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A large diversity of organohalogen contaminants reach the meso- and bathypelagic organisms in the Bay of Biscay (northeast Atlantic)

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ABSTRACT

Deep-sea ecosystems play a key role in the cycling and vertical transfer of matter and energy in oceans. Although the contamination of deep-sea demersal and benthic organisms by persistent organic pollutants has been proven, deep pelagic species have been far less studied. To fill these gaps, we studied the occurrence of a large variety of hydrophobic organic contaminants including polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), legacy and alternative brominated flame retardants (BFRs) and *per*- and polyfluoroalkyl substances (PFASs) in crustaceans and fish species collected in the Bay of Biscay, northeast Atlantic. The results highlighted the global predominance of PCBs in fish, followed by OCPs, PFASs and PBDEs, with highly variable concentrations among species. Most of the chlorinated or brominated contaminants showed increasing concentrations with increasing $\delta^{15}N$ values, while most PFASs showed inverse trends. The contaminant profiles and diagnostic ratios revealed species-specific metabolic capacities and peculiar contribution of highly-brominated BFRs.

1. Introduction

The deep-sea pelagic environment (<200 m) is one of the largest ecosystems on Earth and supports a high diversity and abundance of marine species, especially in the bathyal horizon (<2000 m) (Rogers, 2015). In particular, meso- (<1000 m) and bathypelagic (1000–2000 m) communities represent essential components of oceanic biomass and important prey for higher trophic levels including large pelagic fish, marine mammals and seabirds. They play a key role in the cycling and vertical transfer of matter and energy in oceans (Bernal et al., 2015; Bianchi et al., 2013; Catul et al., 2011). As a consequence of their strong diel vertical migrations (DVM) during which some species (Chouvelon et al., 2022; Eduardo et al., 2021; Takahashi et al., 2000) feed in the epipelagic zone (0–200 m) at night and move back to meso- and bathypelagic zones during the day, meso- and bathypelagic organisms release particulates, organic matter and associated contaminants via faecal pellet egestion and may therefore increase transfers from surface waters to deeper horizons (Belcher et al., 2019; Bernal et al., 2015). Furthermore, their upward migration, during which they become available to surface predators, leads to the transfer of organic matter and contaminants from deep horizons back to epipelagic layers. In addition to their essential role in biogeochemical cycles, mesopelagic organisms have also raised interest regarding their exploitation as new resources for human consumption as well as the fish meal and oil industry (Berntssen et al., 2021; Grimaldo et al., 2020).

Although deep-sea ecosystems have been studied for decades, most studies focused on deep demersal or benthic communities and rarely on deep pelagic organisms, especially those from the ocean "twilight zone" (200–1000 m), which thus remain the most understudied ones. Recent publications have therefore highlighted the urgent need to increase knowledge in various research areas for these deep pelagic ecosystems, including the fate and impact of organic contamination (Martin et al., 2020; Sanganyado et al., 2021). Indeed, oceans and deep waters in particular are final sinks for anthropogenic wastes, including chemicals produced from industrial, urban, domestic and agricultural uses (Froescheis et al., 2000; Looser et al., 2000; Zhang et al., 2019). Although deep-sea ecosystems are remote from direct anthropogenic sources of pollutants, various studies have shown that persistent hydrophobic organic contaminants including the persistent organic pollutants (POPs) and other substances showing similar properties are transported to deep oceanic waters, including the hadal trenches (Cui et al., 2020; Dasgupta et al., 2018; Jamieson et al., 2017; Takahashi et al., 2010; Webster et al., 2014). Various deep-sea species have been shown to be prone to high exposure to these contaminants, leading to their bioaccumulation at

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higher levels than in shallower water organisms (Froescheis et al., 2000; Looser et al., 2000; Mormede and Davies, 2003; Ramu et al., 2006 and references therein) and making chemical contamination by POPs one of the anthropogenic pressures in the deep sea (Stemmler and Lammel, 2013). Among the legacy POPs, polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and polybrominated diphenyl ethers (PBDEs) are by far the mostly-studied organic contaminants in deep ecosystems (Covaci et al., 2008; Ramu et al., 2006; Romero-Romero et al., 2017; Storelli et al., 2009; Takahashi et al., 2010; Webster et al., 2014). However, most of the above-mentioned studies relate to demersal and benthic species living near or on the bottom floor of oceans, while true deep pelagic species are more seldom considered. Other substances with similar properties as POPs are of similar concern and very few data on their occurrence and fate in deep-sea ecosystems are available so far. Among them are alternative flame-retardants such as those used in the replacement of legacy compounds. *Per*- and polyfluoroalkyl substances (PFASs), including the regulated long-chain perfluorocarboxylic acids (PFCAs) in particular, represent another under-studied class of compounds, although increasing concern has been recognised for this vast family of less hydrophobic compounds which are transported to deep seas (Sanchez-Vidal et al., 2015; Sanganyado et al., 2021; Zhang et al., 2019) and for whom oceans constitute the final reservoir (Armitage et al., 2009; Yamashita et al., 2008).

Despite their importance for ocean health and services and their exposure to various anthropogenic threats, deep-sea ecosystems and deep pelagic ones, in particular, are still poorly studied. Specifically, information on organic contaminant occurrence and understanding of their bioaccumulation and biomagnification in deep pelagic food webs are necessary to better assess organic contaminants' ecological impacts (Sanganyado et al., 2021) and assess health risks potentially associated with human exploitation of these deep resources (Grimaldo et al., 2020; Wiech et al., 2020). In this context, this study aimed to investigate the accumulation of a large diversity of organohalogen contaminants (OHCs) in meso- and bathypelagic species from the Bay of Biscay, northeast Atlantic, focusing on crustaceans and fish. The contamination was notably studied using the stable carbon and nitrogen isotope compositions ($\delta^{13}C$ and $\delta^{15}N$ values) concomitantly analysed on organisms, as respective trophic tracers of organic matter sources sustaining them and of their trophic position within the deep pelagic community studied. The results are expected to serve as a benchmark in future studies and are of prime interest to characterise and evaluate the chemical exposure of various organisms having a central role in marine ecosystems. This study provides another light on the contamination of the species living in the twilight zone in addition to the previously published results on major and trace elements analysed in the same samples (Chouvelon et al., 2022).

2. Materials and methods

2.1. Sampling

Samples were collected on the French slope of the Bay of Biscay (NE Atlantic) during the annual EVHOE fishery survey, on board the Ifremer R/V Thalassa in October 2017 (doi:10.17600/17002300). Crustacean and fish specimens of 14 different species (see the detailed species names in Table 1) were collected at night using a 25 m vertical opening pelagic trawl in the deep scattering layer (ca 800 m depth in the water column; 1330 m bottom floor). All samples were collected during the same haul of 60 min at a speed of approximately 4 knots (details are given in the data file deposited in SEANOE system doi:10.17882/90452. The selected fish individuals belonged to the most abundant species, including one species of Sternoptychidae and three species of

Table 1
Biological parameters of deep pelagic samples analysed for OHCs by taxon, family and species collected in the Bay of Biscay in October 2017. The number of individuals used per analysed sample is indicated (e.g.: 12 individuals of *Sergia robusta* were pooled for analysis, *Pasiphaea sivado* were analysed in 3 replicates of 31, 39 and 48 pooled individuals each, etc.…). Lengths (cm) were calculated as mean ± standard deviations of replicate-pooled samples and expressed as cephalothorax length for crustaceans and total length for fish. Minimum and maximum values (min/max) are given when $n = 2$. $\delta^{13}C$ and $\delta^{15}N$ values (in ‰) were measured on muscle subsamples as described in Chouvelon et al., 2022. Maximum length and feeding preferences, when available from the literature, are also indicated.

Taxon/Family	Species	Species code	Individuals per sample	Length	Max length[a]	$\delta^{13}C$	$\delta^{15}N$	Feeding preferences[a]
Crustaceans								
Sergestidae	*Sergia robusta*	Serg_rob	12	0.21	–	−19.90	9.31	Macrozooplankton-micronekton
Pasiphaeidae	*Pasiphaea sivado*	Pasi-siv	31/39/48	0.21 ± 0.01	0.80	−19.57 ± 0.02	9.52 ± 0.08	Euphausiids and calanoids (zooplankton)
Oplophoridae	*Ephyrina figueirai*	Ephy_fig	7	0.17	–	−18.79	10.05	Zooplankton
Fish								
Serrivomeridae	*Serrivomer beanii*	Serr_bea	1/1/1/4	64.3 ± 9.2	78	−19.69 ± 0.16	9.75 ± 0.46	Shrimps, other crustaceans, small fishes
Alepocephalidae	*Xenodermichthys copei*	Xeno_cop	5/5/5	15.3 ± 1.4	31	−19.84 ± 0.20	9.99 ± 0.12	Primarily on crustaceans (euphausiids, copepods, amphipods and decapod zoea), also ostracods and small cephalopods
Paralepididae	*Arctozenus risso*	Arct_ris	5/5	18.3/21.0	30	−19.99/−19.82	10.01/10.60	Fishes and shrimps
Sternoptychidae	*Argyropelecus olfersii*	Argy_olf	3/6	7.5/10.0	9	−19.63/−19.49	10.25/10.33	Crustaceans and small fishes
Myctophidae	*Myctophum punctatum*	Myct_pun	5/5/8	7.7 ± 1.0	11	−20.07 ± 0.22	9.98 ± 0.25	Zooplankton (copepods, euphausiids, larvaceans (appendicularia) zoea stages of brachyura -crustacean-)
	Lampanyctus crocodilus	Lamp_cro	5/5/5	12.8 ± 0.5	30	−19.33 ± 0.06	10.69 ± 0.16	Zooplankton and small crustaceans including copepods, amphipods, euphausiids and decapods
	Notoscopelus kroeyeri	Noto_kro	3/6/9	10.1 ± 2.2	14	−19.27 ± 0.90	11.34 ± 0.17	Crustaceans
Stomiidae	*Chauliodus sloani*	Chaul_slo	3	26.0	35	−19.41	10.80	Midwater fishes and crustaceans, myctophids
	Stomias boa	Stom_boa	1/1/33	31.6 ± 3.7	32	−19.18 ± 0.13	12.02 ± 0.26	Midwater fishes and crustaceans
Trichiuridae	*Aphanopus carbo*	Apha_car	1/1/1	61.7 ± 0.6	151	−19.21 ± 0.03	12.03 ± 0.15	Fish (mainly), crustaceans and cephalopods
Platytroctidae	*Searsia koefoedi*	Sear_koe	3	15.0	15	−19.49	12.22	Cnidarian

[a] Sources: Fishbase; Sealifebase; Bernal et al., 2015; Fanelli et al., 2014; Noël, 2015; Novotny, 2018; Santos et al., 2013.

Myctophidae, two of the most abundant mesopelagic fish families globally (Catul et al., 2011; Valinassab et al., 2007). Handling of samples was conducted on-board using rigorous protocols to avoid external contamination. All individuals were measured, weighed and stored at −20 °C until further processing in the laboratory. Considering data reported in the literature (Table 1), most fish except *Aphanopus carbo* were adult fish.

A total of 137 individuals from 3 crustacean species and 106 individuals from 11 fish species were selected for OHC analyses. To obtain sufficient material for the quantification of OHCs, the whole bodies of individuals belonging to the same species were pooled by specimens of similar sizes (Table 1). When large enough, fish were analysed individually; this was the case for 3 samples of *Serrivomer beanii,* 2 samples of *Stomias boa* and 3 samples of *Aphanopus carbo* (Table 1). In most cases, fish samples (and the crustacean *Pasiphaea sivado, n = 3)* were analysed in replicates ($n = 2$–4). The few cases when only one sample of pooled individuals per species was considered was taken into account in the interpretation of the results.

A small piece of white muscle (<3 % of individual total weight) was also collected for the analysis of stable isotopes of carbon and nitrogen as trophic tracers. Whenever possible, fish individuals' sexes were determined and noted in the composition of each pool; all pools were made of both male and female individuals except for *Serrivomer beanii* (males only) and *Stomias boa* (females only). All fish stomachs were emptied from their major visible content (most stomachs were found to be empty). After pooling, the samples were homogenised using a blender with stainless steel arms, freeze-dried and finely ground up with a ball mill MM400 (Retsch) using bowls and marbles with a zirconium oxide coating. Immediately after freeze-drying, the moisture percentage was determined in each sample; they varied between 69 % and 78 % in crustaceans and between 64 % and 88 % in fish (Table 2).

2.2. Chemical analyses

Extractable organic matter, used as a proxy for total lipid content (TLC), was determined gravimetrically using 500 mg of sample extracted with a mixture of hexane and acetone (80:20 v:v) using pressurised liquid extraction (PLE). The extracts were evaporated to dryness and TLC was expressed in % of dry weight (dw).

PCBs, OCPs and BFRs were determined as described by Munschy et al. (2020a). Briefly, 5–10 g of samples were extracted by PLE with dichloromethane, followed by gel permeation chromatography, a silica and alumina adsorption chromatography column and a two-dimensional HPLC system with two columns coupled in series. Analyses were performed by gas chromatography (Agilent 6890, Palo Alto, CA, USA) coupled to high-resolution mass spectrometry (AutoSpec Ultima, Waters Corp.). BDE-209, DBDPE (decabromodiphenylethane) and BTBPE (1,2-bis(2,4,6-tribromophenoxy)ethane) were analysed using an Agilent 7890B gas chromatograph coupled to a triple quadrupole mass spectrometer Waters Xevo TQS-μ (Millford, US) using atmospheric pressure chemical ionisation operated in the positive mode. The samples were analysed for 35 PCBs ranging from tri- to decachlorinated congeners, including the 12 dioxin-like (dl-) PCBs (CB-77, -81, -105, -114, -118, -123, -126, -156, -157, -167, -169, -189), the 6 indicator (i-) PCBs (CB-28, -52, -101, -138, -153, -180), various OCPs (*p,p′*-DDT, *o,p′*-DDT, *o,p′*-DDD, *p,p′*-DDD, *p,p′*-DDE, dieldrin, aldrin, hexachlorocyclohexanes –HCHs and hexachlorobenzene -HCB, referred to as ∑ OCPs later in the text) and BFRs including 36 PBDE congeners from tri- to decabrominated ones (Table S5) and non-PBDE BFRs (HBB -hexabromobenzene, BB-153 -2,2′,4,4′,5,5′-hexabromobiphenyl, BTBPE and DBDPE).

PFASs were determined according to Munschy et al. (2020a). Briefly, 1 g of sample was extracted using liquid-solid extraction (LSE) with a blend of MeOH/KOH and purified onto two consecutive SPE cartridges: an Oasis WAX weak anion exchange stationary phase and an Envicarb

Table 2
Moisture (%), total lipid contents (TLC in % dw) and concentrations (mean ± standard deviation in ng g^{-1} dw) of ∑PCBs, ∑OCPs, ∑PBDEs and ∑PFASs[a] in crustaceans and fish collected in deep pelagic waters of the Bay of Biscay in October 2017. ∑PCBs, ∑OCPs and ∑PBDE concentrations are additionally given in ng g^{-1} lw (in italic). Minimum and maximum concentrations (noted as min/max) are given when n = 2.

Family	Species	Moisture	TLC	∑ PCBs	∑ OCPs	∑ PBDEs	∑ PFASs
Crustaceans							
Sergestidae	*Sergia robusta*	76	14.7	66.79 *453.84*	12.86 *85.54*	0.65 *4.40*	55.69 –
Pasiphaeidae	*Pasiphaea sivado*	78 ± 0.2	4.3 ± 0.9	11.28 ± 0.69 *273.44 ± 68.51*	1.20 ± 0.13 *28.84 ± 3.06*	0.12 ± 0.05 *2.78 ± 1.18*	23.83 ± 2.70 –
Oplophoridae	*Ephyrina figueirai*	69	51.2	72.34 *141.29*	36.36 *71.02*	nd nd	22.80 –
Fish							
Serrivomeridae	*Serrivomer beanii*	88 ± 1.1	12.4 ± 4.2	51.54 ± 7.37 *442.53 ± 131.23*	19.80 ± 4.90 *175.21* ± 85.53	2.32 ± 0.64 *19.40* ± 5.20	25.80 ± 8.11 –
Alepocephalidae	*Xenodermichthys copei*	87 ± 0.4	6.1 ± 1.1	24.33 ± 5.03 *403.03 ± 92.07*	7.43 ± 0.78 *123.58 ± 20.21*	0.69 ± 0.30 *11.77 ± 6.20*	18.31 ± 0.98 –
Paralepididae	*Arctozenus risso*	72–75	24.5–35.5	22.43/40.42 *91.38/113.75*	6.31/11.71 *25.70/32.96*	0.84/0.87 *2.35/3.56*	3.99/4.73 –
Sternoptychidae	*Argyropelecus olfersii*	74–75	17.0/23.5	21.09/29.89 *123.91/127.05*	5.707/10.90 *33.53/46.32*	0.58/0.84 *3.40/3.57*	6.86/7.81 –
Myctophidae	*Myctophum punctatum*	70 ± 1.7	33.5 ± 4.0	29.81 ± 7.25 *88.04 ± 12.12*	7.75 ± 1.81 *22.93 ± 2.94*	0.60 ± 0.22 *1.78 ± 0.49*	4.31 ± 1.00 –
	Lampanyctus crocodilus	79 ± 0.8	8.9 ± 2.1	66.77 ± 7.89 *768.30 ± 146.26*	23.13 ± 10.84 *257.34 ± 103.77*	0.92 ± 0.35 *10.96 ± 5.09*	11.26 ± 3.82 –
	Notoscopelus kroeyeri	67 ± 3.4	41.9 ± 9.6	62.79 ± 44.23 *152.74 ± 114.86*	17.73 ± 10.66 *42.54 ± 27.07*	1.32 ± 0.98 *3.17 ± 2.53*	3.07 ± 1.34 –
Stomiidae	*Chauliodus sloani*	85	10.5	100.07 *949.47*	38.04 *360.90*	2.45 *23.20*	6.66 –
	Stomias boa	84 ± 1.5	13.3 ± 9.2	91.47 ± 28.43 *1121.99 ± 940.10*	62.49 ± 45.53 *453.74 ± 45.85*	3.49 ± 1.93 *34.24 ± 17.74*	5.92 ± 1.46 –
Trichiuridae	*Aphanopus carbo*	77 ± 2.8	6.4 ± 2.2	69.82 ± 19.81 *1112.38 ± 131.48*	21.12 ± 4.28 *347.08 ± 83.54*	1.68 ± 0.33 *28.06 ± 8.81*	11.84 ± 0.89 –
Platytroctidae	*Searsia koefoedi*	85	17.2	68.63 *398.80*	37.54 *218.15*	3.13 *18.19*	37.25 –

[a] ∑PCBs, ∑OCPs, ∑PBDEs, ∑PFASs = ∑ of all analysed compounds above LOQs taking LOQ = zero.

charcoal stationary phase. Analysis was performed using an Acquity ultra-performance liquid chromatograph (UPLC®, Waters Corp.) coupled to a triple quadrupole mass spectrometer (Xevo® TQ-S micro, Waters Corp.) interfaced with an electrospray ionisation source *Z*-sprayTM (Waters Corp.). The mass spectrometer was operated in negative ionisation mode using multiple reaction monitoring (MRM) with argon as the collision gas. PFASs were analysed for five C_4- to C_{10}-perfluoroalkyl sulfonates (PFSAs) and nine C_6- to C_{14} perfluorocarboxylic acids (PFCAs), namely: perfluorobutane sulfonate (PFBS); perfluorohexane sulfonate (PFHxS); perfluoroheptane sulfonate (PFHpS); perfluorooctane sulfonate (PFOS); perfluorodecane sulfonate (PFDS); perfluorohexanoic acid (PFHxA); perfluoroheptanoic acid (PFHpA); perfluorooctanoic acid (PFOA); perfluorononanoic acid (PFNA); perfluorodecanoic acid (PFDA); perfluoroundecanoic acid (PFUnDA); perfluorododecanoic acid (PFDoDA); perfluorotridecanoic acid (PFTrDA) and perfluorotetradecanoic acid (PFTeDA).

Quality Assurance/Quality Control (QA/QC) procedures were carefully followed during the entire analytical protocol. This included quantification by isotopic dilution using ^{13}C-labeled compounds, five-to-six-point calibration curves in each sequence of samples to calculate relative response factors and check linearity, laboratory blank determination (whole analytical procedure), in-house quality control sample, participation in QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe) and intercomparison exercises with satisfactory Z scores. Detailed analytical parameters and QA/QC results are given in the SI.

2.3. Statistical analyses

All statistical analyses were performed using StatSoft Statistica software v 13.3 with a significance level (α) of <0.05. Concentrations below the limits of quantification (LOQs) were assigned as missing values (i.e. counted as zero in the sums), and only compounds quantified in >50 % of the samples were considered for statistical analyses. Data were tested for normality using the Shapiro-Wilk's test and parametric or non-parametric tests were performed depending on a normal distribution. Correlations (e.g. between total lipid content and POP concentrations and between contaminant concentrations) were tested using simple linear regression coefficients and Spearman's rank correlation test was used to evaluate the strength and direction of relationships. Data comparisons (biological parameters, POP concentrations and ratios) across groups were performed using non-parametric tests (Mann–Whitney: MW- and one-way ANOVA Kruskal-Wallis's: KW -test to compare independent groups) for non-normally distributed data. Results were considered significant only when both tests gave significant results. Principal component analysis (PCA) was performed on normalised concentrations to avoid the concentration effect. All data used in the present study are available under the depository system SEANOE (doi:10.17882/90452).

3. Results and discussion

3.1. Trophic markers

The detailed results obtained for trophic markers on this deep pelagic community were presented in Chouvelon et al. (2022). The values specifically obtained for the samples here analysed for organic contaminants (a selection of those analysed for chemical elements by Chouvelon et al., 2022) are summarised in Table 1. Briefly, δ^{13}C values ranged from −20.30 ‰ to −18.26 ‰, with no significant differences between crustaceans and fish. This relatively small variability for δ^{13}C values (~2 ‰ difference) indicates similar carbon sources sustaining the species (Chouvelon et al., 2022). The δ^{15}N values showed more variability, ranging from 9.24 ‰ to 12.23 ‰ (Table 1). This range suggests a difference of at least one trophic level (following the expected mean difference of 3.4 ‰ per trophic level, Post, 2002) among species of the studied food web. δ^{15}N values were significantly different (MW, $p = 0.0017$) between crustaceans (9.58 ± 0.28 ‰ on average, $n = 5$) and fish (10.76 ± 0.91 ‰, $n = 28$).

3.2. Total lipid contents (TLCs)

The TLC values exhibited high variations among taxa and species, ranging from 4.3 ± 0.9 % dw ($n = 3$) in *Pasiphaea sivado* to 51 % dw in *Ephyrina figueirai* ($n = 1$) for crustaceans and, for fish, from 6.1 ± 0.1 % dw (n = 3) in *Xenodermichthys copei* to 41.9 ± 9.6 % dw (n = 3) in *Notoscopelus kroeyeri* (Table 2). Classically, TLC values showed an inverse significant linear relationship ($p < 0.001$) with humidity percentages (Spitz et al., 2010). TLC values in fish were similar to those reported previously in the literature, even though different extraction methods were used (chloroform:methanol:water (1:2:1) in Şen Özdemir et al., 2019 and ether-ethyl in Spitz et al., 2010 versus hexane and acetone (80:20 v:v) for our samples). The fish species could be distinguished between low-TLC species with TLC values below 15 % dw (the Serrivomeridae *Serrivomer beanii,* the Alepocephalidae *Xenodermichthys copei,* the Myctophidae *Lampanyctus crocodilus,* the Stomiidae *Chauliodus sloani* and the Trichiuridae *Aphanopus carbo*) and the other species (the Papalepididae *Arctozenus risso,* the Sternoptychidae *Argyropelecus olfersii,* the Myctophidaes *Myctophum punctatum* and *Notoscopelus kroeyeri*, the Stomiidae *Stomias boa* and the Platytroctidae *Searsia koefoedi*). These results agree with the energy densities reported by Spitz et al. (2010) in various forage fish species from the Bay of Biscay. Except in *Stomias boa* for which the replicate made from 3 individuals showed extremely different values compared to the two other replicates (made of 1 individual each), TLCs were fairly similar between replicate pool samples of the same species, with an average rsd of 24 % (12–34 % range excluding *Stomias boa*). The TLC values in *Stomias boa* replicates (all female individuals) showed a higher rsd (69 %), with high TLC values of 17.6 % dw and 19.5 % dw for the two 35 cm and 32 cm individuals respectively, while the replicate made from 3 individuals of 27.7 ± 1.5 cm (on average) exhibited a much lower TLC value, i.e. 2.7 % dw. Ontogenic differences may explain the difference observed in the lipid contents of *Stomias boa* samples, as energy reserves were shown to be positively correlated with size at the fish species level (Anthony et al., 2000; Cargnelli and Gross, 1997).

3.3. Bioaccumulation of organohalogen contaminants in the deep pelagic ecosystem

3.3.1. Organic contaminant concentrations and relative contributions show high inter-species variabilities

In both crustaceans and fish species, most PCB congeners showed detection frequencies of 100 %, except for CB-77 and CB-189 (97 %), CB-126 and CB-169 (91 % and 58 % of the samples, respectively), while CB-81 was seldom detected above the LOQ (6 % of the samples) (Table S3). CBs -29, -30, -112 and -114 were never detected. Indicator PCBs (i-PCBs) were 7 times higher than dioxin-like congeners (dl-PCBs) in all species and contributed to 47 % of the $\sum$ PCBs, pointing out the importance of determining more than the classic 18 congeners to better assess total PCB bioaccumulation in marine organisms, while dl-PCBs counted for 7 % of the $\sum$ PCBs. Hexa- and heptachlorinated congeners (i.e. CB-153, CB-138, CB-180 and CB-187, each one contributing to 10–19 % of the $\sum$ PCBs) were the most abundant ones, which is consistent with previous findings in deep-sea ecosystems (Koenig et al., 2013; Romero-Romero et al., 2017; Storelli et al., 2009; Takahashi et al., 2010; Webster et al., 2014).

Among OCPs, dieldrin, endrin, and mirex were detected in all samples, similarly to the DDT isomer *p,p'*-DDE, while the other DDT isomers showed detection frequencies in the 88–94 % range (Table S4). β-HCH was detected in 100 % of the samples, above γ-HCH (73 %), α-HCH (58 %) and δ-HCH (6 %). HCB and PeCB were detected in 97 % and 52 % of the samples, respectively. The other OCPs, namely, aldrin, isodrin,

α-endosulfan, β-endosulfan and endosulfan sulphate, were never detected. DDTs were by far the most abundant OCPs in all species (mean of 16.35 ± 18.90 ng g^{-1} dw), followed by dieldrin (mean of 1.96 ± 1.34 ng g^{-1} dw) > HCB (mean of 1.07 ± 0.91 ng g^{-1} dw) > endrin (mean of 0.25 ± 0.19 ng g^{-1} dw) = ∑ HCHs (mean of 0.20 ± 0.18 ng g^{-1} dw) (Table S4). The (*p,p'*-DDE + *p,p'*-DDD)/Σ DDTs ratio was 0.85 ± 0.06 in all samples, which globally indicates old DDT inputs (Suárez et al., 2013) and is consistent with the ban of DDT usage since the 1970s or 1980s in most countries worldwide (Kalantzi et al., 2001). These results bring evidence that DDT is still a major organochlorine pesticide in marine ecosystems, which is in line with its global past usage and environmental persistence (Li and Macdonald, 2005). ∑ DDTs showed significant positive correlations with all other organochlorine pesticides except ∑ HCHs, possibly related to HCH physico-chemical properties and environmental behaviour (Salvadó et al., 2019). Among DDTs, the *p,p'-DDE* isomer was the most prevalent in all samples (80 ± 11 % of ∑ DDTs), in accordance with the common profiles observed in marine biota including deep-sea organisms (Koenig et al., 2013; Ramu et al., 2006; Storelli et al., 2009). The *o,p'*-DDT/*p,p'*-DDT concentration ratio was 0.36 ± 0.17, on average, in all fish species. This ratio is commonly used to distinguish DDT sources, with values in the 0.2–0.3 range in technical DDT (Kalantzi et al., 2001) whereas a ratio above 0.34 is usually attributed to DDT's origin from dicofol acaricide impurities (Suárez et al., 2013). However, the use of this ratio should be interpreted with caution as species-specific parameters may also influence DDT isomeric profiles.

BDEs -28, -47, -49, -66, -99, -100, -119, -126, -153, -154 and -155 were the most frequently detected PBDE congeners (> 70% of the samples), while BDEs -77, -183, -184, -202, -205, -207 and -209 showed intermediate detection frequencies (27–55%). The other PBDE congeners, i.e. BDEs 30, -71, -85, -138, -171 and -204 were below LOQs in all samples. The non-PBDE BFRs HBB, BB-153 and DBDPE were detected in 61, 73 and 76 % of the samples, respectively. BTBPE was only detected in 7 samples (21 %). Among PFASs, PFOS, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA and PFTeDA were detected in 100 % of the samples. PFOA was detected in 45 % of the samples, PFDS in 27 % and PFHpA was seldom detected (12 %) (Table S6). PFHpS and PFHxA were below LOQs in all samples, PFHxS was detected in one sample only and close to the LOQ. The relative contributions of PFAS and PBDE individual compounds are presented in the following sections.

The concentrations of the various OHC families are presented in Table 2 in both dw (all contaminants) and lw (for PCBs, OCPs and PBDEs) and details for each compound are given in Tables S3 to S6. Mean concentrations in all species (calculated on the mean values of replicates per species) ranged from 11.28 to 100.07 ng g^{-1} dw, 1.20 to 62.49 ng g^{-1} dw, 0.12 to 3.49 ng g^{-1} dw and 3.07 to 55.69 ng g^{-1} dw for ∑ PCBs, ∑ OCPs, ∑ PBDEs and ∑ PFASs, respectively (Table 2).

In crustaceans, the intra-species variability of concentrations was low between the 3 *Pasiphaea sivado* replicate pools (6–11 % rsd for PFASs, PCBs and OCPs and 45 % for PBDEs) but huge variations were found at the inter-species level (rsd values of 55, 67, 107 and 97 % for PFASs, PCBs, OPCs and PBDEs, respectively). PFASs showed the highest contamination levels of all OHC families in *Pasiphaea sivado* (mean of 23.83 ± 2.70 ng g^{-1} dw, representing 65 % ± 2 % of the OHCs) followed by PCBs (11.28 ± 0.69 ng g^{-1} dw, 31 %), OCPs (1.20 ± 0.69 ng g^{-1} dw, 3 %) and PBDEs (0.12 ± 0.05 ng g^{-1} dw, 0.3 %). This species showed the lowest contamination levels of PCBs, OCPs and PBDEs among crustaceans, which can be explained by its lower TLC values. At the opposite, chlorinated OHCs (and PCBs in particular with 49 and 55 % of the OHCs) were predominant in the two other crustacean species *Sergia robusta* and *Ephyrina figueirai*. These differences in the relative contributions of the OHC families can be explained by the biochemical compositions of the studied species; unlike lipophilic contaminants (i.e. PCBs, OCPs and PBDEs), PFASs have an affinity for specific proteins and phospholipids (Armitage et al., 2013; Ng and Hungerbühler, 2013).

In fish, PCBs presented the highest concentrations in all species (mean of 54.42 ± 28.57 ng g^{-1} dw calculated on the individual replicates, representing a relative contribution of 42–76 % of all OHCs), followed by OCPs (21.73 ± 21.26 ng g^{-1} dw, of which 83 % were DDTs), PFASs (11.95 ± 9.58 ng g^{-1} dw) and PBDEs (1.57 ± 1.17 ng g^{-1} dw). The chlorinated and brominated OHC contributions showed significant positive relationships with TLC values ($p = 0.008$) and $\delta^{15}N$ values ($p = 0.003$), and as a result PFAS contribution decreased significantly with both parameters, reflecting their lower trophic magnification factors (TMFs) (Won et al., 2018) and suggesting a potential link with the biochemical compositions of the studied species. However, the two fish species *Serrivomer beanii* and *Xenodermichthys copei,* despite them having been reported to have lower protein contents than the other studied fish species in the Bay of Biscay (Spitz et al., 2010), showed higher PFAS contribution than the other fish (30 ± 8 % versus 10 ± 6 % in the other species), a result that could rather be explained by their lower trophic level (see Section 3.3.6).

The results agree with previous studies showing PCBs and DDTs as the major chlorinated contaminants in deep-sea fish, reflecting their high past usage and persistence, while PBDE levels are usually reported to be several orders of magnitude lower (Koenig et al., 2013; Webster et al., 2014). The intra-species variability of concentrations in all fish species was low for all contaminant families (21 % rsd on average for PFASs and 29, 38 % and 36 % for PCB, OCP and PBDE concentrations in dw) except in *Notoscopelus kroeyeri* (rsd of 44–75 % in dw). The inter-species variability (calculated on the species means) was between 43 % (PCBs) and 87 % (PFASs). A limited number of lipophilic OHCs showed significant ($p < 0.0001$ to 0.022) positive correlations with TLCs (i.e. the less-chlorinated CBs -18, -28, -31, -44, -49, -52 and -66, dieldrin, endrin, ∑ HCHs and each isomer, HCB, BDEs -66 and -77), while ∑ DDTs, PCBs (whether i-PCBs, dl-PCBs or ∑ all congeners were considered) and other PBDEs did not. Despite this lack of significant correlations, concentrations of lipophilic OHCs were normalised to TLCs to compare the concentrations between replicate samples at the intra- and inter-species levels. Normalising the concentrations to TLCs did not decrease the intra-species variability (30, 29 and 39 % for PCBs, OCPs and PBDEs respectively) nor the inter-species variability (73–75 %).

3.3.2. *Chlorinated and brominated contaminant concentrations are in the range of those reported in other deep pelagic species in the NE Atlantic and exceed OSPAR BACs in the majority of samples*

PCB, OCP and PBDE concentrations in deep-sea organisms from various oceanic regions have been published previously, but most of the data refer to bathydemersal and benthopelagic species, i.e. living and feeding close to the bottom seafloor. The studied periods refer to sampling dates of >10 years ago which could bias the comparison for the legacy POPs. Data on contaminants of emerging concern are still very scarce, making comparisons impossible. With these precautions in mind, the following are some comparisons with previously published data focusing specifically on deep pelagic species.

Our concentration ranges (i.e., 31.2–1072.9 ng g^{-1} lw and 0.92–43.37 ng g^{-1} lw for PCBs and PBDEs respectively) compare with the $\sum_6$ PCB and $\sum_{15}$ PBDE concentrations determined in the muscles of 4 deep pelagic fish species similar to ours (namely *Benthosema glaciale, Xenodermichthys copei, Argyropelecus hemigymnus, Gonostoma bathyphilum*) and collected at 1200–1500 m depths in a submarine canyon in the south Bay of Biscay in 2012–2013 (between 134 and 756 ng g^{-1} lw and between <LOD and 23 ng g^{-1} lw, respectively, Romero-Romero et al., 2017). $\sum_7$ ICES PCB (i.e. the International Council for the Exploration of the Sea PCBs, the major and mostly-used congeners) was found in mean concentrations of between 91.6 ± 116 and 613 ± 739 ng g^{-1} lw in the muscle of adult *Aphanopus carbo* (black scabbardfish) collected from the west of Scotland in 2006–2012 and of between 267 ± 150 and 521 ± 301 ng g^{-1} lw in their livers (Webster et al., 2014), which compares closely to our results in whole fish (524–651 ng g^{-1} lw), although younger individuals were considered in our study. In the same individuals, the $\sum_9$ PBDEs were between 1.1 ± 3.0 and 42.5 ± 26.4 ng

g^{-1} lw in the flesh and between 2.4 ± 3.9 and 25.8 ± 8.2 ng g^{-1} lw in the liver (Webster et al., 2014), while our result showed concentrations of a similar order of magnitude with 13.0–17.1 ng g^{-1} lw range in whole fish. Various organic contaminant concentrations (namely, PCBs, HCB, dieldrin and DDTs) were reported in the livers of black scabbardfish collected from the NE Atlantic (from Madeira off the coasts of Marocco to Ireland) in 1999 (Mormede and Davies, 2003). Surprisingly, considering the difference in time of sampling, studied areas and targeted tissues, these contaminant concentrations did not differ drastically from our data ($\sum_7$ ICES PCBs: 91 ng g^{-1} lw to 7660 ng g^{-1} lw versus 524–651 ng g^{-1} lw in our fish samples; HCB: 5.3 to 30.3 ng g^{-1} lw versus 11.4–18.8 ng g^{-1} lw; dieldrin: 22.0–40.9 ng g^{-1} lw versus 20.5–25.0 ng g^{-1} lw) except for the $\sum$ DDT concentration, which was lower in our samples (239.5–397.8 ng g^{-1} lw versus 384–4350 ng g^{-1} lw in Mormede and Davies' study), potentially reflecting a decrease of DDT inputs over the studied periods.

Background Assessment Concentrations (BACs) defined by the Oslo/Paris Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR, 2017) were exceeded in all samples (by a factor of 11–24 on average) for CBs -138 (0.09 ng g^{-1} ww), -153 (0.10 ng g^{-1} ww) and -180 (0.11 ng g^{-1} ww), while CBs -52, -105 and -118 levels were above the BACs in 86 % of the samples (by a factor of 3, 2 and 5, respectively). CBs -28 and -156 were above BACs in 57 % of the samples (3 and 1.5 times respectively). However, BACs were established in the muscle of flatfish in coastal areas as thresholds to assess whether concentrations are close to background levels, while concentrations in whole fish were determined in our study. Similarly, the BACs of 0.065 ng g^{-1} lw recommended for each PBDE congener BDEs -28, -47, -66, -85, -99, -100, -126, -153, -154, -183 and -209 (OSPAR, 2021) were exceeded for BDEs -47, -99, -100 and -154 in >80 % of the samples (by 33, 7, 17 and 27 times, respectively), while BDEs -28, -66, -126, -153 and -209 BACs were exceeded in 34 % to 63 % of the samples (by 1.4–3 times and 100 times for BDE-209). BDE-183 was barely above the BAC (3 % of the sample by 1.6 times) and BDE-85 was never detected above the LOQ. Environmental Assessment Criterias (EACs, in lw) defined as concentrations below which biological effects are unlikely to occur have also been established in fish were exceeded only for CB-118 (25 ng g^{-1} lw) in 30 % of the samples (*Lampanyctus crocodilus*, *Chauliodus sloani*, *Stomias boa* and *Aphanopus carbo*), in agreement with the OSPAR intermediate assessment conducted within the period 1995–2015 (OSPAR, 2017).

3.3.3. Specific congener analysis reveals untypical BFR profiles

BFR profiles were dominated by BDE-209 (mean contribution of 38 % of the 12 summed congeners quantified in >50 % of the samples) followed by BDE-47 (21 %), BDE-155 (19 %) and BDE-154 (14 %). The other mostly-detected congeners (i.e. BDEs -28, -49, -66, -99, -100, 119, -126, -153) contributed each to <10 %, on average, to the summed 12 BDEs. As BDE-47 is generally reported as the major PBDE congener in biota, the present PBDE profile is therefore atypical due to the high contribution of BDE-209. The BDE-209 congener is usually reported as poorly bioaccumulable in fish because of its very high log K_{ow} (12.1, Kelly et al., 2008), high molecular size and degradation propensity via metabolism (Stapleton et al., 2004a; Roberts et al., 2011). This highly brominated congener has also been previously identified at higher abundance (17 % of $\sum$ PBDEs) in deep-sea organisms compared to those from the shelf (Romero-Romero et al., 2017). The presence of a high diversity of several high-brominated congeners, as well as species-specific profiles, suggests BDE-209 bioaccumulation and biotransformation, as detailed in Section 3.3.4 below.

Another peculiar result was the high contribution of DBDPE, quantified in 76 % of the samples at concentrations as high as 16.48 ng g^{-1} dw in *Searsia koefoedi*. Similar to BDE-209, DBDPE is characterised by a very high log K_{ow} (11.1, Covaci et al., 2011) and high molecular size that should limit its bioaccumulation at high levels in fish and generally leads to its biodilution in trophic webs (Tao et al., 2019). However, unlike for BDE-209, no debromination of DBDPE has been reported in fish and its bioaccumulation factor was reported to be 10 times higher than BDE-209's one (He et al., 2012), which would argue in favour of its occurrence at higher levels than BDE-209. Similar to BDE-209, the DBDPE occurrence at particularly high levels in some of the studied samples (i.e. *Serrivomer beanii, Stomias boa, Aphanopus carbo* and *Searsia koefoedi*) could be the result of undigested particles in their digestive tracts. DBDPE sources in the marine environment include plastics from electronic and electrical equipment wastes (Stubbings et al., 2021). However, whether DBDPE found in some fish originated from its adsorption on particulate matter or plastics, or from plastics themselves (eventually extracted following whole fish analyses) cannot be proven. In oceanic waters off the Californian coast (Monterey Bay, US), the highest concentrations of microplastics have been reported between 200 m and 600 m (Choy et al., 2019), and microplastics have been reported in the digestive tracts of mesopelagic fish feeding at these depths, although at very different occurrence frequencies depending on locations (35 % in Boerger et al., 2010; 9 % in Davison and Asch, 2011; 11 % in Lusher et al., 2016; 24 % specifically in Myctophidae in Savoca et al., 2021; 73 % in Wieczorek et al., 2018). Among the various species studied by Wieczorek et al. (2018), *Serrivomer beanii* and *Lampanyctus macdonaldi* were among those presenting the highest frequency of plastic occurrence, while *Serrivomer beanii* and *Myctophum punctatum* were two species presenting the highest number of plastic debris in their gut. Despite the digestive tracts of fish from our study being emptied from visible material before analysis, the presence of small parts of food residues (including those of plastic origin) that could have still be present in the digestive tracts (i.e. not assimilated/absorbed into the organism's tissues) is not unrealistic. The fact that high variations were sometimes found between replicates of the same species (as in *Aphanopus carbo*, with concentrations of 81, 4762 and 7803 pg g^{-1} dw in each of the three replicates made from individual fish, or in *Xenodermichthys copei* with < LOQ, 148 and 1940 pg g^{-1} dw in pooled samples) argues in favour of undigested particles with a short-time and erratic occurrence rather than a long-term accumulation in tissues. On the opposite hand, species such as *Stomias boa* showed high DBDPE concentrations in all replicates (3075, 5963 and 7840 pg g^{-1} dw, rsd = 43 %), while others such as *Myctophum punctatum* or *Lampanyctus crocodilus* exhibited systematically lower concentrations (< LOQ, 42 and 193 pg g^{-1} dw and 436, 682 and 938 pg g^{-1} dw, respectively). Interestingly, the highest DBDPE concentrations (in both dw and lw) were determined in the longest fishes studied, i.e. *Serrivomer beani* (64.3 ± 9.2 cm), *Stomias boa* (31.6 ± 3.7 cm) and *Aphanopus carbo* (61.7 ± 0.6 cm). These species are all characterised by elongate shapes and therefore have potentially longer digestive tracts, which would lead to a higher retention potential in the gut. A higher occurrence of microplastics in larger fish compared to smaller ones has previously been observed in various freshwater species from a lake in Ontario, Canada (McIlwraith et al., 2021). One exception was *Searsia koefoedi*, which exhibited the highest concentration of all species (16.48 ng g^{-1} dw) despite its relatively smaller length (15 cm), but these results would need to be confirmed as only one pool of 3 individuals was analysed. However, this result would be consistent with this species' non-migratory behaviour towards epipelagic waters at night for feeding (but migrations deeper into the bathypelagic zone are possible; Novotny, 2018), as DBDPE has been shown to be high in deeper waters (Zhen et al., 2021). Our results highlight that mesopelagic fish could, in addition to transferring very hydrophobic contaminants to higher trophic level organisms, contribute to their transfer from surface waters to deeper waters and eventually to the bottom sea.

3.3.4. Chlorinated and brominated OHC individual contributions and diagnostic ratios reveal species-specific profiles

Because PCB bioaccumulation depends on their chlorine number and position, which both are source- and metabolism-related, PCB profiles can be examined by their number of Cl atoms and their structure-activity group (SAG) classification (Boon et al., 1997; Yunker et al., 2011). The lower chlorinated congeners are also the most metabolisable ones (SAG

III and IV), while the most refractory ones (6-,7- and 8-Cl) belong to SAG I and II groups. In our samples, 3-Cl showed significant higher contributions to the $\sum$ PCBs in crustaceans compared to fish (10 ± 5 % in crustaceans versus 4 ± 5 % in fish, MW and KW tests), which is consistent with crustaceans' lower trophic levels (i.e. lower $\delta^{15}N$ values). However, inter-species differences were high when only fish were considered, and similar contributions to those of crustaceans were found in some fish species such as *Arctozenus risso*, *Argyropelecus olfersii* and *Myctophum punctatum* (Fig. 1A). These species are among those showing the lowest $\delta^{15}N$ values among the studied fish species, but other species with low $\delta^{15}N$ values (such as *Serrivomer beanii* or *Xenodermichthys copei*) did not show such a high 3-Cl contribution (Fig. 1A), suggesting that the trophic level does not solely explain these results. Although significant metabolic capacities towards PCBs have been evidenced between crustaceans and deep-sea fish (Koenig et al., 2012), no significant differences were observed between taxa regarding SAG groups in our samples. The three crustacean species showed discrepancies in their PCB profiles, with *Sergia robusta* being characterised by higher SAG I and II group contributions (72 % in total, but $n = 1$) than the other two species (52 ± 5 %). Among fish species, *Arctozenus risso*, *Argyropelecus olfersii* and *Myctophum punctatum* showed the highest SAG III and IV (lower chlorinated and metabolisable congeners) and the lowest SAG I and II (hexa- to octachlorinated and refractory congeners) contributions. On the opposite hand, *Serrivomer beanii*, *Xenodermichthys copei*, *Aphanopus carbo*, *Lampanyctus crocodilus* and *Searsia koefoedi* were characterised by higher SAG I and II contributions (Fig. 2). More specifically, the CB-149/CB-153 and CB-132/CB-153 concentration ratios, which could be used as metabolism tracers (i.e. a ratio between hexachlorinated congeners from SAG IV and V and a typical refractory one from SAG I), showed higher values in *Arctozenus risso*, *Argyropelecus olfersii*, *Myctophum punctatum* and *Notoscopelus kroeyeri,* suggesting a lower metabolic activity towards CB-149 and CB-132 in these species (Fig. 2). These results agree with previous findings that different cytochrome P450 activities existed between deep-sea fish species (Koenig et al., 2012).

Similarly to PCBs, some variations in *p,p'*-DDE contributions were observed between fish species (Fig. 1B). Among Myctophidae, both *Myctophum punctatum* and *Notoscopelus kroeyeri* exhibited similar DDT profiles (*p,p'*-DDE and *p,p'*-DDD at 65 ± 5 % and 12 ± 1 %, respectively) and were different from those of the third Myctophid species *Lampanyctus crocodilus*, in which higher *p,p'*-DDE (84 ± 2 %) and lower *p,p'*-DDD (5 ± 1 %) contributions were found. These results could suggest a species-specific DDT profile due to different metabolic capacities, which would be in line with the higher metabolic profile of PCBs observed in *Lampanyctus crocodilus*.

Regarding PBDE profiles (Fig. 1C), the nona-BDEs BDE-207 and BDE-208, the octa-BDEs BDE-202, BDE-205 and BDE-197 and the hepta-BDEs BDE 184 and BDE-183 were quantified in 18–48 % of our samples, although at low levels (their total contribution was 6 % of the sum of all congeners determined above LOQs). In addition, the hexa-BDEs BDE-155 and BDE-154, ranking third and fourth in our samples, have both been reported as debromination products of octa-BDEs (Stapleton et al., 2004b; Zeng et al., 2012). As the nona- to hexa-brominated congeners identified in our samples are not present in technical mixtures (La Guardia et al., 2006; Munschy et al., 2011), their occurrence might be explained by either i) assimilation and further metabolisation by fish or ii) metabolisation by the prey and bioaccumulation in fish via trophic transfer. Indeed, these congeners are among those (ranging from nona- to tetra-BDEs) that have been reported in various fish species following BDE-209 metabolic degradation (Roberts et al., 2011). BDE-154 could result from the debromination in the *meta* position of BDE-183, while

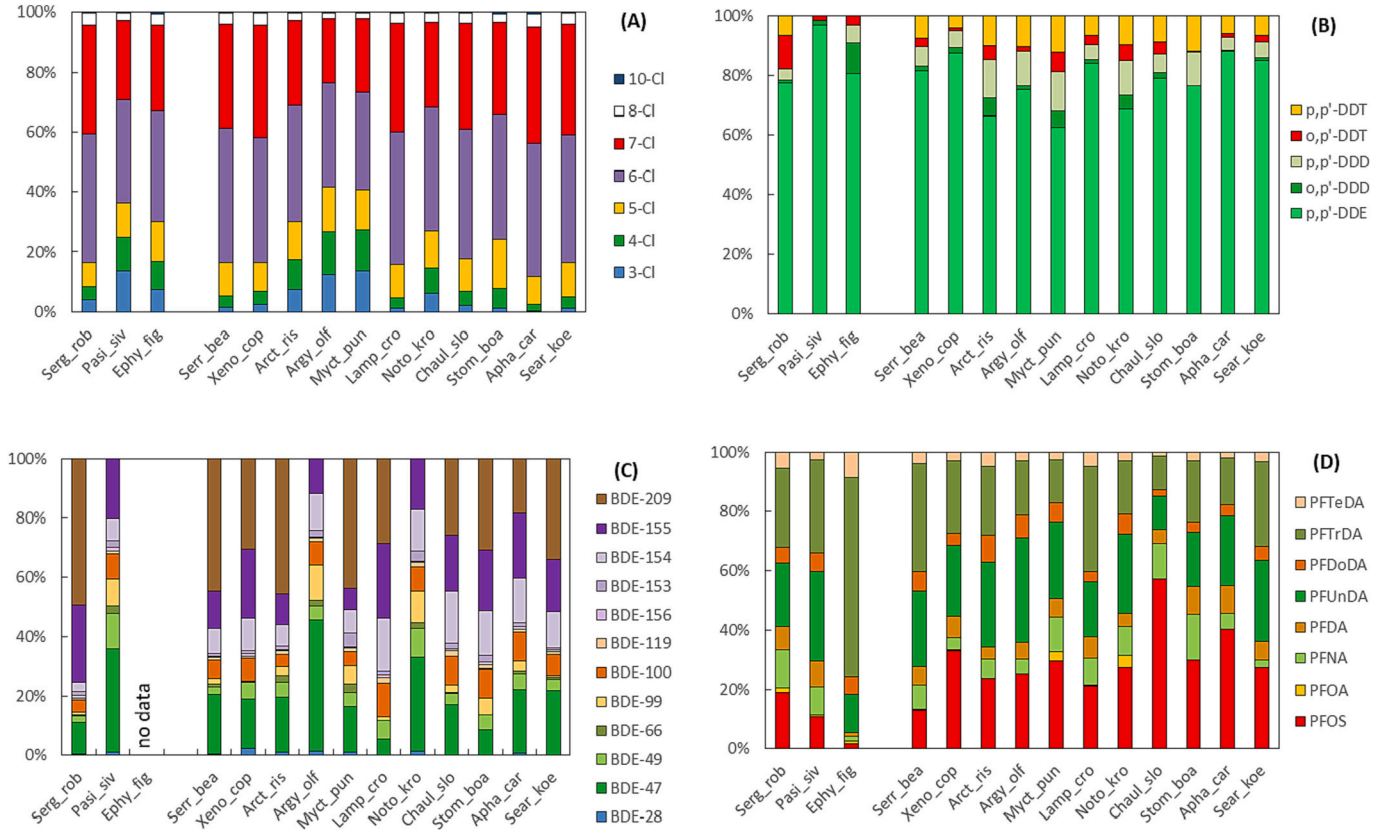

Fig. 1. Relative contributions (% of the summed compounds) of (A) individual PCBs (presented per number of chlorine atoms), (B) DDTs (5 isomers, DDDs in green, DDTs in red/orange), (C) PBDEs (12 congeners, tri-, tetra, penta-, hexa- and deca-Br congeners are presented in blue, green, orange, purple and brown, respectively) and (D) PFASs (odd-chain PFCAs in green, even-chain PFCAs in orange) in deep pelagic crustacean and fish species collected in the Bay of Biscay in October 2017. Species names according to Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

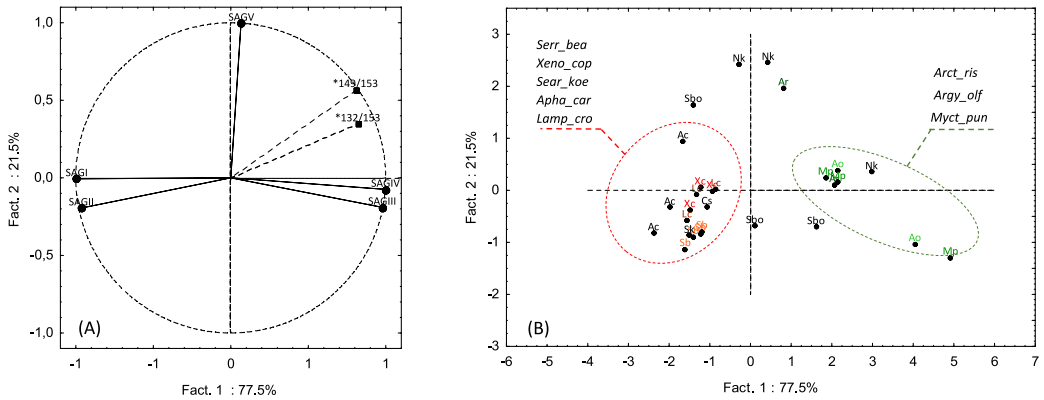

Fig. 2. Projection of the principal component analysis conducted on normalised profiles of PCBs categorized according to their SAG group in deep pelagic fish species collected in the Bay of Biscay in October 2017. Projection of variables (A) and individuals (B) on the first two principal components are presented. CB-149/CB-153 and CB-132/CB-153 were used as supplementary variables. Species abbreviations Ac = *Aphanopus carbo*, Ao = *Argyropelecus olfersii*, Ar = *Arctozenus risso*, Cs = *Chauliodus sloani,* Lc = *Lampanyctus crocodilus,* Mp = *Myctophum punctatum,* Nk = *Notoscopelus kroeyeri,* Sb = *Serrivomer beanii*, Sbo = *Stomias boa, Sk* = *Searsia koefoedi,* Xc = *Xenodermichthys copei.*

BDE-155 could result from successive debrominations in the *meta* positions of BDE-207, BDE-197 and BDE-184, which were all detected in our samples, although at low concentrations compared to their debrominated counterparts. If these congeners originate from BDE-209 metabolism, their detection at concentrations above the LOQs reveals exposure of the studied species to BDE-209. The high variability in PBDE profiles between species could therefore result from species-specific metabolism, as evidenced by various studies in fish (Roberts et al., 2011; Stapleton et al., 2006; Yokota et al., 2021). The results suggest that BDE-183, BDE-197 and BDE-207 could be potential intermediate debromination products of BDE-209 resulting in the formation of BDE-154, and that the removal of Br atoms in the *meta* position was favoured. However, no significant relationship was found between BDE-209 and either BDEs -183, -197 or -207 (although the latter two congeners showed low detection frequencies), nor with BDE-154 or BDE-155. Interestingly, BDE-154 and BDE-155 were highly correlated (n = 28, r = 0.96) in fish, which could reflect their common origin (i.e. BDE-209 biotransformation). Specific ratios such as BDE-99/BDE-100 concentration ratios (BDE-99 being metabolised while BDE-100 is not, a low ratio is indicative of a high metabolic capacity towards BDE-99) or congener relative contributions are commonly used to reveal metabolic capacities in fish (Koenig et al., 2013; Voorspoels et al., 2003). In our samples, BDE-99/BDE-100 ratios were highly variable depending on species and were highly consistent between replicate pools within the same species. The lowest ratios were identified in *Lampanyctus crocodilus* (0.11 ± 0.03), while the highest were found in *Argyropelecus olfersii* (1.56 ± 0.56). In *Xenodermichthys copei*, BDE-99 was below the LOQ in the three replicate pools, suggesting high degradation of BDE-99 in this species. This ratio was not trophic level-dependent but rather highly species-dependent (inter- and intra-family). Indeed, within the Myctophydae family, high variations were found, with both *Myctophum punctatum* and *Notoscopelus kroeyeri* showing high values (1.36 ± 0.23 and 1.29 ± 0.18, respectively), while *Lampanyctus crocodilus* showed a mean ratio of 0.11 ± 0.03 (i.e. a higher metabolism). Concurrently, BDE-154's highest contributions were determined in *Lampanyctus crocodilus* (20 ± 6.2), indicating a higher degradation capacity in this species. Indeed, as BDE-154 could potentially originate from the debromination of higher-brominated congeners, its relative contribution would be indicative of the metabolic capacities of fish, with a high contribution being indicative of a higher degradation capacity into BDE-154.

Although OHC levels and profiles could also be influenced by the vertical migration of organisms and subsequent exposure to different water bodies and prey (Takahashi et al., 2000), this was not observed in our study, which may be due to the small differences in the DVM patterns of the studied species (Chouvelon et al., 2022).

3.3.5. *Perfluorinated substances' molecular profiles are dominated by odd chain length PFCAs*

PFOS was the only PFSA detected in 100 % of the samples, at concentrations ranging from 0.38 to 10.61 ng g^{-1} dw (mean of 3.72 ± 3.96 ng g^{-1} dw) in crustaceans and between 0.35 and 10.04 ng g^{-1} dw (mean of 3.00 ± 2.22 ng g^{-1} dw) in fish (Table S6). PFDS was detected in 27 % of the samples (crustaceans and fish) at levels ranging from 0.04 to 0.09 ng g^{-1} dw (mean of 0.06 ± 0.02 ng g^{-1} dw). Among PFCAs, the long-chain PFCAs perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA) and perfluorotetradecanoic acid (PFTeDA) were detected in 100 % of the samples, while perfluorooctanoic acid (PFOA) was detected in 45 % of the samples. Perfluoroheptanoic acid (PFHpA) was detected in only four samples (*Sergia robusta*, *Xenodermichthys copei* and *Searsia koefoedi*) at a mean concentration of 0.05 ± 0.03 ng g^{-1} dw. The mean concentrations of the most-detected PFCA ranged from 0.18 ± 0.17 ng g^{-1} dw (PFOA) to 4.40 ± 4.51 ng g^{-1} dw (PFTrDA).

In most samples, PFCAs were predominant compared to PFOS (by far the predominant PFSA) with a mean contribution of 75 ± 12 % (mean PFCAs/PFOS ratio = 5.4 considering all species), although some variations were observed within taxon and species (Fig. 1D). PFCA contributions were significantly (p = 0.002) higher in crustaceans (89 ± 6 %) than in fish (72 ± 11 %). The ∑ PFCA contribution was also higher (87 ± 2 %) in *Serrivomer beanii*, the fish species with the lowest $\delta^{15}N$ values and comparable with the ratio determined in crustaceans. PFCA concentrations ranked in the order PFTrDA > PFUnDA > PFNA = PFDA > PFTeDA > PFDoDA > PFOA, showing higher bioaccumulation with increasing carbon chain length, which is consistent with their higher BAF (Houde et al., 2006; Martin et al., 2003; Ng and Hungerbühler, 2014). In fish, all PFCA concentrations except PFOA's were: i) significantly inter-correlated and ii) correlated with PFOS concentrations but iii) not correlated to the other contaminant families. This could reflect different sources and/or bioaccumulation processes.

The occurrence of the longer chain PFCAs (> C_8) in biota is commonly explained by their release in the environment as impurities from fluorotelomer and C_9-based products (Wang et al., 2014) and their subsequent higher bioaccumulation compared to the shorter chain ones (Houde et al., 2006; Conder et al., 2008; Ng and Hungerbühler, 2014). Besides, PFCAs might also originate from the in vivo biotransformation of precursors, which would increase their biomagnification in food webs

(Gebbink et al., 2016). Various experimental studies showed that when selected fluorotelomer alcohol (FTOH) precursors were administrated to fish, PFOA, PFNA, PFDA and PFHpA were formed, although with low yields (Brandsma et al., 2011; Butt et al., 2014). However, recent results showed that PFCAs could also be formed by yet-unidentified precursor degradation (Simmonet-Laprade et al., 2019) and suggest that direct exposure to PFCAs is not a predominant source to explain PFCA profiles in fish. The predominance of the odd-numbered PFCAs PFTrDA (C_{13}) and PFUnDA (C_{11}) has been previously reported in marine fish worldwide (Fujii et al., 2015, 2019; Munschy et al., 2020b; Schultes et al., 2020 and references therein) and explained by direct inputs from industrial releases (Gewurtz et al., 2013; Simmonet-Laprade et al., 2019) and/or by the degradation of precursors (FTOHs), either in the atmosphere, soil or organisms, followed by preferential accumulation of the longer chain length PFCAs (i.e. $C_{11} > C_{10}$ and $C_{13} > C_{12}$). However, FTOH degradation leads to 10-times more abundant even chain length PFCAs than odd chain length PFCAs (Franklin, 2016; Gebbink et al., 2016). In our samples, the mean $(C_{11} + C_{13})/(C_{10} + C_{12})$ ratio was 4.2 ± 1.4, showing a higher occurrence of odd chain length PFCAs in all species (no significant difference was observed between taxa).

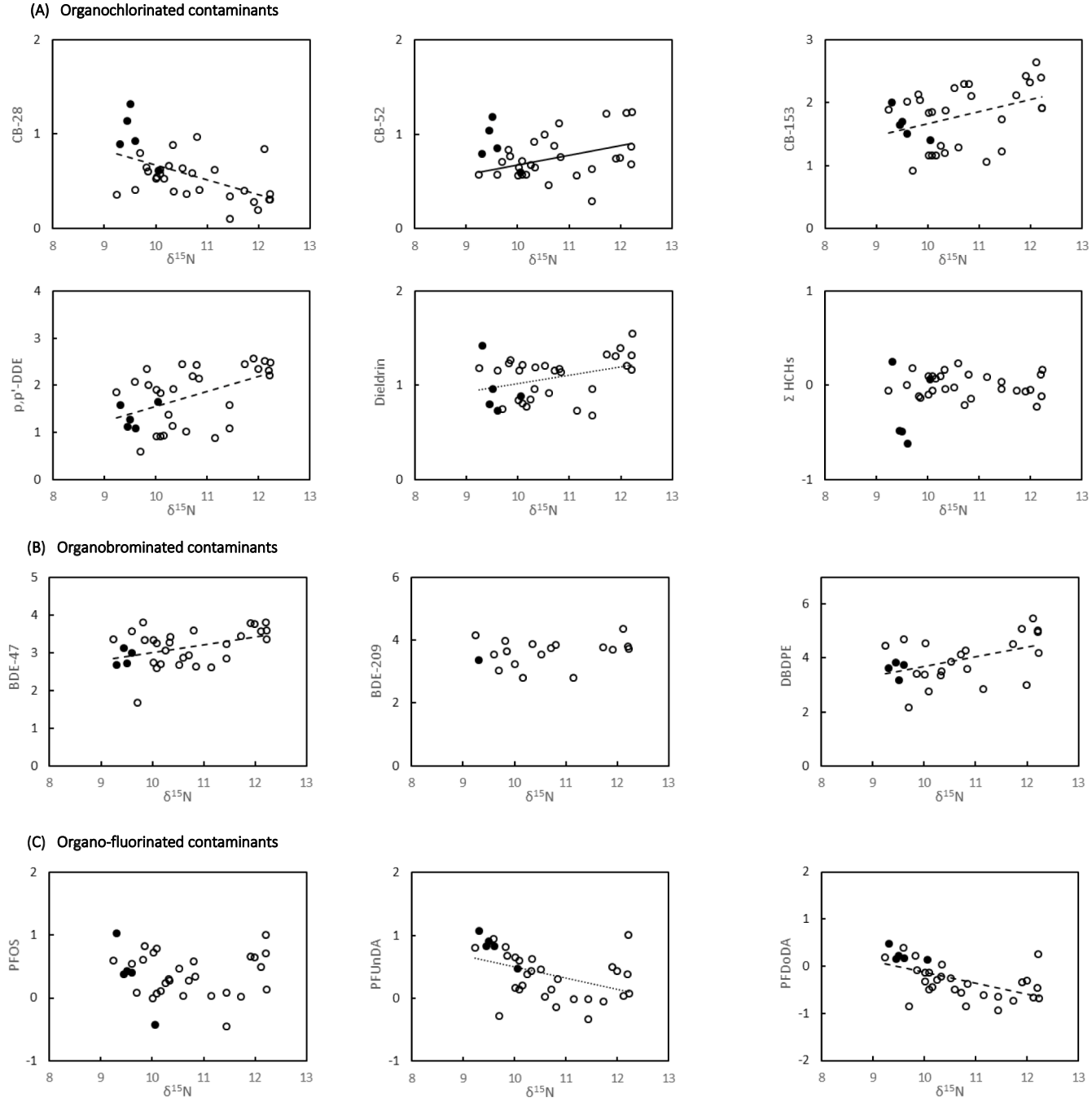

Fig. 3. Relationships between organic contaminant concentrations and $\delta^{15}N$ values (‰) in crustaceans (black dots) and fish (white dots) collected in deep pelagic waters of the Bay of Biscay in October 2017. Concentrations of organochlorinated compounds (A) and organobrominated compounds (B) were log-transformed and expressed in ng g^{-1} lw and pg g^{-1} lw respectively; PFAS concentrations (C) were log-transformed and expressed in ng g^{-1} dw. Significant relationships are indicated by dashed lines when significant for crustaceans and fish together (and for fish alone as well), by continuous lines when significant for fish only, by dotted lines when significant for crustaceans and fish only (i.e. not for fish alone). General linear model statistical parameters are given in Tables S7–S10.

Interestingly, the (C_{11} + C_{13}) - (C_{10} + C_{12}) values showed strong differences between i) the crustaceans and the fish species *Serrivomer beanii* and *Searsia koefoedi* that show high values (14,590 ± 3728 and 14,249 ± 4632, respectively) and ii) the other fish species that presented lower values (3243 ± 2360). This parameter has been used to reveal PFAS sources in soils, allowing them to distinguish between direct release and fluorotelomer degradation (Washington et al., 2020). In biota, this parameter could be influenced by in vivo degradation of precursors into PFCAs, which are not expected to further degrade in organisms. This ratio could therefore give an integrated view of exposure and subsequent bioaccumulation, combined with the effect of precursor biotransformation. The differences observed in our samples would agree with species-specific precursor biotransformation as already reported for PFOS in freshwater invertebrates and fish (Babut et al., 2017).

3.3.6. Most of the chlorinated and brominated OHCs show biomagnification across the studied food chain, while PFASs exhibit a contrasted behaviour

Despite legacy POP concentrations in deep-sea organisms (mostly demersal or benthic) having been reported in the literature, very few studies have examined their biomagnification in deep-sea food webs (but see Cresson et al., 2016) and especially in the deep pelagic. In the present study, significant linear relationships were found between PCB log-transformed concentrations in lw and $\delta^{15}N$ values (Table S7). However, CBs -18, -28 and -31 showed significant negative linear relationships with $\delta^{15}N$ values, indicating their biodilution in the food web, while the higher chlorinated congeners (5 chlorine atoms and above) showed significant positive linear relationships (Fig. 3A, Table S7). Among DDT isomers, *p,p'*-DDE, *p,p'*-DDD and *p,p'*-DDT, but neither *o,p'*-DDT or *o,p'*-DDD, concentrations showed significant positive linear relationships with $\delta^{15}N$ values, as well as dieldrin, endrin, HCB and mirex (Fig. 3A, Table S8). On the opposite hand, neither $\sum$ HCHs nor individual isomers showed any significant relationship. Individual PBDE congeners, except BDEs -28, -66, -77, -183 and -209, showed significant positive linear relationships with $\delta^{15}N$ values (Fig. 3B, Table S9). Among the alternative brominated flame retardants, BB-153 showed significant positive linear relationships ($p = 0.005$), while HBB did not. These results agree with the biomagnification of lipophilic compounds in marine pelagic food webs reported previously, including in deep-sea ecosystems (Romero-Romero et al., 2017; Takahashi et al., 2010). Indeed, the congeners showing no significant relationship with $\delta^{15}N$ values are those with the lowest reported trophic magnification factors (TMFs) (Walters et al., 2016). The lack of biomagnification of the lower-chlorinated PCB congeners such as CBs -18, -28, -31, HCHs and HBB might be related to their moderate hydrophobicity and potential elimination via respiration (Kelly et al., 2007; Takahashi et al., 2010). Besides, low TMFs might also result from the compound hydrophobicity and size, which limit their bioaccumulation (e.g. BDE-209). Peculiar results were obtained for DBDPE that exhibited apparent biomagnification (Fig. 3B, Table S9) (but see Section 3.3.3). The limited extent of the trophic level covered by the food web studied here (i.e. one theoretical trophic level if referring to the average difference of 3.4 ‰ reported between two trophic levels, Post, 2002) might also have restrained the possibility of detecting high contaminant biomagnification for lipophilic compounds (Brandsma et al., 2015; Won et al., 2018).

Among PFASs, individual PFCAs showed a significant decrease with increasing $\delta^{15}N$ values when both crustaceans and fish were considered, while PFOS showed no significant trend. However, no significant trend was detected in fish only, whether individual PFCAs or PFOS were considered (Fig. 3C, Table S10), which shows that the significant relationship was due to the influence of the high concentrations observed in crustaceans. This lack of observed biomagnification for both PFOS and PFCAs in fish contrasts with some previously reported results. Indeed, various PFASs, including PFOS and long-chain PFCAs have been reported to biomagnify in marine trophic webs (Loi et al., 2011; Munoz et al., 2017; Pan et al., 2021; Tomy et al., 2004), but some opposing results lacking evidence of long-chain PFCA biomagnification have also been reported when only piscivore food webs were considered (Du et al., 2021; Kelly et al., 2009; Mazzoni et al., 2020; Miranda et al., 2021; Pan et al., 2021). This has partially been explained by PFCA's high aqueous solubility (due to the carboxylate functional group) and preferential distribution in blood, leading to their efficient respiratory elimination via blood-water exchange in the gills (Kelly et al., 2009). Our results show the complexity of interpretation of PFAS bioaccumulation along marine trophic webs in a given ecosystem, as the observed results could be reflecting different accumulation and/or depuration processes depending on species and potential metabolic capacities towards precursors.

4. Conclusions and implications for higher trophic level consumers

The results obtained in the present study bring evidence of the contamination of deep-sea pelagic organisms from the Bay of Biscay by both legacy POPs and substances of emerging concern, showing that major organohalogen contaminant families (whether chlorinated, brominated or fluorinated) reach meso- and bathypelagic ecosystems. Despite being regulated for decades, PCBs were the major organic contaminant family in fish followed by OCPs, making chlorinated organic contaminants the major contributors to the targeted halogenated ones. On the opposite hand, PBDEs contributed the least to the contaminant load in both taxa. PFASs ranked third in fish while in crustaceans, PFAS and chlorinated contaminant contributions were similar. The significant contribution of PFASs (and among them the long-chain ones) to the load of organic contaminants in the studied deep-sea species, in addition to the lack of data on a large number of emerging contaminants, emphasises the importance of considering this family of compounds together with the legacy POPs in future studies.

Altogether, contaminant profiles and specific ratios suggest that the studied species exhibited metabolic capacities, especially towards PBDEs, and that the metabolic activity was highly species-dependent. Selective bioaccumulation of the investigated OHC families was evidenced and shown to be related to taxa and species, trophic parameters and potential metabolic capacities. While most chlorinated and brominated contaminants showed biomagnification along the studied trophic assemblage, most PFASs showed biodilution.

This high inter-species variability observed in terms of OHC concentrations induce important consequences in terms of matter fluxes in oceanic ecosystems. Variability in species abundance will have a direct impact on the total amount of the different OHCs that will be spatially transferred in the environment during vertical migration, as well as transfer to higher trophic level through food webs.

In conclusion, our results provide essential data for understanding and predicting some impacts of anthropogenic activities on deep pelagic ecosystems, filling a gap regarding the need to increase knowledge on the fate of human-induced organic contamination in the deep ocean. These original results may also allow a better assessment of contaminant vertical transport and transfer to higher trophic levels. In the actual context of climate change and global increase of human pressures, which might affect contaminant cycle dynamics and increase chemical pressures, it appears crucial to better monitor, characterise and understand chemicals' behaviour in offshore marine environments including in the deep sea. To these ends, more efforts are still needed to further assess the impact of the anthropogenic chemical contamination on deep-sea species and, ultimately, on ecosystem functioning.

CRediT authorship contribution statement

Catherine Munschy: Conceptualisation, Investigation, Formal analysis, Writing - original draft, review & editing. Jérôme Spitz: Conceptualisation, Funding acquisition, Sample acquisition, Writing -

review & editing. Nadège Bely: Data acquisition. Karine Héas-Moisan: Data acquisition. Nathalie Olivier: Data acquisition. Charles Pollono: Data acquisition, Validation. Tiphaine Chouvelon: Conceptualisation, Funding acquisition, Sample acquisition, Writing - review & editing.

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Declaration of competing interest

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

Data availability

The data have been deposited on SEANOE https://doi.org/10.17882/90452

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Appendix A. Supplementary data

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