DOCTORAL THESIS

PERSISTENT ORGANIC POLLUTANTS (POPs) IN MARINE SPECIES

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1. Summary/Resumen

Summary

In the last years, there has been an increasing acknowledgment of the vital need to preserve the health of our oceans and the myriad life forms that rely on them. In light of this, the United Nations has included the conservation and the sustainable use of oceans and marine resources into its 17 sustainable development goals (United Nations, 2015). At the same time, with the global recognition of the detrimental impacts caused by anthropogenic pollution on marine ecosystems, the United Nations has issued a call for action, urging the prevention and significant reduction of all kinds of marine pollution by 2025.

It is noteworthy the potential risk to wildlife, in particular for species that face multiple anthropogenic stressors and/or susceptible to accumulate high levels of toxic substances, such as persistent organic pollutants (POPs). Despite being banned for several years, stable and high levels of legacy POPs, such as polychlorinated biphenyls (PCBs), continue to be reported in top predator species, including some cetacean populations (Jepson et al., 2016; Law and Jepson, 2017; Sonne et al., 2018; Stuart-Smith and Jepson, 2017). In certain instances, these documented levels seem to pose substantial risks at both the individual and population levels (Desforges et al., 2018). Simultaneously, specific regions, such as the Mediterranean Sea and to lesser extent the North East Atlantic, have been considered highly polluted areas, where wildlife populations and the entire ecosystems seem to be particularly impacted by the occurrence of these substances (Andersen et al., 2022; Benn et al., 2010; Carpenter, 2019; Danovaro, 2003; El-Kholy et al., 2012; Halpern et al., 2008; Marsili et al., 2018; Merhaby et al., 2019; Nash et al., 2023; Ramos-Miras et al., 2019; Sharma et al., 2021).

All these facts have raised concerns regarding the potential ineffectiveness of previously implemented reduction and elimination measures, while also emphasizing the importance of ongoing monitoring efforts and deeper comprehension of the mechanisms of toxicity, especially in species and regions particularly affected. Within this context, the use of sentinel species capable of providing information about the chemical contamination status of the ecosystems has evolved into a fundamental tool.

Species such as the sperm whale (*Physeter macrocephalus*), beaked whale (*Ziphius cavirostris*), and blue shark (*Prionace glauca*) are recognized as valuable indicators of marine environmental pollution (Alves et al., 2022; Bossart, 2011; Fossi and Panti, 2017). Numerous studies from diverse geographical regions have documented concentrations of POPs in the tissues of these species, sometimes surpassing established toxicity thresholds (Alves et al., 2016; Bachman et al., 2014; de Azevedo e Silva et al., 2007; Evans et al., 2004; Godard-Codding et al., 2011; Knap and Jickells, 1983; Law et al., 2005; Lee et al., 2015b, 2015a; Madgett et al., 2022; Menezes-Sousa et al., 2021; Pinzone et al., 2015; Praca et al., 2011; Storelli et al., 2011b, 2005). It is crucial to emphasize that these species also face a myriad of anthropogenic stressors, including climate change, habitat degradation, acoustic pollution, food scarcity, fishing pressure, collisions, and accidental captures, among others (Cañadas and Notarbartolo Di Sciara, 2018; Notarbartolo Di Sciara, 2014; Sims et al., 2015). Therefore, in the pursuit of safeguarding and preserving marine biodiversity, continual monitoring of pollution levels is paramount, alongside a nuanced understanding of species-specific toxicity mechanisms.

Information regarding the extent of chemical contamination in Mediterranean populations of sperm whales and beaked whales is highly valuable and essential, given the current scarcity of data on this topic. Both populations are undergoing a demographic decline in the Mediterranean region (Cañadas et al., 2018; Podestà et al., 2016); however, the lack of studies prevents an effective evaluation of the potential relationship between this decline and contamination by POPs.

The assessment of exposure to anthropogenic pollutants in sentinel species can also have significant implications for public health, especially in the case of species considered important food sources for humans (Bocio et al., 2007; Gómara et al., 2005; Llobet et al., 2003; Massone et al., 2023; Mikolajczyk et al., 2021; Miniero et al., 2014). In this context, evaluating POP levels in edible species such as sardines (*Sardina pilchardus*), anchovies (*Engraulis encrasicolus*), and bogue (*Boops boops*) in the Mediterranean Sea, and to a lesser extent in the blue shark from the Northeast Atlantic, not only provides data on the pollution status of two important fishing areas, but also sheds light on potential risks to human health from consuming these species.

All the information presented above supports the fundamental objectives of this doctoral thesis, which are:

- To evaluate the levels of POPs in sentinel species inhabiting the Mediterranean Sea and the North East Atlantic Ocean while exploring the potential health risks associated to these species.

- To investigate POP concentrations in important edible fish species (sardines, bogues and anchovies) in order to assess possible risks to human health derived from their consumption.

- To study tissue distribution (muscle and liver) of targeted POPs in blue shark.

- To investigate spatial and temporal trends of POP pollution in both study areas, critically contrasting them with available bibliographic information.

To address the main objectives of this thesis, 61 POPs including 18 polychlorinated biphenyls (PCBs), 26 polybrominated diphenyl ethers (PBDEs), and 17 polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/Fs) have been analyzed and quantified in 31 adipose tissue samples, 108 muscle samples, and 60 liver samples from six different marine species. Specifically, the studied species were sperm whale (n=9), beaked whale (n=22), sardine (n=16), bogue (n=16) and anchovy (n=16), sampled between 2008 and 2017 in different areas of the Mediterranean Sea (Adriatic Sea, Ligurian Sea, and Tyrrhenian Sea), and blue shark (n=60) accidentally captured in coastal waters of Portugal in 2019. For the two cetacean species, analysis was conducted on subcutaneous blubber from stranded individuals (sperm whale) and free-ranging individuals (beaked whale). Regarding the three fish species, the analyses were performed on muscle tissue, while for the blue shark both muscle and liver tissues were investigated.

Fresh (sperm whales, Cuvier's beaked whales, blue sharks) and freeze-dried samples (sardines, anchovies, bogues) were weighed, homogenized with anhydrous sodium sulfate (Na_2SO_4) and spiked with a suite of ^{13}C-labeled standards of target contaminants.
After surrogate addition, samples were Soxhlet extracted for 24h with ~100mL of n-hexane: DCM (9:1) mixture. Extracts were concentrated and purified by using the automated sample preparation system DEXTech+ (LCTech GmbH, Dorfen, Germany) (sperm whales, Cuvier's beaked whales, blue sharks) or by low-pressure chromatography on open columns filled with multilayer-silica gel (sardinas, bogas, anchoas). Final extracts were reconstituted in a few microliters of ^{13}C-

labeled injection standards prior to instrumental analysis. The detection and quantification of POPs were carried out using gas chromatography (GC) coupled with high-resolution mass spectrometry (HRMS). For the quantification of analytes was employed the isotopic dilution technique.

This doctoral thesis has shed light on several key aspects of the presence of POPs in a selection of marine species. The general conclusion of this work emphasizes the pivotal role played by sperm whales, Cuvier's beaked whales, blue sharks, and three fish species as bioindicators of POP pollution in the study areas. These findings specifically underscore the Mediterranean Sea as a contamination hotspot for marine mammals and draw attention to the elevated levels, occasionally indicating an upward trend, in the northeastern region of the Atlantic Ocean.
The specific conclusions of the different studies carried out are detailed below:

- Levels of POPs found in Mediterranean sperm whales tended to be higher than those reported in the same species worldwide. All sperm whales analyzed surpassed the threshold for WHO-TEQs proposed as starting point of immunosuppression in marine mammals (harbour seals).

- POP levels described in Mediterranean Cuvier's beaked whales tended to exceed those observed in the same species globally. The increase in pollutant concentrations in the adipose tissue of males as they age, confirms the bioaccumulation capacity of POPs. At the same time, stable or increased POP levels with age in males and decline in sexually mature females Cuvier's beaked whales confirmed offspring transfer through gestation and lactation. Ultimately, 80% of Cuvier's beaked whales were above the threshold for PCBs responsible for physiological effects.

- PCB levels found in edible fish species (sardines, anchovies and bogues) were generally not different from values reported for the same species and in the same area in recent years. This underpinned the concept of a virtual halt or very slow decline in PCB concentrations in the Mediterranean Sea. Sardines, anchovies and bogues showed concentrations of PCBs in full compliance with European regulated concentrations in diet; consequently, the PCB content of these edible species seemed not to constitute a current hazard for human health.

- Levels of PCBs and PCDD/Fs in blue shark showed relatively steady concentrations, whereas greater loads of PBDEs were detected when comparing with previous studies for the same area (North East Atlantic Ocean). This could be the consequence of the recent and incomplete regulations on PBDEs as well as contamination deriving from plastics and microplastics. PCB and PBDE concentrations in blue shark were below the lowest thresholds identified in marine mammals. PCB and PCDD/F levels detected in blue shark muscle (the most consumed part) were within the acceptable European legal limits; however, the majority of blue shark liver samples exceeded the limits for i-PCBs and WHO-TEQs, which becomes significant when considering the potential implications for the consumption of products derived from shark liver oil. Greater accumulation of PCBs, PBDEs, and PCDD/Fs observed in liver compared to muscle, suggested differences in the toxicokinetics of POPs between the two tissues investigated in blue shark.

Monitoring of legal and emerging POPs is crucial to understand the trends of POPs and to evaluate the efficacy of the reduction and elimination measures implemented so far.

By concurrently exploring toxicity thresholds, we were able to gain insights into the potential risks and adverse effects linked to the observed levels in the species under study. It is clear that gaining deeper insights into the impact of pollution on the health of wildlife, particularly those facing multiple anthropogenic stressors, such as the relatively understudied Cuvier's beak whale, is essential. Additionally, continuous and effective monitoring of POPs in edible fish species is essential for the assessment of risk associated with the consumption of POP-contaminated fish. Toxicity risk assessment represents a crucial tool for regulatory authorities when establishing guidelines and limits for POPs in food to ensure food safety and protect public health.

Additionally, our study highlights the significance of exploring species-specific behaviors in the context of pollutant exposure. The variability observed in data from different species underscore the necessity for further investigation.

As an overarching conclusion, these interconnected findings justify the ongoing concern about POPs and highlight the urgency of addressing chemical pollution as a significant threat to marine species. This, in turn, emphasizes the critical importance of continuous research and monitoring of

POPs, to support conservation initiatives aimed to safeguard our oceans as essential components of global health.

Resumen

En los últimos años ha crecido la conciencia sobre la necesidad de preservar la salud de nuestros océanos y la miríada de formas de vida que dependen de ellos. En este sentido, la conservación y el uso sostenible de los océanos y los recursos marinos se han incluido entre los 17 objetivos de desarrollo sostenible de las Naciones Unidas (United Nations, 2015). Al mismo tiempo, ante el reconocimiento global de los impactos negativos causados por la contaminación antropogénica en los ecosistemas marinos, las Naciones Unidas han emitido una llamada a la acción, exhortando a la prevención y reducción significativa de todo tipo de contaminación marina para el año 2025.
Es importante destacar el riesgo potencial para la fauna silvestre, en particular para las especies que se enfrentan a múltiples factores de estrés antropogénico y/o son susceptibles de acumular altos niveles de sustancias tóxicas, como los contaminantes orgánicos persistentes (COP). A pesar de estar prohibidos desde hace varios años, diferentes especies de depredadores terminales, incluyendo algunas poblaciones de cetáceos, siguen mostrando niveles estables y elevados de COP tradicionales, entre ellos los bifenilos policlorados (PCB) (Jepson et al., 2016; Law and Jepson, 2017; Sonne et al., 2018; Stuart-Smith and Jepson, 2017). En algunos casos, los niveles documentados parecen representar un importante riesgo para estas especies, tanto a nivel individual como poblacional (Desforges et al., 2018). Al mismo tiempo, algunas regiones, entre ellas el mar Mediterráneo y en menor medida el Atlántico Nordeste, se consideran áreas altamente contaminadas, donde las poblaciones de fauna silvestre y los ecosistemas parecen particularmente afectados por la presencia de estas sustancias (Andersen et al., 2022; Benn et al., 2010; Carpenter, 2019; Danovaro, 2003; El-Kholy et al., 2012; Halpern et al., 2008; Marsili et al., 2018; Merhaby et al., 2019; Nash et al., 2023; Ramos-Miras et al., 2019; Sharma et al., 2021).

Todas estas circunstancias han suscitado preocupación con respecto a la posible ineficacia de las medidas de reducción y eliminación implementadas hasta hoy y, al mismo tiempo, han resaltado la importancia de los esfuerzos en la monitorización de estas sustancias, así como de una comprensión más profunda de sus mecanismos de toxicidad, especialmente en especies y

regiones particularmente afectadas. Es por esto que el uso de especies centinela capaces de proporcionar información sobre el estado de contaminación de los ecosistemas se ha convertido en una herramienta fundamental.

Especies como el cachalote (*Physeter macrocephalus*), el zifio (*Ziphius cavirostris*) y el tiburón azul (*Prionace glauca*) se consideran buenos indicadores del estado de contaminación del ambiente marino (Alves et al., 2022; Bossart, 2011; Fossi and Panti, 2017). Así, diversos estudios de diferentes áreas geográficas han reportado niveles de COP en tejidos de estas especies, en algunos casos por encima de los umbrales de toxicidad conocidos (Alves et al., 2016; Bachman et al., 2014; de Azevedo e Silva et al., 2007; Evans et al., 2004; Godard-Codding et al., 2011; Knap and Jickells, 1983; Law et al., 2005; Lee et al., 2015b, 2015a; Madgett et al., 2022; Menezes-Sousa et al., 2021; Pinzone et al., 2015; Praca et al., 2011; Storelli et al., 2011b, 2005). Es esencial destacar que estas especies se enfrentan, además, a múltiples factores de estrés antropogénico, que incluyen el cambio climático, la degradación de sus hábitats, la contaminación acústica, la disminución de alimentos, la presión pesquera, las colisiones y capturas accidentales, entre otros (Cañadas and Notarbartolo Di Sciara, 2018; Notarbartolo Di Sciara, 2014; Sims et al., 2015). Por lo tanto, en un contexto de salvaguarda y protección de la biodiversidad marina, resulta de fundamental importancia la monitorización continua de los niveles de contaminación química, así como una mejor comprensión de los mecanismos de toxicidad específicos para cada especie.
Los datos sobre el grado de contaminación química de las poblaciones mediterráneas de cachalote y de zifio son muy valiosos y necesarios, ya que la información disponible al respecto es muy escasa actualmente. Estas dos poblaciones están experimentando una reducción demográfica en la región del Mediterráneo (Cañadas et al., 2018; Podestà et al., 2016); sin embargo, la carencia de estudios al respeto no permite evaluar eficaciemente la posible relación entre esta disminución y la contaminación por COP.

La evaluación de la exposición a contaminantes antropogénicos en especies centinela puede tener implicaciones significativas también para la salud pública, especialmente en el caso de especies que son consideradas fuentes de alimento importantes para el ser humano (Bocio et al., 2007; Gómara et al., 2005; Llobet et al., 2003; Massone et al., 2023; Mikolajczyk et al., 2021; Miniero et al., 2014). En este contexto, evaluar los niveles de COP en especies de consumo humano como la sardina (*Sardina pilchardus*), la anchoa (*Engraulis encrasicolus*) y la boga (*Boops boops*) en el mar

Mediterráneo, y en menor medida en el tiburón azul del Atlántico Nordeste, no solo proporciona datos acerca del estado de contaminación de dos importantes áreas de pesca, sino que también arroja luz sobre los posibles riesgos para la salud humana derivados del consumo de estas especies.

Toda la información expuesta anteriormente respalda los objetivos fundamentales de esta tesis doctoral que son:

- Evaluar los niveles de contaminación por COP en especies centinela residentes en el mar Mediterráneo y en el Atlántico Nordeste, así como explorar los posibles riesgos para la salud de estas especies.

- Investigar las concentraciones de los COP en especies comestibles de relevancia (sardinas, anchoas y bogas), con el fin de evaluar los posibles riesgos para la salud humana derivados de su consumo.

- Estudiar la distribución tisular (músculo y hígado) específica de diferentes COP en el tiburón azul.

- Investigar las tendencias espaciales y temporales de la contaminación por COP en las dos áreas de estudio, contrastándolas críticamente con la información bibliográfica disponible.

Para abordar los objetivos principales de esta tesis, se han analizado y cuantificado 61 COP, entre ellos 18 policlorobifenilos (PCB), 26 polibromodifenil éteres (PBDE) y 17 policlorodibenzo-p-dioxinas y policlorodibenzofuranos (PCDD/F) en 31 muestras de tejido adiposo, 108 muestras de músculo y 60 muestras de hígado de 6 especies marinas diferentes. Específicamente, las especies objeto de estudio han sido el cachalote (n=9), el zifio (n=22), la sardina (n=16), la boga (n=16) y la anchoa (n=16), muestreadas entre 2008 y 2017 en diferentes áreas del mar Mediterráneo (mar Adriático, mar de Liguria y mar Tirreno), y el tiburón azul (n=60) accidentalmente capturado en las aguas costeras de Portugal, en 2019. En las dos especies de cetáceos se ha analizado la grasa subcutánea procedentes de animales varados (cachalote) y en libertad (zifio). En el caso de las tres especies de pescado, el tejido objeto de estudio ha sido el musculo, mientras que en el tiburón azul se han analizado dos tejidos distintos, el musculo y el hígado.

Las muestras frescas (cachalotes, zifios, tiburones azules) y las muestras liofilizadas (sardinas, bogas, anchoas) han sido pesadas, homogeneizadas con sulfato de sodio anhidro (Na_2SO_4) y fortificadas con una serie de patrones marcados isotópicamente con ^{13}C.

Posteriormente, las muestras fueron sometidas a extracción Soxhlet durante 24 horas utilizando aproximadamente 100 mL de una mezcla de n-hexano: DCM (9:1). Los extractos resultantes fueron concentrados y purificados utilizando el sistema automatizado de preparación de muestras DEXTech+ (LCTech GmbH, Dorfen, Alemania) (cachalotes, zifios, tiburones azules) o mediante cromatografía líquida a baja presión en columnas con sílice multicapa (sardinas, bogas, anchoas). Antes del análisis instrumental, los extractos finales se reconstituyeron entre 10-20 µL de estándares de inyección marcados con ^{13}C. La detección y cuantificación de los COP se llevaron a cabo mediante la técnica de cromatografía de gases (GC) acoplada a espectrometría de masas de alta resolución (HRMS). Para la cuantificación de los analitos, se utilizó la técnica de dilución isotópica.

Los análisis, resultados y discusiones presentados en esta tesis doctoral han proporcionado información valiosa sobre varios aspectos clave relacionados con la presencia de COP en diferentes especies marinas. La conclusión general de esta tesis subraya el papel crucial desempeñado por el cachalote, el zifio, el tiburón azul y las tres especies de peces como bioindicadores de la contaminación por COP en las áreas de estudio. Estos hallazgos resaltan específicamente el mar Mediterráneo como un punto crítico de contaminación para los mamíferos marinos y llaman la atención sobre los niveles elevados, y ocasionalmente en aumento, en la región noreste del Océano Atlántico.

A continuación se presentan las conclusiones específicas de los diferentes estudios realizados:

- Los niveles de COP encontrados en los cachalotes del mar Mediterráneo tienden a ser más altos que los niveles reportados en la misma especie a nivel mundial. Todos los cachalotes analizados superan el límite máximo de WHO-TEQ/g propuesto para la inmunosupresión en mamíferos marinos (foca común).

- Los niveles de COP detectados en los zifios del mar Mediterráneo son generalmente mas elevados que los registrados en dicha especie a escala global. El aumento de las concentraciones de contaminantes en el tejido adiposo de los zifios machos, a medida que aumenta su edad, confirma la capacidad de bioacumulación de los COP. Al mismo tiempo, la estabilidad o el aumento de los niveles de COP con la edad en los machos y la disminución en las hembras sexualmente maduras confirma la transferencia a la progenie a través de la gestación y la lactancia. Finalmente, el 80% de los zifios analizados están por encima del umbral de efectos fisiológicos para PCB.

- Los niveles de PCB encontrados en las sardinas, bogas y anchoas generalmente no difieren de los valores reportados para las mismas especies y en la misma área en años recientes. Esto respalda la idea de un estancamiento aparente o un declive muy lento en las concentraciones de PCB en el mar Mediterráneo. No obstante, las concentraciones en sardinas, bogas y anchoas han resultado en pleno cumplimiento con los valores establecidos a nivel europeo para el consumo humano; en consecuencia, el contenido de PCB en estas especies comestibles no parece representar un riesgo actual para la salud humana.

- Los PCB y PCDD/F en el tiburón azul han mostrado concentraciones relativamente estables, mientras que se han detectado niveles más elevados de PBDE en comparación con estudios previos en la misma área (Atlántico Nordeste). Esta situación podría ser el resultado de regulaciones recientes e incompletas sobre esta familia de COP, así como de la contaminación proveniente de plásticos y microplásticos. Las concentraciones de PCB y PBDE en el tiburón azul han resultado por debajo de los umbrales de toxicidad más bajos establecidos en mamíferos marinos. Las concentraciones de PCB y PCDD/F detectadas en el músculo (la parte más consumida) estan dentro de los límites legales europeos aceptables; sin embargo, la mayoría de las muestras de hígado superan los límites para i-PCBs y WHO-TEQs, lo que cobra importancia al considerar las posibles implicaciones para el consumo de productos derivados del aceite de hígado de tiburón. La mayor acumulación de algunos PCB, PBDE y PCDD/F, observada en el hígado en comparación con el músculo, sugiere la existencia de diferencias en la toxicocinética de los COP en los dos tejidos analizados.

Las actividades de monitorización son fundamentales para comprender las tendencias reales de los COP y evaluar la eficacia de las medidas de reducción y eliminación implementadas hasta la fecha.

Gracias al estudio de los umbrales de toxicidad, hemos adquirido información sobre los potenciales riesgos y efectos adversos vinculados a los niveles observados en las especies objeto de estudio. Resulta fundamental profundizar en la comprensión del impacto de la contaminación en la salud de determinadas especies, especialmente aquellas que enfrentan diversos factores de estrés antropogenicos, como es el caso del zifio, cuya investigación ha sido hasta ahora relativamente limitada. Al mismo tiempo, la monitorización continua y efectiva de estos contaminantes en especies de pescado de interés comercial es esencial para la evaluación de los riesgos asociados con el consumo de pescado. Esto posibilita la adopción de decisiones bien fundamentadas con respecto a las medidas de salud pública. Además, la evaluación de riesgo desempeña un papel fundamental como herramienta crucial para las autoridades regulatorias al establecer directrices y límites en relación a la presencia de COP en los alimentos, con el fin de proteger la salud pública garantizando la seguridad alimentaria.

Por otro lado, este estudio destaca la importancia de explorar los comportamientos específicos de las especies en el contexto de la exposición a la contaminación. La variabilidad observada en los datos de diferentes especies subraya la necesidad de una mayor investigación en este ámbito.

Los resultados derivados de esta tesis doctoral justifican la constante preocupación acerca de los COP y destacan la urgencia de abordar la contaminación química como una amenaza significativa para las especies marinas. Esto, a su vez, enfatiza la importancia vital de la investigación y la monitorización constantes sobre contaminate químicos, y particularmente COP, que ayuden a respaldar iniciativas de conservación destinadas a proteger nuestros océanos, componentes esenciales de la salud global del planeta.

2. Introduction

In the last years, there has been an increasing acknowledgment of the vital need to preserve the health of our oceans and the myriad life forms that rely on them. In light of this, the United Nations has included the conservation and the sustainable use of oceans and marine resources into its 17 sustainable development goals (United Nations, 2015). At the same time, with the global recognition of the detrimental impacts caused by anthropogenic pollution on marine ecosystems, the United Nations has issued a call for action, urging the prevention and significant reduction of all kinds of marine pollution by 2025.

It is noteworthy the potential risk to wildlife, in particular for species that face multiple anthropogenic stressors and/or susceptible to accumulate high levels of toxic substances, such as persistent organic pollutants (POPs). Despite being banned for several years, stable and high levels of legacy POPs, such as polychlorinated biphenyls (PCBs), continue to be reported in top predator species, including some cetacean populations (Jepson et al., 2016; Law and Jepson, 2017; Sonne et al., 2018; Stuart-Smith and Jepson, 2017). In certain instances, these documented levels seem to pose substantial risks at both the individual and population levels (Desforges et al., 2018). Simultaneously, specific regions, such as the Mediterranean Sea and to lesser extent the North East Atlantic, have been considered highly polluted areas, where wildlife populations and the entire ecosystems seem to be particularly impacted by the occurrence of these substances (Andersen et al., 2022; Benn et al., 2010; Carpenter, 2019; Danovaro, 2003; El-Kholy et al., 2012; Halpern et al., 2008; Marsili et al., 2018; Merhaby et al., 2019; Nash et al., 2023; Ramos-Miras et al., 2019; Sharma et al., 2021).

All these facts have raised concerns regarding the potential ineffectiveness of previously implemented reduction and elimination measures, while also emphasizing the importance of ongoing monitoring efforts and deeper comprehension of the mechanisms of toxicity, especially in species and regions particularly affected. Within this context, the use of sentinel species capable of providing information about the contamination status of the ecosystems has evolved into a fundamental tool.

2.1. Persistent Organic Pollutants (POPs)

Persistent organic pollutants (POPs) are a group of toxic chemicals that attract much attention because of their combination of physical-chemical properties and complex effects. In general, they became widely employed during the boom of industrial production after the Second World War (Jones, 2021). Although, in the 1960s, the society started to become aware of the detrimental consequences associated with their use. This growing awareness was catalyzed by the book *Silent Spring* authored by Rachel Carson, which exposed the harmful impact of pesticides on the environment and wildlife. It was during this period that crucial information regarding the presence and adverse effects of POPs came to light, prompting regulatory actions that led to their regulation and banning (Fernández and Grimalt, 2003; Jones, 2021).

Common classes of POPs are families of chlorinated and brominated aromatic compounds, such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), polybrominated diphenyl ethers (PBDEs) and different organochlorine pesticides (e.g. DDT and its metabolites, toxaphene, chlordane, etc.). Some of these compounds are unintentional by-products of combustion or industrial synthesis of other chemicals, such as PCDD/Fs. Others have been manufactured for industrial purposes, such as PCBs, chlorinated paraffins (CPs), and PBDEs, or for agricultural use as pesticides or herbicides, like DDT, lindane, and chlordane. We refer to these contaminants as legacy POPs because their presence in the environment is a “legacy” of previous releases (Rigét et al., 2010). On the other hand, to replace these substances other chemicals have been produced and released into the environment in subsequent years. These chemicals are called “emerging contaminants” because are considered of emerging concern and some are not yet regulated or included in monitoring programs (Lapworth et al., 2012). This is the case of various new brominated flame retardants (BFRs), including pentabromotoluene (PBT) and hexabromobenzene (HBB). Presence and increased levels in recent years of emerging contaminants have been reported for instance in some marine mammal species (Andvik et al., 2023).

POPs are characterized by their resistance to degradation, allowing them to persist in the environment for long periods (Jones and de Voogt, 1999). Nonetheless, they can undergo degradation through a range of processes such as aerobic and anaerobic biodegradation (Abramowicz, 1990; Xiang et al., 2020), atmospheric reactions (Mandalakis et al., 2003; Totten et al., 2002), and abiotic transformations (Xu et al., 2021). Sometimes degradation is responsible for the production of toxic metabolites, as in the case of hydroxylated polychlorinated biphenyls (OH-

PCBs), which result from the oxidation of PCBs and are more toxic than the parent compounds (Tehrani and Van Aken, 2014).

POPs are able to persist in various phases and spread throughout different environmental compartments. They can be found in the atmosphere in gas or particle phases, or sometimes both simultaneously (Jones, 2021). Similarly, in water they can exist in dissolved or particulate states, or both (Fernández et al., 2005). This means that they are subjected to long-range transport (LRT) throughout oceans and the atmosphere; but, at the same time, they can remain close to their sources (Jurado and Dachs, 2008; Scheringer, 2009). Actually, the highest concentrations are usually reported at mid-latitudes, where the majority of chemical use and production have taken place (Gasic et al., 2010). On the other hand, many POPs are semi-volatile and have the ability to undergo exchanges between the atmosphere and various environmental surfaces, producing cycles of evaporation and condensation. This repeated process of volatilization and condensation is known as the grasshopper effect (Gouin et al., 2004; Wania and MacKay, 1996), and it is responsible for the presence of these contaminants in remote areas such as the poles, where they have never been produced or used (Kallenborn, 2006).

POPs are, for the most part, hydrophobic and lipophilic substances (Gaur et al., 2022). This means that in aquatic environments and soils they exhibit a strong affinity to solids, in particular to the organic matter, and tend to avoid aqueous phases; as such, in organisms they preferentially partition into lipids instead of entering the watery environment of cells (Langenbach, 2013). As a result, POPs become stored in fatty tissues with some degree of persistence due to the slowness of metabolism (Daley et al., 2014). At the same time they can biomagnify along the food webs reaching the highest concentrations in top predator species such as orcas (*Orcinus orcas*), which is in fact considered the most contaminated species on earth (Jepson et al., 2016; Kelly et al., 2007).

There is no doubt about the benefits brought by these chemicals, such as pest and disease control, improved standards of living and hygiene, as well as enhancements in certain products' characteristics. On the other hand, as stated by Jones (2021), the development of sensitive analytical methods and the creation of biological monitoring schemes/archives during the 1960/70s have shifted attention to the negative aspects arising from the extensive use of these substances. Since then,the scientific community started to report high levels of POPs and their associated toxicological impacts, especially in top predator species (Peakall and Kiff, 2008; Risebrough et al., 1968). In the 1960s, Rachel Carson revealed the damaging effects of the indiscriminate use of chemical pesticides on the environment and emphasized the need of environmental regulation (Went and Carson, 1963). Specifically, Carson's book *Silent Spring* shed light on the unintended consequences of DDT and other pesticides on non-target organisms and the environment, leading to a greater understanding of the dangers posed by these substances. She stated the case of peregrine falcons exposed to DDT through consumption of contaminated prey and severely affected by the presence of this substance in their tissues. The consequences were so severe that the peregrine falcon populations in many regions, including Northern Europe and North America, faced the risk of extinction (Coristine and Kerr, 2011; Schwarz et al., 2016). Subsequently, DDT was banned or severely restricted in many countries, including the United States, which allowed for the recovery of peregrine falcon populations (Ambrose et al., 2016; Nygård et al., 2019). Another notable case is that of beluga whales (*Delphinapterus leucas*) living in the St. Lawrence Estuary, which are considered one of the most contaminated marine mammals species (Lebeuf et al., 2004; Raach et al., 2011). The apparent failure of its population recovery is attributable to the high levels of various POPs in their tissues (De Guise et al., 1995). As a matter of fact, their bodies were classified as “hazardous waste” when found on the shore, due to their high levels of chemical substances such as DDT and PCBs (Beland, 1996). In addition, some industrial accidents and mass poisonings such as the Yusho (Japan) and Seveso (Italia) episodes, as well as Agent Orange contamination, occurred during the 1960/70s resulting in severe health effects on humans. These effects included respiratory problems, skin disorders, neurological disorders, and even cancer (Guo et al., 2019; Stone, 2007).

As the scientific community has continually reported evidence regarding the worldwide presence and negative impacts of POPs on both humans and wildlife, countries and nations took individual actions and voluntarily started to phase out or restrict some POPs (Jones, 2021). Yet, it was in May 2001 when nations signed the Stockholm Convention, the first truly global cooperation on environmental issues with the objective “to protect human health and the environment from the effects of persistent organic pollutants (POPs)” (UNEP, 2001). In the following years, after voluntary bans and the enforcement of the Stockholm Convention, monitoring programs have shown declines in the concentrations of various POPs in the environment, humans and wildlife (Fång et al., 2015; Jepson et al., 2016; Jepson and Law, 2016; Stuart-Smith and Jepson, 2017).

On the other hand, in the last years different studies still report high and potentially dangerous levels of some legacy POPs in different top predator species (Jepson et al., 2016; Jepson and Law, 2016; Stuart-Smith and Jepson, 2017). PCB concentrations continue to be a significant factor contributing to the decline of cetacean populations in Europe and possibly other top predators in marine ecosystems worldwide (Desforges et al., 2018; Jepson et al., 2016). At the same time, high and potentially toxic levels of PBDEs are still reported in some cetacean species with a global scale impact (Alonso et al., 2014; Bartalini et al., 2022). In addition, studies continue to report the presence and negative effects of emerging POPs such as PFAS (Thilagam and Gopalakrishnan, 2022), CPs (Chen et al., 2023; Wang et al., 2019) and dechlorane plus (DP) (Berger et al., 2023; B. Li et al., 2020; Zafar et al., 2020) in wildlife and humans. In that context, some authors have recently estimated an increase of 66% of human deaths caused by pollution (ambient air pollution and toxic chemical pollution) since 2000, and called for urgent global action to reduce contamination (Fuller et al., 2022). All these facts draw attention to the possible inefficiency of the reduction and elimination measures adopted insofar, and represent a stark reminder of the pressing concern for the management of POPs and for global health protection (Law and Jepson, 2017; Wang et al., 2021).

2.1.1. Polychlorinated biphenyls (PCBs)

Physical and chemical properties

Polychlorinated biphenyls (PCBs) (chemical formula = $C_{12}H_{(10-n)}Cl_n$, where n ranges from 1 to 10) are a family of 209 congeners consisting of two connected benzene rings and 1-10 chlorine atoms. Each congener has a unique arrangement of chlorine atoms, contributing to its specific properties and behavior and directly affecting their persistence, bioaccumulation and toxicity.

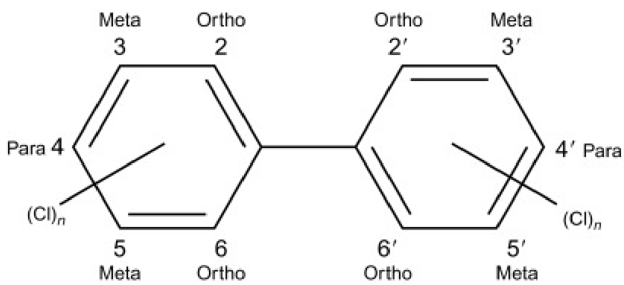


Figure 1. Chemical structure of PCBs (with 1 ≤ n ≤ 10).

Based on the number of chlorine atoms, PCB congeners can be divided into:

- **low-chlorinated PCBs**: when contain four or fewer chlorine substituents. Semi-volatile compounds that undergo rapid metabolism and often characterized by relatively short half-lives.
- **high-chlorinated PCBs**: when contain more than four chlorine atoms. Relatively non-volatile, and more persistent than low-chlorinated PCBs, thanks to their resistance to metabolic degradation.

Because of their molecular configuration, PCBs exhibit hydrophobic properties and are highly resistant to chemical reactions (Shiu and Mackay, 1986). As a consequence of their hydrophobic nature, PCBs have low solubility in water and possess elevated octanol-water partition coefficients (K_{ow}) (Shiu and Mackay, 1986). These range from 3.95 to 8.09, with an increasing value as the number of chlorine substituents increases from mono- to deca-chlorinated (Zhou et al., 2005).

Out of the 209 different congeners, only 20 can achieve coplanarity due to the absence of chlorine substitutions at the *ortho*-positions of the biphenyl rings (Tanabe et al., 1987). However, only PCBs that are substituted in the *para* and *meta* positions exhibit a maximum coplanar conformation, resembling the relatively flat structure of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Safe et al., 1985). We refer to fully coplanar PCBs as four congeners: non-*ortho* substituted PCB-77, -81, -126 and -169.

The coplanar arrangement, characterized by a more "flat" configuration with all 12-carbon atoms in a single plane, gives them the capability to bind the aryl hydrocarbon receptor (Ah receptor) and exhibit biological action and toxicity similar to 2,3,7,8-TCDD (Kafafi et al., 1993). For these reason, coplanar PCBs are also called dioxin-like PCBs and are considered among the most toxic congeners (Giesy and Kannan, 1998).

History and production

These synthetic chemicals were first produced in 1929 (De Voogt and Brinkman, 1989) and were widely used for almost 50 years, with a global production estimated to exceed 1 million metric tons (Breivik et al., 2002). PCBs have been normally produced under different trade names such as Arochlor®, a mixture of up to one hundred different congeners, grouped together based on an average percent weight chlorine basis. Arochlor nomenclature indicated the average % chlorine in the mixture. For instance, Arochlor 1254 contains approximately 54% of chlorine.

Due to their electrical insulation capabilities and flame resistance, PCBs quickly became extensively used as insulators and coolants in transformers and other electrical equipment where these properties were crucial (Breivik et al., 2007). In addition, for an extended period, PCBs were commonly incorporated into the production processes of a diverse range of everyday items. These included plastics, adhesives, paints, varnishes, carbonless copying paper, newsprint, fluorescent light ballasts, and caulking compounds (Ross, 2004). Their lipophilic nature, especially in the case of higher chlorinated congeners, permits their accumulation in the food chain, leading to exposure in both humans and wildlife (Beyer and Biziuk, 2009).

As early as the 1930s, reports began to describe acute health effects in humans caused by occupational exposure to PCBs, such as the development of a skin condition called chloracne (Fischbein et al., 1982; Hens and Hens, 2017). In response, workplace exposure limits were established in the 1940s, and for many years, workers continued to manufacture and use PCBs without significant issues (Ross, 2004). However, in the late 1960s and onwards, concerns regarding the presence of PCBs in the environment began to gain attention. Presence of PCBs was unveiled in the Swedish environment, specifically in eagles, herring, and abiotic matrices that were originally being screened for DDT (Jensen et al., 1969), and in the environment in the United States (Risebrough et al., 1968). In the 1970s and 1980s, thanks to the increasing scientific evidence about the hazards associated with PCBs, many countries took individual measures to restrict their marketing and use (Jones, 2021). In response to the presence of highly chlorinated PCBs in the environment, in 1971 the major US manufacturer of PCBs, Monsanto Chemical Company, took some actions and in 1977 ceased their production (Markowitz and Rosner, 2018). Two years later, the Environmental Protection Agency (EPA) banned PCB manufacture in the United States (Ross, 2004). Europe banned the use and manufacture of PCBs in the mid-1980s (Jepson et al., 2016).

Thanks to scientific evidences about the presence in various environmental compartments as well as their significant adverse health effects in human and wildlife, in 2001, PCBs were classified as persistent organic pollutants (POPs) under the Stockholm Convention (UNEP, 2001). With this first global treaty over 90 countries agreed to ban PCB production, phase out their use in equipments by 2025, and ensure destruction of any remaining PCBs by 2028. Currently, the Convention has garnered the support of 186 Parties.

Even four decades after the cessation of significant PCB production, the impact of these substances may lead to the extinction of more than half of the global killer whale populations (Desforges et al., 2018).

The toxicological risk for human health deriving from consumption of PCB-contaminated fish could, in turn, represents a great concern (Domingo and Bocio, 2007; Marushka et al., 2021; Weintraub and Birnbaum, 2008). In that context, it is somewhat expected that at the current rate of PCB elimination or mitigation, several countries, including certain European nations, are projected to fall short of meeting the targets set by the Stockholm Convention for 2025 and 2028 (Stuart-Smith and Jepson, 2017). This raises the question about the effectiveness of actual global efforts aimed at reducing/eliminating PCBs and emphasizes the need of improving national and international regulations as well as compliance mechanisms.

Presence in the environment

In general, PCB levels in the environment started to increase in the 1930s and peaked in the 1960s (Breivik et al., 2007; Gałuszka et al., 2020). Since the early individual regulations, several authors reported decreasing levels of PCBs in the environment (Bonito et al., 2016; Davis et al., 2007; Fensterheim, 1993; Hammer et al., 2016; Ross et al., 2013; Schneider et al., 2001). For instance, significant declines have been reported in human breast milk in Sweden (Norén and Meironyté, 2000), in Spanish commercial fishes between 1995 and 2003 (Gómara et al., 2005), in salmonids of Lake Michigan between 1972 and 1994 (Conrad et al., 1998), and in Arctic char (*Salvelinus alpinus*) for the period 1960–1996 in Lake Vattern, Sweden (Lindell et al., 2001).

After the Stockholm Convention entered into force in 2004, PCB levels continued to fall in biota and environmental matrices (UNEP, 2017). As a result, numerous scientists and decision-makers might have been under the impression that the issue of PCBs had been adequately resolved, and that the levels of these contaminants would steadily decrease for an extended period. Nonetheless, decreases have slowed in recent years (Hung et al., 2016; Melymuk et al., 2022; Salamova et al., 2013). In this regard, recently different papers have drawn attention to the high and stable PCB concentrations that are still reported in European biota and their implications to the ongoing decline of some European cetacean populations (Jepson et al., 2016; Jepson and Law, 2016; Law and Jepson, 2017) and other top marine predators worldwide (Sonne et al., 2018).

2.1.3. Polybrominated diphenyl ethers (PBDEs)

Physical and chemical properties

Polybrominated diphenyl ethers (PBDEs) (chemical formula = $C_{12}H_{(10-n)}Br_nO$) are a group of brominated flame retardants (BFRs) with a basic structure consisting of two benzene rings connected with an oxygen atom. There exist 209 theoretically possible different congeners defined by the number of bromine atoms (from 1 to 10) and their position in the chemical structure. Congeners are divided into 10 groups based on their levels of halogenation and range from mono- to deca-BDE.

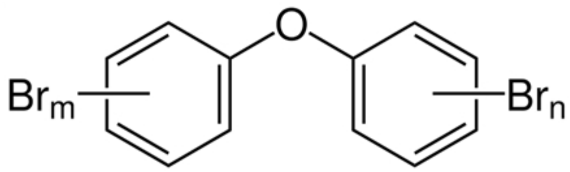


Figure 2. Chemical structure of PBDEs (with 1 ≤ n+m ≤ 10).

PBDEs share similar physical-chemical characteristics with PCBs. They exhibit high hydrophobicity, as evidenced by their logarithms of K_{ow} ranging from 5.08 to 8.07, with the value increasing as the number of bromine substituents rises from monoBDE to decaBDE (Wania and Dugani, 2003). However, as the degree of bromination increases, the water solubility and vapor pressure decrease. As a result, PBDEs have a reduced likelihood of being found dissolved in water or in gas phase. Instead, they tend to be sorbed to particles in the air, sediment, or soil (Wu et al., 2019). PBDEs may exhibit a greater susceptibility to environmental degradation compared to PCBs due to the relatively weaker carbon-bromine bonds as opposed to carbon-chlorine bonds (Siddiqi et al., 2003). Nevertheless, they are considered POPs that endure in the environment for extended periods without significant degradation (Sharkey et al., 2020).

History and production

Production of PBDEs began in the 1970s and peaked in the late 1990s, with an estimated global production of around 40,000 tons per year (European Chemicals Bureau, 2001). From this moment, for more than 10 years the global production remained approximately stable, although there was a shift in use toward the higher brominated preparations (Darnerud et al., 2001).

Initially, the United States and Israel were the main manufacturers of PBDEs; later, China started production in the 1980s, focusing primarily on the production of deca-BDE and became one of the major producers of PBDEs (Abbasi et al., 2019). They have been primarily manufactured as three main technical mixtures: penta-BDE, octa-BDE and deca-BDE -named after the major congeners present in each formula-, and were commonly used as flame-retardants in various applications, including polyurethane (PUR) foams, computers, household appliances, and electrical and electronic products (Jinhui et al., 2017). PBDEs started to capture attention as environmental contaminants in the early 1980s, after being detected in the Sweden's River Viskan (Andersson and Blomkvist, 1981). Since then, various studies started to report PBDEs worldwide in different abiotic and biotic matrices (Darnerud et al., 2001).

By the early 2000s, environmental authorities (e.g., the European REACH regulation; the Stockholm Convention; the US EPA; the Environment and Climate Change Canada) thanks to the growing scientific evidence became aware about the harmful effects of PBDEs, and nations began taking measures to address this issue. In 2004, the United States and Europe voluntarily phased out the production of penta- and octa-BDE mixtures (Hites, 2006), and in 2009, global regulations were implemented through their inclusion in Annex A (elimination) of the Stockholm Convention (UNEP, 2009). The global production of PBDEs between 1970 and 2005 was estimated to be around 1.3 to 1.5 million tons (UNEP, 2010), which is similar in scale to the whole production of PCBs (Breivik et al., 2002).

Despite global efforts to prohibit the production and use of these substances, ongoing production and use still exist in certain regions. Some countries have not ratified all amendments of the Stockholm Convention and lack national regulations regarding PBDE production and use. Brazil and Indonesia are examples of countries without defined regulations or limits on PBDE-containing products or waste (Sharkey et al., 2020). China has set concentration limits for penta- and octa-BDE in newly manufactured goods, but not for deca-BDE (Sharkey et al., 2020). Furthermore, specific exemptions under the Stockholm Convention allow for the use of the three commercial BDE mixtures in certain specific applications. For instance, deca-BDE – only banned in 2019- has exemptions for production until 2036 in some countries, including the European Union (UNEP, 2022a, 2022b).

The most common sources of PBDE contamination are effluents from factories producing flame-retarded polymers, wastewater treatment plants as well as leachates from landfills (Clarke et al., 2008). Currently, products containing PBDEs in use (textile, furniture, plastic and electronics) and accumulated waste stocks are significant sources of contamination (Wu et al., 2019). Improper disposal practices, older landfills in conjunction with higher temperatures and increased rainfall resulting from global warming could enhance PBDE volatilization and transportation further contributing to atmospheric contamination (Gong and Wang, 2022; Ohajinwa et al., 2019; Zhang et al., 2021). These factors are likely to increase the presence of PBDEs in remote areas and their entry into the marine environment (Nadal et al., 2015). Taking into account all these facts, PBDE emissions are expected to continue for the next 30 years (Abbasi et al., 2019).

Presence in the environment

Since the first detection in the Swedish environment in 1981, over the past four decades, PBDEs have been consistently detected in various environmental compartments including air (Bennett et al., 2015; Shoeib et al., 2004; Strandberg et al., 2001; Toms et al., 2009), soil (Wang et al., 2011; Wu et al., 2015), sediment (Liu et al., 2015; Sellström et al., 1998; Voorspoels et al., 2004), water (Ikonomou et al., 2006; Luo et al., 2008; Möller et al., 2011), as well as in biological samples (Akutsu et al., 2003; Król et al., 2012; Law, 2003) and human tissues (Covaci et al., 2008; Mazdai et al., 2003; Schecter et al., 2006), and their ubiquity as contaminants has been acknowledged for the past decade (Abbasi et al., 2019).

Following all the reduction and elimination actions, the levels of most reported PBDEs in the environment started to decline or stabilize worldwide in the early 2000s, although exceptions were observed in certain regions and species (Rotander et al., 2012). This is the case of some top predators in Northern Europe (McKinney et al., 2006; Vorkamp et al., 2005), as well as beluga whales in the Gulf of St. Lawrence (Raach et al., 2011) and Alaska (Hoguet et al., 2013), which continued to show elevated PBDE levels. Notably, the ban on penta- and octa-BDE mixtures resulted in increased production of deca-BDE, which primarily consists of BDE-209 (96.8%). However, in 2019, deca-BDE was also included in Annex A of the Stockholm Convention (UNEP, 2022b). In that context, it should be noted that penta-BDE and octa-BDE congeners are still being detected in marine mammals on a global scale (Alonso et al., 2014; Aznar-Alemany et al., 2021; Kratofil et al., 2020; Simond et al., 2017). Several factors can account for this persistence, including the long-lasting nature of these substances in the environment, the presence of PBDE-containing products still in use, ongoing production of these flame retardants in certain regions, and the degradation of higher brominated congeners (primarily deca-BDE) into lighter, more bioavailable, and more toxic congeners (Abbasi et al., 2019; Bartalini et al., 2022; Sharkey et al., 2020). On the other hand, the extensive use of deca-BDE as an alternative of penta- and octa-BDE led to a shift in the congener distribution pattern of PBDEs in various environmental matrices with increasing levels of higher brominated congeners (Olofsson et al., 2012; Su et al., 2015; Verreault et al., 2018). Studies conducted in Sweden between 2004 and 2011 showed decreasing concentrations of BDE-154 and BDE-183 in sewage sludge, while BDE-209 increased over the same period (Olofsson et al., 2012). In addition, elevated levels of deca-BDE were observed in herring gulls (*Larus argentatus*) from the Laurentian Great Lakes (Su et al., 2015) and top predator birds in the Arctic, sampled in 2012-2013 (Verreault et al., 2018). Although BDE-209 is highly hydrophobic and has low water solubility, suggesting low bioavailability, evidence described biomagnification of this congener in aquatic food webs (Castro-Jiménez et al., 2021; Johnson-Restrepo et al., 2005). Additionally, its affinity for abiotic matrices such as sediments creates a significant contaminant reservoir that poses risks to lower trophic level species and their predators. This holds significance considering the extensive historical production of deca-BDE, which is estimated to be nearly ten times higher than that of penta- and octa-BDE mixtures (Abbasi et al., 2019).

2.1.3. Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/Fs)

Physical and chemical properties

Polychlorinated dibenzo-p-dioxins (PCDDs) (chemical formula = $C_{12}H_{(8-n)}Cl_nO_2$, where n ranges from 1 to 8) and dibenzofurans (PCDFs) (chemical formula = $C_{12}H_{(8-n)}Cl_nO$, where n ranges from 1 to 8) are well-known environmental contaminants. They consist of two benzene rings connected by one or two oxygen atoms and contain one to eight chlorines. Depending on the number and position of chlorine atoms there exist 210 different congeners: 75 possible PCDDs and 135 possible PCDFs. Together, the 210 congeners are commonly referred to as "dioxins," even though there exist more PCDF congeners than PCDDs.

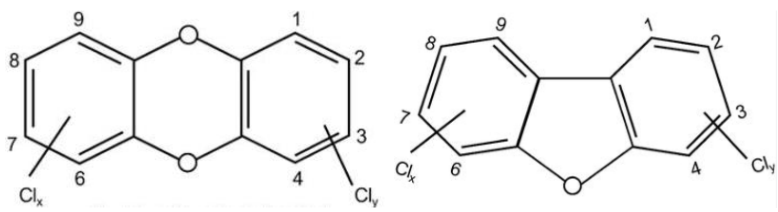


Figure 3. Chemical structure of PCDDs (left) and PCDFs (with 1 ≤ x+y ≤ 8).

PCDD/Fs have high lipophilicity and low water solubility and are, therefore, primarily associated with particulate and organic matter in soil, sediment, and the water column (Baran et al., 2020). Congeners with higher vapor pressures (i.e., the less chlorinated congeners) are found to a greater extent in the gas phase (X. Cao et al., 2018). When sorbed to soil, PCDD/Fs exhibit little potential for significant leaching or volatilization (Kulkarni et al., 2008).

History and production

As other POPs, PCDD/Fs are of significant concern due to their persistence in the environment, bioaccumulative nature, and potential adverse effects on human health and the ecosystems (Hites, 2011).

Opposite to PCBs or PBDEs, PCDD/Fs have never been intentionally produced and are considered unintentional by-products of industrial and combustion processes (Breivik et al., 2004). At the same time, these chemicals can be released into the atmosphere through some naturals processes such as forest fires and volcanic eruptions (Wagner et al., 1990), but their prevalence in the environment is predominantly attributed to various industrial processes (Fiedler, 1996). These comprise waste (hazardous, municipal and medical) incineration, thermal and combustion-related activities, chlorine bleaching of paper pulp, metal smelting, and cement plants, but also traffic, mainly from diesel vehicles (Domingo et al., 2020; Dopico and Gómez, 2015; Kulkarni et al., 2008).

PCDD/Fs have garnered significant public and scientific interest due to the acute toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2378-TCDD) that is often emphasized as one of the most hazardous synthetic compounds, frequently referred to as "the most toxic human-made chemical" (Hites, 2011). Some incidents that occurred during the late 1950s and 1970s led the competent authorities to take certain measures in order to protect human health and the environment. Worthy of note are the case of Chick Edema Disease which killed millions of young chicken in the US (Firestone, 1973), and the use of Agent Orange as an herbicide in Vietnam during the Second World War, responsible for negative effects in human health (soft-tissue sarcoma, non-Hodgkin's lymphoma, Hodgkin's disease, chronic lymphocytic leukemia, and chloracne) and the environment (Stone, 2007). The disaster of Time Beach (Missouri) (Carter et al., 1975; Powell, 1984) and the Seveso incident in Italy (Mocarelli, 2001) represent two other events of important dioxin contamination. In all these incidents, TCDD was the major responsible for causing harm to human health and the environment. It is important to note that TCDD was in most cases present as impurity in commercial products, especially chlorinated products.

Around the mid-1980s, the potential public health concern posed by dioxins became evident. In response to this emerging issue, various countries and different environmental authorities began to take action and limit emissions (Hites, 2011). For instance, in Europe in 1990s, regulations were implemented to control emissions from waste incineration facilities (Quaß et al., 2004), and maximum levels in food were established to protect public health and ensure food safety (Van den Berg et al., 1998). In the US, the EPA developed emission standards for waste incinerators in the 1980s (Liem and van Zorge, 1995). The regulation of dioxins continued to be refined and expanded in the following decades, with the EPA setting stricter standards and guidelines for various industrial processes and waste management practices (McKay, 2002). All these individual efforts were unified internationally in 2004 with the entry into force of the Stockholm Convention (UNEP, 2001).

Presence in the environment

Levels of PCDD/Fs in the environment have been a major concern for several decades. Efforts to measure and monitor these compounds have revealed their presence in air, soil, water, and various biological organisms worldwide (Alcock and Jones, 1996; Demond et al., 2012; Kanan and Samara, 2018; Lohmann and Jones, 1998). As for many POPs, their detection in remote and pristine regions, far from major industrial centers, demonstrates their global distribution and capacity for long-range transport (Lohman and Seigneur, 2001).

During the 20th century, there was a significant fluctuation in the emissions of PCDD/Fs into the environment (Hites, 2011). Before the chemical industry's rise in the 1920s, the presence of PCDD/Fs in the environment was relatively insignificant since their formation is predominantly influenced by human activities. It was during the period from the 1920s to the peak in the 1970s that dioxin emissions and concentrations in the air, soil, and water saw significant escalation (Hites, 2011). The increase can be primarily attributed to the lack of awareness about emission sources until the late 1970, when a study by Olie et al. (1977) described the presence of PCDD/Fs in fly ash from municipal solid waste incinerators. Throughout that period, the resulting absence of adequate control measures and emergency protocols in industrial facilities led to several major accidents with devastating environmental consequences in various regions worldwide (Dopico and Gómez, 2015).

Significant progress in the study of these compounds occurred with advancements in analytical methods. Before the early 1970s, the most commonly used technique for analyzing chlorinated organic compounds was gas-liquid chromatography (GLC) with an electron capture detector (ECD) (Firestone et al., 1972; Ress et al., 1970; Williams and Blanchfield, 1972). However, this method had some limitations, such as an inadequate detection limit for TCDD and lacked specificity. In 1973, a more sensitive and specific method, the congener-specific high-resolution gas chromatography high-resolution mass spectrometry (HRGC-HRMS) was employed for the first time for the analysis of TCDD in human milk and fish exposed to dioxin contaminated Agent Orange in Vietnam (Baughman and Meselson, 1973). Subsequently, during the 1980s, HRGC-HRMS came into use for the identification of different PCDD/F congeners in adipose tissue, human milk and blood (Brouwer et al., 1998; Ryan et al., 1987; Schecter and Ryan, 1992; Schecter and Tiernan, 1985; WHO, 2000), and is now used by most dioxin laboratories worldwide (Kanan and Samara, 2018).

Since the 1970s, there has been a notable shift in trends concerning PCDD/F emission and contamination levels from industrial sources (Alcock and Jones, 1996; Hites, 2011). This transformation can be attributed to increased social awareness, the implementation of more efficient industrial procedures, and the enactment of stricter legislation (Dopico and Gómez, 2015). Nevertheless, despite progress in controlling industrial emissions, nonindustrial sources of PCDD/Fs, such as domestic solid fuel combustion, accidental fires, and illegal incineration of household wastes, have remained relatively stable in recent years and have now become a major focus of concern in terms of dioxin proliferation (Cao et al., 2013; Quaß et al., 2004). In this context, the inventory of dioxin emissions in the United States from 1987 to 2012 indicated a significant reduction of over 95% in emissions from controlled sources, such as waste-to-energy industry, waste incineration, electricity and heat generation, metallurgical processes, cement and asphalt production. However, emissions from open burning sources, such as agricultural burning, construction debris, yard waste and fires, have increased 43% (Dwyer and Themelis, 2015). The authors suggested that some of this increase could be attributed to improved reporting of landfill and forest fires in recent years, along with the actual increase of fires due to higher temperatures and other contributing elements.

Even though dioxins are emitted in relatively low concentrations, they are very persistent and bioaccumulative. It means that even with reduced emissions, PCDD/Fs remain present and

continue to circulate in the environment. Additionally, the legacy of past releases from industrial activities and waste incineration can still contribute to ongoing exposure (Kirkok et al., 2020).
Evidence described the presence and the trophic transfer of dioxins at different levels of the marine food web, from plankton to top predators species (Morales et al., 2016; Romero-Romero et al., 2017; Wan et al., 2005). A recent study by Castro-Jiménez et al. (2021) reported a minor contribution of about <0.5% of PCDD/Fs to the total POP burden in the Mediterranean Sea pelagic food web, with preferential accumulation in lowest trophic levels, such as phytoplankton and zooplankton. Despite this, in the Mediterranean Sea these substances have been reported in many marine species at different trophic levels, such as dolphins (Capanni et al., 2020; Fossi et al., 2004; Pinzone et al., 2015), sharks (Storelli et al., 2011b), tunas (Barone et al., 2018), gulls (Roscales et al., 2016), turtles (Di Renzo et al., 2022; Lambiase et al., 2021; Storelli and Zizzo, 2014), and deep see organisms (Rotllant et al., 2006). In fact, dioxins have been reported in marine organisms, from low trophic levels to top predators species inhabiting the seas and oceans of the whole world (Alves et al., 2023; Domingo and Bocio, 2007; Kim et al., 2019; Strid et al., 2007). As a final note, PCDD/Fs have been also described in fishery products (Baeyens et al., 2007; González and Domingo, 2021; Storelli et al., 2011a), sometimes with levels that exceed the legal limits established for human consumption (Mikolajczyk et al., 2021; Struciński et al., 2013) .

2.2. Toxicity of POPs

Numerous toxicological investigations have provided compelling evidence of the detrimental impacts associated with POP exposure. Although toxicology mechanisms of POPs are very complex, it is widely accepted that the aryl hydrocarbon receptor (AhR) pathway mediates most, if not all, of the toxicological effects of these chemicals (Denison and Nagy, 2003; Safe, 2002; Zhou et al., 2010). The AhR is an intracellular ligand-activated transcription factor that plays a crucial role in regulating the expression of various genes involved in numerous cellular processes (Barouki et al., 2012). When POPs enter the body and bind to the AhR, they activate this receptor, initiating a series of signaling pathways that can have diverse effects on cells and tissues (Rothhammer and Quintana, 2019; Vogel et al., 2020). One significant outcome of AhR activation is the induction of cytochrome P450 enzymes, particularly the CYP1A family, which are responsible for metabolizing a wide range of foreign compounds, including environmental contaminants like POPs (Vogel et al., 2020). While the AhR's primary function is to help the body detoxify harmful substances, it can also lead to the production of toxic intermediates during the metabolism of certain POPs (Esteves et al., 2021). Immune system suppression, reproductive and developmental abnormalities, and carcinogenicity are some adverse effects linked to AhR activation by POPs (Janošek et al., 2006). On the other hand, various POPs do have little or no affinity to bind to the Ah receptor (Kafafi et al., 1993) in mammalian and no mammalians species. Yet, they are still linked to various toxic effects. For instance, some non-coplanar PCBs, also referred to as non-dioxin-like PCBs, have received less attention, but are highly present in the environment and linked to some negative health effects that impact the endocrine and central nervous systems, such as altered thyroid signaling and developmental neurotoxicity (Pessah et al., 2010; Stamou et al., 2013).

In recent times, there has been a significant increase in public concern regarding the toxicity of POPs due to their classification as hormone disruptors (Buha Djordjevic et al., 2020; Kabir et al., 2015; Kumar et al., 2020; Marlatt et al., 2022). In that context, different studies reported POPs' interference with the proper functioning of the endocrine and reproductive systems in both animals (Fair and Houde, 2023; Gaur et al., 2022; Letcher et al., 2015) and humans (Damstra et al., 2002; Damstra, 2002a, 2002b; DiVall, 2013). In addition, on the basis of sufficient evidence of carcinogenicity in humans and experimental animals, the IARC classified TCDD and certain PCB congeners as group 1 "carcinogenic to human" (IARC, 2019). On the other hand, PBDEs has been classified as a Group 3 carcinogen (not classifiable as to its carcinogenicity to humans) based on inadequate evidence of carcinogenicity in humans, and inadequate or limited evidence in experimental animals (IARC, 2016)

2.2.1. Humans

Epidemiological evidence in humans suggests that POPs negatively affect neurodevelopment (Costa et al., 2014; Korrick and Sagiv, 2008; Pham et al., 2019), as well as the thyroid, estrogen (Baba et al., 2018; Cao et al., 2018; Czerska et al., 2013), and immune function (Leijs et al., 2009), and moreover, some POPs have carcinogenetic activity (He et al., 2018; Othman et al., 2022).

All PBDE technical products have shown thyroid disrupting properties (Vonderheide et al., 2008) and some PBDEs have been associated to different breast, ovarian, and cervical cancer cells (Li et al., 2012; Wang et al., 2015). Various studies described potential association between PCB exposure and thyroid (Lerro et al., 2018), testicular (Cheng et al., 2021), prostate (Lim et al., 2017), and breast cancer (Parada et al., 2020). PCBs have been also associated with diabetes mellitus

(Rignell-Hydbom et al., 2007; Turyk et al., 2009). Positive associations between dioxin exposure and invasive breast cancer risk have been also described (VoPham et al., 2020). In addition, a study by Xu et al. (2016) suggest that a high blood level of TCDD was significantly associated with the mortality of different types of cancers.

Oral ingestion, air inhalation and dermal contact represent the most common route exposure for POPs to enter into the human body (Swackhamer et al., 2009). It is noteworthy that dietary intake (dairy products, meat, and fish) is probably the main route through which humans are exposed to these substances (Lee et al., 2014; Wang et al., 2013; Zhang et al., 2012). For instance, studies by Jacobson et al. (1990, 2003) reported lower birth weights, smaller head circumferences, and a shorter attention span in babies born to mothers who consumed large amounts of fish contaminated with PCBs than those from mothers who did not eat such fish. Marushka et al. (2021) confirmed the relationship between dietary exposure to POPs from fish consumption and type 2 diabetes. High levels of POPs in Arctic indigenous populations have been related to frequent cardiovascular diseases and disruption of the immune system, as a consequence of their traditional marine diet mainly relying on contaminated marine organisms such as fish and marine mammals (Johansen et al., 2004; Schæbel et al., 2017). Although conventional wisdom dictates that ingestion is the major source of exposure to POPs, some studies clearly demonstrate that living in proximity of POP-contaminated sites might lead to widespread exposure of the population via air transport of contaminants. Thus, residence in close proximity to hazardous waste sites containing POPs was shown to be a risk factor for cancer (Ozonoff et al., 1994), low birth weights (Baibergenova et al., 2003), respiratory diseases (Kudyakov et al., 2004), and congenital malformations (Geschwind et al., 1992; Marshall et al., 1997).

Other important pathways identified as critical routes of exposure originate from indoor environment and include air inhalation, inhalation/ingestion of dust particle and dermal contact (Dirtu et al., 2012; Harrad et al., 2006; Marek et al., 2017). Vulnerability to these exposure patterns is notably higher for toddlers, infants and children due to their behaviors and the extended time spent at home (Stapleton et al., 2012). In this regard, the study conducted by a Aslam et al. in 2021 assessed health implications derived from the presence of PCBs in indoor dust air, reporting a greater cancer risk for toddlers compared to adults. Furthermore, PBDEs are commonly detected in indoor dust, which has been identified as a one of the most important route of exposure, particularly for children (Klinčić et al., 2020; Zheng et al., 2023). Studies have indicated a correlation between PBDE levels in indoor dust and the presence of these pollutants in breast milk, emphasizing the potential transfer of contaminants from the indoor environment to infants (Coakley et al., 2013; Wu et al., 2007). Moreover, investigations have unveiled a positive correlation between hormone (thyroid and sex hormones) levels and PBDE concentrations found in men, suggesting that exposure to these contaminants through indoor dust may lead to endocrine disruption in men (Johnson et al., 2013; Meeker et al., 2009).

2.2.2. Marine organisms

The presence of POPs in marine organisms is a significant concern due to their ability to accumulate and persist in the marine environment (Wenning and Martello, 2014). Marine organisms can be exposed to POPs through various routes, including direct contact with contaminated water, ingestion of contaminated food, and the uptake of pollutants from sediments (Miglioranza et al., 2023). In general, once entered inside the body, POPs are transported to tissues with high lipid content in which they are stored given that these pollutants are not readily metabolized in fish and marine mammals (Elskus et al., 2005; Tanabe et al., 1994). In times of food scarcity, stored fats (lipids) are mobilized and utilized as a source of energy (La Merrill et al., 2013). As a consequence, POPs can be retained and accumulated in the lipids or can be transported to specific target sites within the organisms where to exert their toxic effects (Bengtson Nash, 2018; Yordy et al., 2010b). Through the process of biomagnification, POP concentrations increases across trophic chain levels, resulting in higher concentrations in top predator species (Castro-Jiménez et al., 2021; Kelly et al., 2007; Madgett et al., 2022). This means that species occupying upper trophic levels in the food chain, like marine mammals and sharks, face significant vulnerability to the adverse effects and toxicological consequences of these substances.

The toxicity of POPs in marine organisms arises from their ability to disrupt various physiological processes. These compounds are known to interfere with hormonal systems, leading to endocrine disruption, reproductive problems, and developmental abnormalities (Harmon, 2015). They can also cause damage to the immune system, making organisms more susceptible to infections and diseases, finally leading to potential effects at population levels (Desforges et al., 2016; Ross, 2002; Segner et al., 2012). Moreover, POPs are associated with oxidative stress and can damage cellular structures, including DNA, proteins, and lipids (Duffy et al., 2002; Kanerva et al., 2012; Li et al., 2005; Valavanidis et al., 2006).

2.2.2.1. Marine mammals

The primary route of POP exposure in marine mammals is diet (Aguilar et al., 1999). Secondly, maternal transfer represents an additional exposure pathway for fetus and calves, while an offloading mechanism for females (Brown et al., 2016; Yordy et al., 2010c). Since most marine mammals reside in higher trophic levels and have long life spans, they can be particularly vulnerable to the effects of bioaccumulation and biomagnification of organic contaminants (Murphy et al., 2018; Ross, 2000). This means that they face a significant risk of pollutants accumulation with detrimental effects at various levels, from cellular to population level (Desforges et al., 2018, 2016; Sonne et al., 2018).

The presence of POPs in marine mammals can lead to reproductive impairments and cause irreversible damage to the animals' immune systems, rendering them more susceptible to life-threatening diseases and potentially posing a threat to their entire populations (Desforges et al., 2016; Reijnders, 1986; Tanabe, 2002; Tsygankov et al., 2015). In that context, several unusual mortality and stranding events have been correlated with high organic contaminant concentrations (Law et al., 2012; Muir et al., 1988; Struntz et al., 2004; Tanabe, 2002; Tilbury et al., 1999). Research conducted on mortalities among marine mammals tried to establish connections between contaminants and disease outbreaks. For instance, the “Baltic Seal Disease Complex” was investigated in the 1970-80s, linking higher POP concentrations in the Baltic grey seal (*Haliocherus grypus*) and the ringed seal (*Pusa hispida*) to greater disease susceptibility (Sonne et al., 2020). Studies showed greater occurrence of lesions, osteoporosis, leiomyoma, stenosis, and occlusions of the uterus, which can affect reproductive success and survival of species (Helle, 1980; Helle et al., 1976a; Olsson et al., 1994; Roos et al., 2012).

In the same period, the endangered beluga whale population inhabiting the St. Lawrence Estuary was shown to have very high organohalogen tissue burdens and a high prevalence of degenerative, infectious, neoplastic, hyperplastic, and necrotic lesions (De Guise et al., 1995; Martineau et al., 1994). During 1990 to 1991, thousands of striped dolphins (*Stenella coeruleolba*) died in the Mediterranean Sea due to a newly discovered morbillivirus known as dolphin morbillivirus (DMV) (Domingo et al., 1990; Van Bressem et al., 1991). Subsequent investigations revealed that the deceased dolphins had higher levels of PCBs compared to free-ranging specimens (Aguilar and Borrell, 1994; Kannan et al., 1993). In recent years there have been increasing reports of marine mammals infected by uncommon diseases (Bossart, 2011; Bossart and Duignan, 2018). A recent research by Desforges et al. (2016) observed a connection between greater contaminant exposure and compromised lymphocyte proliferation and phagocytosis in these animals. This demonstrates that high concentrations of organic contaminants negatively impact both innate and adaptive immunity in marine mammals. In addition, an *in vitro* study with fibroblast cell cultures treated with PCBs and PBDEs showed immunotoxicity with state of stress and a modification of the immune system's response (Marsili et al., 2019). PBDEs have been also associated with cytotoxicity and genotoxicity in pantropical spotted dolphins (*Stenella attenuata*) (Rajput et al., 2021).

Several of these contaminants can act as endocrine disruptors, causing infertility, cancer and other types of diseases (Ross et al., 2000; Stockin et al., 2010; Tanabe, 2002; Ylitalo et al., 2001; Yordy et al., 2010a). PCB exposure has been associated with reduced reproductive outputs, such as increased embryonic loss and increased calf mortality in harbor porpoises (*Phocoena phocoena*) and bottlenose dolphins (*Tursiops truncatus*) (Murphy et al., 2015; Schwacke et al., 2002). A link between affected thyroid hormones circulation levels and exposure to PBDEs has been documented in grey seals (*Halichoerus grypus*) during their first year (Hall et al., 2003). In addition, the beluga whales residing in the St. Lawrence Estuary (Canada) -considered one of the most heavily polluted cetacean populations worldwide (Martineau et al., 2002)- exhibit the highest incidence of cancer among cetaceans, likely due to their high exposure to POPs.

2.2.2.2. Fish

2.2.2.2.1. Bony fish (Osteichthyes)

In fish, POPs have been found to disrupt the reproductive endocrine system, resulting in low hatching success, increased posthatch mortality, and reproductive disruption (Arukwe, 2001; Johnson et al., 2013; Kime, 1995). Particularly, PCB exposure has frequently been associated with depressed sex steroid levels and associated reproductive dysfunction (Arcand-Hoy and Benson, 1998; Simmons et al., 2014). PBDE effects on endocrine and reproductive systems, as well as the interaction between the two, have been reported in captive and wildlife fish (Yu et al., 2015). Although, the thyroid system is the major target of PBDEs in fish, different studies reported that these substances could interfere with activities or expressions of steroidogenic enzymes and consequently alter sex hormone levels, steroidogenesis, and reproduction (Hamers et al., 2006; He et al., 2008; Nakari and Pessala, 2005; Song et al., 2008).

Inhibition of estrogen synthesis, potentially causing fertility defects have been described in rainbow trout (*Oncorhynchus mykiss*) and zebrafish (*Danio rerio*) exposed to TCDD *in vivo* (Hutz et al., 2006, 1999). PCDD/Fs, PCBs, and PBDEs have been associated with changes in thyroid hormones' function resulting in growth and metabolism defects (Dong et al., 2017; Noyes and Stapleton, 2014; Nugegoda and Kibria, 2017; Rolland, 2000; Yu et al., 2015). Additionally, the ability to react normally to environmental stressors could be affected by the interaction between POPs and the hypothalamus-adrenal-pituitary axis (Carr and Patiño, 2011). For instance, Arctic char (*Salvelinus alpinus*) exposed to environmentally relevant concentrations of PCBs had impaired regulation of cortisol production by interrenal tissue, which could lead to a deficiency in adaptation to stress (Aluru et al., 2004; Vijayan et al., 2006).

Early life stages of fish are known to be particularly vulnerable to POPs. In oviparous fish species, adverse effects include decreased hatching success and increased embryo mortality, as well as abnormal development and malformation of larvae, ultimately leading to reduced survival rates (Foekema et al., 2012; Rigaud et al., 2013; Westerlund et al., 2000). For example, delayed hatching and alterations in behavior that included depressed swimming activity and feeding rates in larval mummichog (*Fundulus heteroclitus*) have been reported after exposure to commercial PBDE mixtures (Timme-Laragy et al., 2006). A laboratory study also described teratogenic activity of some PBDE congeners with significant decrease in hatching success, malformations (embryos), and significant increase in embryo mortality in turbot (*Scophthalmus maximus*) (Mhadhbi et al., 2012). Finally, studies reported developmental, reproductive and embryotoxicity in fish with excess mortality, edema, hemorrhages, and craniofacial malformations (Carney et al., 2006; King-Heiden et al., 2012; Tanguay et al., 2005).

Several researches have investigated the impact of pollution on the immune systems of wild fish residing in contaminated water sources (Galloway and Depledge, 2001; Jobling and Tyler, 2003). These findings revealed that fish exposed to these polluted environments exhibited certain adverse effects on their immune functions when compared to fish living in unpolluted reference sites (Arkoosh et al., 1991, 1998; Faisal et al., 1991; Weeks et al., 1986). These effects included a decrease in the secondary *in vitro* antibody response, heightened vulnerability to diseases, reduced cell division (mitogenesis), and impaired functioning of macrophages, which are vital cells involved in the immune response (Arkoosh et al., 1991, 1998; Faisal et al., 1991; Weeks et al., 1986).

2.2.2.2.2. Cartilaginous fish (Chondrichthyes)

Due to their inherent biological and ecological characteristics, such as slow growth, late maturation, and limited reproductive output, elasmobranchs (sharks, rays) - a subclass of chondrichthyes - are particularly vulnerable to POPs' accumulation (Dulvy et al., 2017, 2008; Pierce and Bennett, 2010). While overexploitation and habitat degradation are widely acknowledged as major threats to elasmobranch populations (Dulvy et al., 2014), the potential additional hazard posed by pollution remains poorly understood for this taxonomic group. Most likely because of the growing concern for human exposure to environmental contaminants through fish consumption, most of the studies have mainly focused on the contaminant levels in muscle tissue rather than on the toxicity exerted on these animals. In a review by Gelsleichter and Walker (2010) about pollutant exposure and effects in elasmobranchs it was found that several species, such as Greenland sharks (*Somniosus microcephalus*), bull sharks (*Carcharhinus leucas*), and tiger sharks (*Galeocerdo cuvier*) displayed levels of certain environmental pollutants that were close to or exceeded the limit recommended for human consumption.

As mentioned before, the exploration of detrimental effects of POPs on elasmobranch health is still lacking in comprehensive research. However, some studies have demonstrated that the levels of pollutants present in shark tissues not only exceed the recommended levels for human consumption (Alves et al., 2016), but that they are also greater than the threshold for physiological effects in some teleost species (Lema et al., 2008). In that context, changes in the hypothalamic-pituitary-thyroid axis have been reported in adult fathead minnows (*Pimephales promelas*) exposed to BDE-47 (Lema et al., 2008). Concurrently, Johnson-Restrepo et al. (2005) examined BDE-47 levels in bull sharks and found that this congener was approximately four times higher than the total body burden of the high-dose treatment of fathead minnows in the study by Lema et al. (2008). In addition, POPs have been measured in some shark species such as bull sharks (*Carcharhinus leucas*), Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*), bonnetheads (*Sphyrna tiburo*), sandbar sharks (*C. plumbeus*), and blacktip sharks (*C. limbatus*) in Florida, and were found to accumulate at levels that may elicit adverse biochemical responses in these species (Gelsleichter et al., 2008, 2005; Johnson-Restrepo et al., 2008, 2005). A recent study by Alves et al. (2016) discovered compelling connections between the accumulation levels of contaminants and biochemical responses in blue sharks (*Prionacea glauca*). The researchers observed significant associations with DNA damage, lipid peroxidation levels, inhibition of the antioxidant enzyme glutathione peroxidase, and presence of POPs and metal contamination.

2.3. Marine species as indicators of marine pollution

Understanding and protecting environmental and human health is a multifaceted challenge, given the wide range of anthropogenic and natural stressors that pose risks to the health of exposed organisms. To address these critical questions about the detrimental effects of human activities on biodiversity, scientists have increasingly relied on bioindicators of the ecosystems' health. In general, bioindicators are organisms that provide information on the quality of the environment, from deep marine ecosystems to terrestrial habitats of high altitude (Burger, 2006; Holt and Miller, 2010). The use of bioindicators is based on the idea that impacts of environmental changes are integrated over, and reflected by, the current status or trends in the diversity, abundance, or accumulation of pollutants by one or more species living in that environment (Bartell, 2006; Bonanno and Vymazal, 2017; Zukal et al., 2015).

In the realm of environmental chemical contamination and the potential adverse health impacts linked to exposure, researchers have progressively relied on wildlife as sentinel species, capable of providing insight into the presence, extent and potential consequences arising from the existence of toxic substances (Parmar et al., 2016). In this regard, sentinel organisms are valuable indicators of spatio-temporal trends, long-range transport, distribution, and biomagnification of toxic chemicals. Additionally, they can provide insights into the effects of contaminants through various methods, including *in vitro* exposures, controlled feeding studies, and blood sampling of free-ranging animals. In these scenarios, the development of biomarkers and endpoints of exposure holds significant importance.

To offer valuable information about the contamination status, good sentinels should possess certain fundamental qualities. These include sensitivity to environmental stressors, the ability to accumulate stressors/contaminants in their tissues, a widespread and common distribution, ease of monitoring, well-documented physiology and life history, and a key trophic or ecological role. (Burger and Gochfeld, 2001; Tabor and Aguirre, 2004). Furthermore, they should be preferably large, in order to provide sufficient tissues for the analysis, and have a long lifespan to facilitate long-term studies (Gerhardt, 2011). All these characteristics ensure that selected organisms could be studied and sampled without compromising their populations, allowing comparisons among different areas and providing information about their current and future health conditions.

Considering that they exhibit many of these characteristics, marine mammals and sharks have been identified as valuable sentinel species. (Alves et al., 2022; Bossart, 2011; Desforges et al., 2022). Their high trophic positions in marine food webs leads to the accumulation of high quantities of environmental contaminants (Gray, 2002; Jepson et al., 2016; Ross et al., 2000; Weijs et al., 2015). Moreover, with their fat rich tissues – liver in the case of sharks – they are very susceptible to accumulate lipophilic substances such as POPs (Barrett, 2013; Reijnders et al., 2009). In some cases, especially for some cetacean species, their coastal habitat use brings them into close contact with densely populated and industrialized cities, thereby exposing them to a plethora of human-induced stressors, including chemical pollution (Kucklick et al., 2011; Marsili et al., 2018; Reif et al., 2017). While populations relatively stationary allow for local risk assessments, others with extensive geographic ranges facilitate assessments of exposure across various regions. Ultimately, marine mammals and sharks present a considerable societal relevance since both constitute a significant food resource for many people around the world (Dent and Clarke, 2015; Fielding, 2022; Robards and Reeves, 2011).

Information gained from the study of marine mammals has provided useful perspectives about the severe threat of pollution in different areas of the world (Desforges et al., 2022). Some important examples included the case of beluga whales (*Delphinapterus leucas*) in St. Lawrence Estuary in 1970s-90s (De Guise et al., 1995; Martineau et al., 1994), grey seals (*Phoca vitulina*; *Pusa hispida*; *Halichoerus*) in the Baltic Sea between 1960 and 1980 (Helle et al., 1976b; Olsson et al., 1994; Reijnders, 1986), or sea lions (*Zalophus californianus*) along the California coast in the 1960s-70s (DeLong et al., 1973). In each scenario, studies documented a significant deterioration of the health of individuals resulting in population declines. The primary factor responsible across all three cases has been identified as heightened levels of contaminants accumulated in their tissues, especially POPs.

The sperm whale is widely recognized as sentinel of ocean pollution and health (Fossi and Panti, 2017) and several studies employed this species to monitor contamination and effects of POPs and metals (Godard-Codding et al., 2011; Marsili et al., 2014; Poirier et al., 2021; Savery et al., 2013; Squadrone et al., 2015; Wise et al., 2011; Zaccaroni et al., 2018). Despite the scarcity of information regarding its contamination status and the associated negative effects, studies have identified Cuvier's beaked whale (*Ziphius cavirostris*) as a promising bioindicator of environmental pollution (Bachman et al., 2014; Knap and Jickells, 1983; Law et al., 2005; Peruffo et al., 2023). At the same time, various researches have also highlighted the utility of blue sharks as sentinel organisms for assessing marine pollution in the Atlantic Ocean, Pacific Ocean and the Mediterranean Sea (Alves et al., 2016; Barrera-García et al., 2013, 2012; Storelli et al., 2011b).

In spite of certain limitations, such as their relatively high mobility, fish are highly regarded as valuable organisms for monitoring pollution in aquatic ecosystems. Anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*), due to their high sensitivity to environmental and anthropogenic stressors (Peters et al., 1994; Sala et al., 2022; Sofoulaki et al., 2022), as well as their crucial ecological role in linking planktonic life with predatory species (Lloret-Lloret et al., 2022; Palomera et al., 2007), have been identified as excellent bioindicator species. Fish are omnipresent in aquatic environments and fulfill a vital ecological role within aquatic food webs by transferring energy upwards from lower to higher trophic levels (Beyer, 1996). Furthermore, fish constitute a significant food source for humans in many parts of the world, and therefore, assessing their contamination status can provide insights into the potential risks for human health associated with fish consumption.

It is important to highlight that environmental health extends beyond ecosystems and encompasses various facets of human well-being. Bioindicators can provide insights into human health, the health of the organisms themselves, and the overall health of ecosystems. For instance, when we employ contaminant levels in top-predators as indicators, we obtain information regarding potential risks not only for the species directly involved, but also for humans (who are also top-predators in the food chain) and for species lower down the food chain. This wealth of information allows us to assess the effectiveness of mitigation measures and facilitates well-informed decision-making and targeted actions aimed at preserving both ecosystems' and human health.

2.3.1. Studied species

As stated before, sperm whales, Cuvier's beaked whales, blue sharks and fish encompass a range of characteristics that make them excellent bioindicators of marine ecosystem contamination. However, it is important to emphasize that these species are subject to other anthropogenic stressors that could affect their susceptibility to anthropogenic toxic substances and jeopardize their conservation. In that context, the International Union for Conservation of Nature (IUCN) Red List assessments plays a crucial role in evaluating the conservation status of species worldwide. The assessment process involves the evaluation of various factors that serve for categorizing species based on their risk of extinction. This process helps to identify species requiring urgent

conservation measures, but also plays an important role in monitoring the effectiveness of conservation efforts over time.
The species investigated in this study have been assigned to categories indicating a certain level of extinction risk (Bizsel et al., 2011; Cañadas and Notarbartolo Di Sciara, 2018; Di Natale et al., 2011a, 2011b; Pirotta et al., 2021; Sims et al., 2015).

2.3.1.1. Sperm whale (Physeter macrocephalus)

Figure 4. Depiction of the sperm whale.

The sperm whale is the largest toothed whale and has one of the widest global distribution of any marine mammal species (Whitehead, 2018). This odontocete, feeds mainly on meso- and bathypelagic cephalopods (Wong and Whitehead, 2014). Interestingly, male sperm whales have a preference for larger prey than females, such as demersal fishes, sharks and rays (Wong and Whitehead, 2014). Despite the cessation of commercial whaling, which was once the most prominent threat to sperm whales, other human activities still menace this species. Threats include chemical pollution, entanglement in fishing gear, and noise pollution (Notarbartolo Di Sciara, 2014).
In the Mediterranean Sea, there exist a genetically distinct subpopulation widely distributed within the basin, but geographically isolated from the rest of the oceans (Dulau et al., 2004; Engelhaupt et al., 2009). This subpopulation comprising fewer than 2500 mature individuals is declining in the last years and for this reason it has been listed as endangered by the IUCN (Pirotta et al., 2021). Efforts have been made to safeguard and protect Mediterranean sperm whales, particularly against entanglement in fishing gear and ship collisions (Danovaro et al., 2020). However, other anthropogenic activities such as oil and gas prospecting (seismic airguns), military operations, illegal dynamite fishing, and very specially, chemical contamination, are today sources of concern (Bartalini et al., 2019; Notarbartolo Di Sciara, 2014; Pinzone et al., 2015).

2.3.1.2. Cuvier's beaked whale (Ziphius cavirostris)

Figure 5. Depiction of the Cuvier's beaked whale.

The Cuvier's beaked whale is a deep-diving marine mammal with a cosmopolitan distribution (Baird, 2018; Tyack et al., 2006). Despite their wide distribution, they remain relatively understudied possibly due to their diving behavior, inconspicuous surfacing, and preference for offshore habitats (Hooker et al., 2019). A global assessment has shed light on the presence of a genetically distinct subpopulation of Cuvier's beaked whales in the Mediterranean Sea (Dalebout et al., 2005). With less than 10,000 mature individuals estimated in this subpopulation, there are concerns about a declining trend in their abundance (Cañadas et al., 2018; Podestà et al., 2016). Notably, specific regions within the Mediterranean, such as the Northern Ligurian Sea, Alboran Sea, and Hellenic trench, have been identified as important areas for this species (Cañadas et al., 2018; Tepsich et al., 2014).

The existing research on Mediterranean Cuvier's beaked whales has predominantly focused on assessing the risks associated with noise pollution. Within this framework, studies have identified a potential correlation between Cuvier's beaked whale mass strandings and their exposure to mid- and low-frequency underwater anthropogenic acoustic activities, particularly military activities involving the use of sonar (Cox et al., 2023; Fernández et al., 2005; Frantzis, 1998). Past studies have involved reviewing stranding data (Podesta et al., 2023), studying diving behavior (Tyack et al., 2006), identifying habitat preferences (Azzellino et al., 2011; Tepsich et al., 2014), and estimating density and abundance (Cañadas et al., 2018). All of these were aimed at understanding the impact of anthropogenic noise pollution on these whales. Due to the declining population trend and associated threats, the Mediterranean subpopulation of Cuvier's beaked whale has been classified as Vulnerable by the IUCN Red List of Threatened Species (Cañadas and Notarbartolo Di Sciara, 2018). Consequently, it becomes of crucial importance to investigate other potential stressors, such as POPs, to develop a comprehensive risk assessment and effective conservation strategies for this species.

2.3.1.3. European sardine (Sardina pilchardus), European anchovy (Engraulis encrasicolus) and bogue (Boops boops)

Sardines, anchovies, and bogues are small, pelagic fish species that play a crucial role in marine ecosystems while having significant economic importance in fisheries worldwide. All these three fish species have been assessed for the IUCN Red List of Threatened Species in 2007-2008 and listed as Least Concern in the Mediterranean Sea (Bizsel et al., 2011; Di Natale et al., 2011a, 2011b).

2.3.1.3.1 European sardine (Sardina pilchardus)

Figure 6. Depiction of the European sardine.

The European sardine belongs to the Clupeidae family and is found in temperate and subtropical waters across various regions (Brehmer et al., 2007). The European sardine is one of the well-known sardine species inhabiting the eastern Atlantic Ocean and the Mediterranean Sea (Voulgaridou and Stergiou, 2003). This species forms schools, usually at depths between 25 to 55 or even 100 m by day, rising to 10 to 35 m at night (Di Natale et al., 2011a).

Sardines are ecologically important as a vital prey species for marine mammals, seabirds, and predatory fish. Their high reproductive rates and abundance make them essential components of the marine food web (Cury, 2000). In terms of fisheries, sardines are commercially valuable and have been exploited for centuries. They are caught for human consumption, canning, and fishmeal production, providing employment and economic benefits to many coastal communities. Environmental factors, such as sea surface temperature and plankton availability, influence the dynamics of sardine populations (Véron et al., 2020).

This species has a broad distribution and is abundant in the Mediterranean Sea where it is commercially important (Palomera et al., 2007). In this region the sardine population displayed some fluctuations, but there have been no significant population declines in recent times (Di Natale et al., 2011a).

2.3.1.3.2. European anchovy (Engraulis encrasicolus)

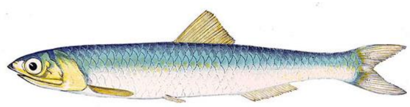


Figure 7. Depiction of the European anchovy.

The European anchovy is a widespread fish species belonging to the Engraulidae family. This pelagic and oceanodromous species is typically found in coastal waters, where it forms large schools (Ventero et al., 2021). It is a very common and abundant species in the Mediterranean Sea, typically inhabiting the entire water column, with the majority of the population concentrated at depths of less than 50 meters (Plounevez and Champalbert, 2000). A genetic study by Tudela et al. (1999) reported the presence of a unique population in the Mediterranean basin, with different spawning grounds. Anchovies are essential components of marine food webs, serving as prey for many marine predators, including larger fish, seabirds, and marine mammals (Free et al., 2021). In commercial fisheries, anchovies are caught mainly for human consumption, processed into canned products, salted, or used for fishmeal and fish oil production.

2.3.1.3.3. Bogue (Boops boops)

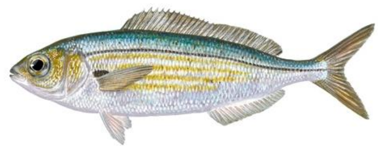


Figure 8. Depiction of the bogue.

The bogue is a small fish species belonging to the Boopsoidea genera, very common and ubiquitous in temperate and tropical waters (El-Maremie and El-Mor, 2015). This species is known for its sociable and schooling behavior and can be found in demersal and semi-pelagic habitats (Arechavala-Lopez et al., 2011). It is capable of ranging as deep as 350 meters, and its presence spans diverse substrates, such as sand, mud, rocks, and seaweeds (Riera et al., 2014; Valle et al., 2003). While it is more frequently encountered at depths shallower than 150 meters, it is also occasionally found in coastal waters (Bizsel et al., 2011). Bogue is a common species in the North East Atlantic and the Mediterranean Sea were, although it is heavily exploited, the population appears to be stable (Bizsel et al., 2011; Cunha et al., 2022). Bogues are ecologically important as prey for various marine predators, contributing to the trophic structure of marine ecosystems. In fisheries, bogues are caught for human consumption, particularly in Mediterranean and Atlantic regions, and are an important component of artisanal fisheries (Azab et al., 2019).

2.3.1.4. Blue shark (Prionace glauca)

Figure 9. Depiction of the blue shark.

The blue shark is a widely distributed and highly abundant pelagic species inhabiting temperate, tropical, and subtropical regions (Sims et al., 2015). With a long lifespan of approximately 20 years, it holds the position of a top predator in the marine ecosystem, primarily preying on pelagic teleost fish and cephalopods, especially squid (da Silva et al., 2021). Researchers have investigated its suitability as a bioindicator species for marine contamination through various studies (Alves et al., 2022, 2016; Barrera-García et al., 2013, 2012; Storelli et al., 2011b) .

Unfortunately, the blue shark faces increasing bycatch in high-seas longline and driftnet fisheries (da Silva et al., 2021). Although once considered less valuable, its meat and fins are now in high demand worldwide, leading to a staggering estimated global catch of nearly 93,000 tons in 2020 (FAO Species Fact Sheets, 2021). This intense exploitation has resulted in its classification as Near Threatened on the IUCN Red List (Sims et al., 2015), with some scientists estimating that its global catch accounts for about 90% of all elasmobranchs caught (Coelho et al., 2012). Consequently, it is crucial to prioritize the analysis of xenobiotics such as POPs in the tissues of this species. This analysis serves as a valuable proxy for assessing the extent and type of contamination present in its environment, and for understanding the potential health impacts associated with human consumption of its meat.

2.4. POPs in the marine environment

The marine environment is considered a global sink for many hydrophobic POPs (Dachs et al., 2002; Jönsson et al., 2003; Lohmann et al., 2006). These substances can enter the marine environment through various pathways comprising atmospheric deposition, runoff, and ocean currents (Wenning and Martello, 2014). Once into the atmosphere, POPs can be transported by air currents, both in gas phase and adsorbed to particles, and deposited into oceans and seas, particularly in regions far from their sources (Gouin et al., 2004; Wania and MacKay, 1996). Agricultural and urban runoffs can transport POPs from the land into water bodies, which eventually reach the marine environment (Fu et al., 2003; Najam and Alam, 2023). Finally, POPs can be transported across vast distances by ocean currents, leading to their global distribution (Iwata et al., 1993; Lohmann et al., 2021, 2007).

Once POPs enter the marine environment, they can undergo several processes. They can bioaccumulate in the tissues of marine organisms through direct uptake from water or ingestion of contaminated preys (Daley et al., 2014; Mackay and Fraser, 2000). Then, POPs can undergo biomagnification in the marine food web, leading to higher concentrations in predators at the top of food chains (Castro-Jiménez et al., 2021; Chiuchiolo et al., 2004; Goerke et al., 2004; Romero-Romero et al., 2017). On the other hand, some POPs have a tendency to adsorb onto sediment particles, leading to their accumulation in marine sediments (Avellan et al., 2022; Gómez-Gutiérrez et al., 2007). PCBs, PBDEs and PCDD/Fs have been broadly described in marine environments, (Domingo and Bocio, 2007; Geyer et al., 1984; Lee and Kim, 2015; Wolska et al., 2012), with the highest levels reported in top predator species, such as orcas and other toothed whales, and in some cases with detrimental concentrations for population conservation (De Guise et al., 1995; Desforges et al., 2018; Marsili et al., 2018; Stuart-Smith and Jepson, 2017).

PCBs are discharged into the aquatic environment mainly with effluents (principally from paper factories), or to lesser extent as a result of local emergencies (e.g., leakage from equipment utilizing transformer oil with added PCBs) (Wolska et al., 2012). In addition, PCB contamination of sediments proximal to harbors has been attributed to the release of various chemical constituents found in marine paints (Lao et al., 2021; Uhler et al., 2021). PBDEs enter coastal waters through discharge from municipal sewage treatment systems (either from households, traffic and/or diffuse releases to the environment), industrial wastewater, leachate from landfills, and deposition from the atmosphere (De Wit, 2002). Some are additives mixed into polymers and are not chemically bound to the plastic or textiles, and therefore may separate or leach from the surface of their product applications into the environment (Turner, 2022). PBDEs may also enter the marine environment with plastic waste. For instance, some sources are microplastics and fibers generated by physical abrasion on disposal (Harrad et al., 2019), fibers discharged to wastewater or the atmosphere on laundering (Dalla Fontana et al., 2020; O'Brien et al., 2020), and larger plastics, including occasional electronic articles, resulting from littering, spillages and improper waste management (Shaw and Turner, 2019). Additionally, plastics and microplastics can serve as a dual source for the presence of PBDEs in the marine environment. On one hand, PBDEs may be released from the plastic matrix into the surrounding water through diffusive migration, becoming a potential source of contamination (Chua et al., 2014; Rochman et al., 2014; Tanaka et al., 2013). On the other hand, plastics can also act as a sink for PBDEs and other organic compounds, with these chemicals being adsorbed and accumulated on the plastic surfaces (Engler, 2012; Gouin et al., 2011; Ogata et al., 2009; Singla et al., 2020; Xu et al., 2019). These interactions provide possible routes of exposure to PBDEs for marine organisms, leading to their potential accumulation in marine life (Avio et al., 2015; Bakir et al., 2014; Teuten et al., 2007).

In the case of PCDD/Fs, the contamination of water primarily results from sources including sewage sludge, paper and pulp industries, fertilizer industries, and others that release these pollutants into the water environment (Jeno et al., 2021). PCDD/Fs have been reported in surface sediments of rivers in China (Ren et al., 2009), Russia (Metelkova et al., 2019), Uruguay, and Argentina (Matta, 2018). Contaminated marine sediments has been described in the Spanish Northern Atlantic Coast (Gómez-Lavín et al., 2011), along the Portugal coast (Nunes et al., 2014), and the Mediterranean Sea (Castro-Jiménez et al., 2013; Eljarrat et al., 2005, 2001; Jimenez et al., 1998).

As mentioned before, after entering the marine environment, POPs are susceptible to biomagnify within the marine food chain (Castro-Jiménez et al., 2021; Chiuchiolo et al., 2004; Goerke et al., 2004; Romero-Romero et al., 2017). The marine food web exhibits a diverse array of both simple and complex food chains, with marine animals occupying various trophic levels. The consumer dynamics, such as the type and length of food webs, can differ among different taxa, which can impact how environmental contaminants are accumulated (Madgett et al., 2019). In addition, due to this variation in trophic dynamics, individuals of the same species may not consistently occupy the same trophic level, owing to differences in age, sex, habitat, seasonal and dietary preferences (Kousteni et al., 2017; Metcalfe et al., 2004; Pauly, 1998).

The marine food web starts with primary producers, such as phytoplankton, algae, and seagrasses, occupying the lowest trophic level. Moving up, the next stage includes first-order consumers like zooplankton and herbivorous feeders, including manatees and dugongs (O'Shea et al., 2018; Trites, 2019). The second order of consumers mainly consists of filter feeders like baleen whales (mysticetes), which consume first-order consumers such as zooplankton and larvae. Mid-trophic pelagic fish such as sardines, anchovies, mackerels, and squids are the most common pelagic species in upwelling systems worldwide (Ceia et al., 2023). In addition, these species hold a crucial position in marine ecosystems, serving as vital connections between zooplankton and apex predators, and they are significant targets for fisheries (Frederiksen et al., 2006). Continuing along the trophic chain, the next level is occupied by intermediate to apex predators, such as toothed whales (odontocetes) and pinnipeds (seals, sea lions, and walruses), which often feed on large fish and even other marine mammals (Bowen, 1997; Nelms et al., 2018). Some of the highest trophic positions are occupied by marine mammals that consume other marine mammals, some shark species, and indigenous communities with a diet relying on top predator species (Bowen, 1997; Heithaus, 2001; Higdon et al., 2012; Nelms et al., 2018).

Both theoretical and assigned trophic levels can be employed to model and estimate the biomagnification of POPs, and to identify species or populations especially prone to the accumulation of these harmful substances. Food web biomagnification of POPs has been widely demonstrated to occur for multiple species from both aquatic (Castro-Jiménez et al., 2021; Nfon et al., 2008; Romero-Romero et al., 2017) and terrestrial environments (Cao et al., 2023; Fremlin et al., 2020). When POPs enter the marine environment, they are often introduced at the base of the food chain by being taken up by small organisms like plankton or algae, and they start to accumulate in their tissues (Chiuchiolo et al., 2004; Li et al., 2020). The next level of the food chain involves small fish or zooplankton that feed on the primary producers (Fisk et al., 2001; Larsson et al., 2000). Since these organisms consume a large number of POP-contaminated plankton, the concentration of POPs in their bodies becomes higher than that in the lowest level. As larger marine predators consume these smaller contaminated organisms, they incorporate all the accumulated POPs from their prey (Gui et al., 2014; Romero-Romero et al., 2017). POPs do not get metabolized or excreted efficiently in the predator's body, which leads to a further increase in the POP concentration in their tissues (Aguilar et al., 2002; Baini et al., 2020; Jeong et al., 2016; Tanabe, 2002; Williams et al., 2020). The biomagnification process continues and POPs move up the food web's trophic levels resulting in higher concentrations in species at the top of the food chains, such as some odontocetes and sharks (Bachman et al., 2014; Jepson and Law, 2016). Additionally, many indigenous communities face the risk of contaminant exposure due to their subsistence consumption of various marine mammal species (Dudarev et al., 2019; Moses et al., 2009). Summing up, the phenomenon of biomagnification poses significant ecological risks and health concerns, above all, for those species occupying higher trophic levels (Leonards et al., 2008; Tsygankov et al., 2015).

2.4.1. Studied areas

Despite the global distribution of POP contamination in marine ecosystems, specific regions, such as the Mediterranean Sea and the North East Atlantic Ocean, exhibit elevated contamination levels that can be attributed to their distinctive geographical and ecological characteristics (Benn et al., 2010; Carpenter, 2019; Danovaro, 2003; El-Kholy et al., 2012; Halpern et al., 2008; Marsili et al., 2018; Merhaby et al., 2019; Nash et al., 2023; Ramos-Miras et al., 2019; Sharma et al., 2021). These factors can amplify the impact of POPs on marine biodiversity and, consequently, on the overall health of ecosystems. Therefore, these areas are of particular concern for conservation and environmental management efforts.

2.4.1.1. The Mediterranean Sea

Figure 10. Depiction of the Mediterranean Sea (34.5531° N, 18.0480° E). (https://earth.google.com/web/)

The Mediterranean Sea is a captivating and culturally significant aquatic system located between Southern Europe, Northern Africa, and Western Asia. It is enclosed by the landmasses of these three continents and connected to the Atlantic Ocean through the Strait of Gibraltar. Known for its historical importance and picturesque landscapes, the Mediterranean Sea has played a crucial role

in the development of human civilizations since ancient times (Bintliff, 2017). Furthermore, its marine biodiversity is one of the most rich and diverse of the world (Coll et al., 2010; Tovar-Sánchez et al., 2020). The sea is home to a wide variety of marine species, ranging from microscopic plankton to large marine mammals, with a high percentage of endemic species (Bianchi and Morri, 2000; Coll et al., 2010; Tortonese, 1985). The Mediterranean Sea also harbors a unique collection of iconic species that require special conservation attention, including sea turtles (Casale et al., 2018), various cetaceans (Pace and Tizzi, 2013), and the critically endangered Mediterranean monk seal (*Monachus monachus*) (Bundone et al., 2019). Moreover, it serves as the primary breeding area for the eastern Atlantic bluefin tuna (*Thunnus thynnus*) (MacKenzie et al., 2009). In addition, coastal areas host diverse ecosystems, including seagrass meadows of the endemic *Posidonia oceanica*, coral reefs, and rocky shores, which serve as important habitats for numerous species (Pinedo et al., 2007; Sbrocca et al., 2021; Tursi et al., 2004; Vacchi et al., 2017).
The rich biodiversity of the Mediterranean Sea is also linked to its role as a migration route for various marine animals, including birds, fishes, and marine mammals (Coll et al., 2010; Jourdain et al., 2007). It serves as an important stopover and breeding ground for numerous migratory species, making it a critical area for conservation efforts (Barboutis et al., 2022; Maggini et al., 2020). However, the Mediterranean ecosystem is facing various challenges and threats (Coll et al., 2010; Danovaro et al., 2020). Pollution from urbanization, industrial activities, shipping, and agricultural runoff poses significant risks to marine life and coastal habitats (Danovaro, 2003; Merhaby et al., 2019; Ramos-Miras et al., 2019; Sharma et al., 2021). Overfishing and destructive fishing practices also put pressure on fish stocks and marine ecosystems (Bearzi et al., 2006; Shumka et al., 2023). Additionally, climate change impacts, such as rising sea temperatures and ocean acidification, further stress the delicate balance of the Mediterranean's biodiversity (Lejeusne et al., 2010; Raitsos et al., 2010). One of the Mediterranean's defining features is its unique hydrodynamics that combined with its semi-enclosed nature, contributes to its distinctive ecological characteristics and susceptibility to pollution (Durrieu de Madron et al., 2011).
This area has become one of the most polluted seas in the world due to a combination of factors and it is considered a sink for many pollutants (El-Kholy et al., 2012; Marsili et al., 2018). One major issue is the increasing population and tourism along the Mediterranean coast (Laubier, 2005; Yıldırım et al., 2023). This leads to higher urbanization and industrial activities, resulting in the discharge of untreated sewage, chemicals, and waste into the sea (El-Kholy et al., 2012; Hassen et al., 2022; Schell et al., 2021). Additionally, coastal development can cause habitat

destruction, affecting marine ecosystems and biodiversity (Claudet and Fraschetti, 2010; Fraschetti et al., 2011; Meinesz et al., 1991; Mejjad et al., 2022).

Shipping is another significant contributor to contamination in the Mediterranean area. This sea serves as a major transportation route, and shipping activities can lead to oil spills, fuel emissions, and the release of ballast water containing invasive species (Flagella and Abdulla, 2005; Kostianoy and Carpenter, 2018; Marmer and Langmann, 2005; Molnar et al., 2008). These incidents not only directly harm marine life, but also have long-term impacts on marine food chains (Deudero et al., 2011).

Agricultural practices, particularly the use of fertilizers and pesticides, contribute to marine pollution through runoff into rivers that eventually reach the sea (Owa, 2013; Zaqoot et al., 2012). This influx of nutrients leads to harmful algal blooms and oxygen-deprived "dead zones," further disrupting the delicate balance of marine ecosystems (Abbas et al., 2022; Barale et al., 2008). Moreover, microplastics have become a widespread issue in the Mediterranean (Sharma et al., 2021). These tiny particles, often from the breakdown of larger plastic debris, are ingested by marine organisms and can make their way up the food chains, posing risks to both marine life and potentially human health (Codina-García et al., 2013; Romeo et al., 2015; Tsangaris et al., 2020).

As mentioned before, the Mediterranean Sea is widely recognized as a vulnerable region particularly susceptible to pollution and it is considered a hot spot area for many POPs, specifically PCBs (Albaigés, 2005; Marsili et al., 2018). In that context, although in recent years several studies have observed a gradual decline in PCB levels in various fish species within the Mediterranean region, other researchers have highlighted elevated PCB concentrations in certain marine mammal populations, confirming the Mediterranean's status as a PCB hotspot for marine mammals (Stuart-Smith and Jepson, 2017). Concentrations of PCBs in different Mediterranean dolphin species have been recently reported surpassing previously toxicity thresholds for marine mammals (Stuart-Smith and Jepson, 2017). Also, PCBs are still detected in human milk and blood among Mediterranean populations (Çok et al., 2012; Ulutaş et al., 2015), in some cases, with no significant decrease over recent years (Consonni et al., 2012). These evidences point to the possibility of new inputs and/or remobilization of these legacy contaminants in the Mediterranean region (Josefsson et al., 2010). This, in turn, implies that the measures taken to mitigate PCB contamination may not be working as effectively as expected on both local and global levels. Various pathways contribute to the influx of POP concentrations into the Mediterranean Sea. These include long-range transportation (LRT) from regions like Asia and different parts of Europe, as well as inputs from river discharges and sedimentation (Albaigés, 2005; Berrojalbiz et al., 2014; Gómez-Gutiérrez et al., 2006; Iacovidou et al., 2009; Lelieveld et al., 2002). As a result, significant levels of POPs are now being reported in various animal species within the Mediterranean Sea (Capanni et al., 2020; Guerranti et al., 2014; Marsili et al., 2018; Muñoz-Arnanz et al., 2011; Pinzone et al., 2015; Roscales et al., 2016; Tekin and Pazi, 2017).

In order to address environmental challenges in this region, the United Nations Environment Programme (UNEP) established The Mediterranean Action Plan (MAP). This comprehensive regional program was launched in 1975 with the aim of protecting and preserving the marine and coastal ecosystems of the Mediterranean Sea, which are under threat from pollution, overfishing, habitat degradation, and other human activities. The primary goal of the MAP is to promote sustainable development and environmental protection in the region through cooperation among the Mediterranean countries. The program focuses on several key areas, including marine pollution, biodiversity conservation, coastal zone management, and sustainable use of marine resources. As a result, there exist currently 1,126 Marine and Coastal Protected Areas (MCPAs) covering 209,303 km^2, including 39 Specially Protected Areas of Mediterranean Importance (SPAMIs). This coverage amounts to approximately 8.3 percent of the Mediterranean Sea area.

2.4.1.2. The North East Atlantic Ocean

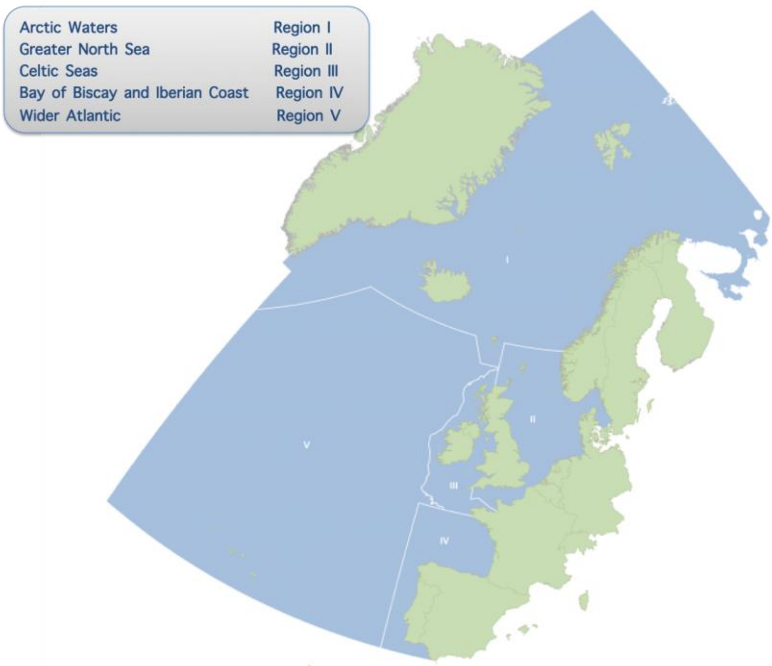


Figure 11. Depiction of North East Atlantic Ocean, subdivided into the different OSPAR areas. (https://www.ospar.org/convention/the-north-east-atlantic)

The North East Atlantic Ocean is a vast and biologically diverse region that stretches from the western coasts of Europe to the eastern coasts of North America. It is one of the most productive marine areas in the world, supporting a rich and varied array of marine life (McQuatters-Gollop et al., 2022). It is home to a wide range of marine species, including fish, marine mammals, seabirds, and invertebrates (Gordó-Vilaseca et al., 2023; Licandro et al., 2010; Waggitt et al., 2020). Its high level of biodiversity is supported by a wide range of habitats, including coastal areas, deep-sea trenches, and underwater mountains (Bongiorni et al., 2010; Henry and Roberts, 2007; Xavier and van Soest, 2007). The North East Atlantic is a significant fishing ground, supporting various commercially valuable fish species such as cod, haddock, herring, and mackerel (Bjørndal, 2009; Hannesson, 2013; Veldhuizen et al., 2015; Zimmermann and Werner, 2019). This region is influenced by the North Atlantic Drift, a warm ocean current that brings mild temperatures to Western Europe, contributing to the temperate climate of countries like the United Kingdom, Ireland, and coastal areas of Scandinavia (Adams et al., 2013; Rasmussen and Thomsen, 2004). Various deep-sea ecosystems in this area are characterized by unique and specialized species that have adapted to the extreme conditions of darkness, cold, and high pressure (Grehan et al., 2017; Grinyó et al., 2022; Kazanidis et al., 2020).

Despite this region is a playground for marine life, providing vital feeding and breeding grounds for numerous species, it is not immune to human impacts, such as coastal development, tourism, overfishing, marine litter, offshore activities, climate change and pollution (McQuatters-Gollop et al., 2022). The coastal areas of the North East Atlantic are heavily populated and developed, leading to habitat destruction, coastal erosion, and loss of natural environments (Halpern et al., 2008; IPBES, 2019). In addition, pollution from coastal urban centers, shipping activities, and oil and gas installations can affect water quality and marine life (Benn et al., 2010; Carpenter, 2019; Nash et al., 2023). In the last years, effects of climate change such rising sea temperatures, ocean acidification, and changes in marine habitats that can have profound effects on marine ecosystems, have been described in this area (Bedford et al., 2020; Hawkins et al., 2009; Poloczanska et al., 2016; Skogen et al., 2018).

In that context, OSPAR (Convention for the Protection of the Marine Environment of the North-East Atlantic) is an international agreement established to protect the marine environment of the North East Atlantic. It was formed in 1992 and includes 15 Contracting Parties, which are the countries bordering the North East Atlantic, along with the European Union. The fifteen Governments are Belgium, Denmark, Finland, France, Germany, Iceland, Ireland, Luxembourg, The

Netherlands, Norway, Portugal, Spain, Sweden, Switzerland, and United Kingdom. The OSPAR Convention aims to prevent and eliminate pollution in the North East Atlantic, conserve biodiversity, and promote sustainable use of marine resources. It addresses various sources of pollution, including chemical substances such as POPs, radioactive substances, and marine litter. It also coordinates efforts to combat pollution from land-based sources, such as agricultural runoff and wastewater discharges. Additionally, this convention focuses on the protection of sensitive habitats and species. It designates and manages marine protected areas to safeguard critical ecosystems and support biodiversity conservation. OSPAR also addresses issues related to offshore oil and gas activities to minimize environmental impacts and ensure responsible resource exploration and extraction.

2.5. The Stockholm Convention

In 1995, the Governing Council of the United Nations Environment Programme (UNEP) urged global action on a list of 12 Persistent Organic Pollutants (POPs). As a result, the Intergovernmental Forum on Chemical Safety (IFCS) was tasked to develop recommendations for international action by 1997, and concluded that immediate global measures were necessary to safeguard human health and the environment from these POPs. These recommendations were approved by the UNEP Governing Council and the World Health Assembly and, in early 1998, negotiations for a legally binding treaty started. Over 120 countries participated in the negotiation meetings of the Stockholm Convention, leading to the adoption of the final document in May 2001 (UNEP, 2001). The convention came into force in 2004 and currently, 186 parties, including 181 states and the European Union, have adopted it. Some countries, such as Israel, Malaysia, and the United States of America (USA), are yet to ratify the Stockholm Convention.

The first objective of the Stockholm Convention is to protect human health and the environment from the harmful effects of POPs by eliminating or restricting their production, use, trade, and release worldwide. The convention requires parties to develop action plans, adopt best available techniques, and promote alternatives to POPs in various sectors such as agriculture, industry, and waste management. For the clear identification of POPs, the Convention outlines specific criteria for classifying substances as POPs, such as chemical identity, evidence of persistence and bio-accumulation, potential for long-range transport, and evidence of adverse effects on human health and the environment. The Stockholm Convention has demonstrated its adaptability, evolving over time to address emerging environmental concerns. Since the Convention's entry into force, more POPs have been added to its scope, and there have been proposals for the consideration of others. Today, more than 30 POPs are listed under the Stockholm Convention.

The initial 12 POPs listed ceased production and new uses in 2009. However, some exemptions allow certain uses to continue for a specified period. For instance, PCBs can remain in existing applications, like transformers or capacitors until 2025 (for all Parties), and PBDEs as well as other newly added substances have time-limited exemptions. Regarding the two POPs listed in Annex B—DDT and PFOS—, safe and affordable alternatives are not yet available. Consequently, they can still be produced or used for specific purposes.

Chemicals listed in Annex C are unintentionally formed and released during the production of other compounds or through thermal processes. To mitigate the unintentional releases of these POPs, Parties are required to develop release inventories, implement best available techniques (BAT), and promote best environmental practices (BEP).

In order to assess changes in POP concentrations in the environment and humans, a Global Monitoring Plan (GMP) for POPs has been established (UNEP, 2007). The GMP establishes a system to consistently collect comparable data about the presence of POPs in different regions. The plan focuses on core matrices such as ambient air and human tissues (human milk or human blood) for all POPs, and surface water for hydrophilic chemicals like PFOS. One notable aspect is the joint monitoring efforts with the World Health Organization (WHO) in assessing human milk, which has provided consistent and high-quality data, proving invaluable in understanding the impact of POPs on human health (van den Berg et al., 2017).

With the intent of promoting an integrated approach to managing regulated substances throughout their life cycle and enhancing the effectiveness management, the Stockholm Convention closely collaborates with the Basel and Rotterdam Conventions. The Basel Convention, established in 1989 and entered into force in 1992, focuses on controlling the transboundary movement of hazardous waste and ensuring its environmentally sound management (UNEP, 1989). On the other hand, the Rotterdam Convention, adopted in 1998 and entered into force in 2004, aims to promote shared responsibility and cooperation among countries in the international trade of certain hazardous chemicals (UNEP, 1999). The Rotterdam Convention established a prior informed consent (PIC) procedure for the trade of specific chemicals, ensuring that these chemicals are not exported to countries that do not wish to receive them. While the three conventions have distinct goals, they often intersect and collaborate in various ways (Fiedler et al., 2019; Weber et al., 2013). For instance, Committees of the different conventions collaborates on reviewing chemicals for listing in the annexes to the respective conventions. At the same time, the Basel Convention's Open-ended Working group develops various technical guidelines for the environmentally sound management of waste, including e-waste and POP wastes. In summary, the Stockholm, Basel and Rotterdam Conventions collaborate by addressing various aspects of hazardous chemicals and waste management, sharing information, building capacity, and promoting cooperation among countries. Together, the three conventions contribute to global efforts to protect human health and the environment form the detrimental impacts of POPs and other hazardous chemical substances.

3. Objectives

The comprehensive coverage of information and arguments within the introduction effectively establishes the foundation for the primary objectives of this doctoral thesis, which are:

- To evaluate the levels of POPs in sentinel species inhabiting the Mediterranean Sea and the North East Atlantic Ocean while exploring the potential health risks associated to these species.

- To investigate POP concentrations in important edible fish species from the Mediterranean Sea to assess possible risks to human health derived from their consumption.

- To study tissue distribution (muscle and liver) of targeted POPs in blue shark.

- To investigate spatial and temporal trends of POP pollution in both study areas, critically constrasting them with available bibliographic information .

4. Material and methods

4.1. Sampling

Seventy-nine blubber/muscle samples of five different marine species, namely sperm whale (*Physeter macrocephalus*), Cuvier's baked whale (*Ziphius cavirostris*), sardine (*Sardina pilchardus*), anchovy (*Engraulis encrasicolus*), and bogue (*Boops boops*) were collected from 2008 to 2017 in different areas of the Mediterranean Sea (Adriatic, Ligurian, Tyrrhenian Seas) (Table 1 and Figure 12). Additionally, seventy-one muscle and seventy-one liver samples were collected from blue shark (*Prionace glauca*) inhabiting the North East Atlantic Ocean (Portuguese coast) in 2019 (Table 1 and Figure 12).

Table 1. Detailed information on the species sampled from 2008 to 2019 in the Mediterranean Sea and Atlantic Ocean.

	Species	N	Tissue	Location	Sampling Date	Sample origin
	Sperm whale (SW) *Physeter macrocephalus*	7	blubber	Adriatic coast	2009	stranded
		2	blubber	Tyrrhenian coast	2008/2016	stranded
	Cuvier's baked whale (CBW) *Zifius cavirostris*	22	blubber	Ligurian Sea	2014/2015	biopsied
	Anchovy (EEN) *Engraulis encrasicolus*	16	muscle	Tyrrhenian Sea	2017	local market
	Sardine (SPC) *Sardina pilchardus*	16	muscle	Tyrrhenian Sea	2017	local market
	Bogue (BBO) *Boops boops*	16	muscle	Tyrrhenian Sea	2017	local market
	Blue shark (BS) *Prionace glauca*	60	muscle/ liver	Portugal coast	2019	by-caught

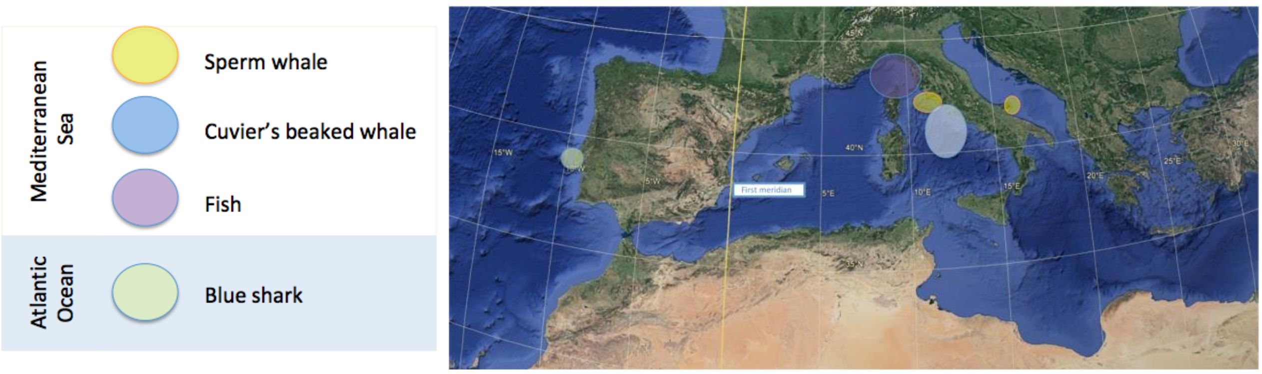


Figure 12. Sampling areas for the six marine species under study. (Map from https://earth.google.com/web/)

4.1.1. Sperm whales

Blubber samples were collected from nine stranded sperm whales (SW), all males. Seven stranded in 2009 during a mass stranding along the Adriatic coast (South East Italy), and two stranded in 2008 and 2016 along the Tyrrhenian coast (North West Italy) (Table 2). As reported by genetic analysis and photo-identification, the seven sperm whales stranded in 2009 belong to the Mediterranean subpopulation and are most likely members of the same pod (Mazzariol et al., 2011).

All samples were taken from the dorsal area, front the cranial insertion of the dorsal fin with a scalpel rinsed with acetone and ethanol between each dissection. Samples were then wrapped in aluminum foil, stored in ice on site and then frozen at −20 °C until residue analysis. For the seven sperm whales involved in the mass stranding, age was determined by counting dentin growth layer groups (GLG), and weight (corrected weight) was calculated considering postmortem body fluid and tissue leakages (Mazzariol et al., 2011). Each of the two sperm whales involved in individual strandings were assigned to a class age according to each specimen's length. Weight was estimated using the animal total length (Lockyer, 1976).

Upon the results of post-mortem investigations (histopathology, virology, bacteriology, parasitology, toxicology, genetic, and screening of veins looking for gas emboli), a multi-factorial cause was proposed as a trigger of this mass stranding (Mazzariol et al., 2011). Detailed information can be found in Bartalini et al. (2019) (Scientific publications 8.1).

Table 2. Detailed information about sperm whales (SW) stranded in 2008, 2009, 2016 in the Mediterranean Sea and discussed in this study.

Sample Code	Stranding date	Area	Size (m)	Weight (t)	Age (years)	Sex	Sampling condition
SW1	12/2009	Adriatic Coast	11.8	14.8	22–25	M	Found dead
SW2	12/2009	Adriatic Coast	12.2	16.0	20	M	Found dead
SW3	12/2009	Adriatic Coast	11.3	14.8	20	M	Found dead
SW4	12/2009	Adriatic Coast	11.4	13.7	20	M	Found dead
SW5	12/2009	Adriatic Coast	10.5	16.0	15	M	Stranded alive
SW6	12/2009	Adriatic Coast	12.1	17.7	20	M	Stranded alive
SW7	12/2009	Adriatic Coast	11.2	15.7	20	M	Stranded alive
SW13	10/2008	Tyrrhenian Coast	4.5	1.3	Young	M	Found dead
SW63806	08/2016	Tyrrhenian Coast	12.8	23.6	Adult	M	Found dead

4.1.2. Cuvier's beaked whales

Twenty-two free-ranging individuals of Cuvier's beaked whales (CBW) (19 males and 3 females) were biopsied in the Ligurian Sea (NW Mediterranean Sea) in 2014 and 2015 (Table 3). Skin biopsies were collected in a specific dorso-lateral area below the dorsal fin using a 68 kg draw weight recurve crossbow (Barnett Panzer V). About 1 g of tissue was obtained using stainless steel biopsy tips (30 mm × 8 mm) attached to 18′′ bolts. Tips were sterilized before use. The tissue samples were stored in liquid nitrogen after biopsy collection and stored at − 80 °C until laboratory analysis.

Biopsied individuals were photographed allowing photo-identification, age and sex classification. Individuals were categorized in three different age classes (juveniles, sub-adults, adults) by estimated size and coloration patterns (Coomber et al., 2016; Rosso et al., 2011).

The sex was first estimated based on size, coloration pattern, natural marking severity and finally individual history when the animals were already present in the photo-id catalogue. The sex was then confirmed by DNA analysis, following the protocol described in Baini et al. (2020) (Scientific publications 8.1).

Table 3. Detailed information about Cuvier's baked whales (CBW) biopsied in 2014 and 2015 in the Ligurian Sea and discussed in this study.

Sample Code	Sampling date	Area	Age (class)	Sex
CBW1_1	22/06/2014	Ligurian Sea	Juvenil	M
CBW1_2	22/06/2014	Ligurian Sea	Juvenil	M
CBW1_3	16/07/2014	Ligurian Sea	Adult	M
CBW1_4	16/07/2014	Ligurian Sea	Adult	F
CBW1_5	25/07/2014	Ligurian Sea	Adult	M
CBW1_6	03/08/2014	Ligurian Sea	Adult	M
CBW1_7	08/08/2014	Ligurian Sea	Adult	M
CBW2_1	02/07/2015	Ligurian Sea	Sub-adult	M
CBW2_2	02/07/2015	Ligurian Sea	Adult	M
CBW2_3	02/07/2015	Ligurian Sea	Sub-adult	M
CBW2_4	04/07/2015	Ligurian Sea	Sub-adult	M
CBW2_5	07/07/2015	Ligurian Sea	Adult	M
CBW2_6	16/07/2015	Ligurian Sea	Sub-adult	M
CBW2_7	17/07/2015	Ligurian Sea	Juvenil	M
CBW2_8	21/07/2015	Ligurian Sea	Sub-adult	M
CBW2_9	21/07/2015	Ligurian Sea	Sub-adult	M
CBW2_10	21/07/2015	Ligurian Sea	Sub-adult	M

CBW2_11	22/07/2015	Ligurian Sea	Adult	M
CBW2_12	13/08/2015	Ligurian Sea	Sub-adult	M
CBW2_13	13/08/2015	Ligurian Sea	Adult	F
CBW2_14	27/08/2015	Ligurian Sea	Sub-adult	M
CBW2_15	28/08/2015	Ligurian Sea	Juvenil	F

4.1.3. Anchovies, sardines and bogues

In spring 2017, three important commercial edible fish species commonly consumed in the Mediterranean region were purchased from local fish markets in Livorno (Italy). The fish species, anchovy (EEN), sardine (SPC) and bogue (BBO), were collected in the Northern Thyrrenian Sea (Table 4). Individuals (sixteen specimens for each species) were stored in ice on site until the arrival at the Department of Physical, Earth and Environmental Sciences of the University of Siena, where they were dissected to generate fillets. Fillets were frozen at −20 °C and then freeze-dried before residue analysis.

Table 4. Detailed information about anchovies (EEN), sardines (SPC) and bogues (BBO) collected in 2017 in the Northern Tyrrhenian Sea and discussed in this study. GSAs: Geographical Subareas of the Mediterranean Sea (FAO, 2009).

Sample Code	Fishing date	Area	Species	GSAs	Weight (g)	Length (cm)
9 EEN 36	26/09/17	Isola d'Elba (Portoferraio)	*Engraulis encrasicolus*	9	10.49	12.4
9 EEN 37	26/09/17	Isola d'Elba (Portoferraio)	*Engraulis encrasicolus*	9	17.55	13.9
9 EEN 40	26/09/17	Isola d'Elba (Portoferraio)	*Engraulis encrasicolus*	9	10.78	12.3
9 EEN 41	26/09/17	Isola d'Elba (Portoferraio)	*Engraulis encrasicolus*	9	12.68	12.9
9 EEN 42	26/09/17	Isola d'Elba (Portoferraio)	*Engraulis encrasicolus*	9	9.90	11.5
9 EEN 43	26/09/17	Isola d'Elba (Portoferraio)	*Engraulis encrasicolus*	9	9.95	12.4
9 EEN 44	26/09/17	Isola d'Elba (Portoferraio)	*Engraulis encrasicolus*	9	9.78	12.0
9 EEN 45	26/09/17	Isola d'Elba (Portoferraio)	*Engraulis encrasicolus*	9	10.37	11.7
9 EEN 52	27/09/17	Isola d'Elba (Portoferraio)	*Engraulis encrasicolus*	9	11.25	12.0
9 EEN 59	27/09/17	Isola d'Elba (Portoferraio)	*Engraulis encrasicolus*	9	11.85	12.3
9 EEN 61	27/09/17	Isola d'Elba (Portoferraio)	*Engraulis encrasicolus*	9	11.56	12.6
9 EEN 63	27/09/17	Isola d'Elba (Portoferraio)	*Engraulis encrasicolus*	9	12.46	12.5
9 EEN 65	27/09/17	Isola d'Elba (Portoferraio)	*Engraulis encrasicolus*	9	10.17	12.1
9 EEN 66	27/09/17	Isola d'Elba (Portoferraio)	*Engraulis encrasicolus*	9	12.10	13.3
9 EEN 67	27/09/17	Isola d'Elba (Portoferraio)	*Engraulis encrasicolus*	9	10.76	12.3
9 EEN69	27/09/17	Isola d'Elba (Portoferraio)	*Engraulis encrasicolus*	9	9.05	11.9
9 SPC 02	10/10/17	Isola d'Elba (Portoferraio)	*Sardina pilchardus*	9	20.66	13.7
9 SPC 03	10/10/17	Isola d'Elba (Portoferraio)	*Sardina pilchardus*	9	21.46	14
9 SPC 04	10/10/17	Isola d'Elba (Portoferraio)	*Sardina pilchardus*	9	25.37	14.5
9 SPC 06	10/10/17	Isola d'Elba (Portoferraio)	*Sardina pilchardus*	9	17.01	13
9 SPC 08	10/10/17	Isola d'Elba (Portoferraio)	*Sardina pilchardus*	9	20.68	14.2
9 SPC 10	10/10/17	Isola d'Elba (Portoferraio)	*Sardina pilchardus*	9	20.21	13.5

9 SPC 13	10/10/17	Isola d'Elba (Portoferraio)	*Sardina pilchardus*	9	17.07	13.3
9 SPC 14	10/10/17	Isola d'Elba (Portoferraio)	*Sardina pilchardus*	9	17.64	13.5
9 SPC 17	10/10/17	Isola d'Elba (Portoferraio)	*Sardina pilchardus*	9	17.1	14.5
9 SPC 20	19/10/17	Isola d'Elba (Portoferraio)	*Sardina pilchardus*	9	20.78	14.3
9 SPC 23	19/10/17	Isola d'Elba (Portoferraio)	*Sardina pilchardus*	9	14.79	12.9
9 SPC 28	19/10/17	Isola d'Elba (Portoferraio)	*Sardina pilchardus*	9	21.25	14
9 SPC 31	19/10/17	Isola d'Elba (Portoferraio)	*Sardina pilchardus*	9	19.9	13.5
9 SPC 32	19/10/17	Isola d'Elba (Portoferraio)	*Sardina pilchardus*	9	23.06	14.5
9 SPC 35	19/10/17	Isola d'Elba (Portoferraio)	*Sardina pilchardus*	9	18.95	12.9
9 SPC 40	19/10/17	Isola d'Elba (Portoferraio)	*Sardina pilchardus*	9	15.85	12.9
9 BBO 01	10/10/17	Isola d'Elba (Portoferraio)	*Boops boops*	9	69.82	20
9 BBO 02	10/10/17	Isola d'Elba (Portoferraio)	*Boops boops*	9	46.43	16.7
9 BBO 03	10/10/17	Isola d'Elba (Portoferraio)	*Boops boops*	9	65.7	19
9 BBO 05	10/10/17	Isola d'Elba (Portoferraio)	*Boops boops*	9	76.66	25
9 BBO 09	10/10/17	Isola d'Elba (Portoferraio)	*Boops boops*	9	77.08	20.5
9 BBO 10	10/10/17	Isola d'Elba (Portoferraio)	*Boops boops*	9	66.11	19.6
9 BBO 14	18/10/17	Isola d'Elba (Portoferraio)	*Boops boops*	9	66.72	19.3
9 BBO 23	18/10/17	Isola d'Elba (Portoferraio)	*Boops boops*	9	62.64	19
9 BBO 24	18/10/17	Isola d'Elba (Portoferraio)	*Boops boops*	9	46.54	16.1
9 BBO 27	18/10/17	Isola d'Elba (Portoferraio)	*Boops boops*	9	60.43	19
9 BBO 28	18/10/17	Isola d'Elba (Portoferraio)	*Boops boops*	9	54.18	17.3
9 BBO 30	18/10/17	Isola d'Elba (Portoferraio)	*Boops boops*	9	65.65	19
9 BBO 31	18/10/17	Isola d'Elba (Portoferraio)	*Boops boops*	9	66.38	18.5
9 BBO 36	19/10/17	Isola d'Elba (Portoferraio)	*Boops boops*	9	76.37	20
9 BBO 38	19/10/17	Isola d'Elba (Portoferraio)	*Boops boops*	9	70.76	20.5
9 BBO 39	19/10/17	Isola d'Elba (Portoferraio)	*Boops boops*	9	66.21	19.6

4.1.4. Blue sharks

Sixty blue shark (BS) specimens (38 males and 22 females) were incidentally caught during swordfish fishing off the coast of Portugal, between March and December 2019. Samples of liver and muscle were obtained from each animal by using inox cutlery, rinsed with acetone and ethanol between each dissection. Samples were wrapped in aluminium foil, placed in individual plastic tubes and preserved at −20 °C until further analysis. Sampling blanks were collected in form of aluminium foil handled and wrapped with the same tools and cleaning protocols. The sex, length (fork length (FL) in cm), and location capture data were registered for all specimens. Assuming sexual maturity for males and females at 180 and 185 cm, respectively (da Silva et al., 2021), 55% of males and 100% of females were juveniles while 45% of males were regarded as adults. Detailed information can be found in Table 5 and in Muñoz-Arnanz et al. (2022) (Scientific publications 8.1).

Table 5. Detailed information about blue sharks (BS) accidentally captured in 2019 off the coast of Portugal and discussed in this study.

Sample Code	Area	Fork Lenght (cm)	Sex	Sample Code	Area	Fork Lenght (cm)	Sex	Sample Code	Area	Fork Lenght (cm)	Sex
BS_1	39°12'N 11°18'W	132	M	BS_22	38º50’N 10º15’W	126	M	BS_42	38º04’N 15º23’W	175	M
BS_2	39°12'N 11°18'W	109	M	BS_23	38º50’N 10º15’W	168	M	BS_46	38º04’N 15º23’W	155	F
BS_3	39°12'N 11°18'W	118	F	BS_24	38º50’N 10º15’W	122	M	BS_47	38º04’N 15º23’W	132	F
BS_4	39°12'N 11°18'W	146	F	BS_25	38º50’N 10º15’W	251	M	BS_48	38º04’N 15º23’W	155	M
BS_5	39°12'N 11°18'W	126	F	BS_26	38º50’N 10º15’W	213	M	BS_49	38º04’N 15º23’W	110	M
BS_6	39°12'N 11°18'W	101	F	BS_27	38º50’N 10º15’W	122	F	BS_50	37º58’N 16º22’W	115	F
BS_7	39°12'N 11°18'W	146	F	BS_28	38º50’N 10º15’W	172	M	BS_51	37º58’N 16º22’W	231	M
BS_8	39°12'N 11°18'W	141	F	BS_29	38º50’N 10º15’W		M	BS_52	37º58’N 16º22’W	135	M
BS_9	39°12'N 11°18'W	115	F	BS_30	38º50’N 10º15’W	147	M	BS_53	37º50’N 15º26’W	115	F
BS_10	39°12'N 11°18'W	130	M	BS_31	39º55’N 13º00’W	230	M	BS_54	37º50’N 15º26’W	135	M
BS_11	39°12'N 11°18'W	129	F	BS_32	39º55’N 13º00’W	147	F	BS_55	37º50’N 15º26’W	130	M
BS_12	39°12'N 11°18'W	121	F	BS_33	39º55’N 13º00’W	234	M	BS_56	37º50’N 15º26’W	135	M
BS_14	39º20’N 11º30’W	247	M	BS_34	39º55’N 13º00’W	143	M	BS_58	37º50’N 15º26’W	130	M
BS_15	39º20’N 11º30’W	221	M	BS_35	39º55’N 13º00’W	247	M	BS_59	37º50’N 15º26’W	160	F
BS_16	39º20’N 11º30’W	247	M	BS_36	39º55’N 13º00’W	242	M	BS_60	37º50’N 15º26’W	112	F
BS_17	39º20’N 11º30’W	218	M	BS_37	39º55’N 13º00’W	201	M	BS_61	37º50’N 15º26’W	140	M
BS_18	39º20’N 11º30’W	234	M	BS_38	39º55’N 13º00’W	205	M	BS_62	37º50’N 15º26’W	155	F
BS_19	39º20’N 11º30’W	135	M	BS_39	39º55’N 13º00’W	226	M	BS_63	37º50’N 15º26’W	105	F
BS_20	39º20’N 11º30’W	164	M	BS_40	39º55’N 13º00’W	143	F	BS_64	37º50’N 15º26’W	125	F
BS_21	39º20’N 11º30’W	242	M	BS_41	39º55’N 13º00’W	193	M	BS_65	37º50’N 15º26’W	150	F

4.2. Analytical procedure

4.2.1. Reagent and standards

High-purity solvents, meeting the standards for pesticide residue grade or equivalent, were employed. Hexane, dichloromethane, and toluene were purchased from J.T. Baker (Deventer, The Netherlands), whereas nonane was acquired from LGC Standards GmbH in Wesel, Germany. Silica gel (70-230 mesh) and sulfuric acid (analytical reagent grade, 95-97%) were supplied by Merck, and anhydrous sodium sulfate by J.T. Baker (Deventer, The Netherlands). The analytical standards used in this study can be found in Table 6.

Table 6. Labeled and native chemical standards used in this study.

Group	Congeners / Isomers	Supplier
native PCBs	PCB-28, -52, -77, -81, -101, -105, -114, -118, -123, -126, -138, -153, -156, -157, -167, -169, -180, -189 (calibration solution **WM48-CVS**)	Wellington Laboratories (Guelph, Ontario, Canada)
labeled PCBs	$^{13}C_{12}$-PCB-28, -52, -77, -81, -101, -105, -114, -118, -123, -126, -138, -153, -156, -157, -167, -169, -180, -189 (calibration solution **WM48-CVS** and PCB mixes **P48-W-ES** and **P48-M-ES**)	
	$^{13}C_{12}$-PCB-70, -111, -170 (PCB mix **P48-RS**)	
native PBDEs	BDE-3, -7,- 15, -17, -28, -47, -49, -66, -71, -77, -85, -99, -100, -119, -126, -138,- 153, -154, -156, -183, -184, -191, -196,-197,-206, -207, -209 (calibration solution **BDE-CVS-G)**	
labeled PBDEs	$^{13}C_{12}$-BDE-79, -138, -206 **(**mix **MBDE-ISS-G)**	
native PCDD/Fs	2,3,7,8-TCDD; 1,2,3,7,8-PeCDD; 1,2,3,4,7,8-/ 1,2,3,6,7,8-/ 1,2,3,7,8,9-HxCDD; 1,2,3,4,6,7,8-HpCDD; OCDD; 2,3,7,8-TCDF; 1,2,3,7,8-/2,3,4,7,8-PCDF; 1,2,3,4,7,8-/ 1,2,3,6,7,8-/ 1,2,3,7,8,9-/ 2,3,4,6,7,8-HxCDF; 1,2,3,4,6,7,8-/ 1,2,3,4,7,8,9-HpCDF; OCDF (calibration solution **EN-1948CVS**)	
labeled PCDD/Fs	2,3,7,8-TC[$^{13}C_{12}$]DD; 1,2,3,7,8-PeC[$^{13}C_{12}$]DD; 1,2,3,4,7,8-/ 1,2,3,6,7,8-HxC[$^{13}C_{12}$]DD; 1,2,3,4,6,7,8-HpC[$^{13}C_{12}$]DD; OC[$^{13}C_{12}$]DD; 2,3,7,8-TC[$^{13}C_{12}$]DF; 2,3,4,7,8-PC[$^{13}C_{12}$]DF; 1,2,3,4,7,8-/ 1,2,3,6,7,8-/ 2,3,4,6,7,8-HxC[$^{13}C_{12}$]DF; 1,2,3,4,6,7,8-HpC[$^{13}C_{12}$]DF; OC[$^{13}C_{12}$]DF (calibration solution **EN-1948CVS** and mix **EN-1948ES**)	
	1,2,3,4-TC[13C12]DD; 1,2,3,7,8,9-HxC[13C12]DD (mix **EN-1948IS**)	

4.2.2. Sample extraction

Fresh (SW, CBW, BS) and freeze-dried samples (EEN, SPC, BBO) (Table 7) were weighed, homogenized with anhydrous sodium sulfate (Na_2SO_4), and spiked with a suite of ^{13}C-labeled standards of target contaminants (Table 8). Detailed information about type and quantity of tissue employed for contaminant analysis can be found in Table 7.

Table 7. Type of tissues, amount (g) of sample and amount of Na_2SO_4 (g) used for POP analysis.

Species	Tissues	Sample (g)	Na_2SO_4 (g)
SW	fresh blubber	~1.7-2	15
CBW	fresh blubber	~ 0.05-0.40	15
Fish	freeze-dried muscle	~ 1	10
BS	fresh liver	~1.5	50
	fresh muscle	~10g	70

Table 8. Used amounts (pg) of surrogate standards spiked prior extraction.

Species	$^{13}C_{12}$-PCBs (**mixes P48-W-ES, P48-M-ES**)	$^{13}C_{12}$-PBDEs (**mix MBDE-MXG**)	$^{13}C_{12}$-PCDD/Fs (**mix EN-1948ES**)
SW	200 pg for all congeners	1000 pg for mono- to penta-brominated congeners 2000 pg for hexa- to octa-brominated congeners 5000 pg for nona- to deca-brominated congeners	2000 pg for tetra- to hexa-chlorinated congeners 4000 pg for hepta- to octa-chlorinated congeners
CBW	200 pg for all congeners	1000 pg for mono- to penta-brominated congeners 2000 pg for hexa- to octa-brominated congeners 5000 pg for nona- to deca-brominated congeners	**-**
SPC, EEN, BBO	200 pg for all congeners	**-**	**-**
BS *liver*	250 pg of dioxin-like (DL)-PCBs (**mix P48-W-ES**) 2500 pg of i-PCBs (**mix P48-M-ES**)	1500 pg for mono- to penta-brominated congeners 3000 pg for hexa- to octa-brominated congeners 7500 pg for nona- to deca-brominated congeners	2000 pg for tetra- to hexa-chlorinated congeners 4000 pg for hepta- to octa-chlorinated congeners
BS *muscle*	100 pg of dioxin-like (DL)-PCBs (**mix P48-W-ES**) 100 pg (muscle) of i-PCBs (**mix P48-M-ES**)	1000 pg for mono- to penta-brominated congeners 2000 pg for hexa- to octa-brominated congeners 5000 pg for nona- to deca-brominated congeners	2000 pg for tetra- to hexa-chlorinated congeners 4000 pg for hepta- to octa-chlorinated congeners

Following a proper equilibrium time after surrogate addition, samples were Soxhlet extracted for 24h with ~100mL of n-hexane: DCM (9:1) mixture.

4.2.3. Sample purification

Extracts were concentrated by rota-evaporation (SW, CBW, EEN, SPC, BBO) or by using a TurboVap® (Zymarck Inc., Hopkinton, MA, USA) (BS), and purified by using the automated sample preparation system DEXTech+ (LCTech GmbH, Dorfen, Germany) (SW, CBW) or by low-pressure chromatography on open columns (EEN, SPC, BBO).

4.2.3.1. DEXTech+ system (SW, CBW)

Final extracts of 10 mL were loaded into the DEXTech+ system (Figure 13), followed by the addition of 500 µL of acetone as a solvent modifier. The DEXTech+ system was utilized in its alumina configuration, which involved the use of three columns: 1) SMART silica gel columns (P/N 14307, LCTech), 2) aluminum oxide (P/N 15433, LCTech), and 3) a carbon cartridge (P/N 15242, LCTech).

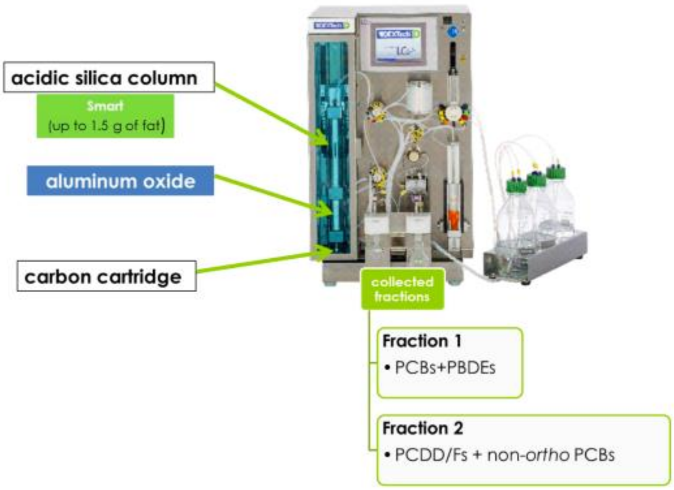


Figure 13. Schematics of the DEXTech+ system used in its alumina configuration.

Three different solvents were employed: 1) n-hexane, 2) n-hexane:dichloromethane (DCM) (1:1), and 3) toluene. This setup in a three-column arrangement resulted in the separation of two fractions: F1, which contained all PCBs except the four non-ortho congeners and all PBDEs, and F2, which contained all 2,3,7,8-PCDD/F congeners and the non-ortho PCB congeners. Each run lasted for 45 minutes, utilizing a total solvent consumption of 205 mL (F1 with a volume of 24 mL of n-hexane:DCM and F2 with a volume of 10 mL of toluene). These fractions were subsequently concentrated using a TurboVap® system to approximately 1 mL, transferred to vials, and taken to nearly dryness under a gentle stream of nitrogen. Vials were reconstituted in a few microliters of ^{13}C-labeled injection standards of DL-PCBs (mix P48-RS), PBDEs (mix MBDE-ISS-G) and PCDD/Fs (mix EN-1948IS) prior to instrumental analysis.

4.2.3.2 Low-pressure chromatography (EEN, SPC, BBO)

Once the extracts were rota-evaporated to ~ 1-2 mL, they were cleaned up by low pressure chromatography on multilayer columns packed with neutral silica (thermically activated at 160°C for 48 h) and acidic silica gel (neutral silica gel modified with H_2SO_4), as it is shown in Figure 14. Each column was initially conditioned with 30 mL of n-hexane:DCM, loaded with an extract, and eluted with 60 mL of n-hexane:DCM. Eluates were evaporated down to ~ 1 mL using a TurboVap®, transferred to vials, and dried under a gentle nitrogen stream. A few microliters of ^{13}C-labeled injection standards of PCBs (mix P48-RS) were used for reconstitution of each sample prior to instrumental analysis.

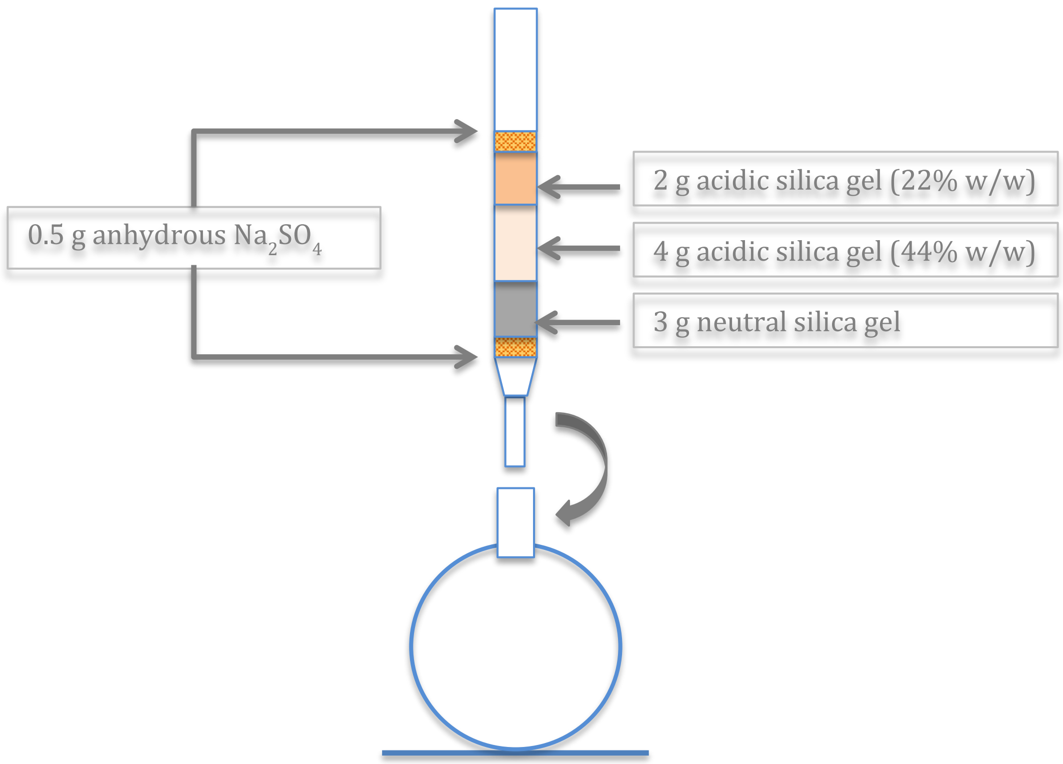


Figure 14. Schematics of the chromatographic column set-up used in the purification process.

4.3. Lipid determination

4.3.1. Sperm whale, Cuvier's baked whale

The lipid content of each sample was determined gravimetrically. Each extract was first rota-evaporated close to dryness. Afterwards, they were transferred to an oven for 30 min at 105°C. They were kept covered overnight and weighed the following day. Weight differences for each flask before and after extraction were assumed as lipid content. The lipid content for each sample is shown in Table 9.

Table 9. Wet weight (w.w.) and lipid weight (l.w.) for the analyzed samples.

	Sample ID	**w.w. (g)**	**l.w. (g)**	**l.w. (%)**
Sperm whale	SW13	1.8033	0.945	**52**
	SW63806	1.9012	0.142	**7.5**
	SW1	1.8788	0.187	**10**
	SW2	1.7274	0.264	**15**
	SW3	1.7880	0.599	**34**
	SW4	1.8771	0.522	**28**
	SW5	1.7217	0.253	**15**
	SW6	1.9652	0.385	**20**
	SW7	1.9294	0.326	**17**
Cuvier's baked whale	CBW1_1	0.1423	0.078	**55**
	CBW1_2	0.0647	0.007	**11**
	CBW1_3	0.1273	0.022	**17**
	CBW1_4	0.3037	0.078	**26**
	CBW1_5	0.1325	0.020	**15**
	CBW1_6	0.1459	0.026	**18**
	CBW1_7	0.0778	0.014	**18**
	CBW2_3	0.2430	0.083	**34**
	CBW2_4	0.3163	0.116	**37**
	CBW2_5	0.1914	0.049	**26**
	CBW2_6	0.3237	0.085	**26**
	CBW2_7	0.1541	0.032	**21**
	CBW2_8	0.1216	0.010	**8.2**
	CBW2_9	0.0517	0.001	**1.9**
	CBW2_10	0.0972	0.028	**29**
	CBW2_11	0.3736	0.122	**35**
	CBW2_12	0.0733	0.007	**10**
	CBW2_13	0.0507	0.016	**32**
	CBW2_14	0.1453	0.052	**36**
	CBW2_15	0.0724	0.017	**24**

4.3.2. Blue shark

After Soxhlet extraction, a one-tenth aliquot of each sample was used for the gravimetric determination of the lipid content. Aliquots were first evaporated close to dryness and placed into vials. Afterwards, vials were transferred to an oven for 30 min at 105°C. They were kept covered overnight and weighed the following day. Weight differences for each vial before and after extraction were assumed as lipid content. A summary of the lipid content (%) for samples of both tissues investigated is shown in Table 10.

Table 10. Mean, standard deviation (SD), median and range of the lipid content (%) of the analyzed samples of liver and muscle of blue shark.

	Tissue	Mean ± SD	Median	Range (min-max)
Blues shark	liver	34.4 ± 9.0	36.2	9.22 – 58.8
	muscle	0.264 ± 0.113	0.259	0.061 – 0.491

4.4. Instrumental determination

SW and BS were analyzed for eighteen PCBs (the six indicators (i-PCBs) congeners # 28, 52, 101, 138, 153, 180 and the twelve dioxin-like (DL-PCBs) including 4 non-ortho congeners # 77, 81, 126, 169 and 8 mono-ortho congeners # 105, 114, 118, 123, 156, 157, 167, 189), twenty-six PBDEs (congeners # 7, 15, 17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 156, 183, 184, 191, 196, 197, 206, 207, 209) and seventeen PCDD/Fs (all 2,3,7,8 – chlorine substituted congeners). CBW underwent analysis for the same eighteen PCBs and twenty-six PBDEs; instead, fish samples were subject to analysis only for the eighteen PCBs.

Analysis was performed by gas chromatography coupled to high-resolution mass spectrometry (GC-HRMS) by means of a Trace GC Ultra gas chromatograph (Thermo Fisher Scientific, Milan, Italy) coupled to a high-resolution mass spectrometer (DFS, Thermo Fisher Scientific, Bremen, Germany). One µL of each extract was injected in splitless mode at a temperature of 260°C using helium as carrier gas at a constant flow mode. GC separation of PBDEs was achieved using a 15 m × 0.25 mm × 0.10 µm Rxi-5Sil MS column (Restek, USA). GC separation of PCBs and PCDD/Fs was achieved using a 60 m × 0.25 mm × 0.25 µm DB-5MS column (Agilent J&W, USA). The different oven temperature programs and carrier flows used for both families of target compounds are shown in Table 11. Positive electron ionization (EI+) was used operating in selected ion monitoring (SIM) mode at a minimum of 10,000 resolving power at 10% valley. The isotopic dilution technique was followed for quantitation purposes. A full description of the instrumental parameters can be found in Baini et al. (2020), Bartalini et al. (2020, 2019), and Muñoz-Arnanz et al. (2022) (Scientific publications 8.1).

Table 11. Oven temperature programs and carrier flow values used in the analysis of the target contaminants.

PCBs			PCDD/Fs			PBDEs		
ramp (°C/min)	T (°C)	hold time (min)	ramp (°C/min)	T (°C)	hold time (min)	ramp (°C/min)	T (°C)	hold time (min)
	140	1		140	1		130	4.2
20	200	3	20	200	3	20	200	3
3	275	-	3	310	8	3	310	8
30	310	10						
helium flow 1.3 mL/min			helium flow 0.8 mL/min			helium flow 1.5 mL/min		

4.5. QA/QC criteria

To guarantee the accuracy of obtained data for the analytes targeted in our study, several quality assurance and quality control measures were undertaken during sample preparation and instrumental analysis. Three solvents of decreasing polarity (acetone, dichloromethane and n-hexane) were used to clean (three times for each solvent) metal and glassware material. A procedural blank was analyzed within each batch of six samples. Care was taken to minimize exposure to UV light throughout the entire analytical procedure.

Quantification of all target analytes was based on the isotopic dilution technique with the following criteria: (a) ratio between the two monitored ions within ±15% of the theoretical value, and (b) limits of quantification (LOQs) corresponding to S/N of 10. Linear calibration curves for PCBs (6 points from 0.1 to 1000 pg/µL, calibration solutions WM48-CVS), PCDD/Fs (7 points from 0.04 to 1280 pg/µL, calibration solutions EN-1948CVS), and PBDEs (5 points from 1.0 to 2000 pg/µL, calibration solutions BDE-CVS-G) were daily checked. When quantifiable levels of a given analyte were found in a procedural blank, they were subtracted from the batch of samples associated to that blank. Concentrations found in field blanks did not indicate any contamination ascribed to the sampling procedure. Average recoveries for the used surrogates and average LOD values (calculated as 3 times the relationship S/N) are described in Table 12, 13, 14 and 15.

Table 12. Recovery values of labelled surrogates and average LODs for target compounds in SW samples.

Compound	**Recovery (%) ± SD**	**Average LOD (pg/g l.w.)**	**Compound**	**Recovery (%) ± SD**	**Average LOD (pg/g l.w.)**
PCBs					
PCB-77		4.29	BDE-184		7.88
$^{13}C_{12}$-PCB-77	75.0± 15.8		BDE-191		7.30
PCB-81		3.93	BDE-196		5.47
$^{13}C_{12}$-PCB-81	76.8± 19.2		BDE-197		5.66
PCB-105		11.4	**$^{13}C_{12}$-BDE-197**	79.4 ± 9.1	
$^{13}C_{12}$-PCB-105	103± 13		BDE-206		3.22
PCB-114		12.1	BDE-207		3.27
$^{13}C_{12}$-PCB-114	97.6± 13.9		**$^{13}C_{12}$-BDE-207**	91.4± 10.3	
PCB-118		11.4	BDE-209		21.5
$^{13}C_{12}$-PCB-118	109± 10		**$^{13}C_{12}$-BDE-209**	55.2± 6.0	
PCB-123		11.7			
$^{13}C_{12}$-PCB-123	107± 15		**PCDDs**		
PCB-126		9.92	2,3,7,8-TCDD		0.626
$^{13}C_{12}$-PCB-126	101± 17		**2,3,7,8-TC[$^{13}C_{12}$]DD**	78.1± 7.8	
PCB-156		4.18	1,2,3,7,8-PeCDD		0.962
$^{13}C_{12}$-PCB-156	103± 12		**1,2,3,7,8-PeC[$^{13}C_{12}$]DD**	71.7± 8.6	
PCB-157		4.28	1,2,3,4,7,8-HxCDD		0.733
$^{13}C_{12}$-PCB-157	97.1± 16.1		**1,2,3,4,7,8-HxC[$^{13}C_{12}$]DD**	88.1± 7.8	
PCB-167		4.15	1,2,3,6,7,8-HxCDD		0.735
$^{13}C_{12}$-PCB-167	90.9 ± 12		**1,2,3,6,7,8-HxC[$^{13}C_{12}$]DD**	82.6± 8.2	
PCB-169		4.40	1,2,3,7,8,9-HxCDD 1,2,3,4,6,7,8-		0.648
$^{13}C_{12}$-PCB-169	110 ± 18		HpCDD		0.351
PCB-189		2.75	**1,2,3,4,6,7,8-HpC[$^{13}C_{12}$]DD**	90.1± 11.2	
$^{13}C_{12}$-PCB-189	106 ± 10		OCDD		0.442
PBDEs			**OC[$^{13}C_{12}$]DD**	74.8± 9.4	
BDE-3		-	**PCDFs**		
$^{13}C_{12}$-BDE-3	55± 14		2,3,7,8-TCDF		1.09

BDE-7		-	**2,3,7,8-TC[$^{13}C_{12}$]DF**	80.6± 9.7	
BDE-15		-	1,2,3,7,8-PCDF		1.12
$^{13}C_{12}$-BDE-15	68± 8		2,3,4,7,8-PCDF		1.11
BDE-17		22.7	**2,3,4,7,8-PC[$^{13}C_{12}$]DF**	82.2± 9.7	
BDE-28		2.08	1,2,3,4,7,8-HxCDF		0.647
$^{13}C_{12}$-BDE-28	102± 12		**1,2,3,4,7,8-HxC[$^{13}C_{12}$]DF**	90.7± 9.8	
BDE-47		3.79	1,2,3,6,7,8-HxCDF		0.599
$^{13}C_{12}$-BDE-47	98.2± 8.7		**1,2,3,6,7,8-HxC[$^{13}C_{12}$]DF**	87.8± 9.3	
BDE-49		5.58	1,2,3,7,8,9-HxCDF		0.664
BDE-66		13.0	2,3,4,6,7,8-HxCDF		0.646
BDE-71		6.01	**2,3,4,6,7,8-HxC[13C12]DF**	82.7± 8.7	
BDE-77		7.63	1,2,3,4,6,7,8-HpCDF		0.236
BDE-85		3.31	**1,2,3,4,6,7,8-HpC[13C12]DF**	87.6± 12.5	
BDE-99		2.99	1,2,3,4,7,8,9-HpCDF		0.242
			OCDF		0.247
$^{13}C_{12}$-BDE-99	97.1± 13.7		**OC[$^{13}C_{12}$]DF**	74.4± 10.6	
BDE-100		2.97			
$^{13}C_{12}$-BDE-100	94.8± 15.6				
BDE-119		-			
BDE-126		-			
$^{13}C_{12}$-BDE-126	88.0 ± 12.4				
BDE-138		29.9			
BDE-153		5.15			
$^{13}C_{12}$-BDE-153	91.4 ± 6.5				
BDE-154		4.50			
$^{13}C_{12}$-BDE-154	88.0 ± 12.4				
BDE-156		23.4			
BDE-183		8.64			
$^{13}C_{12}$-BDE-183	91.4 ± 6.5				

Table 13. Recovery values of labelled surrogates and average LODs for target compounds in CBW samples.

Compound	**Recovery (%) ± SD**	**Average LOD (pg/g l.w.)**	**Compound**	**Recovery (%) ± SD**	**Average LOD (pg/g l.w.)**
PCBs			**PBDEs**		
PCB-28		52.0	BDE-3		ND

$^{13}C_{12}$-PCB-28	74.4± 11.3		**$^{13}C_{12}$-BDE-3**	58.0 ± 12.4	
PCB-52		121	BDE-7		ND
$^{13}C_{12}$-PCB-52	81.4± 11.0		BDE-15		ND
PCB-77		16.8	**$^{13}C_{12}$-BDE-15**	71.7 ± 10.7	
$^{13}C_{12}$-PCB-77	78.3± 9.0		BDE-17		103
PCB-81		15.7	BDE-28		98.4
$^{13}C_{12}$-PCB-81	79.9± 9.2		**$^{13}C_{12}$-BDE-28**	78.5 ± 13.9	
PCB-101		197	BDE-47		65.1
$^{13}C_{12}$-PCB-101	85.3± 8.8		**$^{13}C_{12}$-BDE-47**	94.5 ± 9.8	
PCB-105		165	BDE-49		96.0
$^{13}C_{12}$-PCB-105	88.1± 10.7		BDE-66		104
PCB-114		173	BDE-71		92.4
$^{13}C_{12}$-PCB-114	86.4± 8.1		BDE-77		61.2
PCB-118		185	BDE-85		141
$^{13}C_{12}$-PCB-118	83.2± 9.1		BDE-99		111
PCB-123		170	**$^{13}C_{12}$-BDE-99**	89.9 ± 14.5	
$^{13}C_{12}$-PCB-123	89.0± 10.1		BDE-100		123
PCB-126		34	**$^{13}C_{12}$-BDE-100**	84.8± 13.7	
$^{13}C_{12}$-PCB-126	88.7± 9.7		BDE-119		101
PCB-138		104	BDE-126		ND
$^{13}C_{12}$-PCB-138	104± 14		**$^{13}C_{12}$-BDE-126**	83.1± 11.0	
PCB-153		82.6	BDE-138		126
$^{13}C_{12}$-PCB-153	91.6± 11.2		BDE-153		107
PCB-156		95.9	**$^{13}C_{12}$-BDE-153**	94.5± 14.1	
$^{13}C_{12}$-PCB-156	86.0± 9.3		BDE-154		101
PCB-157		90.5	**$^{13}C_{12}$-BDE-154**	87.0± 14.5	
$^{13}C_{12}$-PCB-157	93.3± 9.6		BDE-156		132
PCB-167		98.7	BDE-183		57.1
$^{13}C_{12}$-PCB-167	82.9 ± 9.9		**$^{13}C_{12}$-BDE-183**	91.9± 13.8	
PCB-169		15.9	BDE-184		52.0
$^{13}C_{12}$-PCB-169	94.2 ± 9.1		BDE-191		51.1
PCB-180		67.7	BDE-196		28.9
$^{13}C_{12}$-PCB-180	91.5 ± 9.4		BDE-197		29.7
PCB-189		56.0	**$^{13}C_{12}$-BDE-197**	87.1± 22.4	
$^{13}C_{12}$-PCB-189	86.3 ± 8.1		BDE-206		57.8
			BDE-207		48.8

			$^{13}C_{12}$-BDE-207	103.9± 18.3	
			BDE-209		210
			$^{13}C_{12}$-BDE-209	66.4± 14.1	

Table 14. Recovery values of labelled surrogates and average LODs for target compounds in fish samples.

Compound	Recovery (%) ± SD	Average LOD (pg/g l.w.)
PCB-28		0.764
$^{13}C_{12}$-PCB-28	74.1± 19.7	
PCB-52		1.43
$^{13}C_{12}$-PCB-52	80.5± 11.1	
PCB-77		0.169
$^{13}C_{12}$-PCB-77	93.1± 12.1	
PCB-81		1.44
$^{13}C_{12}$-PCB-81	91.7± 12.2	
PCB-101		0.824
$^{13}C_{12}$-PCB-101	93.2± 9.5	
PCB-105		0.748
$^{13}C_{12}$-PCB-105	99.2± 11.4	
PCB-114		2.04
$^{13}C_{12}$-PCB-114	91.0± 9.9	
PCB-118		0.643
$^{13}C_{12}$-PCB-118	92.1± 10.4	
PCB-123		0.849
$^{13}C_{12}$-PCB-123	90.3± 9.3	
PCB-126		0.907
$^{13}C_{12}$-PCB-126	89.9± 10.0	
PCB-138		0.166
$^{13}C_{12}$-PCB-138	90.5 ± 10.2	
PCB-153		0.410
$^{13}C_{12}$-PCB-153	89.8 ± 10.5	
PCB-156		0.405
$^{13}C_{12}$-PCB-156	97.0 ± 12.8	
PCB-157		1.56
$^{13}C_{12}$-PCB-157	97.4 ± 12.9	
PCB-167		1.69
$^{13}C_{12}$-PCB-167	98.5 ± 13.0	

PCB-169		0.433
$^{13}C_{12}$-PCB-169	99.0 ± 10.6	
PCB-180		1.62
$^{13}C_{12}$-PCB-180	98.9 ± 11.5	
PCB-189		0.454
$^{13}C_{12}$-PCB-189	93.1 ± 9.7	

Table 15. Recovery values of labelled surrogates and average LODs for target compounds in BS samples.

Compound	Recovery (%) ± SD (liver/muscle)	Average LOD (pg/g l.w.) (liver/muscle)	Compound	Recovery (%) ± SD (liver/muscle)	Average LOD (pg/g l.w.) (liver/muscle)
PCBs			BDE-126		23.1/1.50
PCB-28		0.615/0.028	**$^{13}C_{12}$-BDE-126**	66.1±10.7/61.3±14.1	
$^{13}C_{12}$-PCB-28	61.2±4.7/61.9±16.7		BDE-138		4.28/0.150
PCB-52		1.48/0.050	BDE-153		3.55/0.127
$^{13}C_{12}$-PCB-52	65.0±5.5/69.8±18.1		**$^{13}C_{12}$-BDE-153**	84.0±5.9/72.2±12.3	
PCB-77		0.190/0.013	BDE-154		2.85/0.098
$^{13}C_{12}$-PCB-77	74.8±12.2/73.8±18.1		**$^{13}C_{12}$-BDE-154**	81.0±6.6/73.2±2.6	
PCB-81		0.171/0.012	BDE-156		4.84/0.165
$^{13}C_{12}$-PCB-81	76.4±13.2/73.7±19.0		BDE-183		1.01/0.093
PCB-101		0.171/0.057	**$^{13}C_{12}$-BDE-183**	85.5±9.9/67.3±11.3	
$^{13}C_{12}$-PCB-101	77.7±5.7/80.2±18.9		BDE-184		0.829/0.082
PCB-105		2.08/0.161	BDE-191		0.948/0.083
$^{13}C_{12}$-PCB-105	90.6±9.6/78.2±22.1		BDE-196		1.93/0.346
PCB-114		2.07/0.073	BDE-197		1.83/0.353
$^{13}C_{12}$-PCB-114	93.4±8.7/88.1±26.0		**$^{13}C_{12}$-BDE-197**	80.2±17.0/68.3±9.8	
PCB-118		1.85/0.214	BDE-206		1.58/1.29
$^{13}C_{12}$-PCB-118	87.6± 9.3/73.5± 19.5		BDE-207		1.31/1.18
PCB-123		1.84/0.236	**$^{13}C_{12}$-BDE-207**	84.3±8.5/70.1±11.1	
$^{13}C_{12}$-PCB-123	86.0±9.0/76.0±21.0		BDE-209		14.4/25.9
PCB-126		1.78/0.024	**$^{13}C_{12}$-BDE-209**	59.6±11.9/55.6±13.2	
$^{13}C_{12}$-PCB-126	86.0±12.7/91.2±31.3				
PCB-138		0.416/0.011	**PCDDs**		
$^{13}C_{12}$-PCB-138	98.2±7.3/99.8±23.9		2,3,7,8-TCDD		0.205/0.027
PCB-153		0.389/0.008	**2,3,7,8-TC[$^{13}C_{12}$]DD**	65.7±8.6/73.6±9.8	

$^{13}C_{12}$-PCB-153	78.6±6.5/82.6±22.2		1,2,3,7,8-PeCDD		0.309/0.041
PCB-156		0.266/0.098	**1,2,3,7,8-PeC[$^{13}C_{12}$]DD**	71.6±11.8/80.3±9.3	
$^{13}C_{12}$-PCB-156	84.6±9.9/85.3±24.4		1,2,3,4,7,8-HxCDD		0.222/0.031
PCB-157		1.02/0.097	**1,2,3,4,7,8-HxC[$^{13}C_{12}$]DD**	69.9±10.4/75.0±11.2	
$^{13}C_{12}$-PCB-157	81.3±10.5/83.6±23.6		1,2,3,6,7,8-HxCDD		0.231/0.032
PCB-167		1.27/0.083	**1,2,3,6,7,8-HxC[$^{13}C_{12}$]DD**	62.5±8.9/77.3±10.3	
$^{13}C_{12}$-PCB-167	85.2±11.2/85.9± 23.2		1,2,3,7,8,9-HxCDD		0.242/0.032
PCB-169		1.38/0.012	1,2,3,4,6,7,8-HpCDD		0.209/0.030
$^{13}C_{12}$-PCB-169	88.1±13.5/89.9± 23.9		**1,2,3,4,6,7,8-HpC[$^{13}C_{12}$]DD**	66.7±11.7/82.6±11.2	
PCB-180		0.161/0.008	OCDD		0.300/0.059
$^{13}C_{12}$-PCB-180	85.6±7.0/90.7±19.0		**OC[$^{13}C_{12}$]DD**	69.8±11.5/87.8±12.9	
PCB-189		0.271/0.066	**PCDFs**		
$^{13}C_{12}$-PCB-189	83.5±7.9/85.4±19.0		2,3,7,8-TCDF		0.476/0.028
			2,3,7,8-TC[$^{13}C_{12}$]DF	62.4±7.8/68.9±8.6	
PBDEs			1,2,3,7,8-PCDF		0.364/0.017
BDE-7		-/0.296	2,3,4,7,8-PCDF		0.360/0.017
BDE-15		-/0.153	**2,3,4,7,8-PC[$^{13}C_{12}$]DF**	70.8±11.4/82.3±9.2	
$^{13}C_{12}$-BDE-15	56.6±7.8/55.3±7.4		1,2,3,4,7,8-HxCDF		0.219/0.014
BDE-17		2.18/0.078	**1,2,3,4,7,8-HxC[$^{13}C_{12}$]DF**	68.7±9.0/73.1±11.6	
BDE-28		2.05/0.073	1,2,3,6,7,8-HxCDF		0.217/0.013
$^{13}C_{12}$-BDE-28	76.8±9.9/67.4±14.7		**1,2,3,6,7,8-HxC[$^{13}C_{12}$]DF**	70.4±8.8/75.8±10.3	
BDE-47		3.02/0.109	1,2,3,7,8,9-HxCDF		0.236/0.015
$^{13}C_{12}$-BDE-47	86.2±6.4/68.2±10.2		2,3,4,6,7,8-HxCDF		0.278/0.017
BDE-49		4.96/0.208	**2,3,4,6,7,8-HxC[$^{13}C_{12}$]DF**	66.0±8.3/70.6±9.6	
BDE-66		5.01/0.184	1,2,3,4,6,7,8-HpCDF		0.178/0.014
BDE-71		4.05/0.137	**1,2,3,4,6,7,8-HpC[$^{13}C_{12}$]DF**	63.6±11.0/79.8±10.6	
BDE-77		3.36/0.121	1,2,3,4,7,8,9-HpCDF		0.220/0.017
BDE-85		5.51/0.292	OCDF		0.218/0.031
BDE-99		2.16/0.078	**OC[$^{13}C_{12}$]DF**	65.8±12.3/79.6±12.8	
$^{13}C_{12}$-BDE-99	78.1±8.0/71.0±11.8				
BDE-100		2.46/0.080			
$^{13}C_{12}$-BDE-100	66.9±7.3/61.5±9.6				
BDE-119		2.08/0.066			

The precision for the quantification method was checked by reanalyzing:

- three different blubber samples in three different days obtaining RSDs lower than 8% for all target analytes, for sperm whale.
- three different blubber samples in three different days within two weeks obtaining RSDs lower than 14% for all target analytes, for Cuvier's baked whale.
- one sample of each species in three different days, obtaining RSDs lower than 8% for all PCB congeners, for fish.
- three different liver and three different muscle samples in three different days obtaining RSDs lower than 9% for all target analytes, for blue shark.

When determining pollutants in blubber, we evaluated the accuracy of the analytical method by analyzing a triplicate of the certified standard material SRM 1945 ('Organics in Whale Blubber', NIST). Satisfactory results were obtained for all target compounds and they can be found in Bartalini et al. (2019) (Scientific publications 8.1).

4.6. Data handling

All concentrations are given in ng/g (PCBs and PBDEs), pg/g (PCDD/Fs) or µg/g (PCBs, PBDEs in Cuvier's beaked whale) on lipid weight (l.w.) or wet weight (w.w.) basis. To assess the potential toxicity of DL-PCBs we followed the total toxic equivalency (TEQ) approach, in which each congener's toxicity is relativized to that of the most toxic one, the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) by means of toxic equivalency factors (TEF) (Van den Berg et al., 2006). Toxic equivalent quantities (TEQ) for DL-PCBs and PCDD/Fs were obtained using the World Health Organization (WHO)-2005 toxic equivalency factors (TEF) for mammals.

4.7. Statistical analyses

4.7.1. Cuvier's baked whale

Statistical analyses were conducted using the software RStudio v.1.1.453. Shapiro-Wilks test revealed non-normal distribution for PCBs and PBDEs. Independent Mann–Whitney U tests were used to compare levels of major contaminants among different age groups. If a significant difference was obtained (p value < 0.05), post-hoc Dunn's multiple comparison was performed for individual comparisons of age groups. Spearman's rank correlation was adopted to measure the correlation between variables. All statistical analyses were considered significant at $p < 0.05$.

4.7.2. Blue shark

Statistical analyses were conducted using the software SigmaPlot for Windows version 14.5 (Systat Software Inc, CA, USA) and IBM SPSS Statistics for Windows version 28 (SPSS Inc, IL, USA). Log transformation was used to fulfill the criterion of normality when exploring possible relationships between variables. When log transformation did not achieve normality, nonparametric tests were applied. Pearson or Spearman's correlations were explored among study variables depending on the normality (Pearson) or non-normality of data (Spearman's). No statistically significant differences (p value > 0.05) were found for the lipid content between sexes either on liver (Mann-Whitney, $U_{(22,38)} = 360$, $p = 0.374$) or in muscle (t-test $(58) = -1.525$, $p = 0.133$). No statically significant correlation was found either in males or females between tissues' lipid content and fork length.

5. Results

5.1. Congener profiles

5.1.1. Sperm whales

Most of the congeners of the three POP families targeted in this study (DL-PCBs, PBDEs, PCDD/Fs) (Bartalini et al., 2019 - Scientific publications 8.1) were detected in blubber of all sperm whales analyzed (Table 16), and showed a relative abundance with the following order: DL-PCBs > PBDEs >> PCDD/Fs. Five PBDE congeners, specifically one mono-BDE (BDE-3), two di-BDEs (BDE-7, BDE-15), and two penta-BDEs (BDE-119, BDE-126) were consistently not detected in any sample (Table 16).

Table 16. Detection frequency (% >LOQ) for PCBs, PBDEs, and PCDD/Fs in blubber of sperm whales stranded along the Italian coast in 2008, 2009 and 2016.

Sperm whale (*Physeter macrocephalus*) n=9					
Detection frequency %					
PCBs	*blubber*	**PBDEs**	*blubber*	**PCDD/Fs**	*blubber*
PCB-77	100	**BDE-3**	0	**PCDDs**	
PCB-81	100	**BDE-7**	0	**2378-TCDD**	100
PCB-105	100	**BDE-15**	0	**12378-PeCDD**	100
PCB-114	100	**BDE-17**	11	**123478-HxCDD**	100
PCB-118	100	**BDE-28**	100	**123678-HxCDD**	100
PCB-123	100	**BDE-47**	100	**123789-HxCDD**	44
PCB-126	100	**BDE-49**	100	**1234678-HpCDD**	100
PCB-156	100	**BDE-66**	100	**OCDD**	78
PCB-157	100	**BDE-71**	78		
PCB-167	100	**BDE-77**	100	**PCDFs**	
PCB-169	100	**BDE-85**	22	**2378-TCDF**	100
PCB-189	100	**BDE-99**	100	**12378-PeCDF**	78
		BDE-100	100	**23478-PeCDF**	100
		BDE-119	0	**123478-HxCDF**	100
		BDE-126	0	**123678-HxCDF**	100
		BDE-138	22	**234678-HxCDF**	100
		BDE-153	100	**123789-HxCDF**	0
		BDE-154	100	**1234678-HpCDF**	67
		BDE-156	100	**1234789-HpCDF**	0
		BDE-183	100	**OCDF**	11
		BDE-184	100		
		BDE-191	33		
		BDE-196	67		
		BDE-197	100		
		BDE-206	44		

BDE-207	78
BDE-209	78

The DL-PCB congener profile, showed in Figure 15, was dominated by mono-*ortho* PCBs (105, 114, 118, 123, 156, 157, 167, 189) that accounted for about 99.9% of the total DL-PCBs. Among the targeted DL-PCBs, PCB-118, PCB-156, and PCB-105 were the predominant congeners with average contributions of 58%, 14%, and 12%, respectively. On the other hand, the highly toxic non-*ortho* PCBs (81, 77, 126, 169) made up only a small fraction of total DL-PCBs (~ 0.1%). Among them, the most abundant congener was PCB-126 that accounted for more than half of the non-*ortho* PCB content, followed by PCB-169 (35.7%) and PCB-77 (5.3%), while PCB-81 contributed with <1% (Figure 15).

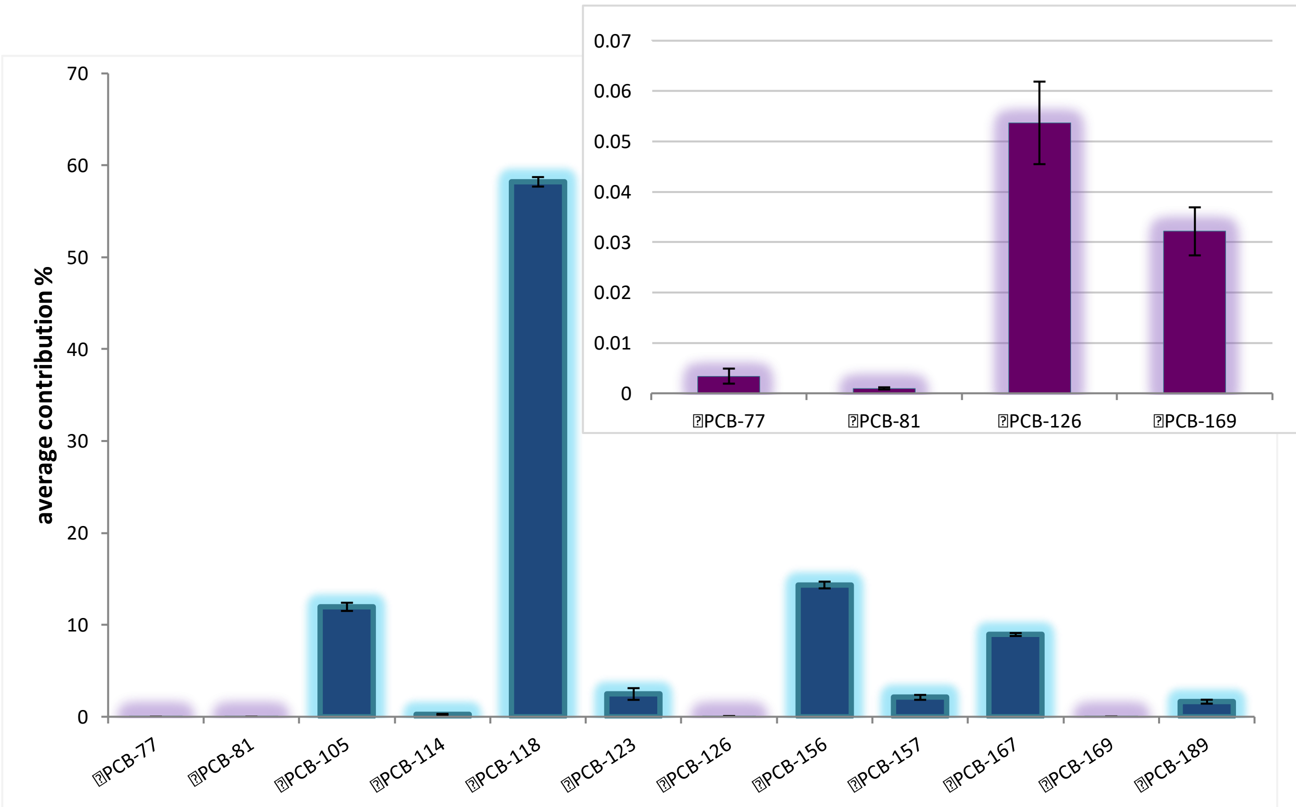


Figure 15. Average contribution of each DL-PCB (non-*ortho* PCB congeners zoomed in) to the total DL-PCB content in Mediterranean sperm whales stranded in 2008, 2009 and 2016. Error bars represent the standard error (SE).

The PBDE congener profile, showed in Figure 16, was dominated by lower-medium brominated (tetra-and penta-BDEs) congeners 47 > 99 > 100, with an average contribution to the total PBDE content of 70%, 10%, and 10%, respectively. On the other hand, higher-brominated congeners, such as BDE-183 (hepta-BDE) or BDE-209 (deca-BDE) represented only a small fraction of the total PBDE content, with an average contribution consistently below 0.1%.

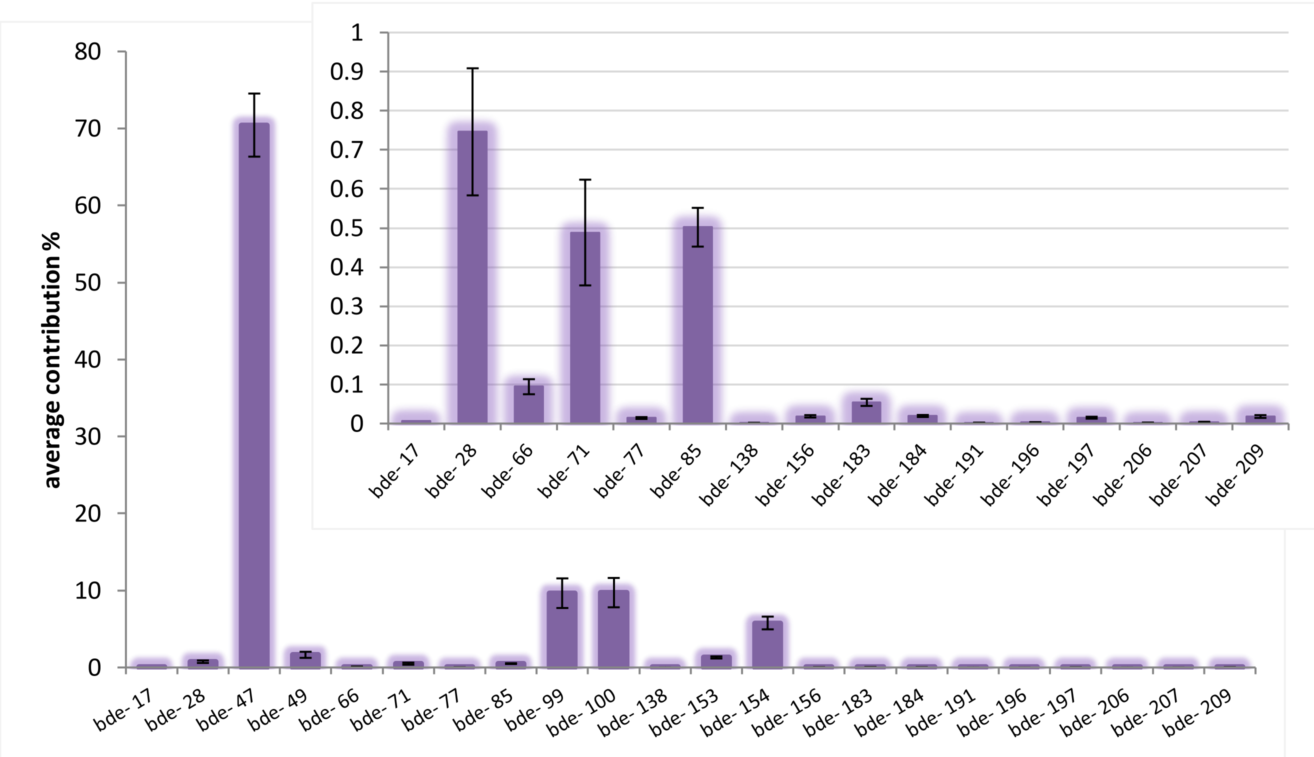


Figure 16. Average contribution of each PBDE congener to the total PBDE content in Mediterranean sperm whales stranded in 2008, 2009 and 2016. Error bars represent the standard error (SE). Congeners with average contribution < 1% are represented on the top.

Dioxins and furans scored average contributions to the total PCDD/F content of 51% and 49%, respectively. The most abundant PCDD/F congener was 2,3,4,7,8-PeCDF followed by 1,2,3,6,7,8-HxCDD, with a relative contribution of 21% and 17%, respectively (Figure 17). Octa-chlorinated congeners (OCDD and OCDF) accounted for the lowest average contributions both for dioxins and furans, with values of 3% and 0.7%, respectively.

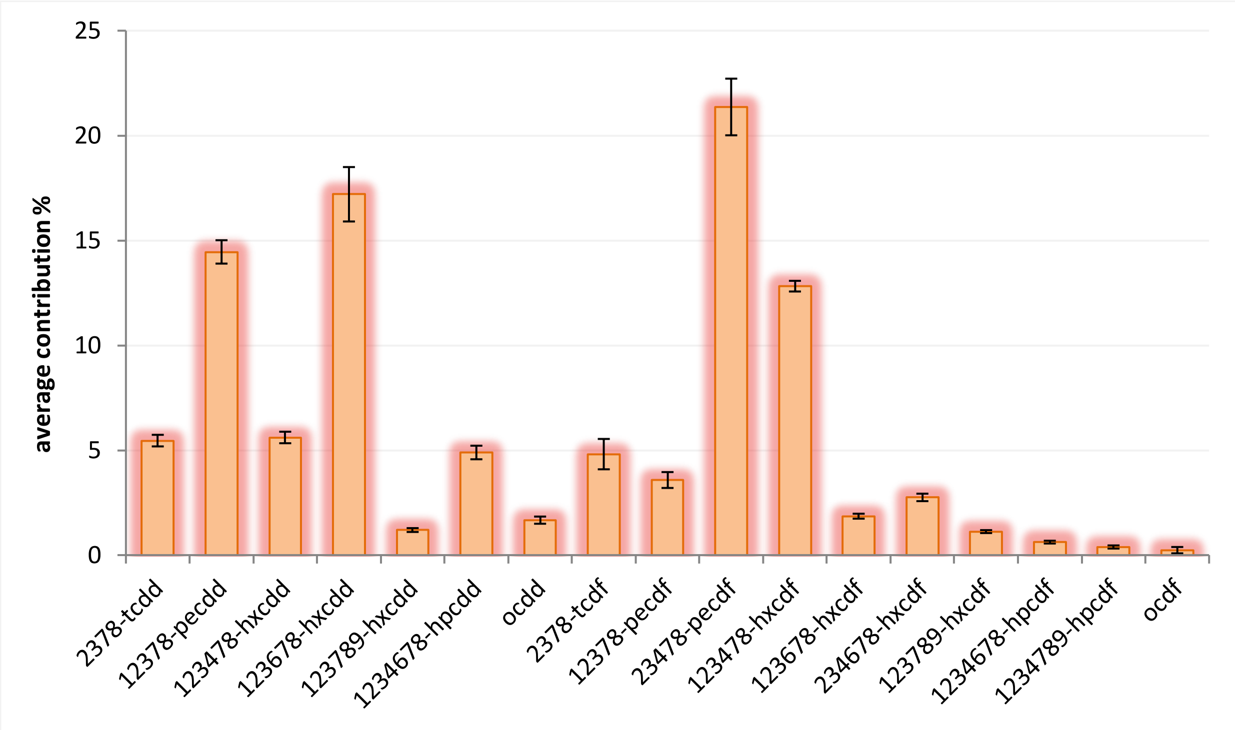


Figure 17. Average contribution of each PCDD/F congener to the total PCDD/F content in blubber of Mediterranean sperm whales stranded in 2008, 2009 and 2016. Error bars represent the standard error (SE).

5.1.2. Cuvier's baked whales

All 18 targeted PCBs were detected in blubber of every Cuvier's beaked whale analyzed (Baini et al., 2020 - Scientific publications 8.1), except for PCB-77, which was not identified in one specimen (Table 17). One mono-BDE (BDE-3), two di-BDE (BDE-7 and BDE-15), and one penta-BDE (BDE-126) were consistently not detected in any sample, and two penta-BDE congeners, specifically BDE-85 and BDE-119, showed low detection frequencies of 10% and 25%, respectively.

Table 17. Detection frequency (% >LOQ) for PCBs and PBDEs in blubber of free-ranging Cuvier's beaked whales biopsied in the Mediterranean Sea waters in 2014.

Cuvier's beaked whales (*Ziphius cavirostris*) n=20			
Detection frequency %			
PCBs	*blubber*	**PBDEs**	*blubber*
PCB-28	100	**BDE-3**	0
PCB-52	100	**BDE-7**	0
PCB-77	90	**BDE-15**	0
PCB-81	100	**BDE-17**	50

PCB-101	100
PCB-105	100
PCB-114	100
PCB-118	100
PCB-123	100
PCB-126	100
PCB-138	100
PCB-153	100
PCB-156	100
PCB-157	100
PCB-167	100
PCB-169	100
PCB-180	100
PCB-189	100

BDE-28	100
BDE-47	100
BDE-49	100
BDE-66	85
BDE-71	55
BDE-77	75
BDE-85	10
BDE-99	100
BDE-100	100
BDE-119	25
BDE-126	0
BDE-138	45
BDE-153	100
BDE-154	100
BDE-156	100
BDE-183	100
BDE-184	100
BDE-191	55
BDE-196	75
BDE-197	100
BDE-206	70
BDE-207	80
BDE-209	80

Cuvier's baked whales showed the following profile dominated by the seven indicator PCBs (ICES7): 153 > 180 > 138 > 118 > 101> 52 > 28. These congeners together contributed to the majority (95%) of the total PCB content (Figure 18). PCBs 153, 180, and 138 accounted for about 38%, 24%, 22%, respectively (l.w.).

On the other hand, DL-PCB congeners accounted only for 10% to the total PCB content, with mono-*ortho* PCBs being the most abundant congeners among them. The predominant DL-PCB congeners were PCB-118, PCB-156, and PCB-105 with an average contribution of 6%, 2%, and 0.4%, respectively (l.w.). Instead, non-*ortho* DL-PCBs exhibited an average contribution to the total PCB content of 0.03% with the following congener profile PCB-77 > PCB-126 > PCB-169 > PCB-81.

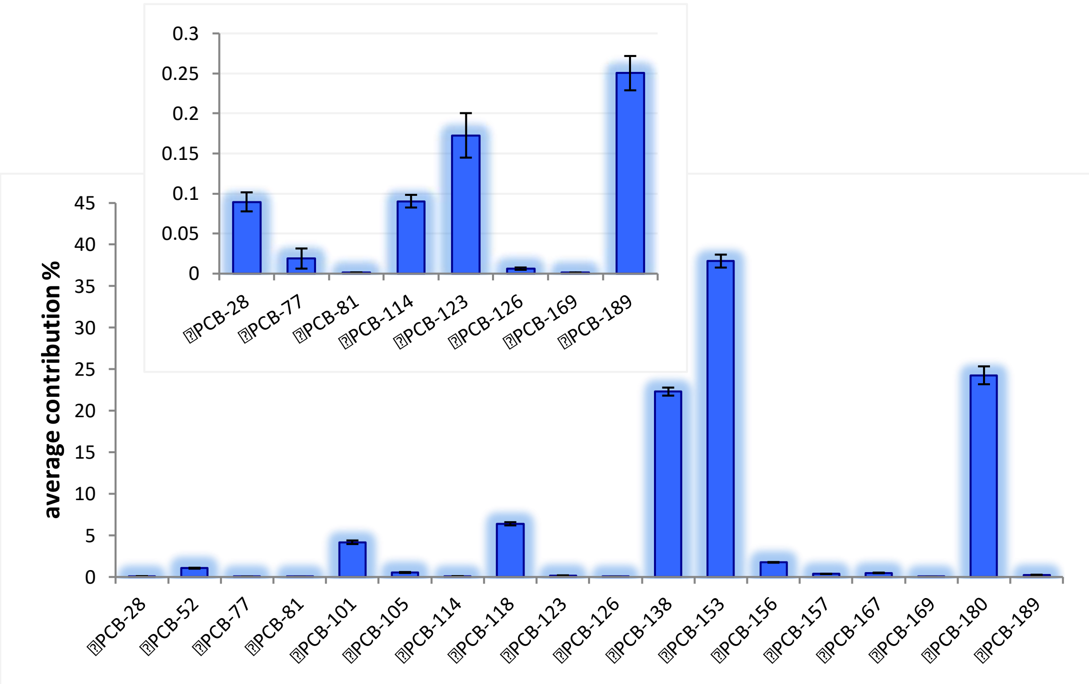


Figure 18. Average contribution of each PCB congener to the total PCB content in blubber of Mediterranean Cuvier’s beaked whales sampled in 2014-2015. Error bars represent the standard error (SE). Congeners with average contribution < 0.5% are represented on the top.

The PBDE congener profile shown in Figure 19 was dominated by lower-medium brominated congeners. The most abundant congeners were BDE-47(Br4), BDE-99(Br5), BDE-154(Br6), BDE-100(Br5), and BDE-153(Br6), whose average contribution to the total PBDE content was 45%, 17%, 14%, 13%, and 5%, respectively.

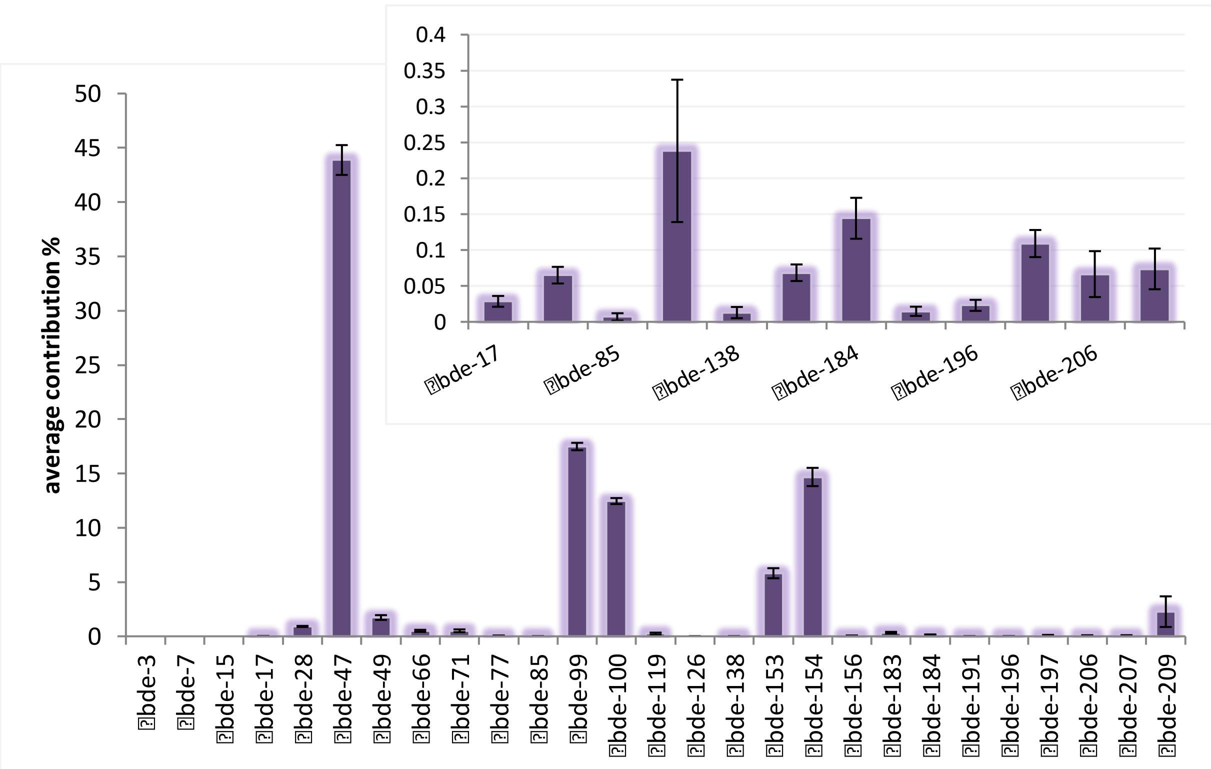


Figure 19. Average contribution of each PBDE congener to the total PBDE content in blubber of Mediterranean Cuvier's beaked whales sampled in 2014-2015. Error bars represent the standard error (SE). Congeners with average contribution < 0.5% are represented on the top.

In order to compare congener profiles among different age classes, PBDEs were grouped according to their respective commercial mixtures penta-, octa- and deca-BDE. Penta-BDE is composed of ten isomers (from tri- to hexa-BDEs) with BDE-99 and BDE-47 being the most abundant congeners contributing with 41.6% and 32.7%, respectively (Sjödin et al., 1998). The major component of octa-BDE are BDE-183 and BDE-197 constituting 42% and 22.2% of the product, respectively. Finally, deca-BDE is almost entirely composed of BDE-209 (96.8%) with a little percentage of BDE-206 (2.18%) (La Guardia et al., 2006). Our results showed that penta-BDE was the most dominant component among all age classes, followed by octa-BDE and deca-BDE (Figure 20). Interestingly, deca-BDE showed decreasing concentrations as the age of specimens increased.

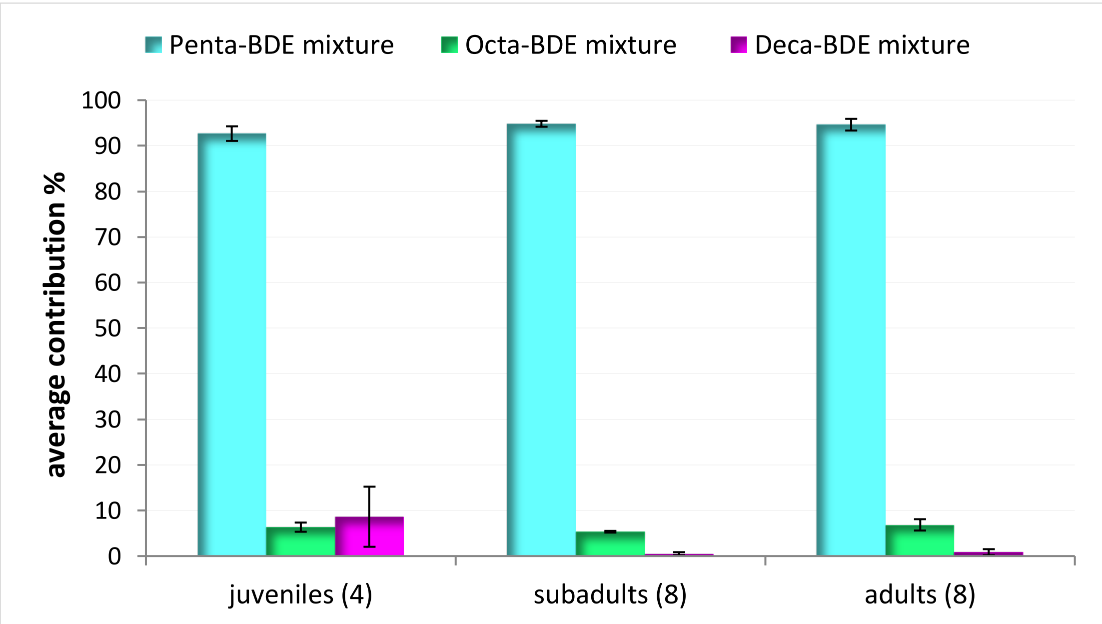


Figure 20. Average contribution of the main BDE congeners from the three technical mixtures (Penta-BDE, Octa-BDE, Deca-BDE) to the total PBDE content in blubber of Mediterranean Cuvier's beaked whales sampled in 2014-2015. Error bars represent the standard error (SE).

Despite the correspondence between PBDE congeners found in blubber of Cuvier's beaked whales and main components in the technical mixtures, the relative abundance of these compounds differed from that found in the commercial formulations. As described in figure 19, BDE-47 showed the highest contribution to the total PBDE content, followed by BDE-99, BDE-154, BDE-100 and BDE-154; instead, in the penta-BDE mixture the pattern of abundance is quite difference with BDE-99 > BDE-47 > BDE-100 > BDE-153 > BDE-154 .

5.1.3. Anchovies, sardines and bogues

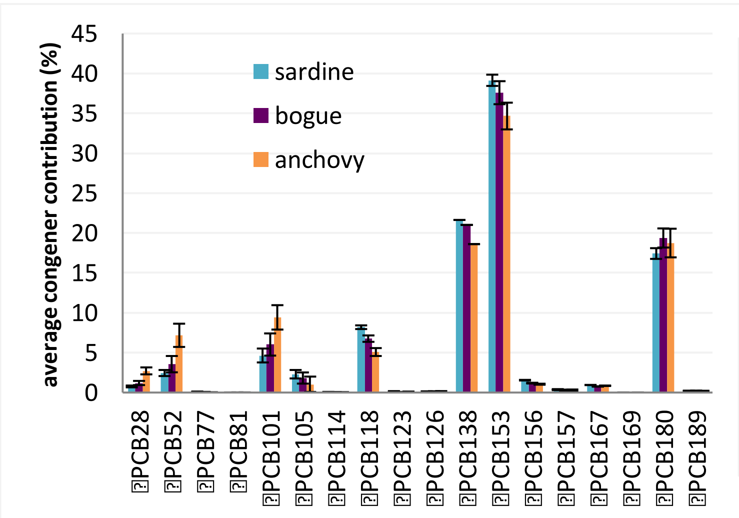
All 18 PCB congeners, with the exception of PCB-81, were detected in the fish species analyzed (sardine, bogue and anchovy) (Bartalini et al., 2020 - Scientific publications 8.1). The detection frequency for the targeted contaminants was in most cases 100% (Table 18). On the other hand, PCB-81 showed a very low detection frequency in sardines (25%) and bogues (～6%), and was not detected in anchovies.

Table 18. Detection frequency (% >LOQ) for PCBs in muscle of sardine, bogue and anchovy from the Mediterranean Sea (2017).

	Sardine (*Sardinas pilchardus*) n=17	**Bogue (*Boops boops*) n=17**	**Anchovy (*Engraulis encrasicolus*) n=17**
	Detection frequency %		
PCBs	*muscle*	*muscle*	*muscle*
PCB-28	100	100	100
PCB-52	100	100	94
PCB-77	100	94	44
PCB-81	25	6	0
PCB-101	100	100	94
PCB-105	100	100	88
PCB-114	100	88	63
PCB-118	100	100	100
PCB-123	100	94	88
PCB-126	100	100	100
PCB-138	100	100	100
PCB-153	100	100	100
PCB-156	100	100	100
PCB-157	100	100	100
PCB-167	100	100	100
PCB-169	88	31	56
PCB-180	100	100	100
PCB-189	100	100	100

Sardines, bogues and anchovies shared the same PCB profile (Figure 21) dominated by the ICES7 PCBs: tri-(#28), tetra-(#52), penta-(#101,118), hexa-(#138,153), and hepta-(#180) chlorinated congeners. The most abundant PCB congeners were PCB-153, PCB-138, and PCB-180 with a similar average contribution in all three species analyzed. The average contribution to the total PCB content was 39%, 38%, 35% for PCB-153, 22%, 21%, 19% for PCB-138, and 17%, 19%, 19% for PCB-180, in sardines, bogues, and anchovies respectively. The most abundant DL-PCB congeners were mono-*ortho* PCBs, specifically PCB-118, PCB-105, and PCB-156, that showed average contributions between 5-8%, 1-2%, and 1-2%, respectively.

The sum of the ICES7 accounted for more than 90% of total PCBs in all species investigated (sardines: 94%; bogues: 96%; anchovies: 98%), while DL-PCBs represented only a small fraction (sardines: 6%; bogues: 4%; anchovies: 2%). As we observed in sperm whale samples, almost the entire DL-PCB content (almost 99%) was made up by mono-*ortho* PCBs (105, 114, 118, 123, 156, 157, 167, 189) with only a minor fraction (1%) represented by non-*ortho* PCBs (77, 81, 126, 169). The same mono-*ortho* congener profile (118 > 105 > 156), similar to that found in sperm whales, was found for all three fish species analyzed in this study. On the other hand, a distinct abundance of non-*ortho* congeners was observed depending on the species. Sardines and bogues exhibited the same profile PCB-77 > 126 > 169 > 81; instead, the pattern of non-*ortho*-PCBs was quite different in anchovies (126 > 77 > 169), with a contribution of PCB-126 about 71% and no detection of PCB-81.



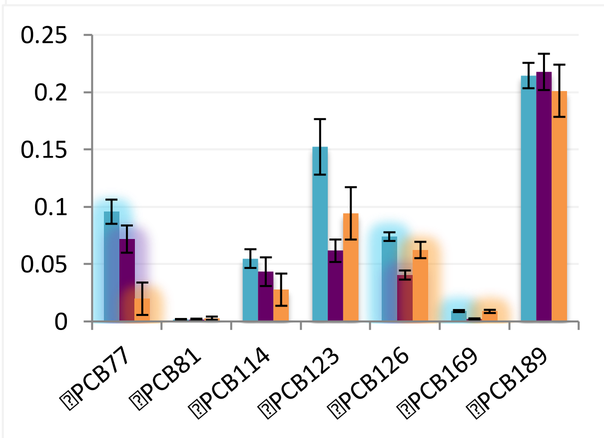


Figure 21. Average contribution of each PCB congener to the total PCB content in sardines, bogues and anchovies from the Mediterranean Sea (2017). Error bars represent the standard error (SE). Congeners with average contribution < 0.3% are zoomed in.

5.1.4. Blue sharks

As reported in Table 19, the three POP families analyzed in muscle and liver of blue sharks, captured in the Northeast Atlantic Ocean (Muñoz-Arnanz et al., 2022 - Scientific publications 8.1), showed important variability in their detection frequency depending on the tissue. PCB congeners were all detected (100%) in each liver sample; instead, muscle samples showed detection frequencies lower than 100% for some DL-PCB congeners, specifically for three non-*ortho* congeners (81, 126, 169), and the mono-*ortho* PCB-189.

Four PBDE congeners (7, 126, 138, 191) were no detected in any sample; BDE-15 and BDE-17 were detected only in muscle with very low detection frequencies of 2% and 7%, respectively.

PCDD/Fs showed highly variable detection frequencies for both tissues (muscle: 2-100%; liver: 2-58%), with 2,3,7,8-TCDF being the only congener detected in all liver samples analyzed.

Table 19. Detection frequency (% >LOQ) for PCBs, PBDEs and PCDD/Fs in liver and muscle of blue sharks captured in 2019 in the Northeast Atlantic Ocean.

Blue shark (*Prionace glauca*) n=60								
Detection frequency %								
PCBs	*liver*	*muscle*	**PBDEs**	*liver*	*muscle*	**PCDD/Fs**	*liver*	*muscle*
PCB-28	100	100	**BDE-7**	0	0			
PCB-52	100	100	**BDE-15**	0	2	**PCDDs**		
PCB-77	100	100	**BDE-17**	0	7	**2,3,7,8-TCDD**	25	16
PCB-81	100	80	**BDE-28**	98	100	**1,2,3,7,8-PeCDD**	47	7
PCB-101	100	100	**BDE-47**	100	100	**1,2,3,4,7,8-HxCDD**	35	4
PCB-105	100	100	**BDE-49**	100	95	**1,2,3,6,7,8-HxCDD**	77	6
PCB-114	100	100	**BDE-66**	98	62	**1,2,3,7,8,9-HxCDD**	13	2
PCB-118	100	100	**BDE-71**	35	90	**1,2,3,4,6,7,8-HpCDD**	68	58
PCB-123	100	100	**BDE-77**	83	0	**OCDD**	12	44
PCB-126	100	80	**BDE-85**	95	37			
PCB-138	100	100	**BDE-99**	100	100	**PCDFs**		
PCB-153	100	100	**BDE-100**	100	100	**2,3,7,8-TCDF**	100	54
PCB-156	100	100	**BDE-119**	80	60	**1,2,3,7,8-PeCDF**	68	16
PCB-157	100	100	**BDE-126**	0	0	**2,3,4,7,8-PeCDF**	98	22
PCB-167	100	100	**BDE-138**	0	0	**1,2,3,4,7,8-HxCDF**	63	20
PCB-169	100	55	**BDE-153**	100	92	**1,2,3,6,7,8-HxCDF**	58	17
PCB-180	100	100	**BDE-154**	100	98	**2,3,4,6,7,8-HxCDF**	70	24
PCB-189	100	95	**BDE-156**	87	2	**1,2,3,7,8,9-HxCDF**	2	15
			BDE-183	73	40	**1,2,3,4,6,7,8-HpCDF**	12	42
			BDE-184	82	5	**1,2,3,4,7,8,9-HpCDF**	2	18
			BDE-191	0	0	**OCDF**	2	30
			BDE-196	2	8			
			BDE-197	27	18			

BDE-206	30	63
BDE-207	40	63
BDE-209	55	62

As described in Figure 22, the PCB congener profile was dominated by ICES7 PCBs exhibiting the following pattern PCB-153 (~ 26%) > PCB-118 (20–21%) ≈ PCB-138 (~ 20%) > PCB-180 (~ 12%), both in liver and muscle.

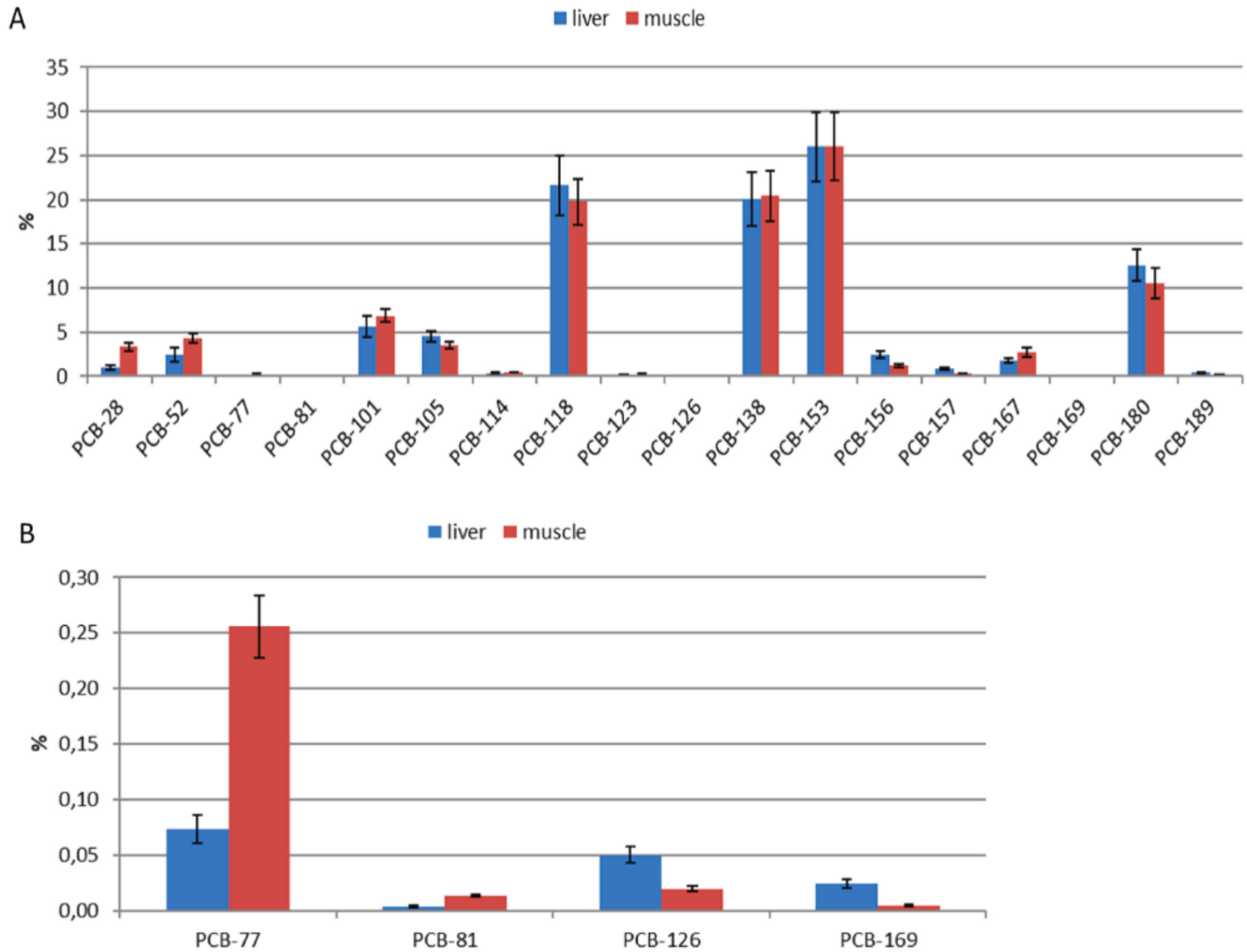


Figure 22. Average contribution of each PCB congener (A) and average contribution of non-*ortho* PCBs (B) to the total PCB content in muscle and liver of Atlantic Ocean blue sharks captured in 2019. Error bars represent the standard error (SE).

Interestingly, many of the less represented congeners showed distinct relative abundances depending on the tissue. The profile for the most toxic non-*ortho* congeners also varied between muscle (PCB-77 > PCB-126 > PCB-81 > PCB-169) and liver (PCB-77 > PCB-126 > PCB-169 > PCB-81).

PCB-77 showed a relative higher contribution in muscle than in liver; although, in both tissues PCB-77 showed the highest contribution, followed by PCB-126.

Liver and muscle of blue sharks showed noticeable differences in the relative abundances of most PBDEs, including the three major BDE congeners (Figure 23): BDE-47 (∼39–50%, muscle-liver) > BDE-100 (∼14–19%) > BDE-154 (∼11–15%).

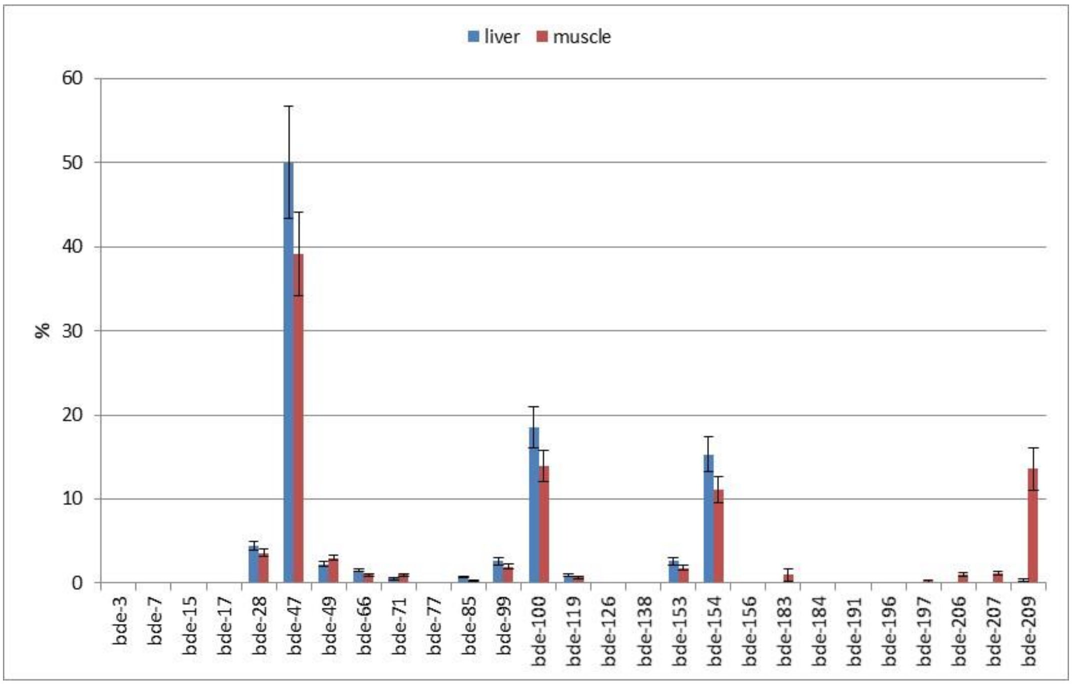


Figure 23. Average contribution of each PBDE congener to the total PBDE content in muscle and liver of Atlantic Ocean blue sharks captured in 2019. Error bars represent the standard error (SE).

On the other hand, higher brominated congeners (hepta- to deca-) showed important differences on tissue abundance, with greater accumulation in muscle. In particular, the decabrominated BDE-209 exhibited relative abundances of 14 and 0.3% in muscle and liver, respectively.

As was observed for PBDEs, although with a much reduced detection rate, especially in muscle, PCDD/F congeners displayed a markedly tissue dependent profile (Figure 24). PCDDs accounted for 23% and 63% of the total PCDD/F content in liver and muscle, respectively. Instead, PCDFs accounted for 77% and 37%, respectively. Predominant congeners in muscle were OCDD (∼22%) and 1,2,3,4,6,7,8-HpCD (∼11%), while a noticeable predominance of 2,3,7,8-TCDF (∼29%) > 2,3,4,7,8-PeCDF (∼23%) > 1,2,3,7,8-PeCDF (∼8%) was found in liver.

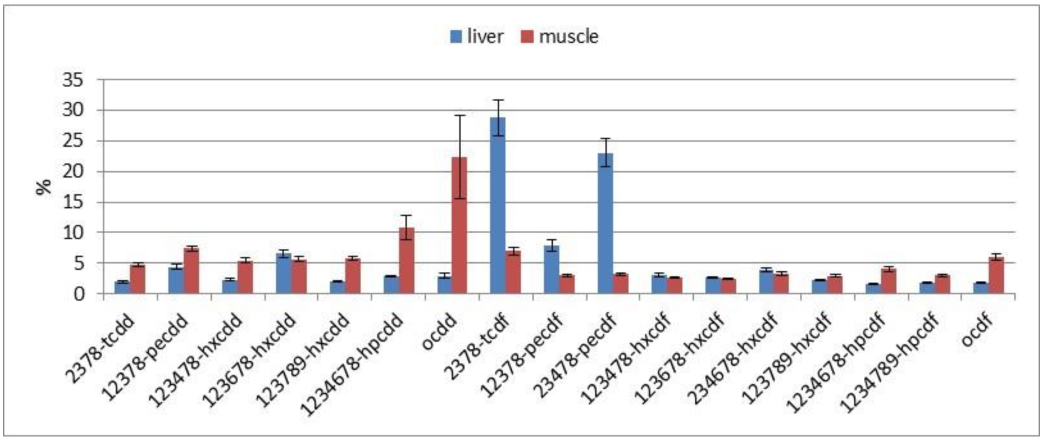


Figure 24. Average contribution of each PCDD/F congener to the total PCDD/F content in muscle and liver of Atlantic Ocean blue sharks captured in 2019. Error bars represent the standard error (SE).

5.2. Concentration values

5.2.1. Sperm whales

Blubber samples of Mediterranean sperm whales showed a wide range of levels for ΣDL-PCBs, ΣPBDEs, ΣPCDDs and ΣPCDFs (Table 20) (Bartalini et al., 2019 - Scientific publication 8.1).

DL-PCBs showed the highest levels among all targeted POP groups, with a median value of 3500 ng/g l.w., followed by PBDEs (356 ng/g l.w.). Mono-*ortho* PCBs were the most abundant congeners among DL-PCBs with a median level of 3490 ng/g l.w. On the other hand, the most toxic non-*ortho* congeners reached a median value of 3.74 ng/g l.w. Considerably lower levels were found for PCDDs (25.5 pg/g l.w.) and PCDFs (27.1 pg/g l.w.). Detailed information on POP concentrations is given in Table 20.

Table 20. Mean, median and range of total DL-PCBs (mono-*ortho* PCBs, non-*ortho* PCBs), PBDEs and PCDD/Fs in blubber of Mediterranean sperm whales sampled in 2009, 2008 and 2016 (concentrations are expressed in ng/g l.w. save for PCDD/Fs, pg/g l.w.).

Sperm whale (*Physeter macrocephalus*)			
Compounds	**Mean**	**Median**	**Range**
Σmono-*ortho* PCBs	6410	3490	2090-20800
Σnon-*ortho* PCBs	4.10	3.74	2.61-7.43
ΣDL-PCBs	6420	3500	2100-20800
ΣPCDDs (pg/g)	29.6	25.5	20.7-47.6
ΣPCDFs (pg/g)	28.2	27.1	23.9-35.9
ΣPCDD/Fs (pg/g)	57.8	56.2	45.4-83.5
ΣPBDEs	612	356	312-1390

5.2.2. Cuvier's baked whales

As reported in Table 21, blubber of Cuvier's baked whales showed a median PCB concentration of 16000 ng/g l.w., and a median PBDE concentration of 300 ng/g l.w., respectively (Baini et al., 2020 - Scientific publications 8.1).

Table 21. Mean, median and range of PCB and PBDE concentrations (in ng/g l.w.) in blubber biopsies from Mediterranean Cuvier's beaked whales sampled in 2014-2015.

Cuvier's baked whale (*Ziphius cavirostris*)			
Compounds	**Mean**	**Median**	**Range**
∑mono-*ortho*-DL-PCBs	2130	1520	259-6370
∑non-*ortho*-DL-PCBs	3.59	1.51	0.570-24.3
∑DL-PCBs	2130	1520	260-6380
∑ICES7	20700	15500	2920-58100
∑PCBs	21400	16000	3060-60600
∑PBDEs	391	300	131-1010

Concentration results for PCBs and PBDEs, divided by sex and age groups are provided in Table 22. In this regard, statistically significant differences were found for PCBs and PBDEs among distinct age classes. The highest PCB concentrations were reported in males, specifically adult males (median: 27100 ng/g l.w.) followed by sub adults (median: 16400 ng/g l.w.) and juveniles (median: 12600 ng/g l.w.), with a significant difference between the latter two (Shapiro-Wilk W=21, p value=0.033). However, no significant differences were found among the other groups. Although no statistical analysis was conducted between sexes in the adult group, the two adult females displayed lower PCB levels (3060 ng/g l.w. and 8220 ng/g l.w.) with a median value (5640 ng/g l.w.) much lower than that of adult males (27100 ng/g l.w.).

Regarding PBDEs, the highest median value was scored by adult males (503 ng/g l.w.) followed by subadult males (351 ng/g l.w.) and juvenile males (271 ng/g l.w.), with a statistically significant difference between adults and juveniles (Shapiro-Wilk W=22, p value =0.0190). No difference was found between the other groups. As highlighted for PCBs, the two adult females showed PBDE values lower than those reported for adult males (54.0 ng/g l.w.; 231 ng/g l.w.).

Table 22. Mean, median and range of PCB and PBDE concentrations (μg/g l.w.) in blubber biopsies of Cuvier's beaked whales sampled in the Mediterranean Sea. Concentrations are divided by class ages (juvenile, subadult, adult). The adult class is further divided by sex.

	Juvenile (n=4)			Subadult (n=8)			Adult male (n=6)			Adult female (n=2)		
	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range
∑PCBs	11.8	12.6	5.44-16.7	25.1	16.4	5.10-60.6	28.2	27.1	10.7-53.9	5.64	5.64	3.06-8.22
NDL-PCBs	7.65	7.89	3.57-11.3	16.9	11.3	3.24-39.9	18.9	17.4	7.07-37.3	3.61	3.61	1.54-5.69
DL-PCBs	1.23	1.30	0.56-1.74	2.54	1.48	0.62-6.38	2.70	2.60	1.11-4.80	0.590	0.590	0.260-0.920
non-*ortho* PCBs	0.00	0.00	0.00-0.01	0.00	0.00	0.00-0.01	0.00	0.00	0.00-0.01	0.010	0.010	0.00-0.0200
mono-*ortho* PCBs	1.22	1.30	0.560-1.74	2.53	1.48	0.620-6.37	2.70	2.60	1.11-4.80	0.580	0.580	0.260-0.890
ΣICES7	8.82	9.43	4.10-12.3	18.3	12.3	3.80-43.7	20.7	19.4	8.05-40.2	4.08	4.08	2.40-5.76
∑PBDEs	0.260	0.271	0.130-0.380	0.620	0.351	0.170-1.93	0.490	0.503	0.250-0.710	0.140	0.140	0.0540-0.231

5.2.3. Anchovies, sardines and bogues

Mediterranean sardines, bogues and anchovies exhibited an ample range of PCB concentrations (Bartalini et al., 2020 - Scientific publication 8.1). The minimum PCB concentration value of 1.01 ng/g w.w. was found for anchovies, meanwhile sardines accounted for the highest concentration of 17.9 ng/g w.w. A detailed description of PCB levels is presented in Table 23.

Table 23. Mean, median and range of PCB concentrations (in ng/g w.w. or pg/g w.w. when indicated) in muscle from the three Mediterranean Sea fish species investigated (2017).

Compounds	Sardine (*Sardina pilchardus*)				Bogue (*Boops boops*)				Anchovy (*Engraulis encrasicolus*)			
	concentration (ng/g w.w.)											
	Mean	Median	Range		Mean	Median	Range		Mean	Median	Range	
∑mono-*ortho*-DL-PCBs	1.35	1.36	0.577	2.62	0.384	0.296	0.174	1.06	0.322	0.272	0.0417	0.757
∑non-*ortho*-DL-PCBs (pg/g w.w.)	17.6	14.4	9.10	40.5	4.12	3.10	0.854	15.3	3.30	3.36	7.76	9.27
∑DL-PCBs	1.37	1.38	0.587	2.66	0.388	0.299	0.176	1.07	0.325	0.276	0.0424	0.776
∑ICES7	9.32	8.56	3.88	16.9	3.27	2.77	1.39	6.90	3.34	2.78	0.991	6.80
i-PCBs *	8.50	7.81	3.57	15.5	3.03	2.62	1.28	6.25	3.15	2.66	0.972	6.44
∑PCBs	9.88	9.12	4.15	17.9	3.42	2.88	1.46	7.22	3.48	2.92	1.01	7.08

* six indicators PCB congeners: 28, 52, 101, 138, 153, and 180 (Commission Regulation (EU) No 277/2012)

Sardine reached the highest median concentration with a value of 9.12 ng/g w.w., followed by anchovy and bogue with median values of 2.92 ng/g w.w. and 2.88 ng/g w.w., respectively. Bogues and anchovies showed similar average levels that were about three times lower than those of sardines.

5.2.4. Blue sharks

In blue sharks captured along the coast of Portugal (Muñoz-Arnanz et al., 2022 - Scientific publications 8.1), the relative abundance of target contaminants followed the order PCBs > PBDEs ≫ PCDD/Fs in liver and muscle. Median concentration values reported for PCBs, PBDEs, and PCDD/Fs in liver were 450 ng/g l.w., 29.7 ng/g l.w., and 27.0 pg/g l.w., respectively, and in muscle 248 ng/g l.w., 28.6 l.w., and 148 pg/g l.w., respectively. More detailed information on pollutant concentrations divided by sex is reported in Table 24.

Table 24. Mean, median and range of total concentrations for PCBs, i-PCBs, DL-PCBs, PBDEs, PCDDs, PCDFs and total PCDD/Fs divided by sex in liver and muscle samples of blue sharks captured in the North East Atlantic Ocean. Values are expressed in ng/g w.w. and (ng/g l.w.) for PCBs and PBDEs while pg/g w.w. and (pg/g l.w.) for PCDDs, PCDFs and PCDD/Fs.

Compound	sex	mean		median		range	
		liver	muscle	liver	muscle	liver	muscle
∑PCBs	both	223 (690)	0.858 (392)	157 (450)	0.530 (248)	12.7–1400 (37.1–4240)	0.0800–4.01 (49.6–2650)
	M	217 (686)	0.831 (364)	163 (497)	0.677 (240)	12.7–785 (37.1–4240)	0.0910–4.01 (67.7–2650)
	F	233 (696)	0.933 (439)	155 (417)	0.453 (354)	193–1400 (64.4–2830)	0.0800–3.54 (49.6–1840)
∑i-PCBs	both	151 (461)	0.613 (282)	97.3 (313)	0.396 (159)	7.38–983 (21.6–2240)	0.0460–3.10 (25.4–1810)
	M	137 (424)	0.558 (253)	102 (321)	0.399 (155)	7.38–684 (21.6–2240)	0.0460–2.44 (29.3–1810)
	F	175 (526)	0.734 (345)	96.8 (261)	0.294 (286)	12.0–983 (40.1–1980)	0.0610–3.10 (25.4–1610)
∑DL-PCBs	both	72.2 (229)	0.245 (110)	47.6 (137)	0.160 (76.7)	5.32–420 (15.5–2320)	0.0190–1.56 (15.3–839)
	M	80.4 (262)	0.273 (117)	57.0 (178)	0.201 (81.9)	5.32–340 (15.5–2320)	0.0310–1.56 (17.8–839)
	F	58.0 (171)	0.199 (98.2)	35.9 (119)	0.141 (64.9)	6.74–420 (21.7–846)	0.0190–0.895 (15.3–507)
∑PBDEs	both	14.9 (46.8)	0.090 (44.0)	10.7 (29.7)	0.0750 (28.6)	1.15–69.7 (3.83–335)	0.004000–0.424 (1.55–316)
	M	15.6 (49.0)	0.101 (49.8)	10.9 (33.0)	0.0890 (30.8)	1.59–57.3 (4.63–335)	0.004000–0.424 (4.08–316)
	F	13.8 (43.0)	0.074 (35.1)	10.4 (29.0)	0.0530 (25.7)	1.15–69.7 (3.83–141)	0.006000–0.245 (1.55–143)
∑PCDDs	both	2.49 (8.53)	0.320 (157)	2.28 (6.26)	0.227 (96.5)	1.07–8.48 (2.37–53.5)	0.0690–2.71 (28.1–1190)
	M	2.37 (7.71)	0.225 (122)	2.10 (6.15)	0.189 (68.9)	1.28–6.86 (3.16–46.8)	0.0690–0.600 (28.1–985)
	F	2.69 (9.94)	0.486 (220)	2.38 (7.00)	0.311 (174)	1.07–8.48 (2.37–53.5)	0.148–2.71 (68.3–1190)
∑PCDFs	both	8.30 (27.2)	0.194 (95.8)	7.35 (19.8)	0.165 (65.8)	1.93–23.0 (5.51–157)	0.0130–0.532 (6.10–456)
	M	7.46 (23.8)	0.166 (86.0)	6.84 (18.2)	0.156 (51.6)	1.93–23.0 (5.51–157)	0.0130–0.296 (6.10–456)
	F	9.75 (33.2)	0.242 (113)	8.81 (24.7)	0.238 (110)	2.16–20.9 (6.55–106)	0.102–0.532 (26.7–181)
∑PCDD/Fs	both	10.8 (35.8)	0.514 (253)	9.64 (27.0)	0.402 (148)	3.27–29.8 (8.67–204)	0.0820–2.96 (37.2–1440)
	M	9.83 (31.5)	0.392 (208)	8.87 (25.2)	0.341 (125)	3.27–29.8 (8.67–204)	0.0820–0.878 (37.2–1440)
	F	12.4 (43.1)	0.727 (333)	11.4 (31.9)	0.549 (315)	3.70–25.8 (8.92–160)	0.306–2.96 (128–1300)

Thanks to the large number of study specimens (38 males and 22 females), a statistical analysis could be explored, in both liver and muscle, among PCBs, PBDEs and PCDD/Fs (in w.w. and l.w.) and factors such as the animal's fork length (FL) as well as the animals' sex and age.

The three families of pollutants showed a positive correlation in liver among them and with FL (Table 25, Spearman's r_s = 0.419–0.948 (w.w.) and 0.281–0.923 (l.w.), $p < 0.05$ marginally in the case of PCDD/Fs with FL, r_s = 0.239, p = 0.068 (w.w.) and 0.0945, p = 0.0547 (l.w.)). On the other hand, in muscle, only PCB and PBDE burdens were positively correlated between them and with FL (Pearson and Spearman's r= 0.286–0.826 (ww) and 0.238–0.835 (l.w.), $p < 0.05$, Table 25), while PCDD/Fs were not correlated with other pollutants (no correlation in w.w. and marginally correlated in l.w.) and negatively correlated with FL (Table 25, Spearman's rs = -0.441 (w.w.) and -0.404 (l.w.), $p < 0.05$).

Table 25. Spearman's (r_s) and Pearson (r) correlations in liver (green) and muscle (grey) of blue shark with concentration values in w.w. (black) and l.w. (blue).

	Log [PBDEs, ng/g]	Log [PCDD/Fs, pg/g]	Log [fork length, cm]
Log [PCBs, ng/g]	**r_s= 0.948** (r= 0.923) **p= 1.698E-30** (9.272E-26)	**r_s= 0.638** (r= 0.645) **p= 4.13E-8** (2.65E-8)	**r_s= 0.422** (r= 0.281) **p= 0.000883** (0.00311)
	r_s= 0.826 (r= 0.835) **p= 2.00E-7** (1.11E-16)	**r_s = 0.0859** (r= 0.327) **p= 0.520** (0.0528)	**r_s= 0.286** (r= 0.238) **p= 0.0283** (0.0690)
Log [PBDEs, ng/g]		**r_s= 0.698** (r= 0.746) **p= 5.81E-10** (7.63E-12)	**r_s= 0.419** (r= 0.354) **p= 0.000969** (0.00591)
		r_s = 0.0238 (r= 0.351) **p= 0.859** (0.0682)	**r_s= 0.339** (r= 0.278) **p= 0.00893** (0.0334)
Log [PCDD/Fs, pg/g]			**r_s= 0.239** (r= 0.0945) **p= 0.0684** (0.0547)
			r_s = -0.441 (r= -0.404) **p= 0.000653** (0.00192)

Differences between adult and subadult specimens could only be explored in males since all females were subadults. In both, liver and muscle, concentrations of pollutants were statistically significantly greater in male adults in comparison to subadult males (in w.w and l.w., Mann-Whitney, $p < 0.05$, see specific U values and significances in Table 26). The exception was the case of PCDD/Fs in muscle, for which higher values were found in subadults (Table 26), even though these differences were not statistically significant (Mann-Whitney U(17,20) = 258, p = 0.073 (w.w.) and U(17,20) = 271, p = 0.168 (l.w.)).

Table 26. Median concentrations (PCBs and PBDEs in ng/g and PCDD/Fs in pg/g) and Mann-Whitney U tests between POP burdens in liver and muscle of adult and subadult male blue sharks. Statistically significance at α=0.05 is shown by *.

Tissue	Pollutants	Median ADULTS (n=17)	Median SUBADULTS (n=21)	Concentration basis (w.w. or l.w.)	Mann-Whitney U (17, 21)	Significance (p)
liver	PCBs	318	78.1	w.w.	52.00	<0.01*
		809	219	l.w.	51.00	<0.01*
	PBDEs	17.9	7.77	w.w.	53.00	<0.001*
		55.6	19.3	l.w.	53.00	<0.001*
	PCDD/Fs	12.3	5.71	w.w.	55.00	=0.01*
		35.3	19.1	l.w.	64.00	<0.001*
muscle	PCBs	0.987	0.440	w.w.	75.00	=0.004*
		285	148	l.w.	91.00	=0.016*
	PBDEs	0.126	0.056	w.w.	81.00	=0.007*
		46.1	22.2	l.w.	104.00	=0.044*
	PCDD/Fs	0.318	0.402	w.w.	105.00	=0.073
		112	139	l.w.	118.00	=0.168

Regarding accumulation dissimilarities between sexes, no statistically significant differences were found for PCBs and PBDEs between males and females, neither in liver nor in muscle concentrations (w.w. and l.w., Table 27). The only difference statistically significant (Mann-Whitney, $p > 0.05$, see specific U values and significances in Table 27) was found for the content of PCDD/Fs in muscle with a higher mean concentration in females (Mann-Whitney, $U(22,38) = 164$ and 163, in w.w. and l.w, both with $p < 0.001$).

Table 27. Median concentrations (PCBs and PBDEs in ng/g and PCDD/Fs in pg/g) and Mann-Whitney U tests between POP burdens in liver and muscle of male and female blue sharks. Statistically significance at α=0.05 is shown by *.

Tissue	Pollutants	Median concentration in males (n=38)	Median concentration in females (n=22)	Concentration basis (w.w. or l.w.)	Mann-Whitney U (17, 21)	Significance (p)
liver	PCBs	163	155	w.w.	328.00	=0.581
		497	417	l.w.	407.00	=0.866
	PBDEs	10.9	10.4	w.w.	389.00	=0.656
		33.0	29.0	l.w.	404.00	=0.830
	PCDD/Fs	8.87	11.4	w.w.	327.00	=0.163
		25.2	31.9	l.w.	335.00	=0.203
muscle	PCBs	0.677	0.453	w.w.	413.00	=0.939

	240	354	l.w.	379.00	=0.550
PBDEs	0.089	0.053	w.w.	336.00	=0.208
	30.8	25.7	l.w.	404.00	=0.803
PCDD/Fs	0.341	0.549	w.w.	164.00	<0.001*
	125	315	l.w.	163.00	<0.001*

Investigating tissue dependency for the targeted contaminants, we found concentration values in wet weight in liver three orders of magnitude greater than in muscle. However, when the concentrations were normalized according to the lipid content, results changed notably. Concentrations of PCBs and PBDEs still tended to be higher in liver, although only for the former the difference was statistically significant (Mann-Whitney, $U(60,60) = 96.00$, $p = 0.009$). Interestingly, the content of PCDD/Fs in l.w. became significantly greater in muscle than in liver (Mann-Whitney, $U(60,60) = 85.00$, $p < 0.001$).

5.3. Toxicity assessment

5.3.1 Sperm whales

Sperm whales analyzed shared the same TEQ pattern (Σnon-*ortho*-DL-PCBs > Σ*ortho*-DL-PCBs > PCDDs > PCDFs), with DL-PCBs contributing the most to TEQs (Bartalini et al., 2019 - Scientific publications 8.1). Total calculated TEQs ranged from 275 to 987 pg TEQs/g l.w. (Table 28), and surpassed the threshold of 210 pg WHO-TEQs/g l.w. in blubber, proposed as starting point of immunosuppression in harbour seals (*Phoca vitulina*) (Ross et al., 1995).

While PBDEs are widely recognized for their toxic effects on cetaceans, it is important to note that the single currently extant upper limit threshold for these contaminants in marine mammals has been established for endocrine disruption in blubber of grey seals (1500 ng/g l.w.) (Hall et al., 2003). This value was employed as a proxy for sperm whales, and we found that total PBDE concentrations (312 to 1390 ng/g l.w.) (Table 5) were always below the above-mentioned reference value, although often in the same order of magnitude.

Table 28. Total TEQs and percentage contribution to total-TEQs in blubber of Mediterranean sperm whales analyzed in this study. Data are expressed in pg TEQs/g l.w, and are showed as mean, median and range.

Compounds	**Average contribution %**	pg TEQs/g l.w. **Mean**	**Median**	**Range**

Σmono-*ortho* PCBs	34.1	192	105	62.9-625
Σnon-*ortho* PCBs	61.7	281	255	188-527
ΣDL-PCBs	**95.8**	**474**	**374**	**261-968**
ΣPCDDs (pg/g)	2.90	13.0	11.9	9.36-21.1
ΣPCDFs (pg/g)	1.20	5.03	4.54	4.26-6.37
ΣPCDD/Fs (pg/g)	**4.20**	**18.1**	**16.3**	**13.9-27.4**
Total TEQs		**492**	**394**	**275-987**

5.3.2. Cuvier's baked whales

As reported in Table 29, DL-PCB concentrations in pg TEQ/g ranged from 36.1 to 983 among Cuvier's beaked whales analyzed in this study (Baini et al., 2020 - Scientific publications 8.1). In juveniles, subadults and female adults, non-*ortho* PCBs accounted for more than 60% to total TEQs (Table 30). In adult males, mono-*ortho* PCBs reached an average percentage contribution of about 51%.

Four individuals reported calculated TEQs above the upper limit of 210 pg WHO-TEQ/g l.w for immunosuppression in harbour seals (*Phoca vitulina*). In addition, we compared average ΣPCB concentrations with two distinct threshold values commonly used for marine mammals. In particular, we considered the lower value of 9 µg/g l.w. proposed as toxicity limit for the onset of physiological effects in marine mammals (Jepson et al., 2016; Kannan et al., 2000), and the higher value of 41 µg/g l.w. suggested as a threshold for reproductive impairment in Baltic ringed seals (*Pusa hispida*) (Helle et al., 1976a). We found that 80% of the sampled individuals exceeded the lower limit, and three of them showed ΣPCB loads above the highest threshold limit, specifically two subadults and one adult.

Table 29. Mean, median and range of TEQ values (pg/g w.w.) of DL-PCBs in blubber biopsies of Mediterranean Cuvier's beaked whales, sampled in 2015.

	TEQs (pg/g w.w.)											
	Juvenile (n=4)			Subadult (n=8)			Adult male (n=6)			Adult female (n=2)		
	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range
∑non-*ortho*-DL-PCBs	65.8	67.7	28.0-99.7	167	85.4	43.2-792	76.4	75.0	39.5-119	145	145	28.3-261
∑mono-*ortho*-DL-PCBs	36.7	39.0	16.9-52.1	76.0	44.4	18.7-191	81.1	77.9	33.4-144	17.3	17.3	7.78-26.8
Total TEQs	102	111	44.9-143	243	129	61.9-983	157	168	88.1-223	162	162	36.1-289

Table 30. Average contribution to total TEQs calculated for Mediterranean Cuvier's beaked whales sampled in 2015.

TEQs	Average contribution (%)			
	Juvenile	Subadult	Adult male	Adult female
Total TEQs	100	100	100	100
∑non-*ortho*-DL-PCBs	64.0	69.0	48.3	89.5
∑mono-*ortho*-DL-PCBs	36.0	31.0	51.7	10.5

To assess the toxicological impact of PBDEs in the Mediterranean Cuvier's beaked whales analyzed, we compared our results with the PBDE threshold value of 1500 ng/g l.w., associated with endocrine disruption in grey seals. Among all individuals analyzed, one specimen (subadult) showed a concentration of PBDEs above this threshold limit.

5.3.3 Anchovies, sardine and bogues

DL-PCB concentrations in pg TEQs/g for all species investigated in this study ranged from 0.0726 to 1.24 pg TEQs/g (Table 31), with non-*ortho*-PCBs accounting for more than 90% of the total TEQ concentration (Table 32).

As sardines, bogues and anchovies represent some of the most commonly consumed fish species in the Mediterranean Sea, we compared our results with regulated levels in diet to assess possible risks to human health derived from their consumption. In this regard, we found that the mean total TEQs and concentrations of the 6 indicators PCB congeners reported for sardines, bogues and anchovies did not exceed regulated levels in diet established by the European Union (Scientific Committee on Food, 2000). Estimated weakly intake (EWI) deriving from the estimated weekly fish consumption in Italy was 1.2, 0.35 and 0.29 for sardine, bogue and anchovy, respectively. These values are far below the tolerable weakly intake (TWI) of 14 pg WHO-TEQ/Kg/week, and are also below the new TWI for dioxins and dioxin-like PCBs in food of 2 pg WHO-TEQ/Kg/week proposed by EFSA (EFSA, 2018).

Table 31. Mean, median and range of TEQs values (pg/g w.w.) of DL-PCBs in muscle from three Mediterranean Sea fish species analyzed in this study.

	Sardine (*Sardina pilchardus*)				Bogue (*Boops boops*)				Anchovy (*Engraulis encrasicolous*)			
	pg WHO-TEQs/g w.w.											
	Mean	Median	Range		Mean	Median	Range		Mean	Median	Range	
∑non-*ortho*-DL-PCBs	0.731	0.704	0.389	1.18	0.124	0.120	0.0637	0.236	0.200	0.208	0.0766	0.387

∑mono-*ortho*-DL-PCBs	0.0405	0.0409	0.0173	0.0787	0.0115	0.00888	0.00522	0.0317	0.00967	0.00816	0.00125	0.0227
Total TEQs	0.771	0.746	0.410	1.24	0.135	0.131	0.0726	0.268	0.210	0.220	0.0778	0.396

Table 32. Average contribution to total TEQs calculated for sardine, bogue and anchovy.

TEQs	Average contribution (%)		
	Sardine	Bogue	Anchovy
Total TEQs	100	100	100
∑non-*ortho*-DL-PCBs	94.8	91.5	95.4
∑mono-*ortho*-DL-PCBs	5.20	8.50	4.60

5.3.4. Blue sharks

In order to assess the potential health risks deriving from the presence of PCBs and PBDEs in blue sharks, we compared our results with available toxicity limits. Due to the absence of specific thresholds for blue shark, we used as a proxy the toxicity upper limit in blubber commonly employed for marine mammals. Regarding PCBs, we compared our results with the value of 9 μg/g l.w. for which onset of physiological impacts have been reported in marine mammals. For PBDEs we referred to the threshold value of 1500 ng/g l.w. set for endocrine disruption in grey seals. All shark specimens analyzed in this study reported average values of PCBs (46.6-2650 ng/g l.w.) and PBDEs (1.55-316 ng/g l.w.) in muscle from one to three orders of magnitude lower than the above-mentioned thresholds.

In order to quantify the potential toxicity of DL-PCBs and PCDD/Fs for humans, we calculated TEQs (Table 33). Important differences in total concentration burdens between tissues (two orders of magnitude higher in liver on average than in muscle), as well as in the relative contributions to total TEQs, were observed. DL-PCBs in liver accounted for ~88% of total TEQs, while PCDD/Fs were responsible for ~77% of total TEQs in muscle, with PCDDs alone responsible for the greatest contribution (~63%).

Table 33. TEQs for DL-PCBs, PCDDs, PCDDs and PCDD/s, and average contribution to total TEQs calculated for liver and muscle samples of blue sharks. Data are given in pg TEQs/g w.w. and (pg TEQs/g l.w.), and are showed as mean, median and range.

TEQs	Average contribution (%)		mean		median		range	
	liver	muscle	liver	muscle	liver	Muscle	liver	muscle
TOTAL	100	100	17.1 (85.8)	0.115 (54.3)	14.4 (66.0)	0.108 (42.3)	2.52-108 (19.1-709)	0.0700-0.340 (16.1-205)

$TEQ_{DL-PCBs}$	88.2	23.0	15.1 (50.0)	0.0270 (12.1)	11.1 (31.5)	0.0220 (9.46)	1.88-101 (4.62-689)	0.0050-0.105 (1.05-80.7)
TEQ_{PCDDs}	4.70	63.3	0.807 (2.81)	0.0730 (34.5)	0.630 (1.81)	0.0640 (24.1)	0.228-3.06 (0.584-20.9)	0.0420-0.244 (9.89-97.1)
TEQ_{PCDFs}	7.10	13.7	1.21 (3.99)	0.0160 (7.61)	1.07 (2.67)	0.0140 (5.01)	0.178-3.70 (0.544-25.3)	0.0080-0.0440 (1.99-30.1)
$TEQ_{PCDD/Fs}$	11.8	77.0	2.02 (6.79)	0.0880 (42.2)	1.61 (5.28)	0.0770 (29.0)	0.529-6.76 (1.57-46.1)	0.0530-0.288 (12.1-125)

To asses the potential risk for human health deriving from the consumption of blue shark meat, we compared PCB, PBDE and PCDD/F concentrations found in analyzed individuals with maximum levels (ML) set for Europe. The European Regulation 1259/2011 and Directive 2013/39/EU established a ML in muscle meat of fish and fishery products of 6.5 pg WHO-TEQ/g (w.w.) for PCDD/Fs and DL-PCBs, and a ML of 75 ng/g (w.w.) for the 6 indicator PCBs. Additionally, Regulation 1259/2011 also establishes a maximum of 3.5 pg WHO-TEQs/g (w.w.) just for PCDD/Fs. All three targeted POP families were found in muscle (∑TEQs: 0.115 pg TEQs/g w.w.; ∑i-PCBs: 0.613 ng/g w.w.; ∑PCDD/Fs: 0.088 pg TEQs/g w.w.) at one to two orders of magnitude lower than the established limits.

If we consider an average value of 0.115 pg TEQs/g in muscle (this study), an average human body weight of 70 Kg, and a per person's consumption of over 1.2 Kg of blue shark muscle per week, our results did not exceed the conservative EFSA's tolerable weekly intake (TWI) of 2 pg WHO-TEQs/Kg body weight/week for dioxins and dioxin-like PCBs in food (Knutsen et al., 2018). To put this figure in context, according to FAOSTAST, the weekly consumption of pelagic fish in 2019 in Portugal and Spain per person was ∼0.22 Kg and ∼0.15 Kg, respectively. On the other hand, although shark livers are not commonly part of the human diet, about 58% of liver samples exceeded the ML for i-PCBs, ∼78% did the same for the 7.5 pg WHO-TEQs/g limit when considering PCDD/Fs + DL-PCBs, and about 38% exceeded the limit of WHO-3.5 pg TEQs/g when considering just PCDD/Fs.

Concerning PBDEs (congeners BDE-28, BDE-47, BDE-99, BDE-10, BDE-153, and BDE-154), all liver samples and 92% of muscle samples analyzed in this study overpassed the *Environmental Quality Standard* limit (EQS) for biota of 0.0085 ng/g w.w. established by the European Water Framework Directive (WFD).

As there is no TWI established for PBDEs, we took into consideration the chronic oral reference doses set out by the US Environmental Protection Agency, and the acute/subchronic minimal risk levels established by the US Agency for Toxic Substances and Disease Registry. Values for these limits were congener-dependent and vary from 100 ng/kg/day (BDE47 and 99) to 7000 ng/Kg/day (BDE-209). We calculated the estimated daily intake (EDI) assuming an average body weight of 70 Kg and the highest daily pelagic fish consumption (Portuguese populations) of 0.03 Kg/day, considering the highest PBDE concentration found in this study for blue shark, which amounted to 69.7 ng/g in liver. The obtained EDI was 29.9 ng/kg/day, which scored below any of the aforementioned limits.

6. Discussion

6.1. Concentrations and trends

6.1.1. PCBs

As described in the Results section (*4.1. Congener profiles*), PCBs were the most abundant POPs family in all investigated species. This result, in line with the majority of published research conducted so far in marine mammals (Green and Larson, 2016; Marsili et al., 2018), sharks (Tiktak et al., 2020), and other fish, was somewhat expected and contributes to reinforce the awareness about the persistent and critical dilemma posed by these legacy contaminants (Jepson and Law, 2016). Several factors are responsible for the higher concentrations of PCBs compared to those of PBDEs and PCDD/Fs. Specifically, PCBs are more persistent in the environment and do not readily break down or degrade (Siddiqi et al., 2003). Although, PBDEs exhibit a great degree of environmental persistence as well, they tend to degrade more rapidly than PCBs (Söderström et al., 2004). PCBs were used in applications with long-life spans (electrical equipment, hydraulic fluids, and heat transfer systems) (Erickson and Kaley, 2011), which allowed these substances to persist in the environment for extended periods even after their production ceased. In comparison, PBDEs were primarily used as flame-retardants in products with shorter lifespans (electronics, textiles, and furniture), and subject to replacement or disposal (Jinhui et al., 2017). This could potentially result in a lower overall abundance of PBDEs compared to PCBs.

In the case of PCDD/Fs, they are not intentionally synthesized chemicals and while estimating their global production is a challenging task, their global production and environmental levels do not approach those seen with PCBs (Song et al., 2023).

Higher chlorinated congeners (hepta- and hexa-) dominated the PCB profiles of the studied species, in accordance with what is usually described in marine organisms (Castro-Jiménez et al., 2021; Madgett et al., 2022; Romero-Romero et al., 2017). Specifically, the same congener profile was found in marine fish (Ben Ameur et al., 2013; Henríquez-Hernández et al., 2017; Herceg Romanić et al., 2021; Milićević et al., 2022; Naso et al., 2005; Vuković et al., 2018), in blue sharks (*Prionace glauca*) worldwide (Alves et al., 2016; de Azevedo e Silva et al., 2007; Lee et al., 2015a; Mol et al., 2016; Storelli et al., 2011b), in Cuvier's baked whales (*Ziphius cavirostris*) (López-Berenguer et al., 2023), and other beaked whales (Anezaki et al., 2016; Hooker et al., 2008). The same congener profile has been described in other cetacean species such as bottlenose dolphin (*Tursiops truncatus*), stripped dolphin (*Stenella coeruleoalba*) (Dron et al., 2022; Jepson et al., 2016; Santos-Neto et al., 2014; Xie et al., 2021), spotted dolphin (*Stenella frontalis*) (Lavandier et al., 2019; Santos-Neto et al., 2014), dugong (*Dugong dugong*) (Weijs et al., 2019), killer whale (*Orcinus orca*) (Jepson et al., 2016), pilot whale (*Globicephala melas*), sperm whale (*Physeter macrocephalus*) (Pinzone et al., 2015), and porpoises (Jepson et al., 2016; Ramu et al., 2006; Xie et al., 2021).

In general, the higher levels of these highly chlorinated congeners could be attributable to two distinct factors: 1) their notably persistence which is closely linked to their heightened tendency to be bioaccumulated and reduced metabolization, owing to their high chlorination degree and lack of adjacent unsubstituted H-atoms in ortho-meta and/or meta-para positions in the aromatic rings (Borja et al., 2005; Bright et al., 1995; Walker, 2008), 2) their abundant contribution to the most common used PCB mixtures, such as Aroclor 1254 and 1260 (M. Frame et al., 1996). In the case of sperm whales and Cuvier's beaked whales, this is supported by several studies that have described a low biotransformation capacity of high chlorinated PCBs by different cetaceans species (Boon et al., 1997; Williams et al., 2020; Yordy et al., 2010c).

As we observed in all the species investigated, almost the entire DL-PCB content (almost 90%) was made up by mono-*ortho* PCBs (105, 114, 118, 123, 156, 157, 167, 189), with only a small fraction represented by non-*ortho* PCBs (77, 81, 126, 169). This fact is in agreement with the major abundance of the former in technical mixtures, and it is also in accordance with previous studies in sperm whales and other marine species where PCB-118 has been reported as the most abundant among DL-PCB congeners (Barraza et al., 2020; Capanni et al., 2020; Castro-Jiménez et al., 2021; Gaus et al., 2005; Pinzone et al., 2015).

6.1.1.1. Sperm whales and Cuvier's beaked whales

In comparison to the most recent available data about PCBs in blubber of Mediterranean sperm whales (Table 34), our results were one order of magnitude higher than those reported for two individuals stranded in 2011 and 2018 (López-Berenguer et al., 2023), and in the same order of magnitude that those described for free-ranging specimens sampled between 2011 and 2013 (Pinzone et al., 2015).

Table 34. PCBs and PBDEs (ng/g l.w.) in blubber samples of sperm whales sampled worldwide. Data are expressed as mean ± SD (when possible). Geographic area of sampling, year of collection and sex (F=female; M=male; U=unidentified) are reported (when possible). Total DL-PCBs are in parenthesis (when possible).

Reference (Year)	Geographic area	Sampling year	Species	N	Sex	ΣPCBs (ΣDL-PCBs)	ΣPBDEs
Aguilar (1983)[p]	North Atlantic	-	SW	8	M	9930	–
				6	F	15550	–
Borrel (1993)[p]	Iceland	1982	SW	10	M	10510±2070	4160±1040
Boer et al. (1998)[r]	NE Atlantic (North sea)	-	SW	3	AM	–	49.6±46.0
Holsbeek et al. (1999) [r]	Southern North Sea	1994-1995	SW	7	M	3032±547	
Law et al. (2003)[q]	Orkney Islands (Scotland)	1994	SW	1		-	42.9
	Netherlands	1995	SW	2		-	80.6±90.7
Evans et al. (2004)[q]	Southern Australia	1998	SW	32	F	800±400	–
				5	M	1300±1200	–
Gaus et al. (2005)[q]	Tasmania (Southern Australia)	-	SW	7	F/M	(28.7±8.73)	–
Godard-Codding et al. (2011)[r]	SC= Sea of Cortez	1999	SW	10		MC: 1514±1693	–
	KR= Kiribati	2000	SW	10		KR: 734±869	–
	GP= Galapagos	2000	SW	10		GP: 1262±1,586	–
	PX1=Pacific Crossing	2000	SW	10		PX1: 777±853	–
	PNG=Papua New Guinea	2001	SW	10		PNG: 1101±1378	–
Bachman et al. (2014)[q]	Pacific Island	2011	SW	1	F	1.470	27.2
Fossi et al. (2014)[r]	Gulf of California (Mexico)	2008-2009	SW	14	M	2193±660	30.8±31.7
					F	2294±1180	283±819
Romero-Romero et al. (2017)[q]	Atlantic Ocean (Cantabrian sea)	-	SW	1		1790	149
Zhan et al. (2019)[q]							
Madgett et al. (2022)[q]	NE Atlantic Ocean	2012-2016	SW	5		821.1-13520	139.4-1888
Megson et al. (2022)[q]	Scottish coast	2010 -2013	SW	1		100000	
Praca et al. (2011)[r]	NWMS	2003-2009	SW	14	1M/13U	107810±108720	–
Marsili et al. (2014)[q]	Italy	2009	SW	7	M	193608±340089	–
Pinzone et al. (2015)[r]	NWMS	2006-2013	SW	32	M	24237±17421	382±176
				11	F	16877±7237	248±106
					F/M	(2120±1490)	
Zaccaroni et al. (2018)[q]	Mediterranean Sea	2014	SW	3	F	-	167±13.9
López-Berenguer et al. (2023)[q]	Mediterranean Sea	2011-2018	SW	2	M	1401±78.4	25.6±11.7
Bartalini et al. (2019)	Mediterranean Sea	2008,2009, 2016	SW	9	M	6421±6150	612±401

[p] Caught animals.
[q] Stranded animals.
[r] Free ranging animals.

When comparing our results with those previously described in sperm whales from outside the Mediterranean Sea, we found PCB concentrations one or more orders of magnitude higher than those reported from 2000 to 2016 for individuals from the Cantabrian Sea, Gulf of California, Galapagos, Papa New Guinea, Hawaii, and other islands in the central and eastern Pacific Ocean

(Fossi et al., 2014; Godard-Codding et al., 2011; Romero-Romero et al., 2017). On the other hand, PCB analysis conducted on sperm whales stranded before 2000 in the North Atlantic described levels two or more times higher than those reported for this study (Aguilar, 1983; Borrell, 1993). This difference could be due to the elapsed sampling years among the studies, along with the fact that the North Atlantic Ocean betwen North America and Europe, two major PCB source regions () (Axelman and Gustafsson, 2002), holds a a position responsible for its role as a major global sink (O'Sullivan, 2013).

To the best of our knowledge, currently only two studies investigated PCB concentrations in blubber of Cuvier's baked whales, and both reported mean/median levels lower than those found in our study (Bachman et al., 2014; Knap and Jickells, 1983) (Table 35).

Table 35. Mean or median (in bold) (ng/g l.w. or ng/g w.w.) of ΣPCB and ΣPBDE concentrations in blubber of Cuvier's beaked whales analyzed in previous studies. Range in parenthesis (when possible).

Reference (Year)	Geographic area	Sampling year	Species	N	Sex	ΣPCBs	ΣPBDEs
Bachman et al. (2014)	Pacific Island	2008, 2011	CBW	2	M	**4250 (3130-5360)** ng/g l.w.	**59.4 (53.6-65.2)** ng/g l.w.
Law et al. (2005)	UK coast	2002	CBW	1	M	-	320 ng/g w.w.
Knap & Jickells (1983)	Unknown	1981	CBW	4	3M-1F	9465 ng/g w.w.	-

Unlike this present study, previous works have been performed in stranded individuals outside the Mediterranean Sea. Specifically, Bachman et al. (2014) documented median levels of PCBs in stranded Pacific Island Cuvier's baked whales (4250 ng/g l.w.) almost four times lower than those in this study. Lower PCB levels (9465 ng/g w.w.) have been also reported for four specimens stranded in 1981 (site of stranding-unknown) (Knap and Jickells, 1983). Lower concentration levels of PCBs have been also indicated for other three species of beaked whales from Japanese (*Berardius bairdii*, *Mesoplodon stejnegeri* and *Mesoplodon carlhubbsi*) (Anezaki et al., 2016; Kajiwara et al., 2006; Kannan et al., 1989; Tanabe et al., 1989) and Canadian waters (*Hyperoodon ampullatus*) (Hooker et al., 2008).

These results are consistent with the fact the Mediterranean Sea, and in particular its western area, is considered for some authors a global PCB "hotspot" for marine mammals (Stuart-Smith and Jepson, 2017).

Comparing the levels of PCBs with those present in the literature for other cetacean species living in the Mediterranean Sea basin, it can be highlighted that Cuvier's beaked whales showed lower concentrations than those in striped dolphins (Fossi et al., 2013; Fossi et al., 2014; Marsili and

Focardi, 1996), common bottlenose dolphins (*Tursiops truncatus*) (Marsili et al., 2018), Risso's dolphins (*Grampus griseus*), and pilot whales (*Globicephala melas*) (Pinzone et al., 2015; Praca et al., 2011). The observed dissimilarities are likely the consequence of distinct trophic levels, prey preferences, feeding habits and feeding ground areas, among species. On the other hand, the comparable levels reported for sperm whales (Bartalini et al., 2019; Pinzone et al., 2015; Praca et al., 2011) are probably due to similar feeding habits (mainly based on mesopelagic squids) of these two species.

When investigating PCB concentrations among different age classes in Cuvier's beaked whales, we found that concentrations in males remained either stable or increased with age. However, in sexually mature females, contaminant burdens generally declined. The concurrent increase in contaminant concentrations in blubber with age has been previously documented in marine mammals (Hickie et al., 2007; Krahn et al., 2009; Murphy et al., 2015). This is typically attributed to the females' detoxification mechanisms, such as gestation and lactation, which enable them to reduce their contaminant body burden by transferring organic pollutants to their offspring (Borrell et al., 1995; Genov et al., 2019; Schwacke et al., 2002; Weijs et al., 2013; Wells et al., 2005). Thanks to the long-term studies conducted on this population, we knew that one of the females had given birth to at least 4 calves since 1999. This whale was, indeed, the one that had the lowest levels of PCBs in the dataset. In addition, detoxification mechanisms are absent in males, which normally accumulate contaminants throughout their entire lives.

As mentioned before, sperm whales and Cuvier's beaked whales share similar DL-PCBs profiles dominated by mono-*ortho* PCBs and with a small contribution of non-*ortho* PCBs. On the other hand, a greater dissimilarity between these two species was observed for the highly toxic non-*ortho* congeners (77, 81, 126, 169). For sperm whales, we found a non-*ortho* PCB profile (126>169>77>81) similar to that described in sperm whales from Australia (Gaus et al., 2005), and the Cantabrian Sea (Romero-Romero et al., 2017), but different from the accumulation patterns found in sperm whales from the Scottish coast (Megson et al., 2022), as well as those generally found in fish and their main prey (e.g. cephalopods) for which PCB-77 represents the predominant congener, followed by PCB-126 and PCB-169 (Cappelletti et al., 2015; Romero-Romero et al., 2017; Storelli, 2008; Tanabe et al., 1987). The prevalence of PCB-126 and PCB-169 could be partially explained by the highest biomagnification potential of these two congeners as reported by Castro-Jiménez et al. (2021) in a pelagic food web from the North Western Mediterranean Sea. The lack of specific studies about the metabolism of non-*ortho* substituted PCBs in sperm whales makes it difficult to find a solid explanation for this distinct profile. We can hypothesize that this species could have an enhanced metabolic capability towards PCB-77, and a reduced or absent metabolic activity towards PCB-126 and PCB-169. However, our hypothesis differs from Boon et al., (2000), who showed that PCB-77 was not metabolised by sperm whales microsomes in vitro. This points out the need of further investigations focused on accumulation and contaminant detoxification pathways in sperm whales and cetaceans in general. Moreover, without information on the specific feeding habits, as well as patterns and pollutant concentrations in their prey, caution is mandatory when trying to understand their contamination profiles. Nonetheless, in general, the average DL-PCB congener profile was alike to that described by Pinzone et al. (2015) for Mediterranean sperm whales sampled between 2008 and 2013.

The abundance profile of non-*ortho*-DL-PCBs in Cuvier's beaked whales (77 ≈ 126 > 169 > 81) was similar to that revealed for others beaked whales (Kannan et al., 1989; Tanabe et al., 1989), but at the same time remarkably different from that reported in blubber of Mediterranean sperm whales analyzed in this study (126 > 169 > 77 > 81). This is interesting, given that diet is the main route of POPs uptake for marine mammals, and both species are deep divers sharing common prey (i.e. mainly mesopelagic squids) (Podestà et al., 2016; Praca et al., 2011). However, it is known that many factors such as lipid content, protein-binding, blood transport, or chlorination level, among others, affect the distribution and partitioning of PCBs, and other POPs, across tissues in marine and terrestrial species (Vijayasarathy et al., 2019). Considering all the above, toxicokinetic differences between these two Mediterranean cetacean species are likely the consequence behind the dissimilar abundance of non-*ortho* congeners, instead of different feeding habits and grounds.

6.1.1.2. Sardines, anchovies and bogues

Among the three fish species investigated, sardines accounted for the highest levels of PCB contamination. This difference was probably due to their higher fat content (Milićević et al., 2022), and is in line with previous studies (Table 36) in which sardines from the Mediterranean and Marmara Seas, and purchased in Spanish markets, seem to be from two to six times more contaminated than anchovies (Coelhan et al., 2006; Herceg Romanić et al., 2021; Milićević et al., 2022; Miniero et al., 2014; Perelló et al., 2015).

Table 36. Mean of ICES7 PCB concentrations (ng/g w.w.) in anchovies, sardines, and bogues from different Mediterranean Sea areas. Median is marked in bold. Range in parenthesis (when possible).

Reference (year)	Geographic Area	Sampling year	Species	ICES7 (ng/g w.w.)
Mauffret et al. (2023)	Bay of Biscay (NE Atlantic)	2014	Sardine	5.41 (1.50-10.7)
Milićević et al. (2022)	Croatian Adriatic Sea	2014-2016	Anchovy	0.000412
			Sardine	0.002754
Romanić et al. (2021)	Eastern Adriatic Sea	2014-2016	Anchovy	**0.380 (0.200-2.35)**
			Sardine	**2.96 (0.400-9.63)**
Castro-Jiménez et al. (2021)	Mediterranean Sea	2013-2016	Bogue	**9.20**
Vuković et al. (2018)	Eastern Adriatic Sea	2014	Anchovy	0.260 (0.210-0.310)
			Sardine	1.57 (0.350-6.32)
		2016	Sardine	3.72 (1.04-9.63)
Perello et al. (2015)	Catalonia markets	2012	Anchovy	13.5
			Sardine	29.0
Miniero et al. (2014)	Southern Adriatic and Tyrrhenian Sea. Ionian Sea	2008	Anchovy	4.51
			Sardine	15.5
Perello et al. (2012)	Catalonia markets	2008	Anchovy	15.8
			Sardine	22.0
Storelli et al. (2011a)	Adriatic Sea		Bogue	111 ng/g l.w.
Çakıroğulları et al. (2010)	Black Sea	2005	Anchovy	17.3
		2006		7.1
Martí-Cid et al. (2007)	Catalonia markets	2005	Sardine	24.3
Coelhan et al. (2006)	Marmara Sea	2003	Anchovy	12.5
			Sardine	22.5
Naso et al. (2005)	Gulf of Naples	2003	Anchovy	35.4 (14.3-85.4)
Stefanelli et al. (2004)	Adriatic Sea	1997	Anchovy	15.6
Perugini et al. (2004)	Adriatic Sea	2002	Anchovy	17.5
			Sardine	15.6
Bayarri et al. (2001)	Adriatic Sea	1998	Anchovy	40.0

* sum of PCB congeners: 52, 101, 118, 138, 153, 180.

Besides the lipid content, an additional factor responsible for the variability of PCB contamination levels could be the species-specific feeding behaviour of these three species. Sardines and anchovies are both considered planktivorous species with a general niche overlapping during some seasons. Yet, several studies also reported differences in feeding strategies and target prey, and therefore, slightly distinct trophic levels (Costalago et al., 2012). Values found in this survey depicted a level of contamination significantly lower (1.6 to 10.6 times lower depending on the species) when comparing with PCB concentrations reported in the late 90s or early 2000s (Table 36). In particular, anchovies caught in the Adriatic Sea in 1998, and in the Gulf of Naples in 2003, reached levels of contamination about two orders of magnitude higher (Bayarri et al., 2001; Naso et al., 2005) than levels found in this study. However, and taking into consideration the wide range of values reported for sardines and anchovies, levels found in this study were not significantly lower – but higher in some occasions - than those measured in last years. Sardines obtained from Spanish markets in 2005, 2008 and 2012 (Martí-Cid et al., 2007; Perelló et al., 2015, 2012), and specimens caught in the Marmara Sea in 2008 (Coelhan et al., 2006), showed contamination levels about two times higher than our values. Moreover, a study conducted on the Adriatic Sea by Vuković et al. (2018) reported PCB concentrations for sardines up to five times higher than those reported in this study. Instead, anchovies analyzed in the same work in 2014 reached values about just one order of magnitude higher. On the other hand, more recent studies on sardines and anchovies caught between 2013 and 2016 in the Adriatic Sea revealed levels between one and four orders of magnitude lower than those described in this study (Herceg Romanić et al., 2021; Milićević et al., 2022). Sardines caught out of the Mediterranean Sea, specifically in the Gulf of Biscay (NE Atlantic Ocean) in 2014, showed levels in the same order of magnitude that those found in this study for Mediterranean Sea sardines (Mauffret et al., 2023).

Bibliographic data on PCB concentrations in bogue are scant in the Mediterranean area. ICES7 values reported in 2011 for fish caught in the Adriatic Sea (Storelli et al., 2011a) were in the same order of magnitude than those reported in this study, six years later. Conversely, study specimens in 2017 from the Atlantic Ocean (Canary Islands) presented levels of PCBs about one order of magnitude higher (Henríquez-Hernández et al., 2017). Finally, a recent study by Castro-Jiménez et al. (2021) in bogues captured in the Mediterranean Sea, between 2013 and 2016, reported median concentrations of ICES7 PCBs about three times higher than those described in our study.

As previously mentioned, sardines, anchovies, and bogues exhibited a congener profile primarily characterized by DL-PCBs. Among these, mono-*ortho* PCBs accounted for the majority, while no-*ortho* PCBs constituted only a small fraction. Specifically, sardines and bogues exhibited the same profile as Cuvier's beaked whales, PCB-77 > -126 > -169 > -81, which is commonly found in other studies conducted on the same and different species in the Mediterranean Sea and other regions across the globe (Gómara et al., 2005; Matthews et al., 2008; Moon and Choi, 2009). Instead, the pattern of non-*ortho*-PCBs was quite different in anchovies (126 > 77 > 169), with a contribution of PCB-126 about 71% and no detection of PCB-81. This profile (126 > 77 > 169) is different from that in anchovies caught in the Adriatic Sea (Bayarri et al., 2001), but similar to that of anchovies caught in the Black Sea (Çakıroğulları et al., 2010), and in the NW Mediterranean Sea, near to the Catalan coast (Llobet et al., 2003).

Dissimilarities in the induction rates of cytochrome P450 enzymes have been explored in the three study fish species based on the ratio PCB-169/PCB-126. Higher values of this ratio have been suggested as a proxy for a higher metabolization of PCB-77 relative to that of other non-*ortho* congeners (Tanabe et al., 1987). However, in this study, very similar average values for this ratio were found for sardines (0.13), bogues (0.10), and anchovies (0.19). Instead, a similar total non-

ortho PCB content was found between bogues and anchovies, while a much higher content of about 4–5 fold was measured in sardines.

Fish can accumulate PCBs from the surrounding environment (Antunes and Gil, 2004) and through prey intake (Mackay and Fraser, 2000). Given that abundance profiles are similar among species for indicator and mono-*ortho* congeners, the differences in non-*ortho* PCBs could be attributed to different feeding habits and grounds. In addition, important dissimilarities in toxicokinetics between species, involving metabolism and selective distribution across tissues, have been described in fish and other species (Albaigés et al., 1987; Kania-Korwel and Lehmler, 2016; Monosson et al., 2003; Ondarza et al., 2014; Reich et al., 1999; Vijayasarathy et al., 2019).

6.1.1.3. Blue sharks

When we compare our results on PCB concentrations with those reported by Alves et al. (2016) in blue sharks sampled in the same area (Portuguese coast), four years before our study, we found quite slightly lower levels, in both tissues (liver: mean=328 ng/g w.w.; muscle: mean=1.12 ng/g w.w.). In addition, previous studies in blue sharks from different parts of the world, and within a time frame of 20 years (Table 37 and 38), described PCB concentrations lower or in the same order of magnitude than those described in our study.

Table 37. Mean ± standard deviation (when possible) and range in parenthesis (when possible) of ΣPCB, ΣPBDE, ΣPCDD, and ΣPCDF concentrations in ***muscle*** of blue sharks sampled worldwide. Data are expressed in ng/g (ΣPCBs, ΣPBDEs) and pg/g (ΣPCDDs and ΣPCDFs). Data expressed in lipid weight basis (* wet weight basis). (M=male; F=female).

Reference (Year)	Geographic area	Sampling year	N	Sex	ΣPCBs	ΣPBDEs	ΣPCDDs	ΣPCDFs
Menezes-Sousa et al. (2021)	Atlantic Ocean	2017	15	M	-	7.40 ± 7.70 (<LOQ-34)	-	-
Alves et al. (2016)	Portuguese coast	2015	20	8M-12F	1.12 ± 1.03 (0.197-4.38)*	0.0543 ± 0.0310 (0.0175-0.131)*	0.0712 ± 0.0788 (0.0251-0.313)*	0.0336 ± 0.0105 (0.0181-0.0563)*
Lee et al. (2015b)	Pacific Ocean	2010	15	-	-	5.70 (0.400-51.4)	-	-
Lee et al. (2015a)	Pacific Ocean	2010	15	-	45.7 ± 39.1 (<LOQ–125)	-	-	-
de Azevedo e Silva et al. (2007)	Brazilian coast	2001	12	4M-8F	430 (185.1–714.7)	-	-	-

Table 38. Mean ± standard deviation (when possible), and range in parenthesis (when possible) of ΣPCB, ΣPBDE, ΣPCDD, and ΣPCDF concentrations in ***liver*** of blue sharks sampled worldwide. Data are expressed in ng/g (ΣPCBs, ΣPBDEs) and pg/g (ΣPCDDs and ΣPCDFs). Data expressed in wet weight basis (* lipid weight basis). (M=male; F=female).

Reference (Year)	Geographic area	Sampling year	N	Sex	ΣPCBs	ΣPBDEs	ΣPCDDs	ΣPCDFs
Alves et al. (2016)	Portuguese coast	2015	20	8M-12F	328 ± 273 (43.2-1160)*	7.63 ± 6.16 (1.35-29.0)*	2.23 ± 1.93 (0.541-8.00)*	6.44 ± 6.04 (1.78-26.9)*
Storelli et al. (2011b)	Mediterranean Sea	2008	22	-	679 (68-1480)	-	189 (68-317)	368 (141-978)
Storelli et al. (2005)	Mediterranean Sea	1999-2001	44	-	2480	-	-	

Specifically, muscle of specimens from the Brazilian coast sampled in 2001 (de Azevedo e Silva et al., 2007), and from coastal waters of Korea sampled in 2010 (Lee et al., 2015a), showed PCB levels of 430 ng/g, l.w. and 45.7 ng/g, l.w, respectively. PCB levels in liver have been studied in blue sharks sampled in 1999-2001 (Storelli et al., 2005) and 2008 (Storelli et al., 2011b) in the Mediterranean Sea, reporting concentrations of 2480 ng/g l.w. and 679 ng/g l.w, respectively. Different pollution status among areas, sampling times, as well as different analytical methods should be factored in when considering the concentration variability among these studies.

As described for Cuvier's beaked whales, we found greater concentrations of PCBs in blue shark male adults in comparison to subadult males. This finding emphasizes an ongoing bioaccumulation of PCBs in blue sharks, along with the absence of offloading mechanisms such as gestation and lactation, present in females. It is known that the reproductive strategy such as oviparity, aplacental or placental viviparity may play an important role in the offloading of contaminant burdens in shark species (Chynel et al., 2021; Lyons and Lowe, 2013). In particular, offloading of organohalogen contaminants (0.03–2.3%) to the embryos has been quantified for hammerhead female sharks (*Sphyrna lewini*) (Lyons and Adams, 2015), which has a placental viviparity strategy as blue sharks (da Silva et al., 2021). At the same time, maternal discharge of POPs in shark species is known to be a route of exposure to offspring (Marler et al., 2018; Mull et al., 2013). While there is evidence suggesting concentration transfer during blue shark gestation, potentially posing a significant contamination risk to the embryos, it is noteworthy that this study did not find differences in PCB concentrations between males and females. This observation draws attention to the presence of limited offloading mechanisms in female blue sharks.

Higher concentrations in liver than in muscle (in wet weight) is in accordance with what has been reported by other authors in blue and other shark species (Alves et al., 2016; Boldrocchi et al.,

2019; Strid et al., 2007). This is likely the consequence of the lipid deficiency in shark muscle (0.26 ± 0.11%) as well as the lipoaffinity of these contaminants, which tend to be preferentially stored in lipid-rich tissues such as liver (34 ± 9%) (Lyons et al., 2021). Additionally, the high metabolic activity and irrigation of this tissue may contribute to these findings.
As stated before, non-*ortho* DL-PCBs represents only a small portion of PCBs profile in blue shark individuals. Particularly, the abundance profile of non-*ortho* PCBs described for liver in blue sharks (PCB-126 > PCB-169 ≥ PCB-81) is similar to that reported in Cuvier's beaked whales, sardines and bogues of the Mediterranean Sea, in blue sharks from the northeast of the Atlantic Ocean (Alves et al., 2016), and is also commonly described in marine species. On the other hand, this abundance profile differs from that found in Mediterranean blue sharks (Storelli et al., 2011b) (PCB-126 > -77> -169), which is likely to reveal differences in regional PCB pollution. On the other hand, PCBs -77 and -81 showed a notably smaller contribution in liver. This, along with the lower ratio PCB126/169, underscores the major metabolic capabilities of this tissue towards these congeners in the study blue sharks (Storelli et al., 2004).

Taking into account that various factors could influence the comparability among different studies, such as the capture location, sampling timing in relation to spawning activity (in the case of fish species), laboratory procedures, and quantification methods, it is important to highlight that our findings generally align with the PCB values reported from other studies for the same species in recent years. Our results support the concept of a virtual halt, or a very slow decrease in PCB concentrations put forth by other researchers (Jepson and Law, 2016; Law and Jepson, 2017; Stuart-Smith and Jepson, 2017). This observation underscores the limited effectiveness of mitigation measures and global efforts to combat PCB contamination, despite their ban in many countries for over four decades, and the establishment of the Stockholm Convention's 2028 deadline for their global elimination.

6.1.2. PBDEs

Sperm whales, Cuvier's beaked whales, and blue sharks shared a similar PBDE congener profile, dominated by lower-medium brominated congeners (BDE-47, BDE-99, BDE-100, BDE-153, and BDE-154). This agrees with the fact that these congeners are generally the most abundant in aquatic food webs (Castro-Jiménez et al., 2021; Fossi et al., 2012; Romero-Romero et al., 2017), with BDE-47 commonly being the predominant congener in most biota samples, including marine mammals (Hites, 2004).

This profile seems to reflect the extensive use of one the most employed BDE formulations, penta-BDE (La Guardia et al., 2006), whose production was voluntarily ceased in 2004 in Europe and USA (Hites, 2006). However, it should still be considered that the presence of BDE-47 in marine organism tissues could be partially due to the debromination of higher brominated congeners (Roberts et al., 2011). BDE-154 levels were also significant in all species investigated. This congener has been suggested to be a debromination product of BDE-183, the predominant congener in the technical octa-BDE mixture (Roberts et al., 2011; Stapleton et al., 2004).

BDE-209, the main component of the commercial deca-BDE mixture (97-98% BDE-209), which was the most intensively used and the latest formulation to be phased out (La Guardia et al., 2006), showed a very low average contribution in all the investigated species. This result could be attributable to two distinct factors. First of all, the highest hydrophobicity of BDE-209 among BDE congeners explains its heightened sequestration into suspended particulate matter and sediments, resulting in low bioavailability (Lee and Kim, 2015). Secondly, highly brominated BDEs as BDE-209 debrominate very rapidly due to sunlight (De Wit, 2002) and they are also poorly bioaccumulated, and easily debrominated in biota into less brominated PBDEs (He et al., 2006; Kierkegaard et al., 2007; Stapleton et al., 2006). In support of this hypothesis, depletion of BDE-209 reported in juvenile carps (*Cyprinus carpio*) has been ascribed to its debromination into penta- to octa-BDEs (Stapleton et al., 2004). Nevertheless, several studies detected BDE-209 in different marine organisms reporting a possible biomagnification of this congener in marine food webs (Castro-Jiménez et al., 2021; Johnson-Restrepo et al., 2005). As in the case of PCBs, PBDE concentrations exhibited an ongoing accumulation in blue sharks and Cuvier's beaked whales. Moreover, PBDE levels seem to be affected by specimen's age, development status as well as presence/absence of detoxification mechanisms such as gestation and lactation in females.

6.1.2.1. Sperm whales and Cuvier's beaked whales

When we compare PBDE concentrations with those described in previous works performed in the Mediterranean Sea, we found that levels in our study were up to three times higher than those reported for sperm whales (three females) stranded along the Adriatic coast in 2014 (Zaccaroni et al., 2018). They were also two orders of magnitude higher than those described by López-

Berenguer et al. (2023) in two individuals stranded along the coastline of South Eastern Spain between 2011 and 2018 (Table 34). On the other hand, the study by Pinzone et al. (2015) reported PBDE levels in the same order of magnitude than those described in our study, in free-ranging specimens sampled in the North Western Mediterranean Sea between 2006 and 2013 (Table 34). We hypothesize that the dissimilarity between concentration values among the aforementioned studies was highly influenced by sex and location. Individuals analyzed by Zaccaroni et al. (2018) were all females, and had already reached sexual maturity and probably given birth, decreasing their contamination loads during gestation and lactation (Reijnders et al., 2009). On the contrary, males analyzed in this study were mostly adults and without off-loading mechanisms as those present in females, they could have been accumulating pollutants - especially those with high hydrophobicity and resistance to metabolism - throughout their entire lives. Regarding PBDE concentrations reported in sperm whales from outside the Mediterranean basin (Table 34), our results were in the same order of magnitude than those described in sperm whales from Gulf of California (Fossi et al., 2014), but six times lower than those reported from North Atlantic specimens (Borrell, 1993). The latter are probably due to the greater historical use of these compounds in North America (Law et al., 2014). Conversely, a recent study performed in sperm whales stranded on the Scottish coast during 2012-2016 (Madgett et al., 2022) reported a range of PBDE levels similar to those of our study.

To the best of our knowledge, only two works investigated PBDE concentrations in blubber of Cuvier's baked whales and reported mean/median levels lower or similar than those found in our study (Bachman et al., 2014; Law et al., 2005) (Table 35). Bachman et al. (2014) documented in stranded Pacific Island Cuvier's baked whales median levels of PBDEs (59.4 ng/g l.w.) almost six times lower, while quite similar PBDE levels (320 ng/g w.w.) were reported for one specimen stranded in 2002 along the UK coast (Law et al., 2005). Considering the limited information available regarding PBDE contamination in Cuvier's beaked whales, our results draw attention to the potential role of the Mediterranean Sea as *hot spot* for PBDEs in marine mammals, and particularly for Cuvier's beaked whales. Comparable levels reported for sperm whales and Cuvier beaked whales are probably due to the similar feeding habits (mainly based on mesopelagic squids) of these two species.

The same increase in contaminant concentrations according to age growing, as in the case of PCBs, has been reported for PBDEs in Cuvier's beaked whales. This fact highlights the potential of bioaccumulation of PBDEs throughout the entire life in this species.

It is worth noting that differences in pollutant concentrations and profiles between our study and previous literature could be linked not only to different geographic areas, time periods and inter-individual differences, but also to distinct analytical methods and type of samples such as free-ranging or stranded specimens. Specifically, it is known that POP concentrations in cetaceans are influenced by blubber thickness (Evans et al., 2003), which could became a significant variable when comparing biopsies taken from free-ranging individuals (few centimetres of the outermost layer) to samples obtained from stranded individuals (usually all three layers of blubber) (Evans et al., 2003; Ryan et al., 2013).

As stated before, sperm whales and Cuvier's beaked whale reported a decrease in bioaccumulation levels along with the bromination degree. This profile has been also observed in different marine mammals species (Alonso et al., 2014; Pinzone et al., 2015; Zaccaroni et al., 2018), including beaked whales (Kajiwara et al., 2006), and in aquatic food webs in general (Johnson-Restrepo et al., 2005). Overall, similar congener profiles of PBDEs have been described in sperm whales inhabiting the Mediterranean Sea (López-Berenguer et al., 2023; Pinzone et al., 2015; Zaccaroni et al., 2018) and the North East Atlantic Ocean (Madgett et al., 2022), as well as in other marine organisms (Capanni et al., 2020; López-Berenguer et al., 2023; Miniero et al., 2014). Although, metabolism plays an important role in the PBDE congener accumulation in aquatic organisms, lower brominated congeners have higher biomagnification potential and, thus, possible toxicological effects (Alonso et al., 2014; Koenig et al., 2013). On the other hand, differences in congener's contribution degree would indicate either exposure to distinct PBDE sources, or different metabolic and elimination capacities among individuals or species (BDE-47: 70% sperm whale *vs.* 45% Cuvier's baked whale). In this regard, a study by Capanni et al. (2020) reported a gradually decline of the BDE-47 relative contribution in older specimens of striped dolphins, probably as a consequence of an easier discharge of less lipophilic and low-molecular weight congeners. A similar hypothesis was proposed for melon-headed whales (*Peponocephala electra*) and arctic beluga whales (*Delphinapterus leucas*), which showed significant inverse correlations between log K_{ow} and mother-fetus ratios of PBDE congeners (Desforges et al., 2012; Kajiwara et al., 2008).

An unexpected increase of higher brominated BDEs in younger Cuvier's beaked whales (juvenile>subadult>adult) was observed. This evidence needs to be further investigated, but a possible explanation could rely on the increasing use of deca-BDE commercial mixture as an alternative of penta- and octa-BDE formulations banned and phased out in 2004 in the European

Union (Directive 2003/11/EC of the European Parliament and of the Council). Juvenile individuals have, therefore, come into direct contact marginally with these commercial mixtures (penta- and octa-), mainly through lactation, having a higher percentage of BDE-209, which constitutes around 92- 97% of the total BDE content in the deca-BDE formulations (La Guardia et al., 2007).

6.1.2.2. Blue sharks

Regarding blue sharks, our study reported higher levels of PBDEs than those described by Alves et al. (2016). Liver and muscle concentrations reported in blue sharks sampled in 2019 (our study) doubled those found in blue sharks sampled in 2015 (liver: mean=7.631 ng/g w.w.; muscle: mean=0.054 ng/g w.w.) in the same area (Alves et al., 2016). This result aligns with the positive correlation between size/age and PBDE accumulation levels reported in the animals sampled in our study. Additionally, the comparison between values reported for blue sharks sampled in 2015 (Alves et al., 2016) and those sampled in 2019 (this study) confirmed a possible increasing trend for PBDEs, as already described in various abiotic and biotic matrices in different areas (Abbasi et al., 2019; Addison et al., 2020; Roscales et al., 2018; Sharkey et al., 2020). PBDE concentrations have been also studied in muscle of blue sharks from coastal waters of Korea and the Equatorial Atlantic Ocean, with levels of 7.70 ng/g l.w. (sampled in 2010) (Lee et al., 2015b) and 7.4 ng/g l.w. (sampled in 2017) (Menezes-Sousa et al., 2021), respectively (Table 37). As mentioned above, when we compare different studies, many variables come into play. Taking this into account, specimens analyzed in our study showed greater levels - between 1 and 2 orders of magnitude higher - suggesting a possible increase of PBDE values in the environment, and specifically for this species in this area.

The abundance pattern of PBDE congeners described in blue shark, dominated by lower-medium brominated congeners, is in line with what has been found for liver and muscle of different shark species from various geographical regions such as waters from South Korea (Lee et al., 2015b), Japan (Nakajima et al., 2022) or Southeastern USA (Weijs et al., 2015). Remarkably, the study by Alves et al. (2016) conducted on blue sharks in the Atlantic Ocean (Portugal) reported a relative abundance profile for each congener similar to that described in our study for blue sharks captured in the same area.

Interestingly, the lower average contribution of BDE-209 in liver (0.3%) than in muscle (14%) emphasizes a possibly enhanced metabolization of BDE-209 in liver of blue shark. Information about the toxicokinetics of BDE-209 in marine species, including sharks, is scant. There are studies on tissue-specific accumulation performed in teleost fish such as common sole (*Solea solea*) (Munschy et al., 2017), but also in marine mammals such as harbor seals (*Phoca vitulina*) (Shaw et al., 2012) that described preferential bioaccumulation of this congener in liver. Yet, a recent study by Nakajima et al. (2022) could not detect BDE-209 in liver from up to eight different species of deep-sea sharks from Japanese waters with an average content of PBDEs relatively comparable with what found in blue sharks' liver in this study. On the other hand, Lee et al. (2015b) detected BDE-209 in just 4.8% of the muscle samples from 13 different shark species investigated, including blue shark. This information suggests that the factor species could play an important role in the toxicokinetics of BDE-209 for each tissue, and emphasizes the need of species-specific investigation, above all in elasmobranchs, which apparently show greater biotransformation of BDE-209 in liver than in muscle.

6.1.3. PCDD/Fs

In our studies, we found always levels of PCDD/Fs lower than those of the other targeted POPs (PCBs and PBDEs). This was anticipated since dioxins and furans, despite their high toxicity degree, are normally present at lower levels than other POPs in the tissues of living organisms. Firstly, they are unintentional products without any commercial value (ATSDR, 1998). They have chemical properties and sources slightly different from those of PCBs, which are mostly associated with industrial processes and products (Ross, 2004). Instead, PCDD/Fs are primarily formed through incomplete combustion processes, as well as during the manufacture of pesticides and other chlorinated substances (Kirkok et al., 2020). Additionally, PCDD/Fs are known to have a higher affinity for particulate matter and tend to bind more strongly to environmental surfaces, resulting in their deposition and accumulation in different environmental compartments (Srogi, 2008).

6.1.3.1. Sperm whales

In comparison to other geographical areas, PCDD/F levels in the sperm whales analyzed in this study were in the same order of magnitude than those reported for sperm whales from Australia (Gaus et al., 2005). On the other hand, the study by Pinzone et al. (2015) reported PCDD/F levels in free-ranging Mediterranean sperm whales one order of magnitude higher (PCDDs: mean=0.36

ng/g l.w.; PCDFs: mean=0.16 ng/g l.w.). The dissimilarities in concentration levels could be attributed to the variation in sampling times – from 3 to 10 years - with our study spanning from 2009 to 2016 and that of Pinzone et al. (2015) covering the period from 2006 to 2013.

In Mediterranean sperm whales we observed a PCDF congener profile (penta > hexa > tetra > hepta > octa) relatively similar to that described in sperm whales from Australia (Gaus et al., 2005) and striped dolphins (*Stenella coeruleoalba*) from the Mediterranean Sea (Fossi et al., 2004). In contrast, the PCDD congener profile (hexa > penta > tetra > hepta > octa) was remarkably different from these two, with a lower concentration of higher chlorinated congeners, and a higher concentration of lower chlorinated congeners.

Owing to the scarcity of information, it is difficult to make solid statements about the contamination status of PCDD/Fs in sperm whales inhabiting the Mediterranean Sea. More studies focused on sperm whale populations and other odontocete species inhabiting the Mediterranean Sea, as well as other regions, are needed in to better understand patterns and trends of these highly toxic contaminants.

6.1.3.2. Blue sharks

When comparing PCDD/F results with those reported in blue sharks sampled in the same area (Portuguese coast) four years before our study (Alves et al., 2016), we found that PCDD/F concentrations were similar in liver (0.541–7.97 pg/g w.w. for PCDDs and 1.78–26.9 pg/g w.w. for PCDFs), but lower in muscle (range: 0.0251–0.313 pg/g ww for PCDDs and 0.0181–0.0563 pg/g w.w. for PCDFs). On the other hand, the study by Storelli et al. (2011b) reported an average of 189 pg/g l.w. and 368 pg/g l.w. for total PCDDs and PCDFs, respectively, one order of magnitude greater than those described in Atlantic specimens of this study. Considering again all the variables involved in direct comparisons among studies, these results seem to reveal differences on the pollution status depending on the sampling area and time, and to confirm the role of the Mediterranean Sea as a sink for these contaminants.

For blue sharks, and to the best of our knowledge, we found an abundance profile of PCDD/Fs similar to that described by Alves et al. (2016) in Atlantic specimens, but different from the only two studies reporting PCDD/Fs in sharks (Storelli et al., 2011b; Strid et al., 2007). In the study by Storelli et al. (2011b), PCDDs and PCDFs accounted for 34% and 66%, respectively, in liver of Mediterranean blue sharks, with 2,3,7,8-TCDF > 2,3,7,8-TCDD > 1,2,3,6,7,8-HxCDD > 2,3,4,6,7,8-

HxCDF as major contributors. Strid et al. (2007) described in Greenland sharks from Icelandic waters a relative abundance in muscle of 22% for PCDDs and 77% for PCDFs, while in liver 13% for PCDDs and 87% for PCDFs. In both tissues there was an overall predominance of 2,3,7,8-TCDF, no detection of octa-congeners, and variable but important contributions of 2,3,4,7,8-PeCDF, 2,3,7,8-TCDD, 1,2,3,7,8-PeCDF, 1,2,3,7,8-PeCDD, and 1,2,3,6,7,8-HxCDD. Apart from the common prevalence of 2,3,7,8-TCDF in liver, and taking into account inter-species differences in the toxicokinetics of these pollutants, the clear dissimilarities for the homolog groups' abundance among these studies are likely the consequence of different regional (and temporal) pollution fingerprints, since all these studies have been performed in different areas and times. PCDD/Fs showed a general lower uptake compared to the other targeted contaminants, with a major propensity of accumulation in liver and a decreasing trend with age in muscle. On the other hand, as described in the Results section (*4.2. Concentration values*), concentrations in lipid weight tended to be higher in muscle than in liver, unlike PCBs and PBDEs for which concentrations still tended to be higher in liver when converted in lipid weight. Although more specific studies are needed, toxicokinetic differences, including distinct metabolism rates, could be behind of most of this heterogeneity found in concentrations among tissues.

Regarding accumulation dissimilarities between sexes, the only difference statistically significant (Mann-Whitney, $p < 0.05$, see specific U values and significances in Table 27) was found for the content of PCDD/Fs in muscle that was higher in females. This result could be also the consequence of distinct toxicokinetics among liver and muscle of males and females. The detection frequency of PCDD/Fs in muscle was notably lower than that in liver, resulting in many values in the muscle corresponding to upperbound values. Even though there were no statistically significant differences in the LODs between both tissues, our primary hypothesis suggests that the overestimation of lipid weight concentrations in muscle samples may be attributed to a biased calculation stemming from the presence of minimal lipid content. This overestimation is expected to affect the concentrations of all contaminants, but it was particularly evident in the case of PCDD/Fs. This is because their total loads were significantly lower than those of PCBs and PBDEs in both tissues, especially in the muscle.

6.2. Toxicity thresholds

Maintaining healthy marine ecosystems requires an adequate risk assessment of the seas and oceans. One of the most important tools needed when studying the potential hazards deriving from the presence of toxic substances, such as POPs, is the establishment of threshold levels. Established toxicity limits inform us about the concentration or level exposure to a substance (or mixture of substances) at which adverse effects or toxicity begins to occur in organisms. In this context, the assessment of risks evaluates and quantifies the potential risks and adverse effects associated with a chemical substance that result of fundamental importance for the management and conservation of marine species, above all, for those populations highly exposed.

For all these reasons, and to gain a better understanding of the potential toxicological risks associated to the presence of PCBs, PBDEs, and PCDD/Fs in the tissues of the studied individuals, we attempted to quantify their toxic potency based on the TEF approach. Due to the absence of species-specific limits, as well as standardized thresholds, we compared our results with toxicity limits established for seals, which are commonly employed for marine mammals in general (Hall et al., 2003; Helle et al., 1976a; Kannan et al., 2000; Ross et al., 1995). In that regard, a certain degree of approximation should be factored in, since marine mammals exhibit some level of variability in their detoxification capabilities and susceptibility to adverse effects (Desforges et al., 2016).

In order to evaluate the potential risk associated with dioxin-like compounds we used the approach of *Toxic Equivalency Factors* (TEFs), in which the concentration of individual compounds present in a mixture are multiplied by their specific TEF value, and the sum is expressed as the TCDD toxic equivalent quotient (TEQ) (van den Berg et al., 2006). This approach, based on the different contribution of each pollutant to the total toxic equivalent (TEQ), makes it possible to carry out a risk assessment for the investigated species.

Taking into account the wide inter-species sensitivity towards different contaminants, it should be highlighted how all analyzed sperm whales surpassed the threshold of 210 pg WHO-TEQ/g l.w. in blubber, proposed as starting point of immunosuppression in harbour seals (Ross et al., 1995). On the other hand, only four Cuvier's beaked whales (20% of the dataset) exceeded the abovementioned limit with the lowest TEQ values amongst those described for other toothed whales in the Mediterranean Sea (Bartalini et al., 2019; Pinzone et al., 2015). The lack of the PCDD/F contribution in the toxicity assessment for Cuvier's beaked whales could be responsible for these lowest levels. However, over 80% of the Cuvier's beaked whales exceeded the lower toxicity threshold of 9 mg/kg l.w. for PCBs, level at which marine mammals suffer physiological effects (Kannan et al., 2000). Furthermore, three animals (15%) exceeded the higher PCB threshold of 41 mg/kg l.w, proposed as a toxicity limit for reproductive impairment in Baltic ringed seals (*Pusa hispida*) (Helle et al., 1976a). While the percentage of animals surpassing these thresholds is lower than what has been observed in striped (Jepson et al., 2016), and bottlenose dolphins (Genov et al., 2019), it is worth noting that some studies have suggested a potential overestimation of the actual risk associated with PCBs when using the lower toxicity threshold (Jepson et al., 2016; Murphy et al., 2018). However, it is crucial to emphasize that various contaminants elicit diverse responses across species. Additionally, the lack of specific investigations on these particular species, along with limited research on marine mammals in general, further complicates the issue.

Interestingly, all sperm whales in this study exhibited the same TEQ pattern (Σnon-*ortho*-DL-PCBs > Σ*ortho*-DL-PCBs > PCDDs > PCDFs), with DL-PCBs as the most abundant pollutants, in agreement with previous investigations on sperm whales, and other cetaceans, from the Mediterranean Sea (Fossi et al., 2004; Pinzone et al., 2015). This draws attention to the fact that, even if PCBs were banned around 40 years ago in most European countries, they continue to pose a significant threat to cetaceans in the Mediterranean Sea, where these contaminants have also been related to cetacean populations' declines (Stuart-Smith and Jepson, 2017).

Numerous negative impacts on mammalian health have been attributed to the presence of PBDEs in their tissues. These include disturbances in the regulation of steroidal and thyroid hormones (Darnerud, 2003; Zhou et al., 2001), immunotoxic effects (Fowles et al., 1994), as well as reproductive disorders and neurological abnormalities (Siddiqi et al., 2003). PBDEs have also been associated to immunotoxicity in harbor porpoises, pantropical spotted dolphins, sperm whales, striped dolphins, killer whales, long-beaked common dolphins, and some mysticete species such as fin whale (*Balaenoptera physalus*), bryed's whale (*Balaenoptera edeni*) and minke whale (*Balaenoptera acutorostrata*) (Beineke et al., 2005; Hall et al., 2003; Huang et al., 2020; Marsili et al., 2019; Simond et al., 2019; Villanger, 2011). Furthermore, genotoxicity and cytotoxicity have been described in pantropic spotted dolphins (Rajput et al., 2021). Currently, despite the extensive evidences about the toxic potential of PBDEs in marine mammals, there is no established specific thresholds neither for sperm whales nor for Cuvier's beaked whales. The only available reference value for marine mammals has been established for grey seals, and refers to a threshold of 1500 ng/g l.w. for endocrine disruption (Hall et al., 2003). In the present study, total PBDE concentrations ranged from 312 to 1390 ng/g l.w. for sperm whales and from 60.0 to 1930 ng/g l.w. for Cuvier's beaked whales. In all age classes of Cuvier's beaked whales, results were below the threshold limit except for one subadult individual. Considering the unknown interspecies sensitivity to these pollutants both in sperm whales and Cuvier's beaked whales, the distinct response to PBDEs among different species cannot be overlooked. Furthermore, apart from interspecies sensitivity, it is important to note that several factors such as age, sex and overall health status can influence the individual response to contaminant concentrations.

Additionally, various factors like habitat loss and degradation, noise pollution, climate related effects, overfishing, entanglement and ship strike, also contribute to the deterioration of these populations. Therefore, it is crucial to gather information about how these chemicals affect poorly studied species and isolated groups of animals. The study of population dynamics, structure and the way they respond to different contamination levels, becomes paramount, particularly in an ecologically significant area like the Mediterranean Sea.

As mentioned before, all Cuvier's beaked whales analyzed in this study (more than 20% of the estimated resident population in the Ligurian Sea) exhibited detectable levels of PCBs, indicating that at least 20% of the population is affected. Furthermore, since 80% of the sampled whales exceeded established toxicity thresholds, it can be inferred that a significant portion of the population may experience detrimental physiological effects, including potential disruptions to their endocrine systems. These findings draw attention to the previously underestimated anthropogenic threat of chemical pollution, which, along with underwater noise disturbance and fishery interaction, emerges as one of the primary threats to the conservation of Cuvier's beaked whales in the Mediterranean Sea (Cañadas et al., 2018; Cañadas and Vázquez, 2014; Podestà et al., 2016) and other areas (Fernández et al., 2005). Recognizing and understanding the exposure and impacts of multiple threats on wild cetaceans is crucial for implementing effective conservation measures for specific populations and, ultimately, safeguarding the survival of the species.

To the best of our knowledge, no toxicity threshold has been established for sardines, anchovies and bogues, until now. Despite POPs having been widely studied for their adverse effects on fish health (Brar et al., 2010; Henry, 2015; Hontela et al., 1992; Teh et al., 1997), determining toxicity thresholds in fish is a complex endeavour due to the wide range of sensitivity of these species. Fish have more aryl hydrocarbon receptor (AhR) genes than other vertebrates, which confer species-

and strain-specific differences in the sensitivity to toxic AhR ligands such as PCDD/Fs and DL-PCBs (Hahn et al., 2005).
Thus, some species have developed adaptive mechanisms and detoxification pathways that allow them to withstand higher levels of contamination, while others may be more susceptible to even low levels of exposure (Hutchinson et al., 2006; Zhou et al., 2010). Berninger and Tillitt (2019) performed a meta-analysis on mortality, growth, and reproduction (MGR) using comprehensive literature data from different fish species. They described a concentration-response threshold of 1 µg/g total PCBs that would result in 17% increase in mortality, 15% inhibition of growth, and 39% inhibition of reproduction. Although this threshold has been established for different species than those considered in our study and therefore with distinct sensitivity to PCB toxicity effects, sardines, anchovies, and bogues inhabiting the Mediterranean Sea appear not to be adversely affected at population level by the presence of these substances.

Species-specific responses to POPs have been previously reported in marine mammals suggesting that some species may be more vulnerable than others. For example, variations in the immune response have been observed for PCBs among different species of odontocetes. Thus, Hammond et al. (2005) demonstrated that exposure to mixed Arochlors at concentrations up to 0.03 µg/ml resulted in a significant reduction (20-30%) in phagocytosis in harbour seals, while no similar effect was observed in grey seals. Additionally, a study by Levin et al. (2004) reported more pronounced impacts of PCB exposure on the immune system of bottlenose dolphins compared to that of belugas. On the other hand, studies on marine mammals show that the range of effects on the immune system, specifically on the lymphocyte function, varies depending on the type of PCB congener (Peñín et al., 2018).
Limited research on the impacts of POPs on elasmobranch health is responsible for the lack of comprehensive understanding and the absence of established toxicity thresholds. For this reason and considering a certain degree of approximation, we compared the concentrations of PCBs and PBDEs in blue sharks with the aforementioned toxicity thresholds established for marine mammals (Hall et al., 2003; Kannan et al., 2000; Ross et al., 1995). None of the animals included in this study exceeded the toxicity limits, as indicated by average PCB and PBDE values that were significantly lower, from one to three orders of magnitude. Our results, however, emphasized an urgent need of further research focused on assessing the potential consequences of POP concentrations on blue shark populations, and elasmobranchs in general.

It is well known that the study of toxicity thresholds in marine mammals -and marine organisms in general-, is a complex task due to various factors at play. First of all, there are various logistical and ethical limitations inherent in studying mammals that predominantly reside in the ocean, often in remote and inaccessible areas that are only available for study during specific periods of the year, and particular stages of the animals' life cycles (Weijs and Zaccaroni, 2016). Similarly, like other indicators of biological responses, the immune function in marine mammals exhibits variations influenced by numerous biological and ecological factors, including age, sex, health status, and exposure to pathogens, among other variables (Desforges et al., 2016). Moreover, synergistic and cumulative effects from multiple pollutants, as well as congener specific effects, further complicate the establishment of clear toxicity thresholds.

Yet, given the increase in diseases and mass mortality events in marine organisms (Gulland and Hall, 2007; Simeone et al., 2015; Tracy et al., 2019; Ward and Lafferty, 2004), it is imperative to understand how contaminants affect their health status and how this is reflected in the conservation of entire populations. Without species-specific and congener-specific studies linking observable effects with contaminant exposure, we would not be able to estimate the true negative impact exerted on marine organisms.

PBDEs do lack of a standardized methodology for risk assessment and, while a group of seven indicator congeners exists for PCBs, there is not a similar counterpart for PBDEs as of yet (Boalt et al., 2013). Although PBDEs have received less attention from a toxicological standpoint, studies have shown that these compounds can disrupt normal biological processes to a similar extent as PCBs (Chen and Bunce, 2003), and in certain cases, they may exhibit even stronger interferences on physiological functions (Hooper and McDonald, 2000). In the last years, due to the extensive use of the deca-BDE mixture, an increasing concentration of higher brominated congeners has been reported for abiotic and biotic matrices (Olofsson et al., 2012; Su et al., 2015; Verreault et al., 2018). In our study, juvenile Cuvier's beaked whales showed higher contributions of BDE-209, which is probably related to the heightened use of deca-BDE in the last years. The shift of distribution patterns towards higher concentrations of more brominated congeners has been reported in marine mammal populations already impacted by other anthropogenic threats (Jeong et al., 2020; Lebeuf et al., 2014; Rotander et al., 2012; Simond et al., 2017). In addition, levels above the existing threshold value established for marine mammals have been described during the last 30 years in different cetacean species (Alonso et al., 2014). Consequently, it is crucial to conduct toxicological investigations specifically addressed to the determination of species-specific thresholds, along with the assessment of various endpoints, which have already been established for other POPs such as PCBs and dioxins (Desforges et al., 2016; Kannan et al., 2000; Ross et al., 1995; Schwacke et al., 2012).

As previously mentioned, threshold values derived from toxicity tests are powerful tools to detect and quantify adverse properties of contaminants; yet, the lack of species-specific and congener-specific limits prevent today from making adequate risk assessments. However, the integration of various advanced omics-based techniques enhances our understanding of the effects of these contaminants. For instance, toxicogenomics is emerging as a crucial tool for understanding how POPs influence genetic responses in marine organisms. Multidisciplinary teams can employ toxicogenomic techniques to explore alterations in gene expression and pathways induced by POP exposure, providing valuable insights into the mechanisms of toxicity (Caballero-Gallardo et al., 2016; Gosse et al., 2008; Liang and Zha, 2016). Metabolomics and proteomics offer complementary perspectives by studying the metabolic and protein changes that can occur in response to POP exposure. These approaches can reveal subtle but critical effects on an organism's physiology that may not be evident through traditional toxicity assessments (Acosta-Tlapalamatl et al., 2022; Liu et al., 2022; Misra et al., 2019; Simond et al., 2022; Zhou et al., 2020). Furthermore, the study of biomarkers, as indicators of biological responses, can reveal the presence of specific POPs, assess oxidative stress levels and identify the activation of detoxification pathways triggered by contamination (Alves et al., 2022; Fossi et al., 2008; Fossi et al., 2014; Panti et al., 2022; Sarkar et al., 2006). Crucially, the integration of these complementary techniques through multidisciplinary studies empowers researchers to develop a holistic understanding of how POPs impact organisms at the molecular, cellular and population levels. This integrated approach could aid in the identification of vulnerable species, the prioritization of mitigation measures, and the formulation of strategies to minimize the detrimental consequences on both ecosystems and human health. Moreover, all this information can contribute to the establishment of regulatory guidelines and monitoring programs to ensure the long-term health and sustainability of marine ecosystems.

6.3. Implications for Human health

Exposure to POPs poses significant concerns for human health due to their potential adverse effects. These toxic compounds accumulated in the environment can enter the human body through various routes, including ingestion of contaminated food, inhalation of contaminated air, and absorption through the skin (Thakur and Pathania, 2020). POPs have been associated with a range of health risks in humans, including endocrine disruption (Ahmed et al., 2019; Damstra, 2002; Gregoraszczuk and Ptak, 2013), developmental abnormalities (Davidsen et al., 2021; Vuong et al., 2018), immune system impairment (Gascon et al., 2013; Kharrazian, 2021), and increased risk of certain cancers (Han et al., 2019; Hardell et al., 2006). For instance, exposure to PCBs has been linked to adverse neurodevelopmental effects such as autism in children (Berghuis and Roze, 2019; Panesar et al., 2020), while exposure to dioxins has been associated with reproductive disorders (Sofo et al., 2015). Additionally, PBDEs have been associated to developmental neurotoxicity (Costa et al., 2014).

It is well known that the main route of exposure to POPs in humans is dietary intake (more than 90%) (Nadal and Domingo, 2013; Negrete-Bolagay et al., 2021; Swackhamer et al., 2009), with fish consumption representing one of the major contributors (70-80%) for some populations (Kiviranta et al., 2004). To mitigate these risks, protect public health and ensure the safety of food consumption, public authorities have established legal limits for POPs in food. These limits, often referred to tolerable daily or weekly intake (TDI/TWI), vary among countries, and are established based on available scientific evidences and risk assessments conducted by international bodies and regulatory authorities. They represent the maximum amount of these substances that can be consumed daily or weekly over a lifetime without posing significant health risks to the general population.

Seafood and fish represent an important source of protein in several countries and play a crucial role in the local diet and culinary tradition in coastal regions such as those from the Mediterranean basin. According to the Food and Agriculture Organization of the United Nations (FAO), in 2020 fisheries and aquaculture production reached an all-time record of 214 million tones. Concurrently, a global fish consumption per capita of 20.2 kg was reported, which is more than double the consumption rate from 50 years ago (FAO, 2022). This figure has been steadily increasing over the years, demonstrating the importance of fish as a protein source in many regions. For example, in coastal nations such as Iceland, Japan, and Portugal, fish and seafood can make up a substantial portion of total protein consumption, accounting for over 20% of the animal protein intake (FAO, 2022). Portugal is the second highest consumer of fish in the European Union with 60.92 kg live-weight per capita consumed annually (Golden et al., 2022). On the other hand, the Mediterranean Sea had a lower annual mean consumption of 33.4 kg of fish per capita. Still, it reported a total fishery production in 2020 of 743,100 tons, with anchovy and sardine as the two most economically valuable commercial species (FAO, 2021).

For all these reasons, and given the increase of fish production and consumption in recent years, we decided to investigate the potential health risk for humans arising from the consumption of the targeted edible species (i.e. sardine, anchovy, bogue, and blue shark). Although blue shark liver is not commonly part of the human diet, shark meat is consumed in many parts of the world. In fact,, blue shark meat and fins contributed to most of the shark consumption worldwide with an estimated global catch of approximately 93,000 tons in 2020 (FAO Species Fact Sheets, 2021).

In this regard, as reported in the Results section (*4.3. Toxicity assessment*), we found that mean total TEQs and concentrations of the 6 indicator PCBs reported for sardines, bogues and anchovies did not exceed regulated levels in diet established by the European Union.

Taking into account the absence in our calculations of PCDD/F contributions to TEQs (that normally includes both PCDD/Fs and DL-PCBs), our results show that the consumption of these important commercial species might not pose a significant risk for human health derived from their DL-PCB content. Interestingly, the comparison of TEQ concentrations described in our study with those previously published for the same species in the Mediterranean Sea highlights a slow decline of TEQs in this area, since very little differences have been reported in the last 20 years (Table 39).

Although we described levels below the legal limit for the three species analyzed, we should emphasize that sardines reported the highest concentration of TEQs, from 5 to 3 times above those of anchovies and bogues. This means that a fish diet based on sardines could lead to a higher intake of POPs than a diet based on the other two species. This finding agrees with a study of Milićević et al. (2022) in which PCBs and organochlorine pesticides (OCPs) were more abundant in those species with the highest fat content such as sardines and garfish (*Belone belone*). According to the mentioned study, the assessment of daily intake values (DI) for organic compounds (OCPs and PCBs) revealed that anchovies and round sardinellas (*Sardinella aurita*) exhibited lower DI for both OCPs and PCBs. Interestingly, these two species also had the highest content of essential fatty acids (EFAs), further highlighting their nutritional value. In summary, anchovies and round sardinella emerged as favourable options for consumption due to their lower intake of harmful organic compounds and their rich EFA composition.

Table 39. Mean TEQ concentrations (pg/g w.w.) of DL-PCBs in sardines and anchovies from the Mediterranean Sea.

Reference (year)	Source	Sampling year	Species	pg TEQs/g w.w.
Llobet et al. (2003)	Spanish markets	2000	sardine	2.5
Gómara et al. (2005)	Spanish markets	1995-2003	sardine	1.91
Bocio et al. (2007)	Spanish markets	2005	sardine-anchovy	1.19-1.24
Miniero et al. (2014)	Southern Adriatic Sea Southern Tyrrhenian Sea Ionian Sea	2007-2008	sardine-anchovy	1.76-0.51

The three-targeted POP families reported values in muscle of blue sharks from one to two orders of magnitude lower than the established limits; therefore, the consumption of shark meat does not seem to pose significant risks for human health. On the other hand, when we analyzed reported values for liver the scenario was quite different. More than half of the analyzed individuals exceeded the threshold limits established at European level both for the six i-PCBs and for the sum of PCDD/Fs and DL-PCBs. Although shark liver is not typically included in human diet, the consumption of products derived from shark liver is not uncommon.

Concerning PBDEs (congeners BDE-28, -47, -99, -10, -153 and -154), all liver samples and 92% of muscle samples overpassed the EQS established by the European Water Framework Directive (WFD). To the best of our knowledge, a TWI for PBDEs has not yet been established. The only limits set to date are for congeners BDE-47, -99, -153, and -209, and refer to chronic oral reference doses (US Environmental protection Agency) and acute/subchronic minimal risk levels (US Agency for Toxic Substances and Disease Registry). As blue sharks analyzed in this study reported concentrations levels below the two limits mentioned above, consumption of this species does not seem to represent a risk for human health.

It is corroborated that the main PBDE congeners in humans are BDE-47, -99 and -153 (Wu et al., 2020); therefore, toxicity limits based on these congeners are a convenient way to obtain a quick and representative picture of the toxicological risk arising from the presence of these contaminants in humans. On the other hand, the shift of PBDE congener profiles to major contributions of higher brominated congeners, reported in different biotic and abiotic matrices in the last years (Olofsson et al., 2012; Su et al., 2015; Verreault et al., 2018), could lead to a change of congener patterns in humans. For instance, in China, a study conducted by Shi et al. (2018) on pooled human milk reported a reduction of BDE-47, -99, -100, and -153, while BDE-183 significantly increased from 2007 to 2011. This is likely due to the increasing consumption of products containing the technical mixture octa-BDE (13-42% BDE183) during that period. Even though specific studies on the toxic effects of BDE-183 in humans are required, it has been demonstrated in oligochaete that the toxicity of BDE-183 was greater than that of BDE-47 (Chiu et al., 2012). Additionally, a study by Louis et al. (2013) on couple fecundity in humans reported that male partners of couples who did not achieve pregnancy had higher concentrations of BDE-183 compared to those who were successful in achieving pregnancy.

More comprehensive and specific PBDE threshold limits should be established in order to better assessing the risk associated with the presence of these substances in human tissues. The use of different mixtures (Al-Omran and Harrad, 2016), as well as distinct capacity of long-range transport, affect the concentrations and profiles of these contaminants both in humans and the environment (Ueno et al., 2004). At the same time, factors such as proximity to pollution sources (urban center, industrial activities, areas with manufacturing or electronic facilities), geographic differences in diet or cultural practices, as well as climate and weather patterns could influence the accumulation of these substances. In that context, depending solely on existing PBDE limits for humans such as the chronic oral reference doses set by US Environmental Protection Agency or the acute/subchronic minimal risk levels defined by the US Agency for Toxic Substances and Disease Registry, may not adequately safeguard certain population groups.

7. Conclusions

This doctoral thesis has shed light on several key aspects of the presence of POPs in a selection of marine species. The general conclusion of the thesis emphasizes the pivotal role played by sperm whale, Cuvier's beaked whales, blue sharks, and three fish species as bioindicators of POP pollution in the the study areas. These findings specifically underscore the Mediterranean Sea as a contamination hotspot for marine mammals and draw attention to the elevated levels, occasionally indicating an upward trend, in the northeastern region of the Atlantic Ocean.

The specific conclusions of the different studies carried out are detailed below:

- Levels of POPs found in Mediterranean sperm whales tended to be higher than those reported in the same species worldwide. All sperm whales analyzed surpassed the threshold for WHO-TEQs proposed as starting point of immunosuppression in marine mammals (harbour seals).

- POP levels described in Mediterranean Cuvier's beaked whales tended to exceed those observed in the same species globally. At the same time, stable or increased POP levels with age in males and decline in sexually mature females Cuvier's beaked whale confirmed offspring transfer through gestation and lactation. Ultimately, 80% of Cuvier's beaked whales were above the threshold for PCBs responsible for physiological effects.

- Levels of PCBs and PCDD/Fs in blue shark showed relatively steady concentrations, whereas greater loads of PBDEs were detected when comparing with previous studies for the same area (North East Atlantic Ocean). This could be the consequence of the recent and incomplete regulations on PBDEs as well as contamination deriving from plastics and microplastics. PCB and PBDE concentrations in blue shark were below the lowest thresholds identified in marine mammals. PCB and PCDD/F levels detected in blue shark muscle (the most consumed part) were within the acceptable European legal limits; however, the majority of blue shark liver samples exceeded the limits for i-PCBs and WHO-TEQs, which becomes significant when considering the potential implications for the consumption of products derived from shark liver oil. Greater accumulation of PCBs, PBDEs, and PCDD/Fs observed in liver compared to muscle, suggested differences in the toxicokinetics of POPs between the two tissues investigated in blue shark.

- PCB levels found in edible fish species (sardines, anchovies and bogues) were generally not different from values reported for the same species and in the same area in recent years. This underpinned the concept of a virtual halt or very slow decline in PCB concentrations in the Mediterranean Sea. Sardines, anchovies and bogues showed concentrations of PCBs in full compliance with European regulated concentrations in diet; consequently, the PCB content of these edible species seemed not to constitute a current hazard for human health.

Monitoring of legacy and emerging POPs is crucial to understand the trends of POPs and to evaluate the efficacy of the reduction and elimination measures implemented so far. By concurrently exploring toxicity thresholds, we were able to gain insights into the potential risks and adverse effects linked to the observed levels in the species under study. It is clear that gaining deeper insights into the impact of pollution on the health of wildlife, particularly those facing multiple anthropogenic stressors, such as the relatively understudied Cuvier's beak whale, is essential.
Additionally, continuous and effective monitoring of POPs in edible fish species is essential for the assessment of risk associated with the consumption of POP-contaminated fish. Toxicity risk assessment represents a crucial tool for regulatory authorities when establishing guidelines and limits for POPs in food to ensure food safety and protect public health.

Additionally, our study highlights the significance of exploring species-specific behaviors in the context of pollutant exposure. The variability observed in data from different species underscore the necessity for further investigation.

As an overarching conclusion, these interconnected findings justify the ongoing concern about POPs and stress the urgency of addressing chemical pollution as a significant threat to marine species. This, in turn, emphasizes the critical importance of continuous research and monitoring of POPs, to support conservation initiatives aimed to safeguard our oceans as essential components of global health.

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9. Annexes

9.1. Scientific publications

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Global PBDE contamination in cetaceans. A critical review☆

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ABSTRACT

This review summarizes the most relevant information on PBDEs' occurrence and their impacts in cetaceans at global scale, with special attention on the species with the highest reported levels and therefore the most potentially impacted by the current and continuous release of these substances. This review also emphasizes the anthropogenic and environmental factors that could increase concentrations and associated risks for these species in the next future. High PBDE concentrations above the toxicity threshold and stationary trends have been related to continuous import of PBDE-containing products in cetaceans of Brazil and Australia, where PBDEs have never been produced. Non-decreasing levels documented in cetaceans from the Northwest Pacific Ocean might be linked to the increased e-waste import and ongoing production and use of deca-BDE that is still allowed in China. Moreover, high levels of PBDEs in some endangered species such as beluga whales (*Delphinapterus leucas*) in St. Lawrence Estuary and Southern Resident killer whales (*Orcinus Orca*) are influenced by the discharge of contaminated waters deriving from wastewater treatment plants.

Climate change related processes such as enhanced long-range transport, re-emissions from secondary sources and shifts in migration habits could lead to greater exposure and accumulation of PBDEs in cetaceans, above all in those species living in the Arctic. In addition, increased rainfall could carry greater amount of contaminants to the marine environment, thereby, enhancing the exposure and accumulation especially for coastal species.

Synergic effects of all these factors and ongoing emissions of PBDEs, expected to continue at least until 2050, could increase the degree of exposure and menace for cetacean populations. In this regard, it is necessary to improve current regulations on PBDEs and broader the knowledge about their toxicological effects, in order to assess health risks and support regulatory protection for cetacean species.

1. Introduction

In recent years there has been growing concern pertaining the stable and high levels of some legacy persistent organic pollutants (POPs) continuously reported in the environment (Jepson et al., 2016; Jones, 2021; Stuart-Smith and Jepson, 2017). At the same time, recent studies have drawn attention to the impact that climate change could have on POPs' cycling and distribution and consequently on exposure and accumulation in living organisms (Borgå et al., 2022; Hung et al., 2022; Nadal et al., 2015). Evidence suggested that climate change-driven processes could enhance long-range transport of POPs (Dalla Valle et al., 2007; Li et al., 2021) and their remobilization from secondary sources, especially in the Arctic zone (Borgå et al., 2022; Chen et al., 2019; Ma et al., 2011; Potapowicz et al., 2019). Undoubtedly, this could translate into enhanced POP exposure and accumulation in cetaceans living in this area. Additionally, some cetacean species inhabiting industrialized regions such as killer whales (*Orcinus orca*) are already facing important anthropogenic pressures and ultimately population depletion, which has been linked to their high body burdens of POPs (Desforges et al., 2018; McGuire et al., 2020).

In this regard, two individual-based models have described the possible negative influence of polychlorinated biphenyls (PCBs) and structurally similar compounds on the potential annual growth rate in the bottlenose dolphin (*Tursiops truncatus*) population of Sarasota Bay (Hall et al., 2006), and on the long-term viability of the world's killer whale population (Desforges et al., 2018). Despite the evidence of the detrimental impact of POPs on marine mammals' health, a comprehensive understanding of toxicological consequences has not yet been

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achieved. In consequence, species-specific threshold values are not well established.

High and stable concentrations, enhanced exposure due to climate change processes and population collapse related to high body burdens for some cetacean species, have led to questioning about the effectiveness of current global efforts aimed at the mitigation and elimination of POPs (Stuart-Smith and Jepson, 2017; Wang et al., 2021; García-Alvarez et al., 2014). Thus, revision of existing multilateral environmental agreements (MEAs), implementation of national legislations and more holistic approaches have become necessary. Special attention should be given to polybrominated diphenyl ethers (PBDEs) for which emissions from in use and waste stocks are expected to continue until 2050 (Abbasi et al., 2019). It is plausible that prohibitions of production and use are not steadily lowering the long-term environmental impact of PBDEs, but shifting instead the issue from more to less industrialized regions where the demand of PBDE-containing products and e-waste import have increased since the late 1990s (Li et al., 2016).

PBDEs are a family of brominated-flame retardants (BFRs) comprising 209 congeners that are divided into 10 groups, from mono-to deca-BDE, according to their different degree of halogenation. The estimated global production from 1970 to 2005 was around 1.3 and 1.5 million of tons (UNEP, 2010), comparable to that of PCBs (Breivik et al., 2002). United States and Israel were the two main manufacturers prior to 1995, while China started its production in the 1980s, principally focused on deca-BDE, and became one of the main producers 20 years later (Abbasi et al., 2019). PBDEs have been commercialized as three technical mixtures, i.e., penta-, octa-, and deca-brominated diphenyl ether, named so after the major congeners present in each mixture. They were primarily used as flame retardants in polyurethane (PUR) foams, computers, housing appliances, electrical and electronic products.

By the beginning of the 21st century, environmental authorities (e.g., the European REACH regulation; the Stockholm Convention; the United States Environmental Protection Agency; Environment and Climate Change Canada) became aware of the harmful effects of PBDEs based on the available scientific evidence, and nations started to take measures. In 2004 the United States and Europe voluntarily phased out the production of penta- and octa-BDE mixtures (Hites, 2006), while in 2009 they were globally regulated with their introduction in the annex A (elimination) of the Stockholm Convention (UNEP, 2009). Accordingly, most reported PBDEs started to decline or level off in the early 2000s in environmental media around the world (Law et al., 2014), with notorious exceptions such as some top predators in Northern Europe (McKinney et al., 2006; Vorkamp et al., 2005) and beluga whales from the Gulf of St. Lawrence (Canada) (Raach et al., 2011) and Alaska (Hoguet et al., 2013). Importantly, the ban on these two mixtures lead to an increased production of deca-BDE formulation, made up of 96.8% BDE-209, until its more recent inclusion in the annex A of the Stockholm Convention in 2019 (UNEP, 2022a). The extensive use of deca-BDE shifted the environmental congener pattern distribution of PBDEs with increasing levels of higher brominated congeners in different abiotic and biotic matrices (Olofsson et al., 2012; Su et al., 2015; Verreault et al., 2018). Thus, Olofsson et al. (2012) reported decreasing concentrations (ca. 20% each year) of BDE-154 and BDE-183 (representative of penta-mix and octa-mix, respectively) in sewage sludge from Sweden during 2004–2011, while BDE-209 increased (ca. 16%) over the same period. Heightened levels of deca-BDE were observed in herring gulls (*Larus argentatus*) from the Laurentian Great Lakes (Su et al., 2015) and top predator birds from the Arctic (Verreault et al., 2018), sampled during 2012–2013. Even if BDE-209 is highly hydrophobic and has low water solubility, making it hardly bioavailable, evidence suggests that its biomagnification may take place in aquatic food webs (Castro-Jiménez et al., 2021; Johnson-Restrepo et al., 2005). In addition, its affinity to abiotic matrices such as sediments generates a large contaminant reservoir that put at risk low-trophic level species and their feeders. Toxicological relevance of higher brominated BDEs has not yet been fully investigated, but their debromination to lighter, more toxic and bioaccumulative congeners have been demonstrated (Eriksson et al., 2004; La Guardia et al., 2007; Rayne et al., 2003; Zhu et al., 2019). This fact is worthy of attention due to the large historical production of deca-BDE estimated to be almost 10 times higher than those of penta- and octa-BDE mixtures (Abbasi et al., 2019).

Even though PBDEs are mostly prohibited today on a global scale, their production and use is still ongoing in some world areas (Sharkey et al., 2020). Several countries have not yet ratified some amendments of the Stockholm Convention and in many cases also lack national regulations for both production and use. For example, in the United States only 13 states have applied limitations on the use/presence of PBDEs in certain goods entering the market, but no federal restrictions are in place. Other examples are those of Brazil and Indonesia where there are no regulations or limits defined for marketable goods or wastes (Sharkey et al., 2020). China has set concentration limits in newly manufactured goods for penta- and octa-BDE, but not for deca-BDE (Sharkey et al., 2020). Moreover, specific exemptions under the Stockholm Convention permit the use of the three commercial BDE mixtures for certain specific applications (UNEP, 2022b; 2022a). For instance, deca-BDE counts on specific exemptions allowing its production up to 2036 in the European Union, Republic of Korea and Switzerland (Sharkey et al., 2020).

Today, in use PBDE-containing products and waste stocks, accentuated by improper disposal in both developed and developing countries, represent important sources of PBDE contamination (Zhang et al., 2021). Due to the lack of chemical bonds and susceptibility to physical, chemical and biological factors, PBDEs can be easily released during waste disposal and therefore contaminate the surrounding environment or reach water masses through leaching and runoff (Danon-Schaffer et al., 2013; Zhang et al., 2021). Additionally, the high temperatures (80°C–90 °C) that can be reached in landfills enhance volatilization of lower brominated congeners (Stubbings and Harrad, 2014). Once in the atmosphere and thanks to their ability for long-range transport, these substances can reach remote areas where they have never been produced (Stubbings and Harrad, 2014; Wang et al., 2017; Zhang et al., 2021). Although recycling infrastructures have been implemented in many countries, older landfills without proper engineered facilities continue to be in use in Europe and United States (Propp et al., 2021), being likely responsible for inadvertent release of PBDEs into the environment (Ohajinwa et al., 2019).

As reported by Abbasi et al. (2019), emissions of PBDEs will continue for the next 30 years. This means that without appropriate control an additional enormous amount of BDE-209 (40 kilotons) may be subject to improper disposal. On top of that, higher temperatures and increased rainfall-runoff expected over the next few years as consequences of global warming could enhance PBDE volatilization and transportation from landfills and dumpsites. These all are likely to increase the amount of these semi-volatile contaminants reaching remote areas and entering the marine environment (Nadal et al., 2015).

Indeed, PBDEs have been detected in various environmental matrices for the last 40 years and since 10 years ago they have been recognized as ubiquitous contaminants (Abbasi et al., 2019). These substances enter the coastal waters through municipal and industrial wastewater outfalls, landfill leachates and atmospheric deposition (De Wit, 2002). Once in the marine environment, thanks to their hydrophobicity and persistency, PBDEs accumulate in biological tissues and biomagnify along the food web (Aznar-Alemany et al., 2019; De Wit, 2002; Noël et al., 2009). Species with large lipid reservoirs, high trophic levels and long-life spans, such as those in the cetacean suborder of odontocetes, tend to accumulate great concentrations of these substances and thus are regarded as bioindicators of POP contamination (Tanabe and Ramu, 2012). In addition, their reduced metabolic ability to eliminate POPs in comparison with carnivorous predators (polar bears, seals and walruses) make toothed whales especially vulnerable to this pollution (McKinney et al., 2011; Sonne et al., 2018). Even if POPs accumulate to a lesser extent in mysticetes, mobilization and redistribution of sequestered contaminants resulting from fasting and associated lipid fluctuations seem to cause a "reexposure" of target tissues (Bengtson Nash, 2018) increasing the likelihood and/or intensity of toxic effects (Aguilar et al., 1999; Bossart, 2011) in this suborder as well.

It has been demonstrated that upon continue exposure, marine mammals could reach potential toxic levels of PBDEs in their tissues. In the last 30 years many studies have reported PBDE levels in species around the world surpassing the threshold value for alteration in thyroid hormone levels in blubber of grey seals (Alonso et al., 2014). Moreover, stable or increasing concentrations of higher brominated congeners have been reported for different species and areas, in some cases in populations already impacted by other anthropogenic threats (Lebeuf et al., 2014; Simond et al., 2017; Jeong et al., 2020; Rotander et al., 2012).

Unlike others contaminants such as PCBs, PBDE effects in marine mammals have not been deeply investigated so far. In spite of the limited information available in literature in this regard, however, PBDEs in marine mammals seem to be associated with different harmful health effects. Particularly, they are responsible for toxicity responses involving the endocrine and immune systems (Simond et al., 2019; Beineke et al., 2005; Huang et al., 2020) as well as for cytotoxicity and genotoxicity to some extent (Rajput et al., 2021).

This review aims to compile the most relevant information on PBDE occurrence and associated health effects in cetaceans. A major objective would be drawing attention on the anthropogenic and environmental factors that could increase PBDE concentrations and associated risks in the next future. The discussion of PBDE levels and trends will be focused on the species with the highest reported levels and therefore the most potentially impacted by the current and continuous release of these substances.

The studies analyzed in this article are performed in blubber or liver of worldwide cetaceans covering a temporal range of about 30 years, since 1992 to 2021. Data are discussed when possible as mean concentration and range, and expressed as ng/g on lipid weight (l.w.) basis. All species investigated and discussed in this work are listed in Fig. 1.

1.1. *Three decades of PBDEs in cetaceans: highest levels reported to date and trends*

Despite their prohibition dating back more than 10 years ago, penta-BDE and octa-BDE congeners are still globally reported in marine mammals (Alonso et al., 2014; Aznar-Alemany et al., 2021; Kratofil et al., 2020; Simond et al., 2017). This can easily be linked to different factors, e.g. the persistence of these substances in the environment, the in use PBDE-containing products, the still ongoing production of these flame retardants in some geographical areas as well as the degradation of higher brominated (mainly deca-BDE) to lighter, bioavailable and more toxic congeners.

Kuehl et al. (1991) published the first study on PBDEs in cetaceans in 1991 reporting a mean value of 200 ng/g l.w. of total tetra-to hexa-BDEs in blubber of bottlenose dolphins collected during 1987–1988 along the Atlantic coast of the U.S. Since then, several studies have been conducted on different tissues and species, most of them focused on blubber of odontocetes inhabiting the Northern Hemisphere (Alonso et al., 2014) and reporting levels one order of magnitude higher than those initially documented in 1991 (Boer et al., 1998; Fair et al., 2007; Lindström et al., 1999; Moon et al., 2010). Values between 1060 and 7900 ng/g l.w., have been reported in more recent years (Kratofil et al., 2020; Noël et al., 2018; Simond et al., 2017) when, *a priori*, it would be expected a greater decrease as a consequence of the ban on production and use of penta- and octa-BDE. The only known threshold value for PBDEs is that of 1500 ng/g l.w., associated to the concentration in blubber of grey seals (*Halichoerus grypus*) eliciting alterations in thyroid hormone levels (Hall et al., 2003). Several studies have documented levels of PBDEs above this threshold (Boer et al., 1998; Fair et al., 2007; Kratofil et al., 2020; Lam et al., 2009; Lindström et al., 1999; Moon et al., 2010; Tuerk et al., 2004), pointing out the hazard these substances pose to cetacean health. Interestingly, some cetacean populations showing the highest concentrations of PBDEs are facing population depletion and lack of recovery. Most of them are classified as endangered species (Kratofil et al., 2020; Noël et al., 2018; Simond et al., 2017), which is likely consequence of

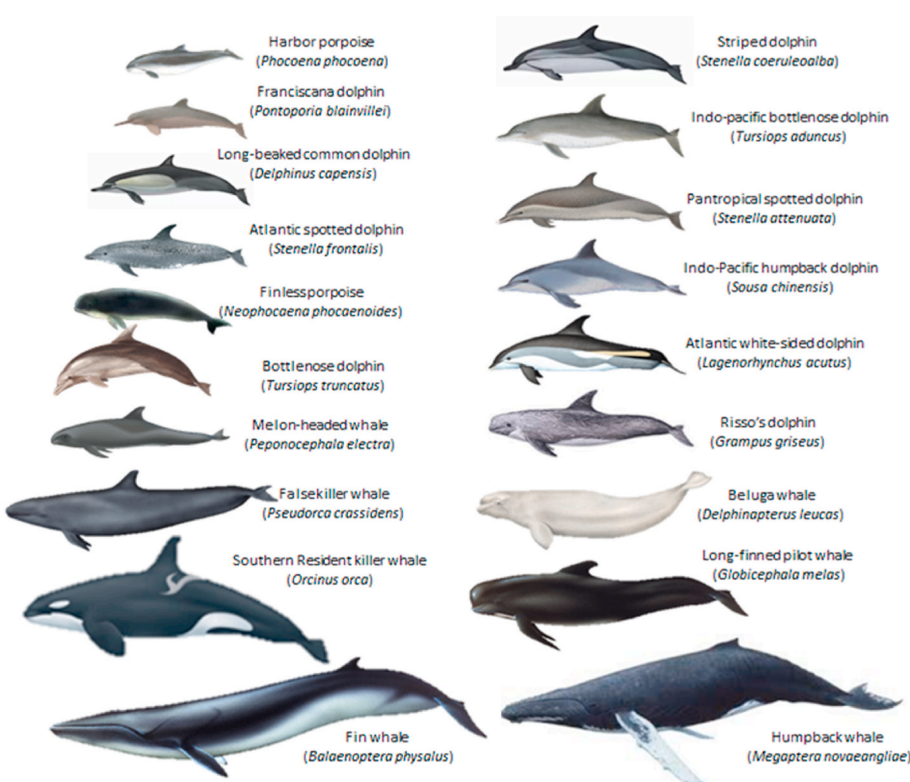

Fig. 1. Cetacean species investigated in this review.

the synergic effects of multiple anthropogenic threats including chemical pollution. Cetacean species living in urbanized coastal regions also deserve special attention due to the constant release of PBDEs in these areas and the subsequent high degree of contamination in their habitats.

To date, the highest concentrations of PBDEs in cetaceans' blubber have been reported for Southern Resident killer whales from Canada (mean: 7900 ng/g l.w.) (Noël et al., 2018), Main Hawaiian Island false killer whales (*Pseudorca crassidens*) (mean: 2900 ng/g l.w.) (Kratofil et al., 2020), Atlantic white-side dolphins (*Leucopleurus acutus*) in the North Sea (mean 7777 ng/g l.w.) (Boer et al., 1998), Indo-pacific humpback dolphin (*Sousa chinensis*) from Hong Kong waters (mean: 3590 ng/g l.w; range: 280-51,100 ng/g l.w.) (Lam et al., 2009) and bottlenose dolphins in the Northwestern Atlantic Ocean (mean: 7850 ng/g l.w.; range: 2680–22,800 ng/g l.w.) (Fair et al., 2007) (Fig. 2). High concentrations of PBDEs, often surpassing the threshold mentioned previously, have also been reported for long-finned pilot whales (*Globicephala melas*) from Faroe Islands (NW Atlantic) (mean: 1939 ng/g l.w.) (Lindström et al., 1999), Atlantic white-sided dolphins of Massachusetts coast (NW Atlantic) (mean: 1607 ng/g l.w.) (Tuerk et al., 2004), long-beaked common dolphins (*Delphinus capensis*) from Korea (NW Pacific) (mean: 1650 ng/g l.w.; range: 140-3100 ng/g l.w.) (Moon et al., 2010) and beluga whales from St. Lawrence Estuary (mean: 1068 ng/g l.w.; range: 666-1287 ng/g l.w) (Simond et al., 2017) (Fig. 2). These high values are to be linked to feeding habits and longer stays in urbanized and industrialized areas such as the California coastline – a feeding area for killer whales (Hanson et al., 2021) - and St. Lawrence Estuary where belugas live.

Different studies revealed the presence of PBDEs in Pacific salmon (*Oncorhynchus* spp) (O'Neill and West, 2009; Shaw et al., 2008; Veldhoen et al., 2010) with higher levels in coastal species, such as Chinook salmon (*Oncorhynchus tshawytscha*) the main prey of southern resident killer whale, than in those species with more oceanic distributions (O'Neill et al., 2006). As reported by the authors, this fact could contribute to the higher PBDE levels found in Southern Resident killer whales in comparison to those reported for other killer whale populations.

PBDE burdens in California's coastline have been found in sediments (Dodder et al., 2012) and wildlife, such as seabirds (Clatterbuck et al., 2018), fish (Maruya et al., 2016) and marine mammals (Shaul et al., 2015; Cossaboon et al., 2019), principally deriving from wastewater and stormwater discharges (Kimbrough, 2009). At the same time, different studies reported the presence of PBDEs in seal species from the Estuary and Gulf of St. Lawrence (Frouin et al., 2011; Soulen et al., 2018) and in fish and invertebrates from the St. Lawrence River, heavily impacted by discharge of treated wastewater, with the Montreal effluent being the most important PBDE source (Marcogliese et al., 2015).

Additionally, important concentrations of PBDEs in Main Hawaiian Island false killer whales have been related to the discharge of contaminated wastewater effluents in the coastal waters of Hawaii (Ylitalo et al., 2009). It is noteworthy that Canadian Southern Resident killer whale and Main Hawaiian Island false killer whale populations are experiencing a population depletion and lack of recovery due to multiple anthropogenic threats, among which chemical contamination may be playing an important role (Bradford et al., 2020; Desforges et al., 2018; Foltz et al., 2014; Hall et al., 2018). Hence, Southern resident killer whales and St. Lawrence beluga whales have been classified as endangered in the United States (Department of commerce. National Oceanic and Atmospheric, 2005) and Canada (SARA, 2017). The Main Hawaiian Island false killer whale stock was listed as endangered under the U.S. Endangered Species Act (ESA) in 2012 as a consequence of a rapid population decline during 1990s (GovInfo Endangered and Threatened Wildlife and Plants, 2012). Thus, the last census counted only 167 individuals, three time less than a previous estimation from 1998 (Bradford et al., 2018). Among the various causes, such as bycatch, decreased prey biomass and size and reduced genetic diversity, exposure to POPs and its related adverse health effects may contribute to the reduction of this population (Foltz et al., 2014; Kratofil et al., 2020; Ylitalo et al., 2009).

Among mysticetes, humpback whales (*Megaptera novaeangliae*) from the Gulf of Maine seem to be the most PBDE contaminated species, with a mean level around 900 ng/g l.w. in specimens sampled during 2005–2006 (Elfes et al., 2010). As suggested by the authors, this is likely a consequence of the great industrialization and human population density of the east coast of the United States.

Liver is the second most investigated tissue for PBDE contamination in cetaceans. PBDE levels in liver at which biological responses or toxicity effects occur have not yet been elucidated. Bearing that in mind, in this work we have also adopted as proxy for a toxicity reference the

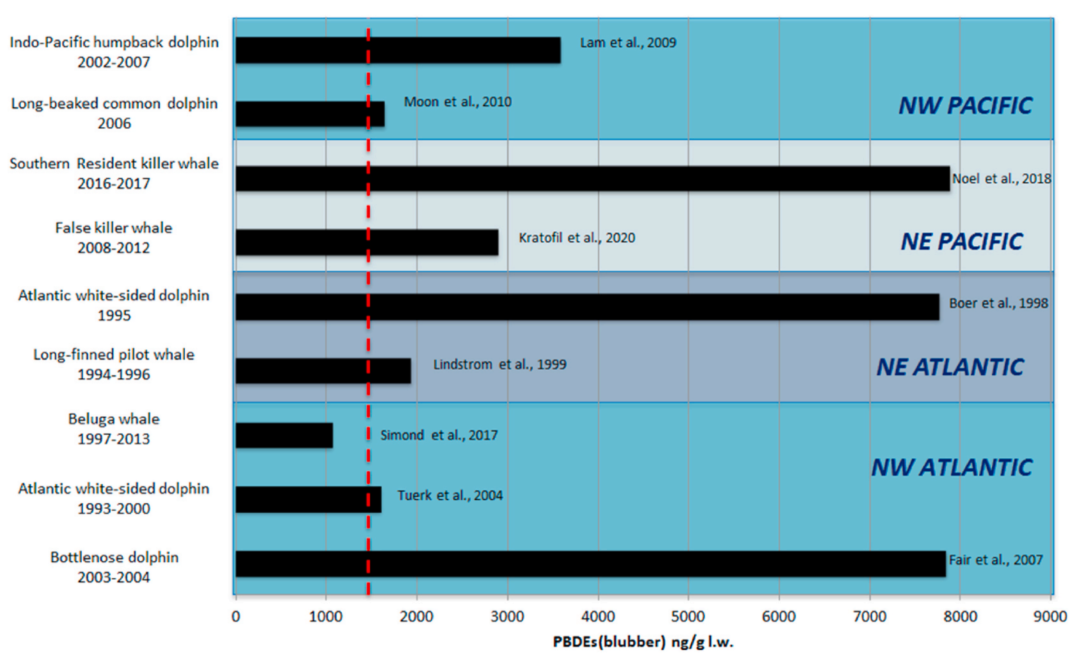

Fig. 2. PBDE highest mean concentrations (ng/g l.w.) in cetaceans' blubber around the world. Dotted line represents the threshold value for alteration of thyroid hormone levels in grey seals (Hall et al., 2003).

threshold established for alteration of thyroid hormone levels in grey seals (Hall et al., 2003).

Notable concentrations surpassing this threshold value of 1500 ng/g l.w., have been reported in liver of striped dolphin (*Stenella coeruleoalba*) (mean: 3624 ng/g l.w.; range: 129-8133 ng/g l.w.) and risso's dolphin (*Grampus griseus*) (mean: 2564 ng/g l.w.; range: 1778–2998 ng/g l.w.) from the Mediterranean Sea (Pettersson et al., 2004), and beluga whale in St. Lawrence Estuary (mean: 2210 ng/g l.w.; range: 246-3030 ng/g l.w.) (McKinney et al., 2006) (Fig. 3). Significant values of PBDEs have been also found in cetaceans inhabiting the highly industrialized and urbanized south-eastern coast of Brazil that represents a feeding and breeding ground for several coastal species such as the Guiana dolphin (*Sotalia guianensis*) (Ribeiro-Campos et al., 2021) and the highly threatened franciscana dolphin (*Pontoporia blainvillei*) (Secchi et al., 2021). Dorneles et al. (2010) found mean PBDE levels in liver of Atlantic spotted dolphins (*Stenella frontalis*), pantropical spotted dolphins (*Stenella attenuata*) and false killer whales of 1150 ng/g l.w., 1215 ng/g l.w. and 3600 ng/g l.w., respectively, which are in the same order of magnitude as those in Mediterranean dolphins (Pettersson et al., 2004) and Canadian Belugas (McKinney et al., 2006) (Fig. 3), dwellers of two well-known contaminated areas. This is in line with the results from a recent study performed in four distinct groups of Atlantic spotted dolphins, stranded between 2005 and 2015 in the Canary Islands, Azores, Caribbean Sea and São Paulo (Méndez-Fernandez et al., 2018). In this study among the four different populations, the highest concentrations of PBDEs in blubber were reported in specimens stranded along the coast of Brazil.

As shown in Fig. 4 the highest PBDE levels have been described for species living in the North Hemisphere and in many cases inhabiting or frequenting recognized contaminated areas such as the Mediterranean Sea, the North American East Coast or the North West Pacific zone. Cetacean species living in Southern Hemisphere waters, on the other hand, appear to be less investigated. The highest PBDE loads in that case have been reported for species inhabiting the Eastern coast of Brazil and Australia, but basically no information exists for the African Coast or the Indian Ocean.

In general, PBDE temporal trends in cetaceans showed remarkable increased concentrations from the late 1980s and earlier 2000s (Hoguet et al., 2013; Kajiwara et al., 2008, 2006; Lebeuf et al., 2004; Leonel et al., 2014; Ramu et al., 2006; Rotander et al., 2012) followed by a slight decrease or level off in subsequent years (Isobe et al., 2011, 2009; Jeong et al., 2020; Kunisue et al., 2021; Rotander et al., 2012; Simond et al., 2017). At the same time, an increased proportion of higher brominated compounds, such as BDE-154 and BDE-153, have been documented in more recent years (Jeong et al., 2020; Kunisue et al., 2021). This tendency probably reflects the regulatory measures implemented in 2009 for penta- and octa-BDE that lead to increased use and production of deca-BDE, only banned in 2019 (UNEP, 2022a, 2009). All of this translated into ongoing inputs of deca-BDE in the environment and a greater bioavailability of higher brominated congeners deriving from debromination processes of BDE-209 (La Guardia et al., 2007; Zhu et al., 2019).

As shown in Table 1, upward trends prior to 1990s and earlier 2000s have been reported in the Northwest Pacific Ocean in finless porpoises (*Neophocaena phocaenoides*) (South China) and melon headed-whales (*Peponocephala electra*) (Japan) (Kajiwara et al., 2008, 2006; Ramu et al., 2006); in the Northeast Pacific and Northwest Atlantic Ocean in beluga whales (Canada, Alaska) (Hoguet et al., 2013; Lebeuf et al., 2004); in the Northeast Atlantic Ocean in long-finned pilot whales (Faroe Islands), white-sided dolphins (Faroe Islands) and fin whales (*Balenoptera physalus*) (Iceland) (Rotander et al., 2012); in the Southwest Atlantic Ocean in franciscana dolphins (*Pontoporia blainvillei*) (Brazil) (Leonel et al., 2014); in Indo-Pacific bottlenose dolphin from South Australia (Weijs et al., 2020). On the other hand, only one study showed clearly downward trends during the 1990s and 2018s in Mediterranean striped dolphins (Aznar-Alemany et al., 2021), whereas Law et al. (2010) reported a decreased trend in harbor porpoises (*Phocoena phocoena*) from United Kingdom between 1998 and 2008. It is worth noting that the majority of these studies did not include BDE-209. This is, however, the dominant component of the deca-BDE mixture and in some cases one of the main BDE congeners of total PBDE contents (Koenig et al., 2013; Muñoz-Arnanz et al., 2011). As mentioned before, while lower brominated congeners such as those predominantly present in the commercial penta-BDE mixture or deriving from debromination processes are decreasing in some cetacean species (Aznar-Alemany et al., 2021; Rotander et al., 2012), increasing concentrations of higher brominated congeners have been reported for different species and areas (Jeong et al., 2020; Kunisue et al., 2021).

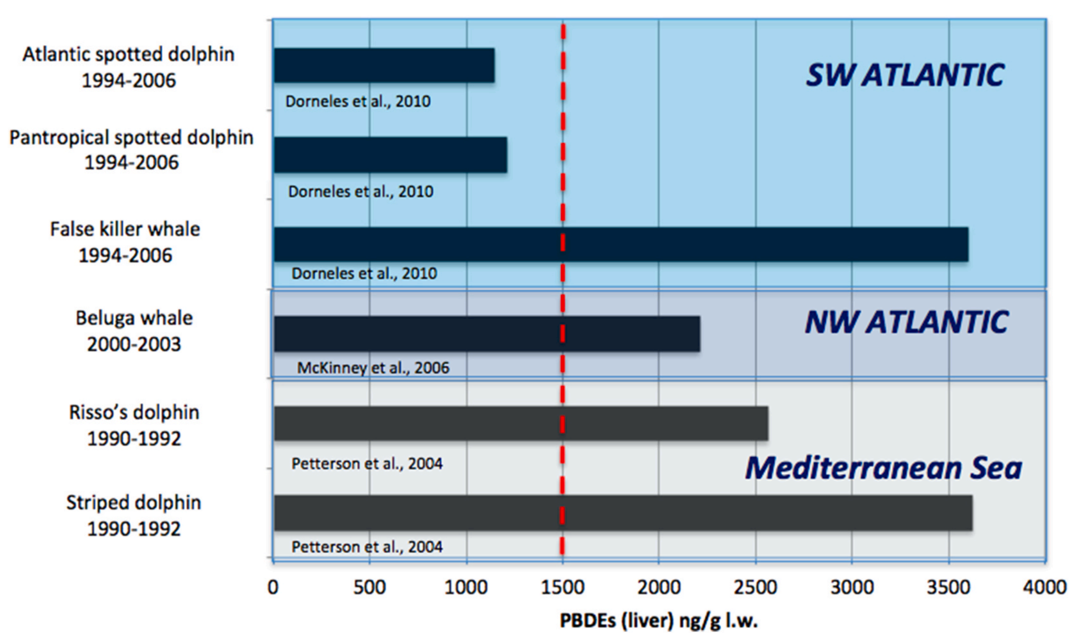

Fig. 3. PBDE highest mean concentrations (ng/g l.w.) in cetaceans' liver around the world. Dotted line represents the threshold value for alteration of thyroid hormone levels in grey seals (Hall et al., 2003).

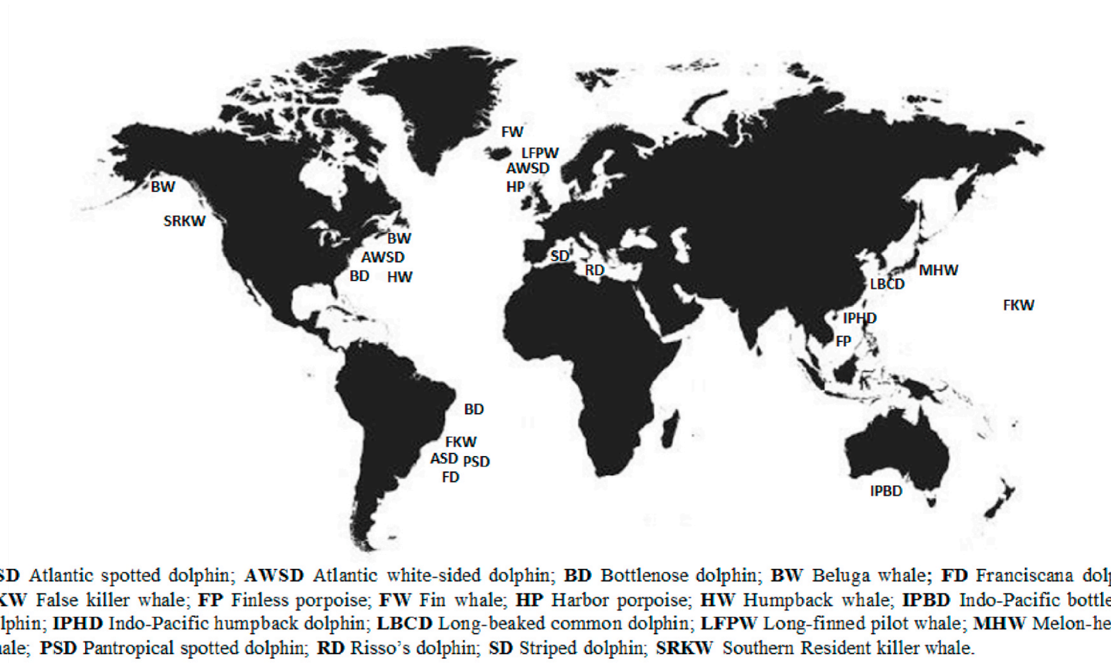

Fig. 4. Map showing locations of cetacean species analyzed in the studies discussed in this review.

Table 1
Most relevant PBDE trends in cetaceans worldwide, according to the levels found in blubber or *liver samples.

Species	Locations	Sampling years	Trends	References	
Finless porpoise	South China	1989–2006	↗ increased	Kajiwara et al., 2006[a]; Ramu et al., 2006[b]	NW Pacific
Finless porpoise	Korea	2003–2010	↘ decreased 2003–2010 ↔ stable 2010–2015	Jeong et al., 2020[c]	NW Pacific
Melon headed-whale	Japan	1982–2015	↗ increased 1982–2011 ↔ stable 2001–2015	Kunisue et al. (2021)[d]	NW Pacific
Melon headed-whale	Japan	1982, 2001, 2002, 2006	↗ increased	Kajiwara et al. (2008)[e]	NW Pacific
Beluga whale	Alaska	1989–2006	↗ increased	Hoguet et al. (2013)[f]	NE Pacific
Beluga whale	St. Lawrence Bay	1987–2013	↗ increased 1987–1997 ↔ stable 1997–2013	Lebeuf et al. (2014)[g]; Simond et al. (2017)[h]	NW Atlantic
Fin whale	Iceland	1986–1989, 2006, 2009	↗ increased	Rotander et al. (2012)[i]	NE Atlantic
Long-finned pilot whale	Faroe Islands	1986, 1997, 2006, 2007	↗ increased	Rotander et al. (2012)[i]	NE Atlantic
White-sided dolphin	Faroe Islands	1997, 2001, 2006	↗ increased 1997–2001/2002 ↘ decreased 2001/2002-2006	Rotander et al. (2012)[i]	NE Atlantic
Harbor porpoise	United Kingdom	1992–2008	↗ peakead around 1998 ↘ decreased until 2008	Law et al. (2010)[j]	NE Atlantic
Franciscana dolphin	Brazil	1994, 1996, 1998, 2000, 2002, 2004	↗ increased	Leonel et al. (2014)[k]	SW Atlantic
Bottlenose dolphin*	Brazil	1994–2012	↔ stable	Dorneles et al. (2010)[l]; Lavandier et al. (2019)[m]	SW Atlantic
Indo-Pacific bottlenose dolphin	South Australia	1989–2014	↗ increased 1989–2005 ↗ increased 2009–2014	Weijs et al. (2020)[n]	SE Indian
Stripped dolphin	Catalan coast	1990, 2004–2009, 2014–2018	↘ decreased trend	Aznar-Alemany et al. (2021)[o]	Mediterranean Sea

PBDE congeners investigated in each study.
[a,b,e] n. 3, 15, 28, 47, 99, 100, 153, 154, 183, 209.
[c] n. 17, 28, 47, 66, 71, 85, 99, 100, 119, 126, 138, 153, 154, 183, 184, 191, 196, 197, 206, 207, 209.
[d] n. 17, 28, 30, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 139, 140, 153, 154, 156/169, 171, 180, 183, 184, 191.
[f] n. 28, 47, 99, 100, 138, 153, 154, 155, 156, 181, 183, 190, 191, 203, 205, 206, 209.
[g] n. 28, 47, 49, 66, 99, 100, 153, 154, 155, 183.
[h] n. 7, 10, 15, 17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 139, 140, 153, 154, 171, 180, 183, 184, 191, 196, 197, 201, 203, 204, 205, 206, 207, 208, 209.
[i] n. 28, 47, 66, 85, 99, 100, 138, 154, 153,183.
[j] n. 28, 47, 66, 85, 99, 100, 138, 153, 154.
[k] n. 1, 2, 3, 7, 8, 11, 10, 12, 13, 15, 17, 25, 28, 30, 32, 33, 35, 37, 47, 49, 66, 71, 75, 77, 85, 99, 100, 116, 118, 119, 126, 138, 153, 154, 155, 166, 181, 183, 190.
[l] n. 28, 47, 66, 85, 99, 100, 153, 154, 183.
[m] n. 47, 100, 99, 154, 153.
[n] n. 47, 99, 100.
[o] n. 28, 47, 99, 100,154, 153, 183, 209.

As reported in Table 1, stable trends of PBDEs have been documented in cetaceans from Northwest Pacific, Northwest Atlantic, and Southwest Atlantic. In addition, increasing proportions of higher brominated congeners have been recently reported in cetaceans from Northwest Pacific. Jeong et al. (2020) investigated blubber of finless porpoises from Korean coastal waters during 2010–2015 and reported a significant decrease of lower brominated congeners (BDE-28 and BDE-47). At the same time, this study documented an increase of more brominated congeners (BDE-153 and BDE-154) during the study period, which is likely related to the greater consumption of deca-BDE in recent years. No significant variation on PBDE concentrations and relatively higher proportions of BDE-154 (equivalent to those of BDE-47) have been reported during 2011–2015 in melon-headed whales of the Japanese coast (Kunisue et al., 2021). Considering that technical penta- and octa- BDE products were banned by the early 2000s, the high proportion of BDE-154 – a minor component of these two mixtures - could be related as well to the increased discharge into the environment of BDE-209 and its debromination processes (La Guardia et al., 2007; Stapleton et al., 2006; Zhu et al., 2019). It is worth noting that China, one of the biggest countries in the Northwest Pacific area and one of the main producers and suppliers of deca-BDE mixture in the last years (Zhang et al., 2017), started the production of this higher brominated product later than Europe and the United States. This could have resulted in increased emissions of this mixture in recent years, and likely in a delay in the effectiveness of global restrictions (An et al., 2022). Even if China has established concentration limits for penta- and octa-BDE in electronics, it has not yet ratified the Stockholm Convention for deca-BDE (Sharkey et al., 2020) and its production, use, import and export will not be banned until 2025 (MEE, 2021). Additionally, China's great degree of industrialization and economic growth as well as e-waste import and inappropriate waste management might have contributed to elevated concentrations of these contaminants in its area, as reported by different studies (Li and Achal, 2020; Luo et al., 2021; Ma et al., 2011; Peng et al., 2019; Zhang et al., 2019). All these facts draw attention to the continuous inputs of PBDEs in the Northwest Pacific zone and the potential impacts on endangered cetaceans inhabiting this area for which information about PBDE contamination is scarce. To date, no studies have investigated PBDEs in North Pacific right whale (*Eubalaena japonica*), Western grey whale (*Eschrichtius robustus*), Northern sei whale (*Balaenoptera borealis*) and Northern blue whale (*Balaenoptera musculus*), all living or frequenting the Northwest Pacific Ocean and classified as endangered by the International Union of Conservation of Nature (IUCN) Red List of Threatened Species (Cooke, 2018a, 2018b; Cooke et al., 2018; Cooke and Clapham, 2018). In Yangtze finless porpoises (*Neophocaena asiaeorientalis asiaeorientalis*), an endemic species of the Yangtze River (China) listed as critically endangered by the IUCN (Wang et al., 2013), a declining population trend has been associated to a high risk of pollution particularly by pesticides. Yet, no information is present about the potential detrimental impact of PBDEs in this species (Zhao et al., 2008).

Continuous exposure to PBDEs has been also reported for St. Lawrence estuary belugas. This population seems to be exposed to continuous inputs of PBDEs as indicated by the high and stable levels of penta-, octa- and deca-BDE from 1997 to 2013, preceded by an increasing trend from 1987 to 1997 (Lebeuf et al., 2014; Simond et al., 2017) (Table 1). Elevated concentrations of PBDEs reported in different abiotic and biotic matrices in this area are to be related to the proximity of North America and the discharge of Montreal municipal effluents - the major source of PBDEs in the St. Lawrence River - along with the Great Lakes water mass (Marcogliese et al., 2015). In consequence, high values of PBDEs continue to be detected in different aquatic species and in a similar fashion as those of Canadian belugas (Travis et al., 2020; Zhou et al., 2019). Zhou et al. (2019) described an increase in PBDE concentrations in predator fishes of the Laurentian Great Lakes from 1990 to 2000, rapidly decreasing concentrations from 2000 to 2007, and unchanged or slightly decreasing levels up to 2015. This tendency probably reflects the voluntary phase out of penta- and octa-BDE commercial mixtures in 2004 and the extensive use of deca-BDE in the following years resulting in increasing concentrations of brominated congeners such as BDE-100 and BDE-154, which possibly stem from debromination processes of BDE-209. This thesis is supported by the increased proportion of BDE-209 - from 72% to 95% - of the total PBDE burdens after 2013 (Zhou et al., 2019).

An increase of PBDE levels was observed from 1989 to 1995 (range: 80–91 ng/g l.w. blubber) and from 2009 to 2014 (range: 23-1851 ng/g l.w. blubber) in Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) from Spencer Gulf and Gulf St Vincent (South Australia), with a difference of at least one order of magnitude between the two study periods (Weijs et al., 2020). Concentrations were in general higher than those reported for other odontocetes from Australia, normally ranging between 4.3 and 440 ng/g l.w. in blubber (Symons et al., 2004; Weijs et al., 2016). These data are not consistent with the results reported for human serum blood in the Australian population, which showed a constant lowering of PBDE levels during 10 years (Drage et al., 2019; Toms et al., 2018). Yet sediments from the Sidney estuary confirmed increased concentrations of these substances in the marine environment from 1990 to 2014, with BDE-209 reaching 97% of the total PBDE concentrations in more recent years (Drage et al., 2015). Prohibition of penta- and octa-BDE and the consequent increment in the use of deca-BDE could be responsible for the rapid increase in the average contribution of BDE-209 to the total PBDE content in sediment. Australia has never manufactured penta- and octa-BDE mixtures and its importation was banned in 2005 (Toms et al., 2009). Despite this, import of PBDE-containing products manufactured elsewhere continues to take place in the country (Drage et al., 2019).

Two different studies on male bottlenose dolphins stranded in Southeast Brazilian coast during 1994–2006 and 2007–2012 reported mean PBDE levels in liver of 982 ng/g l.w. and 960 ng/g l.w., respectively (Dorneles et al., 2010; Lavandier et al., 2019). Even if comparison and interpretation of data generated from different studies is not straightforward, owing to the different variables involved (sampling procedures, analytical methods employed, biological factors, etc.), it suggests that levels of PBDEs in bottlenose dolphins of Southeast Brazil have remained unchanged for nearly 20 years (1994–2012). PBDEs have never been manufactured in Brazil; nevertheless, this country is one of the major global electronic and textile waste producers (Baldé et al., 2017), commercial sectors where large amounts of these substances are employed. In this region, almost 215 thousand tons of municipal solid waste is generated daily and in a great proportion (40%) inadequately disposed (ABRELPE, 2017). This, together with the absence of specific legislation limiting the concentrations of PBDEs in commercial waste goods and a lack of regulation on the import/export of these BFRs (Rodrigues et al., 2015) could favor constant releases of these substances. Moreover, the existing exemptions for the use of penta-/octa-BDEs and deca-BDE in certain applications in Brazil, which will expire in 2030 and 2036, respectively, are likely to delay future decreases in the environmental concentrations of these contaminants (Sharkey et al., 2020).

1.2. Does climate change have an impact on PBDE accumulation in cetaceans?

In recent years, different studies have focused on the influence that climate change-driven processes could have on POPs' fate and distribution and the consequent impact on the global environmental pollution and living organisms (Alava et al., 2017; Chen et al., 2019; de Wit et al., 2022; Gong and Wang, 2022; Hung et al., 2022; Li et al., 2021; Nadal et al., 2015; Potapowicz et al., 2019). It is documented that changes in environmental variables and meteorological conditions brought about by global warming may affect POPs' behavior in the environment and may alter the "grasshopper effect" that is strictly dependent on temperature (Chen et al., 2019; Dalla Valle et al., 2007; Li et al., 2021; Ma et al., 2011; Wania and MacKay, 1996). Enhanced volatilization of POPs between primary and secondary sources, faster degradation in the aquatic ecosystem and distorted partitioning between geochemical spheres, are some processes caused by climate change and potentially determining POPs' fate and distribution (Cai et al., 2014; Macdonald et al., 2005; Nadal et al., 2015; Noyes et al., 2009). A recent study by Gong and Wang (2022) has drawn attention to the increased contribution of "secondary sources" to global contamination and the accelerated cycling of POPs under climate change conditions. It is plausible that increased temperatures are forcing the re-volatilization of POPs stored in pools (glaciers, vegetation and soils), thus converted in secondary sources responsible for the re-emissions of these contaminants into the global cycle (Dalla Valle et al., 2007). Secondary sources represent a threat above all for cold-climate ecosystems, more sensitive to climate change and where increased long-range transport might contribute to accumulation of POPs (IPCC, 2021; Li et al., 2021; Wania and MacKay, 1996). In addition, cryosphere degradation observed both in the Northern and Southern Hemispheres during the last decades could represent another important secondary source of POPs, previously trapped into it (Chadburn et al., 2017; Grannas et al., 2013; Siegert et al., 2019; Stroeve et al., 2012; the IMBIE team, 2018).

As sentinels of marine ecosystem pollution, cetaceans could be affected by changes in POPs' distribution, revealing alterations in the concentration and accumulation pattern of these substances over time. On the other hand, some studies demonstrated alterations in cetaceans' distributions, habitat and migration due to climate change that in some cases could result in increased residence times in more polluted areas (Ramp et al., 2015; Storrie et al., 2018; van Weelden et al., 2021).

Global warming may have an important impact on POPs' occurrence especially to those related to sediments and soils such as BDE-209, which accounts approximately for 75% of the worldwide use of PBDEs (Shi et al., 2015). Increased rainfalls may enhance POPs' deposition into soil, while more intense precipitations and land runoffs could raise the quantity of eroded soil particles reaching the ocean (Dalla Valle et al., 2007). Thus, increased amounts of these toxic and persistent substances could reach the marine environment entering aquatic food webs throughout benthonic organisms feeding on surface sediments or within them. At the same time, increased precipitation and atmospheric aerosols could enhance POPs' deposition to aquatic ecosystems (Castro-Jiménez et al., 2017; Macdonald et al., 2003; Noyes et al., 2009). A high contribution of BDE-209 (70–75% of the detected ΣPBDEs) has been documented in zoo- and phytoplankton as a possible consequence of the constant atmospheric input through dry deposition (Castro-Jiménez et al., 2021; 2017).

Even if BDE-209 is highly hydrophobic with low water solubility that makes it hardly bioavailable, studies have documented accumulation and biomagnification of this congener in cetaceans' tissues (Aznar-Alemany et al., 2019; Noël et al., 2009). It has been also demonstrated that an increase in environmental temperatures and atmospheric concentrations of ozone driven by global warming could enhance PBDE degradation and generation of more toxic and persistent lower brominated congeners (Niu et al., 2015). Keeping in mind the huge historical production and its recent prohibition, deca-BDE is worthy of attention in the next future, above all in those countries where production and use continue, and in urbanized and industrialized coastal areas where great amounts of higher brominated congeners and associated metabolites could be continuously released. It could be argued that the quantity of BDE-209 and other brominated congeners entering the marine food web could increase in the next years due to meteorological changes. This fact could lead to an increase of concentrations in marine top predators such as odontocetes living in coastal areas. On the other hand, as mentioned above, global warming and changes in meteorological conditions have been related to shifts in migration habits of some cetaceans. Thus, some of them began to spend more time in more contaminated lower latitude regions and to act as vectors of contamination to less polluted areas (Borgå et al., 2022; Ramp et al., 2015; Storrie et al., 2018). Species that spend longer periods than usual feeding at southern latitudes are probably increasing their exposure to these substances, as it has been already reported for some seabird species (Bustnes et al., 2010; Elliott et al., 2021; Mora et al., 2016).

A study by Ramp et al. (2015) assigns the earlier ice break-up, caused by global warming, as the plausible cause for fin whales anticipating their date of arrival and increasing their residence time (in about 16 days) in the summer feeding area at St. Lawrence Estuary. This is a well-known contaminated area situated in the North Atlantic Ocean and where the St. Lawrence River pours its waters after receiving PBDE contaminated urban effluents from the Metropolitan Community of Montreal (3.8 million inhabitants) (Marcogliese et al., 2015). Cumulative effects of changes in migrations habits, more intense precipitations and continuous emissions of PBDEs above all in industrialized coastal areas, can result in greater PBDE exposures for fin whales (Ramp et al., 2015). Moreover, higher brominated BDE congeners accumulate preferentially in lower trophic levels such as zooplankton, compartment that includes the main prey of this species and other baleen whales (Castro-Jiménez et al., 2021).

The Cook Inlet Alaskan beluga whale population has increased its residence time in the more industrialized and urbanized upper Cook Inlet during warmer years, suggesting a risk of toxicological implications for this endangered population that is already facing a growth rate reduction and difficult recovery (Ezer et al., 2013). Furthermore, if long-term climatic changes occur in the future, Cook Inlet belugas whale could stay longer in the northernmost part of the inlet facing higher levels of contamination. Hoguet et al. (2013) documented a different degree of PBDE exposure between two distinct populations of Alaskan belugas with the Cook Inlet beluga population being more contaminated than the Bering Sea population. As explained by the authors, the different degree of contamination is probably the consequence of distinct geographic localizations. Cook Inlet is an urbanized and industrialized area where localized inputs of contamination occur, for instance stemming from wastewaters from Anchorage's treatment plant serving more than 200,000 people.

Some species of baleen whales such as humpback whale, fin whale, common minke whale (*Balaenoptera acutorostrata*) and blue whale have increased their annual maximum latitudes between 1° and 4°, apparently due to the reduction of sea ice extent as a consequence of climate warming (Storrie et al., 2018). As cetaceans could act as biovectors of POPs, enhanced ice melt and the subsequent habitat expansion of these species might rise the transportation of persistent and toxic pollutants into polar zones. Furthermore, increasing temperature and sea ice melting could result in remobilization of organic contaminants, such as PBDEs, and increased concentrations in the marine environment (Ma et al., 2011).

1.3. Effects of PBDEs in cetaceans' health: what is already there and what is lacking

A limiting factor in risk assessment of PBDEs in cetaceans is the lack of species-specific toxicity thresholds that can be used to assess potential health risks of these substances. To the best of our knowledge the single upper limit threshold for PBDEs in marine mammals (1500 ng/g l.w.) has been established for endocrine disruption in blubber of grey seals (Hall et al., 2003). To date this threshold is employed for a wide range of species with some degree of approximation (Lam et al., 2009; Lindström et al., 1999; Moon et al., 2010; Tuerk et al., 2004). Toxicokinetic differences as well as distinct physiological characteristics lead to a high level of uncertainty when extrapolating a threshold value for different species, even if among marine mammals. Hence, there is a need for toxicological studies focused on calculating species-specific and tissue-specific threshold values, as well as to assess different endpoints, already settled for other POPs such as PCBs and dioxins (Desforges et al., 2016; Kannan et al., 2000; Ross et al., 1995; Schwacke et al., 2012). PBDEs lack of a unifying methodology like that of the Toxic Equivalent (TEQ) approach used in risk assessment for dioxins, and others dioxin-like compounds. Moreover, while a group of seven indicator congeners exists for PCBs, there is not a similar counterpart yet for PBDEs (Boalt et al., 2013). Despite the lesser attention given to PBDEs, it has been demonstrated that these substances could alter the normal activity of biological systems in the same way as PCBs and in some cases with a higher degree of interference. Studies in rats suggested agonist potency of PBDEs on the aryl hydrocarbon receptor (AHR) comparable to those of some mono-*ortho* PCBs (Meerts et al., 1998). Hooper and McDonald (2000) reported greater ethoxyresorufin-o-deethylase (EROD) induction capacity of commercial penta-BDEs compared to that of Aroclor1254. Potential additive reduction on circulating thyroxin hormone level (T4) induced by coexposure of PCBs and PBDEs have been described by Miller et al. (2012).

Besides being endocrine disruptors (ECD) (Hall et al., 2003; Zhou et al., 2001), PBDEs have been associated to immunotoxicity in harbor porpoises, pantropical spotted dolphins, sperm whales (*Physeter macrocephalus*), striped dolphins, fin whales, Bryde's whales (*Balaenoptera edeni*), killer whales, long-beaked common dolphins, and furthermore, genotoxicity and cytotoxicity in pantropic spotted dolphins (Beineke et al., 2005; Villanger, 2011; Hall et al., 2003; Huang et al., 2020; Marsili et al., 2019; Rajput et al., 2021; Simond et al., 2019). Thymic atrophy and splenic depletion have been significantly correlated with high PBDE concentrations in harbor porpoises (Beineke et al., 2005). Stimulation of innate immune response has been reported in fibroblast cells of pantropical spotted dolphins exposed to BDE-47, BDE-100 and BDE-209 (Huang et al., 2020). A recent study on fibroblast cells of pantropic spotted dolphin described alterations on reactive oxygen species (ROS) production, mitochondrial membrane potential, cellular calcium levels, mitochondrial structure, cell membrane structure and apoptosis, after exposure to BDE-47, BDE-100 and BDE-209 (Rajput et al., 2021). Also, alterations on a polymorphic protein that is induced upon stress, damage or transformation of cells, has been described in fibroblast cells of killer whale, long-beaked common dolphin, Bryde's whale, sperm whale, striped dolphin and fin whale, after treatment with PBDEs (Marsili et al., 2019). Besides, disruptive capacity to thyroid hormones (Villanger, 2011) and alteration of gene transcription involved on the regulation of thyroid and/or steroid hormones (Simond et al., 2019) have been documented in beluga and minke whales.

All these *in vitro* studies, performed mainly in fibroblast cells, have demonstrated the capacity of these contaminants to interfere with hormone production and the immune system response, to negatively affect the structure of the cells and their functions as well as to alter production of reactive oxygen species (ROS) resulting in oxidative stress. Furthermore, it has been shown that the hormone, cellular and immune systems respond to PBDE exposure in a specific way according to different types of congeners, species or tissue analyzed. For example, BDE-47, BDE-99 and BDE-100 have been reported to differently influence oxidative stress, mitochondrial membrane potential (MMP) response, cellular calcium levels and protein expression of apoptosis-associated genes, being BDE-47 the most cytotoxic one (Rajput et al., 2021). At the same time, Marsili et al. (2019) showed differences in the expression of polymorphic proteins in fibroblast cells exposed to a mixture containing 27 PBDEs in different marine mammal species; downregulation has been documented for killer whale and Bryde's whale, while upregulation has been reported for striped dolphin.

Toxicological sensitivity, mechanisms and pathways may vary between species and each congener could interfere with vital systems in multiple ways and at different levels. Therefore, species-specific investigations as well as a broader knowledge about toxicity effects triggered by different substances and congeners result crucial. However, if on one hand it is essential to study negative effects caused by mixtures, as cetaceans are normally exposed to different class of pollutants and various congeners simultaneously, on the other hand, studies on specific substances such as PBDEs could play a key role in management and conservation policies.

2. Conclusions

Despite all regulatory measures taken, PBDEs continue to impact cetaceans at global scale. Climate change and the constant economic growth together with the increase of e-waste trading could lead to higher exposure of cetaceans to PBDEs in the next years, especially in certain areas of the world such as the Arctic and the Northeast Pacific Ocean. Information about negative effects on the health status is still unknown for many species for which toxicological studies have not yet been performed. All of that represents a limiting factor for understanding the real impact exerted by PBDEs on these species both, at individual and population levels.

Canadian Southern Resident killer whales, Atlantic white-sided dolphins in the North Sea, bottlenose dolphins in the Northwest Atlantic Ocean, Indo-Pacific humpback dolphins inhabiting Hong Kong waters, Mediterranean striped dolphins and false killer whales from Hawaii and Brazil showed the highest tissue concentrations of PBDEs. Significant levels and stable trends of PBDEs recently reported in cetaceans of the Southern Hemisphere, where PBDEs have never been produced, highlight the environmental impact of the existing trade of PBDE-containing products. It is noticeable the urgent need for more carefully investigations and monitoring, especially in those countries that have not yet established concentration limits for imported products. The highest levels recently found in species living in urbanized coastal regions have been related to discharge of contaminated waters deriving from wastewater treatment plants, which should improve treatment processes and removal efficiencies of PBDEs. The synergic effects of climate change and ongoing emissions of PBDEs, expected to continue at least until 2050, may result in increased contamination exposure for some species, in particular cetaceans inhabiting the Arctic for which global warming seems to have a greater impact.

In light of this, a greater knowledge about effects both at species and population levels as well as more specific threshold values are strongly needed for cetaceans, in order to assess health risks and support regulatory protection for these species. The lack of generalized decreasing trends in total PBDE concentrations along with the reported changes in congener profiles underpin the need to reevaluate the efficacy of current regulations on PBDEs and to improve waste management, both in developed and developing countries.

Credit author statement

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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.

Data availability

All data are public and properly cited

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Evaluation of PCDD/Fs, dioxin-like PCBs and PBDEs in sperm whales from the Mediterranean Sea

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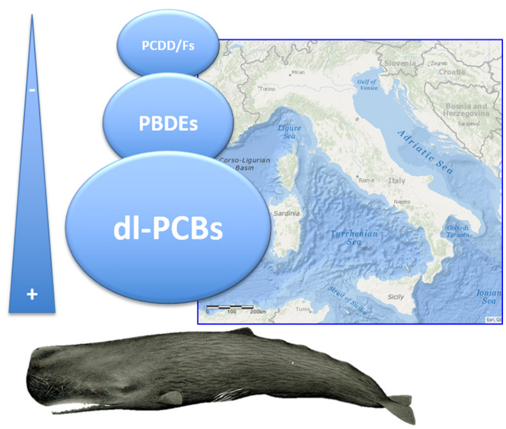
HIGHLIGHTS

- dl-PCBs ranking at the top of the reported values for this species worldwide
- Mediterranean Sea still today a global PCB "hotspot" for marine mammals
- Non-ortho-dl-PCB pattern (126 > 169 > 77)
- TEQ contribution: Σnon-*ortho*-dl-PCBs > Σ*ortho*-dl-PCBs > PCDDs > PCDFs

GRAPHICAL ABSTRACT



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ABSTRACT

Numerous studies to date have reported concentrations of Persistent Organic Pollutants (POPs) in different marine mammal species worldwide. Yet data on sperm whales are scarce from rich and unique biodiverse areas such as the Mediterranean Sea. This work aimed to assess levels of dioxin-like polychlorinated biphenyls (dl-PCBs), polybrominated diphenyl ethers (PBDEs), and polychlorodibenzo-*p*-dioxins and furans (PCDD/Fs) in blubber of sperm whales stranded along the Italian coast between 2008 and 2016. POP mean concentrations (dl-PCBs: 6410 ng/g l.w.; PBDEs: 612 ng/g l.w.; PCDD/Fs: 57.8 pg/g l.w.) were mostly in line with what has been previously reported on the same species in the Mediterranean environment and tended to be higher than those reported from other geographical regions. The relative abundance followed the order dl-PCBs > PBDEs ≫ PCDD/Fs. Interestingly, the non-*ortho* dl-PCB pattern (126 > 169 > 77) was similar to that described in other studies worldwide and different from what is described in its main prey. This could be linked to particular metabolic activities in sperm whales against these highly toxic contaminants. Total TEQs ranged from 275 to 987 pg/g l.w. and showed the pattern Σnon-*ortho*-dl-PCBs > Σ*ortho*-dl-PCBs > PCDDs > PCDFs, with PCBs' contribution about 96%. These findings highlight the high abundance of PCBs still found in the Mediterranean environment despite having been banned for decades. All sperm whales analyzed in this study surpassed the threshold of 210 pg WHO-TEQ/g l.w. proposed as starting point of

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immunosuppression in harbour seals; a level of contamination that may have contributed to an impairment of their immune system.

1. Introduction

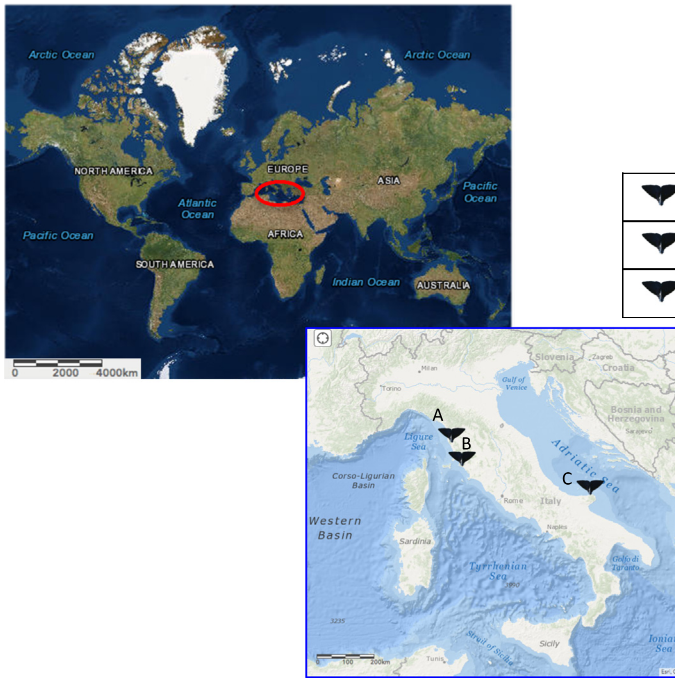
The Mediterranean Sea is a complex and rich ecosystem that accounts for a heightened degree of marine biodiversity (Coll et al., 2012). Concurrently, it is an area under intense anthropogenic pressures (Castro-Jiménez et al., 2013), and as such, it is considered a sink for many environmental contaminants, partially due to its geo-morphological characteristics (e.g. semi-enclosed marine basin), and the high urbanization rate of its coasts along with related activities derived from it (wastewater releases, shipping, fishing and chemical pollution) (Marsili et al., 2018). Persistent organic pollutants (POPs) rank among the most relevant environmental contaminants. Aside from their toxicity, their unique physical-chemical properties are responsible for their global distribution and persistence in the environment. Due to their lipophilic nature they can biomagnify in marine food webs reaching high levels among top predators (Aguilar et al., 2002). Even though the Stockholm Convention banned or restricted the production and use of different POPs since 2004 (UNEP, 2001), they continue to be present in the environment and biota. POP concentrations measured in the Mediterranean Sea reach this area by different pathways; among the most important of these are long-range transportation (LRT) from Asia (Lelieveld et al., 2002) and western, central and eastern Europe (Berrojalbiz et al., 2014; Iacovidou et al., 2009), and from river discharges and sedimentation (Albaigés, 2005; Gómez-Gutiérrez et al., 2006). In consequence, important POP levels are today reported for different animal species (Guerranti et al., 2014; Maisano et al., 2016; Muñoz-Arnanz et al., 2011; Pinzone et al., 2015; Roscales et al., 2016; Tekin and Pazi, 2017), which justifies unceasing research on these pollutants. Concern raises not only for emerging contaminants such as polybrominated diphenyl ethers (PBDEs), for which regulations on their use are more recent, but also for legacy chemicals such as polychlorinated biphenyls (PCBs). Concentrations of the latter in different Mediterranean dolphin species have been recently reported surpassing previously known PCB toxicity thresholds for marine mammals (Stuart-Smith and Jepson, 2017). In consequence, it appears the declining concentrations recorded after the wide ban on PCBs in the European environment in the mid-1980s, followed by the enforcement of the Stockholm Convention in 2004, had somehow halted, leaving in general still high concentration values in most European biota (Jepson et al., 2016; Jepson and Law, 2016; Law and Jepson, 2017; Stuart-Smith and Jepson, 2017).

Cetaceans at high trophic levels, long life spans and large fat deposits tend to accumulate high levels of POPs. Tissue concentrations of environmental contaminants depend on the contamination levels of the source and on many biological factors (diet, body size, body composition, nutritive condition, incidence of disease, age and sex) (Aguilar et al., 1999), and once ingested, the ability of each species to metabolize and excrete contaminants through biotransformation systems (Hakk, 2003; Tanabe et al., 1988). These systems are divided in two phases: phase I, characterized by the introduction or modification of xenobiotics' functional groups, and phase II, involving conjugation or synthetic reactions that increase compounds' solubility. Both phases aim to transform hydrophobic lipid-soluble organic xenobiotics into water-soluble excretable metabolites. In this sense, cetaceans are known to have low phase I biotransformation activity, which translates into low capability to metabolize contaminants (Boon et al., 1997; Hoydal et al., 2018; Tanabe et al., 1988).

Deleterious effects on marine mammals' health from elevated POP concentrations have been strongly suggested in numerous investigations to date, e.g. potential adverse influences on the endocrine and immune systems, and on the reproduction and offspring survivorship rates (Bossart, 2011; Desforges et al., 2016; Hall et al., 2006; Yordy et al., 2010). Thus, high POP levels have been proposed as potential factors involved in mass mortality and stranding events (Evans et al., 2004). However, even when tissue pollutant concentrations and negative health effects have been suggested in pinnipeds and cetaceans (Béland et al., 1993; Reijnders, 1994; Ross et al., 1996; Skaare et al., 2000), establishing a direct association between levels and toxic effects is quite complex owing to the large number of confounding factors involved (e.g. specimen's sex, age, diet, health status, metabolic and excretion capabilities, etc.). This points out the need of a multidisciplinary approach to reach an understanding on the cause of stranding and death (Mazzariol et al., 2016), for which ecotoxicological investigations are essential to understand the real role played by contaminants on these events.

The sperm whale (*Physeter macrocephalus*) is a marine mammal with a cosmopolitan distribution (Whitehead, 2018). This large toothed whale, feeds mainly on meso- and bathypelagic cephalopods (Wong and Whitehead, 2014). Males tend to eat larger prey than females and more likely demersal fishes, such as sharks and rays (Wong and Whitehead, 2014). Although the biggest threat to sperm whales - commercial whaling - has stopped, there are other anthropogenic activities, such as contamination, entanglement in fishing gears and noise pollution that continue to threaten this species. Sperm whales exist as a genetically distinct subpopulation in the Mediterranean Sea, where it is widely distributed and potentially geographically isolated (Dulau et al., 2004; Engelhaupt et al., 2009). This subpopulation accounts for fewer than 2500 mature individuals; a number that is still declining and grouped in one undivided subpopulation, and for all these reasons it is listed by the International Union for Conservation of Nature (IUCN) as endangered (IUCN, 2006). Safeguard and protective measures have been adopted to protect Mediterranean sperm whales, mainly from entanglement in fishing gears and ship collisions. However, other anthropogenic activities such as oil and gas prospecting (seismic airguns), military operations, illegal dynamite fishing and very specially, contamination, are today sources of concern.

Numerous studies have reported to date POP levels in different marine mammal species all over the world (Aguilar et al., 2002; Alonso et al., 2014; Tanabe, 2002). Yet, data on sperm whales are scarce, including the Mediterranean Sea where very few studies have investigated POP contamination on this species. To the best of our knowledge, there exist fifteen studies worldwide from 1993 to 2018 about POPs in sperm whales and only four of them focused on the Mediterranean Sea (Marsili et al., 2014; Pinzone et al., 2015; Praca et al., 2011; Zaccaroni et al., 2018). This work aims to assess levels of dioxin-like polychlorinated biphenyls (dl-PCBs), PBDEs, polychlorodibenzo-*p*-dioxins and furans (PCDD/Fs) and their congener profiles in blubber of sperm whales stranded along the Italian coast. These data will help 1) to expand our knowledge about the current contamination status of the sperm whale Mediterranean sub-population in order to better understand the threat posed by these POPs, 2) to investigate the toxic load represented by dioxin-like PCBs, PCDDs and PCDFs, for which toxic equivalency factors (TEFs) are available. This approach based on the different contribution of each pollutant to total toxic equivalent (TEQ), makes it possible to carry out a risk assessment for this species.



A	63806	Calambrone (PI) 43°35′57.674′′N-10°17′42,255′′E
B	RT13	Castiglione della Pescaia (GR) 42°45′50.021′′N-10°52′30.73′′E
C	PM1	Capoiale-Foce Varano (FG) 41°55′05.08′′N-15°46′23.18′′E
	PM2	Capoiale-Foce Varano (FG) 41°55′03.71′′N-15°46′08.11′′E
	PM3	Capoiale-Foce Varano (FG) 41°55′01.44′′N-15°45′34.69′′E
	PM4	Capoiale-Foce Varano (FG) 41°54′57.44′′N-15°44′32.43′′E
	PM5	Capoiale-Foce Varano (FG) 41°54′54.31′′N-15°44′10.51′′E
	PM6	Capoiale-Foce Varano (FG) 41°54′54.48′′N-15°43′43.73′′E

Fig. 1. Map of the three stranding sites (2008, 2009 and 2016) on the coast of Italy.

2. Material and methods

2.1. Sampling

Blubber was collected from nine stranded sperm whales, all males. Seven stranded in 2009 during a mass stranding along the Apulian coast (southern Adriatic Sea) and two stranded in 2008 and 2016 along the coast of Tuscany (Fig. 1). Detailed information about the nine specimens is listed in Table 1. The seven sperm whales stranded in 2009 were part of the same pod and members of the Mediterranean subpopulation (Mazzariol et al., 2011). A multidisciplinary study (histopathology, virology, bacteriology, parasitology, toxicology, genetic and screening of veins looking for gas emboli) was conducted on these individuals and a multi-factorial cause was proposed for this mass stranding (Mazzariol et al., 2011). Determination of organochlorine compounds (PCBs, HCB and DDTs), polycyclic aromatic hydrocarbons and biomarker responses (CYP1A1 and CYP2B) can be found in (Marsili et al., 2014).

All samples were taken from the dorsal area, front the cranial insertion of the dorsal fin, wrapped in aluminum foil, stored in ice on site and then frozen at −20 °C until residue analysis. For the seven sperm whales involved in the mass stranding, age was determined by counting dentin growth layer groups (GLG), and weight (corrected weight) was calculated considering also postmortem body fluid and tissue leakages (Mazzariol et al., 2011). Each of the two sperm whales involved in individual strandings were assigned to a class age according to each specimen's length; weight was estimated using the animal total length (Lockyer, 1976).

2.2. Analytical procedure

Fresh samples were weighed, homogenized with anhydrous sodium sulfate (Na_2SO_4) and spiked with a suite of ^{13}C-labeled standards of dl-PCBs, PBDEs and PCDD/Fs, prior to Soxhlet extraction (24 h) with a *n*-hexane: DCM (9:1) mixture. Extracts were rota-evaporated and cleaned-up by using the automated sample preparation system DEXTech+ (LCTech GmbH, Dorfen, Germany). Final extracts were evaporated using a TurboVap® system until ~1 mL, transferred to vials, and dried under a gentle nitrogen stream. Fractions were reconstituted in a few microliters of ^{13}C-labeled injection standards of dl-PCBs, PBDEs and PCDD/Fs prior to instrumental analysis. Samples' lipid content was determined gravimetrically. Comprehensive details on the analytical procedure are provided in the Supplementary Information.

2.3. Instrumental determination

Samples were analyzed for 12 dl-PCBs (#77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 189), twenty-seven PBDEs (# 3, 7, 15, 17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 156, 183, 184,

Table 1
Detailed information about sperm whales stranded along the coast of Italy in 2008, 2009 and 2016.

Code	Date of stranding	Site of stranding	Size (m)	Weight (t)	Age (years)	Sex	Remark
PM1	December 2009	Adriatic Coast	11.8	14.8	22–25	M	Found dead
PM2	December 2009	Adriatic Coast	12.2	16.0	20	M	Found dead
PM3	December 2009	Adriatic Coast	11.3	14.8	20	M	Found dead
PM4	December 2009	Adriatic Coast	11.4	13.7	20	M	Found dead
PM5	December 2009	Adriatic Coast	10.5	16.0	15	M	Stranded alive
PM6	December 2009	Adriatic Coast	12.1	17.7	20	M	Stranded alive
PM7	December 2009	Adriatic Coast	11.2	15.7	20	M	Stranded alive
RT13	October 2008	Tyrrhenian Coast	4.5	1.3	Young	M	Found dead
63806	August 2016	Tyrrhenian Coast	12.8	23.6	Adult	M	Found dead

191, 196, 197, 206, 207, 209) and 17 PCDD/Fs (2,3,7,8-susbtituted congeners). Quantification was carried out by the isotopic dilution technique by GC-HRMS on a Trace GC Ultra gas chromatograph (Thermo Fisher Scientific, Milan, Italy) coupled to a high-resolution mass spectrometer (DFS, Thermo Fisher Scientific, Bremen, Germany). A full description of the instrumental parameters can be found in the Supplementary Information.

2.4. QA/QC criteria

Metal and glassware material was cleaned (3×) with three solvents of decreasing polarity: acetone, dichloromethane and *n*-hexane. A procedural blank was analyzed within each batch of six samples. Care was taken to minimize exposure to UV light throughout the entire analytical procedure. Quantification was carried out by the isotopic dilution technique with the following criteria: (a) ratio between the two monitored ions within ±15% of the theoretical value, and (b) limits of quantification (LOQs) corresponding to S/N of 10. Final concentrations were blank corrected. Satisfactory analyses ($n = 3$) of the certified standard reference material SRM 1945 ("Organics in Whale Blubber", NIST) were achieved. Further information related to QA/QC including surrogate recoveries, reference material values and limits of detection of the target compounds is provided in the Supplementary information.

2.5. Data handling

All concentrations are given in ng/g (dl-PCBs and PBDEs) or pg/g (PCDD/Fs) on lipid weight (l.w.) basis. Toxic equivalent quantities (TEQ) for dl-PCBs and PCDD/Fs were determined using the World Health Organization (WHO)-2005 toxic equivalency factors (TEF) for mammals (Van den Berg et al., 2006). Data for PCDD/Fs, dl-PCBs and TEQs are reported in upper bound (i.e. substitution of non-detected compounds for detection limit values).

3. Results and discussions

3.1. Detection frequency

The three POP families (dl-PCBs, PBDEs, PCDD/Fs) were detected in all sperm whale samples analyzed. The relative abundance of the study contaminants followed the order dl-PCBs > PBDEs ≫ PCDD/Fs. Five PBDE congeners (3, 7, 15, 119, 126) were consistently not detected in any sample. The most abundant dl-PCBs congeners were PCB118 (5 Cl), PCB156 (6 Cl) and PCB105 (5 Cl) reaching average contributions of 58%, 14% and 12%, respectively, to the total dl-PCB content. The most abundant PBDE congeners were BDE47 (4 Br), BDE100 (5 Br), BDE99 (5 Br), whose average contribution to the total PBDE content was 70%, 10% and 10%, respectively. The most abundant PCDD/F congeners were 2,3,4,7,8-PeCDF (21%) and 1,2,3,6,7,8-HxCDD (17%).

3.2. Concentration values

The wide range of values found for Σdl-PCBs, ΣPBDEs, ΣPCDDs and ΣPCDFs (Table 2) is likely to reflect on unknown differences in age, sampling year and health status of the sampled specimens. In comparison to the most recent available data of POPs in blubber for this Mediterranean species (Table 3), they were in the same order of magnitude as those reported by Pinzone et al. (2015), save for PCDD/Fs, which were found one order of magnitude lower. Instead, PBDE levels (sum of BDE congeners 28, 47, 100, 99, 154, 153, 209 = 600 ± 399 ng/g l.w.) in our study (all males) were up to three times higher than those reported by Zaccaroni et al. (2018) (167 ± 14 ng/g l.w.) for three Mediterranean sperm whales (all females) stranded along the Adriatic coast in 2014. We can presume this dissimilarity between concentration values was highly influenced by sex since each group was made up by males or females, exclusively. Two of the three females had already reached sexual maturity and probably given birth and decreased their contamination load during gestation and lactation (Reijnders et al., 2009). On the contrary, males analyzed in this study were mostly adults and without offloading mechanisms as gestation and lactation serve to females, they could have been accumulating pollutants - especially those with high hydrophobicity and resistance to metabolism - throughout their entire lives.

In comparison to other geographical areas, dl-PCB and PCDD/F levels in the sperm whales analyzed in this study were two orders higher and in the same order of magnitude, respectively, than those reported for sperm whales from Australia (Gaus et al., 2005). This result is congruent with the fact that the Mediterranean Sea, and in particular its western area, is considered for some authors a global PCB "hotspot" for marine mammals (Stuart-Smith and Jepson, 2017).

Regarding PBDE levels, our results were in the same order of magnitude than those reported for sperm whales from Gulf of California (Fossi et al., 2014), but six times lower than those reported from North-Atlantic specimens (Borrell, 1993), which is probably related to the greater historical use of these compounds in North America (Law et al., 2014).

It is worth noting how differences in pollutant concentrations and profiles between our study and previous literature could be linked not only to different geographic areas and time periods, but also to distinct analytical methods and type of samples. Specifically, it is known that POP concentration in cetaceans is influenced by blubber thickness (Evans et al., 2003), which could became a significant variable when comparing biopsies taken from free-ranging individuals (few centimetres of the outermost layer) to samples obtained from stranded individuals (usually all three layers of blubber) (Evans et al., 2003; Ryan et al., 2013).

3.3. Congener profiles

The dl-PCB congener profile, showed in Fig. 2, was dominated by mono-*ortho* PCBs, that accounted for about 99.9% of the dl-PCBs.

Table 2
Mean, median, range, detection frequencies (% >LOQ) of total dl-PCBs, PBDEs and PCDD/Fs in blubber from Mediterranean sperm whales (concentrations are expressed in ng/g l.w. save for PCDD/Fs, pg/g l.w.); PCDDs, PCDFs, dioxin-like PCBs, total TEQ and percentage contribution to T-TEQ. Data are expressed in pg WHO-TEQ/g l.w and are showed as mean, median and range.

					pg WHO-TEQ/g l.w.			% total TEQ
Compounds	Mean	Median	Range	>LOQ (%)	Mean	Median	Range	
Σmono-*ortho*-dl-PCBs	6410	3490	2090–20,800	100	192	105	62.9–625	34.1
Σnon-*ortho*-dl-PCBs	4.10	3.74	2.61–7.43	100	281	255	188–527	61.7
Σdl-PCBs	**6420**	**3500**	**2100–20,800**	100	**474**	**374**	**261–968**	**95.8**
ΣPCDDs (pg/g)	29.6	25.5	20.7–47.6	100	13.0	11.9	9.36–21.1	2.9
ΣPCDFs (pg/g)	28.2	27.1	23.9–35.9	100	5.03	4.54	4.26–6.37	1.2
ΣPCDD/Fs (pg/g)	**57.8**	**56.2**	**45.4–83.5**	100	**18.1**	**16.3**	**13.9–27.4**	**4.2**
ΣPBDEs	612	356	312–1390	100	–	–	–	–
Total TEQ					**492**	**394**	**275–987**	–

Bold values indicate the total of dl-PCBs, PCDD/Fs and TEQ.

Table 3
PCBs and PBDEs (ng/g l.w.) in blubber samples of sperm whales from different worldwide studies. Data are expressed as mean ± SD (when possible). Geographic area of sampling, year of collection and sex are reported (when possible).

Reference (year)	Geographic area	Sampling year	N	Sex	ΣPCBs (Σdl-PCBs)	ΣPBDEs
Aguilar (1983)[p]	North Atlantic	–	8	M	9930	–
			6	F	15,550	–
Borrell (1993)[p]	Iceland	1982	10	M	10,510 ± 2070	4160 ± 1040
Holsbeek et al. (1999)[r,a]	Southern North Sea	1994–1995	7	M	3032 ± 547	
Evans et al. (2004)[q,b]	Southern Australia	1998	32	F	800 ± 400	–
			5	M	1300 ± 1200	–
Praca et al. (2011)[r,c]	NWMS	2003–2009	14	1 M/13 U	107,810 ± 108,720	–
Marsili et al. (2014)[q,d]	Italy	2009	7	M	193,608 ± 340,089	–
Pinzone et al. (2015)[r,e]	NWMS	2006–2013	32	M	24,237 ± 17,421	382 ± 176
			11	F	16,877 ± 7237	248 ± 106
				F/M	(2120 ± 1490)	
Gaus et al. (2005)[q,f]	Tasmania (Southern Australia)	–	7	F/M	(28.7 ± 8.73)	–
de Boer et al. (1998)[r,g]	NE Atlantic (North sea)	–	3	M	–	49.6 ± 46.0
Bachman et al. (2014)[q,h]	Pacific Island	2011	1	F	1.470	27.2
Fossi et al. (2014)[r,i]	Gulf of California (Mexico)	2008–2009	14	M	2193 ± 660	30.8 ± 31.7
				F	2294 ± 1180	283 ± 819
Godard-Codding et al. (2010)[r,l]	SC = Sea of Cortez	1999	10		MC: 1514 ± 1693	–
	KR = Kiribati	2000	10		KR: 734 ± 869	–
	GP = Galapagos	2000	10		GP: 1262 ± 1586	–
	PX1 = Pacific Crossing	2000	10		PX1: 777 ± 853	–
	PNG = Papua New Guinea	2001	10		PNG: 1101 ± 1378	–
Law et al. (2003)[q,m]	Orkney Islands (Scotland)	1994	1		–	42.9
	Netherlands	1995	2		–	80.6 ± 90.7
Romero-Romero et al. (2017)[q,n]	Atlantic Ocean (Cantabrian sea)	–	1		1790	149
Zaccaroni et al. (2018)[q,o]	Mediterranean Sea	2014	3	F	–	167 ± 13.9
This study[r]	Mediterranean Sea	2008, 2009, 2016	9	M	6420 ± 6150	612 ± 401

[a] Sum of PCB congeners n. 28, 52, 101, 118, 153, 138, 156, 180, 170, 194.
[b] Sum of PCB congeners n. 28, 52, 101, 118, 153, 180.
[c] Sum of PCB congeners n. 28, 44, 52, 101, 118, 128, 138, 153, 170, 180, 187, 195, 206.
[d] Sum of PCB congeners n. 95, 101, 99, 151, 144, 135, 149, 118, 146, 153, 141, 138, 178, 187, 183, 128, 174, 177, 156, 171, 202, 172, 180, 199, 170, 196, 201, 195, 194, 206.
[e] Sum of ndl-PCBs congeners n. 8, 18, 28, 52, 44, 66, 101, 87, 153, 138, 187, 128, 180, 170, 195, 206, 209 and dl-PCBs congeners n. 118, 105, 77, 81, 126, 169, 144, 123, 156, 157, 167, 189.
[f] Sum of PCB congeners n. 77, 81, 126, 169, 105, 114, 118, 123, 156, 157, 167, 189.
[g] Sum of PBDE congeners n. 47, XY‡, 99, 209.
[h] Sum of PCBs congeners n. 8, 18, 28, 29, 31, 44, 45, 49, 50, 52, 56, 63, 66, 70, 74, 79, 82, 87, 92, 95, 99, 101, 104, 105, 106, 107, 110, 112, 114, 118, 119, 121, 127, 128, 130, 132, 137, 138, 146, 149, 151, 153, 154, 156, 157, 158, 159, 163, 165, 166, 167, 170, 172, 174, 175, 176, 177, 178, 180, 183, 185, 187, 188, 189, 191, 193, 194, 195, 196, 197, 199, 200, 201, 202, 203, 205, 206, 207, 208, 209.
[i] Sum of PCB congeners n. 95, 101, 99, 151, 144, 135, 149, 118, 146, 153, 141, 138, 178, 187, 183, 128, 174, 177, 156, 171, 202, 172, 180, 199, 170, 196, 201, 195, 194, 206. Sum of PBDE congeners n. 194, 195, 196, 197, 204, 198, 199, 200, 203, 201, 202, 205, 206, 207, 208, and 209.
[l] Sum of PCB congeners n. 95, 101, 99, 151, 144, 135, 149, 118, 146, 153, 141, 138, 178, 187, 183, 128, 174, 177, 156, 171, 202, 172, 180, 199, 170, 196, 201, 195, 194, 206.
[m] Sum of PBDE congeners n. 28, 47, 66, 71, 75, 77, 85, 99, 100, 119, 138, 153, 154, 190.
[n] Sum of PCB congeners n. (*ortho*) 28, 52, 101, 105, 114, 118, 123, 138, 153, 156, 157, 167, 170, 180, 189, 194; (non-*ortho*) 81, 77, 126, 169. Sum of PBDEs congeners n. 17, 28, 47, 66, 85, 99, 100, 153, 154, 184, 183, 191, 196, 197, 209.
[o] Sum of PBDEs congeners n. 28, 47, 100, 99, 154, 153, 209.
[p] Caught animals.
[q] Stranded animals.
[r] Free-ranging animals.

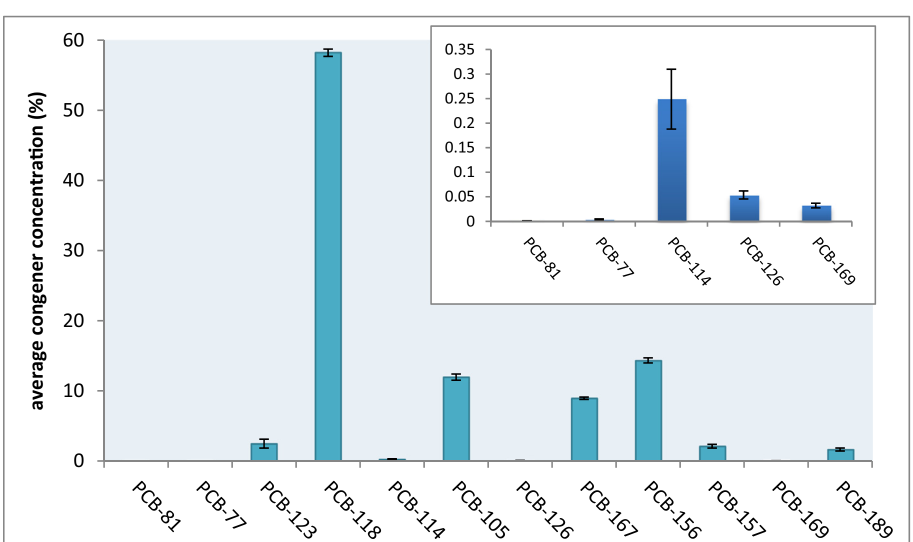


Fig. 2. Average contribution of each dl-PCB congener to the total dl-PCB content. Error bars represent the standard error (SE).

Among them, PCB118 was the most abundant, which is in line with previous studies on sperm whales and other species reporting this congener as frequently the most abundant among dl-PCBs (Bhavsar et al., 2007; Evans et al., 2004; Gaus et al., 2005; Lake et al., 1994; Romero-Romero et al., 2017; Storelli et al., 2011). This was somewhat anticipated since this congener is one of the seven PCBs recommended by The International Council for the Exploration of the Sea (ICES) working groups as indicator congeners (ICES7) for monitoring, based on their relatively clear identification and quantification in gas chromatography and their usual high contribution to the total PCB content in the environmental samples (Boalt et al., 2013).

The non-*ortho* PCBs (81, 77, 126, 169) made up only a small fraction of total dl-PCBs (0.09%). Among these highly toxic congeners, PCB 126 accounted for (58.0%) more than half of the non-*ortho* PCB content, followed by PCB169 (35.7%) and PCB77 (5.3%). On the contrary, PCB81 contributed with <1%. Interestingly, this profile is similar to those found in sperm whales from Australia (Gaus et al., 2005) and the Atlantic Ocean (Romero-Romero et al., 2017), but differs from the accumulation patterns generally found in fish and their main prey, i.e. cephalopods. In these cases, as well as many other instances of abundance patterns in biota, PCB77 is the predominant congener, followed by PCB126 and PCB169 (Cappelletti et al., 2015; Romero-Romero et al., 2017; Storelli, 2008; Tanabe et al., 1987).

Due to the lack of specific studies about the metabolism of non-*ortho* substituted PCBs in sperm whales, we hypothesize with an enhanced metabolic capability of this species towards PCB77. This hypothesis is not supported by Boon et al. (2000), who showed that PCB77 was not metabolised by sperm whales microsomes in vitro. On the contrary, the higher values of PCB126 and 169 suggested reduced or absent metabolic activities towards these congeners. This points out how more focused investigations are needed on accumulation and contaminant detoxification pathways in sperm whales and cetaceans in general. Moreover, without knowing the exact diet of the analyzed individuals as well as patterns and pollutant concentrations in their prey, caution is mandatory when trying to explain their contamination profiles. Nonetheless, in general the average dl-PCB congener profile (Fig. 2) was alike to that described by Pinzone et al. (2015) for Mediterranean sperm whales sampled between 2008 and 2013.

The PBDE congener profile, showed in Fig. 3, was dominated by lower-medium brominated congeners 47 > 99 > 100, which agrees with the fact of these congeners being generally abundant in aquatic food webs (Fossi et al., 2012; Romero-Romero et al., 2017). In fact, BDE47 is commonly the most abundant congener in most biota samples, including marine mammals (Hites, 2004). The low abundance of BDE-209, which is the main component of the commercial mixture deca-BDE (La Guardia et al., 2006), was somewhat anticipated. Its highest hydrophobicity among BDE congeners explains its heightened sequestration into suspended particulate matter and sediments, which results in low bioavailability (Lee and Kim, 2015). Moreover it shows a higher degree of chemical, microbiological degradation, and a higher degree of metabolization than lighter BDE congeners, which is thought to justify its usual absence - or lower concentrations in comparison to other BDE congeners - in marine mammals (Zhang et al., 2016). Overall, the PBDE congener profile was also similar to those described by Pinzone et al. (2015) and Zaccaroni et al. (2018) for Mediterranean sperm whales sampled between 2008 and 2014.

The PCDF congener profile (penta > hexa > tetra > hepta > octa) (Fig. 4) was relatively similar to those reported for sperm whales from Australia (Gaus et al., 2005) and to those reported in blubber of striped dolphin (*Stenella coeruleoalba*) from the Mediterranean Sea (Fossi et al., 2004). In contrast, the PCDD congener profile (hexa > penta > tetra > hepta > octa) was remarkably different from these two, with a lower concentration of higher chlorinated congeners and higher concentration of lower chlorinated congeners.

3.4. Toxicity assessment

It is well established how the toxicity of PCDD/Fs and dl-PCBs is mediated by the cytosolic receptor AhR, based on their planar spatial configuration (Mandal, 2005). Their potential toxicity is commonly assessed by the toxic equivalent quantity (TEQ) approach, in which each's congener toxicity is relativized to that of the most toxic one, the 2,3,7,8-TCDD (Schecter et al., 2006). In this study, TEQs in sperm whale blubber samples were evaluated using the toxic equivalency factors (TEFs) for mammals provided by the World Health Organization (WHO) in 2005 (Van den Berg et al., 2006). Total calculated TEQs ranged from 275 to 987 pg/g l.w.

Regardless of the wide inter-species sensitivity towards different contaminants, it should be highlighted how all analyzed sperm whales surpassed the threshold of 210 pg WHO-TEQ/g l.w. in blubber, proposed as starting point of immunosuppression in harbour seals (Ross et al., 1995).

All sperm whales in this study exhibited the same TEQ pattern (Σnon-*ortho*-dl-PCBs > Σ*ortho*-dl-PCBs > PCDDs > PCDFs), with dl-PCBs as the most abundant pollutants, in agreement with previous investigations about sperm whales and other cetaceans from the

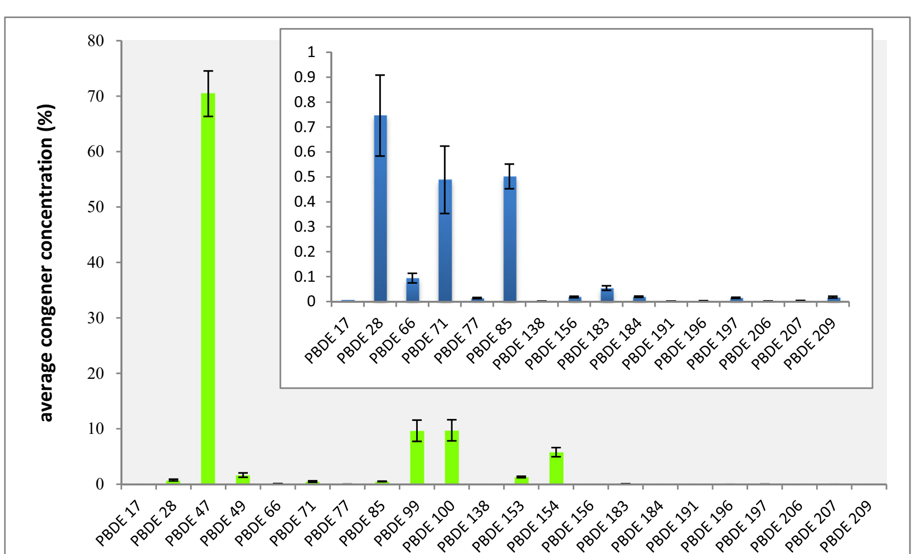


Fig. 3. Average contribution of each BDE congener to the total PBDE content. Error bars represent the standard error (SE).

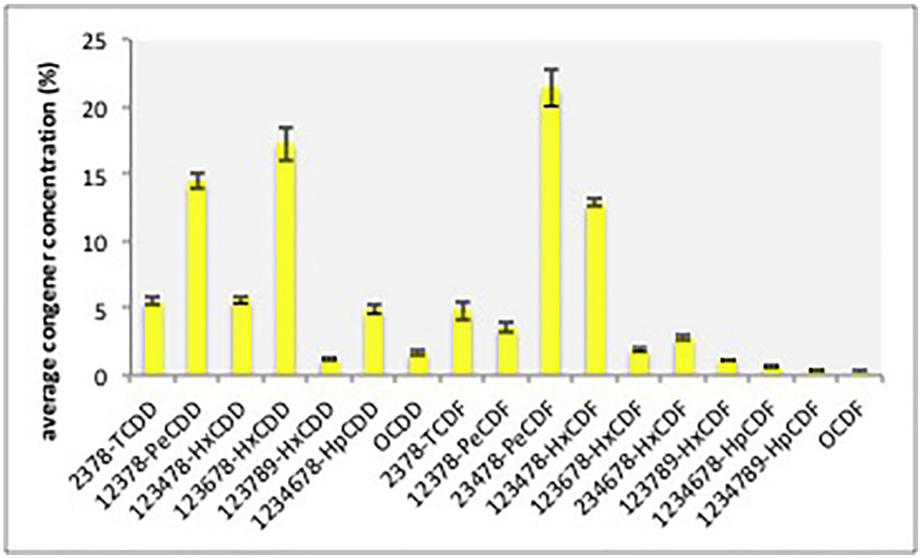


Fig. 4. Average contribution of each PCDD/F congener to the total PCDD/F content. Error bars represent the standard error (SE).

Mediterranean Sea (Fossi et al., 2004; Pinzone et al., 2015). This draws attention to the fact that, even if PCBs were banned around 40 years ago in most European countries, they continue to pose a significant threat to cetaceans in the Mediterranean Sea, where these contaminants have also been related to cetacean populations' declines (Stuart-Smith and Jepson, 2017).

Several adverse health effects have been ascribed to PBDE burdens in mammals; for example, influence on the homeostasis of steroidal and thyroidal hormones (Darnerud, 2003; Zhou et al., 2001), immunotoxicity (Fowles et al., 1994), and reproductive and other neurological disorders (Siddiqi et al., 2003). However, no specific threshold for toxicological effects in sperm whales has been proposed at the moment. Currently, the only available value reported for a marine mammal species is the threshold of 1500 ng/g l.w. established for endocrine disruption in grey seals (Hall et al., 2003). Total PBDE concentrations found in this study ranged from 312 to 1390 ng/g l.w., and therefore were always below the abovementioned reference value, although often in the same order of magnitude. However, the distinct interspecies sensitivity towards these pollutants - currently unknown in the case of sperm whales - cannot be disregarded. Aside from interspecies sensitivity, it is important to underline that, when comparing results with toxicity thresholds, each individual might respond in different ways to contaminant concentrations depending on multiple factors such as sex, age and health status.

4. Conclusions

Concentration levels found in this study were mostly in line with what has been previously reported on the same species in the Mediterranean Sea. Conversely, POPs measured in this study tended to be in higher levels than those reported on sperm whales from other geographical regions. It is important to emphasise that dioxin-like PCBs were, not only found in the highest concentrations among the target POPs, but also ranking at the top of the reported values for the same species worldwide. Our results along with data from recent works, highlight still high levels of PCBs in the Mediterranean Sea biota, which far from showing a decreasing trend during recent years appear to steady linger in this area. This also confirms the role of the Mediterranean Sea as a PCB global "hotspot" for marine mammals, and the need for further mitigation of PCB pollution to protect these sea-dwelling species. As a direct consequence of these concerning high dl-PCB concentrations, all animals in this study surpassed the threshold proposed as starting point of immunosuppression in marine mammals (harbour seals). The lack of a specific threshold for toxicological effects in sperm whales related to the presence of the target POPs does not allow to draw a solid conclusion on the role played by these contaminants in their health status. In turn, this stresses the importance of broadening our knowledge about toxicity, accumulation and detoxification processes, in order to 1) better understand the impact of pollution in the sperm whale's health status and 2) develop more focused conservation strategies, with special attention to the Mediterranean Sea due to its role as a sink for many contaminants of concern.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.10.436.

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Occurrence and distribution of persistent organic pollutants in the liver and muscle of Atlantic blue sharks: Relevance and health risks☆

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ABSTRACT

Blue shark score among the most abundant, widely distributed and worldwide consumed elasmobranchs. In this work contents of PCBs, PCDD/Fs and PBDEs were studied by means of GC-HRMS in muscle and liver of sixty blue sharks from the North East Atlantic sampled in 2019. Concentrations relatively similar were found for PCBs and PCDD/Fs in comparison with those in Atlantic specimens from the same area sampled in 2015. In contrast, PBDE loads doubled, likely mirroring the increased environmental presence of these pollutants. This, together with the different congener profiles reported for the same species in other geographical areas, highlighted the blue shark's potential as bioindicator of the degree and fingerprints of regional pollution by POPs. Interesting dissimilarities between muscle and liver concentrations were detected, most likely ascribed to distinct toxicokinetics involved for the different pollutants. Whereas most POPs preferentially accumulated in liver, some did the opposite in muscle. BDE-209 was the most prominent example, being almost negligible its presence in liver (0.3%) while accounting for ca. 14% of the total PBDE content in muscle. Different findings in this regard described for other shark species call for focused research to ascertain the role of the species in this apparent favored metabolization of BDE-209 in the liver. From a consumption perspective, the concentrations found in muscle -the most relevant part in the human diet-for PCBs and dioxin-like POPs were below the EU maximum allowed levels in foodstuff. Conversely, in liver about 58% and 78% of samples overpassed the European levels for tolerable intake of i-PCBs and dioxin POPs, respectively. Concentrations of PBDEs exceeded EQS (0.0085 ng/g w.w.) established by the European Water Framework Directive in 100% and 92% of liver and muscle samples, respectively, which adds to the open debate of such as a reduce value for this current EQS.

1. Introduction

An increasing awareness about pollution and its relevance to planetary health has progressively strengthened worldwide. As proof, today, pollution can be related to most of the 17 Sustainable Development Goals established by the UN for 2030 (Brusseau, 2019; UNEP, 2017). Persistent organic pollutants (POPs) are one prominent example of chemical pollution posing important risks and pressures to the whole planet (Jones, 2021; Jones and De Voogt, 1999). Their toxic effects along with their chemical-physical characteristics granting them the ability to 1) have long-range transportation and global distribution, 2) undergo bioaccumulation and biomagnification through food webs and 3) be persistent in all environmental compartments, make POPs a class of chemicals for which constant environmental monitoring and research is necessary and encouraged by the Stockholm Convention (SC) (UNEP, 2004; Wang et al., 2022).The existence and interaction among different processes such as wet and dry deposition, air-water exchange, continuous reception of water bodies from land, association with phytoplankton biomass, with settling fluxes of organic matter in the water column (biological pump) and also with plastics and microplastics extant in the marine environment, explain why oceans are both sinks and reservoirs for chemicals such as POPs (Froescheis et al., 2000; Jurado et al., 2004; Morales et al., 2015; Turner, 2022). Among POPs, polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) as

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unintentional substances and polychlorinated biphenyls (PCBs) and polybromodipheyl ethers (PBDEs) as manmade chemicals, are prominent examples (Muñoz-Arnanz et al., 2018; Roscales et al., 2018). The toxicity of the former, make them still today a priority family to monitor in the environment. The great quantities produced and emitted of PCBs (used in transformers, electrical equipment, hydraulic fluids, paints, sealing agents, etc.) and PBDEs (as flame retardants in a wide array of consumer products) justify an ongoing interest for gauging their environmental fate. These pollutants may be bioaccumulated by marine species whose consumption, in turn, might carry risks for other species and ultimately for human beings (Hellberg et al., 2012). These risks unavoidably increase as the POP burdens grow in the consumed species. In that sense, because elasmobranch species, encompassing sharks, rays, and skates, tend to occupy high trophic levels in food webs, they are prone to biomagnification of heightened POP loads, and therefore are among the most prominent sources of risk for human seafood consumers. Yet, currently the occurrence, tissue distribution, and toxicological effects of POPs in sharks are much less known that those in other high trophic aquatic species such as marine mammals (Lyons et al., 2021; Tiktak et al., 2020). While elasmobranchs are not generally preferred as human food source in comparison to other fish species (mainly teleosts), sharks in particular have been and continue to be consumed principally for their meat and fins in numerous areas around the globe (Dent F. and Clarke S., 2015; Tiktak et al., 2020). Additionally, shark meat is often mislabeled not only as different shark species, but also as other seafood products (Bornatowski et al., 2013; Pazartzi et al., 2019; Wainwright et al., 2018). Sharks are also valuable for other industries: their squalene–rich liver oil is coveted, for example, in the textile and tanning fields as a lubricant, in the cosmetics business, and for medicinal purposes (Kibria et al., 2015; Tiktak et al., 2020).

Despite worldwide data inconsistencies, it is certain that global shark populations have been subject to increasing pressures associated with overfishing involved in the supply of all types of shark products (Cardeñosa, 2019; Dent F. and Clarke S., 2015).

The blue shark (*Prionace glauca*) is a pelagic widespread species found in temperate, tropical and subtropical regions with a great overall abundance (IUCN, 2018). It is a long-lived species, with a lifespan of ca. 20 years, behaving as top predator with a diet composed of mainly pelagic teleost fish and cephalopods (mostly squid) (da Silva et al., 2021). Its potential as bioindicator species of marine contamination has been explored by different studies (Alves et al., 2022, 2016; Barrera-García et al., 2013; 2012; Storelli et al., 2011). Additionally, it has been commonly caught as bycatch in high-seas longline and driftnet fisheries in an increasing trend (da Silva et al., 2021). Until not very long ago it was regarded as a not very valuable catch, but its meat and fins are today making up most of the shark consumption worldwide, with an estimated global catch of almost 93,000 tons in 2020 (FAO Species Fact Sheets, 2021). As a highly-exploited species it is regarded as Near Threatened in the International Union for Conservation of Nature (IUCN, 2018) Red List, with some authors estimating that its global catch represents about 90% of the total elasmobranch caught (Coelho et al., 2012). In consequence, the determination of priority xenobiotics such as POPs in tissues of this species becomes of high importance as a proxy for the type and degree of contamination extant in their environment, and to address the potential impacts in public health due to their consumption.

Within the framework of a wider ecotoxicological investigation on blue sharks and their potential as sentinel species for marine pollution monitoring surveys, the main objective of this work was to investigate the levels of selected POPs (PCBs, PCDD/Fs and PBDEs) in two different tissues (i.e. liver and muscle) of specimens from the Northeast Atlantic Ocean, which may pose a risk to the animals, and also evaluate the risks associated to their consumption.

2. Material and methods

2.1. Sampling

Sixty blue shark specimens (38 males and 22 females) were captured as bycatch aboard swordfish fishing vessels operating off the coast of Portugal (Fig. 1) between March and December 2019. Samples of liver and muscle were obtained from each animal by means of inox cutlery, carefully rinsed with acetone and ethanol between dissections. Samples were wrapped in aluminum foil, placed in individual plastic tubes and preserved at −20 °C until further analysis. Sampling blanks were collected in form of aluminum foil handled and wrapped with the same tools and cleaning protocols. The sex and length (fork length (FL) in cm) was registered for all specimens, and data on the locations of captures were also collected (Table S1.1 in the supplementary material (SM)). Out of the 60 specimens, 38 were males with a mean FL of 180,4 cm (range 109–251 cm) and 22 females with a mean FL of 130,9 cm (range 101–160 cm). Assuming sexual maturity for males and females at 180 and 185 cm, respectively (da Silva et al., 2021), 55% of males and 100% of females were juveniles while 45% of males were regarded as adults.

2.2. Analytical procedure

Fresh samples (~1.5 g for liver and ~10 g for muscle) were homogenized and mixed with anhydrous Na_2SO_4, and subsequently spiked with a suite of ^{13}C-labeled standards of PCBs, PCDD/Fs and PBDEs. Soxhlet apparatus were used with a 100 mL of n-hexane:dicloromethane (9:1, v:v) in cycles of 24 h for the extraction of the selected pollutants. The lipid content was gravimetrically determined for each sample using a 10% aliquot of the extracted content. Extracts were purified by means of the automated system DEXTech+ (LCTech GmbH, Dorfen, Germany) that rendered two fractions for each sample. Each one was subsequently evaporated using a TurboVap® system until ~1 mL, transferred to vials, carefully and almost fully dried under N_2, and finally reconstituted in a few microliters of PCB, PCDD/F and PBDE ^{13}C-labeled injection standards in nonane. Details about sample processing are comprehensively described elsewhere (Bartalini et al., 2019) and in the SM.

2.3. Instrumental determination

Targeted analysis was focused on eighteen PCBs (the six indicators (i-PCBs) congeners # 28, 52, 101, 138, 153, 180 and the twelve dioxin-like (DL-PCBs) including 4 non-*ortho* congeners # 77, 81, 126, 169 and 8 mono-*ortho* congeners # 105, 114, 118, 123, 156, 157, 167, 189), twenty-six PBDEs (congeners # 7, 15, 17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 156, 183, 184, 191, 196, 197, 206, 207, 209) and seventeen PCDD/Fs (all seventeen 2,3,7,8 – chlorine substituted congeners). Analysis was performed by gas chromatography coupled to high resolution mass spectrometry (GC-HRMS) by means of a Trace GC Ultra gas chromatograph (Thermo Fisher Scientific, Milan, Italy) coupled to a high-resolution mass spectrometer (DFS, Thermo Fisher Scientific, Bremen, Germany) working at resolution of 10,000 (10% valley). The isotopic dilution technique was followed for quantitation purposes. A full description of the instrumental parameters is provided in the SM.

2.4. Quality assurance and quality control (QA/QC) criteria

All material, metal or otherwise, was cleaned three consecutive times with three solvents of decreasing polarity: acetone, dichloromethane, and n-hexane. Each batch of six samples included one procedural blank. Exposure to UV light was minimized throughout the entire analytical procedure. The isotopic dilution technique was applied for quantitation of the target analytes with the following criteria: (a) ratio between the two monitored ions within ±15% of the theoretical value, and (b) limits of quantification (LOQs) corresponding to S/N of 10. Final

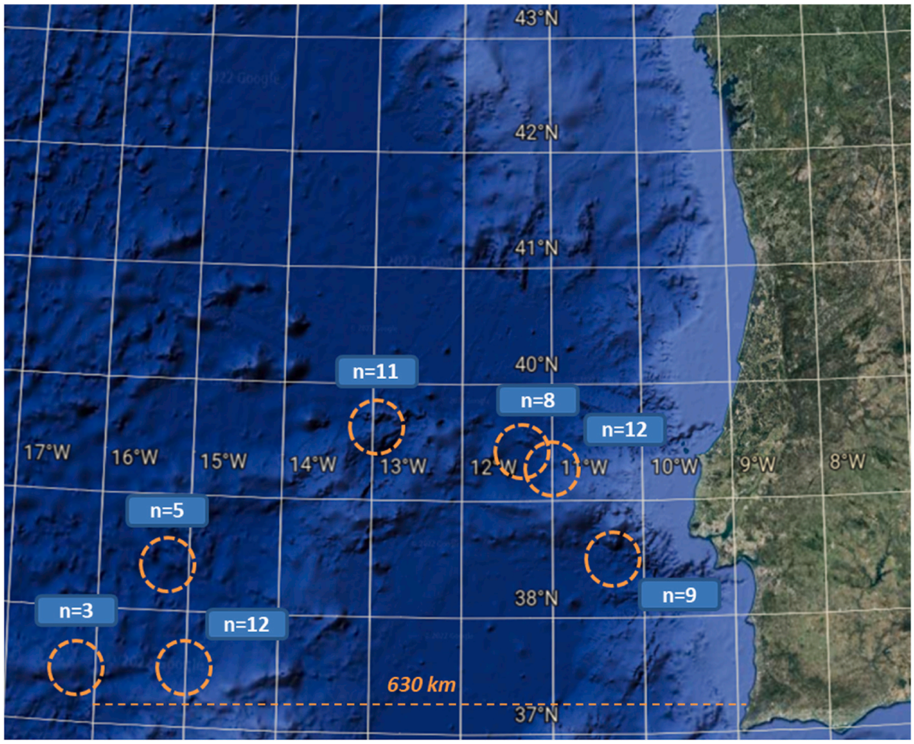


Fig. 1. Map of the locations where blue shark specimens were collected.

concentrations were blank corrected. Additional details in relation to QA/QC are provided in the SM.

2.5. Data handling

Concentrations are given in ng/g (PCBs and PBDEs) or pg/g (PCDD/Fs) on wet weight (w.w.) and lipid weight (l.w.) basis in order to maximize comparability with other studies. Toxic equivalent quantities (TEQ) for DL-PCBs and PCDD/Fs were obtained using the World Health Organization (WHO)-2005 toxic equivalency factors (TEF) for mammals (Van Den Berg et al., 2006). Data for PCDD/Fs, DL-PCBs and TEQs are reported in upper bound (i.e. substitution of non-detected compounds for detection limit values). Statistical analyses were conducted with SigmaPlot for Windows version 14.5 (Systat Software Inc, CA, USA) and IBM SPSS Statistics for Windows version 28 (SPSS Inc, IL, USA).Log transformation was applied when needed to meet the criterion of normality when exploring possible relationships between variables. When log transformation did not achieve normality (Shapiro Wilk's test, section S2.3), nonparametric tests were applied. Pearson or Spearman's correlations were explored among study variables depending on the normality (Pearson) or non-normality of data (Spearman's). A minimum significance level of $\alpha = 0.05$ was set in this study. No statistically

Table 1
Mean, median, and range of total concentrations for PCBs, i-PCBs, DL-PCBs, PBDEs, PCDDs, PCDFs and total PCDD/Fs broken down by specimens' sex in samples of liver and muscle of blue sharks. Values are given in ng/g w.w. and (ng/g l.w.) for PCBs and PBDEs while pg/g w.w. and (pg/g l.w.) for PCDDs, PCDFs and PCDD/Fs.

Compound	sex	mean		median		range	
		liver	muscle	liver	muscle	liver	muscle
∑PCBs	both	223 (690)	0.858 (392)	157 (450)	0.530 (248)	12.7–1400 (37.1–4240)	0.080–4.01 (49.6–2650)
	M	217 (686)	0.831 (364)	163 (497)	0.677 (240)	12.7–785 (37.1–4240)	0.091–4.01 (67.7–2650)
	F	233 (696)	0.933 (439)	155 (417)	0.453 (354)	193–1400 (64.4–2830)	0.080–3.54 (49.6–1840)
∑i-PCBs	both	151 (461)	0.613 (282)	97.3 (313)	0.396 (159)	7.38–983 (21.6–2240)	0.046–3.10 (25.4–1810)
	M	137 (424)	0.558 (253)	102 (321)	0.399 (155)	7.38–684 (21.6–2240)	0.046–2.44 (29.3–1810)
	F	175 (526)	0.734 (345)	96.8 (261)	0.294 (286)	12.0–983 (40.1–1980)	0.061–3.10 (25.4–1610)
∑DL-PCBs	both	72.2 (229)	0.245 (110)	47.6 (137)	0.160 (76.7)	5.32–420 (15.5–2320)	0.019–1.56 (15.3–839)
	M	80.4 (262)	0.273 (117)	57.0 (178)	0.201 (81.9)	5.32–340 (15.5–2320)	0.031–1.56 (17.8–839)
	F	58.0 (171)	0.199 (98.2)	35.9 (119)	0.141 (64.9)	6.74–420 (21.7–846)	0.019–0.895 (15.3–507)
∑PBDEs	both	14.9 (46.8)	0.090 (44.0)	10.7 (29.7)	0.075 (28.6)	1.15–69.7 (3.83–335)	0.004–0.424 (1.55–316)
	M	15.6 (49.0)	0.101 (49.8)	10.9 (33.0)	0.089 (30.8)	1.59–57.3 (4.63–335)	0.004–0.424 (4.08–316)
	F	13.8 (43.0)	0.074 (35.1)	10.4 (29.0)	0.053 (25.7)	1.15–69.7 (3.83–141)	0.006–0.245 (1.55–143)
∑PCDDs	both	2.49 (8.53)	0.320 (157)	2.28 (6.26)	0.227 (96.5)	1.07–8.48 (2.37–53.5)	0.069–2.71 (28.1–1190)
	M	2.37 (7.71)	0.225 (122)	2.10 (6.15)	0.189 (68.9)	1.28–6.86 (3.16–46.8)	0.069–0.600 (28.1–985)
	F	2.69 (9.94)	0.486 (220)	2.38 (7.00)	0.311 (174)	1.07–8.48 (2.37–53.5)	0.148–2.71 (68.3–1190)
∑PCDFs	both	8.30 (27.2)	0.194 (95.8)	7.35 (19.8)	0.165 (65.8)	1.93–23.0 (5.51–157)	0.013–0.532 (6.10–456)
	M	7.46 (23.8)	0.166 (86.0)	6.84 (18.2)	0.156 (51.6)	1.93–23.0 (5.51–157)	0.013–0.296 (6.10–456)
	F	9.75 (33.2)	0.242 (113)	8.81 (24.7)	0.238 (110)	2.16–20.9 (6.55–106)	0.102–0.532 (26.7–181)
∑PCDD/Fs	both	10.8 (35.8)	0.514 (253)	9.64 (27.0)	0.402 (148)	3.27–29.8 (8.67–204)	0.082–2.96 (37.2–1440)
	M	9.83 (31.5)	0.392 (208)	8.87 (25.2)	0.341 (125)	3.27–29.8 (8.67–204)	0.082–0.878 (37.2–1440)
	F	12.4 (43.1)	0.727 (333)	11.4 (31.9)	0.549 (315)	3.70–25.8 (8.92–160)	0.306–2.96 (128–1300)

significant differences were found for the lipid content between sexes either on liver (Mann-Whitney, U(22,38) = 360, p = 0.374) or in muscle (*t*-test(58) = -1.525, p = 0.133). No statiscally significant correlation was found either in males nor females between tissues' lipid content and FL (Table S.2.3).

3. Results and discussion

3.1. Concentration values

In both liver and muscle samples the relative abundance of target contaminants followed the order PCBs > PBDEs ≫ PCDD/Fs. A summary of the pollutant concentrations broken down by specimen' sex is compiled in Table 1 and Figs. S2a–e. The ample range of values for most of these pollutants is posited to partially reflect differences in age, sex, feeding ecology, reproductive status, and the unknown overall health status of the sampled animals, as it has been described for cetaceans (Krahn et al., 2003) and other marine mammals and fish (Borgå et al., 2004). In the liver, loads of the three pollutant families were positively correlated among them (in w.w. and l.w.) and with the animals' fork length (FL) (Table S2.4., Spearman's $r_s = 0.419–0.948$ (w.w.) and 0.281–0.923 (l.w.), $p < 0.05$, marginally in the case of PCDD/Fs with FL, $r_s = 0.239$, $p = 0.068$ (w.w.) and 0.0945, $p = 0.0547$ (l.w.)). In muscle, only PCB and PBDE burdens were positively correlated between them and with FL (Pearson and Spearman's $r/r_s = 0.286–0.826$ (ww) and 0.238–0.835 (l.w.), $p < 0.05$,Table S2.4. Instead, PCDD/Fs were not correlated with other pollutants (no correlation in w.w. and marginally correlated in l.w.) and negatively correlated with FL (Table S2.4., Spearman's $r_s = -0.441$ (w.w.) and −0.404 (l.w.), $p < 0.05$).

Differences between adult and subadult specimens could only be explored in males since all females were subadults. In both liver and muscle samples, concentrations of pollutants were statiscally significantly greater in male adult specimens in comparison to subadult male animals (in w.w and l.w., Mann-Whitney, $p < 0.05$, see specific U values and significances in Table S2.5), except for the case of PCDD/Fs in muscle, for which higher values were found in subadults (Table S2.5) even though there were not statistically significant differences between the two groups (Mann-Whitney U(17,20) = 258, p = 0.073 (w.w.) and U (17,20) = 271, p = 0.168 (l.w.)). Overall, these results were consistent with ongoing bioaccumulation of PCBs and PBDEs that share common sources in contrast with those of PCDD/Fs that, with a general lower uptake, preferentially tend to accumulate in the liver while seemingly decreasing with age in the muscle.

3.2. Sex dependency

No statiscally significant differences (Mann-Whitney, $p > 0.05$, see specific U values and significances in Table S.2.6) were found for POPs depending on sexes, neither in liver or muscle concentrations (w.w. and l.w., Table 1 and S2.6), except for the content of PCDD/Fs in muscle that scored higher in females (Mann-Whitney, U(22,38) = 164 and 163, in w. w. and l.w, both with $p < 0.001$), revealing either tissue-dependent differences in toxicokinetics between males and females for these pollutants specifically, or a strong influence of the specimens' age and developmental status since all study females were subadults while about half of males were adults. Maternal offloading of POPs is known to be a route of exposure to sharks' offspring (Marler et al., 2018; Mull et al., 2013); however, it is also known that sharks' reproductive strategy (e.g. oviparity, aplacental or placental viviparity) may play an important role in the extent and congener profile of the offloading (Chynel et al., 2021; Lyons and Lowe, 2013). For instance (Lyons and Adams, 2015), estimated a 0.03–2.3% offloading of organohalogen contaminants to their embryo in the case of hammerhead female sharks (*Sphyrna lewini*). Alike the hammerhead, the blue shark is a species following a placental viviparity strategy (da Silva et al., 2021). Assuming a similar scale of POPs' mobilization during blue shark's gestation, the transferred concentrations could represent an important load for the embryos, while, at the same time, not being high enough to make a measurable difference between male and females POP burdens. However, as previously stated, it should be stressed that based on their average size (130 ± 17 cm) most study females could not be considered sexually mature (185 to >300 cm) (da Silva et al., 2021). Therefore, they could have not experienced any gestation and thus no maternal offloading is expected yet for these individuals.

3.3. Tissue dependency

Concentration values in wet weight were up to three orders of magnitude greater in liver than in muscle. This was expected upon the lipophilic nature of these contaminants and the high metabolic activity of the liver that count with a high degree of irrigation facilitating the access and accumulation of contaminants. It is also in line with what has been reported by other authors in blue and other shark species (Alves et al., 2016; Boldrocchi et al., 2019; Kibria et al., 2015; Strid et al., 2007). This great difference in concentration values between tissues is accentuated also due to the fact that shark muscle is known to be lipid deficient (Lyons et al., 2021). Thus, while the average lipid content in liver scored 34 ± 9%, the same in muscle reached a reduced value of 0.26 ± 0.11%. However, when the concentrations were normalized according to the lipid content, results changed notably. Concentrations of PCBs and PBDEs still tended to be higher in liver, although only for the former the difference was statistically significant (Mann-Whitney, U (60,60) = 96.00 p = 0.009). Interestingly, the content of PCDD/Fs in l.w. Became significantly greater in muscle than in liver (Mann-Whitney, U (60,60) = 85.00, $p < 0.001$). Differences in toxicokinetics and particularly in metabolism rates for each tissue could be argued as an explanation even though further research is needed to clarify this outcome. The detection frequency of PCDD/Fs in muscle was significantly lower than that in liver (Table S2.1); hence, many values in the former tissue corresponded to upperbound values. Since there were no statistically significant differences between LODs obtained in both tissues, the main explanatory hypothesis leans towards an overestimation of lipid weight concentrations owing to a biased calculation of such small lipid contents in muscle samples. Though this is expected to influence all contaminant concentrations, the effect was more conspicuous in PCDD/Fs for which total loads were well below those of PCBs and PBDEs in both tissues, and, particularly, in the muscle.

3.4. Concentration values in perspective with prior studies in the same area

The blue shark is a highly mobile species undergoing long-distance and complex oceanic migrations influenced by prey availability, age and even sex (da Silva et al., 2010; Queiroz et al., 2005). Rather than reflecting the pollution status linked to specific restricted locations, it appears more convenient to interpret their body burdens as integrators of the extant pollution over wider geographic areas such as, in this case, the Northeast Atlantic Ocean. Interestingly, when comparing concentrations found in this study with those reported in a study conducted on the same species sampled at the Portuguese coast in 2015 (Alves et al., 2016), mean values and ranges [mean; range] of PCBs were overall similar although somewhat smaller in both types of tissues: liver [328; 43.2–1160 ng/g ww] and muscle [1.12; 0.197–4.38 ng/g ww]. In the case of PCDD/Fs, the range of concentrations in this study were similar to that of Alves et al. (2016) in liver [0.541–7.97 pg/g ww for PCDDs and 1.78–26.9 pg/g ww for PCDFs], but higher in muscle [0.0251–0.313 pg/g ww for PCDDs and 0.0181–0.0563 pg/g ww for PCDFs]. Given that the average animals' size in this study (162 ± 46 cm, range 101–251 cm) is greater than that in Alves et al., (2016) (juveniles ranging between 112 and 167 cm), it is unclear the reasons behind this finding, since muscle PCDD/Fs were negatively correlated with total length suggesting biodilution with age. Nevertheless, as PCDD/Fs followed a non-normal distribution with the lowest detection frequency and the lowest abundancy among the study POPs, they are prone to show greater variability in the range of values found as a result of increasing the number of sampled specimens (60 in this study *vs.* 20 in Alves et al., 2016). This, along with a steady state of biotransformation in liver according to a general low uptake of PCDD/Fs, may partially justify the mismatch found between liver and muscle levels found between both studies.

On the other hand, levels of PBDEs (mean and range in ng/g w.w.) basically doubled those found in muscle [0.054; 0.018–0.131] and liver [7.631; 1.346–28.994] reported by Alves et al. (2016). Aside from the increase in bioaccumulation related to a greater size/age of the sampled animals, this picture is in line with some increasing trends observed for PBDEs in different areas, abiotic matrices and species (Abbasi et al., 2019; Addison et al., 2020; Roscales et al., 2018; Sharkey et al., 2020). This, in turn, is likely to reflect the shorter time elapsed since full regulations on PBDEs have taken place compared to those on PCBs and PCDD/Fs. This is especially the case of deca-BDE that is still unregulated in some parts of the globe (BSFE, 2022), accounts for many specific exemptions of use until 2036 from various members of the SC (Sharkey et al., 2020), and for which an increase in its use was anticipated partially driven by the earlier prohibitions on the penta- and octa-formulations. Furthermore, PBDE oceanic levels have been and continue to be heavily influenced by the role of plastics and microplastics as long-term sources of these flame retardants (among other chemicals) owing to their migration towards the marine environment, especially when diffusion from plastic matrices is enhanced by the digestive fluids from marine biota (Turner, 2022).

3.5. *Concentration values in perspective with previous studies from other areas*

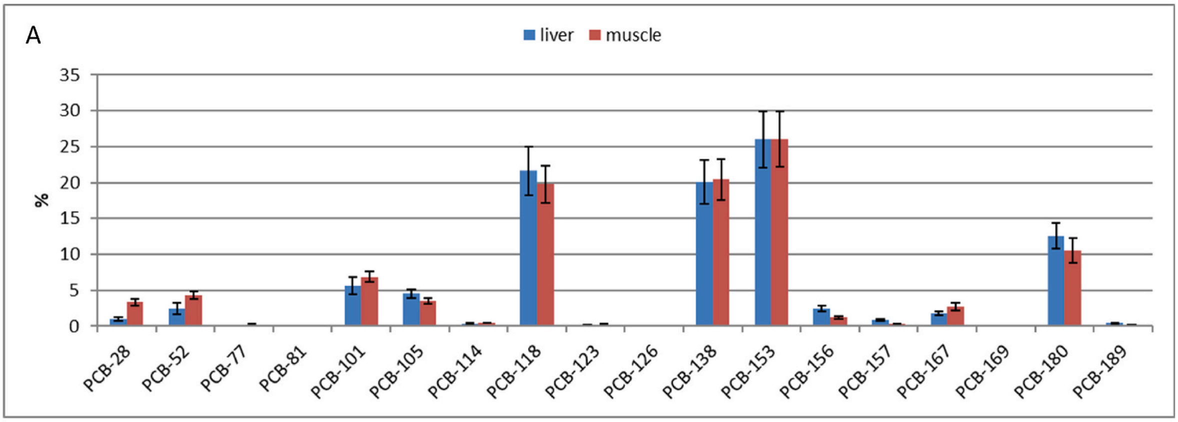
PCBs in blue shark have been reported in muscle of specimens from the Brazilian coast (430 ng/g, l.w., sampled in 2001 (de Azevedo e Silva et al., 2007),) and from coastal waters of Korea (45.7 ng/g, l.w., sampled in 2010 (Lee et al., 2015a),). They have also been reported in liver from Mediterranean specimens (2480 and 679 ng/g l.w.) sampled in 1999–2001 (Storelli et al., 2005) and 2008 (Storelli et al., 2011). This ample variability in concentrations are likely to respond to clear differences in the pollution status among areas and sampling times. Although straightforward comparisons among different studies involving different analytical approaches should be avoided, it is worth noting that PCB values in this study are in the same order of magnitude or even higher than those from the mentioned areas within a temporal arch of around 20 years. As for PBDEs, concentration of these flame retardants have been described in muscle of specimens from coastal waters of Korea (7.70 ng/g lw, sampled in 2010 (Lee et al., 2015b),) and the Equatorial Atlantic Ocean (0.05 ng/g ww, sampled in 2014/2015 (Menezes-Sousa et al., 2021),). As with PCBs, comparisons among different studies must be exerted with caution, but it is worthwhile highlighting the greater values found in the specimens of this study –between 1 and 2 orders of magnitude higher-, in line with the discussed increase of PBDE values in general and for this species in the same area. To the best of our knowledge, besides (Alves et al., 2016) there is only one study reporting data on PCDD/Fs in blue sharks, specifically in liver of animals collected in 2008 in the South-Eastern Mediterranean Sea (Storelli et al., 2011), with an average of 189 pg/g lw and 368 pg/g lw for total PCDDs and PCDFs, respectively These values are one order of magnitude greater than what was found for the Atlantic specimens in this study (Table 1). Bearing again the duly caution in direct comparisons, these results seem to reveal differences on the pollution status depending on the sampling area and time.

3.6. *Variability in congener profiles*

The frequency of detection for each investigated congener is shown in Table S2.1. Most PBDEs and all PCBs presented high detection rates in liver and muscle. For PCBs a shared pattern was found in both tissues for the most abundant congeners (Fig. 1) with PCB153 (~26%) > PCB118 (20–21%) ≈ PCB138 (~20%) > PCB180 (~12%). With some variability, this picture mirrors what has been found for the same species in other studies conducted in different oceans (Alves et al., 2016; de Azevedo e Silva et al., 2007; Gilbert et al., 2015; Lee et al., 2015a; Storelli et al., 2011), highlighting the recalcitrant characteristics of these congeners in general (Borja et al., 2005) and their resistance to metabolization by blue shark (BS) in particular. Interestingly, many of the rest of congeners showed distinct relative abundances depending on the tissue, indicating differences in their toxicokinetics (Fig. 2). The profile for the most toxic non-*ortho* congeners also varied between muscle and liver. In both tissues PCB 77 accounted for the highest contribution, followed by PCB 126 > PCB 169 ≥ PCB 81. This pattern of abundance mostly reflects on that of technical formulations and has been widely described in multiple marine species (e.g fish, shellfish, crustaceans, cephalopods (Bartalini et al., 2020)). It is similar to that reported in BS by Alves et al. (2016) although different to that found in Mediterranean BS by Storelli et al. (2011) (PCB 126 > 77>169), which is likely to reveal differences in regional PCB pollution. It is worth noting, however, that the relative abundances of PCBs 77 and 81 are notably smaller in liver. This along with the lower ratio PCB126/169 underscores the major metabolic capabilities of this tissue towards these congeners (Storelli et al., 2004).

Most PBDEs showed conspicuous differences in their relative abundances in each tissue, including the three mayor BDE congeners (Fig. 3): PBDE 47(~39–50%, muscle-liver) > PBDE 100(~14–19%) > PBDE 154 (~11–15%). The predominance of these three congeners is loosely in consonance with what has been found in liver and muscle for many shark species from different geographical areas such as waters from South Korea (Lee et al., 2015b), Japan (Nakajima et al., 2022) or southeastern USA (Weijs et al., 2015), suggesting a common behavior from a toxicokinetic perspective. Interestingly, the relative abundance profile for each congener in this study was analogous to that reported by Alves et al. (2016) on BS specimens collected in the same area. At the same time, it showed more dissimilarities with those reported for BS from the Southwest Atlantic and Indian-Pacific Oceans (Lee et al., 2015b; Menezes-Sousa et al., 2021), which appears to reflect different PBDE environmental loads.

Differences on tissue abundance heightened in the case of higher brominated congeners (hepta-to deca-). These were markedly bioaccumulated in muscle owing to different toxicokinetic behaviors, most likely pertaining to metabolic capabilities of each tissue. The example of BDE 209 is paradigmatic as it is assumed to have a low potential to bioaccumulate and biomagnify in aquatic food webs derived from its low bioavailability based on its large molecular weight (959 Da) and high hydrophobicity (Lee and Kim, 2015). Due to the enormous production and use of the commercial deca-BDE mix, this congener is today predominantly found in marine abiotic media such as sediments (Zhang et al., 2016). In this study, relative abundances of 14 and 0.3% in muscle and liver, respectively, seem to indicate a readily metabolization of this congener in the liver, despite its high Log K_{ow} (9.8 (Bao et al., 2011),) which, in principle, should favor its accumulation in this organ instead of the lean blue shark' muscle. There is paucity of data pertaining BDE 209 in marine species including sharks; nevertheless, a tissue-specific accumulation of BDE 209 has been reported in teleost fish such as *Solea* (Munschy et al., 2017) and harbor seals (Shaw et al., 2012), in both cases with preferential bioaccumulation in liver. Regarding sharks, a recent study by Nakajima et al. (2022) could not detect BDE 209 in liver from up to eight different species of deep-sea sharks from Japanese waters, though the average content of PBDEs was indeed relatively comparable with what found in blue sharks' liver in this study. Interestingly, Lee et al. (2015b) detected BDE 209 in just 4.8% of the muscle samples from 13 different shark species investigated that included BS; an outcome perhaps explained by the relatively high detection limit reached by these authors (0.63 ng/g w.w. *vs.* 0.006 ng/g w.w. in this



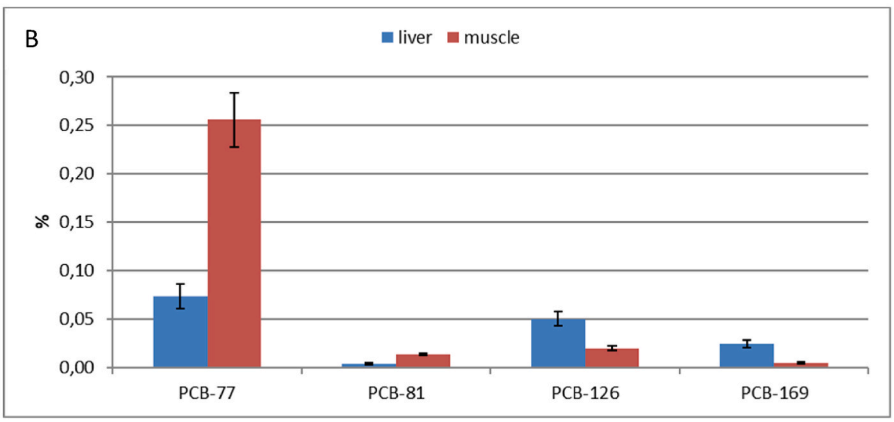


Fig. 2. Average PCB congener profile in muscle and liver of study blue sharks for all PCBs (A) and for non-ortho PCBs (B). Error bars represent standard errors (SE).

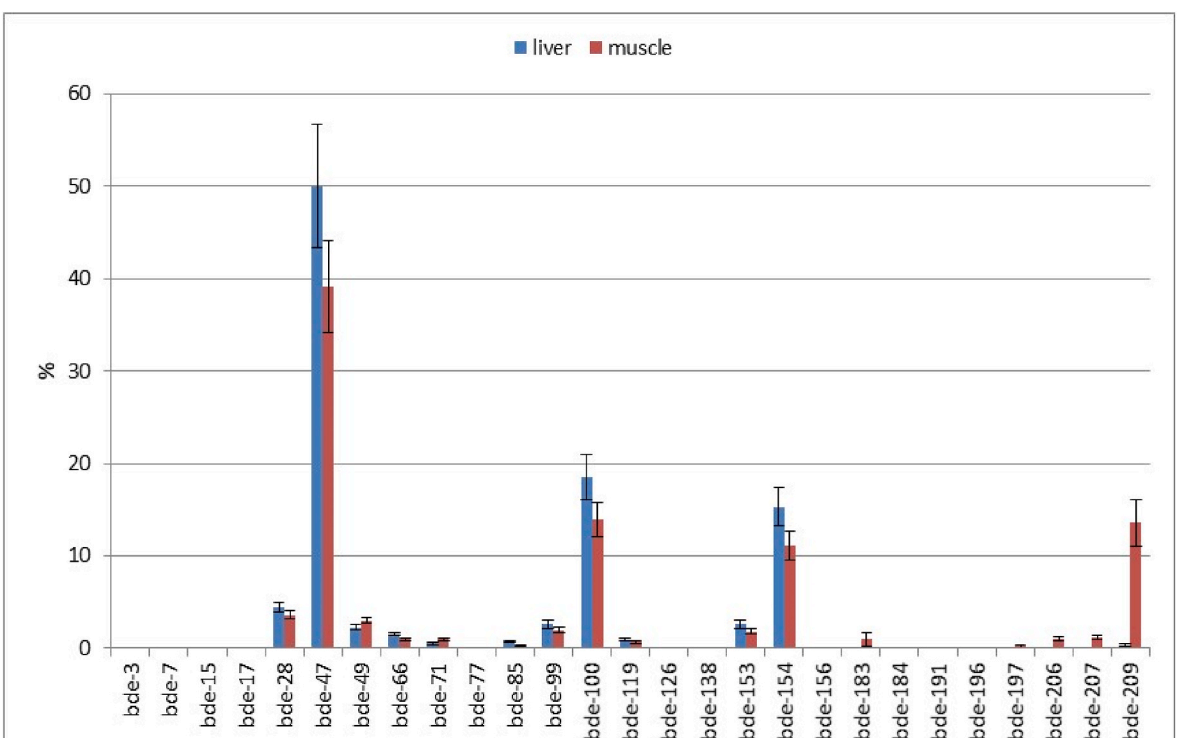


Fig. 3. Average PBDE congener profile in muscle and liver of study blue sharks. Error bars represent standard errors (SE).

study). All in all, data suggest that the factor species may play a vital role in the toxicokinetics involved for this pollutant, paving the need of further investigation in other elasmobranchs for this apparently favored biotransformation of BDE 209 in liver.

As was observed for PBDEs, although with a much reduced detection rate especially in muscle (Table S2.1), the abundance profile for PCDD/F congeners was also markedly tissue dependent (Fig. 4). PCDDs accounted for 23% and 63% of the total PCDD/F content in liver and muscle, respectively, with complementary percentages of 77% and 37% for PCDFs. Congeners OCDD (~22%) and 1,2,3,4,6,7,8-HpCD (~11%) were dominant in muscle, while a noticeable predominance of 2,3,7,8-TCDF (~29%) > 2,3,4,7,8-PeCDF (~23%) > 1,2,3,7,8-PeCDF (~8%) was found in liver. This profile of relative abundance is remarkably close to that found by Alves et al. (2016) in Atlantic specimens before, and yet very different from the only two studies - to the best of our knowledge - reporting PCDD/Fs in sharks: Storelli et al. (2011) in Mediterranean BS (34% and 66% of PCDDs and PCDFs in liver with 2,3,7,8-TCDF > 2,3,7,8-TCDD > 1,2,3,6,7,8-HxCDD > 2,3,4,6,7,8-HxCDF as major contributors) and Strid et al. (2007) in Greenland sharks from Icelandic waters (22% and 77% for PCDDs and PCDFs in muscle while 13% and 87%, respectively, in liver, with overall predominance of 2,3,7,8-TCDF, no detection of octa-congeners, and variable but important contributions of 2,3,4,7,8-PeCDF; 2,3,7,8-TCDD; 1,2,3,7,8-PeCDF; 1,2,3,7,8-PeCDD and 1,2,3,6,7,8-HxCDD). Apart from the common prevalence of 2,3,7,8-TCDF in liver, and without ruling out inter-species differences in the toxicokinetics of these pollutants, the clear dissimilarities for the homolog groups' abundance among these studies are likely to respond to different regional (and temporal) pollution fingerprints.

3.7. Toxicity and compliance with legal limits

Potential adverse effects of POPs on elasmobranch's health remain poorly studied and in consequence toxic thresholds are yet to be established. For marine mammals, in blubber, commonly used toxic thresholds are 1500 ng/g l.w. for PBDEs (set for endocrine disruption in grey seals (Hall et al., 2003)) or 9 μg/g l.w. for PCBs (onset of physiological impacts (Jepson et al., 2016)). No animal in this study reached those limits, with average values of PCBs and PBDEs scoring from one to three orders of magnitude lower instead (Table 1). However, as previously stated, more investigations are needed to evaluate possible impacts of these POP concentrations in BS populations.

Values in upper bound WHO-TEQ_{2005} were calculated based on DL-PCB and PCDD/F concentrations in order to quantify their toxicity potential against humans (Table 2). Due to the conspicuous toxicokinetics' variability discussed for these pollutants on each tissue, notable differences between liver and muscle were observed not only in total values (two orders of magnitude higher in liver on average), but also in the relative contributions to total TEQs. Thus, while DL-PCBs in liver accounted for ~88% of total TEQs, PCDD/Fs were responsible of ~77% of total TEQs in muscle (with PCDDs alone responsible for ~63%). Regarding sharks as food products, at European level Regulation 1259/2011 and Directive 2013/39/EU set a maximum level (ML) in muscle meat of fish and fishery products of 6.5 pg WHO-TEQ/g (w.w.) for PCDD/Fs and DL-PCBs and a ML of 75 ng/g (w.w.) for the six i-PCBs. Additionally, Regulation 1259/2011 also establishes a maximum of 3.5 pg WHO-TEQ/g (w.w.) just for PCDD/Fs. As previously stated, shark livers are not commonly part of the human diet, although the consumption of liver-derived products is not rare. In this study, about 58% of liver samples exceeded the ML for the i-PCBs, ~78% did the same for the 7.5 pg TEQ/g limit when considering PCDD/Fs + DL-PCBs, and about 38% exceeded the limit of 3.5 pg TEQ/g when considering just PCDD/Fs. On the other hand, shark meat in different forms (fins or fillets) is consumed in many parts of the world. Yet values found in muscle for all these parameters fall well below – from one to two orders of magnitude - the established limits, and so do not seem to pose significant risks for human health. For instance, assuming the average value of 0.115 pg WHO-TEQ/g for total TEQs in muscle obtained in this study along with an average human body weight of 70 Kg, a consumption per person of over 1.2 Kg of BS muscle alone per week would be needed to meet the conservative's EFSA's tolerable weekly intake (TWI) of 2 pg WHO-TEQ/Kg body weight/week for dioxins and dioxin-like PCBs in food (Knutsen et al., 2018). To put this figure in context, according to FAOSTAST (https://www.fao.org/faostat/en/#data/FBS), the weekly consumption of pelagic fish in 2019 in Portugal and Spain (top two fish consumers in the European Union) per person was ~0.22 Kg and ~0.15 Kg, respectively.

Pertaining PBDEs (considering congeners BDE-28, -47, −99, −10, −153 and −154), the European Water Framework Directive (WFD) establishes the value of 0.0085 ng/g w.w. Environmental quality standard (EQS) for biota based on human toxicity. As a result of such a reduced value, 100% and 92% of liver and muscle samples, respectively, overpassed this EQS. This picture is common to most studies, raising up criticising about the usefulness of a such small environmental limit that systematically is exceeded (Castro-Jiménez et al., 2021). To the best of our knowledge there is no TWI established for PBDEs. Yet, for congeners BDE-47, -99, −153 and −209 the US Environmental protection Agency set out chronic oral reference doses whereas the US Agency for Toxic Substances and Disease Registry set out acute/subchronic minimal risk levels. Values for these limits were congener-dependent varying from 100 ng/kg/day (BDE-47 and -99) to 7000 ng/Kg/day (BDE-209). The

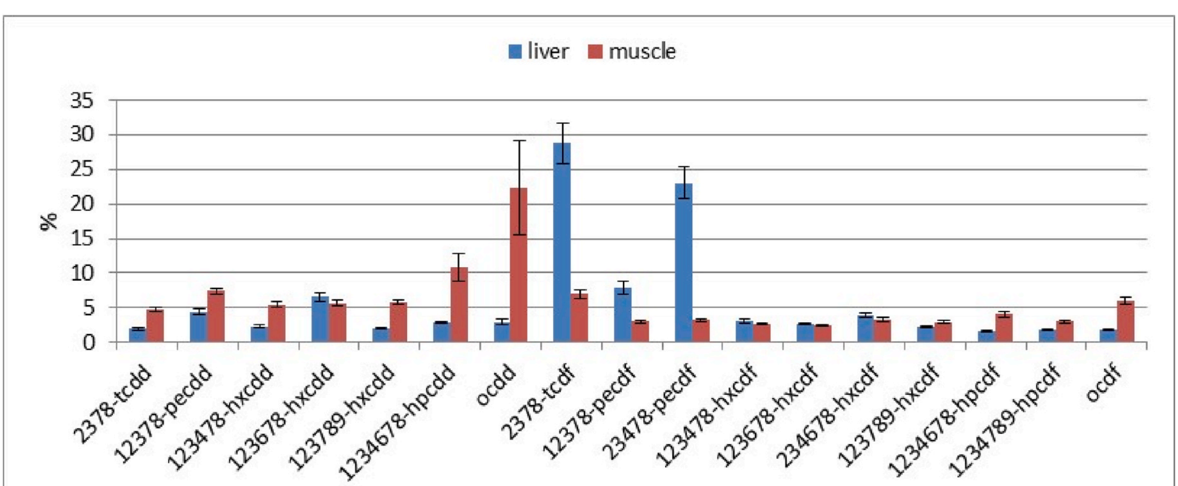


Fig. 4. Average PCDD/F congener profile in muscle and liver of study blue sharks. Error bars represent standard errors (SE).

Table 2
TEQs for DL-PCBs, PCDDs, PCDDs and PCDD/s, and average contribution to total TEQs calculated for samples of liver and muscle of blue sharks. Data are given in pg WHO-TEQ/g w.w and (pg WHO-TEQ/g l.w) and are showed as mean, median and range.

WHO-TEQ_{2005}	Average contribution (%)		mean		median		range	
	liver	muscle	liver	muscle	liver	muscle	liver	muscle
TOTAL	100	100	17.1 (85.8)	0.115 (54.3)	14.4 (66.0)	0.108 (42.3)	2.52–108 (19.1–709)	0.070–0.340 (16.1–205)
$TEQ_{DL-PCBs}$	88.2	23.0	15.1 (50.0)	0.027 (12.1)	11.1 (31.5)	0.022 (9.46)	1.88–101 (4.62–689)	0.005–0.105 (1.05–80.7)
TEQ_{PCDDs}	4.7	63.3	0.807 (2.81)	0.073 (34.5)	0.630 (1.81)	0.064 (24.1)	0.228–3.06 (0.584–20.9)	0.042–0.244 (9.89–97.1)
TEQ_{PCDFs}	7.1	13.7	1.21 (3.99)	0.016 (7.61)	1.07 (2.67)	0.014 (5.01)	0.178–3.70 (0.544–25.3)	0.008–0.044 (1.99–30.1)
$TEQ_{PCDD/Fs}$	11.8	77.0	2.02 (6.79)	0.088 (42.2)	1.61 (5.28)	0.077 (29.0)	0.592–6.76 (1.57–46.1)	0.053–0.288 (12.1–125)

highest value found in this study for total PBDEs in BS was 69.7 ng/g in liver, which would translate into a figure of 29.9 ng/kg/day assuming an average body weight of 70 Kg and the highest Portuguese daily pelagic fish consumption of 0.03 Kg/day; therefore, an absence of risk in regard to PBDEs associated with the consumption of the study BS could be inferred from these values.

4. Conclusions

Today elasmobranchs are facing important pressures such as overfishing and chemical pollution. Overall, this study contributes to enhance our knowledge on the occurrence, distribution, and fate of POPs in Atlantic blue sharks, which are among the most abundant species of elasmobranchs and the most commonly by-catched. Our results suggested differences in POPs' toxicokinetics for the two tissues investigated, i.e. liver and muscle. As expected, a greater accumulation was found for many PCBs, PBDEs and PCDD/Fs in liver. Concurrently, this tissue's higher biotransformation rate seemed to hinder this same pattern for all POPs. Among the most conspicuous cases were those of higher brominated PBDEs, particularly BDE 209 that appeared to be readily metabolized in liver while bioaccumulating in muscle. Inconsistent data from other shark species in this regard calls for further investigation to gauge the species factor for such behavior.

Under the comparative framework drew by previous studies with BS, results from this research showed relatively steady concentrations of PCBs and PCDD/Fs, whereas greater loads of PBDEs. This builds up on the existing data from other media, and particularly in the marine environment. Interestingly, this may also reflect the contribution of multiple factors such as the more recent and globally incomplete regulations on PBDEs, as well as heightened PBDE burdens derived today from plastics and microplastics. All in all, similarities as well as marked differences with pollutants' abundance profiles reported in other studies highlighted the role of BS as bioindicators of the degree and fingerprints of regional pollution by POPs.

The relevance of the POP loads found for the health of the animals is unknown because of the lack of toxic thresholds in BS or any other elasmobranch. Concentrations were, however, below the lowest thresholds identified in marine mammals for PCBs and PBDEs. From a human consumption perspective, the concentrations found in muscle –the most consumed part of sharks – were compliant with European PCB and PCDD/F legal limits. Conversely, most liver samples overpassed limits for i-PCBs and WHO-TEQs, which may be of importance when considering the consumption of oil-related products. Regarding PBDEs, the extremely low values set by the European WFD meant a generalized non-compliance of the samples analysed in this study, which adds to the open debate about the existence of such unreachable legal values.

Credit author statement

Juan Muñoz-Arnanz: Analytical Methodology, Data curation, Investigation, Writing – original draft. Alice Bartalini: Analytical Methodology, Writing – Reviewing and Editing. Luís Alves: Investigation; Writing – Reviewing and Editing. Marco Lemos: Conceptualization, Writing – Reviewing and Editing. Sara Novais: Conceptualization, Supervision, Writing – Reviewing and Editing, Funding acquisition. Begoña Jiménez: Supervision, Writing – Reviewing and Editing, Funding acquisition.

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.

Data availability

Data will be made available on request.

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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.2022.119750.

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Relevance of current PCB concentrations in edible fish species from the Mediterranean Sea

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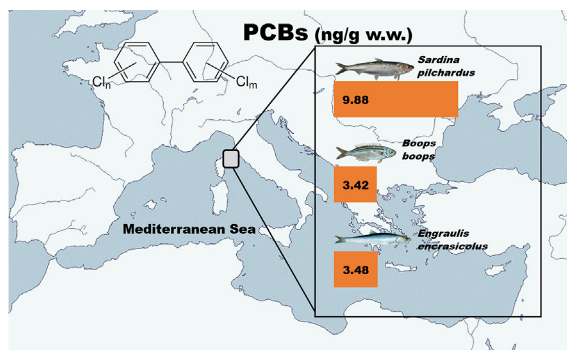
HIGHLIGHTS

- PCBs quantified in three common edible fish species in the Mediterranean Sea
- PCB concentrations in compliance with EU and WHO regulated concentrations in diet
- PCB concentrations were often not different from those reported in last years.
- Inefficient mitigation measurements towards PCB contamination are suggested.

GRAPHICAL ABSTRACT



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ABSTRACT

Legal restrictions and bans have led to a steady decrease in PCB environmental concentrations. Yet, in recent years PCBs have been found at very high levels in the Mediterranean Sea, for instance, in some apex predators. This work aimed to investigate current PCB (eighteen congeners: #28,52,77,81,101,105,114,118,123,126,138,153,156,157,167,169,180,189) concentrations in the Mediterranean Sea and their relevance today, focusing on their occurrence in edible fish species typically consumed in the Mediterranean diet. In spring 2017, a total of 48 fish samples from the Northern Thyrrenian Sea were collected: 16 specimens of sardine (*Sardina pilchardus*), 16 of anchovy (*Engraulis encrasicolus*) and 16 of bogue (*Boops boops*). PCBs were quantified in the muscle of the animals by means of GC-QqQ-MS. They were found in all samples at the greatest concentrations (ng/g w.w.) in sardine (4.15–17.9, range), and very similar values between anchovy (1.01–7.08) and bogue (1.46–7.22). WHO-TEQ PCB values followed the same order, i.e. sardine (0.410–1.24, range in pg/g w.w.) > anchovy (0.0778–0.396) ~ bogue (0.0726–0.268). These concentrations lied below the European limits of 75 ng/g (w. w.) for the six indicator PCBs and 6.5 pg/g WHO-TEQ for dioxins and dioxin-like PCBs in muscle meat of fish. Additionally, estimated weekly intakes (EWI, in pg WHO-TEQ/Kg/week) for sardine (1.2), anchovy (0.29) and bogue (0.35) scored below the safe value proposed by EFSA of 2 pg WHO-TEQ/Kg/week. When comparing with data reported for the same species in previous Mediterranean studies, values found here were lower than those surveyed in the late 90s and early 2000s; however, they were often not notably different from concentrations reported in last years. This builds up on the concept of a current slow decrease of PCBs in the Mediterranean Sea, likely linked to new inputs and/or remobilization of burdens, and reinforces the need of continous monitoring of these legacy contaminants still ubiquitous today.

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1. Introduction

Polychlorinated biphenyls (PCBs) are man-made chemicals first produced as complex mixtures in 1929 (De Voogt and Brinkman, 1989) and for nearly 50 years they were largely used with a worldwide production estimated at over 1 million metric tons (Breivik et al., 2002; WHO, 1993). The commercial utility of these synthetic halogenated aromatic hydrocarbons (e.g. transformers, capacitors, electrical equipment, hydraulic fluids, etc.) was chiefly based on their high physical and chemical stability, which is also responsible for their toxicity and persistency in the environment. In consequence, in the 1970s and 1980s, many countries restricted their marketing and use, and further in 2001, PCBs were classified as persistent organic pollutants (POPs) under the Stockholm Convention (SC) on POPs. The SC entered into force in 2004 with 90 countries agreeing to ban all production of PCBs (UNEP, 2001), to phase out all uses of these chemicals in equipment by 2025, and to ensure the destruction of remaining PCBs by 2028 (Stockholm Convention, Annex A, Part II (a), (e)). Today there are 182 parties in the SC with some relevant exceptions, including Italy and the United States, that have not ratified the Convention (Stockholm Convention, 2019).

In response to the wide ban on these chemicals, most investigations have depicted an overall slow but steady decrease in PCB environmental concentrations (Bonito et al., 2016; Hammer et al., 2016; Ross et al., 2013). Yet, in recent years some authors have focused their attention on high PCB levels still found in different marine mammal species, raising the question about the effectiveness of the current European and global mitigation efforts within a perspective of complete elimination of these harmful substances (Hens and Hens, 2017; Jepson and Law, 2016; Stuart-Smith and Jepson, 2017).

These carcinogenic and mutagenic substances tend to accumulate in fatty tissues and biomagnify along food webs, with deleterious impacts and a potential role in populations' decline especially in species at high trophic levels (Hammond et al., 2009; Jepson et al., 2016). Impair reproduction and disruption of the endocrine and immune systems are among some of the toxic effects observed in vertebrates (Jepson et al., 2005; Kannan et al., 2000; Law et al., 2012; Letcher et al., 2010; Safe et al., 1985). PCBs seem to exert a negative impact also on species at lower levels of trophic webs such as fish. Several studies have shown the presence of these contaminants and their toxic effects on different fish species from all over the world (Brar et al., 2010; Henry, 2015; Hontela et al., 1992; Teh et al., 1997). Among those studies, some have also drawn attention on possible negative impacts on human health resulting from the consumption of contaminated fish (Jiang et al., 2005; Mozaffarian and Rimm, 2006; Sidhu, 2003). Human exposure to these harmful substances start in the early stages of life and continues throughout the entire lifespan, potentially leading to adverse health effects such as cancer (Pavuk et al., 2004), birth defects (Wigle et al., 2008) and dysfunctional immune (Kramer et al., 2012) and reproductive systems (Nicolopoulou-Stamati, 2001). This issue acquires a remarkable relevance in regions where fisheries play an important economic role and fish consumption is an integral part of people's diet, such as the Mediterranean Sea (FAO, 2019).

It is well known that the semi-enclosed Mediterranean Sea is an area particularly susceptible to pollution and it is considered a sink for PCBs and other POPs, probably due to its geographical configuration and location, and the intense anthropogenic pressure to which it is subject (Castro-Jiménez et al., 2013; Marsili et al., 2018). In last years, most researches depicted a slow decrease of PCB levels in different fish species of this area at the same time as other authors reported high levels of these pollutants in some marine mammal populations confirming its role as a marine mammal PCB hotspot (Stuart-Smith and Jepson, 2017). PCBs are still detected in human milk (Çok et al., 2012) and blood (Ulutaş et al., 2015) of Mediterranean human populations, in some cases without a significant decrease in the last few years (Consonni et al., 2012). This picture seems to indicate potential new inputs and/or remobilization of PCBs in the Mediterranean area (Josefsson et al., 2010), which most likely also denotes inefficient mitigation measures and the ineffectiveness of global efforts towards PCB contamination. This is in concert with what is suggested by others authors (Stuart-Smith and Jepson, 2017) who point out the inability of some countries to achieve the 2025 and 2028 targets of the SC at the current rate of mitigation and reduction of PCB environmental levels.

Italian fisheries are among the most important in the whole Mediterranean region with an estimated capture production in 2014–2016 about 185,300 tons (16% of Mediterranean total landings), second only to Turkey (321,800 tons and 32% of Mediterranean total landings) (FAO, 2019). The whole catch composition of the marine Italian fisheries is very heterogeneous, but it is essentially based on two different pelagic species, namely anchovy and sardine (FAO, 2015). These two small pelagic fish species, with high ecological relevance -they transfer energy from lower to upper trophic levels (Costalago et al., 2012) - accounted for 42% (sardine) and 19% (anchovy) of the total landing volume in the central Mediterranean Sea in 2014–2016 (FAO, 2019).

Marine fish and other seafood constitute an important food source for human consumption accounting for about 17% of animal protein consumed by the global population in 2015 (FAO, 2018). Despite the beneficial effects provided by a fish-rich diet (high quality protein, minerals, essential trace elements, fat-soluble vitamins and essential fatty acids), fish consumption is considered one of the most important sources of POP exposure in humans (Pan et al., 2016), far-famed to elicit adverse health effects since early stages of life (Jacobson et al., 1990). In last years, the World Health Organization (WHO) established different toxicological reference values, in order to ensure that people are not exceeding certain body burdens that could adversely affect human health. One of the most often used is the tolerable weekly intake (TWI), recently revised by The Expert Panel on Contaminants in the Food Chain (CONTAM) of the European Food Safety Authority (EFSA). Pointing out how these pollutants remain a serious concern to human health, this panel of experts set a new TWI for dioxins and dioxin-like PCBs in food of 2 pg WHO-TEQ/Kg body weight, seven times lower than the previous TWI (14 WHO-TEQ/Kg body weight) set by the European Commission's former Scientific Committee on food in 2001 (Knutsen et al., 2018). At European level, where attention has been placed in recent years to the ineffectiveness of the efforts towards the elimination of these pollutants, Commission Regulation (EU) No 1259/2011 established a Maximum Level (ML) of 6.5 pg/g WHO-TEQ (w.w.) for dioxins and dioxin-like PCBs in muscle meat of fish and fishery products and a ML of 75 ng/g (w.w.) for the sum of the six indicator PCB congeners (PCB 28, 52, 101, 138, 153 and 180).

This study aimed to investigate current concentrations of PCBs in three fish species of the Mediterranean Sea (anchovy, sardine and bogue) of great commercial interest in order to: 1) further provide data on PCBs in Mediterranean fish species to help understand whether or not a conspicuous decrease trend for these contaminats still holds, and 2) evaluate the potential health risk derived from PCB human intake through diet.

2. Materials and methods

2.1. Sampling

In spring 2017, three important commercial edible fish species commonly consumed in the Mediterranean region were purchased from local fish markets in Livorno. The fish species, sardine (*Sardina pilchardus*), anchovy (*Engraulis encrasicolus*) and bogue (*Boops boops*), were collected in the Northern Thyrrenian Sea. Sixteen specimens for each species were dissected and the flesh weighted and lyophilized. Detailed information about sampling and specimens analyzed, are reported in table S2 in the Supplementary Material (SM).

2.2. Sample processing

Samples were analyzed following the procedure described in Muñoz-Arnanz et al. (2016) with some modifications. Briefly, fresh muscle samples, taken from the dorsal area of all specimens, were freeze-dried during 24–48 h with an Edwards lyophilisation apparatus and their water content was determined gravimetrically. Fresh weight and water content (%) for each sample can be found in table S3. Around 1 g of lyophilized muscle of each sample was homogenized with anhydrous sodium sulfate (Na_2SO_4) and spiked with a suite of ^{13}C-labeled standards of PCBs, prior to Soxhlet extraction (24 h) with a mixture of n-hexane: DCM (9:1). Resulting extracts were rota-evaporated and purified by low-pressure chromatography on open columns packed with different layers of silica gel, i.e. neutral and modified with sulfuric acid (H_2SO_4). Final extracts were evaporated using a TurboVap® (Zymarck Inc., Hopkinton, MA, USA) system until ~1 mL, transferred to vials, and dried by means of a gentle nitrogen stream. Prior to instrumental analysis, each sample was reconstituted with a few microliters of ^{13}C-labeled PCBs. Full details about sample processing can be found in the Supplementary Material (SM).

2.3. Instrumental determination

Eighteen PCBs (#28,52,77,81,101,105,114,118,123,126,138,153,156, 157,167,169,180,189) were analyzed in all samples and quantified by gas chromatography coupled to high resolution mass spectrometry (GC-HRMS) using a Trace GC Ultra gas chromatograph (Thermo Fisher Scientific, Milan, Italy) coupled to a high-resolution mass spectrometer (DFS, Thermo Fisher Scientific, Bremen, Germany). Quantitation was based on the isotopic dilution technique. A comprehensive description of the instrumental parameters can be found in Bartalini et al. (2019) and in the SM.

2.4. QA/QC criteria

To guarantee the accuracy of obtained data for the analytes targeted in our study, several quality assurance and quality control measures were undertaken during sample preparation and analysis. Three solvents of decreasing polarity (acetone, dichloromethane and n-hexane) were used to clean (three times for each solvent) metal and glassware material. A procedural blank was analyzed within each batch of six samples. Care was taken to minimize exposure to UV light throughout the entire analytical procedure. Quantification of all target analytes was carried out by the isotopic dilution technique according to the following criteria: (a) ratio between the two monitored ions within ±15% of the theoretical value, and (b) limits of quantification (LOQs) corresponding to S/N of 10. Final concentrations were blank corrected. Additional details pertaining QA/QC in the quantification procedure including LOD values and surrogate recoveries are provided in the SM.

2.5. Data handling

All concentration values are provided in wet weight (w.w.) basis. Concentrations of mono-*ortho*-dl-PCBs (105, 114, 118, 123, 156, 157, 167, 189) and International Council for the Exploration of the Seas-7 (ICES-7) PCBs (28, 52, 101, 118, 138, 153, 180) are given in ng/g, while concentrations of non-*ortho*-dl-PCBs (77, 81, 126, 169) and TEQ values are given in pg/g.

To assess the potential toxicity of dl-PCBs we followed the total toxic equivalency (TEQ) approach, in which each congener's toxicity is relativized to that of the most toxic one, the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) by means of toxic equivalency factors (TEF) (Van den Berg et al., 2006). Data on dl-PCBs and TEQs are reported in upper bound (i.e. substitution of non-detected compounds for detection limit values). An estimated weekly intake (EWI) of dl-PCBs was calculated by multiplying the weekly pelagic and demersal fish consumption data for Italy, (85 g/week for sardine and anchovy and 135 g/week for bogue (FAOSTAT, 2013)) by the mean WHO-TEQ concentration of dl-PCBs in muscle of analyzed fish (sardine, bogue, anchovy), and dividing by 70 Kg as the average human body weight, as described in the formula below.

$$EWI = \frac{WFC\ X\ WHO-TEQ\ dl-PCBs}{HBW}$$

EWI = estimated weekly intake (pg/week)/KgWFC = weekly fish consumption (g/week)dl-PCBs = dioxin-like-PCB concentration (pg WHO-TEQ/g w.w.) HBW = human body weight (70 Kg)

3. Results and discussion

3.1. Concentration values

All 18 PCBs were detected in all samples analyzed with the exception of PCB81, which was not detected in anchovy. Target PCBs were found at concentrations ranging between 1.01 and 17.9 ng/g w.w. A detailed description of PCB levels is presented in Table 1. Sardine, the species with the highest fat content, showed greater concentrations of contaminants with a mean value of 9.88 ng/g w.w. (median = 9.12 ng/g w.w.; range = 4.15–17.9 ng/g w.w.). Bogue and anchovy showed similar average levels among them, about three times lower than those of sardine, 3.42 ng/g w.w. (median = 2.88 ng/g w.w.; range = 1.46–7.22 ng/g w.w.) and 3.48 ng/g w.w. (median = 2.92 ng/g w.w.; range = 1.01–7.08 ng/g w.w.), respectively. This is in line with previous studies in which sardines from the Mediterranean and Marmara Seas, and purchased in Spanish markets, seem to be from two to three times more contaminated than anchovies (Coelhan et al., 2006; Miniero et al., 2014; Perelló et al., 2015). Differences in PCB contamination between species could be explained by multiple factors; for instance, different lipid content and feeding behavior between species. Even if sardine and anchovy are both considered planktivorous species with a general niche overlapping during some seasons, several studies also reported differences in

Table 1
Mean, median and range of PCB concentrations (in ng/g w.w. or pg/g w.w. when indicated) in muscle from the three Mediterranean Sea fish species investigated.

	Sardine (*Sardina pilchardus*)				Bogue (*Boops boops*)				Anchovy (*Engraulis encrasicolus*)			
	concentration (ng/g w.w.)											
	Mean	Median	Range		Mean	Median	Range		Mean	Median	Range	
∑ICES7	9.32	8.56	3.88	16.9	3.27	2.77	1.39	6.90	3.34	2.78	0.991	6.80
six indicator PCBs*	8.50	7.81	3.57	15.52	3.03	2.62	1.28	6.25	3.15	2.66	0.972	6.44
∑non-*ortho*-dl-PCBs (pg/g w.w.)	17.6	14.4	9.10	40.5	4.12	3.10	0.854	15.3	3.30	3.36	7.76	9.27
∑mono-*ortho*-dl-PCBs	1.35	1.36	0.577	2.62	0.384	0.296	0.174	1.058	0.322	0.272	0.0417	0.757
∑dl-PCBs	1.37	1.38	0.587	2.66	0.388	0.299	0.176	1.07	0.325	0.276	0.0424	0.776
∑PCBs	9.88	9.12	4.15	17.9	3.42	2.88	1.46	7.22	3.48	2.92	1.01	7.08

* Six indicators PCB congeners: 28, 52, 101, 138, 153, and 180 (Commission Regulation (EU) No 277/2012).

feeding strategies and target prey, and therefore, slight distinct trophic levels (Costalago et al., 2012). Values found in this survey depict a level of contamination significantly lower (1.6 to 10.6 times lower depending on the species) when comparing with PCB concentrations reported in the late 90s or early 2000s (Fig. 1 and table S5). In particular, anchovy caught in the Adriatic Sea in 1998 and in the Gulf of Naples in 2003, reached levels of contamination about two orders of magnitude higher (Bayarri et al., 2001; Naso et al., 2005) than current levels found in this study. However, and taking into consideration the wide range of values reported for sardine and anchovy, values found in this study are not significantly lower – but higher in some occasions - than those measured in last years (Fig. 1 and table S5). Sardine obtained from Spanish markets in 2005, 2008 and 2012 (Martí-Cid et al., 2007; Perelló et al., 2012, 2015) and specimens caught in the Marmara Sea in 2008 (Coelhan et al., 2006) showed contamination levels about two times higher than values reported here (Fig. 1 and table S5). Moreover, a study performed in the Adriatic Sea by Vuković et al. (2018) reported PCB values for sardine up to five times higher than those reported here. Instead, anchovies analyzed in this same study in 2014 reached values about just one order of magnitude higher.

Bibliographic data on PCB concentrations in bogue are scant in the Mediterranean Sea. ICES7 values reported in 2011 for caught fish in the Adriatic Sea (Storelli et al., 2011) are in the same order of magnitude than those reported in this study, six years later. Conversely, study specimens in 2017 from the Atlantic Ocean (Canary Island) showed levels of PCBs about one order of magnitude higher (Henríquez-Hernández et al., 2017).

3.2. Congener profiles

The profile of PCB congeners showed in Fig. 2 was dominated by penta-(#101,118), hexa-(#138,153) and hepta-(#180) chlorinated congeners. The same relative abundance PCB-153 > 138 > 180 was observed in the three species, in line with what is commonly found in marine fish (Ben Ameur et al., 2013; Henríquez-Hernández et al., 2017; Naso et al., 2005; Vuković et al., 2018). The presence of these highly

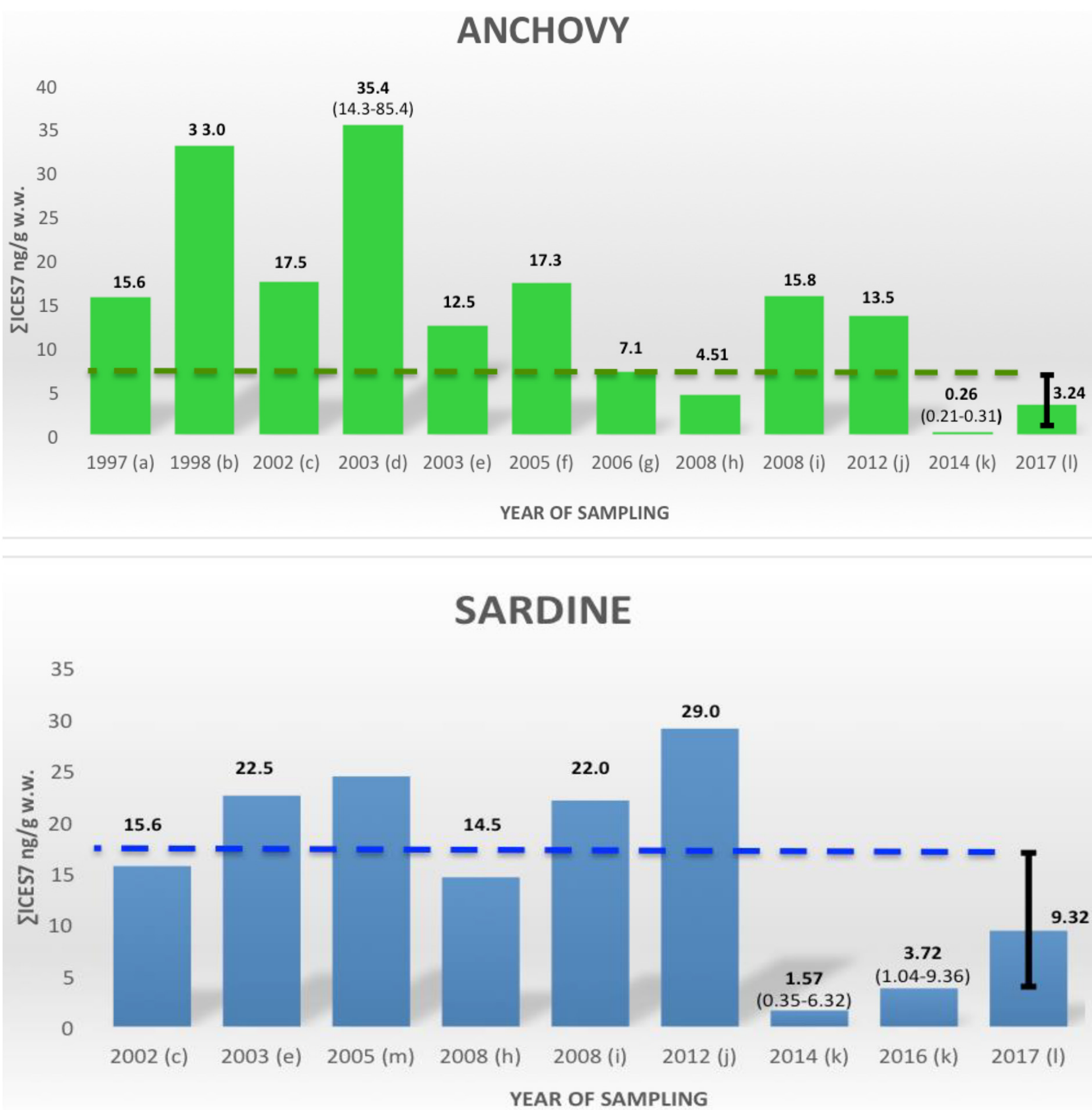

Fig. 1. Reported data on average PCB concentrations (ng/g w.w.) in Mediterranean anchovy and sardine. Values in parenthesis correspond to available range of reported values in a study. Error bars represent the range of values measured in this study. Dash line is intended as visual aid for data comparison.

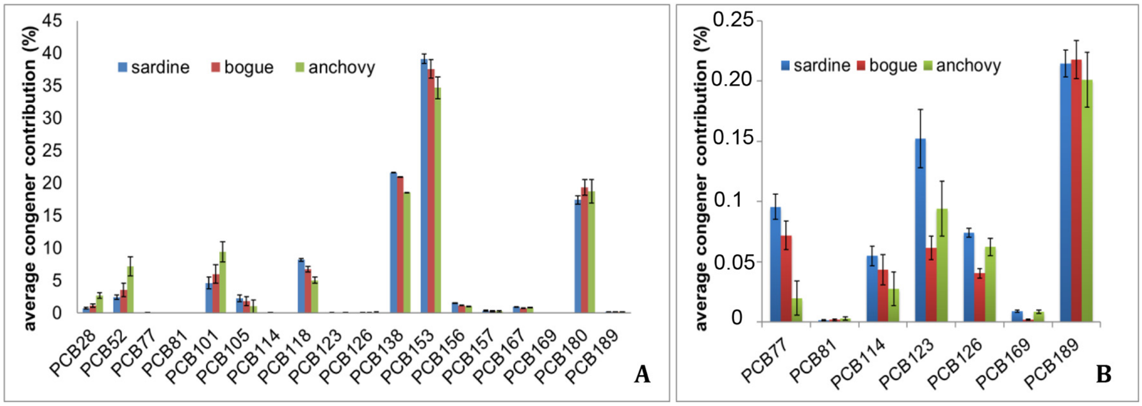

Fig. 2. Average PCB congener contribution of (A) all congeners and (B) non-*ortho* and the least abundant mono-*ortho* congeners. Error bars represent the standard error (SE).

chlorinated congeners at higher levels than others was probably due to their major abundance in commercial PCB mixtures, such as Aroclor 1254 and 1260 (M. Frame et al., 1996) -the most common used in Europe- and also to their high degree of chlorination and lack of adjacent unsubstituted H-atoms in *ortho-meta* and/or *meta-para* positions in the aromatic ring, which make them refractory to metabolic attack (Walker, 2008) and consequently more slowly eliminated and with a heightened tendency to be biaccumulated (Bright et al., 1995).

The sum of the seven indicator congeners (ICES7) accounted for >90% of total PCBs in all species investigated (anchovy: 98%; sardine: 94%; bogue: 96%), while dl-PCBs represented only a small fraction of the total PCB content (anchovy: 2%; sardine: 6%; bogue: 4%). Almost the entire dl-PCB content (almost 99%) was made up by mono-*ortho* PCBs (105, 114, 118, 123, 156, 157, 167, 189) with only a small fraction (1%) represented by non-*ortho* PCBs (77, 81, 126, 169), which is in agreement with the major abundance of the former in the technical mixtures. The three species showed the same mono-*ortho* congener profile (118 > 105 > 156); however, a distinct abundance of non-*ortho* congeners was observed depending on the species. Sardine and bogue exhibited the same profile PCB-77 > 126 > 169 > 81, which is commonly found in other studies in the same and different species in the Mediterranean Sea and other parts of the world (Gómara et al., 2005; Matthews et al., 2008; Moon et al., 2009). Instead, the pattern of non-*ortho*-PCBs was quite different in anchovy (126 > 77 > 169) with a contribution of PCB-126 about 71% and no detection of PCB-81. This profile is different from that in anchovies caught in the Adriatic Sea (Bayarri et al., 2001), but similar to that of anchovies caught in the Black Sea (Çakıroğulları et al., 2010) and in samples collected in Catalonia (Llobet et al., 2003). The ratio of congeners PCB169/PCB126 was explored in order to detect dissimilar induction rates of cytochrome P450 enzymes among the study species. Higher values of this ratio have been suggested as a proxy for a higher metabolization of PCB77 relative to that of the other non-*ortho* congeners (Tanabe et al., 1987). However, in this study, very similar average values for these ratio were found for sardine (0.13), bogue (0.10) and anchovy (0.19). Instead, a similar total non-*ortho* PCB content was found between bogue and anchovy while a much higher content of about 4–5 fold was measured in sardine (Table 1).

Fish accumulate PCBs chiefly through prey intake. Given that abundance profiles are similar among species for the indicator and the mono-*ortho* congeners, the differences in non-*ortho* PCB accumulation patterns could be attributed to different feeding habits and grounds, but also to important dissimilarities in toxicokinetics between species, involving metabolism and selective distribution across tissues as it has been described in fish and other species (Albaiges et al., 1987; Kania-Korwel and Lehmler, 2016; Monosson et al., 2003; Ondarza et al., 2014; Reich et al., 1999; Vijayasarathy et al., 2019).

3.3. Toxicity and risk assessment

Dl-PCB concentrations in pg WHO-TEQ/g are given in Table 2 for all species investigated in this study and ranged from 0.0726 to 1.24 pg WHO-TEQ/g. Non-*ortho*-PCBs accounted for 75% (average of the three species) of total TEQ concentration. As with total PCB concentrations,

Table 2
Mean, median and range of WHO-TEQ values (pg/g w.w.) of dl-PCB in muscle from three Mediterranean Sea fish species.

	Sardine (*Sardina pilchardus*)				Bogue (*Boops boops*)				Anchovy (*Engraulis encrasicolous*)			
	TEQ (pg/g w.w.)											
	Mean	Median	Range		Mean	Median	Range		Mean	Median	Range	
∑non-*ortho*-dl-PCBs	0.731	0.704	0.389	1.18	0.124	0.120	0.0637	0.236	0.200	0.208	0.0766	0.387
∑mono-*ortho*-dl-PCBs	0.0405	0.0409	0.0173	0.0787	0.0115	0.00888	0.00522	0.0317	0.00124	0.00816	0.00125	0.0227
Total TEQs	0.771	0.746	0.410	1.24	0.135	0.131	0.0726	0.268	0.210	0.220	0.0778	0.396

Table 3
Mean WHO-TEQ concentrations (pg/g w.w.) of dl-PCBs in sardine and anchovy from the Mediterranean Sea.

Reference (year)	Source	Sampling year	Species	pg WHO-TEQ/g w.w.
Llobet et al. (2003)	Spanish markets	2000	sardine	2.5
Gómara et al. (2005)	Spanish markets	1995–2003	sardine	1.91
Bocio et al. (2007)	Spanish markets	2005	sardine-anchovy	1.19–1.24
Miniero et al. (2014)	Southern Adriatic Sea Southern Tyrrhenian Sea Ionian Sea	2007–2008	sardine-anchovy	1.76–0.51

WHO-TEQ values in our study were lower but not notably different from most values previously reported in the Mediterranean Sea (Table 3), suggesting, thereby, a steady slow decline of TEQs in this area. Mean total TEQs and concentrations of the 6 PCB indicator congeners (PCB 28, 52, 101, 138, 153 and 180) found in our study for sardine, bogue and anchovy did not exceed regulated levels in diet established by the European Union. EWIs deriving from the estimated weekly fish consumption in Italy were 1.2, 0.29 and 0.35 for sardine, anchovy and bogue, respectively. These values are far below the TWI of 14 pg TEQ/Kg/week and are also below the new TWI for dioxins and dioxin-like PCBs in food of 2 pg WHO-TEQ/Kg/week proposed by EFSA (Knutsen et al., 2018).

Therefore, our results show that the consumption of these important commercial species might not pose a significant risk for human health derived from their dl-PCB content. However, even if PCDD/F contributions to TEQs tend to be generally lower than those of dl-PCBs, they have not been included in the TEQs calculated here, and therefore total (PCDD/F + dl-PCBs) TEQs are expected to be higher than our TEQ values. This is something important to bear in mind when comparing this study results on EWIs and mean levels of total TEQs with the regulated TWI and the European MLs.

4. Conclusions

The three Mediterranean fish species analyzed in this study showed concentrations of PCBs in full compliance with EU (ML of 6.5 pg/g WHO-TEQ w.w.) and WHO (TWI of 2 pg WHO-TEQ/Kg body weight) regulated concentrations in diet; consequently, the PCB content of these edible species seems not to constitute a current hazard for human health. On the other hand, and taking into account that comparability among other studies' values may be highly influenced by variables such as different capture site, year and season of sampling related to the spawning activity, as well as different lab procedures and quantification methods, it is worth noting that our results are generally not different from values reported for the same species in recent years. This underpins the concept of a virtual halt or very slow decline in PCB concentrations, suggested by other authors (Jepson et al., 2016; Jepson and Law, 2016; Law and Jepson, 2017; Stuart-Smith and Jepson, 2017) as a likely consequence of inefficient mitigation measures and ineffectiveness of the global efforts towards PCB contamination, even if PCBs have been banned in many Mediterranean countries for over 40 years and the Stockholm Convention established 2028 as the final date for their global destruction. As ongoing concern about these pollutants is still today justified, it should be underlined the importance of continuous and effective PCB monitoring activities in the Mediterranean Sea, such as the assessment of PCB contamination in edible fish. These research activities play an essential role not only in the context of monitoring programs for PCBs, helping to deepen our knowledge about their occurrence, but also in the field of health risk assessments, aiming to quantify potential risks for fish consumers and, in turn, to take appropriate preventive measures.

Declaration of competing interests

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.

CRediT authorship contribution statement

A. Bartalini: Investigation, Visualization, Writing - original draft, Writing - review & editing. **J. Muñoz-Arnanz:** Conceptualization, Investigation, Visualization, Writing - original draft, Writing - review & editing. **M. Baini:** Writing - review & editing, Resources. **C. Panti:** Writing - review & editing, Resources. **M. Galli:** Resources. **D. Giani:** Resources. **M.C. Fossi:** Conceptualization, Writing - review & editing, Resources, Funding acquisition. **B. Jiménez:** Conceptualization, Writing - review & editing, Resources, Funding acquisition, Supervision.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.139520.

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First assessment of POPs and cytochrome P450 expression in Cuvier's beaked whales (*Ziphius cavirostris*) skin biopsies from the Mediterranean Sea

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The Cuvier's beaked whale (*Ziphius cavirostris*) is one of the least known cetacean species worldwide. The decreasing population trend and associated threats has led to the IUCN categorising the Mediterranean subpopulation as Vulnerable on the Red List of Threatened Species. This study aimed to investigate for the first time the ecotoxicological status of Cuvier's beaked whale in the NW Mediterranean Sea. The study sampled around the 20% of the individuals belonging to the Ligurian subpopulation, collecting skin biopsies from free-ranging specimens. The levels of polychlorinated biphenyl (PCBs), polybrominated diphenyl ethers (PBDEs) and induction of cytochrome's P450 (CYP1A1 and CYP2B isoforms) were evaluated. Results highlighted that the pattern of concentration for the target contaminants was PCBs > PBDEs and the accumulation values were linked to age and sex, with adult males showing significantly higher levels than juvenile. Concerns raised by the fact that 80% of the individuals had PCB levels above the toxicity threshold for negative physiological effects in marine mammals. Therefore, these findings shed light on this silent and serious threat never assessed in the Mediterranean Cuvier's beaked whale population, indicating that anthropogenic pressures, including chemical pollution, may represent menaces for the conservation of this species in the Mediterranean Sea.

Cuvier's beaked whales (*Ziphius cavirostris*) are deep diving marine mammals[1] with an almost sub-polar cosmopolitan global distribution[2]. However, due to its diving behaviour, subtle surfacing and typical offshore habitat, this species, like many other beaked whale species, it remains relatively understudied[3]. A global assessment has highlighted the existence of a genetically distinct subpopulation of Cuvier's beaked whale in the Mediterranean Sea[4]. This subpopulation has been estimated at less than 10,000 mature individuals, with a speculated decreasing trend in abundance[5,6]. To date, three important geographical areas within the Mediterranean Sea have been identified for this species: Northern Ligurian Sea, Alboran Sea and Hellenic trench[5,7].

Most research on Cuvier's beaked whales in the Mediterranean has been driven by the need to assess the risks posed from noise pollution. One of the main threats to this species is exposure to mid- and low frequency underwater sound pollution[8,9]. This threat has been evidenced by multiple records of coincidental mass stranding events[6], the known sensitivity of this species to military sonar[10] and dive behavioural changes caused by passing ships[11]. Research on Cuvier's beaked whales in the Mediterranean basin has been dedicated to reviewing stranding data[12], characterising diving behaviour[1], identifying habitat preferences[7,13,14] and assessing density and abundance estimates[5]. All of which can be used to help identify the risk posed by anthropogenic noise pollution on spatial and behavioural scales.

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The decreasing population trend and associated threats have led to the International Union for Conservation of Nature (IUCN) categorising this Mediterranean subpopulation of Cuvier's beaked whale as Vulnerable on the Red List of Threatened Species[15]. It is, therefore, very urgent to investigate other potential stressors to define an effective risk assessment for the species, such as persistent organic pollutants.

Persistent organic pollutants (POPs) are chemical compounds of global concern due to their persistence in the environment, their ability to be transported over long distances[16] and their effects on natural populations[17,18]. POPs are biologically active chemicals that have the ability to bioaccumulate and biomagnify within marine food webs[19] and have the potential to negatively impact marine organisms[20].

Given the impact and persistence of POPs on and within the environment, the use and manufacture of many of them have already been banned. Polychlorinated biphenyls (PCBs), man-made chlorinated organic chemicals, were banned globally in the 1970s[21] and some polybrominated diphenyl ethers (PBDEs), a class of brominated flame retardants, started being banned around 20 years ago[22].

In the Mediterranean Sea, the environmental concentrations of PCBs does not seem to show any noticeable decline[18,23] and it is also reasonable for PBDEs to be present in the marine environment in large concentrations[22]. These chemicals are considered as legacy contaminants of re-emerging concern since it has been demonstrated to affect natural populations[17,18].

Exposure to xenobiotic compounds, i.e. POPs, can cause immune system suppression[24], and endocrine disruption[25,26] in marine mammals. Concentrations of PCBs in different Mediterranean odontocete species have been reported surpassing toxicity thresholds for marine mammals[27]. Such concentrations can have population level consequences for marine mammals through reduced reproduction and/or survival[28,29].

The induction of cytochrome P450 (CYP450) isoforms has been considered a biomarker of chemical exposure in marine mammals[30]. CYP1A1 and CYP2B have been detected in cetacean skin, and the induction of these isoforms has been related to the exposure to lipophilic contaminants (AhR inducers) such as organochlorine compounds, polycyclic aromatic hydrocarbons (PAHs), and PBDEs both ex vivo and in field studies[31–35].

Since there have been no previous reports on the levels and potential impacts of persistent contaminants in Cuvier's Beaked whale, this paper aims to investigate, for the first time, its ecotoxicological status in the Mediterranean Sea. Sampled animals inhabit the northern Ligurian Sea within the Pelagos Sanctuary, a Specially Protected Areas of Mediterranean Importance (SPAMI) located in the North-western part of the Mediterranean Sea. This is an important area for the species[7] as well as an area subjected to high levels of anthropogenic impact[36–38]. This population is resident and also one of the most studied in the Mediterranean with long-term research starting in the late 1990s. Using photographic mark-recapture methods, the population size, estimated to be roughly 100 individuals[6], and demographic situation is known for a large majority of these animals.

We analysed PCB and PBDE concentrations in blubber portion of biopsy samples collected. Besides, in order to assess the exposure to those anthropogenic contaminants, we evaluate the CYP isoforms (CYP1A1 and CYP2B) expression/induction in skin biopsies by Western Blot (WB) analysis. Contaminant accumulation and CYPs biological responses were further analysed considering demographic variables (i.e. sex and age) to elucidate the levels and the role of persistent contaminant accumulation and their potential effects on the resident Cuvier's beaked whale population.

Results

Sample representativeness. Samples were collected and analysed from 22 different individuals (Fig. 1). These represent 20–28% of the total resident population of Cuvier's beaked whales in the study area (100 individuals with 95% CI = 79–112;[6]). Samples were successfully collected from individuals of three different age classes[39,40]: four juveniles (estimated age < 5-year-old), nine subadults (estimated age 5–10-year-old) and nine adults (estimated age > 10-year-old). Since only three females, one juvenile and two adults, were sampled, it was not possible to perform any statistical analysis between sexes. Therefore, the statistical analysis was focused on the differences between the three male age classes, of which the adult females were not included. The juvenile female (ZCS2_15) was included in the juvenile group, disregarding gender, due to the young age of the specimen (i.e. prepubertal individual). However, two samples, 1 subadult male and one adult male, did not containe enough blubber to analyse the concentrations of PCBs and PBDEs.

Contaminants. All the 18 PCB congeners and 23 out of the 27 (85%) PBDE congeners were detected in every Cuvier's beaked whale sample analysed. PCB concentrations were found to be higher than PBDEs and strong positive correlation was found between PCBs and PBDEs concentrations (rho = 0.97 p value < 0.001).

PCBs. The median ΣPCB in males was found to be highest in adults (ΣPCBs 27.13 mg/kg l.w.) then in subadults (ΣPCBs 16.41 mg/kg l.w.) and lowest in juveniles (ΣPCBs 12.41 mg/kg l.w.), with a significant difference between juveniles and adults (W = 21, p value = 0.033). However, no significant difference was found between the other groups (Fig. 2; Table 1). Although no statistical analysis was conducted between sexes in the adult group, the two adult females (ZCS1_4 = 3.06 mg/kg l.w. and ZCS2_13 = 8.22 mg/kg l.w., Supplementary Information 1, Table S1-1) displayed lower ΣPCBs levels, with a mean value (5.683 mg/kg l.w.) much lower than that of adult males (28.165 mg/kg l.w.: Table 1 and Supplementary Information 1, Table S1-2).

Abundance of PCB congeners in the blubber were PCBs 153 > 180 > 138 > 118 > 101, which together contributed to the majority (95%) of the ΣPCB content in Cuvier's beaked whale samples (Fig. 2). The highly persistent PCB-153 congener represented the main PCB compound, accounting for 38%, 38.6% and 39% of the ΣPCB concentration in juveniles, subadults and adults, respectively. The individual PCB congener profiles also displayed similar patterns to the ΣPCB profile between the age groups with adults generally having higher concentrations than sub-adults and juveniles (Fig. 2).

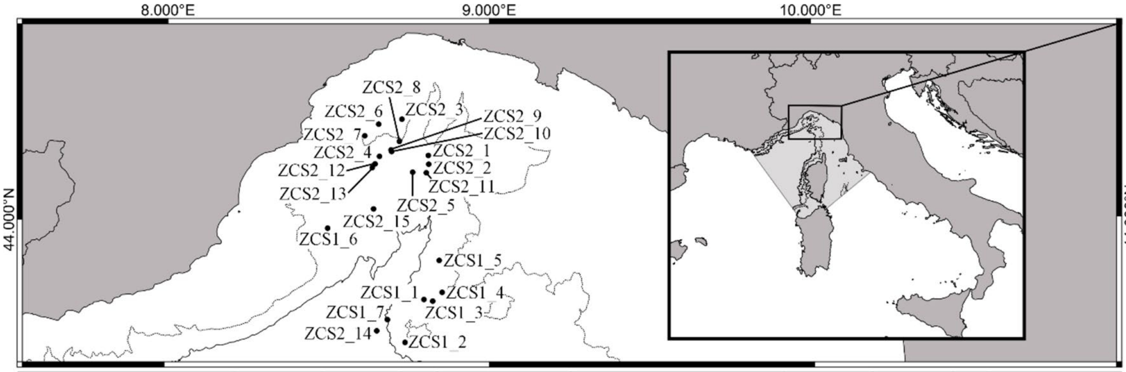

Photo of the individual (at the date of the biopsy)	Biopsy ID & sampling date	Age class	Sex	First photo-id capture	Photo of the individual (at the date of the biopsy)	Biopsy ID & sampling date	Age class	Sex	First photo-id capture
	ZCS1_1 22/06/14	Juvenile	M	22/06/2014		ZCS1_2 22/06/14	Juvenile	M	22/06/2014
	ZCS1_3 16/07/14	Adult	M	24/08/2002		ZCS1_4 16/07/14	Adult	F	15/07/1999
	ZCS1_5 25/07/14	Adult	M	21/08/2002		ZCS1_6 03/08/14	Adult	M	08/06/2004
	ZCS1_7 08/08/14	Adult	M	08/08/2014		ZCS2_1 02/07/15	Subadult	M	01/09/2013
	ZCS2_2 02/07/15	Adult	M	07/08/2013		ZCS2_3 02/07/15	Subadult	M	21/07/2013
	ZCS2_4 04/07/15	Subadult	M	02/07/2015		ZCS2_5 07/07/15	Adult	M	10/09/2003
	ZCS2_6 16/07/15	Subadult	M	26/10/2008		ZCS2_7 17/07/15	Juvenile	M	01/07/2015
	ZCS2_8 21/07/15	Subadult	M	08/08/2014		ZCS2_9 21/07/15	Subadult	M	12/08/2012
	ZCS2_10 21/07/15	Subadult	M	16/08/2013		ZCS2_11 22/07/15	Adult	M	23/08/2003
	ZCS2_12 13/08/15	Subadult	M	14/06/2013		ZCS2_13 13/08/15	Adult	F	27/06/2015
	ZCS2_14 27/08/15	Subadult	M	27/08/2015		ZCS2_15 28/08/15	Juvenile	F	28/08/2015

Figure 1. Map showing the study area, biopsy locations, the extent of the Pelagos Sanctuary, photos of sampled individuals, and information about sampling date, age class and their first photographic capture (Photos by M. Rosso). Map was generated using QGIS Geographic Information System (Version 3.10.10 'A Coruña'; Open Source Geospatial Foundation. http://qgis.org).

To further investigate the PCB results, each congener was assigned to a group. The first groups were based on the degree of chlorination (TriCB, TetraCB, PentaCB, HexaCB and HeptaCB). Then there were the non-*ortho*-dl-PCBs and mono-*ortho*-dl-PCBs. Four Structure Activity Groups (SAGs 1–4) were assigned based on their persistency and capacity for biotransformation as previously defined[41] (Supplementary Information 1). One group was based on the PCB congeners listed by the International Council for the Exploration of the Sea (ICES: Table 1 and Supplementary information 1), here on referred to as ΣICES7.

In all groups, except for TriCB and non-*ortho* PCBs the mean concentration values were highest in adults and lowest in juveniles (Supplementary Information 1), indicating that these PCB group concentrations increased with age. However, due to the high variation among individuals these differences were not statistically significant.

PBDEs. Unlike PCBs not all PBDEs were identified in all the sampled animals. Four PBDE congeners (3, 7, 15, 126) out of the 27 analysed were consistently not detected in any sample.

Regarding the concentrations of ΣPBDE found in the different age groups, the trend was similar to those observed for PCBs. The median value in adults (ΣPBDE = 0.503 mg/kg l.w.) was higher than in subadults (ΣPBDE = 0.351 mg/kg l.w.) and juveniles (ΣPBDE = 0.271 mg/kg l.w.). ΣPBDE were significantly higher in adults than in juveniles (ΣPBDE W = 22, p value = 0.01905), while no difference was found between the other groups (Fig. 3). As highlighted for PCBs, the two adult females showed PBDEs values lower (ZCS1_4 = 0.054 mg/kg l.w.; ZCS2_13 = 0.231 mg/kg l.w.) than those shown in adult males. The PBDEs congener profile shown in Fig. 3, was

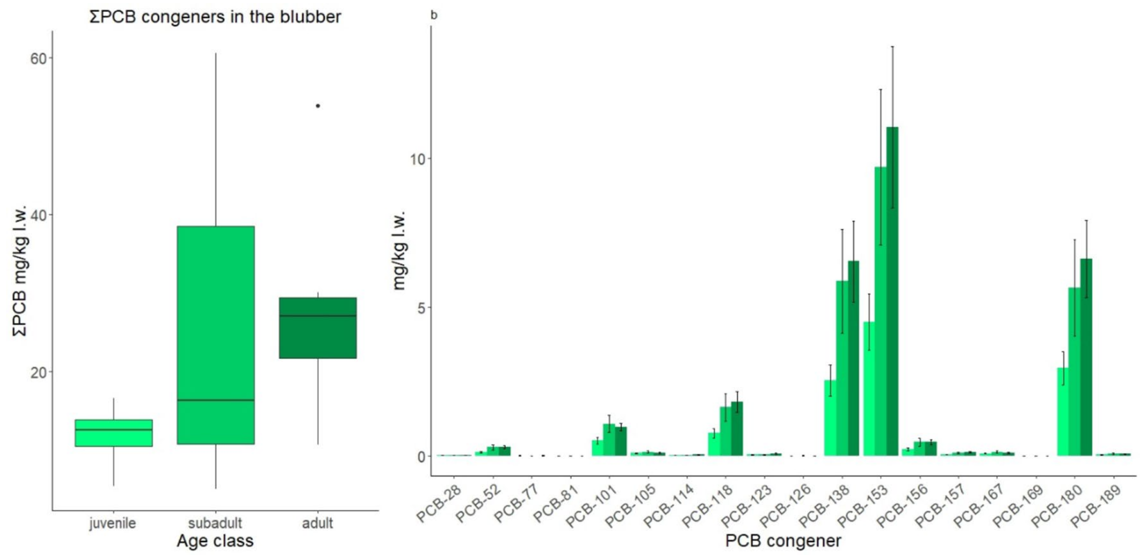

Figure 2. PCB levels in the blubber of Cuvier's beaked whale in the three age classes: juvenile (light green), subadult (medium green) and adult (dark green). (**a**) Boxplots showing ΣPCB concentrations (mg/kg l.w.) in the blubber of juvenile (n = 4), subadult (n = 8) and adult males (n = 6). (**b**) The mean values of each individual PCB congener profiles for all 18 PCB congeners, in the age classes. Error bars represent standard errors (SE).

	Juvenile			Subadult			Adult male			Adult female		
	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD
ΣPCBs	11.82	12.58	4.68	25.11	16.41	19.91	28.17	27.13	14.40	5.64	5.64	3.65
ΣICES7	8.82	9.43	3.44	18.34	12.29	14.24	20.73	19.37	10.77	4.08	4.08	2.38
ΣPBDEs	0.26	0.27	0.11	0.62	0.35	0.60	0.49	0.50	0.15	0.14	0.14	0.13
Total TEQ	102.48	110.89	44.06	242.59	129.15	305.37	157.44	167.51	28.04	162.39	162.39	178.56
BDE-47/PCB-153	0.03	0.03	0.01	0.03	0.03	0.01	0.02	0.03	0.01	0.03	0.03	0.02
BDE-47/BDE-99	2.36	2.59	0.52	2.60	2.63	0.14	2.62	2.58	0.16	2.22	2.22	1.00
BDE-99/BDE-100	1.37	1.31	0.17	1.30	1.28	0.15	1.57	1.55	0.20	1.49	1.49	0.19
BDE-153/BDE-154	0.38	0.37	0.05	0.36	0.35	0.04	0.45	0.45	0.06	0.40	0.40	0.11

Table 1. Mean, median, standard deviation of ΣPCB, ΣICES7, and ΣPBDE, in the blubber of Cuvier's beaked whale of juvenile (n = 4), subadult male (n = 8), adult male (n = 6) concentrations are expressed in mg/kg lipid weight. Total TEQ is expressed in pg/g lipid weight. Mean, median and standard deviation, of BDE-47/PCB-153, BDE-47/BDE-99, BDE-99/BDE-100, BDE-153/BDE-154 ratio.

dominated by lower-medium brominated congeners. The most abundant PBDE congeners were BDE 47, 99, 154, 100, 153 whose average contribution to the total PBDE content was 45%, 17%, 14%, 13%, and 5%, respectively.

The different PBDEs were then grouped in the respective commercial mixtures Deca-, Octa- and Penta-BDE[42] to compare profiles between the age groups. The results show that penta-BDE is the most dominant component among all age classes, followed by Octa-BDE mixture and Deca-BDE mixture. It is interesting to note that the concentrations of the deca-BDE mixture decrease as the age of the whales increases. Despite the correspondence between PBDE congeners found in blubber of Cuvier's beaked whale and main components in the technical mixtures, the relative abundance of these compounds differed from that found in the commercial formulations[42].

Metabolic biotransformation of PCBs and PBDEs. To investigate the metabolic biotransformation capacity of Cuvier's beaked whale for the investigated compounds, the ratio between the most dominant compounds (BDE-47 and PCB-153) was calculated[43]. Results showed that BDE-47/PCB-153 ratios were below 1 in all age classes. The highest values were observed in subadults followed by juveniles and adults (Table 1). BDE-47/PCB-153 ratio did not differ statistically among age classes (subadult vs. adults: $W = 31$, p value = 0.4136; subadult vs juveniles: $W = 20$, p value = 0.5697; adult vs juveniles: $W = 11$, p value = 0.9143).

Regarding PBDE congeners, BDE-99/100, 153/154 and 47/99 ratios have been used to assess possible differences in metabolic capacities of these classes of compounds as well as bioaccumulation patterns[44,45], which may vary among age classes.

BDE-99/100 and BDE-153/154 ratios had similar trends with the highest value in adults (mean value: 1.57 and 0.45, respectively) followed by juveniles (mean value: 1.37; 0.38) and subadults (mean value: 1.30; 0.36). BDE-99/ BDE-100 ratio was significantly higher in adults than subadults ($W = 5$, p value = 0.006) and then juveniles

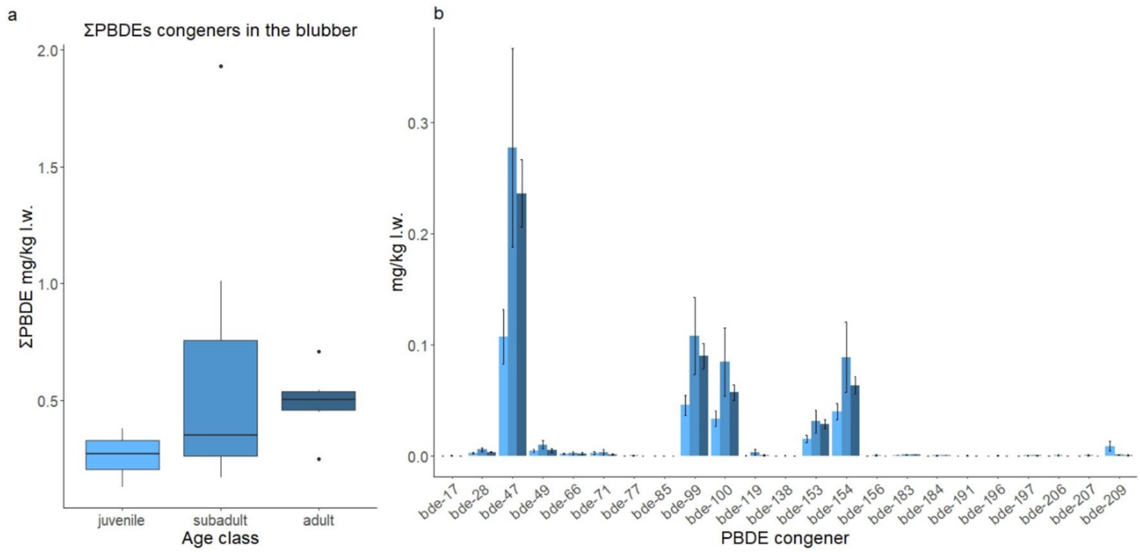

Figure 3. PBDE levels in the blubber of Cuvier's beaked whale in the three age classes: juvenile (light blue), subadult (medium blue) and adult (dark blue). (**a**) Boxplots showing ΣPBDE concentrations (mg/kg lipid weight) in blubber of Cuvier's beaked whale of juvenile (n = 4), subadult male (n = 8) and adult male (n = 6). (**b**) The mean values of the individual PBDE congener for all 27 PBDE congeners, in the age classes. Error bars represent standard errors (SE).

(W = 21, *p* value = 0.033). Concerning BDE-153/ BDE-154 ratio, results were significantly higher in adults than subadults (W = 42, *p* value = 0.010), while no other differences were found among the other groups. BDE-47/99 ratios in the three age classes were higher in adults (mean value: 2.62) and subadults (mean value: 2.60) than in juveniles (mean value: 2.36).

Toxicity assessment. In order to assess the potential toxicological effect of the contaminants analysed, they were evaluated in relation to the threshold limits proposed in the literature. Two PCB toxicity thresholds were used in this study as proposed previously in other toxicological studies on cetaceans[17,18,46]. The lowest value (9.0 mg/kg l.w. ΣPCB) was proposed as the toxicity threshold concentration for the onset of physiologic effects in marine mammals[18,47]. The highest value (41 mg/kg l.w. ΣPCB) was proposed as a toxicity threshold for reproductive impairment in Baltic ringed seals (*Pusa hispida*[48]). The ΣPCB values detected in most of our sampled individuals (80%) exceeded the lower limit, and three of them showed ΣPCB values above the highest threshold limit (two subadults and one adult: Fig. 4a).

Regarding ΣPBDE, we set the threshold at 1.5 mg/kg l.w., which has been found to be the value associated with endocrine disruption in grey seals[49]. ΣPBDE concentrations detected in the different whales belonging to the three age class are all below the threshold limit except for one subadult specimen (Fig. 4b).

The potential toxicity of dl-PCBs is commonly assessed by the Toxic Equivalent Quantity (TEQ) approach, in which each's congener toxicity is relativized to that of the most toxic one, the 2,3,7,8-TCDD[51]. Calculated TEQs ranged from 44 to 982 pg WHO-TEQ/g l.w. It should be highlighted that in the blubber of two adults (ZCS1_3 and ZCS1_13) and two subadults (ZCS2_8 and ZCS2_9) analysed exceed the threshold of 210 pg WHO-TEQ/g l.w. the proposed threshold of immunosuppression in harbour seals[50] (Fig. 4c). However, it is important to stress that there is a wide inter-species sensitivity towards different contaminants and that there are no specific studies on this species, and few on other marine mammals.

Cytochrome P450. The detection of cytochrome CYP450 expression was carried out for the first time on this species. This analysis was successfully accomplished using the Western Blot technique which allowed the identification and quantification of cytochrome-like bands for the CYP1A1 (59 kDa) and CYP2B (56 kDa) in the dermal part of skin biopsies. The protein expression in different age classes was similar for cytochromes 1A1 and 2B with the highest values in subadults followed by adults and juveniles (Fig. 5). CYP1A1 protein expression differed significantly between juvenile and subadult, with subadults having a higher expression (W = 27; *p* value = 0.004), while no differences were noted for CYP2B. Adult females showed CYP1A1 and CYP2B expression more similar to juveniles than adult males.

A significant positive correlation was found also between CYP1A1 and CYP2B concentrations (rho = 0.59, *p* = 0.009). In order to evaluate the influence of PCBs and PBDEs on the expression of CYP1A1 and/or CYP2B, we ran a regression analysis on CYP1A1/CYP2B concentration against single PCB congeners, ΣPCB, different PCB groups (TriCB, TetraCB, PentaCB, HexaCB and Hepta CB, non-*ortho*-dl-PCBs, mono-*ortho*-dl-PCBs and four SAGs), single PBDE congeners, ΣPBDE and Deca-, Octa- and Penta-BDE mixtures. A check for collinearity was conducted to assess the correlation between the PCB groups. Variables showing correlation > 0.8 were not included in the regression analysis. Regression analysis was run using only ΣPCB, TriCB and non-*ortho* PCB,

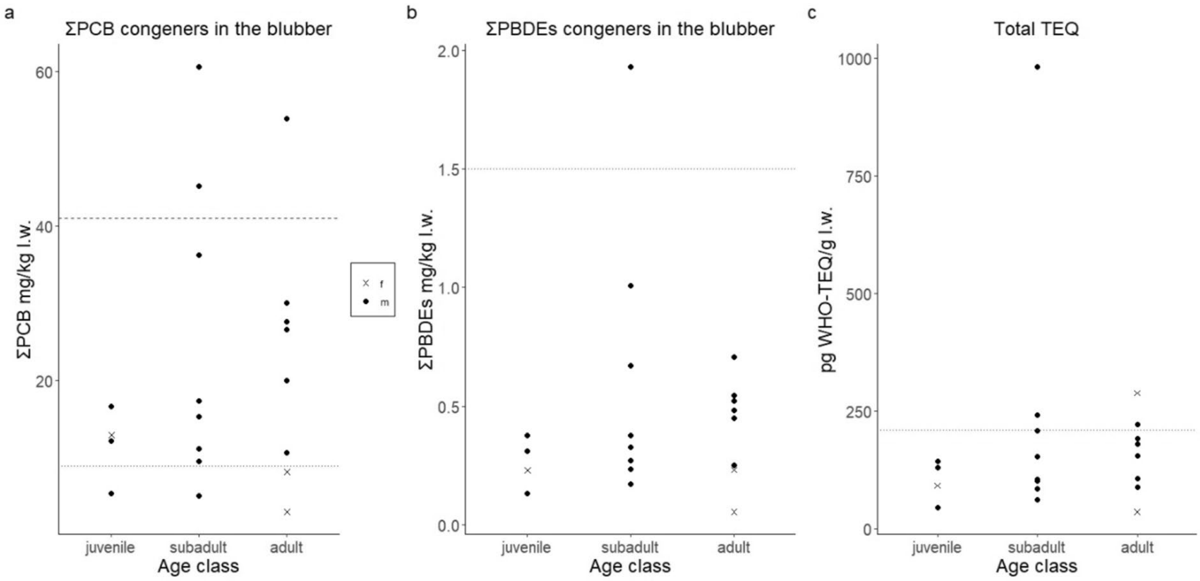

Figure 4. Contaminant concentrations for juvenile (n = 4), subadult male (n = 8), adult male (n = 6) and adult females (n = 2), in relation to ΣPCB, ΣPBDE and TEQs published toxicity threshold. (**a**) ΣPCB (mg/kg l.w.) concentrations in relation to published toxicity thresholds. The lower dotted line represents the lower toxicity threshold (9 mg/kg l.w.) published for marine mammals[18,47]. The dashed line represents the highest threshold (41 mg/kg l.w.) published for marine mammals[48]. (**b**) ΣPBDE (mg/kg l.w.) concentrations in relation to 1.5 mg/kg l.w. published toxicity threshold[49]. (**c**) TEQ concentrations (pg WHO-TEQ/g l.w.) in relation to 210 pg WHO-TEQ/g l.w. published toxicity threshold[50].

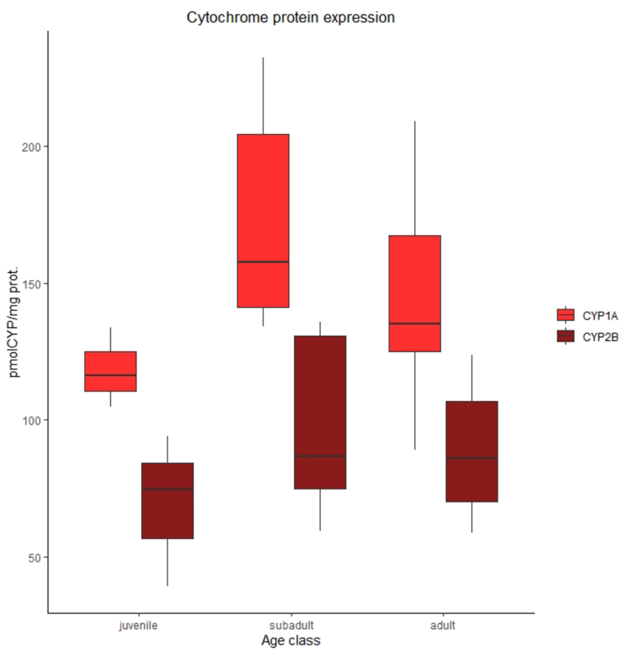

Figure 5. Boxplots showing CYP1A1 and CYP2B expression (pmolCYP/mg protein) in juvenile (n = 4), subadult male (n = 8) and adult male (n = 6). Error bars represent standard errors (SE).

but none of these components showed a statistically significant correlation with CYP1A1/CYP2B. No correlation was found between CYP1A1 and PBDEs. Similarly, no correlation was found when looking at CYP2B and PBDEs concentrations.

Discussion

This represents the first ecotoxicological study on free-ranging Cuvier's beaked whales both in the Mediterranean Sea and worldwide. Sample size (more than 20% of the estimated resident population in the Ligurian Sea) is unique for this kind of study and, above all, for this elusive species. Considering the amount of collected and analysed samples, and also the evenly distribution among age classes, the results may be extrapolated to the entire Cuvier's beaked whale population in the area.

An added value of this study is the analysis of free-ranging individuals from a well-known population, which have been continuously studied (through mark-recapture) in the last twenty-years (since the 1990s). The mark recapture data previously collected allowed an analysis of contaminants concentrations and CYPs expression according to age and life-history of most of the individual sampled and to further provide new insights on the population status.

The analysis of levels and congener profiles of the two classes of contaminants investigated in this study could also provide information about possible biological and environmental differences among age groups. The variation in congener profiles among species and age classes is likely to be driven by several factors such as differences in prey preferences during the different life stages, differences in the physiochemical, physiological and metabolic processes that occur between females and males and age classes from juveniles to adults[25,52].

Currently, few studies on contamination on beaked whales were published. Only three papers specifically report the PCBs and/or PBDEs levels in stranded Cuvier's beaked whales outside the Mediterranean Sea[53–55].

The pollutant load is also reported for other three species of beaked whales that feed at the same trophic level from Japanese waters (*Berardius bairdii*[56,57], *Mesoplodon stejnegeri*[58,59] and *Mesoplodon carlhubbsi*[60]) and from Canadian waters (*Hyperoodon ampullatus*)[34].

Contaminant concentrations found in our study are higher than those reported in previous research for beaked whales, confirming that the Mediterranean Sea represents a contamination hotspot for marine mammals[27,52].

Comparing the levels of ∑PCBs and ∑PBDEs with those present in the literature for the other cetacean species living in the same area, it can be highlighted that Cuvier's beaked whale showed higher levels than fin whale did[52,61,62]. Among the odontocetes the values are lower to those reported for striped dolphin[31,37,63], common bottlenose dolphin[52], Risso's dolphin[64], pilot whale[62,64] and similar to those observed in the sperm whale[62,65]. Differences observed were expected because of trophic level differences among the species, different prey preferences and both feeding habits and feeding ground areas.

In our study a strong positive correlation was found between PCBs and PBDEs concentrations suggesting a similar bioaccumulation of these substances in Cuvier's beaked whales and a possible common source of contamination through their diet, mainly composed by mesopelagic squid[2,6]. The few published data on this species referred to individual specimens[53,54], which does not allow to investigate how sex and age variables affect the accumulation of ∑PCBs and ∑PBDEs in this species. The increase of contaminant concentrations in blubber through the different age classes observed in the male whales, confirms what was previously described in harbour porpoises[66], bottlenose dolphins[29] and killer whales[67,68]. Therefore, while contaminant concentrations in males either remain stable or increase with age, in sexually mature females contaminant burdens generally decline. This may be due to the transfer of organic pollutants from females to offspring through gestation and lactation[46,69–73]. This was also observed in Cuvier's beaked whale where the ∑PCBs and ∑PBDEs levels of the two adult females examined were lower than those of the adult males and similar to those of the young specimens. Thanks to the long-term studies conducted on this population we know that one of the females (ZCS1_4), has given birth to at least 4 calves since 1999 to the sampling date. This whale was, indeed, the one that had the lowest levels of ∑PCBs and ∑PBDEs in the dataset.

Among PCBs, the PCB-153 was the predominant congener in all age groups followed by PCB-180 and 138. This congener distribution and abundance also reflects the high deep-sea sediment and organisms' affinity towards higher chlorinated congeners relative to that of low chlorinated compounds, suggesting the ease for the former to be transported from surface waters to the deep-sea[44]. However, the same trend was also observed in other beaked whales[34,60] and marine mammals in general[46,62,74–76], since these compounds are non biotransformable in cetaceans[41,77,78]. The abundance profile of non-*ortho*-dl-PCBs in the study samples ($77 \approx 126 > 169 > 81$) is similar to other beaked whales[56,57] but is remarkably different from that reported in blubber from Mediterranean sperm whales ($126 > 169 > 77 > 81$[65]). This is interesting given that diet is the main route of POP uptake for marine mammals and both species are deep divers sharing common prey (i.e. mainly mesopelagic squids)[6,64]. However, it is known that many factors such as lipid content, protein-binding, blood transport, or chlorination level, among others, affect the distribution and partitioning of PCBs and other POPs across tissues in marine and terrestrial species[79]. Thus, different toxicokinetics are likely to be considered behind the dissimilar abundance of non-*ortho* congeners in the blubber of these Mediterranean species instead of PCB concentration differences in their feeding habit and grounds.

The most abundant PBDEs were lower-medium brominated congeners BDE-47, BDE-99 and BDE-100, that represent the main components in the commercial penta-BDE formulations[42]. The results of this study were consistent with previous studies that reported a decrease in bioaccumulation with an increase in bromination[62,65,80,81] as observed in other beaked whales[58] and in aquatic food webs in general[82]. Although metabolism plays an important role in the PBDE congener accumulation in aquatic organisms, lower brominated congeners have higher biomagnification potential and, thus, possible toxicological effects[44,80].

BDE-154 levels were also significant in all age classes. BDE-154 has been suggested to be a debromination product of BDE-183, the main congener in the technical octa-BDE mixtures[83,84].

An interesting result was the increase of higher brominated BDEs in younger Cuvier's beaked whales (juvenile > subadult > adult). This evidence needs to be further investigated but a possible explanation could rely on the prohibitions of Penta- and Octa-BDE commercial mixtures since 2004 (Directive 2003/11/EC of the European Parliament and of the Council). Juvenile individuals have therefore never come into direct contact with these commercial mixtures (unless through lactation), and have a higher percentage of BDE-209, which constitutes between 92 and 97% of the total BDE content in the deca-BDE formulations[42]. Possible metabolic differences in biotransformation of PCBs and PBDEs were investigated by calculating the ratios of the most dominant compounds, BDE-47/PCB-153. The lowest ratios were found in the adults, which indicates that the juveniles and subadults have a lesser developed ability for metabolic biotransformation of BDE-47, confirming what was also described in harbour porpoises from the North Sea[43]. With regard only to PBDE metabolism, the results of BDE-99/100, 153/154 and 47/99 ratios suggest that subadults could have greater metabolic capacities of this class of compounds. Moreover, BDE congener ratios could also reflect some differences in the feeding strategies among age classes, as shown for other marine species[44]. However, studies on the feeding ecology of this species in the study area are required to confirm this hypothesis.

The two CYP isoforms protein expression has been evaluated in Cuvier's beaked whale in the present study for the first time. The qualitative and quantitative CYP1A1 and 2B protein analysis showed a correlation between the two isoforms and a significant difference between age classes with the highest levels of expression in subadult males compared to juvenile and adults. A similar trend has been found, for instance, in pilot whales from North-eastern Atlantic[85] and false killer whales from Hawaii[86]. It is worth noting that the levels of CYP1A1 and 2B protein induction in the two adult females are among the lowest of the dataset. This can be related both to the influence of estrogen concentrations on CYP expression due to cross-talk between the Aryl hydrocarbon Receptor (AhR) and estrogen receptor (ER) and the subsequent activation or impairment of estrogen responsive gene expression in the presence of lipophilic compounds[87] and both to the lowest levels of PCB and PBDEs accumulation in adult females due to reproductive and physiological status. However, the regulation of CYP expression patterns in Cuvier's beaked whales may be affected by and be dependent on complex biological and metabolic pathways. These are related to the diet and physiology of this species, which are poorly investigated and need further investigation. No correlation was found between the two classes of compounds detected in the blubber (including any category shown in Table 1) and CYP1A1 and CYP2B analysed in the skin of Cuvier's beaked whale. Also focusing on correlating specific compound which induce specifically the two isoforms, planar compounds (non- and mono-*ortho* PCBs) and CYP1A1 expression, and non-planar compounds (> 2 *ortho* chlorine compounds) with CYP2B, any statistical correlation exists among all age classes. Our data show that the PCBs and PBDEs, which accumulated in the blubber (at least in the outer part of the blubber sampled by skin biopsy remote dart sampling), did not correlate with the induction of CYP isoforms expression. Comparing our data to the only other beaked whale species analysed, *Hyperoodon ampullatus,* was also found to have no correlation between CYP1A1 expression and detected contaminants[34]. However, despite this is true also in some other cetacean species, including deep-divers[33,86], it should be considered that older animals accumulate higher POP levels due to biomagnification but CYPs expression change in the context of maturation and aging being less efficient than younger animals[88] and posing a further risk to the adult males. An additional analysis on the CYP isoforms was performed correlating SAGs with the protein expression. SAG 1, 2 and 5 are considered highly persistent because of their high level of chlorination and, therefore, are poorly metabolized, whereas SAG 3 and 4 are less persistent because they are less chlorinated and more easily metabolized by CYP1A1-mediated enzymes. Thus, this classification appears more appropriate to assess the potential ecotoxicological effects on cetaceans exposed to PCBs. The SAG classification approach became necessary since cetaceans have reduced capability of metabolising non dl-PCBs; therefore, these compounds can accumulate and become toxic to the organisms because of their low CYP2B enzyme activity, compared to other mammal species[41]. Moreover, a wide range of toxic effects were reported for non dl PCBs, which can act through a non-AHR-dependent response[89]. In this study no significant correlations were found between specific SAG congeners (1–4) and CYP1A1 and CYP2B protein expression in all three age classes. This suggests that the protein induction could be due to exposure to other classes of contaminants rather than those measured. It could also be that the levels of exposure that cause effects and negative physiological responses (e.g. age-related CYPs expression) in this species can be different from other cetacean species. Considering also the toxicity assessment of the compounds investigated, any significant relationship between calculated TEQ and CYP1A1 and CYP2B expression were shown. This evidence, already observed in other studies[86], suggests that CYP1A1 and 2B expression can be related to broader and different AhR agonists not measured in the present study.

To gain a better understanding of the significance of the PCB and PBDE findings, toxicity profiles need to be quantified. This is a difficult task due to the vast range of mechanisms, toxicities, and synergistic and antagonistic interactions inter-species sensitivity. This is further confounded by the absence of specific studies on this species and the limited number of similar studies on other marine mammals. The use of Toxic Equivalency Factors (TEFs) to achieve TEQ constitutes an accurate approach for toxic risk assessments associated with dioxin-like compounds. This permits cetacean based evaluations on the different contribution of each pollutant to the TEQ[51].

Overall, in our study only four whales (20% of dataset) exceed the threshold of 210 pg WHO-TEQ/g l.w. in blubber, proposed as the starting point for immunosuppression in harbour seals[50]. TEQ values are the lowest amongst those tested for other toothed whales in the Mediterranean Sea[62,65], but this could be due to lack of PCDD/Fs contribution in our study.

However, over 80% of the whales considered in this study exceeded the lower toxicity threshold of 9 mg/kg l.w. for PCBs, the level at which marine mammals suffer physiological effects[47]. Furthermore, three animals (15%) exceeded the higher PCB threshold of 41 mg/kg l.w.[48]. The percentage of animals surpassing these thresholds is lower than what has been observed in striped[18] and the bottlenose dolphins[46]. As discussed by several scientific papers[18,25], the lower toxicity threshold may overestimate the true PCB risk to cetaceans, however, it is important to stress that there is a wide inter-species sensitivity towards different contaminants and that there are no specific studies on this species, and few on marine mammals[47].

The risk posed to Cuvier's beaked whales from PBDEs may be low as all whales, in all age classes, were below the toxicity threshold limit, unlike other cetacean species in the Mediterranean[52] or elsewhere in the world[80].

However, it is important to understand that the responses to the toxic effects of contaminants is highly specific to individual's physiological status, nutrition state, body size and age. Therefore, caution is needed in applying such threshold levels on large cetaceans[47,70] failure to do so may lead to an over or underestimation of the risk posed to a species or population.

All these data suggest that the health of a population, exposed to a mixture of known and unknown compounds, may depend not only on the levels of contaminants accumulated, but also on the effects which may occur when the levels of accumulation of environmental contaminants are below or near the established threshold levels.

For all these reasons, the integration of the data on the population structure and dynamics and the data on PCBs, PBDEs and CYPs responses on a poorly studied species and genetically isolated population, in an area of high ecological value and, also, taking into consideration the life history of each individual, makes the study unique for living cetaceans in the Mediterranean Sea.

All whales sampled were found to have detectable levels of PCBs, so at a minimum 20% of the population is affected and it could be assumed that all Cuvier's beaked whales in the Pelagos Sanctuary have some levels of POPs. Furthermore, given that 80% were well above toxicity thresholds, it could be said that a large proportion of population could undergo negative physiological effects (including endocrine disruption).

These findings shed light on an additional anthropogenic threat, the chemical pollution, poorly considered so far for Mediterranean Cuvier's beaked whales and its conservation, that should be considered—together with the anthropogenic noise—the main threat for this species in the Mediterranean sea[12]. Characterizing the exposure and effects to a plethora of threats in wild cetaceans can pave the way towards the comprehension of the actions needed for the effective conservation of specific populations and, ultimately, species.

Methods

Permits, ethics statement and approval. Biopsies were obtained in accordance with the relevant guidelines and regulations imposed by the Ministry for Environment, Territory and Sea (MATTM) and under sampling permits n. 0018799/PNM released from the same Italian institute (Div. II) and the Italian National Institute for Environmental Protection and Research (ISPRA). The research permits also included the necessary ethical approval in terms of sample collection, analysis and use for scientific studies.

Biopsy collection. Biopsies of Cuvier's beaked whales were collected in the Ligurian Sea (NW Mediterranean Sea) in 2014 and 2015. A 60-foot sailing vessel was used as a research platform allowing the research crew to reach the core of the study area and stay at sea overnight during consecutive days. The vessel towed a 4.3 m long 20 hp RHIB that was used to approach the whales during biopsy collection operations. Skin biopsies were collected in a specific dorso-lateral area below the dorsal fin using a 68 kg draw weight recurve crossbow (Barnett Panzer V). We obtained about 1 g of tissue samples using stainless steel biopsy tips (30 mm × 8 mm) attached to 18″ bolts. Tips were sterilized before use. The tissue samples obtained were stored in liquid nitrogen a few minutes after biopsy collection and stored at − 80 °C until laboratory analysis.

Age class classification. All the whales biopsied have been sized in situ and photographed in order to allow both age classification and individual identification (i.e. only individuals showing adequate natural marking have been identified). All the whales photographed were separated into three different age classes—juveniles, subadults, adults. Animals were categorized in the age classes by both the estimated size and coloration patterns[39,40]. All the subadult and adult whales biopsied were well-marked allowing for individual identification. The photographs of the individuals identified were compared with each other and to photographs from 158 previously identified individuals collected during previous years (since 1998) in the same study area (CIMA Foundation database) gaining information on the life history of the sampled animals and making a crude estimation of the possible age.

Sex determination. The sex was first estimated in the field based on size, coloration pattern, natural marking severity and individual history if the animals were already present in the photo-id catalogue. Sex was then confirmed by molecular sex determination based on ZFY/ZFX gene[90]. DNA was isolated by 20 mg of dermal tissue homogenised with a Tissue Lyser (Qiagen). The Wizard SV Genomic DNA Purification Kit (Promega) was used for DNA isolation according to the manufacturer's instructions. DNA was quantified by Nano-Drop ND-100 UV–Vis spectrophotometer (NanoDrop Technologies LLC) and purity assessed by 280/260 nm and 260/230 nm ratios. Gender was determined using 50–100 ng of DNA in standard PCR reactions following the protocol described by[90].

Cytochrome analysis: Western Blot. Analysis of cytochrome P450 CYP1A1 and CYP2B, used in this study as marker of POPs exposure, were analysed in the dermal part of the skin biopsies of Cuvier's beaked whale using western-blotting (WB) techniques followed by a semi-quantitative analysis of the data[91]. A triplicate skin standard for odontocetes was used as internal standard during all the WB procedures to monitor the accuracy of the analytical method.

Sub-samples of biopsies (about 30 mg) were weighted and homogenized using a Tissue Lyser (Qiagen) in arylhydrocarbon-receptor (AhR) buffer (1:10)[92]. The homogenates were centrifuged and supernatants (S9 fractions) were collected and immediately frozen at -80 °C until analysis. Proteins were separated by 10% polyacrylamide gels (SDS-PAGE, Criterion XT Precast Gel, Bio-Rad) and blotted onto nitrocellulose sheets for 1 h at 375 mA. The membranes were saturated with a blocking solution for 1 h at room temperature and then incubated over-night with primary polyclonal anti-bodies Goat anti-rabbit CYP1A1 and anti-CYP2B (Oxford Biochemical Research; Oxford MI, USA), diluted 1:5000 and 1:1000 respectively, in TTBS 1% gelatin. Incubation with secondary anti-body anti-goat HRP-labelled (Immun-Star-HRP-Chemiluminescent-Ki, Bio-Rad), diluted 1:3000 in TTBS 1% gelatin, was performed for 90 min at room temperature and protein detection was carried out according to the manufacturer's instruction. Semi-quantitative analysis was performed using the Quantity One software (1-D Analysis Software, Bio-Rad). Molecular weights for CYP1A1 and CYP2B peptide have been calculated by the lane-based functions with multiple regression models using as a Precision Plus Protein Standard (Bio-Rad).

Contaminant analysis. *Sample processing.* PCBs and PBDEs were analysed in blubber samples using the method described in[65]. Briefly, pollutants were extracted in a Soxhlet apparatus (24 h) with a n-hexane: dichloromethane (9:1) mixture. Samples were previously homogenised with anhydrous sodium sulphate (Na_2SO_4) and spiked with a suite of ^{13}C-labeled standards of target contaminants. Resulting extracts were purified by means of the automated sample preparation system DEXTech + (LCTech GmbH, Dorfen, Germany) providing two fractions: F1 containing PBDEs and PCBs (save non-*ortho* PCBs), and F2 containing non-*ortho* PCBs. Fractions were evaporated using a TurboVap (Zymarck Inc., Hopkinton, MA, USA) system until ~ 1 mL and dried under a gentle nitrogen stream. A few microliters of ^{13}C-labeled injection standards of PCBs and PBDEs were used for reconstitution of each sample prior to instrumental analysis. The lipid content of samples was obtained gravimetrically from weight measures before and after extraction. Comprehensive details on the whole sample analysis can be found in the Supplementary Information 2 (SI).

Instrumental determination. Six indicator PCB (PCB-28, 52, 101, 138, 153, 180), twelve dl-PCBs (PCB-77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 189) and twenty-seven PBDE (BDE-3, 7, 15, 17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 156, 183, 184, 191, 196, 197, 206, 207 209) were investigated by means of gas chromatography coupled to high resolution mass spectrometry (GC-HRMS). Specifically, the quantification was carried out by the isotopic dilution technique on a Trace GC Ultra gas chromatograph (Thermo Fisher Scientific, Milan, Italy) coupled to a high-resolution mass spectrometer (DFS, Thermo Fisher Scientific, Bremen, Germany). A full description of the instrumental parameters can be found in the SI.

QA/QC. To avoid contamination all material was cleaned (3X) with the following solvents of decreasing polarity: acetone, dichloromethane and n-hexane. Particular care was taken to minimize exposure to UV light during the whole analytical procedure. A procedural blank was included within each batch of six samples. The isotopic dilution technique was used for quantification according to the following criteria: (a) ratio between the two monitored ions within ± 15% of the theoretical value, and (b) limits of quantification (LOQs) corresponding to S/N of 10. Final concentrations were blank corrected and intrinsically corrected by recoveries. Satisfactory results were obtained from the analyses (n = 3) of the certified standard reference material SRM 1945 ("Organics in Whale Blubber", NIST). Additional data related to QA/QC is provided in the Supplementary Information 2. Toxic Equivalent Quantities (TEQ) for dl-PCBs were determined using the World Health Organization (WHO)-2005 Toxic Equivalency Factors (TEF) for mammals[51].

Non-detected values were calculated according to the medium bound with the substitution of non-detected compounds with the half of the detection limit (LOD).

Statistical analyses. Statistical analyses were conducted using the software RStudio v.1.1.453 and graphic elaborations were realized using ggplot2 package[93]. Shapiro-Wilks test revealed non-normal distribution for PCBs and PBDEs, while a normal distribution was confirmed for CYP1A e CYP2B. Independent Mann–Whitney U tests were used to compare levels of major contaminant and cytochrome activity among different age groups. If a significant difference was obtained (p value < 0.05), post-hoc Dunn's multiple comparison was performed for individual comparisons of age groups. A regression analysis was used to evaluate the influence of PCBs and PBDEs on the expression of CYP1A1 and/or CYP2B. Spearman's rank correlation was adopted to measure the correlation between variables. All statistical analyses were considered significant at $p < 0.05$.

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Author contributions
M.B. participated to the sampling, performed the W.B. analysis, analysed the data, coordinated the writing of the manuscript; C.P. performed sex determination, contributed to the analysis of the data; M.C.F. conceived the study, participated to the sampling, coordinated the experiment; B.J. coordinated the contaminant analysis; P.T. performed the survey at sea, the population study and the statistical analysis; A.M. performed the survey at sea and performed the population study; F.C. performed the sampling and language revision of the ms; A.B. performed the laboratory analysis of chemicals and generated analytical data; J.M.A. generated analytical data and supervised the chemical analysis; M.R. conceived and coordinated the field study, designed the survey, performed most of the sampling and the population study. M.R., B.J. and M.C.F. obtained funding for the data collection and laboratory analysis. All authors contributed to the writing process, reviewed critically the drafts of the manuscript, and gave final approval for publication.

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The authors declare no competing interests.

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9.2. List of acronyms and abbreviations (alphabetical order)

A

AhR: Aryl hydrocarbon receptor

B

BAT: Best available techniques

BBO: Bogue

BEP: Best environmental practices

BFRs: Brominated flame retardants

BS: Blue shark

C

CBW: Cuvier's beaked whale

CPs: Chlorinated paraffins

CYP1A1: Cytochrome P450, family 1, subfamily A, polypeptide 1

D

DDT: Dichlorodiphenyltrichloroethane

DL-PCBs: Dioxin-like polychlorinated biphenyls

DNA: Deoxyribonucleic acid

DP: Dechlorane plus

E

ECD: Electron capture detector

EEN: Anchovy

EI+: Positive electronic ionization

EPA: Environmental Protection Agency

F

F: Female

FAO: Food and Agricultural Organization

FL: Fork length

G

GC-HRMS: Gas chromatography high resolution mass spectrometry

GLC: Gas-liquid chromatography

GLG: Growth layer groups

GMP: Global monitoring plan

GSAs: Geographical Subareas of the Mediterranean Sea

I

IARC: International Agency for Research on Cancer

IFCS: Intergovernmental Forum on Chemical Safety

i-PCBs: Indicator polychlorinated biphenyls

IUCN: International Union for Conservation of Nature

L

LOD: Limit of detection

LOQ: Limits of quantification

LRT: Long-range transport

l.w.: Lipid weight

M

M: Male

MAP: Mediterranean Action Plan

MCPAs: Marine and Coastal Protected Areas

MSFD: Marine strategy framework directive

O

OCs: Organochlorine pesticides

OH-PCBs: Hydroxylated polychlorinated biphenyls

OSPAR: Convention for the Protection of the Marine Environment of the North-East Atlantic

P

PBDEs: Polybrominated diphenyl ethers

PBT: Pentabromotoluene

PCBs: Polychlorinated biphenyls

PCDD/Fs: Polychlorinated dibenzo-*p*-dioxins and –furans

PCDDs: Polychlorinated dibenzo-p-dioxins

PCDFs: Polychlorinated dibenzofurans

PFAS: Per- and polyfluoroalkylated substances

PFOS: Perfluorooctanesulfonic acid

PIC: Prior informed consent

POPs: Persistent organic pollutants

Q

QA/QC: Quality assurance/quality control

R

REACH: Registration, Evaluation, Authorization and Restriction to Chemicals

RSD: Relative standard deviation

S

SD: Standard deviation

SIM: Selected ion monitoring

SPC: Sardine

SRM: Standard Reference Material

SW: Sperm whale

T

TCDD: 2378-tetrachlorodibenzo-p-dioxin

TEF: Toxic equivalency factor

TEQ: Toxic equivalency quotient

U

UNEP: United Nations Environment Programme

US: United States

W

WHO: World Health Organization

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