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INSIGHTS INTO XENOBIOTIC CONTAMINATION IN MEDITERRANEAN DEEP-SEA MEGAFAUNA WITH EMPHASIS ON THE SUBMARINE CANYON DOHRN

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ABSTRACT

The Mediterranean Sea is considered a biodiversity hotspot because it hosts a huge variety of marine species but is also characterized by high amounts of persistent organic contaminants.

The effects of contamination are, in fact, well known in the coastal and pelagic domains, but there's still a lack of information regarding their effects on the deep-water environment, the largest, less know and more vulnerable habitat of the planet.

The focus of this PhD project is to carry out the first assessment of the contamination of the deep waters of the Tyrrhenian Sea, one of the most anthropized area of the Mediterranean basin, by the analysis of the cartilaginous fish fauna, one the most important consumers of marine environments but at the same time one of the most threatened taxa.

The International Union for Conservation of Nature (IUCN) Red List considers pollution threat as, in the Mediterranean subpopulations but also at global level, a threat only for few different chondrichthyan species. One of the first objectives was to highlight the lack of information about pollution in cartilaginous fishes which play a key role in aquatic ecosystem.

Five chondrichthyan species were collected from the deep sea of the Ligurian and North Tyrrhenian Sea (in the Geographic Sub Area 9, GSA9) and of the Dohrn Canyon (Gulf of Naples) and investigated from a toxicological point of view: in particular, we focused our attention on legacy organochlorine compounds (OCs) such as hexachlorobenzene, polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT) and its metabolites. The use and production of these contaminants is banned since the Stockholm Convention on Persistent Organic Pollutants (POPs); despite this, due to their chemical-physical properties (high boiling point, persistence, lipoaffinity, etc.) as well as to the exemptions for their use, their unregulated use in some States, or their marketing with "small" changes in the composition for which they escape the regulations, they still remain a priority for the health status of living organisms.

The toxicological analysis conducted in the sampled species revealed the presences of all the three POPs investigated, both in the Canyon and in the GSA9. The prevalence of PCBs in the samples confirmed that the species are more subjected to an industrial-type of contamination but, one of the principal findings was that from the DDT isomers analysis, resulted the recent use of the industrial DDT, an enriched *op'* isomers formula,

which is still unregulated or is used to produce other pesticides. Moreover, due to the detected maternal transfer of all the three POPs, these species are stressed from the very beginning, causing an additional threat to their conservation. Levels detected in the Dohrn Canyon were significantly lower than those in the GSA9 suggesting that the hydrodynamism of such environment may help in pollutant dispersion, subjecting non-migratory species to minor contamination. However, further studies on different species with different home ranges should be conducted in order to corroborate this hypothesis.

This is the first assessment of the occurrence of organochlorine contaminants in the deep environments of the Tyrrhenian Sea, and it stresses once again the urgency of further focused long term researches, mixing different data from different sources, in order to monitor and better understand the future trends of this impact in the marine environments. Moreover, this study underlines the importance of the role of the marine scientists at the international political level, in order to request further conservation measures for marine species, also for those living in deep sea environments.

STRUCTURE OF THE THESIS

This thesis is structured in seven chapters.

The first chapter is a brief introduction where are presented the main topics discussed in the dissertation.

The second chapter emphasizes the importance of assessing pollution threats in cartilaginous fishes, as well as its scarcity of data on the most important indicator of the health of the world's biodiversity, the International Union for Conservation of Nature's (IUCN) Red List of Threatened Species.

The third chapter describes the methods that have been used throughout this Thesis to achieve the set goals.

Chapters four – six have been prepared as scientific manuscript with the intent to be submitted in the near future. Overall, each of these chapters have a general introduction in which are described the research context and the main objectives, summarized methods, results are presented combined or separate from discussions, and final remarks and conclusions are given at the end. For a better comprehension the list of cited papers is included at the end of each section and chapter.

In these Chapters I present a first toxicological evaluation of organochlorine compounds (OCs) on some deep-sea species opportunistically sampled in areas where anthropic impact can represent a threat.

Specifically, the fourth chapter is the first assessment of some OCs in deep sea chondrichthyans sampled in the Geographic Sub Area 9 (GSA9) (FAO, 2020).

Then, in the fifth chapter the same compounds were investigated in the Dohrn Canyon's megafauna, one of the unexplored Mediterranean submarine canyons.

The comparison between the two areas is addressed in chapter six. To determine if the contamination input was different, a species common to both sampling sites was considered.

The seventh chapter contains concluding considerations to highlight the Thesis' main findings as well as possible future prospects and recommendations in this field.

Chapter 2 of this PhD thesis has already been published in a peer-reviewed journal:

Consales, G., & Marsili, L. (2021). Assessment of the conservation status of Chondrichthyans: underestimation of the pollution threat. The European Zoological Journal, 88(1), 165-180. DOI: 10.1080/24750263.2020.1858981

LIST OF ABBREVIATIONS

AChE – Acetylcholinesterase
BChE – Butyrylcholinesterase
BPA – Bisphenol A
CAT – Catalase
CbE – Carboxylesterase
CR – Critically Endangered
CYP1A – Cytochrome P450 1A
DCBP – Decachlorobiphenyl
DD – Data Deficient
DDD – Dichlorodiphenyldichloroethane
DDE – Dichlorodiphenyldichloroethylane
DDT – Dichlorodiphenyltrichloroethane
EDCs – Endocrine Disruptor Chemicals
EN – Endangered
EOM – Extracted Organic Material
EPA – Environmental Protection Agency
EROD – Ethoxyresorufin – O – Deethylase
F – Female
F% – Frequency
FAC – Fluorescent Aromatic Compounds
GME – *Galeus melastomus*
GPx – Glutathione Peroxidase
GR – Glutathione Reductase
GSA – Geographic Sub Area
GST – Glutathione – S – Transferase
HCB – Hexachlorobenzene
HDA – *Helicolenus dactylopterus*
IDH – Isocitrate Dehydrogenase
IUCN – International Union for Conservation of Nature
IUPAC – International Union of Pure and Applied Chemistry
K – Fulton's condition factor
l.w. – lipid weight
LC – Least Concern
LOD – Limit Of Detection
LP – Lipid Peroxidation
M – Male
N – Number of sampled specimens
ND – Not Determined
OCs – Organochlorine Compounds
PAHs – Polycyclic Aromatic Hydrocarbons
PBDEs – Polybrominated Diphenyl Ethers
PCBs – Polychlorobiphenyls
POPs – Persistent Organic Pollutants
PrChE – Propionylcholinesterase
PSU – Practical Salinity Unit
SD – Standard Deviation
SOD – Superoxide Dismutase
TBARS – Thiobarbituric Acid Reactive Substances

TG – Total Glutathione
THg – Total Mercury
TL – Total Length
TW – Total Weight
Vtg – Vitellogenin
VU – Vulnerable
Zrp – Zona Radiata Proteins

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Chapter 1 ***BRIEF INTRODUCTION***

In the past, deep-sea ecosystems were considered lifeless domains, but over the year awareness of their enormous richness has been increasing (Cartes et al., 2004). Abyssal plains are represented by the oceanic layer that lies at depths greater than 200 m (Sanganyado et al., 2021) and cover more than 60 % of the planet's surface. These ecosystems have important global biogeochemical functions and an important role in driving nutrient cycling (Karl, 2002). These habitats represent exceptional ecosystems since they host rare trophic networks; in the deep waters, cold water coral reefs, cold springs and hydrothermal vents can be found (Sardà et al., 2004).

The Mediterranean Sea is considered a biodiversity hotspot because it hosts a huge variety of marine species (Coll et al., 2010) and it is also characterized by depths of up to 5,000 m and hosts geomorphological structures such as canyons, seamounts, and abyssal plains, which have an enormous biodiversity that is unique and distinctive from other marine regions of the world (Danovaro et al., 2010). The abysses of the Mediterranean Sea have unique characteristics, such as almost constant temperatures (12.5-14.5°C) at all depths, high salinity (38.4-39.0 PSU) and high oxygen levels (4.5-5.0 ml/L). Another aspect that adds to the habitat's uniqueness is the communities' isolation from those in the Atlantic, as well as from those in the eastern and western Mediterranean (Coll et al., 2010). Deep sea species have had to adapt to the unique features of these habitats, such as a lack of light and limited food availability (Kroncke et al., 2003). These ecosystems need greater protection because they appear to be particularly vulnerable to commercial exploitation; around 40% of global trawling takes place in waters deeper than the continental shelf (Roberts, 2002) and many seamount fisheries have been depleted in a relatively short period of time (Lack et al., 2003).

Moreover, the Mediterranean Sea is also characterized by high amounts of persistent organic contaminants (Marsili et al., 2018). This is due to the presence of numerous industrial sites along the coast, as well as being a semi-closed basin; and due to its geomorphology, the water requires more than a century to be completely renewed (Lacombe et al., 1981). Its characteristics facilitate the accumulation rather than dispersion of contaminants, making it a particularly vulnerable and potentially threatened ecosystem (Storelli et al., 2012).

Legacy contaminants such as organochlorine compounds (OCs) are among the substances that are harming marine environments. These are organic chemicals and possess a

particular combination of properties whereby once released into the environment they remain intact for exceptionally long periods of time. These compounds belong to the group of chlorinated hydrocarbon derivatives, which have wide application in the industry and agriculture. Organochlorine insecticides were used in the past to fight diseases such as malaria, but their extensive use and their toxicity to untargeted organisms has led to their being banned in most advanced countries (Aktar et al., 2009). Thanks to their properties are also known as persistent organic pollutants (POPs). These substances exhibit high persistence and volatility, remarkable stability, high capacity to bioaccumulate in the food web, slow degradation, low polarity, low aqueous solubility and high lipid solubility (Jayaraj et al., 2016); moreover, they can be transported over long distances to the most remote areas of the globe (Carrizo and Gustaffson, 2011; Pouch et al., 2021; Wängberg and Björk, 2021). They are dispersed throughout the environment and accumulate in the fatty tissue of living organisms, including humans especially in developing countries (Joseph et al., 2020; Olisah et al., 2020; Oyinloye et al., 2021). Organochlorine compounds are associated with particles suspended in water in the marine environment, which can sink and become a source of contaminants in sediments or be transported (Wania and Mackay, 1993). River discharges, coastal wastewater disposal, the presence of contaminated sediments, agricultural and industrial practices can all cause them to enter aquatic systems (Stortini et al., 2012) and add an additional threat to marine organisms. Among them, the megafauna, which includes animals at the top of the food chain such as cetaceans, cartilaginous fishes, and large pelagic bony fishes, is the one at greatest risk (Colborn and Smolen, 1996; Marsili et al., 2018; Tiktak et al., 2020; Xie et al., 2020). Already threatened by overfishing, bycatch, and habitat depletion, in the last decades deep sea organisms had to face another issue: pollution.

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Chapter 2 ***ASSESSMENT OF THE CONSERVATION STATUS OF CHONDRICHTHYANS: UNDERESTIMATION OF THE POLLUTION THREAT***

Abstract

Cartilaginous fish include sharks, rays, skates, sawfish, and chimaeras. Their habitat ranges from shallow coastal waters to deep ocean floors, estuarine areas as well as rivers and inland waters. Overfishing is considered to be the main threat to their existence, but there are many more stressors that these species face. Pollution is an issue that concerns aquatic organisms at every level, and Chondrichthyans are no exception. Here, we looked at their IUCN Red List assessment and noticed a lack of information regarding anthropogenic contamination for these species. Out of 1124 cartilaginous fish species assessed, only 17 *Selachimorpha* and 32 *Batoidea* species were considered to be facing a "pollution threat"; in most cases, the threat was assigned not from direct ecotoxicological studies of the specimens, but because the species inhabited areas likely to be contaminated. An update on the conservation status of these species is urgently needed. Further, there is a fundamental need to study the effects of contaminants on Chondrichthyans as they play a key role in aquatic ecosystems.

Introduction

There are 1200 species of Chondrichthyans, the majority of which inhabit marine ecosystems (Weigmann, 2016). Sharks, rays, skates, sawfish, and chimaeras belong to this class. Chondrichthyans also occupy a large range of habitats, from shallow coastal waters to deep-sea floors. For this reason, they are subjected to many different threats and stressors. To date, the most prominent threat to cartilaginous fish is overfishing (Dulvy *et al.*, 2014). Since the introduction of large-scale commercial fishing, sharks and rays have been caught indiscriminately in large quantities, despite not being the primary targets of fisheries.

More recently, however, developing markets and depleting numbers of traditionally commercial fish have made these "bycatch" sharks and rays increasingly desirable. Sharks and rays are also intentionally caught and killed because of the perceived threat they pose to humans as well as the incessant demand for shark products, including liver oil, fins, and gills (Fowler *et al.*, 2002; Clarke *et al.*, 2006; Lack and Sant, 2009). Habitat depletion and environmental contamination also represent substantial dangers to Chondrichthyans.

A large portion (~71%) of the Earth's surface is covered with water, and until the 1970s, most toxic wastes were discarded in the oceans (Lumsdaine, 1975) with little understanding of the true negative impacts of such actions. The most common assumption was that the ocean had an unlimited capacity to mix and disperse debris and substances; therefore, after years of uncontrolled dumping, the first effects began to emerge in the 1980s (Lear *et al.,* 1981; Messieh *et al.,* 1991). This led to conventions and international agreements for the protection of the marine environment from human activities and for the production, use, and disposal of toxic substances (Craig, 2004).

The effects of chemicals, especially persistent organic pollutants (*POPs*), are well-known and have been studied in many marine species (Fossi *et al.,* 2013; Marsili *et al.*, 2014; Brown and Takada, 2017; Casini *et al.,* 2018; Mearns *et al.,* 2019; Righetti *et al.,* 2019; Quintanilla-Mena *et al.,* 2020). *POPs* interfere with organisms, compromising multiple physiological processes; they have immunosuppressive properties, are carcinogenic, mutagenic, and teratogenic, and some are known to be endocrine disruptors (Jimenez, 1997; Matthiessen, 2003; Mikula and Svobodova, 2006; IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2016; Centelleghe *et al.,* 2019; Marsili *et al.,* 2019). Even naturally occurring contaminants such as PAHs and heavy metals, which have been made more bioavailable by human activities, are known to be important stress factors for marine organisms (Marsili *et al.,* 2014; Scheuhammer *et al.,* 2015; Marsili *et al.,* 2016; Santana *et al.,* 2018; Cunningham *et al.,* 2019; Lee *et al.,* 2019). Furthermore, most studies have demonstrated the existence of pollutants in Chondrichthyans and their environments (Gelsleichter and Walker, 2010), but few have examined the impact and effects of chemicals on these organisms (Fuentes-Rios *et al.,* 2005; Solè *et al.,* 2010a,b; Barrera-García *et al.,* 2012; Barrera-García *et al.,* 2013; Velez-Alavez *et al.,* 2013*;* Alves *et al.,* 2016; Marsili et al., 2016; Fossi *et al.,* 2017; Cullen et al., 2019; Lyons and Wynne-Edwards, 2019; Ehnert-Russo and Gelsleichter, 2020).

However, environmental contamination is the least studied of the aforementioned threats and stressors, as confirmed by the number of scientific papers on pollution in elasmobranchs. A research conducted on three of the foremost online databases (Scopus, Web of Science, and PubMed) revealed that only approximately 4% of published papers on Chondrichthyes discuss contamination. The research was conducted using keywords such as *"Chondrichthyes," "elasmobranchs," "sharks," "batoids," "contaminants," "organochlorines," "pesticides," "pollution," "plastic," "polychlorobiphenyls (PCBs)," "Polybrominated diphenyl ethers (PBDEs),"*

"Dichlorodiphenyltrichloroethane (DDT)," *"Polycyclic aromatic hydrocarbons (PAHs),"* *"Phthalates,"* *"Bisphenol A (BPA),"* *"heavy metals,"* and *"mercury"* together, in different combinations, or separately to refine results.

The principal aim of this study was to highlight the lack of information regarding pollution in cartilaginous fish, which play a key role in aquatic ecosystems. We reviewed data available from the International Union for Conservation of Nature (IUCN) Red List of Threatened Species, the main database on the conservation status of biological species. Using the "*advanced search*" tool in the IUCN Red List website, which helps to filter data based on multiple categories (species, regions, documents, and Red List Indices), we identified a way to extrapolate information on Chondrichthyes assessed for pollution. In particular, in the section "*Taxonomy*," we ticked the box "*Chondrichthyes*," and in the section "*Threats*," we ticked the box "*Pollution*." With this first step, we want to fill the gap in knowledge regarding publications on the effect of pollutants in Chondrichthyes and the assessment of cartilaginous fish in the IUCN Red List.

Results

The IUCN Red List states that 30% of Chondrichthyans are threatened by extinction; however, "pollution" is mentioned in the threat assessment of only 4%. During species assessment, a scheme must be followed to assign a specific threat to a species, available on the IUCN Red List website. Appendix 1, at the end of this chapter, reports an extract from the IUCN Unified Classification of Direct Threats, mainly focused on the Pollution threat. This scheme suggests six pollution classes; the first four are known for affecting aquatic organisms in general, the fifth involves airborne pollutants, and the last refers to inputs of heat, sound, or light that disturb wildlife or ecosystems. As most of the chemicals that were used and continue to be used by humans unfortunately end up in aquatic ecosystems, it seems logical that at least one pollution class be assigned to Chondrichthyans. After a thorough search on the IUCN Red List website, we found that out of 1124 cartilaginous fish species, only 17 *Selachimorpha* and 32 *Batoidea* species were assigned the "pollution threat". Tables 2.1 and 2.2 list these species with their scientific names, IUCN classification status, assigned pollution class, and last assessment date.

Table 2.1 Species, common name, IUCN Red List classification status, assigned pollution threat, and last assessment date

Selachimorpha

Species	Common name	IUCN Red List status	Pollution threat	Last assessed
Aulohalaelurus kanakorum	New Caledonia Catshark	Data Deficient (DD)	9.2.2. Seepage from mining	20 June 2017
Carcharhinus leiodon	Smoothtooth Blacktip Shark	Endangered (EN)	9.2.1. Oil spills	09 February 2017
Chiloscyllium arabicum	Arabian Carpetshark	Near Threatened (NT)	9.1.2. Run-off 9.2.1. Oil spills	09 February 2017
Glyphis glyphis	Speartooth Shark	Endangered (EN)	9.2.2. Seepage from mining	01 October 2005
Glyphis siamensis	Irrawaddy River Shark	Critically Endangered (CR)	9.1.3. Type Unknown/Unrecorded 9.3.2. Soil erosion, sedimentation 9.3.4. Type Unknown/Unrecorded	01 December 2008
Haploblepharus edwardsii	Puffadder Shyshark	Near Threatened (NT)	9.1.3. Type Unknown/Unrecorded	01 December 2008
Haploblepharus fuscus	Brown Shyshark	Vulnerable (VU)	9.1.3. Type Unknown/Unrecorded	01 December 2008
Haploblepharus kistnasamyi	Natal Shyshark	Vulnerable (VU)	9.1.3. Type Unknown/Unrecorded	25 April 2018
Hemiscyllium hallstromi	Papuan Epaulette Shark	Vulnerable (VU)	9.1.1. Sewage 9.2.2. Seepage from mining	18 February 2015
Hemiscyllium michaeli	Michael's Epaulette Shark	Near Threatened (NT)	9.3.2. Soil erosion, sedimentation	24 January 2012
Hemiscyllium strahani	Hooded Carpetshark	Vulnerable (VU)	9.2.2. Seepage from mining	30 April 2003
Nasolamia velox	Whitenose Shark	Data Deficient (DD)	9.3.2. Soil erosion, sedimentation	01 December 2008
Paragaleus randalli	Slender Weasel Shark	Near Threatened (NT)	9.2.1. Oil spills	01 December 2008
Poroderma pantherinum	Leopard Catshark	Data Deficient (DD)	9.1.3. Type Unknown/Unrecorded	12 May 2004
Rhizoprionodon lalandii	Brazilian Sharpnose Shark	Data Deficient (DD)	9.4. Garbage and solid waste	30 April 2004
Rhizoprionodon longurio	Pacific Sharpnose Shark	Data Deficient (DD)	9.1.3. Type Unknown/Unrecorded	01 December 2008
Schroederichthys tenuis	Slender Catshark	Data Deficient (DD)	9.2.3. Type Unknown/Unrecorded 9.3.3. Herbicides and pesticides	30 April 2004

Table 2.2 Species, common name, IUCN Red List classification status, assigned pollution threat, and last assessment date

Batoidea

Species	Common name	IUCN Red List status	Pollution threat	Last assessed
Anoxypristis cuspidata	Narrow Sawfish	Endangered (EN)	9.1.1. Sewage 9.1.2. Run-off 9.2.1. Oil spills 9.2.2. Seepage from mining 9.3.1. Nutrient loads 9.3.2. Soil erosion, sedimentation 9.3.3. Herbicides and pesticides	07 April 2012
Brevitrygon imbricata	Scaly Whipray	Data Deficient (DD)	9.2.3. Type Unknown/Unrecorded	08 September 2004
Brevitrygon walga	Scaly Whipray	Near Threatened (NT)	9.2.1. Oil spills	09 February 2017
Fluvitrygon kittipongi		Endangered (EN)	9.1.3. Type Unknown/Unrecorded 9.3.3. Herbicides and pesticides	11 July 2007
Fluvitrygon oxyrhyncha	Longnose Marbled Whipray	Endangered (EN)	9.1.1. Sewage 9.2.3. Type Unknown/Unrecorded 9.3.3. Herbicides and pesticides 9.3.2. Soil erosion, sedimentation	03 October 2005
Fluvitrygon signifer	White-edge Freshwater Whipray	Endangered (EN)	9.1.1. Sewage 9.2.3. Type Unknown/Unrecorded 9.3.3. Herbicides and pesticides	03 October 2005
Gymnura crebripunctata	Mazatlan Butterfly Ray	Data Deficient (DD)	9.1.1. Sewage 9.1.2. Run-off	30 April 2011
Gymnura marmorata	California Butterfly Ray	Least Concern (LC)	9.1.1. Sewage 9.1.2. Run-off	30 April 2011
Hemitrygon fluviorum	Estuary Stingray	Vulnerable (VU)	9.2.2. Seepage from mining	02 May 2003
Hemitrygon laevigata	Yantai Stingray	Near Threatened (NT)	9.1.3. Type Unknown/Unrecorded	03 December 2008
Himantura undulata	Bleeker's Variegated Whipray	Vulnerable (VU)	9.1.3. Type Unknown/Unrecorded 9.3.1. Nutrient loads 9.3.2. Soil erosion, sedimentation	12 December 2011
Maculabatis pastinacoides	Round Whipray	Vulnerable (VU)	9.2.2. Seepage from mining	12 September 2004
Maculabatis randalli	Arabian Banded Whipray	Least Concern (LC)	9.2.1. Oil spills	08 February 2017

Mobula birostris	Giant Manta Ray	Vulnerable (VU)	9.1.1. Sewage 9.1.2. Run-off	01 November 2010
Narcine atzi	Oman Numbfish	Data Deficient (DD)	9.1.3. Type Unknown/Unrecorded	12 September 2004
Narcine lingula	Chinese Numbfish	Data Deficient (DD)	9.3.2. Soil erosion, sedimentation	01 January 2007
Pastinachus solocirostris	Roughnose Stingray	Endangered (EN)	9.1.3. Type Unknown/Unrecorded	08 July 2007
Plesiotrygon iwamae	Antenna Ray	Data Deficient (DD)	9.1.1. Sewage 9.2.2. Seepage from mining 9.3.2. Soil erosion, sedimentation	24 June 2003
Potamotrygon brachyura	Giant Freshwater Stingray	Data Deficient (DD)	9.3.3. Herbicides and pesticides	24 June 2003
Potamotrygon castexi	Vermiculate River Stingray	Data Deficient (DD)	9.3.1. Nutrient loads	24 June 2003
Potamotrygon leopoldi	Xingu River Ray	Data Deficient (DD)	9.1.1. Sewage 9.3.2. Soil erosion, sedimentation 9.3.4. Type Unknown/Unrecorded	24 June 2003
Potamotrygon magdalenae	Magdalena Freshwater Stingray	Least Concern (LC)	9.1.2. Run-off 9.3.4. Type Unknown/Unrecorded	08 October 2014
Potamotrygon scobina	Raspy River Stingray	Data Deficient (DD)	9.2.2. Seepage from mining 9.3.3. Herbicides and pesticides	30 April 2004
Potamotrygon yepezi	Maracaibo River Stingray	Data Deficient (DD)	9.3.4. Type Unknown/Unrecorded	05 May 2004
Pristis clavata	Dwarf Sawfish	Endangered (EN)	9.2.2. Seepage from mining 9.2.1. Oil spills	07 May 2012
Pristis pristis	Largetooth Sawfish	Critically Endangered (CR)	9.3.1. Nutrient loads 9.3.2. Soil erosion, sedimentation	01 March 2013
Pristis zijsron	Green Sawfish	Critically Endangered (CR)	9.3.1. Nutrient loads 9.3.2. Soil erosion, sedimentation	20 May 2012
Rhinobatos albomaculatus	White-spotted Guitarfish	Vulnerable (VU)	9.1.3. Type Unknown/Unrecorded 9.2.3. Type Unknown/Unrecorded 9.3.4. Type Unknown/Unrecorded	01 December 2008
Rhinobatos irvinei	Spineback Guitarfish	Vulnerable (VU)	9.1.3. Type Unknown/Unrecorded 9.2.3. Type Unknown/Unrecorded 9.3.4. Type Unknown/Unrecorded	01 December 2008
Torpedo mackayana	West African Torpedo Ray	Data Deficient (DD)	9.1.3. Type Unknown/Unrecorded 9.2.3. Type Unknown/Unrecorded 9.3.4. Type Unknown/Unrecorded	01 January 2007

Urogymnus polylepis	Giant Freshwater Stingray	Endangered (EN)	9.1.3. Type Unknown/Unrecorded 9.2.3. Type Unknown/Unrecorded 9.3.1. Nutrient loads 9.3.3. Herbicides and pesticides 9.3.2. Soil erosion, sedimentation	27 February 2011
Urotrygon nana	Dwarf Round Stingray	Data Deficient (DD)	9.1.1. Sewage 9.2.3 Type Unknown/Unrecorded 9.3.2. Soil erosion, sedimentation	01 December 2008

After evaluating the information provided by IUCN, it has emerged that the species present in Table 2.1 have a fairly limited range; they are not cosmopolitan species but are often endemic. In some cases, the pollution category has been assigned to species that are barely known and studied, such as the New Caledonia Catshark. In fact, this species is known from only one caught specimen and two photographs (Séret, 1990; Ebert *et al.,* 2013; Finucci and Kline, 2018). In most cases, the pollution threat is assigned to the species because they inhabit an area that is known to be, or might be, stressed from anthropogenic factors. Mostly, there is no mention of either the studies conducted in the area or the potential contamination sources. The only ecotoxicological study conducted on the listed species and mentioned in the assessment is the one by Al-Hassan *et al.* (2000), who investigated the presence of PAHs in *Chiloscyllium arabicum.*

To verify whether there were other studies on contamination in these species, we searched for literature regarding pollution for each of the shark species listed in Table 2.1 and found that there were indeed three more species with documented presence of pollutants in their tissues and one more ecotoxicological study on *C. arabicum*. In this species, Adel *et al.* (2018) noted six different metals (cadmium (Cd), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb), and zinc (Zn)) in the liver and muscle of 40 specimens sampled from two sites. The presence of metals in both tissues and liver were higher in specimens collected in the area with more human activity. Adel *et al.* (2018) also performed a risk assessment for food intake; the risk for consumers was low for all the metals, with the exception of total mercury (THg), which was near the risk threshold upon high frequencies of consumption. However, even if the risk for consumers is low, there may still be a risk for the specimen and its physiological status.

The three other species on which ecotoxicological studies were conducted are the smoothtooth blacktip shark (*Carcharhinus leiodon*), the Brazilian sharpnose shark (*Rhizoprionodon lalandii*), and the Pacific sharpnose shark (*R. longurio*).

Moore *et al.* (2015) demonstrated the presence of 11 trace elements in the muscles of five *C. leiodon* juveniles and two adult specimens in northern Kuwait waters. In particular, they looked for arsenic (As), Cd, chromium (Cr), Cu, iron (Fe), Hg, manganese (Mn), Ni, Pb, selenium (Se), and Zn and found that mercury concentrations were higher than the limits imposed by the European Food Safety Authority. The levels of other elements and contaminants may nevertheless act as further stressors to these species.

Three papers have been published on Brazilian sharpnose sharks, in which the presence of *POPs* were investigated, and plastic ingestion and entanglement were documented.

Chlorinated pesticides, *PCBs*, and *PBDEs* were found in the liver of a *R. lalandii* specimen caught during trawling operations off the coasts of Brazil (Cascaes *et al.*, 2014). *PCBs* were present in greater amounts, followed by organochlorine pesticides and *PBDEs.* Miranda *et al.* (2016) documented microplastic pellet ingestion in two specimens upon analyzing stomach content of six individuals. Plastic debris was also found in three juvenile Brazilian sharpnose sharks caught in gillnets in southeast Brazil (Sazima *et al.*, 2002). These sharks presented with plastic collars around their gill region; the tissues were severely damaged by these rings, which probably affected normal feeding and ventilation (Sazima *et al.*, 2002).

In the Pacific sharpnose shark, trace elements were principally investigated. The first study on this species was by Hurtado-Banda *et al.* (2012), who evaluated THg in the muscles and liver of 12 juveniles and 14 adults collected from artisanal fishery landing sites in Sonora (Mexico). Adults had higher THg values than juveniles, and in both age classes, muscle tissue was more contaminated. In the study by Frías-Espericueta *et al.* (2014), Cd, Cu, Pb, and Zn in the liver, muscle, and embryo-related tissues (placenta and umbilical cord) of 15 pregnant females and their embryos were investigated. Cu and Zn had higher values in the placenta and umbilical cord, whereas Pb and Cd were predominant in the maternal muscle and liver, respectively. Another 20 pregnant females and their embryos were sampled, and their blood, placenta, umbilical cord, and embryo livers were analyzed for THg (Frías-Espericueta *et al.*, 2015). Maternal blood had higher values whereas embryonic liver had lower values. They found marked correlations between the THg content in the maternal blood, umbilical cord, and placenta, suggesting transplacental Hg transfer. Frías-Espericueta *et al.* (2019) also conducted a risk assessment for this species. They evaluated the THg in the edible muscles of 15 adult sharks caught by artisanal fisheries. The results showed that only 6.6% of the sharks sampled had mercury levels that exceeded the permissible limit; however, overall, the hazard quotient values for THg and the calculated methylmercury content indicated no risk upon consumption. These levels, however, might represent an additional stressor to the species, on top of bycatch and overfishing.

All these studies, except the one by Moore *et al.* (2015), were published after the last assessment of the species, which indicates that an update is needed.

In addition, it can be seen from Table 2.1 that more than a third of the species are classified as DD, seven out of 17 species belong to threatened categories (VU, EN, and CR), and the remaining four species are not threatened.

Only 10 out of 19 species are considered to be affected by pollution. The main source of contamination for these species is "9.1.3. Type unknown/unrecorded," which falls under the class "9.1. Domestic and Urban Wastewater". This class includes unidentified water-borne sewage and non-point runoff from housing and urban areas (nutrients, toxic chemicals, and/or sediments). The second most abundant class is the one that includes pollution from mining activities, a class assigned to four different species sharing the same area. This is followed by "9.2.1. Oil spills" and "9.3.2. Soil erosion and sedimentation" with three different species under each category. These categories include species who are affected, for example, from coastal sedimentation or from war-related oil releases, as some of these sharks inhabit the Arabian Gulf.

As majority of these pollution classes are associated with the species due to their geographic range, it is not understandable why, for example, in the *Glyphis glyphis* assessment, there is no "Herbicides and pesticides" pollution threat. This species, which was assessed for the last time in 2005, inhabits an area where there is known contamination by several xenobiotics, including *DDT* (Von Westernhagen and Klumpp, 1995; Haynes *et al.*, 2000; Mortimer, 2000).

As already mentioned before, some papers on the presence of pollutants in *Selachimorpha* do exist, although in a very limited number, and, to the best of our knowledge, only 11 of them demonstrate the effects of pollutants on these species. The studied species, their IUCN Red List Status, and the investigated biomarkers are listed in Table 2.3.

Prionace glauca, *Isurus oxyrinchus*, *Carcharodon carcharias*, and the Mediterranean subpopulation of *Galeus melastomus* were assessed after the publication of their respective research papers; however, there is still no mention of pollution in the threat assessments of these species. The other species listed in the table were assessed before the publication of their respective papers; given the information that is now known, their conservation status should be updated.

Table 2.3 Bibliographic research on biomarkers tested in shark species. In brackets, the regional assessment (Mediterranean)

Species	Common name	IUCN Red List status	Investigated biomarkers	Reference
Schroederichthys chilensis	Redspotted catshark	DD	EROD, FAC	Fuentes-Rios et al., 2005
Scyliorhinus canicula	Lesser spotted dogfish	LC, (LC)	AChE, BChE, PrChE, LP	Solè et al., 2010a
Galeus melastomus	Blackmouth catshark	LC, (LC)		
Scyliorhinus canicula	Lesser spotted dogfish	LC, (LC)	CAT, GR, GST, EROD,	Solè et al., 2010b
Galeus melastomus	Blackmouth catshark	LC, (LC)	CbE	
Prionace glauca	Blue shark	NT, (CR)	GR, GPx, GST, CAT, SOD, TBARS	Barrera-García et al., 2012
Prionace glauca	Blue shark	NT, (CR)	GR, GPx, GST, CAT, SOD, TBARS	Barrera-García et al., 2013
Isurus oxyrinchus	Mako shark	EN, (CR)	GR, GPx, GST, CAT, SOD, TBARS	Velez-Alavez et al., 2013
Prionace glauca	Blue shark	NT, (CR)	GST, SOD, CAT, GR, GPx, TG, TBARS, AChE, IDH	Alves et al., 2016
Carcharodon carcharias	Great white shark	VU, (CR)	CYP1A, Vtg, Zrp	Marsili et al., 2016
Rhincodon typus	Whale shark	EN	CYP1A	Fossi et al., 2017
Carcharhinus leucas	Bull shark	NT	EROD, GST	Cullen et al., 2019
Carcharhinus limbatus	Blacktip shark	NT, (DD)		
Sphyrna tiburo	Bonnethead shark	LC		
Rhizoprionodon terraenovae	Atlantic sharpnose shark	LC	TG	Ehnert-Russo and Gelsleichter, 2020

EROD = ethoxyresorufin-O-deethylase; FAC = fluorescent aromatic compounds; AChE = acetylcholinesterase BChE = butyrylcholinesterase; PrChE = propionylcholinesterase; LP = lipid peroxidation; TBARS = thiobarbituric acid reactive substances; CAT = catalase; GR = glutathione reductase; GST = glutathione-S-transferase; CbE = carboxylesterase; GPx = glutathione peroxidase; SOD = superoxide dismutase; TG = total glutathione; IDH = isocitrate dehydrogenase; CYP1A = cytochrome P450 1A; Vtg = vitellogenin; Zrp = zona radiata proteins

Table 2.2 lists 32 *Batoidea* species, of which 34.2% occupy inland waters, 57.9% inhabit marine waters, and the remaining 7.9% are present in both ecosystems, allegedly living in estuarine areas. The predominant Red List category for these species was DD (12 species), followed by EN (seven species), VU (six species), LC (three species), CR, and NT (both two species).

As for *Selachimorpha*, the pollution threat is recognized in species with very restricted ranges, some of which are very rare and are known only from a few specimens in museum collections (Compagno, 2016a; Compagno, 2016b). Although no *Selachimorpha* species is known to have a widespread distribution, in *Batoidea*, *Mobula birostris* represents the only species occurring in tropical, sub-tropical, and temperate waters of the Indian, Atlantic, and Pacific Oceans. Pollution threat is also recognized in other species with fairly wide distribution ranges, but they are limited in number (Carvalho *et al.*, 2009;

Rigby, 2012; D'Anastasi *et al.*, 2013; Kyne *et al.*, 2013; Simpfendorfer, 2013; Manjaji Matsumoto *et al.*, 2016).

Pollution threats have mostly been assigned to species based on some potential and few documented risks (NOAA 2004a,b; IGGC2007; Mudd and Patterson, 2010; Sheppard *et al.*, 2010) associated with the areas they inhabit, as opposed to being based on ecotoxicological studies conducted directly on the specimens. Hence, as was mentioned for the *Selachimorpha*, because all these species inhabit coastal waters, estuarine environments, and freshwater ecosystems in states and regions that were or are known to use chemicals for agriculture (Forget, 1991; Laabs *et al.*, 2002; Hijort *et al.*, 2011; Rao *et al.*, 2017; Mahzabin and Rahman, 2017; Carvalho, 2017; Rivai et al, 2019), they should all be classified under the "Herbicides and Pesticides" pollution class.

We wanted to further verify whether there were scientific papers on contaminants in these species; upon investigating the available literature, it was found that none of the species listed in Table 2.2 had any studies conducted on them concerning pollutants. Nevertheless, ecotoxicological data is available for other species, both on pollutant concentrations and biomarker responses (Bezerra *et al.*, 2019; Cagnazzi *et al.*, 2019a,b; Lyons and Wynne-Edwards, 2019), and their assessments should be updated. Pollution categories assigned by IUCN to assessed *Batoidea* and *Selachimorpha* are summarized in Figure 2.1.

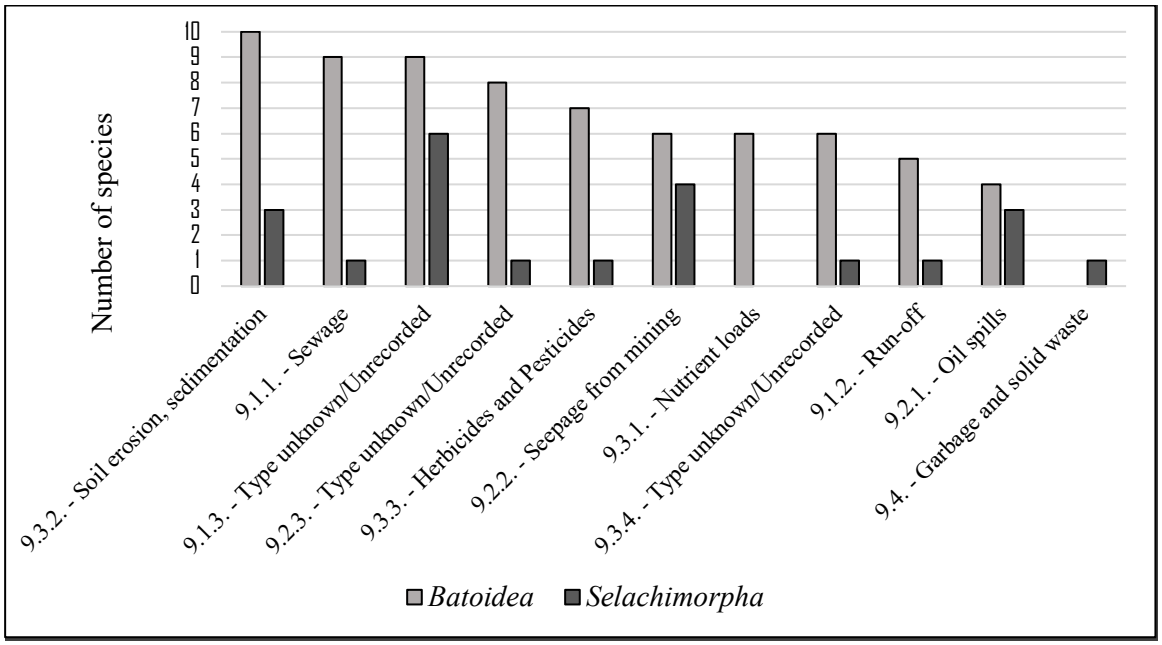

Figure 2.1 Assigned pollution categories to Batoidea and Selachimorpha assessed for pollution in the IUCN Red List. In particular, "9.1.3 - Type unknown/Unrecorded" falls into the "9.1. Domestic and Urban Wastewater" pollution class, "9.2.3 - Type unknown/Unrecorded" falls into the "9.2. Industrial and Military Effluents" pollution class, and "9.3.4 - Type unknown/Unrecorded" falls into the "9.3. Agricultural and Forestry Effluents" pollution class.

Selachimorpha and *Batoidea* have the same pollution threat classes, with the exception of "9.3.1. Nutrient loads" for *Batoidea* and "9.4. Garbage and Solid Waste" for *Selachimorpha.* In particular, these classes are assigned to three sawfish species, three freshwater and inshore ray species, and one coastal shark species. Regarding "Nutrient loads," no records of pollution stress were directly investigated on the animals, whereas the "Garbage and Solid Waste" threat was assigned to the Brazilian sharpnose shark because, as mentioned before, Sazima *et al.* (2002) observed three individuals with plastic pieces around the head and gill region.

The Mediterranean case

As already mentioned, pollution is an issue that concerns majority of the aquatic ecosystems. Some regions are considered more polluted than others; an example is the Mediterranean Sea. It is a landlocked sea, has large urban and industrial concentrations along its shores, and supports heavy maritime traffic; therefore, these conditions make it particularly prone to considerable anthropogenic impact at every marine level (Naso *et al.*, 2005; Fossi *et al*., 2006; Berrojalbiz *et al*., 2011; Bonanno and Raccuia, 2018; Casini *et al.,* 2018; Marsili *et al.*, 2018). Despite its small size, the Mediterranean Sea is considered a biodiversity hotspot. Approximately 10% of the world's marine species are present in its waters and 20% to 30% of the Mediterranean Sea species are endemic (UNEP-MAP, 2010). The Mediterranean Sea is also characterized by a remarkable occurrence of Chondrichthyan species; most of them are considered "Endangered" or "Critically Endangered" as per the last IUCN regional assessment. In terms of the Mediterranean subpopulations, the IUCN Red List currently considers pollution as a threat for only two Chondrichthyan species: the bull ray (*Aetomylaeus bovinus*) and the undulate ray (*Raja undulata*). Previous studies on Mediterranean shark and ray species demonstrated the presence of *POPs* (Storelli and Marcotrigiano, 2001; Storelli *et al.*, 2004; Storelli *et al.*, 2005; Storelli *et al.*, 2011a,b; Cresson *et al.*, 2016) and trace elements (Storelli *et al.*, 2002a,b; Kousteni *et al.*, 2006; Storelli *et al.*, 2011c), posing an additional stressor to their already threatened status. The importance of expanding the knowledge on pollution in these animals is fundamental. Indeed, as shown in Figure 2.2, there is a huge disparity between published papers on Chondrichthyes in general and published papers on pollution in these organisms.

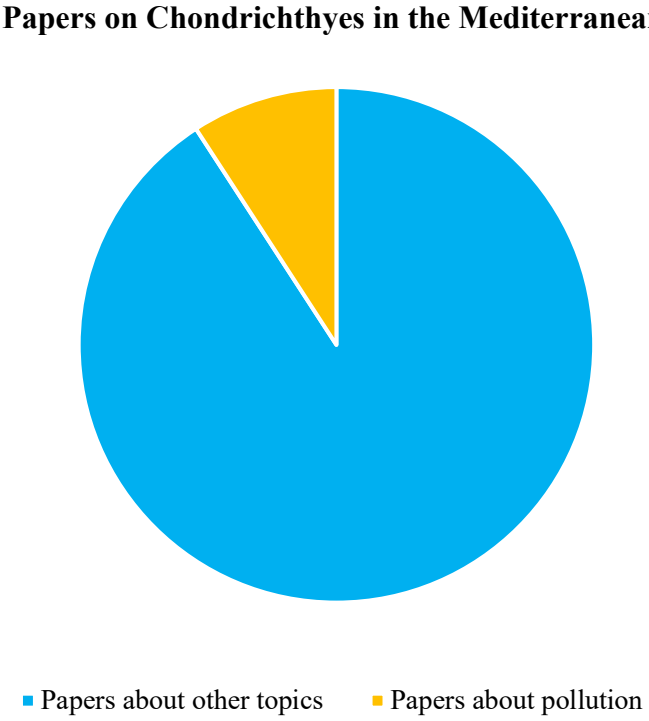

Figure 2.2 Published papers on Chondrichthyes in the Mediterranean Sea. In yellow, the percentage of papers on pollution in general (45 papers, 9%) and, in blue, the percentage of paper on other topics (445 papers, 91%).

The bibliographic research was conducted with two queries—(*Mediterranean sea OR Mediterranean) AND (elasmobranchs OR sharks OR batoids OR chondrichthyes)*—to search for papers on Mediterranean Chondrichthyes in general and *(Mediterranean sea OR Mediterranean) AND (elasmobranchs OR sharks OR batoids OR chondrichthyes) AND (contaminants OR organochlorines OR pollution OR DDT OR PCBs OR PBDEs OR PAHs OR mercury OR heavy metals OR plastic OR phthalates)* to search for papers on pollution in Mediterranean specimens. We refined the results, limiting the research to English articles/reviews in the final publication stage. Articles *in press* were not considered, nor were book chapters, theses, or conference papers/abstracts.

Conclusions

In conclusion, this review aimed to highlight the lack of information regarding pollution in cartilaginous fish. Herein, we demonstrated the need for an update in the conservation status of Chondrichthyes in the IUCN. Contamination is one of the primary stress factors in most marine organisms; it has already been demonstrated to be a substantial threat to cetaceans (Marsili *et al.,* 2019), sea birds (Costantini *et al.,* 2017; Dietz *et al.,* 2019), and

sea turtles (Casini *et al.,* 2018). However, only a few papers exist on Chondrichthyes regarding the effect of pollution. Therefore, it is extremely important that contamination be considered as one of the priority stressors in the evaluation of their assessment. There are several environmental contaminants, most of which are still unknown and others are produced accidentally; many are highly persistent and bioaccumulative. Hence, Chondrichthyes, which are likely at the top of the food chain, are most at risk.

In addition, the number of cartilaginous fish is declining worldwide (Sims, 2015) as they are additionally threatened by factors such as overfishing, bycatch, target fisheries, and illegal trading.

Moreover, given that some sharks and rays are also consumed by humans, it is risky to commercialize products that may be contaminated and lead to undesirable side effects (Mol *et al.,* 2018; Kim *et al.,* 2019; Lara *et al.,* 2020). In addition, for this latter reason, it is fundamental to evaluate pollutant concentrations in edible tissues, both for Chondrichthyes conservation and maintenance of human health.

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Appendix 1 to this Chapter. Extract of the threats' classification scheme proposed by IUCN

IUCN - CMP Unified Classification of Direct Threats					Direct threats are the proximate human activities or processes that have impacted, are impacting, or may impact the the status of the taxon being assessed (e.g., unsustainable fishing or logging). Direct threats are synonymous with sources of stress and proximate pressures. Threats can be past (historical, unlikely to return or historical, likely to return), ongoing, and/or likely to occur in the future.
Level of Classification			**Definition**		
1	**2**	**3**		Examples	Exposition
9. Pollution			Threats from introduction of exotic and/or excess materials or energy from point and nonpoint sources		This class deals with exotic or excess materials introduced to the environment. There is obviously a fine distinction when the pollution comes from another threat - for example, should an oil spill from a pipeline be classified as **4.2 Utility & Service Lines or 9.2 Industrial & Military Effluents**? You will have to exercise some judgement here as to which represents the direct threat in your situation. In some cases, the source of the pollution may be either unknown or from a historical source (e.g., heavy metals buried in sediments). In these cases, you may have to make an educated guess as to which category to assign the pollutant.
	9.1 Domestic & Urban Waste Water		Water-borne sewage and non-point runoff from housing and urban areas that include nutrients, toxic chemicals and/or sediments		This category does not include major industrial discharge, which falls under **9.2 Industrial & Military Effluents.** It does include chemicals and next generation pollutants (caffeine or pharmaceuticals) in household waste streams. Technically, sewage from a pipe is "point-source" whereas a leaking septic system is "nonpoint- source." This category does not include agricultural runoff, which falls under **9.3 Agricultural & Forestry Effluents.**
		9.1.1 Sewage		List the source, and if possible, the specific pollutants of concern e.g., discharge from municipal waste treatment plants, leaking septic systems, untreated sewage, outhouses, etc.	
		9.1.2 Run-off		List the source, and if possible, the specific pollutants of concern e.g., oil or sediment from roads, fertilizers and pesticides from lawns and golf-courses, road salt, etc.	
		9.1.3 Type Unknown/Unrecorded			
	9.2 Industrial & Military Effluents		Water-borne pollutants from industrial and military sources including mining, energy production, and other resource extraction industries that include nutrients, toxic chemicals and/or sediments		The source of the pollution is often far from the system – an extreme example are the heavy metals that migrating eels bring to the Sargasso Sea. Often, the pollutants only become a problem when they bioconcentrate through the food chain. Oil spills from pipelines should generally go here.
		9.2.1 Oil Spills		List the source e.g., leakage from fuel tanks, oil spills from pipelines, PCBs in river sediments, etc.	
		9.2.2 Seepage from Mining		List the specific pollutants if possible e.g., mine tailings, arsenic from gold mining, etc.	
		9.2.3 Type Unknown/Unrecorded			There are other known examples of industrial pollution, which are not specifically captured under the classification scheme. These should be coded here for now, and the type/cause of the pollution noted

					in the text box. Examples include: toxic chemicals from factories, illegal dumping of chemicals, other industrial effluent, ship waste discharge, etc.
	9.3 Agricultural & Forestry Effluents		Water-borne pollutants from agricultural, silivicultural, and aquaculture systems that include nutrients, toxic chemicals and/or sediments including the effects of these pollutants on the site where they are applied		Wind erosion of agricultural sediments or smoke from forest fires goes in **9.5 Air- Borne Pollutants**.
		9.3.1 Nutrient Loads		List the source and specific pollutant of concern: e.g., nutrient loading from fertilizer run-off, manure from feedlots, nutrients from aquaculture, etc.	
		9.3.2 Soil Erosion, Sedimentation		List the source and specific pollutant of concern: e.g., soil erosion from overgrazing, increased run-off and hence sedimentation due to conversion of forests to agricultural lands, etc.	
		9.3.3 Herbicides and Pesticides		List the source and specific pollutant of concern: e.g., herbicide run-off from orchards, etc.	
		9.3.4 Type Unknown/Unrecorded			
	9.4 Garbage & Solid Waste		Rubbish and other solid materials including those that entangle wildlife		This category generally is for solid waste outside of designated landfills - landfills themselves should go in 1.2 Commercial & Industrial Areas. Likewise, toxins leaching from solid waste - for example, mercury leaking out of a landfill into groundwater - should go in 9.2 Industrial & Military Effluents.
		List the type, source, and if possible, the specific pollutants of concern		municipal waste, litter from cars, flotsam & jetsam from recreational boats, waste that entangles wildlife, construction debris, etc.	
	9.5 Air-Borne Pollutants		Atmospheric pollutants from point and nonpoint sources		It may be difficult to determine the sources of many atmospheric pollutants – and thus hard to take action to counter them.
		9.5.1 Acid rain		List the source, and if possible, the specific pollutants of concern e.g., acid rain, excess nitrogen deposition, radioactive fallout, wind dispersion of pollutants or sediments, smoke from forest fires or wood stoves, etc.	
		9.5.2 Smog		List the source, and if possible, the specific pollutants of concern e.g., smog from vehicle emissions, coal burning, wind dispersion of pollutants or sediments, smoke from forest fires or wood stoves, etc.	Smog is a type of air pollution derived from vehicular emission from internal combustion engines and industrial fumes that react in the atmosphere with sunlight to form secondary pollutants that also combine with the primary emissions to form photochemical smog. Smog is also caused by large amounts of coal burning in an area caused by a mixture of smoke, sulphur dioxide and other components.
		9.5.3 Ozone		List the source, and if possible, the specific pollutants of concern e.g., vehicle emissions, factory smoke emissions, smoke from forest fires or wood stoves, wind dispersion of pollutants or sediments, etc.	Ozone is not emitted directly by car engines or by industrial operations, but formed by the reaction of sunlight on air containing hydrocarbons and nitrogen oxides that react to form ozone directly at the source of the pollution or many kilometres down wind.
		9.5.4 Type Unknown/Unrecorded			
	9.6 Excess Energy		Inputs of heat, sound, or light that disturb wildlife or ecosystems		These inputs of energy can have strong effects on some species or ecosystems.

		9.6.1 Light Pollution		List the source, and if possible, the specific pollutants of concern e.g., lamps attracting insects, beach lights disorienting turtles, etc.	
		9.6.2 Thermal Pollution		List the source, and if possible, the specific pollutants of concern e.g., heated water from power plants, damaging atmospheric radiation resulting from ozone holes, etc.	
		9.6.3 Noise Pollution		List the source, and if possible, the specific pollutants of concern e.g., noise from highways or airplanes, sonar from submarines that disturbs whales, etc.	
		9.6.4 Type Unknown/Unrecorded			

Chapter 3 METHODS

Chapter 2 highlighted the lack of information regarding pollutants in cartilaginous fishes. In this chapter are described some of the methods by which this gap should be filled, starting from deep sea and bathyal species which are likely less studied due to the extreme environments they live in.

Impact of the COVID-19 pandemic

Since middle February 2020 COVID-19 pandemic has strongly disturbed the project with a high impact, mainly in the field activities, causing some delay both in the sample collection and in laboratory analysis. Problems have been related to the impossibility to work onboard due the health measures to mitigate the diffusion of the virus COVID-19.

Sampling activities

Samples were collected from two different areas in the Tyrrhenian Sea: the Geographic Sub Area 9 (GSA9), in front of Liguria, Tuscany and Lazio, and the Dohrn Canyon, in front of the Gulf of Naples.

Samples collected in the GSA9

Samples from the GSA9 (Fig. 3.1 A) were collected in June 2019 and October 2020 in the framework of the MEDITS (Mediterranean International Trawl Survey) program, designed from a European Commission's initiative to produce biological data on the demersal resources in the Mediterranean Sea.

Sampling gear, sampling methodology and treatment of the catch are described in the MEDITS Handbook (version 7, 2013). Briefly, trawling hauls at different depth ranges (370m – 656m) in different locations were conducted by the fishing boat S. Anna (Fig 3.1 B).

The MEDITS program is conducted within the Data Collection Framework (DCF) in compliance with the Regulations of the European Council n. 199/2008, the European Commission Regulation n. 665/2008 the Commission Decisions n. 949/2008 and n. 93/2010.

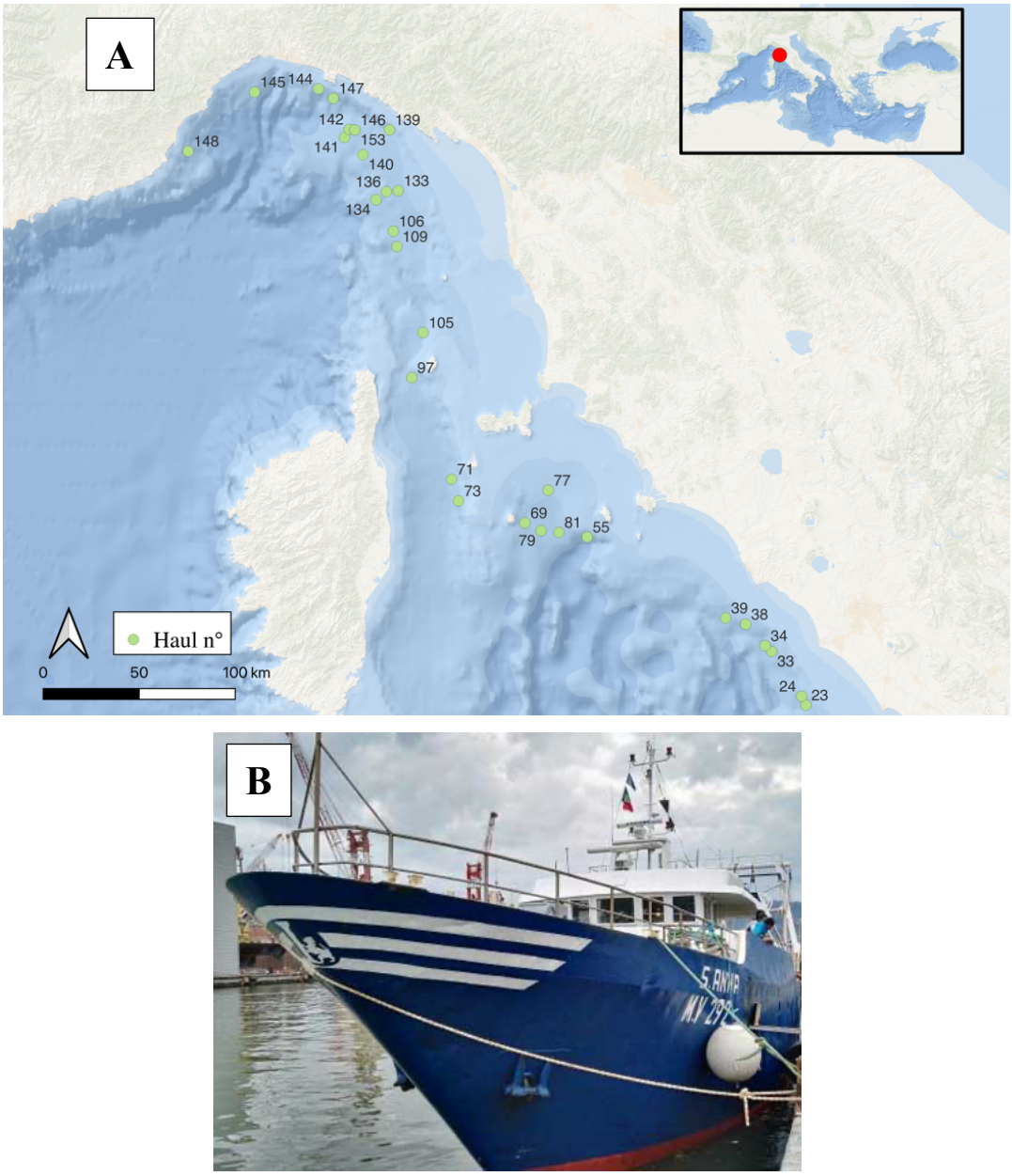

Figure 3.1 Sampling locations in the GSA9 (A) and fishing vessel used during sampling activities conducted during the MEDITS survey (B)

All the single specimens used for this Thesis are reported in Table 3.1 along with their date, coordinates, depth where they were captured, and main biological parameters (length (cm), weight (g), sex and maturation stage according to MEDITS standards)

Table 3.1 Sampled specimens in the GSA9 during MEDITS campaign. The table presents the ID of the sample, the species, the haul number and depth where the specimen has been sampled, the length (cm) and the weight (g) of each sample, sex and maturity stage according to MEDITS standards and if the organochlorine analyses (OCsA) and stomach content analyses (SCA) have been conducted.

ID	Species	Haul n°	Depth	Length	Weight	Sex	Maturity stage	OCsA	SCA
13CMO	*Chimaera monstrosa*	81	597.5	12	103.8	M	2	YES	NO
6CMO		79	560.5	12.5	107.8	F	1	YES	NO
4CMO			560.5	6	12.2	M	1	YES	NO
5CMO			560.5	10.5	62.3	M	1	YES	NO

1CMO73		73	430	18.5	293.6	F	1	YES	NO
2CMO73			430	20.5	495.2	M	3A	YES	NO
3CMO73			430	25.5	807.8	F	3A	YES	NO
7CMO		71	620	6.5	15.3	F	1	YES	NO
11CMO			620	12	88.5	F	1	YES	NO
12CMO			620	13	120.7	F	1	YES	NO
1CMO71			633	24	749.5	F	2	YES	NO
10CMO			620	23.5	733.8	F	3A	YES	NO
9CMO			620	21.5	972.2	F	3B	YES	NO
8CMO			620	18.5	538.3	M	3A	YES	NO
1CMO		109	633	12	114.3	F	1	YES	NO
2CMO			633	24	730.5	F	3A	YES	NO
1CMO109			634.5	14.5	157.8	M	1	YES	NO
3CMO		106	569	15	195.1	F	1	YES	NO
9DLI	*Dalatias licha*	39	535.5	35.5	167.6	F	1	YES	NO
5DLI		55	403	103	6150	F	3C	YES	NO
8DLI		81	597.5	41	265.4	F	1	YES	NO
2DLI		109	633	92	2800	M	3B	YES	NO
2DLI109			634.5	91	2900.5	M	3B	YES	NO
3DLI		106	569	35.5	140.7	M	1	YES	NO
4DLI			569	43	325.6	M	2	YES	NO
1DLI		134	484	33.5	132.2	F	1	YES	NO
2DLI134			489.5	36	192.9	M	1	YES	NO
6DLI		148	462	37	186.8	M	1	YES	NO
7DLI			462	42	299.5	M	1	YES	NO
3DLI139		139	392	37.5	192.2	F	1	YES	NO
1ESP34	*Etmopterus spinax*	34	627.5	27	80.8	F	1	YES	NO
5ESP34			627.5	37.5	247.5	F	3A	YES	NO
2ESP34			627.5	31.5	131	M	2	YES	NO
16ESP		79	560.5	28.5	112.2	M	2	YES	NO
17ESP73		73	430	36	194.4	F	2	YES	NO
3ESP73			430	20.5	41.5	M	1	YES	NO
8ESP73			430	25.5	74.7	M	1	YES	NO
1ESP73			430	11	4	M	1	YES	NO
19ESP		71	620	30.5	123.4	F	1	YES	NO
18ESP			620	23.5	74	F	1	YES	NO
17ESP			620	30	129.4	M	3A	YES	NO
8ESP109		109	634.5	36	205.2	F	3C	YES	NO
12ESP109			634.5	38.5	250.7	F	3C	YES	NO
11ESP109			634.5	37.5	228.9	F	3C	YES	NO
9ESP109			634.5	36	267.4	F	3D	YES	NO
10ESP109			634.5	37	206.9	F	3D	YES	NO
7ESP109			634.5	35	178	F	3D	YES	NO
4ESP109			634.5	30.5	124.4	M	2	YES	NO
10ESP		106	569	32.5	137.8	F	2	YES	NO
8ESP106			577	38	268.7	F	3A	YES	NO
13ESP			569	39	221.1	F	3A	YES	NO
14ESP			569	40.5	280.1	F	3A	YES	NO
11ESP			569	37	187.8	F	3B	YES	NO
12ESP			569	39	230	F	3C	YES	NO
9ESP			569	30.5	108.5	M	2	YES	NO
1ESP134		134	489.5	15	12.2	F	1	YES	NO
8ESP		140	508	29	122.1	M	2	YES	NO
3ESP148		148	471.5	23	56.5	M	1	YES	NO
2ESP148			471.5	5.3	13.1	M	1	YES	NO
8ESP141		141	656	25.5	72.3	F	1	YES	NO
7ESP141			656	23	44.7	M	1	YES	NO
10ESP141			656	28.5	84.5	M	2	YES	NO
9ESP141			656	26	73.9	M	2	YES	NO
7ESP153		153	581.5	31	109.1	F	1	YES	NO
6ESP153			581.5	27.5	91.6	F	1	YES	NO
9ESP153			581.5	32.5	135.3	F	1	YES	NO

11ESP153			581.5	39	256.8	F	2	YES	NO
2ESP			578	34.5	168.9	F	2	YES	NO
3ESP			578	33.5	166.8	F	2	YES	NO
5ESP			578	35.5	178.7	F	2	YES	NO
6ESP			578	36.5	231.2	F	3A	YES	NO
4ESP			578	34.5	194.8	F	3A	YES	NO
8ESP153			581.5	31.5	137.8	M	2	YES	NO
1ESP			578	32	125.3	M	3B	YES	NO
23ESP			605	36	213.5	F	2	YES	NO
22ESP			605	35	170.2	F	2	YES	NO
24ESP		142	605	36.5	188.5	F	2	YES	NO
3ESP142			604.5	36	256.4	F	3A	YES	NO
21ESP			605	33	127	M	3B	YES	NO
7ESP		147	515	38	277.6	F	4B	YES	NO
8ESP145		145	582	37.5	216	F	3A	YES	NO
1GME23			585	51.5	399	F	3A	YES	YES
10GME23			585	44.5	244.6	F	4A	YES	YES
8GME23			585	42.5	192.5	F	4A	YES	YES
11GME23			585	48	301.6	F	4B	YES	YES
9GME23		23	585	43.5	238.5	F	4B	YES	YES
6GME23			585	41.5	211.8	M	3A	YES	YES
12GME23			585	49.5	331.7	F	3A	NO	YES
9GME23			585	43.5	224.8	F	3A	NO	YES
17GME24			497.5	42.5	219.7	F	3A	YES	YES
28GME24			497.5	45	292.1	F	3B	YES	YES
2GME24			497.5	43	208.4	M	3A	YES	YES
4GME24			497.5	46	255.1	M	3B	YES	YES
22GME24			497.5	44	196.1	M	3A	NO	YES
23GME24			497.5	44	232.9	M	3A	NO	YES
25GME24			497.5	44.5	265.1	F	3A	NO	YES
29GME24		24	497.5	45.5	295	F	3B	NO	YES
30GME24			497.5	46	298.5	F	3A	NO	YES
31GME24			497.5	47	297.4	F	3B	NO	YES
32GME24			497.5	47	314	F	3B	NO	YES
33GME24			497.5	47.5	309.6	F	3A	NO	YES
3GME24			497.5	44	301	F	3A	NO	YES
5GME24			497.5	47	251.6	M	3A	NO	YES
6GME24			497.5	50	343.9	F	3B	NO	YES
1GME33	*Galeus melastomus*	33	564	46.5	252.5	M	3B	YES	YES
9GME34			627.5	45.5	258.1	F	3B	YES	YES
2GME34			627.5	42	205	M	3B	YES	YES
7GME34			627.5	42.5	193.7	M	3B	YES	YES
8GME34			627.5	44	251.3	M	3B	YES	YES
1GME34		34	627.5	41.5	182.7	M	3A	NO	YES
3GME34			627.5	43.5	254.1	M	3B	NO	YES
4GME34			627.5	45	256.1	M	3B	NO	YES
5GME34			627.5	48	398.5	F	3B	NO	YES
6GME34			627.5	40.5	180.2	M	3B	NO	YES
191GME			370	41.5	198.5	F	2	NO	YES
190GME		38	370	41.5	184.1	F	4A	NO	YES
188GME			370	40.5	188.8	M	2	NO	YES
217GME		79	560.5	39	162.8	F	2	NO	YES
10GME69			425	48.5	364.8	F	3A	YES	YES
5GME69			425	43	260.7	F	3A	YES	YES
8GME69			425	47	305.9	F	3A	YES	YES
6GME69			425	43.5	265	F	3B	YES	YES
1GME69		69	425	41.5	239.1	F	4A	YES	YES
4GME69			425	42.5	238.8	F	4A	YES	YES
7GME69			425	47	339.9	F	4B	YES	YES
9GME69			425	47	302.5	F	3A	NO	YES
1GME73		73	430	47.5	279.3	F	4B	YES	YES
221GME		77	330	41.5	240.4	F	2	NO	YES

222GME			330	42	237.6	F	2	NO	YES
1GME71		71	633	48	293.3	F	4B	YES	YES
134GME			402	45.5	252.1	F	3A	NO	YES
132GME			402	44	231.8	F	4B	NO	YES
133GME		97	402	44.5	244.6	F	4B	NO	YES
130GME			402	42	217.4	M	2	NO	YES
8GME97			400	44.5	285.6	F	4B	NO	YES
182GME			400.5	44.5	305	F	3A	NO	YES
184GME		105	400.5	49	366.5	F	3A	NO	YES
183GME			400.5	46	274.1	M	3B	NO	YES
1GME105			400.5	46	274.7	F	4B	NO	YES
14GME109			634.5	43	211.3	M	3A	NO	YES
15GME109			634.5	43.5	252.1	M	3B	NO	YES
19GME109			634.5	43.5	266.7	F	4A	NO	YES
1GME109			634.5	44	235.6	F	4A	NO	YES
23GME109		109	634.5	46	262.5	F	3A	NO	YES
23GME109			634.5	45	255.4	M	3B	NO	YES
2GME109			634.5	49	328.6	M	3B	NO	YES
36GME109			634.5	46.5	285.4	F	3B	NO	YES
38GME109			634.5	49.5	319.2	F	3B	NO	YES
39GME109			634.5	50	386.7	F	3B	NO	YES
3GME109			634.5	51	384.3	F	3B	NO	YES
41GME109			634.5	50.5	295.7	F	3A	NO	YES
4GME109			634.5	39.5	149.5	M	3A	NO	YES
5GME109		109	634.5	39.5	158.7	M	3A	NO	YES
6GME109			634.5	40	165.2	M	3A	NO	YES
7GME109			634.5	41	190.7	M	3A	NO	YES
8GME109			634.5	41	198.5	M	3A	NO	YES
151GME			569	44	244.8	F	2	NO	YES
157GME			569	46.5	262.9	F	3A	NO	YES
159GME			569	47	289.7	F	3B	NO	YES
160GME			569	47.5	361.7	F	3B	NO	YES
165GME			569	50	365.4	F	3B	NO	YES
167GME			569	52	356.8	F	3B	NO	YES
143GME			569	40	161.5	M	2	NO	YES
144GME			569	40.5	171.2	M	2	NO	YES
142GME			569	40	167.2	M	3A	NO	YES
145GME			569	42	204.3	M	3A	NO	YES
146GME			569	43	221.9	M	3A	NO	YES
148GME		106	569	43	205.8	M	3A	NO	YES
155GME	*Galeus melastomus*		569	46	246.4	M	3B	NO	YES
158GME			569	47	295.4	M	3B	NO	YES
10GME106			577	48.5	350.5	M	3B	NO	YES
1GME106			577	46	260.2	M	3B	NO	YES
2GME106			577	41.5	190.8	M	3A	NO	YES
3GME106			577	45	236.8	F	4A	NO	YES
4GME106			577	46	254.6	M	3B	NO	YES
5GME106			577	46	305.5	F	3A	NO	YES
6GME106			577	46.5	270.1	M	3B	NO	YES
7GME106			577	46.5	244.1	M	3B	NO	YES
8GME106			577	47.5	332.1	F	3B	NO	YES
9GME106			577	48	293	F	4A	NO	YES
102GME			484	40.5	177	F	2	NO	YES
103GME			484	41.5	204.2	F	2	NO	YES
101GME		134	484	40.5	233	F	4B	NO	YES
3GME134			489.5	49	343.1	F	3B	NO	YES
4GME134			489.5	49	336.3	F	3B	NO	YES
115GME136			371.5	49	320.4	F	3B	YES	YES
39GME136			371.5	45	264.5	M	3A	YES	YES
112GME136		136	371.5	43.5	276.6	F	3A	NO	YES
113GME136			371.5	46.5	304.3	F	3A	NO	YES
114GME136			371.5	49	327.9	F	3B	NO	YES

28GME133		133	412.5	46	354.1	F	3B	YES	YES
30GME133			412.5	47.5	315	F	3B	YES	YES
9GME133			412.5	40	194.2	M	3A	YES	YES
10GME133			412.5	41	205	M	3A	NO	YES
11GME133			412.5	42	217.9	M	3A	NO	YES
12GME133			412.5	50.5	412.1	F	3A	NO	YES
29GME133			412.5	47	285.7	F	4A	NO	YES
1GME140		140	510.5	46	315.8	F	3A	NO	YES
1GME148		148	471.5	47	253.2	M	3B	YES	YES
16GME148			471.5	46.5	290.5	F	4B	NO	YES
17GME148			471.5	47	334.8	F	4A	NO	YES
18GME148			471.5	47.5	296.3	F	4A	NO	YES
5GME153		153	581.5	46	277.1	F	3B	YES	YES
4GME153			581.5	44	260.8	M	3B	YES	YES
17GME			578	46	273.3	M	3B	NO	YES
3GME153			581.5	40.5	180.4	M	3B	NO	YES
14GME		139	393	40	195.5	M	2	NO	YES
15GME			393	41	219.5	M	2	NO	YES
16GME			393	42	223.3	M	2	NO	YES
2GME142		142	604.5	42.5	203.3	M	3A	YES	YES
4GME142			604.5	45	290	M	3A	YES	YES
3GME142			604.5	42	256.1	M	3B	YES	YES
1GME142			604.5	49	358.5	M	3A	NO	YES
1GME145		145	582	45	210.7	F	4A	YES	YES
5GME145			582	47.5	370.8	F	4A	YES	YES
2GME145			582	46	313.9	F	4B	NO	YES
3GME145			582	46	218.8	F	4A	NO	YES
4GME145			582	46.5	304.7	F	3A	NO	YES
6GME145			582	48	280.4	F	4B	NO	YES
1GME144		144	319.5	47	248.6	F	4B	YES	YES

Samples collected in the Dohrn Canyon

Samples from the Dohrn Canyon were collected during 2021 by a collaboration of a selected local fishing team. An artisanal fishing vessel (Fig 3.2) of 6m of total length and 2,43 tons IGT, equipped for demersal longline fishery was used.

Hauls started during the morning, and they had a duration of 24 hours. The experimental demersal longline was constituted of 150 size 7 J-hooks (1 every 15 meters) and the main line was 2,2 nm. The baits were usually given by pieces of *Sarda sarda* and *Boops boops* and set at depth ranging from -445m to -465m along the Canyon Dohrn (Fig 3.2). Commercial catches (*Conger conger*, *Merluccius merluccius* and *Phicys blennoides*) were sold in the market while bycatch or unsellable individuals were kept frozen at -20°C until laboratory analyses.

Figure 3.2 Dohrn Canyon sampling sites and fishing vessel used during sampling activities (in the box on the right)

All the single specimens used for this Thesis are reported in Table 3.2 along with their date, coordinates, depth where they were captured, and main biological parameters (length (cm), weight (g), sex and maturation stage according to MEDITS standards)

Table 3.2 Sampled specimens in the Dohrn Canyon. The table presents the ID of the sample, the species, the date, geographic coordinates, and depth where the specimen has been sampled, the length (cm) and the weight (g) of each sample, sex and maturity stage according to MEDITS standards. All these samples have been through organochlorine and stomach content analyses.

ID	SPECIES	DATA	LATITUDE	LONGITUDE	DEPTH	LENGHT (CM)	WEIGHT (G)	SEX	MATURITY STAGE
9CCO	*Conger conger*	26/04/21	40.694384	14.136798	445	130.5	4500	ND	ND
37ESP	*Etmopterus spinax*	26/04/21	40.694384	14.136798	445	27	84	F	1
14GME	*Galeus melastomus*	26/04/21	40.694384	14.136798	445	48.5	332	F	3A
15GME		26/04/21	40.694384	14.136798	445	41.5	196	M	2
16GME		26/04/21	40.694384	14.136798	445	44	210	M	3A
17GME		26/04/21	40.694384	14.136798	445	42	204	M	3A
18GME		26/04/21	40.694384	14.136798	445	31	80	M	1
19GME		26/04/21	40.694384	14.136798	445	35.5	128	F	1
20GME		26/04/21	40.694384	14.136798	445	37.5	152	F	1
21GME		26/04/21	40.694384	14.136798	445	45	344	F	3A
22GME		26/04/21	40.694384	14.136798	445	34	102	F	1
23GME		26/04/21	40.694384	14.136798	445	33	116	F	1
24GME		26/04/21	40.694384	14.136798	445	43	208	F	2
25GME		26/04/21	40.694384	14.136798	445	30.5	86	M	1
26GME		04/05/21	40.636591	14.164249	465	40	176	F	1
27GME		04/05/21	40.636591	14.164249	465	47	286	F	3B
28GME		04/05/21	40.636591	14.164249	465	48.5	356	F	3A

29GME		04/05/21	40.636591	14.164249	465	46.5	240	M	3A
30GME		04/05/21	40.636591	14.164249	465	49	324	F	3A
31GME		04/05/21	40.636591	14.164249	465	42	210	M	3A
32GME		04/05/21	40.636591	14.164249	465	45	200	M	3A
33GME		04/05/21	40.636591	14.164249	465	35.5	116	M	2
34GME		04/05/21	40.636591	14.164249	465	30	72	M	1
35GME		04/05/21	40.636591	14.164249	465	46.5	302	F	3B
36GME		04/05/21	40.636591	14.164249	465	44	218	F	2
41GME		11/05/21	40.605975	14.148003	450	36	120	F	1
42GME		11/05/21	40.605975	14.148003	450	37	122	F	1
43GME		11/05/21	40.605975	14.148003	450	34	100	F	1
44GME		11/05/21	40.605975	14.148003	450	37.5	130	M	2
45GME		11/05/21	40.605975	14.148003	450	36	116	M	2
46GME		11/05/21	40.605975	14.148003	450	38	158	M	3A
47GME		11/05/21	40.605975	14.148003	450	37.5	118	M	2
48GME		11/05/21	40.605975	14.148003	450	40	154	M	3A
1HDA	*Helicolenus dactylopterus*	04/05/21	40.636591	14.164249	465	19	98	F	2A
2HDA		04/05/21	40.636591	14.164249	465	20.5	118	M	2A
3HDA		04/05/21	40.636591	14.164249	465	17.5	82	M	2A
4HDA		26/04/21	40.694384	14.136798	445	17.5	82	F	2A
6HDA		26/04/21	40.694384	14.136798	445	15	48	M	1
7HDA		26/04/21	40.694384	14.136798	445	17	76	F	2A
8HDA		26/04/21	40.694384	14.136798	445	19	114	ND	ND
10HDA		26/04/21	40.694384	14.136798	445	17	76	M	2A
11HDA		26/04/21	40.694384	14.136798	445	20.5	128	F	2A
12HDA		26/04/21	40.694384	14.136798	445	19	92	ND	ND
38HDA		11/05/21	40.605975	14.148003	450	17	108	ND	ND
39HDA		11/05/21	40.605975	14.148003	450	19	92	ND	ND
40HDA		11/05/21	40.605975	14.148003	450	19	90	ND	ND
5MME	*Merluccius merluccius*	26/04/21	40.694384	14.136798	445	42.5	556	F	2B
13SCN	*Scyliorhinus canicula*	26/04/21	40.694384	14.136798	445	25	42	M	1

Taxonomical identification, morphometric evaluation, and sample preparation

Morphometric and gravimetric parameters (total length (TL); total weight (TW); liver weight (LW); sex) were evaluated in the laboratory according to Fischer et al. (1987) and Serena (2005). Sexual maturity stages were evaluated according to Medits Handbook (version 7, 2013).

To evaluate fishes physiological condition, the Fulton's condition factor (K) was calculated with the following equation:

$$K = 100 * (W * T_L^{-3})$$

whereby W is mass (g) and T_L is total length (cm) (Fulton, 1904).

Muscle tissue 5 – 15 g was collected in the dorsal area while the liver and the stomach were taken as a whole.

Where present, eggs and embryos were collected from mature females. The egg and embryos samples obtained from each specimen were pooled and analyzed in duplicate (eggs) or triplicate (embryos). All samples were stored, in aluminum foil or in a glass jar labeled inside and outside, at −20 °C until toxicological analyses. Stomach contents from each area were pooled and analyzed in triplicate.

Stomach content analysis

The stomachs were removed by cutting above the esophageal sphincter and below the pyloric sphincter. Samples were stored in aluminum foil labeled at −20 °C until the contents were processed and transferred into glass plates. The analysis of the stomach contents was conducted under the stereoscope. The prey was identified at the strictest taxonomical level in the different taxa, evaluating the abundance (N%) and percentage frequency (F%), that is the percentage of stomachs in which at least one individual of a given prey was found.

Due to the extremely small sample size, stomach content analysis in this Thesis represents a preliminary assessment for the diet composition in the animals sampled in the Dohrn Canyon and for the *G. melastomus* sampled in the GSA9. Stomach contents were also used to determine preliminary results on contaminant intake.

Organochlorine compounds determination

Determination of HCB, DDTs and PCBs was performed at the Department of Physical Sciences, Earth and Environment at University of Siena, according to the U.S. Environmental Protection Agency (EPA) 8081/8082 Method modified (Marsili et al., 2016). Specifically, samples (5–20 g) were lyophilized in an Edwards freeze drier for 3 days and extracted with n-hexane (PESTINORM, VWR Chemicals) in a Soxhlet apparatus. VWR cellulose thimbles (internal diameter 25 mm, external diameter 27 mm, length 100 mm) used for extraction of the samples were preheated for about 30 min to 110 °C and pre-extracted for 9 h in a Soxhlet apparatus with n-hexane, in order to remove any organochlorine contamination. Each sample was spiked prior to extraction with 2,4,6-trichlorobiphenyl (International Union of Pure and Applied Chemistry; IUPAC) number 30 Ballschmiter and Zell (1980) as a surrogate compound. The concentration of PCB30 was quantified and its recovery calculated for each sample. After a 9-h extraction with n-hexane, the samples were purified with sulphuric acid to first obtain lipid sedimentation. The extract then underwent liquid chromatography on a column containing florisil that had been dried for 1 h in an oven at 110 °C. This further purified the apolar phase of lipids that could not be saponified, such as steroids like cholesterol. Decachlorobiphenyl (DecaCB - IUPAC number 209) was used as an internal standard, where it was added to each sample prior to the extraction and included in the calibration standard (a mixture of Aroclor 1260, HCB and pp′- and op′-DDT, DDD and DDE). High resolution capillary gas chromatography was performed with an Agilent 6890 N and a 63Ni ECD and an SBP-5 bonded phase capillary column (30 m long, 0.2 mm i.d.). The carrier gas was nitrogen

with a head pressure of 15.5 psi (splitting ratio 50/1). The scavenger gas was argon/methane (95/5) at 40ml/min. Oven temperature was 100 °C for the first 10 min, after which it was increased to 280 °C at 5 °C/min. The injector and detector temperatures were 200 and 280 °C respectively. The extracted organic material (EOM%; lipid content) from freeze-dried samples was calculated in all samples and, then, the results were expressed in ng/g lipid weight (l.w.). A mixture of specific isomers was used to calibrate the system, evaluate recovery and confirm the results.

Capillary gas-chromatography revealed 30 PCB congeners (IUPAC no. 95, 99, 101, 118 – pentachlorobiphenyls; 128, 135, 138, 141, 144, 146, 149, 151, 153, 156 – hexachlorobiphenyls; 170, 171, 172, 174, 177, 178, 180, 183, 187 – heptachlorobiphenyls; 194, 195, 196, 199, 201, 202 – octachlorobiphenyls; 206 – nonachlorobiphenyls). Total PCBs (∑PCBs) were quantified as the sum of all congeners. These congeners constituted 80% of the total peak area of PCBs in the sample. Total DDTs (∑DDTs) were calculated as the sum of the isomers op′DDT, pp′DDT, op′DDD, pp′DDD, op′DDE and pp′DDE. The proportion of endocrine disrupting chemicals (EDCs) was calculated as the sum of the isomers: op′DDT, pp′DDT, op′DDD, pp′DDD, op′DDE, pp′DDE and PCBs IUPAC no. 95, 99, 101, 118, 153 (Fossi and Marsili, 2003; Fossi et al., 2003). Also, pp'DDT, op'DDT, pp'DDE, op'DDE and PCB IUPAC no. 95, 99, 101 and 153 have estrogenic and anti-androgenic capacities and pp'DDE, op'DDT and PCB118 have androgenic and antiestrogenic capacities, affetting both female and male reproductive processes (Fossi and Marsili, 2003). The limit of detection (LOD) for all compounds analysed was 0.1 ng/kg (ppt). The limit of detection (LOD) for all compounds analysed was 0.1 ng/kg (ppt).

Statistical analysis

Unless diversely specified, data were processed with STATISTICA 7.1 Software. Descriptive statistics (mean, standard deviation, minimum and maximum) were used to present the data. To evaluate data distribution was used the Shapiro–Wilk test which uses the null hypothesis principle: the null-hypothesis is that the population is normally distributed ($p>0.05$). All the investigated groups analyzed with Shapiro–Wilk test were non–normal distributed. Then non-parametric tests such as Kruskal–Wallis were performed and then, where possible, specific differences between variables were tested using Kolmogorov-Smirnov test.

Ethics

All experimental protocols followed the recommendations of the University of Siena and of the Committee for the Animal Welfare of the Stazione Zoologica Anton Dohrn (https://www.szn.it/index.php/en/who-we-are/organization/committee-for-the-animal-welfare). Moreover, all methods were carried out in accordance with relevant guidelines and regulations of the European Union.

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*Chapter 4 **PERSISTENT ORGANIC POLLUTANTS (POPS) IN LIGURIAN AND TYRRHENIAN DEEP SEA: POSSIBLE RISK FOR CONSERVATION IN BATHYAL CHONDRICHTHYES?***

Introduction

It was once believed that the sea had very high, if not unlimited, capacity to dissolve substances and solid waste. After years of discharges, a particular attention on negative effects on marine environment started in the 1980s, becoming a priority in scientific research (Lear et al., 1981; Simpson et al., 1981). This has led to the establishment of international conventions and agreements to protect the marine environment from human activities and for the production and proper disposal of toxic substances (Craig, 2004).

Among the substances that were incorrectly discarded and then regulated there are Persistent Organic Pollutants (POPs). These chemicals, which include organochlorine compounds (OCs), due to their chemical structure and properties, enter in the food web very easily, bioaccumulating and biomagnifying in organisms at the top of the food chain (Li et al., 2007). POPs are able to intervene with normal functions of several physiological mechanisms altering the immune system (Centelleghe et al., 2019; Marsili et al., 2019), causing cancer and genetic mutations (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2016), and interfering with endocrine system with androgenic, estrogenic, anti-androgenic and anti-estrogenic properties (Fossi and Marsili, 2003; Fossi et al., 2003). The effects of endocrine disruptive chemicals (EDCs) on marine mammals and teleosts are known (Fossi et al., 2002; Milla et al., 2011; Marsili et al., 2018; Lehnert et al., 2018), but there is little information regarding their effects on cartilaginous fish, (Consales and Marsili, 2021), even though most of Chondrichthyans have a high trophic level and so are one of the groups most exposed to POPs (Tiktak et al., 2020).

Despite being uncommon at depths below 3000 m, a significant number of species lives below 800 m (Hueter et al., 2004; Musick & Cotton 2015; Treberg et al., 2016) and unfortunately little is known about ecotoxicology on deep-water species. Some papers show that the concentrations of POPs are high in the deep sea (Storelli et al., 2009; Mormede and Davies, 2003) making this latter a potential sink for chemical compounds (Looser et al., 2000; Froescheis et al., 2000; Lohmann et al., 2006). In the last two decades few studies were carried out to investigate the presence of POPs in deep sea cartilaginous fishes around the world (Berg et al., 1997; De Brito et al., 2002; Akutsu et al., 2006) and in Mediterranean Sea (Storelli et al., 2004; Storelli et al., 2005).

This chapter focuses on three deep water species of cartilaginous fishes, the ghost shark *Chimaera monstrosa* (Linnaeus, 1758), the kitefin shark *Dalatias licha* (Bonnaterre, 1788) and the velvet belly shark *Etmopterus spinax* (Linnaeus, 1758), widely distributed in the Eastern Atlantic and in the Mediterranean basin between -300 and -1000 m (Fischer et al., 1987; Serena 2005) and commonly found in commercial bycatch of deep-sea fisheries (Holt et al., 2013; Ragonese et al., 2013).

The aim is to assess the prevalence and concentration of some organochlorine compounds such as hexachlorobenzene (HCB), dichlorodiphenyltrichloroethane (DDT) and its metabolites and polychlorinated biphenyls (PCBs) in the su deep water chondrichthyans and to highlight for the first time maternal transfer of these compounds in these animals. Since most of the investigated contaminants are Endocrine Disruptive Chemicals (EDCs), we discuss the potential negative effects on reproduction and population stability of these threatened cartilaginous fish. Moreover, since in the IUCN global and regional assessment for *C. monstrosa, D. licha* and *E. spinax* "Fishing and harvesting aquatic resources" is the only threat considered, this work also aims to be a benchmark for their toxicological assessment.

Methods

Sampling activities and sampled specimens were described in Chapter 3 in "*Sampled collected in the GSA9*" paragraph and in Table 3.1.

Figure 4.1 shows the sampling area with the hauls where the specimens were taken.

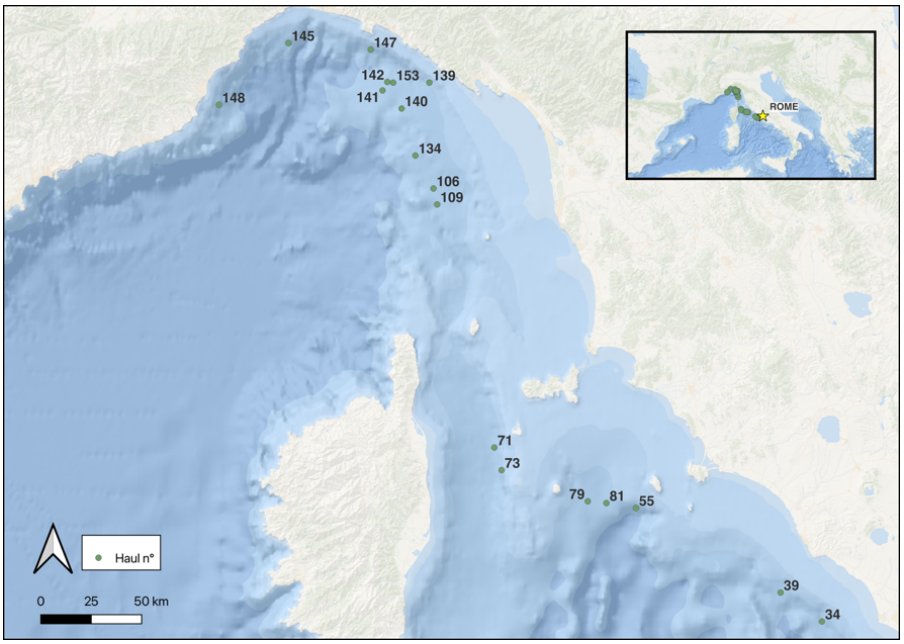


Figure 4.1 Sampling area. Numbers near the green dot indicate the haul number according to the MEDITS program. Detailed information regarding sampled specimens (Chiamera monstrosa n=16; Dalatias licha n=12; Etmopterus spinax n=52) during the hauls are reported in Table 3.1.

Biological parameters were taken according to the paragraph "*Taxonomical identification, morphometric evaluation, and sample preparation*" in Chapter 3.
Collected specimens ranged from 6 cm to 25.5 cm length in size and from 12 g to 972.2 g in weight for Chimaera monstrosa (n=16), from 33 cm to 103 cm total length in size and from 132.2 g to 6150 g in weight for Dalatias licha (n=12) and from 5.3 cm to 42.5 cm total length in size and from 4 g to 289.5 g in weight for Etmopterus spinax (n=52). Table 4.1 summarize collected specimens' mean values of length (cm) and weight (g) divided by sex and their Fulton's condition factor.

Table 4.1 Biological parameters length (cm), weight (g), and Fulton's condition factor (K) measured in the three different species collected in the GSA9. Data are expressed as mean±standard deviation. Range in brackets (minimum – maximum).

SPECIES	*Chimaera monstrosa n=16*		*Dalatias licha n=12*		*Etmopterus spinax n=52*	
SEX	F	M	F	M	F	M
Individuals	11	5	5	7	35	17
LENGTH (cm)	16.77±6.27 (6.50 – 25.50)	14.00±5.89 (6.00 – 20.50)	50.10±29.70 (33.50 – 103.00)	53.79±25.93 (35.50 – 92.00)	34.49±5.39 (15.00 – 42.50)	26.29±6.44 (11.00 – 33.00)
WEIGHT (g)	381.39±355.62 (15.30 – 972.20)	253.16±246.71 (12.20 – 538.30)	1381.48±2666.13 (132.20 – 6150.00)	978.00±1280.96 (140.70 – 2900.50)	188.93±68.63 (12.20 – 289.50)	88.40±44.55 (4.00 – 137.80)
K	5.83±1.41	6.09±1.37	0.41±0.09	0.38±0.04	0.43±0.05	0.41±0.07

Organochlorines determination was conducted according to the paragraph "*Organochlorine compounds determination"* in Chapter 3.
Statistical analysis was carried out with STATA (StataCorp. 2015). Contaminants data were first analysed by summary statistics. Kruskal-Wallis non-parametric test to detect differences among species was conducted and then Dunn's test (with Benjamini–Hochberg correction) for pairwise multiple-comparisons were also conducted. A Mann-Whitney U test was used to test for differences of OCs between sex and to detect differences in OCs between mothers vs embryos and mothers vs eggs. The analysis of maternal transfer was conducted adapting to this framework the approach proposed by Liu et al. 2018. Firstly, for each compound the mass ratio of Egg to Body plus Egg (EBER) and the mass ratio of Embryo to Body plus Embryo were computed using the two following equations (Russell et al., 1999), respectively:

$$EBER\ (\%) = C_{egg} \times M_{egg} / (C_{body} \times M_{body} + C_{egg} \times M_{egg})$$

$$EBER\ (\%) = C_{embryo} \times M_{embryo} / (C_{body} \times M_{body} + C_{embryo} \times M_{embryo})$$

where C_{egg}, C_{embryo}, and C_{body} were concentrations on wet weight basis in eggs, embryos, and maternal bodies and M_{egg}, M_{embryo}, and M_{body} were wet weight of eggs, embryos, and

maternal bodies. Secondly, linear regression analysis was used to test the dependence of EBERs from the octanol water partition coefficient ($LogK_{ow}$) of chemicals.

Results

Organochlorine compounds (OCs)

OCs were detected in all specimens and in all tissues (muscle, eggs, and embryos) (Table 4.2). In *C. monstrosa* and *E. spinax* muscle PCBs were the most present contaminants followed by DDTs and HCB, while in *D. licha* muscle the highest levels were represented by DDTs followed by PCBs and HCB (Fig 4.2 A – B – C). In eggs and embryos of each species the toxicological pattern was the same (PCBs>DDTs>HCB).

The KW test stressed that differences between species were statistically significant for all the three OCs (HCB $\chi2=21.895$, $p<0.0001$; PCBs $\chi2=32.284$, $p<0.0001$; DDTs $\chi2=30.125$ $p<0.0001$), furthermore Dunn's pairwise comparison by pair of species highlighted differences between *C. monstrosa* and *D. licha* (HCB, DDTs and PCBs $p<0.0001$) and between *D. licha* and *E. spinax* (HCB, DDTs and PCBs $p<0.0001$).

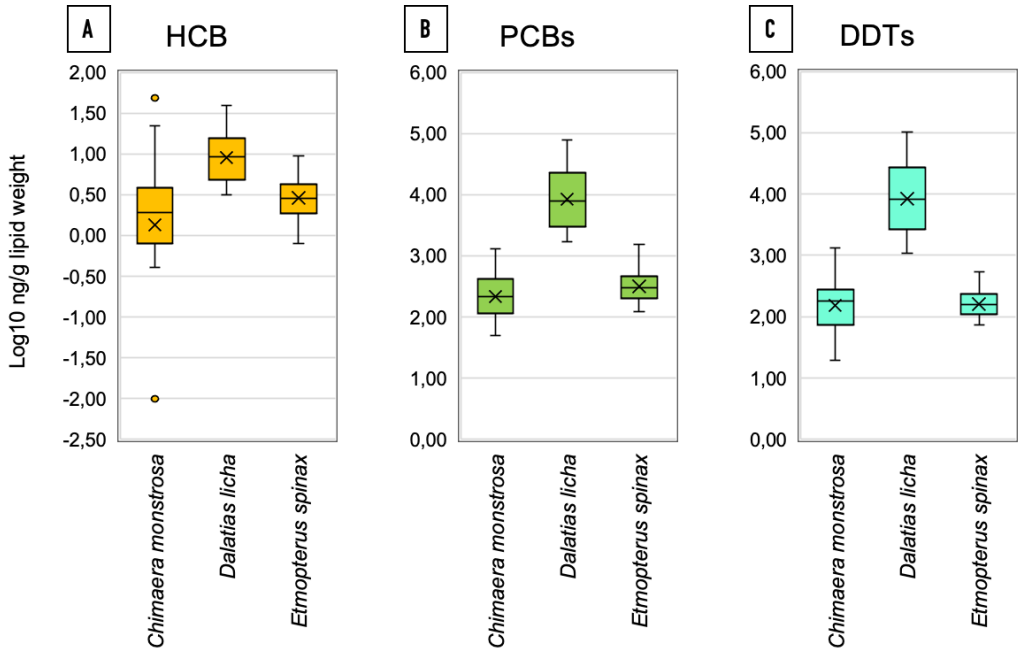


Figure 4.2 Levels of HCB (A), DDTs (B) and PCBs (C) in Chimaera monstrosa (n=16), Dalatias licha (n=12) and Etmopterus spinax (n=51) expressed in log10 ng/g lipid weight (l.w.)

Table 4.2 Concentrations (ng/g lipid weight) of HCB, PCBs and DDTs and ratios (DDTs/PCBs, pp'DDE/pp'DDT, pp'DDE/DDTs, ∑op'DDTs/∑DDTs) in C. monstrosa, D. licha and E. spinax tissues collected in the GSA9. Data are expressed as mean ± standard deviation (minimum – maximum).

		HCB	PCBs	DDTs	DDTs / PCBs	pp'DDE / pp'DDT	pp'DDE / DDTs	∑op'DDTs / DDTs
Chimaera monstrosa	Muscle n=16	6.07±12.16	311.86±306.08	255.32±315.55	0.72	32.67	0.69	0.18
		(0.01 – 48.98)	(48.16 – 1306.47)	(18.27 – 1331.86)				
	Eggs n=3*	7.12±3.64	368.63±168.51	187.52±56.95	0.56	14.87	0.76	0.13
		(3.50 – 12.11)	(183.34 – 591.07)	(136.23 – 266.95)				
Dalatias licha	Muscle n=12	11.67±9.59	17057.56±21877.28	18995.23±27525.89	1.05	6.65	0.70	0.12
		(3.16 – 39.67)	(1728.55 – 77940.06)	(1073.81 – 103921.90)				
	Embryo n=8**	21.71	33402.74	22739.07	0.68	6.99	0.77	0.08
Etmopterus spinax	Muscle n=51	3.24±1.59	381.82±265.08	179.96±96.54	0.54	6.20	0.62	0.23
		(0.79 – 9.44)	(120.17 – 1518.48)	(73.05 – 537.62)				
	Embryos n=42***	6.04±2.03	582.46±345.79	398.28±488.98	0.55	8.62	0.73	0.13
		(4.07 – 10.27)	(268.14 – 1362.42)	(132.16 – 1586.71)				
	Eggs n=6****	5.13±1.05	694.69±405.45	273.28±129.79	0.43	12.68	0.73	0.14
		(3.25 – 6.20)	(279.16 – 1317.84)	(89.47 – 485.33)				

*3 pools from 3 individuals analyzed in duplicate
**8 embryos from 1 individual pooled and analyzed in triplicate
***42 embryos from 7 individuals pooled and analyzed in triplicate
****6 pools from 6 individuals analyzed in duplicate

Table 4.2 also shows ratio between DDT isomers, in particular pp'DDE/pp'DDT, pp'DDE/DDTs and Σop'DDTs/DDTs. The pp'DDE/pp'DDT and pp'DDE/DDTs ratios were high in all the three species both in muscle and in embryonic tissues. The value of ∑op'DDTs/DDTs in all the samples was below 0.20 (0.08 – 0.18) except for *E. spinax* muscle (0.23).

Contamination fingerprints of PCBs and DDTs were calculated for each species and tissue (Fig 4.3 A – B – C – D).

Fingerprints for both classes of contaminants were very similar with very little exceptions for *C. monstrosa* both in muscle and in the eggs for PCB congeners CB-149+118, CB-153 and CB-187. Overall, pp'DDE was the isomer with the highest percentage followed by op'DDT and pp'DDT while for PCBs CB-153, CB-180, CB-149+118, CB-187 and CB-170 were the most present.

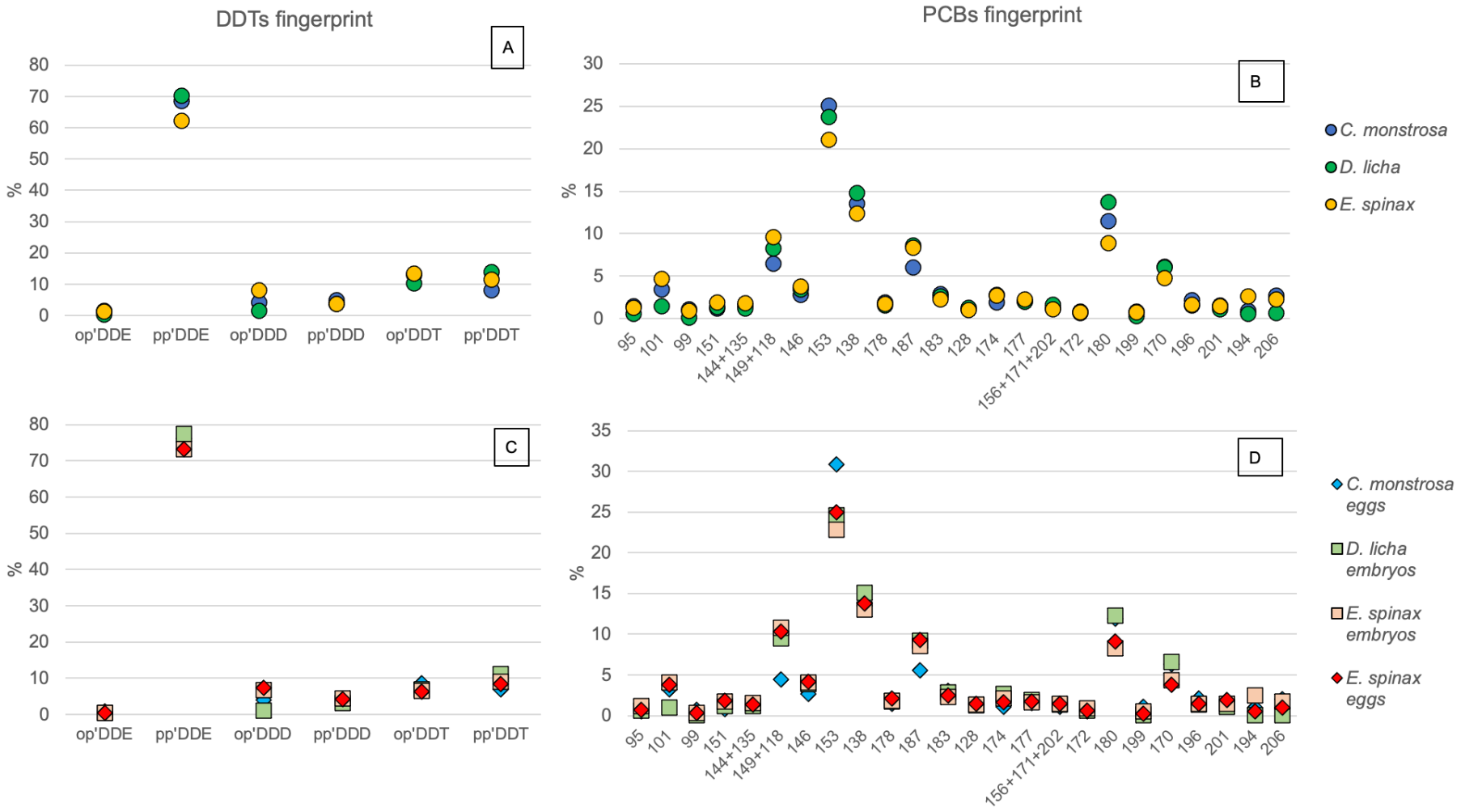


Figure 4.3 DDTs and PCBs fingerprints in muscle tissue (A, B) and in eggs and embryos (C, D) of the three sampled species

Figure 4.4 gives information about the PCB congener composition.

In all the three species (*C. monstrosa* - *D. licha* - *E. spinax*) the abundance of each group of congeners was very similar following the same pattern: hexa-CBs (48.84% - 50.37% - 47.04%) > hepta-CBs (33.38% - 38.72% - 32.13%) > penta-CBs (9.25% - 6.22% - 11.69%) > octa-CBs (5.80% - 4.02% - 6.87%) > nona-CBs (2.73% - 0.66% - 2.26%). The same exact pattern was registered also in the eggs and embryos.

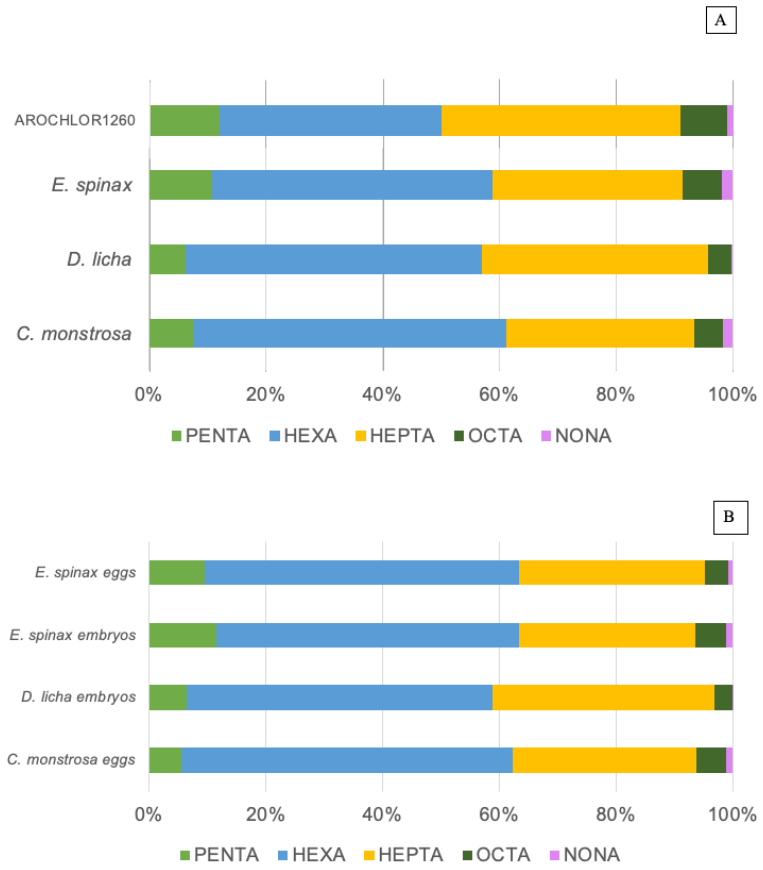


Figure 4.4 Percentage composition of PCBs divided by chlorine content (penta-CBs, hexa-CBs, hepta-CBs, octa-CBs, nona-CBs) on ∑PCBs, in Arochlor 1260 and in n=51 E. spinax, n=12 D. licha, n=16 C. monstrosa muscle tissue (A) and in n=6 E. spinax eggs, n=42 E. spinax embryos, n8 D. licha embryos and n=3 C. monstrosa eggs (B).

Differences between sexes

Mann-Whitney U test results highlighted that there were no statistically significant differences in contaminant accumulation between sexes ($p>0.05$). However, results are summarized in Figure 4.5 A – B – C.

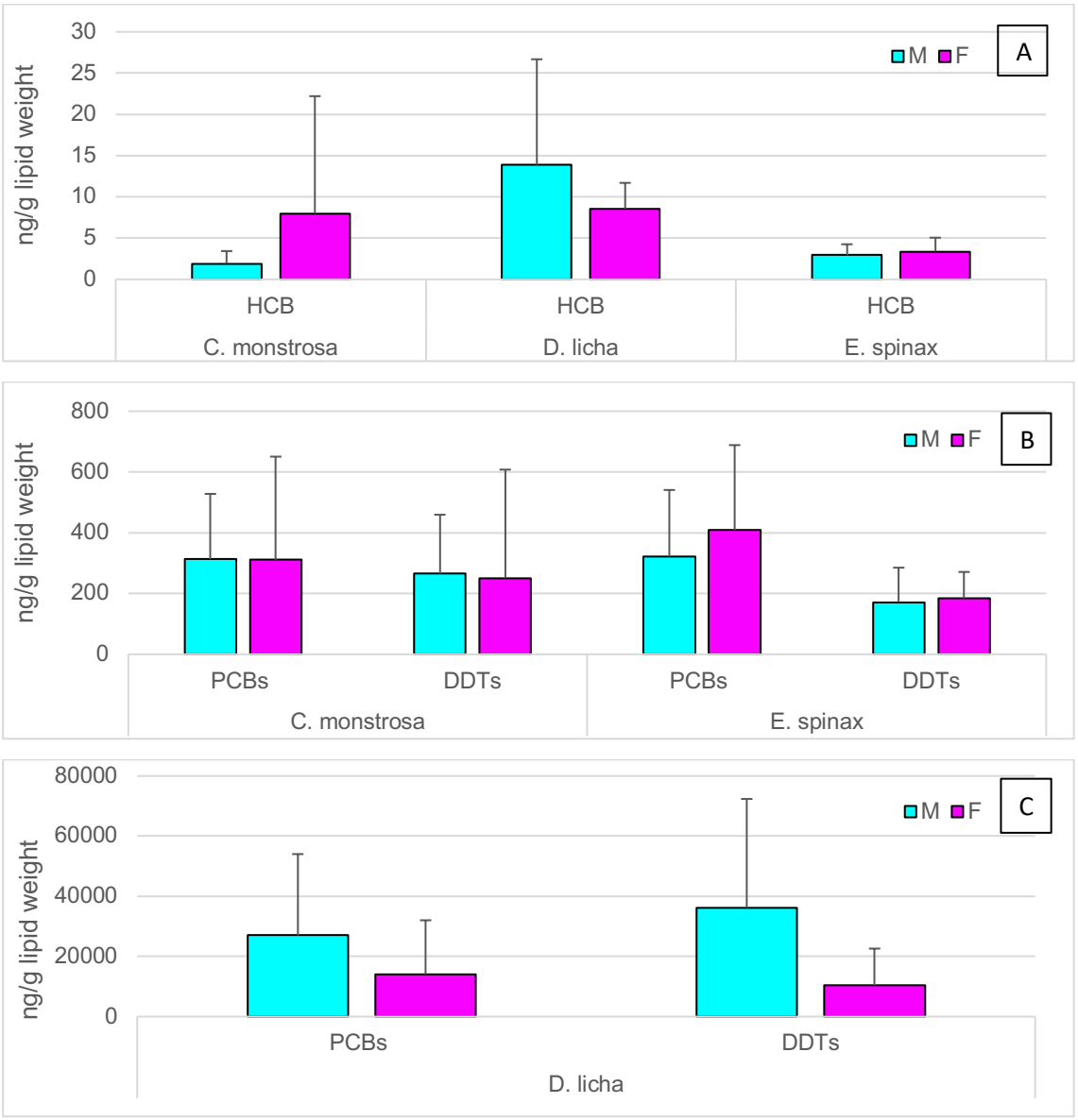


Figure 4.5 Mean concentrations (ng/g lipid weight) of HCB (A), PCBs and DDTs (B, C) in muscle tissue of C. monstrosa (M=5;F=11) D. licha (M=7;F=5) and E. spinax (M=17; F=35) divided by sex. Error bars represent the standard deviation.

Endocrine Disrupting Chemicals (EDCs)

The percentage of EDCs on HCB+PCBs+DDTs (∑OCs) was calculated in all the samples. In all the three different species EDCs represented more than the 50% on ∑OCs. *D. licha* was the species with the highest EDCs percentage (66.69%) followed by *C. monstrosa* (62.52%) and *E. spinax* (58.10%).

EDCs was also calculated for male and female specimens. Males had higher percentage in all the three species (*D. licha*=68.50%; *C. monstrosa*=68.25%; *E. spinax*=59.03%) compared to those detected in females (*D. licha*=64.17%; *C. monstrosa*=59.92%; *E. spinax*=57.67%).

In eggs and embryos EDCs were higher in *D. licha* embryos following the pattern *D. licha* embryos (61.66%)>*C. monstrosa* eggs (60.38%)>*E. spinax* embryos (59.21%)>*E. spinax* eggs (57.34%)

EDCs can also be divided into EDCs with estrogenic and anti-androgenic (E-AA) capacity (pp'DDT, op'DDT, pp'DDE, op'DDE, PCBs n°. 95, 99, 101, 153) and EDCs with androgenic and anti-estrogenic (A-AE) capacity (pp'DDE, op'DDT and PCB n° 118). E-AA were higher in male specimens except for *E. spinax* (*C. monstrosa* M=62.61%, F=61.18%; *D. licha* M=61.12%, F=59.10%; *E. spinax* M=60.20%, F=61.13%) while A-AE EDCs were higher in *C. monstrosa* and *E. spinax* male specimens and *D. licha* female specimens (*C. monstrosa* M=33.41%, F=32.02%; *D. licha* M=34.90%, F=37.41%; *E. spinax* M=32.99%, F=32.17%).

Eggs had higher percentage of E-AA EDCs than embryos (*C. monstrosa* eggs=66.42%; *E. spinax* eggs=61.47%; *D. licha* embryos=60.45%; *E. spinax* embryos=59.51%) while for A-AE EDCs was exactly the opposite with the embryos having highest percentage (*D. licha* embryos=36.76%; *E. spinax* embryos=34.49%; *E. spinax* eggs=32.74%; *C. monstrosa* eggs=29.14%).

Maternal transfer

Due to the small sample size, maternal transfer was calculated only in *Etmopterus spinax,* the most abundant species. Specifically, were analyzed 13 pregnant females, 7 with embryos and 6 with eggs. Concentrations in ng/g wet weight (w.w.) in the whole body and in the embryonic tissues are reported in Table 4.3.

Table 4.3 Sample code, sampled tissue with number of embryos in brackets, length and weight of gravid females, maturity stage according to MEDITS standards, and concentrations (ng/g wet weight) of HCB, PCBs and DDTs in the body/carcass and in the pools of embryos or eggs.

SAMPLE	TISSUE	Length (cm)	Weight (g)	Maturity stage	HCB	PCBs	DDTs
8ESP109	Body	36	177,05	3C	0,44	30,35	19,27
	Embryos (7)		28,15		2,07	157,47	74,29
11ESP109	Body	37,5	206,09	3C	1,61	40,10	23,31
	Embryos (5)		22,81		0,91	82,00	41,97
12ESP109	Body	38,5	216,76	3C	0,63	273,33	71,78
	Embryos (7)		33,94		2,00	193,44	71,94
12ESP	Body	39	204,04	3C	1,76	151,70	40,78
	Embryos (5)		25,96		3,99	439,37	517,53
7ESP109	Body	35	160,26	3D	0,88	51,19	30,48
	Embryos (5)		18,24		1,76	112,01	44,50
9ESP109	Body	36	233,10	3D	0,56	43,47	24,47
	Embryos (6)		34,30		1,75	170,52	86,98

10ESP109	Body	37	188,54	3D	0,57	48,72	29,95
	Embryos (7)		18,36		1,00	89,98	44,58
6ESP	Body	36,5	230,43	3A	1,11	92,45	30,84
	Eggs		0,77		1,58	321,90	86,14
8ESP145	Body	37,5	214,25	3A	0,36	43,92	32,49
	Eggs		1,75		1,13	127,30	79,95
14ESP	Body	40,5	279,13	3A	2,55	257,24	71,56
	Eggs		0,87		0,85	81,87	26,31
9ESP106	Body	42,5	285,86	3A	0,31	74,01	29,37
	Eggs		3,64		1,13	362,47	134,05
5ESP34	Body	37,5	226,90	3A	0,40	42,94	24,58
	Eggs		20,60		1,40	108,95	51,79
3ESP142	Body	36	247,82	3A	0,25	65,08	25,73
	Eggs		8,58		1,95	211,89	106,34

The isomer profiles of DDTs were similar both in mothers with embryos and in mothers with eggs (Fig. 4.6 A – B). ppDDE was the predominant isomer; generally, the abundance of the isomers was higher in the maternal body except for ppDDE and for opDDT where the embryos and the eggs showed greater values. Mann-whitney U test also suggested that these differences were also statistically significant for opDDT between mothers vs embryos (Fig. 4.6 A) and between mothers vs eggs ($p<0.05$) and for ppDDE only in the comparison between mothers vs eggs (Fig. 4.6 B).

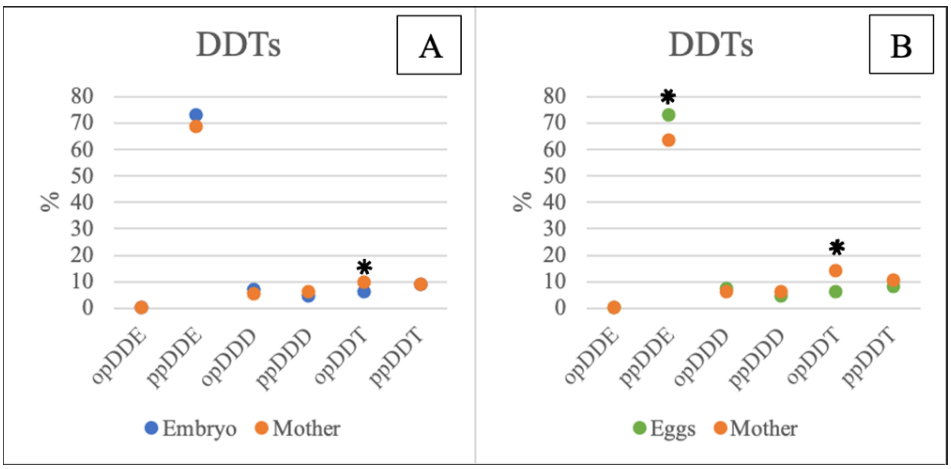


*Figure 4.6 DDTs fingerprints in the maternal body and their embryonic tissues. *= $p<0.05$.*

As for DDTs, congeners profiles of PCBs were similar both in mothers with embryos and in mothers with eggs (Fig. 4.7 A – B). The abundance of the congeners was mostly slightly higher in the embryos rather than in the maternal body, while in the eggs was generally little lower. The differences were statistically significant ($p<0.05$) for PCB n° 128, 172, 180, 196, 201, 206 only between mothers and embryos (Fig. 4.7)

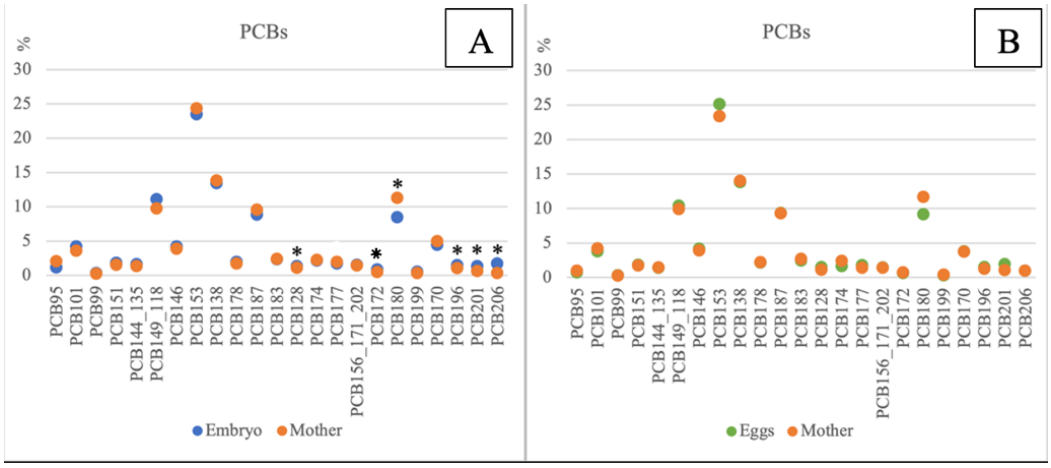
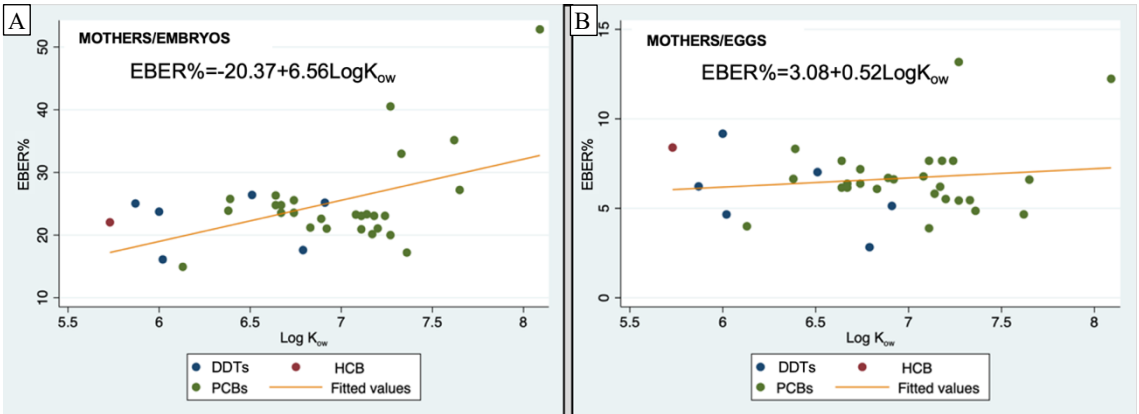


*Figure 4.7 PCBs fingerprints in the maternal body and their embryonic tissues. *= p<0.05*

In Figure 4.8 the scatter plot between EBERs (%) and $LogK_{ow}$ are displayed and the estimated linear regressions are reported. The results stressed a significant relationship between the two variables in *E. spinax* with embryos (p=0.003) while is not in *E. spinax* with eggs (p=0.454).



EBER	Coefficient	Std. err.	t	P>|t|	[95% conf.	interval]
Log K_{ow}	6.56	2.04	3.21	0.003	2.40	10.72
Constant	-20.38	14.04	-1.45	0.157	-48.99	8.23

EBER	Coefficient	Std. err.	t	P>|t|	[95% conf.	interval]
Log K_{ow}	0.52	0.68	0.76	0.454	0.87	1.90
Constant	3.09	4.68	0.66	0.514	-6.45	12.62

Figure 4.8 Relationship between EBER (%) and LogKow for HCB, PCBs, and DDTs in E. spinax. mothers carrying embryos (A) and in mothers carrying eggs (B). *LogKow values were taken from Sangster (1994) and Agudo et al. (2016).*

Discussion

Deep sea chondrichthyans are among the most vulnerable extant deep-sea taxa due to their extremely conservative life histories, and thus slow population rebound rates (Simpfendorfer and Kyne 2009). In recent years catastrophic population declines have been observed across multiple species at numerous locales (White and Kyne 2010; Graham and Daley 2011; Norse et al. 2012; Barbier et al. 2014). The most documented

direct threat for deep water cartilaginous fishes is fishing (Simpfendorfer & Kyne, 2009; Queiroz et al., 2019), but little is known about the possible impact of threats from pollution (Consales & Marsili, 2021). Although the effects of chemicals, especially persistent organic pollutants (POPs), are well known and have been studied in some coastal and open water elasmobranchs (Gelsleichter and Walker, 2010; Alves et al., 2016; Tiktak et al., 2020), our understanding of the occurrence and possible effects on deep sea species is limited (Storelli and Marcotrigiano, 2001; Storelli et al., 2005; Cresson et al., 2016; Salvo et al., 2020). The extreme vulnerability of this group of cartilaginous fishes gives urgency to the need to fill this information gap.

Organochlorine compounds (OCs) occurrence and life history traits

Grater HCB, PCBs and DDTs levels in *D. licha* could be explained by the higher trophic position occupied by this species than *E. spinax* and *C. monstrosa* (Barrìa et al., 2015; Albo-Puigserver et al., 2015, Eronat, 2016). These chondrichthyans' feeding habits vary. *Etmopterus spinax* feeds predominantly on small teleosts, cephalopods and demersal crustaceans (Neiva et al., 2006; Fanelli et al., 2009; Valls et al., 2011; Isbert et al., 2015), *C. monstrosa* main prey are crustaceans, bivalve molluscs and ophiurans (Macpherson, 1980; Eronat, 2016; Tamayo et al., 2021), while *D. licha's* diet is based on other small sharks, fish and crustaceans (Dunn et al., 2010; Navarro et al., 2014; Barrìa et el., 2018), indicating trophic bioaccumulation consistent with the higher OC levels. However, it is very likely that these differences in HCB, PCB, and DDT accumulation in the three species depend on the diet; they are all indeed, as was demonstrated with contaminants fingerprints (Fig 4.3), subjected to the same contamination input.

The predominance of PCBs in all the species is consistent with other studies (Marsili et al., 1997; Fossi et al., 2013; Marsili et al., 2018) in showing that the type of contamination of which our specimens are subjected is mainly from industrial sources. PCB congener composition was similar to the commercial mixture of Arochlor 1260 (the reference standard for PCBs) both in the muscle of the three species and in the eggs and embryos (Fig 4.4). The predominance of Hexa and Hepta -CBs in all the species and tissues could be explained by the resistance to 68etabolization of those congeners which have a high biomagnification potential (Sawhney, 1986; Serrano et al., 2000; Storelli et al., 2005)

The ratios among some DDT isomers (Table 4.2) can give us more information regarding potential historical inputs and its possible illicit use.

The pp'DDE/pp'DDT ratio indicates if there have been recent inputs of the pesticide in the environment (Aguilar 1984). In the commercial mixture this ratio is 0.05; high values

of this ratio indicate no recent inputs because a major part of the active compound must have been degraded. In all the species we found high values both in muscle tissues and in eggs and embryos reflecting an historical contamination. Another indicator of new DDT inputs in the environment is pp'DDE/DDTs. It also gives information of the metabolic "weathering" of DDT: values of 0.6 or below this threshold are considered critical (Tsydenova et al. 2004) and values higher than this imply that there haven't been new inputs. The obtained pp'DDE/DDTs ratio in each species comply with the previous ratio. The value of ∑op'DDTs/DDTs ((op'DDT+op'DDE+op'DDD) /DDTs) reveals which type of DDT was used. Technical DDT (non-insecticidal) has the ∑op'DDTs/DDTs>0.20 (Nowell et al. 1999). While for *C. monstrosa* and *D. licha* this ratio indicates an insecticidal DDT input, for *E. spinax* indicates that it might have been used a technical DDT or at least other pesticides such as Dicofol (Qiu and Zhu, 2010).

Even if not statistically significant, in most cases female specimens had lower OC levels compared to males (Fig 4.5 A – B – C), consistent with other studies in other species (Fossi et al., 2002; Lyons and Adams, 2015; Maisano et al., 2016; Marsili et al., 2018). Lower levels in females could be explained due to the mobilization of lipids during the reproductive period. In fact, the females tend to deploy their fat stocks towards the gonads to support the eggs' development, and along with the lipids, they move also the organochlorines causing a reduction of their levels in other tissues.

Endocrine Disrupting Chemicals (EDCs) distribution and possible negative impact on Chondrichthyes reproduction

All the species possess high levels of endocrine disrupting chemicals (EDC), which can cause negative effects on fish (Milla et al., 2011). EDCs are a structurally diverse group of compounds that may adversely affect the health of humans, wildlife and fisheries, or their progenies, by interaction with the endocrine system (Gillesby and Zacharewski, 1998; Carnevali et al., 2018). Many of the known EDCs are estrogenic, affecting particularly reproductive functions. Because of the lipophilic and persistent nature of most xenobiotic estrogens and their metabolites, many bioaccumulate and biomagnify (Arukwe et al., 1997).

These compounds are considered one of the most dangerous threat for ecosystem functioning due to the potential reproductive alterations in impacted organisms, which can be reflected at the population level (Tanaka, 2003) and in the marine environment generally (Wang & Zhou, 2013). The negative impact of EDCs in the sea is particularly

evident in marine invertebrates (Fernandez, 2019; Katsiadaki, 2019), but also among the top predators such as bony fishes and marine mammals (Fossi et al., 2002; 2003; 2007). The possible impact of the EDCs at population level for these species may be even worse than among other marine organisms: deep sea chondrichthyans are among the most vulnerable extant deep-sea taxa due to their extremely conservative life histories, and thus slow population rebound rates (Simpfendorfer and Kyne 2009). The consequences of EDC contamination on these top predators could compromise their intrinsic low capacity of resilience and could be a relevant driver of population stress and reduction.

Maternal transfer

Our study revealed not only the occurrence of EDCs in all the investigated species of cartilaginous fishes, but also the clear evidence of maternal transfer of these compounds, implying enhanced effects from a more prolonged exposure. Maternal transfer was already demonstrated for other species (Lyons & Lowe, 2013; Lyons & Adams, 2015; Cagnazzi et al., 2019; Chynel et al., 2021); our study represents the first evidence of maternal transfer in deep water species (Table 4.2; Table 4.3).

Even if all the investigated OC compounds were detected in the embryonic tissues of all the species, due to the small sample size and to elucidate maternal transfer mechanism, only *E. spinax* eggs and embryos with their corresponding maternal body were considered (Table 4.3). The velvet-belly shark is a viviparous lecithotrophic species (Musick & Ellis, 2005) which means the embryo development only depend on the yolk sack and not on the maternal nutrient input (Porcu et al., 2014). This also imply that the maternal transfer of contaminants stops when the yolk sack has been fully developed. This could be supported by the results obtained in this study where the positive correlation between EBER (%) and $LogK_{ow}$ was found in mothers with developing and almost fully developed embryos (Fig. 4.8 A) but not in mothers with developing eggs (Fig. 4.8 B). With this relation also seems that transfer of contaminants in this species increases with increasing $LogK_{ow}$ contrary to the studies conducted by Chynel et al (2021) and Lyons and Adams (2015) which considered two placental viviparous species. To corroborate this hypothesis more studies on viviparous lecithotrophic species should be conducted.

Threats of OCs for deep sea cartilaginous fishes and conservation challenges

The deep-sea (> 200 m) has long been considered a pristine environment due to its remoteness from anthropogenic pollution sources. However, there has been growing

concern over the impact of anthropogenic contaminants on deep-sea ecosystems (Ramirez-Llodra et al., 2011). In particular, the deep-sea might act as a sink for highly persistent compounds that enter the marine environment (Kramer et al., 1984; Froescheis et al., 2000; Looser et al., 2000; Scheringer et al., 2004). Organochlorines (OCs) are therefore of particular concern due to their high hydrophobicity, toxicity and persistence (Scheringer et al., 2009).

In the last two decades OC contamination was clearly detected in several deep-water organisms, both invertebrates (Ohkouchi et al., 2016; Lawson et al., 2021) and fishes (Berg, 1999; Takahashi, 2010; Panseri et al., 2019), with negative implications for ecosystems and for human health (Romero-Romero et al., 2017; Panseri et al., 2019). Despite their important role as consumers in deep-water environments (Musick & Cotton, 2015; Treberg et al., 2016), the presence and the impact of OCs in the cartilaginous fishes have been little studied (Storelli et al., 2001; 2005; Davis et al., 2013; Salvo et al., 2019). This reflects a general lack of data regarding anthropic contamination in the chondrichthyes (Tiktak et al., 2020; Consales & Marsili, 2021). Most of deep-water cartilaginous fishes are vulnerable (VU), endangered (EN), or data deficient (DD) by the IUCN (2021). In European seas, five of 30 deep water chondrichthyans species are listed as DD (Nieto et al., 2015). In general, the magnitude of reduction for most of the DD-designated species remains unknown (Leonetti et al., 2020), and it is difficult to understand the real impact of human footprint on these organisms, mainly due to indirect causes such as OC contamination.

Our study confirms the clear occurrence of OC contamination in deep-water cartilaginous fishes, implying possible negative effects at population levels that indicates the urgent need for focused research on this topic (Tiktak et al., 2020; Consales & Marsili, 2021). Effective assessment requires long-term studies, considering all the possible threats for the different species (like contaminant exposure, overfishing, habitat loss) and mixing different data from different sources, like stable isotopes analysis and spatial ecology analysis, as has been done for some marine species of the oceanic megafauna (Le Crozier et al., 2020; Kratofil et al., 2020)

At the same time, a precautionary approach must be stressed by marine scientists at the international political level, to request stricter conservation measures for all marine species yet threatened by several other factors.

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*Chapter 5 **FIRST ASSESSMENT OF ORGANOCHLORINE COMPOUNDS IN DOHRN CANYON'S (NAPLES, ITALY) MEGAFAUNA***

Introduction

The deep-sea (below 200 m depth) is the world's largest ecosystem and one of the least explored and studied; it is rich in biodiversity and supplies a variety of resources, including oil, gas, fisheries, new molecules, and minerals (Ramirez-Llodra et al., 2011). Expanding exploration and industrial exploitation of the vast and unknown deep-ocean environment may act synergistically and span extensive areas, causing regime shifts and altering deep-ocean life-supported services (Danovaro et al., 2017). The deep-sea has also a variety of habitats including abyssal plain, hydrothermal vents, brine lakes, cold seeps, deep sea reefs, seamounts, and canyons.

Submarine canyons are widespread across the globe and typically form U- or V-shaped valleys; the valleys are characterized by vertically developed rock walls and smoother floors in their middle and lower courses (Shepard, 1972). Canyon walls host a wide range of organisms, including sponges, molluscs, polychaetes, crustaceans and echinoderms; Taviani et al. (2019) identified over 60 species distributed on the bottom and walls of these structures. Mediterranean canyons differ from canyons in other oceanic regions; they are usually characterized by a shorter length and shallower average depth and area (Harris and Whiteway, 2011; Harris et al., 2014).

Submarine canyons are considered important biodiversity and biomass hotspots (De Leo et al., 2010, Duffy et al., 2014). In coastal ecosystems, canyons are essential pathways for the movement of nutrient-rich deep water into continental shelf waters (Canals et al., 2006); this allows an increase in local primary productivity and a consequent increase in the amount of resources available to pelagic organisms, which attract a wide variety of predators such as cetaceans, tuna and sharks (De Leo et al., 2010; David and Di-Meglio, 2012; Forrest et al., 2021). Similarly, canyons can act as corridors for the transport of anthropogenic pollutants and litter that are discarded into the seas (Jamieson et al., 2017). This phenomenon is particularly evident in submarine canyons located near rivers or near densely populated and industrialized coastal areas (Richter et al., 2009): the introduction of alien persistent objects and substances to the deep-sea started in the 1970s and it never stopped (Tubau et al., 2015).

The main sources of human impact on these submarine systems are overfishing and destructive fishing, aquaculture, spread of invasive alien species, eutrophication, oil and gas operations, improper disposal of mining wastes, coastal development, ocean

acidification and other stressors related to climate change (Levin and Le Bris, 2015; Fernandez-Arcaya et al., 2017). The impact of marine litter and chemical pollution on the deep-sea is still far to be clearly understood, but recent studies highlighted it can be stronger than every hypothesis (Taylor et al., 2016; Jamieson et al., 2017; Ramirez-Llodra, 2020).

In this framework, the Mediterranean Sea is a landlocked sea with large urban and industrial concentrations along its shores and supports heavy maritime traffic and also remarkable fishing efforts: these conditions make it particularly prone to the accumulation of significant amounts of anthropogenic impact at every marine level (Villasante et al., 2012; Fabri et al., 2014; Tubau et al., 2015). Moreover, despite its limited extension, the basin is rich in a notable and heterogenic number of deep-sea environments, but an important fraction of macrofaunal and megafaunal species remains unknown (Danovaro et al., 2010) and little is known about the anthropogenic impact on the deep Mediterranean Sea (Tubau et al., 2015; Danovaro et al., 2020).

Here the reason to study the Dohrn Canyon, an unexplored and unexploited submarine canyon close to a high urbanized and industrial coastline (the city of Naples) and where recent studies have been highlighted a remarkable presence of dangerous anthropogenic substances (Tornero and d'Alcalà, 2014; Qu et al., 2017).

The Gulf of Naples is a roughly rectangular basin in the south-eastern Tyrrhenian Sea, located among one of the most densely populated regions in Italy.

The Dohrn Canyon is the main canyon that crosses the Gulf of Naples; its width ranges from a few hundred meters to more than 1 km, its depth from 250 m at the shelf edge to some 1300 m at the merging with the bathyal plain (Passaro et al., 2016). It is characterised by two major curved branches; the western is broader than the eastern one, and more deeply incised thus forming a typical Y-structure.

As previously said, submarine canyons are considered important biodiversity and biomass hotspots and the area where the Dohrn Canyon is located is one of the richest in terms of marine biodiversity in the Mediterranean Sea (Psomadakis et al., 2009; Crocetta et al., 2020; Gaglioti et al., 2020).

Unfortunately, for several decades, this canyon has faced several anthropogenic threats, including illegal trash dumping, fishing-related damages to wildlife and flora, and pressures due to its closeness to highly populated areas (Taviani et al., 2019). While for some invertebrates were conducted studies on their abundance and distribution (Gambi et al., 2019; Taviani et al 2019) there is a complete lack of information regarding a Dohrn

Canyon's megafaunal inventory and contamination levels in these organisms. So, the main focus of this study is to assess for the first time legacy contaminants belonging to the class of organochlorine compounds (OCs), such as hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) and its metabolites, in sampled specimens in the Dohrn Canyon. These chemicals, despite their ban in their use and production in most of the countries, continue to threat all marine organisms (Casini et al., 2018; Marsili et al., 2018; Adrogué et al., 2019; Munschy et al., 2019; Quintanilla-Mena et al., 2020; Lawson et al., 2021). Many deep-sea organisms are characterized by high longevity, slow growth, low fecundity, late maturity, and intermittent recruitment, resulting in a high vulnerability, very low resilience, and high chances to bioaccumulate pollutants (Villasante et al., 2012, Koenig et al., 2013a).To date, having a knowledge framework on the evolution of the main threats to these organisms is fundamental, also to ensure adequate protection and risks' management associated not only with the species themselves, but also with their habitats. In fact, environmental contamination is an issue that affects not only the biota, but also the entire surrounding environment and its connections.

Methods

Sampling activities, area and sampled specimens were described in Chapter 3 in "*Sampled collected in the Dohrn Canyon*" paragraph, in Figure 3.2 and in Table 3.2.
Biological parameters were taken according to the paragraph "*Taxonomical identification, morphometric evaluation, and sample preparation*" in Chapter 3.
In total were sampled 48 specimens including three species of osteichthyes and three species of chondrichthyes. Specifically, bony fishes were n=15: European conger (*Conger conger,* n=1), European hake (*Merluccius merluccius,* n=1), blackbelly rosefish (*Helicolenus dactylopterus,* n= 13); and cartilaginous fishes were n=33: small spotted catshark (*Scyliorhinus canicula,* n=1), velvet belly lanternshark (*Etmopterus spinax,* n=1), and blackmouth catshark (*Galeus melastomus,* n=31).
In Table 5.1 are presented both grouped and individual biological parameters (Total Length – TL, Total Weight – TW), the percentage of extracted organic material (%EOM) in the muscle tissue calculated after OCs extraction, and the Fulton's condition factor (K) of the sampled specimens.

Table 5.1 Biological parameters Total Length (cm), Total Weight (g), Extracted Organic Material (%EOM)and Fulton's condition factor (K) measured in the species collected in the Dohrn Canyon. Data are expressed as mean ± standard deviation (SD). Range in brackets (minimum – maximum)

Species Sex (M/F/ND) Code	N	Total Lenght (cm)±SD (min-max)	Total Weight (g)±SD (min-max)	%EOM±SD (min-max)	K±SD (min-max)
Osteichthyes	**15**				
Conger conger	**1**				
ND	1	130.50	4500.00	6.71	0.20
Merluccius merluccius	**1**				
F	1	42.50	556.00	5.79	0.72
Helicolenus dactylopterus	**13**	**18.23±1.50 (15.00-20.50)**	**92.62±20.37 (48.00-128.00)**	**10.76±3.64 (1.79-15.60)**	**1.52±0.23 (1.31-2.20)**
F	4	18.50±1.37 (17.00-20.50)	96.00±20.15 (76.00-128.00)	12.50±1.00 (11.46-13.62)	1.50±0.05 (1.43-1.55)
11HDA		20.5	128	13.62	1.49
1HDA		19	98	11.46	1.43
4HDA		17.5	82	11.54	1.53
7HDA		17	76	13.37	1.55
M	4	17.50±1.97 (15.00-20.50)	81.00±24.92 (48.00-118.00)	11.40±1.47 (9.75-13.09)	1.47±0.09 (1.37-1.55)
10HDA		17	76	12.63	1.55
2HDA		20.5	118	10.14	1.37
3HDA		17.5	82	9.75	1.53
6HDA		15	48	13.09	1.42
ND	5	18.60±0.80 (17.00-19.00)	99.20±9.85 (90.00-114.00)	8.85±5.05 (1.79-15.60)	1.57±0.38 (1.31-2.20)
12HDA		19	92	13.56	1.34
38HDA		17	108	7.08	2.20
39HDA		19	92	6.24	1.34
40HDA		19	90	1.79	1.31
8HDA		19	114	15.60	1.66
Chondrichthyes	**33**				
Etmopterus spinax					
F	1	27.00	84.00	14.71	0.43
Scyliorhinus canicula	**1**				
M	1	25.00	42.00	13.40	0.27
Galeus melastomus	**31**	**39.85±5.55 (30.00-49.00)**	**183.09±82.23 (72.00-356.00)**	**8.58±3.06 (4.26-13.80)**	**0.26±0.05 (0.05-0.32)**
F	16	41.16±5.69 (33.00-49.00)	211.62±93.91 (100.00-356.00)	8.97±3.17 (4.79-13.80)	0.26±0.06 (0.05-0.32)
14GME		48.5	332	8.76	0.29
19GME		35.5	128	11.87	0.29
20GME		37.5	152	7.07	0.29
21GME		45	344	6.38	0.05
22GME		34	102	13.52	0.26
23GME		33	116	9.97	0.32
24GME		43	208	5.19	0.26
26GME		40	176	7.38	0.28
27GME		47	286	10.91	0.28
28GME		48.5	356	12.83	0.31
30GME		49	324	13.80	0.28
35GME		46.5	302	8.71	0.30
36GME		44	218	12.41	0.26
41GME		36	120	4.83	0.26
42GME		37	122	4.79	0.24
43GME		34	100	5.14	0.25
M	15	38.47±5.05 (30.00-46.50)	152.67±52.68 (72.00-240.00)	8.17±2.88 (4.26-13.30)	0.26±0.02 (0.22-0.30)
15GME		41.5	196	8.81	0.27
16GME		44	210	8.64	0.25
17GME		42	204	7.30	0.28
18GME		31	80	10.30	0.27

25GME	30.5	86	7.74	0.30
29GME	46.5	240	6.74	0.24
31GME	42	210	10.75	0.28
32GME	45	200	10.81	0.22
33GME	35.5	116	13.05	0.26
34GME	30	72	13.30	0.27
44GME	37.5	130	4.33	0.25
45GME	36	116	5.66	0.25
46GME	38	158	6.17	0.29
47GME	37.5	118	4.26	0.22
48GME	40	154	4.62	0.24

Stomach content analysis were conducted according to the paragraph "Stomach content analysis" in Chapter 3.

Organochlorines determination was conducted according to the paragraph "*Organochlorine compounds determination"* in Chapter 3.

Data were processed with the Shapiro-Wilk's test to evaluate the distribution using STATISTICA 7.1 software. All the investigated groups analyzed with Shapiro–Wilk test were non–normal distributed, however, due to the small sample size, non-parametric tests (e.g. Kruskal–Wallis) were unable to estimate the statistical significance of the evaluated differences among data groups.

Results and discussion

Sampled specimens

Due to the small sample size no correlation between biological parameters, lipid content and Fulton's condition factor was investigated, and descriptive statistic was used only to present the data.

Overall, the most frequent sampled species were the blackbelly rosefish and the black mouth catshark, nevertheless, all these specimens are very common in Mediterranean deep sea environments including submarine canyons (Sartor et al., 2017; Sion et al., 2019). In general, all the caught specimens could be considered mature/maturing adults possibly due to the type of fishing gear and hook used.

Stomach content analyses

The stomachs of 48 sampled specimens were analyzed to evaluate their contents. The bony fishes' stomachs were all empty except for the *C. conger* were bait and hook were detected. Same for two out of the three shark species: *S. canicula* and *E. spinax*'s stomachs were empty and 26 specimens out of 31 of *G. melastomus* had full stomachs or with traces of prey, while the remaining ones (n=5) were empty or with over digested organic matter. Due to the small sample size, stomach content analysis can give us only little information about their feeding habits, that in any case are extensively studied

(Fanelli et al., 2009; Anastasopoulou et al., 2013a; Bendiab et al., 2016; D'Iglio et al., 2021a). Our findings showed the presence of 63 preys, divided into 6 taxa; 5 unidentified organic elements were considered as organic matter. Cephalopods are the most predated taxa (N% = 50%; F% = 68%), followed by osteichthyes (N% = 29.4%; F% = 56%) and crustaceans (N% = 7.4%; F % = 16%); moreover, remains of *Etmopterus spinax* were found (Fig. 5.2B) in "20GME", a 37.5 cm long female.

Fig. 5.1 shows the abundance (%N) of the preys.

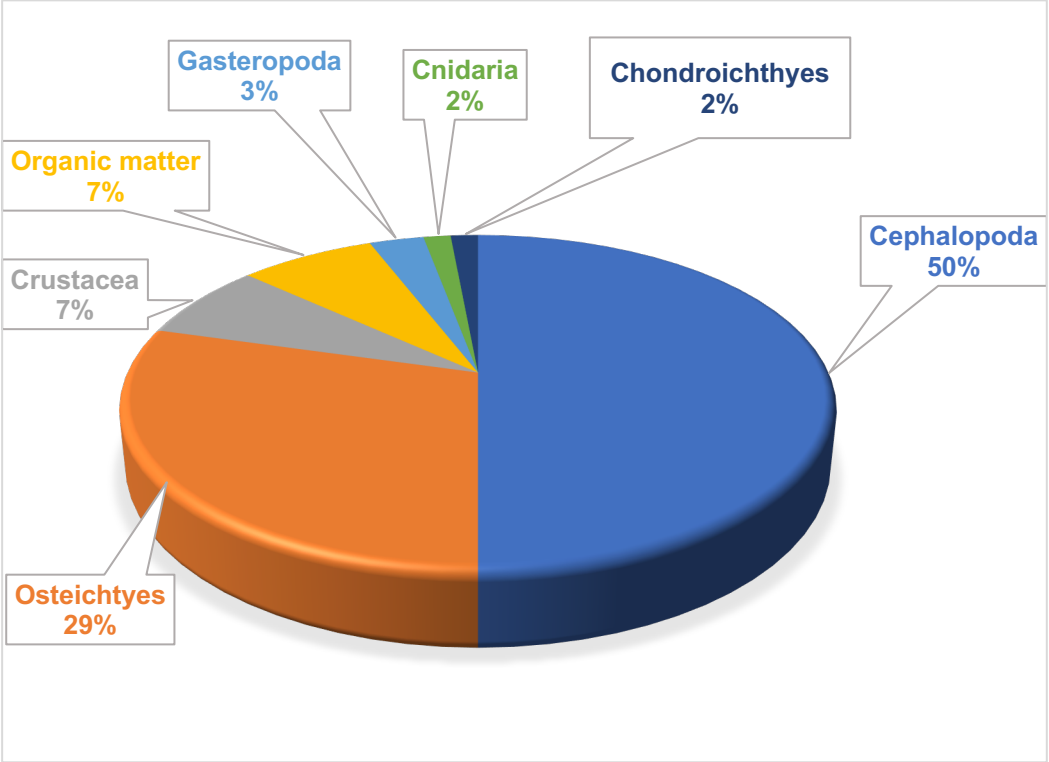

Figure 5.1 Abundance (%N) of the preys recovered in 26 G. melastomus stomach contents.

Other than preys, in two specimens (1GME and 18GME) some fragments of plastic were found (FIG. 5.2A), specifically, two transparent little pieces of plastic bags were found. This was previously documented by several studies in the Mediterranean Sea and linked to feeding strategies and traits (Alomar et al., 2017; Anastasopoulou et al., 2013a,b; Valente et al., 2019; Masacaró, 2020).

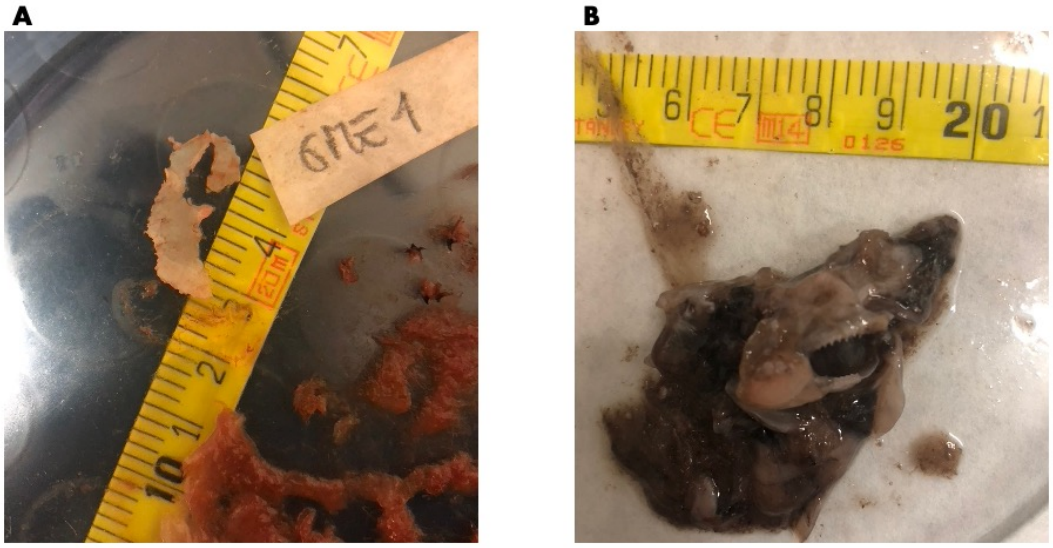

Figure 5.2 Fragment of plastic debris (A) recovered in the stomach content of the specimen 1GME. Head of E. spinax specimen (B) recovered in the stomach content of 20GME.

Organochlorine compounds

HCB, DDTs and PCBs were all detected in the muscle of the sampled 48 specimens. In all the species the OC class with the highest levels was PCBs followed by DDTs and then by HCB. It is important to specify that, due to the small sample size of the most of the sampled species (*C. conger, M. merluccius, E. spinax* and *S. canicula* each represented only by one individual), comparisons among them are not representative thus no statistical analyses were conducted. However, data were presented and was tried to interpret them in the best way possible. The accumulation pattern between the species resulted as follows: *C. conger* < *G. melastomus* < *H. dactylopterus* < *M. merluccius* < *S. canicula* < *E. spinax* for the HCB; *C. conger* < *M. merluccius* < *G. melastomus* < *H. dactylopterus* < *S. canicula* < *E. spinax* for the DDTs; and *C. conger* < *M. merluccius* < *H. dactylopterus* < *G. melastomus* < *S. canicula* < *E. spinax* for the PCBs. Although one specimen is not representative for the species themself, higher levels in *C. conger* and *M. merluccius* could be explained for their dimension, respectively 130.5cm TL and 42.5cm TL, their feeding habits and, high trophic level (Sinopoli et al., 2012; Bănaru et al., 2013; D'Iglio et al., 2022). All the individual HCB, PCB congeners and DDT isomers concentrations are listed in Table 5.2 for bony fishes and Table 5.3 for cartilaginous fishes.

Table 5.2 Concentrations (ng/g lipid weight) of HCB, DDTs and PCBs in Conger conger, Merluccius merluccius and Helicolenus dactylopterus muscle tissue collected in the Dohrn Canyon.

	Conger conger *n=1*	*Merluccius merluccius* *n=1*	*Helicolenus dactylopterus* *n=13*	
			Mean	Standard deviation
HCB	**3.57**	**1.50**	**2.28**	**2.96**
op'DDE	1.09	9.28	3.65	2.81
pp'DDE	254.38	185.29	55.39	32.85
op'DDD	50.75	46.91	15.02	11.84
pp'DDD	8.24	13.25	5.09	3.20
op'DDT	21.38	34.00	13.91	6.90
pp'DDT	49.20	31.49	21.97	24.76
DDTs	**385.04**	**320.22**	**115.03**	**73.33**
95	2.28	3.66	2.51	2.52
101	32.96	36.86	10.15	6.24
99	14.05	5.46	3.63	4.28
151	19.45	16.60	4.32	3.47
144+135	16.03	13.11	5.32	4.60
149+118	154.75	88.59	23.96	15.37
146	86.64	41.45	14.79	9.51
153	569.33	232.75	80.92	39.90
141	23.66	30.30	29.51	61.22
138	283.73	134.94	44.89	23.76
178	38.48	21.12	8.36	4.78
187	196.20	78.96	21.86	12.55
183	68.83	27.41	14.12	12.23
128	30.05	19.67	4.10	1.60
174	40.64	115.51	13.48	19.15
177	40.54	22.25	21.75	56.01
156+171+202	22.79	12.22	6.49	9.79
172	25.73	10.43	5.11	2.66
180	264.03	117.04	38.32	19.20
199	1.02	5.33	1.75	2.05
170	162.13	65.42	41.31	66.41
196	41.90	25.96	8.70	2.64
201	25.22	1.38	4.81	3.48
195	15.56	17.19	45.45	141.48
194	23.73	9.09	3.81	2.61
206	23.68	20.31	9.88	6.08
PCBs	**2223.40**	**1173.01**	**469.29**	**358.86**

Table 5.3 Concentrations (ng/g lipid weight) of HCB, DDTs and PCBs in Scyliorhinus canicula, Etmopterus spinax and Galeus melastomus muscle tissue collected in the Dohrn Canyon.

	Scyliorhinus canicula *n=1*	*Etmopterus spinax* *n=1*	*Galeus melastomus* *n=31*	
			Mean	Standard deviation
HCB	**1.16**	**0.74**	**2.92**	**3.27**
op'DDE	4.20	0.86	6.23	8.82
pp'DDE	59.88	12.74	65.29	40.94
op'DDD	14.71	3.81	18.78	10.21
pp'DDD	3.88	1.58	7.26	6.16
op'DDT	22.55	4.49	31.95	30.98
pp'DDT	17.40	3.98	20.60	12.09
DDTs	**122.62**	**27.46**	**150.09**	**87.07**
95	6.68	0.46	4.28	4.57
101	13.26	1.97	12.49	8.71
99	2.12	0.71	5.58	3.39
151	3.79	1.54	6.47	7.28
144+135	5.94	1.35	5.64	4.04
149+118	16.80	5.21	22.28	19.24
146	14.87	4.20	16.34	13.34
153	67.14	15.80	72.32	70.26
141	7.43	2.06	9.84	6.61
138	58.01	9.24	44.29	41.01
178	12.91	3.18	9.94	7.71
187	29.64	4.91	29.69	25.66
183	7.43	3.15	12.19	9.14
128	2.35	0.57	6.30	6.13
174	3.47	2.43	9.22	6.53
177	4.55	3.10	9.61	7.13
156+171+202	6.54	0.33	7.13	7.30
172	5.45	1.12	5.55	4.34
180	34.12	7.79	33.93	28.82
199	3.66	0.85	2.92	3.77
170	13.81	13.56	25.10	18.97
196	5.42	4.89	12.56	6.56
201	5.40	0.65	4.76	3.75
195	2.18	10.20	12.00	14.95
194	4.77	1.13	4.50	3.60
206	1.96	9.56	7.39	4.03
PCBs	**339.68**	**109.95**	**392.32**	**265.61**

Contaminant ratios were also calculated in order to identify the type and the time of contamination. The ratio of DDTs to PCBs (DDTs/PCBs) has been employed as a marker for shark contamination from agricultural and industrial sources (Aguilar et al. 1999). In all the sampled species the ratio's range vary from 0.17 in *C. conger* to the maximum of 0.40 in *G. melastomus,* which means that the type of contamination to which our specimens are exposed is released from industrial sources. This result is consistent with other studies conducted on edible species in the same area (Naso et al., 2005; Ferrante et al., 2007). PCB pollution in the Gulf of Naples is attributable to multiple sources of industrial and municipal contamination: highly inhabited urban centers, big factories, intense maritime traffic, and numerous waste dumps are all concentrated along the coast. Furthermore, the run-off of the close River Sarno contributes to damage the aquatic ecosystem (Naso et al., 2005; Tornero and d'Alcalà, 2014).

Ratios between DDT isomers gives us information regarding temporal inputs: specifically, pp'DDE/pp'DDT indicates if there have been recent inputs of the pesticide in the environment (Aguilar 1984). In the commercial mixture is 0.05, so high values of this ratio indicate no recent inputs: this is because most of the active compound (pp'DDT) must have been degraded in pp'DDE.

In the sampled specimens this ratio was always above 3, a value not very high compared to other species sampled in the Mediterranean Sea (Corsolini et al., 2008; Storelli et al., 2008; Marsili et al., 2018; Klinčić et al., 2020) but similar to those sampled in the Gulf of Naples (Naso et al., 2005).

Another indicator of new DDT inputs in the environment is pp'DDE/DDTs. It also gives information of the metabolic "weathering" of DDT: values of 0.6 or below this threshold are considered critical (Tsydenova et al. 2004) and values higher than this imply that there haven't been new inputs. Our results showed that only in *C. conger* the value is above the threshold (0.66), while in the other species was below. Specifically, in *M. merluccius* was 0.58, in *H. dactylopterus* and in *S. canicula* was 0.49, in *E. spinax* was 0.46 and in *G. melastomus* was 0.44. This is in accordance with results obtained with pp'DDE/pp'DDT and also with ∑op'DDTs/DDTs ((op'DDT+op'DDE+op'DDD) /DDTs).

This latter reveals which type of DDT was used. In the common and regulated DDT formula, op' isomers account for less than 20% on the total, while in technical DDT (non-insecticidal and unregulated) this ratio is higher than 0.20 (Nowell et al. 1999). In our specimens, except for *C. conger* (∑op'DDTs/DDTs =0.19), the value ranged between 0.28 in *M. merluccius* to 0.37 in *G. melastomus.* Technical DDT is used to produce

Dicofol, an acaricide and miticide frequently used to protect citrus and cotton cultivation. Our findings suggest that there might be a source of contamination by this chemical; the proximity of Sarno River's mouth could explain these data since the pesticide was also detected in another Mediterranean delta ecosystem (Barbieri et al., 2021). Soil erosion as well as atmospheric transport are important carrier of DDT contamination as was previously described by other studies conducted on soils and sediments in the Gulf of Naples (Qu et al., 2016; Qu et al., 2018).

PCB contamination profiles were summarized in Figure 5.3

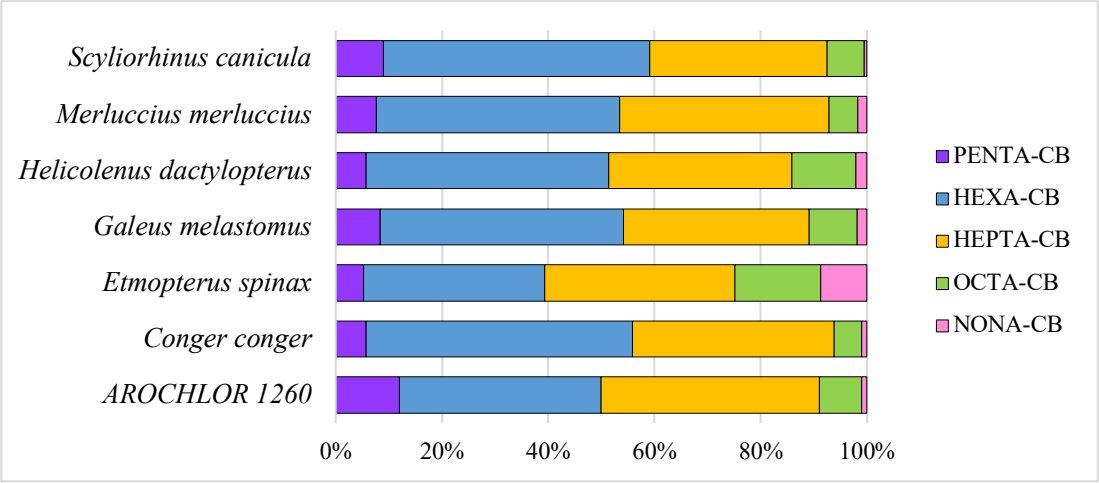

Figure 5.3Percentage composition of PCBs divided by chlorine content (penta-CBs, hexa-CBs, hepta-CBs, octa-CBs, nona-CBs) on ∑PCBs, in AROCHLOR 1260 (reference standard for PCBs) an in n=1 C. conger, n=1 E. spinax, n=31 G. melastomus, n=13 H. dactylopterus, n=1 M. merluccius, n=1 S. canicula muscle tissue.

As for the AROCHLOR 1260 (reference standard for PCBs), hexa- and hepta-CBs were the most abundant in all the species accounting for more than 30% (hexa-CBs 34.08% - 50.10%; hepta-CBs 33.43% - 39.40%) on the total burden of PCBs. Nona-CBs were the less present in all samples except for the specimen of *E. spinax* where penta-CBs represented the group with the minor percentage (5.22%). The higher biomagnification potential, and so the difficulty in the metabolization, of hexa- and hepta-CBs (Sawhney, 1986) explain the major percentage of these PCBs found in our samples. The predominance of highly chlorinated biphenyls was also observed in other studies conducted in the Mediterranean basin (Solé et al., 2001; Koenig et al., 2013a; Cresson et al., 2015; Cresson et al., 2016). Also, the predominance in the tissues of PCB153, PCB138, PCB187 and PCB180 is not only a characteristic of other marine top predators such as cetaceans, pelagic fishes and sharks (Corsolini et al., 1995; Storelli and Marcotrigiano, 2001; Marsili et al., 2016; Marsili et al., 2018) but also of deep sea species (Cresson et al., 2016); this is due to the chemicals' high resistance to breakdown in the environment or in the biota (Storelli et al., 2004; Koenig et al., 2013b). Tolosa et al.

(1995) demonstrated that also the deep sediment profile is dominated by highly chlorinated biphenyls so the resuspension of organic matter could be a source of contamination for deep sea species especially since the Gulf of Naples' sediments are characterized by high levels of pollutants including OCs, heavy metals and polycyclic aromatic hydrocarbons (Tornero and d'Alcalà, 2014; Qu et al., 2018).

Contaminant fingerprints, shown in Fig 3.4, were also calculated in order to see if the species were subjected to the same input of pollution. As we can note from the figure, fingerprints are similar in all the species with only few exceptions for *C. conger, S. canicula* and *E. spinax* in some PCB congeners; this difference could be linked to individual variation since was only analyzed one specimen per species. Apart from that, results suggest that they are all threatened to the same input of pollution.

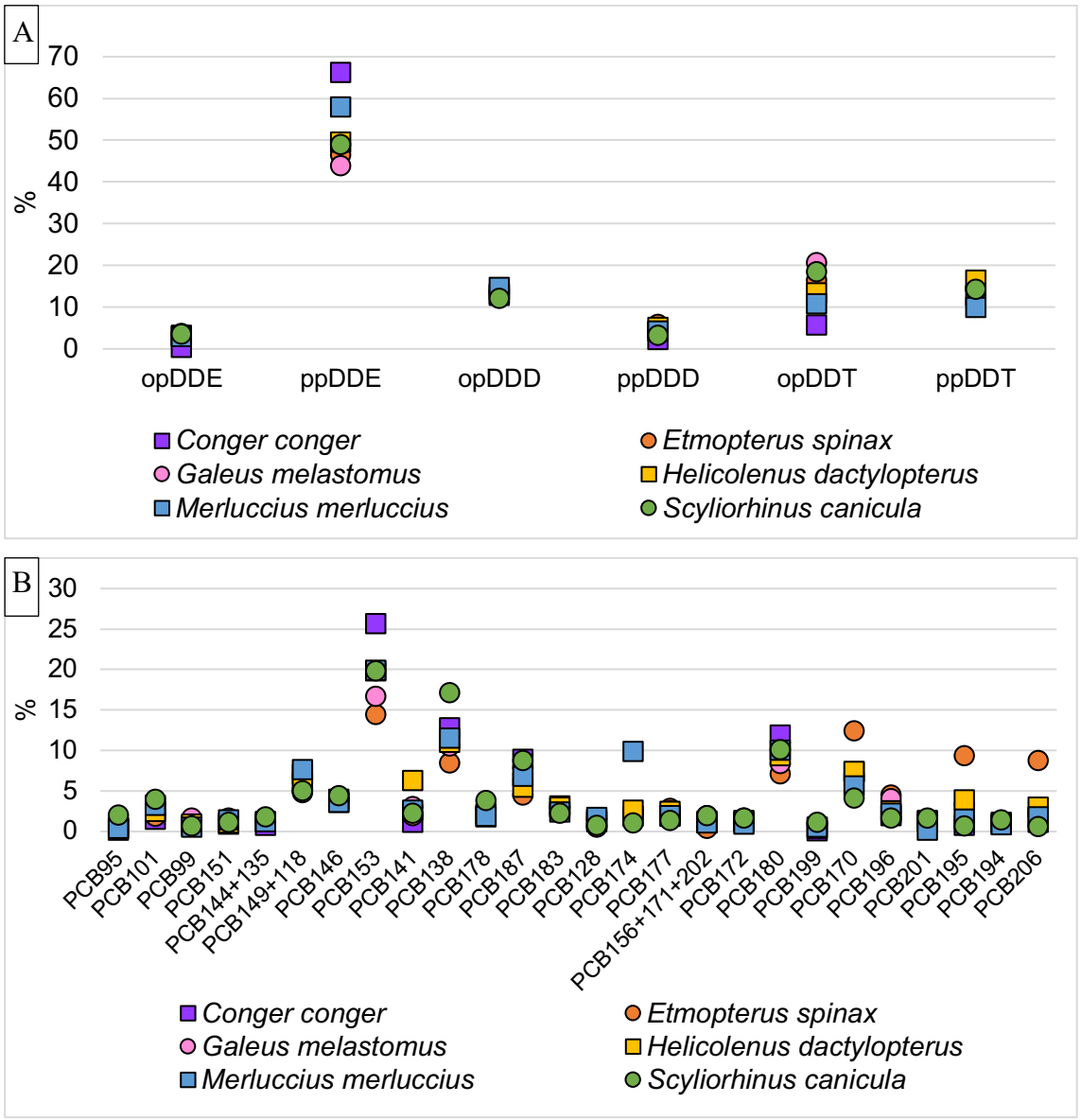

Figure 5.4 DDT (A) and PCB (B) fingerprint in the muscle tissue of the species collected in the Dohrn Canyon.

As previously said the only two species represented by higher number of specimens were *G. melastomus* (n=31) and *H. dactylopterus* (n=13). OCs levels were then calculated according to sex determination (Table 5.4). In *G. melastomus* males showed higher concentrations than females, while in *H. dactylopterus* the undetermined were those having higher levels. As no significant differences of the three OC class (HCB, PCBs, DDTs) across the sexes were found in either species, both sexes and undetermined individuals were considered together for species comparison.

Table 5.4 Concentrations of HCB, PCBs and DDTs with their standard deviation (SD) in G. melastomus (n=31) and Helicolenus dactylopterus (n=13) sampled in the Dohrn Canyon. Samples were also divided by sex: male (M), female (F), not determined (ND)

	N	HCB	SD	PCBs	SD	DDTs	SD
Galeus melastomus	**31**	**2.92**	**3.27**	**392.32**	**265.61**	**150.09**	**87.07**
F	16	2.74	2.90	328.66	167.95	133.11	75.41
M	15	3.12	3.61	460.22	326.76	168.21	94.69
Helicolenus dactylopterus	**13**	**2.28**	**2.96**	**469.29**	**358.86**	**115.03**	**73.33**
F	4	0.62	0.18	371.50	173.90	89.31	28.94
M	4	1.74	0.81	277.91	91.07	82.04	18.40
ND	5	4.04	4.08	700.62	462.09	162.00	97.14

Overall, the levels were very similar in both species (HCB: *G. melastomus*=2.92±3.27 ng/g l.w.*; H. dactylopterus*=2.28±2.96 ng/g l.w.*;* PCBs: *G. melastomus*=392.32±265.61 ng/g l.w.*; H. dactylopterus*=469.29±358.86 ng/g l.w.*;* DDTs: *G. melastomus*=150.09±87.07 ng/g l.w.*; H. dactylopterus*=115.03±73.33 ng/g l.w.) with no statistically significant difference. Their diet, however, differs in terms of preys; while *G. melastomus* mostly feed on Cephalopoda, Osteichthyes, and Crustacea (D'Iglio et al., 2021b), *H. dactylopterus'* diet is mainly composed by crustaceans (Consoli et al., 2010; Capezzuto et al., 2020). For this reason, it is necessary to further investigate contaminant levels in a wider number of individuals.

Lastly, was conducted a bibliographic research to compare the obtained result in this study with the others. Tab 5.5 summarize all the works performed in the Mediterranean Sea in the muscle of the investigated species. Most of the studies focuses on *M. merluccius,* but since our data are represented only by one specimen it is not possible to make any comparison. Variability between individuals in toxicological analysis is always high because their accumulation depends on a large variety of factors, such as habitat, feeding habits, prey availability, age and reproduction. *H. dactylopterus* and *G. melastomus* were the only species in which is possible to do a comparison.

Cresson et al. (2016) conducted a study in the Gulf of Lion, an area characterized by several submarine canyons. The sum of the 7 PCB congeners in *G. melastomus* were slightly higher in our study while those detected in *H. dactylopterus* were lower. As previously mentioned, these differences could be linked to the small number of samples; a comparative and more accurate study between the two areas will be very interesting.

Table 5.5 Bibliographic research on same species as ones collected in the Dohrn Canyon. In particular are specified the sampling area, the number of sampled specimens with sex in brackets (M=male; F=female; ND=not determined), HCB, DDTs and PCBs levels. Values are expressed in ng/g lipid weight (lw) unless specified; ww=wet weight, dw=dry weight.

Species	*Area*	*Sampled specimens (sex)*	*HCB*	*DDTs*	*PCBs*	*Reference*
C. conger	Gulf of Fos, France	6 10 7	/	Not detected	West: 25.4±10.7 Harbor: 24.5±8.2 East: 31.1±11.7 ng/g ww[a]	Dron et al., 2019
C. conger	Ionian Sea, Italy	10 pools from 137 individuals	/	543±201	891±398[b]	Storelli et al., 2012
M. merluccius	Gulf of Naples, Italy	13	29.7	645.1	5572.4[c]	Naso et al., 2005
M. merluccius	Gulf of Naples, Italy	14	15.29±12.66	533.67±470.02	4410.20±3828.37[d]	Ferrante et al., 2007
M. merluccius	Gulf of Lion, France	29 (ND)	/	17.3 ng/g dw (pp'DDE)	78.2 ng/g dw[e]	Bodiguel et al., 2009
		26 (M)	/	32.1 ng/g dw (pp'DDE)	79.1 ng/g dw[e]	
		49 (F)	/	17.3 ng/g dw (pp'DDE)	78.3 ng/g dw[e]	
M. merluccius	Gulf of Lion,	57			PCB153: 51.1±21.4 ng/g dw	Harmelin-Vivien et al., 2012
M. merluccius	Gulf of Lion and Corse, France	85 69 70	/	/	Bastia 13.44 Le Grau du Roi 20.25 Port La Nouvelle 22.01 ng/g dw[d]	Cresson et al., 2015
M. merluccius	Ionian Sea	13	0.6 ng/g ww	3.9 – 12.4 ng/g ww	11.6 – 36.5 ng/g ww[d]	Moraleda-Cibrián et al (2015)
H. dactylopterus	Mar Mediterraneo	19	/	/	25.28 ng/g dw[d]	Cresson et al (2016)
S. canicula	Mar Mediterraneo	6	/	/	17.00 ng/g dw[d]	Cresson et al (2016)
G. melastomus	Mar Mediterraneo	15	/	/	12.76 ng/g dw[d]	Cresson et al (2016)

[a] 42 PCB congeners including the six PCB indicators (PCB 28, 52,101,138, 153, and 180) and the 12 dioxin-like congeners (PCB-DL 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189)
[b] 17 PCB congeners (no. 8, 20, 28, 35, 52, 60, 77, 101, 105, 118, 126, 138, 153, 156, 169, 180 and 209)
[c] 20 PCB congeners (no. 28, 52, 66, 74, 99, 101, 105, 118, 128, 138, 146, 153, 170, 177, 180, 183, 187, 196, 194, and 201)
[d] 7 PCB congeners (no. 28, 52, 101, 118, 138, 153, and 180)
[e] PCB congeners (no. 28, 52, 101, 118, 138, 153, 180, 105, 110, 128, 132, 149, 156, 170, 187, and 194)

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Chapter 6 ***CONTAMINATION STATUS BY PERSISTENT ORGANIC POLLUTANTS OF THE BLACK MOUTH CATSHARK (GALEUS MELASTOMUS) IN TWO DIFFERENT DEEP SEA ENVIRONMENTS***

Introduction

The Blackmouth catshark (*Galeus melastomus*, Rafinesque, 1810) (Scyliorhinidae) is a demersal bottom dwelling species, occurring mainly at depths of 300-400 m (de Sola and Massutì, 2005; Ragonese et al., 2009; Tserpes et al., 2013; Porcu et al., 2020) and distributed in the whole Mediterranean Sea and in the Eastern Atlantic Ocean from Senegal up to Norway (Compagno, 1984). Benthic ecosystems, continental shelf breaks, and slope habitats are all places where it can be found. (Ferretti et al., 2010). The distribution and biology of this species in the Mediterranean Sea have been extensively studied in the central (Ragonese et al., 2009; Rinelli et al., 2005; Bottari et al., 2014; Marongiu et al., 2013; D'Iglio et al., 2021) and western parts (de Sola and Massutì, 2005; Massutì and Moranta 2003; Capapé et al., 2008; Rey et al., 2004).

Studies in the Eastern Mediterranean Sea are still limited and primarily focus on its abundance fluctuations, diet composition, and feeding ecology (Anastasopoulou et al., 2013; Tserpes et al., 2013; Peristeraki et al., 2020). *G. melastomus* is a scavenger and opportunistic predator, and offal and discards from fishing activities can supplement its diet (Olaso et al., 2005). This demersal shark eats a wide variety of food, adapting its diet to seasonal and geographical changes in prey availability in each Mediterranean region (D'Iglio et al., 2021b). Smaller individuals unselectively feed on small crustaceans, fishes and small sepiolids; adults mostly feed on bathypelagic fishes, crustaceans and cephalopods (Fanelli et al., 2009; Valls et al., 2011; Anastasopoulou et al., 2013a).

This species appears still abundant in Mediterranean Sea but it represents a large portion of the bycatch both in demersal trawl and longline fisheries (Abella and Serena, 2005). Individuals taken as bycatch are generally discarded, with low survival rates, but in some areas large individuals are retained and their flesh is used for human consumption (Serena et al., 2005).

According to the IUCN Red List, *G. melastomus* is included in the Least Concern category; the different inhabited bathymetric range leads to a diversification of some ecological factors such as prey, predators and habitat change making its population stable (Abella et al., 2015). Based on trawl fishing catches this species exhibits a consistent size structure, which could be connected to their distribution at deeper depths where trawling is less common, making them less sensitive to fishing (Sion et al., 2004). Anyway, as

previously demonstrated by Sheperd and Meyers (2005), its population growth can be easily reversed by fishing. Moreover, official fishery data in the Mediterranean Sea can underestimate the risk of overexploitation for different species of elasmobranchs and its impact on the life cycle of these marine vertebrates (Cashion et al, 2019; Ramirez-Amaro et al., 2020), placing them in a more critical conservation status. Another component that may affect its abundance could be represented by chemical pollution since is one of the major threats for marine organisms as was formerly proven by other studies (Fossi et al., 2013; Marsili et al., 2014; Brown and Takada, 2017; Casini et al., 2018; Mearns et al., 2019; Righetti et al., 2019; Quintanilla-Mena et al., 2020). Unfortunately, studies on cartilaginous fishes are few (Consales and Marsili, 2021) even though this threat is still a priority especially in the Mediterranean Sea. This basin, besides being a hotspot for its biodiversity (Coll et al., 2010), so is for contamination (Marsili et al., 2018). Once it was believed that deep-sea ecosystems, being far from anthropogenic sources of contamination, were free from xenobiotic substances; but some studies have demonstrated the opposite, pointing out high levels of contaminants and ecological implications (Mormede and Davies 2003; Storelli et al., 2009; Koenig et al., 2013; Romero-Romero et al., 2017; Lawson et al., 2021; Sanganyado et al., 2021).

Principal aim of this chapter is to assess for the first time the presence of some legacy organochlorine contaminants (OCs), in particular hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) and its metabolites in the black mouth catshark*,* chosen as a potential biondicator, which was sampled in two different areas in the Tyrrhenian Sea: the Geographic Sub Area 9 (GSA9) and the Geographic Sub Area 10 (GSA10).

Referring to GSA10, we intend to indicate only the Dohrn Canyon where sampling efforts were mostly focused. The areas of GSA9 (Liguria, Tuscany, Latium) are part of the scientific fishing campaign of the Community Data Collection Framework program (MEDITS), which is focused on the evaluation of the fishery resources of European seas. Since 1994 with the studies carried out by the MEDITS Project it has been possible to evaluate the state of exploitation of fish populations; this has allowed both to address some decisions at the level of the European Commission, but also to understand how the ecosystem is reacting to human pressures in terms of harvesting and impact on biocenosis. In addition, with MEDITS a large portion of the demersal and bathyal megafauna has been characterized, and this information has been an important background to this work,

allowing toxicological focus on deep-sea and common species between the two GSAs (Dohrn Canyon and GSA9).

Methods

Sampling activities, area and sampled specimens were described in Chapter 3 in "*Sampled collected in the GSA9*" and in "*Sampled collected in the Dohrn Canyon*" paragraphs, in Figure 3.1 - 3.2 and in Table 3.1 - 3.2.

However, an overview of the samples taken is showed in Figure 6.1.

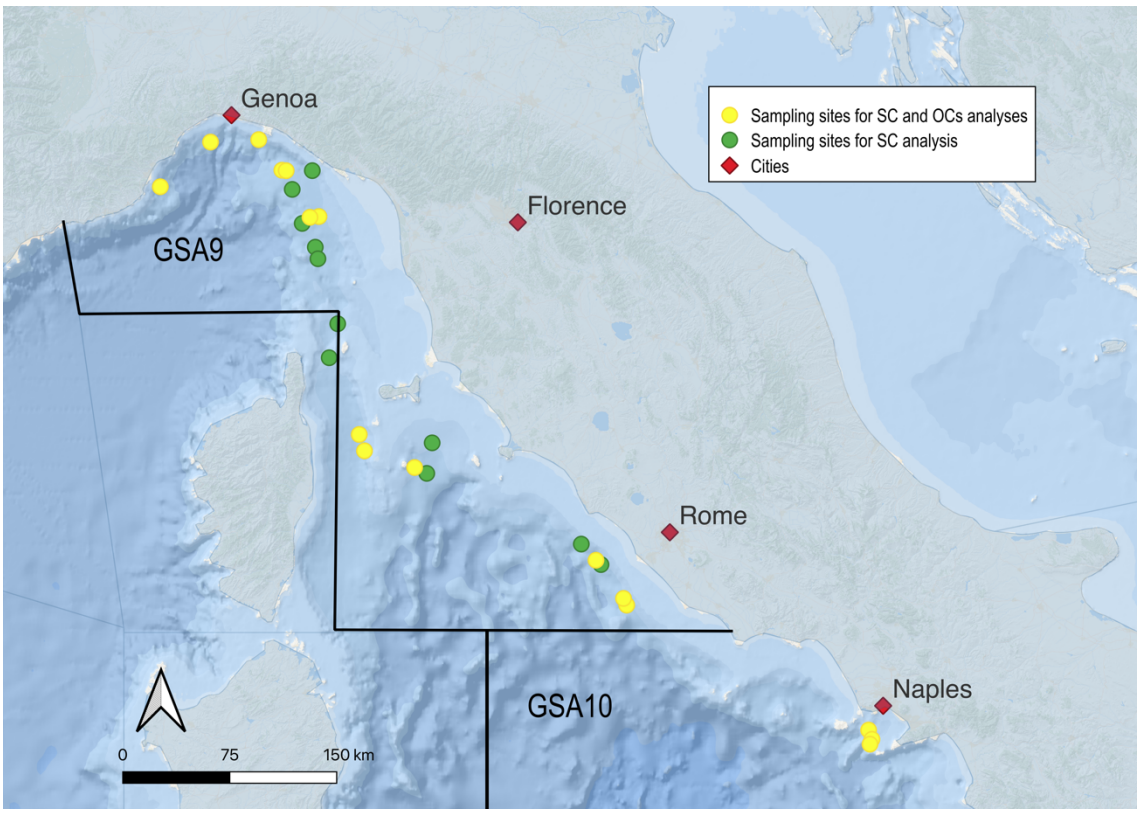

Figure 6.1 Sampling area and sampling sites of G. melastomus in the two Geographic Sub Areas (GSA). Yellow dots represent sampling sites where have been taken G. melastomus for stomach content (SC) and organochlorine (OCs) analyses; green dots represent sampling sites where have been taken G. melastomus only for SC analyses.

Biological parameters were taken according to the paragraph "*Taxonomical identification, morphometric evaluation, and sample preparation*" in Chapter 3.

Biological parameters summarized in the table below (Tab. 6.1) derive from the 69 *G. melastomus* specimens in which ecotoxicological analysis were carried out. From a total of 167 specimens sampled in the GSA9, 38 were chosen randomly between mature individuals. Specifically, 7 males and 7 females from Ligurian area, 7 males and 8 females from Lazio's area and 9 females from Tuscany area. From the Dohrn Canyon, 31 specimens (15 males and 16 females) of different maturation stages were analyzed.

Specifically, 3 immature males Total Length (TL) range: 30 – 31cm, Total Weight (TW) range: 72 – 86 g; 8 immature females TL: 33 – 40 cm, TW: 100 – 176 g; 5 maturing males TL: 35.5 – 41.5 cm, TW: 116 – 196 g; 2 maturing females TL: 43 – 44 cm, TW: 100 – 176 g; 7 mature males TL: 38 – 46.5 cm, TW: 154 – 240 g; 6 mature females TL: 45 – 49 cm, TW: 286 – 356 g.

Table 6.1 Number of sampled specimens (N) their total Length (TL) expressed in cm, Total Weight (TW) expressed in g measured in the species collected in the Dohrn Canyon and in the GSA9. Data are also divided by area and sex (M=male, F=female). SD=Standard Deviation.

(Area/sex)	N	TL (cm)	SD	TW (g)	SD
Dohrn Canyon	**31**	**39.85**	**5.55**	**183.10**	**82.23**
F	16	41.16	5.69	211.63	93.91
M	15	38.47	5.05	152.67	52.68
GSA9	**38**	**45.01**	**2.52**	**266.57**	**51.54**
Liguria	**14**	**45.25**	**2.37**	**272.77**	**51.34**
F	7	46.86	1.22	299.53	53.08
M	7	43.64	2.15	246.01	31.98
Tuscany	**9**	**45.39**	**2.56**	**287.42**	**40.96**
F	9	45.39	2.56	287.42	40.96
Lazio	**15**	**44.57**	**2.57**	**248.26**	**51.29**
F	8	45.38	2.86	268.26	59.60
M	7	43.64	1.81	225.40	24.44

Stomach content analysis were conducted according to the paragraph "Stomach content analysis" in Chapter 3.

Organochlorines determination was conducted according to the paragraph "*Organochlorine compounds determination"* in Chapter 3.

Data were processed according to the paragraph "*Statistical analysis*" in Chapter 3.

Results

Biological parameters

Females showed, on average, lager sizes than males (Table 6.1). Length and weight were positively correlated in both sexes (Fig 6.2); females (R^2=0.91) and males (R^2=0.92).

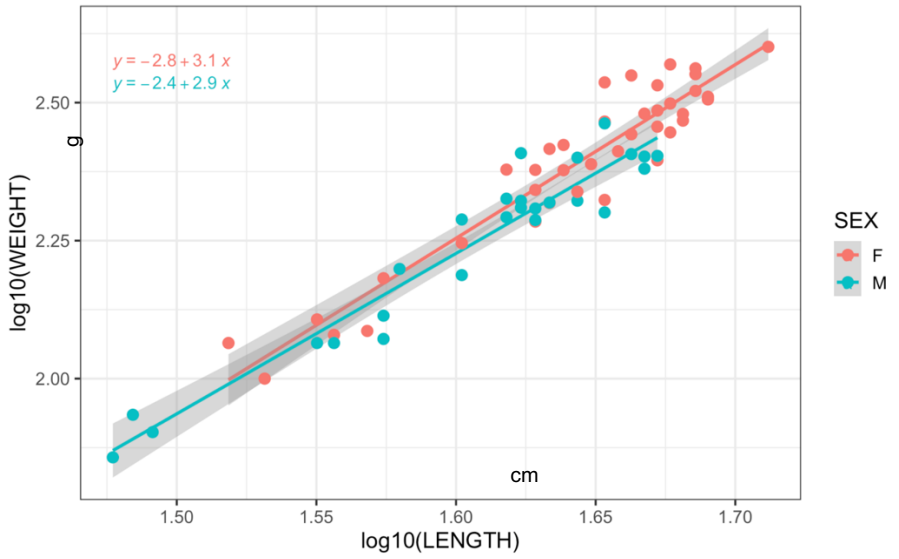

Figure 6.2 log10(Total Length in cm) - log10(Weight in g) relationships of G. melastomus of both sexes females (F, N=40) and males (M, N=29)

Samples were then divided by sampling area, and considering the small sample size in Liguria, Tuscany, and Lazio, these three areas were all considered as GSA9.

Figure 6.3 A – B show again the positive correlation between total length and weight in both sexes in both areas. From these results, specimens sampled in the Dohrn Canyon seems to be bigger than those sampled in the GSA9.

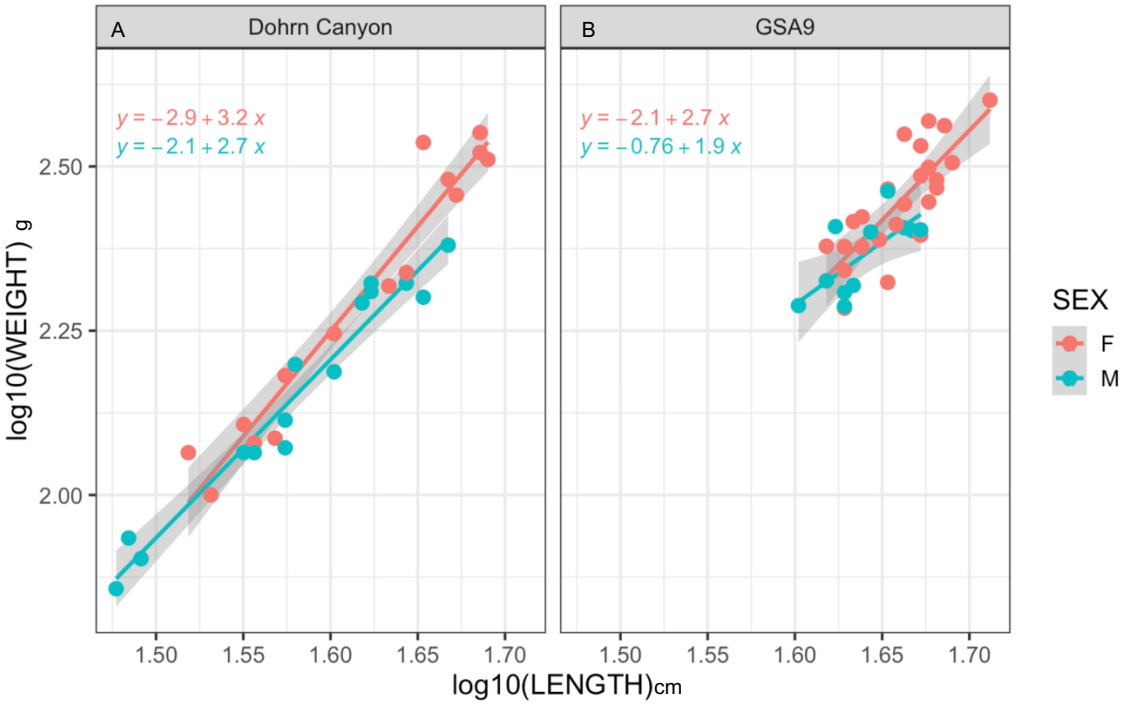

Figure 6.3 log10(Total Length in cm) - log10(Weight in g) relationships of G. melastomus of both sexes in both areas Dohrn Canyon (A) and GSA9 (B).

(A) Dohrn Canyon females (F, N=16; R^2=0.96) and males (M, N=15; R^2=0.95)

(B) GSA9 females (F, N=24; R^2=0.63) and males (M, N=14; R^2=0.53)

Stomach content analysis

For stomach content analysis all the sampled specimens in both areas were investigated and a total of 167 stomachs were analyzed to evaluate their contents. The stomachs collected from the GSA9 belong to 52 males (39.5 cm ≤ TL ≤ 49 cm) and 84 females (39 cm ≤ TL ≤ 52 cm), while those collected from the Dohrn Canyon were belong to 15 males (30 cm ≤ TL ≤ 46.5 cm) and 16 females (33 cm ≤ TL ≤ 49 cm). The analyses carried out showed that 120 specimens from the GSA9 and 26 specimens from the Dohrn Canyon had full stomachs or with traces of prey, while the remaining ones were empty or with digested organic matter. Full stomachs accounted for the 88.23% in the GSA9 and for the 83.87% for the Dohrn Canyon. In Fig. 6.4 A – B is shown the abundance (%N) of the preys and in Table 6.2 are also specified %N and frequency percentage (%F) for each class and prey. From the analyses carried out on the specimens of *G. melastomus* from the GSA9 (Fig. 6.4A and Table 6.2), 325 preys were identified, divided into 4 taxa; 21 different organic elements, like egg capsule of elasmobranch and kitchen scraps, were considered as organic matter. The results obtained show a great abundance and frequency of crustaceans (N% = 44.6%; F% = 79.2%) and cephalopods (N% = 36.3%; F% = 66.7%), followed by a fair presence of osteichthyes (N% = 14.1%; F% = 41.7%). The analyses carried out on the specimens of *G. melastomus* from Dohrn Canyon (Fig 6.4 B and Table 6.2) highlighted the presence of 63 preys, divided into 6 taxa; 5 unidentified organic elements were considered as organic matter. Cephalopods are the most predated taxa (N% = 50%; F% = 68%), followed by osteichthyes (N% = 29.4%; F% = 56%) and crustaceans (N% = 7.4%; F % = 16%); moreover, remains of *Etmopterus spinax* were found in a specimen of *G. melastomus*. During the stomach content analyses, in 8 specimens of *G. melastomus* from the GSA9 and in two specimens from the Dohrn Canyon, some fragments of plastic were found. White and transparent little pieces of plastic bags (N = 7) and green and red fragments of fiber of nets (N = 3) were found in *G. melastomus* from the GSA9, while only two transparent little pieces of plastic bags were found in *G. melastomus* from the Dohrn Canyon.

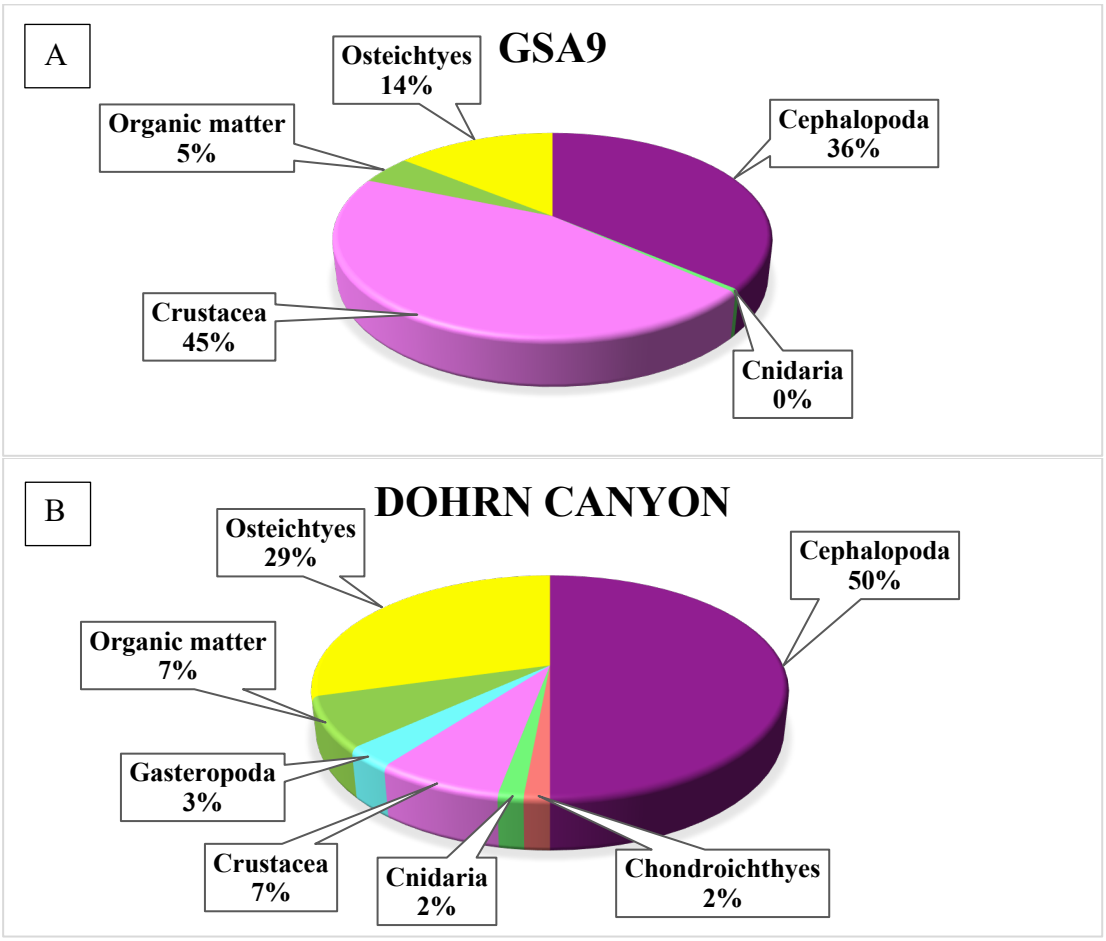

Figure 6.4 Abundance (%N) of the preys recovered in 136 stomach of G. melastomus sampled in the GSA9 (A) and in 26 stomach of G. melastomus sampled in the Dohrn Canyon (B)

Table 6.2 Diet composition of G. melastomus from the GSA9 and Dohrn Canyon. Abundance (N%) and percentage frequency (F%) are expressed for each class and prey. Numbers in bold represents the sum for the class. N.i.= not identified; Cephalopoda/Crustacea/Osteichthyes Type 1= not identified little organisms that the animal might have been ingested as whole. Cephalopoda/Crustacea/Osteichthyes Type 2=pieces of not identified big organisms that the animal might have been ingested.

	%N	%F
GSA9		
CEPHALOPODA	**36,30**	**66,67**
Abralia verany	2,17	6,67
Ancistroteuthis lichtensteinii	0,22	0,83
Eledone cirrhosa	0,22	0,83
Eledone sp.	0,22	0,83
Heteroteuthis dispar	21,52	48,33
Histioteuthis bonnellii	0,43	1,67
Histioteuthis reversa	1,09	3,33
Illex coindetii	1,30	4,17
Oegopsida n.i.	0,22	0,83
Ommastrphidae n.i.	2,17	8,33
Onycoteuthis banksii	1,09	4,17
Sepiolidae n.i.	1,09	3,33
Cephalopoda n.i. Type 1	3,26	10,00
Cephalopoda n.i. Type 2	1,30	4,17

CNIDARIA	**0,43**	**0,83**
Siphonophora n.i.	0,43	0,83
CRUSTACEA	**44,57**	**79,17**
Calocaris macandreae	5,00	15,83
Brachyura n.i.	0,65	2,50
Euphausiidae n.i.	0,43	1,67
Meganyctiphanes norvegica	4,13	4,17
Parapenaeus longirostris	0,22	0,83
Pasiphaea multidentata	0,43	1,67
Pasiphaea sivado	9,57	21,67
Pasiphaea spp.	9,35	20,00
Peneidae n.i.	1,09	4,17
Polychelidae n.i.	0,22	0,83
Crustacea n.i. Type 1	11,30	33,33
Crustacea n.i. Type 2	2,17	5,83
OSTEICHTHYES	**14,13**	**41,67**
Chlorophthalmus agassizi	0,22	0,83
Arctozenus risso	0,43	1,67
Ceratoscopelus maderensis	0,87	3,33
Chauliodus sloani	0,65	2,50
Gadiculus argenteus	0,22	0,83
Gonostoma denudatum	0,22	0,83
Hygophum benoiti	2,17	8,33
Hymenocephalus italicus	0,65	1,67
Lestidiops sphyraenopsis	0,22	0,83
Mullus sp.	0,22	0,83
Myctophidae n.i.	0,65	2,50
Myctophum punctatum	0,87	3,33
Nezumia sp.	0,22	0,83
Notoscopelus elongatus	1,09	4,17
Notoscopelus sp.	0,22	0,83
Osteichthyts n.i. Type 1	4,35	15,83
Osteichthyts n.i. Type 2	0,87	3,33
Organic matter	4,57	10,83
Jelly organisms	1,52	3,33
Shark egg	0,22	0,83
Citrus peel	0,22	0,83
Vegetables	0,65	0,83
Organic matter n.i.	1,74	5,00
Organic matter n.i.	0,22	0,83
Plastic		
Transparent sheet		
White sheet		
Green fiber		
Red fiber		

DOHRN CANYON		
CEPHALOPODA	**50,0**	**68**
Abralia verany	4,4	8
Sepiolidae n.i.	13,2	16
Heteroteuthis dispar	11,8	16
Sepiolidae n.i.	2,9	8
Cephalopoda n.i. Type 1	17,6	28
CONDRICHTHYES	**1,5**	**4**
Etmopterus spinax	1,5	4
CNIDARIA	**1,5**	**4**

Siphonophora nd	1,5	4
CRUSTACEA	**7,4**	**16**
Pasiphaeidae	2,9	8
Crustacea n.i. Type 1	4,4	8
GASTEROPODA	**2,9**	**4**
OSTEICHTHYES	**29,4**	**56**
Gonostoma denudatum	1,5	4
Myctophidae	4,4	12
Osteichthyes n.i. Type 1	23,5	44
ORGANIC MATTER	**7,4**	**20**
Organic matter n.i.	7,4	20
Plastic		
Transparent sheet		

Organochlorine compounds (OCs)

All the three OCs (HCB, PCBs, DDTs) were identified in all the biological materials analyzed (muscle, liver, stomach content). As previously said specimens from GSA9 were sampled in different hauls and since there were no statistical differences between them, they were considered all together. The number of sampled specimens (N), the extracted organic material (EOM%), and detected OC compounds (HCB, DDTs and PCBs) with their standard deviation (SD), are summarized in Table 6.3 divided by area, tissue and sex.

Table 6.3 Number of sampled specimens, Extracted Organic Material (%EOM) and concentrations (ng/g lipid weight) of HCB, PCBs and DDTs and standard deviation (SD) in G. melastomus divided by area of sampling, tissue and sex (M=male, F=female)

Area Tissue Sex	N	EOM%	HCB	SD	PCBs	SD	DDTs	SD
Dohrn Canyon								
Liver	**30**	**83.64**	**17.01**	**5.39**	**4978.74**	**4310.85**	**1124.88**	**628.07**
F	15	83.62	17.47	4.40	4608.67	2049.07	1039.15	431.15
M	15	83.65	16.56	6.20	5348.81	5717.89	1210.61	767.05
Muscle	**31**	**8.58**	**2.92**	**3.27**	**392.32**	**265.61**	**150.09**	**87.07**
F	16	8.97	2.74	2.90	328.66	167.95	133.11	75.41
M	15	8.17	3.12	3.61	460.22	326.76	168.21	94.69
Stomach Content	**pool**	**24.71**	**7.10**	**0.62**	**930.80**	**86.08**	**222.10**	**20.03**
GSA9								
Liver	**35**	**81.02**	**23.32**	**8.40**	**5433.27**	**4839.87**	**1646.83**	**1219.79**
F	24	80.09	21.77	7.01	4258.67	3181.61	1322.94	934.83
M	11	83.05	26.70	10.04	7996.02	6547.39	2353.49	1448.87
Muscle	**38**	**6.32**	**3.85**	**3.46**	**630.51**	**409.03**	**235.58**	**86.12**
F	24	6.01	4.14	4.14	551.28	297.38	239.75	96.54
M	14	6.86	3.35	1.65	766.33	522.79	228.44	63.82
Stomach Content	**pool**	**18.48**	**5.47**	**0.81**	**1283.72**	**300.90**	**474.41**	**25.85**

In general PCBs were the OCs most present in all the biological materials followed by DDTs and HCB. The tissue with the highest levels was the liver while in the muscle were registered the lowest levels for all the three OCs. Overall, the samples from the GSA9 presented higher levels except for HCB in stomach content which was higher in the Dohrn Canyon.

Nonparametric tests were conducted to investigate any differences between sexes and since no significant differences were recorded, in the following analyses sexes were considered all together.

Differences between tissues

Without considering the sampling area, differences between liver and muscle were investigated. As previously said, liver was the tissue with highest levels for HCB, PCBs and DDTs. Kruskal-Wallis test highlighted a statistically significant difference between all the three OCs ($p<0.0001$).

In terms of DDT isomers pp'DDE was the most abundant in both tissues with slight differences in pattern accumulation: specifically, in the liver was

op'DDE<op'DDT<pp'DDD<pp'DDT<op'DDD<pp'DDE

while in the muscle was

op'DDE<pp'DDD<op'DDD<pp'DDT<op'DDT<pp'DDE.

On the contrary the distribution of chlorinated biphenyls classes (penta-, hexa-, hepta-, octa- and nona-CBs) was the same in both tissues as is shown in Fig. 6.5.

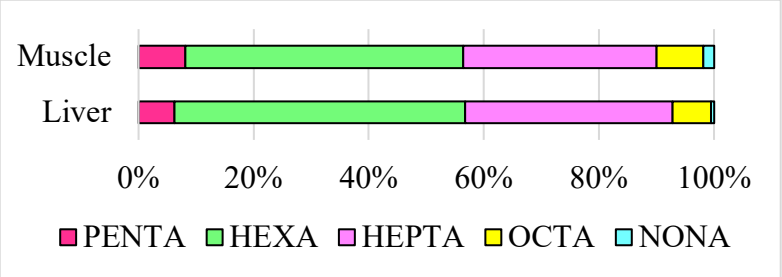

Figure 6.5 Percentage composition of PCBs divided by chlorine content (penta-CBs, hexa-CBs, hepta-CBs, octa-CBs, nona-CBs) on ∑PCBs, analysed in G. melastomus muscle (n=69) and liver (n=65) in GSA9 and Dohrn Canyon

Differences between areas

Muscle and liver from specimens collected in the GSA9 had higher levels compared to those sampled in the Canyon. Considering the area as the independent variable, statistically significant differences were detected in the muscle for all the three OCs (HCB $p<0.001$; PCBs $p<0.001$; DDTs $p<0.0001$) while for the liver only HCB resulted statistically significant ($p<0.001$). Differences in muscle tissue sampled from male specimens between the two areas were significant only for HCB ($p<0.025$) and DDTs

(p<0.005) while for females were statistically significant for all the three pollutants (HCB, DDTs p<0.025; PCBs p<0.05). Since the differences between the areas resulted statistically significant in muscle, this tissue was considered for making comparisons.

In terms of DDT isomers pp'DDE was the most abundant in both areas and the accumulation pattern was the same:

op'DDE<pp'DDD<op'DDD<pp'DDT <op'DDT <pp'DDE.

Also, the PCB congeners composition was similar in the two areas with the predominance of hexa- and hepta-CBs (Fig 6.6)

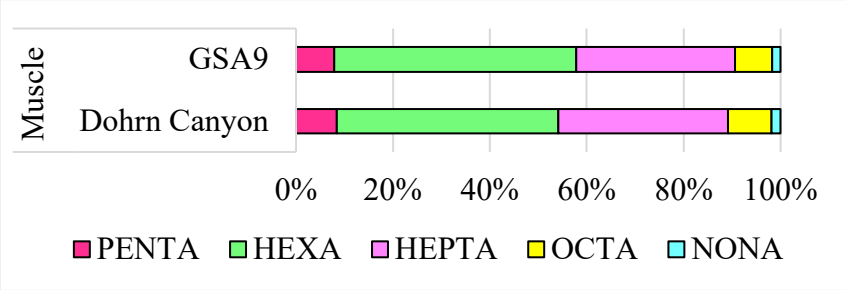

Figure 6.6 Percentage composition of PCBs divided by chlorine content (penta-CBs, hexa-CBs, hepta-CBs, octa-CBs, nona-CBs) on ∑PCBs, analysed in G. melastomus muscle in GSA9 (n=38) and Dohrn Canyon (n=31)

The ratios between OCs (DDTs/PCBs) and DDT isomers (ppDDE/ppDDT, ppDDE/DDTs, ΣopDDTs (opDDT+opDDE+opDDD)/DDTs) and their standard deviation (SD) are summarized in Table 5.3

Table 6.4 Contaminant ratios (DDTs/PCBs, pp'DDE/pp'DDT, pp'DDE/DDTs, ∑op'DDTs/∑DDTs) in G. melastomus muscle sampled in the Dohrn Canyon (n=31) and in the GSA9 (n=38)

	DDTs / PCBs	SD	ppDDE / ppDDT	SD	ppDDE / DDTs	SD	opDDTs / DDTs	SD
Dohrn Canyon	0.40	0.12	3.96	4.48	0.44	0.12	0.37	0.11
GSA9	0.45	0.16	4.73	2.31	0.56	0.09	0.28	0.07

Kruskal-Wallis evidenced statistically significant differences in all the isomer ratios (pp'DDE/pp'DDT p<0.01; pp'DDE/DDTs p<0.001; ∑op'DDTs/∑DDTs p<0.01) between the areas.

Organochlorine determination in the stomach content

The stomach contents from the specimens sampled in the GSA9 were pooled all together as well as those sampled from the Dohrn Canyon. All the three OCs were identified in both pooled samples with the predominance of PCBs followed by DDTs and lastly HCB. PCBs and DDTs were higher in the stomach contents from the GSA9 while HCB levels

were higher in sample from the Dohrn Canyon. Statistically significant differences were only found in DDT levels ($p<0.05$) according to Kruskal-Wallis test.

Discussion

Stomach content analysis

The analysis carried out on the two groups of *G. melastomus* showed mainly the presence of crustaceans, cephalopods and osteichthytes, although in different ratio. The results obtained from the GSA9 specimens reflect those available in the bibliography both for the sampled area (Sartor, 1993; Bulgheri et al., 2008) and for other areas of the Mediterranean Sea (Relini Orsi & Wurtz, 1975; Olaso et al., 2005; Fanelli et al., 2009; Valls et al., 2011; Anastasopoulou et al., 2013; Ait Darna et al., 2018; D'Iglio et al., 2021a).

The greater presence of cephalopods and osteichthyes compared to crustaceans in *G. melastomus* of Dohrn Canyon could be due to the limited availability of samples, even if in this species the proportions of the prey may be different as the season changes (Preciado et al., 2009; Anastasopoulou et al., 2013; Barría et al., 2018), but depends mainly on food availability.

The finding of kitchen scraps in the stomachs analysed confirms that the blackmouth catshark can be considered a generalist opportunistic predator that can adapt its diet to the prey available in different marine environments (D'Iglio et al., 2021b) and sometimes shows scavenger behavior (Bulgheri et al., 2008; D'Iglio et al., 2021a). The presence of other cartilaginous fishes in the stomach contents of *G. melastomus* has already been highlighted by other authors (Bulgheri et al., 2008; Fanelli et al., 2009) as well as the presence of plastic debris (Alomar et al., 2017; Anastasopoulou et al., 2013a,b; Valente et al., 2019).

Organochlorine compounds

Organochlorine compounds are mainly found in tissues with high lipid percentage due to their lipophilic characteristics (Guitart et al., 1996). The extracted organic material (EOM%), which is the lipid content, in the liver was more than 80% compared to less than 10% in the muscle; this will explain the higher concentration of all the three OCs in this fatty tissue.

The black mouth catshark is extensively studied for its feeding habits (D'Iglio et al., 2021b) or its distribution; but scarce information is available regarding pollutants in its tissues. To date, contaminants in *G. melastomus* have never been investigated neither in the GSA nor in the Dohrn Canyon.

In literature there are only two studies available in which PCBs were detected: Storelli et al., (2003) determined the concentrations of 17 PCB congeners in livers of specimens sampled in Adriatic, Ionian and Aegean Sea; Cresson et al., (2016) measured 7 PCB congeners in the muscle of specimens sampled in the Gulf of Lion. Comparing our results with the ones in these two studies, the levels measured in both tissues in the GSA9 and in the Dohrn Canyon were higher than the other areas of the Mediterranean Sea. This is probably due to the presence of densely populated coastlines, the presence of various maritime commercial and touristic ports such as Genoa, Leghorn, Civitavecchia, and Naples but also watercourse discharges from main rivers such as Arno, Tevere, and Sarno. In general, the contamination pattern (HCB<DDTs<PCBs) in our samples reflects prior findings in other species in the same area (Fossi et al., 2007) and also the predominance of highly chlorinated biphenyl congeners in both tissues was consistent with other studies on *G. melastomus* (Storelli et al., 2003; Cresson et al., 2016).

Muscle tissue as indicator of chronic exposure

As suggested by Albaigés et al. (1987) pollutant levels in liver could reflect an acute pollutant input while those in the muscle a chronic one. The liver, indeed, is the major metabolic organ in the body, responsible of the temporary storage, metabolism, and excretion of toxic substances (Henry, 2015). The statistically significant differences highlighted in our study between the two areas for all the OC compounds in muscle tissue and for DDT isomers ratios could confirm the hypothesis that this tissue could be used as indicator for chronic exposure in sharks.

From the DDT isomers ratios, we can assume that there haven't been very recent inputs since the ppDDE/ppDDT is above the level in the commercial mixture (GSA9: 4.73; Dohrn Canyon: 3.96; DDT commercial mixture: 0.05) and high values of this ratio indicate an historical contamination (Aguilar, 1984). Comparing them to others obtained in other areas in the Mediterranean Sea (Corsolini et al., 2008; Storelli et al., 2008; Klinčić et al., 2020) our results are quite low, suggesting a DDT input not very far back in time. Interestingly, though, are the findings from pp'DDE/DDTs and ∑op'DDTs/DDTs ((op'DDT+op'DDE+op'DDD)/DDTs) which denote a possible recent input of pesticides containing DDT or the use of a DDT mixture enriched with op' isomers. Regarding the first one, is below the threshold limit (0.6) proposed by Aguilar (1984), indicating a fresh DDT exposure and the second one, higher than 0.20 in both areas indicating a possible contamination by technical DDT or pesticides such as dicofol (Qiu et al., 2005). In a recent review Thiombane et al. (2018) highlighted in central southern Italy a clear

dominance of historical usage of DDT but also a more recent illicit use of technical DDT or dicofol. Higher levels of this ratio in the samples from the Dohrn Canyon could be explained to the proximity of the sampling site to the coastline, to the influence of the run-off by the near Sarno river (Montuori et al., 2014) and the peculiar marine currents which are characteristic of submarine canyons (Shanmugam, 2008) able to act a sink for pollutants (Froescheis et al, 2000). However, to better assess this factor, it is fundamental to increase the sample effort in both areas, especially where rivers flow into the sea.

The differences in DDT levels between the two sampled areas were also detected in the stomach content pools, strengthening the assumption of a higher contamination input in the GSA9. Although these values reflect a "snapshot" of the contaminants' amount absorbed through the diet, this aspect need to be further investigated with continuous samplings throughout the year both in *G. melastomus* and its preys, since this shark adapt its diet to seasonal and geographical fluctuation of its predated species (Preciado et al., 2009; Anastasopoulou et al., 2013; Barría et al., 2018; D'Iglio et al., 2021b).

G. melastomus has already been proposed as a potential bioindicator for microplastic pollution (Scacco et al., 2022) and with the results obtained in this study could also be considered a valid candidate for chemical pollution.

Our findings also suggest that specimens sampled in the Dohrn Canyon are less subjected to contamination by the OCs investigated compared to those sampled in the GSA9. This latter result, could be also explained as this deep sea habitat is characterized by strong currents which can also influence the primary productivity, increasing the amount and the variety of food resources available to marine predators (Canals et al., 2019). Moreover, the occurrence of local hydrodynamic forcing in the canyon Dohrn (Gravili et al., 2001; Cianelli et al., 2012) may favor the dispersion and dilution of contaminants in the sea water (Piazzolla et al., 2020; Zhang et al., 2021), reducing the pollutant accumulation in the marine organisms inhabiting the seabed of the canyon.

Here as well, to better understand the presence and the influence of pollutants in deep sea organisms, it is necessary to extend this type of research to other mutual species between the two areas, remarking the importance of pollution threat assessment in this biota yet stressed by other multiple factors.

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Chapter 7 ***FINAL CONSIDERATIONS***

Pollution, especially in cartilaginous fishes, is an underestimated threat especially for those inhabiting areas where the anthropic pressure gets stronger. It is also underrated in terms of legislation in establishing conservation measures for the species.

To fill this knowledge gap it is necessary to commit resources at global level and it certainly requires time. By this PhD project I have started to fill this gap, providing the first results on the contaminant levels in some deep sea megafaunal species in two different areas not still investigated from the toxicological point of view: the focus was on the Ligurian and North Tyrrhenian Sea (GSA9) and the Dohrn Canyon.

The main findings throughout this Thesis' chapters can be summarized as follows:

- Information available on contamination status in deep sea species, in particular bony and cartilaginous fishes are scarce, both in the Mediterranean Sea and in the rest of the world;
- Legacy contaminants such as HCB, DDTs and PCBs are still detected despite current legislation;
- The maternal transfer of all the three OCs in the three chondrichthyan species sampled in the GSA9 has been detected, highlighting a possible stress linked to the toxicological properties of these xenobiotics (carcinogenesis, genotoxicity, immunosuppression, endocrine disrupting capacities, etc.) which may pose an additional risk to their conservation, since these species are characterized by long reproductive cycles and low resilience;
- The estrogenic, androgenic, anti-estrogenic, and anti-androgenic compounds percentage was more than a half on the total contaminant burden in the species sampled in the GSA9, highlighting how the threat caused by endocrine disruptor chemicals cannot be neglected at all;
- In the Dohrn Canyon the species are more subjected to an industrial-type of contamination (from PCBs) than an agricultural one (from DDTs)
- In the specimens sampled in the Dohrn Canyon, from the DDT isomers analysis, resulted the recent use of the industrial DDT, an enriched *op'* isomers formula, which is still unregulated or is used to produce other pesticides;
- The mutual species between the two areas, the black mouth catshark (*Galeus melastomus*), showed higher levels for all the three OCs in the GSA9 compared to those in the Dohrn Canyon, both in the liver and the muscle. The differences

were statistically significant only for the muscle tissue, which is considered a good indicator for contaminant's chronic exposure.

This is the first assessment of the occurrence of organochlorine contaminants in the deep environments of the Tyrrhenian Sea, one of the most anthropized area of the Mediterranean basin and it clearly highlights the negative impact on these compounds, despite most of them are banned from a long time.

It stresses once again the urgency of further focused long term researches, mixing different data from different sources, in order to monitor and better understand the future trends of this impact in the marine environments.

Moreover, this study underlines the importance of the role of the marine scientists at the international political level, in order to request further conservation measures for marine species, also for those living in deep sea environments.