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# Plasticizers levels in four fish species from the Ligurian Sea and Central Adriatic Sea (Mediterranean Sea) and potential risk for human consumption

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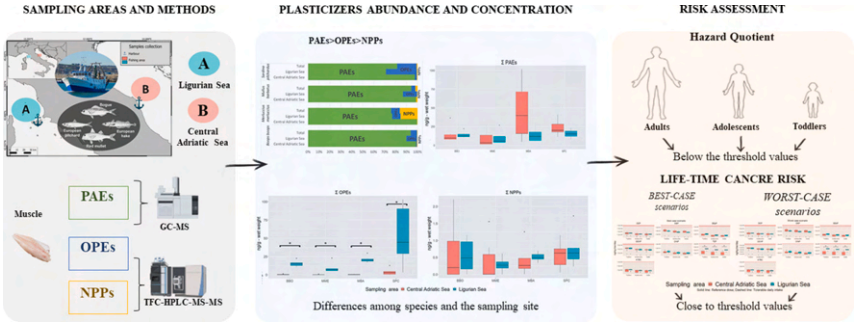
## HIGHLIGHTS

- PAEs, OPEs and NNPs were found in fish muscles from two Mediterranean areas.
- DEHP, DBP, and DIBP show the highest occurrence and concentration in the four fish species.
- Among NPPs, ATBC was detected for the first time in wild fish.
- Plasticizers composition shows differences among species and the sampling site.
- Concentrations of plastics additives in fish were below the threshold limits for human consumption.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Plastic materials contain additives such as plasticizers and flame retardants, which are not covalently bound to plastic polymers and can therefore be unintentionally released into the marine environment. This study investigated three families of compounds, phthalates (PAEs), organophosphate esters (OPEs), and non-phthalate plasticizers (NPPs) currently used as plastic additives, in 48 muscle samples of bogue (*Boops boops*), European hake (*Merluccius merluccius*), red mullet (*Mullus barbatus*), and European pilchard (*Sardina pilchardus*) sampled in the Central Adriatic and the Ligurian Seas. The additional goal of this study is to assess the potential risk to human health from fish consumption with the objective of determining whether the detected levels might potentially pose a concern. PAEs represent the majority of the plastic additives detected in the selected species, with ubiquitous distribution across the study areas, whereas for OPEs and NPPs, there is a more pronounced difference between the two study areas, suggesting that these compounds may represent different exposure levels in the two seas. Among PAEs, bis(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), and diisobutyl phthalate (DIBP) were the most abundant compounds, reaching levels up to 455 ng/g ww. OPEs were detected at

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higher concentrations in samples from the Ligurian Sea, and triethyl phosphate (TEP) was the most abundant compound. Among the NPPs, acetyl tributyl citrate (ATBC) was most frequently detected. From the results obtained, fish consumption may not pose a risk to human health (Hazard Quotient<1) but needs to be considered in future studies. Given the limited number of studies on PAEs, OPEs and NPPs in the Mediterranean Sea, further research is necessary to understand their potential bioaccumulation in marine organisms.

## 1. Introduction

Plastic materials are composed of polymers combined with a mix of chemical compounds that enhance their properties, performance, and manufacturing processes. These additives include plasticizers, flame retardants (FRs), stabilizers, pigments and other chemical compounds (Czogała et al., 2021; Hahladakis et al., 2018; Hermabessiere et al., 2017; Koelmans et al., 2022). Plasticizers are the main additive class and are used to increase the flexibility, softness, and toughness of plastic materials (Czogała et al., 2021). Other significant compounds are FRs additives, which are added to reduce the flammability of materials (Martínez-Carballo et al., 2007). FRs are classified into three categories based on their chemical composition: inorganic FRs, halogenated FRs (for example brominated or chlorinated FRs), and organophosphorus FRs (van der Veen and de Boer, 2012). FRs and plasticizers are added without being covalently bound to the materials (Czogała et al., 2021; Marklund et al., 2005); thus, they, can be unintentionally released in the marine environment by different pathways: leaching, wastewater (Kundu et al., 2022), industrial waste (Panagopoulos and Giannika, 2024) or atmospheric deposition (Hermabessiere et al., 2017). Among these classes of additives, phthalate esters (PAEs), organophosphate esters (OPEs), and non-phthalate plasticizers (NPPs) are widely used in many industrial, urban, and domestic applications.

PAEs are a dominant class of synthetic chemical compounds used as plasticizers (mainly high molecular weight PAEs) in PVC products, building materials, personal-care products, pharmaceuticals, and food packaging (Koniecki et al., 2011; Net et al., 2015). PAEs find application as solvents (mainly low molecular weight) in various industries, such as industrial and cosmetic products (Koniecki et al., 2011). Concerning toxicity, field studies carried out on humans have shown effects on the reproductive system, such as infertility (Rozati et al., 2002; Tranfo et al., 2012), damage to sperm (Rozati et al., 2002), and neurodevelopment effects (Engel et al., 2010; Miodovnik et al., 2011); while in vitro studies have shown anomalies in the reproductive tract in humans (Desdoits-Lethimonier et al., 2012) and in zebrafish (Santangeli et al., 2017). PAEs have also been found to be endocrine disruptors in fish in laboratory studies (Bhatia et al., 2014), and in marine mammals (in the field study of (Routti et al., 2021)). Some PAEs, due to their extensive use and toxicity, are subject to legislative restrictions in Europe (Amberg-Müller et al., 2010; Kyprianou, 2007; Ventrice et al., 2013).

OPEs are employed for different uses, such as flame retardants when chlorinated and brominated (organophosphorus flame retardants, OPFRs), and as plasticizers when they are non-halogenated (Andresen et al., 2004). OPEs are commonly added to materials such as textiles, plastics, electronics, building materials, furniture, lubricants, and varnishes (García-López et al., 2008; Isetun et al., 2004; Martínez-Carballo et al., 2007; Miyake et al., 2018). Several experimental studies have demonstrated the toxicity of some OPEs in different organisms, such as crustaceans, fish, rodents, and humans. These studies have shown that exposure to these substances can result in a number of adverse effects, including alterations to development and reproduction, as well as neurotoxicity, and furthermore some OPEs are also classified as carcinogenic (Chapin et al., 1997; Du et al., 2019; Greaves and Letcher, 2014; Kojima et al., 2013; Li et al., 2015; Meeker and Stapleton, 2010; Schang et al., 2016; van der Veen and de Boer, 2012; Wang et al., 2013; Yuan et al., 2018). NPPs such as adipates, benzoates, citrates, sebacates, and phosphates have been considered less toxic substitutes than PAEs (Bui et al., 2016; Fromme et al., 2016). NPPs have no use limitations (Gugliandolo et al., 2020) in toys, medical devices, and food packaging (LCSP, 2011). Although they are used as safe alternatives to PAEs compounds, some studies have reported their potential adverse effects. NPPs such as acetyl tributyl citrate (ATBC), 1,2 cyclohexanedicarboxilic acid (DINCH), and di(2-ethylhexyl) adipate (DEHA) can be endocrine disruptors (Horie et al., 2022; Sheikh and Beg, 2019; Zughaibi et al., 2022); and DEHA can also cause developmental toxicity (Dalgaard et al., 2003).

Exposure to plastic additives may be related to the direct release of these compounds into the environment. The presence of these chemicals has been identified in different environmental matrices, including sediment (Aznar-Alemany et al., 2018; Schmidt et al., 2021; Souaf et al., 2023), and water (He et al., 2019; Liu et al., 2015; Schmidt et al., 2021). Additives can also leach from plastic debris that come in contact with the organisms through ingestion of macro and microplastics (Fossi et al., 2012; Patsiou et al., 2024; Rios-Fuster et al., 2022). As a result, exposure can occur through inhalation, ingestion, or dermal contact in both marine organisms and humans. Despite the increasing concern of the scientific community, there are limited studies concerning the occurrence and concentration of OPEs in the Mediterranean Sea on fish (Castro-Jiménez et al., 2021; Garcia-Garin et al., 2020; Sala et al., 2022). Similarly, there are very few studies on NPPs (Gugliandolo et al., 2020; Jebara et al., 2021) and PAEs (Gugliandolo et al., 2020; Hidalgo-Serrano et al., 2021; Jebara et al., 2021; Marmara et al., 2024; Squadrone et al., 2023; Squillante et al., 2023). Moreover, there is not much information about the possible risks that the consumption of edible fish contaminated with these compounds can have to human health (Marmara et al., 2024). A diet based on fish species could therefore contribute to exposure PAEs (Das et al., 2014; Squillante et al., 2023), to OPEs (Bekele et al., 2021; Castro et al., 2020) and NPPs (Bui et al., 2016; Zhang et al., 2022), due to the potential bioaccumulation of these compound in fish muscle.

The main aim of this study was to assess the presence and concentration of three classes of plastic additives (OPEs, PAEs and NPPs) in the muscles of commercially important fish species including red mullet (*Mullus barbatus*), bogue (*Boops boops*), European hake (*Merluccius merluccius*), and European pilchard (*Sardina pilchardus*). These selected species were sampled from two areas of the Mediterranean Sea (Ligurian Sea and the Central Adriatic Sea), where there is a knowledge gap regarding these classes of contaminants. Moreover, these species were selected for their distinct ecological niches, habitats and feeding behaviors. The different habitats of the selected species may result in varying rates of OPEs, PAEs and NPPs bioaccumulation.

The additional goal of this study is to assess the potential risk to human health from fish consumption in these two areas and the related exposure in humans of different ages, with the objective of determining whether the detected levels might potentially pose a concern.

## 2. Materials and methods

### 2.1. Study areas

The Central Adriatic Sea and the Ligurian Sea were selected as study areas because little to no investigation has been conducted on the presence of PAEs, OPEs, and NPPs as contaminants in these regions.

The coasts of both the Central Adriatic Sea and the Ligurian Sea are characterized by strong urbanization and industrialization. Moreover, within the Adriatic Sea flows one of the most important rivers of Italy,

the Po River, which passes through some of the most industrialized areas of the country. Along both the Adriatic and Ligurian coasts, there are several plastic manufacturing companies and related industries that could contribute to the release of plastic additives used in the manufacture of their products into the environment. Given their significance as maritime transport routes and the high loads of maritime industrial and tourist activities, the Adriatic and Ligurian Sea waters are exposed to high levels of contamination, including plastic pollution (Galli et al., 2023; Renzi et al., 2010; Strafella et al., 2015; Suaria et al., 2016). A recent study investigating the presence of marine litter in the Adriatic Sea found a predominance of plastic litter in this region, with food packaging products being among the most frequently encountered categories (Pasanisi et al., 2023).

### 2.2. Sample collection

Fish samples of bogue (*Boops boops),* European hake *(Merluccius merluccius),* red mullet *(Mullus barbatus),* and European pilchard (*Sardina pilchardus)* were collected in February–March 2020 using otter trawl fishing vessels and surrounding nets in the Central Adriatic Sea (FAO Geographical Sub-Area, GSA-17) and the Ligurian Sea (FAO GSA-09) (Fig. 1). Six specimens of each species were collected from the two sampling areas for a total of 48 samples. For each fish sample, biometric parameters (total and fork length, total weight) were recorded (details in Table S1). Muscles were carefully collected from each fish specimen, wrapped in pre-cleaned aluminum foil to prevent contamination, and stored at −20 °C, until laboratory analysis. Muscles were then lyophilized and weighed to determine the water content. Subsequently, the samples were divided into two aliquots for the analysis of PAEs and the analysis of OPEs and NPPs.

### 2.3. PAEs analysis

Following the method described by Santini et al. (2024), 0.1 g dw was placed into a 10 mL glass vial, then 100 µL of d4 phthalates mix (1000 ppb) was added to the vial. An amount of 4 mL of acetonitrile was added to the extraction phase, followed by vortex shaking for 1 min. The vial was placed in an ultrasound bath for 15 min at 20 °C, followed by centrifugation for 5 min (4500 rpm at room temperature). Then, the supernatant was transferred to a glass tube, and 2 mL of acetonitrile were added to the residue, extracted again with a vortex for 1 min, and centrifuged for 5 min (4500 rpm). This second phase was performed twice. The samples obtained from the three extractions were purified using Agilent Technologies Bond Elute d-SPE. Upon transfer to the tube, the mixture was stirred with a vortex for 1 min and then centrifuged for 5 min (5000 rpm). To complete the procedure, 3 mL of acetonitrile was added to the dispersive vial, and stirring and centrifugation were repeated. The final extracts were reduced in volume with a gentle stream of ultrapure nitrogen and reconstituted in 0.3 mL of hexane. The instrumental analysis of PAEs was performed using an Agilent 8890 A series gas chromatograph coupled to an Agilent 5977B Inert Plus low-resolution mass spectrometer with a simple quadrupole analyzer equipped with a versatile HP -5MS capillary column (30 m, 0.25 mm). The injection volume was 1 µL (splitless mode), and ultrapure helium

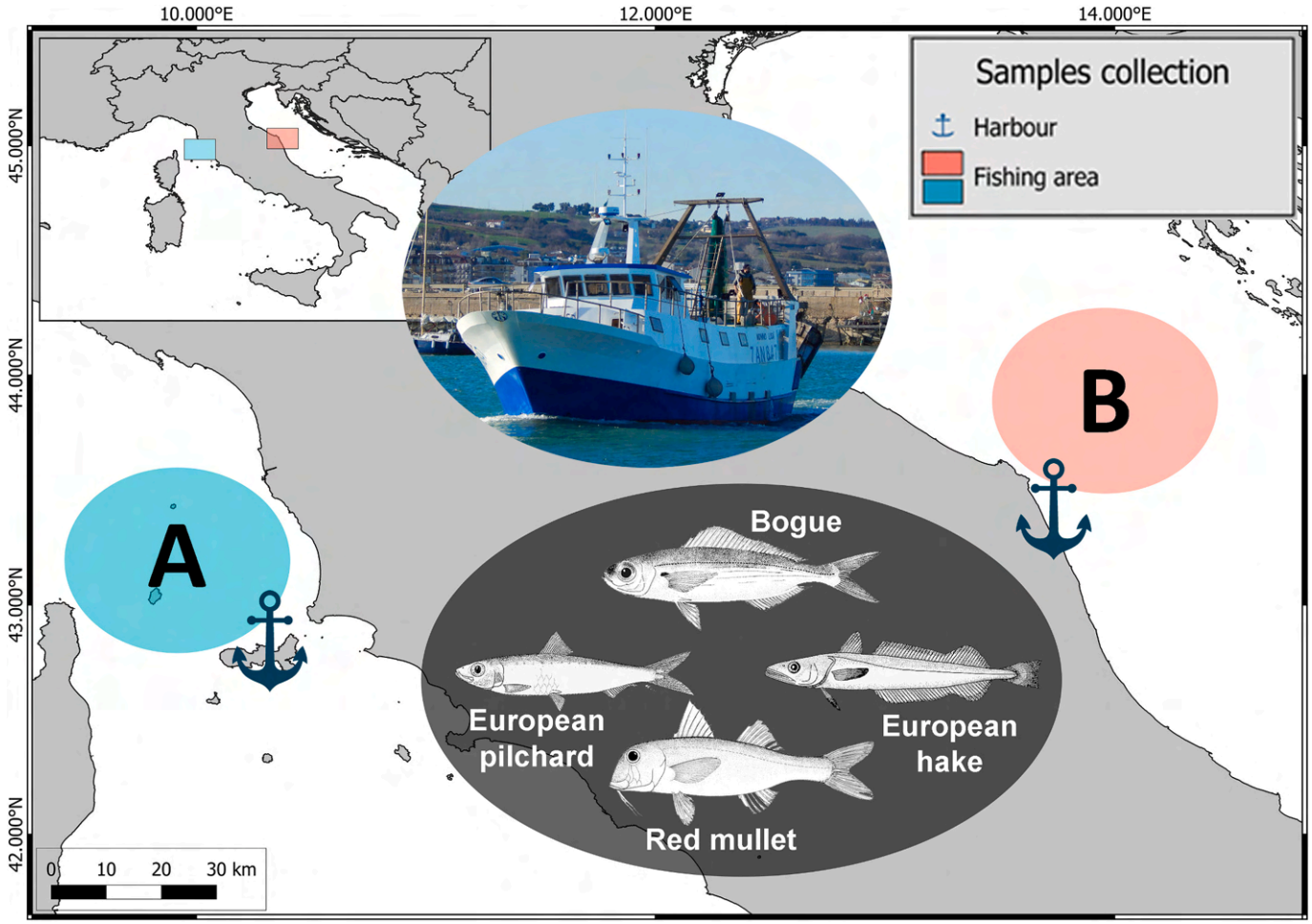


**Fig. 1.** Sampling areas. A = Ligurian Sea; B = Central Adriatic Sea. In the dark circle, the analyzed fish samples: *Boops boops*, *Merluccius merluccius*, *Mullus barbatus* and *Sardina pilchardus*.

with a constant flow rate of 1 mL/min was used as the carrier gas. The oven temperature was set at 80 °C and increased to 210 °C at 20 °C/min and, 240 °C at 15 °C/min. (holding time: 2 min.) and to 310 °C at 15 °C/min. (holding time: 3 min.). Quantitative analysis was performed by single ion monitoring (SIM). For the quantification of PAEs, a calibration curve was made for each compound, consisting of 11 points. The linearity of each calibration curve was assessed by least squares linear regression analysis, without considering the weighing factor. A correlation coefficient ($R^2$) equal to or >0.99 was considered crucial for acceptance.

### 2.4. OPEs and NPPs analysis

Following the methods described by Giulivo et al. (2016) and Fernández-Arribas et al. (2024) 0.8–1.0 g dry weight (dw) of the samples was extracted using 15 mL of a mixture of hexane:acetone (1:1) by ultrasound for 15 min, and the solution was centrifuged for 5 min at 4000 rpm. Extraction was performed twice, and the extracts were combined. The extract was reduced under a gentle nitrogen stream using the Turbovapor at 5 psi and a temperature of 20 °C. Finally, the extract was reconstituted in 2 mL of a mixture of hexane:methanol (1:3) and the solution was centrifuged for 5 min at 4000 rpm. For each sample, an aliquot of 200 μL was used for the instrumental analysis. An amount of 10 μL (1 ng/μL) of the IS mixture was added to this aliquot. Instrumental analysis of OPEs and NPPs was carried out according to Giulivo et al. (2016) and Fernández-Arribas et al. (2024) using turbulent flow chromatography – high pressure liquid chromatography – tandem mass spectrometry (TFC-HPLC-MS-MS), using the system Thermo Scientific TurboFlow™ (Waltham, MA, USA). The two purification columns used were CyclonTM-P (0.5 × 50 mm) and C18-XL (0.5 × 50 mm), and the chromatographic separation column was Purosphere Star RP-18 (125 mm × 0.2 mm), which has a particle size of 5 μm. Purification was achieved using a flow rate of 0.75 mL/min and the solvents (A) water (0,1 % formic acid) and (B) methanol (0.1 % formic acid) as mobile phases, while chromatographic separation was achieved using a flow rate of 0.25 mL/min and the mobile phases of (A) water (0.1 % formic acid) and (B) ammonium acetate in methanol. Mass spectrometric analysis was performed using a system with a triple quadrupole (QqQ) that used a heated electrospray ionization source (HESI) following the detection parameters described in Giulivo et al. (2016). For the quantification of OPEs and NPPs, a calibration curve was created for each compound, consisting of 10 points. The linearity of each calibration curve was assessed by least squares linear regression analysis, without considering the weighing factor. A correlation coefficient ($R^2$) equal to or >0.99 was considered crucial for acceptance. For the twenty OPEs, the area and the amount ratio were calculated considering the IS $^{13}$C2-TBOEP for TBOEP, 2IPPDPP, RDP, 4IPPDPP, TCP, EHDPP, B4IPPPP, IDPP, T2IPP and THP; $d_{27}$-TNBP for the TNBP and DCP; $d_{15}$-TDCIPP for the TPPO, TCIPP and TDCIPP; for the others OPEs was considered the corresponding internal standard. For the four NPPs, the area and the amount ratio were calculated by considering the IS $d_3$-ATBC for the ATBC while for the others NPPs, the IS $d_4$-DEHP was used.

### 2.5. Quality assurance and quality control (QA/QC)

Analytical blanks were prepared using the same sample preparation method to eliminate contamination during the sample preparation. Blank levels were subtracted from the corresponding samples. Analytical parameters such as recoveries, relative standard deviations (RSDs), limits of detection (LODs) and limits of quantification (LOQs) are summarized in Table S2. Details of the standards and reagents used are reported in the Supplementary Materials.

### 2.6. Risk assessment of human consumption

The potential risks due to PAEs, OPEs, and NPPs in fish intake were evaluated based on Tolerable Daily Intake (TDI), Reference Dose (RfD), Life-Time Cancer Risk (LTCR), and cumulative risk as a DEHP equivalent ($GPD_{Eq}$). The first part of the risk assessment involved calculating the edible daily intake (EDI). This value was then necessary for comparing the TDI and RfD values obtained from the literature(Table S5).

$$EDI = (IR \text{ x } CR)/BW$$

· IR = Intake Rate of fish (g/day) in toddlers (median: 50.00, 95th percentile: 94.63), adolescents (median: 56.50, 95th percentile: 177.73), and adult (median: 57.50, 95th percentile: 167.2) (EFSA, 2011)

· CR = Concentration of each contaminant detected in fish muscle (ng/g ww)

· BW = Body Weight ($kg_{bw}$) in toddlers (11.3), adolescents (52.6), adults (69.7) (Leclercq et al., 2009)

To assess the carcinogenic risk of DEHP, the LTCR (dimensionless) was used. This risk was evaluated only for DEHP because the Slope Factor value was available for this compound. Cancer risk was evaluated based using the equation proposed by Conradi et al. (1982).

$$LTCR = ((EDI \text{ x } EF \text{ x } TE)/AT) \text{ x } SF$$

· EF = Exposure Frequency of the contaminant (350 day/year)

· TE = Total Exposure (70 year)

· AT = Average Lifetime time for non-carcinogenic risk (TE x 365 day/year)

· SF = Slope Factor $(mg/kg_{bw}/day)^{-1}$

To evaluate the $GPD_{Eq}$, new EDI values were calculated by normalizing the concentrations of contaminants to DEHP and, multiplying each concentration by a different factor for each compound. The different factors visible are the ratio of the TDI or RfD of DEHP to that of the compound.

$$\text{Compound Factor}_{TDI} = TDI_{DEHP}/TDI_{compound}$$

$$\text{Compound Factor}_{RfD} = RfD_{DEHP}/RfD_{compound}$$

The United States Environmental Protection Agency (USEPA) categorizes an LTCR $>1 \times 10^4$ as an unacceptable risk of cancer development over a human lifetime, whereas values between $1 \times 10^6$ and $1 \times 10^4$ fall within an acceptable risk range (Conradi et al., 1982). Conversely, Health Canada and Alberta Environment and Parks (AEP) suggest a cancer development risk threshold of $1 \times 10^5$ (Health Canada, 2010). Then the hazard quotient (HQ) was calculated. This index, if greater than or equal to 1, represents a potential concern because, it implies the intake of a compound dose greater than that considered safe according to the TDI or RfD.

HQEDI/TDIorEDI/RfD

### 2.7. Statistical analysis

All statistical analyses were performed using R software (R 4.3.1) and Past4 (4.13), with the level of significance being set at $p = 0.05$. The Shapiro-Wilk test was used to assess data normality. The concentration differences of each class of contaminants were analyzed using the Mann-Whitney test for comparison between the two study areas, and the Mann-Whitney pairwise with Bonferroni corrected $p$ values for comparison among species within the same study area. Again, the Mann-Whitney test was used to compare the Edible Intake Values (EDI) for each contaminant between the two study areas. Finally, the Pearson correlation test was conducted to verify the degree of correlation among the pollutants.

## 3. Results and discussions

This study investigated the presence and concentrations of three classes of plastic additives in four edible fish species (European hake,

European pilchard, bogue and red mullet) collected in the Ligurian and Central Adriatic sea (Mediterranean Sea).

The mean concentrations and standard deviations of 11 PAEs, 20 OPEs, and 4 NPPs (ng/g ww) are presented in Table 1. As illustrated in Fig. 2, PAEs constituted the majority of the plastic additive loads detected, with a ubiquitous and similar distribution across study areas. In contrast, for the other additives, OPEs and NPPs, there is a more pronounced difference between the two study areas, suggesting that these compounds may represent disparate impact loads in the Ligurian Sea and the Central Adriatic Sea. Fig. 2 also shows the percentage contributions of PAEs and OPEs compounds detected; NPPs percentages contribution was not reported because only two compounds were detected at low concentrations. PAEs have been previously studied in red mullet (Squillante et al., 2023), European pilchard, and bogue (Marmara et al., 2024), while for European hake, this is the first evaluation as far as we know. OPEs have been previously studied in bogue, European pilchard, and European hake (Garcia-Garin et al., 2020; Sala et al., 2022), but for red mullet, this is the first evaluation. To the best of our knowledge, this is the first study to investigate the presence of NPPs in the selected species.

### 3.1. Phthalates levels

Nine of the eleven PAEs analyzed were detected, with DPrP and DChP below the limit of detection (<LOD) in all samples. The highest occurrence percentages were DEHP (98 %) > DBP (96 %) > DINP (92 %) and DIBP (90 %). The high DEHP concentration is consistent with the literature, as this compound is widespread in the environment, and is the most frequently measured in marine fauna (Squadrone et al., 2023). Despite being listed in the Hazardous Substances Data Bank (HSDB) and its usage being restricted (Carney Almroth and Slunge, 2022), this compound continues to be the most detected. DEHP is one of the most toxic phthalates (Liu et al., 2015), it is crucial to monitor and understand its distribution in organisms and throughout the trophic chain. DBP and

**Table 1**
Mean ± standard deviation values expressed in ng/g ww for the four fish species analyzed in the two study areas. nd = not detected, below LOD; nq = not quantifiable, below the LOQ. Mean and standard deviation values were calculated assigning ½ LOD to nd values, and LOD to nq values.

| | ***Sardina pilchardus*** | | ***Boops boops*** | | ***Mullus barbatus*** | | ***Merluccius merluccius*** | |
|---|---|---|---|---|---|---|---|---|
| | Central Adriatic Sea | Ligurian Sea | Central Adriatic Sea | Ligurian Sea | Central Adriatic Sea | Ligurian Sea | Central Adriatic Sea | Ligurian Sea |
| PAEs | | | | | | | | |
| DMP | 0.633 ± 0.600 | 0.779 ± 0.650 | 0.398 ± 0.300 | 0.201 ± 0.300 | 0.388 ± 0.248 | 0.539 ± 0.649 | 0.131 ± 0.0572 | 0.0452 ± 0.0326 |
| DEP | 2.35 ± 2.27 | 2.62 ± 1.19 | 1.58 ± 1.34 | 3.42 ± 0.994 | 7.69 ± 8.50 | 3.42 ± 0.99 | 3.46 ± 5.89 | 1.47 ± 1.78 |
| DAP | 8.51 ± 10.05 | 0.463 ± 0.562 | 0.760 ± 1.30 | 1.10 ± 0.949 | 5.55 ± 6.05 | 10.0 ± 10.7 | 1.30 ± 1.36 | 0.0966 ± 0.0305 |
| DPrP | nd | nd | nd | nd | nd | nd | nd | nd |
| DIBP | 47.8 ± 31.3 | 23.9 ± 12.8 | 15.1 ± 8.56 | 14.3 ± 5.99 | 176 ± 179 | 14.3 ± 5.99 | 24.5 ± 37.3 | 2.36 ± 4.30 |
| DBP | 100 ± 63.9 | 29.3 ± 10.2 | 30.4 ± 47.7 | 12.2 ± 5.32 | 142 ± 144 | 12.2 ± 5.32 | 17.2 ± 23.4 | 7.38 ± 5.49 |
| BBzP | 1.88 ± 1.15 | 2.15 ± 2.49 | 1.42 ± 1.64 | 5.14 ± 2.78 | 3.10 ± 1.70 | 5.14 ± 2.78 | 1.16 ± 0.96 | 4.23 ± 5.90 |
| DChP | nd | nd | nd | nd | nd | nd | nd | nd |
| DEHP | 57.0 ± 22.7 | 69.7 ± 34.6 | 89.7 ± 58.0 | 93.1 ± 39.1 | 107 ± 74.8 | 93.1 ± 39.1 | 36.6 ± 40.5 | 78.3 ± 91.9 |
| DINP | 9.10 ± 7.58 | 18.7 ± 10.9 | 2.39 ± 1.85 | 4.05 ± 2.35 | 10.4 ± 15.8 | 4.05 ± 2.35 | 1.32 ± 0.537 | 1.36 ± 0.731 |
| DNOP | 6.95 ± 7.60 | 7.52 ± 2.64 | 2.70 ± 2.60 | 7.78 ± 4.17 | 6.08 ± 7.82 | 7.78 ± 4.17 | nd | 0.731 ± 0.491 |
| **ΣPAEs** | **234 ± 106** | **155 ± 54.9** | **144 ± 112** | **141 ± 43.0** | **459 ± 389** | **141 ± 43** | **85.2 ± 106** | **95.7 ± 94.4** |
| OPEs | | | | | | | | |
| TEP | 3.93 ± 4.57 | 39.8 ± 13.2 | 0.262 ± 0.432 | 15.3 ± 4.20 | 0.0378 ± 0.0730 | 19.8 ± 5.11 | nd | 4.91 ± 2.24 |
| TPHP | nd | 0.303 ± 0.466 | nd | nd | nd | nd | nd | 0.0487± 0.0997 |
| TNBP | nd | 0.359 ± 0.425 | 0.676 ± 1.275 | nd | nd | 0.491 ± 0.961 | nd | nq |
| DCP | nd | 1.21 ± 1.83 | nd | nd | 0.0610 ± 0.0372 | nd | nd | 0.180 ± 0.276 |
| TBOEP | nd | nd | nd | nd | nd | nd | nd | 0.0380± 0.0638 |
| 2IPPDPP | nd | 0.556 ± 0.967 | nd | nd | nd | nd | nd | nd |
| RDP | nd | 2.67 ± 4.12 | nd | nd | nd | 0.0172± 0.0274 | nd | 0.208 ± 0.378 |
| 4IPPDPP | nd | 0.791 ± 1.26 | nd | nd | nd | nd | nd | 0.132 ± 0.266 |
| TCP | nd | 1.00 ± 1.42 | nd | nd | nd | nd | nd | 0.139 ± 0.200 |
| EHDPP | nd | 1.20 ± 1.75 | nd | nd | nd | 0.406 ± 0.846 | nd | 0.106 ± 0.142 |
| B4IPPPP | nd | 1.04 ± 1.65 | nd | nd | nd | nd | nd | 0.128 ± 0.279 |
| T2IPPP | 0.04 ± 0.04 | 2.43 ± 4.18 | nd | nd | nd | 0.0267± 0.0163 | nd | 0.222 ± 0.499 |
| THP | nd | 0.87 ± 1.26 | nd | nd | nd | nd | nd | 0.113 ± 0.243 |
| TEHP | nd | 9.47 ± 15.05 | nd | nd | nd | 0.590 ± 0.579 | nd | 3.26 ± 3.52 |
| TCEP | nd | nd | nd | nd | nd | nd | nd | nd |
| TPPO | nd | nd | nq | nd | nd | nd | nd | nq |
| TCIPP | 0.0475 ± 0.0598 | nd | 0.0517 ± 0.0531 | nd | 0.144 ± 0.245 | nd | nd | 0.0408± 0.0344 |
| TDCIPP | nd | nd | nd | nd | nd | nd | nd | nd |
| TPrP | nd | nd | nd | nd | nd | nd | nd | nd |
| IDPP | nd | nd | nd | nd | nd | nd | nd | nd |
| **ΣOPEs** | **3.99 ± 4.56** | **61.2 ± 33.4** | **0.921 ± 1.22** | **15.3 ± 4.20** | **0.184 ± 0.256** | **21.1 ± 4.53** | **nd** | **9.24 ± 6.84** |
| NPPs | | | | | | | | |
| ATBC | 0.626 ± 0.397 | 1.16 ± 0.93 | 0.736 ± 0.837 | 0.550 ± 0.489 | 0.340 ± 0.260 | 0.511 ± 0.128 | 0.271 ± 0.628 | 0.301 ± 0.260 |
| DEHA | 0.853 ± 2.02 | nd | nd | nd | 5.86 ± 13.8 | nd | nd | 18.9 ± 44.1 |
| DINA | nd | nd | nd | nd | nd | nd | nd | nd |
| DINCH | nd | nd | nd | nd | nd | nd | nd | nd |
| **ΣNPPs** | **1.44 ± 2.18** | **1.16 ± 0.93** | **0.731 ± 0.842** | **0.547 ± 0.5492** | **5.98 ± 13.84** | **0.511 ± 0.128** | **0.259 ± 0.634** | **0.256 ± 0.634** |

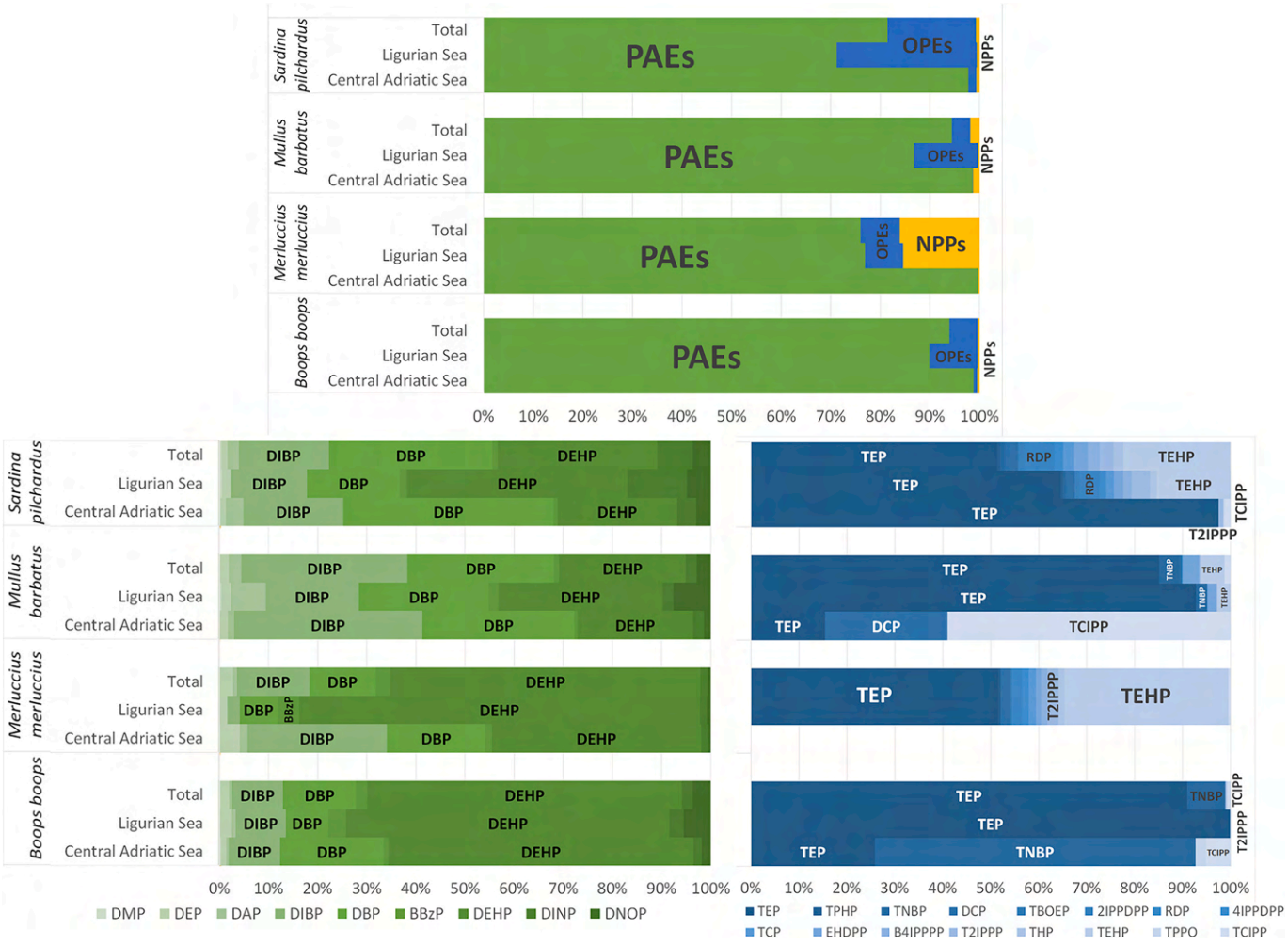


**Fig. 2.** The upper panel shows the percentage of the total load of the three classes of plasticizers analyzed in each species, while the lower panels show the percentage of phthalates (left) and organophosphate esters (right) compounders detected over the total and in the two study areas for each species analyzed.

DIBP have also consistently been reported to exhibit elevated occurrence values, highlighting their significant presence and potential impacts on aquatic ecosystems. They often show high levels of occurrence in relation to fish contamination, as documented by multiple studies analyzing different fish species (Gugliandolo et al., 2020; Jebara et al., 2021; Patsiou et al., 2024). An opposite trend was highlighted in a study by Marmara et al. (2024), in which DBP was found below the limit of quantification in all species analyzed (red mullet, European pilchard and bogue) from the Northern Aegean Sea and the Western Ionian Sea. Regarding DINP, this compound has been investigated in very few studies in the Mediterranean Sea region, and it was detected only in the muscle of three fish species by Squillante et al. (2023) and in the gastrointestinal tract of European anchovy (*Engraulis encrasicolus*) (Marmara et al., 2024). The high occurrence rate could indicate the need for in-depth studies on possible effects of DINP and environmental fate, especially because it is also one of the main substitutes for DEHP (Marmara et al., 2024). The occurrence levels of PAEs in different species and areas are highlighted in Tables S3 and S4. The ΣPAEs had their maximum value in a sample of red mullet from the Central Adriatic Sea, where the species had an average value of 459 ± 390 ng/g ww, while in the Ligurian Sea, the mean value decrease to 141 ± 43.0 ng/g ww. Despite the difference, the large variability of values from the Central Adriatic Sea resulted in no statistically significant differences between the two areas (Mann-Whitney, $p$ value >0.05). Also, for the other three species, no statistically significant differences have been highlighted between the sampling areas or between them (Fig. 3). In the study by Patsiou et al. (2024), from the Ionian Sea, similar levels were found in *Mullus surmuletus,* which has a habitat and feeding habit similar to that of red mullet. In this case, the ΣPAEs mean values was 657 ± 124 ng/g dw in Alykes, 594 ± 147 ng/g dw in Tsilivi and 449 ± 76.6 ng/g dw in the marine protected area of Zakynthos Island. This similarity could suggest that the level of PAEs may depend on the dietary habits and habitats of marine organisms. DIBP, DBP, and DEHP represent >88 % of the total ΣPAEs. These compounds have a molecular weight higher than 270 g/mol that can lead to a relatively higher lipophilicity. This suggests that species with higher lipid content in the muscle which may increase the capability of bioaccumulation in this species. In this study, red mullet, showed the lowest water percentage in the tissue (Table S1), indicating a probable high lipid content, and consequently exhibited the highest sum of mean concentrations of all contaminants. The maximum mean value for DEHP was found in red mullet specimens from Central Adriatic Sea (108 ± 74.8 ng/g ww) (Fig. 3). It has been shown that hydrophobic phthalates such as DEHP are strongly bound to sediment and are detected at higher concentrations in species associated with this environmental matrix, like red mullet (Hu et al., 2016). The detected levels of DEHP were higher than those found by Squadrone et al. (2023), where the presence of DEHP was investigated in various fish species, including European anchovy, where the levels were always below the LOQ. Meanwhile, our results were consistent with those of Squillante et al. (2023), where DEHP levels in fish caught along the coast of Campania ranged between 187 and 776 ng/g. Regarding DBP, a statistically significant difference was observed for European pilchard

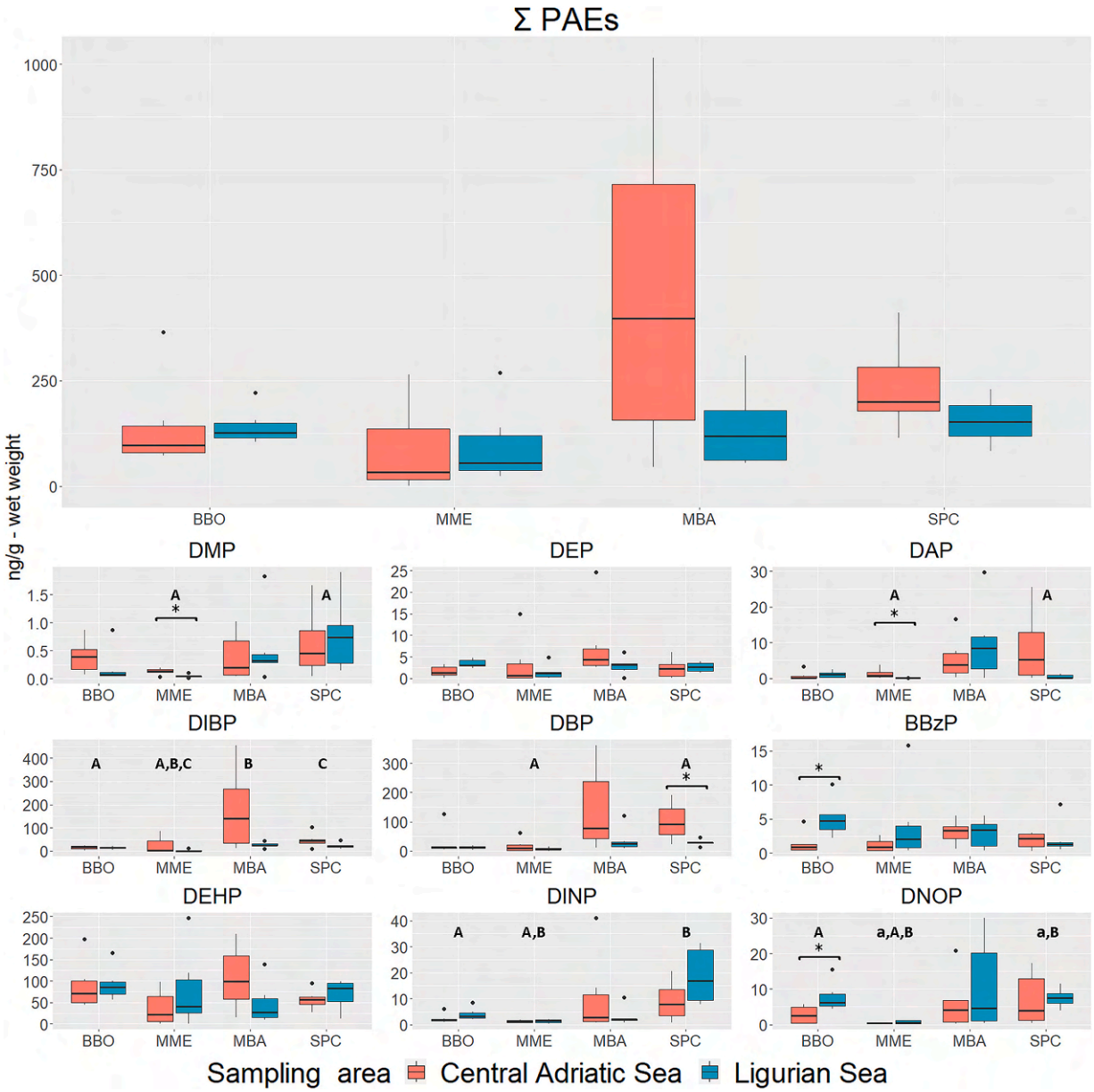


**Fig. 3.** Phthalate concentrations are expressed in ng/g wet weight. Square brackets indicate a statistically significant difference between the two study areas within the same species, while identical letters indicate a statistically significant difference between different species within the same area (lowercase letters for the Central Adriatic Sea and uppercase letters for the Ligurian Sea).

between the two areas ($p$-value = 0.045; Mann-Whitney test), with a mean value of 29.3 ± 10.3 ng/g ww for the Ligurian Sea and 100 ± 63.9 ng/g ww for the Central Adriatic Sea (Fig. 3). Observing the other compounds, we noted statistically significant differences in the concentrations of DMP ($p$ value = 0.020; Mann-Whitney) and DAP (p value = 0.008; Mann-Whitney), two low-molecular compounds, in European hake specimens (Fig. 3). The DMP and DAP concentrations were very low compared with prevalent PAEs, accounting for 0.21 % and 1.90 % of the total ΣPAEs, respectively. In both cases, specimens from the Adriatic Sea were highly contaminated. The results indicate that compounds with low molecular weights show statistically significant differences, with higher values observed in the Central Adriatic Sea. These results show that compounds more closely associated with the water environmental fraction (lower $K_{ow}$), like DMP and DAP, were found at higher concentrations in the Adriatic Sea samples. This could be due to the lower water exchange and characteristics of this semi-closed basin. However, given the limited number of statistically significant differences identified, more in-depth and larger scale analyses are needed. A statistically significant difference with an opposite trend to those previously detected was found in the species bogue for the phthalates BBzP (p value = 0.020; Mann-Whitney) and DNOP (p value = 0.031), where higher values were found in individuals sampled in the Ligurian Sea compared with those in the Adriatic Sea (Fig. 3). The results indicate that compounds with higher molecular weight (high $K_{ow}$ value) show statistically significant differences, with higher values found in the Ligurian Sea. These PAEs, when compared with the more abundant ones, also showed significantly lower values, representing 1.51 % (BBzP) and 2.94 % (DNOP) of the ΣPAEs. Results for other PAEs are summarized in Table 1 and Fig. 3.

### 3.2. Organophosphate esters levels

In this study, OPEs were detected in 79 % of the analyzed samples with a total concentration between 0.13 and 103 ng/g ww (mean 13.99

± 22.5 ng/g ww). Fifteen OPE congeners out of the total twenty analyzed were detected. TCEP, TPPO, IDPP, TPrP, and TDCIPP were below the limit of quantification in all samples. Considering the two study areas separately, OPEs were detected in 100 % of the analyzed samples in the Ligurian Sea with a concentration range between 5.08 and 103 ng/g ww (26.7 ± 26.3 ng/g ww), while in the Central Adriatic Sea, OPEs were detected in 58 % of the analyzed samples with a concentration range between 0.13 and 12.6 ng/g ww (1.27 ± 2.75 ng/g ww). Fig. 2 presents the percentage contribution of congeners in different species and areas.

In the Ligurian Sea, European pilchard exhibited the highest mean concentration of ΣOPEs (61.2 ± 33.4 ng/g ww), followed by red mullet (21.1 ± 4.53 ng/g ww), bogue (15.3 ± 4.20 ng/g ww), and European hake (9.24 ± 6.84 ng/g ww) (Table 1). In the Central Adriatic Sea, European pilchard showed the major concentration of ΣOPEs (3.99 ± 4.56 ng/g ww), followed by bogue (0.92 ± 1.22 ng/g ww) and red mullet (0.18 ± 0.26 ng/g ww) (Table 1). Contrarily, in European hake all compounds were below the LOD or LOQ.

The results show that in the two study areas, European pilchard showed the highest ΣOPEs concentration while European hake showed the lowest ΣOPEs concentration (Table 1). These results are similar to those of Sala et al. (2022) conducted on European pilchard, European anchovy, and European hake from the Spanish coasts, where the highest uptake of OPEs in European pilchard may be attributed to its different

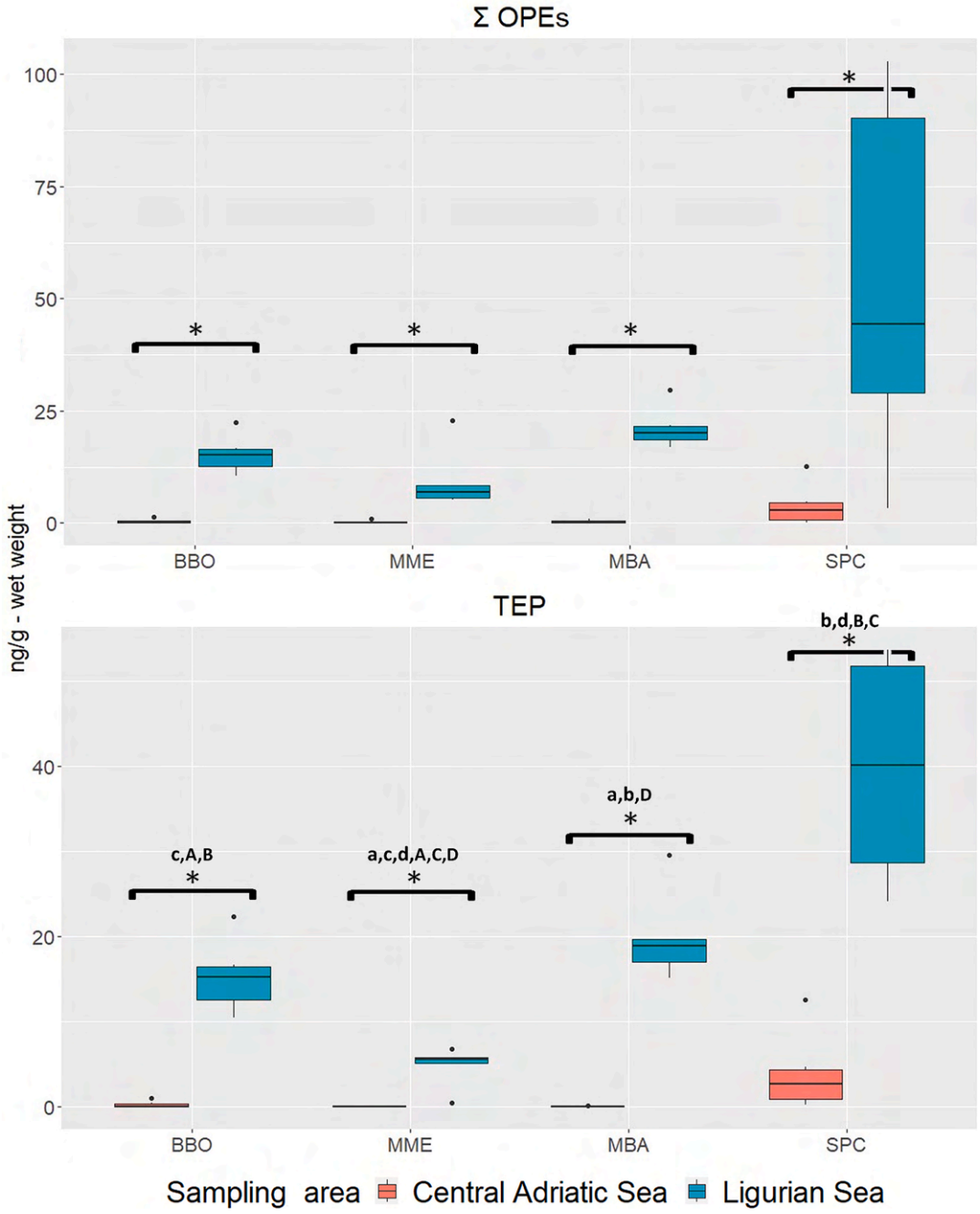


**Fig. 4.** OPE concentrations are expressed in ng/g ww. Statistical differences were obtained through Mann-Whitney pairwaise test ($p$-value <0,05). Square brackets indicate a statistically significant difference between the two study areas within the same species, while identical letters indicate a statistically significant difference between different species within the same area (lowercase letters for the Central Adriatic Sea and uppercase letters for the Ligurian Sea. The abbreviations used in the graph correspond to the following species: BBO=*B. boops*; MME = *M. merluccius*; MBA = *M. barbatus*; SPC=*S. pilchardus*.

diet composition and feeding behavior compared to other species. Indeed, the European pilchard is a filter feeder and feeds on plankton (Costalago et al., 2015; Garrido et al., 2007), whereas the European hake feeds on pelagic preys and during the night on phytoplankton (Buchholz et al., 1995; Fernandes et al., 2015). Therefore, it can be assumed that feeding habits and habitat may exert an influence on the accumulation of these contaminants.

Comparing the mean concentrations of ΣOPEs between this study and that conducted by Sala et al. (2022) in the Western Mediterranean Sea (Spanish coast), the mean values obtained in this study for European pilchard and European hake from the Ligurian Sea were about three times higher than those obtained for samples from Spain. On the contrary, similar concentrations were obtained for bogues from the Ligurian Sea and specimens sampled in Spain in the study of Garcia-Garin et al. (2020). In this study, significative differences were observed when comparing the ΣOPEs concentrations obtained for each individual species between the Ligurian Sea and the Central Adriatic Sea (Mann-Whitney, $p$-value$<0.05$) (Fig. 4).

Although TEP appears to be a hydrophilic compound with a low tendency to accumulate (Log Kow = 0.8 and BCF = 3.88 (van der Veen and de Boer, 2012)), it is interesting to note that it was the most abundant OPE among those analyzed in both the Ligurian Sea and the Central Adriatic Sea. European pilchard exhibited the highest mean concentration of TEP in both study areas (39.8 ± 13.2 ng/g ww in the Ligurian Sea and 3.93 ± 4.57 ng/g ww in the Central Adriatic Sea) (Table 1). Significant differences were observed when comparing TEP concentrations in each species between the two studied areas (Mann-Whitney, $p$-value $<0.05$), as well as among species within the same study area, as shown in Fig. 4.

The TEP results obtained in this study are in contrast with those previously reported in the literature on fish, and the OPEs pattern differs. In the study by Castro et al. (2020) and in Garcia-Garin et al. (2020), TEP was detected at concentrations below the limits of quantification. Given the existing literature on the species under investigation in this study, for European hake, the most frequent compounds were TNBP, DCP, and TBOEP (with the highest concentration), for European pilchard DCP and TCEP, with TDCIPP found at the highest concentration (Sala et al., 2022), for bogue TNBP (found at elevated concentrations), EHDPP and TPPO (Garcia-Garin et al., 2020). In a study by Sala et al. (2021), the OPEs concentrations in the muscles of loggerhead turtles and in its preys (European pilchard, jellyfish and squid) was investigated, and TEP was detected in every sample with higher levels in the muscles of the loggerhead turtles and in plastic debris compared with its preys, showing a possible biomagnification of this compound.

The observed differences in concentrations, including the variation in OPE patterns, can be attributed to the distinct physical-chemical properties of the compounds, as well as to the sources and levels of contamination at the sampling locations. Indeed, as previously highlighted in other studies, the OPE concentrations found in fish can be subject to variations depending on geographical location (Castro et al., 2020; Li et al., 2019; Sala et al., 2022). Additionally, variations in concentrations between the same species or among different species from different areas could also be attributed to differences in feeding habits, body size, and metabolic capacity (Kim et al., 2011; Li et al., 2019; Sundkvist et al., 2010).

TEP is used together with other OPEs such as TCEP, TEHP, TPhP, IDPP, and DCP as a plasticizer in PVC (Björklund et al., 2004; Lassen and Lokke, 1999; WHO, 1990, 1997, 1998, 2000), but also in polyester resins and polyurethane foam (EFRA, 2011; WHO, 1997). TEP is under REACH registration (ECHA, 2023), and its effects remain largely unexplored. Egloff et al. (2014) reported different effects of TEP in developing chicken embryos, particularly at concentrations ≥43,200 ng/g, on the embryo viability, somatic growth, bile acid concentration, and hepatic mRNA expression. Exposure to OPEs could instead be carcinogenic and neurotoxic, affect kidney functioning (van der Veen and de Boer, 2012; WHO, 1998, 2000), cause skin irritation and decrease sperm quality (Meeker and Stapleton, 2010), and act as endocrine disruptors in humans (Heindel et al., 2017).

### 3.3. Non-phthalate plasticizers levels

NPPs were detected in 79 % of the total analyzed samples, with concentrations ranging between 0.13 and 109 ng/g ww (3.73 ± 16.4 ng/g ww). DINA and DINCH were always below the limit of quantification in all samples, while ATBC was detected with an occurrence range between 17 % - 100 % (Table S3). DEHA was detected only in two species from the Central Adriatic Sea and in one species of the Ligurian Sea (see the Table S3 for occurrence details). In the Ligurian Sea specimens, an occurrence of 92 % was obtained for NPPs, with concentration range between 0.13 and 109 ng/g ww (5.36 ± 22.2 ng/g ww). In contrast, specimens from the Central Adriatic Sea an occurrence of 67 % was obtained with concentration range between 0.18 and 34.2 ng/g ww (2.10 ± 6.95 ng/g ww). The highest mean concentration of ∑NPPs was detected in specimens sampled in the Central Adriatic Sea, particularly in red mullet and European pilchard (Table 1).

There are limited studies on the presence of NPPs in fish species. Gugliandolo et al. (2020) and Jebara et al. (2021) investigated the presence of NPPs (DEHA and DEHT) in gilthead seabream (*Sparus aurata)* from Tunisia, reporting a DEHA+DEHT concentration of 26.3 μg/g dw. In contrast, DEHA was detected in a few specimens in this study, at a lower concentration than those reported in other studies. The highest DEHA concentration was detected in European hake sampled from the Ligurian Sea (Table 1).

ATBC (acetyl tributyl citrate) was the NPP detected with the highest occurrence in this study, showing the highest mean value found in European pilchard, followed by bogue, red mullet, and European hake in the Ligurian Sea (Table 1). In the Central Adriatic Sea, ATBC had the highest mean value in bogue, followed by European pilchard, red mullet, and European hake (Table 1). No significant differences were observed in ΣNPPs and ATBC concentration between the two areas (Mann-Whitney, p-value $>0.05$; Fig. 5). Furthermore, no significant differences were observed when comparing the four species sampled within the same area for both the concentration of ΣNPPs and ATBC concentration (Mann-Whitney in pairs, p-value $>0.05$; Fig. 3).

ATBC, a member of the citric acid esters (CAEs) class, is a lipophilic compound with low water solubility. This compound is commonly used in food packaging, cosmetic products, adhesives, and inks (Bui et al., 2016; Johnson, 2002; Stuer-Lauridsen et al., 2001). Although generally considered a non-toxic compound, recent studies demonstrated that it could cause metabolic disturbance and fatty liver induction in rats (Zhang et al., 2023), and interfere with estrogenic and androgen homeostasis (Sheikh and Beg, 2019). To the best of our knowledge, this study is the first to investigated the presence of ATBC in wild fish species, whereas most studies have focused on its presence and other NPPs compounds in food. García Ibarra et al. (2019) studied the migration of plasticizers from plastic packaging into cereal based foods, finding ATBC at higher concentration in all samples. Moreover, human exposure was higher than that of phthalates and other alternative plasticizers. In another study, Zhang et al. (2022) investigated the presence of CAEs in different foods, and their results showed that ATBC had the highest occurrence in sweets, vegetables, eggs/milk, and milk/fish. The presence of these compounds in food is likely due to migration from food packaging. Fernández-Arribas et al. (2024) also detected NPPs, including ATBC, in different matrices such as food, face masks, and indoor air, confirming the widespread presence of these compounds in the environment. These findings underscore the need for increased monitoring to investigate their presence of NPPs in the marine environment and marine organisms, where non-phthalate plasticizers may enter as a result of plastic materials dispersion.

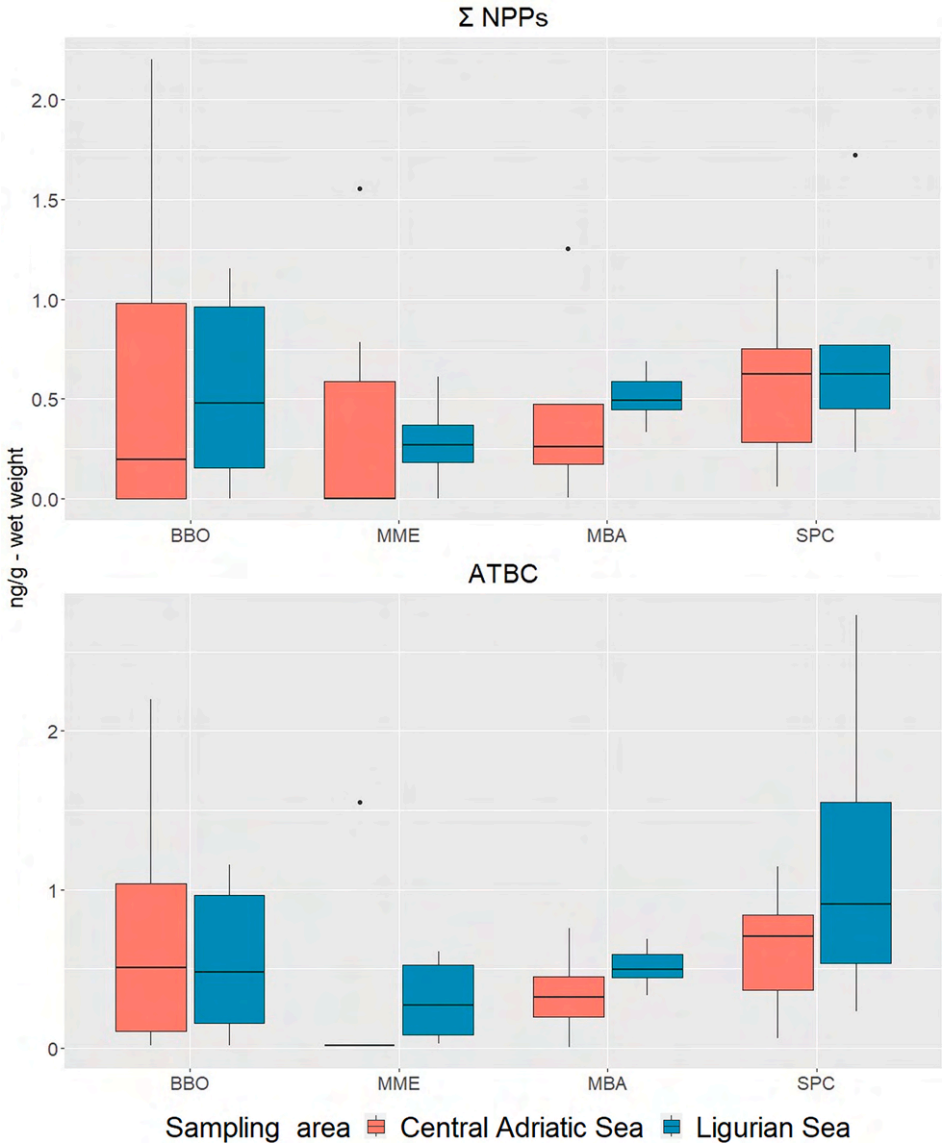


**Fig. 5.** NPPs concentrations are expressed in ng/g ww. For demonstration purpose, the outliers were removed to visualize the boxplot. No statistically significant differences were found between species or areas. The abbreviations used in the graph correspond to the following species: BBO═*B. boops*; MME = *M. merluccius*; MBA = *M. barbatus*; SPC═*S. pilchardus*.

### 3.4. Plastic additives PCA and correlations

PAEs were the most frequently detected class and exhibited the highest concentrations, followed by OPEs and NPPs. A comparison of the species revealed that red mullet was the most affected, with a higher mean concentration. To determine whether the area or species factor exerts any influence on the observed concentrations, we conducted a series of preliminary principal component analyses (PCAs). Subsequently, Pearson correlations coefficients were employed to identify any statistically significant correlations between the three classes of contaminants. Fig. 6 shows PCA and Pearson's correlation for individual species to investigate the differences between study areas. For completeness, the PCA of the two study areas has been reported in supplementary material (S1).

Differences in the accumulation trends of compounds were more pronounced in the Ligurian Sea than in the Central Adriatic Sea. The differences in the concentration of plastic additives detected in the specimens from the two areas may be attributed to the varying levels of plastic waste present in the marine environment. For example, the study by Baini et al. (2017) indicates a positive correlation between the presence of microplastics and the levels of PAEs. Conversely, the study by Schmidt et al. (2021) suggests that, in addition to microplastics, other factors may contribute to the dispersion of plastic additives in the sea, including urban and industrial discharges. These differences could also be attributed to the disparate seabed depths between the two seas or to the varying recirculation of water, with the Adriatic Sea being a semi-enclosed basin. The variation in seafloor depths may determine different feeding behaviors in the same species. The species could also have different metabolic capacities, which could therefore determine disparate levels of plastic additives found in them. As shown in Fig. 2, the congeners that contributed the most to plastic additives concentrations in almost all areas and species were DEHP, DBP, and DIBP. A negative correlation was observed between DEHP and ATBC in red mullet ($p < 0{,}05$, $r = -0{,}68$), whereas for bogue specimens BBzP ($p < 0{,}05$, $r = 0{,}59$) and DNOP ($p < 0{,}05$, $r = 0{,}64$) exhibited a positive correlation with TEP (Fig. 6). The area factor appears to exert the most significant influence on observed trends in compound concentrations within the species. In the Central Adriatic Sea, a greater number of PAEs exhibited correlations among themselves. In contrast, no significant correlation was observed among the different classes of plastic additives.

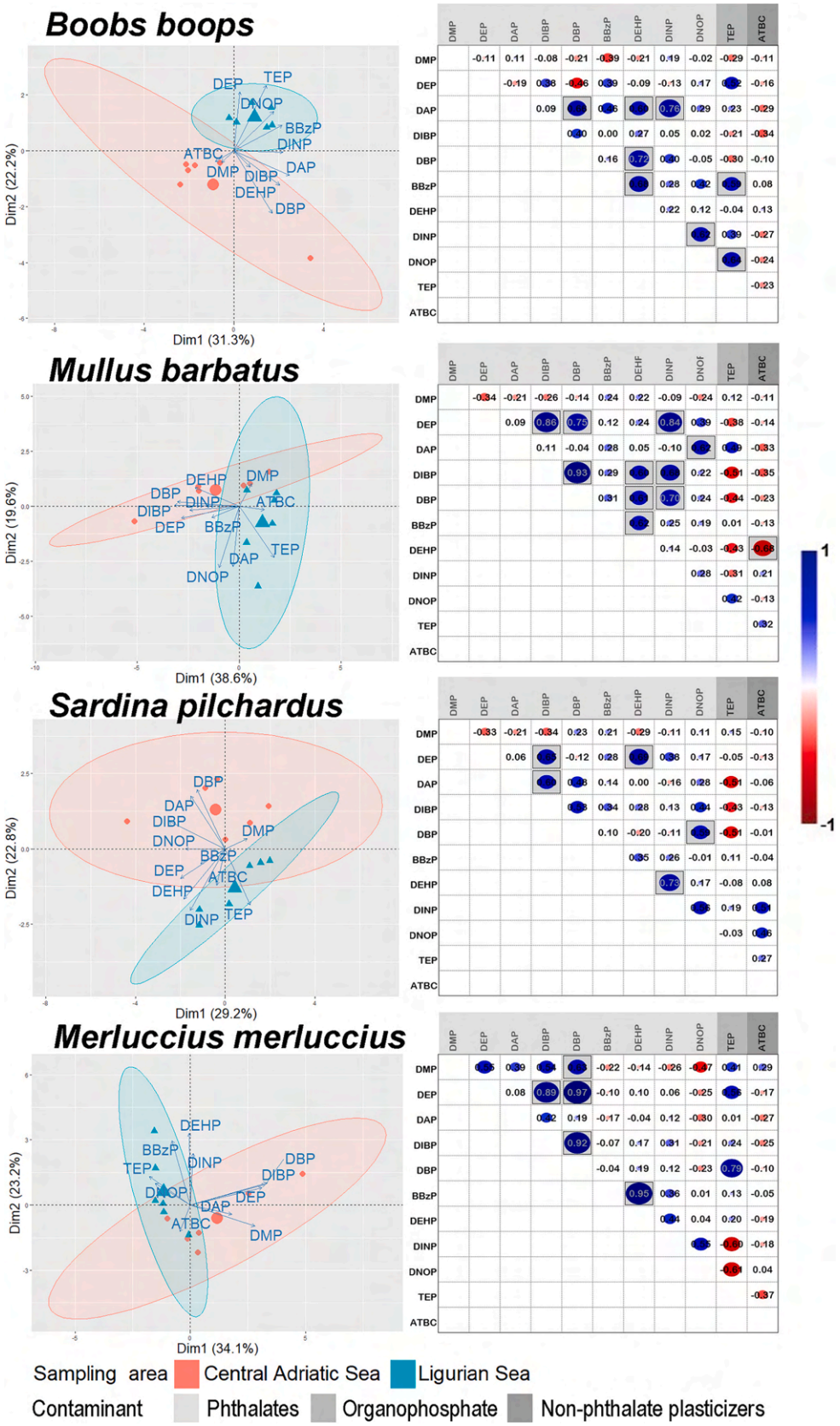


**Fig. 6.** Principal component analysis (PCA) (top) and Pearson correlation (bottom) of PAEs, OPEs and NPPs compounds. Each species was analyzed based on the total number of specimens belonging to the given species that were analyzed in the two areas (i.e. 6 + 6 specimens). In Pearson correlation graphs, the box indicates a statistically significant difference ($p$-value <0.05) and the number within the boxes indicated the degree of correlation.

Regarding the Ligurian Sea, fewer correlations among PAEs were identified, but a correlation was observed between DINP and ATBC. These analyses confirmed that PAEs are a ubiquitous problem in both areas, whereas other plastic additives are more prevalent in the Ligurian Sea. Consequently, a risk assessment was conducted to ascertain the relative risk across the two study areas.

### 3.5. Risk assessment of human exposure

Among the compounds analyzed and detected, DEP, DBP, BBzP, DEHP, DINP, TEP, and ATBC have threshold values that are related to the Tolerable Daily Intake or Reference Dose for humans (see Table S5 for threshold values). These include the most concentrated and prevalent compounds in our study (DEHP and DBP). Subsequently, we investigated the potential risk associated with fish consumption in a relation to the analyzed samples. No distinctions were made among species because we considered all species to be at the same consumption level. The risk assessment was calculated for both the best case (median consumption of fish) and worst case (95th percentile consumption of fish), considering separately toddlers, adolescents, and adults. For each


**Fig. 7.** Estimated Daily Intake (EDI) (best case) on a logarithmic scale of five PAEs, one OPE and one NPP detected in fish muscles compared with respective Tolerable Daily Intake (TDI) (dashed red line) or Reference Dose (RfD) (solid red line). The square brackets indicate statistically significant differences between the two study areas.

contaminant, the value of the EDI, both in the best case and worst case, was found to be lower than the TDI and/or RfD (Table S6 and Fig. 7), which ensured that the HQ was always lower than 1. Also, regarding the cumulative risk of $GPD_{Eq}$ contaminants, the value was found to be lower than that of TDI and RfD (Table S7). Significative differences were obtained only for TEP's EDI values between the two areas for both the best and worst scenarios (Mann-Whitney, p-value<0,05). This result can be related to the highest concentration of TEP in fish sampled in the Ligurian Sea, as reported above in the OPEs level section.

For DEHP, the LTCR mean values were found to be below the threshold of unacceptable risk in both the best-and worst-case scenarios. In detail, we can see how the mean values for toddlers in the best-case scenario and all three categories (toddlers, adolescents, and adults) in the worst scenario are within the acceptable risk range evaluated by USEPA, but are always below the limit set by Health Canada (see Fig. 8). The comparison of our results with the study of Squillante et al. (2023) conducted in the Tyrrhenian Sea, has shown similar EDI values for DBP in both studies, while in our study, smaller values were found for DEP and DINP, and higher EDI values for DEHP, both for the best and worst scenarios. The Hazard Quotient values obtained are in line with other studies conducted in other countries on OPEs, NPPs, and PAEs (Bekele et al., 2021; Sala et al., 2022; Squillante et al., 2023; Zhang et al., 2022). The objective of the risk assessment analysis was to ascertain whether the detected levels might potentially pose a concern. The findings indicated that the fish consumption with the plasticizers levels from the Central Adriatic Sea and the Ligurian Sea may not pose a risk to human health. However, further investigations is recommended, as some values are approaching the limit threshold, particularly LTCR values. Additionally, exposure to these contaminants can also occur through other routes, so it is therefore important to consider all exposure pathways to evaluate a possible human risk.

## 4. Conclusions

In this work, PAEs, OPEs, and NPPs were detected in muscle samples of four fish species of commercial interest (18.5 % of the total fish landed in the Mediterranean Sea)(FAO, 2022): European pilchard, bogue, red mullet, and European hake, which were sampled in the Ligurian and

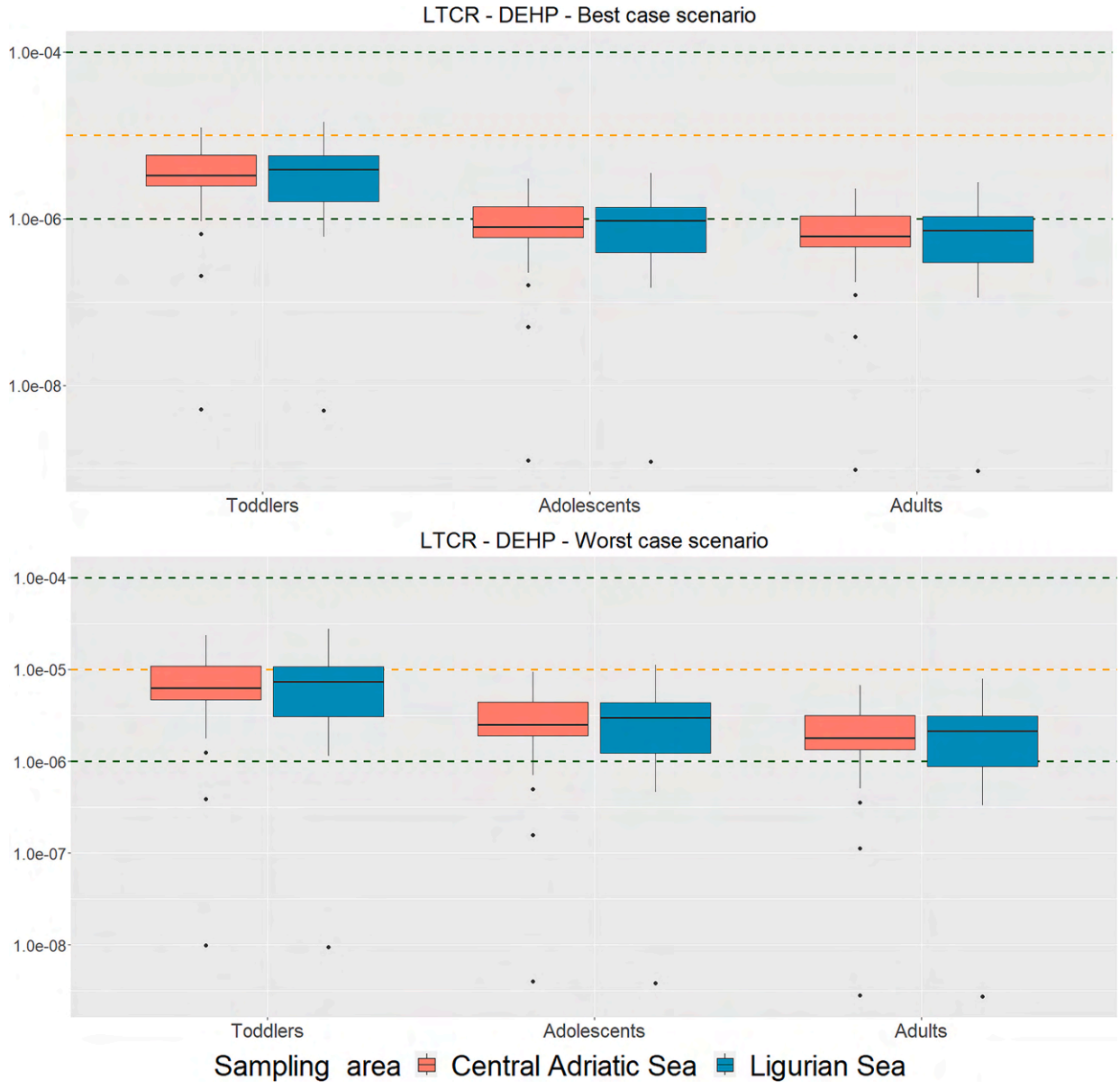


**Fig. 8.** Lifetime cancer risk of DEHP compared with the acceptable interval of risk according to the USEPA (green line) and Health Canada risk threshold (Orange line).

Central Adriatic Sea. PAEs were detected at higher concentration in all samples, followed by OPEs and NPPs. Different accumulation patterns were observed among the different classes of contaminants according to the Kow, of the compounds, sampling area and species characteristics (feeding habits, habitat and lipid composition). The high lipid content contributed the most in the accumulation of PAEs, as showed by the results obtained for the red mullet. OPEs showed the highest occurrence and concentration in specimens from the Ligurian Sea suggesting different sources of theses compound in the two seas. TEP was, instead, the most common compound in both areas. Differently, similar concentration values were obtained between the two areas for NPPs, and ATBC was detected at higher levels. European pilchard was found to be the most affected species in the Ligurian Sea, while for the Central Adriatic Sea was the red mullet. The edible daily intake (EDI) of the three classes of contaminants through fish consumption was assessed, and it showed no significant risk for all groups (children, adolescents and adults) at both study areas also when we considered the cumulative risk using the $GPD_{Eq}$. The lifetime cancer risk values for DEHP were found to be near the threshold values proposed by the USEPA and Health Canada, but, within the acceptable risk level. As some values are close to the limit threshold and exposure to these contaminants can also occur through other routes (ingestion of other food items and beverages, inhalation and dermal contact), it is important to consider all exposure pathways to evaluate possible human risk. The possible impact of unlisted contaminants should also be considered to integrate the GPDEq. Highlighting the necessity to keep monitoring these additives in the environment and in organisms. To gain a deeper understanding of the behavior of these ubiquitous contaminants in the marine environment, it will be essential to monitor the presence of PAEs, OPEs, and NPPs in marine organisms, and other environmental matrix especially considering the limited number of studies on this topic. Future research should therefore focus on assessing the effects of these contaminants on the organisms themselves to understand the impact on marine ecosystems, as these additives are increasingly being detected.

## CRediT authorship contribution statement

**Chiara Dettoto:** Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Andrea Maccantelli:** Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Maria Vittoria Barbieri:** Writing – review & editing, Validation, Methodology, Data curation. **Julio Fernández-Arribas:** Validation, Methodology, Formal analysis. **Cristina Panti:** Writing – review & editing, Validation, Supervision, Funding acquisition, Conceptualization. **Dario Giani:** Investigation, Formal analysis. **Matteo Galli:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Conceptualization. **Ethel Eljarrat:** Writing – review & editing, Supervision, Funding acquisition, Data curation. **Maria Cristina Fossi:** Writing – review & editing, Validation, Supervision, Funding acquisition, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.176442.

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