Marine Pollution Bulletin 220 (2025) 118469

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Trace elements and POPs in baitfish from Madagascar: Implications for whale shark and human exposure

S. Diamant [a], A. Mahevitra [a,b], D. Monticelli [c], D. Banfi [d], B. Villa [c,d], A.R. López [c,e], R. Bettinetti [d], G. Boldrocchi [d,*]

[a] Madagascar Whale Shark Project Foundation, Nosy Be, Madagascar
[b] Institut Halieutique et des Sciences Marines, Toliara, Madagascar
[c] Department of Science and High Technology, University of Insubria, Via Valleggio 11, Como, Italy
[d] Department of Human Sciences, Innovation and Territory, University of Insubria, Via Valleggio 11, Como, Italy
[e] University School for Advanced Studies IUSS Pavia, 27100 Pavia, Italy

ARTICLE INFO

Keywords:
Rhincodon typus
Marine pollution
Sardinella gibbosa
Legacy contaminants
Baitfish consumption
Bioaccumulation
Mercury

ABSTRACT

The rapid increase of human activities is threatening the ocean biodiversity, with marine vertebrates, particularly elasmobranchs, facing higher extinction risks. Among them, the whale shark (*Rhincodon typus*), an endangered migratory filter-feeding species, is threated by multiple anthropogenic pressures, including marine pollution. This study focuses on determining the presence and levels of legacy contaminants (PCBs, DDT) and trace elements (TEs) in baitfish, a primary prey species of whale sharks in Madagascar, and assessing the potential risks to whale sharks and humans through the consumption of contaminated baitfish (*Sardinella gibbosa)*. Results indicate that while levels of DDT were below environmental safety thresholds, Hg levels in baitfish exceeded the acceptable limit, posing significant bioaccumulation risks to higher trophic level organisms, including whale sharks. Furthermore, comparisons with established maximum allowable limits for foodstuffs consumption revealed that *Sardinella gibbosa* may pose both non-carcinogenic and carcinogenic health risks to humans, particularly for pollutants like PCB, Cd, Ni, and Hg. This research highlights the importance of considering baitfish consumption as a significant pathway for pollutant uptake in whale sharks, suggesting that their exposure may be far higher than previously estimated through zooplankton alone. These findings underscore the critical need for continued monitoring of chemical pollution in coastal ecosystems, which are vital hotspot for global marine biodiversity, particularly in regions like Madagascar, where pollution from industrial, agricultural, and mining activities exacerbates the risk to both marine biodiversity and human health.

1. Introduction

The rapid increase of human activities is threatening ocean biodiversity globally. Marine biodiversity loss is directly caused by overexploitation, pollution, habitat destruction, climate change and associated perturbations of the ocean biogeochemistry (Dulvy et al., 2003; Lotze et al., 2006; Worm et al., 2005) and it increasingly diminishes the ocean's ability to provide food, maintain water quality, and other essential ecosystem services (Worm et al., 2005).

Among marine vertebrates, chondrichthyan are considered the group of animals at higher extinction risk, and only one-third of species are considered at minimal risk (Dulvy et al., 2014). Many of these species are intrinsically sensitive to multiple ranges of direct and indirect human pressures, especially where key habitats overlap with highly impacted areas. The whale shark, *Rhincodon typus*, for instance, is a planktivorous elasmobranch species occurring in tropical, subtropical, and warm temperate seas (Rowat and Brooks, 2012; Sequeira et al., 2014; Stevens, 2007). As a highly migratory, long-lived, and slow-growing species that aggregates in coastal marine ecosystems spending significant amounts of time feeding on surface waters (Gleiss et al., 2013; Motta et al., 2010; Rowat and Brooks, 2012; Rowat et al., 2007), the whale shark faces multiple human threats, ranging from vessel collision (Boldrocchi et al., 2020a; Pierce and Norman, 2016; Pirotta et al., 2019; Reynolds et al., 2022a, 2022b; Rowat and Brooks, 2012; Womersley et al., 2024), to fisheries catches, and bycatch in purse-seine nets or gillnets (Di Beneditto et al., 2021; Pierce and

* Corresponding author.
E-mail address: ginevra.boldrocchi@uninsubria.it (G. Boldrocchi).

https://doi.org/10.1016/j.marpolbul.2025.118469
Received 29 March 2025; Received in revised form 14 July 2025; Accepted 16 July 2025
Available online 21 July 2025

Norman, 2016; Reynolds et al., 2022a, 2022b; Rohner et al., 2018; Rowat et al., 2021), which are considered the major contemporary threats to this species (Pierce and Norman, 2016).

A recent studies have highlighted marine pollution as an additional significant risk to the elasmobranch survival (Boldrocchi et al., 2020b, 2021a; Gelsleichter et al., 2020; Gelsleichter et al., 2006; Tiktak et al., 2020), even for lower trophic-level species, such as filter-feeding species (Boldrocchi et al., 2020b, 2022, 2023; Fossi et al., 2014; García-Baciero et al., 2024; Fossi et al., 2017; Pancaldi et al., 2019). Multiple lines of evidence show that even whale sharks can accumulate pollutants at high levels, due to their life history traits and overlap with highly polluted marine ecosystems (Boldrocchi et al., 2020b). Given the conservation status of filter-feeding elasmobranchs and their potential as early indicators of environmental threats, the assessment of potentially rising chemical pollution threats is essential. Still, data on presence and levels of legacy and emerging contaminants in whale sharks is sparse and mainly studied in certain specific regions, such as Djibouti (Boldrocchi et al., 2020b, 2023) and the Gulf of California (Fossi et al., 2014; Villagómez-Vélez et al., 2024; García-Baciero et al., 2024; Pancaldi et al., 2019; 2021), leaving the vast majority of aggregation sites poorly investigated.

In Madagascar, the regular presence of whale sharks has been documented since 2007 (Diamant et al., 2018, 2021; Jonahson and Harding, 2007; Kiszka and van der Elst, 2015), where these animals aggregate in the coastal waters off Nosy Be between September and December (Diamant et al., 2018, 2021). Although multiple works have investigated the ecology of this species, only one study has attempted to indirectly estimate the possible impact of organochlorine accumulation on whale sharks, using zooplankton as target prey (Marsili et al., 2023). However, while whale sharks are known to feed on dense patch of zooplankton in multiple aggregation sites in the Indian Ocean, including Djibouti (Boldrocchi et al., 2018, 2020a; Rowat et al., 2007); Tanzania (Rohner et al., 2015); Mozambique (Rohner et al., 2013), and Qatari waters (Robinson et al., 2013), in Madagascar whale sharks are regularly observed exhibiting "vertical feeding" behavior where they ingest baitfish trapped at the surface, working in collaboration with other predators such as bonito fish (*Euthynus affinis*) and sea birds (Sulidae and Sternidae) in a bait ball dynamic (Diamant et al., 2021; Fontes et al., 2020). This distinctive behavior may have significant implications for the whale shark contamination in this region, but also for humans. Indeed, members of the family Clupeidae comprise the majority of world's landed fish, and *Sardinella gibbosa* is among the most abundant and commercially important species in the Indo-West Pacific sardine fishery (FAO, 2018; Hunnam, 2021).

Accordingly, the present study aims at: 1) evaluating the levels of legacy contaminants (PCBs, DDT) and trace elements (TEs) in *Sardinella gibbosa* collected during the whale shark aggregation season in Madagascar; 2) assessing the potential uptake of pollutants by whale sharks, focusing on contaminants posing a global threat to aquatic biota; 3) estimating the exposure health risks for humans derived from the consumption of baitfish.

2. Materials and methods

2.1. Study site

A total of 12 baitfish samplings of *Sardinella gibbosa* have been carried offshore the island of Nosy Be (13.39° S, 48.20° E) in the

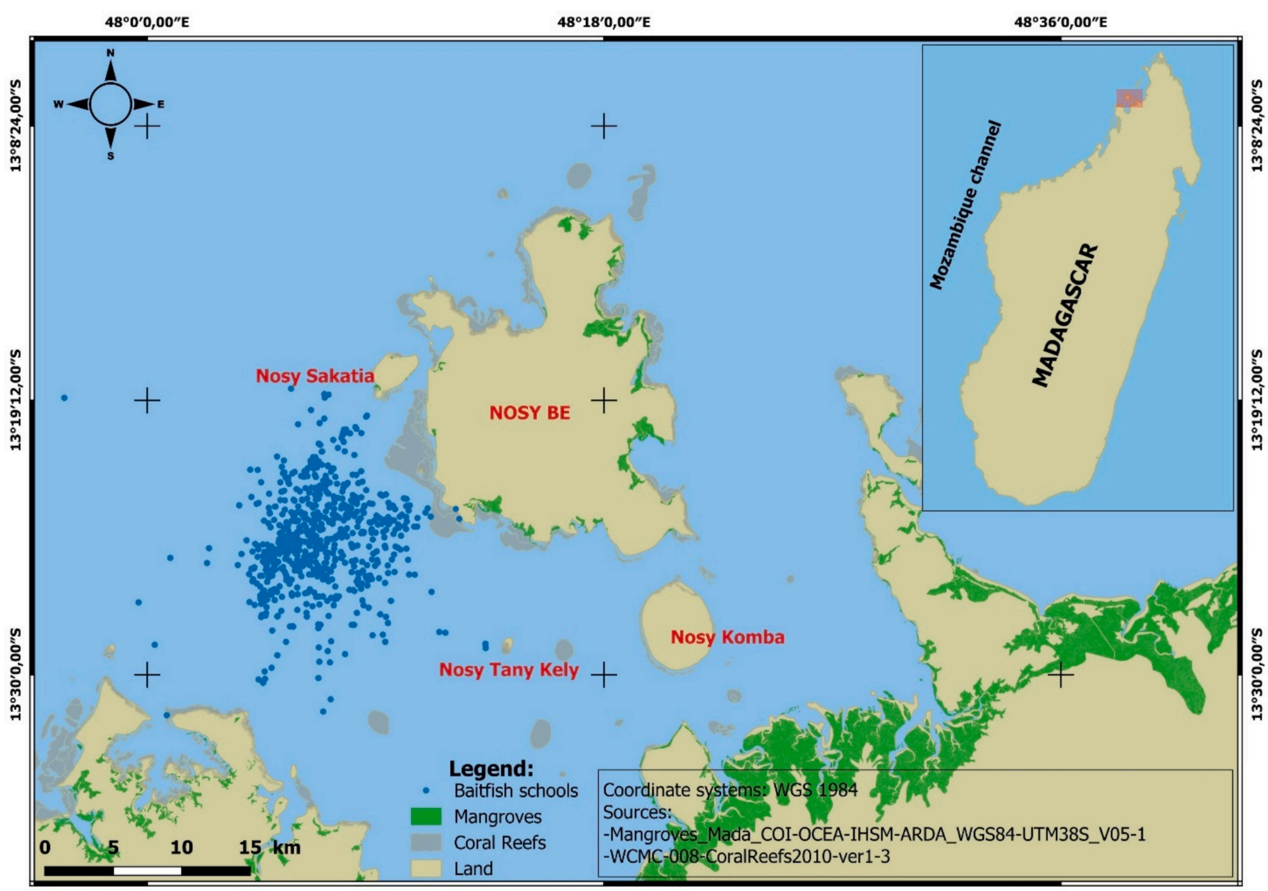

Fig. 1. *Sardinella gibbosa* school area (blue dots) located offshore Nosy Be Island (Madagascar), in September–December 2024. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

northwestern Madagascar (Fig. 1) during the whale shark feeding aggregation, which occurs between September and December. Specifically, 5 baitfish samplings occurred in presence of whale sharks feeding on *Sardinella gibbosa*, while 7 samplings were in the absence of sharks. The samplings were carried out using a small-mesh gillnet (2 mm mesh opening), measuring 7 m in length and 1.5 m in width. The capture technique involved encircling the baitfish schools with the net. Each catch was recorded on board to identify the scientific names of the captured baitfish species (Fig. 2). Species identification was based on the distinctive morphological characteristics of *Sardinella gibbosa*, using the FAO fish identification keys (1984) and the FishBase online database. A total of 72 individuals of *Sardinella gibbosa* from all sampling events were preserved in a 5 mL tube for ecotoxicological analysis.

2.2. Analyses of organochlorine compounds

Sample treatment followed established procedures reported in our previous work (Boldrocchi et al., 2019). Briefly, each analyzed sample ($N = 12$) comprised 6 freeze dried baitfish, homogenized together, for a total of 72 individuals of *Sardinella gibbosa*. Then for each sample, an aliquot (approx. 200 mg) was extracted with 50 mL of 1:1 *v*/v acetone: hexane mixture (pesticide grade, Sigma Aldrich, USA) for 2 h in a Soxhlet apparatus. After concentrating the extract by a rotary evaporator, the solution was digested with 5 mL of concentrated H_2SO_4 (98 %, Carlo Erba, Italy). A purification step using a Florisil column was performed before reducing the extract volume to 0.5 mL under a nitrogen stream.

Organochloride compound concentrations were determined by Gas Chromatography – Mass Spectrometry (GCMS) employing a ISQ 7610 GCMS from Thermo Scientific. Four μL of samples/standards were injected by an AI 1610 autosampler. Separation was performed on a TG-5MS GC column (30 m length, 0.25 mm internal diameter, 0.25 μm film thickness) using the following temperature program: 100 to 120 °C: 20 °C/min; 120 to 320 °C: 10 °C/min; hold at 320 °C for 10 min. Organochloride standards and isotopically labelled standards were purchased from Ultra Scientific Italia. In particular, multistandards for DDT and its degradation products (p,p'-DDT, -DDE, -DDD and o.p'-DDT, -DDE, -DDD) and for PCBs (32 congeners: 18, 28, 31, 44, 52, 77, 81, 95, 99, 101, 105, 110, 114, 118, 123, 126, 128, 138, 146, 149, 151, 153, 156, 157, 167, 169, 170, 177, 180, 183, 187, 189) diluted in isooctane were used for instrument calibration. Isotopically labelled standards (p, p'-DDT D8 and PCB 13C12 labelled congeners 28, 52, 101, 118, 138, 153, and 180) were employed as internal standards. Analytical procedures included analysis of blanks and the standard reference material SRM 1946 - Lake Superior Fish Tissue from the US National Institute of Standards and Technology (NIST), which is certified for DDT and PCB concentrations.

2.3. Analyses of trace elements

Baitfish samples are weighed (approx. 15 mg) and placed into labelled quartz tubes. Each tube receives 0.5 mL of HCl and 0.5 mL of HNO_3. After sealing, three tubes are placed into each microwave vessel (see Spanu et al., 2020) for a detailed description of the procedure). The vessels are positioned in the microwave carousel, and the digestion process is carried out heating up to 150 °C during a 50-min cycle. The tube contents are transferred into pre-weighed HDPE bottles, added with Ge and Rh as internal standards, and brought to a final volume of 15 mL. Analyte determination was performed by an iCAP-Q Inductively Coupled Plasma – Mass Spectrometer from Thermo Scientific calibrating by a multielemental standard (Sigma-Aldrich, multielement standard solution 5 for ICP).

Clean techniques were applied throughout including operating under laminar flow hoods, thorough decontamination of quartz tubes and HDPE bottles, and the use of ultrapure acids obtained by sub-boiling distillation (Monticelli et al., 2019). Quality control was assured by randomly analyzing the certified reference material ERM-BB422 fish muscle, which is certified for trace element concentrations. Analysis of eight replicate aliquots of this material yielded a median recovery of 101 %, with individual recoveries ranging from 94 % to 116 % for the eight elements with certified values.

2.4. Exposure risk assessment for humans

The human health risks of DDT and PCB from consuming *Sardinella gibbosa* were assessed separately for cancer and non-cancer health risks. For non-cancerous risks, the Hazard Ratio (HR) was determined by comparing the estimated daily intake (EDI) with the recommended USEPA reference dose (RfD) as follows:

$$EDI = \frac{C \times DR}{BW} \text{ and } HR = \frac{EDI}{RfD}$$

where: C represents the concentration of organochlorines expressed in ng g^{-1} ww; DR is the estimated daily fish consumption rate (Madagascar's per capita fish consumption of 4 kg per year, according to FAO (2023), and BW is the average adult body weight (70 kg). The RfD values are 500 ng kg^{-1} day^{-1} for DDT (specifically for p,p'DDT; USEPA, 1999a) and 20 ng kg^{-1} day^{-1} for PCB (specifically for Aroclor 1254; USEPA, 1999b).

To evaluate the potential carcinogenic risks from consuming *Sardinella gibbosa*, both cancer risk (CR) and the hazard ratio were calculated following USEPA guidelines. The cancer risk related to OCs was estimated using the following formula:

$$CR = EDI \times CSF$$

where CSF is the cancer slope factor, set at 0.34 and 2 mg kg^{-1} day^{-1} for

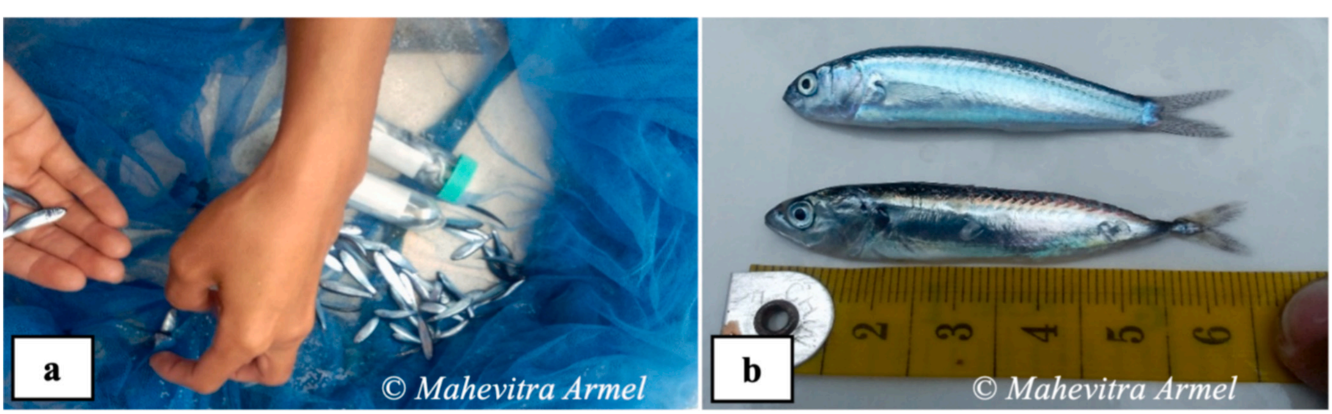

Fig. 2. a: Capture of *Sardinella gibbosa* using a small-mesh gillnet; b: Morphological data recording of *Sardinella gibbosa* in Madagascar.

DDT and PCB, respectively (USEPA, 1999a; USEPA, 1999b). The results are interpreted as follows: a risk of $<10^{-6}$ is deemed acceptable, between 10^{-6} and 10^{-4} is an area of concern, and a risk $>10^{-4}$ is considered unacceptable (USEPA, 2005).

With regards to trace elements, the health risks derived from consuming *Sardinella gibbosa* were calculated using the Target Hazard Quotient (THQ), which quantifies the non-cancer risk, was calculated using the following equations (Chien et al., 2002):

$$THQ = \frac{Ef \ x \ Ed \ x \ FIR \ x \ C}{Wab \ x \ TA \ x \ RfD} \ x \ 10^{-3}$$

where: Ef is the exposure frequency (365 days/year); Ed is the exposure duration (70 years, equivalent to average lifetime); FIR is the fish ingestion rate; C is the concentration of the metal in the food (mg kg^{-1} ww), RfD is the reference dose, Wab is the average body weight (70 kg for an adult), and TA is the averaging exposure time for non-carcinogens (365 days/year × years of exposure).

The carcinogenic risk (CR) was calculated by multiplying the oral carcinogenic potency slope factor (CSF), established by USEPA (0.5 mg kg^{-1} for Cr; 1.7 mg kg^{-1} for Ni; 1.5 mg kg^{-1} for As; 6.3 mg kg^{-1} for Cd; and 0.0085 mg kg^{-1} for Pb), by the EDI. The risk was estimated as the increased probability of an individual developing cancer over a lifetime due to exposure to a potential carcinogen (USEPA, 1989). A risk level $<10^{-6}$ is considered acceptable, between 10^{-6} and 10^{-4} is a concern, and risks $>10^{-4}$ are considered unacceptable (USEPA, 2005). The cancer risk equation used is as follows:

$$CR = CSF \ x \ EDI$$

Data were converted from dry weight (dw) to wet weight (ww) using the wet to dry weight ratio reported in Boldrocchi et al. (2021b) to compare contaminants concentrations found in baitfish with a) the maximum allowable limits for foodstuffs (MAL), b) the European Biota Quality Standards (EQS_{biota}) and c) to estimate the input of contaminants through the ingestion of baitfish.

3. Results and discussion

3.1. Organic and elemental contaminants in baitfish

Among the 32 analyzed PCB congeners, 21 were detected, including PCB 18, 28 + 31, 44, 52, 77, 101, 110, 114, 123 + 118, 126, 128, 138, 149, 153, 156, 157, 167, 169 and 170. The mean concentration of the $sumPCB_{21}$ in baitfish samples from Nosy Be was 57.3 ± 30.2 ng g^{-1} dw, ranging from a minimum of 18.9 ng g^{-1} dw to a maximum of 113 ng g^{-1} dw. The PCB patterns were dominated by higher chlorinated congeners, with Hexa-CBs accounting for an average of 43.7 % of all congener groups, followed by Penta at 20.3 %. Neither Octa-CBs nor Deca-CBs were detected. Tri-CBs were nearly ubiquitous, with PCB 28 + 31 detected in all samples and PCB 18 in 92 % of them. Among the individual PCB congeners, PCB 77, 123(+118), 126 and 169 exhibited the highest concentrations (Fig. 3).

Among the detected PCB congeners, several are of particular toxicological relevance due to their "dioxin-like" properties. These include PCB 77, 126, 169. DL-PCBs are structurally similar to dioxins and furans and exert their toxicity, leading to a range of adverse health effects such as reproductive, developmental, neurodevelopmental, and immunotoxic impacts, as well as carcinogenicity. In particular, PCB 77, 126, and 169 are considered among the most potent DL-PCBs due to their coplanar structure and contribute significantly to the overall toxicity of PCB mixtures (Giesy and Kannan, 1998; Safe, 1994; Van den Berg et al., 2006).

Hexachlorobiphenyls have been previously reported as the dominant congeners in fish. Storelli (2008) analyzed 18 fish species from the Mediterranean Sea and found that Hexa-CBs, along with Penta-CBs, accounted for 70 % to 91 % of the detected congeners. Similarly, Xie et al. (2023) analyzed 22 seafood species from the South China Sea, including round sardinella, and reported Hexa-CB as the predominant PCB congeners. The congeners 149, 153, and 138 were also predominant in round sardinella from the Mediterranean Sea (Vizzini et al., 2010). Similarly, Bartalini et al. (2020) reported a PCB profile in sardines and anchovies dominated by Penta and Hexa-CB. The predominance of these congeners is attributed to their higher chlorination levels and structural characteristics, which makes both groups more resistant to metabolic breakdown, and, as a result, more likely to bioaccumulate in biota compared to the less-chlorinated congeners (Xie et al., 2021, 2023).

The dominance of Hexa-CBs and Penta-CBs in *Sardinella gibbosa* is noteworthy. While their high prevalence is partly due to their persistence and bioaccumulation potential, these congener groups also include compounds with significant toxicological implications. Beyond the dioxin-like effects, certain non-dioxin-like Hexa- and Penta-CBs can

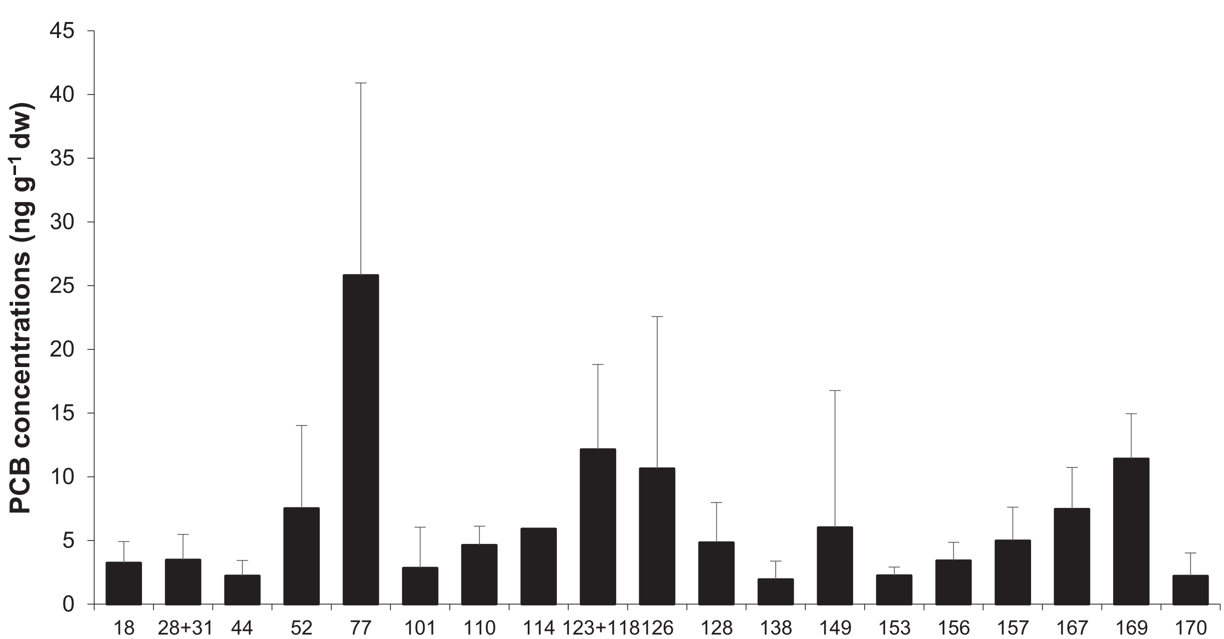

Fig. 3. Accumulation profiles of PCB in *Sardinella gibbosa* ($N = 12$ pool, 72 individual fish). Error bars represent ±1 standard deviation.

also contribute to toxicity, inducing neurotoxic effects, particularly impacting developing organisms (Carpenter, 2006; Bhavsar et al., 2007; Faroon and Ruiz, 2016). The overall toxicological profile of PCBs is complex, with varying effects depending on the specific congener, dose, and duration of exposure, highlighting the potential risks posed by the detected concentrations in *Sardinella gibbosa* to both marine organisms and human consumers.

Total PCB levels of selected congeners in *Sardinella gibbosa* caught in Madagascar were generally comparable to, or slightly higher than, those reported for *Sardinella sindensis* from Pakistan (Munshi et al., 2005). In Senegal, Net et al. (2015) reported $sumPCB_{28}$ levels in *Sardinella aurita* at 10 ± 20 ng g^{-1} dw, which is considerably lower than the mean concentration of the $sumPCB_{21}$ found in baitfish samples in this study (57.3 ± 30.2 ng g^{-1} dw). In Brazil, *Sardinella brasiliensis* accumulates PCB levels much lower than those in *Sardinella gibbosa* caught from Madagascar (<0.38 ng g^{-1} dw for the $sumPCB_{48}$; Santos et al., 2020).

DDT and its degradation products were detected in all samples, except one, with a mean concentration of 11.4 ± 7.9 ng g^{-1} dw in *Sardinella gibbosa*. Among its isomers, p,p'-DDD showed the highest concentration of 9.0 ± 4.6 ng g^{-1} dw, followed by o,p'-DDD and p,p'-DDE with a mean concentration of 5.8 ± 3.9 ng g^{-1} dw and 1.5 ± 0.9 ng g^{-1} dw, respectively.

Once released into the environment, DDT degrades into its more stable and persistent metabolites DDE and DDD. The ratio of p,p'-DDT to p,p'-DDE is widely used to assess recent inputs of DDT into the environment. In this study, the absence of detectable p,p'-DDT, coupled with the presence of p,p'-DDE, suggests that no recent DDT contamination has occurred in the region. Indeed, last time Madagascar was permitted to use DDT for disease vector control was 20 years ago, when DDT was replaced by pyrethroid insecticides (Ratovonjato et al., 2014).

Comparing DDT levels in *Sardinella gibbosa* from Madagascar with other locations of the world, it appears that levels measured in this study were lower than those reported in *Sardinella aurita* from Ghana (Nyarko et al., 2011). For instance, pp'-DDE was reported in concentration of 131.3, 79.7 and 21 ng g^{-1} ww in three different areas (Nyarko et al., 2011), which are higher than the 1.5 ± 0.9 ng g^{-1} ww reported for pp'-DDT in this study. Mean DDT level in this study (3.4 ± 2.4 ng g^{-1} ww) is more in line with those reported in *Sardinella brasiliensis* from Brazil (2.02 ± 4.53 ng g^{-1} ww; Ferreira et al., 2020) were consistent with the total DDT levels reported in this study.

With regards to trace elements, zooplankton showed high levels of Al, Sr and Zn and Fe (Table 1), followed by other TEs, such as Cu, Mn, As, Ni, Se. Non-essential TEs, including Cd, Co, Cr, Pb were measured at lower levels, ranging from a maximum of 1.52 ± 1.41 mg kg^{-1} for Cd to a minimum of 0.3 ± 0.4 mg kg^{-1} for Co (Table 1). Mercury exhibited the lowest concentrations among all TEs, with a mean of 0.08 ± 0.03 mg kg^{-1} (Table 1).

Table 1
Descriptive statistics of trace elements concentrations (mg kg^{-1} dw) in *Sardinella gibbosa* (*N* = 12 pool, 72 individual fish).

Trace Element	Media	Minimum-maximum
Sr	653 ± 948	110–3314
Al	234 ± 452	6.28–1244
Zn	174 ± 133	72.7–574
Fe	51.9 ± 34.7	2.53–66
Cu	15.2 ± 22.1	2.53–66
Mn	9.32 ± 8.2	3.11–32.4
As	9.02 ± 8.66	3.45–36.5
Se	4.17 ± 2.77	1.03–12.7
Ni	3.20 ± 4.73	0.37–15.3
V	1.95 ± 1.30	0.06–3.04
Cd	1.52 ± 1.41	0.39–5.36
Cr	0.95 ± 1.91	0.05–6.59
Pb	0.84 ± 1.43	0.11–4.78
Co	0.30 ± 0.42	0.05–1.27
Hg	0.08 ± 0.03	0.02–0.12

Sardinella gibbosa from Madagascar appeared to bioaccumulate higher concentrations of certain elements than *Sardinella albella* from the South China Sea (Yang et al., 2020). For instance, the mean concentrations of As, Cd and Pb in *S. gibbosa* were 9 ± 8.7 mg kg^{-1}, 1.5 ± 1.4 mg kg^{-1} and 0.8 ± 1.4 mg kg^{-1}, respectively, while concentrations of 1.82 mg kg^{-1}, 0.03 mg kg^{-1} and 0.55 mg kg^{-1} were reported in *S. albella* (Yang et al., 2020). The only exception was Hg which was found in lower concentrations in *S. gibbosa* compared to *S. albella* (Yang et al., 2020). Similarly, TE levels measured in *S. gibbosa* from Pakistan (Ahmed et al., 2015) were generally lower than those observed in Madagascar, with Cd, Ni and Zn median concentrations of 0.65 mg kg^{-1}, 0.6 mg kg^{-1} and 6.6 mg kg^{-1} (Ahmed et al., 2015) compared to 1 mg kg^{-1}, 0.99 mg kg^{-1}, and 123.6 mg kg^{-1} in *S. gibbosa* from Madagascar. When converted to a wet weight basis, the levels measured in Madagascar were comparable to those reported for *S. brasiliensis* from the Southwestern Atlantic for Hg, Ni, Cr, and As (Bauer et al., 2023).

3.2. Whale shark and human exposure to contaminants

To assess potential contaminant risks for whale sharks, and more generally for ecosystems and human health, the concentrations of pollutants measured in baitfish can be compared to the Environmental Quality Standards for biota (EQS_{biota}). These standards have been set for priority substances that pose a global threat, particularly to safeguard the food web from contamination and reduce the risks of adverse effects on ecosystems and human health given their biomagnification potential. Among the analyzed pollutants, EQS_{biota} have been defined for Hg (0.02 mg kg^{-1} ww, EU Directive 2013/39/EC) and DDT (100 μg kg^{-1} ww, Italian Decree 172/2015). Comparison showed that, while mean DDT levels (3.4 ± 2.4 ng g^{-1} ww) were below the corresponding EQS_{biota} limit for all samples, the mean levels of Hg in baitfish, 0.023 ± 0.01 mg kg^{-1} ww, exceeded the EQS_{biota} limit. Specifically, 57 % of the samples exceeding the threshold, underlying that a significant proportion of the baitfish is contaminated with Hg levels above the acceptable limit. This is concerning, as Hg is a toxic pollutant known for its ability to bioaccumulate and biomagnify in the food web, potentially leading to harmful concentrations in organisms at higher trophic levels, including humans. Mercury is well-documented for its neurotoxic effects on fish (Carvalho et al., 2023; Pereira et al., 2016; Puga et al., 2016; Zheng et al., 2019), which could have significant implications for whale shark health, given that these animals may consume large quantities of baitfish daily and the biomagnification of Hg could lead to harmful concentrations.

While the daily food intake in whale sharks has been estimated using zooplankton organisms as main prey target (e.g. Motta et al., 2010), baitfish consumption remains unquantified, despite evidence from multiple studies indicating that whale sharks feed on a broad range of prey beyond pelagic zooplankton (e.g., Boldrocchi and Bettinetti, 2019; Borrell et al., 2011; Lester et al., 2022; Marcus et al., 2016; Montero-Quintana et al., 2021; Rohner et al., 2013).

Marsili et al. (2023) estimated that in Madagascar, the pollutants uptake (DDT + PCB + HCB) through the solely ingestion of zooplankton corresponds to 7555 ng/day. However, based on the authors' energetic demand estimates (101.23 kJ/day) and consistent observations from research teams over seven years of fieldwork, which highlight a close relationship between baitball at the surface and whale shark presence (Fig. 4; Diamant, personal communication), it is clear that whale sharks cannot obtain the necessary energy for survival from plankton alone. Indeed, Motta et al. (2010) estimated that a 6-m whale shark requires a daily food intake of approximately 30,000 kJ/day. Using this value and considering the energy content of a *Sardinella* spp. (~ 5 kJ g^{-1}; Albo-Puigserver et al., 2017; Navarro, 2014), a 6-m whale shark would need to consume around 6 kg of baitfish per day. Through the ingestion of this amount, the daily input of organic contaminants (PCB + DDT ww) via baitfish would be 0.124 mg/day (calculated as 20.6 ng g^{-1} ww * 6000 g/day), which is substantially higher than the intake from zooplankton

Fig. 4. Whale shark specimen feeding at surface on baitfish in Madagascar during September–December 2024 (Photo Credit: Stella Diamant - Madagascar Whale Shark Project).

alone. Assuming that whale sharks feed solely on zooplankton nor on baitfish in Madagascar is not realistic, as its diet would likely include both preys. Still, results from this study clearly demonstrate that relying solely on zooplankton as a prey source underestimates the actual pollutant exposure, suggesting the need for further investigation into the full diet of whale sharks and the associated uptake when considering pollutant uptake assessments.

As regards the possible risks posed to human and environmental health, results were compared with the maximum allowable limit (MALs) established for foodstuffs consumption for both trace elements (Table 2) and organic contaminants. MALs for the former were set by the EU and FAO (European Commission, 2006; FAO, 1983) as follows: Cr (0.1 mg kg^{-1}), Cu (30 mg kg^{-1}), Zn (30 mg kg^{-1}), As (3.5 mg kg^{-1}), Cd (0.05 mg kg^{-1}), Pb (0.3 mg kg^{-1}) and Hg (1 mg kg^{-1}). MALs were exceeded for Cd in all baitfish samples, whose minimum level was 0.12 mg kg^{-1}, in 70 % for Zn, 21 % for Pb and Cr, and 14 % for As (Table 2); figures lower than MALs were measured for Cu and Hg (Table 2). With regards to OCs, the MALs established for foodstuff for DDT and PCBs (sum of the six congeners: 28, 52, 101, 138, 153 and 180) are 50 ng g^{-1} and 75 ng g^{-1} ww, respectively, while the U.S. EPA has set a strict DDT limit of 14.4 ng g^{-1} ww. Baitfish samples collected in Madagascar did not exceed either of these limits.

The hazard ratios were < 1 for non-carcinogenic risk for all analyzed baitfish considering the food consumption rate in Madagascar (11 g per capita per day), but also when considering the global per capita annual fish consumption, which was 20.7 kg in 2022, according to FAO (Table 3). Indeed, *Sardinella gibbosa* is a highly commercialized species,

Table 2
Descriptive statistics of trace elements concentrations (mg kg^{-1} ww) in *Sardinella gibbosa* ($N = 12$ pool, 72 individual fish) and comparison with maximum allowable limits for foodstuff (MAL).

Trace Element	MAL	Baitfish (mean ± sd) ppm ww	Min	Max	% samples exceeding MAL
Cr	0.1	0.28 ± 0.57	0.02	1.98	21
Cu	30	4.6 ± 6.6	0.76	19.80	0
Zn	30	52.3 ± 39.9	21.80	172.06	71
As	3.5	2.7 ± 2.6	1.04	10.94	14
Cd	0.05	0.46 ± 0.42	0.12	1.61	100
Pb	0.3	0.25 ± 0.43	0.03	1.43	21
Hg	1	0.02 ± 0.01	0.01	0.04	0

Table 3
The Hazard Ratio (HR) for non-carcinogenic risk and the carcinogenic risk (CR) for *Sardinella gibbosa* caught in Madagascar.

OCs	Food Consumption	Tissue	HR	CR
DDT	Madagascar	whole fish	0.001	1.8×10^{-7}
DDT	Global	whole fish	0.01	9.4×10^{-7}
PCB	Madagascar	whole fish	0.14	5.4×10^{-6}
PCB	Global	whole fish	0.70	2.8×10^{-5}

and global per capita consumption was also considered. The cancer risk for DDT was $<10^{-6}$, based on both local and global consumption estimates, which is considered acceptable. However, for PCB, the risk ranged between 10^{-6} and 10^{-4} using both local and global consumption estimates, which indicates a level of concern regarding daily exposure to PCB through baitfish consumption (Table 3).

With regards to trace elements, the THQ value was less <1 for all TEs, considering both local and global per capita fish consumption, and thus, people regularly consuming baitfish should not experience any non-carcinogenic adverse health hazard from TE exposure. However, the carcinogenic risks using local per capita fish consumption for Cr and inorganic As (considering two scenarios of 3 % and 10 % of total As (Rahman et al., 2012; Copat et al., 2013) were within 10^{-6} and 10^{-4}, suggesting some level of concern (Table 4). Regarding Ni and Cd, the CRs were even higher than 10^{-4}, which highlights an area of risk (Table 4) (USEPA, 2005). This is even more evident when considering a global per capita fish consumption of 56.7 g per day. In this scenario, only Pb and inorganic As (3 % of total As) were between within 10^{-6} and 10^{-4}, all others including Cr, Ni, Cd and inorganic As (considering 10 % of total As) exceeded 10^{-4}, which are deemed unacceptable (Table 4) (USEPA, 2005).

However, it important to underline that these results represent a scenario in which it has assumed a daily general fish consumption rate of 11 g (in Madagascar) and ~ 57 g (globally) applied to solely *Sardinella gibbosa*, as the only fish source, and assuming an exposure frequency of 365 days per year. Moreover, as showed in this study, a great variability exists in the bioaccumulation of pollutants in *Sardinella* spp. among different locations and species, thus generalization should be avoided.

Nonetheless, the study raised concerns about the daily exposure to PCB, and selected TEs through baitfish consumption at local level, urging further research. Even though the average daily intake is approximately 11 g in Madagascar, the amount of baitfish consumed in a single meal may be higher. This results in temporary exposure to levels that exceed safe limits after a meal. Furthermore, the consumption of fish typically involves a combination of all the analyzed elements, suggesting that regular consumption of these species could pose potential health risks.

These findings align with the status of contamination from Madagascar, where chemical and bacterial pollution originating from industrial and domestic sources represents a significant public health concern (Cooke et al., 2000; Ahamada et al., 2002; Ahamada et al., 2008), and the use of inadequately treated wastewater, which contains high levels pollutants like Cr, contaminates both surface and groundwater systems (Hervé et al., 2010; Ramahazomanana et al., 2023). Pesticide runoff from intensive agricultural practices further impacts coastal ecosystems (Cooke et al., 2000), and extensive Co and Ni mining leads to drinking water contamination exceeding WHO guidelines (Emerman, 2019). Furthermore, aquatic sediment samples in these areas exhibit elevated concentrations of Ni, Cd, and zinc Zn, as mining activities contribute to the accumulation of these heavy metals in river sediments, which are then transported downstream (e.g., Duncan et al., 2018). The absence of sewage treatment leads to the direct discharge of waste, including solid waste, into marine environments (Ahamada et al., 2002; Ahamada et al., 2008). Notably, in Nosy Be, concentrations of various contaminants in coastal sediments are higher than those observed in other parts of Africa, primarily attributed to seaport operations and the discharge of untreated sewage (Hervé et al., 2010).

Table 4
The target hazard quotient (THQ) and the carcinogenic risk (CR) values calculated for each trace element (ppm ww) analyzed in *Sardinella gibbosa* caught in Madagascar.

Trace element	Food Consumption	Mean Concentration	THQ	CR
Hg	Madagascar	0.023	0.012	
V	Madagascar	0.585	0.018	
Co	Madagascar	0.090	0.047	
Cr	Madagascar	0.284	0.015	2.23×10^{-5}
Mn	Madagascar	2.80	0.003	
Ni	Madagascar	0.960	0.008	2.6×10^{-4}
Fe	Madagascar	15.6	0.003	
Zn	Madagascar	52.3	0.027	
Cu	Madagascar	4.56	0.018	
Se	Madagascar	1.25	0.039	
Pb	Madagascar	0.252	0.010	3.4×10^{-7}
Cd	Madagascar	0.457	0.072	4.5×10^{-4}
			0.043*	1.9×10^{-5}*
As	Madagascar	0.08* (0.27)	(0.142)	(6.4×10^{-5})
Hg	Global	0.02	0.06	
V	Global	0.59	0.09	
Co	Global	0.09	0.24	
Cr	Global	0.28	0.08	1.2×10^{-4}
Mn	Global	2.80	0.02	
Ni	Global	0.96	0.04	1.3×10^{-3}
Fe	Global	15.6	0.02	
Zn	Global	52.2	0.14	
Cu	Global	4.56	0.09	
Se	Global	1.25	0.20	
Pb	Global	0.25	0.05	1.7×10^{-6}
Cd	Global	0.46	0.37	2.3×10^{-3}
			0.22*	9.9×10^{-5}*
As	Global	0.08* (0.27)	(0.73)	(3.3×10^{-4})

* Numbers were calculated considering 3 % and10% (in brackets) inorganic As.

4. Conclusion

This study provides important insights into the exposure of marine organisms, particularly whale sharks, but also humans, to chemical pollutants within Madagascar's coastal ecosystems. Our findings indicate that some pollutants, specifically PCB, Cd, Ni and Hg are present in high concentration in *Sardinella gibbosa*, posing potential risks to both whale sharks and human consumers.

This study also reveals that the uptake of pollutants in whale sharks via baitfish consumption is significantly higher than previously reported for the solely zooplankton. This indicates the need of considering a broader range of prey, beyond just zooplankton, when estimating the potential exposure risk for whale sharks. Furthermore, the elevated concentrations of certain pollutants in *Sardinella gibbosa* raise concerns regarding local human consumption, as increased intake could exacerbate both non-carcinogenic and carcinogenic health risks. This research emphasizes the urgent need for continued monitoring of chemical pollution, especially in coastal ecosystems like those off Madagascar, where the convergence of industrial, agricultural, and mining activities poses a severe threat. The study reinforces the critical relevance of coastal ecosystems, which are vital hotspots for global marine biodiversity, and highlights the imperative for targeted conservation and management strategies to protect these invaluable environments and the species that rely on them. The ecological and economic significance of *Sardinella gibbosa* in the region further necessitates comprehensive research to assess the full extent of chemical pollution and its effects on marine biodiversity and human health. In this regard, *Sardinella gibbosa* serves as a valuable environmental sentinel for detecting persistent anthropogenic compounds in the marine environment.

CRediT authorship contribution statement

S. Diamant: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **A. Mahevitra:** Writing – original draft, Visualization, Investigation. **D. Monticelli:** Writing – review & editing, Validation, Methodology, Formal analysis. **D. Banfi:** Writing – review & editing, Methodology, Investigation, Formal analysis. **B. Villa:** Writing – original draft, Visualization, Methodology, Formal analysis. **A.R. López:** Writing – review & editing, Methodology, Formal analysis. **R. Bettinetti:** Writing – review & editing, Supervision, Resources. **G. Boldrocchi:**

Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was carried out under the permit:N°363/24/MEDD/SG/DGGE/DAPRNE/SCBE.Re

S. Diamant thanks the Institut Halieutique et des Sciences Marines (IH.SM) for their support, as well as the Ministry of Environment and Sustainable Development for issuing the research permit. Alejandro R. López acknowledges the support of the Italian inter-university PhD course in Sustainable Development and Climate Change (www.phd-sdc.it). The authors wish to acknowledge the invaluable assistance of Ms. Rossella Perna (University of Insubria), and all the contributing students from University of Insubria for providing support during laboratory analyses.

Data availability

Data will be made available on request.

References

Ahamada, S., Bigot, L., Bijoux, J., Maharavo, J., Meunier, S., Moyne-Picard, M., Paupiah, N., 2002. Status of coral reef in the south west Indian ocean island node: Comoros, Madagascar, Reunion and Seychelles. In: Status of Coral Reef of the Word, pp. 79–100.

Ahamada, S., Bigot, L., Cauvin, B., Hagan, A., Harris, A., Koonjul, M., Meunier, S., Quod, J.P., 2008. Status of the Coral Reefs of the SWIO States: Comoros, Madagascar, Mauritius, Reunion, Seychelles, pp. 73–86.

Ahmed, Q., Benzer, S., Elahi, N., Ali, Q.M., 2015. Concentrations of heavy metals in *Sardinella gibbosa* (Bleeker, 1849) from the coast of Balochistan, Pakistan. Bull. Environ. Contam. Toxicol. 95 (2), 221–225. https://doi.org/10.1007/s00128-015-1572-y.

Albo-Puigserver, M., Muñoz, A., Navarro, J., Coll, M., Pethybridge, H., Sánchez, S., Palomera, I., 2017. Ecological energetics of forage fish from the Mediterranean Sea: seasonal dynamics and interspecific differences. Deep-Sea Research Part II: Topical Studies in Oceanography 140 (March), 74–82. https://doi.org/10.1016/j.dsr2.2017.03.002.

Bartalini, A., Muñoz-Arnanz, J., Baini, M., Panti, C., Galli, M., Giani, D., Fossi, M.C., Jiménez, B., 2020. Relevance of current PCB concentrations in edible fish species from the Mediterranean Sea. Sci. Total Environ. 737, 139520. https://doi.org/10.1016/j.scitotenv.2020.139520.

Bauer, A.B., de Rezende, C.E., Weigert, S., Fischer, L.G., Almeida, M.G., Arueira, T.D., Molisani, M.M., 2023. Assessment of human health risk of metal(loid) content in Brazilian sardine along the southwestern Atlantic. Bull. Environ. Contam. Toxicol. 110 (5), 1–7. https://doi.org/10.1007/s00128-023-03723-7.

Bhavsar, S.P., Fletcher, R., Hayton, A., Reiner, E.J., Jackson, D.A., 2007. Composition of dioxin-like PCBs in fish: an application for risk assessment. Environ. Sci. Technol. 41 (9), 3096–3102.

Boldrocchi, G., Bettinetti, R., 2019. Whale shark foraging on baitfish off Djibouti. Mar. Biodivers. 49 (4). https://doi.org/10.1007/s12526-018-00934-8.

Boldrocchi, G., Moussa Omar, Y., Rowat, D., Bettinetti, R., 2018. First results on zooplankton community composition and contamination by some persistent organic pollutants in the Gulf of Tadjoura (Djibouti). Sci. Total Environ. 627, 812–821. https://doi.org/10.1016/j.scitotenv.2018.01.286.

Boldrocchi, G., Monticelli, D., Omar, Y.M., Bettinetti, R., 2019. Trace elements and POPs in two commercial shark species from Djibouti: implications for human exposure. Sci. Total Environ. 669, 637–648.

Boldrocchi, G., Omar, M., Azzola, A., Bettinetti, R., 2020a. The ecology of the whale shark in Djibouti. Aquat. Ecol. 54 (2). https://doi.org/10.1007/s10452-020-09758-w.

Boldrocchi, G., Monticelli, D., Butti, L., Omar, M., Bettinetti, R., 2020b. First concurrent assessment of elemental- and organic-contaminant loads in skin biopsies of whale sharks from Djibouti. Sci. Total Environ. 722, 137841. https://doi.org/10.1016/j.scitotenv.2020.137841.

Boldrocchi, G., Spanu, D., Mazzoni, M., Omar, M., Baneschi, I., Boschi, C., Zinzula, L., Bettinetti, R., Monticelli, D., 2021a. Bioaccumulation and biomagnification in elasmobranchs: a concurrent assessment of trophic transfer of trace elements in 12 species from the Indian Ocean. Mar. Pollut. Bull. 172 (June), 112853. https://doi.org/10.1016/j.marpolbul.2021.112853.

Boldrocchi, G., Monticelli, D., Mazzoni, M., Spanu, D., Bettinetti, R., 2021b. Accumulation of selected trace elements in shads from three lakes: first insights from Italian pre-alpine area. Biol. Trace Elem. Res. 1–6. https://doi.org/10.1007/s12011-021-02577-6.

Boldrocchi, G., Spanu, D., Polesello, S., Valsecchi, S., Garibaldi, F., Lanteri, L., Bettinetti, R., 2022. Legacy and emerging contaminants in the endangered filter feeder basking shark *Cetorhinus maximus*. Mar. Pollut. Bull. 176, 113466.

Boldrocchi, G., Monticelli, D., Bettinetti, R., 2023. To what extent are filter feeder elasmobranchs exposed to marine pollution? A systematic review. Environ. Pollut. 318, 120881. https://doi.org/10.1016/j.envpol.2022.120881.

Borrell, A., Cardona, L., Kumarran, R.P., Aguilar, A., 2011. Trophic ecology of elasmobranchs caught off Gujarat, India, as inferred from stable isotopes. ICES J. Mar. Sci. 68, 547–554.

Carpenter, D.O., 2006. Polychlorinated biphenyls (PCBs): routes of exposure and effects on human health. Rev. Environ. Health 21 (1), 1–24.

Carvalho, P.S., Fonseca-Rodrigues, D., Pacheco, M., Almeida, A., Pinto-Ribeiro, F., Pereira, P., 2023. Comparative neurotoxicity of dietary methylmercury and waterborne inorganic mercury in fish: evidence of optic tectum vulnerability through morphometric and histopathological assessments. Aquat. Toxicol. 261 (May). https://doi.org/10.1016/j.aquatox.2023.106557.

Chien, L.C., Hung, T.C., Choang, K.Y., Yeh, C.Y., Meng, P.J., Shieh, M.J., Han, B.C., 2002. Daily intake of TBT, Cu, Zn, Cd and As for fishermen in Taiwan. Sci. Total Environ. 285 (1–3), 177–185. https://doi.org/10.1016/S0048-9697(01)00916-0.

Cooke, A.J., Ratomahenina, O., Ranaivoson, E., Razafindrainibe, H., 2000. Madagascar. In: Pergamon Press (Elsevier Science) (Ed.), Seas at the Millenium: An Environmental Evaluation, pp. 113–131.

Copat, C., Arena, G., Fiore, M., Ledda, C., Fallico, R., Sciacca, S., Ferrante, M., 2013. Heavy metals concentrations in fish and shellfish from eastern Mediterranean Sea: consumption advisories. Food Chem. Toxicol. 53, 33–37.

Di Beneditto, A.P.M., Moreira, S.C., Siciliano, S., 2021. Endangered whale sharks in southeastern Brazil: records and management issues. Ocean Coast. Manag. 201 (September 2020). https://doi.org/10.1016/j.ocecoaman.2020.105491.

Diamant, S., Rohner, C.A., Kiszka, J.J., d'Echon, A.G., d'Echon, T.G., Sourisseau, E., Pierce, S.J., 2018. Movements and habitat use of satellite-tagged whale sharks offwestern Madagascar. Endanger. Species Res. 36, 49–58. https://doi.org/10.3354/esr00889.

Diamant, S., Pierce, S.J., Rohner, C.A., Graham, R.T., Guillemain d'Echon, A., Guillemain d'Echon, T., Sourisseau, E., Fidiarisandratra, L.C., Bakary, G., Trélanche, S., Andriananrisoa, F., Kiszka, J.J., 2021. Population structure, residency, and abundance of whale sharks in the coastal waters off Nosy Be, north-western Madagascar. Aquat. Conserv. Mar. Freshwat. Ecosyst. 31 (12), 3492–3506. https://doi.org/10.1002/aqc.3743.

Dulvy, N.K., Sadovy, Y., Reynolds, J.D., 2003. Extinction vulnerability in marine populations. Fish Fish. 4 (1), 25–64. https://doi.org/10.1046/j.1467-2979.2003.00105.x.

Dulvy, N.K., Fowler, S.L., Musick, J.A., Cavanagh, R.D., Kyne, P.M., Harrison, L.R., Carlson, J.K., Davidson, L.N., Fordham, S.V., Francis, M.P., Pollock, C.M., Simpfendorfer, C.A., Burgess, G.H., Carpenter, K.E., Compagno, L.J., Ebert, D.A., Gibson, C., Heupel, M.R., Livingstone, S.R., White, W.T., 2014. Extinction risk and conservation of the world's sharks and rays. ELife 3, 1–34. https://doi.org/10.7554/elife.00590.

Duncan, A.E., de Vries, N., Nyarko, K.B., 2018. Assessment of heavy metal pollution in the sediments of the river Pra and its tributaries. Water Air Soil Pollut. 229, 272. https://doi.org/10.1130/abs/2017se-290796.

Emerman, B.S.H., 2019. Impact on Regional Water Quality of the Rio Tinto QMM Ilmenite Mine, Southeastern.

European Commission, E., 2006. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Off. J. Eur. Union 364, 5–24.

FAO, 1983. Compilation of legal limits for hazardous substances in fish and fishery products. Fish Circular 464, 5–100.

FAO, 2018. The State of World Fisheries and Aquaculture 2018 - Meeting the Sustainable Development Goals.

FAO, 2023. Fishery and Aquaculture Statistics. Food balance sheets of fish and fishery products 1961–2019 (FishStatJ). www.fao.org/fishery/en/statistics/software/fishstatj.

Faroon, O., Ruiz, P., 2016. Polychlorinated biphenyls: new evidence from the last decade. Toxicol. Ind. Health 32 (11), 1825–1847.

Ferreira, V.B., Estrella, L.F., Alves, M.G.R., Gallistl, C., Vetter, W., Silva, T.T.C., Malm, O., Torres, J.P.M., Abadio Finco, F.D.B., 2020. Residues of legacy organochlorine pesticides and DDT metabolites in highly consumed fish from the polluted Guanabara Bay, Brazil: distribution and assessment of human health risk. Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes 55 (1), 30–41. https://doi.org/10.1080/03601234.2019.1654808.

Fontes, J., McGinty, N., Machete, M., Afonso, P., 2020. Whale shark-tuna associations, insights from a small pole-and-line fishery from the mid-north Atlantic. Fish. Res. 229, 105598.

Fossi, M.C., Baini, M., Panti, C., Galli, M., Jiménez, B., Muñoz-Arnanz, J., Ramírez-Macías, D., 2017. Are whale sharks exposed to persistent organic pollutants and plastic pollution in the Gulf of California (Mexico)? First ecotoxicological investigation using skin biopsies. Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol. 199, 48–58.

Fossi, M.C., Coppola, D., Baini, M., Giannetti, M., Guerranti, C., Marsili, L., Panti, C., de Sabata, E., Clò, S., 2014. Large filter feeding marine organisms as indicators of microplastic in the pelagic environment: the case studies of the Mediterranean basking shark (*Cetorhinus maximus*) and fin whale (*Balaenoptera physalus*). Mar. Environ. Res. 100 (2014), 17–24. https://doi.org/10.1016/j.marenvres.2014.02.002.

Gelsleichter, James, Walsh, C.J., Szabo, N.J., Rasmussen, L.E.L., 2006. Organochlorine concentrations, reproductive physiology, and immune function in unique populations of freshwater Atlantic stingrays (*Dasyatis sabina*) from Florida's St. Johns River. Chemosphere 63 (9), 1506–1522. https://doi.org/10.1016/j.chemosphere.2005.09.011.

García-Baciero, A., Rendón-von Osten, J., Pérez-Maturino, V., Ramírez-Macías, D., 2024. Whale shark Rhyncodon typus exposure to organochlorine pesticides in the Southern Gulf of California, Mexico. Mar. Pollut. Bull. 206, 116748.

Gelsleichter, J., Sparkman, G., Howey, L., Brooks, E., Shipley, O., 2020. Elevated accumulation of the toxic metal mercury in the critically endangered oceanic whitetip shark *Carcharhinus longimanus* from the northwestern Atlantic Ocean. Endanger. Species Res. 43, 267–279. https://doi.org/10.3354/esr01068.

Giesy, J.P., Kannan, K., 1998. Dioxin-like and non-dioxin-like toxic effects of polychlorinated biphenyls (PCBs): implications for risk assessment. Crit. Rev. Toxicol. 28 (6), 511–569.

Gleiss, A.C., Wright, S., Liebsch, N., Wilson, R.P., Norman, B., 2013. Contrasting diel patterns in vertical movement and locomotor activity of whale sharks at Ningaloo Reef. Mar. Biol. 160 (11), 2981–2992. https://doi.org/10.1007/s00227-013-2288-3.

Hervé, R.P., Andriamalala, R., Yves, M., Marcellin, R., Christine, R., Andriamandimbisoa, N., 2010. Assessment of heavy metals concentrations in coastal sediments in north-western cities of Madagascar. Afr. J. Environ. Sci. Technol. 4 (2), 51–060. http://www.academicjournals.org/AJEST.

Hunnam, K., 2021. The biology and ecology of tropical marine sardines and herrings in Indo-West Pacific fisheries: a review. Rev. Fish Biol. Fish. 31 (3). https://doi.org/10.1007/s11160-021-09649-9.

Jonahson, M., Harding, S., 2007. Occurrence of whale sharks (*Rhincodon typus*) in Madagascar. Fish. Res. 84 (1), 132–135. https://doi.org/10.1016/j.fishres.2006.11.023.

Kiszka, J., van der Elst, R., 2015. Elasmobranchs (sharks and rays): a review of status, distribution and interaction with fisheries of the Southwest Indian Ocean. In: Offshore Fisheries of the Southwest Indian Ocean, pp. 365–390.

Lester, E., Cannon, T., Lawrence, S., Wilton, J., Araujo, G., 2022. Whale sharks (*Rhincodon typus*) feed on baitfish with other predators at Ningaloo Reef. Pacific Conservation Biology 1–2. https://doi.org/10.1071/PC21033. March 2020.

Lotze, H.K., Lenihan, H.S., Bourque, B.J., Bradbury, R.H., Cooke, R.G., Kay, M.C., Kidwell, S.M., Kirby, M.X., Peterson, C.H., Jackson, J.B.C., Bay, M., 2006. Science 312 (June), 1806–1809. And coastal seas.

Marcus, L., Virtue, P., Pethybridge, H.R., Meekan, M.G., Thums, M., Nichols, P.D., 2016. Intraspecific variability in diet and implied foraging ranges of whale sharks at Ningaloo Reef, Western Australia, from signature fatty acid analysis. Mar. Ecol. Prog. Ser. 554 (July), 115–128. https://doi.org/10.3354/meps11807.

Marsili, L., Consales, G., Romano, P., Rosai, R., Bava, P., Reinero, F.R., Micarelli, P., 2023. A cocktail of plankton and organochlorines for whale shark in the foraging areas of Nosy Be (Madagascar). Diversity 15 (8), 1–16. https://doi.org/10.3390/d15080911.

Montero-Quintana, A.N., Ocampo-Valdez, C.F., Vázquez-Haikin, J.A., Sosa-Nishizaky, O., Osorio-Beristain, M., 2021. Whale shark (*Rhincodon typus*) predatory flexible feeding behaviors on schooling fish. J. Ethol. 39 (3), 399–410. https://doi.org/10.1007/s10164-021-00717-y.

Monticelli, D., Castelletti, A., Civati, D., Recchia, S., Dossi, C., 2019. How to efficiently produce ultrapure acids. International journal of analytical chemistry 2019 (1), 5180610.

Motta, P.J., Maslanka, M., Hueter, R.E., Davis, R.L., de la Parra, R., Mulvany, S.L., Habegger, M.L., Strother, J.A., Mara, K.R., Gardiner, J.M., Tyminski, J.P., Zeigler, L. D., 2010. Feeding anatomy, filter-feeding rate, and diet of whale sharks *Rhincodon typus* during surface ram filter feeding off the Yucatan peninsula, Mexico. Zoology 113 (4), 199–212. https://doi.org/10.1016/j.zool.2009.12.001.

Munshi, A.B., Hina, A.S., Usmani, T.H., 2005. Determination of the level of PCBs in small fishes from three different coastal areas of Karachi, Pakistan. Pak. J. Sci. Ind. Res. 48 (4), 247–251.

Navarro, A.M., 2014. Contenido energético de especies de peces pelágicos de pequeño y mediano tamaño: diferencias intra e interespecíficas y consideraciones metodológicas.

Net, S., Henry, F., Rabodonirina, S., Diop, M., Merhaby, D., Mahfouz, C., Amara, R., Ouddane, B., 2015. Accumulation of PAHs, Me-PAHs, PCBs and total mercury in sediments and marine species in coastal areas of Dakar, Senegal: contamination level and impact. Int. J. Environ. Res. 9 (2), 419–432.

Nyarko, E., Botwe, B., Bampoe, A., Addo, S., Armah, A., Ntow, W., Kelderman, P., 2011. Organochlorine pesticide residues in *Sardinella aurita* from the coastal waters of Accra-Tema, Ghana and their potential health risks. Journal of the Ghana Science Association 13 (1). https://doi.org/10.4314/jgsa.v13i1.69190.

Pancaldi, F., Páez-Osuna, F., Marmolejo-Rodríguez, A.J., Whitehead, D.A., González-Armas, R., Soto-Jiménez, M.F., Galván-Magaña, F., 2021. Variation of essential and non-essential trace elements in whale shark epidermis associated to two different feeding areas of the Gulf of California. Environ. Sci. Pollut. Res. 28 (27), 36803–36816.

Pancaldi, F., Páez-Osuna, F., Soto-Jiménez, M.F., González-Armas, R., O'Hara, T., Marmolejo-Rodríguez, A.J., Vázquez, A., Galván-Magaña, F., 2019. Trace elements in tissues of whale sharks (*Rhincodon typus*) stranded in the Gulf of California, Mexico. Bull. Environ. Contam. Toxicol., 0123456789 https://doi.org/10.1007/s00128-019-02640-y.

Pereira, P., Puga, S., Cardoso, V., Pinto-Ribeiro, F., Raimundo, J., Barata, M., Pousão-Ferreira, P., Pacheco, M., Almeida, A., 2016. Inorganic mercury accumulation in brain following waterborne exposure elicits a deficit on the number of brain cells and impairs swimming behavior in fish (white seabream-*Diplodus sargus*). Aquat. Toxicol. 170, 400–412. https://doi.org/10.1016/j.aquatox.2015.11.031.

Pierce, S., Norman, B., 2016. Rhincodon typus. The IUCN Red List of Threatened Species. https://doi.org/10.2305/IUCN.UK.2016-1.RLTS.T19488A2365291.en.

Pirotta, V., Grech, A., Jonsen, I.D., Laurance, W.F., Harcourt, R.G., 2019. Consequences of global shipping traffic for marine giants. Front. Ecol. Environ. 17 (1), 39–47. https://doi.org/10.1002/fee.1987.

Puga, S., Pereira, P., Pinto-Ribeiro, F., O'Driscoll, N.J., Mann, E., Barata, M., Pousão-Ferreira, P., Canário, J., Almeida, A., Pacheco, M., 2016. Unveiling the neurotoxicity of methylmercury in fish (*Diplodus sargus*) through a regional morphometric analysis of brain and swimming behavior assessment. Aquat. Toxicol. 180, 320–333. https://doi.org/10.1016/j.aquatox.2016.10.014.

Rahman, M.A., Hasegawa, H., Lim, R.P., 2012. Bioaccumulation, biotransformation and trophic transfer of arsenic in the aquatic food chain. Environ. Res. 116, 118–135.

Ramahazomanana, A., Rakotondraibe, J., Rajaona, A.R., Rabefihavanana, L., 2023. Evaluation of the impact of chemical pollution on the quality of the environment in rice fields in the commune of Ankadikely Ilafy, Antananarivo Madagascar. Eur. J. Environ. Earth Sci. 4 (1), 52–61. https://doi.org/10.24018/ejgeo.2023.4.1.324.

Ratovonjato, J., Randrianarivelojosia, M., Rakotondrainibe, M.E., Raharimanga, V., Andrianaivolambo, L., Le Goff, G., Robert, V., 2014. Entomological and parasitological impacts of indoor residual spraying with DDT, alphacypermethrin and deltamethrin in the western foothill area of Madagascar. Malar. J. 13, 1–18.

Reynolds, S.D., Norman, B.M., Franklin, C.E., Bach, S.S., Comezzi, F.G., Diamant, S., Jaidah, M.Y., Pierce, S.J., Richardson, A.J., Robinson, D.P., Rohner, C.A., Dwyer, R. G., 2022a. Regional variation in anthropogenic threats to Indian Ocean whale sharks. Glob. Ecol. Conserv. 33 (December 2021), e01961. https://doi.org/10.1016/j.gecco.2021.e01961.

Reynolds, S.D., Norman, B.M., Franklin, C.E., Bach, S.S., Comezzi, F.G., Diamant, S., Jaidah, M.Y., Pierce, S.J., Richardson, A.J., Robinson, D.P., Rohner, C.A., Dwyer, R. G., 2022b. Regional variation in anthropogenic threats to Indian Ocean whale sharks. Glob. Ecol. Conserv. 33 (August 2021), e01961. https://doi.org/10.1016/j.gecco.2021.e01961.

Robinson, D.P., Jaidah, M.Y., Jabado, R.W., Lee-Brooks, K., Nour El-Din, N.M., Malki, A. A.A., Elmeer, K., McCormick, P.A., Henderson, A.C., Pierce, S.J., Ormond, R.F.G., 2013. Whale sharks, Rhincodon typus, aggregate around offshore platforms in Qatari waters of the Arabian gulf to feed on fish spawn. PloS One 8 (3). https://doi.org/10.1371/journal.pone.0058255.

Rohner, C.A., Couturier, L.I.E., Richardson, A.J., Pierce, S.J., Prebble, C.E.M., Gibbons, M.J., Nichols, P.D., 2013. Diet of whale sharks *Rhincodon typus* inferred from stomach content and signature fatty acid analyses. Mar. Ecol. Prog. Ser. 493, 219–235. https://doi.org/10.3354/meps10500.

Rohner, C.A., Armstrong, A.J., Pierce, S.J., Prebble, C.E.M., Cagua, E.F., Cochran, J.E.M., Berumen, M.L., Richardson, A.J., 2015. Whale sharks target dense prey patches of sergestid shrimp off Tanzania. J. Plankton Res. 37 (2), 352–362. https://doi.org/10.1093/plankt/fbv010.

Rohner, C.A., Richardson, A.J., Jaine, F.R.A., Bennett, M.B., Weeks, S.J., Cliff, G., Robinson, D.P., Reeve-Arnold, K.E., Pierce, S.J., 2018. Satellite tagging highlights the importance of productive Mozambican coastal waters to the ecology and conservation of whale sharks. PeerJ 2018 (1). https://doi.org/10.7717/peerj.4161.

Rowat, D., Brooks, K.S., 2012. A review of the biology, fisheries and conservation of the whale shark *Rhincodon typus*. J. Fish Biol. 80 (5), 1019–1056. https://doi.org/10.1111/j.1095-8649.2012.03252.x.

Rowat, D., Meekan, M.G., Engelhardt, U., Pardigon, B., Vely, M., 2007. Aggregations of juvenile whale sharks (*Rhincodon typus*) in the Gulf of Tadjoura, Djibouti. Environ. Biol. Fishes 80 (4), 465–472. https://doi.org/10.1007/s10641-006-9148-7.

Rowat, D., Womersley, F.C., Norman, B.M., Pierce, S.J., 2021. Global threats to whale sharks. In: Whale Shark Biology, Ecology, and Conservation, pp. 239–265.

Safe, S.H., 1994. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. Crit. Rev. Toxicol. 24 (2), 87–149.

Santos, L.L., Miranda, D., Hatje, V., Albergaria-Barbosa, A.C.R., Leonel, J., 2020. PCBs occurrence in marine bivalves and fish from Todos os Santos Bay, Bahia, Brazil. Mar. Pollut. Bull. 154 (November 2019), 111070. https://doi.org/10.1016/j.marpolbul.2020.111070.

Sequeira, A.M.M., Mellin, C., Fordham, D.A., Meekan, M.G., Bradshaw, C.J.A., 2014. Predicting current and future global distributions of whale sharks. Glob. Chang. Biol. 20 (3), 778–789. https://doi.org/10.1111/gcb.12343.

Spanu, D., Butti, L., Boldrocchi, G., Bettinetti, R., Monticelli, D., 2020. High-throughput, multi-batch system for the efficient microwave digestion of biological samples. Anal. Sci. 36 (7), 889–892.

Stevens, J.D., 2007. Whale shark (*Rhincodon typus*) biology and ecology: a review of the primary literature. Fish. Res. 84 (1), 4–9. https://doi.org/10.1016/j.fishres.2006.11.008.

Tiktak, G.P., Butcher, D., Lawrence, P.J., Norrey, J., Bradley, L., Shaw, K., Preziosi, R., Megson, D., 2020. Are concentrations of pollutants in sharks, rays and skates (Elasmobranchii) a cause for concern? A systematic review. Mar. Pollut. Bull. 160 (September). https://doi.org/10.1016/j.marpolbul.2020.111701.

USEPA, 1989. Risk Assessment Guidance for superfund. Office of Emergency and Remedial Response, US Environmental Protection Agency.

USEPA, 1999a. Integrated Risk Information System (IRIS) on p,p-Dichlorodiphenyldichloroethylene. National Center for Environmental Assessment, Office of Research and Development, Washington, DC.

USEPA, 1999b. U.S. Environmental Protection Agency. Integrated Risk Information System (IRIS) on Aroclor 1254. National Center for Environmental Assessment, Office of Research and Development, Washington, DC.

USEPA, 2005. Guidelines for Carcinogen Risk Assessment. Risk Assessment Forum. US Environmental Protection Agency.

Van den Berg, M., Birnbaum, L.S., Denison, M., De Vito, M., Farland, W., Feeley, M., Peterson, R.E., 2006. The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol. Sci. 93 (2), 223–241.

Villagómez-Vélez, S.I., Noreña-Barroso, E., Galván-Magaña, F., González-Armas, R., Rodríguez-Fuentes, G., Marmolejo-Rodríguez, A.J., 2024. Persistent organic pollutants in whale shark (Rhincodon typus) skin biopsies from Bahía de Los Ángeles, Mexico. Bull. Environ. Contam. Toxicol. 112 (1), 18.

Vizzini, S., Tramati, C., Mazzola, A., 2010. Comparison of stable isotope composition and inorganic and organic contaminant levels in wild and farmed bluefin tuna, Thunnus thynnus, in the Mediterranean Sea. Chemosphere 78 (10), 1236–1243. https://doi.org/10.1016/j.chemosphere.2009.12.041.

Womersley, F.C., Rohner, C.A., Abrantes, K., Afonso, P., Arunrugstichai, S., Bach, S.S., Bar, S., Barash, A., Barnes, P., Barnett, A., Boldrocchi, G., Buffat, N., Canon, T., Perez, C.C., Chuangcharoendee, M., Cochran, J.E.M., de la Parra, R., Diamant, S., Driggers, W., Araujo, G., 2024. Identifying priority sites for whale shark ship collision management globally. Sci. Total Environ. 934 (May). https://doi.org/10.1016/j.scitotenv.2024.172776.

Worm, B., Sandow, M., Oschlies, A., Lotze, H.K., Myers, R.A., 2005. Ecology: global patterns of predator diversity in the open oceans. Science 309 (5739), 1365–1369. https://doi.org/10.1126/science.1113399.

Xie, Q., Yu, R.Q., Yu, R., Wang, Z., Zhang, X., Wu, Y., 2021. Historic changes of polychlorinated biphenyls (PCBs) in juvenile and adult cetaceans from the Pearl River estuary from 2003 to 2020. Sci. Total Environ. 800, 149512. https://doi.org/10.1016/j.scitotenv.2021.149512.

Xie, Q., Liu, F., Zhang, X., Wu, Y., 2023. Fatty acids and organohalogen contaminants in seafood from the Pearl River estuary, China: risk-benefit analyses of seafood consumption. Sci. Total Environ. 900 (January), 165725. https://doi.org/10.1016/j.scitotenv.2023.165725.

Yang, G., Sun, X., Song, Z., 2020. Trophic level and heavy metal pollution of *Sardinella albella* in Liusha Bay, Beibu Gulf of the South China Sea. Mar. Pollut. Bull. 156 (April), 111204. https://doi.org/10.1016/j.marpolbul.2020.111204.

Zheng, N., Wang, S., Dong, W., Hua, X., Li, Y., Song, X., Chu, Q., Hou, S., Li, Y., 2019. The toxicological effects of mercury exposure in marine fish. Bull. Environ. Contam. Toxicol. 102 (5), 714–720. https://doi.org/10.1007/s00128-019-02593-2.