LEGACY AND EMERGING ORGANOHALOGEN COMPOUNDS IN DEEP-SEA PELAGIC ORGANISMS FROM THE BAY OF BISCAY (NORTHEAST ATLANTIC)

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Introduction

Although deep-sea pelagic ecosystems are remote from direct anthropogenic sources of pollutants, various studies produced evidence that hydrophobic contaminants are transported to these environments and bioaccumulate at high levels in deep-sea organisms[1-3]. Because of their high hydrophobicity, long half-lives and long-distance transport, Persistent Organic Pollutants (POPs) are particularly subject to bioaccumulation in deep-sea fauna. In addition, being long-lived and feeding on higher trophic levels, deep-sea fish are more prone to high exposure to POPs[4]. In addition to this, as a consequence of their strong diel vertical migrations, meso- and bathypelagic organisms play an important role in transferring POPs between surface and deep-sea food webs[5]. In this context, this study aimed to investigate the accumulation of selected legacy (polychlorinated biphenyls - PCBs, organochlorine pesticides –OCPs, perfluorooctane sulfonate -PFOS) and emerging (perfluoroalkyl substances -PFASs) POPs in meso- and bathypelagic crustacean and fish species, together with stable isotope ratios of $\delta^{15}N$ and $\delta^{13}C$ as trophic tracers.

Materials and methods

Sampling

Samples were collected on the French slope of the Bay of Biscay (NE Atlantic) during the annual EVHOE survey on board Ifremer R/V Thalassa in October 2017. Fish (11 species) and crustacean (3 species) samples were collected at night using a 25 m vertical opening pelagic trawl in the deep scattering layer (ca 800 m depth ; 1334 m bottom floor). All species were collected during one haul of 60 min at a speed of approximately 4 knots. On-board handling of samples was conducted using rigorous protocols in order to avoid external contamination. Samples were stored in pre-cleaned aluminium foils at -20 °C until further processing in the laboratory.

In order to obtain sufficient material for the quantification of POPs, samples (whole body) belonging to the same species were pooled by individuals of similar sizes (Table 1). Whenever possible, individuals' sex was determined and noted in the composition of each pool. When large enough, fish were also analysed individually. A small piece of white muscle (typically < 3% of individual total weight) was also collected for the analysis of stable isotopes of carbon and nitrogen as trophic tracers. After pooling, the samples were homogenized using a blender, freeze-dried and finely ground up with a ball mill MM400 (Retsch).

Chemical analysis

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 pressurized liquid extraction (PLE). The extracts were evaporated to dryness and TLC was expressed in % of dry weight (dw).

PCBs and OCPs were determined as described by Munschy et al.[6] 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 (Hewlett-Packard 6890) coupled to high resolution mass spectrometry (AutoSpec Ultima, Waters Corp.). 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) and various OCPs (*p,p'*-DDT, *o,p'*-DDT, *o,p'*-DDD, *p,p'*-DDD, *p,p'*-DDE, dieldrin, aldrin, hexachlorobenzene -HCB and hexachlorocyclohexanes –HCHs, referred to as ∑OCPs later in the text).

PFAS were determined according to Munschy et al.[7]. 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 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 ionization 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.

Quality Assurance / Quality Control procedures were carefully followed during the entire analytical protocol. This included quantification by isotopic dilution using ^{13}C-labeled compounds, six-point calibration curves in each sequence of samples in order 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) intercomparison exercises with satisfactory Z scores.

Table 1. List of samples analysed for POPs by taxon and species. The number of individuals per sample and the number of replicates (n) are indicated. Average lengths are in mm for crustaceans (cephalothorax length) and in cm for fish (total length).

Taxon	Family	Species	Species code	Individuals per sample (replicates)	Average length
Crustaceans	Sergestidae	*Sergia robusta*	SERG ROB	12 (n = 1)	21.0
	Pasiphaeidae	*Pasiphaea sivado*	PASI SIV	31-48 (n = 3)	20.3-22.3
	Oplophoridae	*Ephyrina figueirai*	EPHY FIG	7 (n = 1)	16.7
Fish	Serrivomeridae	*Serrivomer beanii*	SERR BEA	1-4 (n = 4)	52.3-72.0
	Alepocephalidae	*Xenodermichthys copei*	XENO COP	5 (n = 3)	14.1-16.9
	Paralepididae	*Arctozenus risso*	ARCT RIS	5 (n = 2)	18.3-21.0
	Sternoptychidae	*Argyropelecus olfersii*	ARGY OLF	3-6 (n = 2)	7.5-10.0
	Myctophidae	*Myctophum punctatum*	MYCT PUN	5-8 (n = 3)	6.5-8.3
		Lampanyctus crocodilus	LAMP CRO	5 (n = 3)	12.3-13.3
		Notoscopelus kroeyeri	NOTO KRO	3-9 (n = 3)	7.9-12.3
	Stomiidae	*Chauliodus sloani*	CHAUL SLO	3 (n = 1)	26.0
		Stomias boa	STOM BOA	1-3 (n = 3)	27.7-35.0
	Trichiuridae	*Aphanopus carbo*	APHA CAR	1 (n = 3)	61.0-62.0
	Platytroctidae	*Searsia koefoedi*	SEAR KOE	3 (n = 1)	15.0

Results and discussion

Total lipid contents (TLCs)

TLCs exhibited high variations between species, ranging from 4.3% ± 0.9% dw (n=3) in *Pasiphaea sivado* to 51% dw in *Ephyrina figueirai* (n = 1). In fish, TLCs varied between 6.1% ± 0.1% dw (n=3) in *Xenodermichthys copei* and 41.9% ± 9.6% dw (n = 3) in *Notoscopelus kroeyeri*. TLCs did not exhibit statistically significant differences between crustaceans and fish. Replicate pool samples of the same species showed average variations of 28% (rsd), on average.

Comparison of the studied POP levels

Among the organohalogen compounds analysed, PCBs presented the highest concentrations in fish (globally), followed by OCPs and PFASs (significant differences, $p < 0.05$), whilst in crustaceans, no significant difference was observed between the three POP families (Fig.1). These results suggest selective bioaccumulation of the investigated POP families depending on taxa and species. PFASs exhibited higher accumulation than PCBs and OCPs in most of the studied crustacean species and also in the lower trophic level fish species (i.e., *Serrivomer beanii and Xenodermichthys copei),* hence suggesting a relationship with the trophic magnification factors (TMFs) of the study contaminants, which are globally lower for PFASs than for PCBs[9].

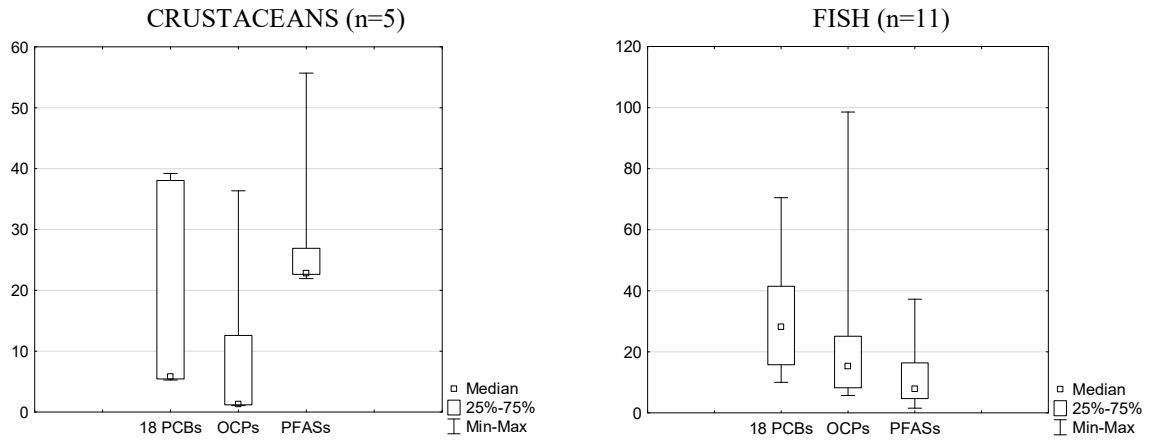

Figure 1. Concentrations (ng g^{-1} dw) of $\sum PCBs_{18}$, $\sum OCPs$ and $\sum PFASs$ in crustaceans and fish collected in deep waters of the Bay of Biscay in October 2017.

Levels and profiles of organochlorinated compounds

The 6 i-PCBs were detected in 100% of the samples at levels ranging from 0.076 ng g^{-1} dw (CB-28) to 23.27 ng g^{-1} dw (CB-153). Major dl-PCBs were detected in 58% (CB-169) to 100% (CB-105, -118, -123, -156, -157, -167) of the samples, at concentrations ranging between 0.0013 ng g^{-1} dw (CB-169) and 7.205 ng g^{-1} dw (CB-118). CB-81 was detected in 2 samples only, while CB-114 was < LOQ (limit of quantification) in all samples. i-PCB and dl-PCB concentrations were highly correlated ($r = 0.93$, $p < 0.05$), with an average ratio (i-PCBs / dl-PCBs) of 7 ± 1. PCBs did not correlate significantly with TLCs. PCB contamination profiles were dominated by hexa- and hepta-chlorinated congeners, which is consistent with previous findings in deep-sea ecosystems[10].

PCB concentrations were compared to OSPAR (Oslo/Paris Convention for the Protection of the Marine Environment of the North-East Atlantic) Background Assessment Concentrations (BACs), which are used to identify areas of potential environmental concern, i.e., those where mean concentrations are above the BACs. All samples showed higher concentrations (by a factor of 10-20) than the corresponding BACs for CB-138, -153, and -180, while CB-28 exhibited higher concentrations than the BAC (by a factor of 3) in 45% of the samples. Environmental Assessment Criteria (EACs), expressed in lipid weight, were exceeded for CB-118 mainly in Stomiidae and Trichiuridae fish.

Among OCPs, *p,p'*-DDE, dieldrin, endrin and β-HCH were quantified in 100% of samples. Other DDT isomers were quantified in 88% (*p,p'*-DDT) to 94% (*o,p'*-DDT and *o,p'*-DDT) of the samples. The highest mean concentrations were recorded for DDTs (0.53-86.23 ng g^{-1} dw range), followed by dieldrin (0.25-6.83 ng g^{-1} dw range) > HCB (0.32-3.93 ng g^{-1} dw range) > endrin (0.03-0.73 ng g^{-1} dw range) = HCHs (0.01-0.61 ng g^{-1} dw range). HCHs, HCB, dieldrin and endrin exhibited significant linear positive correlations with TLCs. DDT profiles showed the predominance of *p,p'*-DDE in all species (Fig. 2), revealing an old input of DDT (average *p,p'*-DDT / *p,p'*-DDE ratio of 0.12 ± 0.05).

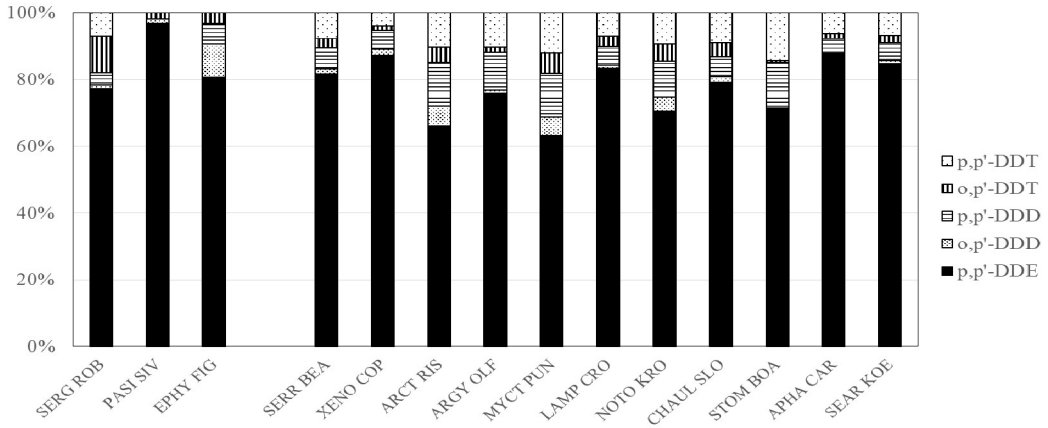

Figure 2. Relative concentration profiles (mean contribution in %) of DDT isomers in crustaceans and fish collected in deep waters of the Bay of Biscay in October 2017.

Levels and profiles of perfluorinated substances

PFOS was the only PFSA detected in 100% of the samples, at concentrations of 0.35-10.61 ng g^{-1} dw. Long-chain PFCAs were detected in 45% (perfluorooctanoic acid -PFOA) to 100% (perfluorononanoic acid -PFNA, perfluorodecanoic acid -PFDA, perfluoroundecanoic acid -PFUnDA, perfluorododecanoic acid -PFDoDA, perfluorotridecanoic acid -PFTrDA, perfluorotetradecanoic acid -PFTeDA) of the samples, at mean concentrations ranging from 0.18 ng g^{-1} dw (PFOA) to 4.40 ng g^{-1} dw (PFTrDA). PFCA concentrations ranked in the order PFTrDA > PFUnDA > PFNA = PFDA > PFTeDA > PFNA > PFOA, showing higher bioaccumulation with increasing carbon chain length. In most samples, PFCAs were predominant compared to PFSAs (average PFCAs / PFSAs ratio = 5), although some variations were observed within species, in particular for *Ephyrina figueirai* and *Chauliodus sloani* (Fig. 3), which showed much higher and lower PFCA contribution than other species (PFCAs / PFSAs ratio of 50 and 0.75, respectively). This could be the result of different accumulation and/or depuration processes depending on species, possibly *via* metabolism capacities.

Relationship with carbon and nitrogen stable isotopes

As expected for lipophilic compounds, significant positive linear relationship was found between PCB concentrations and $\delta^{15}N$ (Fig. 4), indicating their biomagnification in this pelagic deep-sea food web. Conversely, PFAS concentrations showed a significant decrease with increasing $\delta^{15}N$; this lack of biomagnification has been previously observed in a piscivorous food web and related to a high respiratory elimination of PFOS and longer-chain PFCAs *via* gills[8].

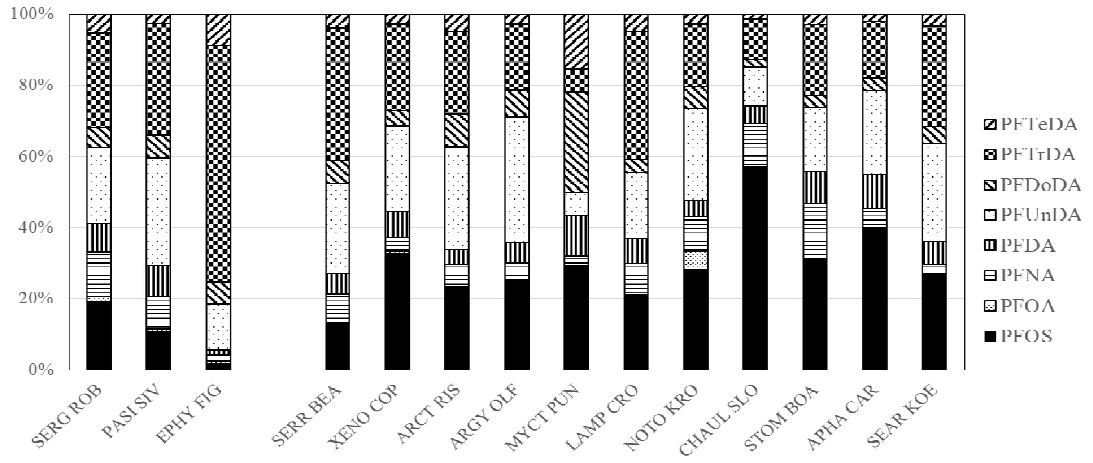

Figure 3. Relative concentration profiles (mean contribution in %) of individual PFASs in crustaceans and fish collected in deep waters of the Bay of Biscay in October 2017. Only compounds with a detection frequency above 40% are presented.

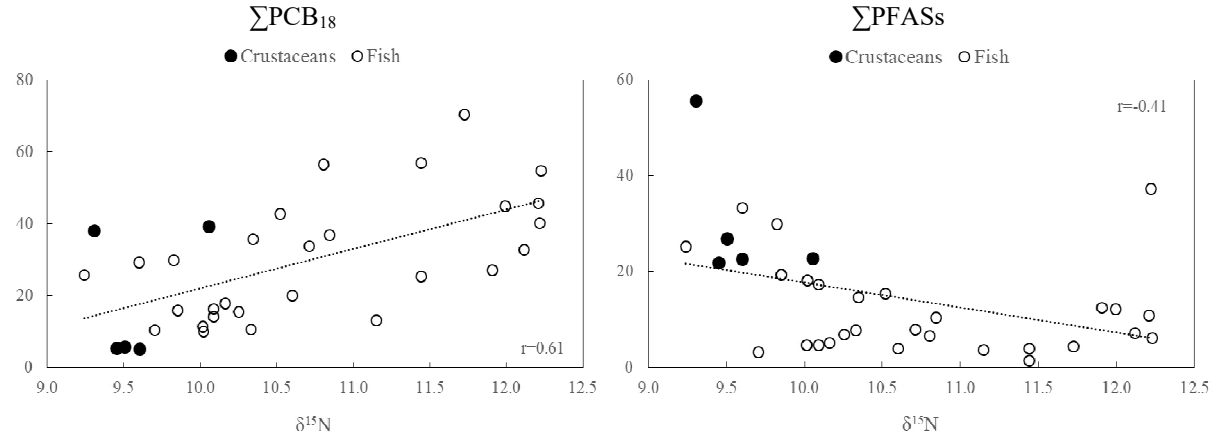

Figure 4. Relationship between $\sum PCB_{18}$ and ∑PFAS concentrations (ng g^{-1} dw) and $\delta^{15}N$ (‰) in crustaceans and fish collected in deep waters of the Bay of Biscay in October 2017.

Conclusions

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. The present results will be included in the next Good Environmental Status assessment of the European Marine Strategy Framework Directive for Descriptor 8 (**"**Concentrations of contaminants give no effects**"**) and will therefore bring key information on the extent of the chemical contamination of open and deep-sea ecosystems.

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