



Non-target analysis of organohalogenated contaminants in deep-sea fishes from the Mediterranean Sea by comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry

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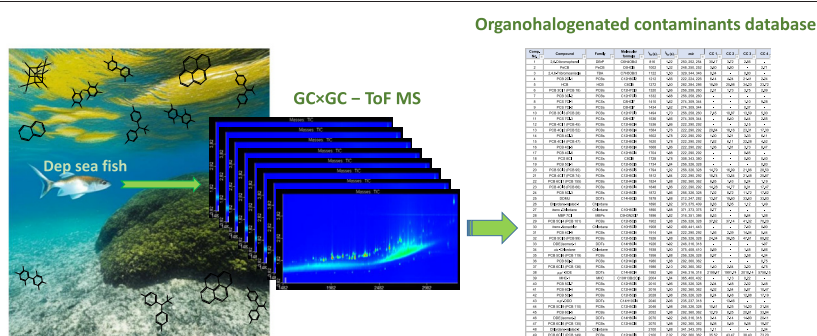
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HIGHLIGHTS

- Suspect and non-target characterization of the organohalogen load in deep-sea fishes
- A total of 116 halogenated compounds identified in two grenadier species
- Non-previously reported POP-like compounds detected in the deep-sea fish species
- New naturally occurring Br- and Cl/Br-compounds identified for the first time
- Different bioaccumulation profiles found in the two studied grenadier species

GRAPHICAL ABSTRACT



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ABSTRACT

The enhanced separation power and identification capabilities make comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry (GC × GC – ToF MS) a valuable instrumental alternative for non-target analysis (NTA). In the present study, GC × GC – ToF MS has been used for the NTA of chlorine- and bromine-containing compounds in composite livers of two scarcely investigated Mediterranean deep-sea fish species, hollowsnout grenadier (*Coelorhynchus caelorhynchus*) and roughsnout grenadier (*Trachyrhynchus trachyrhynchus*). Attention focused on the identification of organohalogenated compounds with structural characteristics similar to those of persistent organic pollutants (POPs). In total, 116 Cl-, Br- and mixed Cl/Br-compounds were either positively or tentatively identified in the analyzed liver samples. Up to 88 of these compounds were legacy POPs, being polychlorinated biphenyls (PCBs) and organochlorinated pesticides (OCPs) the most abundant and frequently detected families. The other 28 identified POP-like compounds were analytes not considered by current regulation and environmental monitoring programs, including, among others, degradation products of specific OCPs, naturally produced organohalogen compounds and several perchlorinated diethyldiphenylmethane-derivatives whose presence in the investigated species is reported here for the first time. The presence of other naturally occurring brominated and mixed halogenated compounds in these fish species is also described for the first time. Our results also showed differences in the accumulation profile of the identified compounds in both species. Thereby, anthropogenic POPs showed higher relative abundances in the livers of roughsnout grenadiers than those in hollowsnout grenadiers, while for naturally occurring compounds the opposite trend or similar levels were found in both species.

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1. Introduction

Persistent organic pollutants (POPs) are defined as toxic and recalcitrant compounds, capable of long-range transport. Their general chemical stability makes them prone to bioaccumulate and bioconcentrate through web chains. Due to these physical-chemical properties, POPs are nowadays ubiquitous contaminants that can be detected far from their emission sources, despite the strong regulation applied to some of these chemicals under the Stockholm Convention (Fiedler, 2008).

The marine environment is considered a relevant sink for POPs (Ge et al., 2021; Looser et al., 2000). It is estimated that up to 70 % of the organohalogen compounds that reach the environment end up in the oceans (Tanabe, 1985). POPs are usually land-sourced pollutants, but they enter the open ocean in a variety of ways, including river runoff, marine current transport, and atmospheric deposition. Their sorption on particulate matter favored their vertical transport through the water column and their final deposition at the bottom of the oceans (Avellan et al., 2021). Although the possibility of remobilization has been demonstrated for specific contaminants (O'Driscoll et al., 2013), several studies reported the presence of POPs in the deep-sea (>200 m), where some of these contaminants reach levels above those of the corresponding shallower marine sediments (Dasgupta et al., 2018; Ge et al., 2021; Sanganyado et al., 2021). As a concomitant impact, POP concentration in deep-seafloor ecosystems may also be favored, and so the bioaccumulation of these compounds by bottom-dwelling organisms (Looser et al., 2000; Mormede and Davies, 2003). This process can be enhanced in the Mediterranean Sea due to limited water exchange and shallower areas than other marine regions (Storelli et al., 2012).

Located in the eastern Mediterranean, the Adriatic Sea is a largely commercially exploited, semi-enclosed area where both legacy and emerging POPs can be traced to human activities in surrounding coastal areas. A number of these pollutants have already been identified as having reached deep-sea regions (Combi et al., 2016; Storelli et al., 2009). Despite these evidences, research on deep-sea fauna, such as benthic and benthopelagic fishes, is relatively infrequent in the literature, probably due to the difficulty of sampling and lower commercial interest of these species that, in general, are not consumed by humans. In this particular Mediterranean region, previous studies have evidenced the presence of selected legacy POPs, including environmental relevant polychlorinated biphenyls (PCBs), diethyl-diphenylmethanes (DDTs), hexachlorobenzene (HCB) and polybrominated diphenyl ethers (PBDEs), in deep-sea fishes such as *Chimaera monstrosa*, *Raja asterias*, *Raja Miraletus* and *Raja clavata* (Storelli et al., 2004), *Phycis blennoides* (Storelli et al., 2008), *Hoplostethus mediterraneus* and *Nettastoma melanurum* (Storelli and Perrone, 2010), *Nezumia sclerorhynchus* (Storelli et al., 2007b), *Coelorrhynchus coelorrhynchus* (Covaci et al., 2008; Storelli et al., 2009; Storelli et al., 2007b) and *Trachyrhynchus trachyrhynchus* (Covaci et al., 2008; Storelli et al., 2009). Information concerning the presence of other emerging and naturally produced POP-like compounds in these species remains essentially unknown. To our knowledge, only an early study from Covaci et al. (Covaci et al., 2008) reported on the presence of selected naturally produced compounds in deep-sea fishes from the marine depression located on front of the Apulia coast (Italy). That pioneer work focused on brominated organic compounds and evaluated the presence of eight widely distributed PBDE congeners (PBDE No. 28, 47, 49, 66, 99, 100, 153 and 154), two methoxylated-PBDEs (MeO-BDE 47 and 68), two polybrominated hexahydroanthene derivatives (i.e., tri- and tetra-BHD), one tribromoanisole (TBA) and a mixed halogenated monoterpene named as MHC-1 in composite liver samples of grenadiers, in particular of the hollowsnout grenadier (*Trachyrhynchus trachyrhynchus*, TT) and roughsnout grenadier (*Coelorrhynchus coelorrhynchus*, CC) species. The authors identified PBHDs as the largest contributors to the total load of organobromines in both species (i.e., 5 to 2500-times above the sum of PBDEs). The PBHD profile was dominated by the tetra-BHD, contrary to that reported for other fish species. This compound also showed higher levels in TT than in CC, while the opposite accumulation trend was observed for all other evaluated brominated families, pointing

to differentiated distribution and bioaccumulation profiles between the two investigated species. In that study, two chromatographic runs on monodimensional gas chromatography coupled to quadrupole mass spectrometry (GC-qMS) operated on electron ionization (EI) or electron-capture negative ionization (ECNI) modes were required for analytes determination. Despite the interest of the reported results regarding the distribution of brominated POPs and POPs-like compounds in these deep-sea fishes, research in this field was, to our knowledge, discontinued.

The current understanding of the contamination process as a global issue has promoted an increasing interest in the development of non-target analyses (NTA), which has become particularly evident during the last decade. NTAs allow for a more realistic idea of the global impact of pollution in the investigated organisms, but also on the transport and distribution of the pollutants in the ecosystems. NTAs typically rely on a generic sample preparation procedure that allows retaining as many compounds as possible (at least, all those belonging to the chemical categories of interest), and on the use of either enhanced separation techniques or high-resolution mass spectrometry instruments. Whatever the instrumental approach selected, the goal is the unambiguous determination of the detected compounds in the complex extracts subjected to analysis. Among the instrumental techniques fulfilling these requirements, comprehensive two-dimensional gas chromatography combined with time-of-flight mass spectrometry (GC × GC-ToF MS) has been demonstrated to be an advantageous option due to its enhanced separation power and identification capabilities. The suitability of GC × GC-ToF MS for the NTA of traces of volatile and semi-volatile organic pollutants in complex environmental extracts has been demonstrated in several studies dealing with matrices of very different nature (Escobar-Arnanz et al., 2018; Hurtado-Fernández et al., 2021; Pena-Abaurrea et al., 2011).

This study reports on the NTA of the organohalogenated compounds present in composite liver samples of grenadiers from the Adriatic Sea using GC × GC-ToF MS. This work represents an extension of previous research done on the presence of selected polybrominated compounds livers of TT and CC species by some of the authors (Covaci et al., 2008) and provides, for the first time, comprehensive information on the presence of anthropogenic and naturally occurring organohalogen contaminants in these two rarely evaluated fish species.

2. Material and methods

A list of the acronyms used throughout this study can be found in Supplementary Information - Part A (SI-A).

2.1. Standards and reagents

A total of 119 pure standards (purity grade above 90 %) of organohalogenated compounds with widely divergent chemical structures and origin were used for the optimization of the GC × GC-ToF MS analysis conditions and, when applicable, positive identification of detected compounds (Pena-Abaurrea et al., 2011). Considered organohalogenated included a mixture of 65 PCBs prepared from individual and commercial standard mixtures obtained from Dr. Ehrenstorfer (Ausburg, Germany) in isooctane and including the 7 priority and 12 toxic PCBs as well as other PCB isomers selected for environmental or chromatographic reasons (i.e., potential coeluting congeners) (Bordajandi et al., 2008). The Pesticide Mix-13 (dissolved in toluene) was obtained from LGC Standards (Teddington, United Kingdom) and included 32 chlorinated compounds, in particular, 6 PCBs (isomers No. 28, 52, 101, 138, 153 and 180), 6 DDTs (isomers 2,4'- and 4,4'-substituted of DDT, DDE and DDD), 5 HCHs (α -, β -, γ -, δ - and ϵ -isomers), 3 chlordane-related compounds (i.e., cis-chlordane, trans-chlordane, oxychlordane), and 12 chlorinated pesticides (aldrin, dieldrin, endrin, α -endosulfan, β -endosulfan, heptachlor, cis-heptachlorepoxyde, trans-heptachlorepoxyde, hexachlorobenzene, isodrin, methoxychlor and mirex). A stock solution of brominated compounds was prepared in isooctane from individual standards purchased from Wellington Laboratories (Guelph, ON, Canada) and Accustandard (New Haven, CT, USA) and

contained 6 PBDEs (No. 28, 47, 99, 153, 154 and 183) and 13 MeO-PBDEs (2-MeO-BDE 3, 2-MeO-BDE 7, 3'-MeO-BDE 7, 4'-MeO-BDE 17, 2'-MeO-BDE 28, 3'-MeO-BDE 28, 3-MeO-BDE 47, 6-MeO-BDE 47, 5-MeO-BDE 47, 4'-MeO-BDE 49, 2'-MeO-BDE 68, 4-MeO-BDE 90 and 6-MeO-BDE 99). Pure standards of tri- and tetra-PBHDs (2,7-dibromo-4a-bromomethyl-1,1-dimethyl-2,3,4,4a,9,9a-hexahydro-1H-xanthene and 2,5,7-dibromo-4a-bromomethyl-1,1-dimethyl-2,3,4,4a,9,9a-hexahydro-1H-xanthene) and MHC-1, in isooctane, were kindly provided by Prof. Dr. A. Covaci (University of Antwerp, Antwerp-Wilrijk, Belgium) (Pena-Abaurrea et al., 2011). PBDE 77 (Wellington Laboratories) dissolved in isooctane was used as an internal standard.

Isooctane, acetone and *n*-hexane (Pestipur quality) were acquired from J. T. Baker (Deventer, Netherlands).

2.2. Samples and sample preparation

Individuals of two deep-sea species, hollowsnout grenadier (CC) and roughsnout grenadier (TT) were caught in the southern Adriatic Sea (Mediterranean Sea). CC individuals usually live at depths between 200 and 500 m and are considered benthic feeders that burrow into sediments to feed on infaunal organisms, such as polychaetes, gastropods, cephalopods and crustaceans (Sever et al., 2008). Meanwhile, TTs preying area are wider than that of CCs. TTs main food consists of pelagic copepods, but individuals also prey on shrimp, fish, cephalopods, polychaetes and gastropods (Cohen, 1990), which makes this species to be considered pelagic and to be found on a level slightly higher than CC in the trophic web (Cohen, 1990).

Samples were prepared by pooling and homogenizing livers from individuals of similar size, thereby resulting in four liver samples of the CC species (mean fat content, 41.2 %, w/w) and six of the TT species (26.7 %, w/w) (Storelli et al., 2009). Pooled samples were prepared according to a methodology previously described (Covaci et al., 2008; Storelli et al., 2009). Briefly, 0.6 g of the composite liver sample was spiked with BDE 77 to act as internal standard, dried by grounding with sodium sulphate and subjected to Soxhlet extraction for 2 h with a mixture of acetone:*n*-hexane (1:3, v/v). The obtained extract was concentrated and eluted through a multilayer column containing activated silica and silica modified with sulphuric acid for fat removal. These extracts were concentrated and further purified by elution through a Florisil Supelclean cartridge (1 g, Supelco, Bornem, Belgium). The Florisil eluate was finally concentrated under a gentle nitrogen current and reconstituted in 50 μ L of isooctane for instrumental analysis. This sample preparation procedure provided clean (i.e., fat-free), ready for analysis extracts and quantitative recoveries for the vast majority of the Cl/Br-containing POP and POP-like families most frequently detected in the fish species investigated. However, the recovery of more polar analytes (e.g., phenols) may be incomplete. This would result in the underestimation of the abundances determined for these types of compounds in the extracts in comparison with those found for less polar analytes, thus preventing a direct comparison among these sets of values. Nonetheless, as this fact would affect all the samples analyzed equally, direct comparison among the abundances determined for a given polar compound in the different individuals was still possible.

2.3. GC \times GC-ToF MS analyses

Standards and samples were analyzed by GC \times GC-ToF MS using a Pegasus 4D instrument (LECO Corp., St. Joseph, MI, USA) consisting of a modified Agilent 6890 GC equipped with a secondary oven and a liquid nitrogen quad-jet dual-stage cryogenic modulator, and hyphenated with a ToF MS with electron impact ionization. An HT-8 (8 % phenyl 92 % methyl polycarborene-siloxane; 30 m \times 0.25 mm i.d. \times 0.25 μ m film thickness) was used as first dimension (1 D) column and coupled to a BPX50 (50 % phenyl 50 % phenyl polysilphenylene-siloxane; 1.9 m \times 0.1 mm i.d. \times 0.1 μ m film thickness) as second dimension (2 D) column. GC columns were acquired from SGE (Melbourne, Australia). The column combination was selected based on previous knowledge of the research group and on the

satisfactory inter- and intra-group separation achieved among evaluated Cl- and Br-containing compounds (Bordajandi et al., 2008; Pena-Abaurrea et al., 2011), and among these and coextracted matrix components under proposed experimental conditions. The optimized GC \times GC method consisted of the injection of 1 μ L of the purified and concentrated liver extracts in the hot splitless mode at 300 $^{\circ}$ C (2 min) and programming the 1 D oven from 80 $^{\circ}$ C (maintained for 2.5 min) to 190 $^{\circ}$ C at 15 $^{\circ}$ C/min, and then to 300 $^{\circ}$ C at 3 $^{\circ}$ C/min. This final temperature was maintained for 20 min. The 2 D oven was programmed to track the main oven but with an offset temperature of 30 $^{\circ}$ C until reaching 300 $^{\circ}$ C; from this temperature, both ovens followed the same program. That is, the 2 D oven program was as follows: 110 $^{\circ}$ C (2.5 min) to 210 $^{\circ}$ C at 15 $^{\circ}$ C/min and finally to 300 $^{\circ}$ C (20 min) at 3 $^{\circ}$ C/min. Helium was used as carrier gas at a head-column constant pressure of 31 psi. The temperature of the modulator was programmed to track that of the 1 D oven but with an offset of 40 $^{\circ}$ C. The modulation period was 6 s with a hot jet pulse of 0.6 s. The transfer line and ion source temperatures were 275 $^{\circ}$ C and 250 $^{\circ}$ C, respectively. The energy of the ionizing electrons was 70 eV, the voltage of the multiplier 1800 eV, and data were acquired at 50 and 100 Hz (Hurtado-Fernández et al., 2021) in full scan mode, in the *m/z* 200–750 range.

LECO ChromaTOF 4.2 software was used for data acquisition and treatment. The software allowed automatic baseline correction, peak deconvolution, and peak area determination. Although the study followed an NTA strategy, the focus was on the presence of Cl- and Br-containing compounds. Thereby, a script function written in Visual basic was used for the automatic identification of halogen-containing analytes on the base of characteristic *m/z* ions and clusters (Escobar-Arnanz et al., 2018). In this study, and following the criteria approach previously applied by the research group (Escobar-Arnanz et al., 2018; Hurtado-Fernández et al., 2021), compounds were considered positively identified when their retention times and mass spectrum agreed with those of the corresponding pure standard analyzed under identical experimental conditions. When appropriate standards were not available, analyte identification followed the multicriteria approach described elsewhere (Escobar-Arnanz et al., 2018) and was in all instances considered tentative.

For each sample, the relative abundance of an identified analyte was calculated by correcting its absolute abundance by that of the internal standard and by the specific fat content of the sample. For a given sample, the normalized contribution of each specific compound within its corresponding class was calculated as the percent contribution of that analyte to the total relative abundance calculated by summing the individual values determined for all analytes belonging to that particular class of compounds. This approach assumed similar instrumental responses for all detected analytes, which is not necessarily correct. However, it was adopted to allow direct comparison among the relative abundances and normalized contributions calculated for the different analytes found in the investigated samples.

2.4. QC & QA

QC and QA included the use of PBDE 77 to control the performance of the sample preparation and instrumental analysis procedures, and the routine analysis of procedure blanks. These blank samples were prepared as livers but without sample. Their analyses demonstrated that no interference affecting the determination of the compounds of interest was introduced by the proposed analytical procedure. Standard mixtures of the 119 evaluated organohalogenated compounds were also routinely analyzed to control the maintenance of their respective retention times and instrumental response throughout the study. The instrumental limits of detection (LODs) were defined as the amount of compound providing an S/N ratio of three for the extracted most abundant ion of the considered analyte in standard solutions. In general, and in agreement with already reported data for similar or close-related pollutants, LODs laid in the 10–100 pg ml $^{-1}$ for a large majority of the evaluated analytes, although slightly higher values were obtained for the most substituted brominated compounds considered in the study (Pena-Abaurrea et al., 2011).

3. Results and discussion

3.1. General overview

Compared to conventional (i.e., one-dimensional) GC, GC \times GC provides increased resolution capability, which is a significant advantage when dealing with the identification of compounds at residue level and when their identification relies on the purity of the recorded mass spectrum. Under experimental conditions proposed in this study, a satisfactory intra- and inter-class separation was achieved for all 119 Cl-/Br-containing compounds considered. The selected column combination also provided structured chromatograms, which facilitated the tentative identification of isomers belonging to a particular family of POPs when the corresponding pure standard was not available (Escobar-Arnanz et al., 2018; Hurtado-Fernández et al., 2021). In addition, a clear separation was achieved among the several families of POP evaluated and remaining co-extracted matrix components, which eluted separated from them and as a relatively compact band at shorter 2D retention times (roughly, 2t_R of 1.00–1.50 s in the 1t_R range of 600–2500 s, Fig. 1). As an example of the typical results obtained in this work, Fig. 1 compares representative GC \times GC–ToF MS chromatograms obtained for liver extracts of the two deep-sea species investigated. Relatively complex chromatograms were obtained in both cases and, although some common structures were identifiable, specific features could also be visualized for each species.

In total, 116 POP-like compounds containing either Cl-, Br- or both halogens were either positively or tentatively identified in the analyzed deep-sea liver samples using the multi-criteria approach detailed in (Escobar-Arnanz et al., 2018; Hurtado-Fernández et al., 2021). Among these analytes, 104 were detected in CC and 107 in the TT samples. These are considered to be relatively large sets of compounds compared to those reported in previous studies involving these fishes, in which a target-oriented strategy was adopted and, consequently, a relatively short number of preselected analytes were investigated (Covaci et al., 2008; Storelli et al.,

2009; Storelli and Perrone, 2010; Storelli et al., 2007b; Storelli et al., 2004). It should be highlighted that, concerning the evaluated see area, except for the above mentioned study by Covaci et al. (Covaci et al., 2008) aiming at the determination of 14 specific legacy and naturally occurring brominated compounds, all other previous studies have essentially focused on the determination of classical legacy chlorinated POPs, in particular PCBs, DDTs and HCB (Storelli et al., 2009; Storelli and Perrone, 2010; Storelli et al., 2007b; Storelli et al., 2004). Therefore, compared to all these previous research works, the present one provides a broader overview of the presence of halogenated compounds, regardless of their potential origin, and of loads of POPs and POP-like compounds in these less investigated deep-sea fishes. Thereby, although in agreement with previously mentioned studies, PCBs and legacy DDTs were identified as the main contributors to the total halogen burden in the liver samples analyzed in the present study (76 %, i.e. 88 of the 116 the identified analytes belonged to these two POP families), the presence of several non-previously detected classes of organohalogenated compounds is reported here for the first time. These classes included various widespread, but less frequently monitored, legacy POPs, like polybrominated biphenyls (PBBs), non-regulated DDT-related compounds, some degradation compounds of specific organohalogenated pesticides (OCs), and certain non-legacy POPs, such as halogenated 1'-methyl-1,2'-bipyrroles (MBPs) and polychlorinated styrenes (PCSs), and mixed Cl/Br micropollutants.

The complete list of identified compounds is presented in Table S1 of SI-B as a searchable excel version. In this table, analytes are sorted according to their corresponding 1t_R . The proposed empirical molecular formula, name, characteristic m/z values and relative abundance in each sample (i.e., relative to the calculated abundance of the internal standard, PBDE 77, and corresponding lipid content) are also provided. The mass spectrum, as experimentally registered for each compound, is accessible as part of a complete mass spectral library in SI-C (for PCBs, only one representative spectrum has been included per homologue group due to the well-known and characterized mass spectra of these analytes).

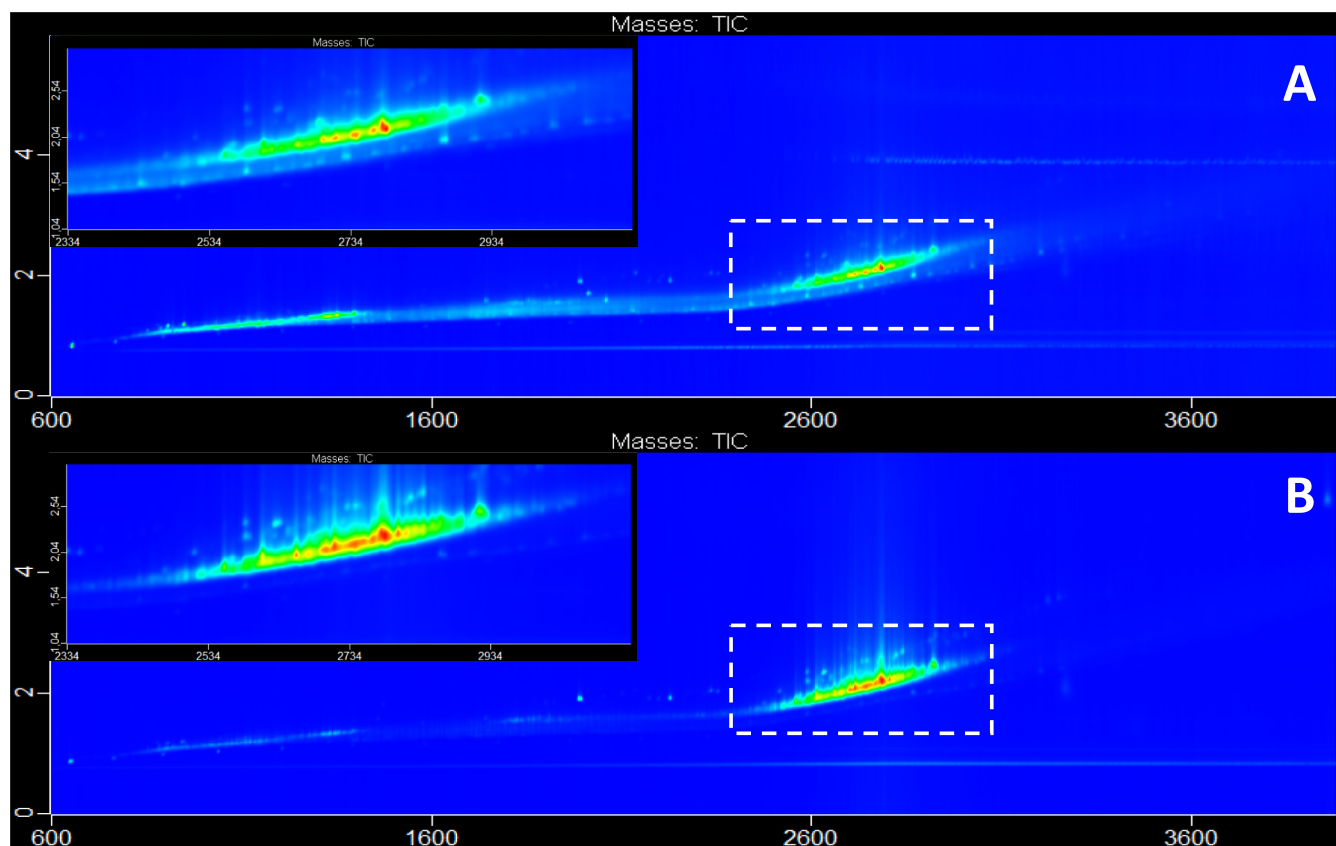


Fig. 1. Typical chromatograms obtained for liver samples of (A) CC and (B) TT. Inserts corresponded to zooms of the indicated areas. No logarithmic scale is applied.

The several classes of organohalogens identified in the livers of the two studied deep-sea fish species, i.e. CC and TT, and their relative contribution to the total load of organohalogens in the samples are graphically shown in Fig. 2 and discussed in the following sections.

3.2. Chlorinated compounds

Legacy Cl-containing POPs are recognized to be ubiquitous pollutants in Mediterranean Sea species (Tanabe, 1985), including the two grenadier species evaluated in this study (Krämer et al., 1984; Storelli et al., 2009; Storelli and Perrone, 2010; Storelli et al., 2007b). The NTA approach applied here confirmed the findings of previous research regarding the presence of PCBs, DDTs, and HCB on livers of CC and TT caught in the same marine depression of the Adriatic Sea. In an early study by Storelli et al. (Storelli et al., 2009), the concentrations of these families of POPs were found to decrease in the order PCBs > DDTs > HCB. The trend was similar in both species, but levels in TTs doubled those found in the CC samples (i.e., 44 pg g⁻¹ vs 20 pg g⁻¹ of toxic tetra-equivalents of 2,3,7,8-tetrachloro-*p*-dioxin, TEQs, respectively). In the present study, PCBs and DDTs were found to be the most prevalent classes of POPs among those detected in CC and TT species. These two families of POPs also showed the highest contributions to the total load of organohalogens detected in the livers, representing at least 96 % of the normalized total abundance calculated for the samples analyzed here. In line with that reported in the above mentioned work (Storelli et al., 2009), in our study, PCB levels were slightly higher in TTs than in CCs (relative abundances about one order of magnitude higher, as shown in Fig. S1 and S2 in SI-A, respectively). In both

species, the main contributors were the priority PCBs No. 138, 151 and 180, a finding that also agreed with previous observations (Storelli et al., 2009). However, while in TT livers the total calculated relative abundance for PCBs was higher than that corresponding to legacy DDTs, the opposite trend was observed in the CC livers, where DDTs showed levels similar to or lower than those detected in TTs. In addition, legacy DDTs exhibited essentially similar profiles in both groups of samples: Except for *o,p'*-DDE and *o,p'*-DDD (the latter was found only in one CC sample), all regulated DDTs were detected in the investigated livers (compounds No. 38, 43, 52, 57 and 68 in Table S1 in SI-B) and, in all instances, the DDTs profile was dominated by *p,p'*-DDE. *p,p'*-DDE is the main expected degradation product of DDT in marine biota not affected by a recent use of DDT. In this study, *p,p'*-DDE normalized contribution to the total load of DDTs in livers accounted for as much as 90 %, a result that would agree with previous observations for CCs and TTs (Storelli et al., 2007a; Storelli et al., 2009) and for other deep-sea species from the investigated area (Storelli et al., 2008; Storelli and Perrone, 2010).

Interestingly, up to ten extra DDT-related compounds, whose presence had not been previously reported in these species, were identified thanks to the application of an NTA approach (analytes No. 25, 33, 46, 73, 90, 94, 95, 97, 103, 108 in Table S1 in SI-B). These analytes included some non-regulated DDT and DDE isomers, DDMU, two TCPMe isomers and four perchlorinated DDE compounds whose presence had been only recently noted in aquatic organisms, in particular in marine mammals (Hoh et al., 2012; Shaul et al., 2015; Trego et al., 2018; Zhou et al., 2016), when using NTA approaches. The overall contribution of this group of DDT-related compounds to the total burden of organohalogens in the

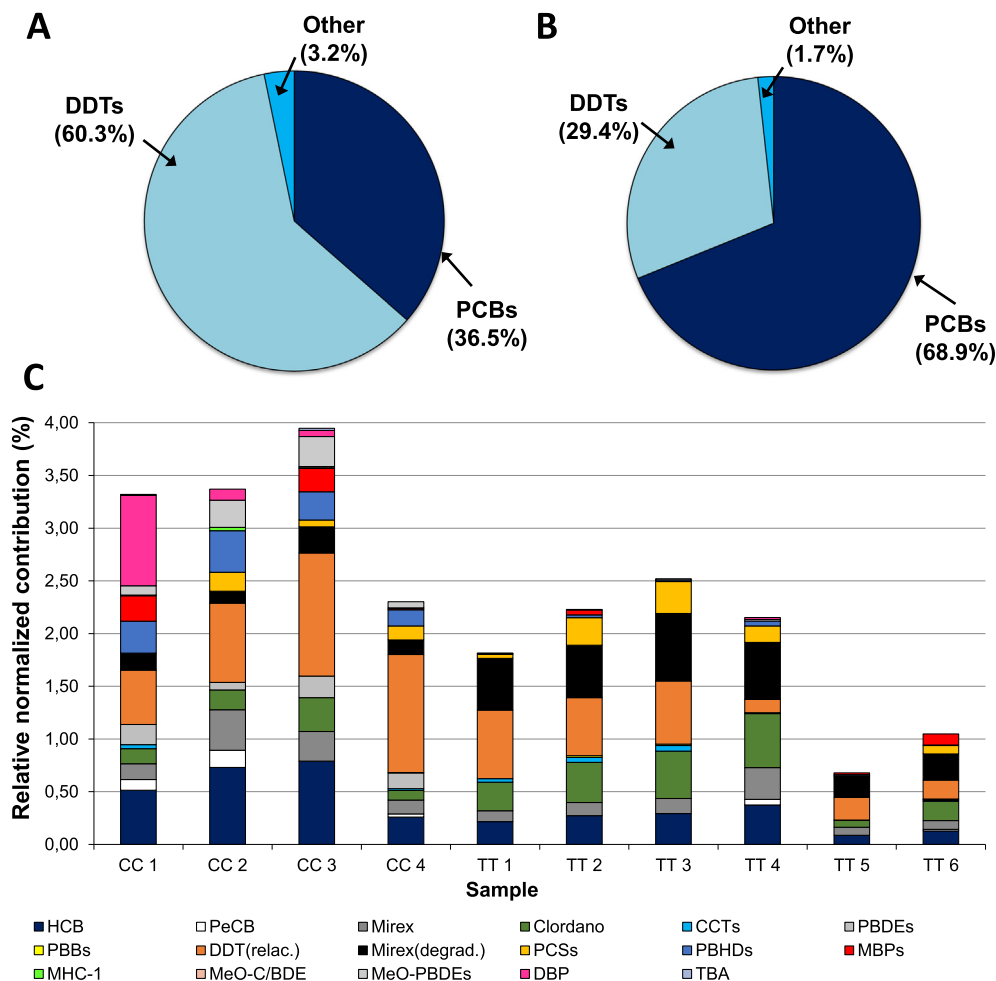


Fig. 2. Relative contribution (as %) of the several classes of compounds identified to the total load of organohalogens in livers of (A) CCs and (B) TTs. (C) shows the contributions calculated for minor classes in the individual samples of grenadier investigated.

investigated livers, evaluated as relative abundances, was similar (for TTs) or higher (for CCs) than the contribution of HCB, which was identified by Storelli et al. (Storelli et al., 2009) as the third most abundant POP after PCBs and DDTs. This finding would evidence the prevalence and environmental relevance of this set of DDT-related compounds which, however, are not considered by current international monitoring programs or legislations on POPs and whose presence in the environment remains largely unknown.

Fig. 3 shows the relative abundances and detection frequency calculated for these DDT-related compounds in the investigated samples. Except for the perchlorinated DDEs, which were almost exclusively detected in the TT livers, all other compounds were found in both species. However, in general, their levels and detection frequencies were higher in TTs than in CCs, a result that could be associated with the different feeding habits and trophic levels occupied by the two species in the food web. Remarkably, in the CC livers, the three non-regulated isomers of DDT and DDE [DDT(isomer), analyte No. 95; DDE(isomer)-1, analyte No. 33, and DDE(isomer)-2, analyte No. 46] were either exclusively or mainly detected in sample CC-4, the one showing the highest concentrations of legacy DDTs. This distribution pattern was different from that observed in TT samples, where DDE(isomer)-2 was also detected in all samples, but DDT(isomer) was detected only in 50 % of the analyzed livers (TT-1, 2 and 3), and DDE(isomer)-1 was never detected. To our knowledge, no previous report on the presence of these DDT-related compounds in deep-sea biota can be found in the literature.

DDMU showed essentially similar relative abundances in all CC samples. However, in the TT livers, the relative abundances of this metabolite covered two orders of magnitude so evidencing a more variable behavior for this compound in this species. *p,p'*-DDMU has been identified in other organisms from remote and deep-sea areas (Ballschmiter et al., 1997; Unger et al., 2008), as well as in benthic organisms from the Bosnian Sea, where this compound showed levels above those of *o,p'*-DDE, *o,p'*-DDT and *p,p'*-DDD (Strandberg et al., 2000). However, as far as we know, no previous information regarding its presence in the two grenadier species evaluated in the present study was available in the specialized literature.

The four perchlorinated DDE-related compounds identified in the analyzed TT livers included one DDE-Cl6 (analyte No. 73 in Table S1-SI-B) and three DDE-Cl7—1 to 3 (analytes No. 90, 94 and 103). Nevertheless, only the latter compound was detected in CCs, in particular in sample CC-4 (Fig. 3). This observation could point to the differentiated distribution of these compounds in both species, as above indicated. Differences in the

prevalence and relative abundance of these four analytes were also observed among the investigated TT samples: DDE-Cl6 and DDE-Cl7—1 were detected in all livers, the highest levels corresponding to TT-6, which was also the sample showing the highest levels of other DDE isomers. To our knowledge, this is the first study reporting on the presence of perchlorinated DDE-related compounds in deep-sea fish species, although these results are considered a further indication of the ubiquity and recalcitrant nature of these analytes.

Regarding the presence of other chlorinated compounds in the livers, a total of 15 chlorinated pesticides were detected in the analyzed samples, including both aromatic and non-aromatic pollutants. Among them, HCB showed the highest relative abundances, a finding that agreed with previously reported results for grenadiers (Storelli et al., 2009) and other deep-sea species from the Mediterranean Sea (Koenig et al., 2013). Other identified contaminants included PeCB, six chlordane-related compounds, one toxaphene-related compound, mirex and five degradation products, and some PCSs isomers. In general, the average contribution of these analytes to the total organohalogen burden calculated for the grenadiers investigated was lower than those of previously discussed families (Fig. S3 in SI-A). Nevertheless, it should be highlighted that the relative abundances calculated for mirex and its degradation products and for chlordane and its related compounds reached those of other chlorinated contaminants with a moderate concentration level in these samples, such as HCB (Storelli et al., 2009).

The six chlordane-related compounds were either tentatively or positively identified as chlordane-related-1, *trans*-chlordane, *trans*-nonachlor, *cis*-nonachlor, *cis*-chlordane, chlordane-related-2 and *cis*-nonachlor (analytes No. 26, 27, 30, 34, 48 and 55 in Table S1 in SI-B, respectively). Some of these compounds have also been determined in grenadiers from the North Atlantic at levels even above those found in other deep-sea species from the area (Froescheis et al., 2000). However, no report of their presence in grenadiers from the Adriatic Sea depression has been found. Results obtained for the evaluated livers evidenced differences among the normalized contributions of the positively identified compounds depending on the considered species (Fig. 4). For both species, *cis*- and *trans*-nonachlor were the highest contributors to the total load estimated for this class of pollutants, in particular in TTs, where they reached contributions of 60 % and above. When detected, *cis*-chlordane showed contributions in the range of 10–20 % in CC, while this isomer was essentially undetected in TTs. *trans*-Chlordane was only identified in sample CC-1. On the other hand, the isomers tentatively identified as chlordane-isomer-1 and chlordane-isomer-2

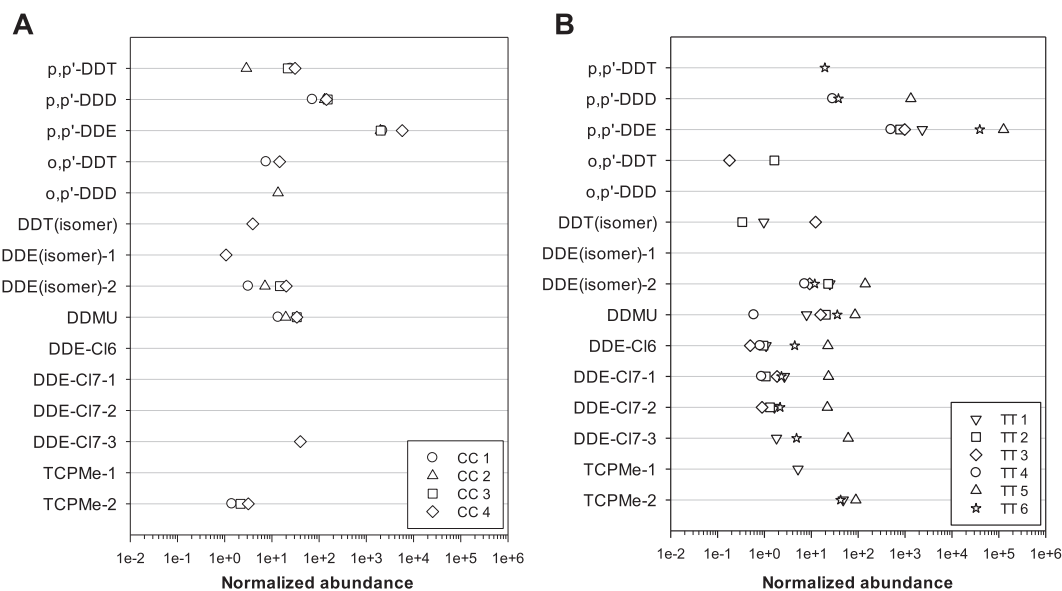


Fig. 3. Relative abundances of legacy DDTs and related compounds identified in the livers of (A) CCs and (B) TTs. Non-detected compounds are not depicted. Note: logarithmic scale.

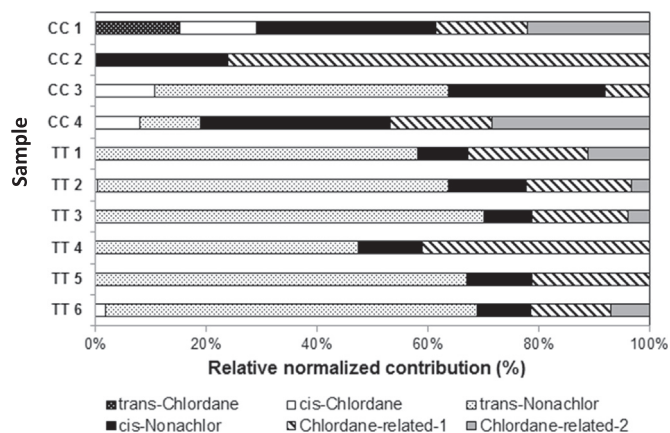


Fig. 4. Normalized contribution, within the “chlordane class”, of the several compounds related to chlordane identified in the samples of grenadier analyzed.

were detected in a large majority of the investigated samples, where they reached average normalized contributions of 20 % and above. These profiles showed marked differences compared to those reported for grenadiers from the North Pacific (Takahashi et al., 2010), which were dominated by *trans*-nonachlor, but with significant contributions also from *cis*-nonachlor and *trans*-chlordane that here showed only minor contributions. In any case, and apart from the possible differences derived from a variable response of the detector to these various compounds, it should be noted that in the mentioned study grenadiers were caught off in a different planet area and that the whole fish was used for analysis.

Mirex (analyte No. 92; Table S.1 in SI-B) has been widely used in the past as a pesticide and flame retardant and its widespread presence in fishes from the Mediterranean Sea is well documented (Rahman et al., 1993). In addition, some studies have suggested that the concentrations of mirex in deep-sea fishes could be higher than in species from shallower waters (Krämer et al., 1984). Our results would contribute to illustrate the ubiquity of mirex because this legacy pesticide was detected in all investigated

livers. In addition, five degradation products of mirex were tentatively identified through their chromatographic behavior and mass spectra (Hoh et al., 2012; Norstrom et al., 1980; Shaul et al., 2015). These compounds included three monohydromirex isomers (analytes No 72, 74 and 83, identified as monohydromirex-1, -2 and -3) and two dihydromirex isomers (analytes No 62 and 66; named as dihydromirex-1 and -2; Table S.1 in SI-B). The relative position of these analytes in the contour plot and their typical profiles in both grenadier species are compared in Fig. 5. The profile of this family of compounds was dominated by the parent compound, mirex, and the species named as monohydromirex-1. These were the only two compounds found in CCs. Meanwhile, TT livers showed minor but consistent contributions of all other identified species (total average normalized contribution within this class, 20 %, Fig. S4 in SI-A). Mirex and its main degradation product, 8-monohydromirex, have been detected in fishes (Shen et al., 2012) and marine organisms (Hoh et al., 2012) from other parts of the earth, as well as in benthic organisms from remote regions, such as the Arctic Ocean (Bidleman et al., 2013). However, no reference regarding the presence of this pesticide and their several photodecomposition products in deep-sea fishes has been found in the literature, despite their relative abundance, in particular in the TT species.

PCSs are spontaneously occurring pollutants formed as byproducts during industrial and technological processes involving chlorine (Carrizo and Grimalt, 2009). Their presence in the environment is not regulated by the Stockholm Convention and data regarding their environmental distribution are scarce compared to other halogenated pollutants. In the present study, three hepta-CSs (analytes No 8, 9 and 11) and one octa-CS isomer (compound No. 18, Table S.1 in SI-B) were identified. In some of the studied grenadier livers, the contribution of these four PCS isomers to the total organohalogenated burden was similar to or higher than that corresponding to other legacy POP classes previously discussed. As observed for other chlorinated compounds, the relative abundances calculated for these PCSs were higher in TT than in CC individuals, and their profile were dominated by PCS 7Cl-1, PCS 7Cl-3 and PCS 8Cl, the latter being the only one detected in all analyzed samples, except for CC-2 and CC-1. Actually, none of the compounds belonging to this family were detected in sample CC-1 (Fig. S5 in SI-A).

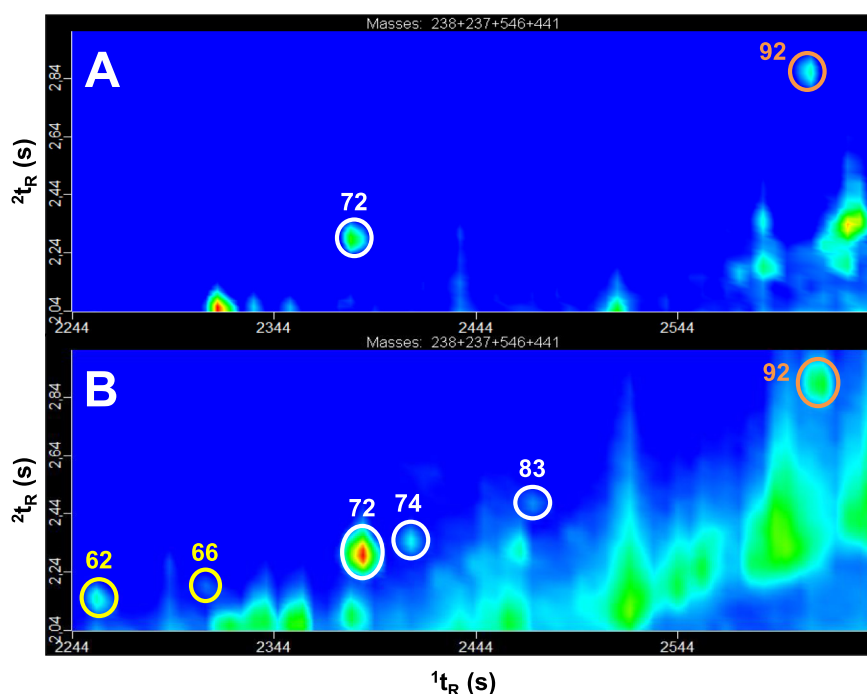


Fig. 5. Typical contour plot of the elution region of mirex (peak # 92) and its degradation products in the investigated livers of (A) CCs and (B) TTs. Monohydromirex isomers are highlighted in white and dihydromirex isomers are marked in yellow. See Table 1 in SI-A for analyte identification.

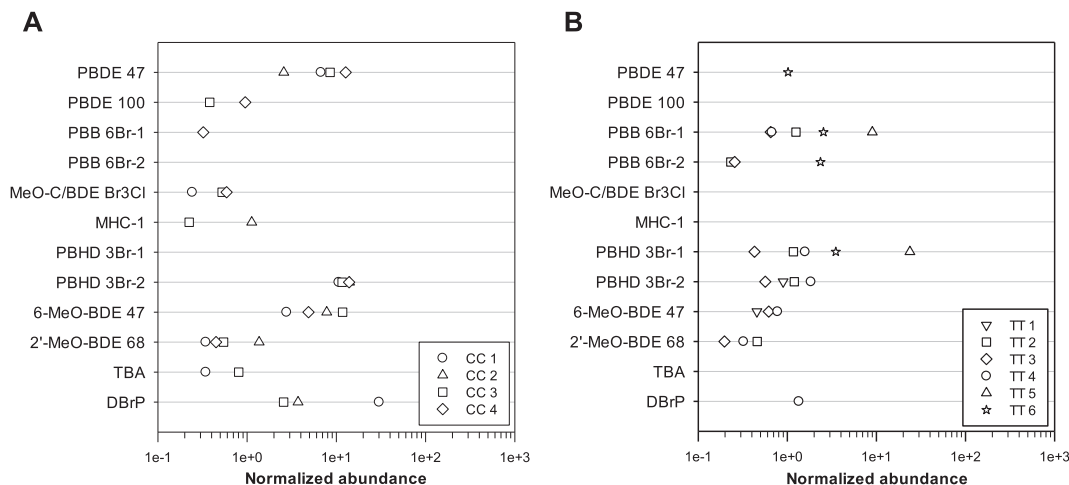


Fig. 6. Relative abundances of brominated compounds identified in the livers of (A) CCs and (B) TTs. Non-detected compounds are not depicted. Note: logarithmic scale.

Finally, it should be mentioned that an MBP-7Cl isomer was detected in three CC samples (CC-1, -3 and -4) and four TT livers (TT-1, -2, -5 and -6). This naturally occurring compound had been found in marine animals occupying high positions in the trophic web (Pangallo and Reddy, 2010). However, there was no evidence of its presence in deep-sea fishes. Unfortunately, and unlike that observed for other chlorinated analytes, its distribution in the investigated samples was rather heterogeneous, which made it difficult to infer any type of accumulation trend, despite its demonstrated biomagnification capability (Pangallo and Reddy, 2010).

3.3. Brominated compounds

The number of brominated compounds detected in the investigated livers was significantly lower than that of the chlorinated analytes discussed above: i.e., 12 Br-containing compounds out of the 116 identified analytes in the samples. The identified organobrominated compounds included legacy POPs, as well as analytes of natural and mixed origin. Among them, 10 were detected in CCs and only 8 TTs. The relative abundances of these compounds were higher in the CCs than in TTs (Fig. 6), which represented the opposite trend to that observed for the chlorinated analytes, but roughly agreed with previous observations for grenadiers from this area (Covaci et al., 2008). The only exception to this general behavior was observed for PBHDs which, in addition, exhibited an unusual profile dominated by the tribrominated isomers (Covaci et al., 2008).

PBDEs were the Br-containing chemical-class exhibiting the highest relative abundances among those identified in livers (Fig. 6), even though only the two congeners, i.e. PBDEs 47 and 100, were detected (probably due to the relatively high LODs obtained for highly substituted PBDE isomers with GC × GC-ToF MS (Pena-Abaurrea et al., 2011)). Among these two congeners, PBDE 47 showed the highest contributions, a finding that agreed with previous results obtained for grenadiers from this (Covaci et al., 2008) and from other parts of the world (Koenig et al., 2013), as well as for other deep-sea fishes from the Mediterranean sea (Koenig et al., 2013) and other regions (Ramu et al., 2006; Takahashi et al., 2010; Webster et al., 2014).

Our results regarding the presence of other organobromines of natural and mixed origin in the grenadiers livers also agreed with those previously reported by Covaci et al. (Covaci et al., 2008) concerning 2'-MeO-BDE 68, 6-MeO-BDE 47, TBA and MHC-1 (Fig. 6). On the other hand, somehow surprisingly, the tetra-BHD that those authors found at concentrations higher than the tri-BHD isomer using a target analysis approach (Covaci et al., 2008) was not detected in the present NTA study. On the other hand, the use of GC × GC-ToF MS allowed the tentative identification of a second tri-BHD isomer, which was not described by these authors (Covaci et al., 2008) (Fig. 6). Other natural occurring brominated and mixed compounds

(Howe et al., 2005; Malmvärn et al., 2005) not previously reported in these fish species but identified in the present study included compounds such as DBrP and MeO-C/BDE Br3Cl (Fig. 6), which were exclusively detected in the CC species.

4. Conclusions

This study took advantage of the enhanced resolution power and identification capabilities provided by GC × GC – ToF MS for the NTA of GC-amenable POPs and POP-like compounds in livers of two scarcely investigated grenadiers from the Adriatic sea. In particular, the focus has been on the exhaustive characterization of the aromatic Cl-, Br- and mixed Cl-/Br-compounds and, for the first time, a comprehensive database on the presence of these analytes in these two deep-sea fish species has been made available. Provided data includes a searchable database summarizing chromatographic and mass spectral information of the 116 organohalogenated compounds either positively or tentatively identified in the samples, and allows the identification of the most prominent families of anthropogenic and naturally occurring compounds detected in these fishes. A 24 % of the identified analytes corresponded to compounds not previously identified in these fishes and whose general environmental distribution and fate remain unclear due to the limited information available in the literature at present. A complete and searchable mass spectral library containing identified analytes is provided to facilitate their identification to other authors interested in these types of determinations. Finally, the reported information pointed to differentiated bioaccumulation profiles for some specific classes of compounds in both species, a topic that may be the subject of future research in this area of knowledge.

CRedit authorship contribution statement

Juan Escobar-Arnanz: Data treatment, Data Curation, Writing-Original Draft. **Miren Pena-Abaurrea:** Methodology, Validation, Formal analysis. **Francisco Javier Santos:** Conceptualization, Writing-Review, Editing. **Lourdes Ramos:** Conceptualization, Supervision, Writing-Review, Editing, Project administration, Funding acquisition.

Additional declarations for articles in life science journals that report the results of studies involving humans and/or animals

Not applicable.

Ethics approval

Not applicable.

Consent to participate

All authors consented to participate in the article preparation.

Consent for publication

All authors give their consent for publication of the article.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare no conflicts of interest.

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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.2023.162873>.

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