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 HNO3 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 13C12-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:H2O (50:50, v/v). 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.

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).

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Parameter | Tissue | Unit | n | | | Median | | | Minimum | | | Maximum | | | | n < LOQ | | | | | Fishing area effect1 | | | Species effect | |
| F | D | S | F | D | S | F | D | S | | F | D | S | | F | D | S | F | | D | S | signif2 | 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 > S3 |
| 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 > S3 |
| 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 | \* | | - | - | - | 2F = 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).

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