



## Baseline

Legacy and emerging contaminants in the endangered filter feeder basking shark *Cetorhinus maximus*G. Boldrocchi<sup>a,\*</sup>, D. Spanu<sup>b</sup>, S. Polesello<sup>c</sup>, S. Valsecchi<sup>c</sup>, F. Garibaldi<sup>d</sup>, L. Lanteri<sup>d</sup>, C. Ferrario<sup>c</sup>, D. Monticelli<sup>b</sup>, R. Bettinetti<sup>a</sup><sup>a</sup> Department of Human Sciences, Innovation and Territory, University of Insubria, Via Valleggio 11, Como, Italy<sup>b</sup> Department of Science and High Technology, University of Insubria, Via Valleggio 11, Como, Italy<sup>c</sup> Water Research Institute, National Research Council of Italy (IRSA-CNR), Brughiero, Italy<sup>d</sup> Department for Earth, Environment and Life Sciences, University of Genoa, Corso Europa 26, Genoa, Italy

## ARTICLE INFO

## Keywords:

PFAS  
PCBs  
Trace elements  
Sharks  
Pollution  
Mediterranean Sea

## ABSTRACT

The status of contamination by chemical pollutants on large filter feeding sharks is still largely unknown. This study investigated for the first time the presence of legacy, emerging contaminants and trace elements in multiple tissues of basking sharks. In general, skin showed higher concentration of legacy and emerging contaminants probably due to pollutants being adsorbed onto the dermal denticles of the skin rather than accumulated in the tissue itself. Contaminants measured in both subcutaneous tissue and muscles appeared to strongly correlate with each other, indicating that the former might be a good proxy of muscle contamination in basking sharks. Considering the migratory nature of this species, longevity and feeding ecology, this species represents the perfect candidate to act as early warning bioindicator of regional contamination. In this context, non-lethal subcutaneous biopsies could allow the early detection of any temporal variation in the bioaccumulation of pollutants in the Mediterranean Sea.

The basking shark (*Cetorhinus maximus*) is the world's second largest fish species with a circumglobal distribution in temperate waters (Compagno, 2001). This filter-feeder is susceptible to several threats and considered Endangered on the International Union for Conservation of Nature (IUCN) Red List throughout their range (Rigby et al., 2021). The basking shark forms seasonal aggregations in several locations including the Atlantic (Couto et al., 2017; Dolton et al., 2020; Miller et al., 2015; Witt et al., 2012), and the Pacific Ocean (Dewar et al., 2018; Finucci et al., 2021). An increasing body of ecological research on this species is being collected at the worldwide level, but information about its status of contamination is scarce. Being particularly prone to bioaccumulation of contaminants, it is important to study pollutants in sharks so as to understand the decline in their populations (Boldrocchi et al., 2019, 2021; Mull et al., 2012). As filter feeders, basking sharks are not expected to accumulate high levels of pollutants via food-web, but recent findings show that even low trophic position species, such as the whale shark, can bioaccumulate levels of contaminants that are comparable to predatory shark species (Boldrocchi et al., 2020). Moreover, filter feeders may act as early warning bioindicators of regional pollution, and

therefore should deserve particular attention. In this context, the aim of the present study is to investigate for the first time the presence of legacy contaminants (PCBs and DDT), trace elements, and emerging contaminants (poli- and per-fluoroalkyl substances, PFAS) in multiple tissues of basking sharks. To the best of our knowledge, the data presented here are among the first providing the status of contamination of this species by multiple chemical pollutants, and one of the few at the worldwide level providing information on PFAS bioaccumulation in sharks. As such, the results could serve as a benchmark for future evaluation of the contamination in the basking shark.

A total of 4 juvenile basking sharks were collected between June 2006 and May 2020 from the Ligurian Sea (Mediterranean Sea) as result of bycatch in trammel net (Table 1, Supplementary material).

Freeze-dried shark samples (skin, subcutaneous, muscle and liver tissues) were digested and analyzed for trace elements following the procedure reported in previous work (Spanu et al., 2020). Briefly, samples were freeze dried for 48 h using a Telstar LyoQuest freeze-drying equipment and grinded. Sample dissolution was achieved by treating ≈100 mg of each sample with 2 ml of ultrapure nitric acid,

\* Corresponding author.

E-mail address: [GINEVRA.BOLDROCCHI@UNINSUBRIA.IT](mailto:ginevra.boldrocchi@uninsubria.it) (G. Boldrocchi).

produced by sub-boiling distillation (Monticelli et al., 2019) in quartz vials in a Milestone ETHOS One microwave under the conditions reported in our previous work (Spanu et al., 2020). Samples were then diluted to 10 g with ultrapure water using a Sartorius Arium Mini Plus and analyzed by Inductively Coupled Plasma-Mass Spectrometry. The resulting concentrations were expressed as mg kg<sup>-1</sup> dry weight (dw). Quality control was performed by randomly including standard reference materials for muscle (ERM®-BB422) and liver (DOLT-5) in the digestion and analysis batches. Recoveries were not statistically different for all the certified elements as reported in our previous work (Spanu et al., 2020).

Concerning the determination of PCBs and DDTs, an aliquot of 0.5 g of freeze-dried shark samples (skin, subcutaneous, muscle and liver tissues) was extracted with 50 ml of a 1:1 v/v acetone/n-hexane mixture following Bettinetti et al. (2016). The extracted samples were then reduced to a final volume of 0.5 ml and analyzed by gas chromatography (GC Carlo Erba, Top 8000) coupled with <sup>63</sup>Ni electron capture detector (Carlo Erba, ECD 80). The temperature program followed Boldrocchi et al. (2019). Quantification was performed using external reference standards of p,p'DDT, p,p'DDE and p,p'DDD (Pestanal, SigmaAldrich, Germany) in isooctane (Carlo Erba, pesticide analysis grade), and Aroclor 1260 (Alltech, IL, USA) with the addition of PCB 28 and 118, respectively. The following congeners were determined: PCB 18, PCB 28 + 31, PCB 44, PCB 52, PCB 101, PCB 118, PCB 138, PCB 149, PCB 153, PCB 170, PCB 180, PCB 194 and PCB 209. Detection limits were 0.1 ng g<sup>-1</sup> for PCBs and 0.05 ng g<sup>-1</sup> for DDT (dw). Quality assurance of the analytical procedure was provided by analyzing the standard reference materials BCR-598 and BCR-349 (Community Bureau of Reference, Brussels) for DDTs and PCBs residues, respectively. The percentages of recovery performed in triplicate were 91.3 ± 1.1% and 102.2 ± 1.6% for PCBs, and 107.5 ± 4% (p,p'DDE), 106.2 ± 4% (p,p'DDD), and 106.2 ± 3% (p,p'DDT) for DDTs.

For the PFAS determination in tissues, few grams of pooled samples were just homogenized, weighed into a 50 ml polypropylene (PP) centrifuge tube and spiked with 100 µL of SIL-IS solution (40 µg/L) before extraction. The extraction and analysis were carried out using the method detailed in Valsecchi et al. (2021). Briefly, sonication extraction was used with acidified water and acetonitrile solution and subsequent purification with MgSO<sub>4</sub>/NaCl. To remove phospholipids, evaporated (1 ml) extracts were filtered through HybridSPE®Phospholipid Ultra cartridges. PFAS in the extract was determined by liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) coupled to a turbulent flow chromatography (TFC) for the online purification of the extracts. A procedural blank was carried out every extraction batch. LODs ranged from 0.01 to 0.1 ng g<sup>-1</sup> wet weight (ww).

The concentrations of selected trace elements in skin, subcutaneous, muscle and liver tissues are presented in Table 1. The Kruskal-Wallis

ANOVA test was used to evaluate possible differences in the mean concentration of trace elements between skin, subcutaneous and muscle tissues. Liver tissue was excluded from this analysis as only one sample was collected for this study. Skin tissue showed higher levels of Cr ( $\chi^2 = 8.35$ , df = 2,  $p = 0.015$ ), Co ( $\chi^2 = 8.35$ , df = 2,  $p = 0.015$ ), Ni ( $\chi^2 = 7.04$ , df = 2,  $p = 0.030$ ), Cu ( $\chi^2 = 6.73$ , df = 2,  $p = 0.035$ ), Zn ( $\chi^2 = 8.00$ , df = 2,  $p = 0.018$ ), Mo ( $\chi^2 = 7.5$ , df = 2,  $p = 0.023$ ), and Pb ( $\chi^2 = 8.77$ , df = 2,  $p = 0.013$ ). The general higher concentration of trace elements in skin tissue could be ascribed to external contamination, as the dermal denticles of the skin are a rough surface that may easily accumulate suspended or sediment particles (Boldrocchi et al., 2021; Corsolini et al., 2014). As a consequence, pollutants may be adsorbed onto the skin surface rather than accumulated in the tissue itself (Boldrocchi et al., 2021; Corsolini et al., 2014).

As skin might be unreliable as a proxy for the internal contamination in sharks (Boldrocchi et al., 2021), the Wilcoxon test has been applied to determine differences in trace element mean values between subcutaneous and muscle tissues. Results showed that only Cr bioaccumulated in higher concentration in subcutaneous tissue ( $\chi^2 = 5.33$ , df = 1,  $p = 0.021$ ). Certain elements measured in both tissues appeared to strongly correlate with each other. For instance, Cd levels in muscle strongly correlated to those measured in the subcutaneous tissue at the 95% significance level (Pearson's correlation,  $r = 0.99$ ,  $p = 0.0072$ ). Similar relationships were observed also for other elements, such as Mn ( $r = 0.95$ ,  $p = 0.0504$ ), Ni ( $r = 0.92$ ,  $p = 0.08$ ), Hg ( $r = 0.93$ ,  $p = 0.07$ ) and Cr ( $r = 0.86$ ,  $p = 0.14$ ), however the relationships were not significant probably due to the small sample size, which call for caution when interpreting these results. Nevertheless, this general tendency suggests that subcutaneous tissue might serve as a proxy for muscle contaminant loads in basking sharks.

The accumulation of 16 PFAS was also investigated. Among the analyzed PFAS, PFUnA, PFDoDA, PFTrDA and PFTeDA were detected in all tissues, PFDA in all except for the subcutaneous tissue, while PFHxA, PFHpA, PFOA, PFNA, C6O4, PFHxS, PFOS, FOSA, 6:2 FTS and 8:2 FTS were lower than the LODs in all samples.

This study showed a predominance of odd-chain length (PFTrDA and PFUnA) versus even-chain compounds (PFDA, PFDoDA and PFTeDA) (Fig. 1a). This supports the absence of PFCA-specific industrial pressures and that atmospheric oxidation and microbial-mediated degradation of fluorotelomers are the dominant sources of perfluoroalkyl carboxylic acids (PFCA) in the feeding environments of the sampled sharks (Casado-Martinez et al., 2021).

PFUnA accounted for a minimum of 0.18 ng g<sup>-1</sup> ww in liver tissue up to 0.61 ± 0.44 ng g<sup>-1</sup> ww in the skin, PFTrDA for a minimum of 0.24 ± 0.1 ng g<sup>-1</sup> ww in the subcutaneous (Fig. 1a). Other commonly found PFAS were represented by PFDoDA, PFTeDA and PFDA: in muscle, they accounted for 0.09 ± 0.03 ng g<sup>-1</sup> ww, 0.06 ± 0.03 ng g<sup>-1</sup> ww and 0.02 ± 0.005 ng g<sup>-1</sup> ww respectively; in skin, they accounted for 0.22 ± 0.72 ng g<sup>-1</sup> ww, 0.09 ± 0.06 ng g<sup>-1</sup> ww and 0.07 ± 0.01 ng g<sup>-1</sup> ww, respectively; in the subcutaneous tissue, they accounted for 0.07 ± 0.03 ng g<sup>-1</sup> ww, 0.02 ± 0.01% and <LOD respectively (Fig. 1a). Similarly, PFTrDA and PFUnA were the most abundant PFAS measured in the liver tissue, 0.27 ng g<sup>-1</sup> ww and 0.18 ng g<sup>-1</sup> ww respectively, followed by PFDoDA, PFTeDA and PFDA measuring 0.08 ng g<sup>-1</sup> ww, 0.05 ng g<sup>-1</sup> ww and 0.01 ng g<sup>-1</sup> ww respectively.

These results are consistent with the higher bioaccumulative abilities of long chain PFAS (C > 8) versus short-chain ones (Chynel et al., 2021; Kelly et al., 2009; Zafeiraki et al., 2019). Indeed, the bioaccumulation factor of PFAS increases with increasing carbon chain length (Lee et al., 2020), which explains the low levels of short-chain compounds found in basking sharks.

Interestingly, previous study on PFAS showed a predominance of PFOS, together with PFTrDA and PFUnA, in elasmobranchs from Greek waters (Zafeiraki et al., 2019), whereas PFOS has never been detected in any specimens or tissues in the present study. Our results are in line with the determination of PFOA and PFOS in big predator fish from the

**Table 1**

Descriptive statistics (mean ± standard deviation) of trace element concentrations (mg kg<sup>-1</sup> dw) in basking sharks based on tissue type.

Trace elements	Tissue			
	Skin	Subcutaneous	Muscle	Liver
	N = 4	N = 4	N = 4	N = 1
Cr	1.41 ± 1.02	0.41 ± 0.12	0.13 ± 0.07	0.11
Mn	4.83 ± 3.14	0.50 ± 0.38	0.33 ± 0.06	1.24
Co	0.07 ± 0.03	0.04 ± 0.02	0.01 ± 0.03	0.02
Ni	0.59 ± 0.49	0.18 ± 0.05	0.09 ± 0.03	0.06
Cu	9.26 ± 12.7	8.0 ± 10.5	0.95 ± 0.20	1.79
Zn	72.7 ± 28.9	17.8 ± 6.55	23.3 ± 0.93	29.9
As	3.13 ± 1.61	1.96 ± 0.97	2.57 ± 0.93	18.2
Se	0.67 ± 0.28	0.66 ± 0.22	0.44 ± 0.10	0.57
Mo	0.09 ± 0.06	0.04 ± 0.03	0.02 ± 0.01	0.20
Cd	0.16 ± 0.18	0.14 ± 0.18	0.03 ± 0.04	5.77
Pb	0.89 ± 0.85	0.10 ± 0.07	0.03 ± 0.02	0.06
Hg	0.27 ± 0.21	0.27 ± 0.20	0.20 ± 0.13	0.04

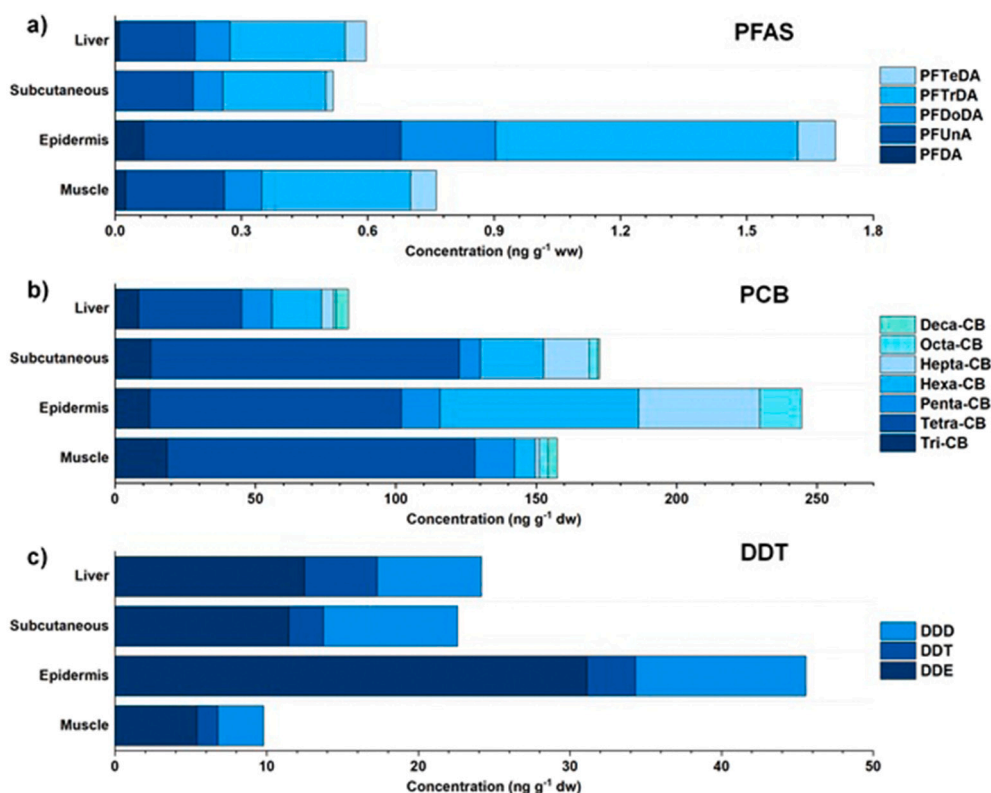


Fig. 1. Accumulation profiles of (a) PFAS, (b) PCBs and (c) DDTs in the basking shark liver ( $N = 1$ ), muscle ( $N = 4$ ), epidermis ( $N = 4$ ) and subcutaneous tissue ( $N = 4$ ) from the Mediterranean Sea.

Mediterranean Sea (Nania et al., 2009), in which these concentrations were always lower than the LOD or very small, even in elasmobranch species (Nania et al., 2009), and in swordfish caught in the Mediterranean Sea (Alessi et al., 2006).

The Kruskal-Wallis ANOVA test showed a significant difference in the mean PFAS concentrations among tissues (subcutaneous, muscle and skin), with skin accumulating higher levels compared to the other two tissue ( $\chi^2 = 7.965$ ,  $df = 2$ ,  $p = 0.0186$ ). Levels of PFAS correlated significantly for each individual contaminant between tissue pairs (Pearson's correlation,  $r = 0.88$ ,  $p < 0.0001$ ) indicating that the subcutaneous tissue might be a good proxy for muscle PFAS contamination in basking sharks.

Among the 13 PCBs investigated in this study, muscles showed higher levels of Tri-CB and Tetra-CB accounting for  $18.4 \pm 7.7$  ng g<sup>-1</sup> dw and  $110 \pm 21.1$  ng g<sup>-1</sup> dw, respectively (Fig. 1b), which is in line to what reported in shark muscles from the Indian Ocean (Boldrocchi et al., 2019). Penta-CB and Hexa-CB were found in medium levels, with a mean of  $14.1 \pm 4.4$  ng g<sup>-1</sup> dw and  $7.3 \pm 3.4$  ng g<sup>-1</sup> dw, respectively. Hepta-CB, Octa-CB and Deca-CB were the less abundant congeners accounting from  $1.5 \pm 0.6$  ng g<sup>-1</sup> dw (Hepta-CB) to  $3.2 \pm 1.5$  ng g<sup>-1</sup> dw (Octa-CB) (Fig. 1b). Subcutaneous tissue showed high levels of Tetra-CB with a mean of  $110 \pm 32$  ng g<sup>-1</sup> dw, followed by Hexa-CB, accounting for  $22.6 \pm 32.4$  ng g<sup>-1</sup> dw, Hepta-CB and Tri-CB, measuring  $16.3 \pm 33.9$  ng g<sup>-1</sup> dw and  $12.5 \pm 3$  ng g<sup>-1</sup> dw respectively. Penta-CB, Octa-CB and Deca-CB ranged from  $7.4 \pm 7$  ng g<sup>-1</sup> dw (Penta-CB) to  $0.7 \pm 0.5$  ng g<sup>-1</sup> dw (Deca-CB) (Fig. 1b). Liver tissue showed higher levels of Tetra-CB ( $36.8$  ng g<sup>-1</sup> dw), followed by Hexa ( $17.7$  ng g<sup>-1</sup> dw) and Penta-CB ( $10.8$  ng g<sup>-1</sup> dw), while less concentrations of Octa ( $1.0$  ng g<sup>-1</sup> dw), Hepta ( $4.2$  ng g<sup>-1</sup> dw) and Deca-CB ( $4.3$  ng g<sup>-1</sup> dw) were found.

Skin tissue showed great variability among specimens ranging from <LOD to  $36.1$  ng g<sup>-1</sup> dw for Tri-CB, from  $22.8$  to  $221$  ng g<sup>-1</sup> dw for Tetra-CB, from  $1.9$  to  $32.9$  ng g<sup>-1</sup> dw for Penta-CB, from <LOD to  $180$  ng g<sup>-1</sup> dw for Hexa-CB, from  $6.7$  to  $141$  ng g<sup>-1</sup> dw for Hepta-CB and

from  $0.2$  to  $33.5$  ng g<sup>-1</sup> dw for Octa-CB. Deca-CB were never detected. As reported for trace element accumulation, this great variability might be explained by skin being a kind of passive sampler for external contamination without the involvement of any internal, i.e., metabolic, process.

A significant correlation was found between each PCB congener levels measured in subcutaneous tissue with those in muscles (Pearson's correlation,  $r = 0.89$ ,  $p < 0.0001$ ) indicating that the subcutaneous tissue might be a good proxy of muscle PCBs contamination in basking sharks.

With regards to DDT, the muscle was the tissues which accumulated lower levels, with a mean of  $9.8 \pm 4.5$  ng g<sup>-1</sup> dw, while skin tissue showed higher levels, with a mean of  $45.6 \pm 69.7$  ng g<sup>-1</sup> dw and up to  $150$  ng g<sup>-1</sup> dw in a specimen. Liver and subcutaneous tissues showed intermediate concentrations accounting for  $24.2$  ng g<sup>-1</sup> dw and  $22.6 \pm 27.2$  ng g<sup>-1</sup> dw respectively.

The predominant metabolite in liver was DDE measuring  $12.5$  ng g<sup>-1</sup> dw, while DDT and DDD accounted for  $4.8$  ng g<sup>-1</sup> dw and  $6.9$  ng g<sup>-1</sup> dw, respectively (Fig. 1c). In muscles, DDE was the predominant metabolite with a mean of  $5.4 \pm 3.6$  ng g<sup>-1</sup> dw, followed by DDT,  $1.4 \pm 0.34$  ng g<sup>-1</sup> dw, and DDD,  $3.0 \pm 1.07$  ng g<sup>-1</sup> dw (Fig. 1c). In subcutaneous tissue, DDE accounted for  $11.4 \pm 18.3$  ng g<sup>-1</sup> dw, while DDD for  $8.9 \pm 8.2$  ng g<sup>-1</sup> dw and DDT for  $2.3 \pm 1.4$  ng g<sup>-1</sup> dw. Finally, in skin, DDE was the predominant metabolite with a mean of  $31.1 \pm 47.8$  ng g<sup>-1</sup> dw, followed by DDD and DDT with a mean of  $11.2 \pm 18.3$  ng g<sup>-1</sup> dw and  $3.2 \pm 3.8$  ng g<sup>-1</sup> dw respectively (Fig. 1c). No significant correlations were found between DDT levels in muscle and subcutaneous tissue.

In all the analyzed tissues, p,p'-DDE was the major metabolite reaching the highest level in epidermis with a mean value of  $26.9 \pm 49.2$  ng g<sup>-1</sup> dw, followed by liver with  $12.5$  ng g<sup>-1</sup> dw, subcutaneous and muscle with a mean levels of  $10.3 \pm 19$  ng g<sup>-1</sup> dw and  $3.9 \pm 2.6$  ng g<sup>-1</sup> dw. A p,p'-DDE/p,p'-DDT ratio can be used to establish whether degradation of DDT occurred recently or in the past. Generally, a high

ratio ( $>0.6$ ) points toward past input of DDT, due to its significant conversion to DDE at the time of analysis. A low ratio ( $<0.6$ ) points toward recent DDT input or inputs of non-degraded DDT (Yogui et al., 2003). Considering only muscles, the relatively high  $p,p'$  DDE/ $p,p'$  DDT ratio in basking sharks (0.6–3.1; mean  $1.6 \pm 1.1$ ) suggests that DDT residues in the analyzed specimens derive mainly from old DDT input.

Basking sharks, together with the whale shark and the megamouth sharks, are the only filter-feeding shark species in existence. With regards to trace element accumulation, basking sharks from the Mediterranean Sea showed considerably lower levels compared to whale sharks from the Indian Ocean. For instance, Boldrocchi et al. (2020) found Cr levels up to  $18.4 \text{ mg kg}^{-1} \text{ ww}$  in the whale shark's subcutaneous tissue from Djibouti, while basking sharks in this study were close to  $0.12 \text{ mg kg}^{-1} \text{ ww}$ , assuming 70% of moisture in the tissue (Bergés-Tiznado et al., 2015). Similarly, Ni and Cd concentrations in whale sharks were up to  $31.5$  and  $0.1 \text{ mg kg}^{-1} \text{ ww}$  respectively, while in basking sharks only  $0.05$  and  $0.04 \text{ mg kg}^{-1} \text{ ww}$ , respectively. In general, trace elements were either in line or much lower than in whale sharks from the Indian Ocean. Similarly, the megamouth sharks from Taiwan appeared to accumulate higher levels of certain toxic elements in their muscles (Ju et al., 2021). For instance, Cr, Ni and As measured  $1.63 \pm 1.25 \text{ mg kg}^{-1} \text{ ww}$ ,  $1.02 \pm 0.74 \text{ mg kg}^{-1} \text{ ww}$  and  $1.37 \pm 0.95 \text{ mg kg}^{-1} \text{ ww}$  respectively, while in this study  $0.04 \pm 0.02 \text{ mg kg}^{-1} \text{ ww}$ ,  $0.03 \pm 0.01 \text{ mg kg}^{-1} \text{ ww}$  and  $0.77 \pm 0.28 \text{ mg kg}^{-1} \text{ ww}$ , respectively.

With regards to OC contamination, only one study evaluated the presence of PCBs and DDT in this species, sharing one sample with the current study (Fossi et al., 2014), and reported mean PCBs value ( $1780 \pm 147 \text{ ng g}^{-1} \text{ lipid weight}$ , Fossi et al., 2014) comparable to those from this study ( $1007 \pm 732 \text{ ng g}^{-1} \text{ lipid weight}$ ). DDTs level from this study, however, was significantly lower ( $\chi^2 = 6.0$ ,  $df = 1$ ,  $p = 0.0143$ ) with a mean value of  $75 \pm 67.4 \text{ ng g}^{-1} \text{ lipid weight}$  versus  $2001 \pm 417 \text{ ng g}^{-1} \text{ lipid weight}$  (Fossi et al., 2014). With regards to PFAS, very few studies have been carried out on sharks in general. Comparing basking sharks to the giant devil ray, the only one filter feeder elasmobranch analyzed from the Mediterranean Sea, levels measured in this study appear to be lower. In giant devil ray,  $\Sigma\text{PFAS}$  value was  $1.5 \text{ ng g}^{-1} \text{ ww}$  in muscle (Zafeiraki et al., 2019) while in the basking sharks  $0.8 \pm 0.2 \text{ ng g}^{-1} \text{ ww}$ .

Several national, regional, and international biodiversity conservation and fisheries management measures protect the basking shark throughout the territorial waters of EU, including multiple countries within the Mediterranean region (Sims et al., 2016). For instance, the basking shark is listed on numerous international agreements, including Appendix II of the Convention on International Trade in Endangered Species, Appendices I and II of the Convention on the Conservation of Migratory Species and the CMS Migratory Sharks Memorandum of Understanding. Considering the migratory nature of this species, longevity and feeding ecology, basking sharks represent the perfect candidates to act as early warning bioindicator for regional contamination. However, since most of the samples are collected opportunistically from stranding or incidental captures, like in this study or in that by Fossi et al. (2014), at present samples size does not allow to evaluate any temporal variation or general trend in the bioaccumulation of pollutants in the Mediterranean Sea. However, as showed in this study, subcutaneous tissue could be used as a proxy for muscle contamination for most contaminants, which allows to collect samples in a non-lethal way and provide an opportunity to monitor the general contamination of legacy and emerging pollutants in the Mediterranean Sea.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2022.113466>.

#### CRediT authorship contribution statement

**Ginevra Boldrocchi:** Conceptualization, Methodology, Investigation, Experiments, Visualization, Data analysis, Formal analysis, Writing – Original Draft, Writing – review and editing, Supervision, Project administration. **Davide Spanu:** Formal analysis, Methodology, Data

analysis, Experiments, Visualization, Writing – Original Draft, Writing – review and editing. **Stefano Polesello:** Data analysis, Validation, Investigation, Resources, Writing – review and editing. **Sara Valsecchi:** Data analysis, Validation, Investigation, Resources, Writing – review and editing. **Fulvio Garibaldi:** Investigation, Validation, Writing – review and editing. **Luca Lanteri:** Investigation, Validation, Writing – review and editing. **Claudia Ferrario:** Data analysis, Writing – review and editing. **Damiano Monticelli:** Formal analysis, Methodology, Investigation, Experiments, Data analyses, Formal analysis, Resources, Writing – Original Draft, Writing – review and editing. **Roberta Bettinetti:** Validation, Resources, Writing – review and editing, Supervision.

#### 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.

#### Acknowledgments

The authors are grateful to Giulia Calogero (Menkab) and Nicola Rasore (University of Genoa) for their support during sample collection, as well as all the assisting students, in particular Gaia Cantù and Arianna Buono (University of Insubria). The authors would like also to thank Prof Carlo Dossi, Dr. Michela Mazzoni, Ms. Rossella Perna (University of Insubria) for their support during data analysis. Finally, the authors are grateful to One Ocean Foundation (<https://www.1ocean.org/>) for assisting in this project.

#### References

- Alessi, E., Tognon, G., Sinesi, M., Guerrenti, C., Perra, G., Focardi, S., 2006. Contaminazione del Mar Mediterraneo: il caso del pesce spada.
- Bergés-Tiznado, M.E., Márquez-Farías, F., Lara-Mendoza, R.E., Torres-Rojas, Y.E., Galván-Magaña, F., Bojórquez-Leyva, H., Pérez-Osuna, F., 2015. Mercury and selenium in muscle and target organs of scalloped hammerhead sharks *Sphyrna lewini* of the SE gulf of California: dietary intake, molar ratios, loads, and human health risks. Arch. Environ. Contam. Toxicol. 69, 440–452. <https://doi.org/10.1007/s00244-015-0226-8>.
- Bettinetti, R., Quadroni, S., Boggio, E., Galassi, S., 2016. Recent DDT and PCB contamination in the sediment and biota of the Como Bay (Lake Como, Italy). Sci. Total Environ. 542, 404–410. <https://doi.org/10.1016/j.scitotenv.2015.10.099>.
- 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 <https://doi.org/10.1016/j.scitotenv.2019.03.122>.
- Boldrocchi, G., Monticelli, D., Butti, L., Omar, M., Bettinetti, R., 2020. 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., 2021. 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, 112853 <https://doi.org/10.1016/j.marpolbul.2021.112853>.
- Casado-Martínez, C., Pascariello, S., Polesello, S., Valsecchi, S., Babut, M., Ferrari, B.J.D., 2021. Sediment quality assessment framework for per- and polyfluoroalkyl substances: results from a preparatory study and regulatory implications. Integr. Environ. Assess. Manag. 17, 716–725. <https://doi.org/10.1002/ieam.4412>.
- Chynel, M., Munsch, C., Bely, N., Héas-Moisano, K., Pollono, C., Jaquemot, S., 2021. Legacy and emerging organic contaminants in two sympatric shark species from Reunion Island (Southwest Indian Ocean): levels, profiles and maternal transfer. Sci. Total Environ. 751, 141807 <https://doi.org/10.1016/j.scitotenv.2020.141807>.
- Compagno, L., 2001. Sharks of the World. An annotated and illustrated catalogue of the shark species known to date. Vol. 2. In: Bullhead, Mackerel and Carpet Sharks (Heterodontiformes, Lamniformes and Orectolobiformes). FAO, Rome.
- Corsolini, S., Ancora, S., Bianchi, N., Mariotti, G., Leonzio, C., Christiansen, J.S., 2014. Organotopism of persistent organic pollutants and heavy metals in the Greenland shark *Somniosus microcephalus* in NE Greenland. Mar. Pollut. Bull. 87, 381–387. <https://doi.org/10.1016/j.marpolbul.2014.07.021>.
- Couto, A., Queiroz, N., Relvas, P., Baptista, M., Furtado, M., Castro, J., Nunes, M., Morikawa, H., Rosa, R., 2017. Occurrence of basking shark *Cetorhinus maximus* in southern portuguese waters: a two-decade survey. Mar. Ecol. Prog. Ser. 564, 77–86. <https://doi.org/10.3354/meps12007>.
- Dewar, H., Wilson, S.G., Hyde, J.R., Snodgrass, O.E., Leising, A., Lam, C.H., Domokos, R., Wraith, J.A., Bograd, S.J., Van Sommeren, S.R., Kohin, S., 2018. Basking shark (*Cetorhinus maximus*) movements in the eastern North Pacific determined using satellite telemetry. Front. Mar. Sci. 5 <https://doi.org/10.3389/fmars.2018.00163>.

- Dolton, H.R., Gell, F.R., Hall, J., Hall, G., Hawkes, L.A., Witt, M.J., 2020. Assessing the importance of Isle of Man waters for the basking shark *Cetorhinus maximus*. *Endanger. Species Res.* 41, 209–223. <https://doi.org/10.3354/esr01018>.
- Finucci, B., Duffy, C.A.J., Brough, T., Francis, M.P., Milardi, M., Pinkerton, M.H., Petersen, G., Stephenson, F., 2021. Drivers of spatial distributions of basking shark (*Cetorhinus maximus*) in the Southwest Pacific. *Front. Mar. Sci.* 8 <https://doi.org/10.3389/fmars.2021.665337>.
- 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, 17–24. <https://doi.org/10.1016/j.marenvres.2014.02.002>.
- Ju, Y.R., Chen, C.F., Chen, C.W., Wang, M.H., Joung, S.J., Yu, C.J., Liu, K.M., Tsai, W.P., Vanson Liu, S.Y., Dong, C.D., 2021. Profile and consumption risk assessment of trace elements in megamouth sharks (*Megachasma pelagios*) captured from the Pacific Ocean to the east of Taiwan. *Environ. Pollut.* 269, 116161 <https://doi.org/10.1016/j.envpol.2020.116161>.
- Kelly, B.C., Ikonou, M.G., Joel, D., Blair, B.S., Hoover, Dale, Grace, Richard, Gobas, F. A.P.C., 2009. Perfluoroalkyl contaminants in an arctic marine food web: trophic magnification and wildlife exposure. *Environ. Sci. Technol.* 43, 4037–4043. <https://doi.org/10.1021/es9003894>.
- Lee, J.W., Lee, H.K., Lim, J.E., Moon, H.B., 2020. Legacy and emerging per- and polyfluoroalkyl substances (PFASs) in the coastal environment of Korea: occurrence, spatial distribution, and bioaccumulation potential. *Chemosphere* 251, 126633. <https://doi.org/10.1016/j.chemosphere.2020.126633>.
- Miller, P.I., Scales, K.L., Ingram, S.N., Southall, E.J., Sims, D.W., 2015. Basking sharks and oceanographic fronts: quantifying associations in the north-east Atlantic. *Funct. Ecol.* 29, 1099–1109. <https://doi.org/10.1111/1365-2435.12423>.
- Monticelli, D., Castelletti, A., Civati, D., Recchia, S., Dossi, C., 2019. How to efficiently produce ultrapure acids. *Int. J. Anal. Chem.* 2019, 8–11. <https://doi.org/10.1155/2019/5180610>.
- Mull, C.G., Blasius, M.E., O'Sullivan, J.B., Lowe, C.G., et al., 2012. Heavy metals, trace elements, and organochlorine contaminants in muscle and liver tissue of juvenile white sharks, *Carcharodon carcharias*, from the Southern California Bight. In: *Global Perspectives on the Biology and Life History of the White Shark*. CRC Press, p. 75.
- Nania, V., Pellegrini, G.E., Fabrizi, L., Sesta, G., Sanctis, P.De, Lucchetti, D., Pasquale, M. Di, Coni, E., 2009. Monitoring of perfluorinated compounds in edible fish from the Mediterranean Sea. *Food Chem.* 115, 951–957. <https://doi.org/10.1016/j.foodchem.2009.01.016>.
- Rigby, C.L., Barreto, R., Carlson, J., Fernando, D., Fordham, S., Francis, M.P., Herman, K., Jabado, R.W., Liu, K.M., Marshall, A., Romanov, E., Kyne, P.M., 2021. *Cetorhinus maximus* (amended version of 2019 assessment). In: *The IUCN Red List of Threatened Species 2021: e.T4292A194720078*. <https://doi.org/10.2305/IUCN.UK.2021-1.RLTS.T4292A194720078.en>. Accessed on 22 December 2021.
- Sims, D., Fowler, S.L., Clò, S., Jung, A., Soldo, A., Bariche, M., 2016. *Cetorhinus maximus*. *The IUCN Red List of Threatened Species 2016: e.T4292A16527877*. Accessed on 22 December 2021.
- 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 <https://doi.org/10.2116/analsci.19A004>.
- Valsecchi, S., Babut, M., Mazzoni, M., Pascariello, S., Ferrario, C., De Felice, B., Bettinetti, R., Veyrand, B., Marchand, P., Polesello, S., 2021. Per- and Polyfluoroalkyl substances (PFAS) in fish from European lakes: current contamination status, sources, and perspectives for monitoring. *Environ. Toxicol. Chem.* 40, 658–676. <https://doi.org/10.1002/etc.4815>.
- Witt, M.J., Hardy, T., Johnson, L., McClellan, C.M., Pikesley, S.K., Ranger, S., Richardson, P.B., Solandt, J.L., Speedie, C., Williams, R., Godley, B.J., 2012. Basking sharks in the Northeast Atlantic: spatio-temporal trends from sightings in UK waters. *Mar. Ecol. Prog. Ser.* 459, 121–134. <https://doi.org/10.3354/meps09737>.
- Yogui, G.T., De Oliveira Santos, M.C., Montone, R.C., 2003. Chlorinated pesticides and polychlorinated biphenyls in marine tucuxi dolphins (*Sotalia fluviatilis*) from the Cananéia estuary, southeastern Brazil. *Sci. Total Environ.* 312, 67–78. [https://doi.org/10.1016/S0048-9697\(03\)00198-0](https://doi.org/10.1016/S0048-9697(03)00198-0).
- Zafeiraki, E., Gebbink, W.A., van Leeuwen, S.P.J., Dassenakis, E., Megalofonou, P., 2019. Occurrence and tissue distribution of perfluoroalkyl substances (PFASs) in sharks and rays from the eastern Mediterranean Sea. *Environ. Pollut.* 252, 379–387. <https://doi.org/10.1016/j.envpol.2019.05.120>.