fire-fighting foams, personal hygiene goods, arthropodicide formulations, and protectors to fabrics, paper, and other surfaces [1]. Due to their environmental persistence, bioaccumulative capacity as well as their broad utilization, PFCs have been of great environmental concern since their global distribution was first demonstrated using samples from marine biota [1]. Despite the awareness of the scientific community about the environmental problem, until recently, when an investigation on perfluorooctane sulfonate (PFOS) dolphins from Rio de Janeiro state was carried out [2], there were no previous studies on PFC levels in environmental samples from the most industrialized regions of the southern half of the planet.

Guanabara Bay (Rio de Janeiro State, Southeast Brazilian region) is the most anthropogenically disturbed area along the lengthy (around 8500 km) Brazilian coastline. The estuary is bordered by 12000 industries and four cities (including Rio de Janeiro metropolitan area) with a total population of about 11 million people [3]. Despite the anthropogenic pressure, Guanabara Bay supplies food and breeding grounds for marine tucuxi dolphins (*Sotalia guianensis*). This dolphin species occurs exclusively in coastal waters of western Atlantic on South and Central America, from southern Brazil (27°35'S, 48°34'W) to Honduras (15°58'N, 79°54'W) [4]. Regarding Guanabara Bay specifically, population assessment studies, accomplished through photo-identification, have reported a population of about 70 marine tucuxi dolphins [5]. Through these photo-id studies, it was possible to verify that this small population exhibits habitat fidelity, since the same individuals are found year-round in this site [5]. The main reason for the marine tucuxi dolphin residence in Guanabara Bay seems to be the presence of food, since feeding-related activities predominate along the entire year [5].

Considering the demonstrated biomagnification capacity of perfluorooctane sulfonate (PFOS), analyses of cetacean tissues are of interest, due to their top position on the food chain and their long life span [3]. The recently published data on PFOS levels of marine tucuxi dolphins from Guanabara Bay [2] has shown that the estuary constitutes a hotspot for environmental contamination by this pollutant. In addition, the possibility that Guanabara Bay works as an exporting system for other organohalogen compounds has been raised [6]. These latter observations strengthen the importance of PFOS determination in top marine predators that occur on the continental shelf frontal to Guanabara Bay and adjacent oceanic region. Therefore, the main objective of the present study was to determine the current concentrations of PFOS in dolphins from neritic and oceanic waters of a region of high industrialization and urbanization in the Southern Hemisphere. Since stable carbon isotopes can be used to point out relative contributions to the diet of different potential primary sources in trophic networks, indicating for example the inshore versus offshore contribution to food intake [7,8], stable carbon isotope measurements were carried out in the same dolphins in order to clarify inter-species differences. To our knowledge, the present study constitutes the first investigation linking stable isotope ratios and pollutants in marine biota samples from Brazil.

To shed light on the placental transfer of PFCs, hepatic PFOS concentrations were determined in a Fraser's dolphin female and its foetus. Hence, the foetus/mother ratio of hepatic PFOS concentrations was compared to the ratio reported in other marine mammal species.

Material and methods

PFOS determination in liver samples from dolphins

In addition to the recent investigation on PFOS levels of marine tucuxi dolphins, in which 23 dolphins from Guanabara Bay [2] were analyzed, PFOS data were obtained through analyses of liver samples that were collected from 32 cetaceans stranded on the beaches of Rio de Janeiro state, in Southeast Brazil, from 1994 to 2006. In total, the 55 dolphins (Table 1) comprised delphinids species that occupy estuarine, continental shelf and oceanic environments. Despite the fact that tissues of ten cetacean species were analysed, only PFOS data from marine tucuxi dolphins, Atlantic spotted dolphins and Fraser's dolphins were used for inter-species statistical comparisons, representing estuarine, continental shelf and oceanic environments, respectively. The remaining species were excluded due to the limited number of individuals of each taxon. The samples were freeze-dried and then ground to a powder. Extraction and measurement methods are described elsewhere [2]. Briefly, an internal standard (^{13}C-PFOS from Wellington Laboratories, Canada) was added to approximately 1.0 g of the ground sample. Spiked samples were used to determine recovery rates for the perfluorochemicals. Concentrations of PFOS were measured combining liquid chromatography and mass spectrometry, using a CapLC system (Waters, USA) connected to a Quadrupole-LIT quadrupole mass spectrometer (Applied Biosystems, UK). Measurement was carried out under (-) electrospray ionization using the transition from mother to daughter ion (499→80/99). The recovery rates varied from 105 to 110 %.

Stable isotope measurements

Measurements of stable isotopes of carbon were carried out in muscle samples from marine tucuxi dolphins (n=20), Atlantic spotted dolphins (n=9), rough-toothed dolphins (n=3), common dolphins (n=1), bottlenose dolphins (n=6), false killer whales (n=2), Fraser's dolphins (n=10), spinner dolphins (n=1), pantropical spotted dolphins (n=2) and Risso's dolphin (*Grampus griseus*, n=1). After being freeze-dried, the samples were ground into a homogeneous powder. As lipids have been shown to be depleted in ^{13}C relatively to the diet [8], they were extracted from samples using repeated rinses with 2:1 chloroform: methanol prior to analysis. Carbonic gas (CO_2) originated from combustion of the sample was analysed on a V.G. Optima (Micromass) IR-MS coupled to an N-C-S elemental analyser (Carlo Erba). Stable isotope ratios were expressed in δ notation according to the following: $\delta^{13}C = [(^{13}C/^{12}C_{sample} / {}^{13}C/^{12}C_{\text{v-PDB standard}}) - 1] \times 1000$.

Results and discussion

Hepatic PFOS concentrations of dolphins from Brazilian waters are presented in Table 1. PFOS was detected in all but one among the 55 dolphins. The presence of PFOS in 31 out of the 32 dolphins from continental shelf and oceanic environments provides additional evidence that PFCs are ubiquitous environmental contaminants. Interestingly, 14 of these 31 mammals belong to species that inhabit an oceanic region in a tropical area of the Southern Hemisphere, which constitutes a finding of importance for the global monitoring of PFCs. Since the crude data on hepatic PFOS concentrations of bottlenose dolphins from Mediterranean Sea have been exposed in literature [9], statistical comparison was carried out to Atlantic spotted dolphins and there was no significant difference (*Mann-Whitney U test*, p= 0.69). The high PFOS levels found in this continental shelf delphinid strengthens the hypothesis of exportation of organohalogen compounds from Guanabara Bay. The fact that the only sample that presented PFOS concentrations below the limit of detection was from a continental shelf species constitutes surprising information. However, the cause of death of this dolphin is unknown. Some investigations have drawn attention to the possibility of interference in PFOS assimilation and metabolization as a consequence of a pathological process [7,10]. Van de Vijver et al. [7] verified that PFOS concentrations in harbour seals with bronchopneumonia were significantly lower than the levels determined in individuals without bronchopneumonia. Kannan et al. [10] observed that hepatic PFOS concentrations were significantly higher in sea otters that died from infectious diseases than in those who died from trauma and other causes.

PFOS levels were higher in coastal than in oceanic species (Fig. 1A). Significant differences in PFOS concentrations were verified both between estuarine (marine tucuxi) and continental shelf (Atlantic spotted)

dolphins (*Student's t-test*, p=0.01) and between Atlantic spotted dolphins and oceanic (Fraser's) dolphins (*Mann-Whitney U test*, p=0.02). These observations corroborate the results of many investigations that have shown higher PFC levels in biological samples from waters close to areas of concentrated industrialization and urbanization [2]. Regarding the Brazilian coast, most delphinid classification studies on coastal or oceanic species have been carried out based either on stomach content analyses or on geographically referenced opportunistic observation of dolphins [11]. Although valuable, dietary studies traditionally performed by digestive content analyses of stranded animals can included a high number of diseased dolphins in the sample set. Unhealthy animals may have fed on prey species that do not represent the diet of the healthy specimens [12]. In the present study, additional information on how far from the coast each delphinid species exerts predation is generated through stable carbon isotope measurements (Fig. 1B). Taking advantage of the continuous variable provided by the stable isotope ratios, correlations were examined between PFOS concentrations and $\delta^{13}C$ values. Significant positive correlations were observed in two different scenarios. At first, all the individuals in which both PFOS concentrations and stable carbon ratios had been determined (n=46) were included in the test (Spearman's correlation test $p<0.0001$). Subsequently, for a better observation of the graph produced, the marine tucuxi dolphin presenting the highest PFOS concentration (2431 ng/g, dry weight) was excluded from the test (Spearman's correlation test $p<0.0001$, Fig. 2). Considering that it is well-known that $\delta^{13}C$ depletion reflects a greater reliance on offshore food [7,8], the positive correlations observed demonstrate that coastal cetaceans are fated to a higher exposure to PFCs due to their proximity to industrial and domestic points of effluent discharge.

The calculation of the foetus/mother (F/M) ratio of hepatic PFOS concentrations in Fraser's dolphins yielded the value of 0.07. A remarkable difference can be observed by comparing this information with data from literature, since F/M=2.75 and F/M=2.62 were calculated for two foetus-mother pairs of marine tucuxi dolphins from Guanabara Bay [2] and F/M=2.57 was found in a harbour porpoise foetus-mother pair [13]. Therefore, further studies are needed to fully elucidate the factors that influence PFOS placental transfer in cetaceans.

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Table 1.Hepatic PFOS concentrations of cetaceans from Brazilian waters.

Habitat	Common Name	Latin Name	Mean ±S.D.	Median	Range	N	Refs.
Guanabara Bay	Marine tucuxi dolphin	*Sotalia guianensis*	772 ±545	735	43 - 2431	23	2
C. Shelf	Bottlenose dolphin	*Tursiops truncatus*	74 ±15	67	63 - 91	3	ps
	Rough-toothed dolphin	*Steno bredanensis*	173 ±84	207	51 - 228	4	ps
	Atlantic spotted dolphin	*Stenella frontalis*	217 ±195	225	40 - 592	7	ps
	False killer whale	*Pseudorca crassidens*	49 ±19	49	35 - 63	2	ps
	Common dolphin	*Delphinus delphis*	113	113	<1.5 - 113	2	ps
Ocean	Pantropical spotted dolphin	*Stenella attenuata*	12	12	xxxx	1	ps
	Spinner dolphin	*Stenella longirostris*	333	333	xxxx	1	ps
	Striped dolphin	*Stenella coeruleoalba*	49	49	xxxx	1	ps
	Fraser's dolphin	*Lagenodelphis hosei*	60 ±93	22	11 - 332	11	ps

C. Shelf, continental shelf; Refs, references; ps, present study.

Fig1A. PFOS concentration (ng/g, dw). Fig1B. Delta ^{13}C values.

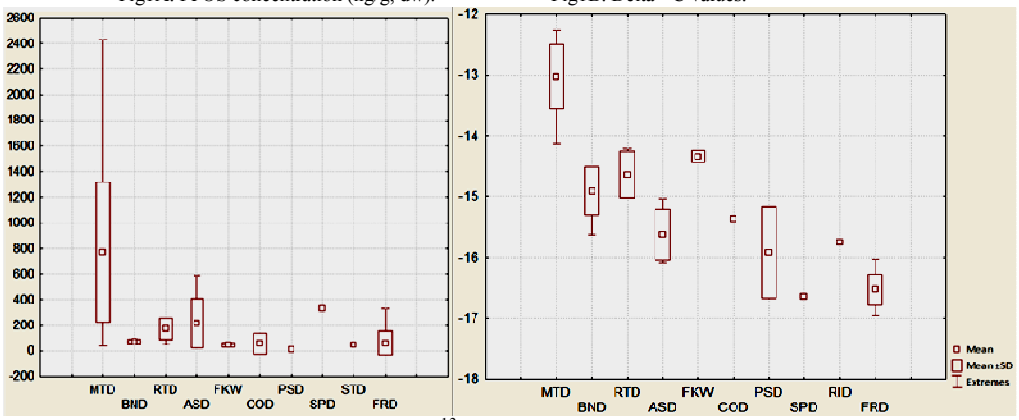

Fig. 1. Hepatic PFOS concentrations and $\delta^{13}C$ values in dolphins from Brazilian waters. MTD, marine tucuxi dolphin; BND, bottlenose dolphin; RTD, rough-toothed dolphin; ASD, Atlantic spotted dolphin; FKW, false killer whale; COD, common dolphin; PSD, pantropical spotted dolphin; SPD, spinner dolphin; STD, striped dolphin; FRD, Fraser's dolphin; and RID, Risso's dolphin.

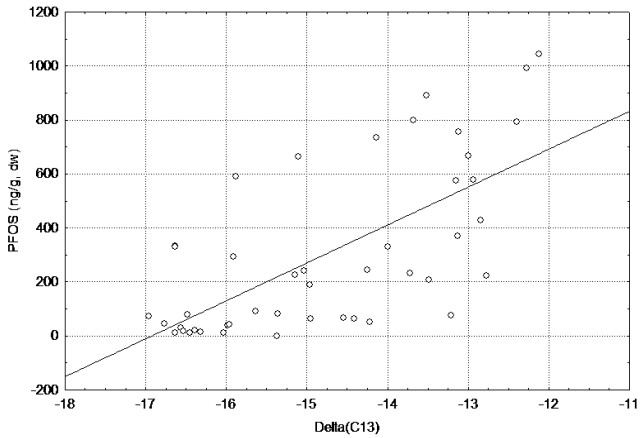

Fig 2. Graphic representation of the statistically significant positive correlation observed between PFOS concentrations and $\delta^{13}C$ values of cetaceans from Brazilian waters.