Environmental Pollution
January 2022, Volume 292, Part B, Pages 118388 (15p.)
https://doi.org/10.1016/j.envpol.2021.118388
https://archimer.ifremer.fr/doc/00732/84400/

Risk and benefit assessment of seafood consumption harvested from the Pertuis Charentais region of France

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Abstract :

Seafood is well recognized as a major source of Long Chain n-3 Polyunsaturated Fatty Acids (LC n-3 PUFA, especially ecosapentaenoic acid, i.e. EPA and docosaheaxaenoic acid, i.e. DHA) and essential trace elements (As, Cu, Fe, Mn, Se, and Zn). It is also a source of non-essential trace elements (Ag, Cd, Hg, Pb) that can be deleterious for health even at low concentrations. Edible parts of sixteen species (fish, cephalopods, crustaceans and bivalves) of great importance in the Pertuis Charentais region, one of the main shellfish farming and fishing areas, were sampled in winter and analyzed to determine their fatty acid (FA) composition and trace element concentrations. Based on these analyses, a suite of indices was calculated to estimate risk and benefit of seafood consumption: the n-6/n-3 ratio, the atherogenic index, the thrombogenic index, the EPA + DHA daily recommended portion, as well as the maximum safe consumption. The results showed that fish contributed the most to LC n-3 PUFA supply, while bivalves and crustaceans were more beneficial in essential trace elements. Whatever the species, the concentrations of non-essential elements were not limiting for seafood consumption, as important amounts of the analyzed species can be eaten daily or weekly before becoming deleterious to consumers. Yet, concentrations of Hg in dogfish and seabass can become a concern for frequent seafood consumers (>three meals a week), confirming that varying seafood items is a key point for consumers to optimize the benefits of diverse seafood resources. Considering FA composition, whiting and pilchard are the most beneficial fish species for human diet, while surmullet was the least beneficial one. However, using an index integrating the relative risk due to Hg content, the surmullet appears as one of the most beneficial. This study provides a temporal shot of the quality of marine resources consumed in winter period in the studied area and highlights the complexity of a quantitative risk and benefit assessment with respect to the biochemical attributes of selected seafood.

Keywords : LC n-3 PUFA, Essential and non-essential trace elements, Mercury, Net risk/benefit index

1. **Introduction**

Seafood (i.e. fish, crustaceans and mollusks) is currently a significant component of food sources for humans worldwide, especially for whose that live in coastal areas (60% of the world's population). The average annual intake per capita had increased from 9.0 kg in 1961, to 20.5 kg in 2017 (FAO, 2018). Seafood is part of a well-balanced human diet with several recognized benefits because, e.g. they are rich in proteins, vitamins, omega-3 fatty acids (FAs) and essential elements such as copper (Cu), iron (Fe), or zinc (Zn).

Seafood is particularly recognized as the main source of long chain highly unsaturated fatty acids of the n-3 series (LC n-3 PUFA, i.e fatty acids with at least 4 double bonds and 20 carbon atoms), also known as LC omega-3 PUFA (*e.g.* Afonso *et al.*, 2013). LC n-3 PUFA, and especially the highly unsaturated ones , are major components of cell membranes but are poorly synthetized *de novo* by vertebrates including humans and thus must be supplied by food. Among these, eicosapentenoic acid (EPA, 20:5n-3) and docosahexanoic acid (DHA, 22:6n-3) in human diet are sourced mostly from seafood (Astorg *et al.*, 2004). EPA and DHA are the most beneficial LC n-3 PUFA. Their benefits on human health, and in particular in cerebral, cardiovascular, and immune functions, are now well-recognized (Gil & Gil, 2015; Mozaffarian & Rimm, 2006; Pike, 1999; Ruxton *et al.*, 2004; Simopoulos, 1991). For example, regular intakes of LC n-3 PUFA help in reducing of risk of death from a coronary heart disease through the reduction of atherogenic and thrombongenic risks (i.e. reduction of the platelet aggregation and subsequent thrombus and atheroma formation in the cardiovascular system; Valfré *et al.*, 2003). LC n-3 PUFA regular intake also induce a drop of dementia disorders and Alzheimer symptoms in elderly people (Hu *et al.*, 2002; Morris *et al.*, 2003; Oomen *et al.*, 2000; Ruxton *et al.*, 2004). The improvement of neuronal development and visual acuity of breast-fed infants by women who regularly consume marine products has also been demonstrated (Fleith & Clandinin, 2005; Koletzko *et al.*, 2008; Simopoulos, 1991). As well as a regular LC n-3 PUFA intake, a balanced dietary n-6/n-3 PUFA ratio between 1:1 and 4:1 has been shown to prevent the onset of coronary heart disease, as n-3 contribute to anti-inflammatory and anti-thrombus process, while n-6 contribute to inflammatory process (Simopoulos *et al.*, 2000; Simopoulos, 2002; 2003).

Seafood intake is, as well, one of the main sources of essential trace elements such as arsenic (As), Cu, Fe, manganese (Mn), selenium (Se) and Zn (*e.g.* Guerin *et al.*, 2011). In organisms, these elements are also cofactors of enzymes involved in antioxidant systems, in the DNA metabolism, and in the oxygen transport (Ansari *et al.*, 2004; Olmedo *et al.*, 2013a; Uthus, 1992). However, these essential elements may be at risk when too high amounts are ingested (Ansari *et al.*, 2004; Ersoy & Çelik, 2009; Frieden, 1985; Goldhaber, 2003; Olmedo *et al.*, 2013a; Storelli, 2009), as they become toxic like other contaminants usually found in seafood. Indeed, mainly fisheries and aquaculture activities occur in coastal waters, where anthropogenic activities (*e.g.* agriculture, industry, urbanization, oil extraction) contribute to the enrichment of waters in essential elements such as As, Cu, Fe, Mn, Se and Zn but also in non-essential elements like silver (Ag), cadmium (Cd), mercury (Hg) and lead (Pb), which are highly toxic even at low concentrations (Leblanc *et al.*, 2006; Maher & Butler, 1988; Naser, 2013; Olmedo *et al.*, 2013b). Some non-essential elements (e.g. Hg or Pb) can also biomagnify along the food chain, reaching elevated concentrations in marine predators regardless of ambient contaminations (Anual *et al.*, 2018; Langston & Bebianno, 1998; Maher & Butler, 1988; Olmedo *et al.*, 2013b, Eagles-Smith *et al.*, 2018). The consumption of seafood contaminated by these trace elements could thus put humans at risk, potentially leading to neurotoxic, carcinogenic or cardiovascular issues (Ansari *et al.*, 2004; Ersoy & Çelik, 2009; Goldhaber, 2003; Leblanc *et al.*, 2006).

The coast of Charente-Maritime and its adjacent Pertuis Charentais area hosts the largest network of intertidal bare mudflats in France, conferring to the area a high primary productivity of the littoral zone that are advantageous for fisheries and shellfish farming (Blanchard *et al.*, 2001). The Marennes-Oléron bay is the first European basin for oyster farming (Miossec *et al.*, 2009). The Pertuis Charentais area and the onshore Bay of Biscay support artisanal fisheries targeting local species such as *Merluccius merluccius, Merlangius merlangus, Lophius piscatorius, Sardina pilchardus, Scomber scombrus and Sepia officinalis* among others (FranceAgriMer, 2017). This highly touristic region is also known for its historical Cd, Cu, and Zn contamination originating from the discharge of a mine treatment wastes upstream from the Gironde River mouth (Grousset *et al.*, 1999; Miquel, 2001; Miramand *et al.*, 2002). Industrial wastes are also an important source of Hg in the Charente River which emerges directly in the Pertuis Charentais (Gagnaire *et al.*, 2003). More recently, an increasing contamination pressure by Ag used as nanoparticles with antimicrobial properties and as hail clouds dispersive agent to protect regional wines also has occurred in coastal waters of this area (Salles *et al.*, 2013).

Despite this recurrent and historic contamination, only few studies consider the nutritional quality of the seafood products from this worldwide important shellfish farming and fishing area (Guérin *et al.*, 2011). In this context, this study assessed the quality of wild or extensive farmed seafood from a unique geographical area, both in terms of FAs and trace elements, and estimated concomitantly the risk and the benefice of their consumption. A total of sixteen marine highly consumed species including fish, crustaceans and mollusks were collected within the Pertuis Charentais area in winter. Their compositions in fatty acids (FA) and trace elements were determined. Based on these data, the exposure of local seafood consumers to beneficial FAs and essential elements, and to potentially non-essential metallic contaminants was assessed. Hazards and benefits related to seafood consumption were characterized using national and international recommendations and by applying composite metrics.

2. Materials and methods

2.1. Ethics statement

The species sampled are not protected or endangered species in the fishing area of the Pertuis Charentais. No field permits or ethical approvals were required for this study, as all species originated from commercial fisheries and were already dead when provided to us. Fish were sacrificed by the commercial fishers at sea using standard fisheries practices (all fish were dead when landed).

2.2. *Sample collection and preparation*

Sixteen marine species were purchased from a local fishmonger between November 2018 and February 2019, including fish (11 species including 1 cartilaginous and 10 teleost species), crustaceans (1 species), cephalopods (2 species) and bivalves (2 species; Table 1). Animals were fished maximum 2 days before being purchased and conserved on ice since fishing. Two brands (“Spéciale”, *i.e.* cultured in coastal waters, and “Fine de Claire”, *i.e.* refined for minimum 4 weeks in saltmarsh clay ponds) of cupped oyster species coming from a shellfish farmer of Oléron Island were purchased. All the specimens were weighed and measured (Table 1). On each individual, the edible part (*i.e.* muscle for fish, cephalopods, crustaceans, and muscle as well as gonad for the great Atlantic scallops) was collected in duplicate. One replicate was used for FAs analyses and the other one for trace element analyses. For oysters, the entire soft edible tissues of two oyster individuals were pooled and split in two subsamples for FAs and trace element analyses. All samples dedicated to further FA analyses were directly dropped into liquid nitrogen and then stored at -80°C. Samples dedicated to trace element analyses were wet weighed (ww) and stored at -20°C.

2.3. *FAs analysis*

The platform “LIPIDOCEAN” (UMR 6539 - Laboratory of Environmental Marine Sciences, Plouzané, France) fulfilled the determination of FAs qualitative and quantitative compositions in specimens, according to the protocol described in Mathieu-Resuge et al. (2020), except that we performed analyses on the total lipid fraction without separating neutral and polar lipids. Briefly, frozen muscles and soft tissues were firstly homogenized by ball mill in liquid nitrogen. Total lipids from approximatively 250 mg of tissue powder were extracted in 6 mL of chloroform-methanol (2:1, v/v). The total lipid extract was then sonicated 5 min at 4°C and stored at -20°C under N_2 gas. An aliquot of the total lipid extract (1 out of 6 mL) was transmethylated for 10 min at 100°C, after evaporation to dryness and addition of 2.3 µg of an internal standard (tricosanoic acid C23:0) and 800 µL of methanol/H_2SO_4 (3.4%; v/v). Resulting FA methyl esters (FAME) were recovered with 800 µL of hexane and washed 3 times with 1.5 mL of hexane-saturated distilled water. FAME were then analyzed by gas chromatography coupled to a flame-ionization detector (GC-FID) on a Varian CP8400 gas chromatograph equipped with splitless injectors. FAME were separated simultaneously on two columns, one polar (ZBWAX: 30 m × 0.25 mm ID × 0.2 µm, Phenomenex) and one apolar (ZB5HT: 30 m × 0.25 mm ID × 0.2 µm, Phenomenex). FAME were identified by comparison of their retention time on both columns with those of commercial standards or lab-made standards mixtures (chromatograms are presented in supplementary materials). FA were named as C :Yn-Z where C is the number of carbon of the aliphatic chain, Y, the number of unsaturation and Z the position of the 1st unsaturation from the terminal carbon.

The FAs analysis procedure was assessed by comparing the quantities measured with C23:0 with theoretical amount of each FA present in a standard mixture of different FA included into different lipid classes in different proportions to attest to the eventual impact of potential bias on the different FA, different class of lipid, proportion considered as well as their initial quantity (50 µg vs 100 µg vs 150 µg). The repeatability was estimated with 4.2% for the GC and 11.9% of variation for the whole FA analysis (Sardenne *et al.*, 2021). A blank was realized for each sample series (1 blank every 14 samples). Blanks follow exactly the same analytical process as samples, from lipid extraction to GC analysis. Blank subtraction was performed as they contained 16:0 and 18:0 traces. The calibration was performed using a FA mixture of known theoretical mass composition of Supelco® 37 Component FAME Mix. The mass percentage values calculated for this certified standard was compared to its known certified values and remained within the 5% of error for the polar column and 6% of error for the apolar column. The GC-FID analyses conducted during our experiment can then be considered as suitable to obtain correct semi-quantitative values for all the fatty acids.

The FAME quantification was made using the standard C23:0 added to each sample before transesterification. The equation used was the following:

Quantity FA (g) = (Area FA X Quantity C23:0) / Area C23:0

Concentration of each FA was estimated by considering the mass of tissue extracted, the total volume of lipid extract and the aliquote volume of the lipid extract used for lipid analyses.

The results of each individual FA were expressed as mass percent of the total FA content (% TFA) and were also given as concentrations (in mg of FA per g of wet sample, mg g^{-1} ww).

2.4. *Trace elements analysis*

Frozen samples were freeze-dried for 36 to 48 hours (Chris® BETA 1-8 LDplus). Then, they were weighed for dry weight (dw) before being homogenized in a porcelain mortar and pestle. Aliquots ~ 200 mg dw tissues were microwave digested with a mixture of 3ml of suprapure nitric acid (VWR/Merck) and 1ml of suprapure chlorhydric acid (VWR/Merck). Trace elements (Ag, As, Cd, Cu, Fe, Mn, Pb, Se, and Zn) were analyzed using an Inductively Coupled Plasma Mass Spectrometry (ICP-MS II Series Thermo Fisher Scientific) and an Inductively Coupled Plasma Atomic Emission Spectrometry (Varian Vista-Pro ICP-AES), as described by Kojadinovic *et al.* (2011). The limits of detection (LOD) ranged from 0.01 μg g^{-1} dw (e.g. Ag, Cd, Pb) to 5 μg g^{-1} dw (Fe). Accuracy and reproducibility were assessed by analyzing procedural blanks and Certified Reference Material (CRM) (DOLT-5 dogfish liver from National Research Council, Canada and IAEA-436 tuna fish flesh homogenate from the International Atomic Energy Agency IAEA). Recovery rates were 96 ± 11 % for DOLT-5 (from 74 % to 118 %) and 103 ± 8 % for IAEA-436 (from 93 % to 120 %).

For Hg analysis, aliquots ranging from 5 to 10 mg dw were analyzed with an Advanced Mercury Analyser spectrophotometer (Altec AMA 254, LOD of 0.05 ng). The accuracy was checked using the CRM DOLT-5 with certified Hg concentration: 0.350 ± 0.005 μg g^{-1} dw. Analyses were repeated, for each individual, twice or three times until getting a relative standard deviation (SD) < 10 %. All the trace elements concentrations were obtained in μg g^{-1} dw and then transformed and expressed in μg g^{-1} ww throughout the manuscript.

2.5. *Data treatment and statistics*

Prior to data analysis, values of trace element concentrations below the limit of detection (LOD) were replaced by the lowest measured value of the corresponding element multiplied by 0.5 (Guérin *et al*., 2011; Olmedo *et al.*, 2013ab).

A Principal Component Analysis (PCA) was fulfilled to study relationships between FA and trace element concentrations in species, using 'FactoMineR' (Le *et al.*, 2008) and 'Factoextra' (Kassambara *et al.*, 2017) packages on R. Individuals with non-available value (NA) of trace elements concentrations were removed from the PCA. PCA was based on correlation matrix and normalized data, centered and divided by the standard deviation, for each variable.

The comparisons of FA and trace element concentrations between the species were tested using means comparison tests, using 'ggrepel' (Slowikowski, 2019), 'tidyverse' (Wickham, 2017), 'cowplot' (Wilke, 2019), and 'multcompView' (Graves *et al.*, 2015) packages on R. One-way ANOVA and Tukey's post-hoc tests were also performed. The conditions of application of parametric tests were determined *a posteriori* by checking the normality and homoscedasticity of data residuals using a Shapiro-Wilk test and a Bartlett test respectively. If these conditions were not respected, non-parametric tests (Kruskal-Wallis and Wilcoxon signed-rank test without adjustment method) were used. The significant level of statistical analyses was set at $\alpha = 0.05$. Species with individuals having trace elements concentrations below the LOD were not considered for the inter-specific comparison. Results presented with boxplots were arranged in descending order for each taxon, based on the means.

The nutritional quantity and quality in terms of FA of the studied species was compared using a hierarchical clustering analyses focusing on (i) two nutritional quantity indices (real DHA and EPA available quantity in biomass), and (ii) six quality indices (LC n-3 PUFA/TFA, n-6/n-3, EPA/TFA, DHA/TFA, the atherogenic index (AI) and the thrombogenic index (TI) – see below) in the different species using 'Pretty Heatmaps' (Kolde, 2019) and 'ColorBrewer Palettes' (Neuwirth, 2014) packages on R.

All data analyses and graphical representations were performed with R version 3.5.0 (R Core Team, 2018).

2.6. *Risk and benefit assessment*

Considering essential and non-essential element concentrations, as well as FA composition, some indices were calculated to estimate risk and benefit of seafood intake.

2.6.1. n-6/n-3 PUFA ratio

The ratio between n-6 and n-3 series concentrations, named as n-6/n-3, was determined for each species. The FA considered were: 16:2n-6, 16:3n-6, 18:2n-6, 18:3n-6, 18:4n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:2n-6, 22:4n-6, 22:5n-6 for n-6 series; and: 16:3n-3, 16:4n-3, 18:3n-3, 18:4n-3, 18:5n-3, 20:3n-3, 20:4n-3, 20:5n-3, 21:5n-3, 22:5n-3, 22:6n-3 for n-3 series. A n-6/n-3 ratio in seafood between 1:1 and 4:1 indicates an improvement in the balance of the contribution of FAs in the human diet and to prevent the onset of coronary heart disease (Simopoulos, 2002).

2.6.2. Atherogenic and thrombogenic indices

The dietary factors involved in the onset of coronary heart disease are directly correlated to qualitative aspects of the lipid fraction. The MUFA and the PUFA of the series n-3 and n-6 seem to have equal role in the prevention of thrombus development, while the n-3 PUFA seem to be more important in the limitation set atheroma. Long-chain SFA (14:0, 16:0, 18:0) accelerate thrombus formation, by reducing the production of arterial prostacylin, a strong antagonist of platelet aggregation, while many studies indicate that long-chain unsaturated fatty acids slow down intra-arterial occlusion and platelet aggregation. Ulbricht and Southgate (1991) summed up these numerous effects through equations for the calculations of the index of thrombogenicity index (TI) and the atherogenicity index (AI). In these indices different weights are attributed to these categories of FA in relation to their different contribution to the prevention or promotion of intra-arterial occlusion and platelet aggregation. The atherogenic (AI) and thrombogenic (TI) potentials of a resource were thus evaluating according to Ulbricht & Southgate (1991) formula:

$$[AI = (12:0 + 4*14:0 + 16:0) / ((n\text{-}6\ PUFA + n\text{-}3\ PUFA) + 18:1n\text{-}9 + other\ MUFA)]\ (in\ mg\ g^{-1}),$$

where 12:0, 14:0, 16:0 are saturated FAs; n-6 PUFA and n-3 PUFA are, respectively, the sum of the polyunsaturated FAs from n-6 series (16:2n-6, 16:3n-6, 18:2n-6, 18:3n-6, 18:4n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:2n-6, 22:4n-6, 22:5n-6) and n-3 series (16:3n-3, 16:4n-3, 18:3n-3, 18:4n-3, 18:5n-3, 20:3n-3, 20:4n-3, 20:5n-3, 21:5n-3, 22:5n-3, 22:6n-3); 18:1n-9 is a monounsaturated FA and MUFA is the sum of all other monounsaturated FAs (14:1n-5, 16:1n-11, 16:1n-9, 16:1n-7, 16:1n-5, 18:1n-11, 18:1n-7, 18:1n-5, 20:1n-11, 20:1n-9, 20:1n-7, 22:1n-11, 22:1n-9, 22:1n-7, 24:1n-9). But the 12:0 was not detected in the present study, so it was not considered in the formulation.

[TI = (14:0 + 16:0 + 18:0) / (0.5*18:1n-9 + 0.5*other MUFA + 0.5*n-6 PUFA + 3*n-3 PUFA + (n-3 PUFA / n-6 PUFA))] (in mg g^{-1}),

where 14:0, 16:0, 18:0 are saturated FAs; 18:1n-9 is a monounsaturated FA; MUFA is the sum of monounsaturated FAs; n-6 PUFA and n-3 PUFA are the sum polyunsaturated FAs from n-6 and n-3 series respectively, as detailed for the AI.

2.6.3. *Daily recommended portion and maximum safe consumption*

A daily recommended portion was determined for each species and corresponded to the intake (expressed in g per day) needed to achieve the 250 mg EPA + DHA daily dietary requirement for an adult (FAO/WHO, 2010). It was thus a function of the sum of EPA and DHA concentrations measured per species. Then, results were compared to the European value of a serving, i.e. 150 g (Roth & Knai, 2003).

The maximum safe consumption of each species considering their supply in each trace elements was estimated through the Maximum Safe Consumption calculation (MSC) (Metian *et al.*, 2013), for a trace element A:

[$MSC_A = (W_{ind} * JL_A) / X_A$] (in g ww per time unit),

where W_{ind} is the mean human body weight (bw, average of 70 kg); JL_A is the Provisional tolerable monthly intake (PTMI, in µg kg^{-1} ww bw) or the Provisional Tolerable Weekly Intake (PTWI, in µg kg^{-1} ww bw) or Provisional Maximum Tolerable Daily Intake (PMTDI, in µg kg^{-1} ww bw) of A; X_A is the mean concentration of A (in µg g^{-1} ww) in seafood. Data under the LOD were not considered in the calculations of indices.

3. Results

3.1. *FA and trace elements and concentrations in seafood*

FAs (TFA, LC n-3 PUFA, DHA, and EPA) and trace elements (Ag, As, Cd, Cu, Fe, Hg, Mn, Pb, Se, and Zn) concentrations in edible tissues of seafood species studied were included in a PCA (Fig. 1). The first and the second principal components, with respectively 43% and 26%, accounted for 69% of the total variation in the analysis. Concentrations of Ag, Cd, Cu, Fe, Mn, Pb, and Zn contributed the most to the first dimension, while TFA, LC n-3 PUFA, EPA, and

DHA concentrations contributed the most to the second principal component. The As, Hg, and Se concentrations were not explained by the two first components according to the correlation circle (Fig. 1A). No correlation was observed between FAs and trace elements concentrations. The projection of individuals showed that the majority of the species were gathered at the origin of the PCA, meaning they were not discriminated by their FA nor trace element contents (Fig. 1B). Nevertheless, the PCA strongly discriminated the cupped oysters “Fine de Claire” (OFC) and “Spéciale” (OSP), the gonad of great Atlantic scallop (GSG) from the other species according to the first component because of their high trace element concentrations. In contrast, the Atlantic mackerel (MKR), the surmullet (SRM), and the European pilchard (PIL) were discriminated according to their high FA concentrations, explained by the second component (Fig. 1B).

3.2. *Comparison of fatty acids profile between seafood species*

The species with the highest TFA concentrations had the highest LC n-3 PUFA and others FA concentrations (Fig. 2; for more detailed see Table S1). The mackerel (MKR), the surmullet (SRM), and the pilchard (PIL) had the highest LC n-3 PUFA concentrations (29.8 ± 14.0 mg g^{-1}, 21.0 ± 8.2 mg g^{-1}, and 10.6 ± 2.7 mg g^{-1}, respectively). In descending order, these species were followed by the meagre (MGR), the seabass (SBS), and the hake (HAK), for which concentrations were around 4.5 mg g^{-1} (Table 1). The black seabream (SBR), the whiting (WTG), the common sole (SOL), the John Dory (JDO), and the dogfish (DOG) were the fish with the lowest LC n-3 PUFA concentration (below 1.9 ± 0.3 mg g^{-1} and up to 0.8 ± 0.1 mg g^{-1}). Concerning cephalopods, the squid (SQD) displayed a higher LC n-3 PUFA content (3.3 ± 0.6 mg g^{-1}) than the cuttlefish (CTF; 1.8 ± 0.1 mg g^{-1}). The LC n-3 PUFA concentration of spider crab (SPI) was 1.0 ± 0.1 mg g^{-1}, similar to those found in the great scallop (GSM) muscle (1.1 ± 0.1 mg g^{-1}). Finally, the two oysters (OSP and OFC) and the scallop gonad (GSG) displayed concentrations of 3.2 ± 1.2 mg g^{-1}, 2.7 ± 1.1 mg g^{-1}, and 4.7 ± 1.2 mg g^{-1} respectively. Proportionally to their TFA content, the muscles of whiting (WTG), cuttlefish (CTF), squid (SQD), and scallop (GSM) were the items with the most important fraction of LC n-3 PUFA (upper than 45 % of TFA, Fig. 3, Table S2). The fraction of LC n-3 PUFA in the other species varied between 30.4 ± 5.3 % of TFA in mackerel (MKR) and 38.3 ± 2 % of TFA in John Dory (JDO). The only exception was surmullet (SRM), which contained the lowest LC n-3 PUFA relative proportion with only 21.4 ± 2.7 % of TFA.

EPA and DHA proportions, which represented an important part of the LC n-3 PUFA, varied with the proportion of LC n-3 PUFA on TFA (Fig. 3, Table S2). Globally, DHA concentrations were higher than EPA concentrations, except for oysters “Spéciale” (OSP), “Fine de Claire” (OFC) and spider crab (SPI). The DHA fraction ranged from 9.6 ± 1.3 % of TFA in surmullet (SRM), to 39.1 ± 2.8 % of TFA in whiting (WTG), while the EPA fraction varied from 4.2 ± 0.6 % of TFA in dogfish (DOG) to 23.7 ± 1.6 % of TFA in spider crab (SPI).

For the cupped oysters, the fraction of LC n-3 PUFA was higher in “Fine de Claire” (OFC; 35 ± 2.1% of TFA) than in “Spéciale” (OSP; 32.7 ± 1.8 % of TFA). The EPA fraction was not significantly different between these two brands, but the DHA fraction was higher in the oyster “Fine de Claire” (OFC; 15.2 ± 1.7 % of TFA) than in the “Spéciale” one (OSP; 13.2 ± 1.2 % of TFA).

3.3. *Risk and benefit assessment of seafood consumption, regarding fatty acid content*

The daily recommended portion of seafood varied logically according to the concentrations of EPA and DHA found in edible part of consumed species (Table 2). Among the seafood species analyzed, only five required more than 240 g of a portion to reach the 250 mg EPA + DHA daily dietary requirement (i.e. the common sole - SOL, the John Dory - JDO, the dogfish - DOG, the spider crab - SPI and the scallop muscle - GSM). Contrasting this, only 15.8 ± 19.6 g of the mackerel (MKR), 19.1 ± 16.7 g of the surmullet (SRM), and 28.1 ± 10.0 g of the pilchard (PIL) were necessary to achieve the recommendation.

The benefit of seafood consumption was assessed through the n-6/n-3 ratio, the AI and the TI calculations (Fig. 3, Table S3). The spider crab (SPI), the seabream (SBR), the common sole (SOL), and the surmullet (SRM) displayed the highest n-6/n-3 ratio (ranging from 0.28 ± 0.04 to 0.33 ± 0.15), whereas the values for the other species ranged from 0.11 ± 0.01 (for the whiting; WTG) to 0.21 ± 0.08 (for the dogfish; DOG). Nonetheless, the pilchard (PIL; 0.10 ± 0.02) and the two cephalopods (SQD; 0.04 ± 0.01 and CTF; 0.08 ± 0.02) had a n-6/n-3 ratio ≤ 0.1. These three species also showed the highest AIs with 0.53 ± 0.07, 0.62 ± 0.03 and 0.45 ± 0.03 for the pilchard, the squid and the cuttlefish, respectively. The lowest AIs were reported for the spider crab (SPI; 0.12 ± 0.01) and the whiting (WTG; 0.26 ± 0.01), while for the other species the AI ranged from 0.029 ± 0.05 (OFC) to 0.40 ± 0.04 (SBR). Concerning the TI, the spider crab (SPI), the whiting (WTG), the cuttlefish (CTF) and the muscle of the scallop (GSM) displayed the lowest values (all equal to 0.06 ± 0.01). The other species had TI comprised between 0.07 ± 0.01 for the squid and 0.25 ± 0.02 for the surmullet.

3.4. *Trace element concentrations in seafood*

The trace element concentrations in edible tissues varied in seafood species from less than 0.001 µg g^{-1} for Ag, Cd, and Pb to more than 200 µg g^{-1} for Zn (Fig. 4A and 4B, Table S4). For all species, the concentrations of non-essential trace elements were lower than the essential ones, with concentrations ranging such as Zn > Fe > As > Cu > Mn > Se > Ag > Hg > Cd, and Pb, based on the overall mean.

The concentrations of non-essential trace elements, Ag and Cd, in the muscles of all fish and cephalopods, were under the limit of detection, and Pb was only significantly measured in the squid (SQD, 0.004 ± 0.001 µg g^{-1}). The highest concentration of Ag was measured in the cupped oysters “Spéciale” (OSP; 0.66 ± 0.52 µg g^{-1}) and “Fine de Claire” (OFC; 0.58 ± 0.68 µg g^{-1}) but was also found in both tissues of the great scallop (GSG; 0.19 ± 0.08 µg g^{-1} and GSM; 0.006 ± 0.002 µg g^{-1}), and in the spider crab (SPI; 0.07 ± 0.02 µg g^{-1}). Cd was detected in the spider crab (SPI; 0.013 ± 0.003 µg g^{-1}) but oysters and scallops had the highest concentrations (OSP; 0.27 ± 0.04 µg g^{-1} > GSG; 0.19 ± 0.16 µg g^{-1} > OFC; 0.18 ± 0.06 µg g^{-1} > GSM; 0.16 ± 0.03 µg g^{-1}). The gonad of the great scallop displayed the highest concentration of Pb (GSG; 0.15 ± 0.17 µg g^{-1}), followed by the oysters, both “Fine de Claire” (OFC; 0.12 ± 0.03 µg g^{-1}) and “Spéciale” (OSP; 0.07 ± 0.02 µg g^{-1}). In a lesser extent, Pb was found in the spider crab (SPI; 0.02 ± 0.003 µg g^{-1}) and in the muscle of the scallop (GSM; 0.009 ± 0.012 µg g^{-1}).

The highest Hg concentrations were measured in the dogfish (DOG; 0.40 ± 0.12 µg g^{-1}) and the seabass (SBS; 0.29 ± 0.07 µg g^{-1}). The flesh of the other fish and cephalopod showed concentrations of Hg comprised between 0.03 ± 0.01 µg g^{-1} in the pilchard (PIL) and 0.09 ± 0.02 µg g^{-1} in the seabream (SBR). The lowest Hg concentrations were measured in the bivalves (the both oyster brands and the both scallop tissues) with a maximum of 0.015 ± 0.003 µg g^{-1} found in the “Fine de Claire” oyster (OFC).

Among the six essential trace elements studied, Cu, Mn and Zn were the most present in the bivalves, especially the oyster “Spéciale” (OSP) and the “Fine de Claire” (OFC) with 18.13 ± 9.19 µg g^{-1} and 9.68 ± 2.99 µg g^{-1} of Cu, respectively, with 7.16 ± 1.87 µg g^{-1} and 4.28 ± 1.83 µg g^{-1} of Mn, respectively, and with 211.7 ± 69.8 µg g^{-1} and 208.6 ± 78.6 µg g^{-1} of Zn, respectively. The results showed that the concentrations of Fe in the hake, the sole, the whiting, and the John Dory were under the LOD, while the highest concentrations of this essential element were found in the gonad of the great scallop (GSG; 32.5 ± 17.8 µg g^{-1}) and in the oysters (OFC; 29.9 ± 6.6 µg g^{-1} and OSP; 24.5 ± 3.4 µg g^{-1}).

It is noteworthy that flesh of the dogfish (DOG) and the spider crab (SPI) displayed the highest concentrations of As, with 37.00 ± 9.31 µg g^{-1} and 21.10 ± 3.05 µg g^{-1}, respectively. Finally, the spider crab (SPI) and the great scallop (GSG) displayed the most important concentration of Se, respectively 1.26 ± 0.16 µg g^{-1} and 1.00 ± 0.24 µg g^{-1}.

3.5. *Risk and benefit assessment of seafood consumption regarding the trace element concentration*

The Maximum Safe Consumption (MSC) showed that the consumption of some species was narrowed by the concentrations of some trace elements, when others did not seem to be restrictive (Table 2). For all the seafood species studied, the MSCs indicated a safe intake of more than (i) 2.0 ± 0.4 kg per day with respect to the Cu and Fe concentrations in the edible tissues, (ii) 6.7 ± 1.0 kg per month regarding to the Cd, and (iii) 15.0 ± 3.0 kg per week regarding to the Pb. The concentrations of Hg were those limiting the most the weekly consumption of the John Dory at 0.87 ± 1.49 kg, the European seabass at 0.40 ± 0.10 kg, and the dogfish at 0.29 ± 0.07 kg. Considering the As concentration, the daily MSCs varied between a consumption of 0.03 ± 0.01 kg of dogfish, and 0.05± 0.01 kg of spider crab, to 1.15 ± 0.50 kg of Atlantic mackerel. Regarding to the Zn concentration, the daily MSCs of the fish, the cephalopods, and the muscle of the great scallop were upper than 2.75 ± 0.40 kg, when MSCs were 0.67 ± 0.05 kg for the spider crab, 0.80 ± 0.30 kg for the gonad of the great scallop, 0.25 ± 0.09 kg for the oyster “Fine de Claire”, and 0.24 ± 0.09 kg for the oyster “Spéciale”.

4. **Discussion**

The consumption of seafood leads to the intake of beneficial and detrimental molecules or elements. This study demonstrates that the concentrations of essential and/or non-essential trace elements, as well as of fatty acids, including LC n-3 PUFA, strongly differed among the different species sampled in the Pertuis Charentais for this study, and were not correlated (Fig. 1). In response to a lack of local data on the seafood quality in one of the most productive and touristic European coastal area, a non-exhaustive baseline and discussion of the risks and benefit for local consumers is presented.

4.1. *Variations in fatty acid contents among seafood species*

For human diet, the highest fish quality in terms of FAs is reflected by a low TFA content, a high quantity of LC n-3 PUFAs, combined with a low content of undesirable FAs, especially saturated FAs, like 14:0 and 16:0, that are considered highly atherogenic (Abrami *et al*., 1992). Considering the TFA content, this study shows that, not surprisingly, the Atlantic mackerel and the surmullet can be considered as fat fish, with TFA concentrations comprised between 80 and 100 mg g^{-1} (Ackman, 1990; Médale, 2009; Sirot *et al*., 2008). Conversely, the other fish species as well as crustaceans, cephalopods, bivalves are considered as intermediaries (25 < TFA < 80 mg g^{-1}) or lean species (TFA < 25 mg g^{-1}). Surprisingly, the European pilchard which is usually considered a fat fish, has in this study a TFA concentration of 28.3 ± 7.2 mg g^{-1}, placing it in the group of intermediaries. The lipid content of muscle tissue of fat species can fluctuate according to age, sexual cycle, trophic ecology, or environmental factors, such as temperature (Médale *et al*., 2009). As an illustration, pilchard caught in November in the Bay of Biscay displayed a lower energy density than fish from the English Channel. This may result from contrasted regional zooplankton productivity and delay in the spawning period (Gatti *et al*., 2018) that occurs later in autumn (October-November) in the Biscay (Coombs *et al*., 2006). More generally, the unique sampling season in winter showed a temporal shot of the lipid composition of marine resources, hiding seasonal variations, including the effect of environmental factors on life history traits, that could be significant in fat species (Sirot *et al*., 2008). Thus, designation of the pilchard of the present study as intermediary fish may be partly attributed to the sampling of post-spawning individuals depleted in TFA (Aidos *et al*., 2002).

The TFA quantity determined in the edible parts of the species does not necessarily inform about the quality of the FAs profile, including the LC n-3 PUFA relative TFA proportion (Fig. 3). The consumption of fat fish such as mackerel and surmullet implies the intake of a high LC n-3 PUFA content, but also an enhanced intake of other PUFAs, monounsaturated FAs (MUFAs), and saturated FAs (SFAs). Contrasting to this, the consumption of lean species such as cuttlefish, squid, and whiting brings an optimal LC n-3 PUFA intake relative to TFA content, and in turn, a lower intake of SFA, making these species qualitatively and relatively more beneficial (Abrami *et al.* 1992). A PUFA/SFA dietary ratio below 0.45 have been often considered undesirable for the human diet because of their potential to increase cholesterol concentrations in the blood (Zhang *et al*., 2020, Ospina *et al*., 2012). In the present study, this ratio ranged from 0.88 to 3.07 (mean ± SD: 1.67 ± 0.40, results not shown), indicating that all species seems beneficial in terms of PUFA proportion supply, while they provide a great disparity of quality with respect to their SFA relative proportion.

In this study, LC n-3 PUFAs are predominantly composed of EPA and DHA. The higher EPA+DHA proportion with respect to the TFA content, the greater the quality of the dietary lipid source for human diet (Abrami *et al.*, 1992). These FAs are both the main structural components of human cell membranes, but they do not play the same physiological role: EPA is mainly involved in immune function and response to stress, while DHA is mostly involved in the development and function of nervous system and brain, as well as in cardiovascular function (Narayan *et al.*, 2006). The present study shows that bivalves, crustaceans, and to a lesser extent, cephalopods, are a greater source of EPA than fish, while DHA is supplied similarly by all taxonomic groups, except by the spider crab, the oysters and the surmullet, which showed the lowest DHA proportion. While it has recently been observed that the EPA/DHA ratio can vary spatially and temporally within a species, and even be reversed, depending on many factors (trophic in particular, in *Sardina pilchardus*, F. Le Grand, pers. com), these results demonstrate the necessity to diversify the consumption of seafood items to allow complete and beneficial intakes, and to optimize the lipid profile for human consumption.

4.2. *Risk and benefit assessment regarding to the fatty acid composition*

Quantitatively, the daily requirement of EPA + DHA has been established at 250 mg, by the JECFA (Joint FAO/WHO Expert Committee on Food Additives). Considering this, a serving of 150 g (usual portion size) of the richest EPA and DHA species (*i.e.* Atlantic mackerel, surmullet, pilchard, meagre, hake, seabass, squid, cuttlefish, oysters, and even the whiting) is enough to reach this recommendation. Noteworthy, a serving of 4-5 scallops (~ 130 g including ~100 g of muscles and ~30 g of gonads) contributes to 94% of the daily requirement of EPA + DHA. However, more than one serving of 150 g of the poorest ones (*i.e.* spider crab, John Dory, common sole and dogfish) are necessary (*i.e.* 275 ± 39 g, 270 ± 120 g, 355 ± 205 g and 391 ± 62 g for these 4 species respectively). Obviously, the recommendations may vary depending on the target population: for example, the requirements for pregnant, breastfeeding women, or elderly would be higher (daily requirement of 300 mg of EPA + DHA; FAO/WHO, 2010), due to the beneficial effects of EPA and DHA on the development of infants or on the decline in the onset of cardiovascular disease (Hellberg *et al.*, 2012; Ruxton *et al.*, 2004). Therefore, these populations should preferably eat mackerel, surmullet, pilchard, meagre, great scallop with gonads, hake, seabass, squid, or even cupped oyster "Spéciale" because a serving of these species is sufficient to achieve their daily requirement.

It is noteworthy that the daily recommended portion for mackerel, sardine and surmullet showed a high variability directly linked to the high inter-individual variability in their FA content. The sampling winter season is a period of spawning for sardine and mackerel in the Bay of Biscay (Alheit *et al.*, 2010). Reproductive status (i.e. gonad maturation stage, pre- or post-spawning status) and sex (and thus the reproductive investment of energy and nutrient in gametes production) could directly modulate the FA content, including EPA and DHA, among individuals (Garrido *et al.*, 2007*;* Caponio *et al.*, 2004). These results raise again the question of seasonal variability of the resources quality and its associated benefit for human consumers with respect to the biology of targeted species.

Western diets are known to be deficient in n-3, and have excessive amount of n-6 (ratio n-6/n-3 between 15:1 and 40:1), while the healthy ratio of n-6/n-3 in the human diet is recommended to be between 1:1 and 4:1 (Simopoulos *et al.*, 2000; Simopoulos, 2003). This imbalance is due to a reduced intake of fish, combined with an excessive amount of vegetable oils rich in linoleic acid (LA; 18:2n-6) (Simopoulos, 2002, 2006). The n-3 and n-6 PUFAs compete for the same enzymes for eicosanoid synthesis, but do not have the same role: n-3 PUFA-derived metabolites have an anti-inflammatory effect, while n-6 PUFA-derived metabolites may have an inflammatory effect (for a review, see Stupin et al. 2019). If the eicosanoid metabolic products from n-6 PUFAs, such as arachidonic acid (20:4n-6) are formed in larger quantities than those formed from EPA (20:5n-3), they will contribute to the formation of thrombus and atheromas, allergic and inflammatory disorders, and cell proliferation. The higher the n-6 /n-3 ratio, the higher the death rate from cardiovascular disease (Simopoulos, 1991, 2006). In this study, the ratios were all much lower than the maximum 4:1 recommended, indicating that the sampled seafood can therefore help to reduce the gap in the total diet by the intake of n-3 PUFA and thus, help to stave off the cardiovascular or inflammatory diseases (Simopoulos, 2002). However, this ratio might lead to simplistic dietary advice, as it does not consider the intake of specific FAs. As mentioned earlier, the highest fish quality is reflected by a simultaneous low TFA content, a high quantity of LC n-3 PUFAs (especially EPA and DHA), as well as a low content of undesirable FAs (*i.e.* SFA, MUFA and n-6 FA; Abrami *et al.*, 1992). For that reason, the AI and TI indices based on functional effects of FAs were also employed in this study to conduct a comprehensive evaluation of the nutritional quality of the studied species.

The AI and TI indices are related to the atherogenicity and thrombogenicity of saturated FAs, such as 16:0 or 18:0. Higher the AI and TI values, higher the platelet aggregation and subsequent thrombus and atheroma formation in the cardiovascular system (Valfré *et al.*, 2003). Among the fish sampled in this study, the whiting and the pilchard can be considered as the two most beneficial fish species for the human diet: they both have the lowest n-6/n-3 ratio. Moreover, the whiting, which is a lean fish with high LC n-3 PUFA fraction, presented the lowest AI and TI indices and covers the EPA+DHA supply in one single meal. In contrast, the pilchard, considered as a fat fish with a lower LC n-3 PUFA proportion is a 5-fold higher source of DHA and EPA, but it presented the highest AI among fish, and one of the highest TI among the studied species. The surmullet, despite providing a high EPA+DHA supply, considered as fat with the lowest LC n-3 PUFA and DHA fraction on TFA, has the highest n-6 /n-3 ratio and the highest TI value, indicating a lower beneficial intake of this species. Concerning other taxa studied here, the squid and the cuttlefish could be considered as beneficial, as they are lean species presenting a high proportion of LC n-3 PUFA (including DHA and EPA), the lowest n-6/n-3 ratio, a low TI, while they also stand out with the highest AI.

4.3. Variation factors of trace element concentrations

The concentration of trace elements varied widely between species, and usually depend on (i) the accumulation pathways (accumulation through contaminated prey consumption versus seawater dissolved contaminant bioconcentration), (ii) the individual characteristics (*e.g.* sex, age), as well as (iii) environmental conditions (*i.e.* seawater temperature) (Rainbow, 1997; Sokolova & Lannig, 2008). The high trace element concentrations found in bivalves is linked to the presence of gills, digestive gland, and gonads, that are known to efficiently concentrate trace elements (Bustamante & Miramand, 2004; Metian *et al.*, 2008) in the edible tissues (El Moshely *et al.*, 2014; Ersoy & Çelik, 2009; Geffard *et al.*, 2001). Also, the gonads of scallop are 1.2-fold more concentrated in Cd to 36-fold more concentrated in Ag than their muscle tissue. Thus, seafood consumed as a whole organism contributes globally much more than muscular flesh of fish, cephalopods and crustaceans to trace elements (except Hg) intake for consumers. Secondly, the filter-feeders such as cupped oysters tend to accumulate more trace elements than other species because they directly filter large volumes of water that can be rich in trace elements from suspended particulate matter (El-Moselhy *et al.*, 2014). Interestingly, the concentration of Cd, Cu, Fe, Mn, and Pb vary significantly among the two brands of cupped oysters and may be attributed to different farming methods. While the oyster “Spéciale” grows up in the open ocean, the “Fine de Claire” finishes its growth in shallow clay ponds in marshes dependent on the arrival of freshwater from the watershed with the presence of navicular microalgae greening the oysters from Marennes-Oléron area. Our results raised the question of the influence of these contrasting environmental conditions on the trace element concentrations in cultured oysters.

It is noteworthy that muscle of carnivorous fish, cephalopods and crustaceans displayed higher concentrations in Hg than bivalves, consistent with Hg biomagnification along the trophic webs (*e.g.* Storelli *et al.*, 2007; Coelho *et al.*, 2010; Ersoy & Çelik, 2009), and to the tropism of methylmercury (MeHg) that binds tightly to the sulfhydryl groups of muscular proteins (Bloom, 1992). Thus, seabass showed the second highest Hg concentrations (0.289 ± 0.071 µg g^{-1}), due to its high trophic position (*e.g.* Chouvelon *et al.*, 2012). Surprisingly, the Hg concentrations in the meagre, *i.e.* a predator in the same trophic position, remained relatively low in comparison (0.065 ± 0.007 µg g^{-1}). Although seabass and meagre individuals were of similar size (475 ± 12 mm and 431 ± 11 mm, respectively), the meagre specimens were considered younger than seabass ones (1 yr *vs.* 5-6 yr old, respectively). Hg concentrations tend to increase with fish age, generally proxied by size (Storelli *et al.*, 2007; Abreu *et al.*, 2000; Chouvelon *et al.*, 2012), as a result of a longer dietary exposure and a poor excretion of assimilated Hg. In addition, young meagre display a trophic regime based on crustaceans, *i.e.* poor Hg prey (Hubans *et al.*, 2017) before switching towards a piscivorous diet (*i.e.* Hg enriched fish prey), limiting again the Hg intake in these individuals. These results raise the question of maximum Hg concentrations recorded in bigger seabass and meagre that could be usually found in seafood markets. In addition, the dogfish showed the highest Hg concentrations, as already observed in the literature (Storelli *et al.*, 2005a; Chouvelon *et al.*, 2012). This species, such as other benthic species living in close association with sediment in which they bury and from where they mainly feed, is more exposed to Hg and MeHg sediment-associated contamination than pelagic species (Storelli *et al.*, 2003c; 2006a). Nevertheless, the general higher Hg concentrations found in Chondrichthyan in comparison with Actinopterygian suggest the influence of metabolic factors, such as specific detoxication mechanisms (Chouvelon *et al.*, 2012).

Finally, it is worth noting that the highest As concentrations were found in benthic and nektobenthic species, i.e. the dogfish, the spider crab, the common sole, the seabream, the surmullet and the cuttlefish. These values are comparable to those reported in previous studies for marine benthic species (Storelli *et al.*, 2005a; Sirot *et al.*, 2009). Such concentrations likely result from their diet based on bottom living invertebrates (Storelli *et al.*, 2005a; Wu *et al.*, 2014), which are enriched by the As trapped in sediment. They also confirm that seafood is an important source of As in human diet. Indeed, in France, seafood contributes to more than 60% of the total dietary As supply (Leblanc *et al.*, 2006).

4.4. *Risk and benefit regarding to the presence of trace elements*

The International and European Regulation publishes the maximum concentrations (*i.e.* maximum permissible levels) of Cd, Hg and Pb that regulates the commercialization and consumption of seafood (EFSA, 2014). These limits established differ among taxa and species. Regarding Cd, the concentrations could not exceed 0.5 µg g^{-1} in crustaceans and 1.0 µg g^{-1} in bivalves and cephalopods. In fish muscle, Cd is permitted at a maximum of 0.25 µg g^{-1} in pilchard, 0.1 µg g^{-1} in mackerel and 0.05 µg g^{-1} in the other species (EC, 2014). None of the individual samples analyzed in this study exceed these limits, with the exception of one scallop gonad that reached 0.52 µg g^{-1}. Concerning Hg, the highest concentration found in dogfish (*i.e.* 0.60 µg g^{-1}) is well below the maximum levels of 1 µg g^{-1} permissible for sharks. Likewise, the seabass individual displaying a Hg concentration of 0.39 µg g^{-1} in muscle did not exceed the 0.5 µg g^{-1} threshold fixed for fish (EC, 2008; EC, 2011). Finally, the value of 0.61 µg g^{-1} Pb recorded in scallop gonad is also below the maximum levels of 1.5 µg g^{-1} authorized for bivalves (EC, 2015).

While seafood is a well-recognized source of proteins and FAs for humans, it also contributes to the chronic intake of potentially harmful trace elements leading the JECFA to establish endpoints representing the permissible human daily, weekly or monthly exposure to both essential and non-essential elements, i.e. As, Cd, Cu, Fe, Hg, Pb, and Zn. Based on these recommendations, the calculations of the maximum safe consumption (MSC) indicate a safety intake with respect to essential Cu, Fe and Zn allowing fish and cephalopod meals of more than 3 kg per day until reaching the established limits. It is noteworthy that frequent seafood consumers could still reach the MSC for Zn when eating ~ 240 g of oyster flesh that corresponds approximatively to two dozen of oysters. In addition, the very high MSCs for Cd and Pb highlighted that local seafood is safe for consumers with respect to these both non-essential metals.

Although it is assumed that As plays an essential role for human health (Mayer *et al.*, 1993), the JECFA established a PTWI at 15 µg kg^{-1} bw, limiting at first glance the consumption of seabream, common sole, dogfish, surmullet, cuttlefish, and spider crab to less than one portion per week (< 150 g). However, this recommendation refers to the toxicity of the inorganic As

(Ansari *et al.*, 2004; Hughes, 2002; Neff, 1997), whereas As found in marine organisms are predominantly organic arsenical compounds (*i.e.* arsenobetaine) known to be far less toxic (Borak & Hosgood, 2007; EFSA, 2009; Olmedo *et al.*, 2013b). Seafood should be thus considered safe for consumers, whereas there is still a lack of data on toxicity of some organic compounds, e.g. As-sugars found in seaweeds, bivalves and crustaceans, and their metabolites produced during the digestive process (Taylor *et al.*, 2017).

This previous point highlights that regulation and recommendation with respect to contaminated seafood is based on the total concentration of contaminant and does not consider its metabolic bioavailability in tissue. Indeed, the subcellular distribution of metals in cytosolic (i.e. metal free in the cytosol) and organelles fractions (e.g. metal bound to metalloproteins, or entrapped in metal rich-granules) drives the proportion of elements available for absorption at the intestinal level, defined as bioaccessibility for consumers (Wallace and Luoma, 2003). Experimental work demonstrated that the bioaccessible fraction rarely exceed 80% of the total concentration for Zn and ranged from 50 to 90% for Cd in raw mussels and oysters (Metian *et al.*, 2009; Gao and Wang, 2014). Consequently, this oral bioaccessibility could help unravel risk associated with consumption of contaminated seafood (Gao and Wang, 2014).

Finally, Hg remains the element of most concern for consumers, and more particularly the MeHg which is the dominant and toxic form in seafood (Andersen & Depledge, 1997; Storelli *et al.*, 2005b). The MSCs calculated on the basis of the PTWI of 1.6 µg kg^{-1} bw of MeHg (JECFA) highlighted that less than 400 g (*i.e.* lower than three portions, ~ 450 g) per week for the European seabass, as well as for the dogfish is enough to exceed a safety intake. Thus, the consumption of these two species may be at risk for high seafood consumers, also considering that the Hg intake might be enhanced when bigger seabass (> 1 kg) are eaten. The essential metal Se is known as a protective antagonist against Hg toxicity (*e.g.* Burger & Gochfeld., 2011), implying that the Se:Hg molar ratios exceeding 1 are protective for adverse Hg effects (Ralston, 2008). The flesh of dogfish and seabass displayed Se in excess in relation to Hg with Se:Hg ratios of 2.6 ± 0.9 and 4.1 ± 1.3, respectively (data not shown), but these values are the lowest compared to those of the other foodstuffs that have a ratio between 13 and 206 (*i.e.* mackerel and scallop gonad, respectively). Even if the mechanisms of Hg toxicity neutralization by Se are clearly known for consumers, these results might indicate the nutritional importance of seafood that would provide enough Se benefit to balance the Hg harm (Ralston, 2008).

4.5. *Application to a concrete case*

Literature reports that MeHg can counteract the cardioprotective effects of fish consumption (Guallar *et al.*, 2002; Salonen *et al.*, 1995) mainly brought by the LC n-3 PUFAs. Considering simultaneously the LC n-3 PUFA and Hg concentrations, two constituents that have a mechanistic basis for influencing cardiovascular outcome, the present analysis makes one to use an index integrating these two parameters in the same calculation to illustrates both the risk and benefit of seafood consumption. In this line, the index of net risk/benefit for cardiovascular endpoints was calculated on a species-specific basis according to Ginsberg & Toal (2009). The method subtracts risk of adult cardiovascular heart disease due to MeHg (23% higher risk/1 ppm hair Hg) from the benefit thanks to PUFA (14.6% lower risk/100 mg EPA+DHA). The calculated index for each species (Fig. 5) showed that the relative risk of consumption increases for species with the highest Hg concentrations, *i.e.* the lesser-spotted dogfish and the European seabass. Surprisingly, the consumption of seabass provides very little benefit, even if consumed as a single portion per week. Fat species, like the Atlantic mackerel or the surmullet seem to provide the best benefit because of their high LC n-3 PUFA concentrations and their low Hg concentrations. However, this index must be considered carefully, as it does not consider the proportion of LC n-3 PUFAs, nor the specific functional role of FAs, making it contradictory to the n-6/n-3 ratio or AI and TI index that could count down the benefit. This is particularly evident concerning the surmullet that we have previously considered not to be beneficial considering only their FA composition and the calculation derived therefrom.

5. Conclusion

The present study highlights the benefit and risks of consuming different seafood varieties from the “Pertuis charentais” area of France, a well-known region for seafood production in Europe. In terms of the FA profile, all species presented a PUFA/SFA ratio, as well as a n-6/n-3 ratio much lower than the threshold from which the rate of cholesterol and cardiovascular disease increase. Considering all of the FA indicators measured in this study, whiting and pilchard appear as the most beneficial fish species for the human diet, while the surmullet is least advantageous. However, using an index that integrates the relative risk due to Hg content, the surmullet and mackerel appear as the seafood with the best effects on the prevention of cardiovascular disease in adults. Overall, the concentration of trace elements with respect to seafood safety recommendations, are such that significant amounts of seafood can be safely eaten on a daily or weekly basis. Yet, levels of Hg in dogfish and seabass can become a concern for frequent seafood consumers (> three meals a week), confirming that diversity in seafood is key for consumers to optimize the benefit of seafood resources.

It is important to note that the risk/benefit assessment for the seafood consumers is strongly believed to vary with season as the lipid content and trace element concentrations may depend on the nutrient availability, the physiological and the reproductive status of marine organisms (Aidos *et al.*, 2002; Lozano-Bilbao *et al.*, 2020). It is also necessary to consider food preparation, as cooking is known to i) damage the LC n-3 PUFAs (Türkkan *et al.*, 2008, Gladyshev *et al.*, 2006, Sardenne *et al.*, 2021), and ii) decrease the bioaccessibility of trace elements (He & Wang, 2011; Houlbrèque *et al.*, 2011), including the levels of other organic contaminants (*i.e.* PCBs, PAHs). In addition, heightened anthropogenic activities may be responsible for increased trace elements concentrations including other contaminants such as persistent organic pollutant (*e.g.* DDT or PCBs) in the environment (Storelli, 2008). Also, global change, through warming, acidification or deoxygenation alter the assemblages and physiology of marine microalgae, leading to an overall reduction in the production of LC n-3 PUFAs at the base of the marine food web (Galloway, 2015, Hixson and Arts 2016). This may have consequences on upper trophic organisms, including seafood species and humans, that may be unable to synthesize these molecules (Hixson & Arts, 2016; Pethybridge *et al.*, 2015).

Acknowledgments

This work is a contribution to the project ECONAT Axe 1 - Ressources Marines Littorales: qualité et éco-valorisation, funded by the Contrat de Plan Etat-Région and the CNRS and the European Regional Development Fund through the project QUALIDRIS (Ressources Marines et Littorales: Qualité et Eco-valorisation). Authors thank Lucas Weppe for its assistance in sample preparation. Thanks to the CPER (Contrat de Projet Etat-Région) and the FEDER (Fonds Européen de Développement Régional) for funding the AMA and the IRMS of LIENSs laboratory. This work benefitted from the French GDR "Aquatic Ecotoxicology" framework which aims at fostering stimulating scientific discussions and collaborations for more integrative approaches. The IUF (Institut Universitaire de France) is acknowledged for its support to PB as a Senior Member.

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Table 1. Scientific and common names, acronym, number (n), length (mm, mean ± SD) and weight (g, mean ± SD), date of procurement of the studied organisms: fish, crustaceans, cephalopods, bivalves from fisheries (white lines) and bivalves from aquaculture (grey lines).

Taxa	Order	Scientific name	Common name	Acronym	*n*	Length (mm)	Weight (g)	Date of procurement	Comment
Fish	*Clupeiformes*	*Sardina pilchardus*	European pilchard	PIL	10	170 ± 9 [a]	53 ± 10	11/2018	not gutted [g]
	Gadiformes	*Merluccius merluccius*	European hake	HAK	9	434 ± 29 [a]	538 ± 116	11/2018	gutted [f]
	Gadiformes	*Merlangius merlangus*	Whiting	WTG	10	316 ± 16 [a]	240 ± 32	11/2018	gutted [f]
	Perciformes	*Scomber scombrus*	Atlantic mackerel	MKR	10	245 ± 20 [a]	142 ± 33	11/2018	not gutted [g]
	Perciformes	*Spondyliosoma cantharus*	Black seabream	SBR	10	220 ± 15 [a]	280 ± 24	02/2019	not gutted [g]
	Perciformes	*Dicentrarchus labrax*	European seabass	SBS	10	475 ± 12 [a]	1223 ± 128	11/2018	not gutted [g]
	Perciformes	*Argyrosomus regius*	Meagre	MGR	10	431 ± 11 [a]	820 ± 55	11/2018	not gutted [g]
	Perciformes	*Mullus surmuletus*	Surmullet	SRM	10	227 ± 10 [a]	206 ± 37	11/2018	not gutted [g]
	Pleuronectiformes	*Solea solea*	Common sole	SOL	10	302 ± 8 [a]	223 ± 39	11/2018	not gutted [g]
	Zeiformes	*Zeus faber*	John Dory	JDO	10	362 ± 21 [a]	620 ± 159	11/2018	gutted [f]
	Carcharhiniformes	*Scyliorhinus canicula*	Lesser-spotted dogfish	DOG	5	641 ± 199 [a]	1149 ± 117	02/2019	not gutted [g]
Crustacean	*Decapoda*	*Maja brachydactyla*	Atlantic spinous spider crab	SPI	5	139 ± 4 [c]	693 ± 66	11/2018	not gutted [g]
Cephalopod	*Myopsida*	*Loligo vulgaris*	European squid	SQD	10	214 ± 17 [b]	241 ± 34	11/2018	not gutted [g]
	Sepiida	*Sepia officinalis*	Common cuttlefish	CTF	10	106 ± 7 [b]	159 ± 38	11/2018	not gutted [g]
Bivalve	*Ostreida*	*Crassostrea gigas* * "Fine de Claire" (green)	Cupped oyster	OFC	20	/	8 ± 2 [e]	02/2019	full [h]
	Ostreida	*Crassostrea gigas* * "Spéciale"	Cupped oyster	OSP	20	/	8 ± 1 [e]	02/2019	full [h]
	Pectinida	*Pecten maximus* (gonad)	Great Atlantic scallop	GSG	10	111 ± 4 [d]	3 ± 1	02/2019	full [h]
	Pectinida	*Pecten maximus* (muscle)	Great Atlantic scallop	GSM	7	111 ± 5 [d]	9 ± 2	02/2019	full [h]

[a]fork length, [b]mantle length, [c]carapace length, [d]shell length, [e]soft tissue weight (without the shell), [f] individuals were bought gutted, [g] individuals were bought not gutted, [h]entire individuals was bought. * The Genus *Crassosstrea* was preferred to *Magallana* in these study because of the current controversy about the designation of the species (Bayne *et al.*, 2017).

Table 2. Daily recommended species consumption (in g wet weight per day, mean ± SD) based on the eicosapentaenoic acid (EPA) + the docosahexanoic acid (DHA) requirement (250 mg per day) by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (FAO/WHO, 2008), considering EPA and DHA concentrations of each studied species (see Table S1 for the EPA and DHA content of each species) and Maximum Safe Consumption (MSC) of trace elements concentrations (expressed in kg per month for Cd; in kg per week for As, Hg, and Pb; in kg per day for Cu, Fe, and Zn), based on provisional tolerable daily, weekly or monthly intake of studied species recommended for an adult of 70 kg body weight.

		EPA + DHA	Non-essential trace elements			Essential trace elements			
Taxa	Species	Daily recommended consumption	MSC$_{\mathrm{Cd}}$	MSC$_{\mathrm{Hg}}$	MSC$_{\mathrm{Pb}}$	MSC$_{\mathrm{As}}$	MSC$_{\mathrm{Cu}}$	MSC$_{\mathrm{Fe}}$	MSC$_{\mathrm{Zn}}$
Fish	Atlantic mackerel	15.8 ± 19.6	1224	1.6	1152	1.150	46	12	10
	Black seabream	156.7 ± 29.0	1576	1.3	1172	0.141	81	23	9.1
	Common sole	354.5 ± 204.5	1540	1.6	1540	0.106	68	22	11
	European hake	68.7 ± 21.8	1758	3.4	1758	0.548	105	64	12
	European pilchard	28.1 ± 10.0	1235	4.8	1096	0.416	33	5.3	8.7
	European seabass	76.1 ± 45.4	1594	0.402	1594	1.340	77	26	11
	John Dory	269.5 ± 119.7	1571	0.874	1571	1.240	72	24	11
	Lesser-spotted dogfish	390.8 ± 62.2	1137	0.294	1351	0.030	114	24	4.4
	Meagre	61.4 ± 8.8	1601	1.8	1601	0.838	81	29	11
	Surmullet	19.1 ± 16.7	1310	1.6	1168	0.163	65	21	11
	Whiting	152.0 ± 20.3	1336	39	1197	0.331	42	9.7	9.4
Crustaceans	Atlantic spinous spider crab	274.8 ± 39.1	142	2.4	98	0.051	6.8	21	0.673
Cephalopods	Common cuttlefish	145.8 ± 10.9	1574	4.2	1574	0.116	25	33	3.7
	European squid	81.0 ± 21.5	1450	2.6	458	0.245	27	21	3.6
Bivalves	Cupped oyster « Fine de Claire »	131.9 ± 92.8	11	8.0	15	0.310	3.9	2.0	0.246
	Cupped oyster « Spéciale »	106.8 ± 68.8	6.7	8.4	28	0.353	2.5	2.3	0.239
	Great Altantic scallop (gonad)	62.0 ± 19.0	17.8	9.8	26	0.408	14	2.4	0.799
	Great Atlantic scallop (muscle)	242.8 ± 22.3	11.2	11	394	0.653	78	14	2.75

Figure Captions

Figure 1. PCA-derived projection of variables and individuals. Variables were defined as the concentrations of Ag, As, Cd, Cu, Fe, Hg, Mn, Pb, Se, and Zn (all expressed of µg g^{-1} wet weight) and the concentrations of total fatty acids (TFA), long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA), EPA, and DHA (all expressed in mg g^{-1} wet weight) in edible parts of fishes, crustacean, cephalopods and bivalves. A) Correlation circle showing the distribution of each variables on the first two components and B) Grouping of all individuals by species (acronym indicated in the white rectangle) on the first two components. Studied species were: for fishes: DOG (lesser-spotted dogfish, *n* = 5), HAK (European hake, *n* = 9), JDO (John Dory, *n* = 10), MGR (meagre, *n* = 10), MKR (Atlantic mackerel, *n* = 10), PIL (European pilchard, *n* = 10), SBR (black seabream, *n* = 10), SBS (European seabass, *n* = 10), SOL (common sole, *n* = 10), SRM (surmullet, *n* = 10), WTG (whiting, *n* = 10); for crustacean: SPI (Atlantic spinous spider crab, *n* = 5); for cephalopods: CTF (common cuttlefish, *n* = 10), SQD (European squid, *n* = 10); for bivalves: GSG (great Atlantic scallop gonad, *n* = 7), GSM (great Atlantic scallop muscle, *n* = 6), OFC (cupped oyster « Fine de Claire », *n* = 10), OSP (cupped oyster « Spéciale », *n* = 10).

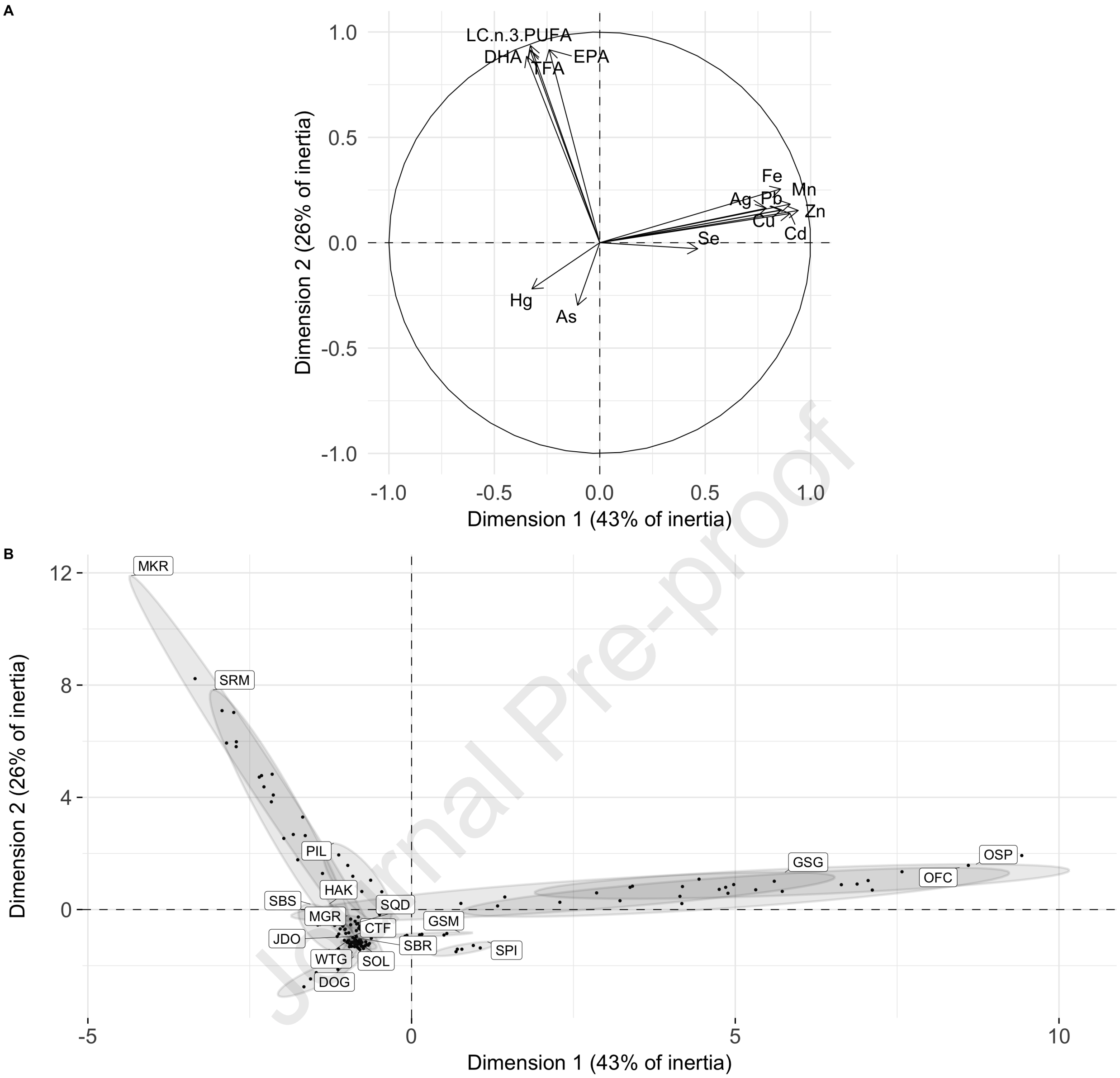
Figure 2. Total fatty acid (TFA, total histogram bars) concentrations (mg g^{-1} wet weight) composed of LC n-3 PUFA (in light grey), others PUFAs (in grey) and others fatty acids (in black) comprising saturated fatty acids, monounsaturated fatty acids, branched fatty acids, and dimethyl acetal fatty acids in edible parts of fishes (blue), crustacean (purple), cephalopods (yellow) and bivalves (red). Studied species were: for fishes: DOG (lesser-spotted dogfish, *n* = 5), HAK (European hake, *n* = 9), JDO (John Dory, *n* = 10), MGR (meagre, *n* = 10), MKR (Atlantic mackerel, *n* = 10), PIL (European pilchard, *n* = 10), SBR (black seabream, *n* = 10), SBS (European seabass, *n* = 10), SOL (common sole, *n* = 10), SRM (surmullet, *n* = 10), WTG (whiting, *n* = 10); for crustacean: SPI (Atlantic spinous spider crab, *n* = 5); for cephalopods: CTF (common cuttlefish, *n* = 10), SQD (European squid, *n* = 10); for bivalves: GSG (great Atlantic scallop gonad, *n* = 7), GSM (great Atlantic scallop muscle, *n* = 6), OFC (cupped oyster « Fine de Claire », *n* = 10), OSP (cupped oyster « Spéciale », *n* = 10).

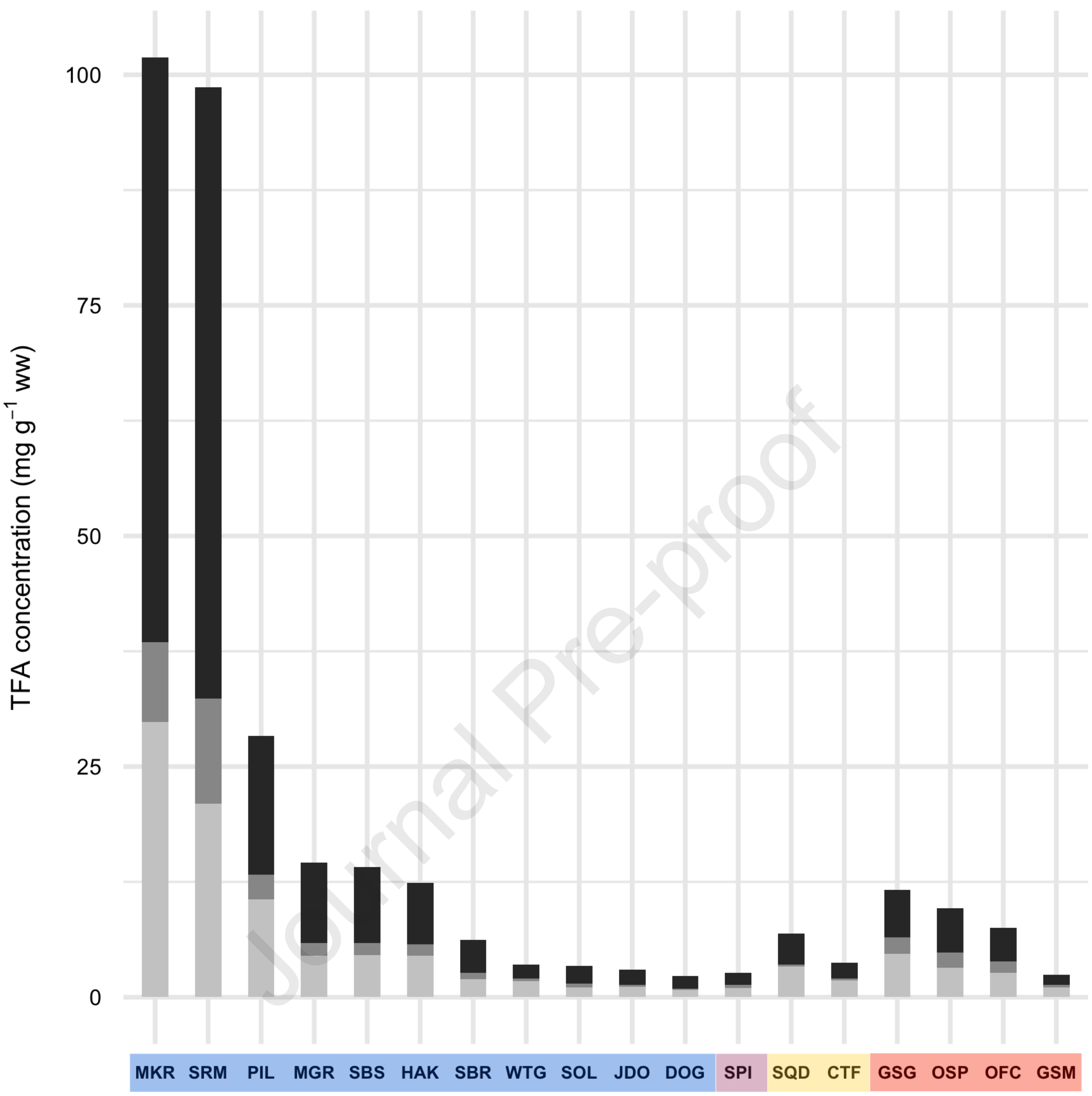
Figure 3. Clustered heatmap showing LC n-3 PUFA/TFA, EPA/TFA, DHA/TFA, n-6/n-3 ratio, atherogenic (AI) and thrombogenic (TI) indices in edible parts of fishes, cephalopods, crustacean and bivalves. Studied species were: for fishes: DOG (lesser-spotted dogfish, *n* = 5), HAK (European hake, *n* = 9), JDO (John Dory, *n* = 10), MGR (meagre, *n* = 10), MKR (Atlantic mackerel, *n* = 10), PIL (European pilchard, *n* = 10), SBR (black seabream, *n* = 10), SBS (European seabass, *n* = 10), SOL (common sole, *n* = 10), SRM (surmullet, *n* = 10), WTG (whiting, *n* = 10); for cephalopods: CTF (common cuttlefish, *n* = 10), SQD (European squid, *n* = 10); for crustacean: SPI (Atlantic spinous spider crab, *n* = 5); for bivalves: GSG (great Atlantic scallop gonad, *n* = 7), GSM (great Atlantic scallop muscle, *n* = 6), OFC (cupped oyster « Fine de Claire », *n* = 10), OSP (cupped oyster « Spéciale », *n* = 10). For all indices, the higher the value, the darker the green.

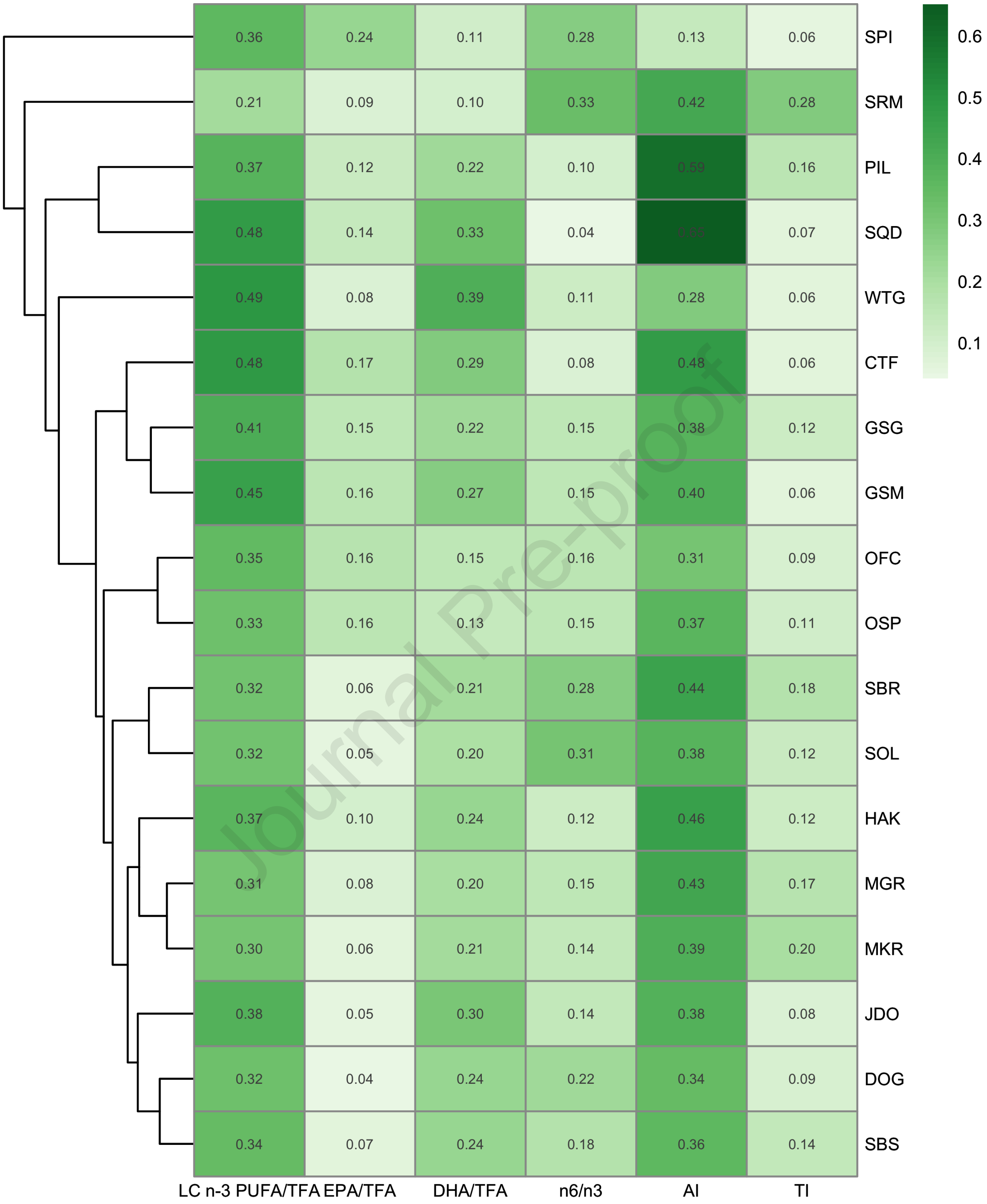
Figure 4. Boxplots showing A) non-essential (Ag, Cd, Hg, and Pb) and B) essential (As, Cu, Fe, Mn, Se, and Zn) trace element concentrations (expressed in µg g^{-1} wet weight) in edible parts of fishes (blue), crustaceans (purple), cephalopods (yellow) and bivalves (red). Studied species were: for fishes: DOG (lesser-spotted dogfish, *n* = 5), HAK (European hake, *n* = 9), JDO (John Dory, *n* = 10), MGR (meagre, *n* = 10), MKR (Atlantic mackerel, *n* = 10), PIL (European pilchard, *n* = 10), SBR (black seabream, *n* = 10), SBS (European seabass, *n* = 10), SOL (common sole, *n* = 10), SRM (surmullet, *n* = 10), WTG (whiting, *n* = 10); for crustaceans: SPI (Atlantic spinous spider crab, *n* = 5); for cephalopods: CTF (common cuttlefish, *n* = 10), SQD (European squid, *n* = 10); for bivalves: GSG (great Atlantic scallop gonad, *n* = 10 except for Hg *n* = 9 and for Mn, and Zn *n* = 8), GSM (great Atlantic scallop muscle, *n* = 7 except for Zn *n* = 6), OFC (cupped oyster « Fine de Claire », *n* = 10), OSP (cupped oyster « Spéciale », *n* = 10). Non-parametric

Kruskal-Wallis tests showed for all elements *p-values* < 0.05 (Ag: χ_2 = 33.749, df = 4; Cd: χ_2 = 20.791, df = 4; Hg: χ_2 = 143.54, df = 17; Pb: χ_2 = 41.48, df = 5; As: χ_2 = 150.75, df = 17; Cu: χ_2 = 155.09, df = 17; Fe: χ_2 = 103.5, df = 13; Mn: χ_2 = 145.31, df = 17; Se: χ_2 = 118.5, df = 17; Zn: χ_2 = 143.34, df = 17). Different letters denote significant differences in trace element concentrations between species (Wilcoxon signed rank tests, without adjust method, *p-value* < 0.05). Blanks correspond to element contents under the limit of detection.

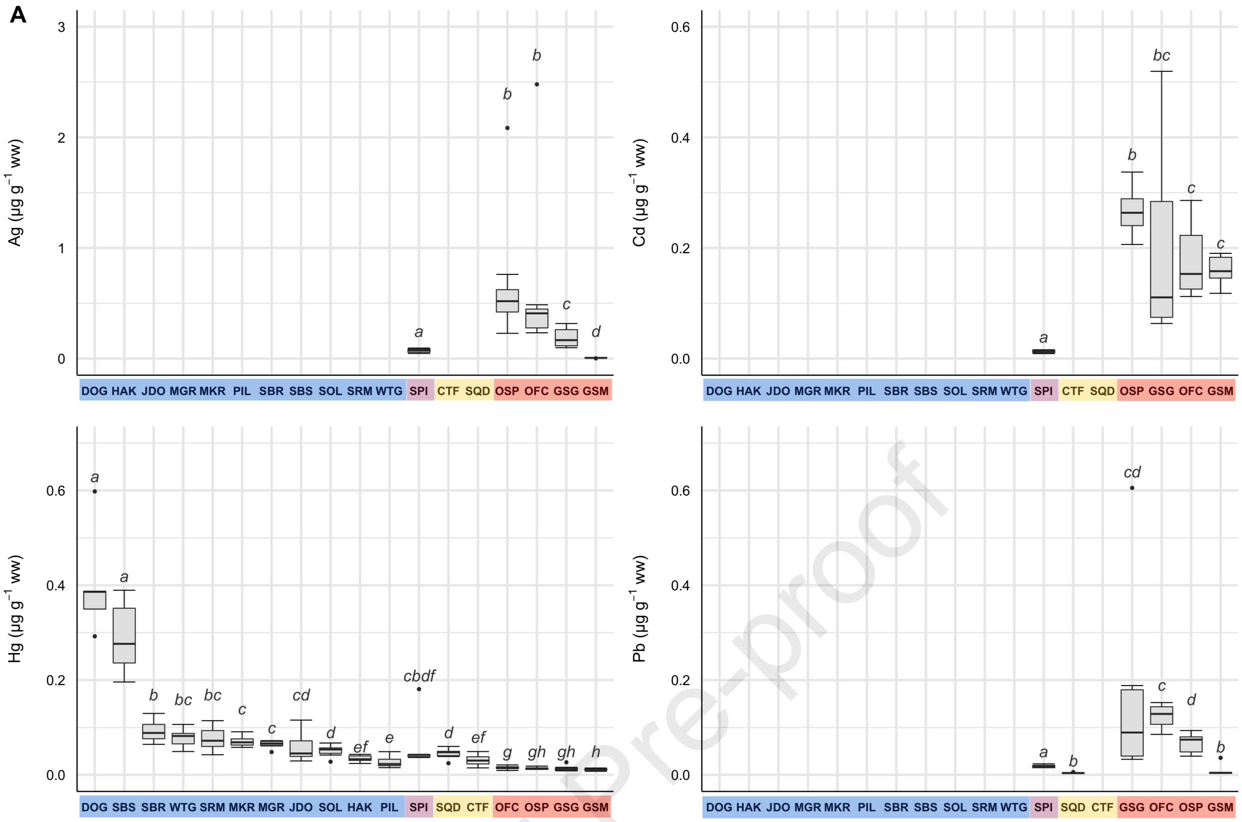
Figure 5. Estimated effect (in %) of Hg and EPA+DHA on cardiovascular heart disease risk, considering one (in black) or two (in grey) 150 g seafood servings per week of the edible parts of fish (blue), crustacean (purple), cephalopods (yellow), and bivalves (red). Studied species were: for fish: DOG (lesser-spotted dogfish, *n* = 5), HAK (European hake, *n* = 9), JDO (John Dory, *n* = 10), MGR (meagre, *n* = 10), MKR (Atlantic mackerel, *n* = 10), PIL (European pilchard, *n* = 10), SBR (black seabream, *n* = 10), SBS (European seabass, *n* = 10), SOL (common sole, *n* = 10), SRM (surmullet, *n* = 10), WTG (whiting, *n* = 10); for crustacean: SPI (Atlantic spinous spider crab, *n* = 5); for cephalopods: CTF (common cuttlefish, *n* = 10), SQD (European squid, *n* = 10); for bivalves: GSG (great Atlantic scallop gonad, *n* = 7), GSM (great Atlantic scallop muscle, *n* = 6), OFC (cupped oyster « Fine de Claire », *n* = 10), OSP (cupped oyster « Spéciale », *n* = 10).

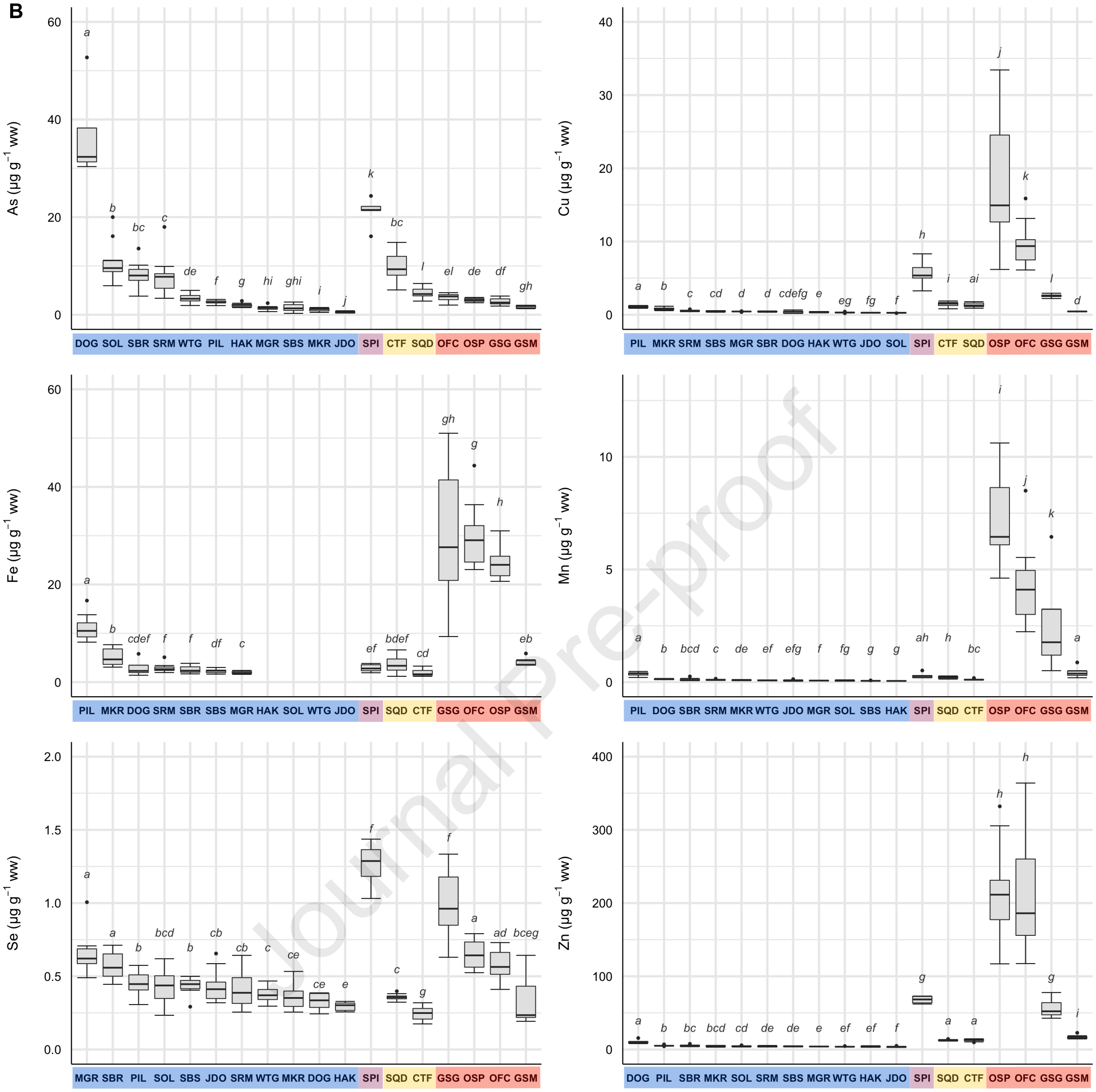

A
LC.n.3.PUFA
DHA
TFA
EPA
Fe
Ag
Pb
Mn
Zn
Cu
Cd
Se
Hg
As
1.0
0.5
0.0
-0.5
-1.0
Dimension 2 (26% of inertia)
Dimension 1 (43% of inertia)
B
MKR
SRM
PIL
HAK
SBS
MGR
SQD
CTF
GSM
JDO
SBR
SPI
WTG
SOL
DOG
GSG
OSP
OFC
12
8
4
0
-5
5
10
Dimension 2 (26% of inertia)
Dimension 1 (43% of inertia)

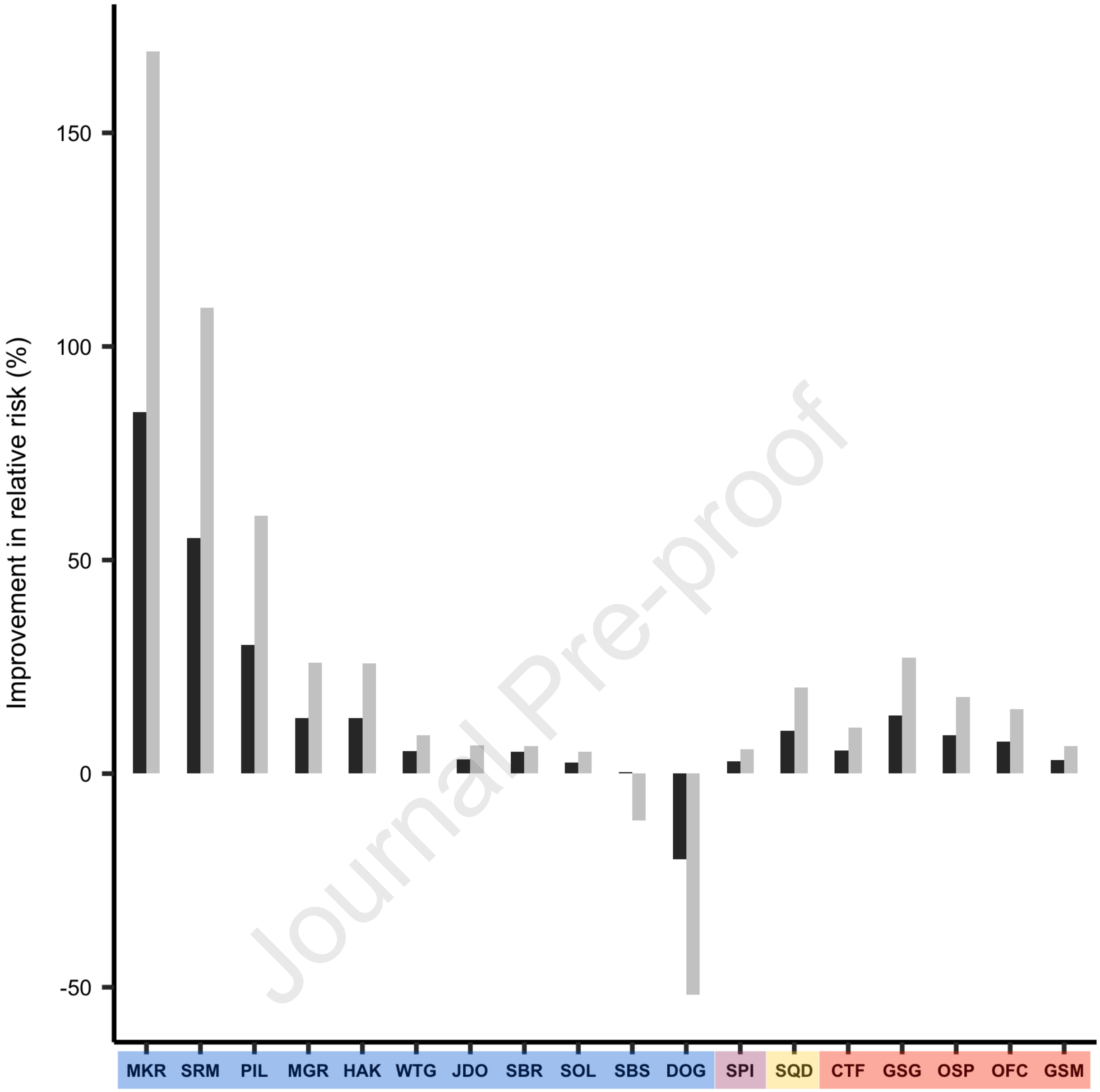

TFA concentration (mg g^{-1} ww)
100
75
50
25
0
MKR
SRM
PIL
MGR
SBS
HAK
SBR
WTG
SOL
JDO
DOG
SPI
SQD
CTF
GSG
OSP
OFC
GSM



	LC n-3 PUFA/TFA	EPA/TFA	DHA/TFA	n6/n3	AI	TI
SPI	0.36	0.24	0.11	0.28	0.13	0.06
SRM	0.21	0.09	0.10	0.33	0.42	0.28
PIL	0.37	0.12	0.22	0.10	0.59	0.16
SQD	0.48	0.14	0.33	0.04	0.65	0.07
WTG	0.49	0.08	0.39	0.11	0.28	0.06
CTF	0.48	0.17	0.29	0.08	0.48	0.06
GSG	0.41	0.15	0.22	0.15	0.38	0.12
GSM	0.45	0.16	0.27	0.15	0.40	0.06
OFC	0.35	0.16	0.15	0.16	0.31	0.09
OSP	0.33	0.16	0.13	0.15	0.37	0.11
SBR	0.32	0.06	0.21	0.28	0.44	0.18
SOL	0.32	0.05	0.20	0.31	0.38	0.12
HAK	0.37	0.10	0.24	0.12	0.46	0.12
MGR	0.31	0.08	0.20	0.15	0.43	0.17
MKR	0.30	0.06	0.21	0.14	0.39	0.20
JDO	0.38	0.05	0.30	0.14	0.38	0.08
DOG	0.32	0.04	0.24	0.22	0.34	0.09
SBS	0.34	0.07	0.24	0.18	0.36	0.14


A
Ag (µg g^{-1} ww)
0
1
2
3
a
b
b
c
d
DOG HAK JDO MGR MKR PIL SBR SBS SOL SRM WTG SPI CTF SQD OSP OFC GSG GSM
Cd (µg g^{-1} ww)
0.0
0.2
0.4
0.6
a
b
bc
c
c
DOG HAK JDO MGR MKR PIL SBR SBS SOL SRM WTG SPI CTF SQD OSP GSG OFC GSM
Hg (µg g^{-1} ww)
0.0
0.2
0.4
0.6
a
a
b
bc
bc
c
c
cd
d
ef
e
cbdf
d
ef
g
gh
gh
h
DOG SBS SBR WTG SRM MKR MGR JDO SOL HAK PIL SPI SQD CTF OFC OSP GSG GSM
Pb (µg g^{-1} ww)
0.0
0.2
0.4
0.6
a
b
cd
c
d
b
DOG HAK JDO MGR MKR PIL SBR SBS SOL SRM WTG SPI SQD CTF GSG OFC OSP GSM


B
As (µg g^{-1} ww)
0
20
40
60
a
b
bc
c
de
f
g
hi
ghi
i
j
k
bc
l
el
de
df
gh
DOG SOL SBR SRM WTG PIL HAK MGR SBS MKR JDO SPI CTF SQD OFC OSP GSG GSM
Cu (µg g^{-1} ww)
0
10
20
30
40
a
b
c
cd
d
d
cdefg
e
eg
fg
f
h
i
ai
j
k
l
d
PIL MKR SRM SBS MGR SBR DOG HAK WTG JDO SOL SPI CTF SQD OSP OFC GSG GSM
Fe (µg g^{-1} ww)
0
20
40
60
a
b
cdef
f
f
df
c
ef
bdef
cd
gh
g
h
eb
PIL MKR DOG SRM SBR SBS MGR HAK SOL WTG JDO SPI SQD CTF GSG OFC OSP GSM
Mn (µg g^{-1} ww)
0
5
10
a
b
bcd
c
de
ef
efg
f
fg
g
g
ah
h
bc
i
j
k
a
PIL DOG SBR SRM MKR WTG JDO MGR SOL SBS HAK SPI SQD CTF OSP OFC GSG GSM
Se (µg g^{-1} ww)
0.0
0.5
1.0
1.5
2.0
a
a
b
bcd
b
cb
cb
c
ce
ce
e
f
c
g
f
a
ad
bceg
MGR SBR PIL SOL SBS JDO SRM WTG MKR DOG HAK SPI SQD CTF GSG OSP OFC GSM
Zn (µg g^{-1} ww)
0
100
200
300
400
a
b
bc
bcd
cd
de
de
e
ef
ef
f
g
a
a
h
h
g
i
DOG PIL SBR MKR SOL SRM SBS MGR WTG HAK JDO SPI SQD CTF OSP OFC GSG GSM


Improvement in relative risk (%)
150
100
50
0
-50
MKR
SRM
PIL
MGR
HAK
WTG
JDO
SBR
SOL
SBS
DOG
SPI
SQD
CTF
GSG
OSP
OFC
GSM

Declaration of interests

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

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: