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Trace metals and persistent organic pollutants contamination in batoids (Chondrichthyes: Batoidea): A systematic review☆

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A B S T R A C T

Batoids (Chondrichthyes: Batoidea; e.g. stingrays, skates, and guitarfish) comprise more than 55% of elasmobranch taxa and represent ecologically important predators in benthic and pelagic habitats. Although overexploitation and habitat degradation are the two biggest threats to batoid populations, coastal and oceanic pollution is also a pervasive potential threat. In this systematic review, we compile published scientific literature on trace metals and persistent organic pollutants (POPs) contamination in elasmobranch species of the Batoidea superorder and present contamination patterns, exposure effects, and potential human exposure risks to most reported contaminants. We found batoids to accumulate a wide range of trace metals, including mercury (Hg), arsenic (As), lead (Pb), copper (Cu), cadmium (Cd) and zinc (Zn). Accumulation of POPs is reported for chlordanes, dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyl (PCB), dieldrin, Heptachlor epoxide, hexachlorobenzene and perfluoroalkyl substances (PFAS). Hg levels in muscle tissue were significantly different among oceanic basins and habitats, consistent with previous global assessments of Hg oceanic background levels. Some batoid species presented Hg levels higher than large pelagic teleost fishes and comparable to sharks. Ecological traits such as, bottom feeding, upper trophic position and elasmobranch-specific physiology and metabolism are discussed as potential factors associated with Hg uptake and accumulation in batoids. Some species exceeded USEPA's maximum contamination safety limits in edible tissues for Hg, As and ΣPCBs. For most trace metals and POPs, there is a lack of studies focusing on contamination levels in batoids. We recommend future research increasing reporting on POPs and trace metals besides Hg in batoids to further investigate the role of Elasmobranch as a bioindicator for marine pollution.

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1. Introduction

Increased nutrient loads, solid waste discharges, oil spills, untreated sewage, radioactive wastes and chemical discharges by industries and urban run-off are all pervasive threats to oceanic and coastal ecosystems (Derraik, 2002; Kitsiou and Karydis, 2011; Vikas and Dwarakish, 2015). Among these, trace metal and persistent organic pollutant (POP) contamination from anthropogenic sources is one of the most widespread human footprints in the Anthropocene (Gałuszka et al., 2014). Anthropogenic activities can alter natural geochemical background and cycling of these elements, increasing uptake of toxic compounds by primary producers and building up along the food web resulting in deleterious effects on aquatic biota (Chopra et al., 2011; de Souza Machado et al., 2016; El-Shahawi et al., 2010; Gałuszka et al., 2014).

Trace metals are metallic elements found in trace amounts in the environment that can be either an essential nutrient (e.g. iron (Fe), zinc (Zn), selenium (Se)) or a toxicant (e.g. mercury (Hg), lead (Pb), cadmium (Cd), arsenic (As)), depending on their concentrations in organisms and/or their specific role in biological metabolism (e.g. copper (Cu) is an essential trace metal because of its role in respiratory proteins found in the Mollusca and Arthropoda) (Morel and Price, 2007). POPs such as, polychlorinated biphenyls (PCBs), chlordanes (CHLs) and dichlorodiphenyl-trichloroethane (DDT) are toxic chemicals, resistant to biodegradation and have no known function in biological metabolism. These pollutants are

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often found accumulating in aquatic biota with a tendency of biomagnification in the food web (Jones and de Voogt, 1999).

Chronic and acute exposure to non-essential trace metals have been studied in many teleost fish species and are known to cause a reduction in reproductive success, immunosuppression and oxidative stress to tissue cells (Baatrup, 1991; Rajeshkumar et al., 2013; Vieira et al., 2009). Similarly, chronic and acute exposures to POPs are hypothesized in association with impaired development, reduced reproductive success and increased carcinogenic effect in many aquatic organisms (Harmon, 2015). Therefore, environmental exposure to trace metals and POPs has the potential to indirectly impact entire populations and communities through the cascading effects of impaired metabolic and reproductive functions of organisms exposed to these pollutants (Fleeger et al., 2003).

Elasmobranchs (e.g. sharks, rays, chimeras) are highly susceptible to accumulation of environmental pollution, in general, due to their intrinsic biological and ecological traits (e.g. slow growth, late maturation, low reproductive output) (Dulvy et al., 2017, 2008; Pierce and Bennett, 2010). Although overexploitation and habitat degradation are suggested as the most significant threats to elasmobranch populations (Dulvy et al., 2014), coastal and oceanic pollution represent a potential additional threat with unknown consequences to this taxonomic group. Similar to teleost fishes, elasmobranchs accumulate water-borne pollutants but likely to a larger extent, as observed for some trace metals, such as silver and copper, under controlled laboratory experiments (De Boeck et al., 2001; Grosell et al., 2003; Webb and Wood, 2000). Paradoxically, there are few numbers of studies investigating trace metal and POP contamination in elasmobranch, and fewer publications focusing only on batoids (Gelsleichter and Walker, 2010).

The term batoids is used here to identify cartilaginous fish encompassing the superorder Batoidea which is further subdivided in four orders: Rajiformes, with suborder of Rhinobatoidei (guitarfishes) and Rajoidea (skates), Pristiformes (sawfishes), Torpediniformes (electric rays), and Myliobatiformes (stingrays) (Aschliman, 2011; Ebert and Compagno, 2007). These organisms differ from sharks by their dorso-ventrally flattened body morphology conferring them a widespread distribution in coastal and oceanic environments, most commonly found in benthic and demersal habitats. Some batoid species play crucial ecological functions in their food webs by directly (e.g. predation) or indirectly (e.g. bioturbation) structuring benthic communities (Bornatowski et al., 2014; Myers et al., 2007; Pierce et al., 2011). Their life history is directly linked to the bottom substrate where trace metals and organic contaminants often accumulate, increasing their exposure potential (de Souza Machado et al., 2016).

In this paper, we conduct an extensive systematic review of the scientific literature on the fate of trace metals and POPs in batoids. Our purpose is to present a general view of contamination in batoid taxa that could help understanding distribution of pervasive pollutants in marine ecosystems. To guide this review, we aim to address the following questions: 1. What are the most common contaminants found in batoids? How do batoid's contamination level differs from teleost fishes occupying similar habitats? 2. Are there geographical differences in contaminant levels found in batoid tissues? 3. What is human's risk of exposure to contaminants through consumption of batoids?

2. Material and methods

The present review identifies research papers related to marine pollution in batoids by adopting a systematic quantitative framework proposed by Pickering & Byrne (2014). Selection criteria included papers published in the English language and institutional reports that provided detailed results of pollutant levels in any batoid species throughout the world. Academic theses, dissertations, and monographs were considered in our discussion but were not included in our meta-analysis as these are not broadly available online and often not subjected to a peer-review process. Online databases such as, Web of Science; Science Direct; Scopus; and Google Scholar, were searched using the following keywords: '*batoids*' '*contamination*' '*copper*' '*elasmobranch*' '*guitarfish*' '*mercury*' '*myliobatodei*' '*organic compounds*' '*pollution*' 'POPs' '*rhinobatodei*' '*rajoidei*' '*rajiformes*' '*skates*' '*stingray*' '*trace metal*' '*torpedinoidei*'. Keywords were used in combination or as a search refining term to improve result outputs. Additional searches were conducted in the reference list of papers to identify studies published in regional journals not included in the mentioned database. Literature surveyed included published papers available at mentioned databases by December 2018.

General recorded information included year, journal, authors, study area, study title, species reported, species size class, sample size, tissue type, pollutant type, biological parameters measured, contamination trends reported, relationship with biological parameters, knowledge gaps highlighted, and additional notes. For trace metals, 24.8% of the results surveyed were reported on a dry weight basis for Hg levels. Because concentrations were commonly reported on a wet weight basis, we converted those reported on a dry weight using the respective tissue moisture content and reported all the Hg concentrations on a wet weight basis in this study. POPs are reported on a lipid weight basis.

We calculated contamination ranges of contaminants using average levels reported in the literature and separated them in 6 independent groups. We used information reported in the respective paper to organize data into groups based on taxa (e.g. sub-order; family; genera and species), area of study (e.g. Barents Sea, China Sea, Laccadive Sea, Mediterranean, North Atlantic, North Pacific, North Sea, South Atlantic and South Pacific) and sex. We also separated species into groups based on preferred prey items (e.g. crustacea, crustacea/fish, fish, invertebrates, mollusk, and zooplankton), habitat zones (e.g. benthic, benthopelagic, and pelagic) and life stage (e.g. adult and juvenile) (Table S3). In addition, we assigned trophic positions to the recorded batoid species based on stomach content and stable isotope ratios previously published in the literature (Barría et al., 2015; Borrell et al., 2011; Ebert and Bizzarro, 2007; Jacobsen and Bennett, 2013; Yemışken et al., 2017).

We followed U.S. EPA and U.S. FDA's published criteria of maximum safety consumption for assessing chemical contaminant data to assess human exposure risk by batoid consumption (Depew et al., 2012; Scheuhammer et al., 2015; USEPA, 2000). A complete list of recorded papers is available in Table S1.

2.1. Statistical analysis

A descriptive statistical approach was used to identify patterns that emerged from the data. Explanatory factors tested included taxonomic groups (e.g. sub-order; family; genera and species), oceanic areas of study (e.g. Barents Sea, Laccadive Sea, Mediterranean, North Atlantic, North Pacific, North Sea, South Atlantic and South Pacific), main prey items (e.g. Crustacea, Crustacea/fish, fish, invertebrates, mollusk and zooplankton), habitat zones (e.g. benthopelagic, demersal and pelagic), life stages (e.g. adult and juvenile) and sex. When not provided by recorded papers, information about species feeding habits and life history was obtained from the literature. We performed parametric (e.g. ANOVA, Student's t) and nonparametric (e.g. Kruskal-Wallis) tests to identify significant differences among groups. We used Generalized Linear Models – GLMs (family = Gamma) to consider factors better explaining Hg concentration variations, and examined potential confounding effects from interactions between the factors “Oceanic area”, “Habitat”, “Sub order”, and “Main prey item. Statistical significance was set at $\alpha = 0.5$. All analysis and plots were conducted using RStudio software (R Core Team, 2017).

3. Results

A total of 47 publications were published between 1981 and December 2018 reporting levels of trace metal and/or POPs in batoids (Fig. 1). A total of 65 batoid species were reported including 25 genera from the suborders Myliobatoidei (35 species), Rajoidei (18 species), Rhinobatoidei (8 species), Torpedinoidei (3 species) and Platyrhinoidei (1 species). Major coastal and oceanic areas identified include the North Pacific Ocean (11 papers), Mediterranean Sea and North Atlantic Ocean (10 papers each), South Pacific Ocean (5 papers), China Sea, North Sea, Barents Sea and South Atlantic Ocean (2 papers each) and Laccadive Sea (1 paper). The vast majority of papers reported exclusively trace metal contamination in batoid species (35 papers). Organic contaminants were reported in 10 papers and 2 studies reported both contaminant types. Trace metal and POPs results will be presented and discussed separately. A detailed list of batoids species surveyed in this study is presented as supplementary material, along with the type of contaminants, study areas and their references (see Table S1).

A variety of tissues has been used to quantify contaminants in batoids, including brachial plate, blood, embryo, eggs, fins, gills, gut, intestine, kidney, liver, muscle, ova, and yolk. Muscle and liver tissues were the most common matrices of analysis being reported in 83% ($n = 39$) and 47% ($n = 22$) of studies, respectively. The genus *Raja* is the most represented batoid taxon, being reported in 36% ($n = 17$) of papers reviewed in this study. Other highly represented genera are *Dasyatis* (note this genus has recently been revised – Last et al. (2016)) and *Myliobatis* being reported in 23% ($n = 11$) and 17% ($n = 8$), respectively. The species *Raja clavata*, also known as Thornback ray, is the most reported batoid species, represented in 21% ($n = 10$) of papers. This species has a wide distribution range occurring in the North and South Eastern Atlantic, the Southwest Indian Ocean and the Mediterranean Sea. We found reported contamination levels for this species mainly in the Mediterranean Sea, but also in North Sea and North Atlantic sites.

3.1. Trace metal contamination in batoids

Twenty-seven trace metals were reported in batoids (Table S1). Among these, mercury (Hg) was the most reported contaminant comprising 84% of papers ($n = 31$) followed by cadmium (Cd) and lead (Pb) ($n = 11$; 31%), zinc (Zn) ($n = 10$; 29%), copper (Cu) ($n = 9$; 26%) and arsenic (As) ($n = 7$; 20%). Contamination levels of most reported trace metals in muscle and liver tissues of batoids are shown in Table 1 along with species presenting the lowest and highest levels. Other trace metals were reported in less than 15% of papers ($n \leq 5$) including chromium (Cr), manganese (Mn), cobalt (Co), iron (Fe), nickel (Ni), selenium (Se), rubidium (Rb), strontium (Sr), palladium (Pd), rhodium (Rh), platinum (Pt), vanadium (V), bismuth (Bi), tin (Sn), antimony (Sb), barium (Ba), thallium (Tl), indium (In), molybdenum (Mo) and silver (Ag).

Due to a very limited number of studies available, we did not observe a clear global trend for most of trace metals except for Hg. Therefore, we focus most of our trace metal contamination discussion primarily on Hg levels. Clear trends of Hg contamination were observed among oceanic basins and animal sizes. Hg levels are discussed here on a wet-weight basis. Hg distributions were significantly different among oceanic basins (Kruskal Wallis chi squared = 51.4; $p < 0.001$) with the Mediterranean Sea presenting consistently higher Hg levels (Fig. 3). Hg levels in muscle tissue of batoid species ranged from 0.086 to 2.42 μg Hg g^{-1} in the Mediterranean Sea (559 specimens of 10 species), 0.011–1.1 μg Hg g^{-1} in the North Pacific (347 specimens of 20 species), 0.039–0.265 μg Hg g^{-1} in the North Atlantic (416 specimens of 6 species), 0.039–0.129 μg Hg g^{-1} in the North Sea (49 specimens of 2 species), 0.096–0.350 μg Hg g^{-1} in the Barents Sea (13 specimens of 2 species), 0.004–2.05 μg Hg g^{-1} in the South Pacific (39 specimens of 8 species), 0.83 to 0.430 μg Hg g^{-1} in the South Atlantic (number of specimens not available; 3 species), 0.019 μg Hg g^{-1} in the Laccadive Sea (15 specimens of 1 species), and 0.040 μg Hg g^{-1} in the China Sea (5 specimens: 1 species). Species were pooled together regardless of their feeding habits and trophic levels to observe how Hg levels varied in areas with different contamination backgrounds.

We have found no significant differences on average trophic position among oceanic basins (Kruskal-Wallis chi-squared = 4.776, df = 3, p-value = 0.189). The Mediterranean Sea dataset included benthic and pelagic species with an average trophic position of 3.43 ± 0.4. The North Pacific basin dataset included benthic and

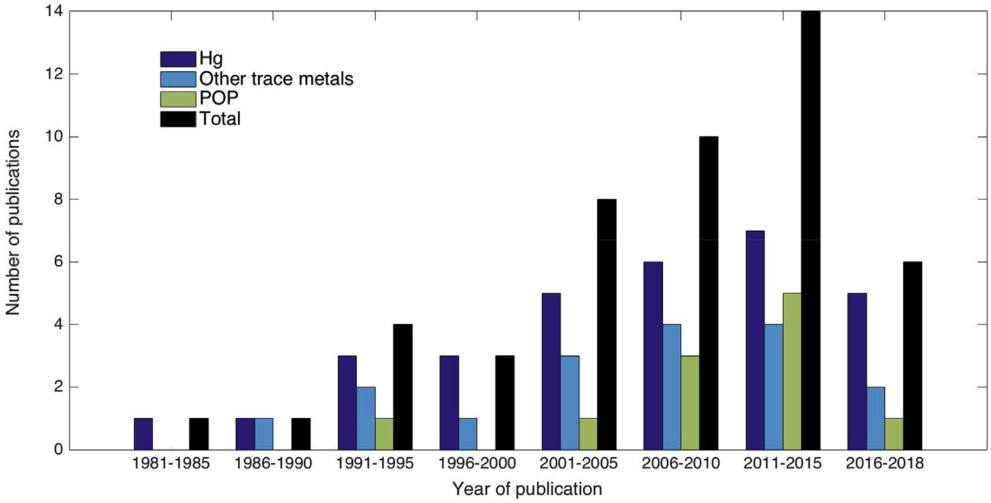

Fig. 1. Number of studies on environmental contamination in batoids categorized by type of contaminant reported. Black bars denote total number of papers published at the respective year period.

Table 1

Contamination levels ($\mu g.g^{-1}$) of most reported trace metals in muscle and liver tissues of batoids. Concentration means were calculated from levels reported in the literature and are shown on a wet weight basis.

Trace metal [d]	Tissue (n)	Mean ± SE (range)	Total species reported	Species with lowest/highest levels
Hg	Muscle [a] (n = 122)	0.35 ± 0.43 (<L.D.[c] − 2.4)	48	*Manta alfredi*/*Torpedo nobiliana*
	Liver [b] (n = 29)	0.32 ± 0.79 (0.01−4.4)	19	*Raja clavata*/*Rhinobatos armatus*
As	Muscle [a] (n = 61)	20.9 ± 19.6 (0.1−94)	10	*Manta alfredi*/*Pteromylaeus bovinus*
	Liver (n = 29)	7.4 ± 3.5 (2.4−16.5)	5	*Pteromylaeus bovinus*
Pb	Muscle [a] (n = 51)	0.31 ± 0.24 (0.01−1.1)	19	*Aptychotrema rostrata*/*Manta alfredi*
	Liver (n = 17)	0.76 ± 0.38 (0.03−1.3)	12	*Raja fyllae*/*Gymnura altavela*
Cu	Muscle [a] (n = 23)	0.74 ± 0.43 (0.12−1.4)	16	*Aptychotrema rostrate*/*Raja radula*
	Liver [b] (n = 18)	5.24 ± 5.51 (1.1−16.5)	11	*Dasyatis pastinaca*/*Raja clavata*
Cd	Muscle [a] (n = 55)	0.06 ± 0.06 (0.03−0.2)	21	*Myliobatis australis*/*Mobula japanica*
	Liver [b] (n = 19)	0.32 ± 0.23 (0.1−0.8)	12	*Raja clavata*
Zn	Muscle [a] (n = 25)	7.2 ± 2.8 (1.7−9.1)	18	*Rhynchobatus australiae*/*Raja miraletus*
	Liver [b] (n = 18)	14.4 ± 6.2 (5.7−29.9)	11	*Dasyatis pastinaca*/*Raja clavata*

[a] Muscle concentrations were converted to wet weight assuming a moisture content of 74% (Escobar-Sánchez et al., 2016).
[b] Liver concentrations were converted to wet weight assuming genus-specific moisture content (Sellami et al., 2018; Tufan et al., 2013).
[c] Limit of detection (L.D.) = 0.004 $\mu g\ g^{-1}$ (Ooi et al., 2015).
[d] See Table S1 for included references.

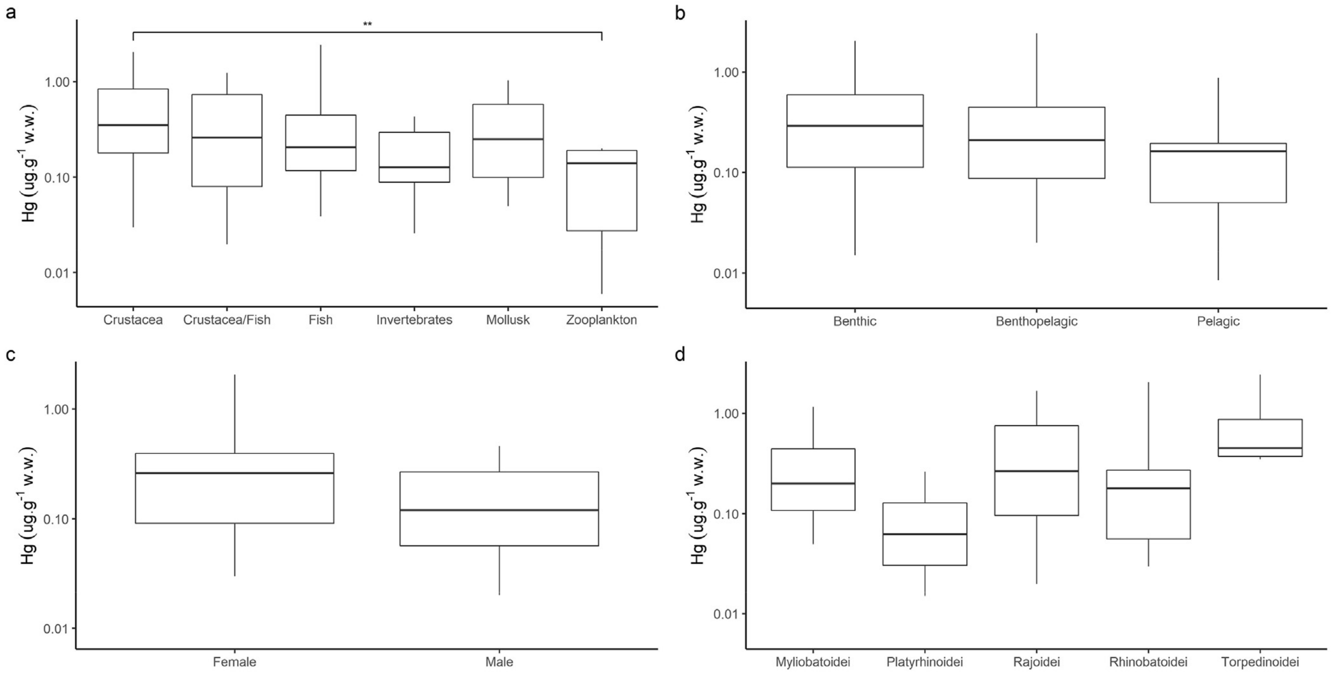

Fig. 2. Boxplot of Hg levels in batoids categorized by (a) main prey item, (b) foraging habitat, (c) sex and (d) sub-order. Asterisks denote statistically significant differences (**0.01).

pelagic species with an average trophic position 3.48 ± 0.4. The North Atlantic basin dataset included benthic and benthopelagic species with an average trophic position of 3.57 ± 0.5. The South Pacific basin dataset included pelagic and benthic species with an average trophic position of 3.47 ± 0.4. In addition, all these oceanic basins shared species of the same genera (i.e. *Dasyatis*, *Myliobatis*, *Raja*, *Torpedo*).

Hg levels in adult specimens were significantly higher compared to juveniles (Student's $t = 3.5$; $p < 0.001$; $df = 1$). Hg concentrations were also significantly different among prey items with “Crustacea” presenting higher concentration compared to “Zooplankton” (GLM, t value = 2.045., $p = 0.04$; Fig. 2a). In contrast, no significant differences were found among sub-orders and foraging habitat (GLM, $p > 0.1$) or between genders (Student's $t = - 1.19$; $p = 0.2$; $df = 1$) (Fig. 2).

3.2. Persistent organic pollutants (POPs) contamination in batoids

There were only 11 papers reporting POPs in batoids. Reported POPs include dichlorodiphenyltrichloroethane (DDTs), chlordanes (CHLs), polychlorinated biphenyls (PCBs), perfluoralkyl substances (PFAS) and polybrominated diphenyl ethers (PBDEs). PCBs, CHLs, and DDTs were the most reported contaminants accounting for 63% of papers in this category (n = 7). PFAS were reported in only two papers but in more species than any other POPs surveyed. The most analyzed tissue was liver accounting for 81% of papers in this category (n = 9). Other reported tissues include muscle, eggs, and embryo. Species were captured from four oceanic basins including the North Atlantic (2 species), the Mediterranean Sea (3 species), the North Pacific (2 species) and the South Pacific (6 species).

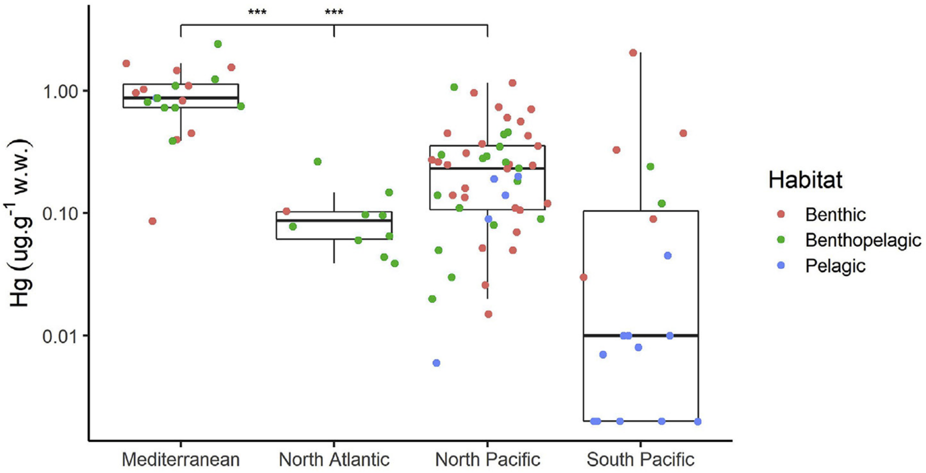

Fig. 3. Boxplot of Hg concentration in muscles of batoid species from major ocean basins. Pooled data include all reported species from each region. Asterisks indicate statistically significant differences (***0.001).

Total PCB levels in liver ranged from 0.2 to 9.5 μg g^{-1} lipid weight (2 species; 274 specimens) (Lyons et al., 2014; Weijs et al., 2015), 0.02–0.07 μg g^{-1} lipid weight for ΣDDTs (4 species; 261 specimens) (Lyons et al., 2014; Miskiewicz and Gibbs, 1994) and 0.02–2.5 μg g^{-1} lipid weight for ΣCHLs (5 species; 276 specimens) (Lyons et al., 2014; Miskiewicz and Gibbs, 1994; Weijs et al., 2015). POP levels in muscle tissue were reported in only four papers and varied from 0.005 to 3.16 μg g^{-1} for total PCBs (6 species; 50 specimens) (Gassel et al., 2013; Johnson-Restrepo et al., 2005; Storelli, 2008). DDTs and CHLs were reported in muscle tissue of one species (e.g. *Rhinobatos productus*) and varied from 0.002 to 0.004 μg g^{-1} of DDTs and non-detectable for CHLs (Gassel et al., 2013). PFAS levels were reported in muscle tissue of one specimen of *Dasyatis americana* (Senthil Kumar et al., 2009).

The highest total PCB levels found in batoids were reported in livers of *Urobatis halleri* (4.5 ± 2.4 μg g^{-1}; n = 208) from Seal Beach, USA (Lyons et al., 2014). The highest mean values of total CHLs were found in livers of *Urolophus kapalensis, Aptychotrema rostrata* and *Myliobatis australis* (2.52 μg g^{-1}; 1.02 μg g^{-1} and 0.99 μg g^{-1}, respectively), all captured in the coast of Sidney, Australia (Miskiewicz and Gibbs, 1994). *M. australis* also presented the highest total DDTs levels (1.48 ± 5.17 μg g^{-1}) (Miskiewicz and Gibbs, 1994). The highest total PFAS concentrations in batoids were found in livers of *D americana* (mean of 0.033 μg g^{-1}; n = 2) and *Neotrygon kuhlii* (0.018 ± 0.027 μg g^{-1}; n = 35) captured at the coasts of Georgia, USA, and Sidney, AUS respectively (Baduel et al., 2014; Senthil Kumar et al., 2009).

3.3. Exposure effects of POPs and trace metal on batoids

We found only five studies reporting the effects of trace metals and POPs contamination on batoids. Assessed contaminants included trace metals (e.g. Cu and Sn) (Dwivedi and Trombetta, 2006; Grosell et al., 2003), an organometallic compound (e.g. tributyltin oxide -TBTO) (Dwivedi and Trombetta, 2006) and persistent organic pollutants (e.g. DDTs, PCBs and CHLs) (Gelsleichter et al., 2006; Lyons et al., 2014; Sawyna et al., 2017). Effects of pollutants were measured through the use of biomarkers (e.g. Na/K-ATPase activity in gill, rectal gland and intestine; EROD activity in liver; enzyme-linked immunosorbent assay (ELISA); CYP1A, heme-oxygenase (HO–1) and Hsp70 proteins expression; lipid peroxidation marker 4-hydroxynonenol) and non-biomarker parameters (e.g. total ammonia, Cl^-, Na^+ and Mg^+ in plasma; immune cell counts; morphological measures of reproductive and respiratory organs; hormone levels; and embryo development).

4. Discussion

The research field of environmental contamination in batoids has received relatively less attention considering that earliest papers were mainly opportunistic studies (i.e. capturing batoids as a bycatch rather than a target species) without asking specific questions about this taxonomic group. That aspect changed in the last decade, with more studies exclusively targeting batoid species to address questions concerning aspects of this taxonomic group. Likely, that shift built on our better understanding of food web function (e.g. trophic interactions associated with ecosystem stability (Gorman and Emmerson, 2009), trophic cascades in food web dynamics (Polis et al., 2000) and the potential role of elasmobranchs in ecosystem stability (i.e. top-down trophic cascades, mesopredator release) (Myers et al., 2007). Studies of environmental contamination in batoids, however, remain relatively limited. This review provides a status quo of pollutant levels in the batoid taxonomic group.

4.1. Trace metal accumulation in batoids

Relatively few studies have investigated the impacts of marine pollution in elasmobranchs (Gelsleichter and Walker, 2010), despite the fact that exposure experiments showed these organisms could be more susceptible to accumulation of and toxicity effects imposed by trace metals. As an example, some species of sharks and skates were found to be 10 times more sensitive to toxicity effects from silver exposure (De Boeck et al., 2001; Webb and Wood, 2000) and accumulated 13 times more copper in gill tissues compared to teleost fish (Grosell et al., 2003). However, there has not been a systematic review that reports trace metals concentrations found in batoids worldwide.

Based on the reported Hg levels in batoids and teleost fishes, our survey revealed that average Hg concentrations were consistently higher in batoids compared to teleost fishes at similar trophic levels and, in some instances, comparable to levels found in top pelagic and benthic predators. In the Gulf of California, average Hg levels from 9 batoid species were found similar to those from 7 large pelagic teleosts (García-Hernández et al., 2007). When comparing fish of similar size, *D. longa*, *Dasyatis brevis*, and *Rhinoptera* *steindachnerii* presented higher, though not statistically significant, average Hg levels than those in the pelagic Indo-Pacific sailfish (*Istiophorus platypterus*), Blue marlin (*Makaira mazara*) and Wahoo (*Acanthocybium solandri*). These pelagic fishes are predators known to accumulate Hg to a great extent due to their upper-level position in the food web (Perelman et al., 2017; Rosas-Alayola et al., 2002). Hg levels in batoids were in the same range compared to four grouper species inhabiting the deep-water or shallow hard bottom reefs (García-Hernández et al., 2007). In another study, a remarkable result was found where *U. halleri*, a medium size invertebrate forager, presented the highest Hg levels among 40 fish species of teleost and elasmobranchs (Jonathan et al., 2015). These authors attributed this high Hg levels to the proximity to bottom sediments and intrinsic physiological traits in elasmobranch taxa.

Denton and Breck (1981) reported average Hg levels in the demersal batoids *G. australis, Himantura uarnak, Rynchobatus dijiddensis* from northeastern Australia. Similar levels were found in teleost fishes of all comparable sizes, all of which were benthic carnivorous predators. In southeast Australia, a similar pattern was also found for *M. australis, Urolophus* sp. *A. rostrata* (Gibbs and Miskiewicz, 1995). In the Mediterranean Sea, specimens of *Raja* genus presented Hg levels comparable to demersal top predators (e.g. *Lophius budegassa, Lepidopus caudatus* and *conger*) but lower than pelagic top predator *Thunnus alalunga* (Storelli et al., 2003b; Storelli, 2008). These findings suggest batoids inherit specific traits that are subject to accumulation of Hg in a greater extent than teleost fishes sharing similar diet and habitats. However, that hypothesis has not been tested and needs further investigation.

It is accepted that trophic position and feeding behavior are two common factors strongly related to Hg accumulation (Hall et al., 1997). Animal physiology and metabolism control elimination process and the accumulation rate of contaminants (Bradley et al., 2017; Trudel and Rasmussen, 1997). These are likely explanations for the apparent higher Hg levels in batoids relative to teleost with comparable diets. Organisms with a long lifespan associated with slow growth rates are known to present increased Hg levels in older/larger individuals (Dang and Wang, 2012). In addition, elasmobranchs present relatively large lipid-rich livers which result in greater bioaccumulation of lipophilic compounds that eventually accrues in muscle tissues due to sulfhydryl groups association to amino acids (Gelsleichter and Walker, 2010).

4.2. Geographic variation of Hg levels batoids

Among reported trace metals, Hg was the only element with enough information to assess accumulation and distribution patterns in batoids from different areas.

Fig. 3 shows the comparison of Hg concentration in batoid species grouped by oceanic basins presenting at least ten Hg observations. Each area is composed of species feeding on various preys (e.g. crustacea, fish, mollusks, and zooplankton) in the benthic, benthopelagic, and pelagic habitats. The intra-basin variability in habitat and main prey items is not strong enough to mask the observed differences among ocean basins. The Mediterranean Sea is a semi-enclosed, Hg-enriched basin, a part of what was known as the Hg mineral belt (Rajar et al., 2007). Riverine inputs and anthropogenic emissions are the main sources of Hg in this basin (AMAP/UNEP, 2013). Rytuba (2003) showed that Mediterranean Sea coastal areas consistently accumulate recently formed submarine deposits enriched in Hg. Average Hg level in *Torpedo* and *Raja* genera in the Mediterranean Sea are four and three times higher, respectively, compared to the Pacific coast of Costa Rica. Sandoval-Herrera et al. (2016) attributed these differences to the deeper distribution of *Torpedo* population in the Mediterranean Sea (200–500 m depth compared to 50–250 m in Costa Rica). Net production of methylated Hg forms occurs primarily in deep hypoxic waters and was found to be higher in the Mediterranean Sea compared to North Pacific basin (Horvat et al., 2003; Sunderland et al., 2009). Moreover, foraging strategy may also explain the Hg content in marine fish. Lacerda et al. (2017) observed that prey type ingested, rather than depth-specific methyl-Hg production, can explain the higher Hg levels found in tuna species feeding at different depths in the Equatorial Atlantic Ocean. This is because deeper water populations are mostly composed of carnivorous species, whereas non-carnivorous prey species predominate in surface waters in their study areas.

Two of the highest Hg levels surveyed in the present study were found in the Gulf of Trieste, Northern Adriatic Sea, an area heavily impacted by Hg mining contamination (Horvat et al., 2014). These authors found that the piscivorous species *Pteroplatytrygon violacea* presented average Hg levels roughly 4 times higher than *Pteromylaeus bovinus* foraging on benthic invertebrates in the same area. Even higher average Hg levels were found in demersal fish consumer *Torpedo nobiliana* from southern Adriatic Sea (Storelli et al., 2002b) despite the fact that Hg mining activities have had relatively small impacts in this area compared to northern Adriatic. In this case, differences in prey preference (deep-water vs. surface water preys) are likely the main driver of observed Hg contents. Also, these authors emphasize that predators closely associated with sediment and consuming a large amount of benthic fish are more susceptible to Hg accumulation than consumers of pelagic fish or benthic invertebrates (Storelli et al., 2002b, 1998). In general, high regional Hg backgrounds and foraging in favor of benthic fish preys are likely causes of high Hg levels in batoid species in these areas.

High average Hg levels were also observed in batoids from sites in the North Pacific basin (Fig. 3), which includes sites in Costa Rica coast, Gulf of California and Baja in Mexico and Southern/Central California coast in the US. The highest Hg concentration in batoid species in this oceanic basin was observed in *Dasyatis longa* from Gulf of California (1.2 $\mu g.g^{-1}$ w. w.) (Ruelas-Inzunza et al., 2013) though it was based on a single specimen. In contrast, García-Hernández et al. (2007) in the same area found an average Hg level of 0.7 ± 0.26 $\mu g.g^{-1}$ w. w. for *D. longa* and with other batoid species presenting lower average Hg levels. The Gulf of California is impacted by a number of anthropogenic Hg sources, in order of importance, including gold mining and refining, Hg mining and refining, chloralkali industry, copper smelting, residential combustion of wood, carboelectric plants and oil refining (Páez-Osuna et al., 2017). That shows there is an important source of Hg to this region, but trophic position and foraging habitat of local biota likely have major role in Hg accumulation as evidenced by the large variability among batoids species and teleost fishes with different life history traits (Escobar-Sanchez et al., 2014; García-Hernández et al., 2007; Ruelas-Inzunza et al., 2013).

The South Pacific and North Atlantic basins presented lower Hg levels on average compared to the Mediterranean and the North Pacific basins (Fig. 3). All but one batoid species analyzed presented Hg levels lower than 0.24 $\mu g \, g^{-1}$. In one large female *Rhinobatos armatus* (>2 m of body length) from Cleveland Bay, Australia, an extremely high Hg level (2.1 $\mu g.g^{-1}$ w. w.) was found, which was comparable to the level found in sharks with similar sizes from the same area (Denton and Breck, 1981).

The observed differences among oceanic basins are consistent with previous surveys of global Hg budgets in oceanic waters (Mason et al., 2012; Sunderland et al., 2009). However, results shown in Fig. 3 should be interpreted with caution because in our analysis, areas were not represented by the same species nor equal number of observations. Future studies need to consider potential interaction effects between interspecific variability in Hg uptake and locations once data become readily available.

We further categorized specimens into juvenile and adult groups when size information was available. We did this by following criteria that separately consider minimum sizes of maturation for each species (Araújo et al., 2016; Babel, 1967; Bizzarro et al., 2007; Cicia et al., 2009; Clarke et al., 2014; Cuevas-Zimbrón et al., 2011; Gadig et al., 2003; Jacobsen et al., 2009; Kyne and Bennett, 2002; López-García et al., 2012; McCully et al., 2012; Oddone and Velasco, 2004; Ramirez Mosqueda et al., 2012; Saglam and Ak, 2012; Smith et al., 2007; Timmons and Bray, 1997; Yeldan et al., 2009). This was consistent with previous findings where positive relationships between Hg levels and animal size have been reported in batoids (Escobar-Sanchez et al., 2014; Gutiérrez-Mejía et al., 2009; Law and Singh, 1991; Lyons et al., 2017; Sandoval-Herrera et al., 2016). All these authors show that habitat use and ontogenetic diet shifts are the major factors explaining this positive correlation.

Ontogenetic shifts are common in elasmobranchs which often is complemented by changing foraging habitat and feeding behavior (Brickle et al., 2003; Grubbs, 2010). Adults present slower metabolism and lower elimination rates of metals compared to juveniles (Gutiérrez-Mejía et al., 2009). Finally, adult elasmobranchs tend to feed on larger preys and in higher quantities (Jacobsen and Bennett, 2013) which also contribute to greater bioaccumulation and higher Hg concentrations.

4.3. Human exposure risk

Essential and non-essential trace metals in batoids, except As and Hg, were found below maximum contamination safety limits for seafood consumption (USEPA, 2000). The maximum contamination limit established for inorganic As is 0.13 μg g^{-1} in edible fish tissues (USEPA, 2000). This is the most conservative limit to avoid potential toxic effects induced by As inorganic forms (e.g. arsenite – As(III) and arsenate – As(V)). In the present survey, the average total As in muscle tissues of batoids was 20.9 ± 19.6 μg.g^{-1} w. w. (Table 1), which is above the safety threshold. However, total As includes organic and inorganic fractions of this element. A major fraction of total As is in harmless organic As forms (e.g. arsenobetaine, arsenocholine, and tetramethylarsonium ion). De Gieter et al. (2002) assessed As speciation in 29 fish species from the North Sea and found an inorganic fraction variation from <1% to 9%. Among these fishes, elasmobranchs (e.g. *Scyliorhinus canicular* and *R. clavata*) presented two of the three highest levels of total As. Nevertheless, the inorganic fraction in these species was less than ~2%, the lowest fraction among all fish species studied by these authors. Assuming an inorganic fraction as low as 2% and an average total As concentration of 20.9 μg g^{-1}, we calculated that batoids surveyed in our study present 0.418 μg g^{-1} of inorganic As, which is roughly 3 times higher than the safety threshold established by USEPA.

In comparison, toxic effects associated with Hg contamination comes from exposure to its organic form, methylmercury (Methyl-Hg), rather than Hg inorganic forms (e.g. Hg^{2+} and Hg^0) (Mason et al., 2012). The great affinity of Methyl-Hg to lipids results in long-term accumulations in biological tissues and deleterious effects from exposure even to relatively low environmental concentrations (Depew et al., 2012; Fitzgerald et al., 2007). In the present study, the proportion of Methyl-Hg in surveyed batoids ranged from 71.6% to 100% (with a mean of 92.8 ± 9.4%) in muscle, 48%–100% (78 ± 20.8%) in liver and 71%–90% (83.8 ± 7.6%) in gill tissues (Baeyens et al., 2003; Horvat et al., 2014; Storelli et al., 2003b, 2003a, 2002b). The proportion of organic Hg in fish is largely related to feeding habit and age (de Pinho et al., 2002), but also strongly influenced by background levels (Lacerda et al., 2014), local geochemistry nature of Hg-associated particles (Lacerda et al., 2007) and phytoplankton assimilation processes occurring in the base of the food web (Mason et al., 1995). Reported Methyl-Hg levels in batoids were comparable with upper-level predatory fishes, such as tuna (Storelli et al., 2002a), mackerel (Hajeb et al., 2010) and sharks (De Carvalho et al., 2014). Therefore, we assume a large proportion of the total Hg in batoids compiled in the present study is Methyl-Hg and, thus toxic to the animal and its consumers.

The maximum total Hg contamination limits for safety consumption is 1.0 μg Hg g^{-1} for predatory fish species (FAO/WHO, 2011; USEPA, 2000). Average Hg concentration found in the present study was below this limit (0.34 ± 0.43 μg.g^{-1} w. w.). However, Hg levels varied greatly among batoid species (Fig. 4), with 18% of surveyed species presenting average Hg levels above the safety limit. Among those, *Leucoraja circulares, P. bovinus, R. clavata, Raja miraleus, Raja oxyrhynchus,* and *T. nobiliana* were captured in the Mediterranean Sea; *D. longa* and *Torpedo peruana*, were captured in the North Pacific; and *R. armatus* was captured in the South Pacific.

Although the worldwide safety limit of 1.0 μg Hg g^{-1} targets to protect humans against Hg exposure, having Hg levels below that threshold does not mean neither the animal nor human consumers are free of risk of experiencing toxicity effects. Recent assessments of Hg toxicity in fish estimated a lowest observable adverse effect level (LOAEL) of about 0.5 μg Hg g^{-1} w. w. in muscle tissues of freshwater and marine fish (Depew et al., 2012; Dillon et al., 2010; Scheuhammer et al., 2015). That means deleterious effects to the fish has been observed under Hg concentrations at this level. Moreover, surveys in the Amazon region observed an impairment of visual capability in a riverine human population with no direct contact with Hg contamination (e.g. gold mining) (Feitosa-Santana et al., 2018). These communities have a diet largely based on fish presenting generally lower-than-LOAEL average Hg concentrations (Azevedo-Silva et al., 2016). If we consider a LOAEL of 0.5 μg Hg g^{-1}, 32% of batoid species surveyed in the present study are at potential risk of adverse effects which may also impair the health of human consumers.

4.4. POPs contamination

The very limited information on POPs contamination in batoids prevented us to observe potential trends with respect to oceanic basins, foraging habitat, gender or animal size. In general, animal size and lipid content are good predictors of PCB variations in some teleost fishes (Gewurtz et al., 2011; Rasmussen et al., 2014). However, positive correlations between animal size and PCB levels are not always observed and trophic ecology seems to be another important factor. As an example, a positive relationship between PCBs levels and animal size has been observed more consistently in upper-level consumers, but not in lower level consumers in freshwater fish species (Gewurtz et al., 2011). In the batoid species *U. halleri*, PCBs, DDTs and CHLs concentrations were shown to increase with size but with different slopes in males (higher) and females (lower) which the authors discuss as a result of maternal offloading of contaminants as an elimination route in females (Lyons et al., 2014; Lyons and Lowe, 2013). A similar result was observed in dolphins where PCBs levels increased with age in males but not in females, highlighting maternal offloading as an important depuration pathway of POPs (Wells et al., 2005). In addition, POP accumulation in *U. halleri* were found to vary by location, sex and age suggesting that similar to Hg, several factors influence POP uptake and accumulation patterns in this species and likely other batoids (Lyons et al., 2014).

Regarding food safety, US EPA's (2000) recommends maximum limits (expressed in a wet weight basis for edible tissues) of 0.38 mg kg^{-1}, 0.94 mg kg^{-1} and 9.4 mg kg^{-1}, for total PCBs, DDTs

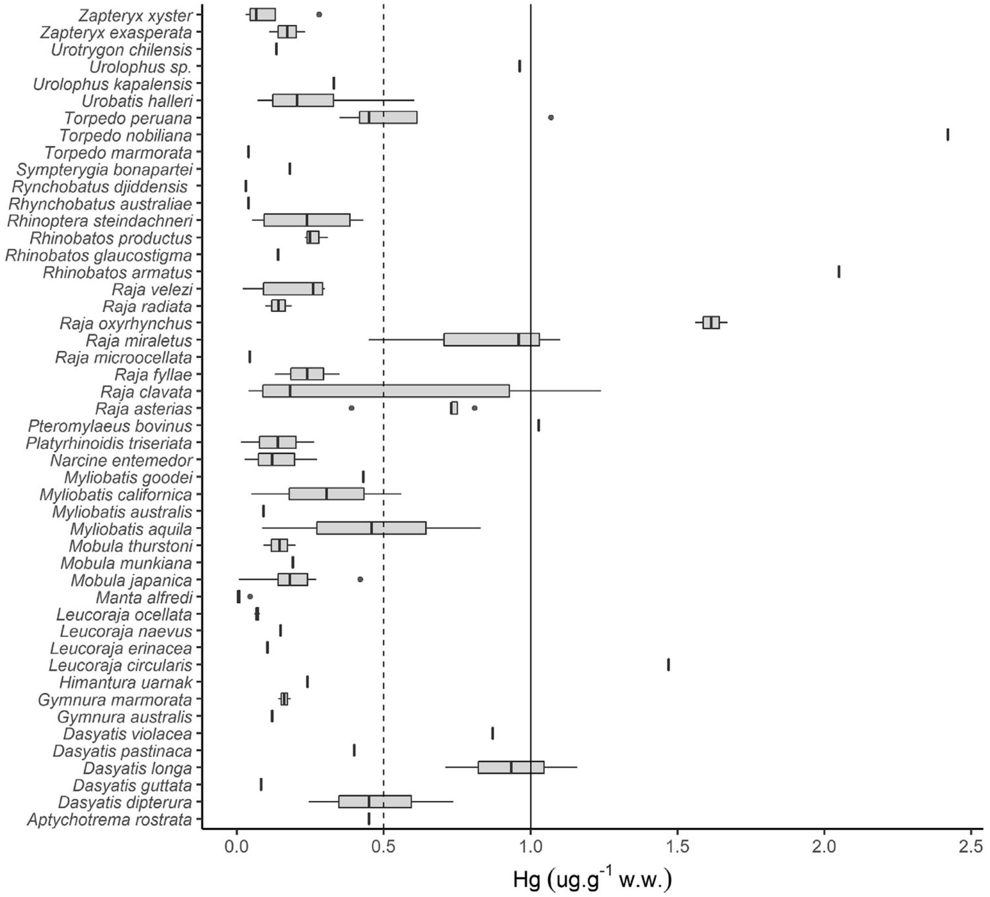

Fig. 4. Hg contamination range reported in batoid species worldwide. The solid line indicates US EPA's recommendation for maximum safety consumption. The dashed line indicates the lowest observable adverse effect level (LOAEL) for freshwater and marine fish (Depew et al., 2012; Dillon et al., 2010; Scheuhammer et al., 2015).

and CHLs, respectively. In the present survey, DDT and CHL levels were always below the safety limits. However, 87.5% of total PCB levels in liver tissue surveyed in the present study exceeded this safety limit. Those samples were juvenile and adult specimens of *U. halleri* from Southern California coast and *D. sabina* from Florida coastal lagoons in the USA (Lyons et al., 2014; Sawyna et al., 2017; Weijs et al., 2015). For edible tissues, such as muscle, 50% of total PCBs levels surveyed in the present study exceeded the safety limits for human consumption (Gassel et al., 2013; Johnson-Restrepo et al., 2005; Storelli, 2008). In Southern California coastal areas, 12 fish species, including batoid species *R. productus*, are currently under restricted consumption advisory due to PCBs contamination (OEHHA, 2009). Weijs et al. (2015) also reported high PCB levels in both liver and muscle tissues of shark species (e.g. *Carcharhinus leucas, Negaprion brevirostris* and *Sphyrna tiburo*) in Florida's coastal lagoons. They found that POPs were more commonly observed in species of higher trophic levels while low trophic level species (e.g. *D. sabina*) had fewer compounds detected. They attributed these differences to a combination of factors including biomagnification, species-specific feeding ecologies and diet, and metabolic traits of sharks and rays.

Johnson-Restrepo et al. (2005) found average ΣPCB levels in *D. sabina* to be higher than in silver perch (*Bairdiella chrysoura*, carnivorous diet) and striped mullet (*Mugil cephalus*, herbivore diet) but lower than spotted seatrout (*Cynoscion nebulosus*, carnivorous diet), red drum (*Sciaenops ocellatus*, carnivorous diet), and hardhead catfish (*Ariopsis felis*, carnivorous diet). In general, our survey suggest that, compared to teleosts, batoids are particularly subjected to accumulation of contaminants mostly due to their close association with sediments, but also because of their feeding ecology associated to varied prey types and elasmobranch physiology that seems to favor accumulation of contaminants (De Boeck et al., 2001; Grosell et al., 2003; Webb and Wood, 2000). However, that hypothesis was not tested in the present study and requires further investigations.

An assessment of PFAS contamination, which has no established maximum safety limit for fish consumption, showed that levels in *D. americana* are among the highest of a fish assemblage including predator teleost fishes and sharks (Senthil Kumar et al., 2009). Among PFA substances, Perfluorooctane sulfonate (PFOS) is one of the most widely detected in biological samples (Houde et al., 2006) and was the main compound found in livers of batoid species surveyed in the present study (Baduel et al., 2014; Senthil Kumar et al., 2009).

Considering POP levels in muscle tissues, Johnson-Restrepo et al. (2005) reported PCB levels in *D. americana* as among the lowest of all organisms including teleost fishes, sharks, and dolphins. These authors highlight that organochlorine uptake is associated to feeding behavior and habitat use, while contaminant accumulation is a tissue-specific process controlled by the metabolic and physiological traits of the organism. This general principle can explain higher POP levels in batoids liver compared to muscle tissues (Johnson-Restrepo et al., 2005; Senthil Kumar et al., 2009).

Information on POP contamination levels in batoids is very limited and values are reported over a wide range. Table S2 shows specific congeners/compounds in reported POP levels in batoids.

This table presents an example of the complexity in reporting POPs, making it very difficult for cross comparison among studies. For instance, PCB is the most reported organic contaminant in batoids. However, studies do not always report comparable PCB congeners. PCBs are synthetic chlorinated aromatic hydrocarbons composed of one to ten chlorine atoms resulting in 209 compounds with different molecular configuration and toxicity. Among these, twelve dioxin-like compounds (77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 189) are recognized as the most toxic to biota (U.S. EPA, 2008). Therefore, direct comparisons among species and areas for total PCB levels summed from varied congeners mixtures may lead to unclear or misleading results (Batang et al., 2016). In the present survey, compound-specific PCB levels were rarely reported and total PCB levels varied greatly among studies. In addition, levels of CHLs and DDTs are sometimes summed together and presented as total pesticides concentrations which further complicate comparisons among studies.

4.5. *Exposure effects of POPs and trace metal on batoids*

The clearnosed skate, *Leucoraja erinacea,* and the Yellow stingray, *Urolophus jamaicensis,* were the only batoid species in which acute exposure was assessed (Dwivedi and Trombetta, 2006; Grosell et al., 2003). In *R. erinacea*, acute exposure to Cu produced alterations of plasma total ammonia but had no effect on plasma electrolytes or Na/K- ATPase enzyme activity (Grosell et al., 2003). In contrast, in *U. jamaicensis*, acute exposure to TBTO resulted in altered behavior and produced stress responses measured by Hsp70 and HO1 proteins expression (Dwivedi and Trombetta, 2006). Two other species, the Atlantic stingray, *Dasyatis sabina* (former *Dasyatis sabina*), and the Round stingray, *U. halleri*, were used to assess the exposure effects of PCBs, DDTs, CHLs and other chlorinated contaminants in the wild. In *U. halleri*, increased EROD and CYP1A enzymes activities corresponded to higher levels of organochlorine compounds (Lyons et al., 2014). Similar correspondence was also found for measured cell proliferation and phagocytosis in whole blood (Sawyna et al., 2017). In contrast, despite the high organochlorine accumulation found in *D. sabina* specimens from two contaminated coastal lakes, the authors found no evidence of impaired reproductive parameters and no clear association with the observed endocrine dysfunction (Gelsleichter et al., 2006). Clearly, there is a need for more research on exposure effects of trace metal and POPs in batoid species as the limited evidence shows contrasting results for trace metal and POPs exposure. Future studies should employ comparable biomarkers such as, leukocytes counting and/or EROD activity and CYP1A mRNA expression to allow interpretation of exposure effects across species. In addition, to better represent real conditions, studies should focus on effects of chronic exposure to environmentally relevant levels of pervasive contaminants, including Hg, As and PCBs, that were observed in the present survey to exceed the action limits in many batoid species.

5. Conclusion

We found batoids accumulating a wide range of trace metals, including mercury (Hg), arsenic (As), lead (Pb), copper (Cu), cadmium (Cd) and zinc (Zn) in our survey. These findings likely represent a common pattern in the upper trophic position of most batoid species in coastal and demersal habitats. Mercury is the most reported contaminant in batoids ranging from moderate to high levels in edible tissues. We found notable differences in batoids Hg levels when the aggregated data were compared among: 1) ocean basins, 2) main prey items, and 3) foraging habitats. Batoids of high Hg levels were mostly associated with habitats affected by natural Hg-rich backgrounds and anthropogenic Hg emissions. These findings were consistent with global Hg budgets in oceanic waters (Mason et al., 2012; Sunderland et al., 2009). Unfortunately, a very limited number of published studies prohibited us from performing meaningful statistical analysis on other trace metals and POPs. Future studies should be encouraged to report contamination loadings in batoids including trace metals besides Hg.

The published data on POPs contamination in batoid taxa are still very limited and for the most part, inconsistent in the way data were reported to draw general patterns. Our findings point to increased susceptibility of contaminant accumulation in batoids because of elasmobranch-specific physiological/metabolic traits that apparently enhance the accumulation of lipophilic contaminants. This hypothesis has not been tested and needs further investigation in future studies.

Regarding human exposure to contaminants by consumption of batoids, our survey showed that contamination levels are generally below recommended safety limits for most contaminants in most species reported. It is advisable, however, to use caution when consuming batoids inhabiting contaminated areas as some species surveyed in the present study were observed to accumulate above-the-limit levels of Hg, As and ΣPCB.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.02.070.

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