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Are whale sharks exposed to persistent organic pollutants and plastic pollution in the Gulf of California (Mexico)? First ecotoxicological investigation using skin biopsies

Maria Cristina Fossi [a], Matteo Baini [a,*], Cristina Panti [a], Matteo Galli [a], Begoña Jiménez [b], Juan Muñoz-Arnanz [b], Letizia Marsili [a], Maria Grazia Finoia [c], Dení Ramírez-Macías [d]

[a] Department of Physical, Earth and Environmental Sciences, University of Siena, Via Mattioli 4, 53100 Siena, Italy
[b] Department of Instrumental Analysis and Environmental Chemistry. Institute of Organic Chemistry (IQOG-CSIC). Juan de la Cierva 3, 28006 Madrid, Spain
[c] ISPRA, Institute for Environmental Protection and Research, Via V. Brancati 48, 00144 Rome, Italy
[d] Tiburon Ballena Mexico proyecto de ConCiencia Mexico AC, La Paz, BCS, Mexico

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ABSTRACT

The whale shark (*Rhincodon typus*) is an endangered species that may be exposed to micro- and macro-plastic ingestion as a result of their filter-feeding activity, particularly on the sea surface. In this pilot project we perform the first ecotoxicological investigation on whale sharks sampled in the Gulf of California exploring the potential interaction of this species with plastic debris (macro-, micro-plastics and related sorbed contaminants). Due to the difficulty in obtaining stranded specimens of this endangered species, an indirect approach, by skin biopsies was used for the evaluation of the whale shark ecotoxicological status. The levels of organochlorine compounds (PCBs, DDTs), polybrominated diphenyl ethers (PBDEs) plastic additives, and related biomarkers responses (CYP1A) were investigated for the first time in the whale shark. Twelve whale shark skin biopsy samples were collected in January 2014 in La Paz Bay (BCS, Mexico) and a preliminary investigation on microplastic concentration and polymer composition was also carried out in seawater samples from the same area. The average abundance pattern for the target contaminants was PCBs > DDTs > PBDEs > HCB. Mean concentration values of 8.42 ng/g w.w. were found for PCBs, 1.31 ng/g w.w. for DDTs, 0.29 ng/g w.w. for PBDEs and 0.19 ng/g w.w. for HCB. CYP1A-like protein was detected, for the first time, in whale shark skin samples. First data on the average density of microplastics in the superficial zooplankton/microplastic samples showed values ranging from 0.00 items/m^3 to 0.14 items/m^3. A focused PCA analysis was performed to evaluate a possible correlation among the size of the whale sharks, contaminants and CYP1A reponses. Further ecotoxicological investigation on whale shark skin biopsies will be carried out for a worldwide ecotoxicological risk assessment of this endangerd species.

1. Introduction

The whale shark (*Rhincodon typus*) has a circumequatorial distribution in all tropical and warm temperate seas (Colman, 1997; Compagno, 1984). This species is epipelagic, oceanic, and coastal, forming seasonal near-shore aggregations in many areas that are related to local seasonal productivity (Rowat and Brooks, 2012; Sequeira et al., 2013). The presence and movements of whale sharks have been linked to the spawning of corals and fishes, upwelling, plankton abundance, and changes in the temperature of water masses (Heyman et al., 2001; Motta et al., 2010; Robinson et al., 2013; Wilson et al., 2001). In the late 90s, some whale shark populations declined drastically (Norman, 2005; Rowat and Brooks, 2012) and, in 2000, the species was listed as vulnerable on the IUCN Red List (Norman, 2000). In 2016, the conservation status was assessed as endangered (Pierce and Norman, 2016). This species has a k-selected life history that makes them vulnerable to exploitation such as large size, slow growth, late maturation, production of few offspring and extended longevity (Colman, 1997; Rowat and Brooks, 2012). Major threats to this species include interaction with fishing activity (direct catches and bycatch), vessel strikes, inappropriate tourism and climate change (Pierce and Norman, 2016). Furthermore, the increasing human activity in whale shark grounds gives rise to chemical pollution from urban wastewaters, vessels, agriculture and waste including plastic debris. During surface ram filter feeding, sharks swam at an average velocity of 1.1 m/s with 85% of their mouth open below the water's surface, as reported by Motta and collaborators (Motta et al., 2010). Whale sharks spend, on average, approximately 7.5 h/day feeding at the

* Corresponding author.
E-mail address: matteo.baini@unisi.it (M. Baini).

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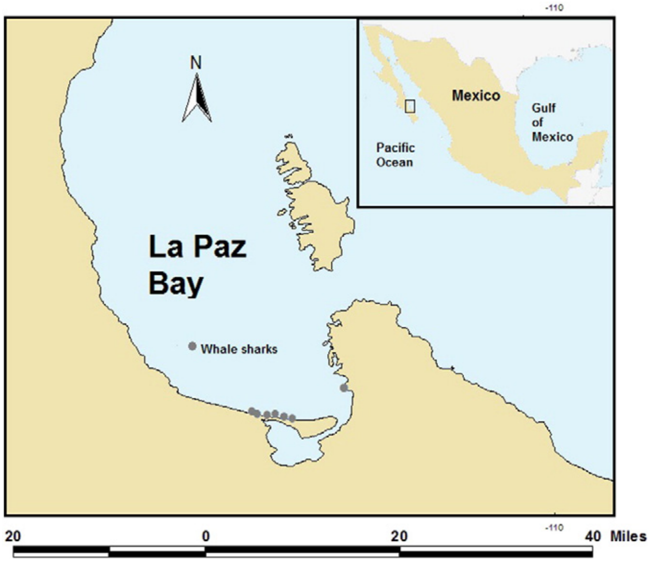

Fig. 1. Gulf of California and La Paz Bay (BCS, Mexico), with grey spots representing juveniles whale shark (*Rhincodon typus*) sampled.

surface on dense plankton dominated by calanoid, copepods, sergestids, chaetognaths and fish larvae (Motta et al., 2010). During the feeding, the whale shark could be exposed to the ingestion of pollutants floating on the sea surface and associated to sea surface microlayer, including floating plastic debris. However, these impacts on filter feeder sharks are largely unknown (Fossi et al., 2014). Juvenile whale sharks (total length < 9 m) aggregate seasonally in different areas of the Gulf of California, specifically in coastal waters of "Bahía de Los Angeles", off the north-central coast of the Baja California Peninsula (Mexico) and "La Paz Bay" off the south-eastern coast of the peninsula (Ramírez-Macías et al., 2012b). Several studies have shown that most sighted aggregations are composed of juvenile male whale sharks (Meekan et al., 2006; Ramírez-Macías et al., 2012a,b; Rowat and Brooks, 2012). In La Paz Bay, a high number of whale sharks aggregate to feed in a predictable manner and for long periods. In this area, the juvenile sharks have showed fidelity to the area remaining in the Bay during the season for up to 135 days and returning during the years, in a season up to 38% of the sharks can be re-sighted from previous years. This shows the importance of this habitat for juvenile sharks (Ramírez-Macías et al., 2012b). La Paz city is one of the most highly populated coastal areas in the Gulf of California and has the highest growth rate (2.6%) in the state. Boat traffic is increasing in the whale shark aggregation area with new marinas, new tourist companies and fisherman's boats. Whale shark tourist activity has also increased, with the government authorizing 109 boats in 2014. Whale sharks represent an important part of the tourist attraction, but their presence imposes also a challenge to protect them. The increasing human impact in whale shark feeding grounds in this area gives rise to urban and industrial waste waters, including macro- and micro-litter.

Marine litter represents a serious concern for the marine environment (Eriksen et al., 2014; Kühn et al., 2015). Presence and distribution of plastic debris in the marine environment has been documented and, it is widely known, that marine debris originates from land; however, the quantity of plastic entering the ocean from mismanaged waste on land is unknown. Jambeck et al. calculated that out of the 275 million MT produced by 192 coastal countries in 2010, 4.8 to 12.7 million metric tons (MT) entering the ocean (Jambeck et al., 2015). Along with the land based sources, other inputs from ocean-based sources include maritime traffic, fishing activities (both commercial and recreational) and aquaculture sites (Galgani et al., 2015). Among marine litter, microplastics, generally defined as fragments <5 mm in dimension (Arthur et al., 2009) represents an emerging worldwide concern for marine organisms as a wide range of organisms, from plankton to larger vertebrates such as turtles or whales, may ingest them (Wright et al., 2013).

Plastic particles can harm marine organisms, causing physical damages (Wright et al., 2013) and/or transporting POPs and partitioning plastic additives (Rochman, 2015). Due to high sorption capacity of plastics for hydrophobic organic chemicals, the chemicals can be transported by microplastics and macroplastics traveling long distances (Lee et al., 2013). Therefore, plastic debris can serve as carrier of persistent organic pollutants (POPs) in marine ecosystems (Besseling et al., 2013; Rochman et al., 2013). In addition, several plastic additives (e.g. flame retardants, stabilizers, and plasticizers) may leach out and become bioavailable to marine organisms (Rochman, 2015).

Despite the growing scientific attention on this issue, little scientific investigation has focused on the potential impact of micro- and macroplastics on large filter feeding marine organisms such as baleen whale and planktivorous sharks (Fossi et al., 2014; Besseling et al., 2015; Fossi et al., 2016). In particular, we lack information about inputs, spatial and temporal distributions and interactions with biota in semi-closed basins, such as the Gulf of California.

In this paper, we perform the first ecotoxicological investigation on whale sharks sampled in the Gulf of California exploring the potential interaction of this species with plastic debris (macro- and micro-plastics), the levels of PBDEs and OCs and related biomarkers responses (CYP1A) using skin biopsies as target tissue due to the lack of stranded organisms and the protected status of the whale shark. Skin biopsy samples were collected from twelve whale sharks in La Paz Bay and a preliminary investigation on microplastic concentration and polymer composition was also carried out in samples collected in the whale shark ground.

2. Material and methods

2.1. Study area and collected samples

La Paz Bay is located in the south of the Gulf of California (BCS, Mexico), with shallow coastal (<50 m) and deep oceanic (>200 m) areas. Juvenile sharks aggregate to feed in the coastal waters of the bay, near to the city. Skin biopsy sample from 12 whale sharks (11 males and 1 female), ranging from 3.5 to 8 m total length, were collected on January and February of 2014, in inshore waters of La Paz Bay (Fig. 1). Biopsies were sampled using biopsy tips mounted on a pole and immediately placed in liquid nitrogen in order to prevent any degradation for biomarker analysis (Ramírez-Macías et al., 2007, 2012b).

Each shark was geo-referenced using a Global Positioning System, and photographed with an underwater camera for future identification. The pattern of lateral markings behind the five gill slits on the left side is unique to each individual and is an effective marker for capture-mark-recapture studies (Taylor, 1994). Scars and other present markings were also recorded. Gender was determined by the presence or absence

Table 1
Size and sex of each whale shark (WS) collected in La Paz Bay (BCS, Mexico) in January and February 2014.

Sample	Date	Sex	Size
WS 1	30/01/2014	M	5.5
WS 2	30/01/2014	M	5
WS 3	30/01/2014	M	4.5
WS 4	30/01/2014	M	4
WS 5	30/01/2014	F	5
WS 6	31/01/2014	M	3.5
WS 7	31/01/2014	M	4
WS 8	31/01/2014	M	7
WS 9	01/02/2014	M	4
WS 10	01/02/2014	M	6
WS 11	01/02/2014	M	4
WS 12	01/02/2014	M	8