DOLPHINS AS INDICATORS OF MICROPOLLUTANT TROPHIC FLOW IN AMAZON BASIN

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ABSTRACT

This study presents an overview of the appropriateness of the use of Amazonian cetaceans as sentinel species for environmental contamination by micropollutants, such as organochlorines and mercury. Due to the top position in food webs occupied by cetaceans and to their long life-span, high micropollutant concentrations have been verified in their tissues, what allows an amplification of the determination capacity by different analytical procedures. Besides providing an analytical advantage, the continuous micropollutant determination in cetaceans has been useful: as a reference of pollutant availability to other organisms; to integrate a complex signal of pollution; to quantify the ecological significance of a contamination. Therefore, published information on the concentrations of the quoted pollutants in the Amazonian cetaceans *Inia geoffrensis* (boto) and *Sotalia fluviatilis* (tucuxi) were gathered herewith. Carrying out comparison with data from Brazilian coastal cetaceans, it became clear how high the levels observed in Amazonian aquatic mammals are. In this context, mercury deserves to be highlighted. Despite the little volume of information on the concentrations of mercury, polychlorinated biphenyls and dichlorodiphenyltrichloroethane in Amazonian cetaceans, the existing information already demonstrates that the levels observed mirror the environmental contamination by such pollutants in distinct Amazonian environments. Perspectives for future investigations are also discussed, including possible ways of overcoming obstacles related to sampling of Amazonian cetaceans.

Keywords: Brazil, Amazon, cetacean, mercury, organochlorines.

RESUMO

BOTOS COMO INDICADORES DO FLUXO TRÓFICO DE MICROPOLUENTES NA BACIA AMAZÔNICA. Este estudo apresenta uma visão geral da adequabilidade do uso de cetáceos amazônicos como espécies sentinelas da contaminação ambiental por micropoluentes como o mercúrio e os organoclorados. Devido ao fato de ocuparem posição de topo nas teias alimentares, bem como à grande longevidade alcançada pelos cetáceos, elevadas concentrações de micropoluentes têm sido verificadas em seus tecidos, o que possibilita uma amplificação da capacidade de determinação de elementos e compostos por diferentes procedimentos analíticos. Além de proporcionar vantagem analítica, a determinação de micropoluentes em cetáceos de forma regular têm sido útil: como avaliação da disponibilidade de poluentes a outros organismos; para integrar um sinal complexo de poluição; bem como para quantificar a significância ecológica de dada contaminação. Desta forma, foram reunidas aqui as informações publicadas sobre as concentrações dos referidos poluentes nos cetáceos amazônicos *Inia geoffrensis* (boto) e *Sotalia fluviatilis* (tucuxi). Efetuando-se comparação com as concentrações observadas em cetáceos costeiros brasileiros, fica patente o quão elevados são os níveis verificados em tais mamíferos aquáticos amazônicos, com destaque para o mercúrio. Apesar do reduzido volume de informação acerca das concentrações de mercúrio, bifenilos policlorados e diclorodifeniltricloroetano em cetáceos amazônicos, as informações existentes já demonstram que os níveis observados nesses animais espelham a contaminação ambiental por tais poluentes em distintos

ambientes amazônicos. Perspectivas para estudos futuros são também discutidas, incluindo possíveis formas de superação de obstáculos relativos à amostragem de cetáceos amazônicos.
Palavras-chave: Brasil, Amazônia, cetáceos, mercúrio, organoclorados.

INTRODUCTION

Aquatic systems have been considered to be ideal final sinks for pollutants, as it was speculated that they would present an infinite dilution capacity (Clark 1992, Jickells 2001). Firstly, pollution problems related to freshwater and coastal zones were identified (Nriagu & Pacyna 1988, Goldberg 1995); however, nowadays it is recognized that disturbed environments are so widely distributed that water pollution can be considered to be global (Nriagu & Pacyna 1988, Walker *et al.* 1997).

A few micropollutants (e.g. polychlorinated biphenyls, chlorinated pesticides and methylmercury) flow through food chains and may go through a concentration increase as they reach high trophic levels, which characterizes the process of biomagnification (Connell 1989, Gray 2002).

Some organisms can be used as biological monitors of pollutant-related environmental alterations. Phillips & Segar (1986) define sentinels as species used for evaluating the bioavailability level of contaminants that are conservative in ecosystems. These species are pollutant accumulators that do not present its effects in their tissues or present sublethal effects, which allow their permanence in the disturbed ecosystem. Besides their use for measuring the totality of a pollutant that is bioavailable, sentinel species amplify, through the accumulation in their tissues, the analytical procedure determination capacity. Frequently, the pollutant concentrations observed in sentinel tissues have been useful as: (1) reference on pollutant availability to other organisms; (2) integrators of complex pollution signals; and (3) to quantify the ecological significance of a contamination (Beeby2001). Aiming to identify potential hazard to humans and wildlife health, sentinels are proposed as systems in which data related to the contamination of animals environmentally exposed to pollutants are regularly and systematically collected and analyzed (National Research Council - NRC 1991).

Cetaceans have been used as indicators of the flow of some elements and pollutant compounds in aquatic ecosystems, due to their long life span as well as to the long biological half-life of pollutants in their tissues (Das *et al*. 2003, Reijnders *et al.* 1999). Thus, these mammals have been used as environmental sentinels of human and aquatic ecosystems health (Reddy *et al.* 2001, Bossart 2006). Regarding the use of organisms as environmental sentinels of the human health specifically, the employment of aquatic mammals with this aim presents a number of advantages over the use of other taxonomic classes, such as aquatic invertebrates and fish. In addition to the fact that they present greater phylogenetic proximity to the human being, pollutant uptake from cetacean respiratory surfaces can be considered to be negligible, given that they breathe atmospheric air, which means that contaminant absorption can be almost exclusively credited to the food intake (Gray 2002, Augier *et al*. 1993).

Predator aquatic mammal contamination by heavy metals and organic compounds has been reported from areas all over the world (O'Shea 1999). It is still a new field of study and the comprehension of the process that governs accumulation and effects over aquatic mammals is still incipient (O'Shea *et al.* 2003).

In South America, some investigations dealt with pollution problem related to cetaceans, however most of the studies are from marine environments (O'Shea *et al.* 1980, Moreno *et al.* 1984, Corcuera *et al.* 1995, Marcovecchio *et al.* 1990,1994, Rosas & Lehti 1996, Lailson-Brito 2000, Lailson-Brito *et al.* 2000, 2002a, 2002b, 2003, 2004, Gerpe *et al.* 2002, Yogui *et al.* 2003, Monteiro-Neto *et al.* 2003, Kunito *et al.* 2004, Kajiwara *et al.* 2004, Torres *et al*. 2006, Dorneles *et al.* 2007a, 2007b, 2007c, 2008a, 2008b, Lailson-Brito 2007, Lailson-Brito *et al.* 2007).

AMAZONIAN CETACEANS

Two cetacean species occur in the Amazon Basin, the boto (*Inia geoffrensis*) and the tucuxi dolphin (*Sotalia fluviatilis*). This latter species is smaller than the former. It is one of the smallest cetaceans, reaching up to 1.6m in length. The tucuxi dolphin is a delphinid that is endemic to the rivers of the Amazon Basin (Da Silva & Best 1996, Cunha *et al*. 2005). Concerning the boto, the currently accepted opinion regards the existence of only one species (*I. geoffrensis*), with three subspecies: *I. geoffrensis geoffrensis*, occurring in all Amazon River Basin, except in the high Madeira River; *I. g. boliviensis*, occurring in the Madeira River above the Teotônio Falls; and *I. geoffrensis humboltiana*, occurring in Orinoco River Basin (Figure 1). The species is classified into the Iniidae Family and is morphologically very distinct from the tucuxi. The total length of adult botos may reach up to 2.3m (females) or 2.8m (males) (Jefferson *et al*. 1993). Although *I. geoffrensis* and *S. fluviatilis* are sympatric species, due to its great flexibility the boto is able to swim in the flooded forest areas formed during the high water level in the Amazon River Basin (Best & Da Silva 1993).

POLLUTION OF AMAZON ECOSYSTEMS

MERCURY

Under the toxicological point of view, mercury can be considered as one of the most dangerous trace elements. It can be naturally found in a number of forms in the environment, such as: elemental mercury (Hg^0); inorganic mercury (Hg^{2+}); monomethylmercury (CH_3Hg^+); and dimethylmercury (CH_3HgCH_3). Mining, fossil fuel combustion, solid waste incineration, fungicide and fertilizing application, as well as solid discharge in sanitary embankment (batteries and thermometers) constitute some of the anthropogenic activities that are responsible for a considerable part of mercury released into the environment (ATSDR 1999).

Most of the mercury found in natural systems is in the inorganic form. However, metallic mercury can be methylated by the action of sulfate-reducing bacteria, which turns mercury in its most toxic form (Miranda *et al*. 2007, Choi & Bartha 1993, 1994). Methylmercury is an organometallic ion generated by the chemically stable binding of mercury to the carbon atom of a short-chain alkyl radical (Miranda *et al*. 2007, Coelho-Souza *et al.* 2007). The concerned compound owns affinity for sulfhydryl, aminocarbonyl and hydroxyl groups present in proteins. In addition, some authors suggest that methylmercury presents high solubility in lipids, which would ease the diffusion of the molecule through biological membrane and hence allow high uptake and accumulation in biological systems, as well as biomagnification (ATSDR 1999).

Brazilian Amazon presents an historical relationship with gold mining activity, which turns the Amazon Basin into a unique area for ecotoxicological studies concerning mercury. The latest gold rush in the Brazilian Amazon, which started in 1979, has turned out to be one of the most destructive activities to the environment and human health in that area. Total environmental mercury emission in the region was estimated to be around 2000 tons from 1979 to 1996 (Malm *et al.* 1998).

Gold miners use Hg for gold recovery due to some of its very unique properties: capacity of forming amalgams with other metals of high density (such as gold), so they both tend to concentrate in the heavier sediment fractions; and volatility, allowing the separation of Hg and gold by volatilization of Hg when burning the amalgam. This process is very attractive due to its simplicity and low cost, and has been widely used in the Brazilian Amazon. Approximately 65-85% of this Hg entered the atmosphere as vapour through burning of amalgam and has been widely dispersed from the gold mining areas. The remaining has entered the aquatic environment directly (Pfeiffer *et al.* 1993).

ORGANOCHLORINE COMPOUNDS

Organochlorine compounds constitute a widely diverse group of molecules of industrial and agricultural use. Among the great variety of properties presented by organochlorine compounds stability is regarded as the most important characteristic of the group (Walker *et al.* 1997).

The entrance of these compounds into the environment occurs through waters from continental drainage (river and lixiviation water discharge), though industrial effluents released in natural bodies of water and through atmospheric precipitation (Clark 1992).

Many organochlorine compounds are liposoluble and present low hydrosolubility. The final destination for many of these compounds is a body of water, where they adsorb to organic matter and are incorporated by plankton (WHO 1976, Newman 1998). Due to the mentioned chemical stability as well as to their resistance to metabolic degradation, some organochlorines are extremely persistent in the environment, which eases their concentration increase through trophic chains (Borga *et al.* 2001). Therefore, fish and mammals are critical groups and may present high concentrations in their tissues.

Concerning biomagnification capacity and effects on organisms, the organochlorine groups that have received most of the attention from the scientific community are the dichlorodiphenyltrichloroethanes (DDTs) and the polychlorinated biphenyls (PCBs).

POLYCHLORINATED BIPHENYLS (PCBS)

PCBs were discovered in the late XIX century and their commercialization started in the 1930s. They are mainly used as dielectric fluid as well as in heat exchange systems (WHO1993).

The concerned compounds are composed by two benzene rings connected by a carbon-carbon binding. They present from 1 to 10 hydrogen or chlorine atoms on positions 2-6 and 2'-6' of the aromatic rings (WHO 1993, 2003). There are 209 possible structures of polychlorinated biphenyls; however, only around 130 of them are likely to occur in commercial mixtures (Pereira 2002).

PCB production was gradually reduced and it is assessed that more than a million tons of PCBs have been produced up to the end of the 1980s (WHO 1993). Legal restrictions to the use of PCBs in Brazil were implemented in 1981, when the prohibition of fabrication, commercialization and use of PCBs was established in the country. However, the same legislation mentioned that equipments that had been already installed, in which PCB was used, could keep on working up to the replacement either of the whole machine or the dielectric fluid by one other than PCB (Penteado & Vaz 2001).

The main PCB human intoxication events has been reported in Japan and Thailand where thousands of people were injured (Safe 1980, Yoshimura 2003). PCBs produce a number of effects in organisms, including hepatomegaly, thymic atrophy, immunosuppression, neurotoxicity, dermal toxicity, etc. (WHO 1993, Hoivik & Safe 1998).

Aquatic organisms, especially those occupying top position on the food chains, tend to present high PCB concentrations in their tissues as a result of the biomagnifications process (Borga *et al.* 2001, Hoekstra *et al.* 2003).

To our knowledge, apart from data generated by analyses of blubber samples from Amazonian dolphins (discussed below), there is no information on PCB levels in environmental matrixes from the Amazon basins.

DICHLORODIPHENYLTRICHLOROETHANE (DDT)

Organochlorine pesticides, such as DDT, besides being cancer promoters, may disrupt endocrine and central nervous systems. In DDT commercial mixtures there is a predominance of *p,p'*-DDT; however, other compounds, such as *o,p'*-DDT; *p,p'*-DDD; *o,p'*-DDD; *p,p'*-DDE; e *o,p'*-DDE, are observed in lower concentrations (WHO 1976).

In 1962, a warning was issued on the possible relation between DDT contamination and wildlife conservation issues. In the book "Silent Spring", Rachel Carson suggested that DDT wide use could constitute the main cause of the population decrease verified in various bird species. In the concerned wildlife health effects of DDT proclaimed by Carson (1962), greater awareness was raised in relation to two top predator species, the peregrine falcon (*Falco peregrinus*) and the bald eagle (*Haliaeetus leucocephalus*). Since then, a series of ecotoxicological studies have been conducted demonstrating DDT toxicity (WHO 2002).

Despite not being as resistant to metabolic degradation as other oganochlorines, one of the DDT degradation products, the DDE, is very persistent in biological systems and may account for more than 80% of the organochlorine compounds present in aquatic organisms (WHO1979, Clark, 1992).

DDT is an organochlorine pesticide that was first synthesized in 1874, but its properties as insecticide were discovered only in the late 1930s by the chemist Paul Muller, who won the Nobel Prize in 1948. Since its discovery, DDT use revolutionized the control concepts against malaria and other tropical insect-borne diseases. A large-scale industrial production started in 1943 and it was used in great quantities mainly for agriculture and forest pest control. A smaller quantity of the world production (20-30%) was used in tropical disease control (Turusov *et al.* 2002). In 1946 a regular system of DDT applications in Amazon houses was established (Wagley 1953). Its use became common in malaria vector control and other tropical diseases, like leishmaniasis. The DDT restrictive measures in Brazil started in 1971 (MA 1971). In 1985 DDT was prohibited for agricultural purposes, but continued to be used for public health campaigns, under the responsibility of FUNASA, the Brazilian National Health Foundation (MA 1985).

PRELIMINARY STUDIES ON MICROPOLLUTANTS IN AMAZONIAN CETACEANS

MERCURY

Lailson-Brito *et al*. (2006) reported mercury concentrations in tissues of four botos (*I. geoffrensis*) and one tucuxi dolphin (*S. fluviatilis*) from Amazon. The tucuxi dolphin and one calf of the species *Inia geoffrensis* were found in Negro River. Two botos, one adult female and a calf, were from Japurá River. The other boto was a calf from Madeira River. Samples from liver, kidney, muscle and, exceptionally brain from the quoted adult female, were analyzed in the study concerned. Mercury concentration (in mg/kg ww) in the analyzed brain was 0.07. Regarding all other concerned organs and tissues from *I. geoffrensis*, concentrations varied from 35.89 in liver of the adult female to 0.09 mg/kg (ww) in kidney of the calf from Japurá River. The most important value to be highlighted was the extremely high hepatic mercury concentration (215.97 mg/kg ww) verified in the tucuxi dolphin from Negro River (Figure 1).

DICHLORODIPHENYLTRICHLOROETHANE (DDT)

Torres *et al*. (2007) carried out DDT determination in blubber samples from four Amazonian dolphins (*I. geoffrensis*). Three of them from Solimões River Basin, Mamirauá Reserve, and one from the Madeira River (Figure 1). Largely forested with numerous channels and lakes, Mamirauá comprises a variety of seasonal floodplain habitats known collectively as várzea. Among the three individuals from Mamirauá Reserve, ΣDDT concentrations in the adipose tissue samples varied from 190 to 3176ng/g lipid weight (lip wt.). Regarding the dolphin from Madeira River, the ΣDDT concentration verified was 2430ng/g lip wt.

POLYCHLORINATED BIPHENYLS (PCBS)

Torres *et al*. (2007) carried out PCB measurement in the same four dolphins mentioned above while describing the preliminary investigations on DDT occurrence in Amazon dolphins. In which concerns the dolphins found in Solimões River Basin, ΣPCB

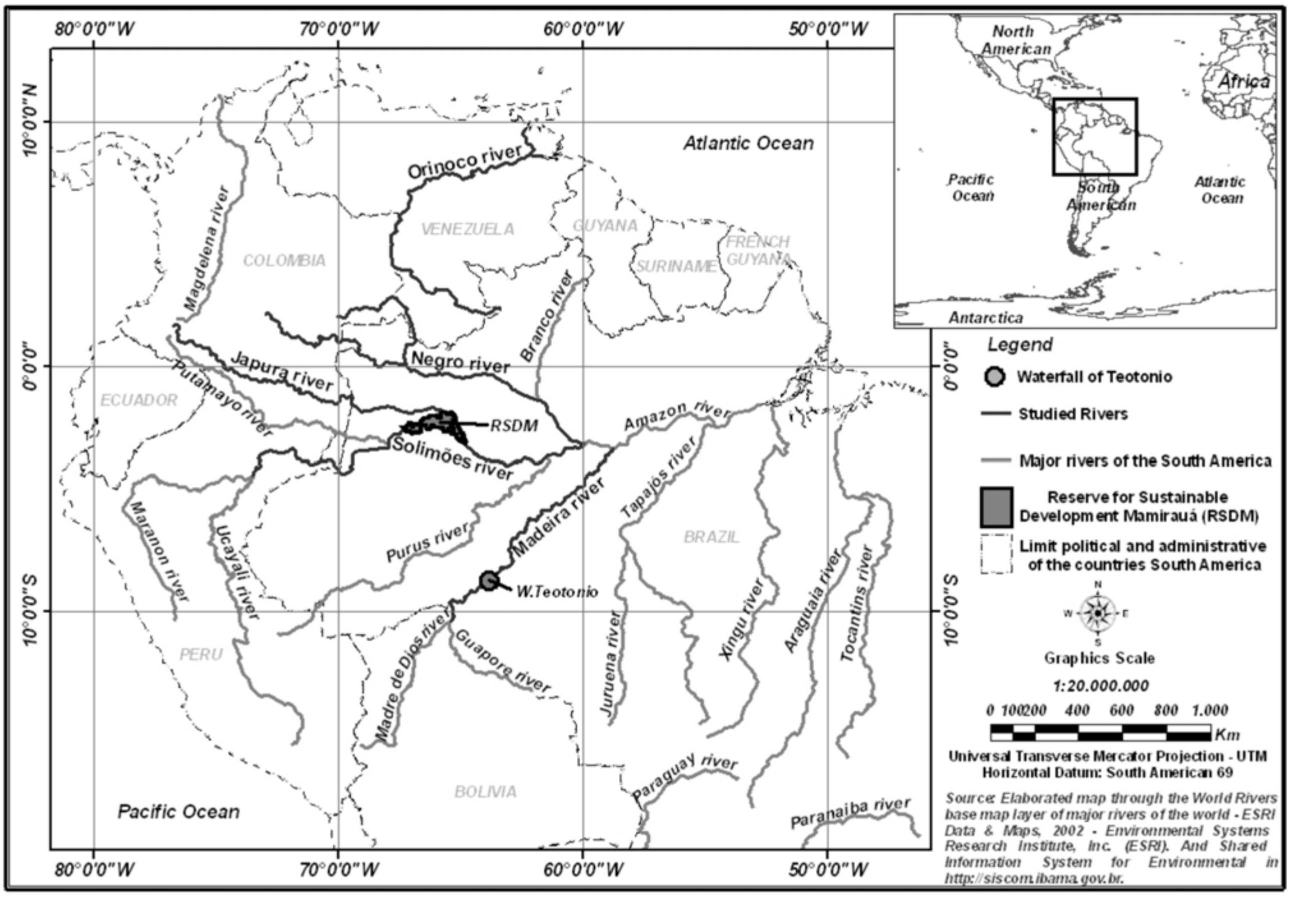

Figure 1. Amazon and Orinoco River Basins, showing the rivers mentioned in the text, as well Teotônio Falls and Mamirauá Sustainable Development Reserve (RSDM).

levels varied between 151 and 1314 ng/g lip wt. Concerning the dolphin found in Madeira River, the ΣPCB concentration verified was 3216 ng/g lip wt.

CETACEANS AS ENVIRONMENTAL SENTINELS FOR MICROPOLLUTANT CONTAMINATION IN AMAZON

Despite the scarcity of scientific information on pollutant levels in cetaceans from Amazon region, the data generated up to now corroborates previously published information regarding other environmental matrixes. The information related to mercury contamination for example clearly illustrates the latter statement. The high mercury concentration (215.97mg/kg ww) verified in liver of a tucuxi dolphin from Negro River, for instance, corroborates published information concerning mercury concentrations in carnivorous fish from different river basins in Brazilian Amazon (Malm *et al.* 1994, Malm *et al.* 1997). Although gold mining activities have been very rare on Negro River basin, Hg concentrations in carnivorous fish are of the same magnitude or even higher than those observed in Tapajós and Madeira River basins, which were both polluted with Hg by gold mining action (Malm *et al.* 1994, Malm *et al.* 1997). In addition, high mercury levels in soil were observed in a recent study carried out on Negro River basin (Azevedo-Silva *et al.* 2006). In fact, some hydrographic basins from the Amazon, present the typical physicochemical conditions that generally favour the formation of methylmercury, which are: low pH and conductivity, poverty in nutrients and richness in dissolved organic matter. The Negro River waters provide these conditions for enhanced production and availability of monomethylmercury (CH_3-Hg^+) (Barbosa *et al.* 2003). The mercury concentration verified in the tucuxi dolphin from Negro River and other findings from literature mentioned above suggest that the Negro River Basin naturally presents mercury-rich soil and that it could constitute a source of the quoted metal to adjacent fluvial bodies of water.

The same holds for the ΣDDT concentration mentioned above, which was verified in the adipose tissue of the boto (*I. geoffrensis*), since these data corroborates the concentrations of the concerned class of organochlorine pesticide in breast milk from women inhabiting the Madeira River Basin (Torres *et al.* 2007). However, the most striking information concerning organochlorine compound contamination in Amazon is related to the ΣPCB levels reported for the same cetacean species and tissue (Torres *et al.* 2007). PCBs constitute a class of organochlorine compounds that is characteristic of urban and industrial environments, as it is expected to be, considering their use and origin. This aspect of PCB distribution on the planet would turn the high PCB levels observed in Amazonian dolphins into surprising information if just the Amazon as a whole was taken into consideration. However, despite the great number of articles that describes Amazon region as a pristine environment, it is important to draw attention to some areas of high demographic concentration in the concerned region, such as Porto Velho and Tefé. The former city (Porto Velho) presents a population of about 370 000 people, while in the latter, the number is > 60 000 (IBGE 2008**)**. Although three out of the four dolphins analyzed by Torres *et al*. (2007) have been found in a preserved environment (Mamirauá Reserve), data obtained from individually marked dolphins demonstrated that Tefé area is within the movement range of the boto population that inhabits the quoted reserve (Martin *et al.* 2004), which could explain the occurrence of high concentrations of a pollutant of industrial and/or urban origin (PCB) in an Amazonian reserve. Even though further investigations comprising a larger sampling are required before strong conclusions can be reached, it is important to highlight that ΣPCB concentrations in the subcutaneous adipose tissue of cetaceans from Amazon seem to mirror human presence in the concerned area.

THE MAGNITUDE OF THE CETACEAN EXPOSURE TO PCB, DDT AND Hg IN AMAZON

Interesting observations on ΣPCB concentration in dolphins that occur in Brazilian waters can be made while comparing fluvial to marine cetaceans. In Brazil, the cetacean species of greatest ecotoxicological concern is the marine tucuxi dolphin (*Sotalia guianensis*). This statement is based on the presence of the species in estuaries and bays of the most urbanized and industrialized region of the Brazilian coast, the Southeast Brazilian Region, as well as on the fact that the species presents habitat fidelity, since the same individuals are found year-round in these sites (Azevedo *et al.* 2004). Due to these features as well as to the mentioned high trophic position and longevity, high levels of persistent bioaccumulative toxicants have been verified in the tissues of the individuals of the species (Yogui *et al.* 2003, Lailson-Brito *et al.* 2003, Kajiwara *et al.* 2004, Lailson-Brito 2007, Torres *et al.* 2006, Dorneles *et al.* 2008b). In a recent investigation on organochlorine levels in marine tucuxi dolphins (Lailson-Brito 2007), the same PCB congeners determined by Torres *et al*. (2007) were used for calculating the ΣPCB, which turn comparison to Amazon dolphins more suitable. Doing so, it is interesting to highlight that the ΣPCB concentration verified in the boto from Madeira River fits within the range of the levels of the quoted pollutant determined in marine tucuxi dolphins from Sepetiba/Ilha Grande Bays Complex (1745 – 25482ng/g lip wt., Lailson-Brito 2007), a degraded estuarine area situated in the most anthropogenically disturbed region of the Brazilian littoral, the coast of the Southeast Region of Brazil.

Comparison of pollutant concentrations between the tucuxi dolphin (*S. fluviatilis*) that occurs in Amazon and the marine tucuxi dolphin (*S. guianensis*), a coastal and estuarine species that occurs from southern Brazil (27°35'S, 48°34'W) to Honduras (15°58'N, 79°54'W) (Da Silva and Best 1996, Flores 2002) can be considered to be suitable for presenting an idea about the magnitude of the micropollutant contamination, especially if the phylogenetic proximity between both species is taken into account. The genetic similarity between both organisms reduces the possibility of a significant difference regarding the toxicokinetic behaviour of a given pollutant in each species. In this context, the genetic closeness between these two dolphins deserves to be highlighted, given that until recently, the genus Sotalia was considered as one single species (*S. fluviatilis*), with marine and riverine ecotypes. However, using mitochondrial DNA sequence data Cunha *et al*. (2005) concluded that marine and riverine *Sotalia* are different species. Therefore, analyzing liver samples from 22 marine tucuxi dolphins that stranded on the beaches of Guanabara Bay, the most degraded estuary along the species distribution (Lailson-Brito 2000), Lailson-Brito (2007) verified that mercury concentrations varied from 0.53 to 132.62mg/kg, wet wt. As it can be observed, none of the 22 marine tucuxis render a hepatic mercury concentration higher than the observed in the tucuxi dolphin from Negro River (215.97mg/kg ww, Lailson-Brito *et al.* 2006). The same holds for another coastal marine mammal, the fransiscana dolphin, since the highest hepatic mercury concentration verified among 17 individuals stranded on Rio de Janeiro state was 47mg/kg, wet wt. (Lailson-Brito *et al.* 2002b).

In which concerns DDT contamination, outstanding information is also observed while carrying out comparison between marine and freshwater dolphins. The ΣDDT concentrations verified in the adipose tissue of two out of the four botos (*I. geoffrensis*), determined by Torres *et al*. (2007), fit within the range of the levels of the quoted pollutant determined in marine tucuxi dolphins from Guanabara Bay (2075–21504 ng/g lip wt., Lailson-Brito 2007). This constitutes remarkable information when it is taken into account that the quoted estuary is the most anthropogenic disturbed habitat in marine tucuxi dolphin distribution (Lailson-Brito 2000).

CONSIDERATIONS FOR FUTURE RESEARCH

The use of stranded dolphins on the Brazilian sea-shore has been shown to be a useful tool for the monitoring of micropollutants in marine environments. This approach allowed the demonstration of which pollutants are going through the system as well as the magnitude of the concentrations that are possible to be observed in top predators of different environments. However, the recovery of the carcasses depends on the notification of stranding events by the population and a quick response from rescue teams. Therefore, a challenge is posed for the use of cetaceans in biomonitoring in Amazon region, considering the non-linear nature of the areas where the dolphins may strand, the difficulties of communication and access, especially in the seasonally flooded areas. One possible solution could be reached through remote biopsy sampling of skin and subcutaneous adipose tissue, which could not only increase the sample size but also drive it to specific areas of interest (areas of higher urbanization and pristine regions, for example). Besides, how representative of the healthy population are the stranded dead dolphin has also been a matter of debate (O'Shea 1999), which turns sampling of live dolphins into an even more attractive approach.

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