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Bioaccumulation of legacy and emerging contaminants in tuna species

Tesi di laurea in

PREVENZIONE E CONTROLLO
DELL'IMPATTO AMBIENTALE

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"Il mare non ha paese nemmeno lui,

ed è di tutti quelli che lo sanno ascoltare."

(Giovanni Verga)

ABSTRACT

Environmental contamination of legacy contaminants has been of great concern worldwide because of their persistence and toxicity to humans and marine species. Only in the last decade, the presence and accumulation of emerging contaminants, and, consequently, their adverse effects in marine biota have been considered. The present research was performed to compare the different distribution of legacy contaminants (Pesticides, PCBs and PAHs) and emerging contaminants (UV filters and Fragrances) in the liver and muscle tissues of three tuna species living in different locations and with different behaviour and feed habits: Sarda sarda, Katsuwonus pelamis from the Atlantic Ocean (Gulf of Càdiz) and Thunnus thynnus from the Strait of Gibraltar. The extraction and quantitative determination of these contaminants from liver and muscle samples have been carried out by Accelerate Solvent Extraction and GC-MS/MS techniques, respectively. From the statistical analysis, significant differences have been found for Fragrances, Pesticides, PCBs and PAHs both among the three tuna species and between the two tissues. Post-hoc analysis showed significant differences between the three tuna species and the tissues, revealing that K. pelamis species differs significantly from the other two species, both in liver and muscle, and that legacy contaminants are responsible for significant differences between and within species. We also explored the similarities between concentrations of PCBs and DDTs in liver and muscle tissue found in samples of T. thynnus and K. pelamis in this study, compared to concentrations of PCBs and DDTs found in specimens of tuna collected in different geographical areas. As a result of the continuous exposure of the marine top predators, such as tuna species, to variable concentrations of emerging and legacy contaminants, their concentration in the marine environment must be constantly monitored.

Table of contents

1. INTRODUCTION

1.1 Preface

The oceans cover three quarters of the earth's surface, contain 97% of the water present on Earth and represent 99% of the space, in terms of volume, occupied on the planet by living organisms.

In recent years, environmental concerns have shifted to the marine environment since the ocean is the final destination for most contaminants produced and spilled by humans.

In particular, among the human activities that produce contaminants we can find: households, industry and agriculture, use of fertilizers and pesticides and wastewater discharge. These activities result in the formation of many pollutants such as Persistent Organic Pollutants (POPs) and Emerging Contaminants (ECs) that represent a threat to water quality and, since being highly persistent and lipophilic, they are also liable to bioaccumulate in fats of species throughout the food chain.

Tuna and tuna-like species are not only of ecological relevance but also of economic importance, as they are species of commercial interest. Economically, they represent a significant source of food, with the so-called principal market tuna species as the most significant in terms of catch weight and trade: Skipjack - SKJ (*Katsuwonus pelamis*), Yellowfin – YFT (*Thunnus albacares*), Bigeye – BET (*Thunnus obesus*), Albacore – ALB (*Thunnus alalunga*), Atlantic bonito – BON (*Sarda sarda*), Bluefin – BFT (*Thunnus thynnus*), including Atlantic bluefin and Pacific bluefin referred as northern species, and southern bluefin tuna.

Several studies (Chiesa et al. 2016; Munschy et al. 2016; Deshpande et al. 2016; Sprague et al. 2012; Dickhut et al. 2009; Storelli et al. 2008) demonstrated that tuna species can record high concentrations of these contaminants in their tissues due to biomagnification and to the high position tuna occupy in the trophic chain.

As a result, this is a reason why many studies have been carried out to assess the state of health of these species and of the habitat in which they live.

1.2 International Conventions and European Regulations

On May 22, 2001 in Stockholm was adopted the Stockholm Convention on Persistent Organic Pollutants (POPs), a global treaty to protect human health and the environment from chemicals.

Considering their long-range transport, no one government acting alone can protect its citizens or its environment from POPs. So, the Stockholm Convention requires its parties to take measures to protect the environment, minimizing the risks through measures to reduce and/or eliminate their emissions or discharges (Halse et al., 2011).

The initial list of POPs contained 12 substances including PCBs, DDT, dioxins and other pesticides, subject to elimination or restriction of use. In 1972, EPA issued a cancellation order for DDT based on its adverse anthropogenic and environmental effects, such as those to wildlife, as well as its potential marine life risks.

Already in 1975, the Barcelona Convention involved 16 Mediterranean countries and the European Community in order to develop the Mediterranean Action Plan (MAP), the first-ever Regional Seas Plan under the control of the United Nations Environment Plan (UNEP) aimed at the protection of the marine environment through a regional approach, and the Mediterranean Marine Pollution Monitoring and Research Plan (MED POL) as a scientific and technical component. The aim of MED POL is the gathering of information about sources, environmental concentrations and effects of pollutants in the region (Nadal, Marquès, Mari, & Domingo, 2015).

Afterwards, in 1995, the Sustainable Development of the Coastal Areas of the Mediterranean (MAP Phase II) was adopted by the Contracting Parties to replace the Mediterranean Action Plan of 1975. The Contracting Parties are now 22, including Spain.

The implementation of the Ecosystem Approach of the MAP system represented an important development tool to support regional and national efforts towards achieving Good Environmental Status (GES) in the Mediterranean, in synergy with relevant global and regional initiatives including the Water Framework Directive (2000/60/EC) and the European Union Marine Strategy Directive (2008/56/EC).

Recently, on a global scale, Agenda 2030 was promoted (Fig. 1). This is an action plan that, for the first time, takes balanced account of the three dimensions of sustainable

development: economic, social and ecological. It was signed in September 2015 by the governments of the 193 members countries of the Organization of United Nations, but it started in the early 2016. It includes 17 Sustainable Development Goals (SDGs) and 169 associated targets. The Countries that have taken part in this plan have the objective of achieving these goals by 2030. Specifically, objective 14 provides for the conservation and sustainable use of oceans, seas and marine resources for sustainable development. In particular, with regard to this study, attention should be paid to the purpose of certain targets:

- **14.1** By 2025, significantly prevent and reduce all forms of marine pollution, particularly from land-based activities, including pollution of marine debris and nutrients.
- **14.2** By 2020, managing and protecting the marine and coastal ecosystem in a sustainable manner to avoid particularly negative impacts, including by enhancing their resilience, and restoring them in order to achieve healthy and productive oceans
- **14.5** By 2020, preserve at least 10% of coastal and marine areas, in accordance with national and international law and based on the most accurate scientific information available.

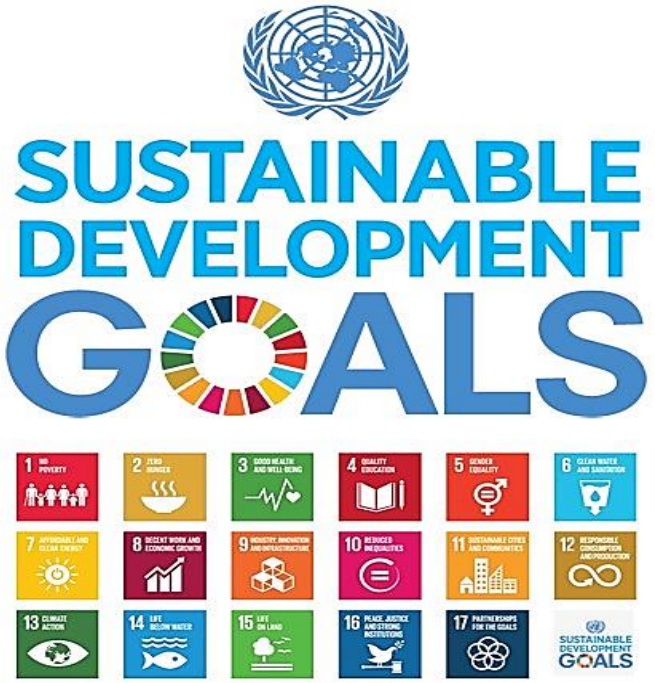


Fig. 1 – Agenda 2030 Sustainable Development Goals (From: https://www.un.org)

Only in the last decade has the problem of pollution caused by emerging contaminants and their toxic effects on organisms emerged. For this reason, regulations on this subject are sometimes absent or still under development, even if Europe has taken good precautions in this regard over the last decade and is developing new methodologies.

Within the Water Framework Directive (WFD) – Directive 2000/60/EC, the European Union introduces a new legislative approach to managing and protecting water, based not on national or political boundaries but on natural geographical and hydrological formations: river basins. It also requires coordination of different EU policies, and sets out a precise timetable for action, with 2015 as the target date for getting all European waters into good ecological and chemical status. Specifically, to define *good chemical status*, environmental quality standards have been established for 33 new and eight previously regulated chemical pollutants of high concern across the EU.

Continuing in the European context, the need to use new methods and new regulations to solve the problems affecting water quality, has led to the European Parliament and the Council of the European Union on June 17, 2008 to issue the Framework Directive 2008/56/EC on Marine Strategy, subsequently implemented in Italy with Legislative Decree no. 190 of 13 October 2010.

The Directive sets the objective for Member States to achieve Good Environmental Status (GES) for their marine waters by 2020. *Good environmental status of marine waters* means the ability to preserve ecological diversity and the vitality of the seas and oceans so that they are clean, healthy and productive while maintaining the use of the marine environment at a sustainable level and safeguarding the potential for the uses and activities of present and future generations. To enable Member States to achieve their objectives, the Directive has developed 11 descriptors describing the ecosystem once good environmental status has been achieved (Fig. 2). With regard to this study, of the 11 descriptors, the interest falls on descriptor 8 and descriptor 9, respectively designed to verify that concentrations of contaminants do not generate levels that give rise to polluting effects and that contaminants in fish and other fishery products at sea destined for human consumption do not exceed the levels laid down in Community legislation or other relevant legislation.

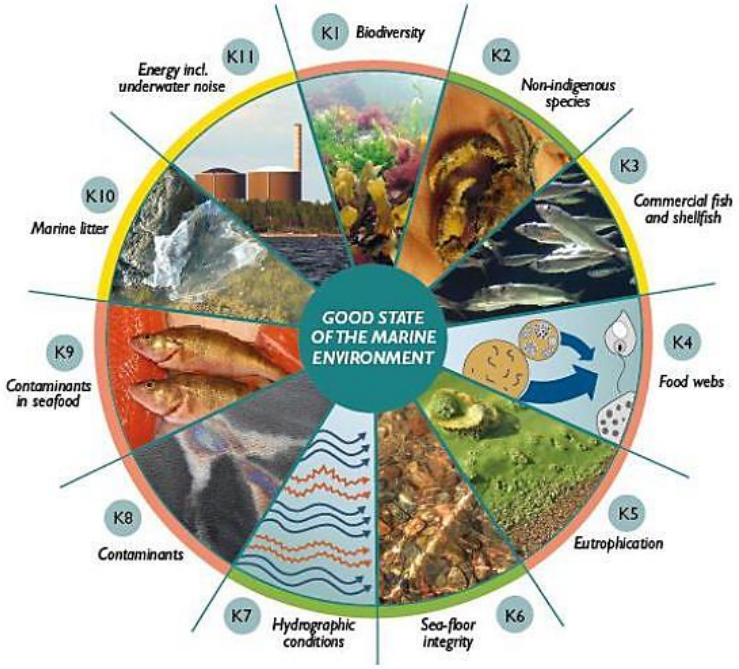


Fig. 2 – Water Framework Directive in Marine Strategy Descriptors (From: www.strategiamarina.isprambiente.it/)

Regarding emerging contaminants legislation, the Directive 2009/1223/EC of the European Parliament and of the Council of 30 November 2009 defines a list of substances, including UV filters, authorized on cosmetic products within a maximum concentration. The UV filters Homosalate, Benzophenone-3, Octocrylene, EHMC and 4-MBC are all part of the list.

In 2008, was introduced the Environmental Quality Standards Directive (EQS) – Directive 2008/105/EC - for priority substances and certain other pollutants as provided for in Article 16 of the Water Framework Directive, with the aim of achieving good surface water chemical status.

Originally, only for 33 priority substances and certain other pollutants within the Annex X of the WFD were calculated the maximum allowable concentration values. Thereafter, on 12 August 2013, within the Directive 2013/39/CE, the list of priority substances, in accordance with Directive 2000/60/EC (WFD) (art. 16) and Directive 2008/105/EC (EQS) (art. 8), was reviewed (reaching the number of 45 substances) and provided for the modification of the EQS for newly identified substances and for certain existing substances in line with new scientific knowledge, including EQS for biota.

In order to identify substances and to include them in the list of priority substances, has been developed, in accordance with the Directive 2008/105/EC, a new mechanism to provide reliable information on the monitoring of substances that have the potential to affect the aquatic environment. This new mechanism, called the “Watch List”, aims to provide support for the prioritisation for emerging substances in line with Article 16 (2) Directive 2000/60/EC and is based on the monitoring of emerging substances, throughout the European territory, at least for a period of 4 years and on a limited number of significant stations. The list of the substances to be monitored is updated every two years and the substances that are not found shall be deleted by the Commission.

Recently, legislative Decree No. 172 of October 13, 2015 implemented Directive 2013/39/EU, which provides for in Article 8, paragraph 1, the establishment of the monitoring of the substances on the Watch List. In this context, the UV filter 2-ethyl-hexyl-4-trimethoxycinnamate (EHMC) was included in the monitoring list as a priority pollutant in surface water.

1.3 Persistent Organic Pollutants (POPs)

In the last decades, even if extensive measures to reduce or eliminate emissions of such substances have already been taken, persistent, bioaccumulative and toxic substances (PBTs) and other substances that behave like PBTs, can be found in the aquatic environment at levels posing a significant risk. For some of those substances there is evidence of long-range transport and long-term ubiquity in the aquatic environment (Friedman & Selin, 2016). So, integrated with chemical monitoring, evaluation of the biological effects in fish and other marine species of these contaminants has also been a major activity in Europe.

Over the years, through directives and conventions, the historic spotlight of concern has focused on certain priority metals and organic chemical contaminants (e.g. cadmium, mercury and organic mercury compounds, polycyclic aromatic hydrocarbons [PAHs], polychlorinated biphenyls [PCBs], etc.). In Europe, these PBT chemicals are often referred to as ‘legacy contaminants’ in the contexts of the Marine Strategy Framework Directive and the Water Framework Directive (Hutchinson, Lyons, Thain, & Law, 2013).

The group of persistent organic pollutants (POPs) includes the polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and other organochlorates like lindane, DDT, aldrin, etc. Main features of these contaminants are their great persistence and hydrophobicity that makes them easily available throughout the food chain, as well as by their toxicity and mutagenic and carcinogenic activity (Pintado-Herrera et al. 2017; Lara-Martín et al. 2005).

The marine ecosystem is particularly exposed to pollution being at the lowest altitude and so acting as an ‘‘end-point’’ of any type of pollutant created on land. The low water solutbility, high lipophilicity and stability of POPs allow these compounds to bioaccumulate and biomagnify along food chains. For example, they tend to retention in the adipose tissues of fish, where they remain apparently without effect if not mobilized. Subsequently, POPs tend to migrate worldwide and can be detected in organisms remote in time and geographical area from the point of exposure. So, fish is a suitable indicator for the environmental pollution monitoring, because fish can bioaccumulate contaminant introduced with their food or bioconcentrate chemicals directly from water via diffusion across the gills and skin (Masci, Orban, and Nevigato 2014; Kalyoncu, Agca, and Aktumsek 2009).

Moreover, these chemicals or their metabolites have a strong persistence in ecosystems, including aquatic organisms and, more specifically, in marine top predators such as marine mammals, seabirds or large pelagic fish (Bogdal et al., 2013). So, the highest concentrations of POPs are found in organisms at the top of the food chain (Fig. 3).

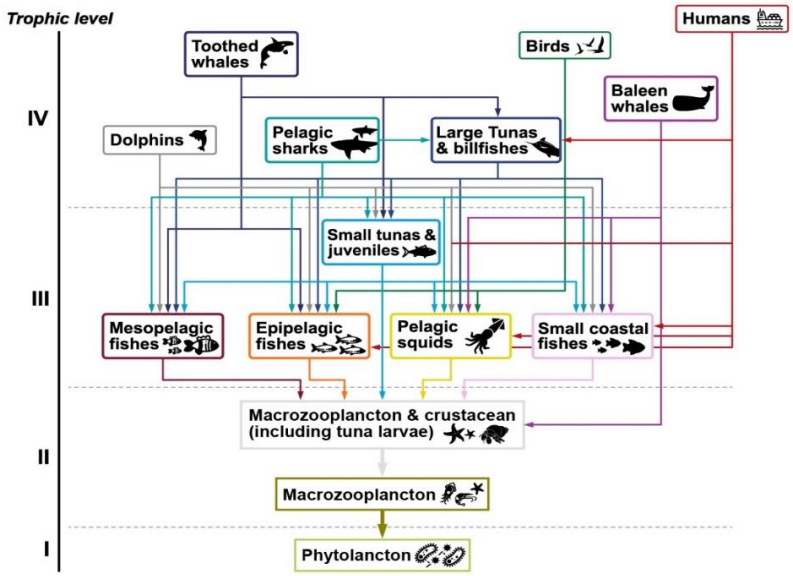


Fig. 3 - Position of tunas and tuna-like species in the marine food web (From: www.fao.org)

1.3.1 Polychlorinated biphenyls (PCBs)

PCBs belong to a broad family of man-made organic chemicals known as chlorinated hydrocarbons, consisting of carbon, hydrogen and chlorine atoms. In 1935, Monsanto Chemical Company (now Solutia Inc) took over commercial production of PCBs from Swann Chemical Company which had begun in 1929. Through the 1960s Monsanto Chemical Company knew increasingly more about PCBs' harmful effects on humans and the environment. PCB manufacture and use continued with few restraints until the 1970s.

Due to their non-flammability, chemical stability, high boiling point and electrical insulating properties, PCBs were used in hundreds of industrial and commercial applications including electrical, heat transfer and hydraulic equipment; plasticizers in paints, plastics and rubber products; pigments, dyes and coats and carbonless copy paper.

Another important source of PCBs in developing countries might be ship-dismantling activities (also referred to as ship breaking or ship wreckage). Old ships containing PCBs in paints, transformer and capacitor oils and sealants, are regularly exported to developing countries or countries with economy in transition, where they are dismantled under primitive conditions (Bogdal et al., 2013) .

E-waste handling (waste of electrical and electronic equipment) and ship-breaking activities in developing countries have been identified as major environmental issues, not only because these practices result in potential environmental emissions, but also because these emissions are further enhanced by climatic conditions. The warm conditions prevailing in tropical regions increase volatilization rates of semivolatile chemicals, because the vapor pressure increases exponentially with temperature (Halse et al., 2011).

Today, PCBs can still be released into the environment from: poorly maintained hazardous waste sites that contain PCBs; illegal or improper dumping of PCB wastes; leaks or releases from electrical transformers containing PCBs; disposal of PCB-containing consumer products into municipal or other landfills not designed to handle hazardous waste; burning some wastes in municipal and industrial incinirators. Once released into the environment they remain for long periods cycling between air, water and soil. In fact, PCBs can travel long distances and have been found in snow and sea water in areas far from where they were released into the environment.

In Artic charr (*Savellinus alpinus*) caught in Lake Ellasjøen, Bjørnøya (situated in the western part of the Barents Sea, at 74° N) were found high levels of PCBs (up to 5 µg/g muscle). Simultaneously, high PCB levels were associated with various adverse effect on physiological activities (i.e., reproductive, endocrine, immunological, behavioral) in several species in the Arctic, such as Northern fur seals (*Callorhinus ursinus*), polar bears, and glaucous gulls (*Larus hyperboreus*) (Jørgensen et al., 2006).As a consequence, they are considered ubiquitous compounds. Generally, the lighter the form of PCB, the further it can be transported from the source of contamination (Friedman & Selin, 2016).

Adverse health effects on marine organisms such as effects on the immune system, reproductive system, nervous system, endocrine system, have been also demonstrated. Durante et al. (2016) found accumulation of PCBs in subcutaneous adipose tissue of common dolphins and Fraser's dolphins. (Perugini et al., 2004) observed a change in the concentration in different organisms, with the contribution of highly-chlorinated PCB congeners increasing, moving up the food chain. Voorspoels et al. (2004), analysing three species of benthic invertebrate organisms, proved that prolonged exposure to these pollutants can interfere with normal physiology and biochemistry.

Tab. 1 - PCBs physico-chemical properties.

PCBs					
Compound	**PCB 52**	**PCB 101**	**PCB 138**	**PCB 153**	**PCB 180**
Chemical formula	$C_{12}H6Cl4$	$C_{12}H_5Cl_5$	$C_{12}H_4Cl_6$	$C_{12}H_4Cl_6$	$C_{12}H_3Cl_7$
Molecular weight (g/mol)	291.98	326.42	360.9	360.88	395.32
Boiling point (at P=1 atm)	-	-	400 °C	-	240-280 °C
Henry's Law constant (atm m^3/mol)	-	-	$0.21\text{-}1.07*10^{-4}$	$1.31\text{-}2.78*10^{-4}$	$1.07*10^{-4}$
Vapor pressure (mmHg at 25 °C)	-	-	$4*10^{-6}$	$3.8*10^{-7}$ - $9*10^{-7}$	-
Solubility in water at 25 °C (mg/L)	-	-	0.0159	0.00086-0.00091	0.0031-0.00656
Log Kow	6.1	6.5	6.5-7.44	8.35-6.72	6.70-7.21

1.3.2 Pesticides and herbicides

There are many different classes of chemical pesticides and herbicides based on chemical composition and on the type of pest they control.

Organochlorine pesticides (OCPs), such as dichlorodiphenyltrichloroethanes (DDTs), hexachlorocyclohexanes (HCHs), and chlordanes (CHLs), are typical persistent organic pollutants of great concern. The production and use of these OCPs have been prohibited in most parts of the world due to their potential acute and chronic health effects (e.g., cancer, disruption of the developmental and endocrine systems, and neurological damage) on non-target organisms, including humans ((Zhou et al., 2018). As a result, concentrations of OCPs in the abiotic and biotic matrices initially decreased but have begun to show signs of leveling off or even increasing recently in marginal sea areas because of land–sea migration (Zhou et al., 2014).

p,p' -DDT (1,1 -[2,2,2-trichloroethylidine]-bis [4-chloro- benzene]) commonly known as DDT (dichloro-diphenyl-trichloroethane) is a chlorinated compound widely used as pesticide, and its metabolites *p,p'* -DDD (1,1-Dichloro-2,2-bis [P-chlorophenyl] ethane) and *p,p'* -DDE (1,1-Dichloro-2,2-bis [p-chlorophenyl] ethylene) are the result of degradation in the environment or in the organisms that metabolize it into these other forms (Tab. 2).

DDT was developed as the first of the modern synthetic insecticides in the 1940s. It was initially used with great effect to combat malaria, typhus, and the other insect-borne human diseases among both military and civilian populations. It also was effective for insect control in crop and livestock production, institutions, homes, and gardens.

The U.S. Department of Agriculture began regulatory actions in the late 1950s and 1960s to prohibit many of DDT's uses because of mounting evidence of the pesticide's declining benefits and environmental and toxicological effects. In 1972, EPA (Environmental Protection Agency) issued a cancellation order for DDT based on its adverse environmental effects, such as those to wildlife, as well as its potential human health risks, but it was produced until middle 1990s in Europe and is still in use for some purposes in some countries of the Mediterranean region (UNEP, 2002). As a result, today, DDT is classified as a probable human carcinogen by the International Agency for Research on Cancer (IARC).

Triazines belong to the class of herbicides. They were first developed in the early 1950s, and all triazines are synthetic. These compounds are known to have potential use as insect chemosterilants. Higher concentrations of these herbicides were found to inhibit plant catabolism pathway, interfering with the electron transport system of photosynthesis process (Jayaraj, Megha, & Sreedev, 2016).

Water often serves as the sink for these chemicals after their application in the different fields. Because of their ubiquitous presence in the environment, non-target species, such as aquatic biota, can be affected. Anthropogenic activities that potentially affect fish lead to changes in their habitats. The responses of fish to such environmental challenges are ultimately reflected as overall alteration in metabolism, such us interfere with the endocrine system (Fatima et al., 2007).

Ametryn is a triazine herbicide and was designed for being phytotoxic to photosystem II in plants. This herbicide is widely used in fields of many crops such as maize, pineapple and sugarcane for many decades. However, overuse of triazine herbicides (such as ametryn and atrazine) not only contaminates soils and waters but potentially affects crop production and food safety as well (Y. Liu et al., 2017).

The environmental fate of ametryn varies based on the site-specific properties of the soil to which it is applied. Ametryn is stable to hydrolysis, degrades slowly by aquatic photolysis and is persistent, so it may leach as a result of high rainfall, floods, and furrow irrigation. Given its persistence and mobility, transport of ametryn to ground water and surface water is expected (Koutnik, Stara, & Velisek, 2015).

Residues of a mixture of pesticides in Great Barrier Reef rivers and creeks during flood events were found. In particular, ametryn was one of those herbicides detected frequently and in relatively high concentrations (Lewis et al., 2009). Furthermore, results from short-term exposure experiments indicates that the herbicide levels from flood-affected coastal waters of the GBR lagoon would negatively affect some marine organisms, at least temporarily (Lewis et al., 2012); Lewis et al. 2009).

Recently, León et al. (2018) conducted a study on plastic debris demonstrating that plastics act as passive samplers in the environment, accumulating hydrophobic organic contaminants which are partially transferred to the marine system. Triazines are included in the category of substances which can be desorbed from plastics to seawater in the first 24h, including ametryn from different polymers.

Although the lethal toxicity of ametryn to fish have been demonstrated, there is a lack of data regarding the effects caused on fish physiology. In Tesolin et al. (2015) an acute exposure of ametryn inhibited cholinesterase activity in juvenile and adult zebrafish. Botelho et al. (2013), showed that the mixture of atrazine and ametryn caused micronuclei formation and erythrocytic nuclear abnormalities in zebrafish.

On May 2006, the Commission regulation (EC) No 777/2006 amending Annex I to Regulation (EC) No 304/2003 of the European Parliament and of the Council concerning the export and import of dangerous chemicals introduced Ametryn as banned in Europe.

Tab. 2 - Pesticides and herbicides physico-chemical properties.

PESTICIDES AND HERBICIDES				
Compound	**p.p´-DDD**	**p.p´-DDE**	**p'p -DDT**	**Ametryn**
IUPAC Name	1.1-Dichloro-2.2-bis [P-chlorophenyl] ethane	1.1-Dichloro-2.2-bis [p-chlorophenyl] ethylene	1.1 -[2.2.2-trichloroethylidine]-bis [4-chloro- benzene]	4-N-ethyl-6-methylsulfanyl-2-N-propan-2-yl-1.3.5-triazine-2.4-diamine
Chemical formula	$C_{14}H_{10}Cl_4$	$C_{14}H_8Cl_4$	$C_{14}H_9Cl_5$	$C_9H_{17}N_5S$
Molecular weight (g/mol)	320.04	318.03	354.49	227.33
Density (g/mL)	1.385	-	1.6	1.18 (at 22 °C)
Boiling point (at P=1 atm)	192.8 °C	316.5 °C	185 °C (0.05 mmHg)	337 °C (at 98.6 kPa)
Henry's Law constant (atm m^3/mol)	$6.60*10^{-6}$	$4.16*10^{-5}$	$8.32*10^{-6}$	$3.9*10^{-9}$
Vapor pressure (mmHg at 25 °C)	$1.35*10^{-6}$	$6.01*10^{-6}$	$1.6*10^{-7}$ (20 °C)	$2.74*10^{-6}$
Solubility in water at 25 °C (mg/L)	0.09	0.04	$5.5*10^{-3}$	209
Log Kow	6.02	6.51	4.89-6.91	2.98

1.3.3 Polycyclic Aromatic Hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are toxic and carcinogenic pollutants resulting from incomplete combustion of carbonaceous materials or high-pressure processes. PAHs are solids with low volatility at room temperature, with relatively high molecular weights; they are soluble in many organic solvents, relatively insoluble in water, and most can be photo-oxidized and degraded to simpler substances (Tab. 3).

PAHs can be generated by both biogenic and anthropogenic processes. Although they can have natural sources (oceanic aerosols, forest fires, etc.), anthropogenic combustion (motor vehicles, domestic burning, power generation via combustion of coal and oil, waste incineration, burning of fossil fuel and natural gas) represent the main source of emissions (F. Liu et al., 2018). They are present in the atmosphere both in the gas phase and associated with particles and can potentially travel long distances reaching remote areas. The main differences between PAHs from other classical POPs are their shorter atmospheric half-lives (range of hours to days), their affinity to soot carbon and the influence of ongoing primary sources on their distribution (Nizzetto et al., 2008).

They have a relatively low water solubility and it decreases approximately logarithmically when molecular mass increases. By contrast, they are highly lipophilic and many of them adsorb to suspended particulate matter, which then settles through the water column, and accumulate in aquatic sediments and marine organisms. Therefore, PAHs may enter the environment either by adsorbing particulate matter through atmosphere and aquatic environments, reaching long distances, or by dissolution, resulting from more local sources (Álvarez-Muñoz, Llorca, Blasco, & Barceló, 2016).

Capolupo et al. (2017) demonstrated lysosomal injuries linked with the increasing concentrations of PAHs in *Mytilus* spp tissue. Cagnazzi et al. (2013) found in humpback and snubfin dolphin tissues high concentrations of PAHs. Among these, naphthalene and pyrene were the most dominant chemicals. (Ferrante, Zanghì, Cristaldi, Copat, Grasso, et al., 2018) showed a greater bioaccumulation profile of PAHs in a bivalve mollusk (*Donax trunculus*) followed by two species of marine teleosts *Sardina pilchardus* and finally *Solea solea*.

Tab. 3 - PAHs physico-chemical properties.

	PAHs		
Compound	**Fluoranthene**	**Phenanthrene**	**Pyrene**
Chemical formula	$C_{16}H_{10}$	$C_{14}H_{10}$	$C_{16}H_{10}$
Molecular weight (g/mol)	202.26	178.2	202.3
Density (g/mL)	-	0.98	1.271
Boiling point (at P=1 atm)	375 °C	340 °C	393 °C
Henry's Law constant (atm m^3/mol)	$6.5*10^{-6}$	$2.56*10^{-5}$	$1.14*10^{-5}$
Vapor pressure (mmHg at 25 °C)	$5*10^{-6}$	$6.8*10^{-4}$	$2.5*10^{-6}$
Solubility in water at 25 °C (mg/L)	0.20-0.26	1.2	0.077
Log Kow	4.9	4.45	4.88

1.4 Emerging contaminants

Emerging contaminants (ECs) are defined as compounds which have been in the environment for a while but for which concerns have been raised much more recently (Sauvé & Desrosiers, 2014).

Several studies (Mackay and Barnthouse 2010; Farré, Pérez, and Kantiani 2008) have proved that ECs are thought to be potential threats to environmental ecosystems and human health and safety. Furthermore, their high production and consumption and their continuous introduction into the environment, ensures that they do not have to be persistent to cause adverse effects.

They include a heterogeneous group of compounds, including pharmaceuticals, drugs of abuse, personal care products (PCPs), steroids and hormones, surfactants, perfluorinated compounds (PFCs), flame retardants like polybrominated diphenyl ethers (PBDEs), industrial additives and agents, and gasoline additives, as well as their transformation products (TPs). In addition, three new classes have to be added to the list of emerging pollutants: nanomaterials, 1,4-dioxane and swimming pool disinfection by-products (DBPs).

Specifically, personal care products (PCPs) include a wide range of compounds such as disinfectants (e.g. triclosan), fragrances (e.g. musks), insect repellants (e.g. DEET), preservatives (e.g. parabens), and ultraviolet (UV) filters (e.g. octocrylene and benzophenone 3). They are products destined to external use on the human body including cosmetics, gels, and soaps among others (Álvarez-Muñoz et al., 2016).

One of the main sources of environmental contamination are Wastewater Treatment Plants (WWTPs) effluents. Other important sources are waste disposal, aquaculture, animal husbandry, and horticulture. They provide a continuous input of these compounds into the environment (e.g., groundwater, surface water and sediment), where different transformations also occur, sometimes producing products that can differ in their environmental behavior and ecotoxicological profile (Petrie, Barden, & Kasprzyk-Hordern, 2015).

PCPs are among the most commonly detected compounds in surface water throughout the world (Peck, 2006). Moreover, due to their lipophilic nature (log K_{OW} values between 4 and 8) and great stability in the environment, these compounds can easily bioaccumulate on animal tissues, reaching several trophic levels (Giokas, Salvador, & Chisvert, 2007).

In comparison to pharmaceuticals, less attention has been placed on determining toxicity and potential risk associated to PCPs release into aquatic environments. Only in the last few years, the organic contamination with personal care products (PCPs) has been of great concern in aquatic systems, and their concentration in the oceans is likely to increase (Brausch & Rand, 2011).

The toxic effects in humans after such a prolonged low dose exposure to UV-Fs and musks have hardly been investigated, showing antiestrogenic, and/or anti-androgenic activity through human estrogen and androgen receptor assays. The toxicity in aquatic organisms and in humans raises legal and health concerns worldwide (Kunz & Fent, 2006).

1.4.1 UV filters

UV filters are used in sunscreen products and cosmetics to protect from UV radiation and can be either organic (absorb UV radiation, e.g. methylbenzylidene camphor) or inorganic micropigments (reflect UV radiation, e.g. ZnO, TiO2) (Brausch & Rand, 2011) (Tab. 4).

Generally, sunscreens contain multiple ingredients for multiple functions. Due to rising concern over sunburn, photoaging, skin cancer and photodermatoses, skin-care products are one of the fastest growing sectors of commodities, which is why is possible to find them in many products of daily use, such as cosmetics, skin creams, body lotions, hair sprays, hair dyes, shampoos, and so forth. This trend is expected to continue because the world's coastal population—who use more of these products than any other sector—is expected to grow from 1.2 to 5.2 billion by 2080 (Marianne E. Balmer, Hans-Rudolf Buser, Markus D. Müller, & Poiger, 2005).

UV filters have been recognized as emerging contaminants (ECs), with potential toxicities in the concentration range of other well-characterized estrogenic chemicals. Several studies Calafat et al. (2008), Danovaro et al., (2008) and Schlumpf and Lichtensteiger

(2001) demonstrated that the UV filter benzophenone-3 (BP-3) and its metabolites are inevitably released into the aquatic environment, causing hazard to organisms, also performing estrogenic and anti- androgenic activities. For this reason, from September 3, 2017, following the opinion of the Scientific Committee on Consumer Safety (SCCS), the EU Commission has published another amendment to the Cosmetic Regulation (EC) n. 1223/2009, restricting the use of Benzophenone-3 as a UV-filter**.** According to the recently published standards, the maximum concentration of BP-3 in cosmetic sunscreen products is defined as 6%, whereas the limit for all the other types of cosmetic products is set at 0.5%.

The extremely large consumption worldwide (10,000 tons annually) of UV filters is contributing to their high detection frequency in various environmental matrices of fresh/sea waters, wastewaters, tap water, groundwater, sewage sludge, sediments, biota and even human bodies (i.e. urine, serum and breast milk), up to µg/L or ng/g levels (Li et al., 2017).

UV-filters can reach the marine environment mainly by two different ways: directly, as consequence of water recreational activities and/or indirectly, from wastewater treatment plants (WWTP) effluents. Some studies (Sánchez-Quiles and Tovar-Sánchez 2015; Marianne E. Balmer et al. 2005) have indicated that there is a direct relationship between beachgoer affluence and the amounts of sunscreens components released into the seawater, mountain lakes and rivers. On the other hand, WWTP effluents could affect areas of high tourism activities. Daily activities like showering, laundering or even urinating are sources of sunscreen components discharged to the WWTP where they are not completely removed.

A study (Marianne E. Balmer et al., 2005) in Switzerland estimated the input of four commonly applied UV filters into WWTPs to be as high as 118 g of 2-ethyl-hexyl-4-trimethoxycinnamate (EHMC), 49 g of 4-methyl-benzilidine-camphor (4MBC), 69 g of benzophenone-3 (BP3), and 28 g of octocrylene (OC) per 10.000 people per day in high use times.

Tab. 4 - UV filters physico-chemical properties.

UV FILTERS						
Compound	**4-Methylbenzylidene Camphor (4MBC)**	**Benzophenone-3 (BP-3)**	**Ethylhexyl methoxycinnamate (EHMC)**	**Ethyl hexyl salycilate (EHS)**	**Homosalate (HMS)**	**Octocrylene (OC)**
IUPAC Name	4.7.7-trimethyl-2-[(4-methylphenyl)methylidene]bicyclo[2.2.1]heptan-3-one	2-Hydroxy-4-methoxybenzophenone	2-ethylhexyl (E)-3-(4-methoxyphenyl)prop-2-enoate	2-ethylhexyl 2-hydroxybenzoate	(3.3.5-trimethylcyclohexyl) 2-hydroxybenzoate	2-ethylhexyl 2-cyano-3.3-diphenylprop-2-enoate
Chemical formula	$C_{18}H_{22}O$	$C_{14}H_{12}O_3$	$C_{18}H_{26}O_3$	$C_{15}H_{22}O_3$	$C_{16}H_{22}O_3$	$C_{24}H_{27}NO_2$
Molecular weight (g/mol)	254.373	228.25	290.403	250.34	262.35	361.49
Density (g/mL)	-	1.32	-	1.014	1.05	1.05 g/cm³ at 25° C
Boiling point (at P=1 atm)	-	-	382 °C	452.60 °C	161.11-165 °C	-
Henry's Law constant (atm m^3/mol)	-	$1.5*10^{-8}$	$8.5*10^{-6}$	-	-	-
Vapor pressure (mmHg at 25 °C)	-	$6.62*10^{-6}$	$2.3*10^{-5}$	$8.07*10^{-5}$	$4.17*10^{-5}$	0.00000000315
Solubility in water at 25 °C (mg/L)	0.57	3.7	0.15	-	insoluble	insoluble
Log Kow	5.47	3.79	5.8	3.77	5	7.1

1.4.2 Fragrances

Fragrances are perhaps the most widely studied class of all PCPs. The most commonly used fragrances are synthetic musks (Tab. 5). Synthetic musks are fragrances used in a wide range of products including deodorants, soaps, and detergents. Synthetic musks are either nitro musks, which were introduced in the late 1800s, or polycyclic musks, introduced in the 1950s (Daughton & Ternes, 1999).

Polycyclic musks are currently used in higher quantities than nitro musks with celestolide (ABDI), galaxolide (HHCB) and tonalide (AHTN) used most commonly and traseloide (ATII), phantolide (AHMI), and cashmeran (DPMI) used less often. HHCB and AHTN production alone has been estimated at about 1 million pounds per year and has thus been placed on the High Production Volume List by the U.S. EPA (Brausch & Rand, 2011).

Like other cosmetics, these chemicals are highly lipophilic and therefore can be expected to bioaccumulate in the environment. The presence of musks have also been reported in fish (Cunha et al., 2015), mussels (Trabalón, Cano-Sancho, et al., 2015), breast milk and human adipose tissue (Rimkus & Wolf, 1996).

Their lipophilic properties (and relative persistence) allow these substances to easily accumulate in various carbon rich environmental compartments including biota (J.-L. Liu & Wong, 2013).

Luckenbach and Epel (2005) revealed that polycyclic musks may act as long-term inhibitors of the activity of multidrug efflux transporters responsible for multixenobiotic resistance (MXR) in gills of the marine mussel *Mytilus californianus*.

Polycyclic musks are more acutely toxic than nitro musks based on published literature. HHCB and AHTN are toxic to aquatic invertebrates at ppb to low ppm levels although they are relatively non-toxic to fish, and for longer exposure periods, invertebrates also appear more sensitive to polycyclic musks than fish (Brausch & Rand, 2011).

Among fragrances, one of the most used is OTNE, commercially known as “ISO E Super”. In recent years this has been the most popular of all fragrances in Europe. This compound has been detected in natural compartments, even at higher concentrations than polycyclic musks (in a range of 29000-810 ppb). The available information about OTNE is limited,

although some data are found in the literature on its bioaccumulation (i.e. 0.037 and 0.049 L kg wet wt^{-1} for fish and Daphnia, respectively) (Sendra et al., 2017). Chronic toxicity of this substance has been also reported in algae, with effects detected at 317 ppb (Ortiz de García, Pinto, García-Encina, & Mata, 2013).

Polycyclic musk compounds such as HHCB (Galaxolide) and AHTN (Tonalide) are used as fragrances in a variety of consumer products, including washing and cleaning agents and personal-care products. Among several polycyclic musks, HHCB and AHTN are the most commonly detected compounds in water and biota. In the United States, HHCB is listed by the US Environmental Protection Agency (EPA), as a high production-volume chemical, which suggests that the production is more than 4500 tons/year, for uses that are reportable under the Toxic Substances Control Act (Kannan et al., 2005). HHCB and AHTN have been shown to accumulate in several aquatic organisms, such as fish and mussels; also in tissues of finless porpoise and shark collected from Japanese coastal waters (Nakata*, 2005).

Regarding marine environment, (Cunha et al., 2015) demonstrated the co-occurrence of musk fragrances and UV-filters on different marine species such as mussels, mullet, clam and seaweeds collected in European hotspots. Among musk fragrances, galaxolide (HHCB) and tonalide (AHTN) were the most frequently quantified in samples from the European hotspots.

Tab. 5 - Fragrances physico-chemical properties.

FRAGRANCES			
Compound	**Octahydrotetramethyl Acetophenone (OTNE)**	**Galaxolide**	**Tonalide**
IUPAC Name	1–(1.2.3.4.5.6.7.8–octahydro–2.3.8.8–tetramethyl–2–naphthalenyl) ethanone	4.6.6.7.8.8-hexamethyl-1.3.4.7-tetrahydrocyclopenta[g]isochromene	1-(3.5.5.6.8.8-hexamethyl-6.7-dihydronaphthalen-2-yl)ethanone
Chemical formula	$C_{16}H_{26}O$	$C_{18}H_{26}O$	$C_{18}H_{26}O$
Molecular weight (g/mol)	234.38	258.44	258.405
Density (g/mL)	0.964 (at 20 °C)	1.0054 (at 20 °C)	-
Boiling point (at P=1 atm)	134 °C	128 °C (at 0.8 mmHg)	326 +/- 4 °C
Henry's Law constant (atm m^3/mol)	-	$1.06*10^{-4}$	$1.4*10^{-4}$
Vapor pressure (mmHg at 25 °C)	0.233 Pa at 23°C	$5.45*10^{-4}$	$5.12*10^{-4}$
Solubility in water at 25 °C (mg/L)	2.68 mg/L at 20°C	1.75	1.25
Log Kow	5.65	5.9	5.7

1.5 Samples biology and bioaccumulation

1) *Sarda sarda* (Bloch, 1793)

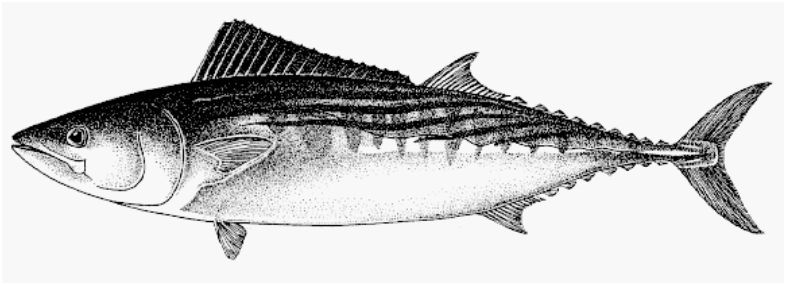


Fig. 4 – *Sarda sarda* (Bloch, 1793) (From: http://www.fao.org)

Geographical Distribution

Tropical and temperate coasts of the Atlantic Ocean, including the Gulf of Mexico and the Mediterranean and Black seas. It is known from Colombia and Venezuela and is much more common south of the Amazon River to northern Argentina. In the eastern Atlantic, it has been taken from near Oslo, Norway south to Port Elizabeth, South Africa.

Habitat and Biology

An epipelagic, neritic schooling species that can adapt to gradual but not sudden changes in the environment and may occur in water temperatures between 12 ° and 27 °C and salinities between 14 and 39 °/oo S, entering estuaries such as Miramichi and the Gulf of St. Lawrence.

In most parts of the Mediterranean spawning occurs between May and July, but off Algeria it extends from March to May. In the eastern Atlantic, it occurs from December to June, including peaks in January and April, off Dakar, and from June to July in Moroccan waters. In the northwestern Atlantic, bonitos spawn in June and July. Adults prey primarily on small schooling fishes, the choice of species depending on the locality. In the Gulf of Mexico, it was also found to feed on a number of invertebrates like squid and shrimps. It can swallow relatively large prey, and both the juveniles and the adults are known to be cannibalistic.

Size

Maximum fork length in the Black Sea is 85 cm and 5 kg weight; in the western Atlantic, the largest fish caught is reported as measuring 91.4 cm fork length and weighing 5.4 kg; common to 50 cm fork length and about 2 kg weight. Minimum length at first maturity is about 39.5 cm in males and 40.5 cm in females.

Interest to Fisheries

The species is particularly important in the Mediterranean and Black seas. Fishing in the Black Sea peaks between May and October, while in the Mediterranean it may vary from area to area or even extend throughout the year. Fishing in the eastern tropical Atlantic takes place between October and May, while it extends throughout the year off Morocco. Peak fishing of the Spanish fleet all around the peninsula is in late spring and in fall.

2) *Katsuwomis pelamis* (Linnaeus, 1758)

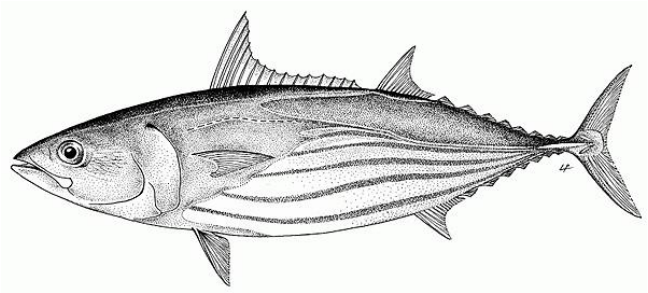


Fig. 5 – Katsuwonus pelamis (Linnaeus, 1758) (From: http://www.fao.org)

Geographical distribution

Cosmopolitan in tropical and warm-temperate waters**;** absent from the Black Sea.

Habitat and Biology

An epipelagic oceanic species with adults distributed roughly within the 15° C isotherm (overall temperature range of recurrence is 14.7° to 30°C), while larvae are mostly restricted to waters with surface temperatures of at least 25°C. Aggregations of this species tend to be associated with convergences, boundaries between cold and warm water masses, upwelling and other hydrographical discontinuities. Depth distribution

ranges from the surface to about 260 m during the day, but it is limited to near surface waters at night.

Skipjack tuna spawn in batches throughout the year in equatorial waters, and from spring to early fall in subtropical waters, with the spawning season becoming shorter as distance from the equator increases.

Food items predominantly include fishes, crustaceans and molluscs. Even though Carangidae and Balistidae are part of the diet of skipjack tuna in all oceans, the wide variety of species taken suggest it to be an opportunistic feeder preying on any forage available. The feeding activity peaks in the early morning and in the late afternoon. Cannibalism is, also, common. The principal predators of skipjack are other tunas and billfishes.

It is hypothesized that the skipjack tuna in the eastern central Pacific originate in equatorial waters, and that the pre-recruits (up to 35 cm fork length) split into a northern group migrating to the Baja California fishing grounds, and a southern group entering the central and south American fishing areas. Having remained there for several months, both groups return to the equatorial spawning areas. A similar migration pattern has been observed in the northwestern Pacific. Skipjack tuna exhibit a strong tendency to school in surface waters. Schools are associated with birds, drifting objects, sharks, whales or other tuna species and may show a characteristic behaviour (jumping, feeding, foaming, etc.).

Size

Maximum fork length is about 108 cm corresponding to a weight of 32.5 to 34.5 kg; common to 80 cm fork length and a weight of 8 to 10 kg. Fork length at first maturity is about 45 cm.

Interest to Fisheries

Skipjack tuna is taken at the surface, mostly with purse seines and pole-and-line gear, but also incidentally by longlines. At present, the major fisheries are the purse seine fisheries, particularly those of Spain, France, Cape Verde, Guatemala and Ghana, followed by baitboat fisheries of Ghana, Spain and France. There has been a recent increase in Skipjack Tuna catchability from 1–13% per year since the early 1980s. (ICCAT, 2008).

3) *Thunnus thynnus* (Linnaeus, 1758)

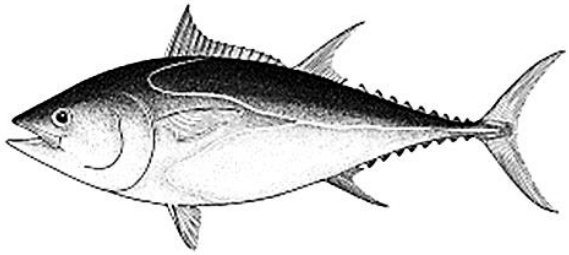


Fig. 6 – *Thunnus thynnus* (Linnaeus, 1758) (From: http://www.fao.org)

Geographical distribution

There are at least two subspecies, one in the Atlantic and one in the Pacific. The Atlantic subspecies is found from Labrador and Newfoundland south into the Gulf of Mexico and the Caribbean Sea and is also known off Venezuela and Brazil in the western Atlantic; in the eastern Atlantic it occurs from the Lofoten Islands off Norway south to the Canary Islands and the Mediterranean Sea. There is also a population off South Africa. The Pacific subspecies is known from the Gulf of Alaska to southern California and Baja California in the eastern Pacific; in the western Pacific, it occurs from Sakhalin Island in the southern Sea of Okhotsk south to the northern Philippines.

Habitat and Biology

Epipelagic, usually oceanic but seasonally coming close to shore. Northern bluefin tuna tolerate a wide range of temperatures. Up to a size of 40 to 80 kg, they school by size, sometimes with other tuna species. Onset of maturity is at about 4 or 5 years, and large adults (age 10 +) are known to spawn in the Gulf of Mexico and in the Mediterranean Sea. In the Pacific, spawning occurs northeast of the Philippines. Females weighing between 270 to 300 kg may produce as many as 10 million eggs per spawning season.

Variations in the food spectrum are attributed primarily to behavioural differences in feeding. 'Vigorous pursuit' would be required to prey on small schooling fishes (anchovies, sauries, hakes) or on squids, while 'modified filter-feeding' is used to feed on red crabs and other less agile organisms. In turn, northern bluefin tuna are preyed upon by killer whales (*Orcinus orca*), pilot whales and blackfish. However, the rather large size of adults drastically reduces the number of potential predator species.

Size

Maximum fork length over 300 cm; common to 200 cm. The biggest fish in the various North Atlantic fisheries range between 540 and 560 kg. In the warmer waters off the Canary Islands, the biggest fish in commercial catches range between 350 and 400 kg.

Interest to Fisheries

Thunnus thynnus is caught with different types of gear, such as trap nets, purse seines, longlines, trolling lines and others. Some of the oldest fisheries documented are Mediterranean trap fisheries. Off Sicily, northern bluefin tuna are traditionally caught in the "tonnare" (Tuna trap fishing), or by harpooning from the "antenna" vessels. Traps similar to the "tonnare" are also used off southern Spain and Morocco.

In particular, *Thunnus thynnus* is worldwide considered one of the most highly valuable fishery resources, particularly for the Japanese market, and its catch and trade support an important production chain. The elevated fishing pressure on this resource has determined a decrease of eastern Atlantic and Mediterranean bluefin tuna stock during the last century even if sustainable management actions for the conservation of this species and management and recommendations on the stock status have been carried out by the International Commission for the Conservation of Atlantic Tunas (ICCAT) (Sumaila and Huang, 2012). As a result, *T. thynnus* has been listed in the International Union for Conservation of Nature (IUCN) Red List as “endangered species”.

The International Commission for the Conservation of Atlantic Tunas (ICCAT) is responsible for the conservation of tunas and tuna-like species in the Atlantic Ocean and adjacent seas, since arrangements are necessary in order to share the available research and fishery information. The organization was established at a Conference of Plenipotentiaries, which prepared and adopted the International Convention for the Conservation of Atlantic Tunas signed in Rio de Janeiro, Brazil, in 1966. After a ratification process, the Convention entered formally into force in 1969. The Commission's work requires the collection and analysis of statistical information relative to current conditions and trends of the fishery resources in the Convention. About 30 species are covered by the Convention, including Atlantic bluefin (*Thunnus thynnus*), Skipjack tuna (*Katsuwonus pelamis*) and Atlantic bonito (*Sarda sarda*).

2. AIM OF THE STUDY

The aim of this study was to evaluate the occurence and levels of different classes of legacy and emerging contaminants and analyse their accumulation in liver and muscle tissues of three tuna species: *Sarda sarda*, *Katsuwonus pelamis* and *Thunnus thynnus*. We focused on substances belonging to both Persistent Organic Pollutants (POPs), such as polychlorinated biphenyls (PCBs), pesticides and herbicides, such as dichlorodiphenyltrichloroethane (DDT) and its metabolites (together referred to as DDTs) and ametryn (triazines), polycyclic aromatic hydrocarbons (PAHs); and the category of Emerging Contaminants (ECs) such as UV filters and Fragrances.

3. MATERIALS AND METHODS

3.1 Study Areas

3.1. 2 Gulf of Cádiz

The Gulf of Cádiz (Fig. 7), wide embayment of the Atlantic Ocean along the southwestern Iberian Peninsula, stretches about 200 miles (320 km) from Cape Saint Vincent (Portugal) to Gibraltar. Continuing southward along the Spanish coast, the most recurrent feature is large marshes (some spreading inland as much as 30 miles [50 km]) behind coastal dunes, which are interrupted by tidal channels, notably of the Guadiana, Tinto-Odiel, Guadalquivir, and Guadalete rivers; along this stretch are many saltworks and occasional settlements, including the city of Cádiz, on the Bay of Cádiz. Farther southward, from Cape Trafalgar to Gibraltar, rocky coasts alternate with beaches.

The exchange of mass energy and sea-land supplied by these channels and the particular weather conditions of the area with the dominance of the eastern winds, provide particularly good conditions for maintaining high rates of primary and secondary production, transforming the proximity of the main mouth of the Gulf in a breeding area of species of commercial interest.

Tuna samples were collected near Puerto de Santa Maria, located on the banks of the Guadalete river, 10 km north of Cádiz.

3.1.3 Strait of Gibraltar

The Strait of Gibraltar (Fig. 7) is a point of natural separation of two seas, the Mediterranean from the East and the Atlantic Ocean from the West. It also separates two continents, Europe and Africa. Because of its geographical characteristics, the Strait has its own unique weather conditions. The two continents channel the wind between its two land masses. It adopts two main directions: east and west. These are known as the winds of levante and poniente.

The Strait of Gibraltar produces a slow exchange of water from the Atlantic Ocean and the Mediterranean. The Atlantic is the only fresh- water resource of the Mediterranean, which takes approximately 100 years to renew itself completely. The Mediterranean is much saltier than the Atlantic, on account of evaporation and high density, so, it flows as an undercurrent as it leaves; while the Atlantic, a more substantial body of water with less salt and density, enters on the surface.

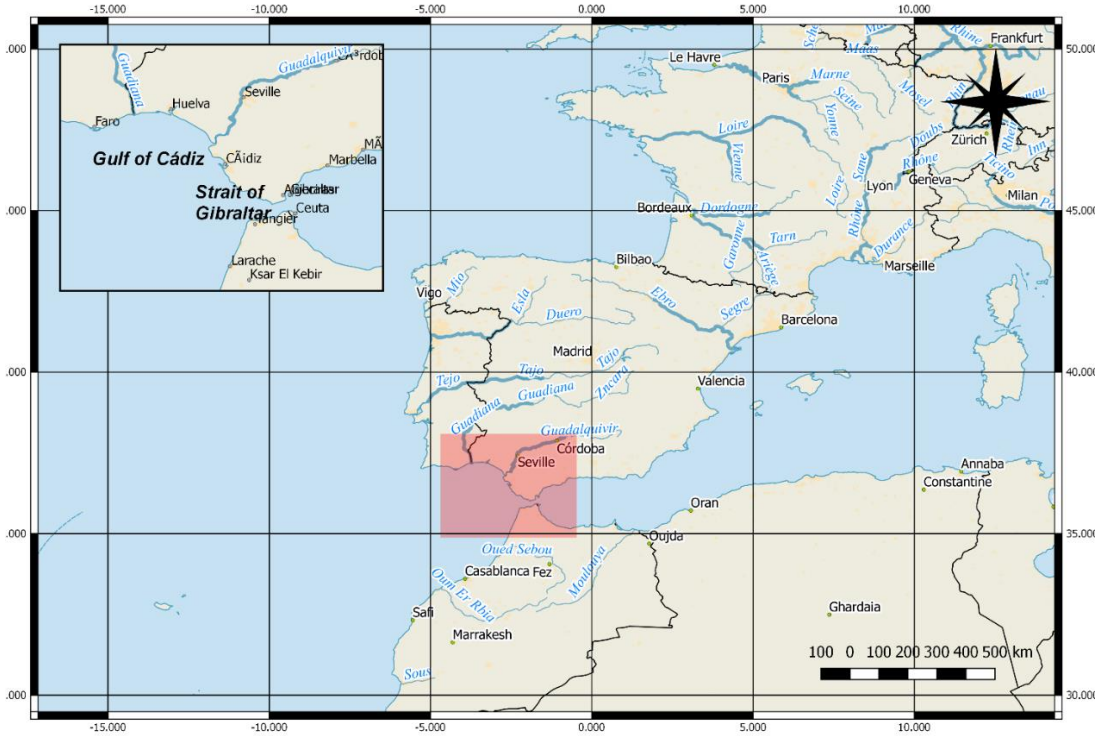


Fig. 7 – Sampling Areas: Gulf of Cádiz, Strait of Gibraltar

3.2 Fishing techniques

The fishing techniques used for the collection of the species concerned are: the “trolling” technique and the “green stick” technique.

The “trolling” technique (Fig. 8) was used for the species places in the Gulf of Cádiz. It is a method of directed fishing for predatory fish and it is practiced using live baits dragged by a boat at very low speed, or artificial baits, dragged at higher speed, around five knots. For catching tuna, among the artificial baits, we can find large octopus of silicone.

The “green stick” (Fig. 9) is a technique for fishing for tuna by trolling synthetic squid from a fiberglass pole around 30 feet (9.1 m) above the water surface. As part of the technique, the squid spend very little time submerged in the water and more of it suspended in the air above, similar to kite fishing. It is named for the green tint of the extremely long fiberglass poles originally made in Japan specifically for this purpose.

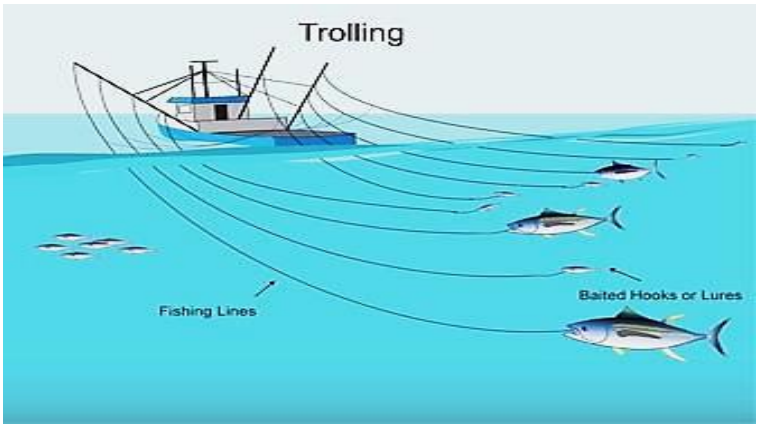


Fig. 8 – Trolling fishing technique (From: http://fish.gov.au/Fishing_Methods/)

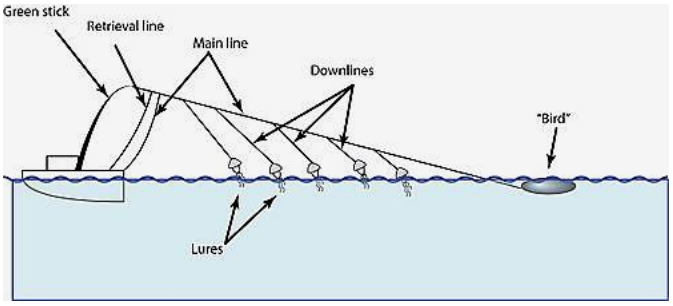


Fig. 9 – Green stick fishing technique (From: https://www.seagrantfish.lsu.edu)

3.3 Samples characteristics and collection

Details of sampling and biometric data are reported in Tab. 10, Tab. 11 and Tab. 12. Three tuna species were collected off the Spanish coasts: *Sarda sarda* (samples of liver n=3; samples of muscle n=3) and *Katsuwonus pelamis* (samples of liver n=6; samples of muscle n=10) were collected off the Atlantic coasts of the Gulf of Cádiz; *Thunnus thynnus* was collected off the Strait of Gibraltar (samples of liver n=22; samples of muscle n=18). Sampling was carried out between May and July 2017. Representative sample from each tuna was obtained by sampling fish tissue from tail muscle and liver.

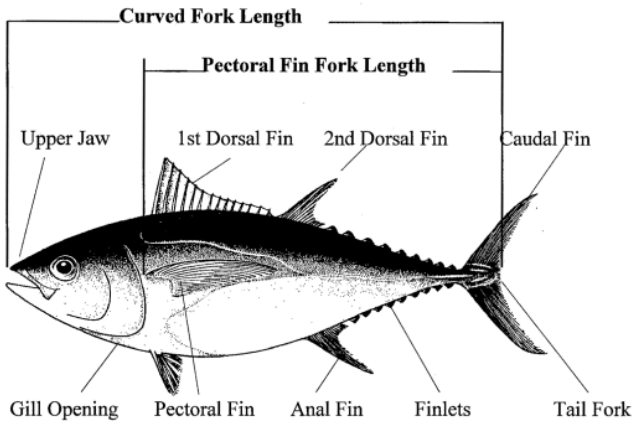


Fig. 10 – Tuna characteristics (From: http://www.marinebasin.com/)

Regarding tuna characteristics (Fig.10), straight fork length (SFL) represents the straight line from the tip of the upper jaw to the posterior of the shortest caudal ray (fork of the caudal fin), while curved fork length (CFL) represents the length from the tip of the upper jaw to the fork over the fish curvature body (Rodriguez-Marin et al., 2015).

The relationship between age and furcal lenght of *S. sarda* is reported in Tab. 6.

First maturity furcal lenght, maximum furcal lenght and common furcal lenght, related to weight of *K. pelamis*, are reported in Tab. 7.

The relationship between size class and weight of *T. thynnus* is reported in Tab. 8; whereas age, size and weight at maturity of the East Atlantic and Mediterranean *T. thynnus* and the West Atlantic *T. thynnus* is reported in Tab. 9.

Tab. 6 –*Sarda sarda* age from western Mediterranean. FL= Furcal length. (Valeiras et al., 2008).

AGE (years)	FL RANGE (cm)
0	< 40
1	40 – 52
2	49 – 55
3	55 – 61

Tab. 7 –*Katsuwonus pelamis* from Atlantic Ocean. FL (Furcal length in cm) (Collette and Nauen, 1983).

MAXIMUM FL (cm)	MOST COMMON FL (cm)	FIRST MATURITY FL (cm)
108 (corresponding to a weight of 32,5 to 34,5 kg)	**80** (corresponding to a weight of 8 to 10 kg)	**45**

Tab. 8 – *Thunnus thynnus* size class and weight (ICCAT, GBYT Sampling Protocol 2008)

SIZE CLASS		WEIGHT RANGE (kg)
V	Larvae	
0	Age 0	**≤ 3**
J	Juveniles	**> 3; ≤ 25**
M	Medium	**> 25; ≤ 100**
L	Large	**> 100**

Tab. 9 - Age, size and weight at maturity for the East Atlantic & Mediterranean bluefin tuna and the West Atlantic bluefin tuna (ICCAT 2006).

	East Atlantic and Mediterranean Sea	West Atlantic	References
First age and size-at-maturity	Age 3 100 cm; 20 kg	Age 5 140 cm; 45 kg	Mather *et al.* 1995
50% maturity	Age 4 115 cm; 30 kg	Age 8 190 cm; 120 kg	Mather *et al.* 1995 ICCAT 1997
100% maturity	Age 5 135 cm; 50 kg	Age 10+ 220 cm; 175 kg	Mather *et al.* 1995; Diaz & Turner 2007

Tab. 10 – Sample *Sarda sarda* (Bloch, 1793) characteristics: Collecting Area; Collection date; Sampling location; Fishing technique; Fork length (LF); Weight.

Sample ID	Collecting Area	Collection date (dd/mm/yyyy)	Sampling location	Fishing technique	LF (cm)	Weight (Kg)
SS 3	Gulf of Cádiz	06/07/2017	Puerto Santa María	Trolling	42.49	1.029
SS 4	Gulf of Cádiz	06/07/2017	Puerto Santa María	Trolling	41.20	0.933
SS 6	Gulf of Cádiz	06/07/2017	Puerto Santa María	Trolling	43.32	1.094
SS 9	Gulf of Cádiz	06/07/2017	Puerto Santa María	Trolling	42.40	1.0215

Tab. 11 – Sample *Katsuwonus pelamis* (Linnaeus, 1758) characteristics: Collecting Area; Collection date; Sampling location; Fishing technique; Fork length (LF); Weight.

Sample ID	Collecting Area	Collection date (dd/mm/yyyy)	Sampling location	Fishing technique	LF (cm)	Weight (Kg)
KP 1	Gulf of Cádiz	06/07/2017	Puerto Santa María	Trolling	45.81	1.890
KP 2	Gulf of Cádiz	06/07/2017	Puerto Santa María	Trolling	43.00	1.538
KP 3	Gulf of Cádiz	06/07/2017	Puerto Santa María	Trolling	43.86	1.640
KP 4	Gulf of Cádiz	06/07/2017	Puerto Santa María	Trolling	45.02	1.785
KP 5	Gulf of Cádiz	06/07/2017	Puerto Santa María	Trolling	44.80	1.757
KP 6	Gulf of Cádiz	06/07/2017	Puerto Santa María	Trolling	44.98	1.781
KP 7	Gulf of Cádiz	06/07/2017	Puerto Santa María	Trolling	45.52	1.851
KP 8	Gulf of Cádiz	06/07/2017	Puerto Santa María	Trolling	47.72	2.1585
KP 9	Gulf of Cádiz	06/07/2017	Puerto Santa María	Trolling	42.91	1.528
KP 10	Gulf of Cádiz	06/07/2017	Puerto Santa María	Trolling	44.84	1.763

Tab. 12 - Sample *Thunnus thynnus* (Linnaeus, 1758) characteristics: Collecting Area; Collection date; Sampling location; Fishing technique; Fork length (LF); Weight.

Sample ID	Collecting Area	Collection date (dd/mm/yyyy)	Sampling location	Fishing technique	LF (cm)	Weight (Kg)
TT 1	Strait of Gibraltar	22/05/2017	Tarifa	Green stick	135.13	49.70
TT 2	Strait of Gibraltar	22/05/2017	Tarifa	Green stick	153.07	71.23
TT 3	Strait of Gibraltar	22/05/2017	Tarifa	Green stick	144.57	60.40
TT 4	Strait of Gibraltar	22/05/2017	Tarifa	Green stick	109.64	27.18
TT 5	Strait of Gibraltar	22/05/2017	Tarifa	Green stick	125.69	40.32
TT 6	Strait of Gibraltar	22/05/2017	Tarifa	Green stick	142.69	58.15
TT 7	Strait of Gibraltar	22/05/2017	Tarifa	Green stick	142.69	58.15
TT 8	Strait of Gibraltar	22/05/2017	Tarifa	Green stick	134.19	48.71
TT 9	Strait of Gibraltar	25/05/2017	Tarifa	Green stick	118.14	33.72
TT 10	Strait of Gibraltar	25/05/2017	Tarifa	Green stick	135.13	49.70
TT 11	Strait of Gibraltar	25/05/2017	Tarifa	Green stick	123.80	38.60
TT 12	Strait of Gibraltar	25/05/2017	Tarifa	Green stick	148.35	65.07
TT 13	Strait of Gibraltar	25/05/2017	Tarifa	Green stick	144.57	60.40
TT 14	Strait of Gibraltar	25/05/2017	Tarifa	Green stick	129.47	43.92
TT 15	Strait of Gibraltar	25/05/2017	Tarifa	Green stick	149.30	66.27
TT 16	Strait of Gibraltar	25/05/2017	Tarifa	Green stick	150.24	67.49
TT 17	Strait of Gibraltar	25/05/2017	Tarifa	Green stick	124.75	39.46
TT 18	Strait of Gibraltar	25/05/2017	Tarifa	Green stick	115.30	31.44
TT 19	Strait of Gibraltar	29/05/2017	Tarifa	Green stick	141.74	57.05
TT 20	Strait of Gibraltar	29/05/2017	Tarifa	Green stick	195.00	143.28
TT 21	Strait of Gibraltar	29/05/2017	Tarifa	Green stick	124.75	39.46
TT 22	Strait of Gibraltar	29/05/2018	Tarifa	Green stick	149.30	66.27

3.4 Sample extraction

3.4.1 Chemicals and solvents

Dichloromethane (DCM), hexane, acetone and ethyl acetate were of chromatography quality, purchased from Sigma–Aldrich (Madrid, Spain) and Scharlau (Barcelona, Spain). Diatomaceous earth (Hydromatrix) was purchased from Agilent Technologies (Madrid, Spain). PTFE centrifuge filters (0.22 m pore size) were purchased from Ciromfg (Florida, United States). A derivatizing agent, N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA) from Sigma–Aldrich (Madrid, Spain), was also used. Sorbent used for sample clean-up was silica (70–230 mesh), provided by Sigma–Aldrich (Madrid, Spain).

Standards (Tab. 13) for galaxolide, polycyclic aromatic hydrocarbons (PAHs) (phenanthrene, pyrene, fluoranthene), triazines (ametryn), organochlorine pesticides (p,p'-DDT, p,p'-DDD, p,p'-DDE), polychlorinated biphenyls (PCB52, PCB138, PCB153, PCB180 and PCB101), were purchased from Dr Ehrenstorfer GmbH (Augsburg, Germany), as well as a deuterated PAH mixture (acenaphthene-d10, chrysene-d12, phenanthrene-d10 and perylene-d12). Benzophenone-3 (BP-3), octocrylene (OC), homosalate (HMS), 2-ethylhexylsalicylate (EHS), 2-ethylhexyl-4-methoxycinnamate (EHMC), 4-methylbenzylidene camphor (4-MBC), and benzophenone d10 were purchased from Sigma–Aldrich (Madrid, Spain). Tonalide was purchased from LGC Standards (Barcelona, Spain). OTNE fragrance was purchased from Bordas Chinchurreta Destilations (Seville, Spain). Triphenylphosphate d15 (TPP-d15) were purchased from Chiron (Trondheim, Norway). Solutions of these chemicals were prepared in acetone and/or ethyl acetate and stored at −20◦C in tightly closed amber vials (Pintado-Herrera, González-Mazo, & Lara-Martín, 2016).

Tab. 13 - List of detected compounds by category: Personal Care Products (PCPs), Polycyclic aromatic hydrocarbons (PAHs), Polychlorinated biphenyls (PCBs), Pesticides, Triazines.

CATEGORY	CLASS	COMPOUNDs
PCPs	**UV filters**	• Benzyl salicylate, ethylhexyl salicylate (EHS) • Homosalate (HMS) • 4-methylbenzylidene camphor (4-MBC) • Octocrylene (OC) • 2-ethylhexyl-4-methoxycinnamate (EHMC) • Benzophenone-3 (BP-3)
	Polycyclic musks	• Galaxolide • Tonalide
	Other frangances	• 1-(1,2,3,4,5,6,7,8-octahydro-2,3,8,8-tetramethyl-2-naphthalenyl-ethanone (OTNE)
PAHs		• Phenanthrene • Pyrene • Fluoranthene
	Deuterated PAH mixture	• Acenaphthene-d10 • Chrysene-d12 • Phenanthrene-d10 • Perylene-d12
PCBs	**Non-dioxin-like PCBs**	• PCB52 • PCB138 • PCB153 • PCB180 • PCB101
PESTICIDES	**Organochlorine Pesticides**	• p,p'-DDT • p,p'-DDD • p,p'-DDE
TRIAZINES		• Ametryn

3.4.2 Pressurized Liquid Extraction (PLE)

PLE technique (Fig. 11 a; b) uses liquid solvents at high temperatures and pressures in order to prepare solid samples for subsequent analysis by gas chromatography (GC). The high temperature allows the analytes to become more soluble and a higher diffusion velocity is achieved, while the high pressure maintains the solvent below its boiling point. At elevated pressures and temperatures, the solvents can penetrate solid samples more efficiently, which reduces the use of the same. A good optimization of PLE method is necessary, considering the high molecular weight matrix components, such as lipids,

present in these samples (tail muscle and liver) that must be eliminated to minimize adverse effects affecting the detection of compounds of interest.

PLE and clean-up procedures extractions were performed on an ASE Dionex-350 system (Thermo Co., Sunyvale, CA, USA) equipped with 11-mL stainless steel extraction cells and 30-mL collection vials (Fig. 12). Sorbent used for sample clean-up were hydromatrix and silica. Silica activation was performed according to Environmental Protection Agency (EPA) proceeding (heated up to 130 °C overnight - 3630C method, EPA); hydromatrix was washed with methanol, dried in a fume hood, purified by heating at 400 °C for 4 hours, and stored at room temperature in desiccators until use. The polarity of the solvent should be close to that of the target compound. Thus, non-polar and water-immiscible solvents or a combination of non-polar with medium-polarity solvents have frequently been used in the extraction of apolar and lipophilic compounds. For this reason, the mixture dichloromethane/hexane (75:25) was selected as the best solvent, using three static extraction cycles of 5 min each (purge time = 60 s, flush volume = 60%), 100 °C and 1500 psi (Pintado-Herrera M. G. et al., 2015).

Twenty-four hours before extraction, 20µL of the surrogates TPP d_{15} (triphenylphosphate d_{15}) were spiked at samples to account for losses during the extraction procedure. The extraction cell (Fig. 12) was loaded by inserting a cellulose filter into the cell outlet, followed by 3 g of activated silica gel, another cellulose filter and a quantity of freeze-dried sample, previously homogenized with 1 g of silica, in a range from a minimum of 0,137 g to a maximum of 0,964 g. When the cell was not full enough because of the little quantity of sample, it was filled with hydromatrix, a high purity, inert diatomaceous earth sorbent, to minimize cell dead volume.

Two types of blanks assays were performed in the same way, one filling the cell with hydromatrix, and another filling the cell with silica, both without tuna sample.

PLE extracts (30 mL) were evaporated to dryness using Büchi Syncore evaporator at different upper and lower plate temperatures, up to 1 mL approximately.

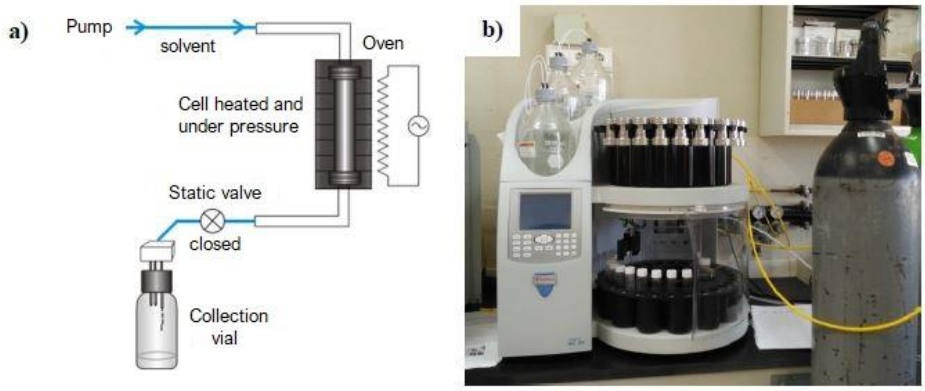


Fig. 11 – a) Extraction process diagram; b) ASE Dionex-350

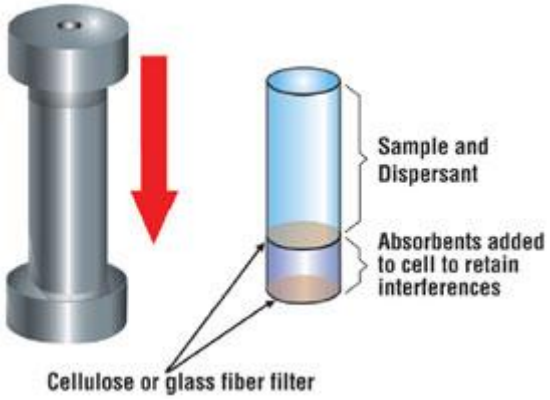


Fig. 12 – PLE cell scheme (From: https://www.americanlaboratory.com/)

3.4.3 Solid Phase Extraction (SPE)

Solid Phase Extraction (SPE) is a routine sample preparation technique used in analytical laboratories for the extraction of analytes from complex matrices.

This sample preparation technique allows the extraction, purification and concentration of analytes that can be isolated from other compounds in the mixture according to their physicochemical properties, before their quantification. In fact, the difference in affinity between analyte and interference present in a liquid matrix allows the separation of the target analyte from the impurities.

The wide use of this procedure is due to the fact that the technique is fast simple and can be automated.

A typical solid phase extraction can be divided into four steps (Fig. 13):

1. First, the columns are balanced or conditioned with solvents in order to moisten the polymer;

2. Subsequently, the loading solution containing the analyte is made to through the polymer. The analyte and some impurities are retained in the column;

3. The polymer is then washed to remove impurities;

4. Finally, the analyte is collected during the elution process, an operation that consists in bringing a substance adsorbed by an adsorbent medium back into solution.

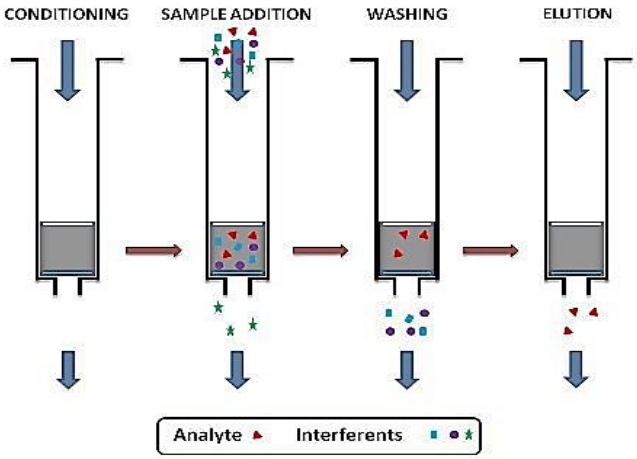


Fig. 13 – SPE clean-up procedure scheme (From: http://cdn.intechopen.com)

The process was performed using Superclean™ LC-Alumina-N SPE 6mL cartridges. The alumina was conditioned with 15 mL of a mixture of hexane/dichlorometane (1:2) as solvent. Then, the sample was percolated through the column. Subsequently, two 20 mL cycles of the mixture hexane/dichlorometane (1:2) were carried out to complete the elution process.

After the SPE technique, the extracts were evaporated to dryness and re-dissolved in 500 µL of ethyl acetate; then, they were sonicated for 3 minutes and filtered in Eppendorf vials through PTFE centrifuge filters (0.22 µm pore size) and centrifuged at 7900 rpm for 10 minutes to eliminate additional lipid interferences.

3.5 Sample analysis

3.5.1 Gas chromatography tandem Mass spectrometry (GC-MS/MS)

Gas chromatography (GC) is a separation technique capable of separating highly complex mixtures based primarily upon differences of boiling point/ vapor pressure and of polarity, while mass spectrometry (MS) is a detection technique that ionizes and fragments molecules.

When combined, these two techniques can be used to cover either a wide range of *m/z* ratios or to gather data for specific masses of interest, respectively. In addition to the quantification, GC-MS/MS is well suited for the identification of unknown volatile components using the mass fragmentation patterns and mass transitions associated with

The final steps of the process involve ion detection and analysis, where the analytes of interest are quantified through comparison to external or internal standards. Compound peaks figure as a function of their *m/z* ratios and peak heights are proportional to the quantity of the corresponding compound. A complex sample will produce several different peaks, due to the presence of many interferences.

The separation, identification and quantification of analytes were performed using gas chromatography (SCION 456-GC, Bruker) coupled to triple quadrupole mass spectrometry (SCION TQ from Bruker with CP 8400 Autosampler) (Fig. 14). Capillary gas chromatography analysis was carried out on a BR-5ms column (30 m × 0.25 mm i.d. × 0.25 µm film thickness), keeping the carrier gas flow (helium) at 1 mL min^{-1}, and the transfer line and the injection port temperatures at 280 °C. The column temperature ramp was as follows: 70 °C for 3.5 min, increased at 25 °C min^{-1} to 180 °C, then at 10 °C min^{-1} to 300 °C, and held for 4 min. Injection volume was 1 µL in splitless mode and the solvent delay was set to 4.5 min. The mass detector was operated in multiple reaction monitoring (MRM) mode using an electron ionization (EI) source set at 70 eV and argon as collision gas (2 mTorr). MS/MS parameters were optimized by injecting standards solutions, using full scan mode (m/z50–500) on a first step to select precursor ions (Q1) that were later fragmented into product ions (Q3) testing different collision energies (from 10 to 40 eV). (Pintado-herrera, González-mazo, & Lara-martín, 2016).

The target compounds were identified and quantified by comparing retention times, two transitions (one for quantification and one for confirmation) and their ion ratio for each analyte to those for commercially available pure standards. Calibration curves were constructed in ethyl acetate for each compound in the range of 5–500 ng mL^{-1}.

Before injection were added to the vials 10 µL of benzophenone deuterated 500 ppb like an internal standard to correct possible fluctuations in the MS signal (ion suppression) by comparing the signal intensities of the internal standards spiked in the calibration curve and in tuna sample extracts (Tab. 14). A derivatizing agent N-tert-Butyldimethylsilyl-N-methyl-trifluoroacetamide (MTBSTFA) was also added (10 µL) to the final extract. All the data were processed using the Bruker MS Workstation.

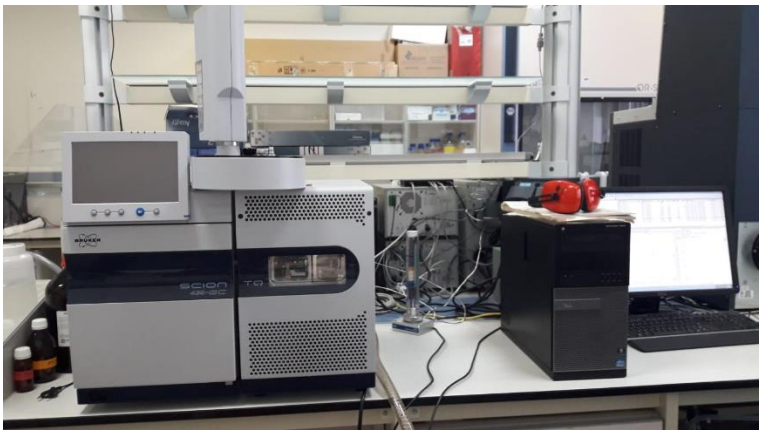


Fig. 14 – SCION TQ (Bruker) with CP8400 Autosampler

3.5.2 Statistical analysis

In order to verify the existence of a significant relation between different species and different tissues, the non-parametric multivariate permutational analysis of variance (PERMANOVA, in PRIMER with PERMANOVA+ v. 6, PRIMER-E Ltd., Plymouth, UK) on the Euclidean distance matrix was employed, calculated after data normalization, transformed to log (x + 1). PERMANOVA calculates a pseudo-F statistic that is directly analogous to the traditional F-statistic for multifactorial univariate ANOVA models and uses permutation procedures (here 9999 permutations) to obtain p-values for each term in the model. Significant differences ($P < 0.05$) were investigated using a post hoc pair-wise

comparisons with the PERMANOVA test. Furthermore, to evaluate similarities between different species and tissues, PCoA analysis was also conducted.

3.5.3 Quality control

The method used in this study allows the simultaneous determination of 98 compounds, priority substances and contaminants of emerging concern (Fig. 15). Only 21 compounds were detected in this study. Additionally, the method provides good sensitivity and selectivity due to the combined use of in-cell clean-up (to remove co-extracted non-target compounds) and tandem mass spectrometry (less susceptible to matrix interferences than most commonly used single quadrupole detectors).

Before the analysis of the samples, the aspects related to the analytical methodology were treated. Primarily, the limits of detection (mLOD) and the limits of quantification (mLOQ) of the method were calculated. They are defined as the lowest concentration of an analyte in a sample that can be detected (mLOD) and the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy (mLOQ). The mLODs were calculated using a signal-to-noise ratio (s/n) of 3:1, whereas mLOQs were calculated using a signal-to-noise ratio of 10:1, respectively by using the lowest concentration value of the analytes in the calibration curve.

Linearity of the GC-MS method was evaluated using a calibration curve with seven concentration levels (5 – 500 ng mL^{-1}). The method showed a good linearity with determination coefficients equal or higher than 0.99 for all the compounds investigated and good repeatability confirming the present method as useful to monitor compounds belonging to different chemical classes (Tab. 14). The recoveries ranged from 40 to 100% for UV filters; from 60 to 100% for pesticides and herbicides and from 86 to 100% for PCBs. Recoveries for fragrances and PAHs were 100%.

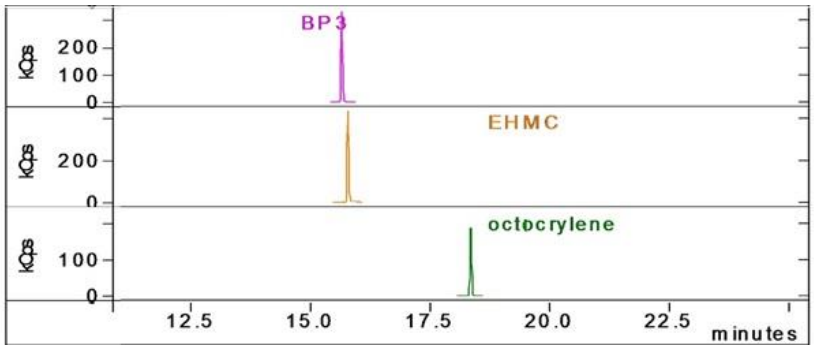


Fig. 15 - Examples of chromatograms of compounds analysed.

Tab. 14 - Analytical parameters: Compound name, Retention time, Precursor ion (Q1 first mass) and Product ions (Q3), Scan time, Calibration curve, Determination coefficients (R^2) and instrumental limits of detection (iLOD) and quantitation (iLOQ). Quantifier transitions are marked in bold.

Compound Name	Retention Time (min)	Q1 First Mass	Q3 First Mass	Collision Energy (V)	Scan Time (ms)	Calibration curve	R^2	iLOD (ng/g)	iLOQ (ng/g)
Polycyclic musks									
Tonalide	12.350	**243**	**187**	10	54	y=1.8712x+0.4712	0.9995	0.05	0.16
		243	159	20					
Galaxolide	12.310	**243**	**213**	15	54	y=1.9849x+0.8444	0.9995	0.04	0.13
		243	171	20					
Other fragrances									
OTNE	10.790	**191**	**109**	10	150	y=0.7719x+0.3023	0.9974	0.24	0.79
		191	121	20					
UV filters									
Homosalate (HMS)	15.590	**195**	**177**	20	54	y=0.9134x-3.0727	0.99	0.05	0.17
		195	159	30					
		195	115	40					
Octocrylene (OC)	18.820	**248**	**165**	30	125	y=0.4926x+0.2168	0.9959	0.16	0.54
		232	176	20					
4-methylbenzylidene camphor (4-MBC)	14.060	**254**	**155**	30	68	y=0.2587x+0.1460	0.9987	0.58	1.92
		254	239	10					
		254	211	10					
		254	105	40					
Benzophenone 3 (BP3)	16.120	**285**	**242**	30	63	y=8.2513x+0.0660	0.9981	0.02	0.06
		285	241	40					
		285	212	40					
Ethyl hexyl salycilate (EHS)	14.870	**195**	**159**	30	58	y=2.6605x+0.2369	0.9993	0.01	0.03
		195	115	40					
2-ethylhexyl methoxycinnamate	16.060	**178**	**132**	20	63	y=2.1894x-0.7777	0.99	1.07	3.57

(EHMC)		178	161	20					
PAHs									
Phenanthrene	11.860	178	152	25	54	y=2.3181-1.4192	0.99	7.5	25
		178	176	30					
		178	177	10					
Fluoranthene	14.26	**202**	**200**	30	58	y=4.2719x-2.0610	0.99	2.5	8.33
		202	152	32					
		202	201	15					
Pyrene	14.740	**202**	**200**	35	58	y=5.8141x-3.0338	0.99	2	6.67
		202	151	45					
		202	201	15					
PCBs									
PCB-101	14.620	**326**	**256**	30	58	y=2.3884x+0.0510	0.9998	0.07	0.23
		326	291	15					
PCB-138	16.100	**360**	**290**	30	63	y=2.3615x+0.1455	0.9997	0.05	0.18
		360	325	15					
PCB-153	17.8	**360**	**325**	15	83	y=0.3114x+0.0501	0.9996	0.33	1.11
		360	290	32					
PCB-180	17.82	**394**	**324**	32	83	y=2.0677x+0.0734	0.999	0.07	0.23
		394	359	15					
PCB-52	13.110	**292**	**222**	30	63	y=2.3079x+0.0191	0.9998	0.09	0.31
		292	257	15					
Organochlorine pesticides									
p,p'-DDD	15.850	**235**	**165**	25	75	y=6.5536x+0.7486	0.9995	0.16	0.55
		235	199	15					
p,p'-DDE	15.100	**246**	**176**	30	58	y=3.7429x+0.3419	0.9998	0.04	0.13
		318	246	25					
p,p'-DDT	16.530	**235**	**165**	15	63	y=1.8081x+0.0113	0.9992	0.21	0.7
		235	199	15					
Triazines									
Ametryn	12.710	**227**	**185**	10	54	y=1.1295x+0.0364	0.9997	0.68	2.27

4. RESULTS AND DISCUSSIONS

4.1 Differences between species and within the same species

The method used was applied to the analysis of 63 tissues of liver and muscle (31 liver; 32 muscle) belonging to three different tuna species: the Atlantic bonito *Sarda sarda*, the Skipjack tuna *Katsuwonus pelamis* and the Atlantic Bluefin tuna *Thunnus thynnus*, in order to evaluate the occurrence of legacy and emerging contaminants (POPs and ECs) in these species and, as a result, in predators and top predators of the marine food chain.

The preliminary PERMANOVA analysis included the comparison between the three species and the two tissues examined. The three species differ significantly, as well as the two tissues (Tab. 15, Fig. 16). Post-hoc analysis showed which species exhibit significant differences (Tab. 16, Tab. 17); and which tissues exhibited significant differences within the same species (Tab. 18).

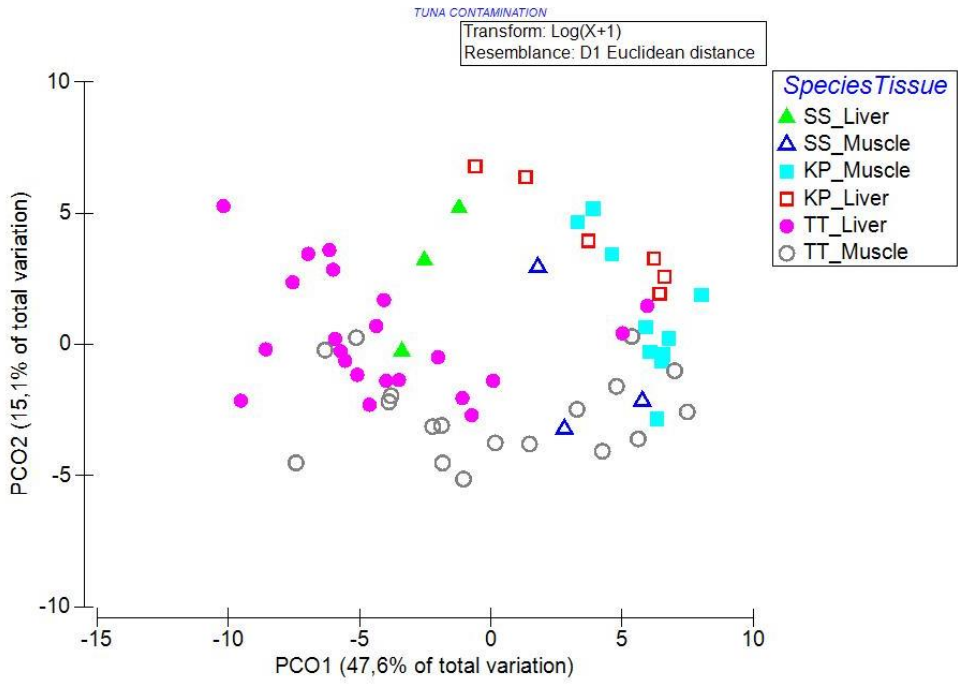


Fig. 16 – Principal Coordinate Analysis (PCoA) regarding the spatial distribution of *T. thynnus* (TT), *K. pelamis* (KP) and *S. sarda* (SS) samples analysed according to species-tissue interaction.

Tab. 15 – Summary of PERMANOVA test on legacy and emerging contaminants mean concentrations (ng g^{-1} d.w.) in tuna species (*Sarda sarda*, *Katsuwonus pelamis*, *Thunnus thynnus*) tissues (liver and muscle). Only significant values (*P* <0.05) are highlighted in bold: * = *P* <0.05; ** = *P* <0.01; * = *P* <0.001.**

PERMANOVA table of results					**UV filters**			**Fragrances**			**Pesticides**			**PCBs**			**PAHs**		
Source	df	SS	Pseudo-F	P(perm)	SS	Pseudo-F	P(perm)	SS	Pseudo-F	P(perm)	SS	Pseudo-F	P(perm)	SS	Pseudo-F	P(perm)	SS	Pseudo-F	P(perm)
Species	2	760.72	9.9475	**0.001**	48.699	16.734	0.158	26.036	27.883	**0.034**	229.01	11.653	**0.001**	390.47	30.913	**0.001**	66.507	11.565	**0.001**
Tissue	1	279.74	7.3161	**0.001**	25.742	17.691	0.155	37.552	80.434	**0.004**	133.08	13.543	**0.001**	71.265	11.284	**0.001**	12.102	4.2091	**0.022**
SpxTi	2	81.548	1.0664	0.37	28.239	0.97035	0.443	13.022	13.946	0.223	8.0092	0.40753	0.834	16.041	1.2699	0.263	16.237	2.8236	**0.033**
Res	56	2141.3			814.84			261.45			550.29			353.67			161.01		
Total	61	3506.3			926.43			356.84			1023.1			939.74			260.21		

Tab. 16 – Summary of PAIR-WISE TEST, Term 'SpxTi' for pairs of levels of factor '*Species*', Within level '*Liver*' of factor '*Tissue*' (SS= *Sarda sarda*; KP= *Katsuwonus pelamis*; TT= *Thunnus thynnus*). Only significant values (*P* <0.05) are highlighted in bold: * = *P* <0.05; ** = *P* <0.01; * = *P* <0.001.**

			Fragrances		**Pesticides**		**PCBs**		**PAHs**	
Groups	t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)
SS, KP	1.8774	**0.033**	1.3121	0.186	3.1681	**0.008**	3.9002	**0.017**	0.59994	0.527
SS, TT	1.3802	0.106	1.7014	0.052	0.96584	0.447	1.7067	0.098	2.8081	0.052
KP, TT	3.2149	**0.001**	1.5994	0.102	3.2106	**0.002**	5.9203	**0.001**	5.5896	**0.001**

Tab. 17 – Summary of PAIR-WISE TEST, Term 'SpxTi' for pairs of levels of factor '*Species*', Within level '*Muscle*' of factor '*Tissue*' (SS= *Sarda sarda*; KP= *Katsuwonus pelamis*; TT= *Thunnus thynnus*). Only significant values (*P* <0.05) are highlighted in bold: * = *P* <0.05; ** = *P* <0.01; * = *P* <0.001.**

			Fragrances		Pesticides		PCBs		PAHs	
Groups	t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)
SS, KP	1.4489	0.058	0.58359	0.878	3.4166	**0.008**	4.3477	**0.004**	0.57176	0.721
SS, TT	1.3241	0.124	1.1542	0.249	1.3156	0.172	1.6313	0.129	1.7861	0.051
KP, TT	2.8705	**0.001**	0.68373	0.643	3.1621	**0.001**	4.7915	**0.001**	2.9384	**0.002**

Tab. 18 – Summary of PAIR-WISE TEST, Term 'SpxTi' for pairs of levels of factor '*Tissue*', Within levels 'SS', 'KP' and 'TT' of factor '*Species*'. Only significant values (*P* <0.05) are highlighted in bold: * = *P* <0.05; ** = *P* <0.01; * = *P* <0.001.**

S. sarda			**Fragrances**		**Pesticides**		**PCBs**		**PAHs**	
Groups	t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)
Liver, Muscle	1.5842	0.097	1.5219	0.203	1.453	0.19	3.1826	0.102	0.70458	0.494

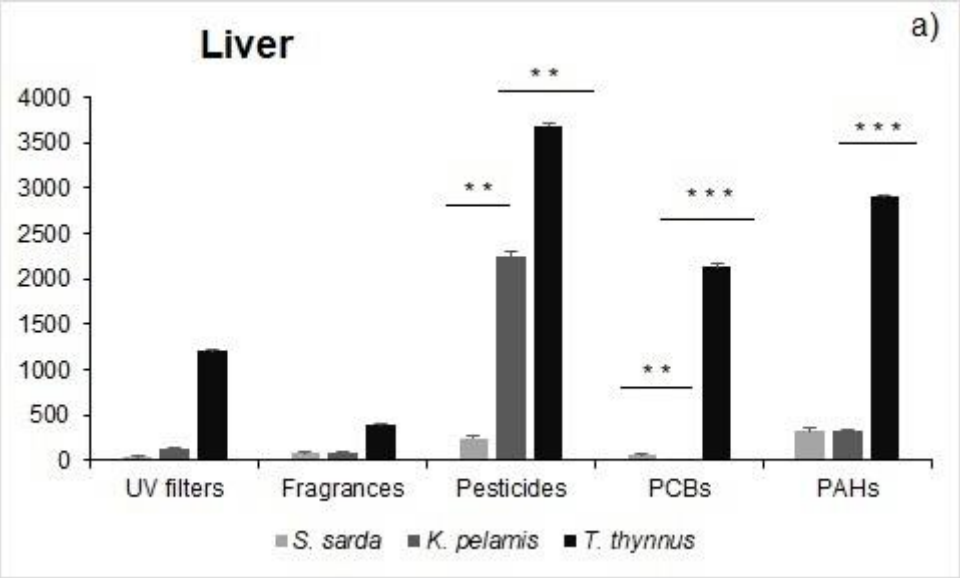
K. pelamis			**Fragrances**		**Pesticides**		**PCBs**		**PAHs**	
Groups	t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)
Liver, Muscle	2.0615	**0.003**	0.96295	0.451	6.4097	**0.001**	2.6643	**0.002**	1.7474	0.053

T. thynnus			**Fragrances**		**Pesticides**		**PCBs**		**PAHs**	
Groups	t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)
Liver, Muscle	2.8177	**0.001**	3.3929	**0.001**	3.3293	**0.001**	3.4134	**0.001**	2.2057	**0.002**

Post-hoc analysis of POPs and ECs showed significantly differences between the three tuna species and the tissues analysed (Tab. 15), revealing that legacy contaminants (POPs) are responsible for significant differences between and within species (Tab. 16, 17, 18).

The differences of ECs and POPs bioaccumulation patterns may have been influenced by the different half-lives of these substances, as well as the the different physico-chemical properties (i.e. partitioning properties: Kow). Tuna can eliminate both ECs and POPs by means of continuous exchange between the blood and seawater through the gills (Corsolini et al., 2014), but due to the different physico-chemical properties of ECs and POPs in this bony fish, ECs seem to be eliminated more efficiently than POPs. Actually, bony fishes can partially metabolize POPs via the detoxifying activity of hepatic enzymes, and also gonads can be a pathway of elimination in large predator fish species (Corsolini et al., 2014).

The physico-chemical properties of the contaminants analysed, their organotropism, the typical characteristics of tissues, biometric parameters, species' different diets and feeding areas are probably the main factors influencing their partitioning into the two tissues of the three tuna fish species (Fig. 17, Fig. 18).



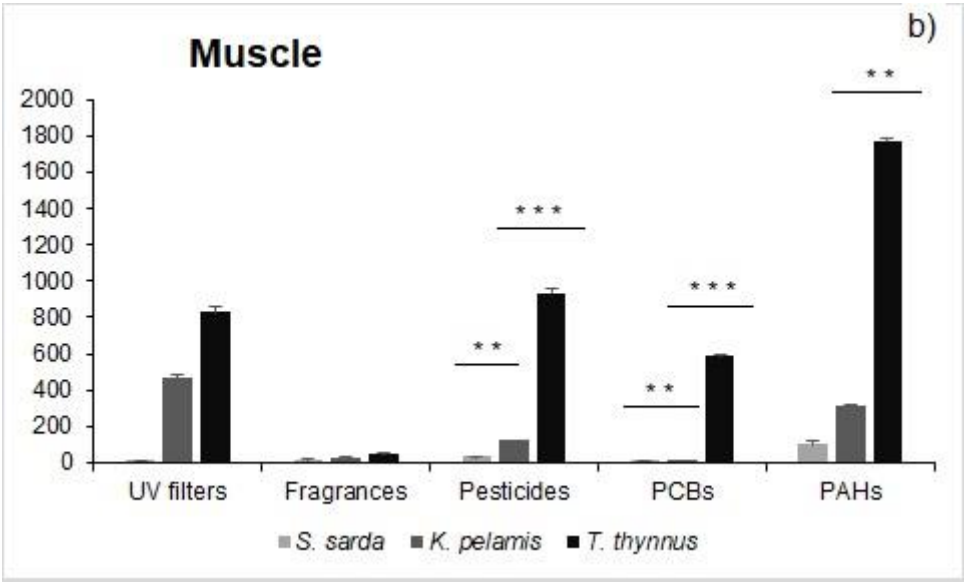
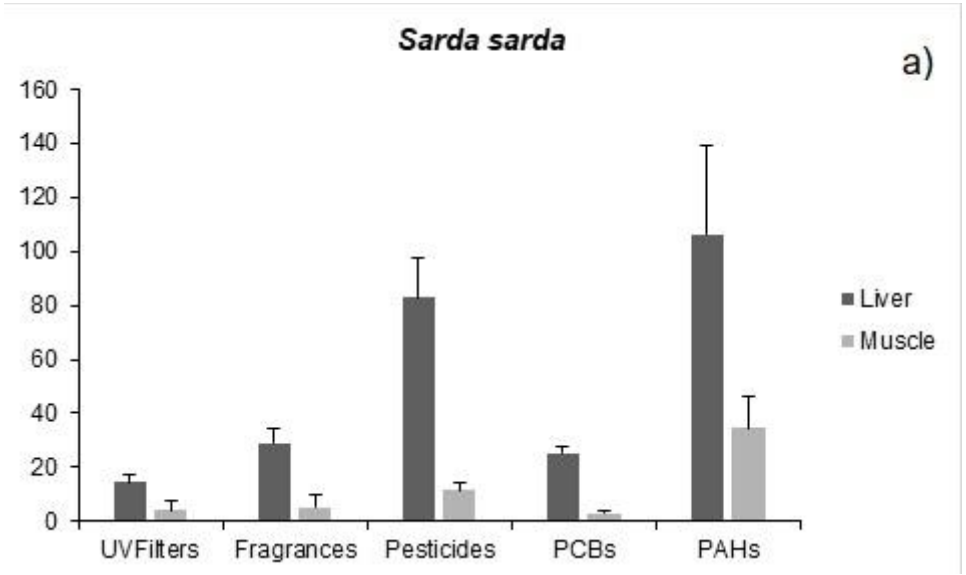


Fig. 17 – Legacy and emerging contaminants (mean concentration ng g^{-1} dw) in liver (a) and muscle (b) of *S. sarda*, *K. pelamis* and *T. thynnus*. Highlighted significant differences between tissues (*= *P* <0.05; ** = *P* <0.01; * = *P* <0.001).**



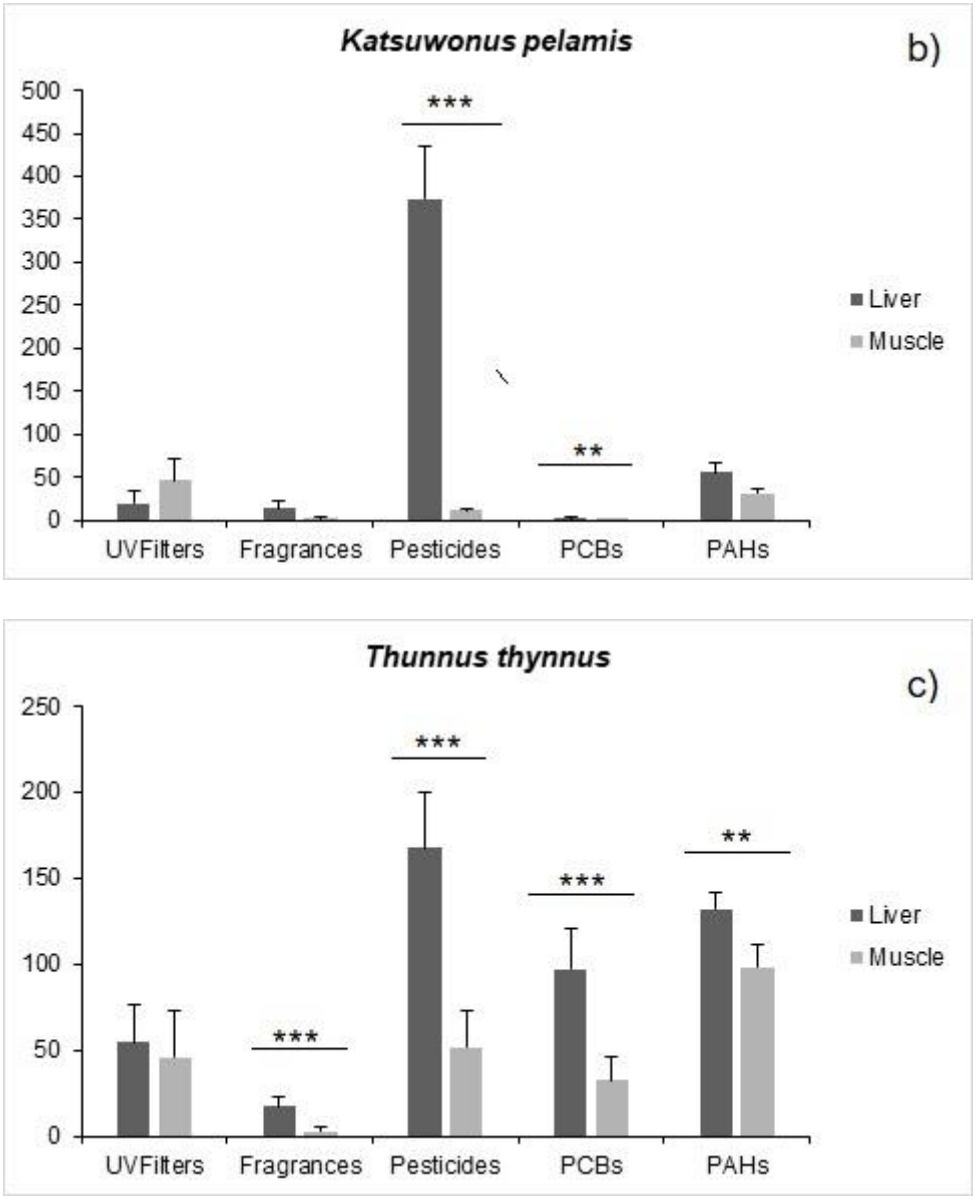


Fig. 18 – Legacy and emerging contaminants (mean concentration ng g^{-1} dw) in liver and muscle of *Sarda sarda* (*a*), *Katsuwonus pelamis* (*b*) and *Thunnus thynnus* (*c*). Highlighted significant differences between tissues (*= *P* <0.05; ** = *P* <0.01; * = *P* <0.001).**

The liver is recognized as the target organ where the contaminants tend to concentrate. It is well known that the liver plays an important role in distribution and detoxification or transformation of xenobiotics, and constitutes an important site of pathological effects induced by these contaminants (Di Bella et al., 2006).

Actually, the liver is the principal organ involved in digestion by secreting enzymes that destroy fats, it also serves as a storage area for fats and carbohydrates. Its functions include detoxification, protein synthesis and the production of biochemicals necessary for digestion. Contamination by organic and inorganic compounds is very frequent in liver as

these compounds can accumulate over time. Because of its detoxification capacity and storage of harmful compounds, the liver is often used as an environmental biomarker (Maisano et al., 2016).

Muscle is the most abundant tissue in fish (about 97%), mainly involved in movement and swimming. In particular, with regard to *Thunnus thynnus* which is a highly migratory species, the red muscles are located near the spinal column and the blood vessels are arranged in such a way as to encourage a countercurrent exchange of heat, leading to an increase in temperature in the central region of the swimming muscles, which is significantly higher than that of external water.

The different distribution of contaminants in the liver and muscle is not only due to the biometric parameters, but also to the species' typical characteristics (Ramos-Miras et al., 2019). Feeding habits and migratory patterns, and therefore the geographical areas that are used by the tuna fish species, are aspects that can contribute to the different accumulation patterns of these substances. The body size and life span order is the following: Bluefin tuna (*Thunnus thynnus*) > Skipjack tuna (*Katsuwonus pelamis*) > Atlantic bonito (*Sarda sarda*). The higher tropic position and longer life span of bluefin tuna are likely to be responsible for the higher levels of ECs and POPs accumulated into its tissues.

Regarding feeding habit and migratory patterns, *Sarda sarda* is a small tuna species with a maximum fork length of 91.4 cm, a maximum body weight of 5.4 kg, with a maximum reported age of 5 years. Average fork length is 50 cm and body weitgh is about 2 kg. *Sarda sarda* is a migratory species distributed in the Western and Eastern tropical and subtropical Atlantic Ocean, from Oslo in Norway to Port Elisabeth in South Africa in the Eastern Atlantic, including the Mediterranean and Black Sea (Relini, Garibaldi, & Lanteri, 2005).

Sarda sarda is an epi-pelagic marine species which lives in schools along the neritic area (up to 80-200 m) and may also enter estuaries. Adult of *S. sarda* primary predatory habits are on adult specimens within small schooling fishes, especially clupeoids such as anchovy, sardine, and sprat, and also on crustaceans (the choice of species depending on the locality). In the eastern Atlantic Ocean, the diet is based on *Sardinella* sp. and *Engraulis* sp. , and both the juveniles and the adults are known to be cannibalistic (Baibbat, Malouli, & Abid, 2016).

Little is known about bonito migration patterns. Some studies suggest that is a resident species in the western Mediterranean Sea all over the year and the mature fish migrate from coastal areas to open sea to spawn. Furthermore, tagged *S. sarda* specimens from the western Mediterranean were found in the Atlantic, both South and North of the Strait of Gibraltar, from Morocco to Portugal (Relini et al., 2005; Valeiras, Jose, & Vives, 2008; Tangen & Bjelland, 2013). Hence, specimens from the Western-Central Mediterranean are apparently not confined to the Mediterranean Sea basin, although it cannot be excluded that Atlantic specimens (which have their spawning grounds along the Atlantic coast of Morocco) move across the Strait Gibraltar toward the Mediterranean basin.

Regarding feeding habit and migratory patterns, *Katsuwonus pelamis* is a cosmopolitan and migratory tuna species, with a maximum fork length of 111 cm and body weight of 34.5 kg. This species is very fast-growing, short-lived (longevity between 6–8 years), and very fecund (size at first maturity: 40–55 cm FL, depending on the area) (Collette and Nauen 1983). *Katsuwonus pelamis* is widely distributed from offshore waters to open seas in tropical and temperate regions in many areas of the world, including the Pacific, Atlantic, and Indian Oceans, but is mostly abundant in the equatorial region throughout the year (Ueno et al., 2006). Their migration rate between areas and their underlying migration route remain a concern to improve fisheries management (Kiyofuji et al., 2019).

Katsuwonus pelamis exhibit a strong tendency to school in surface waters. Aggregations of this species tend to be associated with convergences, boundaries between cold and warm water masses (i.e. the polar front), upwelling and other hydrographical discontinuities. Depth distribution ranges from the surface to about 260 m during the day, but it is limited to near surface waters at night (Benevenuti Soares et al., 2019).

Their principal preys include fishes, crustaceans and molluscs. Even though marine teleosts belonging to the families Carangidae and Balistidae are part of the diet of this tuna species in all oceans, the wide variety of species taken, suggest it to be an opportunistic feeder preying on any forage available. The feeding activity peaks in the early morning and in the late afternoon. Cannibalism is common. The principal predators of skipjack are other tunas and billfishes (Collette and Nauen, 1983).

Regarding feeding habit and migratory patterns, *Thunnus thynnus* is a pelagic species, with a maximum size over 300 cm fork length (average 200 cm) and a maximum body

weight of 600 kg. In the wild, *T. thynnus* are expected to live about 15 years (longest lifespan known in the wild is between 20 and 30 years) (Rooker et al., 2007).

Furthermore, *T. thynnus* usually dive between 200 and 300 m deep and occasionally deeper, up to 1000 m but seasonally, they can be found close to shore and can tolerate a wide range of temperatures (Wilson & Block, 2009).

This species schools by size, sometimes together with other tuna species. It preys on small schooling fishes (anchovies, sauries, hakes) or on squids and red crabs. Juveniles of *T. thynnus* prey mainly on zooplankton and small pelagic coastal fishes, sub-adults prey on medium pelagic fishes, shrimps and cephalopods, while adults prey mainly on cephalopods and larger fishes (Sarà & Sarà, 2007).

Both juvenile and adult tunas are used to spending time on large ocean fronts where abundant primary production, capable of sustaining high levels of biomass (especially small pelagic species and cephalopods), is induced. These upwelling areas are located along the coasts of Morocco and Portugal. In the Mediterranean there are areas where, due to the action of winds or currents, upwelling is possible on a seasonal basis, too.

T. thynnus individuals perform trans-Atlantic migrations between the Mediterranean and western North Atlantic (Gulf of Mexico) stocks. They migrate under the influence of reproductive needs, entering the Mediterranean passing through the Strait of Gibraltar and following the Atlantic current of water, which is shallower, colder and less salty than the waters of the Mediterranean (Rooker et al., 2008; Wilson & Block, 2009). Once the reproduction has taken place, they migrate again towards their native area. The tunas return, for reproduction, just in the same places where they were previously. This fidelity to the spawning site has been demonstrated in both the Mediterranean and the Gulf of Mexico spawning areas (Rooker et al., 2008). In addition, there are genetically recognizable populations within the Mediterranean (Block et al., 2005).

Every spring, *T. thynnus* cross the Strait of Gibraltar from the Atlantic Ocean heading for spawning grounds in the Mediterranean Sea. From mid-July, many of these tunas start a post-reproductive migration back to feeding areas in the North Atlantic Ocean. This *T. thynnus* population may consist of individuals from different sources: I) fish arrived from Mediterranean spawning grounds after completing spawning; II) permanent residents in the area, and III) individuals in excess of the fishing quota that are eventually released after weeks of confinement in traps (Sorell et al., 2017). Besides being a transit passage

for many migratory species such as tuna, the Strait of Gibraltar is also used as an important foraging area by many marine animals. Adult and sub-adult *T. thynnus* use waters of the Gulf of Cadiz including the Strait of Gibraltar as a feeding ground after the spawning season (Sorell et al., 2017)**.**

In tuna fish species, adipose tissue and muscle are the most important energy storage compartments and, consequently, the main tissues legacy contaminants accumulate in. Several physiological mechanisms, such as lipid mobilization during starvation periods, may affect tissue concentrations as the trophic transfer of legacy contaminants and lipids may follow similar pathways (Sprague et al., 2012b). *T. thynnus* entering spawning grounds through the Strait of Gibraltar often arrive with empty stomachs, relying upon accumulated lipid stores to fuel gonadal development and spawning migration (Sorell et al., 2017)**.**

In general, fish store energy primarily in the form of triacylglycerols (TAG) in tissues such as muscle, liver and mesenteric adipose tissue. Lipid, and thus TAG levels within fish tissues varies according to nutritional state based upon age, sex and developmental or reproductive status. Seasonal variations in fish lipid levels are generally related to the reproductive cycle and, prior to sexual maturation, large lipid deposits are accumulated and subsequently mobilized to support gonadal development and spawning migration (Sprague et al., 2012b).

On the contrary, liver tissue in migratory fish such as *Thunnus thynnus* is not a lipid storage site, instead functioning as a high capacity site for lipid processing and *de novo* lipid synthesis. During migration, lipid stores are depleted and used for gonadal development and energy metabolism and, whereas physiologically required lipids are selectively regulated in animals, lipophilic compounds are passively bioaccumulated and their concentrations not regulated. The high lipid normalized content of POPs such as dl-PCBs in the liver may therefore be a result of accumulation after transport of lipid from other tissues for physiological and metabolic processes (Sprague et al., 2012b).

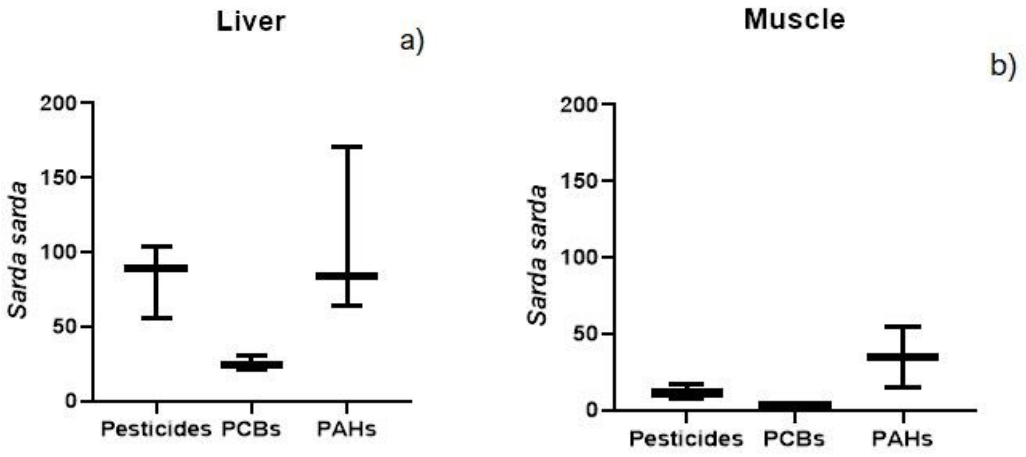
4.2 Occurence of legacy contaminants in tuna species

Post-hoc comparison showed significant differences among different classes of legacy compounds and between tissues (Tab. 16, Tab. 17, Tab. 18). In general, the liver is the tissue displaying the highest concentrations. Moreover, the three tuna species show different concentration patterns in terms of classes of legacy compounds (Tab. 19).

With reference to legacy compound partitioning between tissues in *S. sarda*, PAH and pesticides had higher concentrations than PCBs in the liver tissue, whereas PAHs were higher than pesticides and PCBs in the muscle tissue (Fig. 19, *a* and *b*).

With reference to legacy compound partitioning between tissues in *K. pelamis*, pesticides had higher concentrations than PCBs and PAHs in the liver tissue, whereas PAHs were higher than pesticides and PCBs in the muscle tissue (Fig. 19, *c* and *d*).

With reference to legacy compound partitioning between tissues in *T. thynnus*, pesticides had higher concentrations than PCBs and PAHs in the liver tissue, whereas PAHs were higher than pesticides and PCBs in the muscle tissue (Fig. 19, *e* and *f*).



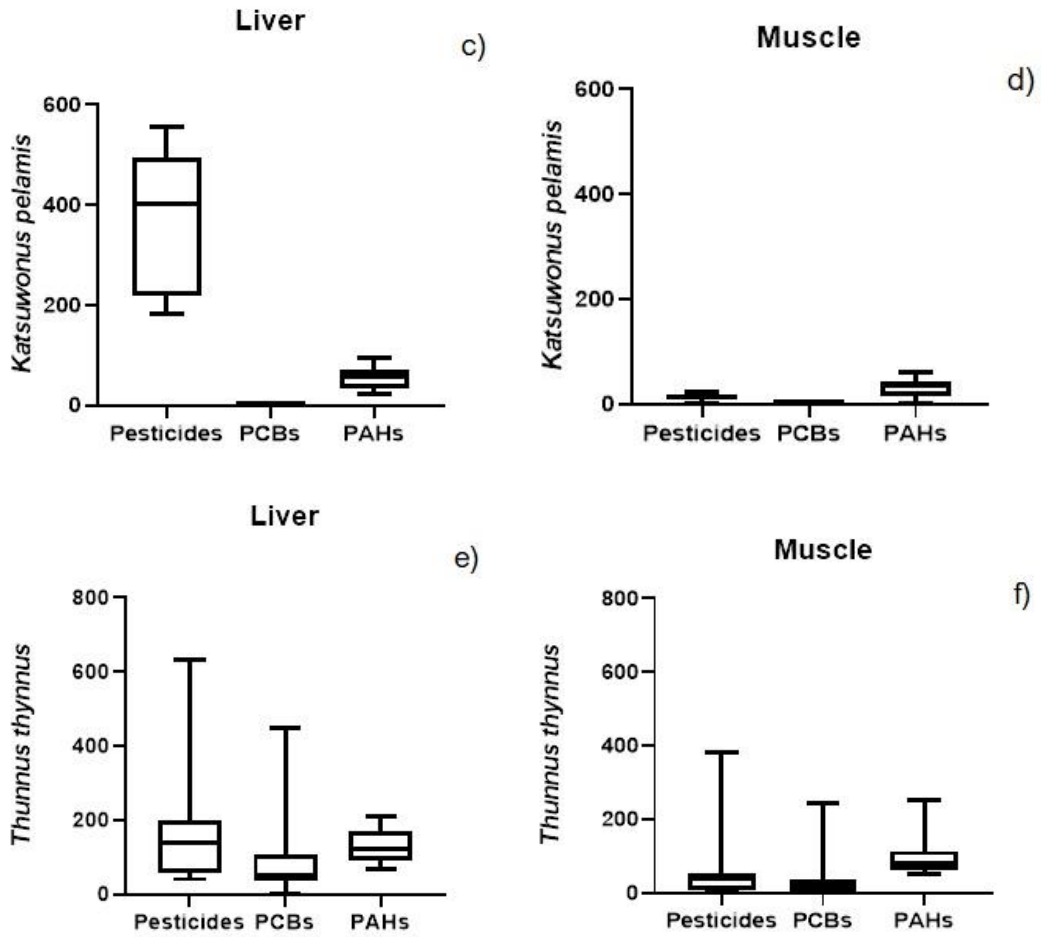


Fig. 19 - Box- plots of legacy contaminants (mean concentration ng g^{-1} d.w.) in liver and muscle of *Sarda sarda* (graphs *a* and *b*), *Katsuwonus pelamis* (graphs *c* and *d*), *Thunnus thynnus* (graphs *e* and *f*).

Tab. 19 – Legacy contaminants concentrations (ng g^{-1} dry weight) in tissues (liver and muscle) of three tuna species (*S. sarda, K. pelamis, T. thynnus*), expressed in minimum value, maximum value, mean value and standard deviation (SD).

			LEGACY CONTAMINANTS											
			PESTICIDES				PCBs					PAHs		
SPECIES	Tissue		p,p'-DDD	p,p'-DDE	p,p' -DDT	Ametryn	PCB101	PCB138	PCB153	PCB180	PCB52	Flu	Phe	Pyr
Sarda sarda	Liver	Min	14.63	188.75	<LOD	<LOD	<LOD	33.00	28.38	25.11	<LOD	45.92	<LOD	31.93
Sarda sarda	Liver	Max	38.72	283.51	17.97	129.61	2.20	66.66	54.58	36.21	8.34	111.48	317.21	84.49
Sarda sarda	Liver	Mean	29.92	220.96	5.99	75.12	1.08	49.44	43.68	28.92	2.78	88.57	163.58	66.58
Sarda sarda	Liver	SD	13.29	54.18	10.37	67.22	1.10	16.84	13.64	6.31	4.82	36.97	158.84	30.02
Sarda sarda	Muscle	Min	6.90	19.65	<LOD	<LOD	<LOD	0.93	<LOD	0.81	1.56	21.27	<LOD	16.03
Sarda sarda	Muscle	Max	10.38	44.73	<LOD	31.40	1.01	9.86	9.90	4.99	2.74	35.71	102.40	26.74
Sarda sarda	Muscle	Mean	8.21	31.71	<LOD	11.58	0.26	5.20	6.90	2.75	1.97	26.33	60.85	20.33
Sarda sarda	Muscle	SD	1.54	11.14	<LOD	14.97	0.50	4.06	4.65	2.23	0.53	6.42	43.38	4.55
Katsuwonus pelamis	Liver	Min	<LOD	23.91	<LOD	693.68	<LOD	<LOD	<LOD	<LOD	<LOD	30.09	<LOD	23.84
Katsuwonus pelamis	Liver	Max	28.82	55.54	<LOD	2181.01	0.80	5.14	15.59	2.34	6.65	101.81	203.07	65.78
Katsuwonus pelamis	Liver	Mean	13.70	37.25	<LOD	1445.68	0.13	1.46	6.09	0.95	2.81	68.44	54.32	45.27
Katsuwonus pelamis	Liver	SD	9.73	12.24	<LOD	589.99	0.33	2.03	7.07	1.13	2.53	27.84	79.23	16.13
Katsuwonus pelamis	Muscle	Min	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Katsuwonus pelamis	Muscle	Max	<LOD	11.22	<LOD	84.72	0.09	0.82	<LOD	0.50	1.34	42.44	109.90	31.13
Katsuwonus pelamis	Muscle	Mean	<LOD	4.61	<LOD	44.96	0.02	0.08	<LOD	0.08	0.46	21.11	55.91	16.40
Katsuwonus pelamis	Muscle	SD	<LOD	3.55	<LOD	25.30	0.04	0.26	<LOD	0.17	0.57	11.07	41.00	8.73
Thunnus thynnus	Liver	Min	<LOD	<LOD	<LOD	<LOD	0.24	4.50	<LOD	<LOD	0.05	0.48	114.21	48.44
Thunnus thynnus	Liver	Max	135.93	2305.78	81.27	861.50	120.06	1059.80	458.09	606.47	8.21	12.31	469.59	108.19
Thunnus thynnus	Liver	Mean	25.16	444.94	24.30	175.85	26.79	235.51	92.03	129.33	2.21	5.45	268.69	72.31
Thunnus thynnus	Liver	SD	37.40	512.31	19.24	269.89	27.91	254.51	121.08	150.50	1.85	4.24	113.64	18.16
Thunnus thynnus	Muscle	Min	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	33.77	<LOD	<LOD
Thunnus thynnus	Muscle	Max	61.36	1354.82	109.68	136.77	67.21	593.17	240.85	317.92	3.97	134.93	450.88	271.87
Thunnus thynnus	Muscle	Mean	4.64	173.56	17.63	12.35	9.07	82.81	26.37	43.79	0.88	63.16	156.32	75.56
Thunnus thynnus	Muscle	SD	15.09	315.59	25.08	32.35	15.38	137.26	56.45	72.94	0.93	30.90	107.42	67.71

As far as Polychlorinated biphenyls (PCBs) are concerned, there are significant differences both between species and between the two tissues (Tab. 16, Tab. 17, Tab. 18).

The 5 PCBs congeners were always found at detectable levels in tuna fish species and tissues, except the congener 153 in *Katsuwonus pelamis* muscle, which was found to be at non detectable concentrations. In general, average levels of PCBs (as a sum of the 5 congeners) found in the three tuna fish species were significantly higher in liver than in the muscle tissue (Fig. 17, Fig. 18; Fig. 20).

Congener levels in *Sarda sarda* are in the following order: PCB 138 (49.4 ± 16.84 ng g^{-1} dw) > PCB 153 (43.7 ± 13.64 ng g^{-1} dw) > PCB 180 (28.9 ± 6.31 ng g^{-1} dw) > PCB 52 (2.8 ± 4.82 ng g^{-1} dw) > PCB 101 (1.1 ± 1.10 ng g^{-1} dw) in the liver tissue, and PCB 153 (6.9 ± 4.65 ng g^{-1} dw) > PCB 138 (5.2 ± 4.06 ng g^{-1} dw) > PCB 180 (2.7 ± 2.23 ng g^{-1} dw) > PCB 52 (1.9 ± 0.53 ng g^{-1} dw) > PCB 101 (1.9 ± 0.50 ng g^{-1} dw) in the muscle tissue.

Congener levels in *Katsuwonus pelamis* are in the following order: PCB 153 (6.1 ± 7.07 ng g^{-1} dw) > PCB 52 (2.9 ± 2.53 ng g^{-1} dw) > PCB 138 (1.5 ± 2.03 ng g^{-1} dw) > PCB 180 (0.9 ± 1.13 ng g^{-1} dw) > PCB 101 (0.1 ± 0.33 ng g^{-1} dw) in the liver tissue, and PCB 52 (0.5 ± 0.57 ng g^{-1} dw) > PCB 138 (0.1 ± 0.26 ng g^{-1} dw) and PCB 180 (0.1 ± 0.17 ng g^{-1} dw) > PCB 101 (0.02 ± 0.04 ng g^{-1} dw) > PCB 153 n.d. in the muscle tissue.

Congener levels in *Thunnus thynnus* are in the following order: PCB 138 (235.5 ± 254.51 ng g^{-1} dw) > PCB 180 (129.3 ± 150.50 ng g^{-1} dw) > PCB 153 (92.1 ± 121.08 ng g^{-1} dw) > PCB 101 (26.8 ± 27.91 ng g^{-1} dw) > PCB 52 (2.2 ± 1.85 ng g^{-1} dw) in the liver tissue, and PCB 138 (82.8 ± 137.26 ng g^{-1} dw) > PCB 180 (43.8 ± 72.94 ng g^{-1} dw) > PCBs 153 (26.4 ± 56.45 ng g^{-1} dw) > PCB 101 (9.1 ± 15.38 ng g^{-1} dw) > PCB 52 (0.88 ± 0.93 ng g^{-1} dw) in the muscle tissue.

Congeners 138 showed the highest concentrations both in the liver and muscle tissue of Thunnus thynnus (235.5 ± 254.51 ng g^{-1} dw and 82.8 ± 137.26 ng g^{-1} dw) when compared to *S. sarda* liver (49.4 ± 16.84 ng g^{-1} dw) and muscle (5.2 ± 4.06 ng g^{-1} dw), and *K. pelamis* liver (1.5 ± 2.03 ng g^{-1} dw) and muscle (0.1 ± 0.26 ng g^{-1} dw).

Conversely, congener 52 concentrations are found at similar low levels in the liver tissue of *S. sarda*, *K. pelamis* and *T. thynnus*, and resulted 1 order of magnitude higher in *S. sarda* (1.9 ± 0.53 ng g^{-1} dw) than in *K. pelamis* (0.5 ± 0.57 ng g^{-1} dw) and *T. thynnus* (0.88 ± 0.93 ng g^{-1} dw) muscle tissue.

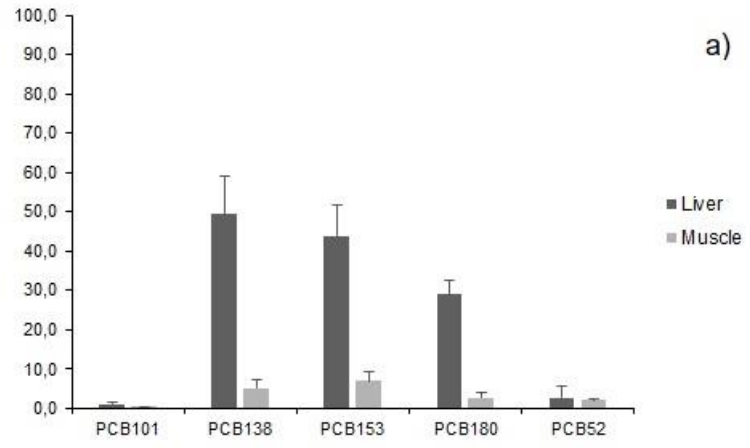
In general, these concentrations of congeners 101, 138, 153, and 180 are 1 to 3 orders of magnitude higher in the liver tissue of *Thunnus thynnus* (26.8 ± 27.91 ng g^{-1} dw; 235.5 ± 254.51 ng g^{-1} dw; 92.1 ± 121.08 ng g^{-1} dw; 129.3 ± 150.50 ng g^{-1} dw, respectively) than in the liver tissue of *Sarda sarda* (1.1 ± 1.10 ng g^{-1} dw; 49.4 ± 16.84 ng g^{-1} dw; 43.7 ± 13.64 ng g^{-1} dw; 2.7 ± 2.23 ng g^{-1} dw, respectively) and *Katsuwonus pelamis* (0.1 ± 0.33 ng g^{-1} dw; 1.5 ± 2.03 ng g^{-1} dw; 6.1 ± 7.07 ng g^{-1} dw; 0.9 ± 1.13 ng g^{-1} dw, respectively)(Tab.5, Fig. 4).

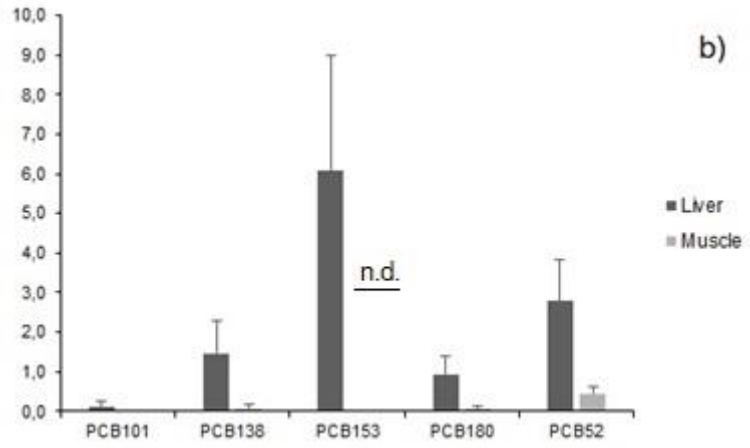
The PCB congeners bioaccumulation trend resulting from this study is in agreement with other studies carried out on specimens of *Thunnus thynnus*. Panseri et al. (2019) found that 138 and 180 congeners were the highest among the 6 indicators PCBs in *Thunnus thynnus* muscle from western Mediterranean. Di Bella et al. (2006) reported PCB138 as the most abundant congener followed by congeners 153 and 180 both in liver and in the muscle tissues of *T. thynnus* from the Straits of Messina, whereas Corsolini et al., 2007 found that congener 153 was predominant with respect to congeners 138 and 180 in *T. thynnus* muscle tissue from the western coast of Sicily. Also Gómara et al. (2005) reported higher concentrations for congener 153 followed by 138 and 180 congeners tin tuna fish muscle from Spanish markets. Literature PCBs data refer to a broad range of tuna size classes and PCB congeners analysed.

Hexa-chlorinated congeners 138 and 153 and hepta-chlorinated congener 180 have a chlorine atom in the 2, 4, or 5 position on one or both of the biphenyl rings, and such chemical structures make the PCB congeners resistant to the metabolic degradation in invertebrates and fish, and thus tend to accumulate mostly in tissues (Corsolini et al., 2014; Corsolini et al., 2007).

PCBs exposure of fish can occur through water via gills, sediment and particularly through contaminated prey. In general, lower chlorinated PCBs are more easily transported via the atmosphere mainly in the gas phase, while heavier PCB congeners are mostly associated with particles and tend to deposit to the surface near sources. The contamination profile depends on the bioconcentration (water–biota transfer) related to the lipophobicity of compounds and the lipid nature of organisms at lower trophic level, while the determining factors are lipophilicity and persistence of compounds, as well as metabolic activity and level of food transfer (Celik & Secer, 2011).

The increasing number of chlorines increases both lipid solubility and bioaccumulation. However, the optimal bioaccumulation capacity is at about six chlorines, probably because higher chlorinated congeners (esp. octa-) are so poorly water soluble that their bioavailability is low (Celik & Secer, 2011). On the other hand, PCBs 101, 138, 153 and 180, being refractory to metabolic attack by monooxygenases, tend to be more slowly eliminated because of their high degree of chlorination and the lack of adjacent unsubstituted H-atoms in ortho e meta and/or meta e para position on the aromatic ring (Chiesa et al., 2016). In fish, PCBs decreased growth; caused ionic imbalance, hyperglycemia, anemia, toxicopathic lesions in tissues, such as gill, liver, and spleen; disrupted reproduction; and ultimately affected population levels (Miranda et al., 2008).





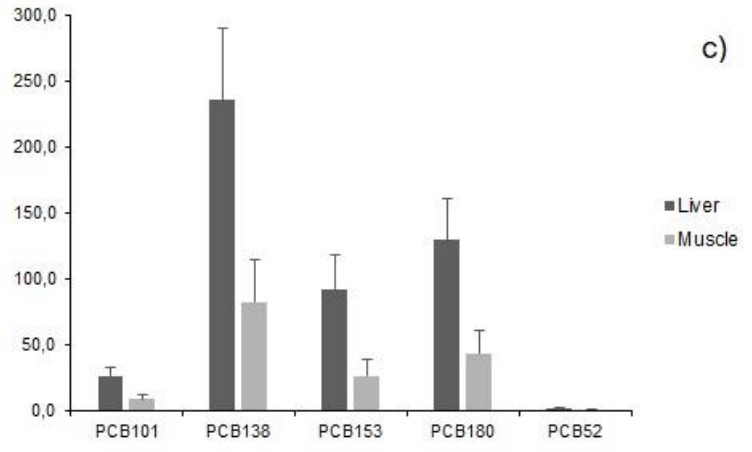


Fig. 20 – PCBs (mean concentration ng g^{-1} d.w., ± s.e.) in two different tissues (liver and muscle) of three tuna species: a) *Sarda sarda*; b) *Katsuwonus pelamis*; c) *Thunnus thynnus*. Reported compounds n.d = non detected.

With reference to organochlorine pesticides (p,p'-DDD, p,p'-DDE and p,p'-DDT) and herbicide (Ametryn), there are significant differences both between species and between the two tissues (Tab. 16, Tab. 17, Tab. 18, Fig. 17, Fig. 18). p,p'-DDT and p,p'-DDD were not always detectable in *Sarda sarda* and *Katsuwonus pelamis* liver and tissue samples (Fig. 21). In contrast, the herbicide Ametryn was always found in detectable concentrations in *Sarda sarda*, *Katsuwonus pelamis* and *Thunnus thynnus* in liver and tissue samples, with much higher concentrations in the liver (75.1 ± 67.22 ng g^{-1} dw; 1445.7 ng g^{-1} dw ± 589.99; 175.9 ng g^{-1} dw ± 269.89, respectively) than in the muscle (11.6 ng g^{-1} dw ± 14.97; 45 ng g^{-1} dw ± 25.30; 12.3 ng g^{-1} dw ± 32.35, respectively).

DDTs levels were higher in liver than in muscle tissue and, generally, the concentration patterns were similar in all three species: p,p'-DDE> p,p'-DDD ≥ p,p'-DDT. Unlike p,p'-DDE andp,p'-DDD metabolites, p,p'-DDT was detected only in liver and muscle tissues of *Thunnus thynnus*, and in *Sarda sarda* liver tissue.

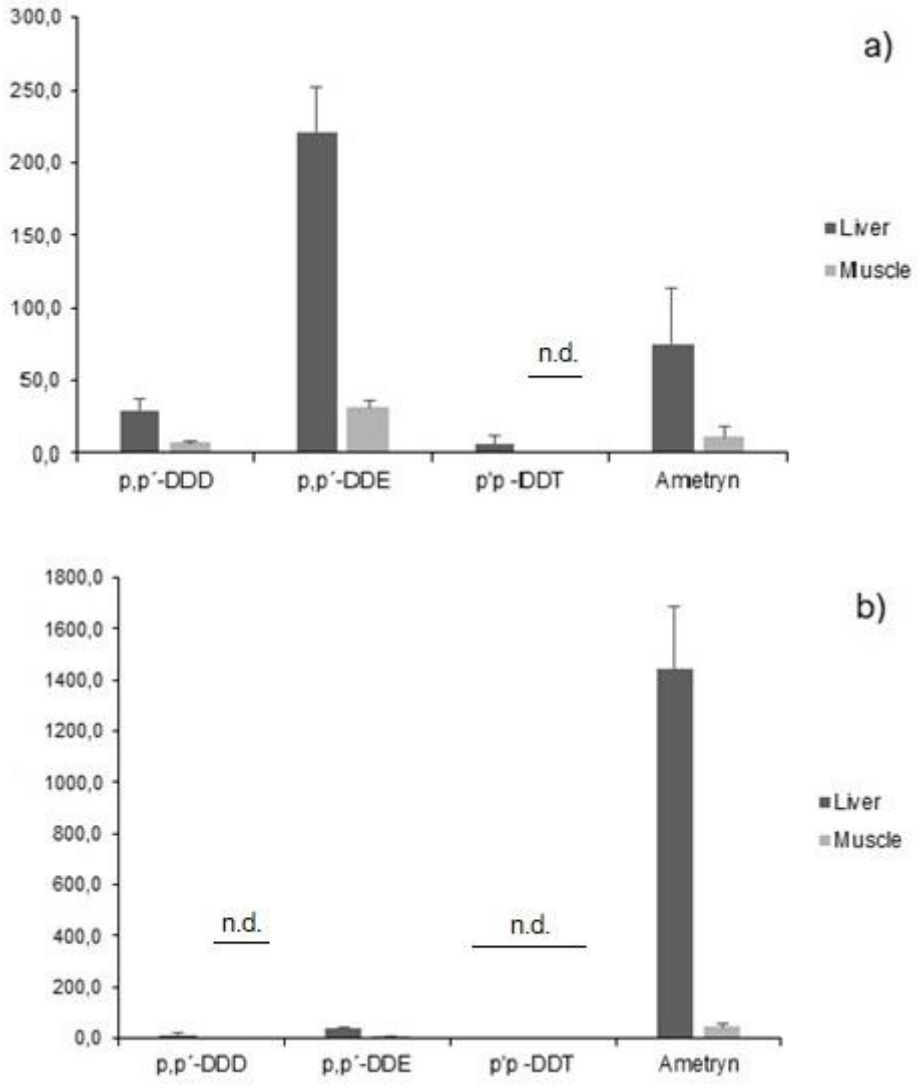
Generally, p,p'-DDE metabolite was always found in detectable concentrations in both tuna liver and muscle tissues. Our results are in agreement with the findings of Storelli et al. (2008b), that reported higher concentration of p,p'-DDE in *T. thynnus* liver tissue (394.2 ng g^{-1} lipid weight) than p,p'-DDT (8.1 ng g^{-1} lipid weight).

Di Bella et al. (2006) and Corsolini et al. (2005) also showed higher concentrations of p,p'-DDE in liver than in muscle of *T. thynnus* from Ionian Sea. In contrast, Vizzini et al. (2010) reported much higher concentration of p,p'-DDE in muscle (2922.98 ng g^{-1} lipid weight)

than in liver (279.32 ng g^{-1} lipid weight) from specimens caught off Favignana (western Sicily).

From the work of Chiesa et al., (2016), p,p'-DDD resulted undetectedable in the muscle tissue of *T. thynnus* from the Indian Ocean, Pacific Ocean and Atlantic Ocean, and the Mediterranean Sea, p,p'-DDE was found to be present only in specimens from the Mediterranean Sea (395.18 ng g^{-1} lipid weight), and p,p'-DDT in specimens from the Atlantic Ocean (149.58 ng g^{-1} lipid weight) and the Mediterranean 155.49 ng g^{-1} lipid weight).

Hisamichi (2010), comparing the results from the muscle of three different tuna species, found the highest concentration of p,p'-DDE in *Thunnus thynnus*, followed by *Thunnus alalunga* and then by *Thunnus albacares*.



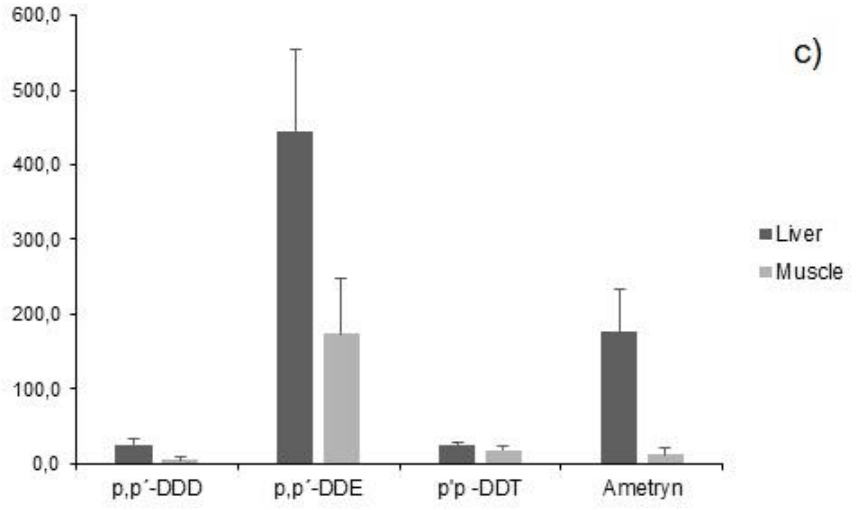


Fig. 21 – Pesticides (mean concentration ng g^{-1} d.w., ± s.e.) in two different tissues (liver and muscle) of three tuna species: a) *Sarda sarda*; b) *Katsuwonus pelamis*; c) *Thunnus thynnus*. Reported compounds n.d = non detected.

With respect to Polycyclic aromatic hydrocarbons (PAHs), there are significant differences both between species and between the two tissues (Tab. 16, Tab. 17, Tab. 18, Fig. 17, Fig. 18)., also in the interaction between the two factors (Tab. 15). Among the 16 EPA priority PAHs, only Phenantrene, Pyrene and Fluoranthene were found always in detectable concentrations in both the liver and muscle tissues of *Sarda sarda, Katsuwonus pelamis,* and *Thunnus thynnus* but with different concentrations patterns (Fig. 22).

Sarda sarda follows the same concentration pattern in both muscle and liver tissue with Phe (163.6 ± 158.84 ng g^{-1} dw in liver; 60.85 ± 158.84 ng g^{-1} dw in muscle) > Flu (88.6 ± 36.97 ng g^{-1} dw in liver; 26.33 ± 6.42 ng g^{-1} dw in muscle > Pyr (66.6 ± 30.02 ng g^{-1} dw in liver; 20.33 ± 4.55 ng g^{-1} dw in muscle). *Thunnus thynnus* also follows the same concentration pattern for both muscle and liver with Phe (268.7 ± 113.64 ng g^{-1} dw in liver; 156.32 ± 107.42 ng g^{-1} dw in muscle) > Pyr (72.3 ± 18.16 ng g^{-1} dw in liver; 75.56 ± 67.71 ng g^{-1} dw in muscle) > Flu (5.45 ± 4.24 ng g^{-1} dw in liver; 63.16 ± 30.90 ng g^{-1} dw in muscle). On the contrary, in *Katsuwonus pelamis* the concentration pattern is different in the two tissues, with Flu (68.4 ± 27.84 ng g^{-1} dw) > Phe (54.3 ± 79.23 ng g^{-1} dw) > Pyr (45.3 ± 16.13 ng g^{-1} dw) in the liver tissue, and Phe (55.9 ± 41.00 ng g^{-1} dw)> Flu (21.1 ± 11.07 ng g^{-1} dw) > Pyr (16.4 ± 8.73 ng g^{-1} dw) in the muscle tissue.

Phe predominated in the liver (163.6 ± 158.84 ng g^{-1} dw) and muscle (60.9± 43.38 ng g^{-1} dw) tissues of *Sarda sarda* and in the liver (268.7 ± 113.64 ng g^{-1} dw) and muscle (156.3 ± 107.42 ng g^{-1} dw) of *Thunnus thynnus*. On the contrary, in *Katsuwonus pelamis* Flu predominated in the liver tissue (68.4 ± 27.84 ng g^{-1} dw) and Phe in the muscle tissue (56 ± 41.00 ng g^{-1} dw).

Pyr was the least concentrated PAHs compound in the liver (66.6 ± 30.02 ng g^{-1} dw) and muscle tissue (20.3 ± 4.55 ng g^{-1} dw) of *Sarda sarda* and in the liver (45.3 ± 6.13 ng g^{-1} dw) and muscle tissue (16.4 ± 8.73 ng g^{-1} dw) of *Katsuwonus pelamis*, whereas Flu was the least concentrated PAHs compound in *Thunnus thynnus* liver (5.4 ± 4.24 ng g^{-1} dw) and muscle tissue (63.1 ± 30.90 ng g^{-1} dw).

From the study (Saija et al., 2016), it can be seen that Fluoranthene and Phenanthrene were not found in all the specimens of *T. thynnus* liver analysed. Whereas, according to our study, Phenanthrene shows higher concentrations than Fluoranthene.

Other studies (Bogdanović et al., 2019; Ranjbar et al., 2019; Ferrante et al., 2018) detected fluoranthene and phenanthrene in fish and fishery products such as fish and bivalve marine species. Marine organisms are exposed to PAHs that are ubiquitously present in the marine environment due to polluted sediments, oil spill residues, shipping activities (de-ballasting waters), industrial and urban run-off, and atmospheric fallout. PAHs uptake depends on the physiology of the taxonomic level of the exposed organism. For example, the metabolic capacity of invertebrates such as bivalves is inferior to that of fish (vertebrates), in which the majority of absorbed PAHs are efficiently bio-transformed by enzymes that increase their water solubility and excretion (Bogdanović et al., 2019); (Perugini et al., 2007).

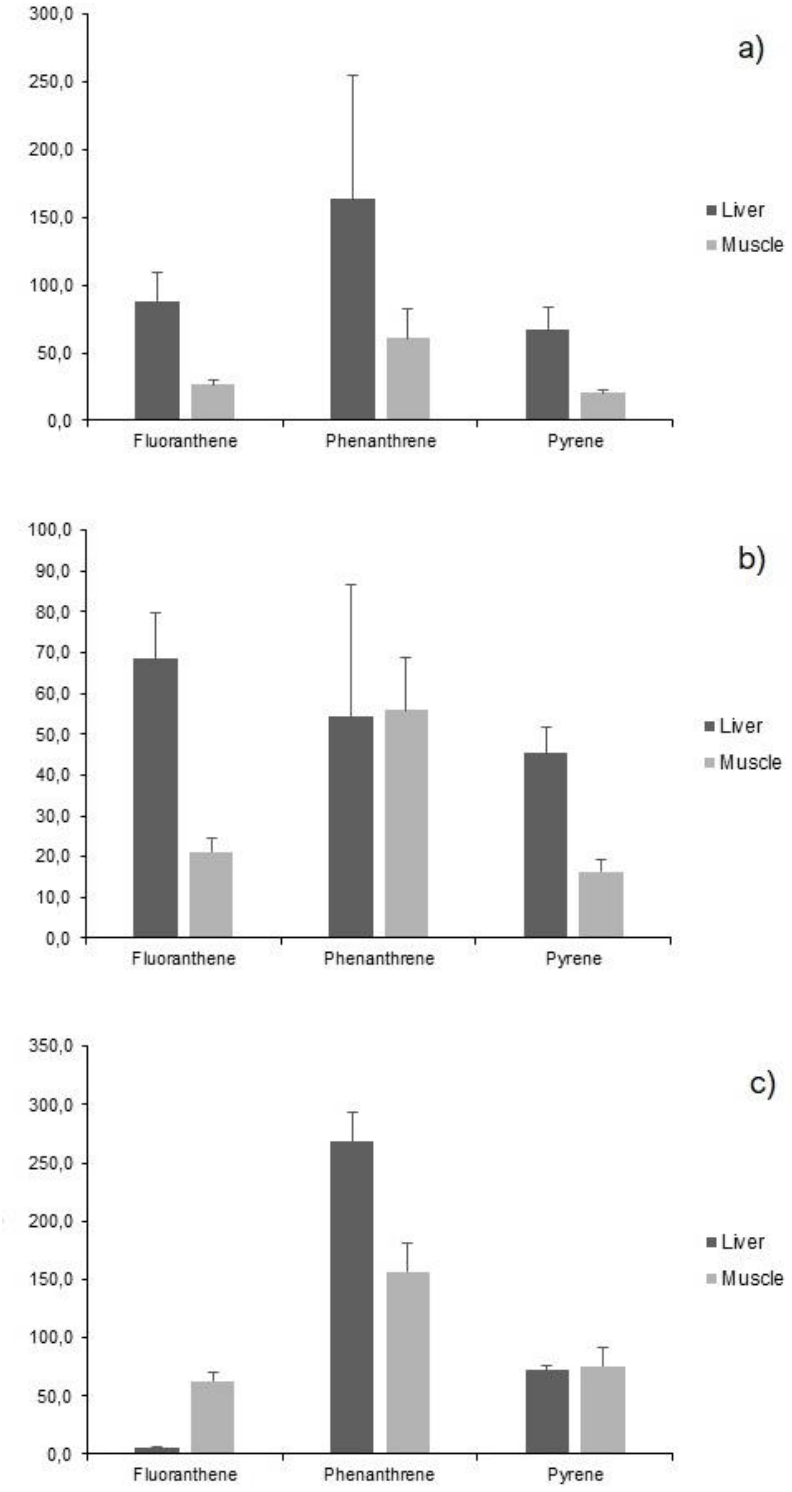


Fig. 22 – PAHs (mean concentration ng g^{-1} d.w., ± s.e.) in two different tissues (liver and muscle) of three tuna species: a) *Sarda sarda*; b) *Katsuwonus pelamis*; c) *Thunnus thynnus*.

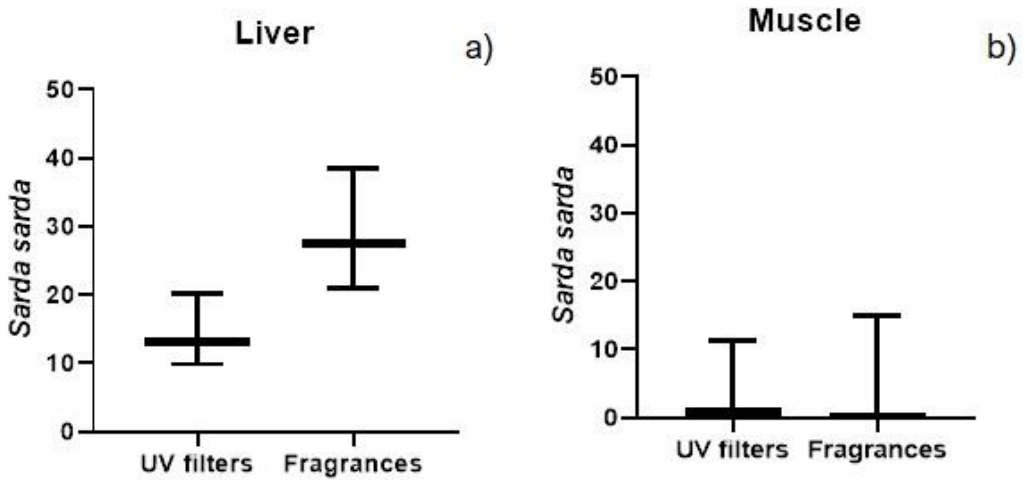
4.3 Occurrence of emerging contaminants in tuna species

The three tuna species show different concentration patterns in terms of classes of emerging compounds (Tab. 20, Fig. 23); but post-hoc comparison showed significant differences between tissues only for fragrances (Tab. 16, Tab. 17, Tab. 18, Fig. 17, Fig. 18).

With reference to emerging compound partitioning between tissues in *S. sarda*, fragrances had higher concentrations than UV filters in the liver tissue, whereas both classes of contaminants exhibit comparable low concentrations in the muscle tissue (Fig. 23, *a* and *b*).

With reference to emerging compound partitioning between tissues in K. *pelamis*, UV filters had higher concentrations than fragrances in the liver tissue, whereas UV filters had higher concentrations than fragrances in the muscle tissue (Fig. 23, *c* and *d*).

With reference to emerging compound partitioning between tissues in *T. thynnus*, UV filters had higher concentrations than fragrances both in the liver and in the muscle tissue, (Fig. 23, *e* and *f*).



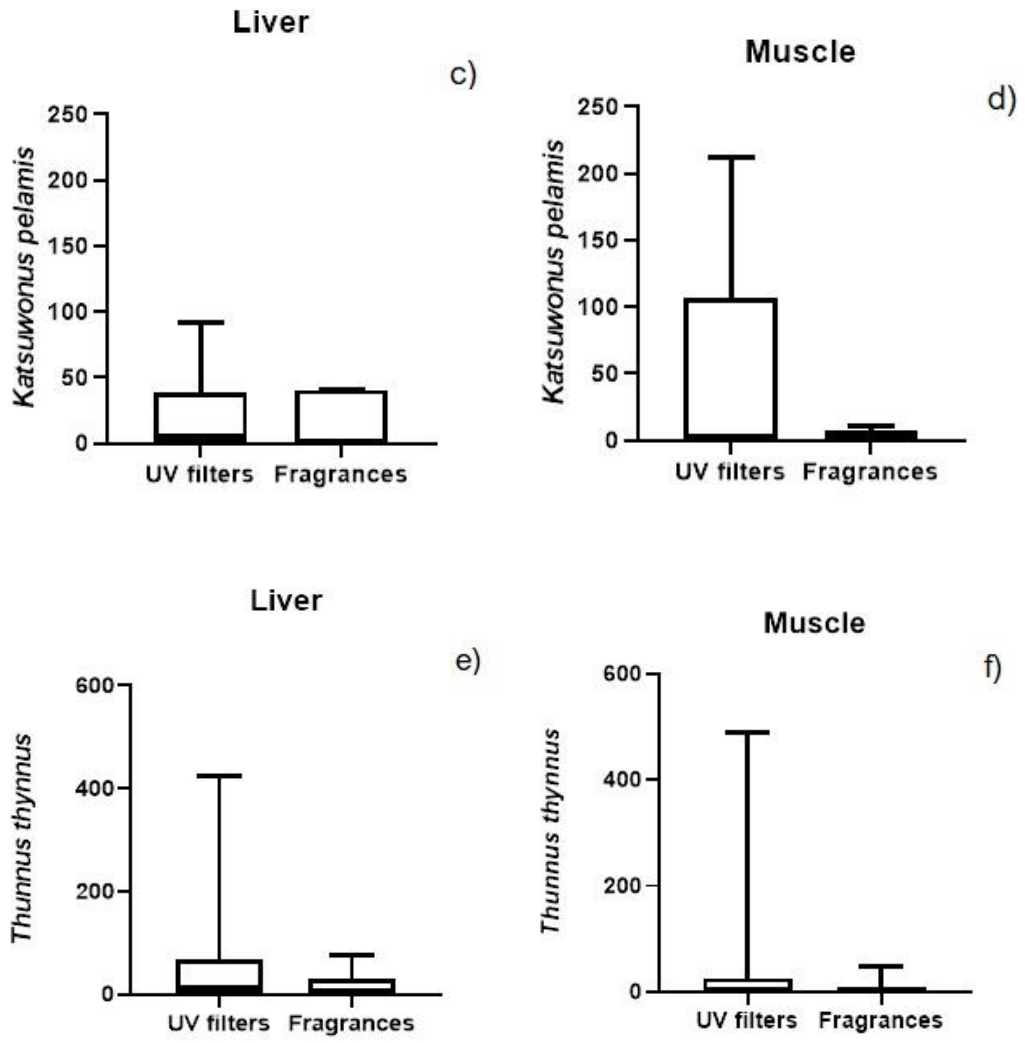


Fig. 23 - Box- plots of emerging contaminants (mean concentration ng g^{-1} d.w.) in liver and muscle of *Sarda sarda* (graphs *a* and *b*), *Katsuwonus pelamis* (graphs *c* and *d*), *Thunnus thynnus* (graphs *e* and *f*).

Tab. 20 – Emerging contaminants (ECs) concentration (ng g^{-1} dry weight) in tissues (liver and muscle) of three tuna species (*S. sarda*, *K. pelamis*, *T. thynnus*), expressed in minimum value, maximum value, mean value and standard deviation (SD).

			EMERGING CONTAMINANTS								
			UV FILTERS						FRAGRANCES		
SPECIES	**Tissue**		**4MBC**	**BP-3**	**EHMC**	**EHS**	**HS**	**OC**	**OTNE**	**Galaxolide**	**Tonalide**
Sarda sarda	Liver	Min	27.90	<LOD	<LOD	<LOD	<LOD	<LOD	20.15	<LOD	<LOD
Sarda sarda	Liver	Max	96.77	<LOD	<LOD	12.64	6.77	31.28	106.82	62.34	8.89
Sarda sarda	Liver	Mean	61.37	<LOD	<LOD	6.04	2.71	16.39	59.34	24.67	2.96
Sarda sarda	Liver	SD	34.47	<LOD	<LOD	6.34	3.58	15.70	43.93	33.14	5.13
Sarda sarda	Muscle	Min	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Sarda sarda	Muscle	Max	6.31	<LOD	<LOD	2.69	0.93	61.56	9.93	25.76	9.17
Sarda sarda	Muscle	Mean	1.58	<LOD	<LOD	0.81	0.25	15.39	2.48	6.44	2.29
Sarda sarda	Muscle	SD	3.15	<LOD	<LOD	1.27	0.46	30.78	4.96	12.88	4.59
Katsuwonus pelamis	Liver	Min	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Katsuwonus pelamis	Liver	Max	49.98	<LOD	28.51	7.71	3.92	470.83	42.23	84.25	12.18
Katsuwonus pelamis	Liver	Mean	14.68	<LOD	4.75	3.25	1.44	98.38	13.05	25.22	2.56
Katsuwonus pelamis	Liver	SD	21.99	<LOD	11.64	3.63	1.68	187.99	20.31	39.44	4.88
Katsuwonus pelamis	Muscle	Min	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Katsuwonus pelamis	Muscle	Max	1180.25	12.54	7.30	10.92	4.64	79.48	21.45	27.79	5.40
Katsuwonus pelamis	Muscle	Mean	251.53	2.21	0.73	2.01	0.57	21.50	3.77	3.29	0.73
Katsuwonus pelamis	Muscle	SD	432.09	4.46	2.31	3.55	1.44	28.26	8.05	8.76	1.72
Thunnus thynnus	Liver	Min	36.26	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Thunnus thynnus	Liver	Max	434.18	79.20	181.13	5.69	1.01	145.42	227.76	24.20	60.97
Thunnus thynnus	Liver	Mean	208.18	5.39	25.79	0.26	0.05	42.98	48.92	1.14	3.30
Thunnus thynnus	Liver	SD	152.12	16.67	51.02	1.21	0.22	42.04	69.01	5.15	13.07
Thunnus thynnus	Muscle	Min	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Thunnus thynnus	Muscle	Max	52.85	9.19	221.36	3.83	<LOD	2697.81	57.77	85.33	<LOD
Thunnus thynnus	Muscle	Mean	7.90	1.30	41.72	0.21	<LOD	224.69	3.65	4.78	<LOD
Thunnus thynnus	Muscle	SD	15.59	2.25	73.22	0.90	<LOD	634.02	13.57	20.10	<LOD

In the context of emerging contaminants, UV filters showed no significant differences, either within the same species and between different species (Tab. 16, Tab. 17, Tab. 18, Fig. 17, Fig. 18).

Among the UV filters analysed, 4-Methylbenzylidene Camphor (4MBC), Benzophenone-3 (BP-3), Ethylhexyl methoxycinnamate (EHMC)**,** Ethyl hexyl salycilate (EHS), Homosalate (HS) and Octocrylene (OC) had different concentrations patterns in both the liver and muscle tissues of *Sarda sarda, Katsuwonus pelamis,* and *Thunnus thynnus* (Tab. 20)

BP-3 and EHMC were not detected in liver and muscle tissues of *Sarda sarda* and in the liver tissue of *Katsuwonus pelamis*; whereas HS was not detected in muscle tissue of *Thunnus thynnus* (Fig. 24).

4MBC predominated in the liver tissue of *S. sarda* (61.4 $\pm$ 34.47 ng g^{-1} dw) and in the liver tissue of *T. thynnus* (208.2$\pm$ 152.12 ng g^{-1} dw), while OC predominated in the muscle tissue of *S. sarda* (15.4 $\pm$ 30.78 ng g^{-1} dw) and in the muscle tissue of *T. thynnus* (224.7 $\pm$ 634.02 ng g^{-1} dw). On the contrary, in *Katsuwonus pelamis* OC predominated in the liver tissue (98.4 $\pm$ 187.99 ng g^{-1} dw) and 4MBC in the muscle tissue (252 $\pm$ 432.09 ng g^{-1} dw).

The higher relative amount of 4MBC and OC in tissues of these fish species could be attributed to their higher octanol-water partition coefficient (log K_{OW} 5.47 for 4MBC and log K_{OW} 7.1 for OC) compared to the other UV filters's octanol-water partition coefficient (Tab. 4).

HS was the least concentrated UV filter in the liver (2.7 $\pm$ 3.58 ng g^{-1} dw) and muscle tissue (0.2 $\pm$ 0.46 ng g^{-1} dw) of *Sarda sarda*; in the liver (1.4 $\pm$ 1.68 ng g^{-1} dw) and muscle tissue (0.6 $\pm$ 1.44 ng g^{-1} dw) of *Katsuwonus pelamis* and in the liver (0.05 $\pm$ 0.22 ng g^{-1} dw) and in muscle tissue (< LOD) of *Thunnus thynnus*.

Cunha et al. (2015) found BP3 and OC in marine species samples (mussel, mullet and clam) from Ebro delta and Tagus estuary Po, but all below the method limit of quantification.

Cunha et al., (2018) also found the same UV filters compounds detected in this study in tuna species, but, in contrast to this study, HS was the most abundant UV filter (20.2 µg kg^{-1} dw), while BP-3 had the lowest concentration (1.3 µg kg^{-1} dw) . Gago-Ferrero et al. (2013) reported higher concentration of EHMC (241 ng g^{-1} dw), followed by OC (30,4 ng g^{-}

^{1} dw) and BP-3 (24,3 ng g^{-1} dw) in the fish species *Luciobarbus sclateri* (Andalusian Barbel) from Guadalquivir river basin (south of Spain).

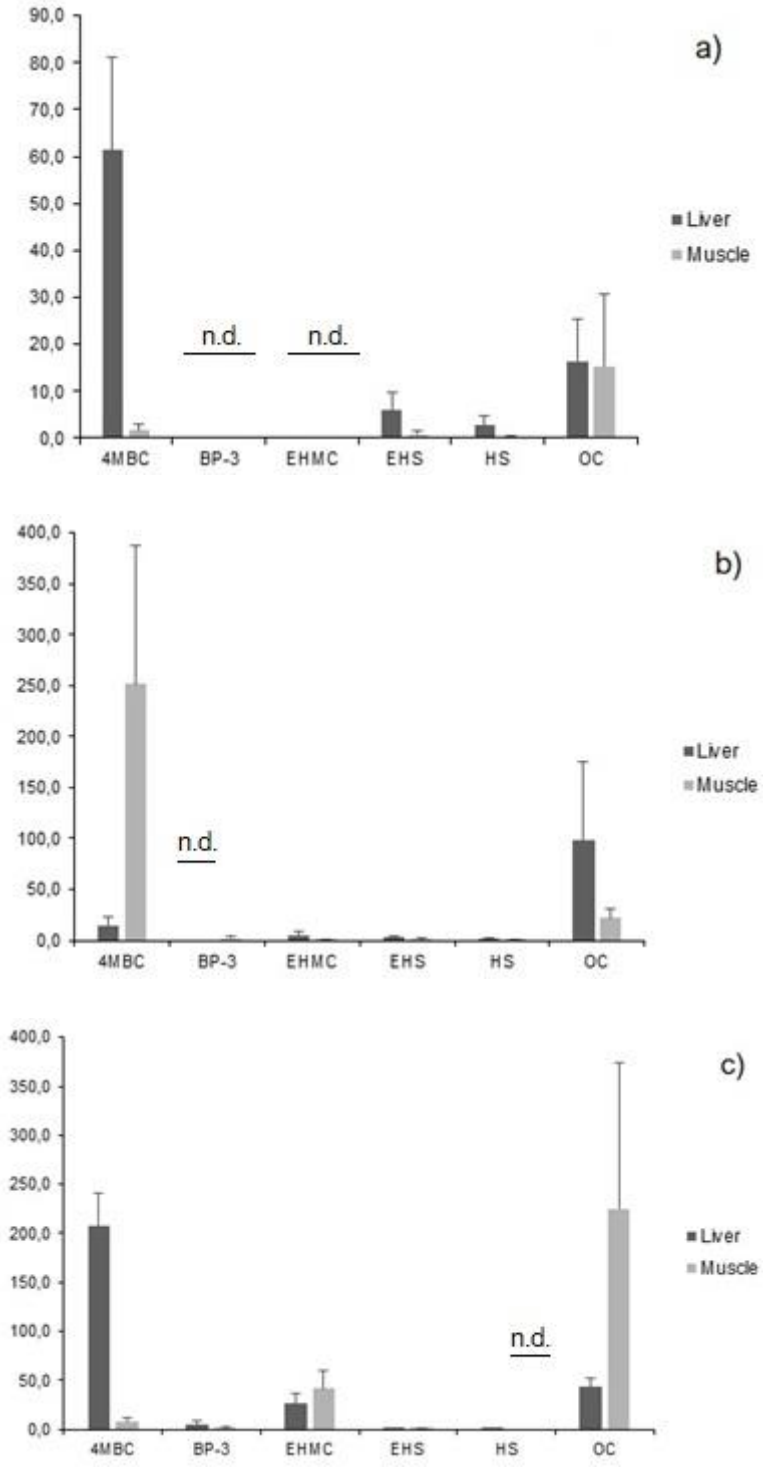


Fig. 24 – UV Filters (mean concentration ng g^{-1} d.w., ± s.e.) in two different tissues (liver and muscle) of three tuna species: a) *Sarda sarda*; b) *Katsuwonus pelamis*; c) *Thunnus thynnus*. Reported compounds n.d = non detected.

With reference to fragrances, there are significant differences both between species and between the two tissues (Tab. 16, Tab. 17, Tab. 18, Fig. 17, Fig. 18).

Among the fragrances analysed, Octahydrotetramethyl Acetophenone (OTNE), and Galaxolide and Tonalide**,** belonging to the class of Polycyclic musks, showed different concentration patterns in both the liver and muscle tissues of *Sarda sarda, Katsuwonus pelamis,* and *Thunnus thynnus* (Tab. 20). In addition, tonalide was always found below the LOD in *Thunnus thynnus* muscle samples (Fig. 25).

OTNE predominated in the liver tissue of *S. sarda* (59.3 $\pm$ 43.93 ng g^{-1} dw) and in the liver tissue of *T. thynnus* (48.9$\pm$ 69.01 ng g^{-1} dw), while Galaxolide predominated in the muscle tissue of *S. sarda* (6.4 $\pm$ 12.88 ng g^{-1} dw) and in the muscle tissue of *T. thynnus* (4.7 $\pm$ 20.10 ng g^{-1} dw). On the contrary, in *Katsuwonus pelamis* Galaxolide predominated in the liver tissue (25.2 $\pm$ 39.44 ng g^{-1} dw) and OTNE in the muscle tissue (3.8 $\pm$ 8.05 ng g^{-1} dw).

Tonalide was the least concentrated compound in the liver (3 $\pm$ 5.13 ng g^{-1} dw) and muscle tissue (2.3 $\pm$ 4.59 ng g^{-1} dw) of *Sarda sarda*; in the liver (2.6 $\pm$ 4.88 ng g^{-1} dw) and muscle tissue (0.7 $\pm$ 1.72 ng g^{-1} dw) of *Katsuwonus pelamis* and n.d. in muscle tissue of *Thunnus thynnus*.

Other studies, among polycyclic musks, agree with the results found in this study. Cunha et al. (2015) found Galaxolide and Tonalide in marine species samples (mussel, mullet, clam and macroalgae) from Ebro delta and Tagus estuary Po, with Galaxolide showing always higher concentrations than Tonalide.

Cunha et al. (2018), found Galaxolide with the highest concentrations among all polycyclic musks analysed, in tuna species (12.8 µg kg^{-1} dw) and other marine organisms. On the contrary, Tonalide showed lower concentrations than Galaxolide in tuna species (2.5 µg kg^{-1} dw) and in other marine organisms. Moreover, Tonalide's use needed risk reduction measures, because it cannot be excluded that photosensitising effects may occur (ECHA, report of Risk Assessment, May 2008).

Also Trabalón et al. (2015) found Galaxolide and Tonalide in seafood species from Tarragona (Spain), with higher concentration of Galaxolide (38.4 ng g^{-1} dw) than Tonalide (5.5 ng g^{-1} dw) in *T. thynnus* samples However, these results are much higher than the concentrations found in *Thunnus thynnus* in this study for Galaxolide (1.14 ng g^{-1} dw in liver; 4.78 ng g^{-1} dw in muscle) and Tonalide (3.30 ng g^{-1} dw in liver; n.d. in muscle).

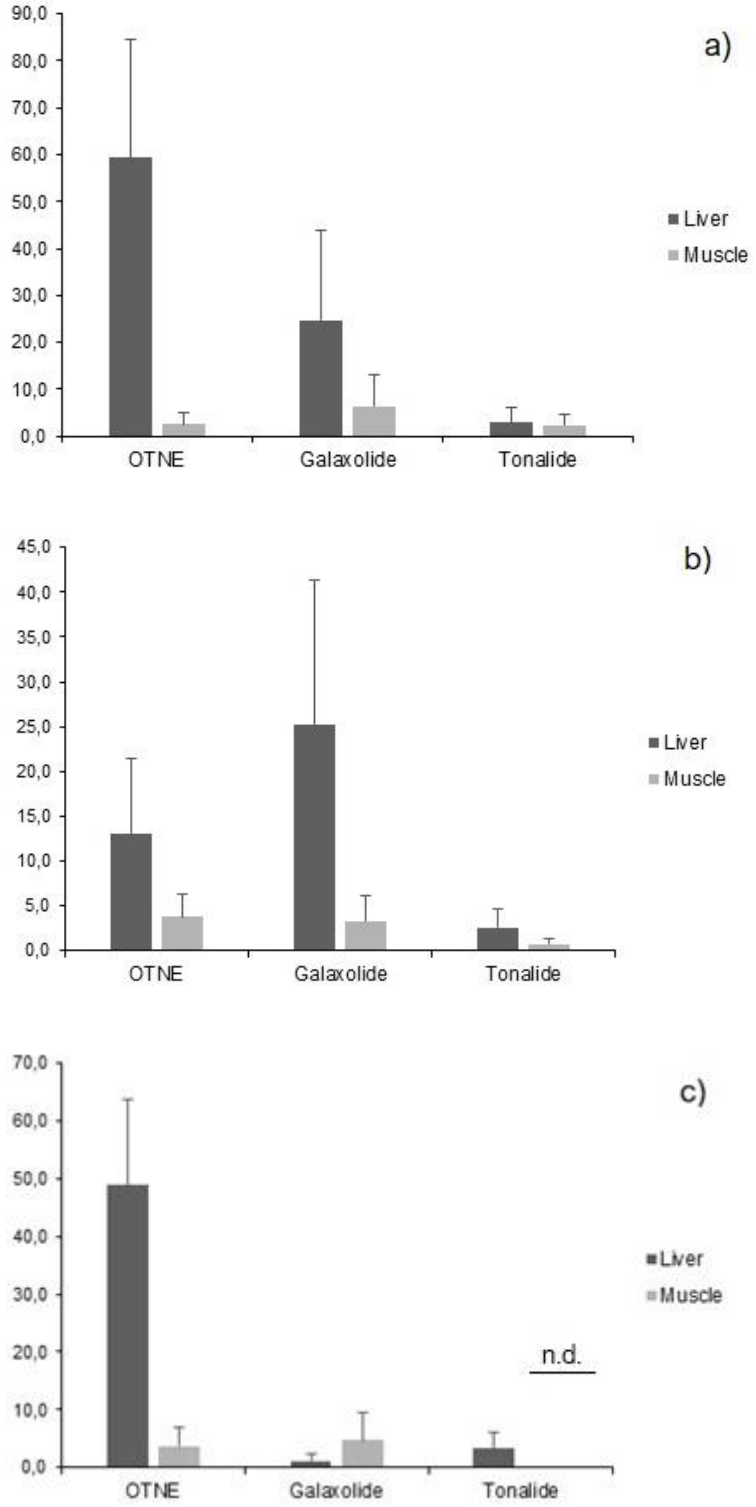


Fig. 25 – Fragrances (mean concentration ng g^{-1} d.w., $\pm$ s.e.) in two different tissues (liver and muscle) of three tuna species: a) *Sarda sarda*; b) *Katsuwonus pelamis*; c) *Thunnus thynnus*. Reported compounds n.d = non detected.

4.4 Correlations of legacy and emerging contaminant concentrations between biometric parameters

Spearman's coefficent correlation (r) between legacy and emerging contaminants in liver and muscle tissues and biometric parameters (furcal lenght and weight) for *S. sarda*, *K. Pelamis* and *T. thynnus* were reported in Tab. 21. Significant Spearman's coefficent correlation (r) in *Sarda sarda* liver and muscle resulted not significant for all classes of compounds analysed.

Significant Spearman's coefficent correlation (r = -0.94) was found in *Katsuwonus pelamis* liver tissue for PAHs ($P < 0.05$).

Significant Spearman's coefficent correlation (r) in *Thunnus thynnus* liver resulted 0.58 for PCBs ($P < 0.01$), 0.56 for DDTs ($P < 0.01$), whereas in muscle resulted 0.47 for DDTs ($P < 0.05$), 0.50 for Fragrances ($P < 0.05$).

Tab. 21 - Spearman's coefficent correlation (r) between legacy and emerging contaminants between biometric parameters (furcal lenght and weight). Significant levels were indicated by the following symbols: ns = not significant (P > 0.05); * = P < 0.05; ** = P < 0.01; *** = P < 0.001.

Sarda sarda				
	Liver		Muscle	
	r		r	
PCBs	-1	ns	0.5	ns
DDTs	-1	ns	-0.5	ns
Herbicide (Ametryn)	-0.5	ns	-0.86	ns
PAHs	-0.5	ns	-0.5	ns
UV Filters	-0.5	ns	-1	ns
Fragrances	-1	ns	-0.86	ns

Katsuwonus pelamis				
	Liver		Muscle	
	r		r	
PCBs	-0.37	ns	-0.05	ns
DDTs	-0.37	ns	-0.08	ns
Herbicide (Ametryn)	-0.31	ns	0.49	ns
PAHs	-0.94	*	0.006	ns
UV Filters	-0.46	ns	0.29	ns
Fragrances	-0.77	ns	0.31	ns

Thunnus thynnus				
	Liver		Muscle	
	r		r	
PCBs	0.58	**	0.43	ns
DDTs	0.56	**	0.47	*
Herbicide (Ametryn)	0.24	ns	-0.005	ns
PAHs	0.14	ns	0.001	ns
UV Filters	0.11	ns	0.12	ns
Fragrances	0.30	ns	0.50	*

4.5 Comparison of PCBs and DDTs concentrations in liver and muscle tissues with literature data

A further interest was to compare and assess similarities between concentrations of PCBs and DDTs, the two classes of legacy contaminants that showed significant differences between liver and muscle tissue in *T. thynnus* and *K. pelamis* in this study, with concentrations of PCBs and DDTs found in *T. thynnus* and *K. pelamis* specimens captured in the Indian Ocean, the Pacific Ocean, the Atlantic Ocean and the Mediterranean Sea (Tab. 25). Emerging contaminants were excluded from this analysis due to the lack of data on their occurrence and levels in tuna specimens from different geographical areas in the World.

Subsequently, a PERMANOVA analysis was carried out with data of PCBs and DDTs concentrations measured in the muscle of *T. thynnus* and *K. pelamis* in this study and in specimen captured in different geographical areas in the World (Tab. 25). Results of *T. thynnus* and *K. pelamis* from different geographic areas showed different concentrations of DDTs and PCBs in their tissues, revealing significant differences between species, and interaction between area and species (Tab. 22). However, the analysis of the individual classes of contaminants shows significant differences between species for DDTs (Tab. 23) and significant differences between areas for PCBs (Tab. 24).

Furthermore, PCoA analysis showed different similarities depending on species, tissue and capture area considered (Fig. 26).

Tab. 22 – Summary of PERMANOVA test on analysis of both DDTs and PCBs concentrations (ng g^{-1} ww) from literature data on tuna species. Only significant values (*P* >0.05) are highlighted in bold: * = *P* <0.05; ** = *P* <0.01; * = *P* <0.001.**

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Ar	2	0.20998	0.10499	2.62E-02	0.999	999
Sp	1	27.312	27.312	6.8178	**0.002**	998
Ti	1	1.05	1.05	0.26211	0.75	999
ArxSp**	1	12.856	12.856	3.2091	**0.045**	999
ArxTi**	1	4.7088	4.7088	1.1755	0.304	999
SpxTi**	0	0		No test		
ArxSpxTi**	0	0		No test		
Res	15	60.089	4.0059			
Total	21	140.45				

Tab. 23 – Summary of PERMANOVA test on DDTs concentrations (ng g^{-1} ww) from literature data on tuna species. Only significant values (*P* >0.05) are highlighted in bold: * = *P* <0.05; ** = *P* <0.01; * = *P* <0.001.**

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Ar	2	3.49E-02	1.74E-02	6.68E-03	0.989	999
Sp	1	17.002	17.002	6.5167	**0.025**	999
Ti	1	0.6218	0.6218	0.23833	0.591	998
ArxSp**	1	0.6561	0.6561	0.25148	0.632	999
ArxTi**	1	3.5137	3.5137	1.3468	0.271	997
SpxTi**	0	0		No test		
ArxSpxTi**	0	0		No test		
Res	14	36.526	2.609			
Total	20	87.475				

Tab. 24 – Summary of PERMANOVA test on PCBs concentrations (ng g^{-1} ww) from literature data on tuna species. Only significant values (*P* >0.05) are highlighted in bold: * = *P* <0.05; ** = *P* <0.01; * = *P* <0.001.**

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Ar	2	7.66E+00	3.83E+00	4.60E+00	**0.017**	998
Sp	1	4.37E-02	4.37E-02	5.25E-02	0.836	997
Ti	1	7.89E-03	7.89E-03	9.47E-03	0.935	997
ArxSp**	0	0		No test		
ArxTi**	1	0.64249	0.64249	0.77131	0.373	998
SpxTi**	0	0		No test		
ArxSpxTi**	0	0		No test		
Res	14	11.662	0.83299			
Total	19	26.638				

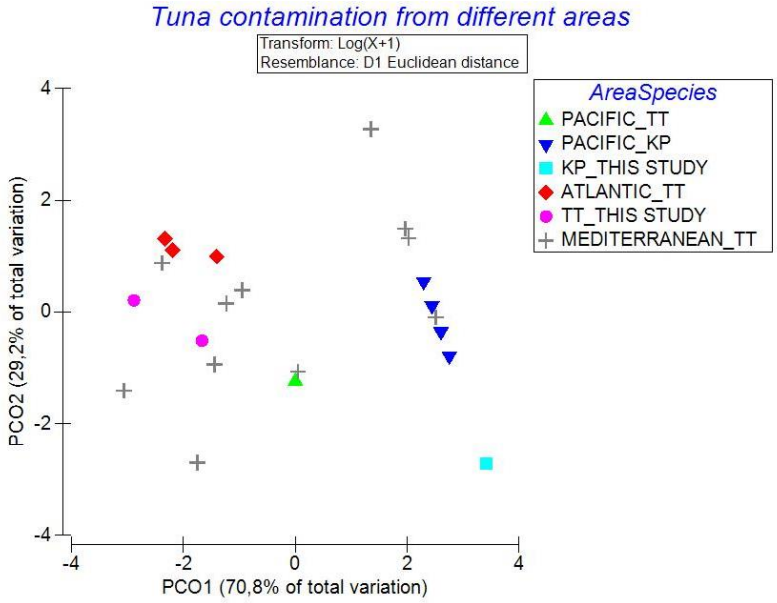


Fig. 26 – Principal Coordinate Analysis (PCoA) regarding the spatial distribution of PCBs and DDTs in *T. thynnus* (TT) and *K. pelamis* (KP) samples from this study and different geographic areas (Pacific Ocean, Atlantic Ocean and Mediterranean Sea).

In order to compare levels of PCBs and DDTs in *T. thynnus* and *K. pelamis* found in this study with other specimen captured in other geographical areas in the World, we assumed a 80% water content in their tissues (Cresson et al., 2017). PCBs concentration found in muscle tissue of *K. pelamis* in our study was 0.12 ± 0.03 ng g^{-1} ww (0 – 0.09). Ueno et al. (2005) found higher concentrations of PCBs (in the range from 8.1 ng g^{-1} ww to 36.25 ng g^{-1} ww) in muscle of *K. pelamis* taken from different areas of the Pacific.

With respect to *T. thynnus*, most of the specimens analysed in this study belong to the medium size class (< 25 kg; ≥100 kg) (Tab. 8 in the Materials and Methods). In the liver tissue, PCBs is 97.2 ± 48.5 ng g^{-1} ww and DDTs is equal to 98.8 ± 18.4 ng g^{-1} ww). Di Bella et al. (2006) found higher concentrations of DDTs than PCBs (299.33 ng g^{-1} for DDTs; 32.76 ng g^{-1} for PCBs), in specimens in the range of 50 to 190 kg captured in the central Mediterranean. In contrast, the opposite pattern is found in other studies on *T. thynnus* liver (Deshpande et al., 2016; Maisano et al., 2016;Storelli et al., 2008; Corsolini et al., 2005). Sprague et al., (2012) reported on average, concentrations of PCBs of one order of magnitude lower (12 ng g^{-1} ww) in the liver tissue of adult individuals caught off Barbate coast (Càdiz, Spain). Conversely, Corsolini et al., (2005) reported the highest concentration for PCBs (233 ng g^{-1} ww) in specimens sampled in the Eastern Mediterranean (Ionian sea).

Generally, the analyses showed higher concentrations of PCBs and DDTs in the liver than in the muscle tissue of *T. thynnus*. In the muscle tissue, PCBs is 32.6 ± 6.5 ng g^{-1} ww and DDTs is 39.2 ± 18.8 ng g^{-1} ww. DDTs had higher concentrations than PCBs also in other studies in individuals from Pacific Ocean, off Japan coasts (Hisamichi, 2010), from western Mediterranean sea (Panseri et al., 2019) and from central Mediterranean, off the Strait of Messina (Di Bella et al., 2006). Furthermore, Gómara et al., (2005) reported very high concentration of PCBs (100.4 ng g^{-1} ww) compared to other studies (Barone et al., 2018; Mezzetta et al., 2011) in which individuals were taken in the Western Mediterranean too.

In contrast, different studies reported higher concentration of PCBs than DDTs, with the highest values showed by Deshpande et al., (2016) in specimens caught in the western Atlantic ocean, off the Gulf of Main (346 ng g^{-1} ww for PCBs; 60.7 ng g^{-1} ww for DDTs) and off Nova Scotia (274 ng g^{-1} ww for PCBs; 56.9 ng g^{-1} ww for DDTs); followed by (Corsolini et al., 2005) in specimens from the eastern Mediterranean (80 ng g^{-1} ww for PCBs; 31 ng g^{-1} ww for DDTs).

However, data between studies are often difficult to compare due to various factors including size and age of fish sampled, although differences in the number and types of congeners examined is usually the most influential (Sprague et al., 2012b).

The European Community Regulation No 1259/2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxin-like PCBs (dl-PCBs) and non-dioxin-like PCBs (ndl-PCBs) in foodstuffs has established maximum levels deriving from the sum of ndl-PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, PCB180): 75 ng g^{-1} wet weight in fish muscle, fishery products and products derived from its processing; 200 ng g^{-1} wet weight in fish liver and products derived from its processing. From the outcomes of this study, few muscle and liver samples of *T. thynnus* exceeded the maximum admissible limits for ndl-PCBs, whereas *K. pelamis* PCBs levels were always below the threshold levels. *T. thynnus* specimens were captured in the Strait of Gibraltar, which is influenced by Atlantic inflows and by the Mediterranean Sea, a semi-enclosed sea surrounded by highly industrialised land masses with high human population densities; this may at least partly explain the high PCB concentrations detected.

"PCB hotspots" marine mammals included the western and central Mediterranean Sea and SW Iberia, the Gulf of Cadiz and the Strait of Gibraltar. Recent findings on PCBs and DDTs occurence in long lived marine apex predators (Jepson et al., 2016) and our study

on tuna fish highlight an ongoing accumulation of these contaminants on top levels of the marine food chain despite the fact that their use and manufacturing was banned over 30 years ago at global and European level.

Tab. 25 - PCBs and OCPs concentrations (ng g^{-1} wet weight and lipid weight) in *K. pelamis* and *T. thynnus* and other tuna species from different global areas. Not detected= n.d.

			Compound				
			PCBs		OCPs		
Sampling site	**Species**	**Tissue**		**Concentration**		**Concentration**	**Reference**
Indian ocean, western (FAO area 51)	*Thunnus thynnus*	Proximal, ventral and caudal anatomic zones	**PCB52**	9.55 ng/g lipid weight	**p,p´-DDD**	n.d.	Chiesa et al., (2016)
Indian ocean, western (FAO area 51)	*Thunnus thynnus*	Proximal, ventral and caudal anatomic zones	**PCB101**	6.39 ng/g lipid weight	**p,p´-DDE**	n.d.	Chiesa et al., (2016)
Indian ocean, western (FAO area 51)	*Thunnus thynnus*	Proximal, ventral and caudal anatomic zones	**PCB138**	6.26 ng/g lipid weight	**p'p -DDT**	158.00 ng/g lipid weight	Chiesa et al., (2016)
Indian ocean, western (FAO area 51)	*Thunnus thynnus*	Proximal, ventral and caudal anatomic zones	**PCB153**	n.d.			Chiesa et al., (2016)
Indian ocean, western (FAO area 51)	*Thunnus thynnus*	Proximal, ventral and caudal anatomic zones	**PCB180**	5.86 ng/g lipid weight			Chiesa et al., (2016)
Indian Ocean	*Thunnus albacares*	Muscle	**PCBs**	0.23 ng/g ww	**DDTs**	0.43 ng/g ww	Nicklisch et al., (2017)
Southwest Indian Ocean, Reunion Island	*Thunnus albacarers*	Muscle	**i-PCBs**	0.20 ng/g ww (± 0.14 SD)	**DDTs**	0.59 ng/g ww (± 0.48 SD)	Munschy et al., (2016)
Southwest Indian Ocean, Reunion Island	*Thunnus albacarers*	Muscle	**i-PCBs**	10.2 ng/g lw (± 7.6 SD)	**DDTs**	28.4 ng/g lw (± 16.1 SD)	Munschy et al., (2016)
Southwest Indian Ocean, Reunion Island	*Thunnus albacarers*	Muscle	**dl- PCBs**	0.03 ng/g ww (± 0.02 SD)			Munschy et al., (2016)
Southwest Indian Ocean, Reunion Island	*Thunnus albacarers*	Muscle	**dl- PCBs**	1.8 ng/g lw (± 1.3 SD)			Munschy et al., (2016)
Indo-Pacific Ocean, off Indonesia	*Katsuwonus pelamis*	Muscle	**PCBs**	6.5 ng/g lipid w			Ueno et al., (2005)
Indo-Pacific Ocean, off Philippines	*Katsuwonus pelamis*	Muscle	**PCBs**	8.5 ng/g lipid w			Ueno et al., (2005)

Indian Ocean, Australia	*Thunnus albacares*	Muscle	**PCBs**	2890 ng/g lipid w (± 3.62)	**p,p´-DDE**	180 ng/g lipid w (± 0.21)	Endo et al., (2016)
Indian Ocean, New Zealand	*Thunnus albacares*	Muscle	**PCBs**	5250 ng/g lipid w (± 3.38)	**p,p´-DDE**	540 ng/g lipid w (± 0.53)	Endo et al., (2016)
Pacific Ocean, western central (FAO area 71)	*Thunnus thynnus*	Proximal, ventral and caudal anatomic zones	**PCB52**	9.07 ng/g lipid weight	**p,p´-DDD**	n.d.	Chiesa et al., (2016)
Pacific Ocean, western central (FAO area 71)	*Thunnus thynnus*	Proximal, ventral and caudal anatomic zones	**PCB101**	5.90 ng/g lipid weight	**p,p´-DDE**	n.d.	Chiesa et al., (2016)
Pacific Ocean, western central (FAO area 71)	*Thunnus thynnus*	Proximal, ventral and caudal anatomic zones	**PCB138**	5.61 ng/g lipid weight	**p'p -DDT**	n.d.	Chiesa et al., (2016)
Pacific Ocean, western central (FAO area 71)	*Thunnus thynnus*	Proximal, ventral and caudal anatomic zones	**PCB153**	n.d.			Chiesa et al., (2016)
Pacific Ocean, western central (FAO area 71)	*Thunnus thynnus*	Proximal, ventral and caudal anatomic zones	**PCB180**	5.21 ng/g lipid weight			Chiesa et al., (2016)
North Pacific Ocean	*Thunnus albacares*	Muscle	**PCBs**	3.40 ng/g ww	**DDTs**	3.40 ng/g ww	Nicklisch et al., (2017)
Notheast Pacific Ocean	*Thunnus albacares*	Muscle	**PCBs**	3.38 ng/g ww	**DDTs**	7.86 ng/g ww	Nicklisch et al., (2017)
Gulf of Mexico	*Thunnus albacares*	Muscle	**PCBs**	8.08 ng/g ww	**DDTs**	1.62 ng/g ww	Nicklisch et al., (2017)
Southeast Pacific Ocean	*Thunnus albacares*	Muscle	**PCBs**	0.55 ng/g ww	**DDTs**	0.49 ng/g ww	Nicklisch et al., (2017)
South China Sea	*Thunnus albacares*	Muscle	**PCBs**	0.69 ng/g ww	**DDTs**	0.93 ng/g ww	Nicklisch et al., (2017)
North China Sea	*Thunnus albacares*	Muscle	**PCBs**	1.25 ng/g ww	**DDTs**	1.42 ng/g ww	Nicklisch et al., (2017)
Northwest Pacific Ocean	*Thunnus albacares*	Muscle	**PCBs**	0.12 ng/g ww	**DDTs**	0.20 ng/g ww	Nicklisch et al., (2017)
Southwest Pacific Ocean	*Thunnus albacares*	Muscle	**PCBs**	0.22 ng/g ww	**DDTs**	0.27 ng/g ww	Nicklisch et al., (2017)

Pacific Ocean, Japan	*Thunnus thynnus*	Muscle	**PCBs**	13.6 ng/g ww (± 17.2 SD)	**p,p´-DDE**	14.6 ng/g ww (± 17.2 SD)	Hisamichi et al., (2010)
		Muscle	**PCBs**	930 ng/g lw (± 600 SD)	**p,p´-DDE**	1040 ng/g lw (± 570 SD)	Hisamichi et al., (2010)
Pacific Ocean, Japan	*Thunnus albacares*	Muscle	**PCBs**	0.31 ng/g ww (± 0.43 SD)	**p,p´-DDE**	0.24 ng/g ww (± 0.26 SD)	Hisamichi et al., (2010)
		Muscle	**PCBs**	230 ng/g lw (± 120 SD)	**p,p´-DDE**	200 ng/g lw (± 100 SD)	Hisamichi et al., (2010)
Pacific Ocean, Japan	*Thunnus alalunga*	Muscle	**PCBs**	1.32 ng/g ww (± 2.10 SD)	**p,p´-DDE**	0.97 ng/g ww (± 1.53 SD)	Hisamichi et al., (2010)
		Muscle	**PCBs**	400 ng/g lw (± 280 SD)	**p,p´-DDE**	290 ng/g lw (± 270 SD)	Hisamichi et al., (2010)
Pacific Ocean, open waters	*Katsuwonus pelamis*	Muscle	**PCBs**	13.43 ng/g lipid w			Ueno et al., (2005)
Pacific Ocean, off Japan	*Katsuwonus pelamis*	Muscle	**PCBs**	22.66 ng/g lipid w			Ueno et al., (2005)
Pacific Ocean, China sea	*Katsuwonus pelamis*	Muscle	**PCBs**	36.25 ng/g lipid w			Ueno et al., (2005)
Pacific Ocean, Bay of Bengal	*Katsuwonus pelamis*	Muscle	**PCBs**	8.1 ng/g lipid w			Ueno et al., (2005)
Pacific Ocean, Japan (Shizuoka)	*Thunnus albacares*	Muscle	**PCBs**	280 ng/g lipid w (± 0.16)	**p,p´-DDE**	200 ng/g lipid w (± 0.11)	Endo et al., (2016)
Pacific Ocean, Japan (Wakayama)	*Thunnus albacares*	Muscle	**PCBs**	230 ng/g lipid w (± 0.14)	**p,p´-DDE**	170 ng/g lipid w (± 0.11)	Endo et al., (2016)
Pacific Ocean, Japan (Ogasawara Islands)	*Thunnus albacares*	Muscle	**PCBs**	550 ng/g lipid w (± 0.26)	**p,p´-DDE**	360 ng/g lipid w (± 0.19)	Endo et al., (2016)
Pacific Ocean, Japan (Okinawa)	*Thunnus albacares*	Muscle	**PCBs**	210 ng/g lipid w (± 0.06)	**p,p´-DDE**	220 ng/g lipid w (± 0.08)	Endo et al., (2016)
Pacific Ocean, Sri Lanka and Maldives	*Thunnus albacares*	Muscle	**PCBs**	330 ng/g lipid w (± 0.42)	**p,p´-DDE**	60 ng/g lipid w (± 0.10)	Endo et al., (2016)

Pacific Ocean, USA (Honolulu)	*Thunnus albacares*	Muscle	**PCBs**	80 ng/g lipid w (± 0.07)	**p,p´-DDE**	30 ng/g lipid w (± 0.07)	Endo et al., (2016)
Atlantic Ocean, eastern central (FAO area 34)	*Thunnus thynnus*	Proximal, ventral and caudal anatomic zones	**PCB52**	9.53 ng/g lipid weight	**p,p´-DDD**	n.d.	Chiesa et al., (2016)
Atlantic Ocean, eastern central (FAO area 34)	*Thunnus thynnus*	Proximal, ventral and caudal anatomic zones	**PCB101**	6.87 ng/g lipid weight	**p,p´-DDE**	n.d.	Chiesa et al., (2016)
Atlantic Ocean, eastern central (FAO area 34)	*Thunnus thynnus*	Proximal, ventral and caudal anatomic zones	**PCB138**	7.82 ng/g lipid weight	**p'p -DDT**	n.d. - 149.58 ng/g al 75° percentile	Chiesa et al., (2016)
Atlantic Ocean, eastern central (FAO area 34)	*Thunnus thynnus*	Proximal, ventral and caudal anatomic zones	**PCB153**	n.d.			Chiesa et al., (2016)
Atlantic Ocean, eastern central (FAO area 34)	*Thunnus thynnus*	Proximal, ventral and caudal anatomic zones	**PCB180**	6.13 ng/g lipid weight			Chiesa et al., (2016)
Northwest Atlatic Ocean	*Thunnus albacares*	Muscle	**PCBs**	3.82 ng/g ww	**DDTs**	1.06 ng/g ww	Nicklisch et al., (2017)
Northeast Atlantic Ocean	*Thunnus albacares*	Muscle	**PCBs**	6.52 ng/g ww	**DDTs**	8.23 ng/g ww	Nicklisch et al., (2017)
Southeast Atlantic Ocean	*Thunnus albacares*	Muscle	**PCBs**	NA (not available)	**DDTs**	3.22 ng/g ww	Nicklisch et al., (2017)
Northwest Atlantic Ocean, Offshore of Virginia	*Thunnus thynnus*	Liver	**PCBs**	189.425 ng/g ww (± 102.3 SD)	**DDTs**	27.6 ng/g ww (± 15,8 SD)	Deshpande et al., (2016)
Northwest Atlantic Ocean, Gulf of Maine	*Thunnus thynnus*	Muscle	**PCBs**	346 ng/g ww (± 223 SD)	**DDTs**	60.7 ng/g ww (± 44.0 SD)	Deshpande et al., (2016)
Northwest Atlantic Ocean, Nova Scotia	*Thunnus thynnus*	Muscle	**PCBs**	274 ng/g ww (± 245 SD)	**DDTs**	56.9 ng/g ww (± 47.6 SD)	Deshpande et al., (2016)
Southeast Atlantic Ocean, South Africa	*Thunnus albacares*	Muscle	**i-PCBs**	0.58 ng/g ww (± 0.22 SD)	**DDTs**	2.09 ng/g ww (± 0.70 SD)	Munschy et al., (2016)
			i-PCBs	8.6 ng/g lw (± 4.7 SD)	**DDTs**	29.9 ng/g lw (± 14.4 SD)	Munschy et al., (2016)
			dl-PCBs	0.14 ng/g ww (± 0.06 SD)			Munschy et al., (2016)

			dl-PCBs	2.1 ng/g lw (± 1.2 SD)			Munschy et al., (2016)
Central Atlantic Ocean, Barbate coast	*Thunnus thynnus*	Liver	**∑Mono-ortho PCBs**	11.886 ng/g ww (± 3347 SD)			Sprague et al., (2012)
	Thunnus thynnus	Muscle	**∑Mono-ortho PCBs**	5.115 ng/g ww (± 878 SD)			Sprague et al., (2012)
	Thunnus thynnus	Liver	**∑Non-ortho PCBs**	0.068 ng/g ww (± 20.67 SD)			Sprague et al., (2012)
	Thunnus thynnus	Muscle	**∑Non-ortho PCBs**	0.044 ng/g ww (± 3.66 SD)			Sprague et al., (2012)
South Atlantic Ocean, off Seychelles	*Katsuwonus pelamis*	Muscle	**PCBs**	4.2 ng/g lipid w			Ueno et al., (2005)
South Atlantic Ocean, off Brazil	*Katsuwonus pelamis*	Muscle	**PCBs**	14 ng/g lipid w			Ueno et al., (2005)
Mediterranean (FAO area 37)	*Thunnus thynnus*	Proximal, ventral and caudal anatomic zones	**PCB52**	25.96 ng/g lipid weight	**p,p´-DDD**	n.d.	Chiesa et al., (2016)
Mediterranean (FAO area 37)	*Thunnus thynnus*	Proximal, ventral and caudal anatomic zones	**PCB101**	35.87 ng/g lipid weight	**p,p´-DDE**	n.d. - 395.18 ng/g al 75° percentile	Chiesa et al., (2016)
Mediterranean (FAO area 37)	*Thunnus thynnus*	Proximal, ventral and caudal anatomic zones	**PCB138**	107.33 ng/g lipid weight	**p'p -DDT**	155.49 ng/g lipid weight	Chiesa et al., (2016)
Mediterranean (FAO area 37)	*Thunnus thynnus*	Proximal, ventral and caudal anatomic zones	**PCB153**	56.22 ng/g lipid weight			Chiesa et al., (2016)
Mediterranean (FAO area 37)	*Thunnus thynnus*	Proximal, ventral and caudal anatomic zones	**PCB180**	32.53 ng/g lipid weight			Chiesa et al., (2016)
Western Mediterranean	*Thunnus thynnus*	Muscle	**PCB52**	0.06 ng/g ww	**DDTs**	130.4 ng/g ww	Panseri et al., (2019)
Western Mediterranean	*Thunnus thynnus*	Muscle	**PCB101**	0.91 ng/g ww			Panseri et al., (2019)
Western Mediterranean	*Thunnus thynnus*	Muscle	**PCB138**	1.46 ng/g ww			Panseri et al., (2019)

Western Mediterranean	*Thunnus thynnus*	Muscle	**PCB153**	n.d.			Panseri et al., (2019)
Western Mediterranean	*Thunnus thynnus*	Muscle	**PCB180**	2.56 ng/g ww			Panseri et al., (2019)
			PCBs	5.53 ng/g ww			Panseri et al., (2019)
Western Mediterranean, Spanish coast	*Thunnus thynnus*	Edible part	**PCB52**	3.14 ng/g ww			Gomara et al., (2005)
			PCB101	3.30 ng/g ww			Gomara et al., (2005)
			PCB138	21.1 ng/g ww			Gomara et al., (2005)
			PCB180	18.0 ng/g ww			Gomara et al., (2005)
			PCBs	100.4 ng/g ww			Gomara et al., (2005)
Western Mediterranean, Thyrrenia sea	*Thunnus thynnus*	Muscle	**PCBs**	5-1327 ng/g ww (min-max)	**p'p- DDE**	0.8-112 ng/g ww	Corsolini et al., (2007)
Mediterranean, Strait of Messina	*Thunnus thynnus*	Liver	**PCB138**	13.76 ng/g ww (± 18.5 SD)	**p'p- DDE**	299.33 ng/g ww (± 1049.18 SD)	Di Bella et al., (2006)
Mediterranean, Strait of Messina	*Thunnus thynnus*	Muscle	**PCB138**	12.43 ng/g ww (±- 20.72 SD)	**p'p- DDE**	54.5 ng/g ww (± 135.3 SD)	Di Bella et al., (2006)
Mediterranean, Strait of Messina	*Thunnus thynnus*	Liver	**PCB153**	11.18 ng/g ww (± 18.21 SD)			Di Bella et al., (2006)
Mediterranean, Strait of Messina	*Thunnus thynnus*	Muscle	**PCB153**	9.11 ng/g ww (± 14.76 SD)			Di Bella et al., (2006)
Mediterranean, Strait of Messina	*Thunnus thynnus*	Liver	**PCB180**	7.82 ng/g ww (± 12.50 SD)			Di Bella et al., (2006)
Mediterranean, Strait of Messina	*Thunnus thynnus*	Muscle	**PCB180**	8.5 ng/g ww (± 15.45 SD)			Di Bella et al., (2006)

Mediterranean, Strait of Messina	*Thunnus thynnus*	Liver	**PCBs**	32.76 ng/g ww			Di Bella et al., (2006)
Mediterranean, Strait of Messina	*Thunnus thynnus*	Muscle	**PCBs**	30.04 ng/g ww			Di Bella et al., (2006)
Mediterranean, western Sicily	*Thunnus thynnus*	Liver	**PCBs**	337 ng/g lipid w (± 94,28 SD)	**p'p- DDE**	279.32 ng/g lipid w (± 67,36 SD)	
Mediterranean, western Sicily	*Thunnus thynnus*	Muscle	**PCBs**	2751.73 ng/g lipid w (± 1276.83 SD)	**p'p- DDE**	2922.98 ng/g lipid w (± 785.85 SD)	Vizzini et al., (2010)
Mediterranean, Thyrrenian sea	*Thunnus thynnus*	Muscle	**PCBs**	666 ng/g ww (± 475 SD)			Focardi (2012)
Mediterranean, Thyrrenian sea	*Thunnus thynnus*	Muscle	**∑DL-PCBs**	17.97 ng/g ww			Mezzetta et al., (2011)
Mediterranenan, Strait of Messina	*Thunnus thynnus*	Liver	**∑NDL-PCBs**	92.02 ng/g ww	**DDTs**	21.5 ng/g ww	Maisano et al., (2016)
Southwestern Mediterranean	*Thunnus thynnus*	Muscle	**∑NDL-PCBs**	84.2 ng/g ww (± 64,3 SD)			Barone et al., (2018)
Mediterranean, Ionian sea	*Thunnus thynnus*	Liver	**PCB138**	147.4 ng/g lipid w (± 50.2 SD)	**p,p'-DDE**	394.2 ng/g lipid w (± 131.4 SD)	Storelli et al., (2008)
Mediterranean, Ionian sea	*Thunnus thynnus*	Liver	**PCB153**	177.0 ng/g lipid w (± 66.0 SD)	**p,p'-DDT**	8.1 ng/g lipid w (± 19.1 SD)	Storelli et al., (2008)
Mediterranean, Ionian sea	*Thunnus thynnus*	Liver	**PCB180**	79.5 ng/g lipid w (± 36.0 SD)	**DDTs**	13.1 ng/g ww (± 5.4 SD)	Storelli et al., (2008)
Mediterranean, Ionian sea	*Thunnus thynnus*	Liver	**PCBs**	15.9 ng/g ww (± 8.0 SD)			Storelli et al., (2008)
Mediterranean, Ionian sea	*Thunnus thynnus*	Muscle	**PCBs**	80 ng/g ww (± 86 SD)	**p,p'-DDE**	31 ng/g ww (± 38 SD)	Corsolini et al., (2004)
Mediterranean, Ionian sea	*Thunnus thynnus*	Liver	**PCBs**	233 ng/g ww (± 76 SD)	**p,p'-DDE**	74 ng/g ww (± 20 SD)	Corsolini et al., (2004)

5. CONCLUSIONS

Environmental contamination by legacy contaminants has been worldwide of great concern because of their persistence and toxicity to humans and wildlife. Only in the last decade, the presence and accumulation of emerging contaminants, and, consequently, their adverse effects in marine biota have been considered.

The present research was performed to compare the occurence and levels of legacy contaminants (DDTs, PCBs and PAHs) and emerging contaminants (UV filters and Fragrances) in the liver and muscle tissues of three tuna species captured in different locations and with different behaviour and feeding habits: *Sarda sarda*, *Katsuwonus pelamis* from the Gulf of Càdiz (Atlantic Ocean) and *Thunnus thynnus* from the Strait of Gibraltar.

From the statistical analysis, significant differences have been found for Fragrances, DDTs, PCBs and PAHs both among the three tuna species and between the two tissues. Post-hoc analysis of legacy and emerging contaminant showed significant differences between the three tuna species and the tissues analysed, revealing that *K. pelamis* species differs significantly from the other two species, both in liver and muscle, and that legacy contaminants are responsible for significant differences between and within species.

Regarding legacy contaminants, the liver is the tissue displaying the highest concentrations. Moreover, the three tuna species show different concentration patterns in terms of classes of legacy compounds. Among PCBs, the congeners 138, 153 and 180 have been identified as most concentrated in both tissues of *S. sarda* and *T. thynnus*, but not in *K. pelamis*, in which muscle tissue, congener 153 was not detected.

DDTs levels were higher in liver than in muscle tissue and, generally, the concentration patterns were similar in all three species: p,p'-DDE> p,p'-DDD ≥ p,p'-DDT. Unlike p,p'-DDE and p,p'-DDD metabolites, p,p'-DDT was detected only in liver and muscle tissues of *Thunnus thynnus*, and in *Sarda sarda* liver tissue.

Phenantrene, belonging to the class of PAHs, predominated in the liver and muscle tissues of *Sarda sarda* and *Thunnus thynnus*. On the contrary, in *Katsuwonus pelamis*, Phenantrene predominated in the muscle tissue.

Regarding emerging contaminants, the three tuna species show different concentration patterns in terms of classes of emerging compounds, but post-hoc comparison showed significant differences between tissues only for fragrances in *T. thynnus*.

4-MBC and Octocrylene, belonging to the class of UV filters, and OTNE and Galaxolide, belonging to the class of Fragrances, have been identified as the most concentrated compounds in both tissues of the three species, with different concentration patterns.

Persistence and lipophilicity of these compounds contributes to their high bioaccumulation potential and trophic transfer along the food chain. Indeed, organisms at the top of the trophic chain, such as tuna species, tend to accumulate more of these substances and can become more vulnerable to their toxic effects.

This research shows that top predators such as tuna species, in particular *S. sarda*, *K. pelamis* and *T. thynnus*, are constantly exposed to and bioaccumulate legacy and emerging contaminants in their tissues, despite regulatory restrictions and ban on POPs have been introduced decades ago. Consequently, their concentrations in these marine top predator species, need to be constantly monitored in the future.

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"It seems to me that the natural world is the greatest source of excitement; the greatest source of visual beauty, the greatest source of intellectual interest. It is the greatest source of so much in life that makes life worth living." (David Attenborough)

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