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Differences in biomarker responses and chemical contamination among three flatfish species in the Bay of Seine (NE Atlantic)

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Abstract :

To assess the potential of the sole as sentinel species for ecotoxicological monitoring, the present study compares contaminant levels and biological responses with two closely related flatfish species: the common dab and European flounder. Trace metals, organic contaminants and biomarkers were measured in the three flatfish species collected during the same oceanographic cruise in the Bay of Seine (France). Overall, sole showed lower concentrations of Hg, met-Hg, Cd, Zn and PBDE (lw), higher concentrations of Ag, Cu, PFOS (ww), PCBs, p,p'-DDE (lw) and OH-pyrene, a higher ability to metabolize PBDEs and higher genotoxic (Comet, Micronuclei) and neurotoxic (AChE inhibition) alterations. Sole was the species most frequently occurring in the bay and appeared sensitive to chemical contamination. We therefore recommend promoting the use of the common sole for ecotoxicological monitoring.

Highlights

► Chemical contaminants alone are not sufficient for assessing the fish health status. ► The species with the most altered biomarker responses was not the most contaminated. ► With regard to pollution, integrated monitoring is key to assess ecosystems' status. ► The common sole is a suitable sentinel species for coastal pollution monitoring.

Keywords : Flatfish, Biomarkers, Trace metals, Organic pollutants, Sentinel species, MSFD

1 Introduction

Impacts of chemical contaminants transported by rivers, atmosphere and directly to the seas and oceans, and the threat they represent to marine organisms is acknowledged as a global concern by authorities and regulators (Tang et al., 2019; Tornero and Hanke, 2016). This is exemplified in international commitments such as the Regional Sea conventions, supporting clean and healthy seas. For instance, the North-East Atlantic Environment Strategy (NEAES) adopted in 2021 by OSPAR includes a dedicated objective on Hazardous Substances for 2030: “Ensure that contaminants do not cause pollution effects, with fish and seafood safe to eat, and that Contracting Parties move towards a cessation of contaminant discharges, emissions, and losses” (OSPAR, 2021). Similarly, the European commission has implemented several actions, including the European Marine Strategy Framework Directive (MSFD) that engages member states to take actions to achieve or maintain a good environmental status (GES) of marine waters. Several descriptors are defining the GES, including one for chemical contamination (Descriptor 8), under which member states should take actions to achieve or maintain contaminants at levels not giving rise to pollution effects. These actions include the monitoring of selected chemicals and their biological effects in sentinel species to support an effective integrated assessment of chemical contamination impact.

Although the monitoring of the health of an ecosystem cannot realistically be limited to a single species (Power and McCarty, 1997), operational constraints including the large number of selected biological

and chemical parameters to be measured in the framework of an integrated approach require focusing on a set of a few pre-selected parameters and sentinel species. These species are selected among those that are widely distributed, easy to sample, and sufficiently sensitive to chemical contamination to provide early warning of contaminant impacts. Flatfish are of particular interest as sentinel species: they live in close contact with and feed near the sediment, where several inorganic and hydrophobic contaminants accumulate, making them likely to be exposed to these contaminants by different exposure routes (dermal, respiratory, trophic). In the North-East Atlantic, the two flatfish species most commonly used for monitoring marine contamination and its effects are the European flounder, *Platichthys flesus*, and the dab, *Limanda limanda*, both from the Pleuronectidae family (Baršienė et al., 2013; Burgeot et al., 2017; Davies and Vethaak, 2012; Hylland et al., 2012; Lang and Wosniok, 2008; Laroche et al., 2013). The common sole, *Solea solea* from the Soleidae family, is phylogenetically more distant and has been less studied for biomonitoring purposes at European scale. From an ecotoxicological point of view, several studies have been conducted with sole and they demonstrated both its ability to metabolize pollutants (Constenla et al., 2022; Wessel et al., 2013), and its sensitivity to chemical pollution (Briaudeau et al., 2021, 2020). Nevertheless, its geographical distribution extends further south than those of the dab and flounder and it has been preferred for monitoring in France, Spain and Portugal and could also be used as such in North African countries (Briaudeau et al., 2019; Cuevas et al., 2015; Cuevas and Zorita, 2018; Mounier et al., 2020; Munschy et al., 2011; Wessel et al., 2010). This species was reported by Walsh et al. (2015) as the third most exploited flatfish by northeast (NE) Atlantic fisheries in 2010, and the fourth fished species in value for North sea, Channel and Atlantic coasts (SIH, 2022). In France in 2020, sole represented a total value of more than 35k€ for 1687 tons, while dab represented about 15 k€ for 1104 tons (France Agrimer, 2022). This is also one of the most widely produced flatfish species in European aquaculture, after the turbot and the Atlantic halibut; aquaculture studies also contribute to the knowledge of the physiology of the species (Cerdà and Manchado, 2013; Howell, 1997; Sardi et al., 2021). Despite the present work focused on inter-flatfish species variability in their response to the chemical contamination, sole may also represent a sanitary issue for human health.

Differences in biological effects and chemical contamination levels are to be expected among flatfish species as (1) their movement and migration, (2) their diet and trophic level and, (3) their physiology and metabolic capacity have been documented as differing. Movement and migration of fish species are largely dependent on environmental parameters (water temperature, salinity, pH, food availability, predation, among others) and vary throughout the species' life cycle and reproduction. With variations across their latitudinal range, flatfish spawn from winter to spring and pelagic larvae dwell during several weeks (ca. 1 to 2 months for *Solea solea*; (Rochette et al., 2012)). Newly metamorphosed flatfish arrive in May-June in nurseries which are mainly located in coastal and estuarine areas, even though, for flounder, nurseries in brackish or even fresh waters have also been observed, and for dab, the question remains open regarding the possible occurrence of open-sea nurseries (Bolle et al., 1994; Daverat et al., 2012; Koutsikopoulos et al., 1995; Rijnsdorp et al., 1992). Then, sexually mature sole and dab, ca. 2-years old, move offshore and reproduce, while flounder tend to remain in coastal areas (Dorel et al., 1991; Koutsikopoulos et al., 1995; Riou et al., 2001).

Flatfish are carnivorous and mostly feed on benthic invertebrates (polychaetes, bivalves, small crustaceans) living on or in the sediment, but also on echinoderms, squids or other fish species (Link et al., 2014). For instance, Piet et al. (1998) reported differences in diet composition among flatfish species from the southern North Sea: adults sole (ca. 20-30 cm) fed mainly on polychaetes (>80% of stomach content) and also on molluscs; flounder fed on both polychaetes and echinoderms and in a smaller proportion fishes (ca. 35%, 60%, 5%, respectively); dab had a broader diet composition as they mainly fed on crustaceans (55%) and in a smaller proportion, polychaetes, fishes, echinoderms and molluscs. The diet thus varies among species, life stages and even populations. Because of these differences in species distribution, diet and physiology, they are exposed to different types of

contamination (nature of contaminants, quantity and exposure patterns) and will exhibit different response capacities to stressors (e.g. Hylland et al., 2017c). The relative importance of these interspecific differences should be considered when assessing contaminant levels and biological responses between species.

Assessment of biological effects of chemical contamination is challenging, as it requires monitoring and integrating different exposure scenarios using different selected sentinel species to represent various modes of exposure to contaminants. Following the development of a biological/chemical integrated assessment method (Vethaak et al., 2017), and within the MSFD context, the field monitoring program SELI (https://doi.org/10.18142/285) has been implemented in France since 2017. It carries out the monitoring of chemical pollution and its potential biological effects in different areas of the French coast under high anthropogenic pressure. A survey took place in 2018 in the Bay of Seine, during which three flatfish species were sampled, namely the common sole, the common dab and the European flounder. While many studies have addressed the effects of contamination on flatfish separately, few have investigated the differences among fish species in an integrated manner, considering both contaminant occurrence and biological effects simultaneously (e.g. Burgeot et al., 2017). The present study therefore aims to compare biomarker responses and contamination levels in these three flatfish species co-occurring in the Bay of Seine, in order to assess the potential of the sole as sentinel species. Benchmarking the response of sole to contamination to the other two species is essential for the development of sole-based indicators to assess the contamination of coastal ecosystems.

2 Materials and Methods

2.1 Fish sampling in the Bay of Seine

Sampling was performed in the Bay of Seine. It is located in the eastern English Channel and is open to Atlantic waters in its western part and is influenced by the inflow of the Seine River in its eastern part (Gasperi et al., 2010; Tronczynski et al., 2004). The bay is subjected to high cumulative anthropogenic pressures that rank it among the most impacted coastal zones in Europe (Dauvin et al., 2020). Despite its chemical contamination, it provides important nurseries for flatfishes, such as the sole, and the plaice, *Pleuronectes platessa* (Le Pape et al., 2007; Rochette et al., 2012), the two most landed species in the Bay of Seine (Riou et al., 2001), and also the flounder (Daverat et al., 2012). The Seine catchment is highly anthropized, inhabited by around 20 million people, with many factories and refineries located upstream (*e.g.* Le Havre harbour and the entire Seine catchment, including the cities of Paris and Rouen) or on the northern shore (*e.g.* Antifer oil terminal) of the estuary, together with high maritime traffic pressure (Miramand et al., 2001; Munschy et al., 2011; Tappin and Millward, 2015). The Bay of Seine has thus been used for the last 3 decades as a pilot area for the study of chemical contamination and its biological effects on marine organisms, especially flatfish species (Akcha et al., 2003; Borcier et al., 2020; Burgeot et al., 2017; Laroche et al., 2013; Laurent et al., 2022).

Flounder, dab and sole were sampled by trawling in September 2018, outside the fishes' reproduction season, on the Oceanographic vessel Antea as part of a SELI campaign (https://doi.org/10.17600/18000585). Four areas around the Seine River mouth were sampled (Fig. 1): the 'Estuary' area in front of the mouth, the 'Open Bay' area further offshore, and two areas on either side of the mouth, the 'North' and 'South' areas. In order to keep fish alive, trawling time and speed were limited to 20 min and 3 knots. A total of 194 fishes were caught during the camping. Sole was the most abundant species and was present in the four studied areas (Table 1). Distributions of flounder and dab were more heterogeneous, with individuals concentrated in two areas: North and Open bay for dab, Estuary and South for the flounder. Flatfish were selected according to their total length, ranging from 22 to 34 cm, to target individuals of an expected age of 2-3-years old. They were kept alive on board in a fish pool during the sampling day, before dissection for sample preparation.

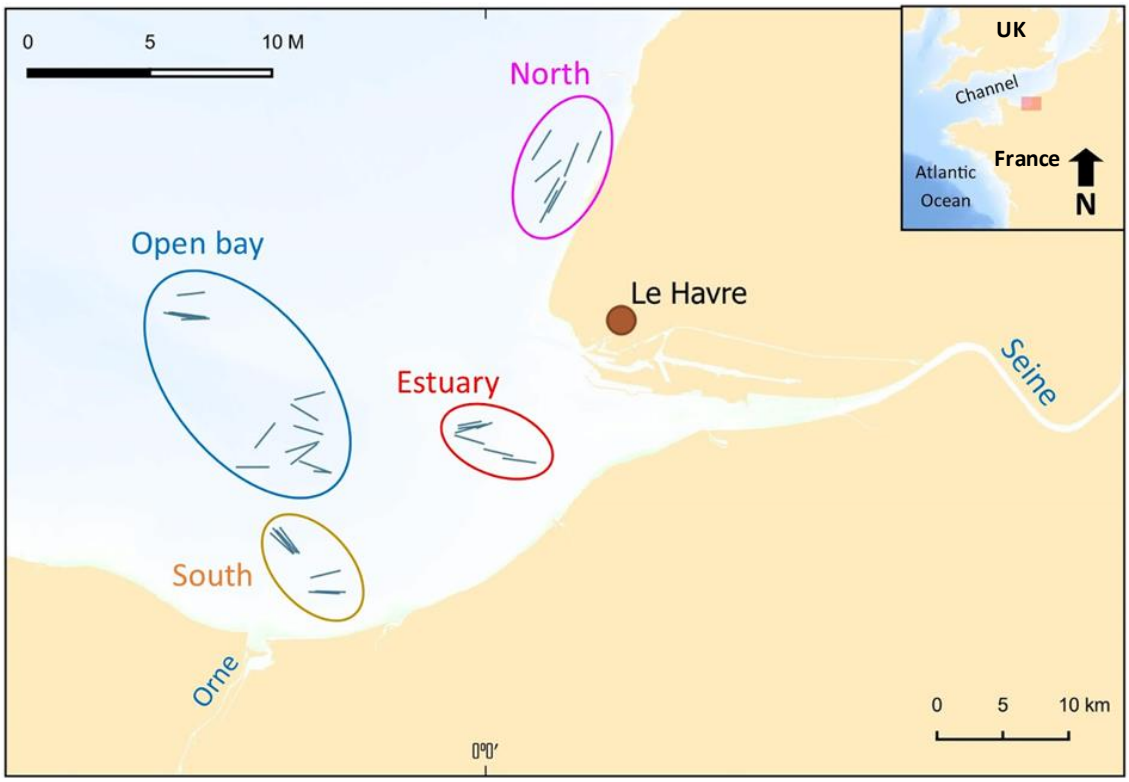

Fig. 1. Map of the Bay of Seine showing the four fishing areas (Estuary, North, South, Open Bay) where flatfishes were caught in September 2018, with indication of trawling lines (in blue).

Table 1. Number of individuals per species used for biomarker (n) and contaminant (n_c) analyses. Number of males (M) and females (F) is indicated in bracket.

Species	Sole		Dab		Flounder	
Fishing area	n(M/F)	n_c(M/F)	n(M/F)	n_c(M/F)	n(M/F)	n_c(M/F)
Estuary	18(7/11)	4(4/0)	5(4/1)	0(NA)	21(11/10)	10(5/5)
North	25(9/16)	0(NA)	15(8/7)	5(5/0)	2(0/2)	0(NA)
South	23(7/16)	5(5/0)	1(0/1)	0(NA)	20(10/10)	7(5/2)
Open Bay	30(15/15)	5(5/0)	34(8/26)	10(5/5)	0(NA)	0(NA)
Total	96(38/58)	14(14/14)	55(0.44)	15(10/5)	43(0.51)	17(10/7)

2.2 On board sample preparation

Once at the dockside, the size and weight of the individuals were determined and blood was sampled with a heparinized syringe through the caudal vein. For each blood sample, one drop was first stored in liquid nitrogen in a cryotube containing RPMI 1640 medium supplemented with fetal calf serum (25%) and dimethyl sulfoxide (20%) for DNA strand breaks analysis by the comet assay. A second drop was then air-dried on a microscope glass slide before fixation in absolute ethanol. Once dried, slides were stored at room temperature prior to microscopic analysis of micronuclei in fish erythrocytes. Then, the fishes were sacrificed and dissected. Sex was determined during the dissection by macroscopic observation of the gonads. Otoliths of each fish were sampled for further age determination by schlerochronology according to Vitale et al. (2019). For each individual, the content of the gallbladder and its content were stored in liquid nitrogen for the measurement of biliary hydroxylated metabolites of Polycyclic Aromatic Hydrocarbons (OH-PAH) concentrations. An aliquot of the liver was stored at -80°C for lysosomal membrane stability assessment. A second aliquot of the liver was stored at - 20°C in a calcinated glass vials for trace metal analysis. One part of fish muscle was

stored in liquid nitrogen for the measurement of acetylcholine esterase activity (AChE). The rest of the fish was saved in calcinated aluminium foil at -20°C. Once in the laboratory, the whole fish muscle was collected under ultra-clean conditions, freeze-dried and ground into a fine powder for measurement of concentrations of mercury and organic contaminants (polychlorobiphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), dichlorodiphenyltrichloroethane and derivates (DDT) and perfluoroalkyl substances (PFASs)).

2.3 Biomarker measurements

The biomarkers measured are among those recommended by the International Council for the Exploration of the Sea (ICES) for the integrated monitoring of contamination effects using fish at the subcellular and tissue levels (Davies and Vethaak, 2012).

2.3.1 General stress and cell integrity - Lysosomal membrane stability

Lysosomal membrane stability (LMS) was assessed in hepatocytes, after demonstration of the acid phosphatase activity, according to Broeg et al. (1999) and UNEP/RAMOGE, 1999. Frozen samples were processed using Tissue Array technology (Array Mold® Kit; n°20,015-A) and Tissue Array blocks were cut at −27 °C using a Leica CM 3050S cryotome. The time of acid labilisation treatment required to produce the maximum staining intensity was assessed under a light microscope as the maximal accumulation of reaction product associated with lysosomes (UNEP/RAMOGE, 1999) and was denoted as the Labilisation Period (LP; in min). LP value is considered as the maximum staining peak in one replicate. Four replicates of LP measurement were made per individual and used to determine the individual LP mean value (Moore et al., 2004).

2.3.2 Neurotoxicity - Acetylcholine esterase activity

Acetylcholine esterase activity (AChE) was measured in fish muscle according to Bocquené and Galgani (1998). Briefly, muscle was thawed before protein extraction by homogenization in Tris buffer (100 mM, pH 8, 0.1% triton, 4°C) using Ultra Turrax (Janke & Kunke IKA Werk®), followed by ultra-centrifugation (9,000 g, 20 min). The protein concentration was measured in the S9-fraction by spectrophotometry according to Bradford (1976). The AChE activity was determined on 100 µg of protein extracts with acetylthiocholine iodide (ACTC, 2.63 mM) as substrate, and dithiobisnitrobenzoate (DNTB, 0.53 mM) for the coloration at 412 nm. The activity is measured after 2 min of incubation, on three replicates per individual, and the final result is expressed by the mean of the three replicates. Activity was expressed in nmol min $^{-1}$ mg^{-1} proteins.

2.3.3 Genotoxicity - DNA strand breaks

DNA strand breaks (SBs) level was measured in fish erythrocytes by applying the comet assay as described by Akcha et al. (2003). Briefly, blood samples were thawed and a cellular suspension (10^6 cell/mL) was prepared for each sample in phosphate-buffered saline (PBS). For each sample, two slides were prepared with three agarose layers each. First layer: slides were immersed in a 0.5% Normal Melting Point (NMP) agarose solution in PBS. Second layer: 85 µL of 0.5% Low Melting Point (LMP) agarose containing cells (30 µL of cellular suspension were previously added to 225 µL agarose) was deposited and spread out using a micro-cover glass. The slides were immediately placed on ice in the dark for 1 min to allow the agarose to solidify. Third layer: once the cover glass was withdrawn, 90 µL of LMP agarose solution were deposited on the slide and spread out using a cover glass. The slides were then stored on ice. Following one-hour lysis step in glacial lysis buffer, and 20 min of pre-incubation in the electrophoresis buffer, DNA migration was performed in the same buffer for 20 min at 23 V. Slides were then washed with Tris buffer, dehydrated in absolute ethanol, dried and stored at room temperature until analysis. Slides were analyzed with an epifluorescence microscope coupled to a CDD camera following staining at least one hour with GelRed™. Images were analyzed with the Komet 6 image-analysis software (Kinetic imaging Ltd.), revealing the percentage of Tail DNA. Analysis was conducted on 50 nuclei per slide (100 nuclei per individual).

2.3.4 Genotoxicity - Micronuclei

Micronuclei (MN) occurrence was measured in fish erythrocytes by applying the micronucleus assay on the slides prepared onboard, as described in Couteau (2020). Blood cells were stained with DAPI, allowing a better visualization of micronuclei compared with Giemsa (Vincent-Hubert et al., 2011) and a semi-automatized process: slides were rinsed twice in PBS buffer, before DAPI coloration (1 µg DAPI/mL in methanol). Slides were then rinsed three times in PBS before a micro-cover slide was fixed on each slide with Mowiol. Slides were saved in the dark before the semi-automatic analysis on 5,000 cells per sample (individual) on a CellInsight CX5 HCS® (Thermo) platform.

2.3.5 PAH metabolization - Hydroxylated PAH metabolites

Concentrations of hydroxylated PAH metabolites (OH-PAH) were measured in the bile of fish using a method of liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS) adapted from Mazéas and Budzinski (2005) and Le Dû-Lacoste et al. (2013). First, samples were sonicated and homogenized in sodium acetate buffer, and an internal standard (1-OH-pyrene-d9 at 0.5 ng/µL) was added to the sample before enzymatic deconjugation. Metabolites were then extracted by solid phase extraction (SPE, C18 cartridge, 500 mg, 3 mL). Extracts were reduced to dryness under nitrogen stream and dissolved in 1 mL methanol/dichloromethane mixture (20/80). The purification step was conducted by a second solid-phase extraction (NH2 SPE cartridge, C18, 500 mg, 3 mL), and concentrated under gas flow at 100 µL in methanol. Finally, the extracts were stored at −20°C until quantification by LC/MSMS in negative ionization mode (Infinity 1290 LC/6460 Triple Quad LC/MS, Agilent Technologies) with an Acquity UPLC BEH column C18 (1.7 µm × 2.1 mm × 50 mm, Waters).

2.4 Contaminant concentrations

Contaminants were analyzed on a sub-sample-set of 2-year-old fish (n = 46), and preferably on males to enhance sample homogeneity (Table 1).

2.4.1 Trace metals

Total mercury (Hg) concentrations were determined in fish muscle according to the standard operating procedure described in the US-EPA method n◦7473 (US EPA, 1998), on aliquots of homogenized powder (~40±10 mg) and by atomic absorption spectrophotometry using an Advanced Mercury Analyzer (ALTEC AMA-254, Altec Ltd). In addition, methyl-mercury (met-Hg) determination was performed in the same tissue according to a method adapted from Azemard and Vassileva (2015) and described in detail in Chouvelon et al. (2018), using liquid-liquid extractions of met-Hg in samples before analysis by AMA-254.

Total silver (Ag), cadmium (Cd), lead (Pb), copper (Cu) and zinc (Zn) concentrations were determined in fish liver, according to Chouvelon et al. (2022), and using a Quadrupole Inductively Coupled Plasma Mass Spectrometer (Q-ICP-MS, ICAP-Qc model from ThermoFisher). The quality assurance of analyses was based on blank and internal standard controls and on the use of certified reference materials (CRMs) analyzed in each analytical run. Limits of detection (LOD) and quantification (LOQ) for each element as well as the CRMs used are specified in Supplemental Material (Annex 1 – Supplementary material 1). Blank values were systematically below the LODs and CRM results concurred with certified concentrations (average recovery rates: 88–109% depending on elements and CRMs).

2.4.2 Organic contaminants and total lipid content

The detailed analytical procedures for the analyses of PCBs, DDTs, PBDEs and PFOS in flatfishes can be found in Munschy et al. (2020) for sole and flounder, and Vaccher et al. (2020) for dab.

The concentrations of the 7 ICES priority PCB congeners (CB-28, -52, -101, -118, -138, -153 and -180) and 8 PBDE congeners (BDE-28, -47, -49, -99, -100, -153, -154, -183) were determined using gas chromatography coupled to high resolution mass spectrometry (GC-HRMS, Hewlett-Packard 6890 GC coupled to a Micromass AutoSpec Ultima MS) by isotopic dilution with each congener's 13C12-labelled isomer.

Among per- and polyfluoroalkyl substances, only the most abundant substance perfluorooctane sulfonate (PFOS) was investigated. Quantification was achieved by isotopic dilution against 13C8-PFOS 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 a Z-spray (Waters Corp.) electrospray ionization source operated in negative mode.

The analyses were conducted in controlled atmosphere laboratories under low dust and positive pressure and Quality assurance and Quality Control (QA/QC) criteria were strictly followed (Munschy et al., 2020). These include procedural blanks, determination of LOQs per sample, determination of labelled compound recoveries, analyses of in-house quality control samples and regular participation in interlaboratory comparison tests.

The extractable organic matter, used as a proxy of the total lipid content to normalize lipophilic contaminant concentrations, was determined gravimetrically in muscle using 0.4-0.8 g dw of the freeze-dried samples extracted with a mixture of hexane/acetone (80/20 v/v) at 100 °C under 100 bars using pressurized liquid extraction (PLE) with a Dionex accelerated solvent extractor (ASE 200, Dionex Corp., USA). The extracts were further evaporated to dryness at 105 °C for 12 h. Total lipid content was used to normalize the concentrations of the lipophilic contaminants (namely PCBs, PBDEs*, p,p'*-DDE).

2.5 Data analysis

2.5.1 Ratios of congeners

Contaminant profiles and various ratios between specific PCB or PBDE congener concentrations are commonly used as tracers of contaminant sources and/or fish diet, physiology and metabolism. Ratios that compare a metabolized congener to a recalcitrant one, or one congener to one of its metabolites, were used to assess one organism's metabolic capacity. For example, BDE-99:BDE-100 concentration ratio decreases when metabolic capacity increases. Voorspoels et al. (2003) reported that in the Western Scheldt Estuary, BDE-99:BDE-100 ratios for organisms such as mussels and shrimps tend to be more BDE-99 dominant (80:20), while those for higher organisms are more BDE-100 dominant (e.g. 20:80 for sole). A higher proportion of BDE-154 among the main PBDEs has been used as indicators of a higher metabolization capacity of PBDEs in various fish species (Voorspoels et al., 2003), given that BDE-154 could originate from higher-brominated congeners, *e.g.* BDE-209 or BDE-183 metabolic degradation (Stapleton et al., 2004).

2.5.2 Statistical analysis

Data analysis was performed using R software (version 4.2.2, R Core Team, 2022).

To improve the representation of biomarker results, 95% confidence intervals for the means of each fishing area or each species were added to the graphs. The confidence intervals were based on log-normal (OH-pyrene and AChE), normal (SB), Poisson (MN) or exponential (LMS) distributions.

Inter-area and interspecific responses differed among biomarkers, hence comparisons were made parameter by parameter in the present work. The interspecific comparisons support our main objective and were therefore more detailed in the present study (three p value levels presented (versus one for spatial effect), test performed with censored data (while no test was performed for spatial effect)). Inter-area comparisons for one species were assessed to determine whether spatial variability was a confounding factor for interspecific comparisons.

Statistical treatments were performed when enough data were available. Area or species were compared among groups of at least eight individuals for biomarkers and three for contaminant levels (α=5%). Therefore, for biomarkers, dabs from the Estuary (n=5) and the South area (n=1), and flounders from the North area (n=2) were not used in the inter-area comparison per species.

Statistical treatments were adapted according to the presence of censored values (i.e. <LOD for metals and <LOQ for organic contaminants). 1) When there is no censored data (the case of metals,

biomarkers, several organic contaminants, sum and ratios), non-parametric Kruskall-Wallis test was applied either for inter-area and interspecific comparisons per species. Pairwise comparisons of parameters between species were performed with the Wilcoxon test with Bonferroni correction. 2) When there were censored data (the case of some organic contaminants), spatial analysis were not performed. For interspecific comparisons, when there were less than 6 censored data (out of 46 samples for organic contaminants), they were substituted to the LOQ and the procedure used for non censored dataset was applied. When there were more than 6 censored data, the Fisher's exact test on the median was applied.

3 Results

3.1 Biomarkers

General health status. Among the five biomarkers measured on fish, lysosomal membrane stability (LMS) appeared similar among fishing areas for each species separately or among species at the scale of the Bay of Seine (Fig. 2).

Neurotoxicity. In sole, Acetylcholine esterase (AChE) activity was lower in individuals from the Estuary than in the 3 other stations. In dab, a lower AChE activity was measured in individuals from the Estuary and the Open bay than in individuals from the North. In flounder, AChE activity was similar among the 2 stations where flounders were sampled. At the scale of the Bay of Seine, flounder showed a threefold higher activity than the other two species (median: 227, 79 and 67 nmol/min/mg of proteins for flounder, dab and sole, respectively, Table 2).

Genotoxicity. In sole, the highest levels of DNA strand breaks (SBs) were observed in individuals from the Estuary and the lowest in those from the North (Fig. 2, Table 2). Levels of SBs were similar among area for flounder and dab. Levels of SBs were significantly higher in sole than in flounder and dab (median: 25, 16 and 16% DNA tail for sole, flounder and dab, respectively, Table 2). Micronuclei (MN) had low frequencies in all three species. However, they were more frequent in sole (Fig. 2) and scarcer in flounder (median: 0.15 and 0.03‰, for sole and flounder, Table 2).

Exposure to PAHs. Among the different hydroxylated PAH metabolites, the pyrene metabolite (OH-pyrene) was the most quantified metabolite in the bile for the three species (177 samples quantified out of the 178 analyzed). Hydroxy-phenanthrene and hydroxy-benzo(a)pyrene were quantified in less than 70% of the samples (quantified in 85, 121, 16 and 1 out of the 178 samples for 2+3-OH-phenanthrene, 1+9-OH-phenanthrene, 4-OH-phananthrene and 3-OH-B(a)P, respectively). The results for these less quantified PAH metabolites are not further discussed in this paper. OH-pyrene concentrations were higher in sole and flounder from the Estuary than in those from the South, and were higher in dabs from the North than in those from the Open bay (Fig. 2, Table 2). At the scale of the Bay of Seine, OH-pyrene concentrations were the highest in flounder followed by sole and then dab.

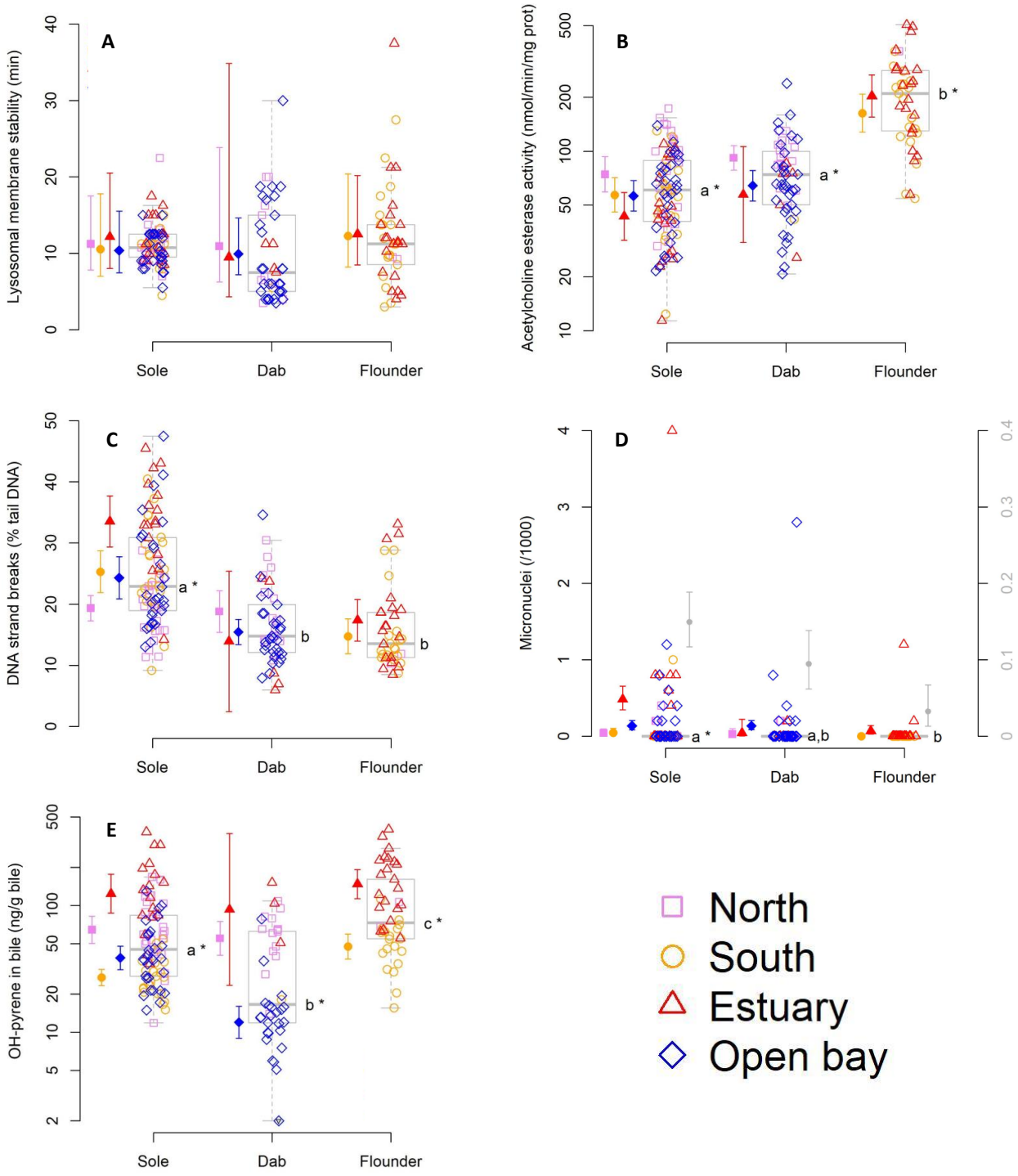

Fig. 2. Biomarker levels in three flatfish species: lysosomal membrane stability in the liver (A), acetylcholine esterase activity in the muscle (B), DNA strand breaks levels in the erythrocytes (C), micronuclei frequency in the erythrocytes (D) concentration of pyrene metabolite in the bile (E). Empty symbols are individual data, full symbols are the means of each species and fishing area. Bars indicate 95 % confidence intervals around the mean for each fishing area and, for micronuclei mean, for all fishing areas in grey (right axis). Letters (a, b, c) indicate significant differences among species. Stars (*) indicate, for a species, significant differences among fishing areas.

3.2 Trace metals

Trace metals were quantified in all samples, except Pb which was quantified in nearly half of the samples (49% of the samples) and especially in dab (1/14 dab samples), and Ag which was quantified

in 5/15 dab samples. However, trace metals were detected in all the samples and estimated values between LOD and LOQ were used in the statistical analysis.

Differences in trace metal concentrations among fishing areas were observed for Zn and Ag in flounder, in which higher concentrations were measured in the South than in the Estuary area. In other words, Hg, Pb, Cu concentrations in the three species, and Zn and Ag in sole and dab were similar among sampling stations. Although no significant difference was observed for Hg, an effect of the fishing area was observed for the methylated fraction (met-Hg) concentrations in sole: met-Hg concentrations measured in the Open bay were higher than in the South area. Concentrations of met-Hg were not measured in the other fishing areas for sole.

At the Bay of Seine scale, Hg and Cd concentrations were lowest in sole and highest in dab (Fig. 3, Table 2). The methylated form of Hg represented about two thirds of total muscle Hg and its proportion did not vary among species (Table 2). Liver Pb concentrations were similar among the three species, while liver Ag concentrations were significantly higher in sole than in dab or flounder (Fig. 3). Regarding liver Cu and Zn concentrations, the least contaminated species were dab and sole, respectively.

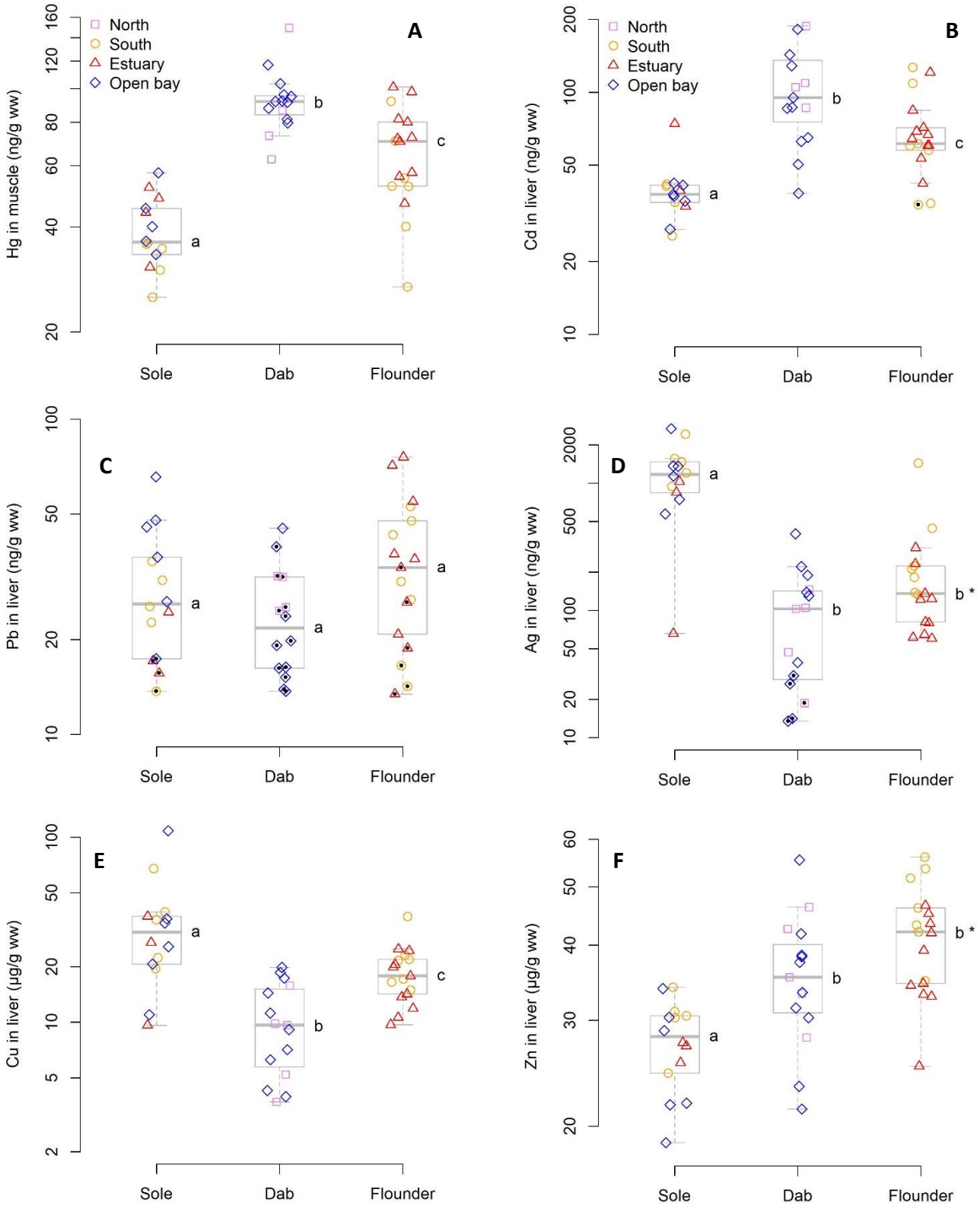

Fig. 3. Trace metal concentrations in fish muscle (Hg) and liver (other trace metals): mercury (A), cadmium (B), lead (C), silver (D), copper (E) and zinc (F). A dot inside a symbol indicates a concentration estimated below LOQ (all values were >LOD). Letters (a, b, c) indicate significant differences among species. Stars (*) indicate significant differences among fishing areas for a species.

3.3 Organic contaminants

All PCB congeners, most BDE congeners (BDE-28, -47, -49, -100 and -154) and PFOS were quantified in 100% of the analyzed samples (Table 2, Table S1). PBDE detection frequencies were lower in sole than in flounder and dab (in sole: BDE-99, -153, -183 were quantified in 11, 8 and 8 samples out of 14 (Table S1).

For organic contaminants, the only significant differences in concentrations were observed for PFOS in sole, and for ∑ PCB (lw) in flounder, with lower concentrations in the Open bay (sole) or the South area (flounder) compared to the Estuary. The BDE-99:BDE-100 ratio was significantly higher in flounders from the estuary than in those from the South area.

When the PCB and PBDE concentrations (sum of congeners) were expressed on a wet weight basis, sole was the least contaminated species (Fig. 4 A, B). However, in relation with the significantly higher lipid content in dab (1.5, 0.7 and 0.4% ww for dab, flounder and sole respectively, Table 2), concentrations of these lipophilic compounds expressed per lipid weight (lw) were the lowest in dab. The contamination (in lw) ranking order was similar for PCBs and PBDEs, *i.e.,* flounder > sole > dab (Fig. 4 A, B). Among PCBs, the dominant compound was CB-153 in the three studied species. Among PBDEs, the BDE-47 was dominant when all species were considered together. However, differences were observed between species for PBDEs (Fig. 4). BDE-47 represented 50 and 54% of the sum of the 6 PBDE congeners in flounder and dab while it was in similar proportion as BDE-154 in sole (32 and 31% for BDE-47 and BDE-154, respectively). Concentration ratios of PBDE congeners were different among fish species (Fig. 4 C,D). The BDE-99:BDE-100 ratio was higher in flounder than in sole and dabs (Fig. 4, Table 2). The proportion of BDE-154 was significantly higher in sole than in the two other species.

Concerning the two other families of organic contaminants, concentrations of *p,p'*-DDE (in lw) and PFOS (in ww) were the lowest in dab and did not differ significantly between sole and flounder (Fig. 4 E,F).

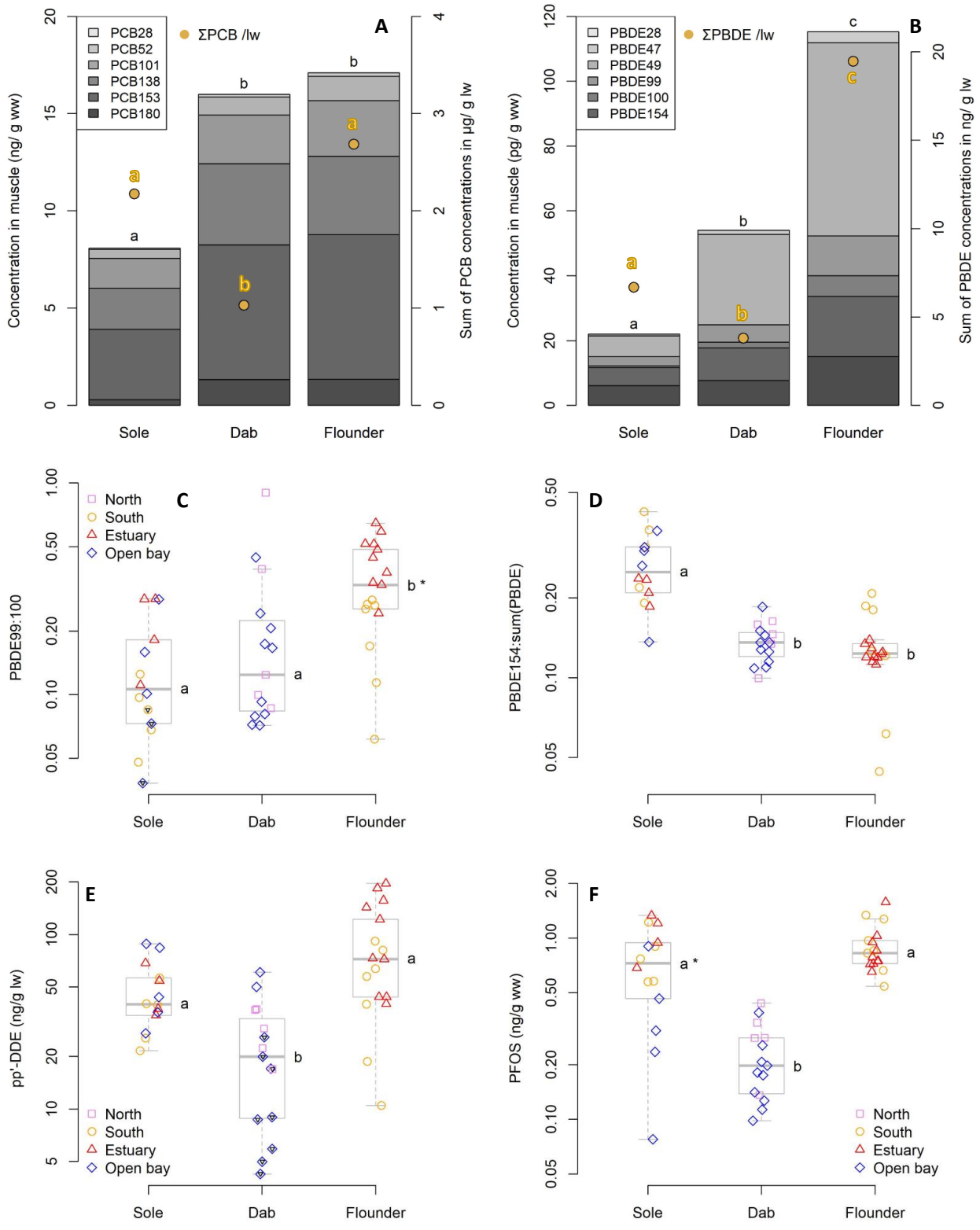

Fig. 4. PCB and PBDE congeners' median concentrations (ng/g ww, histograms) in fish muscle; ∑ PCB (CB-28, -52, -101, -118, -138, -153, -180) and ∑ PBDE (BDE-28, -47, -49, -99, -100, -153, -154, -183) concentrations (ng/g lw, yellow dots, A, B). Grey and yellow letters indicate significant differences among species considering the sum of congeners in wet weight (ww) or lipid weight (lw), respectively. Individual ratios: BDE-99:BDE-100 (C) and BDE-154 over the sum of PBDE congener concentrations (28+47+49+99+100+153+154+183, D). p,p'-DDE (E in ng/g lw) and PFOS (F in ng/g ww) concentrations. For p,p'-DDE in dab, a triangle inside a symbol indicates that for these individuals, the concentration is below LOQ (reported value). Letters (a, b, c) indicate significant differences among species. Stars (*) indicate significant differences among fishing areas for a species.

Table 2. Parameters measured in the flatfish including trace metal and organic contaminant concentrations, and biomarker responses. For each parameter, descriptive statistics (n =number of individuals, n < LOQ = number of measurements below the quantification limit) and significant differences among fishing areas and species are indicated (F =flounder, D =dab and S =sole).

Parameter	Tissue	Unit	n			Median			Minimum			Maximum			n < LOQ[1]			Fishing area effect[2]			Species effect	
			F	D	S	F	D	S	F	D	S	F	D	S	F	D	S	F	D	S	Signif[3]	Ranking
Biological parameters																						
Length		cm	43	55	96	28.5	27	26.5	23.5	22	22	34	33.5	32	-	-	-	ns	ns	ns	***	F > D = S
Age		year	39	54	90	2	2	2	1	1	1	3	5	4	-	-	-	ns	ns	ns	ns	ns
Biomarkers																						
LMS	liver	min	42	45	89	12.26	9.99	11.0	3.00	3.50	4.50	37.50	30.0	22.50	-	-	-	ns	ns	ns	*	S = D = F
AChE	muscle	nmol/min/ mg prot	43	55	95	226.7	78.6	66.6	54.5	20.7	11.4	755.9	238.3	173.1	0	0	0	ns	*	*	***	F > D = S
SBs (Comet)	blood	% tail DNA	41	49	89	15.84	16.3	24.9	8.5	6.0	9.2	33.1	34.6	47.5	-	-	-	ns	ns	*	***	S > D = F
MN	blood	‰	43	55	95	0.033	0.09	0.15	0.00	0.00	0.00	1.20	2.80	4.00	-	-	-	ns	ns	*	*	S > F[6]
OH-Pyr	bile	ng/mL bile	42	42	94	114.1	37.0	68.5	15.6	2.0	11.9	400.1	152.1	381.6	0	1	0	*	*	*	***	F > S > D
Matrix composition																						
% dry matter	muscle	%	17	15	14	26	25	23	23	21	23	30	30	26	0	0	0	ns	ns	ns	**	F = D > S
% dry matter	liver	%	17	15	13	38	51	28	29	44	24	56	65	32	0	0	0	ns	ns	ns	***	D > F > S
Lipid Content	muscle	% (ww)	17	15	14	0.7	1.5	0.4	0.2	0.8	0.1	2.3	4.2	0.7	0	0	0	ns	ns	ns	***	D > F = S
Contaminants expressed in wet weight																						
Hg	muscle	ng/g ww	17	15	14	70.4	91.7	36.2	26.9	62.6	25.1	101.0	149.3	57.2	0	0	0	ns	ns	ns	***	D > F > S
met-Hg	muscle	ng/g ww	8	15	10	32.9	63.1	24.0	16.2	26.0	15.2	67.4	127.4	36.2	0	0	0	ns	ns	*	***	D > S[6]
Cd	liver	ng/g ww	17	15	12	61.4	95.1	37.6	34.4	38.3	25.6	126.7	235.1	74.3	1	0	0	ns	ns	ns	***	D > F > S
Pb	liver	ng/g ww	17	14	14	33.8	21.8	25.9	13.4	13.7	13.7	75.8	45.1	65.5	6	13	4	ns	ns	ns	ns	ns
Ag	liver	ng/g ww	17	15	13	136	103	1136	60	13	66	1444	401	2680	0	5	0	*	ns	ns	***	S > F = D
Cu	liver	µg/g ww	17	15	13	17.8	9.6	34.4	9.7	3.7	9.6	37.3	19.8	108.3	0	0	0	ns	ns	ns	***	S > F > D
Zn	liver	ng/g ww	17	15	13	42.1	35.4	28.8	25.2	21.4	18.8	56.1	55.4	34.1	0	0	0	*	ns	ns	***	F = D > S
p,p'-DDE	muscle	ng/g ww	17	15	14	0.41	0.25	0.14	0.16	0.09	0.08	0.57	1.32	0.34	0	8	0	ns	-	ns	-	[3,6]F > S
PFOS	muscle	ng/g ww	17	15	14	0.83	0.20	0.73	0.54	0.10	0.08	1.58	0.44	1.33	0	0	0	ns	ns	*	***	F = S > D
Contaminants expressed in lipid weight																						
∑ PCBs	muscle	pg/g lw	17	15	14	2214	934	1990	343	244	858	9508	1840	4225	-	-	-	*	*		***	F = S > D
∑ PBDEs[4]	muscle	pg/g lw	17	15	14	19673	4098	6159	3054	1325	2742	65635	7183	13904	-	-	-	*	*		***	F > S > D
p,p'-DDE	muscle	ng/g lw	17	15	14	72.2	19.9	39.8	10.5	4.2	21.5	196.0	60.8	88.4	0	8	0	ns	-	ns	-	[3]S = F > D

Contaminants ratios																				
met-Hg:Hg	muscle	8	15	10	0.62	0.67	0.63	0.56	0.41	0.58	0.68	0.85	0.77	-	-	-	ns	ns	*	ns	ns
BDE-99:BDE-100	muscle	17	15	14	0.33	0.12	0.11	0.06	0.07	0.04	0.65	0.90	0.28	-	-	-	*	ns	-	-	[3]F > D = S
BDE-154:ΣPBDE[5]	muscle	17	15	14	0.12	0.14	0.25	0.04	0.10	0.14	0.21	0.19	0.42	-	-	-	ns	ns	ns	***	S > D = F

[1] For trace metals, all value were >LOD. Estimated values between LOD and LOQ were used in the statistical analysis

[2] Spatial effect per species, when there are no censored data: Kruskall-Wallis test and Wilcoxon with Bonferroni correction as post hoc test: ns: non significant, * p<0.05

[3] Species effect at the scale of the Bay of Seine: when there are ≤ 6/46 censored data, when censored data are substituted by the LOQ: Kruskall-Wallis test and Wilcoxon with Bonferroni correction as post hoc test: ns: non significant, * p<0.05, ** p<0.01, *** p<0.001; when there are > 6/46 censored data: Median exact test of Fischer (p < 0.05)

[4] ΣPBDE = BDE-28+47+49+99+100+154

[5] ΣPBDE = BDE-28+47+49+99+100+153+154+183

[6] When only 2 species are indicated, the third one has a value intermediate and similar to both other species

-: not applicable (when there are censored data for the spatial effect and >8/46 censored data for the species effect)

4 Discussion

4.1 Biological effects

Lysosomal functional integrity is a generic common target for environmental stressors in all eukaryotic organisms, from yeast and protozoans to humans, and has been highly conserved in the course of evolution (Davies and Vethaak, 2012). Lysosomal membrane stability (LMS) is therefore considered as a universal biomarker of organism health and its response range is expected to be similar in all species (Moore, 2012). Animals are considered to be healthy if the labilisation period, used as the proxy of lysosomal stability, is >20 min (Background Assessment Criteria, BAC); stressed, but compensating if >10 min (Environmental Assessment criteria, EAC) and severely stressed and probably exhibiting pathology if <10 min (Moore, 2012). In this study, with median values of 12.3, 10 and 11 min for flounder, dab and sole, respectively, LMS were indeed similar among species and indicated that all three species were exposed to a general stress but at a level that can be compensated. Similarly low values were recorded in previous studies in flounder after an oil spill (Baršienė et al., 2006), in dab from the Seine estuary (Burgeot et al., 2017) and sole (*Solea senegalensis*) from laboratory experiments exposed to metals and organic model contaminants (Briaudeau et al., 2023, 2021). In the present study, the lysosomal biomarker did not allow for site differentiation for any of the three species.

Acetylcholine esterase (AChE) activity differed among species, with flounder showing higher (possibly less impacted) activity than dabs and soles. The higher activity measured in the flounder than in dab has already been observed (Burgeot et al., 2017, 2001), and is illustrated by the derivation of species-specific BAC and EAC (Vethaak et al., 2017). In this study, flounder (together with sole) also showed higher concentrations of PFOS (ww) than dab, which is known to be neurotoxic (Gaballah et al., 2020). The higher basal AChE value in flounder could result from a possible adaptation to higher concentrations of land-based neurotoxic compounds, as this species lives further upstream in the estuary than the other two. The AChE activity was similar in sole and dab, and in both cases, greater enzymatic inhibition was detected for individuals from the Estuary. These similar values and tendencies suggest that in absence of sole-specific assessment criteria, BAC and EAC developed for dab could also be applied to sole.

The level of DNA strand breaks (SBs) and the occurrence of micronuclei (MN) are both indicators of the effects of genotoxic compounds but at different time scales (Bolognesi and Cirillo, 2014; Costa et al., 2008; Martins and Costa, 2015): SBs inform on reversible primary DNA lesions depending on repair capabilities and are more representative of recent genotoxicants exposure, and MNs inform on irreversible and integrative chromosome damage. DNA damages occur upon exposure to contaminants that induce the production of reactive oxygen species (ROS). For instance, metals are known to induce ROS production in fish (Lee et al., 2019; Sevcikova et al., 2011), but also organic compounds such as glyphosate-based herbicides (Cavalcante et al., 2008). PAHs and especially their metabolites have genotoxic effects; correlations between bile concentrations of PAH metabolites and SBs have been reported in flatfishes (Dévier et al., 2013; Wessel et al., 2010). In the present study, no correlation was observed between these two parameters among sole individuals (r spearman = - 0.03). In the present study, the micronuclei frequency was lower in flounder than in dab. This observation is consistent with the background levels proposed for monitoring: the BAC for flounder (0-0.3 ‰) is also lower than that for dab (0.5 ‰) (Vethaak et al., 2017). There is no proposed sole-specific BAC for MN frequency. The mean MN frequency in sole from the Basque continental shelf (southern Bay of Biscay) was 0.19 ‰ in September, the same month as in the present study (Cuevas and Zorita, 2018). In the latter study, September was the month showing the lowest frequency over the year.

The higher SBs and MN values in soles than in dabs and flounders in the present study suggests either that the sole is more sensitive to contamination in the Bay of Seine than the other studied species, or that it has higher baseline levels that should be considered when deriving environmental assessment criteria. To study the differences in species sensitivity in background level derivation, species responses

to contamination should be compared in a site impacted by chemical contamination (such as the Bay of Seine) and a reference site, *i.e.* with very low contamination levels (e.g. Hylland et al. (2017). This might be explored with experimental studies, but this might lack of realism. However, the identification of such sites (similar environmental conditions but contrasting contamination history) in the field is challenging.

4.2 Contaminant concentrations

While the liver Cd and Pb concentrations measured in flounder were in the same range as those reported for individuals collected in 2010 in the Seine estuary by Kerambrun et al. (2013), the Cu and Zn concentrations found here were lower, but the individuals considered were also larger than those of Kerambrun et al. (2013). Furthermore, concentrations found in soles in the present study were likely to be similar or higher for Hg and Zn than those reported in sole from polluted reservoirs from southwest Spain, but they were lower for Cd, Pb and Cu (in muscle: 36 versus 12 to 17 ng/g ww, in liver: Cd: 38 versus 80 to 430 ng/g ww, Pb: 26 versus 200 to 420 ng/g ww, Cu: 35 versus 51 to 129 µg/g ww and Zn: 29 versus 15 to 27 µg/g ww in Seine (present study) and Spain, respectively; fish size were not indicated in Usero et al. (2004). There was no effect of the fishing areas, except for met-Hg in sole, and Ag and Zn in flounder. However, significant concentration differences among species were found for all metals analyzed except Pb, with an order of magnitude in the range of 1 to 3 times differences among species, and a 10 times higher concentration in sole for Ag. Sole seems to accumulate less Hg, Cd and Zn, but more Ag and Cu than the other two species. These results are in accordance with a previous study analyzing different fish species from another French estuary, the Gironde estuary, which also reported higher Hg concentrations in the muscle and higher Cd and Zn concentrations in the liver of flounder compared to sole, whereas sole had higher liver Cu concentrations (Durrieu et al., 2005). Overall, the present study suggests that species are able to develop species-specific mechanisms for metal bioaccumulation, regulation and/or excretion, despite probably similar exposure to metal contamination in a same site, although potential differences in their diet and of the contamination of their prey (not considered here) may also partly explain these observed differences among species.

Bioaccumulation of lipophilic organic contaminants is strongly dependent on the fish lipid content. The differences in lipid content found among the three fish species are consistent with previous studies on the same species (Bodin et al., 2014; Voorspoels et al., 2003; Westernhagen et al., 1999). Sole were leaner than dab and the least contaminated by PCBs, PBDEs and *p,p'*-DDE when concentrations were expressed in wet weight. When normalized to lipids, concentrations of these contaminants were the lowest in dab, the fattest species, while PCBs and *p,p'*-DDE concentrations were similar in flounder and sole. PBDE concentrations were higher in flounder in both wet weight and lipid weight.

PCB concentrations higher in flounder than in sole was previously observed in the Gironde estuary with ca. 5 and 2 µg/g lw (185 and 50 µg/g dw) in the muscle of flounder and sole, respectively, for individuals of similar size range as in the present study (Bodin et al., 2014). Also, PBDE concentrations higher in flounder than in sole (in both ww and lw) as in the present study were reported in the Loire estuary by Bragigand et al. (2006) thought no statistics were done to confirm the significance of the difference between flounder and sole (ca. 280 and 150 pg BDE-47/g ww in sole and flounder muscle, respectively).

PFOS is a persistent contaminant that tends to accumulate in protein-rich tissues (Martin et al., 2003). The fatty composition of the dab may not favour the accumulation of PFOS, unlike for the lipophilic contaminants discussed above. The lower PFOS concentrations in sole from the Open bay area suggested reduced exposure to PFOS in this area, which could also explain the lower PFOS contamination levels in dabs (mostly originating from this area). It was indeed previously shown that river discharge is the main source of PFOS to the marine environment (Lindim et al., 2016), which supports the existence of a concentration gradient from the estuary to more open waters. Similar concentrations of PFOS were found in flatfishes from the Dutch coast (0-1 ng/g ww), with a slightly lower concentration in dab than sole (Zafeiraki et al., 2019).

The present work focuses on inter-flatfish species variability in their response to the chemical contamination. However, sole as one of the highest consumed fish in Europe, may represent a sanitary issue for human health in the case of threshold exceedance. This was the case for PBDE (EQS(human health) = 0.0085 ng/g ww, refers to the sum of 6 PBDE) which was exceeded in 44 out of 46 samples, including 12 out of the 14 soles analysed. However, maximum permissible concentration (MPC) set by the commission regulation 2023/915 were not exceeded in any of the samples: 1) PCB (Sum of 6 PCBs 28, 52, 101, 138, 153 and 180) concentrations were 1 to 2 orders of magnitude lower than 75 ng/g ww; 2) PFOS concentrations in sole muscle were in same order of magnitude but lower than newly set MPC (2 ng/g ww) 3) Cd and Pb concentrations in liver were considered more related to the bivalve threshold than the muscle one and were several orders of magnitude lower than bivalve MPC: 1 and 1.5 mg/kg ww for Cd and Pb respectively. 4) Finally, concentrations in Hg in muscle were ca. one order of magnitude lower than MPC in sole (0.5 µg/g ww). These comparisons are related to human health assessment and related to descriptor 9 of the MSFD. The present data are also the basis for D8 indicators (environmental health assessment) proposed to the French authority for GES assessment in 2024.

4.3 Metabolization capacities

PAHs are efficiently metabolized by fish (Wessel et al., 2013) so that their metabolites excreted in the bile are used as biomarkers of recent PAH exposure (Hylland et al., 2012). PAH biotransformation and elimination of their metabolites via the bile is a rapid process (Budzinski et al., 2004; Krahn et al., 1992; Wessel et al., 2012). Due to the role of bile in digestion processes, with episodic release of bile into the oesophagus, the density and volume of bile differ according to the feeding status (Collier and Varanasi, 1991). Indicators of recent PAH exposure is hence dependent on the feeding status of the fish (Hylland et al., 2012). This biomarker is also influenced by PAH concentrations, food availability and metabolization capacities of fish that can vary depending on the species (Krahn et al., 1992; Luthe et al., 2002; Solbakken and Palmork, 1981). In the present study, PAH concentrations were higher at the Estuary than in the North and South areas. These results are in line with previous studies in the same area, where higher OH-pyrene concentrations were up to threefold higher in the Estuary than in the Open bay (dab in 2005 and 2006: ca. 20 to 100 $ng.g^{-1}$ bile in the Open Bay and ca. 80 to 435 $ng.g^{-1}$ bile in the Estuary area, (Dévier et al., 2013); dab and flounder in 2008 and 2009: 534 and 290 $ng.g^{-1}$ bile in dab from the Estuary and Open Bay, respectively and 348 and 225 ng/g bile in dab from the Estuary and Open Bay, respectively, (Burgeot et al., 2017)). The most significant interspecific difference in the present study for OH-pyrene levels was observed in the Open bay area, where OH-pyrene concentrations in sole were twice higher in sole than in dab. This might be related to 1) heterogeneity in exposure between populations of both species in the Open bay, the largest area among the 4 investigated in the present study and/or to 2) higher metabolization capacity in sole, as suggested by PBDEs ratio in the present study. In the present study, flounder also presented higher concentration in OH-pyrene than dab, regardless of the area. In contrast, in 2008/2009, OH-pyrene concentrations in both species from the Bay of Seine in 2008/2009 were statistically similar (348 ± 183 and 534 ± 305 ng/g bile for OH-pyrene in flounder and dab from the Seine estuary, respectively, (Burgeot et al., 2017)). This last study also reported inter-annual variation by a factor up to 10 (e.g. mean OH-pyrene concentration in dab from the Open bay: 29 and 290 ng/g in 2008 and 2009 respectively) which the authors linked to a weaker Seine flow in 2009 when the lowest PAH concentrations were observed.

Ratios between PBDE congeners were used to assess metabolization capacities among species, assuming similar environmental contaminants profiles between the living areas of the different species. Although BDE-99 and BDE-100 are both pentabrominated PBDEs, it has been observed that BDE-99 could be metabolized in fish but not BDE-100 (Roberts et al., 2011; Stapleton et al., 2004). The results of the present study suggest that BDE-99 was metabolized in all three species and more efficiently in sole and dab than in flounder. Ratios measured in the present study were within the range of those previously reported in sole on the French Atlantic coast (median: 0.18, Munschy et al., 2011). Voorspoels et al. (2003) showed that the BDE-99:BDE-100 ratio had comparable values in sole and dab,

which is consistent with the present study. The highest proportion of BDE-154 in sole is in favour of a greater metabolic capacity of sole (Stapleton et al., 2004). High contribution of BDE-154 has also been reported in marine fish species especially in sole (Munschy et al., 2011; Voorspoels et al., 2004). Although it is difficult to infer metabolic capacity solely from congener profiles determined in samples collected in the wild, the sole may have a higher ability to metabolize PBDEs than the dab and the flounder.

4.4 Relationship to phylogenic distances

In the present study, flatfish from different phylogenetic families were studied. The sole belongs to a different family of flatfish (Soleidae) than the dab and the flounder (Pleuronectidae). Phylogenetic distances between species may explain physiological differences, particularly in the processes involved in the uptake, distribution and biotransformation of contaminants and their biological effects (e.g. Dallas et al. (2013). This was recently shown in bivalves with differences in chemical bioaccumulation between species and their hybrids (Sussarellu et al., 2022). However, only a few biomarker responses and bioaccumulation levels appeared more similar between dab and flounder (Pleuronectidae) than with sole (Soleidae): DNA strand breaks and Ag concentrations which were lower in dab and flounder than in sole, while Hg, Cd and Zn concentrations were higher in dab and flounder than in sole.

5 Conclusion

In the Bay of Seine, a human-impacted habitat, comparison of contaminant levels and biological responses between sole, dab and flounder did not lead to a clear species differentiation (e.g. one species far above the two others in terms of contaminant levels or effects). Among the three studied flatfish, sole was the most abundant species in the prospected areas of the Bay of Seine, whereas dab and flounder were mainly found in half of the surveyed areas. Sole showed higher levels of genotoxic alteration (SBs, MN) than dab and flounder, and they showed among the highest levels of neurotoxic alteration (AChE inhibition) and PAH exposure (OH-pyrene). Regarding the interspecific differences in contamination, sole concentrations in Hg, met-Hg, Cd Zn were lower than in dab and flounder, sole concentrations in PBDEs (lw) were intermediate between dab and flounder, while concentrations in other lipophilic organic contaminants (PCBs and DDT, in lw) and PFOS were amongst the highest together with flounder. PBDE profiles in sole suggested that sole may have a higher ability to metabolize PBDEs compared to flounder and dab. This metabolization ability usually enables individuals to excrete contaminants and reduce the contaminant loads but also requires the organism to allocate energy for metabolization.

In brief, the present study highlighted that sole is widely distributed in the Bay of Seine, and sensitive to the chemical contamination even if it does not always show the highest contamination levels. Sole is therefore recommended as a sentinel species for the integrated study and monitoring of biological effects of contaminants. The use of sole as sentinel species might require the derivation of species-specific thresholds for biomarkers unless a pragmatic level of biological complexity is found for biological effect thresholds.

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6 References

Akcha, F., Vincent Hubert, F., Pfhol-Leszkowicz, A., 2003. Potential value of the comet assay and DNA adduct measurement in dab (Limanda limanda) for assessment of in situ exposure to genotoxic compounds. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 534, 21–32. https://doi.org/10.1016/S1383-5718(02)00244-9

Azemard, S., Vassileva, E., 2015. Determination of methylmercury in marine biota samples with advanced mercury analyzer: Method validation. Food Chemistry 176, 367–375. https://doi.org/10.1016/j.foodchem.2014.12.085

Baršienė, J., Lehtonen, K.K., Koehler, A., Broeg, K., Vuorinen, P.J., Lang, T., Pempkowiak, J., Šyvokienė, J., Dedonyte, V., Rybakovas, A., Repečka, R., Vuontisjärvi, H., Kopecka, J., 2006. Biomarker responses in flounder (Platichthys flesus) and mussel (Mytilus edulis) in the Klaipėda-Būtingė area (Baltic Sea). Marine Pollution Bulletin, The BEEP Project: Biological Effects of Environmental Pollution in Marine Coastal Ecosystems: Biomonitoring in the Baltic Sea 53, 422–436. https://doi.org/10.1016/j.marpolbul.2006.03.009

Baršienė, J., Rybakovas, A., Lang, T., Andreikėnaitė, L., Michailovas, A., 2013. Environmental genotoxicity and cytotoxicity levels in fish from the North Sea offshore region and Atlantic coastal waters. Marine Pollution Bulletin 68, 106–116. https://doi.org/10.1016/j.marpolbul.2012.12.011

Bocquené, G., Galgani, F., 1998. Biological effects of contaminants: Cholinesterase inhibition by organophosphate and carbamate compounds (report). ICES Techniques in Marine Environmental Science (TIMES). https://doi.org/10.17895/ices.pub.5048

Bodin, N., Tapie, N., Ménach, K.L., Chassot, E., Elie, P., Rochard, E., Budzinski, H., 2014. PCB contamination in fish community from the Gironde Estuary (France): Blast from the past. Chemosphere 98, 66–72. https://doi.org/10.1016/j.chemosphere.2013.10.003

Bolle, L.J., Dapper, R., Witte, J.I.J., Van Der Veer, H.W., 1994. Nursery grounds of dab (Limanda limanda L.) in the southern North Sea. Netherlands Journal of Sea Research 32, 299–307. https://doi.org/10.1016/0077-7579(94)90007-8

Bolognesi, C., Cirillo, S., 2014. Genotoxicity biomarkers in aquatic bioindicators. Current Zoology 60, 273–284.

Borcier, E., Charrier, G., Couteau, J., Maillet, G., Le Grand, F., Bideau, A., Waeles, M., Le Floch, S., Amara, R., Pichereau, V., Laroche, J., 2020. An Integrated Biomarker Approach Using Flounder to Improve Chemical Risk Assessments in the Heavily Polluted Seine Estuary. Journal of Xenobiotics 10, 14–35. https://doi.org/10.3390/jox10020004

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72, 248–254. https://doi.org/10.1006/abio.1976.9999

Bragigand, V., Amiard-Triquet, C., Parlier, E., Boury, P., Marchand, P., Hourch, M.E., 2006. Influence of biological and ecological factors on the bioaccumulation of polybrominated diphenyl ethers in aquatic food webs from French estuaries. Science of The Total Environment 368, 615–626. https://doi.org/10.1016/j.scitotenv.2006.04.001

Briaudeau, T., Alves Dos Santos, L.A., Zorita, I., Izagirre, U., Marigómez, I., 2021. Biological responses and toxicopathic effects elicited in Solea senegalensis juveniles by waterborne exposure to benzo[a]pyrene. Marine Environmental Research 170, 105351. https://doi.org/10.1016/j.marenvres.2021.105351

Briaudeau, T., Guerrero-Limon, G., Zorita, I., Marigómez, I., Izagirre, U., 2023. Toxicopathic effects of waterborne Cd in sole juveniles, a prospective pollution monitoring sentinel for estuarine ecosystems.

Briaudeau, T., Zorita, I., Cuevas, N., Franco, J., Marigómez, I., Izagirre, U., 2019. Multi-annual survey of health status disturbance in the Bilbao estuary (Bay of Biscay) based on sediment chemistry and juvenile sole (Solea spp.) histopathology. Marine Pollution Bulletin 145, 126–137. https://doi.org/10.1016/j.marpolbul.2019.05.034

Briaudeau, T., Zorita, I., Izagirre, U., Marigómez, I., 2020. Biological responses and toxicopathic effects elicited in Solea senegalensis juveniles on exposure to contaminated sediments under laboratory conditions. Science of The Total Environment 731, 138849. https://doi.org/10.1016/j.scitotenv.2020.138849

Broeg, K., Zander, S., Diamant, A., Körting, W., Krüner, G., Paperna, I., Westernhagen, H. v., 1999. The use of fish metabolic, pathological and parasitological indices in pollution monitoring. Helgol Mar Res 53, 171–194. https://doi.org/10.1007/s101520050023

Budzinski, H., Mazéas, O., Tronczynski, J., Désaunay, Y., Bocquené, G., Claireaux, G., 2004. Link between exposure of fish (Solea solea) to PAHs and metabolites: Application to the "Erika" oil spill. Aquat. Living Resour. 17, 329–334. https://doi.org/10.1051/alr:2004040

Burgeot, T., Akcha, F., Ménard, D., Robinson, C., Loizeau, V., Brach-Papa, C., Martínez-Gòmez, C., Goff, J.L., Budzinski, H., Menach, K.L., Cachot, J., Minier, C., Broeg, K., Hylland, K., 2017a. Integrated monitoring of chemicals and their effects on four sentinel species, Limanda limanda, Platichthys flesus, Nucella lapillus and Mytilus sp., in Seine Bay: A key step towards applying biological effects to monitoring. Marine Environmental Research 124, 92–105. https://doi.org/10.1016/j.marenvres.2016.10.009

Burgeot, T., Bocquené, G., His, E., Vincent, F., Geffard, O., Beiras, R., Quiniou, F., Goraguer, H., Galgani, F., 2001. Technical Annex (Chapter 7) - Procedures for Cholinesterase Determination in Fish and Mussel, in: Garrigues, P., Barth, H., Walker, C.H., Narbonne, J.F. (Eds.), Biomarkers in Marine Organisms. Elsevier Science, Amsterdam, pp. 487–489. https://doi.org/10.1016/B978-044482913-9/50033-X

Cavalcante, D.G.S.M., Martinez, C.B.R., Sofia, S.H., 2008. Genotoxic effects of Roundup® on the fish Prochilodus lineatus. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 655, 41–46. https://doi.org/10.1016/j.mrgentox.2008.06.010

Cerdà, J., Manchado, M., 2013. Advances in genomics for flatfish aquaculture. Genes Nutr 8, 5–17. https://doi.org/10.1007/s12263-012-0312-8

Chouvelon, T., Cresson, P., Bouchoucha, M., Brach-Papa, C., Bustamante, P., Crochet, S., Marco-Miralles, F., Thomas, B., Knoery, J., 2018. Oligotrophy as a major driver of mercury bioaccumulation in medium-to high-trophic level consumers: A marine ecosystem-comparative study. Environ Pollut 233, 844–854. https://doi.org/10.1016/j.envpol.2017.11.015

Chouvelon, T., Munschy, C., Bruzac, S., Caurant, F., Churlaud, C., Crochet, S., Guillou, G., Mauffret, A., Méndez-Fernandez, P., Niol, J., Sireau, T., Steinberg, C., Wessel, N., Spitz, J., 2022. High inter-species variability in elemental composition of the twilight zone fauna varies implications for predators and exploitation by humans. Environmental Research 204, 112379. https://doi.org/10.1016/j.envres.2021.112379

Collier, T.K., Varanasi, U., 1991. Hepatic activities of xenobiotic metabolizing enzymes and biliary levels of xenobiotics in english sole (Parophrys vetulus) exposed to environmental contaminants. Arch. Environ. Contam. Toxicol. 20, 462–473. https://doi.org/10.1007/BF01065834

Constenla, M., Soler-Membrives, A., Besada, V., Carrassón, M., 2022. Impact assessment of a large river on the sediments and fish from its continental shelf: using Solea solea as sentinel in the Ebro river mouth (NW Mediterranean, Spain). Environ Sci Pollut Res 29, 15713–15728. https://doi.org/10.1007/s11356-021-16408-7

Costa, P.M., Lobo, J., Caeiro, S., Martins, M., Ferreira, A.M., Caetano, M., Vale, C., DelValls, T.Á., Costa, M.H., 2008. Genotoxic damage in Solea senegalensis exposed to sediments from the Sado Estuary (Portugal): Effects of metallic and organic contaminants. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 654, 29–37. https://doi.org/10.1016/j.mrgentox.2008.04.007

Couteau, J., 2020. MESURE DE BIOMARQUEURS SUR PLATICHTHYS FLESUS PRELEVES EN ESTUAIRE DE SEINE EN SEPTEMBRE 2019.

Cuevas, N., Zorita, I., 2018a. Baseline levels of environmental genotoxicity and potential confounding factors using common sole (Solea solea) as sentinel organism. Marine Environmental Research 138, 1–8. https://doi.org/10.1016/j.marenvres.2018.03.008

Cuevas, N., Zorita, I., Costa, P.M., Quincoces, I., Larreta, J., Franco, J., 2015. Histopathological indices in sole (Solea solea) and hake (Merluccius merluccius) for implementation of the European Marine Strategy Framework Directive along the Basque continental shelf (SE Bay of Biscay). Marine Pollution Bulletin 94, 185–198. https://doi.org/10.1016/j.marpolbul.2015.02.030

Dallas, L.J., Cheung, V.V., Fisher, A.S., Jha, A.N., 2013. Relative sensitivity of two marine bivalves for detection of genotoxic and cytotoxic effects: a field assessment in the Tamar Estuary, South West England. Environ Monit Assess 185, 3397–3412. https://doi.org/10.1007/s10661-012-2800-0

Dauvin, J.-C., Raoux, A., Pezy, J.-P., Baux, N., Niquil, N., 2020. The Bay of Seine: A Resilient Socio-Eco-System Under Cumulative Pressures, in: Ceccaldi, H.-J., Hénocque, Y., Komatsu, T., Prouzet, P., Sautour, B., Yoshida, J. (Eds.), Evolution of Marine Coastal Ecosystems under the Pressure of Global Changes. Springer International Publishing, Cham, pp. 95–109. https://doi.org/10.1007/978-3-030-43484-7_7

Daverat, F., Morais, P., Dias, E., Babaluk, J., Martin, J., Eon, M., Fablet, R., Pécheyran, C., Antunes, C., 2012. Plasticity of European flounder life history patterns discloses alternatives to catadromy. Mar. Ecol. Prog. Ser. 465, 267–280. https://doi.org/10.3354/meps09910

Davies, I., M., Vethaak, A., D., 2012. Integrated marine environmental monitoring of chemicals and their effects (ICES cooperative research report No. 315). ICES.

Dévier, M.-H., Le Dû-Lacoste, M., Akcha, F., Morin, B., Peluhet, L., Le Menach, K., Burgeot, T., Budzinski, H., 2013. Biliary PAH metabolites, EROD activity and DNA damage in dab (Limanda limanda) from Seine Estuary (France). Environ Sci Pollut Res 20, 708–722. https://doi.org/10.1007/s11356-012-1345-7

Dorel, D., Koutsikopoulos, C., Desaunay, Y., Marchand, J., 1991. Seasonal distribution of young sole (Solea solea (L.)) in the nursery ground of the bay of Vilaine (Northern bay of Biscay). Netherlands Journal of Sea Research, Proceedings of the First International Symposium on Flatfish Ecology 27, 297–306. https://doi.org/10.1016/0077-7579(91)90032-V

Durrieu, G., Maury-Brachet, R., Girardin, M., Rochard, E., Boudou, A., 2005. Contamination by heavy metals (Cd, Zn, Cu, and Hg) of eight fish species in the Gironde estuary (France). Estuaries 28, 581–591. https://doi.org/10.1007/BF02696069

France Agrimer, 2022. Consommation des produits de la pêche et de l'aquaculture 2020.

Gaballah, S., Swank, A., Sobus, J.R., Howey, X.M., Schmid, J., Catron, T., McCord, J., Hines, E., Strynar, M., Tal, T., 2020. Evaluation of Developmental Toxicity, Developmental Neurotoxicity, and Tissue Dose in Zebrafish Exposed to GenX and Other PFAS. Environmental Health Perspectives 128, 047005. https://doi.org/10.1289/EHP5843

Gasperi, J., Gromaire, M.C., Kafi, M., Moilleron, R., Chebbo, G., 2010. Contributions of wastewater, runoff and sewer deposit erosion to wet weather pollutant loads in combined sewer systems. Water Research 44, 5875–5886. https://doi.org/10.1016/j.watres.2010.07.008

Howell, B.R., 1997. A re-appraisal of the potential of the sole, Solea solea (L.), for commercial cultivation. Aquaculture, Proceedings of the fish and shellfish Larviculture Symposium LARVI '95 155, 355–365. https://doi.org/10.1016/S0044-8486(97)00103-8

Hylland, K., Skei, B.B., Brunborg, G., Lang, T., Gubbins, M.J., le Goff, J., Burgeot, T., 2017. DNA damage in dab (Limanda limanda) and haddock (Melanogrammus aeglefinus) from European seas. Marine Environmental Research, The ICON Project (the trans-European research project on field studies related to a large-scale sampling and monitoring 124, 54–60. https://doi.org/10.1016/j.marenvres.2016.01.001

Hylland, K., Vethaak, A., Davies, I., 2012. Background document: polycyclic aromatic hydrocarbons metabolites in fish bile. ICES Cooperative Research Report 315, 18–25.

Kerambrun, E., Henry, F., Cornille, V., Courcot, L., Amara, R., 2013. A combined measurement of metal bioaccumulation and condition indices in juvenile European flounder, Platichthys flesus, from European estuaries. Chemosphere 91, 498–505. https://doi.org/10.1016/j.chemosphere.2012.12.010

Koutsikopoulos, C., Dorel, D., Desaunay, Y., 1995. Movement of Sole (Solea Solea) in the Bay of Biscay: Coastal Environment and Spawning Migration. Journal of the Marine Biological Association of the United Kingdom 75, 109–126. https://doi.org/10.1017/S002531540001523X

Krahn, M.M., Burrows, D.G., Ylitalo, G.M., Brown, D.W., Wigren, C.A., Collier, T.K., Chan, S.L., Varanasi, U., 1992. Mass spectrometric analysis for aromatic compounds in bile of fish sampled after the Exxon Valdez oil spill. Environ. Sci. Technol. 26, 116–126. https://doi.org/10.1021/es00025a012

Lang, T., Wosniok, W., 2008. The Fish Disease Index: a method to assess wild fish disease data in the context of marine environmental monitoring. ICES CM 2008/D:01.

Laroche, J., Gauthier, O., Quiniou, L., Devaux, A., Bony, S., Evrard, E., Cachot, J., Chérel, Y., Larcher, T., Riso, R., Pichereau, V., Devier, M.H., Budzinski, H., 2013. Variation patterns in individual fish responses to chemical stress among estuaries, seasons and genders: the case of the European flounder (Platichthys flesus) in the Bay of Biscay. Environ Sci Pollut Res 20, 738–748. https://doi.org/10.1007/s11356-012-1276-3

Laurent, J., Lavergne, E., Couteau, J., Le Floch, S., Ouddane, B., Cachot, J., Davail, B., Clérandeau, C., Devin, S., Fisson, C., Devaux, A., Amara, R., Diop, M., Pichereau, V., Laroche, J., 2022. Impacts of chemical stress, season, and climate change on the flounder population of the highly anthropised Seine estuary (France). Environ Sci Pollut Res 29, 59751–59769. https://doi.org/10.1007/s11356-022-20000-y

Le Dû-Lacoste, M., Akcha, F., Dévier, M.-H., Morin, B., Burgeot, T., Budzinski, H., 2013. Comparative study of different exposure routes on the biotransformation and genotoxicity of PAHs in the flatfish species, Scophthalmus maximus. Environ Sci Pollut Res 20, 690–707. https://doi.org/10.1007/s11356-012-1388-9

Le Pape, O., Baulier, L., Cloarec, A., Martin, J., Le Loc'h, F., Désaunay, Y., 2007. Habitat suitability for juvenile common sole (Solea solea, L.) in the Bay of Biscay (France): A quantitative description using indicators based on epibenthic fauna. Journal of Sea Research 57, 126–136. https://doi.org/10.1016/j.seares.2006.08.011

Lee, J.-W., Choi, H., Hwang, U.-K., Kang, J.-C., Kang, Y.J., Kim, K.I., Kim, J.-H., 2019. Toxic effects of lead exposure on bioaccumulation, oxidative stress, neurotoxicity, and immune responses in fish: A review. Environmental Toxicology and Pharmacology 68, 101–108. https://doi.org/10.1016/j.etap.2019.03.010

Lindim, C., van Gils, J., Cousins, I.T., 2016. Europe-wide estuarine export and surface water concentrations of PFOS and PFOA. Water Research 103, 124–132. https://doi.org/10.1016/j.watres.2016.07.024

Link, J.S., Smith, B.E., Packer, D.B., Fogarty, M.J., Langton, R.W., 2014. The trophic ecology of flatfishes, in: Gibson, R.N., Nash, R.D.M., Geffen, A.J., Van Der Veer, H.W. (Eds.), Flatfishes. John Wiley & Sons, Ltd, Chichester, UK, pp. 283–313. https://doi.org/10.1002/9781118501153.ch11

Luthe, G., Stroomberg, G.J., Ariese, F., Brinkman, U.A.T., van Straalen, N.M., 2002. Metabolism of 1-fluoropyrene and pyrene in marine flatfish and terrestrial isopods. Environmental Toxicology and Pharmacology 12, 221–229. https://doi.org/10.1016/S1382-6689(02)00093-5

Martin, J.W., Mabury, S.A., Solomon, K.R., Muir, D.C.G., 2003. Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout (Oncorhynchus mykiss). Environmental Toxicology and Chemistry 22, 196–204. https://doi.org/10.1002/etc.5620220126

Martins, M., Costa, P.M., 2015. The comet assay in Environmental Risk Assessment of marine pollutants: applications, assets and handicaps of surveying genotoxicity in non-model organisms. Mutagenesis 30, 89–106. https://doi.org/10.1093/mutage/geu037

Mazéas, O., Budzinski, H., 2005. Solid-phase extraction and purification for the quantification of polycyclic aromatic hydrocarbon metabolites in fish bile. Anal Bioanal Chem 383, 985–990. https://doi.org/10.1007/s00216-005-0096-4

Miramand, P., Guyot, T., Rybarczyk, H., Elkaim, B., Mouny, P., Dauvin, J.C., Bessineton, C., 2001. Contamination of the biological compartment in the Seine estuary by Cd, Cu, Pb, and Zn. Estuaries 24, 1056–1065. https://doi.org/10.2307/1353017

Moore, M.N., 2012. Background document: lysosomal stability as a global health status indicator in biomonitoring. ICES Cooperative Research Report 68–93.

Moore, M.N., Lowe, D., Köhler, A., 2004. Biological effects of contaminants: measurement of lysosomal membrane stability. (Report). International Council for the Exploration of the Sea (ICES). https://doi.org/10.25607/OBP-227

Mounier, F., Loizeau, V., Pecquerie, L., Drouineau, H., Labadie, P., Budzinski, H., Lobry, J., 2020. Dietary bioaccumulation of persistent organic pollutants in the common sole Solea solea in the context of global change. Part 2: Sensitivity of juvenile growth and contamination to toxicokinetic parameters uncertainty and environmental conditions variability in estuaries. Ecological Modelling 431, 109196. https://doi.org/10.1016/j.ecolmodel.2020.109196

Munschy, C., Bely, N., Héas-Moisan, K., Olivier, N., Pollono, C., Hollanda, S., Bodin, N., 2020. Tissue-specific bioaccumulation of a wide range of legacy and emerging persistent organic contaminants in swordfish (Xiphias gladius) from Seychelles, Western Indian Ocean. Marine Pollution Bulletin 158, 111436. https://doi.org/10.1016/j.marpolbul.2020.111436

Munschy, C., Héas-Moisan, K., Tixier, C., Boulesteix, L., Morin, J., 2011a. Classic and novel brominated flame retardants (BFRs) in common sole (Solea solea L.) from main nursery zones along the French coasts. Science of The Total Environment 409, 4618–4627. https://doi.org/10.1016/j.scitotenv.2011.07.021

Piet, G.J., Pfisterer, A.B., Rijnsdorp, A.D., 1998. On factors structuring the flatfish assemblage in the southern North Sea. Journal of Sea Research 40, 143–152. https://doi.org/10.1016/S1385-1101(98)00008-2

Power, M., McCarty, L.S., 1997. Environmental Policy Analysis, Peer Reviewed: Fallacies in Ecological Risk Assessment Practices. Environ. Sci. Technol. 31, 370A-375A. https://doi.org/10.1021/es972418b

Rijnsdorp, A., Vethaak, A., Van Leeuwen, P., 1992. Population biology of dab Limanda limanda in the southeastern North Sea. Mar. Ecol. Prog. Ser. 91, 19–35. https://doi.org/10.3354/meps091019

Riou, P., Le Pape, O., Rogers, S.I., 2001. Relative contributions of different sole and plaice nurseries to the adult population in the Eastern Channel: application of a combined method using generalized linear models and a geographic information system. Aquatic Living Resources 14, 125–135. https://doi.org/10.1016/S0990-7440(01)01110-X

Roberts, S.C., Noyes, P.D., Gallagher, E.P., Stapleton, H.M., 2011. Species-specific differences and structure-activity relationships in the debromination of PBDE congeners in three fish species. Environmental science & technology 45 5, 1999–2005.

Rochette, S., Huret, M., Rivot, E., Le Pape, O., 2012. Coupling hydrodynamic and individual-based models to simulate long-term larval supply to coastal nursery areas. Fisheries Oceanography 21, 229–242. https://doi.org/10.1111/j.1365-2419.2012.00621.x

Sardi, A.E., Bégout, M.-L., Cousin, X., Labadie, P., Loizeau, V., Budzinski, H., 2021. A review of the effects of contamination and temperature in Solea solea larvae. Modeling perspectives in the context of climate change. Journal of Sea Research 176, 102101. https://doi.org/10.1016/j.seares.2021.102101

Sevcikova, M., Modra, H., Slaninova, A., Svobodova, Z., 2011. Metals as a cause of oxidative stress in fish: a review. Vet. Med. 56, 537–546. https://doi.org/10.17221/4272-VETMED

SIH, 2022. Synthèse des flottilles de pêche - Flotte de la façade Mer du Nord - Manche - Atlantique, 2021.

Solbakken, J.E., Palmork, K.H., 1981. Metabolism of phenantherene in various marine animals. Comparative Biochemistry and Physiology Part C: Comparative Pharmacology 70, 21–26. https://doi.org/10.1016/0306-4492(81)90073-3

Stapleton, H.M., Letcher, R.J., Baker, J.E., 2004. Debromination of Polybrominated Diphenyl Ether Congeners BDE 99 and BDE 183 in the Intestinal Tract of the Common Carp (Cyprinus carpio). Environ. Sci. Technol. 38, 1054–1061. https://doi.org/10.1021/es0348804

Sussarellu, R., Chouvelon, T., Aminot, Y., Couteau, J., Loppion, G., Dégremont, L., Lamy, J.-B., Akcha, F., Rouxel, J., Berthelin, C., Briaudeau, T., Izagirre, U., Mauffret, A., Grouhel, A., Burgeot, T., 2022. Differences in chemical contaminants bioaccumulation and ecotoxicology biomarkers in Mytilus edulis and Mytilus galloprovincialis and their hybrids. Environ Pollut 292, 118328. https://doi.org/10.1016/j.envpol.2021.118328

Tang, Y., Yin, M., Yang, W., Li, H., Zhong, Y., Mo, L., Liang, Y., Ma, X., Sun, X., 2019. Emerging pollutants in water environment: Occurrence, monitoring, fate, and risk assessment. Water Environment Research 91, 984–991. https://doi.org/10.1002/wer.1163

Tappin, A.D., Millward, G.E., 2015. The English Channel: Contamination status of its transitional and coastal waters. Marine Pollution Bulletin, The English Channel and it's catchments: Status and Responses to Contaminants 95, 529–550. https://doi.org/10.1016/j.marpolbul.2014.12.012

Tornero, V., Hanke, G., 2016. Chemical contaminants entering the marine environment from sea-based sources: A review with a focus on European seas. Marine Pollution Bulletin 112, 17–38. https://doi.org/10.1016/j.marpolbul.2016.06.091

Tronczynski, J., Munschy, C., Moisan, K., 2004. Les contaminants organiques qui laissent des traces.

UNEP/RAMOGE, 1999. Galdies: Manual on the biomarkers recommended for... - Google Scholar [WWW Document]. URL https://scholar.google.com/scholar_lookup?title=Manual%20on%20the%20Biomarkers%20Recommended%20for%20the%20MED%20POL%20Biomonitoring%20Programme&publication_year=1999&author=UNEP%2FRAMOGE (accessed 2.7.23).

US EPA, O., 1998. EPA Method 7473 (SW-846): Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry [WWW Document]. URL https://www.epa.gov/esam/epa-method-7473-sw-846-mercury-solids-and-solutions-thermal-decomposition-amalgamation-and (accessed 7.17.23).

Usero, J., Izquierdo, C., Morillo, J., Gracia, I., 2004. Heavy metals in fish (Solea vulgaris, Anguilla anguilla and Liza aurata) from salt marshes on the southern Atlantic coast of Spain. Environment International 29, 949–956. https://doi.org/10.1016/S0160-4120(03)00061-8

Vaccher, V., Ingenbleek, L., Adegboye, A., Hossou, S.E., Koné, A.Z., Oyedele, A.D., Kisito, C.S.K.J., Dembélé, Y.K., Hu, R., Adbel Malak, I., Cariou, R., Vénisseau, A., Veyrand, B., Marchand, P., Eyangoh, S., Verger, P., Dervilly-Pinel, G., Leblanc, J.-C., Le Bizec, B., 2020. Levels of persistent organic pollutants (POPs) in foods from the first regional Sub-Saharan Africa Total Diet Study. Environment International 135, 105413. https://doi.org/10.1016/j.envint.2019.105413

Vethaak, A.D., Davies, I.M., Thain, J.E., Gubbins, M.J., Martínez-Gómez, C., Robinson, C.D., Moffat, C.F., Burgeot, T., Maes, T., Wosniok, W., Giltrap, M., Lang, T., Hylland, K., 2017. Integrated indicator framework and methodology for monitoring and assessment of hazardous substances and their effects in the marine environment. Marine Environmental Research 124, 11–20. https://doi.org/10.1016/j.marenvres.2015.09.010

Vincent-Hubert, F., Arini, A., Gourlay-Francé, C., 2011. Early genotoxic effects in gill cells and haemocytes of Dreissena polymorpha exposed to cadmium, B[a]P and a combination of B[a]P and Cd. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 723, 26–35. https://doi.org/10.1016/j.mrgentox.2011.03.008

Vitale, F., Worsøe Clausen, L., Ní Chonchúir, G., 2019. Handbook of fish age estimation protocols and validation methods (report). ICES Cooperative Research Reports (CRR). https://doi.org/10.17895/ices.pub.5221

Voorspoels, S., Covaci, A., Maervoet, J., Schepens, P., 2004. PCBs and OCPs in marine species from the Belgian North Sea and the Western Scheldt Estuary. ORGANOHALOGEN COMPOUNDS 66.

Voorspoels, S., Covaci, A., Schepens, P., 2003. Polybrominated diphenyl ethers in marine species from the Belgian North Sea and the Western Scheldt Estuary: levels, profiles, and distribution. Environ Sci Technol 37, 4348–4357. https://doi.org/10.1021/es034503r

Walsh, S.J., Astarloa, J.M.D. de, Poos, J.J., 2015. Atlantic flatfish fisheries, in: Flatfishes: Biology and Exploitation, 2nd Edition. pp. 346–394. https://doi.org/10.1002/9781118501153.ch13

Wessel, N., Le Dû-Lacoste, M., Budzinski, H., Burgeot, T., Akcha, F., 2013. UPLC MS/MS Quantification of Primary Metabolites of Benzo[a]pyrene and Fluoranthene Produced In Vitro by Sole (Solea solea) Liver Microsomal Activation. Polycyclic Aromatic Compounds 33, 52–71. https://doi.org/10.1080/10406638.2012.725197

Wessel, N., Ménard, D., Pichavant-Rafini, K., Ollivier, H., Le Goff, J., Burgeot, T., Akcha, F., 2012. Genotoxic and enzymatic effects of fluoranthene in microsomes and freshly isolated hepatocytes from sole (Solea solea). Aquatic Toxicology, Proceedings from the 16th International Symposium on Pollutant Responses in Marine Organisms (PRIMO16) 108, 33–41. https://doi.org/10.1016/j.aquatox.2011.09.024

Wessel, N., Santos, R., Menard, D., Menach, K.L., Buchet, V., Lebayon, N., Loizeau, V., Burgeot, T., Budzinski, H., Akcha, F., 2010. Relationship between PAH biotransformation as measured by biliary metabolites and EROD activity, and genotoxicity in juveniles of sole (Solea solea). Marine Environmental Research 69, S71–S73. https://doi.org/10.1016/j.marenvres.2010.03.004

Westernhagen, H. v., Krüner, G., Broeg, K., 1999. Ethoxyresorufin O-deethylase (EROD) activity in the liver of dab (Limanda limanda L.) and flounder (Platichthys flesus L.) from the German Bight. EROD expression and tissue contamination. Helgoland Marine Research 53, 244–249. https://doi.org/10.1007/s101520050027

Zafeiraki, E., Gebbink, W.A., Hoogenboom, R.L.A.P., Kotterman, M., Kwadijk, C., Dassenakis, E., van Leeuwen, S.P.J., 2019. Occurrence of perfluoroalkyl substances (PFASs) in a large number of wild and farmed aquatic animals collected in the Netherlands. Chemosphere 232, 415–423. https://doi.org/10.1016/j.chemosphere.2019.05.200

Differences in biomarker responses and chemical contamination among three flatfish species in the Bay of Seine (NE Atlantic)

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Supplementary material

SM 1: Supplementary material 1: Methods used for the determination of trace metal concentrations in flatfish

Total mercury (Hg) concentrations were determined in fish muscle according to the standard operating procedure described in the US-EPA method N◦7473 (US EPA, 1998) on aliquots of homogenized powder (~40±10 mg), by atomic absorption spectrophotometry using an Advanced Mercury Analyzer (ALTEC AMA-254, Altec Ltd). Limits of detection (LOD) and quantification (LOQ) were 0.005 and 0.015 mg kg^{-1} dry weight (dw), respectively. In addition to total Hg concentrations, methyl-mercury (met-Hg) determination was performed in fish muscle according to a method adapted from Azemard and Vassileva (2015), which uses liquid-liquid extractions of met-Hg in samples before analysis by AMA-254. Briefly with this method, aliquots of powder (10–200 mg, depending on total Hg concentrations) were acidified with 5 mL of HCl (25% v/v, prepared with ultrapure HCL) to solubilize Hg, 10 mL volume of toluene were then added and both phases were homogenized. After centrifugation, a fraction (5 mL) of the upper organic phase (i.e. toluene containing extracted met-Hg) was transferred to a second tube containing the same volume of a 0.002 M sodium thiosulfate solution. This tube was vigorously shaken, centrifuged, and an aliquot of a known volume (50–500 µL) of the lower phase (containing the back-extracted methyl-Hg) was finally directly analyzed with AMA-254.

Total silver (Ag), cadmium (Cd), lead (Pb), copper (Cu) and zinc (Zn) concentrations were determined in fish liver, according to an in-laboratory approved method and using a Quadrupole Inductively Coupled Plasma Mass Spectrometer (Q-ICP-MS, ICAP-Qc model from ThermoFisher). Briefly with this method, aliquots of homogenized powder (~200 mg) were placed in Teflon bombs and mineralized with a mixture of ultrapure HNO_3 acid and milli-Q water using a microwave (ETHOS-UP model from Milestone). Then, the digests were diluted to 50 mL with ultrapure water before analyses with Q-ICP-MS. The LODs and LOQs were respectively 0.02 and 0.07 mg kg^{-1} dw for Ag, Cd and Pb, 0.3 and 0.9 mg kg^{-1} dw for Cu, 2.8 and 9.3 mg kg^{-1} dw for Zn.

The quality assurance of all metal analyses relied on blank and internal standard controls and on the accuracy and reproducibility of data relative to certified reference materials (CRMs) analyzed in each analytical run. The CRMs used were i) for total Hg: IAEA-142 (mussel homogenate, International Atomic Energy Agency/IAEA) and IAEA-407 (whole-fish homogenate, IAEA); ii) for met-Hg: IAEA-407; iii) for Ag, Cd, Pb, Cu and Zn: DORM-4 (fish protein, National Research Council Canada/NRCC), DOLT-5 (dogfish liver, NRCC) and CE-278k (mussel tissue, Joint Research Centre-European Commission). Blank values

were systematically below the LODs and CRM results concurred with certified concentrations, with average recovery rates ranging between 88 and 109% for the different metals and CRMs.

Supplementary material 2: Methods used for the determination of organic contaminant concentrations in flatfish

Organic contaminant analyses in sole and flounder were carried out in IFREMER, Nantes, France and have been fully described elsewhere (Munschy et al., 2020). PCB, OCP and PBDE analyses were performed on 4-5 g dw of the freeze-dried muscle samples extracted by dichloromethane using pressurized liquid extraction (PLE) with a Dionex ASE 300 (ASE, Dionex Corp., USA), followed by several purification steps including gel permeation chromatography, a silica and alumina column, and a two-dimensional HPLC system with two columns coupled in series: a nitrophenylpropylsilica column (Nucleosil, 5 µm particles, 250x4.6 mm, Interchim, France) in series with a 2-(1-pyrenyl)ethyldimethylsilylated silica (PYE) column (Cosmosil, 5 µm particles, 150x4.6 mm, Promochem, France). The concentrations of the 7 ICES priority PCB congeners (CB-28, -52, -101, -118, -138, -153 and -180) and 8 PBDE congeners (BDE-28, -47, -49, -99, -100, -153, -154, -183) were determined using gas chromatography coupled to high resolution mass spectrometry (GC-HRMS, Hewlett-Packard 6890 GC coupled to a Micromass AutoSpec Ultima MS) by isotopic dilution with each congener's $^{13}C_{12}$-labelled isomer.

Among per- and polyfluoroalkyl substances, only the most abundant substance perfluorooctane sulfonate (PFOS) was investigated. PFOS analyses were conducted on 1 g dw of freeze-dried muscle samples extracted using liquid-solid extraction with MeOH/KOH (0.01 M of KOH), purified onto two consecutive SPE cartridges (an OASIS WAX weak anion exchange stationary phase, Waters, and an ENVI-Carb graphite stationary phase, Sigma-Aldrich), evaporated to dryness and reconstituted in 200 µL of a mixture of MeOH:H_2O (50:50, v/v). Quantification was achieved by isotopic dilution against $^{13}C_8$-PFOS 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 a Z-spray (Waters Corp.) electrospray ionization source operated in negative mode.

Organic contaminant analyses in dabs were carried out in LABERCA (Laboratoire d'étude des Résidus et Contaminants dans les Aliments), ONIRIS, France and have been fully described elsewhere (Vaccher et al., 2020). Briefly, PCBs and PBDEs were extracted from freeze-dried sample using PLE with a mix of toluene and acetone (70/30; v/v) and purified using three successive purification columns: a multi-layer acidified (22% and 44% sulfuric acid) silica column eluted with hexane, a florisil column eluted with hexane and toluene and a carbon column eluted with toluene. All analyses were done by GC-HRMS. OCP concentrations were determined after extraction using DCM/hexane followed by purification on a florisil column eluted with hexane, DCM and ethyl acetate, and injection into a GC-MS/MS system. PFOS was analyzed by liquid-solid extraction followed by purifications onto two consecutive SPE cartridges (WAX and ENVI-Carb) and injected into a LC-ESI(-)-MS/MS system. Analytical performances were validated according to accredited methods.

Table S1. PCB and PBDE congeners measured in the flatfish. For each parameter, descriptive statistics (n =number of individuals, n < LOQ = number of measurements below the quantification limit) and significant differences among fishing areas and species are indicated (F =flounder, D =dab and S =sole).

Parameter	Tissue	Unit	n			Median			Minimum			Maximum			n < LOQ			Fishing area effect[1]			Species effect	
			F	D	S	F	D	S	F	D	S	F	D	S	F	D	S	F	D	S	signif[2]	Ranking
Congeners expressed in wet weight																						
CB-28	muscle	ng/g ww	17	15	14	0.18	0.13	0.06	0.08	0.05	0.02	0.33	0.32	0.16	0	0	0	*	ns	*	***	F = D > S
CB-52	muscle	ng/g ww	17	15	14	1.25	0.92	0.46	0.45	0.25	0.16	2.23	2.11	1.22	0	0	0	*	ns	*	**	F > S[3]
CB-101	muscle	ng/g ww	17	15	14	2.86	2.51	1.54	1.12	0.80	0.60	5.87	6.65	3.39	0	0	0	*	ns	*	**	F > S[3]
CB-118	muscle	ng/g ww	17	15	14	2.78	2.28	1.32	1.16	0.79	0.58	4.28	5.90	2.51	0	0	0		ns		**	F = D > S
CB-138	muscle	ng/g ww	17	15	14	4.03	4.17	2.11	1.79	1.42	0.95	7.43	10.86	4.45	0	0	0	*	ns	*	**	D = F > S
CB-153	muscle	ng/g ww	17	15	14	7.43	6.93	3.62	3.00	2.57	1.64	12.78	20.60	7.77	0	0	0	*	ns	*	**	F = D > S
CB-180	muscle	ng/g ww	17	15	14	1.35	1.33	0.30	0.27	0.40	0.11	2.61	3.97	1.79	0	0	0	*	ns	*	***	F = D > S
BDE-28	muscle	pg/g ww	17	15	14	3.39	1.31	0.50	0.88	0.22	0.16	9.06	2.34	1.58	0	0	0	*	ns	*	***	F > D > S
BDE-47	muscle	pg/g ww	17	15	14	59.53	27.88	6.39	27.25	11.44	2.08	189.34	61.52	22.84	0	0	0	ns	ns	*	***	F > D > S
BDE-49	muscle	pg/g ww	17	15	14	12.31	5.34	2.86	7.83	1.77	0.77	22.02	11.35	11.55	0	0	0	*	ns	*	***	F > D = S
BDE-99	muscle	pg/g ww	17	15	14	6.40	1.74	0.60	1.18	0.31	0.17	15.98	12.97	2.74	0	0	3	*	ns	-	***	F > D = S
BDE-100	muscle	pg/g ww	17	15	14	18.49	10.08	5.55	10.34	4.32	2.15	37.90	24.83	14.53	0	0	0	ns	ns	*	***	F > D > S
BDE-153	muscle	pg/g ww	17	15	14	3.58	1.76	0.38	0.85	0.43	0.18	6.32	5.91	1.57	0	0	6	*	ns	-	***	F = D > S
BDE-154	muscle	pg/g ww	17	15	14	15.11	7.73	6.10	6.84	2.94	2.56	24.06	20.55	16.96	0	0	0	ns	ns		***	F > D = S
BDE-183	muscle	pg/g ww	17	15	14	0.67	0.58	0.17	0.19	0.23	0.10	1.78	13.36	0.78	0	2	6	*	-	-	-	[2]F = D; F > S
Congeners expressed in lipid weight																						
CB-153	muscle	ng/ g lw	17	15	14	1081	458	956	171	118	438	4667	974	2224	0	0	0	*	*		***	F = S > D
BDE-47	muscle	pg/ g lw	17	15	14	10025	2054	2171	1392	658	850	32414	3531	4530	0	0	0	ns	*		***	F > S = D

[1] Spatial effect per species, when there are no censored data: Kruskall-Wallis test and Wilcoxon with Bonferroni correction as post hoc test: ns: non significant, * p<0.05

[2] Species effect at the scale of the Bay of Seine: when there are ≤ 6/46 censored data, when censored data are substituted by the LOQ: Kruskall-Wallis test and Wilcoxon with Bonferroni correction as post hoc test: ns: non significant, * p<0.05, ** p<0.01, *** p<0.001; when there are > 6/46 censored data: Median exact test of Fischer (p < 0.05)

[3] When only 2 species are indicated, the third one has a value intermediate and similar to both other species.

-: not applicable (when there are censored data for the spatial effect and >8/46 censored data for the species effect).

References

Azemard, S., Vassileva, E., 2015. Determination of methylmercury in marine biota samples with advanced mercury analyzer: method validation. Food Chem 176, 367–375. https://doi.org/10.1016/j.foodchem.2014.12.085

Munschy, C., Vigneau, E., Bely, N., Héas-Moisan, K., Olivier, N., Pollono, C., Hollanda, S., Bodin, N., 2020. Legacy and emerging organic contaminants: Levels and profiles in top predator fish from the western Indian Ocean in relation to their trophic ecology. Environmental Research 188, 109761. https://doi.org/10.1016/j.envres.2020.109761

US EPA, O., 1998. EPA Method 7473 (SW-846): Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry [WWW Document]. URL https://www.epa.gov/esam/epa-method-7473-sw-846-mercury-solids-and-solutions-thermal-decomposition-amalgamation-and (accessed 7.17.23).

Vaccher, V., Ingenbleek, L., Adegboye, A., Hossou, S.E., Koné, A.Z., Oyedele, A.D., Kisito, C.S.K.J., Dembélé, Y.K., Hu, R., Adbel Malak, I., Cariou, R., Vénisseau, A., Veyrand, B., Marchand, P., Eyangoh, S., Verger, P., Dervilly-Pinel, G., Leblanc, J.-C., Le Bizec, B., 2020. Levels of persistent organic pollutants (POPs) in foods from the first regional Sub-Saharan Africa Total Diet Study. Environ Int 135, 105413. https://doi.org/10.1016/j.envint.2019.105413