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Baseline

Assessment of trace elements, POPs, ^{210}Po and stable isotopes (^{15}N and ^{13}C) in a rare filter-feeding shark: The megamouth

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ABSTRACT

With less than 60 records being reported worldwide, the megamouth (*Megachasma pelagios*) is today one of the least known shark species inhabiting our oceans. Therefore, information concerning the biology and ecology of this enigmatic organism is very scarce and limited to feeding behaviour and preferred habitat. The present work reports new data on the concentrations of trace elements, organic mercury, POPs and ^{210}Po in hepatic and muscular tissues of a specimen found stranded in the southeastern coast of Brazil. Additionally, we provide new evidence based on stable isotope analysis (δ^{15}N and δ^{13}C) confirming the preference for the pelagic habitat and the zooplanktivorous feeding behaviour of the megamouth. These results are consistent with the low concentrations of organic pollutant compounds and other elements measured in our samples.

The megamouth, (*Megachasma pelagios*) is a pelagic filter-feeding shark, distributed in circumtropical and warm temperate waters (Taylor et al., 1983). Since the discovery of this enigmatic shark in Hawaii in 1976, approximately 60 records have been reported and documented, principally on the east and west coasts of the Pacific Ocean (FLMNH, 2014). In the Atlantic Ocean, only three occurrences have been reported to date (Séret, 1995; Amorim et al., 2000; FLMNH, 2014). In general, there is very scarce information about this species regarding its biology, ecology, and conservation status (Compagno, 2001). The very few studies existing on feeding behaviour have shown that the megamouth predates on epipelagic and mesopelagic euphausiid shrimps, copepods, and jellyfish, and that it may even undertake vertical movements following the migratory pathway of euphausiid preys (Taylor et al., 1983; Compagno, 2001; Sawamoto and Matsumoto, 2012). Recent descriptions of records suggest that bycatch in pelagic fishing gears is a potential threats to the conservation of the megamouth (Nelson et al., 1997; Morrissey and Elizaga, 1999; Amorim et al., 2000; Sawamoto and Matsumoto, 2012; Tomita et al., 2014).

Marine pollution is also an issue of great concern. There is a range of persistent chemical contaminants ubiquitously distributed in marine environments that have been detected in a wide variety of marine organisms (Chiuchiolo et al., 2004). Such chemical pollutants (including, heavy metals and organic pollutants) impact mainly organisms inhabiting coastal areas, although also organisms from pelagic and other remote environments (e.g. Antarctica), far from the source of emissions, appear affected

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(Davis, 1993; Chiuchiolo et al., 2004; Braune et al., 2005). However, no study to date has investigated the level of contamination of megamouth sharks.

The objectives of this paper are (1) to describe, using stable isotope analysis, the habitat preferences of a megamouth specimen found stranded on the Brazilian coast and (2) to conduct the first assessment of organic pollutants, trace elements, organic mercury (Hg-Org), and ^{210}Po in this shark.

On 09 July 2009, an adult male of megamouth was found stranded in Praia Grande, Arraial do Cabo, located on the east coast of the Rio de Janeiro state, Brazil (22°57′S; 42°04′W, Fig. 1). The total body length of the shark was 539 cm. The region around Arraial do Cabo is conspicuously distinct from the surrounding areas. The water temperature can drop from an average annual value of 22 °C to less than 17 °C due to the Cabo Frio upwelling system. This region presents high primary productivity, complex biodiversity and abundance of the aquatic organisms (Moser and Gianesella-Galva~o, 1997). The areas surrounding Arraial do Cabo present a relatively narrow shelf, with the coastline changing abruptly from north–south to east–west direction (Valentin, 2001).

The specimen was found by a beach-monitoring program conducted regularly on the central-north coast of the Rio de Janeiro by the Group of Marine Mammal Research from Região dos Lagos (Grupo de Estudos de Mamíferos Marinhos da Região dos Lagos, GEMM-Lagos). The sex of the specimen was determined from the examination of the carcass. The visual assessment of the stomach chamber revealed the absence of food remains. Samples of liver and of muscle taken from the dorsal part of the shark were collected and preserved frozen immediately after the visual inspection of the carcass. These hepatic and muscular samples were used to perform the analyses presented in this study.

The species was identified through genetic analysis using the mitochondrial cytochrome c oxidase subunit I (COI) gene as molecular marker. For this purpose, genomic DNA extraction was performed according to a modified protocol from D'Amato and Corach (1996). COI was amplified using primers HCO2198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′) and LCO1490 (5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′) (Folmer et al., 1994). Amplifications were carried out in 10 mL reaction flasks containing about 250 ng of DNA, dNTPs (0.025 mM), primers (1 μM each), Taq DNA polymerase (Invitrogen, 1 U), $MgCl_2$ (2.5 μM) and amplification was buffered under the following conditions: 94 °C (5 min), 35 cycles at 94 °C (1 min), 36 °C (1 min) and 72 °C (90 s), and a final step at 72 °C (7 min). The amplified fragment of about 800 base pairs (bp) was purified using GFX™ PCR DNA and a gel band purification kit (GE Healthcare), following manufacturer's instructions. Direct DNA sequencing was performed with the ET Dye terminator cycle sequence kit (GE Healthcare) and analysed in a MegaBace 1000 automated sequencer (GE Healthcare). Sequences were edited with the BioEdit Sequence Alignment Editor – Version 7.0.1 (Hall, 1999) and a consensus sequence was produced. Then, a blast search using the nucleotide–nucleotide basic local alignment (BLASTn) search tool was conducted using the consensus generated.

Stable isotope ratios (δ^{13}C and δ^{15}N) were obtained from the dry muscle sample of the megamouth using a Thermo QuestFinnigan Delta Plus isotope ratio mass spectrometer (Finnigan MAT) interfaced to an Elemental Analyzer (Carlo Erba). Pee Dee Beleminite carbonate and atmospheric nitrogen were used as standard values and an analytical precision of ±0.1‰ for δ^{13}C and of ±0.2‰ for δ^{15}N (triplicate samples). The results were expressed in parts per thousand (‰). Lipids were not extracted from the muscle sample prior to analysis; however, the C:N ratio (1.9) was lower than 3.5, indicating low levels of lipids. Thus, the interpretation of the δ^{13}C result was not compromised (Post et al., 2007).

The muscle and liver samples were analysed for the presence of 21 different trace elements (Ag, Al, As, B, Bi, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Pb, Se, Sr, and Zn) using a methodological procedure previously described by Moura (2013). The concentration of these elements was determined by ICP mass spectrometry (ICP–MS), using a PerkinElmer Nexlon 300× spectrometer. Rhodium (^{103}Rh) was used as internal standard and, to ensure

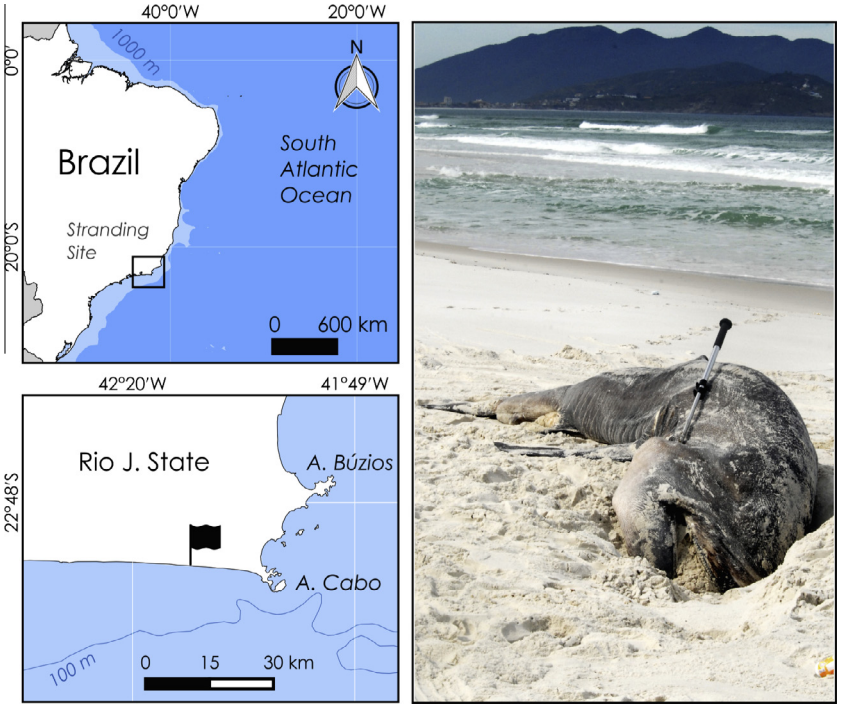

Fig. 1. Picture of the megamouth shark (*Megachasma pelagios*) analysed in this study and map showing the location where it was found stranded (black flag), in Praia Grande, Arraial do Cabo, southeastern Brazil. Photo credit: Bruno C. Rennó, under the project *Habitats PETROBRAS/GEMM-Lagos*.

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